Studies Toward the Synthesis of a Novel Diamine Scaffold

Joseph Edward Quinlan III
Abstract:

The orexin system in humans contains receptors Orexin 1 (OX1) and Orexin 2 (OX2). These receptors are involved in numerous physiological processes including wake/sleep cycling, energy homeostasis, and motivation/reward. In scientific literature, it is known that antagonism of these receptors exhibits therapeutic effects in the realm of cocaine and alcohol addiction, as well as sleep disorders. On the market today exist drugs for treatment of sleep disorders through orexin receptor antagonization. Here we report a synthesis toward novel conformationally rigid antagonists of the orexin receptor system that may provide improved affinity and/or selectivity compared to known compounds. We report the synthesis of a conformationally-restricted bicyclic diamine scaffold with intrinsic structural properties that could assist in improving the binding affinity of known orexin receptor antagonists by enabling the optimization functionalization of chemically distinguishable endocyclic nitrogen atoms.
MONTCLAIR STATE UNIVERSITY

STUDIES TOWARD THE SYNTHESIS OF A NOVEL DIAMINE SCAFFOLD

By

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Introduction:

Since their near simultaneous discovery by de Lecea *et al.* and Sakurai *et al.* in 1998, the Orexin/Hypocretin pair of G-protein coupled receptors (GPCRs) have been the epicenter of much active research (de Lecea *et al.* 1998, Sakurai *et al.* 1998). Due to their involvement in numerous physiologic pathways, research into development of novel pharmacotherapies is actively underway (Kukkonen 2014). The orexin system includes receptors Orexin 1 (OX1) and Orexin 2 (OX2), which are activated by neuropeptides orexin A (OX-A) and orexin B (OX-B) (Nuñez *et al.* 2009) and largely reside in the lateral hypothalamus. It has since been discovered that OX-A has reasonable binding affinity for not only OX1 but OX2 as well, though preferential binding affinity is for OX1. Conversely, OX-B has significant binding affinity only for OX2. This difference in binding plays a large role in the receptors’ value as drug targets and their downstream effects. In 1954, the lateral hypothalamus was identified as an important region of the brain for reward and self-stimulation (Olds & Milner, 1954). Though they maintain the highest density within the lateral hypothalamus, the orexin receptor system exhibits an extensive reach through neural networks, providing the neurochemical basis for this system to play a large role in regulation of a wide range of central nervous system (CNS) functions (Chen *et al.* 1999).

Many neurological diseases have been associated with the lateral hypothalamus and, more specifically, the orexin receptor system. Extensive research is currently ongoing to treat chronic sleep disorders such as narcolepsy through targeting of the orexin system. Indeed, much of the understanding of the orexin receptor system’s hand in sleep-wake regulation has come as a result of narcolepsy-cataplexy research (Chow & Cao 2016). Early research in the orexin receptor system have linked canine narcolepsy sleep disorder as a mutation in the OX-2 receptor (Lin *et al.* 1999). Multiple studies have shown that deficiencies in expression of orexin receptors and neuropeptides
has been linked with narcolepsy in humans (Chen et al. 2018), where some 90% of narcoleptics with cataplexy exhibit orexin neuropeptide deficiency (Zeitzer et al. 2006). This has been identified largely through disruption of the orexin receptor system, which has shown to produce narcoleptic symptoms in animal models (Lin et al. 1999). Though OX1 antagonism can exhibit a nonnegligible effect on sleep-wake cycling, more substantial response is demonstrated through OX2 (Beuckmann et al. 2004) or OX1/OX2 antagonism, with double antagonism exhibiting the most drastic phenotype (Scammell & Winrow, 2011).

Similarly, the OX-A neuropeptide has been found to exist in neural fibers associated with sleep-wake regulation. Owing to this, the orexin receptor system is dutifully tied to initiating and maintaining sleep and has substantial links in the pathogenesis of insomnia (de Lecea et al. 2005). Largely characterized by symptoms of difficulty falling asleep and maintaining sleep, insomnia is a pervasive disorder in much of the adult human population. Studies into the cause of insomnia showed that loss of orexinergic neurons resulted in extreme sleepiness in animals (Liblau et al. 2016). OX-A levels had been found to directly correlate with wakefulness, showing lower OX-A levels upon awakening, a gradual increase as the day progressed, and a tapering of OX-A nearing nightfall (Zeitzer et al. 2003). Much like issues with narcolepsy, the opposite problem of orexin expression also seems likely to be one of the roots of insomnia. Not only have OX-A levels been associated with time of day, but direct testing of the effects of OX-A levels and wakefulness have been researched. When administered near the onset of normal sleep periods, OX-A produced a dose-dependent increase in the time rats spent awake during the second and third hour after dosing (Piper et al. 2000). This fundamental research has given hope that insomnia can be combatted through antagonism of the orexin receptor system.
Orexinergic stimulation has been shown to promote reward-seeking behavior in animal models as well. This includes not only seeking of highly palatable foods, but also drugs and alcohol. Inactivation of the lateral hypothalamus has been shown to cease resumption of addictive or reward-generating behaviors such as alcohol and sucrose consumption (Marchant et al. 2009). The increased indulgence in reward-generating behaviors as a result of stimulation by OX-A was shown when mice and rats were injected with OX-A and exhibited a higher rate of ingestion of highly palatable foodstuffs when compared to control groups. In the same paper, Martin-Fardon showed that after repeated self-administration, activation of orexinergic neurons was substantially higher for rats self-administering cocaine than rats self-administering sweetened condensed milk, showing the likelihood of relapse in repeated exposure of non-natural stimulants (Martin-Fardon et al. 2016). Similarly, injection of OX-A has been shown to promote cocaine-seeking behavior in mice with a history of cocaine dependence and that the effects of the OX-A injection are mediated by OX2 (Matzeu et al. 2016). In the same vein of thought, the work of Schneider et al showed that administration of OX-A into the lateral hypothalamus was met with ethanol intake by ethanol-drinking rats (Schneider et al 2007). Hypothalamic orexinergic neurons appeared in higher numbers in animal models who are exposed to ethanol availability (Dayas et al. 2008). As one would expect, administration of OX1 and OX2 antagonists has seen a marked decrease in consumption of alcohol in high-drinking rodent models (Anderson et al. 2014). As a result of these findings, the orexin receptor system has been an emerging area of focus for combatting drug and alcohol addiction.

Development of dual orexin receptor antagonists (DORAs) and OX2 selective orexin receptor antagonists (2-SORAs) for treatment of sleep disorders and other neurological diseases has been fruitful in the 22 years since the discovery of the orexins. However, development of OX1
SORAs (1-SORAs) has proven to be much more elusive. For the most part, clinical stage orexin antagonists have been developed in absence of well-defined structures of these receptors. Without knowledge of the specific discrepancies between the two receptors, the development of nonspecific DORAs has been substantially more successful than selective antagonists. Despite this, a wide variety of orexin antagonists share general structural motifs. Common orexin antagonists follow a general structural formula of two aromatic moieties joined by a scaffold (Lawrence 2017). It has since been determined the importance of not only these chemical motifs but also the general structure these ligands must adopt in order to successfully bind to the active sites of the receptors.

The first orexin receptor antagonist approved by the FDA, suvorexant (MK-4305) (1) was approved for use in the United States in 2014 for treatment of sleep disorders (mrknewsroom.com). As a trailblazer for the viability of orexin antagonists for treatments of neurological diseases, 1 has been the point of focus for many orexin antagonism studies. As a dual antagonist, 1 exhibits reasonable binding affinity and antagonistic effects to both OX1 and OX2. IC$_{50}$ values were determined for 1 in both OX1 and OX2, valuing 0.68 nM and 0.42 nM, respectively (Jenck et al. 2017). Multiple studies have been conducted to observe what conformation 1 adopts within the active sites of both of these receptor (Christopher et al. 2020). Using the flexibility of the homopiperazine scaffold, 1 adopts a cis conformation where both aromatic groups fold toward each other, giving the overall molecule a “U” shape where the toluene and benzoazole moieties are in close proximity.
Much like 1, filorexant (MK-6096) (2) was developed by Merck in the exploration of orexin antagonists useful in combatting insomnia. Due to its general structural similarity to 1, 2 also exhibits binding affinity to both OX-1 and OX-2. Though not as groundbreaking as 1 for success due to it failing clinical trials, much can be learned from 2’s similar mode of binding to OX1 and OX2, with $K_i$ values of 11 nM for both OX1 and OX2 (Winrow et al. 2011). Despite having limited success for mitigating side effects, 2 exhibited firm binding to both orexin receptors via a similar conformation to 1, indicating conformation may play a role in binding affinity of orexin antagonists in OX-1 and OX-2.

Lastly, daridorexant (ACT-541468) (3), developed by Idorsia Pharmaceuticals, carries regiochemical, conformational, and binding affinity similarities to both 1 and 2. IC$_{50}$ values were determined to be 0.52 and 0.78 nM for OX1 and OX2, respectively (Jenck et al. 2017). Intended to treat insomnia much like the aforementioned DORAs, 3 sought to tackle the issue of daytime somnolence that is seen in patients who take 1 through a faster metabolic half-life (Jenck et al. 2017).

All of these orexin antagonists include structural similarities that result in affinity for the receptors. Structural homology includes of two aromatic moieties bridged by a conformationally flexible scaffold and is pervasive among SORAs and DORAs alike. These structural motifs have
been later shown to have properties that greatly affect ligand binding. Specifically, the flexibility of the scaffolds enables these molecules to adopt the necessary “U” shape to effectively bind to the active site of both OX1 and OX2. Binding occurs as a result of the ability of the molecule in its bound conformation to engage in π-stacking both intramolecularly and with nearby histidine and phenylalanine residues on the receptor (Christopher et al. 2020). This observation is consistent with a paper from Merck that employed NMR to observe chemical shifts of specific protons suggesting ORX antagonists adopt U-shaped conformations to create an intramolecular π-stacking between the aromatic regions of the molecule. However, the existence of aromatic moieties alone is not the sole reason behind the adoption of a U shape. Through synthetic deduction, the conformation of similar structured molecules that lack either an aromatic moiety or the carbonyl group on the amide linker was determined. It was seen that missing either piece of the structure significantly reduced the molecule’s binding affinity for the orexin receptors (Cox et al. 2009). More notably, in solution phase, molecules with homopiperazine scaffolds, like 1, are likely to adopt chair or twist-chair conformations. As a result, in order to adopt the boat conformation that enables more successful π-stacking, the molecule must spend an energetic penalty to alter the scaffold conformation to bring the aromatic moieties together. However, π-stacking alone is not suitable enough stability to promote this change alone (Cox et al. 2009). The implementation of flexible scaffolds remains consistent among numerous known orexin antagonists, likely for their ability to bend to the necessary shape but does not represent an energetically ideal scaffold for orexin antagonists.

Orexin-targeting pharmaceuticals are on the forefront for discovery as novel treatments of many neurological diseases. Although these drugs are relatively new in the field, they possess great potential to treat a swath of neurological disorders. Despite the wealth of research being conducted
on the development of SORAs and DORAs, only 2 such drugs have been approved by the FDA to date. The need for new and better orexin antagonists is growing as we more fully understand their potential to treat disease.

In order to create the most ideal orexin antagonist, the design of the drug must aide in bringing the drug closer to the biologically active conformation. Size of the scaffold is also important, as variations in size can lead to unfavorable interactions with the receptor as was seen with alkyl linkers in the synthesis proposed by Cox et al (Cox et al. 2009). With these parameters in mind, implementation of a conformationally rigid diamine scaffold should best suit the biologically active conformation necessary in orexin antagonists. Like the scaffold for 1, the ethylene diamine derivative enables a myriad of functions that can apply far beyond the applications of orexin antagonists. As a result, construction of a conformationally rigid ethylenediamine derivative for use as a scaffold will create a diverse template from which numerous drugs of all varieties can be designed.

Our hypothesis is that a template that is conformationally rigid that incorporates known orexin receptor fragments will lead to novel orexin antagonists that may display improved potency and/or selectivity for one or both receptors. The implementation of a more conformationally rigid scaffold that adopts the necessary cis structure should aide in ligand binding to the receptor and molecular features that influence receptor selectivity.

In this thesis, we report progress toward the synthesis of a conformationally rigid diamine scaffold and apply it to the discovery of novel orexin antagonists. Our synthetic plan employs a chiral starting material ((S)-proline) and proceeds in a stereospecific manner that will result in a chiral scaffold. This will allow us to prepare chiral ligands in a straightforward manner to assess
the effect of stereochemistry in the template to provide a more complete picture of ligand-receptor interactions.

**Discussion:**

Synthesis of the rigid scaffold, in part, followed a synthetic route for stereoselective β-lactams via amination of unactivated C(sp³)-H bonds of carboxamides (Wu et al. 2016). This synthetic route was the inspiration for construction of a rigid ethylenediamine derivative scaffold for use in orexin antagonists.

**Scheme 1**

Despite the reported reaction conditions in the synthesis of the β-lactams, several changes to reaction conditions were assessed and employed throughout the synthesis of the rigid scaffold. Amide coupling to form intermediate 4 went as planned in high yield. C-H bond activation for cyclization of the prolineamide intermediate 4 was originally carried out in a microwave reactor at 160 °C for 90 minutes. However, this reaction proved to be problematic. Issues downstream in the synthesis and inconsistent yield were the largest factors for attempting alternative reaction
conditions, but variable reaction pressures and stability also played a role. Scaling up the microwave reaction proved to be capricious, leading to complex mixtures, difficult chromatography and low yields. Relatively higher yields were observed on smaller scales, requiring numerous small-scale reactions to produce a meaningful quantity of intermediate 5. After numerous attempts to investigate changing stoichiometry, temperature, and reaction time, the viability and scalability of the microwave reaction was remarkably limited.

When using the microwave, the reaction time was very specific, any longer under that temperature and the pressure of the vial would increase too greatly. If the reaction were left to the typical 90-minute heating time, a substantial amount of unreacted starting material remained. This made purification via flash column chromatography challenging due to the high degree of similarity in chromatographic mobility between 4 and 5. Purification proved excessively difficult and often-times coelution would occur even under the most careful of scrutiny. As a result, yields were never more than 60%, and often the eluted fractions came impure. When these impure fractions were carried forward in the sequence, only the lactam reacted, leaving behind the prolineamide starting material 4 which was easily separated from the desired lactam 6. However, this process exhibited exceptionally poor yield and thus alternate methods were explored (see below).

Following the central synthesis scheme, oxidative cleavage of the aminoquinoline directing group to generate 6 was performed initially at room temperature for 45 minutes following a protocol from the literature (Wu et al. 2016). In our hands this proved to be deleterious to the starting material, exhibiting over oxidation upon contact and complete degradation of the starting material. This appeared to be the case after numerous attempts and variations of ratio of oxidant/reductant, water, and reaction time. To address this, lower temperatures were investigated,
beginning at -45 °C. However, after 8 hours, the reaction using 3 equivalents of ceric ammonium nitrate showed no reaction and complete integrity of the starting material. Thus, higher reaction temperatures were investigated. We were pleased to observe rapid (2 hours), complete, clean reaction at -20 °C in an ice/salt bath.

**Scheme 2**

An alternative synthetic route to form intermediate 7 was also explored as a means to circumvent the need for oxidative cleavage to create 6, which proved to be a reasonably low yield reaction at the time. In place of 4, commercially available (S)-1-Cbz-2-aminomethylpyrrolidine was acylated with picolinic acid to form 8 Further C-H bond activated cyclization was attempted in modeling our reaction conditions after the work of Chen *et al.*, who showed the success of converting (S)-valine into β lactams through the use of acetic acid, PhI(OAc)₂, and palladium(II) acetate (Chen *et al.* 2012).
Several reaction conditions and reagents were assessed in the direction of this synthesis, particularly in the cyclization to form intermediate 9. Following the procedure from Chen et al, we did not observe the desired product 24 hours in refluxing toluene. We then assessed if the cyclization would proceed using the iodopentafluorobenzene solvent in the microwave, however this reaction, too, produced no desirable product. As a result, we abandoned this synthetic route in favor of optimization of the original synthesis.

After failed attempts at alternative synthetic routes, we explored the viability of closed-system reflux under thermal conditions for cyclization to generate 5. Interestingly, we were able to acquire the same product in better yield, about 90%, through a thermal reflux using the same catalyst and solvent system as the previous microwave conditions. After initial reaction preparation akin to normal microwave cyclization, the sealed vial was instead placed into an oil bath and heated to 160 °C. The vial was covered in aluminum foil to ensure constant temperature and left to react for 18 hours. After 18 hours, TLC analysis showed formation of 5 with a small amount of unreacted starting material. The reaction was left to reflux for another 12 hours in an attempt to consume the remaining starting material. Much to our disappointment, this resulted in total reactant and product degradation. Ultimately, the reaction was left with an 18-hour reaction time to deliver significantly higher yield than under the microwave conditions.

Reduction of the lactam ring in 6 to form 4 was performed using borane dimethyl sulfide as a 2M solution in refluxing THF. TLC analysis, accompanied by ninhydrin staining, revealed the presence of the desired product 7. This reaction went reasonably smoothly, however we decided to attempt reduction using LiAlH₄ in refluxing diethyl ether as a means to get better yield and a possibly faster reaction time. However, issues with solubility of starting material in ether as
well as unclean NMR spectra warranted changing conditions back to borane for the completed synthesis of the ethylene diamine scaffold.

**Structural Analysis**

![Filorexant bound to OX1 active site (PDB ID 6TP6)](image1)

![Daridorexant bound to OX1 active site (PDB ID 6TP3)](image2)

![Suvorexant bound to OX2 active site (PDB ID 4S0V)](image3)

![Suvorexant bound to OX1 active site (PDB ID 4ZJ8)](image4)

Based on crystal structure of OX1 fitted with model drug 1 (PDB ID 4ZJ8), a *cis* (boat) conformation is observed in the homopiperazine ring when the drug is bound to the active site of OX1. This conformation is important as it allows for placement of the benzoazole ring, or more importantly, the meta chlorine, into a hydrophobic subpocket of residues W112 and V106. This
interaction greatly aids in binding affinity and subsequent orientation of the drug. In this position, it is reported in the literature that a π-stacking interaction occurs between the benzoxazole and the toluene group, which is further stabilized by an offset π-stacking interaction with H344 (Christopher et al. 2020). This π-stacking not only enforces stabilization of the conformation of the molecule but stabilization of the drug within the active site.

Being a dual antagonist, the binding of 1 to OX2 has also been crystallographically studied (PDB ID 4S0V) (Christopher et al. 2020). Much like the binding to OX1, 1 in OX2 adopts the characteristic cis conformation of the spacer group, positioning both aromatic groups into hydrophobic pockets of the active site. Moreover, intramolecular π-stacking is exhibited when bound to the OX2 active site. This nearly identical conformation of 1 is greatly indicative of the high conservation of the structures of OX1/OX2 and also exhibits the difficulty for the development of 1- and 2- SORAs.

The structure of 2 has also been well defined inside the OX1 receptor. Akin to 1, 2 also adopts a cis conformation when bound to the active site of OX1 (PDB ID 6TP6) (Christopher et al. 2020). Indeed, its mode of binding is extremely similar to that of 1. Intramolecular π-stacking between the fluoropyridine and the benzamide substituents creates a stable conformation which is further stabilized by offset π-stacking with H344. The fluorine atom on the ether-linked fluoropyridine moiety, the analogue to the chlorobenzoxazole of 1, binds to the same subpocket of W112 and V106.

Similarly, the structure of 3 has been identified inside the OX1 receptor (PDB ID 6TP3) (Christopher et al 2020). As has been seen with 1 and 2, the cis conformation of the spacer group remains imperative to the binding of 3. This common binding method is indicative of the success in intramolecular π-stacking combined with hydrophobic interactions and hydrogen bonding. The
*cis* structure of the scaffold of 3 differs from that of 2 and 1 in that the aromatic arms are attached to a substantially smaller scaffold. This forces the scaffold to adopt a twist shape in the pyrrolidine ring, adding reasonable stress to the overall structure.

Looking at structural views of 1, 2, and 3 bound in both OX1 and OX2, the most notable pattern is the adoption of a *cis* conformation of the spacer groups, resulting in an overall “U” shape of each ligand. It has proven fundamental to ligand binding that these drugs must adopt a *cis* conformation within the spacer group in order to properly orient the molecule into the correct subpockets for good binding. Yet, the inclusion of a conformationally flexible scaffold has many detriments. Not only does it contain numerous degrees of conformational freedom to adopt the necessary one for binding within the active site of the orexin receptors, but the mode of binding causes angular strain on the molecule and is energetically unfavorable compared to the native conformation in solution. Though these groups contain functionalities that direct the scaffold into a *cis* conformation (i.e., an endocyclic methyl stereocenter), they remain able to adopt various other conformations due to the steric size of the auxiliary groups.

Thus, the introduction and substitution of these cores with a core that has a more rigid and fixed conformation should assist in ligand binding and thus improve the affinity of the drug overall. Conformationally restricted orexin antagonists have been synthesized before to observe the effects on potency. Analogues of 1 were synthesized as a macrocycle that connects the ends of the two aromatic groups, forcing the molecule to adopt a *cis* structure (Cox *et al* 2009). The closed-loop
macrocycle was assayed for binding affinity in both OX1 and OX2 versus its precursor lacking the closed loop, and thus the conformational restriction. As they hypothesized, enforcing a conformational restriction on the structure of the antagonist showed increased potency in terms of $K_i$ from 63 nM and 120 nM for the flexible antagonist for OX1 and OX2, respectively, to 51 nM and 42 nM for the conformationally restricted macrocycle on the same receptors (Cox et al. 2009). The authors speculate that due to molecular design, the alkyl linker between aromatic moieties, which creates the macrocycle, can cause steric interference in the receptor and thus does not fully represent the benefit of conformationally restricted scaffolds in orexin binding and specificity.

To circumvent this issue, the adoption of a conformationally restricted scaffold should force the molecule closer to the bioactive conformation while creating as little steric hinderance as possible. By altering the core of the molecule, the relative size compared to the non-restricted model should be commensurate. This enables the benefits of having conformationally restricted drugs without the deficits of poor binding affinity due to steric hinderance. Design of an appropriate conformationally restricted core could allow higher binding affinity, as the molecule will need to move through fewer degrees of freedom to adopt the necessary conformation to bind
to the receptor. A conformationally restricted linker should also allow for less movement within the binding pocket itself, allowing for better binding affinity of the aromatic regions to their respective subpockets and more concrete π-stacking interaction.

Based on predictive software of the 3-dimensional geometry of the bicyclic diamine, we can see that the system has an intrinsic cis structure. This is advantageous to orexin antagonist design due to the similarity between its native conformation and the biologically active conformation the antagonists adopt within the receptor active site.

In terms of size, the distance between both nitrogen atoms varies by only 0.02 nm between the predicted 3-dimensional geometry and the found distance of the nitrogen atoms in bound 1 in OX1 and OX2. The potential for this scaffold is evident by its thermodynamic favorability compared to conformationally flexible scaffolds. By already adopting a similar conformation to bound 1 in its native state, the conformationally rigid scaffold affords the drug the opportunity to have improved binding affinity, and thus better potency as a pharmacotherapy for neurological disorders. For 2 and 3, the distance between the auxiliary groups differs from the synthesized core by 0.126 Å and 0.0129 Å, respectively. The close proximity to the size of the scaffolds in their
biologically active conformations facilitates the conformationally rigid scaffold to replace the current flexible scaffolds to improve potency of these molecules, and may aide in improving binding specificity for one or both receptors as well.

The introduction of two chemically distinguishable nitrogen atoms within the scaffold affords the opportunity to attune and optimize ligand affinity for the receptor. Compounding the fact that the scaffold is already in a more ideal conformation is the ability to functionalize the nitrogen atoms on the scaffold easily with a myriad of various moieties. This is powerful as the ligand binding can be attenuated easily to create more tightly binding and more specific ligands for both OX1 and OX2.

**Future Work and Conclusions:**

Nitrogen heterocycles demonstrate great versatility as frameworks for many bioactive compounds. The application of conformationally rigid scaffolds for orexin antagonists has an unforeseen number of possible applications. Suitable as an initial application can be implementing this scaffold to known orexin antagonists such as 1, 2, and 3. Not only does this ensure a model of known antagonists that implies binding success to the receptors but also enables these drugs to be used as references to identify the degree to which conformationally rigid scaffolds improve potency and binding specificity.
With 1, 2, and 3 as models, two options for inclusion of the novel spacer group can be envisioned. Both regioisomers of the individual model drugs contain the intrinsic cis conformation, allowing for probable binding within OX1 and OX2 receptors. By exploring both regioisomers, we will be better suited for constructing a more ideal ligand for the receptors given these specific aromatic functional groups. This path of future work will also aide in showing which form of the rigid scaffold shows better binding by placement of the aromatic arms in their preferred subpockets. Ultimately, the goal is to observe the difference in binding modes between conformationally rigid and conformationally flexible scaffolds. We anticipate that the substitution of a conformationally rigid scaffold will increase binding affinity for the orexin receptors given the conformational restraints observed by other researchers.

To completely investigate the effects of conformationally rigid scaffolds, we propose future work in investigating the enantiomer of the starting material, (R)-proline, as the base for creation of the conformationally rigid scaffold. By exploring the enantiomer, we can observe the
effects that stereochemistry has in the binding affinity of these drugs to the orexin receptors. In terms of synthesis, the chemistry described above should translate completely to the construction of the enantiomer.

The orexin receptor system illustrates great potential as drug targets to treat many neurological diseases. By intelligent design, the implementation of conformationally rigid scaffolds in known orexin antagonists can lead to not only the improvement of potency and binding affinity for these drugs, but also to the discovery of novel antagonists for these receptors as well. There is much evidence in the literature to suggest constructing ligands that more closely adopt the biologically active conformation in their native state should increase affinity of the drug due to more efficient binding.

**Experimental:**

All $^1$H NMR spectra was resolved using a 400MHz Bruker NMR Spectrometer in either CDCl$_3$, D$_6$-DMSO, or D$_4$-Methanol. Microwave reactions were performed using the Biotage Initiator+ Microwave Reactor. Flash Chromatography was performed using silica gel columns on the CombiFlash Rf autocolumn. Crystal structures were observed on the Chimera software. Predicted 3D structures were visualized using MolView software. Thin Layer Chromatography (TLC) was conducted on commercially available silica on alumina plates and visualization was performed using UV (254 nm and 365 nm) light, or by dipping in phosphomolybdic acid, ninhydrin, or KMnO$_4$ followed by heating.
The amide coupling to form intermediate 4 was performed at room temperature in methylene chloride using 1.5 mol equivalents of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and a catalytic amount of dimethylaminopyridine. To a 250 mL round-bottom flask was added 2.49 grams of Cbz-L-Proline and 100 mL of methylene chloride. 1.91 grams of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and 0.12 grams of dimethylaminopyridine were then added and allowed to dissolve over the span of 2 minutes. 8-Amino-5-methoxyquinoline was added to the solution and was allowed to stir overnight at room temperature for about 18 hours forming intermediate 4.

Cyclization to form intermediate 5a was formed in a microwave reactor in three stages over the course of 94 minutes. Reactions were executed using a Biotage Initiator+ microwave reactor. Reaction was prepped using a 0.5-2.0 ml Biotage reaction vial fitted for the instrument. To the vial was added 0.5 g of intermediate 4 and 0.5 ml of solvent iodopentafluorobenzene. The reaction was allowed to stir until well dissolved. Next was added 80 mg of Pd(OAc)\(_2\) into the stirring solution. The powder was well mixed into the solution. Finally, the remaining 0.5ml of
iodopentafluorobenzene was added to the solution, followed by 250 mg of Ag(OAc)$_2$. The reaction was well mixed for 30 minutes before being deposited into the microwave reactor. Failure to do so results in inconsistent heating and explosion is probable. The vial was inserted to the microwave reactor and was heated to 50°C for 60 seconds, 120°C for 3 minutes, and finally 160°C for 90 minutes. After heating, reaction was cooled, unsealed, and extracted from the vial using 50 ml of ethyl acetate or methylene chloride and purified via flash chromatography to produce intermediate 5a.

\[
\begin{align*}
\text{C-H bond activation} & \hspace{1cm} \text{and subsequent cyclization} \\
\text{was also determined to work} & \hspace{1cm} \text{under} \\
\text{thermal reflux. Reaction was prepped} & \hspace{1cm} \text{in similar fashion to the method for constructing} \\
\text{intermediate 5a. To a 0.5-2.0 ml biotage} & \hspace{1cm} \text{intermediate 4 and 0.5ml} \\
\text{reactor vial was added 0.5 g of intermediate} & \hspace{1cm} \text{of iodopentafluorobenzene. The solution was allowed to stir to completely homogenize. Next was} \\
\text{4 and 0.5ml} & \hspace{1cm} \text{added 80 mg of Pd(OAc)$_2$ into the stirring solution. The powder was well mixed into the solution.} \\
\text{of iodopentafluorobenzene. The solution} & \hspace{1cm} \text{Finally, the remaining 0.5ml of iodopentafluorobenzene was added to the solution, followed by} \\
\text{was allowed to stir to completely homogenize. Next was} & \hspace{1cm} \text{250 mg of Ag(OAc)$_2$. The vial was sealed, set in an oil} \\
\text{added 80 mg of Pd(OAc)$_2$ into the stirring} & \hspace{1cm} \text{bath, covered in foil, and heated to 160°C} \\
\text{solution. The powder was well mixed into the solution.} & \hspace{1cm} \text{bath temperature. The solution was stirred on reflux for 18 hours to produce intermediate 5b.}
\end{align*}
\]
Oxidative cleavage to generate intermediate 6 was performed at -20°C using ceric ammonium nitrate. To 84 ml of acetonitrile in a scintillation vial was added 1.0 g of intermediate 5. The flask was added to acetone/water/NaCl ice bath at -20°C and allowed to cool. Separately, 4.08 g of ceric ammonium nitrate was dissolved completely in 4.5 ml of deionized water. The solution of ceric ammonium nitrate was added dropwise to the cooled acetonitrile solution over a period of 5 minutes and was left to stir at -20°C for 2 hours generating intermediate 6.

To an oven dried round bottom flask was added a solution of 50mg of intermediate 6 in THF. The solution was concentrated on the rotary evaporator and dried on high vacuum pump overnight. The following morning, the flask was removed from the pump and evacuated of air under dry N₂ gas. Sealed, dry THF was added to the flask containing intermediate 6 and was left to fully dissolve. The resulting solution was transferred to another oven dried round bottom flask cooled under dry N₂ gas. To the flask was then added 2 equivalents of borane dimethyl sulfide in THF dropwise, over a period of 5 minutes. The solution was left to stir at room temperature for 4 hours to produce intermediate 7.
To a round bottom flask was added 66 mg of picolinic acid and 100 mg of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide dissolved in methylene chloride. The solution was allowed to stir until homogenous followed by addition of 5 mg of dimethylaminopyridine and 100 mg of (S)-1-Cbz-2-aminomethylpyrrolidine over the span of 2 minutes. The solution was stirred for 24 hours at room temperature to produce intermediate 8.
Spectra:
References:

- Jamie M. Zeitzer, PhD, Alberto Morales-Villagran, PhD, Nigel T. Maidment, PhD, Eric J. Behnke, BS, Larry C. Ackerson, BS, Faustino Lopez-Rodriguez, MD, PhD, Itzhak Fried, MD, PhD, Jerome Engel, Jr., MD, PhD, Charles L. Wilson, PhD, “Extracellular Adenosine in the Human Brain During Sleep and Sleep Deprivation: An in Vivo Microdialysis Study”, *Sleep*, **2006**, 29(4): 455–461


• de Lecea L., Gregor Sutcliffe J., “The hypocretins and sleep” *FEBS J.*, **2005**, 272(22)


• “FDA Approves Belsomra (suvorexant) for the Treatment of Insomnia” *mrknewsroom.com*, Wednesday, **August 13, 2014**.
• Alexander Treiber, Ruben de Kanter, Catherine Roch, John Gatfield, Christoph Boss, Markus von Raumer, Benno Schindelholz, Clemens Muehlan, Joop van Gerven and Francois Jenck, “The Use of Physiology-Based Pharmacokinetic and Pharmacodynamic Modeling in the Discovery of the Dual Orexin Receptor Antagonist ACT-541468”, *J. Pharmacol. Exp. Ther.* 2017, 362 (3) 489-503


• Christopher J. Winrow, Anthony L. Gotter, Christopher D. Cox, Pamela L. Tannenbaum, Susan L. Garson, Scott M. Doran, Michael J. Breslin, John D. Schreier, Steven V. Fox, Charles M. Harrell, Joanne Stevens, Duane R. Reiss, Donghui Cui, Paul J. Coleman, John J. Renger, “Pharmacological characterization of MK-6096 – A dual orexin receptor antagonist for insomnia”, *Neuropharmacology* 2011


