Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Sommer S. Zimmerman **Date**

Design, Synthesis, and Biological Evaluation of Subunit-Selective *N*-Methyl-D-Aspartate Receptor Modulators

By

Sommer S. Zimmerman
Doctor of Philosophy

Chemistry

Dennis C. Liotta
Advisor

Lanny S. Liebeskind
Committee Member

Stefan A. Lutz
Committee Member

Stephen F. Traynelis
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

Design, Synthesis, and Biological Evaluation of Subunit-Selective *N*-Methyl-D-Aspartate Receptor Modulators

By

Sommer S. Zimmerman

B.S., Florida State University, 2006 & 2008

Advisor: Dennis C. Liotta, Ph.D.

An abstract of

A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

2014

Abstract

Design, Synthesis, and Structure-Activity Relationship of Subunit-Selective *N*-Methyl-D-Aspartate Receptor Modulators

Chapter 1: Design, Synthesis, and Structure-Activity Relationship of GluN2C/GluN2D-Selective NMDA Receptor Antagonists

Chapter 2: Design, Synthesis, and Structure-Activity Relationship of Novel GluN1/GluN2C-Selective NMDA Receptor Positive Allosteric Modulators

By: Sommer S. Zimmerman

Chapter 1: *N*-Methyl-D-aspartate (NMDA) receptors are members of the family of ionotropic glutamate receptors that mediate excitatory neurotransmission. The overactivation of NMDA receptors has been associated with a range of neurological conditions including Parkinson's disease (PD), Alzheimer's disease (AD), stroke, epilepsy, neuropathic pain, and traumatic brain injury (TBI). In an effort to discover a treatment for these neurological insults, a number of antagonists of the NMDA receptor have been developed.

A fluorescence-based primary screen revealed a class of antagonists selective for GluN2C- and GluN2D-containing receptors over other NMDA receptor subtypes, with selectivity greater than 500-fold. Evaluation of a series of analogs resulted in compounds with potency in the low micromolar range (IC_{50} =1-5 μM) and high selectivity (> 500-fold) for GluN2C- and GluN2D-containing NMDA receptors over GluN2A- and GluN2B-containing NMDA receptors. These analogs represent a novel series of allosteric inhibitors that are selective for GluN2C- and GluN2D-containing NMDA receptors.

Chapter 2: NMDA hypofunction contributes to the psychosis observed in various neuropsychiatric diseases, such as schizophrenia. Potentiators of NMDA function may therefore offer therapeutic potential for such diseases of psychosis. In addition, an increasing amount of research has indicated that potentiation of NMDA receptors may find utility towards the treatment of anxiety disorders, as well as towards the enhancement of learning and memory.

A series of novel compounds that selectively potentiate GluN2C-containing NMDA receptors were developed based on a screening hit identified in a fluorescence-based primary screen. The most active analogs tested were over 100-fold selective for recombinant GluN2C-containing receptors over GluN2A/B/D-containing NMDA receptors. These analogs represent a novel class of NMDA receptor modulators that are highly selective for one NMDA receptor subunit (GluN2C) and provide a useful tool with which to evaluate the role of GluN2C in normal and neuropathological function.

Design, Synthesis, and Biological Evaluation of Subunit-Selective *N*-Methyl-D-Aspartate Receptor Modulators

By

Sommer S. Zimmerman

B.S., Florida State University, 2006 & 2008

Advisor: Dennis C. Liotta, Ph.D.

A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

2014

Table of Contents

List of Illustrations

List of Figures **Page**

Chapter 1:

Chapter 2:

List of Tables **Page**

Chapter 1:

Chapter 2:

List of Schemes **Page**

Chapter 1:

Chapter 2:

List of Abbreviations

AD: Alzheimer's Disease

ACN: Acetonitrile

AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ATD: Amino Terminal Domain

BBB: Blood-Brain-Barrier

CNS: Central Nervous System

CS: Conditioned Stimulus

CTD: Carboxy Terminal Domain

DCC: *N,N'*-Dicyclohexylcarbodiimide

DCM: Dichloromethane

DCS: D-Cycloserine

DLB: Dementia with Lewy bodies

DMAP: 4-Dimethylaminopyridine

DMF: Dimethylformamide

DMSO: Dimethylsulfoxide

***ee*:** Enantiomeric Excess

EC_{50}: Half-Maximal Excitatory Concentration

EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EPSP: Excitatory Postsynaptic Potential

EtOAc: Ethyl Acetate

FDA: Food and Drug Administration

GABA: Gamma-Aminobutyric Acid

GPCR: G-Protein Coupled Receptor

GoF: Gain of Function

HD: Huntington's Disease

h(s): hour(s)

HPLC: High Performance Liquid Chromatography

HRMS: High Resolution Mass Spectrometry

IC_{50}: Half-Maximal Inhibitory Concentration

IR: Infrared Radiation

iGluR: Ionotropic Glutamate Receptor

LBD: Ligand Binding Domain

LCMS: Liquid Chromatography-Mass Spectrometry

LoF: Loss of Function

MeOH: Methanol

mGluR: Metabotropic Glutamate Receptor

min.: minutes

mRNA: Messenger Ribonucleic Acid

M.W.: Microwave

NAAG: *N*-Acetylaspartylglutamate

nACh: Nicotinic Acetylcholine Receptor

NBS: *N*-Bromosuccinimide

NMDA: *N*-Methyl-D-aspartate

NMR: Nuclear Magnetic Resonance

PCP: Phencyclidene

PD: Parkinson's Disease

PDD: Parkinson's Disease Dementia

PPTS: Pyridinium *p*-Toluene Sulfonic Acid

PTSD: Post-Traumatic Stress Disorder

rt: room temperature

SAR: Structure Activity Relationship

SEM: Standard Error of the Mean

SSRI: selective serotonin reuptake inhibitor

$t_{1/2}$: Half-life

TBI: Traumatic Brain Injury

***t*-BuOH:** *tert*-Butanol

TEA: Triethylamine

TFA: Trifluoroacetic Acid

TIPSCl: Triisopropylsilyl Chloride

TLC: Thin Layer Chromatography

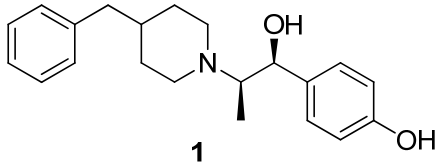
TMD: Transmembrane Domain

US: Unconditioned Stimulus

Chapter 1: Design, Synthesis, and Structure-Activity Relationship of GluN2C/GluN2D-Selective NMDA Receptor Antagonists

1.1 STATEMENT OF PURPOSE

The first small molecule demonstrating subunit-selective inhibition of NMDA receptors was originally discovered in 1988.[1] Ifenprodil (**1**) is able to selectively inhibit GluN2B-containing receptors over GluN2A-, GluN2C-, and GluN2D-containing receptors (Figure 1).[2] This remarkable discovery triggered resurgence in the search for subunit-selective modulators of NMDA function. Indeed, prior to 2010, little progress had been made towards the identification of antagonists selective for any other subunit.[3-7]



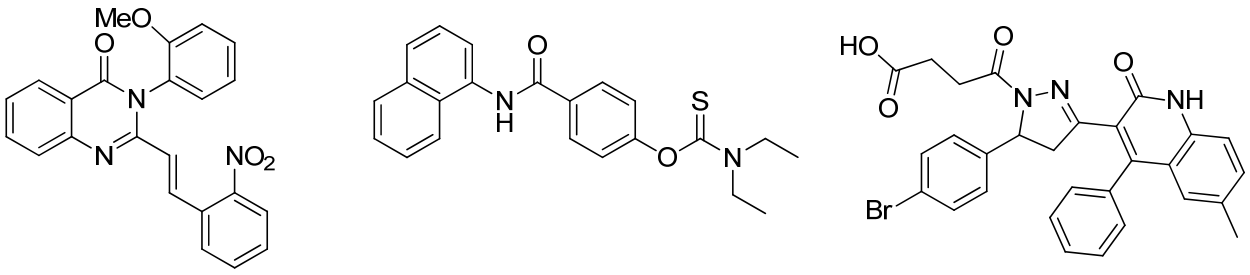
1

Figure 1. Structure of Ifenprodil

The Traynelis lab has conducted an extensive search for allosteric modulators of GluN2C- and GluN2D-containing NMDA receptors.[8] Subunit-selective antagonists could potentially serve as pharmacological tools to better understand the contribution of GluN2C- and GluN2D-containing receptors to brain function. The significant role that NMDA receptors play in a variety of neurological conditions, including Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD), could also allow for the development of novel therapeutics.[9,10]

In their quest to identify subunit-selective modulators, the Traynelis lab conducted a fluorescence-based primary screen of nearly 100,000 compounds. Of these, thee classes

were identified for further examination (Figure 2). These included quinazolin-4-ones (987 series, **987**), phenyl alkylcarbamothioates (1063 series, **1063**), and dihydropyrazoquinolines (1105 series, **1105**). Their activity was confirmed using two-electrode voltage clamp recordings of *Xenopus laevis* oocytes expressing recombinant GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, and GluN1/GluN2D receptors.



987 **1063** **1105**

Figure 2. Screening hits identified in a fluorescence-based assay

Each of these series have become a focal point of structure-activity relationship efforts in our lab in collaboration with the Traynelis lab. Class 1063 offered a remarkable selectivity not observed for the other classes. Specifically, compound **1063** was more than 500-fold selective for GluN2C- and GluN2D-containing receptors over GluN2A- and GluN2B-containing receptors. We sought to increase the inhibitory actions of the 1063-series through an extensive analysis of the SAR. The goals of this project were accomplished using the following strategy:

1. Design and synthesis of novel 1063-series analogs with an emphasis on enhancing potency and maintaining selectivity.

2. Determination of the central binding interactions of 1063 analogs within the GluN2D-containing NMDA receptor *in vitro*.

3. Determination of preliminary *in vivo* pharmacokinetic properties of the 1063-series.

1.2 INTRODUCTION AND BACKGROUND

1.2.1 NMDA Receptor Structure, Function, and Localization

L-Glutamate is the major excitatory neurotransmitter within the mammalian central nervous system (CNS), the actions of which are mediated via activation of two distinct families of receptors: metabotropic and ionotropic.[10-12] Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors (GPCRs) primarily responsible for regulating synaptic transmission, plasticity, and neuronal excitability. This class consists of eight receptor types (mGluR1-8) which are further divided based on structural homology, pharmacology, and secondary messenger coupling into three unique categories (Group I, II and III).[11] In contrast, ionotropic glutamate receptors (iGluRs) are integral membrane proteins that comprise an ion channel pore. These receptors are subdivided into three distinct classes based on structure homology and pharmacology, including 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA), *N*-methyl-D-aspartate (NMDA), and kainate receptors. Each class of iGluRs are activated by glutamate, and are named based on the synthetic glutamate mimic to which they selectively bind (Figure 3).[10,12]

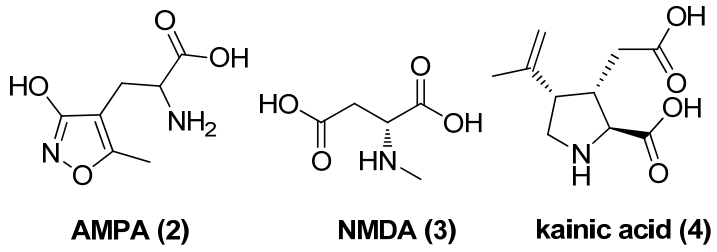


Figure 3. Synthetic agonists which selectively bind to iGluRs

NMDA receptors are ligand-gated ion channels that mediate a slow component of the excitatory post synaptic potential (EPSP). Each receptor resides as a tetrameric assembly composed of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits (Figure 4). The GluN1 subunit is encoded by eight different splice variants (a-h) of a single gene, whereas the GluN2 subunit is composed of four distinct gene products (GluN2A, GluN2B, GluN2C, and GluN2D).[13,14]

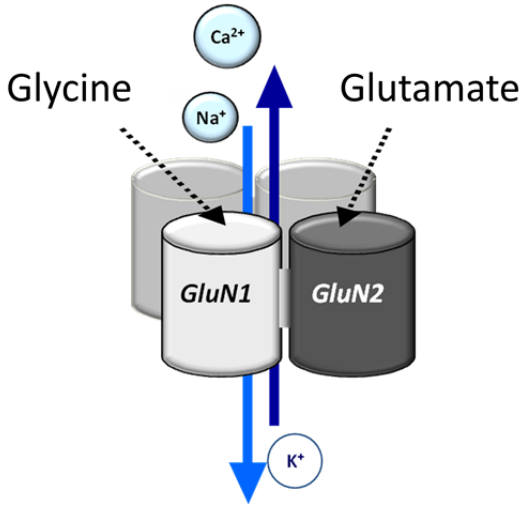


Figure 4. NMDA receptor subunit structure

Functional NMDA receptors require a tetrameric assembly of two GluN1 and two GluN2 subunits arranged such that an ion conduction pore is formed between the subunits, allowing for cation permeability. Each GluN1 and GluN2 receptor subunit has a modular structure composed of an extracellular amino-terminal domain (ATD), a ligand-binding domain (LBD), a transmembrane domain (TMD), and an intracellular carboxy-terminal domain (CTD) (Figure 5). The LBD is further defined by two amino acid segments referred to as S1 and S2. These semiautonomous domains can fold correctly as isolated, purified polypeptides, and are connected by flexible linkers.[10,13]

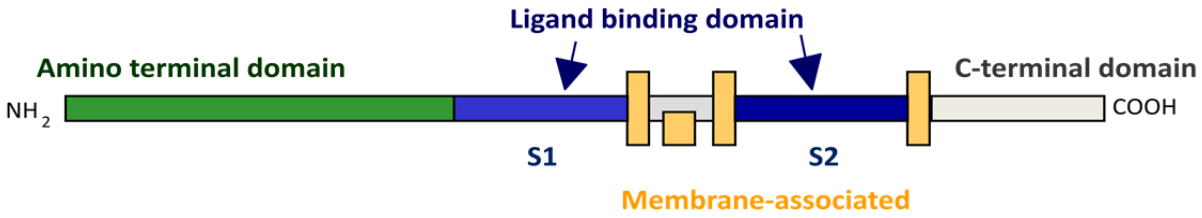


Figure 5. Domains of a single NMDA receptor subunit

NMDA receptors are unique compared to other iGluRs in that they require the binding to co-agonists, glycine and glutamate. Glycine binds the LBD of GluN1 subunits and glutamate binds the LBD of GluN2 subunits (Figure 6). Following the binding of both agonists, a conformational shift of the receptor opens the channel pore. When this occurs at the same time as neuronal depolarization, block of the channel by extracellular Mg^{2+} is reduced and cations (e.g., Na^{+} and Ca^{2+}) will flow into the cell. This process plays an important role in neuron to neuron synaptic transmission.[15,16]

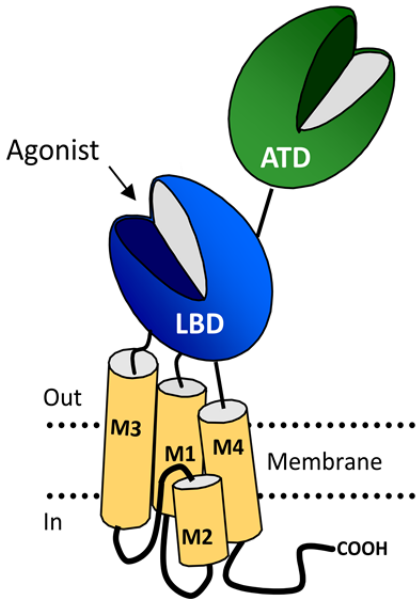


Figure 6. Cartoon representation of a single NMDA receptor subunit

The four distinct NMDA receptor subtypes display unique pharmacological and electrophysiological properties. The channel deactivation time-course for a brief glutamate pulse, in the continuous presence of glycine, spans a ~50-fold range for the different GluN2 subunits. For example, GluN2D-containing NMDA receptors display extremely slow decay times for ligand-gated receptors, ranging from about 4 to 5 s.[17-19] GluN2A-containing receptors, in contrast, display considerably more rapid channel kinetics. Other properties, including sensitivity to magnesium block, single-channel conductance, and open probability, also vary markedly by GluN2 subunit composition.[10,17,18]

NMDA receptor expression is controlled both spatially and temporally throughout development. *In situ* hybridization of the mRNA for GluN1 reveals that GluN1 is ubiquitously expressed throughout the CNS during the developmental period from postnatal day 1 into adulthood. GluN2A and GluN2C mRNA expression is only minimally observed at postnatal day 1, but increases substantially over the lifetime of the rat. GluN2B and GluN2D mRNA expression, however, predominate in early postnatal development, with decreases in expression observed after postnatal day 11. Additionally, the four GluN2 subunits display precise anatomical localization patterns in the adult rat brain. The GluN2A mRNA resides primarily in the cerebral cortex, including the hippocampus, the GluN2B mRNA is predominantly expressed in the telencephalic regions and thalamus, the GluN2C mRNA is concentrated in the cerebellum, and the GluN2D mRNA is highly expressed in the brainstem and basal ganglia, including diencephalon.[20,21]

1.2.2 Therapeutic Rationale for NMDA Receptor Antagonists

A large amount of data collected over decades shows that glutamate rises in injured tissue during ischemia, stroke, and traumatic brain injury (TBI) in both animal models[22-30] and human patients.[31-35] Overactivation of NMDA receptors by this glutamate has been strongly correlated with neurotoxicity in the CNS due to influx or release of excess Ca^{2+}.[9,36-41] The excessive or prolonged exposure of NMDA receptors to glutamate can be triggered by a variety of insults. For example, damaged cells release extremely high levels of glutamate following ischemic stroke or TBI. This triggers a cascade of cell death which can continue for hours and sometimes days after the injury has occurred.[36]

Several NMDA receptor antagonists have demonstrated neuroprotective effects in preclinical models of acute trauma. However, the success of these compounds has not transferred to clinical trials.[42-50] This discrepancy can be explained by several factors, namely adverse side effects and timing of administration. Competitive antagonists at the glutamate site, such as Selfotel, and various channel blockers, such as Aptiganel, demonstrate significant psychomimetic and cardiovascular effects at the effective concentrations.[10,44,51] In addition, the window of efficacy for acute brain trauma is extremely narrow.[10,44] Studies have indicated that the effectiveness of NMDA receptor antagonists for treatment of TBI can be realized only when administered within two hours of injury. The neuroprotective effect observed in clinical trials was thus significantly reduced when the treatment window was extended.[10]

Overactivation in NMDA activity has also been linked to many chronic neurodegenerative diseases including PD, AD, HD, dementia, and amyotrophic lateral sclerosis, as well as chronic pain.[36,37,51-53] For example, memantine is a moderate affinity, non-competitive NMDA receptor channel blocker that is FDA approved for use in

moderate to severe AD.[54-56] Memantine may also have therapeutic utility towards the treatment of Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB),[57] and vascular dementia.[58] This is in stark contrast to the low tolerability profiles of other known NMDA receptor channel blockers including ketamine, phencyclidine, and MK-801.[10,51,59] Compared to these first generation channel blockers, memantine is mechanistically unique, offering a lower affinity, faster dissociation kinetics, and a distinct binding mechanism.[10,59,60]

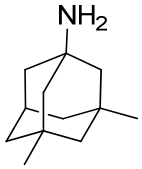
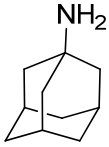
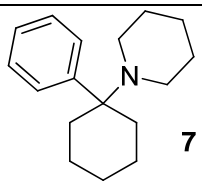
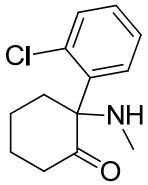
1.2.3 Classes of NMDA Receptor Antagonists

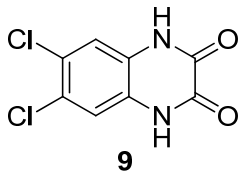
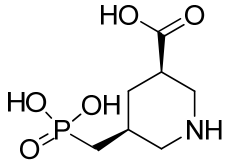
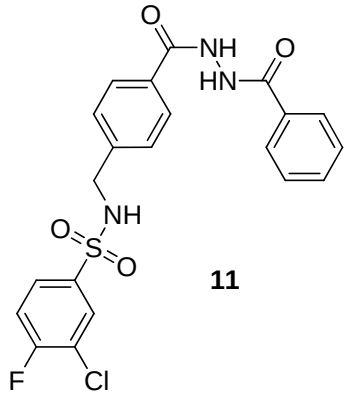
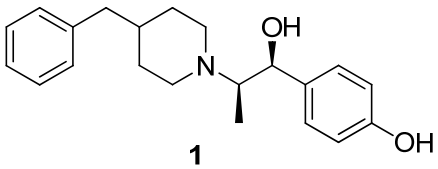
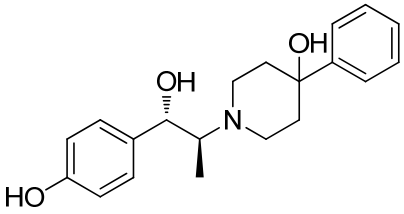
The NMDA receptor contains several known modulatory sites capable of binding small molecules which have been exploited in the search for NMDA receptor antagonists. Three distinct classes of compounds have been outlined based on their pharmacological site of action. These include channel blockers, competitive antagonists, and allosteric modulators acting at various locations.[60-63]

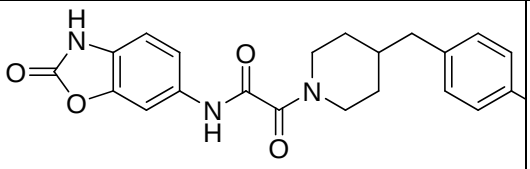
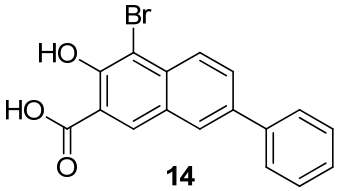
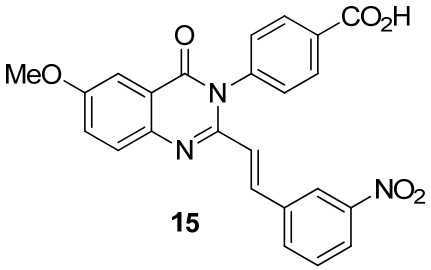
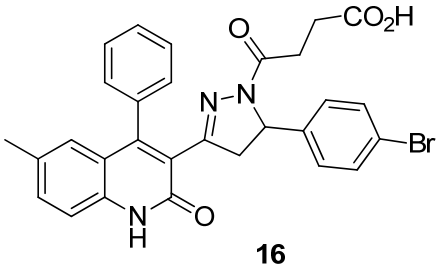
Channel blockers bind within the ion conduction pore and exhibit a voltage-dependent inhibition of calcium influx. Because the activity of these analogs requires prior activation of the receptor by glutamate and glycine they are also sometimes referred to as uncompetitive antagonists.[64] Examples of NMDA receptor channel blockers include memantine (Namenda®, **5**), amantadine (Symmetrel®, **6**), phencyclidine (PCP, **7**), and ketamine (**8**). Memantine and amantadine are FDA approved for use in moderate to severe Alzheimer's disease and for treatment of Parkinson's disease, respectively.[65] PCP was originally commercialized for use as a general anesthetic. The hallucinogenic and psychomimetic properties led to significant abuse, causing PCP to be withdrawn from the market. Ketamine, which suffers from less pronounced psychic effects, was later developed

as a replacement analog.[66] The chronic use of most NMDA receptor channel blockers has continued to be limited due to adverse side effects (e.g., hallucinations, confusion, and agitation) believed to stem from a lack of subunit specificity.[37] The success of memantine is largely attributed to its unique mechanism of action including low-affinity binding and fast off-rate kinetics.[67]

Table 1. Classes of NMDA receptor antagonists with exemplary analogs shown

Class of NMDA receptor antagonist	Compound Name	Structure	Intended Use
Channel blocker (uncompetitive antagonist)	Memantine	 **5**	Alzheimer's disease
	Amantadine	 **6**	Parkinson's disease
	Phencyclidine	 **7**	Anesthesia
	Ketamine	 **8**	Anesthesia

Class of NMDA receptor antagonist	**Compound Name**	**Structure**	**Intended Use**
Competitive glycine antagonist	Licostinel	 **9**	Stroke
Competitive glutamate antagonist	Selfotel	 **10**	Stroke, Head Injury
Allosteric GluN2A-selective antagonist	TCN201	 **11**	--
Allosteric GluN2B-selective antagonist	Ifenprodil	 **1**	Depression, Stroke, TBI
	Taxoprodil	 **12**	Head Injury

Class of NMDA receptor antagonist	Compound Name	Structure	Intended Use
	Radiprodil	 **13**	Neuropathic Pain
Allosteric GluN2C/GluN2D-selective antagonist	UBP618	 **14**	--
	QNZ46	 **15**	--
	DQP1105	 **16**	--

Competitive antagonists refer to a class of compounds that bind at either the glycine binding site at GluN1 or the glutamate binding site at GluN2. Block by competitive antagonists is surmountable by increasing agonist concentration.[46,62] Antagonists binding at the glycine site can be exemplified by licostinel (ACEA-1021, **9**).[68-70] Despite offering a relatively high potency against NMDA receptors (IC_{50} = 0.17 μM), licostinel and related

analogs also demonstrate activity against other ionotropic glutamate receptors, including AMPA (IC_{50} = 1.7 μM) and kainate (IC_{50} = 0.76 μM).[68] Pursuit of this compound was discontinued after crystals were discovered in the urine of patients during clinical trials, indicating poor aqueous solubility and metabolism.[69] An example of a competitive antagonist at the glutamate binding site is Selfotel (**10**).[46,71] Selfotel progressed to a Phase III study of ischemic stroke but the trial was terminated prematurely due to the risk-benefit ratio.[45,72,73]

It is noteworthy that NMDA receptors contain a number of extracellular modulatory sites, distinct from the agonist-binding sites, capable of binding small molecules.[74] In addition, the identification of four distinct GluN2 subunits potentially allows for subunit-selective modulation of NMDA receptor function.[62] A classic example of a non-competitive, allosteric modulator of NMDA function is ifenprodil (**1**). Ifenprodil is a selective antagonist of GluN2B-containing NMDA receptors. A large number of related analogs have also been studied, including taxoprodil (CP-101,606, **12**) and radiprodil (RGH-896, **13**). The subunit-selectively is believed to contribute to the lack of adverse events associated with this class of compounds.[74] A range of negative allosteric modulators selective for other subunits have also more recently been pursued. Examples of these include TCN201 (selective for GluN2A-containing receptors, **11**),[7] UBP618 (selective for GluN2C/GluN2D-containing receptors, **14**),[6] QNZ46 (selective for GluN2C/GluN2D-containing receptors, **15**)[4] and DQP1105 (selective for GluN2C/GluN2D-containing receptors, **16**).[3] Despite these developments, there remains a need for potent, subunit-selective antagonists to better assess the physiological role of each individual receptor subtype.[62]

1.2.4 Rationale for Antagonist Design

A series of naphthylphenyl carbamothioate antagonists was first identified in a GluN2C/D screen performed by the Traynelis lab (Figure 7). The initial lead compound, **1063**, emerged as an attractive candidate for medicinal chemistry efforts for a variety of reasons. Specifically, this compound offered selectivity greater than 500-fold for GluN2C/GluN2D-containing NMDA receptors over GluN2A/GluN2B-containing NMDA receptors, estimated by fitting concentration-effect curves with minimum fixed to 0. Previous SAR efforts had resulted in analogs no more than 50-fold selective. In addition, preliminary studies indicated that **1063** efficiently permeates the blood-brain-barrier (S. Traynelis, personal communication). Other properties, including a low molecular weight (378.49 Da) and novel binding site (as determined by mutagenesis experiments), also made this compound desirable for further pursuit.

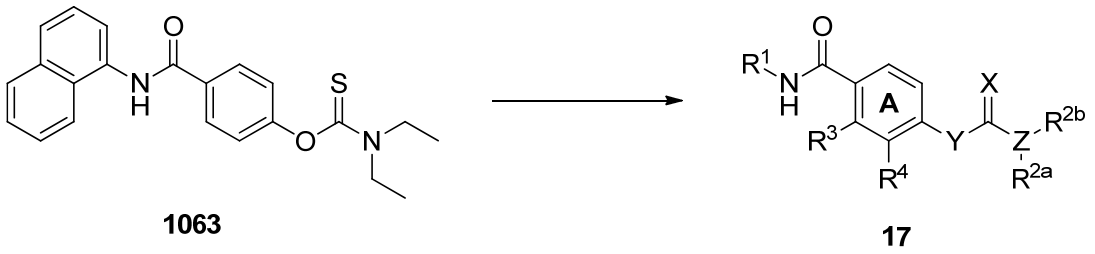
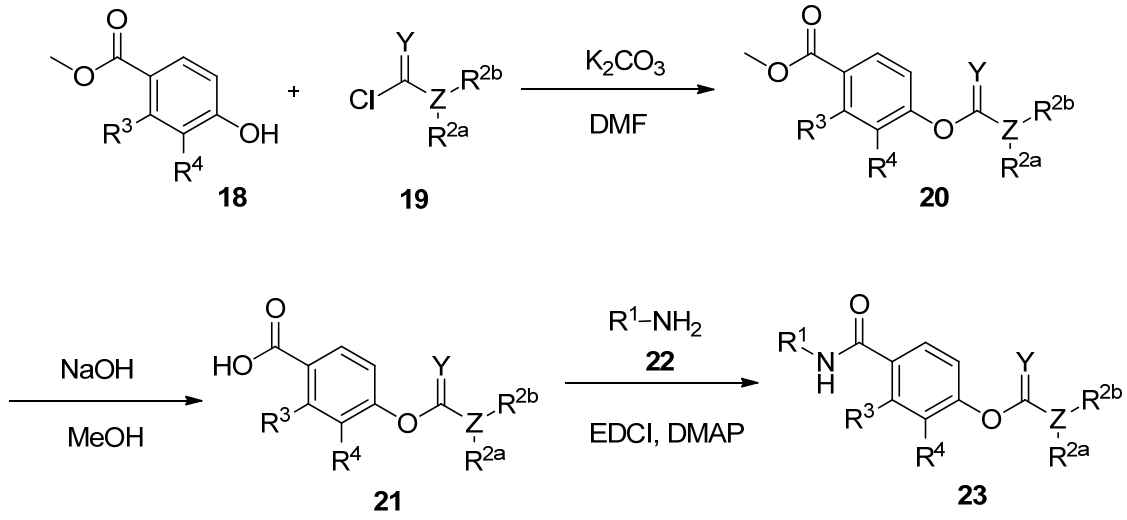


Figure 7. Initial screening hit and generic structure for SAR development

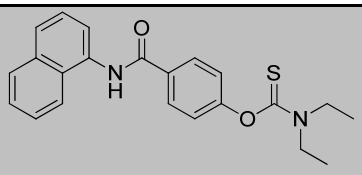
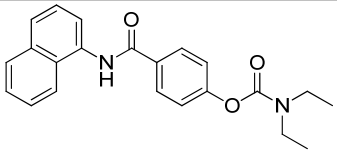
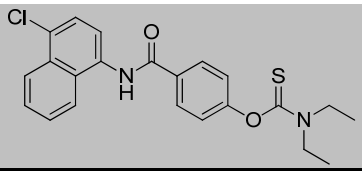
The synthetic route to 1063 analogs and SAR rationale were developed by Dr. Cara Mosley (Scheme 1). Initial modifications revealed that replacing the sulfur present in the carbamothioate with an oxygen atom, as in **1063-2,** led to modest decreases in potency and selectivity (Table 2), but significantly improved the stability in buffer solution (data not shown). Additionally, a slight preference for bulky and branched carbamates (e.g., *N,N*-

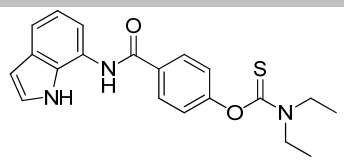
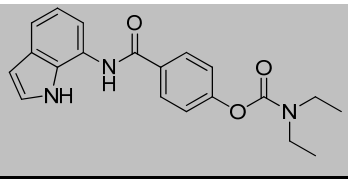
diisopropyl) compared to small (e.g., *N,N*-dimethyl) and linear substituents (e.g., *N,N*-dimethyl and *N,N*-dibutyl) was observed (Mosley dissertation, 2010).



Scheme 1. General synthetic route towards 1063 analogs developed by Dr. Cara Mosley (Mosley dissertation, 2010)

Table 2. Lead analogs to come out of previous SAR efforts

ID	Structure	IC_{50} (μM)[a]				(2A IC_{50})/ (2D IC_{50})
		GluN2A	GluN2B	GluN2C	GluN2D	
1063		NE	NE	2.2	1.2	1100
1063-2		NE	NE	5.6	4.2	220
1063-12		NE	NE	25	100	--

ID	Structure	IC_{50} (μM)[a]				(2A IC_{50})/ (2D IC_{50})
		GluN2A	GluN2B	GluN2C	GluN2D	
1063-21		NE	NE	15	4.1	--
1063-20		NE	NE	5.3	2.6	--

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from between 4-22 oocytes from 1-4 frogs for each receptor tested. NE indicates less than 15% inhibition at 10 μM.

We envisioned analysis of a series of analogs that would allow us to further explore the conformational flexibility and stability of the carbamate substituent (Figure 8). Specifically, rigidification would be analyzed by preparation of thiazole and oxazolidinone analogs. Previous efforts had demonstrated that replacement of the carbamate with either an ester or urea led to inactivity (Mosley dissertation, 2010). Additional modifications were hypothesized to probe stability (X = OCH_2 or is absent) and linker flexibility (X = CH_2O).

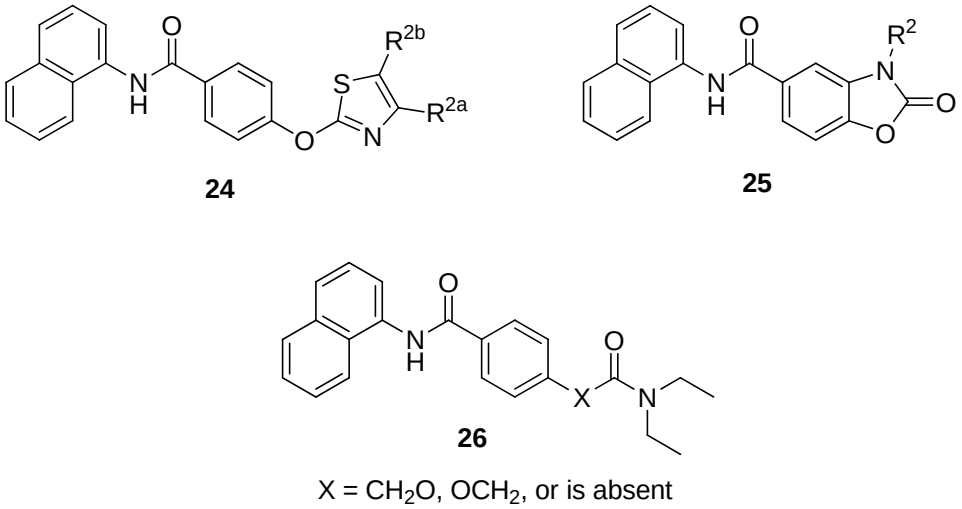


Figure 8. Proposed analogs for exploration of conformational flexibility and stability of the carbamate

Earlier studies revealed that replacement of the *N,N*-diethylcarbamate with an *N,N*-dimethylcarbamate led to complete inactivity. All additional modifications to the carbamate involved symmetrical substitutions (Mosley dissertation, 2010). To this end, we were interested to learn whether both ethyl groups were required for activity. We thus envisaged preparing an asymmetrically substituted *N,N*-dialkylcarbamate (Figure 9).

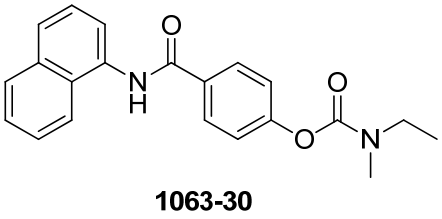


Figure 9. Proposed asymmetrical carbamate

Modifications to the central phenyl ring were previously unexplored. We were interested in analyzing ring substitutions and positional isomers that would affect electronic and steric properties. This included the introduction of halogens to influence electron

density and positional preference. Preparation of *ortho*- and *meta*-substituted analogs would allow us to probe various conformations as well.

A series of analogs with modifications at R^1 were developed by Dr. Timothy Acker. These efforts revealed that substituted naphthyl derivatives led to inactivity or, in the case of **1063-12** (see Table 2), which contains a 4-chloronaphthyl moiety, a modest preference for GluN2C-containing receptors over GluN2D-containing receptors. We were also interested to learn whether the naphthyl ring could be replaced with other aryl groups, as it was hypothesized that this substituent could become a metabolic liability. For example, cleavage of the amide bond *in vivo* would generate 1-naphthylamine, a known carcinogen. Previous efforts revealed that replacement with an indole ring linked at the seven-position, as in **1063-21** and **1063-20**, maintained both potency and selectivity compared to the original lead (Acker dissertation, 2013). Additional replacements for the naphthyl ring would be analyzed to further evaluate the effect on potency (Figure 10).

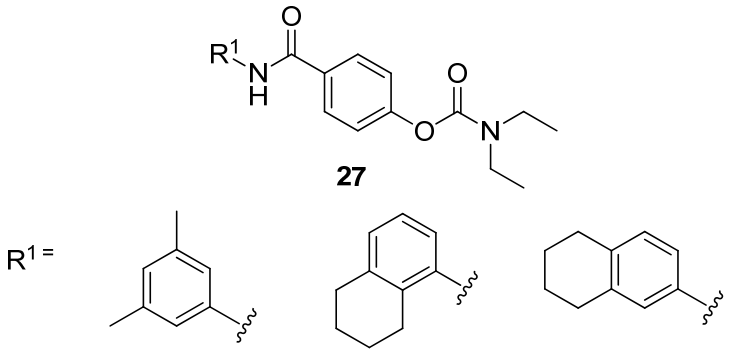


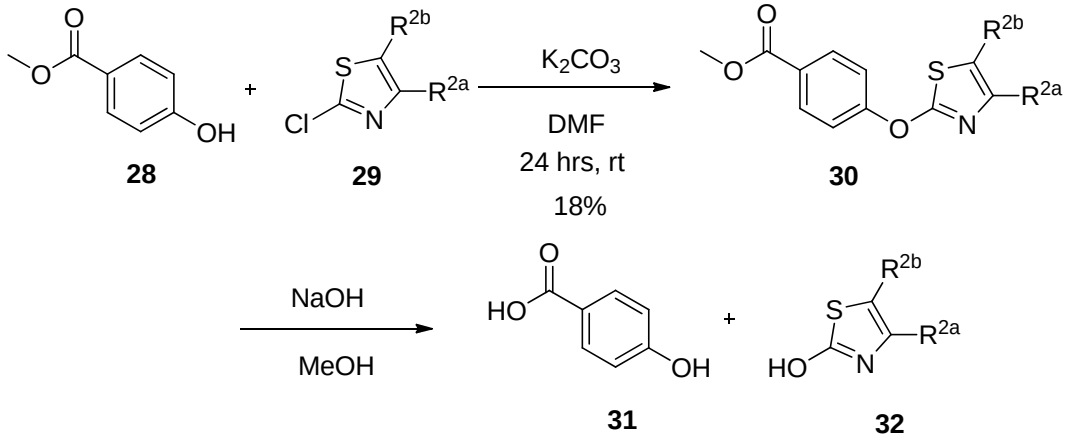
Figure 10. Proposed R^1 substitutions

The current work focused on the synthesis and biological evaluation of the additional analogs described above.

1.3 SYNTHESIS OF 1063-SERIES ANALOGS

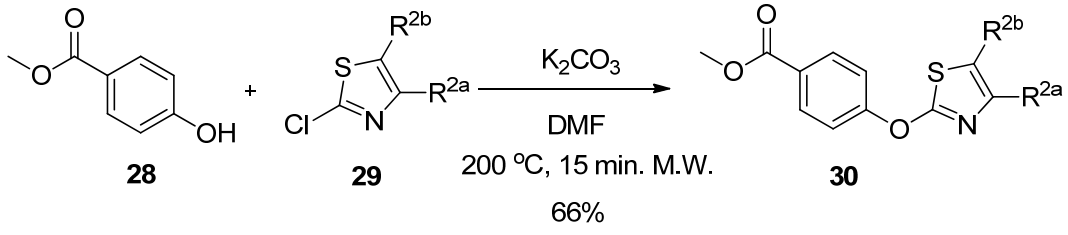
Analogs containing an asymmetrical carbamate, modifications to the central phenyl ring, or modifications at R^1 were prepared as illustrated in Scheme 1 above (see Chapter 1, *1.2.4*).

Initial attempts to synthesize the thiazole analogs by this route were low yielding and led to cleavage of the thiazole ring, affording carboxylic acid **31** and hydroxy-thiazole **32** (Scheme 2).



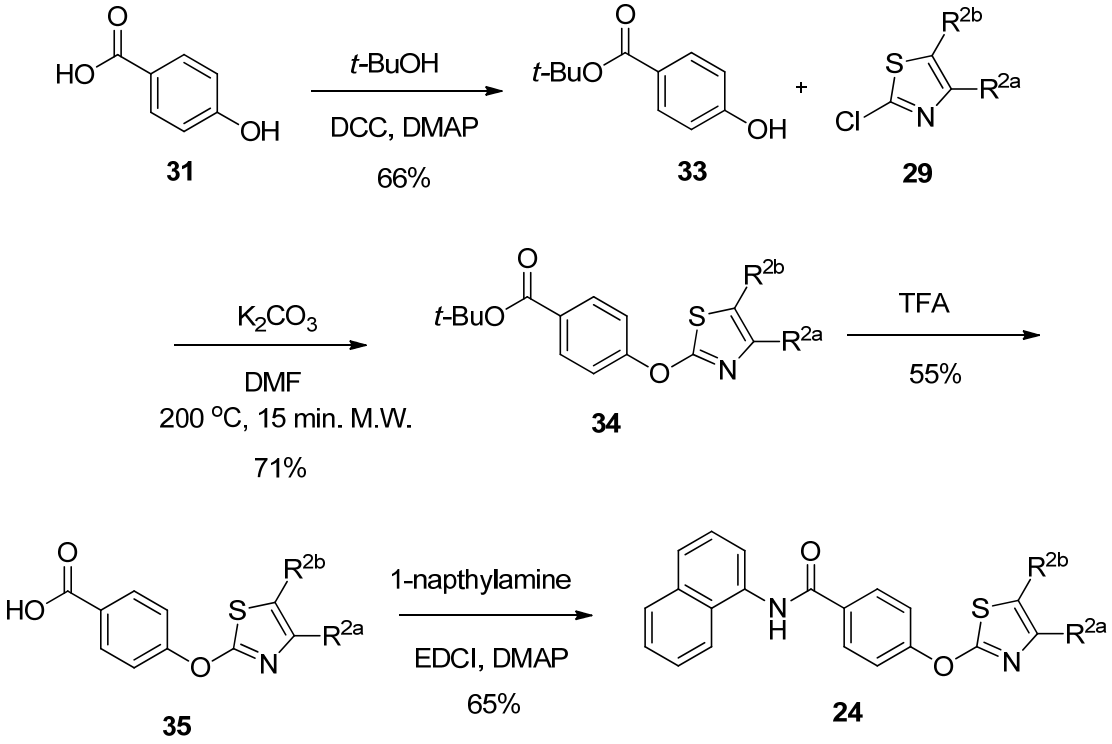
Scheme 2. Failed synthesis of thiazole derivatives using basic conditions

Significant improvements in yield and speed were realized by heating the reaction at 200 °C in the microwave reactor; the reaction was complete by TLC in just 15 minutes, compared to the original conditions of 24 hours (Scheme 3).



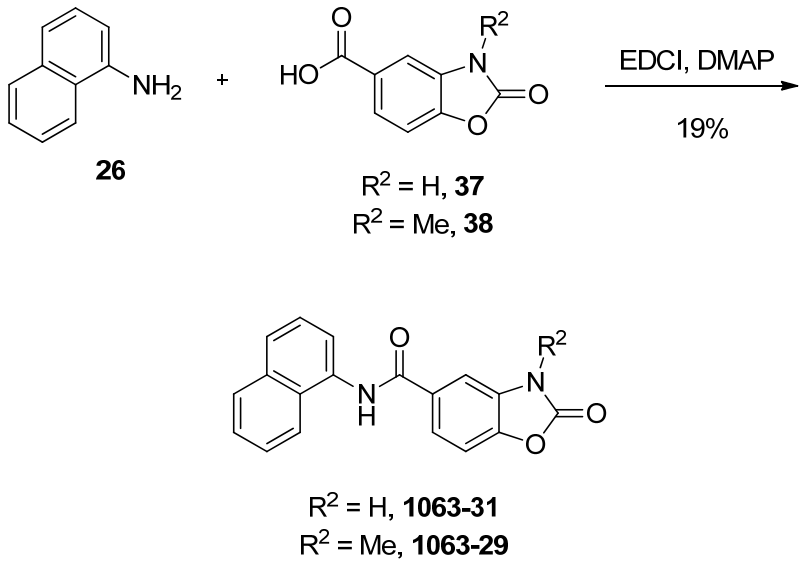
Scheme 3. Optimized thiazole conditions

To avoid cleavage of the thiazole, a *tert*-butyl ester was installed in place of the methyl ester, and deprotection was afforded under acidic conditions (Scheme 4). Thus, coupling of acid **31** with *t*-butanol afforded phenol **33**, which was then heated with the desired thiazole in the microwave reactor to yield ester **34** in 71% yield. Hydrolysis with trifluoroacetic acid afforded acid **35**. Final 1063 thiazole analogs were then generated by reacting acid **35** with 1-napthylamine under standard carbodiimide coupling conditions.



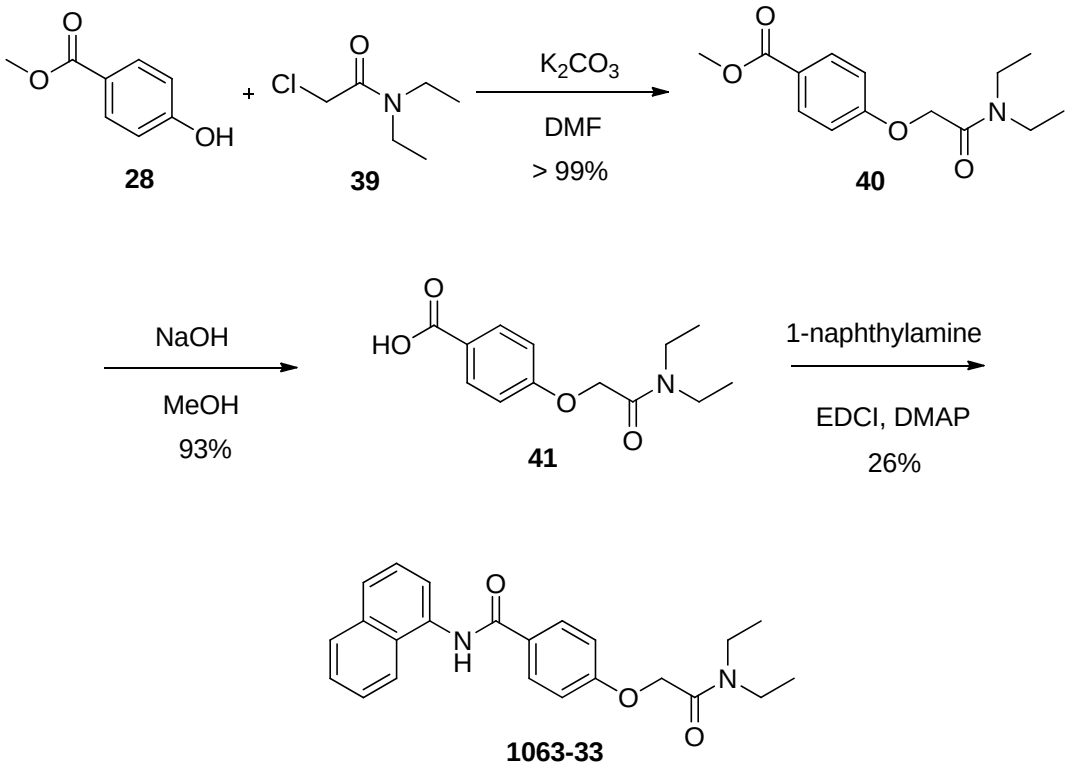
Scheme 4. Synthesis of thiazole analogs

Oxazolidinone analogs were prepared in a single step from the commercially available carboxylic acid (Scheme 5).



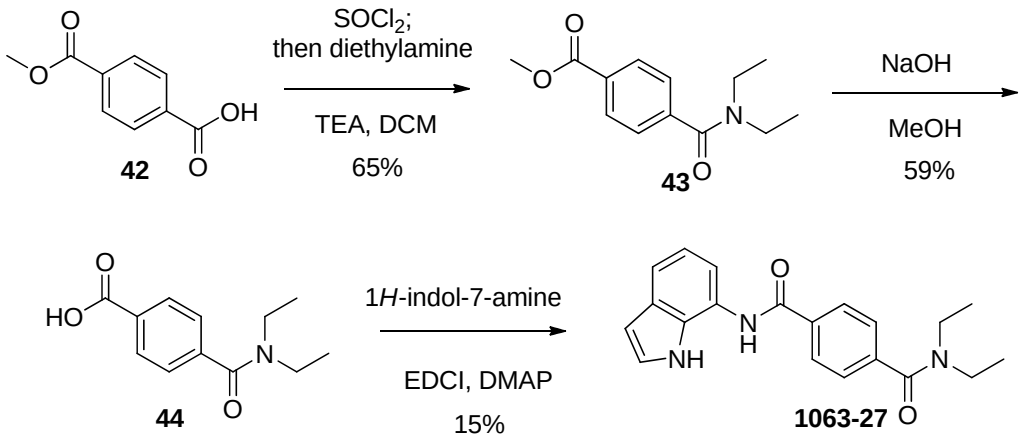
Scheme 5. Synthesis of oxazolidinone analogs

Compounds containing modified linkers were synthesized using several different methods. For example, ether **1063-33** was prepared similarly to what was previously described (Mosley dissertation, 2011) (Scheme 6). 2-Chloro-*N,N*-diethylacetamide was reacted with phenol **28** to yield ester **40**. Saponification generated acid **41**, which was then coupled to 1-naphthylamine to afford the desired ether analog **1063-33**.



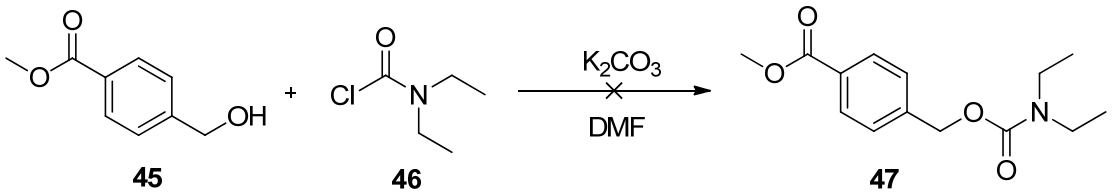
Scheme 6. Synthesis of ether analog 1063-33

Amide **1063-27** was prepared from commercially available carboxylic acid **42** (Scheme 7). Reaction of acid **42** with thionyl chloride afforded the desired acid chloride, which was subsequently coupled with diethylamine to yield amide **43**. Cleavage of the ester was achieved using sodium hydroxide in methanol to afford acid **44**. Final amide analog **1063-27** was prepared by coupling of acid **44** with 1*H*-indol-7-amine.



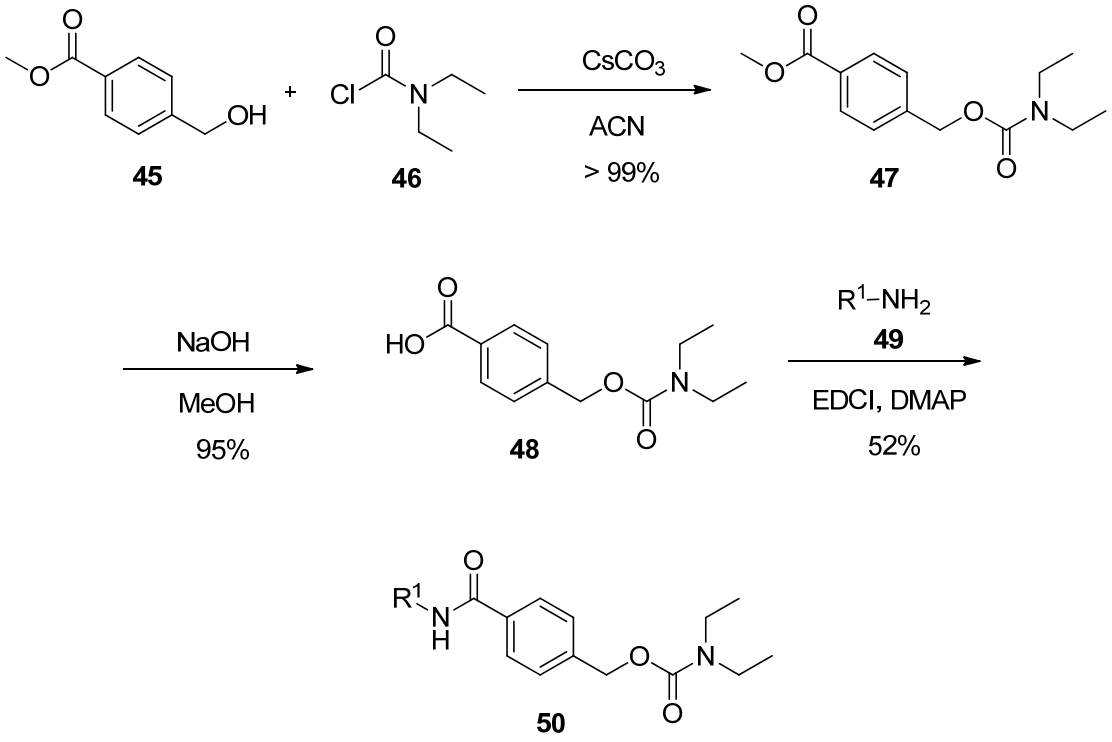
Scheme 7. Preparation of amide 1063-27

Initial efforts to synthesize carbamate analogs with an extended linker using the previously developed route revealed that the decreased acidity of the alcohol required the use of a more reactive base (Scheme 8).



Scheme 8. Failed synthesis of carbamate 47

The reaction of alcohol **45** with diethylcarbamic chloride proceeded in excellent yield, however, in the presence of cesium carbonate, to yield carbamate **47** (Scheme 9). Subsequent saponification afforded acid **48**, which was then coupled to the desired amine to generate the final carbamate analog.



Scheme 9. Synthesis of extended linker analogs

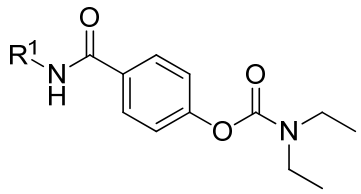
1.4 RESULTS AND DISCUSSION

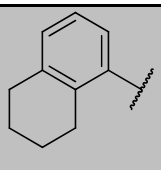
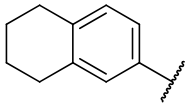
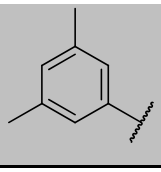
1.4.1 Structure-Activity Relationship of 1063-Series Analogs

All 1063 analogs were evaluated in *Xenopus laevis* oocytes over-expressing GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, and GluN1/GluN2D receptors.

Replacement of the naphthyl substituent at R^1 with a cyclohexene ring led to compounds with multi-dimensional conformations (Table 3). Both analogs revealed decreases in potency, with **1063-19** offering a slight preference for GluN2D-containing receptors (IC_{50} = 26 μM) over GluN2C-containing receptors (IC_{50} = 58 μM). A similar preference was observed for **1063-17**.

Table 3. Optimization of R^1



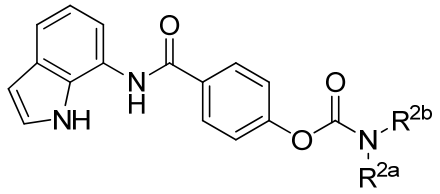
Compound ID	R^1	IC_{50} (μM)[a]			
		GluN2A	**GluN2B**	**GluN2C**	**GluN2D**
1063-18		NE	NE	23	20
1063-19		NE	NE	58	26
1063-17		NE	NE	18	7.8

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from between 2-3 oocytes from 1 frog for each compound and receptor tested. NE indicates less than 15% inhibition at 10 μM.

The result of asymmetrical carbamate substituents is illustrated in Table 4.

Unfortunately, no activity was observed for asymmetric dialkylamine **1063-30** at all subunits tested.

Table 4. Asymmetric evaluation of R^{2a} and R^{2b}

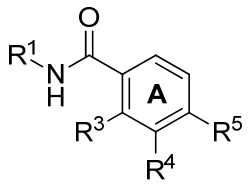


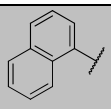
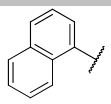
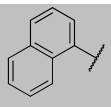
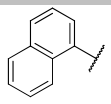
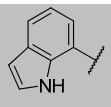
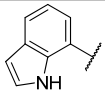
ID	R^{2a}	R^{2b}	IC_{50} (μM)[a]			
			GluN2A	GluN2B	GluN2C	GluN2D
1063-30	Me	Et	NE	NE	NE	NE

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from 2 oocytes from 1 frog for each receptor tested. NE indicates less than 15% inhibition at 10 μM.

Both the *ortho*- (**1063-25**) and *meta*-isomer (**1063-24**) revealed no inhibitory effect up to 100 μM (Table 5). A slight trend appeared to emerge from the biological data of halogen-containing analogs. Compound **1063-39**, which contains an iodo-substituent *ortho* to the carbamate, was inactive, while analogs containing halogen atoms with smaller van der Waals radii (e.g., Cl or F) led to modest activity. In addition, the increased electronegativity of fluorine analog **1063-40** offered decreases in potency compared to chlorine analogs **1063-42** and **1063-41**. These data indicate that a balance between steric and electronic properties is required on the A-ring for optimal activity of substituted phenyl derivatives.

Table 5. Optimization of A-ring substituent position and identity



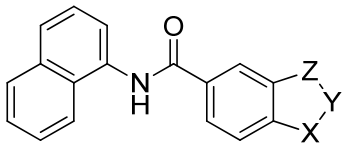
ID	R1	R3	R4	R5	IC50 (μM)[a]			
					GluN 2A	GluN 2B	GluN 2C	GluN 2D
1063-25		$OC(O)NEt_2$	H	H	NE	NE	NE	NE
1063-24		H	$OC(O)NEt_2$	H	NE	NE	NE	NE
1063-39		H	I	$OC(O)NEt_2$	NE	NE	NE	NE
1063-42		H	Cl	$OC(O)NEt_2$	NE	NE	45	21
1063-41		H	Cl	$OC(O)NEt_2$	NE	NE	NE	NE
1063-40		H	F	$OC(O)NEt_2$	NE	NE	NE	NE

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from 2 oocytes from 1 frog for each compound and receptor tested. NE indicates less than 15% inhibition at 10 μM.

The result of modifying the linker is illustrated in Tables 6 and 7. All thiazole (**1063-22** and **1063-23**) and oxazolidinone (**1063-31** and **1063-29**) analogs showed no biological effect. **1063-27**, which contains an amide linker, demonstrated weak selectivity for

GluN1/GluN2A. All other analogs containing modified linkers led to inactivity. This data suggests that the carbamate linker is critical for the potency of the series.

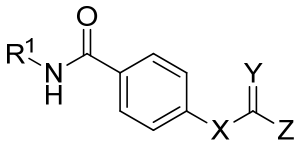
Table 6. Optimization of the linker

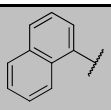
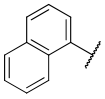
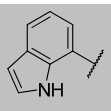


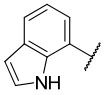
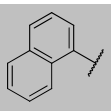
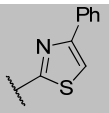
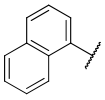
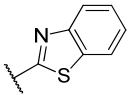
ID	X	Y	Z	IC_{50} (μM)[a]			
				GluN2A	GluN2B	GluN2C	GluN2D
1063-31	O	C=O	NH	NE	NE	NE	NE
1063-29	O	C=O	NMe	NE	NE	NE	NE

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from 2 oocytes from 1 frog for each compound and receptor tested. NE indicates less than 15% inhibition at 10 μM.

Table 7. Optimization of the linker



ID	R1	X	Y	Z	IC_{50} (μM)[a]			
					GluN2A	GluN2B	GluN2C	GluN2D
1063-33		OCH_2	C=O	NEt_2	NE	NE	NE	NE
1063-43		CH_2O	C=O	NEt_2	NE	NE	NE	NE
1063-38		CH_2O	C=O	NEt_2	NE	NE	NE	NE

ID	R^1	X	Y	Z	IC_{50} (μM)[a]			
					GluN2A	GluN2B	GluN2C	GluN2D
1063-27		--	C=O	NEt_2	497	NE	NE	NE
1063-22		O			NE	NE	NE	NE
1063-23		O			NE	NE	NE	NE

[a] IC_{50} values were obtained by fitting the Hill equation to the average concentration-effect curves. Data are from between 2-3 oocytes from 1 frog for each compound and receptor tested. NE indicates less than 15% inhibition at 10 μM.

1.4.2 In vitro Analysis of 1063-Series Mechanism of Action

The inhibitory effect of **1063-2** at different splice variants of GluN1- (GluN1-1a, GluN1-1b, GluN1-3a, and GluN1-3b) and GluN2D-containing receptors was evaluated (Figure 11). However, no significant difference was observed at any of the splice variants tested.

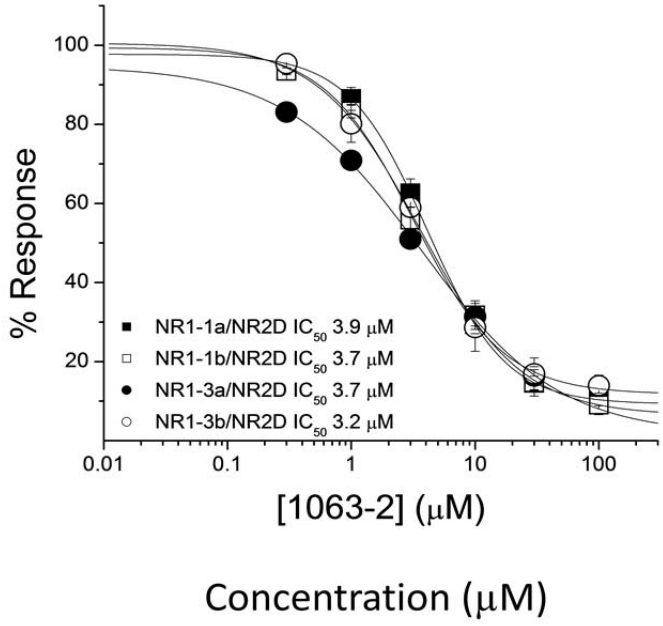


Figure 11. Dose response curve of 1063-2 at various splice variants of GluN1 and GluN2D-containing NMDA receptors

In order to evaluate the molecular determinants of activity of 1063 analogs, a series of GluN2A-GluN2D chimeras were generated by Dr. Katie Vance (Figure 12). These data revealed that the transmembrane domain was important for the inhibitory action of this series (Figure 13). Specifically, replacement of the transmembrane region of GluN2A with that of GluN2D (2A-(2D M1M2M3)) demonstrated inhibition comparable to that of GluN2D-containing receptors. Construction of smaller chimeras further localized the important residues for activity to the M1 helix of the transmembrane domain (Vance dissertation, 2012).

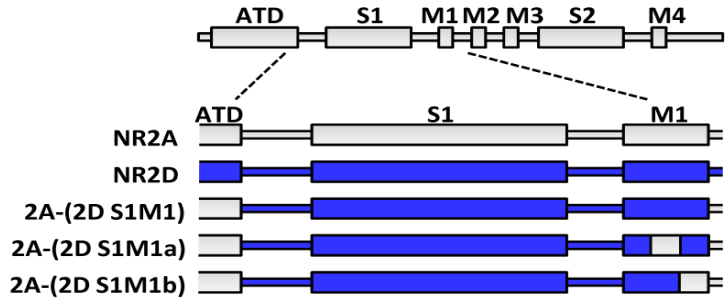


Figure 12. Schematic diagram of GluN1/GluN2A and GluN1/GluN2D chimeras that were prepared by Dr. Katie Vance (Vance dissertation, 2012)

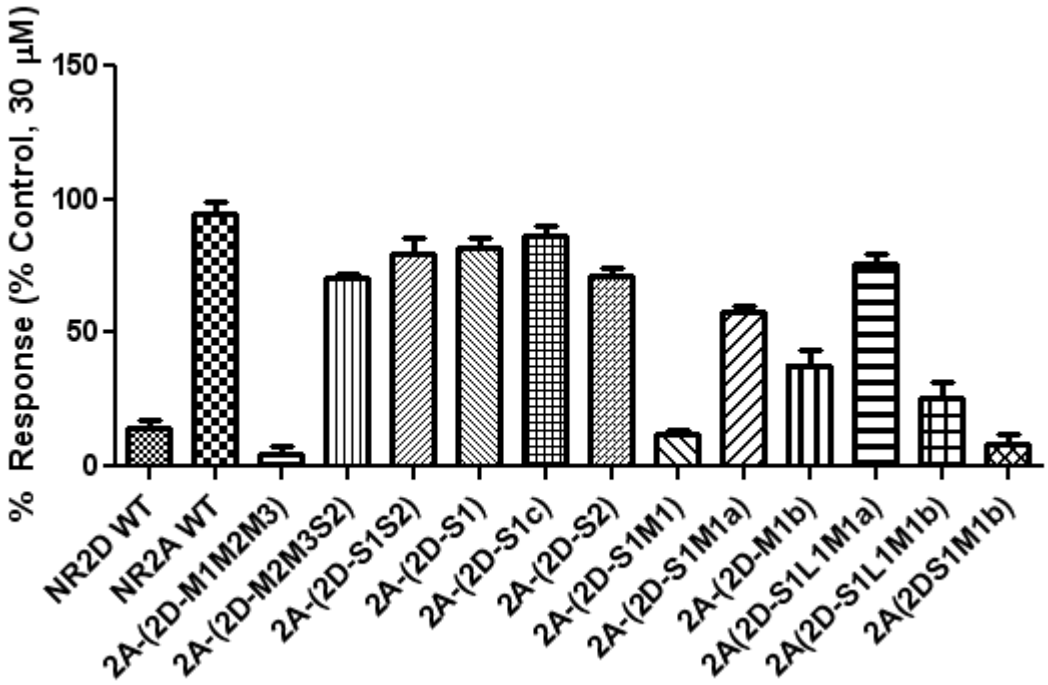


Figure 13. Mutagenesis studies evaluating the effect of 1063-2 at GluN1/GluN2D receptors

Site directed mutagenesis of residues in the M1b transmembrane region from GluN2A into GluN2D revealed a single residue that was critical for activity of the 1063-series (Figure 14). Replacement of a cysteine residue at position 590 in GluN2D with a lysine residue, as in GluN2A, led to nearly a complete loss of inhibition (Vance dissertation,

2012). Interestingly, this residue is located only one helical turn deeper than the binding site of CIQ, the selective potentiator of GluN2C- and GluN2D-containing receptors previously reported by our lab.[5]

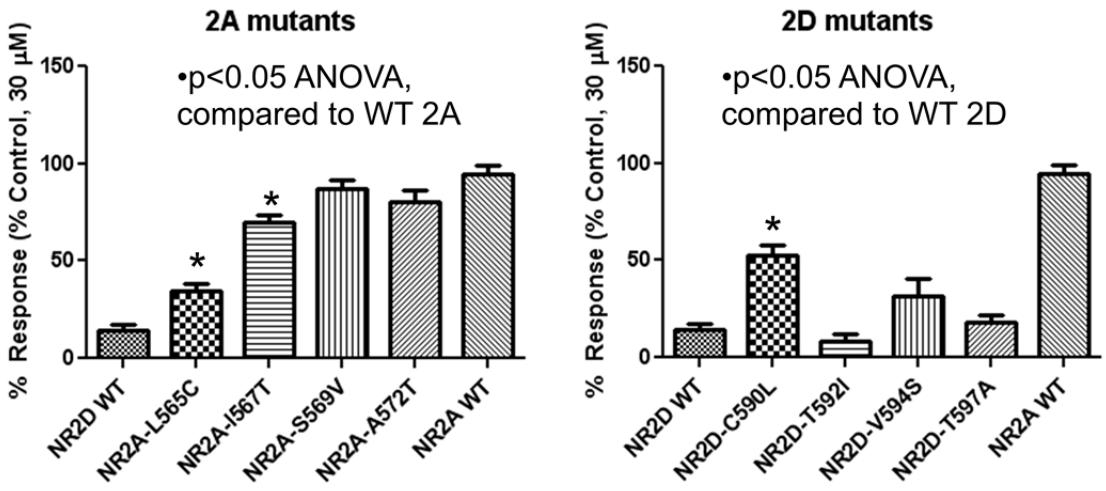


Figure 14. Site directed mutagenesis evaluating the effect of 1063-2 at GluN1/GluN2D receptors

The binding region of the 1063 class is illustrated in Figure 15. Two subunits are shown as a GluN1/GluN2 heterodimer. The ligand binding domain of each subunit is based off of molecular dynamic simulations derived from a model. The lower lobe of this domain is known to vary the most between the GluN2A and GluN2D subunit (region highlighted in red).[75] The transmembrane region is shown as a cartoon representation. 1063 class antagonists were determined to bind within the M1 helix of the transmembrane domain.

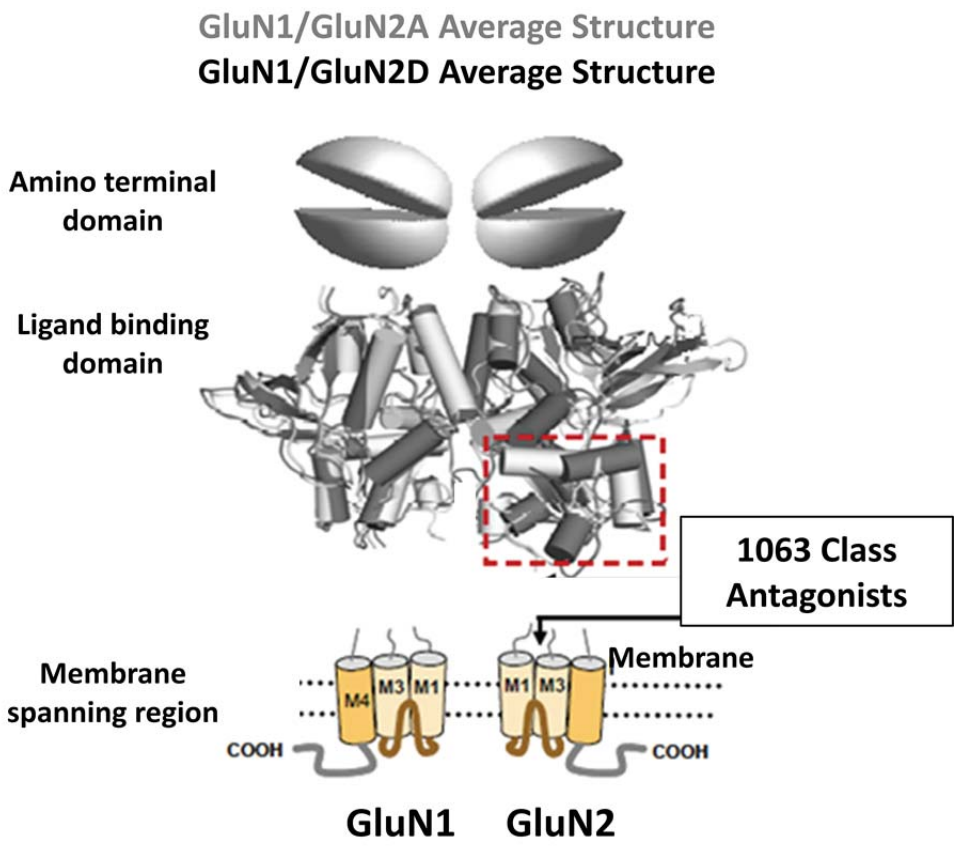


Figure 15. Structural determinants of activity for the 1063-series

1.4.3 In vivo Analysis of Pharmacokinetic Properties of 1063

Several of the most potent analogs, **1063**, **1063-2**, **1063-4** and **1063-20**, were evaluated *in vivo* to determine pharmacokinetic properties, including half-life ($t_{1/2}$) and peak plasma concentration (Table 8). While both **1063** and **1063-2** were found to have relatively short half-lives ($t_{1/2}$ = < 30 min. for each), **1063-2** was found to sufficiently enter the plasma, with a peak plasma concentration of 146 ng/mL (0.40 μM) after oral dosing compared to **1063** (9.7 ng/mL, 0.03 μM). **1063-20** revealed slight improvements in both half-life ($t_{1/2}$ = < 1 h) and peak plasma concentration (470 ng/mL, 1.34 μM). Increases in peak plasma

concentration were demonstrated for both **1063-2** (909 ng/mL, 2.51 μM) and **1063-20** (692 ng/mL, 1.97 μM) in a rat model. No plasma or brain binding data was obtained.

Table 8. *In vivo* pharmacokinetic properties of 1063 analogs

No.	Species	Peak Plasma (ng/mL)	Peak Plasma (μM)	$t_{1/2}$	Brain: Plasma
1063	Mouse	9.7	0.03	< 30 min	0.30
	Rat	27.6	0.07	< 30 min	3.00
1063-2	Mouse	146	0.40	< 30 min	0.56-0.92
	Rat	909	2.51	1 h	1.70
1063-4	Mouse	39	0.12	< 1 h	4.30
	Rat	--	--	--	--
1063-20	Mouse	470	1.34	< 1 h	0.20
	Rat	692	1.97	< 1 h	0.10

Compound **1063-2** was found to partition adequately into the brain (brain : plasma, 3:2) (see Table 8). A rat model of **1063-2** was used to observe the absorption and

distribution of the drug over time (Figure 16). Analysis of the data revealed an increase in the concentration of compound in the brain over a three hour span, indicating that this analog is sufficiently lipophilic to offer relatively high permeability.

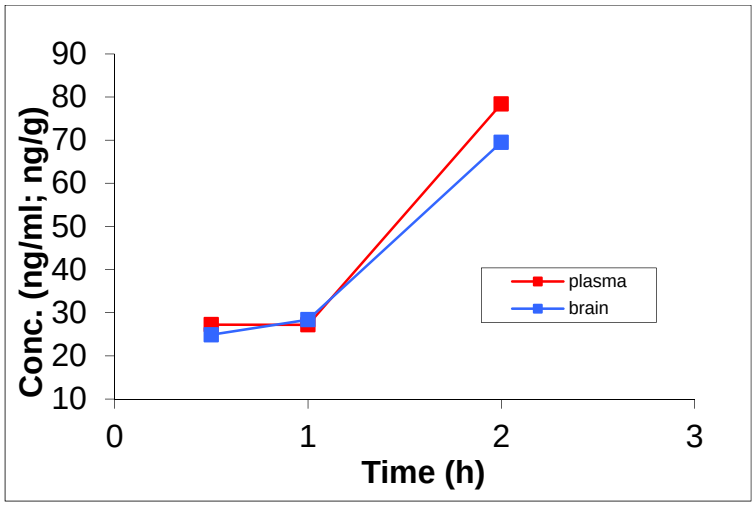


Figure 16. Concentration of 1063-2 in rat plasma and brain over time

1.5 CONCLUSIONS

A class of subunit-selective antagonists were developed with high selectivity for GluN1/GluN2C and GluN1/GluN2D, and a unique scaffold. These compounds were found to bind within a novel site on the receptor that is similar to the tetrahydroisoquinoline site,[5] but one helical turn deeper. The 1063-series is highly selective for GluN2C- and GluN2D-containing NMDA receptors, with selectivity greater than 500-fold. The drug-like properties of this series, including penetration of the blood-brain-barrier (BBB) and plasma absorption, make 1063 analogs extremely attractive from a medicinal chemistry perspective.

Previous efforts involved the introduction of a carbamate to afford compound **1063-2**, a highly selective inhibitor of GluN2C- and GluN2D-containing receptors with increased

in vitro stability compared to **1063**. Replacement of the naphthyl moiety with an indole ring led to **1063-20**, which has an IC_{50} of 2.6 ± μM at GluN1/GluN2D and a selectivity nearly 1000-fold.

Despite work on the extensive SAR, advances in activity have yet to be realized. The additional substitutions and modifications explored have not demonstrated any clear position or functionality that enhances potency compared to the lead analog, **1063**. These compounds represent a novel class of antagonists with high selectivity for GluN1/GluN2C and GluN1/GluN2D that effectively permeate the blood-brain-barrier. This series of molecules could serve as pharmacological tools to evaluate the contribution of GluN2C- and GluN2D-containing receptors, and potentially allow for the development of novel therapeutics.

1.6 CHEMISTRY EXPERIMENTAL DATA

All dry solvents were obtained from a Glass Contour System. Reagents used were acquired from commercial suppliers and utilized without additional purification. Pre-coated glass plates (silica gel 60 F254, 0.25 mm) were used to monitor the progress of reactions by thin layer chromatography (TLC). Purification by flash column chromatography was performed on a Teledyne ISCO Combiflash Companion using prepackaged Teledyne RediSep disposable normal phase silica columns. ^{1}H and ^{13}C NMR were each carried out on an INOVA-400 (400 MHz), VNMRS-400 (400 MHz), INOVA-600 (600 MHz), Unity-600 (600 MHz), or Mercury 300 (300 MHz). All chemical shifts are reported in parts per million and referenced to the residual solvent peak. All coupling constants are reported in Hertz (Hz). The IR spectra were acquired with a Nicolet Avatar 370 DTGS. Mass spectra were

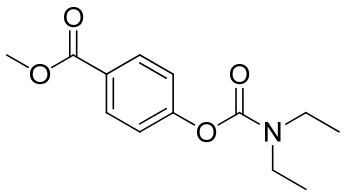
performed by the Emory University Mass Spectrometry Center on a VG 70-S Nier Johnson or JEOL instrument. Purity of all final compounds was found to be ≥ 95% unless otherwise noted.

General Preparation of Carbamate and Thiocarbamate Compounds (Procedure I). To a solution of the corresponding hydroxybenzoate (1.0 mmol) in DMF (0.26 M) was added finely ground potassium carbonate (2.0 equiv) which had been oven dried for 24 h. The mixture stirred at 24 °C for 1 h before carbamoyl chloride (1.1 equiv) was added. After stirring for 24 h, the mixture was diluted with distilled water and extracted with Et_2O (2x). The organic phase was separated and was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1).

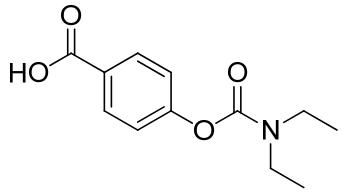
General Preparation of Benzoic Acid Compounds (Procedure II). To a solution of the corresponding methyl ester (1.0 mmol) in MeOH (0.063 M) was added 1.0 M NaOH (3.8 equiv). The reaction mixture was allowed to stir for 24 h before the pH was adjusted to 3 via addition of 1.0 M HCl. The crude product was then further purified as necessary. The combined organic layers were washed with brine, filtered, and concentrated *in vacuo* to give the desired product.

General Preparation of Phenyl Alkylcarbamate and Alkylcarbamothioate Compounds (Procedure III). To a solution of the corresponding carboxylic acid (1.0 mmol) in DMF (0.16 M) at 0 °C was added DMAP (1.1 equiv) and EDCI (1.0 equiv). The reaction mixture was allowed to continue stirring at 0 °C for 45 min. The mixture was then treated with the corresponding amine (1.1 equiv) before slowly warming to room temperature and stirring 24 h. The reaction was concentrated *in vacuo* and partitioned between 1.0 M HCl and EtOAc. The resulting biphasic solution was extracted with EtOAc (2x). The organic layers were

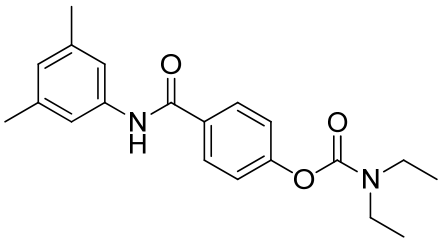
combined and washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 2/1).



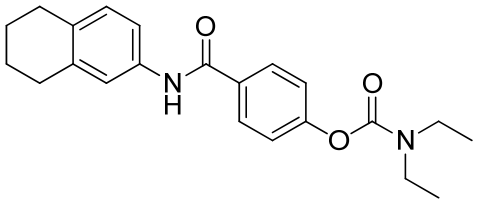
*Methyl 4-(diethylcarbamoyloxy)benzoate (**1063-17a**).* Compound **1063-17a** was prepared via Procedure I from methyl 4-hydroxybenzoate (0.50 g, 3.3 mmol) and *N,N*-diethylcarbamoyl chloride (0.96 mL, 7.7 mmol, 1.1 equiv) to give a colorless oil (0.74 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.9 Hz, 2H), 3.78 (s, 3H), 3.30-3.27 (m, 4H), 1.16-1.08 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 155.1, 153.1, 130.7, 126.6, 121.4, 51.8, 42.1, 41.8, 14.0, 13.1.



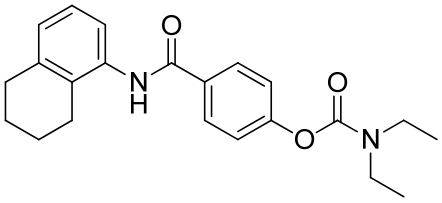
*4-(Diethylcarbamoyloxy)benzoic acid (**1063-17b**).* Compound **1063-17b** was prepared via Procedure II from **1063-17a** (0.50 g, 2.0 mmol) to give a white solid (0.35 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (br s, 1H), 7.97 (d, *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H), 3.41-3.30 (m, 4H), 1.19 (t, *J* = 6.7 Hz, 3H), 1.12 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 154.9, 152.7, 130.7, 127.5, 121.9; HRMS calcd for $C_{12}H_{15}NO_4$ 238.10804; found 238.21147 $[M+H]^+$.



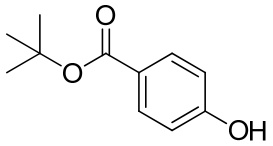
*4-((3,5-Dimethylphenyl)carbamoyl)phenyl diethylcarbamate (**1063-17**).* Compound **1063-17** was prepared via Procedure III from **1063-17b** (0.30 g, 1.3 mmol) and 3,5-dimethylaniline (0.17 g, 1.4 mmol) to give a white solid (0.30 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87-7.84 (m, 2H), 7.73 (s, 1H), 7.28 (s, 2H), 7.25-7.23 (m, 2H), 6.81 (s, 1H), 3.50-3.38 (m, 4H), 2.34 (s, 6H), 1.32-1.22 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 154.3, 135.9, 138.8, 138.1, 132.1, 128.6, 126.4, 122.0, 118.2, 42.6, 42.2, 21.6, 14.4, 13.6; HRMS (APCI) calcd for $C_{20}H_{24}O_3N_2$ 341.18604; found 341.18597 $[M+H]^+$.



*4-((5,6,7,8-Tetrahydronaphthalen-1-yl)carbamoyl)phenyl diethylcarbamate (**1063-18**).* Compound **1063-18** was prepared via Procedure III from **1063-17b** (0.30 g, 1.3 mmol) and 5,6,7,8-tetrahydronaphthalen-1-amine (0.19 mL, 1.4 mmol, 1.1 equiv) to give a pale pink solid (0.34 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.0 Hz, 1H), 7.58 (s, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 8.0 Hz, 1H), 6.97 (d, J = 12 Hz, 1H), 3.48-3.39 (m, 4H), 2.82 (t, J = 6.4 Hz, 2H), 2.67 (t, J = 6.4 Hz, 2H), 1.89-1.86 (m, 2H), 1.82-1.78 (m, 2H), 1.30-1.21 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 138.4, 135.6, 132.1, 128.5, 126.6, 126.1, 122.2, 120.7, 42.2, 30.0, 24.8, 23.1, 22.7, 14.5, 13.5; HRMS (APCI) calcd for $C_{22}H2_6O_3N_2$ 367. 20162; found 367.20128 $[M+H]^+$; Anal. ($C_{22}H_{26}O_3N_2$) C: 71.93, H: 7.10, N: 7.61.

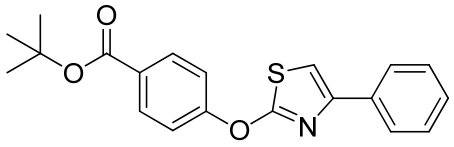


*4-((5,6,7,8-Tetrahydronaphthalen-2-yl)carbamoyl)phenyl diethylcarbamate (**1063-19**).* Compound **1063-19** was prepared via Procedure III from **1063-17b** (0.30 g, 1.3 mmol) and 5,6,7,8-tetrahydronaphthalen-2-amine (0.20 g, 1.4 mmol) to give a gray solid (0.29 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.82 (m, 3H), 7.40 (s, 1H), 7.31 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.22-7.20 (m, 2H), 7.05 (d, *J* = 8.4 Hz, 1H), 3.48-3.41 (m, 4H), 2.78-2.75 (m, 4H), 1.82-1.78 (m, 4H), 1.30-1.21 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 154.4, 138.1, 135.5, 135.1, 133.8, 132.1, 129.7, 128.5, 122.1, 120.9, 118.0, 42.6, 42.2, 29.8, 29.2, 23.4, 23.3, 14.5, 13.6; HRMS (APCI) calcd for $C_{22}H_{26}O_3N_2$ 367.20162; found 367.20138 $[M+H]^+$; Anal. ($C_{22}H_{16}O_3N_2$) C: 71.84, H: 7.09, N: 7.69.

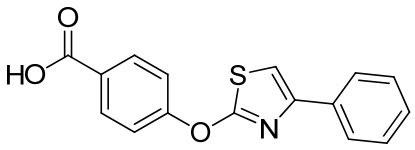


*tert-Butyl 4-hydroxybenzoate (**33**).* To a solution of methyl 4-hydroxybenzoate (2.5 g, 18 mmol) in DCM (75 mL, 0.24 M) was added DMAP (0.090 g, 0.71 mmol, 0.039 equiv) and *t*-BuOH (50 mL, 18 mmol, 0.36 M). A 1.0 M solution of DCC in DCM (27 mL, 27.2 mmol, 1.5 equiv) was added dropwise and the reaction mixture was stirred for 24 h. The mixture was filtered and concentrated *in vacuo*. The resulting residue was diluted with EtOAc and washed with saturated aqueous sodium bicarbonate (2x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 10/3) to yield a white solid (2.9 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92-7.88 (mult, 2H), 6.87-6.84

(mult, 2H), 1.61 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 160.2, 131.9, 124.20, 115.30, 81.2, 28.45; HRMS Calcd for $C_{11}H_{14}O_3$ 193.0870; found 193.0869 $[M-H]^+$.

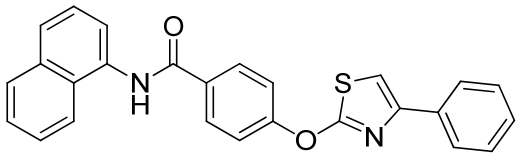


*tert-Butyl 4-(4-phenylthiazole-2-yloxy)benzoate (**1063-22b**).* To a solution of **33** (0.97 g, 5.0 mmol) in DMF (19 mL) was added finely ground potassium carbonate (1.4 g, 10.0 mmol) which had been oven dried for 24 h. The reaction mixture stirred at rt for 1 h before 2-chloro-4-benzylthiazole (1.1 g, 5.5 mmol) was added. Heating for 15 min at 200 °C μW afforded a liquid which was diluted with distilled water and extracted with Et_2O (2x). The organic phase was separated and was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a colorless oil (0.2 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97-7.94 (mult, 2H), 7.70-7.67 (mult, 2H), 7.30-7.18 (mult, 5H), 6.90 (s, 1H) 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 158.7, 150.0, 134.2, 131.60, 129.0, 128.9, 128.8, 128.4, 126.3, 126.1, 119.3, 114.4, 107.1, 81.4, 28.4; HRMS Calcd for $C_{20}H_{19}NO_3S$ 354.1158; found 354.1158 $[M+H]^+$.

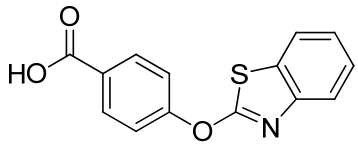


*4-(4-Phenylthiazol-2-yloxy)benzoic acid (**1063-22c**).* A solution of **1063-22b** (1.3 g, 3.6 mmol) in TFA (14.5 mL) was allowed to stir for 1 h at rt. The reaction mixture was filtered while washing with DCM to afford a white solid (0.56 g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 7.5 Hz, 2H), 7.73 (s, 1H), 7.53 (d, J = 9.0 Hz, 2H), 7.44-7.33 (mult, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.9, 166.5, 158.2, 148.6, 133.7, 131.6,

128.8, 128.2, 125.6, 119.7, 110.8, 109.2; HRMS Calcd for $C_{16}H_{11}NO_3S$ 298.0532; found 298.0532 $[M+H-C_4H_8]^+$.

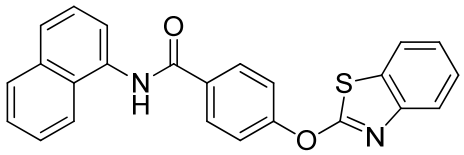


*N-(Napthalen-1-yl)-4-(4-phenylthiazol-2-yloxy)benzamide (**1063-22**).* Compound **1063-22** was prepared via Procedure III from **1063-22c** (0.51 g, 1.7 mmol) to give a white solid (0.45 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.214 (br s, 1H), 8.08-8.01 (mult, 3H), 7.94-7.91 (mult, 2H), 7.85-7.76 (mult, 3H), 7.56-7.51 (mult, 5H), 7.44-7.34 (mult, 3H), 7.101 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.4, 171.4, 165.3, 157.3, 148.6, 133.8, 132.0, 130.1, 129.2, 128.8, 128.2, 128.1, 126.4, 126.1, 126.0, 125.7, 125.6, 123.9, 123.4, 119.8, 110.8, 108.9; HRMS Calcd for $C_{26}H_{18}N_2O_2S$ 423.1162; found 423.1164 $[M+H]^+$.

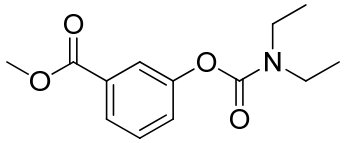


*4-(Benzo[d]thiazol-2-yloxy)benzoic acid (**1063-23c**).* To a solution of **33** (0.97 g, 5.0 mmol) in DMF (19 mL) was added finely ground potassium carbonate (1.4 g, 10 mmol) which had been oven dried for 24 h. The reaction mixture stirred at rt for 1 h before 2-chloro-benz[*d*]thiazole (0.68 mL, 5.5 mmol, 1.1 equiv) was added. Heating for 15 min at 200 °C μW afforded a liquid which was diluted with distilled water and extracted with Et_2O (2x). The organic phase was separated and was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a yellow oil (1.2 g, 71%) which was carried on without purification. A solution of the *tert*-butyl ester (1.2 g, 3.5 mmol) in TFA (14 mL) was allowed to stir for 1 h at rt. The reaction mixture was filtered while washing with DCM to afford a white solid (0.96 g, 45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.5 (br s, 1H), 8.05 (d, J = 8.7

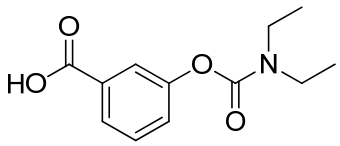
Hz, 2H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.2, 175.9, 170.4, 158.8, 148.5, 132.6, 127.0, 126.7, 124.8, 122.2, 121.6, 120.5; HRMS Calcd for $C_{14}H_9NO_3S$ 272.0376; found 272.0377 $[M+H]^+$.



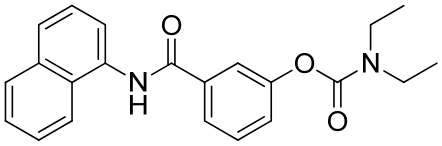
*4-(Benzo[d]thiazol-2-yloxy)-N-(napthalen-1-yl)benzamide (**1063-23**).* Compound **1063-23** was prepared via Procedure III from **1063-23c** (0.33 g, 1.2 mmol) to afford a white solid (0.15 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (br s, 1H), 8.096 (d, *J* = 9.0 Hz, 2H), 8.03 (d, *J* = 7.5 Hz, 1H), 7.94-7.91 (mult, 2H), 7.79-7.73 (mult, 3H), 7.58-7.52 (mult, 5H), 7.47-7.41 (mult, 1H), 7.37-7.31 (mult, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.1, 186.7, 171.0, 157.3, 149.0, 134.4, 132.6, 132.4, 129.4, 129.1, 127.8, 126.72, 126.66, 126.6, 126.3, 126.0, 124.7, 122.1, 121.7, 121.6, 121.0, 120.9; HRMS Calcd for $C_{24}H_{16}N_2O_2S$, 397.1005; found 397.1006 $[M+H]^+$.



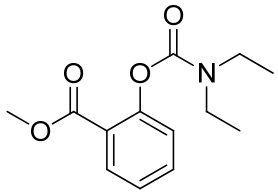
*Methyl 3-(diethylcarbamoyloxy)benzoate (**1063-24a**).* Compound **1063-24a** was prepared via Procedure I using methyl 3-hydroxybenzoate (1.0 g, 6.6 mmol) and diethylcarbamoyl chloride (0.92 mL, 7.2 mmol, 1.1 equiv) to yield a colorless oil (1.5 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.79 (t, *J* = 6.3 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.35 (dq, *J* = 8.4, 1.2 Hz, 1H), 3.92 (s, 3H), 3.43 (q, *J* = 7.2 Hz, 4H), 1.30-1.19 (mult, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 153.8, 151.5, 131.4, 129.2, 126.5, 126.2, 123.0, 52.1, 42.3, 42.9, 14.2, 13.3; HRMS Calcd for $C_{13}H_{17}NO_4$ 252.1230; found 252.1231 $[M+H]^+$.



*3-(Diethylcarbamoyloxy)benzoic acid (**1063-24b**).* Compound **1063-24b** was prepared via Procedure II from **1063-24a** (1.5 g, 6.1 mmol) to give a colorless oil (1.3 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (br s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.85 (s, 1H), 7.49-7.37 (mult, 2H), 3.52-3.40 (mult, 4H), 1.30-1.20 (mult, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.0, 154.2, 151.7, 130.9, 129.5, 127.6, 127.1, 123.7, 42.6, 42.2, 14.4, 13.5; HRMS Calcd for $C_{12}H_{15}NO_4$ 238.1074; found 238.1074 $[M+H]^+$.

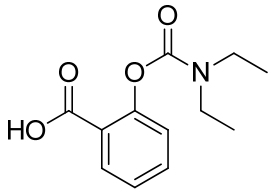


*3-(Napthalen-1-ylcarbamoyl)phenyl diethylcarbamate (**1063-24**).* Compound **1063-24** was prepared via Procedure III from **1063-24b** (1.1 g, 4.5 mmol) and 1-napthylamine (0.70 g, 4.9 mmol, 1.1 equiv) to afford a viscous, pink oil was obtained (0.82 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (br s, 1H), 7.94-786 (mult, 3H), 7.80-7.75 (mult, 3H), 7.54-7.48 (mult, 3H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.38-7.34 (mult, 1H), 3.49-3.38 (mult, 4H), 1.31-1.20 (mult, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 154.0, 151.6, 135.9, 134.1, 132.6, 129.4, 128.44, 128.38, 126.4, 126.1, 126.0, 125.6, 125.2, 123.9, 122.5, 121.9, 121.2, 42.3, 42.0, 14.2, 13.3; HRMS Calcd for $C_{22}H_{22}N_2O_3$ 363.1703; found 363.1704 $[M+H]^+$.

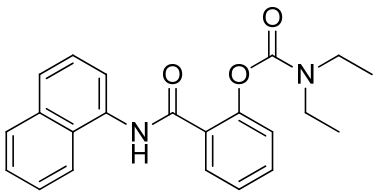


*Methyl 2-(diethylcarbamoyloxy)benzoate (**1063-25a**).* Compound **1063-25a** was prepared via Procedure I using methyl 2-hydroxybenzoate (1.0 g, 6.6 mmol) and diethylcarbamoyl

chloride (0.92 mL, 7.2 mmol, 1.1 equiv) to yield a colorless oil (1.7 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.53 (td, *J* = 7.8, 1.8 Hz, 1H), 7.27 (td, *J* = 7.5, 0.9 Hz, 1H), 7.16 (dd, *J* = 8.1, 1.2 Hz, 1H), 3.86 (s, 3H), 3.51 (q, *J* = 7.2 Hz, 2H), 3.40 (q, *J* = 6.9 Hz, 2H), 1.32-1.20 (mult, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 153.6, 150.9, 133.1, 131.1, 124.9, 123.8, 51.7, 42.0, 41.7, 13.7, 13.0; HRMS Calcd for $C_{13}H_{17}NO_4$ 252.1230; found 252.1231 $[M+H]^+$.

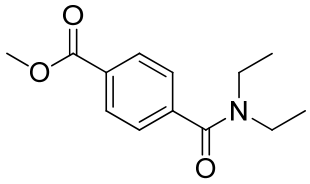


*2-(Diethylcarbamoyloxy)benzoic acid (**1063-25b**).* Compound **1063-25b** was prepared via Procedure II from **1063-25a** (1.6 g, 6.2 mmol). After acidification, the residue was then diluted with distilled water and extracted with Et_2O (2x). The combined organic layers were washed with brine, filtered and concentrated *in vacuo* to give a colorless oil (1.5 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.59 (td, *J* = 8.1, 1.8 Hz, 1H), 7.30 (td, *J* = 7.8, 0.9 Hz, 1H), 7.19 (dd, *J* = 8.4, 0.9, 1H), 3.50 (q, *J* = 7.2 Hz, 2H), 3.40 (q, *J* = 7.2 Hz, 2H), 1.31-1.19 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 154.0, 151.7, 134.2, 132.0, 125.3, 124.2, 123.3, 42.2, 41.9, 13.9, 13.2; HRMS Calcd for $C_{12}H_{15}NO_4$ 238.1074; found 238.1074 $[M+H]^+$.

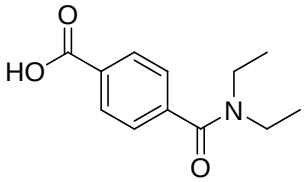


*2-(Napthalen-1-ylcarbamoyl)phenyl diethylcarbamate (**1063-25**).* Compound **1063-25** was prepared via Procedure III from **1063-25b** (1.0 g, 4.4 mmol) and 1-napthylamine (0.69 g, 4.8 mmol, 1.1 equiv) to afford a pink solid (0.66 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (br s,

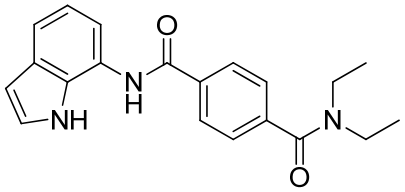
1H), 8.19 (d, *J* = 7.5 Hz, 1H), 7.98-7.94 (m, 1H), 7.90-7.87 (m, 2H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.56-7.49 (m, 4H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 3.42 (q, *J* = 6.9 Hz, 2H), 3.29 (q, *J* = 7.2 Hz, 2H), 1.15 (t, *J* = 7.2 Hz, 3H), 0.97 (t, *J* = 6.9 Hz, 3H; ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.3, 154.9, 148.5, 134.3, 132.8, 132.0, 130.7, 130.3, 128.8, 127.0, 126.40, 126.36, 126.1, 126.0, 125.8, 123.4, 121.2, 120.2, 42.6, 42.3, 14.2, 13.2; HRMS Calcd for $C_{22}H_{22}N_2O_3$ 363.1703; found 363.1704 $[M+H]^+$.



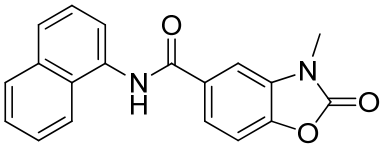
*Methyl 4-(diethylcarbamoyl)benzoate (**43**).* Thionyl chloride (2.8 mL, 5.6 mmol, 2.0 M) was added to 4-(methoxycarbonyl)benzoic acid (1.0 g, 5.6 mmol) and the reaction was brought to reflux. After refluxing for 1 h, the resulting mixture was concentrated *in vacuo*. To a stirred solution of acyl chloride (0.96 g, 4.9 mmol) in DCM (9.7 mL, 0.5 M) was added diethylamine (0.55 mL, 5.3 mmol, 1.1 equiv) and TEA (0.85 mL, 6.1 mmol, 1.25 equiv). The reaction was allowed to stir for 20 min at rt before being treated with DCM and diluted with distilled water. After extracting with DCM (2x), the combined organic layers were washed with brine, dried over ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to yield a pale pink oil (0.79 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, *J* = 8.1, 0.9 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 3.75 (s, 3H), 3.38 (q, *J* = 6.3 Hz, 2H), 3.03 (q, *J* = 6.9 Hz, 2H), 1.07 (t, *J* = 6.6 Hz, 3H), 0.914 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 166.6, 141.7, 130.8, 129.9, 126.4, 52.4, 43.4, 39.5, 14.3, 13.01; HRMS Calcd for $C_8H_6BrFO_2$ 232.9608; found 232.9609 $[M+H]^+$.



*4-(Diethylcarbamoyl)benzoic acid (**44**).* Compound **44** was prepared via Procedure II from **43** (0.79 g, 3.4 mmol). After acidification the mixture was concentrated *in vacuo*. Distilled water was added to create a suspension. The suspension was stored at 0 °C for approximately 30 min before being filtered. Washing the crystals with distilled water afforded a white solid (0.44 g, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 3.58 (q, *J* = 6.6 Hz, 2H), 3.23 (q, *J* = 6.9, 2H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.12 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.3, 142.0, 130.6, 130.5, 126.5, 43.6, 39.7, 14.4, 13.1; HRMS Calcd for $C_{12}H_{15}NO_3$ 222.1125; found 222.1128 $[M+H]^+$.

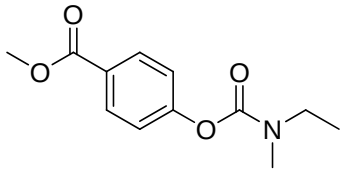


*N^1,N^1-Diethyl-N^4-(1H-indol-7-yl)terephthalamide (**1063-27**).* Compound **1063-27** was prepared via Procedure III from **44** (0.34 g, 1.6 mmol) to give a brown solid (0.076 g, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (br s, 1H), 9.13 (br s, 1H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.23-7.17 (mult, 4H), 7.01 (t, *J* = 8.0 Hz, 1H), 6.51 (t, *J* = 2.4 Hz, 1H), 3.49 (q, *J* = 6.8 Hz, 2H), 3.12 (q, *J* = 6.8 Hz, 2H), 1.19 (t, *J* = 6.8 Hz, 3H), 1.02 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 165.6, 139.8, 135.6, 130.8, 128.3, 128.1, 126.5, 125.1, 123.1, 119.7, 118.4, 114.1, 102.8, 43.7, 39.8, 14.4, 13.1; HRMS Calcd for $C_{20}H_{21}N_3O_2$ 336.1707; found 336.1709 $[M+H]^+$.

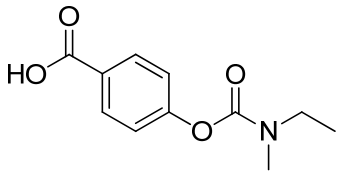


*3-Methyl-N-(napthyalen-1-yl)-2-oxo-2,3-dihydrobenzo[d]oxazole-5-carboxamide (**1063-29**).*

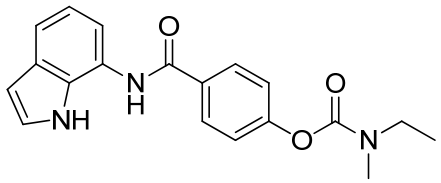
Compound **1063-29** was prepared via Procedure III from 3-methyl-2-oxo-2,3-dihydrobenzo[*d*]oxazole-5-carboxylic acid (0.19 g, 1.0 mmol) and 1-napthylamine (0.14 g, 1.0 mmol, 1.0 equiv) to afford a white, amorphous solid (0.0066 g, 2%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.42 (br s, 1H), 8.0 (mult, 2H), 7.94 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 7.2 Hz, 1H), 7.77 (s, 1H), 7.60-7.55 (mult, 3H), 7.52 (d, J = 9.0 Hz, 1H), 7.42 (d, J = 9.0 Hz, 1H), 3.43 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.2, 165.4, 154.1, 144.3, 133.8, 132.0, 130.3, 129.2, 128.1, 126.4, 126.1, 126.0, 125.6, 124.0, 123.3, 122.5, 109.2, 108.7, 28.3; HRMS Calcd for $C_{19}H_{14}N_2O_3$ 319.1077; found 319.1077 $[M+H]^+$.



*Methyl 4-(ethyl(methyl)carbamoyloxy)benzoate (**1063-30a**).* Compound **1063-30a** was prepared via Procedure I from methyl 4-hydroxybenzoate (1.0 g, 6.6 mmol) and *N*-ethyl-*N*-methyl carbamoyl chloride (0.80 mL, 7.2 mmol, 1.1 equiv) to give the desired benzoate (1.2 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$, 1:1 ratio of rotamers) δ 8.07-8.03 (mult, 4H), 7.20 (dd, J = 8.4, 2.7 Hz, 4H), 3.91 (s, 6H), 3.52-3.39 (mult, 4H), 3.08 (s, 3H), 3.00 (s, 3H), 1.30-1.19 (mult, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.0, 166.7, 155.5, 131.2, 127.1, 121.8, 52.3, 44.4, 13.4, 12.6; HRMS Calcd for $C_{12}H_{15}NO_4$ 238.1074; found 238.1074 $[M+H]^+$.

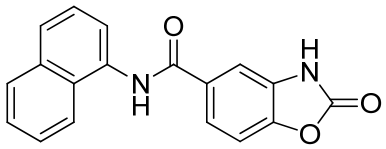


*4-(Ethyl(methyl)carbamoyloxy)benzoic acid (**1063-30b**).* Compound **1063-30b** was prepared via Procedure II from **1063-30a** (1.2 g, 5.1 mmol). After acidification, the residue was then diluted with distilled water and extracted with Et_2O (2x). The combined organic layers were washed with brine, filtered, and concentrated *in vacuo* to give a white solid (0.81 g, 72%). ^{1}H NMR (400 MHz, DMSO-d_6, 1:1 ratio of rotamers) δ 7.96-7.94 (mult, 4H), 7.24 (d, J = 8.4 Hz, 4H), 3.41 (q, J = 8.0 Hz, 2H), 3.34 (q, J = 7.2 Hz, 2H), 3.03 (s, 3H), 2.91 (s, 3H), 1.18 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.7, 166.7, 154.8, 130.7, 127.5, 121.9, 43.6, 17.2, 12.2; HRMS Calcd for $C_{11}H_{13}NO_4$ 224.0917; found 224.0916.

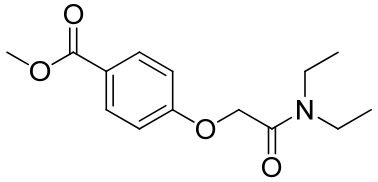


*4-(1H-Indole-7-ylcarbamoyl)phenyl ethyl(methyl)carbamate (**1063-30**).* Compound **1063-30** was prepared via Procedure III from **1063-30b** (0.11 g, 0.5 mmol) and 1*H*-indole-7-amine (0.066 g, 0.5 mmol, 1.0 equiv) to give a pink solid (071 g, 42%). ^{1}H NMR (600 MHz, $CDCl_3$, 1:1 mixture of rotamers) δ 9.91 (br s, 2H), 8.21 (s, 2H), 8.11 (d, J = 9.0 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.28-7.23 (mult, 4H), 7.07 (t, J = 7.8 Hz, 2H), 6.91 (d, J = 7.2 Hz, 2H), 6.59 (t, J = 1.8 Hz, 2H), 3.57-3.55 (mult, 2H), 3.55-3.41 (mult, 2H), 3.11 (d, J = 12.6 Hz, 3H), 3.04 (d, J = 11.4 Hz, 3H), 1.29-1.26 (mult, 3H), 1.26-1.21 (mult, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, 1:1 mixture of rotamers) δ 165.0, 153.9, 153.4, 153.3, 131.8, 129.9, 129.5, 129.4, 129.3, 125,6, 125.0, 123.3, 123.2, 122.1, 121.5, 119.1, 118.6, 117.7, 117.2,

116.2, 115.7, 101.8, 101.4, 43.7, 34.0, 33.7, 13.3; HRMS Calcd for $C_{19}H_{19}O_3N_3$ 338.1499; found 338.1499 $[M+H]^+$.

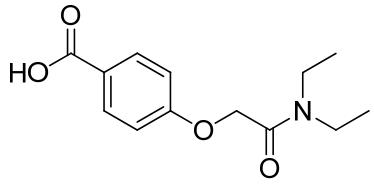


*N-(Napthalen-1—yl)-2-oxo—2,3,-dihydrobenzo[d]oxazole-5-carboxamide (**1063-31**).* Compound **1063-31** was prepared via Procedure III from 2-oxo-2,3-dihydrobenzo[*d*]oxazole-5-carboxylic acid (0.18 g, 1.0 mmol) and 1-napthylamine (0.14 g, 1.0 mmol, 1.0 equiv) to afford a brown, amorphous solid (0.058 g, 19%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (br s, 1H), 7.96-7.95 (mult, 2H), 7.85 (t, J = 7.8 Hz, 2H), 7.76 (s, 1H), 7.55-7.52 (mult, 4H), 7.44 (d, J = 8.4 Hz, 1H), 3.33 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.5, 154.5, 145.7, 133.9, 133.8, 130.5, 130.2, 129.3, 128.1, 126.4, 126.1, 126.0, 125.6, 124.0, 123.4, 122.4, 109.29, 109.27; HRMS Calcd for $C_{18}H_{12}N_2O_3$ 305.0921; found 305.0921 $[M+H]^+$.

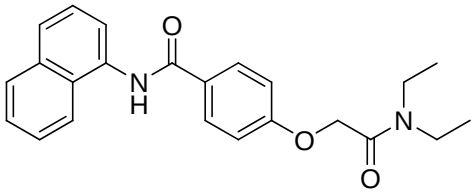


*Methyl 4-(2-(diethylamino)-2-oxoethoxy)benzoate (**40**).* Compound **40** was prepared via Procedure I from methyl 4-hydroxybenzoate (0.15 g, 1.0 mmol) and *N,N*-diethylacetamide (0.17 g, 1.1 mmol, 1.1 equiv). After stirring for 24 h, the mixture was diluted with distilled water and extracted with Et_2O (2x). Upon separating the organic phase, the solution was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the desired benzoate (0.29 g, >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98-7.95 (mult, 2H), 6.96-6.93 (mult, 2H), 4.71 (s, 2H), 3.85 (s, 3H), 3.39-3.35 (mult, 4H), 1.20 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.2 Hz, 3H); ^{13}C

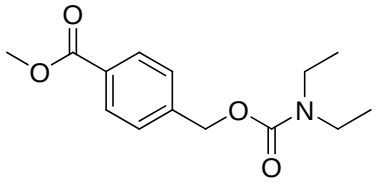
NMR (100 MHz, $CDCl_3$) δ 166.8, 166.4, 161.9, 131.7, 123.5, 114.4, 67.4, 52.0, 41.7, 40.5, 14.4, 12.9; HRMS Calcd for $C_{14}H_{19}NO_4$ 266.1387; found 266.1384 $[M+H]^+$.



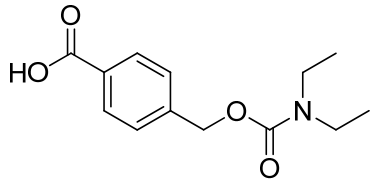
*4-(2-(Diethylamino)-2-oxoethoxy)benzoic acid (**41**).* Compound **41** was prepared via Procedure II from **40** (0.29 g, 1.1 mmol) to give a white solid (0.25 g, 93 %). ^{1}H NMR (600 MHz, DMSO – d_6) δ 7.87-7.86 (mult, 2H), 6.98-6.96 (mult, 2H), 4.89 (s, 2H), 3.80 (br s, 1H), 3.32 (q, J = 7.2 Hz, 2H), 3.28 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H), 1.03 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 166.7, 162.3, 132.3, 131.7, 123.0, 114.43, 114.39, 67.2, 41.7, 40.6, 14.3, 12.8; HRMS Calcd for $C_{13}H_{17}NO_4$ 252.1230; found 252.1230 $[M+H]^+$.



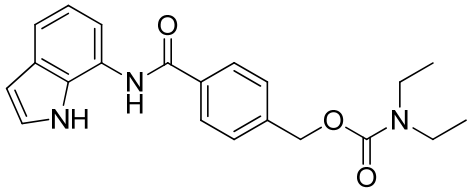
*4-(2-(Diethylamino)-2-oxoethoxy)-N-(naphthalen-1-yl)benzamide (**1063-33**).* Compound **1063-33** was prepared via Procedure III from **41** (0.25 g, 1.0 mmol) and 1-napthylamine to give the desired benzamide (0.097 g, 25%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (br s, 1H), 8.00-7.96 (mult, 3H), 7.91 (t, J = 7.8 Hz, 2H), 7.74 (d, J = 9.0 Hz, 1H), 7.53-7.52 (mult, 3H), 7.06 (d, J = 8.4 Hz, 2H), 4.76 (s, 2H), 3.42-3.40 (mult, 4H), 1.24 (t, J = 6.6 Hz, 3H), 1.15 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.6, 161.3, 146.4, 134.4, 129.4, 129.0, 128.1, 126.6, 126.2, 126.17, 126.0, 121.5, 121.0, 119.1, 115.0, 109.6, 67.5, 41.8, 40.6, 14.6, 13.0; HRMS Calcd for $C_{23}H_{24}N_2O_3$ 377.1860; found 377.1856 $[M+H]^+$.



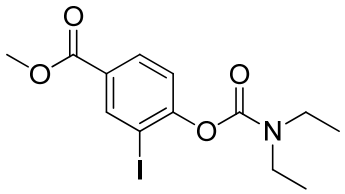
*Methyl 4-(((diethylcarbamoyl)oxy)methyl)benzoate (**47**).* To a solution of methyl 4-(hydroxymethyl)benzoate (0.17 g, 1.0 mmol) in acetonitrile (2.6 mL, 0.32 M) was added cesium carbonate (0.80 g, 4.17 mmol, 5.0 equiv). The mixture stirred at rt for 3 h before diethyl carbamoyl chloride (0.11 mL, 0.83 mmol, 1.0 equiv) was added. After stirring for an additional 24 h, the mixture was diluted with saturated NH_4Cl and extracted with EtOAc (2x). Upon separating the organic phase, the solution was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a white solid (0.23 g, >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.8 Hz, 2H), 5.18 (s, 2H), 3.91 (s, 3H), 3.31 (q, *J* = 5.2 Hz, 4H), 1.13 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 155.7, 142.5, 129.9, 129.7, 127.4, 66.1, 52.3, 42.2, 41.5, 14.3, 13.6; HRMS Calcd for $C_{14}H_{19}NO_4$ 266.1387; found 266.1385 $[M+H]^+$.



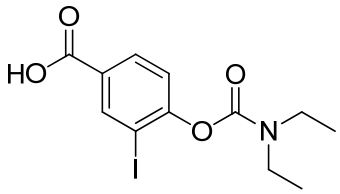
*4-(((Diethylcarbamoyl)oxy)methyl)benzoic acid (**48**).* Compound **48** was prepared via Procedure II from **47** (0.69 g, 2.6 mmol) to give a white solid (0.62 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 5.13 (s, 2H), 3.24 (mult, 4H), 1.06 (mult, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.4, 154.7, 141.9, 130.8, 129.4, 127.1, 65.4, 41.5, 40.9, 14.1, 13.4; HRMS Calcd for $C_{13}H_{17}NO_4$ 250.1085; found 250.1084 $[M-H]^+$.



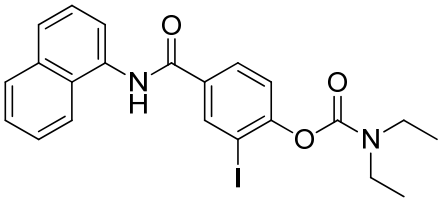
*4-((1H-Indol-7-yl)carbamoyl)benzyl diethylcarbamate (**1063-38**).* Compound **1063-38** was prepared via Procedure III from **48** (0.26 g, 1.0 mmol) and 1*H*-indol-7-amine (0.15 g, 1.1 mmol, 1.1 equiv) to give a white solid (0.20 g, 52%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.98 (br s, 1H), 8.16 (br s, 1H), 7.93 (d, *J* = 7.8 Hz, 2H), 7.56-7.50 (mult, 3H), 7.29 (t, *J* = 2.4 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 1H), 6.59 (t, *J* = 2.4 Hz, 1H), 5.22 (s, 2H), 3.40-3.39 (mult, 4H), 1.17 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 165.9, 155.8, 141.3, 134.0, 130.8, 128.5, 127.7, 125.1, 122.7, 119.6, 118.5, 114.1, 102.7, 66.2, 42.2, 41.5, 14.3, 13.6; HRMS Calcd for $C_{21}H_{23}N_3O_3$ 366.1812; found 366.1809 $[M+H]^+$.



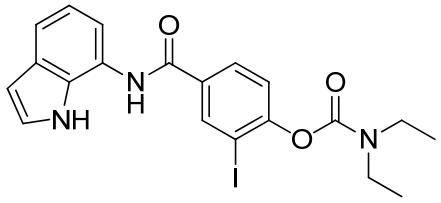
*Methyl 4-((diethylcarbamoyl)oxy)-3-iodobenzoate (**1063-39a**).* Compound **1063-39a** was prepared via Procedure I from methyl 4-hydroxy-3-iodobenzoate (1.0 g, 3.6 mmol) to give a white solid (1.24 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (d, *J* = 2.1 Hz, 1H), 8.02 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 3.91 (s, 3H), 3.53 (q, *J* = 7.5 Hz, 2H), 3.41 (q, *J* = 7.2 Hz, 2H), 1.33 (t, *J* = 6.9 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 155.6, 152.5, 140.8, 130.9, 128.7, 123.1, 90.5, 52.6, 42.6, 42.4, 14.6, 13.5; HRMS Calcd for $C_{13}H_{16}INO_4$ 378.0197; found 378.0198 $[M+H]^+$.



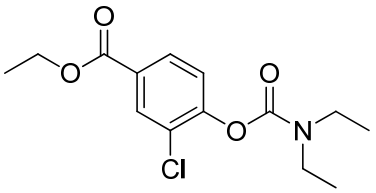
*4-((Diethylcarbamoyl)oxy)-3-iodobenzoic acid (**1063-39b**).* Compound **1063-39b** was prepared via Procedure II from **1063-39a** (0.95 g, 2.5 mmol). After concentrating *in vacuo*, the crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 2/1) to give a white solid (0.81 g, 88%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.33 (d, J = 1.2 Hz, 1H), 7.94 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 3.47 (q, J = 7.2 Hz, 2H), 3.32 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H), 1.14 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 155.1, 151.8, 139.7, 130.5, 129.5, 123.6, 92.0, 42.0, 41.8, 14.3, 13.2; HRMS Calcd for $C_{12}H_{14}INO_4$ 361.9895; found 361.9893 $[M-H]^+$.



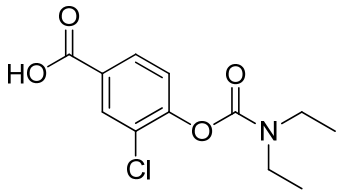
*2-Iodo-4-(naphthalen-1-ylcarbamoyl)phenyl diethylcarbamate (**1063-39**).* Compound **1063-39** was prepared via Procedure III from **1063-39b** (0.36 g, 0.99 mmol) and 1-naphthylamine (0.16 g, 1.1 mmol) to give the desired product (0.077 g, 16%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (br s, 1H), 8.41 (d, J = 2.1 Hz, 1H, 7.87-7.84 (m, 3H), 7.73 (d, J = 8.7 Hz, 2H), 7.52-7.43 (m, 3H), 7.22 (d, J = 8.4 Hz, 1H), 3.52 (q, J = 7.2 Hz, 2H), 3.34 (q, J = 6.9 Hz, 2H), 1.33 (t, J = 6.9 Hz, 3H), 1.14 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 154.5, 152.8, 138.8, 134.3, 133.3, 132.6, 128.7, 128.5, 128.4, 126.7, 126.4, 126.2, 125.7, 123.2, 122.6, 121.8, 91.1, 42.6, 42.3, 14.5, 13.3; HRMS (APCI) calcd for $C_{22}H_{21}IN_2O_3$ 489.06614; found 489.06633.



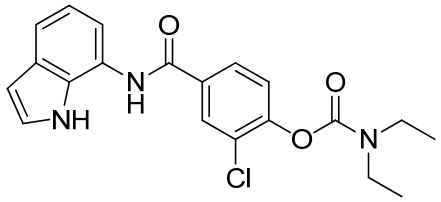
*4-((1H-Indol-7-yl)carbamoyl)-2-iodophenyl diethylcarbamate (**1063-40**).* Compound **1063-40** was prepared via Procedure III from **1063-39b** (0.34 g, 0.94 mmol) and 1*H*-indol-7-amine (0.14 g, 1.0 mmol, 1.1 equiv) to give a pale yellow solid (0.14 g, 32%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.81 (br s, 1H), 8.34-8.31 (mult, 2H), 7.82 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.27-7.25 (mult, 2H), 7.07 (t, J = 7.8 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.58 (t, J = 3.0 Hz, 1H), 3.57 (q, J = 7.2 Hz, 2H), 3.46 (q, J = 6.6 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H), 1.28 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 163.8, 154.6, 153.1, 138.8, 133.1, 130.8, 128.7, 125.2, 123.3, 122.5, 119.6, 118.6, 114.4, 102.8, 91.2, 42.7, 42.5, 14.6, 13.6; HRMS Calcd for $C_{20}H_{20}IN_3O_3$ 478.0622; found 478.0619 $[M+H]^+$.



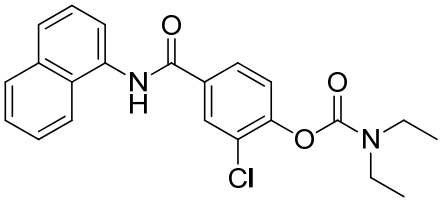
*Ethyl 3-chloro-4-((diethylcarbamoyl)oxy)benzoate (**1063-41a**).* Compound **1063-41a** was prepared via Procedure I from ethyl 3-chloro-4-hydroxybenzoate (1.0 g, 5.0 mmol) to give a white solid (1.1 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 2.0 Hz, 1H), 7.95 (dd, J_1 = 2.0 Hz, J_2 = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 4.37 (q, J = 6.8 Hz, 2H), 3.49 (q, J = 6.8 Hz, 2H), 3.40 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H), 1.30 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 152.6, 151.5, 131.6, 129.1, 128.7, 127.5, 124.1, 61.5, 42.7, 42.3, 14.6, 14.3, 13.4; HRMS Calcd for $C_{14}H_{18}ClNO_4$ 300.0997; found 300.1000 $[M+H]^+$.



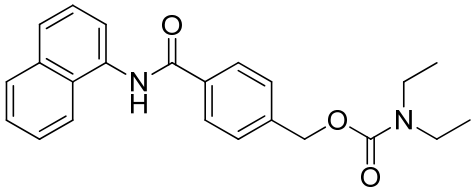
*3-Chloro-4-((diethylcarbamoyl)oxy)benzoic acid (**1063-41b**).* Compound **1063-41b** was prepared via Procedure II from **1063-41a** to give a white solid (0.78 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, J = 1.6 Hz, 1H), 7.91 (dd, J_1 = 2.0 Hz, J_2 = 8.4 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 3.44 (q, J = 6.4 Hz, 2H), 3.31 (q, J = 7.2 Hz, 2H), 1.24 (t, J = 6.8 Hz, 3H), 1.12 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 151.7, 150.7, 130.6, 129.4, 129.3, 126.6, 124.8, 42.0, 41.7, 14.1, 13.2; HRMS Calcd for $C_{12}H_{14}ClNO_4$ 270.0539; found 270.0538 $[M-H]^+$.



*4-((1H-Indol-7-yl)carbamoyl)-2-chlorophenyl diethylcarbamate (**1063-41**).* Compound **1063-41** was prepared via Procedure III from **1063-41b** (0.55 g, 2.0 mmol) and 1*H*-indol-7-amine (0.29 g, 2.2 mmol) to give a gray solid (0.78 g, >99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (br s, 1H), 8.31 (br s, 1H), 7.98 (d, J = 2.0 Hz, 1H), 7.77 (dd, J_1 = 1.6 Hz, J_2 = 8.8 Hz, 1H) 7.55 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.07 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.58 (d, J = 2.0 Hz, 1H), 3.53 (q, J = 6.8 Hz, 2H), 3.45 (q, J = 7.2 Hz, 2H), 1.34 (t, J = 6.8 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 153.1, 150.6, 132.7, 130.9, 129.8, 128.5, 128.1, 126.9, 125.3, 124.4, 122.5, 119.6, 118.7, 114.2, 102.8, 42.8, 42.5, 14.3, 13.5; HRMS Calcd for $C_{20}H_{20}ClN_3O_3$ 386.1266; found 386.1268 $[M+H]^+$.



*2-Chloro-4-(naphthalen-1-ylcarbamoyl)phenyl diethylcarbamate (**1063-42**).* Compound **1063-42** was prepared via Procedure III from **1063-41b** (0.24 g, 1.3 mmol) and 1-naphthylamine (0.20 g, 1.4 mmol) to give the desired product (0.21 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (br s, 1H), 8.00 (d, *J* = 1.8 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.75-7.68 (m, 2H), 7.59 (dd, *J* = 2.7 Hz, *J* = 6.6 Hz, 1H), 7.48-7.37 (m, 3H), 7.16 (dd, *J* = 1.8 Hz, *J* = 1.8 Hz, 1H), 3.45 (q, *J* = 6.9 Hz, 2H), 3.27 (q, *J* = 7.2 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.05 (t, *J* = 7.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 152.8, 150.2, 134.2, 132.7, 132.6, 129.8, 128.6, 128.4, 127.5, 126.8, 126.7, 126.2, 126.0, 125.5, 123.9, 123.0, 122.2, 42.5, 42.2, 14.1, 13.2; HRMS (APCI) calcd for $C_{22}H_{21}ClN_2O_3$ 397.13135; found 397.13105; Anal. ($C_{22}H_{21}ClN_2O_3$) C: 66.78, H: 5.26, N: 6.79.



*4-(Naphthalen-1-ylcarbamoyl)benzyl diethylcarbamate (**1063-43**).* Compound **1063-43** was prepared via Procedure III from **48** (0.26 g, 1.0 mmol) and 1-naphthylamine (0.16 g, 1.1 mmol) to give the desired product (0.19 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (br s, 1H), 7.95-7.86 (m, 5H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.49-7.38 (m, 5H), 5.16 (s, 2H), 3.32 (q, *J* = 6.8 Hz, 4H), 1.14 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CDCl3) δ 182.2, 174.2, 155.7, 141.2, 134.3, 132.7, 130.4, 128.8, 127.8, 127.7, 127.3, 126.4, 126.2, 125.8, 121.9, 121.3, 66.2, 42.1, 41.5, 14.3, 13.6; HRMS (APCI) calcd for $C_{23}H_{24}N_2O_3$ 377.18597; found 377.18577; Anal. ($C_{23}H_{24}N_2O_3$) C: 72.87, H: 6.60, N: 7.38.

1.7 BIOLOGY EXPERIMENTAL DATA

1.7.1 In vitro Analysis of 1063-Series Analogs (Dr. Stephen Traynelis)

All biological experiments were performed in the lab of Dr. Stephen Traynelis in the Department of Pharmacology at Emory University's School of Medicine.

Glutamate receptor expression in Xenopus laevis oocytes

All procedures involving the use of animals were reviewed and approved by Emory University Institutional Animal Care and Use Committee. cRNA was synthesized by *in vitro* transcription according to the manufacturer's specifications (Ambion). The cRNA quality was determined by gel electrophoresis. The quantity of the synthesized cRNA was estimated using both spectroscopy and gel electrophoresis. Stage IV and stage V oocytes were removed by surgical methods from the ovaries of healthy *Xenopus laevis* previously anesthetized with 3-amino-benzoic acid ethylester (1 gm/l) (see Traynelis et al. 1998). Oocyte clusters containing ~30 cells were alternatively incubated with 292 U/mL Worthington (Freehold, NJ) type IV collagenase or 1.3 mg/mL collagenase (Life Technologies, Gaithersburg, MD; 17018-029) for a period of 2 h in Ca^{2+}-free solution at pH = 7.5 with slow agitation to remove the follicular cell layer. The Ca^{2+}-free solution was further comprised of (in mM) 89 NaCl, 2.5 KCl, and 10 HEPES. Extensive washing of the oocytes was performed in the same solution supplemented with 1.8 mM $CaCl_2$. The oocytes were then maintained in Barth's solution consisting of (in mM) 88 NaCl, 1 KCl, 24 $NaHCO_3$, 10 HEPES, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, and 0.91 $CaCl_2$ and supplemented with 100 μg/mL gentamycin, 40 μg/mL streptomycin, and 50 μg/mL penicillin. Within 24 h of isolation, the oocytes were manually injected with 3-5 ng of GluN1-1a (hereinafter GluN1)

subunit and 5 ng of the desired GluN2 subunit in a 50 nL volume and incubated in Barth's solution at 15 °C for 1-7 days.

Voltage-clamp recordings from Xenopus laevis oocytes

Two-electrode voltage-clamp recordings were made from *Xenopus laevis* oocytes expressing recombinant GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, GluN1/GluN2D, GluA1, or GluK2 receptors 2-7 days post-injection of cRNA. Voltage-clamp recordings from oocytes were made during perfusion 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 (23 °C). Glass microelectrodes with a tip resistance of 0.3-1.0 MΩ were pulled from thin-walled glass capillary tubes using a PP-83 puller (Narashige). Current electrodes were filled with 3.0 M KCl and voltage electrodes were filled with 0.3 M KCl. The membrane potential was held at -40 mV for all recordings. Compounds were made as 20 mM stock solutions in DMSO, and diluted to the final concentration in recording solution containing 100 μM glutamate and 30 μM glycine. Final DMSO content was 0.05-0.5% (vol/vol). Oocytes expressing GluK2 receptors were pre-treated with 10 μM concanavalin-A for 10 minutes. NMDA receptors were activated by 100 μM glutamate and 30 μM glycine; AMPA and kainate receptors were activated by 100 μM glutamate. In order to prevent a gradual increase in current response over the course of the experiment, some oocytes expressing GluN1/GluN2A were either pretreated with 50 μM BAPTA-AM for 10 minutes or injected with 50 nl of 2 mM K-BAPTA.

Dose response curves were generated by applying a maximally effective concentration of glutamate and glycine, followed by application of glutamate and glycine containing variable concentrations of test compound up to 100 μM. The inhibitory response

evoked by test compounds was given as a percentage of the initial response to glutamate and glycine alone. This percentage is expressed as an average of recordings from oocytes obtained from a single frog.

The IC_{50} (half-maximally effective concentration of inhibitor) was determined by fitting the equation:

$$Response = (100 - minimum) / (1 + ([I] / IC_{50})^{N}) + minimum$$

to the average composite concentration-response data normalized to the current in the absence of inhibitor (100%) where *N* is the Hill slope, *[I]* is the inhibitor concentration, and *minimum* is the minimum response predicted for saturating concentrations of inhibitor. *Minimum* was restricted to greater than or equal to 0 for all fitted curves. 1-10 mM 2-hydroxypropyl-β-cyclodextrin was added to the recording solution for several analogs to ensure all compound remained in solution. No detectable effect on NMDA receptor response is observed for 2-hydroxypropyl-β-cyclodextrin alone (data not shown).

1.7.2 In vivo Analysis of 1063-Series Analogs for Pharmacokinetic Properties

Measurement of test compound concentrations in mouse plasma and brain following oral administration of 1063-2 was performed by Lundbeck. These experiments were performed on 3 groups of 2 C57BI/6 mice. Control animals received 20 mg/kg 100% PEG400, while test animals received 20 mg/kg 1063-2 dosed by intraperitoneal injection. Samples were collected at 0.5, 1, and 3 h after administration (6 mice per time point) from plasma and brain after being subject to isofluorane anesthesia. Isolation of the plasma from whole blood was achieved via centrifugation (approx. 1500 x g) for 10 min at 5 °C. LC-MS/MS was used to determine the concentration of 1063-2 present.

1063-2 concentrations in rat plasma and brain were measured following subcutaneous injection of 1063-2 by Lundbeck. 6 groups of 2 wistar rats were used. Control animals received 20 mg/kg 100% PEG400 and test animals received 20 mg/kg 1063-2 dosed by intraperitoneal injection. Following administration of isofluorane anesthesia, samples were collected at 0.5, 1, and 3 h after administration (12 rats per time point) from plasma and brain. Centrifugation (approx. 1500 x g) for 10 min at 5 °C was used to isolate the plasma from whole blood. The concentration of 1063-2 present in the sample was determined by LC-MS/MS.

REFERENCES

(1) Carter, C.; Benavides, J.; Legendre, P.; Vincent, J. D.; Noel, F.; Thuret, F.; Lloyd, K. G.; Arbilla, S.; Zivkovic, B. *Journal of Pharmacology and Experimental Therapeutics* **1988**, *247*, 1222.

(2) Williams, K. *Molecular Pharmacology* **1993**, *44*, 851.

(3) Acker, T. M.; Yuan, H.; Hansen, K. B.; Vance, K. M.; Ogden, K. K.; Jensen, H. S.; Burger, P. B.; Mullasseril, P.; Snyder, J. P.; Liotta, D. C.; Traynelis, S. F. *Molecular Pharmacology* **2011**, *80*, 782.

(4) Mosley, C. A.; Acker, T. M.; Hansen, K. B.; Mullasseril, P.; Andersen, K. T.; Le, P.; Vellano, K. M.; Bräuner-Osborne, H.; Liotta, D. C.; Traynelis, S. F. *Journal of Medicinal Chemistry* **2010**, *53*, 5476.

(5) Mullasseril, P.; Hansen, K.; Vance, K.; Ogden, K.; Yuan, H.; Kurtkaya, N.; Santangelo, R.; Orr, A.; Le, P.; Vellano, K.; Liotta, D.; Traynelis, S. *Nature Communications* **2010**.

(6) Costa, B. M.; Irvine, M. W.; Fang, G.; Eaves, R. J.; Mayo-Martin, M. B.; Skifter, D. A.; Jane, D. E.; Monaghan, D. T. *Journal of Pharmacology and Experimental Therapeutics* **2010**, *335*, 614.

(7) Bettini, E.; al., e. *J. Pharmacol. Exp. Ther.* **2010**, *335*, 636.

(8) Hansen, K. B.; Mullasseril, P.; Dawit, S.; Kurtkaya, N.; Yuan, H.; Vance, K.; Orr, A.; Kvist, T.; Ogden, K.; Le, P. *Journal of Pharmacology and Experimental Therapeutics* **2010**, *333*, 650.

(9) Danysz, W.; Parsons, C. *Neurotoxicity Research* **2002**, *4*, 119.

(10) Traynelis, S.; Wollmuth, L.; McBain, C.; Menniti, F.; Vance, K.; Ogden, K.; Hansen, K.; Yuan, H.; Myers, S.; Dingledine, R. *Pharmacological Reviews* **2010**, *62*, 405.

(11) Kew, J. N.; Kemp, J. A. *Psychopharmacology* **2005**, *179*, 4.

(12) Mayer, M. L.; Armstrong, N. *Annual Review of Physiology* **2004**, *66*, 161.

(13) Mori, H.; Mishina, M. *Neuropharmacology* **1995**, *34*, 1219.

(14) Chatterton, J. E.; Awobuluyl, M.; Premkumar, L. S.; Takahashi, H. *Nature* **2002**, *415*, 793.

(15) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacological Reviews* **1999**, *51*, 7.

(16) Javitt, D. C.; Zylberman, I.; Zukin, S. R.; Heresco-Levy, U.; Lindenmayer, J.-P. *The American Journal of Psychiatry* **1994**, *151*, 1234.

(17) Cull-Candy, S. G.; Leszkiewicz, D. N. *Science STKE* **2004**, *2004*, re16.

(18) Vicini, S.; Wang, J. F.; Li, J. H.; Zhu, W. J.; Wang, Y. H.; Luo, J. H.; Wolfe, B. B.; Grayson, D. R. *J Neurophysiology* **1998**, *72*, 555.

(19) Wyllie, D. J. A.; Behe, P.; Colquhoun, D. *J Physiology* **1998**, *510*, 1.

(20) Akazawa, C.; Shigemoto, R.; Bessho, Y.; Nakanishi, S.; Mizuno, N. *The Journal of Comparative Neurology* **1994**, *347*, 150.

(21) Monyer, H.; Burnashev, N.; Laurie, D. J.; Sakmann, B.; Seeburg, P. H. *Neuron* **1994**, *12*, 529.

(22) Benveniste, H.; Drejer, J.; Schousboe, A.; Diemer, N. H. *Journal of Neurochemistry* **1984**, *43*, 1369.

(23) Baethmann, A.; Maier-Hauff, K.; Schürer, L.; Lange, M.; Guggenbichler, C.; Vogt, W.; Jacob, K.; Kempski, O. *Journal of Neurosurgery* **1989**, *70*, 578.

(24) Choi, D. W.; Rothman, S. M. *Annual Review of Neuroscience* **1990**, *13*, 171.

(25) Nilsson, P.; Hillered; Ponten, U.; Ungerstedt, U. *J Cereb Blood Flow Metab* **1990**, *10*, 631.

(26) Beal, M. F. *The FASEB Journal* **1992**, *6*, 3338.

(27) Rodríguez-Ithurralde, D.; Olivera, S.; Vincent, O.; Maruri, A. *Journal of the Neurological Sciences* **1997**, *152, Supplement 1*, s54.

(28) Adachi, H.; Fujisawa, H.; Maekawa, T.; Yamashita, T.; Ito, H. *International Journal of Hyperthermia* **1995**, *11*, 587.

(29) Kostandy, B. *Neurological Sciences* **2012**, *33*, 223.

(30) Qureshi, A. I.; Ali, Z.; Suri, M.; Fareed, K.; Shuaib, A.; Baker, G.; Todd, K.; Guterman, L. R.; Hopkins, L. N. *Critical Care Medicine* **2003**, *31*, 1482.

(31) Persson, L.; Hillered, L. *Journal of Neurosurgery* **1992**, *76*, 72.

(32) Baker, A. J.; Moulton, R. J.; MacMillan, V. H.; Shedden, P. M. *Journal of Neurosurgery* **1993**, *79*, 369.

(33) Yamamoto, T.; Rossi, S.; Stiefel, M.; Doppenberg, E. M.; Zauner, A.; Bullock, R.; Marmarou, A. In *Neuromonitoring in Brain Injury, Proceedings of the International Conference on Neurochemical Monitoring in the Intensive Care Unit, 2nd*; Bullock, R., Ed. Williamsburg, VA, 1997, p 17.

(34) Srinivasan, R.; Sailasuta, N.; Hurd, R.; Nelson, S.; Pelletier, D. *Brain: a journal of neurology* **2005**, *128*, 1016.

(35) Wagner, A. K.; Fabio, A.; Puccio, A. M.; Hirschberg, R.; Li, W.; Zafonte, R. D.; Marion, D. W. *Critical Care Medicine* **2005**, *33*, 407.

(36) Majdi, M.; Chen, H.-S. V. *Journal of Receptor, Ligand and Channel Research* **2009**, *2*, 59.

(37) Kalia, L. V.; Kalia, S. K.; Salter, M. W. *The Lancet Neurology* **2008**, *7*, 742.

(38) Novelli, A.; Reilly, J. A.; Lysko, P. G.; Henneberry, R. C. *Brain Research* **1988**, *451*, 205.

(39) Mody, I.; MacDonald, J. F. *Trends in Pharmacological Sciences* **1995**, *16*, 356.

(40) Kaindl, A. M.; Degos, V.; Peineau, S.; Gouadon, E.; Chhor, V.; Loron, G.; Le Charpentier, T.; Josserand, J.; Ali, C.; Vivien, D. *Annals of Neurology* **2012**, *72*, 536.

(41) Wroge, C. M.; Hogins, J.; Eisenman, L.; Mennerick, S. *J Neuroscience* **2012**, *32*, 6732.

(42) Vemuganti, L.; Raghavendra, R.; Aclan, D.; Todd, K. G.; Bowen, K. L.; Dempsey, R. J. *Brain Research* **2001**, *911*, 96.

(43) Green, A. R. *Clinical and Experimental Pharmacology and Physiology* **2002**, *29*, 1030.

(44) Dirnagle, U.; Iadecola, C.; Moskowitz, M. A. *Trends in Neuroscience* **1999**, *22*, 391.

(45) Davis, S. M.; Albers, G. W. *Lancet* **1997**, *349*, 32.

(46) Lees, K. R. *Neurology* **1997**, *49*, S66.

(47) Grotta, J.; Clark, W.; Coull, B.; Pettigrew, L. C.; Mackay, B.; Goldstein, L. B.; Meissner, I.; Murphy, D.; LaRue, L. *Stroke* **1995**, *26*, 602.

(48) Bullock, M. R.; Merchant, R. E.; Carmack, C. A.; Doppenberg, E.; Shah, A. K.; Wilner, K. D.; Ko, G.; Williams, S. A. *Annals of the New York Academy of Sciences* **1999**, *890*, 51.

(49) Merchant, R. E.; Bullock, M. R.; Carmack, C. A.; Shah, A. K.; Wilner, K. D.; Ko, G.; Williams, S. A. *Annals of the New York Academy of Sciences* **1999**, *890*, 42.

(50) Morris, G. F.; Bullock, R.; Marshall, S. B.; Marmarou, A.; Maas, A.; Marshall, L. F. *J Neurosurgery* **1999**, *91*, 737.

(51) Muir, K. W. *Current Opinion in Pharmacology* **2006**, *6*, 53.

(52) Paoletti, P.; Neyton, J. *Current Opinion in Pharmacology* **2007**, *7*, 39.

(53) Milnerwood, A. J.; Raymond, L. A. *Trends in Neuroscience* **2010**, *33*, 513.

(54) Winblad, B.; Jones, R. W.; Wirth, Y.; Stoffler, A.; Mobius, H. J. *Dementia and Geriatric Cognitive Disorders* **2007**, *24*, 20.

(55) Parsons, C.; Danysz, W.; Quack, G. *Neuropharmacology* **1999**, *38*, 735.

(56) Reisberg, B.; Doody, R.; Stoffler, A.; Schmitt, F.; Ferris, S.; Mobius, H. J. *The New England Journal of Medicine* **2003**, *348*, 1333.

(57) Aarsland, D.; Ballard, C.; Walker, Z.; Bostrom, F.; Alves, G.; Kossakowski, K.; Leroi, I.; Pozo-Rodriguez, F.; Minthon, L.; Londos, E. *Lancet Neurology* **2009**, *8*, 613.

(58) Mobius, H. J.; Stoffler, A. *International Psychogeriatrics* **2003**, *15 Suppl 1*, 207.

(59) Lipton, S. A. *Current Alzheimer's Research* **2005**, *2*, 155.

(60) Lipton, S. A. *Nature Reviews* **2006**, *5*, 160.

(61) Gerber, A. M.; Vallano, M. L. *Mini-Reviews in Medicinal Chemistry* **2006**, *6*, 805.

(62) Ogden, K. K.; Traynelis, S. F. *Trends in Pharmacological Sciences* **2011**, *32*, 726.

(63) Santangelo, R. M.; Acker, T. M.; Zimmerman, S. S.; Katzman, B. M.; Strong, K. L.; Traynelis, S. F.; Liotta, D. C. *Expert Opinion on Therapeutic Patents* **2012**, *22*, 1337.

(64) Lipton, S. A. *Journal of Alzheimer's Disease* **2004**, *6*, S61.

(65) Kornhuber, J.; Weller, M. *Biological Psychiatry* **1997**, *41*, 135.

(66) Mion, G.; Villevielle, T. *CNS Neuroscience and Therapeutics* **2013**, *19*, 370.

(67) Lipton, S. A. *NeuroRx* **2004**, *1*, 101.

(68) Woodward, R. M.; Huettner, J. E.; Guastella, J.; Keana, J. F.; Weber, E. *Molecular Pharmacology* **1995**, *47*, 568.

(69) Petty, M. A.; Weintraub, P. M.; Maynard, K. I. *CNS Drug Reviews* **2004**, *10*, 337.

(70) Cai, S. X. *Curr. Top. Med. Chem.* **2006**, *6*, 651.

(71) Lehmann, J.; Hutchinson, A. J.; McPherson, S. E.; Mondadori, C.; Schmutz, M.; Sinton, C. M.; Tsai, C.; Murphy, D. E.; Steel, D. J.; Williams, M. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 65.

(72) Dawson, D. A.; Wadsworth, G.; Palmer, A. M. *Brain Research* **2001**, *892*, 344.

(73) Keyser, J. D.; Sulter, G.; Luiten, P. G. *Trends in Neuroscience* **1999**, *12*, 535.

(74) Mony, L.; Kew, J. N.; Gunthorpe, M. J.; Paoletti, P. *Br. J. Pharmacol.* **2009**, *157*, 1301.

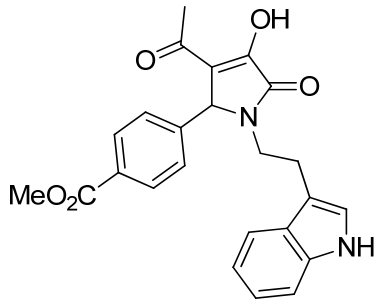
(75) Erreger, K.; Geballe, M. T.; Kristensen, A.; Chen, P. E.; Hansen, K. B.; Lee, C. J.; Yuan, H.; Le, P.; Lyuboslavsky, P. N.; Micale, N.; Jørgensen, L.; Clausen, R. P.; Wyllie, D. J. A.; Snyder, J. P.; Traynelis, S. F. *Molecular Pharmacology* **2007**, *72*, 907.

Chapter 2: Design, Synthesis, and Structure-Activity Relationship of Novel GluN1/GluN2C-Selective NMDA Receptor Positive Allosteric Modulators

2.1 STATEMENT OF PURPOSE

A series of adverse symptoms have been linked to hypofunction of NMDA receptor activity, including memory deficits and behavioral dysfunction. Excessive release of glutamate could reflect NMDA receptor hypofunction in inhibitory interneurons. The reduced inhibition could lead to overactivation of postsynaptic activity in principle neurons. This cascade is believed to influence cognitive and behavioral activity.[1] Indeed, glutamatergic hypofunction is implicated in the pathophysiology of schizophrenia.[2-6] The significant role of NMDA receptors in these cognitive and behavioral processes makes these receptors an important therapeutic target.

In an effort to identify small molecules capable of selectively modulating NMDA receptor function, a GluN1/GluN2C cell line and multi-well fluorescence-based assay were developed by the Traynelis laboratory. Two commercial diversity libraries were screened to identify potential lead compounds that modulate GluN2C-containing NMDA receptors by non-competitive mechanisms. Screening of these diverse compound libraries yielded a novel class of potentiators for recombinant GluN1/GluN2C NMDA receptors (1616 Series, Figure 1, **1616**).



1616

Figure 1. Screening hit identified in a GluN1/GluN2C screening effort

Compound **1616** demonstrated remarkable selectivity for GluN2C-containing receptors, with no activity observed at GluN2A-, GluN2B- or GluN2D-containing receptors. In collaboration with the Traynelis laboratory, we envisaged developing an extensive structure-activity relationship around this class of potentiators. Additionally, we sought to improve the on-target potency of this series. The goals of this project were accomplished using the following strategy:

1. Design and synthesis of novel 1616-series analogs with an emphasis on enhancing potency.

2. Determination of the structural determinants of activity of 1616 within the GluN1/GluN2C receptor.

3. Determination of preliminary pharmacokinetic properties of the 1616-series *in vivo*.

2.2 INTRODUCTION AND BACKGROUND

2.2.1 Subunit-Selective Modulators of NMDA Receptor Function

Several polyamines and neurosteroids were identified in the early 1990's as exogenous compounds capable of enhancing NMDA receptor function.[7-10] Spermine, for example, selectively potentiates GluN2B-containing receptors with minimal effect observed at GluN2A-, GluN2C-, and GluN2D-containing receptors.[11,12] Spermidine offers similar selectivity.[11] The neurosteroid pregnenolone sulfate demonstrates both potentiation at GluN2A- and GluN2B-containing NMDA receptors and minimal inhibition at GluN2C- and GluN2D-containing NMDA receptors.[13] In addition, aminoglycoside antibiotics have demonstrated selective potentiation of GluN1/GluN2B receptors.[14]

The first small molecule subunit-selective potentiator of NMDA function, typified by CIQ (**1**) , which contains a dihydroisoquinoline core, was recently discovered collaboratively by the Traynelis and Liotta groups (Figure 2). This class of compounds demonstrates remarkable selectivity for GluN2C- and GluN2D-containing receptors over all other receptor subtypes. Extensive SAR efforts were performed, resulting in analogs with potency in the low micromolar range.[15,16] A variety of structurally unrelated molecules were also reported by Costa et al. that demonstrated potentiation of NMDA receptors at concentrations above 30 μM. For example, UBP710 (**2**) was able to selectively potentiate GluN2A- and GluN2B-containing NMDA receptors.[17]

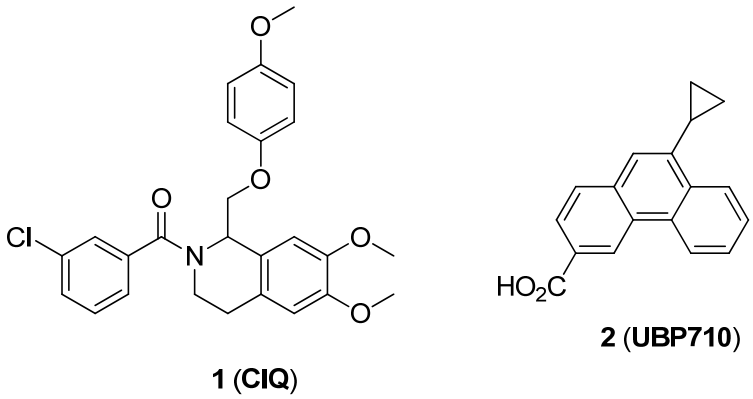


Figure 2. Subunit-selective potentiators of NMDA receptor function

In contrast to the limited literature on subunit-selective positive modulators of NMDA receptor function, a host of research has been documented on subunit-selective antagonists (see Chapter 1, *1.2.3* for subunit-selective antagonists). The discovery of GluN2B-selective compounds, including ifenprodil and related analogs, aided significantly in the understanding of the pharmacological role of this subunit in normal brain function and disease. Analogs selective for other subunits including GluN2A[18] and GluN2C/GluN2D[17,19-21] have also been reported by our lab and others.

Despite these advances, there remains a need for potent and selective modulators of NMDA function. The distinct anatomical locations and pharmacological properties of the GluN2 subunits (see Chapter 1, *1.2.1*) have led many to hypothesize that each subunit may contribute differently to the pathophysiology of various condition states. The lack of subunit-selective modulators has made this concept ever elusive, with little known about the precise biological function of each GluN2 subunit. Additionally, proof-of-concept studies to examine whether the actions of one subunit may be exploited for therapeutic gain, reducing side effects by avoiding all other subunits via selectivity, could also be explored. The identification of positive and negative modulators of NMDA activity selective for a single

subunit remains an important strategy for understanding the role of individual receptor subunits in normal brain function and development.

2.2.2 Therapeutic Rationale for GluN2C-Selective Agonists

NMDA receptor hypofunction is associated with a range of adverse symptoms, including cognitive impairments and psychotic symptoms, in animal and human studies.[1,4,22] An important consequence of blockade of NMDA function is excessive release of glutamate leading to overactivation of postsynaptic neurons. It is theorized that this cascade may contribute to the psychosis, cognitive impairments, and negative symptoms observed in a variety of neuropsychiatric disease states.[1,4]

An abundance of literature implicates glutamatergic hypofunction in the etiology and pathophysiology associated with schizophrenic patients.[2,3,6] More recently genetic linkage studies identified several genes for schizophrenia, including neuregulin-1, G72, RGS4, DTNBP1, PPP3CC, and GRIN1.[23-28] Despite serving a variety of different roles, most appear to function via a common pathway involving modulation of NMDA receptors.[6] For example, neuregulin-1, first identified as a susceptibility gene for schizophrenia in 2002,[23] regulates expression of GluN2C-containing NMDA receptors.[29,30]

Further analysis has demonstrated distinct expression patterns of mRNAs for individual NMDA receptor subtypes in the brains of schizophrenic patients.[30-33] These studies reveal a 53% increase in GluN1/GluN2D mRNA expression.[31] Additionally, a significant decrease in the expression of the GluN2C subunit was observed.[30,32,33] It is hypothesized that this deficiency leads to a decrease in overall glutamatergic transmission and reduced synaptic sprouting.[30,34]

2.2.3 Glutamate Hypofunction Hypothesis

A central discovery in 1980 revealed significantly decreased levels of glutamate in the cerebrospinal fluid of 20 schizophrenic patients.[35] Although future studies were unable to replicate this finding, a reduction in the concentration of γ-glutamylglutamine, an amino acid known to be involved in glutamate uptake and/or release, was observed. [36-38] In addition, despite not being significantly reduced, glutamate was found to be one of five substances (including γ-glutamylglutamine) required to distinguish between schizophrenic patients and controls.[39]

Additional evidence implicating glutamatergic dysfunction as a critical component of schizophrenia stems from evaluation of the post-mortem brains of individuals with schizophrenia. Frozen brain tissue was assayed for alterations in brain levels of several neuropeptides and amino acids including *N*-acetylaspartylglutamate (NAAG), aspartate and glutamate, with particular emphasis on the prefrontal cortex and hippocampus.[40] NAAG is a neuropeptide localized to subpopulations of glutamatergic neurons.[41] Although the precise mechanism is unclear, it is hypothesized that NAAG may act as an antagonist of NMDA receptor function.[42,43] Thus, excessive accumulation of NAAG may result in hypofunction of NMDA activity.[42] Data from post-mortem studies found increased levels of NAAG in the brains of schizophrenic patients compared to controls.[40]

The ability of the NMDA antagonist PCP to induce a psychotic state reminiscent of prominent symptoms of schizophrenia was originally documented in the 1950's.[44] It was not until decades later, however, that studies indicated a central role of NMDA receptors in the mechanism of action by which PCP disrupts normal brain function.[45,46] This finding

triggered the emergence of the glutamate hypofunction hypothesis, in which inhibition of NMDA function is believed to play a crucial role in the pathophysiology of schizophrenia.[47] Similar effects were later demonstrated by the NMDA receptor channel blocker, ketamine.[48] In an effort to better understand the etiology and pathophysiology of schizophrenia, animal models involving administration of PCP were developed. It is hoped that these models may provide additional insight into the neurobiological basis of schizophrenic brain functioning.[49]

Treatment of schizophrenia most commonly relies on D2 dopamine receptor antagonists. Unfortunately, nearly one third of patients fail to experience relief from the positive or negative symptoms associated with the disease.[50] A common strategy is to augment antipsychotic treatment with a drug known to target neurotransmission systems, in order to enhance the observed effect.[51] Specifically, the glutamatergic system is believed to be involved in the disease pathophysiology, with a substantial amount of data supporting this view.[24,52-58]

Mice mutated to diminish NMDA receptor glycine site occupancy were found to exhibit behavioral dysfunctions cognizant of many negative and cognitive symptoms of schizophrenia. These disturbances were reversed upon treatment with D-serine, a known NMDA receptor agonist at the glycine site.[59] Agonists of NMDA activity have also demonstrated beneficial effects in schizophrenic patients when administered in combination with antipsychotic medication. Activation of the glycine site at GluN1 resulted in a reduction of negative symptoms,[3] whereas the NMDA agonist D-serine offered significant improvements in positive, negative, and cognitive symptoms of the disease.[2]

2.2.4 Enhancement of Learning and Memory

NMDA receptors are known to play an important role in cognitive tasks, including learning and memory acquisition.[60,61] Furthermore, studies involving CA1-specific NMDA receptor knockout mice have indicated that NMDA enrichment can rescue memory deficits.[62,63] Transgenic mice with over-expression of GluN2B-containing receptors demonstrate superior learning and memory in behavioral tasks.[64,65] More recently, a study of GluN2C knockout mice revealed impaired working memory in knockout mice compared to controls.[66] This data suggest that potentiation of NMDA receptor function may enhance learning and memory in subjects, as well.

Several studies of NMDA receptor antagonists in humans have demonstrated impaired memory processes. The competitive antagonist SDZ EAA 494 led to significant impairment of both verbal and non-verbal memory test performance in healthy subjects. Spatial memory test performance remained mostly unaffected.[67] Administration of ketamine, a NMDA receptor channel blocker, in a double-blind, placebo-controlled study of healthy volunteers revealed deficits in episodic and working memory. Impairments in recognition memory and procedural learning were also observed.[68] Taken together these data are consistent with the idea that NMDA receptors represent a potential target for improving cognitive processes such as learning and memory.

2.2.5 Extinction of Fear

Generalized anxiety disorder (GAD) is an extremely common condition that remains largely undiagnosed.[69,70] Current treatment typically involves administration of selective serotonin reuptake inhibitors (SSRIs) or benzodiazepines. Despite being frequently

prescribed, benzodiazepines suffer from adverse events such as tolerance and withdrawal and are thus recommended only for short-term treatment.[71] There are also various psychosocial approaches involving prolonged exposure to the feared stimulus. While both of these techniques are independently beneficial, combination treatment methods have failed to offer additional improvements in efficacy.[72,73]

A relationship between exposure to a traumatic event and the development of anxiety disorders including post-traumatic stress disorder (PTSD) has been hypothesized.[74,75] This would suggest an associative learning mechanism similar to that observed in Pavlovian fear conditioning. If circuitry and receptor expression remain conserved between humans and the animal model, psychotherapeutic approaches may translate from animal models involving experimentally-induced extinction of fear. In addition, the combination of pharmacological agents which enhance learning and exposure therapy could potentially serve as effective treatments for anxiety disorders. The enhancement of learning could essentially aid in "unlearning" of the negative association.[76,77]

The role of NMDA receptors in some forms of learning and memory is well documented.[60,61] Several studies have coupled modulation of the NMDA receptor with psychotherapy. For example, the NMDA partial agonist D-cycloserine (DCS) has been shown to improve extinction of fear in human patients.[78,79] DCS is a partial agonist at GluN2A-, GluN2B-, and GluN2D-containing receptors. Binding of DCS to GluN2C leads to more current than is observed with saturating glycine concentrations.[80,81] DCS has also demonstrated beneficial effects towards the treatment of a variety of anxiety-related disorders including social anxiety disorder,[82] obsessive-compulsive disorder,[83] and phobias.[78] A recent study reported by Ogden et al. demonstrated that small molecule potentiators selective for GluN2C- and GluN2D- containing NMDA receptors can increase fear

extinction and enhance fear acquisition in mice.[84] Additionally, deficits in fear acquisition were observed in a behavioral study of GluN2C knockout mice.[66] Taken together, this evidence indicates that potentiation of GluN2C-containing receptors may offer a potential treatment path for anxiety-related disorders.

Inhibition of NMDA receptors has been shown to block acquisition,[85] consolidation,[86] reconsolidation,[87] and extinction[88] of fearful memories in animal models based on Pavlovian fear conditioning. Pavlovian fear conditioning is a form of learning involving exposure to an unconditioned aversive stimulus (US) paired with a neutral conditioned stimulus (CS) in order to evoke a learned fear response to the originally neutral stimulus. Thus, exposure of an animal to a naturally aversive stimulus (i.e. a shock) paired with a CS (i.e. a light or tone) causes the animal to associate the two stimuli. Eventually, exposure to the CS alone will result in behaviors indicative of a fearful response in the form of elevated blood pressure, tachycardia, or potentiated startle response. The learned fear response may, in turn, be extinguished by repeated exposure to the CS alone.

Despite being highly studied, the extinction of fear in Pavlovian conditioning is still not fully understood. Experimental extinction refers to the presentation of the conditioned stimulus in the absence of the unconditioned stimulus with which it was previously paired, resulting in a decrease or loss of the conditioned response.[89] It was originally suggested that this disassociation arises from an active form of unlearning.[90] Later research, however, appears to indicate a more direct role for the conditioned stimulus in the extinction process. Specifically, extinction may arise from depreciation of the conditioned stimulus,[91] and learning of a new, competing association.[89] These data support the observation that the fear response may be reinstated following extinction.[92] While the precise mechanisms underlying the extinction of fear are still not fully understood, it is clear that cognitive processes such as

learning and memory are directly involved. Having been implicated in both fear extinction and fear conditioning, the NMDA receptor may offer a novel target for further examination of the fear response and treatment of anxiety disorders associated with such.

2.2.6 Biological Screening Hit and Rationale for 1616-Series Analogs

A GluN1/GluN2C cell line was developed and used to optimize a multi-well fluorescence-based assay for NMDA receptor modulators. Two commercial diversity libraries were screened to identify potential lead compounds that modulate GluN2C-containing NMDA receptors by non-competitive mechanisms.[93] Screening of these compound libraries yielded a novel class of potentiators typified by compound **1616** selective for recombinant GluN1/GluN2C NMDA receptors (Figure 3). Structural modifications to **1616** were designed to develop an understanding of the SAR for this class of molecules.

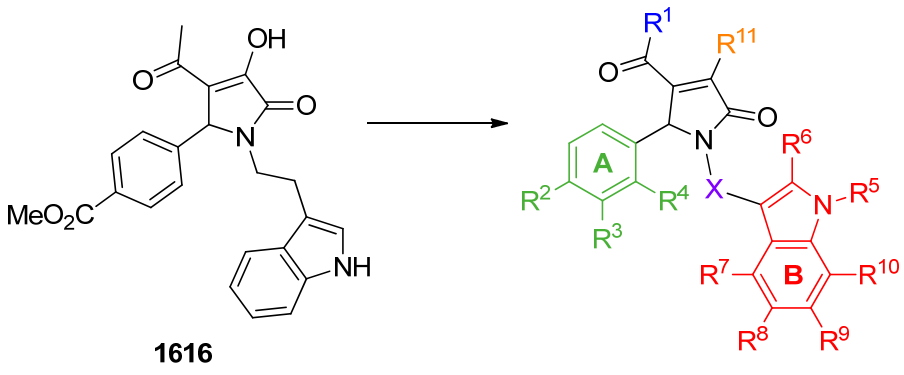


Figure 3. Initial screening hit (1616) and generic structure for SAR development

Compound **1616**, which contains a pyrrolidinone core motif, potentiated GluN1/GluN2C responses with a potentiation of 237 ± 8.2 % of control at 100 μM with an EC_{50} of 23 ± 2.4 μM (n = 12) (Figure 4). Increasing the concentration of compound **1616** to

100 μM did not reveal any potentiating actions at GluN1/GluN2A, GluN1/GluN2B, or GluN1/GluN2D (n = 11-15). In addition, 30 μM of compound **1616** had no effect on homomeric recombinant GluA1 AMPA receptor responses (87 ± 1.5% control, n = 12) or homomeric GluK2 recombinant kainate receptors (93 ± 2.5% at 120 μM control, n = 5) expressed in *Xenopus laevis* oocytes (data not shown).

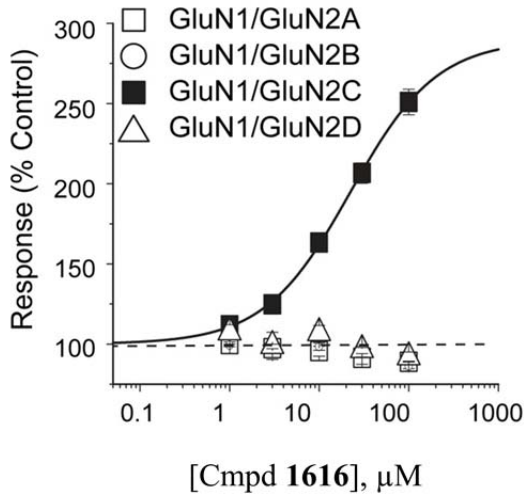


Figure 4. Concentration-effect curves of 1616 at GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, and GluN1/GluN2D

A series of modifications at R^1 were envisioned to explore the available space within the binding pocket. Specifically, analogs containing additional steric bulk (e.g., Et, *i*Pr, *t*Bu, Ph) would be prepared (Figure 5). The introduction of heteroaromatic systems would be pursued if large substituents are well tolerated.

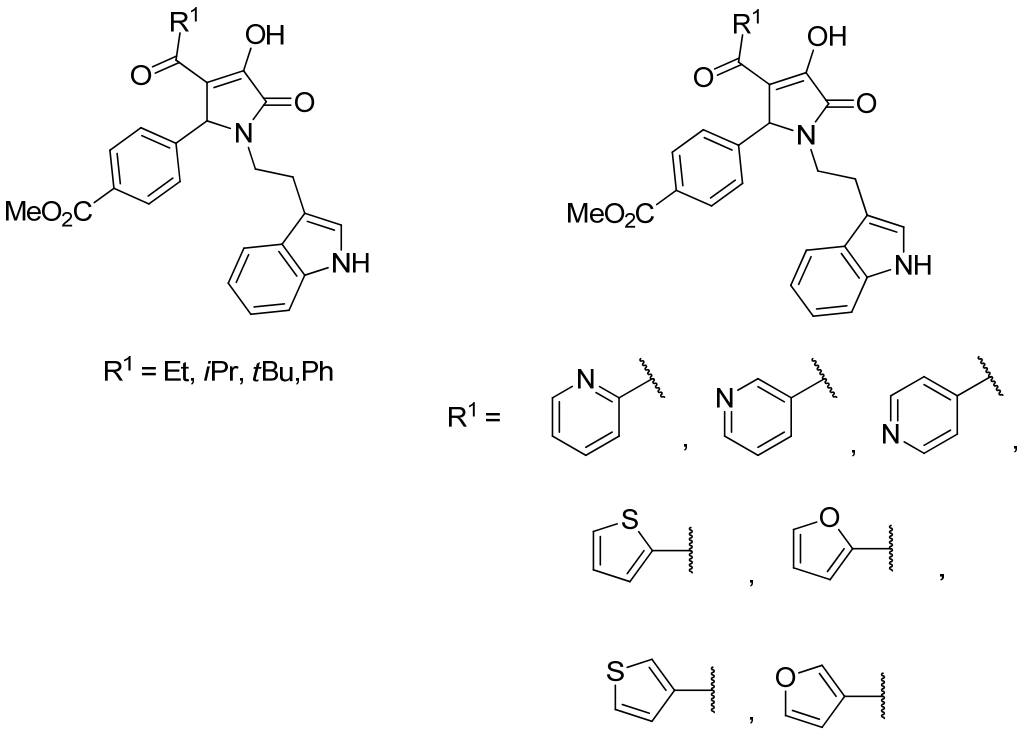
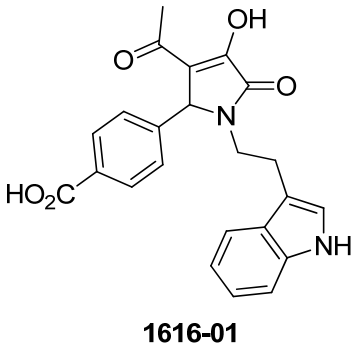


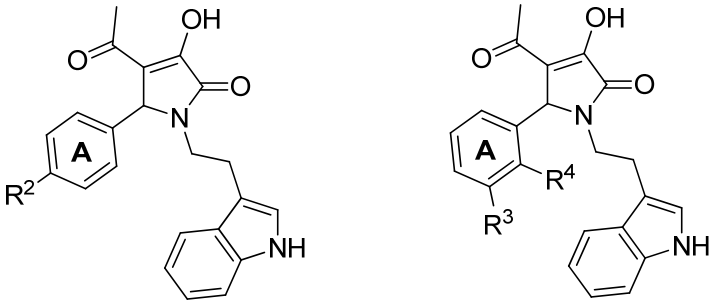
Figure 5. Proposed R^1 modifications

We were concerned that the methyl ester may act as a metabolic liability, due to cleavage *in vivo*. Initial efforts demonstrated that conversion to a carboxylic acid, as in **1616-01**, led to inactivity at all subunits tested (Figure 6, data not shown). A series of analogs were envisioned in which the ester was replaced with a variety of isosteres (Figure 7). Positional isomers and analogs in which bulkier esters were present would also be explored.



1616-01

Figure 6. Benzoic acid 1616-01, prepared by Dr. Ethel Garnier

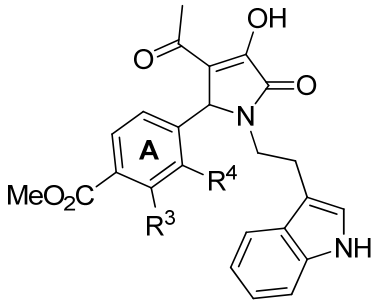


R^2 = CO_2Et, CO_2iPr, CO_2tBu
or
R^2 = NO_2, CN, $CONH_2$,
CONHMe, $CONMe_2$, SO_2H, SO_2Me

R^3 = CO_2Me, R^4 = H
R^3 = H, R^4 = CO_2Me

Figure 7. Proposed ester and isostere analogs

We envisioned making modifications to the A-ring to probe electronic and steric effects (Figure 8). Specifically, we were interested in holding the *para*-methyl ester constant while placing an additional substituent on the ring (e.g., OH, OMe, Me, Cl, F). Additional analogs in which the A-ring is replaced with a heteroaromatic ring were envisaged to probe the structural determinants of activity within the binding pocket (Figure 9).



R^3 = H, R^4 = OH, OMe, Me, Cl, F
R^3 = OH, OMe, Me, Cl, F, R^4 = H

Figure 8. Proposed A-ring substitutions

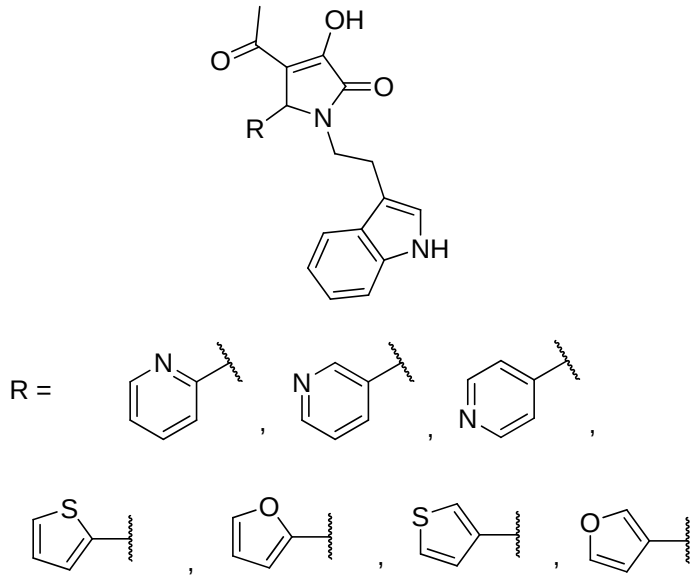


Figure 9. Proposed heteroaryl A-ring replacements

We were interested in exploring the role of the B-ring in potency and selectivity. This included replacement of the B-ring with a number of aryl and heteroaryl groups (Figure 10). Steric and electronic properties of the indole would be probed via ring substitution (Figure 11).

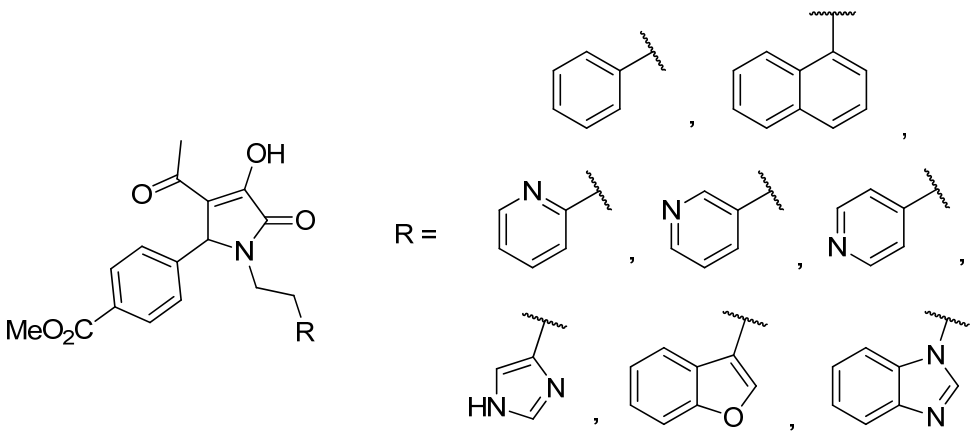


Figure 10. Proposed B-ring replacement analogs

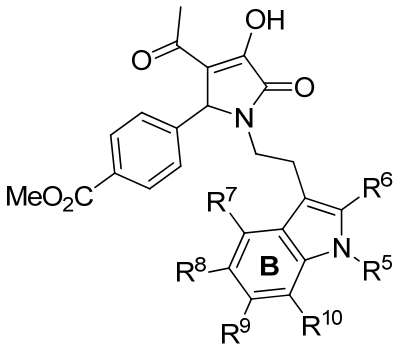
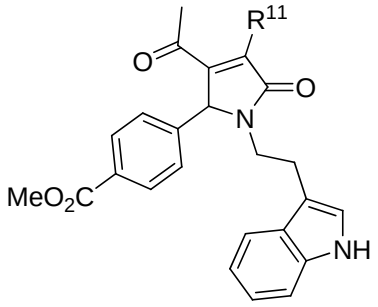


Figure 11. Proposed analogs containing substituted indoles

Modifications at R^{11} were envisaged to determine the significance of the enol in controlling potency and selectivity (Figure 12). In particular, this would be explored by replacement of the hydroxyl (e.g., with an amine) or protection of the alcohol (e.g., OMe, OAc).



R^{11} = NH_2, OMe, OAc

Figure 12. Proposed modifications at R^{11}

We also envisioned analysis of the linker (Figure 13). Specifically, we wanted to probe multiple conformations of the indole. This would be accomplished by rigidification, or shortening, of the linker; or alternatively, by allowing for additional flexibility (e.g., lengthening) of the linker.

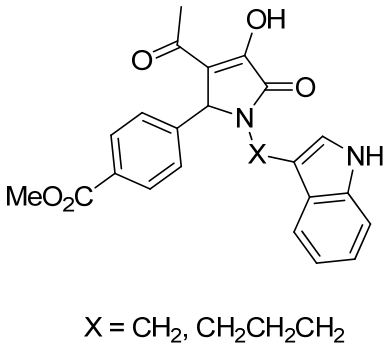
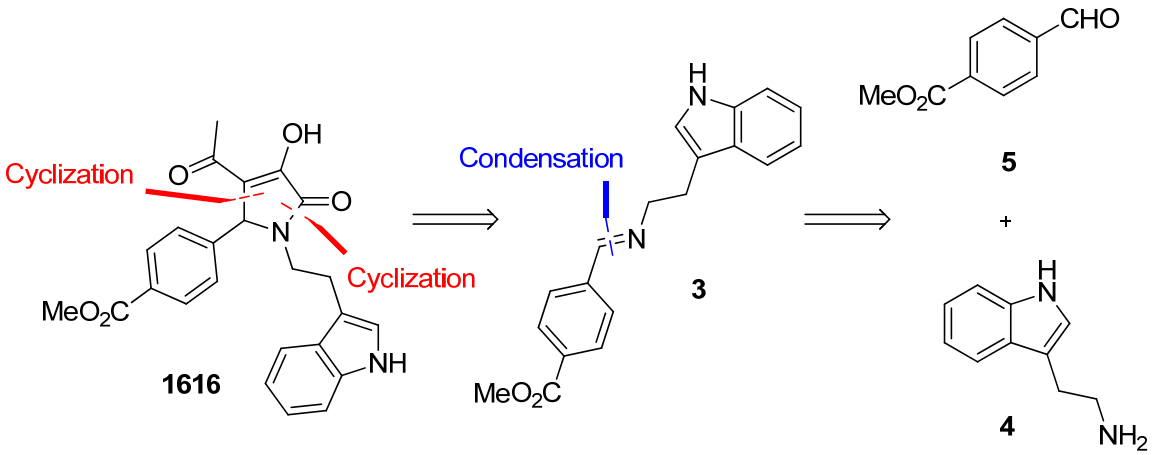


Figure 13. Proposed linker modifications

2.3 SYNTHESIS OF 1616-SERIES ANALOGS

2.3.1 Synthesis of Pyrrolidinone Analogs

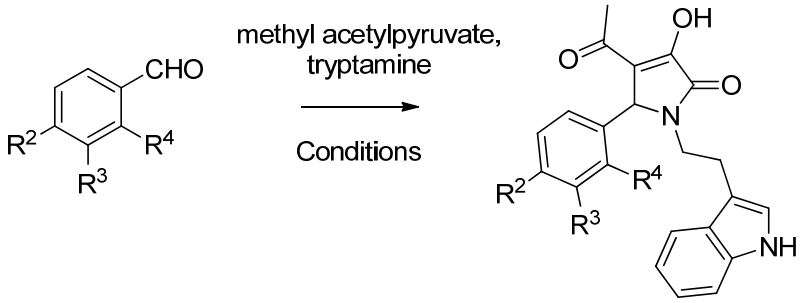
The retrosynthetic analysis of the 1616-series is illustrated in Scheme 1. It was envisioned that 1616 analogs could be formed via a one-pot Biginelli-like reaction. The pyrrolidinone core would arise from cyclization of the corresponding pyruvate onto imine (**3**), which could be generated by condensation of tryptamine (**4**) onto the appropriate benzaldehyde (**5**). Pyruvate, benzaldehyde, and amine derivatives with varying substitution were synthesized as needed (see Chapter 2, *2.3.2, 2.3.3,* and *2.3.4* for synthesis).



Scheme 1. Retrosynthetic analysis of 1616-series using Biginelli-like reaction

Thermal conditions were explored based on literature precedent[94] using a variety of benzaldehyde derivatives to access the pyrrolidinone core. Refluxing in dioxane appeared to be extremely substrate dependent. In most cases, only starting material was able to be isolated (Table 1).

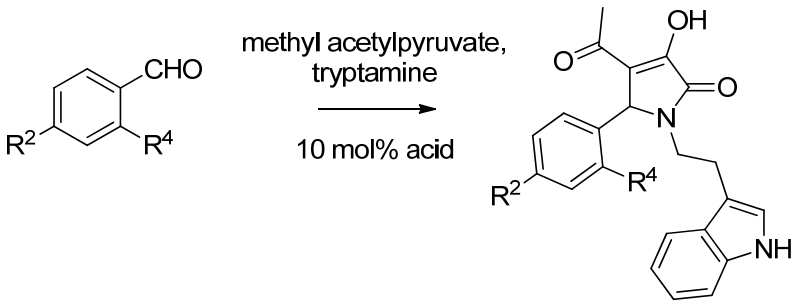
Table 1. Heat catalyzed Biginelli-like conditions attempted



Starting Material ID	R^2	R^3	R^4	Temperature (°C)	Time (h)	Yield (%)	Product ID
7	CO_2t-Bu	H	H	100	2	--	**1616-03**
8	H	CO_2Me	H	100	0.4	60	**1616-02**
9	H	H	CO_2Me	100	2	--	**1616-05**

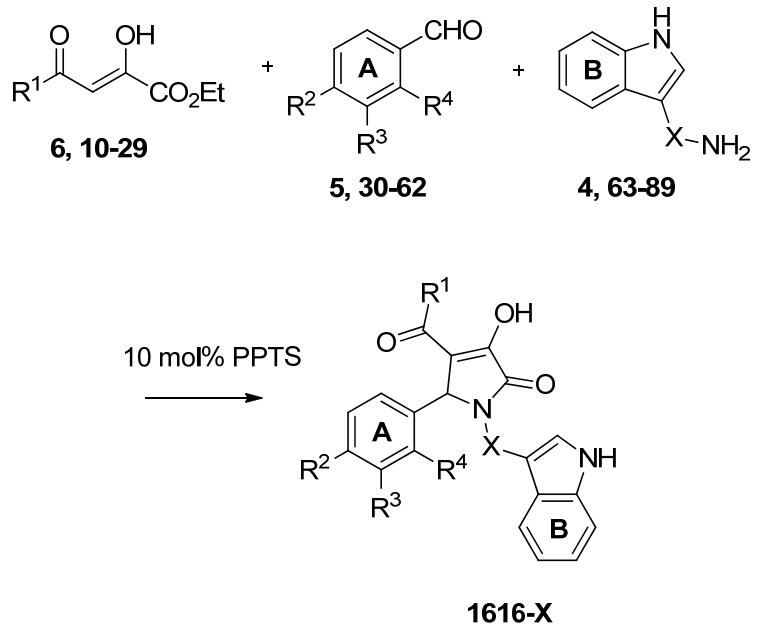
Standard Biginelli reaction conditions employ an acid catalyst. A series of acids were subsequently screened for their ability to effectively catalyze formation of the pyrrolidinone core (Table 2). Treatment of benzaldehyde **5** or **9** with 10 mol% pyridinium *p*-toluenesulfonic acid (PPTS) afforded the desired product in good yield.

Table 2. Acid catalyzed Biginelli-like conditions attempted



Starting Material ID	R^2	R^4	Acid	Time	Yield (%)	Product ID
9	H	CO_2Me	PTSA	< 1 min.	65	**1616-05**
5	CO_2Me	H	PTSA	12 h	--	**1616**
9	H	CO_2Me	PPTS	< 1 min.	61	**1616-05**
5	CO_2Me	H	PPTS	5 min.	72	**1616**

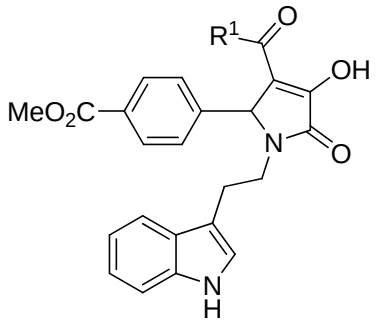
Thus, reaction of the appropriate pyruvate, benzaldehyde and amine derivative in the presence of 10 mol% PPTS afforded the desired pyrrolidinone analogs (Scheme 2).



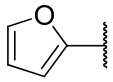
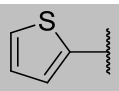
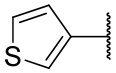
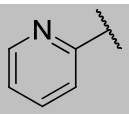
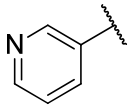
Scheme 2. Synthetic route to access pyrrolidinone analogs

All of the pyrrolidinone analogs prepared as described above are detailed in Tables 3-10.

Table 3. Summary of pyrrolidinone analogs with modifications at R^1



Compound ID	R^1
1616-29	Et
1616-32	*i*-Pr

Compound ID	R^1
1616-33	*t*-Bu
1616-46	Ph
1616-62	*m*-OH-Ph
1616-34	*m*-OMe-Ph
1616-38	*m*-Me-Ph
1616-36	*m*-Cl-Ph
1616-35	*m*-F-Ph
1616-40	*o*-OMe-Ph
1616-39	*o*-Me-Ph
1616-42	*o*-Cl-Ph
1616-41	*o*-F-Ph
1616-27	
1616-37	
1616-54	
1616-30	
1616-28	

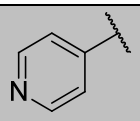
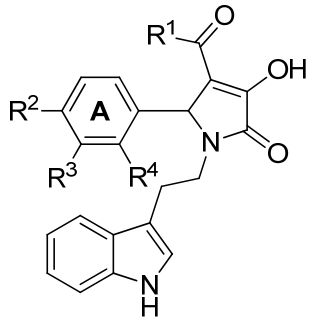
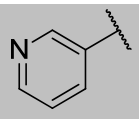
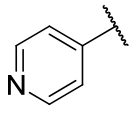
Compound ID	R^1
1616-50	

Table 4. Summary of pyrrolidinone analogs with A-ring substitutions



Compound ID	R^1	R^2	R^3	R^4
1616-02	Me	H	CO_2Me	H
1616-05	Me	H	H	CO_2Me
1616-16	Me	CO_2Et	H	H
1616-44	Me	CO_2Et	H	OH
1616-48		CO_2Et	H	H
1616-51		CO_2Et	H	H
1616-15	Me	CO_2iPr	H	H
1616-03	Me	CO_2tBu	H	H

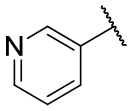
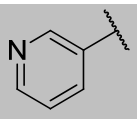
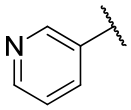
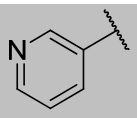
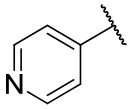
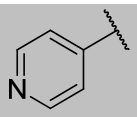
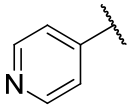
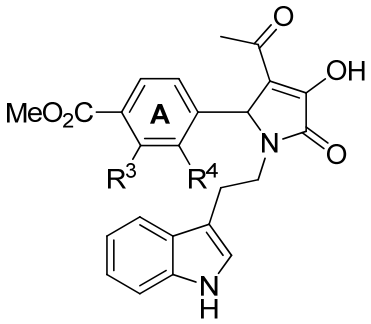
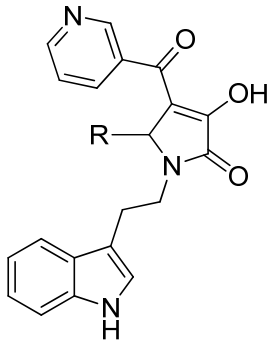
Compound ID	R^1	R^2	R^3	R^4
1616-08	Me	NO_2	H	H
1616-28		CN	H	H
1616-72		CF_3	H	H
1616-90		SO_2NH_2	H	H
1616-97		SO_2NHMe	H	H
1616-60		$C(O)NH_2$	H	H
1616-52		C(O)NHMe	H	H
1616-53		$C(O)NMe_2$	H	H

Table 5. Summary of pyrrolidinone analogs containing modifications at A-ring positions R^3 and R^4



Compound ID	R^3	R^4
1616-07	OH	H
1616-11	OMe	H
1616-23	Me	H
1616-24	Cl	H
1616-25	F	H
1616-12	H	OH
1616-04	H	OMe
1616-18	H	Me
1616-17	H	Cl
1616-26	H	F

Table 6. Summary of pyrrolidinone analogs containing A-ring replacements



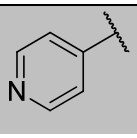
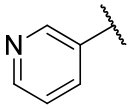
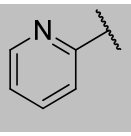
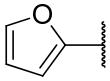
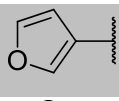
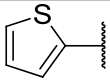
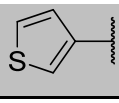
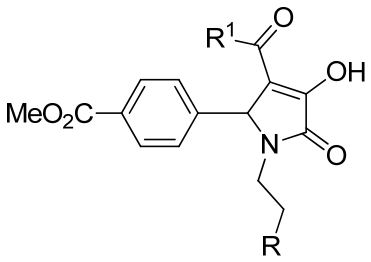
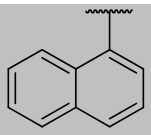
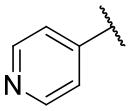
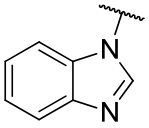
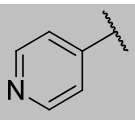
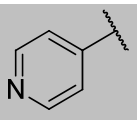
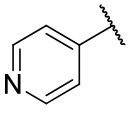
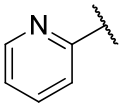
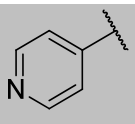
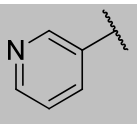
Compound ID	R
1616-73	
1616-74	
1616-75	
1616-82	
1616-76	
1616-81	
1616-83	

Table 7. Summary of pyrrolidinone analogs containing B-ring replacements



Compound ID	R^1	R
1616-66	Me	Ph
1616-67	Me	*m*-Me-Ph
1616-68	Me	*m*-Cl-Ph
1616-69	Me	*m*-F-Ph
1616-70	Me	*m*-OMe
1616-71	Me	*m*-OH
1616-63	Me	
1616-56		
1616-57		
1616-58		
1616-59		

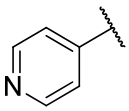
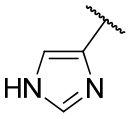
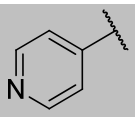
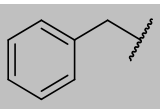
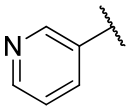
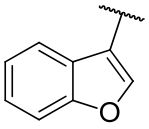
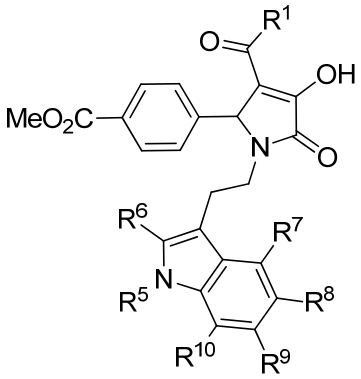
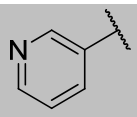
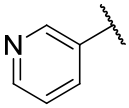
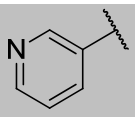
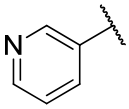
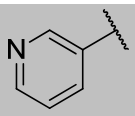
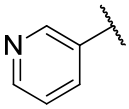
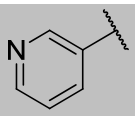
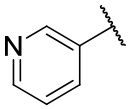
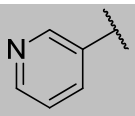
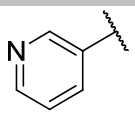
Compound ID	R^1	R
1616-64		
1616-88		
1616-93		

Table 8. Summary of pyrrolidinone analogs containing B-ring substitutions



Compound ID	R^1	R^5	R^6	R^7	R^8	R^9	R^{10}
1616-65	Me	Boc	H	H	H	H	H
1616-79	Me	Me	H	H	H	H	H
1616-47		Me	H	H	H	H	H

Compound ID	R^1	R^5	R^6	R^7	R^8	R^9	R^{10}
1616-19		H	Me	H	H	H	H
1616-86		H	H	Me	H	H	H
1616-77		H	H	H	OMe	H	H
1616-89		H	H	H	H	OMe	H
1616-85		H	H	H	H	Me	H
1616-80		H	H	H	H	Cl	H
1616-84		H	H	H	H	F	H
1616-78		H	H	H	H	H	OMe
1616-13		H	H	H	H	H	Me

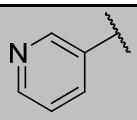
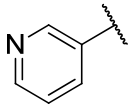
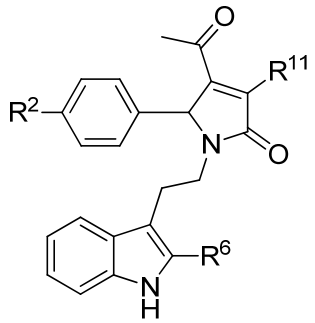
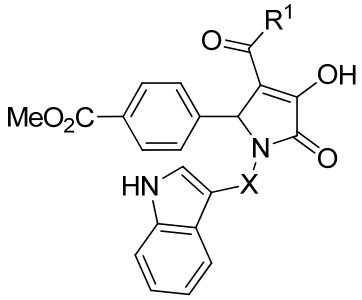
Compound ID	R^1	R^5	R^6	R^7	R^8	R^9	R^{10}
1616-91		H	H	H	H	H	Cl
1616-94		H	H	H	H	H	F

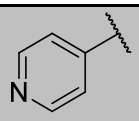
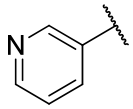
Table 9. Summary of pyrrolidinone analogs containing modifications at R^{11}



Compound ID	R^2	R^6	R^{11}
1616-43	CO_2Et	Me	OH
1616-14	CO_2Me	H	OMe
1616-20	CO_2Me	H	OC(O)Me
1616-21	CO_2Me	H	NH_2
1616-95	CO_2Me	Me	$OC(O)CH_2CH_2CH_3$
1616-96	CO_2Me	Me	$OC(O)CH=CH_2$

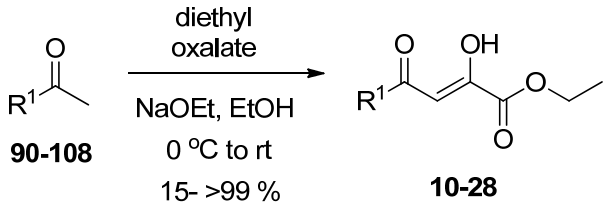
Table 10. Summary of pyrrolidinone analogs containing modified linkers



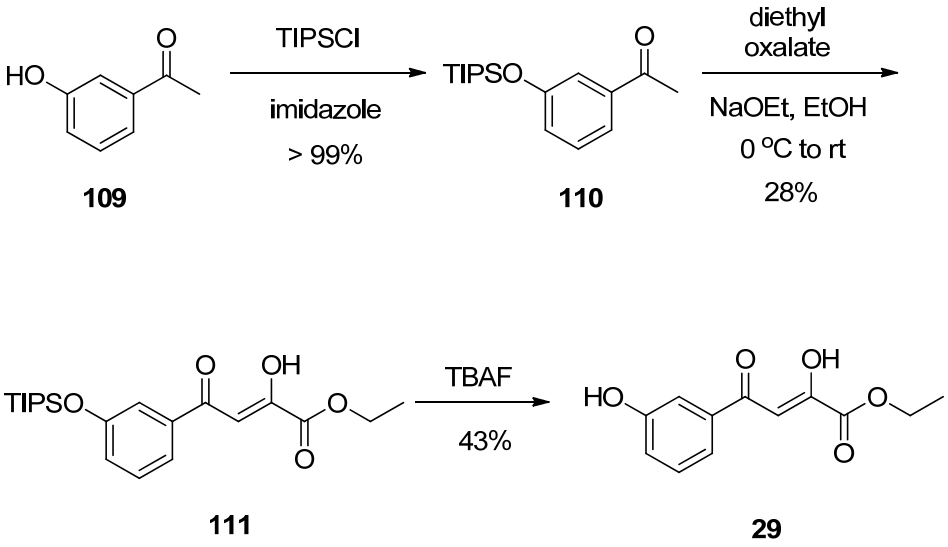
Compound ID	R^1	X
1616-55		$-CH_2-$
1616-87		$-CH_2CH_2CH_2-$

2.3.2 Synthesis of Pyruvate Analogs

Pyruvate derivatives were accessed starting from the methyl ketone. Addition of diethyl oxalate and sodium ethoxide to a methyl ketone generated a series of pyruvate analogs containing modifications at R_1 (Scheme 3). In the case of *ortho*-hydroxy analog **28**, it was necessary to first protect the hydroxyl with triisopropyl chloride (TIPSCl) before the addition of diethyl oxalate (Scheme 4). Standard deprotection afforded the target pyruvate **29**. All of the methyl ketone substrates and pyruvate derivatives generalized by Schemes 3 and 4 are detailed in Table 11.

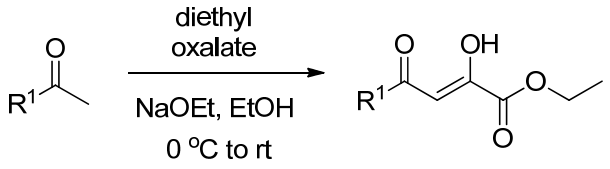


Scheme 3. Generalized synthesis of pyruvate derivatives

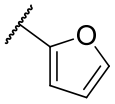
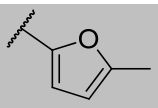
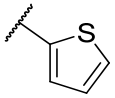
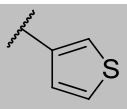
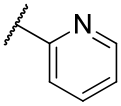
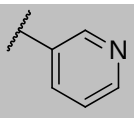
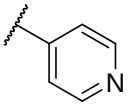


Scheme 4. Synthesis of pyruvate analog 29

Table 11. Summary of pyruvate analogs

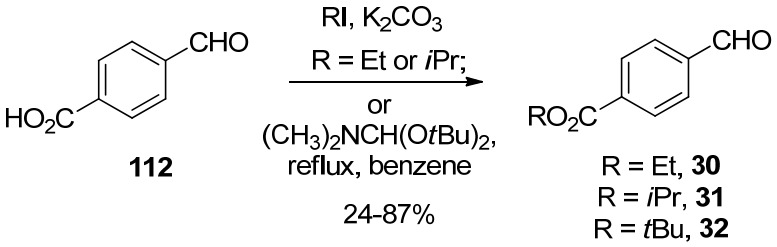


Starting Material ID	R^1	Product ID
10	Et	**1616-29a**
11	*i*-Pr	**1616-32a**
12	*t*-Bu	**1616-33a**
13	Ph	**1616-46a**

Starting Material ID	R^1	Product ID
29	*m*-OH-Ph	**1616-40a**
14	*m*-OMe-Ph	**1616-39a**
15	*m*-Me-Ph	**1616-42a**
16	*m*-Cl-Ph	**1616-41a**
17	*m*-F-Ph	**1616-62c**
18	*o*-OMe-Ph	**1616-34a**
19	*o*-Me-Ph	**1616-38a**
20	*o*-Cl-Ph	**1616-36a**
21	*o*-F-Ph	**1616-35a**
22		**1616-27a**
23		**1616-31a**
24		**1616-37a**
25		**1616-54a**
26		**1616-30a**
27		**1616-28a**
28		**1616-50**

2.3.3 Synthesis of Benzaldehyde Analogs

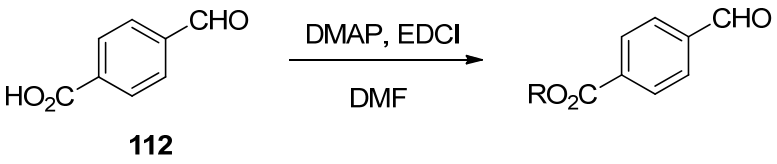
Starting from benzoic acid **112**, standard alkylation conditions afforded the ethyl and isopropyl esters (Scheme 5). In contrast, *tert*-butyl ester **32** was accessed using *N,N*-dimethylformamide di-*tert*-butyl acetal.



Scheme 5. Synthesis of *para*-ester benzaldehydes 30-32

In order to synthesize amide isosteres, standard amide coupling conditions were explored (Table 12). Treatment of benzoic acid **112** with DMAP, EDCI, and the appropriate amine afforded secondary amide **34** and tertiary amide **35**. Only starting material was isolated in an attempt to generate primary amide **33** under similar conditions.

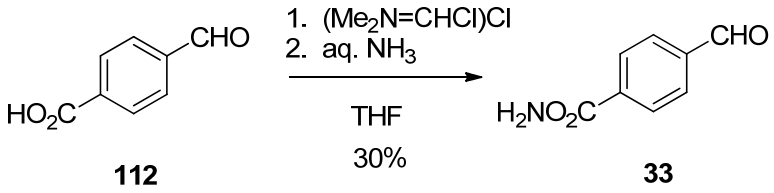
Table 12. Amide coupling conditions



R	Amine	Yield	Product ID
NH_2	NH_3	--	**33**
NHMe	$MeNH_2$	14	**34**

R	Amine	Yield	Product ID
NMe_2	Me_2NH	41	**35**

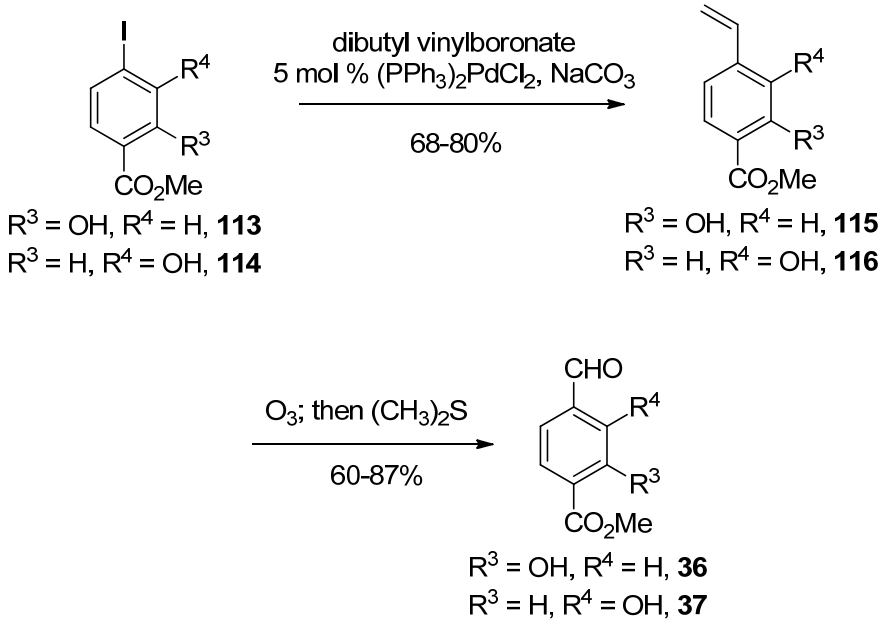
As an alternative route to the desired primary amide, the carboxylic acid was first converted to the more reactive acid chloride (Scheme 6). Thus, addition of the Vilsmeier reagent to acid **112**, followed by reaction with aqueous ammonia, afforded primary amide **33** in a one pot procedure.



112 **33**

Scheme 6. Synthesis of primary amide 33

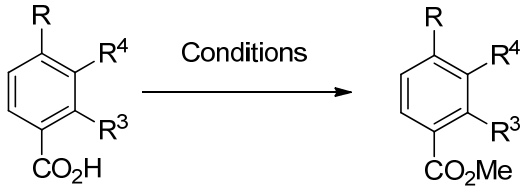
Di-substituted benzaldehyde analogs were synthesized using several different procedures. Phenols **36** and **37** were prepared via Suzuki coupling of dibutyl vinylboronate and the appropriately substituted methyl 4-iodobenzoate, followed by ozonolysis (Scheme 7).



Scheme 7. Synthesis of phenols 36 and 37

Methoxy derivative **38** could be generated by di-methylation of the corresponding carboxylic acid precursor (Table 13). Applying the same conditions to benzoic acid **118** resulted only in methylation of the carboxylic acid. The reaction proceeded smoothly, however, in the presence of a stronger methylating agent (e.g, dimethyl sulfate as shown).

Table 13. Di-alkylation of benzoic acid derivatives

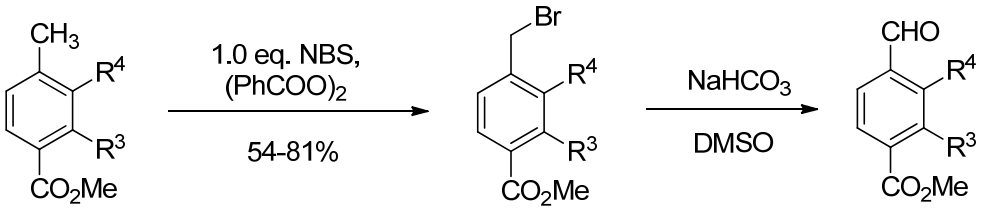


Starting Material ID	R	R^3	R^4	Conditions	Yield (%)	Product ID
117	CHO	H	OMe	CH_3I, K_2CO_3	58	**38**
118	Me	OMe	H	CH_3I, K_2CO_3	--	**119**

Starting Material ID	R	R^3	R^4	Conditions	Yield (%)	Product ID
118	Me	OMe	H	$(CH_3O)_2SO_2$, K_2CO_3	77	**119**

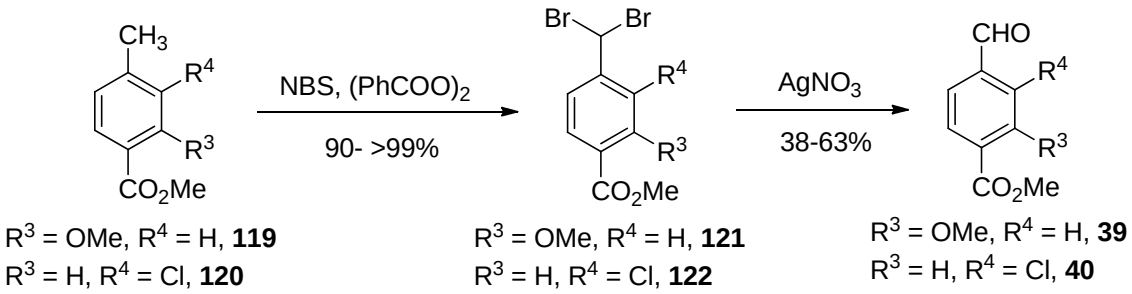
In an attempt to access the desired benzaldehyde, methoxy **119** was treated with 1.0 equivalent of *N*-bromosuccinimide (NBS), followed by formylation as previously described (Table 14).[95] Unfortunately, this route afforded none of the desired methoxy analog and proved low yielding for chloro derivative **120**, as well.

Table 14. Initial attempts towards benzaldehyde derivatives 39 and 40



Starting Material ID	R^3	R^4	Yield (%)	Product ID
119	OMe	H	--	**39**
120	H	Cl	17	**40**

Alternatively, addition of 2.0 equivalents of NBS afforded dibromo analogs **121** and **122** in excellent yield (Scheme 8). Subsequent formylation using silver nitrate resulted in the desired benzaldehydes.



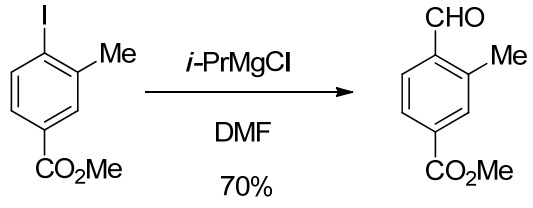
R^3 = OMe, R^4 = H, **119**
R^3 = H, R^4 = Cl, **120**

R^3 = OMe, R^4 = H, **121**
R^3 = H, R^4 = Cl, **122**

R^3 = OMe, R^4 = H, **39**
R^3 = H, R^4 = Cl, **40**

Scheme 8. Synthesis of benzaldehyde derivatives 39 and 40

Toluene analog **41** was prepared from commercially available iodide **123** by reaction with a Grignard reagent in dimethylformamide (DMF) as described by Mottram et al. (Scheme 9).[96]

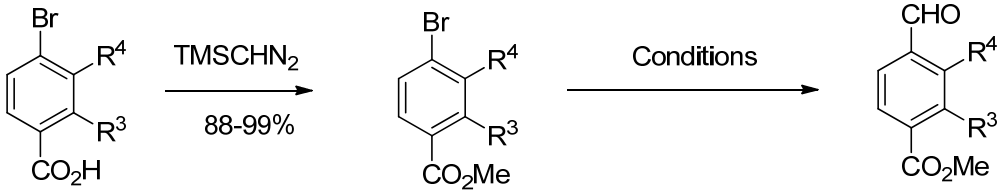


123 **41**

Scheme 9. Synthesis of benzaldehyde 41

Benzaldehyde derivatives containing an *ortho*-fluoro or a *meta*-methyl, -chloro, or -fluoro were readily available as the bromine precursor. Methylation of the corresponding carboxylic acid afforded the desired esters (Table 15). Initial attempts for conversion of the bromine resulted in extremely low yields. In an alternative route, bromine analogs **124**, **125**, **126**, and **127** were alkylated and washed with a steady stream of carbon monoxide in the presence of a palladium catalyst and sodium formate. This led to the desired benzaldehydes, with modest improvements in yield.

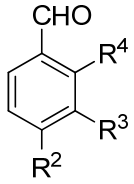
Table 15. Attempted formylation conditions of bromine analogs



Starting Material ID	R^3	R^4	Conditions	Yield (%)	Product ID
124	F	H	1. ZnCN, $(PPh_3)_4Pd$ 2. H_2, Raney Ni	6	**42**
125	H	F	1. ZnCN, $(PPh_3)_4Pd$ 2. H_2, Raney Ni	5	**43**
124	F	H	CO, $(PPh_3)_2PdCl_2$, HCOONa	11	**42**
125	H	F	CO, $(PPh_3)_2PdCl_2$, HCOONa	8	**43**
126	Cl	H	CO, $(PPh_3)_2PdCl_2$, HCOONa	24	**44**
127	Me	H	CO, $(PPh_3)_2PdCl_2$, HCOONa	19	**45**

All of the benzaldehyde derivatives generalized above are detailed in Table 16.

Table 16. Summary of benzaldehyde derivatives prepared

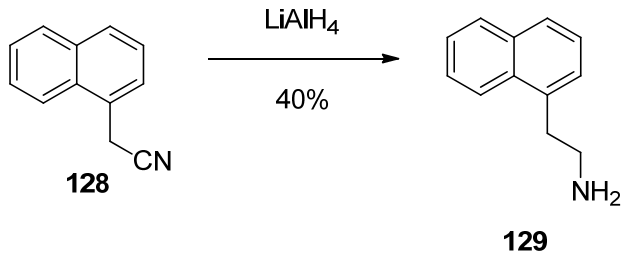


R^2	R^3	R^4	Compound ID
CO_2Et	H	H	**30**
CO_2iPr	H	H	**31**

R^2	R^3	R^4	Compound ID
CO_2tBu	H	H	**32**
CO_2NH_2	H	H	**33**
CO_2NHMe	H	H	**34**
CO_2NMe_2	H	H	**35**
CO_2Me	H	OH	**37**
CO_2Me	H	OMe	**38**
CO_2Me	H	Me	**41**
CO_2Me	H	Cl	**40**
CO_2Me	H	F	**43**
CO_2Me	OH	H	**36**
CO_2Me	OMe	H	**39**
CO_2Me	Me	H	**45**
CO_2Me	Cl	H	**44**
CO_2Me	F	H	**42**

2.3.4 Synthesis of B-Ring Modifications

Naphthylene **129** was synthesized from cyanide **128** (Scheme 10). Reduction with lithium aluminum hydride afforded the desired amine in moderate yield.



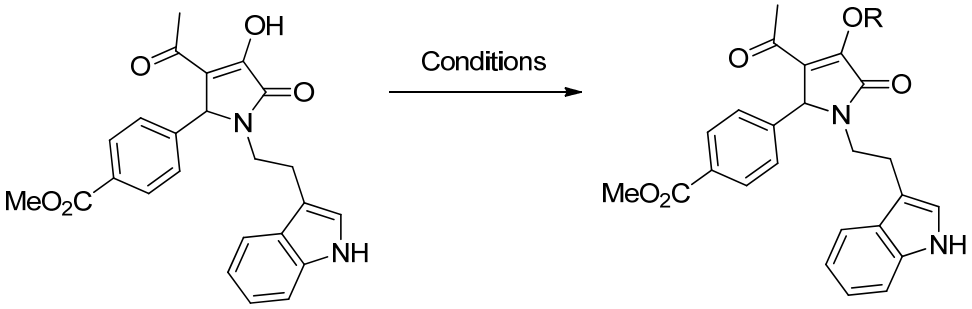
128 129

Scheme 10. Synthesis of naphthylene 129

2.3.5 Synthesis of R^{11} Modifications

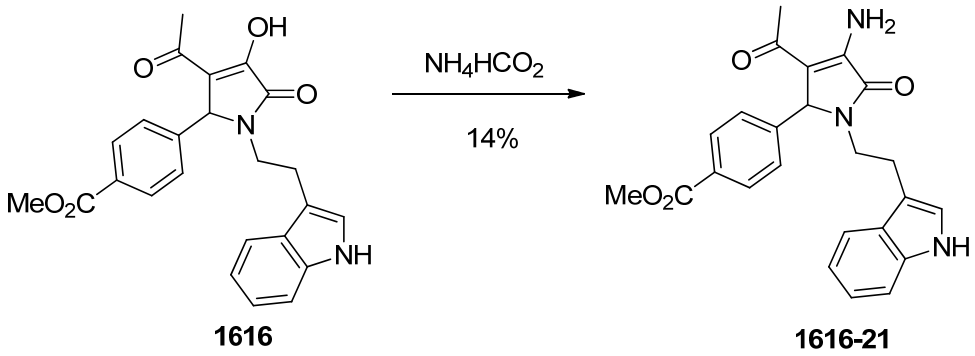
It was envisioned that a variety of conditions could be applied to lead compound **1616** in order to generate a series of analogs containing modifications at R^{11} (Table 17). In order to assess whether the enol might be acting as a hydrogen donor, protected enol derivatives were explored. Methoxy **1616-14** was synthesized in modest yield by reacting enol **1616** with (diazomethyl)trimethylsilane. Reaction with acetic anhydride and pyridine afforded acetyl ester **1616-20**.

Table 17. Conditions for preparation of protected enols 1616-14 and 1616-20



Starting Material ID	R	Conditions	Yield (%)	Product ID
1616	Me	$TMSCHN_2$	46	**1616-14**
1616	C(O)Me	acetic anhydride, pyridine (1.5 eq.)	7	**1616-20**

It was also anticipated that compounds in which the enol was replaced with alternative electron donating groups would be explored. **1616** was reacted with ammonium formate to yield amine **1616-21** (Scheme 11).



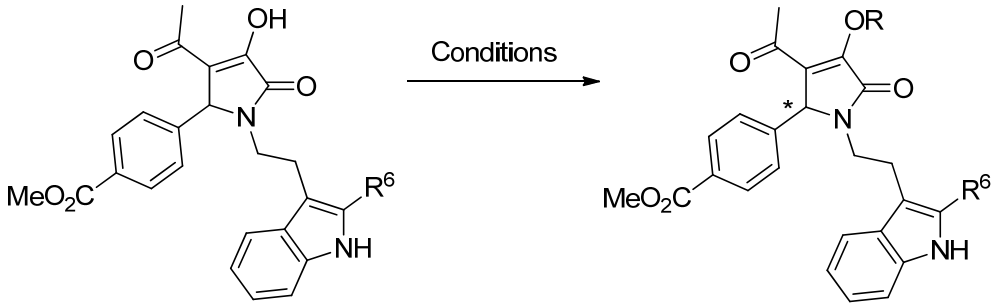
Scheme 11. Synthesis of amine 1616-21

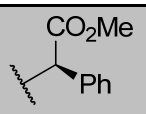
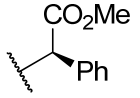
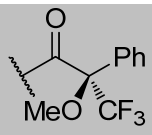
2.3.6 Separation of 1616-Series Enantiomers

All previous compounds were prepared and tested as racemic mixtures. It was therefore critical that the enantiomers be isolated and evaluated separately, as well. Lead analogs **1616** and **1616-19** were selected for attempts at separation.

Previous literature reported the separation of compounds containing a pyrrolidinone core as diastereomers via normal phase column chromatography.[97] Reaction of **1616** with methyl (*S*)-(+)-mandelate under Mitsonobu conditions afforded ester **130** as a diastereomeric mixture (Table 18). Unfortunately, conditions capable of separating these diastereomers were unable to be identified. Similarly, ester **131** proved equally inseparable. Mosher's ester **132**, synthesized from **1616-19** using standard coupling conditions, was also inseparable by normal phase column chromatography.

Table 18. Attempts at diastereomeric separation via normal phase column chromatography

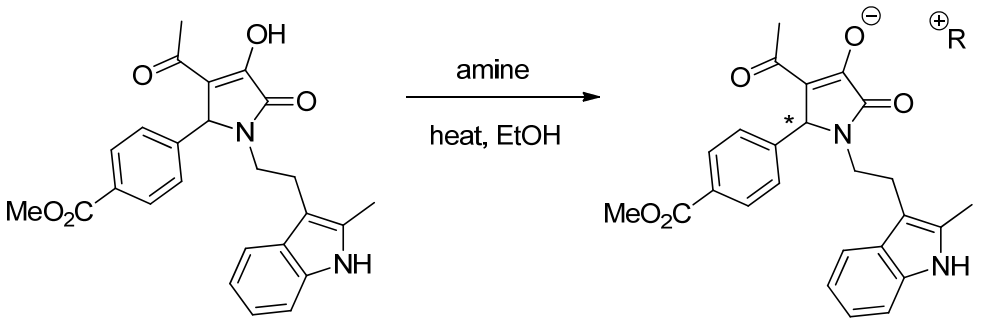


Starting Material ID	R	R^6	Conditions	Overall Yield (%)	Separation Achieved (Y/N)	Product ID
1616		H	methyl (*S*)-(+)-mandelate, 0 °C, PPh_3, DIAD	30	N	**130**
1616		Me	methyl (*S*)-(+)-mandelate, 0 °C, PPh_3, DIAD	70	N	**131**
1616-19		Me	(*R*)-Mosher's acid, 0 °C, DCC, DMAP	79	N	**132**

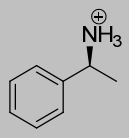
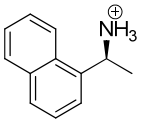
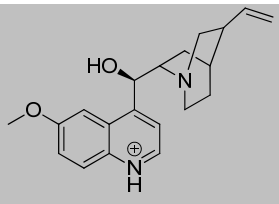
Recrystallization methods were also explored for the separation of **1616-19** enantiomers (Table 19). Addition of (*S*)-1-phenylethanamine afforded diastereomeric salt **133**. Several solvents were attempted for crystallization, including dichloromethane, ethyl acetate, and ether, but all led only to a 1:1 mixture. Similar results were observed for naphthylene salt **134**. Cinchonidine salt **135** was slightly enriched in one enantiomer (49% *ee*) after only one crystallization. Additional crystallization attempts in dichloromethane led only

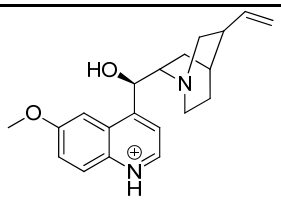
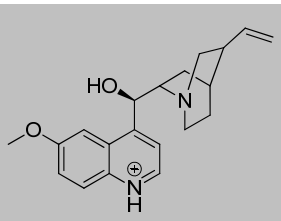
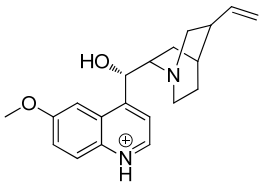
to 70% *ee*. At this point, alternative solvent systems were explored. Re-crystallization in methanol gave one enantiomer in 90% *ee*. In an attempt to obtain the other enantiomer, cinchonine salt **137** was prepared. Unfortunately, only a 1:1 mixture was obtained.

Table 19. Attempts at separation as diastereomeric salts



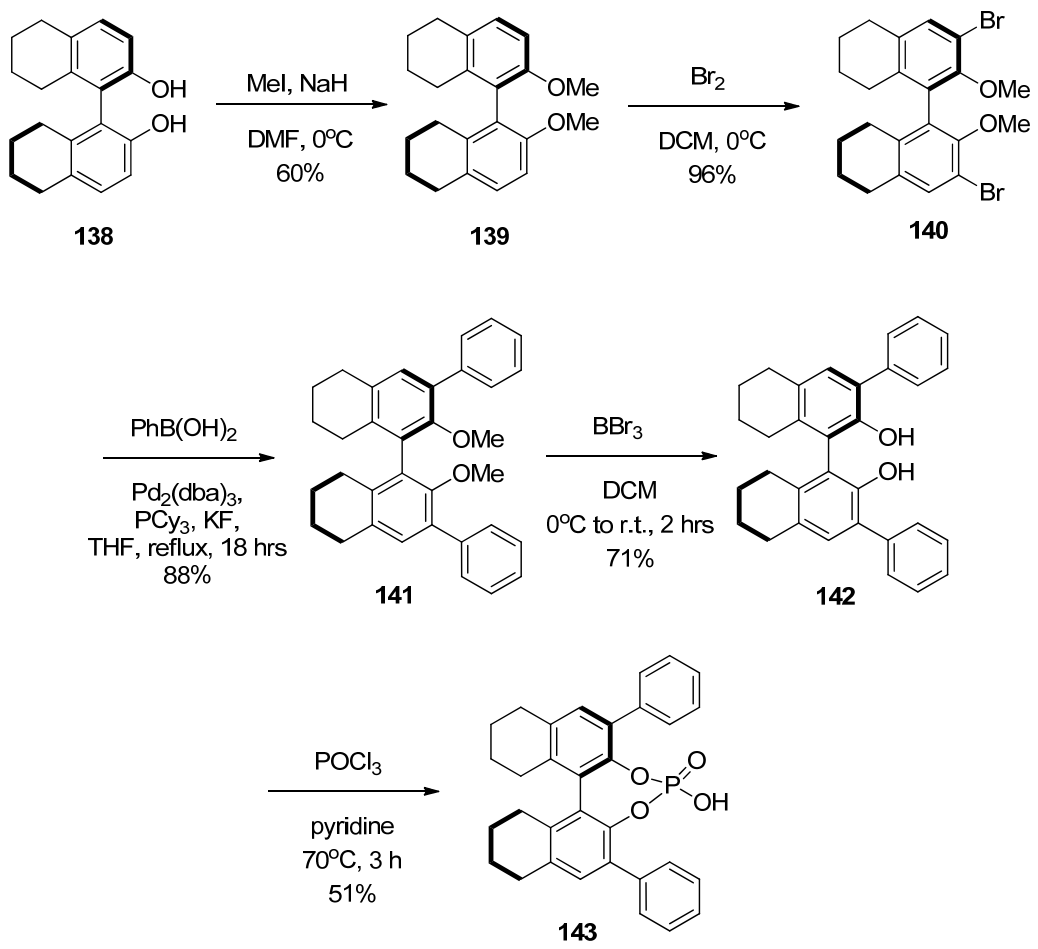
1616-19

Amine	R	Crystallization Method	*ee* (%)	Product ID
(*S*)-1-phenylethanamine		Dissolve in (1) DCM, (2) EtOAc, or (3) Et_2O with heating and store for up to 3 days at rt	--	**133**
(*S*)-1-(naphthalene-1-yl)ethanamine		Dissolve in either (1) DCM, (2) EtOAc, or (3) Et_2O with heating and store for up to 3 days at rt	--	**134**
Cinchonidine		Dissolve in DCM with heating and stored at 0 °C for 1 week	49	**135**

Amine	R	Crystallization Method	*ee* (%)	Product ID
Cinchonidine		Dissolve in DCM with heating and stored at 0 °C for 1 week (2x)	70	**136**
Cinchonidine		Dissolve in DCM with heating and stored at 0 °C for 1 week (2x); then re-crystallize in MeOH	90	**1616-19-1**
Cinchonine		Dissolve in either (1) DCM, (2) MeOH, or (3) EtOH with heating and store for up to 3 days at 0 °C	--	**137**

The racemic mixture of **1616-19** was capable of being resolved into two peaks using an analytical OD-RH chiral HPLC column (44% isocratic ACN with 0.1% formic acid). Evaluation of diastereomeric salt **1616-19-1** (peak 1) revealed 95% purity and only 90% *ee*.

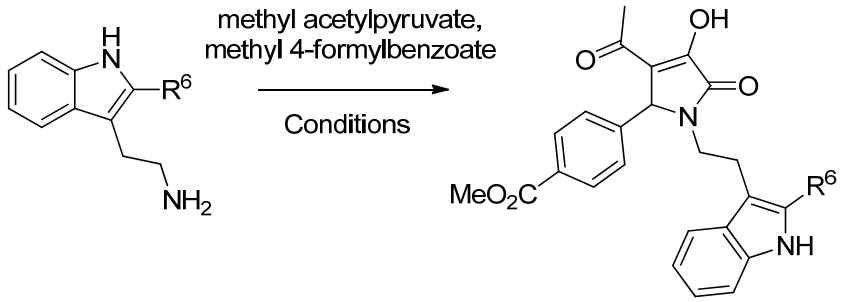
Recent literature has reported the use of chiral phosphoric acid catalysts for enantioselective Biginelli reactions.[98-100] Following this precedent, a chiral phosphoric acid catalyst was prepared in an attempt to synthesize **1616** and **1616-19** enantioselectively (Scheme 12). Protection of (*R*)-BINOL derivative **138** with methyl iodide and sodium hydride afforded methoxy **139**. Subsequent bromination followed by Suzuki coupling with phenyl boronic acid led to binol-based **141**. Compound **141** was then deprotected to yield hydroxy **142** before reacting with phosphorous oxychloride to generate acid **143**.



Scheme 12. Synthesis of chiral phosphoric acid catalyst 143

Amine **4** was then reacted with phosphoric acid catalyst **143** under the optimized Biginelli conditions (Chapter 2, *2.3.1*) in an attempt to synthesize **1616** enantioselectively (Table 21). These conditions led to an extremely low yield. While excess methyl acetylpyruvate afforded significantly improved yields for both **1616** and **1616-19**, no enantioselectivity was observed.

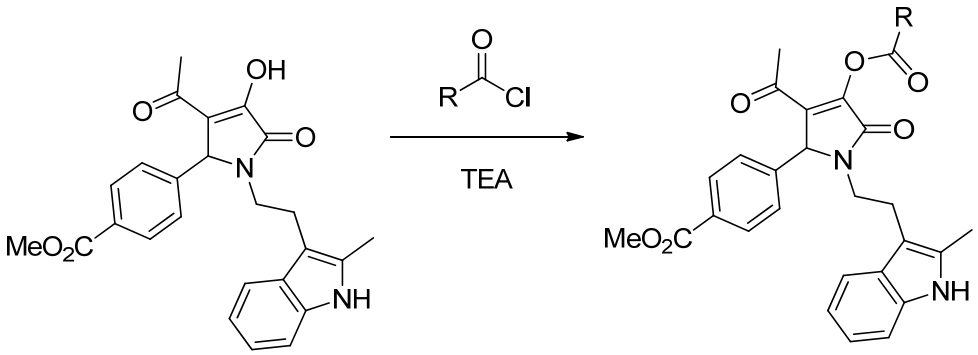
Table 20. Attempted enantioselective synthesis of 1616 and 1616-19



Starting Material ID	R^6	Conditions	Yield (%)	*ee*	Product ID
4	H	10 mol% **143**, dioxane, overnight	10	--	**1616**
4	H	excess pyruvate, 10 mol% **143**, DCM, overnight	82	--	**1616**
62	Me	excess pyruvate, 10 mol% **143**, DCM, overnight	37	--	**1616-19**

In order to discover whether the enantiomers of 1616 analogs might be accessed via enzymatic resolution, esters **1616-95** and **1616-96** were prepared. Reaction of **1616-19** with the appropriate acid chloride and triethylamine afforded esters **1616-95** and **1616-96** (Table 22).

Table 21. Synthesis of ester derivatives for enzymatic resolution



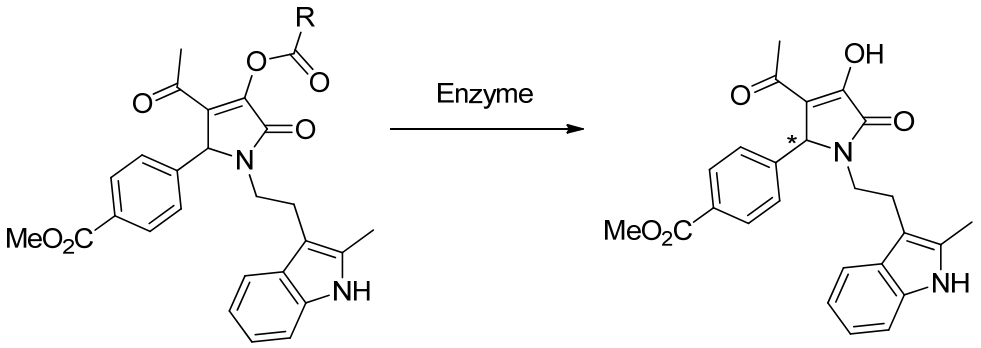
1616-19

R	Yield (%)	Product ID
$-CH_2CH_2CH_3$	31	**1616-95**
$-CH{=}CH_2$	20	**1616-96**

A variety of enzymes known to cleave butyryl and vinyl esters were chosen to screen for their ability to selectively react with one enantiomer over the other. Specifically, esterase from porcine liver, lipase from *C. antartica*, and lipase from *C. antartica* engineered in the Lutz laboratory at Emory University were evaluated (Table 23). Esterase from porcine liver offered no selectivity, cleaving both the ester at R^{11} and the methyl ester of both enantiomers. For all other enzymes tested, a 1:1 mixture of **1616-19** was isolated.

Both **1616-95** and **1616-96** appeared to crash out of solution upon addition of the lipase from *C. antartica* in methanol. For this reason, a biphasic reaction was also explored. These conditions offered no improvement in selectivity. In addition, starting material was still observed even after 24 h.

Table 22. Attempted enzymatic resolution of 1616-19



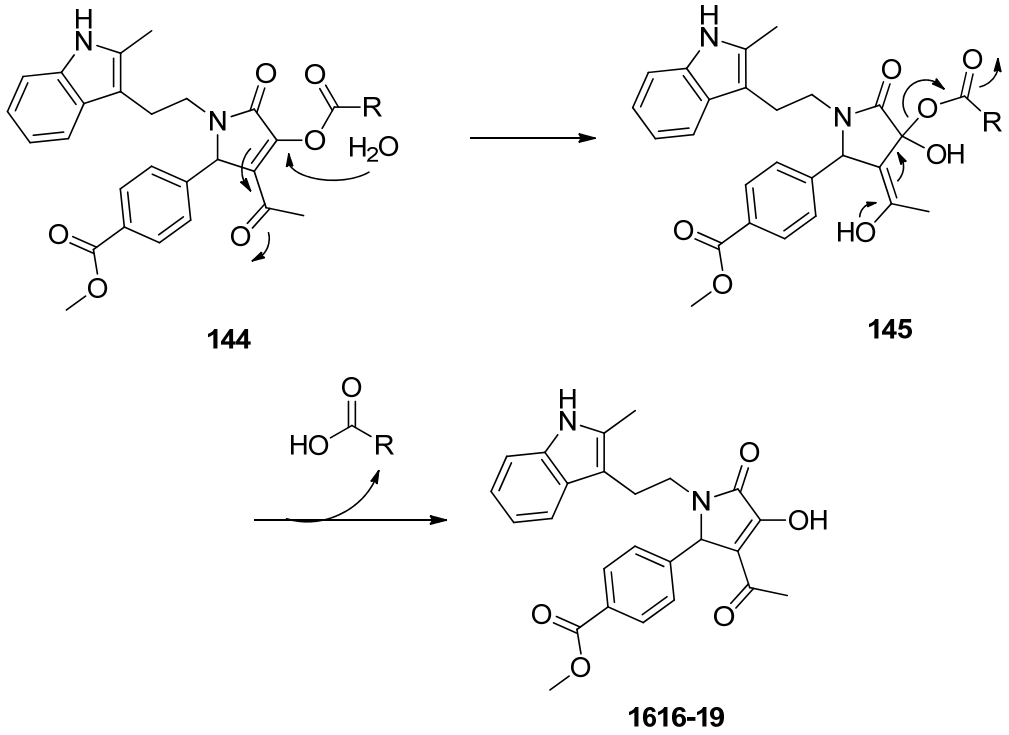
1616-19

Starting Material ID	R	Enzyme	Solvent (2.3:1, pH=7)	Substrate Observed (Y/N)	1616-19
1616-95	$CH_2CH_2CH_3$	esterase from porcine liver	MeOH: phosphate buffer	N	--*
1616-95	$CH_2CH_2CH_3$	lipase from *C. antartica*	MeOH: phosphate buffer	N	1:1
1616-95	$CH_2CH_2CH_3$	lipase from *C. antartica* (Lutz)	MeOH: phosphate buffer	N	1:1
1616-95	$CH_2CH_2CH_3$	control	MeOH: phosphate buffer	N	1:1
1616-96	$CH{=}CH_2$	esterase from porcine liver	MeOH: phosphate buffer	N	1:1
1616-96	$CH{=}CH_2$	lipase from *C. antartica*	MeOH: phosphate buffer	N	1:1
1616-96	$CH{=}CH_2$	lipase from *C. antartica* (Lutz)	MeOH: phosphate buffer	N	1:1

Starting Material ID	R	Enzyme	Solvent (2.3:1, pH=7)	Substrate Observed (Y/N)	1616-19
1616-96	$CH{=}CH_2$	control	MeOH: phosphate buffer	N	1:1
1616-95	$CH_2CH_2CH_3$	lipase from *C. antartica*	DCM: phosphate buffer	Y	1.2:1
1616-95	$CH_2CH_2CH_3$	control	DCM: phosphate buffer	Y	1:1
1616-96	$CH{=}CH_2$	lipase from *C. antartica*	DCM: phosphate buffer	Y	1.1:1
1616-96	$CH{=}CH_2$	control	DCM: phosphate buffer	Y	1.2:1

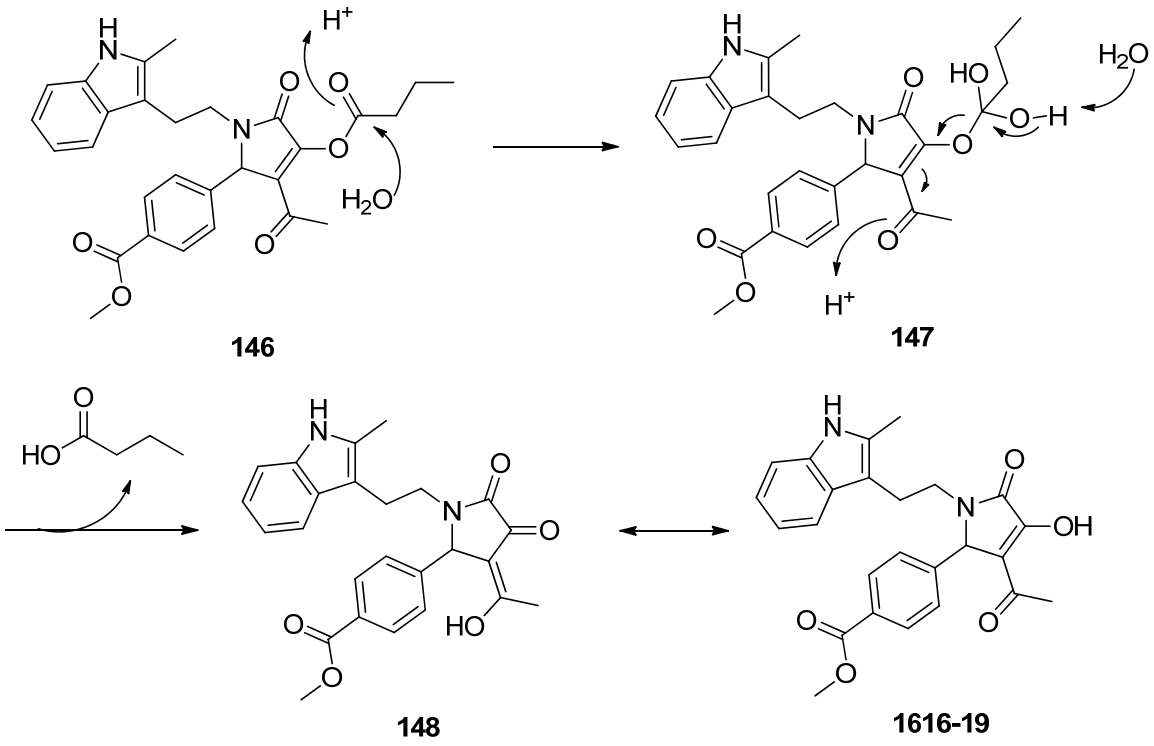
*Butyryl and methyl ester cleaved to the acid, in a 1:1 enantiomeric ratio

Interestingly, all controls demonstrated comparable results to the enzymes, with the reaction proceeding to completion in methanol, and with starting material remaining in biphasic systems. It was hypothesized that in aqueous environments, Michael addition of water may occur, facilitating the loss of butyric or acrylic acid (Scheme 13).



Scheme 13. Proposed mechanism of hydrolysis in aqueous conditions

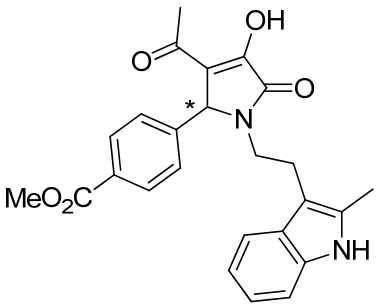
Separation of **1616** and **1616-19** via semi-preparatory HPLC was previously not pursued due to solubility concerns. Interestingly, ester **1616-95** demonstrated considerable improvements in solubility in organic solvents. The racemic mixture of **1616-95** was capable of being resolved into two peaks using an analytical OD-RH chiral HPLC column (44% ACN/H_2O w/ 0.1% formic acid). This method was transferred to a semi-prep OD-RH column using ester **1616-95**. Under these aqueous acidic conditions, **1616-95** was found to decompose into **1616-19**. The presence of acid may promote the Michael addition of water, generating increased formation of the enol (Scheme 13, above). Alternatively, cleavage of the ester may occur (Scheme 14). Thus, subjecting ester **1616-95** to a semi-preparatory HPLC reverse phase column afforded the separation of **1616-19** enantiomers.



Scheme 14. Alternative mechanism of hydrolysis in aqueous acidic medium

Each enantiomer was accessed for purity and enantiomeric excess using an analytical HPLC chiral OD-RH column as described above (Table 24). The compounds were then subjected to two-electrode voltage clamp analysis in *Xenopus* oocytes. As anticipated, the activity of **1616-19-1** was comparable to the previously isolated enantiomer (see Table 20), offering a potency of 18 ± 0.6 μM (n = 6). **1616-19-2** was inactive (n = 6), confirming that **1616-19-1** is responsible for the activity of **1616-19**.

Table 23. HPLC and biological data from 1616-19 enantiomers

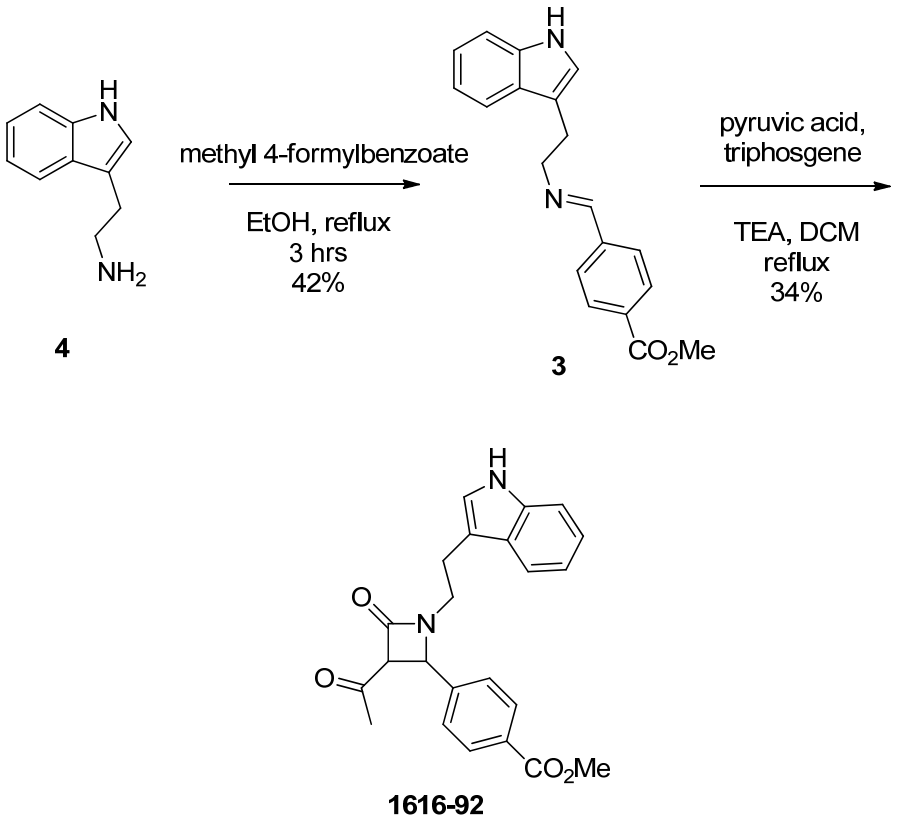


Peak	Retention Time (min.) [a]	Purity	*ee* (%)	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[b]
				GluN2 A	GluN2 B	GluN2 C	GluN2 D	GluN2C
1	26.082	99.5	99	106 ± 3.7	101 ± 2.1	259 ± 7.8	95 ± 1.8	18 ± 0.6 (259)
2	29.071	98	96	103 ± 2.7	96 ± 1.3	105 ± 3.7	117 ± 3.7	--
Racemic	--	--	--	97 ± 3.8	88 ± 1.6	179 ± 3.4	95 ± 2.5	16 ± 0.5 (217)

[a] Retention time was obtained on a ChiralPak OD-RH 4.6 mm x 150 mm, 5 μm column. Mobile phase was 44% isocratic ACN with 0.1% formic acid over 40 minutes. [b] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes were between 1.3-1.9. Data for active compounds at GluN1/GluN2C are from between 6-13 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 2-5 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 2 oocytes for all compounds).

2.3.7 Synthesis of β-Lactam Analog

The synthesis of β-lactam **1616-92** is illustrated in Scheme 15. Briefly, reaction of amine **4** with methyl 4-formylbenzoate in refluxing ethanol afforded imine **3**. Cyclization with pyruvic acid and triphosgene led to the desired β-lactam.



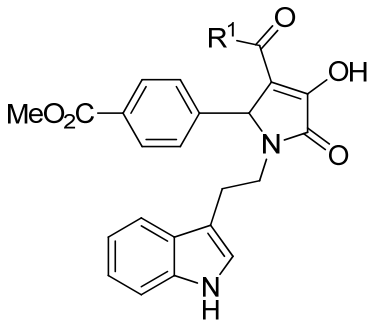
Scheme 15. Synthesis of β-lactam 1616-92

2.4 RESULTS AND DISCUSSION

2.4.1 Structure-Activity Relationship of 1616 Series – R^1 Modifications

A series of analogs containing various alkyl substituents were synthesized. The result of this effort is summarized in Table 25. Ethyl derivative **1616-29** offered a comparable potency to lead compound **1616**. Indeed, replacement of R^1 with a phenyl group, as in **1616-46**, also revealed modest potency (EC_{50} = 17 ± 2.3) with only minimal decreases in maximal potentiation (161%).

Table 24. Optimization of potency though evaluation of keto-linked substituents

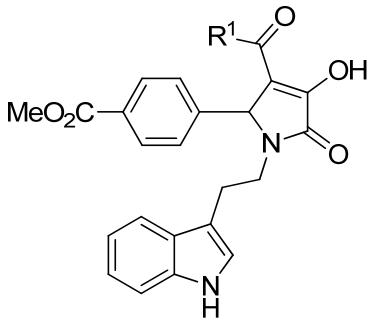


Compound ID	R¹	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM %)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-29	Et	85 ± 2.8	77 ± 2.4	160 ± 3.0	78 ± 2.0	24 ± 2.5 (204)
1616-32[b]	*i*Pr	95 ± 0.2	87 ± 1.8	119 ± 1.1[b]	89 ± 2.0	61 ± 10 (170)[b]
1616-33	*t*Bu	87 ± 4.8	93 ± 3.6	129 ± 5.8	79 ± 2.0	52 ± 6.4 (187)
1616-46	Ph	116 ± 5.4	85 ± 2.3	143 ± 2.7	79 ± 1.4	17 ± 2.3 (161)

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant figures when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes varied between 1.2-2.0. Data for active compounds at GluN1/GluN2C are from between 6-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 3-15 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 3 oocytes all compounds). For all tables, GluN2 subunits were co-expressed with GluN1 in *Xenopus* oocytes and evaluated using two-electrode voltage-clamp recordings. [b] The response to 100 μM of test compound was greater than 140% of control.

To further investigate the structural determinants of activity at R^1, a series of analogs with substituted phenyl rings were prepared (Table 26). Notably, **1616-62**, with a *meta* hydroxyl group, displayed a considerably higher potency at GluN2C-containing receptors (7.0 ± 0.92 μM), but caused significant inhibition of GluN2B- and GluN2D-containing receptors at 100 μM (42.1 ± 1.6 % and 48.1 ± 2.4 %, respectively, normalized to agonist activated current); such mixed-action modulators that potentiate one subunit while inhibiting another are intriguing but of little utility as pharmacological probes.

Table 25. Effect of aryl substituent position and identity at R^1



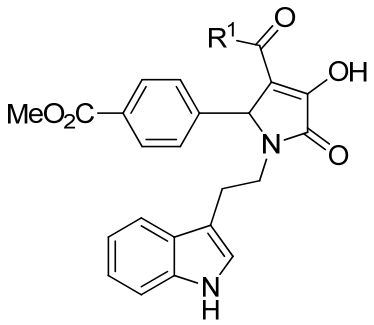
Compound ID	R^1	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-40	*o*-OMe-Ph	98 ± 2.2	84 ± 1.6	115 ± 3.8	83 ± 3.4	--
1616-39	*o*-Me-Ph	97 ± 6.1	95 ± 3.2	132 ± 3.5	82 ± 1.2	9.7 ± 0.6 (135)
1616-42	*o*-Cl-Ph	76 ± 6.3	89 ± 3.2	107 ± 4.7	108 ± 2.4	--
1616-41	*o*-F-Ph	89 ± 4.8	85 ± 4.7	134 ± 3.2	70 ± 2.2	12 ± 0.6 (141)
1616-62	*m*-OH-Ph	89 ± 1.3	65 ± 2.4	169 ± 8.9	69 ± 2.2	7.0 ± 0.92 (176)
1616-34	*m*-OMe-Ph	92 ± 1.1	90 ± 1.5	109 ± 2.3	80 ± 4.0	--

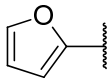
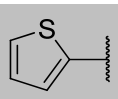
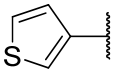
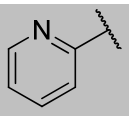
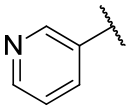
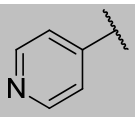
Compound ID	R^1	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-38	*m*-Me-Ph	97 ± 2.1	90 ± 2.8	122 ± 3.2	84 ± 0.7	16 ± 1.9 (131)
1616-36	*m*-Cl-Ph	107 ± 6.4	83 ± 3.1	145 ± 3.4	83 ± 2.1	8.7 ± 0.3 (145)
1616-35	*m*-F-Ph	98 ± 6.5	83 ± 2.4	133 ± 3.5	78 ± 2.4	11 ± 1.1 (136)
1960	*p*-OMe-*Ph*	92 ± 3.9	91 ± 1.9	134 ± 4.1	115 ± 12	9.1 ± 0.14 (137)
1986	*p*-Me-Ph	103 ± 4.4	92 ± 2.0	120 ± 5.0	87 ± 0.5	--
1959	*p*-Cl-Ph	99 ± 4.2	96 ± 2.1	120 ± 2.2	83 ± 0.8	--
1979	*p*-F-Ph	87 ± 2.2	77 ± 1.8	136 ± 2.3	87 ± 2.2	8.0 ± 0.66 (134)

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant figures when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes varied between 1.2-2.0. Data for active compounds at GluN1/GluN2C are from between 6-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n=3-15 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n≥3 oocytes all compounds). Data for compound **1616** from Table 25 is shown here to facilitate comparison.

A number of analogs were prepared in which the phenyl ring was replaced with a heteroaromatic ring (Table 27). Two compounds containing a pyridine ring at R^1 potentiated responses up to ~200% with EC_{50} values of 12 ± 1.9 μM (**1616-28**) and 8.9 ± 1.3 μM (**1616-50**). Interestingly, **1616-30**, which contained a 2-substituted pyridine ring, was inactive at all receptor subunits.

Table 26. Effect of heteroaromatic substitution at R^1



Compound ID	R^1	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-27		99 ± 4.9	82 ± 0.5	124 ± 2.8	78 ± 1.8	21 ± 2.1 (138)
1616-37		106 ± 3.5	81 ± 3.7	117 ± 1.8	79 ± 1.6	--
1616-54		110 ± 4.8	85 ± 1.5	134 ± 4.6	79 ± 1.9	15 ± 2.2 (150)
1616-30		106 ± 3.5	78 ± 4.4	97 ± 3.0	75 ± 3.9	--
1616-28		97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-50		92 ± 5.9	87 ± 2.9	186 ± 3.7	84 ± 1.6	8.9 ± 1.3 (196)

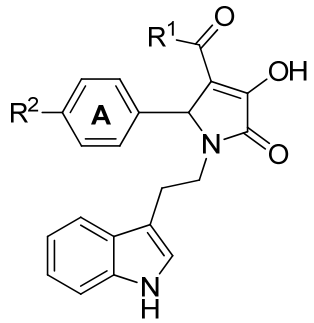
[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant figures when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and

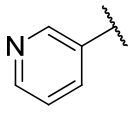
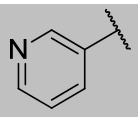
glycine (30 μM) response. Hill slopes varied between 1.2-2.0. Data for active compounds at GluN1/GluN2C are from between 6-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 3-15 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 3 oocytes all compounds). Data for compound **1616** from Table 25 is shown here to facilitate comparison.

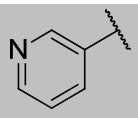
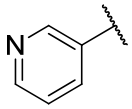
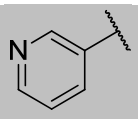
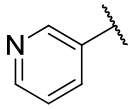
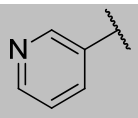
2.4.2 Structure-Activity Relationship of 1616-Series – A-Ring Modifications

Due to the high potency observed in pyridine derivatives **1616-28** and **1616-50**, many future analogs replaced the methyl at R^1 with a pyridine ring. A series of analogs containing ester isosteres at A-ring position R^2 were therefore prepared (Table 28). Unfortunately, none of these compounds exhibited any activity.

Table 27. Effect of ester isosteres at R^2



ID	R^1	R^2	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	CO_2Me	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-28		CO_2Me	97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-72		CF_3	86 ± 3.6	74 ± 4.5	86 ± 2.2	58 ± 2.7 [c]	--

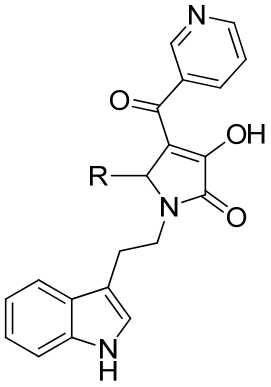
ID	R^1	R^2	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-08	Me	NO_2	106 ± 3.4	83 ± 2.0	94 ± 0.3	77 ± 1.1	--
1616-49		CN	98 ± 6.8	89 ± 0.8	100 ± 2.7	94 ± 1.8	--
1616-60		$C(O)NH_2$	101 ± 4.2	92 ± 4.5	90 ± 2.7	91 ± 0.8	--
1616-52		C(O)NHMe	96 ± 1.3	105 ± 5.7	93 ± 2.8	89 ± 2.4	--
1616-53		$C(O)NMe_2$	118 ± 3.2	86 ± 1.2	101 ± 1.8	89 ± 0.8	--
1616-90		SO_2H	86 ± 2.0	82 ± 1.9	123 ± 2.2	83 ± 1.5	16 ± 0.76 (130)
1616-97	Me	SO_2Me	74 ± 2.9	88 ± 1.6	114 ± 3.8	73 ± 0.6	--

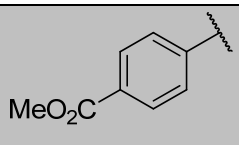
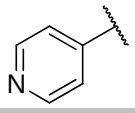
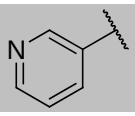
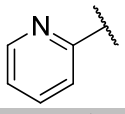
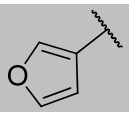
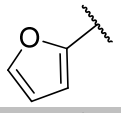
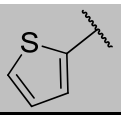
[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant figures when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes varied between 1.3-1.7. Data for active compounds at GluN1/GluN2C are from between 8-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 3-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 3 oocytes all compounds). [b] Inhibited GluN1/GluN2D with a mean IC_{50} value of 41 μM. Data for compounds **1616** from Table 25 and **1616-28** from Table 27 are shown here to facilitate comparison.

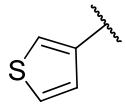
A series of heteroaromatic rings were also evaluated for their ability to selectively potentiate GluN2C-containing receptors (Table 29). For all analogs tested, no detectable activity at recombinant NMDA receptors was observed. This suggested that placement of

the phenyl ring substituted with a *para*-methyl or -ethyl ester at the A-ring appears to be essential for the activity in this structural series.

Table 28. Effect of A-ring replacement



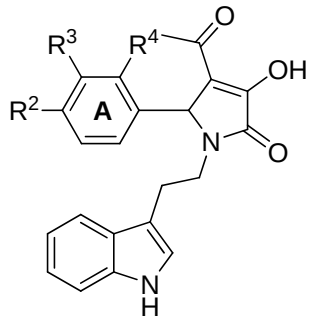
Compound ID	R	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-28		97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-73		95 ± 1.8	92 ± 0.3	96 ± 1.3	95 ± 0.7	--
1616-74		86 ± 2.1	93 ± 2.0	110 ± 2.1	93 ± 2.1	--
1616-75		77 ± 1.2	86 ± 1.3	103 ± 1.9	96 ± 2.7	--
1616-76		92 ± 2.3	79 ± 3.2	95 ± 2.2	98 ± 1.9	--
1616-82		99 ± 2.3	86 ± 3.9	108 ± 2.0	96 ± 1.5	--
1616-81		84 ± 7.7	87 ± 3.2	110 ± 4.0	89 ± 3.1	--

Compound ID	R	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
		GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-83		96 ± 2.0	89 ± 5.5	102 ± 3.0	93 ± 2.4	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. All data are from 4-20 oocytes; the lack of effect was confirmed by testing all compounds at 100 μM (data not shown, n ≥ 3 oocytes for all compounds). Data for compound **1616-28** from Table 27 is included to facilitate comparison.

Positional isomer analogs of the methyl ester were inactive at GluN1/GluN2C (Table 30). One compound, **1616-16**, which contained an ethyl ester at ring position R^2, displayed comparable potency to screening hit **1616**. Analogs containing bulkier ester substituents were also inactive.

Table 29. Effect of A-ring modifications



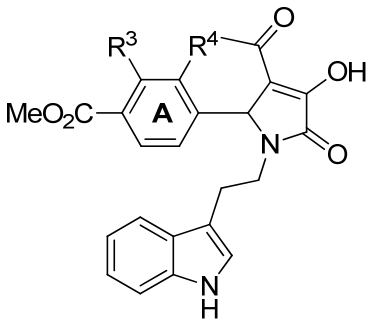
Compound ID	R^2	R^3	R^4	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
				GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	CO_2Me	H	H	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-02	H	CO_2Me	H	99 ± 2.6	88 ± 0.91	94 ± 1.2	94 ± 1.5	--
1616-05	H	H	CO_2Me	88 ± 3.1	78 ± 0.6	99 ± 4.5	93 ± 7.0	--

Compound ID	R^2	R^3	R^4	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
				GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-16	CO_2Et	H	H	104 ± 5.8	83 ± 2.0	201 ± 4.5	91 ± 2.2	15 ± 1.1 (237)
1616-15	CO_2i-Pr	H	H	96 ± 0.9	95 ± 3.2	102 ± 2.0	83 ± 4.9	--
1616-03	CO_2t-Bu	H	H	99 ± 4.1	81 ± 4.3	85 ± 2.8	66 ± 2.8	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant figures when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes varied between 1.3-1.7. Data for active compounds at GluN1/GluN2C are from between 8-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 3-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 3 oocytes all compounds). Data from compound **1616** from Table 25 is shown here to facilitate comparison.

A variety of substituents at A-ring positions R^3 and R^4 were systematically tested while holding the *para*-methyl ester constant at R^2 (Table 31). Substitution at the *meta* position (R^3) revealed either a loss of potency or complete inactivity. Evaluation of a series of *ortho* (R^4) ring substituents demonstrated a preference for electron donating groups. For example, analogs containing an *ortho*-hydroxy (**1616-12**) exhibited modest potentiation with increased potency, whereas analogs containing an *ortho*-chloro (**1616-17**) or -fluoro (**1616-26**) were slightly less active.

Table 30. Optimization of A-ring substituents



Compound ID	R^3	R^4	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	H	H	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-07	OH	H	81 ± 1.7	79 ± 1.2	123 ± 2.5	90 ± 3.0	29 ± 2.8 (151)
1616-11	OMe	H	98 ± 3.3	80 ± 1.8	92 ± 2.4	86 ± 1.4	--
1616-23	Me	H	108 ± 3.4	91 ± 2.5	88 ± 2.1	86 ± 0.4	--
1616-24	Cl	H	88 ± 4.3	95 ± 5.1	113 ± 4.2	83 ± 1.5	--
1616-25	F	H	99 ± 4.1	83 ± 2.2	114 ± 3.3	93 ± 5.3	--
1616-12	H	OH	107 ± 3.9	86 ± 3.8	173 ± 3.0	88 ± 1.9	15 ± 0.6 (202)
1616-04	H	OMe	102 ± 6.1	83 ± 0.3	132 ± 3.3	100 ± 3.1	46 ± 19 (183)
1616-18	H	Me	101 ± 1.8	95 ± 4.2	129 ± 3.6	85 ± 1.9	35 ± 1.4 (165)
1616-17	H	Cl	103 ±	87 ± 1.7	139 ± 2.8	90 ± 0.6	36 ± 3.0

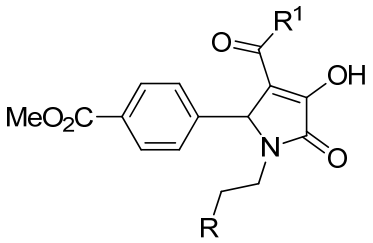
Compound ID	R^3	R^4	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
			3.9				(191)
1616-26	H	F	93 ± 2.5	96 ± 2.0	123 ± 3.4	91 ± 1.1	37 ± 2.6 (155)

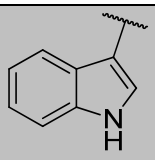
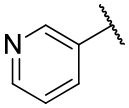
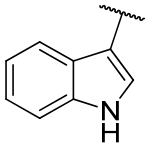
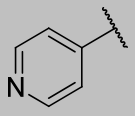
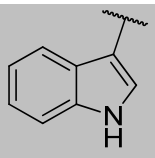
[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response; Hill slopes ranged between 1.3-1.8. Data for active compounds at GluN1/GluN2C are from between 3-12 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 3-15 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 5 oocytes for all compounds). Data for compound **1616** from Table 25 is included to facilitate comparison.

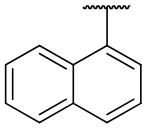
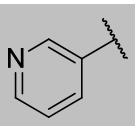
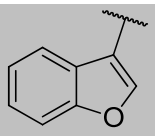
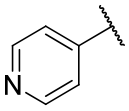
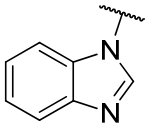
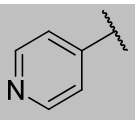
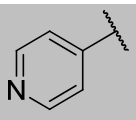
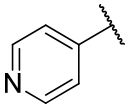
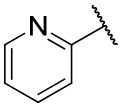
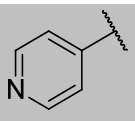
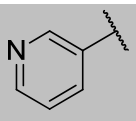
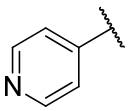
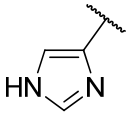
2.4.3 Structure-Activity Relationship of 1616-Series – B-Ring Modifications

An assortment of acyclic, cyclic, and heterocyclic systems at the B-ring were evaluated for potency and subunit-selectivity while retaining optimal R^1 (Me, *meta*-pyridine, or *para*-pyridine) and R^2 (CO_2Me) substitutions (Table 32). Replacement of the indole –NH- with –O- led only to weak activity (**1616-93**), suggesting the presence of a hydrogen bond in the binding pocket. In all other instances, removal of the indole led to complete inactivity. Interestingly, substitution with a napthyl derivative, as in **1616-63**, led to strong inhibition at all four subunits. These data suggest that the indole functionality is preferred for activity.

Table 31. Effect of replacing the B-ring



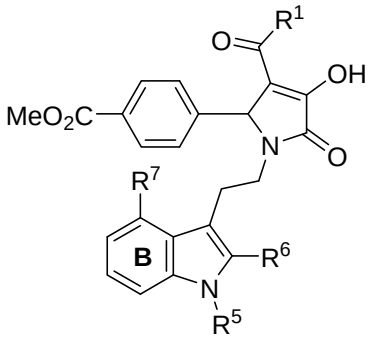
Compound ID	R[1]	R	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) µM (%)[a]
			GluN2 A	GluN2 B	GluN2 C	GluN2 D	GluN2 C
1616	Me		95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-28			97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-50			92 ± 5.9	87 ± 2.9	186 ± 3.7	84 ± 1.6	8.9 ± 1.3 (196)
1616-66	Me	Ph	108 ± 1.7	117 ± 4.9	95 ± 2.8	99 ± 3.7	--
1616-67	Me	*m*-Me-Ph	108 ± 3.5	92 ± 3.0	96 ± 3.3	102 ± 4.3	--
1616-68	Me	*m*-Cl-Ph	97 ± 2.4	94 ± 2.2	96 ± 2.2	89 ± 2.1	--
1616-69	Me	*m*-F-Ph	112 ± 2.7	108 ± 1.8	98 ± 1.4	96 ± 0.8	--

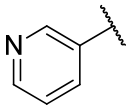
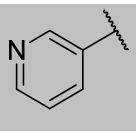
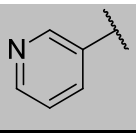
Compound ID	R^1	R	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-70	Me	*m*-OMe	110 ± 4.2	116 ± 4.0	97 ± 1.9	91 ± 1.6	--
1616-71	Me	*m*-OH	96 ± 6.1	106 ± 3.7	99 ± 2.7	91 ± 2.6	--
1616-63[b]	Me		44 ± 2.6 *b*	20 ± 1.6 *b*	34 ± 2.5 *b*	18 ± 1.7 *b*	--
1616-93			96 ± 1.1	83 ± 2.6	113 ± 3.8	80 ± 2.7	--
1616-56			104 ± 5.8	107 ± 2.7	84 ± 4.1	89 ± 3.0	--
1616-57			109 ± 2.9	103 ± 4.5	100 ± 3.5	97 ± 0.8	--
1616-58			100 ± 4.3	101 ± 1.0	97 ± 2.1	98 ± 0.5	--
1616-59			118 ± 4.0	104 ± 5.6	78 ± 4.9	92 ± 1.4	--
1616-64			101 ± 2.6	95 ± 1.0	89 ± 6.1	94 ± 2.0	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. All data are from 3-14 oocytes from 2-3 frogs. When no effect was found, the lack of effect was confirmed by testing at 100 μM (data not shown, $n \geq 3$ oocytes for all compounds). Data for compound **1616** from Table 25 and compounds **1616-28** and **1616-50** from Table 27 are included to facilitate comparison. [b] Inhibited at GluN1/GluN2A with an IC_{50} of 18 μM, at GluN1/GluN2B with an IC_{50} of 7.2 μM, at GluN1/GluN2C with an IC_{50} of 11 μM, and GluN1/GluN2D with an IC_{50} of 5.7 μM.

This led to the examination of substituted indoles as an alternative strategy to access increased potency. The data describing these compounds is summarized in Tables 33 and 34. Methylation of the indole nitrogen led to inactivity (**1616-79**), further suggesting the importance of a hydrogen atom at this position in the binding pocket. The best potency was obtained for analogs with substitutions at R^9. Compound **1616-85** demonstrated an ability to selectively potentiate GluN2C-containing NMDA receptors up to 218% with an EC_{50} value of 4.3 ± 0.3 μM. It is unclear whether the increase in potency observed for **1616-85** can be ascribed to a steric effect or, alternatively, to a mildly electropositive effect. Consistent with a steric effect, analogs which contain larger R^9 substituents, such as **1616-89** (R^9 = OMe), revealed a slight loss of potentiation compared to **1616-85**. Analogs containing strongly electron withdrawing R^9 substituents such as **1616-84** (R^9 = F) also decrease the observed activity.

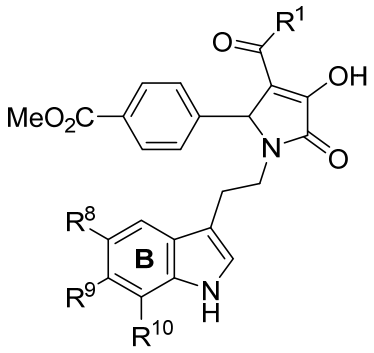
Table 32. Optimization of B-ring substituents

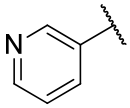
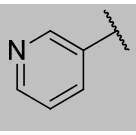
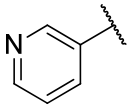
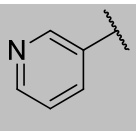
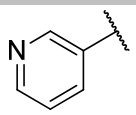
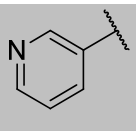
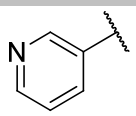


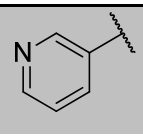
#	R^1	R^5	R^6	R^7	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
					GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	H	H	H	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-28		H	H	H	97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-79		Me	H	H	111 ± 5.4	87 ± 2.5	106 ± 3.3	85 ± 1.9	--
1616-19	Me	H	Me	H	97 ± 3.8	88 ± 1.6	179 ± 3.4	95 ± 2.5	16 ± 0.5 (217)
1616-86		H	H	Me	112 ± 0.8	70 ± 3.7	85 ± 2.7	74 ± 1.4	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes were between 1.3-1.9. Data for active compounds at GluN1/GluN2C are from between 6-27 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 4-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 4 oocytes for all compounds). Data for compound **1616** from Table 25 and compound **1616-28** from Table 28 are included to facilitate comparison.

Table 33. Optimization of B-ring substituents



#	R[1]	R[8]	R[9]	R[10]	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
					GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	H	H	H	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-28		H	H	H	97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-77		OMe	H	H	112 ± 1.6	90 ± 3.7	94 ± 2.6	91 ± 4.3	--
1616-84		H	F	H	94 ± 4.3	64 ± 3.3	163 ± 11	82 ± 1.7	13 ± 1.8 (167)
1616-80		H	Cl	H	93 ± 8.1	66 ± 1.4	170 ± 9.2	86 ± 3.0	8.5 ± 1.0 (204)
1616-85		H	Me	H	103 ± 1.1	82 ± 1.5	219 ± 5.4	86 ± 1.7	4.3 ± 0.3 (218)
1616-89		H	OMe	H	84 ± 1.8	98 ± 7.6	194 ± 2.8	85. ± 0.7	8 ± 1.3 (204)
1616-94		H	H	F	105 ± 2.4	84 ± 1.2	161 ± 3.2	86 ± 4.7	18 ± 1.8 (191)

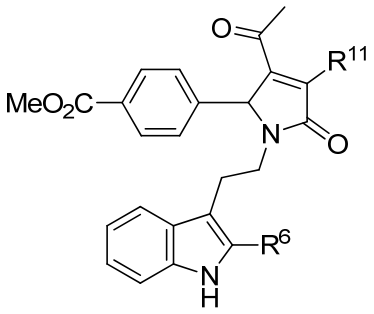
#	R^1	R^8	R^9	R^{10}	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
					GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-91		H	H	Cl	90 ± 3.4	85 ± 3.7	127 ± 2.3	78 ± 1.5	7 ± 2.1 (128)
1616-13	Me	H	H	Me	98 ± 5.1	81 ± 2.4	121 ± 3.7	92 ± 1.1	25 ± 2.9 (139)

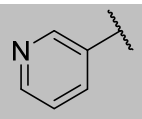
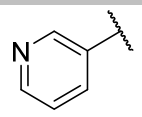
[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes were between 1.3-1.9. Data for active compounds at GluN1/GluN2C are from between 6-27 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 4-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 4 oocytes for all compounds). Data for compound **1616** from Table 25 and compound **1616-28** from Table 28 are included to facilitate comparison.

2.4.4 Structure-Activity Relationship of 1616-Series – R^{11} Modifications

Several modifications were made at R^{11} to determine the significance of the enol in controlling potency and selectivity (Table 35). Replacement with an amine, as in **1616-21**, led to a complete loss of potentiation at concentrations up to 100 μM. In most instances, compounds containing a protected alcohol led to less potent analogs. For example, a 10-fold decrease in potency was observed for acetate **1616-20**. In contrast, buytryl ester **1616-95** maintained activity comparable to lead analog **1616**, with an EC_{50} of 14 ± 1.9 μM. These data suggest that enhancements in potency cannot be gained though modifications of the enol.

Table 34. Optimization of potency though modification of R^{11} substitutions



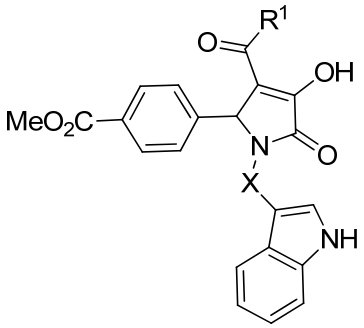
#	R^1	R^6	R^{11}	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
				GluN2 A	GluN2 B	GluN2 C	GluN2 D	GluN2 C
1616	Me	H	OH	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616 -21	Me	H	NH_2	93 ± 4.4	85 ± 5.8	102 ± 1.2	75 ± 1.1	--
1616 -14	Me	H	OMe	109 ± 4.1	91 ± 3.5	127 ± 3.0	82 ± 2.8	37 ± 2.2 (163)
1616 -20	Me	H	OAc	95 ± 1.7	90 ± 1.2	120 ± 3.2	92 ± 1.3	52 ± 5.8 (173)
1616 -95		Me	$OC(O)CH_2CH_2CH_3$	112 ± 3.2	93 ± 5.0	186 ± 11	93 ± 2.3	14 ± 1.9 (184)
1616 -96		Me	$OC(O)CH=CH_2$	106 ± 6.0	104 ± 2.5	125 ± 5.0	100 ± 4.5	105 ± 25 (208)

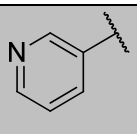
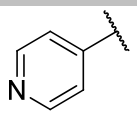
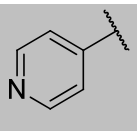
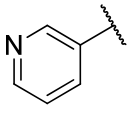
[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Data for active compounds at GluN1/GluN2C are from between 5-9 oocytes from 2-3 frogs for each compound tested. The Hill slope varied between 1.2-1.8, and was fixed to be 1.5 for less potent analogs (**1616-20**). When no effect was found (n = 3-9 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 4 oocytes for all compounds). Data for compound **1616** from Table 25 is included to facilitate comparison.

2.4.5 Structure-Activity Relationship of 1616 Series – Linker Modifications

The original screening hit, **1616**, contains a two carbon region linking the B-ring with the core pyrrolidinone. The linker modifications explored are illustrated in Table 36. Both shortening (**1616-55**) and extension (**1616-87**) of the linker resulted in elimination of all activity. These data suggest that the potency of pyrrolidinone analogs is highly dependent on the length of the carbon linkage.

Table 35. Effects of linker modifications



#	R1	X	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616-28		CH_2CH_2	97 ± 1.7	76 ± 3.0	180 ± 7.1	78 ± 1.2	12 ± 1.9 (201)
1616-50		CH_2CH_2	92 ± 5.9	87 ± 2.9	186 ± 3.7	84 ± 1.6	8.9 ± 1.3 (196)
1616-55		CH_2	106 ± 1.1	101 ± 1.1	84 ± 1.9	87 ± 1.7	--
1616-87		$CH_2CH_2CH_2$	96 ± 1.3	93 ± 3.1	84 ± 2.1	98 ± 1.9	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Data for active compounds at GluN1/GluN2C are from between 7-8 oocytes from 2 frogs for each compound tested; the Hill slope varied between 1.3-1.4. When no effect was found at 30 μM (n = 3-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 4 oocytes for all compounds). Data for compounds **1616-28** and **1616-50** from Table 27 are included to facilitate comparison.

2.4.6 Rationale and Results for β-Lactam Analog

Preliminary assessment of β-lactam **1616-92** indicated a high degree of structural similarity (Tanimoto shape = 0.731) and minimal electrochemical similarity (Tanimoto color = 0.372) (Figure 14). β-Lactams are commonly observed in biologically active compounds.[101-107] In addition, there is an abundance of literature regarding the preparation of β-lactam rings.[107-111] For these reasons, **1616-92** was pursued for synthesis and biological evaluation.

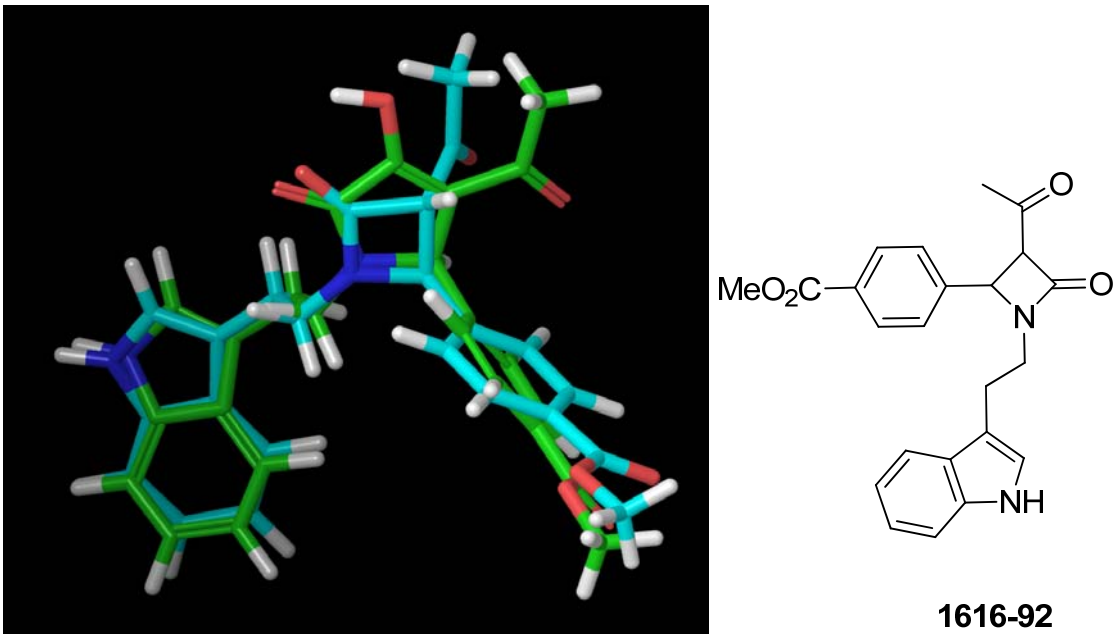


Figure 14. Overlaid structures of 1616 (green) and 1616-92 (blue)

1616-92 was evaluated via two-electrode voltage clamp analysis for its ability to selectively potentiate GluN2C-containing NMDA receptors over GluN2A-, GluN2B-, and GluN2D-containing NMDA receptors (Table 37). Unfortunately, no activity was observed at any subunit combinations tested. Further pursuit of this scaffold was abandoned due to lack of efficacy.

Table 36. Biological data from scaffold hopping analog, 1616-92

Compound ID	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
	GluN2A	GluN2C	GluN2C	GluN2D	GluN2C
1616-92	94 ± 3.4	94 ± 6.2	99 ± 2.3	99 ± 4.1	--

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Data are from 2-3 oocytes from 1 frog for each receptor tested.

These data suggest that the points of structural diversity between the two structures may play an important role in the activity of 1616 analogs. The computational overlay indicates two significant differences (Figure 15). Firstly, while a large degree of variation is tolerated at R^1, the role of the ketone has yet to be fully explored. The ketones of **1616** and **1616-92** appear to face near opposite directions. Additionally, 1616 analogs previously evaluated indicate that the oxygen of the enol is central for activity. This region of space is empty for **1616-92**. Taken together these results indicate that both the enol and the ketone near R^1 play an important role in the activity of 1616 analogs.

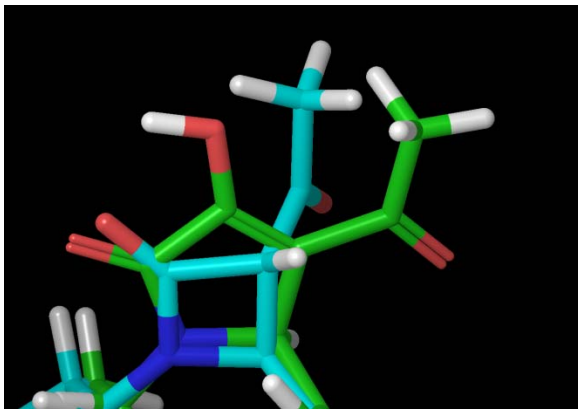


Figure 15. Points of structural diversity between 1616 and 1616-92

2.4.7 Off-Target Effects of 1616-Series Analogs

The activity of **1616** and **1616-19** was evaluated against several ionotropic receptors including, AMPA, kainate, GABA, nicotinic acetylcholine (nACh) receptors and glycine receptors (Figures 16 and 17). Compounds **1616** and **1616-19** indicated no effect at AMPA, kainate, GABA and glycine receptors. Minimal inhibition of nicotinic acetylcholine receptors by **1616-19** was observed. These data demonstrate the ability of 1616 analogs to selectively potentiate GluN2C-containing NMDA receptors over other ion channel receptors.

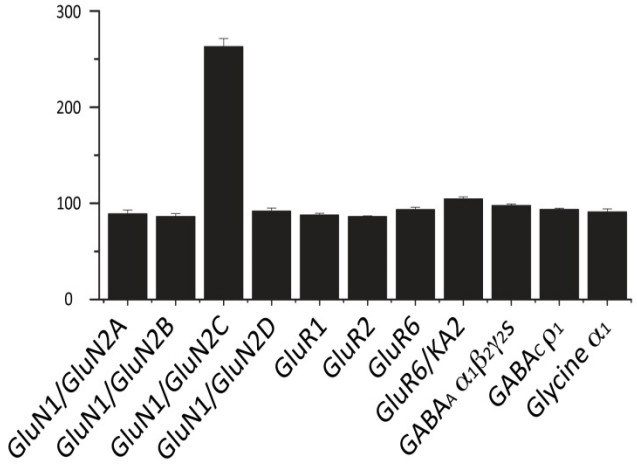


Figure 16. Off-target responses to 1616

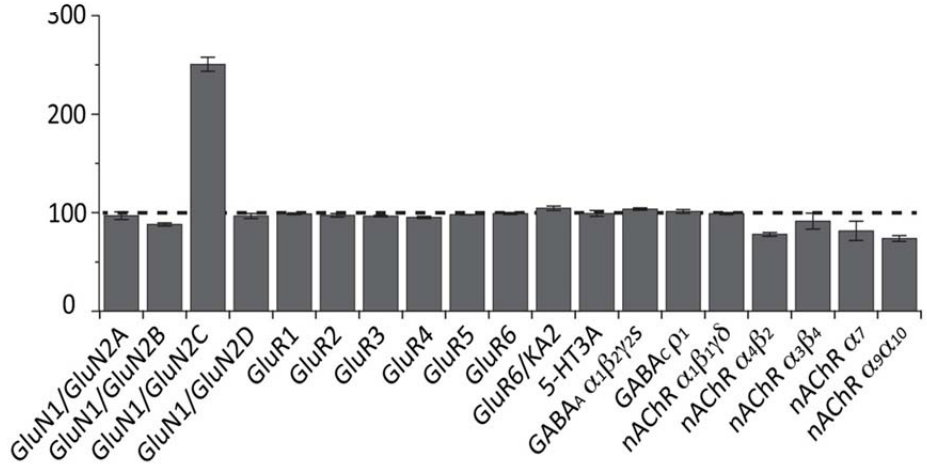


Figure 17. Off-target responses to 1616-19

2.4.8 In vitro Analysis of 1616-Series Mechanism of Action and Structural Determinants of Activity

Two-electrode voltage clamp recordings for **1616-19** (1, 3, 10, 30, and 100 μM) at all four GluN2 subunits are illustrated in Figure 18. Concentration-effect curves for **1616-19** administered *in vitro* further demonstrate the selectivity for GluN1/GluN2C NMDA receptors.

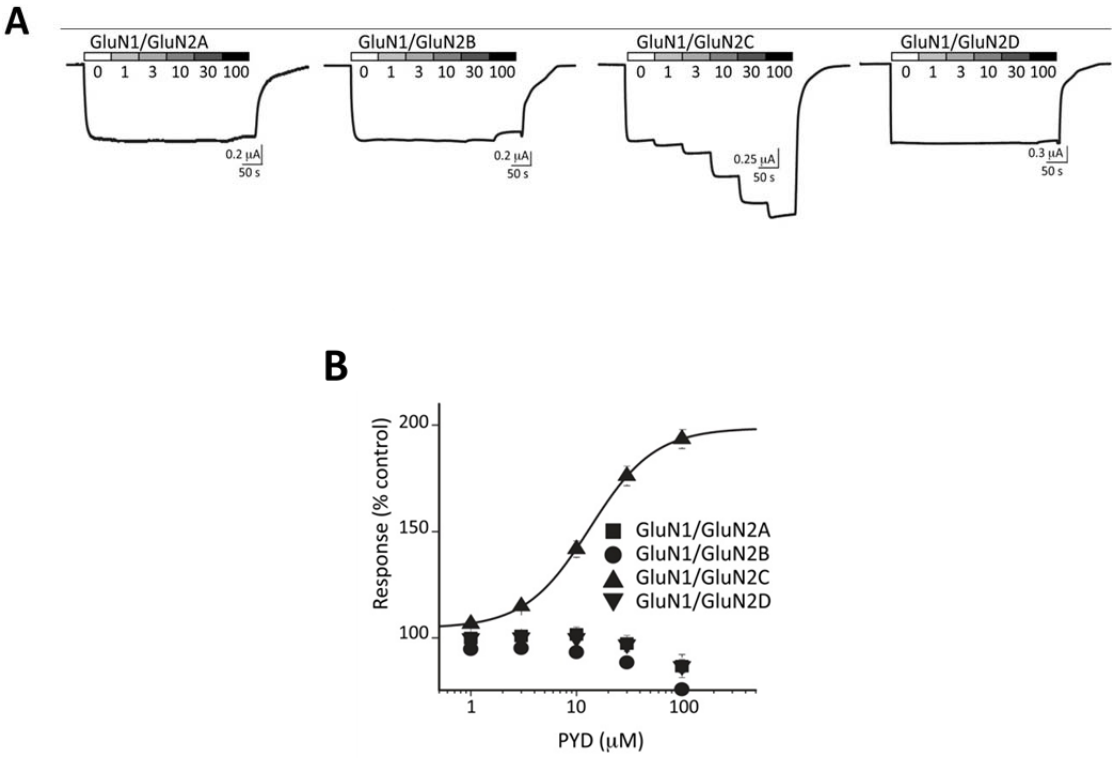


Figure 18. *In vitro* analysis of 1616 analogs: (A) Representative current recordings from two-electrode voltage clamp recordings of *Xenopus laevis* oocytes expressing recombinant NMDA receptors activated by 100 μM glutamate and 30 μM glycine in the presence of increasing concentrations of 1616-19 (1-100 μM). (B) Composite concentration-effect curves for 1616-19 at GluN2A-, GluN2B-, GluN2C- and GluN2D-containing NMDA receptors.

In order to identify the structural determinants of activity of 1616 analogs, a series of chimeras were generated by the Traynelis lab and tested against **1616**. Specifically, chimeric GluN1/GluN2C and chimeric GluN1/GluN2A receptors were prepared (Figure 19). Gain-of-function (GoF) chimeras were generated by inserting portions of GluN2C into GluN2A and loss-of-function (LoF) chimeras were generated by inserting portions of GluN2A into GluN2C.

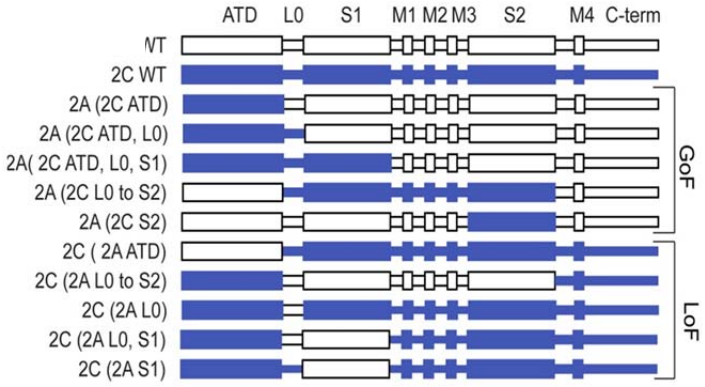


Figure 19. Schematic diagram of GluN1/GluN2A and GluN1/GluN2C chimeras that were prepared.

The gain-of-function chimera in which the ATD, L0, and S1 region were transferred from GluN2C to GluN2A (2A (2C ATD, L0, S1)) effectively resulted in potentiation of the GluN2A subunit (Figure 20). Loss-of-function chimeras were subsequently prepared in which the ATD, L0, or S1 of GluN2A was transferred to GluN2C. Replacement of either the ATD (2C (2A ATD)) or S2 (2C (2A L0)) domain of GluN2C with that of GluN2A resulted in complete loss of activity. While activity was observed for the loss-of-function chimera GluN2C (2A L0), the maximum potentiation was significantly reduced (147 ± 2.2%, n = 2).

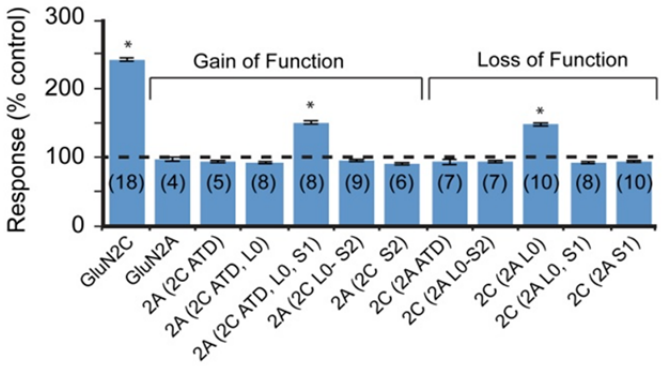


Figure 20. Chimeric receptors indicate that the ATD and S1 regions of the GluN2C subunit are essential for activity of 1616 (Khatri, Zimmerman, Liotta, Traynelis, unpublished data).

A series of 61 point mutations made in the ATD, L0 and S1 region were evaluated for their ability to effect potentiation by **1616**. These data revealed two residues in the S1 domain, K470 and S472, which are required for activity (Figure 21). In addition, residues S393 and R401 which are located in the L0 domain, also effect the potentiating actions of **1616** (Figure 22).

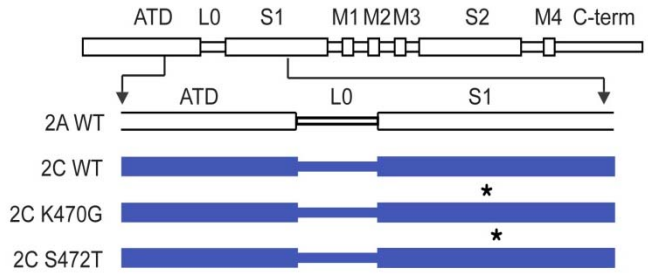


Figure 21. Point mutations in the ATD, L0 and S1 domains indicate two residues in the S2 domain that are essential for activity of 1616

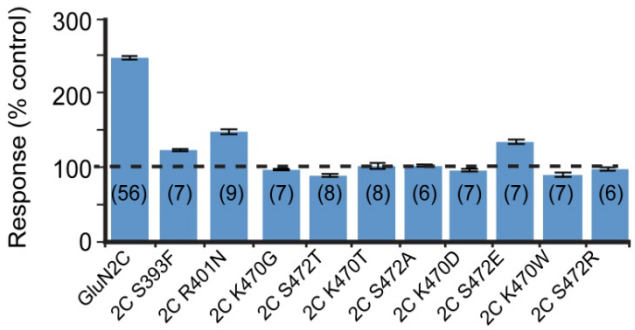


Figure 22. Residues K470, S472, S393 and R401 alter potentiation of 1616. (Khatri, Zimmerman, Liotta, Traynelis, unpublished data).

Figure 23A illustrates a homology model of the GluN1 (blue) and GluN2C (orange) subunits based on a crystal structure of both an AMPA and a NMDA receptor. The two residues required for the activity of **1616** are indicated and may account for the potentiation of the 1616-series.

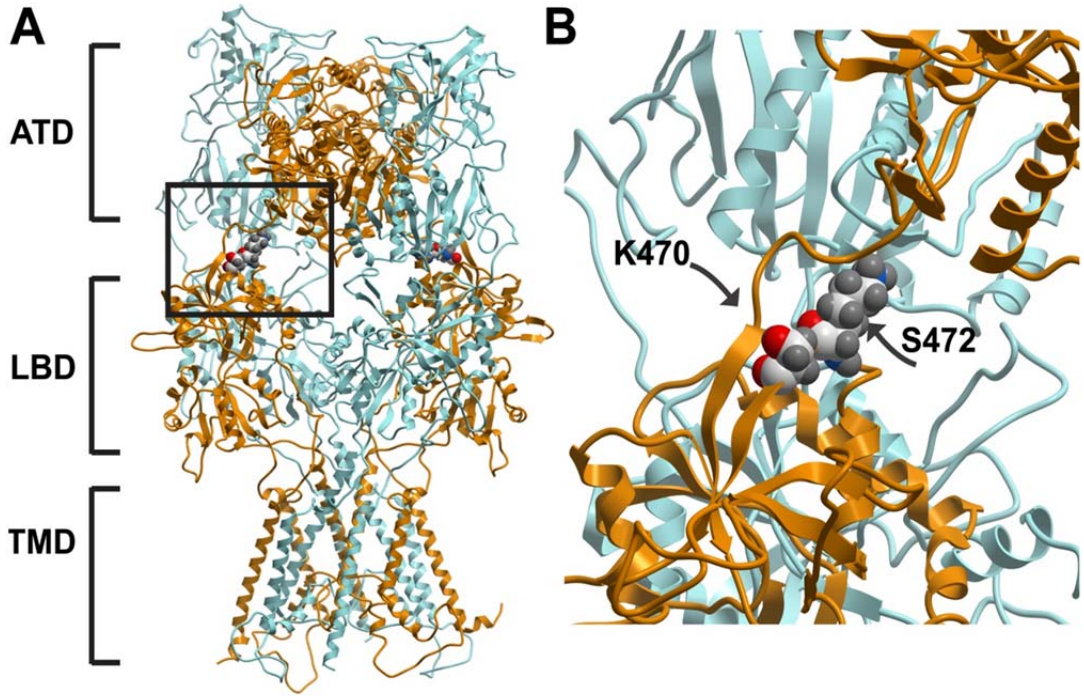


Figure 23. (A) Homology model of GluN1/GluN2C subunits. (B) Residues K470 and S472 are located at the top of the S1 domain, near the ATD. (Khatri, unpublished data)

2.4.9 In vivo Analysis of 1616-Series Analogs

Pharmacokinetic properties of **1616**, including half-life and bioavailability, were evaluated by the Lundbeck A/S. Wistar rats were injected subcutaneously (5 mg/kg) with **1616** and the concentration measured in plasma. **1616** demonstrated a peak plasma concentration of 289 ng/mL (0.69 μM) and a half-life ($t_{1/2}$) of ~2.5 h. No detectable levels were observed in the brain (C. Bundgaard, personal communication).

2.5 CONCLUSIONS

Inhibition of NMDA activity has been linked to many neurological diseases. Here, we describe the first class of positive allosteric modulators that are selective for GluN2C-containing NMDA receptors over GluN2A-, GluN2B- and GluN2D-containing NMDA receptors.

A significant number of structurally similar analogs were synthesized based on the screening hit **1616**. The result of these efforts was the development of an extensive structure-activity relationship (Figure 24). While a range of substituents were well-tolerated at R^1, more potent analogs contained a *meta*-pyridine ring. Modification at R^{11} demonstrated that the hydrogen was not essential for activity, but larger ether or ester substituents failed to increase potency. All changes in the linker led to inactive compounds. Modifications of the A-ring indicated that a *para*-methyl or –ethyl ester is required for activity of the series. Nearly all other substitutions or replacements led to a complete loss of activity. In contrast, substitution on the B-ring generally resulted in active compounds, with substituents at R^9 offering the most significant improvements in potency. Specifically, substitution at R^9 with a chlorine or methyl led to the most potent analogs, **1616-80** and **1616-85**, respectively.

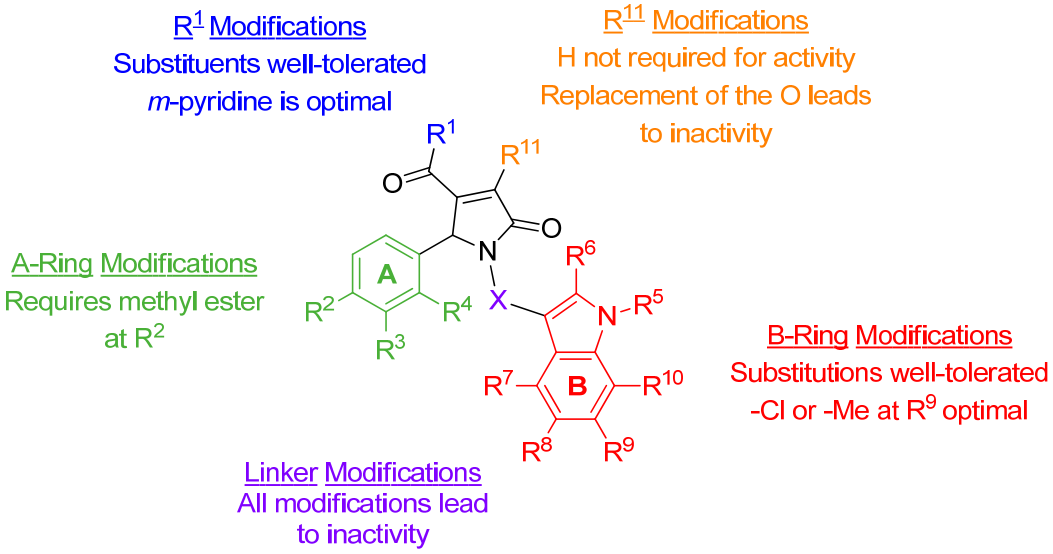
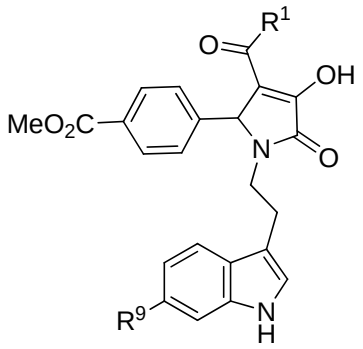


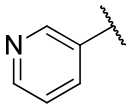
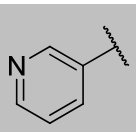
Figure 24. SAR summary of 1616-series

Separation of the enantiomers of **1616-19** demonstrated that the activity of the series could be attributed to only one enantiomer. **1616-19-1** resulted in an IC_{50} of 18 ± 0.6 μM and a maximum potentiation of 259%, while **1616-19-2** was inactive. The enantiomers were obtained in 99% *ee* (inactive) and 96% *ee* (active) using a ChiralPak OD-RH column (44% isocratic acetonitrile with 0.1% formic acid).

Through these efforts a novel modulator with structural determinants of selectivity residing within the linker (L0) between the ATD and S1 domains of the GluN1/GluN2C receptor has been established. Compounds **1616-80** and **1616-85** demonstrate significant improvements in potency compared to **1616** (Table 38). Both analogs have IC_{50}'s in the low single digit micromolar range and potentiate at ~200% at GluN2C-containing NMDA receptors.

Table 37. Comparison of the most potent 1616 analogs with the initial screening hit



Compound ID	R^1	R^9	$I_{30\ \mu M}/I_{CONTROL}$ (mean ± SEM)				EC_{50} (max.) μM (%)[a]
			GluN2A	GluN2B	GluN2C	GluN2D	GluN2C
1616	Me	H	95 ± 4.4	96 ± 4.1	196 ± 7.4	96 ± 3.0	24 ± 2.4 (275)
1616-80		Cl	93 ± 8.1	66 ± 1.4	170 ± 9.2	86 ± 3.0	8.5 ± 1.0 (204)
1616-85		Me	103 ± 1.1	82 ± 1.5	219 ± 5.4	86 ± 1.7	4.3 ± 0.3 (218)

[a] Fitted EC_{50} values are shown for GluN1/GluN2C to two significant digits when potentiation at 30 μM of the test compound exceeded 120%; values in parentheses are the fitted maximum response as a percentage of the initial glutamate (100 μM) and glycine (30 μM) response. Hill slopes were between 1.3-1.9. Data for active compounds at GluN1/GluN2C are from between 6-27 oocytes from 2-3 frogs for each compound tested. When no effect was found (n = 4-11 oocytes), the lack of effect was confirmed by testing at 100 μM (data not shown, n ≥ 4 oocytes for all compounds).

This class of potentiators offer a marked selectivity for GluN2C-containing NMDA receptors, with no activity observed at GluN2A-, GluN2B-, and GluN2D-containing NMDA receptors. These compounds may serve as a pharmacological tool to evaluate the role of the GluN2C subunit in normal and neuropathological function.

2.6 CHEMISTRY EXPERIMENTAL DETAIL

All dry solvents were obtained from a Glass Contour System. Reagents used were acquired from commercial suppliers and utilized without additional purification. Pre-coated glass plates (silica gel 60 F254, 0.25 mm) were used to monitor the progress of reactions by thin layer chromatography (TLC). Purification by flash column chromatography was performed on a Teledyne ISCO Combiflash Companion using prepackaged Teledyne RediSep disposable normal phase silica columns. ^{1}H and ^{13}C NMR were each carried out on an INOVA-400 (400 MHz), VNMRS-400 (400 MHz), INOVA-600 (600 MHz), Unity-600 (600 MHz), or Mercury 300 Vx (300 MHz). All chemical shifts are reported in parts per million and referenced to the residual solvent peak. All coupling constants are reported in Hertz (Hz). The IR spectra were acquired with a Nicolet Avatar 370 DTGS. Mass spectra were performed by the Emory University Mass Spectrometry Center on a VG 70-S Nier Johnson or JEOL instrument. Microanalyses were executed by Atlantic Microlab, Inc (Norcross, GA) for C, H, N, O. Purity of all final compounds was established by LCMS (Agilent), unless otherwise noted. The conditions used for purity determination are listed for each individual compound.

2.6.1 Chemistry Experimental Detail for 1616-Series

General Preparation of Pyrrolidinones (Procedure I). To a stirred solution of aldehyde (1.0 mmol) in dioxane (1.0 M) was added tryptamine (1.0 equiv) and 10 mol % pyridinium 4-methylbenzenesulfonate. Upon the formation of a slurry, methyl acetopyruvate (1.0 equiv) was added. The resulting mixture was allowed to stir at rt for up to 12 h. In most instances a precipitate had crashed out of solution, which was collected via filtration and washed with

Et_2O. The solid was dissolved in an appropriate solvent and washed with saturated ammonium chloride and brine, before being dried over $MgSO_4$, filtered and concentrated *in vacuo*. Additional purification was achieved via recrystallization with an appropriate solvent system to afford the desired pyrrole. If a precipitate did not form, the mixture was concentrated before being subjected to the work-up as described above. Purification was achieved via flash column chromatography on SiO_2 (MeOH/DCM) to afford the desired pyrrole. Additional purification was obtained by HPLC (85% CAN with 0.1% formic acid) as needed.

General Preparation of Methyl Hydroxy-4-vinylbenzoates (Procedure II). To a solution of methyl hydroxy-4-iodobenzoate (1.0 mmol) in THF:H_2O (4:1, 0.13 M) was added dibutyl vinylboronate (1.5 equiv), sodium carbonate (7.0 equiv) and 5 mol % dichloro-bis(triphenylphosphine)palladium. The reaction mixture was purged with N_2 (g) for 5 min before being refluxed for 2 h. The resulting mixture was concentrated *in vacuo*, diluted with EtOAc and washed with water and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to yield the product.

General Preparation of Methyl 4-Formylbenzoates (Procedure III). Methyl hydroxyl-4-vinylbenzoate (1.0 mmol) was dissolved in DCM (0.4 M) in a flask open to air. The reaction mixture was cooled to -78 °C and a stream of O_2 (g) was passed though it for 5 min. At this time, O_3 (g) was bubbled into the mixture until the color turned blue. The resulting solution was then purged with O_2 (g) for an additional 5 min before being treated with dimethylsulfane (3.0 equiv) and allowed to warm to rt overnight. The mixture was concentrated *in vacuo* and purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to yield the desired product.

General Preparation of 4-Formylbenzoates (Procedure IV). To a solution of 4-formylbenzoic acid (1.0 mmol) in DMF (0.26 M) was added finely ground potassium carbonate (2.0 equiv) and alkyl halide (2.5 equiv). The reaction stirred at rt until completion was indicated by TLC before being diluted with water and extracted with Et_2O (2x). The combined organic layers were then washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to afford the product.

General Preparation of Methyl 4-(Dibromomethyl)benzoates (Procedure V). To a solution of methyl benzoate (1.0 mmol) in carbon tetrachloride (0.1 M) was added *N*-bromosuccinimide (2.3 equiv) and benzoic peroxyanhydride (0.04 equiv). The reaction mixture was refluxed for 4 h. At this time the resulting solution was cooled to rt and filtered. The filtrate was collected, quenched with water and washed with saturated sodium thiosulfate (2x). The combined organic layers were then dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the desired methyl 4-(dibromomethyl)benzoate as a yellow oil. The crude material was then dissolved in acetone: water (5:1, 0.35 M) and silver nitrate (2.0 equiv) was added. The flask was covered with foil before being allowed to stir at rt for 3 h. The reaction mixture was then filtered through celite, diluted with EtOAc and extracted with saturated sodium bicarbonate (2x). The combined organic layers were washed with water and brine before being dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the product. Purification was achieved as needed via flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1).

General Preparation of Methyl 4-Formylbenzoates (Procedure VI). To a solution of methyl 4-bromobenzoate (1.0 mmol) in DMF (0.6 M) was added 17 mol% bis(triphenylphosphine)palladium (II) dichloride and sodium formate (1.5 equiv). The

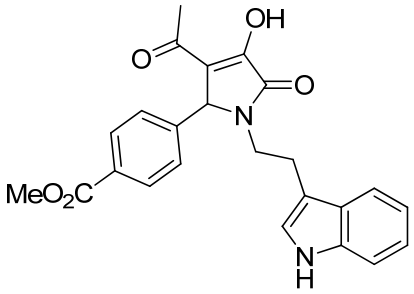
reaction mixture was stirred at 110 °C under a steady stream of CO for 2 h. At this time, the mixture was cooled to rt, diluted with saturated sodium carbonate and extracted with EtOAc (2x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 3/1) to yield the desired product, which was taken on without further purification. The crude material was then dissolved in acetone/water (83/17, 0.35 M) and silver nitrate (2.0 equiv) was added. The flask was covered with foil before being allowed to stir at rt for 3 h. The resulting mixture was filtered through celite, diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to give the product.

General Preparation of Methyl 4-Formylbenzoates (Procedure VII). To a solution of 4-bromobenzoic acid (1.0 mmol) in THF: MeOH (4:1, 0.3 M) at 0 °C was added (diazomethyl)trimethylsilane (2.4 equiv). The reaction was allowed to warm to rt over the period of 1 h. At this time the mixture was concentrated *in vacuo* and 1.0 M HCl was added. The mixture was extracted with EtOAc (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the product.

General Preparation of Pyruvates (Procedure VIII). To a solution of sodium ethanolate (1.0 equiv) in EtOH (0.72 M) at 0 °C was added a mixture of diethyl oxalate (1.0 equiv) and ethanone (1.0 mmol) over 20 min. The mixture was allowed to stir at rt for 4 h. In most instances a precipitate had formed which was collected via filtration and washed with absolute EtOH. If no precipitate was evident a minimal amount of water was added and the mixture was concentrated *in vacuo*. The residue was dissolved in water, neutralized with acetic acid and extracted with Et_2O (3x). The combined organic layers were dried over

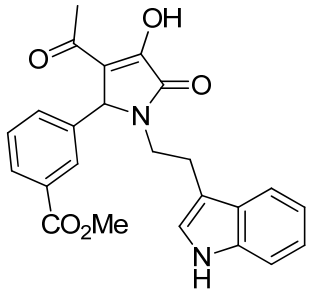
$MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved as needed via flash column chromatography on SiO_2 (Hexanes/EtOAc: 4/1) to obtain the product.

General Preparation of 4-Formylbenzamides (Procedure IX). To a solution of 4-formylbenzoic acid (1.0 mmol) in DMF (0.61 M) at 0 °C was added DMAP (1.1 equiv) and EDCI (1.0 equiv). The reaction mixture was stirred at 0 °C for 45 minutes. At this time the corresponding amine (1.0 equiv) was added and the mixture was warmed to room temperature and stirred overnight. The resulting mixture was concentrated *in vacuo*, partitioned between 1.0 M HCl and EtOAc and extracted with EtOAc (2x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 1/1) to afford the product.

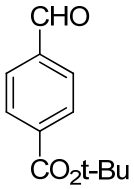


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-00**).* Compound **1616-00** was prepared via Procedure I from methyl 4-formylbenzoate (3.0 g, 18 mmol), tryptamine (2.9 g, 18 mmol) and methyl acetopyruvate (2.6 g, 18 mmol) to yield a cream colored solid (5.5 g, 72 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.33-7.24 (m, 4H), 7.12-7.03 (m, 2H), 6.91 (t, J = 7.6 Hz, 1H), 5.17 (s, 1H), 3.83 (s, 3H), 3.83-3.77 (m, 1H), 3.00-2.90 (m, 1H), 2.87-2.80 (m, 1H), 2.74-2.67 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 165.1, 142.5, 136.3, 129.3, 128.1, 126.9,

125.5, 122.9, 121.2, 121.1, 118.4, 118.3, 118.1, 111.6, 111.5, 110.8, 66.4, 59.8, 52.2, 40.8, 23.6; mp 99-105 °C; HRMS (APCI) Calcd for $C_{24}H_{22}N_2O_5$ 419.1607; found 419.1606 $[M+H]^+$.

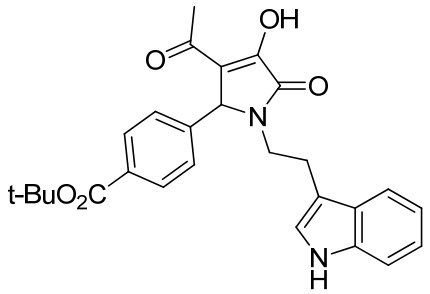


*Methyl 3-(1-2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-02**).* Compound **1616-02** was prepared via Procedure I from methyl 3-formylbenzoate (0.50 g, 3.1 mmol), tryptamine (0.49 g, 3.1 mmol) and methyl acetopyruvate (0.44 g, 3.1 mmol) to yield a pale pink solid (0.77 g, 60 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.73 (s, 1H), 7.45 (t, J = 7.2 Hz, 1H), 7.39-7.26 (m, 3H), 7.10 (d, J = 2.0 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 6.89 (t, J = 8.0 Hz, 1H), 5.24 (s, 1H), 3.85-3.76 (m, 4H), 2.98-2.91 (m, 1H), 2.87-2.80 (m, 1H), 2.72-2.65 (m, 1H), 2.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.5, 166.0, 165.1, 154.8, 137.8, 136.3, 132.5, 129.9, 129.1, 129.0, 128.4, 126.9, 122.9, 121.1, 119.8, 118.3, 118.1, 111.5, 110.8, 59.8, 52.3, 40.8, 29.8, 23.7; mp 218-220 °C; HRMS (APCI) Calcd for $C_{24}H_{22}N_2O_5$ 419.1593; found 419.1596 $[M + H]^+$; Anal. ($C_{24}H_{22}N_2O_5$) C, H, N.

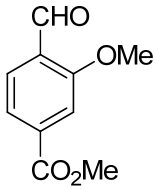


*tert-Butyl-4-formylbenzoate (**1616-03a**).* To a solution of 4-formylbenzoic acid (1.0 g, 6.7 mmol) in refluxing benzene (12.6 mL, 0.50 M) was added 1,1-di-*tert*-butoxy-*N,N*-dimethylmethanamine (6.4 mL, 26.6 mmol, 4.0 equiv) over a period of 1 h. The reaction

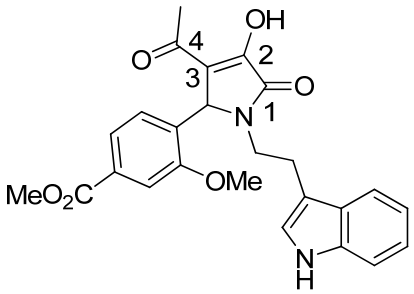
was then allowed to continue refluxing for 30 min before being cooled to rt and diluted with water. After washing with saturated sodium bicarbonate (2x), the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to yield a white solid (1.1 g, 81 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.10 (s, 1H), 8.14 (dd, J = 1.6 Hz, J = 6.8 Hz, 2H), 7.93 (dt, J = 1.6 Hz, J = 7.6 Hz, 2H), 1.62 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 164.9, 139.0, 137.2, 130.2, 129.6, 82.2, 28.3; HRMS (APCI) Calcd for $C_{12}H_{14}O_3$ 207.1016; found 207.1016 $[M+H]^+$.



*tert-Butyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl) benzoate (**1616-03**).* Compound **1616-03** was prepared via Procedure I from **1616-03a** (0.50 g, 2.4 mmol), tryptamine (0.39 g, 2.4 mmol) and methyl acetopyruvate (0.35 g, 2.4 mmol) to yield a pale yellow solid (0.92 g, 83 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.81 (d, J = 8.4 Hz, 2H), 7.33-7.22 (m, 4H), 7.10 (d, J = 2.0 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 6.91 (t, J = 7.6 Hz, 1H), 5.17 (s, 1H), 3.83-3.76 (m, 1H), 2.96-2.89 (m, 1H), 2.86-2.81 (m, 1H), 2.79-2.67 (m, 1H), 2.26 (s, 3H), 1.53 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.5, 165.1, 164.6, 154.4, 142.0, 131.1, 129.1, 127.9, 126.9, 126.2, 122.8, 121.0, 119.8, 118.2, 118.1, 111.4, 110.7, 80.7, 59.8, 40.8, 29.7, 27.8, 23.6; mp 145-150 °C; HRMS (APCI) Calcd for $C_{27}H_{28}N_2O_5$ 461.2063; found 461.2065 $[M+H]^+$.

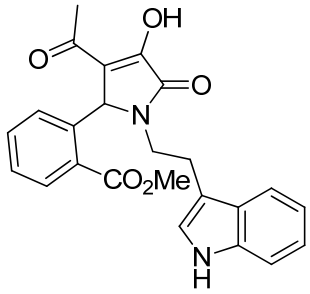


*Methyl 4-formyl-3-methoxybenzoate (**1616-04a**).* To a solution of 4-formyl-3-hydroxybenzoic acid (0.5 g, 3.0 mmol) in DMSO (5.2 mL, 0.60 M) was added finely ground potassium carbonate (2.6 g, 19 mmol) and methyl iodide (0.65 mL, 3.0 mmol, 1.0 equiv). The reaction mixture was allowed to stir at rt for 3 h before being diluted with water and extracted into EtOAc. The organic layer was washed with water (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was then purified using flash column chromatography on SiO_2 (5% MeOH: DCM) to yield a white solid (0.34 g, 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (s, 1H), 7.88 (dd, J = 0.8 Hz, J = 8.0 Hz, 1H), 7.70-7.67 (m, 2H), 4.00 (s, 3H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6, 166.3, 161.6, 136.6, 128.7, 127.7, 121.8, 113.0, 56.2, 52.8; mp 80-83 °C; HRMS (APCI) Calcd for $C_{10}H_{10}O_4$ 195.0652; found 195.0650 $[M+H]^+$.

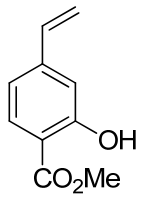


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl)-3-methoxybenzoate (**1616-04**).* Compound **1616-04** was prepared via Procedure I from **1616-04a** (0.20 g, 1.0 mmol), tryptamine (0.17 g, 1.0 mmol) and methyl acetopyruvate (0.15 g, 1.0 mmol) to yield an off-white solid (0.16 g, 36 %). ^{1}H NMR (600 MHz, DMSO-d_6, 56°C) δ 10.68 (s, 1H), 7.53 (s, 1H), 7.48 (dd, J = 1.6 Hz, J = 8.0 Hz, 1H), 7.32-7.28 (m, 2H), 7.06-

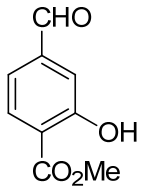
7.02 (m, 3H), 6.92 (td, J = 7.2 Hz, J = 0.8 Hz, 1H), 5.59 (s, 1H), 3.86 (s, 6H), 3.81-3.72 (m, 1H), 2.97-2.85 (m, 2H), 2.84-2.69 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.5, 165.9, 165.2, 157.9, 154.8, 136.2, 130.6, 126.9, 126.6, 122.7, 121.9, 121.0, 118.3, 117.8, 111.7, 111.5, 110.7, 56.0, 52.3, 41.0, 40.1, 29.7, 23.4 (Note: Carbon 3 and either Carbon 1 or 2 are absent); mp 103-107 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_6$ 449.1707; found 449.1704 $[M+H]^+$.



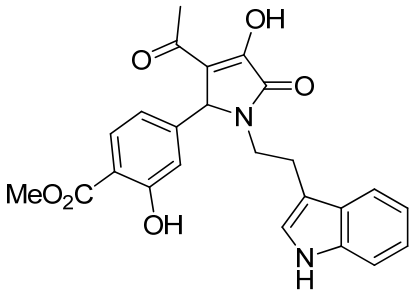
*Methyl 2-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl) benzoate (**1616-05**).* Compound **1616-05** was prepared via Procedure I from methyl 2-formylbenzoate (0.10 g, 0.61 mmol), tryptamine (0.098 g, 0.61 mmol) and methyl acetopyruvate (0.088 g, 0.61 mmol) to yield a white solid (0.18 g, 72 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.72 (s, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.32-7.25 (m, 2H), 7.10 (d, J = 1.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.89 (t, J = 7.6 Hz, 1H), 5.24 (s, 1H), 3.86 (s, 3H), 3.82-3.75 (m, 1H), 2.97-2.90 (m, 1H), 2.86-2.79 (m, 1H), 2.71-2.66 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.6, 166.0, 165.0, 154.5, 137.8, 136.2, 132.5, 129.8, 129.0, 128.9, 128.3, 126.8, 122.9, 121.0, 119.9, 118.2, 118.0, 111.4, 110.7, 59.7, 52.2, 40.8, 29.8, 23.6; mp 210-218 °C; HRMS (APCI) Calcd for $C_{24}H_{22}N_2O_5$ 419.1602; found 419.1599 $[M+H]^+$.



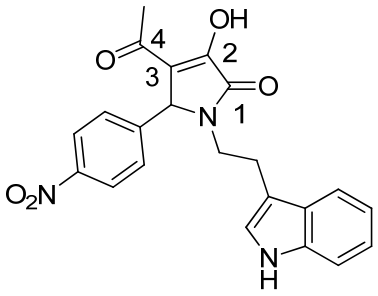
*Methyl 2-hydroxy-4-vinylbenzoate (**1616-07a**).* Compound **1616-07a** was prepared via Procedure II from methyl 2-hydroxy-4-iodobenzoate (1.0 g, 3.6 mmol) to yield a clear oil (0.51 g, 80 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 1.6 Hz, 1H), 6.95 (dd, *J* = 1.6 Hz, *J* = 8.4 Hz, 1H), 6.68 (dd, *J* = 10.8 Hz, *J* = 17.6 Hz, 1H), 5.87 (d, *J* = 17.6 Hz,1H), 5.40 (d, *J* = 10.4 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 161.9, 145.0, 136.1, 130.2, 117.5, 117.3, 115.1, 111.7, 52.5; HRMS (APCI) Calcd for $C_{10}H_{10}O_3$ 179.0703; found 179.0701.



*Methyl 4-formyl-2-hydroxybenzoate (**1616-07b**).* Compound **1616-07b** was prepared via Procedure III from **1616-07a** (0.49 g, 2.8 mmol) to afford a white solid (0.30 g, 60 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 10.02 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.41 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 4.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 170.1, 162.1, 141.6, 131.0, 119.6, 119.0, 117.0, 53.1; HRMS (APCI) Calcd for $C_9H_8O_4$ 181.0495; found 181.0493 $[M+H]^+$.

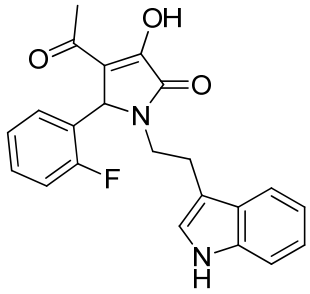


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl)-2-hydroxybenzoate (**1616-07**).* Compound **1616-07** was prepared via Procedure I from **1616-07a** (0.15 g, 0.83 mmol), tryptamine (0.13 g, 0.83 mmol) and methyl acetopyruvate (0.12 g, 0.83 mmol) to yield a brown solid (0.10 g, 27 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 10.49 (s, 1H), 7.68 (dd, J = 8.0 Hz, J = 2.4 Hz, 1H), 7.34-7.30 (m, 2H), 7.10 (s, 1H), 7.05 (t, J = 6.8 Hz, 1H), 6.92 (t, J = 6.8 Hz, 1H), 6.84 (s, 1H), 6.64 (d, J = 8.0 Hz, 1H), 5.12 (s, 1H), 3.86 (s, 3H), 3.83-3.76 (m, 1H), 2.98-2.83 (m, 2H), 2.75-2.68 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.7, 168.8, 165.0, 159.8, 154.4, 154.1, 136.0, 130.3, 126.9, 122.8, 121.0, 119.6, 118.3, 118.2, 118.1, 117.0, 112.8, 111.4, 110.7, 59.6, 52.4, 40.9, 29.8, 23.5; mp 188-190 °C; HRMS (APCI) Calcd for $C_{24}H_{22}N_2O_6$ 435.1551; found 435.1549 $[M+H]^+$. Anal. ($C_{24}H_{22}N_2O_6$) C, H, N.



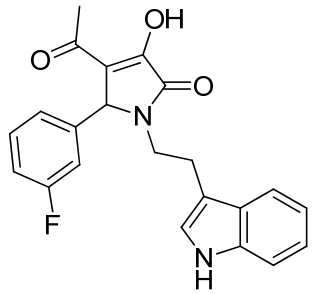
*1-(2-(1H-Indol-3-yl)ethyl)-4-acetyl-3-hydroxy-5-(4-nitrophenyl)-1H-pyrrol-2(5H)-one (**1616-08**).* Compound **1616-08** was prepared via Procedure I from 4-nitrobenzaldehyde (0.50 g, 3.3 mmol), tryptamine (0.53 g, 3.3 mmol) and methyl acetopyruvate (0.48 g, 3.3 mmol) to yield a pale yellow solid (1.0 g, 75 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (br s, 1H), 10.82 (s,

1H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 1.6 Hz, 1H), 7.04 (t, *J* = 8.0 Hz, 1H), 6.91 (t, *J* = 7.6 Hz, 1H), 5.26 (s, 1H), 3.85-3.78 (m, 1H), 2.97-2.84 (m, 2H), 2.80-2.77 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.6, 165.2, 154.7, 147.1, 144.9, 136.2, 129.0, 126.8, 123.5, 122.9, 121.0, 118.2, 118.0, 111.4, 110.7, 59.4, 41.0, 29.8, 23.5 (Note: Carbon 3 is absent); mp 142-150 °C; HRMS (APCI) Calcd for $C_{22}H_{19}N_3O_5$ 406.1398; found 406.1395 $[M+H]^+$.



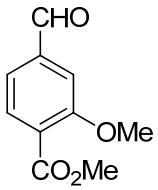
*1-(2-(1H-Indol-3-yl)ethyl)-4-acetyl-5-(2-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (**1616-09**).*

Compound **1616-09** was prepared via Procedure I from 2-fluorobenzaldehyde (0.42 mL, 4.0 mmol), tryptamine (0.65 g, 4.0 mmol) and methyl acetopyruvate (0.58 g, 4.0 mmol) to yield a cream colored solid (1.0 g, 67 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (br s, 1H), 10.83 (s, 1H), 7.38-7.27 (m, 3H), 7.22-7.03 (m, 5H), 6.92 (t, *J* = 6.8 Hz, 1H), 5.41 (s, 1H), 3.83-3.76 (m, 1H), 2.98-2.86 (m, 2H), 2.85-2.67 (m, 1H), 2.29 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 197.3, 165.1, 161.0 (d, *J* = 246.5 Hz), 154.7, 136.2, 130.1, (d, *J* = 7.8 Hz), 128.0, 126.9, 125.5, 124.7, 123.5, 122.8, 121.0, 118.3, 117.8, 115.7 (d, *J* = 21.3 Hz), 111.4, 110.5, 40.9, 40.0, 29.8, 23.4; mp 180-182 °C; HRMS (APCI) Calcd for $C_{27}H_{19}FN_2O_3$ 379.1444; found 379.1448 $[M+H]^+$.

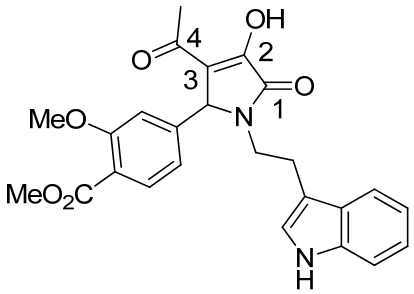


*1-(2-(1H-Indol-3-yl)ethyl)-4-acetyl-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (**1616-10**).*

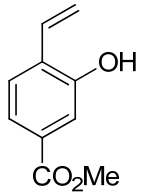
Compound **1616-10** was prepared via Procedure I from 3-fluorobenzaldehyde (0.43 mL, 4.0 mmol), tryptamine (0.65 g, 4.0 mmol) and methyl acetopyruvate (0.58 g, 4.0 mmol) to yield a light brown solid (0.87 g, 57 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (br s, 1H), 10.83 (s, 1H), 7.37-7.31 (m, 3H), 7.13-7.04 (m, 3H), 6.98-6.91 (m, 3H), 5.13 (s, 1H), 3.84-3.77 (m, 1H), 2.98-2.82 (m, 2H), 2.75-2.71 (m, 1H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.6, 164.9, 162.1 (d, J = 242.6), 154.4, 139.9, 136.2, 130.4 (d, J = 7.8 Hz), 128.0, 126.9, 125.5, 123.5, 122.8, 121.0, 119.7, 118.2 (d, J = 25.2 Hz), 114.9 (d, J = 20.4 Hz), 114.7 (d, J = 18.5 Hz), 111.1 (d, J = 100.8 Hz), 59.6, 40.7, 40.0, 23.6; mp 172-178 °C; HRMS (APCI) Calcd for $C_{22}H_{19}FN_2O_3$ 379.1453; found 379.1449 $[M+H]^+$. Anal. ($C_{22}H_{19}FN_2O_3$) C, H, N.



*Methyl 4-formyl-2-methoxybenzoate (**1616-11a**).* Compound **1616-11a** was prepared via Procedure V from methyl 2-methoxy-4-methylbenzoate (0.50 g, 2.8 mmol) to yield a yellow oil (0.21 g, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.49-7.47 (m, 2H), 3.97 (s, 3H), 3.93 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.7, 166.2, 159.3, 139.9, 132.1, 125.9, 122.9, 110.8, 56.4, 52.7. HRMS (APCI) Calcd for $C_{10}H_{10}O_4$ 193.0495; found 193.0495 $[M-H]^-$.

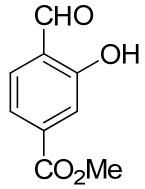


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl)-2-methoxybenzoate (**1616-11**).* Compound **1616-11** was prepared via Procedure I from **1616-11a** (0.1 g, 0.52 mmol), tryptamine (0.083 g, 0.52 mmol) and methyl acetopyruvate (0.074 g, 0.52 mmol) to yield a cream colored solid (0.17 g, 74 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.34-7.31 (m, 2H), 7.11 (s, 1H), 7.06 (t, J = 6.8 Hz, 1H), 6.94-6.91 (m, 2H), 6.70 (d, J = 8.0 Hz, 1H), 5.14 (s, 1H), 3.83-3.76 (m, 7H), 2.99-2.84 (m, 2H), 2.75-2.69 (m, 1H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.9, 165.0, 158.1, 136.2, 130.9, 128.1, 126.9, 125.5, 125.0, 122.9, 121.0, 119.6, 118.6, 118.3, 118.1, 111.5, 110.8, 60.0, 55.9, 51.9, 40.9, 40.0, 23.6 (Note: Carbons 3 and 4 are absent); mp 130-135 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_6$ 449.1707; found 449.1709 $[M+H]^+$.

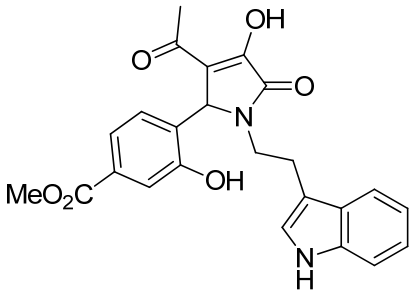


*Methyl 3-hydroxy-4-vinylbenzoate (**1616-12a**).* Compound **1616-12a** was prepared via Procedure II from methyl 3-hydroxy-4-iodobenzoate (0.5 g, 1.8 mmol) to yield a pale yellow solid (0.22 g, 68 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (dd, J = 1.2 Hz, J = 7.8 Hz, 1H), 7.55 (d, J = 1.2 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.00 (dd, J = 10.8 Hz, J = 18.0 Hz, 1H), 5.88 (d, J = 18.0 Hz, 1H), 5.59 (br s, 1H), 5.47 (d, J = 11.4 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (150 MHz,

$CDCl_3$) δ 167.5, 153.3, 131.0, 130.2, 129.9, 127.2, 122.1, 117.8, 117.2, 52.6; mp 85-89 °C; HRMS (APCI) Calcd for $C_{10}H_{10}O_3$ 179.0703; found 179.0703 $[M+H]^+$.

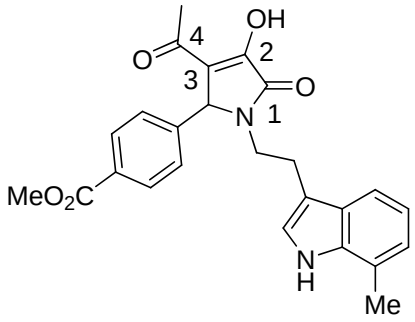


*Methyl 4-formyl-3-hydroxybenzoate (**1616-12b**).* Compound **1616-12b** was prepared via Procedure III from **1616-12a** (0.22 g, 1.2 mmol) to afford a pale yellow solid (0.19 g, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (s, 1H), 10.00 (s, 1H), 7.67-7.66 (m, 3H), 3.96 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.7, 165.9, 161.4, 137.5, 133.8, 123.1, 120.6, 119.3, 52.9; HRMS (APCI) Calcd for $C_9H_8O_4$ 181.0495; found 181.0496 $[M+H]^+$.

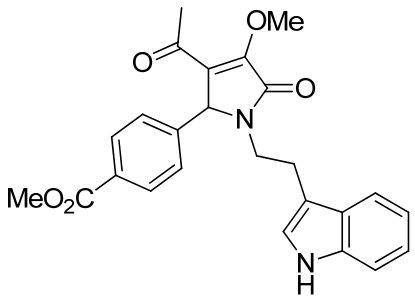


*Methyl 4-(1-(2-(1H-indol-3-yl) ethyl)-3-acetyl-4-hydroxy-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl)-3-hydroxybenzoate (**1616-12**).* Compound **1616-12** was prepared via Procedure I from **1616-12b** (0.1 g, 0.56 mmol), tryptamine (0.089 g, 0.52 mmol) and methyl acetopyruvate (0.080 g, 0.56 mmol) to yield an off-white solid (0.020 g, 8 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1H), 10.07 (br s, 1H), 7.47 (s, 1H), 7.35-7.29 (m, 3H), 7.05-7.02 (m, 3H), 6.91 (t, J = 10.8 Hz, 1H), 5.58 (s, 1H), 3.83 (s, 3H), 3.80-3.74 (m, 1H), 3.02-2.88 (m, 2H), 2.76-2.68 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 196.6, 190.2, 163.6, 151.5, 136.4, 136.2, 127.0, 126.9, 123.5, 123.0, 122.5, 121.0, 118.32, 118.25, 118.0, 111.4, 111.2, 110.4, 94.5, 52.7, 52.2,

44.8, 29.3, 26.7; mp 194-197 °C; HRMS (APCI) Calcd for $C_{24}H_{22}N_2O_6$ 435.1551; found 435.1552 $[M+H]^+$.

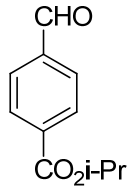


*Methyl 4-(3-acetyl-4-hydroxy-1-(2-(7-methyl-1H-indol-3-yl) ethyl)-5-oxo-2, 5-dihydro-1H-pyrrol-2-yl) benzoate (**1616-13**).* Compound **1616-13** was prepared via Procedure I from methyl 4-formylbenzoate (0.094 g, 0.57 mmol), 2-(7-methyl-1*H*-indol-3-yl) ethanamine (0.10 g, 0.57 mmol) and methyl acetopyruvate (0.083 g, 0.57 mmol) to yield a white solid (0.16 g, 66 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 10.78 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.12-7.07 (m, 2H), 6.83-6.82 (m, 2H), 5.20 (s, 1H), 3.83-3.76 (m, 4H), 2.98-2.80 (m, 2H), 2.80-2.66 (m, 1H), 2.41 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.9, 165.0, 142.4, 135.7, 129.3, 128.1, 126.5, 125.5, 122.5, 121.5, 120.5, 118.5, 115.7, 111.1, 109.7, 59.7, 52.1, 40.8, 40.0, 23.7, 16.7 (Note: Carbons 3 and 4 are absent); mp 220-227 °C; HRMS (APCI) Calcd from $C_{25}H_{24}N_2O_5$ 433.1758; found 433.1758 $[M+H]^+$.

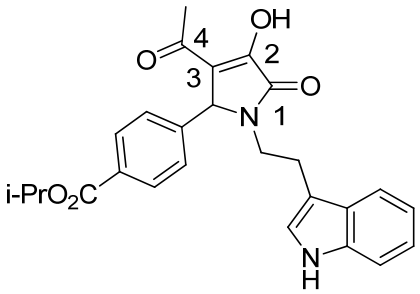


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-14**).* To a solution of **1616-14a** (0.50 g, 1.2 mmol) in DCM: MeOH (1:1, 0.13 M) was added (diazomethyl)trimethylsilane (0.72 mL, 1.4 mmol). The reaction mixture continued to

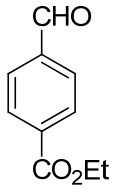
stir at rt for 5 h before being concentrated *in vacuo*. The crude residue was then purified using flash column chromatography on SiO_2 (3% MeOH: DCM) to yield a pale yellow solid (0.24 g, 46 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 7.88 (dd, J = 1.6 Hz, J = 8.0 Hz, 2H), 7.33-7.24 (m, 4H), 7.09-7.03 (m, 2H), 6.92 (t, J = 7.6 Hz, 1H), 5.20 (s, 1H), 4.36 (s, 3H), 3.84 (s, 3H), 3.80-3.71 (m, 1H), 2.96-2.89 (m, 1H), 2.84-2.77 (m, 1H), 2.72-2.63 (m, 1H), 2.25 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.9, 165.9, 164.2, 154.0, 141.7, 136.2, 129.5, 129.3, 128.2, 126.8, 126.2, 122.9, 121.0, 118.3, 118.0, 111.5, 110.7, 59.5, 59.1, 52.2, 40.9, 30.3, 23.6; mp 40-45 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_5$ 433.1763; found 433.1761 $[M+H]^+$; Anal. ($C_{25}H_{24}N_2O_5$) C, H, N.



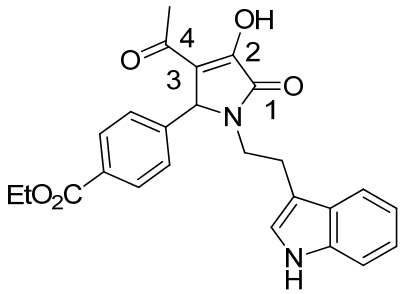
*Isopropyl 4-formylbenzoate (**1616-15a**).* Compound **1616-15a** was prepared via Procedure IV from 4-formylbenzoic acid (1.0 g, 6.7 mmol) and 2-iodopropane (1.7 mL, 17 mmol, 2.5 equiv) to yield a white solid (0.30 g, 24 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.09 (s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 5.28 (sept, J = 6.6 Hz, 1H), 1.39 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.0, 165.2, 139.2, 136.1, 130.3, 129.6, 69.4, 22.1; HRMS (APCI) Calcd for $C_{11}H_{12}O_3$ 193.0859; found 193.0860 $[M+H]^+$.



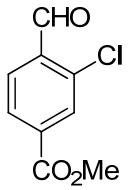
*Isopropyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-15**).* Compound **1616-15** was prepared via Procedure I from **1616-15a** (0.20 g, 1.0 mmol), tryptamine (0.17 g, 1.0 mmol) and methyl acetopyruvate (0.15 g, 1.0 mmol) to yield an orange, amorphous solid (0.028 g, 6 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.33-7.23 (m, 3H), 7.11-7.00 (m, 3H), 6.91 (t, J = 7.6 Hz, 1H), 5.16-5.11 (m, 2H), 3.82-3.77 (m, 1H), 3.02-2.85 (m, 2H), 2.76-2.71 (m, 1H), 2.26 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.0, 164.9, 136.2, 129.2, 128.1, 126.8, 125.5, 123.5, 122.8, 121.2, 121.0, 118.5, 118.3, 118.1, 111.5, 110.8, 68.1, 59.8, 40.9, 39.9, 23.6, 21.6 (Note: Carbons 1 and 2 are absent); HRMS (APCI) Calcd for $C_{26}H_{26}N_2O_5$ 447.1928; found 447.1922 $[M+H]^+$.



*Ethyl 4-formylbenzoate (**1616-16a**).* Compound **1616-16a** was prepared via Procedure IV from 4-formylbenzoic acid (1.0 g, 6.7 mmol) and iodoethane (1.3 mL, 17 mmol, 2.5 equiv) to yield a yellow oil (1.0 g, 87 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.11 (s, 1H), 8.21 (d, J = 8.4 Hz, 2H), 7.96 (dd, J = 1.8 Hz, J = 7.2 Hz, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.9, 165.8, 139.3, 135.7, 130.4, 129.7, 61.8, 14.5; HRMS (APCI) Calcd for $C_{10}H_{10}O_3$ 179.0703; found 179.0703 $[M+H]^+$.

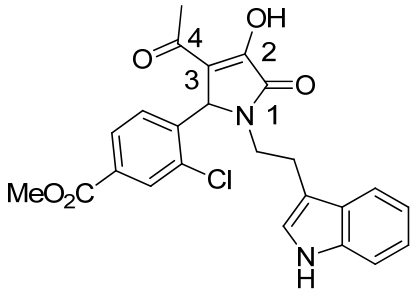


*Ethyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-16**).* Compound **1616-16** was prepared via Procedure I from **1616-16a** (0.20 g, 1.1 mmol), tryptamine (0.18 g, 1.1 mmol) and methyl acetopyruvate (0.16 g, 1.1 mmol) to yield a pale pink solid (0.20 g, 42 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 10.82 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.33-7.24 (m, 4H), 7.09 (d, J = 2.4 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 6.91 (t, J = 7.6 Hz, 1H), 5.18 (s, 1H), 4.30 (q, J = 7.2 Hz, 2H), 3.83-3.76 (m, 1H), 2.97-2.89 (m, 1H), 2.87-2.80 (m, 1H), 2.74-2.67 (m, 1H), 2.26 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.6, 165.4, 165.1, 142.4, 136.2, 129.6, 129.3, 128.1, 126.9, 122.9, 121.0, 118.3, 118.1, 111.5, 110.7, 60.7, 59.8, 40.8, 39.9, 23.6, 14.2 (Note: Carbon 3 and either Carbon 1 or 2 are absent); mp 180-183 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_5$ 433.1771; found 433.1765 $[M+H]^+$.

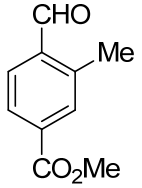


*Methyl 3-chloro-4-formylbenzoate (**1616-17a**).* Compound **1616-17a** was prepared via Procedure V from methyl 3-chloro-4-methylbenzoate (1.0 g, 5.4 mmol) to yield a white solid (0.61 g, 63 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.13 (d, J = 1.8 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.2 Hz, 1H), 3.97 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.4, 173.1,

165.2, 136.1, 135.3, 132.0, 129.6, 128.3, 53.1; mp 47-50°C; HRMS (APCI) Calcd for $C_9H_7ClO_3$ 197.0000; found 197.0000 $[M-H]^-$.

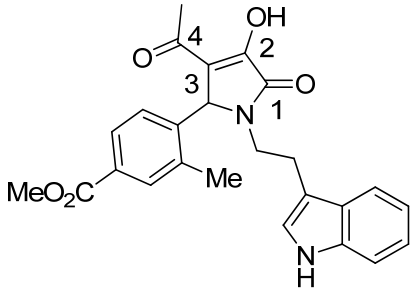


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-3-chlorobenzoate (**1616-17**).* Compound **1616-17** was prepared via Procedure I from **1616-17a** (0.10 g, 0.50 mmol), tryptamine (0.081 g, 0.50 mmol) and methyl acetopyruvate (0.073 g, 0.50 mmol) to yield a pale yellow solid (0.080 g, 35 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 10.81 (s, 1H), 7.95 (d, J = 1.6 Hz, 1H), 7.76 (dd, J = 1.2 Hz, J = 8.0 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 7.09-7.01 (m, 3H), 6.91 (t, J = 7.6 Hz, 1H), 5.68 (s, 1H), 3.85 (s, 3H), 3.80-3.73 (m, 1H), 2.97-2.84 (m, 2H), 2.77-2.72 (m, 1H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.5, 170.4, 165.5, 164.8, 139.8, 136.2, 134.7, 130.8, 129.8, 128.2, 127.8, 126.9, 122.8, 121.0, 118.3, 117.7, 111.5, 110.4, 59.8, 55.6, 52.6, 41.5, 23.5 (Note: Carbon 3 is absent); mp 55-60 °C; HRMS (APCI) Calcd for $C_{24}H_{21}ClN_2O_5$ 453.1225; found 453.1222 $[M+H]^+$.



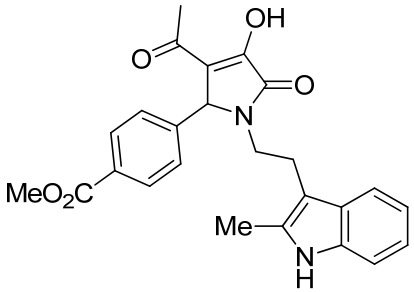
*Methyl 4-formyl-3-methylbenzoate (**1616-18a**).* To a solution of methyl 4-iodo-3-methylbenzoate (1.0 g, 3.6 mmol) in THF (24 mL, 0.15 M) at -15 °C was added isopropylmagnesium chloride (7.2 mL, 14.5 mmol, 4.0 equiv). The reaction mixture was allowed to continue stirring at -15

°C for 2 h before *N,N*-dimethylformamide (1.4 mL, 18 mmol, 5.0 equiv) was added. The mixture was warmed to room temperature over a period of 1 h. At this time the reaction was quenched with HCl and extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved using flash column chromatography on SiO_2 (Hexanes/EtOAc: 6/1) to yield a white solid (0.45 g, 70 %) which was taken on without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.10-8.00 (m, 1H), 7.95-7.87 (m, 2H), 3.96 (s, 3H), 2.73 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.5, 166.5, 140.8, 137.1, 134.3, 133.1, 131.9, 127.5, 52.8, 19.7; HRMS (APCI) Calcd for $C_{10}H_{10}O_3$ 177.0546; found 177.0541 $[M+H]^+$.

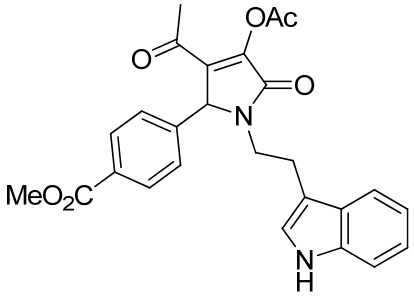


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-3-methylbenzoate (**1616-18**).* Compound **1616-18** was prepared via Procedure I from **1616-18a** (0.10 g, 0.56 mmol), tryptamine (0.090 g, 0.56 mmol) and methyl acetopyruvate (0.081 g, 0.56 mmol) to yield an orange solid (0.12 g, 50 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.75 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 1.8 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 6.90 (t, J = 7.8 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.27 (s, 1H), 3.81 (s, 3H), 3.75 (dt, J = 8.4 Hz, J = 13.8 Hz, 1H), 2.94-2.89 (m, 1H), 2.76-2.69 (m, 2H), 2.31 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.0, 165.5, 140.6, 138.2, 136.3, 131.0, 128.8, 127.0, 126.8, 125.4, 122.9, 121.1, 118.3, 117.7, 111.5,

110.7, 55.6, 41.5, 40.1, 23.6, 18.5, 14.1 (Note: Carbon 3,4 and either Carbon 1 or 2); mp 60-70 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_5$ 433.1763; found 433.1764 $[M+H]^+$.

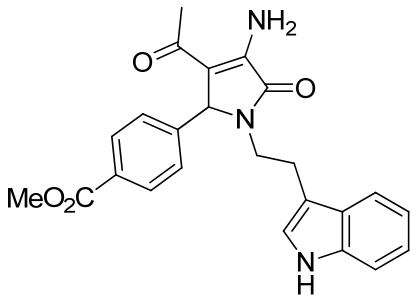


*Methyl 4-(3-acetyl-4-hydroxy-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-19**).* Compound **1616-19** was prepared via Procedure I from methyl 4-formylbenzoate (0.094 g, 0.57 mmol), 2-(2-methyl-1*H*-indol-3-yl)ethanamine (0.10 g, 0.57 mmol) and methyl acetopyruvate (0.083 g, 0.57 mmol) to yield a cream colored solid (0.18 g, 73 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.73 (s, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.21 (d, J = 7.8 Hz, 1H), 7.18-7.15 (m, 3H), 6.97 (t, J = 7.8 Hz, 1H), 6.86 (t, J = 7.8 Hz, 1H), 5.03 (s, 1H), 3.83 (s, 3H), 3.64-3.59 (m, 1H), 2.92-2.87 (m, 1H), 2.77-2.72 (m, 1H), 2.60-2.56 (m, 1H), 2.26 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.1, 165.9, 165.0, 142.4, 135.1, 132.2, 129.8, 129.7, 129.3, 128.1, 127.9, 125.6, 120.1, 118.2, 117.0, 110.5, 106.5, 60.1, 52.1, 40.9, 39.9, 24.5, 10.9; mp 182-187 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_5$ 433.1758; found 433.1759 $[M+H]^+$.



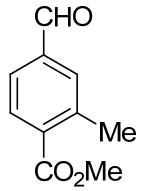
*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-acetoxy-3-acetyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-20**).* To a solution of **1616** (0.50 g, 1.2 mmol) in DCM (11 mL, 0.11 M) was added acetic

anhydride (0.14 mL, 1.4 mmol, 1.2 equiv) and pyridine (0.14 mL, 1.8 mmol, 1.5 equiv). The reaction mixture was stirred at rt for 6 ½ h before being concentrated *in vacuo*. The crude material was then purified by flash column chromatography on SiO_2 (3% MeOH: DCM). Additional purification was achieved using HPLC (ACN/Water: 3/1, isocratic) to give a yellow oil (0.038 g, 7 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.94-7.92 (m, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.21 (t, J = 8.0 Hz, 1H), 7.10-7.05 (m, 3H), 6.98 (d, J = 2.0 Hz, 1H), 4.93 (s, 1H), 4.07-4.00 (m, 1H), 3.91 (s, 3H), 3.08-2.89 (m, 3H), 2.47 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.5, 167.0, 166.6, 163.8, 147.7, 139.2, 137.0, 136.6, 131.0, 130.4, 128.1, 127.4, 122.6, 122.2, 119.9, 118.7, 112.6, 111.5, 62.3, 62.4, 41.5, 30.1, 24.4, 20.8; HRMS (APCI) Calcd for $C_{26}H_{24}N_2O_6$ 461.1721; found 461.1717 $[M+H]^+$.

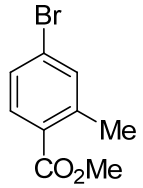


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-amino-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-21**).* To a solution of **1616** (0.50 g, 1.2 mmol) in 2-methoxyethanol (8.36 mL, 0.14 M) was added ammonium formate (0.11 mL, 2.2 mmol, 1.8 equiv). The reaction mixture was refluxed for 3 h before being concentrated *in vacuo*, ground with a mortar and pestle and triturated with Et_2O. Further purification was achieved via flash column chromatography on SiO_2 (MeOH/DCM: 1/6) to yield a pale yellow solid (0.070 g, 14 %). ^{1}H NMR (400 MHz, $CDCl_3$, 56 °C) δ 10.02 (br s, 1H), 8.36 (br s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.39-7.34 (m, 2H), 7.17 (t, J = 7.6 Hz, 1H), 7.08-7.02 (m, 3H), 6.97 (s, 1H), 6.44 (br s, 1H), 4.77 (s, 1H), 4.04-3.97 (m, 1H), 3.91 (s, 3H), 3.09-2.99 (m, 2H), 2.92-2.85 (m, 1H), 1.56 (s, 3H); ^{13}C NMR (150

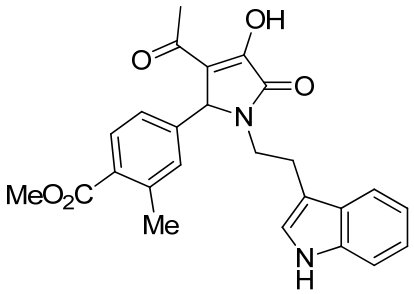
MHz, DMSO-d_6) δ 177.8, 165.9, 164.4, 163.2, 144.7, 136.2, 129.7, 129.5, 128.3, 126.9, 122.7, 121.0, 118.2, 118.0, 111.4, 110.8, 105.6, 58.0, 52.1, 48.6, 41.0, 23.2; mp 50-54 °C; HRMS (APCI) Calcd for $C_{24}H_{23}N_3O_4$ 418.1766; found 418.1766 $[M+H]^+$.



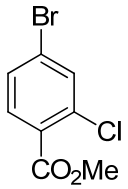
*Methyl 4-formyl-2-methylbenzoate (**1616-23b**).* Compound **1616-23b** was prepared via Procedure VII from **1616-23a** (1.0 g, 4.4 mmol) to yield a clear oil (0.15 g, 19 %) which was taken on without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.76-7.75 (m, 2H), 3.94 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 167.5, 141.0, 138.3, 132.8, 131.3, 130.7, 126.9.52.5, 21.7; HRMS (APCI) Calcd for $C_{10}H_{10}O_3$ 179.0703; found 179.0703 $[M+H]^+$.



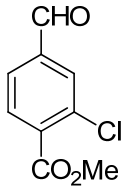
*Methyl 4-bromo-2-methylbenzoate (**1616-23a**).* Compound **1616-23a** was prepared via Procedure VIII from 4-bromo-2-methylbenzoic acid (1.0 g, 4.7 mmol) to give a yellow oil (0.98 g, 92 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.39 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 3.89 (s, 3H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 142.6, 134.8, 132.3, 129.2, 128.5, 126.9, 52.2, 21.8; HRMS (APCI) Calcd for $C_9H_9BrO_2$ 228.9859; found 228.9860 $[M-H]^-$.



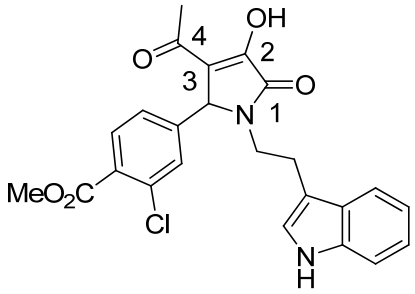
*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-2-methylbenzoate (**1616-23**).* Compound **1616-23** was prepared via Procedure I from **1616-23b** (0.10 g, 0.56 mmol), tryptamine (0.090 g, 0.56 mmol) and methyl acetopyruvate (0.081 g, 0.56 mmol) to yield a pale orange solid (0.13 g, 53 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.40-7.36 (m, 2H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.00 (s, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.74 (s, 1H), 4.76 (s, 1H), 4.07-4.02 (m, 1H), 3.90 (s, 3H), 3.11-2.93 (m, 3H), 2.51 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.6, 167.6, 164.6, 159.8, 141.4, 139.0, 136.5, 131.5, 131.3, 130.5, 127.2, 125.2, 122.6, 122.3, 119.8, 119.5, 118.7, 112.4, 111.6, 61.4, 52.2, 41.3, 28.2, 24.4, 21.9; mp 40-43 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_5$ 433.1745; found 433.1745 $[M+H]^+$.



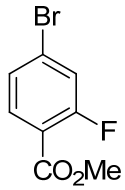
*Methyl 4-bromo-2-chlorobenzoate (**1616-24a**).* Compound **1616-24a** was prepared via Procedure VIII from 4-bromo-2-chlorobenzoic acid (2.0 g, 8.5 mmol) to give an orange oil (2.1 g, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.47 (dd, *J* = 2.0 Hz, *J* = 8.4 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 135.2, 134.0, 132.8, 130.2, 128.9, 126.7, 52.8; HRMS (APCI) Calcd for $C_8H_6BrClO_2$ 248.9312; found 248.9313 $[M-H]^-$.



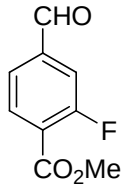
*Methyl 2-chloro-4-formylbenzoate (**1616-24b**).* Compound **1616-24b** was prepared via Procedure VII from **1616-24a** (1.0 g, 4.0 mmol) to yield a yellow oil (0.19 g, 24 %) which was taken on without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 7.97-7.94 (m, 2H), 7.84-7.81 (m, 1H), 3.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.4, 162.7, 139.0, 135.3, 134.7, 132.0, 131.9, 127.4, 53.1; HRMS (APCI) Calcd for $C_9H_7ClO_3$ 197.0000; found 197.0000 $[M-H]^-$.



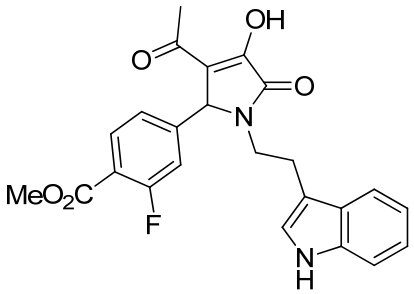
*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-2-chlorobenzoate (**1616-24**).* Compound **1616-24** was prepared via Procedure I from **1616-24b** (0.09 g, 0.45 mmol), tryptamine (0.073 g, 0.45 mmol) and methyl acetopyruvate (0.065 g, 0.45 mmol) to yield a pale orange, amorphous solid (0.14 g, 68 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.40-7.23 (m, 4H), 7.14-7.03 (m, 2H), 6.93 (t, J = 7.6 Hz, 1H), 5.13 (s, 1H), 3.83-3.78 (m, 4H), 2.98-2.82 (m, 2H), 2.75-2.68 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.8, 165.2, 142.8, 136.2, 131.8, 131.2, 130.5, 129.4, 128.0, 126.8, 126.0, 122.9, 121.0, 118.3, 118.0, 111.4, 110.7, 59.1, 52.5, 48.6, 40.8, 23.6 (Note: Carbons 1 and 2 are absent); HRMS (APCI) Calcd for 453.1225; found 453.1219 $[M+H]^+$.



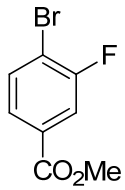
*Methyl 4-bromo-2-fluorobenzoate (**1616-25a**).* Compound **1616-25a** was prepared via Procedure VII from 4-bromo-2-fluorobenzoic acid (1.0 g, 4.6 mmol) to give an off-white solid (0.93 g, 88 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.81 (m, 1H), 7.39-7.34 (m, 2H), 3.94 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2 (d, J = 6.3 Hz), 163.5 (d, J = 269.7 Hz), 133.3, 128.1 (d, J = 8.3 Hz), 127.7, 120.8 (d, J = 26.9 Hz), 117.8 (d, J = 10.2 Hz), 52.7; HRMS (APCI) Calcd for $C_8H_6BrFO_2$ 232.9608; found 232.9609 $[M+H]^+$.



*Methyl 2-fluoro-4-formylbenzoate (**1616-25b**).* Compound **1616-25b** was prepared via Procedure VI from **1616-25a** (0.91 g, 3.9 mmol) to yield a white solid (0.080 g, 11 %) which was taken on without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.12 (td, J = 0.8 Hz, J = 7.6 Hz, 1H), 7.74 (dd, J = 1.6 Hz, J = 8.0 Hz, 1H), 7.66 (dd, J = 1.6 Hz, J = 10.0 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.3, 164.1, 162.1 (d, J = 261 Hz), 141.0 (d, J = 6.7 Hz), 133.2 (d, J = 9.7 Hz), 125.1 (d, J = 4.4 Hz), 120.8 (d, J = 25.3 Hz), 117.3 (d, J = 23.1 Hz), 53.0; HRMS (APCI) Calcd for $C_9H_7FO_3$ 183.0353; found 183.0352 $[M+H]^+$.

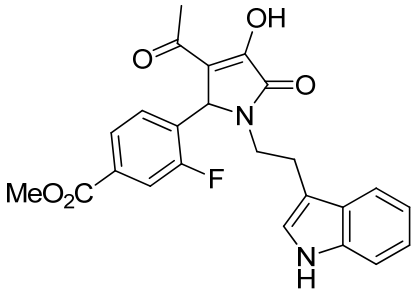


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-2-fluorobenzoate (**1616-25**).* Compound **1616-25** was prepared via Procedure I from **1616-25b** (0.08 g, 0.44 mmol), tryptamine (0.070 g, 0.44 mmol) and methyl acetopyruvate (0.063 g, 0.44 mmol) to yield a pale orange solid (0.096 g, 50 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.40-7.27 (m, 2H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.14-7.06 (m, 1H), 6.95 (d, *J* = 1.2 Hz, 1H), 6.78 (dd, *J* = 1.2 Hz, *J* = 7.6 Hz, 1H), 6.70 (dd, *J* = 1.2 Hz, *J* = 10.8 Hz, 1H), 4.80 (s, 1H), 4.09-4.03 (m, 1H), 3.92 (s, 3H), 3.10-2.96 (m, 3H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.1, 193.6, 165.5, 162.0 (d, *J* = 260.4 Hz), 142.8 (d, *J* = 7.4 Hz), 136.5, 132.8, 127.0, 123.5, 123.4 (d, *J* = 26.0 Hz), 122.5, 122.3, 122.1, 120.1, 119.7, 118.7, 118.5, 111.9, 111.7, 61.2, 52.6, 41.6, 29.2, 24.4; mp 40-45 °C; HRMS (APCI) Calcd for $C_{24}H_{21}FN_2O_5$ 437.1512; found 437.1512 $[M+H]^+$.

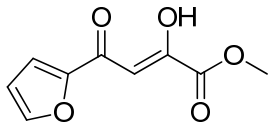


*Methyl 4-bromo-3-fluorobenzoate (**1616-26a**).* Compound **1616-26a** was prepared via Procedure VII from 4-bromo-3-fluorobenzoic acid (2.0 g, 9.1 mmol) to give a yellow oil (2.1 g, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 2.0 Hz, *J* = 8.8 Hz, 1H), 7.67 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.63-7.59 (m, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 159.0

(d, *J* = 247.4 Hz), 138.8, 131.5 (d, *J* = 6.4 Hz), 126.3 (d, *J* = 1.9 Hz), 117.5 (d, *J* = 12.0 Hz), 114.9 (d, *J* = 21.3 Hz), 52.7; HRMS (APCI) Calcd for $C_8H_6BrFO_2$ 232.9608; found 232.9609.

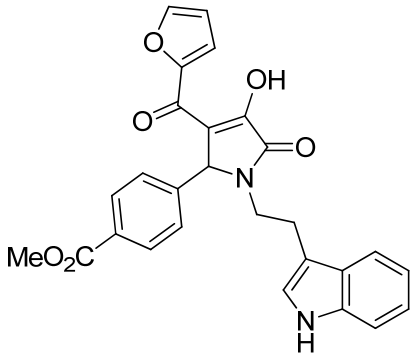


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-3-fluorobenzoate (**1616-26**).* Compound **1616-26** was prepared via Procedure VI from **1616-26a** (2.0 g, 8.7 mmol) to yield methyl 3-fluoro-4-formylbenzoate as a clear oil. The crude material was then combined with tryptamine (0.11 g, 0.67 mmol) and methyl acetopyruvate (0.097 g, 0.67 mmol) and carried on though Procedure I to yield an orange, amorphous solid (0.033 g, 11 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.31-7.27 (m, 2H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 6.93 (s, 1H), 5.52 (s, 1H), 4.02-3.97 (m, 1H), 3.88 (s, 3H), 3.02-2.87 (m, 3H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.9, 191.4, 171.1, 167.1 (d, *J* = 186.7 Hz), 162.7, 136.4, 132.6, 130.9, 127.4, 125.4, 122.3, 121.9, 119.2, 118.7, 116.9 (d, *J* = 23.8), 114.0, 112.5, 111.3, 102.3, 52.4, 45.9, 41.3, 28.4, 24.1; HRMS (APCI) Calcd for $C_{24}H_{21}FN_2O_5$ 437.1507; found 437.1509 $[M+H]^+$.

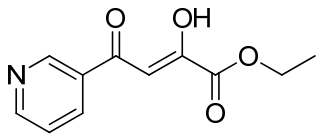


*(Z)-Methyl 4-(furan-2-yl)-2-hydroxy-4-oxobut-2-enoate (**1616-27a**).* Compound **1616-27a**was prepared via Procedure VIII from 1-(furan-2-yl)ethanone (1.0 g, 9.1 mmol) in MeOH (4.1 mL, 2.2 M) to yield a yellow solid (1.1 g, 61 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, *J* =

1.2 Hz, 1H), 7.33 (d, *J* = 3.6 Hz, 1H), 6.93 (s, 1H), 6.61-6.61 (m, 1H), 3.92 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.2, 165.6, 162.6, 151.0, 148.0, 118.8, 113.3, 99.3, 53.3; HRMS (APCI) Calcd for $C_9H_8O_5$ 197.0445; found 197.0443 $[M+H]^+$.

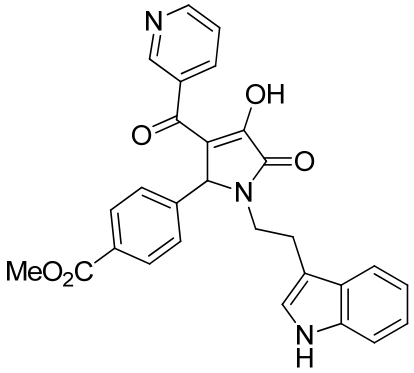


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-(furan-2-carbonyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-27**).* Compound **1616-27** was prepared via Procedure I from methyl 4-formylbenzoate (0.42 g, 2.6 mmol), tryptamine (0.41 g, 2.6 mmol) and **1616-27a** (0.50 g, 2.6 mmol) to yield an orange solid (0.079 g, 7 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.05 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.43-7.41 (m, 2H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.16 (t, *J* = 7.2 Hz, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 6.94 (s, 1H), 6.39 (dd, *J* = 1.8 Hz, *J* = 3.6 Hz, 1H), 5.39 (s, 1H), 4.04-3.99 (m, 1H), 3.88 (s, 3H), 3.07-2.96 (m, 2H), 2.93-2.89 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 167.1, 153.1, 145.1, 136.5, 129.8, 129.7, 129.2, 128.4, 128.2, 127.4, 122.3, 122.2, 119.5, 118.9, 117.1, 114.2, 112.7, 111.7, 111.4, 111.3, 61.3, 52.2, 41.3, 24.3; mp 60-65 °C; HRMS (APCI) Calcd for $C_{27}H_{22}N_2O_6$ 471.1551; found 471.1547 $[M+H]^+$.

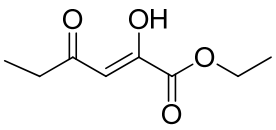


*(Z)-ethyl 2-hydroxy-4-oxo-4-(pyridin-3-yl)but-2-enoate (**1616-28a**).* Compound **1616-28a** was prepared via Procedure VIII from 1-(pyridin-3-yl)ethanone (9.0 mL, 83 mmol) to yield a pale

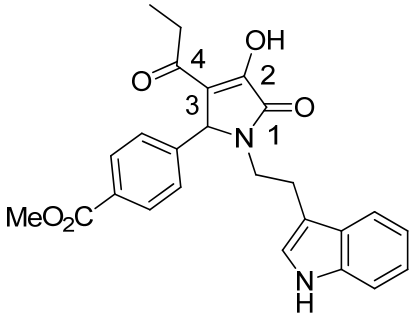
yellow solid (7.3 g, 40 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.19 (t, *J* = 1.2 Hz, 1H), 8.81 (d, *J* = 4.8 Hz, 1H), 8.26 (dt, *J* = 1.2 Hz, *J* = 8.4 Hz, 1H), 7.46 (dd, *J* = 4.8 Hz, *J* = 7.8 Hz, 1H), 7.07 (s, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.8, 181.1, 171.0, 161.9, 154.1, 149.3, 135.3, 130.7, 123.9, 63.0, 14.2; mp 43-45 °C; HRMS (APCI) Calcd for $C_{11}H_{11}NO_4$ 222.0761; found 222.0759 $[M+H]^+$.



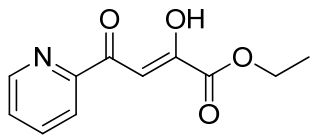
*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-28**).* Compound **1616-28** was prepared via Procedure I from methyl 4-formylbenzoate (0.079 g, 0.48 mmol), tryptamine (0.077 g, 0.45 mmol) and **1616-28a** (0.10 g, 0.48 mmol) to yield a yellow solid (0.11 g, 49 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.80 (s, 1H), 8.69 (d, *J* = 4.4 Hz, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 7.2 Hz, 2H), 7.43-7.42 (m, 2H), 7.34-7.29 (m, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 7.06 (t, *J* = 6.8 Hz, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 5.42 (s, 1H), 3.87-3.82 (m, 4H), 3.02-2.89 (m, 2H), 2.79-2.74 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.8, 165.9, 165.0, 152.1, 149.1, 142.1, 136.3, 133.9, 129.5, 129.4, 128.3, 128.1, 126.9, 125.5, 123.5, 122.9, 121.0, 118.3, 118.1, 111.5, 110.7, 109.5, 60.4, 52.2, 41.1, 23.7; mp 199-205 °C; HRMS (APCI) Calcd for $C_{28}H_{23}N_3O_5$ 482.1711; found 482.1707 $[M+H]^+$.



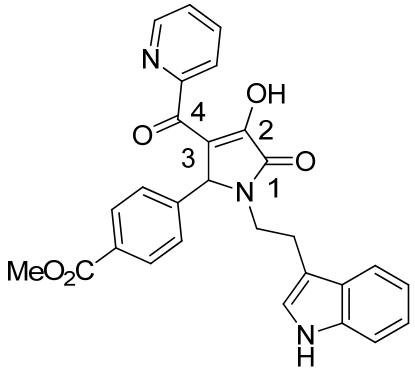
*(Z)-Ethyl 2-hydroxy-4-oxohex-2-enoate (**1616-29a**).* Compound **1616-29a** was prepared via Procedure VIII from butan-2-one (1.2 mL, 14 mmol) to yield a yellow oil (0.58 g, 24 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.33 (s, 1H), 4.31 (q, *J* = 5.4 MHz, 2H), 2.50 (q, *J* = 7.2 Hz, 2H), 1.33 (t, *J* = 9.0 Hz, 3H), 1.13 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 204.2, 181.2, 162.2, 101.4, 62.5, 34.3, 14.1, 8.7; HRMS (APCI) Calcd for $C_8H_{12}O_4$ 173.0808; found 173.0805 $[M+H]^+$.



*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-5-oxo-3-propionyl-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-29**).* Compound **1616-29** was prepared via Procedure I from methyl 4-formylbenzoate (0.095 g, 0.58 mmol), tryptamine (0.93 g, 0.58 mmol) and **1616-29a** (0.10 g, 0.58 mmol) to yield a cream colored solid (0.16 g, 64 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.33-7.23 (m, 4H), 7.09 (d, *J* = 2.4 Hz, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.91 (t, *J* = 6.8 Hz, 1H), 5.17 (s, 1H), 3.83-3.76 (m, 4H), 2.96-2.89 (m, 1H), 2.86-2.79 (m, 1H), 2.75-2.57 (m, 3H), 0.85 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.9, 165.1, 142.5, 136.2, 129.7, 129.3, 128.1, 126.9, 125.5, 122.9, 121.0, 118.2, 118.1, 111.5, 110.7, 59.8, 54.9, 52.2, 40.8, 40.0, 23.6 (Note: Carbons 1 and 2 are absent); mp 175-180 °C; HRMS (APCI) Calcd $C_{25}H_{24}N_2O_5$ 433.1758; found 433.1756 $[M+H]^+$.

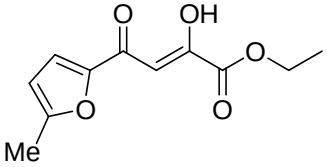


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(pyridin-2-yl)but-2-enoate (**1616-30a**).* Compound **1616-30a** was prepared via Procedure VIII from 1-(pyridin-2-yl)ethanone to yield a dark red solid (0.61 g, 33 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (d, *J* = 4.8 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.92 (td, *J* = 1.8 Hz, *J* = 7.2 Hz, 1H), 7.59 (s, 1H), 7.53 (ddd, *J* = 1.8 Hz, *J* = 4.8 Hz, *J* = 7.8 Hz, 1H), 4.40 (q, *J* = 6.6 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 218.3, 181.2, 173.1, 151.9, 149.3, 137.7, 127.7, 123.2, 98.9, 62.8, 14.3; HRMS (APCI) Calcd for $C_{11}H_{11}NO_4$ 222.0761; found 222.0759 $[M+H]^+$.

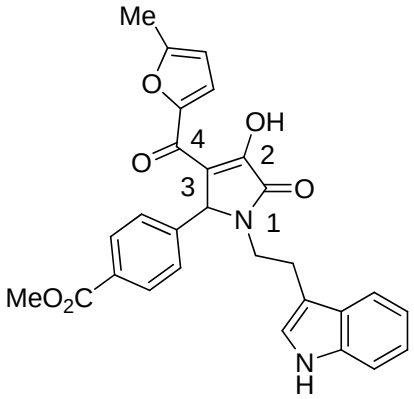


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-5-oxo-3-picolinoyl-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-30**).* Compound **1616-30** was prepared via Procedure I from methyl 4-formylbenzoate (0.074 g, 0.45 mmol), tryptamine (0.072 g, 0.45 mmol) and **1616-30a** (0.10 g, 0.45 mmol) to yield a yellow, amorphous solid (0.037 g, 17 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (d, *J* = 4.2 Hz, 1H), 8.21 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 8.07 (dt, *J* = 1.2 Hz, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.71 (dt, *J* = 0.6 Hz, *J* = 6.0 Hz, 1H), 7.39 (d, *J* = 7.2 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.21-7.17 (m, 3H), 7.07 (t, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 1.8 Hz, 1H), 5.14 (s, 1H), 4.15-4.10 (m, 1H), 3.89 (s, 3H), 3.11-2.95 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.8, 173.2, 166.8, 165.7, 151.7, 145.3, 142.5, 140.6, 136.5, 130.3, 130.0, 128.3, 128.1, 127.3,

125.2, 122.3, 119.6, 118.7, 112.5, 111.5, 109.6, 61.6, 41.4, 29.9, 24.3 (Note: Carbon 3 is absent); HRMS (APCI) Calcd for $C_{28}H_{23}N_3O_5$ 482.1710; found 482.1708 $[M+H]^+$.

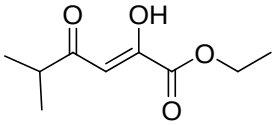


*(Z)-Ethyl 2-hydroxy-4-(5-methylfuran-2-yl)-4-oxobut-2-enoate (**1616-31a**).* Compound **1616-31a** was prepared via Procedure VIII from 1-(5-methylfuran-2-yl)ethanone (1.0 g, 8.1 mmol) to yield a black solid (0.53 g, 29 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (br s, 1H), 7.28 (d, *J* = 3.6 Hz, 1H), 6.87 (s, 1H), 6.26 (d, *J* = 3.2 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 2.45 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 165.0, 162.4, 159.8, 149.8, 120.9, 110.3, 99.3, 62.7, 14.4, 14.3; HRMS (APCI) Calcd for $C_{11}H_{12}O_5$ 225.0758; found 225.0754 $[M+H]^+$.

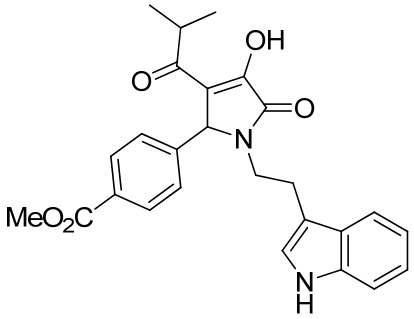


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(5-methylfuran-2-carbonyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-31**).* Compound **1616-31** was prepared via Procedure I from methyl 4-formylbenzoate (0.073 g, 0.45 mmol), tryptamine (0.071 g, 0.45 mmol) and **1616-31a** (0.10 g, 0.45 mmol) to yield a yellow solid (0.081 g, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 3.2 Hz, 1H), 7.02 (s, 1H), 6.99 (d, *J* = 8.4

Hz, 2H), 6.06 (d, *J* = 3.2 Hz, 1H), 5.14 (s, 1H), 4.07-4.01 (m, 1H), 3.89 (s, 3H), 3.17-3.10 (m, 1H), 3.07-2.93 (m, 2H), 2.15 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.0, 174.2, 166.6, 163.1, 159.1, 148.9, 141.3, 136.6, 130.7, 130.3, 130.0, 127.3, 122.6, 121.7, 119.8, 119.0, 115.4, 112.7, 111.5, 110.2, 61.6, 52.5, 41.3, 24.5, 14.3 (Note: Carbon 3 is absent); mp 180-183 °C; HRMS (APCI) Calcd for $C_{26}H_{24}N_2O_6$ 485.1712; found 485.1712 $[M+H]^+$.

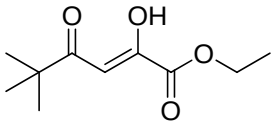


*(Z)-Ethyl 2-hydroxy-5-methyl-4-oxohex-2-enoate (**1616-32a**).* Compound **1616-32a** was prepared via Procedure VIII from 3-methylbutan-2-one (1.0 g, 12 mmol) to yield a black oil (1.8 g, 83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 2.62 (sept, *J* = 7.2 Hz, 1H), 1.32 (t, *J* = 6.4 Hz, 3H), 1.13 (d, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CDCl3) δ 207.4, 167.1, 162.3, 100.2, 62.6, 39.1, 18.7, 14.1, 14.0; HRMS (APCI) Calcd for $C_9H1_4O_4$ 187.0965; found 187.0963 $[M+H]^+$.

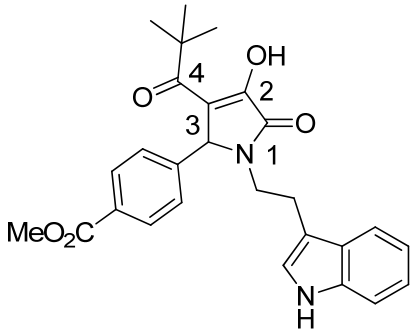


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-isobutyryl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-32**).* Compound **1616-32** was prepared via Procedure I from methyl 4-formylbenzoate (0.088 g, 0.54 mmol), tryptamine (0.086 g, 0.54 mmol) and **1616-32a** (0.10 g, 0.54 mmol) to yield a light brown, amorphous solid (0.067 g, 28 %). ^{1}H NMR (600 MHz, DMSO-d_6, 70°C) δ 10.65 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.06-7.03 (m, 2H), 6.92 (t, *J* = 8.4 Hz, 1H), 5.18 (s, 1H), 3.83 (s, 3H), 3.79-3.75 (m, 1H),

3.40-3.20 (m, 2H), 2.98-2.93 (m, 1H), 2.91-2.86 (m, 1H), 2.72-2.67 (m, 1H), 0.86 (d, J = 4.8 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.1, 166.0, 136.2, 129.2, 128.1, 128.0, 126.9, 125.5, 123.4, 122.8, 121.1, 121.0, 118.5, 118.2, 118.1, 111.6, 111.5, 110.9, 109.5, 60.0, 52.1, 40.9, 23.7, 23.2, 18.5, 17.7; HRMS (APCI) Calcd for $C_{26}H_{26}N_2O_5$ 447.1915; found 447.1916 $[M+H]^+$.

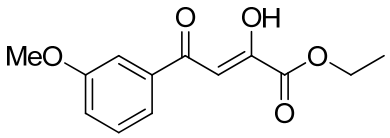


*(Z)-Ethyl 2-hydroxy-5,5-dimethyl-4-oxohex-2-enoate (**1616-33a**).* Compound **1616-33a** was prepared via Procedure VIII from 3,3-dimethylbutan-2-one (1.0 g, 10 mmol) to yield a yellow oil (0.62 g, 31 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (s, 1H), 4.30 (q, *J* = 6.8 Hz, 2H), 1.32 (t, *J* = 6.8 Hz, 3H), 1.16 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.0, 167.4, 162.0, 97.7, 62.2, 41.4, 26.5, 13.9; HRMS (APCI) Calcd for $C_{10}H_{16}O_4$ 201.1121; found 201.1119 $[M+H]^+$.

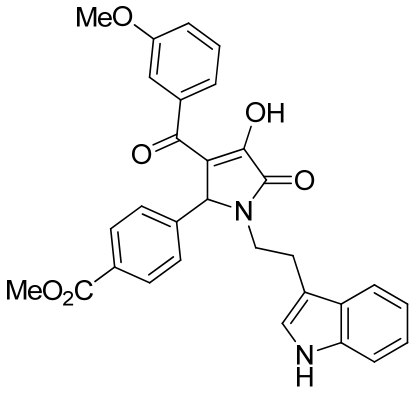


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-5-oxo-3-pivaloyl-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-33**).* Compound **1616-33** was prepared via Procedure I from methyl 4-formylbenzoate (0.082 g, 0.50 mmol), tryptamine (0.080 g, 0.50 mmol) and **1616-33a** (0.10 g, 0.50 mmol) to yield an orange oil (0.092 g, 40 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (br s, 1H), 7.93 (d, *J* = 7.8 Hz, 2H), 7.40-7.35 (m, 2H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 7.04 (dd, *J* = 1.2 Hz, *J* = 7.8 Hz, 2H), 6.96 (s, 1H), 5.04 (s, 1H), 4.02-3.99 (m, 1H), 3.91 (s, 3H), 3.09-

3.00 (m, 2H), 2.95-2.91 (m, 1H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.2, 181.2, 166.7, 141.2, 136.5, 130.6, 130.2, 128.0, 127.2, 122.5, 122.2, 119.8, 118.8, 118.7, 111.6, 111.4, 63.0, 52.4, 41.7, 27.7, 25.3, 24.5 (Note: Either Carbon 1 or 2 is absent); HRMS (APCI) Calcd for $C_{27}H_{28}N_2O_5$ 461.2071; found 461.2077 $[M+H]^+$.

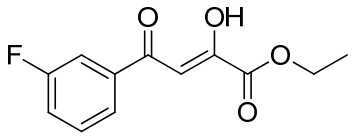


*(Z)-Ethyl 2-hydroxy-4-(3-methoxyphenyl)-4-oxobut-2-enoate (**1616-34a**).* Compound **1616-34a** was prepared via Procedure VIII from 1-(3-methoxyphenyl)ethanone (1.0 g, 6.7 mmol) to yield a brown-yellow oil (0.96 g, 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.48 (s, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.03 (s, 1H), 4.38 (q, *J* = 7.6 Hz, 2H), 3.85 (s, 3H), 1.39 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.9, 169.4, 162.3, 160.1, 136.4, 130.0, 120.6, 120.3, 112.4, 98.3, 62.7, 55.6, 14.2; HRMS (APCI) Calcd for $C_{13}H_{14}O_5$ 251.0914; found 251.0911 $[M+H]^+$.

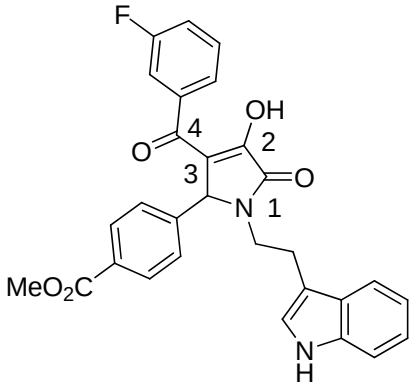


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(3-methoxybenzoyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-34**).* Compound **1616-34** was prepared via Procedure I from methyl 4-formylbenzoate (0.066 g, 0.40 mmol), tryptamine (0.064 g, 0.40 mmol) and **1616-34a** (0.10 g, 0.40 mmol) to yield a pale yellow, amorphous solid (0.046 g, 23 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80°C) δ 10.64 (s, 1H), 7.89-7.82 (m, 3H), 7.35-7.22 (m, 6H), 7.07-7.05 (m, 2H),

6.98-6.91 (m, 2H), 5.34 (s, 1H), 4.26 (m, 1H), 3.82 (s, 3H), 3.74 (s, 3H), 3.01-2.90 (m, 2H), 2.78-2.75 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 188.6, 165.9, 165.1, 158.9, 141.9, 139.3, 136.3, 129.6, 129.4, 129.3, 128.3, 128.1, 126.9, 125.5, 122.9, 121.2, 121.1, 119.0, 118.3, 118.1, 113.5, 111.5, 110.8, 60.7, 55.3, 52.2, 41.1, 23.8; HRMS (APCI) Calcd for $C_{30}H_{26}N_2O_6$ 511.1877; found 511.1871 $[M+H]^+$.

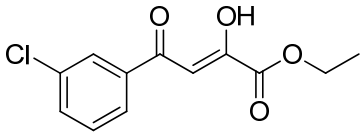


*(Z)-Ethyl 4-(3-fluorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1616-35a**).* Compound **1616-35a** was prepared via Procedure VIII from 1-(3-fluorophenyl)ethanone (1.0 g, 7.2 mmol) to yield a light brown solid (1.1 g, 61 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (br s, 1H), 7.78 (dt, *J* = 1.6 Hz, *J* = 7.6 Hz, 1H), 7.68 (dt, *J* = 1.6 Hz, *J* = 9.2 Hz, 1H), 7.49 (td, *J* = 6.0 Hz, *J* = 8.4 Hz, 1H), 7.31 (ddd, *J* = 1.2 Hz, *J* = 2.8 Hz, *J* = 8.4 Hz, 1H), 7.04 (s, 1H), 4.41 (q, *J* = 7.6 Hz, 2H), 1.42 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4 (d, *J* = 2.2 Hz), 170.3, 163.0 (d, *J* = 24.7 Hz), 162.1, 137.2 (d, *J* = 7.4 Hz), 130.7 (d, *J* = 7.4 Hz), 123.7 (d, *J* = 2.2 Hz), 120.9 (d, *J* = 20.8 Hz), 114.8 (d, *J* = 22.3 Hz), 98.1, 62.9, 14.2; mp 32-34 °C; HRMS (APCI) Calcd for $C_{12}H_{11}FNO_4$ 239.0714; found 239.0710 $[M+H]^+$.

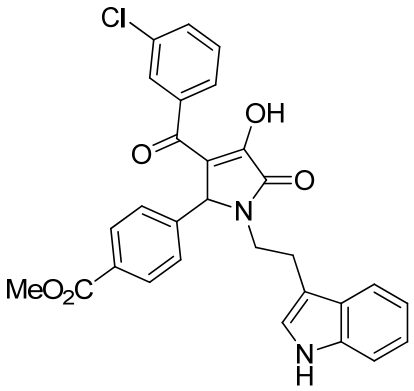


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-(3-fluorobenzoyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-35**).* Compound **1616-35** was prepared via Procedure I from methyl 4-

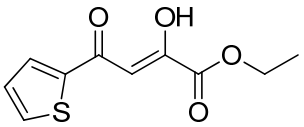
formylbenzoate (0.069 g, 0.42 mmol), tryptamine (0.067 g, 0.42 mmol) and **1616-35a** (0.10 g, 0.42 mmol) to yield a yellow, amorphous solid (0.028 g, 14 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80°C) δ 10.66 (s, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.53-7.45 (m, 2H), 7.34 (dd, J = 3.0 Hz, J = 8.4 Hz, 2H), 7.29-7.26 (m, 1H), 7.22 (d, J = 7.8 Hz, 2H), 7.13-7.10 (m, 1H), 7.07-7.04 (m, 2H), 6.93 (t, J = 7.8 Hz, 1H), 5.32 (s, 1H), 3.84-3.79 (m, 4H), 3.01-2.96 (m, 1H), 2.92-2.88 (m, 1H), 2.76-2.71 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.7, 167.0, 161.5 (d, J = 241.7 Hz), 143.0, 137.6, 136.2, 129.3, 128.9, 128.6, 128.1, 127.9, 126.9, 125.5, 124.1, 122.8, 121.0, 118.3, 118.1, 116.6, 116.5, 111.5, 110.9, 61.0, 52.0, 41.2, 23.5 (Note: Carbon 3 is absent); HRMS (APCI) Calcd for $C_{29}H_{23}FN_2O_5$ 497.1510; found 497.1513 $[M-H]^-$.



*(Z)-Ethyl 4-(3-chlorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1616-36a**).* Compound **1616-36a** was prepared via Procedure VIII from 1-(3-chlorophenyl)ethanone (1.0 g, 6.5 mmol) to yield a brown-green solid (1.1 g, 65 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (br s, 1H), 7.96 (t, J = 1.6 Hz, 1H), 7.87 (dt, J = 1.2 Hz, J = 7.6 Hz, 1), 7.58 (ddd, J = 1.2 Hz, J = 1.6 Hz, J = 8.0 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 7.04 (s, 1H), 4.41 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.3, 170.3, 162.1, 126.7, 135.4, 133.8, 130.3, 128.0, 126.1, 98.1, 62.9, 14.2; mp 33-37°C; HRMS (APCI) Calcd for $C_{12}H_{11}ClO_4$ 255.0419; found 255.0416 $[M+H]^+$.

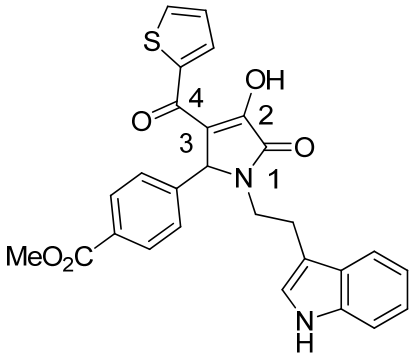


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-(3-chlorobenzoyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-36**).* Compound **1616-36** was prepared via Procedure I from methyl 4-formylbenzoate (0.064 g, 0.39 mmol), tryptamine (0.063 g, 0.39 mmol) and **1616-36a** (0.10 g, 0.39 mmol) to yield a pale yellow, amorphous solid (0.045 g, 22 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80°C) δ 10.69 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 7.58 (d, J = 6.0 Hz, 1H), 7.35-7.32 (m, 3H), 7.29-7.26 (m, 3H), 7.07-7.05 (m, 2H), 6.93 (t, J = 7.2 Hz, 1H), 5.28 (s, 1H), 3.82-3.79 (m, 4H), 2.98-2.94 (m, 1H), 2.88-2.84 (m, 1H), 2.74-2.68 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.1, 166.1, 145.4, 137.9, 136.4, 136.3, 129.2, 128.2, 127.0, 126.8, 125.5, 123.44, 123.43, 122.8, 121.2, 121.0, 118.5, 118.3, 118.1, 111.6, 111.5, 111.0, 109.5, 60.5, 52.1, 23.2, 20.8; HRMS (APCI) Calcd for $C_{29}H_{23}ClN_2O_5$ 513.1223; found 513.1219 [M-H]$^-$.

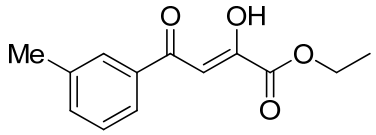


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(thiophen-2-yl)but-2-enoate (**1616-37a**).* Compound **1616-37a** was prepared via Procedure VIII from 1-(thiophen-2-yl)ethanone (0.89 mL, 7.9 mmol) to yield a yellow oil (0.71 g, 40 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, J = 1.2 Hz, J = 4.0 Hz, 1H), 7.74 (dd, J = 0.8 Hz, J = 4.8 Hz, 1H), 7.18 (dd, J = 4.0 Hz, J = 5.2 Hz, 1H), 6.91 (s, 1H), 4.38 (q, J = 7.6 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.3,

164.8, 162.2, 142.2, 135.4, 132.8, 128.9, 99.6, 62.8, 14.2; HRMS (APCI) Calcd for $C_{10}H_{10}O_4S$ 227.0370; found 227.0370 $[M+H]^+$.

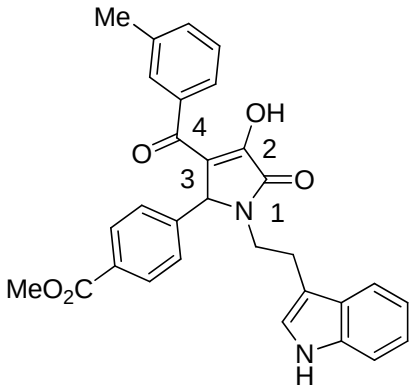


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-5-oxo-3-(thiophene-2-carbonyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-37/SSZ-1616-141**).* Compound **1616-37** was prepared via Procedure I from methyl 4-formylbenzoate (0.073 g, 0.44 mmol), tryptamine (0.071 g, 0.44 mmol) and **1616-37a** (0.10 g, 0.44 mmol) to yield a yellow solid (0.043 g, 20 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 4.0 Hz, 1H), 7.34-7.29 (m, 4H), 7.06-7.02 (m, 3H), 6.92 (t, J = 7.2 Hz, 1H), 5.80-5.70 (m, 1H), 5.28 (s, 1H), 3.86-3.74 (m, 4H), 3.00-2.89 (m, 1H), 2.85-2.78 (m, 1H), 2.73-2.67 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.8, 144.7, 136.2, 129.0, 128.2, 126.9, 126.8, 123.4, 122.9, 121.2, 121.0, 118.5, 118.3, 118.1, 111.6, 111.5, 111.1, 109.5, 52.0, 48.6, 40.0, 23.5 (Note: Carbons 1,2 and 4 are absent); mp 190-195 °C; HRMS (APCI) Calcd for $C_{27}H_{22}N_2O_5S$ 485.1177; found 485.1173 $[M-H]^-$.

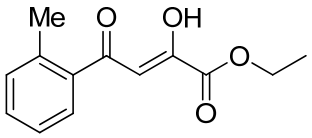


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(m-tolyl)but-2-enoate (**1616-38a**).* Compound **1616-38a** was prepared via Procedure VIII from 1-*m*-tolylethanone (1.0 mL, 7.5 mmol) to yield a brown-yellow oil (0.26 g, 15 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.77 (m, 2H), 7.41-7.35 (m, 2H), 7.05 (s, 1H), 4.39 (q, J = 7.2 Hz, 2H), 2.42 (s, 3H), 1.40 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 191.1, 169.7, 162.4, 138.9, 135.0, 134.8, 128.9, 128.5, 125.3, 98.2, 62.7, 21.5, 14.2; HRMS (APCI) Calcd for $C_{13}H_{14}O_4$ 235.0965; found 235.0963 $[M+H]^+$.

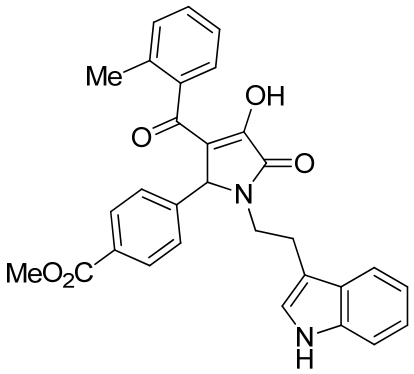


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(3-methylbenzoyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-38**).* Compound **1616-38** was prepared via Procedure I from methyl 4-formylbenzoate (0.17 g, 1.0 mmol), tryptamine (0.17 g, 1.0 mmol) and **1616-38a** (0.24 g, 1.0 mmol) to yield a pale yellow, amorphous solid (0.029 g, 6 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80°C) δ 10.67 (s, 1H), 7.72 (m, 2H), 7.36-7.33 (m, 2H), 7.28-7.21 (m, 2H), 7.11-7.04 (m, 6H), 6.93 (t, J = 7.2 Hz, 1H), 5.29 (s, 1H), 3.86-3.78 (m, 4H), 3.01-2.96 (m, 1H), 2.91-2.86 (m, 1H), 2.76-2.72 (m, 1H), 2.23 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.2, 190.0, 182.1, 182.0, 166.0, 145.4, 144.7, 136.2, 128.8, 127.9, 126.9, 122.9, 122.8, 121.0, 118.3, 118.1, 111.5, 111.1, 109.2, 52.0, 48.6, 41.1, 23.6, 20.9 (Note: Carbons 1, 2, 3, and 4 are absent); HRMS (APCI) Calcd for $C_{30}H_{26}N_2O_5$ 493.1769; found 493.1768 $[M-H]^-$.

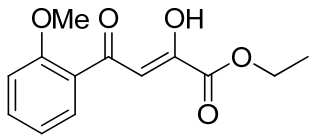


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(o-tolyl)but-2-enoate (**1616-39a**).* Compound **1616-39a** was prepared via Procedure VIII from 1-*o*-tolylethanone (0.98 mL, 7.5 mmol) to yield an orange oil (1.8 g, >99 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 7.2 Hz, 1H), 7.30-7.24 (m, 3H), 6.84 (s, 1H), 4.38 (q, J = 7.2 Hz, 2H), 2.54 (s, 3H), 1.39 (t, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz,

$CDCl_3$) δ 195.8, 168.2, 162.2, 138.4, 135.7, 132.2, 132.0, 129.1, 126.1, 101.8, 62.6, 21.1, 14.1; HRMS (APCI) Calcd for $C_{13}H_{14}O_4$ 235.0965; found 235.0963 $[M+H]^+$.

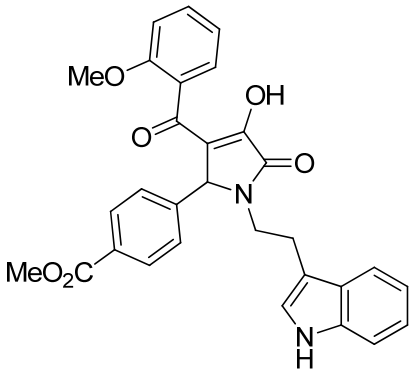


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(2-methylbenzoyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-39**).* Compound **1616-39** was prepared via Procedure I from methyl 4-formylbenzoate (0.14 g, 0.85 mmol), tryptamine (0.14 g, 0.85 mmol) and **1616-39a** (0.20 g, 0.85 mmol) to yield a pale yellow, amorphous solid (0.040 g, 9 %). ^{1}H NMR (600 MHz, DMSO-d_6, 70°C) δ 10.71 (br s, 1H), 7.67 (d, J = 7.2 Hz, 2H), 7.37 (d, J = 9.0 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.13-6.99 (m, 4H), 6.94-6.91 (m, 2H), 6.84-6.74 (m, 3H), 4.96 (s, 1H), 3.82-3.76 (m, 4H), 2.97-2.92 (m, 1H), 2.82-2.71 (m, 2H), 1.78 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.1, 182.1, 182.03, 182.01, 165.9, 144.4, 139.4, 136.3, 134.2, 129.3, 128.8, 128.3, 127.9, 127.0, 124.7, 123.0, 122.7, 121.1, 118.3, 118.1, 111.6, 111.2, 108.9, 60.3, 52.0, 48.6, 41.1, 23.6; HRMS (APCI) Calcd for $C_{30}H_{26}N_2O_5$ 493.1761; found 493.1763 $[M-H]^-$.

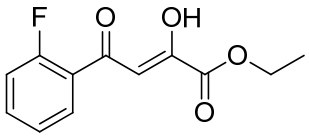


*(Z)-Ethyl 2-hydroxy-4-(2-methoxyphenyl)-4-oxobut-2-enoate (**1616-40a**).* Compound **1616-40a** was prepared via Procedure VIII from 1-(2-methoxyphenyl)ethanone (1.0 g, 6.7 mmol) to yield a yellow solid (0.89 g, 53 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98-7.87 (m, 1H), 7.52-7.48 (m, 1H), 7.31 (s, 1H), 7.06-6.98 (m, 2H), 4.41-4.34 (m, 2H), 3.93 (s, 3H), 1.42-1.38 (m, 3H); ^{13}C

NMR (150 MHz, $CDCl_3$) δ 190.4, 169.1, 162.6, 159.2, 134.7, 130.7, 124.2, 120.9, 111.8, 103.2, 62.4, 55.7, 14.1; HRMS (APCI) Calcd for $C_{13}H_{14}O_5$ 249.0769; found 249.0769 $[M-H]^-$.

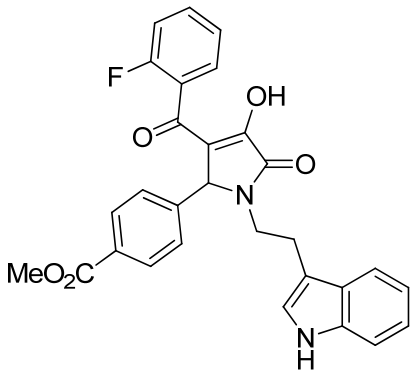


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(2-methoxybenzoyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-40**).* Compound **1616-40** was prepared via Procedure I from methyl 4-formylbenzoate (0.13 g, 0.80 mmol), tryptamine (0.13 g, 0.80 mmol) and **1616-40a** (0.20 g, 0.80 mmol) to yield a pink, amorphous solid (0.028 g, 7 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.89 (s, 1H), 7.50 (d, J = 6.0 Hz, 1H), 7.34-7.18 (m, 5H), 7.09-7.04 (m, 3H), 6.96-6.88 (m, 3H), 5.27 (s, 1H), 3.83-3.74 (m, 6H), 2.99-2.87 (m, 2H), 2.73-2.65 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.1, 182.1, 166.2, 156.0, 146.1, 137.3, 132.7, 129.1, 128.8, 128.4, 128.1, 128.0, 127.7, 127.0, 125.6, 122.8, 121.0, 119.7, 118.2, 118.1, 111.5, 111.1, 111.0, 60.3, 56.1, 55.2, 52.1, 40.9, 23.6, 18.6; HRMS (APCI) Calcd for $C_{30}H_{26}N_2O_6$ 509.1710; found 509.1711 $[M-H]^-$.

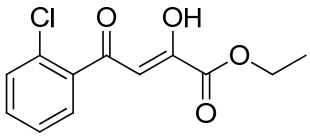


*(Z)-Ethyl 4-(2-fluorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1616-41a**).* Compound **1616-41a** was prepared via Procedure VIII from 1-(2-fluorophenyl)ethanone (0.88 mL, 6.7 mmol) to yield a yellow solid (0.48 g, 29 %). ^{1}H NMR (600 MHz, $CDCl_3$, 60°C) δ 7.98-7.94 (m, 1H), 7.56-7.54 (m, 1H), 7.30-7.26 (m, 1H), 7.19-7.15 (m, 1H), 7.10-7.09 (m, 1H), 4.42-4.38 (m, 2H),

1.43-1.40 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.7, 170.4, 162.2, 161.9 (d, J = 255.8 Hz), 135.4 (d, J = 8.3 Hz), 130.7, 125.0, 123.7, 117.1 (d, J = 24.8 Hz), 102.5 (d, J = 12.3 Hz), 62.9, 14.3; HRMS (APCI) Calcd for $C_{12}H_{11}FO_4$ 239.0714; found 239.0718 $[M+H]^+$.

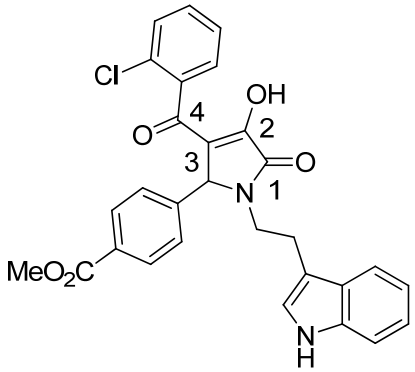


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-(2-fluorobenzoyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-41**).* Compound **1616-41** was prepared via Procedure I from methyl 4-formylbenzoate (0.19 g, 0.84 mmol), tryptamine (0.14 g, 0.84 mmol) and **1616-41a** (0.20 g, 0.84 mmol) to yield a brown, amorphous solid (0.070 g, 17 %). ^{1}H NMR (600 MHz, DMSO-d_6, 70°C) δ 10.68 (s, 1H), 7.80-7.54 (m, 2H), 7.35-7.30 (m, 4H), 7.11-6.78 (m, 7H), 5.07 (s, 1H), 3.81-3.74 (m, 4H), 2.97-2.92 (m, 1H), 2.87-2.82 (m, 1H), 2.72-2.67 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.6, 165.9, 161.2, 156.0, 155.1, 139.5, 136.3, 135.0, 130.0, 129.6, 128.6, 126.8, 126.7, 126.3, 125.3, 124.8, 123.1, 121.1, 119.3, 118.3, 118.0, 111.5, 110.6, 59.1, 52.3, 41.2, 23.8; HRMS (APCI) Calcd for $C_{29}H_{23}FN_2O_5$ 497.1510; found 497.1513 $[M-H]^-$.

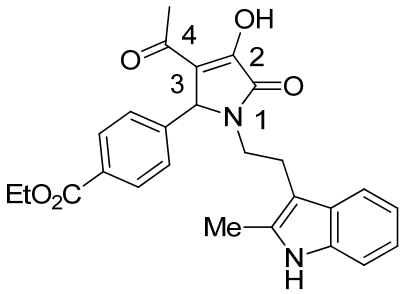


*(Z)-Ethyl 4-(2-chlorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1616-42a**).* Compound **1616-42a** was prepared via Procedure VIII from 1-(2-chlorophenyl)ethanone (1.0 g, 6.5 mmol) to yield a yellow oil (0.58 g, 35 %). ^{1}H NMR (600 MHz, $CDCl_3$, 60 °C) δ 7.65 (t, J = 6.6 Hz, 1H),

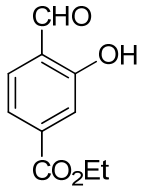
7.47-7.44 (m, 2H), 7.37 (d, *J* = 6.6 Hz, 1H), 6.95 (d, *J* = 6.6 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.8, 168.1, 161.9, 135.8, 132.9, 132.2, 131.1, 130.3, 127.2, 103.1, 62.7, 14.1; HRMS (APCI) Calcd for $C_{12}H_{11}ClO_4$ 255.0419; found 255.0417 $[M+H]^+$.



*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-(2-chlorobenzoyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-42**).* Compound **1616-42** was prepared via Procedure I from methyl 4-formylbenzoate (0.13 g, 0.79 mmol), tryptamine (0.13 g, 0.79 mmol) and **1616-42a** (0.20 g, 0.79 mmol) to yield a cream colored solid (0.27 g, 66 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 10.64 (s,1H), 7.67 (m, 2H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.24-7.18 (m, 2H), 7.10-7.03 (m, 4H), 6.92 (t, *J* = 7.2 Hz, 2H), 6.78 (m, 1H), 5.04 (s, 1H), 3.86-3.75 (m, 4H), 2.97-2.92 (m, 1H), 2.85-2.83 (m, 1H), 2.73-2.68 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 185.2, 165.9, 144.1, 136.3, 130.0, 129.5, 129.1, 128.8, 128.7, 128.6, 128.1, 128.0, 126.9, 126.5, 123.0, 121.0, 118.3, 118.1, 111.5, 111.0, 67.1, 52.1, 40.0, 25.2 (Note: Carbons 1,2 and 3 are absent); mp 248-253 °C; HRMS (APCI) Calcd for $C_{29}H_{23}ClN_2O_5$ 513.1214; found 513.1215 $[M-H]^-$.

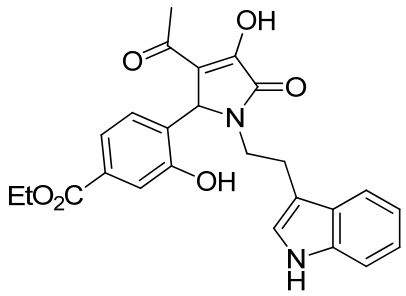


*Ethyl 4-(3-acetyl-4-hydroxy-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-43**).* Compound **1616-43** was prepared via Procedure I from **1616-16a** (0.15 g, 0.84 mmol), 2-(2-methyl-1*H*-indol-3-yl)ethanamine (0.15 g, 0.84 mmol) and methyl acetopyruvate (0.12 g, 0.84 mmol) to yield a cream colored solid (0.078 g, 21 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 10.5 (br s, 1H), 7.83 (d, J = 7.2 Hz, 2H), 7.23-7.18 (m, 4H), 6.95 (t, J = 7.2 Hz, 1H), 6.86 (t, J = 7.2 Hz, 1H), 5.09 (s, 1H), 4.30 (q, J = 7.2 Hz, 2H), 3.61-3.56 (m, 1H), 2.92-2.87 (m, 1H), 2.80-2.75 (m, 1H), 2.57-2.53 (m, 1H), 2.19 (s, 3H), 2.05 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.3, 189.3, 165.5, 135.1, 132.1, 129.1, 127.9, 120.1, 118.1, 117.0, 112.1, 110.5, 108.9, 106.6, 98.5, 90.2, 54.9, 48.7, 48.6, 40.0, 29.0, 14.2, 11.0 (Note: One of either carbon 1, 2, or 4 is absent); mp 190-195 °C; HRMS (APCI) Calcd for $C_{26}H_{26}N_2O_5$ 447.1928; found 447.30 $[M+H]^+$.

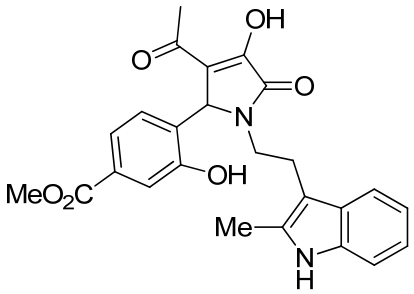


*Ethyl 4-formyl-3-hydroxybenzoate (**1616-44a**).* To a solution of 4-formyl-3-hydroxybenzoic acid (0.43 g, 2.6 mmol) in DMF (0.52 mL, 5.0 M) was added cesium fluoride (0.59 g, 3.9 mmol) and iodoethane (0.23 mL, 2.9 mmol, 1.1 equiv). The reaction mixture stirred at rt for 6 days before being concentrated *in vacuo*, diluted with water and extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*.

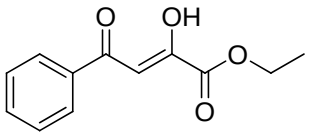
Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 1/1) to afford a white solid (0.19 g, 39 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.95 (s, 1H), 9.99 (s, 1H), 7.68-7.65 (m, 3H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.2, 165.4, 161.4, 137.9, 133.8, 133.0, 120.6, 119.362.0, 14.4; HRMS (APCI) Calcd for $C_{10}H_{10}O_4$ 195.0652; found 195.0649 $[M+H]^+$.



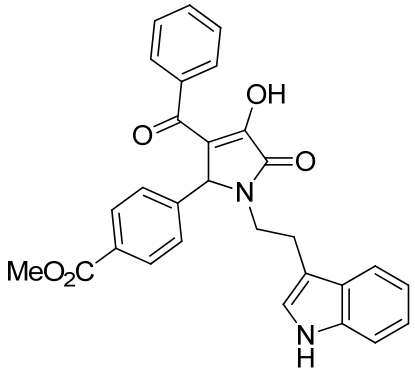
*Ethyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-3-hydroxybenzoate (**1616-44**).* Compound **1616-44** was prepared via Procedure I from **1616-44a** (0.15 g, 0.77 mmol), tryptamine (0.12 g, 0.77 mmol) and methyl acetopyruvate (0.11 g, 0.77 mmol) to yield a cream colored solid (0.20 g, 57 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 10.42 (s, 1H), 7.59-7.46 (m, 1H), 7.34-7.23 (m, 3H), 7.12-7.02 (m, 2H), 6.97-6.89 (m, 2H), 5.76 (s, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 3.78-3.74 (m, 1H), 2.98-2.85 (m, 2H), 2.73-2.68 (m, 1H), 2.28 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.7, 165.5, 165.1, 156.2, 154.8, 136.2, 130.5, 128.1, 127.0, 126.7, 125.5, 122.7, 121.0, 120.2, 118.3, 118.0, 116.1, 111.4, 110.8, 60.7, 41.0, 40.0, 29.7, 23.4, 14.2.; mp 200-205 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_6$ 449.1721; found 449.1723 $[M+H]^+$.



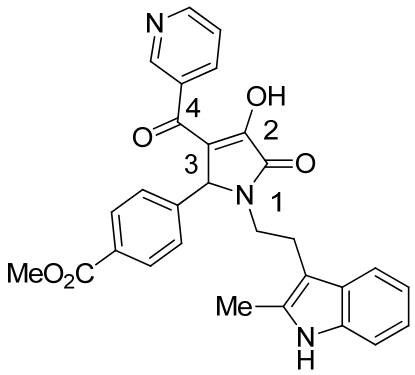
*Methyl 4-(3-acetyl-4-hydroxy-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-3-hydroxybenzoate (**1616-45**).* Compound **1616-45** was prepared via Procedure I from **1616-12b** (0.19 g, 1.0 mmol), 2-(2-methyl-1*H*-indol-3-yl)ethanamine (0.18 g, 1.0 mmol) and methyl acetopyruvate (0.15 g, 1.0 mmol) to yield a red solid (0.085 g, 18 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 10.62 (br s, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 6.6 Hz, 1H), 7.18 (d, J = 7.2 Hz, 2H), 6.93 (t, J = 6.6 Hz, 1H), 6.85-6.82 (m, 2H), 5.73 (s, 1H), 3.82 (s, 3H), 3.73-3.67 (m, 1H), 2.89-2.85 (m, 1H), 2.75-2.69 (m, 1H), 2.65-58 (m, 1H), 2.20 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 204.8, 188.7, 166.0, 163.2, 156.4, 145.5, 137.9, 135.2, 132.2, 128.2, 128.0, 125.6, 120.5, 120.1, 118.3, 117.0, 111.9, 110.6, 106.3, 52.2, 41.5, 40.0, 22.4, 20.8, 11.0; mp >250 °C; HRMS (APCI) Calcd for $C_{25}H_{24}N_2O_6$ 447.1567; found 447.1565 $[M-H]^-$.



*(Z)-Ethyl 2-hydroxy-4-oxo-4-phenylbut-2-enoate (**1616-46a**).* Compound **1616-46a** was prepared via Procedure VIII from acetophenone (1.0 g, 8.3 mmol) to yield an orange oil (0.80 g, 44 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, J = 7.8 Hz, 2H), 7.55 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.8 Hz, 2H), 7.03 (s, 1H), 4.35 (q, J = 6.6 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.7, 169.8, 162.1, 134.8, 133.8, 128.9, 127.9, 97.9, 62.6, 14.1; HRMS (APCI) Calcd for $C_{12}H_{12}O_4$ 221.0808; found 221.0806 $[M+H]^+$.

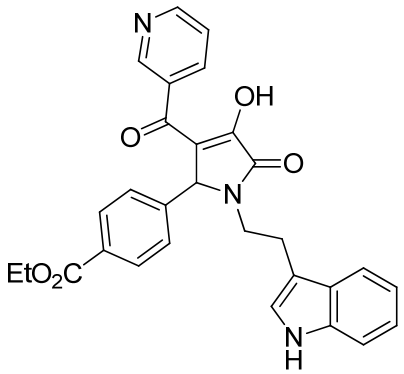


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-benzoyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-46**).* Compound **1616-46** was prepared via Procedure I from **1616-46a** (0.15 g, 0.68 mmol), tryptamine (0.11 g, 0.68 mmol) and methyl 4-formylbenzoate (0.11 g, 0.68 mmol) to yield a cream colored solid (0.031 g, 9 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.59-7.48 (m, 2H), 7.40-7.28 (m, 3H), 7.24-7.21 (m, 3H), 7.11-7.04 (m, 2H), 6.92 (t, J = 7.6 Hz, 1H), 6.74 (s, 1H), 5.25 (s, 1H), 3.80-3.74 (m, 4H), 3.00-2.90 (m, 1H), 2.82-2.67 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.1, 188.9, 165.9, 165.4, 142.1, 138.2, 136.4, 132.4, 129.6, 129.5, 128.7, 128.3, 128.1, 127.0, 123.0, 121.1, 118.9, 118.4, 118.2, 111.6, 110.9, 60.8, 52.1, 41.2, 23.8; mp 200-205 °C; HRMS (APCI) Calcd for $C_{29}H_{24}N_2O_5$ 481.1771; found 481.1765 $[M+H]^+$.



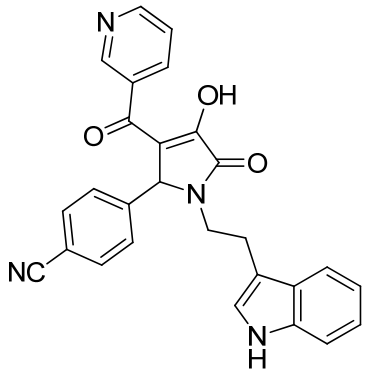
*Methyl 4-(4-hydroxy-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-47**).* Compound **1616-47** was prepared via Procedure I from **1616-28a** (0.15 g, 0.68 mmol), 2-(2-methyl-1*H*-indol-3-yl)ethanamine (0.12 g, 0.68 mmol) and methyl 4-

formylbenzoate (0.11 g, 0.68 mmol) to yield an orange solid (0.046 g, 14 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 8.73 (s, 1H), 8.48 (d, J = 4.4 Hz, 1H), 7.93 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.30-7.17 (m, 5H), 6.97 (t, J = 6.4 Hz, 1H), 6.86 (t, J = 7.2 Hz, 1H), 5.23 (s, 1H), 3.81 (s, 3H), 3.63-3.56 (m, 1H), 2.96-2.88 (m, 1H), 2.76-2.69 (m, 1H), 2.57-2.50 (m, 1H), 2.18 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.6, 166.0, 150.4, 150.1, 149.2, 136.8, 136.1, 135.5, 135.2, 132.2, 129.0, 128.6, 128.0, 123.6, 122.6, 120.1, 118.2, 117.0, 110.6, 106.7, 61.2, 52.0, 41.3, 22.6, 11.0 (Note: 2 of Carbons 1, 2, 3, or 4 are absent); mp >250 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1867; found 496.1872 $[M+H]^+$.

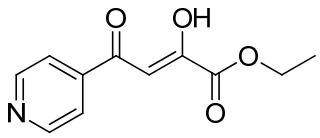


*Ethyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-48**).* Compound **1616-48** was prepared via Procedure I from **1616-28a** (0.15 g, 0.68 mmol), tryptamine (0.11 g, 0.68 mmol) and **1616-16a** (0.12 g, 0.68 mmol) to yield a yellow solid (0.027 g, 8 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.75 (s, 1H), 8.48 (d, J = 3.2 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.34-7.27 (m, 5H), 7.08-7.04 (m, 2H), 6.92 (t, J = 7.2 Hz, 1H), 5.33 (s, 1H), 4.28 (q, J = 7.6 Hz, 2H), 3.84-3.77 (m, 1H), 3.00-2.93 (m, 1H), 2.87-2.80 (m, 1H), 2.73-2.66 (m, 1H), 1.28 (t, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.8, 182.1, 162.9, 155.0, 148.3, 148.1, 136.4, 136.3, 127.0, 123.8, 123.4, 123.2, 123.1, 121.5, 121.3, 120.8, 118.5, 118.2, 117.8, 111.8, 111.2, 109.5, 60.6,

45.3, 23.6, 23.3, 14.0; mp >250 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1867; found 496.1872 $[M+H]^+$.

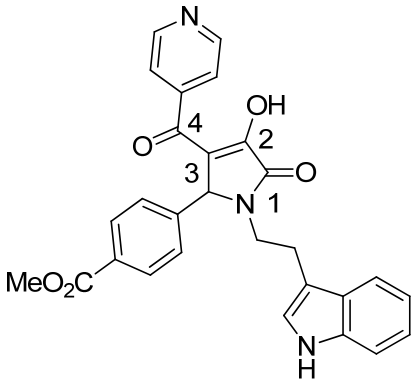


*4-(1-(2-(1H-Indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzonitrile (**1616-49**).* Compound **1616-49** was prepared via Procedure I from 4-formylbenzonitrile (0.15 g, 1.1 mmol), tryptamine (0.18 g, 1.1 mmol) and **1616-28a** (0.25 g, 1.1 mmol) to yield a yellow solid (0.084 g, 17 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.81 (s, 1H), 8.69 (d, J = 3.6 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.8 Hz, 2H), 7.50-7.47 (m, 3H), 7.33 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.13 (s, 1H), 7.07 (t, J = 7.8 Hz, 1H), 6.94 (t, J = 7.2 Hz, 1H), 5.44 (s, 1H), 3.88-3.83 (m, 1H), 3.01-2.90 (m, 2H), 2.79-2.75 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.3, 165.1, 152.1, 150.2, 149.2, 142.5, 136.3, 133.9, 132.4, 129.0, 126.9, 123.9, 123.4, 122.9, 121.1, 118.6, 118.3, 118.1, 117.7, 111.5, 111.0, 110.7, 60.3, 41.2, 23.7; mp >250 °C; HRMS (APCI) Calcd for $C_{27}H_{20}N_4O_3$ 449.1608; found 449.1607 $[M+H]^+$.

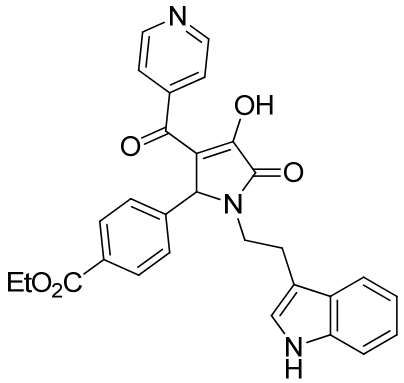


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(pyridin-4-yl)but-2-enoate (**1616-50a**).* Compound **1616-50a** was prepared via Procedure VIII from 1-(pyridine-4-yl)ethanone (0.55 mL, 8.3 mmol) to yield an orange solid (0.29 g, 16 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (d, J = 6.0 Hz, 2H), 7.77 (d, J = 6.4 Hz, 2H), 7.07 (s, 1H), 4.41 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR

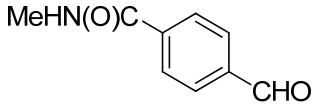
(100 MHz, $CDCl_3$) δ 187.1, 173.4, 161.7, 151.1, 141.4, 120.8, 98.0, 63.1, 14.2; HRMS (APCI) Calcd for $C_{11}H_{11}NO_4$ 222.0761; found 222.0759 $[M+H]^+$.



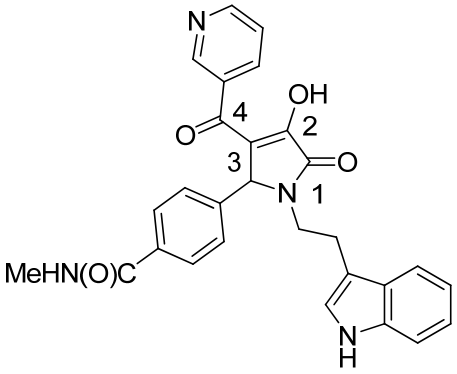
*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-isonicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-50**).* Compound **1616-50** was prepared via Procedure I from methyl 4-formylbenzoate (0.15 g, 0.9 mmol), tryptamine (0.15 g, 0.9 mmol) and **1616-50a** (0.20 g, 0.9 mmol) to yield a yellow solid (0.13 g, 30 %). ^{1}H NMR (600 MHz, DMSO-d_6, 80°C) δ 10.63 (s, 1H), 8.47 (d, J = 2.8 Hz, 1H), 7.76-6.92 (m, 12H), 5.27 (s, 1H), 3.82-3.78 (m, 4H), 3.00-2.95 (m, 1H), 2.91-2.86 (m, 1H), 2.74-2.72 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.3, 166.0, 149.5, 149.0, 146.6, 136.3, 128.9, 128.5, 128.0, 127.0, 122.8, 122.3, 121.1, 120.7, 118.3, 118.1, 111.5, 111.0, 52.0, 48.6, 41.1, 23.5 (Note: Carbons 1 and 2 are absent); mp >250 °C; HRMS (APCI) Calcd for $C_{28}H_{23}N_3O_5$ 480.1570; found 480.1568 $[M-H]^-$.



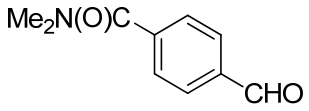
*Ethyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-isonicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-51**).* Compound **1616-51** was prepared via Procedure I from **1616-16a** (0.40 g, 2.3 mmol), tryptamine (0.36 g, 2.3 mmol) and **1616-50a** (0.50 g, 2.3 mmol) to yield a yellow solid (0.019 g, 2 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.68 (d, J = 5.4 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 5.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 1.8 Hz, 1H), 7.06 (t, J = 7.8 Hz, 1H), 6.92 (t, J = 7.2 Hz, 1H), 5.40 (s, 1H), 4.29 (q, J = 7.2 Hz, 2H), 3.88-3.82 (m, 1H), 3.00-2.90 (m, 2H), 2.78-2.73 (m, 1H), 1.29 (7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.0, 165.4, 165.0, 149.6, 145.8, 142.2, 136.2, 129.8, 129.7, 129.3, 128.3, 126.9, 122.9, 121.8, 121.1, 118.3, 118.1, 117.35, 111.5, 110.7, 60.7, 60.3, 41.1, 23.6, 14.2; mp 169-172 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1880; found 496.1877 $[M+H]^+$.



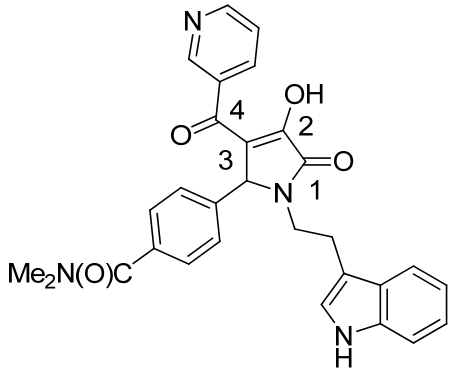
*4-Formyl-N-methylbenzamide (**1616-52a**).* Compound **1616-52a** was prepared via Procedure IX from methanamine (3.3 mL, 2.0 M in MeOH, 6.7 mmol) to yield a white solid (0.14 g, 13 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.08 (s, 1H), 7.96-7.92 (m, 4H), 6.28 (br s, 1H), 3.06 (d, J = 4.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.7, 167.3, 140.0, 138.3, 130.1, 127.8, 27.2; HRMS (APCI) Calcd for $C_9H_9NO_2$ 164.0706; found 164.0708 $[M+H]^+$.



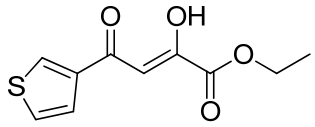
*4-(1-(2-(1H-Indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-N-methylbenzamide (**1616-52**).* Compound **1616-52** was prepared via Procedure I from **1616-52a** (0.15 g, 0.93 mmol), tryptamine (0.15 g, 0.93 mmol) and **1616-28a** (0.21 g, 0.93 mmol) to yield a yellow solid (0.33 g, 73 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.79 (s, 1H), 8.67 (d, J = 4.8 Hz, 1H), 8.24 (d, J = 3.6 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.8 Hz, 2H), 7.45 (t, J = 5.4 Hz, 1H), 7.39-7.34 (m, 4H), 7.11-7.05 (m, 2H), 6.94 (t, J = 7.2 Hz, 1H), 5.38 (s, 1H), 3.88-3.84 (m, 1H), 3.11-3.09 (m, 1H), 3.02-2.94 (m, 2H), 2.76 (d, J = 4.8 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.3, 165.0, 152.3, 149.3, 136.3, 136.1, 134.5, 128.1, 127.9, 127.3, 125.5, 123.4, 122.4, 122.9, 121.0, 118.5, 118.3, 118.14, 118.1, 111.6, 110.8, 60.4, 41.0, 26.2, 23.7 (Note: Carbon 4 is absent); mp 240-245 °C; HRMS (APCI) Calcd for $C_{28}H_{24}N_4O_4$ 481.1876; found 481.1879 $[M+H]^+$.



*4-Formyl-N,N-dimethylbenzamide (**1616-53a**).* Compound **1616-53a** was prepared via Procedure IX from dimethylamine (6.7 mL, 2.0 M in THF, 13 mmol) to yield an opaque oil (1.2 g, 51 %). ^{1}H NMR (400 MHz, $CDCl_3$, 60 °C) δ 10.01-9.99 (s, 1H), 7.89-7.85 (m, 2H), 7.54-7.50 (m, 2H), 3.07 (s, 3H), 2.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 142.1, 136.9, 129.9, 127.7, 39.4, 35.4; HRMS (APCI) Calcd for $C_{10}H_{11}NO_2$ 178.0863; found 178.0864 $[M+H]^+$.

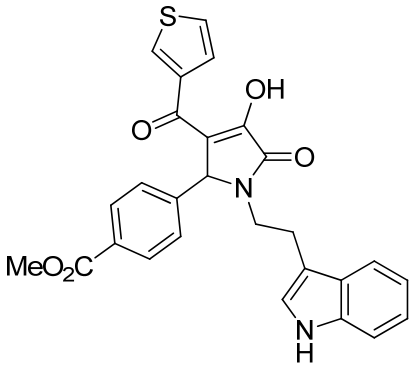


*4-(1-(2-(1H-Indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-N,N-dimethylbenzamide (**1616-53**).* Compound **1616-52** was prepared via Procedure I from **1616-28a** (0.38 g, 1.7 mmol), tryptamine (0.27 g, 1.7 mmol) and **1616-53a** (0.30 g, 1.7 mmol) to yield a yellow solid (0.33 g, 39 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.81 (s, 1H), 8.69 (d, J = 4.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.49-7.47 (m, 2H), 7.36-7.29 (m, 4H), 7.14-7.10 (m, 2H), 7.06 (t, J = 7.8 Hz, 1H), 6.92 (t, J = 7.2 Hz, 1H), 5.42 (s, 1H), 3.88-3.82 (m, 1H), 3.02-2.95 (m, 5H), 2.84 (s, 3H), 2.77-2.71 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.71, 164.9, 152.3, 149.3, 136.4, 136.3, 136.2, 128.1, 127.8, 127.2, 125.5, 123.4, 122.9, 121.2, 121.0, 118.5, 118.3, 118.1, 111.6, 111.5, 110.8, 60.5, 41.0, 40.0, 23.8, 23.2 (Note: Carbon 4 is absent); mp 210-212 °C; HRMS (APCI) Calcd for $C_{29}H_{26}N_4O_4$ 495.2040; found 495.2036 $[M+H]^+$.

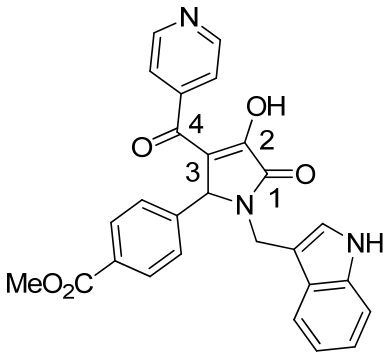


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(thiophen-3-yl)but-2-enoate (**1616-54a**).* Compound **1616-54a** was prepared via Procedure VIII from 1-(thiophen-3-yl)ethanone (1.0 g, 7.9 mmol) to yield a cream colored solid (1.2 g, 68 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (d, J = 3.0 Hz, 1H), 7.55 (d, J = 5.4 Hz, 1H), 7.37 (dd, J = 3.0 Hz, J = 5.4 Hz, 1H), 6.86 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 185.6, 168.7, 162.3, 139.6,

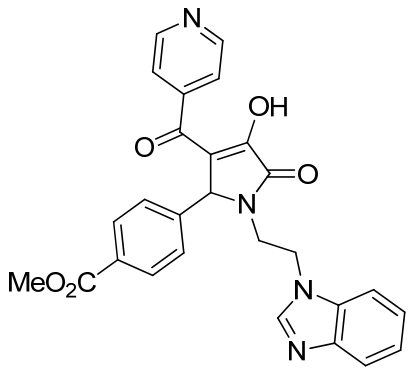
132.7, 127.3, 126.4, 99.3, 62.7, 14.2; HRMS (APCI) Calcd for $C_{10}H_{10}O_4S$ 227.0373; found 227.0377 $[M+H]^+$.



*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-5-oxo-3-(thiophene-3-carbonyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-54**).* Compound **1616-54** was prepared via Procedure I from methyl 4-formylbenzoate (0.36 g, 2.2 mmol), tryptamine (0.35 g, 2.2 mmol) and **1616-54a** (0.50 g, 2.2 mmol) to yield a cream colored solid (0.70 g, 65 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.38 (d, J = 2.8 Hz, 1H), 7.85 (d, J = 7.6 Hz, 2H), 7.49 (t, J = 4.0 Hz, 1H), 7.37-7.31 (m, 4H), 7.13 (s, 1H), 7.07 (t, J = 7.6 Hz, 1H), 6.93 (t, J = 6.8 Hz, 1H), 5.76 (d, J = 1.2 Hz, 1H), 5.44 (s, 1H), 3.89-3.81 (m, 4H), 3.02-2.90 (m, 2H), 2.80-2.74 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.8, 165.9, 142.1, 141.6, 136.3, 134.2, 129.5, 129.4, 128.3, 128.1, 127.3, 126.9, 126.3, 125.5, 122.9, 121.1, 118.3, 118.1, 111.5, 110.8, 60.7, 52.1, 41.1, 23.8; mp 189-192 °C; HRMS (APCI) Calcd for $C_{27}H_{22}N_2O_5S$ 487.1336; found 487.1335 $[M+H]^+$.

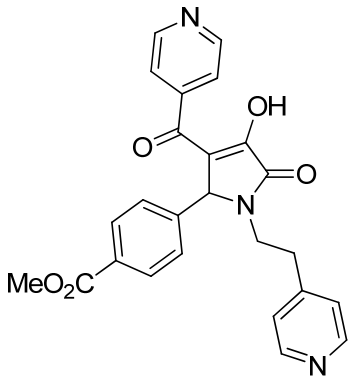


*Methyl 4-(1-((1H-indol-3-yl)methyl)-4-hydroxy-3-isonicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-55**).* Compound **1616-55** was prepared via Procedure I from methyl 4-formylbenzoate (0.28 g, 1.7 mmol), (1*H*-indol-3-yl)methanamine (0.25 g, 1.7 mmol) and **1616-50a** (0.38 g, 1.7 mmol) to yield an orange solid (0.78 g, 98 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.66 (d, J = 5.6 Hz, 2H), 7.92 (d, J = 8.0 Hz, 2H), 7.51-7.37 (m, 6H), 7.13-7.08 (m, 2H), 6.97 (t, J = 7.2 Hz, 1H), 5.11-5.07 (m, 2H), 3.84 (s, 3H), 3.80 (d, J = 14.8 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.0, 165.1, 149.2, 129.45, 129.36, 128.2, 128.1, 126.2, 125.5, 125.1, 122.0, 121.7, 121.5, 119.0, 118.9, 118.6, 118.2, 111.7, 109.3, 59.2, 52.2, 35.5 (Note: Carbon 4 is absent); mp 240-243 °C; HRMS (APCI) Calcd for $C_{27}H_{21}N_3O_5$ 468.1567; found 468.1566 $[M+H]^+$.

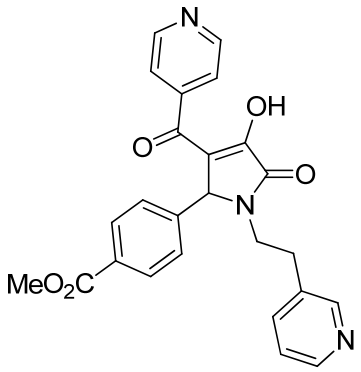


*Methyl 4-(1-(2-(1H-benzo[d]imidazol-1-yl)ethyl)-4-hydroxy-3-isonicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-56**).* Compound **1616-56** was prepared via Procedure I from methyl 4-formylbenzoate (0.19 g, 1.1 mmol), 2-(1*H*-benzo[*d*]imidazol-1-yl)ethanamine (0.18 g, 1.1

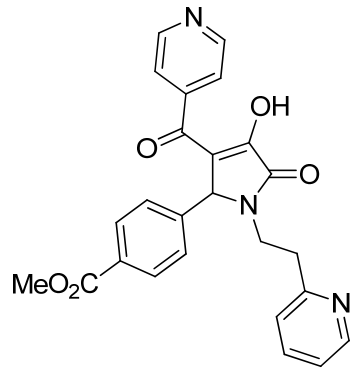
mmol) and **1616-50a** (0.25 g, 1.1 mmol) to yield a yellow solid (0.40 g, 73 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 1.3 Hz, 2H), 8.59 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.67 (t, J = 5.2 Hz, 1H), 7.61 (t, J = 5.2 Hz, 1H), 7.52 (d, J = 5.6 Hz, 2H), 7.33-7.29 (m, 4H), 5.37 (s, 1H), 4.61-4.54 (m, 1H), 4.48-4.44 (m, 1H), 4.03-3.98 (m, 1H), 3.82 (s, 3H), 3.08-3.04 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.2, 166.4, 165.9, 149.2, 146.4, 143.4, 142.6, 139.6, 132.9, 129.4, 128.4, 128.14, 128.06, 125.5, 123.5, 123.1, 122.1, 118.2, 111.0, 60.08, 52.2, 43.1 (Note: One sp^3 Carbon is under the DMSO peak); mp 135-140 °C; HRMS (APCI) Calcd for $C_{27}H_{22}N_4O_5$ 483.1676; found 483.1674 $[M+H]^+$.



Methyl 4-(4-hydroxy-3-isonicotinoyl-5-oxo-1-(2-(pyridin-4-yl)ethyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate ***(1616-57)***. Compound **1616-57**was prepared via Procedure I from methyl 4-formylbenzoate (0.19 g, 1.1 mmol), 2-(pyridin-4-yl)ethanamine (0.14 g, 1.1 mmol) and **1616-50a** (0.25 g, 1.1 mmol) to yield a yellow solid (0.47 g, 95 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (d, J = 6.0 Hz, 2H), 8.49-8.48 (m, 2H), 7.92 (d, J = 8.0 Hz, 2H), 7.56-7.53 (m, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.29-7.26 (m, 2H), 5.47 (s, 1H), 3.91-3.83 (m, 4H), 2.88-2.78 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.1, 185.8, 182.0, 166.3, 166.0, 149.5, 149.2, 148.3, 146.6, 143.4, 129.4, 128.2, 124.6, 122.2, 115.6, 59.9, 52.2, 40.6, 32.7; mp 126-129 °C; HRMS (APCI) Calcd for $C_{25}H_{21}N_3O_5$ 444.1567; found 444.1566 $[M+H]^+$.

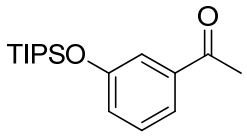


*Methyl 4-(4-hydroxy-3-isonicotinoyl-5-oxo-1-(2-(pyridin-3-yl)ethyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-58**).* Compound **1616-58** was prepared via Procedure I from methyl 4-formylbenzoate (0.19 g, 1.1 mmol), 2-(pyridin-3-yl)ethanamine (0.14 g, 1.1 mmol) and **1616-50a** (0.25 g, 1.1 mmol) to yield a yellow solid (0.47 g, 93 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (d, J = 6.0 Hz, 2H), 8.44 (dd, J = 1.2 Hz, J = 4.8 Hz, 1H), 8.41 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 6.0 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.35 (dd, J = 4.8 Hz, J = 7.6 Hz, 1H), 5.51 (s, 1H), 3.91-3.83 (m, 4H), 2.91-2.77 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 206.6, 186.6, 165.9, 165.6, 149.3, 148.9, 146.9, 146.1, 142.7, 137.3, 134.7, 129.5, 128.2, 128.1, 123.8, 122.0, 116.6, 59.9, 52.2, 41.3, 30.5; mp 160-163 °C; HRMS (APCI) Calcd for $C_{25}H_{21}N_3O_5$ 444.1567; found 444.1565 $[M+H]^+$.

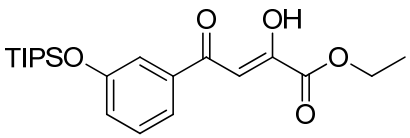


*Methyl 4-(4-hydroxy-3-isonicotinoyl-5-oxo-1-(2-(pyridin-2-yl)ethyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-59**).* Compound **1616-59** was prepared via Procedure I from methyl 4-formylbenzoate (0.19 g, 1.1 mmol), 2-(pyridin-2-yl)ethanamine (0.14 g, 1.1 mmol) and **1616-50a** (0.25 g, 1.1

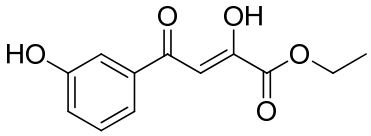
mmol) to yield a yellow solid (0.50 g, >99 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (d, J = 6.0 Hz, 2H), 8.48 (dd, J = 1.6 Hz, J = 5.2 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.73 (td, J = 2.0 Hz, J = 7.6 Hz, 1H), 7.53 (dd, J = 1.2 Hz, J = 4.4 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.27 (s, 1H), 7.25 (t, J = 3.6 Hz, 1H), 5.44 (s, 1H), 4.00-3.93 (m, 1H), 3.82 (s, 3H), 3.07-2.97 (m, 2H), 2.92-2.84 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 206.6, 186.7, 165.9, 165.5, 158.1, 156.6, 149.4, 148.7, 146.1, 142.6, 137.3, 129.5, 128.2, 125.6, 123.6, 122.0, 116.9, 60.1, 52.2, 40.3, 35.4; mp 187-190 °C; HRMS (APCI) Calcd for $C_{25}H_{21}N_3O_5$ 444.1567; found 444.1564 $[M+H]^+$.



*((Triisopropylsilyl)oxy)phenyl)ethanone (**1616-62a**).* To a solution of 1-(3-hydroxyphenyl)ethanone (3.0 g, 22 mmol) in DCM (59 mL, 0.38 M) was added 1*H*-imidazole (2.4 mL, 44 mmol, 2.0 equiv) and chlorotriisopropylsilane (8.7 mL, 41 mmol, 1.8 equiv). The resulting mixture was stirred at rt for 6 h before being diluted with water and extracted with DCM (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the desired product as a yellow oil (6.4 g, >99 %) which was taken on without further attempts at purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 7.2 Hz, 1H), 7.47 (s, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.07 (dd, J = 0.8 Hz, J = 2.4 Hz, 1H), 2.57 (s, 3H), 1.26 (sept, J = 8.0 Hz, 3H), 1.11 (d, J = 8.0 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.1, 156.5, 138.6, 129.6, 124.9, 121.4, 119.3, 26.7, 18.0, 17.82, 17.76; HRMS (APCI) Calcd for $C_{17}H_{28}O_2Si$ 293.1931; found 293.1932 $[M+H]^+$.

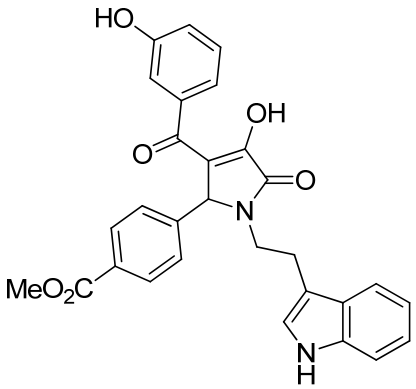


*(Z)-Ethyl 2-hydroxy-4-oxo-4-(3-((triisopropylsilyl)oxy)phenyl)but-2-enoate (**1616-62b**).* Compound **1616-62b** was prepared via Procedure VIII from **1616-62a** (8.8 g, 30 mmol) to yield a yellow oil which was taken on without further attempts at purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, *J* = 1.2 Hz, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 1.6 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.05 (s, 1H), 4.40 (q, *J* = 6.8 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H), 1.28 (sept, *J* = 6.4 Hz, 3H), 1.12 (d, *J* = 4.0 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8, 169.5, 162.3, 156.8, 136.5, 130.0, 125.5, 120.8, 118.9, 98.3, 62.7, 18.0, 17.8, 17.6, 14.2; HRMS (APCI) Calcd for $C_{21}H_{32}O_5Si$ 393.2092; found 393.2091 $[M+H]^+$.

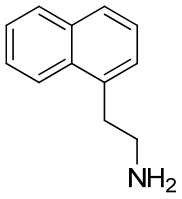


*(Z)-Ethyl 2-hydroxy-4-(3-hydroxyphenyl)-4-oxobut-2-enoate (**1616-62c**).* To a solution of **1616-62b** (3.3 g, 8.5 mmol) in THF (150 mL, 0.057 M) at 0 °C was added a solution of TBAF in THF (25 mL, 1.0 M, 3.0 equiv). The reaction mixture was stirred for 30 min at 0 °C before being warmed to rt and stirred for an additional 35 min. At this time the resulting solution was diluted with water and extracted with EtOAc (4x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 1/1) to afford a pale yellow solid (0.86 g, 43 %) which was taken on without further attempts at purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.50 (m, 2H), 7.37-7.31 (m, 1H), 7.15-7.12 (m, 1H), 7.06 (s, 1H), 4.40 (q, *J* = 6.8 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8,

169.6, 162.8, 156.8, 136.2, 130.3, 121.3, 120.3, 114.7, 98.4, 63.2, 14.2; HRMS (APCI) Calcd for $C_{12}H_{12}O_5$ 237.0758; found 237.0758 $[M+H]^+$.

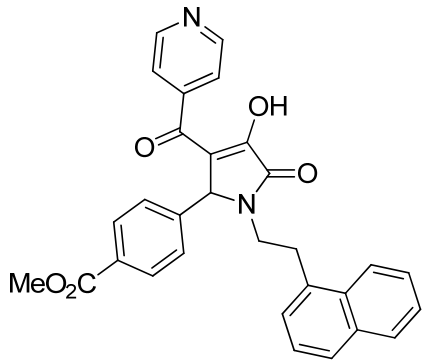


*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-3-(3-hydroxybenzoyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-62**).* Compound **1616-62** was prepared via Procedure I from methyl 4-formylbenzoate (0.35 g, 2.1 mmol), tryptamine (0.34 g, 2.1 mmol) and **1616-62c** (0.50 g, 2.1 mmol) to yield a cream colored solid (1.0 g, 96 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.37-7.29 (m, 6H), 7.13-7.12 (m, 1H), 7.07-7.03 (m, 3H), 6.91 (t, J = 7.8 Hz, 1H), 5.36 (s, 1H), 3.87-3.81 (m, 4H), 3.00-2.93 (m, 2H), 2.78-2.73 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 198.0, 166.0, 157.1, 136.40, 136.3, 129.4, 128.4, 128.2, 125.6, 123.4, 122.9, 121.2, 121.1, 119.5, 118.6, 118.4, 118.25, 118.19, 115.2, 111.7, 111.6, 110.9, 109.7, 66.5, 52.2, 41.2, 23.8; mp 78-80 °C; HRMS (APCI) Calcd for $C_{29}H_{24}N_2O_6$ 497.1707; found 497.1707 $[M+H]^+$.



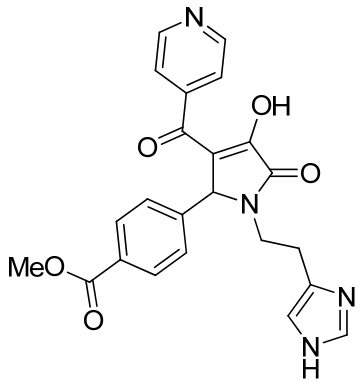
*2-(Naphthalen-1-yl)ethanamine (**1616-63a**).* 2-(Naphthalen-1-yl)acetonitrile (0.89 mL, 6.0 mmol) in diethyl ether (5.0 mL, 1.2 M) was added dropwise to a solution of lithium aluminum hydride (12 mL, 12 mmol, 2.0 equiv) in diethyl ether (20 mL, 0.30 M). The suspension was

then allowed to stir at rt for 12 h. Water was added dropwise until no more gas was given off, at which point 1.0 M NaOH was added to pH = 9. The mixture was extracted with Et_2O (2x) and washed with brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (10% MeOH/DCM) to yield a yellow oil (0.41 g, 40 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 7.2 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.50-7.44 (m, 2H), 7.37 (t, *J* = 6.6 Hz, 1H), 7.30 (d, *J* = 6.6 Hz, 1H), 3.18 (t, *J* = 7.2 Hz, 2H), 3.04 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.3, 134.0, 131.9, 128.8, 127.2, 126.8, 126.0, 125.6, 125.5, 123.6, 42.4, 36.6; HRMS (APCI) Calcd for $C_{12}H_{13}N$ 172.1121; found 172.1119 $[M+H]^+$.

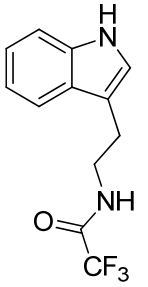


*Methyl 4-(4-hydroxy-3-isonicotinoyl-1-(2-(naphthalen-1-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-63**).* Compound **1616-63** was prepared via Procedure I from methyl 4-formylbenzoate (0.40 g, 2.4 mmol), **1616-63a** (0.41 g, 2.4 mmol) and **1616-50a** (0.53 g, 2.4 mmol) to yield a yellow solid (0.67 g, 56 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.70 (d, *J* = 3.6 Hz, 2H), 7.93-7.84 (m, 4H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.57-7.40 (m, 7H), 7.30 (d, *J* = 6.6 Hz, 1H), 5.40 (s, 1H), 3.84-3.80 (m, 4H), 3.37-3.34 (m, 1H), 3.13-3.08 (m, 1H), 3.03-3.00 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 196.1, 165.9, 162.1, 160.1, 136.4, 136.3, 130.7, 129.5, 128.2, 128.0, 125.5, 123.4, 122.8, 121.2, 120.8, 118.48, 118.45, 118.2, 118.1, 115.2, 111.6,

111.3, 61.5, 52.2, 48.6, 26.2; mp 221-224 °C; HRMS (APCI) Calcd for $C_{30}H_{24}N_2O_5$ 493.1758; found 493.1756 $[M+H]^+$.

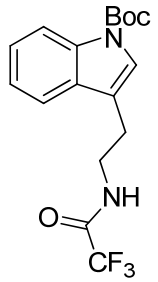


*Methyl 4-(1-(2-(1H-imidazol-4-yl)ethyl)-4-hydroxy-3-isonicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-64**).* Compound **1616-64** was prepared via Procedure I from methyl 4-formylbenzoate (0.37 g, 2.3 mmol), 2-(1*H*-imidazol-4-yl)ethanamine (0.25 g, 2.3 mmol) and **1616-50a** (0.50 g, 2.3 mmol) to yield an orange solid (0.031 g, 3 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.50 (d, J = 5.2 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 5.2 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.26 (s, 1H), 7.11 (s, 1H), 5.27 (s, 1H), 3.90-3.82 (m, 4H), 2.83-2.76 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.5, 169.1, 166.1, 148.8, 148.0, 134.0, 131.2, 129.0, 128.5, 128.1, 127.8, 127.6, 122.4, 116.4, 111.6, 66.3, 52.0, 40.0, 22.3; mp 64-70 °C; HRMS (APCI) Calcd for $C_{23}H_{20}N_4O_5$ 433.1512; found 433.1513 $[M+H]^+$.



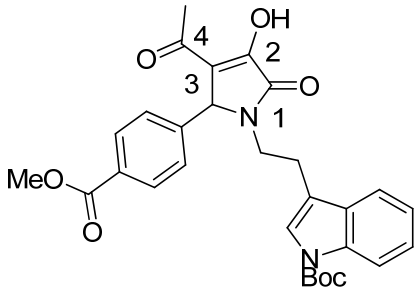
*N-(2-(1H-indol-3-yl)ethyl)-2,2,2-trifluoroacetamide (**1616-65a**).* To a solution of 2-(1*H*-indol-3-yl)ethanamine (2.0 g, 12 mmol) in DCM (112 mL, 0.11 M) at 0 °C was added pyridine (11 mL, 140 mmol, 11 equiv). 2,2,2-Trifluoroacetic anhydride (1.9 mL, 13 mmol, 1.1 equiv) was

added dropwise and the reaction continued to stir at 0 °C for 5 min before being warmed to rt and stirred for 2 h. The resulting mixture was diluted with DCM and washed with saturated sodium bicarbonate, brine and water. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 3/1) to yield a pale yellow solid (2.5 g, 79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.23 (t, *J* = 6.8 Hz, 1H), 7.15 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.48 (br s, 1H), 3.67 (q, *J* = 6.8 Hz, 2H), 3.05 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 157.2, 136.6, 127.1, 122.6, 122.5, 119.9, 118.6, 111.9, 111.6, 40.3, 24.9; HRMS (APCI) Calcd for $C_{12}H_{11}F_3N_2O$ 279.0716; found 279.0716 $[M+Na]^+$.

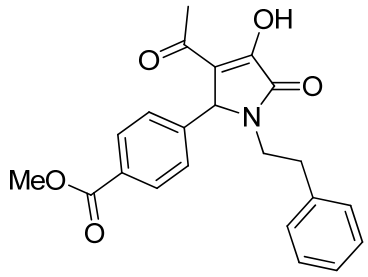


*tert-Butyl 3-(2-(2,2,2-trifluoroacetamido)ethyl)-1H-indole-1-carboxylate (**1616-65b**).* To a solution of **1616-65a/1616-287** (2.5 g, 9.8 mmol) in THF (98 mL, 0.10 M) was added di-*tert*-butyl dicarbonate (2.7 mL, 12 mmol, 1.2 equiv) and *N,N*-dimethylpyridin-4-amine (0.060 g, 0.49 mmol). The resulting mixture was warmed to 40 °C and stirred for 1 h. The reaction was diluted with DCM and washed with water before being dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (Hexanes/EtOAc: 10/1) to yield a yellow residue (2.7 g, 76 %) which was carried on without further attempts at purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (br s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.26 (t, *J* = 8.8 Hz, 1H), 6.59 (s, 1H), 3.68 (q, *J* = 6.4 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H), 1.67 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ

171.4, 157.3, 149.8, 135.8, 130.1, 125.0, 123.6, 122.9, 118.8, 116.6, 115.6, 84.0, 39.8, 28.4, 24.7; HRMS (APCI) Calcd for $C_{17}H_{19}F_3N_2O_3$

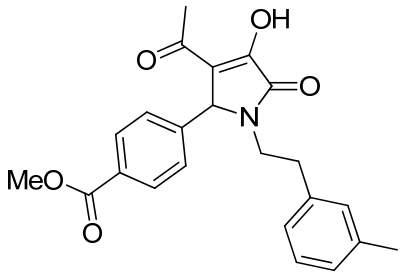


*tert-Butyl-3-(2-(3-acetyl-4-hydroxy-2-(4-(methoxycarbonyl)phenyl)-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-1H-indole-1-carboxylate (**1616-65**).* To a solution of **1616-65b** (2.7 g, 7.5 mmol) in MeOH: Water (2:1, 0.10 M) was added finely ground potassium carbonate (3.7 g, 27 mmol). The resulting mixture was stirred at rt for 48 h before being diluted with water and extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield an orange oil which was taken on without further purification. The crude material was then combined with methyl 4-formylbenzoate (1.1 g, 6.9 mmol) and methyl acetopyruvate (1.0 g, 6.9 mmol) and carried on though Procedure I to yield a cream colored solid (2.2 g, 60 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.39 (s, 1H), 7.33-7.27 (m, 3H), 7.17 (t, J = 7.6 Hz, 1H), 5.35 (s, 1H), 3.88-3.76 (m, 4H), 2.93-2.87 (m, 2H), 2.80-2.73 (m, 1H), 2.28 (s, 3H), 1.60 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.0, 165.9, 165.2, 149.0, 142.4, 134.7, 129.8, 129.7, 129.3, 128.1, 125.5, 124.4, 123.2, 122.4, 119.0, 117.2, 114.7, 83.5, 59.5, 52.6, 52.2, 29.8, 27.7, 22.9 (Note: Either Carbon 1 or 2 is absent); mp 142-146 °C; HRMS (APCI) Calcd for $C_{29}H_{30}N_2O_7$ 541.1945; found 541.1970 $[M+Na]^+$.



*Methyl 4-(3-acetyl-4-hydroxy-5-oxo-1-phenethyl-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-66**).*

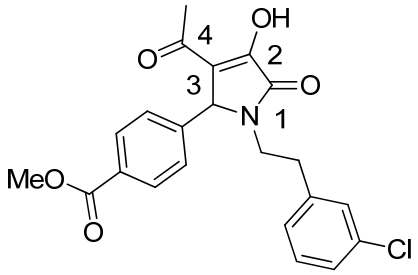
Compound **1616-66** was prepared via Procedure I from methyl 4-formylbenzoate (0.34 g, 2.1 mmol), ethyl acetopyruvate (0.33 g, 2.1 mmol) and 2-phenylethanamine (0.25 g, 2.1 mmol) to yield a white solid (0.73 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, J = 8.4 Hz, 2H), 7.31-7.24 (m, 4H), 7.19 (d, J = 7.2 Hz, 1H), 7.10 (d, J = 6.8 Hz, 2H), 5.15 (s, 1H), 3.83-3.76 (m, 4H), 2.82-2.60 (m, 3H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.9, 165.9, 165.0, 142.4, 138.5, 129.4, 129.4, 128.6, 128.5, 128.1, 126.4, 125.6, 119.7, 66.4, 59.7, 52.2, 41.5, 33.5; mp 123-128 °C; HRMS (APCI) Calcd for $C_{22}H_{21}NO_5$ 380.1493; found 380.1494 $[M+H]^+$.



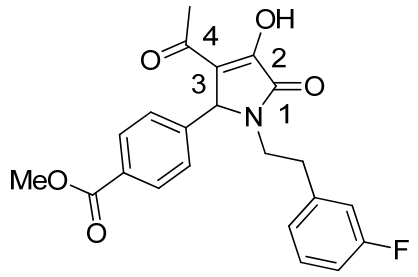
*Methyl 4-(3-acetyl-4-hydroxy-1-(3-methylphenethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-67**).*

Compound **1616-67** was prepared via Procedure I from methyl 4-formylbenzoate (0.30 g, 1.8 mmol), ethyl acetopyruvate (0.29 g, 1.8 mmol) and 2-(*m*-tolyl)ethanamine (0.25 g, 1.8 mmol) to yield a white solid (0.56 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, J = 7.6 Hz, 2H), 7.28 (d, J = 7.6 Hz, 2H), 7.12 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 7.6 Hz, 1H), 6.88-6.86 (m, 2H), 5.13 (s, 1H), 3.83 (s, 3H), 3.79-3.73 (m, 1H), 2.77-2.70 (m, 2H), 2.61-2.57 (m, 1H), 2.26

(s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.5, 166.0, 165.1, 142.4, 138.4, 137.5, 129.4, 129.2, 128.4, 128.1, 127.1, 126.9, 125.62, 125.56, 119.7, 66.4, 59.7, 52.2, 41.6, 33.4, 21.0; mp 118-123 °C; HRMS (APCI) Calcd for $C_{23}H_{23}NO_5$ 394.1649; found 394.1651 $[M+H]^+$.

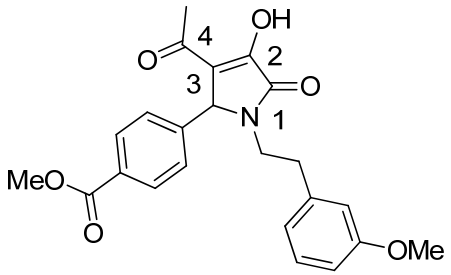


*Methyl 4-(3-acetyl-1-(3-chlorophenethyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-68**).* Compound **1616-68** was prepared via Procedure I from methyl 4-formylbenzoate (0.26 g, 1.6 mmol), ethyl acetopyruvate (0.25 g, 1.6 mmol) and 2-(3-chlorophenyl)ethanamine (0.25 g, 1.6 mmol) to yield a pale yellow solid (0.50 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, J = 8.4 Hz, 2H), 7.32-7.20 (m, 5H), 7.08 (d, J = 7.2 Hz, 1H), 5.22 (s, 1H), 3.83-3.79 (m, 4H), 2.79-2.69 (m, 3H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 165.1, 142.3, 141.2, 133.0, 130.5, 130.2, 129.4, 128.5, 128.1, 127.6, 127.4, 126.4, 125.5, 59.5, 52.2, 41.1, 32.9, 29.7 (Note: Carbon 4 is absent); mp 118-122 °C; HRMS (APCI) Calcd for $C_{22}H_{20}ClNO_5$ 414.1108; found 414.1109 $[M+H]^+$.

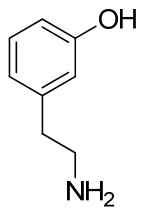


*Methyl 4-(3-acetyl-1-(3-fluorophenethyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-69**).* Compound **1616-69** was prepared via Procedure I from methyl 4-formylbenzoate (0.30 g, 1.8

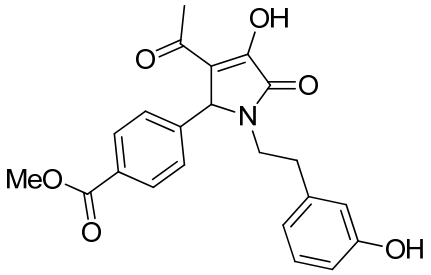
mmol), ethyl acetopyruvate (0.28 g, 1.8 mmol) and 2-(3-fluorophenyl)ethanamine (0.25 g, 1.8 mmol) to yield an off-white solid (0.57 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.29-7.25 (m, 1H), 7.00-6.94 (m, 3H), 5.22 (s, 1H), 3.83-3.79 (m, 4H), 2.81-2.69 (m, 3H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 165.1, 162.2 (d, J = 241.8 Hz), 142.3, 141.6, 141.5, 130.3 (d, J = 8.1 Hz), 129.44, 129.40, 128.1, 125.6, 124.8, 115.3 (d, J = 20.9 Hz), 113.2 (d, J = 20.9 Hz), 59.5, 52.2, 41.1, 33.0, 15.2 (Note: Carbon 4 is absent); mp 114-119 °C; HRMS (APCI) Calcd for $C_{22}H_{20}FNO_5$ 398.1403; found 398.1404 $[M+H]^+$.



*Methyl 4-(3-acetyl-4-hydroxy-1-(3-methoxyphenethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-70**).* Compound **1616-70** was prepared via Procedure I from methyl 4-formylbenzoate (0.27 g, 1.7 mmol), ethyl acetopyruvate (0.26 g, 1.7 mmol) and 2-(3-methoxyphenyl)ethanamine (0.25 g, 1.7 mmol) to yield an off-white solid (0.52 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 6.68-6.66 (m, 2H), 5.15 (s, 1H), 3.83-3.76 (m, 4H), 3.69 (s, 3H), 2.77-2.70 (m, 2H), 2.65-2.60 (m, 1H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 165.0, 159.3, 145.8, 142.4, 140.1, 129.5, 129.42, 129.38, 128.1, 125.5, 120.8, 114.1, 111.9, 59.6, 54.9, 52.2, 41.4, 33.4, 29.7 (Note: Carbon 4 is absent); mp 150-153 °C; HRMS (APCI) Calcd for $C_{23}H_{23}NO_6$ 410.1603; found 410.1604 $[M+H]^+$.

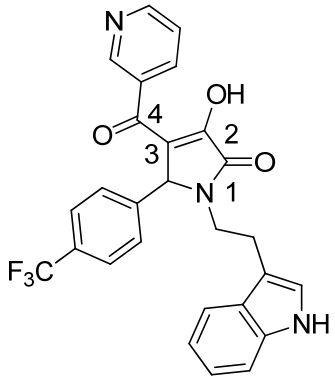


*3-(2-Aminoethyl)phenol (**1616-71a**).* To a solution of 2-(3-methoxyphenyl)ethanamine (1.0 g, 6.6 mmol) in acetic acid (3.97 mL, 1.7 M) was added 48 % hydrogen bromide solution (4.0 mL, 35 mmol, 5.3 equiv). The resulting mixture was brought to reflux and stirred for 4 h. The mixture was then cooled to rt and concentrated *in vacuo*. The residue was dissolved in MeOH and concentrated down 4 times to afford a brown crystalline solid. The solid was dissolved in minimal DCM and triethylamine (2.8 mL, 20 mmol, 3.0 equiv) was added. After stirring for 2 h the mixture was washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford an orange oil (0.46 g, 51 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, J = 8.0 Hz, 1H), 6.71-6.68 (m, 2H), 6.65 (s, 1H), 4.01 (br s, 3H), 3.02 (t, J = 6.4 Hz, 2H), 1.15 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 140.6, 130.2, 119.8, 116.3, 114.3, 42.7, 38.5; HRMS (APCI) Calcd for $C_8H_{11}NO$ 138.0913; found 138.0913 $[M+H]^+$.

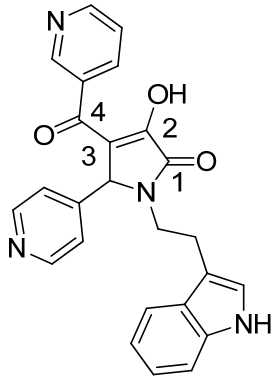


*Methyl 4-(3-acetyl-4-hydroxy-1-(3-hydroxyphenethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-71**).* Compound **1616-71** was prepared via Procedure I from methyl 4-formylbenzoate (0.55 g, 3.4 mmol), ethyl acetopyruvate (0.53 g, 3.4 mmol) and **1616-71a** (0.46 g, 3.4 mmol) to yield a cream colored solid (1.0 g, 78 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (br s, 1H), 7.93

(d, *J* = 8.8 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.08 (t, *J* = 7.6 Hz, 1H), 6.61 (dt, *J* = 1.6 Hz, *J* = 6.8 Hz, 1H), 6.54-6.52 (m, 2H), 5.16 (s, 1H), 3.87-3.83 (m, 4H), 2.74-2.69 (m, 2H), 2.56-2.52 (m, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.0, 166.0, 165.0, 157.5, 142.4, 139.9, 129.9, 129.7, 129.5, 129.2, 128.1, 127.0, 119.2, 115.4, 113.5, 59.7, 52.2, 48.7, 41.6, 33.6; mp 98-104 °C; HRMS (APCI) Calcd for $C_{22}H_{21}NO_6$ 396.1447; found 396.1446 $[M+H]^+$.

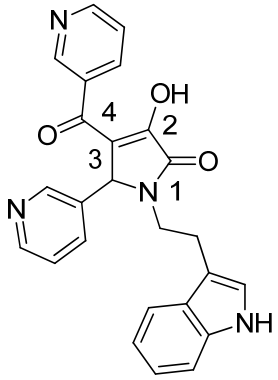


*1-(2-(1H-Indol-3-yl)ethyl)-3-hydroxy-4-nicotinoyl-5-(4-(trifluoromethyl)phenyl)-1H-pyrrol-2(5H)-one (**1616-72**).* Compound **1616-72** was prepared via Procedure I from 4-(trifluoromethyl)benzaldehyde (0.17 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield a yellow solid (0. 24 g, 49 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.83 (d, *J* = 1.2 Hz, 1H), 8.70 (dd, *J* = 1.8 Hz, *J* = 4.8 Hz, 1H), 8.20 (br s, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.54-7.48 (m, 3H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.13 (s, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 6.91 (t, *J* = 7.2 Hz, 1H), 5.46 (s, 1H), 3.88-3.83 (m, 1H), 3.02-2.92 (m, 2H), 2.78-2.73 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.2, 165.3, 151.8, 149.0, 141.9, 136.6, 136.3, 134.2, 128.9, 128.2, 126.9, 125.5, 125.4, 123.5, 122.9, 121.2, 121.1, 118.2, 118.0, 111.5, 110.7, 60.3, 41.2, 23.7 (NOTE: Either Carbon 1 or 2 is absent); mp 235-240 °C; HRMS (APCI) Calcd for $C_{27}H_{20}F_3N_3O_3$ 492.1535; found 492.1532 $[M+H]^+$.



*1-(2-(1H-Indol-3-yl(ethyl)-3-hydroxy-4-nicotinoyl-5-(pyridine-4-yl)-1H-pyrrol-2(5H)-one (**1616-73**).*

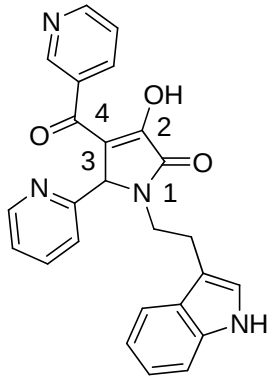
Compound **1616-73** was prepared via Procedure I from isonicotinaldehyde (0.11 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield a mustard-colored solid (0.32 g, 76 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.84 (d, J = 1.8 Hz, 1H), 8.67 (dd, J = 1.8 Hz, J = 4.8 Hz, 1H), 8.53 (d, J = 5.4 Hz, 2H), 8.05 (dt, J = 2.4 Hz, J = 8.4 Hz, 1H), 7.49-7.47 (m, 2H), 7.42-7.34 (m, 3H), 7.30 (br s, 1H), 7.13 (d, J = 1.8 Hz, 1H), 7.06 (t, J = 7.8 Hz, 1H), 6.94 (t, J = 8.4 Hz, 1H), 5.34 (s, 1H), 3.92-3.87 (m, 1H), 3.02-2.91 (m, 2H), 2.82-2.77 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 185.5, 166.1, 151.3, 148.9, 148.4, 136.7, 136.3, 134.5, 126.9, 125.5, 123.6, 123.4, 123.0, 121.1, 118.3, 118.1, 116.1, 111.6, 110.8, 59.7, 41.2, 23.7 (NOTE: Either Carbon 1 or 2 is absent); mp 225-230 °C; HRMS (APCI) Calcd for $C_{25}H_{20}N_4O_3$ 425.1608; found 425.1610 $[M+H]^+$.



*1-(2-(1H-Indol-3-yl)ethyl)-3-hydroxy-4-nicotinoyl-5-(pyridine-3-yl)-1H-pyrrol-2(5H)-one (**1616-74**).*

Compound **1616-74** was prepared via Procedure I from nicotinaldehyde (0.11 g, 1.0 mmol),

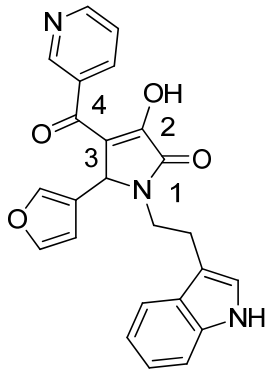
1616-28a (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield an orange solid (0.29 g, 67 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.90 (s, 1H), 8.83 (d, J = 2.4 Hz, 1H), 8.68 (dd, J = 1.8 Hz, J = 4.8 Hz, 1H), 8.57 (d, J = 1.8 Hz, 1H), 8.49 (dd, J = 2.4 Hz, J = 4.8 Hz, 1H), 8.04 (dt, J = 1.8 Hz, J = 7.8 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.49-7.47 (m, 1H), 7.36-7.32 (m, 3H), 7.24 (s, 1H), 7.13 (d, J = 1.8 Hz, 1H), 7.06 (t, J = 7.8 Hz, 1H), 6.94 (t, J = 8.4 Hz, 1H), 5.39 (s, 1H), 3.89-3.85 (m, 1H), 3.02-2.93 (m, 2H), 2.79-2.75 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.3, 151.9, 149.3, 149.1, 148.9, 136.4, 136.3, 135.7, 134.1, 128.1, 126.9, 125.5, 124.0, 123.4, 122.9, 121.1, 118.3, 118.1, 111.5, 110.7, 58.4, 41.0, 23.7 (NOTE: Carbons 1 and 2 are absent); mp 225-227 °C; HRMS (APCI) Calcd for $C_{25}H_{20}N_4O_3$ 425.1608; found 425.1610 $[M+H]^+$.



*1-(2-(1H-Indol-3-yl)ethyl)-3-hydroxy-4-nicotinoyl-5-(pyridine-2-yl)-1H-pyrrol-2(5H)-one (**1616-75**).*

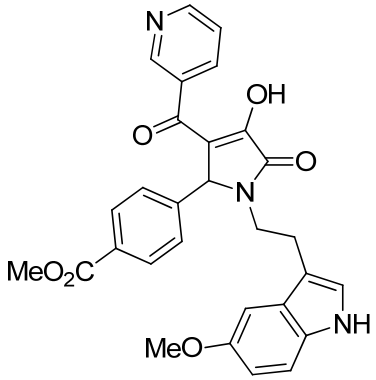
Compound **1616-75** was prepared via Procedure I from picolinaldehyde (0.11 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield a yellow solid (0.30 g, 71 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.80 (d, J = 1.2 Hz, 1H), 8.69 (dd, J = 1.2 Hz, J = 4.8 Hz, 8.55 (d, J = 4.2 Hz, 1H), 8.00 (d, J = 7.2 Hz, 1H), 7.77 (td, J = 1.2 Hz, J = 7.2 Hz, 1H), 7.50-7.46 (m, 2H), 7.38-7.30 (m, 4H), 7.12 (d, J = 1.8 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 6.95 (t, J = 7.8 Hz, 1H), 5.54 (s, 1H), 3.87-3.82 (m, 1H), 3.01-2.91 (m, 2H), 2.72-2.67 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.2, 165.4, 156.1, 151.9, 149.4, 149.0,

137.2, 136.3, 134.2, 128.2, 126.9, 125.5, 123.8, 123.4, 122.8, 121.0, 118.3, 118.1, 111.5, 110.8, 62.1, 41.4, 23.6 (NOTE: Either Carbon 1 or 2 and Carbon 3 are absent); mp 218-223 °C; HRMS (APCI) Calcd for $C_{25}H_{20}N_4O_3$ 425.1613; found 425.1613 $[M+H]^+$.

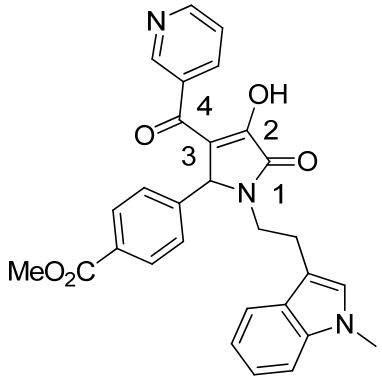


*1-(2-(1H-indol-3-yl)ethyl)-5-(furan-3-yl)-3-hydroxy-4-nicotinoyl-1H-pyrrol-2(5H)-one (**1616-76**).*

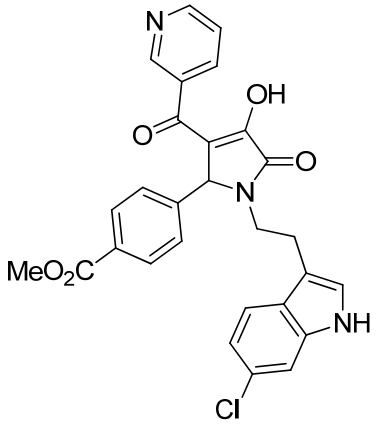
Compound **1616-76** was prepared via Procedure I from furan-3-carbaldehyde (0.10 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield an orange solid (0.12 g, 28 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.85 (d, J = 1.8 Hz, 1H), 8.71 (dd, J = 1.2 Hz, J = 4.8 Hz, 1H), 8.05 (dt, J = 1.8 Hz, J = 7.8 Hz, 1H), 7.74 (s, 1H), 7.50-7.48 (m, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.18 (s, 1H), 7.15 (d, J = 1.8 Hz, 1H), 7.10 (s, 1H), 7.07 (t, J = 7.2 Hz, 1H), 6.98 (t, J = 7.8 Hz, 1H), 6.48 (d, J = 1.2 Hz, 1H), 5.40 (s, 1H), 3.89-3.84 (m, 1H), 3.12-3.00 (m, 2H), 2.84-2.80 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.4, 152.4, 149.5, 144.1, 142.7, 136.3, 133.8, 127.0, 125.5, 123.4, 122.9, 121.1, 120.8, 118.3, 118.2, 117.5, 111.5, 110.9, 108.6, 52.4, 40.7, 23.7 (NOTE: Carbon 1 and 2 are absent); mp 211-217 °C; HRMS (APCI) Calcd for $C_{24}H_{19}N_3O_4$ 414.1448; found 414.1449 $[M+H]^+$.



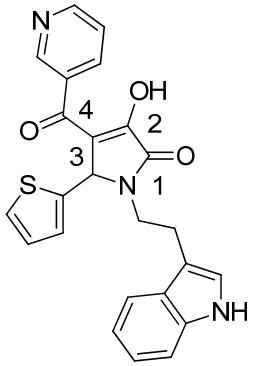
*Methyl 4-(4-hydroxy-1-(2-(5-methoxy-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-77**).* Compound **1616-77** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(5-methoxy-1*H*-indol-3-yl)ethanamine (0.19 g, 1.0 mmol) to yield a yellow solid (0.35 g, 68 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.79 (d, J = 1.8 Hz, 1H), 8.68 (dd, J = 1.8 Hz, J = 4.8 Hz, 1H), 7.99 (dt, J = 1.8 Hz, J = 7.8 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.48-7.46 (m, 1H), 7.44 (d, J = 7.8 Hz, 2H), 7.22-7.21 (m, 2H), 7.09 (d, J = 2.4 Hz, 1H), 6.75 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 2.4 Hz, J = 9.0 Hz, 1H), 5.44 (s, H), 3.85-3.80 (m, 4H), 2.68 (s, 3H), 2.98-2.89 (m, 2H), 2.73-2.70 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.7, 181.1, 165.9, 165.2, 153.0, 152.0, 149.1, 142.4, 136.3, 134.1, 131.4, 129.5, 129.4, 128.3, 127.2, 123.6, 123.5, 117.9, 112.2, 111.3, 110.5, 99.7, 60.4, 55.2, 52.2, 41.1, 23.8; mp 222-225 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_6$ 512.1816; found 512.1822 $[M+H]^+$.



*Methyl 4-(4-hydroxy-1-(2-(1-methyl-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-79**).* Compound **1616-79** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(1-methyl-1*H*-indol-3-yl)ethanamine (0.17 g, 1.0 mmol) to yield cream colored solid (0.21 g, 43 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.81 (d, J = 1.8 Hz, 1H), 8.70 (dd, J = 1.8 Hz, J = 4.8, 1H), 8.01 (dt, J = 1.8 Hz, 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.50-7.46 (m, 3H), 7.36 (d, J = 9.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.14-7.10 (m, 2H), 6.96 (t, J = 7.8 Hz, 1H), 5.50 (s, 1H), 3.85-3.80 (m, 4H), 3.70 (s, 3H), 3.00-2.92 (m, 2H), 2.76-2.72 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.2, 165.9, 165.0, 153.9, 152.2, 149.1, 142.0, 136.6, 136.3, 133.9, 129.5, 129.4, 128.4, 127.3, 127.2, 123.5, 121.2, 118.4, 118.3, 110.1, 109.7, 60.4, 52.2, 41.3, 32.2, 23.5 (NOTE: Either Carbon 1 or 2 is absent); mp 215-218 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1867; found 496.1872 $[M+H]^+$.

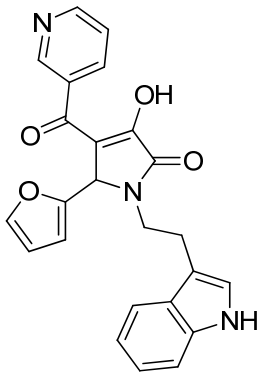


*Methyl 4-(1-(2-(6-chloro-1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-80**).* Compound **1616-80** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(6-chloro-1*H*-indol-3-yl)ethanamine (0.20 g, 1.0 mmol) to yield a yellow solid (0.44 g, 85 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.80 (s, 1H), 8.68 (d, J = 3.6 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.77 (br s, 1H), 7.48-7.41 (m, 3H), 7.37 (d, J = 1.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.17 (s, 1H), 6.94 (dd, J = 1.2 Hz, J = 8.4 Hz, 1H), 3.87-3.82 (m, 4H), 2.97-2.89 (m, 2H), 2.79-2.76 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.2, 165.9, 165.3, 149.2, 136.6, 136.2, 129.44, 129.36, 128.3, 128.1, 125.8, 125.7, 125.5, 124.7, 124.1, 123.3, 119.5, 118.8, 118.6, 111.2, 111.1, 109.8, 60.3, 52.1, 41.0, 23.4; mp 184-189 °C; HRMS (APCI) Calcd for $C_{28}H_{22}ClN_3O_5$ 516.1321; found 516.1324 $[M+H]^+$.



*1-(2-(1H-indol-3-yl)ethyl)-3-hydroxy-4-nicotinoyl-5-(thiophen-2-yl)-1H-pyrrol-2(5H)-one (**1616-81**).*

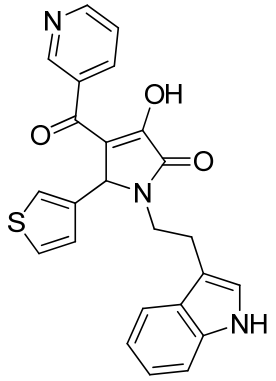
Compound **1616-81** was prepared via Procedure I from thiophene-2-carbaldehyde (0.11 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield an orange solid (0.07 g, 16 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.81 (s, 1H), 8.72 (d, J = 4.8 Hz, 1H), 8.02 (dd, J = 1.8 Hz, J = 6.0 Hz, 1H), 7.53-7.51 (m, 1H), 7.48-7.44 (m, 2H), 7.34 (d, J = 7.8, 1H), 7.20 (d, J = 3.0 Hz, 1H), 7.15 (d, J = 1.8 Hz, 1H), 7.07 (t, J = 7.8 Hz, 2H), 7.00-6.96 (m, 2H), 5.76 (s, 1H), 3.87-3.83 (m, 1H), 3.14-3.09 (m, 1H), 3.05-3.00 (m, 1H), 2.77-2.72 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.2, 152.4, 149.2, 140.2, 136.3, 133.8, 130.3, 128.7, 127.02, 126.96, 126.5, 123.6, 122.9, 121.1, 118.3, 118.2, 111.5, 110.8, 56.1, 40.9, 23.6 (NOTE: Carbons 1, 2 and 3 are absent); mp 150-155 °C; HRMS (APCI) Calcd for $C_{24}H_{19}N_3O_3S$ 430.1226; found 430.1223 $[M+H]^+$.



*1-(2-(1H-indol-3-yl)ethyl)-5-(furan-2-yl)-3-hydroxy-4-nicotinoyl-1H-pyrrol-2(5H)-one (**1616-82**).*

Compound **1616-82** was prepared via Procedure I from furfural (0.10 g, 1.0 mmol), **1616-28a**

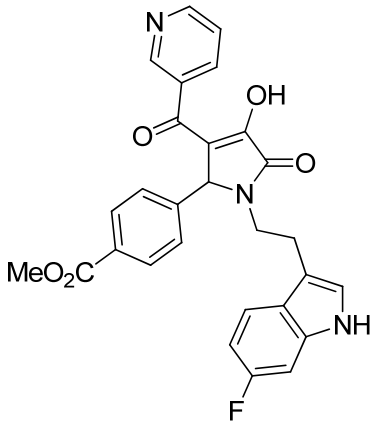
(0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield a mustard colored solid (0.06 g, 14 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.82 (d, J = 1.2 Hz, 1H), 8.72 (dd, J = 1.8 Hz, J = 4.8 Hz, 1H), 8.03 (dt, J = 1.8 Hz, J = 5.4 Hz, 1H), 7.62 (t, J = 0.6 Hz, 1H), 7.53-7.52 (m, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.18 (s, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.10-7.06 (m, 2H), 6.99 (t, J = 6.6 Hz, 1H), 6.52 (d, J = 3.0 Hz, 1H), 6.433-6.425 (m, 1H), 3.82-3.77 (m, 1H), 3.19-3.14 (m, 1H), 3.00-2.95 (m, 1H), 2.66-2.61 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.9, 164.5, 152.3, 149.1, 148.9, 143.4, 143.2, 136.3, 134.0, 127.0, 123.5, 122.9, 122.8, 121.0, 118.4, 118.3, 118.1, 115.0, 111.5, 110.8, 110.2, 54.5, 41.4, 23.7; mp 211-216 °C; HRMS (APCI) Calcd for $C_{24}H_{19}N_3O_4$ 414.1448; found 414.1452 $[M+H]^+$.



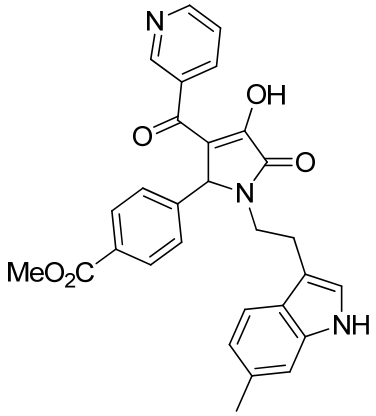
*1-(2-(1H-Indol-3-yl)ethyl)-3-hydroxy-4-nicotinoyl-5-(thiophen-3-yl)-1H-pyrrol-2(5H)-one (**1616-83**).*

Compound **1616-83** was prepared via Procedure I from thiophene-3-carbaldehyde (0.11 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield a yellow solid (0.11 g, 25 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.86 (s, 1H), 8.71 (d, J = 4.8 Hz, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 1.8 Hz, 1H), 7.50-7.47 (m, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.25 (s, 1H), 7.14 (s, 1H), 7.08-7.06 (m, 2H), 6.96 (t, J = 7.8 Hz, 1H), 5.55 (s, 1H), 3.86-3.80 (m, 1H), 3.06-2.98 (m, 2H), 2.76-2.70 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 187.4, 181.2, 164.5, 152.5, 137.2, 136.3, 133.8, 127.05, 126.98,

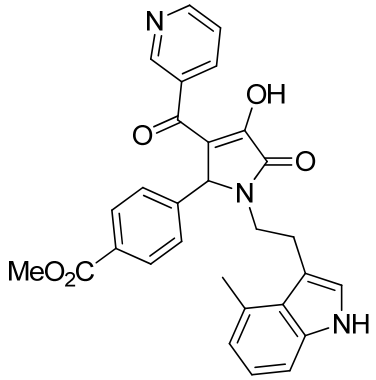
126.2, 126.0, 125.4, 123.5, 122.9, 122.8, 121.1, 118.4, 118.2, 118.0, 111.5, 110.9, 55.0, 41.2, 23.9; mp 238-242 °C; HRMS (APCI) Calcd for $C_{24}H_{19}N_3O_3S$ 430.1198; found 430.1201 $[M+H]^+$.



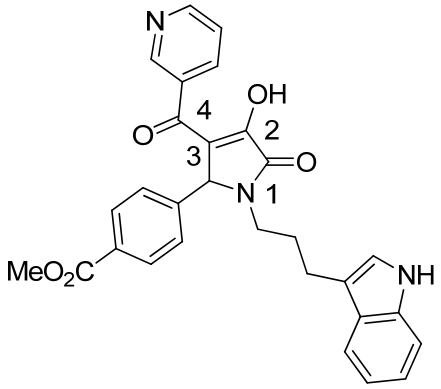
*Methyl 4-(1-(2-(6-fluoro-1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-84**).* Compound **1616-84** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 3-(6-fluoro-1*H*-indol-3-yl)ethanamine (0.18 g, 1.0 mmol) to yield a pale orange solid (0.44 g, 87 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.82 (d, J = 0.6 Hz, 1H), 8.69 (d, J = 4.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.80 (br s, 1H), 7.49-7.47 (m, 1H), 7.43 (d, J = 7.8 Hz, 2H), 7.33-7.30 (m, 1H), 7.31-7.10 (m, 2H), 6.79 (td, J = 9.6 Hz, J = 1.8 Hz, 1H), 5.44 (s, 1H), 3.89-3.82 (m, 4H), 2.98-2.91 (m, 2H), 2.80-2.76 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.9, 181.1, 165.9, 165.1, 159.7, 158.1, 152.3, 142.2, 136.3, 136.1, 133.9, 129.5, 129.3, 128.3, 125.5, 123.8, 123.4, 119.1, 118.1, 111.1, 106.7, 106.6, 60.4 (d, J = 14.4 Hz), 52.1, 41.1, 23.1; mp 162-167 °C; HRMS (APCI) Calcd for $C_{28}H_{22}FN_3O_5$ 500.1595; found 500.1600 $[M+H]^+$.



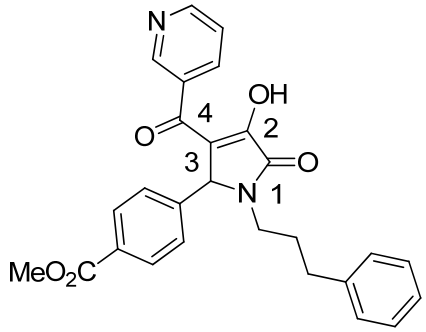
*Methyl 4-(4-hydroxy-1-(2-(6-methyl-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-85**).* Compound **1616-85** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(6-methyl-1*H*-indol-3-yl)ethanamine (0.17 g, 1.0 mmol) to yield a cream colored solid (0.10 g, 20 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.79 (s, 1H), 8.68 (d, J = 3.6 Hz, 1H), 7.99 (d, J = 6.6 Hz, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.47 (t, J = 6.0 Hz, 1H), 7.42 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 1H), 7.11 (s, 1H), 7.02 (s, 1H), 6.75 (d, J = 7.8 Hz, 1H), 5.40 (s, 1H), 3.90-3.82 (m, 4H), 2.99-2.89 (m, 2H), 2.74-2.71 (m, 1H), 2.37 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.7, 165.9, 165.1, 152.1, 149.1, 142.3, 136.7, 136.2, 134.0, 130.0, 129.5, 129.4, 128.4, 128.1, 125.5, 124.9, 123.4, 122.2, 120.0, 117.8, 111.3, 110.6, 60.4, 52.2, 41.1, 23.8, 21.4; mp 202-207 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1867; found 496.1868 $[M+H]^+$.



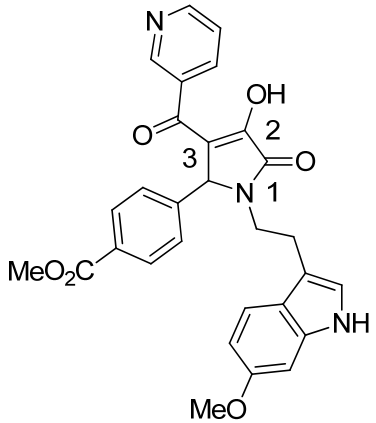
*Methyl 4-(4-hydroxy-1-(2-(4-methyl-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-86**).* Compound **1616-86** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(4-methyl-1*H*-indol-3-yl)ethanamine (0.17 g, 1.0 mmol) to yield a pale yellow solid (0.05 g, 11 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84 (d, J = 1.8 Hz, 1H), 8.82 (d, J = 1.8 Hz, 1H), 8.70 (dd, J = 1.8 Hz, J = 4.8 Hz, 1H), 8.01 (dt, J = 1.8 Hz, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.49-7.44 (m, 3H), 7.15 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.92 (t, J = 6.6 Hz, 1H), 6.67 (d, J = 7.2 Hz, 1H), 5.48 (s, 1H), 3.89-3.81 (m, 4H), 3.14-3.09 (m, 1H), 2.98-2.87 (m, 2H), 2.44 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.9, 165.9, 165.1, 152.3, 149.3, 142.2, 136.7, 136.2, 133.9, 129.5, 129.4, 128.3, 125.4, 123.4, 123.1, 122.9, 121.1, 120.1, 119.9, 118.2, 111.5, 109.6, 66.4, 60.4, 52.2, 25.5, 19.7; mp 170-175 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1845; found 496.1850 $[M+H]^+$.



*Methyl 4-(1-(3-(1H-indol-3-yl)propyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-87**).* Compound **1616-87** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 3-(1*H*-indol-3-yl)propan-1-amine (0.17 g, 1.0 mmol) to yield a yellow solid (0.35 g, 71 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.75 (s, 1H), 8.82 (s, 1H), 8.69 (d, J = 4.8 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.49-7.47 (m, 3H), 7.37 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.06-7.03 (m, 2H), 6.92 (t, J = 7.2 Hz, 1H), 5.54 (s, 1H), 3.62-3.55 (m, 4H), 2.83-2.79 (m, 1H), 2.62-2.55 (m, 2H), 1.84-1.80 (m, 1H), 1.73-1.69 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.9, 165.9, 165.2, 154.4, 152.1, 149.2, 142.4, 136.3, 134.0, 129.5, 128.3, 127.0, 123.5, 122.4, 122.3, 120.8, 118.3, 118.0, 113.4, 111.4, 60.4, 52.3, 40.7, 28.0, 22.1 (NOTE: Either Carbon 1 or 2 and Carbon 3 are absent); mp 221-226 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_5$ 496.1845; found 496.1852 $[M+H]^+$.

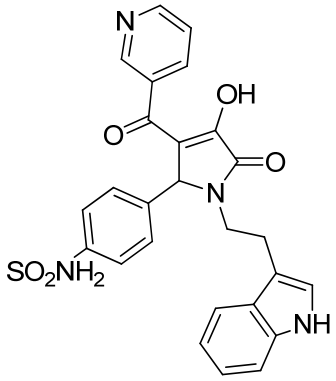


*Methyl 4-(4-hydroxy-3-nicotinoyl-5-oxo-1-(3-phenylpropyl)-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-88**).* Compound **1616-88** was prepared via Procedure I from methyl 4-formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 3-phenylpropan-1-amine (0.14 g, 1.0 mmol) to yield a pale yellow solid (0.07 g, 16 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.68 (d, J = 3.6 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.52 (d, J = 7.8 Hz, 2H), 7.47 (dd, J = 4.8 Hz, J = 7.8 Hz, 1H), 7.22 (d, J = 7.2 Hz, 2H), 7.16-7.12 (m, 3H), 5.54 (s, 1H), 3.82 (s, 3H), 3.59-3.54 (m, 2H), 2.73-2.69 (m, 2H), 1.74-1.65 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 186.5, 165.9, 165.5, 152.0, 149.2, 142.7, 141.1, 136.2, 134.2, 129.4, 128.5, 128.2, 126.1, 125.8, 123.3, 60.1, 52.2, 40.0, 32.2, 28.9 (NOTE: Carbons 1, 2 and 3 are absent) ; mp 172-177 °C; HRMS (APCI) Calcd for $C_{27}H_{24}N_2O_5$ 457.1750; found 457.1746 $[M+H]^+$.



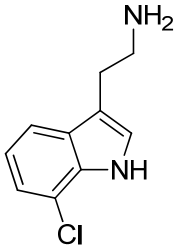
*Methyl 4-(4-hydroxyl-1-(2-(6-methyoxy-1H-indol-3-yl)ethyl)-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-89**).* Compound **1616-89** was prepared via Procedure I from methyl 4-

formylbenzoate (0.16 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and 2-(6-methoxy-1*H*-indol-3-yl)ethanamine (0.19 g, 1.0 mmol) to yield a pale orange solid (0.39 g, 76 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.78 (s, 1H), 8.67 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 7.2 Hz, 1H), 7.41 (d, J = 7.8 Hz, 2H), 7.11 (d, J = 7.8 Hz, 1H), 6.96 (s, 1H), 6.83 (s, 1H), 6.58 (d, J = 7.8 Hz, 1H), 5.39 (s, 1H), 3.82 (s, 3H), 3.75-3.70 (m, 4H), 2.94-2.90 (m, 2H), 2.71-2.69 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.8, 155.6, 145.7, 137.6, 137.4, 137.0, 130.8, 130.4, 129.5, 129.4, 128.3, 128.11, 128.07, 125.5, 121.3, 118.7, 110.8, 108.5, 107.5, 94.5, 60.4, 55.2, 40.9, 23.8, 20.8 (NOTE: Two of either Carbon 1, 2, or 3 are absent); mp 195-200 °C; HRMS (APCI) Calcd for $C_{29}H_{25}N_3O_6$ 512.1816; found 512.1821 $[M+H]^+$.

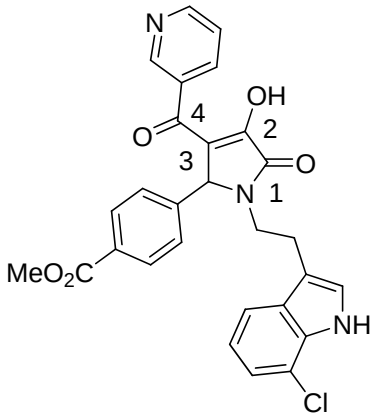


*4-(1-(2-(1H-Indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5,-dihydro-1H-pyrrol-2-yl)benzenesulfonamide (**1616-90**).* Compound **1616-90** was prepared via Procedure I from 4-formylbenzenesulfonamide (0.19 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol), and tryptamine (0.16 g, 1.0 mmol) to yield a pale orange solid (0.024 g, 5 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.81 (s, 1H), 8.69 (d, J = 4.2 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 9.0 Hz, 2H), 7.50-7.47 (m, 3H), 7.34 (d, J = 7.8 Hz, 2H), 7.32 (s, 2H), 7.14 (d, J = 1.8 Hz, 1H), 7.07 (t, J = 8.4 Hz, 1H), 6.96 (t, J = 7.2 Hz, 1H), 5.41 (s, 1H), 3.91-3.86 (m, 1H), 3.03-2.98 (m, 1H), 2.92-2.88 (m, 1H), 2.81-2.76 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.3,

165.0, 149.3, 149.1, 143.9, 140.6, 136.3, 133.9, 131.8, 128.6, 126.9, 125.9, 123.4, 122.9, 121.1, 118.4, 118.2, 111.5, 110.8, 60.1, 41.0, 23.7 (NOTE: Two of either Carbons 1,2 or 3 are absent); mp >250 °C; HRMS (APCI) Calcd for $C_{26}H_{22}N_4O_5S$ 503.1384; found 503.1381 $[M+H]^+$.

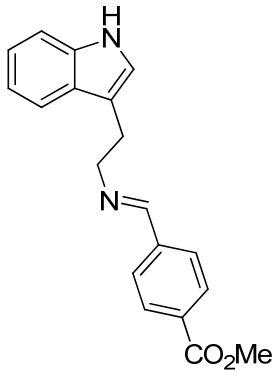


*2-(7-Chloro-1H-indol-3-yl)ethanamine (**1616-91a**).* To a slurry of 2-(7-chloro-1*H*-indol-3-yl)ethanamine hydrochloride (0.15 g, 0.65 mmol) in MeOH (0.072 mL) was added triethylamine (0.11 mL, 0.78 mmol). Ether (3.62 mL) was added and the mixture was stirred at -10 °C for 1 h. The resulting triethylamine hydrochloride salt was filtered off and the filtrate was concentrated *in vacuo* to afford a white solid (0.074 g, 58 %) which was carried on immediately.

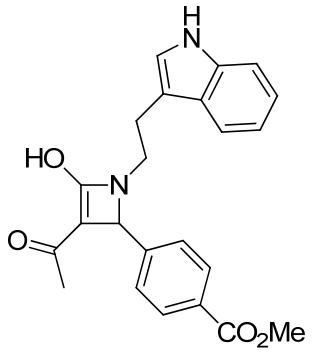


*Methyl 4-(1-(2-(7-chloro-1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-91**).* Compound **1616-91** was prepared via Procedure I from methyl 4-formylbenzoate (0.064 g, 0.39 mmol), **1616-28a** (0.086 g, 0.39 mmol) and **1616-91a** (0.076 g, 0.39 mmol) to yield a yellow solid (0.009 g, 5 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.22 (s,

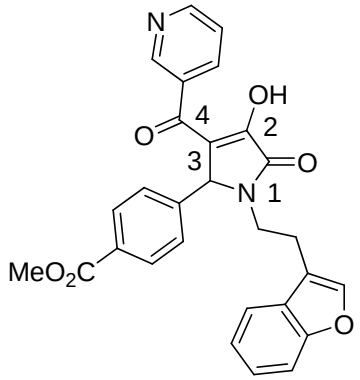
1H), 8.80 (s, 1H), 8.69 (d, *J* = 4.2 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.49-7.44 (m, 3H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 1.8 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 5.49 (s, 1H), 3.89-3.82 (m, 4H), 3.00-2.91 (m, 2H), 2.83-2.78 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.3, 165.9, 165.0, 152.4, 149.3, 142.0, 136.2, 133.9, 133.0, 129.5, 129.4, 129.0, 128.3, 124.4, 123.5, 120.6, 119.4, 118.2, 117.3, 115.9, 112.3, 60.2, 52.2, 41.0, 23.6 (NOTE: One of either Carbon 1, 2 or 3 are absent); mp 246-249 °C; HRMS (APCI) Calcd for $C_{28}H_{22}ClN_3O_5$ 516.1321; found 516.1325 $[M+H]^+$.



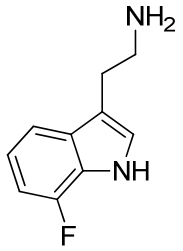
*(E)-Methyl 4-(((2-(1H-indol-3-yl)ethyl)imino)methyl)benzoate (**1616-92a**).* Methyl 4-formylbenzoate (0.33 g, 2.0 mmol) and 2-(1*H*-indol-3-yl)ethanamine (0.32 g, 2.0 mmol) were combined in EtOH (10 mL, 0.2 M) and refluxed for 3 h. At this time, the resulting mixture was cooled to rt and concentrated *in vacuo*. Purification was achieved via recrystallization with EtOH to yield an off-white, crystalline solid (0.26 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.14 (br s, 1H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 7.01 (s, 1H), 3.99 (t, *J* = 7.2 Hz, 2H), 3.95 (s, 3H), 3.21 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 160.7, 140.3, 136.4, 131.8, 130.0, 128.1, 127.6, 122.4, 122.1, 119.4, 119.1, 113.9, 111.3, 62.4, 52.5, 26.9; HRMS (APCI) Calcd for $C_{19}H_{18}N_2O_2$ 307.1441; found 307.1440 $[M+H]^+$.



*Methyl 4-(1-(2-(1H-indol-3-yl)ethyl)-3-acetyl-4-hydroxyl-1,2-dihydroazet-2-yl)benzoate (**1616-92**).* To a solution of pyruvic acid (0.10 mL, 1.3 mmol) in DCM (4.2 mL) was added triphosgene (0.13 g, 0.42 mmol). The resulting mixture was refluxed for 30 min. At this time, a solution of **1616-92a** (0.26 g, 0.85 mmol) in DCM (0.85 mL) was added dropwise to the refluxing solution. Triethylamine (0.36 mL, 2.6 mmol) was added and the mixture continued to reflux for 5 h before being brought to rt and stirred overnight. The mixture was then washed with water (2x) and saturated sodium bicarbonate (2x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (3:1 Hexanes/EtOAc) to afford a pale yellow, crystalline solid (0.11 g, 34 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.86 (s, 1H), 3.93-3.90 (m, 5H), 3.39 (td, *J* = 4.2 Hz, *J* = 12.6 Hz, 1H), 3.07 (td, *J* = 6.0 Hz, *J* = 16.8 Hz, 1H), 2.91 (dd, *J* = 3.6 Hz, *J* = 15.6 Hz, 1H), 2.47 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.2, 166.9, 166.1, 143.8, 136.6, 130.5, 130.2, 129.5, 129.0, 128.5, 126.6, 123.0, 120.2, 118.6, 111.4, 110.4, 52.5, 52.2, 40.6, 28.1, 22.5; mp 70-75 °C; HRMS (APCI) Calcd for $C_{23}H_{22}N_2O_4$ 391.1652; found 391.1653 $[M+H]^+$.

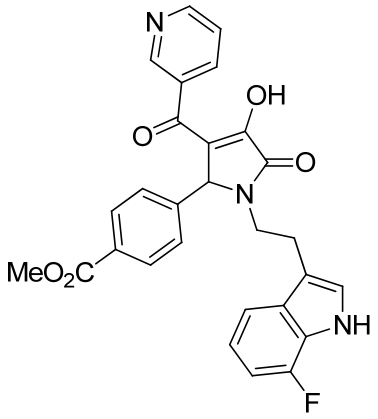


*Methyl 4-(1-(2-(benzofuran-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-93**).* Compound **1616-93** was prepared via Procedure I from methyl 4-formylbenzoate (0.20 g, 1.2 mmol), **1616-28a** (0.27 g, 1.2 mmol) and 2-(benzofuran-3-yl)ethanamine (0.20 g, 1.2 mmol) to yield a pale yellow solid (0.028 g, 4.7 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.57 (s, 1H), 7.99 (d, J = 7.2 Hz, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.77 (s, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.47-7.42 (m, 3H), 7.37 (t, J = 5.4 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.20 (t, J = 7.2 Hz, 1H), 5.44 (s, 1H), 3.87-3.82 (m, 4H), 2.97-2.92 (m, 2H), 2.76-2.75 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.3, 181.3, 166.0, 154.6, 150.6, 149.5, 142.4, 135.9, 129.1, 128.8, 128.1, 127.5, 124.4, 122.7, 122.4, 119.6, 117.0, 115.6, 111.3, 60.2, 52.1, 40.0, 21.7 (NOTE: Carbons 1, 2 and 3 are absent); mp 225-230 °C; HRMS (APCI) Calcd for $C_{28}H_{22}N_2O_6$ 481.1397; found 481.1396 $[M-H]^-$.

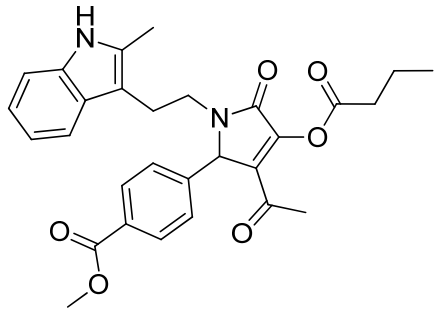


*2-(7-Fluoro-1H-indol-3-yl)ethanamine (**1616-94a**).* To a slurry of 2-(7-fluoro-1*H*-indol-3-yl)ethanamine hydrochloride (0.05 g, 0.23 mmol) in MeOH (0.026 mL) was added triethylamine (0.039 mL, 0.28 mmol). Ether (1.3 mL) was added and the mixture was stirred

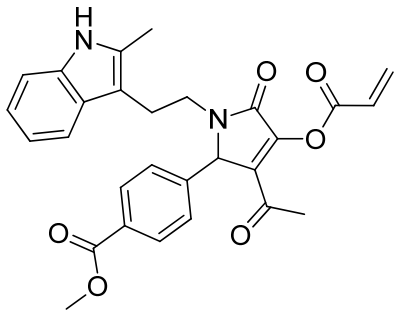
at -10 °C for 1 h. The resulting triethylamine hydrochloride salt was filtered off and the filtrate was concentrated *in vacuo* to afford a white solid (0.040 g, >99 %) which was carried on immediately.



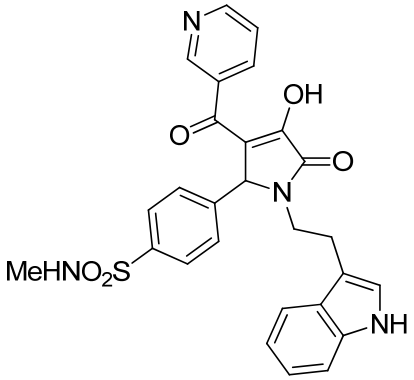
*Methyl 4-(1-(2-(7-fluoro-1H-indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-94**).* Compound **1616-94** was prepared via Procedure I from methyl 4-formylbenzoate (0.039 g, 0.24 mmol), **1616-28a** (0.052 g, 0.24 mmol) and **1616-94a** (0.042 g, 0.24 mmol) to yield a pale yellow solid (0.11 g, 97 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.30 (s, 1H), 10.11 (s, 1H), 8.74 (m, 1H), 8.46 (m, 1H), 7.98-7.97 (m, 1H), 7.87-7.86 (m, 1H), 7.37-7.33 (m, 2H), 7.28 (m, 1H), 7.16-7.14 (m, 2H), 6.89-6.87 (m, 2H), 5.23 (s, 1H), 3.84-3.76 (m, 4H), 2.92 (m, 1H), 2.79 (m, 1H), 2.73-2.68 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 193.0 (d, J = 13.4 Hz), 169.4, 166.2, 165.5, 150.0, 149.5, 148.4, 147.7, 139.1, 136.6, 134.3, 131.1, 129.7, 129.0, 128.7, 128.3, 127.7, 124.0, 118.6, 114.7, 112.4, 105.8, 60.0 (d, J = 45.5 Hz), 52.0 (d, J = 41.2 Hz), 40.1 (d, J = 21.8 Hz), 23.3; mp 65-69 °C; HRMS (APCI) Calcd for $C_{28}H_{22}FN_3O_5$ 500.1616; found 500.1616 $[M+H]^+$.



*Methyl 4-(3-acetyl-4-(butyrloxy)-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-95**).* To a solution of **1616-19** (0.30 g, 0.69 mmol) and triethylamine (0.19 mL, 1.4 mmol, 2.0 equiv) in THF (0.69 mL, 1.0 M) at -30 °C was added butyryl chloride (0.072 mL, 0.69 mmol, 1.0 equiv) dropwise over 20 min. The mixture was allowed to stir at -30 °C for 2 h before being concentrated *in vacuo.* The crude material was dissolved in EtOAc, washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (10% EtOAc: DCM) to afford a yellow, amorphous, crystalline solid (0.12 g, 35 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 7.2 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 2H), 4.78 (s, 1H), 3.89 (s, 3H), 3.87-3.82 (m, 1H), 3.02 (dt, *J* = 13.8 Hz, *J* = 8.4 Hz, 1H), 2.91 (dt, *J* = 13.8 Hz, *J* = 7.8 Hz, 1H), 2.82-2.78 (m, 1H), 2.70 (t, *J* = 7.8 Hz, 2H), 2.24 (s, 3H), 2.23 (s, 3H), 1.85 (sextet, 7.8 Hz, 2H), 1.09 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.6, 170.0, 166.6, 163.7, 147.9, 139.1, 136.9, 135.4, 132.1, 130.8, 130.2, 128.3, 128.0, 121.5, 119.7, 117.8, 110.6, 108.0, 62.5, 52.4, 41.4, 35.9, 30.2, 23.3, 18.4, 13.7, 11.5; HRMS (APCI) Calcd for $C_{29}H_{30}N_2O_6$ 503.2168; found 503.2172 $[M+H]^+$.



*Methyl 4-(3-acetyl-4-(acryloyloxy)-1-(2-(2-methyl-1H-indol-3-yl)ethyl)-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)benzoate (**1616-96**).* To a solution of **1616-19** (3.0 g, 6.9 mmol) and triethylamine (1.9 mL, 14 mmol, 2.0 equiv) in THF (6.9 mL, 1.0 M) at -30 °C was added acryloyl chloride (0.72 mL, 6.9 mmol, 1.0 equiv) dropwise over 20 min. The mixture was allowed to stir at -30 °C for 2 h before being concentrated *in vacuo*. The crude material was dissolved in EtOAc, washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification was achieved via flash column chromatography on SiO_2 (10% EtOAc: DCM) to afford a yellow, amorphous, crystalline solid (0.68 g, 20 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.27-7.20 (m, 2H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.03-6.94 (m, 3H), 6.71 (d, *J* = 0.9 Hz, 1H), 6.43 (d, *J* = 10.2 Hz, 1H), 6.20 (d, *J* = 0.6 Hz, 1H), 4.81 (s, 1H), 3.89-3.81 (m, 4H), 3.08-2.76 (m, 3H), 2.24 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.1, 191.6, 186.7, 166.6, 163.6, 162.2, 147.6, 139.0, 137.3, 135.7, 135.4, 132.1, 130.8, 130.2, 128.3, 128.0, 126.0, 121.5, 119.6, 117.7, 110.7, 107.9, 62.6, 52.4, 41.5, 30.2, 23.3, 11.5; HRMS (APCI) Calcd for $C_{28}H_{26}N_2O_6$ 487.1837; found 487.1860 $[M+H]^+$.



*4-(1-(2-(1H-Indol-3-yl)ethyl)-4-hydroxy-3-nicotinoyl-5-oxo-2,5-dihydro-3-nicotinoyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-N-methylbenzenesulfonamide (**1616-97**).* Compound **1616-97** was prepared via Procedure I from 4-formyl-*N*-methylbenzenesulfonamide (0.20 g, 1.0 mmol), **1616-28a** (0.22 g, 1.0 mmol) and tryptamine (0.16 g, 1.0 mmol) to yield an orange-yellow solid (0.11 g, 21 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.80 (s, 1H), 8.50 (d, J = 4.8 Hz, 1H), 8.02-8.00 (m, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 4.8 Hz, 1H), 7.34-7.29 (m, 3H), 7.09-7.05 (m, 2H), 6.94 (t, J = 7.8 Hz, 1H), 3.84-3.79 (m, 1H), 3.01-2.95 (m, 1H), 2.89-2.85 (m, 1H), 2.70-2.65 (m, 1H), 2.39 (d, J = 4.8 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.7, 149.9, 149.6, 149.5, 146.1, 138.0, 136.4, 136.3, 135.9, 128.5, 128.1, 127.0, 126.5, 125.6, 123.4, 122.7, 122.5, 121.0, 118.5, 118.3, 118.1, 111.5, 111.1, 60.3, 41.1, 28.7, 23.6; mp >220 °C; HRMS (APCI) Calcd for $C_{27}H_{24}N_4O_5S$ 517.1540; found 517.1545 $[M+H]^+$.

2.6.2 Separation of Enantiomers of 1616-19

The separation of the enantiomers of **149** was obtained using a ChiralPak OD-RH 30 mm x 250 mm, 5 μm column with the following conditions: flow rate 10 mL/min, injection volume 1-2 mL (5 mg/mL), 44% acetonitrile/66% water with 0.1% formic acid; **149a** t_R = 121.3 min; **149b** t_R = 129.3 min. Enantiomeric excess (ee) of both enantiomers **149a** and

149b was determined using a ChiralPak OD-RH 4.6 mm x 150 mm, 5 μm column with the following conditions: flow rate 0.5 mL/min, injection volume 10 μL, 44% acetonitrile/66% water with 0.1% formic acid; **149a** $[\alpha]_D^{20}$ -18 (*c* = 0.10, methanol), t_R = 26.1 min, 98% *ee* **149b** $[\alpha]_D^{20}$ +9 (*c* = 0.10, methanol), t_R = 29.1 min, 96% *ee* A Perkin-Elmer 314 instrument was used to obtain optical rotation data.

2.7 BIOLOGY EXPERIMENTAL DETAIL

2.7.1 In vitro Analysis of 1616-Series Analogs (Dr. Stephen Traynelis)

All protocols involving *Xenopus laevis* were approved by the Emory University Institutional Animal Care and Use Committee. Two-electrode voltage-clamp recordings were made from *Xenopus laevis* oocytes expressing recombinant GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, GluN1/GluN2D, GluA1, or GluK2 receptors following injection of cRNA. cDNAs for rat GluN1-1a (GenBank accession numbers U11418 and U08261; hereafter GluN1), GluN2A (D13211), GluN2B (U11419), GluN2C (M91563), GluN2D (L31611), GluA1 (X17184), and GluK2 (Z11548) were provided by Drs. S. Heinemann (Salk Institute), S. Nakanishi (Kyoto University), and P. Seeburg (University of Heidelberg). Oocyte isolation, cRNA synthesis, and cRNA injection have been previously described (Traynelis et al. 1998); some experiments were performed with oocytes obtained from Ecocyte (Austin, TX). Voltage-clamp recordings from oocytes were made during perfusion with recording solution containing 90 mM NaCl, 1.0 mM KCl, 0.5 mM $BaCl_2$, 0.005 mM EDTA, and 10 mM HEPES at pH 7.4 (23°C). Glass microelectrodes had resistances of 0.3-1.0 MΩ and were filled with 3.0 M KCl; the membrane potential was held

at -40 mV for all recordings. Compounds were made as 20 mM stock solutions in DMSO, and diluted to the final concentration in recording solution; final DMSO content was 0.05-0.5% (vol/vol). Oocytes expressing GluK2 receptors were pre-treated with 10 μM concanavalin A for 10 minutes. NMDA receptors were activated by 100 μM glutamate plus 30 μM glycine; GluA1 and GluK2 receptors were activated by 100 μM glutamate. In order to prevent a gradual increase in current response over the course of the experiment of GluN1/GluN2A receptor responses in oocytes, some oocytes expressing GluN1/GluN2A were injected with 50 nl of 2 mM K-BAPTA (potassium 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid). The response to glutamate and glycine in the absence and presence of 5-7 concentrations of 1616 analogs were recorded in multiple oocytes obtained from two different frogs for all experiments. The EC_{50} (half-maximally effective concentration of potentiator) was determined by fitting the equation

$$Response = (100 - maximum) / (1 + (EC_{50}/[\text{concentration}])^{N}) + maximum \qquad (1)$$

to the average composite concentration-response data normalized to the current in the absence of potentiator (100%). *N* is the Hill slope, which ranged between 1 and 2 and is not reported; *maximum* is the fitted maximal response to a saturating concentration of potentiator.

To generate a cell line with inducible NMDA receptor expression, we used a previously described Tet-On (tetracycline-inducible promoter; Clontech, Mountain View, CA) baby hamster kidney (BHK-21, ATCC CCL-10) cell line (Hansen et al., Comb. Chem. High. Though. Screen., 2008). The BHK-21 Tet-On cell line was maintained at 37 °C, 5% CO_2 and 95% relative humidity in culture medium composed of Dulbecco's modified eagle medium (DMEM) containing GlutaMAX-I, 4500 mg/L glucose and 110 mg/L sodium

pyruvate (Invitrogen, Carlsbad, CA) supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL) (Invitrogen, Carlsbad, CA), 10% dialyzed fetal bovine serum (Invitrogen, Carlsbad, CA), and 1 mg/mL G418 (Invitrogen, Carlsbad, CA). The selection marker G418 was always included to provide continuous selection of Tet-On-compatible BHK-21 cells. The cells were co-transfected with rat GluN1-1a (GenBank accession no. U11418) in the inducible pTRE2 vector and rat GluN2C (GenBank accession no. D13212) in the pCI-IRES-bla vector (See Hansen et al., Comb. Chem. High. Though. Screen., 2008 for details on this vector) using Fugene 6 transfection reagent (Promega, Madison, WI). The ratio of GluN1 and GluN2C DNA used for transfection was 10:1. The NMDA receptor antagonists DL-2-amino-5-phosphonopentanoate (AP5) (200 μM; Abcam, Cambridge, MA) and 7-chloro-kynurenate (7-CKA) (200 μM; Abcam, Cambridge, MA) were added to the culture medium to prevent NMDA receptor-mediated cell death. The following day, the cells were diluted 1:1000 and 1:10,000 and seeded in 144 mm dishes. The next day, two days after transfection, 10 μg/mL blasticidin S (Invivogen, San Diego, CA) was added to the culture medium to select for transfected cells. Unless otherwise stated, the culture medium for the cell lines always contained 1 mg/mL G418 and 10 μg/mL blasticidin S for selection, as well as 200 μM AP5 and 200 μM DCKA to prevent NMDA receptor-mediated cell death. The media was changed every 2-3 days, and blasticidin S-resistant clones were isolated 10-20 days after transfection, and evaluated for their response properties. Fluorescence-based assays were conducted as previously described (Hansen et al., 2010) and test compounds were screened at 10 μM. For some compounds, visual detection of precipitation led to inclusion of 1-10 mM 2-hydroxypropyl-β-cyclodextrin in the recording solution to enhance solubility and enable generation of the full concentration-response data.

REFERENCES

(1) Newcomer, J. W.; Krystal, J. H. *Hippocampus* 2001, *11*, 529.

(2) Tsai, G.; Yang, P.; Chung, L.-C.; Lange, N.; Coyle, J. T. *Biological Psychiatry* 1998, *44*, 1081.

(3) Heresco-Levy, U.; Javitt, D. C.; Ermilov, M.; Mordel, C.; Silipo, G.; Lichtenstein, M. *Archives of General Psychiatry* 1999, *56*, 29.

(4) Coyle, J. T.; Basu, A.; Benneyworth, M.; Balu, D.; Konopaske, G. *Handbook of Experimental Pharmacology* 2012, *213*, 267.

(5) Balu, D. T.; Coyle, J. T. *Neuroscience & Biobehavioral Reviews* 2011, *35*, 848.

(6) Moghaddam, B. *Neuron* 2003, *40*, 881.

(7) Williams, K.; Dawson, V. L.; Romano, C.; Dichter, M. A.; Molinoff, P. B. *Neuron* 1990, *5*, 199.

(8) Ransom, R. W.; Stec, N. L. *J Neurochemistry* 1988, *51*, 830.

(9) Wu, F.; Gibbs, T. T.; Farb, D. H. *Molecular Pharmacology* 1991, *40*, 333.

(10) Park-Chung, M.; Wu, F. S.; Farb, D. H. *Molecular Pharmacology* 1994, *46*, 146.

(11) Williams, K.; Zappia, A. M.; Pritchett, D. B.; Shen, Y. M.; Molinoff, P. B. *Molecular Pharmacology* 1994, *45*, 803.

(12) Traynelis, S. F.; Hartley, M.; Heinemann, S. F. *Science* 1995, *268*, 873.

(13) Horak, M.; Vlcek, K.; Chodounska, H.; Vyklicky Jr, L. *Neuroscience* 2006, *137*, 93.

(14) Masuko, T.; Kuno, T.; Kashiwagi, K.; Kusama, T.; Williams, K.; Igarashi, K. *Journal of Pharmacology and Experimental Therapeutics* 1999, *290*, 1026.

(15) Mullasseril, P.; Hansen, K.; Vance, K.; Ogden, K.; Yuan, H.; Kurtkaya, N.; Santangelo, R.; Orr, A.; Le, P.; Vellano, K.; Liotta, D.; Traynelis, S. *Nature Communications* 2010.

(16) Santangelo Freel, R. M.; Ogden, K. K.; Strong, K. L.; Khatri, A.; Chepiga, K. M.; Jensen, H. S.; Traynelis, S. F.; Liotta, D. C. *Journal of Medicinal Chemistry* 2013, *56*, 5351.

(17) Costa, B. M.; Irvine, M. W.; Fang, G.; Eaves, R. J.; Mayo-Martin, M. B.; Skifter, D. A.; Jane, D. E.; Monaghan, D. T. *Journal of Pharmacology and Experimental Therapeutics* 2010, *335*, 614.

(18) Bettini, E.; al., e. *J. Pharmacol. Exp. Ther.* 2010, *335*, 636.

(19) Mosley, C. A.; Acker, T. M.; Hansen, K. B.; Mullasseril, P.; Andersen, K. T.; Le, P.; Vellano, K. M.; Bräuner-Osborne, H.; Liotta, D. C.; Traynelis, S. F. *Journal of Medicinal Chemistry* 2010, *53*, 5476.

(20) Acker, T. M.; Yuan, H.; Hansen, K. B.; Vance, K. M.; Ogden, K. K.; Jensen, H. S.; Burger, P. B.; Mullasseril, P.; Snyder, J. P.; Liotta, D. C.; Traynelis, S. F. *Molecular Pharmacology* 2011, *80*, 782.

(21) Acker, T. M.; Khatri, A.; Vance, K. M.; Slabber, C.; Bacsa, J.; Snyder, J. P.; Traynelis, S. F.; Liotta, D. C. *Journal of Medicinal Chemistry* 2013, *56*, 6434.

(22) Balu, D. T.; Li, Y.; Puhl, M. D.; Benneyworth, M. A.; Basu, A. C.; Takagi, S.; Bolshakov, V. Y.; Coyle, J. T. *Proceedings of the National Academy of Sciences* 2013, *110*, E2400.

(23) Stefansson, H.; Sigurdsson, E.; Steinthorsdottir, V.; Bjornsdottir, S.; Sigmundsson, T.; Ghosh, S.; Brynjolfsson, J.; Gunnarsdottir, S.; Ivarsson, O.; Chou, T. T. *American Journal of Human Genetics* 2002, *71*, 877.

(24) Chumakov, I.; Blumenfeld, M.; Guerassimenko, O.; Cavarec, L.; Palicio, M.; Abderrahim, H.; Bougueleret, L.; Barry, C.; Tanaka, H.; La Rossa, P. *Proc Natl Acad Sci USA* 2002, *99*, 13675.

(25) Chowdari, K. V.; Mirnics, K.; Semwal, P.; Wood, J.; Lawrence, E.; Bhatia, T.; Deshpande, S. N.; Thelma, B. K.; Ferrell, R. A.; Middleton, F. A. *Human Molecular Genetics* 2002, *11*, 1373.

(26) Straub, R. E.; Jiang, Y.; MacLean, C. J.; Ma, Y.; Webb, B. T.; Myakishev, M. V.; Harris-Kerr, C.; Wormley, B.; Sadek, H.; Kadambi, B. *American Journal of Human Genetics* 2002, *71*, 337.

(27) Gerber, D. J.; Hall, D.; Miyakawa, T.; Demars, S.; Gogos, J. A.; Karayiorgou, M.; Tonegawa, S. *Proc Natl Acad Sci USA* 2003, *100*, 8993.

(28) Martucci, L.; Wong, A. H. C.; Trakalo, J.; Cate-Carter, T.; Wong, G. W. H.; Macciardi, F. M.; Kennedy, J. L. *Am J Med Genet* 2003, *119B*, 24.

(29) Ozaki, M.; Tohyama, K.; Kishida, H.; Buonanno, A.; Yano, R.; Hashikawa, T. *J Neuroscience Research* 2000, *59*, 612.

(30) Schmitt, A.; Koschel, J.; Zink, M.; Bauer, M.; Sommer, C.; Frank, J.; Treutlein, F.; Schulze, T.; Schneider-Axmann, T.; Parlapani, E.; Rietschel, M.; Falkai, P.; Henn, F. A. *Eur Arch Psychiatry Clin Neurosci* 2010, *260*, 101.

(31) Akbarian, S.; Sucher, N. J.; Bradley, D.; Tafazzoli, A.; Trinh, D.; Hetrick, W. P.; Potkin, S. G.; Sandman, C. A.; Bunney, J., W. E.; Jones, E. G. *J Neuroscience* 1996, *16*, 19.

(32) Weickert, C. S.; Fung, S. J.; Catts, V. S.; Schofield, P. R.; Allen, K. M.; Moore, L. T.; Newell, K. A.; Pellen, D.; Huang, X.-F.; Catts, S. V.; Weickert, T. W. *Molecular Psychiatry* 2012, 1.

(33) Beneyto, M.; Meador-Woodruff, J. H. *Neuropsychopharmacology* 2008, *33*, 2175.

(34) Lipton, S. A.; Kater, S. B. *Trends in Neuroscience* 1989, *12*, 265.

(35) Kim, J. S.; Kornhuber, H. H.; Schmid-Burgk, W.; Holzmuller, B. *Neuroscience Letters* 1980, *20*, 379.

(36) Gattaz, W. F.; Gattaz, D.; Beckmann, H. *Archiv fur Psychiatrie und Nervenkrankheiten* 1982, *231*, 221.

(37) Korpi, E. R.; Kaufmann, C. A.; Marnela, K. M.; Weinberger, D. R. *Psychiatry Research* 1987, *20*, 337.

(38) Perry, T. L. *Neuroscience Letters* 1982, *28*, 81.

(39) Do, K. Q.; Lauer, C. J.; Schreiber, W.; Zollinger, M.; Gutteck-Amsler, U.; Cuenod, M.; Holsboer, F. *J Neurochemistry* 1995, *65*, 2652.

(40) Tsai, G.; Passani, L. A.; Slusher, B. S.; Carter, R.; Baer, L.; Kleinman, J. E.; Coyle, J. T. *Arch General Psychiatry* 1995, *52*, 829.

(41) Tsai, G.; Slusher, B. S.; Sim, L.; Coyle, J. T. *J Chemical Neuroanatomy* 1993, *6*, 277.

(42) Puttfarken, P. S.; Handen, J. S.; Montgomery, D. T.; Coyle, J. T.; Werling, L. L. *J Pharmacol Exp Ther* 1993, *266*, 796.

(43) Bergeron, R.; Coyle, J. T. *Current Medicinal Chemistry* 2012, *19*, 1360.

(44) Luby, E.; Cohen, B. D.; Rosenbaum, G.; Gottlieb, J. S.; Kelley, R. *Archives of Neurology And Psychiatry* 1959, *81*, 363.

(45) Lodge, D.; Anis, N. A. *European Journal of Pharmacology* 1982, *77*, 203.

(46) Lodge, D.; Aram, J. A.; Church, J.; Davies, S. N.; Martin, D.; O'Shaughnessey, C. T.; Zeman, S. In *Excitatory amino acid transmission*; Alan R. Liss Inc.: New York, 1987, p 83.

(47) Olney, J. W.; Newcomer, J. W.; Farber, N. B. *Journal of Psychiatric Research* 1999, *33*, 523.

(48) Lahti, A. C.; Koffel, B.; LaPorte, D.; Tamminga, C. A. *Neuropsychopharmacology* 1995, *13*, 9.

(49) Jentsch, J. D.; Roth, R. H. *Neuropsychopharmacology* 1999, *20*, 201.

(50) de Bartolomeis, A.; Sarappa, C.; Magara, S.; Iasevoli, F. *European Journal of Pharmacology* 2012, *682*, 1.

(51) Leucht, S.; Heres, S.; Kissling, W.; Davis, J. M. *International Journal of Neuropsychopharmacology* 2011, *14*, 269.

(52) Krystal, J. H.; D'Souza, D. C.; Mathalon, D.; Perry, E.; Belger, A.; Hoffman, R. *Psychopharmacology* 2003, *169*, 215.

(53) Deakin, J. F.; Simspon, M. D. *J Psychiatric Research* 1997, *31*, 277.

(54) Meador-Woodruff, J. H.; Healy, D. J. *Brain Research Reviews* 2000, *31*, 288.

(55) Stone, J. M. *Therapeutic Advances in Psychopharmacology* 2011, *1*, 5.

(56) Egerton, A.; Stone, J. M. *Current Pharmaceutical Biotechnology* 2012, *13*, 1500.

(57) Nunes, E. A.; MacKenzie, E. M.; Rossolatos, D.; Perez-Parada, J.; Baker, G. B.; Dursun, S. M. *Expert Review of Neurotherapeutics* 2012, *12*, 801.

(58) Schwartz, T. L.; Sachdeva, S.; Stahl, S. M. *Current Pharmaceutical Design* 2012, *18*, 1580.

(59) Labrie, V.; Lipina, T.; Roder, J. C. *Psychopharmacology* 2008, *200*, 217.

(60) Nakanishi, S. *Science* 1992, *258*, 597.

(61) Nicoll, R. A.; Malenka, R. C. *Annals of the New York Academy of Sciences* 1999, *868*, 515.

(62) Rampon, C.; Tang, Y. P.; Goodhouse, J.; Shimizu, E.; Kyin, M.; Tsien, J. Z. *Nature Neuroscience* 2000, *3*, 238.

(63) Rampon, C.; Tsien, J. Z. *Hippocampus* 2000, *10*, 605.

(64) Tang, Y.-P.; Shimizu, E.; Dube, G. R.; Rampon, C.; Kerchner, G. A.; Zhuo, M.; Liu, G.; Tsien, J. Z. *Nature* 1999, *401*, 63.

(65) Tang, Y. P.; Wang, H.; Feng, R.; Kyin, M.; Tsien, J. Z. *Neuropharmacology* 2001, *41*, 779.

(66) Hillman, B. G.; Gupta, S. C.; Stairs, D. J.; Buonanno, A.; Dravid, S. M. *Neurobiology of Learning and Memory* 2011, *95*, 404.

(67) Rockstroh, S.; Emre, M.; Pokorny, R.; Tarral, A. *Psychopharmacology* 1996, *124*, 261.

(68) Morgan, C. J. A.; Riccelli, M.; Maitland, C. H.; Curran, H. V. *Drug and Alcohol Dependence* 2004, *75*, 301.

(69) Somers, J. M.; Goldner, E. M.; Waraich, P.; Hsu, L. *Canadian Journal of Psychiatry* 2006, *51*, 100.

(70) Starcevic, V.; Portman, M.; Beck, A. *The Journal of Nervous and Mental Disease* 2012, *200*, 664.

(71) Allgulander, C.; Bandelow, B.; Hollander, E.; Montgomery, S. A.; Nutt, D. J.; Okasha, A.; Pollack, M. H.; Stein, D. J.; Swinson, R. P. *CNS spectrums* 2003, *8*, 53.

(72) Foa, E. B.; Hembree, E. A.; Cahill, S. P.; Rauch, S. A.; Riggs, D. S.; Feeny, N. C.; Yadin, E. *Journal of Consulting and Clinical Psychology* 2005, *73*, 953.

(73) Barlow, D. H.; Gorman, J. M.; Shear, M. K.; Woods, S. W. *JAMA: The Journal of the American Medical Association* 2000, *283*, 2529.

(74) Mineka, S.; Zinbarg, R. *American Psychologist* 2006, *61*, 10.

(75) Yehuda, R.; LeDoux, J. *Neuron* 2007, *56*, 19.

(76) Davis, M.; Ressler, K.; Rothbaum, B. O.; Richardson, R. *Biological Psychiatry* 2006, *60*, 369.

(77) Hofmann, S. G. *Behaviour Research and Therapy* 2007, *45*, 1987.

(78) Ressler, K. J.; Rothbaum, B. O.; Tannenbaum, L.; Anderson, P.; Graap, K.; Zimand, E.; Hodges, L.; Davis, M. *Archives of General Psychiatry* 2004, *61*, 1136.

(79) Davis, M. *Dialogues in clinical neuroscience* 2011, *13*, 463.

(80) Dravid, S. M.; Burger, P. B.; Prakash, A.; Geballe, M. T.; Yadav, R.; Le, P.; Vellano, K.; Snyder, J. P.; Traynelis, S. F. *J Neuroscience* 2010, *30*, 2741.

(81) Shenin, A.; Shavit, S.; Benveniste, M. *Neuropharmacology* 2001, *41*, 151.

(82) Hofmann, S. G.; Meuret, A. E.; Smits, J. A. J.; Simon, N. M.; Pollack, M. H.; Eisenmenger, K.; Shiekh, M.; Otto, M. W. *Archives of General Psychiatry* 2006, *63*, 298.

(83) Wilhelm, S.; Buhlmann, U.; Tolin, D. F.; Meunier, S. A.; Pearlson, G. D.; Reese, H. E.; Cannistraro, P.; Jenike, M. A.; Rauch, S. L. *American Journal of Psychiatry* 2008, *165*, 335.

(84) Ogden, K.; Khatri, A.; Traynelis, S. *Neuropsychopharmacology* 2013.

(85) Kim, J. J.; DeCola, J. P.; Landeira-Fernandez, J.; Fanselow, M. S. *Behavioral Neuroscience* 1991, *105*, 126.

(86) Shimizu, E.; Tang, Y. P. *Science* 2000, *290*, 1170.

(87) Lee, J. L. C.; Milton, A. L.; Everitt, B. J. *The Journal of Neuroscience* 2006, *26*, 10051.

(88) Szapiro, G.; Vianna, M. R. M.; McGaugh, J. L.; Medina, J. H.; Izquierdo, I. *Hippocampus* 2003, *13*, 53.

(89) Delamater, A. R. *The Quaterly Journal of Experimental Psychology* 2004, *57B*, 97.

(90) Rizley, R. C.; Rescorla, R. A. *Journal of Comparative and Physiological Psychology* 1972, *81*, 1.

(91) Rescorla, R. A. *Journal of Comparative and Physiological Psychology* 1973, *82*, 137.

(92) Bouton, M. E.; Bolles, R. C. *Journal of Experimental Psychology* 1979, *5*, 368.

(93) Hansen, K. B.; Mullasseril, P.; Dawit, S.; Kurtkaya, N.; Yuan, H.; Vance, K.; Orr, A.; Kvist, T.; Ogden, K.; Le, P. *Journal of Pharmacology and Experimental Therapeutics* 2010, *333*, 650.

(94) Gein, V. L.; Mihalev, V. A.; Kasimova, N. N.; Voronina, E. V.; Vakhrin, M. I.; Babushkina, E. B. *Pharmaceutical Chemistry Journal* 2007, *41*, 208.

(95) Lu, R.-J.; Tucker, J. A.; Pickens, J.; Ma, Y.-A.; Zinevitch, T.; Kirichenko, O.; Konoplev, V.; Kuznetsova, S.; Sviridov, S.; Brahmachary, E.; Khasanov, A.; Mikel, C.; Yang, Y.; Liu, C.; Wang, J.; Freel, S.; Fisher, S.; Sullivan, A.; Zhou, J.; Stanfield-Oakley, S.; Baker, B.; Sailstad, J.; Greenberg, M.; Bolognesi, D.; Bray, B.; Koszalka, B.; Jeffs, P.; Jeffries, C.; Chucholowski, A.; Sexton, C. *Journal of Medicinal Chemistry* 2009, *52*, 4481.

(96) Mottram, L. F.; Maddox, E.; Schwab, M.; Beaufils, F.; Peterson, B. R. *Organic Letters* 2007, *9*, 3741.

(97) Zou, D.; Zhai, H. X.; Eckman, J.; Higgins, P.; Gillard, M.; Knerr, L.; Carre, S.; Pasau, P.; Collart, P.; Grassi, J.; Libertine, L.; Nicolas, J. M.; Schwartz, C. E. *Letters in Drug Design & Discovery* 2007, *4*, 185.

(98) Chen, H.-S. V.; Lipton, S. A. *Journal of Neurochemistry* 2006, *97*, 1611.

(99) Li, N.; Chen, X.-.-H.; Song, J.; Luo, S.-W.; Fan, W.; Gong, L.-Z. *J. Am. Chem. Soc.* 2009, *131*, 15301.

(100) Xu, F.; Huang, D.; Lin, X.; Wang, Y. *Org. Biomol. Chem.* 2012, *10*, 4467.

(101) Altmann, K.-H. *Mini-Reviews in Medicinal Chemistry* 2003, *3*, 149.

(102) Contopoulos-Ioannidis, D. G.; Ioannidis, J. *Current allergy and asthma reports* 2004, *4*, 471.

(103) Mascaretti, O. A.; Danelon, G. O.; Laborde, M.; Mata, E. G.; Setti, E. L. *Current Pharmaceutical Design* 1999, *5*, 939.

(104) Lodise, T. P.; Low, D. E. *Drugs* 2012, *72*, 1473.

(105) Perez-Llarena, F. J.; Bou, G. *Current Medicinal Chemistry* 2009, *16*, 3740.

(106) Bonfiglio, G.; Russo, G.; Nicoletti, G. *Expert Opinion on Investigational Drugs* 2002, *11*, 529.

(107) Kidwai, M.; Sapra, P.; Bhushan, K. R. *Current Medicinal Chemistry* 1999, *6*, 195.

(108) Parrick, J.; Mehta, L. K. *Progress in Heterocyclic Chemistry* 1996, *8*, 66.

(109) Stavila, E.; Loos, K. *Tetrahedron Letters* 2013, *54*, 370.

(110) Ornelas, M. A.; Gonzalez, J.; Sach, N. W.; Richardson, P. F.; Bunker, K. D.; Linton, A.; Kephart, S. E.; Pairish, M.; Guo, C. *Tetrahedron Letters* 2011, *52*, 4760.

(111) Mickel, S. J.; Hsaio, S.-N.; Miller, M. J. *Organic Synthesis* 1987, *65*.