Synthesis of Allosteric Modulators for Nicotinic Acetylcholine Receptors

Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation
"with Honors Research Distinction" in the undergraduate
colleges of The Ohio State University

by
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June 2011

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Abstract

Nicotinic acetylcholine receptors (nAChR) can be found throughout the human nervous system. The receptors regulate a multitude of functions, including development, inflammation, and movement. They also serve as the receptor site for nicotine, an extremely addictive drug. Novel therapeutic strategies for breaking this addiction involve synthesis of negative allosteric modulators that could deactivate the binding site for nicotine on these receptors. However, because many subtypes of the nAChR exist, it is difficult to target one without affecting others. This study aims to synthesize a series of analogs of compound **16**, an arylsulfonyl piperazine-containing compound that was previously shown to display selectivity for the Hα4β2 nAChR compared to the Hα3β4 nAChR receptor subtype.[4] Synthetic pathways are focused on amide bond formation between substituted arylsulfonyl piperazines and aryl amines. Nine derivatives of **16** have been synthesized. Results confirm the identity of these compounds by ^{1}H, ^{13}C, *m/z*, and elemental analyses. Biological testing of these compounds has, thus far, shown that the analogs retain the potency of **16** for the Hα4β2 nAChR, but have lost selectivity for that receptor subtype. Future work will focus on exploring different hypotheses regarding the basis of **16** receptor subtype selectivity through the synthesis and evaluation of additional analogs.

Acknowledgements

First and foremost, I would like to thank my research advisor, Dr. Karl Werbovetz, for giving me the opportunity to do research in his lab for the past few years. His guidance and knowledge has allowed me to develop my skills in organic chemistry and my ability for critical thought in the field of medicinal chemistry.

I would also like to thank Mr. Julian Richard for his assistance at the start of the project and with research in general. His explanations of organic chemistry lab techniques and reactions have been invaluable in the completion of this project. I'd like to thank all of the other members of the Werbovetz lab, including Dr. Sihui Long, Dr. Xiohua Zhu, Mona Desai, Trupti Pandharkar, and Shanshan He, for creating an atmosphere conducive to research and a fun learning environment.

I would like to thank Bitna Yi, Dr. Tatiana Gonzalez-Cestari, and Dr. Brandon Henderson for their assistance in testing the biological activity of my compounds in their system. I would also like to thank Ryan Pavlovicz for his computational structures.

I would like to thank Dr. Christopher Callam, for sparking my initial interest in organic chemistry and for organic lab research. Through his courses, I learned that organic chemistry can be an enjoyable and educational experience.

Last, but certainly not least, I would like to thank my family, who always supported what I was doing. Even though they didn't understand what I was doing, they were always happy to listen to me ramble on about my research. They gave me the opportunity to even attend The Ohio State University in the first place and for that I am forever grateful.

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1. Introduction

The nicotinic acetylcholine receptor (nAChR) is a ligand gated ion channel found in locations throughout the peripheral and central nervous systems. When binding occurs with the endogenous ligand or agonistic molecules, these ion channels open to allow movement of cations such as Ca^{2+} and Na^{+}. Nicotinic acetylcholine receptors are responsible for modulation of the release of several neurotransmitters and have been implicated in neurological diseases such as Parkinson's, Alzheimer's, and schizophrenia.[5] As the name suggests, the receptor is activated by nicotine, an active ingredient in the tobacco plant. The receptor was first isolated from the Pacific electric ray in 1970 and visualized by electron microscopy of crystallized receptors in the late 1980s.[3]

The structure of the receptors includes five individual α and β monomers that combine in varying ratios to form different receptor subtypes. Several different α/β monomers can combine to form even more subtypes. It is between these α and β subunits, or two α subunits depending on receptor, that the binding site for acetylcholine and receptor agonists is found. In the brain, two of the primary receptor subtypes are Hα3β4 (human α3β4) and Hα4β2 (human α4β2). The Hα4β2 receptor subtype is generally accepted to be involved in nicotine addiction.[1] The Hα3β4 receptor, however, has been associated with the undesired side-effects of nAChR antagonists.[6] The high level of homology at the orthosteric site of the receptors makes it difficult to target a single subtype with agonistic or antagonistic drugs.

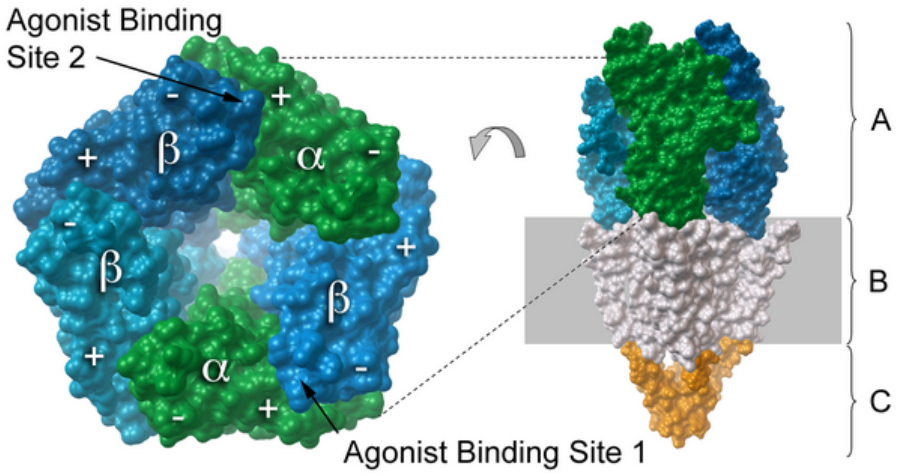
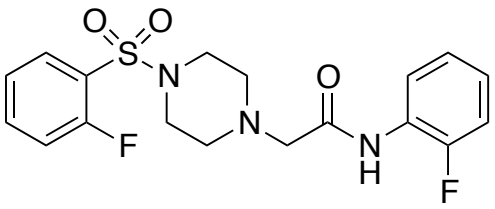


Figure 1.1. The nicotinic acetylcholine receptor is made up of both α and β subunits with binding sites at the α/β interface. The receptor contains an extracellular domain (A), a transmembrane domain (B), and an intracellular domain (C). Image reproduced from [6].

More recent efforts in the field have instead focused on negative allosteric modulators to treat nicotine addiction. These molecules do not act at the binding site of the endogenous ligand, but rather bind to a site nearby, changing the conformation of the receptor and reducing its activity. This approach allows for exploitation of minor differences between the α/β subunits that make up each receptor. Selective modulation of the receptors through the allosteric site provides the ultimate goal of the ability to treat nicotine addiction without any unwanted side-effects. At the moment, only four non-nicotine containing drugs are FDA approved and regularly used to treat nicotine addiction. With nicotine addiction affecting millions worldwide, it is clear that finding alternative treatment options is of great importance.

Recent identification of a negative allosteric site on the Hα3β4 and Hα4β2 receptors has provided a new target for drug discovery. A computational model of the nAChR subtypes has been developed based on homologous structures found in molluskan species.[6] Using this model in collaboration with the several other labs, molecules that may negatively modulate the nAChR have been synthesized and identified.

Previous work has shown that the arylsulfonyl piperazine core could act as a promising scaffold in the synthesis of a nAChR antagonist. Using the computer model of the nAChR, possible molecular interactions between the drug and receptor can be identified. Upon identification, molecules can be synthesized and manipulated to optimize the binding energy of the drug to the receptor. Synthesis and subsequent *in vitro* studies of a particular arylsulfonyl piperazine, compound **16**, have shown that the molecule has inhibitory activity as well as selectivity for the Hα4β2 receptor subtype.[2] Some researchers have suggested that the ortho substitution on the arylsulfonyl ring is involved in steric interactions that force the molecule into a particular conformation that is favorable in docking with the allosteric site of the Hα4β2 receptor. Published data only explore the activity of a single ortho substituted arylsulfonyl piperazine. Using this molecule as a lead, analogs have been synthesized to study the possibility of using alternatively substituted arylsulfonyl piperazines as potential targets for selective inhibition of the nAChR.



	Hα4β2	Hα3β4
IC_{50} Value (μM)	8.0 (5.4-11.8)	99.8 (57.2-174)

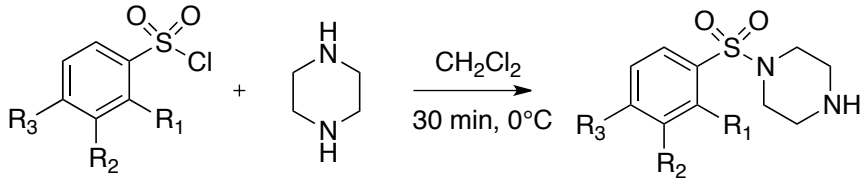
Figure 1.2. Compound **16** contains the arylsulfonyl piperazine pharmacophore that forms the basis of all compounds synthesized in this study. The compound shows both biological activity and selectivity.

2 Synthetic Methods and Results

2.1 Nucleophilic Acyl Substitution. The sulfonyl piperazine building block present in the target compound was synthesized from the corresponding sulfonyl chlorides and piperazine (Scheme 2.1). An excess of piperazine was used to drive the reaction toward the desired product. It also acted as the additional base necessary in the reaction. The reaction is a favorable one to use in this synthesis considering the short time and mild conditions required for the reaction.

For this reaction, piperazine (6 eq) in dichloromethane was cooled to 0°C, then the sulfonyl chloride (1 eq) was added. The resulting mixture was reacted at 0°C for 30 min to produce the desired sulfonyl piperazine. The reaction was monitored by TLC to ensure completion. Following completion, the reaction was diluted with additional dichloromethane. The organic solution was then quenched with aqueous sodium bicarbonate, washed with brine, and dried with sodium sulfate. The resulting solution was concentrated *in vacuo* to provide the crude product, which was then dissolved in minimal dichloromethane and the pure product precipitated by the addition of hexanes. The purity of the compounds was confirmed by TLC and NMR analyses. Using this procedure, the desired product was obtained in moderate to high yields (49%-86%, Table 2.1).

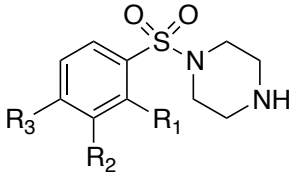
Precipitation was not a viable option to obtain desired products **2.2b**, **2.2c**, and **2.2e**. These materials were initially obtained as oils and were allowed to stand at rt for ~24 hr, allowing crystallization to occur. The purity of these compounds was confirmed by TLC and NMR analyses. Compound **2.2d** would neither precipitate upon the addition of hexane nor crystallize upon standing at rt or at -4°C and had to be isolated as the hydrochloride salt. To accomplish this, the crude oil was dissolved in ethanol and ethanolic-HCl was added until the solution was acidic. The solution was then concentrated *in vacuo* and the desired salt was isolated by filtration to provide a moderate yield.



2.1a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
2.1b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
2.1c: $R_1 = Br$ $R_2 = H$ $R_3 = H$
2.1d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$
2.1e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$
2.1f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$
2.1g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$

2.2a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
2.2b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
2.2c: $R_1 = Br$ $R_2 = H$ $R_3 = H$
2.2d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$
2.2e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$
2.2f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$
2.2g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$

Scheme 2.1. Nucleophilic acyl substitutions using sulfonyl chlorides and piperazine.



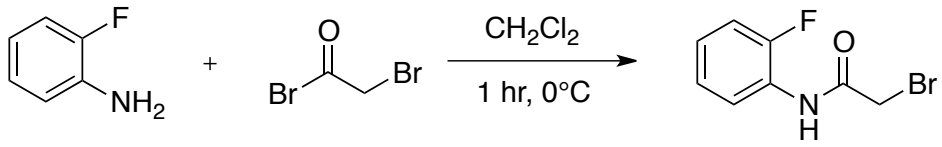
Compound	Yield
2.2a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$	76%
2.2b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$	67%
2.2c: $R_1 = Br$ $R_2 = H$ $R_3 = H$	66%
2.2d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$	49%
2.2e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$	72%
2.2f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$	86%
2.2g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$	80%

Table 2.1. Isolated yields of nucleophilic acyl substitution reactions.

2.2 Fluoroaniline acyl substitution reaction. The second molecule necessary in synthesizing the target compound was made from 2-fluoroaniline and bromoacetyl bromide (Scheme 2.2). This building block remained constant for each compound synthesized in this study. To reduce the likelihood of a second substitution at the methylene carbon, the stoichiometry is controlled and the reaction is performed at a low temperature.

2-Fluoroaniline (1 eq) was initially mixed with TEA in dichloromethane and cooled to 0°C. To this solution, bromoacetyl bromide (1 eq) was added dropwise over 5 min. The resulting solution was reacted at 0°C for 1 hr. The progress of the reaction was monitored by TLC to ensure completion. Upon completion, the reaction was diluted with additional dichloromethane, washed with NH_4Cl, and dried with magnesium sulfate. The resulting solution was then concentrated *in vacuo* to produce the crude product, which was then dissolved in minimal dichloromethane and the pure product precipitated by the addition of hexanes. The purity of the compound was determined by TLC and NMR analyses. With this procedure, the desired product was obtained in moderate yield (62%).

Initial attempts to recrystallize the product were mostly unsuccessful and resulted in poor isolated yield. Precipitation from DCM/hexanes resulted in a much higher yield of the product without sacrificing purity. In some cases, following dissolution in minimal DCM, hexanes were added in a higher proportion than DCM. The similar boiling points of the compounds allowed DCM to evaporate along with hexanes, but hexanes, being in greater quantity, would be left, forcing the product out of solution.



2.3

62%

Scheme 2.2. Nucleophilic acyl substitution between fluoroaniline and bromoacetyl bromide and the corresponding yield.

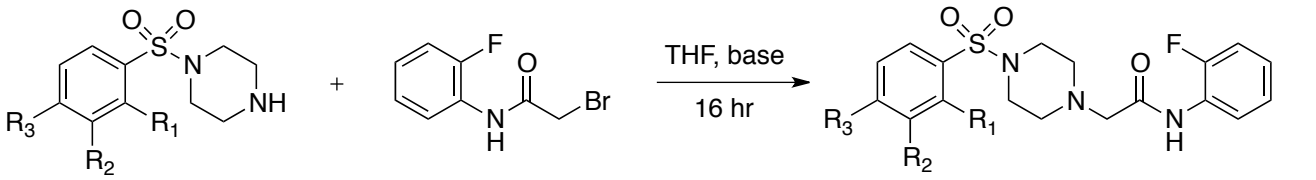
2.3 Bimolecular Nucleophilic Substitution. The target molecules were synthesized through bimolecular substitution reactions between the secondary amine in compounds **2.2a-g** and the

bromoacetyl group in **2.3** (Scheme 2.3). This reaction allows for the coupling of the two previously synthesized molecules and provides the amide bond connection formed in a prior reaction. The reaction was done under an inert atmosphere, N_2, to prevent any possibilities of oxidation or unwanted side-products.

Compounds **2.2a-g** (1 eq) were first combined with compound **2.3** (1 eq) in THF at room temperature. To this mixture, triethylamine (TEA) or Na_2CO_3 (2 eq) was added and the resulting mixture was reacted at room temperature overnight. The progress of the reaction was monitored by TLC to ensure completion. Upon completion, the reaction was diluted with DCM, filtered if necessary, then concentrated *in vacuo* to produce the crude product, which was dissolved in minimal DCM. The compound was then applied to a silica column and eluted (hexanes/EtOAc) as the pure compound. Following elution, the fractions were concentrated *in vacuo* and the resulting solid was recrystallized in EtOAc to yield the pure target compound. The purity of the compound was determined by TLC, NMR, and MS analyses. Using this procedure, the desired product was obtained in moderate to high yield (41%-75%, Table 2.2).

In most cases, it was determined that TEA was the more convenient base to use in the reaction, as it did not require filtering following dilution with DCM. While most compounds were effectively crystallized in EtOAc, some were crystallized in a mix of EtOAc with several drops of hexanes to reduce the polarity of the crystallization solvent. This was particularly useful in crystallization of compound **2.4e**.

Before biological testing, all compounds were converted to the hydrochloride salt. To do this, the compounds were dissolved in ethanol and ethanolic-HCl was added until the solution was acidic. The solution was then concentrated *in vacuo* and the desired salt was isolated by filtration to provide moderate to high yields.

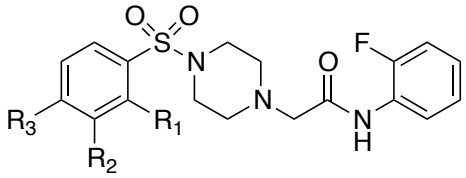


2.2a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
2.2b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
2.2c: $R_1 = Br$ $R_2 = H$ $R_3 = H$
2.2d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$
2.2e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$
2.2f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$
2.2g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$

2.3

2.4a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
2.4b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
2.4c: $R_1 = Br$ $R_2 = H$ $R_3 = H$
2.4d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$
2.4e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$
2.4f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$
2.4g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$

Scheme 2.3. Bimolecular substitution reaction between the secondary amine of the sulfonyl piperazines and the bromofluorophenylacetamide.



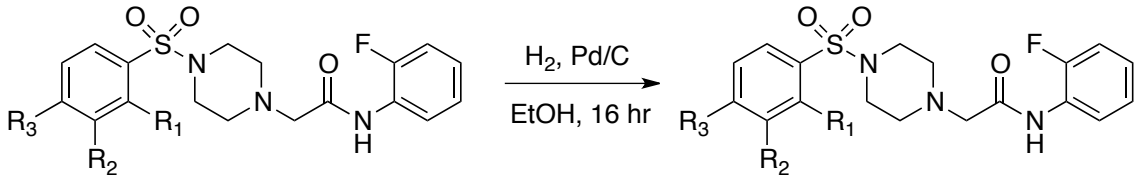
Compound	Yield
2.4a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$	73%
2.4b: $R_1 = CH_3$ $R_2 = H$ $R_3 = H$	41%
2.4c: $R_1 = Br$ $R_2 = H$ $R_3 = H$	75%
2.4d: $R_1 = OCH_3$ $R_2 = H$ $R_3 = OCH_3$	62%
2.4e: $R_1 = R_2 = -(CH)_4-$ $R_3 = H$	75%
2.4f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$	62%
2.4g: $R_1 = H$ $R_2 = H$ $R_3 = NO_2$	69%

Table 2.2. Isolated yields of bimolecular substitution reactions.

2.4 Reduction Reactions. Using synthesized nitroaromatic compounds as precursors, two molecules containing hydrogen bond donord were synthesized via catalytic hydrogenation reactions conducted on **2.4a** and **2.4f** (Scheme 2.4). Ethanol was the solvent of choice as its low vapor pressure prevented combustion of Pd/C upon addition to the flask.

Compounds **2.4a** and **2.4f** (1 eq) were first dissolved in EtOH at room temperature. To this solution, 10% Pd/C (10% w/w) was slowly added. The reaction flask was evacuated of air and placed under 20 psi H_2 overnight. Progress of the reaction was measured by TLC to ensure completion. Upon completion, the mixture was filtered through celite and concentrated *in vacuo*. When necessary, the resulting product was purified by column chromatography and recrystallization. With this procedure, the desired product was obtained in low to moderate yield (Table 2.3). Before biological testing, **2.5a** and **2.5b** were converted to the hydrochloride salts in the same manner as **2.4a-g**.

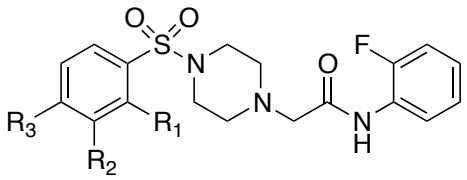
Initial attempts at hydrogenation were unsuccessful. Hydrogenation for a shorter time at high and low pressures resulted in incomplete reactions. It was ultimately determined that hydrogenation at a lower pressure overnight provided the best results. Purification also proved to greatly reduce yields. Compound **2.5b** was isolated in poor yield (17%), likely due to the purification procedures used. In support of this hypothesis, **2.5a** was synthesized using the same conditions and was obtained in 58% yield, but no additional purification was necessary after filtering through celite.



2.4a: $R_1 = NO_2$ $R_2 = H$ $R_3 = H$
2.4f: $R_1 = H$ $R_2 = NO_2$ $R_3 = H$

2.5a: $R_1 = NH_2$ $R_2 = H$ $R_3 = H$
2.5b: $R_1 = H$ $R_2 = NH_2$ $R_3 = H$

Scheme 2.4. Catalytic hydrogenation of nitro-substituted arylsulfonyl piperazines to synthesize amine-substituted molecules.



Compound	Yield
2.5a: $R_1 = NH_2$ $R_2 = H$ $R_3 = H$	58%
2.5b: $R_1 = H$ $R_2 = NH_2$ $R_3 = H$	17%

Table 2.3. Isolated yields of amine-substituted arylsulfonyl piperazines.

3 Biological Methods and Results

3.1 Biological Assays. Following synthesis, the target molecules were converted to hydrochloride salts and tested *in vitro* by the McKay Lab. The purpose of converting the compounds to the salt was to increase solubility in the aqueous environment of the biological assays.

The McKay lab has developed an assay using HEK cells that selectively express the α4β2 and the α3β4 nAChR subtypes.[2] The cells in were treated with Fluo-4-AM, which acts as a fluorescent indicator for calcium, thus allowing for calcium flow to be measured. The synthesized molecules are indirect modulators and must be compared to a baseline measurement. For this reason, epibatidine is used as an orthosteric agonist to induce calcium influx. Epibatidine treated cells were compared to cells treated with both epibatidine and the synthesized molecules to determine an IC_{50}. These values were used to determine the activity and relative selectivity of each compound (Table 3.1).

	Hα4β2[t]	Hα3β4[t]
Name	IC_{50} (μM)	IC_{50} (μM)
16 (Lead)	8.0 (5.4-11.8)	99.8 (57.2-174)
2.4a	9.3 (7.2-12.11)	11.5 (7.5-17.5)
2.4b	5.6 (4.2-7.4)	5.7 (4.0-7.9)
2.4c	6.2 (4.6-8.2)	6.6 (4.4-9.7)
2.4d	13.6	9.3 (2.7-32.5)
2.4e	8.1 (5.4-12.2)	5.8 (5.3-6.3)
2.4f	26.2 (20.4-33.7)	21.8 (19.5-24.4)
2.4g	>100	>100
2.5a	14.5 (8.9-23.8)	23.3 (13.3-40.7)
2.5b	10.9	12

[t] Values represent geometric means (confidence limits)

Table 3.1. IC_{50} values for the synthesized compounds. The IC_{50} value is defined as the concentration of compound at which 50% of the nAChR activity is inhibited.

4 Discussion of Activity

4.1 Structure at the Docking Site. It is important to first note that this study is based on a theoretical structure of the α4β2 and α3β4 nAChR subtypes. This crystal structure of the receptors are known in the pacific ray, which provided a foundation for the computationally determined human model. It may or may not represent the actual allosteric site, but for the purposes of this study, it has been assumed that this structure is accurate.

The allosteric site for both receptor subtypes was initially determined through blind docking techniques to be at an alpha-beta interface within the receptor. This means that the alpha subunit contributes the primary portion of the docking site, whereas the beta subunit contributes the complementary interface. Differences in structure at this particular site were modeled for both the α4β2 and α3β4 receptor subtypes (Figure 4.1). Mutation studies done by the McKay lab, however, showed that there was not a change in the binding of arylsulfonyl piperazines at this site, suggesting that the initially determined docking site was incorrect.

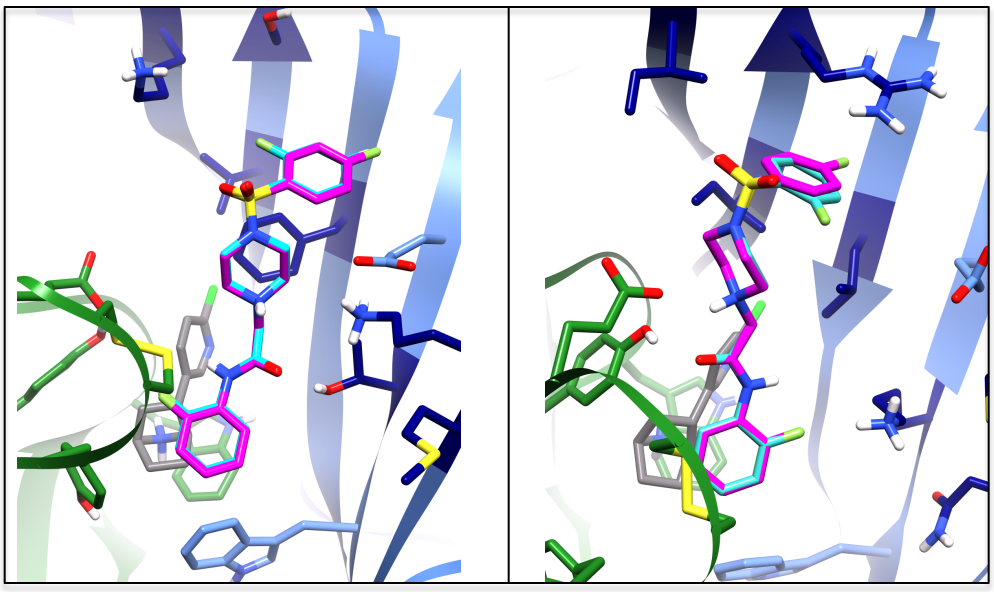


Figure 4.1. The allosteric site was initially believed to be at this alpha-beta interface in the α4β2 (left) and the α3β4 (right) receptors. Compound **16** (cyan) can be seen compared to its para-substituted counterpart (magenta) within this docking site. Image reproduced with permission from Ryan Pavolicz.

New blind docking studies done by Ryan Pavlovicz identify a new docking site, this time at a beta-alpha interface (Figure 4.2). This site provides new insights as to the possible mode of binding for the synthesized compounds. Possible areas of importance within the binding site include amino acids that lack conservation between the subtypes, allowing for selectivity in intermolecular interactions to occur. Using this model, possible interactions between the synthesized compound and receptor have been analyzed.

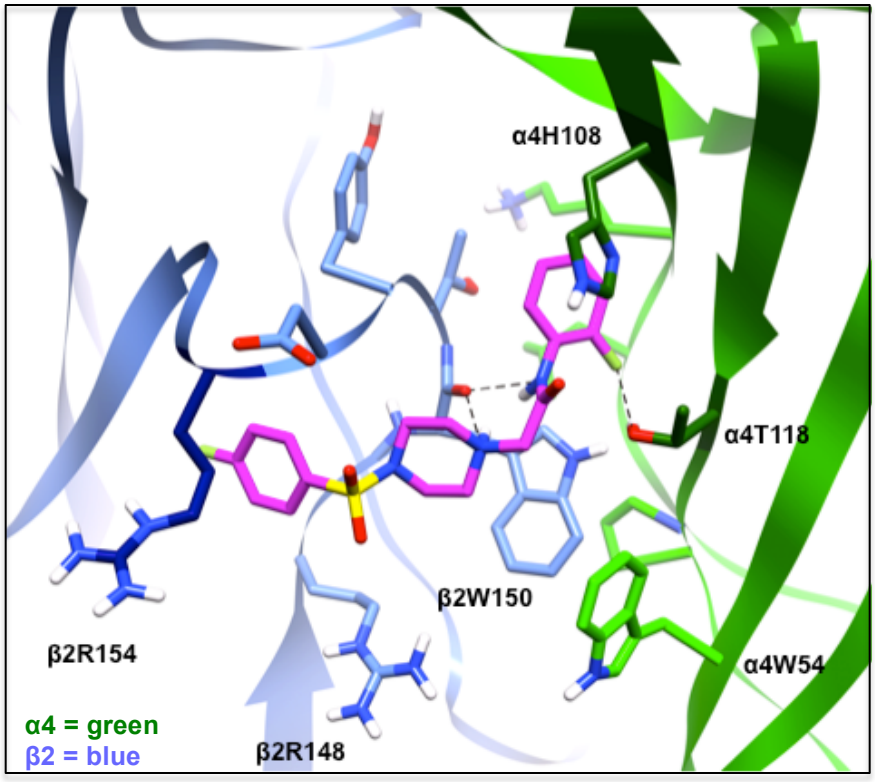


Figure 4.2. The new allosteric site is at this beta-alpha interface in the nAChR subtypes. Nonconserved amino acids are labeled. A para-substituted arylsulfonyl piperazine is shown (magenta) in the α4β2 receptor. Image reproduced with permission from Ryan Pavolicz.

4.2 Molecular Binding Interactions. The initial goal of this study was to analyze the effects of placing various ortho-substituents on the arylsulfonyl ring. However, it is worthwhile to analyze all possible interactions between the ligand and the receptor (Figure 4.3). Compound **16**, an ortho-fluoro arylsulfonyl piperazine, showed high selectivity for α4β2 over α3β4. It has been previously theorized that this may be due to a change in conformation due to the sterics between the *o*-fluorine and the sulfonyl group. If this is indeed the case, then larger steric interactions may provide similar results. Nitro substitution at the ortho (**2.4a**), meta (**2.4f**), and para (**2.4g**) positions show that the position on the

ring is important, as a drop in activity is noted for the meta-substitution and a lack of activity is noted for the para-substitution.

The electron withdrawing properties of fluorine in **16** seem to be relatively unimportant, as the more electron withdrawing nitro substituent does not convey any additional activity. The methyl-substituent in **2.4b**, a mildly electron donating group, actually seems to increase activity in the α4β2 receptor with respect to **16**. However, all selectivity is lost. Compound **2.4c** possesses approximately the same activity as **16**, but selectivity is lost. Considering these observations, there does not seem to be a clear trend that deems importance for the electronic properties of the ortho-substitution.

The size of the fluorine atom may be important. In the docking site, a nearby aspartic acid residue provides the possibility for polar interactions, but also steric clashes. Ortho and meta substitution may be in the appropriate positions to form polar interactions with this side chain, whereas the para position cannot. The ortho position also provides the additional interactions with the sulfonyl group, perhaps partially explaining the greater activity in ortho-substitution. To explain the selectivity though, amino acids unique to the α4β2 should be noted. The flexibility and length of side chains in arginine154 and 148 may allow them to take multiple configurations that could be involved in some steric clashes with larger substituents. It is possible that fluorine is small enough to avoid these steric clashes while still allowing for polar or even hydrogen bonding interactions with the side chains. Some kind of steric interactions seem to play a role here, as having no substituents on the ring results in high activity, but no selectivity.

Testing was also done to determine the role of hydrogen bonding interactions at the arylsulfonyl ring. Two molecules, **2.5a** and **2.5b** contained amines at varying positions. These amines were able to act as hydrogen bond donors and should have conveyed additional activity if there were important hydrogen bond acceptors in the area. If these hydrogen bond acceptors were unique to one receptor over

the other, it would be expected that selectivity would also be observed. The IC_{50} values of these compounds did not show an increase in activity nor selectivity. It can therefore be assumed that hydrogen bonding does not play an important role in this part of the molecule.

Away from the arylsulfonyl ring, there are a few other properties that seem to be important for binding. The tertiary amine (not the sulfonamide) has a pK_a of about 10, meaning that it will be protonated at biological pH. This protonated amine can act as a hydrogen bond donor for a nearby carbonyl in the protein chain. Also interacting with this carbonyl is the amide, donating a second hydrogen bond. It is important to realize that these interactions will contribute only to activity and not selectivity because the hydrogen bond acceptor here is common to both receptor subtypes. The fluoro-substituent on the amide aromatic ring may be involved in halogen or hydrogen bonding with a nearby threonine residue unique to the α4β2 receptor. This, however, would seem to contribute little to overall selectivity considering that fluorine has relatively weak halogen bonding properties and acts as a poor hydrogen bond acceptor when it is an aromatic substituent. While they do not explicitly seem to act as such, the sulfonyl and carbonyl can both act as hydrogen bond acceptors, perhaps adding to the activity at both subtypes.

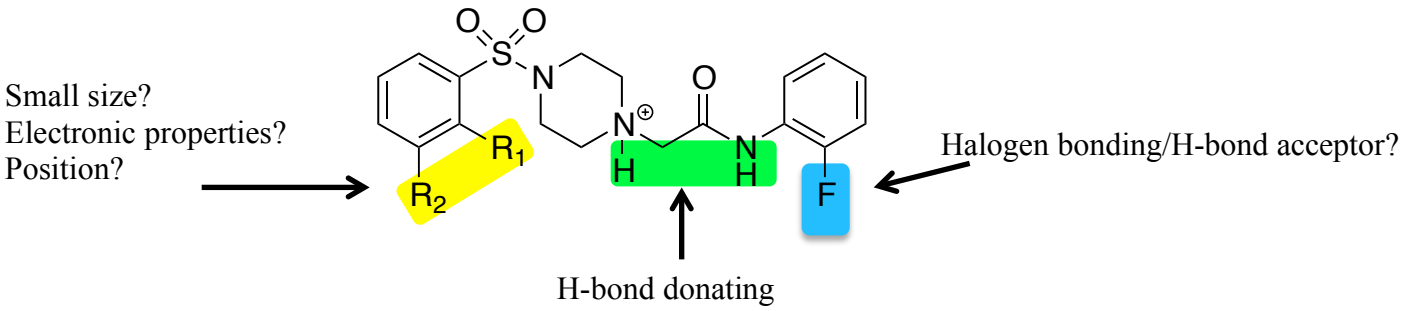
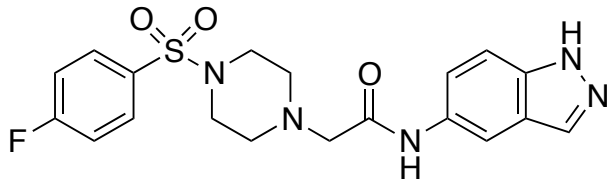


Figure 4.3. The activity of the molecule appears to be most dependent on the highlighted areas. The selectivity however, appears to be limited to the substitution in the yellow region.

4.2 Future Directions. Studies based on derivatives of compound **16** do not appear to provide any promising data with which to continue research. A lack of clear trends in the data as well as uncertainty in the actual structure of the allosteric site, however, prevents easy determination of alternative synthetic analogs. A previously synthesized molecule, compound **11**, has both biological activity and selectivity for the α4β2 receptor (Figure 4.4). Unique to this compound is the presence of an indazole ring system in place of the flurophenylamide found in **16**. Also worth noting in this particular compound is the presence of a p-fluoro substituent on the arylsulfonyl ring.



	Hα4β2	Hα3β4
IC_{50} Value (μM)	6.6 (3.8-11.3)	23.8 (13.1-43.3)

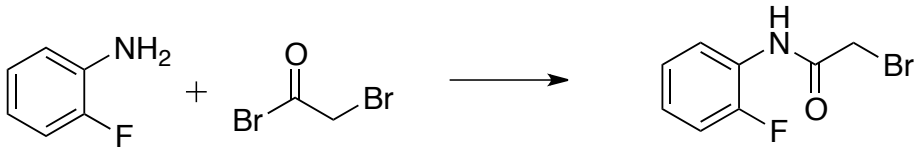
Figure 4.4. Compound **11** shows both biological activity and selectivity for the α4β2 subtype. Unique to this compound is the presence of the indazole ring system.

Compounds based on **16** have shown that the para-substitution has the least activity out of the three positions on the ring. Compounds **2.4g**, a p-nitroaromatic compound, did not show any activity. This was very different in the meta and particularly ortho-substituted compounds **2.4f** and **2.4a**, respectively, which showed a much greater activity. It has therefore been concluded that the activity and selectivity in this molecule may be mostly due to the presence of the indazole.

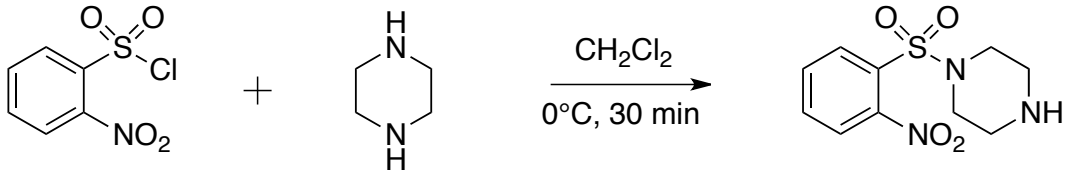
Current and future efforts will focus on synthesizing ortho-substituted arylsulfonyl piperazines containing this indazole ring system, particularly an o-fluoro substituted compound. By combining what

is known about activity and selectivity in these molecules, additional derivatives can be synthesized in hopes of creating a new molecules that is both highly active and highly selective.

5 Experimental Procedures

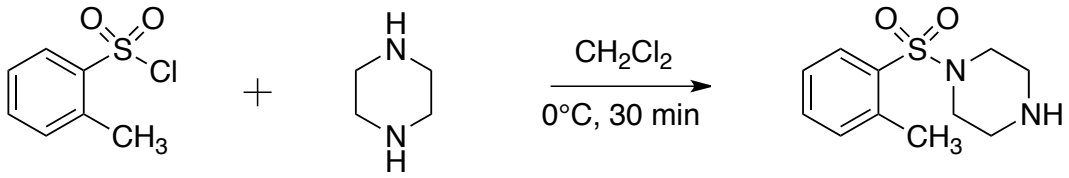


2-bromo-*N*-(2-fluorophenyl)acetamide (2.3): A solution of 2-fluoroaniline (0.50 g, 4.50 mmol, 1 eq) and triethylamine (0.50 g, 4.95 mmol, 1.1 eq) in DCM (25 mL) was prepared in a 100 mL round bottom flask and cooled to 0° C. Bromoacetyl bromide (0.908 g, 4.50 mmol, 1 eq) was added dropwise over 5 min to the reaction mixture and was stirred at 0° C for 1 hr. Upon completion, the reaction mixture was diluted with DCM (25 mL), washed with 10% NH_4Cl (3 x 25 mL), dried with $MgSO_4$, and concentrated *in vacuo* to provide crude product. The crude product was purified by precipitation (DCM/Hexanes) to give **2.3** as a light pink solid (0.65 g, 2.80 mmol, 62%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.40 (s, 1H), 8.25-8.31 (m, 1H), 7.11-7.21 (m, 3H), 4.07 (s, 2H).

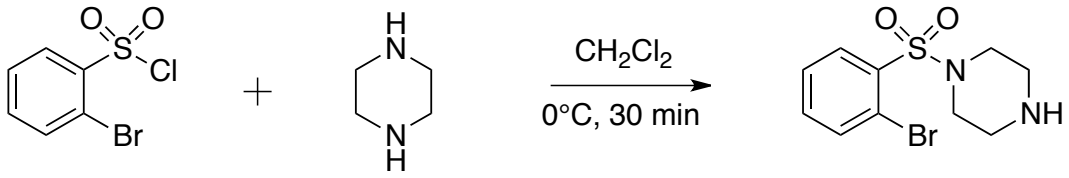


1-((2-nitrophenyl)sulfonyl)piperazine (2.2a): A solution of piperazine (1.63 g, 18.92 mmol, 6 eq) dissolved in DCM (50 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1a** (0.70 g, 3.16 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (50 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (50 mL), washed with brine (50 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield the crude product. The crude product was dissolved in minimal DCM and precipitated by the addition of hexanes to provide **2.2a** as a light yellow solid (0.65 g, 2.40 mmol,

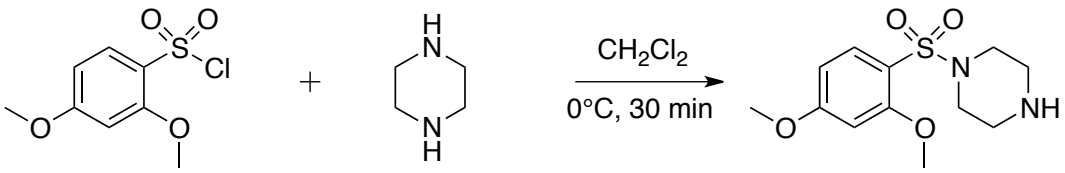
76%): mp 144.5-145.5°C (lit mp 146-148°C); ^{1}H NMR (DMSO, 300 MHz) δ = 7.84–8.01 (m, 4H), 3.03-3.06 (m, 4H), 2.71-2.74 (m, 4H), 2.34 (s, NH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 7.65-7.73 (m, 4H), 3.27-3.31 (m, 4H), 2.94-2.97 (m, 4H), 1.62 (s, 1H).



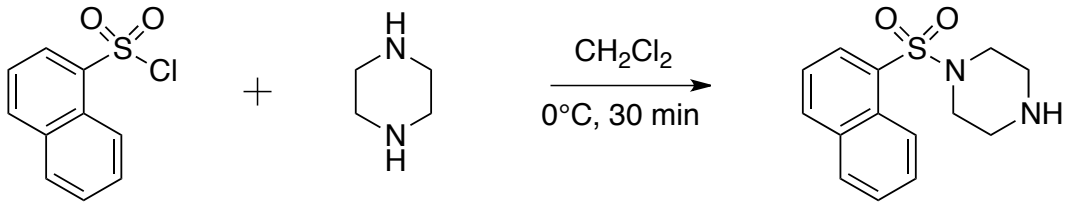
1-(*o*-tolylsulfonyl)piperazine (2.2b): A solution of piperazine (0.82 g, 9.53 mmol, 6 eq) dissolved in DCM (50 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1b** (0.30 g, 1.57 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (50 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (50 mL), washed with brine (50 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield the crude product. The crude product was left to sit and, after solidifying, recrystallized from EtOAc to give **2.2b** as a colorless crystalline solid (0.25 g, 1.04 mmol, 67%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 7.84 (d, J = 9 MHz, 1H), 7.42 (dd, 1H), 7.25-7.29 (dd, 2H), 3.07-3.10 (m, 4H), 2.83-2.86 (m, 4H), 2.59 (s, 3H), 1.92 (s, 1H).



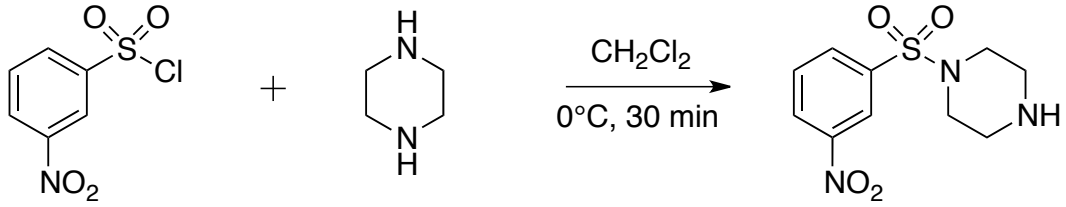
1-((2-bromophenyl)sulfonyl)piperazine (2.2c): A solution of piperazine (0.51 g, 5.92 mmol, 6 eq) dissolved in DCM (20 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1c** (0.25 g, 0.98 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (20 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (30 mL), washed with brine (30 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield a colorless oil, which crystallized upon standing overnight to give **2.2c** as a colorless crystalline solid (0.20 g, 0.66 mmol, 66%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.10 (d, J = 6 MHz, 1H), 7.78 (d, J = 9 MHz, 1H), 7.39-7.50 (m, 2H), 3.28-3-32 (m, 4H), 2.92-2.95 (m, 4H), 1.68 (s, 1H).



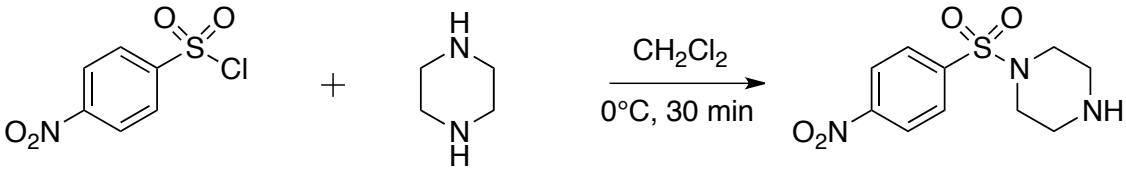
1-((2,4-dimethoxyphenyl)sulfonyl)piperazine (2.2d): A solution of piperazine (0.66 g, 7.63 mmol, 6 eq) dissolved in DCM (45 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1d** (0.30 g, 1.27 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (45 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (30 mL), washed with brine (30 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield a colorless oil. Ethanolic HCl was added to the oil until the solution became acidic and was concentrated *in vacuo* to yield **2.2d** as a light yellow hydrochloride salt (0.20 g, 0.62 mmol, 49%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 7.68 (d, J = 6 MHz, 1H), 6.43 (d, J = 6 MHz, 1H), 3.76-3.79 (m, 6H), 3.07-3.08 (4H), 2.81-2.82 (m, 5H).



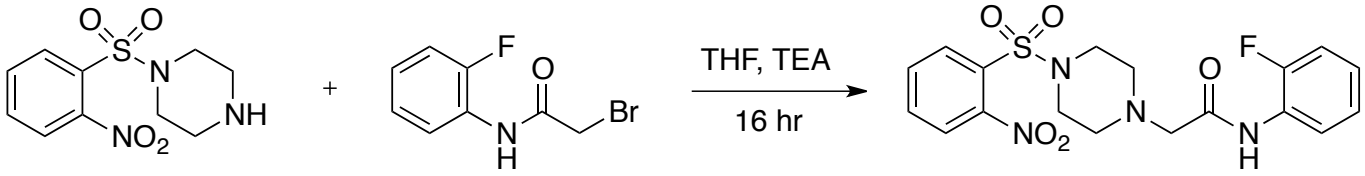
1-(naphthalenesulfonyl)piperazine (2.2e): A solution of piperazine (0.69 g, 7.94 mmol, 6 eq) dissolved in DCM (45 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1e** (0.30 g, 1.32 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (90 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (40 mL), washed with brine (40 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield a light yellow oil, which crystallized upon standing for 48 h to give **2.2e** as a light yellow solid (0.26 g, 0.94 mmol, 72%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.75 (d, J = 9 MHz, 1H), 8.17 (d, J = 6 MHz, 1H), 8.04 (d, J = 6 MHz, 1H), 7.89 (d, J = 9 MHz, 1H), 7.49-7.64 (m, 3H), 3.11-3.12 (m, 4H), 2.76-2.84 (m, 4H), 2.36 (s, 1H).



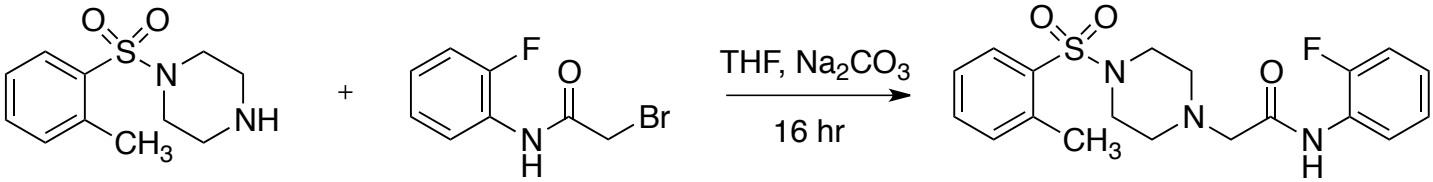
1-((3-nitrophenyl)sulfonyl)piperazine (2.2f): A solution of piperazine (1.17 g, 13.58 mmol, 6 eq) dissolved in DCM (45 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1f** (0.50 g, 2.26 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (45 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (30 mL), washed with brine (40 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield the crude product. The crude product was dissolved in minimal DCM and precipitated by the addition of hexanes to provide **2.2f** as a white solid (0.53 g, 1.95 mmol, 86%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.61 (s, 1H), 8.49 (d, J = 9 MHz, 1H), 8.09-8.12 (s, 1H), 7.77-7.82 (dd, 1H), 3.06-3.08 (m, 4H), 2.97-2.99 (m, 4H).



1-((3-nitrophenyl)sulfonyl)piperazine (2.2g): A solution of piperazine (1.17 g, 13.58 mmol, 6 eq) dissolved in DCM (45 mL) was prepared in a 100 mL round bottom flask and cooled to 0°C. Compound **2.1g** (0.50 g, 2.26 mmol, 1 eq) was added to the reaction mixture and stirred at 0°C for 30 min. Upon completion, the mixture was diluted with DCM (45 mL), quenched with the addition of saturated aqueous $NaHCO_3$ (30 mL), washed with brine (40 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to yield the crude product. The crude product was dissolved in minimal DCM and precipitated by the addition of hexanes to provide **2.2g** as a light yellow solid (0.49 g,1.81 mmol, 80%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.39-8.42 (m, 2H), 7.94-7.97 (m, 2H), 3.04-3.08 (m, 4H), 2.94-2.97 (m, 4H).

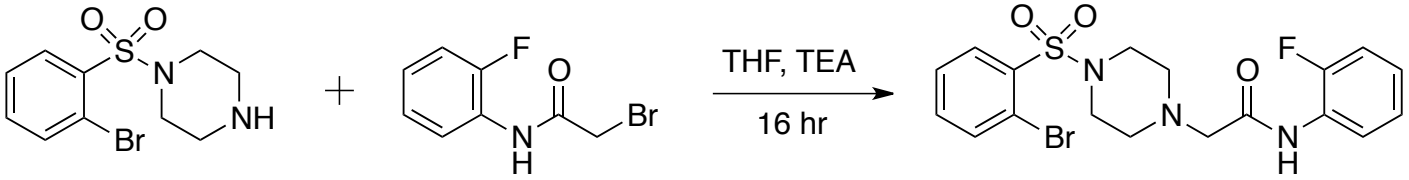


***N*-(2-fluorophenyl)-2-(4-((2-nitrophenyl)sulfonyl)piperazin-1-yl)acetamide (2.4a):** A mixture of **2.2a** (0.63 g, 2.34 mmol, 1 eq), **2.3** (0.54 g, 2.32 mmol, 1 eq), and TEA (0.62 mL, 4.45 mmol, 2 eq) in THF (16 mL) was created in a 250 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (50 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by recrystallization from EtOAc to provide **2.4a** (0.72 g, 1.70 mmol, 73%) as a light yellow crystalline solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 9.27 (s, 1H), 8.32-8.37 (dd, 1H), 8.04 (d, J = 9 MHz, 1H), 7.67-7.80 (m, 3H), 7.06-7.17 (m, 3H), 3.46 (d, J = 6 MHz, 4H), 3.24 (s, 2H), 2.73-2.76 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.51, 154.05, 150.83, 148.36, 133.93, 131.69, 131.44, 131.02, 125.91, 125.77, 124.71, 124.66, 124.55, 124.45, 124.30, 121.39, 121.38, 114.92, 114.67, 61.75, 52.85, 45.98; *m/z* calculated for $C_{18}H_{19}FN_4O_5S$: 422.11; found: 422.06.

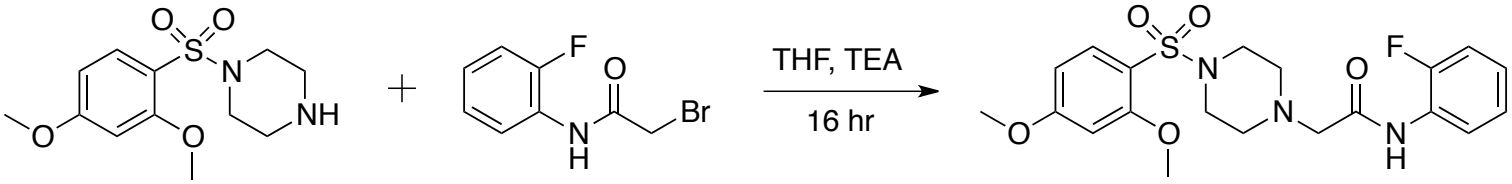


***N*-(2-fluorophenyl)-2-(4-(*o*-tolylsulfonyl)piperazin-1-yl)acetamide (2.4b):** A mixture of **2.2b** (0.29 g, 1.19 mmol, 1 eq), **2.3** (0.17 g, 0.71 mmol, 1 eq), and Na_2CO_3 (0.25 g, 2.40 mmol, 2 eq) in THF (8 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (50 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by recrystallization from EtOAc to provide **2.4b** (0.11 g, 0.29 mmol, 41%) as a light brown crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.29 (s, 1H), 8.31-8.36 (dd, 1H), 7.96 (d, J = 9 MHz, 1H), 7.49-7.54 (dd, 1H), 7.38 (d, J = 3 MHz, 2H), 7.08-7.17 (m, 3H), 3.32 (s, 4H), 3.22 (s, 2H), 2.67-2.73 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.59, 154.08, 150.85, 138.08, 135.54,

133.12, 132.91, 130.34, 126.18, 125.93, 125.79, 124.67, 124.62, 124.53, 124.43, 121.38, 121.37, 114.95, 114.70, 61.77, 52.84, 45.24, 20.69; *m/z* calculated for $C_{19}H_{22}FN_3O_3S$: 391.14; found: 391.07.

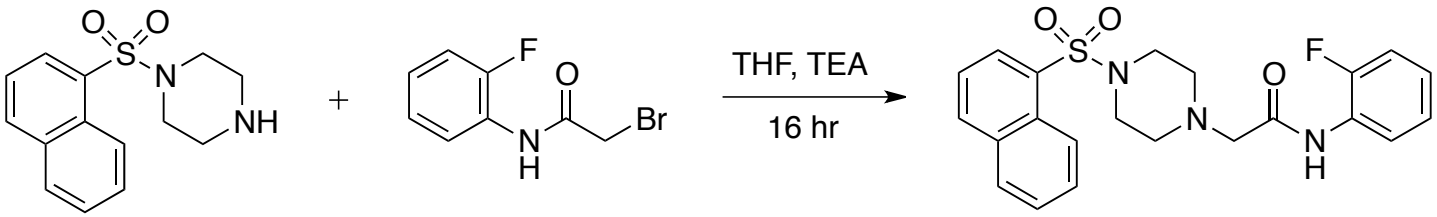


2-(4-((2-bromophenyl)sulfonyl)piperazin-1-yl)-*N*-(2-fluorophenyl)acetamide (2.4c): A mixture of **2.2c** (0.20 g, 0.65 mmol, 1 eq), **2.3** (0.15 g, 0.65 mmol, 1 eq), and TEA (0.18 mL, 1.30 mmol, 2 eq) in THF (4 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (15 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by recrystallization from EtOAc to provide **2.4c** (0.22 g, 0.48 mmol, 75%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.32 (s, 1H), 8.32-8.37 (dd, 1H), 8.15 (d, J = 9 MHz, 1H), 7.81 (d, J = 9 MHz, 1H), 7.44-7.52 (m, 2H), 7.08-7.18 (m, 3H), 3.44-3.47 (m, 4H), 3.23 (s, 2H), 2.71-2.74 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.61, 154.07, 150.85, 137.68, 135.90, 133.86, 132.34, 127.61, 125.95, 125.81, 124.68, 124.63, 124.52, 124.42, 121.36, 121.35, 120.53, 114.95, 114.70, 61.83, 52.92, 45.67; *m/z* calculated for $C_{18}H_{19}BrFN_3O_3S$: 455.03; found: 454.95.

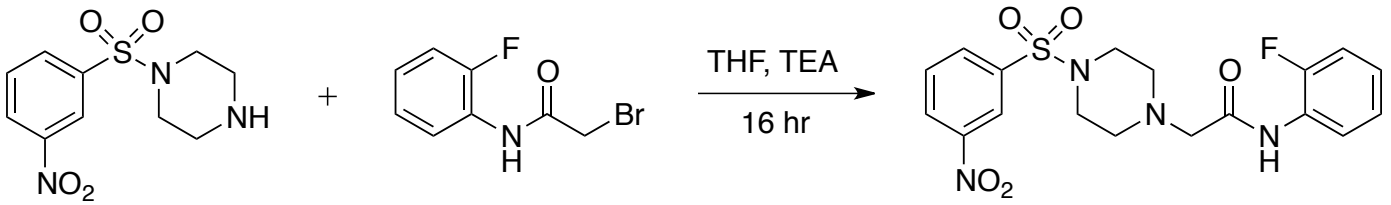


2-(4-((2,4-dimethoxyphenyl)sulfonyl)piperazin-1-yl)-*N*-(2-fluorophenyl)acetamide (2.4d): A mixture of the hydrochloride salt of **2.2d** (0.18 g, 0.57 mmol, 1 eq), **2.3** (0.13 g, 0.57 mmol, 1 eq), and TEA (0.24 mL, 1.71 mmol, 3 eq) in THF (3 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (15 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by precipitation from DCM/hexanes to provide **2.4d** (0.15 g, 0.34 mmol, 62%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.35 (s, 1H) 8.33-8.38 (dd, 1H), 7.86 (d, J = 12 MHz, 1H), 7.07-7.17 (m, 3H), 6.56 (d, J = 6 MHz, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 3.34 (s, 4H), 3.21 (s, 2H), 2.70-2.72 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.83,

166.87, 158.45, 154.02, 150.08, 133.53, 126.00, 125.87, 124.73, 124.68, 124.43, 124.33, 121.35, 118.41, 114.87, 114.62, 104.36, 99.56, 61.75, 55.93, 55.73, 53.19, 45.98; *m/z* calculated for $C_{20}H_{24}FN_3O_5S$: 437.14; found: 437.15.

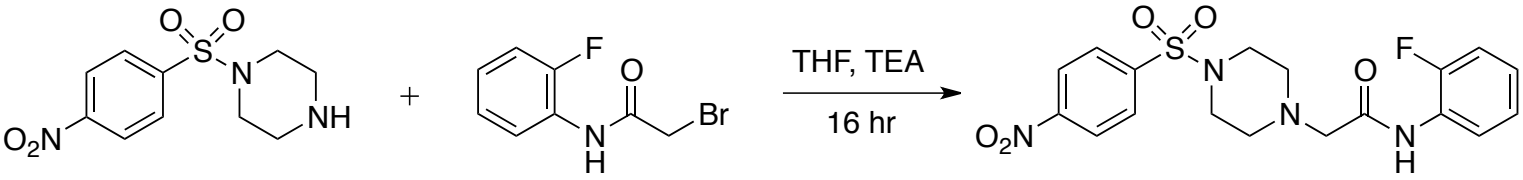


***N*-(2-fluorophenyl)-2-(4-(naphthalen-1-ylsulfonyl)piperazin-1-yl)acetamide (2.4e):** A mixture of **2.2e** (0.26 g, 0.95 mmol, 1 eq), **2.3** (0.22 g, 0.95 mmol, 1 eq), and TEA (0.26 mL, 1.90 mmol, 2 eq) in THF (5 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (15 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by drying on a high vacuum to provide **2.4e** (0.31 g, 0.72 mmol, 75%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.18 (s, 1H), 8.74 (d, J = 9 MHz, 1H), 8.30 (d, J = 15 MHz, 2H), 8.15 (d, J = 9 MHz, 1H), 7.99 (d, J = 6 MHz, 1H), 7.58-7.72 (m, 3H), 7.04-7.15 (m, 3H), 3.36 (s, 4H), 3.15-3.18 (s, 2H), 2.61-2.68 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.57, 154.05, 150.82, 134.78, 134.42, 132.52, 130.74, 129.03, 128.89, 128.23, 126.98, 125.86, 124.73, 125.03, 124.63, 124.58, 124.53, 124.43, 124.14, 121.43, 121.42, 114.92, 114.67, 61.71, 52.77, 45.48; *m/z* calculated for $C_{22}H_{22}FN_3O_3S$: 427.14; found: 427.04.

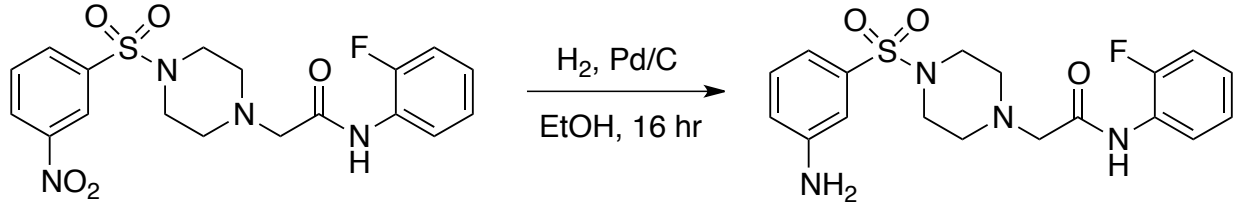


***N*-(2-fluorophenyl)-2-(4-((3-nitrophenyl)sulfonyl)piperazin-1-yl)acetamide (2.4f):** A mixture of **2.2f** (0.40 g, 1.47 mmol, 1 eq), **2.3** (0.34 g, 01.47 mmol, 1 eq), and TEA (0.41 mL, 2.95 mmol, 2 eq) in THF (5 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (15 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica

column chromatography followed by recrystallization from EtOAc to provide **2.4f** (0.39 g, 0.92 mmol, 62%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.08 (s, 1H), 8.65 (s, 1H), 8.54 (d, J = 6 MHz, 1H), 8.27-8.32 (dd, 1H), 8.14 (d, J = 6 MHz, 1H), 7.82-7.87 (dd, 1H), 7.03-7.17 (m, 3H), 3.23 (s, 6H), 2.76-2.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.29, 153.98, 150.76, 148.47, 138.34, 133.09, 130.70, 127.58, 125.74, 125.61, 124.75, 124.70, 124.64, 124.54, 122.76, 121.45, 114.89, 114.64, 61.62, 52.49, 46.11; *m/z* calculated for $C_{18}H_{19}FN_4O_5S$: 422.11; found: 422.11.

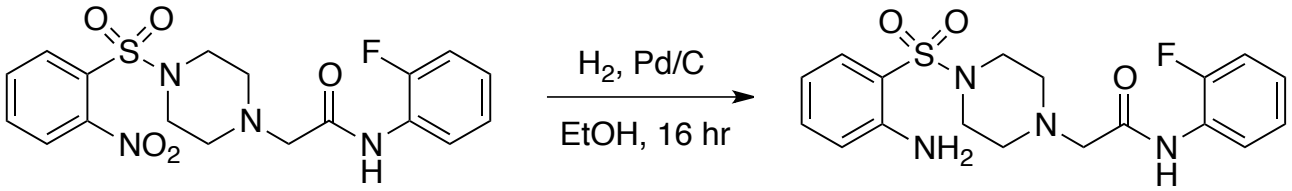


***N*-(2-fluorophenyl)-2-(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)acetamide (2.4g):** A mixture of **2.2g** (0.45 g, 1.65 mmol, 1 eq), **2.3** (0.38 g, 1.65 mmol, 1 eq), and TEA (0.46 mL, 3.30 mmol, 2 eq) in THF (7.5 mL) was created in a 100 mL round bottom flask. The mixture was left to react overnight under an N_2 atmosphere. Upon completion, the reaction mixture was diluted with DCM (80 mL) and then concentrated *in vacuo* to provide the crude product. The product was purified by silica column chromatography followed by recrystallization from EtOAc to provide **2.4g** (0.48 g, 1.13 mmol, 69%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.08 (s, 1H), 8.46 (d, J = 9 MHz, 2H), 8.27-8.32 (dd, 1H), 8.00 (d, J = 9 MHz, 2H), 7.11-7.15 (m, 1H), 7.04-7.08 (m, 2H), 3.23 (s, 6H), 2.75-2.78 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.26, 153.98, 150.76, 150.37, 141.90, 128.87, 125.73, 125.60, 124.76, 124.71, 124.67, 124.57, 124.53, 121.45, 114.90, 114.65, 61.62, 52.51, 46.07; *m/z* calculated for $C_{18}H_{19}FN_4O_5S$: 422.11; found: 422.10.



2-(4-((3-aminophenyl)sulfonyl)piperazin-1-yl)-*N*-(2-fluorophenyl)acetamide (2.5a): A solution of **2.4f** (0.70 g, 1.66 mmol, 1 eq) dissolved in ethanol was created at rt in a 250 mL round bottom flask. To the reaction flask, 10% Pd/C (0.07 g, 10% w/w) was added. The resulting mixture was placed under 20 psi H_2 and reacted overnight. Upon completion, the reaction was filtered through celite and

concentrated *in vacuo* to provide the crude product. The crude product was purified via silica column chromatography and recrystallized from EtOAc/hexanes to yield **2.5a** (0.11 g, 0.28 mmol, 17%) as a white crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.14 (s, 1H), 8.27-8.32 (dd, 1H), 7.28-7.37 (dd, 1H), 7.12-7.14 (m, 2H), 7.03-7.08 (m, 3H), 6.92 (d, J = 9 MHz, 1H), 4.00 (s, 2H), 3.16-3.20 (m, 6H), 2.71-2.74 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 167.64, 154.08, 150.86, 147.25, 136.53, 130.09, 125.85, 125.72, 124.66, 124.61, 124.58, 124.47, 121.56, 121.55, 119.23, 117.25, 114.92, 114.66, 113.29, 61.67, 52.62, 46.07; *m/z* calculated for $C_{18}H_{21}FN_4O_3S$: 392.13; found: 392.13.



2-(4-((2-aminophenyl)sulfonyl)piperazin-1-yl)-*N*-(2-fluorophenyl)acetamide (**2.5b):** A solution of **2.4a** (0.70 g, 1.66 mmol, 1 eq) dissolved in ethanol was created at rt in a 250 mL round bottom flask. To the reaction flask, 10% Pd/C (0.07 g, 10% w/w) was added. The resulting mixture was placed under 20 psi H_2 and reacted overnight. Upon completion, the reaction was filtered through celite and concentrated *in vacuo* to provide the product as an oil. The oil was placed on a high vacuum with no further purification to yield **2.5b** (0.37 g, 0.95 mmol, 58%) as a brown crystalline solid: ^{1}H NMR ($CDCl_3$, 300MHz) δ = 9.20 (s, 1H), 8.27-8.33 (dd, 1H), 7.54-7.61 (m, 1H), 7.34-7.39 (dd, 1H), 7.05-7.15 (m, 3H), 6.76-6.83 (m, 2H), 5.07 (s, 2H), 3.19-3.26 (m, 6H), 2.69-2.72 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 171.20, 167.71, 167.66, 154.09, 150.86, 146.37, 134.53, 130.31, 125.85, 125.72, 124.66, 124.61, 124.59, 124.49, 121.51, 121.50, 117.79, 117.40, 117.37, 114.95, 114.69, 61.68, 52.58, 45.92; *m/z* calculated for $C_{18}H_{21}FN_4O_3S$: 392.13; found: 392.12.

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