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Ernest Etchegaray Murray

Part I: Design and Synthesis of NMDA Receptor Antagonists

Part II: Design and Synthesis of Measles Virus Inhibitors

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2010

Part I: Design and Synthesis of NMDA Receptor Antagonists

Part II: Design and Synthesis of Measles Virus Inhibitors

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B.S. Wake Forest University 2004

Advisor: Dr. Dennis C. Liotta

An Abstract of
A thesis submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the Degree of Masters of Science

Department of Chemistry

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Abstract

Part I: Design and Synthesis of NMDA Receptor Antagonists

Part II: Design and Synthesis of Measles Virus Inhibitors

Ernest Murray

Part I of this thesis elaborates on the design and synthesis of new N-methyl D-aspartate (NMDA) receptor antagonists in the effort to treat stroke. The NMDA receptor is a glutamate receptor that has been found to be sensitive to pH fluctuations in the extracellular space. During a stroke, ischemia causes elevated proton concentration allowing the opportunity to tune receptor antagonist design towards neuroprotection in vulnerable conditions. Phenylethanolamines (like ifenprodil) are known NMDA subunit NR2B antagonists that allosterically affect the gate opening at different pHs. Our chiral 1-(phenethylamino)-3-phenoxypropan-2-ol scaffold compounds (also known as "93 series") act similarly and have been found to utilize the proton sensor of the NMDA receptor to provide up to a 17 fold inhibition at pH 6.9 compared to the physiological pH of 7.3. Our newest scaffold the 96 series is similar to the 93 series but lacks chirality, and has also exhibited potent IC_{50} values at pH 6.9.

Part II discusses new efforts in resisting measles virus proliferation through the use of novel small molecules. The measles virus (of the *paramyxovirus* family) is a serious concern in third world countries where vaccination systems are not at the 95% coverage rate required to stop the spread. No effective retroviral treatments have been developed for victims of this disease. Fusion (MV-F) protein, a protein associated with viral entry into the host cell, may be an effective path to treatment. Data from the mutagenesis of MV-F along with computational homology models have indicated the possible location of an allosteric inhibitor binding site. Three new compounds were synthesized with pyridinyl moeties designed to increase potency. Another likely target for the measles virus life cycle is the RNA-dependent RNA polymerase (RDRP). Though the mechanism action is not known, we have development several very potent RDRP

inhibitors based on a lead compound discovered in a screening library. Our most potent inhibitors come from targeting the RDRP, and these molecules can inhibit measles virus viability in the nanomolar concentrations.

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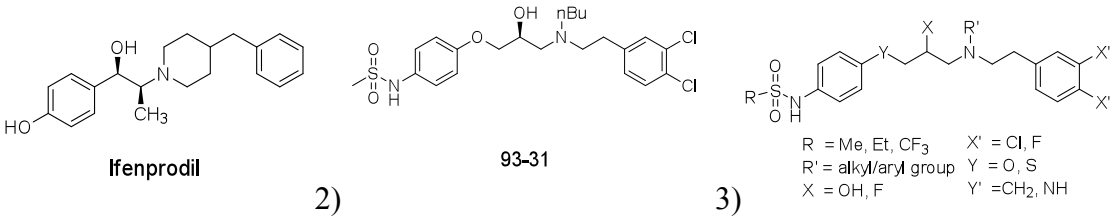
I. Design, Synthesis, and Biological Activity of NMDA Receptor Antagonists

1.1. Statement of Purpose

The N-methyl D-aspartate (NMDA) receptor is a glutamatergic receptor found in the central nervous system that controls the flow of ions such as Ca^{2+} at the synaptic junction. Overactivation of this receptor has been implicated in neurological diseases such as stroke, Alzheimer's disease, Parkinson's disease, and epilepsy.[i] While it is an intriguing biological target, nonselective antagonism of NMDA receptor activity with receptor blockers can result in severe side effects. Our goal has been to design and synthesize selective NMDA receptor antagonists that utilize receptor subunit specificity and cellular conditions to impede neurodegenerative propagation.

During a stroke, hypoxia and ischemia cause neuronal death releasing excessive amount of cellular contents, including glutamate, into the extracellular space.[ii] Excessive



1) 2) 3)

Figure 1. 1 Development of NR2B Selective NMDA Antagonists 1) NR2B antagonist Ifenprodil. 2) pH sensitive NR2B receptor antagonist. 3) Template for NMDA receptor antagonist analogues

glutamate can over-stimulate NMDA receptors causing damaging amounts of calcium to enter cells. This calcium influx can trigger excitotoxic signaling that propagates neuronal death, glutamate release, and subsequent apoptosis of nearby cells. Our rationale involves inhibiting the NMDA receptor during stroke to prevent this propagation of neuronal death caused by excessive glutamate release.

In addition to excessive glutamate, ischemic stroke is associated with an elevated proton concentration in the area of the surrounding infarct.[iii] Phenylethanolamines (like ifenprodil) are allosteric, subunit-specific antagonists that differentially affect the NMDA receptor channel, opening under acidic and basic pH conditions. Given the conditions found to exist in the extracellular space during a stroke, combined with a history of compounds known to inhibit the NMDA receptor at different proton concentrations, we have a unique opportunity to tune receptor antagonist design towards neuroprotection when CNS cells are most vulnerable.

Our 1-(phenethylamino)-3-phenoxypropan-2-ol scaffold compounds (referred to herein as "93 series" compounds) are phenylethanolamines, and as such, have been found to provide up to a 17-fold greater inhibition of NMDA receptors at pH 6.9 compared to the physiological pH of 7.6. Our new series of compounds, the 96 series, has also exhibited potent IC_{50} values at pH 6.9 yet lack the chiral hydroxyl group which is present in the 93 series. Our goal is to design and synthesize potent and selective small molecules that can work prophylactically to inactivate the NMDA receptor during an ischemic episode such as stroke.

1.2 Background and Introduction

1.2.1 Neurological Disorders and the NMDA Receptor

Alzheimer's disease, Parkinson's disease, epilepsy, and stroke are examples of neurodegenerative disorders that are characterized by excessive glutamatergic activation that results in neuronal damage. The N-methyl D-aspartate (NMDA) receptor is a ubiquitous ionotropic receptor that is critical to normal synaptic transmissions, allowing calcium influx following co-agonist binding of glutamate and glycine to the receptor.[i,iv] Overactivation of the NMDA receptor results in increased intracellular calcium concentration that can accumulate to toxic levels, ultimately leading to neuronal death.

Structurally, the NMDA receptor exists as two heterodimers of NR1 and NR2 subunits that form a tetramer containing four transmembrane domains and two extracellular domains in each subunit (Figure 1.2).[v] Once glutamate and glycine bind to the Ligand Binding Domains (LBDs) of NR2 and NR1 subunits respectively, a series of conformational changes occur that allow the conductance channel to open.[vi] Magnesium stabilizes patency of the ion pore allowing calcium influx to occur. Four distinct NR2 subunit gene products A-D are expressed differentially with the NR1 subunit. Thus, NR1/NR2A, NR1/NR2B, NR1/NR2C, and NR1/NR2D receptors are found in the nervous system, with each receptor exhibiting unique pharmacology in terms of ion conductance, kinetics, affinity for ligands, and expression patterns.

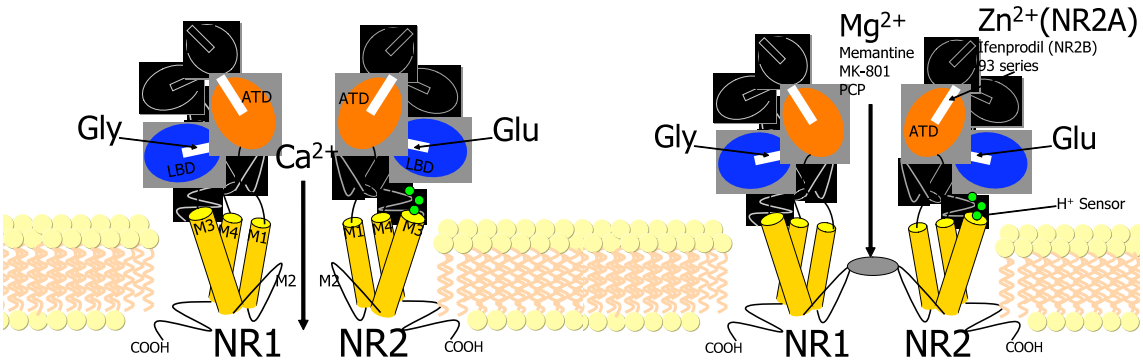


Figure 1. 2 The NMDA Receptor. (*left)* Representation of the NMDA receptor activation in presence of agonist glutamate and coagonist glycine; (*right*) NMDA receptor inactivation by channel blocking or allosteric regulation.

The first compounds discovered to inhibit NMDA receptor antagonist were non-selective channel blockers. Currently FDA approved for Alzheimer's disease under the trade name Namenda©, memantine is one of the few successes from this class and acts by weakly interacting with the ion pore at the magnesium binding site.[vii,viii] More potent channel blockers such as MK-801 have been clinically unsuccessful due to severe psychotomimetic side effects.[ix] Other classes of NMDA inhibitors include glycine site antagonists, glutamate site antagonists, and subunit-selective antagonists. One of the earliest examples of NR1/NR2B subunit-selective antagonists were the phenylethanolamines like ifenprodil (Figure 1.1 a) which binds to the Amino Terminal Domain (ATD).[x]

Some NR1/NR2B-selective NMDA receptor antagonists, like ifenprodil, were discovered to inhibit receptors via a novel mechanism involving a proton sensor region.[xi] Administration of ifenprodil was believed to increase endogenous proton inhibition by increasing receptor sensitivity. Ifenprodil decreases NR1/NR2B receptor-mediated ion

current by 2.4 fold when the pH is shifted from 7.5 to 6.8.[xi] The discovery of the proton sensor opened the field of research towards developing small molecules that selectively inhibit receptor overactivation by utilizing proton-mediated inhibition.

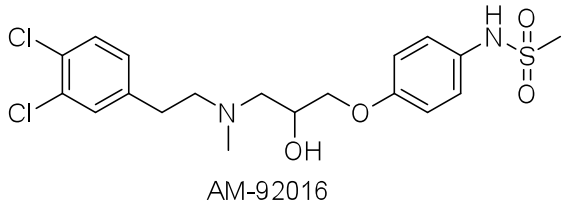
1.2.2 Development of NR2B Selective NMDA Receptor Antagonists

In 2001, the Liotta lab collaborated with Stephen Traynelis and Ray Dingledine of the Emory University Pharmacology Department and initiated a project to develop selective NR1/NR2B NMDA receptor inhibitors. These inhibitors were designed to exhibit similar properties to ifenprodil but with a greater difference in inhibition between acidic and basic pHs.[iii] The activity of the analogues was measured in NR2B containing NMDA receptors at two pH values. The pH boost, or fold shift, is defined as the ratio of IC_{50} values at pH 7.6 and pH 6.9:

Fold Shift= (IC_{50} value at pH 7.6) / (IC_{50} value at pH 6.9)

The more potent a compound is at acidic pH compared to the alkaline pH, the larger the fold shift. Consequently, the larger the magnitude of the fold shift, the greater likelihood of having deceased side effects resulting from physiologic NMDA receptor blockage.

The search for new compounds with ifenprodil-like fold shift characteristics lead to screening hit AM-92016 (Figure 1.3).[iii] A library of compounds was designed (Figure



AM-92016

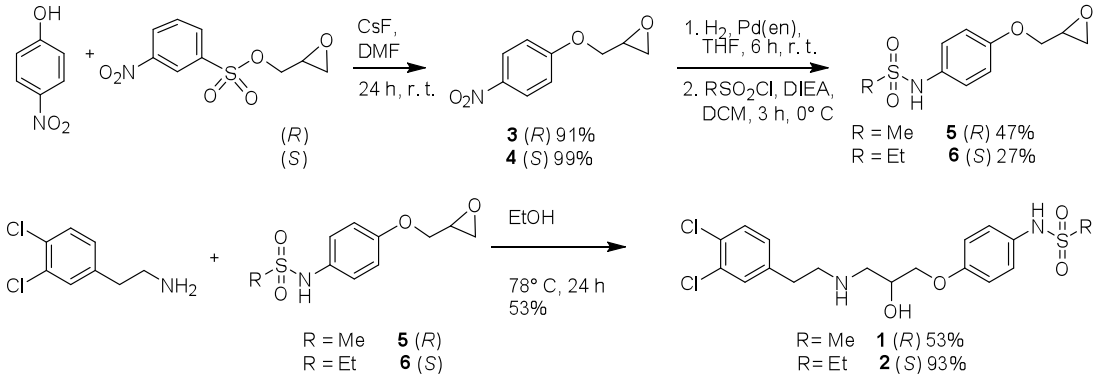
Figure 1.3 NR1/NR2B selective NMDA receptor antagonist screening hit. AM-92016 also known as 93-1.[iii]

1.1 c) based on this lead. Design goals included increasing diversity at the sulfonamide R group, the amino R' group, and converting the alcohol to fluorine, which is isosteric to oxygen in bond length but has unique electronic properties. Initial efforts led to compound 93-31 that exhibited a fold shift of 17 (Table 1.1).[xii]

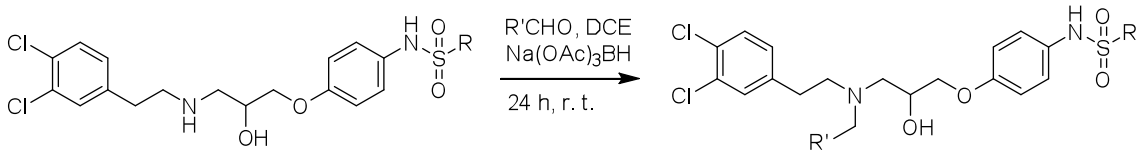
Development of the NR1/NR2B NMDA receptor antagonist library was based on previous work derived from common amino alcohol precursors **1** and **2** (Scheme 1.1). Regioselective addition of nitrophenol to the C1 carbon of chiral, non-racemic glycidyl nosylate[xiii] in the presence of cesium fluoride[xiv] afforded the nitrophenyl glycidol ethers (**3**(*R*) and **4**(*S*)) in high yield. The nitro group was reduced to the aniline in the presence of a poisoned palladium catalyst[xv] to avoid hydrogenolysis of the epoxide. The aniline was found to be unstable to air and silica gel and was immediately protected with alkyl sulfonyl chlorides to afford the alkyl sulfonamide epoxides (**5**(*R*) and **6**(*S*)). The epoxide was opened in an S_N2 fashion with dichlorophenethylamine to afford amino alcohols **1** and **2**.[xvi] These amino alcohols served as scaffolds for further functionalization to develop NR1/NR2B NMDA receptor libraries.

Amino alcohols **1**, **2**, and **7**[xvi] were reacted with various commercially available

Scheme 1.1 Syntheses of Amino Alcohols 1 and 2.



Scheme 1.2 Synthesis of Tertiary Amines via Reductive Amination



R = Me **1**(*R*)
R = Me **7**(*S*)
R = Et **2**(*S*)

Table 1. 1 Results and Biological Data from Scheme 1.2

	R group	R' group	Series name(*R/S*)	Percent Yield	pH 7.6 IC_{50} (μM)	pH 6.9 IC_{50} (μM)	Fold Shift
1	Me	H	93-3 (*R*)	--	0.22	0.05	4
7	Me	H	93-4 (*S*)	--	0.04	0.02	2
2	Et	H	93-89 (*S*)	--	0.70	0.22	3
8	Me	C_3H_7	93-31 (*S*)	60 %	0.45	0.03	17
9	Me	C_3H_7	93-88 (*R*)	36 %	1.45	0.23	6
10	Me	C_2H_5	93-6 (*S*)	79 %	0.50	0.05	10
11	Me	C_6F_5	93-39 (*S*)	13 %	0.40	0.04	10
12	Me	C_5H_9	93-153(*S*)	82 %	1.52	1.45	1
13	Et	C_3H_7	(*S*)	28%			
14	Et	C_6F_5	(*S*)	23%			

aldehydes to afford tertiary amines **8-14** (Scheme 1.2, Table 1.1)**.**

All compounds **1**, **2**, and **7-14** were submitted for biological analysis and tested by collaborators in the Traynelis lab group.[xii] As evident in Table 1.1, the (*S*) enantiomer is superior in activity and selectivity compared to its (*R*) counterpart. The compound **7**(*S*) exhibits twice the fold shift as the enantiomer **1**(*R*). Compound **8**(*S*) demonstrated a fold shift of 17 compared the enantiomer **9**(*R*) with a fold shift of 3. As a consequence of these early studies, further lead optimization and design focused on the (*S*) enantiomer.

1.3 Results and Discussions

1.3.1 Replacement of Chiral Hydroxyl Group with Fluorine

Replacing the alcohol on the 93 series scaffold with fluorine proved to be challenging due to the presence of the nitrogen located within close proximity to the hydroxyl group. Attempts in our group to replace the hydroxyl group with fluorine proved to be unsuccessful using the fluorinating agent diethylaminosulfur trifluoride (DAST), most likely due to the presence of the nucleophilic nitrogen within close proximity of the carbon which would serve as an electrophile.[xvii] The secondary amine

Scheme 1.3 Attempted synthesis of Amino Fluoride via 2

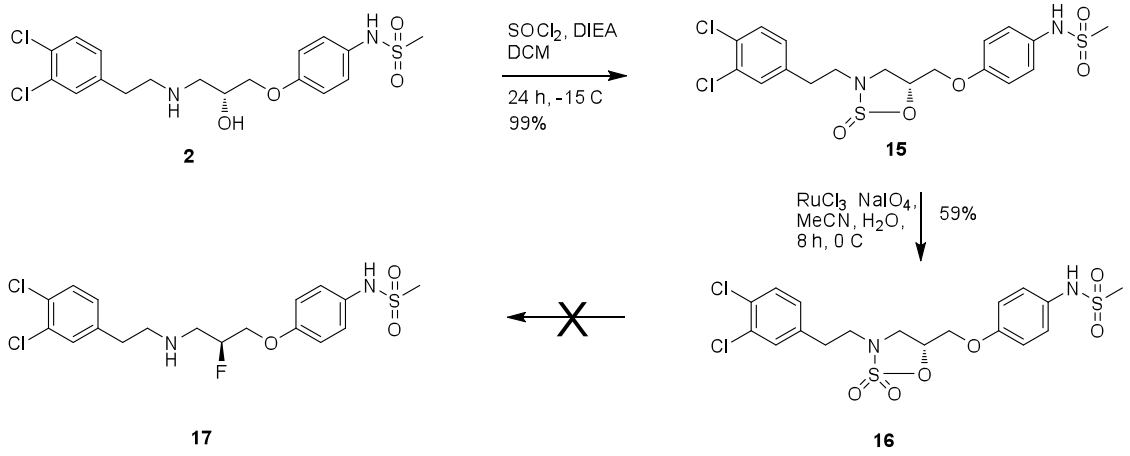


Table 1.2. Unsuccessful fluorination conditions

Reagent	TBAF	TBAF	TBAT	TBAT	TBAT	HF pyr.	KF 18-c-6	KF 18-c-6	KF 18-c-6	KF 18-c-6	KF Kryptofix
Equiv.	1	1.5	1	4	6	1	0.5	0.1	0.25	1.5	1.5
Time Temp	24 h 82 °C	24 h 82° C	24 h 82° C	24 h 82° C	24 h 82° C	24 h rt	24 h 82° C	48 h 82° C	48 h 82° C	48 h 82° C	24 h 82° C

was protected with conventional nitrogen protecting groups such as the tert-butyloxycarbonyl (Boc) group, but the desired fluoride product was not formed.[xviii]

Alternative protecting group strategies were subsequently explored that would utilize the proximal placement of two functional groups of **2**, namely the secondary amine and the chiral hydroxyl group. A promising method for stereoselectively substituting hydroxyl group two to three atoms away from an amino group is initially forming a cyclic sulfamidate.[xix] This acts as a dual functional-group conversion which protects the nitrogen and increases the electrophilicity of carbon bearing the hydroxyl group. Sulfamidate intermediates have been used to produce 1,2-amino fluorides from amino alcohols (Figure 1.4). [xx] An additional advantage to this strategy is that same pot deprotection occurs following treatment with 20% sulfuric acid.

The attempted synthesis of **17** incorporating the sulfamidate chemistry is shown in Scheme 1.3. Formation of the cyclic sulfamidite by adding thionyl chloride to the amino alcohol in the presence of excess triethylamine afforded the product **15** as a mixture of diastereomers. Oxidation of the thiazolodinone oxide **15** to the dioxide **16** was accomplished using the $RuCl_3 \cdot H_2O/NaIO_4$ in acetonitrile system at moderate yields.[xxi,xxii] A variety of fluorinating agents used in the attempted conversion of the sulfamidate species to the amino fluoride (Table 1.2) include tetrabutylammonium fluoride (TBAF), tetrabutylammonium triphenyl difluorosilicate (TBAT), HF in pyridine, and a combination of anhydrous potassium fluoride salts with crown ethers. Attempts to

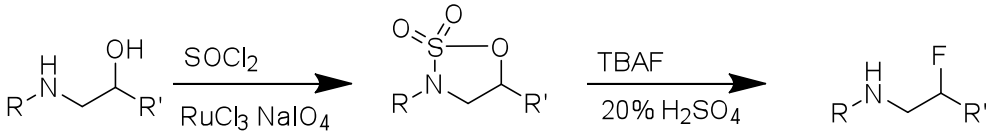
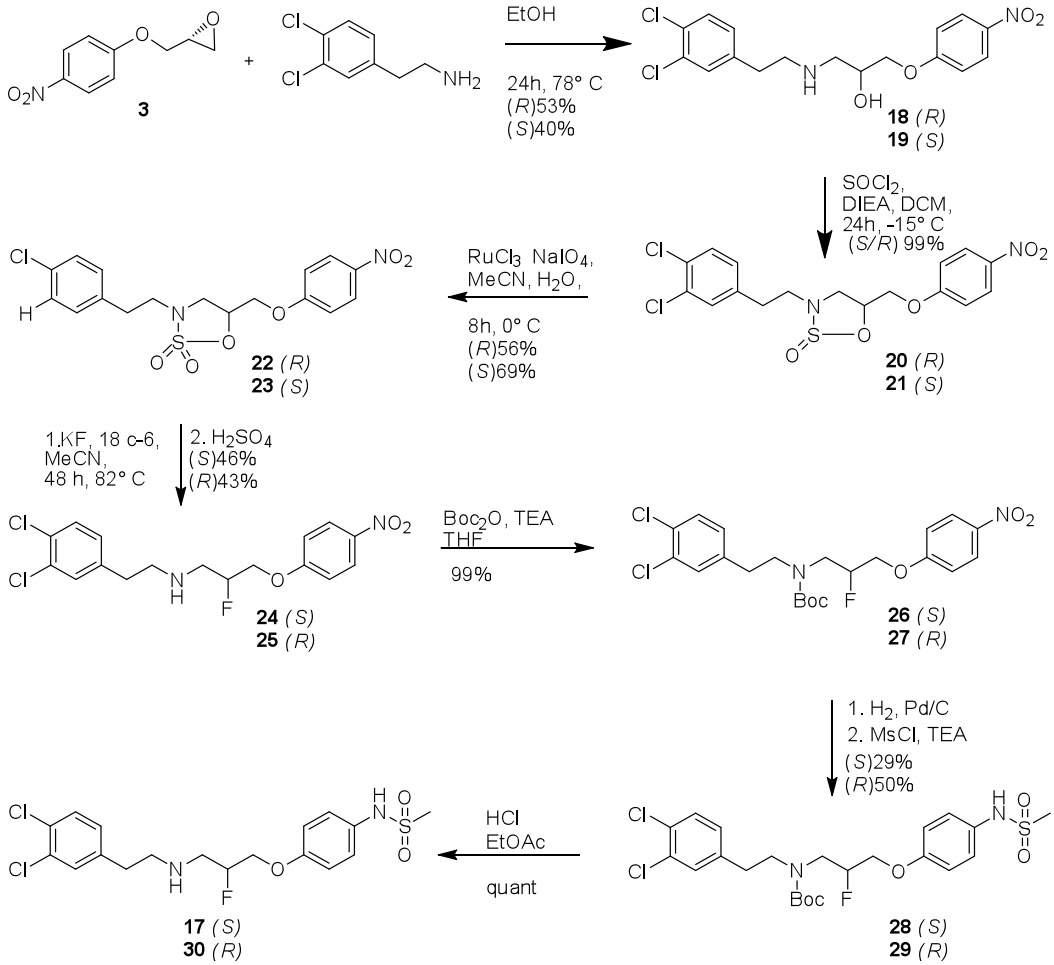


Figure 1.4. Cyclic sulfamidate formation and fluorination.[xx]

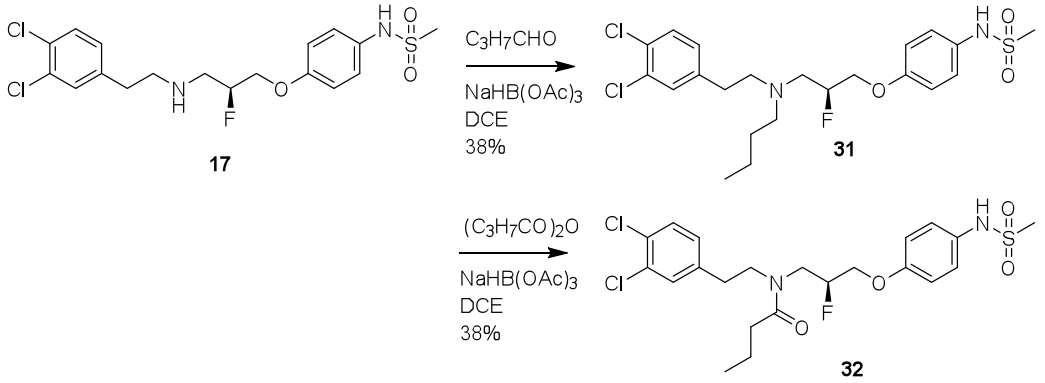
separate the desired product from side products by HPLC were unsuccessful.

The revised synthetic approach towards sulfamidate **17** involved incorporating fluorine before mesylation of the aniline (Scheme 1.4). The nitro amino alcohol **18** was created by opening nitro epoxide **3** with dichlorophenethylamine. Amino alcohol **18** reacted with thionyl chloride to form sulfamidite **20** and was then oxidized to afford nitro sulfamidate **22** in yields analogous to methanesulfonamide derivatives. The nitrofluoroamine **24** was formed by treatment with the potassium fluoride

Scheme 1.4. **Fluorination of the 93 series scaffold**



Scheme 1.5. Derivatives of Fluoroamine 17

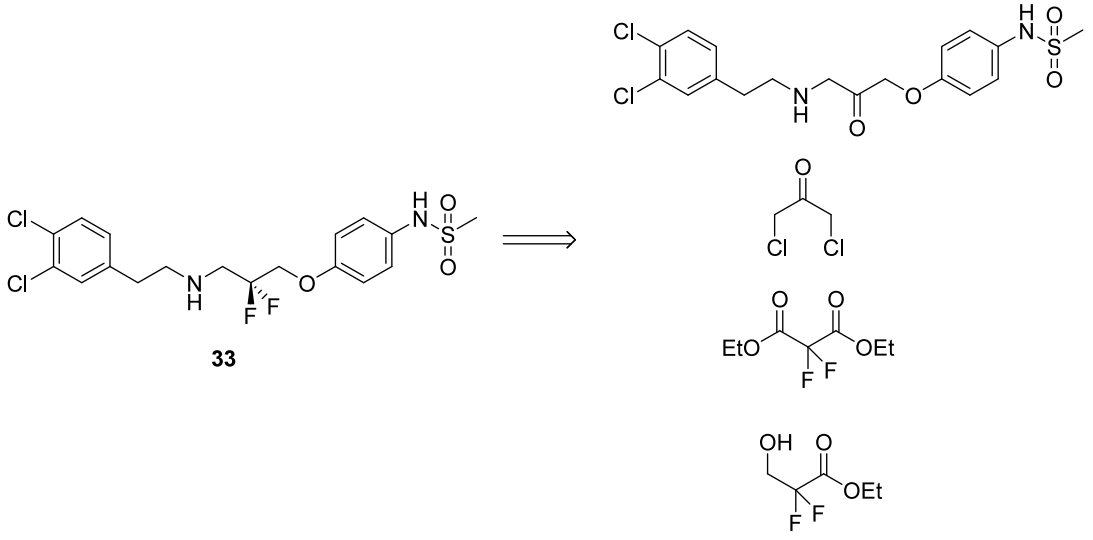


formed by treatment with the potassium fluoride and 18-crown-6. Nitro fluoroamine **24** was protected with Boc anhydride to form **26.** Compound **26** was reduced to the aniline with palladium catalyst and then treated with methanesulfonyl chloride to form **28**. Finally, compound **28** was deprotected with hydrochloric acid to give **17**. Enantiomer **30** was synthesized in the same fashion of **17**. The fluoroamine **17** was further derivatized by reductive amination with butyraldehyde to give tertiary amine **3**. Fluoroamine **17** was also acylated with butyric anhydride to give **32**.

1.3.2 Synthesis of Difluorinated NR2B NMDA Receptor Antagonists

The modest biological activity of chiral monofluoro derivative (discussed in section 1.4) spurred interest in development of an achiral difluoro compound that could be made analogously to the retrosynthetic route depicted in Scheme 1.6. The most obvious method would be a late stage addition of the difluoro functionality to the amino ketone structure of the same oxidation state using a conventional fluorinating reagent such as DAST. Oxidation of amino alcohol **1** and amino protected (Boc and fluorenylmethyl carbamate (Fmoc)) derivatives to the corresponding ketone precursor were unsuccessful. Furthermore, alkylation reactions with 1,3-dichloroacetone (a three carbon chain with the crucial carbon in the same oxidation state as amino ketone) failed using a multitude of conditions. Diethyl difluoromalonate, a synthetic fragment

Scheme 1.6. Retrosynthetic Approach for the Difluoro analogue

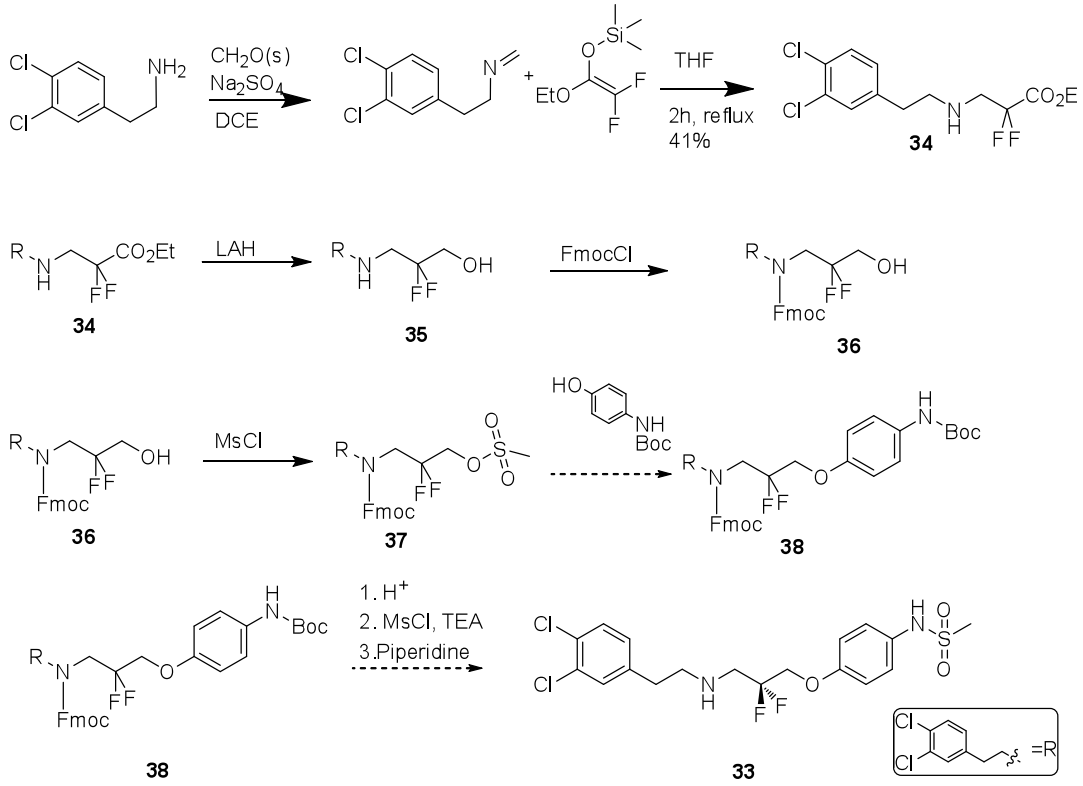


decorated with the difluoro functionality installed, could not be used in a large scale.

The most fruitful efforts in synthesizing the difluoro analogue were by functionalizing ethyl difluoro acetate shown in Scheme 1.7. Though initial strategies included an aldol reaction that coupled various difluoro acetic acid derivatives with formaldehyde equivalents, literature revealed that difluoro enol ether can be effectively formed under Reformatsky[xxiii] conditions and coupled to various aldehyde synthons.

The condensation of formaldehyde with dichlorophenethylamine afforded the imine that was coupled to the silyl enol ether of ethyl difluoro acetate in one pot to afford compound **34** (Scheme 1.7). Preliminary efforts suggested that there was success in reducing the ester and protecting the amine, but reaction of compound **38** with N-Boc protected aminophenol was unsuccessful.

Scheme 1.7. Incomplete Synthesis of the Difluoro Analogue



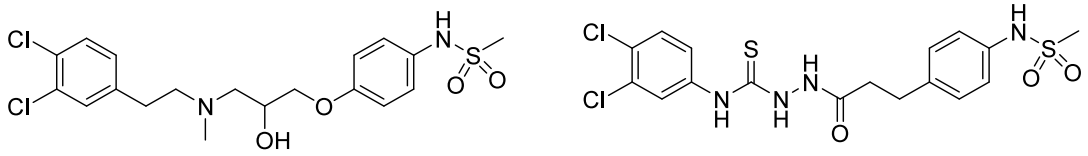


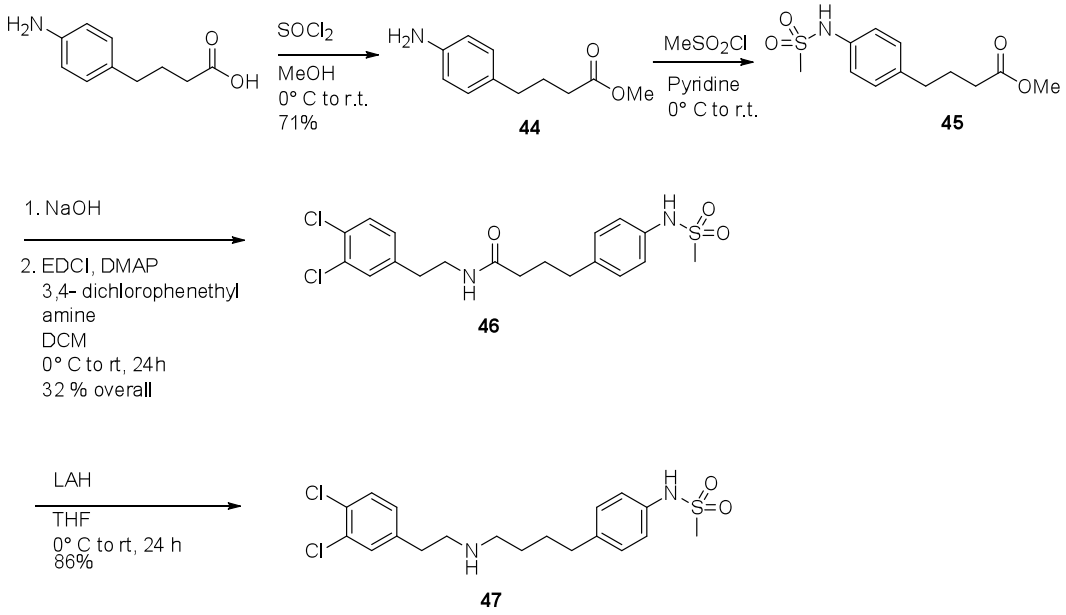
Figure 1.5 93 and 96 series Seminal Compounds. 93-1 has an IC_{50} of 39 nM at pH 6.9 and a boost of 7 fold compared to pH7.6 (*left*) 96-1 has an IC_{50} of 180 nM and no fold shift (*right)*.

1.3.3 Synthesis of Achiral NR2B NMDA Receptor Antagonists

The 96 series scaffold is an interesting variant of its chiral precursor, in that potent analogues have shown minimal interaction with undesirable targets such as the hERG receptor (discussed briefly in section 1.4).[xxiv] Structurally, the 96 series analogues differ from the 93 series in that the achiral analogues contain a unique amide in its internal scaffold (Figure 1.4).[xxiv] Considerable effort has been done in our lab to probe the active pharmacophores in each series to reveal which structure-activity relations affect potency, boost, and safety. In this work, two compounds are included which contain two aromatic rings similar to the 93 series with a seven atom linker interrupted with an amide or basic nitrogen. The synthesis of these molecules, shown in Scheme 1.8, utilized conventional chemistry and occurred with few complications.

Synthesis of **46** and **47** starts with functionalization of 4(4-aminophenyl) butyric acid. The carboxylic acid is reacted with thionyl chloride in methanol to form ester **44.** Compound **44** is then reacted with methanesulfonyl chloride to afford **45**. The ester is hydrolyzed to reform the acid which is coupled to the commercially available dichlorophenethylamine to form product **46** using 1-ethyl-3-(3'-dimethylaminopropyl)

Scheme 1.8. Synthesis Achiral NR2B NMDA Receptor Antagonists



carbodiimide (EDCI). Amide **46** is reduced using lithium aluminum hydride (LAH) to form the free amine **47**.

1.4 Biological Results and Conclusions

Biological testing of these analogues indicate some interesting trends that suggest modification to the current hypothesis about how these molecules serve as NMDA receptor antagonists (Table 1.3). The fluoro compound **17** proved to be both potent and have a reasonable fold shift, but its (*R)* enantiomer lacked similar activity, which is consistent with previous studies (see section 1.2.2). The 96 series compounds **46** and **47** reveal that a basic amine may not be required for the boost to be exhibited.

This is an exciting finding since amides are more stable, and they weakly interact with the ubiquitous human-ether-a-go-go related gene (hERG) receptor compared to 93 series analogues, a significant consideration for potential therapeutic compounds.[xxv,xxvi] The hERG receptor is an ion channel that is located in the heart, and its inhibition has recently been recognized by the FDA as potential hazard. Inhibition of the hERG receptor has been implicated in causing Torsades de Pointes, a condition that may result in heart attack or death. Molecules that inhibit this receptor have little chance in becoming a viable clinical candidate. With this in mind, the 96 series molecules have a favorable safety profile that is a continually being explored by our lab.[xxiv]

Table 1.3. Biological Results for NMDA Receptor Antagonists

compound	series	Potency (in μmol)		
		pH 7.6	pH 6.9	Fold shift
17	93	2.03	0.23	9
30	93	1.42	4.40	3
31	93	50.8	3.86	13
32	93	1.19	0.86	1
46	96	1.82	0.26	7
47	96	0.15	0.03	5

II. Design and Synthesis of Measles Virus Inhibitors

2.1 Statement of Purpose:

Infections derived from the measles virus (*paramyxovirus* family) are a serious concern, especially in third world countries. Vaccination systems must include 95% of a population in order to stop the spread of the virus by herd immunity. No effective retroviral treatments have been developed for victims of this disease. Targeting the measles virus, like any other single strand RNA virus, can be accomplished by inhibiting any one of the many steps of the viral life cycle.

There are several interesting targets for inhibition of the viral life cycle, such as measles virus fusion (MV-F) protein, a protein associated with viral entry into the host cell. Another classical anti-viral target is the viral RNA-dependent RNA polymerase (RDRP), a crucial enzyme specific to negatively stranded RNA viruses. Our goal is to use medicinal chemistry methods, computational chemistry techniques, and high-throughput biological screening results to quickly find an effective small molecule treatment for the disease induced by measles virus infection.

2.2 Introduction and Background

The measles virus (MV) is a single stranded RNA virus from the family *paromyxoviridae* and is responsible for one million deaths annually throughout the world.[xxvii] Despite effective vaccination systems employed since the 1960s that have almost eliminated the threat in some countries, most developing nations lack the 95% coverage rate required to stop the spread of the virus. Although vaccinations begin as early as nine months of age, infants under the age of one year account for a third of all measles-related deaths.[xxviii] In addition, recent concerns over vaccination safety have lowered vaccination rates, increasing the susceptibility to infection in some developed nations. No effective retroviral treatments have been developed for victims of this disease, and targets need to be discovered to arrest MV virulence in infected victims.

Fusion entry inhibition is a possible therapeutic target and has been proven effective in other viruses such as HIV.[xxix,xxx] Fusion of the viral envelope with host cell membranes begins with recognition of a cellular receptor on the host cell by hemagglutinin (H), neuraminidase/hemagglutinin (NH), and glycoproteins located on the extracellular surface of the viral envelope.[xxxi] Receptor recognition and viral association is followed by insertion of the

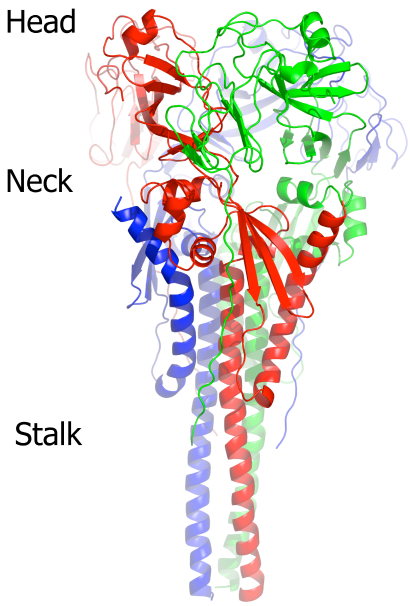


Figure 2.1. Measles Virus Fusion Protein Structure[xxxiv]

lipophilic stalk of the MV fusion (F) protein into the host cell membrane, where the two membranes are fused to allow the contents of the viral envelope to enter the cytoplasm. Within this step in the viral cycle, inhibition of the MV-F protein is an ideal target for arresting the viral entry of MV into a host cell and ultimately the spread of the virus.

Another interesting target is the viral RNA dependent RNA polymerase (RDRP) which is specific to the viral life cycle of single stranded RNA viruses (Figure 2.3).[xxxii] In order to produce viral proteins, RDRPs convert the non-coding viral RNA strand to the complementary coding RNA sequences that are used synthesize peptides within the host's cytoplasm. The RDRP is a combination of polypeptides derived from the L (Large) and P (phospho) genes on the small 20 kb genome.[xxxiii] This protein is not exclusively targeted at its allosteric binding site; it is also susceptible to a small molecule interaction that can interfere with subunit assembly of the holoenzyme.

1.2.2 History of Small Molecule Target Development

The Measles Virus Fusion (MV-F) protein exists as a trimer that has a globular head, a lipophilic stalk, and a neck that connects two other two features (Figure 2.1).[xxvii,xxxiv]

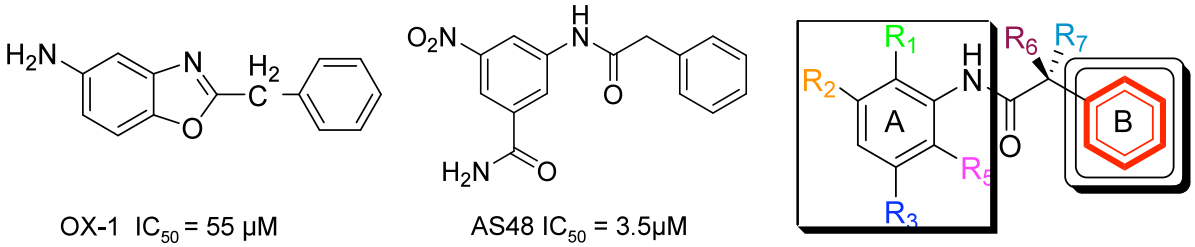


Figure 2.2. MV-F Inhibitor Development. OX-1 Initial hit found from screening library *(left)* AS48, second generation MV-F inhibitor *(middle)* Template for lead optimization *(right)*.

Although no crystal structures exist for the MV-F protein, homology models have been developed based on known structures of the Newcastle Disease virus fusion (NDV-F) protein.[xxvii, xxxv] Development of the hit compound OX-1 (Figure 2.2),[xxxvi] which shows good activity against viral entry (IC_{50}= 55 μM), and protein mutation studies paved the way to finding a binding model. Mutation of Val94 of the MV-F protein has indicated the possible location of an allosteric inhibitor binding site.[xxxvi]

Using molecular modeling techniques, classical synthesis, and biological assays with collaborators, our lab worked to develop novel MV-F inhibitors. One of our first leads, AS-48 (Figure 2.2) exhibited 100 times more potency than OX-1. Since the initial research in this project, a library of compounds have been developed which revealed information about ligand interactions in the protein binding site. The left hand side of the molecule (A ring) has been extensively investigated; however, very few details are known about developing right hand side (B ring) of the molecule (Figure 2.2). Spatially, the model indicates the side of the binding pocket associated with the right, B-ring, is very unforgiving of most steric additions to an unfunctionalized phenyl ring.[xxxvi] Efforts have therefore been focused on developing heterocyclic analogues of this B-ring in order to increase the number of hydrogen bonding interactions between the ligand and the protein backbone.

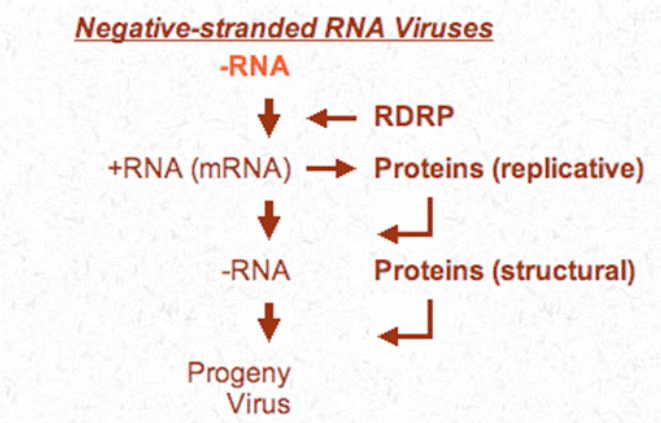
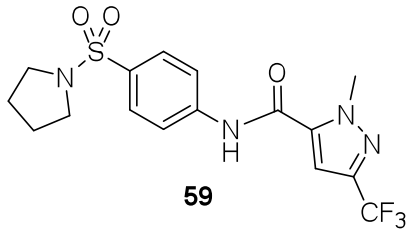


Figure 2.3. RDRP role in Viral Life Cycle

In parallel to MV-F protein inhibitor development, a screening library was devised to discover new leads to inhibit the viral life cycle. Potent lead compound **59** has been discovered by our molecular screening library to inhibit the measles virus RDRP in the 0.1 μm concentration (Figure 2.4), and synthetic SAR development has been employed in order to increase potency. Although the mechanism of action for this series of molecules is unknown, the target MV-RDRP is believed to be inhibited. When treated with these compounds, measurements of viral peptides and viral mRNA levels are decreased though viral entry still takes place.[xxxvii]



59

Figure 2.4 Screening Library Lead.

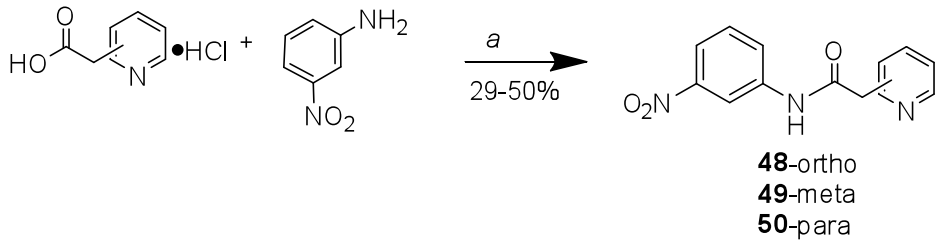
2.3 Results and Discussion

2.3.1 MV-F Inhibitors

In these analogues, the left-hand side A ring is a simple *m*-nitroaniline that has good viral inhibition when coupled to a B ring phenyl (IC_{50}~3 μM).[xxxvi] Docking models suggested that the biological activity of these analogues might be sensitive to variations in the B ring that utilized isomeric pyridinyl groups.[xxxviii] Synthesis of three of these pyridine analogues are shown in Scheme 2.1 starting with the commercially available pyridinyl acetic acid hydrochloride salts.

In order to form the amide bond linking the two sides of the molecule, many conditions were attempted with thionyl chloride to try to make the acid chloride, but this approach was abandoned for the superior carbodiimide coupling reagents. Initially, acid chlorides were used to form the amide bond for this type of coupling. Multiple attempts with thionyl chloride proved to be unsuccessful in the formation of acid chlorides from pyridinyl acetic acid. Fortunately, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was successful as a coupling reagent with catalytic dimethylaminopyridine (DMAP) and stoichiometric quantities of triethylamine (TEA) as seen in Scheme 2.1.

Scheme 2.1. Synthesis of MV-F inhibitors



48-ortho
49-meta
50-para

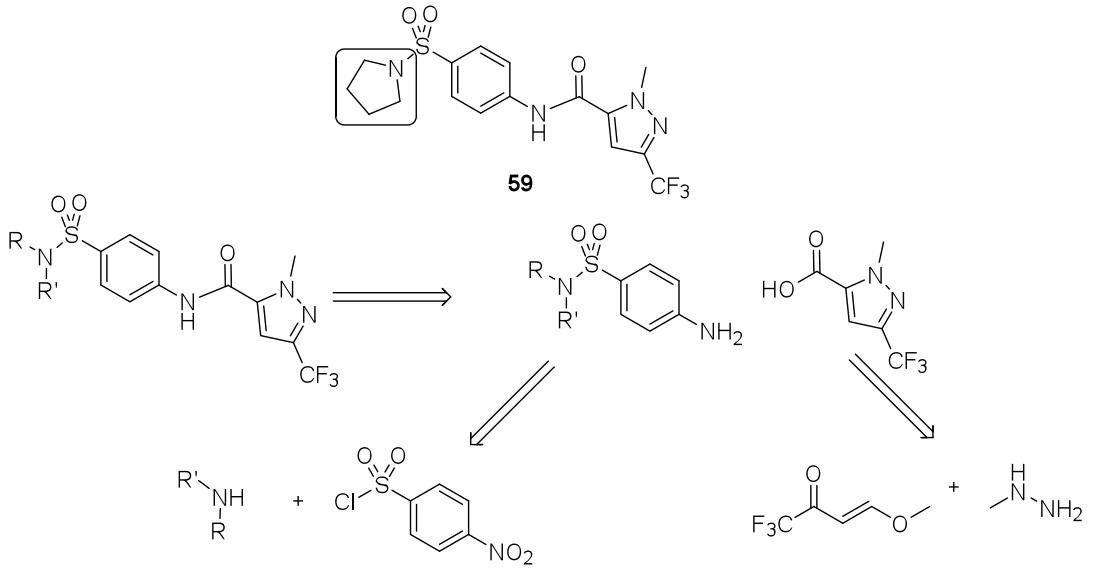
[a]EDCI, DMAP, TEA, DCM, 24 h, 0° C.

Unfortunately, compounds **48-50** exhibited IC_{50} values greater than 77 μM. This data reveals that other variables such as solvation energy may be participating in activity. The affinity of these molecules towards water may be preventing the ligand from interacting with the binding site. The penalty of desolvation has been determined to be as high as 4 kcal/mole using computational techniques.[xxxix] Desolvation of a more hydrophobic moiety would require less energy and therefore might be a better candidate for this model.

2.3.2 RDRP Inhibitors

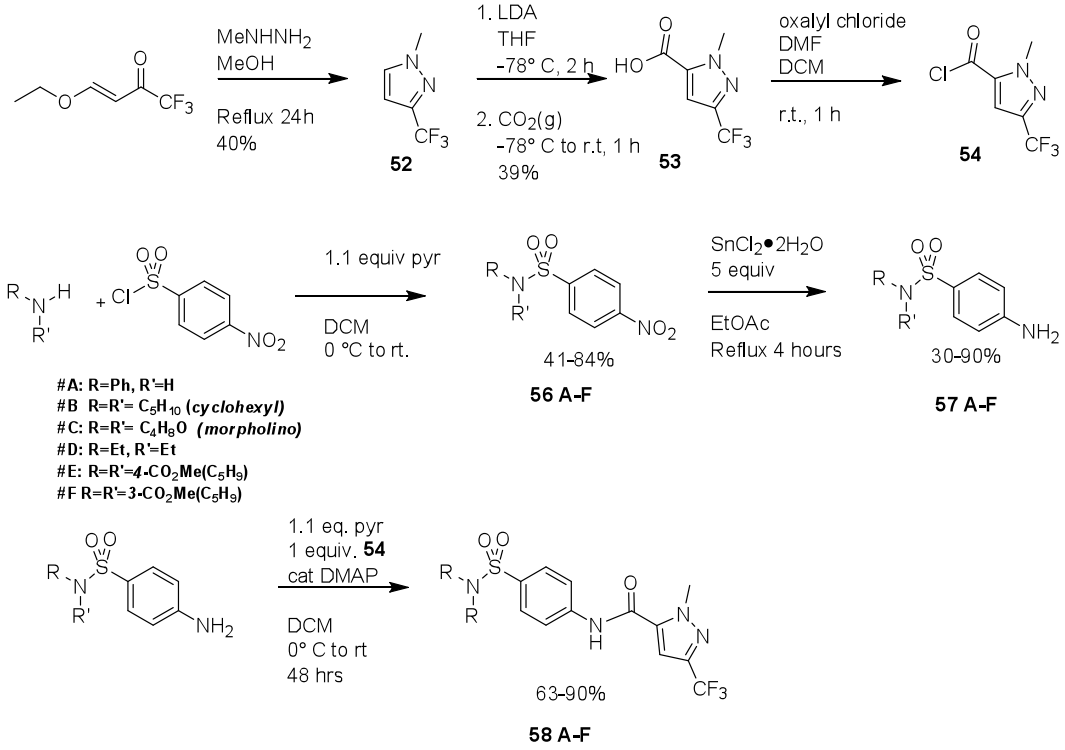
A retrosynthetic approach to lead compound **59** shows several disconnections that would allow development of a diverse library of compounds (Scheme 2.2). This work utilizes alkyl sulfonamide derivatives that are formed via an early connection of various amines with nitrobenzene sulfonyl chloride (Scheme 2.3). Once the sulfonamide is in place, the nitro group is reduced using tin via the Bellamy procedure[xl] to the aniline and then coupled to the acid chloride of the 1-methyl-3-trifluoromethyl-pyrazole.[xli]

Scheme 2.2 Retrosynthetic approach to Measles RDRP inhibitors



A variety of commercially available amines were considered. Various cyclic and acyclic derivatives were explored including the 6-membered ring piperidine, the 6-membered unsaturated phenyl ring, and dimethyl amine. Larger ring systems were also examined. Unfortunately, attempts to couple the amine with more highly developed sulfonyl chlorides to form the sulfonamide at a later stage in the synthesis proved to be difficult and were not fully explored.

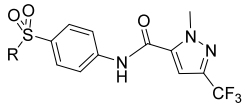
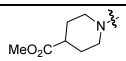
Scheme 2.3. **Synthesis of Measles Virus RDRP Inhibitors**



2.4 Biological Results

Several tests were run to determine the ability of these molecules to inhibit viral proliferation.[xxxvii] These compounds were tested against two measles virus strains (Edmonston and Anchorage) which are the two most commons strains vaccinated for in the United States. The most interesting results were observed when the steric bulk around the sulfonamide alkyl chains was increased. Significant increases in potency were noticed when the 5-membered ring was increased to a 6-membered ring (Table 2.1). The potency was found to be in the nanomolar range. Subsequent diethyl derivatives also proved to be quite active. Currently, animal studies are ongoing with the piperidine derivative to test the molecule's pharmacokinetic profile and efficacy *in vivo.*

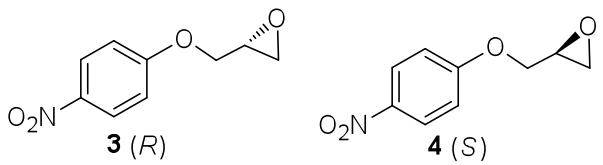
Table 2.1. MV RDRP inhibitors Biological Results

	 R=	Edmonston Strain (μM)	Anchorage Strain (μM)
Lead		1.2	N/A
58 A		toxic	toxic
58 B		0.95	0.005
58 C		5.0	NA
58 D		0.4	0.0007
58 E		1.8	NA
58 F		11	NA

3. Experimental

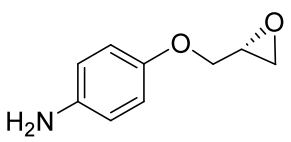
General: All reagents were obtained from Aldrich, TCI America, and Transworld. Reactions requiring anhydrous conditions were performed in oven-dried glassware under dry argon. The following solvent abbreviations may be used: dichloromethane (DCM), diethyl ether (ether), hexane (Hex), ethyl acetate (EtOAc) and tetrahydrofuran (THF). Reaction progress was monitored via thin-layer chromatography (TLC) on pre-coated glass-backed plates and pre-coated aluminum (silica gel 60 Å F_{254}, 0.25 mm thickness backed plates, 20x20 aluminum sheets). Chromatography was carried out with silica gel 60 Å (230 - 400 mesh) from Sorbent Technologies. ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz Mercury, 400 MHz Inova, 600 MHz Inova, or 600 MHz Unity spectrometer in deuterated chloroform ($CDCl_3$) or deuterated methyl sulfoxide (DMSO d_6) and referenced to the residual solvent peak (^{1}H δ 7.27 ppm, ^{13}C δ 77.23 ppm for $CDCl_3$; ^{1}H δ 2.50 ppm, ^{13}C δ 39.51 ppm for DMSO d_6). Chemical shifts are reported in parts per million (δ), and coupling constants are reported in hertz (Hz). The following abbreviations will be used: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), broad singlet (bs), and multiplet (m). Mass spectra were obtained on either a VG 70-S Nier Johnson or JEOL Mass Spectrometer. IR was recorded on a Nicolet Avata 370 DTC-S by Thermo.

(*R and S*) 2-((4-Nitrophenoxy)methyl)oxirane (3)(4)

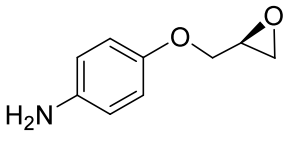


To a 100 mL oven dried round bottom flask, 8.79 g of CsF (57.9 mmol, 3 equivalents) was added to a solution of 2.68 g of 3-nitrophenol in 15 mL of dry DMF with a small magnetic stir bar under argon. After 1.5 hours of stirring, 5.00 g (19.3 mmol, 1 equiv.) of (*R)* glycidyl nosylate was added to the bright yellow mixture, and it continued to stir at room temperature for 24 hours under argon. The reaction mixture was added to 50 mL of crushed ice and warmed slowly to room temperature while stirring. The organic layer was extracted with EtOAc (3 x 15 mL) and dried with $MgSO_4$. The solution was concentrated to afford a light yellow solid. Column separation was performed using EtOAc: Hex system (1:1) to afford yellow solid of mass 3.44 g (91.4 %). The (*S*) enantiomer **2** was also prepared in 99% yield. NMR data coincided with literature.[iii] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.80 (q, *J*= 2.5, 1H), 2.96 (t, *J*=4.5, 1H), 3.41 (m, 1H), 4.02 (dd, *J*=11.1, 5.9, 1H), 4.39 (dd, *J*= 11.1, 2.6, 1H), 7.01 (d, *J*=9 Hz, 2H), 8.22 (d, *J*= 11 Hz, 2H).

4-(Oxiran-2-ylmethoxy)benzenamine



(*R*)



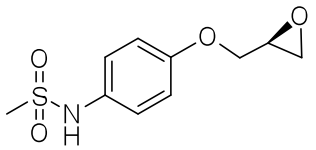
(*S*)

800 mg (4.10 mmol) of 2-((4-nitrophenoxy) methyl) oxirane was dissolved with 80 mg (10% weight of starting material) of Pd/C (en) in 25 mL of anhydrous THF while stirring in a clean dry 250 mL round bottom flask. One atmosphere of hydrogen gas was added, and the reaction mixture was stirred at room temperature for 6 hours. The catalyst was filtered off and then excess THF was removed by rotary evaporation to afford a brown oil. Crude oil was directly added into next step. NMR values were same as literature. ^{1}H-NMR (400 MHz $CDCl_3$) δ 2.69 (dd, 1H, *J*=2.4, 4.5 Hz), 2.83 (t, 1H, *J*=4.5 Hz), 3.26-3.30 (m, 1H), 3.43 (m, 2H), 3.83 (dd, 1H, 5.9, *J*=11.1 Hz), 4.1 (dd, 1H, *J*= 3.1, 11.1 Hz), 6.59 (dd, 2H, *J*= 2.4, 6.8 Hz), 6.72 (dd, 2H, *J*=2.4, 6.8 Hz).

General procedure for synthesis of alkyl sulfonamide phenyl glycidyl ethers. 5 mL of anhydrous DCM under argon was added to the crude oil (677 mg, 4.20 mmol of 4-(oxiran-2-ylmethoxy)benzenamine in an ice bath. 0.75 mL (4.50 mmol, 1.1 equivalents) of diisopropylethylamine was added while stirring. After 5 minutes of stirring, 0.317 mL (4.20 mmol, 1 equivalent) of methanesulfonyl chloride was added dropwise with stirring, and continued to stir at 0° C for 3 hours. 10 mL of distilled H_2O was added to the reaction mixture, then extracted with DCM (3x15 mL) and dried with $MgSO_4$. The

solution was concentrated then let dry in vacuo overnight. Chromatographic separation is performed on a 9 DCM: 1 EtOAc system to afford a white solid.

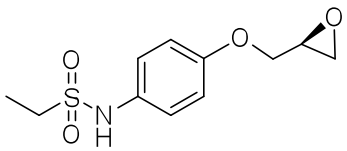
(*R*)-N-(4-(Oxiran-2-ylmethoxy)phenyl)methanesulfonamide (5)



5 (*R*)

Product was isolated in 42 % yield. NMR data coincided with literature. ^{1}H-NMR ($CDCl_3$ 300MHz) δ 2.76 (dd, 1H, *J*= 2.4, 5.2 Hz), 2.92 (t, 1H, *J*= 4.4 Hz), 2.95 (s, 3H), 3.34-3.36 (m, 1H), 3.92 (dd, 1H, *J*= 5.6, 11.2 Hz), 4.24 (dd, 1H, *J*= 2.8, 11.2 Hz), 6.36 (s, 1H), 6.91 (dd, 2H, *J*= 2.0, 6.9 Hz), 7.19 (dd, 2H, *J*= 2.0, 6.9 Hz). ^{13}C-NMR ($CDCl_3$ 75 MHz) δ 39.19, 44.84, 50.31, 69.30, 115.85, 124.81, 129.77, 157.18.

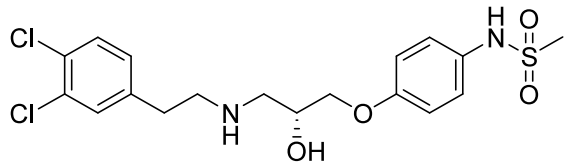
(*S*)-N-(4-(Oxiran-2-ylmethoxy)phenyl)ethanesulfonamide (6)



6 (*S*)

Product was recovered with 26.5% yield. ^{1}H NMR ($CDCl_3$ 600 MHz) δ 1.39 (t, *J*= 7.3, 3H), 2.77 (dd, *J*=2.8, 5, 1H), 2.93 (t, *J*=4.5, 1H), 3.07 (q, *J*=7.5, 2H), 3.93 (dd, *J*=5.8, 16.0, 1H), 4.24 (dd, *J*=3.0, 11, 1H), 6.91(d, *J*=8.8, 2H), 7.19 (d, *J*=8.8, 2H) ^{13}C NMR ($CDCl_3$ 150 MHz) δ 8.49, 44.84, 45.77, 50.29, 69.31, 115.82, 124.57, 129.77.

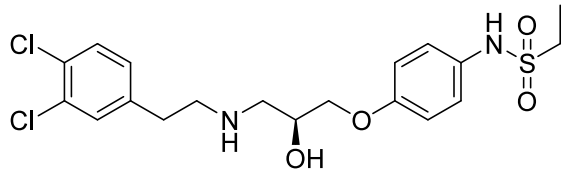
(*R*) N-(4-(3-(3,4-Dichlorophenethylamino)-2-hydroxypropoxy)phenyl)methane-sulfonamide (1)



1(R)

To a 100 mL clean dry round bottom flask, 2.00 g (8.22 mmol , 1 equivalent) of N-(4-(oxiran-2-ylmethoxy)phenyl) methanesulfonamide was added to 15 mL of hot EtOH. 1.22 mL (8.22 mmol, 1 equivalent) of 3,4-dichlorophenethyl amine was added dropwise while stirring. The reaction mixture was refluxed for 6 hours. The EtOH was completely removed by rotary evaporation to reveal a dark brownish oil. Column chromatography was performed in a 10% solution of MeOH in DCM. After chromatography, a yellowish foam was obtained after drying under vacuum of mass 1.78 g (50.0%). ^{1}H NMR ($CDCl_3$ 300 MHz) δ 2.75-2.94 (m, 6H), 2.95 (s, 3H), 3.94, (m, 1H), 3.96 (s,1H), 3.99-4.05 (m, 1H), 6.87 (dd, *J*= 2.0, 6.8, 2H), 7.04 (dd, *J*=2.4, 8.0, 1H,), 7.18 (dd, *J*=2.4, 6.8, 2H,), 7.30 (d, , *J*= 2.0, 1H), 7.35 (d, 1H, *J*= 8.4). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 35.82, 39.24, 50.71, 51.65, 68.35, 70.91, 115.68, 124.92, 128.39, 129.63, 130.49, 130.63, 130.85, 140.26, 157.31.

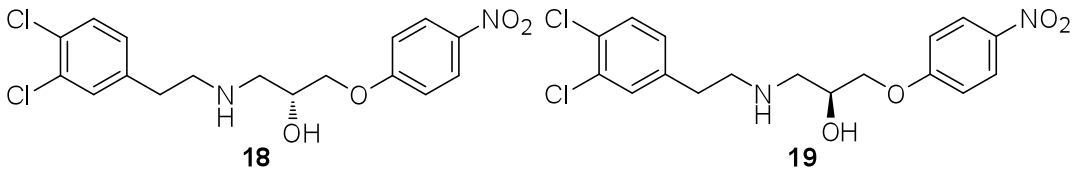
(*S*)N-(4-(3-(3,4-Dichlorophenethylamino)-2-hydroxypropoxy)phenyl)ethane sulfonamide (2)



2(S)

Add 1.78 g (7.02 mmol, 1 equivalent) of N-(4-(oxiran-2-ylmethoxy) phenyl) ethanesulfonamide into 30 mL of hot EtOH in a 100 mL clean dry round bottom flask. Let cool then add 1.04 mL (8.22 mmol, 1 eq) of 3,4-dichlorophenethylamine dropwise while stirring. Reflux for 24 hours, and then remove the EtOH completely by rotary evaporation to reveal a dark brownish oil. Purify by column chromatography in a 10% iPrOH in DCM system. After chromatography, a white solid is obtained after drying under vacuum of mass 2.93 g (93%) mp 119-121° C .^{1}H NMR ($CDCl_3$ 600 MHz) δ 1.38 (t, *J*=7.5, 3H), 2.76-2.80(m, 3H), 2.87-2.95 (m, 3H), 3.07 (q, *J*=7.5, 2H), 3.96 (m, 2H), 4.02 (m, 1H), 6.87 (d, *J*=8.8, 2H), 7.05 (dd, *J*=1.8, 8.0, 1H), 7.18 (d, *J*=8.8, 2H), 7.31 (d, *J*=1.8, 1H), 7.36 (d, *J*=8, 1H). ^{13}C NMR ($CDCl_3$ 600 MHz) δ 8.49, 35.85, 45.77, 50.72, 51.65, 68.34, 70.90, 115.67, 124.65, 128.39, 130.03, 130.86, 131.66, 140.29, 144.37, 157.13.

(*R*)-1-(3,4-Dichlorophenethylamino)-3-(4-nitrophenoxy)propan-2-ol (18)

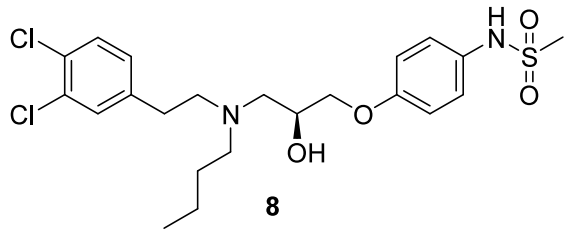


18 **19**

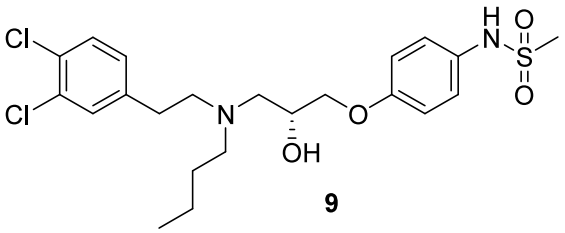
1.00 g (4.11 mmol) of (*R*) nitrophenyl glycidyl ether is dissolved in 15 mL of hot EtOH. 0.610 mL (4.11 mmol, 1 equivalent.) of dichlorophenethylamine is added dropwise while stirring. Reflux for 6 hours and then remove the EtOH completely. Column chromatography is performed in a 10% solution of MeOH in DCM to reveal a white solid of 1.02g (55.6%). The enantiomer **19** from (*S*) nitrophenyl glycidyl ether was recovered in 40% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.77-2.93 (m, 4H), 2.89-2.97 (m, 4H), 4.05 (m, 4H), 6.96 (d, *J*=7.2, 2H), 7.06 (dd, *J*=1.8, 8.1, 1H), 7.32 (d, *J*=2.0, 1 H), 7.37 (d, *J*=8.4, 1H).

General procedure for preparation of tertiary amines. 1 mmol of amino alcohol (**1**, **7**, or **2**) and 1 mmol of appropriate aldehyde were dissolved in 10 mL 1,2-dichloroethane and treated with 2.8 mmol of sodium triacetoxyborohydride. After stirring overnight at room temperature, the reaction mixture was quenched with saturated sodium bicarbonate. Water phase is extracted with DCM. Organic phase is dried by $MgSO_4$ and evaporated. The residue is purified with 1 EtOAc: 1 Hex system by column chromatography to yield a colorless oil.

(*S*)-N-(4-(3-((3,4-Dichlorophenethyl)(butyl)amino)-2-hydroxy propoxy) phenyl) methanesulfonamide (8)



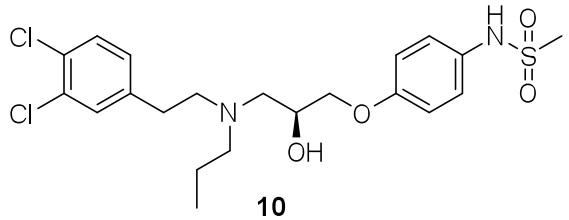
8



9

Product is isolated with 60% yield. The (*R*) enantiomer **9** was found in 36 % yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.88 (t, *J*=7.2, 3H), 1.26 (m, 2H), 1.41 (m, 2H), 2.47-2.79 (m, 8H), 2.90 (s, 3H), 3.91 (m, 3H), 6.83 (d, *J*= 8.4, 2H), 6.99 (dd, *J*=2.1, 7.8, 1H), 7.15 (d, *J*=8.4, 2H), 7.24 (d, *J*=2.1, 1H), 7.31 (d, *J*=7.8, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.20, 20.68, 29.35, 32.97, 38.95, 54.04, 55.69, 57.02, 66.37, 70.60, 115.67, 120.78, 124.71, 128.36, 129.67, 130.21, 132.42, 140.55, 157.28.

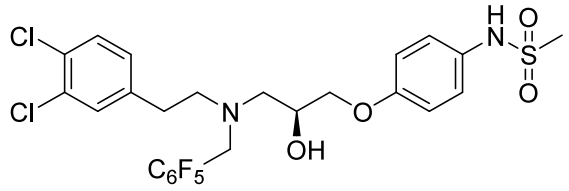
(*S*)-N-(4-(3-((3,4-Dichlorophenethyl)(propyl)amino)-2-hydroxypropoxy)phenyl) methanesulfonamide (10)



10

Product is isolated with 79% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*= 7.2, 3H), 1.48 (m, 2H), 2.5-2.8 (m, 8H), 2.94 (s, 3H), 3.92 (m, 3H), 6.87 (d, *J*=8.8, 2H), 7.00 (dd, J = 2, 8.4, 1H), 7.19 (d, *J*=8.8, 2H), 7.27(t, *J*=1.8, 1H), 7.34 (d, *J*=8.4, 1H).

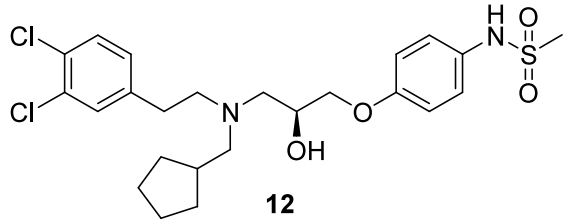
(*S*)-N-(4-(3-((3,4-Dichlorophenethyl)(pentafluorobenzyl)amino)-2-hydroxypropoxy) phenyl) methanesulfonamide (11).



11

Product is isolated with 13% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.77-3.98 (m, 6H), 3.07 (m, 4H), 3.83-4.00 (m, 4H), 4.12 (m, 1H), 6.92 (d, *J*=8.8, 2H), 7.05 (dd, *J*=1.6, 8, 1H), 7.20 (d, *J*=8.0, 2H), 7.27 (m, 2H), 7.35 (d, *J*=8, 1H).

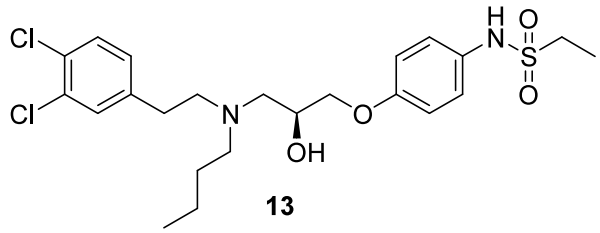
(*S*)-N-(4-(3-((3,4-dichlorophenethyl)(cyclopentylmethyl)amino)-2-hydroxypropoxy)-phenyl)methanesulfonamide (12)



12

Product was recovered with 81% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.14 (m, 2H), 1.52-1.73 (m, 6H), 2.05 (septet, *J*=6.8) , 1H), 2.48 (m, 2H) 2.64 (d, *J*=6.4, 2H) , 2.69-2.85 (m, 4H), 2.95 (s, 3H), 3.96 (m, 3H), 6.91 (d, *J*= 8.8, 2H), 7.05 (dd, *J*=1.6, 8.0, 1H), 7.19 (d, *J*=8.8, 2H), 7.30 (d, *J*=2.0, 1H), 7.35 (d, *J*=8.0, 1H). ^{13}C NMR δ 25.38, 31.35, 32.85, 38.13, 39.07, 56.19, 57.40, 60.44, 66.37, 70.67, 115.64, 124.81, 128.42, 129.61, 130.27, 130.54, 130.82, 132.73, 140.59, 157.41. Anal. Calcd for $C_{24}H_{33}Cl_3N_2O_4S \cdot ½H_2O$: C, 51.38 ; H, 6.11; N, 4.99. Found C, 51.49; H, 6.07; N, 4.99.

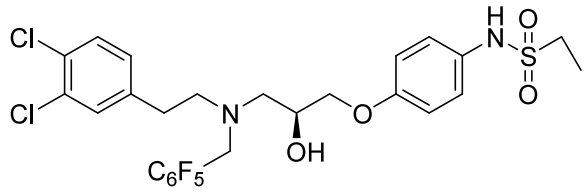
N-(4-(3-((3,4-Dichlorophenethyl)(butyl)amino)-2-hydroxypropoxy)phenyl)ethane sulfonamide (13)



13

Product is isolated in 28.4% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.89 (t, *J*=7.2, 3H), 1.27-1.45 (m, 7H), 2.54-2.79 (m, 8H), 3.05 (q, *J*=7.2, 2H), 3.92 (m, 3H), 6.86 (d, *J*=9.0, 2H), 6.99 (dd , *J*=2.0, , 1H), 7.18 (d, *J*=9.0, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 8.51, 14.32, 20.79, 29.44, 33.07, 45.67, 54.12, 55.77, 57.15, 66.36, 70.64, 115.56, 124.37, 128.30, 129.59, 130.46, 130.73, 140.44, 157.03.

N-(4-(3-((3,4-Dichlorophenethyl)(perfluorobenzyl)amino)-2-hydroxypropoxy)-phenyl)ethanesulfonamide(14).

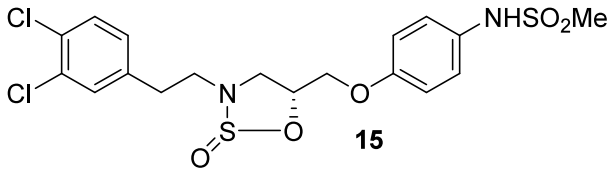


14

Product is isolated in 23% yield. ^{1}H NMR ($CDCl_3$ 600 MHz) δ 1.38 (t, *J*=7.6, 3H), 2.69-2.79 (m, 6H), 2.86 (bs, 1H), 3.08 (q, *J*=7.1, 2H), 3.81-3.91 (m, 4H), 3.99 (m, 1H), 6.40 (s, 1H), 6.84 (d, *J*=11.0, 2H), 6.96 (dd, *J*= 1.9, 8.1, 1H), 7.19 (m, 3H), 7.32 (d, *J*=8.1, 1H). ^{13}C NMR ($CDCl_3$ 600 MHz) δ 8.43, 32.92, 45.35, 45.69, 55.57, 56.69, 66.90, 69.87, 115.48, 124.45, 128.37, 129.37, 130.51, 130.61, 132.54, 140.11, 156.93.

General procedure for preparation of cyclic sulfamidite: One mmol of amino alcohol **1**, **2**, or **7** is dissolved in 10 mL DCM and cooled to -15° C. 2 mmol of triethylamine is added and stirred. In a dropwise fashion, add 1 mmol of thionyl chloride in DCM, followed another 2 equivalents of triethylamine. Stirring is continued at -15 °C for 24 hours. Evaporate the solvent to reveal a light brown oil. Product is purified with column chromatography using 10 % MeOH in DCM system to reveal a yellow foam in 98+% yield.

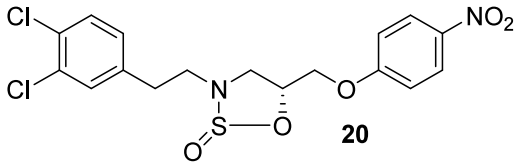
(*R*)-N-(4-(3-(3,4-Dichlorophenethyl)-2-oxo-oxathaizolidin-5-yl)methoxyphenyl) methanesulfonamide (15)



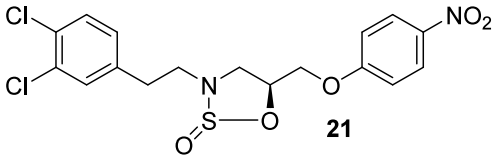
15

The mixture of diastereomers forms in a ratio of 4:3. The mixture of diastereomers was continued to next step without further characterization.

(*R*)-3-(3,4-dichlorophenethyl)-5-((4-nitrophenoxy)methyl)oxathiazolidin-2-oxide (20 and 21)



20



21

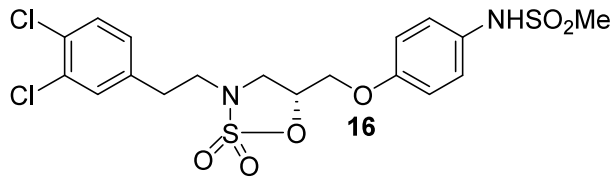
A mixture of diastereomers forms in a 1:1 ratio.

Both diastereomers are isolated in 98 to 99% yields. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 2.92-2.98 (m, 4H), 3.09-3.20 (m, 2H), 3.36-3.5 (m, 4H) , 3.59-3.68(m, 2H), 4.01 (dd, *J*=6.6,

9.6, 1H), 4.15 (dd, *J*=5.1, 8.7, 1H), 4.33 (dd, *J*=5.7, 10.2, 1H), 4.43 (dd *J*=5.7, 7.5), 4.97 (m, 1H), 5.31 (m,1H), 6.93-7.09 (m, 6H), 7.30-7.41 (m, 4H), 8.23 (m, 4H). IR 1156 (S=O asym), 1344 (NO_2).

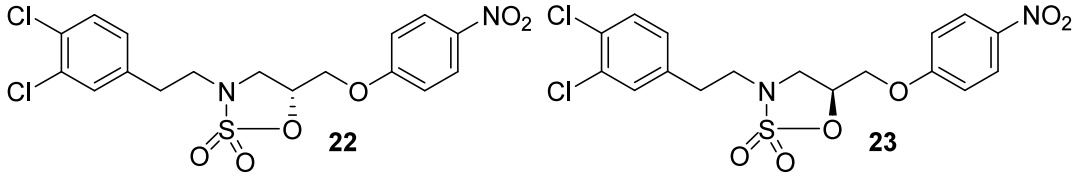
General procedure for preparation of cyclic sulfamidate: 1 mmol of sulfamidite was dissolved in 10 mL of acetonitrile and cooled to 0 °C. To this solution was added 1.5 mmol of sodium periodate and 0.01 mmol of ruthenium chloride hydrate. Stir for 5 minutes then add 5 mL distilled water. Continue to stir 24 hours at 0 °C. Extract with 3x10 mL of EtOAc, dry with $MgSO_4$ and concentrated by rotary evaporation.

N-(4-((3-(3,4-Dichlorophenethyl)-2-dioxo-oxathaizolidin-5-yl)methoxy)phenyl) methanesulfonamide.**(16)**



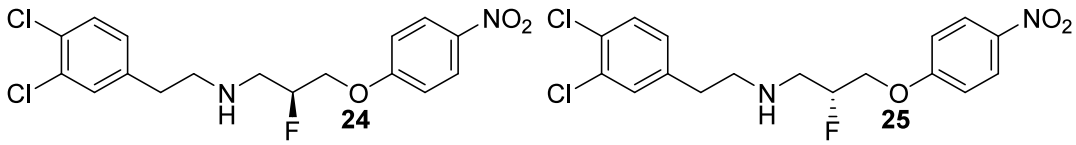
Purification was performed by silica gel chromatography with a 3 EtOAc: 7 DCM system to afford a pink foam in 59.3% yield. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 2.91-3.01 (m,5H), 3.33-3.50 (m, 4H), 3.63 (m, 2H), 4.12 (dd, *J*= 5.4, 10.4, 1H), 4.23 (dd, *J*=5.2, 10.4), 5.00 (p, J = 6.0, 1H), 6.88 (d, *J*=6 , 2H), 7.10 (dd, *J*=1.8, 7.8 1H), 7.22 (d, *J*=6 , 2H), 7.36 (m, 2H). IR (in DCM solution) 1183 (SO_2 Asym).

3-(3,4-Dichlorophenethyl)-5-((4-nitrophenoxy)methyl)oxathiazolidin-2-dioxide (22 and 23)



Purification was performed on a 10% MeOH in DCM solution to afford a cream colored solid **22** in 58.5 % yield. **23** was recovered in 69% yield. ^{1}H NMR ($CDCl_3$ 400 MHz) δ 2.98 (t, *J*=7.2, 2H), 3.39 (t, *J*=7.2, 2H), 3.47 (dd, *J*=6.2, 9.4), 3.65 (dd, *J*=7.0, 9.4), 4.24 (dd, *J*= 5.4,10.4, 1H), 4.33 (dd, *J*=5.2, 10.4), 5.07 (p, *J*=7.2, 1H), 6.98 (d, *J*=9.2, 2H), 7.10 (dd, *J*=2.4, 8.4, 1H), 7.35 (d, *J*=2.4, 1H), 7.38 (d, *J*=8.4, 1H), 8.25 (d, *J*=9.2, 2H) IR 1184 (SO_2 asym) .

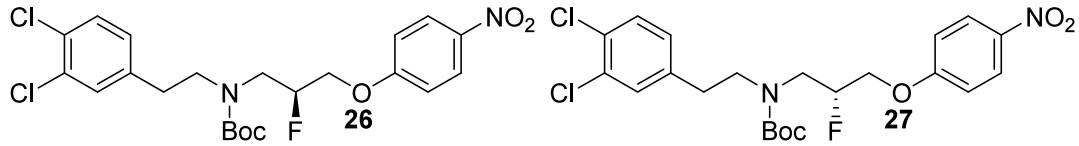
N-(3,4-Dichlorophenethyl)-2-fluoro-3-(4-nitrophenoxy)propan-1-amine (24 and 25)



Dissolve sulfamidate **22** (515 mg, 1.04 mmol) in 10 mL of dry acetonitrile with 275 mg (1.04 mmol, 1.0 equiv.) of 18 crown-6 in a clean dry 100 mL round bottom flask. Add about 1.1 equivalents of solid anhydrous KF (66 mg, 1.14 mmol). Reaction turns from a light brown to yellowish brown upon addition. Reflux (in a dried condenser) under argon for 48 hours. Add 3 mL of EtOAc and 3 mL of 20% H_2SO_4 and stir for up to 24 hours. Basify the solution to pH 11-14 with solid sodium carbonate and extract with 3 x 10 mL of EtOAc. Dry with $MgSO_4$ and concentrate by rotary evaporation. Silica gel chromatographic separation was performed in 3 EtOAc: 7 Hexanes system to afford a

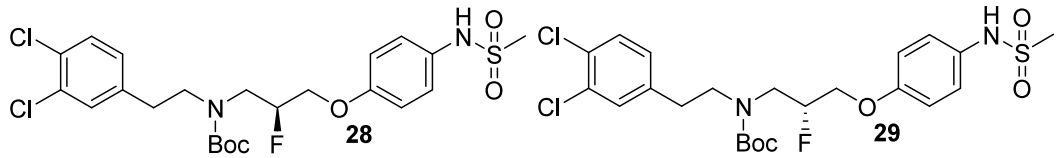
yellow oil **24** of mass 210 mg (46.4% yield). **25** was recovered in 43% yield. ^{1}H NMR ($CDCl_3$ 600MHz) δ 2.80 (t, *J*=7.1, 2H) , 2.93-3.07(m, 4H), 4.20-4.28 (m, 2H), 4.98 (doublet of quintets, *J*= 4.5 , 48.2, 1H), 6.9 (d, *J*=9.5, 2H), 7.06 (dd, *J*=1.9, 8.1, 1H), 7.32 (d, *J*=1.9, 1H), 7.36 (d, *J*=8.1, 1H), 8.23 (d, *J*=9.1, 2H). ^{13}C NMR ($CDCl_3$ 100 MHz) δ 35.70, 50.10 (d, *J*=22), 50.92, 68.87(d, *J*=23), 91.09 (d, *J*=172), 114.74, 126.16, 128.37, 130.58, 130.84, 132.55, 140.20, 142.12, 163.46 ^{19}F NMR ($CDCl_3$), uncalibrated, 1 signal -192.45 (m).

tert-Butyl-3,4-dichlorophenethyl(2-fluoro-3-(4-nitrophenoxy)propyl)carbamate (26 and 27)



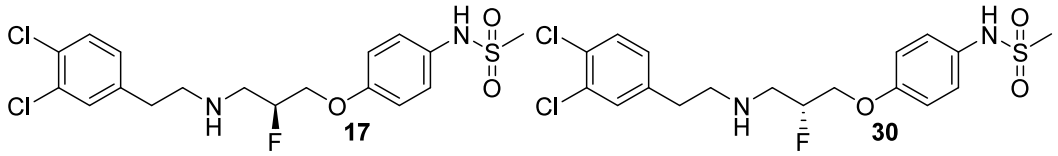
1.66 g (4.29 mmol) of secondary amine **24** was dissolved in 40 mL of THF, then 1.2 equivalent of Boc_2O and 1.0 equivalent of TEA was added and stirred at room temperature for 3 hours. Reaction was monitored by TLC in a 1:1 EtOAc: Hex system. The product is the material that has migrated furthest (rf >0.8). Add 30 mL of saturated ammonium chloride, 30 mL of $NaHCO_3$, extract with 3 x 50 mL of DCM and dry over $MgSO_4$. Separation is performed by silica gel column in a 1:3 EtOAc: Hexanes system. The first spot is collected to reveal a yellow foam 2.08 g (99% yield). Enantiomer **27** was recovered at 98% yield. ^{1}H NMR ($CDCl_3$ 400 MHz) recovered as a mixture of rotamers : 1.15 (s, 9H), 2.83 (m, 2H), 3.35-3.75 (m, 4H), 4.10-2.33(m, 2H), 4.87-5.11(m, 1H), 6.97-7.01(m, 3H), 7.28 (m, 1H) 7.36 (d, *J*=8.4, 1H) 8.22 (d, *J*=9.2, 2H).

tert-Butyl-3,4-dichlorophenethyl(2-fluoro-3-(4-(methylsulfonamido)phenoxy)-propyl)carbamate (28 and 29)



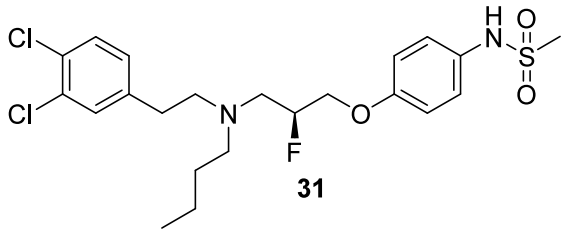
Dissolve 2.08 g (4.3 mmol) of the carbamate ((*S*)**26** or (*R*)**27)** in 50 mL of EtOH and add 10% by weight (200 mg) of 5% Pd on carbon. Add 1 atm of H_2 gas and stir overnight (check by TLC). Filter off catalyst and remove solvent by rotary evaporation. Chromatographic separation was performed on a silica gel column in 9:1 DCM: EtOAc (w/ 1% TEA) to obtain the aniline as a reddish oil (0.80 g, 41% yield). To the 0.370 mg (0.81 mmol) of the aniline, 20 mL of DCM was added. Then the solution was cooled to 0°C and 0.169 mL (0.27 mmol , 1.1 equivalent) of DIEA was added followed by the slow, dropwise addition of 0.079 mL (0.24 mmol, 1.0 equivalent) MsCl. The reaction mixture was allowed to stir at 0 C for 3 hours. Add 10 mL of distilled H_2O and stir for thirty minutes. Then extract 3x15 mL of DCM and dry the organic layer with $MgSO_4$ for an hour. Concentrate then dry in vacuo overnight. Chromatographic separation was performed on a silica gel column in a 9: 1 DCM: EtOAc solvent system to yield 300 mg of a yellow oil (29% overall yield). Enantiomer **29** *(R)* was recovered in 50 % overall yield. ^{1}H NMR ($CDCl_3$ 400 MHz) δ 1.44 (s, 9H), 2.83 (m, 2H), 2.96 (s, 3H), 3.40-3.75 (m, 4H), 4.05-4.25 (m, 2H), 4.97 (m, 1H), 6.23 (s, 1H), 6.91(d, *J*= 8.8, 2H), 7.02 (m, 1H), 7.20 (m, 3H), 7.36(d, *J*=8.4 , 1H).

(S)-N-(4-(3-(3,4-Dichlorophenethylamino)-2-fluoropropoxy)phenyl) methane-sulfonamide (17 and 30)



Stir 300 mg (0.56 mmol) of carbamate ((*S*)**29** or (*R*)**28)** in 6 mL of concentrated HCl and 12 mL of EtOAc for 30 minutes. Check formation of baseline species by TLC. Neutralize with sodium bicarbonate and extract with DCM 3 x 10 mL. 180 mg (68%) of product was recovered. Analytical separation was achieved on a Reverse Phase column 40 minute method gradient of 25% MeCN in H_2O to 50% for 30 minutes then from 50% to 100 % MeCN for 10 more minutes. The product was collected at 21.05 minutes. Preparative HPLC separation was achieved on the same schedule over 80 minutes. Isolation of the product revealed a clear oil. ^{1}H NMR ($CDCl_3$ 400 MHz) 2.77-2.84 (m, 2H), 2.87-3.08 (m, 7H), 4.09-4.12 (m, 1H) 4.13-4.16 (m, 1H) 4.92 (doublet of quintets, *J*=47.6, 4.6, 1H), 6.89 (dd, *J*=6.8, 2.0, 2H), 7.05 (dd, *J*=8.0, 2.0, 1H), 7.21(dd, *J*=6.8, 2.0, 2H), 7.31 (d, *J*=1.6, 1H), 7.35 (d, *J*=8.0, 1H) ^{13}C 35.6, 39.1, 50.3 (d, *J*=21.36) , 50.9, 68.5 (d, *J*=24.4), 91.3 (d, *J*=172.4), 115.7, 124.8, 128.4, 128.7, 128.9, 130.6, 130.8., 140.2, 157.0. HRMS Calc 434.06 Found (M+H) 435.07046. Hydrochloride salt formation was quantative. HCl gas is bubbled to a solution of the product in 10 mL of EtOH for 1 hour. EtOH is removed by rotary evaporation and the product is dried overnight under high vacuum to afford a white powder. Anal. Calcd for $C_{18}H_{22}Cl_3FN_2O_3S$: C, 45.82; H, 4.70; N, 5.94; Found: C, 45.84, H, 4.76; N, 5.90.

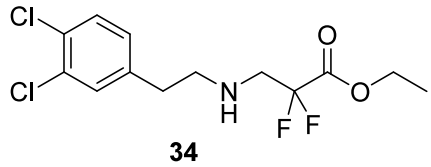
(S)-N-(4-(3-((3,4-dichlorophenethyl)(butyl)amino)-2-fluoropropoxy)phenyl) methanesulfonamide -31



31

Dissolve 100 mg of HCl salt into 20 mL DCM and add 1 equivalent of triethylamine. Add 10mL of distilled H_2O and extract 3x20 mL of DCM. Dry the combined organic layers with $MgSO_4$ and remove volume by rotary evaporation until free amine is an oil. Then add 10 mL of DCE to the oil and add the aldehyde. Stir for 20 minutes at room temperature, and then add sodium triacetoxyborohydride. Stir overnight or until reaction is complete. Purify by column chromatography in a 3:7 EtOAc: Hex solvent system. Collect the top spot. ^{1}H NMR (400 MHz in $CDCl_3$) δ 0.90 (t, *J*=7.6, 3H), 1.25-1.34 (m, 2H), 1.38-1.46 (m, 2H), 2.55 (t, *J*=7.4, 2H), 2.72 (m, 2H), 2.76 (m,2H), 2.84-2.92 (m, 2H), 2.98 (s, 3H) 3.96-4.11 (m, 2H), 4.81 (doublet of quintets, *J*=49.7, 1.5, 1H), 6.62 (s, 1H), 6.88 (d, *J*=8.8, 2H), 7.03 (dd, *J*=7.6, 2, 1H), 7.22 (d, *J*=8.8, 2H), 7.28 (d, *J*=2.4, 1H), 7.32 (d, *J*=8.4, 1H) ^{13}C NMR (100 MHz in $CDCl_3$) δ 14.3, 20.7, 29.5, 33.0, 39.1, 54.5 (d, *J*=23.2), 54.8, 56.5, 68.4 (d, *J*=23.2), 91.0 (d, *J*=173.5), 115.6, 124.8, 128.5, 129.7, 130.0, 130.4, 130.9, 132.2, 141.0, 157.1.

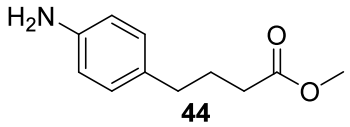
Ethyl-3-(3,4-dichlorophenethylamino)-2,2-difluoropropanoate (34)



34

Add 85 mg (2.8 mmol) of solid paraformaldehyde and 1.12 grams (7.8 mmol) of Na_2SO_4 to a solution of 500 mg (2.6 mmol) of 3,4 dichlorophenethylamine in 10 mL of dry DCE using a clean dry flask and reflux for 30 minutes. Reaction is completed by TLC - higher rf large streak. Cool down, filter, and remove volume of the Schiff base to an oil by rotary evaporation. In a separate clean dry flask, stir 275 mg of zinc dust metal (4.2 mmol) in 100 mL of refluxing dry THF for 5 minutes before adding 802 mg of ethyl bromodifluoroacetate (3.9 mmol). Then add TMSCl (458 mg 4.2 mmol) dropwise and stir for two minutes. Then add the Schiff base dissolved in 25 mL of THF. Reflux for two hours then add 100 mL of saturated sodium bicarbonate and extract with 3x 20 mL of DCM. Remove volume by rotary evaporation to yield a yellow oil. Crude yield is 700 mg. Chromatographic separation is achieved with 1:1 EtOAc: Hex to collect spot with high rf. Top spot was collected to yield the product as a yellow oil in 26% yield. ^{1}H (400 MHz in $CDCl_3$) δ 7.35 (d, *J* = 8.3 Hz, 1 H), 7.29 (d, *J* = 1.9 Hz, 1H), 7.03 (dd, *J* = 7.9; 1.9 Hz, 1H), 4.31 (q, *J* = 7.3Hz, 2H), 3.22 (t, *J* = 13.5 Hz, 2H), 2.94 (t, *J* = 7.0 Hz, 2H), 2.71 (2, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100MHz in $CDCl_3$) δ 163 (t, *J*=32 Hz), 140.09, 132.32, 130.72, 130.40, 130.22, 128.34, 115.48 (t, *J*=252 Hz), 62.91, 51.60 (t, *J*=26 Hz), 50.54, 35.56, 14.01. 19F -110.83 (t, *J*=13 Hz) no standard.

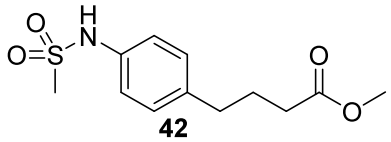
Methyl-4-(4-aminophenyl)butanoate (44)



44

Add 2.00 (11.2 mmol) grams of the 4-4aminophenyl butanoic acid into a solution of 50 mL of dry methanol in a dry 250 mL round bottom flask. Then add 5.31 grams of sulfonyl chloride at 0° C dropwise. Allow the reaction to warm to room temperature and then remove solvent by rotary evaporation. Add 30 mL of 1 M NaOH, and extraction 3x50 mL EtOAc. Dry the organic layer with $MgSO_4$ and remove solvent until a solid is revealed. Continue to the next step without further purification. ^{1}H NMR (400 MHz in $CDCl_3$) δ 1.90 (quintet, *J*=8 Hz, 2H), 2.32 (t, *J*=7.6, 2H), 2.55 (t, *J*=6.0, 2H), 3.66 (s, 3H), 6.64 (d, *J*=8.6, 2H), 6.97 (d, *J*=8.6, 2H).

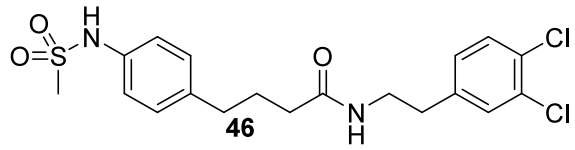
Methyl-4-(4-(methylsulfonamido) phenyl)butanoate (45)



42

Add 1.00 gram (5.6 mmol) of aniline to 15 mL of pyridine at room temperature and add 0.475 mL 6.1 mmol) of methanesulfonyl chloride and stir for 24 hours. Add 30 mL of water and extract 3x30 mL of DCM. Dry the combined organic layers with $MgSO_4$ and remove solvent until material becomes a red oil. Put under high vacuum overnight. Purify using column chromatography in a 1:1 EtOAc Hexane solvent system. ^{1}H (400 MHz in $CDCl_3$) δ 1.92 (quintet, *J*=8 Hz, 2), 2.33 (t, *J*=7.3, 2H), 2.6 (t, *J*=7.9, 2H), 2.98 (s, 3H), 3.66 (s, 3H), 7.13-7.22 (m, 4H). ^{13}C NMR (100 MHz in $CDCl_3$) δ 26.5, 33.4, 34.5, 51.8, 121.6, 129.8, 134.9, 138.9, 174.2.

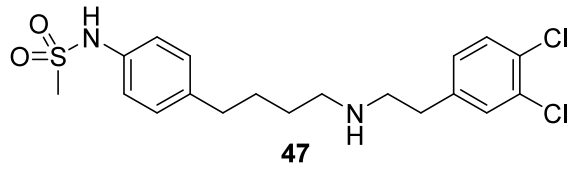
N-(3,4-Dichlorophenethyl)-4-(4-(methylsulfonamido)phenyl)butanamide (46)



46

Add 20 mL of MeOH to a flask containing 400 mg (1.5 mmol) of methyl 4-(4-(methylsulfonamido) phenyl) butanoate with 3 equivalents of 1 M NaOH in water. Stir 24 hours. Acidify with HCl to pH 3 or lower. Remove volume by rotary evaporation until crystals precipitate out of solution. Filter, wash, and dry the crystals under high vacuum and use directly in coupling reaction. Add the 150 mg (0.58 mmol) carboxylic acid, 112 mg (0.58 mmol) of EDCI, and 111 mg (0.58 mmol) of DMAP into the DCM at 0°C. Add a drop of DMF to get the starting material to dissolve. Let warm up to room temperature and stir overnight. Add 20 mL of 1N HCl and extract with 3x30 mL of EtOAc. Dry with $MgSO_4$ and reduce volume by rotary evaporation. Directly make a silica cake with the product and proceed to separate using chromatography in a 1:1 EtOAc: DCM solvent system. Gradually add 10% MeOH in DCM. Collect second spot with RF of approx. 0.25 in 1:1 EtOAc: DCM. Collect white solid in 64 % yield. mp 101-103° C. ^{1}H NMR (400 MHz in $CDCl_3$) δ 1.90 (quintet, *J*=7.7, 2H), 2.15 (t, 7.6, 2H), 2.60 (t, *J*=7.6), 2.79 (t, *J*=7.0, 2H), 2.99 (s, 3H), 6.82 (s, 1H), 7.04 (dd, *J*=8.3, 1.9, 1H), 7.11-7.17 (m, 4H), 7.29 (d, *J*=1.9, 1H), 7.37 (d, J=9.2, 1H), 8.02 (s, 1H). ^{13}C (100 MHz in $CDCl_3$) δ 173.09, 139.32, 139.01, 134.95, 132.62, 134.95, 132.62, 130.91, 130.73, 129.78, 128.42, 121.60, 40.42, 39.37, 35.91, 35.00, 34.63, 27.23.

N-(4-(4-(3,4-Dichlorophenethylamino)butyl)phenyl)methanesulfonamide (47)



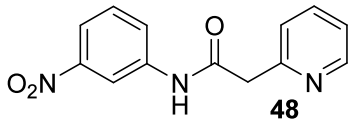
47

In a solution of 5 mL of THF with 100 mg (0.5 mmol) starting material, add 3 equivalents of LAH in 1.0 M THF at room temperature and stir for 24 hours. Spot can be seen by 10% MeOH in DCM as a large tail below starting material. Add 5 mL of dH_2O very carefully (in a dropwise fashion to neutralize LAH) and stir for 30 minutes. Extract with 3x20 mL of DCM (add 5 more mL of water to extraction mixture and a little bit of saturated sodium bicarbonate wash to facilitate separation) and dry organic layer with $MgSO_4$. Reduce volume using rotary evaporation and put under high vacuum. Separate using a very small column and start with 1:1 EtOAc Hex to remove more non polar spots, then add 10% MeOH in DCM. HCl salt prepared. Anal. Calcd for $C_{19}H_{25}Cl_3N_2O_2S$. , 48.10; H, 4.46; N, 11.81 Found: C, 48.12; H, 4.36; N, 11.52 ^1H (400 MHz in $CDCl_3$) δ 7.35 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 1.9 Hz, 1H), 7.14(m, 4H), 7.04 (dd, *J* = 8.2 Hz, 1.9 Hz, 1H).

General Procedure for synthesis of nitrophenyl pyridinyl acetamides: Dissolve the pyridylacetic acid hydrochloride (200 mg, 1.13 mmol) into 5 mL of DCM and add 1 equivalent of triethylamine (0.16 mL) at ambient temperature. Upon addition the reaction mixture turns red. Then add 250 mg (1.1 equivalents) of dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). Add 15 mg of DMAP and stir for 15 minutes. Add nitro aniline and stir for 24 hrs at 0 °C. Add 5 mL of saturated sodium bicarbonate

solution and extract with 3x10 mL of EtOAc. Dry the organic layer with $MgSO_4$ and concentrated with rotary evaporations. Wash with cold chloroform to a yellow powder.

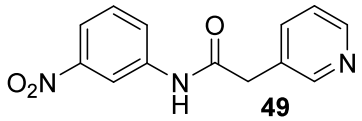
N-(3-Nitrophenyl)-2-(pyridin-2-yl)acetamide (48).



48

Product is isolated with 40.5% yield. ^{1}H NMR ($CDCl_3$ 300MHz) δ 3.94 (s, 2H), 7.34 (m, 2H), 7.48 (t, *J*=8.4, 1H), 7.76 (t, *J*=7.5, 1H), 7.93 (d, *J*=7.5, 1H), 8.01 (d, *J*=8.0, 1H), 8.40 (t, *J*=2.3, 1H), 8.65 (d, *J*=5.1, 1H), 10.60 (bs, 1H) ^{13}C NMR ($CDCl_3$ 100MHz) δ 45.37, 114.63, 118.63, 122.76, 124.70, 125.64, 129.80, 137.97, 139.46, 148.61, 148.96, 154.92. 167.86. Anal. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33. Found: C, 60.74; H, 4.35; N, 16.09.

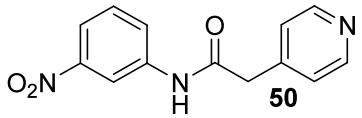
N-(3-Nitrophenyl)-2-(pyridin-3-yl)acetamide (49)



49

Product is isolated in 28% yield. ^{1}H NMR (DMSO d_6 600 MHz) δ 3.77 (s, 2H), 7.37 (dd, *J*=4.8, 7.2, 1H), 7.61 (t, *J*=1.8, 1H), 7.75 (d, *J*=7.8, 1H), 7.91 (dd, *J*=2, 8.1, 2H), 8.47 (dd, *J*=1.8, 4.8, 1H), 8.53 (d, *J*=1.8, 1H), 8.62 (t, *J*=2, 1H) ^{13}C NMR (DMSO d_6 150 MHz) δ 40.10, 113.24, 117.91, 123.46, 125.11, 130.27, 131.13, 136.93, 140.15, 147.98, 150.29, 169.39. Anal. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33 Found: C, 59.54; H, 4.33; N, 15.92.

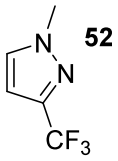
N-(3-Nitrophenyl)-2-(pyridin-4-yl)acetamide (50)



50

Product is isolated in 51% yield. ^{1}H NMR (DMSO d_6 400 MHz) δ 3.76 (s, 2H), 7.36 (d, J=6.0, 2H), 7.61(t, J=8.2, 1H), 7.92 (m, 2H), 8.52 (d, J=6.0, 2H), 8.62 (t, J=2.2, 1H) ^{13}C NMR (DMSO d_6 100 MHz) δ 41.97, 112.90, 117.63, 124.40, 124.77, 129.94, 139.72, 143.82, 147.61, 149.20, 151.93, 168.32. Anal. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33. Found: C, 60.83; H, 4.36; N, 16.16.

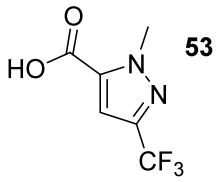
1-Methyl-3-(trifluoromethyl)-1H-pyrazole (52)



52

Procedure is followed according to reference.[xli] A solution of methyl hydrazine (2.7 g, 59 mmol) and 1,1,1-trifluoro-4-ethoxy-3-buten-2-one (10.00 g, 59 mmol.) in 100 mL of dry MeOH were allowed to reflux overnight.. Add 60 mL of brine water and then extract with 3 x 50 mL of Et_2O. The oil was then purified by vacuum distillation to afford product as a yellow oil (3.50 g, 39.2% yield). ^{1}H ($CDCl_3$, 600 MHz): δ 7.41 (d, J=1.3, 1H), 6.52 (d, J=2.3, 1H), 3.98 (s, 3H).

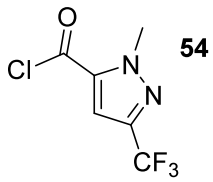
1-Methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxylic acid (53)



53

Add 3.26 mL (23 mmol, 2.3 g) of redistilled dried diisopropylamine to a stirring solution of 14.5 mL (23 mmol) of 1.6 M n-butyl lithium in hexanes with 40.0 mL of dry THF under argon at -78° C. Then add 3.50 g (23 mmol) of pyrazole in to the solution and stir for 2 hours. The solution turns yellowish. Bubble CO_2 into the colored solution (it will turn clear). Continue bubbling for 1 hour, and then remove volume by rotary evaporation. 80 mL of 1 M NaOH was added to the residue and the layer was washed 2 x 50 mL with ether. The aqueous layer acidified with concentrated HCl to pH of 2 and then extracted 4x50 mL with ether. The combined layers were washed with brine and the dried with $MgSO_4$. The organic solvent was removed by rotary evaporation and the remaining solid was recrystallized in EtOAc: Hex solvent system to afford product as white crystals (1.15 grams, 26% yield). This material was used directly in the following reaction.

1-Methyl-3-(trifluoromethyl)-1H-pyrazole-5-carbonyl chloride



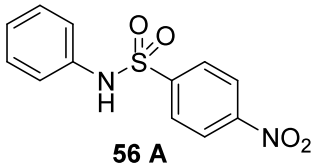
54

Oxalyl chloride (108 mg, 0.8 mmol) and 5 drops of DMF were added to a solution of 150 mg (0.77 mmol) of carboxylic acid in 20 mL of methylene chloride. The solution was stirred for one hour at room temperature. Organic solvents were removed by rotary

evaporation and then under high vacuum for 1 hour. The yellow oil was used directly in the following reaction.

General procedure for synthesis of nitrobenzenesulfonamides. To a stirring solution of 4 nitrobenzenesulfonylchloride in DCM (1 mL/mmol) at 0° C, add 1.1 equivalent of pyridine and stir for ten minutes. Then add 1 equivalent of amine dropwise. The reaction is allowed to warm up to room temperature and stir for 24 hours or until reaction is completed by TLC. Add saturated sodium bicarbonate (1.5 mL/mmol) solution and extract 3 times with 2 mL/mmol of DCM. Wash the organic layer with 1N HCl solution (1.5 mL/mmol). Dry organic layer with $MgSO_4$, and then remove volume by rotary evaporation. Dry in vacuo overnight. No further purification is necessary.

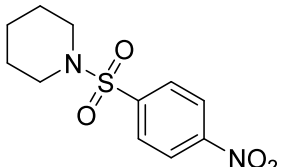
4-Nitro-N-phenylbenzenesulfonamide (56 A)



56 A

A bright red powder was recovered in 67% yield. ^1H ($CDCl_3$, 400 MHz): δ 8.28 (m, 2H), 7.96 (m, 2H), 7.29 (m, 2H), 7.09(dd, *J*=8.6, 1.0, 2H), 6.89 (bs, 1H).

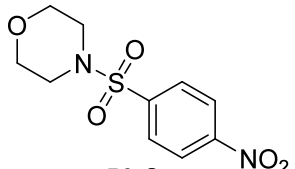
1-(4-Nitrophenylsulfonyl)piperidine (56 B)



56 B

A white powder is isolated in 40% yield. ^{1}H ($CDCl_3$, 600 MHz) δ 8.40 (dd , *J*= 8.5, 1.7, 2H), 7.96 (dd , *J*= 8.5, 1.7, 2H), 3.06 (t, *J*=5.5, 4H), 1.68 (quintet, *J*=5.7, 4H), 1.47 (quintet, *J*=5.8 Hz, 2H) .

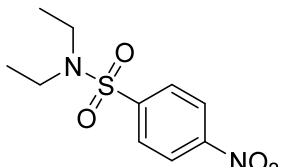
4-(4-Nitrophenylsulfonyl)morpholine (56 C)



56 C

A white solid is isolated in 37% yield. ^{1}H ($CDCl_3$, 400 MHz) δ 8.42 (dd , *J*= 7.2, 2.0, 2H), 7.96 (dd , *J*= 7.2 , 2.0 , 2H), 3.78 (t, *J*=3.2 , 4H), 1.68 (t , *J*=4.6 , 4H).

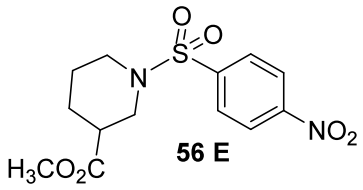
N,N-Diethyl-4-nitrobenzenesulfonamide (56 D)



56 D

A white solid is isolated in 40% yield. ^{1}H ($CDCl_3$, 600 MHz) δ 8.36 (dd, *J*= 7.1, 1.0, 2H), 8.01 (dd , *J*= 7.1 , 1.0 , 2H), 3.30 (q, *J*=7.0 , 4H), 1.17 (t , *J*=6.0 , 6H).

Methyl 1-(4-nitrophenylsulfonyl) piperidine-3-carboxylate (56 E)



56 E

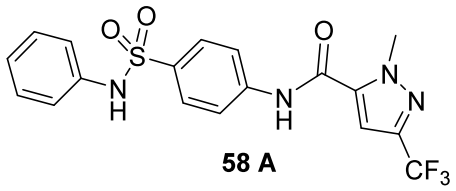
The material is recovered in 43.5% yield. ^{1}H ($CDCl_3$, 400 MHz) δ 8.39 (dd, *J*= 6.8 , 2.0, 2H), 7.95 (dd , *J*= 6.8 , 2.0, 2H), 3.67 (m, 4H), 2.60 (m, 2H), 2.33 (m, 1H) 2.02 (m, 2H), 1.85 (m, 2H) 1.56 (m , 1H).

General procedure for anilinobenzenesulfonamides. The procedure is followed according to reference.[xl] In a solution nitrobenzenesulfonamide in 2 mL/mmol of EtOAc is added 5 equivalents of $SnCl_2$ $(H_2O)_2$. The reaction is then heated to reflux for 3 hours or until reaction is completed by TLC. Reaction is then cooled down, and 2 mL/mmol of saturated sodium bicarbonate is added. The mixture is extracted with 3x3 mL/mmol of EtOAc and then washed with brine. The organic layer is dried with $MgSO_4$, and solvent is removed using rotary evaporation. The product is dried under high vacuum for 24 hours. The resulting amine is used in the following reaction without purification.

General procedure for amide bond coupling. Stir a solution of acid chloride and 1.1 equivalents of pyridine with 0.1 equivalents DMAP at 0° C. Add the amine in solution dropwise to the solution of the acid chloride. Stir for 24 hours or until the reaction is complete by TLC. Add 2 mL/mmol of saturated sodium bicarbonate solution and extract with 3x4 mL/mmol of EtOAc. Dry with $MgSO_4$, filter and remove volume by rotary

evaporation. Dry en vacuo overnight, then purify via column chromatography with 1:1 EtOAc: Hex solvent system.

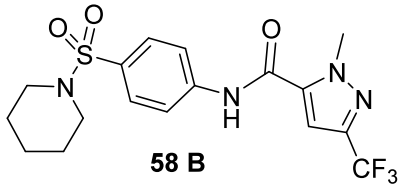
N-((4-(Anilin-yl)-sulfonyl)phen-1-yl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (58 A)



58 A

Reddish Solid is isolated in 88% yield. mp 209-211° C. ^{1}H (*d6* DMSO, 400 MHz) δ 10.69 (s, 1H), 10.22 (s, 1H), 7.86 (d, *J*=9.0, 2H), 7.75 (d, *J*=9.0, 2H), 7.50 (s, 1H), 7.21 (m, 2H), 7.09 (d, *J*=7.8, 2H), 7.07 (t, *J*=7.0, 1H), 4.14 (s, 3H).

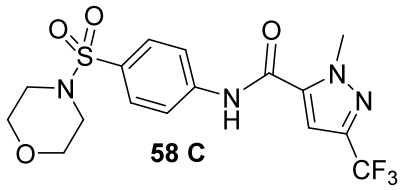
N-((4-Piperidin-1-ylsulfonyl)phen-1-yl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (58 B)



58 B

A white solid is isolated in 85% yield. Mp 232-234° C ^{1}H (*d6* DMSO, 400 MHz) : δ 10.76 (s, 1H) 7.99 (d, *J*=8.6 , 2H), 7.75 (d, *J*=8.6, 2H), 7.55 (s, 1H), 4.17 (s, 3H), 2.86 (m, 4H), 1.53 (m, 4H), 1.33 (m, 2H) ^{13}C ($CDCl_3$, 150 MHz): δ 157.27, 141.09, 136.28, 132.27, 129.13, 120.39, 105.67, 47.16, 40.64, 25.23, 23.64. Anal. Calcd. For $C_{17}H_{19}F_3N_4O_3S$: C, 49.03; H, 4.60; N, 13.45, F, 13.69. Found C, 49.14; H, 4.61; N, 13.45; F 13.69.

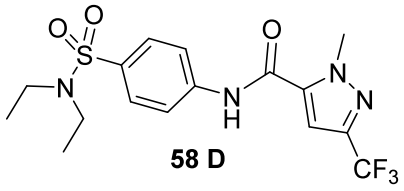
N-((4-(Morpholin-4-yl)-sulfonyl)phen-1-yl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (58 C)



58 C

A white solid is isolated in 92% yield. mp 244-246° C. ^{1}H (CD_3OD, 400 MHz) : δ 8.00 (d, *J*=9.0, 2H), 7.78 (d, *J*=9, 2H), 7.34 (s, 1H), 4.23 (s, 3H), 3.71 (t, *J*=5.7, 4H), 2.94 (t, *J*=4.7, 4H)) ^{13}C (Acetone d_6, 150 MHz): δ 158.38, 143.62, 137.85, 131.37, 130.01, 120.93, 107.14, 104.10, 69.27, 66.67, 47.15.

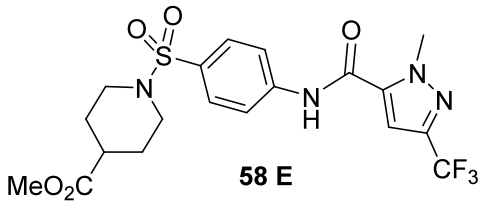
N-((4-(Diethyl-amin-yl)-sulfonyl)phen-1-yl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (58 D)



58 D

A white solid is isolated in 63% yield. mp 169-170° C. ^{1}H ($CDCl_3$, 600 MHz) : δ 7.93 (d, *J*=8.7, 2H), 7.81 (d, *J*=8.7, 2H), 7.35 (s, 1H), 4.23 (s, 3H), 3.24 (q, *J*=7.5, 4H), 1.13 (t, *J*=6.0, 6H).) ^{13}C ($CDCl_3$, 150 MHz): δ 157.38, 141.04, 141.41 (q, *J*=39.4 Hz) , 136.35, 136.01, 128.28, 120.91 (d, *J*=270.0 Hz), 120.68, 105.95, 42.26, 40.50, 14.24. Anal. Calcd for $C_{16}H_{19}F_3N_4O_3S$: C, 47.52; H, 4.74; N, 13.85. Found: C, 47.58; H, 4.81; N, 13.85.

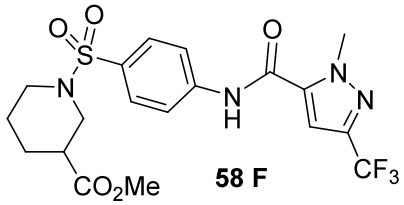
N-((4-(4-Methoxycarbonyl-piperidin)-1-ylsulfonyl) phen-1-yl)-1-methyl-3-(trifluoro-methyl) -1H-pyrazole-5-carboxamide (58 E)



58 E

White solid isolated in 83 % yield. mp 171-172° C ^{1}H ($CDCl_3$, 300 MHz) : δ 8.08 (s, 1H), 7.78 (m, 4H), 7.04 (s, 1H), 4.28 (s, 3H), 3.68 (m, 4H), 2.57 (m, 2H), 2.37 (m, 1H), 1.98 (m, 1H), 1.81 (m, 1H), 1.69 (m, 1H) 1.40 (m, 1H) ^{13}C ($CDCl_3$, 150 MHz): δ 174.50, 157.31, 141.49, 140.90 (q, *J*=39 Hz), 136.24, 131.77, 129.03, 120.86 (d, *J*=268 Hz), 105.82, 52.25, 45.57, 40.64, 39.95, 27.50. Anal. Calcd for $C_{19}H_{21}F_3N_4O_5S$: C, 48.10; H, 4.46; N, 11.81 Found: C, 48.06; H, 4.48; N, 11.64.

N-((4-(3-Methoxycarbonyl-piperidin)-1-ylsulfonyl) phen-1-yl)-1-methyl-3-(trifluoro-methyl)-1H-pyrazole-5-carboxamide (58 F)



58 F

White foam isolated in 65 % yield. ^{1}H ($CDCl_3$, 300 MHz) : δ 7.97 (s, 1H), 7.77 (m, 4H), 7.02 (s, 1H), 4.29 (s, 3H), 3.68 (s, 3H), 3.60 (m, 1H), 2.54 (m, 2H), 2.30 (s, 1H), 1.97 (m, 3H), 1.85 (m, 2H)) ^{13}C ($CDCl_3$, 150 MHz): δ 173.46, 157.41, 141.67, 140.93 (q, *J*=38.1 Hz), 136.32, 131.68, 129.02, 120.55, 120.93 (d, *J*=268 Hz), 105.94, 53.31, 47.84, 46.51, 45.59, 40.65, 27.53, 26.51, 24.09. Anal. Calcd for $C_{19}H_{21}F_3N_4O_5S$: C, 48.10; H, 4.46; N, 11.81. Found: C, 48.12; H, 4.36; N, 11.52.

4. References and Notes

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