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Timothy M. Acker Date

Structural Determinants of Activity, Mechanism and Structure Activity Relationships of Novel GluN2C/D Subunit Selective Antagonists of the N-methyl-D-Aspartate Receptor

By

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Doctor of Philosophy

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An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
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Molecular Systems Pharmacology
2013

Abstract

Structural Determinants of Activity, Mechanism and Structure Activity Relationships of Novel GluN2C/D Subunit Selective Antagonists of the N-methyl-D-Aspartate Receptor

By Timothy M. Acker

The N-methyl-D-Aspartate (NMDA) receptors are ionotropic glutamate receptors whose family members, identified by sequence homology and pharmacology, comprise the 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA), kainate and delta receptors. The NMDA receptors respond to the co-agonists glycine and glutamate and mediate the slow component of excitatory neurotransmission throughout the central nervous system (CNS). The functional NMDA receptor is a hetero-tetramer consisting of two GluN1 subunits which bind to glycine and two GluN2 subunits (GluN2A-D). Both the Glun1 subunits, which have eight different splice variants, and the GluN2 subunits, which are encoded by four distinct genes, can impart various unique functional and pharmacological properties to the functional receptor, with the GluN2 subunits having a greater impact on the various different properties. While the receptors have been known and studied intensely for several decades, until recently, subunit-selective pharmacological tools remained elusive since the 1980s when the first selective agent targeting GluN2B receptors was discovered and characterized. This dissertation describes novel subunit-selective allosteric modulators which target the GluN2C- and GluN2D-containing NMDA receptors. The findings include the identification of key structural determinants of activity for one of the classes described, the identification of highly potent and selective congeners within the same class, the stereochemical preference of one of the more potent and selective members of the small molecules, the beginning of the physicochemical property optimization of the molecules and data and hypotheses suggesting that distinct classes of molecules bind to a shared, or overlapping site at the GluN2D containing receptors.

Structural Determinants of Activity, Mechanism and Structure Activity Relationships of Novel GluN2C/D Subunit Selective Antagonists of the N-methyl-D-Aspartate Receptor

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Figures and Tables

List of Abbreviations

5,7-DCKA	5,7-dichlorokynurenic acid
ACBC	1-aminocyclobutane-1-carboxylic acid
ACEA-1011	5-nitro-6,7-dichloro-1,,4-dihydro-2,3-quinoxalinedione
ACN	Acetonitrile
ACPC	1-aminocyclopropane-1-carboxylic acid.
aCSF	artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
Anal.	Analytical elemental analysis
APV (or AP5)	2-amino-5-phosphonopentanoate
AP7	2-amino-7-phosphonopentanoate
ATD	Amino terminal domain
BBB	Blood-brain barrier
BH3-DMS	Borane-dimethyl sulfide
BHK	Baby hamster kidney
Boc	Tert-butyl carbamate
Cacld	Calculated
$CDCl_3$	Deuterated chloroform
CGS-19755	(2R, 4S)-4-(phosphonomethyl)piperidine-2-carboxylic acid
cis-ACPD	(1R,3R)-aminocyclopentane-cis-dicarboxylate
CLogP	Calculated log P
CNS	Cenral nervous system
CNS-1102	N-(1-naphthyl)-N'-(3-ethylphenyl)-N'-methylguanidine hydrochloride
CP 101,606	(S,S)-1-(4-hydroxyphenyl)-2-[4-hydroxy-4-phenylpiperidin-1-yl)-1-propanol methanesulfonate trihydrate
R-CPP	(R)-4-(3-phosphonopropyl) piperazine-2-carboxylic acid
CTD	Carboxyl-terminal domain
CIQ	3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone
DCM	dichloromethane
DMAP	Dimethyl amino pyridine
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DQP	Dihydro-quinolone pyrazoline
DQP-1105	4-(5-(4-bromophenyl)-3-(6-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxobutanoic acid
EC_{50}	Half-maximal effective concentration
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide)
e.e.	Enantiomeric excess
EPSC	Excitatory postsynaptic current
ET_3N	Triethylamine

EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and drug administration
GPe	External globus pallidus
GPi	Internal globus pallidus
GV150,526A	(3-[2-(phenylaminocarbonyl)ethyl]-4,6-dichloroindole-2-carboxylic acid
HA 966	(+)-(1-hydroxy-3-aminopyrrolidine-2-one)
HRMS	High resolution mass spectrometer
IC_{50}	Half-maximal inhibitory concentration
iGluR	Ionotropic glutmate receptor
KA	Kainate
L-689,560	4-trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline
L-CCG-IV	(2S,3R,4S)-2-(carboxycyclopropyl)glycine
LC-MS/MS	liquid chromatography mass spec-mass spec
LBD	Ligand-binding domain
LTD	long-term depression
LTP	long-term potentiation
LIVBP	Leucine/isoleucine/valine binding protein
MDR1-MDCK	Madin-Darby canine kidney cells transfected with the human MDR1 gene
MeOH	Methanol
MHz	Megahertz
MK-801	(5R,10S)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine
mRNA	Messenger ribonucleic acid
MsCl	Methanesulfonyl-chloride
NaOH	Sodium hydroxide
NHP5G	(RS)-2-(N-hydroxypyrazol-5-yl)glycine
NIMH	National Institute of Mental Health
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
NVP-AAM077	(R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid
PBPD	(2S,3R)-1-(biphenyl-4-carbonyl)piperazine-2,3-dicarboxylic acid
Papp	Permeability coefficient
PCA	1-phenylcyclohexylamine
PCP	phencyclidine
PDSP	Psychoactive Drug Screening Program
Pgp	P-glycoprotein
PMPA	(RS)-4-(phosphonomethyl)-piperazine-2-carboxylic acid
PPDA	(2S,3R)-1-(phenanthren-2-carbonyl)piperazine-2,3-dicarboxylic acid
(R)-NHP4G	(R)-2-(N-hydroxylpyrazol-4-yl)glycine

Ro 25-6981	[R-(R*,S*)]-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride
QNZ	Qunazilinone-4-one
QNZ46	(E)-4-(6-methoxy-2-(3-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)-benzoic acid
QSAR	Quantitative structure activity relationship
Ro5	Lipinski's rule of five
SAR	Structure activity relationship
STN	Subthalamic nucleus
SYM-2081	(2*S*,4*R*)-4-methylglutamate
TCN-201	3-Chloro-4-fluoro-*N*-[4-[[2-(phenylcarbonyl)hydrazino]carbonyl]benzyl]benzenesulfonamide
TEVC	Two-electrode voltage clamp
TFA	Trifluoro acetic acid
THF	Tetrahydrofuran
TMD	Transmembrane domain
TPSA	Topological polar surface area
trans-ACBD	Trans-1-aminocyclobutane-1,3-dicarboxylate
UBP141	(2R,3S)-1-(phenanthrenyl-3-carbonyl)piperazine-2,3-dicarboxylic Acid

Chapter 1: Introduction

1.1. Abstract

The NMDA receptor family is crucial to excitatory neurotransmission throughout the central nervous system. The GluN2C- and GluN2D-containing NMDA receptors are temporally and spatially regulated in their expression throughout development and persist into the adult brain. The availability of highly potent and selective pharmacological probes with which to study these receptors has remained elusive until recently. This thesis describes the structural determinants of selectivity for a representative member of a novel class of negative allosteric modulators of the GluN2C- and GluN2D-containing receptors and the development of highly potent and selective members of the same class of compounds. I develop a quantitative-structure activity relationship (QSAR) model for another class of subunit-selective modulators, compare the chemical similarity between the two classes of compounds and contribute to the structure-activity relationship of a third class of molecules. The compounds discovered in this thesis will be invaluable pharmacological probes with which to dissect the roles the GluN2C- and GluN2D-containing receptors have in the regions of the brain where they are expressed and functional.

1.2 Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is a member of the ionotropic glutamate receptor (iGluR) family. The iGluR family also comprises the related glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate, which are distinguished on the basis of sequence identity and pharmacology

(Traynelis et al., 2010). NMDA receptors mediate a slow, Ca^{2+}-permeable component of excitatory neurotransmission throughout the central nervous system (CNS). Functional NMDA receptors consist of two glycine-binding GluN1 subunits encoded by eight splice variants of a single gene, and two glutamate-binding GluN2 (A-D) subunits, arising from four genes (Chatterton et al., 2002; Durand et al., 1992; Hollmann and Heinemann, 1994; Monyer et al., 1994; Santangelo et al., 2012; Traynelis et al., 2010).

Amongst the iGluR family, NMDA receptors uniquely require both glutamate, which binds at the GluN2 subunit, and glycine, which binds the GluN1 subunit, as co-agonists for receptor activation (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). Following channel opening, membrane depolarization is required to relieve voltage-dependent Mg^{2+} block before ions can permeate the channel pore (Mayer et al., 1984; Nowak et al., 1984; Santangelo et al., 2012). The NMDA receptor subtypes each have diverse functional and pharmacological properties (Neyton and Paoletti, 2006; Ogden and Traynelis, 2011; Paoletti, 2011; Santangelo et al., 2012; Traynelis et al., 2010).

The GluN1 subunit has been shown to affect the deactivation time course, pharmacology and intracellular binding proteins of the functional NMDA receptors (Ehlers et al., 1998; Hollmann et al., 1993; Johnson et al., 1996; Logan et al., 1999; Traynelis et al., 2010; Zukin and Bennett, 1995). However, it is generally thought that the GluN2 subunit has a more profound influence on the functional differences between NMDA receptors (Erreger et al., 2004; Monyer et al., 1994; Traynelis et al., 2010; Vicini et al., 1998). For example, GluN2D-containing receptors deactivate 50 times more slowly than GluN2A-containing receptors (Monyer et al., 1994; Vance KM, 2011; Vicini

et al., 1998; Wyllie et al., 1998; Yuan et al., 2009). GluN2D-containing receptors also have a much higher affinity for both glutamate and glycine than any other receptor combination (Chen et al., 2008; Erreger et al., 2007; Matsui et al., 1995; Traynelis et al., 2010).

In this thesis, I will describe the mechanism and structural determinants of selectivity for a novel class of GluN2C- and GluN2D-containing subunit-selective antagonists of the NMDA receptor. I will also describe the synthesis and structure activity relationships of the same class of compounds, identify several highly potent and selective congeners, show the stereo-chemical preference for a representative member of the class and describe a preliminary pharmacophore model based on my findings. I will further describe a series of computational and synthetic experiments that evaluate the hypothesis that two structurally distinct classes of compounds share an overlapping binding site at the receptor. Finally, I will describe the synthesis and rationale for developing another class of ligands, which are also GluN2C- and GluN2D-preferring subunit-selective antagonists. The compounds and pharmacophores developed in this thesis will allow for the rapid expansion of our understanding of the physiological and patho-physiological roles that GluN2C- and GluN2D-containing receptors play in the CNS.

The remainder of the introduction to this thesis will summarily describe key aspects of NMDA receptor function and pharmacology as well as explain the rationale for development of these agents and their potential utility. I will also provide a background on general approaches employed in this body of work, which aimed to

improve upon the pharmacological activity and profile of these novel negative allosteric modulators.

1.3 NMDA Receptor Topology

1.3.a. Subunit arrangement and stoichiometry

Each subunit of the NMDA receptors is made up of semi-autonomous domains (**Figure 1.1**). The amino terminal domain (ATD), the ligand binding domain (LBD) and the transmembrane domain (TMD) are each thought to have binding sites and structural determinants that are responsible for the actions of endogenous and exogenous modulatory ligands (Traynelis et al., 2010). The carboxy terminal domain (CTD) is known to bind intracellular proteins such as calmodulin and PSD-95, and is subject to post-translational modifications that alter the function of the receptor (**Figure 1.1**) (Traynelis et al., 2010).

Two GluN1 subunits and two GluN2 subunits assemble to form the functional NMDA receptor (Monyer et al., 1992; Schorge et al., 2005). Uniquely among the iGluR family, NMDA receptors require the co-agonist binding of glutamate and glycine, with the GluN2 subunits binding glutamate (Furukawa et al., 2005; Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Nicholls, 1989). Two GluN1 subunits may combine with two different GlunN2 so that a tri-heteromeric receptor is formed, however, the functional, pharmacological and physiological implications of these arrangements are less well understood than that of the di-heteromeric receptors comprised of two GluN1 and two similar GluN2 subunits (Chazot and Stephenson, 1997; Dunah et al., 1998; Hatton and Paoletti, 2005; Luo et al., 1997; Pina-Crespo and Gibb, 2002; Sheng et al., 1994; Sundstrom et al., 1997; Traynelis et al., 2010).

Figure 1.1. Linear amino acid sequence and structural homology model of an NMDA Receptor.

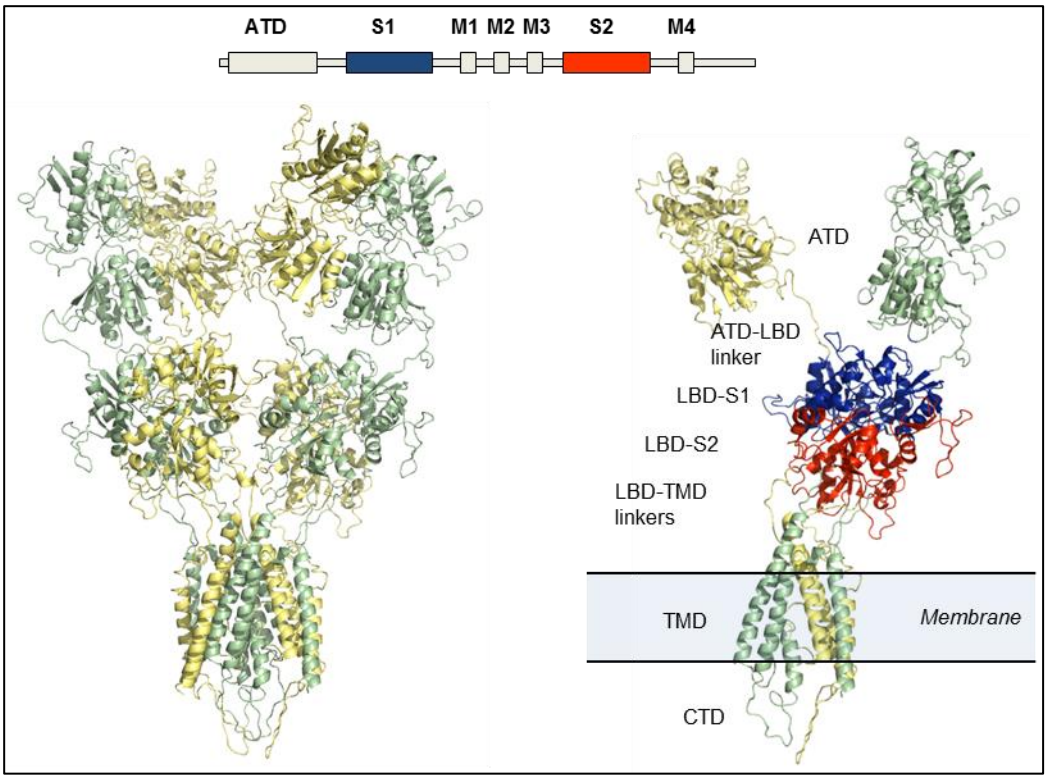


Figure 1.1. Linear amino acid sequence and structural homology model (Built by Pieter Burger; Acker et al., 2011) of an NMDA receptor depicting semi-autonomous domains. A. Linear diagram of the NMDA receptor subunit amino acid sequence. B. Homology model ribbon depiction of a full-length tetrameric NMDA receptor. The green subunits represent GluN2D and the yellow represent GluN2A. C. One GluN1 subunit and one GluN2 subunit depicting the symmetry and symmetry mismatch of the assembly as well as the semi-autonomous domains. (Reprinted from Santangelo et al., 2012, with permission).

An X-ray crystal structure of a full-length AMPA receptor has been diffracted at a resolution of 3.6 Å (Sobolevsky et al., 2009). The overall symmetry observed in the AMPA receptors is thought to be conserved with the NMDA receptors in the following way. The four subunits are thought to form a 4-fold axis of symmetry through the lipid bilayer which arises from three transmembrane helices and a re-entrant loop from each subunit (M1, M3, M4 and M2, respectively, **Figure 1.1**). The LBD is thought to have a 2-fold axis of symmetry and there is thought to be a symmetry mismatch that occurs between the ATD and LBD domains in the following way. There may be a subunit swap of amino terminal domains such that the LBD dimers are A/D, B/C (where A and C would each represent GluN1 subunits, while D and B would represent GluN2 subunits); the ATD dimers are thought to be A/C, B/D, also with an overall 2-fold axis of symmetry (**Figure 1.1**) (Sobolevsky et al., 2009).

1.3.b. The amino-terminal domain

The amino-terminal domain (ATD) has the lowest sequence homology of the semi-autonomous domains between the four GluN2 subunits (**Table 1.1**)(Traynelis et al., 2010). The ATD controls important pharmacological and functional properties (open probability, deactivation time course, agonist EC_{50}, and binding sites for endogenous and exogenous modulators) among the four GluN2 subunits (Gielen et al., 2009; Traynelis et al., 2010; Vance KM, 2011; Yuan et al., 2009).

The X-ray crystal structure of isolated and solubilized GluN2B ATD domain and GluN1/GluN2B heteromeric ATD dimers have been resolved (Karakas et al., 2009; 2011).

Table 1.1. Sequence identity and conservation of residues in glutamate receptor subunits.

Receptor subunits	ATD	S1	S2	LBD	M1M2M3	M4	TMD	CTD	All
GluN1 GluN2A-D GluN3A-B	1 (24)	19 (61)	18 (76)	19 (68)	16 (81)	10 (83)	14 (81)	0.0 (2.9)	5 (29)
GluN2A-D	19 (76)	60 (94)	66 (99)	63 (96)	75 (99)	69 (100)	73 (99)	2 (47)	25 (70)

Table 1.1. Sequence identity and conservation of residues in NMDA receptor subunits. Numbers are the percentage of residues in the regions that are identical in all subunits within the group. Numbers in parenthesis are the percentage of residues that are identical in 50% of the subunits in the group (i.e. conserved). ATD includes the signal peptide, M1M2M3 includes pre-M1 and intracellular loops, LBD is S1 and S2. TMD is M1M2M3 and M4. (Adapted with permission from Traynelis et al., 2010.)

These structural studies reveal a bi-lobed and clamshell like structure which shows topological similarity to both the leucine/isoleucine/valine binding protein (LIVBP) and the metabotropic glutamate receptor mGluR1a (Karakas et al., 2009; 2011; Kumar et al., 2009; Masuko et al., 1999; O'Hara et al., 1993; Traynelis et al., 2010).

The crystal structure of the isolated GluN2B ATD revealed several key features of the domain. When compared to the crystal structures of non-NMDA glutamate receptors, the GluN2B ATD crystal structure was significantly different in the overall architecture; the root-mean-square deviation comparison to that of the crystal structures for the AMPA and kainate amino-terminal domains suggests that the NMDA receptor ATD is rotated by 45º as compared to the AMPA receptor ATD and 54º as compared to the kainate receptor ATD (Karakas et al., 2009). This observation could help explain the regulatory roles of the ATD in the NMDA receptors as compared to those of the non-NMDA receptors, which are not known to regulate receptor function (Karakas et al., 2009).

Both GluN2A- and GluN2B-containing receptors are partially inhibited by zinc at physiological concentrations (Fayyuzuddin et al., 2000; Low et al., 2000; Paoletti and Neyton, 1997). The GluN2B ATD crystal structure also revealed that the binding site for this endogenous modulator is within the inter-domain clamshell region of the isolated domain (Karakas et al., 2009). This endogenous site of modulation is distinct from the binding site for the GluN2B-containing receptor subunit selective antagonist ifenprodil, which binds within the dimer interface between the GluN1 and GluN2B ATD (Karakas et al., 2011).

It has been shown that splice variants of the GluN1 subunit which include exon 5, a 21-amino acid segment located in the ATD, controls inhibition of GluN2B receptors by protons and zinc, as well as potentiation by spermine, glutamate EC_{50} and deactivation time-course of those receptors (Rumbaugh et al., 2000; Traynelis et al., 1998; Traynelis et al., 1995; Traynelis et al., 2010). Inclusion of exon 5 in GluN1 receptors also doubles the open probability of GluN1/GluN2D-containing receptors while accelerating the deactivation time course (Vance et al., 2011).

It has also been shown that the identity of the GluN2 subunit ATD has a profound effect on the functional and pharmacological properties of NMDA receptors. While the GluN2 receptor ATD is not required for recombinantly expressed receptors to reach the cell surface, these receptors have different functional and pharmacological features than those receptors with an ATD intact (Gielen et al., 2009; Yuan et al., 2009). Furthermore, replacement of the ATD of one GluN2 receptor subunit with that of another GluN2 subunit causes drastic changes in the agonist EC_{50} values, deactivation time course and open-probability of the receptors toward that of the receptor whose ATD was installed on the other GluN2 subunit (Gielen et al., 2009; Yuan et al., 2009). These observations suggest that while the domain is not required, its presence is crucial to the functional properties and pharmacology of the native receptors.

1.3.c. The ligand binding domain

The ligand binding domains of one GluN1 and one GluN2 subunit form a back-to-back dimer (Furukawa et al., 2005) (**Figure 1.2**).

Figure 1.2. Homology model of GluN1/GluN2D ligand binding domain interface and transmembrane linker regions.

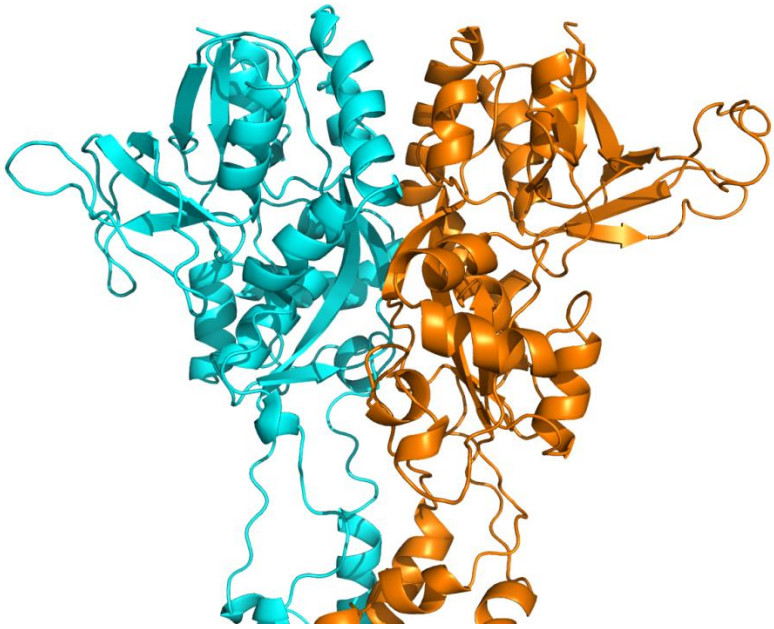


Figure 1.2. The homology model of GluN1/GluN2D was derived from the crystallographic coordinates of the full length AMPA receptor, the GluN1-GluN2B ATD and the GluN1-GluN2D-LBD by Dr. Pieter Burger as described in Acker et al., 2011. GluN2D is represented in blue and GluN1 in orange. The cartoon illustration of the back to back interface of a GluN1-GluN2D ligand binding domain and the transmembrane linker regions is shown. Upon agonist binding to the clamshell region, a conformational shift occurs and the gating steps are initiated. Image created with PyMol (PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).

The arrangement of the dimer is similar to that observed for AMPA and kainate receptors (Armstrong and Gouaux, 2000; Furukawa et al., 2005; Kumar et al., 2011; Sobolevsky et al., 2009). The LBD is formed by two segments of the polypeptide chain that are separated by regions encoding the S1-M1, S2-M4 and M3-S2 linker regions connecting the ligand binding domain as well as the trans-membrane helices (**Figure 1.1 and 1.2**).

Agonist binding to the GluN1 and GluN2 subunits is well understood and has been studied structurally (Armstrong and Gouaux, 2000; Furukawa et al., 2005; Mayer et al., 2006; Vance et al., 2011). Agonist binding at both GluN1 and GluN2 subunits occurs at the cleft between the S1 and S2 domains (**Figure 1.2**) (Furukawa and Gouaux, 2003; Furukawa et al., 2005; Inanobe et al., 2005). Upon agonist binding, the LBD is thought to undergo a conformational shift and domain closure that transmits the consequences of the binding event to the transmembrane region, allowing for the channel pore to open (Erreger et al., 2004; Furukawa and Gouaux, 2003; Inanobe et al., 2005; Mayer et al., 2006; Traynelis et al., 2010; Vance et al., 2011). The binding of agonist (partial or full) and the corresponding degree of domain closure does not correlate with the degree of channel opening, rather partial agonists are hypothesized to have a smaller effect on the equilibrium constant from the bound-closed conformation to the bound-open conformation than that of full-agonists (Inanobe et al., 2005).

1.3.d. The trans-membrane linker regions and membrane spanning domains

The membrane spanning helices plus a re-entrant loop of the AMPA receptor subunits are arranged in a fashion similar to an inverted potassium channel, and the high degree of sequence similarity (**Table 1.1**) in this region across the glutamate receptor

family suggests a similar arrangement for the NMDA receptor channel pore (Kuner et al., 2003; Santangelo et al., 2012; Sobolevsky et al., 2009; Wo and Oswald, 1995). Each receptor subunit has three transmembrane helices and a re-entrant loop which lines the channel pore (**Figure 1.1**). It is thought that the conformational changes associated with ligand binding and domain closure of the LBD cause movement in the LBD-TM linker regions and subsequent gating of the four overlapping M3-transmembrane helices which form and open the channel pore (Jones et al., 2002; Sobolevsky et al., 2009; reviewed in Traynelis et al., 2010).

The linker regions connecting the LBD and trans-membrane regions have been proposed to comprise/influence the binding of ligands that could either positively or negatively modulate receptor function (Acker et al., 2011; Krupp et al., 1998; Santangelo et al., 2012; Sobolevsky et al., 2009; Talukder et al., 2010; Yuan et al., 2005). Observations made using small cysteine modifying reagents in these regions can have differential effects on the receptor's open probability (Jones et al., 2002; Sobolevsky et al., 2002; Talukder et al., 2010; Yuan et al., 2005). That is, certain cysteine mutations within these regions, when reacted with a modifying reagent, will increase the open probability while other residues can cause inhibition of the response to maximally effective concentrations of glutamate and glycine when reacted with the same, or similar reagents (Jones et al., 2002; Sobolevsky et al., 2002; Talukder et al., 2010; Yuan et al., 2005). These regions have also previously been implicated as structural determinants of activity for endogenous modulatory ions; tonic inhibition of NMDA receptors by protons is influenced by mutations of residues within the linker regions connecting the LBD to

the TM domains, as well as within the LBD dimer interface (Gielen et al., 2009; Low et al., 2003; Santangelo et al., 2012).

1.3.e. The carboxy-terminus domain

The C-terminal domain shows low sequence homology between various GluNX subunits (**Table 1.1**) (Traynelis et al., 2010). This region of the receptor can bind to a variety of intracellular scaffolding proteins and is a target for post-translational modification, such as phosphorylation (Santangelo et al., 2012; Traynelis et al., 2010). Modulation of the protein-protein interactions that occur between the intracellular C-terminus and multiple binding partners can impact cellular localization and thus represent an intriguing new therapeutic strategy for regulating NMDA receptors (Cook et al., 2012; Kornau et al., 1995; Niethammer et al., 1996; Santangelo et al., 2012; Traynelis et al., 2010).

1.4. NMDA receptor pharmacology

1.4.a. NMDA receptor agonists

The ligand binding domain has been studied and explored thoroughly for agonists, partial and full, that act at either the glutamate binding site of the GluN2 subunits, or the glycine binding site of the GluN1 subunits. Since the identification of glycine as a requisite co-agonist, many efforts have been aimed at exploiting the pharmacological potential of the agonist binding pocket (Kleckner and Dingledine, 1988). The co-crystal structure of glycine and other glycine site modulators bound to the GluN1 ligand binding domain revealed the key structural determinants of binding between the subunit and the obligate co-agonist (Furukawa and Gouaux, 2003). Compounds acting at the glycine binding site of the GluN1 subunit have a broad range of potency and efficacy and include

endogenous and exogenous ligands (**Table 1.2**) (Traynelis et al., 2010). Notably, D-serine, which has a similar potency and efficacy profile to that of glycine, may be the principle glycine site agonist in certain regions of the brain (Basu et al., 2009; McBain et al., 1989; Panatier et al., 2006; Pullan et al., 1987). Interestingly, the identity of the GluN2 subunit present in the functional receptor has a profound impact on the potency and efficacy of the glycine site agonists (**Table 1.2**) (Chen et al., 2008; Furukawa and Gouaux, 2003; Kuryatov et al., 1994; Traynelis et al., 2010; Wafford et al., 1995).

Extensive studies have also identified endogenous and exogenous ligands that are partial or full agonists at the glutamate binding site of GluN2 subunits (**Table 1.3**) (Clausen et al., 2008; Fleck et al., 1993; Patneau and Mayer, 1990; Priestley et al., 1995; Shinozaki et al., 1989; Sivaprakasam et al., 2009; Traynelis et al., 2010). The co-crystal structures of agonist bound GluN1-GluN2 LBD heterodimers has revealed the structural determinants of agonist binding (Furukawa and Gouaux, 2003; Vance et al., 2011). While L-glutamate is thought to be the principle excitatory agonist in the brain for the GluN2 subunits, D- and L-aspartate, cysteinesulfinate and homocysteate are also endogenous agonists of GluN2 subunits (Do et al., 1988; Do et al., 1986; Nicholls, 1989; Olney et al., 1987; Patneau and Mayer, 1990; Traynelis et al., 2010).

Table 1.2. Glycine site agonists**.**

Glycine-site agonist	GluN2A μM (efficacy)	GluN2B μM (efficacy)	GluN2C μM (efficacy)	GluN2D μM (efficacy)
Glycine	1.1 (100)	0.72 (100)	0.34 (100)	0.13 (100)
L-serine	212 (95)	77 (98)	27 (110)	15 (98)
D-serine	1.3 (98)	0.65 (96)	0.32 (110)	0.16 (93)
L-alanine	96 (79)	36 (65)	28 (92)	13 (97)
D-alanine	3.1 (96)	0.89 (84)	0.56 (96)	0.22 (99)
D-cycloserine	19 (90)	8.2 (65)	3.3 (190)	2.9 (94)
HA 966	12 (12)	4.6 (14)	--	--
β-Cl-D-alanine	21 (84)	9.9 (88)	3.7 (79)	1.7 (81)
β-F-DL-alanine	11 (92)	0.98 (88)	0.40 (84)	0.40 (91)
tri-F-DL-alanine	1.3 (130)	0.65 (64)	0.32 (110)	0.16 (93)
ACPC	1.3 (79)	0.65 (89)	0.35 (88)	0.083 (89)
ACBC	45 (13)	6.6 (33)	--	--

Table 1.2. Percentage relative efficacy (in parenthesis) is the current response to a maximally effective concentration of agonist relative to the response to a maximally effective concentration of glycine. All values are from recombinant rat NMDA receptors expressed in *X. laevis* oocytes and coactivated by glutamate (Chen et al., 2008) except HA 966 and ACBC (Priestley et al., 1995). Values are given to 2 significant digits. Abbreviations: HA 966, (+)-(1-hydroxy-3-aminopyrrolidine-2-one); ACPC, 1-aminocyclopropane-1-carboxylic acid; ACBC, 1-aminocyclobutane-1-carboxylic acid. (Adapted from Traynelis et al., 2010, wtih permission)

Table 1.3. Glutamate site agonists**.**

Glutamate-site agonist	GluN2A μM (efficacy)	GluN2B μM (efficacy)	GluN2C μM (efficacy)	GluN2D μM (efficacy)
L-glutamate	3.3 (100)	2.9 (100)	1.7 (100)	0.51 (100)
D-glutamate	250 (99)	160 (120)	110 (100)	42 (110)
L-aspartate	48 (99)	14 (78)	41 (110)	12 (91)
D-aspartate	30 (103)	10 (91)	9.3 (99)	2.1 (90)
***N*-methyl-L-aspartate**	580 (99)	130 (69)	150 (66)	40 (75)
***N*-methyl-D-aspartate**	94 (93)	30 (78)	22 (86)	7.3 (92)
SYM2081	140 (72)	25 (89)	18 (71)	3.2 (75)
***cis*-ADA**	890 (100)	220 (95)	80 (81)	32 (140)
***trans*-ADC**	470 (38)	170 (48)	95 (73)	50 (80)
***cis*-ACPD**	61 (76)	21 (75)	22 (49)	11 (77)
***Cis*-2,3-piperidinedicarboxylic acid**	21 (3)	38 (7)	--	--
(*R*)-NHP4G	150 (33)	61 (76)	120 (54)	48 (77)
(*RS*)-ethyl-NHP5G	47 (5)	68 (45)	91 (52)	43 (70)
(*R*)-propyl-NHP5G	NE	105 (6)	429 (22)	153 (37)

Table 1.3. Percentage relative efficacy (in parenthesis) is the current response to a maximally effective concentration of agonist relative to the response to a maximally effective concentration of glutamate. All values are from recombinant rat NMDA (GluN1 plus the indicated GluN2) receptors expressed in *X. laevis* oocytes activated by agonist plus maximally effective concentration of glycine. Data are EC_{50} in μM. Efficacy is given as the current response to a maximally effective concentration of agonist relative to the response to maximally effective concentration of glutamate. All values are from recombinant rat NMDA (GluN1 plus the indicated GluN2) receptors expressed in *Xenopus laevis* oocytes activated by agonist plus maximally effective concentration of glycine. NHP5G, Et-NHP5G, and Pr-NHP5G values from (Clausen et al., 2008). L-*trans*-ADC values from (Sivaprakasam et al., 2009). *Cis*-2,3-piperidinedicarboxylic acid from (Priestley et al., 1995). All remaining values from (Erreger et al., 2007). Values are given to 2 significant digits. Abbreviations: SYM2081, (2S,4R)-methylglutamate; L-CCG-IV, (2S,3R,4S)-2-(carboxycyclopropyl)glycine; *trans*-ACBD, trans-1-aminocyclobutane-1,3-dicarboxylate; *cis*-ADA, cis-azetidine-2,4-dicarboxylic acid; *trans*-ADC, trans-azetidine-2,3-dicarboxylic acid; *cis*-ACPD, (1R,3R)-aminocyclopentane-cis-dicarboxylate; (*R*)-NHP4G, (*R*)-2-(*N*-hydroxylpyrazol-4-yl)glycine; NHP5G, (RS)-2-(*N*-hydroxypyrazol-5-yl)glycine. (Adapted from Traynelis et al., 2010, wtih permission).

Synthetic efforts around L-glutamate have led to the identification of a host of compounds that have varying potency and efficacy profiles that are similar to those with the glycine site agonists in that the glutamate site agonists are typically most potent when GluN2D is present and least potent when GluN2A is present (**Table 1.3**)(Clausen et al., 2008; Sivaprakasam et al., 2009; Traynelis et al., 2010). While subunit selectivity has remained a challenge for the glutamate site agonists due to the high sequence similarity within the agonist binding domain (**Table 1.1**), some subunit selectivity can be seen with the N-hydroxypyrazol-5-yl glycine (NHPG) analogs and the azetidine containing analogs (**Table 1.3.**) (Clausen et al., 2008; Sivaprakasam et al., 2009; Traynelis et al., 2010). For example, the racemic mixture of Amino-(1-hydroxy-4-ethyl-pyrazol-3-yl)-acetic Acid Hydrochloride ((*RS*)-ethyl-NHP5G; **Table 1.3**) exhibits less than two-fold selectivity for GluN2A- and GluN2D-containing receptors over GluN2C-containing receptors with similar efficacy at GluN2B-, GluN2C- and GluN2D- containing receptors and reduced efficacy at GluN2A-containing receptors (Clausen et al., 2008). The (*R*)-enantiomer of 2-(*N*-hydroxylpyrazol-4-yl)glycine ((*R*)-NHP4G; **Table 1.3**) exhibits close to three-fold selectivity for GluN2D-containing receptors over GluN2C- and GluN2A-containing receptors and has increased efficacy at GluN2A-containing receptors as compared to the racemic (*RS*)-ethyl-NHP5G (Clausen et al., 2008). The potency profiles of the azetidine analogs (*cis*-azetidine-2,4-dicarboxylic acid; *cis*-ADA and *trans*-azetidine-2,3-dicarboxylic acid; *trans*-ADC; **Table 1.3**) have a clear trend preferring GluN2C- and GluN2D-containing receptors over GluN2A- and GluN2B-containing receptors (Sivaprakasam et al., 2009).

1.4.b Competitive antagonists of the NMDA receptor

Beginning in the 1980s and persisting into the early 1990s, pharmaceutical companies pursued competetive antagonists of the NMDA receptors as a therapeutic strategy for indications such as traumatic brain injury and ischemic cell death (Foster and Kemp, 1989; Leeson et al., 1991). These pursuits were based on the observations that glutamate and related analogs were excitotoxic and caused neuronal cell death that is Ca^{2+} dependent (Rothman and Olney, 1986). The initial attempts to develop these drugs were slowed by unsuccesful clinical trials, which can be partly attributed to the adverse side-effects of global antagonism of the NMDA receptors, but also had low-patient inclusion and flawed dosing regimens (Chen and Lipton, 2006; Gladstone et al., 2002; Kalia et al., 2008; Muir, 2006).

As a result of these efforts however, numerous different competetive antagonists exist for both the glutamate binding site of GluN2 subunits and the glycine binding site of GluN1 subunits (**Table 1.4**)(Traynelis et al., 2010). The conserved sequence in the glutamate binding sites across the GluN2 subunits (**Table 1.1**) has impeded the development of subunit-selective competitive antagonists that bind within the LBD of NMDA receptors (Furukawa et al., 2005; Kinarsky et al., 2005). Modest subunit selectivity has been obtained in some of the phosphonate containing compounds such as (*R*)-2-amino-5-phosphonopentanoate (AP5; **Table 1.4**) and (*R*)-2-amino-7-phosphonopentanoate (AP7; **Table 1.4**) (Olverman et al., 1988).

Table 1.4. Competitive antagonists of the NMDA receptor**.**

Competitive Antagonist	Site	GluN2A	GluN2B	GluN2C	GluN2D
			Ki, μM		
7-CKA[a]	GluN1	0.6	0.2	--	--
5,7-DCKA[b]	GluN1	0.03	0.05	0.17	0.09
CGP-61594(K_b)[b]	GluN1	0.43	0.045	0.16	0.34
CGP-58411(K_b)[c]	GluN1	0.24	0.13	--	--
ACEA-1011(K_b)[e]	GluN1	0.33	0.46	0.21	0.74
ACEA-1021[f]	GluN1	0.004	0.004	0.003	0.011
L-689,560(K_b)[c]	GluN1	0.004	0.02	--	--
L-701,324[a]	GluN1	0.005	0.005	--	--
(R)-AP5[g]	GluN2	0.28	0.46	1.6	3.7
(R)-AP7[g]	GluN2	0.49	4.1	6.4	17
PMPA[g]	GluN2	0.84	2.7	3.5	4.2
(R)-CPP[g]	GluN2	0.041	0.27	0.63	1.99
NVP-AAM077(K_b)[h]	GluN2	0.015	0.078	--	--
UBP141[j]	GluN2	14	19	4.2	2.8
CGS-19755 (selfotel)[g]	GluN2	0.15	0.58	0.58	1.1

Table 1.4. Data presented as K_i in μM, except where indicated K_b. Abbreviations: 7-CKA, 7-chlorokynurenic acid; 5,7-DCKA, 5,7-dichlorokynurenic acid; CGP 61594, (±)-trans-4-[2-(4-azidophyenyl)acetylamino]-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid; GV150,526A, (3-[2-(phenylaminocarbonyl)ethyl]-4,6-dichloroindole-2-carboxylic acid; ACEA-1011, 5-Chloro-7-trifluoromethyl-1,4-dihydro-2,3-quinoxalinedione; ACEA-1021, 5-nitro-6,7-dichloro-1,,4-dihydro-2,3-quinoxalinedione; (R)-AP5, (R)-2-amino-5-phosphonopentanoate; L-689,560, 4-*trans*-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline; (R)-AP7, (R)-2-amino-7-

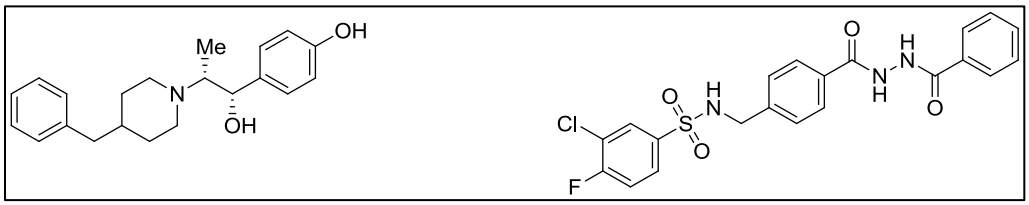
phosphonopentanoate; PMPA, (RS)-4-(phosphonomethyl)-piperazine-2-carboxylic acid; (R)-CPP, (R)-4-(3-phosphonopropyl) pizerazine-2-carboxylic acid; NVP-AAM077, (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid; PPDA, (2S,3R)-1-(phenanthren-2-carbonyl)piperazine-2,3-dicarboxylic acid; (R)-α-AA, (R)-a-aminoadipate; PBPD, (2S,3R)-1-(biphenyl-4-carbonyl)piperazine-2,3-dicarboxylic acid; UBP141, (2R,3S)-1-(phenanthrenyl-3-carbonyl)piperazine-2,3-dicarboxylic Acid; CGS-19755, (2R, 4S)-4-(phosphonomethyl)piperidine-2-carboxylic acid. *a* K_B values from Cheng-Prusoff correction of IC_{50} values measured for inhibition of glycine-activated currents in mouse L(tk–) cells (Priestley et al., 1995), *b* (Hess et al., 1998), *c* (Hess et al., 1996), *d* (Chopra et al., 2000), *e* (Woodward et al., 1995b), *f* (Woodward et al., 1995a), *g* (Feng et al., 2005), *h* (Frizelle et al., 2006), *i* (Feng et al., 2004), *j* (Morley et al., 2005)(Adapted from Traynelis et al., 2010, wtih permission).

1.4.c. Noncompetitive modulators of the NMDA receptor

A number of endogenous and exogenous modulatory ligands are thought to bind to the extracellular ATD of the various GluN1-GluN2 receptors. The discovery of Ifenprodil (**Figure 1.3**) as a subunit-selective antagonist of GluN2B-containing receptors and the development of analogs (**Table 1.5**) related to the compound has allowed for many studies that have deepened our understanding of the GluN1-GluN2B containing NMDA receptors, as well as numerous clinical trials investigating the utility of GluN2B-subunit selective antagonists (Mony et al., 2009; Traynelis et al., 2010; Williams, 1993). The compounds have been used to understand the biophysical properties of the channels, investigate the contributions of the receptor in long-term potentiation (LTP), long-term depression (LTD), learning and memory formation, ischemic cell death, Parkinson's disease among many other observable phenomena, many of which suggest the NMDA receptors could be a tractable drug target (Dogan et al., 1997; Hoffmann et al., 2000; Mony et al., 2009; Neyton and Paoletti, 2006; Nikam and Meltzer, 2002; Traynelis et al., 2010; Zhou and Baudry, 2006).

Ifenprodil (**1; Figure 1.3**), an anti-hypertensive agent, binds selectively to the ATD of GluN2B-containing receptors and the co-crystal structure of this complex shows the ligand binding between the GluN1-GluN2B ATD heterodimer interface (Karakas et al., 2011). The more potent analogs that have been developed, including CP-101,606 and Ro 25-6981 (**Table 1.5**) are thought to bind to the same site at the receptor (Chenard et al., 1995; Fischer et al., 1997).

Figure 1.3. GluN2A and GluN2B subunit-selective modulators**.**



Ifenprodil (**1**) TCN-201 (**2**)

Figure 1.3. Subunit selective allosteric modulators of GluN2B and GluN2A containing receptors.

Table 1.5. IC_{50} values for noncompetitive GluN2B-selective NMDA receptor antagonists**.**

Noncompetitive antagonist	GluN2A (μM)	GluN2B (μM)	GluN2C (μM)	GluN2D (μM)
Ifenprodil	39	0.15	29	76
Ro 25-6981	52	0.0090	--	--
CP 101,606	>100	0.039	>100	>100
Eliprodil	>100	3.02	--	--
Felbamate	2600	520	2400	--
Haloperidol	NE	3	NE	NE

Table 1.5. Data are from rat recombinant receptors, except ifenprodil which was characterized on human recombinant receptors. NE, <10% inhibition at 300 μM. Data for ifenprodil are from (Hess et al., 1998). Values for Ro 25-6981 from (Fischer et al., 1997), CP 101,606 from (Mott et al., 1998), eliprodil from (Avenet et al., 1997), felbamate from (Harty and Rogawski, 2000), haloperidol from (Ilyin et al., 1996). Abbreviations: Ro 25-6981, [R-(R*,S*)]-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride; CP 101,606 (Traxoprodil mesylate), (S,S)-1-(4-hydroxyphenyl)-2-[4-hydroxy-4-phenylpiperidin-1-yl)-1-propanol methanesulfonate trihydrate (Adapted from Traynelis et al., 2010, wtih permission).

These more potent classes of compounds and other similar scaffolds have limited some of the off-target effects such as binding to the human ether-a-go-go channel and activity at the α1-adrenergic channel (Kawai et al., 2007; Mosley et al., 2009; Traynelis et al., 2010). While ifenprodil and closely related analogs generally do not exhibit the psycho-mimetic effects of phencyclidine and other non-selective inhibitors of NMDA receptor function, approved use of these drug-like compounds has yet to be achieved (discussed in ***Chapter 1.5.b***).

Recently, a class of compounds typified by TCN-201 (**Figure 1.3**), has been discovered and shown to be highly selective for GluN1-GluN2A containing receptors (Bettini et al., 2010). These compounds are glycine sensitive, negative allosteric modulators that have structural determinants of activity at the interface of the GluN2A-GluN1 LBD (Hansen et al., 2012). The mechanism of inhibition relies on conformational changes within the receptor that are glutamate dependent in that the onset of TCN-201 inhibition is significantly increased when glutamate is pre-applied to HEK cells that are recombinantly expressing the GluN1-GluN2A receptors (Hansen et al., 2012). However, it is glycine's affinity for the receptor that is reduced in the presence of increasing concentrations of the compound and vice-versa (Hansen et al., 2012). The implication of a modulatory binding site within the ligand binding domain interface of the NMDA receptors is a promising finding, as such a site on the AMPA receptors has yielded compounds that are or have been pursued in the clinic for several indications including depression and attention deficit/hyperactivity disorder (Chang et al., 2012; Hansen et al., 2012; Jin et al., 2005; Lees, 2000; Ward et al., 2010).

Recently, several classes of subunit-selective allosteric modulators have now been identified and characterized, including the previously identified AMPA antagonizing quinazolin-4-one (QNZ) scaffold, the dihydroquinilone-pyrazoline (DQP) class of compounds described in this thesis and the phenanthrene- or naphthalene-containing modulators (UBP) (**Figure 1.4**) (Acker et al., 2011; Costa et al., 2010; Hansen and Traynelis, 2011; Mosley et al., 2010). The unique structural determinants of action of these ligands suggests unique binding sites, which could establish a precedent for therapeutically relevant modulatory sites in regions other than the ATD (Acker et al., 2011; Fayyazuddin et al., 2000; Hansen and Traynelis, 2011; Horak et al., 2006; Jang et al., 2004).

The UBP-class of molecules exhibits complex pharmacology and SAR at the NMDA receptors (**Figure 1.4**; Costa et al., 2010). For example, UBP-512 (**Figure 1.4**) has been shown to be inhibitory at all four GluN2 receptor subtypes at low micromlar concentrations, and then also potentiate GluN2A- and GluN2B-containing receptors at higher micromolar concentrations (Costa et al., 2010). Some compounds in the phenanthranene series are negative allosteric modulators with modest subunit-selectivity for inhibition, however the subunit selectivity is variable; some compounds show preferential inhibition of GluN2C- and GluN2D-containing receptors while others show slight preferential inhibition of GluN2A-containing receptors over the other subtypes (Costa et al., 2010). The potency of compounds in the class is also variable from low to high micromolar ranges with no obvious trend that develops for either potentiation or inhibition (Costa et al., 2010).

Figure 1.4 Negative allosteric modulators of GluN2C- and Glun2D-containing receptors.

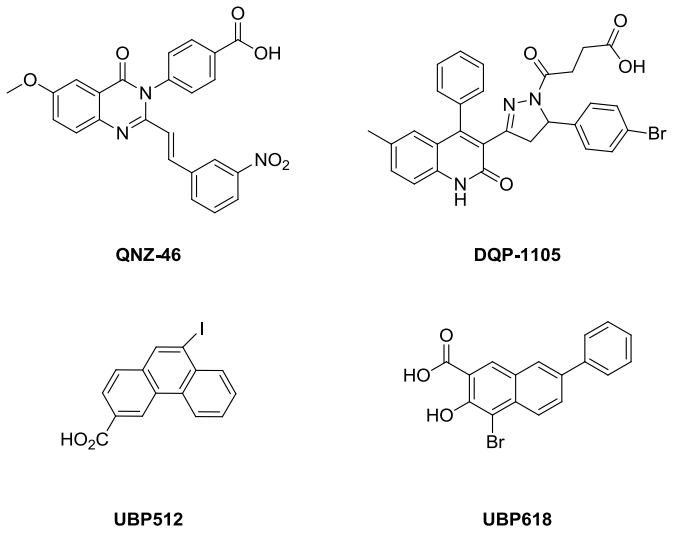


Figure 1.4 Negative allosteric modulators of GluN2C- and Glun2D-containing receptors. (Top Left) (E)-4-(6-methoxy-2-(3-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)-benzoic acid (QNZ-46) is a representative member of the quinazolin-4-one class of compounds. (Top Right) 4-(5-(4-bromophenyl)-3-(6-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (DQP-1105) is a representative member of the DQP-class of compounds. (Bottom left) 9-iodophenanthrene-3-carboxylic acid (UBP-512) is a phenanthranene containing representative member of the UBP-class of compounds. (Bottom Right) 1-bromo-2-hydroxy-6-phenylnaphthalene-3-carboxylic acid (UBP618) is a representative naphthalene containing member of the UBP-class of molecules.

A class of GluN2C- and GluN2D-subunit selective potentiators typified by (3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)-3,4-dihydroisoquinolin-2(1 H)-yl)methanone (CIQ) has been described mechanistically and through SAR efforts in the Traynelis and Liotta labs at Emory University (Mullasseril et al., 2010; Santangelo et al., 2013). CIQ has structural determinants of activity that reside within the M1 trans-membrane helix, a region not previously identified with any other modulatory ligands in the glutamate receptor family (Mullasseril et al., 2010). Analogs of CIQ also exhibit potentiation of only GluN2C- and GluN2D-containing receptors with potencies in the mid-nanomolar range (CIQ EC_{50}; 3μM) (Santangelo et al., 2013). This finding suggests that an allosteric modulatory site might exist for ligands that interact with this or adjacent portions of the NMDA receptor (Mullasseril et al., 2010). It remains unclear whether these compounds access a binding site in the lipid bilayer, or interact with elements in or near the transmembrane helices from the extracellular side. As was discussed above, potentiators within the UBP-class of compounds can favor GluN2C- and GluN2D-containing receptors with complex structure-activity relationships, which may involve the S2 region of the LBD (Costa et al., 2010).

1.4.d. Uncompetitive antagonists of the NMDA receptor

The organic channel blockers memantine and amantadine (**Figure 1.5**) as well as other channel blockers are thought to have partially overlapping binding sites within the channel pore (Burnashev et al., 1992; Jin et al., 2007; Kashiwagi et al., 2002; LePage et al., 2005; Santangelo et al., 2012; Yamakura et al., 1993). Examples of organic cationic channel blockers include phencyclidine (PCP), ketamine, MK-801, as well as Cerestat (CNS1102) (**Table 1.6** and **Figure 1.5**).

Figure 1.5. Some clinically relevant NMDA receptor antagonists.

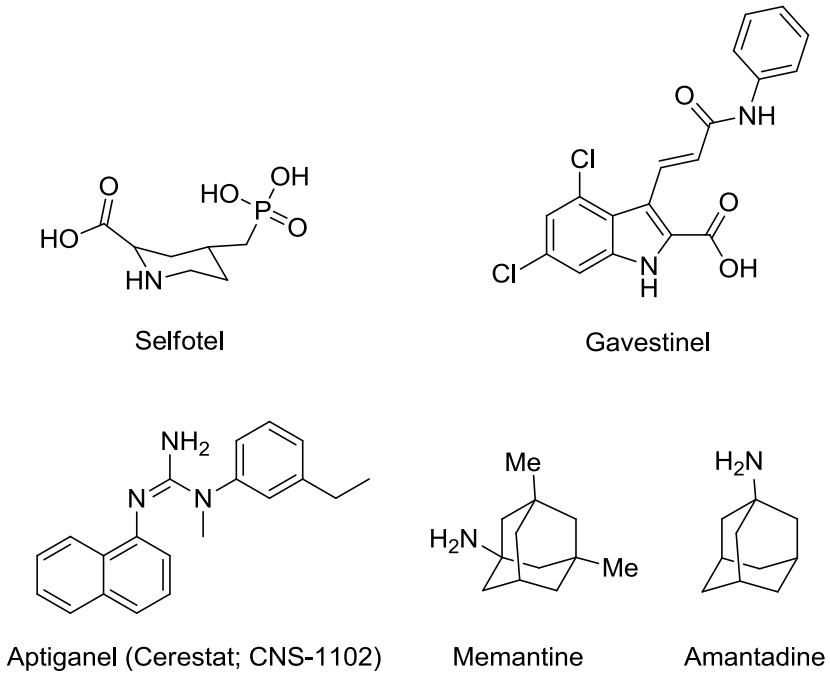


Figure 1.5. Structures of several clinically pursued modulators of the NMDA receptor.

Table 1.6. Uncompetitive antagonists of the NMDA receptor.

Uncompetitive Antagonist	GluN2A (μM)	GluN2B (μM)	GluN2C (μM)	GluN2D (μM)
(+)-MK-801	0.015	0.009	0.024	0.038
(-)-MK-801	0.35	0.32	0.038	0.17
(-)-ketamine	16	1.6	1.1	1.5
(±)-norketamine	51	8.7	5.6	7.5
Dextromethorphan	11	3.7	1.1	5.4
PCA	19	3.9	1.6	1.7
CNS-1102	0.13	0.068	0.087	0.14
Amantadine	130	70	35	38
Memantine	0.80	0.57	0.52	0.54
Memantine – 1 mM Mg^{2+}	13	10	1.6	1.8
(±)-ketamine	0.33	0.31	0.51	0.83
(±)-ketamine– 1 mM Mg^{2+}	5.4	5.08	1.2	2.9

Table 1.6. Uncompetitive antagonists of the NMDA receptor. Data are IC_{50} in μM. All values were measured in 0 Mg^{2+}, unless otherwise indicated. Values for memantine and (±)-ketamine are from (Kotermanski and Johnson, 2009). All remaining values from (Dravid et al., 2007). Abbreviations: MK-801 (Dizocilpine), (5R,10S)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; PCA, 1-phenylcyclohexylamine; CNS-1102 (Aptiganel), N-(1-naphthyl)-N'-(3-ethylphenyl)-N'-methylguanidine hydrochloride. (Adapted from Traynelis et al., 2010, wtih permission).

Both memantine and amantadine, approved for use in the treatment of Alzheimer's disease and Parkinson's disease respectively, have low affinity and rapid dissociation rates which may improve their clinical utility by preferentially inhibiting overactive NMDA receptors (Blanpied et al., 1997; Blanpied et al., 2005; Chen and Lipton, 2006; Lipton, 2007; Parsons et al., 1995; Santangelo et al., 2012). It is also notable that amantadine shows some subunit-selectivity across the GluN2 members (**Table 1.6**). PCP and ketamine on the other hand have slower dissociation rates which could explain their dissociative and schizophrenic like symptoms (Jentsch and Roth, 1999; Luby et al., 1959; Parsons et al., 1995).

1.5. Anatomical location, physiological function and therapeutic rationale

1.5.a. Anatomical location and physiological function

The expression of functional NMDA receptors is both spatially and temporally controlled through development. *In situ* hybridization of the mRNA for each the GluN1 and four GluN2 subunits shows that GluN1 is ubiquitously expressed throughout development and into the adult rat brain, while GluN2B and GluN2D predominate in both embryonic and early postnatal development (**Figure 1.5**) (Monyer et al., 1994; Standaert et al., 1994; Wenzel et al., 1996).

Figure 1.6. *In Situ* hybridization of the NMDAR subunit mRNA throughout rat development.

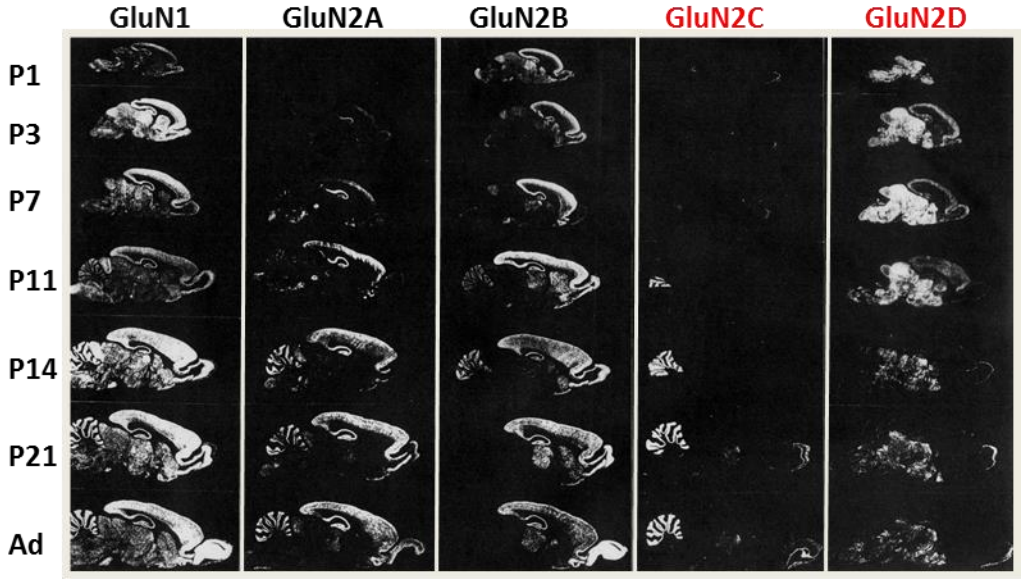


Figure 1.6. *In situ* hybridization of NMDAR subunit mRNA at various times beginning at postnatal (P) day one. It can be seen that the GluN1 subunit is ubiquitously expressed throughout development, while the GluN2 subunits are both temporally and spatially distinct. As can be seen in the adult (Ad) brain, GluN2C-mRNA is primarily located within the cerebellum while the GluN2D-mRNA is primarily located in the lower brain-stem region of the brain where the basal ganglia circuit is located. Adapted from (Akazawa et al., 1994)).

While there appears to be a subunit switch from GluN2B and GluN2D to GluN2A and GluN2C in the adult brain, GluN2B and Glun2D are still present, and the localization of GluN2D to the basal ganglia makes it an attractive target for diseases such as Parkinson's which arises from an imbalance within the basal ganglia circuitry due to the loss of dopaminergic projecting neurons within the substantia nigra (**Figure 1.5**) (Hallett and Standaert, 2004; Monyer et al., 1994; Standaert et al., 1994; Wenzel et al., 1996). Within the basal ganglia, the Glun2D subunit is found in the striatum, nucleus accumbens, thalamus, globus pallidus and substantia nigra (Monyer et al., 1994; Standaert et al., 1994; Wenzel et al., 1996).

The location of the GluN2D subunit in the subthalamic nuclei makes blockade of this NMDA receptor subtype in Parkinson's disease particularly interesting. Normal dopaminergic neuronal output from the striatum has an overall inhibitory effect on glutamatergic output from the subthalamic nuclei to the globus pallidus (Hallett and Standaert, 2004). Decreased inhibition of excitatory glutamatergic output in this region of the basal ganglia circuitry leads to overactivation of the GluN2D-containing NMDA receptors localized in this region. It has been shown that the non-selective glutamate site competitive antagonist, CPP (**Table 1.4**) decreased the firing rate and pattern of the globus pallidus after electrophysiological stimulation of the cortical region of the brain, which is thought to mimic the loss of dopaminergic neurons (Nambu et al., 2000). Furthermore, male Sprague-Dawley rats with 6-hydroxy dopamine induced lesions to the striatum show improvements in behavior when treated with the uncompetitive channel blocker, MK-801 (**Table 1.6**) (Blandini et al., 2001). These observations and others suggest agents that act at the GluN2D-containing NMDA receptor could be useful in

understanding and potentially treating symptoms of Parkisnon's disease such as the loss of coordinated motor control of the limbic system (Hallett and Standaert, 2004).

The development of the subunit selective pharmacological probes in this thesis has allowed for functional characterization of GluN2D containing receptors in the subthalamic nuclei and other regions in which the GluN2C- and GluN2D-subunits are expressed (Vance et al., 2013). It has been proposed that GluN1-GluN2B-GluN2D containing hetero-trimers are found in the substantia nigra pars compacta; NMDA evoked excitatory post-synaptic currrents (EPSCs) are only partially inhibited by the GluN2B subunit selective antagonist ifenprodil (**Table 1.5**) and are modestly inhibited by UBP141 (**Table 1.4**) which shows slight subunit selectivity (7.9-fold) for GluN2D-containing receptors over GluN2B-containing receptors (Brothwell et al., 2008). These observations suggest that a pharmacological probe that can inhibit the hetero-trimers could be particularly useful.

1.5.b. Therapeutic rationale

The NMDA receptor has been pursued as a therapeutic target for decades. Over-activation of ionotropic glutamate receptors leads to increased Ca^{2+} influx which can be toxic to neuronal populations. For this reason, NMDA receptor antagonists have been pursued heavily for indications such as traumatic brain injury and ischemic cell death where the over-activation of the glutamate receptors is thought to be directly involved with the loss of local neuronal populations. However, simple blockade of the NMDA receptors, while efficacious in animal models, has yet to translate into positive clinical outcomes. Clinical trials have been carried out with the competitive antagonists selfotel

(glutamate site; **Table 1.4**; **Figure 1.4**) and gavestinel (glycine site; GV150,526A, **Table 1.4**; **Figure 1.4**), numerous channel blockers including aptiganel (CNS-1102, **Table 1.6**; **Figure 1.4**) and GluN2B subunit-selective antagonists, including traxoprodil (CP-101,606; **Table 1.5**) (Albers et al., 2001; Davis et al., 1997; Lees et al., 2000; Morris et al., 1999; Yurkewicz et al., 2005). Unfortunately, for numerous reasons including adverse effects and low patient inclusion, each of the aforementioned clinical trial failed to reach a statistically significant improvement at the end-point for the desired indications in ischemic stroke or TBI (Kalia et al., 2008; Traynelis et al., 2010).

More recent clinical trials and positive clinical outcomes for compounds targeting the NMDA receptor have come about with compounds that have a variety of mechanisms. First, the channel blockers, memantine and amantadine (**Figure 1.3**, **Table 1.6**), have both been approved by the food and drug administration (FDA) for use in Alzheimer's disease and Parkinson's disease, respectively (Kalia et al., 2008). The efficacy of these agents is not fully understood, however their tolerability is attributed to their use dependence and fast off-rate kinetics (Chen and Lipton, 2006; Danysz et al., 2000; Parsons et al., 1999). The unique mechanism of both of these approved drugs, while they are not subunit-selective, may allow specific targeting of overly-active NMDA receptors (Chen and Lipton, 2006). The positive clinical outcomes with these channel blockers supports the idea that subunit-selective agents targeting the specific NMDA receptor subtypes involved in the diseases could be a valuable strategy for intervention.

One of the more recent and remarkable findings related to the use of NMDA receptor modulators has been the efficacy of ketamine in depression. While the dissociative effects of channel blockers such as PCP and ketamine are well documented,

ketamine has recently been used in short-term treatment regimens at low doses in patients with treatment resistant depression and relieves the depression for long periods of time after treatment (Muir, 2006; Wolff and Winstock, 2006). The mechanism of ketamine's efficacy is beginning to become clear and is thought involve synaptogenesis that is dependent on the activation of the mammalian target of rapamycin (Duman and Aghajanian, 2012; Li et al., 2011). These findings further highlight the utility of pharmacological agents targeting the NMDA receptor.

1.6. Structure activity relationship rationale

Goodman and Gillman's The Pharmacological Basis of Therapeutics explains simply: "Exploitation of structure-activity relationships on many occasions has led to the synthesis of valuable therapeutic agents…. Given adequate information about both the molecular structures and the pharmacological activities of a relatively large group of congeners, it is possible to identify the chemical properties (*i.e.*, the pharmacophore) required for optimal action at the receptor….." (Goodman and Gilman's, 11ed). Of course, generating a structure activity relationship relies on the ability to synthesize the compounds of interest. In this thesis, my principal goal has been to lay the foundations for such understanding, aiding in the development of optimized chemical compounds that could have therapeutic potential in any number of potential diseases and conditions involving the GluN2C- or GluN2D-containing NMDA receptors.

Modern day medicinal chemistry efforts often benefit from crystallographic data or reliable homology models which can greatly expedite the discovery and optimization of ligands against a target of interest (Congreve et al., 2011; Liao, 2007). However, in

the absence of such data, one can often rely upon well understood physical properties associated with functionality contained within a class of compounds which can be used to describe a pharmacophore for the compounds (Topliss, 1977; Van Drie, 2012).

Several factors contribute to a medicinal chemistry program. Beginning from a hit series which can be identified using numerous methodologies (high-throughput screening, etc.), the medicinal chemist will have several objectives before the hit series can identify a lead compound for further development (Lipinski and Hopkins, 2004). The primary objective after hit identification is to identify congeners that are highly potent and selective agents acting at the desired target. If sufficient potency is attained, metabolic liabilities, membrane permeability, including blood brain barrier penetration, can begin to be addressed and optimized for "drug-likeness."

Arguably, the most notable work quantifying "drug-likeness" evaluated the physico-chemical properties of compounds that were either approved for use in humans, or in late-stage development and extracted the properties most closely related to compounds used in man (Lipinski et al., 1997). The rule of five (Ro5) states that "poor absorption or permeation are more likely when: there are more than 5 H-bond donors, the molecular weight is greater than 500, the Log P is greater than 5 and there are more than 10 H-bond acceptors; compound classes that are substrates for biological transporters are exceptions to the rule" (Lipinski et al., 1997). In practice, a compound should usually not fail more than one of these guidelines when being carried forward from "hit" to "lead" (Lipinski et al., 1997). Because high-throughput screening libraries are often assembled with compounds that fail more than one or more of these guidelines and the biological "hits" from these screens rarely possess the optimal activity profiles, these considerations

are often optimized once a congener of sufficient potency and selectivity has been identified.

One strategy that is often used to move from a hit series to a lead series relies on computational modeling of the structure activity relationships derived in the initial search for potent and selective congeners in the form of QSAR modeling (Hansch and Fujita, 1964). The methods available for deriving QSAR models attempt to extract the pharmacophore of the compounds being studied by correlating biological activity to the shape and electrostatics of the series of compounds, and by defining the geometric, or spatial relationships between the pharmacophore features (Leach et al., 2009). "A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response" (Leach et al., 2009; Wermuth et al., 1998). The QSAR models that are derived from the pharmacophore hypotheses can then be used to perform high-throughput *in silico* screens of compounds in an attempt to identify compounds which contain the requirements of the pharmacophore, but differ in their chemical scaffold. These experiments can generate new hits that can be further evaluated for their drug-likeness, tested for biological activity and incorporated appropriately into a medicinal chemistry program.

In this thesis, I will identify structural determinants of activity and the mechanism of action for the DQP-1105 class of molecules which are negative allosteric modulators of GluN2C- and GluN2D-containing receptors. I will also describe a systematic SAR which identifies highly potent and selective congeners within the class of compounds, allows for assignment of the absolute stereochemistry of the preferred enantiomer for a

representative member of the class and allows for pharmacophore features to be deduced. I will describe the advances made toward making the compounds penetrable to the BBB and attempts to attenuate the metabolism of one of the more promising congeners. I will further describe computational experiments that attempt to define the minimal pharmacophore of the QNZ containing compounds and compare the shape and electrostatic similarity of two classes of molecules to each other. I also describe synthetic efforts that aim to test the hypothesis that two distinct classes of molecules share an overlapping site at the receptors. I will further describe the synthesis and SAR of a novel class of carbamate containing derivatives that were identified in the Traynelis lab and are also subunit selective allosteric modulators of GluN2C- and GluN2D-containing NMDA receptors.

Chapter 2: Materials and Methods

2.1. Molecular Biology

cDNAs for rat wild type NMDA receptor subunits GluN1-1a (GenBank U08261; hereafter GluN1), GluN2A (GenBank D13211), GluN2B (GenBank U11419), GluN2C (GenBank M91563), and GluN2D (GenBank L31611, see (Monyer et al., 1994), GluA1 (GenBank X17184), GluK2 (GenBank Z11548) were provided by Drs. S. Heinemann (Salk Institute), S. Nakanishi (Kyoto University), and P. Seeburg (University of Heidelberg. Site-directed mutagenesis was accomplished using the Quikchange approach (Agilent Technologies, Santa Clara, CA). All DNA constructs were verified by sequencing (SeqWright, Houston, TX).

2.2. Two-electrode voltage-clamp recording from Xenopus laevis oocytes

Two-electrode voltage-clamp recordings were performed in *Xenopus laevis* oocytes expressing recombinant rat GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C and GluN1/GluN2D. In some experiments, recordings were performed in oocytes expressing recombinant human GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, or GluN1/GluN2D (Acker et al., 2011). Oocytes were isolated from *Xenopus laevis* as approved by the Emory University IACUC, and treated according to methods previously described (Dravid et al., 2007). The pipettes for cRNA injection were filled with mineral oil and attached to an automatic injector (Nanoject II, Drummond Scientific). The cRNA was transcribed in vitro using the mMessage Machine kit (Ambion), diluted with nuclease free water, and injected at a ratio of 1:2 GluN1:GluN2 (5-10 ng total cRNA). The oocytes were kept in Barth's solution comprised of (in mM) 88 NaCl, 5 Tris-HCl,

2.4 $NaHCO_3$, 1 KCl, 0.84 $MgSO_4$, 0.41 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 0.1 mg/ml gentamycin sulfate, 1 U/ml penicillin, and 1 µg/ml /streptomycin at pH 7.4 at 15-17ºC for two to five days before experiments. Oocytes were placed into a perfusion chamber and continually washed with recording solution containing (in mM) 90 NaCl, 1.0 KCl, 0.5 $BaCl_2$, 0.005 EDTA, and 10 HEPES at pH 7.4 (23ºC). Glass electrodes with a tip resistance of 0.5-2.5 MΩ were pulled from thin-walled glass capillary tubes (World Precision Instruments, Cat. No. TW-150F-4, Sarasota, FL, USA). Voltage electrodes were filled with 0.3 M KCl and current electrodes were filled with 3.0 M KCl. Voltage clamp recordings were conducted at a holding potential of -40 mV using an OC-725 amplifier (Warner Instrument Co). For some experiments the current-voltage relationship was evaluated by stepping the membrane potential in 10 mV increments from -60 mV to +30 mV. All compounds were made as 20 mM stock solutions in dimethyl sulfoxide DMSO and dissolved to reach the final concentration in recording solution. Final DMSO content was 0.05-0.5% (vol/vol).

The receptor binding profiles and K_i determinations for compounds (26) **997-2**3 and (58) **997-33** was generously provided by the National Institute of Mental Health Psychoactive Drug Screening Program, Contract # HHSN-271-2008-025C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. Data was collected using 5 µM of compounds (26)**997-23** and (58) **997-33**.

2.3. Compound Solubility

Compound solubility was obtained on a Nepholostar Plus instrument (BMG labtech, Nephlostar plus) using Costar 96-well plates (black with clear bottom). Briefly, stock compound solutions of 20 mM in 100% DMSO were serially diluted 1:1 in DMSO down to 0.8 µM. 3 µl of each concentration was then pipetted into a well of the 96 well plate, which contained 297 µl of the assay buffer. Compounds were tested in triplicate with oocyte recording buffer used in two-electrode voltage clamp recordings (see Methods and Materials). Data were normalized to blank values from oocyte recoding solution and fit using segmental linear regression with GraphPad Prism (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com) and the solubility was approximated to the X-intersect of the line intersecting the highest concentration assayed. I also evaluated the percent response of GluN1/GluN2D containing receptors expressed in *Xenopus laevis* oocytes in the presence of 10 µM DQP-1105 in recording buffer (0.1% DMSO) as a function of time. The bioassay evaluated responses at 0, 1, 3, 6, 12 hours (23^{o}C) using unfiltered solutions and solutions that had been filtered through a 0.20 µm filter (Millex-FG, Millipore Corporation, Billerica, MA, USA).

2.4. MDR1-MDCK permeability

The MDR1-MDCK permeability assays were performed by Absorption Systems. Cell monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell® plates. The permeability assay buffer was Hanks Balanced Salt Solution containing 10 mM HEPES and 15 mM glucose

at pH 7.4. The buffer in the receiver chamber also contained 1% bovine serrum albumin. The dosing solution concentration in the assay buffer was 5µM for each compound tested. The cell monolayers were dosed on the apical side (A-to-B) or basolateral side (b-TO-a) and incubated at 37ºC with 5%CO_2 in a humidified incubator. Samples were taken from the donor and receiver chambers at 120 minutes. Each determination was performed in duplicate. The co-dosed lucifer yellow flux was also measured for each monolayer to ensure no damage was inflicted to the cell monolayers during the flux period. All samples were assayed by LC-MS/MS using eletrospray ionization. The apparent permeability (P_{app}) and percent recovery were calculated as follows.

$$P_{app} = (dC_r/dt) \times V_r/(A \times C_A) \quad (1)$$

$$\textit{Percent recovery} = 100 \text{ X } ((V_r \text{ X } C_r^{final}) + (V_d \text{ X } C_d^{final})) + (V_d \text{ X } C_d^{final}))/(V_d \text{ X } C_N) \quad (2)$$

Where:

dC_r/dt is the slope of the cumalitive concentration in the receiver compartment versus time in µM s^{-1};

V_r is the volume in the receiver compartment in cm^3; V_d is the volume in the donor compartment in cm^3; A is the area of the insert (1.13 cm^2 for12-well Transwell®); C_A is the average of the nominal dosing concentration and the measured 120 minute donor concentration in µM; C_N is the nominal concentration of the dosiung solution in µM; C_r^{final} is the cumulative receiver concentration in µM at the end of the incubation period; C_d^{final} is the concentration of the donor in µM at the end of the incubation period.

2.5. Human liver microsomal stability

The human liver microsomal stability assays were performed by Absorption Systems, and human liver microsomes were obtained from XenoTech. The reaction mixture was prepared with 0.5 mg/mL human liver microsomes, 100 mM potassium phosphate (pH 7.4), 5 mM magnesium chloride, 1 µM test compound. The reaction mixture was incubated in a shaking water bath at 37ºC for 3 minutes prior to the addition of NADPH (1mM). Testosterone was run simultaneously in a separate vessel as a control. 100 µl aliquots were taken at 0, 10, 20, 30 and 60 minutes for both test compound and testosterone. The aliquots were combined immediately with 400 µl of ice cold 50/50 acetonitrile (ACN)/water (H_2O) ACN/*d*H_2O containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate microsolmal proteins. All samples were assayed by liquid chromatography mass spectrometery-mass spectrometry (LC-MS/MS) using electrospray ionization and multiple reaction monitoring and the peak area responses to internal standard of the compounds at each time point was compared to the peak area response at time 0 to determine the percent compound remaining.

2.6. Reagents

DQP-1105 (**19**; 4-(5-(4-bromophenyl)-3-(6-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid) was obtained from Pharmeks (Moscow, Russia); The compound was more than 90% pure as determined by the chemical supplier. Glutamate, glycine, and all other chemicals were from Sigma-Aldrich (St Louis, MO). DQP-1105 had a molecular weight of 558.42, a

maximum solubility at 1 hour in oocyte recording buffer plus 0.1% DMSO of 27 µM, and maximum solubility at 24 hours in phosphate buffered saline (no DMSO) of 6.5 µM. DQP-1105 is also known as DC060015, and was identified in a virtual screen as a low potency (K_D 18.6 µM) inhibitor of SARS-Coronavirus 3CL, where it was reported to have a CLogP value of 6.55 (Chen et al., 2006).

2.7. Chemistry Experimental

All reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. Reaction progress was monitored by thin layer chromatography (TLC) on precoated glass plates (silica gel 60 F254, 0.25 mm). Proton, carbon and fluorine NMR spectra were recorded on INOVA-400 Megahertz (400 MHz), VNMRS 400 (400 MHz), UNITY-600 (600 MHz), or INOVA-600 (600 MHz) instruments. Proton and carbon spectra were referenced to the residual solvent peak while fluorine spectra were referenced to trifluoroacetic acid (TFA) residual peak. The Emory University Mass Spectrometry Center collected mass spectral data on either a VG 70-S Nier Johnson or JEOL instrument. Compound purity was assessed by reverse phase liquid chromatography (two solvent systems) using an Agilent Zorbax, 3.5 µm, XD B-C18 column, 4.6 X 50 mm, or by elemental analyses, performed by Atlantic Microlab Inc. Purity for all compounds tested was at or above 95% unless otherwise noted. Flash chromatography was performed on a Teledyne ISCO Combiflash Companion with pre-packaged Teledyne RediSep disposable normal phase silica columns. General synthetic procedures are described in the chapter they are first employed.

2.8. Computational Analysis

Structures used in computational modeling experiments were first prepared using LigPrep (LigPrep, version 2.5, Schrödinger, LLC, New York, NY, 2011) within Maestro (Maestro, version 9.2, Schrödinger, LLC, New York, NY, 2011). Energy minimized conformations and low energy conformers were generated using the OPLS_2005 force field in MacroModel (MacroModel, version 9.9, Schrödinger, LLC, New York, NY, 2011). The topological polar surface area (TPSA) approximations were obtained from QikProp (QikProp, version 3.4, Schrödinger, LLC, New York, NY, 2011) using energy minimized conformations, as above. Pharmacophore hypotheses and QSAR models were generated using Phase (Phase, version 3.3, Schrödinger, LLC, New York, NY, 2011) (Dixon et al., 2006). ROCS overlays were created using vROCS (Release 3.1.2, OpenEye Software, Inc, 2011) and viewed using VIDA (Release 4.2.1, OpenEye Software, Inc. 2011)(Grant et al., 1996).

2.9. Data analysis

Antagonist IC_{50} values were determined by non-linear least squares fitting of the equation:

$$Response = 100 / (1 + ([inhibitor\ concentration] / IC_{50})^{N}) \quad (3)$$

to concentration-response data from individual experiments normalized to the response in the absence of inhibitor (100%), where IC_{50} is the concentration that inhibits the response half-maximally and N is the Hill slope; inhibition was assumed to be complete at saturation. Some IC_{50} values were determined by fitting equation (2) to mean composite data (Data describing the structure-activity relationship for multiple ligands). Only

compounds that produced at least 25% inhibition of NMDA receptors at 30 μM were studied further. Agonist concentration-response data were fitted by the equation

$$\textit{Percent Response} = 100 / (1 + (EC_{50} / [\textit{agonist concentration}])^{N}), \quad (4)$$

where EC_{50} is the concentration of agonist that produces a half-maximally effective response and *N* is the Hill slope.

Chapter 3: Mechanism and structural determinants of activity for DQP-1105

3.1. Abstract

The dihydroquinilone-pyrazoline scaffold was identified as an inhibitor of NMDA receptors from a multiwell Ca^{2+}-based screen of approximately 100,000 compounds from ChemDiv and Asinex diversity libraries against GluN1/GluN2C or GluN1/GluN2D expressing BHK cell lines (Hansen et al., 2010b; Mosley et al., 2010). Evaluation of a subset of analogues sharing this scaffold led to the identification of DQP-1105 (data not shown), which is the primary focus of this chapter. I have evaluated the mechanism of action and structural determinants of selectivity for DQP-1105. My results suggest that the class of compounds could share a binding site at the GluN2D-containing NMDA receptor with that of the previously described QNZ containing compounds. Furthermore, the results from this study suggest a similar mechanism of action to that of the QNZ compounds; both classes of compounds are negative allosteric modulators of GluN2D-containing NMDA receptors that are glutamate dependent. These conclusions indicate that a previously unknown modulatory site on the receptor could be exploited through structure activity relationships in order to discover highly potent and selective compounds against GLuN2C- and GluN2D-containing receptors.

3.2. Introduction

The N-Methyl-D-Aspartate (NMDA) receptors belong to the family of ionotropic glutamate receptors that mediate the majority of excitatory neuronal transmission within the central nervous system (Traynelis et al., 2010). NMDA receptors comprise four subunits that combine to form functional ion channels. Each subunit folds into four semi-

autonomous domains, including the amino-terminal domain, the ligand-binding domain, the transmembrane domain and the carboxy-terminal domain. Functional NMDA receptors are formed by the assembly of two GluN1 subunits that bind the co-agonist glycine together with two GluN2 subunits that bind the co-agonist glutamate. Four independent genes encoding GluN2 subunits have been identified (GluN2A-D) and account for differences in functional properties of the NMDA receptors, including deactivation time course, agonist potency, mean open time, and open probability (Chen and Wyllie, 2006; Dingledine et al., 1999; Paoletti and Neyton, 2007; Traynelis et al., 2010). The GluN2 subunit also controls the sensitivity of the receptor to Mg^{2+} block, proton inhibition, modulation by Zn^{2+}, as well as potency and efficacy of the endogenous agonists glutamate and glycine (Traynelis et al., 2010).

The spatial and temporal expression of the various GluN2-containing NMDA receptors has led to hypotheses concerning specific roles for the various NMDA receptor subunits in certain pathophysiological conditions, including Parkinson's disease and schizophrenia (Chen and Lipton, 2006; Hallett and Standaert, 2004). Despite considerable progress in our understanding of ionotropic glutamate receptor structure and function, the lack of subunit-selective pharmacological tools targeting NMDA receptors has impeded advances in understanding the roles of different GluN2-containing NMDA receptor subtypes both in normal brain function and in various disease states. Here we report a new structural class of antagonists, typified by 4-(5-(4-bromophenyl)-3-(6-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxobutanoic acid (DQP-1105), which is approximately 50-fold selective for NMDA receptors containing GluN2C or GluN2D subunits over GluN2A- or GluN2B-containing

receptors. The mechanism of action and structural determinants of subunit-selectivity for DQP-1105 are described below. The results from this study will facilitate the development of pharmacological probes with increased potency and selectivity among GluN2 subunits.

3.3. Results

3.3.a. Subunit selectivity of DQP-1105 inhibition

The actions of DQP-1105 against current responses from recombinant NMDA, AMPA, and kainate receptors expressed in *Xenopus laevis* oocytes were evaluated (**Fig. 3.1B, Table 3.1**) to determine its potency and selectivity across the glutamate receptor ion channel family. When co-applied in solutions containing maximally effective concentrations of glutamate and glycine, DQP-1105 inhibited recombinant NMDA receptors containing the GluN1/GluN2C and GluN1/GluN2D subunits with IC_{50} values that were lower than that for inhibition of GluN1/GluN2A (**Table 3.1**). For example, in *Xenopus* oocytes, DQP-1105 inhibited GluN1/GluN2D receptors with an IC_{50} value of 2.7 $\pm$ 0.2 μM (mean $\pm$ SEM; n=44), while it had minimal effects on GluN1/GluN2A. The IC_{50} value at GluN1/GluN2A determined by fitting each concentration-effect curve was at a minimum 206 $\pm$ 36 μM, and might be higher as responses from some oocytes showed no inhibition and thus could not be fitted and included in this analysis (n=13; **Fig. 3.1.B**, **Table 3.1**). IC_{50} values determined at recombinant human and rat NMDA receptors were similar (**Table 3.1**).

Figure 3.1. DQP-1105 inhibition and subunit-selectivity.

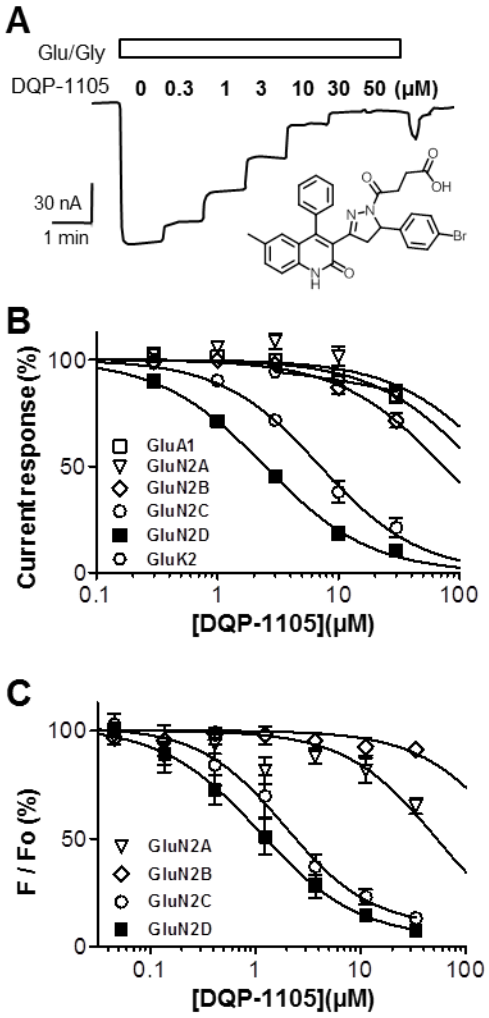


Figure 3.1. Subunit selectivity of DQP-1105. *A,* Representative current response of GluN1/GluN2D receptors expressed in *Xenopus* oocyte recording during co-application of 100 μM glutamate, 30 μM glycine (Glu/Gly), and the designated concentration of DQP-1105 (structure shown in *inset*). *B,* Composite concentration-effect curves determined using two-electrode voltage-clamp electrophyiology for DQP-1105 against recombinant AMPA, kainate, and NMDA receptors expressed in *Xenopus* oocytes. *C,* Concentration-effect curves determined using fluorescence-based measurements of intracellular calcium expressed as a percent of baseline fluorescence (F/Fo) for DQP-1105 inhibition of recombinant GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, or GluN1/GluN2D stably expressed in BHK cells. IC_{50} values are listed in Table 3.1. (BHK data from Lundeck)(Acker et al., 2011).

Table 3.1. Concentration-response data for DQP-1105 at ionotropic glutamate receptors

	Agonist	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)	GluA1 IC_{50} (μM)	GluK2 IC_{50} (μM)
Oocytes TEVC (rat)	Glutamate	206 ± 36	121 ± 32	8.5 ± 1.2	2.7 ± 0.2	198 ± 44	153 ± 9.1
Oocytes TEVC (rat)	NMDA	135 ± 66	43 ± 4	2.4 ± 0.2	1.5 ± 0.2	--	--
Oocytes TEVC (human)	Glutamate	NE	206 ± 137	5.4 ± 1.0	2.2± 0.1	--	--
BHK Ca^{2+} Imaging* (rat)	NMDA	85 ± 24	NE	1.4 ± 0.5	1.6 ± 0.5	--	--

Table 3.1. Mean IC_{50} values (± SEM) for inhibition of responses in oocytes to 100 μM glutamate and 30 μM glycine were determined. Oocyte experiments were performed in 3-44 oocytes from 1-4 frogs. Oocytes assayed using NMDA as agonist were activated using 50 – 1000 μM NMDA and 50 μM glycine. BHK cell imaging results are from 3 or more independent experiments. Mean fitted Hill slopes for GluN1/GluN2C and GluN1/GluN2D were between 0.87-1.14; Hill slope was fixed to 1.0 for GluN1/GluN2A, GluN1/Glun2B, GluA1, and GluK2. NE indicates that there was no detectable effect at the concentrations evaluated; -- indicates that conditions were not assayed (Acker et al., 2011). *Data from Henrik Jensen, included for comparison.

DQP-1105 had minimal effects on current responses of homomeric GluA1 AMPA receptors or homomeric GluK2 kainate receptors (**Table 3.1**.), and no effect on the leak currents in uninjected *Xenopus* oocytes (n=4; data not shown), or on the leak currents from oocytes expressing NMDA receptors (n=4; data not shown).

In order to determine whether DQP-1105 is selective for GluN2C/D- over GluN2A/B-containing NMDA receptors expressed in mammalian cells, the actions of this compound on recombinant NMDA receptor responses in BHK cells was evaluated (Data collected by Lundbeck). IC_{50} values for DQP-1105 inhibition of the fluorescent signal in BHK cell lines stably expressing GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, or GluN1/GluN2D loaded with the Ca^{2+}-sensitive dye Fluo4 were determined (Hansen et al., 2008; Hansen et al., 2010b). DQP-1105 inhibited responses of GluN1/GluN2C and GluN1/GluN2D receptors activated by an EC_{80} concentration of NMDA plus glycine with IC_{50} values that were slightly more potent than those obtained from oocyte recordings (**Table 3.1**., **Figure 3.1.C**). GluN1/GluN2A-containing NMDA receptor responses were inhibited to only 65 ± 3% of control by 33 μM DQP-1105, which allowed us to estimate a mean IC_{50}, whereas GluN1/GluN2B responses were negligibly inhibited (**Table 3.1**, **Figure 3.1.C**). Thus, DQP-1105 exhibited a similar level of selectivity for GluN2C/D in intact mammalian cells as was observed in *Xenopus* oocytes.

3.3.b. Mechanism of action of DQP-1105

To begin to determine the mechanism of DQP-1105 inhibition, we evaluated whether the actions of DQP-1105 were dependent on the agonist concentration for

GluN1/GluN2D receptor responses recorded in *Xenopus* oocytes. Sub-maximal inhibition of GluN1/GluN2D receptor responses by 5 µM DQP-1105 could not be surmounted by 10-fold increases in either glutamate or glycine concentration, suggesting that DQP-1105 acts by a non-competitive mechanism (**Fig. 3.2A**). Furthermore, the EC_{50} values for glutamate and glycine at GluN1/GluN2D receptors expressed in *Xenopus* oocytes were not significantly altered in the presence of an IC_{75} concentration of DQP-1105 (5 µM). Glutamate EC_{50} values in 30 µM glycine were 0.30 ± 0.02 and 0.27 ± 0.03 µM in the absence and presence of DQP-1105, respectively ($p > 0.05$, unpaired t-test, n=10-11). Glycine EC_{50} values in 100 µM glutamate were 0.11 ± 0.01 and 0.11 + 0.01 µM in the absence and presence of DQP-1105 , respectively ($p > 0.05$, unpaired t-test, n=4). In order to determine whether DQP-1105 inhibition involved the permeation pathway, we evaluated the inhibition at various membrane potentials. The degree of inhibition produced by DQP-1105 was the same at all voltages tested ($p = 0.86$, 1-way ANOVA; n=5), suggesting that DQP-1105 inhibition is voltage-independent. In addition, the mean reversal potential (V_{REV}) was not significantly different between control response to glutamate plus glycine (V_{REV} -0.31 $\pm$ 1.4 mV; n=5) and the response to glutamate and glycine plus 3-5 µM DQP-1105 (V_{REV} -0.81 $\pm$ 3.3 mV; n=5; $p = 0.76$; t-test; Fig. 3.2.B). We interpret these data to suggest that DQP-1105 neither inhibits receptor function by competing with glutamate or glycine at the agonist binding domain, nor inhibits receptor function by interacting with the channel pore in a manner that is influenced by the transmembrane electric field.

Figure 3.2. DQP-1105 inhibits recombinant GluN1/GluN2D receptors through a non-competitive and voltage-independent mechanism.

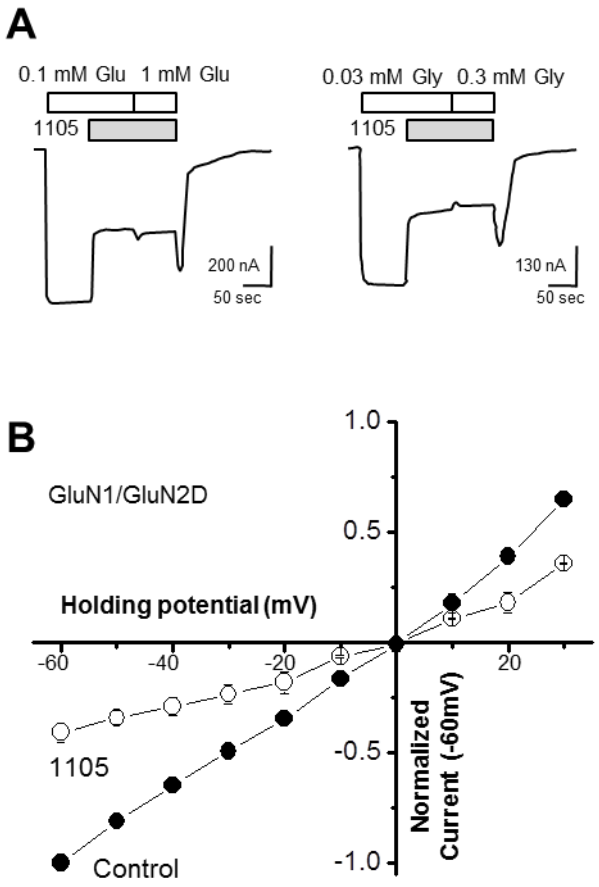


Figure 3.2. DQP-1105 inhibits recombinant GluN1/GluN2D receptors through a non-competitive and voltage-independent mechanism. *A*, GluN1/GluN2D responses to 100 μM glutamate and 30 μM glycine were inhibited by co-application of glutamate, glycine, and 5 μM DQP-1105, and the glutamate concentration was subsequently increased 10-fold to 1000 μM (*left panel*). GluN1/GluN2D responses elicited by 100 μM glutamate and 30 μM glycine were inhibited by co-application of 5 μM DQP-1105, and the glycine concentration was subsequently increased 10-fold to 300 μM glycine (*right panel*). Neither the increase of glutamate or glycine altered the level of inhibition, suggesting a non-competitive mechanism. *B*, The mean current-voltage relationship of recombinant GluN1/GluN2D receptors was determined in the absence and presence of 3-5 μM DQP-1105 (10 mV steps from -60 mV to +30 mV, n=5). Error bars are SEM and shown when larger than symbol size. (Some data in 3.2.B. was collected by Praseeda Mullaseril)(Acker et al., 2011).

3.3.c. Structural determinants of DQP-1105 activity

DQP-1105 shows sufficient selectivity for GluN2D over GluN2A to allow use of a chimeric strategy in which domains of subunits are swapped to identify the divergent structural elements of the GluN2D subunit that might account for the observed selectivity. A series of GluN2A and GluN2D chimeric receptors that transferred different portions of GluN2D into GluN2A in order to test for gain of function for novel GluN2C/D preferring modulators has previously been published (Hansen and Traynelis, 2011; Mullasseril et al., 2010). This set of chimeric receptors was used to evaluate the structural determinants of DQP-1105 action, and found that transferring the ATD, S1 region of the ligand-binding domain, or the trans-membrane elements by themselves (including two trans-membrane helices and a reentrant loop) did not render GluN2A containing receptors sensitive to DQP-1105 (**Fig 3.4.A** and **3.4.B**). By contrast, transferring either the S1-S2 regions or the S2 domain alone from GluN2D to GluN2A completely transferred the DQP-1105 sensitivity to GluN2A (**Fig. 3.4.A** and **3.4.B**), suggesting structural determinants that define subunit selectivity may be contained within the S2 region of GluN2D.

Figure 3.4. Chimeric receptor data using DQP-1105.

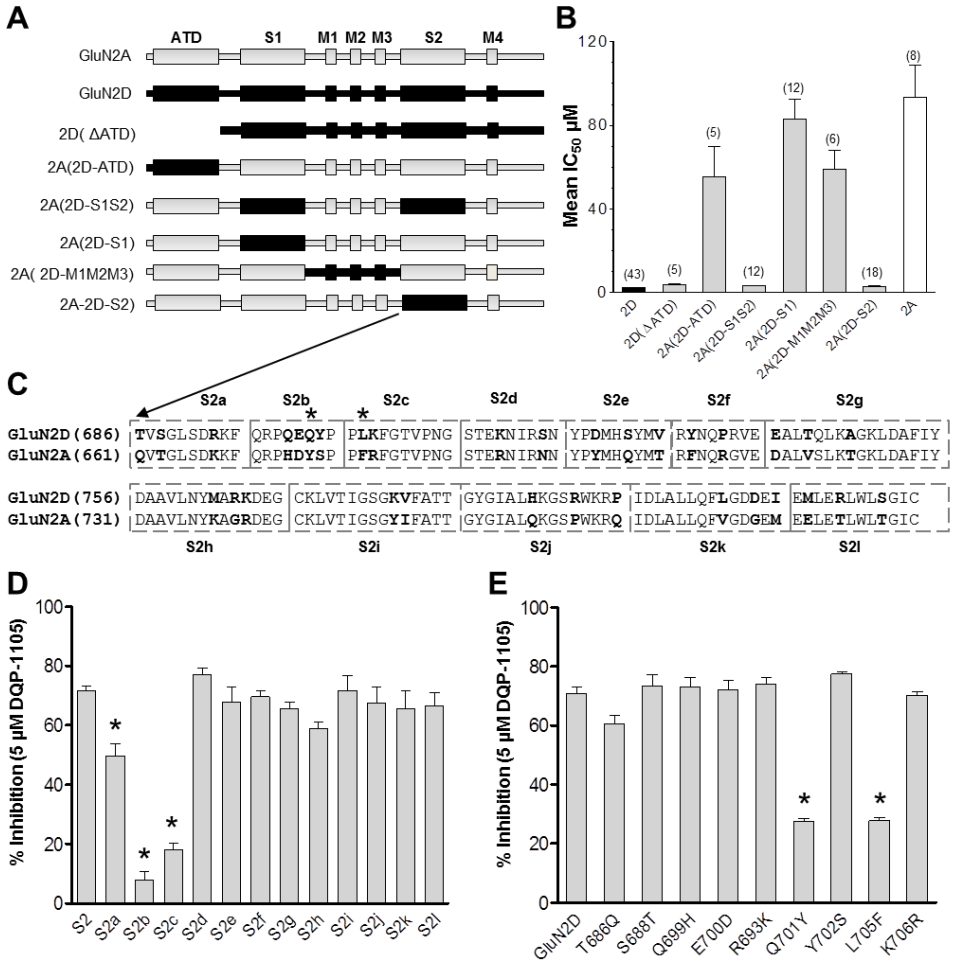


Figure 3.4. Identification of structural determinants of GluN2D-selective inhibition using chimeric GluN2A-GluN2D receptors. *A*, Indicated regions of the polypeptide were exchanged between wild type GluN2A and GluN2D. Concentration-effect curves at GluN1/GluN2D, GluN1/GluN2A, GluN1/GluN2A(2D-S1S2), GluN1/GluN2A(2D-S1), GluN1/GluN2A(2D-S2), *B*, GluN1/GluN2A(2D-M1M2M3), GluN1/GluN2A(2D-ATD), and GluN1/GluN2A(ΔATD) receptors were analyzed, and the mean IC_{50} values (±SEM) are shown. These data suggest that the S2 region transfers DQP-1105 sensitivity to GluN2A. *C*, Linear representation of the S2 regions of GluN2A(2D-S2) in which regions of GluN2D-S2 have been reverted to that of GluN2A to probe for loss of inhibition of DQP-1105. *D*, The inhibition produced by 5 μM DQP-1105 in the presence of 1000 μM glutamate and 300 μM glycine is shown as a percent of control. Three chimeric receptors show significantly reduced inhibition as compared to GuN2A (S2) chimera ($p<0.05$, 1-way ANOVA with Tukey's test, n=5-13 oocytes per chimeric reeptor). *E*, Site-directed mutagenesis of residues within GluN2D S2 chimeric regions identified Gln701 and Leu705 (asterisks in *B*) as key structural determinants for antagonist activity of DQP-1105 at GluN2D receptors ($p<0.05$, 1-way ANOVA with Tukey's test; n=4-11 oocytes per mutant receptor) (Acker et al., 2011).

This result is surprisingly similar to the structural determinants for neurosteroids (Horak et al., 2006; Jang et al., 2004) and the structurally unrelated quinazolin-4-ones (Hansen and Traynelis, 2011). Consistent with this result, experiments in which the GluN2D subunit ATD has been removed show that the ATD is not involved in the selectivity of DQP-1105 for GluN2D over GluN2A (**Fig. 3.4.B**). To identify specific divergent structural elements within the S2 region that might define the subunit selectivity, I utilized 12 additional chimeric receptors (Hansen and Traynelis, 2011) that revert non-conserved S2 residues within the GluN2A(2D-S2) chimera back to wild type GluN2A residues (see **Fig. 3.4.C** and **3.4.D**). This strategy allowed me to examine whether clusters of residues that are divergent between GluN2D and GluN2A impact DQP-1105 selectivity. I found that the S2a, S2b, and S2c chimeric receptors exhibited reduced sensitivity to DQP-1105 when tested at a single concentration, suggesting that some of the nine divergent residues between GluN2A and GluN2D within these three regions control DQP-1105 potency at the receptor. Subsequent mutation of each of these divergent residue in GluN2D to that observed in Glun2A identified two amino acids (GluN2D Gln701 and Leu705) that significantly reduced inhibition (**Fig. 3.3.E**). Concentration-effect curves showed that GluN2D(Q701Y) and GluN2D(L705F) decreased the potency of DQP-1105 more than 6-fold to 19 $\pm$ 2 μM (n=8) and 17 $\pm$ 2 μM (n=8), respectively. These data suggest that DQP-1105 shares structural determinants with quinazolin-4-ones (Hansen and Traynelis, 2011) and perhaps neurosteroids (Horak et al., 2006), identifying this region as a site at which structurally diverse endogenous and exogenous ligands can interact.

3.4. Discussion

A novel class of GluN2C/D preferring non-competitive antagonists that inhibit NMDA receptors has been mechanistically explored. I show that the structural determinants of selectivity for DQP-1105 reside within a portion of the S2 region of GluN2D that overlaps closely with previously identified structural determinants important in the selectivity of quinazolin-4-ones, another class of GluN2C/D preferring antagonists (Hansen and Traynelis, 2011). In addition, the shared reliance of quinazolin-4-ones, dihydroquinilone-pyrazolines, and neurosteroids on the lower lobe of the ligand binding domain clamshell indicate that this region is an important site at which channel gating can be controlled (Jang et al., 2004). These data also show that the allosteric binding site(s) in this region can accommodate a wide range of structurally diverse, negative modulators, which increases the likelihood that well-tolerated and potentially therapeutically useful subunit-selective NMDA receptor antagonists can be developed. Furthermore, the findings presented here provide support for a previously unknown antagonist binding site that does not involve the amino terminal domain, the well-conserved agonist recognition sites, or residues within the channel pore. That is, this new site is distinct from GluN2B-selective negative allosteric modulators (Hansen et al., 2010a), competitive antagonists, and channel blockers.

Several studies have recently identified positive and negative allosteric modulators that act with enhanced potency at the GluN2C/D subunits (Costa et al., 2010; Mosley et al., 2010; Mullasseril et al., 2010). Of these, only one other class of non-competitive GluN2C/D antagonist has been reported with potency in the low micromolar range and greater than 20-fold selectivity over NMDA receptors comprised of other

GluN2 subunits, AMPA receptors, and kainate receptors. That class, which contains the previously identified AMPA antagonist quinazolin-4-one backbone (Mosley et al., 2010), is chemically distinct from DQP-1105 yet shares similar structural determinants of action, and a similar mechanism in that glutamate/NMDA binding enhances antagonist potency (Hansen and Traynelis, 2011). Structural determinants within NMDA receptor subunits have not been defined for other GluN2C/D-preferring antagonists, apart from the suggestion that the S2 region of the ligand binding domain is important in their actions (Costa et al., 2010; Horak et al., 2006). Thus, it is presently unclear whether the neurosteroids and phenanthrene derivatives share structural determinants with DQP-1105 and the quinazolin-4-ones.

It is known that the ligand binding domain undergoes a significant conformational change upon binding of agonist (Furukawa and Gouaux, 2003). It has been previously proposed that the GluN2C/D ligand binding domain closure around glutamate can increase the potency for quinazolin-4-one antagonists (Hansen and Traynelis, 2011), which share structural determinants of selectivity with DQP-1105. In this model, residues implicated by site-directed mutagenesis as important for the actions of inhibitors, including the residues identified in this study, are in sufficiently close proximity to the linkers (S1-M1 and M3-S2) connecting the transmembrane helices to the proximal region of the ligand binding domain to influence their movement (**Fig. 3.5**). Numerous studies have shown that structural determinants near or within the linker regions modulate receptor gating (Krupp et al., 1998; Talukder et al., 2010; Yuan et al., 2005). Most recently, specific residues within the S1-M1, M3-S2 and S2-M4 linker regions were suggested to serve as potential targets for allosteric modulators (Talukder et al., 2010).

Our identification of residues in the vicinity of the extracellular end of the transmembrane linkers raise the possibility that DQP-1105 and related analogues could interact with both the LBD and portions of the linker regions in order to negatively modulate the receptors.

Figure 3.5. Cartoon illustration of structural determinants of selectivity for DQP-1105.

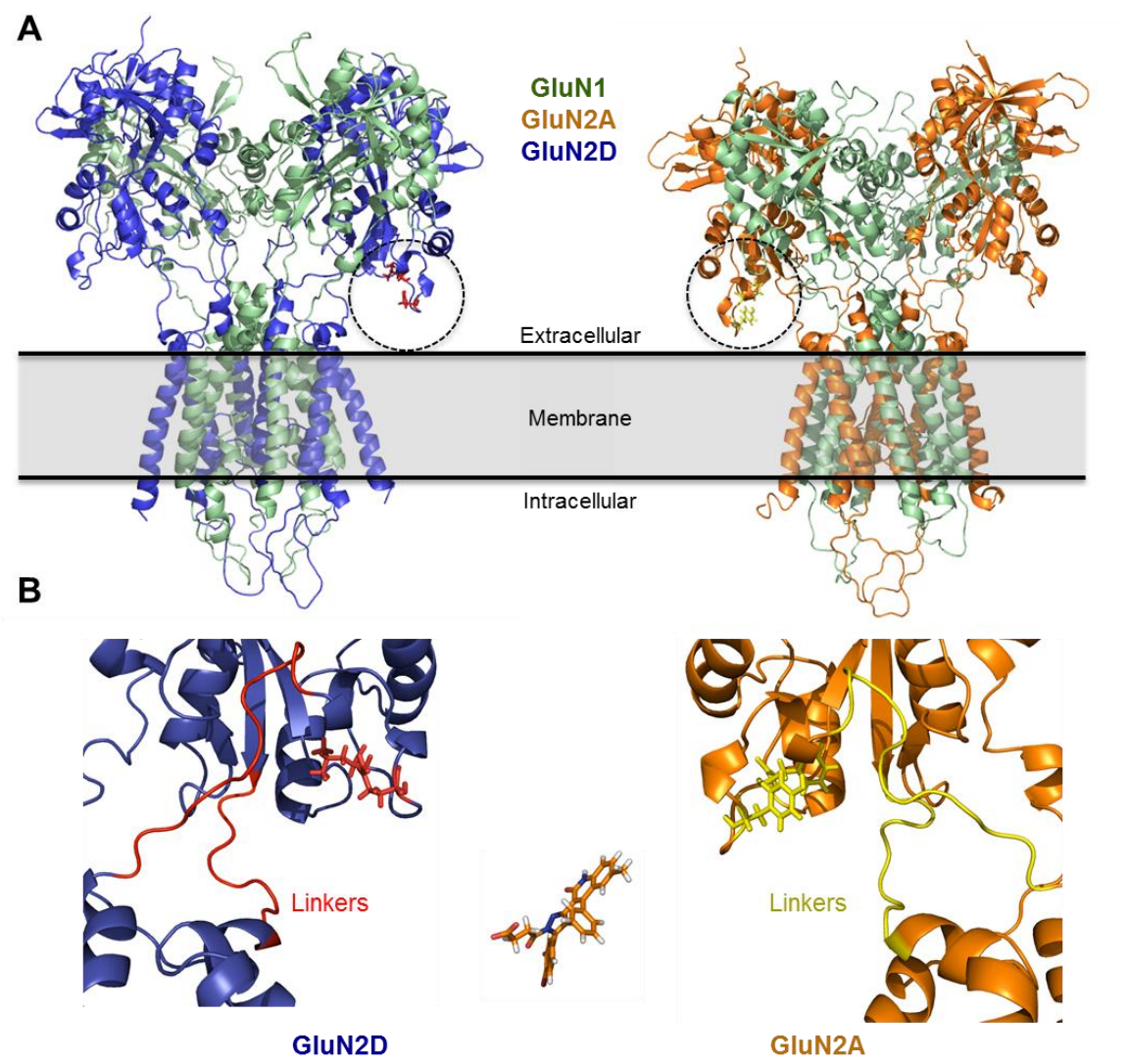


Figure 3.5. Homology models (Built by Dr. Pieter B. Burger) of GluN1-GluN2 receptors shows that residues identified in lower lobe of the clamshell shaped ligand binding domain are localized near each other in three-dimensional space, and in close proximity to the linkers that connect the ligand binding domain to the transmembrane helices. *A*, The *left panel* shows a GluN1-GluN2D receptor homology model (ATD omitted); the *right panel* shows a GluN1-GluN2A homology model (ATD omitted). *B*, Individual GluN2D-subunit are expanded (*left panel*) to show the lower portion of the ligand binding domain containing residues Q701 and L705 (red), which are critical divergent structural determinants for the antagonist activity of DQP-1105. The S1-M1 and M3-S2 linker regions are also shown in red. Individual GluN2A-subunits are expanded (*right panel*) to show the same region with the residues corresponding to GluN2D-Gln701(Tyr in GluN2A, yellow) and GluN2D- Leu705 (Phe in GluN2A yellow); linkers are also shown in yellow. Compound DQP-1105 is shown in the lower panel on the same scale (Acker et al., 2011).

While the residues implicated by mutagenesis data in DQP-1105 selectivity are not immediately adjacent on the polypeptide chain, homology models of GluN1/GluN2 place these residues close in three dimensional space (**Fig. 3.5.**). However, no crystallographic data exists for GluN2C, and the region identified in chimeric receptors shows a high degree of chain flexibility in the crystallographic structure of the isolated GluN2D ligand binding domain (Vance et al., 2011). Moreover, whereas I conclude that GluN2D residues Gln701 and Leu705 control the selectivity between GluN2A and GluN2D, the determinants of GluN2C selectivity have yet to be explored in depth. One of the residues important for GluN2D selectivity (Leu705) is a conserved Phe in GluN2A/B/C, suggesting this residue does not contribute to selectivity for GluN2C over GluN2A. Nevertheless, it is tempting to speculate that these residues may form or influence part of the binding pocket for DQP-1105 and related analogues. The finding that the DQP class of molecules shares structural determinants with the previously identified quinazolin-4-ones suggests that potent and selective molecules incorporating functionality from both groups might be developed through medicinal chemistry efforts.

Chapter 4. Structure activity relationship of DQP-1105 class of compounds.

4.1. Abstract

After describing the pharmacological mechanism of a representative member of the dihydroquinilone-pyrazoline (DQP) class of GluN2C/D subunit-selective antagonists, I set out to improve upon the selectivity and potency of the compounds using synthetic chemistry (Acker et al., 2011). In this chapter, I provide an extensive exploration of the structure-activity relationship (SAR), employing a traditional medicinal chemistry approach. I further provide an analysis of the stereochemical preference of one enantiomer for a representative member of the class and show that the *S* enantiomer of one of the more potent and selective compounds discovered is at least two-fold more potent than the racemic mixture and proves to be 10-fold more potent than the *R* enantiomer. I also describe the initial results regarding metabolic stability and BBB penetration. These efforts have yielded a 10-fold enhancement in potency and up to 200-fold selective analogues as well as insight into the requirements of the emerging pharmacophore for these compounds.

4.2. Introduction

Glutamatergic neurotransmission through ionotropic-glutamate receptors is the primary means of excitatory synaptic transmission in the mammalian central nervous system (CNS). The receptor family is comprised of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors (Mayer, 2005; Traynelis et al., 2010). NMDA receptors are widely expressed in the central nervous system and are thought to be involved in a range of important

physiological processes including axonal guidance, synaptic plasticity, and memory formation (Cull-Candy and Leszkiewicz, 2004; Pérez-Otaño and Ehlers, 2005; Traynelis et al., 2010; Wang et al., 2011). NMDA receptors are also thought to play an important role in pathophysiological conditions including Parkinson's disease, schizophrenia, depression, and ischemia (Hallett and Standaert, 2004; Huei-Sheng Vincent and Stuart, 2006; Mony et al., 2009; Traynelis et al., 2010).

NMDA receptors mediate the slow component of excitatory synaptic transmission and require the binding of both glutamate and glycine for channel activation. Glycine binds to the GluN1 subunit, which has eight splice variants encoded by a single gene (Durand et al., 1992; Furukawa and Gouaux, 2003; Hollmann and Heinemann, 1994). The GluN2 subunits (GluN2A-D) bind glutamate, and are encoded by four distinct genes (Furukawa et al., 2005; Hollmann and Heinemann, 1994; Vance KM, 2011). The GluN2 subunits control many of the functional and pharmacological properties of the receptor, including agonist EC_{50}, single channel open time and open probability, as well as deactivation-time course following removal of glutamate (Chen and Wyllie, 2006; Gielen et al., 2009; Paoletti and Neyton, 2007; Traynelis et al., 2010; Vance KM, 2011; Yuan et al., 2009). NMDA receptor deactivation time course determines the time course for the slow, Ca^{2+} -permeable component of synaptic transmission (Lester et al., 1990). Typically, NMDA receptors are blocked by extracellular Mg^{2+} at resting membrane potentials, and the requirements of the glutamate release and depolarization-induced relief of Mg^{2+} block have led to the idea that the NMDA receptors act as coincidence detectors in the brain (Mayer et al., 1984; Nowak et al., 1984). The Mg^{2+} IC_{50} and the

kinetics of block and unblock also vary according to the GluN2 subunit (Clarke and Johnson, 2006).

The GluN1 subunits are expressed throughout the CNS, but GluN2 subunit composition and expression vary both during development as well as anatomically (Akazawa et al., 1994; Bräuner-Osborne et al., 2000; Galvan and Wichmann, 2008; Hallett and Standaert, 2004; Laurie and Seeburg, 1994; Monyer et al., 1994; Standaert et al., 1993; Standaert et al., 1994). The spatially-restricted expression patterns, together with distinct functional and pharmacological differences imparted by the GluN2 subunits make NMDA receptor subunit-selective modulators of therapeutic interest for several neurological disorders, including stroke, schizophrenia, treatment resistant depression, Parkinson's disease (Bräuner-Osborne et al., 2000; Chen and Lipton, 2006; Goff et al., 2008; Hallett and Standaert, 2004). Subunit-selectivity will restrict modulator actions to brain regions that express the subunit of interest, potentially limiting side effects that occur as a result of global NMDA receptor block.

The discovery and pharmacological mechanism of a representative member of the DQP class of GluN2C/D subunit-selective antagonists was previously reported (Acker et al., 2011). In this report I provide an extensive exploration of the structure-activity relationship (SAR), an analysis of the stereoselectivity for a representative member of the class and initial results regarding metabolic stability and BBB penetration. These efforts have yielded significantly more potent and selective analogues as well as insight into the pharmacophore for these pyrazoline-containing compounds.

4.3. RESULTS

4.3.a. Chemistry

The structure-activity relationship around the quinolone-pyrazoline core was probed by testing the potency and selectivity of analogues with aromatic rings containing a variety of substitutions in combination with perturbations of the acyl chain moiety (**Figure 4.1.**, **Scheme 4.1.**). Anthranillic acids **1a-d** were reacted with triphosgene under standard conditions to yield the isatoic anhydrides **2a-d**. These compounds were then converted to the appropriate benzophenones via a known two-step sequence (Frye et al., 1991). The substituted quinolone core **4a-z** was accessed by condensation with ethyl acetoacetate using microwave irradiation. The resultant methyl ketone underwent base-catalyzed condensation with an appropriate aryl aldehyde **5a-z** yielding the α,β-unsaturated ketone compounds **6a-z**. These intermediates could be treated with hydrazine monohydrate in ethanol, utilizing microwave irradiation, to yield the pyrazoline containing compounds **7a-z**. The pyrazoline amine was then functionalized with anhydride derivatives **8, 9** or **10** to yield the fully saturated or *cis*-double bond derivatives.

Figure 4.1. General structure of DQP-class of compounds.

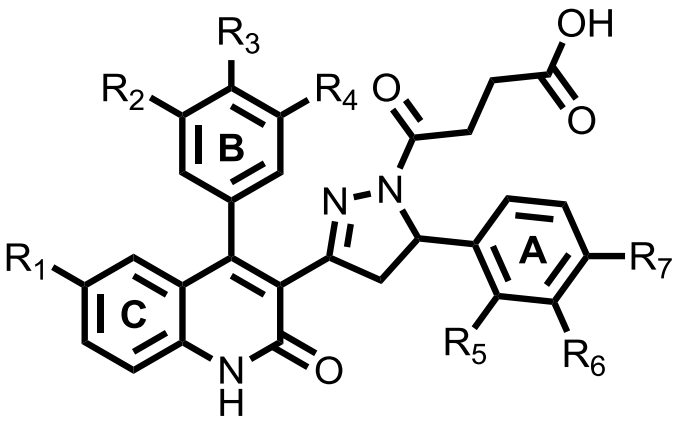
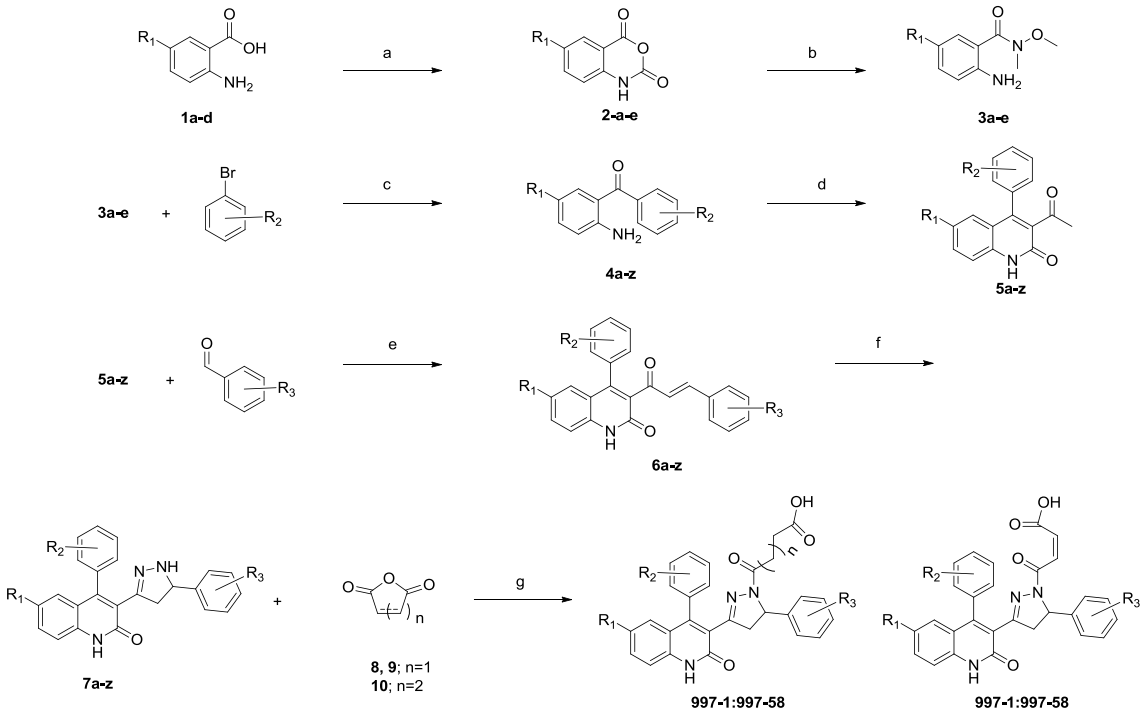


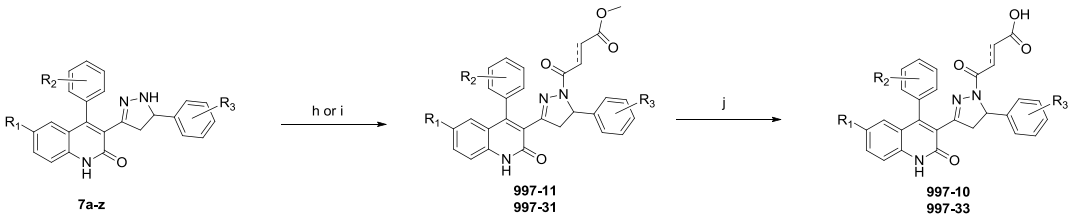
Figure 4.1 The structure of a general analogue with numbered substituents where modifications could readily be accessed is shown.

Scheme 4.1. Synthesis of dihydro-quinolone-pyrazoline derivatives.



Scheme 4.1. (a) Anhydrous THF, Triphosgene (warning, triphosgene is toxic, see methods), reflux. (b) EtOH, Weinreb's HCl salt, reflux. (c) Anhydrous THF, -78 °C. (d) ethylacetoacetate, DMF, 4Å molecular sieves, 180 °C, µW (e) 4:3 EtOH:H2O (0.05 M), 0 °C to r.t. (f) EtOH, 110 °C, µW (g) Anhydrous THF, 4Å molecular sieves, 165 °C, µW

Scheme 4.2. Synthesis of unsaturated and alkyl chain containing compounds.

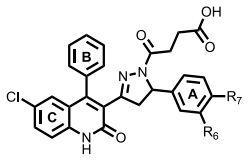


Scheme 4.2. (h) EDCI, DMAP. (**11**)-mono-methyl succinic acid, DCM, 0 °C to r.t. (i) Anhydrous THF, (**12**)- (E)-methyl 4-chloro-4-oxobut-2-enoate, 4Å molecular sieves, 165 °C, µW. (j) NaOH, EtOH:H_2O.

The unsaturated *trans*-derivatives could be accessed under standard amide coupling conditions with **11,** or with the acid chloride **11**, which could be saponified under basic conditions yielding the target scaffold (**Scheme 4.2**). These compounds were evaluated for activity at recombinantly rexpressed NMDA receptors in *Xenopus* oocytes as described in the methods section.

We first evaluated the effect of substituents on the **A**-phenyl ring (**Figure 4.1**) by holding the chlorine substitution on the quinolone core constant and evaluating the substitutions shown in **Tables 4.1** and **4.2**. Although substitution at any of the three available positions is tolerated, substitution at either the R_5 or R_6 position showed no improvement in potency or selectivity with any of the analogs tested (**Table 4.2**). The 4-substituted phenyl derivatives result in the best potency and selectivity. This observation led to the identification of **2023** (**Table 4.1**), which has a *para*-nitro group at R_7, as the most potent *para*-substituted analogue compared to the unsubstituted compound (1.1 μM vs. 88 μM respectively, **1149, Table 4.1**). We therefore explored bio-isosteres of the nitro substitution; replacing this group with a carboxylic acid (**997-5, Table 4.1**) which showed no activity. By contrast, the methyl ester (**997-6, Table 4.1**) remained active but had decreased potency (32 μM vs 1.1 μM). Interestingly, sp^3 hybridization is tolerated, but not preferred, as can be seen with the tri-fluoromethyl-containing compound **997-8** (IC_{50} 4.1 μM, GluN2D). A simplified Hansch analysis of the *para*-σ and *para*-π parameters of the A-ring at GluN2D-containing receptors suggests that the σ contribution is more directly associated (r^2=0.75, $p < 0.05$ Pearson two-tailed correlation analysis) with the IC_{50} values than the relative hydrophobicity (r^2= 0.21, $p > 0.29$, Pearson two-tailed correlation analysis) (**Figure 4.2.a** and **4.2.b**) (Topliss, 1977).

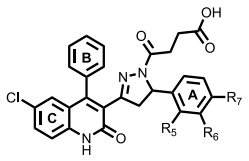
Table 4.1. Evaluation of A-Ring *para* substitutions**.**



DQP-	R_6	R_7	2A IC_{50} 2D IC_{50}	2B IC_{50} 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
1149	H	H	-	-	NE	NE	86	88
997-19	H	F	9	6	128	87	23	14
997-12	H	Cl	-	-	NE	NE	5.5	4.5
1179	H	Br	-	7	NE	22	3.6	3.1
2023	H	NO_2	85	42	91	45	0.9	1.1
997-5	H	COOH	-	-	NE	NE	NE	NE
997-6	H	COOMe	-	-	NE	NE	93	32
997-8	H	CF_3	20	13	80	54	5	4.1
997-17	H	OMe	9	9	197	187	28	21
2022	H	NMe_2	-	-	NE	NE	39	19
2006	-OCH_2O-		-	4	NE	90	23	23

Table 4.1. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 7-18 oocytes between 2-4 frogs; NE indicates less than 30% inhibition at 100 μM.

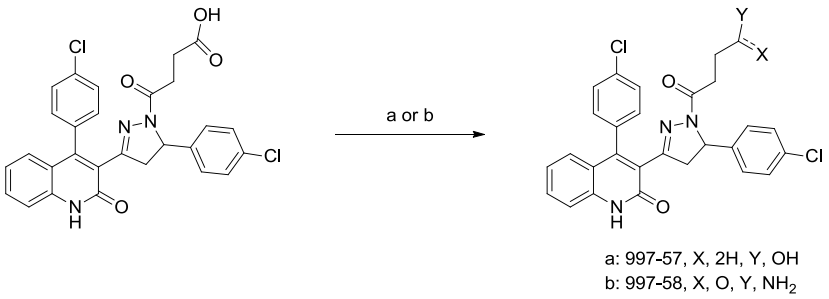
Table 4.2. Evaluation of A-Ring *ortho*- and *meta*- substitutions**.**



DQP-	R_5	R_6	R_7	2A IC_{50} 2D IC_{50}	2B IC_{50} 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-16	H	F	H	4	4	114	111	32	26
997-13	H	Cl	H	5	5	43	43	14	8.8
1176	H	Br	H	6	7	24	30	6.8	4.0
997-56	H	OMe	H	9	7	162	129	38	19
2021	H	NO_2	H	-	6	NE	109	22	19
2065	F	H	H	6	4	281	203	54	88
997-14	Cl	H	H	7	6	109	92	26	16
997-15	Br	H	H	8	6	102	80	21	13
2004	Cl	H	Cl	9	9	28	28	3.4	3.1
2062	OMe	H	H	-	4	NE	251	43	65

Table 4.2. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 6-10 oocytes between 2 frogs; NE indicates less than 30% inhibition at 100 μM.

Scheme 4.3. Synthesis of primary alcohol and amide derivatives.



a: 997-57, X, 2H, Y, OH
b: 997-58, X, O, Y, NH_2

Scheme 4.3. (a) BH_3-DMS, Anhydrous THF, 0 °C. (b) EDCI, DMAP, NH_3 in dioxane (0.5M), THF.

Figure 4.2. Correlation between C-ring σ and π parameters to potency.

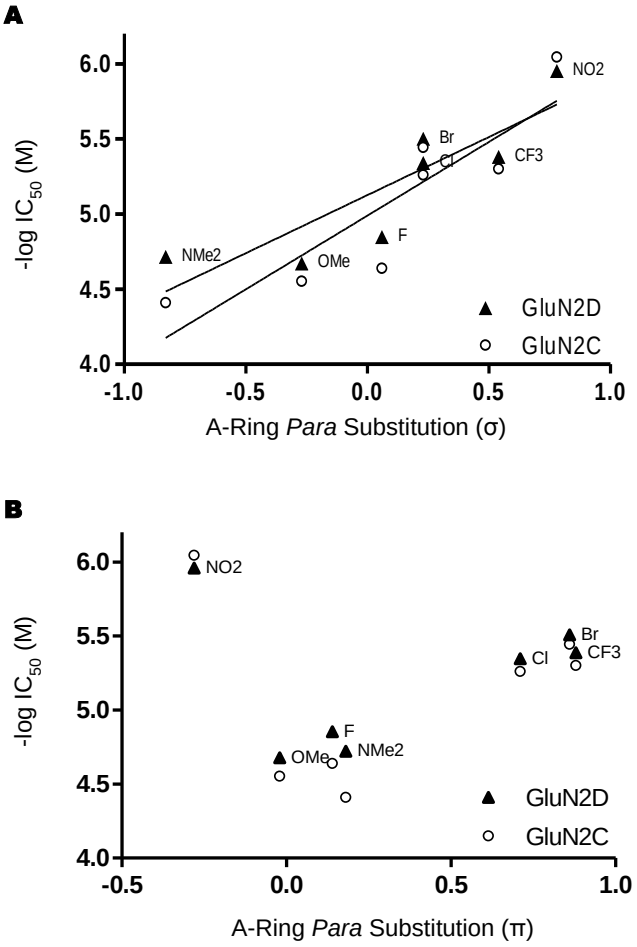
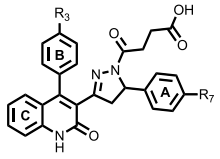


Figure 4.2. Correlation between C-ring σ and π paramaters to potency. A. The σ contribution of the *para*-substitution vs. log IC_{50} values of the A-ring shows a correlation for GluN2C- and GluN2D-containing receptors. (GluN2D r^2=0.75, $p < 0.05$ Pearson two-tailed correlation analysis; GluN2C r^2 =0.79, $p < 0.05$ Pearson two-tailed correlation analysis). B. The π contribution of the *para*-substitution vs. log IC_{50} values of the A-ring shows no correlation for GluN2C- and GluN2D-containing receptors (Pearson two-tailed correlation analysis $p > 0.05$ for both).

I next modified the B-ring substituents, employing a Hansch-type approach, with the aim of understanding the physical properties of the substituent effects at the *meta*- and *para*-positions (Topliss, 1977). *Para*-B-ring substitutions showed enhanced potency at GluN2D- and GluN2C-containing receptors, with IC_{50} values of 0.71 μM and 0.39 μM for **997-7** inhibition of GluN2C- and GluN2D-containing receptors, respectively (**Table 4.3**). Interestingly, this compound also showed less selectivity for GluN2D-containing over GluN2A-containing receptors (33-fold) as compared to GluN2B-containing receptors (67-fold), suggesting a slightly more favorable interaction with GluN2A-containing receptors.

Co-varying the A-ring *para*-substituents with the *para*-bromine B ring substitution determined the *para*-chlorine A-ring substitution to be optimal for potency; therefore, this was used for further SAR elaboration (**997-7, -20, -29, -55**). A similar trend was observed using the *para*-chlorine substitution on the B-ring while co-varying the A-ring substituents (**997-54, -23, -52, -53**). The evaluation of the *para*-σ substituent effects at the B-ring show a seemingly parabolic relationship when compared to potency for GluN2A-, GluN2C- and GluN2D-containing receptors with an optimal σ value corresponding to the bromine and chlorine substitutions at all three receptors (**Figure 4.3.A** and **4.3.B**). At the *meta*-position on the B ring, the correlation between the potency and the hydrophobic π value for substitutions at GluN2A-containing receptors also appears parabolic, but similar results were not found with GluN2B-, GluN2C-, or GluN2D-containing receptors (**Figure 4.3.C** and **4.3.D**). The decrease in potency at GluN2A-containing receptors in compounds that are *meta*-substituted with the CF_3 group

Table 4.3. A and B Ring Modifications.



DQP-	R_3	R_7	2A IC_{50} 2D IC_{50}	2B IC_{50} 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-7	Br	Br	33	59	13	23	0.71	0.39
997-20	Br	Cl	34	79	10	23	0.56	0.29
997-29	Br	F	12	26	34	75	3.8	2.9
997-55	Br	H	7	12	64	113	10	9.1
997-54	Cl	Br	37	67	19	34	0.95	0.51
997-23	Cl	Cl	48	50	21	22	0.77	0.44
997-52	Cl	F	14	26	47	90	4.1	3.4
997-53	Cl	H	4	13	49	143	13	11

Table 4.3. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 8-18 oocytes between 2-3 frogs.

Figure 4.3. Evaluation of substituent effects of B-ring modifications.

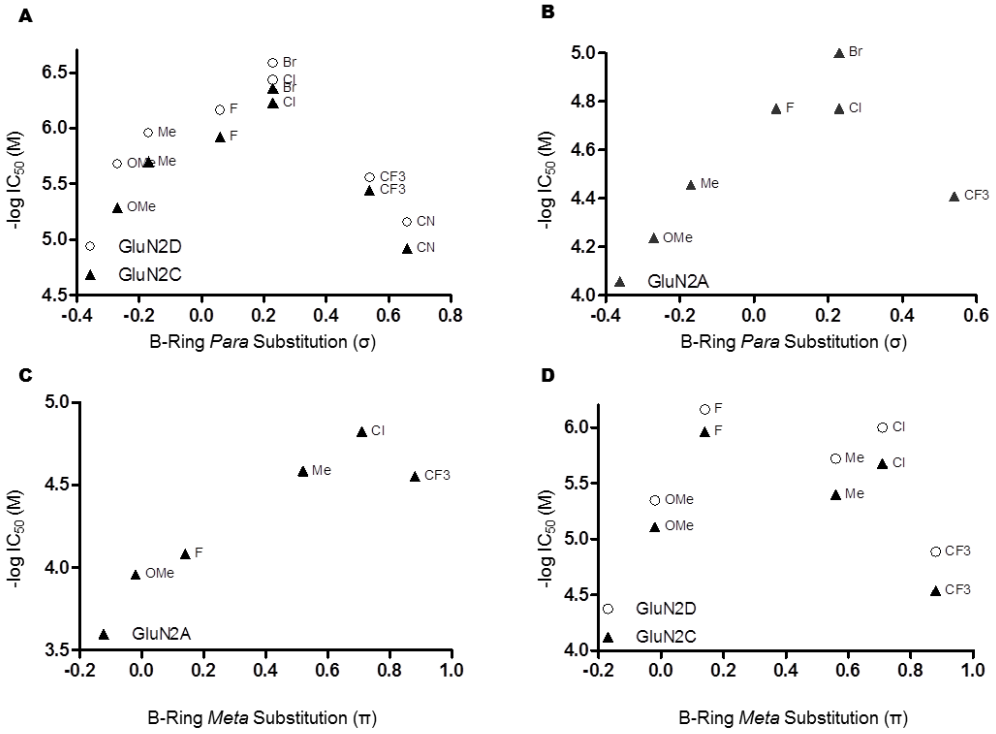


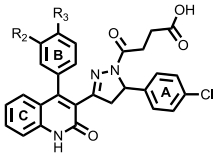
Figure 4.3. Evaluation of substituent effects for B-ring modifications. A. The analysis of the *para*-substituents at GluN2C- and GluN2D-containing receptors appears parabolic with respect to the σ coefficient, with an optimal value close to that of the chloro and bromo substitution. B. The analysis of the *para*-substituents at GluN2A-containing receptors shows a similar parabolic relationship as observed at the GluN2C- and GluN2D-containing receptors with the σ coefficient. C. The analysis of the *meta*-substituent effects showed no clear correlation with the π coefficient for GLuN2C- or GluN2D-containing receptors. D. The analysis of the *meta*-substituent effects appears parabolic with respect to the π coefficient for GluN2A-containing receptors, suggesting an optimal interaction close to that of the chlorine substitution.

(**997-51**, **Table 4.4** and **Figure 4.3.d**) could be a result of steric clashes with the receptor, or as was observed with the *para*-σ substituent, could suggest that the optimal hydrophobicity at the GluN2A-containing receptors is attained with the chlorine substitution.

From this analysis of *meta*- and *para*-substitutions, we hypothesized that combining an optimally parameterized *para*-substitution for potency at GluN2D-containing receptors with a *meta*-substitution that was less active at GluN2A-containing receptors might improve selectivity. Both Cl and F substitutions gave optimal activity, thus these groups were co-varied (**Table 4.5**). I therefore synthesized compound **997-38,** which maintained potency but did not increase selectivity (**Table 4.5**). Several other compounds that were di-substituted on the B-ring exhibited submicromolar potency at GluN2D-containing receptors, but all showed modest selectivity over GluN2A-containing receptors (**997-34, 41, 42, 35, 37**, **Table 4.5**). Compounds that were di-substituted did not improve compound properties over mono-substituted analogues.

I next made a series of substitutions to the C-ring on the quinolone core (**Table 4.6**). Beginning with the a methyl group at R_1 in combination with either the *para*-chlorine or the *meta*-fluoro substitution on the B-ring, I synthesized compounds **997-43** and **997-44,** which decreased the potency as compared to the more favorable compounds with only B-ring and A-ring substitution. Interestingly, the modifications showed variability with regards to the relative selectivity for GluN2A- over GluN2B-containing receptors, suggesting that there remains room in this portion of the binding pocket for potential optimization of selectivity.

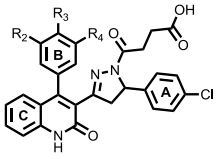
Table 4.4. B Ring Modifications**.**



DQP-	R_2	R_3	2A IC_{50} 2D IC_{50}	2B IC_{50} 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-24	H	F	36	98	21	57	1.0	0.58
997-23	H	Cl	48	50	21	22	0.77	0.44
997-26	H	Me	24	30	33	42	2.5	1.4
997-21	H	OMe	27	33	62	75	5	2.3
997-27	H	CN	22	-	156	NE	12	7
997-36	H	CF_3	10	14	29	39	3.6	2.8
997-28	F	H	67	101	46	70	1.1	0.69
997-22	Cl	H	20	43	20	43	2.1	1.0
997-25	Me	H	13	37	24	71	4.0	1.9
997-39	OMe	H	24	34	110	152	7.8	4.5
997-40	CN	H	-	-	NE	NE	19	13
997-51	CF_3	H	11	18	28	47	3.4	2.6

Table 4.4. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 6-14 oocytes between 2-3 frogs; NE indicates less than 30% inhibition at 100 μM. Data for 997-23 is presented in **Table 4.3** and repeated here to facilitate comparisons.

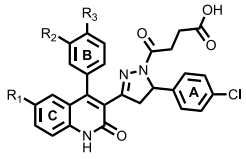
Table 4.5. B-Ring Di-substitutions**.**



DQP-	R_2	R_3	R_4	2A IC_{50} / 2D IC_{50}	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-38	F	Cl	H	23	53	12	28	0.91	0.53
997-34	Cl	F	H	19	34	19	34	1.4	1.0
997-41	Cl	Cl	H	8	22	7.7	20	0.79	0.91
997-42	F	F	H	32	108	21	71	0.78	0.66
997-35	F	H	F	26	123	21	100	1.1	0.81
997-37	Cl	H	Cl	8	19	5.5	13	0.78	0.70

Table 4.5. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves from oocyte recordings (see Methods). Data are from 8-15 oocytes between 2 frogs.

Table 4.6. C-Ring Modifications**.**

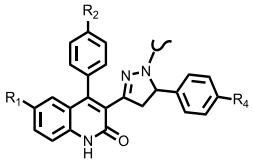


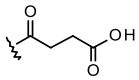
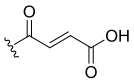
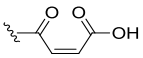
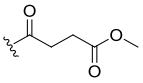
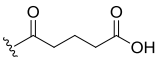
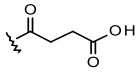
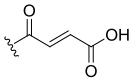
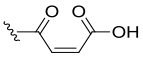
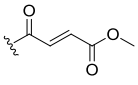
DQP-	R_1	R_2	R_3	2A IC_{50} / 2D IC_{50}	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-43	CH_3	H	Cl	42	82	54	107	1.5	1.3
997-44	CH_3	F	H	28	32	128	144	5.9	4.5
997-46	Cl	H	Cl	11	9	14	12	2.2	1.3
997-45	Cl	F	H	11	10	57	51	7.3	5.3
997-49	F	H	Cl	10	16	14	23	2.5	1.4
997-50	F	F	H	10	16	43	69	3.4	4.3
997-47	OMe	H	Cl	10	27	50	140	3.8	5.2
997-48	OMe	F	H	10	13	100	132	14	10

Table 4.6. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see Methods). Data are from 7-12 oocytes between 2 frogs.

I subsequently evaluated a series of perturbations of the acyl chain of the pyrazoline nitrogen (**Table 4.7**). Restricting the conformation to a *cis*-configuration (**997-30**) with the maleate derivative maintained potency as compared with the parent compound in each instance tested (**Table 4.7**). The *trans*-fumaric derivatives (**997-33 and 997-10; Scheme 4.2**) were slightly more potent at GluN2D-containing receptors than the saturated derivatives, but no more selective than the saturated derivatives over GluN2A- and GluN2B-containing receptors (**Tables 4.7, 4.4, and 2**). The fumaric ester was inactive, as was the succinic ester (**997-31** and **997-11; Table 4.7**) and these substitutions were not explored further. I also evaluated several glutaric derivatives such as compound **997-32**, which showed similar potencies to that of the succinic derivative (**Table 4.7 and 997-20** in **Table 4.3**) at all receptors tested, suggesting that the length of the acyl chain is not crucial for activity. Reduction of the acid to the primary alcohol led to **997-57** (**Table 4.7, Scheme 4.3**) which retained activity at GluN2C- and GluN2D-containing receptors while improving selectivity over GluN2A-containing receptors to 90-fold as compared to the acid containing compound (**997-23**, **Table 4.2**). The primary amide derivative of the succinate acyl chain was then synthesized (**997-58, Scheme 4.3**), and found to retain activity but showed decreased potency and selectivity compared to the alcohol and acid moieties. Replacing the amide linkage with the alkyl derivative in compound **997-62** diminished potency at GluN2D-containing receptors slightly over the parent compound **997-23**; however, selectivity over the other receptor subtypes was maintained (**Table 4.7, Scheme 4.3**). Replacing the primary alcohol with the mono-fluorine isostere led to compound **997-64** (**Scheme 4.4**), which was inactive at the receptors but is described in more detail below (Kim et al., 2008; O'Hagan, 1989).

Table 4.7. Acyl chain perturbations**.**



DQP	R_1	R_2	R_4	Acyl Chain	2A IC_{50} / 2D IC_{50}	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
1179	Cl	H	Br		-	7	NE	22	3.6	3.1
997-10	Cl	H	Br		-	-	NE	NE	2.1	1.4
997-9	Cl	H	Br		15	7	74	37	8.9	5
997-11	Cl	H	Br		-	-	NE	NE	NE	NE
2066	Cl	H	Br		20	23	78	90	6.4	4.0
997-20	H	Br	Cl		34	79	10	23	0.56	0.29
997-33	H	Br	Cl		23	62	4.3	11.7	0.20	0.19
997-30	H	Br	Cl		21	30	12	17	1.0	0.57
997-31	H	Br	Cl		-	-	NE	NE	59	95

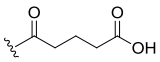
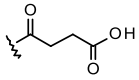
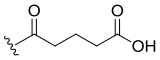
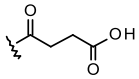
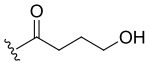
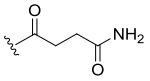
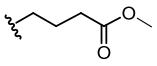
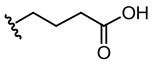
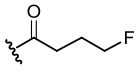
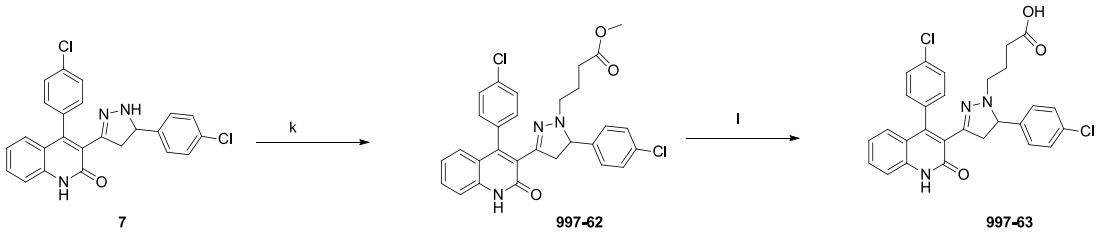
997-32	H	Br	Cl		33	91	10.4	29	0.6	0.32
2023	Cl	H	NO_2		85	42	91	45	0.9	1.1
997-4	Cl	H	NO_2		10	8	109	92	10	11
997-23	H	Cl	Cl		48	50	21	22	0.77	0.44
997-57	H	Cl	Cl		90	48	62	33	1.7	0.69
997-58	H	Cl	Cl		31	18	34	20	2.0	1.1
997-62	H	Cl	Cl		-	-	NE	NE	NE	NE
996-63	H	Cl	Cl		34	31	58	53	3	1.7
997-64	H	Cl	Cl		-	-	NE	NE	NE	NE

Table 4.7. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 5-24 oocytes between 2-4 frogs; NE indicates less than 30% inhibition at 100 μM. Data for compounds 1179, 997-20, 2023, 997-23, and were presented in preceding tables, and are shown here for comparison.

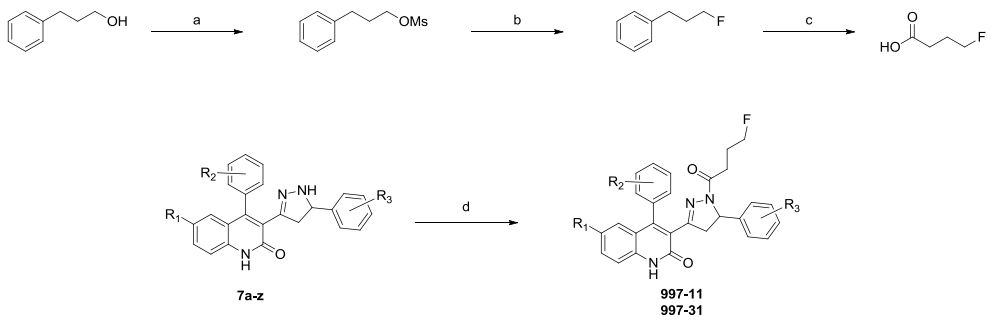
Scheme 4.3. Synthesis of alky chain derivatives**.**



7 997-62 997-63

Scheme 4.3. Synthesis of alkyl chain derivatives. (k) methyl 4-oxobutanoate, BH_3-DMS, THF (i) NaOH, EtOH:H_2O.

Scheme 4.4. Synthesis of mono-fluoro isostere of the alcohol derivative.

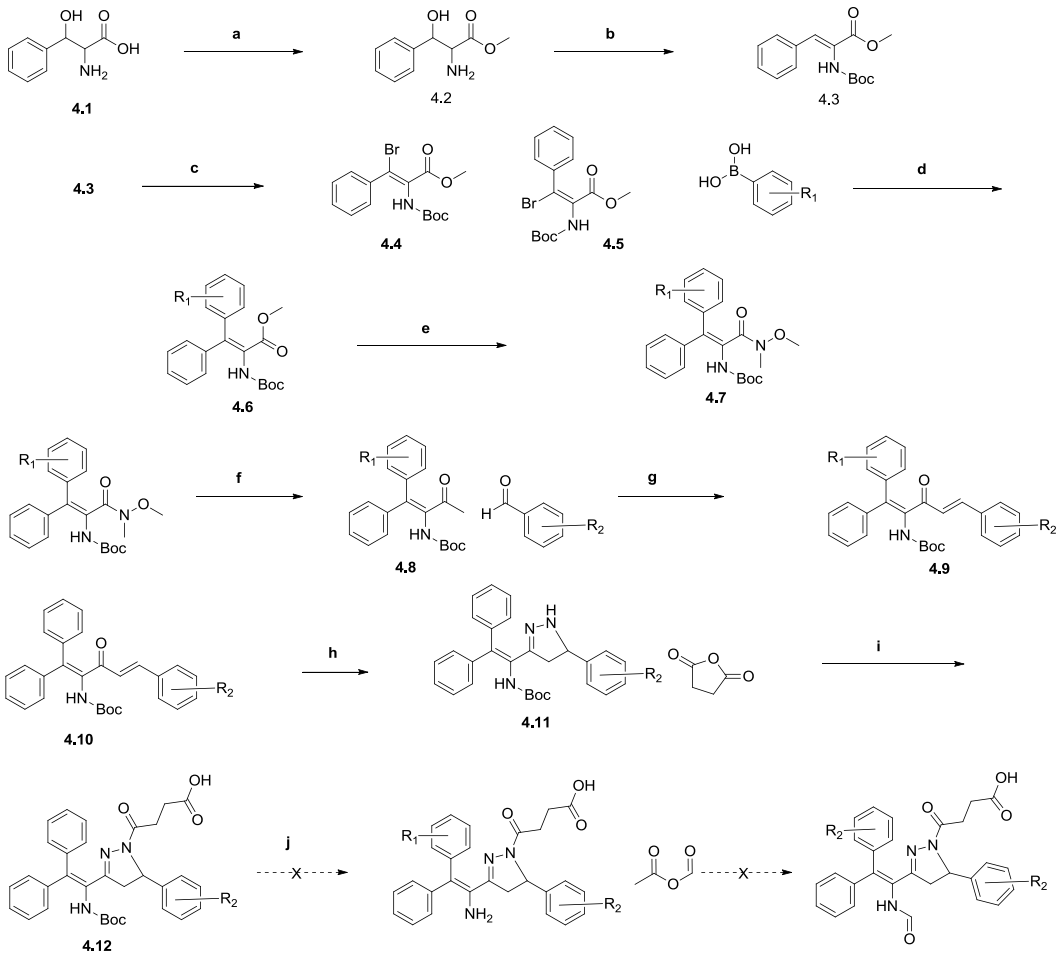


Scheme 4.4. (a) MsCl, Et_3N, DCM. (b) CsF, t-BuOH. (c) $RuCl_3$, $KNaIO_4$, CCl_4:ACN:H_2O. (d) EDCI, DMAP, DCM.

These observations suggest that the negative charge of the succinic acyl moiety is not required.

I also designed a synthetic scheme aimed at better understanding the contributions of the quinolone core to the activity of the compounds. The target molecules were envisioned to allow for breaking the aniline linkage and amide bond contained within the quinolone core while allowing for other functionality that was important for activity to remain geometrically similar (**Scheme 4.5**). The beta-hydroxy amino acid **4.1** could be converted to the methyl ester derivatives under standard conditions. A two-step sequence was required to obtained the Boc-protected de-hydro compound, **4.2**. The resultant double bond could be brominated to give a 3:1 mixture of separable isomers **4.4** and **4.5**. Suzuki coupling conditions afforded the di-phenyl containing **4.6** which underwent transformation to the Weinreb amide **4.7**. Grignard addition gave the methyl ketone **4.8** in moderate yields and these compounds could be treated with the general conditions outlined in **scheme 4.1** to give the Boc-protected precursor to the desired target, **4.12**. Unfortunately, the de-protected enamine was subject to rapid hydrolysis in the presence of tri-fluoro acetic acid and the compounds were no longer pursued. While the Boc-protected compound **4.12** showed only minimal activity at GluN2D-containing receptors, the Boc-carbamate is a relatively large protecting group. The observation that some inhibition occurred (approx. 50% at 100 μM) suggests that further manipulation at this region of the compounds could yield better results.

Scheme 4.5. Scaffold-hopping synthesis.



Scheme 4.5. Synthetic conditions for scaffold hopping compounds. (a) HCl, MeOH. (b) i. Boc_2O, pyridine ii. Boc_2O, DMAP iii. Tetratmethyl guanidine. (c) i. NBS ii. Et_3N, DCM (d) $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O. (e) Weinreb HCl salt, *n*-BuLi. (f) methylmagnesium bromide, THF, 0ºC. (g) KOH/EtOH/H_2O. (h) hydrazine monohydrate, µW. (i) µW, THF, molecular sieves. (j) TFA.

Finally, I evaluated the selectivity and potency of purified enantiomers of a representative member of this class of compound. The enantiomers of the racemic final product (**997-23**) were separable via reverse phase chiral chromatography using an OD-RH column (**Figure 4.3,** see *Methods*). Absolute stereochemistry of the second peak to elute was assigned using X-ray crystallography (**Figure 4.3**). Evaluation of the purified enantiomers showed that the *S* enantiomer is 13 times more potent at GluN2D-containing receptors (IC_{50} 0.17 μM) than the *R* enantiomer (IC_{50} 1.9 μM; **Table 4.8**). In addition, the *S* enantiomer shows enhanced selectivity for GluN2C/D over GluN2A/B compared to the racemic mixture (**Table 4.8**).

Figure 4.3. Enantiomeric resolution of 997-23.

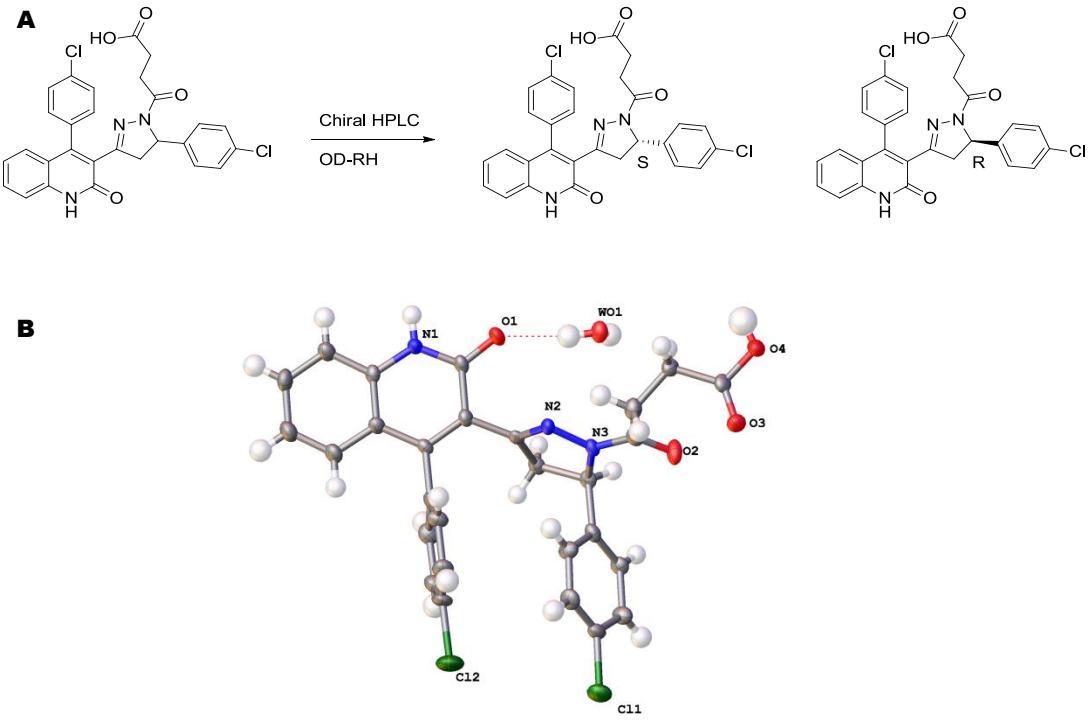
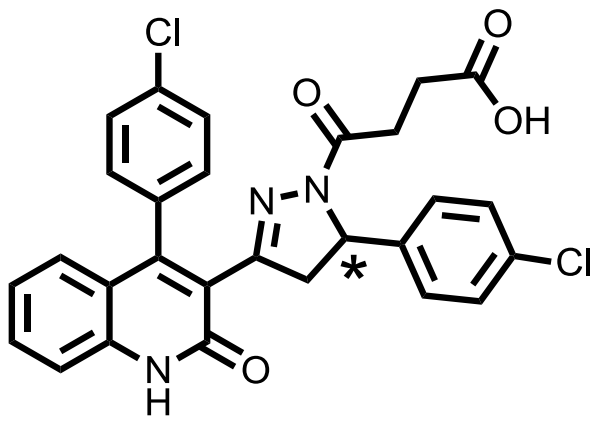


Figure 4.3. Separation of enantiomers and crystallographic data for *R* configuration. A. The enantiomers of the final compound, **997-23**, could be separated using reverse phase chiral chromatography. B. The crystal structure of the inactive enantiomer was solved using X-ray diffraction and has the *R* configuration (Data and analysis from X-ray crystallography core; Chemistry Dept.).

Table 4.8. Stereochemistry preference of purified enantiomers**.**



DQP	R_2	R_4	2A IC_{50} / 2D IC_{50}	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
997-23	Cl	Cl	48	50	21	22	0.77	0.44
***S* 997-59**	Cl	Cl	78	156	13	26	0.22	0.17
***R* 997-60**	Cl	Cl	23	49	45	52	2.1	1.9

Table 4.8. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 8-17 oocytes between 2-4 frogs; data for compound 997-23, which was presented in preceding tables, is shown here for comparison.

4.3.b. Evaluation of off-target effects

The actions of these compounds on 42 different ion channels, G-protein coupled receptors, and transporters were tested via the National Institutes of Mental Health (NIMH) psychoactive drug screening program (PDSP; **Table 4.9**). The primary binding assay demonstrated that compounds **997-23** and **997-33** had a minimal effect on the receptors and transporters, with initial screens showing inhibition of 3 receptors by **997-23** (5HT6, H2, kappa-opoid) and 4 receptors by **997-33** (5-HT_{1E}, 5-HT_6, kappa-opoid, mu-opoid). The *Ki* values at these receptors were greater than 10 μM for both compounds on all receptors. The data collected from the PDSP demonstrate the utility of this class of compounds to selectively inhibit the GluN2C- and Glun2D-containing NMDA receptors.

Table 4.9: Off-target responses for compounds 997-23 and 997-33.

Receptor	$I_{TEST(23)}$ / $I_{CONTROL}$ (%)	$I_{TEST(33)}$ / $I_{CONTROL}$ (%)	n
Serotonin receptor 5-HT1A	79	83	4
Serotonin receptor 5-HT1B	84	88	4
Serotonin receptor 5-HT1D	111	119	4
Serotonin receptor 5-HT1E	117	115	4
Serotonin receptor 5-HT2A	110	113	4
Serotonin receptor 5-HT2B	80	76	4
Serotonin receptor 5-HT2C	82	96	4
Serotonin receptor 5-HT3	110	114	4
Serotonin receptor 5-HT5A	103	92	4
Serotonin receptor 5-HT6	45	46	4
Serotonin receptor 5-HT7	121	132	4
Adrenergic receptor α1A	117	111	4
Adrenergic receptor α1B	85	74	4
Adrenergic receptor α1D	114	115	4
Adrenergic receptor α2A	76	99	4
Adrenergic receptor α2B	89	90	4
Adrenergic receptor α2C	68	41	4
Adrenergic receptor β2	105	88	4
Adrenergic receptor β3	113	73	4
Benzodiazepine site in Rat Brain	108	119	4
Dopamine receptor D1	98	85	4
Dopamine receptor D2	85	73	4
Dopamine receptor D3	50	109	4
Dopamine receptor D4	77	65	4
Dopamine receptor D5	93	59	4
Opioid receptor (δ)	91	75	4
Opioid receptor (κ)	114	14	4
Opioid receptor (μ)	48	50	4
Histamine receptor H1	114	83	4
Histamine receptor H2	48	80	4
Histamine receptor H3	89	90	4
Muscarinic receptor M1	111	115	4
Muscarinic receptor M2	120	115	4
Muscarinic receptor M3	110	110	4
Muscarinic receptor M4	110	120	4
Muscarinic receptor M5	112	110	4
Sigma receptor 1	89	57	4
Sigma receptor 2	106	117	4
PBR	96	88	4
Norepinephrine transporter	87	84	4
Dopamine transporter	51	50	4
Serotonin transporter	38	68	4

Table 4.9. Receptors for which the primary screen showed more than 50% inhibition of binding were subject to determination of Ki, which was >10 μM for all receptors and transporters tested. Compounds **997-23** and **997-33** were tested at 5 μM for all targets.

4.3.c. Aqueous solubility, BBB penetration and human liver microsomal stability

Finally, three of the most potent analogs, **997-33**, **997-23** and **997-57** were evaluated for plasma stability. The compounds showed minimal degradation in human, rat or mouse plasma over a two-hour time-course (data not shown). I evaluated the aqueous solubility of compound **997-23** in oocyte recording buffer using nephelometry to be > 80 μM.

The topological polar surface area (TPSA) of the carboxylic acid compounds was calculated to be outside the optimal range (< 90 $Å^2$) for blood-brain barrier (BBB) penetration (**997-23** TPSA**,** 129.5 $Å^2$, QikProp). However, reduction of the acid to the alcohol moves the properties of this class closer to a typical range for CNS penetration (**997-**57; 102.0 $Å^2$, QikProp) (Hitchcock and Pennington, 2006). In order to assess the potential BBB penetration, compounds **997-23** and **997-57** were selected for evaluation in the MDR1-MDCK permeability assay (**Table 4.10**)(Wang et al., 2005). The MDR1-MDCK assay is a permeability assay which assesses the potential for BBB penetration using dog kidney cells which overexpress p-glycoprotein (P-gp) and have high transepithelial resistance (Wang et al., 2005). As was anticipated with the carboxylic acid containing **997-**23, the potential for BBB penetration was low. The results for the alcohol-containing compound also suggested low BBB potential, however, the permeability coefficient (Papp (A-B)) was

Table 4.10. MDR1-MDCK permeability assay.

Test Compound	Direction	Recovery (%)	$P_{app}(10^{-6}cm/s)$			Efflux Ratio
			1	2	Avg	
(64)997-57	A-to-B	43	2.45	2.48	2.46	26
	B-to-A	73	66.2	62.7	64.5	
(26)997-23	A-to-B	73	0.47	0.47	0.47	55
	B-to-A	76	33.4	17.9	25.6	
(68)997-64	A-to-B	43	4.51	3.24	3.88	2.5
	B-to-A	67	9.32	9.72	9.52	

Table 4.10. The P_{app} and efflux ratio were calculated as described in the Methods section. Compounds displaying a $P_{app} < 3.0 \times 10^{-6}$ cm/s and an efflux ratio > 10 are interpreted to have a low potential for crossing the BBB. Compounds with $P_{app} > 3.0 \times 10^{-6}$ cm/s and an efflux ratio < 10 are expected to have high brain penetration.

Table 4.11. Human liver microsomal stability.

Test Compound	% Remaining of initial					Half-life[a]	CL_{int}[b] (ml/min/mg protein)
	0 min	10 min	20 min	30 min	60 min		
(64)997-57	100	52	31	24	6.5	13	0.110
(26)997-23	100	101	100	115	86	>60	<0.02
(68)997-64	100	67	49	49	39	35	0.040

Table 4.11. [a] Half-life was calculated based on $t_{1/2} = 0.693/k$, where k is the elimination rate constant based on the slope of the natural logarithom percent remaining versus incubation time. [b] Intrinsic clearance (CL_{int}) was calculated on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

much closer to the recommended 3.0 X 10^{-6} cm/s (**997-57**, (Papp A-B; 2.46 X 10^{-6} cm/s)) than that of the carboxylic acid containing compound (**997-23**, (Papp A-B; 0.47 X 10^{-6} cm/s)) (**Table 4.10**)(Wang et al., 2005). From the results of the assay, it is unclear whether Pgp-efflux, or low membrane permeability remains to be optimized. The same compounds were also evaluated for metabolic stability in human liver microsomes. While the carboxylic acid containing **997-23** showed minimal degradation over the 60 minute assay, the alcohol derivative, **997-57**, had a half-life of 13 minutes (**Table 4.11**).

In order to evaluate an analog with lower TPSA, the mono-fluoro **997-64** (TPSA 79.08 $Å^2$; QikProp; **Table 4.7**) was assayed in the MDR1-MDCK assay and was classified as being highly brain penetrable (Papp A-B; 3.88 X 10^{-6} cm/s, (Papp B-A; 9.52 X 10^{-6} cm/s)) (**Tables 4.7** and **4.10**). This compound was also less susceptible to degradation in the human liver microsomal assay than the alcohol derivative (**997-57**) (**Table 4.11**). Unfortunately, this compound did not retain the activity of the alcohol derivative at the receptors; however, this data suggests that the acyl-chain is a candidate for further optimization. Taken together, the physico-chemical properties of the class will need to be improved upon before broad *in vivo* utility is warranted.

4.4. Discussion

This study describes the development of potent, selective, and soluble negative allosteric modulators for GluN2C- and GluN2D-containing NMDA receptors that act on the membrane proximal lobe of the GluN2 glutamate binding domain. I describe here several compounds with IC_{50} values in the 100-500 nanomolar range that show 50-200 fold selectivity over GluN2A- and GluN2B-containing receptors (see **Figure 4.5**).

I have taken a classical approach to the SAR, which has allowed me to propose features of a hypothetical pharmacophore (**Figure 4.6)**. My results suggest there is a conserved portion of the binding pocket between GluN2A-, GluN2C- and GluN2D-containing receptors with respect to the *para*-position of the B-ring, specifically with regard to the σ coefficient. The observation that there is optimal activity with a chlorine or bromine at this position has led to the hypothesis that there could be a halogen bond being formed at the receptors. Furthermore, the SAR has revealed that rigidifying the acyl-chain in the trans-conformation can enhance potency at the GluN2D-containing receptors over, while the length of the chain was found not to be crucial for activity. Furthermore, the finding that **997-57** (**Table 4.7**) both retained potency and selectivity suggests that the charge on the carboxylic acid is not crucial, however, the data suggest that a hydrogen bond donor may be required at that position (**Figure 4.6**). The substituents that were explored on the A-ring allowed for an analysis that directly correlated potency to the σ coefficients, when the R_8 position was substituted with chlorine (**Figure 4.1**). Thus, the electronics of the A-ring are the most important factor for activity at the desired GluN2C- and GluN2D-containing receptors when the quinolone core is substituted with chlorine.

Figure 4.5. Improvements in selectivity and potency.

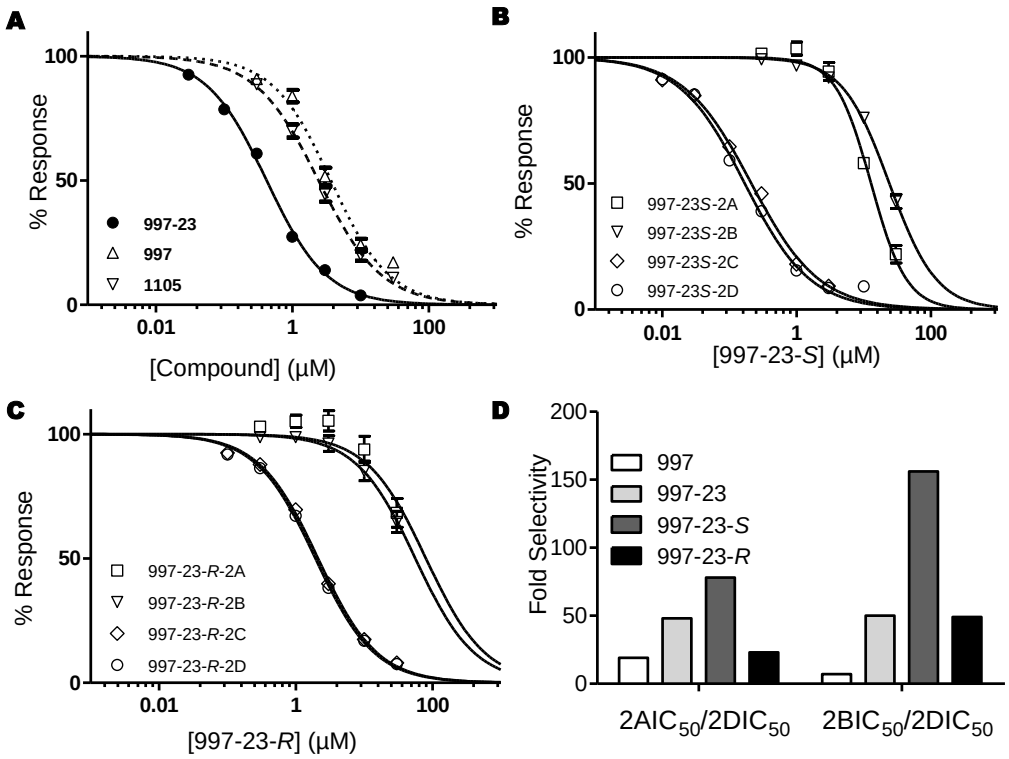


Figure 4.5. A. The potency of the racemic compounds at GluN2D-containing receptors was improved 10-fold over the previous members in the class. B. The potency of the S-enantiomer of compound 997-23 is two-fold more potent than the racemic mixture at GluN2D-containing receptors while the potency at GluN2A- and GluN2B containing receptors is unaffected. C. The potency of the *R*-enantiomer at GluN2C- and GluN2D-containing receptors is diminished as compared to the racemate making it much less selective over GluN2A- and GluN2B-containing receptors. D. Bar graph showing the fold-selectivity improvements attained through SAR.

Figure 4.6 Pharmacophore features elucidated through SAR**.**

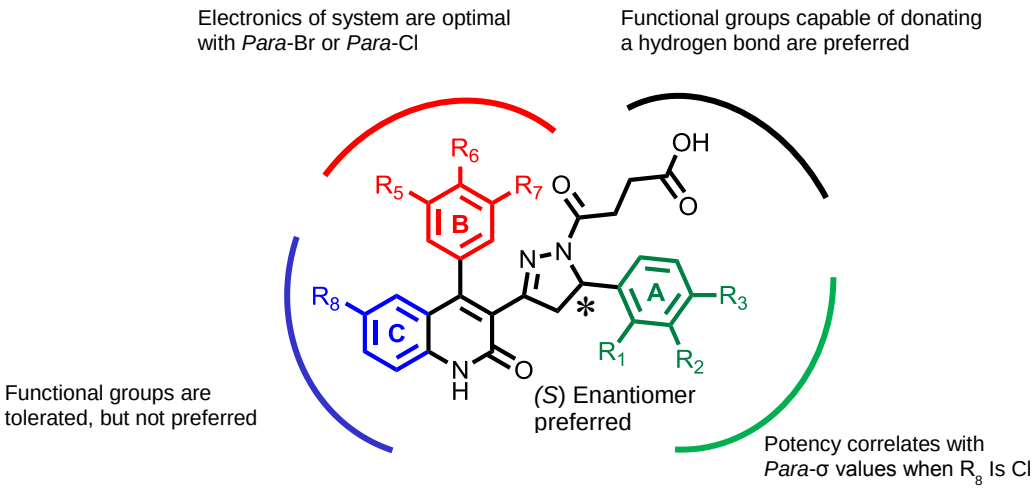


Figure 4.6. Proposed pharmacophore features describing key findings from the SAR**.** The *para*-substitution of the A-ring shows correlation between the sigma coefficient and activity when R_8 is a chlorine; The length and configuration of the acyl-chain is flexible, with the *trans*-configuration improving potency at GluN2C/D-containing receptors; B-ring modification shows an optimal *para*-sigma coefficient close to that of chlorine and bromine for GluN2A-, GluN2C- and GluN2D-containing receptors, suggesting a conserved nature of the binding interaction at each of the three receptors and possibly a halogen bond. The C-ring substitutions explored are consistent with this portion of the molecule interacting with a hydrophobic pocket.

I expect a reduction in molecular weight in conjunction with further optimization of the topological polar surface area will be required to obtain optimal BBB penetration. These observations and the improvement in potency suggest this class of compounds might be useful as pharmacologic probes to evaluate the contributions of the GluN2C- and GluN2D-containing NMDA receptors in normal and patho-physiologic processes.

The off-target actions for the racemic compounds **997-23** and **997-33** were evaluated in a series of two-electrode voltage-clamp recordings using recombinant ligand-gated ion channels expressed in *Xenopus* oocytes in the Traynelis lab. Compounds **997-23** and **997-33** were tested at 3 μM on GluA1-4, GluK1-2, GluK2/GluK5, $5HT_{3A}$, $GABA_A$, $GABA_C$, glycine α1, nicotinic acetylcholine receptors, and purinergic $P2_{X2}$ receptors. Of the ion channel classes evaluated, compounds **997-23** and **997-33** altered agonist-induced currents by less than 10%, with the exception of the nicotinic acetylcholine receptors, which exhibited 13-28% inhibition by **997-23** and **997-33** (data collected in Traynelis lab and not shown). These results strengthen the conclusion that the compounds are selective for GluN2C- and Glun2D-containing receptors.

The GluN2C and GluN2D NMDA receptor subunits remain understudied, largely because of a lack of potent and selective pharmacological tools. However, these NMDA receptor subunits reside in a number of brain regions that are highly relevant for neurological disease. For example, expression of functional GluN2D in neurons in the subthalamic nuclei raises the possibility that GluN2D-selective inhibitors could attenuate neuronal firing rate and possibly alter firing patterns in subthalamic neurons, which could be of utility in Parkinson's disease (Bolam et al., 2000; Galvan and Wichmann, 2008;

Monyer et al., 1994; Mullasseril et al., 2010; Standaert et al., 1993; Standaert et al., 1994; Wichmann and DeLong, 1998; Wilson and Bevan, 2011). In addition, expression of GluN2D in SNc neurons raises the possibility that GluN2D-selective antagonists might serve neuroproteective roles in Parkinson's disease by diminishing Ca^{2+} influx into the dopaminergic SNc neurons, which may lead to neuronal death (Hallett and Standaert, 2004; Surmeier et al., 2005). GluN2C is expressed widely in the cerebellum, and has also been suggested to have a role in both emotional learning and schizophrenia (Dravid et al., 2010; Lisman et al., 2008). The compounds described here could therefore be tools with which to evaluate GluN2C- and GluN2D-containing receptor function in specific circuits implicated in these conditions.

4.5. Chemistry Experimental

4.5.a. Evaluation of Enantiomers

Separation of the final compounds used for biological testing from the racemic **997-23** was obtained using a ChiralPak OD-RH 30 mm X 250 mm, 5μm column using the following conditions: Flow rate 10 ml/min, injection volume 4-6 ml (2 mg/ml), 60% ACN (0.1% Formic acid) : 40% H_2O (0.1% Formic acid); (66) ***S*-997-23**, R_t, 21.8; (67) ***R*-997-23** R_t, 25.1 minutes. The enantiomeric excess (e.e.) of the final products, (67) ***R*-997-23** and (66) ***S*-997-23** was determined using an Agilent 1200 HPLC pump on a ChiralPak OD-RH column (4.6 mm X 150 mm, 5μm) using the following condititions: Flow rate 1 ml/min, injection volume 10 μl, 60% ACN (0.1% Formic acid) : 40% H_2O (0.1% Formic acid); (66)***S*-997-23,** $[\alpha]_D^{20}$ -34.0 (c = 0.32 mg/ml, chloroform), R_t, 7.47 min, 100% e.e. (67)***R*-997-23** $[\alpha]_D^{20}$ + 36.0 (c = 0.245 mg/ml, MeOH), R_t, 8.79 min, 100% e.e.. Optical rotation data was collected using a Perkin-Elmer 314 instrument. NMR spectrum was identical to that of racemic (26) **997-23** for each enantiomer.

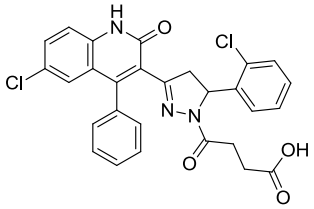
Single crystals of peak two from the separation of racemic (26) **997-23** were grown by slow evaporation of a solution of the compound in a mixture of methanol and water. Crystal data: $C_{28}H_{23}Cl_2N_3O_5$, (M =552.39): 1.124 × 0.087 × 0.056, orthorhombic, space group $P\ 2_12_12_1$ (no.19), a = 8.0529(5) Å, b = 10.2097(5) Å, c = 31.2978(13) Å, V = 2573.2(2) $Å^3$, Z = 4, μ(MoKα)=0.315 mm^{-1}, *Dcalc* =1.490 g/mm^3. Intensity data were collected on a Bruker APEX II CCD diffractometer with monochromated MoKα radiation (λ = 0.71073 Å) at 173 K, in the 2θ range 2.6-53.4°. The user interface Olex2 was used for the crystallographic calculations and crystal structure visualization

(Dolomanov et al., 2009). The structure was solved with Superflip by charge flipping and refined by least squares minimization using Shelxl (Petříček et al., 2006; Sheldrick, 2008). A total of 15745 reflections were measured ($2.602 \leq 2\theta \leq 53.41$) while 5408 unique data (R_{int}=0.124) were used in the refinements. The final R_1 was 0.0590 ($I > 2\sigma(I)$) and the weighted R value wR_2 was 0.0874 (all data) (Crystals grown and data collected and analyzed by Cathryn Slabber and John Bacsa Ph.D. at the Emory X-crystallography core facility).

4.5.b. Synthetic Procedures

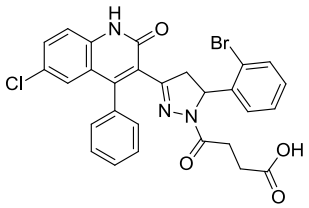
General procedure for the synthesis of acylated quinolone-pyrazoline products

(Procedure G). In an appropriately sized microwaveable vessel, the pyrazol-3-yl-quinolin-2(1H)-one intermediate (1.00 equiv.) was dissolved in anhydrous THF (0.15 M) with 4 Å molecular sieves present. The appropriate anhydride (1.00 equiv.) was added. The solution was microwaved with stirring for 20 minutes at 165 °C. The THF was removed under vacuum, the organics were dissolved in DCM, washed three times with acidified brine (pH 2, HCl), dried over magnesium sulfate, filtered, concentrated under reduced pressure and subjected to flash column chromatography using 0-10% MeOH:DCM gradient unless otherwise noted.



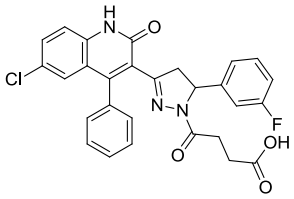
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-14g). Compound **(997-14)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.046 g, 0.460 mmol) and 6-chloro-3-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one 0.200 g, 0.460 mmol. After removal of the THF, the title compound was obtained by trituration from EtOAc and hexanes as a yellow solid. Yield

0.136 g, 55.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 12.13 (s, 1H), 7.63 (dd, *J*= 8.6, 1.6 Hz, 1H), 7.56-7.49 (m, 2H), 7.45-7.41 (m, 4H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.19-7.15 (m, 2H), 6.93 (s, 1H), 6.41 (d, *J* = 7.4 Hz, 1H), 3.87 (dd, *J*=18.4, 12.4 Hz, 1H), 2.75 (dd, *J* = 18.2, 4.7 Hz, 1H), 2.60-2.54 (m, 1H), 2.46-2.32 (m, 2H), 2.39-2.30 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.55, 168.78, 160.02, 152.56, 150.10, 138.66, 134.68, 131.27, 130.52, 129.36, 128.78, 128.37, 128.58, 126.13, 125.90, 124.33, 120.66, 117.64, 56.40, 44.20, 28.43, 28.22. HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{21}Cl_2N_3O_4Na$, 556.08013; found 556.08038. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.30H_2O$: C, 62.30; H, 4.03; N, 7.78. Found C, 62.27; H, 4.32; N, 7.49.



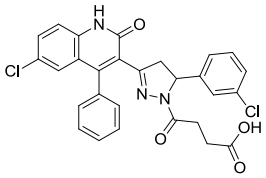
4-(5-(2-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-15). Compound **(997-15)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.063 g, 0.627 mmol) and 3-(5-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (0.300 g, 0.627 mmol). After removal of the THF, the title compound was obtained by trituration from EtOAc and hexanes as a yellow solid. Yield 0.140 g, 38.6 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 12.05 (s, 1H), 7.64 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.53 (p, *J* = 7.6 Hz, 2H), 7.42 (dd, *J* = 11.1, 7.9 Hz, 3H), 7.26 – 7.15 (m, 3H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.39 – 6.32 (m, 1H),

5.48 (dd, *J* = 12.1, 4.6 Hz, 1H), 3.87 (dd, *J* = 18.3, 12.0 Hz, 1H), 2.73 (dd, *J* = 18.4, 4.6 Hz, 1H), 2.62 – 2.51 (m, 1H), 2.49 – 2.28 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.53, 168.76, 160.06, 152.42, 150.11, 140.19, 137.33, 132.58, 131.25, 129.08, 128.41, 126.12, 124.30, 120.64, 117.63, 58.69, 44.33, 28.41, 28.21. HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{21}ClBrN_3O_4Na$; 600.02962, found 600.02945. Anal. Calcd for $C_{28}H_{21}ClBrN_3O_4$: C, 58.10; H, 3.66; N, 7.26. Found C, 54.48; H, 3.54; N, 6.55. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.17 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 1.17 min; > 95% purity.



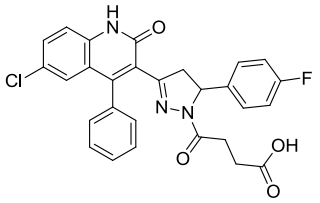
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-16). Compound **(997-16)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.120 g, 1.20 mmol) and 6-chloro-3-(5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.500 g, 0.1.20 mmol). After removal of the THF, the title compound was obtained by trituration from EtOAc and hexanes as a yellow solid. Yield 0.380 g, 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 12.15 (s, 1H), 7.65 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.53 (m, 2H), 7.44 (m, 3H), 7.26 (d, *J* = 7.0 Hz, 2H), 7.03 (t, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.64 (dd, *J* = 12.3, 8.8 Hz, 2H), 5.35 (dd, *J* = 12.3, 4.4 Hz, 1H), 3.75 (dd, *J* = 18.6, 12.1 Hz, 1H), 2.81 (dd, *J* = 17.7, 4.7 Hz, 1H), 2.60 – 2.23

(m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.57, 168.75, 163.36, 160.94, 160.12, 152.47, 149.98, 144.95, 144.88, 137.33, 134.56, 131.25, 130.52, 130.45, 129.40, 128.56, 128.42, 128.38, 128.29, 126.14, 126.07, 124.53, 121.50, 120.65, 117.65, 114.00, 113.79, 112.34, 112.13, 58.41, 45.14, 28.53, 28.20. HRMS (*m/z)*: [M-H]$^-$ calcd for $C_{28}H_{20}ClN_3O_4F$; 516.11319; found, 516.11239. Anal. Calcd for $C_{28}H_{21}ClN_3O_4F$: C, 64.93; H, 4.09; N, 8.11. Found C, 64.11; H, 4.03; N, 7.94. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.97 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 0.73 min; > 95% purity.



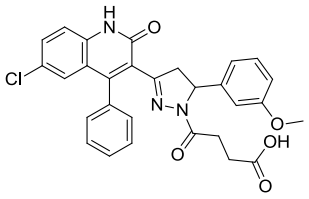
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-13). Compound **(997-13)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.046 g, 0.460 mmol) and 6-chloro-3-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.200 g, 0.460 mmol). After removal of the THF, the title compound was obtained by trituration from hexanes as a yellow solid. Yield 0.156 g, 63.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 12.04 (s, 1H), 7.64 – 7.57 (m, 1H), 7.56 – 7.34 (m, 5H), 7.27 – 7.17 (m, 3H), 6.97 – 6.87 (m, 2H), 6.68 (d, *J* = 6.8 Hz, 1H), 5.29 (dd, *J* = 12.2, 4.8 Hz, 1H), 3.71 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.84 – 2.73 (m, 1H), 2.50 – 2.32 (m, 2H), 2.25 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.55, 168.78, 152.53, 150.00, 144.54, 137.35, 134.62, 133.05, 130.47, 129.37, 128.60,

128.45, 127.14, 126.15, 125.51, 124.51, 124.11, 120.64, 58.40, 45.10, 28.53, 28.18. HRMS (*m/z)*: $[M+Na]^+$ calcd for $C_{28}H_{21}Cl_2N_3O_4Na$; 556.08013; found, 556.07988. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.20H_2O$: C, 61.68; H, 4.10; N, 7.71. Found C, 61.74; H, 4.27; N, 7.32.



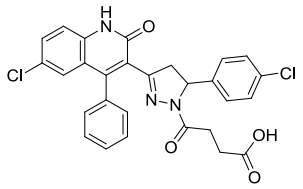
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-19). Compound **(997-19)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.062 g, 0.622 mmol) and 6-chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.260 g, 0.622 mmol). After removal of the THF, the title compound was obtained by trituration from hexanes as a yellow solid. Yield 0.236 g, 73.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 12.15 (s, 1H), 7.68 – 7.38 (m, 6H), 7.27 (d, J = 7.4 Hz, 1H), 7.04 (td, J = 8.8, 2.4 Hz, 2H), 6.93 (d, J = 2.5 Hz, 1H), 6.85 – 6.76 (m, 2H), 5.35 - 5.30 (m, 1H), 3.80 – 3.67 (m, 1H), 2.83 - 2.74 (m, 1H), 2.48 – 2.39 (m, 2H), 2.34 – 2.25 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.59, 168.66, 160.13, 152.45, 149.96, 138.39, 137.34, 134.56, 131.25, 129.47, 128.51, 127.55, 126.09, 124.64, 120.68, 117.65, 115.26, 115.05, 58.24, 45.23, 28.92, 28.59, 28.22. ^{19}F NMR (376 MHz, DMSO-d_6) δ -116.13 – -116.20 (m). HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{20}ClN_3O_4F$, 516.11319; found, 516.11246. Anal. Calcd for $C_{28}H_{21}ClN_3O_4F$: C,

64.93; H, 4.09; N, 8.11. Found C, 61.01; H, 4.22; N, 7.15. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.95 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 0.72 min; > 95% purity.

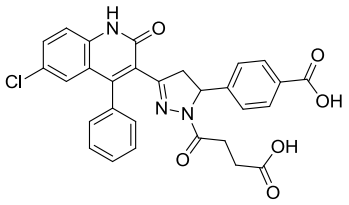


4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-56). Compound **(997-56)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.029 g, 0.29 mmol) and 6-chloro-3-(5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.13 g, 0.29 mmol). The title compound was purified using flash chromatography (2-10% MeOH:DCM) and isolated as a yellow solid. Yield 0.090 g, 58.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 12.08 (s, 1H), 7.64 (dt, J = 8.8, 2.1 Hz, 1H), 7.58 – 7.42 (m, 3H), 7.39 (d, J = 7.3 Hz, 1H), 7.27 (d, J = 7.3 Hz, 1H), 7.13 (t, J = 7.9 Hz, 1H), 6.93 (d, J = 2.2 Hz, 1H), 6.77 (dd, J = 8.2, 2.4 Hz, 1H), 6.59 (s, 1H), 6.32 (d, J = 7.7 Hz, 1H), 5.27 (dd, J = 12.0, 4.7 Hz, 1H), 3.77 – 3.63 (m, 5H), 2.85 (dd, J = 18.4, 4.7 Hz, 1H), 2.48 – 2.36 (m, 2H), 2.28 (t, J = 6.8 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.54, 168.64, 160.16, 159.25, 152.47, 149.91, 143.75, 137.32, 134.64, 131.21, 129.59, 129.15, 128.59, 128.48, 128.37, 128.30, 126.09, 124.56, 120.68, 117.63, 117.39, 112.37, 111.35, 58.88, 54.98, 45.23, 28.56, 28.22. HRMS (*m*/*z*): [M-H]$^-$

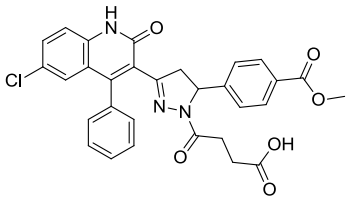
calcd for $C_{29}H_{23}ClN_3O_5$, 528.13317; found, 528.13351. Anal. Calcd for $C_{29}H_{23}Cl_1N_3O_5 \cdot 0.70H_2O$: C, 64.32; H, 4.54; N, 7.76. Found C, 64.38; H, 4.67; N: 7.67.



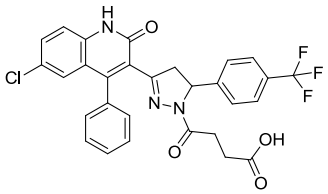
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-12). Compound **(997-12)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.046 g, 0.460 mmol) and 6-chloro-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.200 g, 0.460 mmol). The title compound was obtained by dissolving the crude mixture into hot EtOAc and adding small portions of hot hexanes until a yellow precipitate formed. The mixture was allowed to cool and filtered to give the title compound as a yellow solid. Yield 0.187 g, 76.0 %. ^{1}H NMR (400 MHz DMSO-d_6) δ 12.42 (s, 1H), 12.15 (s, 1H), 7.64 (dd, J = 8.7, 2.5 Hz, 1H), 7.61 – 7.38 (m, 5H), 7.27 (d, J = 7.9 Hz, 3H), 6.94 (d, J = 2.5 Hz, 1H), 6.79 (d, J = 8.2 Hz, 2H), 5.33 (dd, J = 12.1, 4.7 Hz, 1H), 3.75 (dd, J = 18.5, 12.0 Hz, 1H), 2.78 (dd, J = 18.4, 4.7 Hz, 1H), 2.49 – 2.23 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.55, 168.67, 160.09, 152.42, 149.96, 141.13, 137.32, 134.54, 131.59, 131.24, 129.47, 128.57, 128.46, 128.37, 127.38, 126.13, 126.06, 124.56, 120.66, 117.64, 58.29, 45.12, 28.57, 28.23. HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{22}Cl_2N_3O_4$, 534.09819, found; 534.09774. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.40H_2O$: C, 62.09; H, 4.06; N: 7.76. Found C, 61.90; H, 4.13; N: 7.64.



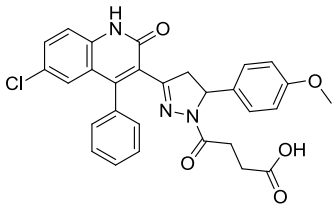
4-(1-(3-Carboxypropanoyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzoic acid (997-5). Compound **997-5** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.045 g, 0.451 mmol) and **6f** 4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzoic acid (0.200 g, 0.451 mmol). The mixture was allowed to cool and filtered to give the title compound as a white solid. Yield 0.040 g, 16.3 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (bs, 3H), 7.79 (d, J = 8.0 Hz, 2H), 7.64 (dd, J = 8.7, 2.4 Hz, 1H), 7.60 - 7.41 (m, 5H), 7.27 (d, J = 7.4 Hz, 1H), 6.96 - 6.82 (m, 3H), 5.39 (dd, J = 12.0, 4.8 Hz, 1H), 3.79 (dd, J = 18.4, 12.2 Hz, 1H), 2.79 (dd, J = 18.4, 4.7 Hz, 1H), 2.61 - 2.37 (m, 2H), 2.30 (t, J = 6.9 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.51, 172.19, 168.72, 167.04, 160.09, 152.41, 149.99, 146.92, 137.33, 134.56, 131.25, 129.68, 129.55, 129.51, 128.52, 128.48, 128.38, 126.12, 126.07, 125.54, 124.53, 120.68, 117.65, 58.71, 45.12, 28.53, 28.22. HRMS (*m/z)*: $[M+Na]^+$ calcd for $C_{29}H_{22}ClN_3O_6Na$, 566.10893; found, 566.10923. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.71 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 0.53 min; > 95% purity.



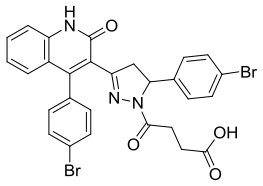
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(methoxycarbonyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-6). Compound **997-6** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.055 g, 0.546 mmol) and methyl 4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzoate (0.250 g, 0.546 mmol). The title compound was purified using flash chromatography (2-10% MeOH:DCM), followed by trituration using hot hexanes from hot EtOAc. Yield 0.040 g, 13.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 12.06 (s, 1H), 7.84 – 7.72 (m, 2H), 7.64 – 7.35 (m, 6H), 7.22 (d, J = 7.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 3H), 5.36 (dd, J = 12.3, 4.6 Hz, 1H), 3.90 – 3.69 (m, 4H), 2.74 (dd, J = 18.5, 4.6 Hz, 1H), 2.51 – 2.33 (m, 2H), 2.30 – 2.21 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.53, 168.77, 165.96, 160.10, 152.43, 150.02, 147.43, 137.34, 134.59, 131.29, 129.44, 128.41, 126.14, 125.76, 124.50, 120.67, 117.67, 58.68, 52.16, 45.09, 28.52, 28.22. HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{30}H_{24}ClN_3O_6Na$, 580.12458; found; 580.12484. Anal. Calcd for $C_{30}H_{24}ClN_3O_6 \cdot 0.80H_2O$: C, 62.95; H, 4.51; N, 7.34. Found C, 63.04; H, 4.55; N, 7.36.



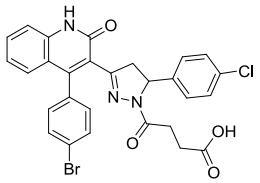
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-8). Compound **997-8** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.043 g, 0.427 mmol) and 6-chloro-4-phenyl-3-(5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.200 g, 0.427 mmol). The title compound was obtained after flash chromatpography (2-10% MeOH:DCM) as a yellow solid. Yield 0.030 g, 12.4 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 12.04 (s, 1H), 7.65 (dd, J = 8.7, 2.4 Hz, 1H), 7.61 – 7.40 (m, 7H), 7.26 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 8.0 Hz, 2H), 6.93 (d, J = 2.3 Hz, 1H), 5.43 (dd, J = 12.2, 4.7 Hz, 1H), 3.79 (dd, J = 18.5, 12.2 Hz, 1H), 2.80 (dd, J = 18.6, 4.7 Hz, 1H), 2.60 – 2.40 (m, 2H), 2.29 (t, J = 6.8 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.14, 169.46, 160.72, 153.09, 150.68, 147.32, 138.01, 135.23, 131.92, 130.09, 129.22, 129.12, 129.05, 126.88, 126.79, 126.72, 126.05, 125.13, 121.31, 118.31, 59.19, 45.74, 29.19, 28.89. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{22}ClN_3O_4F_3$, 568.12455; found, 568.12554. $C_{29}H_{21}ClN_3O_4F_3 \cdot 0.07EtOAc$; HPLC 85% MeOH:$H_2O$ (0.1% Formic Acid) R_t 1.1 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.86 min; > 95% purity.



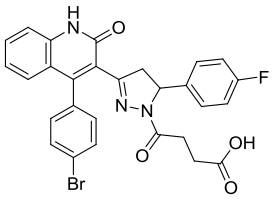
4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-17). Compound **997-17** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.058 g, 0.582 mmol) and 6-chloro-3-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.250 g, 0.582 mmol). After removal of the THF, the title compound was obtained by dissolving the crude mixture into hot EtOAc and adding small portions of hot hexanes until a yellow precipitate formed, the mixture was allowed to cool and filtered to give the title compounds as a yellow solid. Yield 0.132 g, 42.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 12.16 (s, 1H), 7.64 (dd, J = 8.9, 2.4 Hz, 1H), 7.60 - 7.49 (m, 3H), 7.45 (d, J = 8.7 Hz, 1H), 7.43 - 7.38 (m, 1H), 7.29 (d, J = 7.0 Hz, 1H), 6.93 (d, J = 2.5 Hz, 1H), 6.82-6.66 (m, 4H), 5.24 (dd, J = 12.1, 4.6 Hz, 1H), 3.89 - 3.62 (m, 4H), 2.78 (dd, J = 18.3, 4.6 Hz, 1H), 2.45 (t, J = 7.0 Hz, 2H), 2.27 (t, J = 6.9 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.58, 168.49, 160.14, 158.22, 152.39, 149.86, 137.30, 134.58, 134.31, 131.22, 129.42, 128.57, 128.43, 128.35, 126.77, 126.09, 126.02, 124.74, 120.71, 117.63, 113.71, 58.47, 58.37, 55.10, 54.99, 45.27, 28.63, 28.24. HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{24}ClN_3O_5Na$, 552.12967; found, 552.13018. Anal. Calcd for $C_{29}H_{24}ClN_3O_5 \cdot 1.00H_2O$: C, 63.56; H, 4.78; N, 7.67. Found C, 63.68; H, 4.57; N, 7.59.



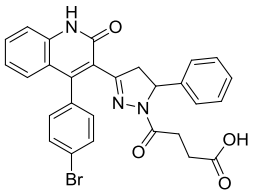
4-(5-(4-Bromophenyl)-3-(4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-7). Compound **997-7** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.057 g, 0.573 mmol) and 4-(4-bromophenyl)-3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.300 g, 0.573 mmol). There was a yellow solid present in the reaction vessel which was filtered, dissolved in DCM, washed three times with brine, dried and determined to be the title compound. Yield 0.320 g, 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 12.18 (s, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.48 – 7.39 (m, 3H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.15 (t, *J* = 7.7 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 2H), 5.33 (dd, *J* = 11.8, 4.4 Hz, 1H), 3.74 (dd, *J* = 18.7, 12.1 Hz, 1H), 2.76 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.65 – 2.39 (m, 2H), 2.39 – 2.30 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.52, 168.65, 160.14, 152.69, 149.89, 141.52, 138.55, 134.40, 131.73, 131.45, 131.24, 130.82, 127.63, 127.28, 123.41, 122.41, 121.82, 120.09, 119.07, 115.59, 58.28, 45.14, 28.49, 28.23. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{20}Br_2N_3O_4$, 619.98260; found, 619.98231. Anal. Calcd for $C_{28}H_{21}Br_2N_3O_4$: C, 53.96; H, 3.40; N, 6.74. Found C, 52.23; H, 3.67; N, 5.55. HPLC 85% MeOH:H_2O 0.1% Formic Acid) R_t 1.2 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 0.84 min; > 94% purity.



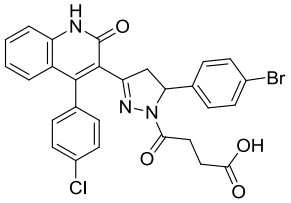
4-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-20). Compound **997-20** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.105 g, 1.04 mmol) and 4-(4-bromophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.500 g, 1.044 mmol). The title compound was obtained after column chromatpgraphy using 10% MeOH:DCM, followed by trituration from EtOAc using hexanes as a yellow solid. Yield 0.240 g, 39.7 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 12.05 (s, 1H), 7.74 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.65 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.36 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.34 - 7.25 (m, 2H), 7.22 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.85 - 6.75 (m, 2H), 5.35 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.73 (dd, *J* =18.4, 12.1 Hz, 1H), 2.76 (dd, *J* = 18.6, 4.4 Hz, 1H), 2.65 - 2.42 (m, 2H), 2.34 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.54, 168.69, 160.17, 152.70, 149.90, 141.10, 138.58, 134.42, 131.61, 131.45, 131.27, 130.84, 128.34, 127.30, 123.44, 122.42, 119.08, 115.60, 58.23, 45.16, 28.48, 28.23. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{20}BrClN_3O_4$, 576.03312; found, 576.03267. Anal. Calcd for $C_{28}H_{21}BrClN_3O_4 \cdot 0.50H_2O$: C, 57.21; H, 3.77; N, 7.15. Found C, 57.02; H, 3.72; N, 7.05.



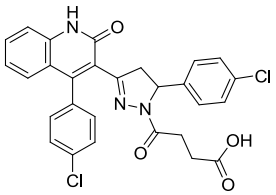
4-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-29). Compound **997-29** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.054 g, 0.541 mmol) and 4-(4-bromophenyl)-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.250 g, 0.541 mmol). The title compound, a yellow solid, was obtained after purifying using flash chromatography (0-10% MeOH:DCM). Yield 0.100 g, 32.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 12.11 (s, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.05 (t, J = 8.9 Hz, 3H), 6.83 (dd, J = 8.4, 5.2 Hz, 2H), 5.35 (dd, J = 12.0, 4.4 Hz, 1H), 3.72 (dd, J = 18.5, 12.0 Hz, 1H), 2.78 (dd, J = 18.4, 4.4 Hz, 1H), 2.65 - 2.43 (m, 2H), 2.34 (t, J = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.56, 168.64, 160.16, 152.71, 149.87, 138.56, 138.36, 134.42, 131.69, 131.46, 131.24, 130.86, 127.46, 127.38, 127.29, 123.47, 122.43, 121.80, 119.08, 115.59, 115.18, 114.96, 58.15, 45.26, 28.50, 28.23. ^{19}F NMR (376 MHz, DMSO-d6) δ -116.06 – -116.19 (m). HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{22}BrFN_3O_4$, 562.07722, found; 562.07669. Anal. Calcd for $C_{28}H_{21}BrFN_3O_4$: C, 59.80; H, 3.76; N, 7.47. Found C, 50.97; H, 3.55; N, 6.00. HPLC 75-95% MeOH:H_2O (0.1% Formic Acid) R_t 0.89 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.67 min; > 95% purity.



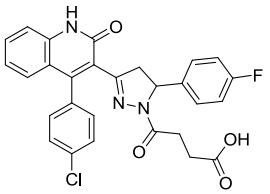
4-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-55). Compound **997-55** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.023 g, 0.23 mmol) and 4-(4-bromophenyl)-3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.10 g, 0.23 mmol). The title compound was obtained after purifying using flash chromatography (0-10% MeOH:DCM) as an off-white solid. Yield 0.110 g, 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 12.20 (s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.67 – 7.54 (m, 2H), 7.47 – 7.34 (m, 2H), 7.23 (d, J = 5.4 Hz, 4H), 7.15 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 6.77 (d, J = 6.7 Hz, 2H), 5.32 (dd, J = 11.8, 4.7 Hz, 1H), 3.75 (dd, J = 18.2, 11.9 Hz, 1H), 2.77 (dd, J = 18.3, 4.5 Hz, 1H), 2.62 – 2.41 (m, 2H), 2.33 (t, J = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.54, 168.57, 160.17, 152.68, 149.84, 142.25, 138.54, 134.42, 131.74, 131.40, 131.25, 131.16, 130.84, 128.36, 127.28, 127.03, 125.33, 123.55, 122.39, 121.77, 119.11, 115.57, 58.87, 45.39, 28.54, 28.24. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{21}BrN_3O_4$, 542.07209; found, 542.07235. Anal. Calcd for $C_{28}H_{22}BrN_3O_4$: C, 61.89; H, 3.89; N, 7.73. Found C, 56.02; H, 4.02; N, 6.78. HPLC 95% MeOH:H_2O (0.1% Formic Acid) R_t 0.91 min; > 95% purity; 65% ACN: H_2O (0.1% Formic Acid) R_t 0.70 min; > 95% purity.



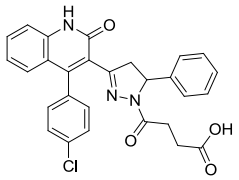
4-(5-(4-Bromophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-54). Compound **997-54** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.031 g, 0.31 mmol) and 3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-chlorophenyl)quinolin-2(1H)-one (0.15 g, 0.31 mmol). The title compound was obtained after purifying using flash chromatography (0-10% MeOH:DCM) as an off-white solid. Yield 0.048 g, 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 7.64 – 7.48 (m, 2H), 7.42 (t, *J* = 7.9 Hz, 5H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 2H), 5.33 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.72 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.79 – 2.69 (m, 1H), 2.53 – 2.36 (m, 2H), 2.32 (t, *J* = 6.9 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.14, 169.65, 160.19, 152.36, 149.87, 141.71, 138.60, 133.96, 133.25, 131.59, 131.44, 131.23, 130.56, 128.37, 127.64, 127.26, 123.65, 122.43, 120.06, 119.18, 115.66, 58.23, 45.12, 30.27, 29.69. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{22}BrClN_3O_4$, 578.04767; found, 578.04719. Anal. Calcd for $C_{28}H_{21}BrClN_3O_4 \cdot 1.20H_2O$: C, 56.01; H, 3.93; N, 7.00. Found C, 56.02; H, 4.02; N, 6.78.



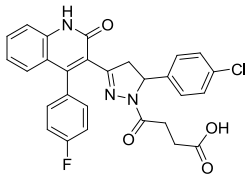
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-23). Compound **997-23** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.046 g, 0.460 mmol) and 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.200 g, 0.460 mmol). The title compound was obtained after purifying using flash chromatography (2-10% MeOH:DCM) followed by trituration with hexanes from EtOAc as a yellow solid. Yield 0.054 g, 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 12.06 (s, 1H), 7.65 – 7.55 (m, 2H), 7.52 (dd, J = 8.2, 2.3 Hz, 1H), 7.48 – 7.38 (m, 2H), 7.34 – 7.24 (m, 3H), 7.15 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 5.35 (dd, J = 11.9, 4.5 Hz, 1H), 3.73 (dd, J = 18.5, 12.0 Hz, 1H), 2.77 (dd, J = 18.4, 4.4 Hz, 1H), 2.65 – 2.43 (m, 2H), 2.34 (t, J = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.54, 168.67, 160.17, 152.72, 149.89, 141.09, 138.57, 134.04, 133.22, 131.59, 131.46, 130.55, 128.32, 127.31, 122.42, 119.15, 115.59, 58.21, 45.16, 28.47, 28.20. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{22}Cl_2N_3O_4$, 534.09819; found, 534.09787. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4$: C, 62.93; H; 3.96; N, 7.86. Found C, 62.38; H, 4.03; N, 7.73. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.95 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.61 min; > 95% purity.



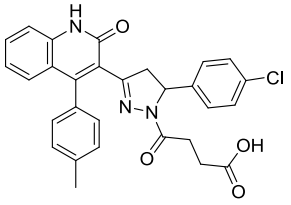
4-(3-(4-(4-Chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-52). Compound **997-52** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.029 g, 0.287 mmol) and 4-(4-chlorophenyl)-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.120 g, 0.287 mmol). The title compound was obtained after purifying using flash chromatography (2-10% MeOH:DCM). Yield 0.085 g, 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 12.08 (s, 1H), 7.65 – 7.49 (m, 3H), 7.47 – 7.34 (m, 2H), 7.34 – 7.26 (m, 1H), 7.15 (t, *J* = 7.7 Hz, 1H), 7.10 – 7.00 (m, 3H), 6.88 – 6.80 (m, 2H), 5.35 (dd, *J* = 11.7, 4.3 Hz, 1H), 3.72 (dd, *J* = 18.1, 12.0 Hz, 1H), 2.78 (dd, *J* = 18.5, 4.3 Hz, 1H), 2.64 – 2.42 (m, 2H), 2.33 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.19, 169.29, 160.82, 153.38, 150.51, 139.22, 138.99, 134.70, 133.85, 132.08, 131.24, 128.97, 128.11, 128.05, 127.94, 124.20, 123.06, 119.81, 116.25, 115.76, 115.62, 58.80, 45.91, 29.16, 28.87. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{20}ClFN_3O_4$, 516.11319; found, 516.11362. Anal. Cacld for $C_{28}H_{21}ClFN_3O_4 \cdot 0.70DCM$: C, 59.70; H, 3.91; N, 7.28. Found C, 59.54; H, 4.15; N, 7.20.



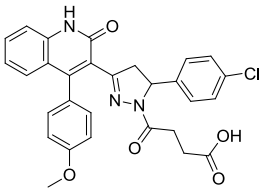
4-(3-(4-(4-Chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-53). Compound **997-53** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.055 g, 0.550 mmol) and 4-(4-chlorophenyl)-3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.220 g, 0.550 mmol). The title compound was obtained by filtering from DCM after removal of the THF *in vacuo*. Yield 0.204 g, 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 12.13 (s, 1H), 7.66 – 7.54 (m, 2H), 7.54 – 7.39 (m, 3H), 7.32 – 7.11 (m, 5H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.77 (dd, *J* = 6.5, 3.0 Hz, 2H), 5.32 (dd, *J* = 11.9, 4.3 Hz, 1H), 3.75 (dd, *J* = 18.4, 12.1 Hz, 1H), 2.77 (dd, *J* = 18.3, 4.3 Hz, 1H), 2.64 – 2.39 (m, 2H), 2.33 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.55, 168.57, 160.17, 152.70, 149.84, 142.23, 138.54, 134.03, 133.17, 131.48, 131.40, 130.57, 128.33, 128.25, 127.28, 127.03, 125.33, 123.62, 122.39, 119.18, 115.57, 58.86, 45.39, 28.53, 28.23. HRMS (*m*/*z)*: $[M-H]^-$ calcd for $C_{28}H_{21}ClN_3O_4$, 498.12261; found, 498.12276. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.87 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.69 min; > 95% purity.



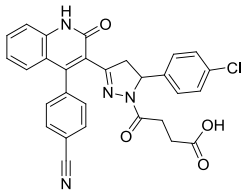
4-(5-(4-Chlorophenyl)-3-(4-(4-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-24). Compound **997-24** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.072 g, 0.718 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-fluorophenyl)quinolin-2(1H)-one (0.300 g, 0.718 mmol). The title compound was obtained by filtering from DCM after removal of the THF *in vacuo*. Yield 0.210 g, 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 12.09 (s, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.40 - 7.25 (m, 5H), 7.15 (t, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.34 (dd, *J* = 11.9, 4.6 Hz, 1H), 3.74 (dd, *J* = 18.5, 11.9 Hz, 1H), 2.79 (dd, *J* = 18.4, 4.6 Hz, 1H), 2.64 - 2.42 (m, 2H), 2.33 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.54, 168.67, 160.21, 152.82, 150.17, 141.11, 138.53, 131.57, 131.44, 130.80, 128.30, 127.36, 123.62, 122.38, 119.40, 115.57, 115.31, 115.10, 58.21, 45.16, 28.50, 28.20. HRMS (*m/z)*: $[M+Na]^+$ calcd for $C_{28}H_{21}ClFN_3O_4Na$, 540.10968; found, 540.10938. Anal. Calcd for $C_{28}H_{21}ClFN_3O_4 \cdot 0.90H_2O$: C, 62.96; H, 4.30; N, 7.86. Found C, 62.80; H, 4.06; N: 7.80.



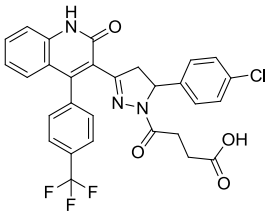
4-(5-(4-Chlorophenyl)-3-(2-oxo-4-p-tolyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-26). Compound **997-26** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.060 g, 0.604 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-p-tolylquinolin-2(1H)-one (0.250 g, 0.604 mmol). The title compound was obtained as a yellow solid after purifying using flash chromatography (2-10% MeOH:DCM) and trituration from EtOAc using hexanes. Yield 0.260 g, 84%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.24 (s, 1H), 12.08 (s, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.36 – 7.21 (m, 5H), 7.18 – 7.04 (m, 3H), 6.84 (dd, *J* = 8.5, 2.3 Hz, 2H), 5.32 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.68 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.74 (dd, *J* = 18.4, 4.5 Hz, 1H), 2.63 – 2.39 (m, 5H), 2.31 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.55, 168.65, 160.25, 152.86, 151.26, 141.13, 138.55, 137.66, 132.18, 131.51, 131.28, 129.24, 128.79, 128.69, 128.54, 128.26, 127.45, 123.25, 122.23, 119.43, 115.52, 58.17, 45.20, 28.79, 28.60, 28.24, 20.90. HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{25}ClN_3O_4$, 514.15281; found, 514.15260. Anal. Cacld for $C_{29}H_{24}ClN_3O_4 \cdot 0.80H_2O$: C, 65.92; H, 4.88; N, 7.95. Found C, 65.86; H, 4.84; N, 7.75.



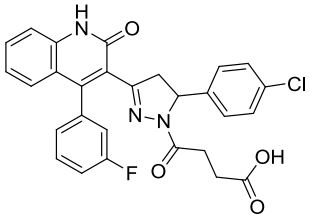
4-(5-(4-Chlorophenyl)-3-(4-(4-methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-21). Compound **997-21** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.047 g, 0.465 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-methoxyphenyl)quinolin-2(1H)-one (0.200 g, 0.465 mmol). The title compound was obtained after trituration from EtOAc using hexanes as a yellow solid. Yield 0.165 g, 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.05 (s, 1H), 12.24 (s, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.38 – 7.06 (m, 6H), 7.01 (d, J = 8.5 Hz, 1H), 6.83 (d, J = 7.8 Hz, 3H), 5.33 (dd, J = 11.8, 4.4 Hz, 1H), 3.85 (s, 3H), 3.67 (dd, J = 18.4, 12.1 Hz, 1H), 2.78-2.53 (m, 2H), 2.41 – 2.22 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.22, 173.68, 168.75, 160.42, 160.28, 159.20, 152.91, 151.06, 141.19, 138.54, 131.50, 131.26, 131.01, 130.92, 130.00, 128.39, 128.24, 127.68, 127.44, 127.00, 123.52, 122.23, 119.66, 115.54, 113.68, 113.56, 58.16, 55.17, 45.24, 32.60, 28.76, 28.44. HRMS (*m/z)*: $[M+K]^+$ calcd for $C_{29}H_{24}ClN_3O_5$, 568.10361; found, 568.10341. Anal. Calcd for $C_{29}H_{24}ClN_3O_5$: C, 65.72; H, 4.56; N, 7.92. Found C, 57.27; H, 4.20; N, 6.50. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.89 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.67 min; > 95% purity.



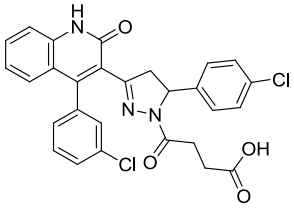
4-(5-(4-Chlorophenyl)-3-(4-(4-cyanophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-27). Compound **997-27** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.059 g, 0.588 mmol) and 4-(3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (0.150 g, 0.588 mmol). The title compound was obtained as a yellow solid after trituration from EtOAc using hexanes. Yield 0.150 g, 48.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.09 (s, 1H), 7.97 (ddd, *J* = 11.8, 7.7, 1.7 Hz, 2H), 7.64 – 7.56 (m, 2H), 7.50 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.14 (t, *J* = 7.7 Hz, 1H), 6.95 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.89 – 6.84 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.79 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.89 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.57 – 2.23 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.45, 168.70, 160.12, 152.50, 149.37, 140.99, 140.55, 138.61, 132.20, 131.62, 130.59, 130.50, 129.79, 128.37, 128.25, 127.32, 127.23, 123.18, 122.52, 118.75, 118.63, 115.65, 111.18, 58.30, 45.00, 28.77, 28.22. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{29}H_{20}ClN_4O_4$, 523.11786; found, 523.11828. Anal. Calcd for $C_{29}H_{21}ClF_3N_3O_4$: C, 66.35; H, 4.03; N, 10.67. Found C, 63.51; H, 4.39; N, 9.33. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.67 min; 87% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.89 min; 85% purity.



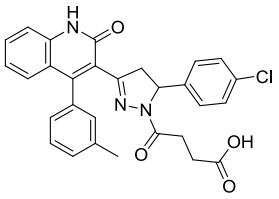
4-(5-(4-Chlorophenyl)-3-(2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-36). Compound **(997-36)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.039 g, 0.385mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.180 g, 0.220 mmol). The title compound was obtained after flash column chromatography using 0-10% MeOH:DCM as a yellow solid. Yield 0.125 g, 57.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 12.07 (s, 1H), 7.88 (dd, *J* = 19.4, 8.1 Hz, 2H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.15 (t, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.36 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.80 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.91 (dd, *J* = 18.5, 4.8 Hz, 1H), 2.52 - 2.39 (m, 1H), 2.40 - 2.21 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.42, 168.68, 160.19, 152.58, 149.61, 141.06, 139.85, 138.62, 131.62, 130.35, 129.69, 128.31, 127.27, 125.20, 123.28, 122.54, 118.95, 115.63, 58.23, 45.04, 28.32, 28.05. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.595 (s). HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{29}H_{20}ClF_3N_3O_4$, 566.10999; found, 566.11036. Anal. Clacd for $C_{29}H_{21}ClF_3N_3O_4 \cdot 0.40H_2O$: C, 60.56; H, 3.82; N, 7.31. Found C, 60.57; H, 4.00; N: 7.23.



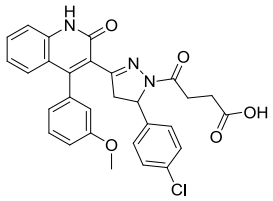
4-(5-(4-Chlorophenyl)-3-(4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-28). Compound **997-28** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.060 g, 0.598 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)quinolin-2(1H)-one (0.250 g, 0.367 mmol). The title compound was obtained after flash column chromatography using 10% MeOH:DCM, followed by trituration from EtOAc using hexanes as a yellow solid. Yield 0.190 g, 61.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 12.08 (s, 1H), 7.57 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.36 - 7.21 (m, 5H), 7.18 - 7.04 (m, 3H), 6.84 (d, J = 8.2 Hz, 2H), 5.32 (dd, J = 12.0, 4.5 Hz, 1H), 3.68 (dd, J = 18.5, 12.0 Hz, 1H), 2.74 (dd, J = 18.5, 4.5 Hz, 1H), 2.52 - 2.39 (m, 4H). ^{13}C NMR (100 MHz DMSO-d_6) δ 173.48, 168.60, 160.19, 149.62, 141.15, 138.53, 131.53, 131.45, 130.35, 128.37, 127.33, 127.16, 122.44, 119.07, 115.57, 58.27, 45.11, 28.79, 28.47, 28.18. HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{21}ClFN_3O_4Na$, 540.10968; found, 540.11014. Anal. Calcd for $C_{28}H_{21}ClFN_3O_4 \cdot 0.90H_2O$: C, 62.96; H, 4.30; N, 7.87. Found C, 63.04; H, 4.38; N, 7.55.



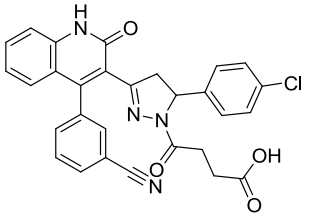
4-(5-(4-Chlorophenyl)-3-(4-(3-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-22). Compound **997-22** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.069 g, 0.691 mmol) and 4-(3-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.300g, 0.691 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.170 g, 46.1%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (s, 1H), 12.04 (s, 1H), 7.58 – 7.51 (m, 2H), 7.51 – 7.46 (m, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.36 – 7.32 (m, 1H), 7.25 (ddt, J = 7.1, 4.9, 2.5 Hz, 2H), 7.19 (d, J = 7.5 Hz, 1H), 7.12 (t, J = 7.7 Hz, 1H), 7.01 – 6.94 (m, 1H), 6.81 – 6.73 (m, 2H), 5.34 – 5.28 (m, 1H), 3.83 – 3.74 (m, 1H), 2.84 – 2.70 (m, 1H), 2.52 – 2.34 (m, 2H), 2.31 – 2.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.50, 168.64, 160.20, 152.66, 149.46, 141.16, 138.53, 137.44, 137.28, 133.18, 131.56, 131.47, 130.15, 130.00, 129.59, 128.49, 128.40, 127.29, 127.16, 123.43, 122.48, 119.17, 115.58, 58.30, 45.10, 28.78, 28.49, 28.19. HRMS (*m/z)*: $[M+Na]^+$ calcd for $C_{28}H_{21}Cl_2N_3O_4Na$, 556.08013; found, 556.07990. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4 \cdot 0.70H_2O$: C, 61.48; H, 4.13; N, 7.68. Found C, 61.51; H, 4.11; N: 7.48.



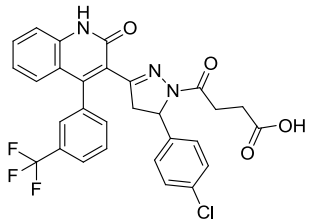
4-(5-(4-Chlorophenyl)-3-(2-oxo-4-(m-tolyl)-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-25). Compound **997-25** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.060 g, 0.604 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(m-tolyl)quinolin-2(1H)-one (0.250g, 0.604 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.240 g, 77.0%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.21 (s, 1H), 12.05 (s, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.27 (m, 3H), 7.27 – 7.17 (m, 2H), 7.16 – 7.06 (m, 2H), 7.00 (q, *J* = 10.2, 8.9 Hz, 2H), 6.80 – 6.67 (m, 2H), 5.33 – 5.23 (m, 1H), 3.80 – 3.62 (m, 1H), 2.76 (dd, *J* = 18.4, 4.6 Hz, 0.5H), 2.64 (dd, *J* = 18.5, 4.6 Hz, 0.5H), 2.57 – 2.34 (m, 5H), 2.31 – 2.18 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.61, 173.54, 168.63, 168.57, 160.29, 152.88, 152.70, 151.33, 151.24, 141.25, 141.20, 138.52, 137.49, 137.43, 135.17, 135.03, 131.53, 131.30, 130.25, 128.84, 128.36, 128.32, 128.13, 127.50, 127.41, 127.34, 127.26, 126.39, 125.46, 123.16, 122.28, 119.42, 115.55, 115.51, 58.28, 58.23, 45.25, 28.90, 28.79, 28.67, 28.57, 28.35, 28.24, 28.14, 21.09, 21.02, 20.97, 20.91. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{25}ClN_3O_4$, 514.15281; found, 514.15263. Anal. Calcd for $C_{29}H_{24}ClN_3O_4 \cdot 1.50H_2O \cdot 0.06EtOAc$: C, 64.29; H, 5.07; N, 7.69. Found C, 64.65; H, 4.90; N, 7.29.



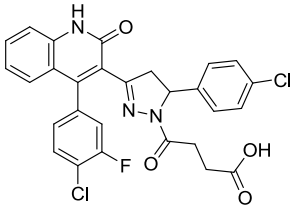
4-(5-(4-Chlorophenyl)-3-(4-(3-methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-39). Compound **997-39** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.070 g, 0.698 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-methoxyphenyl)quinolin-2(1H)-one (0.300g, 0.698 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.090 g, 24.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.23 (s, 1H), 12.03 (s, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.48 – 7.35 (m, 2H), 7.26 (dd, J = 22.1, 8.1 Hz, 2H), 7.18 – 7.12 (m, 1H), 7.09 (t, J = 8.3 Hz, 2H), 6.95 (d, J = 9.9 Hz, 1H), 6.85 – 6.72 (m, 3H), 5.37 – 5.30 (m, 1H), 3.84 – 3.64 (m, 4H), 2.83 – 2.72 (m, 1H), 2.60 – 2.51 (m, 2H), 2.31 (t, J = 7.1 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.52, 168.60, 160.26, 159.03, 152.90, 150.95, 141.24, 141.19, 138.52, 138.50, 136.50, 136.46, 131.56, 131.48, 131.33, 131.31, 129.43, 129.38, 128.35, 128.21, 128.14, 127.46, 127.43, 127.19, 123.24, 123.14, 122.32, 121.65, 120.70, 119.31, 119.27, 115.51, 115.13, 114.02, 113.88, 113.58, 58.25, 55.26, 54.99, 45.30, 45.19, 28.59, 28.22. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{25}ClN_3O_5$, 530.14773; found, 530.14715. Anal. Calcd for $C_{29}H_{24}ClN_3O_5$: C, 65.72; H, 4.56; N, 7.93. Found C, 65.52; H, 4.72; N, 7.97.



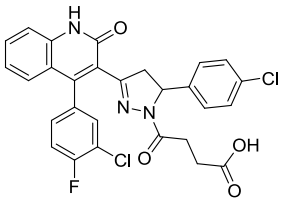
4-(5-(4-Chlorophenyl)-3-(4-(3-cyanophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-40). Compound **997-40** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.021g, 0.212mmol) and 3-(3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (0.090g, 0.212 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.034 g, 31.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (d, J = 3.6 Hz, 1H), 12.10 (s, 1H), 8.04 – 7.91 (m, 1H), 7.84 – 7.67 (m, 2H), 7.65 – 7.55 (m, 2H), 7.43 (d, J = 8.2 Hz, 1H), 7.31 (d, J = 8.3 Hz, 2H), 7.16 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.82 (dd, J = 13.4, 8.2 Hz, 2H), 5.40 – 5.32 (m, 1H), 3.96 – 3.81 (m, 1H), 2.87 (dt, J = 18.3, 4.1 Hz, 1H), 2.42 (d, J = 7.6 Hz, 2H), 2.35 – 2.26 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.58, 173.42, 168.61, 160.13, 152.58, 148.90, 141.02, 138.54, 136.80, 136.70, 134.66, 133.48, 133.05, 132.18, 132.09, 131.73, 131.57, 129.51, 129.32, 128.47, 128.41, 127.34, 127.25, 127.04, 123.64, 123.53, 122.54, 119.03, 118.52, 115.59, 111.60, 111.40, 58.27, 45.03, 28.75, 28.32, 28.10. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{29}H_{20}ClN_4O_4$, 523.11786; found, 523.11778. Anal. Calcd for $C_{29}H_{21}ClN_4O_4$: C, 66.35; H, 4.03; N, 10.67. Found C, 63.97; H, 4.59; N, 9.39. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.68 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.59 min; > 95% purity.



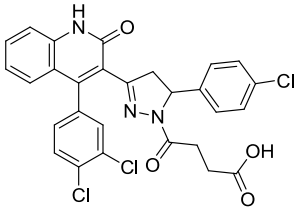
4-(5-(4-Chlorophenyl)-3-(2-oxo-4-(3-(trifluoromethyl)phenyl)-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-51). Compound **997-51** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.054g, 0.53mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.25 g, 0.53 mmol). The title compound was obtained as a yellow solid using a 0-8% MeOH gradient in DCM. Yield 0.073 g, 24%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 12.06 (s, 1H), 7.96-7.84 (m, 1H), 7.82 – 7.66 (m, 2H), 7.59 (t, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.20 – 7.13 (m, 1H), 6.95 (dd, *J* = 23.9, 8.2 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 5.34 (dt, *J* = 12.2, 4.3 Hz, 1H), 3.95 – 3.70 (m, 1H), 3.00 – 2.74 (m, 1H), 2.54 – 2.23 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.40, 173.34, 168.62, 168.52, 160.20, 160.17, 152.67, 152.61, 149.38, 141.10, 141.03, 138.57, 136.57, 136.47, 133.59, 132.57, 131.60, 131.51, 129.50, 129.33, 129.15, 128.92, 128.49, 128.38, 128.30, 127.67, 127.25, 127.17, 127.13, 126.47, 125.07, 123.62, 123.45, 122.54, 122.36, 119.26, 119.11, 115.61, 58.26, 58.21, 45.12, 45.01, 28.37, 28.29, 28.19, 28.08. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{22}ClF_3N_3O_4$, 568.12455; found, 568.12417. Anal. Calcd for $C_{29}H_{21}ClF_3N_3O_4 \cdot 0.20H_2O$; C, 60.94; H, 3.77; N, 7.35. Found C, 60.79; H, 3.96; N, 7.33.



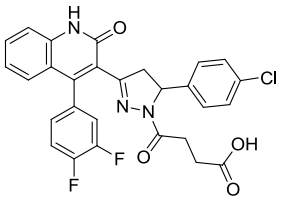
4-(3-(4-(4-Chloro-3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-38). Compound **997-38** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.040 g, 0.398 mmol) and 4-(4-chloro-3-fluorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.180g, 0.398 mmol). The title compound was obtained after flash column chromatography using 0-10%MeOH:DCM as a yellow solid. Yield 0.096 g, 43.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.12 (s, 1H), 7.79 – 7.64 (m, 1H), 7.62 – 7.51 (m, 2H), 7.46 – 7.38 (m, 2H), 7.33 – 7.23 (m, 2H), 7.21 – 7.00 (m, 2H), 6.93 – 6.78 (m, 2H), 5.39 (dt, *J* = 12.1, 4.4 Hz, 1H), 3.85 – 3.72 (m, 1H), 2.91 – 2.79 (m, 1H), 2.66 – 2.39 (m, 2H), 2.34 (td, *J* = 7.0, 2.2 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.61, 173.47, 173.44, 168.68, 168.66, 168.09, 168.05, 160.11, 157.84, 156.19, 152.77, 152.70, 152.63, 148.67, 141.03, 140.99, 138.55, 136.45, 136.36, 136.30, 131.63, 131.57, 130.63, 130.53, 128.34, 128.28, 127.32, 127.21, 127.12, 127.00, 126.21, 123.57, 123.45, 122.54, 119.69, 119.58, 118.92, 117.28, 115.58, 58.28, 58.17, 45.15, 28.77, 28.39, 28.17. ^{19}F NMR (376 MHz, DMSO-d_6) δ -116.40. HRMS (*m/z)*: $[M+K]^+$ calcd for $C_{28}H_{20}Cl_2FN_3O_4K$, 590.04465; found, 590.04631. Anal. Calcd for $C_{28}H_{20}Cl_2FN_3O_4 \cdot 0.80H_2O \cdot 0.10EtOAc$: C, 59.26; H, 3.92; N, 7.30. Found C, 59.16; H, 4.21; N, 7.14.



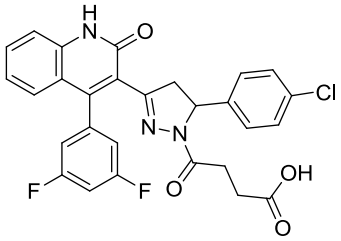
4-(3-(4-(3-Chloro-4-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-34). Compound **(997-34)** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.040 g, 0.398 mmol) and 4-(3-chloro-4-fluorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.180g, 0.398 mmol). The title compound was obtained after flash column chromatography using 0-10%MeOH:DCM as a yellow solid. Yield 0.120 g, 54.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 12.12 (s, 1H), 7.73 – 7.50 (m, 3H), 7.44 (dd, *J* = 8.2, 1.7 Hz, 2H), 7.29 (td, *J* = 8.8, 2.0 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.09 – 7.01 (m, 1H), 6.88 (dt, *J* = 8.6, 2.4 Hz, 2H), 5.38 (dd, *J* = 10.4, 4.6 Hz, 1H), 3.90 – 3.76 (m, 1H), 2.93 – 2.77 (m, 1H), 2.66 – 2.40 (m, 2H), 2.40 – 2.28 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.63, 173.48, 173.44, 168.68, 160.15, 155.93, 152.68, 152.60, 148.76, 148.72, 141.01, 138.50, 133.00, 132.90, 131.93, 131.62, 131.57, 131.50, 130.63, 130.51, 129.53, 128.39, 128.34, 127.38, 127.23, 127.11, 123.74, 123.66, 122.50, 119.77, 119.46, 119.24, 116.87, 116.66, 115.56, 58.25, 45.10, 28.78, 28.43, 28.38, 28.15. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{21}Cl_2FN_3O_4$, 552.08877; found, 552.09030. Anal. Calcd for $C_{28}H_{20}Cl_2FN_3O_4$: C, 60.88; H, 3.64; N, 7.61. Found C, 58.29; H, 4.04; N, 6.66. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.93 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.55 min; > 95% purity.



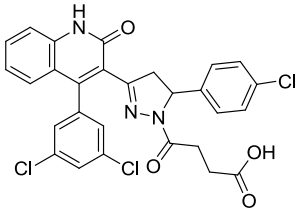
4-(5-(4-Chlorophenyl)-3-(4-(3,4-dichlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-41). Compound **997-41** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.0427 g, 0.427 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (0.200g, 0.427 mmol). The title compound was obtained after flash column chromatography using 0-10%MeOH:DCM as a yellow solid. Yield 0.102 g, 42.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 12.10 (s, 1H), 7.83 – 7.70 (m, 1H), 7.63 – 7.54 (m, 2H), 7.46 – 7.37 (m, 2H), 7.32 – 7.22 (m, 2H), 7.16 (t, J = 7.7 Hz, 1H), 7.04 (t, J = 7.0 Hz, 1H), 6.87 – 6.80 (m, 2H), 5.37 (dd, J = 12.0, 4.3 Hz, 1H), 3.88 – 3.74 (m, 1H), 2.90 – 2.74 (m, 1H), 2.53 – 2.39 (m, 2H), 2.38 – 2.29 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.04, 169.33, 160.76, 153.24, 149.13, 141.66, 139.20, 136.68, 132.40, 132.27, 132.19, 131.96, 131.08, 130.76, 129.73, 129.05, 128.98, 127.99, 127.83, 127.74, 123.18, 119.69, 116.23, 58.93, 45.75, 29.62, 29.11, 29.07, 28.86. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{21}Cl_3N_3O_4$, 568.05922; found, 568.06168. Anal. Calcd for $C_{28}H_{20}Cl_3N_3O_4 \cdot 0.80H_2O$: C, 57.66; H, 3.73; N, 7.20. Found C, 57.70; H, 3.65; N: 6.94.



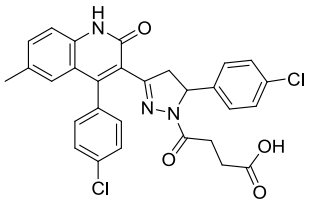
4-(5-(4-Chlorophenyl)-3-(4-(3,4-difluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-42). Compound **997-41** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.0427 g, 0.427 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (0.200g, 0.427 mmol). The title compound was obtained after flash column chromatography using 0-10%MeOH:DCM as a yellow solid. Yield 0.110 g, 68.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 12.13 (s, 1H), 7.63 – 7.52 (m, 3H), 7.46 – 7.39 (m, 1H), 7.35 – 7.28 (m, 1H), 7.29 – 7.23 (m, 2H), 7.20 – 7.10 (m, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 6.95 – 6.84 (m, 2H), 5.43 – 5.33 (m, 1H), 3.88 – 3.73 (m, 1H), 2.86 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.66 – 2.29 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.08, 169.36, 160.79, 153.45, 149.53, 141.69, 139.20, 139.20, 132.28, 132.14, 128.99, 128.91, 127.98, 127.80, 124.26, 123.12, 119.83, 116.22, 58.92, 45.79, 29.07, 28.84. ^{19}F NMR (376 MHz, DMSO-d6) δ -5.88 – -6.23 (m), -6.44 – -6.75 (m). HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{19}ClF_2N_3O_4$, 534.10376; found, 534.10358. Anal. Calcd for $C_{28}H_{20}ClF_2N_3O_4 \cdot 0.40H_2O$: C, 61.92; H, 3.86; N, 7.74. found C, 61.76; H, 4.15; N, 7.52.



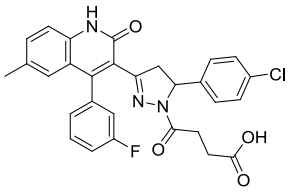
4-(5-(4-Chlorophenyl)-3-(4-(3,5-difluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-35). Compound **997-35** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.041 g, 0.413 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,5-difluorophenyl)quinolin-2(1H)-one (0.180g, 0.413 mmol). The title compound as a yellow solid was obtained after flash column chromatography using 0-10% MeOH:DCM. Yield 0.133 g, 56.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.07 (s, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.47 - 7.33 (m, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.1 Hz, 2H), 5.40 (dd, *J* = 12.0, 4.6 Hz, 1H), 3.86 (dd, *J* = 18.4, 12.0 Hz, 1H), 2.90 (dd, *J* = 18.5, 4.6 Hz, 1H), 2.59 - 2.41 (m, 2H), 2.33 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.65, 173.46, 168.64, 163.55, 163.43, 161.11, 160.95, 160.11, 152.68, 148.60, 141.09, 139.05, 138.95, 138.52, 131.58, 128.55, 128.40, 127.26, 127.13, 123.41, 122.57, 118.79, 115.58, 113.31, 113.12, 112.32, 112.12, 103.85, 58.30, 45.02, 28.80, 28.37, 28.37, 28.15. ^{19}F (376 MHz, DMSO-d_6) δ -109.98 - -110.05 (m). HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{28}H_{19}ClF_2N_3O_4$, 534.10376; found, 534.10402. Anal. Calcd for $C_{28}H_{20}ClF_2N_3O_4 \cdot 0.50H_2O$: C, 61.71, H: 3.88, N: 7.71; C: 61.52, H: 4.05, N: 7.42.



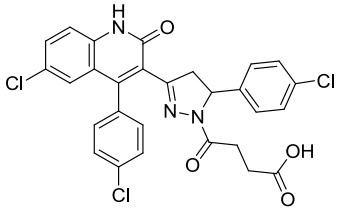
4-(5-(4-Chlorophenyl)-3-(4-(3,5-dichloro (phenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-37). Compound **997-37** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.038 g, 0.384 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (0.180g, 0.384 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.080 g, 36.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.10 (s, 1H), 7.84 - 7.71 (m, 1H), 7.64 - 7.56 (m, 2H), 7.51 - 7.39 (m, 2H), 7.29 (t, *J* = 7.3 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 6.9 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 2H), 5.39 (dd, *J* =12.0, 4.3 Hz, 1H), 3.89 - 3.75 (m, 1H), 2.85 – 2.67 (m, 1H), 2.64 - 2.30 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.46, 168.66, 160.11, 152.60, 152.53, 148.48, 141.01, 138.52, 136.01, 131.77, 131.59, 131.31, 131.11, 130.39, 130.13, 129.07, 128.40, 128.33, 127.35, 127.17, 127.06, 122.55, 119.02, 115.57, 58.24, 45.09, 28.38, 28.17. HRMS calcd for $C_{28}H_{19}Cl_3N_3O_4$ [M-H]$^-$; 566.04466, found; 566.04483. Anal. $C_{28}H_{20}Cl_3N_3O_4 \cdot 0.30H_2O$; C, 58.57; H, 3.62; N, 7.32. Found C, 58.58; H, 3.75; N, 7.23.



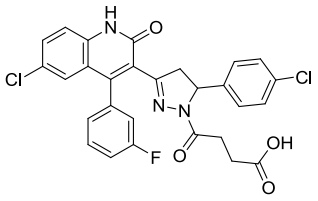
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-43). Compound **997-43** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.039 g, 0.39 mmol) and 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-methylquinolin-2(1H)-one (0.175 g, 0.39 mmol). The title compound was obtained after flash column chromatography using 0-8% MeOH:DCM as a yellow solid. Yield 0.114 g, 53.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 11.79 (s, 1H), 7.56 (dt, *J* = 8.2, 2.3 Hz, 1H), 7.48 (dt, *J* = 8.2, 2.3 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.30 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.27 – 7.20 (m, 3H), 6.80 – 6.72 (m, 3H), 5.35 – 5.25 (m, 1H), 3.75 – 3.59 (m, 1H), 2.76 – 2.65 (m, 1H), 2.61 – 2.33 (m, 2H), 2.30 (m, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.54, 168.64, 160.00, 152.85, 149.66, 141.10, 136.63, 134.07, 133.16, 132.79, 131.56, 131.48, 131.40, 130.53, 128.37, 128.30, 127.30, 126.53, 123.47, 119.05, 115.59, 58.18, 45.18, 28.47, 28.19, 20.58. HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{29}H_{22}Cl_2N_3O_4$, 546.09929; found, 546.09872. Anal. Calcd for $C_{29}H_{23}Cl_2N_3O_4 \cdot 0.50H_2O$: C, 62.49; H, 4.34; N, 7.54. Found C, 62.44; H, 4.55; N 7.39.



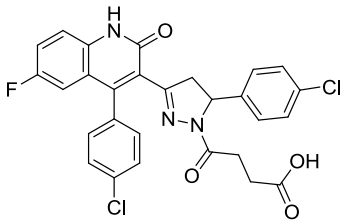
4-(5-(4-Chlorophenyl)-3-(4-(3-fluorophenyl)-6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-44). Compound **997-44** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.037 g, 0.37 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (0.160 g, 0.37 mmol). The title compound was obtained after flash column chromatography using 0-10% MeOH:DCM as a brownish solid. Yield 0.094 g, 47.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 12.12 (s, 1H), 7.64 – 7.47 (m, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.40 – 7.32 (m, 2H), 7.32 – 7.22 (m, 3H), 7.11 (dd, *J* = 23.8, 8.5 Hz, 1H), 6.86 – 6.77 (m, 3H), 5.35 (dt, *J* = 12.5, 3.4 Hz, 1H), 3.80 (dd, *J* = 18.6, 12.1 Hz, 1H), 2.81 (dt, *J* = 18.5, 5.2 Hz, 1H), 2.53 – 2.39 (m, 2H), 2.31 (t, *J* = 6.8 Hz, 2H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.46, 168.57, 162.67, 161.11, 160.03, 152.88, 152.81, 149.39, 141.15, 137.59, 136.61, 132.77, 131.58, 131.52, 131.38, 130.25, 128.36, 128.20, 127.33, 127.15, 126.57, 126.52, 125.83, 124.79, 123.38, 123.30, 118.98, 116.66, 116.52, 115.57, 115.19, 115.05, 58.25, 45.16, 28.98, 28.47, 28.19, 20.61. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.28 – -113.50 (m). HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{29}H_{22}ClFN_3O_4$, 530.12884; found, 530.12883. Anal. Calcd for $C_{29}H_{23}ClFN_3O_4 \cdot 1.20H_2O$: C, 62.92; H, 4.62; N, 7.59. Found C, 62.82; H, 4.37; N, 7.34.



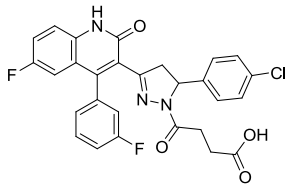
4-(3-(6-Chloro-4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-46). Compound **997-46** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.038 g, 0.38 mmol) and 6-chloro-4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.18 g, 0.38 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-8% MeOH:DCM. Yield 0.033g, 15.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 12.08 (s, 1H), 7.69 – 7.58 (m, 2H), 7.54 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.44 (dd, *J* = 8.5, 2.7 Hz, 2H), 7.35 – 7.23 (m, 3H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.85 – 6.76 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.72 (dd, *J* = 18.5, 12.0 Hz, 1H), 2.76 (dd, *J* = 18.5, 4.4 Hz, 1H), 2.64 – 2.38 (m, 2H), 2.33 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.50, 168.71, 159.96, 152.33, 148.76, 141.00, 137.33, 133.52, 133.36, 131.61, 131.48, 131.38, 130.52, 128.52, 128.32, 127.27, 126.25, 125.91, 124.80, 120.43, 117.67, 58.25, 45.08, 28.47, 28.19. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{21}Cl_3N_3O_4$, 568.05922; found, 568.05881. Anal. Calcd for $C_{28}H_{20}Cl_3N_3O_4 \cdot 0.40H_2O$: C, 58.38; H, 3.64; N, 7.29. Found C, 58.29; H, 3.73; N, 7.24.



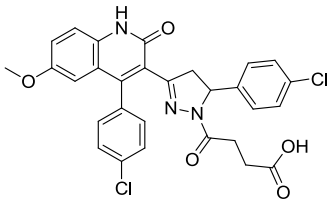
4-(3-(6-Chloro-4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-45). Compound **997-45** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.035 g, 0.35 mmol) and 6-chloro-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)quinolin-2(1H)-one (0.16 g, 0.35 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-8% MeOH:DCM. Yield 0.039 g, 20.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.45 (s, 1H), 12.05 (s, 1H), 7.65 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.63 – 7.53 (m, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.42 – 7.32 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.19 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.83 (dd, *J* = 11.9, 8.3 Hz, 2H), 5.36 (dt, *J* = 12.2, 4.6 Hz, 1H), 2.88 – 2.80 (m, 1H), 2.51 – 2.44 (m, 2H), 2.31 (t, *J* = 6.9 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.13, 169.33, 163.45, 161.82, 160.66, 153.02, 152.95, 149.14, 141.73, 137.95, 137.52, 132.03, 131.21, 129.05, 127.96, 127.78, 126.91, 126.57, 125.39, 125.30, 121.01, 118.31, 116.19, 58.96, 45.71, 29.12, 28.85. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{19}Cl_2FN_3O_4$, 550.07421; found, 550.07485. Anal. Calcd for $C_{28}H_{20}Cl_2FN_3O_4 \cdot 1.00H_2O$: C, 58.96; H, 3.89; N, 7.37. Found C, 58.98; H, 3.78; N, 7.06.



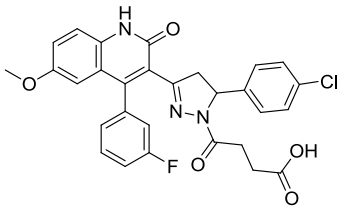
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-6-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-49). Compound **997-49** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.035 g, 0.35 mmol) and 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoroquinolin-2(1H)-one (0.19 g, 0.42 mmol). The title compound was obtained after flash column chromatography using 0-10% MeOH:DCM as a yellow solid. Yield 0.151 g, 65.1%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.37 (s, 1H), 12.07 (s, 1H), 7.61 (dd, J = 8.2, 2.4 Hz, 1H), 7.56 – 7.41 (m, 4H), 7.34 – 7.25 (m, 3H), 6.85 – 6.78 (m, 2H), 6.71 (dd, J = 9.7, 2.9 Hz, 1H), 5.36 (dd, J = 12.0, 4.5 Hz, 1H), 3.73 (dd, J = 18.5, 12.1 Hz, 1H), 2.78 (dd, J = 18.5, 4.4 Hz, 1H), 2.62 – 2.54 (m, 1H), 2.53 – 2.40 (m, 1H), 2.33 (t, J = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.48, 168.69, 159.89, 157.84, 156.25, 152.46, 149.03, 141.01, 135.30, 133.52, 133.45, 131.59, 131.40, 130.47, 128.48, 128.30, 127.28, 124.74, 119.94, 119.78, 119.61, 117.67, 117.62, 111.93, 111.77, 58.24, 45.07, 28.46, 28.18. ^{19}F NMR (376 MHz, DMSO-d_6) δ -120.13 – -120.29 (m). HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{28}H_{19}Cl_2FN_3O_4$, 550.07421; found, 550.07419. Anal. Calcd for $C_{28}H_{20}Cl_2FN_3O_4$: C, 60.88; H, 3.65; N, 7.61. Found C, 60.16; H, 3.98; N, 7.28. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.10 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.81 min; > 95% purity.



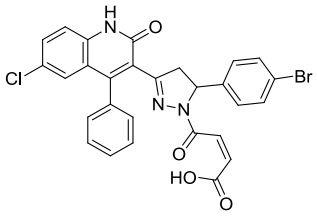
4-(5-(4-Chlorophenyl)-3-(6-fluoro-4-(3-fluorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-50). Compound **997-50** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.041 g, 0.41 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (0.18 g, 0.41 mmol). The title compound was obtained after flash column chromatography using 0-10% MeOH:DCM as a yellow solid. Yield 0.076 g, 34.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.34 (s, 1H), 12.01 (s, 1H), 7.59 – 7.39 (m, 3H), 7.37 – 7.19 (m, 3H), 7.14 (t, J = 7.8 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 6.83 – 6.75 (m, 2H), 6.74 – 6.63 (m, 1H), 5.40-5.33 (m, 1H), 3.81 (dd, J = 18.5, 12.1 Hz, 2H), 2.85 – 2.75 (m, 1H), 2.46 – 2.36 (m, 2H), 2.27 (t, J = 7.0 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.47, 168.66, 162.80, 161.17, 161.11, 159.93, 157.85, 156.26, 152.52, 152.44, 148.77, 141.08, 137.08, 137.02, 136.97, 135.29, 131.62, 131.56, 130.55, 128.39, 127.39, 127.25, 127.21, 127.08, 125.75, 124.69, 124.59, 119.90, 119.83, 117.66, 116.65, 115.50, 115.36, 111.87, 58.36, 58.24, 45.07, 45.00, 28.46, 28.18. ^{19}F NMR (376 MHz, DMSO-d_6) δ -112.89 – -113.28 (m), -120.04 – -120.29 (m). HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{19}ClF_2N_3O_4$, 534.10376; found, 534.10345. Anal. Calcd for $C_{28}H_{20}ClF_2N_3O_4$: C, 62.75; H, 3.76; N, 7.84. Found C, 61.88; H, 3.98; N: 7.67.



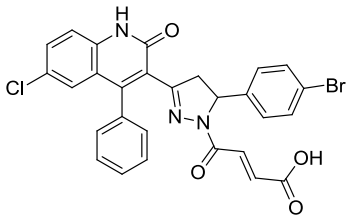
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-47). Compound **997-47** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.039 g, 0.39 mmol) and 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-methoxyquinolin-2(1H)-one (0.18 g, 0.39 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.095 g, 43.4%. ^{1}H NMR (600 MHz, DMSO-d6) δ 12.19 (s, 1H), 12.09 (s, 1H), 7.61 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.52 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.42 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.32 – 7.25 (m, 4H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 2.8 Hz, 1H), 5.34 (dd, *J* = 11.9, 4.5 Hz, 1H), 3.73 (dd, *J* = 18.4, 12.0 Hz, 1H), 3.60 (s, 3H), 2.77 (dd, *J* = 18.4, 4.4 Hz, 1H), 2.63 – 2.44 (m, 2H), 2.33 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.52, 168.66, 159.69, 154.24, 152.88, 149.28, 141.09, 134.02, 133.23, 133.15, 131.57, 131.42, 130.51, 128.39, 128.29, 128.12, 127.31, 123.95, 120.17, 119.68, 117.01, 109.05, 58.20, 55.32, 45.15, 28.48, 28.21. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{29}H_{22}Cl_2N_3O_5$, 562.09420; found, 562.09430. Anal. Calcd for $C_{29}H_{23}Cl_2N_3O_5$: C, 61.71; H, 4.11; N, 7.44. Found C, 61.47; H, 4.06; N, 7.36.



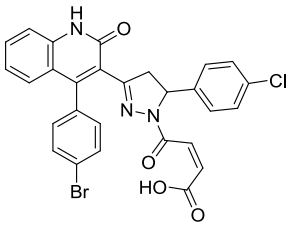
4-(5-(4-Chlorophenyl)-3-(4-(3-fluorophenyl)-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-48). Compound **997-48** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.027 g, 0.27 mmol) and 3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (0.12 g, 0.27 mmol). The title compound was obtained as a yellow solid after flash column chromatography using 0-10% MeOH:DCM. Yield 0.040 g, 27.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 7.64 – 7.48 (m, 1H), 7.43 – 7.07 (m, 6H), 6.87 – 6.77 (m, 2H), 6.45 – 6.40 (m, 1H), 5.40 – 5.30 (m, 1H) 3.87 – 3.74 (m, 2H), 3.60 (s, 3H), 2.83 (dt, J = 18.4, 5.2 Hz, 1H), 2.54 – 2.39 (m, 2H), 2.31 (dd, J = 15.3, 8.6 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.55, 168.62, 162.77, 162.71, 161.15, 161.08, 159.73, 154.23, 152.93, 152.85, 149.03, 141.16, 137.56, 137.51, 133.12, 131.59, 131.52, 130.38, 128.41, 128.32, 127.44, 127.26, 127.07, 125.78, 124.73, 123.87, 123.78, 120.06, 119.63, 116.98, 115.17, 109.18, 58.35, 58.17, 55.38, 55.20, 45.10, 28.49, 28.23. ^{19}F NMR (376 MHz, DMSO-d6) δ -113.15 – -113.37 (m). HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{29}H_{22}ClFN_3O_5$, 546.12375; found, 546.12384. Anal. Calcd for $C_{29}H_{23}ClFN_3O_5$: C, 63.56; H, 4.23; N, 7.67. Found C, 52.15; H, 3.91; N, 5.95. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.809 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.625 min; > 95% purity.



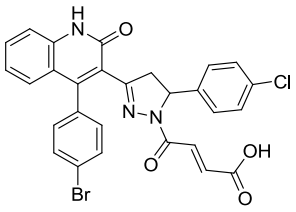
(Z)-4-(5-(4-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoic acid (997-9). Compound **997-9** was prepared according to general procedure G using furan-2,5-dione (0.061 g, 0.63 mmol) and 3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (0.30 g, 0.63 mmol). The title compound was obtained after filtration from the cooled reaction medium and rinsed with THF. Yield 0.200 g, 55.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 12.41 (s, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.54 (q, J = 9.5, 6.9 Hz, 3H), 7.40 (dt, J = 13.4, 7.9 Hz, 4H), 7.25 (d, J = 7.2 Hz, 1H), 6.92 (s, 1H), 6.78 (d, J = 8.1 Hz, 2H), 6.40 (d, J = 12.1 Hz, 1H), 6.16 (d, J = 12.1 Hz, 1H), 5.37 (dd, J = 12.0, 4.9 Hz, 1H), 3.78 (dd, J = 18.5, 12.0 Hz, 1H), 2.79 (dd, J = 18.7, 4.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.77, 161.77, 160.01, 153.58, 150.11, 140.93, 137.36, 134.39, 131.31, 129.57, 129.46, 128.64, 128.43, 127.96, 126.16, 126.08, 124.26, 120.62, 120.29, 117.67, 58.43, 45.17. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{28}H_{18}ClBrN_3O_4$, 574.01747; found, 574.01654. Anal. Calcd for $C_{28}H_{29}BrClN_3O_4$: C, 58.30; H, 3.32; N, 7.28. Found C, 43.78; H, 3.00; N: 5.57. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.18 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.06 min; > 95% purity.



(E)-4-(5-(4-Bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoic acid (997-10). Compound **997-10** was prepared from (E)-methyl 4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoate (0.190 g, 0.32 mmol) using the following method. In a 25 mL round-bottomed flask, ethanol (10 mL), (E)-methyl 4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoate (190 mg, 0.322 mmol) and 1M NaOH (1.222 mL, 1.222 mmol) were stirred to give a yellow solution. After four hours, 1M HCl (1.22 mL) was added and a yellow solid precipitated. The solid was filtered and rinsed with water to give the title compound as a yellow solid. Yield 0.170 g, 92.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.03 (s, 1H), 12.46 (s, 1H), 7.66 (dd, J = 8.8, 2.4 Hz, 1H), 7.61 – 7.38 (m, 7H), 7.31 (dt, J = 5.6, 2.5 Hz, 1H), 7.26 (d, J = 15.7 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.78 (d, J = 8.3 Hz, 2H), 6.45 (d, J = 15.7 Hz, 1H), 5.45 (dd, J = 11.8, 4.5 Hz, 1H), 3.80 (dd, J = 18.7, 11.9 Hz, 1H), 2.88 (dd, J = 18.7, 4.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.81, 166.11, 160.30, 159.96, 155.82, 154.41, 150.24, 140.70, 137.39, 134.48, 132.67, 131.83, 131.42, 129.19, 128.63, 128.51, 128.36, 127.93, 127.83, 126.17, 126.05, 124.09, 120.60, 120.44, 117.66, 58.69, 58.63, 45.11. HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{28}H_{18}ClBrN_3O_4$, 574.01747; found, 574.01750. Anal. Calcd for $C_{28}H_{19}ClBrN_3O_4 \cdot 1.00H_2O$: C, 56.54; H, 3.56; N, 7.06. Found C, 56.66; H, 3.76; N: 6.93.

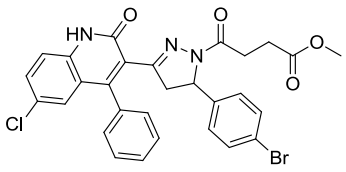


(Z)-4-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoic acid (997-30). Compound **997-30** was prepared according to general procedure G using furan-2,5-dione (0.061 g, 0.63 mmol) and 4-(4-bromophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.30 g, 0.63 mmol). The THF was removed under vacuum and the resultant residue was dissolved in hot EtOAc. A yellow solid was present upon cooling which was filtered and determined to be the desired product. Yield 0.171 g, 47.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 12.30 (s, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.32 (t, J = 8.6 Hz, 3H), 7.22 (d, J = 8.2 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 8.1 Hz, 2H), 6.48 (d, J = 12.2 Hz, 1H), 6.20 (d, J = 12.1 Hz, 1H), 5.47 – 5.38 (m, 1H), 3.76 (dd, J = 18.8, 11.9 Hz, 1H), 2.84 – 2.74 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.80, 161.77, 160.05, 153.90, 150.03, 140.50, 138.60, 134.27, 131.76, 131.69, 131.55, 131.28, 130.82, 129.68, 129.41, 128.36, 127.51, 127.32, 123.10, 122.47, 121.89, 119.04, 115.61, 93.88, 67.04, 58.28, 45.27, 25.15. HRMS (*m*/*z*): $[M-H]^-$ calcd for $C_{28}H_{18}ClBrN_3O_4$, 576.03202; found, 576.03371. Anal. Calcd for $C_{28}H_{19}ClBrN_3O_4 \cdot 0.60H_2O$: C, 57.23; H, 3.46; N, 7.15. Found C, 57.22; H, 3.54; N, 6.91.



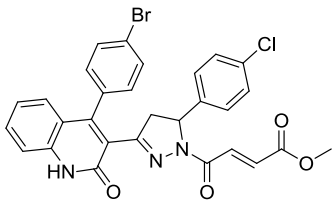
(E)-4-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoic acid (997-33). Compound **997-33** was prepared from (E)-methyl 4-(3-(4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoate (0.500 g, 0.85 mmol) using the following method. In a 50 mL round-bottomed flask, ethanol (28.2 mL), **60** (*E*)-methyl 4-(3-(4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoate (500 mg, 0.85 mmol) and 1M NaOH (3.22 mL, 3.22 mmol) were stirred to give a yellow solution. After four hours, 1M HCl (3.22 mL) was added and a yellow solid precipitated. The solid was filtered and rinsed with water. The resulting solid was dissolved in DCM, washed with brine and the organics were dried over magnesium sulfate *in vacuo*. The title compound was obtained from column chromatography (0-10% MeOH in DCM) as a yellow solid. Yield 0.320 g, 65.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 12.28 (s, 1H), 7.71 – 7.51 (m, 3H), 7.48 – 7.19 (m, 6H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.44 (d, *J* = 15.7 Hz, 1H), 5.45 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.73 (dd, *J* = 18.7, 11.8 Hz, 1H), 2.89 (dd, *J* = 18.6, 4.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.08, 166.05, 160.24, 160.04, 154.75, 152.81, 152.77, 150.29, 140.25, 138.65, 134.49, 132.80, 131.95, 131.60, 131.36, 131.26, 130.74, 128.51, 127.55, 127.43, 122.92, 122.47, 121.84, 119.01, 115.61, 58.50, 45.19. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{20}ClBrN_3O_4$, 576.03202; found, 576.03294. Anal. Calcd for

$C_{28}H_{19}ClBrN_3O_4 \cdot 0.40H_2O$: C, 57.58; H, 3.42; N, 7.19. Found C, 57.46; H, 3.50; N, 7.23.



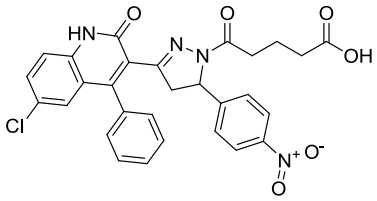
Methyl 4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoate (997-11). Compound **997-11** was prepared from 4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (0.40 g, 0.69 mmol) in the following manner. The 4-(5-(4-bromophenyl)-3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid was dissolved in 6.9 mL THF and freshly prepared HCl in MeOH was added dropwise to the reaction vessel with stirring until TLC indicated completion. Upon completion, the THF was removed under vacuum, the residue dissolved in DCM, washed 3X with brine and column chromatographed using 0-10% MeOH gradient in DCM to give the title compound as a yellow solid. Yield 0.100 g, 24.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 7.64 (dd, J = 8.7, 2.4 Hz, 1H), 7.54 (dtt, J = 12.7, 6.4, 3.9 Hz, 3H), 7.48 – 7.38 (m, 4H), 7.31 – 7.24 (m, 1H), 6.93 (d, J = 2.3 Hz, 1H), 6.77 – 6.70 (m, 2H), 5.31 (dd, J = 12.0, 4.6 Hz, 1H), 3.76 (dd, J = 18.5, 12.2 Hz, 1H), 3.59 (s, 1H), 3.54 (s, 2H), 2.79 (dd, J = 18.5, 4.6 Hz, 1H), 2.61 – 2.42 (m, 2H), 2.37 (t, J = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.44, 168.37, 168.08, 160.09, 152.81, 152.61, 149.98, 141.49, 137.32, 134.58, 131.30, 129.45, 128.48, 128.36, 127.78, 127.67, 126.13, 126.01, 124.50, 120.65, 120.14, 117.65, 58.43, 58.30, 51.35, 51.24, 45.08, 28.46, 27.96. HRMS (*m/z)*: $[M-H]^-$ calcd for $C_{29}H_{22}ClBrN_3O_4$, 590.04877; found, 590.04851. Anal. Calcd for

$C_{29}H_{23}ClBrN_3O_4$: C, 58.75; H, 3.91; N, 7.09. Found C, 58.35; H, 4.08; N, 6.66.

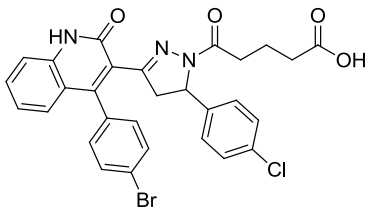


(*E*)-Methyl 4-(3-(4-(4-bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobut-2-enoate (997-31). Compound **997-31** was prepared from 4-(4-bromophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one(0.750g, 1.6 mmol) and (*E*)-methyl 4-chloro-4-oxobut-2-enoate (0.28 g, 1.9 mmol) using standard procedure G. The THF was removed under vacuum, the residue dissolved in DCM, washed 3X with brine and the organics concentrated. The title compound was obtained as a yellow solid by flash chromatography using a 0-10% MeOH gradient in DCM. Yield 0.546 g, 59.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.70 (dt, *J* = 8.3, 1.9 Hz, 1H), 7.67 (dt, *J* = 8.1, 2.0 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.37 – 7.32 (m, 3H), 7.30 (dt, *J* = 8.2, 2.0 Hz, 1H), 7.27 – 7.22 (m, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 2H), 6.53 (dd, *J* = 15.5, 1.0 Hz, 1H), 5.49 (dd, *J* = 11.6, 4.3 Hz, 1H), 3.86 – 3.73 (m, 4H), 3.03 (dd, *J* = 18.6, 4.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.13, 160.11, 159.92, 154.93, 150.40, 140.16, 138.71, 134.71, 133.36, 132.02, 131.69, 131.37, 131.30, 131.11, 130.80, 129.93, 128.65, 128.56, 127.57, 127.46, 122.77, 122.50, 121.77, 119.01, 115.63, 58.56, 52.16, 45.15. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{22}ClBrN_3O_4$, 590.04767; found, 590.04887. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.56 min; > 90% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.39

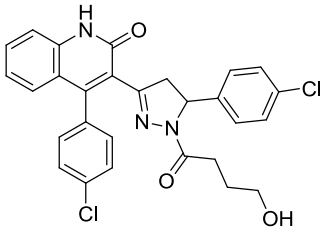
min; > 90% purity.



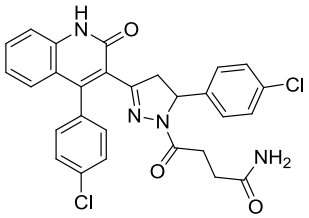
5-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-oxopentanoic acid (997-4). Compound **997-4** was prepared according to general procedure G using dihydrofuran-2,5-dione (0.077 g, 0.67 mmol) and 6-chloro-3-(5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.30 g, 0.67 mmol). The title compound was obtained after being dissolved in hot EtOAc followed by slow addition of hot hexanes to yield the title compound as a yellow solid. Yield 0.220 g, 58.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 12.12 (s, 1H), 8.12 (d, J = 8.3 Hz, 2H), 7.70 – 7.51 (m, 4H), 7.51 – 7.38 (m, 2H), 7.28 (d, J = 6.6 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 2.5 Hz, 1H), 5.50 (dd, J = 12.3, 4.8 Hz, 1H), 3.83 (dd, J = 18.6, 12.2 Hz, 1H), 2.86 (dd, J = 18.6, 4.9 Hz, 1H), 2.45 – 2.30 (m, 1H), 2.29 – 2.17 (m, 1H), 2.13 (t, J = 7.4 Hz, 2H), 1.65 – 1.45 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.07, 169.49, 160.10, 152.49, 149.59, 146.59, 137.35, 131.30, 129.39, 128.47, 126.68, 126.08, 124.34, 123.82, 120.63, 117.67, 58.32, 44.90, 32.94, 32.53, 19.70. HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{24}ClN_4O_6$, 559.13789; found, 559.13826. Anal. Calcd for $C_{29}H_{23}ClN_4O_6$: C, 62.31; H, 4.15; N, 10.02. Found C, 62.42; H, 4.39; N, 9.72.



5-(3-(4-(4-Bromophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-oxopentanoic acid (997-32). Compound **997-32** was prepared according to general procedure G using dihydro-2H-pyran-2,6(3H)-dione (0.071 g, 0.63 mmol) and 4-(4-bromophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.30 g, 0.63 mmol). Yield 0.204 g, 54.9%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.29 (s, 1H), 12.09 (s, 1H), 7.77 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.70 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.36 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.23 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.86 – 6.76 (m, 2H), 5.36 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.81 – 3.68 (m, 1H), 2.80 (dd, *J* = 18.7, 4.3 Hz, 1H), 2.40 (dt, *J* = 15.3, 7.4 Hz, 1H), 2.29 – 2.06 (m, 3H), 1.68 – 1.51 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.11, 169.29, 160.18, 152.73, 149.89, 141.29, 138.57, 134.59, 131.73, 131.64, 131.47, 131.30, 131.25, 130.88, 128.40, 127.31, 127.27, 123.41, 122.44, 121.77, 119.09, 115.60, 58.13, 45.10, 32.96, 32.58, 19.82. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{29}H_{24}ClBrN_3O_4$, 592.06332; found, 592.06461. Anal. Calcd for $C_{29}H_{23}ClBrN_3O_4$: C, 58.75; H, 3.91; N, 7.09. Found C, 58.47; H, 3.96; N, 6.91.

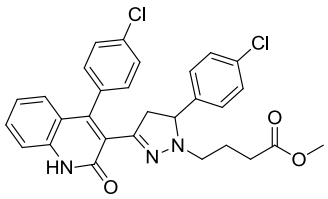


4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-1-(4-hydroxybutanoyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-57). In a flame dried 25 mL round bottomed flask, (5-(4-chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (0.30 g, 0.56 mmol) was dissolved in THF (10 mL) and cooled on an ice bath to 0 °C under nitrogen and with stirring. BH_3-DMS (2.0M in Hexanes, 0.561 ml, 2 eq.) was added dropwise. The reaction was stirred for thirty minutes, quenched with MeOH and the solvent removed under vacuum. The resultant residue was dissolved in DCM, washed three times with brine and the organics combined, dried over magnesium sulfate, concentrated *in vacuo* and column chromatographed using a 0-8% gradient of MeOH in DCM to give the title compound as a yellow solid. Yield 0.063 g, 21.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.65 – 7.49 (m, 3H), 7.46 – 7.24 (m, 5H), 7.20 – 7.00 (m, 2H), 6.89 – 6.78 (m, 2H), 5.35 (dd, *J* = 12.0, 4.4 Hz, 1H), 4.45 (t, *J* = 5.2 Hz, 1H), 3.74 (dd, *J* = 18.5, 12.1 Hz, 1H), 3.41 – 3.27 (m, 2H), 2.80 (dd, *J* = 18.5, 4.5 Hz, 1H), 2.44 – 2.19 (m, 2H), 1.59 – 1.39 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.82, 160.17, 152.52, 149.83, 141.31, 138.53, 134.18, 133.14, 131.58, 131.43, 131.39, 130.57, 129.11, 128.33, 127.28, 123.50, 122.41, 119.14, 115.57, 60.19, 58.10, 45.06, 30.14, 27.71. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{24}Cl_2N_3O_3$, 520.11892; found, 520.11993. Anal. Calcd for $C_{28}H_{23}Cl_2N_3O_3 \cdot 0.40H_2O$: C, 63.74; H, 4.55; N, 7.96. Found C, 63.75; H, 4.33; N, 7.89.



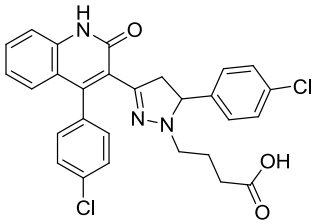
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanamide (997-58). In a flame dried 25 mL round bottomed flask, 4-(5-(4-chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (0.30 g, 0.56 mmol), 4-dimethylaminopyridine (0.069 g, 0.56 mmol) and N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine (0.096 g, 0.618 mmol) were added to THF(11.23 mL) at 0 °C and stirred for 45 minutes. Ammonia (0.5 M in dioxane, 1.0 eq, 1.1 ml) was added to the flask and the reaction mixture was stirred overnight while being allowed to warm to room temperature. The reaction was quenched with dilute HCl (0.1 M) and the organics were removed under vacuum. The resultant residue was dissolved in DCM, washed 3X with brine and the organics dried over magnesium sulfate and concentrated *in vacuo* prior to column chromatography using a 0-8% MeOH gradient in DCM (0.1 % Et_3N). The title compound was obtained as a white solid. Yield 0.019 g, 6.35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.66 – 7.56 (m, 2H), 7.55 – 7.49 (m, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.28 (dd, *J* = 8.2, 4.4 Hz, 3H), 7.24 (s, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.73 (s, 1H), 5.34 (dd, *J* = 12.1, 4.5 Hz, 1H), 3.71 (dd, *J* = 18.4, 12.2 Hz, 1H), 2.72 (dd, *J* = 18.4, 4.5 Hz, 1H), 2.59 – 2.50 (m, 2H), 2.20 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.69, 169.78, 160.79,

153.18, 150.49, 141.85, 139.21, 134.63, 133.87, 132.22, 132.19, 132.09, 131.23, 128.99, 128.95, 127.94, 124.23, 123.07, 119.80, 116.24, 58.83, 46.27, 45.80, 29.94, 29.44. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{23}Cl_2N_4O_3$, 533.11417; found, 533.11517. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.89 min; > 90% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.00 min; > 95% purity.



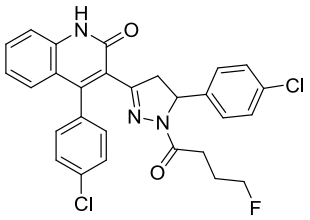
Methyl 4-(5-(4-chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)butanoate (997-62). In a 20 mL round-bottomed flask, 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.500 g, 1.151 mmol) and methyl 4-oxobutanoate (0.121 ml, 1.151 mmol) were dissolved in DCE (11.51 ml). The mixture was allowed to stir at room temperature for four hours and sodium triacetoxyborohydride (0.293 g, 1.381 mmol) was added in one portion. The reaction was monitored by TLC and HPLC-MS. Upon completion, the DCE was removed under vacuum and the residue diluted with DCM and washed 3X with brine. The organics were concentrated and the title compound was obtained from flash column chromatography using 0-10% MeOH in DCM and trituration of the compound from ether as a yellow solid. Yield 0.150 g, 24.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.22 (s, 1H), 7.55 – 7.45 (m, 3H), 7.40 (s, 1H), 7.34 – 7.26 (m, 5H), 7.22 (s, 2H), 7.13 (t, *J* = 7.6 Hz, 1H), 4.11 (dd, *J* = 13.6, 10.1 Hz, 1H), 3.62 (s, 3H), 3.41 (dd, *J* = 16.4, 10.1 Hz,

1H), 2.91 – 2.78 (m, 1H), 2.70 – 2.53 (m, 2H), 2.30 – 2.08 (m, 2H), 1.84 – 1.66 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.18, 162.94, 150.63, 146.34, 139.59, 138.08, 135.07, 134.23, 133.49, 131.12, 130.90, 129.08, 128.89, 128.51, 128.33, 127.82, 124.96, 122.96, 120.65, 116.18, 70.81, 52.20, 51.66, 46.60, 31.30, 23.04. HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{26}Cl_2N_3O_3$, 534.13457; found, 534.13624. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 2.35 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 2.09 min; > 95% purity.



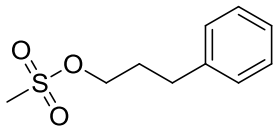
4-(5-(4-Chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)butanoic acid (997-63). In a 10 mL round-bottomed flask, **66** methyl 4-(5-(4-chlorophenyl)-3-(4-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)butanoate (0.100 g, 0.187 mmol) was dissolved in NaOH (0.711 ml, 0.711 mmol), H_2O (10.00 ml) and Ethanol (6.24 ml). The mixture was stirred at room temperature for four hours and monitored by TLC/LC-MS. Upon completion, HCl (0.711 ml, 0.711 mmol) was added, giving a bright yellow solid which was filtered, dissolved in DCM and washed 3X with brine. The organics were combined, dried over magnesium sulfate and concentrated *in vacuo*. The title compound was obtained by flash chromatography using 0-8% MeOH in DCM. Yield 0.050 g, 51.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (s, 1H), 11.92 (s, 1H), 7.59 – 7.49 (m, 3H), 7.42 – 7.37 (m, 3H), 7.35 – 7.29 (m, 4H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 4.03 (dd, *J* = 13.8, 10.2

Hz, 1H), 3.33 (dd, *J* = 16.4, 10.2 Hz, 1H), 2.73 (dd, *J* = 16.5, 13.8 Hz, 1H), 2.54 – 2.46 (m, 1H), 2.40 – 2.31 (m, 1H), 2.12 – 1.96 (m, 2H), 1.55 – 1.39 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.25, 160.57, 148.75, 146.65, 140.21, 138.31, 135.02, 132.63, 131.86, 131.23, 131.19, 130.84, 130.75, 129.09, 128.44, 128.08, 127.81, 127.04, 125.16, 122.15, 119.31, 115.43, 69.48, 52.16, 46.06, 30.83, 22.35. HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{24}Cl_2N_3O_3$, 520.11892; found, 520.11970. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.33 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.98 min; > 95% purity.



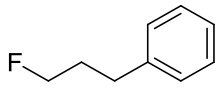
4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-1-(4-fluorobutanoyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-64). In a 10 mL round-bottomed flask, 4-fluorobutanoic acid (0.100 g, 0.943 mmol), DMAP (0.127 g, 1.037 mmol) and EDC (0.199 g, 1.037 mmol) were dissolved in DCM (9.43 ml) which had been pre-cooled to 0°C. The mixture was stirred for 45 minutes prior to the addition of the **26f** 4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (0.409 g, 0.943 mmol). The reaction was allowed to warm to room tempereature, and monitored by TLC. The reaction was quenched with 0.2 N HCl and extracted into DCM. The organics were washed 3X with brine, dried over magnesium sulfate and concentrated. The title compound was obtained as a yellow solid after column

chromatography using a gradient of 0-50% EtOAc/DCM as a yellow solid. Yield 0.050 g, 21.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.16 (s, 1H), 7.62 – 7.43 (m, 4H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.34 – 7.16 (m, 5H), 7.02 (d, *J* = 8.3 Hz, 2H), 5.42 (dd, *J* = 11.8, 4.0 Hz, 1H), 4.44 (dtd, *J* = 47.3, 5.8, 1.7 Hz, 2H), 3.70 (dd, *J* = 18.2, 11.8 Hz, 1H), 3.15 (dd, *J* = 18.3, 4.1 Hz, 1H), 2.68 – 2.45 (m, 2H), 2.18 – 1.82 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.45, 162.91, 152.52, 151.98, 151.92, 140.41, 138.42, 135.43, 134.99, 133.97, 133.56, 132.00, 130.81, 130.72, 130.68, 130.36, 129.05, 128.96, 128.85, 128.73, 128.70, 128.04, 127.60, 127.52, 126.86, 123.47, 123.09, 120.36, 116.41, 84.06, 82.97, 59.35, 45.44, 29.91, 29.88, 25.69, 25.62, 25.56. ^{19}F NMR (282 MHz, $CDCl_3$) δ -220.53 (tt, *J* = 47.3, 26.3 Hz). HRMS (*m/z)*: $[M+H]^+$ calcd for $C_{28}H_{23}Cl_2N_3O_2F$, 522.11459; found, 522.11462. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.46 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.32 min; > 95% purity.

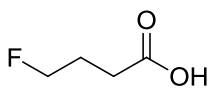


3-phenylpropyl methanesulfonate. In a 500 mL round-bottomed flask, 3-phenylpropan-1-ol (10.00 ml, 73.4 mmol) and triethylamine (12.28 ml, 88 mmol) were added to DCM (300ml). The methanesulfonyl chloride (6.87 ml, 88 mmol) was added slowly and allowed to stir for 2 hrs. until TLC indicated completion. The reaction was then washed with brine 3X and the organics dried over magnesium sulfate and concentrated *in vacuo*. The title compound was isolated using column chromatography 0-20% EtOAc in hexanes as a colorless oil. Yield 14.0 g, 89%. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 7.35 – 7.17 (m, 5H), 4.21 (t, J = 6.0 Hz, 2H), 3.27 – 3.09 (m, 3H), 2.68 (t, J = 7.8 Hz, 2H), 2.02 – 1.93 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 140.77, 128.40, 128.34, 126.01, 69.73, 36.56, 30.97, 30.30. HRMS calcd for $C_{10}H_{15}O_2S$ $[M+H]^+$; 215.07364 found; 215.07352.



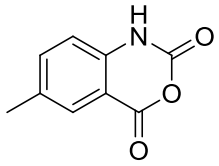
(3-fluoropropyl)benzene. In a 250 mL round bottom flask, 3-phenylpropyl methanesulfonate (14 g, 65.3 mmol) in tert-butanol (88 ml, 920 mmol) was stirred with cesium fluoride (29.8 g, 196 mmol) at 80 °C for 3 hours. Upon cooling, diethyl ether was added and filtered to remove the inorganics. The organics were concentrated and the resultant residue partitioned between EtOAc and brine. The organics were washed 3X, dried over magnesium sulfate adn concentrated *in vacuo*. The title compound was obtained after flash chromatography using a 0-20% gradient of EtOAc/hexanes as a colorless oil. Yield 2.40 g, 26.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.13 (m, 5H), 4.42 (dt, J = 47.3, 6.0 Hz, 2H), 2.72 (t, J = 7.8, 2H), 2.14 – 1.79 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.28, 128.65, 128.62, 126.20, 84.06, 82.42, 32.32, 32.12, 31.51, 31.46. ^{19}F NMR (376 MHz, $CDCl_3$) δ -220.87 (tt, J = 47.1, 25.4 Hz). HRMS calcd for $C_9H_{12}F$ $[M+H]^+$; 139.09176 found; 139.09166.



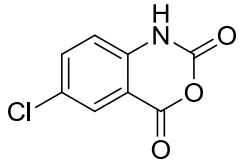
4-fluorobutanoic acid. In a 250 mL round-bottomed flask, (3-fluoropropyl)benzene (2.40 g, 17.37 mmol), potassium periodate (14.98 g, 65.1 mmol), and ruthenium(III) chloride (0.036 g, 0.174 mmol) in Acetonitrile (24.12 ml), Water (72.4 ml) and CCl4 (24.12 ml). The mixture was stirred vigorously overnight. Upon completion, the aqueous layer was separated and acidified with HCl until pH 2. The acidic layer was extracted 3X with ethyl acetate. The ethyl acetate was then extracted 3X with a pH 10 NaOH solution which was re-acidified with HCl and then extracted with EtOAc 3X. The resultant organics from the acid/base extraction were dried over magnesium sulfate and concentrated to give the title compound as a brownish oil. Yield 0.140 g, 7.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (s, 1H), 4.51 (dt, *J* = 47.1, 5.8 Hz, 2H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.23 – 1.92 (m, 2H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 179.65, 83.73, 82.09, 29.95, 25.69, 25.49. ^{19}F NMR (282 MHz, $CDCl_3$) δ -221.27 – -222.29 (m). HRMS calcd for $C_4H_6O_2F$ $[M-H]^-$; 105.03573 found; 105.03580.

General procedure for the synthesis of isatoic anhydride intermediates

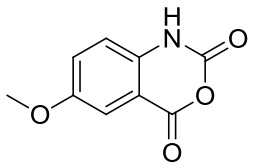
(Procedure A). A mixture of an appropriately substituted 2-aminobenzoic acid (**1**, 1.0 equiv.) and triphosgene (0.34 equiv. WARNING, triphosgene is toxic and should be handled with care, refer to MSDS prior to handling) in THF (0.23 molar) was stirred at 70 °C for approximately two hours. The mixture was then poured onto an ice bath (200 mL) and the resultant solid was filtered and washed with MeOH to give the desired products, unless otherwise noted.



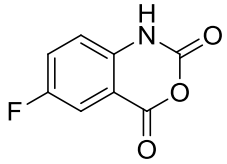
6-Methyl-1H-benzo[d][1,3]oxazine-2,4-dione (997-43a). Compound **997-43a** was synthesized via general procedure A using 2-amino-5-methylbenzoic acid (10.0 g, 66.2 mmol). The desired product was obtained as a white solid. Yield 8.80 g, 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 7.71 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.90, 147.12, 139.22, 137.92, 132.93, 128.30, 115.27, 109.99, 20.08. HRMS calcd for $C_9H_8NO_3$, 178.04987 $[M+H]^+$; found; 178.04980.



6-Chloro-1H-benzo[d][1,3]oxazine-2,4-dione (997-45a). Compound **997-45a** was synthesized via general procedure A using 2-amino-5-chlorobenzoic acid (10.0 g, 58.3 mmol). The desired product was obtained as a white solid. Yield 10.0 g, 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (s, 1H), 7.88 (d, J = 2.5 Hz, 1H), 7.79 (dd, J = 8.7, 2.5 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.96, 146.77, 140.29, 136.63, 127.67, 127.13, 117.45, 112.03. HRMS calcd for $C_8H_5NO_3Cl$, 197.99525 $[M+H]^+$; found; 197.99519.



6-Methoxy-1H-benzo[d][1,3]oxazine-2,4-dione (997-47a). Compound **997-47a** was synthesized via general procedure A using 2-amino-5-methoxybenzoic acid (5.5 g, 33 mmol). The desired product was obtained as a white solid. Yield 5.30 g, 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 7.43 - 7.31 (m, 2H), 7.11 (dd, J = 9.0, 2.5 Hz, 1H), 3.81 (d, J = 2.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.17, 146.97, 135.51, 125.74, 116.96, 110.70, 109.81, 55.75. HRMS calcd for $C_9H_8NO_4$, 194.04478 $[M+H]^+$; found; 194.04469.

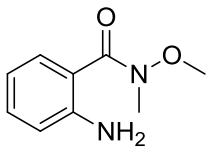


6-Fluoro-1H-benzo[d][1,3]oxazine-2,4-dione (997-49a). Compound **997-49a** was synthesized via general procedure A using 2-amino-5-fluorobenzoic acid (10.0 g, 65 mmol). The desired product was obtained as a white solid. Yield 10.1 g, 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 7.76 - 7.60 (m, 2H), 7.18 (dd, J = 8.9, 4.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.20, 158.71, 156.32, 146.81, 138.13, 124.91, 124.66, 117.61, 117.53, 114.13, 113.89, 111.55, 111.47. ^{19}F NMR (376 MHz, DMSO-d_6) δ -119.32 - -119.43 (m). HRMS calcd for $C_8H_5FNO_3$, 182.02480 $[M+H]^+$; found; 182.02466.

General procedure for the synthesis of N-methoxy-N-methylbenzamide intermediates

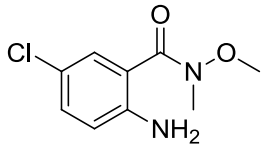
(Procedure B). In a round-bottomed flask, triethylamine (1.5 equivalents) and N,O-dimethylhydroxylamine hydrochloride (1.5 equiv.) in Ethanol (2.5 M, 90%) were stirred for 10 minutes. The appropriate isatoic anhydride (1.0 equiv.) was added slowly. The reaction mixture was heated to reflux for two hours. Upon completion, the mixture was poured onto an ice/saturated sodium bicarbonate mixture. The ethanol was removed *in vacuo* and the mixture was extracted with ethyl acetate. The organics were washed 3X with brine, dried over magnesium sulfate, concentrated *in vacuo* and purified using a Teledyne ISCO Combiflash Companion and an appropriately sized column 20-50% EtOAc:Hexanes gradient unless otherwise noted.(Frye et al., 1991)

2-Amino-N-methoxy-N-methylbenzamide (997-23b). Compound **997-23b** was synthesized via general procedure B using 1H-benzo[d][1,3]oxazine-2,4-dione (10.00 g, 61.3 mmol). Purification with flash chromatography using 20% EtOAc:Hexanes yielded the title compound as a brown oil. Analytics match literature.

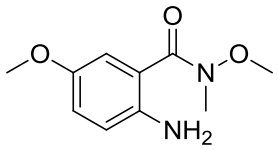


2-Amino-N-methoxy-N,5-dimethylbenzamide (997-43b). Compound **997-43b** was synthesized via general procedure B using 6-methyl-1H-benzo[d][1,3]oxazine-2,4-dione

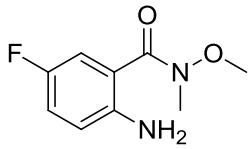
(8.0g, 45.2 mmol). Upon work-up, the title compound was obtained as a yellow oil (6.00g, 68.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 7.00 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 4.43 (s, 2H), 3.61 (s, 3H), 3.33 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.23, 144.17, 132.26, 129.24, 126.30, 117.92, 116.94, 61.25, 34.58, 20.48. HRMS calcd for $C_{10}H_{15}N_2O_2$, 195.11280 $[M+H]^+$; found; 195.11277.



2-Amino-5-chloro-N-methoxy-N-methylbenzamide (997-46b). Compound **997-46b** was synthesized via general procedure B using 6-chloro-1H-benzo[d][1,3]oxazine-2,4-dione (5.53 g, 28.0 mmol). Upon work-up, the title compound was obtained as a brown oil without purification. Yield 3.98 g, 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 2.4 Hz, 1H), 7.14 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 4.70 (s, 2H), 3.59 (s, 3H), 3.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.83, 145.66, 131.56, 129.02, 118.32, 118.13, 61.56, 34.17. HRMS calcd for $C_9H_{12}N_2O_2Cl$, 215.05818 $[M+H]^+$; found; 215.05823.



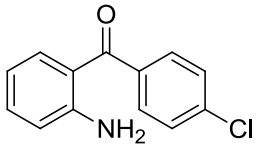
2-Amino-N,5-dimethoxy-N-methylbenzamide (997-47b). Compound **997-47b** was synthesized via general procedure B using 6-methoxy-1H-benzo[d][1,3]oxazine-2,4-dione (4.9 g, 25.4 mmol). Upon work-up, the mixture was chromatographed using a 50-75% gradient of EtOAc in hexanes and the title compound was obtained as a brown oil. Yield 2.64 g, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, *J* = 3.0 Hz, 1H), 6.82 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.66 (d, *J* = 8.8 Hz, 1H), 4.06 (s, 2H), 3.74 (s, 3H), 3.60 (s, 3H), 3.34 (s, 3H). HRMS calcd for $C_{10}H_{15}N_2O_3$, 211.10772 $[M+H]^+$; found; 211.10776.



2-Amino-5-fluoro-N-methoxy-N-methylbenzamide (997-49b). Compound **997-47b** was synthesized via general procedure B using 6-fluoro-1H-benzo[d][1,3]oxazine-2,4-dione (9.0 g, 49.7 mmol). Upon work-up, the mixture was chromatographed using a 50-65% gradient of EtOAc in hexanes and the title compound was obtained as a brown oil. Yield 3.90 g, 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.10 - 6.95 (m, 2H), 6.80 - 6.66 (m, 1H), 5.27 (s, 2H), 3.54 (d, *J* = 1.5 Hz, 3H), 3.23 (d, *J* = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.69, 154.11, 151.80, 143.52, 117.90, 117.68, 117.62, 117.55, 116.96, 116.89, 114.23, 114.00, 74.41, 60.76, 33.27. ^{19}F NMR (376 MHz, DMSO-d_6) δ 2.63 - 2.51 (m). HRMS calcd for $C_9H_{12}N_2O_2F$, 199.08773 $[M+H]^+$; found; 199.08765.

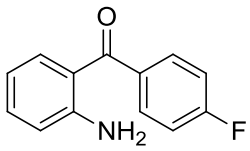
General procedure for the synthesis of 2-aminobenzophenone intermediates

(Procedure C). In a flame dried round-bottomed flask at -78 °C, the appropriate N-methoxy-N-methylbenzamide (1 equiv.) and an appropriately substituted bromobenzene (1 equiv.) in Tetrahydrofuran (0.17 M) were stirred vigorously under nitrogen. N-butyllithium (2 equiv., 2.5M in Hexanes), was added at a rate of 8 ml/hour. After the addition was complete, 2 ml of 1N HCl per ml of n-Butyllithium was added to the flask. The THF was removed *in vacuo* and the mixture was extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, and concentrated. Purification with flash column chromatography (10-20% gradient) EtOAc:Hexanes gave the desired products, unless otherwise noted.

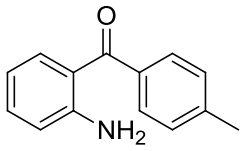


(2-Aminophenyl)(4-chlorophenyl)methanone (997-23c). Compound **997-23c** was prepared via general procedure C using 1-bromo-4-chlorobenzene (5.00g, 27.7 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.31g, 27.7 mmol). Purification by flash chromatography using 20% isocratic EtOAc:Hexanes yielded the title compound as a yellow solid. Yield 3.20 g, 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, J = 1.1 Hz, 4H), 7.32 - 7.20 (m, 2H), 7.15 (s, 2H), 6.87 (dd, J = 8.4, 1.1 Hz, 1H), 6.49 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.18, 196.54, 171.14, 151.96, 151.07, 138.61, 135.71, 134.40, 133.89, 133.68, 131.25, 130.46, 128.31, 126.95, 116.94,

116.52, 115.99, 114.31, 114.18, 26.73, 21.94, 13.91. HRMS calcd for $C_{13}H_{11}ClNO$, 232.05237 $[M+H]^+$; found; 232.05206.

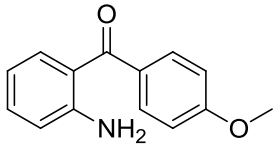


(2-Aminophenyl)(4-fluorophenyl)methanone (997-24c). Compound **997-24c** was prepared via general procedure C using 1-bromo-4-fluorobenzene (5.83g, 3.66 ml, 33.3 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (6.00 g, 33.3 mmol). Purification with flash chromatography using a 10-20% EtOAc:Hexanes gradient and concentration *in vacuo* yielded the title compound as a yellow solid. Yield 3.20 g, 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (dd, J = 8.3, 5.4 Hz, 2H), 7.37 - 7.22 (m, 4H), 7.10 (s, 2H), 6.87 (d, J = 8.3 Hz, 1H), 6.49 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.45, 164.84, 162.36, 151.85, 136.37, 136.34, 134.22, 133.64, 131.40, 131.31, 116.92, 116.28, 115.31, 115.10, 114.15. ^{19}F NMR (400 MHz, DMSO-d_6), δ -109.669. HRMS calcd for $C_{13}H_{11}FNO$, 216.08192 $[M+H]^+$; found; 216.08163.

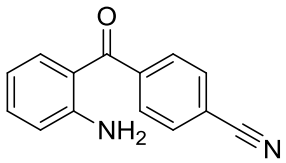


(2-Aminophenyl)(p-tolyl)methanone (997-26c). Compound **997-26c** was prepared via general procedure C using 1-bromo-4-methylbenzene (4.75g, 3.41 ml, 27.7 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol). The title compound was obtained as a bright yellow solid. Yield 2.50 g, 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, J = 7.4 Hz, 2H), 7.29 (d, J = 8.2 Hz, 4H), 7.06 (s, 2H), 6.92 -

6.84 (m, 1H), 6.55 - 6.45 (m, 1H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.61, 151.68, 141.05, 137.11, 133.95, 133.66, 128.87, 128.71, 116.82, 116.66, 114.05, 81.43, 21.02. HRMS calcd for $C_{14}H_{14}NO$, 212.10699 $[M+H]^+$; found; 212.10672.

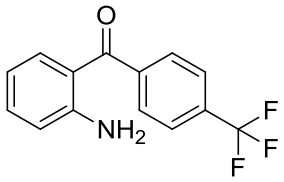


(2-Aminophenyl)(4-methoxyphenyl)methanone (997-21c). Compound **997-21c** was prepared via general procedure C using 1-bromo-4-methoxybenzene (5.19g, 3.47 ml, 27.7 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol). The title compound was obtained as a bright yellow solid. Yield 2.45 g, 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 7.9 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 7.02 (d, J = 8.1 Hz, 2H), 6.90 - 6.80 (m, 3H), 6.51 (t, J = 7.5 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.55, 161.77, 151.31, 133.58, 133.29, 131.95, 131.25, 117.22, 116.76, 114.09, 113.47, 55.35. HRMS calcd for $C_{14}H_{14}NO_2$, 228.10191 $[M+H]^+$; found; 228.10162.

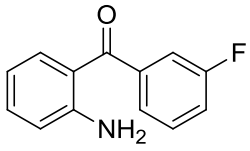


4-(2-Aminobenzoyl)benzonitrile (997-27c). Compound **997-27c** was prepared via general procedure C using 4-bromobenzonitrile (5.05 g, 27.7 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol). The title compound was

obtained as a bright yellow solid. Yield 3.00 g, 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, J = 7.8 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.57 - 7.03 (m, 4H), 6.97 - 6.76 (m, 1H), 6.61 - 6.37 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.39, 152.35, 144.18, 134.92, 133.88, 132.33, 128.92, 118.40, 117.06, 115.35, 114.30, 112.93, 14.04. HRMS calcd for $C_{14}H_{11}N_2O$, 223.08659 $[M+H]^+$; found; 223.08626.

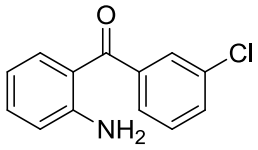


(2-Aminophenyl)(4-(trifluoromethyl)phenyl)methanone (997-36c). Compound **997-36c** was prepared via general procedure C using 1-bromo-4-(trifluoromethyl)benzene (3.75 g, 16.7 mmol) and 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.00 g, 16.7 mmol). The title compound was obtained as a yellow solid after column chromatography. The material was impure by NMR, and carried forward without further purification. HRMS calcd for $C_{14}H_{11}NOF_3$, 266.07873 $[M+H]^+$; found; 266.07916.

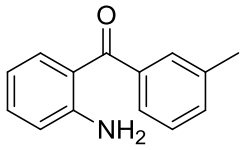


(2-Aminophenyl)(3-fluorophenyl)methanone (997-28c). Compound **997-28c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol) and 1-bromo-3-fluorobenzene (3.05 ml, 27.7 mmol). Crude yield, 3.20 g, 54%. Material carried forward without purification. HRMS calcd for

$C_{13}H_{11}NOF$, 216.08192 $[M+H]^+$; found; 216.08171.

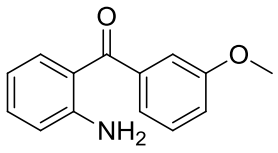


(2-Aminophenyl)(3-chlorophenyl)methanone (997-22c). Compound **997-36c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol) and 1-bromo-3-chlorobenzene (5.31 g, 3.26 ml, 27.7 mmol). The title compound was obtained as a yellow solid. Yield 3.30 g, 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (d, J = 8.0 Hz, 1H), 7.61 - 7.45 (m, 3H), 7.38 - 7.27 (m, 1H), 7.27 - 7.22 (m, 3H), 6.89 (d, J = 8.4 Hz, 1H), 6.51 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.12, 152.12, 141.98, 134.60, 133.77, 133.09, 130.53, 130.15, 127.93, 127.05, 116.96, 115.69, 114.22. HRMS calcd for $C_{13}H_{11}NOCl$, 232.05237 $[M+H]^+$; found; 232.05211.

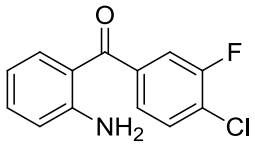


(2-Aminophenyl)(m-tolyl)methanone (997-25c). Compound **997-25c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol) and 1-bromo-3-methylbenzene (4.75 g, 3.37 ml, 27.7 mmol). The title compound was obtained as a yellow solid. Yield 4.10 g, 70%. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 7.38 (t, J = 4.8 Hz, 3H), 7.35 - 7.31 (m, 1H), 7.31 - 7.25 (m, 2H), 7.14 (s, 2H), 6.88 (d, J = 8.0 Hz, 1H), 6.58 - 6.45 (m, 1H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.06, 151.86, 140.05, 137.55, 134.14, 133.85, 131.45, 128.88, 128.01, 125.74, 116.84, 116.41, 114.06, 20.91. HRMS calcd for $C_{14}H_{14}NO$, 212.10699 $[M+H]^+$; found; 212.10684.

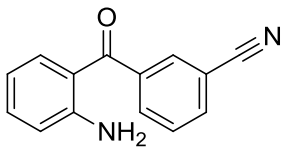


(2-Aminophenyl)(3-methoxyphenyl)methanone (997-39c). Compound **997-39c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.00 g, 16.65 mmol) and 1-bromo-3-methylbenzene (3.11 g, 2.11 ml, 16.65 mmol). The title compound was obtained as a yellow oil. Yield 1.80 g, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, J = 8.2, 1.6 Hz, 1H), 7.41 - 7.24 (m, 2H), 7.22 - 7.15 (m, 2H), 7.09 - 7.04 (m, 1H), 6.73 (dd, J = 8.2, 3.6 Hz, 1H), 6.64 - 6.55 (m, 1H), 6.10 (s, 2H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.99, 159.50, 151.10, 141.56, 134.76, 134.47, 129.23, 121.82, 118.21, 117.42, 117.13, 115.67, 113.88, 55.59. HRMS calcd for $C_{14}H_{14}NO_2$, 228.10191$[M+H]^+$; found; 228.10232.

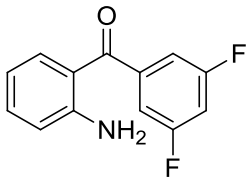


(2-aminophenyl)(4-chloro-3-fluorophenyl)methanone (GLuN2D-206). Compound

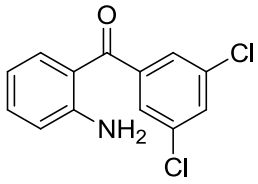
GluN2D-206 was prepared via general procedure C using 2-Amino-N-methoxy-N-methylbenzamide (4.30 g, 23.87 mmol) and 4-bromo-1-chloro-2-fluorobenzene (5.00 g, 23.87 mmol). The title compound was obtained as a yellow solid, with impurities present; the material was carried forward without further purification. Yield 2.00 g, 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 - 7.46 (m, 1H), 7.44 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.38 (td, *J* = 7.7, 1.8 Hz, 2H), 7.32 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1H), 6.78 - 6.68 (m, 1H), 6.69 - 6.53 (m, 1H), 6.11 (s, 2H). ^{19}F NMR (376 MHz, DMSO-d_6) δ -116.01- -116.05 (m). HRMS (*m/z)*: $[M+H-H_2]^+$ calcd for $C_{13}H_8NOFCl$, 248.02730; found, 248.02775.



3-(2-Aminobenzoyl)benzonitrile (997-40c). Compound **997-40c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (5.00 g, 27.7 mmol) and 3-bromobenzonitrile (5.05 g, 27.7 mmol). The title compound was obtained as a yellow solid. Yield 2.20 g, 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, *J* = 7.8 Hz, 1H), 7.98 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.38 - 7.25 (m, 3H), 7.19 (d, *J* = 7.9 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.50 (t, *J* = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.74, 152.29, 141.08, 134.85, 134.14, 133.90, 132.86, 131.78, 129.58, 118.31, 117.02, 115.44, 114.36, 111.52. HRMS calcd for $C_{14}H_9N_2O$, 221.07094 $[M+H-H_2]^+$; found; 221.07073.

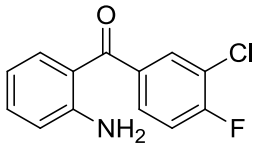


(2-Aminophenyl)(3,5-difluorophenyl)methanone (997-35c). Compound **997-35c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.00 g, 16.65 mmol) and 1-bromo-3,5-difluorobenzene (3.21g, 1.92 ml, 16.65 mmol). The title compound was obtained as a yellow solid. Yield, 1.15 g, 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.14 (h, *J* = 5.1 Hz, 2H), 7.02 - 6.92 (m, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.66-6.57 (m, 1H), 6.16 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.08, 164.01, 163.89, 161.51, 161.39, 151.45, 143.28, 135.17, 134.30, 131.41, 117.38, 117.03, 115.92, 112.32, 112.25, 112.14, 112.06, 106.63, 106.38, 106.13. HRMS calcd for $C_{13}H_{10}NOF_2$, 234.07250 $[M+H]^+$; found; 234.07287.

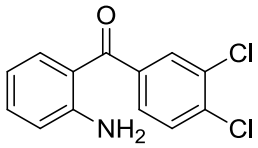


(2-Aminophenyl)(3,5-dichlorophenyl)methanone (997-37c). Compound **997-37c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.00 g, 16.65 mmol) and 1-bromo-3,5-dichlorobenzene (3.76g, 2.14ml, 16.65 mmol). The title compound was isolated as a yellow solid. Yield, 1.50 g, 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 1.9 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.44 (dd, *J*

= 8.3, 2.0 Hz, 1H), 7.36 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.32 - 7.25 (m, 1H), 6.72 (d, *J* = 8.3 Hz, 1H), 6.60 (t, *J* = 7.5 Hz, 1H), 6.14 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.20, 151.28, 139.83, 135.45, 134.92, 134.22, 134.14, 132.71, 131.34, 131.06, 130.35, 130.28, 128.39, 117.30, 117.25, 115.80. HRMS calcd for $C_{13}H_{10}NOCl_2$, 266.01340 $[M+H]^+$; found; 266.01392.

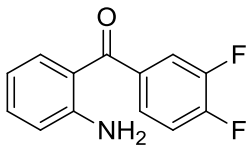


(2-Aminophenyl)(3-chloro-4-fluorophenyl)methanone (997-38c). Compound **997-38c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.00 g, 16.65 mmol) and 4-bromo-2-chloro-1-fluorobenzene (3.49g, 2.02ml, 16.65 mmol). The NMR of the title compound showed significant impurities after column chromatography. The material was carried forward without further attempts to purify. Yield 0.420 g, 57% with impurity. HRMS calcd for $C_{13}H_{10}NOClF$, 250.04295 $[M+H]^+$; found; 250.04338.

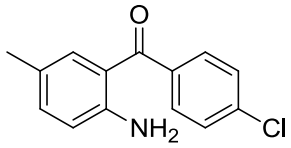


(2-Aminophenyl)(3,4-dichlorophenyl)methanone (997-41c). Compound **997-41c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (3.99 g, 22.13 mmol) and 4-bromo-1,2-dichlorobenzene (5.00 g, 22.13 mmol). The title compound was isolated as a yellow solid. Yield 1.50g, 26%. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.80 - 7.67 (m, 2H), 7.55 - 7.42 (m, 1H), 7.38 - 7.15 (m, 4H), 6.83 (d, *J* =

8.4 Hz, 1H), 6.46 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.04, 152.16, 140.33, 134.74, 133.72, 133.51, 131.26, 130.53, 130.21, 128.62, 116.99, 115.52, 114.30. HRMS calcd for $C_{13}H_8NOCl_2$, 263.99775 $[M+H]^+$; found; 263.99833.

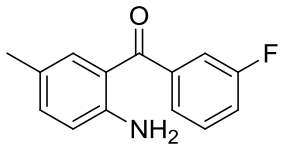


(2-Aminophenyl)(3,4-difluorophenyl)methanone (997-42c). Compound **997-42c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide**997-23b** (4.67 g, 25.9 mmol) and 4-bromo-1,2-difluorobenzene (5.00 g, 25.9 mmol). The compound showed significant impurities after column chromatography. The material was carried forward without further attempts to purify. Yield 2.20 g, 36% with impurities. HRMS calcd for $C_{13}H_8NOF_2$, 232.05685 $[M+H-H_2]^+$; found; 232.05730.

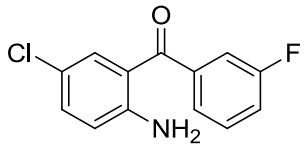


(2-Amino-5-methylphenyl)(4-chlorophenyl)methanone (997-43c). Compound **997-43c** was prepared via general procedure C using 2-amino-N-methoxy-N,5-dimethylbenzamide **997-43b** (3.00 g, 15.45 mmol) and 1-bromo-4-chlorobenzene (2.96 g, 15.45 mmol). The title compound was obtained as a yellow solid after purification . Yield 2.05 g, 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 - 7.54 (m, 2H), 7.49 - 7.39 (m, 2H), 7.19 - 7.09 (m, 2H), 6.67 (dd, *J* = 8.3, 1.6 Hz, 1H), 5.93 - 5.88 (m, 2H), 2.17 (d, *J* =

1.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.81, 149.00, 138.63, 137.42, 135.83, 135.46, 133.86, 131.00, 130.75, 128.56, 124.88, 118.00, 117.41, 20.51. HRMS calcd for $C_{14}H_{13}NOCl$, 246.06802 $[M+H]^+$; found; 246.06812.

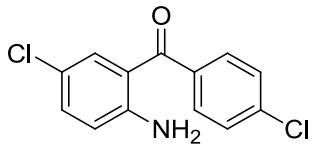


(2-Amino-5-methylphenyl)(3-fluorophenyl)methanone (997-44c). Compound **997-44c** was prepared via general procedure C using 2-amino-N-methoxy-N,5-dimethylbenzamide **997-43b** (1.90 g, 9.78 mmol) and 1-bromo-3-fluorobenzene (1.71 g, 9.78 mmol). The title compound was obtained as a yellow solid after purification. Yield 0.800 g, 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 - 7.37 (m, 2H), 7.33 (dt, *J* = 9.0, 2.2 Hz, 1H), 7.26 - 7.09 (m, 3H), 6.68 (d, *J* = 8.3 Hz, 1H), 5.95 (s, 2H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.15, 136.01, 133.97, 129.97, 129.89, 124.92, 118.17, 117.96, 117.41, 116.24, 116.01, 20.55. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.02 - -113.14 (m). HRMS calcd for $C_{14}H_{13}NOF$, 230.09757 $[M+H]^+$; found; 230.09762.

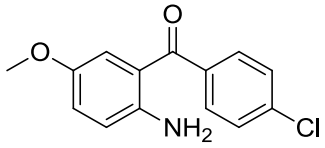


(2-Amino-5-chlorophenyl)(3-fluorophenyl)methanone (997-45c). Compound **997-45c** was prepared via general procedure C using 2-amino-5-chloro-N-methoxy-N-methylbenzamide **997-46b** (3.00 g, 13.98 mmol) and 1-bromo-3-fluorobenzene (2.45 g, 13.98 mmol). The title compound was obtained as a yellow solid after column chromatography. Yield 2.20 g, 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 8.0,

5.4 Hz, 1H), 7.41 - 7.36 (m, 2H), 7.33 (dt, *J* = 8.9, 2.0 Hz, 1H), 7.24 (dq, *J* = 9.0, 2.4 Hz, 2H), 6.69 (d, *J* = 8.7 Hz, 1H), 6.12 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.53, 163.83, 161.36, 149.71, 141.55, 141.48, 134.75, 133.28, 133.14, 130.22, 130.14, 124.94, 124.91, 120.17, 118.75, 118.72, 118.65, 118.51, 118.29, 116.19, 115.97. ^{19}F NMR (376 MHz, DMSO-d_6) δ -112.75 (td, *J* = 9.1, 5.7 Hz). HRMS calcd for $C_{13}H_{10}NOClF$, 250.04295 $[M+H]^+$; found; 250.04303.

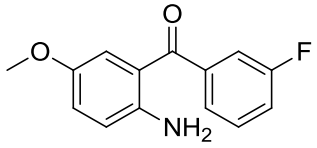


(2-Amino-5-chlorophenyl)(4-chlorophenyl)methanone (997-46c). Compound **997-44c** was prepared via general procedure C using 2-amino-5-chloro-N-methoxy-N-methylbenzamide **997-46b** (3.00 g, 13.98 mmol) and 1-bromo-4-chlorobenzene (2.68 g, 13.98 mmol). The title compound was obtained as a yellow solid after column chromatography. Significant impurities remained and the material was carried forward without further purification. Yield 2.20 g, 59% with impurities. HRMS calcd for $C_{13}H_{10}NOCl_2$, 266.01340 $[M+H]^+$; found; 266.01321.

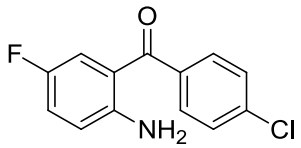


(2-Amino-5-methoxyphenyl)(4-chlorophenyl)methanone (997-47c). Compound **997-**

47c was prepared via general procedure C using 2-amino-N,5-dimethoxy-N-methylbenzamide **997-47b** (1.2 g, 5.7 mmol) and 1-bromo-4-chlorobenzene (1.1g, 5.7 mmol). The title compound was obtained as a brown oil after column chromatography. Yield 1.00 g, 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 - 7.46 (m, 4H), 7.06 (dd, J = 9.0, 3.1 Hz, 1H), 6.85 (d, J = 9.0 Hz, 1H), 6.76 (d, J = 3.5 Hz, 2H), 6.73 (d, J = 3.0 Hz, 1H), 3.56 (d, J = 2.3 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 195.94, 148.26, 146.70, 138.41, 135.87, 130.61, 128.40, 123.66, 118.48, 115.64, 115.28, 55.36. HRMS calcd for $C_{14}H_{13}NO_2Cl$, 262.06293 $[M+H]^+$; found; 262.06289.

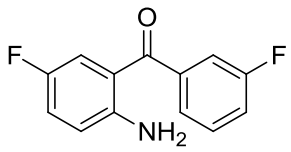


(2-Amino-5-methoxyphenyl)(3-fluorophenyl)methanone (997-48c). Compound **997-48c** was prepared via general procedure C using 2-amino-N,5-dimethoxy-N-methylbenzamide **997-47b** (1.3 g, 6.2 mmol) and 1-bromo-3-fluorobenzene (1.1 g, 6.2 mmol). The title compound was obtained as a brown oil after column chromatography. Significant impurities remained and the material was carried forward without further purification. Yield 1.20 g, 79%. HRMS calcd for $C_{14}H_{13}NO_2F$, 246.09248 $[M+H]^+$; found; 246.09244.

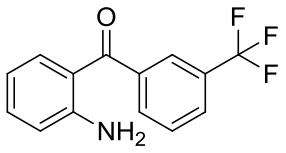


(2-Amino-5-fluorophenyl)(4-chlorophenyl)methanone (997-49c). Compound **997-49c** was prepared via general procedure C using 2-amino-5-fluoro-N-methoxy-N-

methylbenzamide **997-49b** (1.5 g, 7.6 mmol) and 1-bromo-4-chlorobenzene (1.4 g, 7.6 mmol). The title compound was obtained as a yellow solid after column chromatography. Yield 0.534 g, 28%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 - 7.52 (m, 4H), 7.25 (td, *J* = 8.5, 3.0 Hz, 1H), 7.00 (s, 2H), 6.99 - 6.95 (m, 1H), 6.92 - 6.88 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 195.53, 152.33, 150.79, 148.69, 137.90, 136.12, 130.54, 128.50, 122.79, 122.63, 118.58, 118.53, 117.51, 117.36, 115.25. ^{19}F NMR (376 MHz, DMSO-d6) δ 2.55 - 2.41 (m). HRMS calcd for $C_{13}H_{10}NOClF$, 250.04295 $[M+H]^+$; found; 250.04285.



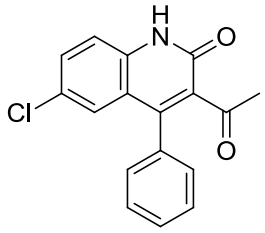
(2-Amino-5-fluorophenyl)(3-fluorophenyl)methanone (997-50c). Compound **997-49c** was prepared via general procedure C using 2-amino-5-fluoro-N-methoxy-N-methylbenzamide **997-49b** (1.5 g, 7.6 mmol) and 1-bromo-3-fluorobenzene (1.3 g, 7.6 mmol). The title compound was obtained as a yellow solid after column chromatography. Yield 0.580 g, 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 - 7.52 (m, 1H), 7.50 - 7.32 (m, 3H), 7.27 (td, *J* = 8.6, 3.1 Hz, 1H), 7.10 (s, 2H), 6.96 (dd, *J* = 9.9, 3.1 Hz, 1H), 6.90 (dd, *J* = 9.2, 4.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 195.28, 148.90, 130.55, 124.63, 123.04, 122.88, 118.59, 118.11, 117.96, 117.56, 117.42, 115.18, 115.03, 39.93, 39.79, 39.65, 39.51, 39.38, 39.24, 39.10. ^{19}F NMR (376 MHz, DMSO-d_6) δ 2.59 - 2.47 (m), -112.76 - -112.88 (m). HRMS calcd for $C_{13}H_{10}NOF_2$, 234.07250 $[M+H]^+$; found; 234.07242.



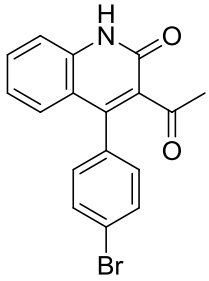
(2-Aminophenyl)(3-(trifluoromethyl)phenyl)methanone (997-51c). Compound **997-51c** was prepared via general procedure C using 2-amino-N-methoxy-N-methylbenzamide **997-23b** (4.81 g, 26.7 mmol) and 1-bromo-3-(trifluoromethyl)benzene (6.00 g, 26.7 mmol). The title compound was obtained as a yellow solid after column chromatography. Yield 2.88 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, J = 7.7 Hz, 1H), 7.86 (d, J = 8.2 Hz, 2H), 7.76 (t, J = 7.5 Hz, 1H), 7.38 - 7.15 (m, 4H), 6.93 (d, J = 8.4 Hz, 1H), 6.59 - 6.46 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.20, 152.28, 140.93, 134.72, 133.74, 132.43, 129.45, 127.26, 124.80, 117.08, 115.64, 114.28. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.91 (s). HRMS calcd for $C_{14}H_{11}NOF_2$, 266.07873 $[M+H]^+$; found; 266.07847.

General procedure for the synthesis of quinolin-2(1H)-one intermediates

(Procedure D). An appropriate 2-aminobenzophenone (1.0 equiv.) and the ethyl acetoacetate (1.5 equiv.) were dissolved in DMF (1.00 M) in an appropriately sized microwaveable vessel and microwaved at 180°C for 8 minutes in the presence of 4 Angstrom sieves. The DMF was removed under vacuum and EtOAc was added, an insoluble material was present, filtered, washed with EtOAc and determined to be the desired product, unless otherwise noted.

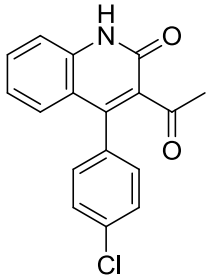


3-Acetyl-6-chloro-4-phenylquinolin-2(1H)-one (997-14d). Compound **997-14d** was prepared via procedure D with (2-amino-5-chlorophenyl) (phenyl)methanone (4.00 g, 17.27 mmol). The reaction was carried out at 210 °C for 8 minutes. Yield 3.30 g, 62%. ^{1}H NMR 400 MHz (DMSO-d_6) δ 12.42 (bs, 1H), 7.64 (dd, J = 2.35, 8.61, 1H), 7.55-7.51 (m, 3H), 7.44 (d, J = 9.0, 1H), 7.35-7.33 (m, 2H), 6.95 (d, J = 2.35, 1H), 2.22 (s, 3H) ^{13}C NMR 100 MHz (DMSO-d_6) δ 201.39, 159.12, 145.87, 137.24, 134.35, 133.50, 131.22, 129.03, 128.72, 126.25, 120.25, 117.76 HRMS calcd for $C_{17}H_{13}ClNO_2$, 298.06293 $[M+H]^+$; found; 298.06263.

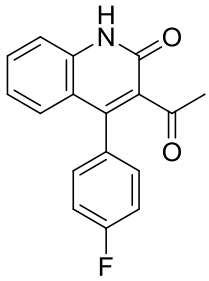


3-Acetyl-4-(4-bromophenyl)quinolin-2(1H)-one (997-7d). Compound **997-7d** was prepared with (2-aminophenyl)(4-bromophenyl)methanone (2.00g, 7.24 mmol). The reaction was carried out at 140 °C for 75 minutes, using THF as solvent. The product was obtained by filtration as a white solid. Yield 1.00 g, 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 8.06 - 6.86 (m, 8H), 2.29 (s, 3H). ^{13}C NMR (100 MHz,

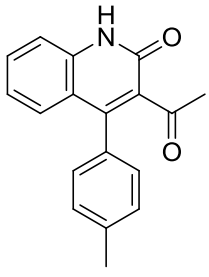
DMSO-d_6) δ 201.79, 159.28, 146.12, 138.51, 133.54, 133.38, 131.51, 130.98, 127.06, 122.52, 122.21, 118.66, 115.72, 31.56. HRMS calcd for $C_{17}H_{13}BrNO_2$, 342.01242 $[M+H]^+$; found; 342.01235.



3-Acetyl-4-(4-chlorophenyl)quinolin-2(1H)-one (997-23d). Compound **997-23d** was prepared with (2-aminophenyl)(4-chlorophenyl)methanone **997-23c** (2.45g, 10.58 mmol). The reaction was carried out using THF as solvent at 160 °C for 30 minutes. Filtration of the title compound from EtOAc yielded the title compound as a white solid. Yield 1.320 g, 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 7.58 (d, J = 7.8 Hz, 3H), 7.48 - 7.33 (m, 3H), 7.16 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.73, 159.26, 146.06, 138.48, 133.53, 133.14, 131.38, 130.70, 128.59, 127.01, 122.47, 118.72, 115.71, 92.91, 31.53. HRMS calcd for $C_{17}H_{13}ClNO_2$, 298.06293 $[M+H]^+$; found; 298.06254.

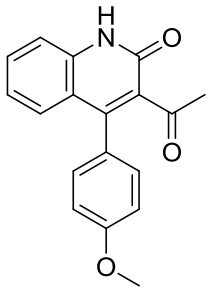


3-Acetyl-4-(4-fluorophenyl)quinolin-2(1H)-one (997-24d). Compound **997-24d** was prepared via procedure D with (2-aminophenyl)(4-fluorophenyl)methanone **997-24c** (3.00 g, 13.95 mmol). The reaction was carried out at 210 °C for 8 minutes. Following removal of the DMF under vacuum, the title compound was obtained via filtration from EtOAc as an off-white solid. Yield 1.60 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.48 - 7.31 (m, 5H), 7.16 (t, J = 7.7 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.80, 159.30, 146.23, 138.48, 133.62, 131.32, 131.12, 131.04, 130.49, 127.03, 122.45, 118.97, 115.702, 115.66, 115.44, 31.51. ^{19}F NMR (400 MHz DMSO-d_6) δ -113.57- -113.610 (m). HRMS calcd for $C_{17}H_{13}FNO_2$, 282.09248 $[M+H]^+$; found; 282.09201.

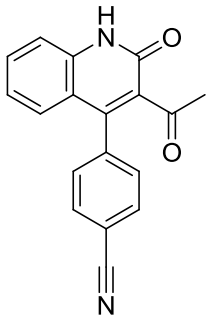


3-Acetyl-4-(p-tolyl)quinolin-2(1H)-one (997-26d). Compound **997-26d** was prepared via procedure D with (2-aminophenyl)(p-tolyl)methanone **997-26c** (2.40g, 11.36 mmol).

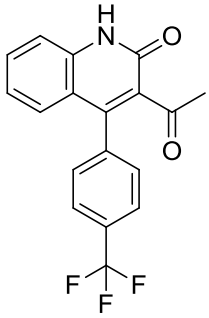
Filtration from EtOAc yielded the title compound as a yellow solid. Yield 1.45 g, 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.61 - 7.52 (m, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 7.7 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H), 7.18 - 7.05 (m, 2H), 2.39 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.39, 159.95, 147.70, 139.09, 138.76, 133.98, 131.88, 131.82, 129.73, 129.33, 127.74, 122.98, 119.67, 116.29, 32.20, 21.54. HRMS calcd for $C_{18}H_{16}NO_2$, 278.11756 $[M+H]^+$; found; 278.11816.



3-Acetyl-4-(4-methoxyphenyl)quinolin-2(1H)-one (997-21d). Compound **997-21d** was prepared via procedure D with (2-aminophenyl)(4-methoxyphenyl)methanone **997-21c** (1.56g, 6.86 mmol). The title compound was obtained after removal of the DMF *in vacuo* and filtration from EtOAc as a white solid. Yield 0.898 g, 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.61 - 7.52 (m, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.25 (d, J = 8.1 Hz, 4H), 7.18 - 7.11 (m, 1H), 7.07 (d, J = 8.1 Hz, 1H), 3.84 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.84, 159.40, 159.31, 146.81, 138.45, 133.45, 131.13, 130.23, 127.09, 126.08, 122.30, 119.19, 115.65, 113.95, 55.18, 31.53. HRMS calcd for $C_{18}H_{16}NO_3$, 294.11247 $[M+H]^+$; found; 294.11207.

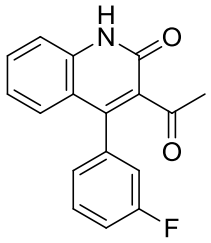


4-(3-Acetyl-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-27d). Compound **997-27d** was prepared via procedure D with 4-(2-aminobenzoyl)benzonitrile **997-27c** (2.50 g, 11.25 mmol). The title compound was obtained by filtration from EtOAc as a yellow solid. Yield 1.15 g, 36%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.37 (s, 1H), 8.00 (d, *J* = 7.9 Hz, 2H), 7.59 (q, *J* = 7.7 Hz, 3H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.60, 159.23, 145.99, 139.47, 138.57, 133.17, 132.41, 131.58, 129.89, 126.98, 122.59, 118.50, 118.32, 115.76, 111.56, 31.51. HRMS calcd for $C_{18}H_{13}N_2O_2$, 289.09715 $[M+H]^+$; found; 289.09670.



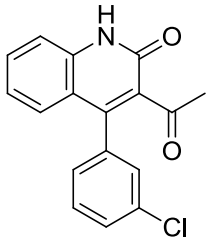
3-Acetyl-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-36d). Compound **997-36d** was prepared via procedure D with (2-aminophenyl)(4-

(trifluoromethyl)phenyl)methanone **997-36c** (1.20 g, 4.52 mmol). The title compound was obtained by filtration from EtOAc as a yellow solid. Yield 0.455 g, 30%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.63 - 7.52 (m, 3H), 7.43 (d, J = 8.2 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.68, 180.73, 159.25, 146.07, 138.74, 138.54, 133.33, 131.54, 129.75, 127.04, 125.42, 125.37, 122.61, 118.49, 115.74, 31.52. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.69 (s). HRMS calcd for $C_{18}H_{13}NO_2F_3$, 332.08929 $[M+H]^+$; found; 332.08990.

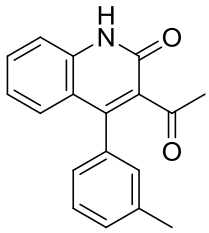


3-Acetyl-4-(3-fluorophenyl)quinolin-2(1H)-one (997-28d). Compound **997-28d** was prepared via procedure D with (2-aminophenyl)(3-fluorophenyl)methanone **997-28c** (3.00 g, 13.94 mmol). The reaction was removed from the microwave reactor, vented, and re-submitted to microwave irradiation for eight minutes at the same temperature. The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 1.60 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.57 (p, J = 7.5 Hz, 2H), 7.43 (d, J = 8.3 Hz, 1H), 7.41 - 7.12 (m, 4H), 7.06 (d, J = 8.2 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.63, 163.07, 159.27, 152.13, 145.83, 138.48, 136.59, 136.51, 134.59, 133.83, 133.34, 131.40, 130.73, 130.65,

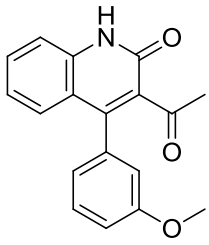
127.01, 125.06, 124.58, 122.51, 118.64, 116.96, 116.01, 115.78, 115.70, 115.49, 114.22, 31.49. HRMS calcd for $C_{17}H_{13}NO_2F_1$, 280.09248 $[M+H]^+$; found; 280.09237.



3-Acetyl-4-(3-chlorophenyl)quinolin-2(1H)-one (997-22d). Compound **997-22d** was prepared via procedure D with (2-aminophenyl)(3-chlorophenyl)methanone (3.00 g, 12.95 mmol). The reaction was removed from the microwave reactor, vented, and re-submitted to microwave irradiation for eight minutes at the same temperature. The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 2.10 g, 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.68 - 7.50 (m, 3H), 7.43 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 6.7 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.64, 159.26, 145.72, 138.48, 136.38, 133.37, 133.18, 131.42, 130.42, 128.69, 128.46, 127.57, 127.00, 122.54, 118.65, 115.70, 31.52. HRMS calcd for $C_{17}H_{13}NO_2Cl$, 298.06293 $[M+H]^+$; found; 298.06273.

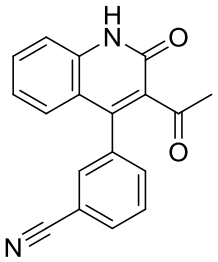


3-Acetyl-4-(m-tolyl)quinolin-2(1H)-one (997-25d). Compound **997-25d** was prepared via procedure D with (2-aminophenyl)(m-tolyl)methanone (3.00 g, 14.20 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 2.13 g, 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.16 - 7.02 (m, 4H), 2.34 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.83, 159.40, 147.20, 138.48, 137.86, 134.22, 133.25, 131.29, 129.43, 129.20, 128.49, 127.20, 125.90, 122.46, 119.01, 115.73, 31.65, 21.04. HRMS calcd for $C_{18}H_{16}NO_2$, 278.11756 $[M+H]^+$; found; 278.11801.

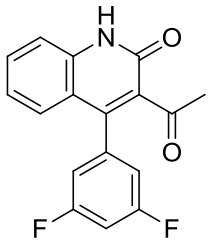


3-Acetyl-4-(3-methoxyphenyl)quinolin-2(1H)-one (997-39d). Compound **997-39d** was prepared via procedure D with (2-aminophenyl)(3-methoxyphenyl)methanone (1.80 g, 7.92 mmol). The DMF was removed *in vacuo* and DCM was added. The organics were washed 3X with brine, concentrated and purified by flash chromatography using a 0-10%

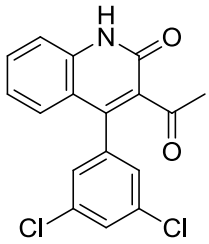
MeOH:DCM gradient. The title compound was obtained as a yellow solid. Yield 1.40 g, 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.41 (t, J = 8.3 Hz, 2H), 7.19 - 7.09 (m, 2H), 7.05 (d, J = 8.3 Hz, 1H), 6.91 - 6.84 (m, 2H), 3.78 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.65, 159.28, 159.05, 146.76, 138.43, 135.53, 133.18, 131.23, 129.74, 127.12, 122.40, 120.99, 118.81, 115.63, 114.48, 114.05, 55.20, 31.51. HRMS calcd for $C_{18}H_{16}NO_3$, 295.11247 $[M+H]^+$; found; 294.11310.



3-(3-Acetyl-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-40d). Compound **997-40d** was prepared via procedure D with 3-(2-aminobenzoyl)benzonitrile (2.40 g, 10.80 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 1.50 g, 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 7.97 (dt, J = 7.3, 1.7 Hz, 1H), 7.89 (d, J = 1.7 Hz, 1H), 7.78 - 7.65 (m, 2H), 7.60 (d, J = 7.1 Hz, 1H), 7.43 (d, J = 8.2 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 6.98 (d, J = 6.9 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.67, 159.25, 145.51, 138.52, 135.67, 133.70, 133.48, 132.52, 132.19, 131.59, 129.76, 127.09, 122.64, 118.59, 118.40, 115.71, 111.66, 31.52. HRMS calcd for $C_{18}H_{13}N_2O$, 289.09715 $[M+H]^+$; found; 289.09786.

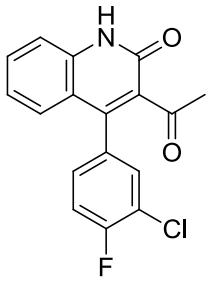


3-Acetyl-4-(3,5-difluorophenyl)quinolin-2(1H)-one (997-35d). Compound **997-35d** was prepared via procedure D with (2-aminophenyl)(3,5-difluorophenyl)methanone (1.00 g, 4.29 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.525 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 7.55 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.36 - 7.31 (m, 1H), 7.18 - 7.09 (m, 3H), 7.03 (d, J = 8.1 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.55, 163.37, 161.04, 160.91, 159.22, 145.13, 138.48, 137.91, 133.22, 131.58, 127.05, 122.65, 118.37, 115.69, 112.65, 112.39, 104.28, 31.45. HRMS calcd for $C_{17}H_{12}NO_2F_2$, 300.08306 $[M+H]^+$; found; 300.08364.

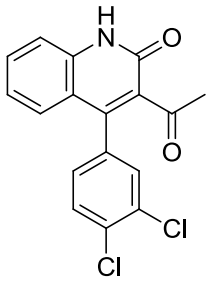


3-Acetyl-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (997-37d). Compound **997-37d** was prepared via procedure D with (2-aminophenyl)(3,5-dichlorophenyl)methanone (1.30 g, 4.88 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.552 g, 34%. ^{1}H NMR (400 MHz,

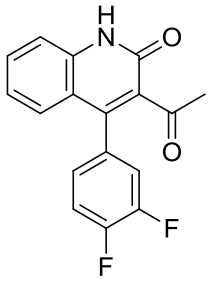
DMSO-d_6) δ 12.34 (s, 1H), 7.77 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.68 (t, *J* = 1.6 Hz, 1H), 7.59 (t, *J* = 8.6 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.35 (dt, *J* = 8.2, 1.7 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.69, 159.23, 145.01, 138.49, 135.03, 133.40, 131.55, 131.32, 130.74, 130.70, 129.25, 127.10, 122.61, 118.54, 115.68, 31.52. HRMS calcd for $C_{17}H_{12}NO_2Cl_2$, 332.02396 $[M+H]^+$; found; 332.02462.



3-Acetyl-4-(3-chloro-4-fluorophenyl)quinolin-2(1H)-one (997-38d). Compound **997-38d** was prepared via procedure D with (2-aminophenyl)(3-chloro-4-fluorophenyl)methanone (0.580 g, 2.32 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.420 g, 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.68 - 7.52 (m, 3H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.40 - 7.35 (m, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.72, 159.24, 158.48, 156.01, 145.13, 138.46, 133.59, 132.02, 131.97, 131.49, 130.94, 129.86, 129.78, 127.09, 122.58, 119.84, 119.67, 118.76, 117.23, 117.02, 115.67, 31.51. ^{19}F NMR HRMS calcd for $C_{17}H_{12}NO_2ClF$, 316.05351 $[M+H]^+$; found; 316.05412.

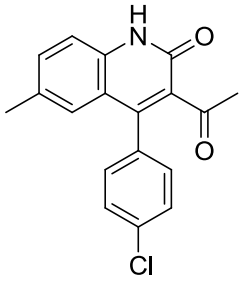


3-Acetyl-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (997-41d). Compound **997-41d** was prepared via procedure D with (2-aminophenyl)(3,4-dichlorophenyl)methanone (1.4 g, 5.26 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.620 g, 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.66 - 7.52 (m, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.35 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.69, 159.23, 145.01, 138.48, 135.04, 133.39, 131.56, 131.32, 130.75, 130.70, 129.26, 127.11, 122.62, 118.54, 115.68, 31.52. HRMS calcd for $C_{17}H_{12}NO_2Cl_2$, 332.02396 $[M+H]^+$; found; 332.02481.

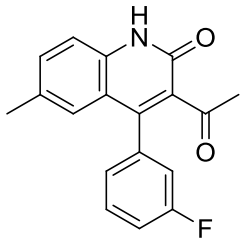


3-Acetyl-4-(3,4-difluorophenyl)quinolin-2(1H)-one (997-42d). Compound **997-42d** was prepared via procedure D with (2-aminophenyl)(3,4-difluorophenyl)methanone (1.80

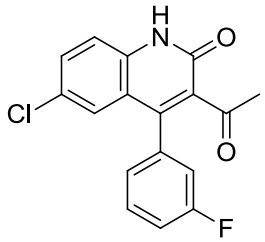
g, 7.72mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as an off-white solid. Yield 0.480 g, 20.9%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.31 (s, 1H), 7.63 - 7.50 (m, 3H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.30 - 7.12 (m, 2H), 7.14 - 7.01 (m, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.69, 159.24, 145.22, 138.45, 133.55, 131.48, 127.09, 126.15, 122.57, 118.71, 118.50, 118.31, 117.77, 115.67, 31.49. ^{19}F NMR (400 MHz, DMSO- d_6) δ HRMS calcd for $C_{17}H_{12}NO_2F_2$, 300.08306 $[M+H]^+$; found; 300.08375.



3-Acetyl-4-(4-chlorophenyl)-6-methylquinolin-2(1H)-one (997-43d). Compound **997-43d** was prepared via procedure D with (2-amino-5-methylphenyl)(4-chlorophenyl)methanone (1.80 g, 7.33 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 1.05 g, 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.46 - 7.39 (m, 1H), 7.38 - 7.31 (m, 3H), 6.83 (s, 1H), 2.27 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.86, 159.11, 145.80, 136.57, 133.46, 133.21, 132.70, 131.48, 130.69, 128.59, 126.26, 118.61, 115.71, 31.54, 20.55. HRMS calcd for $C_{18}H_{15}NO_2Cl$, 312.07858 $[M+H]^+$; found; 312.07868.

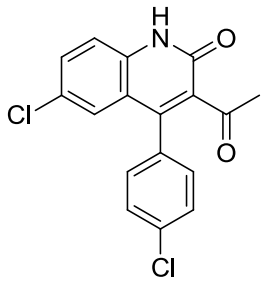


3-Acetyl-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (997-44d). Compound **997-44d** was prepared via procedure D with (2-amino-5-methylphenyl)(3-fluorophenyl)methanone (1.20 g, 5.23 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.676 g, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.56 (q, J = 6.9 Hz, 1H), 7.46 - 7.29 (m, 3H), 7.24 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 4.8 Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.73, 163.08, 160.63, 159.13, 145.57, 136.69, 136.57, 133.37, 132.69, 131.49, 130.71, 130.62, 126.23, 125.06, 118.55, 116.00, 115.78, 115.70, 115.47, 31.50, 20.57. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.06 - -113.18 (m). HRMS calcd for $C_{18}H_{15}NO_2F$, 296.10813 $[M+H]^+$; found; 296.10797.

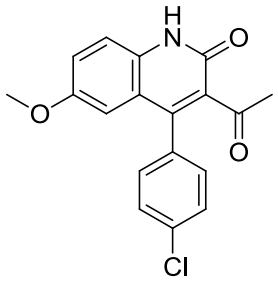


3-Acetyl-6-chloro-4-(3-fluorophenyl)quinolin-2(1H)-one (997-45d). Compound **997-45d** was prepared via procedure D with (2-amino-5-chlorophenyl)(3-fluorophenyl)methanone (1.10 g, 5.23 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.630 g,

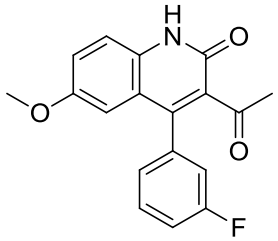
45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H), 7.74 - 7.53 (m, 2H), 7.45 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.36 (tt, *J* = 8.7, 2.4 Hz, 1H), 7.32 - 7.25 (m, 1H), 7.20 (d, *J* = 7.4 Hz, 1H), 6.96 (t, *J* = 2.3 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.29, 163.11, 160.67, 159.06, 144.68, 137.22, 135.81, 135.72, 134.39, 131.34, 130.94, 130.86, 126.38, 125.65, 125.04, 119.93, 117.74, 116.05, 115.85, 115.80, 31.37. ^{19}F NMR (376 MHz, DMSO-d_6) δ -112.77 (td, *J* = 9.2, 5.7 Hz). HRMS calcd for $C_{17}H_{12}NO_2FCl$, 316.05351 $[M+H]^+$; found; 316.05338.



3-Acetyl-6-chloro-4-(4-chlorophenyl)quinolin-2(1H)-one (997-46d). Compound **997-46d** was prepared via procedure D with (2-amino-5-chlorophenyl)(4-chlorophenyl)methanone (1.75 g, 6.58 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.580 g, 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H), 7.68 - 7.63 (m, 1H), 7.62 - 7.58 (m, 2H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.41 - 7.37 (m, 2H), 6.98 - 6.93 (m, 1H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.44, 159.08, 144.94, 137.25, 134.51, 133.86, 132.39, 131.36, 130.71, 128.81, 126.37, 125.67, 120.04, 117.77, 31.43. HRMS calcd for $C_{17}H_{12}NO_2Cl_2$, 332.02396 $[M+H]^+$; found; 332.02385.

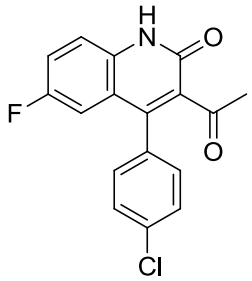


3-Acetyl-4-(4-chlorophenyl)-6-methoxyquinolin-2(1H)-one (997-47d). Compound **997-47d** was prepared via procedure D with (2-amino-5-methoxyphenyl)(4-chlorophenyl)methanone (0.80 g, 3.1 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.387 g, 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 7.58 (d, J = 8.0 Hz, 2H), 7.38 (dt, J = 8.0, 4.5 Hz, 3H), 7.28 (dd, J = 8.9, 2.8 Hz, 1H), 6.44 (d, J = 3.0 Hz, 1H), 3.61 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.92, 158.80, 154.34, 145.45, 133.91, 133.56, 133.12, 133.03, 130.69, 128.67, 120.16, 119.27, 117.12, 108.72, 55.31, 31.53. HRMS calcd for $C_{18}H_{15}NO_3Cl$, 328.07350 $[M+H]^+$; found; 328.07332.

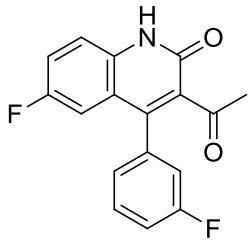


3-Acetyl-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (997-48d). Compound **997-48d** was prepared via procedure D with (2-amino-5-methoxyphenyl)(3-fluorophenyl)methanone (0.90 g, 3.7 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.387 g, 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.57 (q, J = 7.3 Hz, 1H), 7.43 -

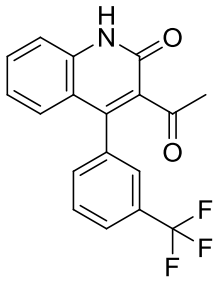
7.22 (m, 4H), 7.18 (d, *J* = 7.7 Hz, 1H), 6.47 - 6.42 (m, 1H), 3.61 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.79, 158.80, 154.33, 145.21, 136.50, 133.78, 133.02, 130.78, 125.04, 120.09, 119.22, 117.10, 115.95, 115.75, 108.80, 55.28, 31.48. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.00 - -113.10 (m). HRMS calcd for $C_{18}H_{15}NO_3F$, 312.10305 $[M+H]^+$; found; 312.10288.



3-Acetyl-4-(4-chlorophenyl)-6-fluoroquinolin-2(1H)-one (997-49d). Compound **997-49d** was prepared via procedure D with (2-amino-5-fluorophenyl)(4-chlorophenyl)methanone (0.450 g, 1.8 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.296 g, 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.56 - 7.43 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 6.74 (dd, *J* = 9.7, 2.8 Hz, 1H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.59, 159.02, 158.33, 155.95, 145.19, 135.26, 134.49, 133.79, 132.57, 130.67, 128.77, 119.79, 119.60, 119.54, 117.83, 111.77, 111.52, 31.44. ^{19}F NMR (376 MHz, DMSO-d_6) δ -120.10 (td, *J* = 8.9, 5.0 Hz). HRMS calcd for $C_{17}H_{12}NO_2ClF$, 316.05351 $[M+H]^+$; found; 316.05334.



3-Acetyl-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (997-50d). Compound **997-50d** was prepared via procedure D with (2-amino-5-fluorophenyl)(3-fluorophenyl)methanone (0.540 g, 2.3 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.306 g, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 7.62 - 7.42 (m, 3H), 7.40 - 7.31 (m, 1H), 7.30 - 7.24 (m, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 6.73 (dd, *J* = 9.7, 2.9 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.10, 159.65, 145.59, 136.62, 136.54, 135.85, 135.03, 131.57, 131.50, 125.66, 120.47, 120.23, 118.36, 116.64, 116.42, 112.42, 32.05. ^{19}F NMR (376 MHz, DMSO-d_6) δ -112.87 (td, *J* = 9.3, 5.8 Hz), -120.07 (td, *J* = 8.9, 5.0 Hz). HRMS calcd for $C_{17}H_{12}NO_2F_2$, 300.08306 $[M+H]^+$; found; 300.08289.

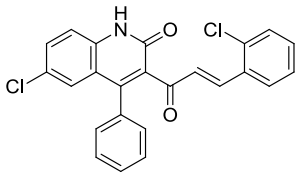


3-Acetyl-4-(3-(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-51d). Compound **997-51d** was prepared via procedure D with (2-aminophenyl)(3-(trifluoromethyl)phenyl)methanone (2.5 g, 9.4 mmol). The title compound was obtained by filtration from EtOAc after removal of DMF *in vacuo* as a yellow solid. Yield 0.847

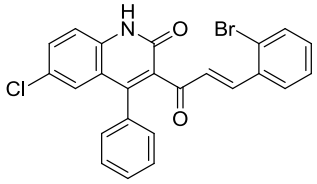
g, 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.80 - 7.71 (m, 2H), 7.68 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.77, 171.99, 159.31, 145.86, 138.58, 135.46, 133.63, 133.02, 131.51, 129.67, 126.96, 125.47, 122.64, 118.70, 115.79, 31.48. ^{19}F NMR (376 MHz, DMSO-d6) δ -61.68 (s). HRMS calcd for $C_{18}H_{13}NO_2F_3$, 332.08929 $[M+H]^+$; found; 332.08907.

General procedure for the synthesis of quinolin-2(1H)-one acrolyl intermediates

(Procedure E). In a round bottom flask, the quinolin-2(1H)-one (1.00 equiv.) and potassium hydroxide (25 equiv.) were stirred in EtOH/H_2O (4:3, 0.05 M) at 0°C for 45 minutes prior to addition of an appropriately substituted benzaldehyde (1 equiv.). The reaction was stirred overnight. The reaction was quenched by slow addition of acetic acid (equimolar to KOH) at which point a solid appeared and was filtered and carried forward unless otherwise noted. **Alternate 1**. In a round bottom flask, the quinolin-2(1H)-one (1.00 equiv.) and potassium hydroxide (50 equiv.) were stirred in EtOH/H2O (4:3, 0.05 M) at 0°C for 45 minutes prior to addition of an appropriately substituted benzaldehyde (1 equiv.). The reaction was stirred for overnight. The reaction was quenched by slow addition of acetic acid (equimolar to KOH) at which point a solid appeared and was filtered and carried forward unless otherwise noted.

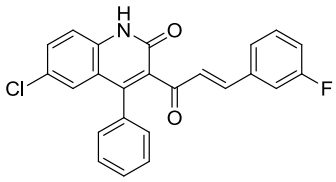


(E)-6-chloro-3-(3-(2-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-14e). Compound **997-14e** was prepared via general procedure E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (1.00g, 3.36 mmol) and 2-chlorobenzaldehyde (0.472 g, 3.36 mmol). The reaction yielded the title compound as a yellow solid. Yield 1.20 g, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.66 - 7.55 (m, 2H), 7.43 (m, 5H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.28 (d, *J* = 7.1 Hz, 2H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.89 (d, *J* = 16.1 Hz, 1H), 5.72 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.32, 159.35, 139.55, 137.56, 134.04, 132.24, 131.64, 131.35, 130.06, 129.65, 128.98, 128.62, 128.38, 127.78, 126.32, 125.71, 120.15, 117.85, 54.93. HRMS calcd for $C_{24}H_{16}Cl_2NO_2$ $[M+H]^+$; 420.05526, found; 420.05475.

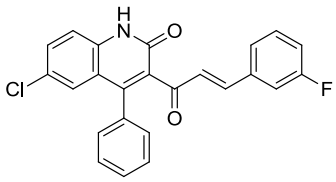


(E)-3-(3-(2-Bromophenyl)acryloyl)-6-chloro-4-phenylquinolin-2(1H)-one (997-15e). Compound **997-15e** was prepared via general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (0.800g, 2.69 mmol) and 2-bromobenzaldehyde (0.497 g, 2.69 mmol). Filtration and drying from DCM using magnesium sulfate yielded the title compound as a yellow solid. Yield 0.900g, 72.1%, yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.82 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.73 - 7.65 (m, 2H), 7.62 (d, *J*

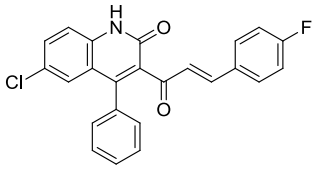
= 16.1 Hz, 1H), 7.55 - 7.33 (m, 8H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.92 (d, *J* = 16.1 Hz, 1H). ^{13}C NMR 100 MHz (DMSO-d_6) δ 193.31, 159.35, 147.24, 142.28, 137.56, 133.32, 133.26, 132.41, 132.36, 131.36, 129.73, 129.02, 128.63, 128.47, 128.31, 126.34, 125.71, 125.15, 120.11, 117.87. HRMS calcd for $C_{24}H_{16}BrClNO_2$ $[M+H]^+$; 464.00474, found; 464.00460.



(E)-6-Chloro-3-(3-(3-fluorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-16e, GluN2D-077). Compound **997-16e** was prepared via general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (0.800 g, 2.69 mmol) and 3-fluorenzaldehyde (0.333g, 0.283 ml, 2.69 mmol). Yield 0.700g, 65%, yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.61 - 7.38 (m, 7H), 7.36 - 7.31 (m, 3H), 7.24 (td, *J* = 8.5, 2.6 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.84 (d, *J* = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.03, 161.14, 159.42, 146.92, 144.88, 137.56, 133.56, 132.33, 131.02, 128.90, 128.59, 128.51, 126.13, 125.61, 125.01, 120.58, 117.74, 114.97. ^{19}F (376 MHz DMSO-d_6) -112.802 (m). HRMS calcd for $C_{24}H_{16}ClFNO_2$ $[M+H]^+$; 404.08481, found; 404.08463.

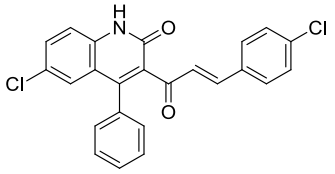


(E)-6-Chloro-3-(3-(3-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-13e, Glun2D-066). Compound **997-13e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (1.00g, 3.36 mmol) and 3-chlorobenzaldehyde (0.472 g, 3.36 mmol). Yield 0.600 g, 43%, yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 7.80 (s, 1H), 7.70 - 7.63 (m, 2H), 7.56 - 7.37 (m, 7H), 7.36 - 7.30 (m, 2H), 6.98 (d, J = 2.5 Hz, 1H), 6.85 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.03, 159.42, 144.65, 137.56, 136.51, 133.72, 133.56, 132.32, 131.01, 130.66, 130.31, 128.89, 128.65, 128.50, 126.93, 125.61, 120.59, 117.73. HRMS calcd for $C_{24}H_{16}Cl_2NO_2$ $[M+H]^+$; 420.05526, found; 420.05589.

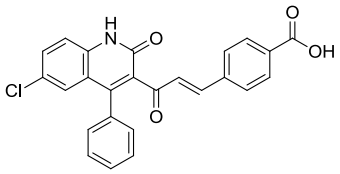


(E)-6-Chloro-3-(3-(4-fluorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-19e). Compound **997-19e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one(0.800 g, 2.69 mmol) and 4-fluorobenzaldehyde (0.333g, 0.283 ml, 2.69 mmol). Filtration from reaction medium followed by drying over magnesium sulfate from DCM yielded the title compound as a yellow solid. Yield 0.705 g, 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 7.75 (dd, J = 8.7, 5.6 Hz, 2H), 7.65 (dd, J = 8.6, 2.5 Hz, 1H), 7.54 - 7.37 (m, 5H), 7.32 (dd, J = 7.5, 1.9 Hz, 2H), 7.22 (t, J = 8.7 Hz, 1H), 6.97 (d, J = 2.4 Hz, 1H), 6.73 (d, J = 16.4 Hz, 1H). ^{13}C NMR 100 MHz (DMSO-d_6)

δ 193.95, 159.45, 146.79, 145.19, 137.55, 133.63, 132.47, 131.13, 131.05, 128.90, 128.49, 127.27, 126.11, 125.61, 120.61, 117.74, 116.09, 115.87. ^{19}F NMR (376 MHz, DMSO-d_6) δ -109.74 - -109.87 (m). HRMS calcd for $C_{24}H_{16}ClFNO_2$ $[M+H]^+$; 404.08481, found; 404.08451.

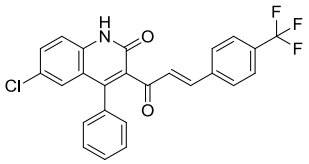


(E)-6-Chloro-3-(3-(4-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-12e). Compound **997-12e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (1.00 g, 3.36 mmol) and 4-chlorobenzaldehyde (0.472, 3.36 mmol). Filtration yielded the title compound as a yellow solid. Yield 0.600 g, 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.6 Hz, 1H), 7.56 - 7.36 (m, 7H), 7.32 (d, J = 7.2 Hz, 2H), 6.97 (s, 1H), 6.78 (d, J =16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.92, 159.42, 146.87, 144.85, 137.55, 135.33, 133.58, 133.22, 132.37, 130.99, 130.36, 128.96, 128.88, 128.49, 127.96, 126.10, 125.60, 120.58, 117.73. HRMS calcd for $C_{24}H_{16}Cl_2NO_2$ $[M+H]^+$; 420.05526, found; 420.05574.



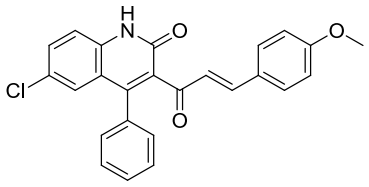
(E)-4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-3-oxoprop-1-enyl)benzoic acid (997-5e). Compound **997-5e** was prepared via the general method E

using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one(0.90 g, 3.02mmol) and 4-formylbenzoic acid (0.454, 3.02 mmol). The reaction was quenched by slow addition of 1N HCl until the pH had reached 2. The resultant solid was filtered, dissolved into hot EtOAc and washed 3X with brine. The organics were dried over magnesium sulfate, and then adhered to silica under vacuum. The title compound was obtained after column chromatography using 2-10% MeOH:DCM as a white solid. Yield 0.470g, 36%. ^{1}H NMR 400 MHz (DMSO-d_6) δ 13.14 (bs, 1H), 12.39 (bs, 1H), 7.92 (d, *J* = 8.22, 2H), 7.86 (d, *J* = 8.22, 2H), 7.17 (dd, *J* = 10.96, 1.96, 1H), 7.58 (d, *J* = 16.43, 1H), 7.49-7.40 (m, 4H), 7.34 (d, *J* = 7.24, 2H), 6.99 (d, *J* = 2.35, 1H), 6.87 (d, *J* = 16.43, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.55, 168.72, 160.10, 152.43, 149.99, 146.89, 137.33, 134.57, 131.26, 129.55, 128.49, 128.39, 126.13, 126.08, 125.53, 124.54, 120.68, 117.66. HRMS calcd for $C_{25}H_{17}ClNO_4$ $[M+H]^+$; 430.08406, found; 430.08382.

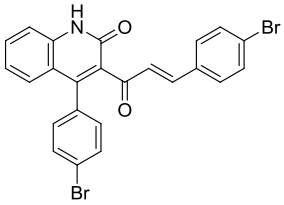


(E)-6-Chloro-4-phenyl-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)quinolin-2(1H)-one (997-08e). Compound **997-8e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (1.00 g, 3.36 mmol) and 4-(trifluoromethyl)benzaldehyde (0.585, 0.449 ml 3.36 mmol). The title compound was obtained after dissolving the filtrate in DCM, drying over magnesium sulfate, and removal of the DCM *in vacuo* as a yellow solid. Yield 0.900 g, 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.71 – 7.65 (m, 1H), 7.60 (d, *J* = 16.5 Hz, 1H), 7.45 (dt, *J* = 13.8, 7.9 Hz, 4H), 7.34 (d, *J* = 6.8

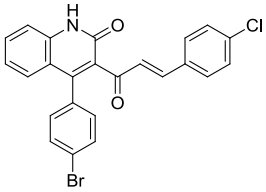
Hz, 2H), 7.02 – 6.96 (m, 1H), 6.91 (d, *J* = 16.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 194.02, 159.45, 144.18, 138.31, 137.61, 133.56, 132.23, 131.08, 129.66, 129.24, 128.92, 128.70, 128.52, 126.16, 125.71, 120.57, 117.77, 38.88. HRMS calcd for $C_{25}H_{16}ClF_3NO_2$ $[M+H]^+$; 454.08162, found; 454.08114.



(E)-6-Chloro-3-(3-(4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-17e,). Compound **997-17e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (0.800 g, 2.69 mmol) and 4-mehtoxybenzaldehyde (0.366, 0.307 ml 2.69 mmol). The title compound was obtained after dissolving the filtrate in DCM, drying over magnesium sulfate, and removal of the DCM *in vacuo* as a yellow solid. Yield 0.721g, 65%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.36 (s, 1H), 7.69 - 7.59 (m, 3H), 7.54 - 7.36 (m, 4H), 7.33 (dd, *J* = 7.4, 1.9 Hz, 2H), 7.03 - 6.90 (m, 3H), 6.62 (dd, *J* = 16.4, 2.4 Hz, 1H), 5.76 (d, *J* = 2.6 Hz, 1H), 3.79 (d, *J* = 2.4 Hz, 3H). ^{13}C NMR 100 MHz (DMSO-*d*$_6$) 193.731, 161.506, 159.482, 146.506, 137.502, 133.715, 132.696, 130.873, 130.620, 128.909, 128.447, 126.810, 126.066, 125.575, 125.166, 120.642, 117.703, 114.414, 55.366, 54.942. HRMS calcd for $C_{25}H_{19}ClNO_3$ $[M+H]^+$; 416.10480, found; 416.10567.

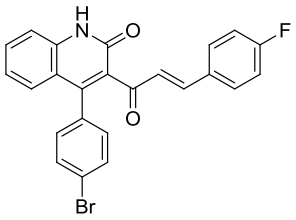


(E)-4-(4-Bromophenyl)-3-(3-(4-bromophenyl)acryloyl)quinolin-2(1H)-one (997-7e). Compound **997-7e** was prepared via the general method E, alternate 1, using 3-acetyl-4-(4-bromophenyl)quinolin-2(1H)-one (0.900g, 2.63 mmol) and 4-bromobenzaldehyde (0.487g, 2.63 mmol). The product was obtained after filtration as a white solid. Yield 0.700 g, 52.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.68 - 7.55 (m, 7H), 7.53 - 7.44 (m, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 16.4 Hz, 1H). ^{13}C NMR 100 MHz (DMSO-d_6) δ 194.23, 159.49, 146.99, 144.65, 138.79, 133.58, 133.53, 131.88, 131.46, 131.34, 131.22, 131.11, 130.60, 128.18, 126.84, 124.24, 122.35, 122.13, 118.94, 115.74. HRMS calcd for $C_{24}H_{16}Br_2NO_3$ $[M+H]^+$; 507.95423, found; 507.95457.

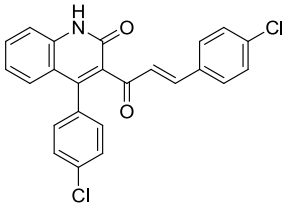


(E)-4-(4-Bromophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (997-20e). Compound **997-20e** was prepared via the general method E, using 3-acetyl-4-(4-bromophenyl)quinolin-2(1H)-one (3.500 g, 10.23 mmol) and 4-chlorobenzaldehyde (1.44g, 10.23 mmol). The product was obtained after filtration as a white solid. Yield 4.24 g, 89%. ^{1}H NMR (600 MHz, DMSO-d_6)) δ 7.72 - 7.64 (m, 2H), 7.61 (dd, J = 8.2, 2.7 Hz, 2H), 7.55 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 10.1 Hz, 1H), 7.47 - 7.40 (m, 3H), 7.25

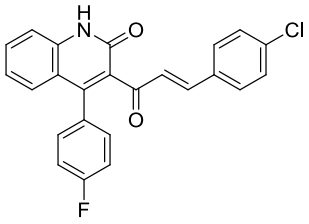
(td, *J* = 7.9, 2.7 Hz, 2H), 7.12 (td, *J* = 8.6, 4.2 Hz, 1H), 7.06 - 6.98 (m, 1H), 6.81 (dd, *J* = 16.4, 2.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6)) δ 195.04, 160.41, 153.58, 153.52, 147.47, 144.98, 139.92, 135.94, 134.33, 133.95, 132.14, 132.03, 131.94, 131.81, 131.10, 129.63, 128.84, 127.40, 122.73, 119.60, 116.75. HRMS calcd for $C_{24}H_{16}ClBrNO_2$ $[M+H]^+$; 464.00474, found; 464.00592.



(E)-4-(4-Bromophenyl)-3-(3-(4-fluorophenyl)acryloyl)quinolin-2(1H)-one (997-29e). Compound **997-29e** was prepared via the general method E, alternate 1, using 3-acetyl-4-(4-bromophenyl)quinolin-2(1H)-one (0.800g, 2.34 mmol) and 4-fluorbenzaldehyde (0.290g, 0.247 ml, 2.63 mmol). Filtration from the neutralized reaction medium yielded the title compound as a yellow solid. Yield 0.930 g, 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (dd, *J* = 8.5, 5.4 Hz, 3H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.34 - 7.15 (m, 6H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.87 - 6.74 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.29, 165.68, 164.31, 161.83, 148.80, 143.16, 140.84, 135.80, 131.50, 131.42, 130.88, 130.65, 130.59, 130.51, 128.72, 128.54, 125.62, 122.21, 120.83, 119.25, 118.11, 115.97, 115.75. ^{19}F NMR (376 MHz, DMSO-d_6) δ -111.00. HRMS calcd for $C_{24}H_{16}BrFNO_2$ $[M+H]^+$; 448.03429, found; 448.03547.

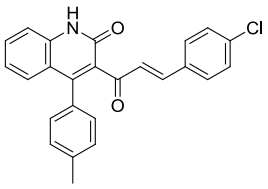


(E)-4-(4-Chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (997-23e). Compound **997-23e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)quinolin-2(1H)-one (0.800g, 2.69 mmol) and 4-chlorobenzaldehyde (0.378g, 2.69 mmol). The title compound was obtained after filtration from the reaction medium followed by extraction from DCM and a 3X wash with brine, drying over magnesium sulfate and concentration *in vacuo* as a yellow solid. Yield 0.760 g, 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (d, J = 12.7 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.65-7.55 (m, 2H), 7.55 - 7.43 (m, 5H), 7.41-7.32 (m, 2H), 7.22-7.02 (m, 2H), 6.84 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.23, 159.51, 146.98, 144.58, 138.79, 135.32, 133.45, 133.27, 133.16, 131.56, 131.24, 130.88, 130.71, 130.41, 128.96, 128.60, 128.45, 128.17, 128.00, 126.87, 122.37, 119.03, 115.73. HRMS calcd for $C_{24}H_{14}Cl_2NO_2$ $[M+H-H_2]^+$; 418.03961, found; 418.04002.



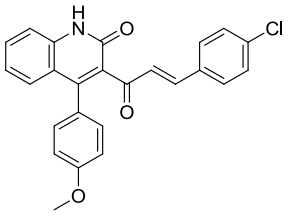
(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(4-fluorophenyl)quinolin-2(1H)-one (997-24e). Compound **997-24e** was prepared via the general method E using 3-acetyl-4-(4-fluorophenyl)quinolin-2(1H)-one (0.800g, 2.84 mmol) and 4-chlorobenzaldehyde

(0.400g, 2.84 mmol). The title compound was obtained after filtration from the reaction medium followed by extraction from DCM and a 3X wash with brine, drying over magnesium sulfate and concentration *in vacuo* as a yellow solid. Yield 0.950 g, 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.73 (d, *J* = 8.2 Hz, 2H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.53 (s, 1H), 7.52 - 7.44 (m, 3H), 7.38 (dd, *J* = 8.3, 5.1 Hz, 2H), 7.28 (t, *J* = 8.6 Hz, 2H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.83 (d, *J* = 16.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.32, 160.81, 159.55, 147.19, 144.50, 138.76, 135.29, 133.28, 131.65, 131.26, 131.18, 130.57, 130.37, 128.94, 128.14, 126.88, 122.32, 119.26, 115.70, 115.49, 115.27. ^{19}F NMR (400 MHz, DMSO-d_6) δ (-113.75 - -113.78) (m). HRMS calcd for $C_{24}H_{16}ClFNO_2$ $[M+H]^+$; 404.08481, found; 404.08515.

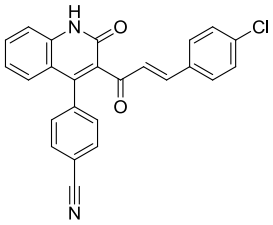


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-p-tolylquinolin-2(1H)-one (997-26e). Compound **997-26e** was prepared via the general method E using 3-acetyl-4-p-tolylquinolin-2(1H)-one (0.800g, 2.88 mmol) and 4-chlorobenzaldehyde (0.406 g, 2.88 mmol). The title compound was obtained after filtration from the reaction medium as a white solid. Yield 1.00 g, 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 2H), 7.47 - 7.40 (m, 2H), 7.20 (q, *J* = 7.3 Hz, 4H), 7.15 - 7.07 (m, 2H), 6.80 (d, *J* = 16.4 Hz, 1H), 2.30 (d, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.45, 159.73, 148.04, 144.13, 138.96, 137.86, 135.19, 133.30, 131.30, 130.94, 130.33, 128.93, 128.86, 128.29, 126.93, 122.07, 119.35, 115.84, 20.82. HRMS calcd for $C_{25}H_{18}ClFNO_2K$ $[M+K]^+$; 438.06577, found;

438.06555.

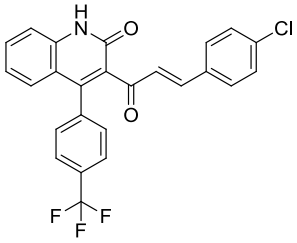


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(4-methoxyphenyl)quinolin-2(1H)-one (997-21e). Compound **997-21e** was prepared via the general method E using 3-acetyl-4-(4-methoxyphenyl)quinolin-2(1H)-one (0.800 g, 2.73 mmol) and 4-chlorobenzaldehyde (0.383 g, 2.73 mmol). The title compound was obtained by filtration of the neutralized reaction medium as a white solid. Yield 0.950 g, 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.57 (dt, *J* = 8.4, 4.2 Hz, 1H), 7.53 - 7.40 (m, 3H), 7.24 (d, *J* = 8.7 Hz, 2H), 7.15 (d, *J* = 4.2 Hz, 3H), 7.01 - 6.92 (m, 2H), 6.80 (d, *J* = 16.4 Hz, 1H), 3.75 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.61, 159.80, 159.20, 147.80, 144.06, 139.05, 135.18, 133.34, 131.39, 130.89, 130.38, 130.33, 128.93, 128.30, 126.93, 126.31, 122.03, 119.51, 115.91, 113.73, 55.10. HRMS calcd for $C_{25}H_{19}ClNO_3$ $[M+H]^+$; 416.10480, found; 416.10517.



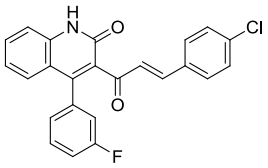
(E)-4-(3-(3-(4-Chlorophenyl)acryloyl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-27e). Compound **997-27e** was prepared via the general method E using 4-(3-acetyl-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (0.800 g, 2.77 mmol) and 4-

chlorobenzaldehyde (0.390 g, 2.77 mmol). The title compound was obtained by filtration of the neutralized reaction medium as a yellow solid. Yield 0.620 g, 54%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.28 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.76 - 7.70 (m, 3H), 7.66 - 7.39 (m, 7H), 7.25 - 7.11 (m, 1H), 7.10 - 6.93 (m, 1H), 6.86 (d, J = 16.3 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 193.93, 159.39, 146.66, 139.34, 138.82, 135.36, 133.22, 132.29, 131.48, 130.35, 130.09, 130.02, 128.95, 128.15, 126.80, 122.48, 118.41, 115.77, 111.52, 98.78. HRMS calcd for $C_{25}H_{16}ClN_2O_2$ $[M+H]^+$; 411.08948, found; 411.09043.

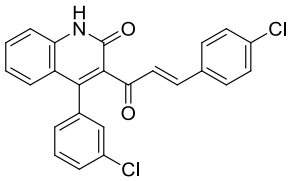


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-36e). Compound **997-36e** was prepared via the general method E using 3-acetyl-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.375 g, 1.13 mmol) and 4-chlorobenzaldehyde (0.159 g, 1.13 mmol). The title compound was obtained by filtration of the neutralized reaction medium as a white solid. Yield 0.448 g, 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 7.65 - 7.54 (m, 3H), 7.51 (d, J = 5.4 Hz, 1H), 7.47 (t, J = 7.4 Hz, 3H), 7.16 (t, J = 7.7 Hz, 1H), 7.00 (d, J = 8.0 Hz, 1H), 6.86 (d, J =16.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.17, 159.62, 146.75, 144.50, 139.13, 138.75, 135.31, 133.28, 131.58, 131.26, 130.42, 129.93, 128.95, 128.20, 126.77, 125.40, 125.28, 122.33, 118.80, 116.01.

^{19}F NMR (376 MHz, DMSO-d_6) δ -61.69 (s). HRMS calcd for $C_{25}H_{16}Cl\ F_3NO_2$ $[M+H]^+$; 454.08162, found; 454.08184.

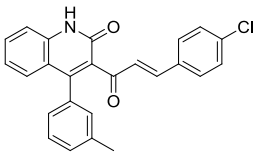


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-fluorophenyl)quinolin-2(1H)-one (997-28e). Compound **997-28e** was prepared via the general method E using 3-acetyl-4-(3-fluorophenyl)quinolin-2(1H)-one (0.800 g, 2.84 mmol), and 4-chlorbenzaldehyde (0.400 g, 2.84 g). The title compound was obtained by filtration of the neutralized reaction medium as a yellow solid. Yield 0.800 g, 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.72 (d, J = 8.2 Hz, 2H), 7.64 - 7.41 (m, 6H), 7.39 - 7.06 (m, 5H), 6.83 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.09, 162.86, 160.42, 159.49, 146.68, 144.60, 138.77, 136.59, 136.51, 135.32, 133.25, 131.43, 131.24, 130.58, 130.50, 130.35, 128.95, 128.06, 126.84, 125.26, 122.39, 118.90, 116.20, 115.97, 115.72, 115.63, 115.43, 54.92. ^{19}F NMR (376 MHz DMSO-d_6) δ -113.290 - -113.355 (m). HRMS calcd for $C_{24}H_{16}ClFNO_2$ $[M+H]^+$; 404.08481, found; 404.08510.



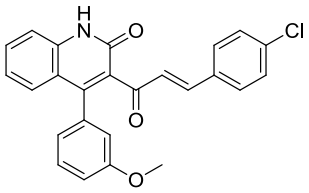
(E)-4-(3-Chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (997-22e). Compound **997-28e** was prepared via the general method E using 3-acetyl-4-(3-

chlorophenyl)quinolin-2(1H)-one (0.800g, 2.69 mmol) and 4-chlorobenzaldehyde (0.378 g, 2.69 mmol). The title compound was obtained after filtration from the reaction medium followed by extraction from DCM and a 3X wash with brine, drying over magnesium sulfate and concentration *in vacuo* as a yellow solid. Yield 0.670 g, 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.52 (d, J = 16.4 Hz, 1H), 7.49 - 7.41 (m, 6H), 7.33 - 7.23 (m, 1H), 7.19 (t, J = 7.7 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.14, 159.51, 146.56, 144.77, 138.80, 136.39, 135.35, 133.29, 132.99, 131.51, 131.31, 130.40, 130.32, 128.99, 128.79, 128.65, 128.10, 127.75, 126.85, 122.47, 118.95, 115.77. HRMS calcd for $C_{24}H_{16}Cl_{2}NO_2$ $[M+H]^+$; 417.05560, found; 417.05599.

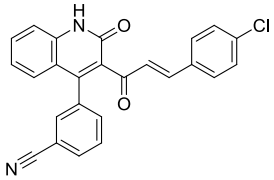


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-m-tolylquinolin-2(1H)-one (997-25e). Compound **997-25e** was prepared via the general method E using 3-acetyl-4-m-tolylquinolin-2(1H)-one (0.800g, 2.88 mmol) and 4-chlorobenzaldehyde (0.406 g, 2.88 mmol). The title compound was obtained after filtration from the neutralized reaction medium. Yield 0.790 g, 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.57 (t, J = 7.6 Hz, 1H), 7.51 - 7.41 (m, 4H), 7.28 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.15 - 7.04 (m, 4H), 6.79 (d, J = 16.4 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.25, 159.64, 148.10, 144.22, 138.81, 137.41, 135.19, 134.27, 133.31, 131.16, 131.04, 130.28, 129.49, 128.93, 128.19, 127.02, 125.99, 122.19, 119.23, 115.69, 20.94. HRMS calcd for $C_{25}H_{18}ClNO_2K$ $[M+ K]^+$; 438.06577, found;

438.06566.

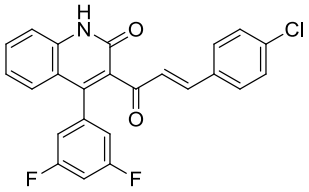


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-methoxyphenyl)quinolin-2(1H)-one (997-39e). Compound **997-39e** was prepared via the general method E using 3-acetyl-4-(3-methoxyphenyl)quinolin-2(1H)-one (0.500 g, 1.71 mmol) and 4-chlorobenzaldehyde (0.240 g, 1.71 mmol). The title compound was obtained after filtration from the neutralized reaction medium as a yellow solid. Yield 0.640 g, 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 7.73 (dd, J = 8.4, 1.4 Hz, 2H), 7.60 - 7.54 (m, 1H), 7.54 - 7.49 (m, 2H), 7.45 (dd, J = 8.6, 1.4 Hz, 2H), 7.37 - 7.28 (m, 1H), 7.21 - 7.10 (m, 2H), 6.94 (dd, J = 8.3, 2.5 Hz, 1H), 6.90 (d, J = 2.2 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.81 (d, J = 16.5, 1H), 3.66 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.49, 173.28, 159.84, 158.80, 147.58, 144.24, 139.27, 135.71, 135.19, 133.34, 131.14, 130.92, 130.32, 129.49, 128.95, 128.29, 126.88, 122.03, 121.24, 119.09, 116.07, 114.64, 114.03, 55.04, 25.58. HRMS calcd for $C_{25}H_{18}Cl\ NO_3K$ $[M+K]^+$; 454.06068, found; 454.06177.

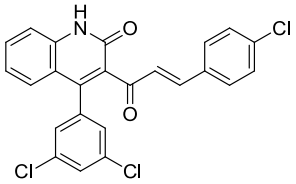


(E)-3-(3-(3-(4-Chlorophenyl)acryloyl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-40e). Compound **997-40e** was prepared via the general method E using 3-(3-acetyl-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (0.500 g, 1.73 mmol) and 4-

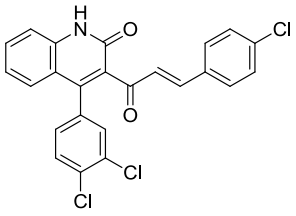
chlorobenzaldehyde (0.244 g, 1.73 mmol). The title compound was obtained after filtration from the neutralized reaction medium as a yellow solid. Yield 0.310 g, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 - 7.86 (m, 1H), 7.85 (s, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 6.9 Hz, 2H), 7.64 - 7.57 (m, 1H), 7.57 - 7.51 (m, 2H), 7.50 - 7.41 (m, 2H), 7.16 (t, J = 7.6 Hz, 1H), 7.08 - 6.89 (m, 1H), 6.84 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.19, 159.75, 145.95, 144.44, 135.77, 135.34, 133.85, 133.25, 132.56, 132.43, 131.24, 130.38, 129.70, 128.98, 128.14, 126.73, 122.26, 118.35, 116.26, 111.42, 71.87, 25.29. HRMS calcd for $C_{25}H_{14}ClN_2O_2$ $[M+H-H_2]^+$; 409.07383, found; 409.07499.



(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3,5-difluorophenyl)quinolin-2(1H)-one (997-35e). Compound **997-35e** was prepared via the general method E using 3-acetyl-4-(3,5-difluorophenyl)quinolin-2(1H)-one (0.375 g, 1.25 mmol) and 4-chlorobenzaldehyde (0.176 g, 1.25 mmol). The title compound was obtained after filtration from the neutralized reaction medium as a yellow solid. Yield 0.425 g, 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 7.74 (d, J = 8.2 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 16.4 Hz, 1H), 7.47 (d, J = 8.1 Hz, 3H), 7.32 (t, J = 9.5 Hz, 1H), 7.24 - 7.10 (m, 4H), 6.87 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.93, 159.44, 144.73, 138.89, 137.86, 135.37, 133.24, 131.48, 131.38, 130.38, 129.00, 127.99, 126.79, 122.48, 118.58, 115.85, 112.87. HRMS calcd for $C_{24}H_{15}ClNO_2F_2$ $[M+H]^+$; 422.07539, found; 422.07647.

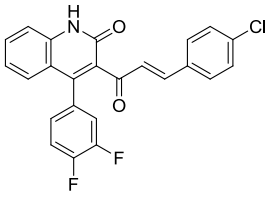


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (997-37e). Compound **997-37e** was prepared via the general method E using 3-acetyl-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (0.375 g, 1.13 mmol) and 4-chlorobenzaldehyde (0.159 g, 1.13 mmol). The title compound was obtained after filtration from the neutralized reaction medium as a yellow solid. Yield 0.375 g, 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H), 7.79 - 7.72 (m, 3H), 7.72 - 7.65 (m, 1H), 7.66 - 7.56 (m, 1H), 7.54 (d, J = 16.3 Hz, 1H), 7.51 - 7.44 (m, 3H), 7.33 (dt, J = 8.3, 1.6 Hz, 1H), 7.23 - 7.02 (m, 2H), 6.87 (d, J = 16.4, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.75, 160.18, 146.29, 139.66, 136.01, 135.69, 133.93, 132.33, 131.97, 131.33, 131.06, 130.04, 129.63, 128.78, 127.50, 123.06, 116.56. HRMS calcd for $C_{24}H_{15}Cl_3NO_2$ $[M+H]^+$; 454.01629, found; 454.01727.

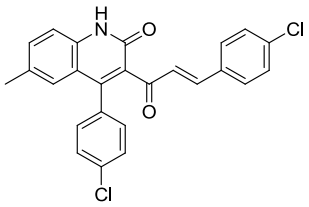


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (997-41e). Compound **997-41e** was prepared via the general method E using 3-acetyl-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (0.520 g, 1.57 mmol) and 4-chlorobenzaldehyde (0.220 g, 1.57 mmol). The title compound was obtained via filtration after the reaction was quenched, followed by addition of 1N HCl until the pH reached 4 as a yellow solid.

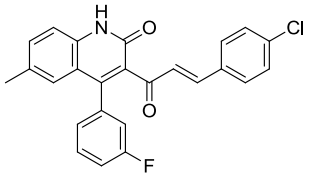
Yield 0.580 g, 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.73 (t, J = 9.4 Hz, 3H), 7.67 (s, 1H), 7.62 (t, J = 7.7 Hz, 1H), 7.56 (d, J = 16.4 Hz, 1H), 7.52 - 7.44 (m, 3H), 7.33 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.7 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 6.88 (d, J = 16.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.01, 159.40, 145.70, 144.79, 138.77, 135.37, 134.97, 133.24, 131.68, 131.53, 131.37, 131.15, 131.01, 130.68, 130.40, 129.36, 128.96, 128.08, 126.86, 122.49, 118.78, 115.74. HRMS calcd for $C_{24}H_{15}Cl_3NO_2$ $[M+H]^+$; found;



(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3,4-difluorophenyl)quinolin-2(1H)-one (997-41e). Compound **997-41e** was prepared via the general method E using 3-acetyl-4-(3,4-difluorophenyl)quinolin-2(1H)-one (0.360 g, 1.20 mmol) and 4-chlorobenzaldehyde (0.169 g, 1.20 mmol). The title compound was obtained after filtration from the neutralized reaction medium as a yellow solid. Yield 0.420 g, 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.67 - 7.43 (m, 7H), 7.32 - 7.03 (m, 3H), 6.85 (d, J = 16.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.16, 159.53, 145.94, 144.59, 138.94, 135.33, 133.26, 131.74, 131.25, 130.38, 128.98, 128.09, 126.83, 126.32, 122.36, 118.95, 118.68, 118.50, 117.85, 117.68, 115.88. ^{19}F NMR (376 MHz, DMSO-d6) δ -5.88 - -6.17 (m). HRMS calcd for $C_{24}H_{15}ClF_2NO_2$ $[M+H]^+$; 422.07539, found; 422.07588.

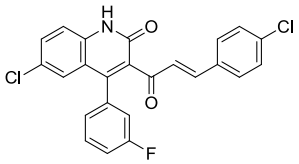


(E)-4-(4-Chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-methylquinolin-2(1H)-one (997-43e). Compound **997-43e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)-6-methylquinolin-2(1H)-one (0.350 g, 1.12 mmol) and 4-chlorobenzaldehyde (0.158 g, 1.12 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.320 g, 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H) 7.82 - 7.67 (m, 2H), 7.64 - 7.27 (m, 9H), 6.89 - 6.77 (m, 2H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.61, 159.90, 146.38, 143.92, 137.83, 135.20, 133.46, 133.31, 133.25, 132.29, 131.52, 130.91, 130.36, 128.95, 128.53, 128.40, 128.31, 125.95, 118.90, 116.44, 26.01. HRMS calcd for $C_{25}H_{18}Cl_2NO_2$ $[M+H]^+$; 434.07091, found; 434.07161.

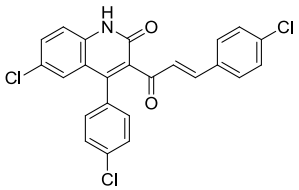


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (997-44e). Compound **997-44e** was prepared via the general method E using 3-acetyl-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (0.40 g, 1.36 mmol) and 4-chlorobenzaldehyde (0.190 g, 1.36 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.320 g, 65.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.67 (s, 1H), 7.47 - 7.25 (m, 8H), 7.23 - 6.98 (m, 4H), 6.80

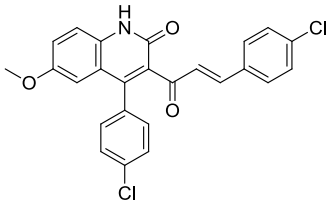
(d, *J* = 16.3 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.60, 161.74, 149.16, 144.04, 136.83, 136.71, 133.44, 133.10, 133.04, 130.40, 129.81, 129.37, 127.95, 126.90, 125.17, 119.58, 116.85, 116.43, 76.90, 21.32. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.29 - -113.41 (m). HRMS calcd for $C_{25}H_{18}ClFNO_2$ $[M+H]^+$; 418.10046, found; 418.10027.



(E)-6-Chloro-3-(3-(4-chlorophenyl)acryloyl)-4-(3-fluorophenyl)quinolin-2(1H)-one (997-45e). Compound **997-44e** was prepared via the general method E using 3-acetyl-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (0.40 g, 1.36 mmol) and 4-chlorobenzaldehyde (0.190 g, 1.36 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield quantitative. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (dd, *J* = 8.5, 1.7 Hz, 2H), 7.64 - 7.54 (m, 2H), 7.54 - 7.41 (m, 4H), 7.30 - 7.20 (m, 2H), 7.16 (d, *J* = 7.6 Hz, 1H), 6.97 (t, *J* = 2.1 Hz, 1H), 6.83 (d, *J* = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.24, 162.88, 160.44, 160.17, 144.97, 144.48, 139.02, 136.19, 135.29, 133.25, 132.42, 130.73, 130.63, 130.34, 128.97, 128.03, 125.57, 125.29, 125.20, 120.19, 118.84, 116.00. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.07 (td, *J* = 9.3, 5.9 Hz). HRMS calcd for $C_{24}H_{15}Cl_2FNO_2$ $[M+H]^+$; 438.04584, found; 438.04648.

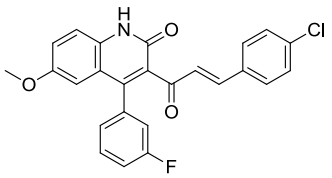


(E)-6-Chloro-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (997-46e). Compound **997-46e** was prepared via the general method E using 3-acetyl-6-chloro-4-(4-chlorophenyl)quinolin-2(1H)-one (0.350 g, 1.05 mmol) and 4-chlorobenzaldehyde (0.148 g, 1.05 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield quantitative. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (dd, J = 8.5, 1.8 Hz, 2H), 7.65 - 7.60 (m, 1H), 7.56 - 7.43 (m, 6H), 7.41 - 7.29 (m, 2H), 6.96 (t, J = 2.1 Hz, 1H), 6.83 (d, J = 16.5, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.25, 160.02, 145.36, 144.53, 138.75, 135.30, 133.59, 133.25, 132.71, 132.55, 130.88, 130.79, 130.37, 128.95, 128.58, 128.07, 125.66, 125.25, 120.32, 118.63. HRMS calcd for $C_{24}H_{15}Cl_3NO_2$ $[M+H]^+$; 454.01629, found; 454.01755.

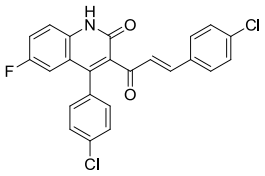


(E)-4-(4-Chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-methoxyquinolin-2(1H)-one (997-47e). Compound **997-47e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)-6-methoxyquinolin-2(1H)-one (0.300 g, 0.92 mmol) and 4-chlorobenzaldehyde (0.129 g, 0.92 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.35 g, 85%. ^{1}H NMR

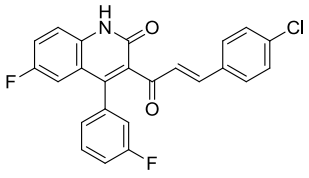
(400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 7.72 (dd, J = 8.5, 1.4 Hz, 2H), 7.61 - 7.19 (m, 9H), 6.83 (d, J = 16.3 Hz, 1H), 6.50 - 6.42 (m, 1H), 3.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.35, 159.10, 154.29, 146.34, 144.33, 133.46, 133.26, 133.18, 132.03, 130.85, 130.40, 128.96, 128.51, 128.16, 119.94, 119.52, 117.25, 108.62, 55.38. HRMS calcd for $C_{25}H_{18}Cl_2NO_3$ $[M+H]^+$; 450.06583, found; 450.06603.



(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (997-48e). Compound **997-48e** was prepared via the general method E using 3-acetyl-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (0.300 g, 0.96 mmol) and 4-chlorobenzaldehyde (0.135 g, 0.96 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.296 g, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.61 - 7.37 (m, 5H), 7.39 - 7.11 (m, 4H), 6.83 (d, J = 16.4 Hz, 1H), 6.49 (d, J = 2.8 Hz, 1H), 3.62 (s, 3H). ^{13}C NMR (100 MHz, C) δ 194.22, 159.10, 154.29, 146.05, 144.38, 136.61, 136.53, 135.28, 133.45, 133.25, 131.90, 130.64, 130.35, 128.97, 128.06, 125.23, 119.87, 119.43, 117.21, 115.90, 115.50, 108.70, 55.36. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.13 - -113.26 (m). HRMS calcd for $C_{25}H_{18}ClFNO_3$ $[M+H]^+$; 434.09538, found; 434.09533.

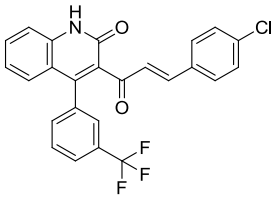


(E)-4-(4-Chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-fluoroquinolin-2(1H)-one (997-49e). Compound **997-49e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)-6-fluoroquinolin-2(1H)-one (0.270 g, 0.86 mmol) and 4-chlorobenzaldehyde (0.120 g, 0.86 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.294 g, 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, J = 8.6 Hz, 3H), 7.54 - 7.43 (m, 7H), 7.34 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 16.4 Hz, 1H), 6.75 (dd, J = 9.7, 2.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 194.20, 167.95, 159.67, 157.75, 156.19, 145.80, 144.51, 136.29, 135.28, 133.58, 133.23, 132.77, 132.59, 132.56, 130.81, 130.42, 130.31, 128.93, 128.56, 127.98, 119.75, 118.26, 111.24. HRMS calcd for $C_{24}H_{15}Cl_2FNO_2$ $[M+H]^+$; 438.04584, found; 438.04544.



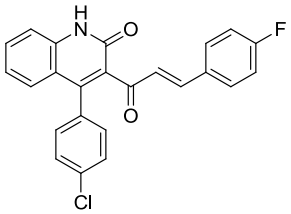
(E)-3-(3-(4-Chlorophenyl)acryloyl)-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (997-50e). Compound **997-50e** was prepared via the general method E using 3-acetyl-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (0.270 g, 0.90 mmol) and 4-chlorobenzaldehyde (0.127 g, 0.90 mmol). The title compound was obtained after

filtration from the neutralized medium as a yellow solid. Yield 0.352 g, 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 - 7.66 (m, 2H), 7.54 - 7.48 (m, 3H), 7.46 (d, J = 7.8 Hz, 3H), 7.36 - 7.10 (m, 3H), 6.83 (d, J = 16.4 Hz, 1H), 6.75 (d, J = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 194.31, 173.20, 168.00, 167.92, 162.48, 160.86, 160.01, 157.66, 156.08, 150.76, 145.31, 144.34, 136.93, 136.32, 135.26, 133.26, 132.41, 130.64, 130.37, 130.27, 128.98, 128.95, 128.00, 125.24, 119.63, 119.58, 119.21, 119.19, 118.67, 116.15, 115.99, 115.57, 111.10. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.07 - -113.19 (m), -121.00 - -121.12 (m). HRMS calcd for $C_{24}H_{15}ClFNO_2$ $[M+H]^+$; 422.07539, found; 422.07541.

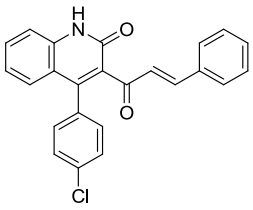


(E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-51e). Compound **997-51e** was prepared via the general method E using 3-acetyl-4-(3-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.40 g, 1.2 mmol) and 4-chlorobenzaldehyde (0.17g, 1.2 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield quantitative. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 7.83 - 7.56 (m, 6H), 7.50 - 7.34 (m, 5H), 7.00 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 6.84 (dd, J = 16.3, 2.3 Hz, 1H). ^{13}C NMR (151 MHz, dmso) δ 196.25, 163.00, 145.39, 143.41, 137.11, 135.56, 134.13, 133.88, 131.95, 130.77, 130.65, 129.96, 129.53, 129.36, 126.72, 126.47, 125.44, 121.08, 119.68. ^{19}F NMR (376

MHz, DMSO-d6) δ -61.78 (s). HRMS calcd for $C_{25}H_{16}ClF_3NO_3$ $[M+H]^+$; 454.08162, found; 454.08178.

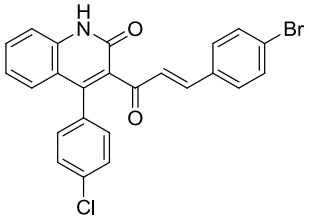


(E)-4-(4-Chlorophenyl)-3-(3-(4-fluorophenyl)acryloyl)quinolin-2(1H)-one (997-52e). Compound **997-52e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)quinolin-2(1H)-one (0.30 g, 1.0 mmol) and 4-fluorobenzaldehyde (0.13g, 1.0 mmol). The title compound was obtained after filtration from the neutralized medium as an off-white solid. Yield 0.29 g, 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.82 - 7.73 (m, 2H), 7.60 (t, J = 7.8 Hz, 1H), 7.56 - 7.48 (m, 3H), 7.45 (d, J = 8.3 Hz, 1H), 7.38 - 7.31 (m, 2H), 7.24 (t, J = 8.6 Hz, 2H), 7.17 (t, J = 7.7 Hz, 1H), 7.07 (d, J = 8.2 Hz, 1H), 6.78 (d, J = 16.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 194.19, 159.50, 146.86, 144.86, 138.76, 133.42, 133.18, 131.64, 131.20, 131.13, 131.07, 130.96, 130.87, 128.43, 127.45, 126.84, 122.35, 119.04, 116.02, 115.88, 115.71. ^{19}F NMR (376 MHz, DMSO-d_6) δ -109.80 - -109.89 (m). HRMS calcd for $C_{24}H_{16}ClFNO_2$ $[M+H]^+$; 404.08481, found; 404.08513.

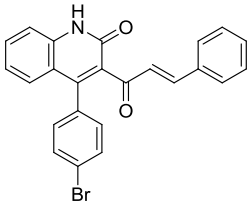


4-(4-Chlorophenyl)-3-cinnamoylquinolin-2(1H)-one (997-53e). Compound **997-53e**

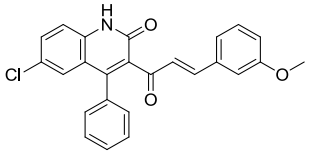
was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)quinolin-2(1H)-one (0.30 g, 1.0 mmol) and benzaldehyde (0.11 g, 1.0 mmol). The title compound was obtained after filtration from the neutralized medium as an off-white solid. Quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.69 (d, J = 7.8 Hz, 2H), 7.65 - 7.58 (m, 1H), 7.56 - 7.48 (m, 3H), 7.48 (d, J = 4.4 Hz, 1H), 7.46 - 7.32 (m, 5H), 7.17 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 16.4, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.31, 159.53, 146.88, 146.13, 138.79, 134.29, 133.44, 133.20, 131.24, 130.90, 128.92, 128.74, 128.47, 127.55, 126.87, 122.38, 119.04, 115.74. HRMS calcd for $C_{24}H_{17}ClNO_2$ $[M+H]^+$; 386.09423, found; 386.09430.



(E)-3-(3-(4-Bromophenyl)acryloyl)-4-(4-chlorophenyl)quinolin-2(1H)-one (997-54e). Compound **997-54e** was prepared via the general method E using 3-acetyl-4-(4-chlorophenyl)quinolin-2(1H)-one (0.50 g, 1.7 mmol) and 4-bromobenzaldehyde (0.31 g, 1.7 mmol). The title compound was obtained after filtration from the neutralized medium as an off-white solid. Yield 0.58 g, 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.62 - 7.56 (m, 3H), 7.56 - 7.45 (m, 4H), 7.34 (d, J = 8.4 Hz, 2H), 7.20 - 7.11 (m, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.85 (d, J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.35, 159.65, 146.82, 144.50, 139.11, 133.60, 133.38, 133.23, 131.87, 131.11, 130.87, 130.58, 128.42, 126.74, 124.19, 122.19, 118.99, 116.03, 40.13. HRMS calcd for $C_{24}H_{16}ClBrNO_2$ $[M+H]^+$; 464.00474, found; 464.00561.



4-(4-Bromophenyl)-3-cinnamoylquinolin-2(1H)-one (997-55e). Compound **997-55e** was prepared via the general method E using 3-acetyl-4-(4-bromophenyl)quinolin-2(1H)-one (0.30 g, 0.88 mmol) and benzaldehyde (0.093 g, 0.88 mmol). The title compound was obtained after filtration from the neutralized medium as an off-white solid. Yield 0.270 g, 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.76 - 7.54 (m, 5H), 7.56 - 7.35 (m, 5H), 7.29 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 16.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.29, 159.52, 146.90, 146.10, 138.78, 134.28, 133.58, 131.60, 131.36, 131.22, 131.14, 130.83, 128.91, 128.74, 127.56, 126.86, 122.37, 122.14, 118.96, 115.74, 38.89. HRMS calcd for $C_{24}H_{17}BrNO_2$ $[M+H]^+$; 430.04372, found; 430.04398.

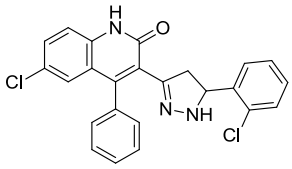


(E)-6-Chloro-3-(3-(3-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (997-56e). Compound **997-56e** was prepared via the general method E using 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (0.50 g, 1.7 mmol) and 3-methoxybenzaldehyde (0.23 g, 1.7 mmol). The title compound was obtained after filtration from the neutralized medium as a yellow solid. Yield 0.404 g, 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.66 - 7.56 (m, 1H), 7.52 - 7.35 (m, 5H), 7.29 (d, J = 7.3 Hz, 2H), 7.27 - 7.17 (m,

3H), 7.00 - 6.87 (m, 2H), 6.75 (d, J = 16.4 Hz, 1H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.19, 159.60, 159.54, 146.66, 146.36, 137.82, 135.70, 133.68, 132.48, 130.89, 129.89, 128.93, 128.84, 128.49, 127.71, 126.00, 125.55, 121.11, 120.60, 117.97, 117.07, 113.29, 55.24. HRMS calcd for $C_{25}H_{19}ClNO_2$ $[M+H]^+$; 416.10480, found; 416.10453.

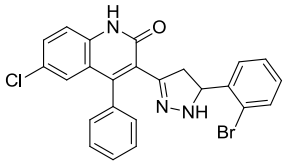
General procedure for the synthesis of pyrazol-3-yl-quinolin-2(1H)-one intermediates

(Procedure F). In an appropriately sized microwaveable vessel, the quinolin-2(1H)-one acrolyl intermediate (1 equiv.) was dissolved in EtOH (10 mL, 190 proof or 200 proof) and hydrazine monohydrate (1.5 equiv.) was added. The mixture was microwaved for 20 minutes at 110 °C. The EtOH was removed under vacuum, followed by the addition of DCM and filtration to yield the desired intermediates, which were carried forward without further purification unless otherwise noted.

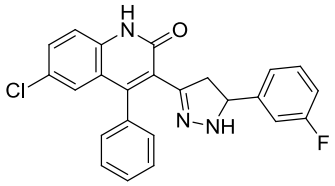


6-Chloro-3-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-14f). Compound **(997-14F)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(2-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.600

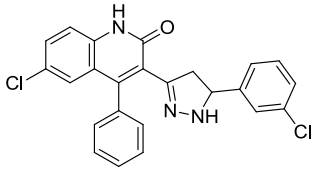
g, 1.428 mmol). Filtration from DCM yielded the title compound as a bright yellow solid. Yield 0.424 g, 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 7.61 - 7.31 (m, 7H), 7.31 - 7.09 (m, 5H), 6.90 (d, *J* = 2.4 Hz, 1H), 4.93 - 4.83 (m, 1H), 3.37 (dd, *J* = 11.1, 5.3 Hz, 1H), 2.43 (dd, *J* = 16.5, 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.48, 148.49, 145.29, 140.59, 136.98, 135.16, 131.43, 130.42, 129.27, 129.00, 128.52, 128.21, 127.72, 127.36, 125.66, 120.87, 117.37, 59.54. HRMS calcd for $C_{24}H_{18}Cl_2N_3O$ $[M+H]^+$; 434.08214, found; 434.08169.



3-(5-(2-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-chloro-4-phenylquinolin-2(1H)-one (997-15f). Compound **(997-15f)** was prepared according to general procedure F using (E)-3-(3-(2-bromophenyl)acryloyl)-6-chloro-4-phenylquinolin-2(1H)-one (0.500g, 1.076 mmol). Filtration from the reaction vessel yielded the title compound as a yellow solid. Yield 0.458 g, 89%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.24 (s, 1H), 7.60 – 7.39 (m, 6H), 7.35 (d, *J* = 7.4 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.19-7.14 (m, 3H), 6.91 (d, *J* = 2.5 Hz, 1H), 4.90 – 4.79 (m, 1H), 3.48 – 3.36 (m, 1H), 2.41 (dd, *J* = 16.5, 9.0 Hz, 1H). ^{13}C NMR 100 MHz (DMSO-d_6) δ 160.509, 152.317, 148.529, 145.122, 142.257, 136.996, 135.181, 132.257, 130.434, 129.318, 128.871, 128.537, 128.306, 128.2224, 127.934, 126.476, 125.813, 125.672, 121.981, 120.873, 117.383, 61.973, 43.602. HRMS calcd for $C_{24}H_{18}BrClN_3O$ $[M+H]^+$; 478.03163, found; 478.03170.

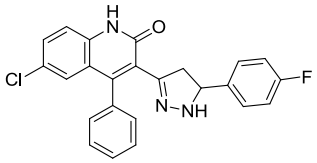


6-Chloro-3-(5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-16f). Compound **(997-16f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(3-fluorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.500 g, 1.238 mmol). Filtration from DCM yielded the title compound as a bright yellow solid. Yield 0.398 g, 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H), 7.57 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.55 - 7.45 (m, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.37 - 7.32 (m, 1H), 7.32 - 7.28 (m, 1H), 7.28 - 7.20 (m, 2H), 7.04 (td, *J* = 8.6, 2.6 Hz, 1H), 6.98 - 6.88 (m, 3H), 4.69 - 4.58 (m, 1H), 3.27 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.58 (dd, *J* = 16.5, 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.56, 148.46, 145.48, 136.99, 135.17, 130.43, 129.20, 128.63, 128.25, 125.81, 122.56, 120.88, 117.38, 113.25, 113.03, 62.18, 44.45, 40.14, 39.92, 39.72, 39.62, 39.51, 39.30, 39.09, 38.88. ^{19}F (376 MHz DMSO-d_6) HRMS calcd for $C_{24}H_{18}ClFN_3O$ $[M+H]^+$; 418.11169, found; 418.11151.

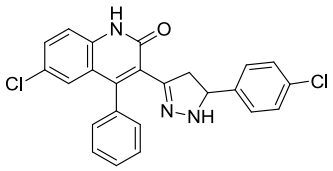


6-Chloro-3-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-13f). Compound **(997-13f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(3-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.600 g, 1.43 mmol). Filtration from DCM yielded the title compound as a slightly yellow

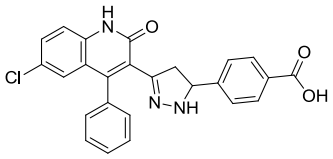
solid. Yield 0.441 g, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 7.58 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.55 - 7.44 (m, 3H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 7.4 Hz, 1H), 7.31 - 7.20 (m, 4H), 7.10 - 6.99 (m, 1H), 6.91 (d, *J* = 3.0 Hz, 2H), 4.68 – 4.57 (m, 1H), 3.26 (dd, *J* = 16.5, 11.1 Hz, 1H), 2.58 (dd, *J* = 16.5, 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.54, 146.19, 136.99, 135.17, 132.91, 130.15, 129.14, 128.64, 128.26, 126.85, 126.39, 125.81, 125.64, 125.23, 120.85, 117.38, 62.08, 44.46. HRMS calcd for $C_{24}H_{18}Cl_2N_3O$ $[M+H]^+$; 434.08214, found; 434.08153.



6-Chloro-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-19f). Compound **(997-19f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(4-fluorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.500 g, 1.238 mmol). Filtration from DCM yielded the title compound as a yellow solid. Yield 0.350 g, 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.61 - 7.45 (m, 4H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 7.0 Hz, 1H), 7.27 - 7.02 (m, 6H), 6.91 (s, 1H), 4.66-4.55 (m, 1H), 3.25 (dd, *J* = 16.4, 10.9 Hz, 1H), 2.55 (dd, *J* = 16.2, 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.56, 148.40, 145.48, 139.72, 136.98, 135.18, 130.41, 129.27, 128.69, 128.40, 128.31, 128.23, 126.76, 125.80, 125.65, 120.90, 117.38, 115.02, 114.81, 62.09, 44.56. HRMS calcd for $C_{24}H_{18}ClFN_3O$ $[M+H]^+$; 418.11169, found; 418.11139.

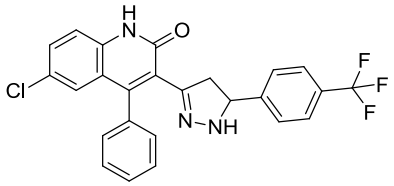


6-Chloro-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-12). Compound **(997-12f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(4-chlorophenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.600 g, 1.428 mmol). Filtration from DCM yielded the title compound as a yellow solid. Yield 0.347 g, 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.58 (dd, J = 8.7, 2.4 Hz, 1H), 7.56 - 7.45 (m, 3H), 7.41 (d, J = 8.9 Hz, 1H), 7.34 (dd, J = 6.6, 2.0 Hz, 1H), 7.32 - 7.27 (m, 2H), 7.26 - 7.18 (m, 2H), 7.13 - 7.04 (m, 2H), 6.89 (d, J = 2.3 Hz, 1H), 4.65 - 4.54 (m, 1H), 3.26 (dd, J = 16.5, 11.0 Hz, 1H), 2.59 - 2.47 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.53, 148.43, 145.45, 142.59, 136.98, 135.16, 131.37, 130.44, 129.27, 128.67, 128.34, 128.16, 126.68, 125.79, 125.65, 120.90, 117.38, 62.03, 44.51. HRMS calcd for $C_{24}H_{18}Cl_2N_3O$ $[M+H]^+$; 434.08214, found; 434.08269.

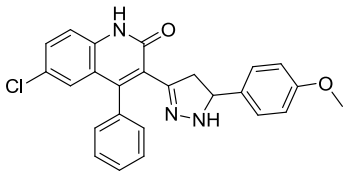


4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzoic acid (997-5f). Compound **(997-5f)** was prepared according to general procedure F using (E)-4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-3-oxoprop-1-enyl)benzoic acid (0.300g, 0.698 mmol). The title compound was obtained by

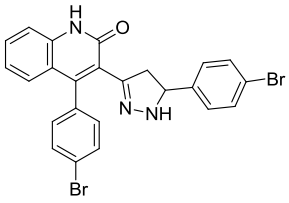
filtration from DCM as a bright yellow powder. Yield 0.270 g, 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (d, *J* = 7.9 Hz, 2H), 7.63 - 7.46 (m, 4H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 7.1 Hz, 1H), 7.24 (dd, *J* = 5.9, 3.3 Hz, 1H), 7.17 (s, 1H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 4.60 (t, *J* = 10.4 Hz, 1H), 3.26 (dd, *J* = 16.4, 11.0 Hz, 1H), 2.68 - 2.38 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.17, 160.61, 148.39, 145.51, 144.99, 137.04, 135.23, 129.12, 128.73, 128.33, 128.23, 126.84, 125.80, 125.69, 120.95, 117.45, 62.81, 44.56. HRMS calcd for $C_{25}H_{19}ClN_3O_3$ $[M+H]^+$; 444.11095, found; 444.11068.



6-Chloro-4-phenyl-3-(5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (**997-5f**). Compound **(997-5f)** was prepared according to general procedure F using (E)-6-chloro-4-phenyl-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)quinolin-2(1H)-one (0.800g, 0.962 mmol). The title compound was obtained by filtration from DCM as a yellow solid. Yield 0.825 g, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 7.63 – 7.58 (m, 2H), 7.58 – 7.44 (m, 2H), 7.42 (d, *J* = 8.7 Hz, 3H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.30 (dd, *J* = 7.9, 5.6 Hz, 3H), 7.25 – 7.20 (m, 1H), 6.89 (d, *J* = 2.5 Hz, 1H), 4.75 – 4.64 (m, 1H), 3.38 – 3.26 (m, 1H), 2.57 (dd, *J* = 16.5, 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.53, 148.53, 148.40, 145.47, 137.00, 135.15, 130.48, 129.28, 128.33, 128.27, 127.38, 127.25, 127.22, 126.60, 125.81, 125.76, 125.68, 125.58, 125.23, 125.16, 125.11, 120.88, 117.40, 62.21, 44.61. HRMS calcd for $C_{25}H_{18}ClF_3N_3O$ $[M+H]^+$; 468.10850, found; 468.10856.

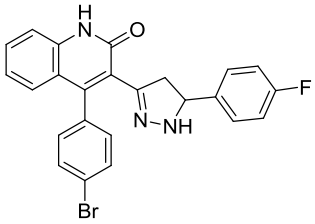


6-Chloro-3-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-17). Compound **(997-17f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(4-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.400g, 0.962 mmol). The title compound was obtained by filtration from DCM as a bright yellow solid. Yield 0.384 g, 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 7.59 - 7.55 (m, 1H), 7.55 - 7.46 (m, 3H), 7.41 (dd, *J* = 8.9, 1.3 Hz, 1H), 7.36 - 7.31 (m, 1H), 7.23 (dd, *J* = 5.8, 2.9 Hz, 1H), 7.04 (d, *J* = 3.1 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.90 (t, *J* = 1.8 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 2H), 4.57 - 4.46 (m, 1H), 3.72 (s, 3H), 3.18 (dd, *J* = 16.5, 10.8 Hz, 1H), 2.61 - 2.50 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.54, 158.24, 148.27, 145.49, 136.96, 136.95, 135.35, 135.20, 130.34, 129.22, 128.72, 128.27, 128.17, 127.57, 125.75, 125.62, 120.91, 117.33, 113.58, 62.43, 55.02, 44.47. HRMS calcd for $C_{25}H_{21}ClN_3O_2$ $[M+H]^+$; 430.13168, found; 430.13233.



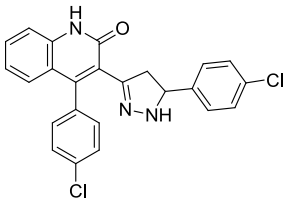
4-(4-Bromophenyl)-3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-7f). Compound **(997-7f)** was prepared according to general procedure F using (E)-4-(4-bromophenyl)-3-(3-(4-bromophenyl)acryloyl)quinolin-2(1H)-one (0.500 g, 0.982 mmol). The title compound was obtained via filtration from the reaction

medium as a white solid. Yield 0.400 g, 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 7.70 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.63 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.29 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.22 (d, *J* = 3.0 Hz, 1H), 7.17 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.10 (t, *J* = 7.7 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.99 (d, *J* = 8.2 Hz, 1H), 4.65 - 4.55 (m, 1H), 3.35 - 3.26 (m, 1H), 2.57 (dd, *J* = 16.5, 8.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.60, 148.39, 142.97, 138.23, 135.08, 131.63, 131.18, 131.07, 130.97, 130.66, 128.66, 126.85, 126.73, 125.47, 122.09, 121.34, 119.91, 119.27, 115.38, 61.87, 44.48. HRMS calcd for $C_{24}H_{18}Br_2N_3O$ $[M+H-H_2]^+$; 521.98111, found; 521.98154.



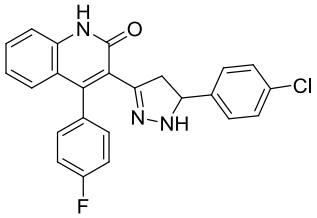
4-(4-Bromophenyl)-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-20f). Compound **(997-20f)** was prepared according to general procedure F using (E)-4-(4-bromophenyl)-3-(3-(4-fluorophenyl)acryloyl)quinolin-2(1H)-one (0.500 g, 1.12 mmol). The title compound was obtained after removal of the EtOH, followed by addition of DCM, wash 3X with brine, drying over magnesium sulfate, followed by concentration *in vacuo* as a yellow solid. Yield 0.366 g, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.22 - 7.04 (m, 7H), 6.99 (d, *J* = 8.2 Hz, 1H), 4.68 - 4.57 (m, 1H), 3.33 - 3.24 (m, 1H), 2.59 (dd, *J* = 16.6, 8.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.33, 149.00, 146.44, 140.44, 138.88,

135.81, 132.28, 131.73, 131.29, 129.02, 128.94, 127.51, 126.29, 122.74, 121.97, 119.96, 116.03, 115.65, 115.44, 62.63, 55.59, 45.20. ^{19}F NMR (376 MHz, DMSO-d_6) HRMS calcd for $C_{24}H_{18}BrFN_3O$ $[M+H]^+$; 460.04553, found; 460.04687.

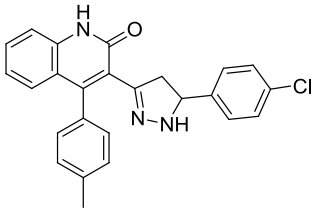


4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one

(997-23f). Compound **(997-23f)** was prepared according to general procedure F using (E)-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (0.500 g, 1.19 mmol). The title compound was obtained via filtration from the reaction medium as a yellow solid. Yield 0.224 g, 43%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.12 (s, 1H), 7.60 – 7.47 (m, 3H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.27 – 7.19 (m, 2H), 7.19 – 7.03 (m, 3H), 6.99 (d, *J* = 8.2 Hz, 1H), 4.62 (t, *J* = 10.1 Hz, 1H), 3.39 – 3.26 (m, 1H), 2.59 (dd, *J* = 16.5, 8.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.66, 148.37, 145.81, 142.63, 138.23, 134.75, 132.67, 131.39, 131.32, 130.70, 130.63, 128.29, 128.18, 128.13, 126.85, 125.63, 122.07, 119.36, 115.38, 61.97, 44.50. HRMS calcd for $C_{24}H_{18}Cl_2N_3O$ $[M+H]^+$; 434.08214, found; 434.08248.

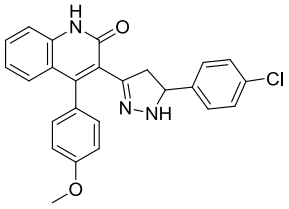


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-fluorophenyl)quinolin-2(1H)-one (997-24f). Compound **(997-24f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(4-fluorophenyl)quinolin-2(1H)-one (0.500g, 1.24 mmol). The title compound was obtained via filtration from the DCM as a yellow solid. Yield 0.410 g, 79.0 %.^{1}H NMR (400 MHz, DMSO-d6) δ 12.09 (s, 1H), 7.56 – 7.47 (m, 1H), 7.43 – 7.21 (m, 6H), 7.23 – 7.06 (m, 5H), 7.00 (d, *J* = 8.1 Hz, 1H), 4.66 – 4.55 (m, 1H), 3.28 (dd, *J* = 16.5, 11.0 Hz, 1H), 2.57 (dd, *J* = 16.5, 9.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.70, 148.69, 145.98, 142.62, 138.22, 132.12, 131.56, 131.47, 131.39, 130.89, 130.61, 128.35, 128.14, 126.92, 125.78, 122.04, 119.61, 115.37, 115.37, 115.20, 114.99, 62.03, 44.56. ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.821. HRMS calcd for $C_{24}H_{18}ClN_3O$ $[M+H]^+$; 418.11169, found; 418.11202.

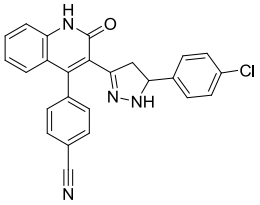


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-p-tolylquinolin-2(1H)-one (997-26f). Compound **(997-26f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-p-tolylquinolin-2(1H)-one (0.500g, 1.25 mmol). There was a solid present in the reaction vial which was filtered to give the title

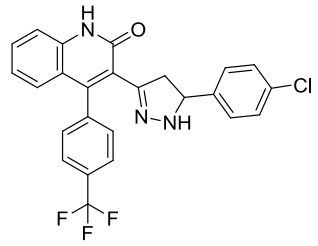
compound as a white solid. Yield 0.340 g, 66%.^{1}H NMR (400 MHz, DMSO-d6) δ 12.05 (s, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.32 – 7.27 (m, 3H), 7.27 – 7.17 (m, 2H), 7.17 – 7.05 (m, 5H), 7.02 (d, *J* = 8.2 Hz, 1H), 4.58 (dt, *J* = 10.9, 6.0 Hz, 1H), 3.22 (dd, *J* = 16.4, 11.0 Hz, 1H), 2.57 – 2.46 (m, 1H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.73, 149.72, 146.00, 142.72, 138.23, 137.06, 132.79, 131.29, 130.46, 129.19, 128.73, 128.62, 128.36, 128.08, 127.00, 125.41, 121.87, 119.67, 115.29, 61.87, 44.65, 20.94. HRMS calcd for $C_{25}H_{21}ClN_3O$ $[M+H]^+$; 414.13677, found; 414.13687.



3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-methoxyphenyl)quinolin-2(1H)-one (997-21f). Compound **(997-21f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(4-methoxyphenyl)quinolin-2(1H)-one 90.500g, 1.20 mmol). The title compound was obtained via filtration from DCM as a white solid. Yield 0.291 g, 56%.^{1}H NMR (400 MHz, DMSO-d6) δ 12.30 (s, 1H), 7.55 – 7.38 (m, 1H), 7.32 – 7.20 (m, 2H), 7.18 – 6.95 (m, 10H), 4.65 – 4.54 (m, 1H), 3.84 (s, 3H), 3.22 (dd, *J* = 16.5, 11.0 Hz, 1H), 2.59 – 2.42 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.82, 158.81, 149.48, 149.48, 146.18, 142.79, 138.40, 131.30, 130.68, 130.40, 130.09, 128.37, 128.08, 127.82, 126.98, 125.61, 121.78, 119.84, 115.46, 113.54, 61.86, 55.11, 44.70. HRMS calcd for $C_{25}H_{21}ClN_3O_2$ $[M+H]^+$; 430.13168, found; 430.13204.

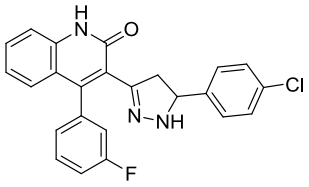


4-(3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-27f). Compound **(997-27f)** was prepared according to general procedure F using 4-(3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile 0.500 g, 1.22 mmol). The title compound was obtained as a white solid. Yield 0.350 g, 68%. ^{13}C NMR (101 MHz, dmso) δ 160.58, 147.78, 145.67, 142.46, 141.27, 138.25, 132.06, 131.44, 130.76, 130.70, 130.52, 129.98, 128.32, 128.26, 128.18, 128.09, 126.69, 125.43, 122.20, 118.93, 115.45, 115.39, 110.68, 62.13, 44.31. HRMS calcd for $C_{25}H_{18}ClN_4O$ $[M+H]^+$; 425.11637, found; 425.11754.

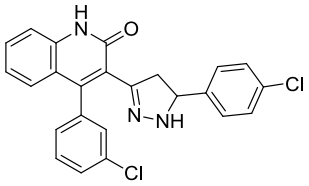


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-36f). Compound **(997-36f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(4-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.300 g, 0.661 mmol). The title compound was obtained after removal of the EtOH *in vacuo* as a yellow solid. Yield 0.277 g, 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.81 (s, 1H), 7.73 (t, *J* = 8.6 Hz, 2H), 7.56 - 7.32 (m, 5H), 7.25 (d, *J* = 4.3 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 3H), 7.08 (t, *J* = 8.3 Hz, 1H), 4.72 (dd,

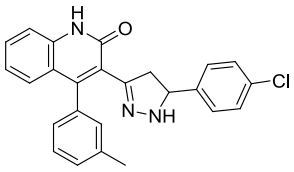
J = 10.9, 6.3 Hz, 1H), 3.29 (dd, *J* = 16.8, 11.0 Hz, 1H), 2.90 (dd, *J* = 16.8, 6.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.05, 150.33, 147.81, 141.98, 139.78, 138.26, 133.56, 131.39, 129.95, 129.87, 129.05, 128.24, 127.81, 127.50, 125.45, 124.78, 123.15, 120.22, 116.42, 63.26, 44.82. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.42 (s). HRMS calcd for $C_{25}H_{18}ClF_3N_3O$ $[M+H]^+$; 468.10850, found; 468.10933.



3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)quinolin-2(1H)-one (997-28f). Compound 997-28f was prepared according to general procedure F using (E)-3-(3-(4-Chlorophenyl)acryloyl)-4-(3-fluorophenyl)quinolin-2(1H)-one (0.500 g, 1.24 mmol). The title compound was obtained after removal of the EtOH in vacuo as a yellow solid. Yield 0.350 g, 68%. 1H NMR (400 MHz, DMSO-d6) δ 11.64 (s, 1H), 7.63 – 7.45 (m, 2H), 7.41 (d, J = 8.1 Hz, 1H), 7.34 – 7.27 (m, 3H), 7.24 – 7.08 (m, 5H), 7.06 – 6.98 (m, 2H), 4.69 – 4.57 (m, 1H), 3.42 – 3.27 (m, 1H), 2.70 – 2.55 (m, 1H). 13C NMR (100 MHz, DMSO-d) δ 163.05, 160.67, 160.62, 160.59, 148.16, 148.14, 145.77, 142.68, 142.65, 138.27, 138.22, 138.19, 131.39, 130.63, 130.23, 130.14, 128.32, 128.24, 128.17, 126.83, 125.61, 125.57, 125.52, 124.99, 122.09, 119.29, 116.57, 116.35, 115.92, 115.70, 115.38, 114.91, 114.87, 114.71, 114.67, 62.05, 61.99, 44.56, 44.51. HRMS calcd for C24H18ClFN3O [M+H]+; 418.11169, found; 418.11207.

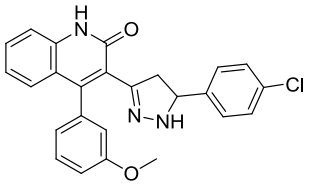


4-(3-Chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-22f). Compound **(997-22f)** was prepared according to general procedure F using (E)-4-(3-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (0.500 g, 1.19 mmol). The title compound was obtained by filtration from DCM after removal of the EtOH as a yellow solid. Yield 0.425 g, 82%. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.85 (s, 1H), 7.52 (dd, *J* = 16.0, 7.3 Hz, 3H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.31 (dd, *J* = 8.1, 3.2 Hz, 3H), 7.26 – 7.16 (m, 1H), 7.16 – 7.09 (m, 3H), 6.98 (d, *J* = 8.1 Hz, 1H), 4.68 – 4.58 (m, 1H), 3.43 – 3.32 (m, 1H), 2.67 – 2.54 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.66, 145.77, 142.76, 142.61, 138.22, 138.02, 132.92, 131.39, 130.65, 130.00, 129.10, 128.33, 128.22, 127.94, 127.55, 12707, 126.80, 125.61, 122.14, 119.30, 115.40, 62.09, 44.54. HRMS calcd for $C_{24}H_{18}Cl_2N_3O$ $[M+H]^+$; 434.08214, found; 434.08249.

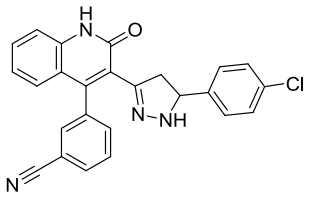


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-m-tolylquinolin-2(1H)-one (997-25f). Compound **(997-25f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-m-tolylquinolin-2(1H)-one (0.500 g, 1.25 mmol). The title compound was obtained by fitration from DCM after removal of the EtOH as a yellow solid. Yield 0.380 g, 73%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.05 (s, 1H), 7.51

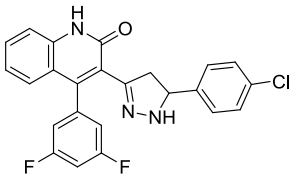
(t, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.34 - 7.24 (m, 4H), 7.16 - 6.95 (m, 6H), 4.65 - 4.52 (m, 1H), 3.34 – 3.19 (m, 1H), 2.58 - 2.50 (m, 1H), 2.38 (s, 1.5 H), 2.30 (s, 1.5 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.19, 163.16, 152.33, 152.26, 148.57, 148.51, 142.35, 138.22, 138.18, 138.11, 135.88, 135.83, 133.38, 133.35, 131.09, 129.95, 129.70, 129.32, 129.29, 129.16, 129.02, 128.90, 128.46, 128.37, 128.01, 127.98, 127.93, 126.52, 126.47, 124.43, 122.86, 120.79, 116.30, 63.18, 45.21, 45.09, 21.80, 21.76. HRMS calcd for $C_{25}H_{21}ClN_3O$ $[M+H]^+$; 414.13677, found; 414.13735.



3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-methoxyphenyl)quinolin-2(1H)-one (997-39f) Compound **(997-39f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3-methoxyphenyl)quinolin-2(1H)-one (0.550 g, 1.32 mmol). The title compound was obtained by filtration from DCM as a yellow solid. Yield 0.470 g, 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 7.61 - 7.21 (m, 5H), 7.20 - 7.00 (m, 6H), 6.97 - 6.70 (m, 2H), 4.60 (t, *J* = 10.1 Hz, 1H), 3.84 - 3.60 (m, 3H), 3.36 - 3.22 (m, 1H), 2.61 - 2.43 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.80, 158.86, 149.31, 145.96, 142.88, 142.82, 138.40, 137.11, 131.30, 130.46, 129.27, 128.38, 128.23, 128.14, 126.92, 125.35, 121.87, 121.03, 119.41, 115.49, 115.05, 114.18, 113.44, 61.92, 61.85, 55.18, 55.00, 44.67. HRMS calcd for $C_{25}H_{21}ClN_3O_2$ $[M+H]^+$; 430.13168, found; 430.13231.

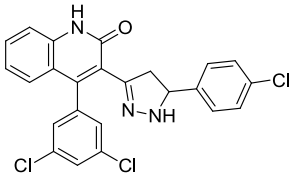


3-(3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (997-40f). Compound **(997-40f)** was prepared according to general procedure F using (E)-3-(3-(3-(4-chlorophenyl)acryloyl)-2-oxo-1,2-dihydroquinolin-4-yl)benzonitrile (0.250 g, 0.608 mmol). The title compound was obtained after removal of the EtOH, followed by addition of DCM, wash 3X with brine, drying over magnesium sulfate, and concentration *in vacuo* as a yellow solid. Yield 0.110 g, 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 8.11 - 7.79 (m, 1H), 7.77 - 7.65 (m, 2H), 7.59 - 7.51 (m, 2H), 7.43 (d, J = 8.3 Hz, 1H), 7.35 - 7.30 (m, 2H), 7.28 - 7.21 (m, 1H), 7.21 - 6.82 (m, 4H), 4.69 - 4.55 (m, 1H), 3.51 - 3.34 (m, 1H), 2.74 - 2.59 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.62, 147.34, 145.72, 145.58, 142.71, 142.49, 138.28, 137.42, 137.34, 134.47, 133.77, 132.94, 132.13, 131.77, 131.41, 130.71, 129.40, 129.33, 128.36, 128.28, 128.20, 126.78, 125.81, 125.77, 122.19, 119.19, 118.68, 115.47, 111.34, 111.24, 62.11, 61.88, 44.33. HRMS calcd for $C_{25}H_{18}ClN_4O_1$ $[M+H]^+$; 425.11637, found; 425.11710.



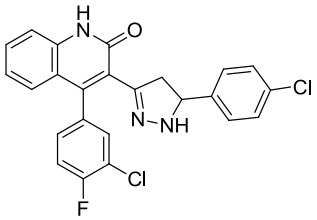
3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,5-difluorophenyl)quinolin-

2(1H)-one (997-35f). Compound **(997-35f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3,5-difluorophenyl)quinolin-2(1H)-one (0.300 g, 0.711 mmol). The title compound was obtained by filtration from DCM as a yellow solid. Yield 0.280 g, 90%. ^{1}H NMR (400 MHz, CDCl3) δ 12.76 (s, 1H), 7.58 - 7.46 (m, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.34 - 7.28 (m, 2H), 7.27 - 7.22 (m, 2H), 7.16 (d, *J* = 4.0 Hz, 2H), 6.98 - 6.89 (m, 1H), 6.86 (t, *J* = 9.7 Hz, 2H), 5.84 (s, 1H), 4.79 (dd, *J* = 10.9, 6.3 Hz, 1H), 3.39 (dd, *J* = 16.8, 10.9 Hz, 1H), 3.02 (dd, *J* = 16.8, 6.4 Hz, 1H). ^{13}C NMR (100 MHz, CDCl3) δ 162.98, 149.29, 147.68, 141.96, 138.18, 133.57, 131.46, 129.11, 127.83, 127.36, 124.78, 123.24, 119.93, 116.41, 112.70, 104.09, 63.41, 44.83. ^{19}F NMR (376 MHz, DMSO-d6) δ -110.39 – -110.57 (m). HRMS calcd for $C_{24}H_{17}ClN_3OF_2$ $[M+H]^+$; 436.10227, found; 436.10308.

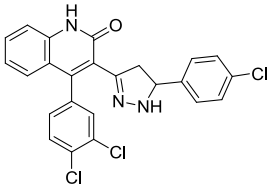


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (997-37f). Compound **(997-37f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3,5-dichlorophenyl)quinolin-2(1H)-one (0.300 g, 0.660 mmol). The title compound was obtained by filtration from DCM as a yellow solid. Yield 0.220 mg, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.82 - 7.60 (m, 1H), 7.58 - 7.48 (m, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.36 - 7.25 (m, 4H), 7.22 (dt, *J* = 8.5, 2.3 Hz, 1H), 7.12 (ddd, *J* = 10.8, 6.9, 2.2 Hz, 3H), 7.00 (d, *J* = 8.1 Hz, 1H), 4.70 – 4.58 (m, 1H), 3.47 - 3.32 (m, 1H), 2.72 - 2.52 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.60, 146.89, 145.53, 138.22, 136.79, 131.40, 131.01, 130.69, 130.32,

130.04, 128.25, 128.16, 126.80, 122.21, 119.14, 115.41, 61.86, 44.56. HRMS calcd for $C_{24}H_{17}Cl_3N_3O$ $[M+H]^+$; 468.04317, found; 468.04453.

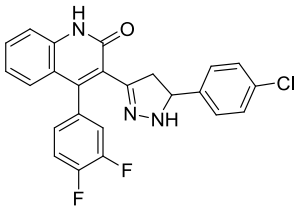


4-(3-Chloro-4-fluorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-38f). Compound **(997-38f)** was prepared according to general procedure F using (E)-4-(3-chloro-4-fluorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (0.300 g, 0.684 mmol). The title compound was obtained as a yellow solid. Yield 0.250 g, 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.61 – 12.51 (m, 1H), 7.60 – 7.46 (m, 1H), 7.48 – 7.10 (m, 11H), 4.84 – 4.72 (m, 1H), 3.52 – 3.23 (m, 1H), 3.06 – 2.92 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.54, 147.93, 142.02, 141.92, 138.13, 133.60, 131.43, 129.16, 129.08, 127.89, 127.79, 127.53, 125.14, 123.23, 120.39, 116.72, 116.37, 63.40, 63.34, 44.99, 44.83. HRMS calcd for $C_{24}H_{15}Cl_2FN_3O$ $[M+H]^+$; 450.05707, found; 450.05800.



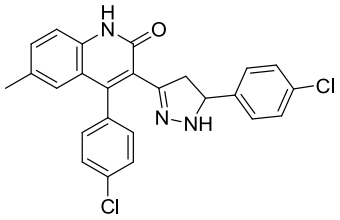
3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,4-dichlorophenyl)quinolin-2(1H)-one (997-41f). Compound **(997-41f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3,4-dichlorophenyl)quinolin-

2(1H)-one (0.400 mg, 0.880 mmol) . The title compound was obtained after removal of the EtOH, followed by addition of DCM, wash 3X with brine, drying over magnesium sulfate, followed by concentration *in vacuo* as a yellow solid. Yield 0.301 g, 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 7.82 – 7.64 (m, 1H), 7.68 – 7.47 (m, 2H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.36 – 7.26 (m, 3H), 7.22 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.13 (dd, *J* = 12.3, 8.1 Hz, 3H), 7.01 (d, *J* = 8.2 Hz, 1H), 4.74 – 4.60 (m, 4H), 3.48 – 3.36 (m, 1H), 2.72 – 2.61 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.58, 146.86, 145.52, 142.68, 142.50, 138.19, 136.78, 131.40, 130.70, 130.56, 130.32, 130.03, 128.25, 128.18, 128.14, 126.81, 122.22, 119.15, 115.38, 62.05, 61.87, 44.36. HRMS calcd for $C_{24}H_{15}Cl_3N_3O$ $[M+H-H_2]^+$; 466.02784, found; 466.02752.



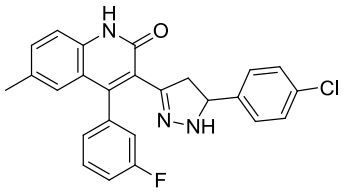
3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3,4-difluorophenyl)quinolin-2(1H)-one (997-42f). Compound **(997-42f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3,4-difluorophenyl)quinolin-2(1H)-one (0.300 g, 0.711 mmol). Upon completion of the reaction, there was a precipitate forming in the reaction vessel. A small amount of water was added to the

mixture and the title compound was filtered from the medium to give the title compound as a white solid. Yield 0.160 g, 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 7.62 – 7.22 (m, 7H), 7.20 – 6.99 (m, 5H), 4.64 (t, J = 10.4 Hz, 1H), 3.45 – 3.27 (m, 1H), 2.73 – 2.54 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.61, 150.22, 147.36, 145.79, 142.55, 138.17, 133.38, 131.40, 130.68, 128.30, 128.20, 128.14, 128.11, 126.86, 122.15, 119.32, 115.35, 62.08, 61.99, 44.43. ^{19}F NMR (376 MHz, DMSO-d_6) δ -6.46 - -6.75 (m), -7.34 - -7.63 (m). HRMS calcd for $C_{24}H_{16}ON_3ClF_2K$ $[M+K]^+$; 474.05816, found; 474.05829.

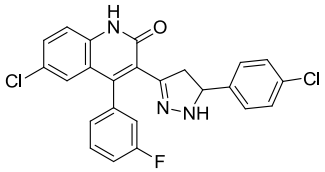


4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-methylquinolin-2(1H)-one (997-43f). Compound **(997-43f)** was prepared according to general procedure F using (E)-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-methylquinolin-2(1H)-one (0.250 g, 0.576 mmol). Upon completion of the reaction, the ethanol was removed under vacuum and DCM was added, the resultant solid was filtered to yield the title compound as a yellow solid. Yield 0.220 g, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 7.62 – 7.44 (m, 1H), 7.45 – 7.25 (m, 5H), 7.27 – 7.15 (m, 4H), 7.14 – 7.06 (m, 1H), 6.76 (s, 1H), 4.66 – 4.55 (m, 1H), 3.30 (dd, J = 16.5, 11.0 Hz, 1H), 2.62 – 2.47 (m, 1H), 2.21 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.54, 148.15, 146.00, 142.70, 136.40, 134.84, 132.61, 131.90, 131.36, 130.92, 130.69, 128.30, 128.18, 128.13, 126.13, 125.64, 119.26, 115.51, 61.90, 44.55, 20.63. HRMS calcd for

$C_{25}H_{20}ON_3Cl_2$ $[M+H]^+$; 448.09779, found; 448.09865.

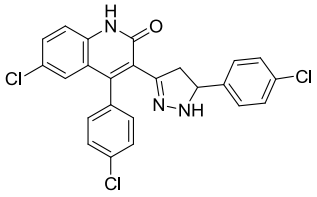


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (997-44f). Compound **(997-44f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3-fluorophenyl)-6-methylquinolin-2(1H)-one (0.250 g, 0.598 mmol). Upon completion of the reaction, the ethanol was removed under vacuum and DCM was added, the resultant solid yield the title compound as a yellow solid. Yield 0.190 g, 74%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.61-7.46 (m, 1H), 7.44 - 7.26 (m, 5H), 7.24 - 7.01 (m, 5H), 6.77 (s, 1H), 4.65 – 4.57 (m, 1H), 3.38 – 3.27 (m, 1H), 2.65 – 2.50 (m, 1H), 2.21 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.53, 147.90, 145.93, 142.71, 138.33, 136.37, 131.88, 131.35, 130.89, 130.18, 130.13, 128.31, 128.23, 128.15, 126.10, 125.60, 125.50, 124.96, 119.17, 115.46, 114.85, 61.98, 61.93, 44.59, 44.54. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.67 - -113.80 (m). HRMS calcd for $C_{25}H_{20}ON_3ClF$ $[M+H]^+$; 432.12734, found; 432.12741.



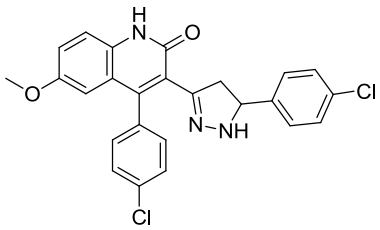
6-Chloro-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)quinolin-2(1H)-one (997-45f). Compound **(997-45f)** was prepared according to general procedure F using (E)-6-chloro-3-(3-(4-chlorophenyl)acryloyl)-4-(3-

fluorophenyl)quinolin-2(1H)-one (0.250 g, 0.570 mmol). Upon completion of the reaction, DCM was added, the organics were washed 3X with brine, dried over magnesium sulfate, followed by concentration *in vacuo* to give an orange solid. Yield 0.188 g, 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 7.58 (dd, *J* = 8.6, 2.4 Hz, 2H), 7.56 - 7.46 (m, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.30 (tdt, *J* = 13.4, 6.9, 3.4 Hz, 3H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.15 - 7.09 (m, 2H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.95 - 6.82 (m, 1H), 4.68 - 4.57 (m, 1H), 3.34 (dt, *J* = 16.5, 11.0 Hz, 1H), 2.69 - 2.59 (m, 1H). ^{13}C NMR (151 MHz, dmso) δ 160.75, 146.72, 145.51, 142.61, 131.40, 130.36, 128.71, 128.30, 128.22, 128.17, 126.80, 125.63, 125.37, 125.00, 120.57, 117.84, 62.03, 61.99, 44.42, 44.35. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.37 - -113.51 (m). HRMS calcd for $C_{24}H_{17}ON_3Cl_2F$ $[M+H]^+$; 452.07272, found; 452.07317.



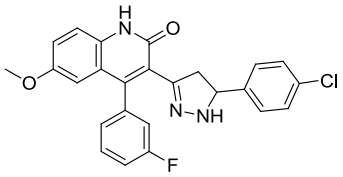
6-Chloro-4-(4-chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-46f). Compound **(997-46f)** was prepared according to general procedure F using (E)-6-chloro-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)quinolin-2(1H)-one (0.250 g, 0.550 mmol). Upon completion of the reaction, the ethanol was removed under vacuum and DCM was added, the resultant solid yield the title compound as a yellow solid. Yield 0.217 g, 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 7.62 - 7.55 (m, 2H), 7.53 (dt, *J* = 8.4, 2.4 Hz, 1H), 7.43

(dd, *J* = 8.9, 2.0 Hz, 1H), 7.39 - 7.34 (m, 1H), 7.31 (dd, *J* = 8.4, 2.2 Hz, 2H), 7.29 - 7.24 (m, 2H), 7.14 - 7.05 (m, 2H), 6.92 - 6.85 (m, 1H), 4.67 - 4.57 (m, 1H), 3.31 (dd, *J* = 15.9, 11.9 Hz, 1H), 2.59 (dd, *J* = 16.6, 8.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.47, 147.13, 145.33, 142.52, 137.04, 134.13, 132.93, 131.39, 131.31, 130.68, 130.51, 128.35, 128.26, 128.14, 126.91, 125.87, 125.47, 120.66, 117.48, 61.97, 44.33. HRMS calcd for $C_{24}H_{17}ON_3Cl_3$ $[M+H]^+$; 468.04317, found; 468.04333.



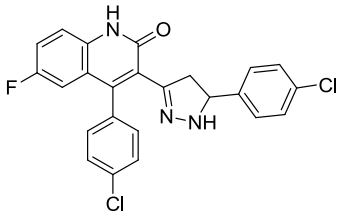
4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-methoxyquinolin-2(1H)-one (997-47f). Compound **(997-47f)** was prepared according to general procedure F using (E)-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-methoxyquinolin-2(1H)-one (0.300 g, 0.67 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, the organics were washed 3X with brine, filtered over magnesium sulfate followed by removal of the solvent *in vacuo* to yield the title compound as a yellow solid. Yield 0.220 g, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.56 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.50 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.41 - 7.33 (m, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.27 - 7.22 (m, 2H), 7.20 (dd, *J* = 8.0, 3.0

Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.40 (d, *J* = 2.6 Hz, 1H), 4.61 (td, *J* = 10.0, 8.8, 2.9 Hz, 1H), 3.59 (s, 3H), 3.31 (dd, *J* = 16.5, 11.0 Hz, 1H), 2.59 (dd, *J* = 16.5, 8.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.22, 154.01, 147.76, 146.03, 142.66, 132.65, 131.37, 131.29, 130.67, 128.31, 128.22, 128.13, 126.10, 119.88, 119.16, 116.84, 108.85, 61.94, 55.26, 44.50. HRMS calcd for $C_{25}H_{20}O_2N_3Cl_2$ $[M+H]^+$; 464.09271, found; 464.09224.

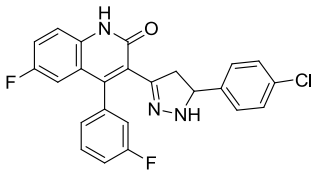


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (997-48f). Compound **(997-48f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3-fluorophenyl)-6-methoxyquinolin-2(1H)-one (0.250 g, 0.58 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, the organics were washed 3X with brine, filtered over magnesium sulfate followed by removal of the solvent *in vacuo* to yield the title compound as a yellow solid. Yield 0.147 g, 57%. ^{1}H NMR (400 MHz, , DMSO-d_6) δ 11.86 (s, 1H), 7.60 - 7.45 (m, 1H), 7.36 (d, *J* = 9.0 Hz, 1H), 7.34 - 7.28 (m, 3H), 7.25 - 7.15 (m, 3H), 7.12 (dd, *J* = 8.4, 2.0 Hz, 2H), 7.09 - 7.00 (m, 1H), 6.40 (t, *J* = 2.6 Hz, 1H), 4.67 - 4.56 (m, 1H), 3.59 (s, 3H), 3.49 - 3.27 (m, 1H), 2.68 - 2.43 (m, 1H). ^{13}C NMR (100 MHz, , DMSO-d_6) δ 160.86, 154.68, 146.58, 143.31, 133.42, 132.00, 130.82, 128.98, 128.90, 128.81, 128.66, 125.59, 120.49, 119.76, 117.38, 109.58, 62.63,

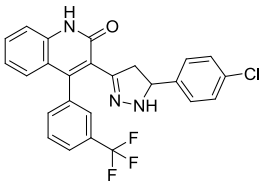
55.88, 45.22. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.59 - -113.71 (m). HRMS calcd for $C_{25}H_{19}O_2N_3ClFK$ $[M+K]^+$; 486.07814, found; 486.07837.



4-(4-Chlorophenyl)-3-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoroquinolin-2(1H)-one (997-49f). Compound **(997-49f)** was prepared according to general procedure F using (E)-4-(4-chlorophenyl)-3-(3-(4-chlorophenyl)acryloyl)-6-fluoroquinolin-2(1H)-one (0.300 g, 0.68 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, the organics were washed 3X with brine, filtered over magnesium sulfate followed by removal of the solvent *in vacuo* to yield the title compound as a yellow solid. Yield 0.211 g, 68%. ^{1}H NMR (600 MHz, DMSO-d6) δ 12.40 (s, 1H), 7.59 – 7.40 (m, 4H), 7.36 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.33 – 7.18 (m, 4H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.65 (dd, *J* = 9.9, 2.8 Hz, 1H), 4.66 – 4.59 (m, 1H), 3.32 (dd, *J* = 16.6, 11.1 Hz, 1H), 2.61 (dd, *J* = 16.5, 8.9 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.52, 157.67, 156.09, 147.34, 145.65, 142.56, 135.25, 134.37, 132.86, 131.40, 131.26, 130.66, 128.34, 128.29, 128.14, 126.86, 120.17, 120.11, 118.78, 118.61, 117.65, 117.60, 111.42, 111.27, 61.99, 44.36. ^{19}F NMR (376 MHz, DMSO-d_6) δ -120.67 - -120.78 (m). HRMS calcd for $C_{24}H_{17}ON_3Cl_2F$ $[M+H]^+$; 452.07272, found; 452.07348.

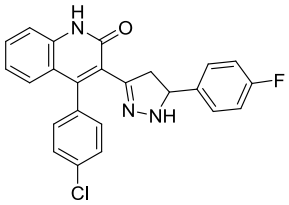


3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (997-50f). Compound **(997-50f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-6-fluoro-4-(3-fluorophenyl)quinolin-2(1H)-one (0.300 g, 0.68 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, the organics were washed 3X with brine, filtered over magnesium sulfate followed by removal of the solvent *in vacuo* to yield the title compound as a yellow solid. Yield 0.211 g, 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 7.67 - 6.99 (m, 11H), 6.65 (dt, J = 9.9, 2.3 Hz, 1H), 4.68 - 4.57 (m, 1H), 3.50 - 3.27 (m, 1H), 2.70 - 2.55 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.53, 158.08, 155.71, 147.12, 145.60, 142.61, 137.86, 137.78, 135.24, 131.40, 130.40, 130.32, 128.32, 128.25, 128.18, 126.81, 124.96, 120.11, 118.82, 118.58, 117.69, 115.68, 115.13, 111.44, 111.20, 62.01, 44.38. ^{19}F NMR (376 MHz, DMSO-d_6) δ -113.44 - -113.58 (m), -120.87 (td, J = 8.8, 3.2 Hz). HRMS calcd for $C_{24}H_{16}ON_3ClF_2K$ $[M+K]^+$; 474.05816, found; 474.05829.



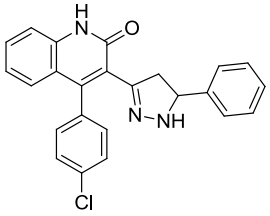
3-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(3-

(trifluoromethyl)phenyl)quinolin-2(1H)-one (997-51f). Compound **(997-51f)** was prepared according to general procedure F using (E)-3-(3-(4-chlorophenyl)acryloyl)-4-(3-(trifluoromethyl)phenyl)quinolin-2(1H)-one (0.300 g, 0.68 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, the organics were washed 3X with brine, filtered over magnesium sulfate followed by removal of the solvent *in vacuo* to yield the title compound as a yellow solid. Yield 0.330 g, 80%. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.85 - 7.60 (m, 3H), 7.57 - 7.32 (m, 3H), 7.28 (dd, *J* = 13.2, 8.0 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 7.09 - 6.94 (m, 2H), 6.88 (dd, *J* = 8.1, 4.3 Hz, 1H), 4.60 (dd, *J* = 11.9, 8.4 Hz, 1H), 3.48 - 3.31 (m, 1H), 2.67 - 2.53 (m, 1H). HRMS calcd for $C_{25}H_{18}ON_3ClF_3$ $[M+H]^+$; 468.10850, found; 468.10800.



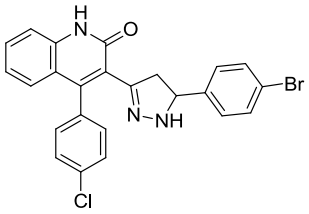
4-(4-Chlorophenyl)-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-52f). Compound **(997-52f)** was prepared according to general procedure F using (E)-4-(4-chlorophenyl)-3-(3-(4-fluorophenyl)acryloyl)quinolin-2(1H)-one (0.25 g, 0.62 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, DCM was added, and the resultant solid was filtered to yield the title compound as a yellow solid. Yield 0.150 g, 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 7.59 - 7.54 (m, 1H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.35 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.25 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.21 - 7.04 (m, 6H), 6.99 (d, *J* = 8.1 Hz, 1H),

4.67 - 4.57 (m, 1H), 3.30 (dd, *J* = 16.5, 10.9 Hz, 1H), 2.60 (dd, *J* = 16.5, 9.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.69, 148.35, 145.84, 139.76, 138.23, 134.80, 132.66, 131.33, 130.74, 130.65, 128.39, 128.31, 128.20, 126.88, 125.71, 122.10, 119.39, 115.39, 115.01, 114.79, 62.02, 44.56. ^{19}F NMR (376 MHz, DMSO-d_6) δ -115.35 - -115.47 (m). HRMS calcd for $C_{24}H_{18}ON_3ClF$ $[M+H]^+$; 418.11169, found; 418.11180.

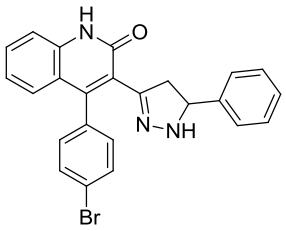


4-(4-Chlorophenyl)-3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-53f). Compound **997-53f** was prepared according to general procedure F using 4-(4-chlorophenyl)-3-cinnamoylquinolin-2(1H)-one (0.30 g, 0.78 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, MeOH was added and the resultant solid was filtered to yield the title compound as a yellow solid. Yield 0.280 g, 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 7.57 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.53 (d, *J* = 7.1 Hz, 1H), 7.52 - 7.48 (m, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.36 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.31 - 7.18 (m, 4H), 7.16 (s, 1H), 7.14 - 7.06 (m, 3H), 6.99 (d, *J* = 7.9 Hz, 1H), 4.60 (t, *J* = 10.2 Hz, 1H), 3.37 - 3.23 (m, 1H), 2.61 (dd, *J* = 16.5, 9.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.65, 148.29, 145.80, 143.54, 138.20, 134.78, 132.62, 131.35, 130.70, 130.59, 128.20, 128.15, 126.91, 126.49, 126.44, 126.39, 125.77, 122.05,

119.38, 115.35, 62.78, 44.57. HRMS calcd for $C_{24}H_{17}ON_3Cl$ $[M+H-H_2]^+$; 398.10547, found; 398.10549.

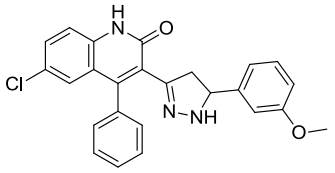


3-(5-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-(4-chlorophenyl)quinolin-2(1H)-one (997-54f). Compound **997-54f** was prepared according to general procedure F using (E)-3-(3-(4-bromophenyl)acryloyl)-4-(4-chlorophenyl)quinolin-2(1H)-one (0.30 g, 0.65 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, MeOH was added and the resultant solid was filtered to yield the title compound as a yellow solid. Yield, 0.190 g, 62%. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.65, 148.38, 145.80, 143.08, 138.23, 134.76, 132.67, 131.33, 131.09, 131.07, 131.02, 130.70, 128.67, 128.22, 128.14, 126.88, 126.83, 125.63, 122.09, 119.88, 119.37, 115.38, 62.04, 61.94, 44.47. HRMS calcd for $C_{24}H_{17}ON_3Cl$ $[M+H]^+$; 478.03163, found; 478.03228.



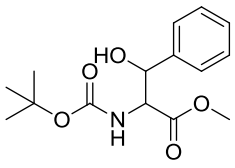
4-(4-Bromophenyl)-3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (997-55f). Compound **997-55f** was prepared according to general procedure F using 4-(4-bromophenyl)-3-cinnamoylquinolin-2(1H)-one (0.25 g, 0.58 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, MeOH was added

and the resultant solid was filtered to yield the title compound as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 7.71 (dd, J = 8.1, 2.4 Hz, 1H), 7.63 (dd, J = 8.1, 2.3 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 8.3 Hz, 1H), 7.33 - 7.21 (m, 4H), 7.20 - 7.15 (m, 2H), 7.15 - 7.06 (m, 3H), 6.99 (d, J = 8.0 Hz, 1H), 4.66 - 4.55 (m, 1H), 3.39 - 3.26 (m, 1H), 2.60 (dd, J = 16.5, 9.1 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.67, 148.30, 145.75, 143.60, 138.22, 135.17, 131.66, 131.33, 131.07, 131.00, 130.60, 128.22, 126.91, 126.84, 126.45, 125.72, 122.07, 121.31, 119.33, 115.37, 62.77, 44.59. HRMS calcd for $C_{24}H_{17}ON_3Br$ $[M+H-H_2]^+$; 442.05495, found; 442.05458.

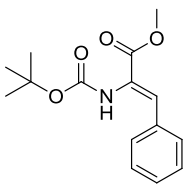


6-Chloro-3-(5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-56f). Compound **997-56f** was prepared according to general procedure F using (E)-6-chloro-3-(3-(3-methoxyphenyl)acryloyl)-4-phenylquinolin-2(1H)-one (0.30 g, 0.72 mmol). Upon completion of the reaction, the ethanol was removed under vacuum, MeOH was added and the resultant solid was filtered to yield the title compound as a yellow solid. Yield 0.180 g, 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.70 – 7.36 (m, 6H), 7.37 – 7.06 (m, 4H), 7.01 – 6.73 (m, 2H), 6.70 – 6.63 (m, 1H), 4.63 – 4.48 (m, 1H), 3.85 – 3.63 (m, 4H), 3.25 – 3.11 (m, 0.5H), 2.66 – 2.53 (m, 0.5H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.05, 160.56, 159.20, 148.35, 145.61, 145.03, 137.00, 135.23, 130.41, 129.32, 129.26, 129.06, 128.79, 128.24, 126.79, 125.78, 125.69, 125.57, 120.87, 118.78, 118.67, 117.38, 112.59, 112.47, 112.05, 111.92, 63.06, 62.89, 55.02, 54.89, 44.40, 44.36. HRMS calcd for $C_{25}H_{21}O_2N_3Cl$ $[M+H]^+$; 430.13168; found;

430.13141.

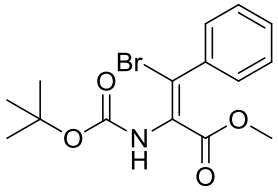


Methyl 2-(tert-butoxycarbonylamino)-3-hydroxy-3-phenylpropanoate. In a 250 mL round-bottomed flask, methyl 2-amino-3-hydroxy-3-phenylpropanoate (0.300 g, 1.537 mmol), 4-dimethylaminopyridine (0.038 g, 0.307 mmol) and BOC-Anhydride (0.714 ml, 3.07 mmol) were dissolved in Pyridine (15.37 ml). The reaction was stirred for 4 hours until TLC/MS indicated completion. 2N HCl was diluted 3-fold and used to wash the reaction mixture after removal of the pyridine *in vacuo* and re-solvation in DCM. Sodium bicarbonate was then used to wash the DCM. The organics were dried over magnesium sulfate, dried *in vacuo* and determined to be the mono-Boc protected product. Yield 0.380 g, 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 – 7.19 (m, 5H), 6.63 (d, J = 9.0 Hz, 1H), 5.78 – 5.64 (m, 1H), 5.09 – 4.98 (m, 1H), 4.32 (dd, J = 9.2, 3.9 Hz, 1H), 3.61 (d, J = 3.0 Hz, 3H), 1.28 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.12, 155.38, 149.62, 141.52, 127.86, 127.17, 126.10, 123.91, 78.43, 72.28, 60.19, 51.91, 40.18, 28.03. HRMS calcd for $C_{15}H_{22}O_5N$ $[M+H]^+$; 296.14925; found; 196.14995.



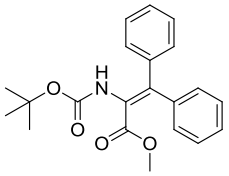
(*Z*)-Methyl 2-(tert-butoxycarbonylamino)-3-phenylacrylate. In the 500 mL round-bottomed flask that was used to make the mono-Boc protected product, DMAP (0.786 g,

6.43 mmol) and methyl 2-(tert-butoxycarbonylamino)-3-hydroxy-3-phenylpropanoate (19 g, 64.3 mmol) in acetonitrile (129 ml) were added after the pyridine had been removed under vacuum, to give a orange solution. BOC-Anhydride (14.94 ml, 64.3 mmol) was added and the reaction mixture was stirred at room temperature, monitored by TLC with diethyl ether/hexanes (1:1) until all reactant was consumed (stirring overnight). 1,1,3,3-tetramethylguanidine (8.14 ml, 64.3 mmol) was then added and the mixture was stirred for three hours. After drying *in vacuo*, the residue was dissolved in ether and washed with dilute HCl, sodium bicarbonate, and brine. Removal of solvent afforded product. Analytics match literature (Ferreira et al., 2007).

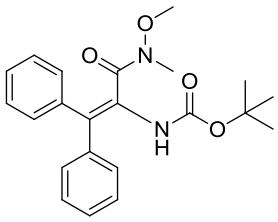


(Z)-Methyl 3-bromo-2-(tert-butoxycarbonylamino)-3-phenylacrylate. In a 500 mL round-bottomed flask (t=g) was (Z)-methyl 2-(tert-butoxycarbonylamino)-3-phenylacrylate (4.6 g, 16.59 mmol) and N-bromosuccinimide (3.54 g, 19.91 mmol) in DCM (166 ml). The mixture was stirred for 16 hours until TLC indicated that the starting material had been consumed. Triethylamine (3.47 ml, 24.88 mmol) was then added and the mixture was stirred for another hour. The mixture was diluted with DCM and washed 3x with brine. The isomers were seperated using an 80 g column (Pet ether (35 °C-60°C) : Diethyl ether 0-20%). (E)-methyl 3-bromo-2-(tert-butoxycarbonylamino)-3-phenylacrylate (1.00 g, 2.81 mmol, 51.3 % yield) and (Z)-methyl 3-bromo-2-(tert-butoxycarbonylamino)-3-phenylacrylate (1.90 g, 5.33 mmol, 48.7

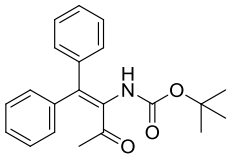
% yield) as a 2:1 mixture, 50% overall yield (Abreu et al., 2004). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.24 (m, 5H), 6.52 (s, 1H), 3.50 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 129.34, 129.15, 128.45, 28.30. HRMS calcd for $C_{15}H_{19}O_4NBr$ $[M+H]^+$; 356.04920; found; 356.05015.



Methyl 2-((tert-butoxycarbonyl)amino)-3,3-diphenylacrylate. To a solution of (Z)-methyl 3-bromo-2-((tert-butoxycarbonyl)amino)-3-phenylacrylate (6 g, 16.84 mmol) and phenylboronic acid (2.259 g, 18.53 mmol) in DME (135 ml) and Water (33.7 ml) and sodium carbonate (3.57 g, 33.7 mmol) were added with heating to 90°C. The mixture was stirred for four hours until TLC indicated completion. The DME was removed under vacuum and EtOAc was added to the vessel. The organics were washed 3X with water and brine, dried over magnesium sulfate and column chromatographed using a 0-20% gradient of EtOAc in hexanes to give methyl 2-((tert-butoxycarbonyl)amino)-3,3-diphenylacrylate as a white solid. Yield 3.69 g, 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.19 (m, 8H), 7.17 – 7.07 (m, 2H), 6.07 (s, 1H), 3.53 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.08, 129.34, 128.96, 128.51, 128.30, 128.05, 125.90, 28.37. HRMS calcd for $C_{21}H_{23}O_4NNa$ $[M+Na]^+$; 376.15193; found; 376.15180.

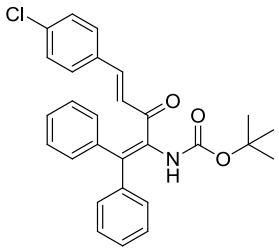


Tert-butyl (3-(methoxy(methyl)amino)-3-oxo-1,1-diphenylprop-1-en-2-yl)carbamate. In a 10 mL round-bottomed flask, N,O-dimethylhydroxylamine hydrochloride (0.290 g, 2.97 mmol) was added to a flame dried flask at -78°C under argon in THF (14.15 ml). N-butyllithium (1.188 ml, 2.97 mmol) was then added dropwise. Mixture was stirred for 5 min., ice bath removed for 15 min., and the bath replaced for 10 minutes prior to addition of methyl 2-((tert-butoxycarbonyl)amino)-3,3-diphenylacrylate (0.500 g, 1.415 mmol). Butyllithium (1.132 ml, 2.83 mmol) was then added to the reaction vessel dropwise. Upon completion of the addition, the reaction was determined to be complete and quenched at -78°C with the addition of 4 ml 1N HCl. The THF was removed under vacuum and the organics extracted from the HCl with DCM, washed with brine, dried over magnesium sulfate and columned with a 0-1% MeOH:DCM gradient. Yield 0.240 g, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.15 (m, 10H), 6.36 – 5.89 (m, 1H), 3.58 (s, 1.5 H), 3.41 (s, 1.5 H), 3.08 (s, 1.5 H), 3.04 (s, 1.5H), 1.63 (s, 1H), 1.47 – 1.42 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.31, 129.93, 129.79, 129.10, 128.40, 128.20, 128.07, 59.70, 28.45, 28.41.

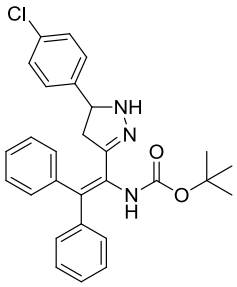


Tert-butyl (3-oxo-1,1-diphenylbut-1-en-2-yl)carbamate. Solution of tert-butyl (3-(methoxy(methyl)amino)-3-oxo-1,1-diphenylprop-1-en-2-yl)carbamate (0.880 g, 2.301 mmol) in THF (23.01 ml) added to flame-dried flask with stir bar. Cooled to 0ºC and stirred under argon. Methylmagnesium bromide (7.67 ml, 23.01 mmol) was added dropwise at 0ºC and the mixture was monitored by TLC. Quenched dropwise with 5%

HCl. Yield 0.300 g, 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 5H), 7.29 – 7.20 (m, 2H), 7.18 – 7.06 (m, 2H), 6.07 (s, 1H), 1.97 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.38, 130.16, 128.92, 128.78, 128.74, 30.26, 28.42.

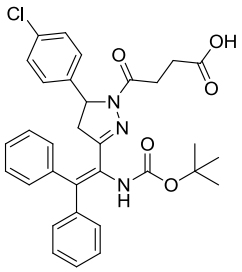


(*E*)-Tert-butyl (5-(4-chlorophenyl)-3-oxo-1,1-diphenylpenta-1,4-dien-2-yl)carbamate. In a 25 ml round bottom flask, the tert-butyl (3-oxo-1,1-diphenylbut-1-en-2-yl)carbamate (0.240 g, 0.711 mmol), 4-chlorobenzaldehyde (0.100 g, 0.711 mmol) were dissolved in EtOH/H2O (4:3) at 0°C. The reaction was stirred for seven hours. The reaction was quenched by slow addition of acetic acid (0.814 ml, 14.23 mmol) and filtered. Yield 0.250 g, 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.33 (m, 5H), 7.35 – 7.07 (m, 10H), 6.41 (d, *J* = 15.9 Hz, 1H), 6.13 (s, 1H), 1.49 – 1.39 (m, 9H).



Tert-butyl(1-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2-diphenylvinyl)carbamate. The (E)-tert-butyl (5-(4-chlorophenyl)-3-oxo-1,1-diphenylpenta-1,4-dien-2-yl)carbamate (0.55 g, 1.20 mmol) was dissolved in ethanol (10.00) with the hydrazine monohydrate (0.64 g, 1.20 mmol The mixture was

microwaved for 20 minutes at 110 °C. The reaction was checked by TLC, and LC-MS and was determined to be complete. The ethanol was removed under vacuum, DCM was added, the organics washed three times with brine, dried over magnesium sulfate and concentrated before column chromatography using a 0-5% MeOH-DCM gradient. Yield 0.332 g, 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.06 (m, 14H), 6.13 (s, 1H), 5.85 (s, 1H), 4.68 – 4.58 (m, 1H), 2.58 – 2.44 (m, 1H), 2.14 – 2.01 (m, 1H), 1.49 – 1.32 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.33, 130.84, 130.38, 130.17, 129.92, 128.92, 128.74, 128.38, 128.25, 128.13, 128.01, 127.57, 64.71, 42.61, 28.42, 28.34.



4-(3-(1-((Tert-butoxycarbonyl)amino)-2,2-diphenylvinyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid. In a 20 mL microwaveable vial, dihydrofuran-2,5-dione (63.3 mg, 0.633 mmol) and tert-butyl (1-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2-diphenylvinyl)carbamate (300 mg, 0.633 mmol) were dissolved in Tetrahydrofuran (6.5 ml) with molecular sieves. The reaction was heated to 165 °C for 20 minutes. Upon completion, the THF was removed under vacuum and the residue was partitioned between DCM and brine. The organics were washed three times with brine, dried over magnesium sulfate, concentrated and subjected to column chromatography using a 0-8% MeOH:DCM gradient. The title compound was obtained as a yellow solid. Yield 0.290 g, 80%. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.81 (s, 1H),

8.67 (s, 1H), 7.40 – 7.00 (m, 13H), 6.97 (d, J = 7.3 Hz, 1H), 5.32 – 5.21 (m, 1H), 2.95 (dd, J = 17.8, 11.8 Hz, 1H), 2.80 – 2.41 (m, 2H), 2.44 – 2.28 (m, 2H), 1.98 – 1.83 (m, 1H), 1.51 – 1.10 (m, 9H). ^{13}C NMR (100 MHz, dmso) δ 174.28, 169.47, 141.57, 141.33, 140.99, 132.27, 130.50, 130.38, 130.29, 130.10, 129.65, 129.58, 129.29, 129.25, 129.19, 129.10, 128.94, 128.93, 128.89, 128.73, 128.59, 128.32, 128.27, 128.22, 128.02, 126.62, 79.39, 59.43, 43.91, 29.32, 28.95, 28.73, 28.72.

Chapter 5. QSAR and ROCS computational modeling

5.1. Abstract

The observation that the QNZ-class of compounds and the DQP-class of compounds share both structural determinants of activity and a mechanism of action led me to explore how similar the molecules are computationally (Acker et al., 2011; Hansen and Traynelis, 2011). A hybrid compound was designed in order to evaluate whether functionality from the QNZ class of molecules which improved potency within that class could improve potency when the same functionality was installed on the DQP-class of compounds. Furthermore, the published data for the QNZ class of compounds, with an extensive SAR data surrounding the scaffold, was used to attempt QSAR modeling of the pharmacophore and *in silico* screening for compounds that have novel scaffolds.

5.2. Introduction

Ionotropic glutamate receptors mediate excitatory synaptic transmission in the mammalian central nervous system (CNS). The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors comprise the family (Mayer, 2005; Traynelis et al., 2010). The NMDA receptor family is responsible for the time-course of deactivation for glutamate-evoked currents throughout the central nervous system and are involved in important physiological processes including axonal guidance, synaptic plasticity, and memory formation (Cull-Candy and Leszkiewicz, 2004; Pérez-Otaño and Ehlers, 2005; Traynelis et al., 2010; Wang et al., 2011).

Functional NMDA receptor subunit composition and expression vary both temporally and spatially throughout development (Akazawa et al., 1994; Bräuner-Osborne et al., 2000; Galvan and Wichmann, 2008; Hallett and Standaert, 2004; Laurie and Seeburg, 1994; Monyer et al., 1994; Standaert et al., 1993; Standaert et al., 1994). Subunit-selective modulators of the NMDA receptor are particularly interesting from a drug-development perspective for neurological disorders, including stroke, schizophrenia, treatment resistant depression, Parkinson's disease due to the unique pharmacology and differential expression of the various subtypes throughout the CNS (Bräuner-Osborne et al., 2000; Chen and Lipton, 2006; Goff et al., 2008; Hallett and Standaert, 2004). Subunit-selective modulators of the NMDA receptor should limit side effects that occur as a result of global antagonism of the NMDA receptor.

To this end, we have been focused on understanding and improving the activity of small molecules which target GluN2C- and GluN2D-containing receptors for several years (Mosley et al., 2010, Santangelo et al., 2013, Acker et al., 2013). To date, our efforts have yielded the most potent and selective negative allosteric modulators of the GluN2C- and GluN2D-containing receptors that have been reported (Acker et al., 2013). However, in the absence of crystallographic information regarding the binding site, rational medicinal chemistry efforts aimed at improving the physico-chemical properties in order to achieve broad *in vivo* applications have been limited. A full-length crystal structure of the AMPA receptor has been resolved and numerous studies have reported x-ray crystallographic data from isolated ligand binding domains of the various subunits from the NMDA receptor family (Furukawa et al., 2005; Sobolevsky et al., 2009; Vance et al., 2011). Unfortunately, the QNZ and DQP classes of compounds have structural

determinants of activity at the lower lobe of the D2 domain within the ligand binding domain, in close proximity to the transmembrane linker regions (Acker et al., 2011; Hansen and Traynelis, 2011). This region was poorly resolved or not visible in the AMPA receptor x-ray crystallographic study and is replaced with a glycine-threonine linker in the isolated NMDA ligand binding domain studies (Furukawa and Gouaux, 2003; Sobolevsky et al., 2009; Vance KM, 2011). The absence of crystallographic data at this portion of the receptor or at a closely related homologous receptor was thought to be prohibitive in terms of docking the compounds as a means of elucidating binding modes.

However, intrigued by the shared structural determinants of activity and similar mechanism of action for the two distinct classes of molecules, I hypothesized that the compounds could bind to an overlapping site at the receptor, as has been observed for distinct classes of molecules that act at the closely related AMPA receptors (Menniti et al., 2000). I first compared the overall shape (Gaussian function for overlapping volume) and color (Gaussian function for atom-type or atom-similarity; i.e. -NH and -OH are both hydrogen bond donors and acceptors and therefore the fitting function will try and overlay these similar "colors" in conjunction with the overall shape) similarities of the two classes using ROCS software (OpenEye) and designed and synthesized a hybrid compound which contained a moiety that had improved potency in one of the classes (Grant et al., 1996; Nicholls et al., 2010).

Next, I utilized the published SAR data from the QNZ class of compounds to build QSAR models in an attempt to both define the pharmacophore for the class and to expand the chemical space of modulatory ligands available by identifying novel scaffolds

through *in silico* screening (Mosley et al., 2010). One other study utilized the same data set to build QSAR models around this class of molecules (Radchenko et al., 2012). That report, however, did not attempt to validate the models they had built with external data sets, rather was focused on determining the factors controlling selectivity and homology modeling of a potential binding site (Radchenko et al., 2012).

While the attempts to engineer a hybrid compound did not bring drastic changes in potency which might suggest exact atomic contacts at the receptor were being shared, the substitution was tolerated and did improve potency over the parent compound, suggesting that the binding pocket is similar in nature, if not shared at the receptor. The QSAR models derived from the previously published data were validated for compounds that share a similar backbone and identified numerous novel scaffolds using *in silico* screening, as potential novel modulators.

5.3. Results

5.3.a. Tanimoto comparison of distinct classes and synthesis of hybrid compounds

The structural determinants of activity at the receptor for both classes of compounds reside in the lower D2 portion of the ligand binding domain (Acker et al., 2011; Hansen and Traynelis, 2011). I hypothesized that the compounds might have shape and electrostatic similarities that could lead to the design of novel chemical entities containing the most preferential pharmacophore features from each class of compound. The structures of compound 1936 and 987-8 were compared for their overall Tanimoto similarity with both 1179 *S* and 1179 *R* using ROCS software (OpenEye). The energy minimized conformation of 1179 *S* (Relative potential energy OPLS-2005, water solvated; 0.00) was queried against the database of low-energy conformations available

for compound 1936, the quinolone core was overlapped with the hydroxyl–napthyl moiety of 1936 and the quinazilinone-4-one core overlaid with the *para*-bromine phenyl moiety of 1179*S* (Tanimoto combo score 0.733, **Figure 5.1**). Similar results were found when the energy minimized conformation of 1179*R* (Relative potential energy OPLS-2005, water solvated; 0.00) was queried against the database of 1936 conformers (Tanimoto combo score 0.734, **Figure 5.2**).

Figure 5.1. ROCS overlay of energy minimized conformation of 1179 *S* with the most similar 1936 conformation found in ROCS.

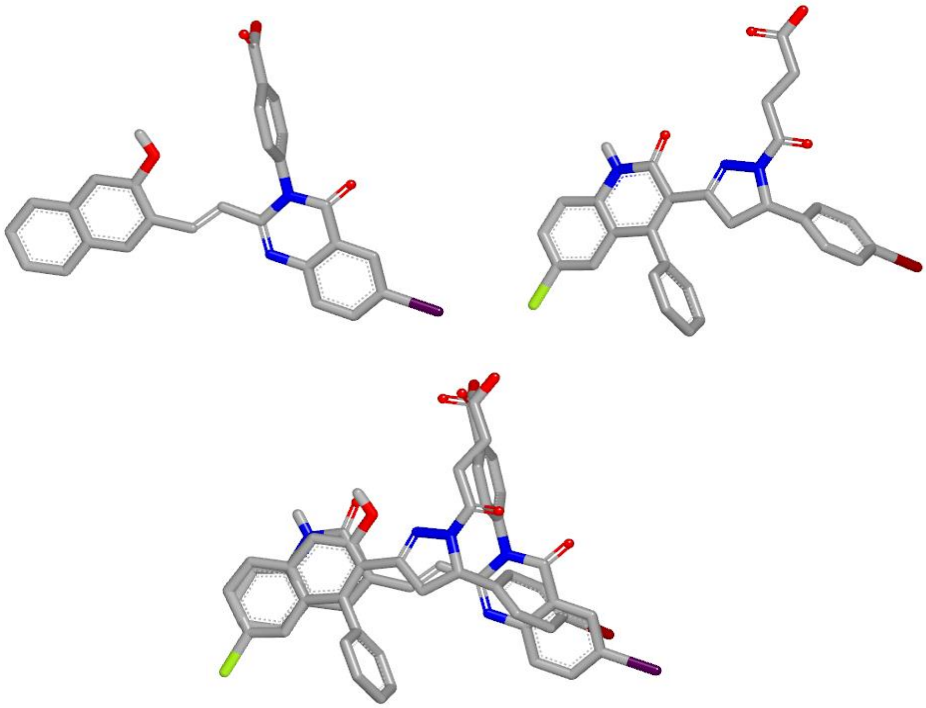


Figure 5.1. 1179*S* (top-right) energy minimized conformation overlaid with the most similar 1936 conformation found when the available conformers were used as a database (combo Tanimoto 0.733).

Figure 5.2. ROCS overlay of energy minimized conformation of 1179 *R* with most similar conformation of 1936.

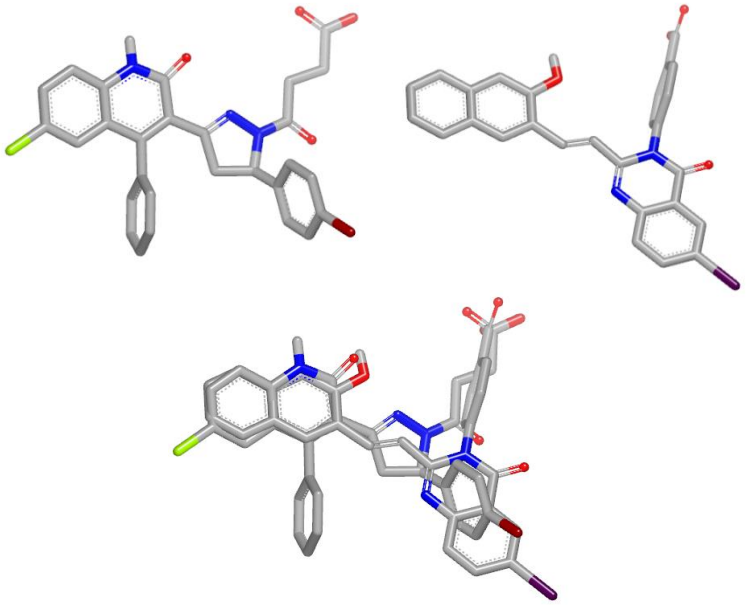


Figure 5.2. 1179R energy minimized structure (top-left) overlaid with the most similar 1936 conformation found (top-rigth) when the available conformers were used as a database (combo Tanimoto score 0.734).

Figure 5.3. ROCS overlay of energy minimized compound 1936 and most similar 1179 *S* conformation.

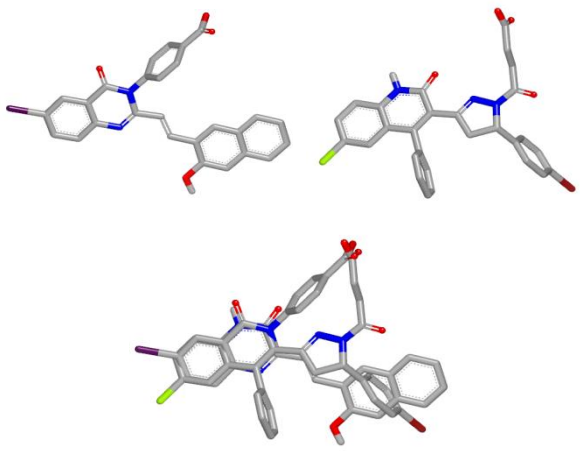


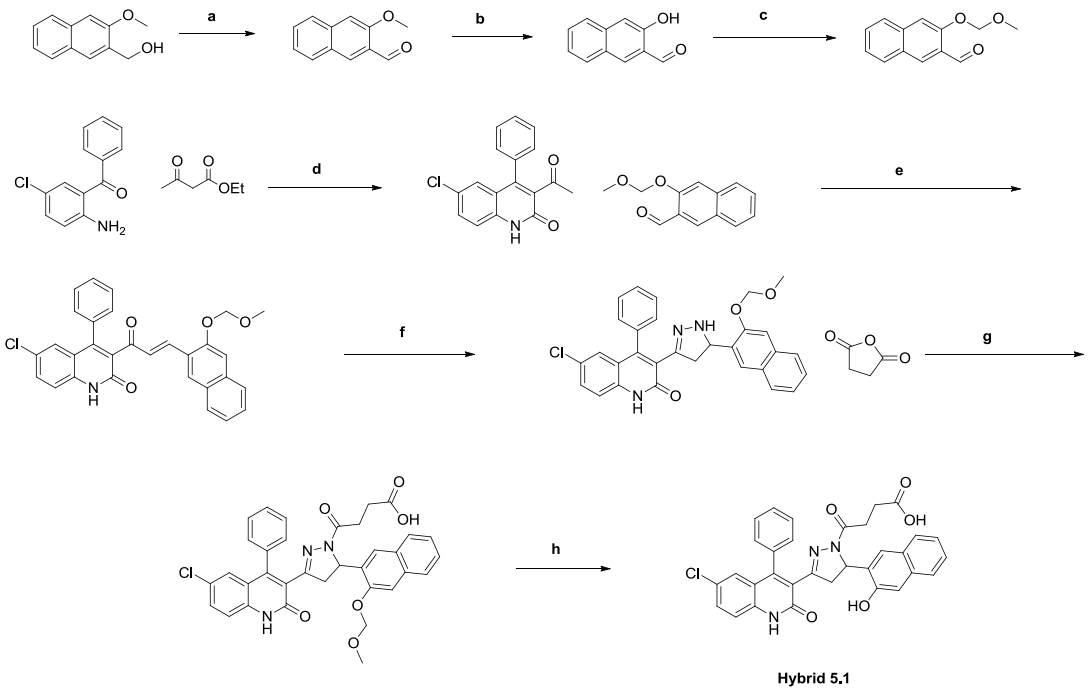
Figure 5.3. 1936 energy minimized structure overlaid (Relative potential energy OPLS-2005) with the most similar 1179*S* conformation found when the available conformers were used as a database (Tanimoto combo score of 0.681).

Using the energy minimized structure of 1936 (Relative potential energy OPLS-2005; 0.00) and assessing the overall similarity to a database of 1179 *S* low-energy conformations overlaid the quinolone and quinazilinone-4-one cores; the hydroxyl–napthyl moiety of 1936 also overlaid with the *para*-bromine phenyl moiety of 1179*S* (Tanimoto combo score of 0.681 **Figure 5.3**). Similar results were found when the database of 1179 *R* conformations was queried against the same energy minimized query for 1936 (Relative potential energy OPLS-2005, water solvated; 0.00) (Tanimoto combo score of 0.721). Neither the query using the energy minimized conformations of the 1179 compounds, nor the query of the energy minimized 1936 found the lowest energy conformation within the databases being mined. I also evaluated the overall similarity of compound 987-8 to that of 1179*S* (Tanimoto combo score 0.696) and 1179*R* (Tanimoto combo score 0.696), and all overlays suggested that the *meta*-substituted NO_2-phenyl moiety of 987-8 overlaid with the *para*-substituted phenyl ring of the 1179 molecules (data not shown).

I hypothesized that the hydroxyl-napthyl moiety of 1936 could occupy a similar space at the receptor as the A-ring of compound 1179 at the binding site of the receptor. I therefore designed a dihydroquinilone-pyrazoline compound that contained this moiety (997-01; **Scheme 5.1**). The synthesis of the compound began with the oxidation of (3-methoxynaphthalen-2-yl)methanol with PCC (**Scheme 5.1**). Subsequent de-protection of the OMe with BBr_3 gave compound 997-01b, and protection with MOM-Cl gave compound 997-01c. Condensation conditions with 3-acetyl-6-chloro-4-phenylquinolin-2(*1H*)-one gave the α-β-unsaturated methyl ketone pre-cursor (997-01d) to the pyrazoline containing compound which is accessed by treatment with hydrazine monohydrate (997-

01e). Acylation of the pyrazoline with succinic anhydride gave compound 997-01f, which could be de-protected under acidic conditions to give compound 997-01. The potency of the compound at GluN2D-containing receptors was similar to the parent compound, 1179, suggesting that the hybrid compound did not share the exact atomic contacts at the receptor with 1936 (**Table 5.1**).

Scheme 5.1. Synthesis of hybrid compound.



Scheme 5.1. Synthesis conditions for hybrid compound. (a) PCC, DCM. (b) BBr_3, Solvent. (c) i. Huning's base ii. MOM-Cl. (d) DMF, µW, molecular sieves. (e) KOH/EtOH/H_2O. (f) Hydrazine monohydrate, MeOH, µW. (f) i. HCl, ii. NaOH.

Table 5.1. Potency of novel hydroxy-napthyl containing compounds at recombinant NMDA receptors.

QNZ	2A IC_{50} / 2D IC_{50}	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
1936	9	8	0.66	0.58	0.094	0.074
1978	5	4	0.53	0.39	0.083	0.100
1179	NA	NA	-	-	7.0	2.7
Hybrid-5.1	16	6	41	15	3.9	2.6

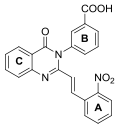
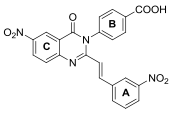
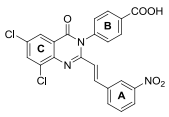
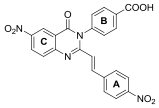
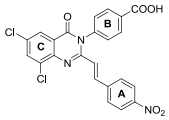
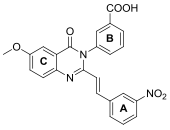
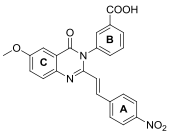
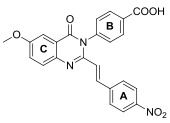
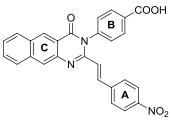
Table 5.1. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 7-20 oocytes between 2-4 frogs.

I also synthesized a number of analogues within the 987 series of compounds utilizing synthetic methodology developed by Dr. Cara Mosley (Mosley et al., 2010). These compounds were designed to systematically explore the SAR of this class of compounds and the results for the compounds that I synthesized are summarized in **Table 5.2** and described in the chemistry experimental section. Several of these compounds were utilized in designing the QSAR models described below.

5.3.b. QSAR Modeling

Analysis of the published SAR for the QNZ-class of molecules led to the rational design and synthesis of compounds 1936 and 1978 as potentially improved congeners within the QNZ class of compounds (Traynelis lab; **Figure 5.4**). These hydroxy-napthyl containing compounds were the most potent congeners identified in the QNZ series with potencies of 0.074 μM and 0.100 μM respectively at GluN2D-containing receptors (**Table 5.1**). The observed selectivity for GluN2D-containing receptors over GluN2A- and GluN2B-containing compounds, however, was diminished over other congeners in the class (**Table 5.1**). The QSAR models for the QNZ series of compounds at GluN2D receptors were derived from the IC_{50} values that have been previously reported and including the newly discovered compound 1936 (Acker et al., 2011; Mosley et al., 2010). The QSAR model chosen for the QNZ class of molecules has six features (ANRRRR) in the pharmacophore, a Q^2 of 0.509 and an R^2 of 0.888 for the 4 partial least squares fit that was used for the screening (**Figure 5.4; Tables 5.3, 5.4** and **5.5**). Compounds from the Zinc library, Princeton database, Chembridge library and compound 1978 were screened.

Table 5.2. Structural modifications made to the QNZ class of molecules.

QNZ-	Structure	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
987-25		17.0	19.7	8.2	6.7
987-29		-	-	204	90
987-30		224	109.0	16.0	10.0
987-33		-	-	266.0	79.0
987-34		-	-	9.2	5.4
987-46		208.0	378.0	23.8	13.0
987-47		238.0	238.0	21.4	15.7
987-48		197.0	206.0	13.4	7.2
987-51		-	-	10.4	14.4

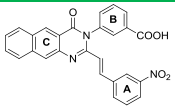
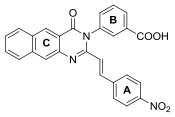
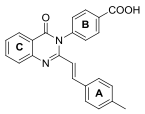
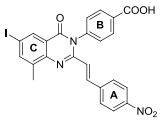
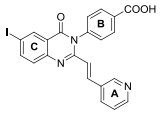
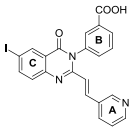
987-52		136.0	31.0	10.8	4.4
987-53		-	62.0	9.8	9.0
987-58		41	138.0	28.0	17.0
987-59		-	-	-	-
987-56		26.0	31.0	5.7	3.9
987-57		45.0	49.0	7.4	4.3

Table 5.2. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 4-12 oocytes between 1-43 frogs; - indicates less than 30% inhibition at 100 μM.

Figure 5.4. Schematic representation of QSAR model.

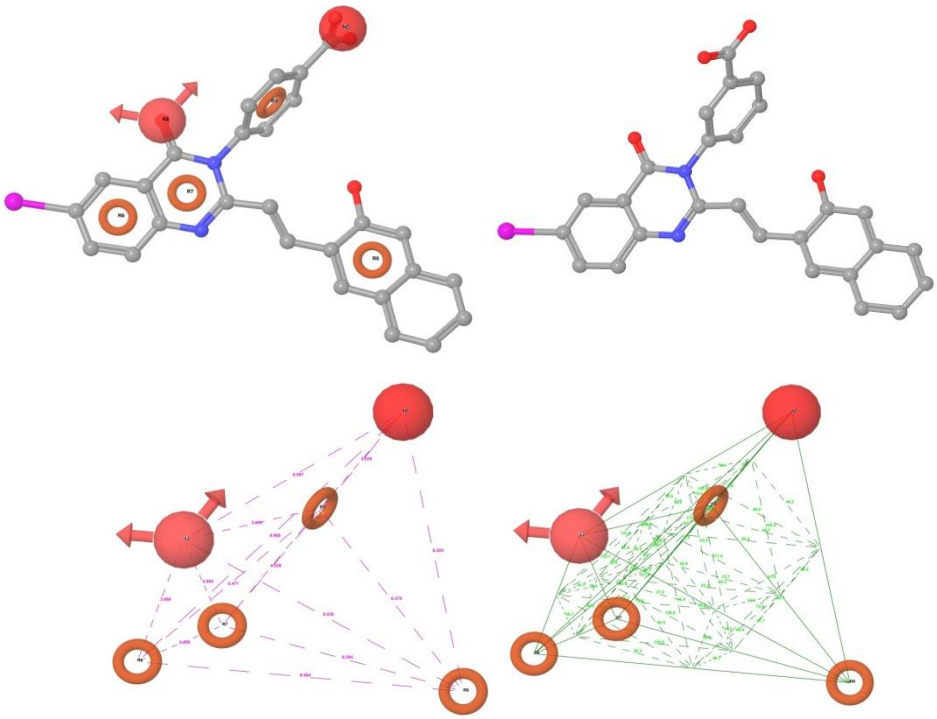


Figure 5.4. A. Compound 1936 is shown overlaid with the pharmacophore features of the QSAR developed from the published SAR (data include IC_{50} values for 1936). B. Compound 1978 is shown, which is a positional isomer of 1936 (*meta*-carboxylic acid vs, *para*-carboxylic acid respectively). C. QSAR model with distances between pharmacophores (see Table 5.5). D. QSAR model with angles between pharmacophore features (see table 5.5).

Compound 1978 was predicted to be active at 6 µM, which is considerably less potent than its measured value at GluN2D containing receptors (0.100 µM, **Table 5.1**). These results were considered to be reasonable, however, as only compound 1936 and one other compound had sub micro-molar activity within the QSAR model. The first series of compounds purchased were selected from the ZINC library screen and matched five pharmacophore features of the QSAR. These compounds were closely related to the quinazilinon-4-one backbone and were reasonably well predicted by the model (**Figure 5.5**).

Table 5.3. Analytical data for QSAR models derived from QNZ data set**.**

ID	# Factors	SD	R-squared	F	P	Stability	RMSE	Q-squared
AANRRR.4	4	0.277	0.887	78.5	2.1 -018	0.536	0.506	0.492
ANRRRR.35	4	0.262	0.899	89	2.3e-019	0.534	0.578	0.338
ANRRRR.32	4	0.264	0.897	87.1	3.4e-019	0.546	0.569	0.359
AANRRR.31	4	0.265	0.896	86	4.0e-019	0.527	0.583	0.327
AANRRR.32	4	0.267	0.895	85.4	4.9e-019	0.585	0.510	0.484
ANRRRR.36	4	0.277	0.888	78.9	2.0e-018	0.581	0.497	0.509

Table 5.3. Statistics and QSAR models derived from the QNZ data set, including IC_{50} data for compound 1936. Model ANRRRR.36 was chosen for the subsequent screening as it shows the most favorable combination of fitness.

Table 5.4. Ligand data set used to derive QSAR models.

Ligand	QSAR set	Activity $-\log(IC_{50})$	# Factors (PLS)	Predicted Activity $-\log(IC_{50})$
987.mol	test	-0.724	4	-1.7
987_5.mol	training	-0.342	4	-0.32
987_6.mol	training	-0.061	4	-0.23
987_08.mol	training	-0.53	4	-1.17
987_11.mol	training	-0.322	4	-0.46
987_13.mol	test	-0.971	4	-1.26
987_15.mol	training	-1.719	4	-1.96
987_16.mol	training	-2.117	4	-1.63
987_18.mol	training	-2.16	4	-2.11
987_19.mol	training	-2.477	4	-2.18
987_20.mol	training	-2.477	4	-2.53
987_23.mol	training	-0.803	4	-0.31
987_24.mol	training	-0.994	4	-1.47
987_25.mol	training	-0.826	4	-0.63
987_26.mol	training	-2.44	4	-2.36
987_27.mol	training	-2.17	4	-2.24
987_28.mol	test	-1.14	4	-1.1
987_29.mol	test	-1.954	4	-1.21
987_31.mol	training	-1.164	4	-1.14
987_32.mol	test	-0.963	4	-1.04
987_35.mol	test	-0.447	4	-0.33
987_36.mol	training	-0.826	4	-0.35
987_43.mol	training	-0.903	4	-0.77
987_44.mol	training	0.046	4	-0.37

987_45.mol	training	-0.531	4	-0.72
987_46.mol	training	-1.113	4	-0.98
987_47.mol	test	-1.2	4	-1.41
987_48.mol	test	-0.857	4	-1.71
1043.mol	training	-0.946	4	-0.87
1045.mol	test	-1.252	4	-1.29
1116.mol	test	0.222	4	-0.3
1159.mol	training	-1.72	4	-1.79
1160.mol	training	-1.164	4	-0.79
1206.mol	training	-1.176	4	-1.4
1208.mol	training	-1.424	4	-1.2
1427.mol	training	-2.233	4	-2.17
1436.mol	test	-1.561	4	-1.48
1437.mol	training	-0.771	4	-1.21
1443.mol	training	-1.204	4	-1.31
1453.mol	training	-1.176	4	-1.24
1455.mol	training	-1.491	4	-1.61
1456.mol	training	-1.176	4	-1.36
1457.mol	test	-1.447	4	-0.97
1460.mol	training	-2.447	4	-1.89
1461.mol	training	-0.963	4	-1.02
1463.mol	training	-1.529	4	-1.74
1465.mol	training	-1.362	4	-1.2
1465.mol	training	-1.36	4	-1.13
1466.mol	training	-1.23	4	-1.06
1467.mol	training	-1.204	4	-0.93
1468.mol	training	-1.342	4	-1.5

1470.mol	training	-0.415	4	-0.63
1472.mol	test	-2.477	4	-1.94
1493.mol	training	-2.462	4	-2.38
1494.mol	test	-2.269	4	-1.79
1503.mol	training	-2.225	4	-2.48
1504.mol	test	-2.245	4	-1.68
1505.mol	training	-2.477	4	-2.43

Table 5.4. Ligands, activity and related data used to build and validate the QSAR. The group assignment (training or test) is designated, as is the activity of the designated compound (Activity threshold set to -1.00)

Table 5.5. QSAR model ANRRRR.36 site measurements and angles.

Site1	Site2	Site3	Angle	Site1	Site2	Distance
N5	A2	R6	141.8	A2	N5	6.807
N5	A2	R7	100.5	A2	R6	3.688
N5	A2	R8	63.5	A2	R7	2.684
N5	A2	R9	18.6	A2	R8	8.536
R6	A2	R7	41.3	A2	R9	3.67
R6	A2	R8	78.3	N5	R6	9.968
R6	A2	R9	123.1	N5	R7	7.757
R7	A2	R8	37	N5	R8	8.205
R7	A2	R9	81.8	N5	R9	3.529
R8	A2	R9	44.9	R6	R7	2.438
A2	N5	R6	13.2	R6	R8	8.584
A2	N5	R7	19.9	R6	R9	6.471
A2	N5	R8	68.6	R7	R8	6.593
A2	N5	R9	19.4	R7	R9	4.228
R6	N5	R7	6.7	R8	R9	6.475
R6	N5	R8	55.4			
R6	N5	R9	6.3			
R7	N5	R8	48.7			
R7	N5	R9	0.6			
R8	N5	R9	49.2			
A2	R6	N5	25			
A2	R6	R7	46.7			
A2	R6	R8	76.8			

A2	R6	R9	28.4
N5	R6	R7	21.8
N5	R6	R8	51.8
N5	R6	R9	3.4
R7	R6	R8	30.3
R7	R6	R9	18.4
R8	R6	R9	48.5
A2	R7	N5	59.6
A2	R7	R6	92
A2	R7	R8	128.8
A2	R7	R9	59.2
N5	R7	R6	151.5
N5	R7	R8	69.2
N5	R7	R9	0.5
R6	R7	R8	139
R6	R7	R9	151.2
R8	R7	R9	69.6
A2	R8	N5	47.9
A2	R8	R6	24.9

Table 5.5. QSAR model ANRRRR.36 pharmacophore site angles and distances. Key: A= hydrogen-bond acceptor, N=negative, R=ring.

Figure 5.5. Compounds identified as active in the initial screening using QNZ-QSAR.

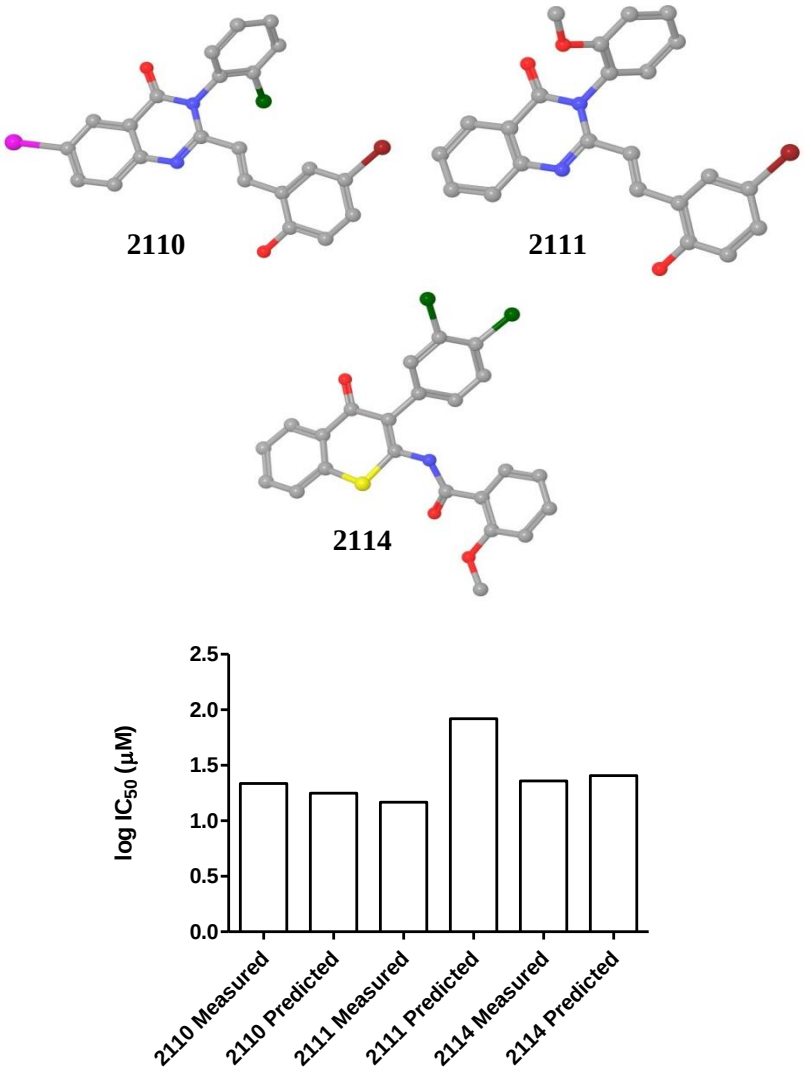


Figure 5.5. Compounds identified as active in the initial screening of the Princeton database using QNZ-QSAR ANRRRR.36. A. Compounds 2110 and 2111 (Top) share the quinazilinone-4-one backbone with the series of compounds that were used to develop the pharmacophore hypotheses and QSAR models. Compound 2114 contains a thiochromone backbone in place of the quinazilinone-4-one- backbone, a common medicinal chemistry isostere.

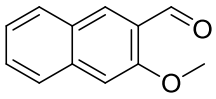
5.4. Discussion

The most important finding from these sets of experiments is a validated QSAR model that is presumed to be able to predict the activity of compounds that share the pharmacophore of the QNZ-class of molecules. The results of the screening performed could lead to novel chemical space that would allow for more desirable physico-chemical properties to be built into compounds sharing a binding site and mechanism of action. The comparison of the overall shape and color of the two classes of compounds supports the hypothesis that these compounds could act at a similar site on the receptor. Furthermore, it is tempting to speculate that the hydroxyl-napthyl moiety of compounds 1936 and 1978 could be positioned within the binding site where the quinolone core is for the DQP-class of compounds; in effect suggesting that compound 1936 contains a feature of the DQP-class of molecules.

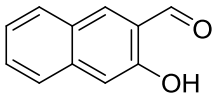
Expanding the number of known modulatory compounds that act at this site will allow for pharmacological studies to explore the contributions of GluN2C- and GluN2D-containing receptors in normal and patho-physiological conditions. Furthermore, elucidation of the binding mode for these compounds would be a great advancement in our ability to better design more drug-like molecules and could validate whether all or part of a binding site might be overlapping. In the absence of crystallographic information and/or higher affinity ligands, it will remain challenging to determine whether these compounds do indeed share a binding site at the receptor.

5.5. Chemistry Experimental

5.5a. Synthesis of hybrid molecule:

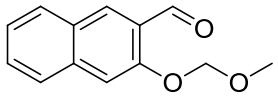


3-Methoxy-2-naphthaldehyde (997-01a). In a 100 mL round-bottomed flask, (3-methoxynaphthalen-2-yl)methanol (1.00 g, 5.31 mmol) in dichloromethane (40 ml) was added PCC (2.290 g, 10.63 mmol) in one portion. The reaction was stirred at room temperature for one hour, filtered over a pad of celite, rinsed with DCM, and concetrated *in vacuo* to give 3-methoxy-2-naphthaldehyde (0.880 g, 4.73 mmol, 89 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.37 (s, 1H), 8.05 (dd, J = 8.2, 1.1 Hz, 1H), 7.93 – 7.86 (m, 1H), 7.66 – 7.57 (m, 1H), 7.53 (s, 1H), 7.47 – 7.40 (m, 1H), 4.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.29, 199.19, 166.62, 146.78, 139.94, 139.86, 139.39, 138.88, 136.77, 134.72, 134.71, 134.22, 116.58, 65.50, 65.34. HRMS calcd for $C_{12}H_{11}O_2$ $[M+H]^+$; 187.07536 found; 187.07529.

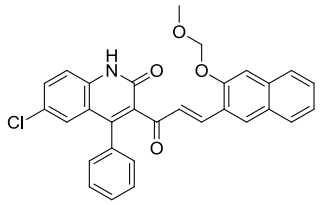


3-Hydroxy-2-naphthaldehyde (997-01b). In a 250 mL round-bottomed flask, 3-methoxy-2-naphthaldehyde (2.00 g, 10.74 mmol) was dissolved in DCM (107 ml) at -10 °C with a salt/ice bath. BBr_3 (1.218 ml, 12.89 mmol) was added drop-wise to the solution. The reaction was stirred, monitored by TLC, and sluggish. The reaction was quenched with MeOH, extracted with DCM, and washed with 1N HCl (50 mL), sat. sodium bicarbonate (50 mL) and brine. 300 mg of product and 1.4 g of statrting material were recovered by flash column chromatography using Hexanes:EtOAc (0-20%) . The

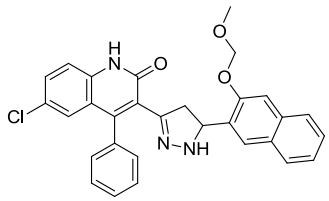
starting material was re-dissolved in DCM as above, and re-subjected to an excess of BBR3 (5ml). The reaction was quenched as above, extracted as above, and columned as above. The product was obtained and combined with the previously purified material. Yield 1.40 g, 76%. ^{1}H NMR 400 MHz (DMSO-d_6) δ 10.62 (s, 1H), 10.43 (s, 1H), 8.36 (s, 1H), 7.99 (d, J = 8.22, 1H), 7.78 (d, J = 8.61, 1H), 7.57-7.53 (m, 1H), 7.38-7.341 (m, 1H), 7.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.99, 155.78, 137.53, 129.36, 126.88, 124.24, 124.02, 110.63. HRMS calcd for $C_{11}H_9O_2$ $[M+H]^+$; 173.05971, found 173.05958.



3-(Methoxymethoxy)-2-naphthaldehyde (997-01c). In a 250 mL round-bottomed flask (t=g) was 3-hydroxy-2-naphthaldehyde (2.00 g, 11.62 mmol), MOM-Cl (0.882 ml, 11.62 mmol) and Hunig's base (2.029 ml, 11.62 mmol) were dissolved in DCM (116 ml). The mixture was refluxed for 30 minutes and cooled to room temperature. The mixture was then washed with 1% HCl and the organics collected, dried over magnesium sulfate and concentrated to give 3-(methoxymethoxy)-2-naphthaldehyde (2.12 g, 9.80 mmol, 84 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.37 (s, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.64 – 7.54 (m, 2H), 7.43 (ddd, J = 8.1, 6.8, 1.2 Hz, 1H), 5.44 (s, 2H), 3.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.74, 154.40, 136.88, 130.27, 129.77, 129.31, 127.74, 126.82, 125.64, 125.05, 110.28, 94.45, 56.13. HRMS calcd for $C_{13}H_{12}O_3Na$ $[M+Na]^+$; 239.06787, found 239.06758.

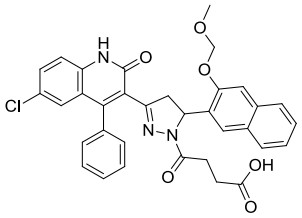


(E)-6-Chloro-3-(3-(3-(methoxymethoxy)naphthalen-2-yl)acryloyl)-4-phenylquinolin-2(1H)-one (997-01d). In a 250 mL round-bottomed flask , 3-acetyl-6-chloro-4-phenylquinolin-2(1H)-one (1.252 g, 4.20 mmol) and KOH (11.79 g, 210 mmol) were dissolved in EtOH (50.5 ml) and water (33.6 ml) at 0°C. 3-(methoxymethoxy)-2-naphthaldehyde (1.00 g, 4.62 mmol) was then added and the mixture was allowed to warm to room temperature. The reaction mixture was quenched with acetic acid and filtered to give (E)-6-chloro-3-(3-(3-(methoxymethoxy)naphthalen-2-yl)acryloyl)-4-phenylquinolin-2(1H)-one (1.500 g, 3.02 mmol, 71.9 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H), 8.31 (s, 1H), 7.92 – 7.59 (m, 4H), 7.55 – 7.30 (m, 9H), 7.08 – 6.98 (m, 2H), 5.37 (s, 2H), 3.35 (d, J = 1.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.05, 159.67, 152.75, 146.84, 137.87, 134.96, 133.61, 132.62, 131.15, 128.99, 128.60, 128.42, 126.15, 125.63, 124.56, 120.38, 118.06, 94.41, 55.95. HRMS calcd for $C_{30}H_{23}O_4NCl$ $[M+H]^+$; 496.13101, found 496.13126.



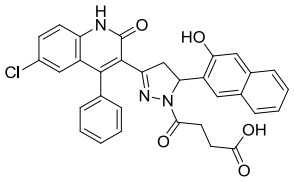
6-Chloro-3-(5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (997-01e). In a 20 ml microwaveable vessel, (E)-6-chloro-3-(3-(3-(methoxymethoxy)naphthalen-2-yl)acryloyl)-4-phenylquinolin-2(1H)-one (1.00

g, 2.016 mmol) and hydrazine monohydrate (0.117 ml, 2.420 mmol) were dissolved in Ethanol (15 ml). The mixture was heated in the microwave reactor to 110°C for 15 minutes. Upon completion there was a yellow solid in the vessel which was filtered and rinsed with ethanol. The solid was determined to be the desired product, 6-chloro-3-(5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.700 g, 1.373 mmol, 68.1 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.80 – 7.68 (m, 3H), 7.57 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.52 – 7.29 (m, 8H), 7.22 – 7.14 (m, 2H), 6.89 (d, *J* = 2.3 Hz, 1H), 5.34 (q, *J* = 6.6 Hz, 2H), 4.92 (td, *J* = 10.2, 3.3 Hz, 1H), 3.48 – 3.25 (m, 4H), 2.54 – 2.47 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.54, 152.23, 148.31, 145.84, 136.97, 135.23, 133.06, 132.92, 130.38, 129.08, 128.66, 128.44, 128.20, 128.14, 127.54, 126.76, 126.44, 125.96, 125.76, 125.63, 125.39, 123.96, 120.89, 117.34, 108.18, 93.76, 57.72, 55.85, 43.07. HRMS calcd for $C_{30}H_{25}O_3N_3Cl$ $[M+H]^+$; 510.15790, found 510.15800.



4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-01f). The 6-chloro-3-(5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-phenylquinolin-2(1H)-one (0.600 g, 1.177 mmol) was added to DMF (11.77 ml) in a microwaveable vessel with molecular sieves present. The reaction was heated with stirring to 180°C for ten minutes. The DMF was removed under vacuum, the

residue partitioned between DCM and brine, washed three times, dried over magnesium sulfate and concentrated to give 4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (0.300 g, 0.492 mmol, 41.8 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 12.09 (s, 1H), 7.82 – 7.55 (m, 3H), 7.45 (td, *J* = 19.4, 17.3, 7.9 Hz, 7H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.1 Hz, 2H), 5.61 (dd, *J* = 11.9, 4.7 Hz, 1H), 5.43 – 5.33 (m, 2H), 3.83 (dd, *J* = 18.3, 11.9 Hz, 1H), 3.44 (d, *J* = 1.7 Hz, 3H), 2.95 – 2.85 (m, 1H), 2.84 – 2.68 (m, 1H), 2.38 (dtd, *J* = 18.3, 12.5, 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.53, 152.25, 148.32, 145.85, 136.99, 135.24, 133.075, 132.93, 130.40, 129.10, 128.68, 128.46, 128.22, 127.55, 126.77, 125.98, 125.77, 125.64, 125.40, 123.98, 120.90, 117.35, 108.19, 93.77, 57.73, 55.86, 43.07. HRMS calcd for $C_{34}H_{28}O_6N_3ClNa$ $[M+Na]^+$; 632.15588, found 632.15922.



4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-hydroxynaphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (997-01). In a 100 mL round-bottomed flask, 4-(3-(6-chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-(methoxymethoxy)naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (0.400 g, 0.656 mmol) was dissolved in tetrahydrofuran (50 ml), HCl in MeOH was added until TLC indicated completion. The carboxylic acid was esterified in the reaction, so the THF was removed under vacuum and NaOH (2.62 ml, 2.62 mmol) in 10 ml MeOH was added with stirring for two hours until TLC indicated completion. The

reaction was neutralized with the slow addition of HCl and the MeOH removed under vacuum. The residue was dissolved in DCM and washed three times with brine. The organics were combined and dried over magnesium sulfate and concentrated under vacuum. 4-(3-(6-Chloro-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-5-(3-hydroxynaphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid was isolated as a yellow solid after column chromatography using 0-8% MeOH in DCM. Yield 0.075 g, 20.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 12.06 (s, 1H), 10.07 (s, 1H), 7.69 – 7.58 (m, 2H), 7.51 (t, J = 7.6 Hz, 1H), 7.40 (ddt, J = 20.7, 13.4, 5.9 Hz, 5H), 7.27 (q, J = 6.9 Hz, 2H), 7.14 (d, J = 10.2 Hz, 2H), 6.93 (d, J = 2.4 Hz, 1H), 6.84 (s, 1H), 5.54 (dd, J = 11.8, 4.5 Hz, 1H), 3.84 – 3.71 (m, 1H), 2.87 (dd, J = 18.2, 4.7 Hz, 1H), 2.69 (d, J = 6.4 Hz, 1H), 2.53 – 2.47 (m, 1H), 2.42 – 2.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.65, 169.47, 168.80, 161.51, 160.45, 160.13, 153.01, 152.36, 150.01, 137.35, 134.83, 133.58, 131.20, 129.87, 129.13, 128.44, 128.29, 127.77, 127.36, 126.13, 126.09, 125.94, 125.80, 125.54, 124.57, 123.54, 122.79, 120.68, 117.63, 108.77, 54.59, 44.41, 28.46, 28.36. HRMS calcd for $C_{32}H_{25}O_5N_3Cl$ $[M+H]^+$; 566.14733, found 566.14756. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.978 min; > 91% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.564 min; > 95% purity.

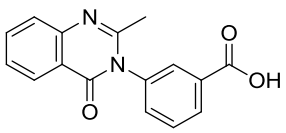
5.5.b. Synthesis of analogs in the QNZ-class

General procedure for synthesis of benzoxazine (5) intermediates (General procedure 5.A). The anthranilic acid (**4**, 1.0 mmol) was suspended in acetic anhydride (8.7 equiv). Upon refluxing all material went into solution, and the mixture was refluxed until TLC indicated the reaction was finished (generally 1 hr). The solvent was removed *in vacuo* to give a solid. The crude material was recrystallized using either hexanes or

hexanes and ethyl acetate. The product was collected by filtration and washed with hexanes (Mosley et al., 2010).

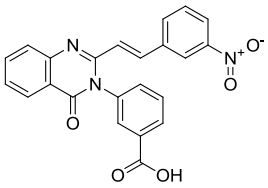
General procedure for synthesis of quinazolinone (987-1) intermediates (General procedure 5.B). The benzoxazine (**5**, 1.0 mmol) and aniline (**6**, 1.2 equiv) were dissolved in glacial acetic acid (56 equiv). The solution was refluxed until TLC indicated the reaction was finished (generally 6-12 hours). After cooling to room temperature, the mixture was concentrated *in vacuo* to give a solid. The crude material was recrystallized and the product was collected by filtration and washed with ethyl acetate (Mosley et al., 2010) .

General procedure for synthesis of (*E*)-3-phenyl-2-styrylquinazolin-4(3H)-one (987-2) products (General procedure 5.C). The quinazolinone (**7**, 1.0 mmol), benzaldehyde (**8**, 1.33 equiv), and sodium acetate (1.63 equiv) were suspended in a mixture of glacial acetic acid (58 equiv) and acetic anhydride (7.0 equiv). The mixture was refluxed until TLC indicated the reaction was finished (generally 12-18 hours). After cooling to room temperature, the mixture was filtered and washed with methanol. Further purification was performed (chromatography or recrystallization) as necessary (Mosley et al., 2010).

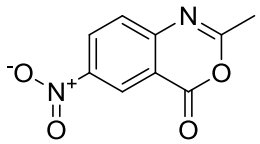


3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-25b). Compound **987-25b** was prepared by general method 5.B using 2-methyl-4H-benzo[d][1,3]oxazin-4-one (1.00g, 6.21 mmol) and 3-aminobenzoic acid (1.02g, 7.45 mmol, 1.20 equiv.). The solid was re-crystallized using Ethyl Acetate and Methanol to give a white solid (1.00 g, 57%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (bs, 1H), 8.01-8.12 (m, 2H), 8.02 (t, J =1.6, 1H), 7.83-7.87 (m, 1H), 7.66-7.76 (m, 3H), 7.50-7.54 (m, 1H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.58, 161.45, 154.13, 147.38, 138.19, 134.68, 133.08, 132.39, 130.02, 129.83, 129.53, 126.72, 126.48, 126.33, 120.49, 24.12. HRMS calculated for $C_{16}H_{13}N_2O_3$, $[M+H]^+$281.09123; found 281.09159.

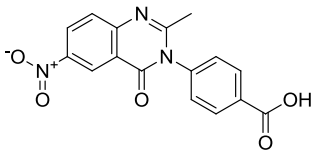


(*E*)-3-(2-(3-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-25). Compound **987-25** was prepared via procedure 5.C using **987-25b** (0.500 g, 1.78 mmol), and *meta*-nitrobenzaldehyde (0.359 g, 2.37 mmol, 1.3 equiv.), to give a yellow solid (0.409 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (bs, 1H), 8.10-8.18 (m, 3H), 8.04 (d, J = 8.2, 2H), 7.91 (t, J = 7.6, 1H), 7.81 (d, J = 7.8, 1H), 7.73-7.77 (m, 2H), 7.66-7.71 (m, 1H), 7.56-7.62 (m, 2H), 7.42 (d, J = 7.4, 1H), 6.34 (d, J = 15.7, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.56, 161.30, 150.47, 148.18, 147.15, 137.03, 134.94, 133.97, 133.40, 132.6, 130.46, 130.07, 129.92, 128.57, 127.47, 127.11, 126.53, 124.80, 124.45, 120.84. HRMS calculated for $C_{23}H_{16}N_3O_5$ $[M+H]^+$; 414.108; found 414.10778. Anal. ($C_{23}H_{15}N_3O_5$) C, H, N.

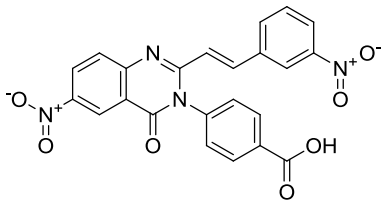


2-methyl-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one (987-29a). Compound **987-29a** was prepared via procedure 5.A using 2-amino-5-nitrobenzoic acid (2.00g, 11.0 mmol). The

product was purified by recrystallization in hexanes to give an orange solid. Yield 0.80 g, 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (d, *J* = 2.8, 1H), 8.61 (dd, *J* = 6.4, 2.4, 1H), 7.72 (d, *J* = 8.8, 1H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.802, 158.01, 150.88, 130.94, 128.35, 124.85, 117.39, 21.89.

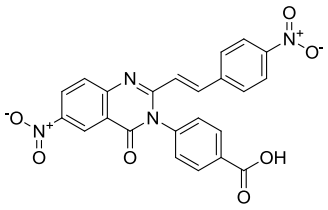


4-(2-methyl-6-nitro-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-29b). Compound **987-29b** was prepared via procedure 5.B using compound **987-29a** (0.80 g, 4.00 mmol) and *para*-aminobenzoic acid (0.60 g, 5.0 mmol, 1.2 equiv.). The crude material was re-crystallized with ethyl acetate and methanol (0.54 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (bs, 1H), 8.79 (d, *J* = 2.8, 1H), 8.59 (dd, J_1 = 6.8, 2.4, 1H), 7.87 (d, *J* = 8.8, 1H), 7.65 (dd, *J* = 4.8, 1.6, 2H), 2.19 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.64, 160.57, 158.14, 151.52, 144.80, 141.08, 131.72, 130.72, 128.78, 128.57, 122.44, 120.63.

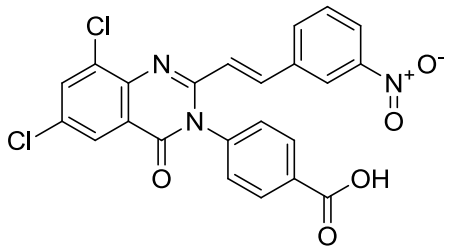


(*E*)-4-(6-nitro-2-(3-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid. Compound **987-29** was prepared via procedure 5.C using compound **987-29b** (0.250 g, 0.769 mmol) and 3-nitrobenzaldehyde (0.154 g, 1.33 equiv., 1.02 mmol). The crude material was purified by re-crystallization in Ethyl Acetate and Hexanes to give a yellow solid (0.321 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (bs, 1H), 8.84 (d, *J* = 3.0, 1H), 8.65

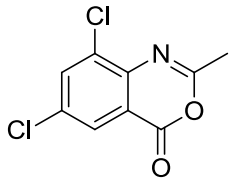
(dd, *J* = 9.0, 2.6, 1H), 8.32 (s, 1H), 8.15-8.21 (m, 4H), 7.98 (d, *J* = 9.0, 1H), 7.91 (d, *J* = 8.1, 1H), 7.63-7.77 (m, 3H), 6.54 (d, *J* = 15.4, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.68, 160.59, 153.93, 151.45, 148.30, 144.84, 140.01, 139.11, 136.19, 133.58, 131.88, 130.82, 130.59, 129.30, 129.02, 128.92, 124.51, 122.88, 122.58, 122.18, 120.81. HRMS calcd for $C_{23}H_{14}N_4O_7$, 458.0862; found 459.09363 $[M+H]^+$. Anal. ($C_{23}H_{14}N_4O_7$) C, H, N. MP > 260 °C.



(*E*)-4-(6-nitro-2-(4-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid (987-33). Compound **987-33** was prepared via procedure 5.C using compound **987-29b** (0.250 g, 0.769 mmol) and 3-nitrobenzaldehyde (0.154 g, 1.33 equiv., 1.02 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 8.81 (d, *J* = 2.7 Hz, 1H), 8.63 (dd, *J* = 9.0, 2.7 Hz, 1H), 8.19 (dd, *J* = 8.5, 2.5 Hz, 4H), 8.12 (dd, *J* = 15.4, 3.0 Hz, 1H), 7.99 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.73 (dd, *J* = 8.9, 2.7 Hz, 2H), 7.67 (dd, *J* = 8.4, 2.8 Hz, 2H), 6.55 (dd, *J* = 15.6, 3.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.74, 160.56, 153.78, 151.39, 147.83, 144.91, 140.70, 139.72, 138.83, 132.48, 130.81, 129.17, 129.11, 128.90, 124.21, 123.47, 122.57, 120.89. Anal. Calc ($C_{23}H_{14}N_4O_7$) C, 60.27; H, 3.08; N, 12.22, found; C, 59.75; H, 2.99, N, 12.05.

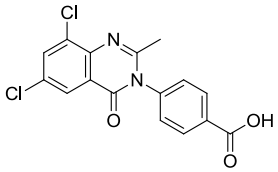


(*E*)-4-(6,8-dichloro-2-(3-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-30). Compound **987-30** was prepared via procedure 5.C using **987-34b** (0.250 g, 0.716 mmol) and *meta*-nitrobenzaldehyde (0.144 g, 0.952 mmol, 1.3 equiv) to give the title compound as a yellow solid, which was obtained by filtration (0.139 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (bs, 1H), 8.29 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 8.16-8.20 (m, 3H), 8.08 (s, 1H), 8.04-8.05 (m, 1H) 7.88 (d, J = 7.8 Hz, 1H), 7.62-7.78 (m, 3H), 6.51 (d, J = 15.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ166.7, 159.9, 151.7, 148.3, 142.8, 140.2, 138.2, 136.2, 134.5, 133.4, 131.8, 130.8, 130.7, 130.6, 129.3, 124.7, 124.3, 123.2, 122.8, 122.4. HRMS calcd for $C_{23}H_{13}Cl_2N_3O_5$, 481.0232; found 482.03068 $[M+H]^+$. MP > 260.

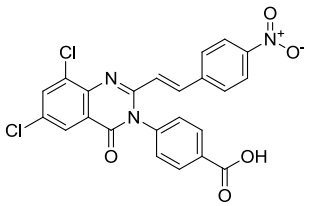


6,8-Dichloro-2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one (987-34a). Compound **987-34a** was prepared via procedure 5.A using 3-5-dichloroanthranilic acid (2.00 g, 9.71 mmol). The crude material was purified via recrystallization using hexanes and ethyl acetate to give a brown solid which was obtained by filtration (1.00 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 2.4 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 2.51 (s, 1H). ^{13}C NMR (100

MHz, $CDCl_3$) δ161.6, 158.1, 142.2, 136.9, 133.8, 132.5, 126.8, 119.1, 21.9. HRMS calc. for $C_9H_5Cl_2N_2O_2$, 228.9697; found, 229.97685 $[M+H]^+$.

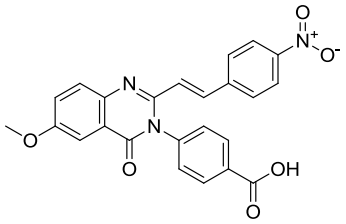


4-(6,8-dichloro-2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-34b). Compound **987-34b** was prepared via procedure 5.B using compound **987-34a** (1.00 g, 4.35 mmol) and *para*-aminobenzoic acid (0.715 g, 5.22 mmol, 1.2 equiv.). The crude material was recrystallized with ethyl acetate and methanol to give a tan solid (0.44 g, 29%). ^{1}H NMR (400 Mhz, DMSO-d_6) δ 13.30 (bs, 1H), 8.19 (d, J = 2.4 Hz, 1H), 8.63 (d, J = 8.22 Hz, 2H), 8.01 (d, J = 2.4 Hz, 1H), 7.63 (d, J = 8.2 Hz, 2H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.66, 159.94, 155.81, 142.77, 141.26, 134.30, 131.82, 131.67, 130.69, 130.36, 128.76, 124.56, 123.00, 24.45. HRMS calc. for $C_{16}H_{10}Cl_2N_2O_3$, 348.0068; found, 349.01383 $[M+H]^+$.

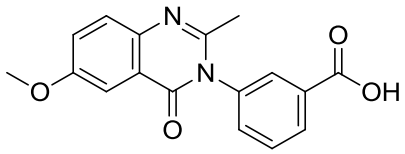


(*E*)-4-(6,8-dichloro-2-(4-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-34). Compound **987-34** was prepared via procedure 5.C using compound **987-34b** (0.250 g, 0.716 mmol) and *para*-nitrobenzaldehyde (0.144 g, 0.952 mmol, 1.3 equiv.) to yield the title compound as a yellow solid (0.05 g, 14%). ^{1}H NMR (400 Mhz, DMSO-d_6) δ 13.34 (bs, 1H), 8.24 (d, J = 2.7, 1H) 8.16-8.20 (m, 4H), 8.05 (d, J = 2.4, 1H), 8.02 (d, J = 15.7,

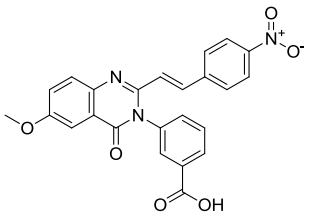
1H), 7.71 (d, *J* = 9.0, 2H), 7.65 (d, *J* = 8.6, 2H), 6.53 (d, *J* = 15.3, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.68, 159.89, 151.59, 147.72, 142.70, 140.78, 140.09, 138.00, 134.50, 132.38, 131.84, 130.88, 130.81, 129.25, 129.00, 124.72, 124.21, 123.69, 123.22. HRMS calc. for $C_{23}H_{13}Cl_2N_3O_5$, 481.0232; found, 482.0974 $[M+H]^+$. MP > 260 °C.



(*E*)-4-(6-methoxy-2-(4-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-48). Compound **987-48** was prepared via procedure 5.C using compound **987-8b** (0.730 g, 2.35 mmol) and *para*-nitrobenzaldehyde (0.473 g, 3.13 mmol, 1.3 equiv) to yield the title compound as a yellow solid (0.559 g, 54%). ^{1}H NMR (400 Mhz, DMSO-d_6) δ 13.32 (bs, 1H), 8.17 (t, *J* = 8.8 Hz, 4H), 7.92 (d, *J*=15.3, 1H), 7.78 (dd, *J* = 10.0, 4 Hz, 1H), 7.68 (d, *J* = 8.9 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 1H) 7.58-7.52 (m, 2H), 6.51 (d, *J* = 15.7 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 158.4, 148.3, 147.4, 141.7, 141.7, 141.3, 140.7, 135.7, 130.7, 129.4, 128.6, 124.5, 124.2, 106.6, 55.8. HRMS calc for $C_{24}H_{17}N_2O_6$, 443.1117; found, 444.11978 $[M+H]^+$. Anal.($C_{24}H_{17}N_2O_6$) C, H, N. MP > 260 °C.

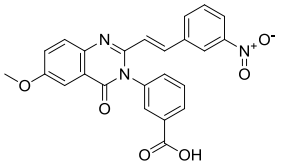


3-(6-methoxy-2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-47b). Compound **987-47b** was prepared via procedure 5.B using 6-methoxy-2-methyl-4H-benzo[d][1,3]oxazin-4-one (0.400 g, 2.09 mmol) and 3-aminobenzoic acid (0.344g, 2.51 mmol, 1.2 equiv.) to yield a white solid which was re-crystallized from EtOAc and MeOH. Yield 0.730g, 60%. ^{1}H NMR (400 Mhz, DMSO-d_6) δ 13.26 (bs, 1H), 8.06-8.08 (m, 1H), 7.98 (s, 1H), 7.70-7.74 (m, 2H), 7.63 (d, J = 9.0 Hz, 1H), 7.47 (s, 1H) 3.86 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), 166.53, 157.56, 130.03, 129.79, 121.19, 55.67, 23.87.

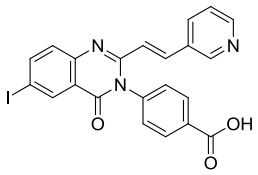


(*E*)-3-(6-methoxy-2-(4-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-47). Compound **987-47** was prepared via procedure 5.C using compound **987-47b** (0.730 g, 2.35 mmol) and 4-nitrobenzaldehyde (0.473 g, 3.13 mmol, 1.3 equiv) to yield the title compound as a yellow solid (0.728g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (bs, 1H), 8.13-8.18 (m, 3H), 8.04 (s, 1H), 7.91 (d, J = 15.7 Hz, 1H), 7.74-7.79 (m, 3H), 7.65 (d J = 9.0 Hz, 2H), 7.51-7.54 (m, 2H), 6.50 (d, J = 15.7 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.54, 160.96, 158.19, 148.57, 147.39, 141.68, 141.35, 137.14, 135.54, 133.43, 132.45, 130.11, 129.97, 129.24, 128.54, 124.42, 124.22, 124.16, 121.67,

106.57, 55.78. HRMS calcd. for $C_{24}H_{17}N_3O_6$, 443.1117; found, 444.11978$[M+H]^+$. MP > 260 °C.

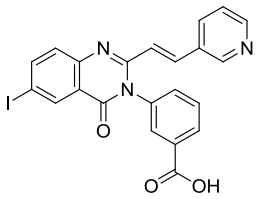


(*E*)-3-(6-methoxy-2-(3-nitrostyryl)-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (987-46). Compound **987-46** was prepared via procedure 5.C using compound **987-47b** (0.194 g, 0.626 mmol) and 3-nitrobenzaldehyde (0.126 g, 0.833 mmol, 1.3 equiv.) to yield the title compound as a yellow solid (0.087g, 33%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (bs, 1H), 8.21 (s, 1H), 8.13-8.15 (m, 2H), 8.03 (s, 1H), 7.92 (d, *J* = 15.7 Hz, 1H), 7.82 (d, *J* = 7.83, 1H), 7.73-7.77 (m, 3H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.51-7.53 (m, 2H), 6.47 (d, *J* = 15.7 Hz, 1H), 3.89 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), 166.56, 161.01, 158.10, 148.72, 148.28, 141.74, 137.21, 136.75, 135.67, 133.49, 133.24, 132.35, 130.50, 130.09, 129.99, 129.17, 124.44, 123.81, 122.93, 122.14, 121.60, 106.56, 55.78. HRMS calcd. for $C_{24}H_{17}N_3O_6$, 443.1117; found, 444.11957 $[M+H]^+$. MP > 260 °C.



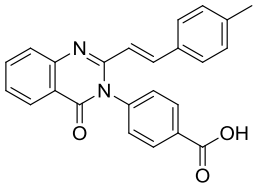
(*E*)-4-(6-iodo-4-oxo-2-(2-(pyridin-3-yl)vinyl)quinazolin-3(4*H*)-yl)benzoic acid (987-56). Compound **987-56** was prepared via procedure 5.C using 4-(6-iodo-2-methyl-4-oxoquinazolin-3(4H)-yl)benzoic acid (0.240 g, 0.591 mmol) and nicotinaldehyde (0.084 g, 0.074 mL, 0.786 mmol, 1.33 equiv.). The reaction mixture was filtered and washed

with MeOH to give the title compound as a yellow solid (0.170 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ13.31 (bs, 1H), 8.67 (d, *J* = 2.4, 1H), 8.52 (dd, *J* = 4.7, 1.6, 1H), 8.41 (d, *J* = 2.0, 1H), 8.19 (dd *J* = 8.4, 2.1, 1H), 8.15 (d, *J* = 8.6, 2H), 7.93 (d, *J* = 15.7, 1H), 7.79 (d, *J* = 8.2, 1H), 7.58-7.63 (m, 3H), 7.37 (dd *J* = 8.02, 4.9, 1H), 6.43 (d, *J* = 15.7, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 166.71, 159.92, 151.34, 149.54, 146.58, 143.29, 140.46, 136.27, 133.90, 131.66, 130.71, 130.50, 129.34, 124.09, 122.47, 121.57, 91.89. HRMS calcd for $C_{22}H_{14}IN_3O_3$ 495.0080; found, 494.00150 $[M-H]^-$. Anal. ($C_{22}H_{14}IN_3O_3$) C, H, N. MP>260 °C.

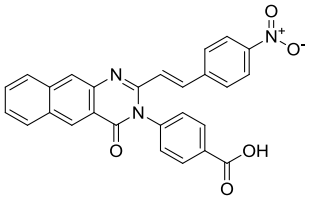


(*E*)-3-(6-iodo-4-oxo-2-(2-(pyridin-3-yl)vinyl)quinazolin-3(4*H*)-yl)benzoic acid (987-57). Compound **987-57** was prepared via procedure 5.C using compound **987-43b** (0.300 g, 0.739 mmol) and nicotinaldehyde (0.105 g, 0.092 mL, 0.982 mmol, 1.3 equiv.) The reaction mixture was re-crystallized from EtOAc/Hexanes to give the title compound as a yellow solid (0.111 g, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (bs, 1H), 8.65 (s, 1H), 8.51 (d, *J* = 3.9, 1H), 8.39 (s, 1H), 8.16 (t, *J* = 9.4, 2H), 8.05 (s, 1H), 7.92 (d, *J* = 15.7, 1H), 7.74-7.78 (m, 3H), 7.58 (d, *J* = 8.6, 1H), 7.34-7.37 (m, 1H), 6.42 (d, *J* = 15.7, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.54, 160.09, 151.56, 150.52, 149.48, 146.6, 143.21, 136.88, 136.13, 134.69, 133.86, 133.35, 132.43, 130.52, 130.20, 130.11, 129.93, 129.43, 124.05, 122.55, 121.71, 91.76. HRMS calcd for $C_{22}H_{14}IN_3O_3$ 495.0080; found,

494.00165 $[M-H]^-$. Anal. ($C_{22}H_{14}IN_3O_3$) C 53.35, H 2.85, N 8.48; found C 45.18, H 2.23, N 7.16. MP > 260 °C.

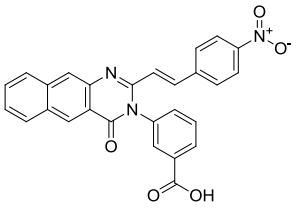


(*E*)-4-(2-(4-Methylstyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid (987-58). Compound **987-58** was prepared via procedure 5.C using 4-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzoic acid (1.00 g, 3.57 mmol) and 4-methylbenzaldehyde (0.561 ml, 4.75 mmol). The product was crystallized with EtOAC/Hexanes. Yield 0.480 g, 35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (s, 1H), 8.20 – 8.10 (m, 3H), 7.94 – 7.73 (m, 3H), 7.66 – 7.50 (m, 3H), 7.32 – 7.25 (m, 2H), 7.17 (d, J = 7.9 Hz, 2H), 6.26 (d, J = 15.5 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.72, 161.20, 151.10, 147.43, 140.97, 139.82, 139.20, 134.91, 132.03, 131.45, 130.63, 129.68, 129.63, 129.45, 127.67, 127.62, 127.20, 126.61, 126.46, 120.46, 118.65, 20.96. HRMS calcd for $C_{24}H_{19}O_3N_2$ $[M+H]^+$; 383.13818, found 383.13843. Anal. ($C_{24}H_{18}O_3N_2$) C, H, N.

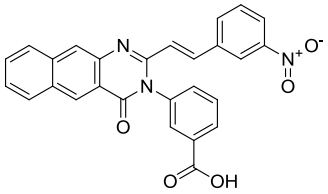


(*E*)-4-(2-(4-Nitrostyryl)-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (987-51). Compound **987-51** was prepared via procedure 5.C using 4-(2-methyl-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (0.360 g, 1.09 mmol) and 4-nitrobenzaldehyde (0.219 g, 1.45 mmol). The product was obtained after column

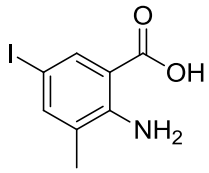
chromatography using 1:1 EtOAC/Hexanes. Yield 0.236 g, 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (s, 1H), 8.90 (s, 1H), 8.41 (s, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.24 – 8.13 (m, 5H), 8.02 (d, J = 15.6 Hz, 1H), 7.77 – 7.58 (m, 6H), 6.57 (d, J = 15.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.40, 162.22, 150.14, 148.15, 143.18, 141.88, 141.44, 137.10, 136.96, 132.16, 131.86, 131.35, 130.30, 130.07, 129.46, 129.34, 128.77, 128.70, 128.64, 127.28, 125.69, 124.95, 124.84, 120.42. HRMS calcd for $C_{27}H_{18}O_5N_3$ $[M+H]^+$; 464.12410, found 464.12486.



(*E*)-3-(2-(4-Nitrostyryl)-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (987-53). Compound **987-53** was prepared via procedure 5.C using 3-(2-methyl-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (0.345 g, 1.04 mmol) and 4-nitrobenzaldehyde (0.210 g, 1.39 mmol). The product was obtained after column chromatography using 1:1 EtOAC/Hexanes. Yield 0.263 g, 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.09 (s, 1H), 8.85 (s, 1H), 8.39 (s, 1H), 8.28 – 8.08 (m, 5H), 8.00 (d, J = 15.6 Hz, 1H), 7.85 – 7.57 (m, 7H), 6.55 (d, J = 15.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.60, 161.73, 149.71, 147.45, 142.57, 141.23, 137.16, 136.41, 136.14, 133.67, 132.38, 131.16, 130.20, 130.12, 129.39, 128.75, 128.60, 127.96, 126.56, 124.98, 124.42, 124.15, 119.86. HRMS calcd for $C_{27}H_{18}O_5N_3$ $[M+H]^+$; 464.12410, found 464.12473.

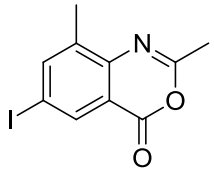


(*E*)-3-(2-(3-nitrostyryl)-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (987-52). Compound **987-51** was prepared via procedure 5.C using 3-(2-methyl-4-oxobenzo[g]quinazolin-3(4H)-yl)benzoic acid (0.345 g, 1.04 mmol) and 3-nitrobenzaldehyde (0.210 g, 1.39 mmol). The product was obtained after column chromatography using 1:1 EtOAC/Hexanes. Yield 0.208 g, 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (s, 1H), 8.85 (s, 1H), 8.37 (s, 1H), 8.28 – 8.21 (m, 2H), 8.20 – 8.08 (m, 4H), 8.02 (d, J = 15.6 Hz, 1H), 7.88 – 7.57 (m, 6H), 6.52 (d, J = 15.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.60, 161.78, 149.92, 149.88, 148.29, 142.65, 137.22, 136.69, 136.44, 136.28, 133.67, 133.37, 132.42, 131.13, 130.53, 130.20, 130.06, 129.42, 128.74, 127.99, 126.53, 124.86, 123.20, 122.19, 119.89. HRMS calcd for $C_{27}H_{18}O_5N_3$ $[M+H]^+$; 464.12410, found 464.12464. Anal. ($C_{27}H_{17}O_5N_3$) C, H, N.

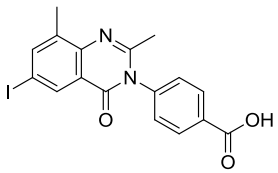


2-Amino-5-iodo-3-methylbenzoic acid (987-59a). In a 500 mL round-bottomed flask at 60°C, 2-amino-3-methylbenzoic acid (1.00 g, 6.62 mmol) was dissolved in 6% aqueous HCl (100 mL) to give an colorless solution. Iodine chloride (192 ml, 19.18 mmol, 0.1M) was added, and the reaction mixture was stirred for 1 hour. The mixture was poured onto sodium sulfite (19.18 mmol) in crushed ice and neutralized with sodium hydroxide

pellets with cooling. The pale precipitate was filtered, washed with ice-water and MeOH to give 2-amino-5-iodo-3-methylbenzoic acid. Yield 1.00 g, 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 2.2 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.79, 149.38, 136.75, 126.44, 111.59, 74.51. HRMS calcd for $C_8H_9O_2N_1I$ $[M+H]^+$; 277.96726, found 277.96689.

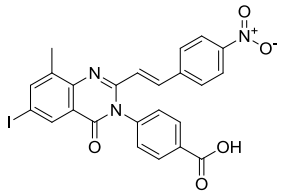


6-Iodo-2,8-dimethyl-4H-benzo[d][1,3]oxazin-4-one (987-59b). Compound **987-59a** was prepared via procedure 5.A using 2-amino-3-methylbenzoic acid (0.750 g, 2.7 mmol). The product was filtered from the reaction medium as a yellow solid. Yield 0.600 g, 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 – 8.09 (m, 2H), 2.42 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.80, 158.28, 145.18, 137.27, 133.24, 118.16, 92.64, 21.38, 16.17. HRMS calcd for $C_{20}H_9O_2N_1I$ $[M+H]^+$; 301.96726, found 301.96686.



4-(6-Iodo-2,8-dimethyl-4-oxoquinazolin-3(4H)-yl)benzoic acid (987-59c). Compound 987-59c was prepared via procedure 5.B using **987-59b** (1.70 g, 5.65 mmol) and 4-aminobenzoic acid (0.344g, 2.51 mmol, 1.2 equiv.) to yield a white solid which was re-crystallized from EtOAc and MeOH. Yield 1.30 g, 55%. ^{1}H NMR (400 MHz, DMSO-

d$_6$) δ 12.77 (s, 1H), 8.24 – 8.17 (m, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 8.06 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 2.53 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.05, 166.66, 160.20, 153.66, 145.17, 142.90, 141.60, 137.69, 132.17, 131.44, 130.59, 128.86, 122.06, 91.08, 24.48, 16.56. HRMS calcd for $C_{17}H_{14}O_3N_2I$ $[M+H]^+$; 421.00437, found 421.00442.



(E)-4-(6-Iodo-8-methyl-2-(4-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid (987-59). Compound **987-59** was prepared via procedure 5.C using **987-59** (0.500 g, 1.19 mmol) and 4-nitrobenzaldehyde (0.239 g, 1.58 mmol). The product was obtained after crystallization first using 1:1 EtOAC/Hexanes and then MeOH/H_2O. Yield 0.140 g, 21%. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.33 (s, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 8.23 – 8.10 (m, 5H), 8.05 (d, *J* = 15.4 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.1 Hz, 2H), 6.51 (d, *J* = 15.5 Hz, 1H), 2.67 (s, 3H). HRMS calcd for $C_{24}H_{17}O_5N_3I$ $[M+H]^+$; 554.02075, found 554.02078.

Chapter 6. 1063 Series of Antagonists

6.1. Abstract

One other class of compounds discovered within the Traynelis' group was referred to as the 1063 series of compounds. Initially, this class of compounds was particularly attractive from a medicinal chemistry perspective because the molecules were considerably smaller than the other classes identified, the synthesis was relatively straightforward and early congeners could cross the blood brain barrier. This class of compounds was probed for structure activity relationships with the hopes of improving potency and selectivity while maintaining BBB penetration. The initial SAR was undertaken to explore the compounds tolerability to modification in three different areas of the molecules and below, I describe my contributions to the project.

6.2. Introduction

The initial biological results concerning the hit compound in the 1063 series made it an attractive target for further medicinal chemistry efforts for a number of reasons (**Figure 6.1**). First, the compound was much more selective (>500-fold) for GluN2C- and GluN2D-containing receptors than the other hits identified in the screen (data not shown). Second, the compound has a low molecular weight (378.49 Da) and crosses the blood brain barrier (data not shown).

Figure 6.1. Screening hit and general structure for 1063-series SAR.

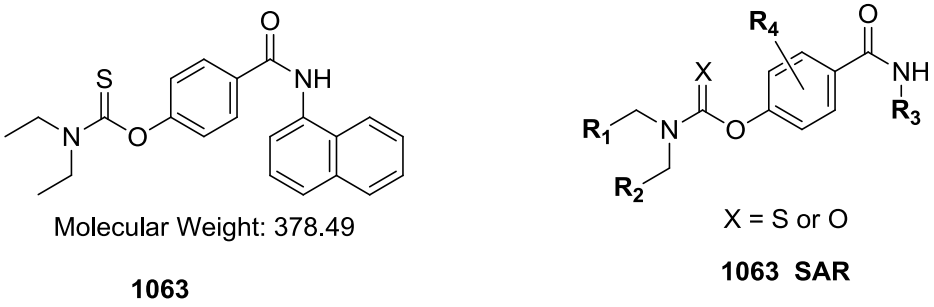
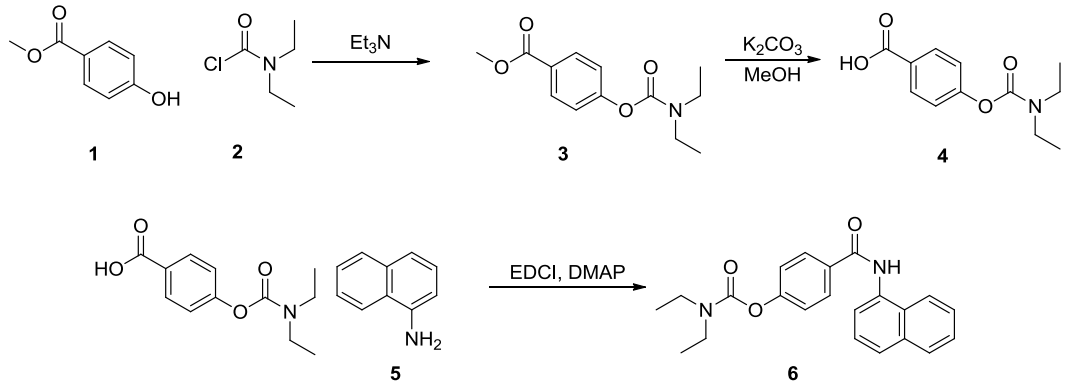


Figure 6.1. Compound 1063 was identified as a GluN2C- and GluN2D-containining NMDA receptor antagonist. The SAR was envisaged to come from the general structure **1063 SAR**.

The initial synthesis and rationale for the SAR was developed by Dr. Cara Mosley in the Liotta group (**Figure 6.1** and **Scheme 6.1**, Mosley dissertation, 2010).

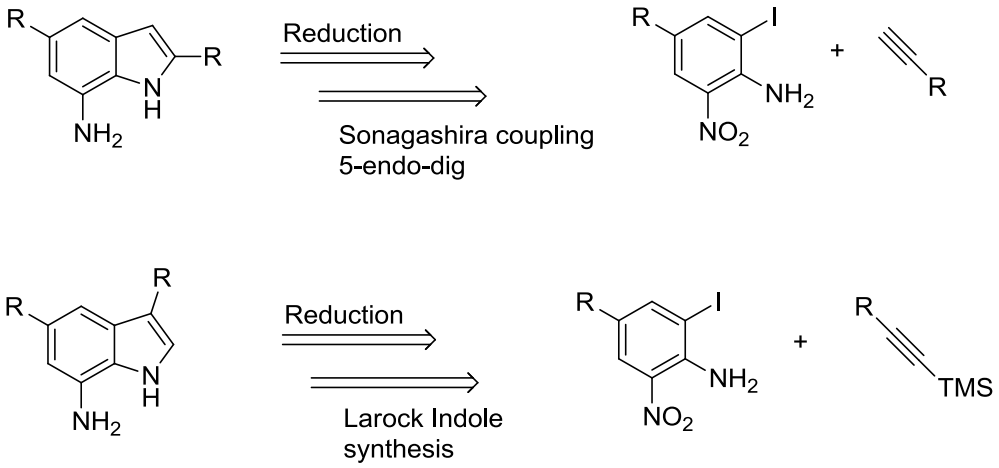
The modifications that were made by Dr. Mosley were primarily focused on the carbamate moiety. Her contributions led to more stable compounds, as the carbamothioate (X = S) was thought to be oxidized to the carbamate (X = O) (Mosley, thesis, 2010). However, perturbations to the linker phenyl ring or modifications to the napthyl moiety were unexplored. I imagined that substitutions to this region of the compound could be beneficial to potency and remove a potential metabolic liability with the napthyl group; 1-napthylamine is known to be a carcinogen and, due to the amide linkage, would be a likely site of metabolism *in vivo*. The substitutions that were envisaged included probing the napthyl ring with substitutions and also replacing the napthyl ring with an indole ring (**Figure 6.2** and **Scheme 6.2**). The indole is a well-studied, privileged structure that is often found in biologically active organic compounds (Welsch et al., 2010). The indole ring has been thoroughly investigated synthetically and can be synthesized via a number of different routes (Taber and Tirunahari, 2011). As is shown in **Scheme 6.2**, I imagined that the first modifications would probe the linkage of the amine and then subsequently move to positional modifications using either the Larock indole synthesis, or a Sonagashira cross-coupling followed by a 5-endo-dig cyclization, which had significant precedence in the synthetic literature (Ezquerra et al., 1996; Larock et al., 1998; Rodriguez et al., 2000). Next, the amide linkage was thought to be of concern from a metabolic perspective, so a route exploring the directionality of the linkage was designed (**Scheme 6.2**). Finally, substitutions to the phenyl linker were imagined to come from commercially available benzoic acids or benzoic methyl esters.

Scheme 6.1. General synthetic scheme for 1063-analogs.



Scheme 6.1. The general synthesis of the 1063-series of compounds (Originally developed by Dr. Cara Mosley).

Scheme 6.2 Retro-synthetic route to differentially substituted indoles.



Scheme 6.2. Retro-synthetic route to differentially substituted indoles. Top: 2,5, 2 or 5 substituted indoles can be accessed via Sonagashira cross-coupling followed by 5-endo-dig cylization. Bottom: 3-5, or 3-substituted indoles were thought to come from Larock indole synthesis. Both methods share a common intermediate.

6.3. Results

Several compounds were synthesized from commercially available starting materials and are summarized in **Table 6.1**. The substituted napthyl derivative 1063-012 shows selectivity for GluN2C-containing receptors over GluN2D-containing receptors, however, potency was low (**Table 6.1**). Napthyl esters with free amine groups as in 1063-13 and 1063-14 were inactive (**Table 6.1**). Linking an indole through either the five or six position was deleterious to activity, while linkage through the seven position to either the carbamate or carbamothioate containing final compounds (1063-20 and 1063-21 respectively) maintained potency at GluN2C- and GluN2D-containing receptors and excellent selectivity over GluN2A- or GluN2B-containing receptors (**Table 6.1**). The 4-amino indole linkage did not improve potency over the 7-substituted compound (1063-37, **Table 6.1**). The 7-amino indole linkage was therefore chosen for further SAR development and substitutions to the core of the indole were made accordingly.

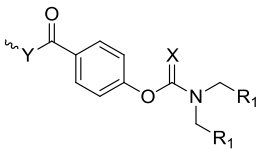


Table 6.1. Substitutions to the terminal aryl ring.

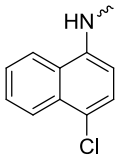
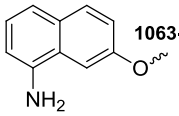
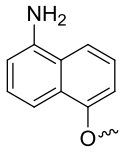
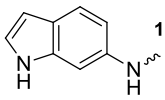
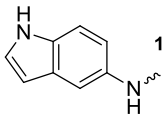
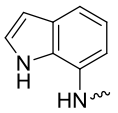
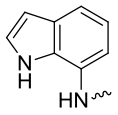
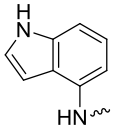
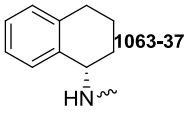
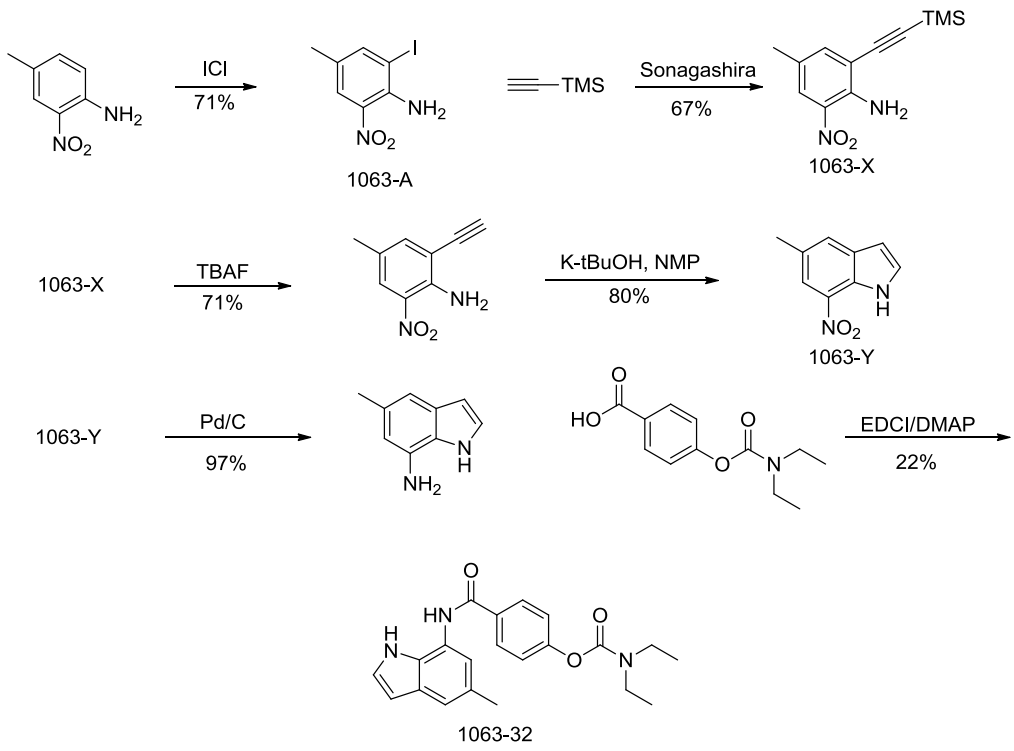
R_2	R_1	X	2A IC_{50} 2D IC_{50}	2B IC_{50} 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
 **1063-12**	$(H_3)_2$	O	N/A	N/A	-	-	25.0	100.0
 **1063-13**	$(Me)_2$	O	N/A	N/A	-	-	385.0	54.6
 **1063-14**	$(Me)_2$	O	N/A	N/A	-	-	-	-
 **1063-15**	$(Me)_2$	O	N/A	21	-	44.0	11.0	11.6
 **1063-16**	$(Me)_2$	O	N/A	N/A	-	-	30.2	44.0
 **1063-20**	$(H_3)_2$	O	928	358	2415.0	933.0	5.3	2.6
 **1063-21**	$(H_3)_2$	S	N/A	N/A	-	-	14.9	4.1
 **1063-26**	$(H_3)_2$	O	N/A	100	-	560.0	7.8	5.5
 **1063-37**	$(H_3)_2$	O	N/A	N/A	-	-	-	-

Table 6.1. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 4-10 oocytes and between one and two frogs; - indicates less than 30% inhibition at 100 μM.

The 5-methyl, 7-amino indole containing compound showed reduced potency as compared to the un-substituted compound (1063-32; **Scheme 6.3; Table 6.2**). Similar results were observed with the 3, 5-dimethyl-1*H*-indole containing compound (1063-34; **Scheme 6.4; Table 6.2**). Placement of a hydrogen bond acceptor with the methyl ester at the 5- position of the indole (Compound 1063-35; **Scheme 6.5**) did not improve potency over the 3, 5 dimethyl containing 1063-34, although the results are complicated due to inseparable regio-isomers obtained during the Larock indole step. Removal of the methyl ester giving the 3-methyl substituted containing compound was slightly more potent, but suffered from the same complication, as the 2-methyl regio-isomer was inseparable (Compound 1063-47; **Scheme 6.6**). However, the 2-methyl substituted indole containing compound was obtained as a single regio-isomer and was inactive (1063-48; **Scheme 6.7**).

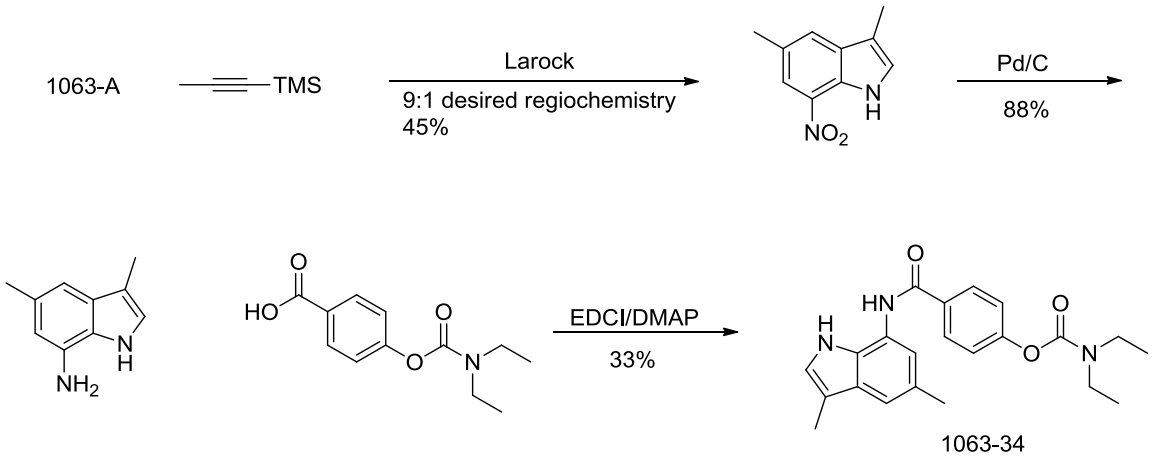
Finally, substitutions to the phenyl linker of the compound and the orientation of the amide linkage were investigated. Placing a methoxy-group *meta* to the carbamate led to compound 1063-46 (**Scheme 6.8**), which was inactive. Placing a chlorine *meta* to the amide linkage led to compound 1063-45 which maintained potency and selectivity (**Scheme 6.9**). Finally, the orientation of the amide bond was reversed as compared to the hit compound, which decreased activity as is seen with compound (1063-44; **Scheme 6.10**).

Scheme 6.3 Sonagashira methodology



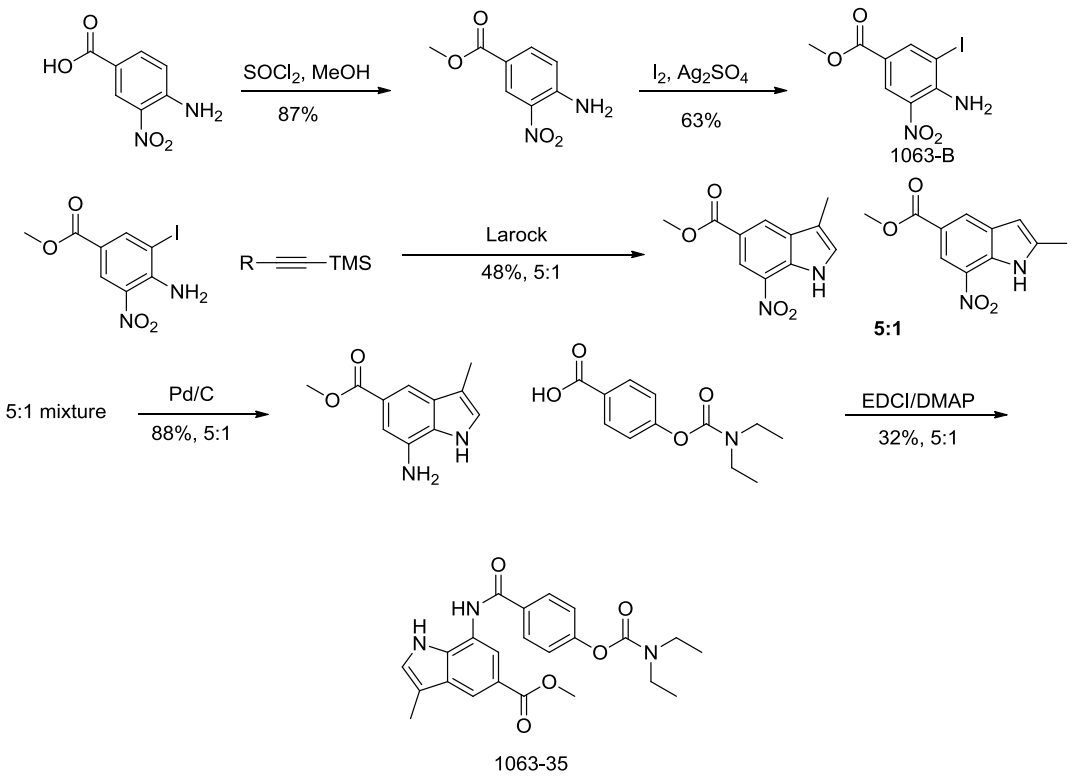
Scheme 6.3. Synthesis of compound 1063-32. The Sonagashira method followed by cyclization gave the desired indole.

Scheme 6.4. Larock indole synthesis of 3, 5-dimethyl substituted indole containing compounds.



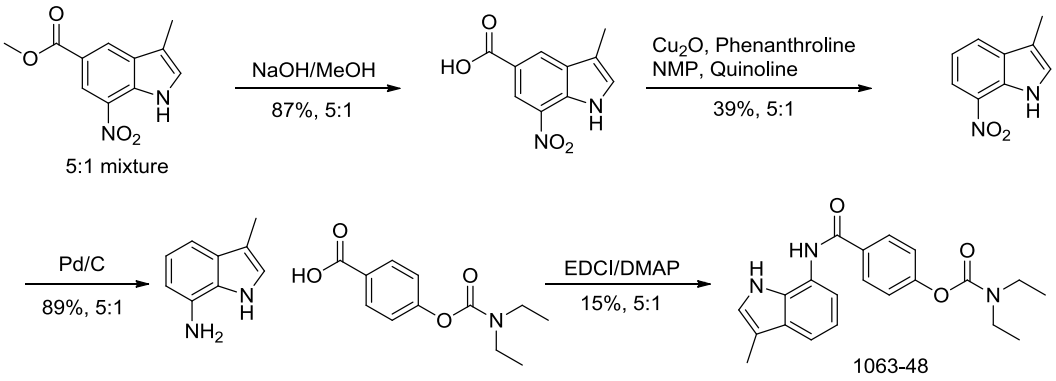
Scheme 6.4 Using the common intermediate, 1063-A, the Larock indole synthesis of the 3,5-disubstituted containing 1063-34 was accomplished.

Scheme 6.5. Larock indole synthesis of 3-methyl, 5-caroxylate derivative.



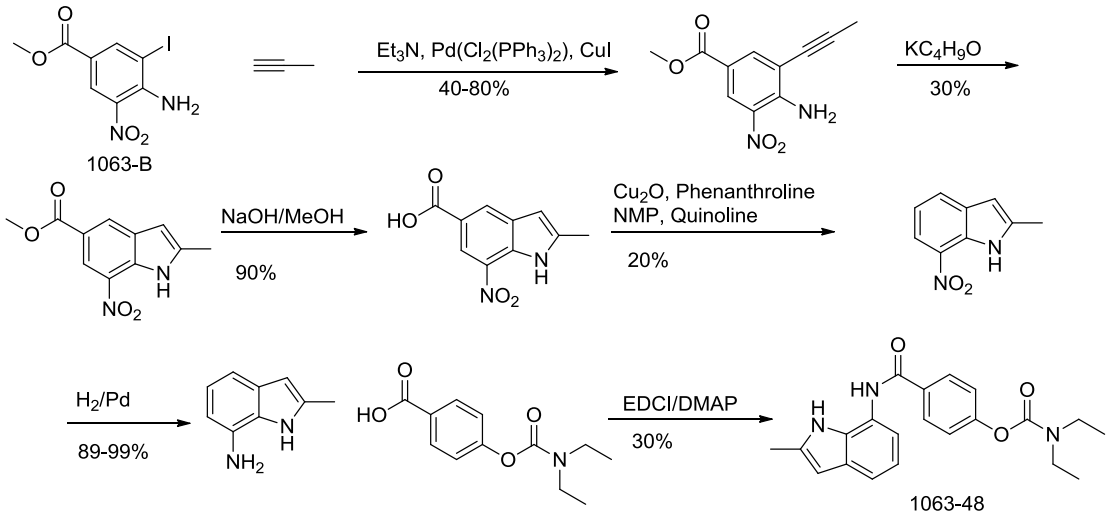
Scheme 6.5. The Larock indole synthesis was employed to obtain 1063-35. However, the regio-selectivity observed with the densely functionalized iodo-containing phenyl ring was not as high as expected.

Scheme 6.6 Larock methodology for mono-substituted indole compounds.



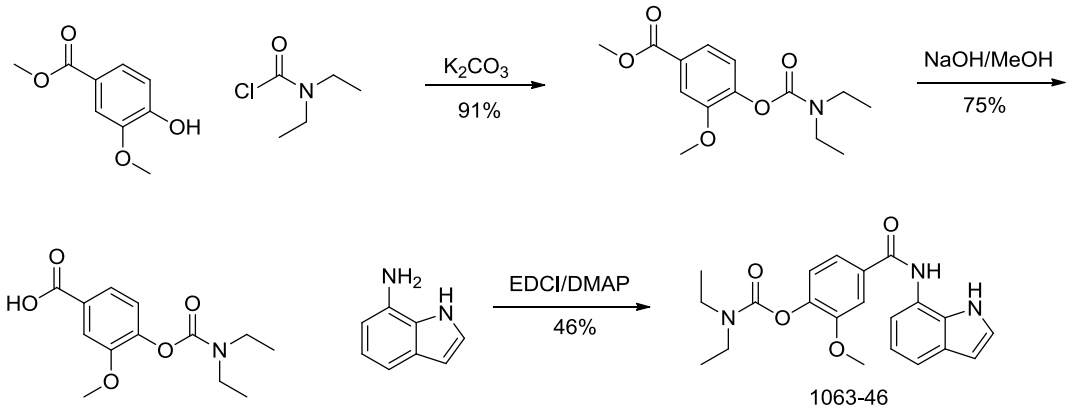
Scheme 6.6. Using the 5:1 mixture of 1063-C, compound 1063-48 was obtained as a mixture of regio-isomers.

Scheme 6.7. Sonagashira method access to 2-substituted indoles.



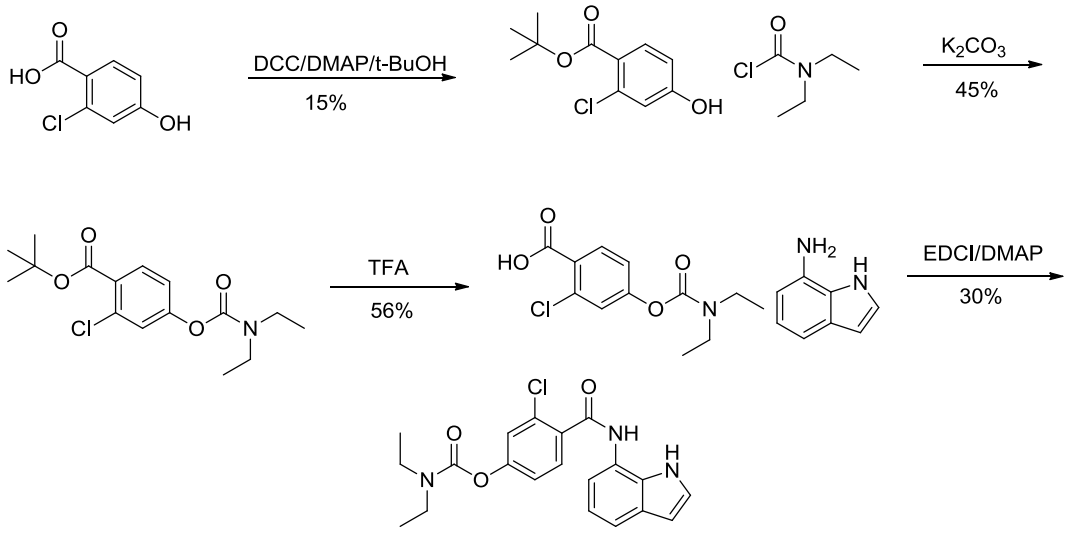
Scheme 6.7. Sonagashira method allows access to the 2-methyl substituted indole containing final compound 1063-48.

Scheme 6.8 Substitution to the phenyl linker.



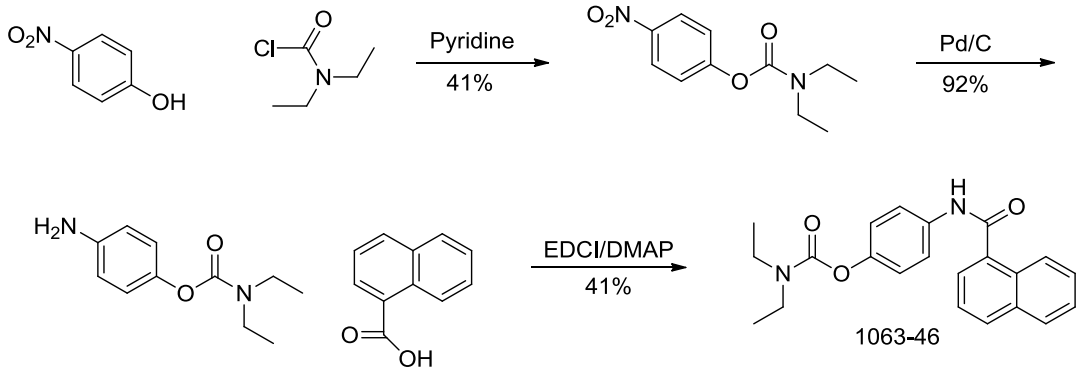
Scheme 6.8. Compound 1063-46 was designed to test a substitution *ortho* to the amide linkage on the phenyl linker region in combination with the optimal substitutions elsewhere.

Scheme 6.9. Further substitution to the phenyl linker.



Scheme 6.9. Compound 1063-45 was designed to probe the position *meta* to the amide linkage.

Scheme 6.10 Orientation of the amide linkage.



Scheme 6.10. Compound 1063-46 was synthesized to probe the effects of reversing the amide linkage within the class of compounds.

Table 6.2. Potency of 1063 compounds against NMDA receptors.

1063-	2B IC_{50} / 2D IC_{50}	GluN2A IC_{50} (μM)	GluN2B IC_{50} (μM)	GluN2C IC_{50} (μM)	GluN2D IC_{50} (μM)
32	27	-	215	20.0	7.9
34	4	-	365	102.0	93.0
35	N/A	177.0	238.0	-	-
47	N/A	-	-	26.0	20.0
48	N/A	-	-	-	320.0
46	N/A	-	-	-	310.0
45	N/A	-	-	7.7	6.0
44	N/A	-	-	15.0	15.0

Table 6.2. IC_{50} values were obtained by fitting the Hill equation to the average composite concentration-effect curves (see methods). Data are from 4-10 oocytes and between one and two frogs; - indicates less than 30% inhibition at 100 μM.

6.4. Discussion

The synthetic efforts aimed at elucidating a SAR for this class of compounds yielded modest improvements over the hit compound in the series. These results suggest that regions of the small molecules which may yield desirable improvements have yet to be found. This was not only the observation with the compounds that I contributed to, but also with the other regions of the molecule (data not shown). However, removing the potential liability of napthylamine as a metabolite by installing an indole as a replacement is an advance for the series. These compounds not only suffer from a small window of opportunity from and SAR perspective, but have challenging pharmacology as well (data not shown). It remains to be seen whether this seemingly attractive drug-like scaffold will yield useful pharmacological probes.

The observations regarding the regio-chemical outcome of the Larock indole synthesis are instructive. Previous work has shown that the use of the TMS-substituted propyne should install the smaller alkyl group at the three position of the resultant indole, however the starting material aniline in that study was un-substituted (Larock et al., 1998). An alternative strategy would be to use the Bartoli method of indole synthesis in order to make the 3-methyl substitution selectively, which has precedence with the desired 7-NO_2 group in the final product (Dydio et al., 2009).

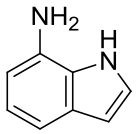
In conclusion, this class of compounds remains an interesting prospect from a medicinal chemistry perspective but more work is necessary to fully understand the pharmacology of the compounds. The SAR has not revealed any single position or type

of substitution that seems to enhance potency, albeit selectivity in the class for GluN2C- and GluN2D-containing receptors remains high.

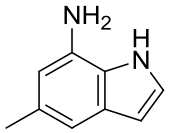
6.5. Chemistry Experimental

General Method for reduction of nitro containing compounds to amino compounds (procedure 1063a).

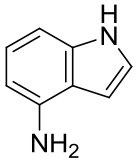
An appropriately substituted nitro-indole was dissolved in MeOH (0.1M) and 10% wet Pd/C (0.1 eq) was added. The reaction was carried out at 40 psi on a hydrogenator for 45 minutes. The solution was then filtered over a pad of celite, dried *in vacuo* and characterized unless otherwise noted.



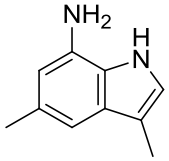
7-Aminoindole. 7-aminoindole was synthesized from 7-nitroindole (1.00g, 6.17mmol) using method 1063a to give a deep blue solid. Yield 0.800 g, 98.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (bs, 1H), 7.24 (t, J = 2.74 Hz, 1H), 6.78-6.69 (m, 2H), 6.31-6.28, m, 2H), 5.04 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 133.67, 128.57, 123.75, 119.93, 108.60, 104.56, 101.45. HRMS calcd for $C_8H_9N_2$ $[M+H]^+$; 133.07602, found; 133.07580.



5-Methy-1*H*-indol-7-amine. 5-Methy-1*H*-indol-7-amine was synthesized from 5-methyl-7-nitro-1*H*-indole (0.248 g, 1.41 mmol) using method 1063a. Yield 0.200 g, 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (bs, 1H), 7.17 (t, J = 2.74 Hz, 1H), 6.56 (s, 1H), 6.19-6.14 (m, 2H), 6.31-6.28, m, 2H), 4.93 (s, 2H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 133.30, 128.41, 128.19, 124.04, 123.68, 108.38, 106.31, 100.96, 21.56. HRMS calcd for $C_9H_{11}N_2$ $[M+H]^+$; 147.09168, found; 147.09160.

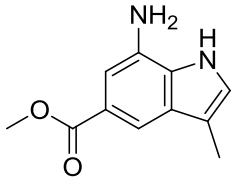


1*H*-Indol-4-amine. 1*H*-indol-4-amine was synthesized from 4-nitro-1*H*-indole (1.00 g, 6.17 mmol) using method 1063a to give a white solid. Yield 0.700 g, 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (bs, 1H), 7.10 (t, J = 3.5 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 6.86 (t, J = 7.8 Hz, 1H), 6.85 (d, J = 6.45 Hz, 1H), 6.47-6.45 (m, 1H), 6.40 (d, J = 8.0 Hz, 1H), 3.91 (s, 2H). HRMS calcd for $C_8H_9N_2$ $[M+H]^+$; 133.07602, found; 133.07580.

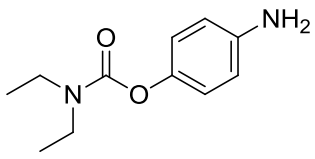


3,5-Dimethyl-1*H*-indol-7-amine. 3,5-Dimethyl-1*H*-indol-7-amine was synthesized from 3, 5-dimethyl-7-nitro-1*H*-indole (0.620 g, 3.26 mmol) using method 1063a. The title compound was isolated after column chromatography using a 20-80% gradient of EtOAc

in hexanes. Yield 0.460 g, 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 6.92 (s, 1H), 6.73 (s, 1H), 6.32 (s, 1H), 3.60 (s, 2H), 2.40 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.96, 129.41, 129.25, 125.19, 121.79, 121.71, 111.59, 110.40, 110.33, 110.04, 110.00, 21.39, 9.69. HRMS calcd for $C_{10}H_{13}N_2$ $[M+H]^+$; 161.10733, found; 161.10693.

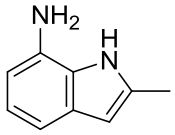


Methyl 7-amino-3-methyl-1*H*-indole-5-carboxylate. Methyl 7-amino-3-methyl-1*H*-indole-5-carboxylate was synthesized from methyl 3-methyl-7-nitro-1*H*-indole-5-carboxylate (0.260 g, 1.11 mmol) using method 1063a. 5:1 mixture remained. Yield, 0.200 g, 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.94 – 7.87 (m, 1H), 7.25 (d, J = 1.4 Hz, 1H), 6.97 – 6.91 (m, 1H), 3.90 (s, 3H), 3.75 (s, 2H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.94, 130.95, 130.15, 129.03, 122.75, 122.63, 122.08, 114.48, 114.42, 114.22, 109.12, 109.08, 52.17, 9.96. HRMS calcd for $C_{11}H_{13}N_2O_2$ $[M+H]^+$; 205.09715, found; 205.09721.

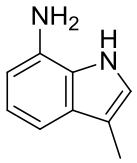


4-aminophenyl diethylcarbamate. 4-aminophenyl-diethylcarbamate was synthesized from 4-nitrophenyl deithylcarbamate (0.250 g, 1.05 mmol) using method 1063a. The title compound was obtained using flash chromatography with a 20%-50% EtOAc in hexanes

gradient. Yield 0.200 g, 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.84 – 6.68 (m, 2H), 6.55 – 6.46 (m, 2H), 4.96 (s, 2H), 3.34 (s, 4H), 1.20 – 1.04 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.94, 143.87, 143.58, 122.44, 122.36, 115.59, 115.51, 77.54, 77.22, 76.90, 42.17, 41.78, 14.26, 13.49. HRMS calcd for $C_{11}H_{17}N_2O_2$ $[M+H]^+$; 209.12845, found; 209.12836.



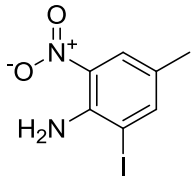
2-Methyl-1*H*-indole-7-amine. 2-Mehtyl-1*H*-indole-7-amine was synthesized from 2-methyl-7-nitro-1*H*-indole (0.160 g, 0.908 mmol) using method 1063a in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.17 – 7.02 (m, 1H), 6.90 (t, *J* = 7.6 Hz, 1H), 6.49 (t, *J* = 6.7 Hz, 1H), 6.15 (d, *J* = 7.1 Hz, 1H), 3.56 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.23, 130.27, 130.12, 127.06, 120.44, 112.14, 108.31, 101.24, 13.70. HRMS calcd for $C_{19}H_{11}N_2$ $[M+H]^+$; 147.09168, found; 147.09150.



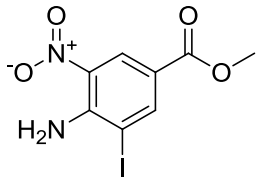
3-Methyl-1*H*-indol-7-amine. 3-methyl-1*H*-indol-7-amine was synthesized from 3-methyl-7-nitro-1*H*-indole (0.310 g, 1.76 mmol) using method 1063-a. Yield 0.230 g, 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.00 (s, 1H), 6.75 – 6.59 (m, 2H), 6.33 – 6.26 (m, 1H), 4.94 (s, 2H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 133.57, 128.47, 125.80, 121.14, 119.26, 109.54, 104.64, 99.59, 9.91. HRMS calcd for $C_{19}H_{11}N_2$ $[M+H]^+$; 147.09168, found; 147.09152.

General method (1) for iodination (1063-b). An appropriately substituted nitro-aniline was dissolved in 6% aqueous HCl (100ml) at 60ºC and 0.1M ICl was added (2.9eq.). The reaction mixture was stirred for one hour and then poured onto sodium sulfite in crushed as and neutralized with NaOH. The precipitate was filtered and rinsed with water and MeOH, dried *in vacuo* and characterized unless otherwise noted.

General method (2) for iodination (1063-b2). An appropriately substituted nitro-aniline was dissolved in ethanol (0.1M). Silver sulfate (1.4eq) and diodine (1.4eq) were added and the reaction mixture was stirred for 36 hours. The mixture was concentrated under vacuum, dissolved in DCM and washed with brine three times. The organics were combined and the compounds isolated by column chromatography as noted.

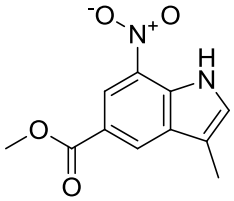


2-Iodo-4-methyl-6-nitroaniline (1063-32b). 2-Iodo-4-mehtyl-6-nitroaniline was synthesized via general method 1063b1 using 4-mehtyl-2nitroaniline (1.00 g, 6.57 mmol). The title compound was isolated as a pale solid. Yield 1.30 g, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, J = 1.96, 1H), 7.88 (s, 1H), 6.90 (bs, 2H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.24, 142.64, 130.53, 126.90, 125.72, 88.19, 18.96. HRMS calcd for $C_7H_8N_2O_2I$ $[M+H]^+$; 278.96251, found; 278.96241.



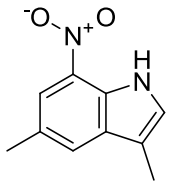
Methyl 4-amino-3-iodo-5-nitrobenzoate (1063-35b). Methyl 4-amino-3-iodo-5-nitrobenzoate was synthesized using methyl-4-amino-3-nitrobenzoate (4.67 g, 23.81 mmol) via general method 1063-b2. The title compound was isolated via chromatography using DCM as a yellow/orange solid. Yield 3.32 g, 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 – 8.52 (m, 1H), 8.43 – 8.37 (m, 1H), 7.60 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.74, 147.34, 144.92, 130.01, 128.15, 117.87, 87.94, 52.35. HRMS calcd for $C_8H_8N_2O_4I$ $[M+H]^+$; 322.95234, found; 322.95221.

General Larock indole synthesis (1063-c). An appropriately substituted nitro-aniline (1.0 eq.) was dissolved in DMF (0.100M) with lithium chloride (1.00eq.), an appropriate alkyne (5.0 eq.), finely ground potassium carbonate (5.0 eq.), and palladium acetate (0.1 eq.). The reaction mixture was heated to 100ºC for five hours. The mixture was then concentrated *in vacuo*, partitioned between DCM and brine and washed three times. The organics were combined and concentrated and the title compounds were obtained by flash chromatography using 0-5% MeOH in DCM, unless otherwise noted.



Methyl 3-methyl-7-nitro-1*H*-indole-5-carboxylate (1063-35c). Methyl 3-methyl-7-nitro-1*H*-indole-5-carboxylate was synthesized using methyl 4-amino-3-iodo-5-nitrobenzoate (0.750 g, 2.33 mmol) via general procedure 1063-c. The resultant product was a mixture of 2- and 3-substituted indole (1:5) that was inseparable by

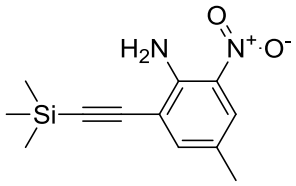
chromatography. The mixture was carried forward. Yield 0.260 g, 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.58 – 8.49 (m, 2H), 7.42 (s, 1H), 3.92 (s, 3H), 2.35 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.60, 132.42, 132.01, 130.24, 127.75, 127.39, 119.57, 118.93, 113.41, 52.42, 9.06. HRMS calcd for $C_{11}H_{11}N_2O_4$ $[M+H]^+$; 235.07133, found; 235.07132.



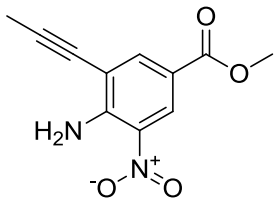
3,5-Dimethyl-7-nitro-1*H*-indole (1063-34c). 3,5-Dimethyl-7-nitro-1*H*-indole was prepared from 2-Iodo-4-mehtyl-6-nitroaniline (2.00 g, 7.19 mmol) and trimethyl(prop-1-ynyl)silane (4.04 g, 36.0 mmol) using general method 1063-c. the product was a 9:1 mixture of the desired 3-substituted indole to the undesired 2-substituted indole that were inseparable. Yield 0.620 g, 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.99 – 7.93 (m, 1H), 7.71 – 7.66 (m, 1H), 7.09 (s, 1H), 2.50 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.81, 128.73, 127.84, 124.61, 124.54, 120.43, 120.37, 113.05, 21.67, 9.98. HRMS calcd for $C_{10}H_{11}N_2O_4$ $[M+H]^+$; 191.08150, found; 191.08139.

General Sonagashira coupling conditions (1063-d). In a flame dried flask, an appropriately substituted iodo-aniline was dissolved in THF (1.4 M) with copper iodide (0.05 eq.), triethylamine (1.00 eq.), and palladium (bis-triphenylphosphine)(di-chloride) (0.05 eq.). The mixture was cooled to 0ºC and an appropriate alkyne (1.1 eq.) was added dropwise to the flask. The mixture was allowed to warm and stirred for two hours. The

THF was removed *in vacuo* and ether added to the residue. The organics were filtered over a pad of celite and washed three times with brine. The organics were collected, dried over magnesium sulfate and concentrated *in vacuo* prior to column chromatography using a 0-20% EtOAc:hexanes method, unless otherwise noted (Ezquerra et al., 1996).



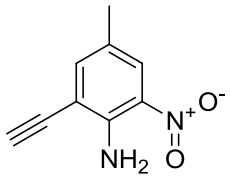
4-methyl-2-nitro-6-((trimethylsilyl)ethynyl)aniline (1063-32d). 4-methyl-2-nitro-6-((trimethylsilyl)ethynyl)aniline was prepared from using method 1063-d 2-Iodo-4-mehtyl-6-nitroaniline (1.00g, 3.60 mmol) and ethynyltrimethylsilane (0.389 g, 3.96 mmol). The title compound was obtained as an orange solid. Yield 0.600 g, 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 – 7.88 (m, 1H), 7.42 – 7.36 (m, 1H), 6.57 (s, 2H), 2.22 (t, J = 0.7 Hz, 3H), 0.26 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.82, 140.15, 126.64, 125.50, 112.16, 103.10, 99.31, 0.13. HRMS calcd for $C_{12}H_{17}N_2O_2Si$ $[M+H]^+$; 249.10538, found; 205.10535.



Methyl 4-amino-3 nitro-5-(prop-1-ynyl)benzoate (1063-48d). In a flame dried 100 mL round-bottomed flask, triethylamine (1.731 ml, 12.42 mmol), copper(I) iodide (0.237 g, 1.242 mmol), palladium(bis-triphenylphosphine)(di-chloride) (0.436 g, 0.621 mmol), and methyl 4-amino-3-iodo-5-nitrobenzoate (2.00g, 6.21 mmol) were added and stirred at

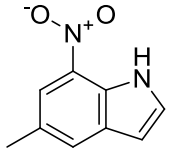
room temperature. Prop-1-yne (0.274 g, 6.83 mmol) was bubbled in for 10 minutes and the mixture was stirtred at rt until TLC indicated completion, within an hour. The solvents were removed *in vacuo*, and ether was added to the mixture prior to filtering over a pad of celite. The organics were washed three times with brine, dried over magnesium sulfate and concentrated *in vacuo*. The title compound was obtained after column chromatography using 20% EtOAc in hexanes as yellow solid (Ghilagaber et al., 2007). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, J = 2.0 Hz, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.73 (s, 2H), 3.82 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.31, 148.14, 137.05, 130.45, 127.28, 115.89, 112.93, 96.03, 73.30, 52.24, 4.55. HRMS calcd for $C_{11}H_9N_2O_4$ $[M-H]^-$; 233.05678, found; 233.05652.

Compounds not synthesized via general methods:

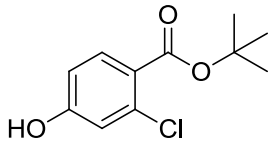


2-ethynyl-4-methyl-6-nitroaniline (1063-32x). In a 25 mL round-bottomed flask, 4-methyl-2-nitro-6-((trimethylsilyl)ethynyl)aniline (1.00 g, 4.03 mmol) was dissolved in THF (Volume: 6.00 ml). TBAF (4.43 ml, 4.43 mmol) was added and the mixture was stirred at rt for 1 hr. The THF was removed *in vacuo* and the crude material washed with brine and DCM. the product was purified by flash chromatography (Hexanes/ 20 % EtOAc)to give 2-ethynyl-4-methyl-6-nitroaniline (0.500 g, 2.84 mmol, 70.5 % yield). ^{1}H NMR (400 MHz) $CDCl_3$ δ 7.94 (s, 1H), 7.41(s, 1H), 6.58 (bs, 2H), 3.50 (s, 1H), 2.24 (s,

3H). ^{13}C NMR (100 MHz) $CDCl_3$ δ 144.05, 140.53, 127.07, 125.28, 110.94, 85.16, 85.04, 78.57, 20.16.

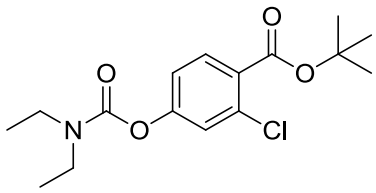


5-methyl-7-nitro-1H-indole (1063-32Z). In a 10 ml round-bottomed flask, potassium tert-butoxide (0.573 g, 5.11 mmol) was dissolved in 1.35 ml NMP. A solution of 2-ethynyl-4-methyl-6-nitroaniline (0.500 g, 2.84 mmol) in the remaing NMP was added dropwise to the basic solution at rt. The mixture was stirred for 1.5 hours. Reaction performed under inert conditions. The reaction mixture was quenched with water, extracted with ether and dried over magnesium sulfate. The product was purified using flash chromatography Hexanes/Ethyl Acetate (0-20 % gradient). Product was not pure after column. Attempts at crystallization were unsuccessful as well, starting material remained at approximately 80-20 mixture. Product was carried on to the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) d 9.802 (bs, 1H), 7.94 (s, 1H), 7.2 (s, 1H), 7.320 (t, *J* = 2.74, 1H), 6.582-6.569 (m, 1H), 2.471(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d 131.993, 129.929, 129.158, 127.901, 126.904, 126.844, 120.133, 103.525, 21.143.



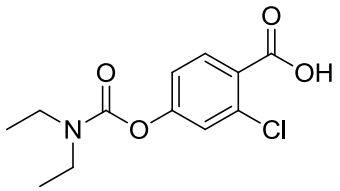
Tert-butyl 2-chloro-4-hydroxybenzoate (1063-45x). 2-Chloro-4-hydroxybenzoate (2.5 g, 14.49 mmol) was dissolved in DCM (Volume: 60.4 ml) and combined with DMAP (0.069 g, 0.565 mmol) and tBuOH (40.2 ml, 14.49 mmol) under Argon at 24°C. DCC

(21.73 ml, 21.73 mmol) was added in to the solution dropwise. The reaction was stirred overnight before being filtered and concentrated. The mixture was diluted with EtOAc and washed two times with sodium bicarbonate. The combined organic layers were then washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was then dry loaded on a 120g RediSep column and run in a 10:3 Hexanes:EtOAc mix. Yield 0.50 g, 15%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.67 (m, 1H), 6.92 (d, J = 2.4 Hz, 1H), 6.81 – 6.71 (m, 1H), 6.62 (s, 1H), 1.60 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.58, 159.24, 135.38, 133.48, 123.47, 118.16, 114.02, 82.55, 28.38. HRMS calcd for $C_{11}H_{12}O_3Cl$ $[M-H]^-$; 227.04805, found; 227.04803.

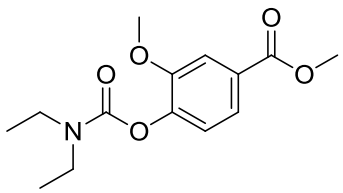


Tert-butyl 2-chloro-4-(diethylcarbamoyloxy)benzoate (1063-45Y). In a 10 mL round-bottomed flask, tert-butyl 2-chloro-4-hydroxybenzoate (0.500 g, 2.187 mmol), and pottasium carbonate (0.604 g, 4.37 mmol) were stirred in DMF (4.99 ml) for 1 hr at 23 °C to give an opaque suspension. Diethylcarbamic chloride (0.277 ml, 2.187 mmol) was added. The mixture was stirred for four hours at room temperature. The reaction mixture was dried *in vacuo* and then diluted with 75 mL water and extracted with DCM. The combined extracts were washed with water and brine and then dried over $MgSO_4$ and concentrated. The title compound was obtained by column chromatography using 0-20% EtOAc in hexanes. Yield 0.322 g, 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.6 Hz, 1H), 7.22 (d, *J* = 2.3 Hz, 1H), 7.07 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.38 (dq, *J* = 14.4, 7.1 Hz, 4H), 1.57 (s, 9H), 1.20 (dt, *J* = 15.9, 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ

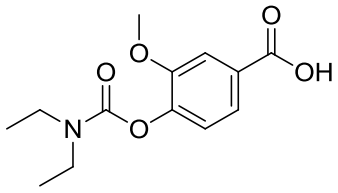
164.69, 153.86, 153.23, 134.26, 132.24, 128.56, 124.39, 124.32, 120.11, 82.49, 42.61, 42.20, 28.42, 28.32, 14.43, 13.49. HRMS calcd for $C_{12}H_{15}O_4NCl$ $[M+H]^+$; 272.06841, found; 272.06840.



2-Chloro-4-(diethylcarbamoyloxy)benzoic acid (1063-45Z). In a 10 mL round-bottomed flask, tert-butyl 2-chloro-4-(diethylcarbamoyloxy)benzoate (0.322 g, 0.982 mmol) was dissolved in TFA (Volume: 3.93 ml). The mixture was stirred until TLC indicated completion. The reaction mixture was diluted with diethyl ether, washed three times with brine, dried over magnesium sulfate and concentrated. The residue was then partitioned between sodium bicarbonate and DCM and washed three times with sodium bicarbonate. The organics were dried over magnesium sulfate and concentrated to yield the desired product. Yield 0.150 g, 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.28 (d, *J* = 2.3 Hz, 1H), 7.13 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.47 – 3.32 (m, 4H), 1.28 – 1.15 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.79, 154.85, 153.64, 136.10, 133.84, 125.16, 124.96, 124.92, 120.36, 42.82, 42.44, 14.25, 13.31. HRMS calcd for $C_{12}H_{13}O_4NCl$ $[M-H]^-$; 270.05386, found; 270.05432.

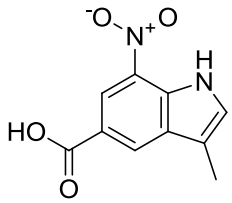


Methyl 4-(diethylcarbamoyloxy)-3-methoxybenzoate (1063-56Y). In a 50 mL round-bottomed flask, methyl 4-hydroxy-3-methoxybenzoate (2.5 g, 13.72 mmol), and pottasium carbonate (3.79 g, 27.4 mmol), were stirred in DMF (31.3 ml) for 1 hr at 23 °C to give an opaque suspension. Diethylcarbamic chloride (1.739 ml, 13.72 mmol) was added. The mixture was stirred overnight at room temperature. The reaction mixture was dried *in vacuo* and then diluted with 75 mL water and extracted with DCM. The combined extracts were washed with water and brine and then dried over $MgSO_4$ and concentrated to give the title compound without further purification. Yield 3.50 g, 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.39 (dq, *J* = 28.2, 7.1 Hz, 4H), 1.21 (dt, *J* = 27.3, 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.75, 153.60, 151.74, 144.80, 128.09, 123.31, 122.73, 113.47, 56.30, 56.18, 52.46, 52.33, 42.52, 42.28, 14.20, 13.57. HRMS calcd for $C_{14}H_{20}O_5N$ $[M+H]^+$; 282.13360, found; 282.13349.

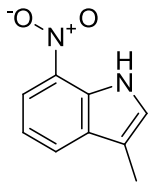


4-((Diethylcarbamoyl)oxy)-3-methoxybenzoic acid (1063-46Z). The Reactant 1 (3.50 g, 12.44 mmol) was dissolved in 13 ml MeOH and 8.00 ml 1N NaOH was added and heated to 120 °C in the microwave for 10 minutes. The mixture was checked by TLC which indicated completion. The mixture was acidified to pH 3 and then filtered to give the product as a white solid. Yield 2.50 g, 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.60 (m, 2H), 7.17 (d, *J* = 8.2 Hz, 1H), 3.87 (s, 3H), 3.41 (dq, *J* = 26.7, 7.1 Hz, 4H), 1.23 (dt, *J* = 26.9, 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.06, 153.72, 151.83,

145.41, 127.43, 123.58, 113.92, 56.33, 56.21, 42.59, 42.36, 14.17, 13.51. HRMS calcd for $C_{13}H_{17}O_5NNa$ $[M+Na]^+$; 290.09989, found; 290.09981.

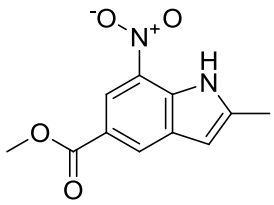


3-Methyl-7-nitro-1H-indole-5-carboxylic acid (1063-47X). The methyl 3-methyl-7-nitro-1H-indole-5-carboxylate (1.200 g, 5.12 mmol) was dissolved in 13 ml MeOH and 8.00 ml 1N NaOH and heated to 120 °C in the microwave for 10 minutes. The mixture was checked by TLC which indicated completion. The mixture was acidified to pH 3 and then filtered to give the product as a yellow solid. Yield 1.00 g, 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 11.96 (s, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.55 – 8.53 (m, 1H), 7.44 – 7.39 (m, 1H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.35, 166.73, 132.38, 131.98, 130.17, 127.52, 120.92, 119.25, 113.28, 9.09. HRMS calcd for $C_{10}H_7O_4N_2$ $[M-H]^-$; 219.04113, found; 219.04112.



3-Methyl-7-nitro-1*H*-indole (1063-47Y). A flame dried flask was charged with 1,10-phenanthroline (0.082 g, 0.454 mmol), 3-methyl-7-nitro-1H-indole-5-carboxylic acid (1.00 g, 4.54 mmol), and copper(I) oxide (0.032 g, 0.227 mmol). N-methylpyrolidone (5.77 ml, 60.0 mmol) and quinoline (1.921 ml, 16.21 mmol) were added via syringe into

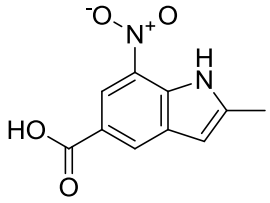
the flask and the mixture was heated to 170 °C. The reaction was stirred and monitored by TLC until completion. The reaction mixture was poured onto 2N HCl (5ml), and extracted with diethyl ether. The organics were washed with brine, dried over Magnesium Sulfate, filtered and dried *in vacuo (*Gooßen *et al., 2007).* The product was purified using column chromatography EtOAc/Hexanes 0-20% gradient on a Redi-Sep column. Yield 0.310 g, 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 8.09 (dd, *J* = 8.7, 3.9 Hz, 1H), 8.06 – 7.99 (m, 1H), 7.31 (s, 1H), 7.25 – 7.19 (m, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 132.43, 132.31, 128.41, 127.11, 126.08, 118.51, 117.96, 111.58, 9.21. HRMS calcd for $C_9H_7O_2N_2$ [M-H]$^-$; 175.05130, found; 175.05104.



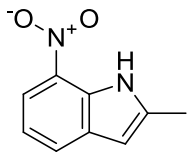
Methyl 2-methyl-7-nitro-1*H*-indole-5-carboxylate (1063-48x).

A flame-dried round-bottomed flask was charged with potassium tert-butoxide (2.50 g, 22.29 mmol) and 5.0 ml N-methylpyrolidone. A solution of methyl 4-amino-3-nitro-5-(prop-1-ynyl)benzoate (2.90 g, 4.27 mmol) in 6.0 ml N-methylpyrolidone was added dropwise to the basic solution at rt. The mixture was stirred overnight. Reaction performed under inert conditions. The reaction was quenched with water and DCM was added to the mixture. After several washes, the organics were combined and dried *in vacuo*. The mixture was columned using a Redi-Sep column and a 0-20% EtOAc/Hexanes gradient. The final product was obtained by crystallization in EtOAc/Hexanes; 1:9 ratio of solvents. Yield 0.600 g, 21%. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 9.79 (s, 1H), 8.74 (d, *J* = 1.4 Hz, 1H), 8.58 – 8.48 (m, 1H), 6.46 (dq, *J* = 2.1, 1.1 Hz, 1H), 3.95 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.55, 139.57, 132.80, 131.98, 128.98, 121.92, 119.68, 103.24, 103.13, 52.55, 14.03. HRMS calcd for $C_{11}11O_4N_2$ $[M+H]^+$; 235.07133, found; 235.07134 (Rodriguez et al., 2000).



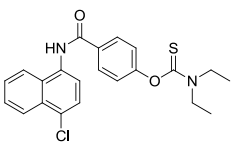
2-Methyl-7-nitro-1H-indole-5-carboxylic acid (1063-48y). The methyl 2-methyl-7-nitro-1H-indole-5-carboxylate (0.600 g, 2.56 mmol) was dissolved in 10 ml MeOH and 4.34 ml 1N NaOH was added and heated to 120 °C in the microwave for 10 minutes. The mixture was checked by TLC indicated completion. The mixture was acidified to pH 3 and filtered to give the desired product. Yield 0.490 g, 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 12.07 (s, 1H), 8.50 (s, 1H), 8.47 (s, 1H), 6.57 (s, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.73, 141.37, 132.79, 131.47, 130.38, 127.98, 121.34, 118.13, 102.50, 13.44. HRMS calcd for $C_{10}H_7O_4N_2$ $[M-H]^-$; 219.04113, found; 219.04103.



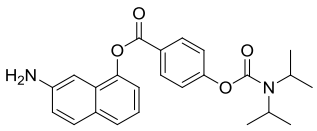
2-Methyl-7-nitro-1H-indole (1063-48z). A flame dried flask was charged with 1,10-phenanthroline (0.040 g, 0.223 mmol), 2-methyl-7-nitro-1H-indole-5-carboxylic acid (0.490 g, 2.225 mmol), and copper(I) oxide (0.016 g, 0.111 mmol). NMP (2.83 ml, 29.4

mmol) and quinoline (0.941 ml, 7.94 mmol) were added via syringe into the flask and the mixture was heated to 170 °C. The reaction was stirred and monitored by TLC until completion. The reaction mixture was poured onto 2N HCl (5ml) and extracted with diethyl ether. The organics were washed with brine, dried over magnesium sulfate, filtered and dried *in vacuo*. The product was purified using column chromatography EtOAc/Hexanes 0-20% gradient on a Redi-Sep column. Yield 0.160 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 8.04 – 7.88 (m, 2H), 7.22 – 7.11 (m, 1H), 6.47 – 6.40 (m, 1H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 139.79, 132.86, 132.83, 131.86, 128.53, 127.42, 118.51, 117.17, 101.10, 101.02, 13.39. HRMS calcd for $C_{19}H_7O_2N_2$ [M-H]$^-$; 175.05130, found; 175.05130.

General Method 1063-final The carboxylic acid was dissolved in DCM (0.100 mmol) and cooled to 0 °C in an ice bath. DMAP (1.1 eq.) and EDCI (1.0 eq) were then added to give a suspension and stirred for 45 minutes. The appropriate amine or alcohol (1.1 eq.) was then added to the flask and the mixture was stirred overnight, while being allowed to warm to room temperature. Upon completion, the solvent was evaporated and the residue was partitioned between 1.0N HCl and EtOAc. The mixture was extracted two times with EtOAc and the organics washed three times with brine, dried over magnesium sulfate, and concentrated under vacuum.

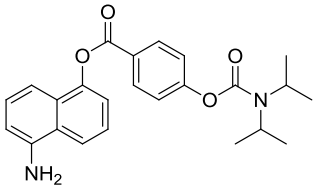


O-(4-((4-Chloronaphthalen-1-yl)carbamoyl)phenyl) diethylcarbamothioate (1063-12). Compound **1063-12** was prepared via General Method 1063-final using 4-((diethylcarbamothioyl)oxy)benzoic acid (0.400g, 1.58 mmol) and 4-chloronaphthalen-1-amine (0.309 g, 1.74 mmol). The title compound was obtained as a pink solid after column chromatography (0-30% EtOAc:hexanes) and crystallization from EtOAc and hexanes. Yield 0.21 g, 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.24 (dd, J = 8.3, 1.1 Hz, 1H), 8.17 – 8.06 (m, 3H), 7.81 – 7.59 (m, 4H), 7.32 – 7.23 (m, 2H), 3.85 (q, J = 7.1 Hz, 2H), 3.72 (q, J = 7.1 Hz, 2H), 1.27 (dt, J = 18.2, 7.1 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 185.10, 165.55, 156.18, 133.55, 131.45, 130.31, 129.10, 129.04, 128.30, 127.84, 127.64, 127.09, 126.95, 126.08, 124.24, 124.13, 124.05, 123.91, 122.96, 122.87, 47.89, 44.12, 13.51, 13.43, 11.64, 11.53. HRMS calcd for $C_{22}H_{22}N_2O_2ClS$ $[M+H]^+$; 413.10850, found; 413.10870. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 1.04 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.68 min; > 95% purity.

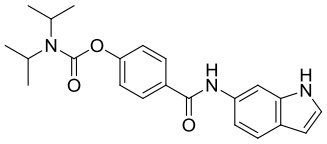


7-Aminonaphthalen-1-yl 4-((diisopropylcarbamoyl)oxy)benzoate (1063-10). Compound **1063-10** was prepared via general method 1063-final using 4-((diisopropylcarbamoyl)oxy)benzoic acid (.40 g, 1.5 mmol) and 8-aminonaphthalen-2-ol (0.264 g, 1.66 mmol). The title compound was obtained as a brown solid after column chromatography (0-30% EtOAc:hexanes). Yield 0.31 g, 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 – 8.22 (m, 2H), 8.05 (d, J = 8.4 Hz, 1H), 7.46 – 7.30 (m, 4H), 7.23 (t, J = 7.9 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.72 (d, J = 7.4 Hz, 1H), 5.92 (s, 2H), 4.09 – 3.97 (m, 2H), 1.33 – 1.22 (m, 12H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.82, 156.38,

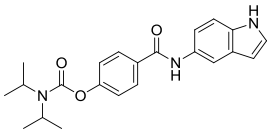
152.66, 147.08, 145.92, 132.18, 132.03, 128.42, 128.35, 128.31, 125.89, 124.50, 123.63, 122.98, 122.93, 121.43, 118.98, 118.94, 108.71, 108.51, 108.34, 47.09, 46.61, 21.94, 20.73. HRMS calcd for $C_{24}H_{27}N_2O_4$ $[M+H]^+$; 407.19653, found; 407.19653. Anal. C, H, N.



5-Aminonaphthalen-1-yl 4-((diisopropylcarbamoyl)oxy)benzoate (1063-14). Compound **1063-14** was prepared via General Method 1063-final using 4-((diisopropylcarbamoyl)oxy)benzoic acid (0.400g, 1.51 mmol) and 5-aminonaphthalen-1-ol (0.264 g, 1.66 mmol). The title compound was obtained as a red solid after column chromatography (0-30% EtOAc:hexanes) and crystallization from EtOAc and hexanes. Yield 0.11 g, 18%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 1H), 7.43 – 7.28 (m, 4H), 7.19 (t, J = 7.9 Hz, 1H), 6.95 (d, J = 8.3 Hz, 1H), 6.69 (d, J = 7.5 Hz, 1H), 5.88 (s, 2H), 4.06 – 3.94 (m, 2H), 1.25 (d, J = 15.7 Hz, 12H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.82, 156.38, 152.66, 147.08, 145.92, 132.18, 132.03, 128.42, 128.34, 128.30, 125.89, 124.50, 123.63, 122.98, 122.93, 121.42, 118.98, 118.93, 108.71, 108.51, 108.33, 47.10, 46.68, 21.94, 20.73. HRMS calcd for $C_{24}H_{27}N_2O_4$ $[M+H]^+$; 407.19653, found; 407.19718. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.762 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.924 min; > 95% purity.

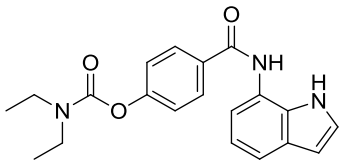


4-((1H-Indol-6-yl)carbamoyl)phenyl diisopropylcarbamate (1063-15) Compound **1063-15** was prepared via General Method 1063-final using 4-((diisopropylcarbamoyl)oxy)benzoic acid (0.400g, 1.51 mmol) and 1*H*-indol-6-amine (0.219 g, 1.66 mmol). The title compound was obtained as a white solid after column chromatography (0-30% EtOAc:hexanes) and crystallization from EtOAc and hexanes. Yield 0.324 g, 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.27 (s, 1H), 8.13 (s, 1H), 7.97 – 7.88 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.27 – 7.13 (m, 3H), 7.04 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.51 (t, *J* = 2.5 Hz, 1H), 4.18 (tt, *J* = 7.2, 4.0 Hz, 1H), 4.03 (s, 1H), 1.44 – 1.34 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.86, 154.28, 154.02, 136.43, 133.00, 132.53, 129.00, 125.46, 125.34, 122.45, 120.95, 114.16, 104.36, 102.34, 47.62, 46.76, 22.07, 20.94. HRMS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$; 380.19687, found; 380.19717. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.995 min; > 95% purity; 75% ACN:H_2O (0.1% Formic Acid) R_t 1.722 min; > 95% purity.

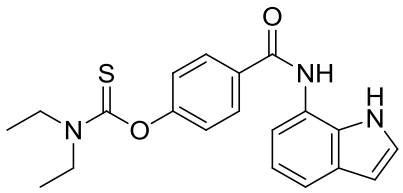


4-((1H-Indol-5-yl)carbamoyl)phenyl diisopropylcarbamate (1063-16) Compound 1063-16 was prepared via General Method 1063-final using 4-((diisopropylcarbamoyl)oxy)benzoic acid (0.400g, 1.51 mmol) and 1*H*-indol-5-amine (0.219 g, 1.66 mmol). The title compound was obtained as a white solid after column

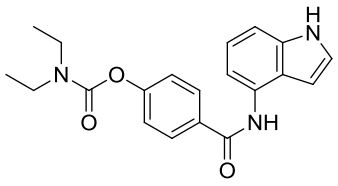
chromatography (0-30% EtOAc:hexanes) and crystallization from EtOAc and hexanes. Yield 0.19 g, 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 10.09 (s, 1H), 8.04 – 7.96 (m, 3H), 7.43 – 7.30 (m, 3H), 7.25 (d, J = 8.3 Hz, 2H), 6.41 (t, J = 2.3 Hz, 1H), 4.02 (p, J = 7.0 Hz, 2H), 1.32 – 1.23 (m, 12H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.00, 154.06, 153.04, 133.64, 132.62, 131.57, 130.06, 129.58, 129.48, 129.00, 128.06, 126.58, 126.51, 122.21, 122.16, 122.12, 116.76, 112.94, 112.81, 111.67, 111.64, 101.81, 47.00, 46.50, 21.97, 20.79. HRMS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$; 380.19687, found; 380.19695. Anal. C, H, N.



4-((1*H*-Indol-7-yl)carbamoyl)phenyl diethylcarbamate (1064-20). Compound 1063-20 was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.40 g, 1.7 mmol) and 1*H*-indol-7-amine (0.22 g, 1.7 mmol). The title compound was obtained as a purple solid after column chromatography (0-30% EtOAc:hexanes) and crystallization from DCM and hexanes. Yield 0.34 g, 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 10.14 (s, 1H), 8.11 – 8.03 (m, 2H), 7.42 (d, J = 7.8 Hz, 1H), 7.34 (dd, J = 5.4, 2.5 Hz, 1H), 7.30 (d, J = 8.8 Hz, 3H), 7.00 (t, J = 7.7 Hz, 1H), 6.47 (t, J = 2.4 Hz, 1H), 3.44 (q, J = 7.1 Hz, 2H), 3.33 (q, J = 7.1 Hz, 2H), 1.23 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.81, 153.74, 152.96, 131.61, 129.82, 129.26, 129.20, 123.06, 121.70, 118.71, 117.38, 115.95, 101.43, 41.83, 41.58, 14.25, 13.32. HRMS calcd for $C_{20}H_{22}N_3O_3$ $[M+H]^+$; 352.16557, found; 352.16948. Anal. calc. for $C_{20}H_{21}N_3O_3 \cdot 0.3\ H_2O$; C, H, N.

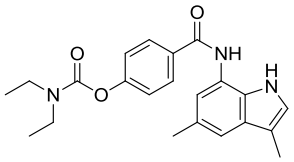


O-(4-((1H-Indol-7-yl)carbamoyl)phenyl) diethylcarbamothioate (1063-21). Compound 1063-31 was prepared via general method 1063-final using 4-((diethylcarbamothioyl)oxy)benzoic acid (0.40 g, 1.7 mmol) and 1*H*-indol-7-amine (0.22 g, 1.7 mmol). The title compound was obtained as a purple solid after column chromatography (0-30% EtOAc:hexanes) and crystallization from DCM and hexanes. Yield 0.34 g, 57%. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.89 (s, 1H), 10.13 (s, 1H), 8.08 – 8.00 (m, 2H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.34 – 7.18 (m, 4H), 6.96 (t, *J* = 7.7 Hz, 1H), 6.44 (dd, *J* = 3.1, 1.9 Hz, 1H), 3.81 (q, *J* = 7.0 Hz, 2H), 3.68 (q, *J* = 7.0 Hz, 2H), 1.23 (dt, *J* = 18.5, 7.0 Hz, 6H). ^{13}C NMR (151 MHz, dmso) δ 185.84, 165.46, 156.61, 132.80, 130.49, 129.84, 129.78, 125.92, 125.85, 123.71, 123.44, 123.36, 119.44, 119.30, 118.04, 116.64, 116.58, 102.13, 102.02, 48.54, 44.76, 14.18, 14.09, 12.31, 12.20. HRMS calcd for $C_{20}H_{22}N_3O_2S$ $[M+H]^+$; 368.14272, found; 368.14281. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.740 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.939 min; > 95% purity.



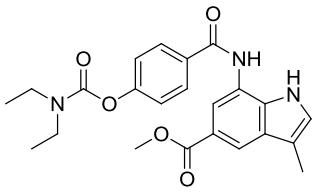
4-(1H-Indol-4-ylcarbamoyl)phenyl diethylcarbamate (1063-26).

Compound **1063-26** was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.41 g, 1.7 mmol) and 1*H*-indol-4-amine (0.25 g, 1.8 mmol). The title compound was obtained as a an off-white solid after crystallization from DCM and hexanes. Yield 0.39 g, 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 10.10 (s, 1H), 8.03 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 7.6 Hz, 1H), 7.34 – 7.22 (m, 4H), 7.08 (t, J = 7.8 Hz, 1H), 6.59 (t, J = 2.5 Hz, 1H), 3.43 (q, J = 7.3 Hz, 2H), 3.36 – 3.28 (m, 2H), 1.18 (dt, J = 34.8, 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.78, 153.63, 152.95, 136.82, 131.86, 130.23, 129.17, 124.38, 122.21, 121.65, 120.89, 113.18, 108.50, 99.96, 41.81, 14.22, 13.30. HRMS calcd for $C_{20}H_{22}N_3O_3$ $[M+H]^+$; 352.16557, found; 352.16537. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.618 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.685 min; > 95% purity.

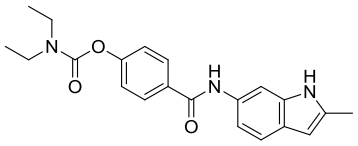


4-(3,5-Dimethyl-1H-indol-7-ylcarbamoyl)phenyl diethylcarbamate (1063-34). Compound **1063-062** was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.74 g, 3.1 mmol) and 3,5-dimethyl-1H-indol-7-amine (0.46 g, 2.9 mmol). The title compound was obtained as a brown solid after crystallization from DCM and hexanes. Yield 0.330 g, 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 – 10.37 (m, 1H), 10.04 (s, 1H), 8.09 – 8.01 (m, 2H), 7.33 – 7.25 (m, 2H), 7.20 (d, J = 1.4 Hz, 1H), 7.13 (s, 1H), 7.06 (t, J = 1.7 Hz, 1H), 3.43 (q, J = 7.1 Hz, 2H), 3.38 – 3.28 (m, 2H), 2.40 (s, 3H), 2.24 (s, 3H), 1.23 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.37, 154.35, 153.60, 132.29, 130.29,

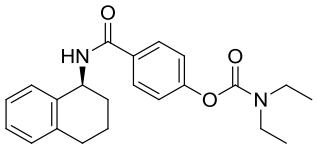
129.87, 128.91, 127.25, 123.49, 123.34, 123.19, 122.34, 122.26, 117.86, 117.77, 115.86, 115.75, 109.76, 42.46, 42.23, 22.00, 21.90, 14.94, 14.82, 14.03, 13.87, 10.42, 10.26. HRMS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$; 380.19687, found; 380.19642. Analytical calculated for $C_{22}H_{26}N_3O_3 \cdot 0.92\ H_2O$; C, H, N.



Methyl 7-(4-(diethylcarbamoyloxy)benzamido)-3-methyl-1H-indole-5-carboxylate (1063-35). Compound **1063-35** was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.25 g, 1.1 mmol) and methyl 7-amino-3-methyl-1H-indole-5-carboxylate (0.20 g, 0.98 mmol). The title compound was obtained as a brown solid after crystallization from MeOH:H_2O. Yield 0.377 g, 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.24 (s, 1H), 8.08 (d, J = 8.5 Hz, 3H), 8.02 (s, 1H), 7.31 (d, J = 8.2 Hz, 2H), 7.26 (s, 1H), 3.86 (s, 3H), 3.43 (q, J = 7.1 Hz, 2H), 3.33 (q, J = 7.2 Hz, 2H), 2.32 (s, 3H), 1.23 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.14, 164.92, 153.86, 152.92, 132.56, 131.28, 129.32, 129.28, 129.03, 124.48, 124.41, 122.84, 121.72, 119.75, 118.09, 116.45, 111.44, 51.79, 41.81, 41.58, 14.22, 13.29, 9.47. HRMS calcd for $C_{23}H_{26}N_3O_5$ $[M+H]^+$; 423.18670, found; 423.18661. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.945 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.141 min; > 95% purity.

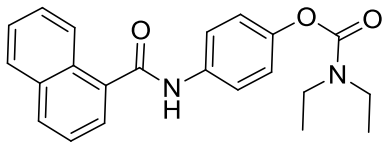


4-(2-Methyl-1H-indol-6-ylcarbamoyl)phenyl diethylcarbamate (1063-36). Compound **1063-36** was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.41 g, 1.7 mmol) and 2-methyl-1H-indol-5-amine (0.25 g, 1.7 mmol). The title compound was obtained as a brown solid after crystallization from DCM and hexanes. Yield 0.21 g, 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 – 10.83 (m, 1H), 10.04 (s, 1H), 8.03 – 7.95 (m, 2H), 7.82 (d, J = 1.9 Hz, 1H), 7.33 – 7.18 (m, 4H), 6.13 – 6.07 (m, 1H), 3.42 (q, J = 7.1 Hz, 2H), 3.31 (t, J = 7.1 Hz, 2H), 2.37 (s, 3H), 1.18 (dt, J = 34.6, 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.90, 154.10, 153.60, 136.88, 133.82, 132.84, 131.37, 129.48, 129.03, 122.30, 115.55, 112.02, 110.71, 99.90, 42.45, 42.22, 14.13, 13.92. HRMS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$; 366.18122, found; 366.18144. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.796 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.918 min; > 95% purity.



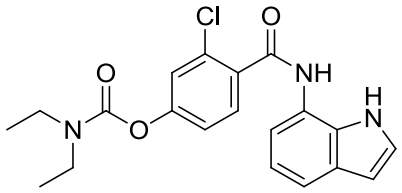
(S)-4-(1,2,3,4-Tetrahydronaphthalen-1-ylcarbamoyl)phenyl diethylcarbamate (**1063-37**). Compound **1063-37** was prepared via general method1063-final using 4-((diethylcarbamoyl)oxy)benzoic acid (0.40 g, 1.7 mmol) and (S)-1,2,3,4-tetrahydronaphthalen-1-amine2-methyl-1H-indol-5-amine (0.25 g, 1.7 mmol). The title compound was obtained as a brown solid after crystallization from DCM and hexanes.

Yield 0.261 g, 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, J = 8.6 Hz, 1H), 7.98 – 7.90 (m, 2H), 7.24 – 7.08 (m, 6H), 5.29 – 5.19 (m, 1H), 3.46 – 3.25 (m, 4H), 2.87 – 2.69 (m, 2H), 2.05 – 1.91 (m, 2H), 1.88 – 1.69 (m, 2H), 1.20 (t, J = 7.0 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.82, 154.11, 153.58, 138.33, 137.85, 131.89, 129.47, 129.27, 128.49, 127.28, 126.46, 122.25, 122.14, 47.95, 47.80, 42.19, 30.57, 29.59, 21.19, 14.79, 13.85. HRMS calcd for $C_{22}H_{27}N_2O_3$ $[M+H]^+$; 367.20162, found; 367.20144. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.661 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.730 min; > 95% purity.

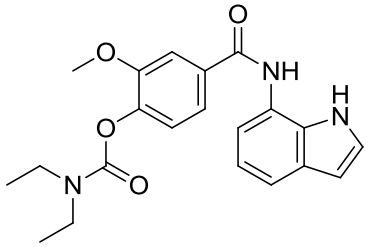


4-(1-Naphthamido)phenyl diethylcarbamate (**1063-44**). Compound 1063-44 was prepared via general method 1063-final using 4-aminophenyl diethylcarbamate (0.18 g, 0.84 mmol) and 1-naphthoic acid (0.15 g, 0.84 mmol). The title compound was obtained as an off-white solid after crystallization from MeOH and water. Yield 0.12 g, 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.23 – 8.15 (m, 1H), 8.09 (d, J = 8.2 Hz, 1H), 8.06 – 8.01 (m, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.76 (d, J = 6.9 Hz, 1H), 7.67 – 7.54 (m, 3H), 7.12 (d, J = 8.7 Hz, 2H), 3.52 – 3.22 (m, 4H), 1.21 (t, J = 7.0 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.16, 153.52, 147.04, 136.35, 134.71, 133.16, 130.16, 130.08, 129.64, 128.38, 127.03, 126.40, 125.48, 125.16, 125.07, 122.24, 122.15, 122.10, 120.53, 41.71, 41.45, 14.24, 13.34. HRMS calcd for $C_{22}H_{23}N_2O_3$ $[M+H]^+$; 363.17032, found; 363.16994. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t

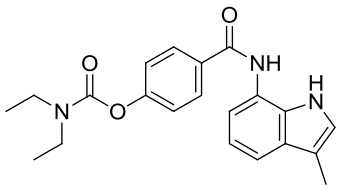
0.918 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.046 min; > 95% purity.



4-(1H-indol-7-ylcarbamoyl)-3-chlorophenyl diethylcarbamate (**1063-45**). Compound 1063-45 was prepared via general method 1063-final using 1H-indol-7-amine (0.07 g, 0.55 mmol) and 2-chloro-4-(diethylcarbamoyloxy)benzoic acid (0.15 g, 0.55 mmol). The title compound was obtained as a brown solid after crystallization from MeOH and water. Yield 0.063 g, 30%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 10.28 (s, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.29 (dd, J = 8.4, 2.3 Hz, 1H), 7.00 (t, J = 7.7 Hz, 1H), 6.47 (dd, J = 3.0, 1.9 Hz, 1H), 3.43 (q, J = 6.9 Hz, 2H), 3.33 (q, J = 7.2 Hz, 2H), 1.22 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.45, 152.71, 152.44, 133.67, 130.54, 130.02, 129.19, 128.19, 125.29, 123.33, 123.01, 120.90, 118.86, 117.06, 113.91, 41.89, 41.60, 14.14, 13.22. HRMS calcd for $C_{20}H_{20}N_3O_3Cl$ $[M+H]^+$; 386.12660, found; 386.12640. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.791 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 1.015 min; > 95% purity.

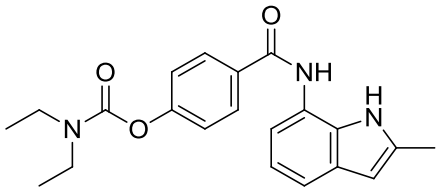


4-(1H-indol-7-ylcarbamoyl)-2-methoxyphenyl diethylcarbamate (**1063-46**). Compound **1063-46** was prepared via general method 1063-final using 1H-indol-7-amine (1.19 g, 8.98 mmol) and 4-(diethylcarbamoyloxy)-3-methoxybenzoic acid (2.4 g, 8.98 mmol). The title compound was obtained as an off-white solid after crystallization from MeOH and water. Yield 1.56g, 46%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 8.55 (s, 1H), 7.50 (dd, *J* = 7.1, 1.7 Hz, 1H), 7.45 (d, *J* = 2.1 Hz, 1H), 7.36 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.29 – 7.20 (m, 1H), 7.08 – 6.97 (m, 3H), 6.55 (dd, *J* = 3.1, 2.1 Hz, 1H), 3.74 (s, 3H), 3.52 – 3.37 (m, 4H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.21, 125.08, 123.39, 119.80, 119.58, 118.30, 114.10, 112.00, 102.72, 56.00, 42.63, 42.43. 14.20, 13.65. HRMS calcd for $C_{21}H_{24}N_3O_4$ $[M+H]^+$; 382.17613, found; 382.17599. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.710 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.822 min; > 95% purity.



4-(3-methyl-1H-indol-7-ylcarbamoyl)phenyl diethylcarbamate (**1063-47**). Compound **1063-47** was prepared via general method 1063-final using 3-methyl-1H-indol-7-amine (0.20 g, 1.37 mmol) and 4-(diethylcarbamoyloxy)-3-methoxybenzoic acid (0.33 g, 1.37 mmol). The title compound was obtained as a pink solid after crystallization from MeOH and water, HPLC using 75% MeOH:water and a second crystallization from MeOH : water. NMR shows a 6:1 mixture of the 3-substituted : 2-substituted indole product. Yield 0.075 g, 15%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 10.09 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.38 – 7.19 (m, 4H), 7.12 (s, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 3.49 –

3.39 (m, 2H), 3.38 – 3.28 (m, 2H), 2.27 (s, 3H), 1.27 – 1.10 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.77, 153.72, 152.95, 152.17, 131.63, 130.05, 130.00, 129.49, 129.26, 122.87, 122.68, 121.69, 115.85, 115.53, 109.63, 41.81, 41.59, 14.25, 13.30, 9.69. HRMS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$; 366.18122, found; 366.18095.



4-(2-methyl-1H-indol-7-ylcarbamoyl)phenyl diethylcarbamate (**1063-48**). Compound **1063-48** was prepared via general method 1063-final using 2-methyl-1H-indol-7-amine (0.13 g, 0.91 mmol) and 4-(diethylcarbamoyloxy)-3-methoxybenzoic acid (0.22 g, 0.91 mmol). The title compound was obtained as an off-white solid after crystallization from MeOH and water. Yield 0.18 g, 54%. ^{1}H NMR (400 MHz DMSO-d_6) δ 10.71 (s, 1H), 10.04 (s, 1H), 8.06 (d, J = 8.2 Hz, 2H), 7.33 – 7.19 (m, 4H), 6.92 (t, J = 7.7 Hz, 1H), 6.15 (s, 1H), 3.43 (q, J = 7.2 Hz, 2H), 3.38 – 3.28 (m, 2H), 2.39 (s, 3H), 1.23 (t, J = 6.9 Hz, 3H), 1.14 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.75, 153.70, 152.97, 135.49, 131.70, 130.05, 130.00, 129.25, 122.18, 121.68, 116.30, 115.36, 99.54, 41.80, 41.58, 13.44, 13.31. HRMS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$; 366.18122, found; 366.18094. HPLC 85% MeOH:H_2O (0.1% Formic Acid) R_t 0.784 min; > 95% purity; 75% ACN: H_2O (0.1% Formic Acid) R_t 0.917 min; > 95% purity.

Chapter 7. Discussion and Conclusion

7.1. Summary

The GluN2C- and GluN2D-containing NMDA receptors remain largely understudied in relation to the GluN2A- and GluN2B-containing receptors. In part, this is due to the lack of subunit selective pharmacological probes that can assist in isolating and understanding the subunit contributions to both normal and patho-physiological states in which the GluN2C- and GluN2D-containing receptors are involved. I show that the DQP-class of molecules shares some structural determinants of selectivity at the GluN2D receptor with the QNZ class of compounds. While not presented in this thesis, the two-classes of compounds are also glutamate dependent which has implications both in terms of their potential therapeutic relevance and also in terms of the likelihood that they may act at a similar or shared site at the receptors. I provide an extensive structure activity relationship for the DQP-class of compounds which both improved the potency and selectivity of the class as well as provided a framework for moving the compounds toward therapeutically relevant entities. I also evaluated the overall shape (volume distribution) and color (similarity of atom-type) similarity of the compounds which supports the hypothesis that the compounds could act at a similar site as not only are they pharmacologically similar, they have three-dimensional similarity as well. The synthetic efforts and advances that have been made in the pharmacological profile of these compounds have provided highly potent and selective negative allosteric modulators that are being used to dissect the role GluN2D-containing receptors have in the subthalamic nucleus.

Finally, I designed and implemented a strategy aimed at elucidated the structure activity relationship of another class of GluN2C- and GluN2D-containing receptors which has unique structural determinants of activity.

7.2. DQP-1105 as a representative member of the class of compounds

The identification of residues Q701 and L705 as structural determinants of selectivity for the DQP-class of compounds has a number of implications (Acker et al., 2011). First, the lower lobe of the ligand binding domain where these residues are located may represent an endogenous modulatory site for the steroid pregenenolone sulfate (Horak et al., 2006). The implication that the DQP-class of molecules bind to an endogenous allosteric modulatory site is that optimization of the molecules through synthetic efforts could lead to highly potent and selective pharmacological probes.

Furthermore, the LBD-TM linker regions are responsible for transmitting gating information upon binding of agonist to the receptor (Krupp et al., 1998; Talukder et al., 2010; Yuan et al., 2005). Excised outside-out single-channel patch clamp experiments on recombinantly expressed GluN1-GluN2D NMDA receptors evaluating the response to maximally effective concentrations of glutamate and glycine in the presence or absence of various concentrations of DQP-1105 did not significantly affect the mean open time or the open duration, but rather, significantly reduced the open probability of the receptors (experiments conducted and analyzed by Kevin Ogden; Acker et al., 2011). This observation leads to the conclusion that the activation energy for a conformational change necessary for channel opening is raised in the presence of DQP-1105 (Acker et al., 2011).

Second, data collected by Dr. Katie Vance in HEK patch-clamp recordings from cells recombinantly expressing GluN1-GluN2D receptors during these studies showed that there is a use dependence for the antagonist effects of DQP-1105 (data not shown) (Acker et al., 2011). That is, glycine evoked peak responses with pre-application of DQP-1105 and NMDA were significantly less than that of glycine evoked currents in the absence of DQP-1105 (Acker et al., 2011). However, pre-application of DQP-1105 and glycine exhibited similar peak responses to that of NMDA evoked currents in the absence of DQP-1105 (Acker et al., 2011). The conclusion of these experiments is that DQP-1105 is use-dependent in that the binding of the modulator to the receptor requires NMDA, but not glycine. Thus, it appears that the well documented domain closure and subsequent conformational changes associated with channel opening either influence a binding site for the DQP-1105 class of molecules, or allow access of the molecules to a site which, once bound, disallows channel opening (Erreger et al., 2004; Furukawa and Gouaux, 2003; Inanobe et al., 2005; Mayer et al., 2006; Vance et al., 2011).

Huei-Sheng Vincent Chen and Stuart A. Lipton have proposed that the ideal clinical candidate targeting the NMDA receptor for treating excitotoxicity would preferentially block excessive activation (Chen and Lipton, 2006). Furthermore, a use-dependent antagonist that is also subunit-selective might be particularly efficacious in specific brain regions where aberrant activation of the receptor is either causing or involved in pathophysiological disease states. These observations and hypotheses provided a framework upon which to utilize synthetic chemistry in order to create more potent and selective molecules that exploit what appears to be a prime pharmacological target.

7.3. Optimizing the DQP-class of compounds through synthetic chemistry

While the selectivity of the DQP-class was relatively high at the beginning of this project, the low potency and solubility of the compounds that were tested through the initial SAR efforts left ample room for improvement. Given that the synthesis of the more diverse compounds required more than just a few steps, it was not surprising that the first efforts to identify more potent and selective congeners by evaluating commercially available sources fell short. I followed a strategy that would enable conclusions regarding the nature of activity to be made in a manner that should continue to be useful as the compounds are moved into the next phase of development.

Furthermore, the highly potent and selective congeners that were discovered through the SAR are significantly more soluble and have allowed for full pharmacological profiles against all four GluN2-receptor subtypes to be realized in a number of instances. The data support the hypothesis that a similar binding site for these compounds exist at all four receptor subtypes and is more conserved across GluN2A, GluN2C and GluN2D receptors in some region of the pocket than the same site at GluN2B-containing receptors. While no compounds in this study exhibited preference for GluN2A- or GluN2B-containing receptors over GluN2C- and GluN2D-containing receptors, the full-inhibition that was observed in many instances at all four subtypes raises the possibility that subunit-selectivity could be tuned with the appropriate modifications to the small molecules.

Also, because NMDA receptors are thought to exist as hetero-trimeric complexes, the ability of these compounds to inhibit responses from receptor populations that are tri-

heteromeric in nature is encouraging. It has been shown that the GluN2B-subunit selective antagonist ifenprodil is not as potent at GluN1-GluN2A-GluN2B containing receptors as it is at GluN1-GluN2B containing receptors (Hatton and Paoletti, 2005). Ifenprodil and its closely related analogs have been the standard for subunit-selective NMDA receptor antagonists; however, tri-heteromeric receptors are hypothesized to be more predominant than previously appreciated. Therefore, pharmacological probes such as the ones that I have developed in this chapter, which exhibit some affinity for each receptor subtype, could be more useful in dissecting the roles these receptor combinations play in normal and pathophysiological conditions. For example, GluN2D has been hypothesized to form heteromeric complexes with both GluN2A and GluN2B in certain regions of the adult rat cortex, striatum and thalamus (Dunah et al., 1998). It is tempting to speculate that a tool compound or set of tool compounds whose affinities or IC_{50} for each different potential receptor sub-population was known could be invaluable in understanding the biology of these receptors.

My studies have also allowed for the absolute stereochemistry of the purified enantiomers for a representative of the DQP-class of compounds to be assigned. Not only was the *S* enantiomer of 997-23 more potent than the racemic mixture, but it was also considerably more selective than the *R* enantiomer. It is known that the stereochemistry of a compound can not only affect its biological activity, but can also have profound effects on metabolism and off-target effects (Evans et al., 1988; Wienkers et al., 2011). These considerations can be crucially important as a class of compounds is evaluated for *in vivo* experimentation and my findings could be of considerable utility moving forward.

The carboxylic acid motif in the compounds was perceived as one of the major impediments to obtaining broad *in vivo* utility and allowing for proof of concept studies testing hypotheses related to the normal and pathophysiological contributions of these receptor subtypes. My studies have shown that the acid motif itself is not required for activity. Paradoxically, the data from the SAR suggests that the carboxylic acid motif could act as a hydrogen bond donor within the binding site, and not a hydrogen bond acceptor as one would expect. Only the acid, alcohol and primary amide retained potency at the receptors (see chapter 4) and the one common feature those three active compounds (-COOH, -OH and $CONH_2$) share is the potential to donate a hydrogen bond when bound at the receptor. While this is counterintuitive for the carboxylic acid which would be normally be deprotonated at physiological pH, it is well known that local environments within a protein can vary (Alexov et al., 2011). Therefore, it could be that this region of the binding pocket is sufficiently acidic such that the acid is protonated when bound and capable of donating a hydrogen bond, or that the acid donates a hydrogen bond through a water mediated network.

The preliminary data regarding both the potential for blood-brain barrier penetration and metabolism regarding the mono-fluoro compound that I synthesized (see chapter 4; 997-64) is also particularly instructive from a development perspective. Most importantly, this isosteric replacement of the alcohol suggests that, in principle, the compounds can be made to be blood-brain barrier penetrable by reducing the topological polar surface area of the compounds (Hagmann, 2008). Furthermore, while P-glcyoprotein affinity was not determined for the alcohol containing compound (see chapter 4; 997-57), the efflux ratio in the MDR1-MDCK assay suggests strongly that the

compound is a reasonably good substrate. Importantly, the efflux ratio for the mono-fluoro compound was only slightly higher than the passive diffusion of the compounds in the opposite direction. These observations are promising in that the acyl-chain, which is installed late in the synthesis, seems to be able to tune properties that will affect *in vivo* utility. The right combination of substituents in this region of the molecules could retain the desirable potency and selectivity profiles observed through my SAR and move the compounds toward a broader application.

Furthermore, the observed metabolism for the alcohol containing compound as compared to that of the mono-fluoro compound is also instructive. While the metabolite identification was not obtained for the alcohol containing compound, it was assumed that the rapid elimination was most likely due to an oxidation. This is supported by the increase in half-life of the compound when the fluorine was installed (see chapter 4). Taken together, these observations support the hypothesis that, not only can an appropriate substitution on the acyl-chain moiety attenuate metabolism and increase passive blood-brain barrier penetration, but it can also mitigate undesirable affinity for efflux. Now, the challenge is to discover the appropriate combination of modifications which retain potency and selectivity while meeting these objectives.

While instructive, the progress made through this study undoubtedly represents a small fraction of what remains to be learned before a compound, or compounds, are discovered that will meet the criterion for being a drug. However, the amount of knowledge that can be obtained through pharmacological isolation, manipulation and investigation of these receptors using compounds such as those that I have developed

could improve our understanding of the role these receptors play in physiological and patho-physiological conditions.

7.4. QSAR and ROCS computational modeling

The pharmacological data surrounding the QNZ- and DQP-class provides a compelling rationale for these two distinct classes of compounds binding to an overlapping site at the receptor. In the context of closely related ionotropic glutamate receptors, this phenomenon has been described and known since the quinazilinone-4-one-backbone containing molecules were first described as high affinity negative allosteric modulators of the AMPA receptors (Menniti et al., 2000). The discovery and characterization of CP-465, 022 led to the observation that the previously described AMPA antagonists containing the 2, 3-benzodiazepine scaffold (GYKI) could displace a tritiated analog within the quinazilinone-4-one class of compounds (Donevan and Rogawski, 1993; Menniti et al., 2000). The structural determinants of activity at the AMPA receptors for both the quinazilinone-4-one and benzodiazepine compounds resides in the linker regions at the lower lobe of the D2-domain, in close proximity to structural determinants of activity that have been described for both the QNZ- and DQP-class of compounds (Acker et al., 2011; Balannik et al., 2005; Hansen and Traynelis, 2011). Notably, for the two-classes of AMPA receptor antagonists and for the two-classes of NMDA receptor antagonists, some structural determinants were the same while others were different.

The results from the ROCS comparisons and hybrid molecule that was synthesized continue to support the pharmacological observations and hypothesis that the

two classes of compounds share an overlapping site at the receptors. If the small molecule overlays bear any similarity to the conformations of the molecules in the binding site, compound 1936 may be interacting with residues at the receptor that interact with the quinolone-amide core of the DQP-class of molecules, a hypothesis that is supported by both the higher Tanimoto combo similarity scores for that overlay and by the observation that installing the hydroxy-napthyl moiety onto the DQP-class of compounds did not improve potency (see chapter 5). The one clear difference between the compounds that is apparent in the modeling experiments and, now, more so that there are structure activity relationships around both scaffolds of compounds, is the B-ring of the DQP-class (see chapter 4). Modifications to this ring improved potency and selectivity for the class of compounds and clearly does not overlay with any of the QNZ compounds. It would be interesting to design an analog within the QNZ class of compounds that installs this functionality onto the hydroxyl napthyl ring of the QNZ class of compounds.

The QSAR model that was developed can predict the activity of compounds that either share the QNZ-backbone or are closely related to the scaffold, but it remains to be seen whether the model adequately describes a minimal pharmacophore model that can be used to find novel modulators of the NMDA receptors. Interestingly, while not all of the predicted hits from the high-throughput *in silico* screening based on the QNZ-QSAR have been purchased or tested, there are several hits predicted to be active at the receptors which have benzodiazepine like structures, similar to that of the GYKI compound. It will be interesting to determine whether compounds similar this scaffold can interact with the binding site at the NMDA receptors as the benzodiazepine compounds have not

previously been shown to inhibit glutamate evoked responses from the NMDA receptors (Donevan and Rogawski, 1993).

If these modeling efforts expand the number of known modulators that act at an overlapping site, the likelihood of developing more drug-like compounds can only increase. Furthermore, the functionality available within the suites of software that are used for these experiments can assist in moving the compounds closer to drug-likeness.

7.5. 1063 Series of Antagonists

This class of compounds appeared to be a highly attractive project to pursue. The compounds identified in the screening effort carried out by the Traynelis lab are highly selective for GluN2C- and GluN2D-containing receptors (100-fold), low molecular weight (< 400 dalton) and brain penetrable (the original screening hit). The original scaffold did prove to have a serious liability with the 1-napthylamine linkage into the amide bond of the connecting phenyl ring (see chapter 6). While my efforts to remove that liability by replacing the napthyl group with an indole were successful from a potency and selectivity perspective, the indole containing compound had significantly less blood-brain barrier penetration (data collected by Lundbeck and not shown).

From a synthetic medicinal chemistry perspective, the compounds offer a variety of opportunities and challenges. For example, achieving the desired regio-selectivity with appropriately functionalized indole ring remains to be evaluated. Furthermore, the compounds exhibited decreased potency as a function of time when left in Oocyte recording buffer, presumably due to degradation (data not shown; collected in Traynelis lab). This was originally thought to be due to oxidation of the thio-carbamate, however,

the same observation was subsequently made with compounds which simply contained the carbamate. Identifying the exact degradation products and finding appropriate substitutions which maintain potency and selectivity while ameliorating the labile functionality remains another goal.

7.6. Conclusion

The ability to pharmacologically isolate GluN2C- and GluN2D-containing receptors has been hindered by the lack of potent, selective probes with which to study the receptors. This thesis describes the structural determinants of selectivity for a representative member of a novel class of negative allosteric modulators of the GluN2C- and GluN2D-containing NMDA receptor. Synthetic chemistry efforts led to the identification of several of the most potent and selective negative allosteric modulators of the GluN2C- and GluN2D-containing receptor yet to be described. Further characterization of the isolated enantiomers for one of the more potent and selective congeners shows that one enantiomer (997-23-*S*) is considerably more potent and selective than the other (997-23-*R*). The discoveries made in this thesis will allow for the scientific community to isolate and functionally characterize these under-studied receptors. Furthermore, the findings described here lay the foundation for compounds to be developed which contain the pharmacophore requirements of the potent and selective congeners of the 997-class of compounds, but are more drug-like in nature. I also describe a similarity comparison between two-distinct classes of compounds which may be binding to an overlapping site at the receptors along with a QSAR model for the 987-class of compounds. The findings from these efforts could lead to the discovery of novel scaffolds of compounds and be utilized to a greater extent should the binding mode of the

compounds become known. Finally, I conclude my work with a description of the SAR around another class of subunit selective compounds which may provide yet another set of compounds for investigating these receptors and their contributions to normal and patho-physiological conditions.

Chapter 8 References

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