Combating malaria: a drug discovery approach using thiazole derivatives against PfPKG enzyme

Hari Bezwada*[1], Michelle Cheon*[2], Ryan Divan*[3], Hannah Escritor*[4], Michelle Kagramian*[5], Isha Korgaonkar*[6], Maya MacAdams*[7], Udgita Pamidigantam*[8], Richard Pilny*[9], Eleanor Race*[10], Angadh Singh*[11], Nathan Zhang*[12], LeeAnn Nguyen[13], Dr. Fina Liotta[14]

[1] Moorestown High School, Moorestown, New Jersey

[2] Fort Lee High School, Fort Lee, New Jersey

[3] Saint Peter's Preparatory School, Jersey City, New Jersey

[4] Eastern Regional High School, Voorhees, New Jersey

[5] Marine Academy of Technology and Environmental Science, Stafford Township, New Jersey

[6] Northern Highlands Regional High School, Allendale, New Jersey

[7] Kingsway Regional High School, Woolwich, New Jersey

[8] Thomas Edison Energysmart Charter School, Somerset, New Jersey

[9] North Warren Regional High School, Blairstown, New Jersey

[10] Clearview Regional High School, Mullica Hill, New Jersey

[11] Wayne Hills High School, Wayne, New Jersey

[12] Watchung Hills Regional High School, Watchung, New Jersey

[13] Department of Chemistry, Drew University, Madison, New Jersey

[14] Department of Chemistry, Rutgers University, Newark, New Jersey

*These authors contributed equally to this work

SUMMARY

Malaria is a deadly disease caused by the *Plasmodium* parasite, which continues to develop resistance to current antimalarial drugs. In this research project, the effectiveness of numerous thiazole derivatives was explored in inhibiting the PfPKG, a crucial part of the *Plasmodium* life cycle. This study involved the synthesis of six thiazole-derived amides to inhibit the PfPKG pathway. Nuclear Magnetic Resonance (NMR) spectroscopy and Infrared (IR) spectroscopy were used to characterize these compounds. Furthermore, AutoDocking software was used to predict binding affinities of these thiazole-derived amides in silico. In silico, compound 6 exhibited the highest predicted binding affinity to PfPKG, while compound 5 had the lowest affinity. Compounds 1-4 displayed varying degrees of predicted binding affinity. In-vitro, it was found that compound 4 had the best percent inhibition, while compound 5 had the worst percent inhibition. Overall, all six compounds had weak inhibition (approximately 30-39% at 10 μM), but these results provide foundation for future drug discovery experiments.

INTRODUCTION

Malaria is a parasite-based disease that is transmitted via the bites of infected female *Anopheles* mosquitoes. In 2020, an estimated 627,000 people died of malaria—most being young children in sub-Saharan Africa (1). Malaria is caused by the *Plasmodium* parasite, which depends on a number of metabolic pathways in its human host in order to survive and procreate. Current treatments include antimalarial drugs such as artemether-lumefantrine (Coartem); however, a growing problem is *Plasmodium*'s development of resistance to these drugs (2). This, in conjunction with the fact that mosquitoes continue to show insecticide resistance, which ultimately reduces the effectiveness of preventative measures like indoor spraying (coating the walls and other surfaces of a house with a residual insecticide), necessitates the development of alternative treatment methods (3).

Creating a small molecule drug targeted explicitly against Plasmodium could be crucial in malaria control and eradication efforts. In addition to allowing for more targeted treatment, it would be a cost-effective measure that could be provided even to these developing areas of the world, bringing the overall fatality and severity of malaria down. The Plasmodium parasite chiefly depends on the PfPKG enzyme, a cyclic GMP (cGMP) activated serine/threonine protein kinase found within malaria parasites, specifically in the P. falciparum parasite (4). The malaria parasite uses the PfPKG enzyme in the folate synthesis pathway, which is essential for the synthesis of DNA and RNA. The folate synthesis pathway also proves essential for the growth, replication, and survival of the parasite, allowing it to continue reinfecting in the human body. The inhibition

of PfPKG leads to the alteration of several key processes in the parasite's life cycle including: blood stage replication, erythrocyte invasion, and gametogenesis and ookinete motility (4).

In conducting research to provide preclinical validation of PfPKG as a target for antimalarial therapy, published studies reported that a PfPKG inhibitor based on imidazopyridine successfully cleared *P. falciparum* infection in mice engrafted with human erythrocytes (5). Another current intervention is derived from selective inhibitors of PKG from *Eimeria* and *Plasmodium* which are believed to interact with a small hydrophobic pocket near the ATP-binding site (5). The presence of a specific gatekeeper residue (T618 in PfPKG) in these parasites' PKGs allows access to the pocket, making them sensitive to these inhibitors and essential for various stages of the *Plasmodium* life cycle (6). One of the first potent inhibitors of PfPKG was an isoxazole-based scaffold, 4-[2-(4-fluorophenyl)5(1-methylpiperidine-4-yl)-1H pyrrol-3-yl]pyridine [Compound L] (Figure 1) (6). Isoxazoles and isoxazoline are five-membered heterocyclic molecules containing nitrogen and oxygen, a popular heterocyclic compound for developing novel drug candidates (6). The molecule can have a broad range of bioactivity used for anti-tumor, antibacterial, anti-inflammatory, antidiabetic, cardiovascular, and other activities (6).

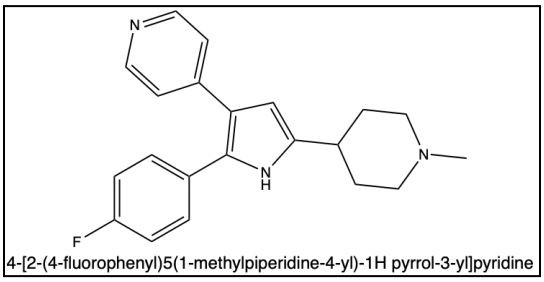


Figure 1. Isoxazole-based scaffold, 4-[2-(4-fluorophenyl)5(1-methylpiperidine-4-yl)-1H pyrrol-3-yl]pyridine [Compound L].

Existing literature has primarily focused on experiments conducted with thiazole analogs, such as the chloroquine-sensitive *Plasmodium falciparum* 3D7 strain. The studies found that modifying the N-aryl amide group linked to the thiazole ring had shown substantial in vitro antimalarial activity (7).

Thiazole, a heterocyclic compound composed of a five-membered ring containing three carbon atoms, one nitrogen atom, and one sulfur atom, is a versatile biological scaffold in the field of medicinal chemistry. Its electron-rich sulfur and nitrogen atoms enable crucial interactions with viral enzymes, disrupting viral replication as effective antiviral agents. In

anticancer research, thiazole derivatives have exhibited cytotoxic potential against cancer cells, inducing apoptosis and inhibiting tumor growth pathways (3). Thiazole holds potential as a starting compound in synthetic chemistry, in which chemists can expedite drug development with a privileged structure disrupting the parasite's life cycle.

While many citizens of the Western World view malaria as a far-off threat that only affects developing countries and avid travelers, the recent rise in United States based malaria cases proves that this disease is a prevalent issue around the world. Malaria is a parasitical, mosquito-borne disease and ranks as the fifth deadliest disease worldwide (1). The US spent an average of 206 million dollars each year from 2012 to 2022 on malaria treatments and control; this spending does not include the money dedicated to research for a malaria vaccine (17). While malaria mainly impacts Sub-saharan Africa, that does not make the U.S. immune to its infection. During the summer of 2023 in the U.S. multiple locally acquired cases have been recorded in Texas and Florida (18).

In this study, the synthesis of six differing thiazole-derived compounds in order to target the PfPKG pathways contribute positive data for drug discovery and the overall goal of combating malaria. Usage of amides can be synthesized based on thiazole as a starting compound in hopes of inhibiting the PfPKG pathway. This was done by first synthesizing the thiazole, then the related pyridinyl/pyrimidinyl compounds, and then the six different amides. For the analysis portion of the project, a Nuclear Magnetic Resonance (NMR) spectroscopy was then performed to observe the position of protons in the synthesized amide compounds as well as Infrared (IR) spectroscopy to identify the functional groups. These compounds were then docked in an AutoDocking software that allowed for the visualization of the compounds' binding to the PfPKG active site and the determination of quantifiable predicted binding affinities.

RESULTS

The end result of synthesis yielded six distinct amide products to be sent out for biological testing through a partner laboratory at Montclair State University (Table I.). Each compound contains a thiazole and unique associated derivative in hopes of yielding different data due to their R groups. In order to confirm the end products, two methods were utilized: infrared spectroscopy (IR) and nuclear magnetic resonance (NMR) spectroscopy.

Table I. Final Synesized Amide Product Table

Final Amide	Structure	Compound #

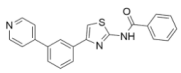
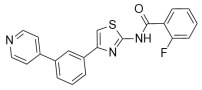
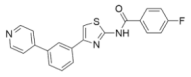
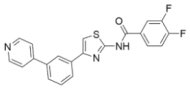
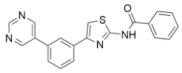
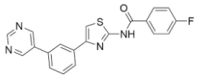
N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl)benzamide		1
2-fluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl) benzamide		2
4-fluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl) benzamide		3
3,4-difluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl)be nzamide		4
N-(4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-yl) benzamide		5
4-fluoro-N-(4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-yl)be nzamide		6

Table III. NMR data for Associated Compounds Synthesized in Laboratory. Cumulative of degrees of unsaturation, chemical shift, spin multiplicity, integral, and coupling constants. Refer to Appendices G-N and X for NMR Spectra

Compound	NMR signals
A	1H NMR (600 MHz, cdcl3) δ 7.92 (s, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.39 (d, J = 1.9 Hz, 1H), 7.30 – 6.93 (m, 2H), 6.73 (s, 1H), 5.10 (s, 3H)
B	1H NMR (600 MHz, cdcl3) δ 8.66 (d, J = 5.2 Hz, 1H), 8.07 (s, 0H), 7.82 (d, J = 7.9 Hz, 0H), 7.57 – 7.52 (m, 1H), 7.48 (t, J = 7.7 Hz, 0H), 7.24 (s, 0H), 6.80 (s, 0H), 5.03 (s, 1H).
C	1H NMR (500 MHz, Acetone) δ 9.17 (s, 1H), 9.11 (s, 2H), 8.23 (t, J = 1.8 Hz, 1H), 8.00 (dt, J = 7.8, 1.4 Hz, 1H), 7.70 – 7.63 (m, 2H), 7.56 (t, J = 7.7 Hz, 1H), 7.51 (dd, J = 5.9, 2.2 Hz, 1H), 7.16 (s, 1H), 6.47 (s, 2H).

1	1H NMR (500 MHz, Acetone) δ 8.72 – 8.65 (m, 1H), 7.99 (s, 0H), 7.97 (s, 0H), 7.94 (d, J = 7.1 Hz, 2H), 7.83 – 7.78 (m, 0H), 7.69 (dd, J = 8.0, 1.6 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.58 (d, J = 6.2 Hz, 1H), 7.54 (d, J = 7.6 Hz, 2H), 7.49 (d, J = 7.7 Hz, 0H).
2	1H NMR (600 MHz, cd3od) δ 8.63 – 8.55 (m, 1H), 7.85 (d, J = 6.5 Hz, 1H), 7.70 (dt, J = 7.8, 1.3 Hz, 1H), 7.64 – 7.60 (m, 1H), 7.52 (ddt, J = 10.1, 4.8, 2.1 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.27 (td, J = 7.6, 1.0 Hz, 1H), 7.15 – 7.06 (m, 1H), 4.08 (q, J = 7.1 Hz, 0H), 3.29 (s,0H), 1.99 (s, 0H), 1.21 (t, J = 7.1 Hz, 0H).
3	1H NMR (600 MHz, cd3od) δ 4.07 (d, J = 7.1 Hz, 1H), 3.47 – 3.10 (m, 3H), 1.99 (s, 2H), 1.21 (t, J = 7.1 Hz, 2H).
4	1H NMR (500 MHz, Acetone) δ 10.10 (s, 1H), 8.77 – 8.66 (m, 1H), 8.35 (s, 0H), 8.26 – 8.16(m, 1H), 8.12 (s, 0H), 8.07 (ddd, J = 7.8, 3.1, 1.6 Hz, 1H), 8.01 (d, J = 8.3 Hz, 2H), 7.94 –7.85 (m, 2H), 7.83 – 7.70 (m, 5H), 7.67 (d, J = 8.1 Hz, 2H), 7.60 (dt, J = 18.7, 8.0 Hz, 2H),7.35 (d, J = 7.8 Hz, 3H), 7.17 (d, J = 7.6 Hz, 1H), 7.03 (s, 1H).
5	1H NMR (500 MHz, Acetone) δ 9.19 (s, 1H), 9.15 (s, 1H), 9.07 (s, 1H), 9.01 (s, 1H), 8.33 (s, 1H), 8.23 (m, 2H), 8.10 (dt, 2H), 8.06 (m, 3H), 7.94 (m, 5H), 7.89 (m, 4H), 7.79 (dd, 4H), 7.76 (s, 1H), 7.74 (s, 1H), 7.69 (m, 6H), 7.63 (dt, 6H), 7.52 (dd, 5H), 7.48 (s, 1H), 7.43 (m, 3H), 7.38 (m, 4H)
6	1H NMR (500 MHz, Acetone) δ 9.20 (s, 1H), 9.15 (s, 2H), 8.36 – 8.23 (m, 4H), 8.10 (d, J = 7.8 Hz, 1H), 7.77 (s, 1H), 7.74 (s, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.38 (t, J = 8.6 Hz, 2H), 7.21 (s, 0H).

Docking Studies

Compounds 5 and 6 had the lowest in silico binding affinity of the six amides, while Compound 2 had the best in silico binding affinity (Table IV). Compounds 5 and 6 were both pyrimidinyl compounds, which could indicate that pyrimidinyl compounds are not as effective as pyridinyl compounds in inhibiting the PfPKG enzyme. It must also be noted that Compounds 1 and 2 took up a unique orientation in docking through AutoDock Vina which yielded a greater binding affinity, which can be observed by comparing Appendices P and Q to the other, typical

orientations in the remaining docking simulations. However, as these different orientations are only observed in silico, their affinity in an orientation similar to the Compound L, which has been derived from previous literature, will be used primarily.

Table IV. In-Silico Binding Affinity of Amides as determined by the AutoDock Vina Score. Refer to Appendices O-U for enzyme-inhibitor complex visualizations.

Drug	AutoDock Vina Score
4-[2-(4-fluorophenyl)-5-(1-methylpiperidine-4-yl)-1H pyrrol-3-yl]pyridine	-8.7
Compound 1	-9.6 (-10.1*)
Compound 2	-9.7 (-10.3*)
Compound 3	-9.8
Compound 4	-10.1
Compound 5	-9.5
Compound 6	-9.7

Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311.

Percent (%) Inhibition Data

Table V. Percent inhibition at 10uM for six thiazole-based compounds and starting material, 2-aminothiazole. Refer to Appendix V for control IC50 data.

Compound	% Inhibition at 10 μM	STDev
N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl)benzamide	32.3	26.2
2-fluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl) benzamide	34	19.8

4-fluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl) benzamide	30.1	5.5
3,4-difluoro-N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl)benzamide	38.6	11.2
N-(4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-yl) benzamide	30.2	15.4
4-fluoro-N-(4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-yl)benzamide	37.9	24
2-aminothiazole	34	2.8

DISCUSSION

Only five of the hundred existing species of malaria have been known to cause disease in humans (15). The specific species of *Plasmodium* that causes malaria targeted in this study was *P. falciparum.* cGMP, a second messenger in eukaryotic cells, is key in amplifying cellular responses. *P. falciparum* PKG (PfPKG), the cGMP-dependent protein kinase, is responsible for triggering the cGMP signaling in *Plasmodium* (16). In other words, PKG is required for the activation of P*lasmodium*. This research worked to synthesize new and more effective malaria combative drugs by employing various functional groups in combination with either 4-(3-(pyridin-4-yl)phenyl)thiazol-2-amine or 4-(3-(pyrimidin-5-yl)phenyl)thiazol-2-amine. This approach to malaria treatment emphasizes the importance of understanding molecular chemistry and biology as a mechanism of disease remedy.

Based on the NMR results acquired, Compounds 4 and 5 were produced with contamination. This could have been due to excess solvent, residual material from previous experiments left in the glassware, or an impurity in the nitrogenous atmosphere. For this reason, the results generated by the AutoDock are only representative of the most optimal conditions for a reaction to occur (ex. compound orientation in enzyme active binding site) (Table V). Impurities found in our compounds, whether from previous reactions or contamination, could be representative of a synergic effect. Although Compound 4 is associated with the highest corresponding affinity, its non-pure product sent out for analysis can not confirm its full binding potential due to the product mixture. The Percent (%) Inhibition Data affirmed that compound 5

had the lowest % Inhibition at 10 uM (Table V). Generally, the % inhibition data of the six compounds was around 30-39%, so it was not considered potent. However, the compounds are binding to the enzyme and inhibiting to some extent, so future drug models can be built upon the current structures.

Determining the best binding affinity between the six synthesized compounds and the PfPKG active site was the overall goal of this experiment. Each synthesized compound was considered to dock more favorably than Compound L, showing an improvement in inhibitory efficacy with a slightly different orientation (Figure 7). The pyridyl-based compounds tended to have better results than the pyrimidyl-based compounds with the same amides, and amides with greater amounts of fluorine atoms binded more favorably. It should be noted that the docking studies yielded unique binding orientations for Compounds 1 and 2, leading to a higher possible binding affinity in silico. The docking results also showed that all the synthesized amides had four torsions, which resulted in activity and one that led to inactivity, showing that there is a possibility for unfavorable orientation leading to a lower probability of collision in a proper orientation and thus an expected longer average time for inhibition.

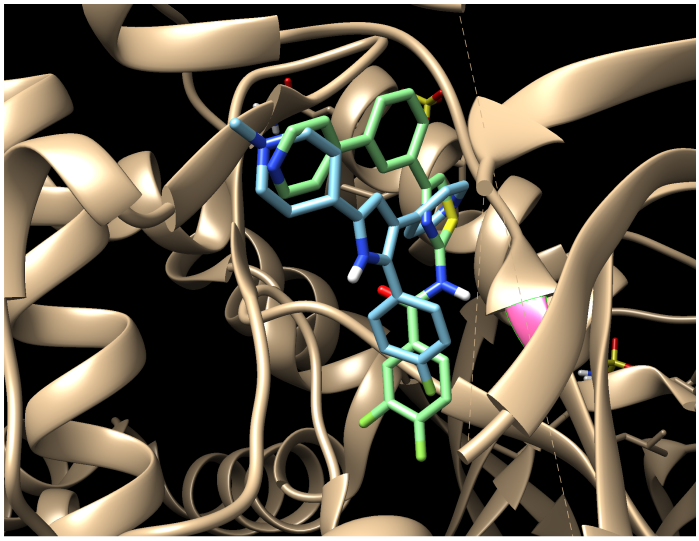


Figure 7. Ligand binding at PfPKG enzyme active site. Chimera model of Compound L (blue) vs. Compound 4 (green) orientation in enzyme-inhibitor complex with gatekeeper residue Thr618 (pink).

Though in silico results were favorable, even yielding more favorable docking for the synthesized compounds than Compound L, in vitro results showed rather low inhibition; even at 10 μM, none of the compounds were able to reach 50% inhibition, remaining between 30-39%. The control compounds, however, reached 50% inhibition at approximately 60 nm (Appendix

W), showing that these specific thiazole-based inhibitors, although active, are not as potent as the controls. Just as in the docking simulations, Compound 4 was the most effective inhibitor out of the synthesized compounds. Additionally, the pyrimidinyl-based compounds were less effective than the pyridyl-based ones across the board, and the variation in inhibition across these structures can guide future studies and synthesis.

Limitations

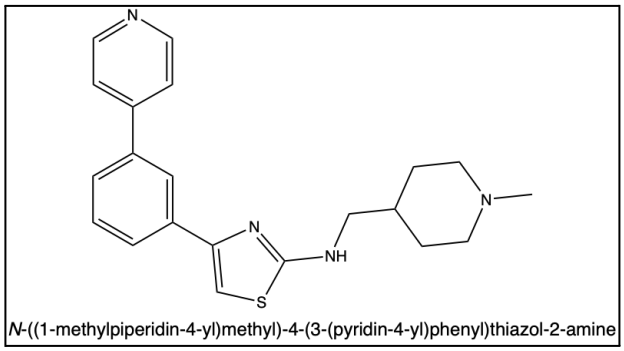
There were several limitations to this series of experiments. Firstly, time was a limiting factor as there were only three weeks and eight total team project sessions to synthesize and evaluate the compounds. Two of these meetings were spent synthesizing base compounds such as the thiazole core, leaving even less time to synthesize the final product. Due to these restrictions, there was not enough time to conduct multiple trials to reduce error. If time were not a constraint, it would have been better to dock the substances before the synthesis. Preliminary docking would have been advantageous due to allowing more efficient and targeted synthesis of compounds that are effective in silico. It would have been ideal to include more replicates of the six different compounds instead of generating only one sample of each for biological processing. If there was an error in the synthesis of one compound as determined by results from NMR/IR, there would be no way to compare the PfPKG inhibition of this compound to the other five. Additionally, small scale reagents were another limiting factor. The functional group and base pairings of these compounds was largely determined by what products were available, not by which had the best predicted outcomes. These reactions were executed on a micro scale due to the toxicity of the reagents, which overall negatively impacted the product yield. In other words, there was not enough reagent to generate large amounts of product.

The programs used to dock the synthesized compounds were “Chimera” and “AutoDock Vina,” both of which are primarily used for protein modeling and synthesis. These programs were used following compound synthesis to test the binding affinity of each amide. It is important to acknowledge that the team had limited experience with using these docking tools. This could be attributed to the fact that computer programs can not account for all the possible situations (ex: attaching to a variant active site) which may occur out of simulation. Due to inexperience, there might have been a possibility of overlooking some potential interactions between the compounds and the target protein (PfPKG enzyme). One alternative option to consider for more accurate analysis would be to send the compounds for docking on external sites like "Swissdock,” which is a well-established online platform that specializes in molecular docking.

For Future Reference:

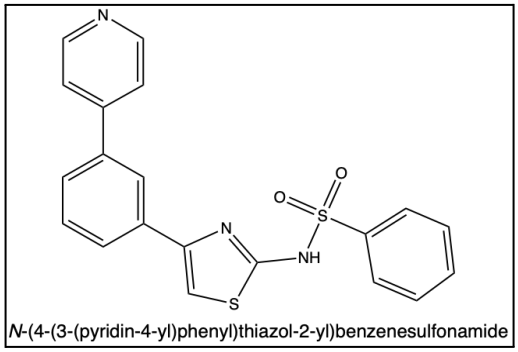
In future versions of this study, it would be ideal for researchers to dock their compound prior to synthesis, so that they would be able to select the best hypothetical base and functional group pairing to ensure the best inhibition of the PfPKG enzyme. In other words, reviewing the various target-compound interactions of the potential compounds prior to synthesis can aid in maximizing the positive results and also minimize the amount of wasted product and base.

For future studies, the focus of the research should be to follow the compounds of the best PfPKG inhibition to maximize their suitability for the human body. The amides synthesized in this research focused on having the highest possible affinity, this however would not be suitable as a drug in their current condition given that they are not soluble and would expose the patient to a greater risk of complications. To improve solubility for future drug potential, some approaches could include adjusting the pH, reducing bulkiness, or exploring alternative functional groups. Also, the compounds in their current states do not have the ability to be easily excreted from the body, consequently they would be forced to stay within the body increasing the risk for toxicity. To alleviate this, a hypothetical soluble drug was considered, *N*-((1-methylpiperidin-4-yl)methyl)-4-(3-(pyridin-4-yl)phenyl)thiazol-2-amine) (Figure 8.A), using the same pyridinyl and 2-aminothiazole core but replacing the benzene ring with a cyclohexane, to which a nitrogen is attached. The carbonyl group in the original compound would impact the solubility through interactions with the nitrogen, and replacing it in this new compound would allow for greater solubility. When docking this compound, it receives an AutoDock score of -8.9, showing considerable potency while hypothetically having more potential as a drug. Simulations also show no inactive torsions, meaning that there is a higher probability for successful collision than the synthesized compounds.



N-((1-methylpiperidin-4-yl)methyl)-4-(3-(pyridin-4-yl)phenyl)thiazol-2-amine

A



N-(4-(3-(pyridin-4-yl)phenyl)thiazol-2-yl)benzenesulfonamide

B

Figure 8. Comparison of compound solubility based on their chemical structures from literature versus laboratory synthesis. **A.** Represents the structure of soluble malaria drug currently being studied in literature, used as a control and comparative model for the docking simulation. **B.** Desired chemical structure for sulfonamide synthesis.

This experiment initially sought to consider sulfonamides with the 2-aminothiazole cores. However, due to the high complexity of synthesis, individual steps in the process failed to yield sufficient product due to limitations of scale, rendering the sulfonamides unable to be produced. Though in vitro results are not available due to such limitations, docking through AutoDock showed that the compound had a score of -9.8, which is greater than the comparable Compounds 1 and 5. This compound also has no inactive torsions, so it similarly has a higher probability for successful collisions than the amides. Further exploration of sulfonamides could be advantageous, though considerations of solubility must remain.

CONCLUSIONS

Among the six compounds synthesized, the AutoDock showed that Compound 6 resulted in the highest binding affinity (Table IV). A high affinity allows for the drug to have a stronger bond to the scaffold structure. This competitive inhibitor thereby has the greatest ability to bind to the substrate without allowing the enzyme PfPKG to catalyze the reaction that causes malaria. Compound 5 had the lowest affinity according to the docking, thus it is the least effective of the synthesized compounds in silico. In vitro, however, these compounds all proved to be relatively ineffective when compared to the controls, requiring more than 150 times more substance while still bringing about lower inhibition. All the compounds achieved an average of 37% inhibition at 10 μM, and when considering their insolubility alongside this low efficacy, they prove to be unreasonable to use as antimalarial drugs, though these results may help guide future explorations in PfPKG inhibition. A possible reason for this weak potency could be the lack of alignment between the synthesized compounds and the literary compound, as they take up different spaces which may have affected the capability for inhibition. This difference in interactions with the enzyme means that compounds with a more similar orientation may be prospective targets for synthesis.

In forming all of these compounds, it is evident that there are similarities and differences on the molecular level that alter how the compounds react. The basic structure of all the drugs consists of cyclopentane. This is a five sided figure of carbon bonds with hydrogens bonded to each carbon. This structure, prevalent throughout all the drugs, is helpful because the

hydrogens on the edges of the molecule allow for it to react with other molecules, in a potential hydrogen bond, while still producing dispersion forces as well. All of the structures also contain a carbon with a double oxygen bond and a bond with NH.

Along with similarities, these molecules also share some differences. Compounds 1-4 (Table I) all have a cyclopentane with a nitrogen, which is the pyridine that was used during the experiment. On the other hand, compounds 5-6 both have rings with two nitrogens, which is the pyrimidine that was used in those two reactions. Both of these compounds act as ligands, which have the purpose of attaching to the protein in order to stabilize it and react with outside molecules. Another difference is in the amount of fluorine bonded to each compound. Compound 1 and 5 both have no fluorine, whereas 2, 3, and 6 have one, and Compound 4 has two. In addition, each compound has fluorine in a different point in the carbon ring. Fluorine is able to create dipole bonds and hydrogen bonds when binding with hydrogen, which makes it good for a drug to have because it allows the molecule to react better. Overall, these differences in structure cause each of the molecules to behave differently.

In order to get stronger results in the future, limitations must be addressed and efforts should be made to not only increase the potency of the compounds, but also their solubility and excretability. In this way, progress can be made towards the most effective inhibition of the PfPKG enzyme and a more efficient and reliable treatment for malaria.

MATERIALS AND METHODS

Summation of Methods

The synthesis of thiazole amides 1-6 was accomplished in three steps from the a-bromoketone, 2, 3'-dibromoacetophenone. Thus, the condensation reaction of the dibromoacetophenone with thiourea in refluxing ethanol led to the formation of 2-aminothiazole A in excellent yield. The subsequent Suzuki cross coupling reaction of compound A with both pyridin-4-ylboronic acid and pyrimidin-3,5-ylboronic acid afforded the corresponding biaryl coupled products B and C in modest yields. Amide formation of compounds B and C with a series of substituted benzoyl chlorides gave rise to the target thiazole amides 1-6. Each synthesis was characterized by a similar methodology for working up the product using thin layer chromatography and column chromatography.

Synthesis of the Thiazole Core

The first priority of this project is to synthesize the thiazole core, 2-aminothiazole. This core is a scaffold to which more functional groups may be added to achieve more potent

compounds. The following reaction scheme is used to synthesize the thiazole core, 4-(3-bromophenyl)thiazol-2-amine, or Compound A.

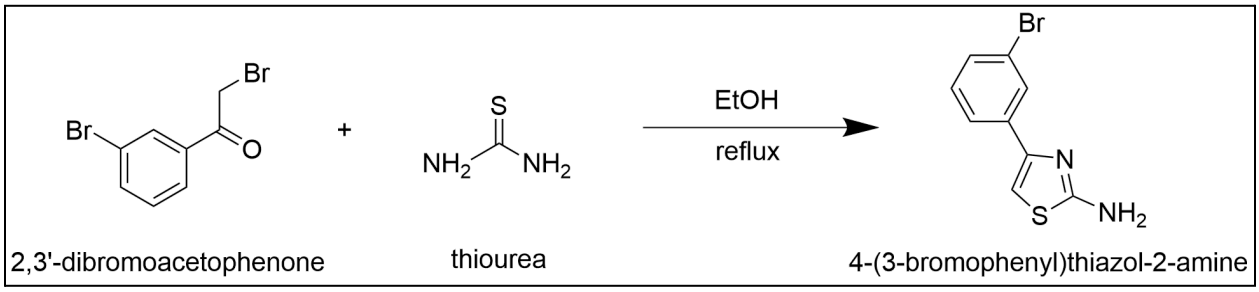


Figure 2. Synthesis of 4-(3-bromophenyl)thiazol-2-amine (Compound A) Reaction Scheme. Refer to Appendix A for Reagent Table

A 2-necked 50 mL round bottom reaction vessel equipped with a reflux condenser and a rubber septum was set up and placed under a nitrogen atmosphere. 2,3'-dibromoacetophenone, thiourea, and ethanol were added to the reaction vessel. The homogenous solution was then mixed at reflux for 90 minutes. Once the reaction was cooled, the solution was diluted with a saturated solution of $NaHCO_3$ to a pH of 8-9. The crude product was extracted with 2 portions of 25 mL of dichloromethane. The combined organic layers were washed with brine solution and then dried with $MgSO_4$. The product was concentrated in a rotary evaporator, and it was diluted using petroleum ether. The solid product was filtered using a sintered glass funnel.

Suzuki Cross-Coupling Reaction

During this reaction, the pyridyl and pyrimidinyl groups must be coupled with the thiazole core . Both products with either pyridyl or pyrimidinyl groups are needed for the final reaction scheme. Suzuki's Cross-Coupling method was implemented in order to take advantage of organometallic chemistry to push a reaction forward to obtain the desired product. This reaction combines boronic acid, and organohalide and a palladium catalysis for product coupling (21).

Figure 3. Synthesis of 4-(3-(pyridin-4-yl)phenyl)thiazol-2-amine (Compound B). Addition of 4-(3-bromophenyl)thiazole-2-amine, pyridin-4-ylboronic acid and Pd(dppf)Cl_2 catalyst to push for cross coupled product. Refer to Appendix B for Reagent Table

A 2-necked 50 mL round bottom reaction vessel equipped with a reflux condenser and a rubber septum was set up and placed underneath a nitrogen atmosphere. 4-(3-bromophenyl)thiazol-2-amine, 4-pyridylboronic acid, K2CO3, THF, and water were added to the reaction vessel. Pd(dppf)Cl2 was added to the homogenous solution. The homogenous solution was stirred at reflux for 4 hours. The reaction mixture was diluted with water. The reaction mixture was extracted with ethyl acetate. The product was concentrated under a rotor evaporator and purified with silica gel.

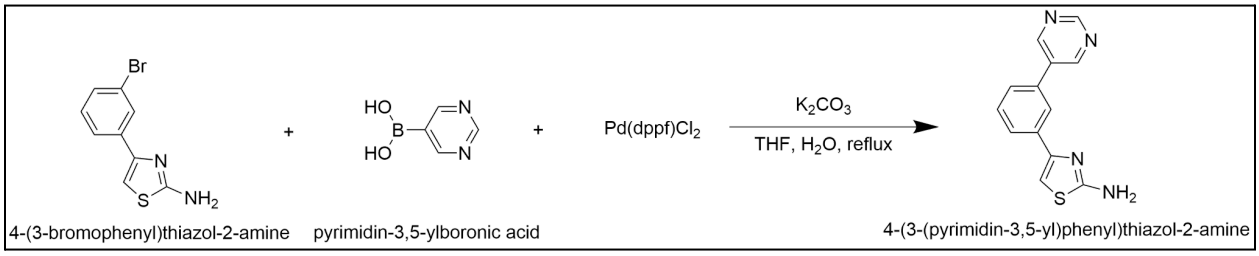


Figure 4. Synthesis of 4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-amine (Compound C). Addition of 4-(3-(pyrimidin-3,5-yl)phenyl)thiazol-2-amine, pyridin-4-ylboronic acid and Pd(dppf)Cl_2 catalyst to push for cross coupled product. Refer to Appendix B for Reagent Table

A 2-necked 50 mL round bottom reaction vessel equipped with a reflux condenser and a rubber septum was set up and placed underneath a nitrogen atmosphere. 4-(3-bromophenyl)thiazol-2-amine, 3,5-pyrimidinylboronic acid, K2CO3, THF, and water were added to the reaction vessel. Pd(dppf)Cl2 was added to the homogenous solution. The homogenous solution was stirred at reflux for 4 hours. The reaction mixture was diluted with water. The reaction mixture was extracted with ethyl acetate. The product was concentrated under a rotor evaporator and purified with silica gel.

Synthesis of the Amide

Once the pyridin-4-yl and pyrimidin-3,5-yl groups have been coupled with the thiazole core, various amides must be synthesized using the available benzoyl chloride, where R-groups are: H, F-2, F-4, diF-3,4

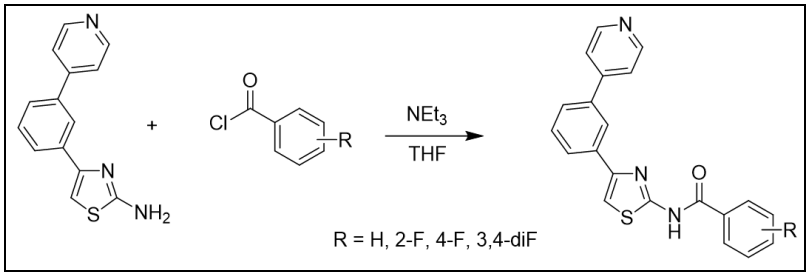


Figure 5. Amide Reaction Scheme. Synthesis of Compound 1-4 requires the use of pyridin-4-yl and associated side groups with THF and triethylamine. Refer to Appendix C for Reagent Table

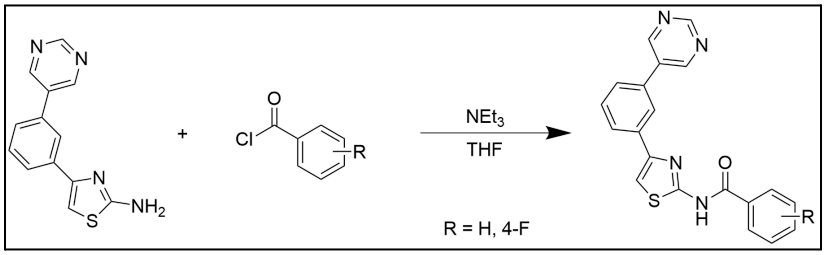


Figure 6. Amide Reaction Scheme. Synthesis of Compounds 5-6 requires the use of pyrimidin-3,5-yl and associated side groups with THF and triethylamine. Refer to Appendix C for Reagent Table

A 2-necked 50 mL round bottom reaction vessel equipped with a reflux condenser and a rubber septum was set up and placed underneath a nitrogen atmosphere. An acyl chloride, a thiazol-2-amine, triethylamine, and THF were added to the reaction vessel. The mixture was stirred at reflux until no starting material remained. Water was added, and the solution was extracted twice with dichloromethane. The combined organic layers were dried with Na_2SO_4 and concentrated using a rotor evaporator. The crude product was concentrated using column chromatography.

Docking Analysis:

In order to consider which of the six amides synthesized would be the most effective in inhibiting the PfPKG enzyme, consideration of the predicted binding affinity was an important step. Table IV details the predicted binding affinities of the six amides in addition to the binding affinity of 4-[2-(4-fluorophenyl)-5-(1-methylpiperidine-4-yl)-1H pyrrol-3-yl]pyridine, through a docking process in UCSF Chimera (10). Vina was utilized for the docking calculations, and its output score was used to determine binding affinity in silico (11, 12). The AutoDock Vina Score

details the energy released due to the bond formation between the ligand and the protein. Thus, a greater negative score represents a more effective in silico binding affinity between the protein and the amide. Prior to docking, each compound was additionally prepped through Chimera's Dock Prep, using the Dunbrack 2010 rotamer library and charges calculated by ANTECHAMBER (13, 14).

PfPKG Enzymatic Assay

An additional area of investigation was performing structure-activity relationship (SAR) studies, through a partner facility at Montclair State University. *Plasmodium falciparum* PKG (PfPKG) and human PKG (hPKG) kinase activity was assayed by a team of researchers, using a commercial immobilized metal ion affinity-based fluorescence polarization (IMAP) assay according to the manufacturer's protocol (Molecular Devices). Briefly, kinase assays (20 µl in black half volume 96 well microtiter plates) contained; 10 mM Tris-HCl, pH 7.2, 10mM MgCl2, 0.05% NaN3, 0.01% Tween®20, 10 uM ATP, 1 uM cGM and 21 ng of recombinant enzyme per well. Inhibitors were preincubated with enzyme at 25^0C for 15 minutes and reactions were initiated with addition of 120 nM fluorescent peptide substrates, FAM-PKAtide for PfPKG and FAM-IP3R for hPKG (Molecular Devices). Fluorescent polarization was measured using a Synergy 2 Microplate reader (BioTek, Winooski, VT). Fluorescent polarization was read in parallel and perpendicular with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. IC50 data were analyzed using a four parameter logistic curve fit using Microsoft Excel Solver.

ACKNOWLEDGEMENTS:

The work done on this project would not have been possible without funding from the Office of the Secretary of Higher Education of New Jersey, Overdeck Family Foundation, Novartis, Rutgers University of Newark, and New Jersey's Governor's School of the Sciences (NJGSS). We'd also like to thank NJGSS Program Directors Dr. Adam Cassano and Dr. Stephen Dunaway, Dr. Mary-Ann Pearsall, fellow alumnae and parents of alumni, as well as Drew University. Major contributions to the enzymatic inhibition data provided by Dr. John Siekierka and his research team at Montclair State University. Finally, our teaching assistant, for their support, advice, and insightful comments.

REFERENCES

1. "Malaria Fact Sheet." www.health.ny.gov, www.health.ny.gov/diseases/communicable/malaria/fact_sheet. Accessed 29 July 2023
2. "CDC - Malaria - Malaria Worldwide - Impact of Malaria." www.cdc.gov, www.cdc.gov/malaria/malaria_worldwide/impact. Accessed 29 July 2023
3. Singh, Inder Pal, Gupta, S. K., Kumar, S. "Thiazole Compounds as Antiviral Agents: An Update." 2020, vol. 16, no. 1, pp. 4-23. doi:10.2174/1573406415666190614101253
4. Rotella, D., Siekierka, J., Bhanot, P. "Plasmodium Falciparum CGMP-Dependent Protein Kinase – a Novel Chemotherapeutic Target." Frontiers in Microbiology, 2021, vol. 11. doi:10.3389/fmicb.2020.610408.
5. Tsagris, D. J., Birchall, K., Bouloc, N., et al. "Trisubstituted Thiazoles as Potent and Selective Inhibitors of Plasmodium Falciparum Protein Kinase G (PfPKG)." Bioorganic & Medicinal Chemistry Letters, 2018, vol. 28, no. 19, pp. 3168-3173. doi:10.1016/j.bmcl.2018.08.028.
6. Mahmood, S. U., Cheng, H., Tummalapalli, S. R., et al. "Discovery of isoxazolyl-based inhibitors of Plasmodium falciparum cGMP-dependent protein kinase." RSC Medicinal Chemistry, 2020, vol. 11, no. 1, pp. 98-101. doi:10.1039/C9MD00511K. https://pubs.rsc.org/en/content/articlelanding/2020/md/c9md00511k.
7. Bueno, J. M., Carda, M., Crespo, B., et al. "Design, synthesis and antimalarial evaluation of novel thiazole derivatives." Bioorganic & Medicinal Chemistry Letters, 2016, vol. 26, no. 16, pp. 3938-3944. doi:10.1016/j.bmcl.2016.07.010.
8. Tyner, T., Francis, J. "Infrared Spectroscopy." ACS Reagent Chemicals, 2017 Jan. doi:10.1021/acsreagents.2008.
9. "What is NMR?" chemchhujiacil. chem.ch.huji.ac.il/nmr/whatisnmr/whatisnmr.html. Accessed 2 August 2023
10. Pettersen, E. F., et al. "UCSF Chimera--a visualization system for exploratory research and analysis." J Comput Chem, 2004 Oct;25(13):1605-12.
11. Eberhardt, J., Santos-Martins, D., Tillack, A. F., Forli, S. "AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings." J Chem Inf Model, 2021.
12. Trott, O., Olson, A. J. "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading." J Comput Chem, 2010;31(2):455-461.

13. Shapovalov, M. V., Dunbrack, R. L. Jr. "A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions." Structure, 2011 Jun 8;19(6):844-58. doi: 10.1016/j.str.2011.03.019. PMID: 21645855; PMCID: PMC3118414.
14. Wang, J., Wang, W., Kollman, P. A., Case, D. A. "Automatic atom type and bond type perception in molecular mechanical calculations." J Mol Graph Model, 2006 Oct;25(2):247-60. doi: 10.1016/j.jmgm.2005.12.005. Epub 2006 Feb 3. PMID: 16458552.
15. "Malaria | Osmosis Study Video." Medscape, 2020. YouTube. www.youtube.com/watch?v=3_2TnCqBFcY. Accessed 2 August 2023
16. Baker, D. A., et al. "A potent series targeting the malarial cGMP-dependent protein kinase clears infection and blocks transmission." Nature Communications, 2017, vol. 8, no. 1. doi:10.1038/s41467-017-00572-x.
17. National Institute of Health. "RePORT." reportnihgov, 2020 Feb 24. Accessed 1 August 2023. report.nih.gov/funding/categorical-spending#/.
18. Connolly, L. "What you need to know about malaria in the U.S." 2023 Jul 10. health.ucdavis.edu/news/headlines/what-you-need-to-know-about-malaria-in-the-us/2023/07.
19. Diagana, T., & Jones, C. "Hitting malaria where it hurts." Nature Microbiology, 2017, vol. 2, no. 10, pp. 1336–1337. doi:10.1038/s41564-017-0036-z.
20. Borcea, A.-M., Ionuț, I., Crișan, O., Oniga, O. "An Overview of the Synthesis and Antimicrobial, Antiprotozoal, and Antitumor Activity of Thiazole and Bisthiazole Derivatives." Molecules, 2021, vol. 26, no. 3, p. 624. doi:10.3390/molecules26030624.
21. Rossi, R., Bellina, F., Carpita, A. "Palladium Catalysts for the Suzuki Cross-Coupling Reaction: An Overview of Recent Advances." Synthesis, 2004(15):2419–2440. doi:10.1055/s-2004-8312

APPENDICES

A. Synthesis of the Thiazole Core - Reagent Table

Reagent	Molar Mass (g/mol)	Amount Needed (g or mL)	Equivalence
2,3'-dibromoacetophenone	277.94	1.00 g	1
Thiourea	76.12	0.301 g	1.1
Ethanol (EtOH)	46.08	10 mL	N/A
Sodium Bicarbonate (Na_2SO_4)	84.01	10 mL *(saturated solution)*	N/A
Dichloromethane	84.93	50 mL	N/A
Petroleum Ether	86.18	10 mL	N/A
Magnesium Sulfate ($MgSO_4$)	120.37	1 scoopula	N/A
Reagent	**Melting and Boiling Point**	**Density (g/mL)**	**Toxicity**
2,3'-dibromoacetophenone	47°C; 52°C	N/A	Irritant. Do not breathe dust. Do not get in eyes, on skin, or on clothing
Thiourea	170°C; 176°C	N/A	Irritant. Wear PPE/face protection. Ensure ventilation. Avoid dust formation. Avoid ingestion and inhalation. Do not get in eyes, on skin, or on clothing
Ethanol (EtOH)	-117°C; 78°C	0.789	Irritant. Do not breathe vapors. Do not get in eyes, on skin, or on clothing.
Sodium Bicarbonate (Na_2SO_4)	N/A	N/A	Eye and skin irritant. Do not breathe dust.

Dichloromethane	-95°C; 40°C	1.33	Irritant. Do not breathe vapors. Do not get in eyes, on skin, or on clothing. Possible carcinogen.
Petroleum Ether	60°C - 95°C	0.700	Irritant. Do not breathe vapors. Do not get in eyes, on skin, or on clothing.
Magnesium Sulfate ($MgSO_4$)	N/A	N/A	Irritant. Do not breathe dust. Do not get in eyes, on skin, or on clothing.

B. Cross-Coupling Reaction - Reagent Table

Reagent	Molar Mass (g/mol)	Amount Needed (g or mL)	Equivalence
4-(3-bromophenyl)thiazol-2-amine	255.14	0.400 g	1
4-pyridylboronic acid	122.92	0.600 g	3.1
3,5-pyrimidinylboronic acid	123.91	0.600 g	N/A
Potassium Carbonate (K_2CO_3)	138.21	0.650 g	N/A
THF	72.11	16 mL	N/A
Water	18.02	24 mL	N/A
$Pd(dppf)Cl_2$	821.80	0.040 g	N/A
Ethyl Acetate	88.11	20 mL	N/A
Reagent	Melting and Boiling Point	Density (g/mL)	Toxicity
4-(3-bromophenyl)thiazol-2-amine	N/A	N/A	Irritant. Do not inhale vapors or get substance in eyes or on skin.
4-pyridylboronic acid	N/A	N/A	Irritant and corrosive.

			Do not inhale vapors or get substance in eyes or on skin.
3,5-pyrimidinylboronic acid	N/A	N/A	Irritant and corrosive. Do not inhale vapors or get substance in eyes or on skin.
Potassium Carbonate (K_2CO_3)	N/A	N/A	Irritant. Do not inhale vapors or get substance in eyes or on skin.
THF	-109°C; 65°C	0.888	Irritant and flammable. Do not inhale vapors or get substance in eyes. Possible carcinogen.
Water	0°C; 100°C	1.000	Nontoxic unless consumed in abnormally high quantities.
$Pd(dppf)Cl_2$	N/A	N/A	Irritant. Do not inhale vapors or get substance in eyes or on skin.
Ethyl Acetate	-84°C; 77°C	0.902	Narcotic and flammable Do not get substance in eyes.

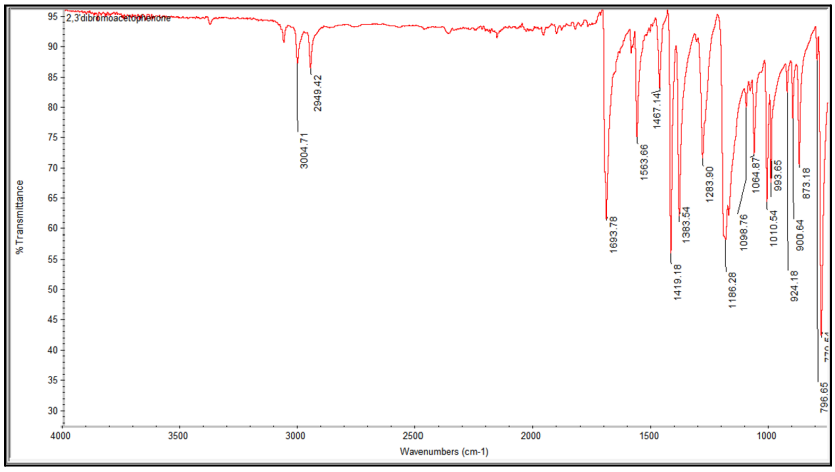
C. Synthesis of the Amide - Reagent Table

Reagent	Molar Mass (g/mol)	Amount Needed (g or mL)	Equivalence
4-(3-(pyridin-4-yl)phenyl) - thiazol-2-amine	253.00	0.210 g	1
4-(3-(pyrimidin-3,5-yl) - phenyl)thiazol-2-amine	254.00	0.211 g	1
Benzoyl Chloride	140.45	0.212 g	N/A
4-fluorobenzoyl Chloride	158.45	0.239 g	N/A
3,4-fluorobenzoyl Chloride	176.45	0.266 g	N/A

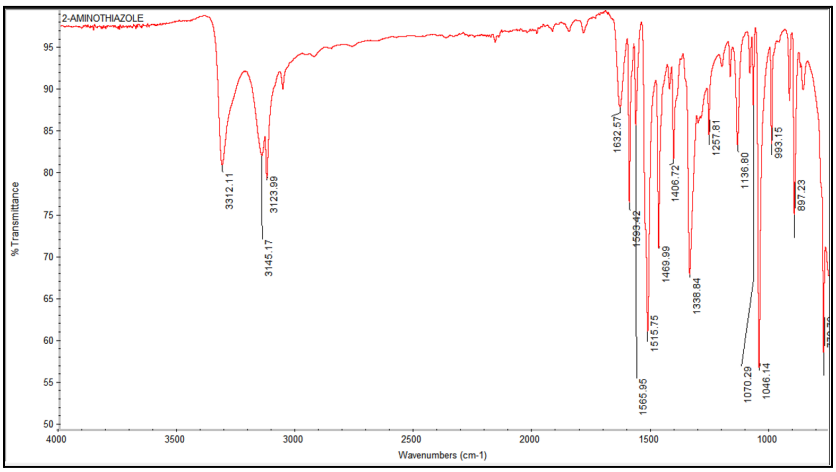
Triethylamine	101.19	0.253 g	N/A
THF	72.11	5.00 mL	N/A
Water	18.02	15.o mL	N/A
Dichloromethane	84.93	20.0 mL	N/A
Sodium Sulfate (Na_2SO_4)	142.04	N/A	N/A
Reagent	Melting and Boiling Point	Density (g/mL)	Toxicity
4-(3-(pyridin-4-yl)phenyl) - thiazol-2-amine	N/A	N/A	Irritant. Do not get substance in eyes or on skin.
4-(3-(pyrimidin-3,5-yl) - phenyl)thiazol-2-amine	N/A	N/A	Irritant. Do not get substance in eyes or on skin.
Benzoyl Chloride	-1ºC; 386.6ºC	1.21	Carcinogenic. Do not inhale or touch
4-fluorobenzoyl Chloride	9.0ºC; 82ºC	1.21	Irritant. Do not get substance in eyes or on skin.
3,4-fluorobenzoyl Chloride	11ºC; 82ºC	1.34	Irritant. Do not get substance in eyes or on skin.
Triethylamine	-115°C; 89°C	0.729	Irritant and flammable. Do not inhale vapors, get substance in eyes or on skin.
THF	-109°C; 65°C	0.888	Irritant and flammable. Do not inhale vapors or get substance in eyes. Possible carcinogen.
Water	0°C; 100°C	1.00	Nontoxic unless consumed in abnormally high quantities.
Dichloromethane	-95°C; 40°C	1.33	Irritant. Do not breathe vapors. Do not get in

			eyes, on skin, or on clothing. Possible carcinogen.
Sodium Sulfate (Na_2SO_4)	884°C;26 1,429°C	2.67	Generally nontoxic, however it can cause temporary asthma and eye irritation.

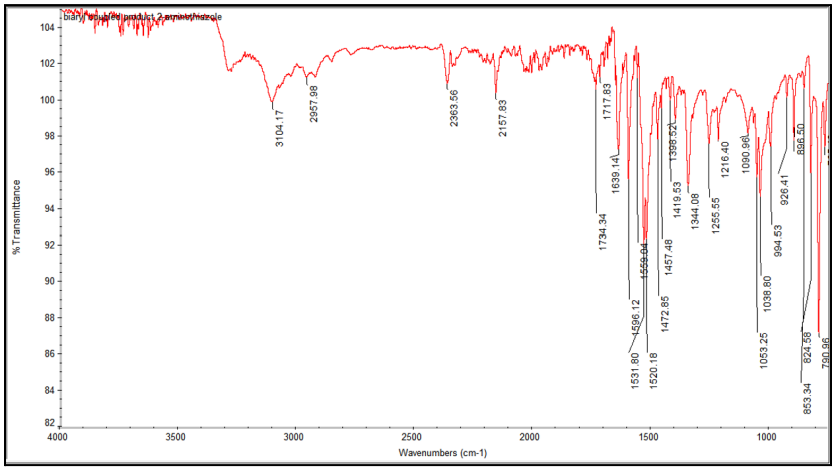
D. IR Spectrum of 2,3'-dibromoacetophenone (Starting Material)



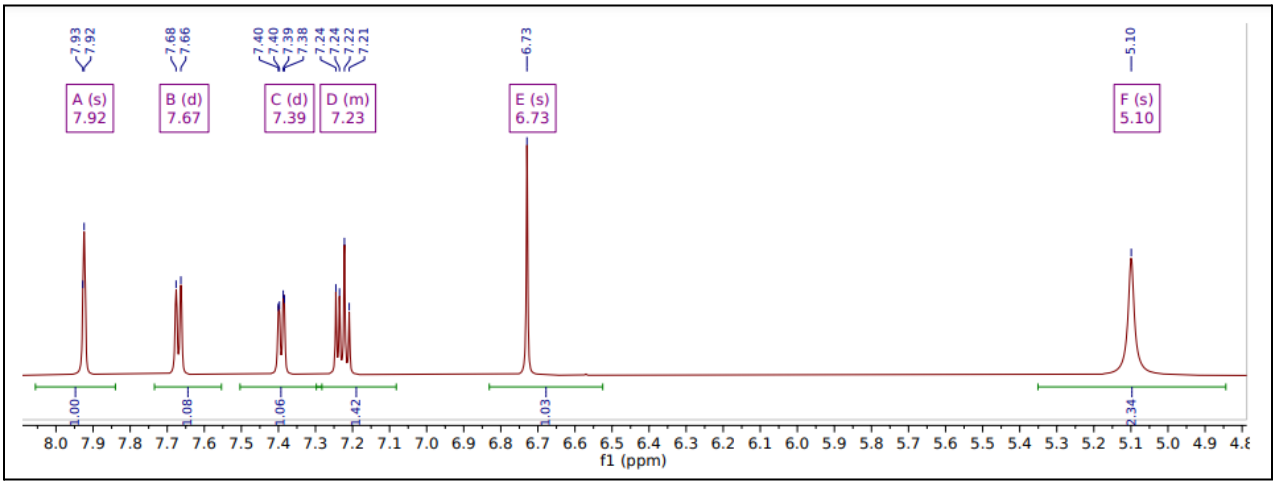
E. IR Spectrum of 4-(3-bromophenyl)thiazol-2-amine (Compound A)



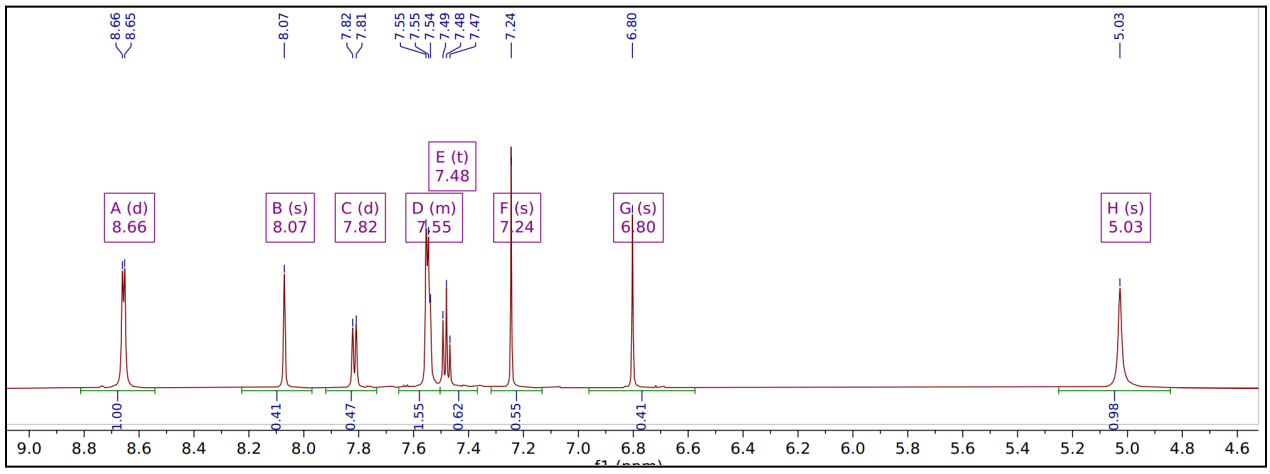
F. IR Spectrum of 4-(3-(pyridin-4-yl)phenyl)thiazol-2-amine (Compound B)



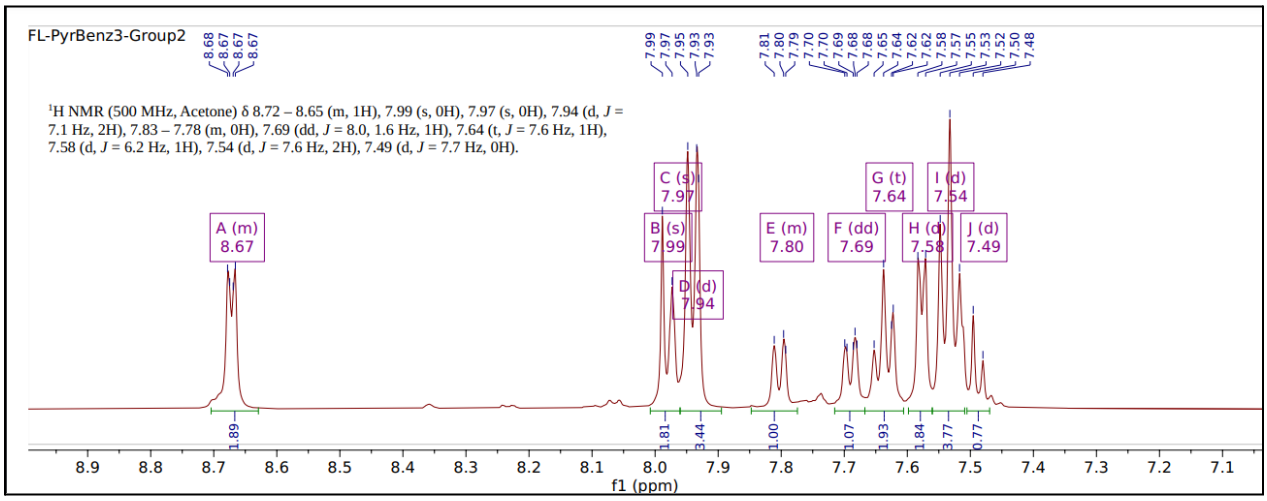
G. NMR Spectrum of Compound A



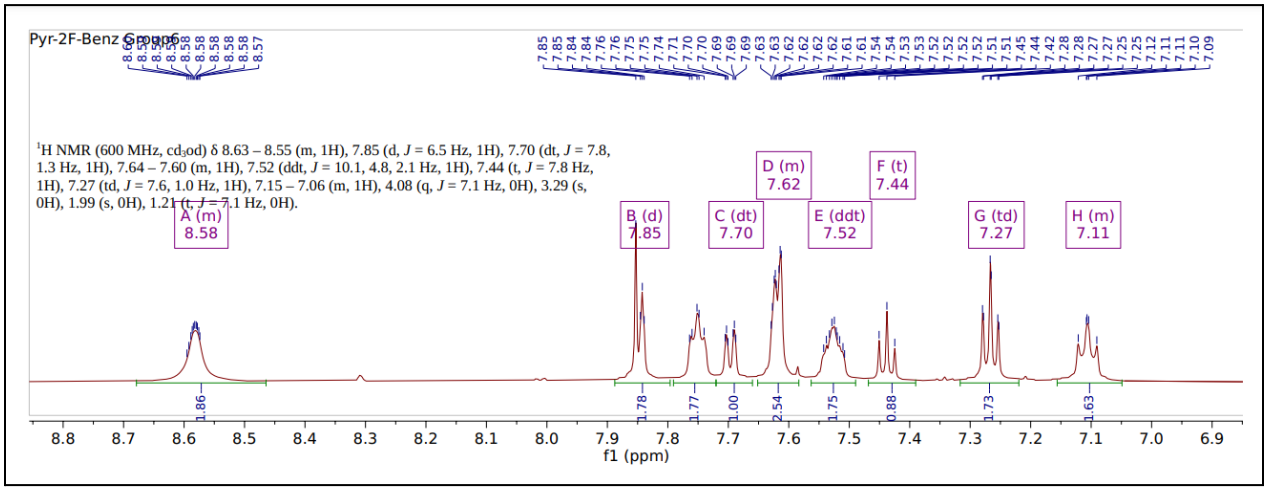
H. NMR Spectrum of Compound B



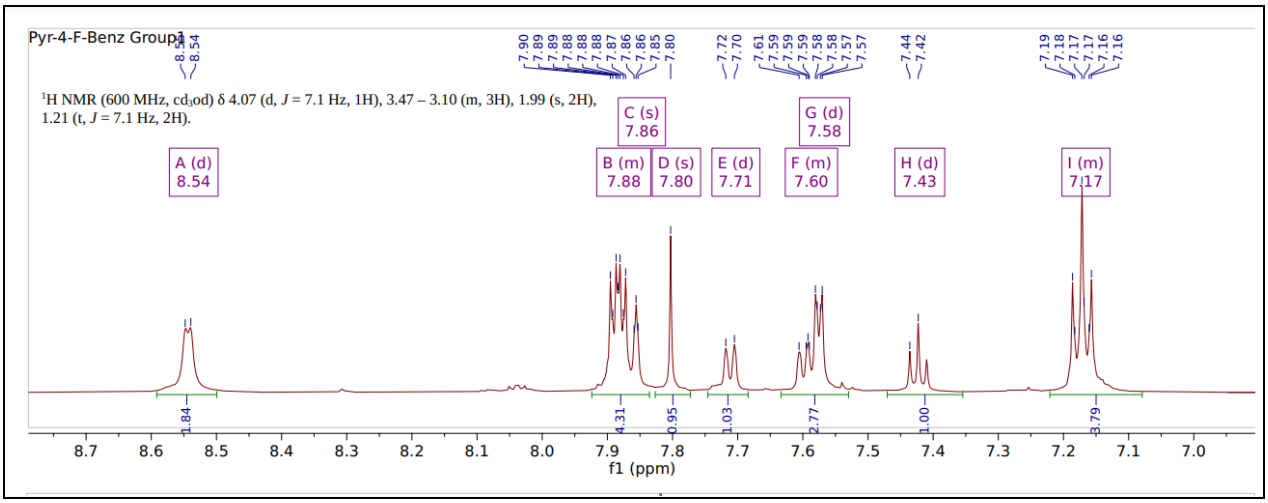
I. NMR Spectrum of Compound 1



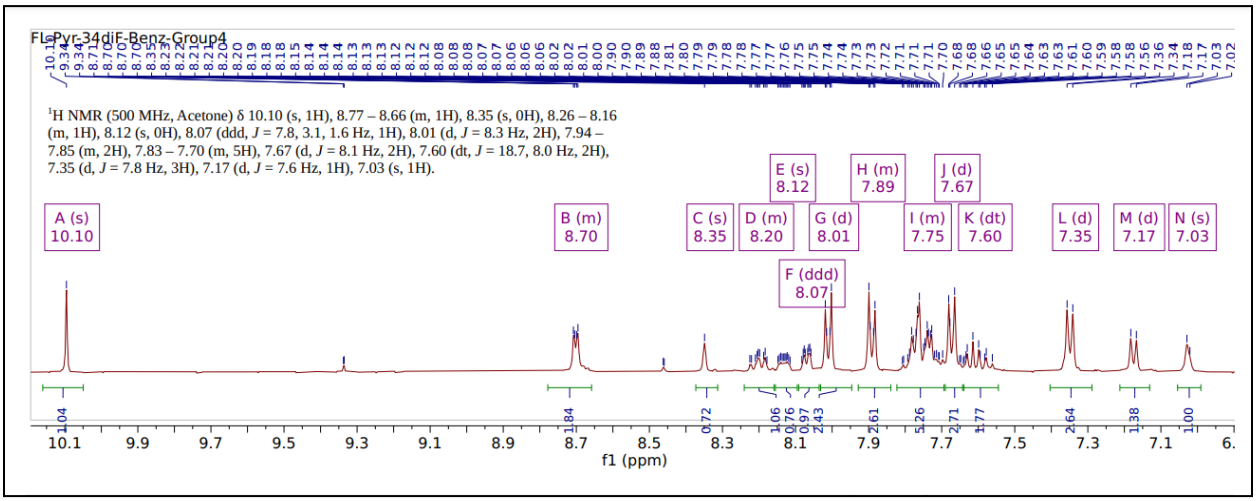
J. NMR Spectrum of Compound 2



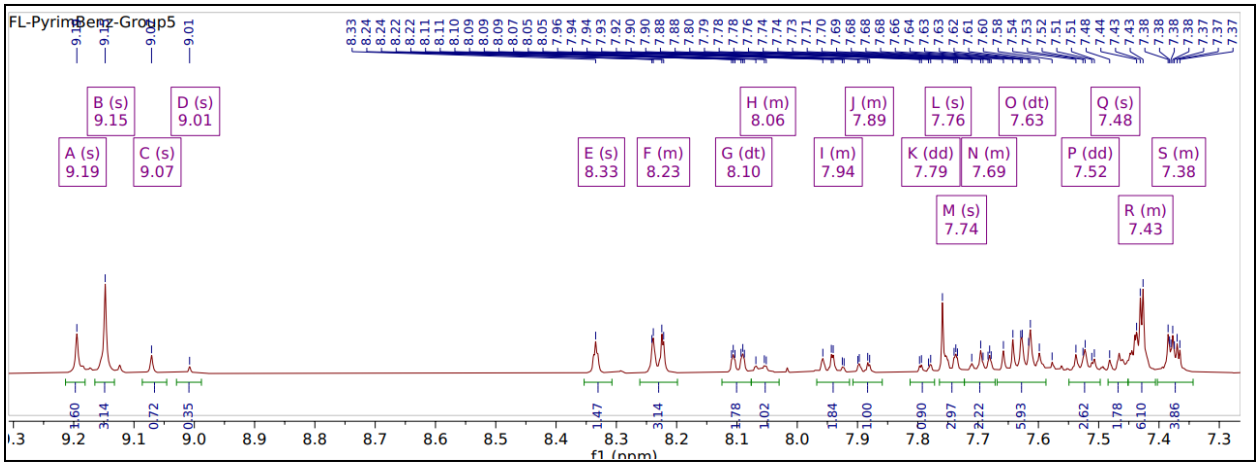
K. NMR Spectrum of Compound 3



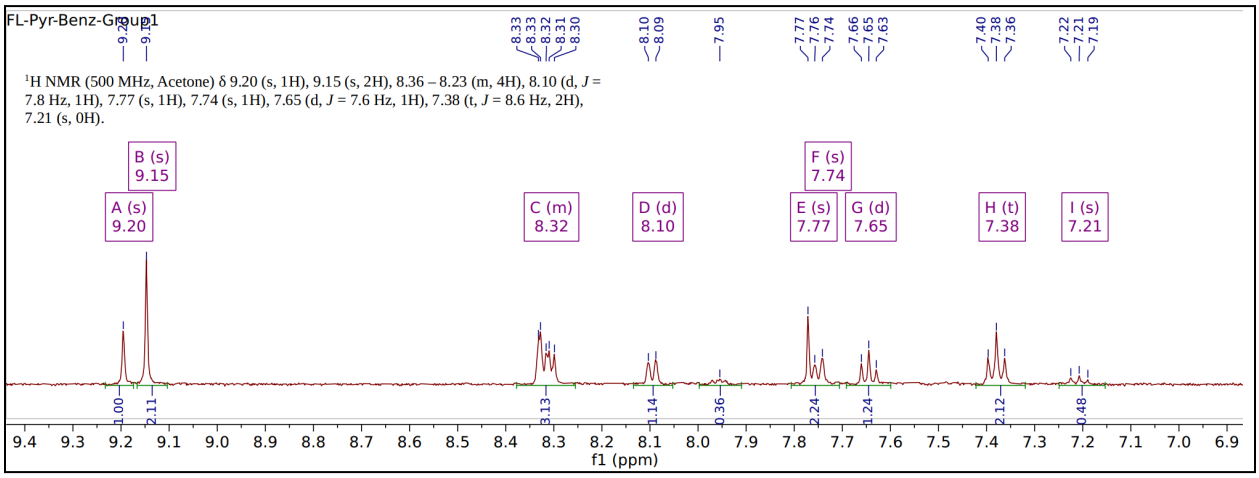
L. NMR Spectrum of Compound 4



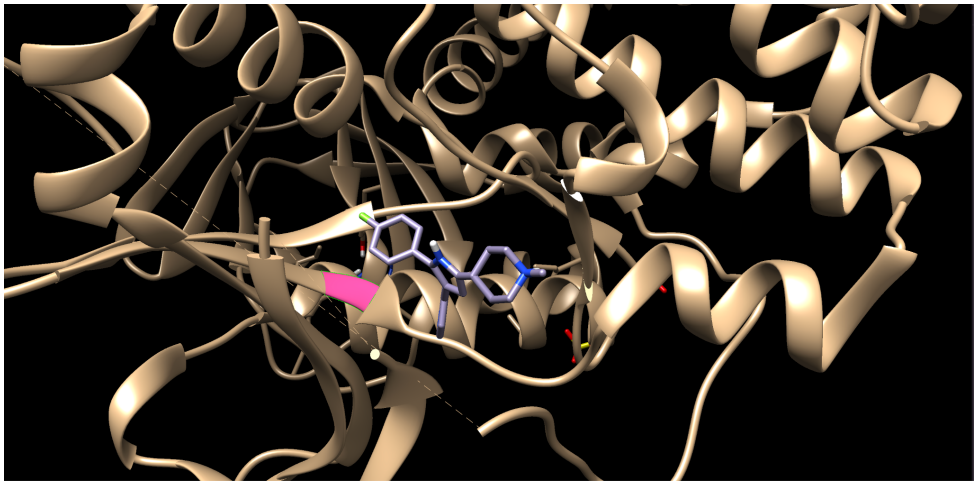
M. NMR Spectrum of Compound 5



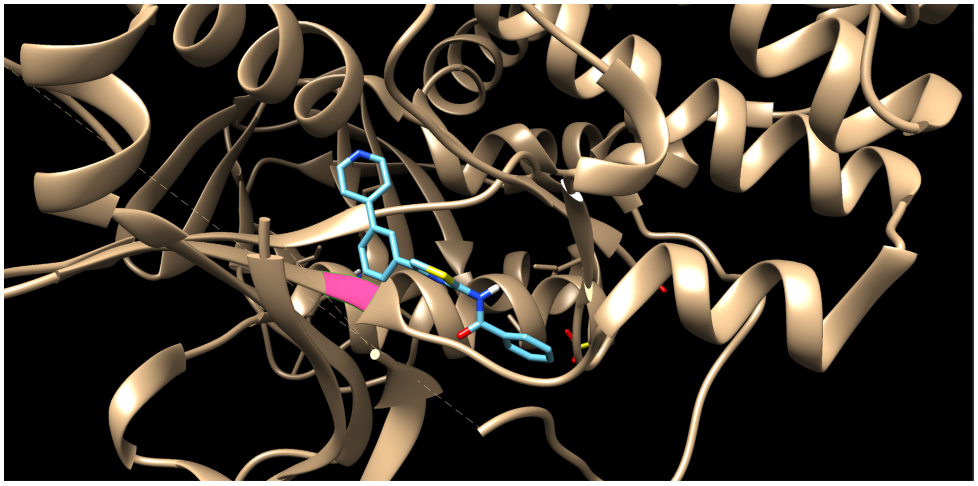
N. NMR Spectrum of Compound 6



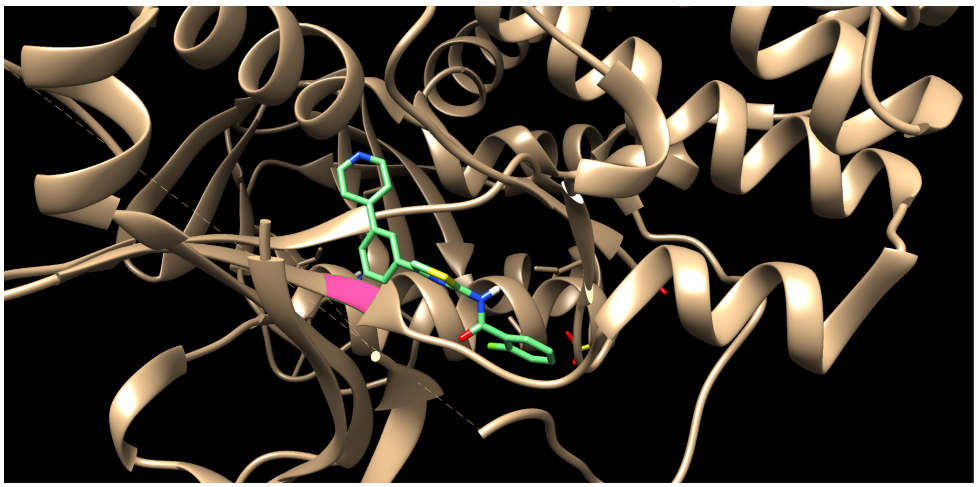
O. Compound L enzyme-inhibitor complex



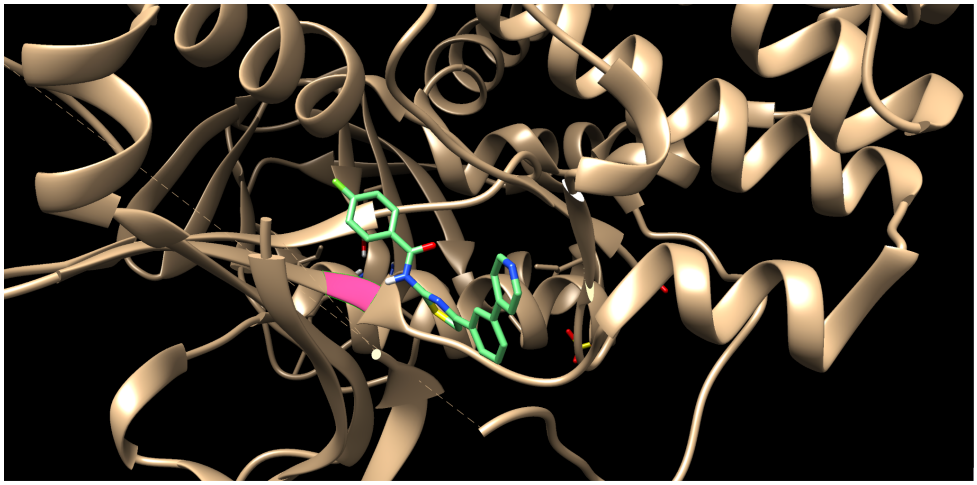
P. Compound 1 enzyme-inhibitor complex (idiosyncratic orientation)



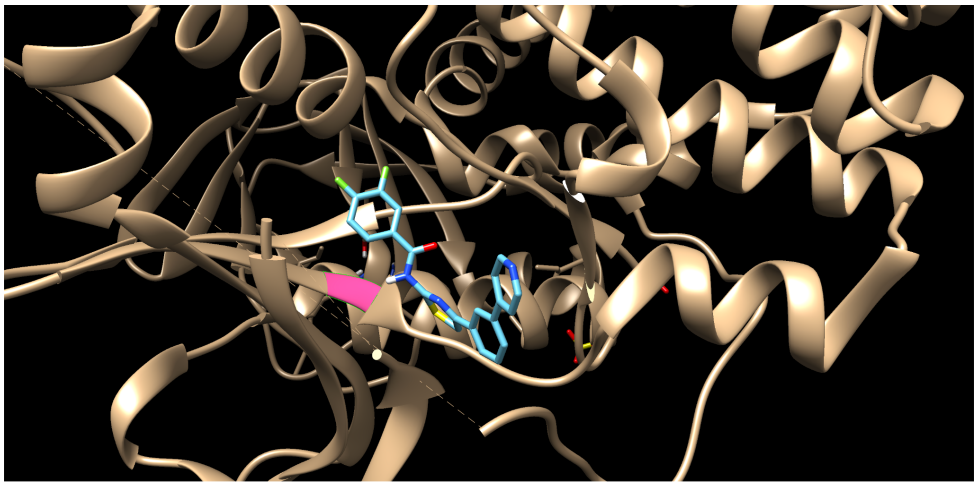
Q. Compound 2 enzyme-inhibitor complex (idiosyncratic orientation)



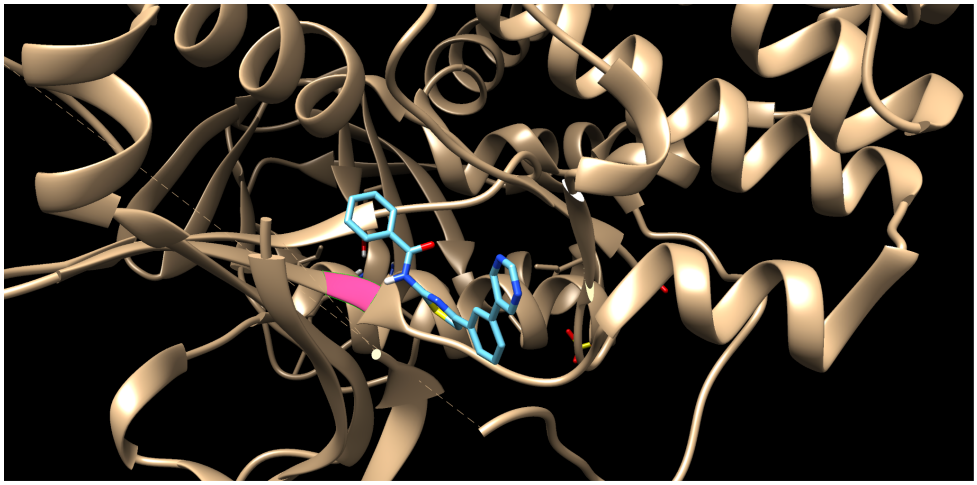
R. Compound 3 enzyme-inhibitor complex



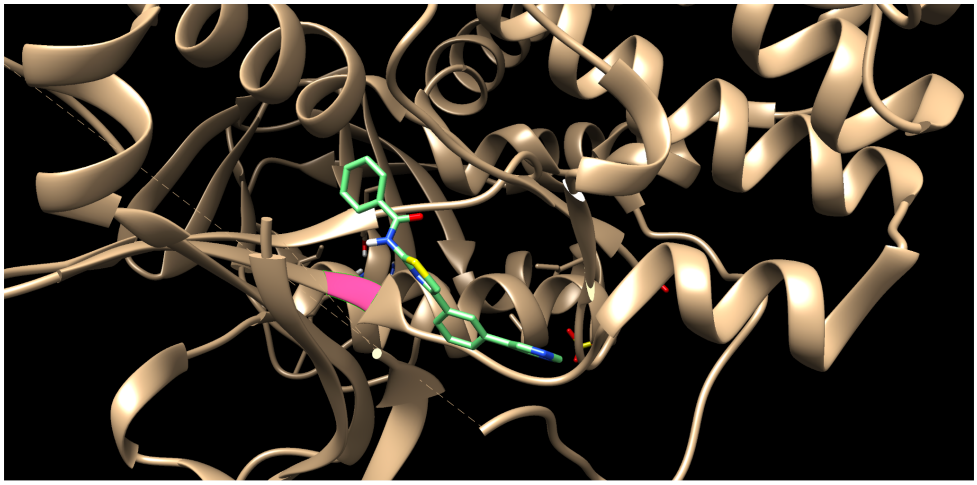
S. Compound 4 enzyme-inhibitor complex



T. Compound 5 enzyme-inhibitor complex



U. Compound 6 enzyme-inhibitor complex

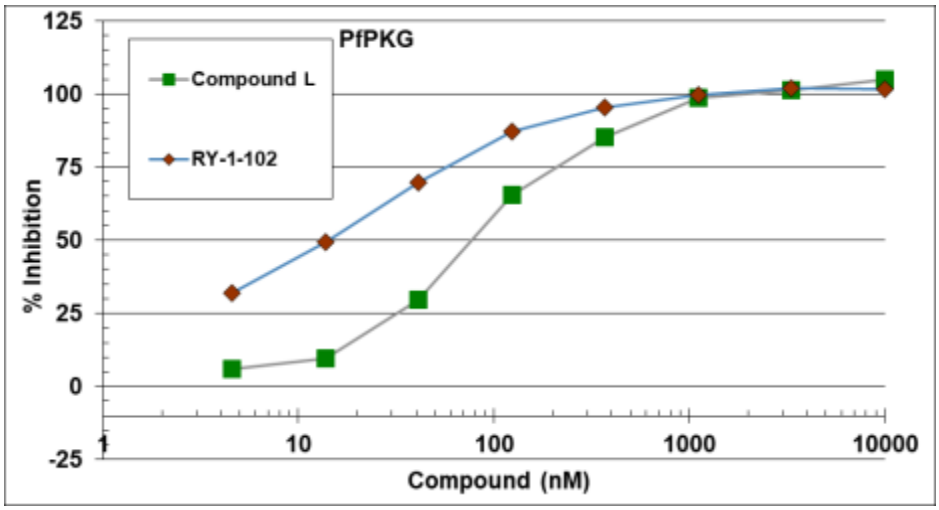


V. Percent inhibition at 60nM for two active control compounds.

Compound 1	% inhibition	RY-1-102	% inhibition
10,000	105.0535988	10,000	101.608
3333.333333	101.3782542	3333.333333	102.0674
1111.111111	98.62174579	1111.111111	99.54058
370.3703704	85.29862175	370.3703704	95.40582
123.4567901	65.54364472	123.4567901	87.13629
41.15226337	29.70903522	41.15226337	69.90812

13.71742112	9.494640123	13.71742112	49.2342
4.572473708	5.819295559	4.572473708	32.00613

W. Compound L and RY-1-102 % inhibition



X. NMR Spectrum of Compound C