# DESIGN OF AN OPTIMIZED COMPOUND AGAINST CRYPTOCCOCAL MENINGITIS: SELECTIVITY PROFILE VERSUS SEROTONIN NEURORECEPTOR

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## Abstract
*Cryptococcal meningitis remains overlooked in modern-day medicine. Caused by the fungus C. neoformans, this opportunistic fungal infection afflicts the lives of hundreds of thousands of people annually, particularly for populations with limited access to medicinal resources. The current treatment options for cryptococcal meningitis lack when it comes to their widespread availability and administration approach. This research project delves into the advancement of phenothiazines as a cheaper medicinal alternative, which would seek to act as a more effective fungicidal that would also be available as an orally administrable medication as opposed to the standard IV administration technique most commonly used today. This project aimed to reduce our lead compound's binding affinity towards various serotonin neuroreceptors, as the unintentional inhibition of these neuroreceptors could make way for undesirable side effects. The undesirable side effects are reason for concern regardless of the compound's current promising antifungal properties. Through the use of molecular modeling software, we showcase the initial finding of patterns in molecular analogs that could lead to a substantial lowering of affinity toward serotonin neuroreceptors. Lowering those receptors' affinity will potentially allow us to continue the pursuit of a more logistically effective treatment option for cryptococcal meningitis.*

*Keywords: Cryptococcal meningitis, Cryptococcus neoformans, antifungal, lead optimization, phenothiazine, selectivity profile, serotonin, molecular modeling, structural based drug design*

## Introduction
When it comes to the advancements of modern-day medicine, cryptococcal meningitis remains a piece of the collective puzzle that has not entirely been solved. Caused by the fungus *Cryptococcus neoformans*, cryptococcal meningitis is an opportunistic fungal infection, meaning that it predominantly afflicts those with previously compromised immune systems, more often than not targeting individuals with HIV/AIDS. On top of this increased susceptibility to populations with high HIV/AIDS rates, cryptococcal meningitis currently has an absurdly high mortality rate upwards of 90%. The CDC estimates that of the 220,000 cases of cryptococcal meningitis that occur every year, 181,000 of them are fatal ("C. neoformans Infection Statistics," 2018). Most of these cases, and by extension, most of these deaths, originate in regions of the world with limited access to medicinal resources. Sub-Saharan Africa leads the world in cryptococcal meningitis, with over 160,000 cases annually, it is followed by Asia and the Pacific with just over 40,000 cases annually.

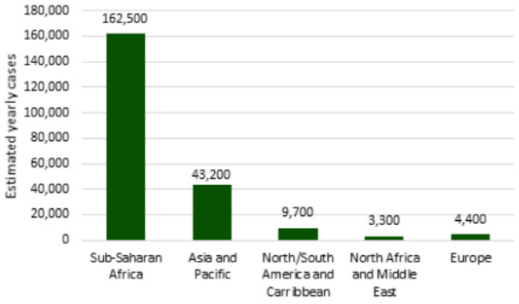


*Figure 1: This chart shows the estimated number of cases of cryptococcal meningitis in varied regions of the world ("C. neoformans Infection Statistics," 2018)*

*Current medicinal limitations*

To those unfortunate enough to fall ill with cryptococcal meningitis, they are usually treated with an initial dose of amphotericin B, which requires close medical supervision due to the drug's high level of toxicity. Following amphotericin administration, the patient receives an extended dose of fluconazole to flush the remainder of the fungus out of the body ("C. neoformans Infection," 2019). This standard treatment method's effectiveness varies, and not everyone can afford the medication. Additionally, this treatment option is only administrable intravenously (IV), which complicates matters further for those who do not live near a medical facility or do not have the means to travel to one for treatment. This project seeks to eventually create a medicinal alternative that would possess a more powerful antifungal profile at a lower cost to patients. Furthermore, there is the potential to make a more widespread delivery method, such as introducing an oral agent that would eliminate many of the unnecessary costs of being treated in a medical facility.

*Phenothiazines*

Recent studies have shown that phenothiazines, a class of compounds most typically used as antipsychotics, are a potential antifungal alternative (Montoya et al., 2017). The testing of numerous analogs of phenothiazines against *C. neoformans*, has shown advancements towards a drug compound with antifungal properties. These properties are necessary for the drug to act as an alternate medication for cryptococcal meningitis. This project focuses on the lead compound **17e** and the synthesis and testing of analogs based on the original molecule's antifungal properties against *C. neoformans*.

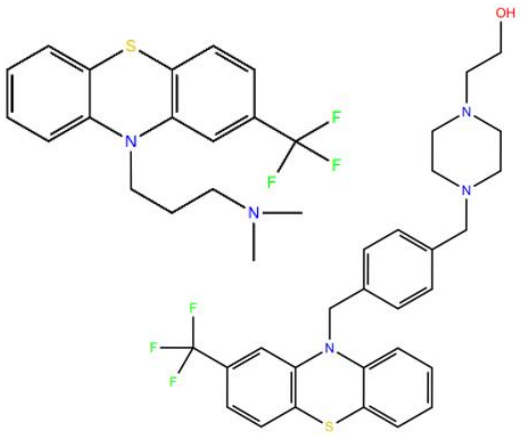


*Figure 2: This figure shows several phenothiazine lead compounds (**8** [left] and **17e** [right]) that have shown promising levels of antifungal activity against C. neoformans.*

*Reason for concern*

Despite the high levels of antifungal activity against *C. neoformans*, there is one critical issue that halts the advancement of these otherwise encouraging lead compounds. The binding affinity of some of these lead compounds towards several serotonin neuroreceptors is too high. The affinity must be lowered to avoid potentially undesirable side effects via unintentional inhibition of these vital hormonal neuroreceptors. This dilemma stems from the structural similarities between the serotonin neuroreceptor's original ligand and our lead compound. This dilemma brings us to this project's driving question: How can we improve our lead compound's selectivity profile against the serotonin neuroreceptor while also retaining as much of the previously observed antifungal activity against cryptococcal meningitis?

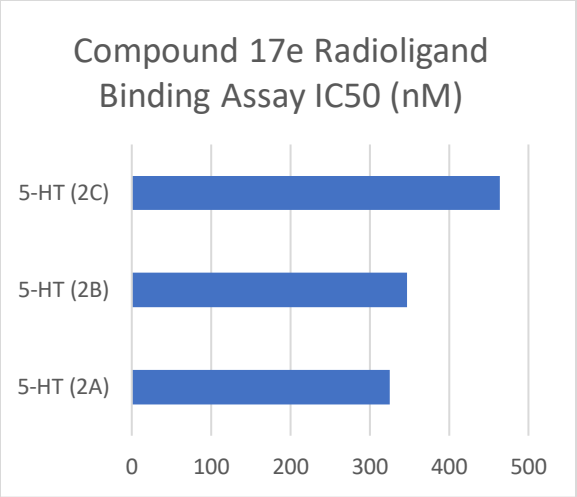


*Figure 3: This chart shows our lead compound 17e being tested against three serotonin neuroreceptors by showcasing their respective $IC_{50}$ values (the minimum concentration needed to inhibit at least 50% of a given receptor). (Montoya, et al., 2017).*

**Methods**

This project had to be modified significantly due to the challenges posed by the SARS-CoV-2 pandemic. Instead of the in-lab research initially planned**,** the project promptly shifted to utilizing the latest molecular modeling software offered by Schrödinger's main interface: Maestro (Release 2020-2). This molecular modeling software is routinely implemented in different research fields, ranging from materials science research to biotechnology research. In its essence and for this project's purposes, Maestro allows us to visualize the intermolecular and intramolecular interactions that are likely to occur between molecules. More specifically, it enabled us to roughly calculate the numerical data points needed to predict whether a ligand and its associated ligand binding site have a high affinity for one another. Its use of high-level physics-based algorithms allows scientists to dictate what would be worth experimenting on in a laboratory setting. Predictions made by Maestro allow researchers to spread out the allocation of resources. For this project, the software would primarily serve to provide the ability to formulate substantiated hypotheses from the results obtained. Some hypotheses will then be tested in a laboratory setting to affirm results when lab work can safely resume. It allows us to perform structural based drug design (SBDD) to accurately predict which molecular models would likely do well in a real-life setting.

*Docking Studies*

The primary methodology employed throughout this project's duration were studies called docking studies. Docking studies are studies between two or more molecular structures and how well they bind to a designated biological target. More specifically, we observed the numerical "docking score" after each of the studies conducted. A docking score is a relative score that accounts for all the positive interactions (hydrogen bonds, salt bridges, etc.) and all the negative interactions (steric clashes, repulsive net charges, etc.) between a ligand and its respective binding site. This score will determine whether or not the introduction of that ligand into that binding site lowered the system's overall energy, which is typically a favorable outcome. The lower and more negative the score, the greater the affinity of the ligand to the binding site. We wanted to lower the affinity of our lead compound toward the serotonin neuroreceptor. Therefore, we looked for less negative docking scores that indicated that more detrimental interactions were occurring.

*Working Knowledge of Interactions*

In this project, we have the advantage of more or less knowing what interactions are likely to occur if we were to add or remove a substituent from our molecule of interest.

We have conducted studies to show how the change in Gibbs free energy is affected when various substituents are added or subtracted to a base molecule (Bissantz et al., 2010). From this, we can reasonably infer the energetic changes that would arise in the molecules central to our project and perhaps allow that to assist us in making critical decisions in our molecular synthesis.

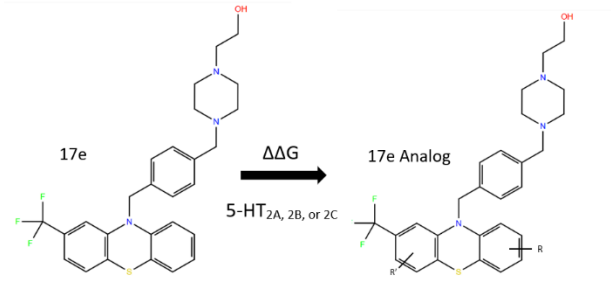


*Figure 4: This figure showcases how the addition of different substituents to our lead compound 17e at the R and/or the R' positions changes ΔΔG*

On top of this working knowledge of organic chemistry principles, we had the opportunity to explore a new feature of Maestro, Ligand Designer, which allows us to gain essential information from our lead compound. It does so by providing us color-coded areas around our ligand when it embeds itself in the binding site. The colored areas, or lack thereof, indicate that the protein accommodates or does not accommodate the addition of a substituent at the various positions of the molecule based on the available space remaining in the binding site. Since we want to reduce the affinity of our lead compound towards the serotonin neuroreceptor, we can take the areas of the ligand which are predicted not to accept an addition favorably and work from there by adding substituents at that spot to see if it generates worse docking scores or prevents binding in that orientation altogether.

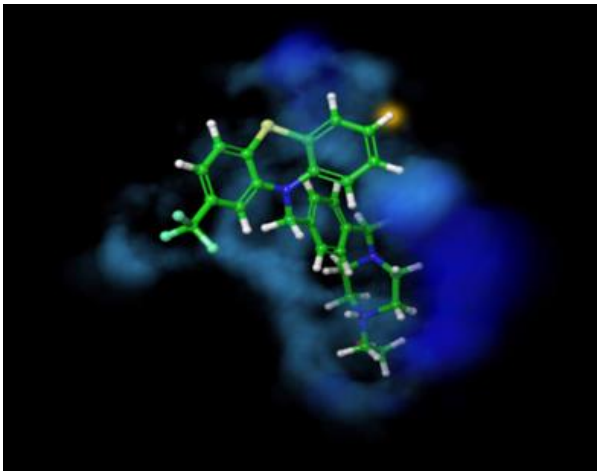

*Figure 5: This is a screenshot of the new feature we are utilizing on our lead compound inserted into the docking site. The blue areas indicate more space to be filled and, therefore, likely accommodate a substituent. The areas in yellow, by contrast, indicate that an addition of a substituent at that position would reduce binding affinity, likely for steric hindrance reasons.*

## Results

From the studies that we have run so far, we have several hypotheses for moving forward with in-lab synthesis and testing our molecular analogs. Several patterns have emerged, suggesting that we can reduce affinity toward the serotonin neuroreceptor. A couple of factors played a role in considering whether any given molecule would be worth synthesizing and testing in the laboratory setting.

### *Docking score*

Naturally, this was the basis for what constituted a good molecular candidate or not. The less negative the docking score, the more likely the analog would bind poorly in the serotonin neuroreceptor binding assay. We were hoping to observe some docking scores that indicated that a particular analog would outright not bind to the neuroreceptor, but we did not observe such results, at least not yet at the time of this publication.

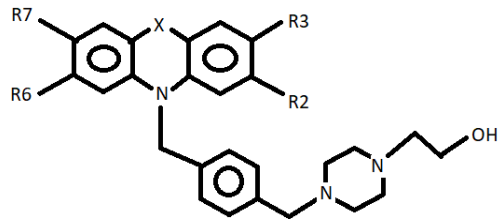


*Figure 6: This shows the backbone template of our lead compound, highlighting the $R_1$-$R_4$ groups that can be changed to create a new molecular analog.*

*Orientation of the Ligand*

The orientation of the ligand analogs into the binding site became essential to us when we saw the appearance of alternate binding orientations. We decided to compare the orientations and their prevalence from each respective analog to the original serotonin ligand's orientation in the binding site. Knowing that the original ligand bound to the docking site in a specific orientation that was also observed in our docking studies, we concluded that whose most stable binding orientations were different from that of the original ligand may have reduced affinity for the receptor.

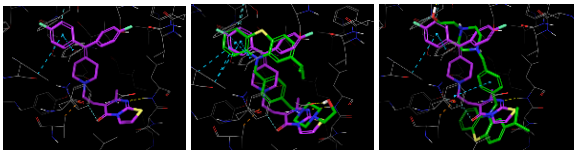

*Figure 7: These screenshots showcase the original ligand (left) and its respective orientation (labeled the RIT orientation) along with several of our lead compound analogs superimposed on that aforementioned original ligand, showing similar or alternate orientations (middle and right) that have been observed in this docking study (RIT orientation and a flipped orientation).*

Since we cannot definitively know that any alternative orientation other than what we have observed in the original ligand docking would occur in a real-life setting, we have reason to prioritize analogs that only give back orientations that differ from the original ligand binding orientation. It is important to note that the software algorithm attempts to fit any given ligand into the binding site provided, and this process is theoretically susceptible to returning orientations that might not be realistic. From this reasoning, we have found analogs that have primarily exhibited this behavior and are potentially usefulz analogs to synthesize in the laboratory setting to test this hypothesis of reduced affinities towards the serotonin neuroreceptor.

| Docking Study | Relative Ranking | Compound | Docking Score | Orientation |
|---|---|---|---|---|
| PTZ (2Me) Retest | Best | PTZ_2Me | -8.806 | RIT Twisted |
| | 2nd Best | PTZ_2Me | -7.307 | RIT |
| | 3rd Best | PTZ_2Me | -6.822 | Flipped |
| | 3rd Worst | PTZ_2Me | -3.136 | RIT |
| | 2nd Worst | PTZ_2Me | -2.983 | RIT |
| | Worst | PTZ_2Me | -2.951 | RIT |
| PTZ (2CF3/7Me) Retest | Best | PTZ_2CF3_7Me | -6.776 | Flipped |
| | 2nd Best | PTZ_2CF3_7Me | -6.705 | Flipped |
| | 3rd Best | PTZ_2CF3_7Me | -6.398 | Flipped |
| | 3rd Worst | PTZ_2CF3_7Me | -2.867 | Flip Twisted |
| | 2nd Worst | PTZ_2CF3_7Me | -2.752 | Flip Twisted |
| | Worst | PTZ_2CF3_7Me | -2.742 | Flip Twisted |

*Figure 8: This chart shows the results of testing two for their docking score and orientation. The second analog shows that of the three best docking scores it gave back, all of them were in a flipped orientation relative to the original "RIT" orientation (PDB 6BQH; Peng et al., 2018). This observation leads us to the prioritization of this compound for in-lab synthesis.*

## Conclusion/Discussion

As mentioned before, the SARS-CoV-2 pandemic prevented us from initiating the originally intended synthesis for this ongoing project. However, the modified project showed us just how valuable the use of molecular modeling software could be on the pathway to helping people who suffer on an annual basis from this specific fungal infection. Our results from the software are not a perfect representation of reality. As long as we instill some cognizance of those limitations, software use will continue to play a vital role in this project's progress towards achieving our goal. Resources to synthesize and test a vast multitude of

molecular analogs are limited. By limiting the number of analogs that we synthesize in the laboratory setting, we prioritize our resources and focus on what could get us to where we want to be more efficiently and cost-effectively. Much work still needs to be done on both the virtual and real-life interface of this project, but the results obtained to date are encouraging towards our end goal of finding a new treatment for cryptococcal meningitis.

*Next Steps*

The next step for this project is to synthesize some of these molecular analogs in the laboratory setting to test them for their respective selectivity profiles against the serotonin neuroreceptor and their antifungal activity against *C. neoformans*. These syntheses will involve Ullmann-type CuI-catalyzed reactions between an aryl ortho-dihalide and an aryl ortho-aminobenzenethiol (Dai et al., 2012). Once complete, two nucleophilic substitution reactions with the amine portions of our initial product and a reagent will follow for the last step of the molecule synthesis. By utilizing $^{1}$H NMR spectroscopy and HPLC coupled mass spectrometry, we can analyze and test our synthesized products' purity based on the chemical shift values we have on record (Montoya, et al., 2017).

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