UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

SYNTHESIS AND EVALUATION OF PIPERAZINE-CARBOXAMIDE DERIVATIVES AS POTENTIAL ANTIMALARIAL /ANTIMYCROBIAL AGENTS

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL CHEMISTRY

JULY 2018

DECLARATION

I hereby declare that this submission is my own work which I undertook under supervision in the Department of Chemistry, University of Ghana towards the award of MPhil in Chemistry and that to the best of my knowledge, it has neither been partially nor wholly presented anywhere else for another degree and contains no materials previously published by another person except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to the glory of the Almighty God for how far He has brought me in life. I also want to dedicate this work to my lovely family especially my mother, Catechist Gladys Aboagye who loves me very much and whom I also truly love.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Dr. Richard Amewu for his immense dedication, patience, supervision, care and guidance throughout the project.

My sincere thanks also go to my colleagues who helped me in the lab especially Mr. Henry Onyame, Miss. Henrietta Attram, Fritz Plange and Crystal F. Ade.

I also want to thank Mr. Samuel Owusu-Atuah (General) for running the IR spectra of all my samples, Mr. Bob Essien for his time and the department Secretaries, Miss. Victoria Honu and Mrs. Dorothy Akaho for their advice, encouragement and sumptuous meals.

My profound gratitude also goes to the staff members of La Anglican '1' Junior High School for their kindness, support and tolerance especially Headmaster, Mr. Edward Bernard Quaye during the course of this work. I am grateful for every help offered.

And to my dearest and fondest friend, Elikplim Ophelia Yawa Dogbey, thank you for being my friend through the changing scenes of life. You are unquantifiably more valuable to me than you know. God richly bless you, Eli.

I wish to express my profound gratitude to the Medicines for Malaria Ventures providing the funding, the staff of the Drug Discovery Unit at University of Dundee for the antimalarial testing and the Department of Chemistry, UG for the spectroscopic analysis including the NMR, FTIR etc.

Finally, I would like to express my appreciation to the entire Faculty of the Department of Chemistry, University of Ghana for their correctional inputs and criticisms during my earlier presentations.

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ACT	Artemisinin-Based Combination Therapy
AMK	Amikacin
CAP	Capreomycin
CIP	Ciprofloxacin
CHIKV	Chikungunya Virus
CLR	Clarithromycin
DHPMS	3,4-Dihydropyrimidin-2(1H)-Ones
DOTS	Directly Observed Treatment Short
DSC	Differential Scanning Calorimeter
EMB	Ethambutol
ETH	Ethionamide
INH	Isoniazid
KAN	Kanamycin
LEV	Levofloxacin
LLIN	Long-Lasting Insecticidal Net
LZD	Linezolid
MABSC	*Mycobacterium abscessus* Complex
MDR	Multi Drug Resistant
MDT	Multi Drug Therapy
MMV	Medicine For Malaria Ventures
MOX	Moxifloxacin
NMCP	National Malaria Control Programme
NTD	Neglected Tropical Diseases
NTM	Non-Tuberculous Mycobacteria
NTP	National Tb Control Programme
OFX	Ofloxacin

OPD	Out Patient Department
PAS	Para-Aminosalicylic Acid
PSA	Polar Surface Area
PTH	Prothionamide
PZA	Pyrazinamide
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Tests
RIF	Rifampicin
SAR	Structure And Activity Relationship
TDR	Tertiary Drug Resistant
VCCLAB	Virtual Computational Chemistry Laboratory
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR-TB	Extensive Drug Resistant TB

ABSTRACT

Malaria and tuberculosis continue to be endemic in Africa and several other regions across the globe. Malaria is caused by a parasite transmitted by the female anopheles' mosquito. *Plasmodium falciparum* is responsible for human malaria in most part of Africa and *Plasmodium vivax* causes malaria in Asia. Tuberculosis on the other hand is caused by *Mycobacterium tuberculosis* which is propelled when an infected person coughs, sneezes or even talks. Together, these two diseases are responsible for high mortality in the global population including infants. The current mainstay of malaria chemotherapy are the Artemisinin-based combination therapies (ACTs) while tuberculosis is treated with four main drugs known as the frontline drugs including Isoniazid, rifampicin, ethambutol and pyrazinamide. But the emergence of resistance, partly as a result of patient non-compliance, towards the frontline drugs by all pathogens is rendering the fight more challenging. There are no clinically approved vaccines available for the immunization of both disease and no new drug has been introduced to combat tuberculosis for over 50 years now. Carboxamide and urea compounds are emerging as potential drug candidates and were identified to possess antiproliferative, antimalarial and antitubercular activities. This project focused on the synthesis of aryl carboxamides incorporating urea motif as well as analogues devoid of the urea functionality to establish how critical the urea motif is to observed biological activity based MMV676444. Overall, **26** urea and **9** non-urea derivatives were synthesized. Preliminary antimalarial testing of the compounds showed that six (**6**) possess antimalarial activity in the sub-micromolar to micromolar ranges. The most active compound was **102** with *in vitro* antimalarial activity of 0.574 μM against the 3D7 strain of the malaria parasite. It was generally observed that the compounds containing the urea moiety were more potent than the non-urea compounds. The

compounds have also been submitted for anti-tubercular testing, but results were not available at time of compiling the thesis.

CHAPTER ONE

1.0 INTRODUCTION

1.1 **Background**

Neglected Tropical Diseases (NTDs) are an array of communicable diseases that are found in tropical and subtropical countries. They include Buruli ulcer, Chagas disease, Dengue, Leprosy, Rabies and several others. They are caused by pathogens such as protozoa, bacteria, viruses and helminths. A total of 149 countries with over a billion people have been plagued with these class of diseases (Hotez & Kamath, 2009). Because they are communicable diseases, poor sanitation increases the rate of spread leading to heavy economic burden on governments. They are mostly referred to as "neglected" because they have largely been eradicated from most of the first world countries and they persist only among heavily impoverished countries. People who are affected by this array of diseases are poor and marginalized with some living in conflict-prone zones. Affected people are unable to work for long periods of time and this further leads to unemployment and family problems.

Another reason why they are classified as neglected is the fact that research and funding for research into drug and vaccine discovery for these diseases is very low and investors do not see it as a viable enterprise mainly because the targeted group will not be able to afford the drugs even if they were manufactured and made available. Some of the diseases, however, have had some substantive work done on them and some vaccines and drugs exist for their treatment and control. **Table 1.1** below summarizes a list of NTDs, a brief description of them, their treatment and mode of transmission.

Table 1.1: List of 20 Neglected Tropical Diseases.

Disease	Short description	Mode of transmission	Treatment available	Source
Buruli Ulcer	A chronic debilitating disease that affects the skin, bones and can lead to permanent disfigurement and disability	No known mode of transmission. Caused by *Mycobacterium ulcerans* that produce mycolactone	Treated with regular anti-biotics such as Rifampicin and streptomycin	(Nakanaga et al., 2013)
Chagas Disease	A tropical parasitic disease that causes severe fever, swollen lymph nodes and headaches	It is spread by an insect known as Triatominae through its bites	Can be treated with Benznidazole, and nifurtimox	(Rassi, Rassi, & Marcondes de Rezende, 2012)
Cysticercosis	A painful infectious disease that causes swellings in the brain	Caused by young form of pork tapeworms	No known medication but can generally be treated by anti-biotics	(García, Gonzalez, Evans, Gilman, & Cysticercosis Working Group in Peru, 2003)
Dengue fever	A mosquito–borne tropical disease caused by dengue virus.	By female mosquito mainly the *Aedes aegypti* and *Albopicto.*	Supportive care, intravenous fluids, blood transfusion. Has no known drugs but only vaccines.	(Kularatne, 2015) (WHO 2012)
Dracunculiasis (Guinea worm disease)	An infection that comes about when a person drinks water containing copepods infected with guinea worm larvae.	Guinea worms spread by water fleas	Supportive care	(Cairncross, Tayeh, & Korkor, 2012)

Echinococcosis	A parasitic disease that affects the alveoli and brains	Humans are infected through ingestion of parasite eggs through contact with animal host	Surgery and general anti-biotics (abendazole)	(GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016)
Fascioliasis	A parasitic worm infestation disease which causes abdominal pain, anemia, jaundice, and urticaria.	The liver fluke mainly attacks cattle and sheep but also humans	It is mainly treated with traclabendazole	(Mas-Coma, Bargues, & Valero, 2005)
Human African Trypanosomiasis (Sleeping sickness)	It is a vector–borne parasitic disease that manifests in fever, swollen lymph glands, blood in urine and aching muscles	Transmitted to humans through infected tsetse fly bites which have acquired their infection from other infected humans and animals	Can be treated with pentamidine or suramin through intravenous injection	(Maya et al., 2007)
Leishmaniasis	It is a parasitic disease which causes skin ulcers on the hands, mouth and nose	It is caused and spread by sandflies infested with leishmania	Can be treated with liposomal amphotericin B	(Sundar & Chakravarty, 2013)
Leprosy (Hansen's disease)	It is a chronic infectious disease that affects the nerves, respiratory tracts and eyes	It is caused and transmitted by *Mycobacterium leprae.* spread through cough and contact with nasal discharge from an infected person	Can be treated with multi drug therapy (MDT) using rifampicin, dapsone and clofazimine	(Rassi et al., 2012)
Lymphatic filariasis (Elephantiasis)	Also known as Elephantiasis. It causes severe swelling of the	It is caused by microscopic parasitic	Can be treated with abendazole	(GBD, 2016)

	arms, legs, breasts or genitals in some cases.	filarial worms and it is spread by mosquitoes		
Onchocerciasis	Also known as river blindness and is a parasitic worm infestation that causes itching, skin nodules, disfiguring skin and blindness	It is caused by *onchocerca volvulus* through repeated bites of blackflies.	Can be treated with Ivermectin and Doxycycline	(GBD, 2016)
Rabies	A vaccine-preventable infection from dogs to humans which causes headache, excessive salivation, muscle spasms and mental confusion	Dogs infected with rabies transfer the disease to humans through bites	Immediate washing of wound with soap and vaccination of dogs	(WHO, 2017)
Schistosomiasis	An acute and chronic parasitic disease that causes abdominal pain, diarrhea and blood in stool.	People get infected when they come in contact with water infested with fluke (trematode)	Can be treated with praziquantel	(WHO, 2017)
Soil transmitted Helminths (Hookworm, whipworm)	An intestinal disease caused by roundworms found in soil contaminated with fecal matter	Caused by worms found in fecal matter	Mass treatment with drugs and surgery	(Ziegelbauer et al., 2012)
Trachoma	A bacterial infection that causes blindness by roughening the inner surface of the eyes	Caused by *Chlamydia trachomatis* which can easily spread in crowded dwellings	Surgery	(Evans & Solomon, 2011)

Yaws	An infection that affects the skin, bones and joints	It caused by *Treponema pollidum*	Azithromycin, benzanthine penicillin	(Mitjà, Asiedu, & Mabey, 2013)
Chikungunya	A viral disease which manifests in fever, and severe joint pain.	Transmitted from human to human by mosquitoes.	No known medicine or vaccines. Symptoms are treated with anti-viral.	(WHO, 2017)
Foodborne trematodiases	An infection that causes severe liver and lung diseases often resulting in disability and death.	Caused by trematode worms and affects humans when they consume raw or uncooked fish, crustaceans and vegetables that harbour the larval stage of the parasite.	No particular known medication. Patients are treated individually.	(WHO, 2017)
Snakebite Envenoming	250 of the more than 3,000 species of snakes that exist are considered dangerous to human and they live in over 160 countries causing over 81,000 deaths annually through venomous bites	Caused by the venom of a snakes	Tetanus vaccine or the use of antivenom to counter the snake bite symptoms	(Healthline, 2018)

The World Health Organization (WHO) has set up a roadmap towards the complete elimination and eradication of two NTDs by the year 2020. In 2017, the Strategic and Technical Advisory Group for Neglected Tropical Diseases added three more diseases to make a total of 20 NTDs as listed in **Table 1.1** (Hotez, 2017). Malaria and tuberculosis are however no longer classified as

neglected tropical diseases because they affect people of all continents, albeit in different proportions but remain the persistent tropical diseases that continue to claim lives across Africa, Asia and Latin America.

Tuberculosis and malaria have been around for millennia and their eradication and treatment have been numbered among the major health issues in most African countries and other parts of the world where they are endemic. In Ghana and other African countries, they are the commonest diseases known to cause increase in morbidity and mortality rates of infants, women and children under 5 years (Vector, Disease, Progamme, & Marg, 2008). These diseases have also been found to be endemic in both tropical and Southeast Asia. Whereas HIV and malaria have been concentrated in Africa in most cases (>80 % incidence), tuberculosis has mainly been concentrated in Asia and Southern Africa. Most of the countries that have suffered significantly from these diseases include low and middle-income nations. Notwithstanding, some developed countries have also received their fair share of the disease burden. Globally, tuberculosis alone is responsible for 1.3 million deaths annually. Ghana is among the top 30 countries with high TB/HIV burden (WHO, 2016). Six (6) of every 1000 deaths in Ghana is due to HIV/TB infection (WHO, 2016).

Although there has been some improvement in the rate of mortality of malaria, much room still remains for improvement. For example, the rate of child deaths from malaria since 2004 in sub-Saharan Africa has reduced from a stunning 44.1 % to 15.7 % (Murray et al., 2014). Generally, the incidence and rate of mortality of malaria, tuberculosis and HIV/AIDS has been on the decrease globally since 1990 due to the pragmatic efforts of all stakeholders in the health industry.

1.2 **Malaria**

Malaria is a parasitic disease and is caused by five (5) known parasites namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi.* All five known *Plasmodium* parasites affect humans in different regions of the world. For example, malaria in Africa is caused mainly by the *Plasmodium falciparum* whereas malaria in Asia is mainly caused by *Plasmodium vivax.* The main vector for the transmission of the disease from person to person is the female *Anopheles* mosquito (**Figure 1.1**).

Figure 1.1: A Mature *Anopheles* Mosquito (source - Tactical Mosquito Control website)

There are about 400 different known species of mosquitoes of which about 30 are of biological importance (WHO, 2017). The three main types of mosquitoes found in Ghana are the *culex, Aedes* and the *Anopheles* which is the vector for the malaria parasite. The disease can be transferred from an infected person to a healthy person through the bite of the mosquito mostly during the night. The initial symptoms include fever, headache, and chills which may be mild and difficult to recognize. Severe anaemia is also a common symptom in children. If not treated, this develops into severe illness often leading to death (Sánchez, Patterson, & Ahmed, 2012).

In 2015, an estimated 216 million cases were recorded which resulted in 445,000 deaths; most of these cases occurred in sub-Saharan Africa (Moss, Shah, & Morrow, 2017). Children under five years, infants and pregnant women were the most affected. Between 2000 and 2016, the WHO estimated that the mortality rate of malaria has dropped by 29 % and case incidence by 21 % globally. This feat was achieved because of the scale up of the diagnostic and preventive measures and interventions put in place to help curb the disease (WHO, 2017).

An estimated 3.2 million people are at risk of being infected with malaria annually. The year 2016 alone recorded nearly half a million deaths. The WHO's Global Technical Strategy for malaria control and eradication has set a target to eliminate malaria in at least 10 countries by 2020, 20 countries by 2025 and 30 countries by 2030. According to the classification of malaria infection by WHO in 2016, 88 % of all cases occurred in Africa, 10 % in Southeast Asia and 2 % in the Mediterranean region. India is the country with the most malaria cases in Southeast Asia. **Figure 1.2** shows the global distribution of malaria for 2016 (Bloland, 2001).

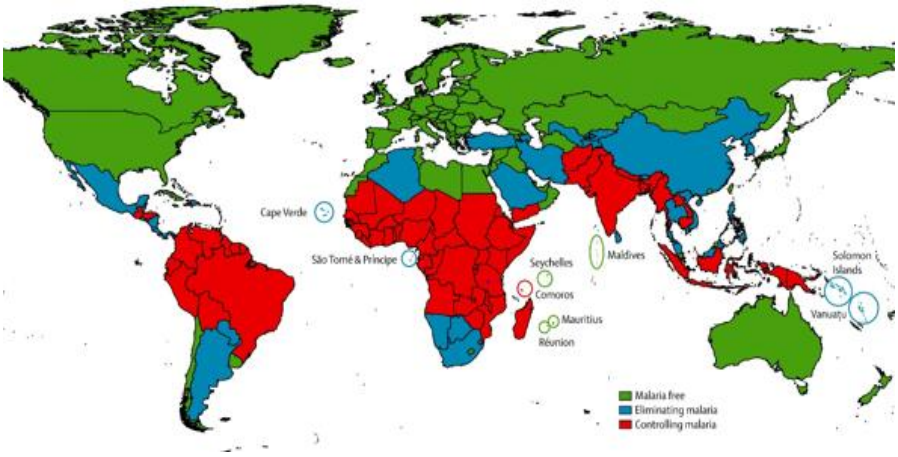


Figure 1.2: Global distribution of malaria for 2015

1.2.1 Malaria in Ghana

Malaria is currently the leading cause of morbidity in Ghana. In 2009 for example, malaria accounted for 32.5 % of all Out Patient Department (OPD) cases and also 48.8 % of all children under five admissions in the entire country (UNICEF Ghana Fact sheet, 2009). Attempts have been made over the years to control the disease since the early 1950s. In an attempt to reduce the incidence of the disease, interventions such as the application of insecticides, chemoprophylaxis using pyrimethamine medicated salts and also improving the drainage systems were employed. In 1999, the National Malaria Control Programme (NMCP) set out to reduce malaria incidence to 50 % by the year 2010 using the Roll Back Malaria (RBM) initiative (Ghana Health Services, 2017). This initiative was to be achieved by inter-sector partnerships which was geared towards treatment and prevention strategies by making information more readily available to the general public. Strategies employed to reduce the occurrence of malaria include; improvement of malaria prevention by intensified education and usage of treated insecticide nets, and evaluation, improving in-service training to health service personnel among others. All these interventions culminated into the reduced incidence. Funding strengthening monitoring for malaria prevention and treatment in Ghana is mainly sponsored by the Government (Ghana Health Services, 2017). The NMCP also receives direct financial and logistics support from the Global fund for Malaria, TB and HIV/AIDS. It also partners with WHO, UNICEF, USAID/PMI DFID, etc. (Abdul-Fatawu Adam Wunizoya, 2016).

It has been reported that the prevalence of malaria in Ghana has reduced from 50 % in 2012 to 20.4 % in 2016 as a result of the strategies employed (Ghana Health Services, 2016). To date, Northern region continues to be the region with most cases of malaria occurrence.

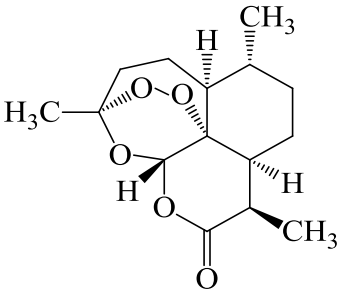
1.2.2 Treatment and Control measures

Malaria prevention is the most cost-effective way to reduce the rate of infection. Preventive mechanisms include early detection. The main diagnostic tools are the Rapid Diagnostic Tests (RDT) and slide test (Vector et al., 2008).

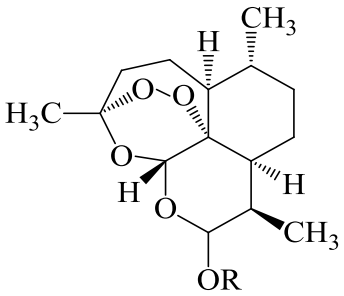
The main methods adopted by the WHO for the prevention of malaria are the use of treated mosquito nets (Long-Lasting Insecticidal Net, LLIN), indoor residual spraying and chemoprophylaxis (WHO, 2017). There are several available medications for the treatment of malaria. Some of the medications include chloroquine, artemether-lumefantrine (Coartem), artesunate-amodiaquine (Amonate), artesunate-mefloquine, atovaquone, quinine and several others (**Figure 1.3**). Most of these drugs are available as tablets and pills but patients who are unable to take drugs orally may have to resort to intravenous treatment options. Choosing a drug depends on the type of parasite causing the disease, region where the malaria infection was acquired, drug resistance history, age and sex (Shanks, 2016). In most sub-Saharan countries of Africa, chloroquine is no longer the drug of choice in treating malaria (Douglas, Anstey, Angus, Nosten, & Price, 2010). This is because the *P. falciparum* parasite has become resistant to chloroquine. It is, however, the drug of choice for treating *P. vivax* and *P. ovale* malaria (Malaria consortium, 2017) (Douglas et al., 2010).

The best available treatment for malaria, especially *P. falciparum* malaria however is the Artemisinin-based Combination Therapy (ACT) (Iwalokun, Udoh, & Balogun, 2017). ACTs are drugs made by combining fast acting artemisinin-based compounds (derivatives) with other drugs such as lumifantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone (Malaria consortium, 2017.) (**Table 1.2**). They are known to be effective

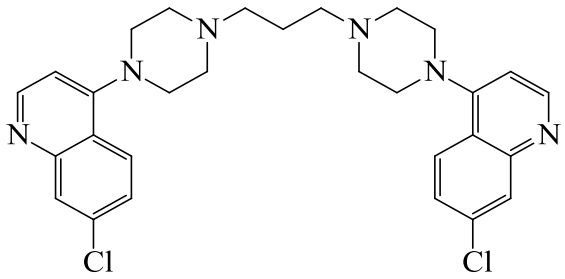
in completely treating patients with full blown malaria coupled with their ability to delay and prevent resistance (Douglas et al., 2010).



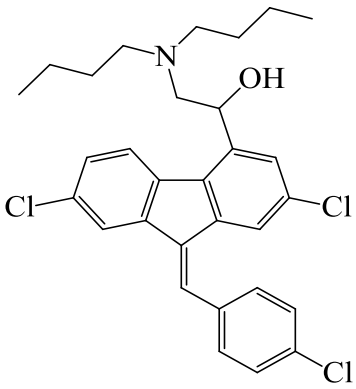
1, Artemisinin



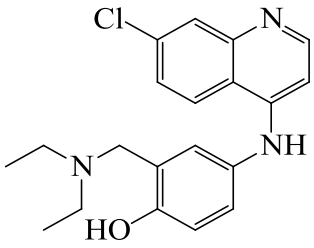
2, Dihydroartemisinin, R = H
3, Artemether, R = CH_3
4, Arteether, R = CH_2CH_3
5, Artesunate, R = $COCH_2CH_2COOH$



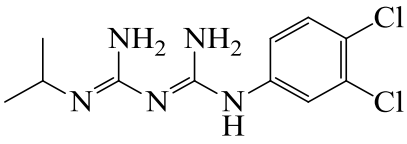
6, Piperaquine



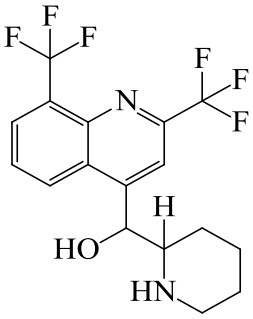
7, Lumifantrine



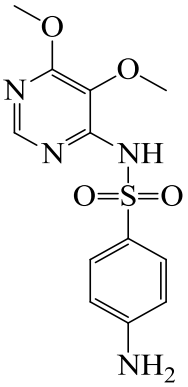
8, Amodiaquine



9, Chlorproguanil



10, Mefloquine



11, Sulfadoxine

Figure 1.3: Selected medications for the treatment of malaria

Table 1.2: Artemisinin-based Combination Therapy

Artemisinin Combination	**Trade name**
Artesunate-Amodiaquine	Camoquine
Artemether-Lumefantrine	Co-artem, Riamet
Artesunate-Mefloquine	Lariam, Mephaquine
Artesunate-Pyronaridine	Pyramax
Dihydroartemisinin-Piperaquine	Dihydroqinghaosu, Artenimol
Artesunate-Chlorproguanil-Dapsone	Lapdap + Artesunate

1.2.3 Resistance to Antimalarials

A parasite is said to have developed resistance towards a particular drug if "the drug gains access to the parasite for a time duration within which it is supposed to act and yet the parasite multiplies despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance level of the drug" (Wangdi *et al.*, 2016).

Of the five known human malaria parasites, two of them *P. falciparum* and *P. vivax* have developed resistance towards various antimalarial drugs. Some factors that account for the spread of drug-resistant malaria include:

1. Unusual genetic structure of malaria parasites in regions known for antimalarial drug resistance.
2. Counterfeit or substandard treatments.
3. Unregulated or poorly administered antimalarial drugs.

4. Artemisinin-based drug use without a complementary combination treatment, such as lumefantrine (Hanboonkunupakarn & White, 2016) (UNICEF Ghana Fact sheet, 2007).

Chloroquine was the first to lose its efficacy against *P. falciparum.* Chloroquine was discovered in 1934 by a German scientist in his quest to replace quinine which was used as an anti-fever drug from the bark of *Remijia sp* (Prevention, 2017). It was initially called rosochin and was used as an antimalarial by British and U. S. scientists in 1946 during the Second World War. Resistance to chloroquine was first observed in the Thai-Cambodian border in the year 1957 and later in Colombia and Venezuela in 1960. Currently *P. falciparum* has developed resistance to chloroquine in all known endemic regions and in some cases *P. vivax* as well (Venture, 2018). The underlining reason of *P. vivax* resistance to chloroquine is not known. (White, 2004). *P. falciparum* malaria is currently being treated successfully by the use of the ACTs in many endemic regions. The problem with ACTs in recent times is the surge in resistance observed in four countries namely Cambodia, Myanmar, Thailand and Vietnam (Birkett, 2016). *P. falciparum* resistant strains are treated with high dose of mefloquine and artesunate for three days or artesunate alone for seven days.

Chloroquine is still used to treat uncomplicated *P. vivax* malaria while primaquine is used to eliminate the liver stage of the parasite and also prevent relapse. Very recently, *P. vivax* has also developed resistance to primaquine in some regions. These, in general, pose a threat to the global effort to eliminate malaria by the year 2030.

Sulfadoxine/pyrimethamine, a fixed dose combination is increasingly being relied upon as a replacement for chloroquine in the treatment of *P. vivax* malaria. A combination of quinine and tetracycline or mefloquine is used to treat *P. vivax* resistant strains. A combination of primaquine and chloroquine is used to treat chloroquine-resistant *P. vivax* malaria.

Antimalarial drug resistance can be prevented when pragmatic efforts are put in place to ensure that correct medications are issued, proper diagnostic tests are performed, and patients are monitored to ensure adherence to prescriptions.

1.2.4 Vaccine development

There has been a steady increase in the research to establish a vaccine for malaria. Vaccines are needed that target the different developmental stages of the *plasmodia*. Because *P. falciparum* is mainly concentrated in Africa and *P. vivax* has a wider geographical distribution, there will be the need to design specific vaccines to tackle them both since there is no homology between them (Birkett, 2016).

The focus in recent years has been an increase in the development of vaccines that prevent transmission of the disease rather than treating clinical cases. In October 2015, RTS,S/AS01 (RTS,S) a recombinant protein-based malaria vaccine completed clinical trials and was recommended to be a complementary, preventive, diagnostic and treatment vaccine. RTS,S was to be rolled out in November 2016 on a pilot project in three selected countries in Africa after funding has been sorted. The countries include Ghana, Kenya and Malawi (WHO, 2016).

1.3 **Tuberculosis**

Tuberculosis (TB) is a bacterial disease and is caused by *Mycobacterium tuberculosis*. It is one of the important world health threatening diseases. It mostly affects the lungs (pulmonary TB) and other parts of the body as well (extra-pulmonary TB). Extra-pulmonary TB affects the brain, spines or even the kidney and may manifest in different ways (Mitjà et al., 2013). Without proper treatment, TB can be fatal. Tuberculosis is an air-borne disease and therefore spreads rapidly.

When an infected person coughs, sneezes or spits, aerosols of the bacilli are propelled and carried around. A healthy person needs to inhale only few droplets of these germs to get infected with the disease. Of the scores of people exposed to the TB bacilli, only about 10 % get infected with the bacteria (Breman & Brandling-Bennett, 2011) . The first stage of infection is called the latent stage. At this stage, an infected person is not visibly sick with the disease and cannot transmit it to others. Only about 10 % of persons infected with latent tuberculosis progress to develop the full-blown disease. The most common symptom of lung TB is prolonged cough (over three weeks), chest pain, weakness, weight loss, fever and night sweat. More likely than not, people with compromised immune systems are at a higher risk of developing the disease. Of the 10 % that get infected, some can treated and they recover fully and some also die notwithstanding while some also develop complications like the drug-resistant form of the disease (WHO, 2016). Complications may result in patients with a history of tobacco use, malnutrition, diabetes, or HIV. For those who never get treated, most of them die within two years and some just survive although no treatment was administered.

Paediatric TB is another problem the WHO is battling with. It is difficult to detect TB in children left alone resistant forms of it. Multi Drug Resistant TB (MDR-TB) can be transmitted from adults to other adults and also to children.

The main diagnostic tool for TB is the sputum microscopy test. This method involves careful observation of the sputum of a patient under the microscope for the presence of the bacteria. The greatest disadvantage of this method for many decades was its inability to detect the presence of MDR forms of TB. It is also able to detect only about half the number of TB bacteria present. The development of the Xpert MTB/RIF® assay helped to detect the resistant forms of it. Until 2016, only the Xpert MTB/RIF assay has been successful at diagnosing and detection of MDR-TB in

children. Almost in all cases, the detection of pediatric cases of MDR–TB in households with an adult suffering from MDR–TB was a strong indication of the possible transmission of MDR-TB to children. Most TB programs are reluctant to provide complex treatment regimen to children suffering from MDR-TB although there is the possibility of an excellent outcome. (Outhred, Britton, & Marais, 2017).

In general, *M. tuberculosis* is a tenacious pathogen. Even when the primary infection is contained, the bacteria within the granuloma can survive for decades, persisting in a special dormant state (Fitzgerald & Musser, 2001). When the immune system is compromised by such factors as malnutrition, HIV infection, diabetes, renal disease, chemotherapy, or extensive corticosteroid therapy, reactivation of the disease can occur (Alexander & Liu, 2006). The protective granuloma disintegrates, and the long dormant *M. tuberculosis* revives and spreads unchecked (Alexander & Liu, 2006).

1.3.1 Tuberculosis in Ghana

In 2016, 156 of every 100,000 OPD cases were TB-related cases (WHO, 2018). In 2017, the country recorded 14,550 new cases of TB against the 15,606 cases in 2013. The Eastern and Western regions continue to hold the highest incidence of cases in the country. The Ghana Society for the Prevention of Tuberculosis was established in 1954 to champion government efforts to combat the menace of TB. Government subsequently sent out nurses on sponsorship basis to Israel to be educated on mass TB screening. The National TB control Programme (NTP) was established in 1992 to help with ongoing works on eradication and control of TB in the country. Ghana enrolled on the WHO Directly Observed Treatment, Short course (DOTS) strategy which was based on

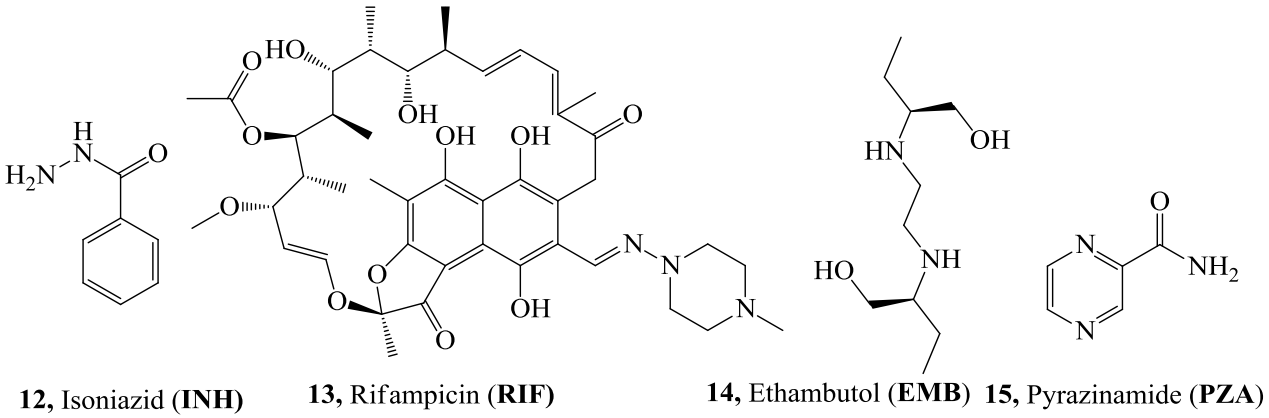
standard diagnosis, treatment, surveillance, drug supply, and recording system. Ghana achieved 100 % DOTS coverage in the year 2000 (WHO, 2001).

1.3.2 Treatment.

TB is treatable and preventable. The prevalence rate of TB has dropped drastically in this decade. Over 51 million people have been treated successfully in over 47 countries that have adopted the WHO strategy towards eradication of TB since 1995 (Schito, Hanna, & Zumla, 2017). TB treatment is complicated and requires the use of a combination of various drugs. The effective treatment of TB with the frontline drugs require several months of chemotherapy to eliminate the persistent bacteria (Chetty, Ramesh, Singh-Pillay, & Soliman, 2017). No new drugs have been introduced for more than 50 years after the discovery of ethambutol ('Global tuberculosis report WHO Library Cataloguing-in-Publication Data'). Resistance to existing antibiotics, with the subsequent emergence of Multi-Drug Resistant mycobacteria strains, together with an increasing economic burden, has urged the development of new anti-TB drugs (Chetty et al., 2017).

Streptomycin was the first anti-tuberculosis agent to be discovered. The first large scale clinical trial of the drug was performed in 1948 (Chetty et al., 2017). This led to the subsequent release of thiacetazone and para-aminosalicylic acid (PAS), onto the market (Briggs et al., 1968). The combined use of both drugs recorded high successful treatment rates and reduced propensity for antibiotic resistance. However, the vast appearance of drug-resistance to streptomycin in later times lead to the design, discovery and development of new anti-TB drugs (Coreil & Dyer, 2017). These include: isonicotinic acid (isoniazid-INH) – discovered in 1951, pyrazinamide (PZA) and cycloserine – 1952, ethionamide – 1956, rifampicin (RIF) – 1957, and ethambutol (EMB) – 1962 (E. White, 1989).

The current frontline drugs used for the treatment of the disease are Isoniazid (**INH**) Rifampicin (**RIF**), Pyrazinamide (**PZA**) and Ethambutol (**EMB**) (**Figure 1.4**).



12, Isoniazid (**INH**) **13,** Rifampicin (**RIF**) **14,** Ethambutol (**EMB**) **15,** Pyrazinamide (**PZA**)

Figure 1.4: Frontline Drugs for the Treatment of Tuberculosis

These are the mainstay for treating tuberculosis for the first six to nine months after diagnosis. The first two months of treatment is called the **intensive phase** and all four frontline drugs are used in combination. The next four months of treatment is called the **continuous phase** and is treated with only **INH** and **RIF** (Ying Zhang, 2005).

In many cases, the bacteria become resistant to the frontline drugs when the prescribed regimen and dosage is not followed to the letter. This occurs when patients stop the course of treatment either due to cost of purchasing the drugs or when they no longer experience pronounced expression of the symptoms. Below are second line drugs for treating the drug resistant forms of TB. They have been sub divided into three:

i. Fluoroquinolones- Ofloxacin (**OFX**), levofloxacin (**LEV**), moxifloxacin (**MOX**) and ciprofloxacin (**CIP**).

ii. Injectable anti-tuberculosis drugs- Kanamycin (**KAN**), amikacin (**AMK**) and capreomycin (**CAP**).

iii. Less-effective second-line anti-tuberculosis drugs- Ethionamide (**ETH**)/Prothionamide (**PTH**), Cycloserine (**CS)/**Terizidone, P-aminosalicylic acid (**PAS**). (Nath & Ryoo, 2012)

In case the bacteria develop resistance to the second line drugs, as has been observed in some cases, there are third line drug regimen that could be used. These drugs include Rifabutin, Macrolides such as clarithromycin (**CLR**), Linezolid (**LZD**), Thioacetazone (**T**), Thioridazine, Arginine, Vitamin D, R207910. ("Third Line Drugs for Tuberculosis | Tuberculosis Treatment," 2012). The success rate for complete healing is between 30 to 80 % and it takes over two years of treatment (Franz et al., 2017). All these drugs are not to be used in isolation but as a combination therapy. Simply put, treatment of TB is complicated. The duration of treatment is crucial.

Recently, there have been reports of few cases in which patients have been resistant to all available anti-TB drugs, known as Tertiary Drug Resistant-TB (TDR-TB). Cases of TDR-TB resistance have become prevalent in China, India, Africa and Eastern Europe (Xiang et al., 2011)**.**

1.3.3 Mode of Action

Due to the complexity associated with the TB bacilli, different drugs are used to target different stages of its life cycle. Some drugs are administered as prodrugs and are only activated by the enzymes specific to TB.

1.3.3.1 Isoniazid

M. tuberculosis is highly susceptible to INH (MIC 0.02–0.2 μg/mL). INH is only active against growing tubercle bacilli, and is not active against non-replicating bacilli or under anaerobic conditions. INH enters the mycobacterial cell by passive diffusion. The most significant adverse reactions associated with isoniazid administration are hepatotoxicity and neurotoxicity. INH is a prodrug that is activated by the mycobacterial enzyme KatG. Activated INH inhibits the synthesis of essential mycolic acids by inactivating the NADH-dependent enoyl-acyl carrier protein reductase encoded by *inhA* (Chan & Iseman, 2002).

1.3.3.2 Rifampicin

Rifampicin (RIF) was introduced in 1957 as an anti-tuberculosis drug and has excellent sterilizing activity. RIF acts by binding to the *β*-subunit of RNA polymerase (*rpoB*) the enzyme responsible for transcription and expression of mycobacterial genes, resulting in inhibition of the bacterial transcription activity and thereby killing the organism. An important characteristic of RIF is that it is active against actively growing and slowly metabolizing (non-growing) bacilli (Chan & Iseman, 2002). Hepatotoxicity occurs less frequently than with isoniazid administration. RIF MICs range from 0.05 to 1 μg/mL on solid or liquid media, but the MIC is higher in egg media (MIC = 2.5–10 μg/mL).

1.3.3.3 Pyrazinamide

One key characteristic of pyrazinamide (PZA) is its ability to inhibit semi-dormant bacilli residing in acidic environments (Mitchison, 1979). Pyrazinamide is a structural analogue of nicotinamide

and is a pro-drug that needs to be converted into its active form, pyrazinoic acid, by the enzyme pyrazinamidase/nicotinamidase (PZase). PZA is only active against *M. tuberculosis* at acid pH (e.g., 5.5). MIC for PZA at pH 5.5 is 50.0 μg/mL. Hypersensitivity reactions and gastrointestinal upset may occur with PZA administration.

1.3.3.4 Ethambutol

Ethambutol (EMB) plays an important role in the chemotherapy of drug-resistant TB. EMB is also an important antimycobacterial drug as it enhances the effect of other companion drugs including aminoglycosides, rifamycins and quinolones. The most common side effects observed with EMB are dizziness, blurred vision, color blindness, nausea, vomiting, stomach pain, loss of appetite, headache, rash, itching, breathlessness, swelling of the face, lips or eyes, numbness or tingling in the fingers or toes. Patients taking EMB should have their visual acuity and color vision checked at least monthly. The MICs of EMB for *M. tuberculosis* are in the range of 0.5–2 μg/mL. EMB is a bacteriostatic agent that is active for growing bacilli and has no effect on non-replicating bacilli. EMB interferes with the biosynthesis of cell wall arabinogalactan. It inhibits the polymerization of cell-wall arabinan of arabinogalactan (Nath & Ryoo, 2012).

1.3.4 Drug resistance

The treatment of TB can fail which then leads to the drug resistant form. A patient suffering from drug resistant form of TB has essentially developed resistance towards the first-line TB drugs, most importantly Isoniazid and Rifampicin (Franz et al., 2017). The main cause of this condition is the noncompliance of the patient to strict regimen, poor quality of drugs and incorrect

prescription by health care providers. This condition can be treated with WHO approved second line drugs. In the event that the second line drugs also fail, there are few third line drugs used as a combination therapy. The recovery rate of patients is usually very slow and most do not make it through the therapy. There is also the possibility of the patients developing Extensive Drug Resistant TB (XDR-TB) which is a more serious form of the MDR-TB. The patient at this stage is left with no further treatment options as yet. Bedaquiline and Delamanid have recently been approved to treat MDR-TB (Ben-Kahla & AL-Hajoj, 2016) (Schito et al., 2017) (Hong et al., 2017).

1.3.5 Co-Infection of Tuberculosis with HIV

With all the achievements made so far in battling the disease, the burden of TB still remains on the high side. Patients who have been co-infected with HIV have had the worst hits. In 2011, of the estimated 8.7 million new cases recorded, 13 % were co-infected with HIV of which 1.4 million died due to TB. Also in 2013, there was a total of 1.5 million deaths out of the 9 million new cases recorded. Half a million of these new cases also had HIV infection concurrently (Chetty et al., 2017). The continual persistence of TB lies in factors such as the spread of HIV, emergence of drug resistant strains, reinfections and failure of public health programs. Over half a million of all cases reported in 2015 were Multi drug resistant cases. The patients were resistant towards the two frontline drugs, rifampicin and isoniazid. Most of these cases were recorded in India. The top seven countries with the highest TB burden according to the WHO 2016 report are India, Indonesia, China, Nigeria, Pakistan, South Africa and Bangladesh (WHO, 2016).

Because there are no vaccines currently available to be administered as prophylactics, people living in endemic communities are at a greater risk of contracting the disease (Ben-Kahla & AL-

Hajoj, 2016). Also, the high cost of treatment, coupled with the long duration of treatment also eventually culminates in the continual prevalence of the disease.

Because HIV weakens the immune system, persons suffering from HIV who contract Malaria and TB are at a greater risk of losing the fight for their lives due to their already weakened immune systems. Altogether, HIV, TB and malaria kill approximately 5 million people a year globally. This ought not to be because this fight can be won. Current advances in drug medications make it possible to extend the lives of those infected with HIV, while TB and malaria are both curable and preventable. In China for example, about 30,000 deaths were averted by the adoption of the WHO's DOTS programme (Hotez, 2015). New infection of HIV can also be reduced, if not averted, if the proper mechanisms which are already available are appropriately used.

1.3.6 *Mycobacterium tuberculosis* and the Genus *Mycobacterium*

The genus *Mycobacterium* comprises more than 70 species. A few, notably *M. tuberculosis*, *M. leprae*, *M. bovis* and *M. ulcerans* cause significant morbidity and mortality. Others, including *M. kansasii*, *M. fortuitum*, *M. abscessus*, *M. xenopi*, *M. chelonae*, and the *M. avium* complex are responsible for occasionally lethal opportunistic infections. However, the vast majority are harmless environmental organisms, common in water and soil. Under the microscope, mycobacteria are small, rod-shaped bacteria. They are Gram-positive organisms but are best distinguished by their characteristic acid-fast staining. This acid-fastness is a property of the mycobacterial cell wall, an unusual, lipid-rich structure that forms a hydrophobic, low permeability barrier and provides innate protection against many antimicrobial agent (Pagel & Pomiankowski, 2002).

1.3.6.1 Genomics study of *Mycobacterium tuberculosis*

Genomics is the study of the molecular organization of genomes, their information content and the gene products they encode. *M. Tuberculosis* genome is one of the largest, exceeded only by *E. coli* and *Pseudomonas aeruginosa.* Using bio-informatics, more than 3,920 genes of *M. tuberculosis* have been identified that encode proteins more than 80 amino-acids in length. (Pagel & Pomiankowski, 2002). The combination of genomics and bioinformatics has the potential to generate the information and knowledge that will enable the conception and development of new therapies and interventions needed to treat this airborne disease and to elucidate the unusual biology of its etiological agent, *Mycobacterium tuberculosis* (Wengenack et al., 1998).

The complete genome sequence of the best-characterized strain of *M. tuberculosis*, **H37Rv,** has been determined and analyzed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new therapeutic interventions. The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine + cytosine content that is reflected in the amino-acid biased content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation (Cole et al., 1998).

Figure 1.5*:* Represents the complete genome of *M. tuberculosis* (H37Rv). The outer circle shows the scale in Megabase (Mb), with 0 representing the origin of replication. The first ring from the exterior denotes the positions of stable RNA genes (tRNAs are blue, others are pink) and the direct repeat region (pink cube); the second ring inwards shows the coding sequence by strand (clockwise, dark green; anticlockwise, light green).

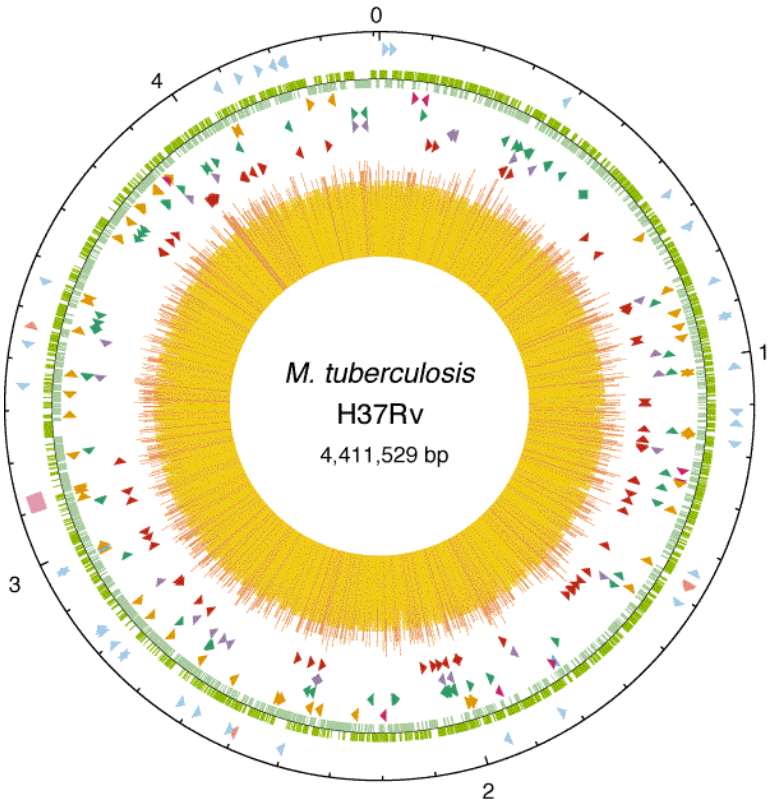


Figure 1.5: The genome of *Mycobacterium tuberculosis*

The third ring depicts repetitive DNA (insertion sequences, orange; 13E12 replication-associated protein (REP) family, dark pink; prophage, blue); the fourth ring shows the positions of the pseudoproxy experiment (PPE) family members (green); the fifth ring shows the PE family members (purple, excluding PGRS); and the sixth ring shows the positions of the (polymorphic G + C-rich sequences) PGRS sequences (dark red). The histogram (center) represents G + C content, with, 65 % G + C in yellow, and 65 % G + C in red.

The figure was generated with software from DNASTAR. DNASTAR is a global bioinformatics software company incorporated in 1984 that is headquartered in Madison, Wisconsin. (DNASTAR develops and sells software for sequence analysis in the fields of genomics, molecular biology, and structural biology) (Schwei, 2017).

It is thought that the progenitor of the *M. tuberculosis* complex, comprising *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum* and *M. microti*, arose from a soil bacterium and that the human bacillus may have been derived from the bovine form following the domestication of cattle. The complex lacks inter strain genetic diversity, and nucleotide changes are very rare. This is important in terms of immunity and vaccine development as most of the proteins will be identical in all strains and therefore antigenic drift will be restricted. On the basis of the systematic sequence analysis of 26 loci in a large number of independent isolates, it was concluded that the genome of *M. tuberculosis* is either unusually inert or that the organism is relatively young in evolutionary terms (Sreevatsan et al., 1997).

In recent years, due to excessive antibiotic use, multidrug-resistant tuberculosis has become a serious public health threat in many countries and a major obstacle to disease control. Identifying the differences between drug-resistant and sensitive strains is of great help in drug resistance site identification. Toward this end, Zhang *et al* used two closely related strains, CCDC5079 and CCDC5180, to study the whole genome by the shotgun strategy (Zhang et al., 2011).

Whole genome sequencing (WGS) has been shown to provide a rapid and comprehensive view of the genotype of the organism, and thus enable reliable prediction of the drug susceptibility phenotype within a clinically relevant timeframe (Witney et al., 2016).

There are several problems associated with the currently available TB treatment. Firstly, the duration and complexity of treatment results in non-adherence to treatment. This leads to suboptimal response (failure and relapse), the emergence of resistance, and continuous spread of the disease (Volmink & Garner, 2007). Secondly, adverse events in response to anti-TB drugs are common and contribute to the problem of non-adherence (Volmink & Garner, 2007). Third, the increasing incidence of multidrug-resistant (MDR; resistance to at least rifampin and isoniazid)

and extensively drug-resistant (XDR; MDR resistance plus resistance to a fluoroquinolone and an aminoglycoside) TB is a serious concern. Resistant TB occurs in the presence of partially suppressive drug concentrations that enable replication of bacteria, the formation of mutants, and overgrowth of wild-type strains by mutants (selective pressure) (Chan & Iseman, 2002). Resistance to anti-tuberculosis drugs has been a problem since the era in which chemotherapy began. After dramatic outbreaks of MDR-TB in the early 1990s, resistance became recognized as a global problem. MDR-TB now threatens the inhabitants of countries in Europe, Asia, Africa, and the Americas (WHO, 2014). An understanding of the molecular basis of drug resistance may contribute to the development of new drugs.

Management of MDR-TB relies on prompt recognition and treatment with at least 3 drugs to which an isolate is susceptible. During the last decade, there has been a marked increase in the number and gravity of tuberculosis cases both in developing countries and in industrialized nations (Ahmed & Hasnain, 2004). The rise in tuberculosis (TB) incidence over the last two decades is partly due to TB deaths in HIV-infected patients and partly due to the emergence of multidrug resistant strains of the bacteria. Due to its slow growth and high virulence, it is extremely difficult to work with the TB bacterium (Ahmed & Hasnain, 2004).

1.4 **Problem Statement**

Both malaria and TB can be cured and prevented. The major problem with the current treatment of both diseases is the fact that both require a combination of two or more drugs for effective therapeutic results. Incidentally, the causative agent for both diseases has developed resistance to the all currently available drugs treatment options. TB bacilli is particularly notorious for developing resistance towards all known therapeutics agents due to the long duration of treatment.

There is therefore the need to develop more effective drug medications to replace the current ones.

1.5 **Overall Aim of the Project**

The rationale for the study is to synthesize more effective drugs for the treatment of malaria and TB based on MMV676444 template. Compound in the MMV676444 scaffold contain urea functionality and there is evidence in literature showing that urea containing compounds versatile and are reported to possess antimalarial and anti-tuberculosis activities among others.

The overall aim of this project is to synthesize possible potent anti-tubercular and antimalarial agents based on the MMV676444 template.

1.6 **Objectives**

- Design and synthesize a library of compounds based on the MMV676444 template.
- Characterize the synthesized compounds using NMR and other spectroscopic tools.
- Test them for *in vitro* anti-malarial and anti-tubercular activities.

1.7 **Justification**

The Medicine for Malaria Ventures (MMV) recently established the "pathogen box" which contains approximately 400 chemical compounds with activity against several diseases including tuberculosis, malaria, toxoplasmosis, dengue, helminthes and several others **Figure 1.6**. The aim

of establishing the pathogen box was to increase research activity towards finding new drugs for treating neglected diseases.

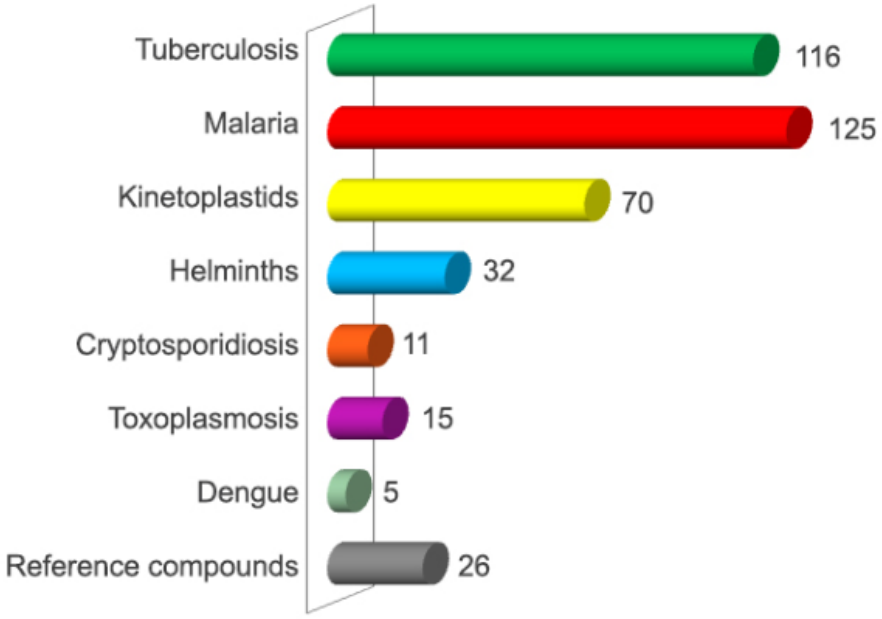
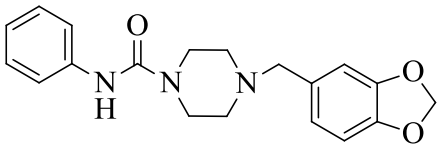


Figure 1.6: Number of Compounds with activity towards various diseases in the Pathogen Box

One of the compounds in the "pathogen box" from the MM676444 chemical scaffold **16** was found to possess antitubercular activity. The Minimum Inhibition Concentration (MIC) was determined for **16** against two strains of mycobacterium GAST/Fe and 7H9/ADC/Tw using Alarmar Blue assay over two weeks and the results obtained are shown in **Table 1.3**. It is anticipated that synthesis of analogues of MMV676444 could lead to the discovery of potent leads for development into drug candidates towards the treatment of malaria and tuberculosis.



16

Figure 1.7: Representative Lead compound MMV676444 in the pathogen box

Table 1.3: Anti - *Mycobacterium tuberculosis* activity data for MMV676444

1-week GAST/Fe* MIC (μM)	2-week GAST/Fe MIC (μM)	1-week 7H9/ADC/Tw** MIC (μM)	2-week 7H9/ADC/Tw MIC (μM)	2-week Alamar Blue 7H9/ADC/Tw MIC (μM)
0.8	1.2	>50	>50	12.5

* Glycerol-alanine-salts with Tween 80 and iron.
** **7H9 broth** is a liquid growth medium specially used for culture of *Mycobacterium* species; 7H9 containing 10% ADC (human gene) and 0.05% Tween 80

1.8 **Hypothesis**

Although the compounds from the MMV676444 scaffold was only found to possess antitubercular activity, it was hypothesized that they may have antimalarial activity as well because other carboxamides of similar structures have some antimalarial properties.

Two series of compounds will be synthesized (**Figure 1.7**). **Series 1** compounds will have urea motif as in **16** using already established synthetic methods. **Series 2** compounds will be devoid of the urea functionality to establish the importance of the urea functionality found in **Series 1** compounds.

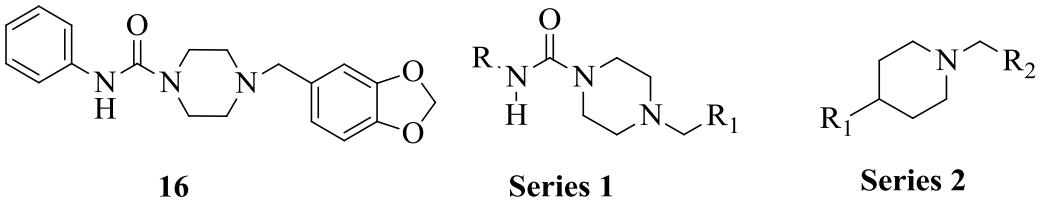


Figure 1.8: Chemical scaffolds to be synthesized

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 **Carboxamides**

Carboxamides are a class of organic compounds with the functional group R-CO-NR'R''. R', R'' may be hydrogen, an alkyl or an aryl substituent on the amide nitrogen (Fletcher *et al*, 1974). They are part of a sub-group under the major amide functional group. Most of the time, when amides are being described, they are generally referred to as carboxamides. Other examples of sub-functional groups under amides are the sulphonamides and phosphoramides. Carboxamides are able to undergo hydrogen bonding between the carbonyl oxygen with high electron density and the hydrogens attached to the nitrogen atom (**Figure 2.1)**.

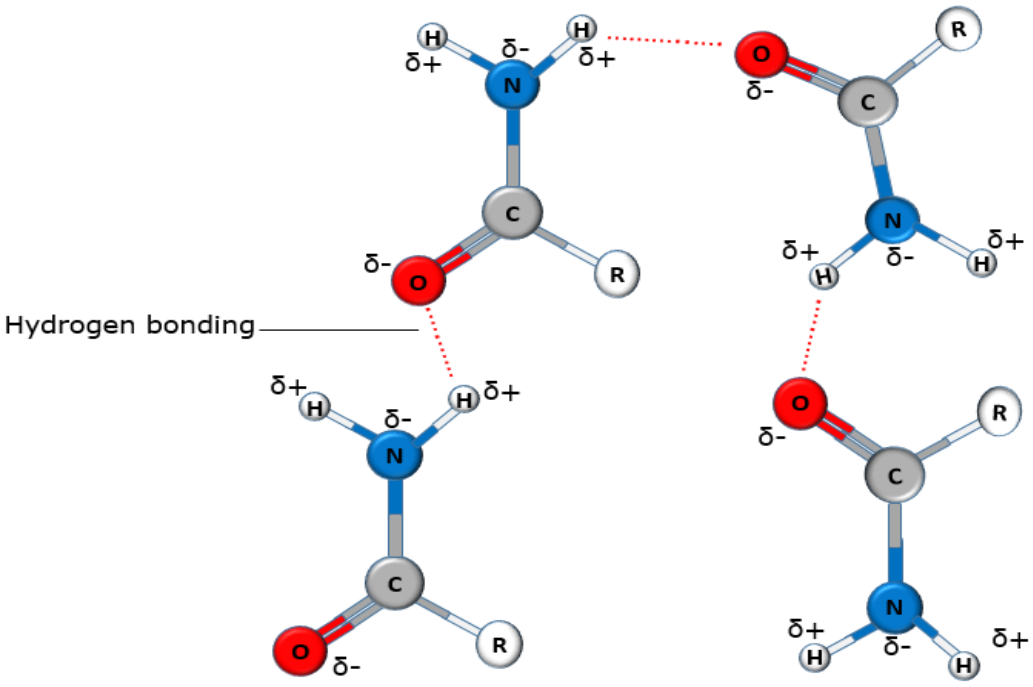


Figure 2.1: Amide hydrogen bonding

Carboxamides are generally bases. The basic character and their ability to undergo protonation and hydrogen bond formation is centered around the carbonyl oxygen based on a study conducted by Shainyan *et al* in 2011 (Shainyan, Chipanina, & Oznobikhina, 2012). It is worth noting that the basicity of carboxamides is higher than that of ketones due to the presence of conjugation that allows electrons to be shifted to the more electronegative oxygen in the amide group. Carboxamides have been well-studied and have been proven to be a key pharmacophore in drug design with a wide range of application in drug synthesis. **Figure 2.2** shows the structure of some carboxamide derivatives.

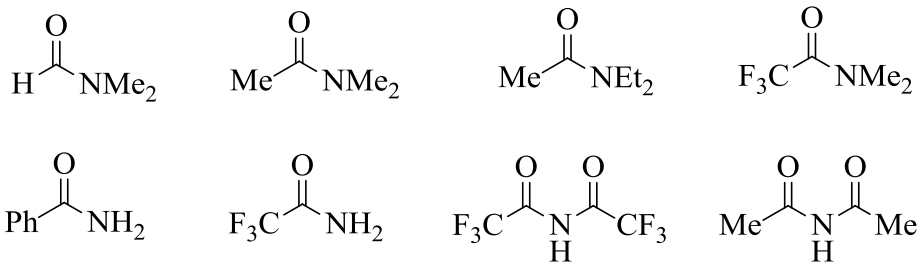
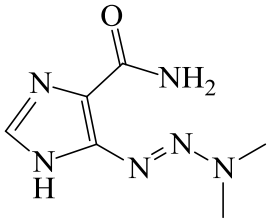
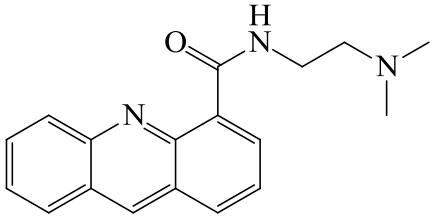


Figure 2.2: List of carboxamide derivatives

Carboxamides appear in a good number of drug candidates. Over 25 % of all commercial chemotherapeutic agents are carboxamides (Ugwu et al., 2017). This makes them very important and worth studying. **Figure 2.3** shows representative drugs with carboxamide functional groups. Dacarbazine **17** also known as imidazole carboxamide, is used in treating melanoma and Hodgkin's disease (Serrone, Zeuli, Sega, & Cognetti, 2000) while acridine carboxamide **18** is a topoisomerase inhibitor that was developed to treat cancers (Dittrich et al., 2003).



17, Dacarbazine



18, Acridine carboxamide

Figure 2.3: Examples of carboxamide drugs

Raffa *et al* (2017) synthesized a group of carboxamides (**Figure 2.4)** and tested them for their antiproliferative activity against human leukemia K562 cells. The compounds displayed very encouraging results when various substitutions such as R^1 = I, R^2 = H, and R^3 = cinnamido, propioamido, phenoxyacetamido groups were used (Raffa et al., 2017).

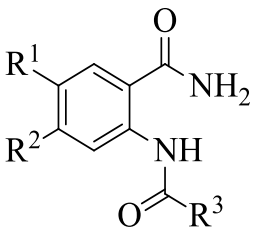


Figure 2.4. Generic Structure of biologically active carboxamide investigated Raffa et al

The bioactivity results showed that superior activities were obtained for the benzamides with various substitutions on the aromatic ring especially when iodine or 4,5-dimethoxy were used. While activity of 0.57 µM and 1.2 µM, were obtained when R^1 = I and R^3 = cinnamido groups respectively, activities of 0.40 µM and 0.43 µM, respectively were obtained when R^1 = I and R^3 = propioamido group and 0.16 µM and 0.58 µM respectively when R^1 = R^2 = methoxy and R^3 = phenoxyacetamido groups.

It was further noticed that substituents on the benzamido group reduced the inhibition of K562 cell growth although the anti-proliferative activity was maintained. Structure-Activity Relationship (SAR) studies of the compounds showed that changing from cinnamido to propioamido group, resulted in increased activity. (Raffa et al., 2017).

Additional analogues from the benzamido series revealed **19, 20** and **21** (**Figure 2.5**) as the frontrunners with excellent anti-proliferative activities against K562 leukemia cell lines.

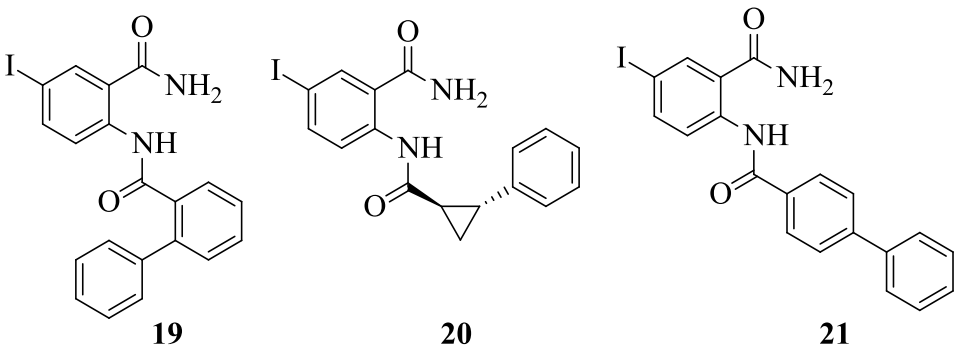


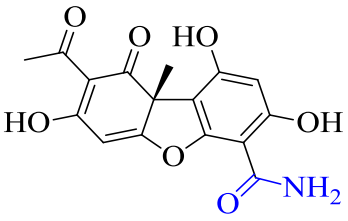
Figure 2.5: Structures of biologically active compounds by Raffa et al

Compound **21** was highly cytotoxic to the cancer cell lines with 0.5 mM IC_{50} value. The mechanism of action studies showed it induced the double strand DNA and caused damage to the cancerous cells leading to apoptosis of the cell after 48 hours (Raffa et al., 2017).

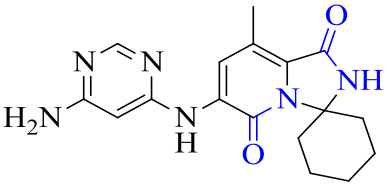
2.1.1 Carboxamides as anti-cancer agents

Cancers have been difficult to treat due to the evasive nature they possess. Malignant cancerous cells grow and progress because of the presence of Mitogen-activated protein kinase (MAPK)-interacting kinases (Mnks). Mnks are a family of serine/threonine kinases that are responsible for the transformation and progression of cancerous cells (Chen et al., 1999; Zhang et al., 2007). They regulate the initiation and further translation by exclusively phosphorylating the eukaryotic

initiation factor 4E (eIF4E). It is this Mnks-mediated complex that promotes the progression of cancers. In normal noncancerous tissues, this Mnks–mediated complex is not necessary for cell development (Diab et al., 2014). Because of the pharmacological role Mnks play in the growth and development of cancers, they can be targeted as an ideal nontoxic therapeutic strategy for the onward treatment of cancers. Carboxamides **22** and **23** in **Figure 2.6** are known Mnks inhibitors.



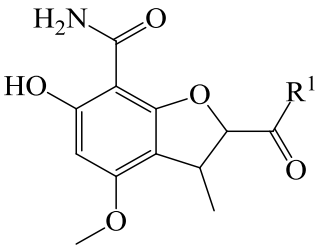
22, Cercosporamide



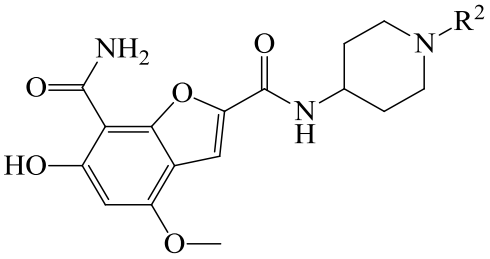
23, eFT508

Figure 2.6: Chemical structures of known Mnks inhibitors

Wang et al (2018) synthesized novel benzofurans as possible potent Mnks inhibitors. Three of the synthesized compounds in the series had IC_{50} values in the micromolar concentrations ranging from 1.16 – 3.55 µM. The most potent (**24**) with IC_{50} of 1.16 µM, was obtained when R^1 was 1-ethyl-4-methylpiperazine. They further expanded the scaffold to compounds belonging to **25** from which the most active possess IC_{50} value of 0.27 µM when R^2 was 1-ethyl-3-(trifluoromethyl)benzene (Wang et al., 2018).



24, R^1 = 1-ethyl-4-methylpiperazine



25, R^2 = 1-ethyl-3-(trifluoromethyl)benzene

Figure 2.7: Mnk Inhibitors synthesized by Wang et al

In a separate research, Zhao et. al. synthesized some carboxamides and screened them as possible anticancer agents. They synthesized 6-chloro-2-(propylthio)-8,9-dihydro-7H-purine derivatives through intramolecular cyclization techniques. Of the many compounds they investigated, compound **26** (**Figure 2.8**) was the most potent with IC_{50} of 2.80 µM against A549 cell lines (2.80 µM) and also anti-proliferative activity. It was also selective between cancer and normal cells and induced morphological changes and apoptosis of cancer cells. When a thiophene group was substituted in place of the benzene ring (**27**), the resultant compound showed the highest activity towards PC-3 cell lines (5.02 µM) (Zhao et al., 2018).

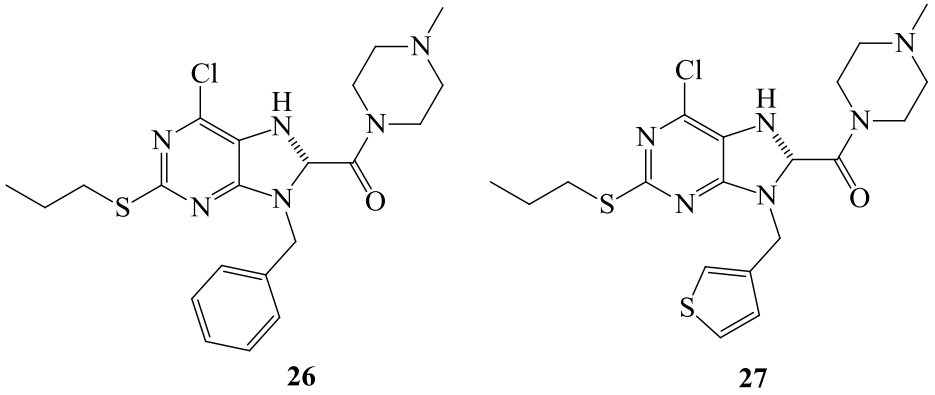
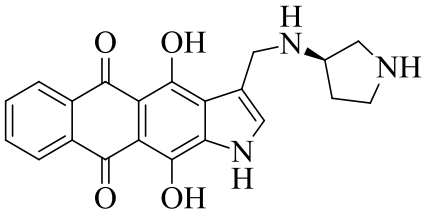


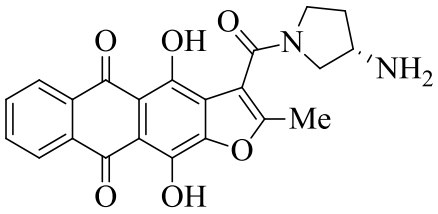
Figure 2.8: Scaffold from the work of Zhao et al

Tikhomirov et al, (2018) synthesized novel pyrrole and furan containing anthraquinones and evaluated them for their antitumor properties and found compound **28 (**naphtho[2,3-f]indole-5,10-dione) **(Figure 2.9)** to possess moderate antitumor properties. Through 'Scaffold hopping' method they replaced some of the substituent and discovered an alternative anthra[2,3-b]furan-3-carboxamide scaffold which led to the discovery of compound **29**. This new compound demonstrated multiple effects including binding to DNA, poisoning of topoisomerases 1 and 2 and

inhibition of protein kinases. It also had excellent antitumor efficacy *in vivo* as well as demonstrated activity against drug resistant P388/ADR leukemia B16/F10 melanoma models.



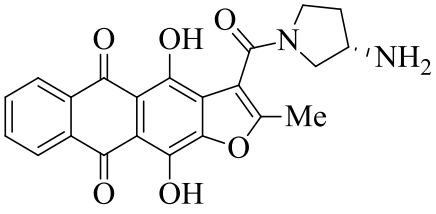
28, Naphtho[2,3-f]indole-5,10-dione



29, Anthra[2,3-b]furan-3-carboxamide

Figure 2.9: Structures of antitumor carboxamide-anthraquinones

New derivatives of compound **29** were designed and synthesized with substitutions made at the *peri*-position of the heterocyclic core to give new derivatives (**Figure 2.10)**. Although all the new derivatives were less cytotoxic than the reference drug doxorubicin, they still inhibit the growth of human and murine tumor cells at micro molar concentrations. It was observed that the presence of 4,11-hydroxyl groups was crucial for the cytotoxicity effect. They also had an added advantage of being able to circumvent drug resistance that is mediated by Pgp expression and non-functional p53. They also possessed all the characteristics of the reference compound **29**. Compounds **30** and **31** were very active against gastric cancer, notwithstanding their p53 status which was in line with previous observations (Tikhomirov et al., 2018).



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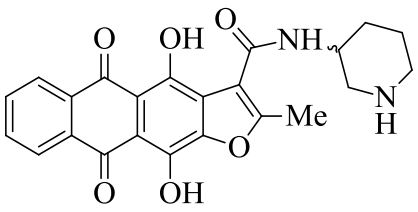


31

Figure 2.10: Structure of active carboxamide anthraquinones from the work of Tikhomirov et al

2.1.2 Anti-fungal Carboxamides

Carboxamides have also been established as potent antifungal agents. Most of the known clinically used amide fungicides are synthetic and have a broad spectrum of antifungal activity including compounds **32 – 37** (Wehrstedt et al, 2005, Borges et al, 2005) (**Figure 2.11*)*** used to control a variety of plant diseases.

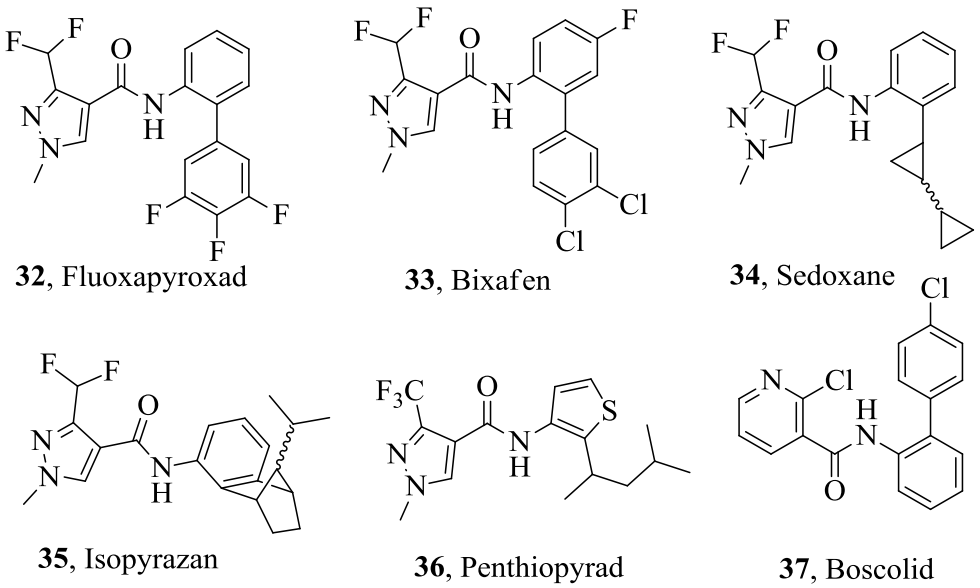
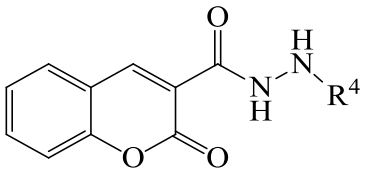


Figure 2.11: Representative Structures of carboxamide fungicides

One of the most studied carboxamides are the coumarins, e. g warfarin and acenocoumarol, used as anticoagulating agents to inhibit production of vitamin K. Coumoxystrobin is a strobilurin fungicide that possesses antifungal activity. Based on the chemical properties of carboxamides, hydrazides and coumarins, Yu *et al* decided to synthesize hybrid compounds with both functionalities to enhance activity and improve efficacy as possible control for some phytopathogenic diseases in plants. (Yu et al., 2018)

The library of compounds (**Figure 2.12)** were tested against six phytopathogenic fungi namely: *Botrytis cinerea, Alternaria solani, Rhizoctorzia solani, Cucumber anthrax* and *Alternaria leaf spot.* Initial screening showed that none of coumarin–carboxamide coupled derivatives were active against the six pathogens that were used compared to osthole and boscalid which were known antifungal agents. In contrast, the coumarin–hydrazide coupled derivatives had very low EC_{50} values against the same pathogens. Two of the coumarin–hydrazide coupled derivatives (**38** and **39**) had EC_{50} values as low as 1.57 µg/mL and 1.65 µg/mL respectively. Clinically used boscalid with EC_{50} of 0.51 µg/mL was still a little more effective than the two. Compound **38** (1.8 µg/mL) however exhibited superior activity than the control boscalid (2.98 µg/mL) against *Rhizoctorzia solani* (Yu et al., 2018).



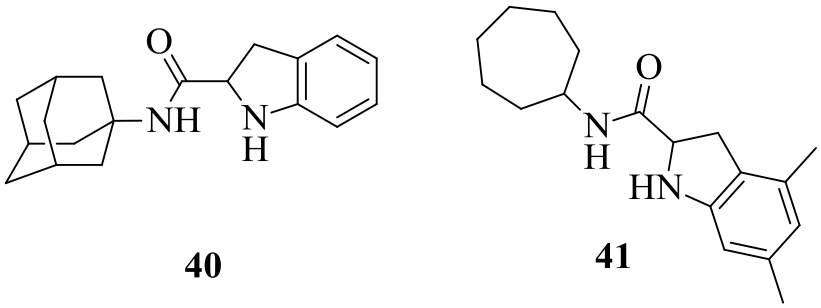
38, R^4 = *o*-Chlorophenyl
39, R^4 = *m*-Fluorophenyl

Figure 2.12: Structures of coumarin-carboxamide and coumarin- hydrazide derivatives

2.1.3 Anti-tubercular Carboxamides

A number of drugs in development as well as clinically used compounds containing the carboxamide functionality have been reported for use as anti-tubercular agents. First, indole carboxamides were investigated as potential antitubercular agents by various researchers (Kondreddi et al., 2013; Lun et al., 2013; Onajole et al., 2013). They demonstrated activity against drug resistant strains and *in vivo* efficacy assay in mouse models. In a separate research, Franz *et al* explored the possibility of increasing the antitubercular activity of carboxamides by fusing

indole and carboxamide functionalities. Their findings (**Figure 2.13)** showed that most of the synthesized compounds were inactive against *M. avium* except for compound **40** (8 µM) and **41** (0.15 µM). They also demonstrated *in vivo* efficacy in infected mouse models with excellent pharmacokinetics/pharmacodynamics. Both compounds had intriguing activities against *Mycobacterium abscessus* complex (MABSC) 0.063-0.25 µM. [*M. abscessus* is a group of mycobacteria that causes chronic lung infections and skin or soft tissue infections in humans. They are classified under non-tuberculous mycobacteria (NTM) organisms (Kusunoki & Ezaki, 1992). *Mycobacterium avium* is also classified as an NTM. They are known to cause respiratory infection in birds, pigs and humans (Horsburgh, 1991)] (Franz et al., 2017).



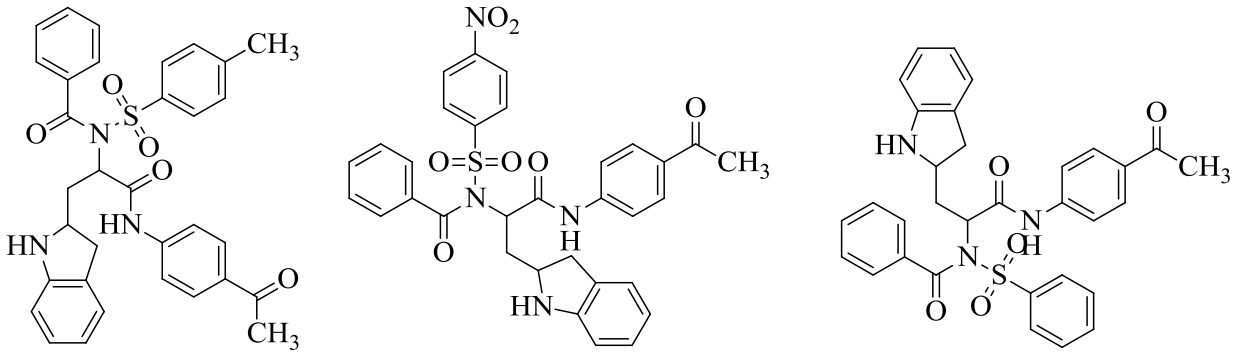
40 **41**

Figure 2.13: Structures and biological activities of active carboxamides by Franz et al

2.1.4: Anti-malarial Carboxamides

Some carboxamides in development and clinical usage have been described as potent inhibitors of drug sensitive *P. falciparum* malaria strains. Ugwu et al (2017) synthesized a library of carboxamides incorporating the sulphonamide functionalities from un-activated carboxylic acids with boric acid as the catalyst (Ugwu et al., 2017). The compounds (**Figure 2.14**) were then screened for their *in vitro* antimalarial and antioxidant activity and compounds **42, 43, 44** exhibited antimalarial activity of 0.03, 0.02, and 0.05 µM as well as 39.47, 49.24, 61.24 % inhibition

respectively, at 5 mg/mL of DPPH. Molecular docking experiments of the compounds with plasmepsin II showed a good binding profile that suggests inhibition of the enzyme as a possible mode of action. Compounds **42** and **43** recorded fascinating IC_{50} for the free radical scavenging activity that makes them promising antioxidants (0.045 mM and 0.73 mM) when compared to ascorbic acid which had an IC_{50} value of 0.34 mM (Ugwu et al., 2017).



42 **43** **44**

Figure 2.14: Structures of biologically active sulphonamides.

2.2 **Urea**

Urea, also called carbamide, is a carbonyl functional group joined to two amines. They have a general molecular formula $R^1R^2NCONR^3R^4$. They are very important to metabolism in animals and the main nitrogen compound in urine. Urea compounds are normally solids at room temperature and are mostly soluble in water. They are produced by the liver as a detoxifier and then excreted. Urea compounds are used in chemical industry to make explosives, in the automobile industry as anti-pollutants and also in the pharmaceutical industry to produce drugs. A major use of urea, outside of pharmacological applications, is in the production of fertilizers. Urea fertilizers are environmentally friendly since most soil organisms have the enzyme called urease

for the conversion of urea to ammonia or ammonium or bicarbonate ion (Decaux et al., 2010; Kurzer & Sanderson, 1956; Meessen, 2010).

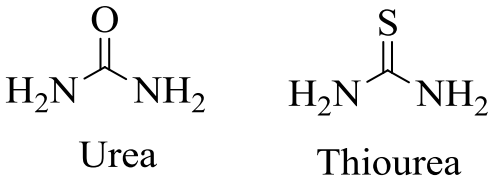
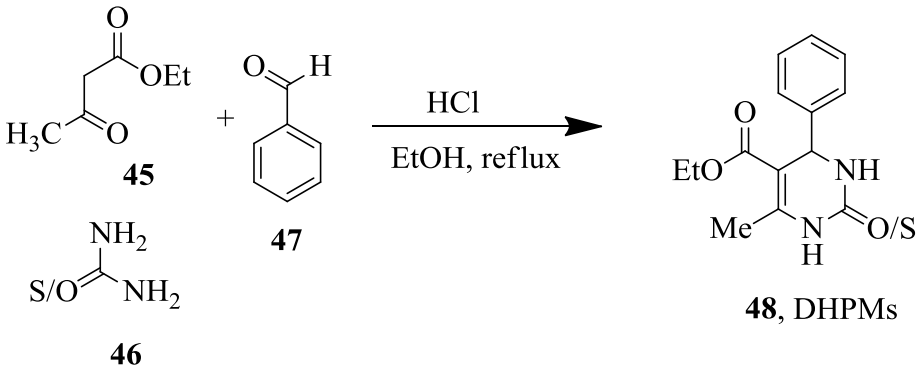


Figure 2.15: General structure of Urea and Thiourea

Pharmacologically, urea serves as a linker between different moieties and helps to build bulky compounds.

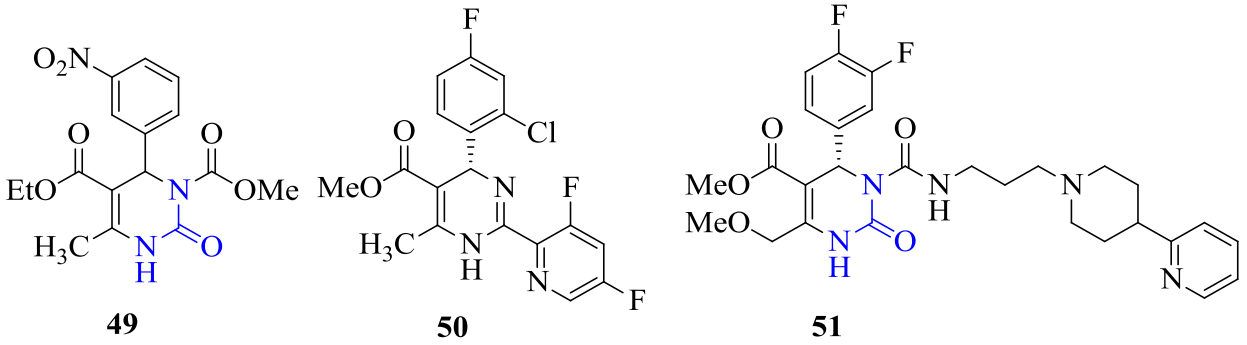
2.2.1 Application of Urea

Urea compounds are used in multicomponent reactions in which two or more reactants are placed in a single vessel to produce products that have components of all the reactants. This is the main means by which heterocyclic compounds are generated. Multicomponent reactions are sometimes called cascade domino or one-pot reaction processes. One application of multicomponent reaction is observed in the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) through the Biginelli reaction in **Scheme 2.1** The advantage for these type of reactions are that they start with readily available small molecules (Nagarajaiah, Mukhopadhyay, & Moorthy, 2016).



Scheme 2.1: Synthesis of DHMPs by multicomponent reaction (Biginelli reaction)

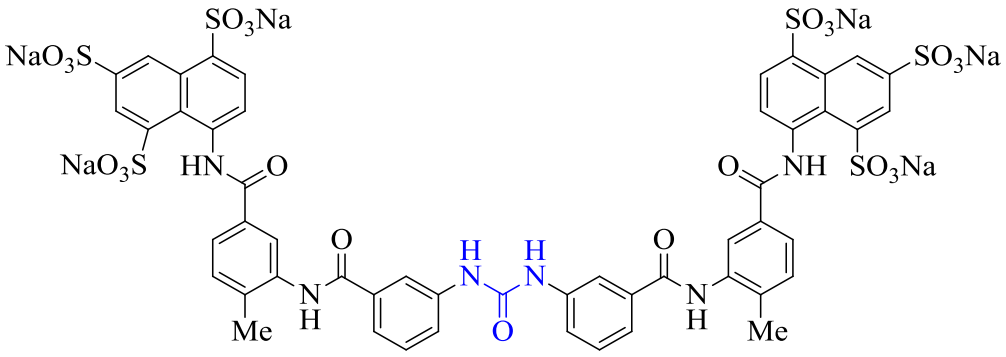
Biginelli reactions usually result in racemic mixtures that have wide range of pharmacological applications (Nagarajaiah et al., 2016). For example, compound **49** is a known calcium channel blocker (Atwal et al., 1990), **50** is an anti-inflammatory and antiviral (Greenhalfjo & Diggory, 1971) and **51** is receptor antagonist agent (Barrow et al., 2000).



49 **50** **51**

Figure 2.16: Examples of biologically active DHPMs

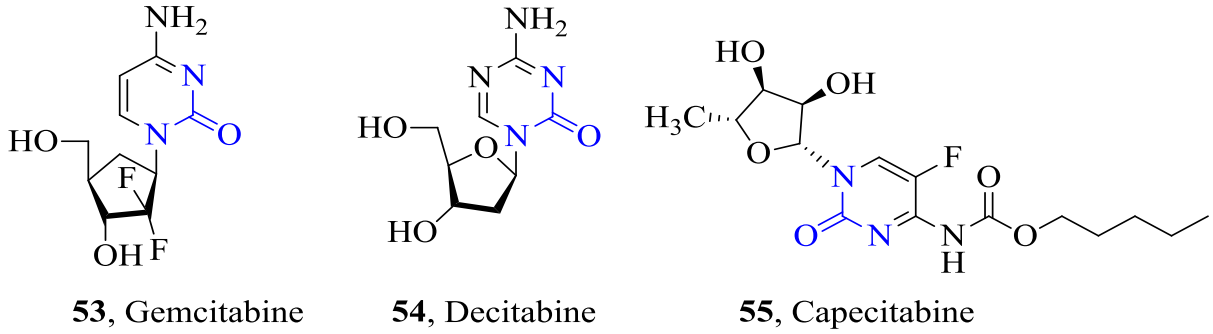
One of the urea based compounds with important pharmacological applications is Suramin (**52**). Suramin is used to treat Chikungunya virus (CHIKV) which has affected millions of people in Asia, Africa and, recently, in the Caribbean. It inhibits CHIKV replication through multiple mechanism and is also reported to inhibit Zika virus replication (Hwu et al., 2017).



52, Suramin

Figure 2.17: Structure of Suramin

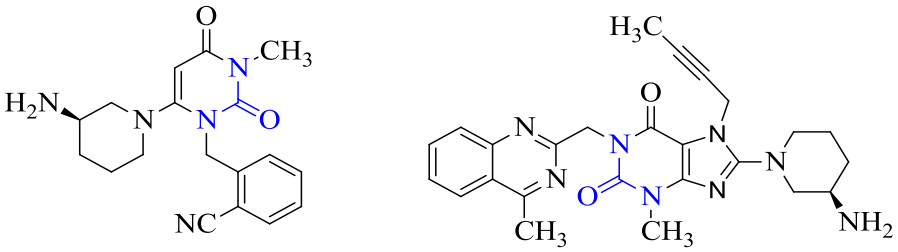
Urea containing compounds have been used as anticancer agents. Examples include gemcitabine **53**, dectabine **54** and capecitabine **55** which are clinically used anticancer agents.



53, Gemcitabine **54**, Decitabine **55**, Capecitabine

Figure 2.18: Some anticancer agents containing urea functionality

Urea-containing compounds such as alogliptin **56** and linagliptin **57**, are orally available anti diabetic drugs that inhibit dipeptidyl peptidase-4 in type 2 diabetic patients. **Figure 2.19** represents ureas used as antidiabetics.



56, Alogliptin **57**, Linagliptin

Figure 2.19: Some antidiabetic drugs with urea functionality

Further, urea-containing compounds have also been used to inhibit the synthesis of some important proteins that account for the continual existence of HIV/AIDS. Compounds in **Figure 2.20** are urea containing compounds that are known to inhibit the CYP3A proteins (Xu et al., 2014).

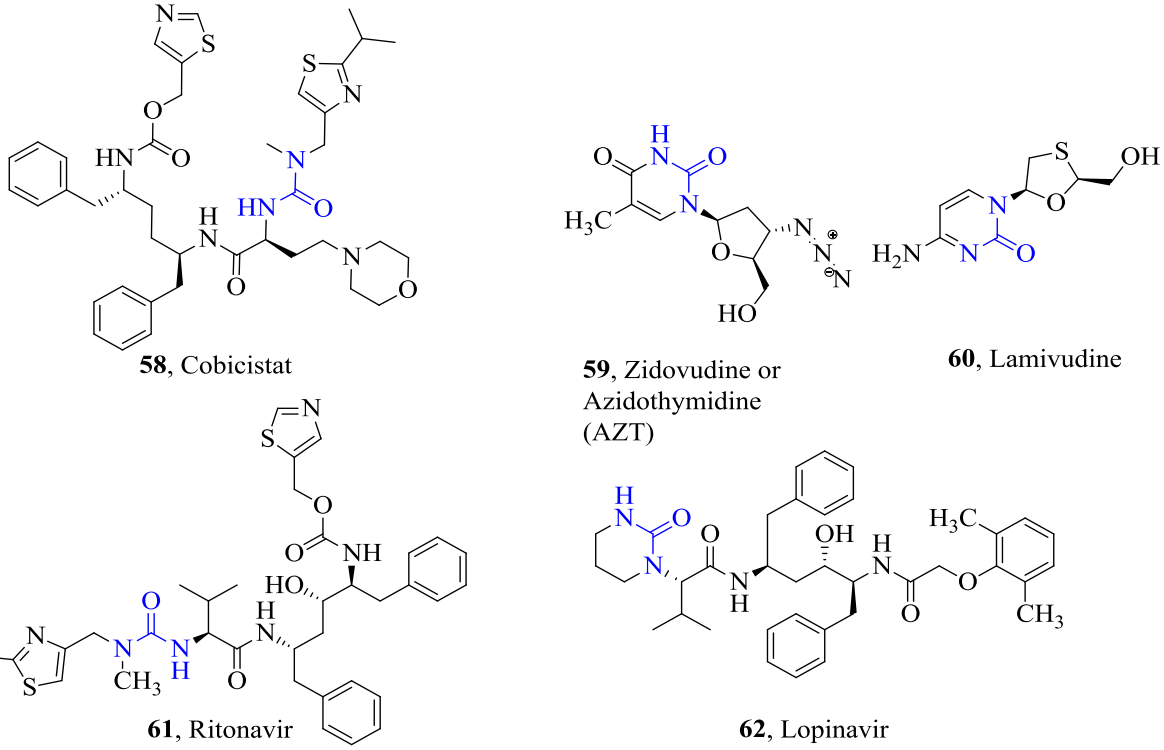


Figure 2.20: Some frontline anti-HIV drugs containing drugs containing urea functionality (Bandyopadhyay & Banik, 2015; Xu et al., 2014)

Cafedrine **63** and theodrenaline **64,** are both urea containing compounds that are used as a combinational therapy for cardiovascular disease. They increase the pressure of the heart in hypotension patients.

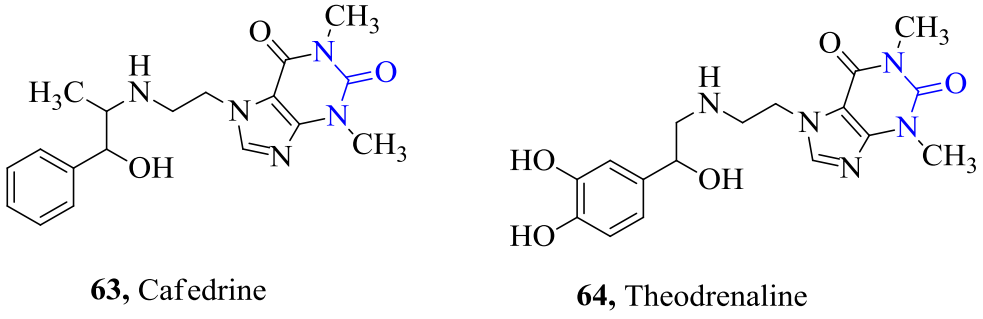
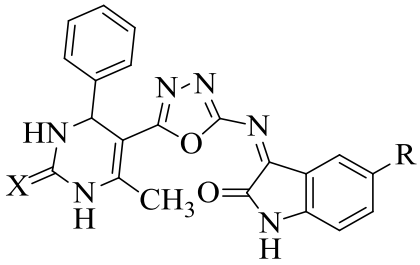


Figure 2.21: Some frontline drugs for cardiovascular disease containing urea

Akhaja et al synthesized 1,3-Dihydro-2H-indol-2-one derivatives and tested them for antitubercular and antimalarial activities and found that they were active. Two of the compounds **65** and **66** had both antitubercular and antimalarial activities in micro molar range. They postulated that the antitubercular and antimalarial activity might be due to sufficient hydrogen bond acceptors and donors with desirable lipophilicity (Akhaja & Raval, 2012).

Table 2.1: Structures and biological activities of Urea compounds



Target	Compound	X	R	MIC values (µg/mL)
Antitubercular activity	**65**	O	NO_2	6.25
	66	S	Cl	6.25
Antimalarial activity	**65**	O	NO_2	0.177
	66	S	Cl	0.035

CHAPTER THREE

3.0 MATERIALS AND METHODS

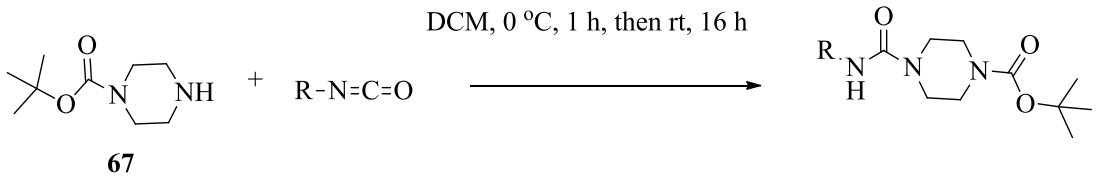
3.1 **Synthesis of Compounds**

With exception of those stated, all reagents were obtained from commercial suppliers. Sure seal dichloromethane, hexane, methanol, acetyl chloride and ethyl acetate were used throughout the experiments. Analytical thin layer chromatography was performed on pre-coated silica gel (0.25 mm layer of silica gel F254) aluminium sheets. UV light (254 nm) was used for all visualizations and flash column chromatography was performed using silica gel 60 Å particle size 35-70 micron, Davisil® Chromatography grade. FTIR spectra were run using a Perkin-Elmer FTIR spectrophotometer spectrum 2. Solid samples were applied neat on to sodium chloride discs.

^{1}H-NMR spectra were recorded using a Bruker 500 MHz NMR spectrometer situated at the Department of Chemistry, University of Ghana. Spectra were referenced to the residual solvent peak and chemical shifts expressed in ppm from TMS as the internal reference peak. All NMR experiments were performed at room temperature. The following annotations are used to describe multiplicity: s, singlet; bs, broad-singlet; d, doublet; t, triplet; q, quartet; m, multiplet and coupling constants *J*, are expressed in Hertz.

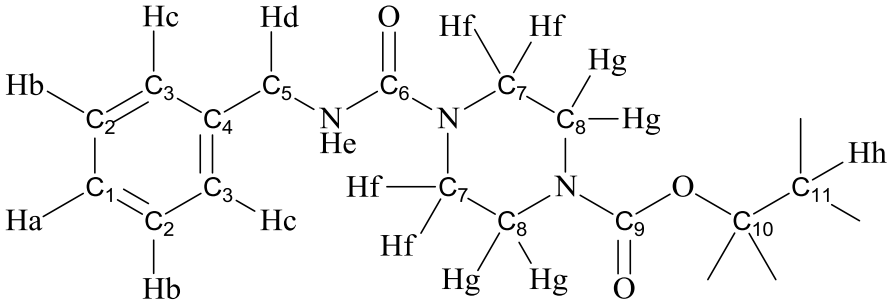
Melting points are expressed in degree Celsius (°C) and performed using the Gallemkamp melting point apparatus and capillary tubes.

3.1.1 **General procedure for making the Boc protected ureas**



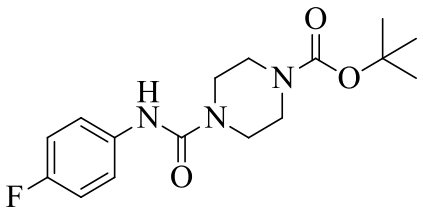
A solution of piperazine-1-carboxylic acid tert-butyl ester **67,** (26.85 mmol, 1.1 eq) in DCM (10 mL) was cooled in an ice bath and treated with the isocyanate (27.39 mmol, 1 eq). After 1 h, the bath was removed and the mixture stirred at room temperature for 15 h. The resulting mixture was filtered and the solid was washed with dichloromethane (DCM, 2×10 mL), giving the title compound as an amorphous solid.

3.1.1.1 Preparation of **tert-butyl 4-(benzylcarbamoyl)piperazine-1-carboxylate, 68**



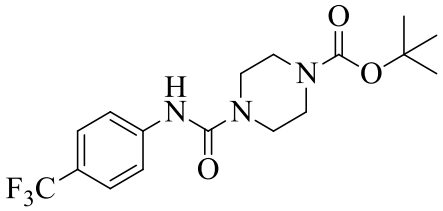
This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (1.54 g, 8.26 mmol, 1.1 eq) and isocyanatomethyl)benzene (1 g, 7.51 mmol, 1 eq)] in 92 % yield as a white amorphous solid. Mpt 189-191 °C. V_{max} (neat) cm^{-1} (N-H) 3179.8, (C-H) 2922.9, (C-H) 2862.5, (C=O) 1715.5, (C-O) 1632.3, (C=C) 1512.8, 1414.9, (C-N) 1302.6, 1130.9. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (**Ha**) 7.42 (dd, *J* = 6.3, 6.8 Hz, 1H), (**Hb**) 7.36 (t, *J* = 6.8 Hz, 2H), (**Hc**) 7.12 (d, *J* = 6.3 Hz, 2H), (**Hd**) 4.43 (s, 1H), (**He**) 6.0 (s, 1H), (**Hf**) 3.46-3.42 (m, *J* = 8.1 Hz, 4H), (**Hg**) 3.39-3.35 (m, *J* = 8.1 Hz, 4H), (**Hh**) 1.46 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C (**C6**) 161.3, (**C9**) 157.6, (**C4**) 134.7, (**C2**) 129.2, (**C3**) 126.7, (**C1**) 124.8, (**C10**) 81.4, (**C7**) 72.1, (**C8**) 71.5, (**C5**) 38.0 (**C11**) 23.4.

3.1.1.2 Preparation of **tert-butyl 4-((4-fluorophenyl)carbamoyl)piperazine-1-carboxylate, 69**



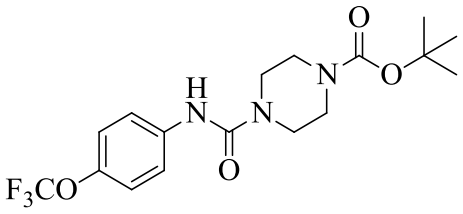
This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (1.36 g, 7.28 mmol, 1.1 eq) and 1-fluoro-4-(isocyanatomethyl)benzene (1 g, 6.62 mmol, 1 eq)] in 95 % as a white powder. Mpt 178-179 ^{o}C. V_{max} (neat) cm^{-1} 3146.8, 3064.9, 2973.32, 2860.1, 1689.0, 1631.1, 1538.2, 1225.6, 1157.6, 1102.3, 1001.2. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.28 (d, J = 8.2 Hz, 2H), 6.99 (d, J = 8.2 Hz, 2H), 6.43 (s, 1H), 3.48 (s, 8H), 1.48 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 159.9, 158.1, 155.2, 154.6, 134.5, 122.3, 80.3, 66.0, 43.6, 28.5.

3.1.1.3 Preparation of **tert-butyl 4-((4-(trifluoromethyl)phenyl)carbamoyl)piperazine-1-carboxylate, 70**



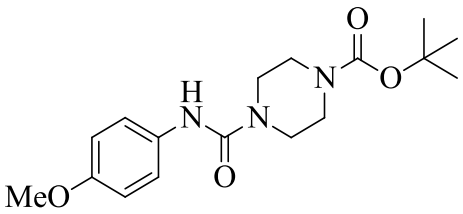
This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (1.01 g, 5.47 mmol, 1.1 eq) and 1-(isocyanatomethyl)-4-(trifluoromethyl)benzene (1 g, 4.97 mmol, 1 eq)] in 91 % yield as a white amorphous solid. Mpt 239-241 ^{o}C. V_{max} (neat) cm^{-1} 3136.0, 3062.5, 2860.1, 1683.9, 1536.9, 1327.3, 1175.4. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.45 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 6.71 (s, 1H), 3.42 (s, 8H), 1.41 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.5, 157.5, 154.3, 142.0, 126.0, 119.2, 80.4, 28.3.

3.1.1.4 Preparation of **tert-butyl 4-((4-(trifluoromethoxy)phenyl)carbamoyl)piperazine-1-carboxylate, 71**



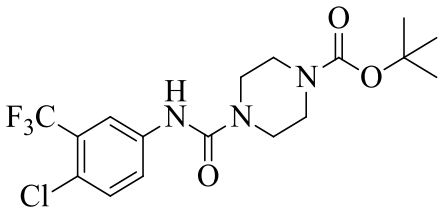
This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (0.94 g, 5.07 mmol, 1.1 eq) and 1-(isocyanatomethyl)-4-(trifluoromethoxy)benzene (1 g, 4.61 mmol, 1 eq)] in 93 % as a white powder. Mpt 248-249 ^{o}C. V_{max} (neat) cm^{-1} 3102.2, 2921.8, 1632.9, 1567.6, 1324.2, 1147.9, 1129.4. ^{1}H-NMR (500 MHz, DMSO) δ_H 8.19 (s, 1H), 7.61 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 3.42 (s, 8H), 1.41 (s, 9H). ^{13}C-NMR (126 MHz, DMSO) δ_C 161.0, 155.1, 143.2, 140.1, 135.4, 128.3, 121.6, 121.2, 111.7, 42.8, 41.2.

3.1.1.5 Preparation of **tert-butyl 4-((4-methoxyphenyl)carbamoyl)piperazine-1-carboxylate, 72**



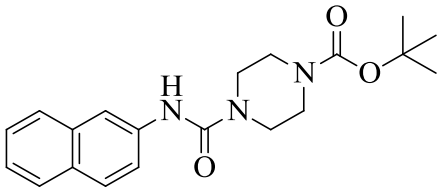
This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (1.26 g, 6.74 mmol, 1.1 eq) and 1-(isocyanatomethyl)-4-methoxybenzene (1 g, 6.13 mmol, 1 eq)] in 92 % yield as a white amorphous solid. Mpt. 205-207 ^{o}C. V_{max} (neat) cm^{-1} 3106.8, 3004.9, 2953.32, 2860.1, 1689.0, 1611.1, 1530.2, 1220.6, 1107.6, 1102.3, 1000.2. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.25 (d, J = 6.1 Hz, 2H), 6.86 (d, J = 6.1, 2H), 6.49 (s, 1H), 3.49 (s, 8H), 1.51 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.8, 156.0, 155.6, 154.6, 131.7, 122.6, 114.1, 80.3, 79.7, 55.5, 45.7, 43.7, 28.4.

3.1.1.6 Preparation of **tert-butyl 4-((4-chloro-3-(trifluoromethyl)phenyl)carbamoyl) piperazine-1-carboxylate, 73**



This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (0.87 g, 4.68 mmol, 1.1 eq) and 1-chloro-4-(isocyanatomethyl)-2-(trifluoromethyl)benzene (1 g, 4.26 mmol, 1 eq)] in 90 % yield as a white amorphous solid. Mpt. 145-146 ^{o}C. V_{max} (neat) cm^{-1} 3242.0, 3012.5, 2870.1, 1703.9, 1636.9, 1327.3, 1205.4, 1203.4, 1103.5. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.67 – 7.64 (s, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.7 Hz, 1H), 6.29 (s, 1H), 3.48 (s, 8H), 1.47 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 159.6, 154.4, 137.8, 131.9, 131.7, 128.6, 128.3, 125.6, 124.0, 123.7, 121.5, 118.9, 118.8, 80.6, 53.4, 43.8, 28.3.

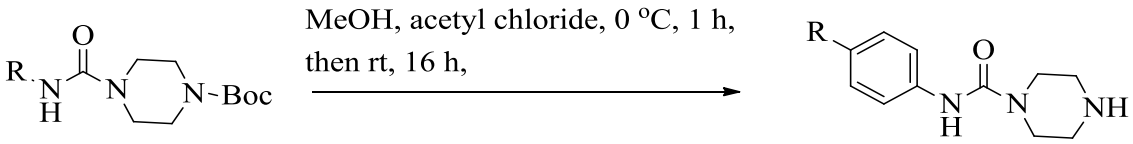
3.1.1.7 Preparation of **tert-butyl 4-(naphthalen-2-ylcarbamoyl)piperazine-1-carboxylate, 74**



This compound was prepared according to the general procedure for making boc-protected urea [piperazine-1-carboxylic acid tert-butyl ester (1.12 g, 6.01 mmol, 1.1 eq) and 2-(isocyanatomethyl)naphthalene (1 g, 5.46 mmol, 1 eq)] in 91 % as a white powder. Mpt 261-262 ^{o}C. V_{max} (neat) cm^{-1} 3189.0, 2930.1, 2814.3, 2722.7, 1635.5, 1543.3, 1418.4, 1248.6, 1035.7. ^{1}H-NMR (500 MHz, DMSO) δ_H 8.18 (s, 1H), 8.04 (d, J = 6.2 Hz, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.67 (dd, J = 8.9, 2.1 Hz, 1H), 7.45 – 7.43 (d, J = 6.2 Hz,1H), 7.37 – 7.33 (s,

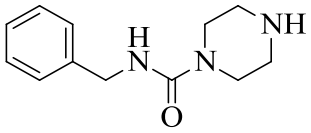
1H), 3.58 (s, 8H), 1.68 (s, 9H). ^{13}C-NMR (126 MHz, DMSO) δ_C 161.6, 155.3, 138.5, 133.9, 131.9, 129.6, 128.1, 127.7, 127.4, 126.6, 124.4, 121.5, 115.5, 43.0, 41.4.

3.1.2 **General procedure for the de-protection of the boc-protected ureas**



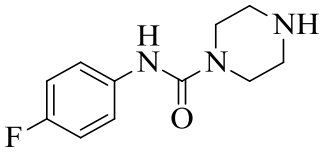
A solution of boc-protected urea (1 eq) in MeOH (30 mL) was treated with acetyl chloride (10 eq) at 0 °C and the resulting mixture stirred at this temperature for 20 min. The reaction mixture was then warmed to room temperature, stirred for 16 h and the resulting suspension was filtered. The solid was washed with Et_2O (3×100 mL) and dried in vacuum, giving a white powder. This powder was partitioned between DCM and 10 % aq. KOH. The aqueous phase was extracted with DCM (3 x 10 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated to give the title compound as a white amorphous solid.

3.1.2.1 Preparation of ***N*-benzylpiperazine-1-carboxamide, 75**



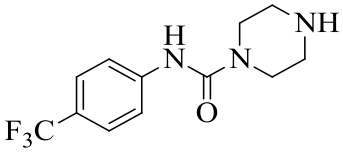
This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 93 % as a white powder. Mpt 231-232 °C. V_{max} (neat) cm^{-1} 3123.3, 2946.8, 2917.9, 2804.6, 2766.1 1617.0, 1539.3, 1511.0, 1345.5, 1263.2, 1002.3. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.52 (dd, *J* = 6.6, 6.9 Hz, 2H), 7.39 (t, *J* = 6.9 Hz, 1H), 7.12 (d, *J* = 6.6 Hz, 2H), 6.31 (s, 1H), 4.41 (s, 2H), 3.36-3.31 (m, *J* = 8.0 Hz, 4H), 2.86 – 2.78 (m, *J* = 8.0 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.5, 157.8, 139.5, 128.6, 127.7, 127.3, 65.8, 45.3, 44.5, 14.8.

3.1.2.2 Preparation of ***N*-(4-fluorophenyl)piperazine-1-carboxamide, 76**



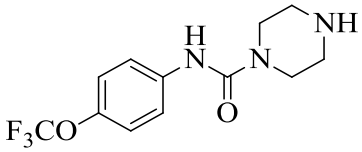
This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 92 % as a colourless oil. V_{max} (neat) cm^{-1} 3028.7, 2255.1, 2127.4, 1662.3, 1023.8, 1003.8. ^{1}H-NMR (500 MHz, DMSO) δ_H 7.50 (d, *J* = 7.2 Hz, 2H), 6.99 (d, *J* = 7.2 Hz, 2H), 6.36 (s, 1H), 3.72 (t, *J* = 8.4 Hz, 4H), 3.10 (t, *J* = 8.4 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 161.8, 158.4, 156.8, 155.7, 137.1, 121.7, 115.0, 80.4, 42.8, 41.2.

3.1.2.3 Preparation of ***N*-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 77**



This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 88 % yield as a white amorphous solid. Mpt 249-250 ^{o}C. V_{max} (neat) cm^{-1} 3236.0, 3162.5, 2880.1, 1693.9, 1516.9, 1407.3, 1105.4, 998.6. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.45 (d, *J* = 8.6 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 6.71 (s, 1H), 3.83 (t, *J* = 6.3 Hz, 4H), 3.09 (t, *J* = 6.3 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 161.0, 154.3, 142.0, 131.5, 126.0, 119.2, 101.6, 80.4, 28.3.

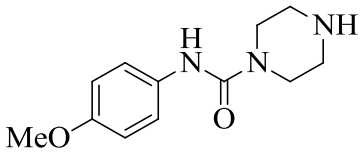
3.1.2.4 Preparation of ***N*-(4-(trifluoromethoxy)phenyl)piperazine-1-carboxamide, 78**



This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 90 % as a white powder. Mpt 254-256 ^{o}C. V_{max} (neat) cm^{-1} 3225.5, 3102.2, 2921.8, 1682.9, 1567.6, 1324.2, 1147.9, 1129.4. ^{1}H-NMR (500 MHz, DMSO) δ_H 8.19 (s, 1H),

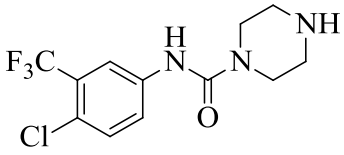
7.61 (d, *J* = 8.3 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 3.83 (t, *J* = 8.0 Hz, 4H), 3.12 (t, *J* = 8.0 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 160.8, 155.1, 143.2, 140.1, 121.6, 121.2, 42.8, 41.2.

3.1.2.5 Preparation of ***N*-(4-methoxyphenyl)piperazine-1-carboxamide, 79**



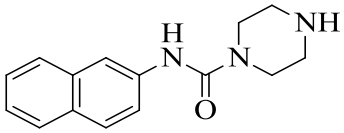
This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 86 % as a colourless oil. V_{max} (neat) cm^{-1} 3196.8, 2963.32, 2840.1, 1669.0, 1601.1, 1530.2, 1210.6, 1102.3, ^{1}H-NMR (500 MHz, DMSO) δ_H 8.37 (s, 1H), 7.39 – 7.30 (d, *J* = 2.3 Hz, 2H), 6.87 – 6.78 (2, *J* = 2.3 Hz, 2H), 3.68 (t, *J* = 5.2 Hz, 4H), 3.09 (t, *J* = 5.2 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 160.4, 155.1, 133.5, 122.1, 114.0, 79.4, 55.1, 42.9, 41.2, 40.3, 39.6, 39.5.

3.1.2.6 **Preparation of *N*-(4-chloro-3-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 80**



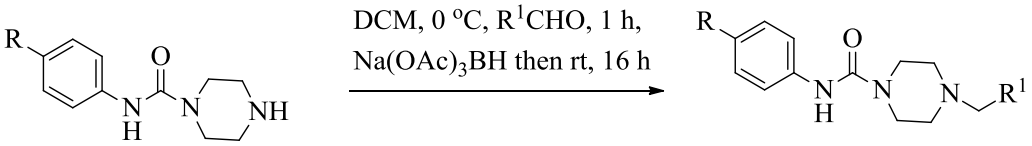
This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 86 % as a colourless oil. V_{max} (neat) cm^{-1} 3206.8, 3104.9, 2913.32, 2810.1, 1719.0, 1641.1, 1536.2, 1102.3, 1001.6 ^{1}H-NMR (500 MHz, DMSO) δ_H 7.82 (s, 1H), 7.61 (d, *J* = 2.6 Hz, 1H), 7.43 (d, *J* = 2.6 Hz, 1H), 6.44 (s, 1H), 3.74 (t, *J* = 6.8 Hz, 4H), 3.12 (t, *J* = 6.8 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 161.0, 154.2, 140.0, 131.5, 126.4, 126.1, 123.9, 122.3, 121.7, 118.0, 117.9, 42.4, 40.8.

3.1.2.7 Preparation of ***N*-(naphthalen-2-yl)piperazine-1-carboxamide, 81**



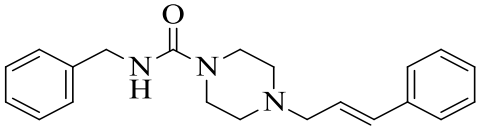
This compound was prepared according to the general procedure for the de-protection of the boc-protected ureas in 78 % as a white powder. Mpt 271-272 ^{o}C. V_{max} (neat) cm^{-1} 3189.0, 2930.1, 2814.3, 2722.7, 1635.5, 1543.3, 1418.4, 1248.6, 1035.7. ^{1}H-NMR (500 MHz, DMSO) δ_H 8.08 (s, 1H), 8.00 (d, *J* = 6.7 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 2.5 Hz, 1H), 7.47 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.45 – 7.33 (d, *J* = 6.7 Hz,1H), 7.30 – 7.27 (s, 1H), 3.81 – 3.73 (t, *J* = 6.6 Hz, 4H), 3.16 – 3.08 (t, *J* = 6.6 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 158.9, 155.3, 138.5, 133.9, 129.6, 128.1, 127.7, 127.4, 126.6, 124.4, 121.5, 115.5, 43.0, 41.4.

3.1.3 **General procedure for the synthesis of the piperazine-1-carboxamides**



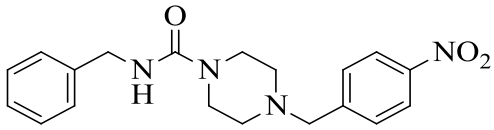
To a solution of the de-protected urea (1.16 mmol, 1 eq) in DCM (20 mL) was added the aldehyde (1.16 mmol, 1 eq) and the resulting mixture stirred at rt for 30 min. Sodium triacetoxy borohydride (1.74 mmol, 1.5 eq) was then added, stirred at rt for 16 h. The reaction was then quenched by adding saturated $NaHCO_3$ and extracted with DCM (3 x 20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography afforded the required amine product.

3.1.3.1 Preparation of ***N*-benzyl-4-cinnamylpiperazine-1-carboxamide, 82**



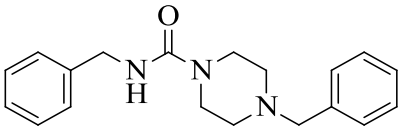
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 83 % yield as crystalline solid. Mpt 130-131 ^{o}C. V_{max} (neat) cm^{-1} 3131.8, 3028.8, 2922.7, 2857.6, 2809.4, 2766.1, 1616.1, 1533.4, 1262.9. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.61 (d, *J* = 2.2 Hz, 2H), 7.52 (d, *J* = 4.2 Hz, 2H), 7.42 (dd, *J* = 2.2, 4.8 Hz, 2H), 7.31 (dd, *J* = 4.2, 5.1 Hz, 2H), 7.21 (d, *J* = 4.8 Hz, 1H), 7.17 (d, *J* = 5.1 Hz, 1H), 6.52 (d, *J* = 15.8 Hz, 1H), 5.01 (d, *J* = 15.8 Hz, 2H), 4.85 (td, *J* = 6.2, 6.8 Hz, 1H), 4.41 (s, 2H), 3.41 (t, *J* = 8.8 Hz, 4H), 3.16 (d, *J* = 6.2 Hz, 2H), 2.47 (t, *J* = 8.8 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 162.0, 157.6, 139.5, 136.7, 133.5, 128.6, 127.8, 127.7, 127.3, 126.4, 126.0, 61.0, 53.0, 45.3, 43.3.

3.1.3.2 Preparation of ***N*-benzyl-4-(4-nitrobenzyl)piperazine-1-carboxamide, 83**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 80 % yield as a crystalline solid. Mpt 177-178 ^{o}C. V_{max} (neat) cm^{-1} 3158.9, 3323.4, 2917.9, 2804.6, 2766.1, 1617.0, 1539.3, 1511.0, 1263.2, 1002.3. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.20 (d, *J* = 8.1 Hz, 2H), 7.8 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 3.3, 4.2 Hz, 2H), 7.29 (t, *J* = 4.2 Hz, 1H)7.22 (d, *J* = 3.2 Hz, 2H), 4.42 (d, *J* = 5.3 Hz, 2H), 3.75 (d, *J* = 5.3 Hz, 1H), 3.66 (s, 2H), 3.46 (t, *J* = 8.2 Hz, 4H), 2.49 (t, *J* = 8.2 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.7, 157.0, 139.2, 128.7, 127.8, 127.5, 123.8, 123.6, 61.7, 5.35, 52.5, 44.7, 43.6.

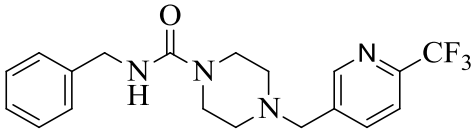
3.1.3.3 Preparation of ***N*, 4-dibenzylpiperazine-1-carboxamide, 84**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 75 % yield as a white solid. Mpt. 153-155 ^{o}C. V_{max} (neat) cm^{-1} 3195.9, 3117.9,

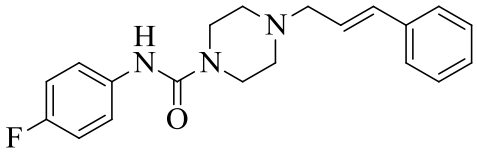
2923.7, 2855.2, 1729.7, 1644.3, 1602.0, 1556.2, 1151.8. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.41 – 7.39 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.29 (dd, *J* = 8.2, 7.8 Hz, 2H), 7.27 (dd, *J* = 8.6, 7.4 Hz, 2H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.67 (s, 1H), 3.56 (s, 2H), 3.51 (s, 2H), 3.42 (t, *J* = 8.9 Hz, 4H), 2.47 (t, *J* = 8.9 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 161.3, 157.5, 139.3, 129.3, 128.7, 128.4, 127.8, 127.3, 63.1, 52.5, 45.0, 43.7.

3.1.3.4 Preparation of ***N*-benzyl-4-((6-(trifluoromethyl)pyridin-3-yl)methyl)piperazine-1-carboxamide, 85**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 81 % yield as a white solid. Mpt. 163-164 ^{o}C. V_{max} (neat) cm^{-1} 3125.9, 2913.7, 2815.2, 1709.7, 1624.3, 1598.0, 1526.2, 1191.8. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.53 (d, *J* = 8.1 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.32 (s, 1H), 7.31 – 7.27 (d, *J* = 8.2 Hz, 1H), 7.23 (dd, *J* = 8.1, 7.7 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 2H), 6.94 (s, 1H), 3.61 (s, 2H), 3.55 (s, 2H), 3.49 – 3.46 (t, *J* = 12.1 Hz, 4H), 2.47 – 2.43 (t, *J* = 12.1 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.5, 156.1, 152.2, 141.1, 122.1, 122.1, 115.7, 115.5, 62.4, 52.8, 44.2, 43.4.

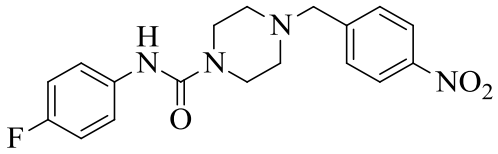
3.1.3.5 Preparation of **4-cinnamyl-*N*-(4-fluorophenyl)piperazine-1-carboxamide, 86**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 78 % yield as white solid. Mpt 173-175 ^{o}C. V_{max} (neat) cm^{-1} 3167.4, 3057.7, 2915.5, 2809.4, 2768.47, 1623.8, 1539.5, 1511.2, 1300.7, 1203.5. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.71 (d, *J* = 2.2 Hz, 2H), 7.62 (d, *J* = 4.4 Hz, 2H), 7.42 (dd, *J* = 2.2, 4.8 Hz, 1H), 7.38 (dd, *J* = 4.4,

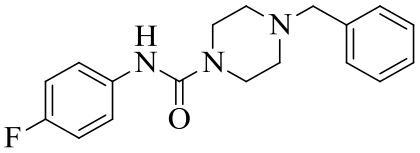
5.3 Hz, 2H), 7.21 (d, *J* = 4.8 Hz, 2H), 7.14 (d, *J* = 5.3 Hz, 1H), 6.55 (s, 1H), 3.61 (d, *J* = 7.9 Hz, 2H), 3.59 (td, *J* = 7.9, 6.2 Hz, 1H), 3.53 (d, *J* = 6.2 Hz, 1H), 3.51 – 3.45 (t, *J* = 10.1 Hz, 4H), 2.47–2.33 (t, *J* = 10.1 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 158.1, 155.0, 136.7, 134.8, 133.4, 128.8, 127.8, 126.6, 125.6, 122.1, 115.4, 60.8, 52.7, 43.9.

3.1.3.6 Preparation of ***N*-(4-fluorophenyl)-4-(4-nitrobenzyl)piperazine-1-carboxamide, 87**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 75 % yield Mpt 143-145 ^{o}C. V_{max} (neat) cm^{-1} 3114.3, 2922.7, 2855.2, 2773.3, 1630.1, 1507.1, 1341.9, 1208.4. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.54 (d, *J* = 5.5 Hz, 2H), 7.32-7.24 (d, *J* = 5.5 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.97 (d, *J* = 7.6 Hz, 2H), 6.40 (s, 1H), 3.65 (s, 2H), 3.52 (t, *J* = 12.7 Hz, 4H), 2.51 (t, *J* = 12.7 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 159.8, 158.1, 154.9, 147.3, 134.6, 129.6, 123.60, 122.0, 115.5, 61.45, 52.5, 44.0.

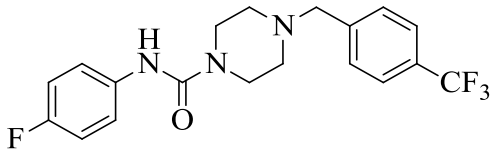
3.1.3.7 Preparation of **4-benzyl-*N*-(4-fluorophenyl)piperazine-1-carboxamide, 88**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 79 % yield as a white powder Mpt 125-127 ^{o}C. V_{max} (neat)cm^{-1} 3144.5, 2920.3, 2814.3, 1624.2, 1541.0, 1509.5, 1301.6, 1204.1, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.22 (d, *J* = 8.4 Hz, 2H), 8.34 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.31 (dd, *J* = 8.6, 4.7 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.34 (s, 1H), 3.66 (s, 2H), 3.56 – 3.50 (t, *J* = 11.7 Hz, 4H), 2.55 – 2.48 (t, *J* = 11.7

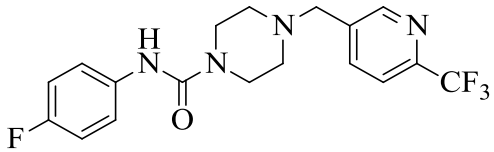
Hz,4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.0, 158.0, 154.9, 147.2, 145.6, 134.6, 129.4, 123.6, 121.8, 115.3, 62.0, 52.8, 43.6.

3.1.3.8 Preparation of ***N*-(4-fluorophenyl)-4-(4-(trifluoromethyl)benzyl)piperazine-1-carboxamide, 89**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 84 % yield as a white powder. Mpt. 175-176 ^{o}C. V_{max} (neat) cm^{-1} 3106.8,, 2973.32, 2880.1, 1749.0, 1621.1, 1536.2, 1124.3, 1101.6, 1003.4, 1000.9 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.55 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.35 – 7.24 (d, *J* = 8.2 Hz, 2H), 7.21 (dd, *J* = 8.2, 8.9 Hz, 2H), 6.94 (s, 1H), 3.48 – 3.45 (t, *J* = 8.7 Hz, 4H), 2.47 – 2.43 (t, *J* = 8.7 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.1, 158.1, 155.2, 142.1, 134.9, 129.9, 129.6, 129.3, 125.4, 125.4, 125.4, 122.1, 122.1, 115.7, 115.5, 62.4, 52.8, 44.2, 43.4.

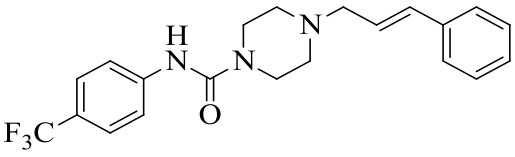
3.1.3.9 Preparation of ***N*-(4-fluorophenyl)-4-((6-(trifluoromethyl)pyridin-3-yl)methyl) piperazine-1-carboxamide, 90**



This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 74 % yield as a yellowish powder. Mpt. 141-143 ^{o}C. V_{max} (neat) cm^{-1} 3115.8,, 2951.3, 2820.1, 1620.1, 1556.2, 1204.3, 1114.3, 1101.5, 1013.4, 1002.2 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.60 (d, *J* = 2.1 Hz, 1H), 7.80 (d, *J* = 2.1 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.20 – 7.19 (d, *J* = 8.0 Hz, 2H), 7.18 (s, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.47 (s, 1H), 3.54 (s, 2H), 3.41 (t, *J* =

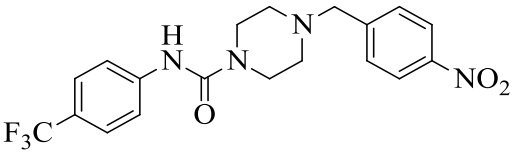
5.1 Hz, 4H), 2.39 (t, *J* = 5.1 Hz, 4H), ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 159.9, 158.0, 155.1, 150.2, 147.1, 137.9, 136.8, 134.8, 122.2, 120.2, 115.5, 59.5, 52.7, 43.9, 29.6, 22.8

3.1.3.10 Preparation of **4-cinnamyl-*N*-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 91**



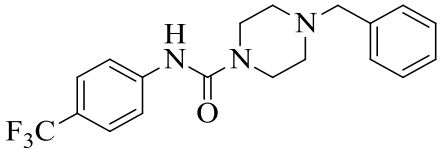
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 82 % yield as a white solid. Mpt 151-153 ºC. V_{max} (neat) cm^{-1} 3197.5, 2862.5, 2816.7, 2773.3, 1667.0, 1525.5, 1332.4, 1238.6, 1106.8, 1153.7. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.30 (d, *J* = 7.7 Hz, 1H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.25 (d, *J* = 6.6 Hz, 2H), 6.61 (d, *J* = 15.8 Hz, 1H), 6.33 (dt, *J* = 15.8, 6.6 Hz, 1H), 3.19 (t, *J* = 9.0 Hz, 4H), 2.53 – 2.46 (t, *J* = 9.0 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 154.5, 144.5, 137.2, 132.4, 128.6, 127.3, 126.8, 126.2, 125.6, 118.8, 60.4, 52.5, 43.9.

3.1.3.11 Preparation of **4-(4-nitrobenzyl)-*N*-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 92**



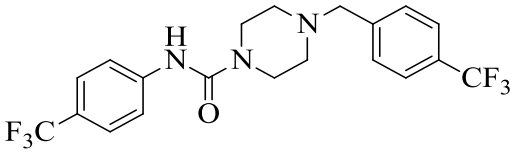
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 77 % yield as a grey solid. Mpt 156-158 ºC. V_{max} (neat) cm^{-1} 3151.7, 2946.8, 2903.4, 2857.6, 2797.4, 1650.0, 1602.2, 1511.9, 1315.6, 1208.0, 1112.7, 1182.8. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.23 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.6 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 6.52 (s, 1H), 3.72 (s, 2H), 3.63 – 3.57 (t, *J* = 12.0 Hz, 4H),), 2.56 – 2.48 (t, *J* = 12.0 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 154.9, 147.3, 146.5, 144.3, 129.5, 125.7, 123.3, 118.7, 61.7, 52.5, 43.9.

3.1.3.12 Preparation of **4-benzyl-*N*-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 93**



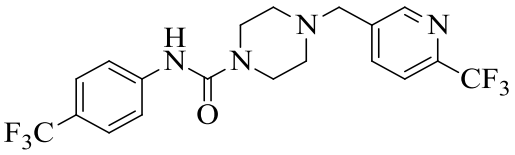
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 78 % yield as an amorphous solid. Mpt 141-143 ^{o}C. V_{max} (neat) cm^{-1} 3113.1, 2922.7, 2852.8, 2814.3, 1643.1, 1602.0, 1537.7 1313.4, 1107.4, 1065.3. ^{1}H-NMR (500 MHz, Acetone) δ 8.20 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 4.6 Hz, 2H), 7.24-7.17 (dd, *J* = 3.4, 4.6 Hz, 2H), 7.15-7.10 (d, *J* = 3.4 Hz, 1H), 6.59 (s, 1H), 3.44-3.84 (t, *J* = 11.0 Hz, 4H), 3.08 (s, 2H), 2.35 – 2.26 (t, *J* = 11.0 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 154.5, 144.4, 138.3, 128.9, 128.2, 127.0, 125.5, 118.9, 62.3, 52.3, 44.0.

3.1.3.13 Preparation of **4-(4-(trifluoromethyl)benzyl)-*N*-(4-(trifluoromethyl)phenyl) piperazine-1-carboxamide, 94**



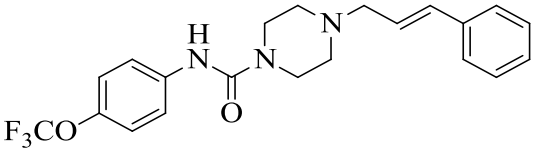
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 84 % yield as a grey solid. Mpt 161-162 ^{o}C. V_{max} (neat) cm^{-1} 3151.7, 2936.8, 2913.4, 2887.6, 1720.0, 1622.2, 1501.9, 1228.0, 1122.7, 1122.8. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.20 (d, *J* = 8.2 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 6.45 (s, 1H) 3.62 (s, 2H), 3.63 – 3.57 (t, *J* = 11.0 Hz, 4H),), 2.56 – 2.48 (t, *J* = 11.0 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 159.9, 153.2, 148.3, 146.9, 144.3, 128.5, 126.7, 121.3, 119.7, 62.7, 52.6, 44.9.

3.1.3.14 Preparation of ***N*-(4-(trifluoromethyl)phenyl)-4-((6-(trifluoromethyl)pyridin-3-yl)methyl)piperazine-1-carboxamide, 95**



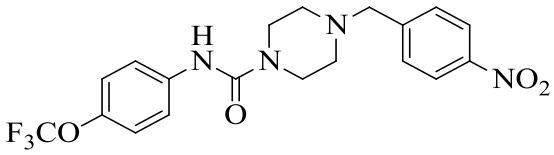
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 73 % yield as a white powder. Mpt. 124-125 ºC. V_{max} (neat) cm^{-1} 3105.8, 2951.3, 2860.1, 1709.0, 1620.1, 1506.2, 1134.3, 1101.6, 1010.4, 1000.1 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.68 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 2.1 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.31 (d, *J* = 2.1 Hz, 1H), 6.66 (s, 1H), 3.62 (s, 2H), 3.52 (t, *J* = 5.0 Hz, 4H), 2.50 (t, *J* = 5.0 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 161.4, 154.4, 150.5, 147.6, 147.4, 142.3, 137.9, 136.9, 126.3, 120.4, 119.9, 59.6, 52.8, 44.2, 32.0, 30.3, 29.8, 29.9, 22.8, 14.2.

3.1.3.15 Preparation of **4-cinnamyl-*N*-(4-(trifluoromethoxy)phenyl)piperazine-1-carboxamide, 96**



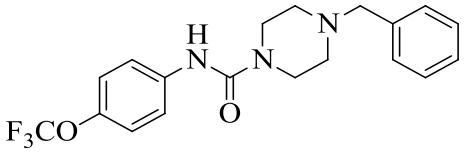
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 90 % yield as a white powder Mpt 128-129 ºC. V_{max} (neat) cm^{-1} 3134.3, 2925.1, 2857.6, 2807.0, 1729.7, 1638.5, 1509.4, 1155.4. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.02 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 7.4 Hz, 2H), 7.19 (t, *J* = 7.6 Hz, 2H), 7.10 (t, *J* = 7.3 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.46 (d, *J* = 15.9 Hz, 1H), 6.19 (dt, *J* = 15.9, 6.6 Hz, 1H), 3.45 – 3.37 (m, 1H), 3.07 – 3.03 (m, 4H), 3.07 – 3.00 (m, 2H), 2.41 – 2.33 (m, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 154.8, 143.3, 140.1, 137.1, 132.7, 128.5, 127.4, 126.7, 126.2, 121.1, 120.4, 60.4, 52.5, 44.0.

3.1.3.16 Preparation of **4-(4-nitrobenzyl)-*N*-(4-(trifluoromethoxy)phenyl)piperazine-1-carboxamide, 97**



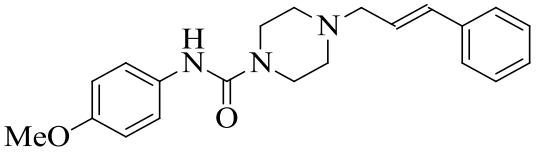
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 81 % yield as a white solid. Mpt 127-129 ^{o}C. V_{max} (neat) cm^{-1} 3110.7, 2942.0, 2802.2, 1637.2, 1513.0, 1347.5, 1239.4, 1002.3. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.23 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 6.56 (s, 1H), 3.60 – 3.54 (t, J = 9.0 Hz, 4H), 2.88 (s, 2H), 2.56 – 2.48 (t, J = 9.0 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 154.7, 147.3, 146.7, 143.3, 139.9, 129.7, 123.2, 121.2, 120.4, 61.7, 52.29, 44.0.

3.1.3.17 Preparation of **4-benzyl-*N*-(4-(trifluoromethoxy)phenyl)piperazine-1-carboxamide, 98**



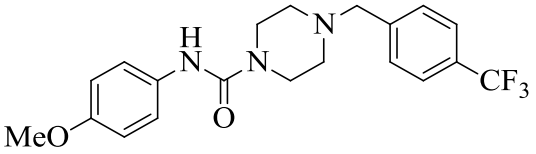
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 84 % yield as a white solid. Mpt 128-130 ^{o}C. V_{max} (neat) cm^{-1} 3119.2, 2925.1, 2816.67, 1637.9, 1234.6, 1198.7, 1150.8. ^{1}H-NMR (500 MHz, Acetone) δ_H 8.15 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 6.8 Hz, 2H), 7.39-7.31 (dd, J = 7.7, 6.8 Hz, 2H), 7.27 (d, J = 7.7 Hz, 1H), 7.19 (d, J = 9.0 Hz, 2H), 6.55 (s, 1H), 3.55 (t, J = 14.2 Hz, 4H), 2.88 (s, 2H), 2.48 – 2.42 (t, J = 14.2 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 156.5, 140.0, 138.2, 128.9, 128.1, 127.0, 121.2, 120.4, 62.3, 52.3, 43.6.

3.1.3.18 Preparation of **4-cinnamyl-*N*-(4-methoxyphenyl)piperazine-1-carboxamide, 99**



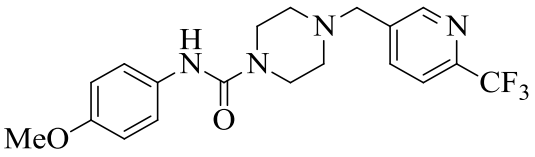
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 94 % yield as a yellowish powder. Mpt. 139-141 ^{o}C. V_{max} (neat) cm^{-1} 3146.8, 2923.32, 2810.1, 1679.0, 1641.1, 1586.2, 1261.5, 1124.3, 1102.4, 1001.5, 933.8 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.53 (d, *J* = 7.0 Hz, 2H), 7.39 (d, *J* = 7.0 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 2H), 7.29 (dd, *J* = 8.6, 7.6 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H), 6.56 (d, *J* = 6.5 Hz, 1H), 6.36 – 6.23 (d, *J* = 6.5 Hz, 2H), 3.77 (dt, *J* = 6.5, *J* = 6.8 Hz, 1H), 3.55 (t, *J* = 5.1 Hz, 4H), 3.23 (d, *J* = 6.8 Hz, 1H), 2.58 (t, *J* = 5.1 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 160.4, 156.1, 155.6, 136.6, 132.0, 128.8, 127.9, 126.5, 122.8, 122.5, 114.3, 114.2, 60.9, 55.5, 52.7, 43.9, 43.7.

3.1.3.19 Preparation of ***N*-(4-methoxyphenyl)-4-(4-(trifluoromethyl)benzyl)piperazine-1-carboxamide, 100**



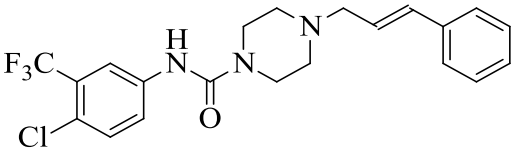
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 81 % yield as a creamy crystalline solid. Mpt.169-171 ^{o}C. V_{max} (neat) cm^{-1} 3236.8, 2903.32, 2810.1, 1649.0, 1601.1, 1546.2, 1124.3, 1111.6, 1100.4, 1001.5, 939.8. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.45 (d, *J* = 2.3 Hz, 2H), 7.24 (d, *J* = 2.3 Hz, 2H), 6.83 (d, *J* = 2.8 Hz, 2H), 6.82 (d, *J* = 2.8 Hz, 2H), 6.26 (s, 1H), 3.58 (s, 2H), 3.48 (t, *J* = 5.8, Hz, 4H), 2.49 – 2.46 (t, *J* = 5.8, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 162.0, 156.1, 155.6, 142.2, 132.0, 129.8, 129.5, 126.9, 125.4, 125.4, 123.2, 122.5, 114.2, 62.6, 55.6, 52.8, 44.2.

3.1.3.20 Preparation of ***N*-(4-methoxyphenyl)-4-((6-(trifluoromethyl)pyridin-3-yl)methyl) piperazine-1-carboxamide, 101**



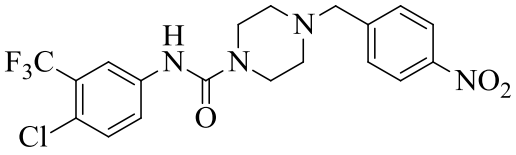
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 90 % yield as a white powder. Mpt. 152-153 ^{o}C. V_{max} (neat) cm^{-1} 3111.4, 2951.3, 2820.1, 1610.1, 1556.2, 1204.3, 1114.3, 1101.5, 1013.4, 1002.2 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.68 (d, *J* = 2.1 Hz, 1H), 8.32 (d, *J* = 2.1 Hz, 1H) 7.87 (s, 1H), 7.66 (d, *J* = 9.7 Hz, 2H), 7.24 – 7.20 (d, *J* = 9.7 Hz, 2H), 6.31 (s, 1H), 3.61 (s, 2H), 3.48 (t, *J* = 5.1 Hz, 4H), 2.48 (t, *J* = 5.1 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 159.6, 156.1, 155.6, 150.5, 147.6, 147.3, 137.9, 137.0, 131.9, 129.1, 122.8, 122.6, 120.6, 120.3, 114.2, 59.6, 55.6, 52.8, 44.1, 29.8.

3.1.3.21 Preparation of ***N*-(4-chloro-3-(trifluoromethyl)phenyl)-4-cinnamylpiperazine-1-carboxamide, 102**



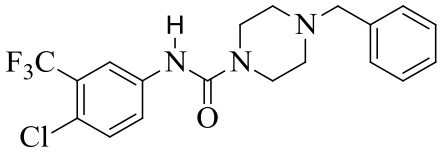
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 94 % yield as a white powder. Mpt. 98-99 ^{o}C. V_{max} (neat) cm^{-1} 3201.4, 3015.8,, 2881.3, 2820.1, 1702.1, 1602.2, 1204.3, 1013.4, 1003.2 ,989.3 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.60 (d, *J* = 8.6 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.28 (dd, *J* = 8.0, 8.2 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.21 (s, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 15.0 Hz, 2H), 6.14 (dt, *J* = 6.9, 15.0 Hz, 1H), 3.48 (t, *J* = 11.4 Hz, 4H), 3.13 (d, *J* = 6.9 Hz, 1H), 2.47 (t, *J* = 11.4 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 161.1, 154.7, 138.3, 136.4, 134.6, 131.6, 128.7, 128.4, 128.0, 126.5, 125.4, 124.4, 121.7, 119.1, 60.7, 52.5, 43.7, 32.0, 29.7, 22.7, 14.2.

3.1.3.22 **Preparation of *N*-(4-chloro-3-(trifluoromethyl)phenyl)-4-(4-nitrobenzyl)piperazine-1-carboxamide, 103**



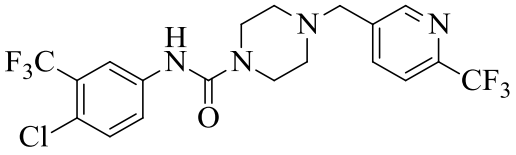
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 86 % yield as a white powder. Mpt. 134-136 ^{o}C. V_{max} (neat) cm^{-1} 3146.8, 2963.2, 2840.1, 1699.0, 1621.1, 1536.2, 1281.5, 1123.3, 1101.4, 1001.7, 933.8, 914.6. ^{1}H-NMR (500 MHz, Acetone-d_6)) δ_H 8.19 – 8.14 (s, 1H), 8.04 (d, J = 11.3 Hz, 2H), 7.77 (d, J = 8.8, Hz, 2H), 7.62 (d, J = 11.3, Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 6.55 (s, 1H), 3.67 (s, 2H), 2.02 (t, J = 4.4 Hz, 4H), 1.26 (t, J = 4.4 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 155.3, 151.4, 148.1, 147.5, 141.2, 132.4, 132.4, 130.5, 127.8, 125.1, 124.5, 124.2, 118.8, 63.6, 62.3, 53.7, 44.6.

3.1.3.23 **Preparation of 4-benzyl-*N*-(4-chloro-3-(trifluoromethyl)phenyl)piperazine-1-carboxamide, 104**



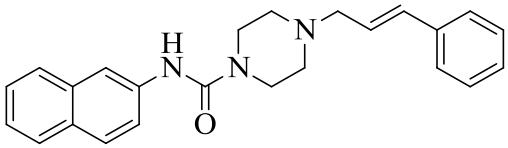
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 89 % yield as a white powder. Mpt 125-127 ^{o}C. V_{max} (neat) cm^{-1} 3155.4, 2893.2, 2831.1, 1698.9 1609.0, 1546.2, 1261.5, 1103.3, 1190.4, 1000.5, 918.9. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.66 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 7.45 (s, 1H), 7.37 (s, 1H), 7.33 (d, J = 6.1 Hz, 2H), 7.29 (dd, J = 6.1, 2.6 Hz, 2H), 7.14 (d, J = 2.6 Hz, 2H) 6.65 (s, 1H), 3.55 (s, 2H), 2.49 (t, J = 9.9 Hz, 4H), 1.26 (t, J = 9.9 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 162.0, 154.3, 138.0, 137.3, 131.7, 129.8, 128.4, 127.1, 125.4, 123.8, 123.7, 121.5, 118.7, 62.8, 52.5, 44.0, 31.3, 29.0.

3.1.3.24 Preparation of ***N*-(4-chloro-3-(trifluoromethyl)phenyl)-4-((6-(trifluoromethyl)pyridin-3-yl)methyl)piperazine-1-carboxamide, 105**



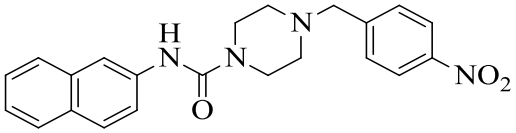
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 83 % yield as a grey coloured powder. Mpt. 187-189 ^{o}C. V_{max} (neat) cm^{-1} 3126.8, 2883.2, 2810.1, 1689.0, 1601.1, 1556.2, 1231.5, 1133.3, 1100.4, 1001.7, 901.6, 871 ^{1}H-NMR (500 MHz, Acetone-d_6) δ_H 8.73 (s, 1H), 8.15 – 7.99 (d, J = 2.8 Hz, 1H), 7.88 (d, J = 2.8 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 6.67 (s, 1H), 3.73 (s, 2H), 3.61 – 3.58 (t, J = 2.2 Hz, 4H), 2.06 (t, J = 2.2 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 155.2, 151.5, 149.4, 147.0, 141.0, 139.0, 136.5, 132.4, 125.2, 124.3, 124.5, 123.0, 121.1, 118.9, 61.7, 59.9, 53.6, 44.9, 44.6.

3.1.3.25 Preparation of **4-cinnamyl-*N*-(naphthalen-2-yl)piperazine-1-carboxamide, 106**



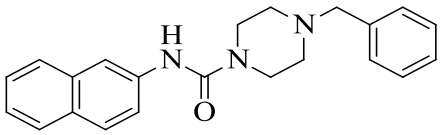
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 90 % yield as a white solid. Mpt 188-190 ^{o}C. V_{max} (neat) cm^{-1} 3031.2, 2942.0, 2910.7, 2761.2, 1601.1, 1635.8, 1543.1, 1276.6, 1003.9, ^{1}H-NMR (500 MHz, DMSO) δ_H 8.72 (s, 1H), 8.02 (s, 1H), 7.78 (dd, J = 8.4, 5.0 Hz, 2H), 7.73 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.8, 1.4 Hz, 1H), 7.46 (d, J = 7.7 Hz, 2H), 7.42 (t, J = 8.8 Hz, 1H), 7.33 (t, J = 7.7 Hz, 2H), 7.24 (d, J = 1.4 Hz, 1H), 6.57 (d, J = 15.8 Hz, 1H), 6.33 (dt, J = 15.8, 6.5 Hz, 1H), 3.55 – 3.47 (t, J = 9.8 Hz, 4H), 3.15 (d, J = 6.5 Hz, 2H), 2.48 – 2.42 (t, J = 9.8 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 161.2, 155.5, 138.7, 137.1, 134.0, 132.6, 129.5, 129.1, 128.2, 127.9, 127.8, 127.4, 127.3, 126.7, 126.5, 124.2, 121.5, 115.3, 60.1, 52.8, 44.0.

3.1.3.26 Preparation of ***N*-(naphthalen-2-yl)-4-(4-nitrobenzyl)piperazine-1-carboxamide, 107**



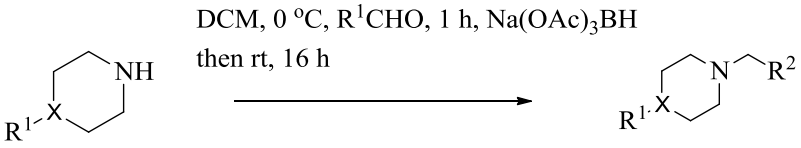
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 92 % yield as a white solid. Mpt 227-229 ºC. V_{max} (neat) cm^{-1} 3240.8, 2946.8, 2896.2, 2795.0, 1636.1, 1502.9, 1342.1, 1277.2. ^{1}H-NMR (500 MHz, DMSO) δ_H 8.73 (s, 1H), 8.22 (d, *J* = 8.3 Hz, 2H), 8.01 (s, 1H), 7.77 (dd, *J* = 11.7, 6.5 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 1H), 7.67 (d, *J* = 11.7 Hz, 2H), 7.55 (d, *J* = 6.5 Hz, 2H), 7.53 – 7.50 (m, 3H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.34 (t, *J* = 7.0 Hz, 1H), 6.77 (s, 1H), 3.67 (s, 2H), 3.52 (t, *J* = 7.7 Hz, 4H), 2.44 (t, *J* = 7.7 Hz, 4H). ^{13}C-NMR (126 MHz, DMSO) δ_C 155.4, 147.1, 146.8 138.6 134.0, 130.3, 129.5, 128.2, 127.8, 127.4, 126.5, 124.2, 123.8, 121.4, 115.3, 61.5, 53.1, 43.9.

3.1.3.27 Preparation of **4-benzyl-*N*-(naphthalen-2-yl)piperazine-1-carboxamide, 108**



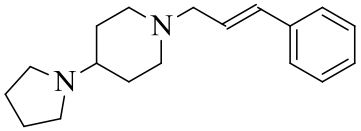
This compound was prepared according to the general procedure for making piperazine-1-carboxamides in 84 % yield as a white solid. Mpt 189-191 ºC. V_{max} (neat) cm^{-1} 3060.1, 3026.3, 2799.8, 2758.8, 1635.8, 1541.4, 1496.9, 1248.1, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.83 (s, 1H), 8.62 (d, *J* = 8.3 Hz, 2H), 8.01 (s, 1H), 7.77 (dd, *J* = 11.7, 6.5 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 1H), 7.67 (d, *J* = 11.7 Hz, 2H), 7.55 (d, *J* = 6.5 Hz, 2H), 7.53 – 7.50 (d, *J* = 4.6 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.34 (t, *J* = 7.0 Hz, 1H), 7.31 (dt, *J* = 4.6, 3.4 Hz, 1H), 7.18 (d, *J* = 3.4 Hz, 2H), 6.57 (s, 1H), 3.59 – 3.53 (t, *J* = 7.8 Hz, 4H), 2.55 – 2.48 (t, *J* = 7.8 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 162.2, 155.0, 136.5, 134.0, 130.1, 129.2, 128.6, 1284, 127.5, 127.3, 126.3, 124.5, 120.5, 115.9, 62.8, 52.8, 44.5.

3.1.4 **General procedure for making the non-urea compounds**



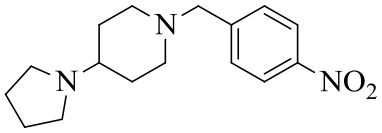
To a solution of the 4-(1pyrrolidinyl)piperidine (0.3 g 1.945 mmol, 1eq) in 10 mL of DCM was cooled in an ice bath and treated with 4-nitrobenzaldehyde (0.294 g, 1.945 mmol, 0.19 mL, 1 eq) after 1 hour the bath was removed and the resulting mixture stirred at room temperature for 30 min. Sodium triacetoxy borohydride (0.615 g 2.9 mmol, 1.5 eq) was then added, stirred at rt for 16 hours. The reaction was quenched by adding saturated $NaHCO_3$ and extracted with DCM (3 X 20 mL). The combined organic extracts was washed with brine, dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography afforded the required amine product.

3.1.4.1 Preparation of **1-cinnamyl-4-(pyrrolidin-1-yl)piperidine, 109**



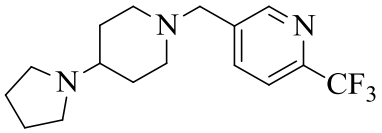
This compound was prepared according to the general procedure for making the non-urea derivatives in 79 % yield as a pale yellow powder that decomposed at 294 °C. V_{max} (neat) cm^{-1} 2903.2, 2830.1, 1609.0, 1516.2, 1201.5, 1113.3, 1005.7, 944.6. ^{1}H-NMR (500 MHz, , Acetone-d_6) δ_H 7.51 (t, J = 7.8 Hz, 2H), 7.38 (dt, J = 7.8, 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 1H), 6.84 (d, J = 15.8 Hz, 2H), 6.36 (dt, J = 15.8, 7.2 Hz, 2H), 3.40 (d, J = 17.4 Hz, 2H), 3.34 (p, J = 4.6 Hz, 4H), 2.82 (t, J = 13.3 Hz, 2H), 2.38 (dt, J = 13.3, 3.2 Hz, 1H), 2.12 (qd, J = 7.2, 4.6, 3.2 Hz, 4H), 2.06 – 1.98 (t, J = 7.2 Hz, 4H). ^{13}C-NMR (126 MHz, Acetone) δ_C 139.7, 137.1, 129.7, 129.6, 127.8, 120.5, 61.2, 60.1, 53.0, 51.5, 49.5, 48.4, 28.2, 23.9.

3.1.4.2 Preparation of **1-(4-nitrobenzyl)-4-(pyrrolidin-1-yl)piperidine, 110**



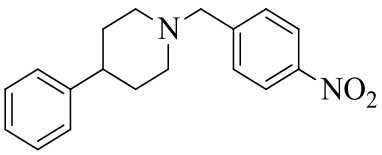
This compound was prepared according to the general procedure for making the non-urea derivatives in 75 % as a pale grey powder. Mpt. 88-89 ºC. V_{max} (neat) cm^{-1} 2893.2, 2870.1, 1516.2, 1261.5, 1126.3, 1015.7, 949.6 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.51 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 3.80 (s, 2H) 3.48 (td, *J* = 12.4, 5.2 Hz, 4H), 3.34 (t, *J* = 5.2 Hz, 1H), 2.81 (t, *J* = 12.4 Hz, 4H), 2.48 (t, *J* = 9.1 Hz, 4H), 2.12 (t, *J* = 9.1 Hz, 4H), ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 138.1, 131.1, 125.6, 124.3, 122.4, 120.1, 80.2, 67.1, 59.5, 48.4, 29.1, 26.7.

3.1.4.3 Preparation of **5-((4-(pyrrolidin-1-yl)piperidin-1-yl)methyl)-2-(trifluoromethyl) pyridine, 111**



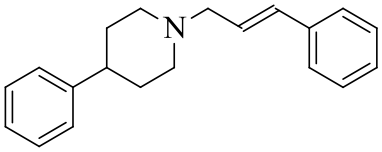
This compound was prepared according to the general procedure for making the non-urea derivatives in 74 % yield as a white powder. Mpt. 163-165 ºC. V_{max} (neat) cm^{-1} 2833.2, 2830.1, 1609.5, 1506.2, 1201.5, 1003.3, 1100.5 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.59 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 3.68 (s, 2H), 3.41 (t, *J* = 6.4 Hz, 4H), 2.95 (dq, *J* = 2.7, 5.8 Hz, 4H), 2.55 (q, *J* = 6.4 Hz, 4H), 2.08 (d, *J* = 2.7 Hz, 1H), 2.07 (t, *J* = 5.8 Hz, 4H), 2.05 (s, 1H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 150.3, 147.6, 147.3, 137.9, 137.8, 122.7, 120.5, 120.4, 120.4, 120.4, 62.4, 58.9, 51.7, 50.8, 27.8, 23.8.

3.1.4.4 Preparation of **1-(4-nitrobenzyl)-4-phenylpiperidine, 112**



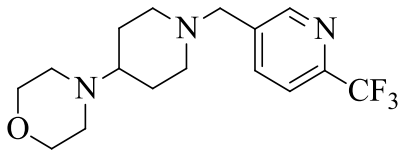
This compound was prepared according to the general procedure for making the non-urea derivatives in 73 % as a white powder. Mpt. 97-98 ^{o}C. V_{max} (neat) cm^{-1} 2883.2, 2830.1, 1689.0, 1651.1, 1506.2, 1201.5, 1113.3, 1111.4, 1100.5 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.20 (d, *J* = 2.0 Hz, 2H), 8.18 (d, *J* = 2.0 Hz, 2H), 7.57 – 7.52 (m, 1H), 7.31 (t, *J* = 10.4 Hz, 2H), 7.25 (d, *J* = 1.6 Hz, 1H), 7.21 – 7.18 (m, 1H), 3.63 (s, 2H), 3.00 – 2.94 (m, 3H), 2.52 (td, *J* = 10.4, 5.3 Hz, 1H), 2.16 (td, *J* = 10.4, 3.7 Hz, 4H), ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 148.1, 147.0, 146.6, 146.5, 129.3, 128.3, 126.8, 126.7, 126.0, 123.6, 123.3, 63.8, 62.4, 54.3, 42.3, 33.3,

3.1.4.5 Preparation of **1-cinnamyl-4-phenylpiperidine, 113**



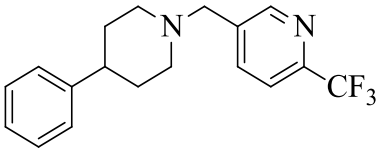
This compound was prepared according to the general procedure for making the non-urea derivatives in 71 % yield as a colourless oil. V_{max} (neat) cm^{-1} 2913.2, 2810.1, 1699.0, 1601.1, 1506.2, 1230.5, 1103.3, 1110.4, 1002.7, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 7.41 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 1H), 7.24 (d, *J* = 7.3 Hz, 1H), 7.24 – 7.18 (d, *J* = 8.5 Hz, 1H), 3.20 – 3.14 (m, 2H), 2.54 (dt, *J* = 9.1, 6.6 Hz, 1H), 2.15 (dd, *J* = 8.5, 6.6 Hz, 1H), 1.88 (m, *J* = 9.1, 3.6 Hz, 4H), 1.28 (t, *J* = 3.6 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 146.8, 136.9, 133.3, 128.7, 128.6, 128.4, 128.4, 127.5, 126.9, 126.4, 126.9, 126.2, 61.3, 54.2, 42.9 3.3, 29.7, 22.7.

3.1.4.6 Preparation of **4-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)piperidin-4-yl)morpholine, 114**



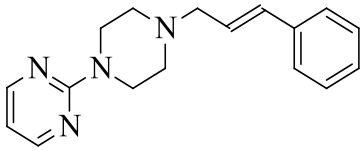
This compound was prepared according to the general procedure for making the non-urea derivatives in 72 % yield as a white powder. Mpt 86-87 ^{o}C. V_{max} (neat) cm^{-1} 2923.2, 2831.1, 1651.1, 1506.2, 1206.5, 1103.3, 1100.5, 1004.9 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.63 (s, 1H), 7.83 (d, *J* = 8.1, Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 3.74 – 3.66 (t, *J* = 2.6 Hz, 4H), 3.55 (s, 2H), 2.87 (dq, *J* = 11.5, 2.5, 2.1 Hz, 2H), 2.57 – 2.48 (m, 4H), 2.18 (t, *J* = 11.8, Hz, 1H), 2.02 (td, *J* = 11.8, 2.5 Hz, 2H), 1.80 (dq, *J* = 12.0, 2.5 Hz, 2H), 1.53 (qd, *J* = 12.0, 2.1 Hz, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 150.4, 147.2, 146.9, 137.9, 137.7, 125.0, 122.8, 120.6, 120.2, 67.4, 62.5, 59.7, 53.1, 49.9, 29.8, 28.3.

3.1.4.7 Preparation of **5-((4-phenylpiperidin-1-yl)methyl)-2-(trifluoromethyl)pyridine, 115**



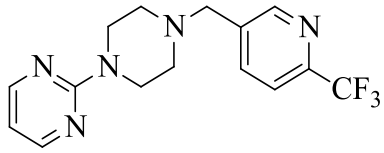
This compound was prepared according to the general procedure for making the non-urea derivatives in 78 % yield as a white powder. Mpt. 102-104 ^{o}C. V_{max} (neat) cm^{-1} 2983.2, 2891.1, 1649.0, 1556.2, 1231.5, 1101.3, 1130.4, 1010.5, 916.6, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.69 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 2H), 7.21 (d, *J* = 7.2 Hz, 2H), 3.70 (s, 2H), 3.04 (d, *J* = 10.5 Hz, 1H), 2.58 – 2.45 (m, 1H), 2.22 (td, *J* = 10.5, 6.9 Hz, 1H), 2.15 (d, *J* = 1.8 Hz, 4H), 1.84 (tt, *J* = 9.0, 3.4 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 175.5, 150.8, 147.4, 145.7, 138.4, 136.2, 128.5, 126.8, 126.4, 122.7, 120.3, 59.8, 54.2, 42.1, 32.8, 30.9, 29.7, 21.4.

3.1.4.8 Preparation of **(*E*)-2-(4-cinnamylpiperazin-1-yl)pyrimidine, 116**



This compound was prepared according to the general procedure for making the non-urea derivatives in 81 % yield as a white powder. Mpt. 70-72 ^{o}C. V_{max} (neat) cm^{-1} 2943.2, 2820.1, 1709.0, 1621.1, 1536.2, 1230.5, 1103.3, 1015.7, 948.6 ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.30 (dd, *J* = 6.9, 1.3 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.33 – 7.29 (m, 1H), 7.24 (dt, *J* = 8.0, 7.2 Hz, 2H), 6.57 – 6.44 (m, 2H), 6.31 (td, *J* =, 6.9, 1.3 Hz, 1H), 3.86 (t, *J* = 5.1 Hz, 4H), 3.20 (dd, *J* = 7.2, 1.5 Hz, 1H), 2.57 (t, *J* = 5.1 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 162.0, 158.0, 137.2, 133.6, 128.9, 128.7, 127.9, 126.9, 126.3, 126.6, 110.2, 61.5, 53.4, 44.6.

3.1.4.9 Preparation of **2-(4-((6-(trifluoromethyl)pyridin-3-yl)methyl)piperazin-1-yl)pyrimidine, 117**



This compound was prepared according to the general procedure for making the non-urea derivatives in 78 % yield as a creamy powder. Mpt 99-100 ^{o}C. V_{max} (neat) cm^{-1} 2880.2, 2810.1, 1609.0, 1506.2, 1231.5, 1163.3, 1123.4, 1000.5. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 8.68 (d, *J* = 3.6 Hz, 1H), 8.31 (d, *J* = 3.6 Hz, 1H), 8.28 (d, *J* = 4.4 Hz, 2H), 7.93 – 7.82 (m, 1H), 7.71 – 7.62 (s, 1H), 6.47 (d, *J* = 4.4 Hz, 1H), 3.82 (q, *J* = 4.5 Hz, 4H), 3.61 (s, 2H), 2.50 (q, *J* = 4.5 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ_C 157.7, 150.4, 148.4, 147.3, 147.0, 137.8, 137.9, 135.5, 122.7, 120.5, 120.2, 109.9, 61.8, 59.7, 53.0.

3.2 ***P. falciparum* (3D7) *in vitro* assay**

Widely used malaria reference strain Chloroquine-sensitive *P. falciparum strain* 3D7 cultures were maintained in a 5 % suspension of human red blood cells culture in RPMI 1640 medium (pH 7.3). These were supplemented with 0.5% Albumax II, 12 mM sodium bicarbonate, 0.2 mM hypoxanthine, and 20 mg/L gentamicin at 37°C, in 1% O_2, 3% CO_2 atmosphere and a nitrogen

balance (Trager & Jensen, 1976). Fluorescence assay was used to quantify growth inhibition, utilising the binding of SYBR Green (Bennett et al., 2004) to double stranded DNA which after excitation at 485 nm emitted a fluorescent signal at 528 nm. Mefloquine was the control drug used to monitor the quality of the assay. Compound bioactivity was expressed as IC_{50}. The most potent hits from each series identified by the primary screening were reconfirmed by testing in a [^{3}H]-Hypoxanthine incorporation assay (Jiang et al., 2016)

3.3 *In vitro* Cell Assay Data Analysis

All data were processed using IDBS ActivityBase. Raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: z'> 0.5, S:B> 3, $\%CV_{(\text{no inhibition control})}$ < 15. Z' was calculated with;

$$1 - \frac{3 \text{ x } (StDev\_high + StDev\_low)}{\text{ABS(Mean\_high- Mean\_low)}}$$

Curve fitting was calculated with the formula;

$$y = A + \frac{B - A}{1 + (C/x)^D}$$

Where A = % inhibition at bottom, B = % inhibition at the top, C = IC_{50}, D = slope, x = inhibitor concentration and y = % inhibition. B was fixed to 100 if curve definition was poor. (Baragaña et al., 2015)

3.4 **Physicochemical parameters**

Physicochemical parameters of all synthesized compounds were calculated using the Virtual Computational Chemistry Laboratory (VCCLAB) http://www.vcclab.org

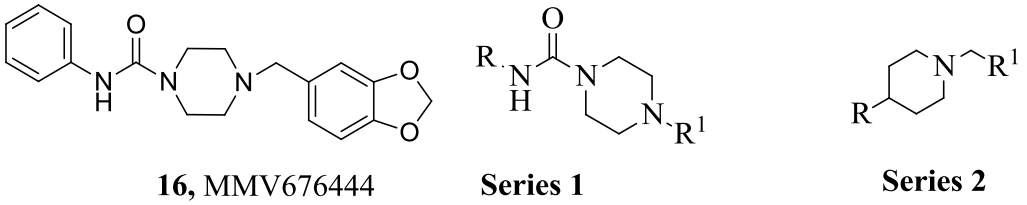
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter focuses on the results of the various experiments conducted and their meaning, significance and how they compare with theoretical data.

4.1 **Chemistry**

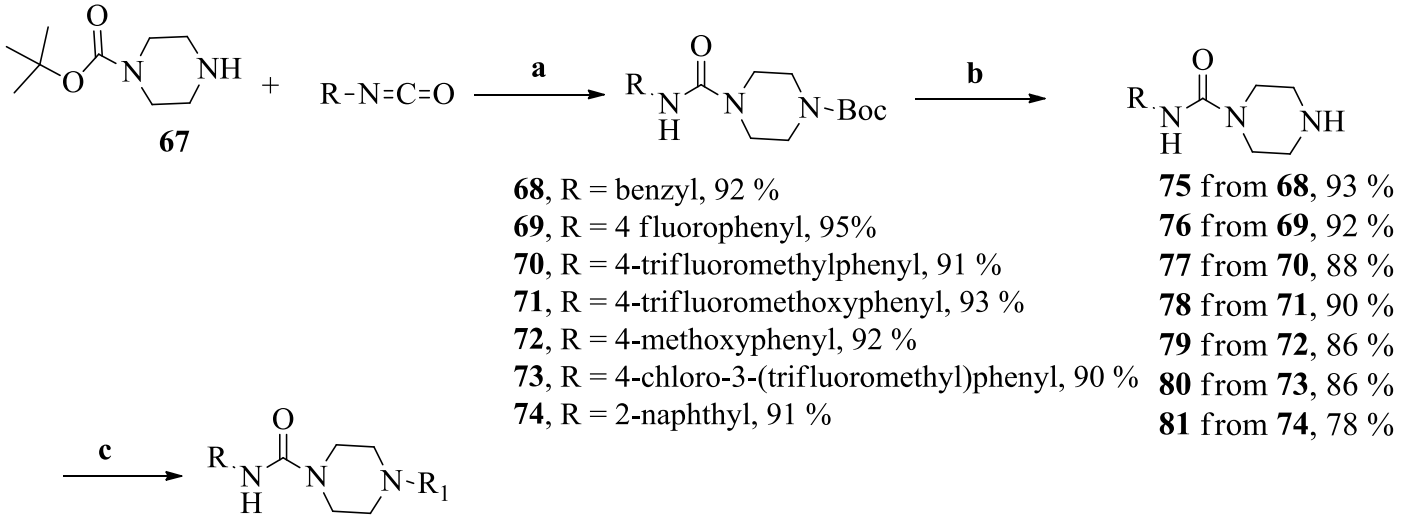
Two series of compounds were synthesized in 1-3 chemical steps according to available literature procedures (Takahashi et al., 2013) in very high to excellent yields. The **Series 1** compounds were based on the **MMV676444** scaffold containing the urea functionality while **Series 2** were designed without the urea functionality found in **Series 1**. MMV676444 which is a scaffold selected from the pathogen box possess anti-TB activity and it is expected that analogues of it will have even better MIC values.



16, MMV676444 **Series 1** **Series 2**

Figure 4.1: Series of compounds synthesized

The **Series 1** compounds were synthesized according to **Scheme 4.1** below. First, Boc-protected piperazine **67** was reacted with a variety of substituted isocyanates according to the method developed by Takahashi and co-workers (Takahashi et al., 2013) to give intermediates (6**8-74**) in excellent yields.



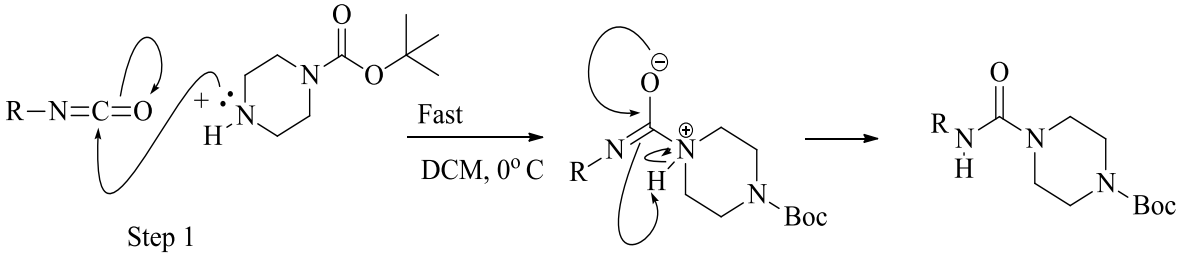
68, R = benzyl, 92 %
69, R = 4 fluorophenyl, 95%
70, R = 4-trifluoromethylphenyl, 91 %
71, R = 4-trifluoromethoxyphenyl, 93 %
72, R = 4-methoxyphenyl, 92 %
73, R = 4-chloro-3-(trifluoromethyl)phenyl, 90 %
74, R = 2-naphthyl, 91 %

75 from **68**, 93 %
76 from **69**, 92 %
77 from **70**, 88 %
78 from **71**, 90 %
79 from **72**, 86 %
80 from **73**, 86 %
81 from **74**, 78 %

82, JAK 001, R = benzyl, R^1 = cinnamyl, 83 %
83, JAK 002, R = benzyl, R^1 = 4-nitrobenzyl, 80 %
84, JAK 003, R = benzyl, R^1 = benzyl, 75 %
85, JAK 004, R = benzyl, R^1 = 6-(trifluoromethyl)pyridin-3-yl)methyl), 81 %
86, JAK 005, R = 4-fluorophenyl, R^1 = cinnamyl, 78 %
87, JAK 006, R = 4-fluorophenyl, R^1 = 4-nitrobenzyl, 75 %
88, JAK 007, R = 4-fluorophenyl, R^1 = benzyl, 79 %
89, JAK 008, R = 4-fluorophenyl, R^1 = 4-(trifluoromethyl)benzyl, 84 %
90, JAK 009, R = 4-fluorophenyl, R^1 = 6-(trifluoromethyl)pyridin-3-yl)methyl, 74 %
91, JAK 010, R = 4-(trifluoromethyl)phenyl, R^1 = cinnamyl, 82 %
92, JAK 011, R = 4-(trifluoromethyl)phenyl, R^1 = 4-nitrobenzyl, 77 %
93, JAK 012, R = 4-(trifluoromethyl)phenyl, R^1 = benzyl, 78 %
94, JAK 013, R = 4-(trifluoromethyl)phenyl, R^1 = 4-(trifluoromethyl)benzyl, 84 %
95, JAK 014, R = 4-(trifluoromethyl)phenyl, R^1 = 6-(trifluoromethyl)pyridin-3-yl)methyl, 73 %
96, JAK 015, R = 4-(trifluoromethoxy)phenyl, R^1 = cinnamyl, 90 %
97, JAK 016, R = 4-(trifluoromethoxy)phenyl, R^1 = 4- nitrobenzyl, 81 %
98, JAK 017, R = 4-(trifluoromethoxy)phenyl, R^1 = benzyl, 84 %
99, JAK 018, R = 4-methoxyphenyl, R^1 = cinnamyl, 94 %
100, JAK 019, R = 4-methoxyphenyl, R^1 = 4-(trifluoromethyl)benzyl, 81 %
101, JAK 020, R = 4-methoxyphenyl, R^1 = 6-(trifluoromethyl)pyridin-3-yl)methyl, 90 %
102, JAK 021, R = 4-chloro-3-(trifluoromethyl)phenyl, R^1 = cinnamyl, 94 %
103, JAK 022, R = 4-chloro-3-(trifluoromethyl)phenyl, R^1 = 4-nitrobenzyl, 86 %
104, JAK 023, R = 4-chloro-3-(trifluoromethyl)phenyl, R^1 = benzyl, 89 %
105, JAK 024, R = 4-chloro-3-(trifluoromethyl)phenyl, R^1 = 6-(trifluoromethyl)pyridin-3-yl)methyl, 83 %
106, JAK 025, R = 2-naphthyl, R^1 = cinnamyl, 90 %
107, JAK 026, R = 2-naphthyl, R^1 = 4-nitrobenzyl, 92 %
108, JAK 027, R = 2-naphthyl, R^1 = benzyl, 84 %

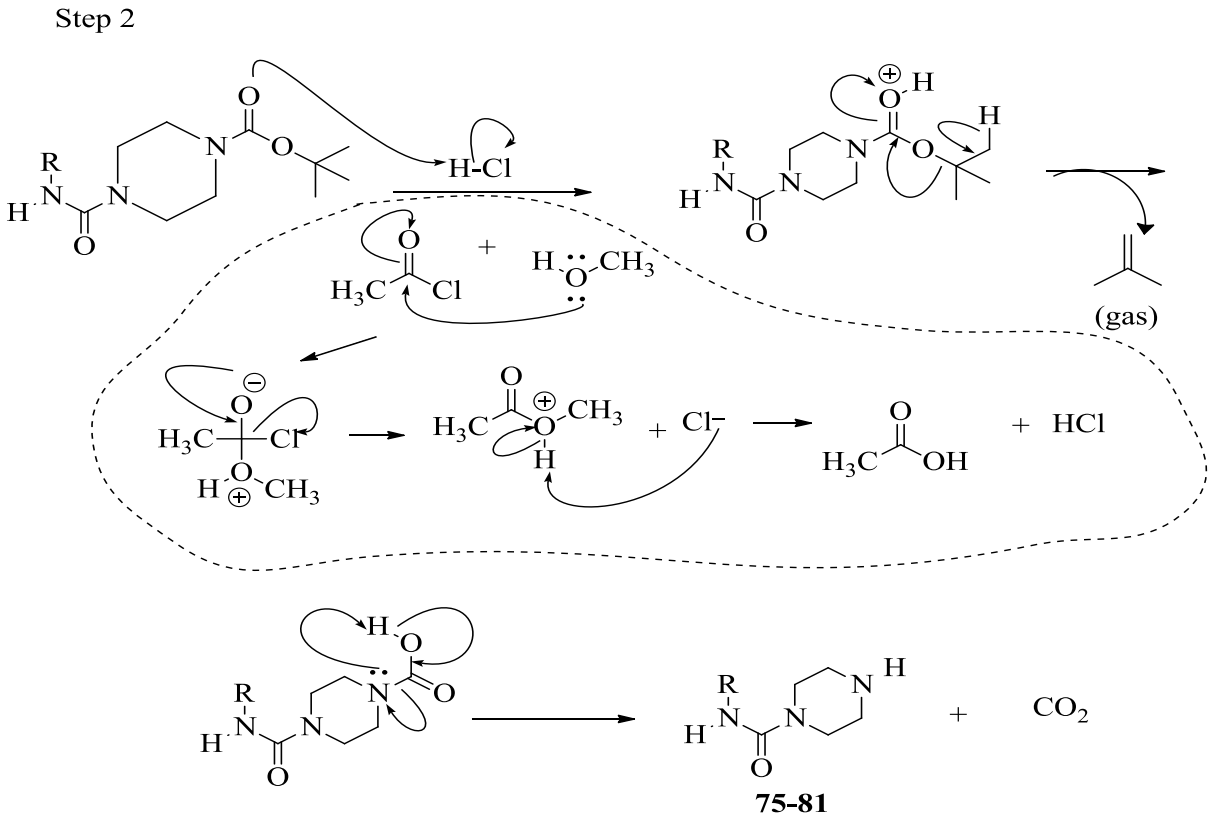
Scheme 4.1: a) DCM, 0 ^{o}C, 1 h, then rt, 16 h b) MeOH, acetyl chloride, 0 ^{o}C, 1 h, then rt, 16 h, c) DCM, 0 ^{o}C, R^{1}CHO, 1 h, $Na(OAc)_3BH$ then rt, 16 h

The mechanism for the reaction is shown in **Scheme 4.2** below. The lone pairs on the nitrogen in the boc-protected piperazine attack the carbonyl of the isocyanate which leads to the formation of the carboxylate ion. The double bond of the carbonyl reforms and the double bond between the nitrogen and the carbonyl carbon breaks to pick a proton from the nitronium ion of the piperazine ring resulting in the formation of the urea function (Making Polyurethane Foams, 2005).



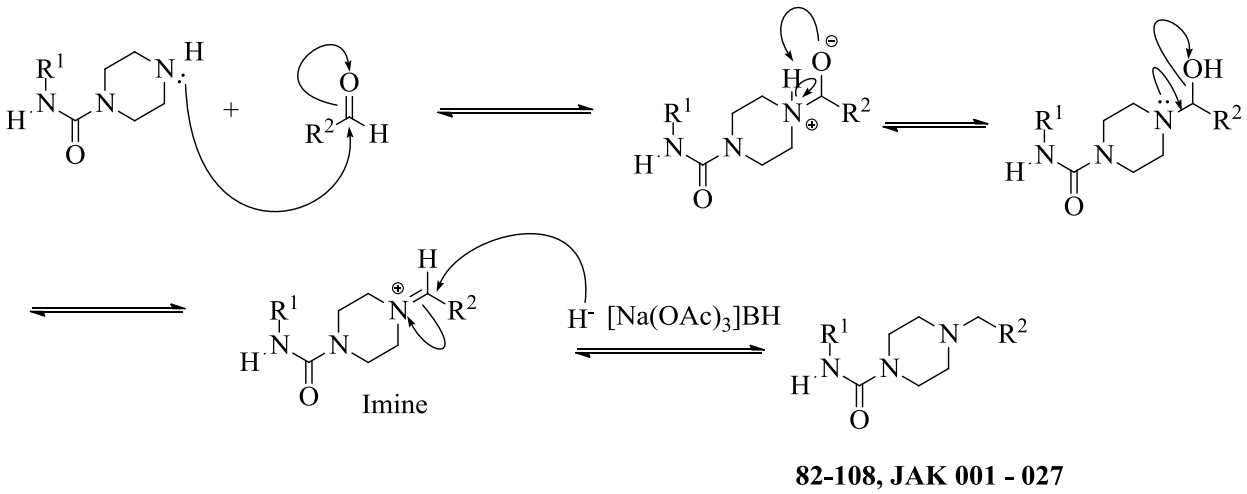
Scheme 4.2: Preparation of Boc-protected urea piperazine

This was followed by acid catalyzed de-protection of the Boc-group **Scheme 4.3** (Reaction, 2011; Goto et al., 2013)**.** The acid (HCl) required for the hydrolysis was generated *in situ* by reacting MeOH with Acetyl chloride. Two gases (2-methylprop-1-ene and carbon dioxide) were evolved in the exothermic reaction process to give the amines which were either used for the next step as hydrochloride salt or neutralized with a base to give the free amine (**75-81**).



Scheme 4.3: Mechanism for the de-protection reaction

Finally, the targeted compounds were synthesized according to **Schemes 4.4**. The resultant amines (free base or hydrochloride salts) generated in the previous step were reacted with different aldehydes by reductive amination to give the titled compounds **82-108**. The aldehydes used included benzaldehyde, cinnamaldehyde, 4-nitrobenzaldehyde, 4-(trifluoromethyl)benzyldehyde and for bioiosteric purposes, 6-(trifluoromethyl)nicotinaldehyde. The process involves two steps; imine formation when the piperazine derivative reacts with the aldehyde followed by reduction with a strong base sodium triacetoxyborohydride [$Na(OAc)_3BH$] (Takahashi et al., 2013)..



Scheme 4.4: Reductive amination of free amine to form title compound

All the synthesized compounds were characterized by 1D and 2D NMR and FTIR. The NMR was used to ascertain the purity and to confirm that the titled compounds were duly synthesized by intergration of the peak and chemical shifts. The FTIR on the other hand was to confirm presence of functional groups in the target compounds.

The ^{1}H-NMR of compound **100** as in **Figure 4.2** gave a doublet at chemical shift of 7.59 and 7.46 ppm with coupling constants of 8.1 Hz corresponding to the aromatic protons in ring A (**Ha** and **Hb**). Doublets at chemical shifts of 7.23 and 6.83 ppm with coupling constant of 9.0 Hz correspond to the other aromatic protons **Hg** and **Hh** respectively**.** The methoxy protons **Hi** occurred as a singlet at the chemical shift of 3.77 ppm while the benzylic protons **Hc** occurred as a singlet at 3.58 ppm. The piperizinyl protons **He** and **Hd** occurred as multiplets at chemical shifts between 3.53–3.46 and 2.51–2.44 respectively. The proton on the amide nitrogen **Hf** occurred as a singlet at the chemical shift of 6.26 ppm.

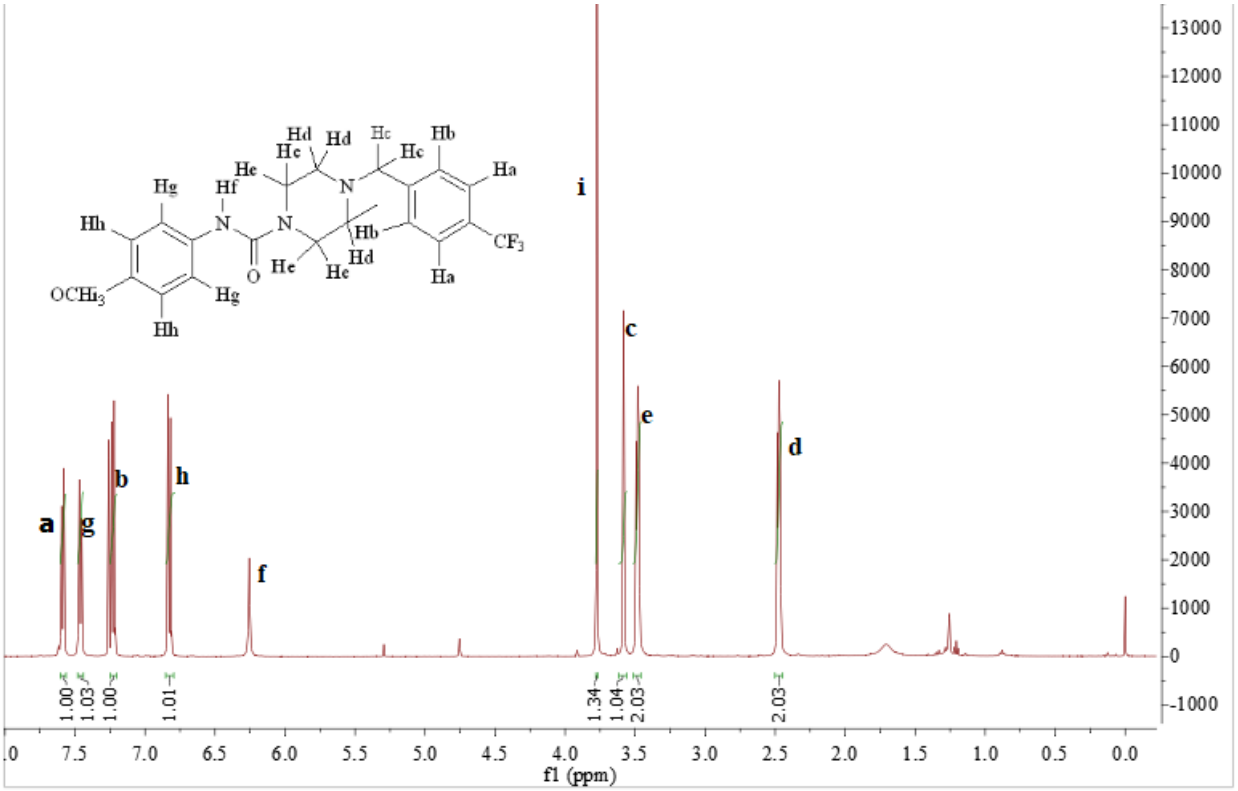


Figure 4.2: ^{1}H-NMR of 100

The ^{13}C-NMR of compound **100 Figure 4.3** is shown below. There were 14 distinct peaks at the chemical shifts of 156.1 (**Ca**), 155.4 (**Cb**), 136.6 (**Cc**), 131.7 (**Cd**), 128.6 (**Ce**), 127.8 (**Cf**), 126.4 (**Cg**), 122.5 (**Ch**), 114.2 (**Ci**), 61.0 (**Cj**), 55.9 (**Ck**), 52.3 (**Cm**) and 43.6 (**Cn**).

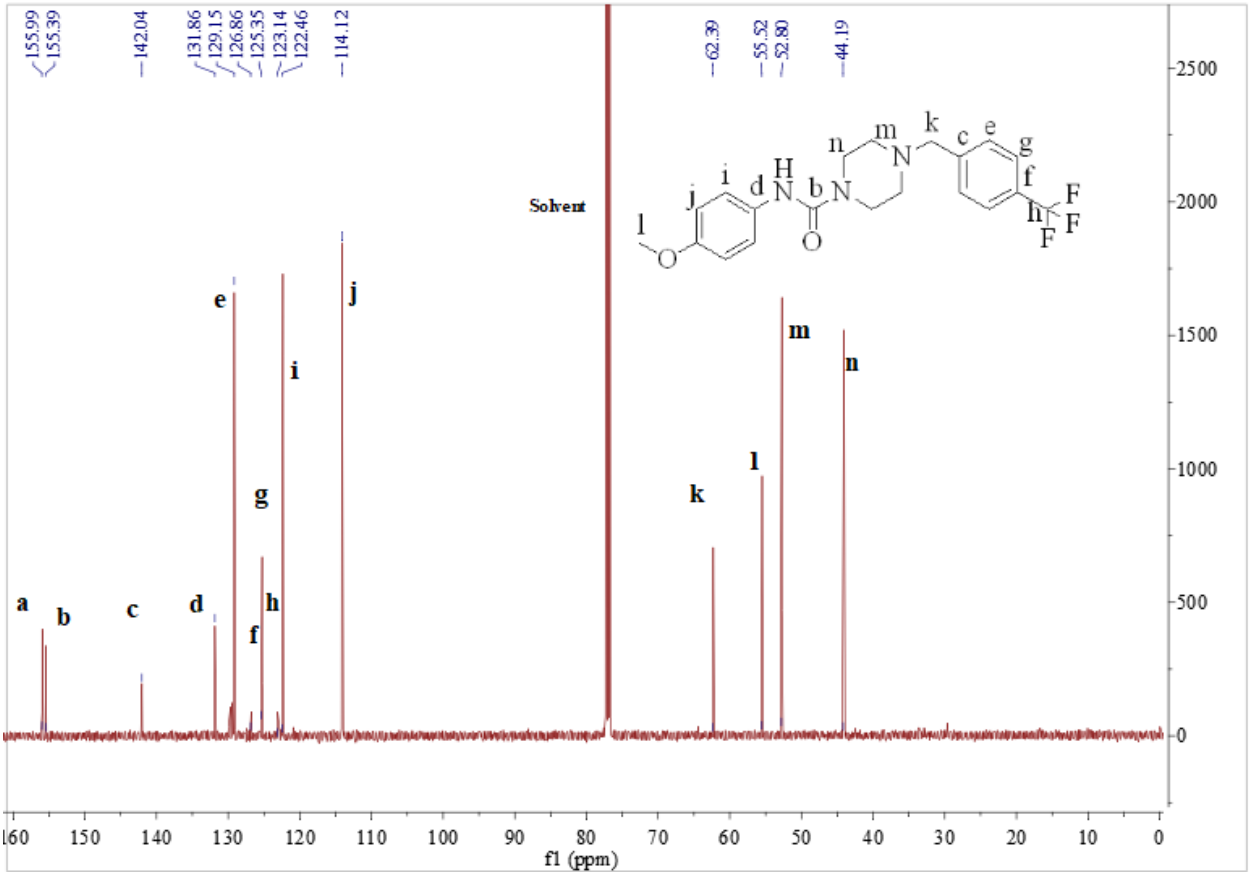


Figure 4.3: ^{13}C-NMR of 100

Figure 4.4 is the FTIR spectrum of **100**. The broad prominent absorption peak at 3333.45 cm^{-1} is the N-H peak of the urea functionality. Also, bands appearing at 2927.35, 1728.79, 1577.79 are characteristic of C-H, C=C and C=O stretch vibrations. Other bands at 1453.82, 1152.33 and 952.28 are characteristic of C-H bending and C-O, C-F bond vibrations. The fingerprint region is also in agreement with the mono-substituted aromatic region of the compound.

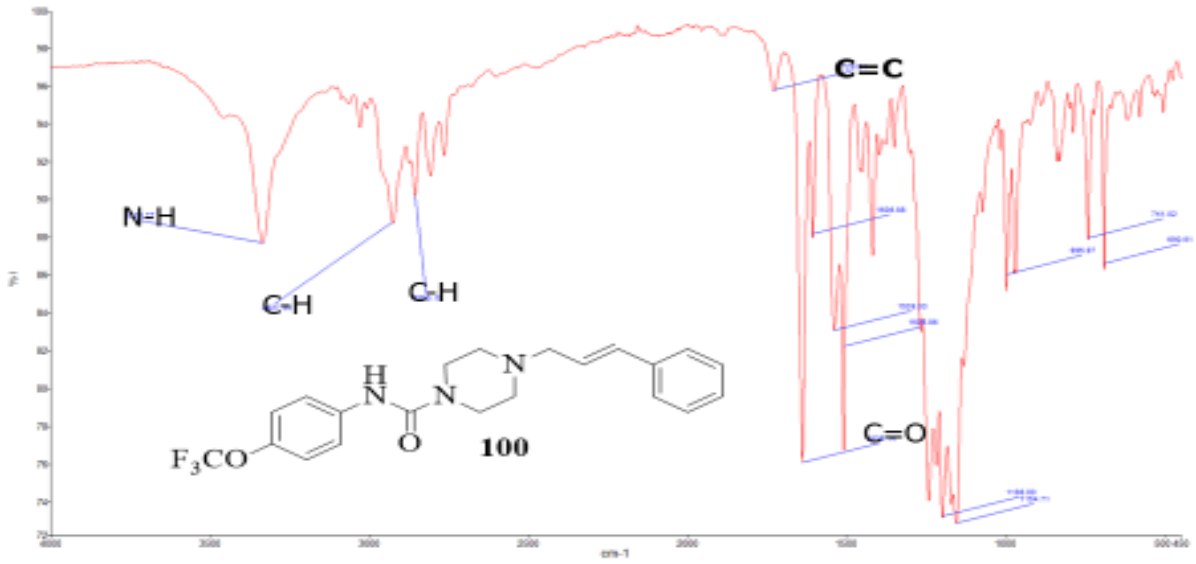
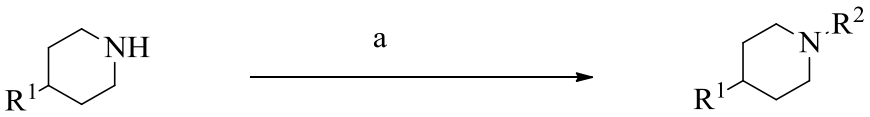


Figure 4.4: FTIR spectrum of 100

Series 2 compounds**, 109-117** were synthesized via reductive amination of bicyclic amines with a variety of aldehydes **Scheme 4.5**. They were designed to establish the importance of the urea functionality found in **Series 1** compounds due to the limitations of urea containing compounds. Bicyclic amines such as 4-(pyrrolidin-1-yl)piperidine, 4-phenyl piperidine, and 2-(piperazin-1-yl)pyrimidine were used while the aldehydes were benzaldehyde, cinnamaldehyde, 4-nitrobenzaldehyde, 4-(trifluoromethyl)benzyldehyde and for bioiosteric purposes, 6-(trifluoromethyl)nicotinaldehyde. (Takahashi et al., 2013).



109, **JAK 028**, X = C, R^1 = 1-pyrrolidinyl , R^2 = cinnamyl, 79 %
110, **JAK 029**, X = C, R^1 = 1-pyrrolidinyl , R^2 = 4-nitrobenzyl, 75 %
111, **JAK 030**, X = C, R^1 = 1-pyrrolidinyl , R^2 = 6-(trifluoromethyl)pyridin-3-yl)methyl), 74 %
112, **JAK 031**, X = C, R^1 = phenyl, R^2 = nitrobenzyl, 73 %
113, **JAK 032**, X = C, R^1 = phenyl, R^2 = cinnamyl, 71 %
114, JAK 033, X = C, R^1 = morpholinyl, R^2 = 6-(trifluoromethyl)pyridin-3-yl)methyl), 72 %
115, **JAK 034**, X = C, R^1 = phenyl, R^2 = 6-(trifluoromethyl)pyridin-3-yl)methyl), 78 %
116, **JAK 035**, X = N, R^1 = 2-pyrimidinyl, R^2 = cinnamyl, 81 %
117, **JAK 036**, X = N, R^1 = 2-pyrimidinyl, R^2 = 6-(trifluoromethyl)pyridin-3-yl)methyl), 78 %

Scheme 4.5: a) DCM, 0 ^oC, R^1CHO, 1 h, $Na(OAc)_3BH$ then rt, 16 h

4.2 **Physicochemical Parameters**

The physicochemical parameters of all the synthesized compounds were calculated to establish which of them will influence biological activity the most. Parameters such as AlogP, LogD, Polar Surface area (PSA), Solubility as well as their Molecular weights and the number of the Lipinski rule of 5 obeyed were investigated **Table 4.1**.

Table 4.1: Table of results of the calculated physicochemical properties for all the synthesized compounds

Compound	Code	ALogP	LogD	PSA	Solubility	Mw
82	JAK 001	3.17	3.16	35.58	-4	335.44
83	JAK 002	2.60	1.24	81.4	-4	354.40
84	JAK 003	2.71	1.34	35.58	-4	309.41
85	JAK 004	3.19	2.65	47.94	-5	378.39
86	JAK 005	3.37	3.36	35.58	-4	339.41
87	JAK 006	2.80	1.44	81.4	-4	358.37
88	JAK 007	2.90	1.54	35.58	-3	313.38
89	JAK 008	3.84	3.84	35.58	-5	381.37
90	JAK 009	3.12	3.12	48.47	-5	382.36
91	JAK 010	4.01	5.03	35.58	-4	389.17
92	JAK 011	2.90	4.25	87.39	-3	408.37
93	JAK 012	3.49	4.50	35.58	-6	363.16
94	JAK 013	4.58	4.58	35.58	-6	431.37
95	JAK 014	3.86	3.86	48.47	-6	432.36
96	JAK 015	4.62	4.83	44.81	-5	405.41
97	JAK 016	2.74	4.04	96.62	-4	424.37
98	JAK 017	4.10	4.30	44.81	-6	379.38
99	JAK 018	3.14	1.58	44.81	-4	351.45
100	JAK 019	3.62	3.62	44.81	-5	393.40
101	JAK 020	2.92	2.90	57.70	-4	394.39
102	JAK 021	4.77	4.77	35.58	-6	423.86
103	JAK 022	4.20	4.20	81.40	-6	442.82
104	JAK 023	4.30	2.94	35.58	-5	397.82
105	JAK 024	4.52	4.52	48.47	-7	466.81
106	JAK 025	4.07	4.06	35.58	-6	371.47
107	JAK 026	3.50	2.14	81.40	-5	390.44
108	JAK 027	3.61	2.25	35.58	-5	345.44
109	JAK 028	2.95	1.49	6.48	-4	270.41
110	JAK 029	2.37	0.82	52.30	-3	289.37
111	JAK 030	2.70	1.16	19.37	-4	313.36
112	JAK 031	4.00	3.97	49.06	-5	296.36
113	JAK 032	4.74	4.99	3.24	-5	277.40
114	JAK 033	1.93	0.41	28.60	-3	329.36
115	JAK 034	4.33	4.04	16.13	-6	320.35
116	JAK 035	2.75	2.75	32.26	-3	280.37
117	JAK 036	2.51	2.50	45.15	-3	323.32

- Calculated using the Virtual Computational Chemistry Laboratory (VCCLAB); http://www.vcclab.org.

For the **Series 1** compounds (**82-108**), AlogP in the range of **2.6-4.77**, LogD in the range of **1.24-4.77** and PSA in the range of **35.58-96.62** were obtained. While the **Series 2** compounds (**109-117**) gave AlogP in the range of **1.93-4.74**, LogD in the range of **0.41-4.99** and PSA in the range of **3.24-81.40** respectively.

4.3 **Antimalarial Screening**

The compounds from this scaffold were primarily intended for antitubercular use but were also tested for their antimalarial activity **Table 4.4**. All the synthesized compounds were submitted for anti-TB but the results were not ready as at time of writing this thesis. The results will be published in a later communication. However, all the compounds were evaluated for their *in vitro* antimalarial activity against *P. falciparum* 3D7 strain from clinical isolates using *P. falciparum* 384-well SYBR Green assay. Mefloquine was used as the positive control. Plates were inoculated and incubated at 37 ^{o}C for 72 hours. The antimalarial activities obtained were in the micromolar range 0.574 - 25.11 µM. The most active compound was **86** with IC_{50} of 0.574 µM. Other active compounds included **95**, **102**, **89**, **105** and **109** with IC_{50} values of 3.110, 4.155, 4.277, 4.439 and 5.432 µM, respectively. Though the *in vitro* antimalarial activity of the synthesized compounds were not superior to the clinically used mefloquine (0.388 µM), the results represent an encouraging start since this class of compounds were not originally intended for antimalarial activity.

Table 4.4: Antimalarial results for synthesized compounds

COMPOUND	CODE	IC_{50} µM
82	JAK 001	>10
83	JAK 002	>10
84	JAK 003	>10
86	JAK 005	0.574
87	JAK 006	>3.467
88	JAK 007	>10
89	JAK 008	4.277
90	JAK 009	>25.11
94	JAK 013	8.574
95	JAK 014	3.110
99	JAK 018	>25.11
100	JAK 019	>25.11
101	JAK 020	>25.11
102	JAK 021	4.155
103	JAK 022	10.53
104	JAK 023	10.59
105	JAK 024	4.439
106	JAK 025	>10
107	JAK 026	>10
108	JAK 027	>10
109	JAK 028	5.432
110	JAK 029	>25.11
111	JAK 030	>25.11
112	JAK 031	>25.11
114	JAK 033	>25.11
115	JAK 034	>25.11
116	JAK 035	>25.11
117	JAK 036	>25.11
Mefloquine	-	0.388

4.4 **Structure and Activity Relationship**

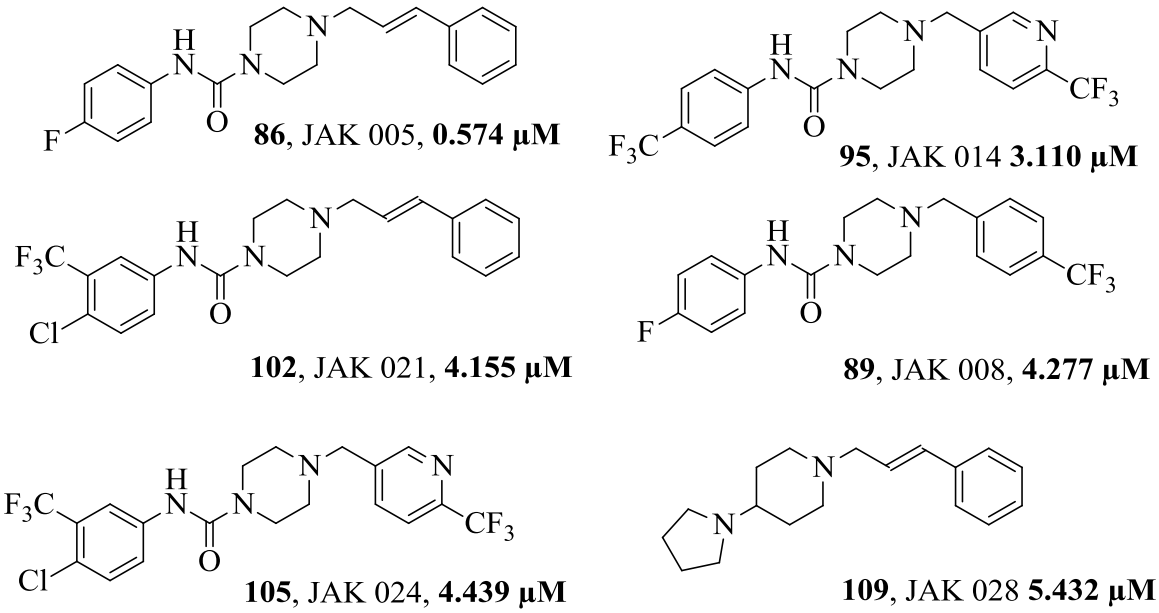


Figure 4.5: List of most active compounds and their antimalarial activities

Figure 4.4 shows that all the active urea compounds have phenyl groups attached directly to the urea functionality with electron withdrawing groups attached to the phenyl groups. Both **86** and **102** have cinnamido groups, but the pyridine group with the trifluoromethyl substituent in **109** could be the reason for the higher activity observed compared to **102**. Although **89** has two phenyl rings and two electron withdrawing groups just as **105**, there is a missing cinnamido group which could be the reason for the reduced activity. **109,** with the least observed activity is a non-urea derivative and the total absence of the electron withdrawing groups, and the urea functionality accounts for the reduced activity. This shows the relevance of the urea function in drug discovery.

There was no traceable correlation between the calculated physicochemical parameters and the antimalarial activities as observed in **Table 4.4**.

CHAPTER FIVE

4.1 CONCLUSIONS

A total 36 compounds have been designed and synthesized in high to excellent yields and their antimalarial activities evaluated. **Series 1** derivatives containing urea moiety were synthesized in three reaction steps; first, by treating Boc-protected piperazine with a number of isocyanates followed by acid catalyzed hydrolysis to furnish the corresponding amines. The resultant amines were then reacted with a variety of aldehydes *via* reductive amination reactions to give the targeted piperazine-1-carboxamides **82-108**.

Series 2 analogues, **109-117** which were amines devoid of the urea functionality were synthesized using a similar approach as **Series 1** by treating the respective amines with selected aldehydes. Purification of all the intermediates and final compounds was done by column chromatography and the purity ascertained by ^{1}H-NMR, ^{13}C-NMR and melting point determination. FTIR was used to detect diagnostic functional groups that indicated formation of new bonds.

Preliminary antimalarial testing of the synthesized compounds against the 3D7 strain of the malaria parasite revealed a number of hit compounds including **86**, **95**, **102**, **89**, **105** and **109.** Compound **86** was the most active of the series with an IC_{50} of 0.574 μM against the malaria parasite, but its activity was less than the control Mefloquine (0.388 μM).

Overall, several hit compounds have been identified for development into potential drug candidates.

4.2 RECOMMENDATIONS

- Analogues containing the piperonal moiety contained in the lead compound **16** should be synthesized and tested for both antimalarial and antitubercular activity
- ADME (i.e. absorption, distribution, metabolism and excretion) of the compounds should be studied to determine mode of action.
- The antitubercular activity of all the synthesized compounds should be performed.
- Biological testing should be done locally if the platform exists to avoid delay in data collection.
- Mass spectrometric data should be acquired to enable full characterization of the molecules.
- Financial support should be provided to facilitate the research work.

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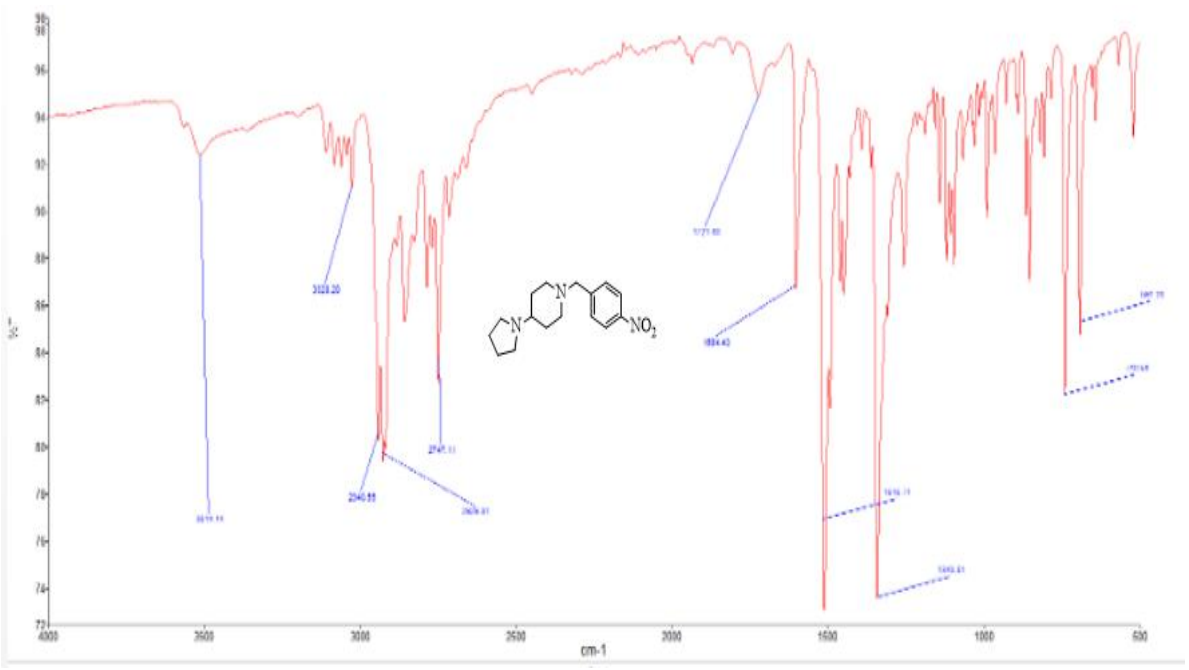
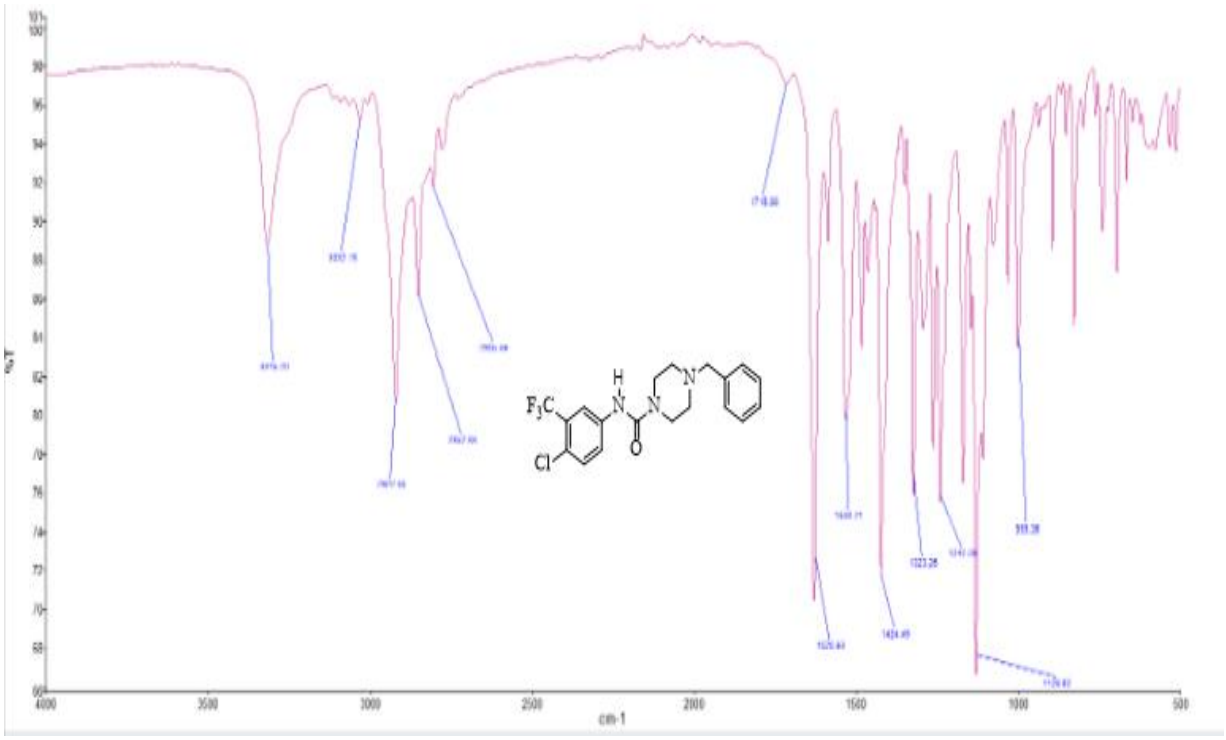
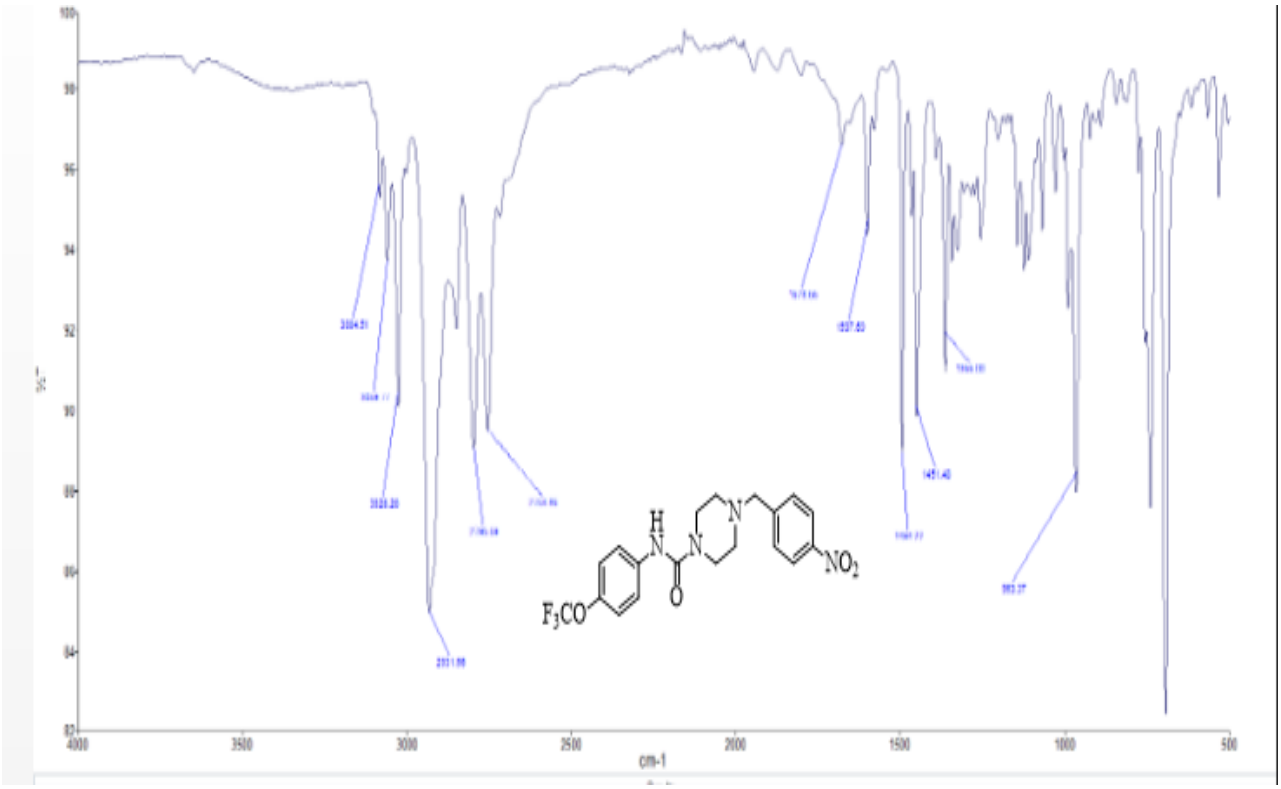
APPENDIX A

IR spectra of some selected compounds

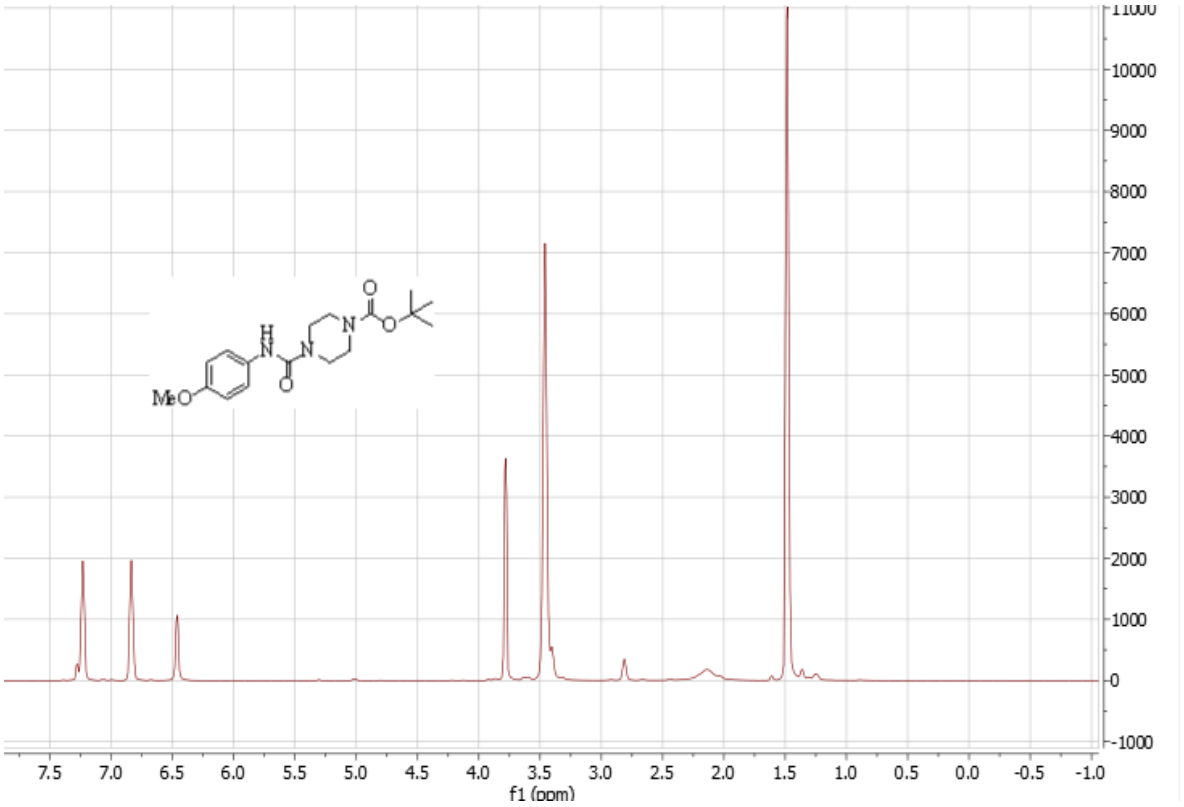


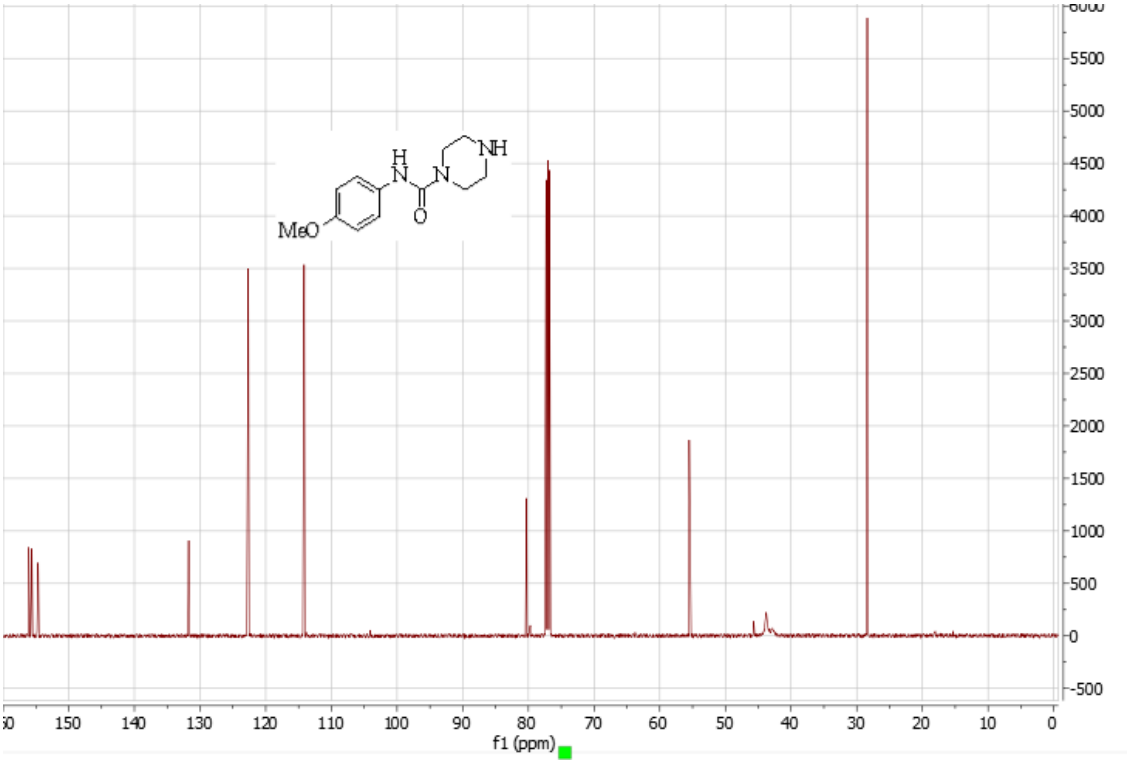


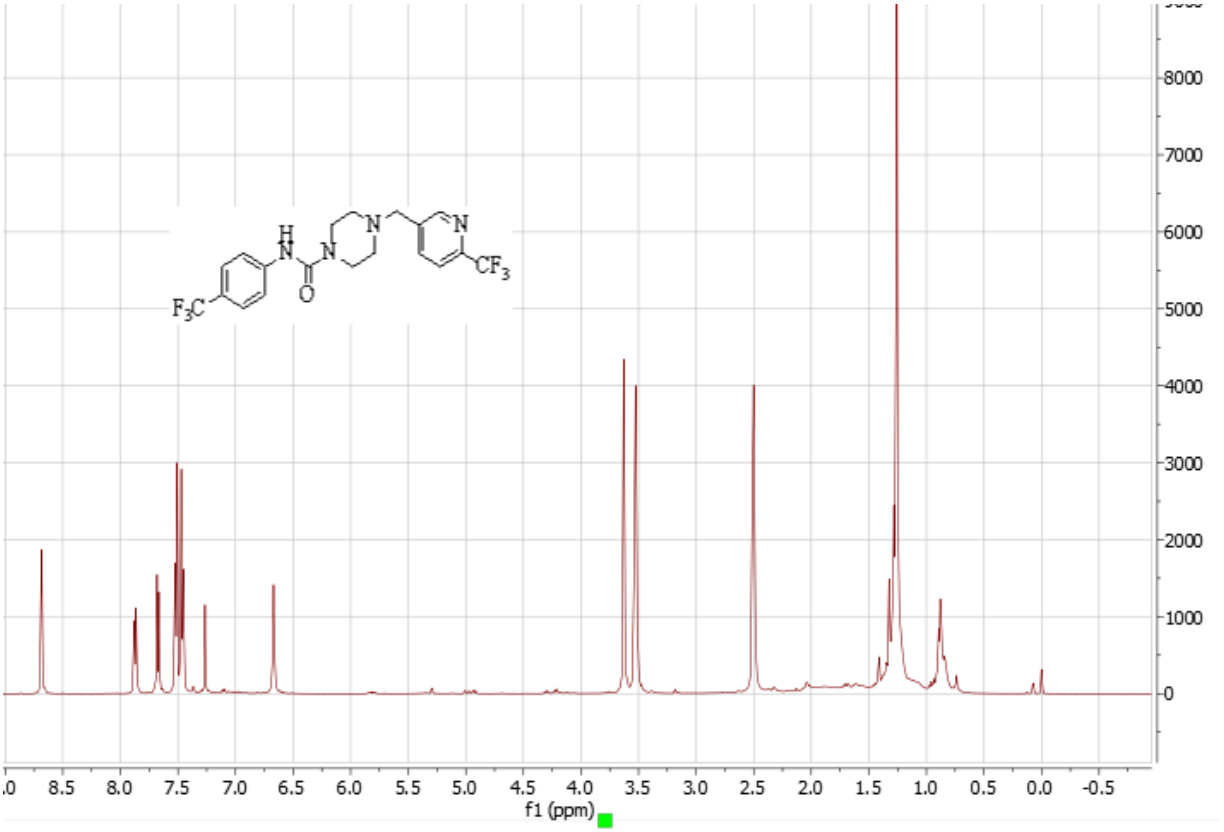


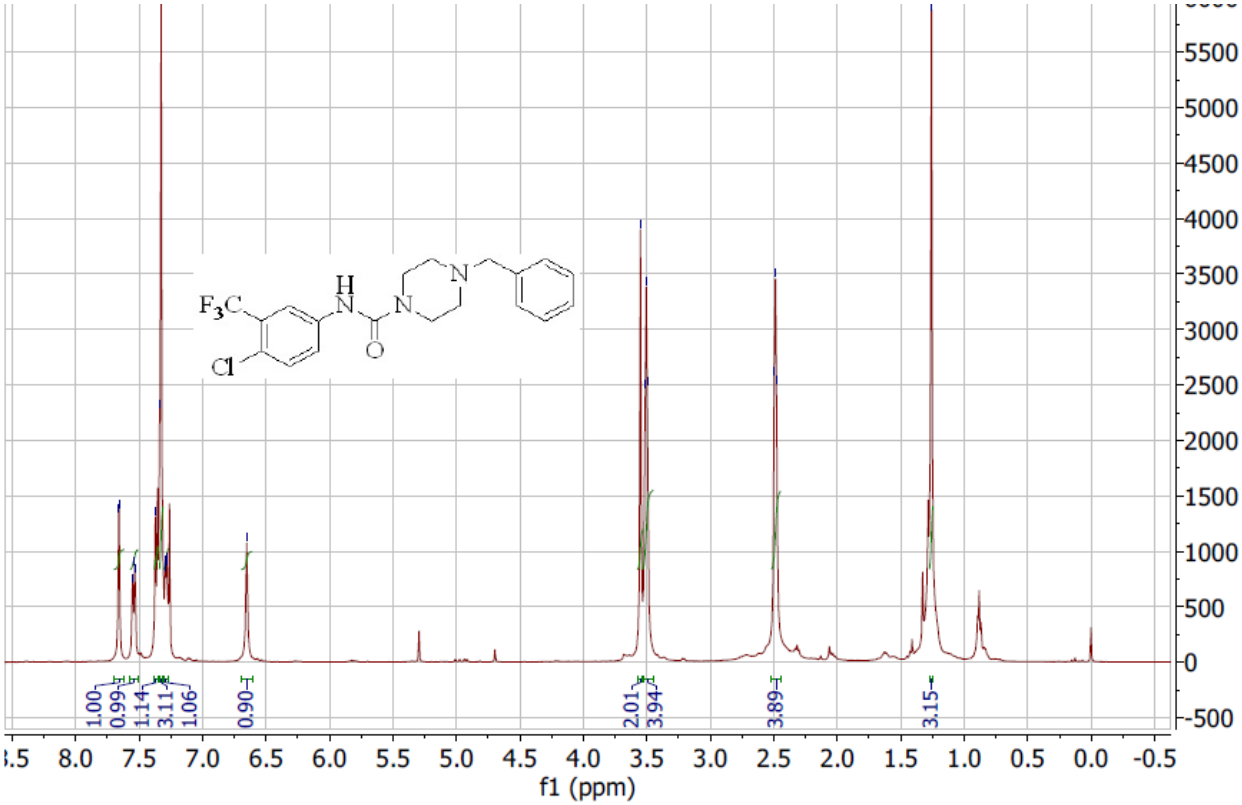
APPENDIX B

^{1}H-NMR of some selected compounds









APPENDIX C

^{13}C of some selected compounds