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Modified Chromenes as Precursors to TAAR Regulators

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Abstract

200 million people worldwide are living with a thyroid disorder related to a hormonal imbalance. Symptoms of this imbalance include deviations from normal heart rates and metabolic rates. A chemical messenger known as T3 has been shown to raise cardiac and metabolic rates, but this takes place on a timescale of hours. However, recent discoveries have revealed that a different thyroid hormone known as T1AM is capable of affecting some of these same physiological conditions in a matter of minutes. T1AM is a potent, selective agonist for the Trace Amine Associated Receptor (TAAR). Several studies have probed the structural basis of T1AM activation, but these studies have not yet provided a complete picture. Based on these findings, we have developed a new molecular scaffold that incorporates key structural features in a conformationally-restricted arrangement. For example, the incorporation of a six carbon ring in our target compounds will confine the location of the amine and even create multiple stereocenters. Furthermore, several chiral locations on this scaffold will have structural variability. To date, our research has focused on optimizing the reactions that produce 3-nitro-2H-chromenes, the chemical precursors to these target compounds. The successful production of a panel of these chromenes has set the stage for subsequent reactions that will allow us to generate many T1AM analogues. By understanding the regulation of TAAR we may gain a greater understanding of its role in biology and human physiology.

Background

The thyroid gland in vertebrates regulates normal physiology by secreting hormones, the predominant one being Thyroxine (T4, figure 1). In target tissues T4 is modified by the removal of an iodine atom to generate 3,5,3'-triiodothyronine (T3, figure 2), a high affinity ligand for nuclear receptors TRα and TRβ (Scanlan 2004). The effects of T3 include increased heart rate, increased metabolic rate, and raised core body temperature. Because the effects of T3 are communicated through a transcription pathway, they are manifested over a period of hours or days (Scanlan 2004). Overproduction of T3 is known as hyperthyroidism and is a common hormonal imbalance marked by increasing cardiac rate, metabolic rate, and core body temperature.

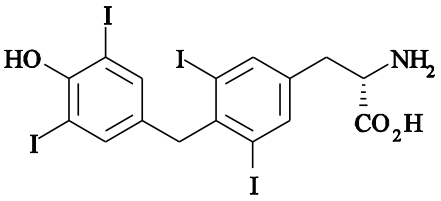


Figure 1. Thyroxine (T4)

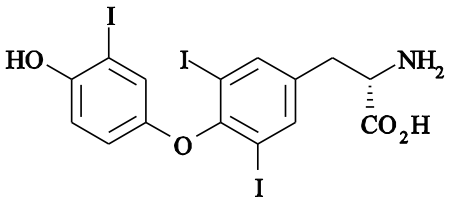


Figure 2. 3,5,3'-Triidothyronine (T3)

T3 can be modified still further by decarboxylation and the removal of two more iodine atoms to form 3-iodothyronamine (T1AM, figure 3), another hormone with an important role in vertebrate homeostasis. When T1AM was administered to mice, they showed immediate inactivity, ptosis (drooping eyelids) and reduced body temperature. Furthermore, the injection of T1AM into isolated rat hearts induced bradycardia in approximately ten minutes (Scanlan 2004). These effects constitute the opposite of T3 but occur in a matter of minutes, an indication that T1AM communicates through a direct pathway, unlike that of T3. Thus it came as no surprise when T1AM was shown to not have an affinity for the receptors TRα or TRβ. Instead, administering T1AM to HEK293 cells expressing the rat Trace Amine Associated Receptor

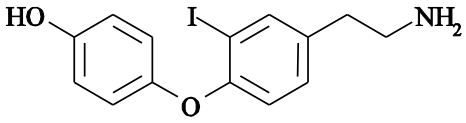


Figure 3. 3-Iodothyronamine (T1AM)

(rTAAR) resulted in cAMP accumulation (Scanlan 2004). Further tests confirmed that T1AM does indeed bind preferentially to TAAR from species of rats and mice (mTAAR), while T3 and T4 do not (Scanlan 2004). Based on these findings, it is believed that TAAR is responsible for communicating the effects of T1AM such as ptosis and bradycardia in mice.

The Trace Amine Associated Receptor is a G protein-coupled receptor (GPCR) similar to β_2 adrenergic (β_2AR), dopamine, and serotonin receptors. Since these receptors all belong to the same subfamily of class A rhodopsin-like GPCRs, the structure of β_2AR can serve as an model for understanding the structure of TAAR (Tan 2008). A GPCR like β_2AR is composed of seven transmembrane (TM) proteins and contains a binding site located among TM 3, 5, 6, and 7 (figure 4). Projecting from TM1 there is an amino terminus, and TM7 ends in a carboxy terminus (Tan 2008).

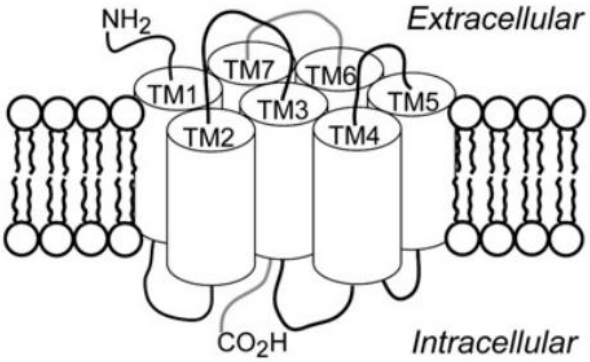


Figure 4. Cross Section of GPCR

In the inactive state of β_2AR, there is ionic lock between TM6 and TM3 composed of a glutamic acid residue in TM6 (E6.30) and aspartic acid and arginine residues (D3.49 and R3.50) in TM3 (Tan 2008). When an agonist enters the binding pocket, it induces a conformational change that breaks this ionic lock, allowing TM6 to pivot away from TM3 (figure 5). As a result, the G protein recognition sites on the intracellular surface of the receptor are exposed, initiating a signaling cascade (Tan 2008). Since

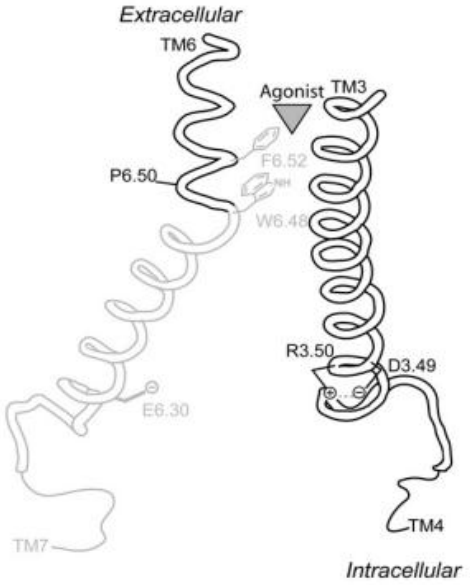


Figure 5. Active Conformation of β_2AR

TAAR contains the same pertinent amino acid residues, the mechanism for agonist binding likely occurs the same way. Interestingly, binding is not simply a one step process but rather a sequence of increasing interactions between the agonist and intermediate conformational states of the receptor that culminate when the fully active state is reached (Tan 2008). TAAR may prove to have similarly complex ligand receptor interactions.

To better understand these interactions, the structure activity relationship (SAR) of rTAAR and mTAAR was probed by various synthetic T1AM analogues. Since one of the distinguishing features of T1AM relative to T3 is the number and location of iodine atoms, thyronamines were synthesized with anywhere from zero to four iodine atoms at various locations on the aryl rings. It was found that any change to the number or position of iodine atoms relative to T1AM reduced TAAR activation (Hart 2006). Still, a T1AM analogue without any iodine atoms (T_0AM) did exhibit enough TAAR activation to serve as template for further SAR investigations. In addition, removal of the hydroxyl from T_0AM (figure 6) was beneficial for both rTAAR and mTAAR affinity relative to T_0AM (Hart 2006). This established a convenient, elementary scaffold for variance in future analogues (figure 6).

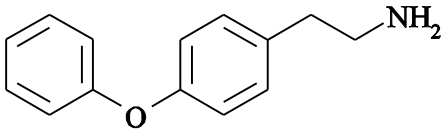


Figure 6. New Elementary Scaffold

Two different sites of modification relative to this scaffold were the bridges, one a biaryl ether connecting the two rings and the other an ethyl group supporting the amine. Lengthening the biaryl ether by adding methylene groups generally improved mTAAR activation but was detrimental to rTAAR activation (Hart 2006). Interestingly, when a long enough bridge had been reached (figure 7), mTAAR and rTAAR activation stabilized, suggesting that the compound was able to adopt

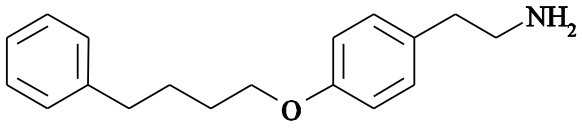


Figure 7. Long Biaryl Bridge

whatever conformation best suited the receptor. Based on these results, the original biaryl ether bridge appeared to be sufficient for future analogues. Modifications were also made to the ethyl bridge supporting the amine by attaching an amide to the ring and adjusting the linkage length to the amine, as in figure 8. Generally, these compounds were poor agonists for both mTAAR and rTAAR (Tan 2007), although bridges of sufficiently long length gradually improved activation potential, again suggesting that enough flexibility was achieved to adopt whatever conformation was needed to match the TAAR binding site. Preserving the original bridge supporting the amine in T1AM was the most rational structure for further agonists.

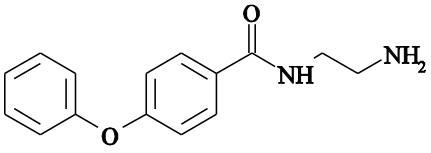


Figure 8. Poor TAAR Agonist

One final modification of obvious significance is the basic amine. Replacing the amine with a hydroxyl group abolished rTAAR and mTAAR affinity, demonstrating that this functional group is essential for TAAR activation (Hart 2006). Different alkylation states of the amine were also explored. In general, monomethylation (figure 9) was tolerated for both rTAAR and mTAAR, but longer alkyl chains and dimethylation of any sort were detrimental (Hart 2006).

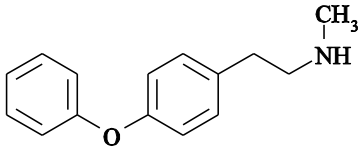


Figure 9. Monomethylation

These findings provided a foundation for developing rTAAR superagonists, or compounds that have an affinity for the receptor that exceeds T1AM. The rational basis for making further modifications involved a proposed binding orientation and its surrounding amino acid residues in rTAAR. Appending a phenyl group to the carbon in the β position to the amine provided a strategic location for attaching polar functional groups to interact with asparagines (N7.35 and N7.39), methionine (M6.55) and cysteine (C6.54) (Tan 2008). This new structural scaffold, shown in figure 10, exhibited unprecedented

affinity for rTAAR. Interestingly, monomethylation of the amine also improved activation for this compound (Tan 2008). This method of design, introducing functional groups to accommodate the residues surrounding a proposed binding orientation, was very successful at generating superagonists.

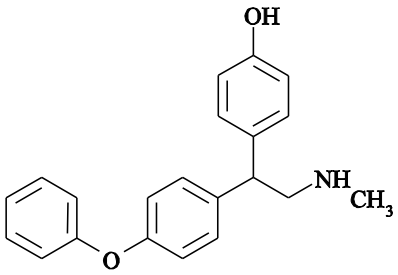


Figure 10. rTAAR Superagonist

Another avenue of SAR research involves antagonists, compounds that inhibit receptor activation. Antagonists are structurally similar to agonists since they still must have an affinity for the receptor, but they differ in key ways in order to prevent activation upon binding. Figure 11 shows a potent rTAAR antagonist that contains two crucial structural modifications from the superagonist scaffold. First of all, the R group has been apended in a precise location where it can interfere with the motion of the rotamer toggle switch. This steric obstruction encumbers the conformational change that would normally accompany activation. As a result, compounds containing especially bulky R groups, such as pentane or hexane ethers, were the most capable of sterically impeding this motion and performed the best as antagonists. The second essential modification was repositioning the phenyl ether, which was necessary to eliminate the symmetry of the molecule and restrict the binding orientation of the compound. Antagonists based on this structure exhibited inhibition of T1AM activation in competition assays, albiet in modest concentrations (Tan 2008).

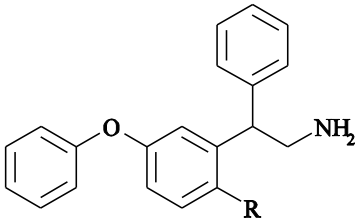


Figure 11. R = OHex, OPent

Based on all these SAR studies, we have developed a novel molecular scaffold that incorporates these components in a new arrangement (figure 12). Essential features including the basic amine and biaryl linkage have been conserved, while nonessential moities such as the hydroxyl group and iodine have not. However, the most significant structural revision is the cyclization of the ring connected to the

α carbon to the amine. This increases the rigidity of the compound and confines the location of the amine, which can be controlled even further because it is attached to a chiral carbon. Two crucial points of variation also exist on this scaffold at the R1 and R2 positions. Based on past superagonist structures, appending a phenyl ring at the R1 position provides a location for polar functional groups that can have interactions with the receptor. Furthermore, the variability at the R2 position could be exploited to generated antagonists by appending bulky groups such as hexane ethers. The potential for stereoisomers involving three chiral centers, at the amine, R1, and R2 positions, allows us to exert a tremendous amount of control over the exact spatial positioning of all these groups.

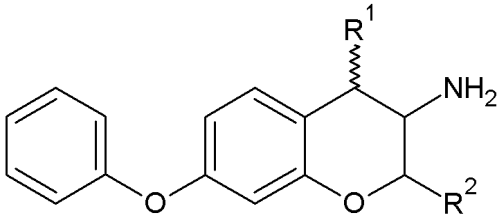
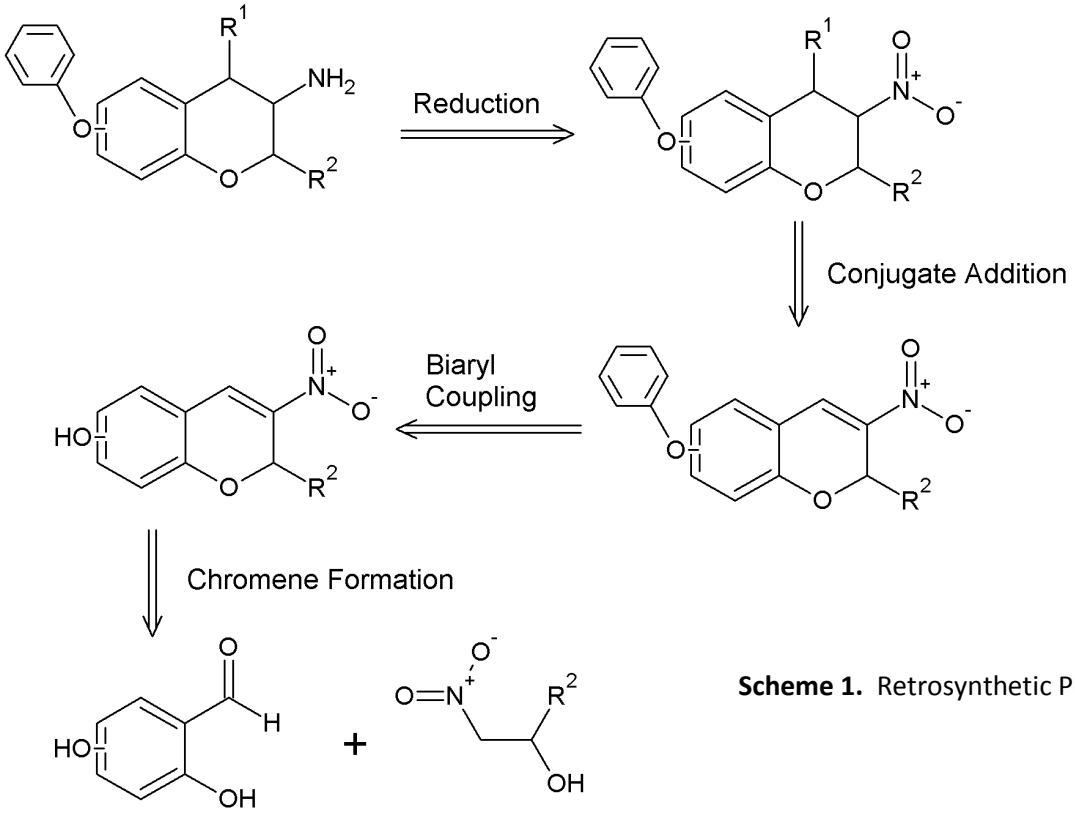


Figure 12. Novel Scaffold

Results and Discussion

Our laboratory work has been focused on optimizing the synthesis of this scaffold (scheme 1).

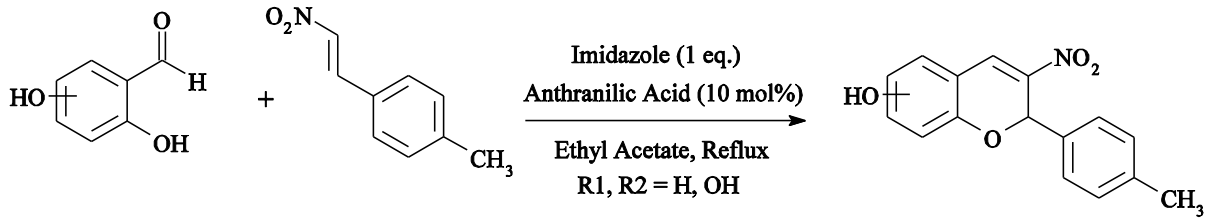


Scheme 1. Retrosynthetic Plan

The first step in this strategy is the production of 3-nitro-2H-chromenes, compounds that contain nitro groups that serve as precursors to amines. These nitrochromenes also have hydroxyl groups that will later facilitate the attachment of phenyl ethers. To date, two different reactions have been employed to generate a panel of nitrochromenes varying at the R2 position and at the location of the –OH group that will later be replaced by a phenyl ether.

The first successful procedure for synthesizing these nitrochromenes utilizes the same substrates as shown in scheme 1. Salicylaldehyde (1 eq.) and nitroethanol (1.6 eq.) are to be mixed with di-*n*-butylammonium chloride (DBAC, 0.5 eq.) in the solvent isopentyl acetate (Dauzonne 1984). Instead of using DBAC, we employed dibutylamine and then generated HCl in situ by mixing acetyl chloride and isopentanol. Another important factor was the utilization of a Dean Stark apparatus, which was essential for collecting the water produced by the reaction. By Le Chatelier's Principle, this removal of product helped push the reaction toward completion. We were unable to duplicate the yield reported in this paper, generating only a 16% yield instead of the reported 50%. Next, this reaction was applied to different substrates, including 2,4-dihydroxybenzaldehyde and 2,5-dihydroxybenzaldehyde, with the yield for the latter reaching 24.4%.

The second successful method of nitrochromene formation was based on the Morita-Baylis-Hillman reaction (scheme 2). According to the literature, the best mixture for this reaction was 1

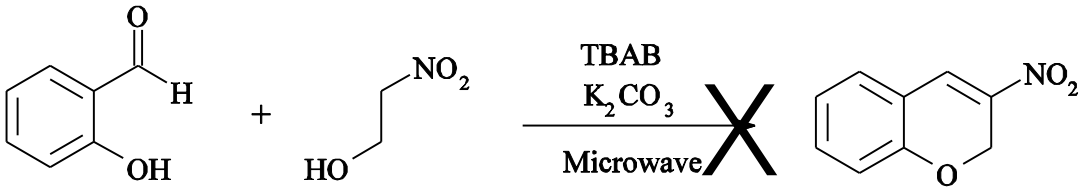


Scheme 2. Morita-Baylis-Hillman Based Nitrochromene formation

equivalent of the nitroalkene, 1 equivalent of formaldehyde, 1 equivalent of imidazole, and 10 mol % of

anthranilic acid in THF at room temperature (Rastogi 2004). We changed this procedure significantly by employing salicylaldehyde instead of formaldehyde and refluxing the reaction in ethyl acetate and later propyl acetate (scheme 2). These temperatures proved conducive to chromene formation and yields of approximately 20% were reached, although the product was difficult to purify by column chromatography because the reactants had the same Rf as the product. We also discovered that using a Dean Stark apparatus to remove water improved yields for this reaction as well.

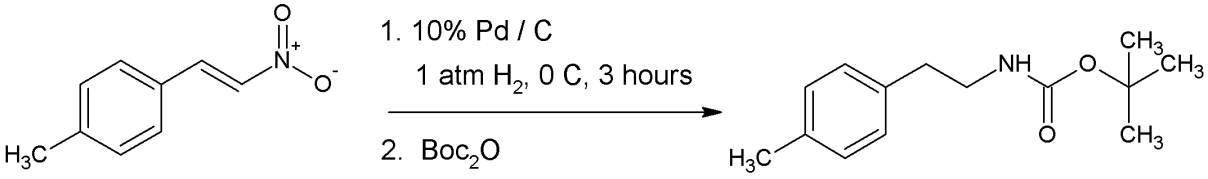
Another chromene formation tactic that we attempted was solvent free and facilitated by a regular household microwave (Scheme 3). We were unable to generate any nitrochromenes by following the synthesis outlined in the article (Koussini 2008) or by making modifications including



Scheme 3. Microwave Assisted Chromene Formation

raising the amount of TBAB to a stoichiometric quantity and adjusting the microwaving time intervals. However, based on how the Dean Stark apparatus was necessary for removing water from the one-pot synthesis reaction and Morita-Baylis-Hillman reaction, adding molecular sieves to remove water from this reaction might push it to completion in a similar way. We intend to try this in the future while we continue to optimize the other chromene formation reactions that have succeeded.

The last step in scheme 1 is reduction of the amine, but testing this chemistry on a model system provided a cheaper more precedented way to optimize it (scheme 4). The literature used 3,4-



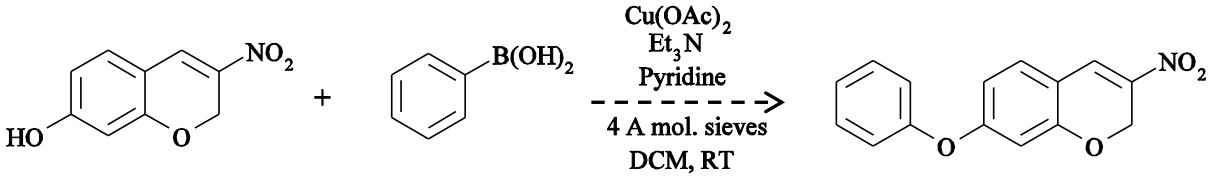
Scheme 4. Model System of Hydrogenation

methylenedioxy-β-nitrostyrene (Kohno 1990), a trans-nitrostyrene with a different pattern of aromatic substitution than the substrate shown in scheme 4. In addition, the specific catalyst called for, 5% Pd/C K-type was not readily available, so 10% Pd/C was used instead. After the reaction was stopped, boc anhydride was added to protect the amine from decomposition. Even with all of these modifications, promising peaks were still visible on NMR, although further optimization and purification steps are required before any yields can be reported.

The success of the first stage of this synthesis includes the utilization of two different chromene formation reactions and the production of a panel of nitrochromenes. Exploring the reduction of these nitrochromenes with a model system was the first step in converting these precursors into TAAR regulators. However, much more still needs to be done. Based on the retrosynthetic plan (scheme 1), several more reactions are required to append other moieties and complete the target scaffold. These challenges will provide plenty of inquiries for further laboratory investigations over the next year.

Conclusion and Future Directions

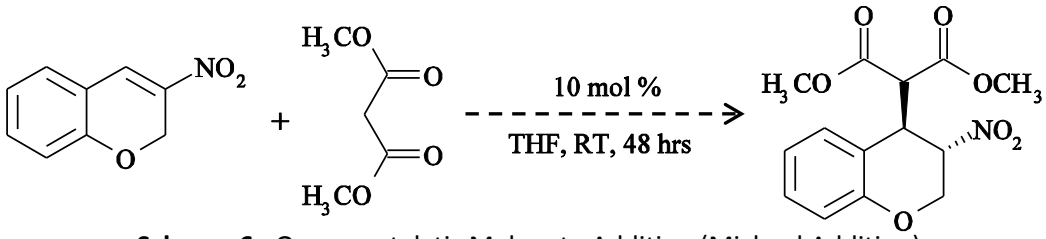
A very important step in the completion of this scaffold is replacement of the hydroxyl group with a phenyl ether, which is accomplished by a copper coupling reaction (scheme 5). This chemistry



Scheme 5. Copper Coupling

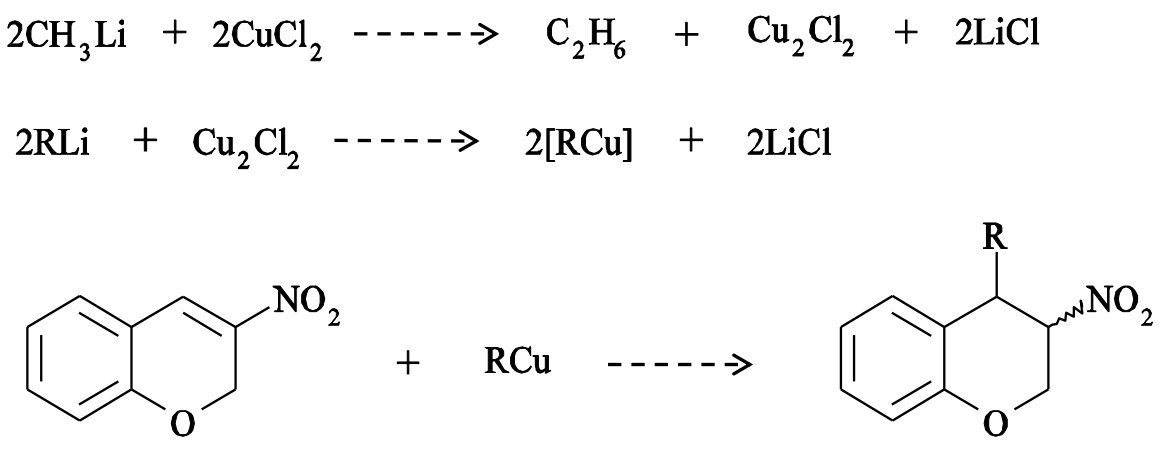
has already been utilized in the lab, and will be extended in the nitrochromene case. Still, the creation of novel TAAR regulators requires this structural alteration.

Conjugate addition to the nitrochromene provides variation at the R1 position, a modification that could be used to generate superagonists and lead antagonists. One method of conjugate addition



Scheme 6. Organocatalytic Malonate Addition (Michael Addition)

is organocatalytic malonate addition (scheme 6). While the range of groups that can be added to nitroalkenes is limited, the literature on this reaction reports achievement of enantioselectivity (Chen 2011). The second method of conjugate addition that could be employed is organocopper addition, a multistep process inolving organometallics (scheme 7). A wide variety of substrates are compatible with this reaction, providing a great potential for variation at a position that has already been utilized in the development of superagonists. Enantioselectivity of this step will also be explored.



Scheme 7. Organocopper addition

TAAR performs a crucial role in normal vertebrate physiology that is still being resolved. Upon activation by the selective agonist T1AM, TAAR initiates rapid and acute effects on cardiac rate, metabolic rate, and body temperature. SAR studies of this receptor with T1AM analogues have shown what structural features are essential for TAAR affinity, and rational molecular design based on binding orientation has led to highly effective agonists and antagonists. We hope to produce novel TAAR regulators by incorporating these moieties into a rigid, conformationally-restricted scaffold. Even though our synthesis has not been completed yet, the successful production of a panel of substituted 3-nitro-2H-chromenes has provided many precursors for the creation diverse panel of target compounds. As we continue optimizing the synthesis of TAAR regulators, we expect to learn more about the nature of this important receptor.

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Plans for Dissemination

Presentations

- Student Summer Showcase
- 2011 West Michigan Undergraduate Research Symposium at Van Andel
- *American Chemical Society National Meeting* or a *Council on Undergraduate Research Meeting*- when data is sufficient

Publication

- When sufficient research has been completed, the S3 report will serve as the basis for submission to a peer-reviewed journal (e.g. *Journal of Organic Chemistry* or *Journal of Medicinal Chemistry*).