Title: A Computational and Synthetic Approach for the development of the first selective agonists towards the 5-Hydroxytryptamine2A receptor

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Abstract:

Drug discovery is a significant process that enables scientists to discover medications for the human population. Over the course of the last century or two, drug development has led to cures for numerous ailments. However, there still are many unanswered and incurable conditions that exist today, emphasizing the continued importance of these research efforts. One major target class for drug development involves the G-protein coupled receptors (GPCRs). Because GPCRs are the largest class of membrane receptors, they are in turn the largest class of druggable targets. One particular GPCR of interest is the 5-hydroxytryptamine2A receptor, which is known to be the canonical target for serotonin, but also psychedelic drugs such as LSD. Targeting 5-HT2A has the potential to treat neuropsychiatric disorders, such as PTSD and depression, but selectivity issues in drug development have hindered the production of reliable medications. Namely, lack of selectivity in drugs such as LSD leads to undesirable hallucinogenic effects, in addition to the therapeutic antidepressant effects. Our goal in this research review paper is to outline the steps for drug development by highlighting the specific example of designing potent and selective agonists towards the 5-HT2A receptor.

Background/Introduction/Significance:

Drug discovery and development describes the process of identifying a relevant biological target and designing a potent and selective compound that elicits therapeutic effects towards a particular condition or illness. In the twentieth century, drug discovery has allowed scientists to more efficiently investigate, run tests, and develop medications for diseases around the world.

A major consideration for drug discovery scientists is to design *selective* compounds towards therapeutically relevant targets. To date, some FDA-approved drug compounds are very selective, meaning that they have minimal side effects and are generally safe to use. On the contrary, other drugs produced are not as selective, and can cause adverse side effects, which limit their utility. For example, LSD has very good anti-depressant effects and is known to treat cluster headaches, but it is currently illegal for general use because of severe adverse effects including hallucinations and fatigue. However, current efforts in drug development are continually improving and scientists are utilizing structure-aided drug design to increase drug selectivity.

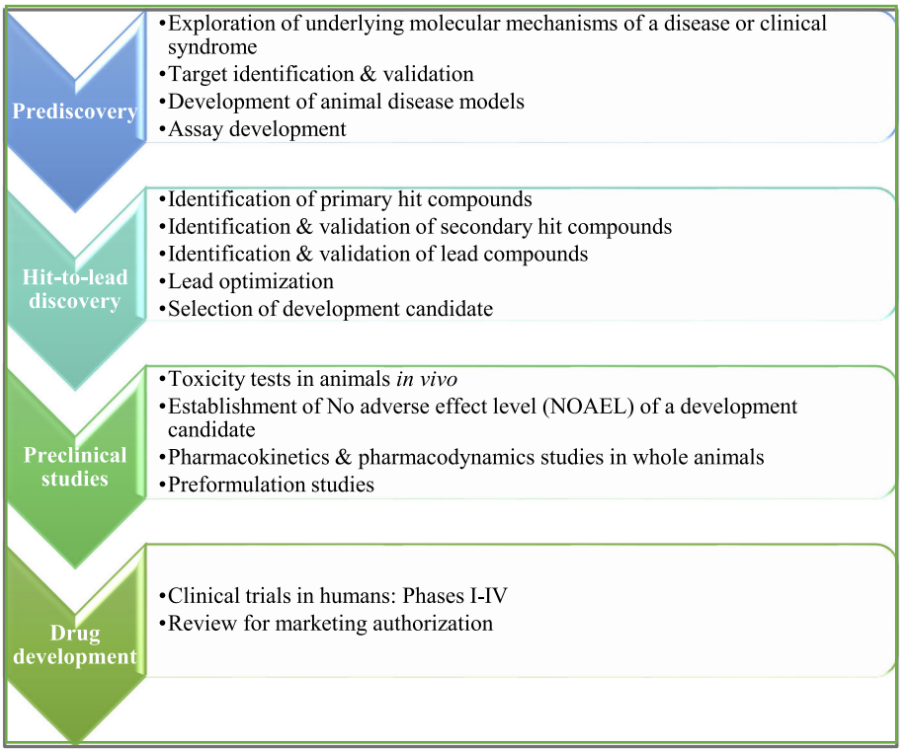

Fig. 1 *Generic outline of the drug discovery and development process*[1]

Figure 1 outlines the general developmental process for drug discovery. This process commences with the identification of the problem. In this research, we will focus on the development of selective drug candidates for neuropsychiatric diseases such as depression, anxiety, PTSD, and schizophrenia. The second step is identifying the relevant target that could alleviate the disease. This is the receptor that the ligand would have to bind to. Drug discovery can be carried out in either one of two ways: phenotypic drug discovery or target-based drug discovery.

In a phenotypic based drug discovery approach, researchers are more focused on the fact that a drug can provide therapeutic relief than on the details of the mechanism of action. Drug repurposing is a prime example of phenotypic based drug discovery. For example, in the past three years, drugs like hydroxychloroquine and remdesivir were repurposed. After a significant number of deaths from the Covid-19 virus, there was a desperate need to find a potent and effective drug in the shortest time possible. Originally, hydroxychloroquine is a drug designed to prevent and treat malaria. Remdesivir is another FDA approved drug for the treatment of Ebola and Marburg virus infections. These two drugs were repurposed using the phenotypic drug discovery approach and tested as potential treatments for Covid-19 before the production of vaccines.

On the other hand, in a target-based drug discovery approach, researchers are more interested in uncovering the detailed mechanism of action of a ligand to a particular target. The process starts with choosing the most appropriate screening technique to find a good starting hit molecule. Usually, either high throughput screening (HTS) or virtual screening practices are utilized. HTS is the more traditional approach that utilizes 'in stock' libraries to test against a new target receptor. Usually, the HTS process screens compounds on the scale of a few million molecules. On the other hand, virtual screening is a newer technique that screens compounds on the scale of billions. It is generally a much more efficient and cost-effective approach over

HTS, and consists of programs that filter compounds by comparing their binding abilities to the target receptor in virtual space.

Other testing techniques on the primary hit compounds are used to further eliminate molecules with a weaker binding ability in order to discover the most potent and selective compounds. Two factors are considered throughout this process: selectivity and potency. Potency describes the consideration that the smallest amount of a drug should be given for the largest pharmacological effect. Selectivity describes the consideration that the drug molecule should ideally bind to only one target receptor. A potent and selective compound should limit the side effects of a potential drug.

Further studies are carried out *in vitro* where a drug is assayed in well plates outside of an organism. Structural aided drug design is applied to lead compounds to modify molecules and improve their overall potency and selectivity. After numerous *in vitro* tests, *in vivo* testing can be carried out on animals, monitoring the effects of a drug in living organisms.

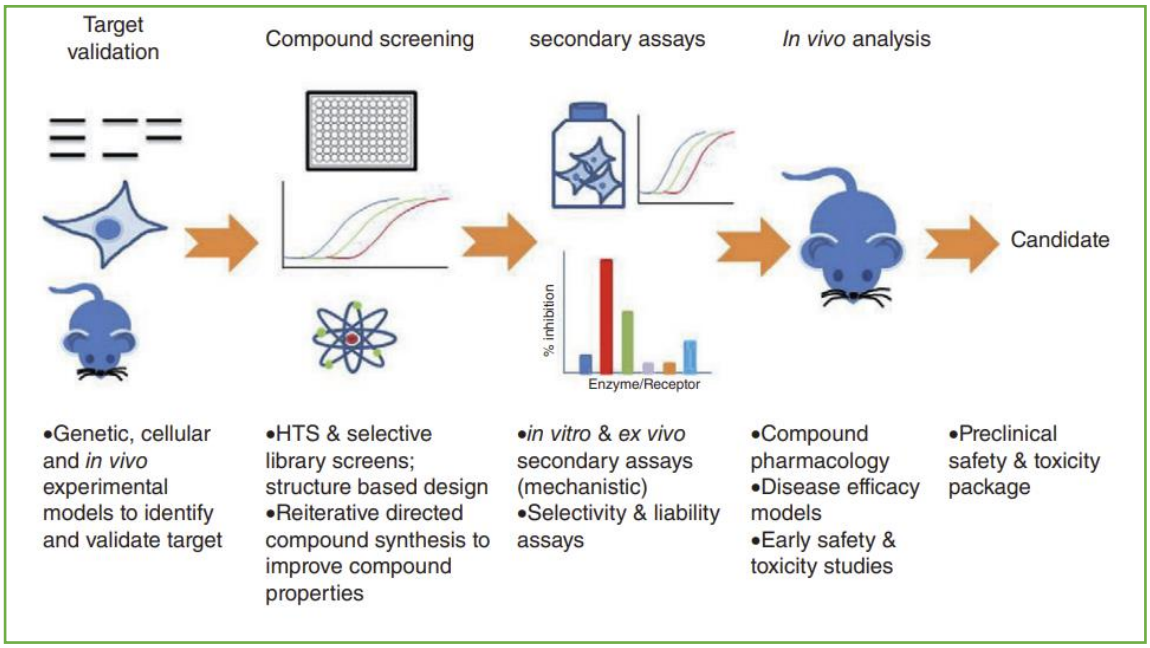

Fig. 2 *Overview of drug discovery screening assays.*[2]

In the final stages of drug development, human clinical trials are run, which consists of 4 phases based on efficacy and safety. In the first phase, a drug is given to a small number of healthy volunteers who are closely monitored to test safety. Secondly, testing is carried out on a larger number of patients to monitor how a drug is metabolized and gather initial data on efficacy. After the second phase, an even larger trial in patients helps to affirm efficacy and safety. Lastly, studies are undertaken after a drug has been licensed, to further ensure safety, efficacy, and effectiveness data in routine clinical use. Figure 2, again, highlights the critical stages of the drug development process.

As mentioned previously, in this project, we sought to develop potent and selective drug candidates for the 5-HT2A receptor, a well-known serotonergic G-protein coupled receptor (GPCR). GPCRs form the largest family of membrane receptors in humans, thus making them the largest family of druggable targets. They are seven-transmembrane receptors (7 TMRs) as

they pass through the cell membrane seven times and detect molecules outside the cell to activate cellular responses within.

When a ligand binds to the GPCR, signaling will progress via the G-protein mediated pathway and/or the beta Arrestin pathway. In the G-protein mediated pathway, the ligand binds to the GPCR. The complex undergoes a conformational change, and the GPCR then binds to the heterotrimeric G-protein. GDP on the alpha subunit is exchanged into GTP because it receives an extra phosphate group. The G-protein splits into its alpha, beta, and gamma subunits, and the alpha subunit leads to further downstream signaling. A particular example of this process is demonstrated by serotonin, which binds to 5-hydroxytryptamine2A to signal via the alpha(q) pathway, at the end of which calcium ions flux.

GPCRs can alternatively follow the Beta Arrestin pathway. This occurs when a ligand binds to the GPCR and is eventually desensitized and is unable to further influence the G-protein mediated signaling pathway. This makes another protein known as beta Arrestin to bind to the GPCR. The GPCR becomes more stable inside the cell so it moves into the cell where it can be either recycled or degraded. The details of GPCR signaling can be seen in Figures 3 and 4.

Normally, when a ligand binds to a GPCR, both pathways are activated at the same time. However, there is a way of making the ligand function by only one of the pathways. This idea is known as biased agonism, and describes that a drug molecule can be designed to favor signaling via either the G-protein mediated pathway ***or*** the beta Arrestin pathway.

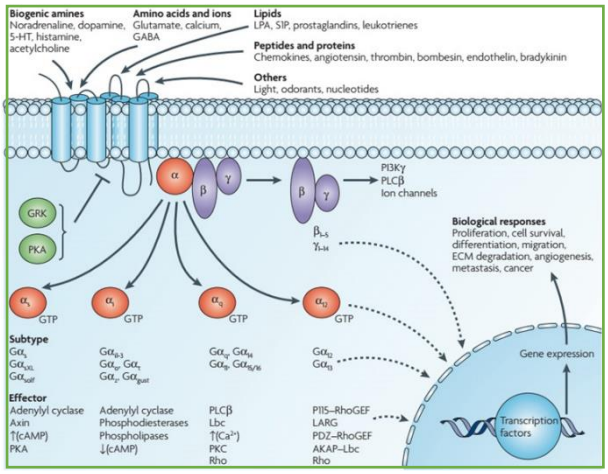

Fig. 3 *G-protein mediated pathway* [3]

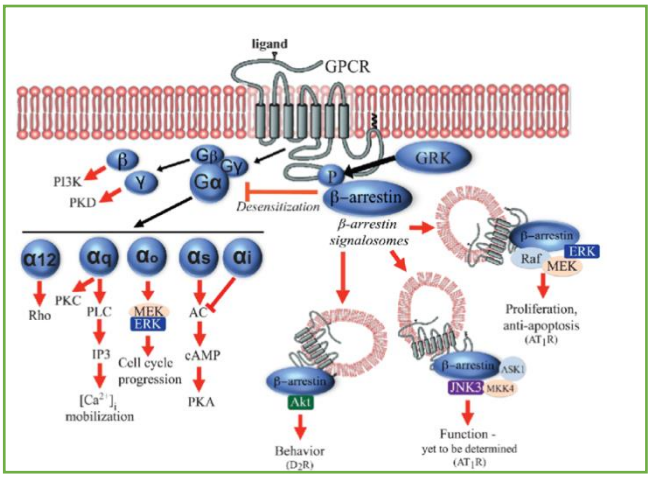

Fig. 4 *Beta-Arrestin pathway* [3]

In this project, we sought to develop a potent and selective agonist towards a specific GPCR known as 5-hydroxytryptamine2A (5-HT2A). 5-hydroxytryptamine (serotonin) is a neurotransmitter produced from 5-hydroxytryptophan within the central nervous system (CNS) that binds to the 5-HT2A receptor. This interaction contributes to feelings of satiety/contentment.

Scientists have discovered that tryptamines including LSD, a semi-synthetic member of a larger class of ergolines, bind to the 5HT2A receptor very potently. The nitrogen atom in the tryptamine moiety is known to form a salt bridge with an aspartic acid residue in the 5-HT2A binding pocket. LSD and other agonists towards 5-HT2A are well known to possess anti-depressant effects, and have even been approved for cluster headaches. However, LSD (Figure 5) is not selective because it simultaneously binds to other proteins, which in turn causes psychoactive side effects and thus limits the drug's utility to a larger population. Additionally, LSD strongly activates both the GPCR and beta-Arestin pathways, and so, shows poor functional selectivity as well. This is generally true for other tryptamine-based psychedelics, including psilocybin and dimethyltryptamine.

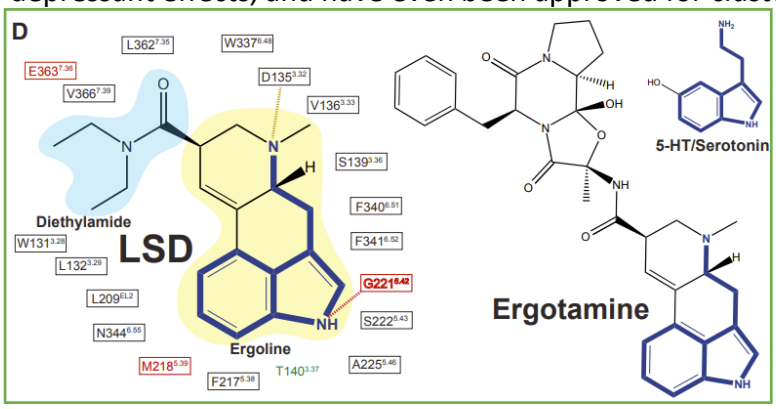

Fig. 5 *Structure of LSD, ergotamine and serotonin* [4]

Considering all of the factors mentioned above, the overall goal for this project is to harness the therapeutic properties of such hallucinogenic drugs while getting rid of the psychoactive side effects. To achieve this, we can design a potent and selective biased agonist towards the 5-HT2A receptor. Accomplishing this goal would be a great contribution to addressing the growing problem of neuropsychiatric diseases.

In order to design a potent and selective agonist, we sought to include nitrogen heterocycles into our substrates. In particular, tetrahydropyridine (THP) derived ligands were designed and synthesized. N-heterocycles are highly common and desirable in both natural products and pharmaceutical drugs because the lack of free rotation of the molecule in the binding pocket facilitates strong intermolecular and ionic interactions. In other words, this feature ensures stronger bonding of the ligand to the protein because the ligand is held tightly in place. The presence of the nitrogen atom allows for hydrogen bonding, the strongest type of intermolecular force to occur between the ligand and the amino acid residues of the protein, as well as salt bridges. As we sought to develop more novel classes of drug molecules towards 5-HT2A, THPs were selected as desirable substrates over the more common pyridine and piperidine derived molecules.

In order to produce THPs, the transition metal-catalyzed C-H bond functionalization method can be used. An amine, an enal/enone and an alkyne are catalyzed with Rh(I) to undergo a C-H bond addition and alkenylation, followed by 6-π electrocyclization to produce DHPs (1,2 dihydropyridine). This is an intermediate that can follow various reaction pathways; scientists can selectively obtain different THP products from DHPs via modulation of the strength of acids added (Figure 6).

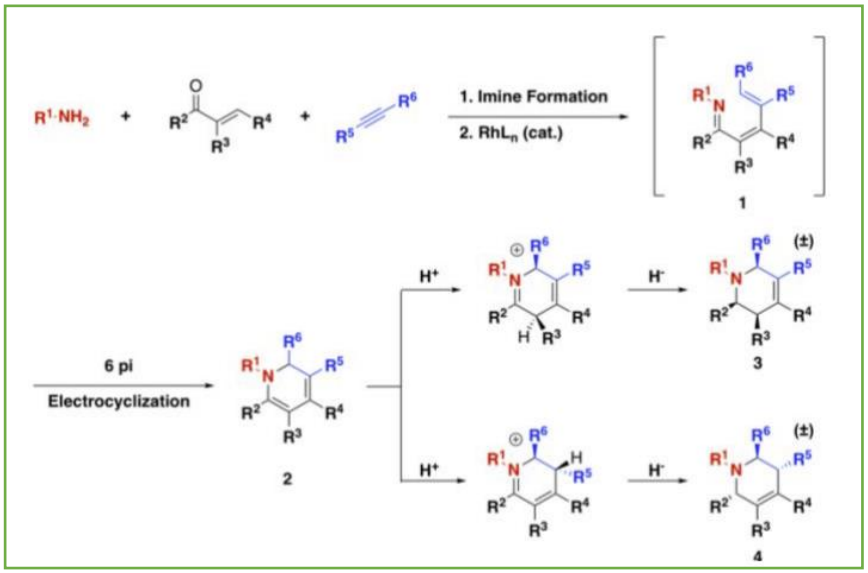

Fig. 6 *Rh(I)-cat. DHP formation and protonation/reduction to THP* [5]

In order to find a suitable starting point for synthesizing potent and selective THP-derived ligands towards 5-HT2A, virtual screening was utilized for this project and was conducted by implementing the ZINC database. ZINC is a database of commercially available compounds where 75 million are tetrahydropyridine compounds containing the essential fragments for targeting the serotonin receptors. Using three-dimensional conformations, the 75 million THP compounds were docked into the receptor and only 5 million compounds were successfully docked and scored. The docking and scoring process considered the strength of the Van der Waals interactions between receptor and ligand, electrostatic interactions, and cost of ligand desolvation. The top 300,000 scored compounds were clustered off into 14,959 cluster heads using a similarity coefficient called the Tanimoto Coefficient (Tc) threshold of 0.5. The top 4,000 cluster heads were then manually selected from the 14,959 cluster heads. Key interactions were observed between the ligand and the receptor in the 4,000 cluster heads where 205 compounds had the strongest interactions. Finally, the 205 compounds were assessed using factors such as the cost of starting materials and the similarity to already existing drugs. After the final experimental observations, 30 primary hit compounds were obtained, and 17 (Figure 8) were synthesized via the C-H activation process described previously.

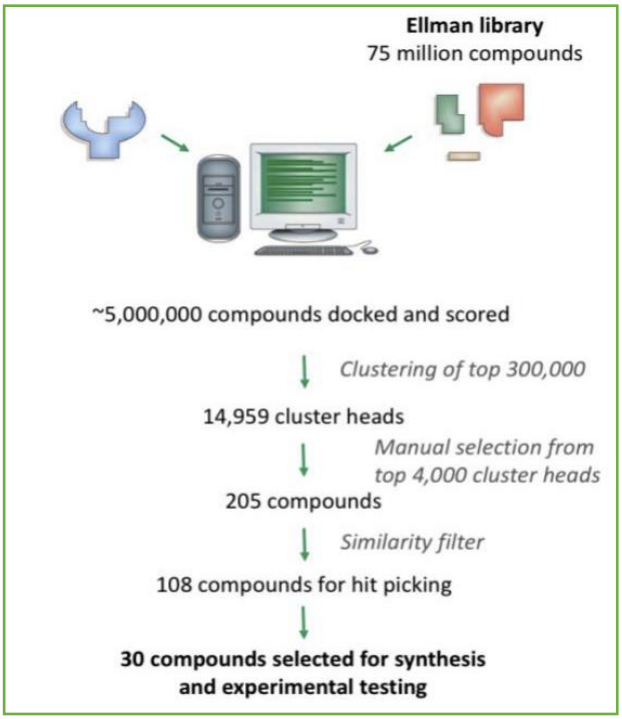

Fig. 7 *Filtering Process and Selection of Top Compounds* [6]

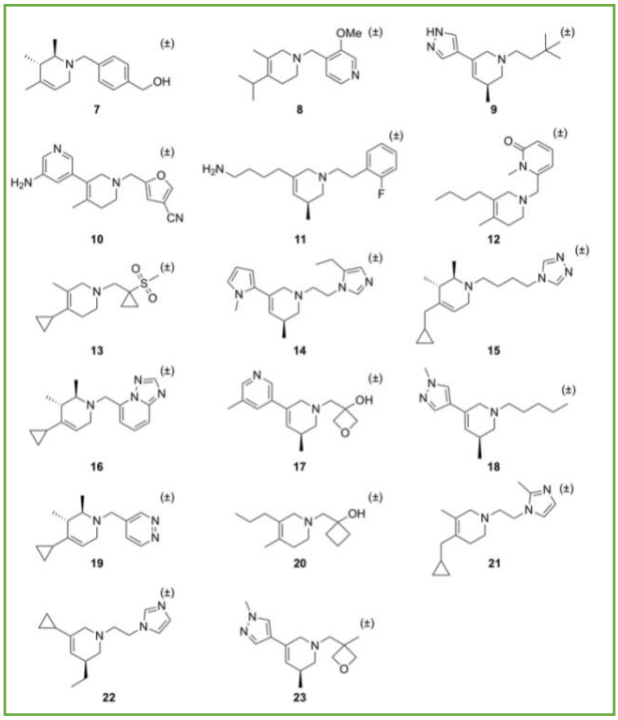

Fig. 8 *THP compounds synthesized from original top hits* [6]

The 17 synthesized compounds were assessed for biological activity utilizing calcium flux assays. These molecules were tested in both agonist and antagonist-based assays. For agonist mode (Figure 11), the black curve on each of the graphs serves as the control. The black curve denotes serotonin binding the 5-HT2 receptors. The same amount of the receptor was added into each well plate. Increasing concentrations of a particular drug were added to the well plates containing the receptor. The points that make the black curve represent the percentage of maximum effect produced by different concentrations of the same drug. For small concentrations, the effect of the drug was recorded to be close to 0 because the number of serotonin molecules relative to the total number of binding sites was very low. As concentration increases, percentage of Emax also increases until it reaches a certain concentration (e.g., -7 log [drug M] for 5-HT_{2B}). At this concentration, all binding sites are occupied. Further increase in concentration doesn't affect the percentage of Emax at this point because we are already at maximum. The potency of a particular drug is quantified by the EC_{50}. EC_{50} describes the concentration of the drug that produces 50% of the Emax. This is the most accurate representation of the potency of a certain drug. One of the main molecules of interest was compound #11 (Fig 10). As the graphs show, this compound did not possess the qualities of an agonist towards the 5-HT2 receptors.

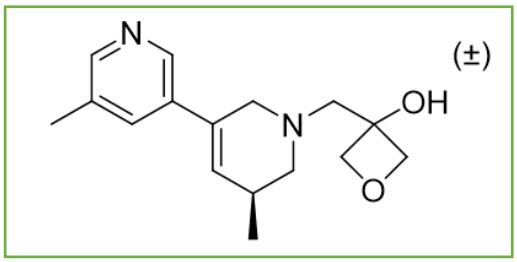

Fig. 10 *The structure of compound #11*[6]

Agonist mode:

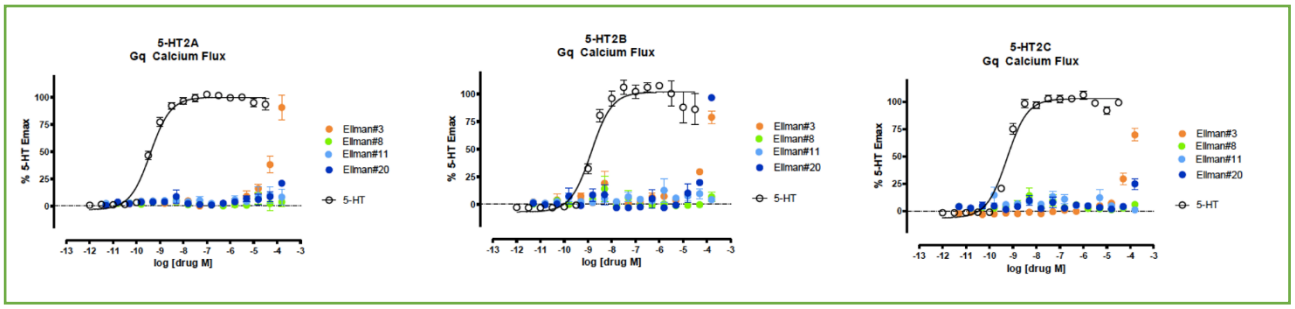

Fig. 11 *Compound #11 exhibits very weak agonist activity at all 3 receptors*

Compound #11 was also tested in antagonist mode. In the antagonist mode (Figure 12), a known agonist is first added to each well plate containing the receptor, effectively inducing 100% Emax as a starting point. Increasing concentrations of the molecules of interest are subsequently added into the well-plates. The effect of each concentration was recorded and plotted on the graph (light blue line for Compound 11). As the concentration of the inhibitor increases, the percentage of Emax decreases and reaches almost zero at -4 log [drug M]. At this concentration, almost all of the function of the receptors is blocked. Further increase in concentration doesn't affect the percentage of Emax at this point because we are already at minimum. The IC_{50} for Compound #11 against the 5-HT2B receptor (related to 5-HT2A) was discovered to be 268 nM. This is a promising start to then conduct SAR to further develop a more potent and selective ligand towards the 5-HT2 receptors.

Antagonist mode:

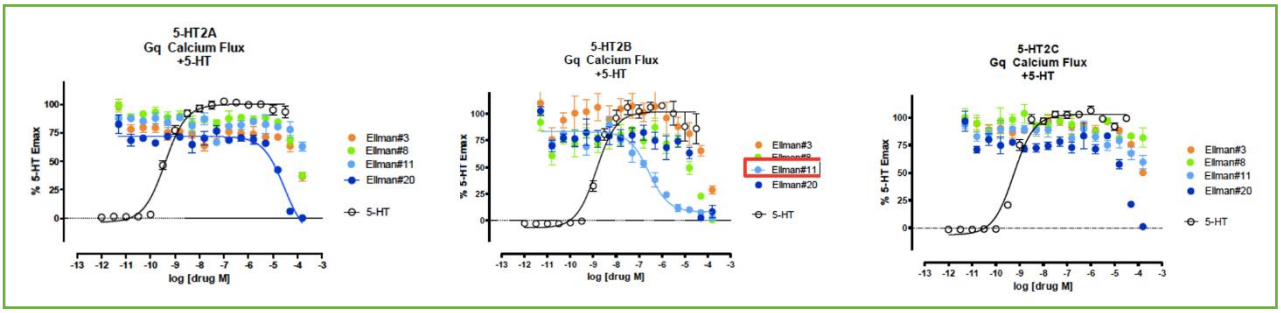

Fig. 12 *Compound #11 exhibits selective antagonist activity at* $5 - HT_{2B}$ *with* EC_{50}*= 268 nM*

In conclusion, here we have demonstrated the discovery of potent and selective ligands towards the 5-HT2 receptors, with one molecule even achieving an IC_{50} of 268 nm. This can provide a good starting place to now conduct SAR and develop even more potent and selective agonists that may provide the groundwork for treating neuropsychiatric disorders. More generally, this review describes the application of virtual screening to synthetic chemistry to efficiently discover drug candidates towards a particular target. This is a generalizable strategy that can be applied to many targets to potentially treat a wide variety of diseases and disorders. Drug discovery is an ongoing development that will continue to grow and evolve as more technology become incorporated with the fundamental science.

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