UNIVERSITY OF CALIFORNIA SAN DIEGO

Organic Synthesis and Testing of Novel Compounds in Search of New Broad-Spectrum Antibacterial and Liver-Stage Antimalarial Drugs

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science

in

Biology

by

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Professor Michael David, Co-Chair
Professor Randolph Hampton

2019

The thesis of Jmelle Joseph Gentry is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-chair

_____

Chair

University of California San Diego

2019

DEDICATION

I would like to dedicate my thesis to my parents who gave me everything when they had nothing. It is because of their love and support that I am who I am today. I would not have made it this far without them. I also want to dedicate my thesis to my friends, family, and professors who have been there to mentor and teach me.

TABLE OF CONTENTS

Signature Page…… iii

Dedication……..... iv

Table of Contents…… v

List of Figures…… vi

List of Schemes…….. vii

List of Tables……. viii

Acknowledgements…… ix

Abstract of the Thesis…… x

Introduction……..... 1

Chapter 1: Methods…….. 5

1.1: Antibacterials……. 5

1.2: Antimalarials…….. 20

Chapter 2: Results…….…….... 30

2.1: Antibacterials…… 30

2.2: Antimalarials…… 33

Chapter 3: Discussion……...…… 40

3.1: Antibacterials…… 40

3.2: Antimalarials……. 44

References…… 47

LIST OF FIGURES

Figure 2.1.1: Proton NMR of Compound 2..…… 31

Figure 2.1.2: Proton NMR of Compound 3..…… 32

Figure 2.1.3: Proton NMR of Compound 6..…… 33

Figure 2.2.1: Proton NMR of MP 22…… 35

Figure 2.2.2: Proton NMR of MP 23…… 36

Figure 2.2.3: Proton NMR of MP 28…… 37

Figure 2.2.4: Proton NMR of MP 46…… 38

Figure 2.2.5: Proton NMR of MP 51…… 39

LIST OF SCHEMES

Scheme 1.1.1: Reaction 1…… 5

Scheme 1.1.2: Reaction 2…… 7

Scheme 1.1.3: Reaction 3…… 8

Scheme 1.1.4: Reaction 4…… 9

Scheme 1.1.5: Reaction 5…… 10

Scheme 1.1.6: Reaction 6…… 11

Scheme 1.1.7: Reaction 7…… 13

Scheme 1.1.8: Reaction 8…… 14

Scheme 1.1.9: Reaction 9…… 15

Scheme 1.1.10: Reaction 10…… 17

Scheme 1.1.11: Reaction 11…… 18

Scheme 1.2.1: Formation of MP 22 and MP 28…….. 20

Scheme 1.2.2: Formation of MP 23 and MP 29…….. 22

Scheme 1.2.3: Formation of MP 31…… 24

Scheme 1.2.4: Formation of MP 46 and MP 51…….. 25

LIST OF TABLES

Table 1.1.1: Optimization Table from Reaction 1 …… 6

Table 1.2.1: Optimization Table from MP 46…… 26

Table 1.2.2: Table of Compounds……..…… 27

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Last but not least, I would like to acknowledge Professor Michael David and Professor Randolph Hampton for being on my thesis committee as well as teaching me their knowledge of pharmacology, physiology and metabolic biochemistry. Their teachings have help shape me as the student that I am today.

The Methods section of this thesis is coauthored with Jianxi Zhang.

ABSTRACT OF THE THESIS

Organic Synthesis and Testing of Novel Compounds in Search of New Broad-Spectrum Antibacterial and Liver-Stage Antimalarial Drugs

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Jmelle Joseph Gentry

Master of Science in Biology

University of California San Diego, 2019

Professor Dionicio Siegel, Chair
Professor Michael David, Co-chair

The war between humans and microbes is as old as our existence. Humans have created antibiotics since 1928 to protect ourselves however, bacteria have consistently evolved to develop resistance to these antibiotics. While bacteria are a major threat to humans, they are not

the only microbes that pose a threat to our survival. Protozoan parasites of the Plasmodium species cause 219 million cases of malaria annually. Malaria continues to threaten nearly half of the world's population. In this research, two types of compounds were synthesized. Compound 7, a molecule that is similar in structure to tricyclic gyrase inhibitors was synthesized in hopes of discovering the next broad-spectrum, dual action antibiotic. Minimum Inhibitory Concentration assays were used to test the compound against six antibiotic-resistant bacterial strains. The tricyclic gyrase inhibitors were difficult to synthesized due to the benzylic properties of the starting materials. Compound 7 was synthesized but did not demonstrate antibiotic activity when tested against the bacteria. Other dicyclic amines were synthesized in hopes of creating a compound that selectively target Plasmodium parasites in the asexual liver stage over the asexual blood stages because it is less likely for the parasite to develop resistance. MP 28 and MP 51 were successfully synthesized. The Half Maximal Inhibitory Concentration (IC50) values will be used to determine the efficacy of the compounds against the Plasmodium falciparum parasite. The synthesized antimalarial compounds have not yet been tested.

INTRODUCTION

According to the World Health Organization, antibiotic resistant bacteria are becoming a worldwide crisis. Bacteria, as well as other microorganisms, are rapidly developing resistance to the treatments currently available to control these pathogens. Drug resistance develops when the efficacy of a drug is compromised and the therapeutic dose of that drug no longer has the ability to halt microorganismal growth (WHO 2014). Antibiotic overuse and the shortage of new antibiotic development by pharmaceutical companies are two primary factors responsible for the large increase in antibiotic resistance seen around the world (Ventola 2014). Physicians in The United States over-prescribe antibiotics to treat common infections, such as the common cold, that can be passed on their own. For example, penecillins are being prescribed to treat of pharyngitis, or a common sore throat in children; only 37% of cases are caused by a streptococcus bacterium while viral infections account for most of the cases, yet 60% of pharyngitis patients were prescribed narrow-spectrum penecillins (Dooling, et al. 2014). The US is not the only source of the problem, many developing countries have less strict regulations and sell antibiotics without the need of a prescription, which promotes overuse when antibiotics are not necessary (Blair, et al. 2015). Bacteria are especially resourceful due to their vast array of mechanisms to develop and spread resistance to antibiotics (Gold & Moellering 2012). Overexposure and use of the incorrect antibiotic to bacteria allows them to develop resistance to these drugs; these bacteria are then able to spread their resistance to other bacteria (Ventola 2014). Furthermore, single mutations in a gene can allow bacteria to become resistant without exposure to these antibiotics. Bacteria then proliferate and spread their resistance genes to future generations (Gold & Moellering 2012). These are just a few of the many different ways that

bacteria are able to gain and spread antibiotic resistance. The antimicrobial resistance crisis continues to rapidly escalate and should be faced with a significant sense of urgency.

While antibiotic resistant bacteria remain a major problem in the world, protozoan parasites are another important subset of infectious pathogens that require immediate attention because they cause deadly diseases such as malaria. There were 219 million malaria cases reported around the world in 2017 alone, and malaria continues to threaten nearly half of the world's population (WHO 2018). According to the Center for Disease Control, Malaria is a disease that affects large populations in tropical and subtropical areas around the world that have low socioeconomic status, including South America, Southern Asia, and Africa where the disease is the leading cause of death in many countries (CDC 2018). Malaria is caused by one of five Plasmodium species that that include P. *falciparum*, P. *malariae*, P. *vivax*, P. *ovale*, and P. *knowlesi*. Plasmodium parasites are spread through blood transfer by a bite from an infected female *Anopheles* spp. mosquito (Phillips 2017). When an infected mosquito bites a person, a Plasmodium sporozoite, or motile spore-like infective agent, is injected into the dermis of the individual (Cowman, et al. 2016). From there the sporozoite penetrates blood vessels and travels through the bloodstream to infect liver hepatocytes. The sporozoites undergo asexual reproduction in the liver hepatocytes until they form tens of thousands of merozoites that rupture the hepatocytes and then move on to endocytose and infect circulating red blood cells (RBCs) in the bloodstream (Cowman, et al. 2016). The merozoites asexually reproduce inside of RBCs where they can safely divide and hide from the host's immune response (Cowman, et al. 2016). A new female Anopheles mosquito will then ingest these blood stage parasites from the infected host and the cycle continues. Visitors to endemic regions can use short-term prophylactic drugs to prevent infections but that is not feasible for large populations due to limited resources

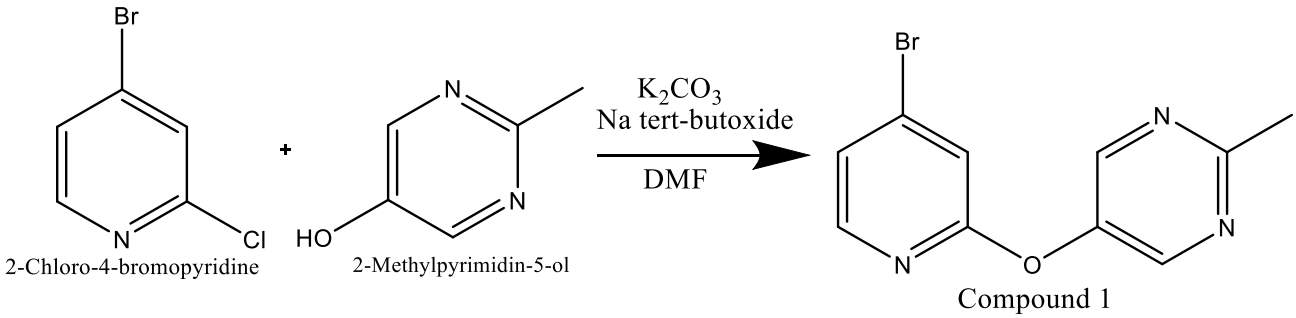
(Antonova-Koch, et al. 2018). Individuals who live in these areas must rely on more long-term antimalarial methods such as vector control, including insecticide-treated nets. The current methods for malaria treatments are sufficient but are slowly declining due to resistance to insecticides and other current treatments (Antonova-Koch, et al. 2018). Artemisinin is a plant-based compound that is the current treatment for malaria. Artemisinin combination therapies, which use a combination of fast acting artemisinin and a drug from a different class, are the first-line defenses against malarial infections due to the decreased risk of antimalarial resistance if two or more drug classes are used (WHO 2018). Plasmodium parasites, especially *P. falciparum*, are slowly showing increased resistance to many different drug classes in many South Asian countries (Bloland 2001).

With the growing antimicrobial resistance crisis, the need for new treatments is evident. At the Siegel lab in the Skaggs School of Pharmacy we are looking to design and synthesize compounds that will be tested for antibiotic and antimalarial activity. During phase one of the research we synthesized tricyclic aryl amines to be tested as tricyclic gyrase (GyrB/ParE) inhibitors against bacteria because this particular drug type has a dual action mechanism, which gives bacteria a lower chance of developing antibiotic resistance. These aryl amines were synthesized by executing palladium-based cross coupling. These compounds were tested for antibiotic activity against six different bacterial strains by Jianxi Zhang in the Siegel lab. The Minimum Inhibitory Concentration (MIC) assays were used to determine the efficacy of the synthesized compounds towards the bacteria. During phase two of the research, we looked toward making antimalarial compounds that selectively target Plasmodium parasites in the asexual liver stage over the asexual blood stages because it is less likely for the parasite to develop resistance to liver stage drugs. These compounds were synthesized by executing an aldol

reaction, followed by the addition of a hydrazine compound. These compounds were chosen based on compounds similar in structure that have previously demonstrated antimalarial activity. The synthesized compounds will be sent to the Winzeler Lab in the Skaggs School of Pharmacy to be tested to determine if they exhibited antimalarial activity. The Half Maximal Inhibitory Concentration (IC50) values will be used to determine the efficacy of the compounds. The IC50 is used to measure of potency of a compound to inhibit the binding of another compound. Our hope is that one of the compounds that we synthesize could potentially go on to be a candidate for a broad-spectrum antibiotic or an antimalarial compound that can function as a both prophylactic and long-term treatment.

CHAPTER 1: METHODS

Chapter 1.1: Antibiotic Methods

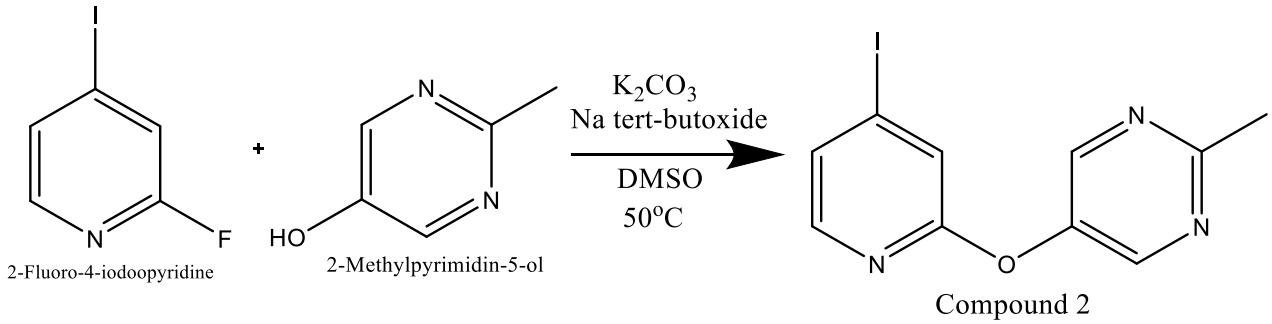


Scheme 1.1.1: Reaction 1 for creating backbone of tricyclic gyrase inhibitor.

A reaction was set up by adding 1.2ml of Dimethylformamide (DMF) to a round-bottom flask followed by the addition of 60mg of 2-Methylpyrimidin-5-ol. 50mg of Sodium tert-butoxide and 72mg of Potassium carbonate were added to the solution followed by the addition 100mg of 2-Chloro-4-bromopyridine. The reaction was sealed off from oxygen to progress in an inert (N_2) atmosphere. The reaction was stirred at room temperature overnight and its progress was confirmed by thin layer chromatography (TLC). The product was partitioned by using liquid-liquid extraction. The organic layer was composed of 3:1 DCM:Ethylacetate and the aqueous layer was composed of brine. The organic layer was extracted and the excess water was dried over anhydrous sodium sulfate. The solvent was separated from the crude product by using a rotary evaporator and a high-pressure vacuum pump. The product was then separated from the crude mixture by column chromatography and confirmed by nuclear magnetic resonance (NMR).

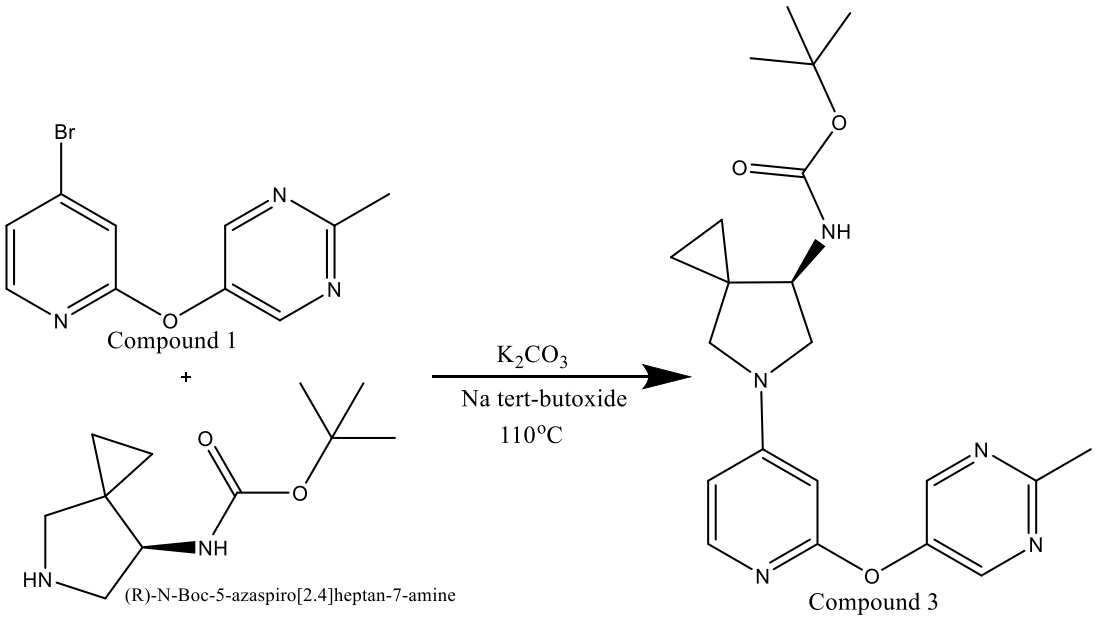
Table 1.1.1: Optimization Table form the reaction in Scheme 1.1. The reaction from scheme 1 was run under different conditions to optimize the yield because high amounts of compound 1 were needed for the next step of tricyclic gyrase inhibitor synthesis. These conditions include the bases, the solvent, and the temperature.

Base	Solvent	Temperature	Yield
Na tert-butoxide, K_2CO_3	DMF	room temperature	42%
NaH, K_2CO_3	DMF	room temperature	42%
Na tert-butoxide, K_2CO_3	DMSO	room temperature	48%
NaH, K_2CO_3	DMSO	room temperature	49%
NaH, K_2CO_3	DMSO	50°C	65%
Na tert-butoxide, K_2CO_3	DMSO	50°C	66%



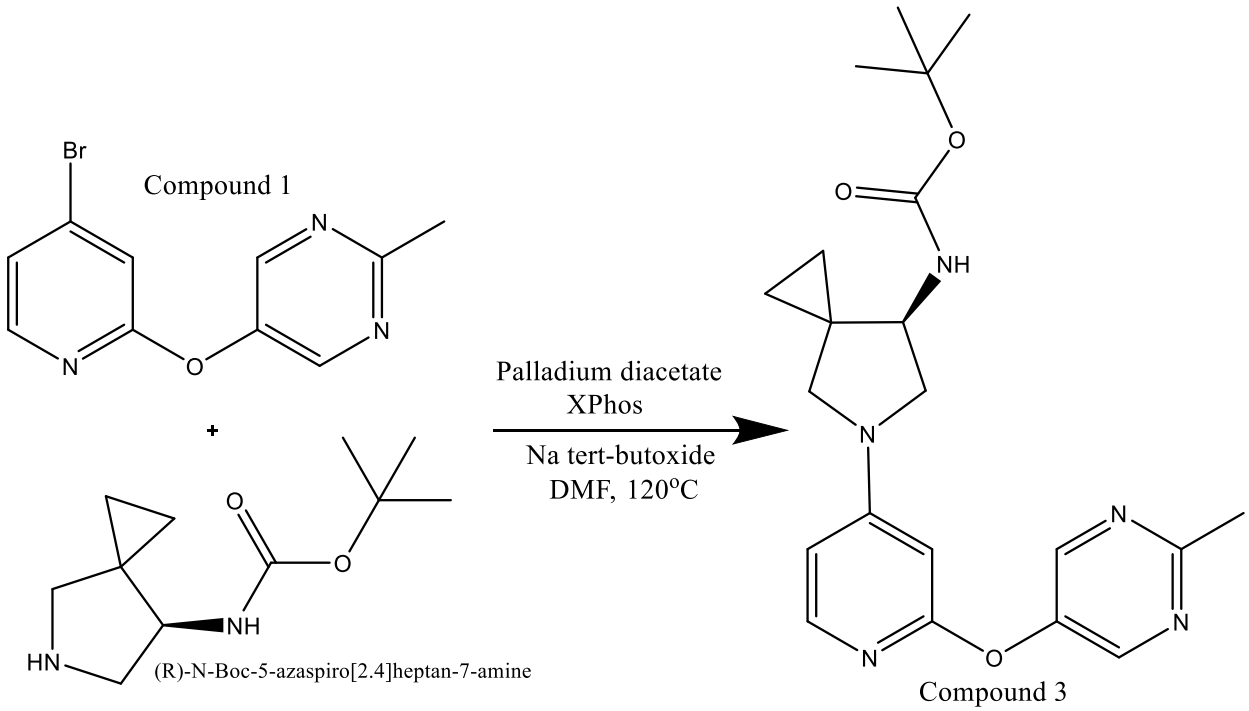
Scheme 1.1.2: Reaction 2.

A reaction was set up by adding 3.5ml of Dimethylsulfoxide (DMSO) to a round-bottom flask followed by the addition of 150mg of 2-Methylpyrimidin-5-ol. 150mg of Sodium tert-butoxide and 220mg of Potassium carbonate were added to the solution followed by the addition 300mg of 2-Fluoro-4-iodoopyridine. The reaction was sealed off from oxygen to progress in an inert (N_2) atmosphere. The reaction was stirred at room temperature overnight and its progress was confirmed by TLC. The product was partitioned by using liquid-liquid extraction. The organic layer was composed of 3:1 DCM:Ethylacetate and the aqueous layer was composed of brine. The organic layer was extracted and the excess water was dried over anhydrous sodium sulfate. The solvent was separated from the crude product by using a rotary evaporator and a high-pressure vacuum pump. The product was then separated from the crude mixture by column chromatography and confirmed by NMR.



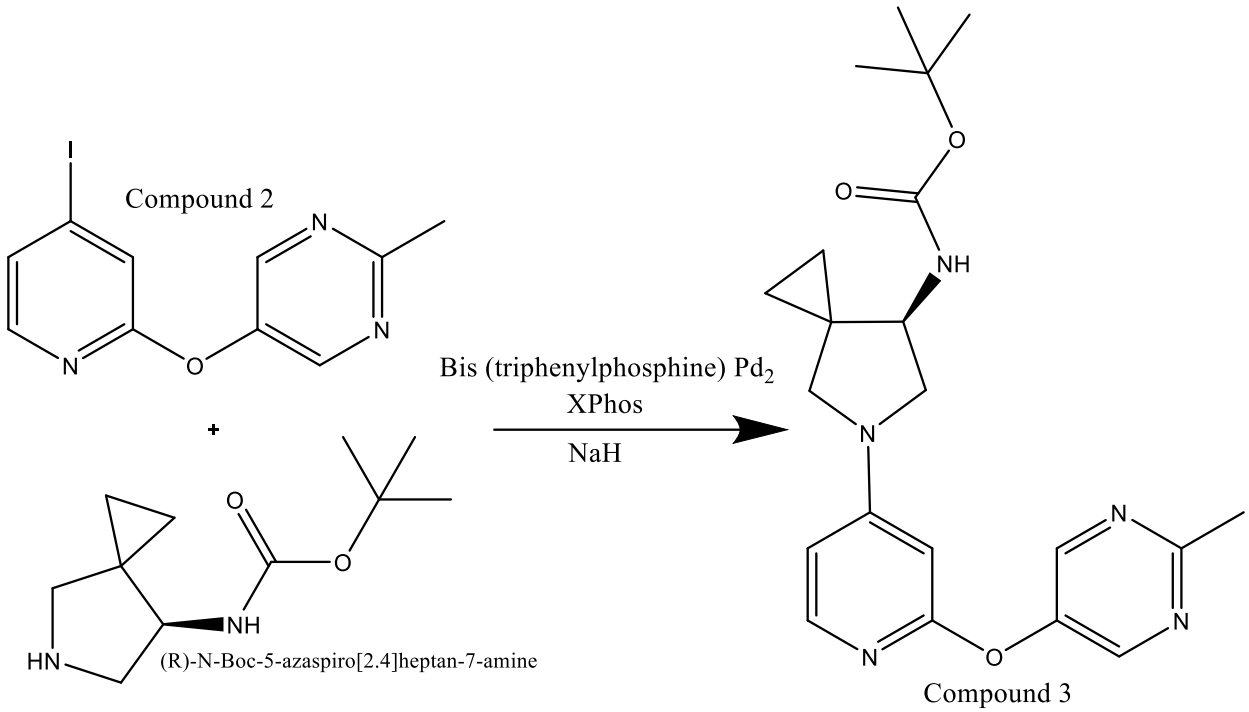
Scheme 1.1.3: Reaction 3.

A reaction was set up by adding 1ml of DMSO to a round-bottom flask followed by the addition of 18mg of (R)-N-Boc-5-azaspiro[2.4]heptan-7-amine and 25mg of Compound 1. 54mg of potassium carbonate and 33mg of sodium tert-butoxide were added to the solution. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 110^{o}C for 14 hours. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



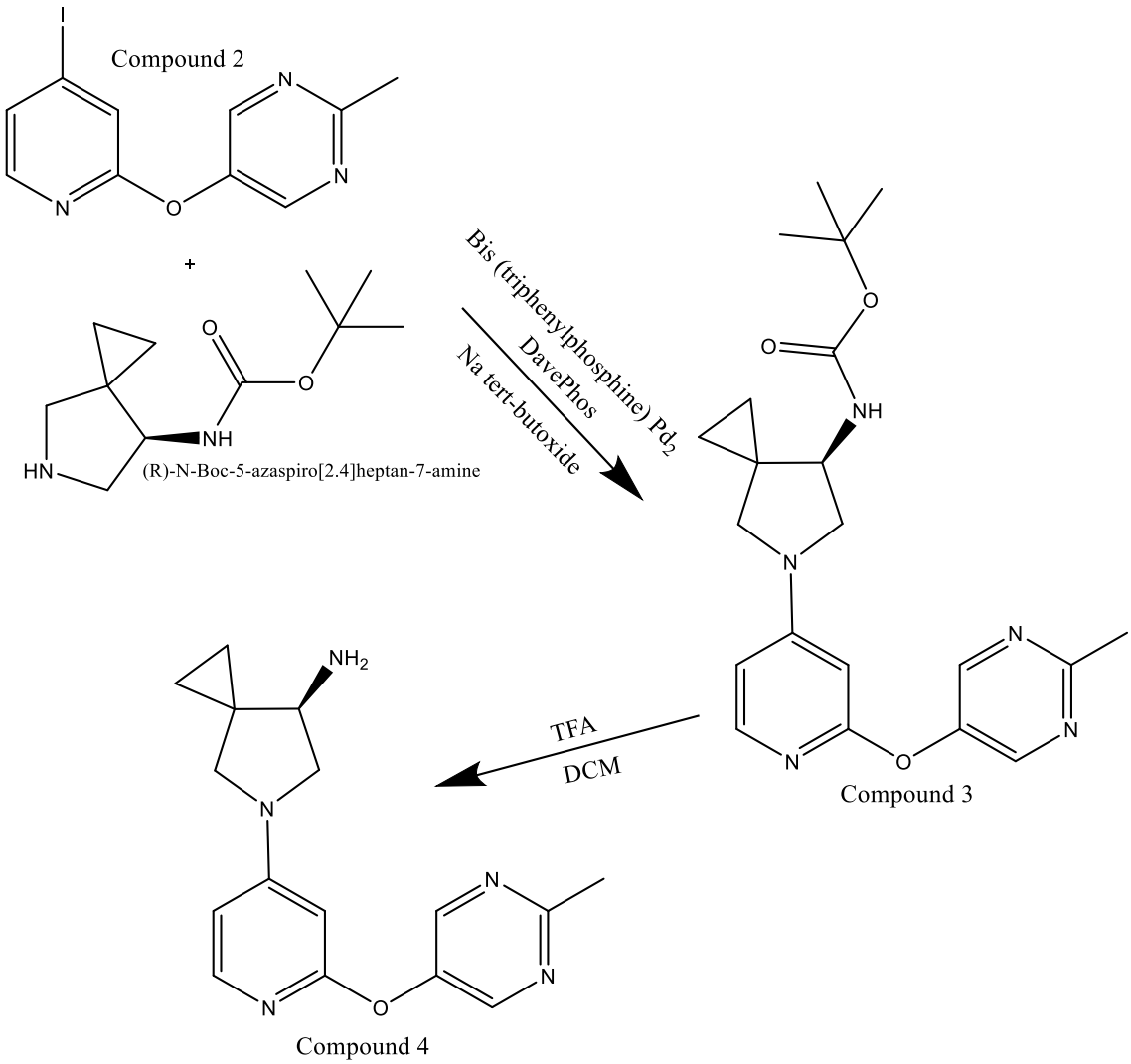
Scheme 1.1.4: Reaction 4.

A reaction was set up by adding 1.5ml of Tetrahydrofuran (THF) to a round bottom flask followed by the addition of 8.6mg of (R)-N-Boc-azaspiro[2.4]heptan-7-amine and 14mg of sodium tert-butoxide. The reaction was stirred for 30 minutes. 11.5mg of Compound 1, 1.7mg of palladium diacetate, and .9mg of XPhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 120°C overnight. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.1.5: Reaction 5.

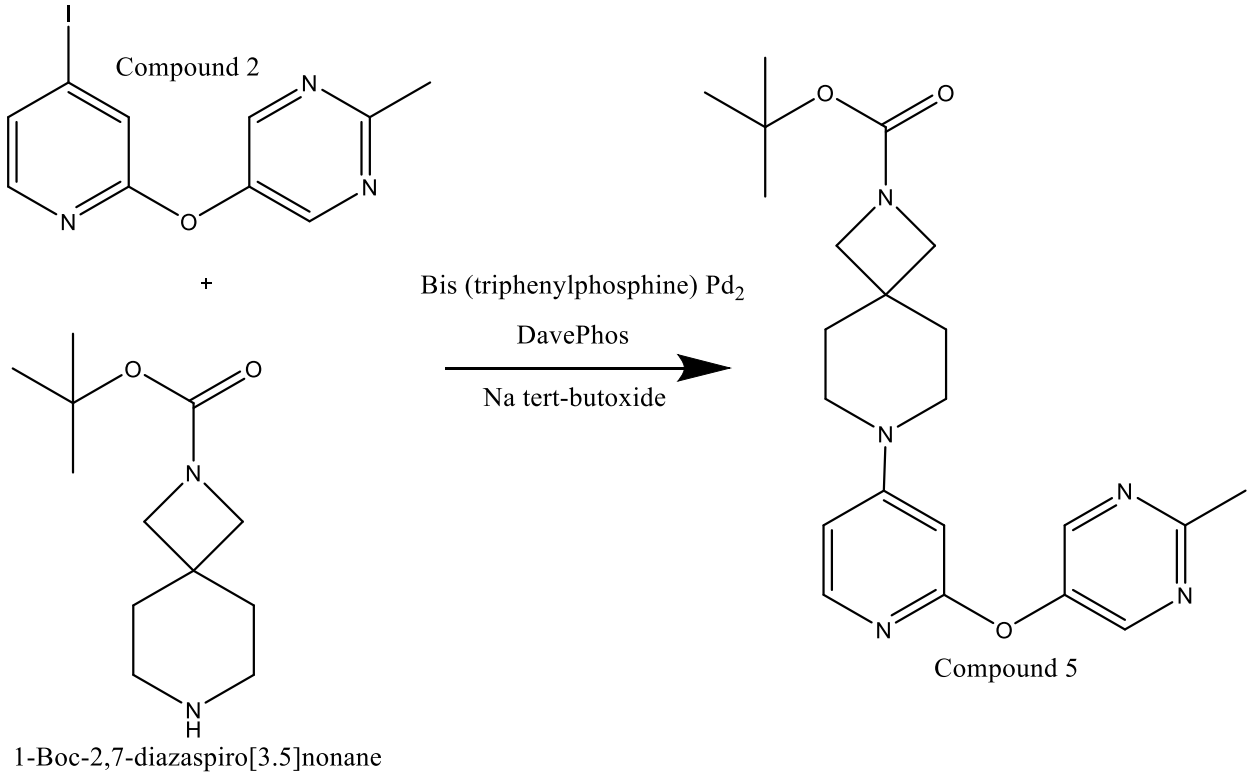
A reaction was set up by adding 2ml of Toluene to a round-bottom flask followed by the addition of 18.4mg of (R)-N-Boc-azaspiro[2.4]heptan-7-amine and 9mg of sodium hydride. The reaction was stirred for 30 minutes. 14mg of Compound 2, 1.9mg of Bis (triphenylphosphine) Pd_2, and 1mg of XPhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 120^{o}C overnight. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.1.6: Reaction 6.

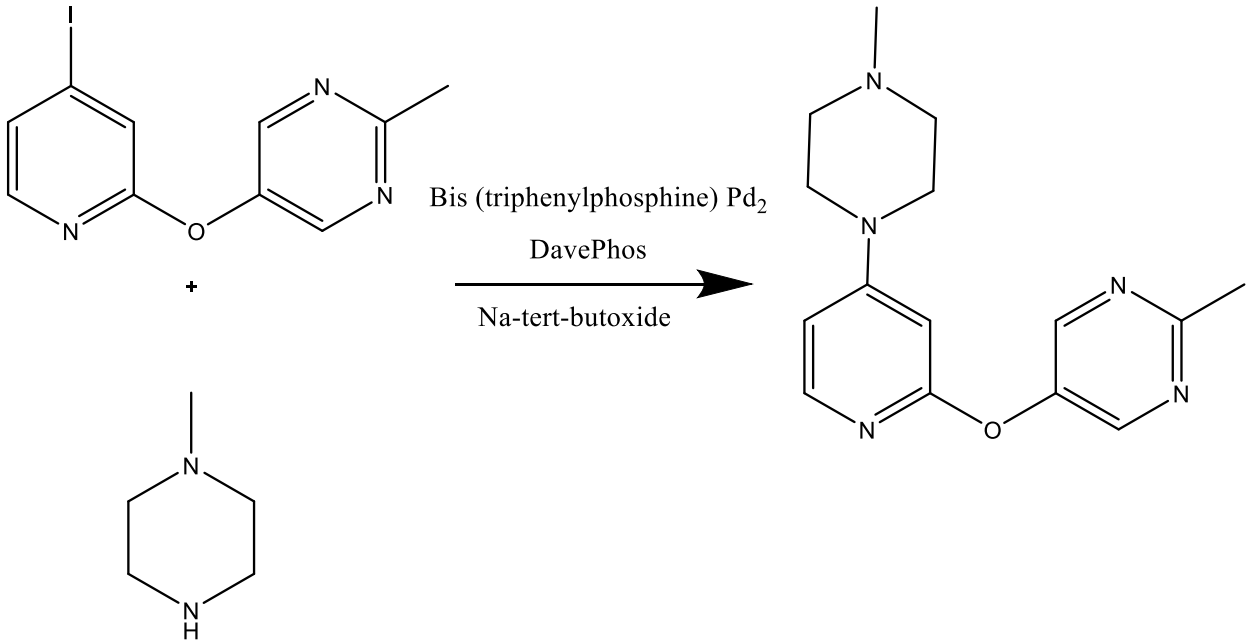
A reaction was set up by adding 5ml of Toluene to a round-bottom flask followed by the addition of 174mg of (R)-N-Boc-azaspiro[2.4]heptan-7-amine and 108mg of sodium tert-butoxide. The reaction was stirred for an hour. 200mg of Compound 2, 35.8mg of Bis (triphenylphosphine) Pd_2, and 39.4mg of DavePhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 110^{o}C for 14 hours. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer.

The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present. The yield was 9%. 4ml of 1:1 Trifluoroacetic acid (TFA) and Dichloromethane (DCM) were added to a small vial containing compound 3. The solvents were then evaporated and an NMR of the product was taken. The NMR revealed multiple smaller compound but the desired compound was not present.



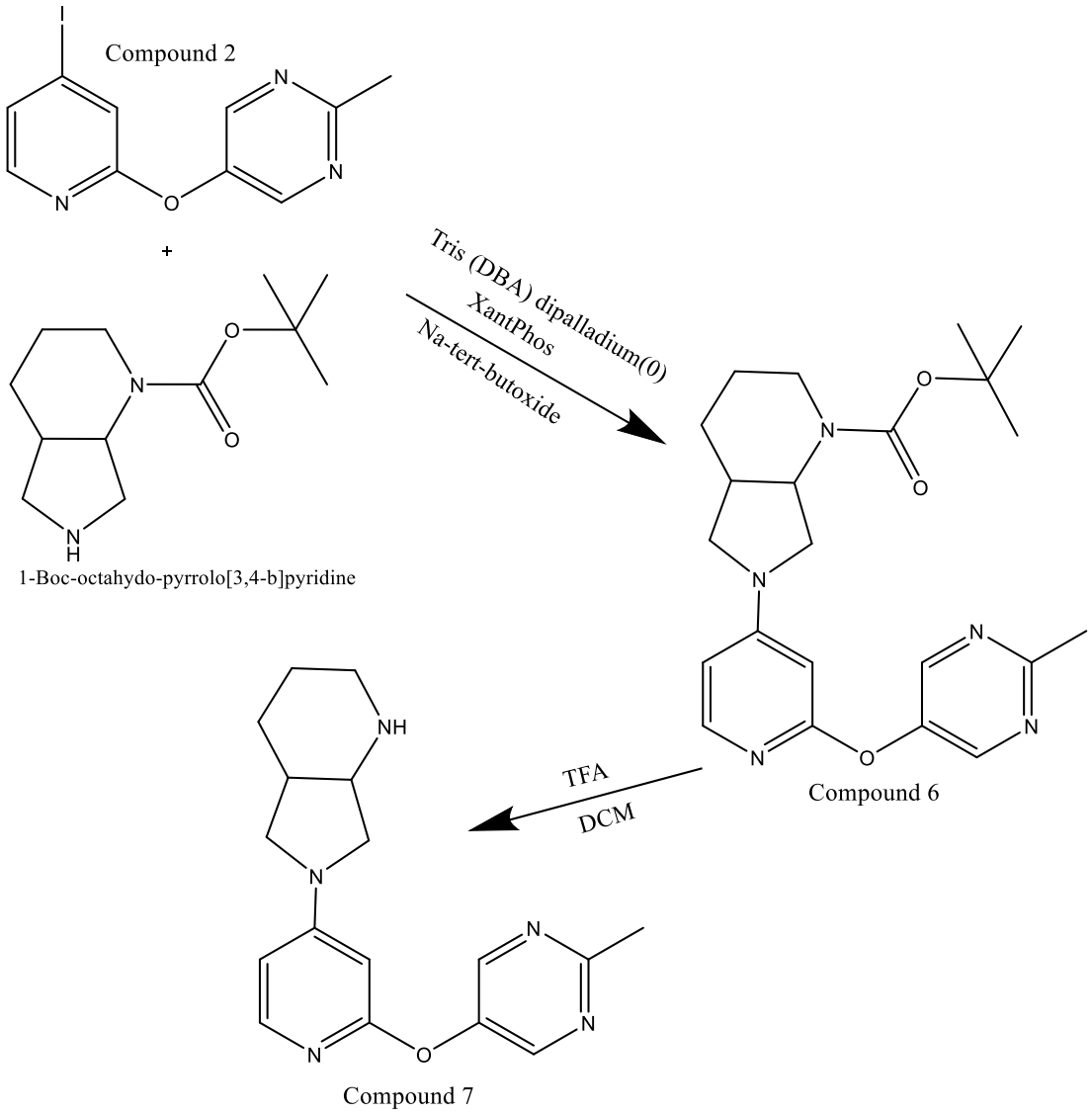
Scheme 1.1.7: Reaction 7.

A reaction was set up by adding 5ml of Toluene to a round-bottom flask followed by the addition of 122mg of 1-Boc-2,7-diazaspiro[3.5]nonane and 91mg of sodium tert-butoxide. The reaction was stirred for an hour. 169mg of Compound 2, 31.2mg of Bis (triphenylphosphine) Pd_2, and 35.2mg of DavePhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 110^{o}C for 14 hours. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.1.8: Reaction 8.

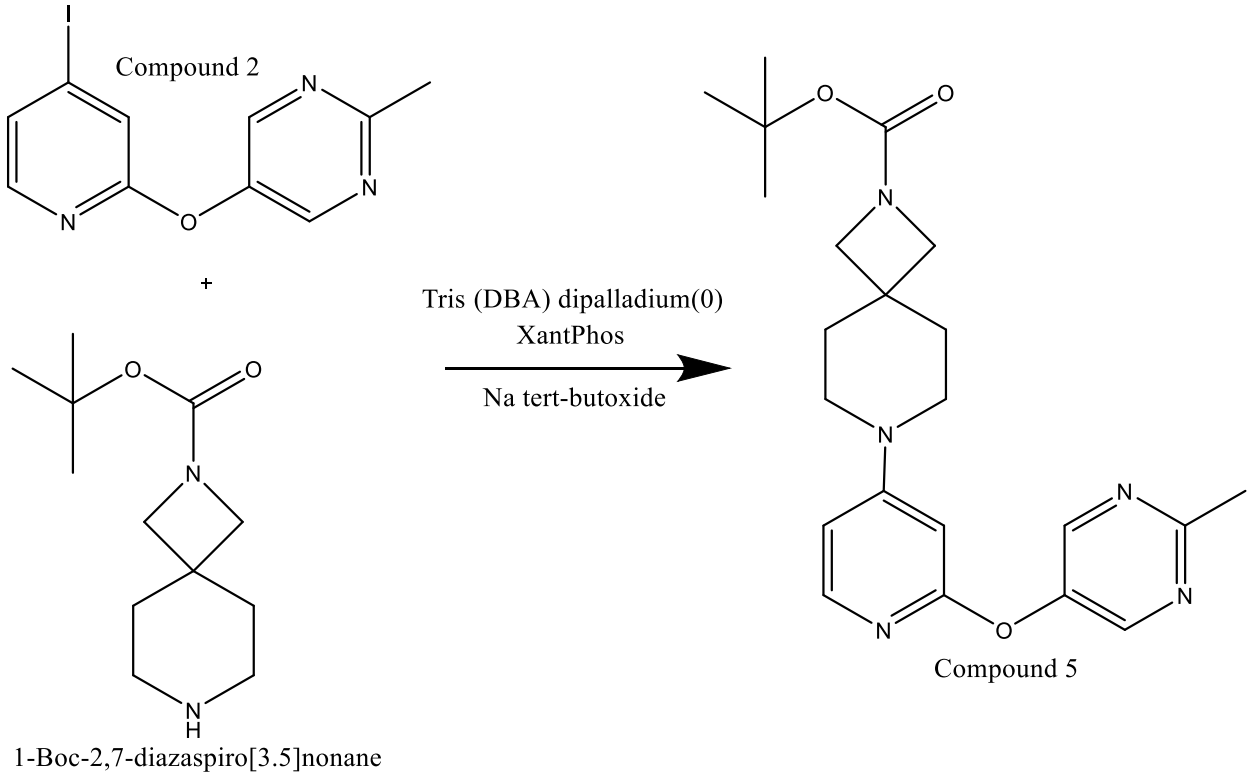
A reaction was set up by adding 5ml of Toluene to a round-bottom flask followed by the addition of 96mg of 1-Methylpiperazine and 184mg of sodium tert-butoxide. The reaction was stirred for an hour. 300mg of Compound 2, 55.4mg of Bis (triphenylphosphine) Pd_2, and 88mg of DavePhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 110^{o}C for 14 hours. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.1.9: Reaction 9.

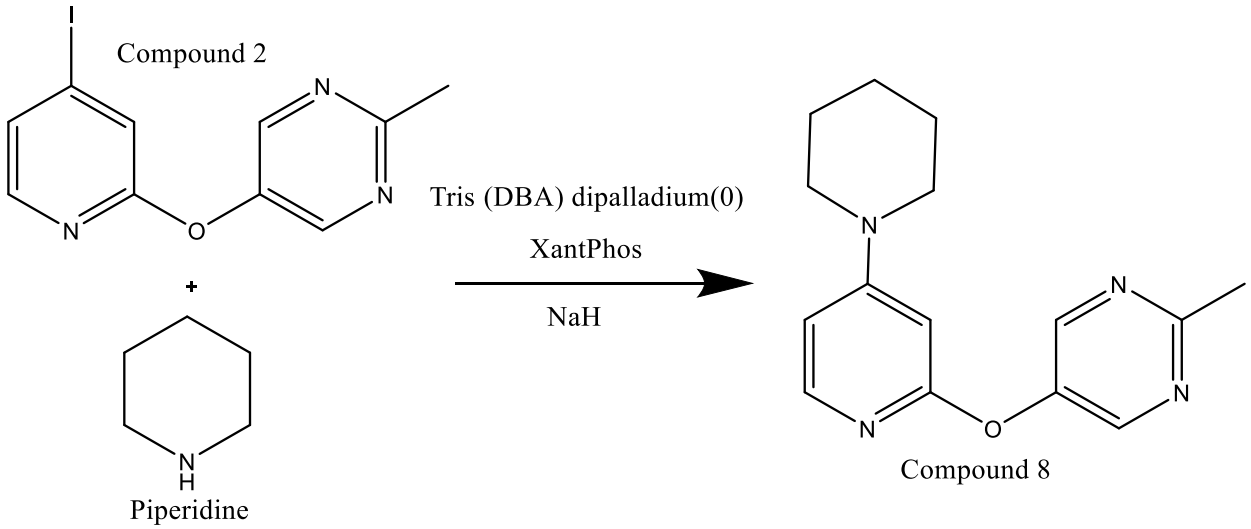
A reaction was set up by adding 4ml of Toluene to a round-bottom flask followed by the addition of 215mg of 1-Boc-octohydro-pyrrolo[3,4-b]pyridine and 183mg of sodium tert-butoxide. The reaction was stirred for an hour. 297mg of Compound 2, 55mg of Tris (dibenzylideneacetone) palladium(0), and 87mg of XantPhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction

flask. The reaction was stirred at 100^{o}C overnight. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present. The yield was 11%. 4ml of 1:1 Trifluoroacetic acid (TFA) and Dichloromethane (DCM) were added to a small vial containing compound 3. The solvents were then evaporated and an NMR of the product was taken. The NMR revealed that the compound was present.



Scheme 1.1.10: Reaction 10.

A reaction was set up by adding 3ml of Toluene to a round-bottom flask followed by the addition of 135mg of 1-Boc-2,7-diazaspiro[3.5]nonane and 116mg of sodium tert-butoxide. The reaction was stirred for an hour. 250mg of Compound 2, 49mg of Tris (dibenzylideneacetone) palladium(0), and 61mg of XantPhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 100^{o}C overnight. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.1.11: Reaction 11.

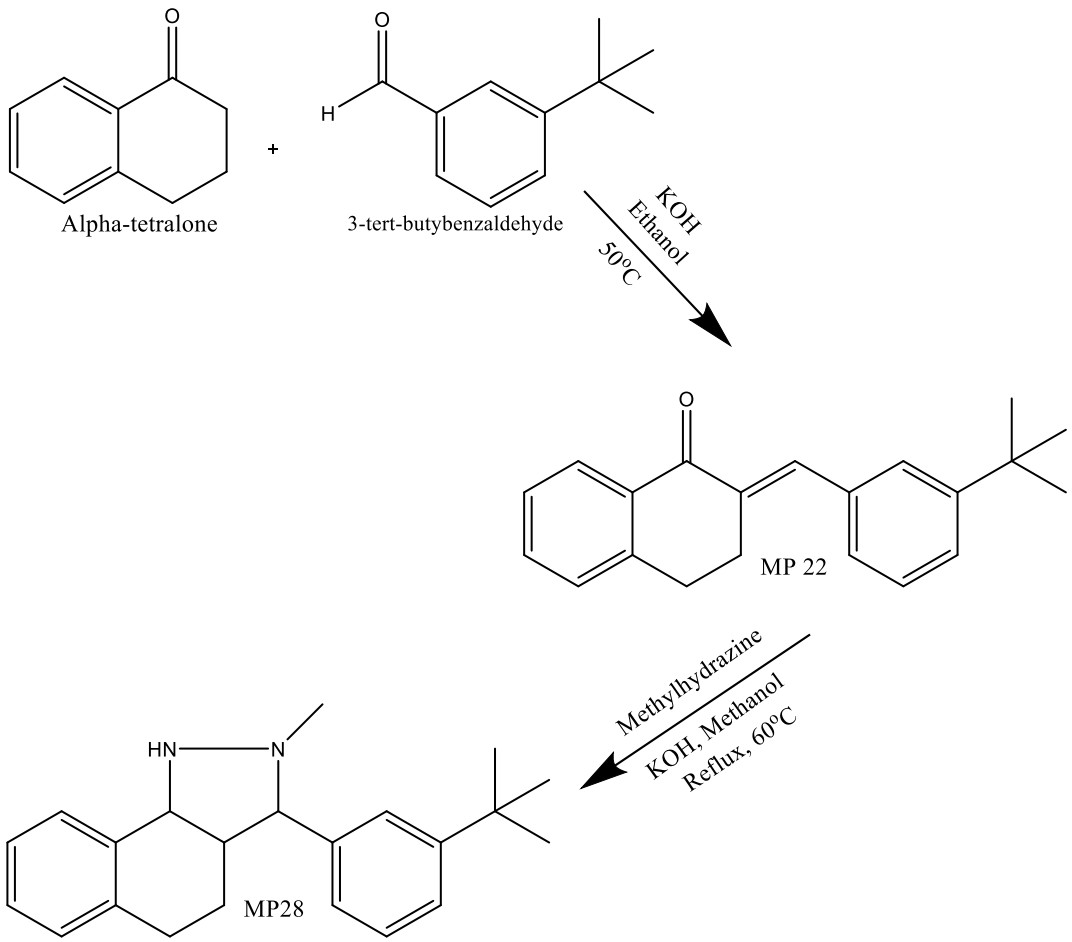
A reaction was set up by adding 3ml of Toluene to a round-bottom flask followed by the addition of 93mg of piperidine and 74mg of sodium hydride. The reaction was stirred for an hour. 200mg of Compound 2, 33mg of Tris (dibenzylideneacetone) palladium(0), and 47mg of XantPhos were added to the reaction. The reaction was sealed off from oxygen and an inert (N_2) atmosphere was added to the reaction flask. The reaction was stirred at 100^{o}C overnight. The reaction was partitioned by using liquid-liquid extraction. Chloroform was used for the organic layer and brine was used for the aqueous layer. The chloroform layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.

Testing of the Synthesized Compound

The test compound was prepared as a stock solution by dissolving into 100% dimethylsulfoxide (DMSO). Broth microdilution was used to determine MIC values, and Ca-MHB (Mueller Hewitt broth) was used as media according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Then bacteria were diluted in MHB to $2x10^6$ cfu/ml, and 10 μl of it was added to each well of a 96-well assay plate containing 170 μl of MHB. Stock solutions were diluted in dilution plates by two-fold dilution. 20 μl of compound solution at each concentration were added to the assay plate to give a final compound concentration ranges from 100 μM to 0.05 μM. Each well with the same concentration was repeated three times to eliminate experimental uncertainty. Assay plates were covered with parafilm and incubated at 37°C for 24 hours. After incubation, plates were read at OD_{600nm} using a VersaMax plate reader. The MIC values were determined by recording the lowest concentration of compound which inhibited bacteria growth (Zhang 2019).

The Methods section of this thesis is coauthored with Jianxi Zhang.

Chapter 1.2: Antimalarial Methods

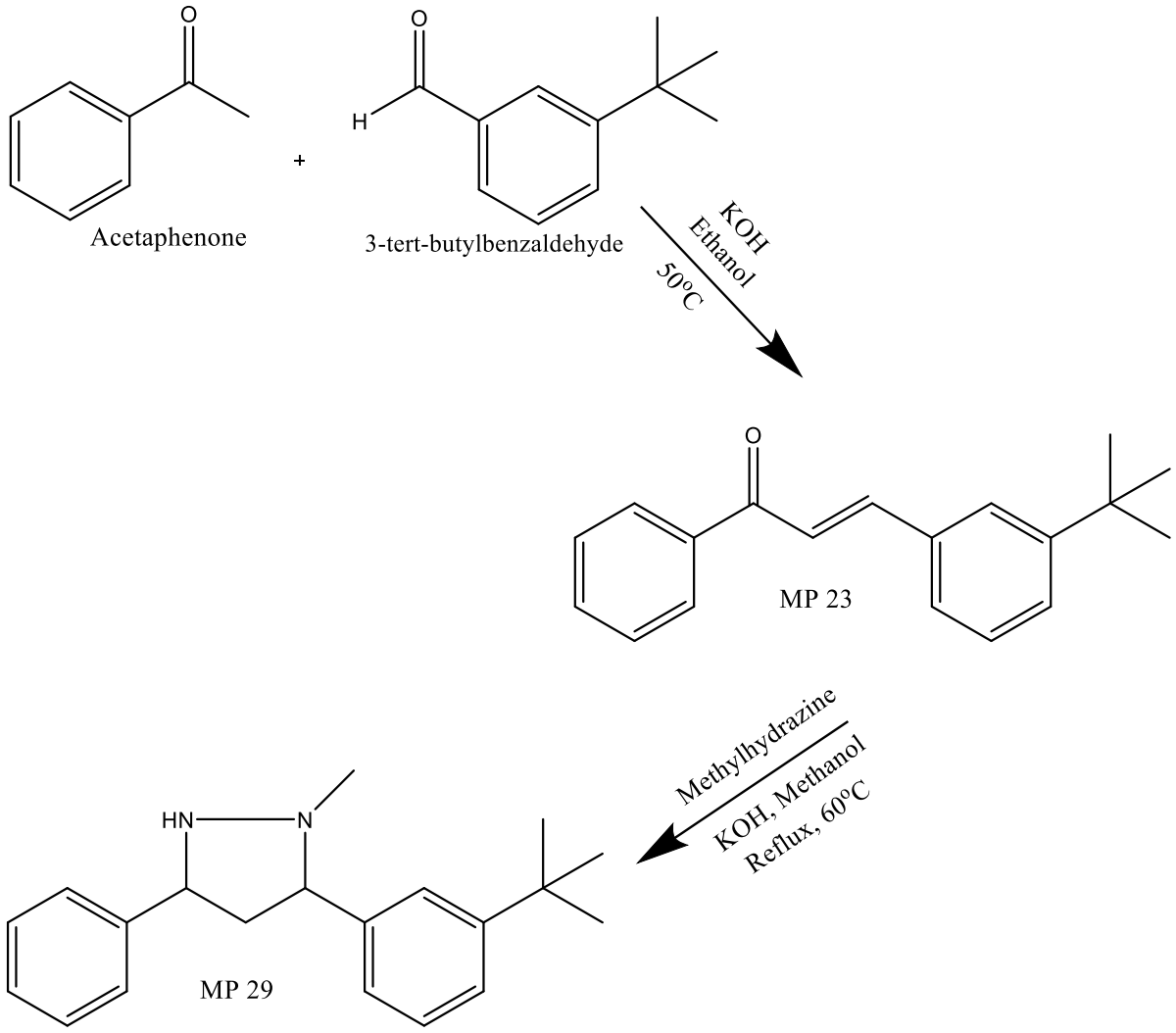


Scheme 1.2.1: Formation of MP 22 and MP 28.

A reaction was set up by adding 1.66ml of ethanol to a small vial followed by the addition of 0.94ml of Alpha-tetralone, 0.15ml of 3-tert-butylbenzaldehyde, and 0.26ml of Potassium hydroxide. The vial was sealed and the reaction allowed to run at 50^{o}C overnight. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the

solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present.

A second reaction was set up by adding 7ml of methanol followed by the addition of 145.6mg of MP 22 and 0.52ml of Methylhydrazine to a medium vial. The vial was sealed and the reaction allowed to run at 60^{o}C for 48 hours. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present.

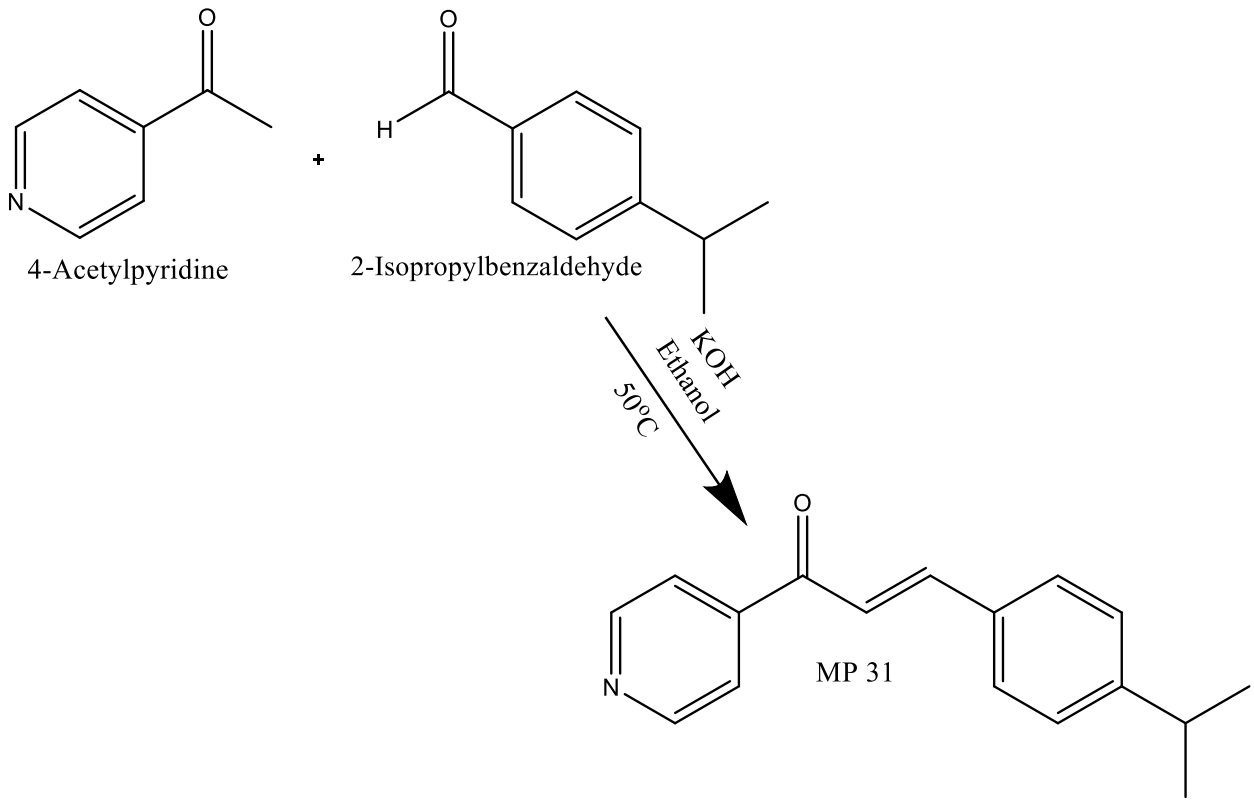


Scheme 1.2.2: Formation of MP 23 and MP 29.

A reaction was set up by adding 1.66ml of ethanol to a small vial followed by the addition of 0.98ml of Acetophenone, 0.15ml of 3-tert-butylbenzaldehyde, and 0.31ml of Potassium hydroxide. The vial was sealed and the reaction allowed to run at 50^{o}C overnight. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the

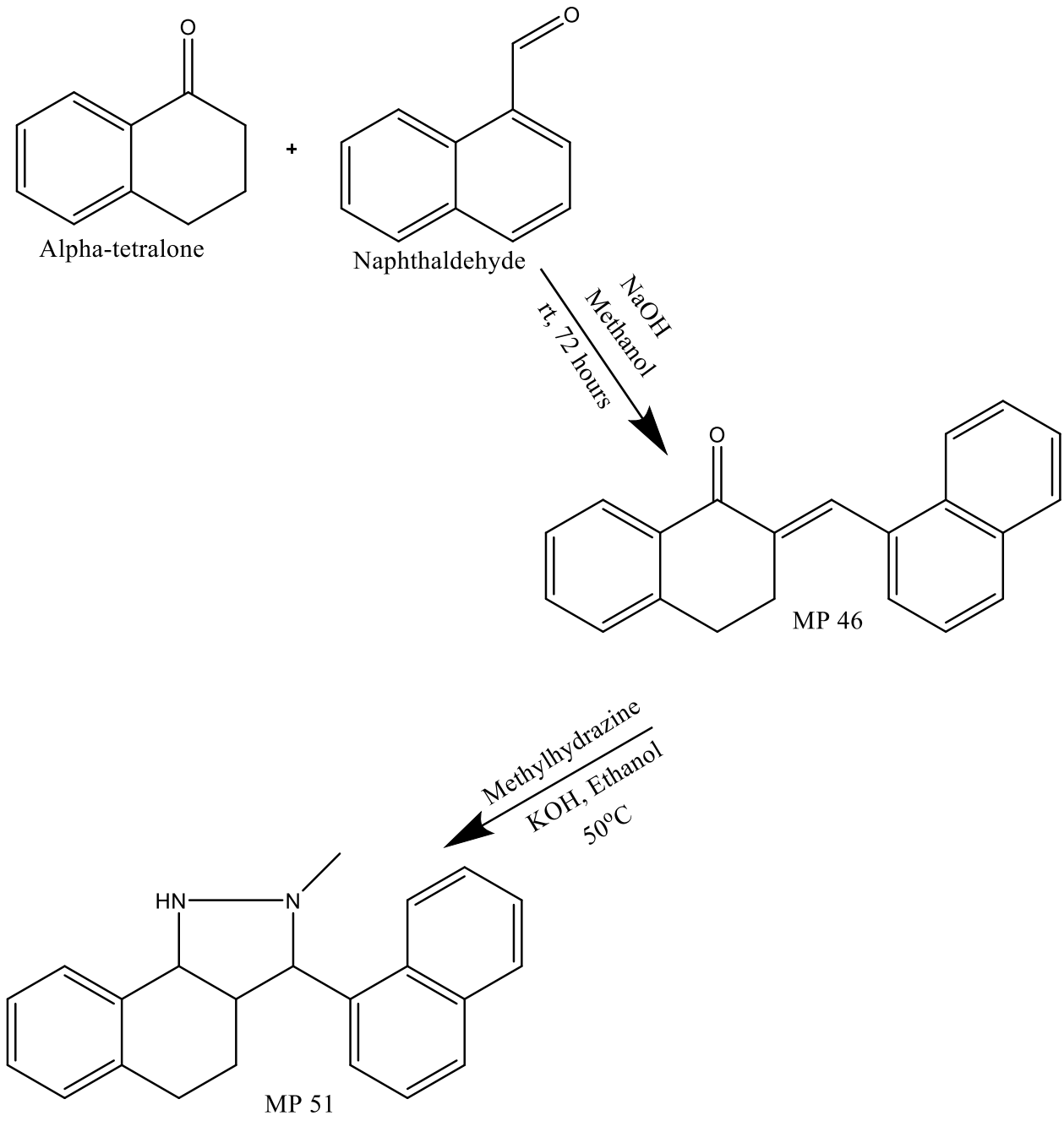
solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present.

A second reaction was set up by adding 7ml of methanol followed by the addition of 149.2mg of MP 23 and 0.59ml of Methylhydrazine to a medium vial. The vial was sealed and the reaction allowed to run at 60^{o}C for 48 hours. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.2.3: Formation of MP 31.

A reaction was set up by adding 1.65ml of ethanol to a small vial followed by the addition of 0.91ml of 4-Acetylpyridine, 0.14ml of Isopropylbenzaldehyde, and 0.31ml of Potassium hydroxide. The vial was sealed and the reaction allowed to run at 50^{o}C overnight. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.



Scheme 1.2.4: Formation of MP 46 and MP 51.

A reaction was set up by adding 4.6ml of methanol to a medium vial followed by the addition of 0.182ml of Alpha-tetralone, 0.27ml of 1-Naphthaldehyde, and 0.41ml of Sodium

hydroxide. The vial was sealed and the reaction allowed to run at room temperature for 72 hours. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was present. The same reaction was run with Ethanol as the solvent and same conditions. NMR analysis revealed that the desired product was present.

A second reaction was set up by adding 9ml of methanol followed by the addition of 313mg of MP 46 and 0.41ml of Methylhydrazine to a medium vial. The vial was sealed and the reaction allowed to run at 50^{o}C for 48 hours. The product was partitioned by using liquid-liquid extraction. DCM was used for the organic layer and brine was used for the aqueous layer. The DCM layer was separated and the excess water was dried over sodium sulphate. The product was isolated by column chromatography and the solvent was evaporated using a rotary evaporator and a high-pressure vacuum pump. NMR analysis revealed that the desired product was not present.

Table 1.2.1: Optimization Table from MP 46. The reaction to form MP 46 was run twice, both times using different solvents. This table shows the yield for both reactions.

Solvent	Yield
Methanol	80%
Ethanol	87%

Table 1.2.2: Table of Compounds. List of all the synthesized compounds for reader's reference.

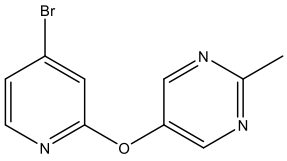
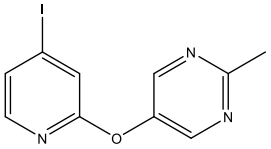
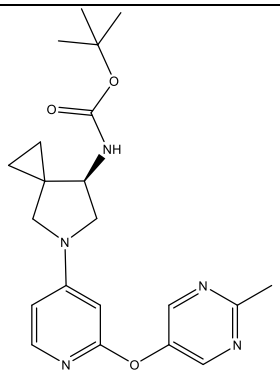
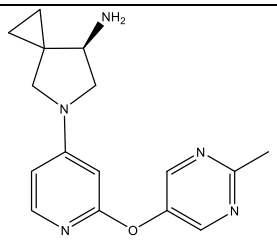
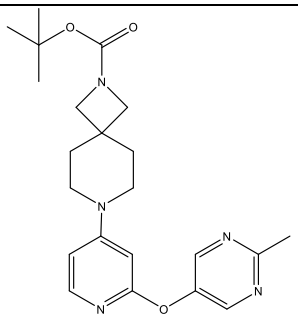
Compound Name	Structure of Compound
Compound 1	
Compound 2	
Compound 3	
Compound 4	
Compound 5	

Table 1.2.2: Table of Compounds. List of all the synthesized compounds for reader's reference, Continued

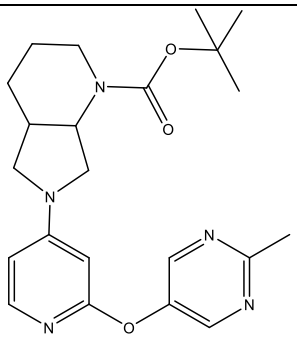
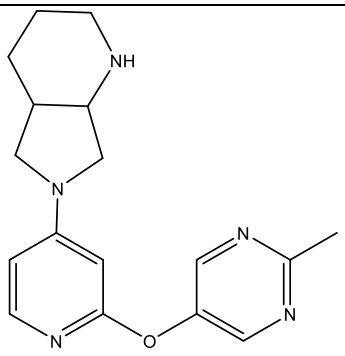
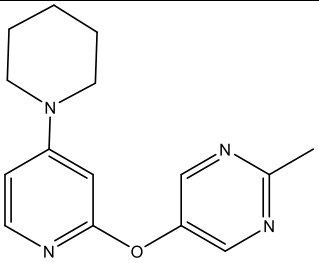
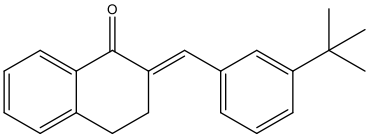
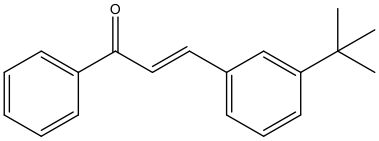
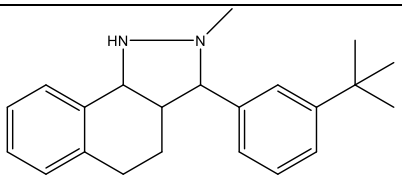
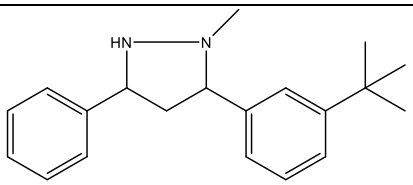
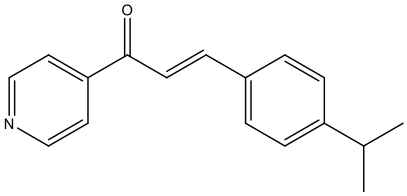
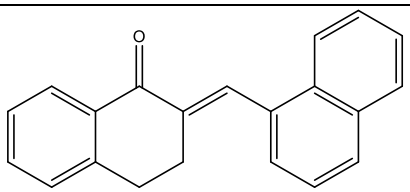
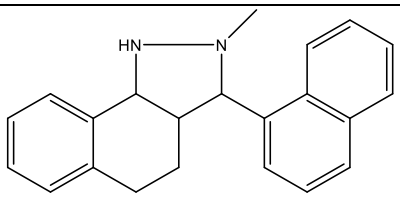
Compound 6	
Compound 7	
Compound 8	
MP 22	
MP 23	
MP 28	

Table 1.2.2: Table of Compounds. List of all the synthesized compounds for reader's reference, Continued.

MP 29	
MP 31	
MP 46	
MP 51	

CHAPTER 2: RESULTS

Chapter 2.1: Antibiotic Results

Reaction 1 initially produced a 42% yield when it was run in DMF at room temperature. The reaction was later optimized to produce a 66% when the solvent was changed to DMSO, the reaction was run at 50^{o}C (Reaction 2), and the starting material (2-Chloro-4-bromopyridine) was changed to 2-Fluoro-4-iodopyridine. The next step of adding cyclic amines presented a struggle. Most of the reactions did not run with the exception of Reaction 6 and Reaction 9. Reaction 6 produced Compound 3 with a 9% yield. The compound was then placed in DCM and TFA to attempt to remove the Boc protecting group but NMR analysis indicated that the desired compound had broken into smaller compounds. Reaction 9 produced Compound 6 with an 11% yield. Removal of the Boc protecting group worked as expected and Compound 7 was completed. Following its completion, Compound 7 was then sent to Jianxi Zhang in the Siegel lab to be tested for antibiotic activity against six different bacterial strains. The bacterial strains that the compound was tested against were *Vancomycin-resistant Enterococcus*, *Staphylococcus aureus* (TCH 1516), Group A *Streptococcus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The tested compound showed little, to no antibiotic activity towards these bacteria compared to the control.

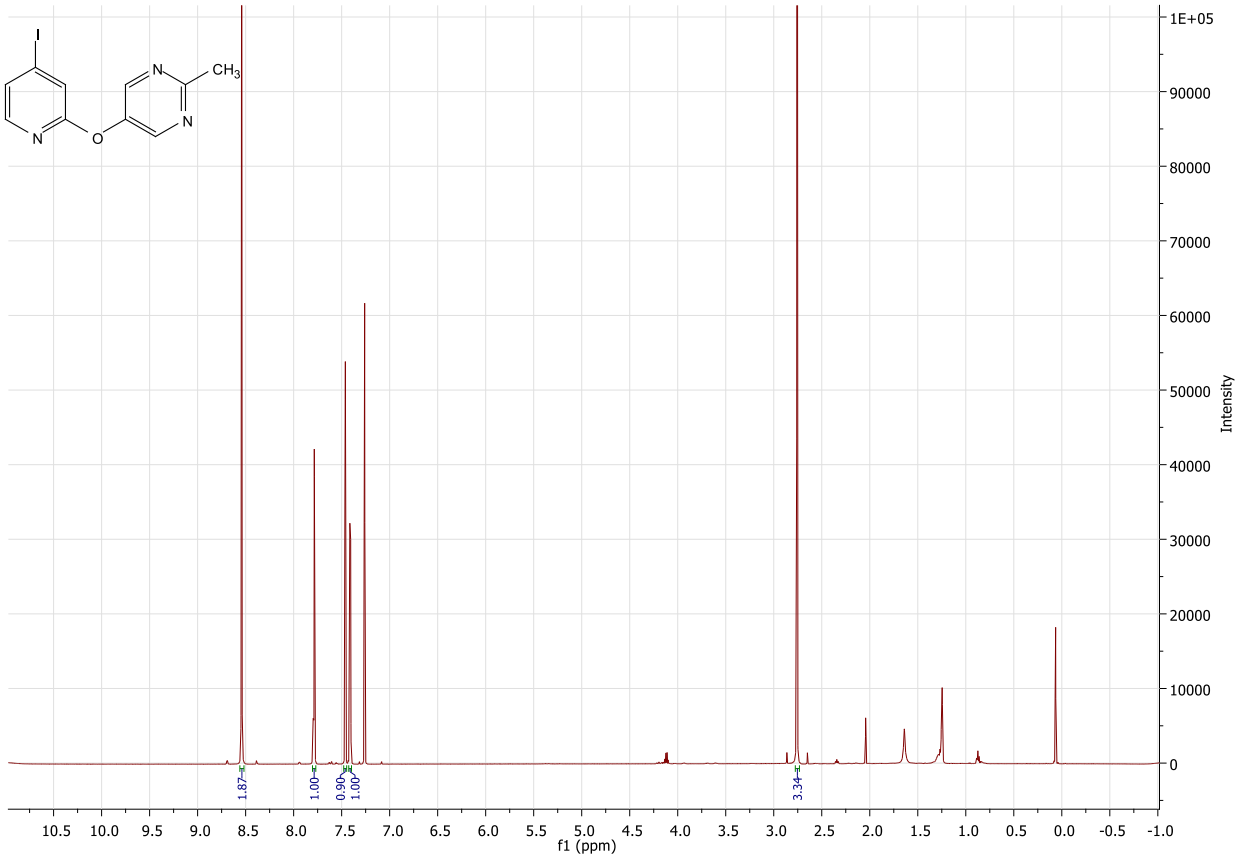


Figure 2.1.1: Proton NMR of Compound 2. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of Compound 2.

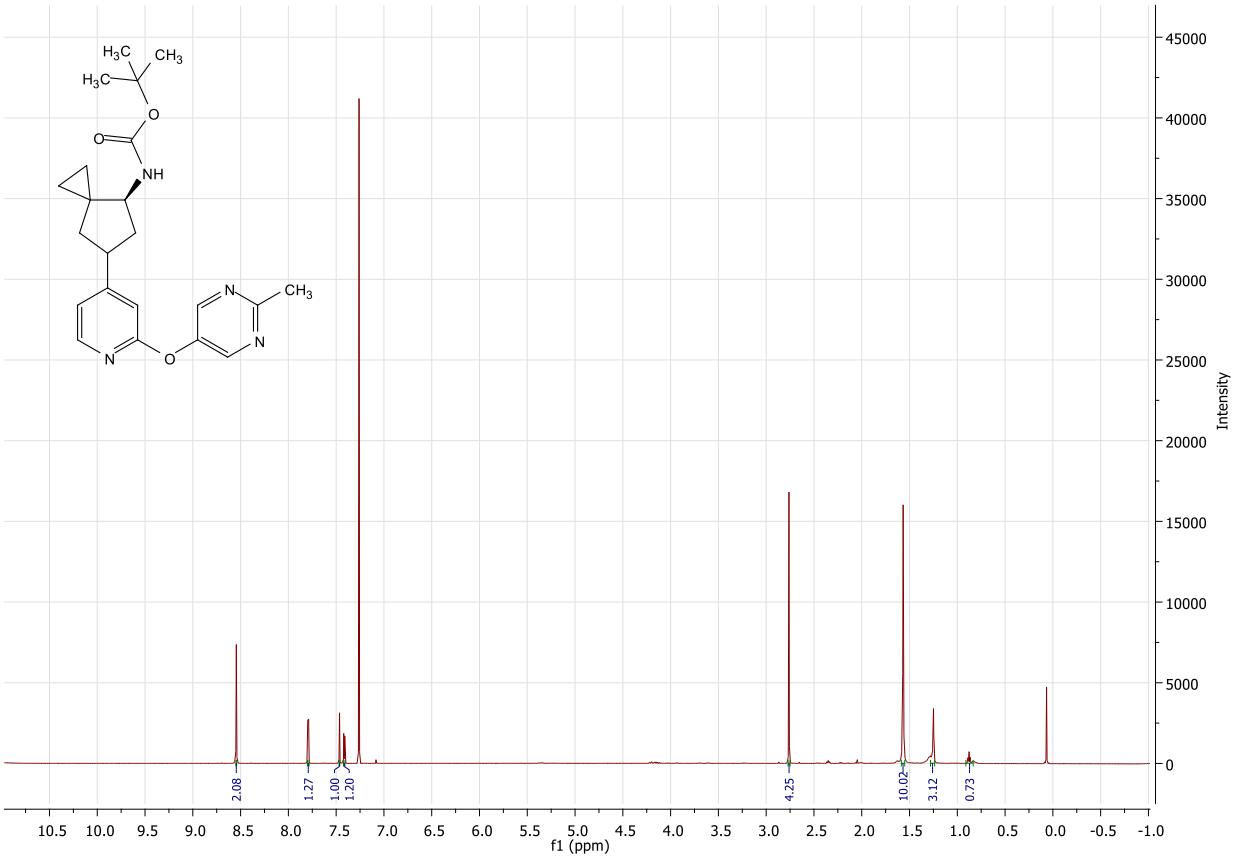


Figure 2.1.2: Proton NMR of Compound 3. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of Compound 3.

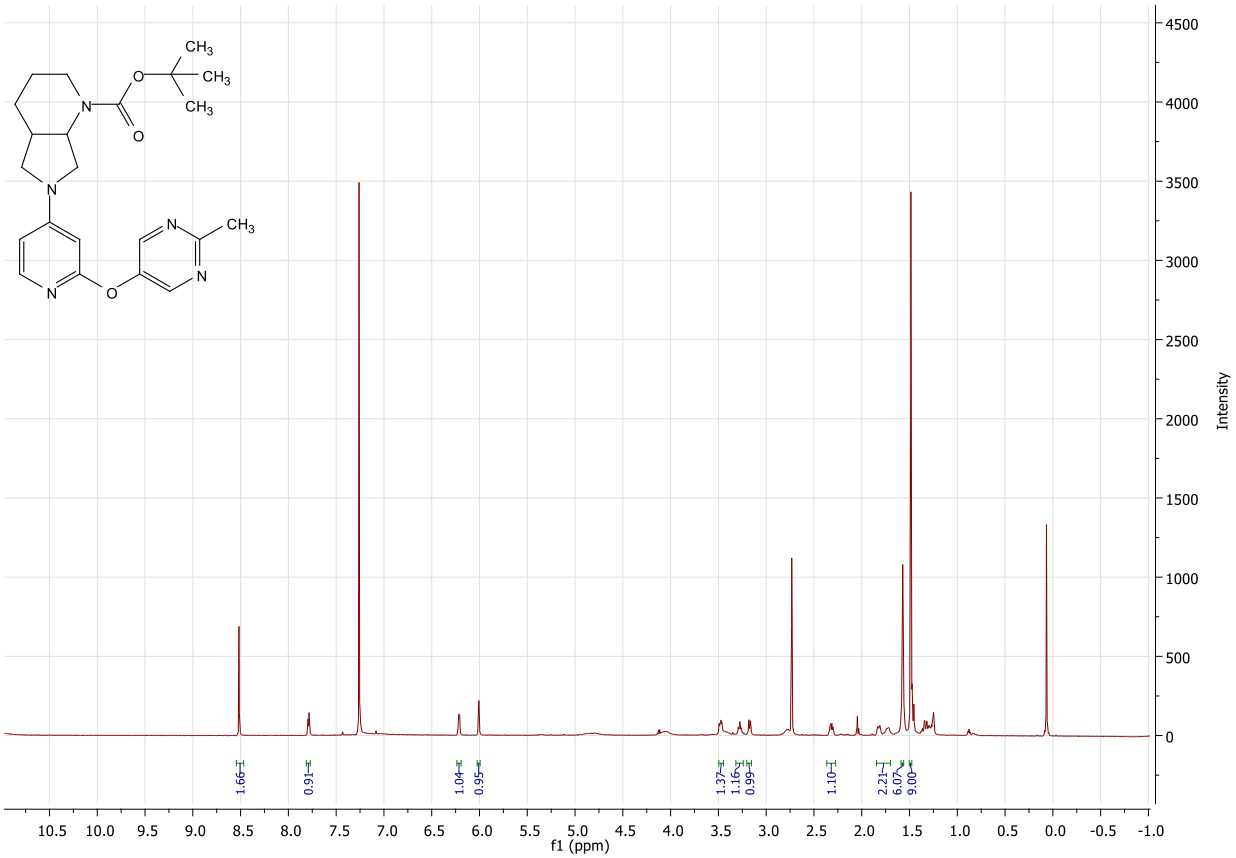


Figure 2.1.3: Proton NMR of Compound 6. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. There were extra peaks at 1.26, 2.05, and 4.12ppm, which indicates that there was excess ethyl acetate in the sample. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of Compound 6.

Chapter 2.2: Antimalarial Results

NMR analysis confirmed that the reactions that formed MP 22 and MP 28 were successful. MP 22 produced a yield of 73%. MP 28 produced a yield of 59%. MP 28 will be sent to the Winzeler Lab in the Skaggs school of pharmacy to be tested for antibiotic activity. Results for the antimalarial properties are yet to come. NMR analysis confirmed that the reaction that formed MP 23 was successful. MP 23 produced a yield of 68%. The following step of adding methyl hydrazine was not successful. The reaction that formed MP 31 formed a solid with the solvent after 24 hours and formed back into a solution the next day. NMR analysis revealed that the desired product was not synthesized. The reaction was run again with the same parameters and the solid did not form but the results were still the same. NMR analysis confirmed that the reaction that formed MP 46 was successful. MP 46 produced a yield of 80%. The same reaction was run but ethanol was used as the solvent instead of methanol, this reaction produced a yield of 87%. NMR analysis revealed that the desired product was present. The following step of adding methylhydrazine to MP 46 was successful and MP 51 was synthesized. This reaction produced a yield of less than 1%. NMR analysis revealed that the desired product was synthesized.

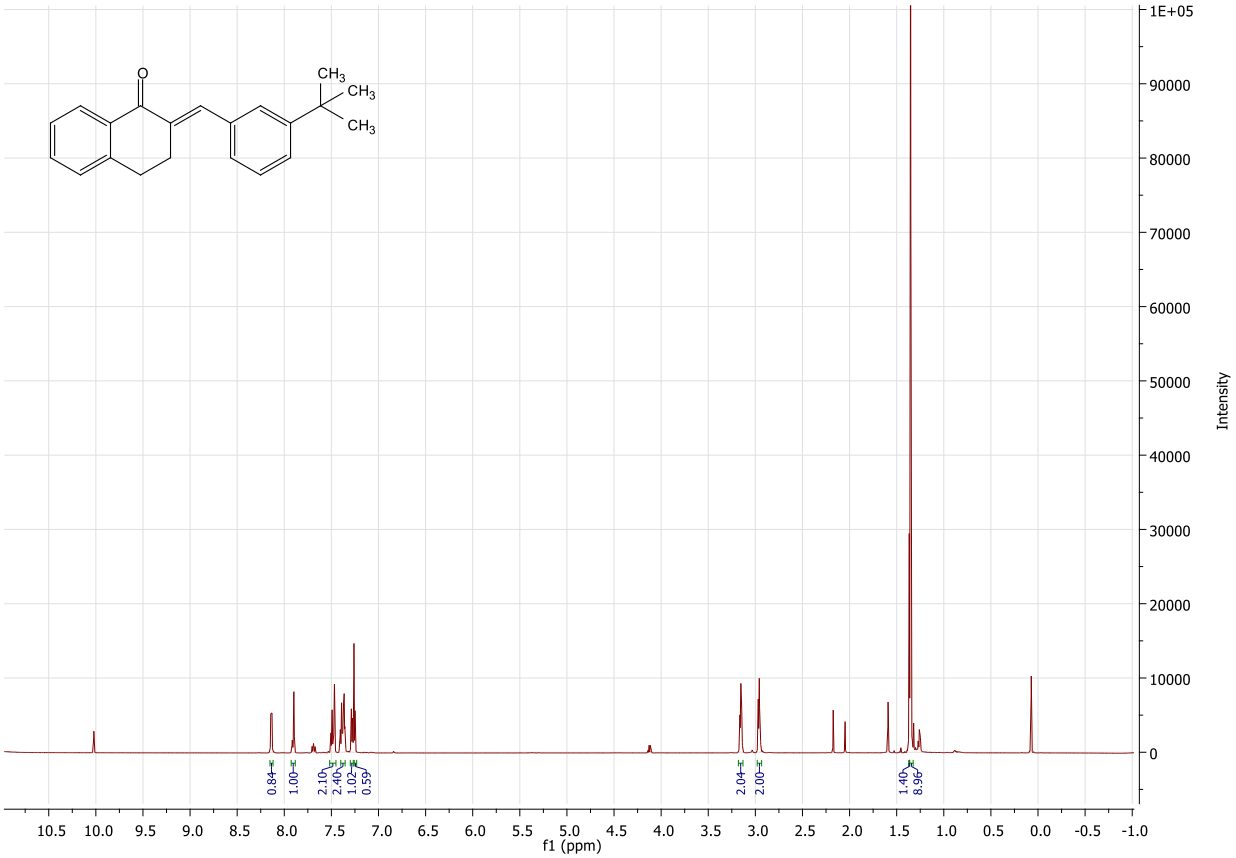


Figure 2.2.1: Proton NMR of MP 22. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of MP 22.

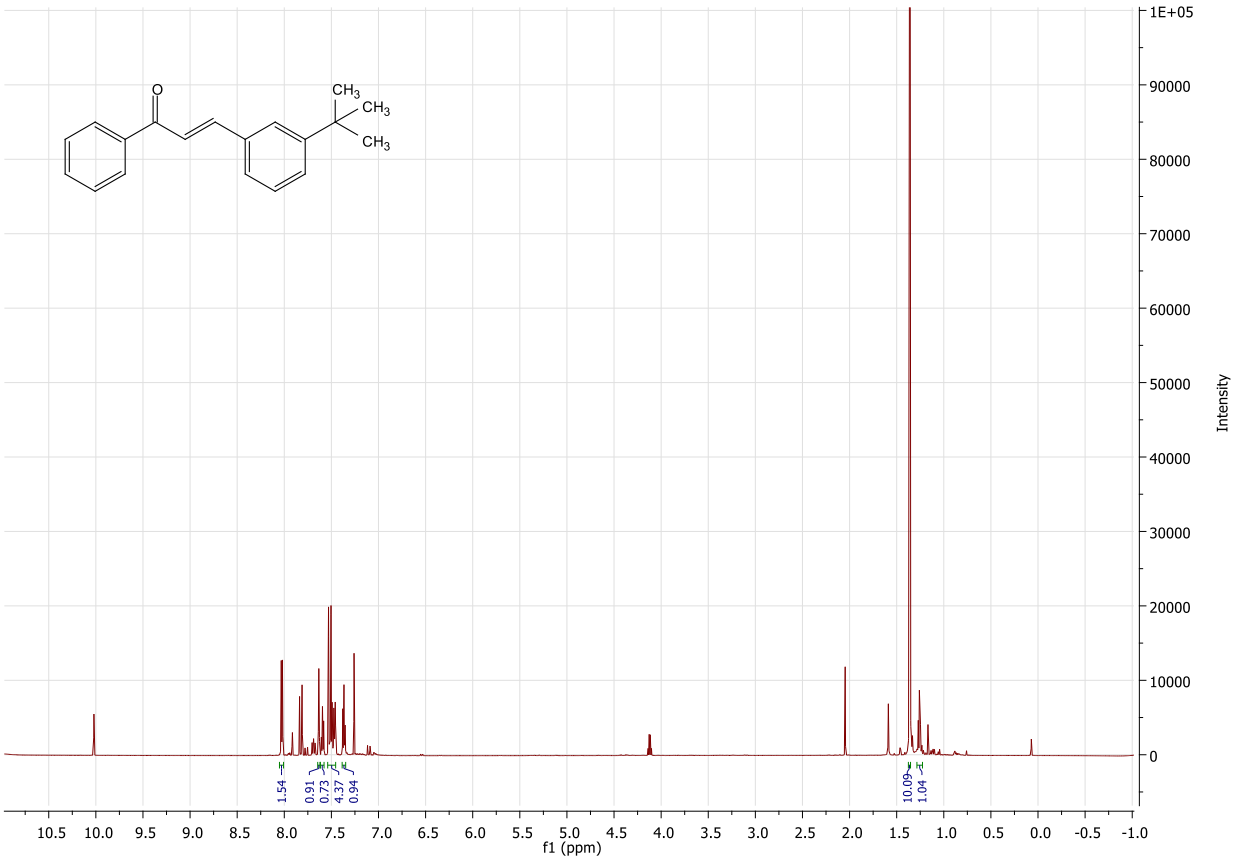


Figure 2.2.2: Proton NMR of MP 23. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of MP 23.

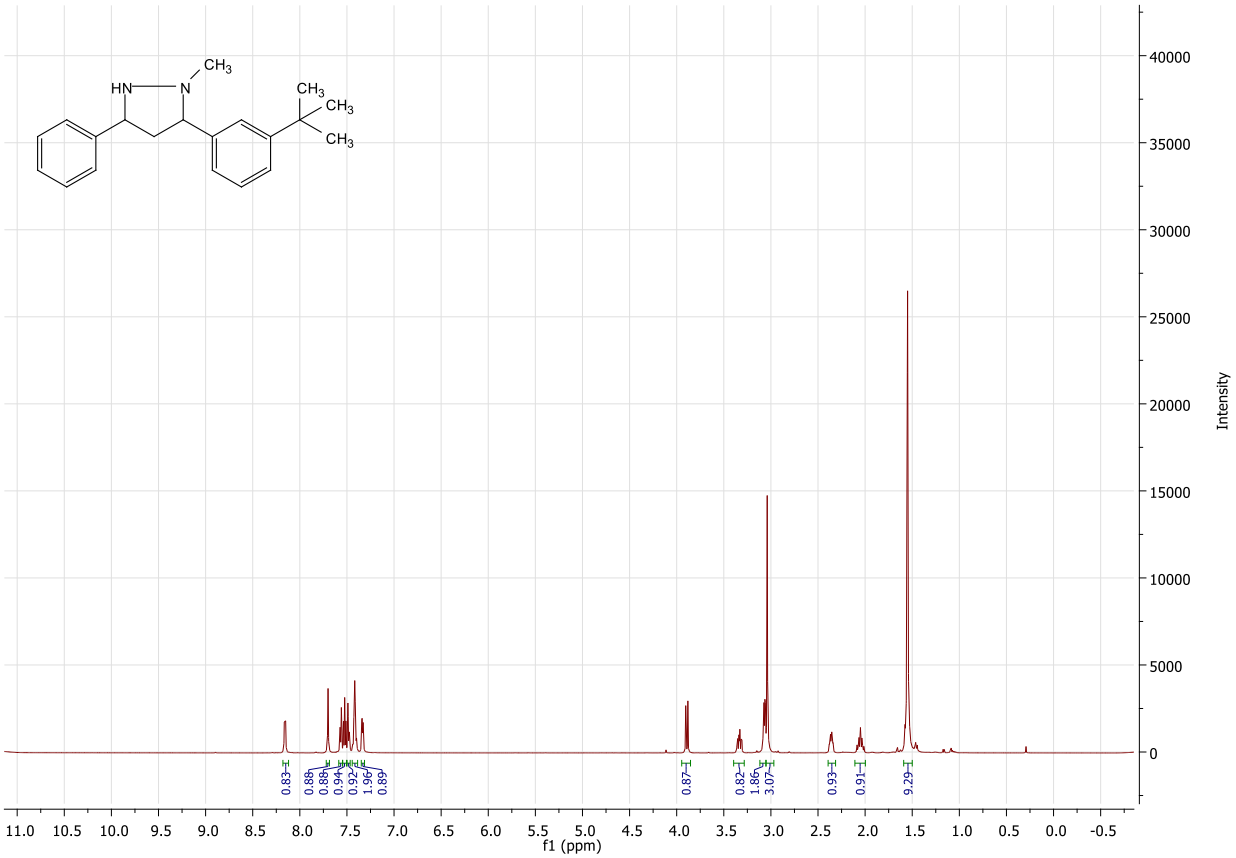


Figure 2.2.3: Proton NMR of MP 28. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of MP 28.

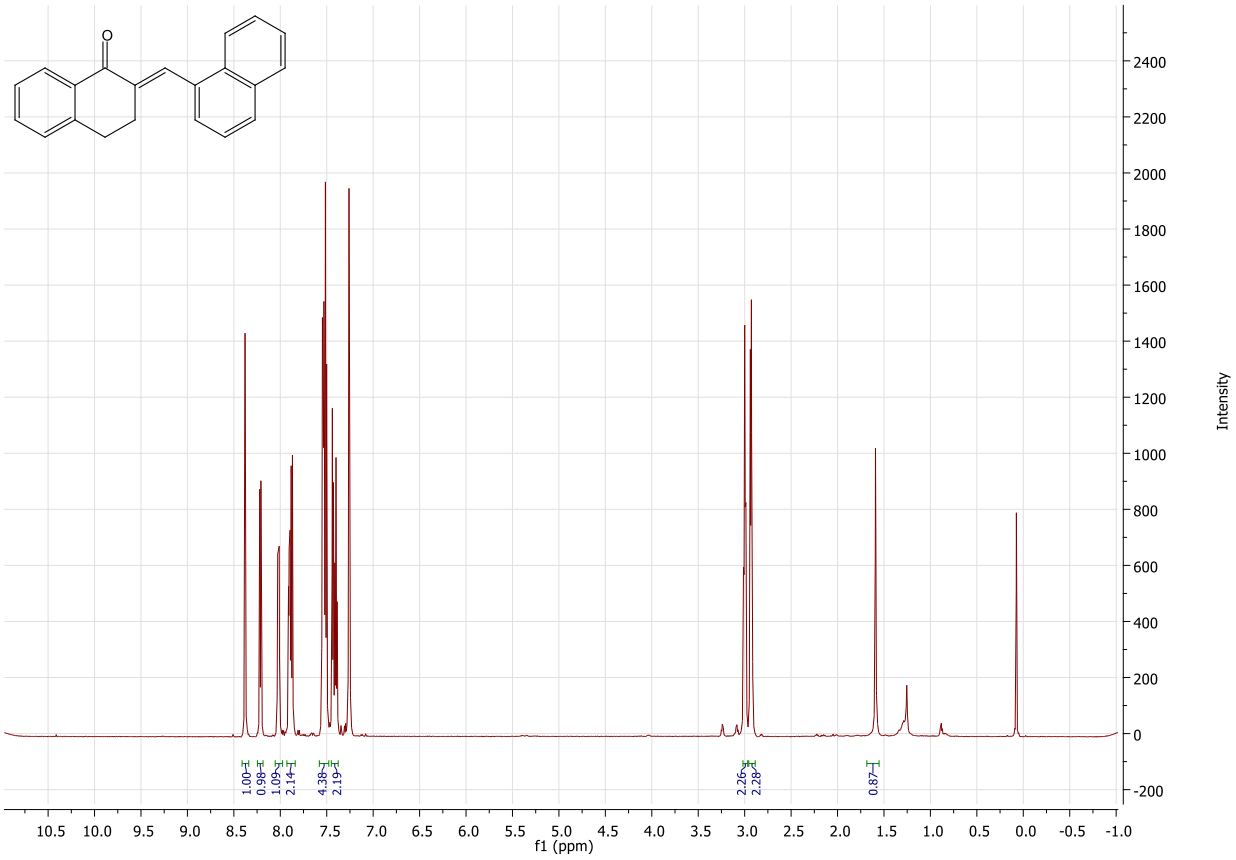


Figure 2.2.4: Proton NMR of MP 46. The NMR was run in deuterated chloroform. The solvent peak occurs at 7.26ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of MP 46.

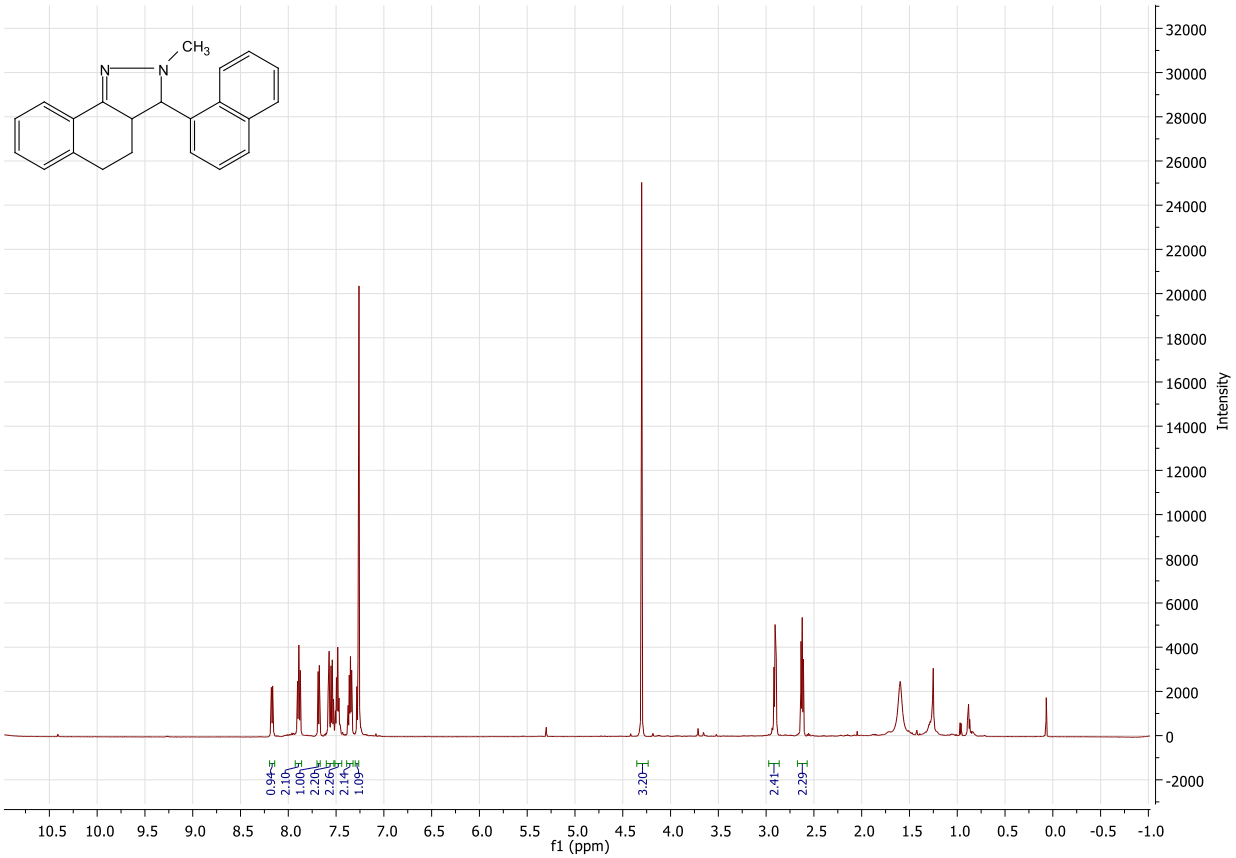


Figure 2.2.5: Proton NMR of MP 51. The NMR was run in deuterated chloroform. The solvent peaks occur at 7.26 and 1.6ppm. All of the protons in the desired compound are present, indicating that the NMR analysis confirms the synthesis and isolation of MP 51.

CHAPTER 3: DISCUSSION

Chapter 3.1: Antibiotic Discussion

The Sn2 substitution reaction that was used to create the tryciclic gyrase (GyrB/ParE) inhibitor backbone was an effective reaction. Compounds similar to this structure were synthesized due to their dual action mechanism and lack of currently existing antibiotic resistance. Initially, 2-Chloro-4-bromopyridine was used to react with 2-Methylpyrimidin-5-ol to create the structure, but 2-Fluoro-4-iodopyridine was substituted for 2-Chloro-4-bromopyridine due to the fluoride atom's higher reactivity in the Sn2 reaction. Another reason for shifting to the Iodopyridine was due to the iodide atom's higher reactivity in Buchwald-Hartwig coupling, which was the next step of the synthesis. The increased reactivity of the halide atoms is due to the electronegativity difference. Higher electronegative halides are more reactive in Sn2 reactions and lower electronegative halides are more reactive in Buchwald-Hartwig aminations. The reaction was initially run at room temperature but was later run at 50^{o}C which resulted in a higher yield. This was expected because higher temperatures increase the frequency of collisions of the molecules. The base and the solvent that were used were also variables that were changed in order to optimize the yield. Sodium hydride was used in place of sodium tert-butoxide as the base in the reaction but the yield was not significantly different. DMSO was used instead of DMF as the solvent and as a result it produced a higher output. The reaction was reported to have an 85% yield but we were only able to optimize the reaction up to 72%. That could be due to different sources of the reactants or insufficient ability to maintain an inert atmosphere for the duration of the reaction. A rubber stopper and a nitrogen-filled balloon were used to uphold an inert atmosphere in this experiment compared to a three-neck flask with a constant flow of nitrogen being used in previous experiments.

Following the synthesis of compound 2, Buchwald-Hartwig amination was used to couple the addition of multiple different di- and tricyclic amines to the halide position of the synthesized compound. Potassium carbonate and sodium tert-butoxide in DMSO was the first attempt to catalyze the cross coupling but the reaction did not progress (Reaction 3). This could be due to the low reactivity of the aryl halide or lack of catalysts in the reaction. The next attempt to catalyze the amination included the use of palladium diacetate, XPhos, and sodium tert-butoxide in DMSO but the reaction did not progress (Reaction 4). This could be attributed to the fact that the catalysts were old and could have expired. The same reaction was run with Bis(triphenylphosphine)Pd_2, XPhos, and sodium hydride in DMSO. The reaction seemed to produce many different products but the desired product was not able to isolated (Reaction 5). The same reaction was run using Bis(triphenylphosphine)Pd_2, DavePhos, and sodium tert-butoxide in DMSO and the reaction was successful. The desired product was able to be purified and isolated (Reaction 6). These particular catalysts were chosen because they were able to produce a similar reaction in the past and they did not appear to be expired. The compound broke into many smaller compounds when the Boc group was attempted to be removed as indicated by TLC and NMR. This data suggests that the compound could have been acid labile or could have reacted with the DCM and TFA in an undesired way. Another explanation for the breakdown of the compound could have been that it was left in the DCM/TFA for too long.

Since Reaction 6 was successful in coupling the amination of Compound 2 to the desired amine, the same reactants were used in the next reaction. Bis(triphenylphosphine)Pd_2, DavePhos, and sodium tert-butoxide in DMSO were used to catalyze the coupling of 1-Boc-2,7-diazaspiro[3.5]nonane to Compound 2 but the reaction was not successful (Reaction 7). Because of the success from reaction 6, the same catalysts were used in an attempt to cross couple 1-

Methylpiperazine to Compound 2 but again, the reaction was not successful (Reaction 8). 1-Methylpiperazine was used as a control because it is much less complex than the other amines and therefore could possibly give us some information about the reactivity of the amine and the success rate of the reaction. The results were inconclusive. There was no clear explanation to why the same reaction worked with a larger amine and not 1-Methylpiperazine.

Following the lack of success from Reaction 8, Tris(dibenzylideneacetone)palladium(0), XantPhos, and sodium tert-butoxide in DMSO was used in attempt to catalyze the amination of 1-Boc-octohydro-pyrrolo[3,4-b]pyridine to Compound 2 (Reaction 9). This reaction was successful and the compound was able to be purified and isolated. The Boc group was then able to be successfully removed from the desired compound. The sudden success of the reaction could be due to the catalyst being purchased specifically for the reaction. Palladium catalysts can lose their potency as time goes on but these catalysts were new. Following the success of Reaction 9, the same catalysts were used in an attempt to catalyze the coupling of 1-Boc-2,7-diazaspiro[3.5]nonane to Compound 2 (Reaction 10). The reaction was not successful. Following the lack of success for Reaction 10, pieridine was attempted to be coupled to Compound 2 using the same catalysts but the reaction was also not successful (Reaction 11). Piperidine was used as a control to see if the simplest of cyclic amines could be coupled onto Compound 2. Failure to catalyze Reaction 11 showed that Buchwald-Hartwig might not be the best way to create the desired compounds.

The synthesized Compound 7 was isolated, purified, and sent to Jianxi Zhang in the Siegel lab to be tested for antibiotic activity. After testing, the results proved that the compound in question did not exhibit antibiotic activity according to the minimum inhibitory concentration (MIC) assays that were performed. The goal was to synthesize compounds that were similar in

structure to Novobiocin, a tricyclic gyrase (GyrB/ParE) inhibitor that was synthesized by a pharmaceutical company by the name of Trius. Novobiocin was a first line antibiotic that was used in the 1960's but usage began to rapidly decline following the report of adverse side effect by patients. These drugs are extremely effective because the have a dual mechanism of action. On one hand they inhibit the ATPase activity on the GyrB subunit of bacterial DNA gyrase, while also targeting the ParE subunits of Topoisomerase IV. This dual action inhibition significantly decreases the rate of resistance acquisition. A bacteria would have to be multi-drug resistant in order to survive such antibiotics (Zhang 2019).

Chapter 3.2: Antimalarial Discussion:

Synthesis of the malaria project (MP) compounds were much more successful than the synthesis of antibacterial compounds. Synthesis of the MP compounds required a two-step synthesis that involved an aldol condensation to form an enone, and the addition of a hydrazine to that enone. Methylhydrazine was used as the hydrazine for this research. An aldehyde, a ketone, a strong base, and heat were used to catalyze the synthesis of each enone. Alpha-tetralone and 3-tert butylbenzaldehyde were the reactants used to produce MP 22. Methyl hydrazine was added to the enone to produce MP 28. Acetophenone and 3-tert butylbenzaldehyde were the reactants used to synthesize MP 23. The reaction to couple methylhydrazine to MP 23 was not successful. A possible reason that the reaction did no progress could be due to the low reactivity of MP 23 to methylhydrazine. 4-acetylpyridine were and 4-isopropylbenzaldehyde were used to catalyze the formation of MP 31 but the reaction was unsuccessful. The reaction formed into a solid for two days before going back to the liquid state. A possible reason that the reaction did not progress could be because of the properties of the pyridine as the aldehyde. For the other reactions, the aldehyde had a benzene ring but this aldehyde had an amine group in the ring to form a pyridine. Reactivity of pyridines in the aldol condensation remain to be unknown and did not produce the desired reaction. That was the last time that a pyridine was used in the reaction. Alpha-tetralone and 1-Naphthaldehyde were used to perform the aldol condensation in MP 46. The reaction was successful and MP 46 was synthesized. Alpha-tetralone seems to be a good ketone when preforming an aldol condensation. This same reaction was run twice, using both methanol and ethanol as the solvent. The reaction produced a yield of 80% when run in methanol and a yield of 87% when run in ethanol. This experiment was the only time that methanol was used as the solvent and these results suggest that

ethanol is the solvent of choice when running the aldol condensations with an aryl ketone and a benzaldehyde. The following step of adding methyl hydrazine to MP 46 was a bit more difficult because MP 46 was not fully soluble in ethanol. DCM was added to the reaction to increase the solubility of MP 46. During the 48-hour reaction, the DCM was evaporated and escaped the reaction vial. The yield of this reaction was less than 1%. This could be attributed to the decreased solubility of MP 46 in ethanol combined with the evaporation of the DCM. Another possible explanation for the low yield is that the reaction was run at 50^{o}C compared to the hydrazine additions being run at 60^{o}C in past experiments.

The synthesized compounds have yet to be tested by the Winzeler lab for antimalarial activity. These compounds will be tested against the Plasmodium falciparum species using the half maximal inhibitory concentration (IC50). IC50 measures the potency of the compounds to inhibit the growth of Plasmodium falciparum by 50%. The growth of the parasite will be tested in human liver cells in attempts to find a treatment that will terminate the parasite in the liver-stage. Most current treatments for malaria affect the parasite in the blood stages. The plasmodium species, particularly Plasmodium falciparum which is the deadliest, is evolutionarily prepared to resist drugs that target the blood stages of malaria. It is the hope that the synthesis and testing of the compounds made in this research can go on to show positive activity towards the plasmodium parasites and could go on to being an analog for the next treatment for malaria. It is the impression that liver stage drugs give the parasite less of a chance of developing resistance than blood stage treatments due to the majority of current treatments being blood stage drugs. The synthesized compounds have yet to be tested by the Winzeler lab, however each compound that is tested adds to a greater project known as the Malaria Drug Accelerator (MalDA). The MalDA was founded and is funded by the Bill and Melinda Gates Foundation and

includes the research from 13 labs around the US and China, including the Winzeler lab. Although the results are inconclusive, this research will aid in the discovery of the next treatment, even if the compounds don't directly inhibit malarial growth.

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