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An Investigation Towards the Synthesis of a Novel Conformationally Restricted Ethylenediamine Scaffold

Fanny Mai

Abstract

This study reports the synthesis of a novel conformationally restricted ethylenediamine scaffold that can be explored for drug discovery. There was significant progress in synthesizing the target scaffolds, but future studies are needed to finish this synthesis. This potential ethylenediamine compound may offer new scaffolds for exploration in drug design and orexin receptor antagonists with improved selectivity for OX1R and OX2R.

MONTCLAIR STATE UNIVERSITY

AN INVESTIGATION TOWARDS THE SYNTHESIS OF A NOVEL CONFORMATIONALLY RESTRICTED ETHYLENEDIAMINE SCAFFOLD

by

Fanny Mai

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Introduction:

It has been shown that a drug's chemical structure determines its physicochemical properties, such as its appearance, boiling point, density, and ADME properties[1]. Therefore, structural modifications of drugs are likely to alter their interactions with target proteins and change their pharmaceutical properties such as solubility, metabolism, and pharmacokinetics[1]. Conformationally restricted molecules possess structural features that decrease the freedom of intramolecular motions by holding atoms at well-defined distances and orientations in space[2]. The molecule's rigidity may be beneficial for achieving efficient and selective binding to its biological protein targets in specific regions of space. This is due to the restricted scaffolds holding nitrogen atoms at an optimal distance so that the nitrogen atoms can achieve efficient intramolecular interactions with other molecules.

In medicinal chemistry, diamines can be used to evaluate ligands for G-protein coupled receptors and enzyme inhibitors[3]. Ethylenediamine is an organic compound used as a building block to produce many other chemical products such as aminophylline[4]. Ethylenediamine is a conformationally flexible bidentate chelating ligand with two nitrogen atoms donating their lone pairs of electrons. Due to its bifunctional nature, the two amines readily form heterocycles by reacting to itself[5]**.** Today, many drugs on the market have more rigid ethylenediamine units as building blocks for biologically active molecules[4]. *Figure 1* below illustrates different points at which this diamine can be restricted. The synthesis of new rigid ethylenediamine scaffolds may provide templates for new drug designs and show potential for drug design. These scaffolds permit exploration of stereochemistry and geometry of ethylenediamine to influence drug-target interactions.

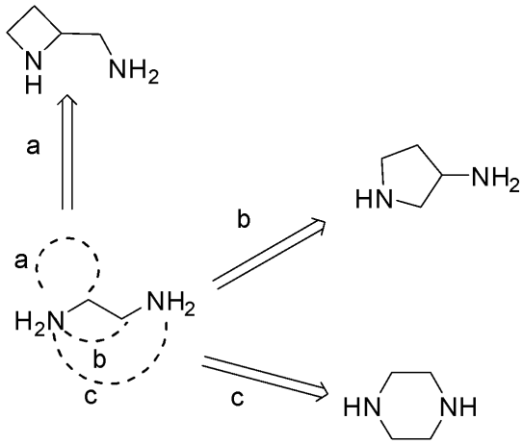


Figure 1. The ethylenediamine structure can be conformed into different restricted cyclic structures.

One example of a case where a conformationally restricted diamine was used to improve the affinity of an enzyme inhibitor is shown in *Figure 2*. The flexible sulfur-based linker in BPTES (**2**) was replaced by a 3-amino-pyrrolidine moiety[6] (**4**).

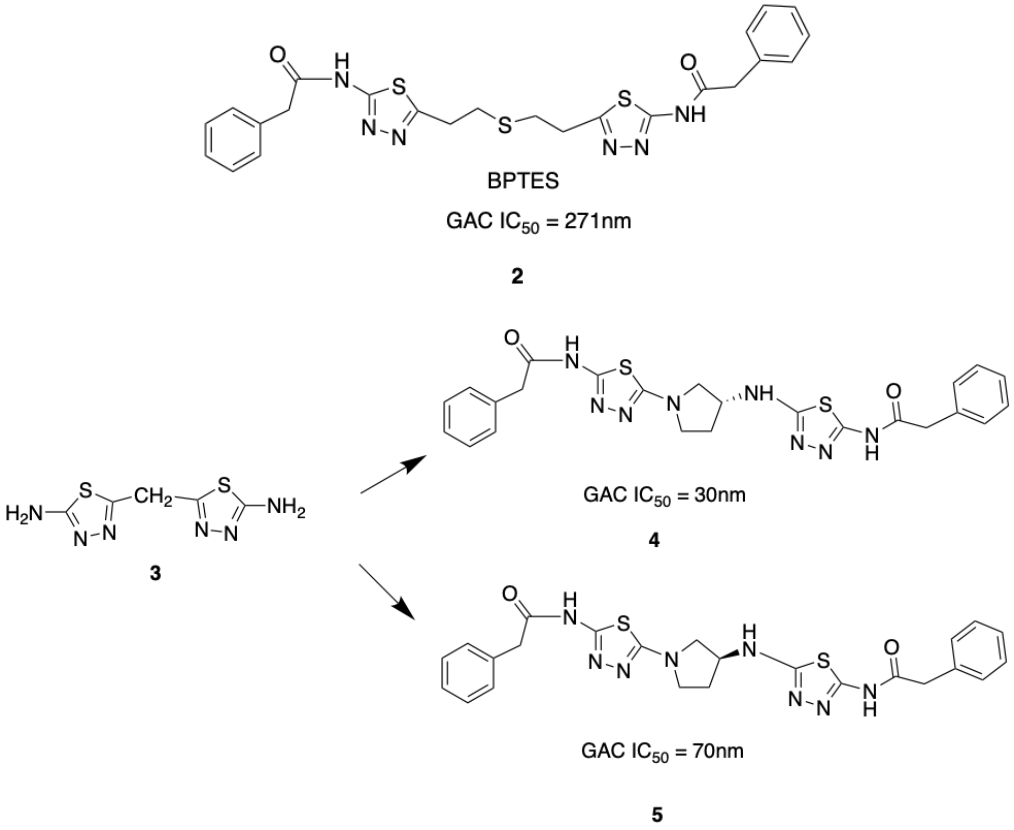


Figure 2. Ethylenediamine core showed increased activity compared to the leading known GAC inhibitor BPTES by 9x fold.

The ethylenediamine structure was investigated as a scaffold during screening for leads to address different diseases. Inhibition of glutaminase (GAC) is an anticancer target that leads to the reduction of tumor cells. The GAC inhibitors contain a lipophilic chain that connects two aromatic heterocycles with an ethylenediamine core structure used for screening and further optimization *(Fig 2)*. Ligand and lipophilic efficiencies were used as the scoring methods to measure GAC inhibitors' potency and physicochemical properties. This leads to improved cell-based ligand efficacy (LE), lipophilic efficiency (LipE), and GAC IC_{50} compared to the known GAC inhibitor BPTES[6] **(2)**. Both isomers of the amino pyrrolidine were evaluated for their stereoselectivity and showed that the one enantiomer isomer, **4** has a better inhibition than the other enantiomer **5**. Two lead structure designs from the vital feature scaffold went through further optimizations, which increased the activity of the microsomal stability assay, thus leading to the formation of improved GAC inhibitors.

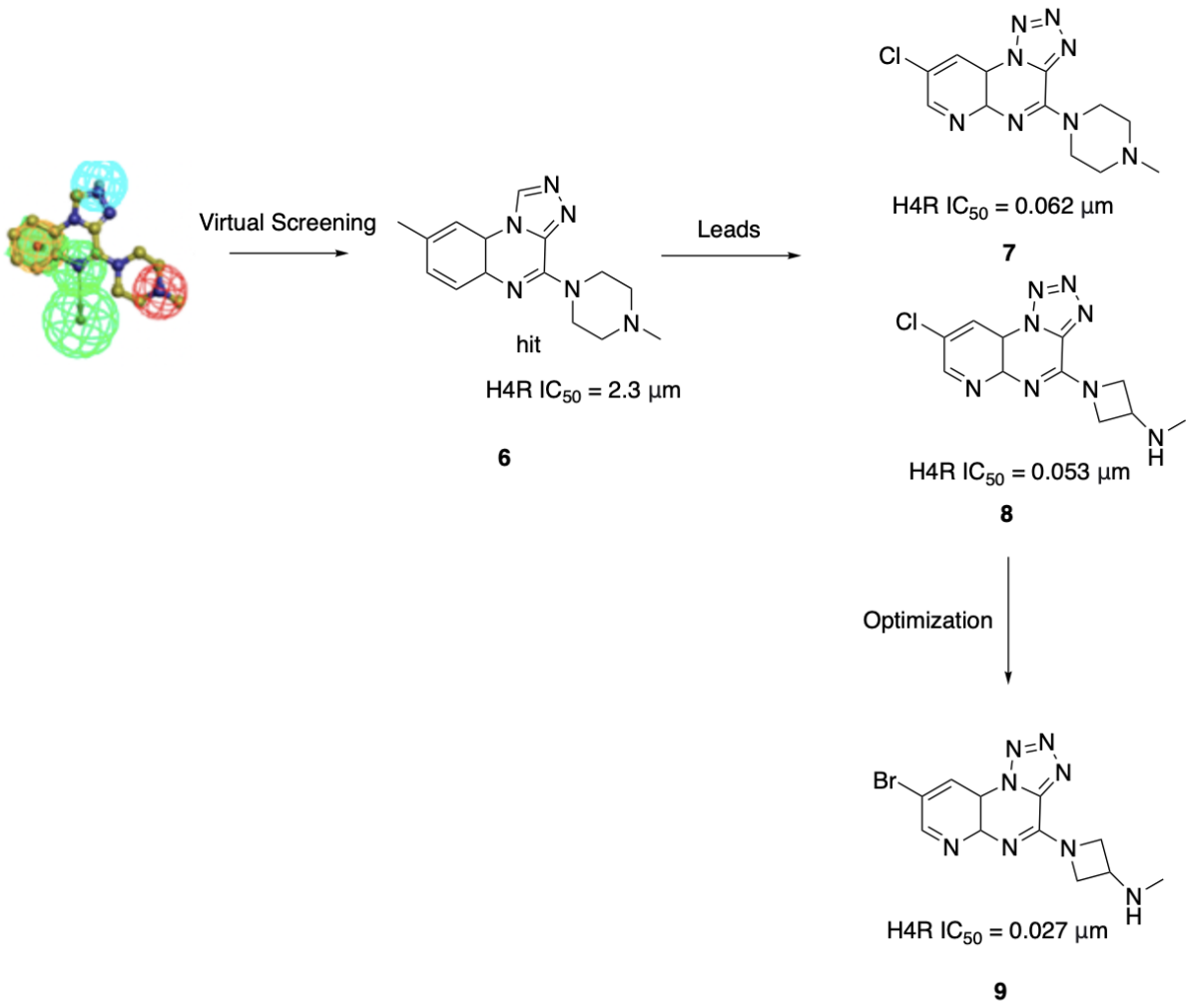


Figure 3: Variation in the ethylenediamine fragment during optimization led to IC_{50} activity to increase 10x fold.

A different mode of ethylenediamine modification was used by Ko and coworkers, who carried out H4 receptor antagonist pharmacophore screening to obtain hits for treating atopic dermatitis[7]. A hit compound is a molecule that exhibits the desired activity at a given target molecule in a screening assay. Compound **6** was among these initial hits and served as the basis for continued optimization because it displayed good inhibitory activity (H4R IC_{50} >100 µM) and selectivity over H3R (2.3 µM). However, this hit structure had poor metabolic stability and modest potency. The team investigated changes in structure and alternative diamines to optimize

these properties. The authors modified the methyl group to a chlorine group and changed Triazole to a Tetrazole (*Fig 3*). This modification increases the potency of the H4 receptor antagonist. In addition, the H4 receptor antagonist changes its alternative ethylenediamine structure, thus leading to compound **8**. Compound **8** dramatically improved inhibitory activity for H4R, metabolic stability, and selectivity issues for other histamine receptors. Eventually, the team derived compound **9** as a lead compound by introducing bromine instead of the chlorine atom, which exhibits strong inhibitory activity against H4R and excellent selectivity over H3R. Compound **9** is a highly potent and selective H4R antagonist without noticeable action on off-targets, including other histamine receptors and 5-HT3R, and exhibited an excellent pharmacokinetic profile[7].

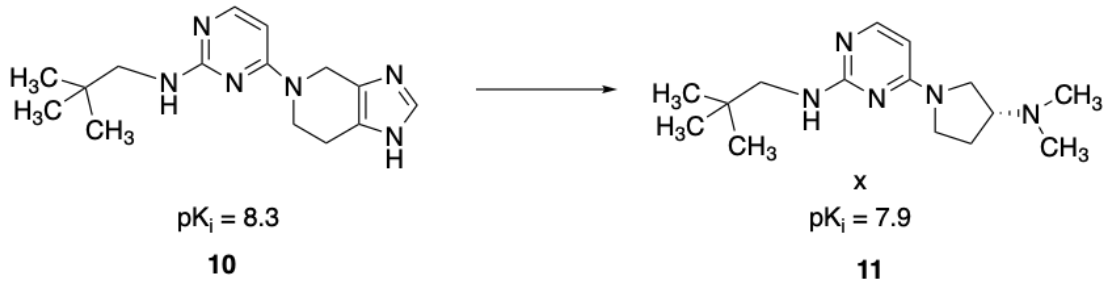


*Figure 4. Radiolabeling binding studies found and improved pK$_i$, leading to compound **11**.*

A different approach to investigating conformationally restricted diamines and their influence on affinity and selectivity for H4 receptor ligands was used by Bartole and colleagues[8]. The human histamine H4 receptor (hH4R) is a promising target for treating immune system disorders such as rheumatoid arthritis and bronchial asthma. Radioligand binding studies on humans, mice, and rats were tested with different structures to improve H4 receptor binding. After radiolabeling, binding studies with **10**, the addition of an ethylenediamine structure showed an improvement in the pK$_i$ value in the human receptors *(Fig 4)*. The **11** compound structures at the human, mouse, and rat histamine H4 receptors revealed comparable Kd values (41/17/22

nM), low nonspecific binding (11−17%), and fast associations/dissociations8 (25−30 min). Compound **11** has the potential for pharmacological studies on the H4R related to translational animal models.

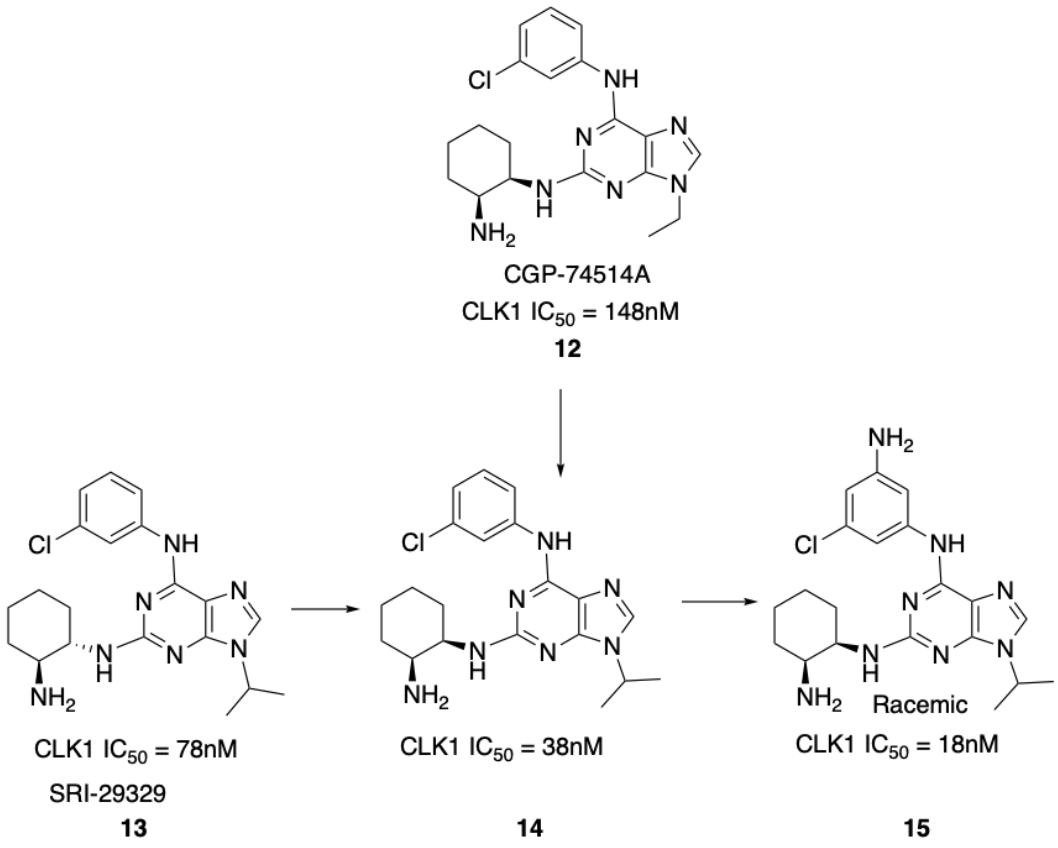


Figure 5. Active CLK inhibitors profiled from CGP-74514A led to the racemic CLK inhibitor with improved IC_{50}.

A triple exon-skipping luciferase reporter assay identified new CLK (CDC2-like protein kinases) inhibitor pharmacophore hits. Increasing evidence links the CLKs to cancer with CLK1, and CLK2 inhibition may benefit the treatment of triple-negative breast cancer[9]. The screening identified several active hits, the most potent of which was CGP-74514A (**12**), which has been reported to be cyclin-dependent kinases (CDKs) inhibitors. The CGP-74514A (**12**) shows significant CLK inhibition and clear structure-activity relationships (SAR) at CLKs[10]. Using this

approach, the dual CLK2/CDK1 inhibitors CGP-74514A were optimized to yield more specific CLK inhibitors SRI-29329 (**13)**. Analogs of SRI-29329 with changed stereochemistry, such as compound **15,** increased CLKL IC_{50} *(Fig 5).*

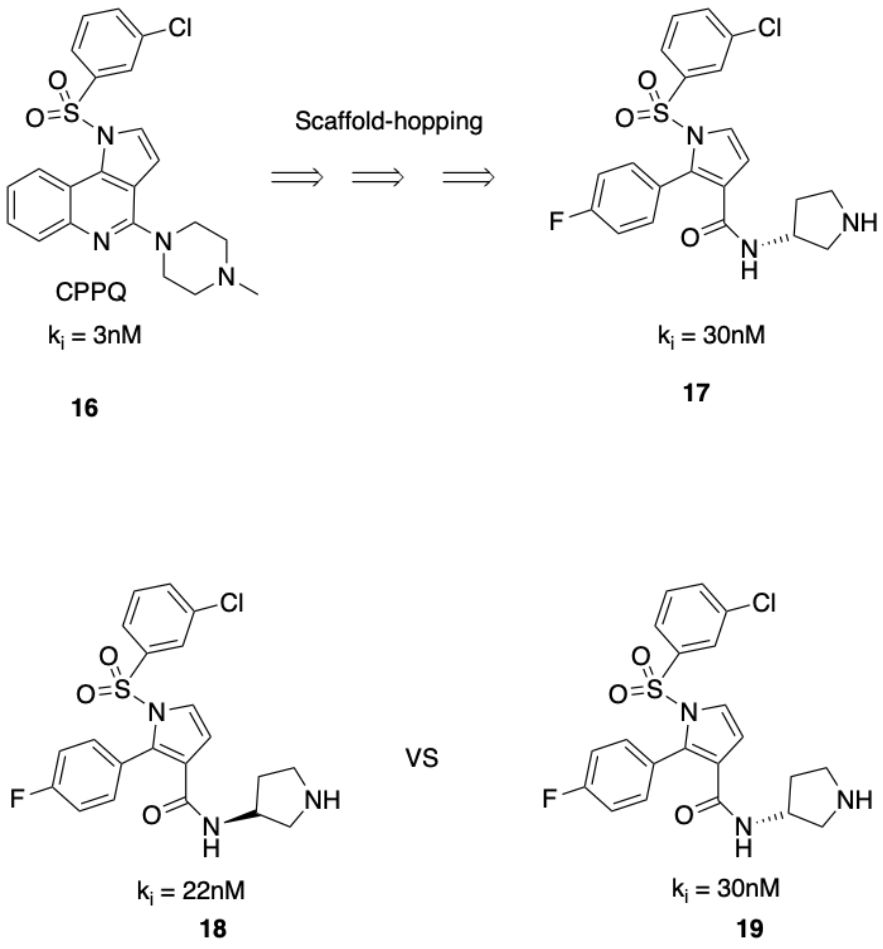
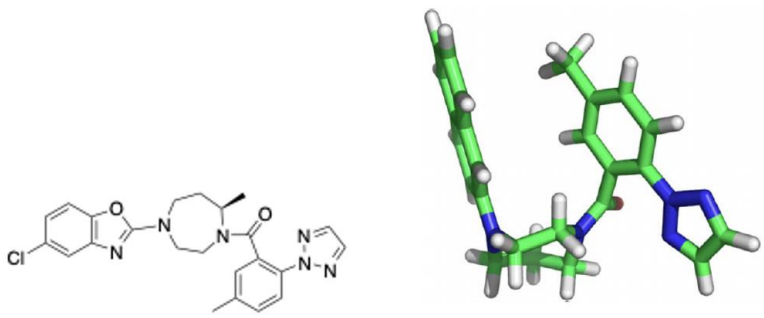


Figure 6. Scaffold-hopping changed the ethylenediamine structure of the control core and its stereochemistry, leading to increased k_i activity of 5-HT6R

The serotonin type 6 receptor (5-HT6R) is a promising target for treating cognitive impairments[11]. The scaffold-hopping approach around pyrroloquinoline derivatives CPPQ **(16)** containing an ethylenediamine structure was applied to modify the central core structure for developing 5-HT6R antagonists (*Fig 6*). This modification has changed the compound's activity

at 5-HT6R. The enantiomeric diversity was further evaluated for their selectivity over serotonin receptors. A comparison of the binding mode of **18** and **19** demonstrated differences in the orientation of the enantiomers of the ethylenediamine group. Studies revealed that the structural requirements for higher affinity for the 5-HT6R lead to compound **19** with higher metabolic stability and brain penetrant. This compound might be considered a new cognition-enhancing agent.

Furthermore, rigid ethylenediamine structures can create an orexin receptor antagonist. The orexins stimulate two distinct G-protein coupled receptors, orexin-1 (OX1R) and orexin-2 (OX2R), associated or selectively located in specific brain areas and expressed extensively across the central nervous system. The role of orexin-1 receptors is believed to play a role in addiction, panic, anxiety, and sleep. At the same time, the role of the orexin-2 receptor is an essential modulator of sleep. The antagonist's inhibition of both OX1R and OX2R showed effective treatment of insomnia[12].



20 **21**

*Figure 7. Structure of suvorexant commonly known as Belsomra **(20)**. Suvorexant folds into a twist-boat ring conformation **(21)**.*

The development of small-molecule dual orexin receptor antagonists (DORAs) demonstrated the importance of the orexin pathway for modulating sleep. The DORAs, suvorexant (Belsomra), became the first orexin antagonist approved by the FDA to treat insomnia[13]. To further optimize potent and brain penetrant DORAs, a scaffold-hopping approach using known orexin ligands as a template searched for new lead structures. The restricted ethylenediamine scaffold may contain structural properties that improve the binding affinity of orexin receptor antagonists.

Suvorexant and several other orexins ligands fold into a twist-boat ring conformation, with intramolecular π-stacking suggesting a low-energy conformation in orexin receptor antagonists[14]. The U-shaped conformation **21** elicits bioactivity through orexin receptors with improved antagonistic potency for the OX1 and OX2 receptors compared to open conformation[15]. The orexin antagonist structure needs to fit the active conformation better of orexin receptors for further optimization.

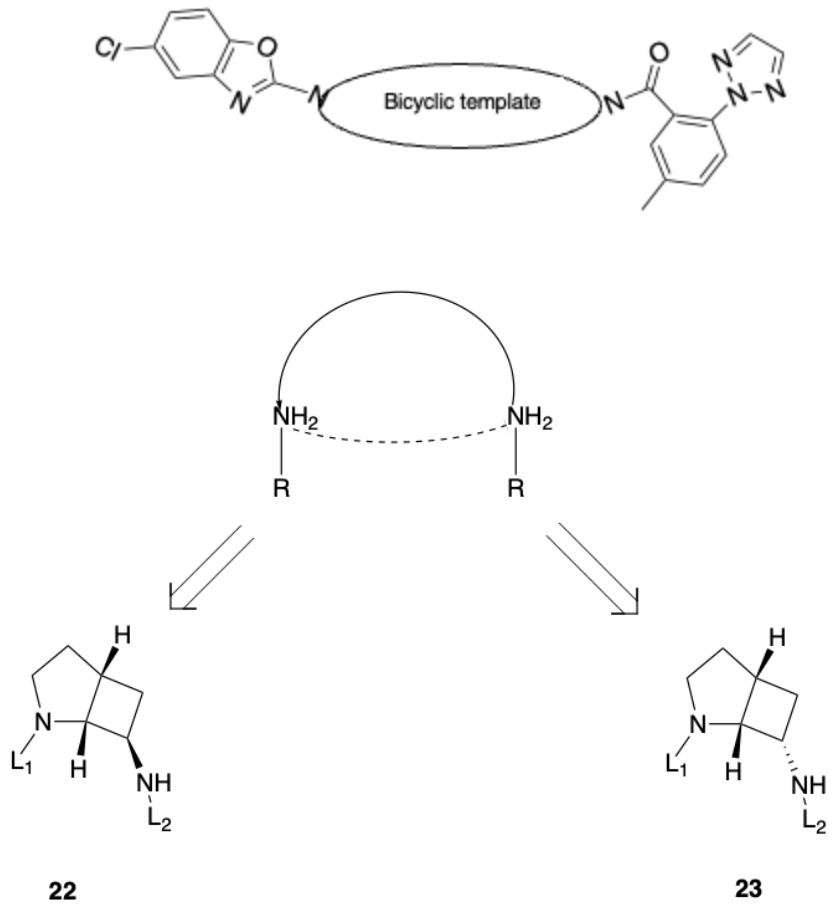


Figure 8. The design of scaffolds for potential antagonists or agonists for OXR1/OXR2 for this study.

This potential diamine rigid scaffold was derived by the ligand-based drug design approach carrying the U-shaped conformation, with which known potent orexin antagonists, such as suvorexant, are known to bind to the OXRs. The racemic bicyclic template will contain a new restricted ethylenediamine structure unknown in the literature to be tested with OXRs (*Fig 8*). The various intermolecular interactions of the different enantiomers of the scaffold may form a different morphology of the orexin receptors[16].

This study hypothesizes that conformationally restricted ethylenediamine is used in drug design as a focal point for further optimization. These results may offer new scaffolds unknown

in the literature for exploration in drug design research. Moreover, the restricted ethylenediamine scaffold containing known orexin receptor ligands may lead to orexin antagonists or agonists with improved selectivity for OX1R and OX2R.

Discussion

Scheme 1

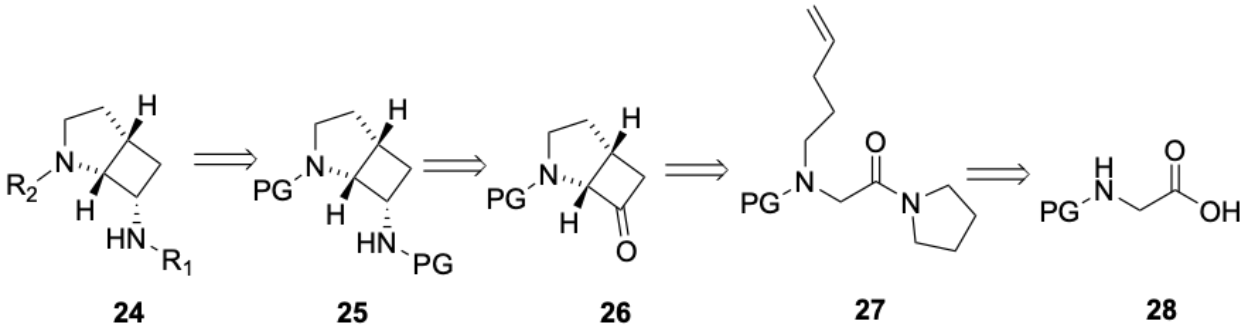


Figure 9. Scheme for retrosynthesis for the target diamine structure.

To achieve the rigid diamine scaffolds for potential antagonists or agonists for OXRs, the retrosynthetic analysis for the target diamine **24** is shown in *Figure 9*. Starting from a commercially available glycine derivative, the necessary carbons can be added and cyclized to give ketone **26**. The second nitrogen can be added in a stereoselective manner by reductive amination with the expectation that the rigid fused natural of the bicyclic will provide the desired stereochemistry. The rigidity of this racemic bicyclic template **25** displays the two nitrogen atoms in a specific orientation with a particular angle between them. The two nitrogen atoms are differentiated, permitting the installation of the R group pairs on each nitrogen in a particular stereochemistry.

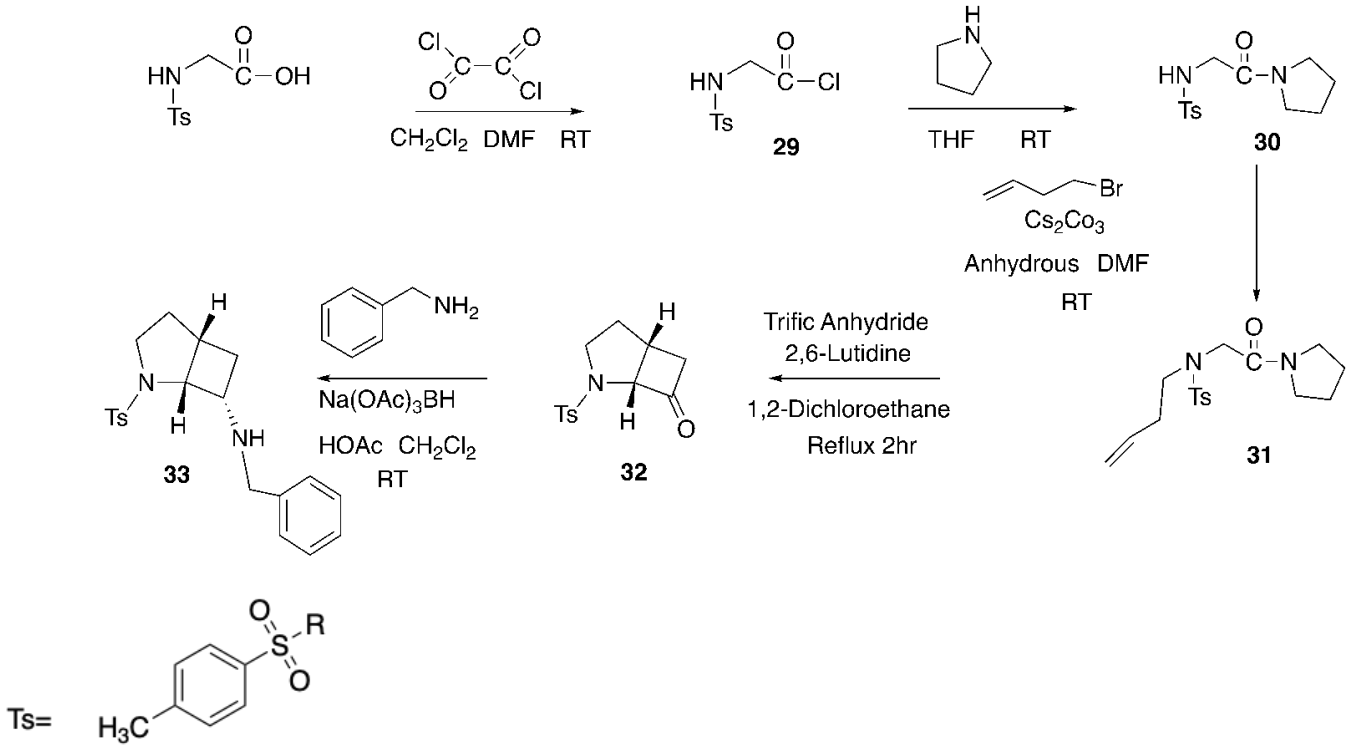


Figure 10. Scheme of the synthesis conditions of the target diamine structure.

This bicyclic ketone is a known compound and was synthesized using the procedure of Marko 1985[17]. This cyclization was amenable to scale up and provided a good yield (70-85%) on a milligram and gram scale.

Reductive amination using $Na(OAc)_3BH$ and benzylamine in dichloromethane at room temperature furnished two ninhydrin positive products with similar R_f values on silica gel TLC. Ninhydrin detects basic nitrogen atoms in molecules on a TLC plate. Purification of the crude material was attempted to separate these two products; however, this proved to be difficult, and this mixture was used without additional purification. The proton NMR spectrum and MS of the mixture are shown below. The NMR spectrum suggests this TLC observation may be misleading, as each signal can be assigned to specific protons in the target structure.

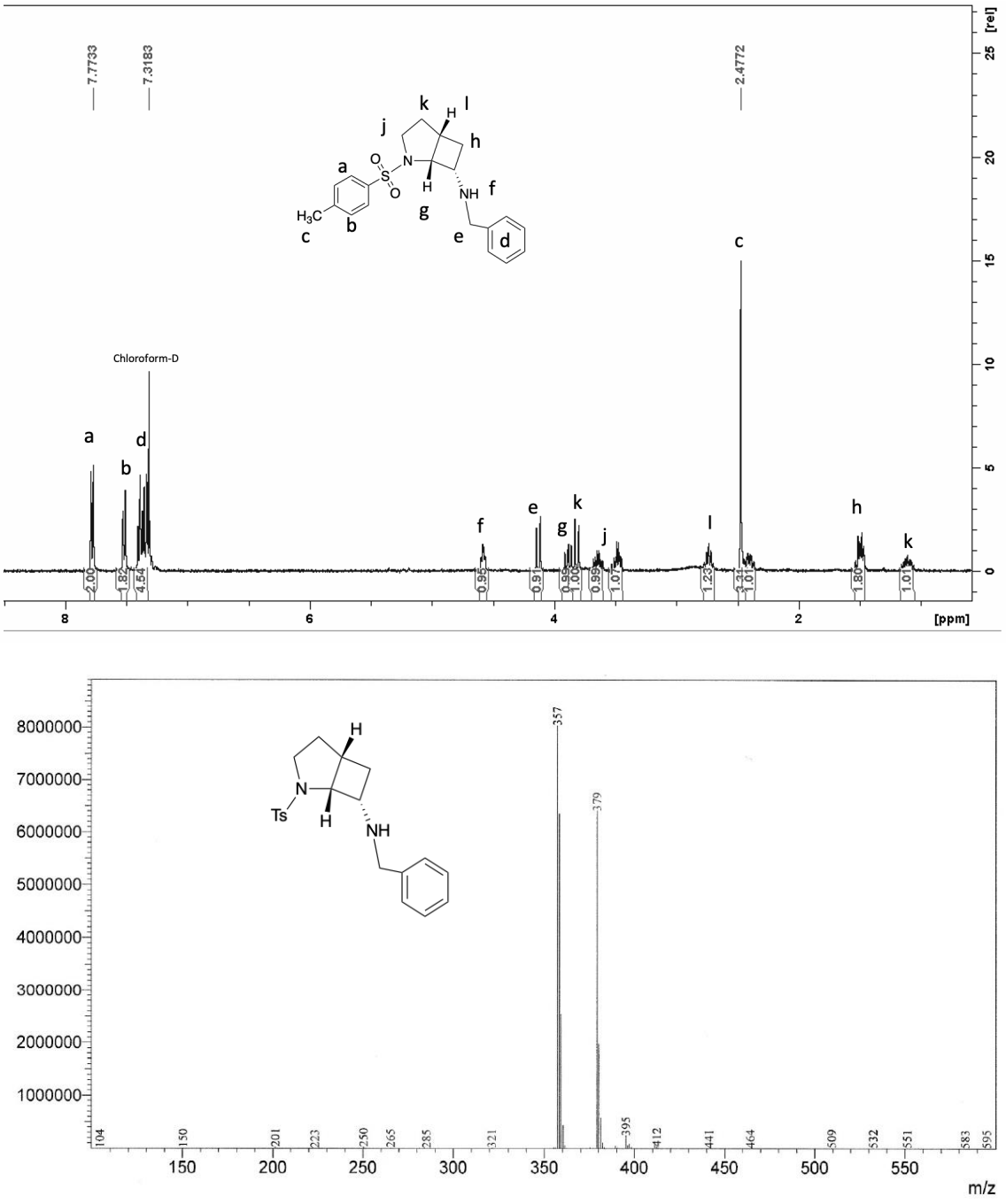
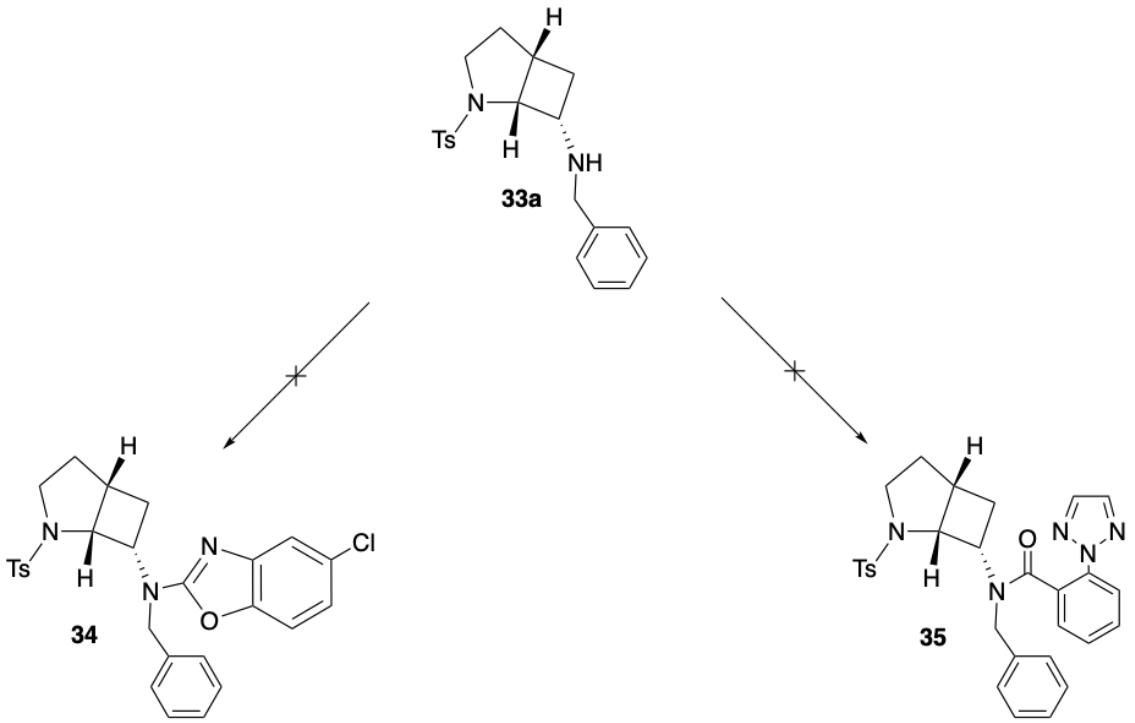


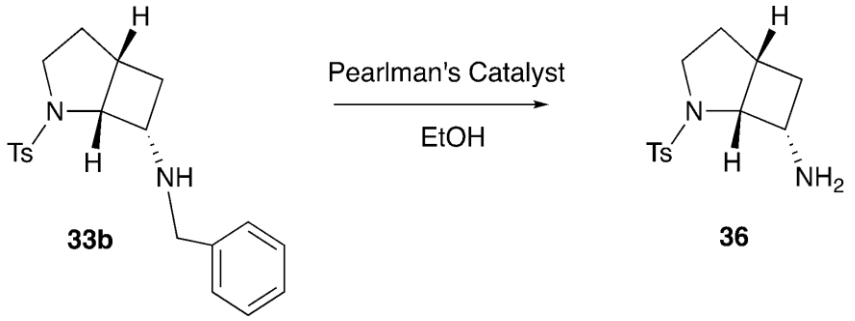
*Figure 11. [1]H NMR and MS of compound **33**,*

The structure of the major product was assigned by a combination of [1]H NMR and LCMS spectra (*Fig 11*). The hydrogen signals were assigned and correlated to the major product **33**. The LCMS spectrum showed the major product **33** with a molecular ion peak of 357 m/z.

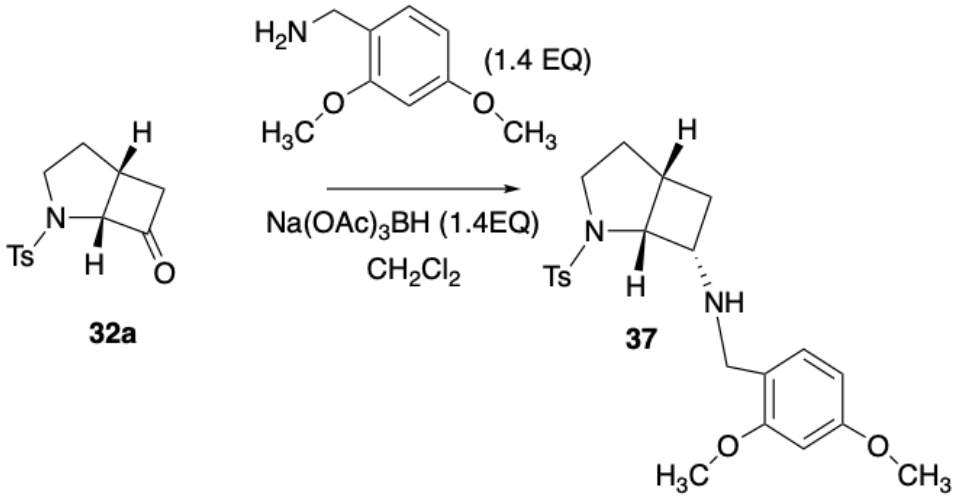
The minor unknown product gave a molecular ion peak at 379 m/z, indicating no amine stereoisomer**.** Therefore, the structure of this minor compound is unknown.



Subsequent displacement and amidation reactions were tested with **33a**. Introduction of the 5-chlorobenzoxazole by displacement from 2,5-dichlorobenzooxazole in various solvents with a range of bases and amidation using HATU, Et_3N, and DMF with 2-(2, H,1,2,3 triazol-2-yl) benzoic acid. Curiously, amidation of **33a** was unsuccessful under different conditions. Similarly, reactions to afford **34** from **33a** were also unsuccessful with K_3CO_3 and Et_3N in DMF, CH_3CN, and EtOH. As a result, the benzyl protecting group was removed prior to either amide formation or chloro displacement from 2,5-dichlorobenzoxazole.

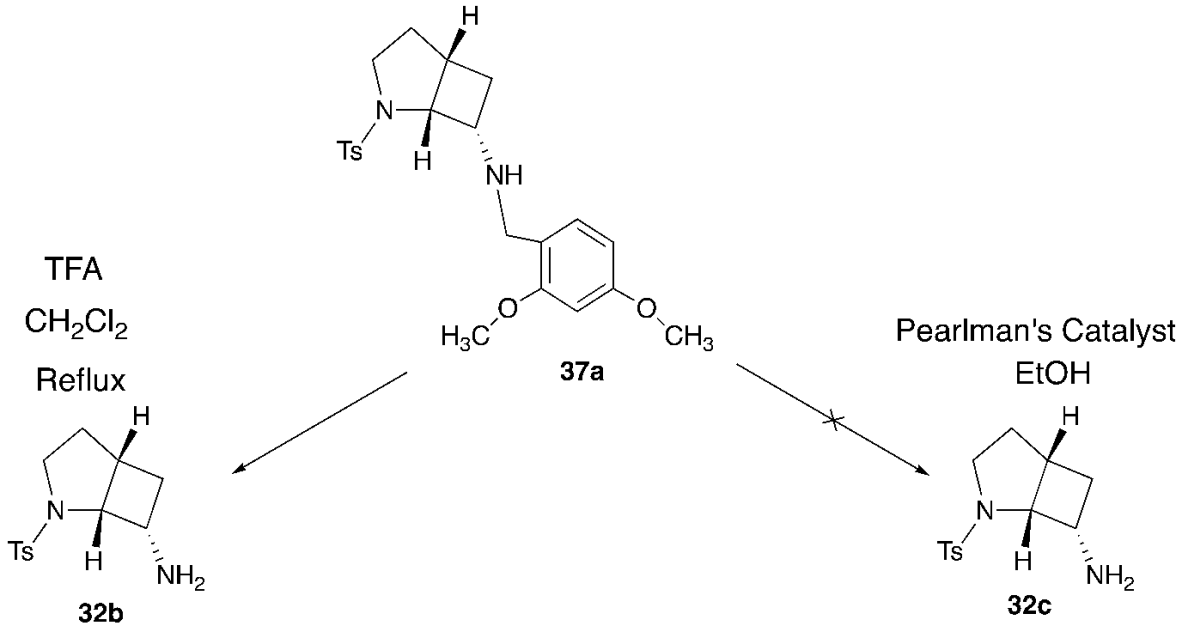


Hydrogenation in ethanol at room temperature using Pearlman's catalyst ($Pd(OH)_2$ on carbon) proved the most suitable catalyst and solvent. This reaction was limited in scale because we observed decreased product yield if more than 1 gram of benzylamine was used. On a 1-gram scale, this reaction can typically obtain a yield of 56% in crude form. However, the primary amine product **36** is hard to purify due to high polarity, and as a result, the crude material was used for future reactions.

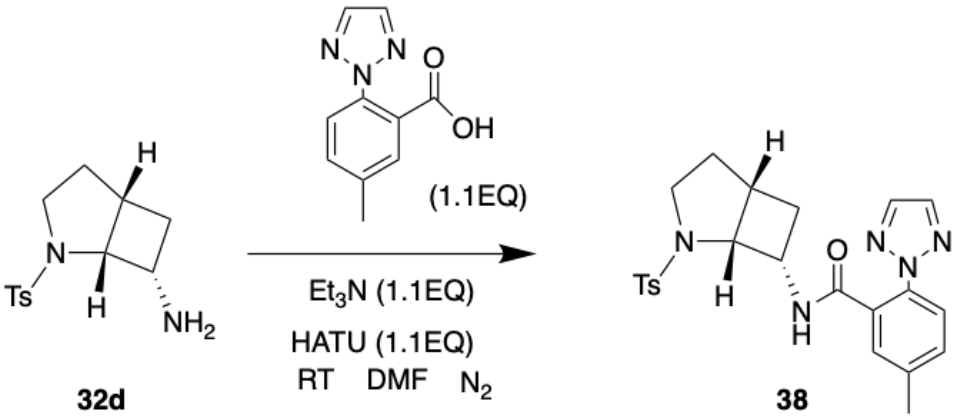


Because this hydrogenation reaction was difficult to scale up, we briefly explored using 2,4-dimethoxylbenzylamine based on the anticipated ability to remove the electron-rich dimethoxybenzyl group with trifluoroacetic acid. However, the crude dimethoxylbenzylamine product **37** was hard to purify to homogeneity. Silica gel chromatography with different solvents

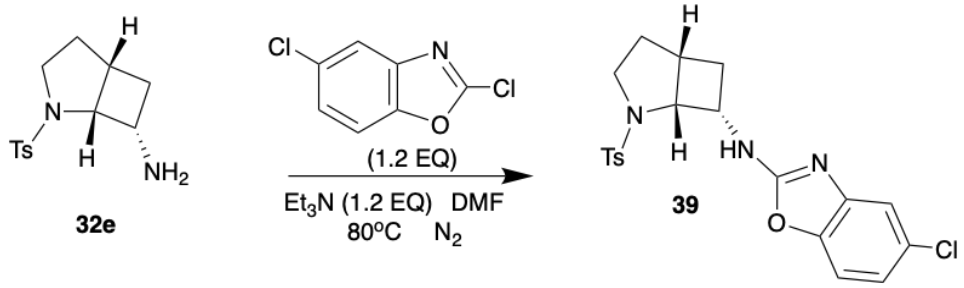
and attempted recrystallization proved unsuccessful, and as a result, it was elected to attempt removal of the dimethoxybenzyl group from crude **37** using trifluoroacetic acid.



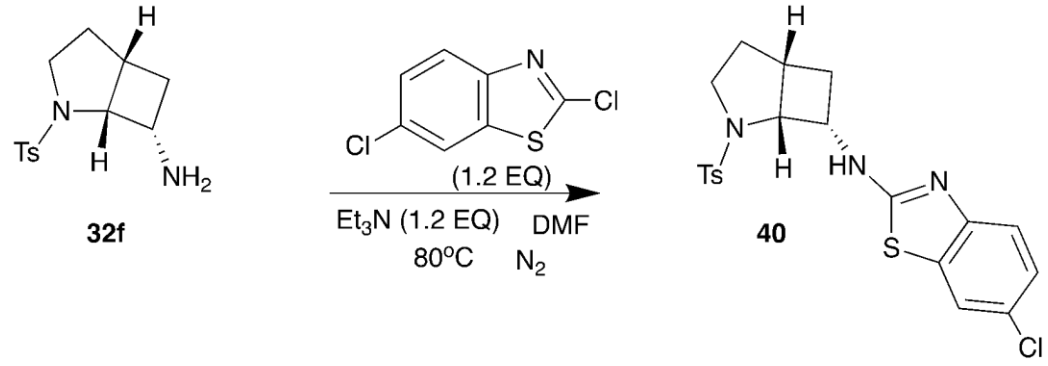
Following the Nussbaumer et al. procedure[18], removing the dimethoxybenzyl with trifluoroacetic acid in DCM was successful in a small-scale (25mg) reaction. The ^{1}H NMR of the crude material **32b** clearly showed product formation by comparison to the crude NMR of the hydrogenation reaction **32**. However, like with the hydrogenation reaction, scale-up proved difficult. Different solvents, temperature, and stoichiometry were explored, and observed a complex mixture of products and/or incomplete reactions. Attempted hydrogenation also failed, and this effort was stopped.



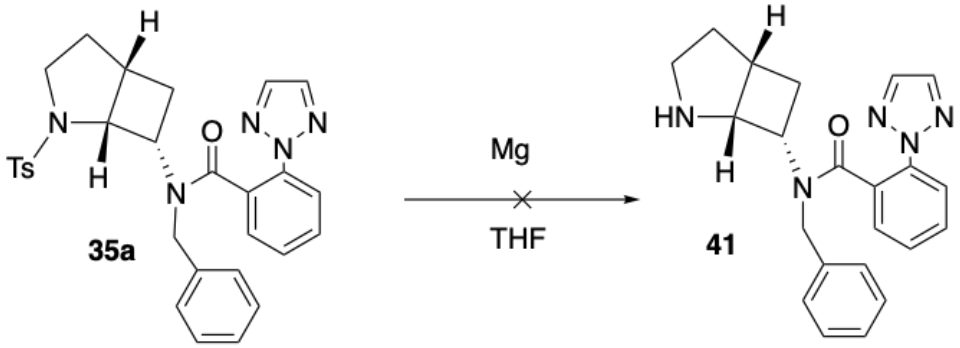
The amidation reaction following Chen et al.'s procedure[19] was carried out using primary amine **32d**. After investigating a variety of coupling reagents and bases, the most efficient combination was HATU, Et_3N, in DMF with a slight excess of carboxylic acid (~1.1 equivalents). The reaction was successful, and the yield for compound **38** obtained was 40-55%. The yield of this reaction is variable based on the use of crude material **32d**.



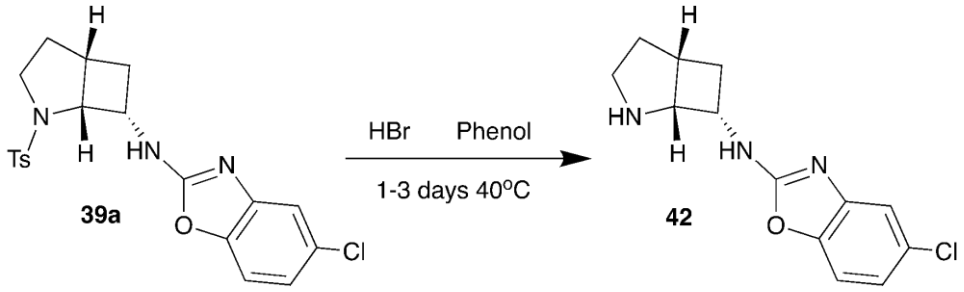
Following the procedure of Boss et al., primary amine **32e** was reacted with a known orexin fragment, 2,5-dicholorobenzooxazole[20]. Using conditions such as K_2CO_3 in DMF at 60°C**,** chloro displacement by **32e** was unsuccessful. Different bases were investigated, such as K_2CO_3 and Et_3N, and solvents such as THF, DMF, CH_3N, and EtOH. Increasing the temperature from 60°C to 80°C provided an increase in product yield. The ^1H NMR and LCMS are consistent with the expected product **39**. The displacement reaction was proven successful, with a 50% yield.



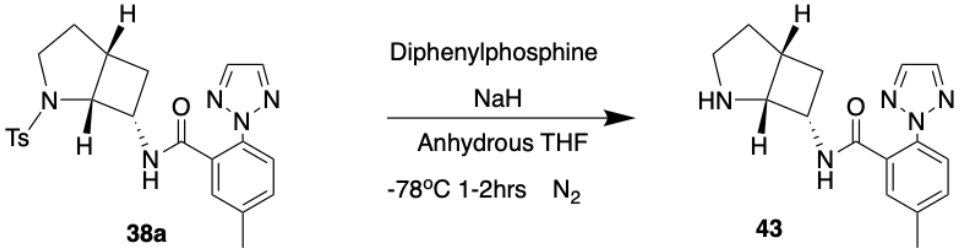
A second orexin building block, 2,6-dichlorobenzothiazole, was used under the same conditions. Again, the reaction was successful, with a 50% yield.



With one of the nitrogen atoms containing orexin fragments in place, the focus is on removing the tosyl amide protecting group to enable the second orexin building block installation. Sonification of **35a** using magnesium turnings in methanol was attempted to cleave the tosyl protecting group[21]. Despite a couple of changes to improve the reaction conditions by using different solvents and washed Mg, the reaction remained unsuccessful. This approach was abandoned.



Weisblat's experimental procedure[22], hydrogen bromide in acetic acid with phenol removed the tosyl protecting group in a small-scale (50mg) reaction. The ^{1}H NMR and LCMS spectra of compound **42** showed evidence for the deprotected product. Removal of HBr in acetic acid on a larger scale proved challenging, making it difficult to scale this reaction up. The polarity of the product **42** made purification by standard silica gel chromatography methods difficult, leading us to investigate other routes for tosyl amide deprotection.



From the procedure reported by Yoshida et al. [23]., the deprotection of the tosyl protection group using diphenylphosphine anion was tested. The reaction was carried out with diphenylphosphine and NaH in THF under N_2 at -78°C for 2 hours. Following an exploratory reaction on a small scale (25mg), TLC analysis showed a presence of a spot with an Rf value similar to that observed following the HBr/phenol reaction mentioned above. The ^{1}H NMR of the crude product indicated the tosyl group had been removed. However, LCMS analysis of the sample suggested a mass of 272g/mol rather than the expected 263g/mol for the deprotected

product **43**. Different conditions need further investigation because this deprotection reaction may potentially remove the protecting group efficiently.

Scheme 2

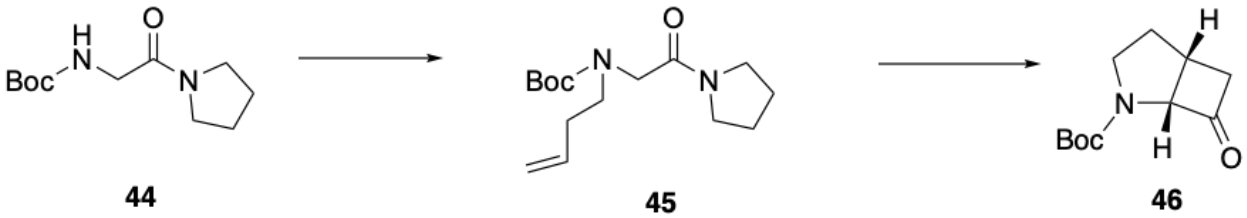


Figure 12. Scheme for the target diamine structure with Boc protecting group.

The removal of the tosyl protecting group posed a challenge during this study. Thus, an alternative route was considered using the tert-butyloxycarbonyl (Boc) protecting group. The removal of the Boc protecting group can be easily removed using TFA. Several approaches and reaction conditions were assessed to obtain the desired ketone **46**.

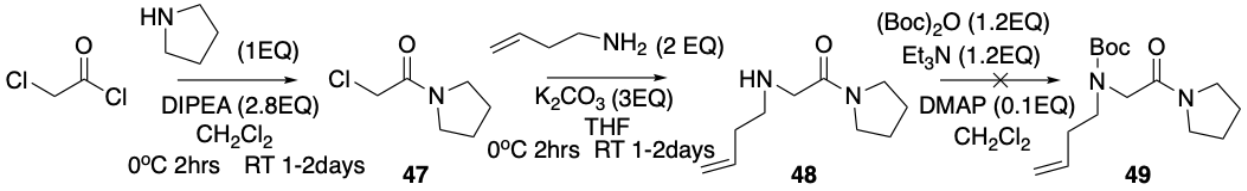


*Figure 13. Synthesis route for the targe structure **49.***

Using the procedure of Robertiet al. procedure[24], the synthesis of chloroacetamide **47** was accomplished in 85% yield. Following chloro displacement with butenyl amine, the presumed product **48** was unreactive in the presence of Boc-anhydride. This suggested product was not the

desired secondary amine and instead was another unidentified compound. Because this issue could not be easily solved, this scheme was abandoned.

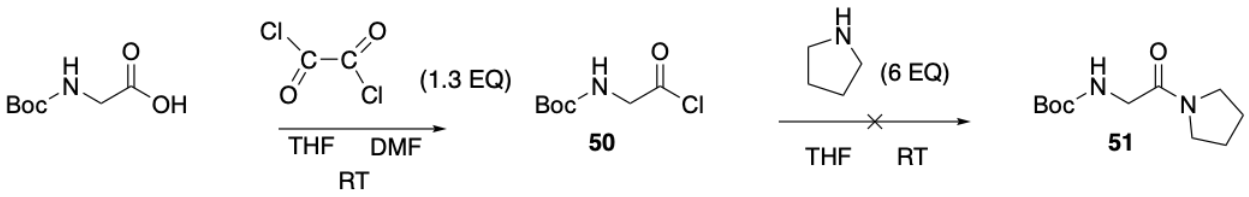


*Figure 14. Synthesis route for target structure **51**.*

This led to the investigation of an alternative approach beginning with Boc-protected glycine. Following a similar route to *Figure 9,* we attempted to prepare acid chloride **50** using oxalyl chloride in THF. However, we discovered that the attempted reaction of the product with pyrrolidine to form amide **51** was unsuccessful. This may be due to either rapid decomposition of the acid chloride or some other undesired reaction to furnish another byproduct. After a couple of unsuccessful attempts to optimize pyrrolidine formation reaction, this route was discontinued.

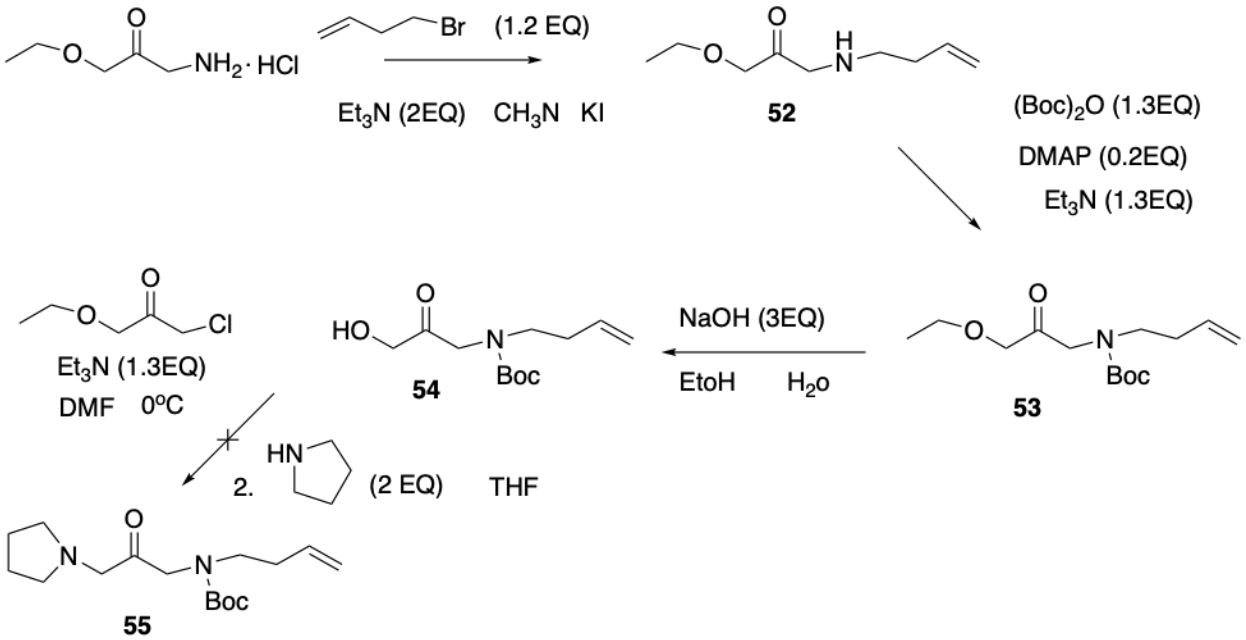


*Figure 15. Synthesis route for the target structure **55**.*

Using Lijun's (2013) procedure[25], the formation of intermediates **52-54** was successful, with a good overall yield of 70-80%. However, there was little to no reaction when adding the pyrrolidine with compound **54**, and this route was discontinued.

Overall, the inability to form the intermediate **45** showed that this Boc protecting route was unsuccessful and incomplete. Further studies are needed to determine the optimal conditions to synthesize intermediate **45**. The succession of obtaining this intermediate will potentially form the desired rigid ethylenediamine scaffold due to the ease of removing the Boc protecting group.

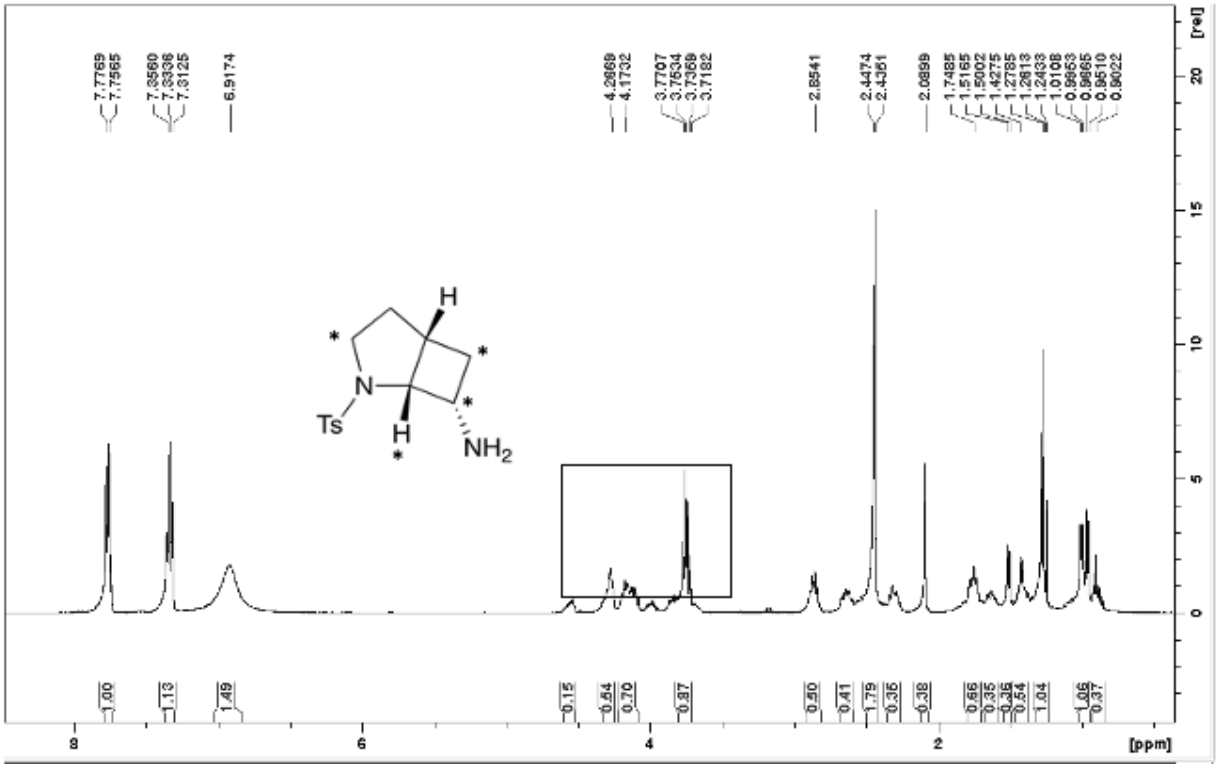
Conclusion and Future Research

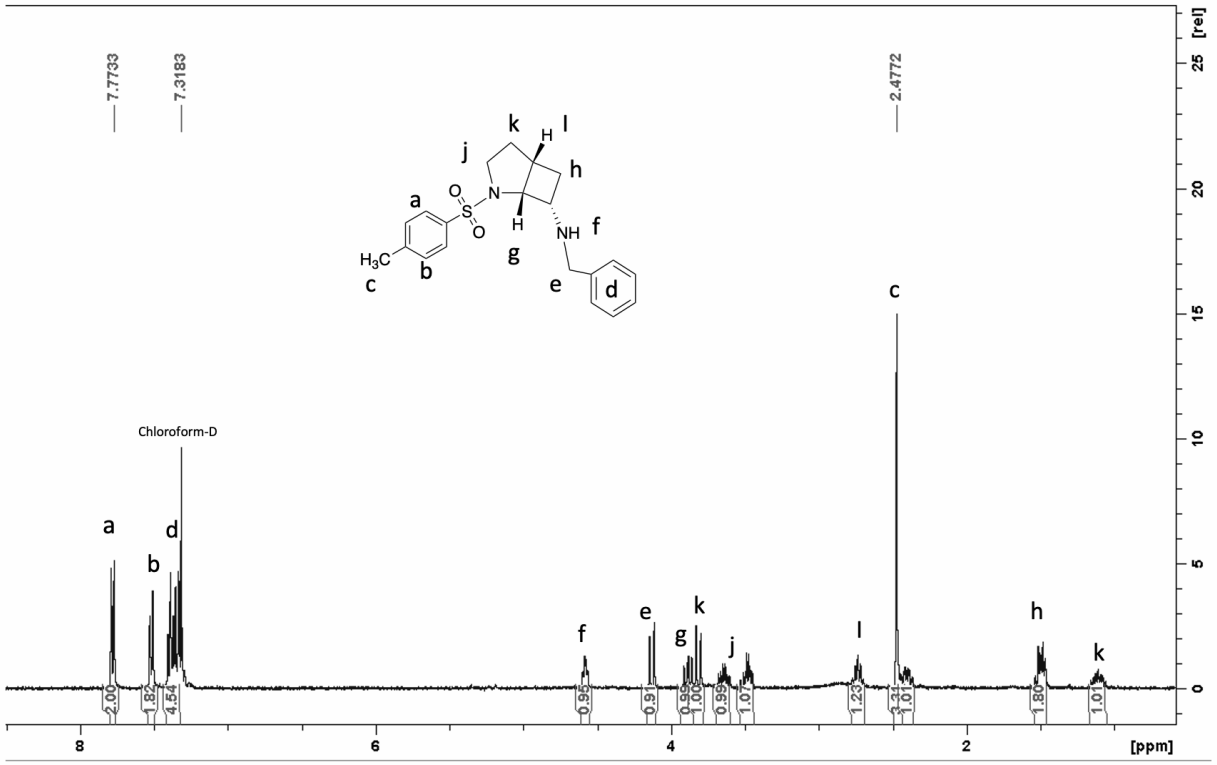
For future consideration, the application of conformationally rigid ethylenediamine scaffolds can potentially be studied for orexin antagonists for improved binding specificity and potency. Exploring the racemic template of the ethylenediamine scaffolds may create an ideal enantiomer(s) for the orexin ligand. This could bring potential drug targets for orexin receptors to treat neurological diseases. However, some issues need to be addressed before testing the ethylenediamine scaffold's activity. These issues include continuing the investigation of removing the tosyl protecting group and installing a second different orexin ligand fragment. In addition, to further investigate the Boc protection group synthesis route as an alternative to forming rigid ethylenediamine scaffolds. The synthesis of a new conformationally rigid ethylenediamine derivative scaffold can create diverse orexin ligand drug designs template. These studies may open new possibilities for conformationally rigid ethylenediamine synthesis and its application to orexin receptor antagonist receptors.

Results

^{1}H NMR

(Crude product)

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2.4772
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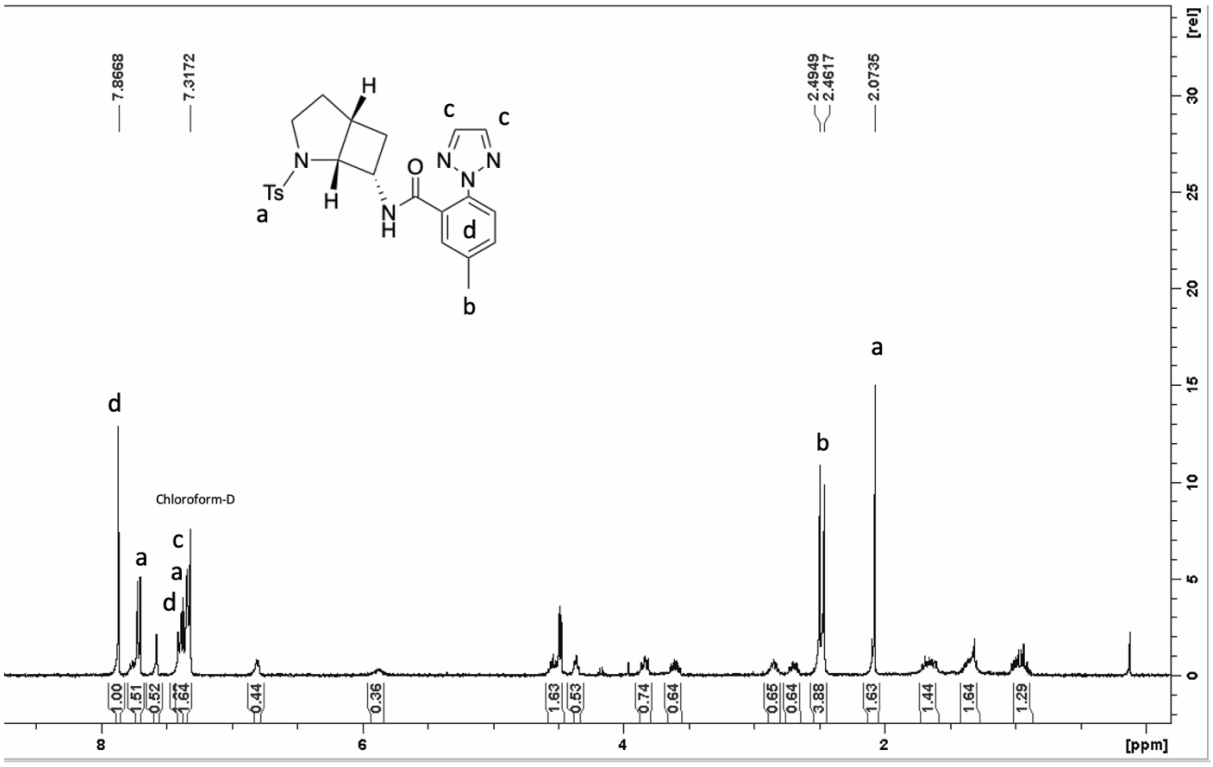
[rel]
[ppm]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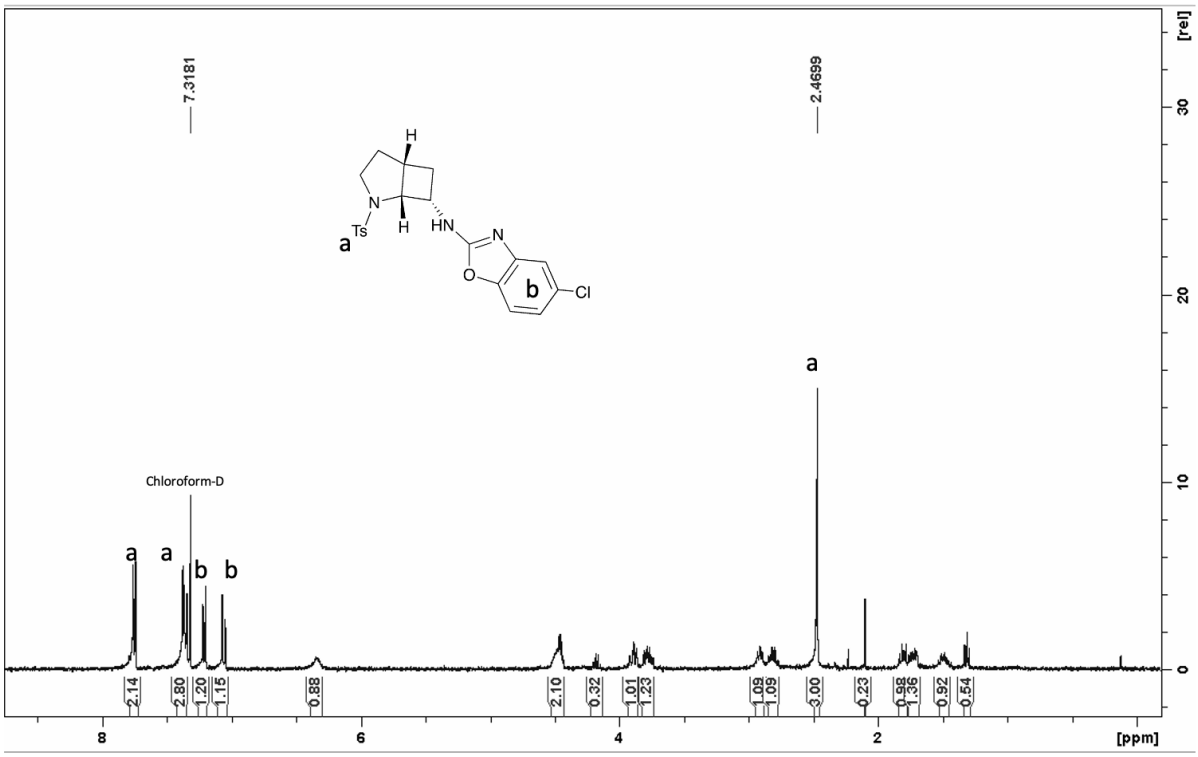
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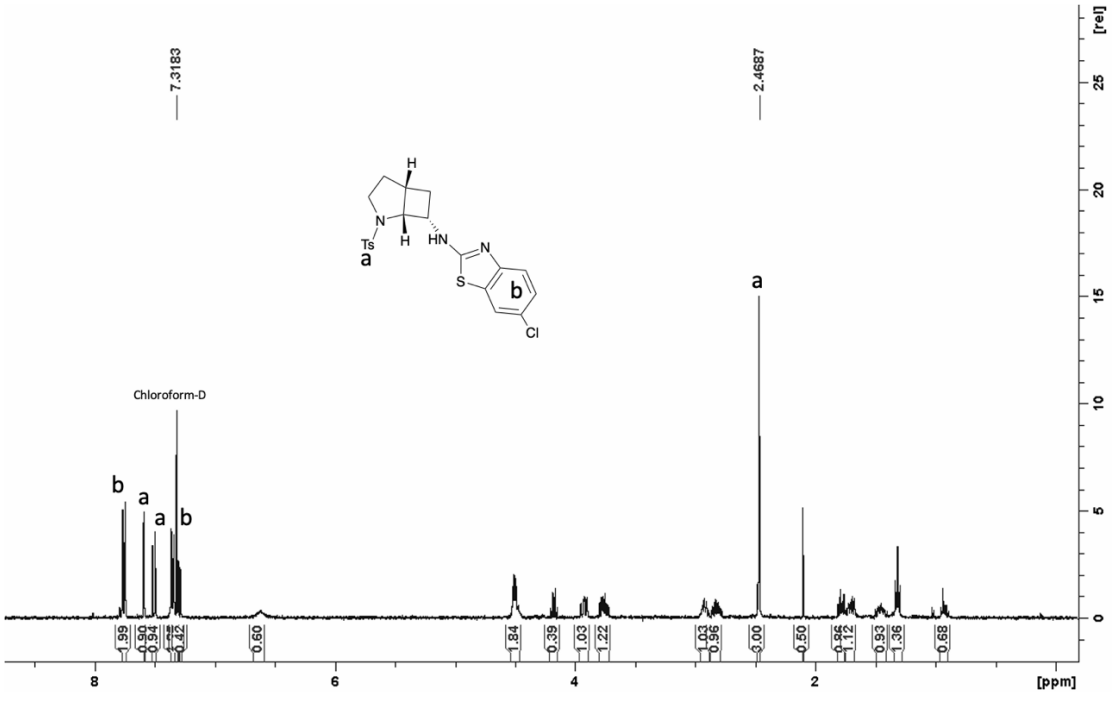
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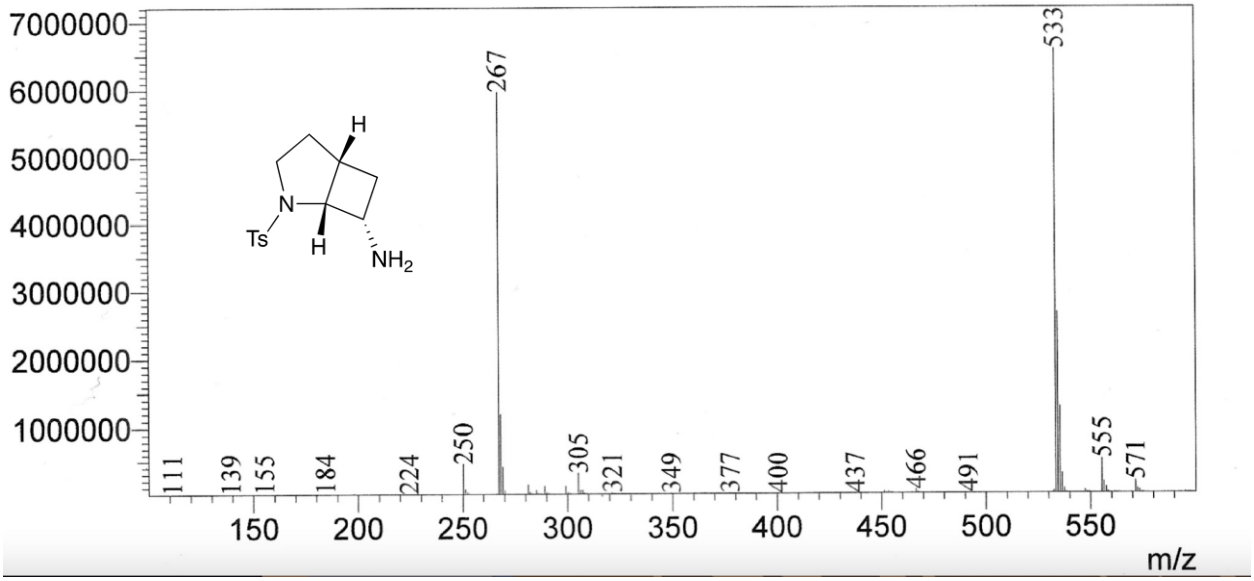
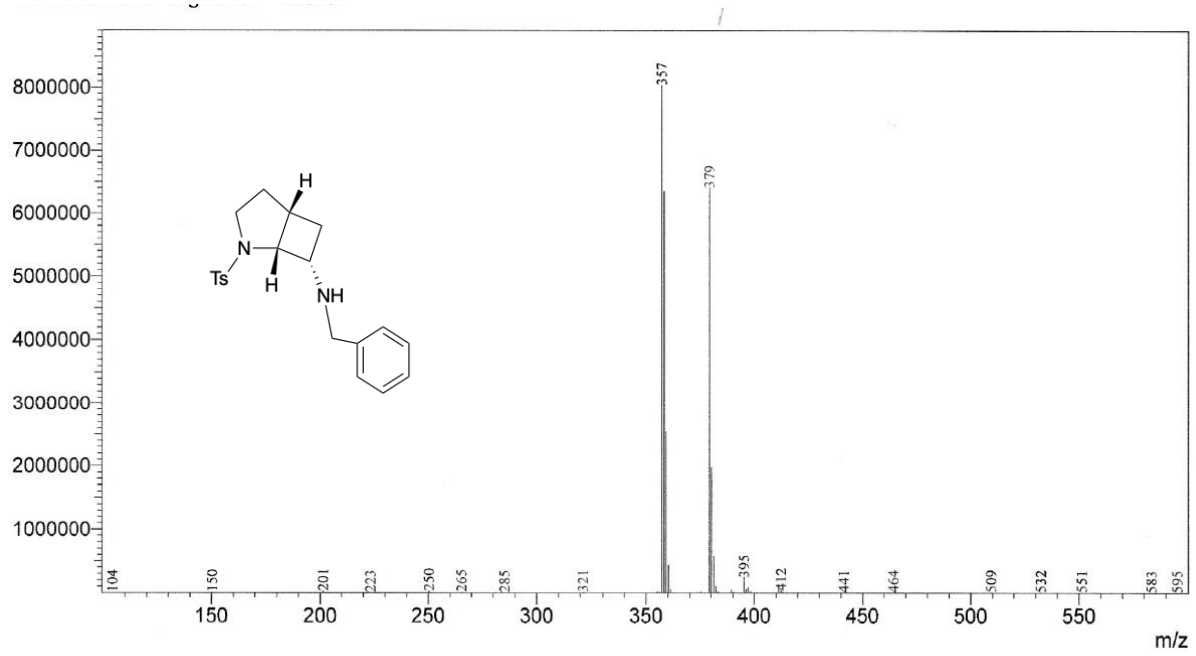
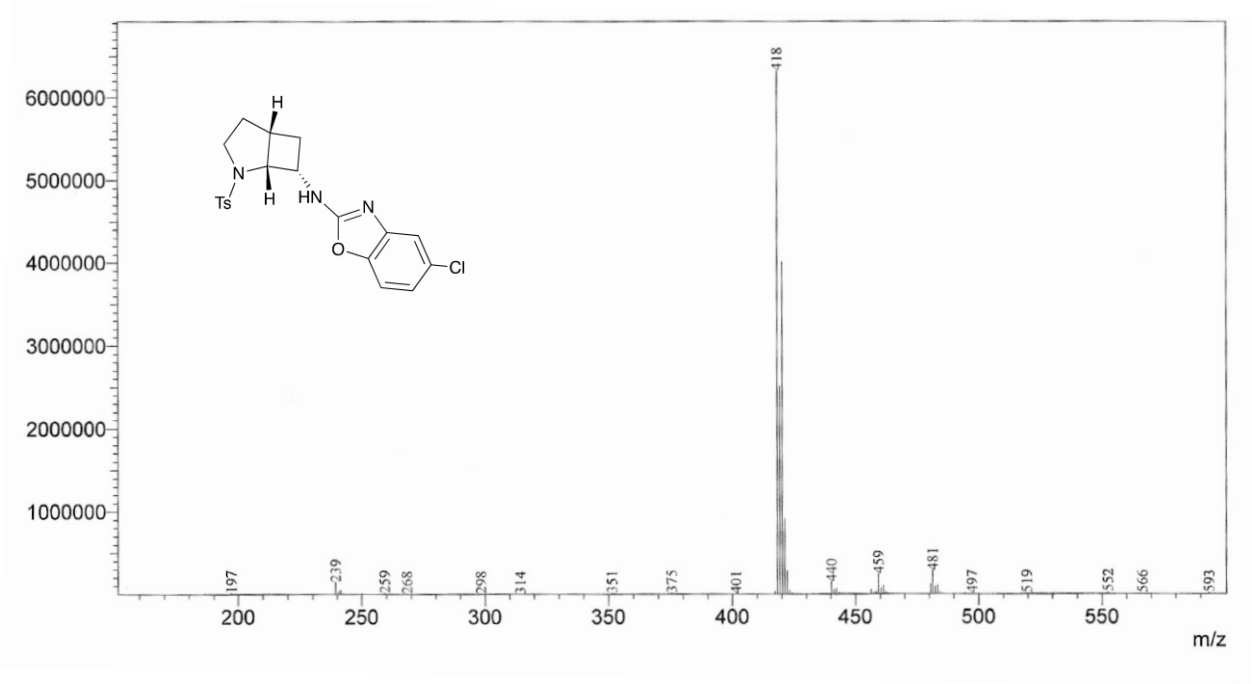
Liquid Chromatography-Mass Spectrometry (LC-MS)



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298
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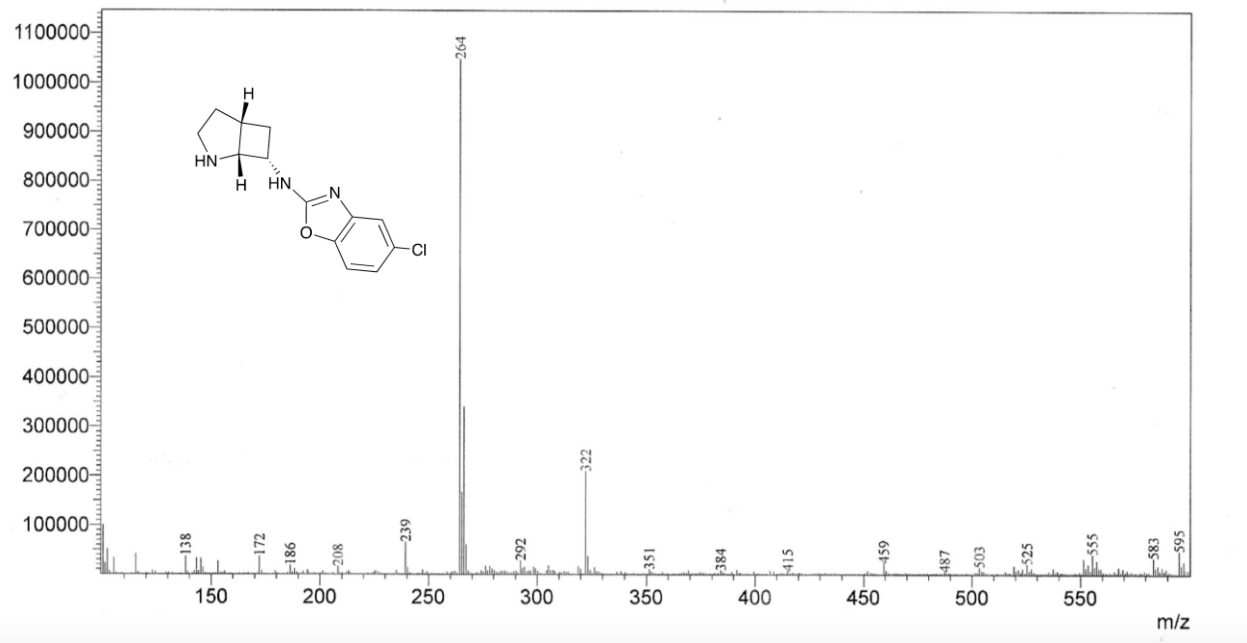
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172
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208
239
292
351
384
415
459
487
503
525
555
583
595
H
HN
H
HN
N
O
Cl

Experimental Method

All ^{1}H NMR spectra were obtained using a 400MHz Bruker NMR Spectrometer in $CDCl_3$, D_6-DMSO, or Acetone-6. The LCMS spectra were recorded using the Shimadzu LC-MS system in methanol. Flash Chromatography was performed using silica gel columns and run using CombiFlash Rf auto column. Thin Layer Chromatography (TLC) was conducted on commercially available silica on alumina plates. The Thin Layer Chromatography (TLC) visualization was done by PMA, $KMnO_4$, Ninhydrin stains, and UV light (254 nm and 365 nm).

Compound 29
Np-Tosyl Glycine (15g, 65.5mmol), oxalyl chloride (16.9ml, 131 mmol), DMF, and CH_2Cl_2(300ml) was stirred at RT for 2-3hrs to form an acid chloride. The reaction was evaporated and put on the pump overnight.

Compound 30
Compound 29(15g, 60.7mmol), pyrrolidine (50.7ml, 607mmol), and THF (300ml) were mixed overnight under N_2. The next day, the reaction mixture was put under the rotavapor to evaporate the THF. The reaction was diluted with 200mL of EtOAc and washed with HCL and brine. The mixture was dried with Na_2SO_4 and concentrated using a rotavapor. Purification occurred on an 80g column and a 3:1 EtOAc: Hexane solvent system. The percent yield was 85%

Compound 31
Compound 30(14.5g, 51.42mmol), K_2CO_3 (4.3g, 30.93mmol), 4-Bromo-but-1ene (3.7ml, 36.12mmols), and DMF were stirred at RT overnight under N_2. The reaction was diluted with 300ml distilled water and extracted with EtOAc. The extracted mixture was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 79%

Compound 32
Under anhydrous conditions, triflic anhydride (366ul, 2.24mmol) was mixed with 1,2-dichloroethane and 1,6-lutidine (173ul, 1.64mmol) was mixed with 1,2-dichloroethane in a separate flask. The compound 7(500mg, 1.49mmol)/1,2-dichloroethane mixture was added dropwise to the mixed triflic anhydride/1,2-dichloroethane mixture, and lastly, the 1,6-lutidine mix was added. The reaction was heated to reflux and stirred under N_2 for 2-3 hours. The mixture was removed from heat and was cooled and concentrated using a rotavapor. 10mL of chloroform and 1mL of acetone were added and stirred at reflux under N_2 for 1 hour. The mixture was cooled, and the chloroform was extracted. The remaining mixture was extracted with DCM and combined with the extracted chloroform mixture. The organic mixture was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred on a 12g column and a 1:1 EtOAc: Hexane solvent system. The percent yield was 76%.

Compound 32b

Compound 37(25mg) was treated with TFA(2ml) in DCM overnight at reflux. The reaction mixture was diluted with sodium bicarbonate and was extracted with DCM. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor.

Compound 33

A mixture of compound 32 (100mg, 0.377mmol), benzylamine (58uL, 0.528mmol), $Na(OAC)_3BH$(112mg, 0.528mmol), a drop of HOac and CH_2Cl_2 was stirred at room temperature for 2-3 hours. The reaction was then washed with sodium bicarbonate, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred with a 95:5 DCM: Methanol solvent system. The percent yield was 85%.

Compound 36

Pearlman's catalyst (150mg, 30% of compound 32) was added to a dry glass vial, then 100ml of ethanol was added, and lastly, intermediate 33(500mg, 1.40mmol). The mixture was stirred and put on the hydrogenator overnight at RT. The reaction was filtered with diatomaceous earth and concentrated using a rotavapor. The water was removed using ethanol and acetone washes. The percent yield was 56%.

Compound 37

Compound 32 (250mg, 0.943mmol), was dissolved in CH_2Cl_2 and 2,4- dimethoxybenzylamine (170ul, 1.13mmol), then $Na(OAC)_3BH$(240mg, 1.13mmol) was added. The mixture was left to stir at room temperature for 3 hours. The reaction mixture was washed with sodium bicarbonate, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 63%.

Compound 38

A mixture of compound 32(360,1.35mmol), 5-methyl-2-(2H-1,2,3, triazol-2-yl) (282mg, 1.49mmol), HATU (566mg, 1.49mmol), Et_3N (101.19ul, 1.49mmol) and DMF was stirred at RT overnight. The reaction was diluted with brine and extracted with EtOAc, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred in a 1:1 EtOAc: Hexane solvent system. The percent yield was 45%.

Compound 39

A mixture of compound 32(360mg,1.35mmol), 2,5-Dichlorobenzooxazole(305mg,1.62mmol), Et_3N (226ul,1.62mmol), and DMF was stirred and heated to 80^oC under N_2 overnight. The reaction was extracted with EtOAc, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred in a 1:1 EtOAc: Hexane solvent system. The percent yield was 55%.

Compound 40

A mixture of compound 32(180mg, 0.80mmol),2,6-dichlorobenzothiazole (196mg,0.96mmol), Et_3N (134ul, 0.96mmol), and DMF was stirred and heated to 80^oC under N_2 overnight. The reaction was extracted with EtOAc, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred in a 1:1 EtOAc: Hexane solvent system. The percent yield was 55%.

Compound 42

Compound 39(500mg, 1.2mmol) was dissolved in 15ml of HBR, and Phenol (475mg, 4.8mmol) was added. The reaction stirred at 40^oC for three days. The mixture was neutralized with sodium bicarbonate until pH reached ~7. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 63%

Compound 47

A mixture of pyrrolidine (671ul, 8.04mmol), DIPEA (4.3ml, 24.78mmol) in CH_2Cl_2(20ml) was stirred at 0^oC. Then chloroacetyl chloride (705ul, 8.85mmol) was added dropwise and was stirred at 0^oC for 1 hour. The reaction mixture was diluted with CH_2Cl_2 and washed with sodium bicarbonate and HCl. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 85%

Compound 48

A 0.3M mixture of 1-amino-3-butene hydrochloride, K_2Co_3 and THF was cooled in an iced bath at 0^oC. Then 0.5M solution of compound 47 and THF was added slowly to the reaction mixture and stirred at 0^oC for 2 hours and was warmed to room temperature and stirred for 1-3days. The reaction mixture was diluted with sodium bicarbonate and was extracted with CH_2Cl_2. The resulting organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 79%

Compound 50

A mixture of N-Boc-Glycine (1g, 5.71mmol) in 20ml of THF with a drop of DMF and oxalyl chloride (641ul, 7.42mmol) in a drop-by-drop and stirred for 2hours and concentrated and was put on the vacuum pump overnight.

Compound 52

A mixture of glycine ethyl ester hydrochloride (30g, 214.8mmol) and CH_3N was stirred in an ice bath at 0^oC, and Et_3N (60ml, 450mmol) was added slowly drop by drop; the reaction was stirred for 10 minutes at 0^oC. Then a spoonful of KI and 4-Bromo-but-lene (24ml, 236.4mmol) was added, and the reaction was left to stir at RT for 48hours under N_2. The reaction was filtered

through celite (Diatomaceous earth) and concentrated using a rotavapor. The percent yield was 79%

Compound 53

A mixture of compound 52(500mg, 3.18mmol), Et_3N (576ul, 4.13mmol), and DMAP (77.7mg, 0.63mmol), and Di-tert-butyl dicarbonate (902.4mg, 4.13mmol) in CH_2Cl_2(10ml) was stirred overnight. The reaction was diluted with CH_2Cl_2 and washed with HCl, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. Purification occurred on a 6:1 Hexane: EtOAc solvent system. The percent yield was 75%.

Compound 54

A mixture of compound 53(5.3g, 21.6mmol), NaOH (2.6g, 64.83mmol), in EtOH(100ml), and H_2O (25ml) was stirred at reflux for 2 hours. The reaction mixture was cooled and concentrated, and drops of 2N HCL were added until a cloudy precipitate was formed. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotavapor. The percent yield was 80%.

References

1. Mao, Fei, et al. “Chemical Structure-Related Drug-Like Criteria of Global Approved Drugs.” Molecules (Basel, Switzerland) vol. 21,1 75. 12 Jan. 2016, doi:10.3390/molecules21010075

2. Grygorenko, Oleksandr O., et al. “Bicyclic Conformationally Restricted Diamines.” Chemical Reviews, vol. 111, no. 9, 2011, pp. 5506–68. Crossref, https://doi.org/10.1021/cr100352k.

3. Tummalapalli, Sreedhar Reddy, et al. “Synthesis and Computational Analysis of Conformationally Restricted [3.2.2]- and [3.2.1]-3-Azabicyclic Diamines.” Tetrahedron Letters, vol. 58, no. 43, 2017, pp. 4087–89. Crossref, https://doi.org/10.1016/j.tetlet.2017.09.033.

4.https://go.drugbank.com/drugs/DB14189.

5. Stability of Metal Complexes and Chelation. The University of the West Indies at Mona, 15 Aug. 2020, https://chem.libretexts.org/@go/page/10912.

6. McDermott, Lee A., et al. “Design and Evaluation of Novel Glutaminase Inhibitors.” Bioorganic & Medicinal Chemistry, vol. 24, no. 8, 2016, pp. 1819–39. Crossref, https://doi.org/10.1016/j.bmc.2016.03.009.

7. Ko, Kwangseok, et al. “Discovery of a Novel Highly Selective Histamine H4 Receptor Antagonist for the Treatment of Atopic Dermatitis.” Journal of Medicinal Chemistry, vol. 61, no. 7, 2018, pp. 2949–61. Crossref, https://doi.org/10.1021/acs.jmedchem.7b01855.

8. Bartole, Edith, et al. “[3H]UR-DEBa176: A 2,4-Diaminopyrimidine-Type Radioligand Enabling Binding Studies at the Human, Mouse, and Rat Histamine H4 Receptors.” Journal of Medicinal Chemistry, vol. 62, no. 17, 2019, pp. 8338–56. Crossref, https://doi.org/10.1021/acs.jmedchem.9b01342.

9. Uzor, Simon, et al. “CDC2-like (CLK) Protein Kinase Inhibition as a Novel Targeted Therapeutic Strategy in Prostate Cancer.” Scientific Reports, vol. 11, no. 1, 2021. Crossref, https://doi.org/10.1038/s41598-021-86908-6

10. Shi, Yihui, et al. “A Triple Exon-Skipping Luciferase Reporter Assay Identifies a New CLK Inhibitor Pharmacophore.” Bioorganic & Medicinal Chemistry Letters, vol. 27, no. 3, 2017, pp. 406–12. Crossref, https://doi.org/10.1016/j.bmcl.2016.12.056.

11. Drop, Marcin et al. “2-Phenyl-1h-Pyrrole-3-Carboxamide as a New Scaffold for Developing 5-HT6 Receptor Inverse Agonists with Cognition-Enhancing Activity.” ACS Chemical

Neuroscience, vol. 12, no. 7, 2021, pp. 1228–1240., https://doi.org/10.1021/acschemneuro.1c00061.

12. Préville, Cathy, et al. “Substituted Azabicyclo[2.2.1]Heptanes as Selective Orexin-1 Antagonists: Discovery of JNJ-54717793.” ACS Medicinal Chemistry Letters, vol. 11, no. 10, 2020, pp. 2002–09. Crossref, https://doi.org/10.1021/acsmedchemlett.0c00085.

13. Stump, Craig A., et al. “Discovery of Highly Potent and Selective Orexin 1 Receptor Antagonists (1-SORAs) Suitable for in Vivo Interrogation of Orexin 1 Receptor Pharmacology.” Bioorganic & Medicinal Chemistry Letters, vol. 26, no. 23, 2016, pp. 5809–14. Crossref, https://doi.org/10.1016/j.bmcl.2016.10.019.

14. Cox, Christopher D., et al. “Conformational Analysis of N, N-Disubstituted-1,4-Diazepane Orexin Receptor Antagonists and Implications for Receptor Binding.” Bioorganic & Medicinal Chemistry Letters, vol. 19, no. 11, 2009, pp. 2997–3001. Crossref, https://doi.org/10.1016/j.bmcl.2009.04.026.

15. Futamura, Aya, et al. “Identification of Highly Selective and Potent Orexin Receptor 1 Antagonists Derived from a Dual Orexin Receptor 1/2 Antagonist Based on the Structural Framework of Pyrazoylethylbenzamide.” Bioorganic & Medicinal Chemistry, vol. 25, no. 20, 2017, pp. 5203–15. Crossref, https://doi.org/10.1016/j.bmc.2017.07.051.

16. Sans, M., et al. “Organobridged Silsesquioxanes Based on Cyclobutane Diamines: Influence of the Stereochemistry on the Morphology of the Materials.” Tetrahedron, vol. 72, no. 22, 2016, pp. 2913–19. Crossref, https://doi.org/10.1016/j.tet.2016.04.001.

17. Marko, Istvan, et al. “Intramolecular [2+2] Cycloadditions of Ketenes and Keteniminium Salts to Olefins.” Journal of the American Chemical Society, vol. 107, no. 7, 1985, pp. 2192–94. Crossref, https://doi.org/10.1021/ja00293a073.

18. Nussbaumer, Peter, et al. “Highly Selective TFAA-Cleavage of Tertiary 2,4-Dimethoxybenzylamines and Its Use in the Synthesis of Secondary Amines.” Tetrahedron, vol. 47, no. 26, 1991, pp. 4591–602. Crossref, https://doi.org/10.1016/s0040-4020(01)86465-6.

19. Chen, Yin, et al. “Facile Synthesis of Suvorexant, an Orexin Receptor Antagonist, via a Chiral Diazepane Intermediate.” Chinese Chemical Letters, vol. 26, no. 1, 2015, pp. 103–07. Crossref, https://doi.org/10.1016/j.cclet.2014.09.020.

20. Boss, C., et al. "3, 8-Diazabicyclo [4.2. 0] octane derivatives as orexin receptor antagonists and their preparation and use for the treatment of diseases." WO 2012085857 (2012).

21. Martín-Encinas, Endika, et al. “Synthesis of Novel Hybrid Quinolino[4,3-b][1,5]Naphthyridines and Quinolino[4,3-b][1,5]Naphthyridin-6(5H)-One Derivatives and Biological Evaluation as Topoisomerase I Inhibitors and Antiproliferatives.” European Journal of Medicinal Chemistry, vol. 195, 2020, p. 112292. Crossref, https://doi.org/10.1016/j.ejmech.2020.112292.

22. D. I. Weisblat, B. J. Magerlein, and D. R. Myers Journal of the American Chemical Society 1953 75 (15), 3630-3632 DOI: 10.1021/ja01111a007

23. Yoshida, Suguru, et al. “Nucleophilic Substitution Reaction at the Nitrogen of Arylsulfonamides with Phosphide Anion.” Journal of the American Chemical Society, vol. 134, no. 47, 2012, pp. 19358–61. Crossref, https://doi.org/10.1021/ja309642r.

24. Roberti, Marinella, et al. “Rad51/BRCA2 Disruptors Inhibit Homologous Recombination and Synergize with Olaparib in Pancreatic Cancer Cells.” European Journal of Medicinal Chemistry, vol. 165, 2019, pp. 80–92. Crossref, https://doi.org/10.1016/j.ejmech.2019.01.008.

25. Sun, L., et al. “Preparation of macrocyclic compounds for the treatment of cancer.” W2013013240(2013).