UNIVERSITY OF KWAZULU-NATAL

Novel quinolone substituted urea and thiourea derivatives as anti-microbial and anti-cancer agents: Design, synthesis and biological screening

MASTERS THESIS

2015

Mbuso Faya

Novel quinolone substituted urea and thiourea derivatives were synthesized and evaluated for their antimicrobial potential against H37Rv tuberculosis strain and a panel of pathogenic microorganism. PASS online tool was also used to predict potential anticancer activity of these compounds.

Thesis submitted in partial fulfillment of the requirements
For the award of the degree in

MASTER OF MEDICAL SCIENCE
(Pharmaceutical Chemistry)

College of Health Sciences,
University of KwaZulu-Natal

By

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(B.Sc)

Supervisor

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Preface

This study represents original work by the author and has not been submitted in any other form to another university. Where the use of work pertaining to others has been duly acknowledged in the text.

Candidate: Mbuso Faya

Signed: **Name:** **Date:**

As the Candidate's supervisor, I have approved this thesis for submission.

Supervisor: Dr. Rajshekhar Karpoormath

Signed:

Name:

Date:

Declaration – Plagiarism

I, **Andile Mbuso Faya,** declare that the experimental work described in this dissertation was carried out at the School of Pharmacy, College of Health Sciences, University of KwaZulu-Natal, Westville campus under the supervision of Dr. Rajshekhar Karpoormath, and that:

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List of Publications

Current Perspective of Natural Alkaloid Carbazole and its Derivatives as Antitumor Agents

Author(s): Mahamadhanif S. Shaikh, Neeta Thapliyal, Rajesh A. Rane, Mahesh B. Palkar, A. M. Faya, Harun M. Patel, Wesam S. Alwan, Kavita Jain, Girish A. Hampannavar and RajshekharKarpoormath

Journal: Anti-Cancer Agents in Medicinal Chemistry

My contribution to this paper was drawing of structures and tables and sorting out the relevant information for the primary author.

Acknowledgements

My first and sincere appreciation goes to my family for their support and guidance throughout the course of my MSc degree. I would like to express my appreciations towards my friends who encouraged me to further my studies. I would also like to thank my supervisor Dr. Rajshekhar Karpoormath for his support and guidance and my fellow colleagues, Wesam, Thabiso, Afsana, Siva, Hanif, Ali, Kavita as well as the postdoctoral students Dr Rajesh Rane, Mahesh Palkar, Dr Haroon Patel and Dr Neeta for going all out in their efforts to impart relevant laboratory skills and knowledge until the end of my studies.

Finally I would like to acknowledge Inkosi Albert Luthuli (Department of Microbiology) for availing their research facilities to my research work, NIH (USA) for providing anti-tuberculosis testing and Dr Rajeh Rane for assisting with anti-Cancer activity with his colleagues in India. I would also like to acknowledge the national Research Foundation (NRF) and the College of Health Science for their financial assistance towards my research as well as living expenses and also a special thanks to the University of KwaZulu-Natal for giving me the opportunity to carry out my research work and add to the on-going knowledge development sphere.

List of Abbreviations

°C	Degrees Celsius
^{13}C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
CDCl3	Deuterated chloroform
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
d	Doublet
EtOAc	Ethyl actetate
EtOH	Ethanol
FT-IR	Fourier transform infrared spectroscopy
Hz	Hertz
m.p.	Melting point
MIC	Minimum inhibitory concentration
min	Minutes
m	Multiplet
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
RBF	Round Bottom Flask
S	Singlet
TLC	Thin layer chromatography
t	Triplet
UV-VIS	Ultraviolet-visible spectroscopy

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Abstract

Cancer which is also referred to as malignant neoplasm is a group of diseases involving abnormal cell growthwith the capacity to invade and spread throughout the body. The most frequently diagnosed cancer has been found to be breast cancer and it is also the leading cause of death by cancer among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. The ongoing discovery of newer cancer as a result of mutations has been a driving force in the design, synthesis and development of newer chemo-therapeutic agents which combat cancer. As a contribution to these efforts, we synthesized compounds specifically targeting cancerous cells. A series of eleven 2 quinolone-urea/thiourea hybrid molecules were synthesized by a simple Pachman condensation reaction followed by the reaction of the newly synthesized 2-quinolone with various substituted isocynates and isothiocyantes leading to the lead compounds which were evaluated for their activity potential against TB H_{37}Rv strain and bacterial clinical strains. Pass online tool (***Prediction of activity spectra for substances***) was also used to predict the activity potential of these compounds and the reflected high probability of these compounds to be used as anticancer agents. The compounds also reflected low to moderate activity of these compounds as antimicrobial agents as well as antitubercular agents which. The PASS results were confirmed by our experimental work. The synthesized compounds were confirmed by melting point, FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The yield of these compounds obtained ranged from 40% to 80%. The synthesized compounds displayed moderate activity towards *E.coli* ATCC35218 and *S.pneuminiae* ATCC49619 strains with highest MIC of 12.5µg/mL respectively. PASS activity prediction also showed good Pa (probability to be active) indexes which reflected that the compounds are mostly active as anticancer agents.

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Chapter 1
Thesis Overview

1.1 BACKGROUND AND RATIONAL OF THIS STUDY

The work in this thesis is an effort towards understanding the 2-quinolone moiety in drug discovery and its anticancer activity and additionally its antitubercular and antimicrobial activities. Cancer which is also referred to as malignant neoplasm is a disease which can affect any part of the body and it is characterized by cell growth which is unregulated and out of control[1,2]. There are many different types of cancers which affect different parts of the body such as breasts, prostrate, lymph glands, bone marrow etc[3]. Cancer becomes harmful to the body when cells continue to divide uncontrollably and form tumours. Tumours have the ability to grow and further divide and consequently affect body systems such as the circulatory, digestive and nervous systems and they can also discharge hormones that alter normal body functions[4]. Benign tumours are localised in a specific part of the body and demonstrate limited growth[5]. Many drugs have been designed and used to combat cancer. These drugs were designed with intent to target specific sites within the cancerous cell or mechanisms which are responsible for causing cancer when unregulated. There are various drug targets including telomerase, topoisomerase and NF-κB in which many synthesized drugs act[6,7]. The drugs which act on these target sites are effective and have been used to treat various cancers. However, due to mutations and the emergence of different environmental factors have made it difficult to treat cancer and the current drugs are quickly becoming less significant in the treatments of cancers. Hence, there is a great need to develop new compounds which target different receptors and specific target-sites in cancerous cells for effective treatment[8].

Quinolones are a family of synthetic broad-spectrum antibacterial agents which exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating. Some quinolones selectively inhibit the topoisomerase II ligase domain[9,10]. This modification on the topoisomerase II ligase domain leads to DNA disintegration via the nuclease activity of the intact enzyme domains[11,12]. Some quinolone analogues have been enhanced to be more selective for topoisomerase IV ligase domain, and this has given them improvedGram-positive coverage[13]. The protein target of quinolones in many Gram-negative bacteria is DNA gyrase and topoisomerase IV for many Gram-positive bacteria[14]. Some derivatives of quinolones display high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases which are toxic to cultured mammalian cells and *in vivo* tumour models[15]. Therefore these cytotoxic classes of quinolone derivatives indicate an exploitable source of new anticancer agents. The quinolone pharmacophore has also been found to inhibit

NF-κB which is a protein complex that controls transcription of DNA. Incorrect regulation of NF-κB has been linked to cancer, inflammatory, autoimmune diseases, septic shock, viral infection and improper immune development[16,17].

Therefore with the above evidences in mind, this thesis is directed at investigating the anticancer potential of the 2-quinolone pharmacophore and its novel derivatives. The novel compounds will also be subjected to *in vitro* screening against tuberculosis (H37Rv strain) and bacterial clinical isolates to further evaluate their pharmacological activity.

1.1 AIMS OF THIS STUDY

1.2.1 To synthesize 7-amino-4-methyl-2-quinolone urea/ thiourea derivatives

1.2.2 To evaluatethe synthesised compounds against a panel of cancer cell lines

1.2.3 To further screen the synthesized compounds against *Mycobacterium tuberculosis* and Gram-positive and Gram-negative bacterial clinical isolates.

1.3 OBJECTIVES OF THIS STUDY

1.3.1 To synthesize 7-amino-4-methyl-2-quinolone urea/ thiourea derivatives by a reaction between an intermediate 7-amino-4-methyl-2-quinolone with different isocyanates and isothiocyanates.

1.3.2 To carry out anticancer evaluation using MTT assay against eleven cancer cell lines (**MCF7**, **CaCO2**, **K-562**, **ECV-304**, **UACC-257**, **PC-3**, **WRL68**, **PA1**, **TK-10**, **HOP-62**, and**KB403)**

1.3.3 To carry out antitubercular testing against H37Rv strain.

1.3.4 To carry out antimicrobial screening against a panel of Gram-positive as well as Gram-negative bacteria employing the minimal inhibitory concentration (MIC) technique.

1.4 NOVELTY AND SIGNIFICANCE OF THIS STUDY

Previous studies on the 2-quinolone ring reported the synthesis of this pharmacophore coupled with amino acids, thiazolidinones, thiazoles, mannich bases etc. However, no investigations have been reported to date on the reaction of 7-amino-4-methyl-2-quinolone with isocyanates and isothiocyanates to yield quinolone urea/thiourea derivatives. The current

study is the first of its kind and the findings of these hybrid molecules are reported in this thesis. Another important aspect, which highlights the novelty of this work, is evaluating 2-quinolone and its derivatives as potential anticancer agents. Further no study has been reported on urea coupled to the amino group at C-7 of the 2-quinolone ring as evidenced by literature search till date. The work presented will contribute further towards understanding of anticancer properties. Also, this study could serve as a road map for further target-based anticancer drug design and development.

1.5 OVERVIEW OF THIS THESIS

This thesis is divided into 5 chapters, including its overview.

Chapter 1: This chapter addresses the background, aim and objectives, significance as well as the general outline and structure of the thesis.

Chapter 2: It provides a general overview on cancer, cancer drugs and their target sites as well as brief account on current marketed drugs for cancer chemotherapy. The chapter starts with a historic background of cancer followed by the pathophysiology of cancer and the different mechanisms of cancer formation including some updated statistics. The chapter also highlights many aspects of quinolone scaffold and its wide range of pharmacological activities, chemistry and quinolones coupled to other pharmacophores. The topoisomerase enzyme and NF-κB enzyme are crucial drug targets and the main focus of the work towards anticancer evaluation. Secondly the DNA gyrase and tuberculosis H37Rv strain are also mentioned as drug targets for the synthesized novel compounds.

Chapter 3: This chapter provides experimental data, description of different spectroscopic techniques employed to confirm the formation of the desired novel target compounds and their schematic representation. The chapter also displays concise synthetic strategy and mechanisms of the entire reaction leading to the formation of the novel compounds. Procedures or assay methods employed to evaluate the biological activity have also been explained in this chapter.

Chapter 4: This chapter highlights the results obtained during the course of the research and their discussion. Physico-chemical properties of the synthesized derivatives such as purity, appearance, molecular weight and percentage yield observed have been reported. The chapter also focuses on spectroscopic characterization data to verify the formation of the compounds and lists some examples of spectrums obtained with respect to synthesized compounds.

Chapter 5: This chapter focuses on general concluding remarks and future scope of the research work which includes different possibilities on chemical modifications of the 2-quinolone pharmacophore.

Appendix: This section reflects all the NMR and IR spectra obtained for all synthesized derivatives during the course of the research work.

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Chapter 2
Introduction

2.1 Introduction to Antimicrobial Agents

Antimicrobial drugs have played a critical role in the treatment of infectious diseases and have caused major changes in how we treat and halt the propagation of pathogenic microorganisms thereby giving us hope in fighting pandemics which have proven to have devastating effects upon humanity, however drug resistant microorganisms remain an important problem in clinical practice that has proven difficult to solve[1]. If it happens that an improper antibiotic happens to be chosen for the treatment of infection with drug-resistant microorganisms, the therapy may lead to a worse prognosis. In the case of epidemics, there may be quite a limited choice of agents for antimicrobial therapy. Currently fewer new antimicrobial agents are being developed and the rate of antimicrobial production is devastatingly less than the emergence of drug resistant microorganisms. With this situation in mind, we are now facing a situation of severely limited options among antimicrobial agents.

An **antimicrobial agent** is an agent that kills microorganisms or inhibits their growth. Antimicrobial drugs can be categorized according to the microorganisms they act against. For example, antibacterial agents are used against bacteria and antifungal agents are used against fungi. They can also be classified according to their function. Agents that kill microbes are called *microbicidal* and those that inhibit their growth are called *microbiostatic*[2]. The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones term also used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials[3].

2.2 History of Antimicrobial agents

Infectious diseases have accounted for a very large proportion of diseases as a whole. It was during the 19th century that microorganisms were found to be responsible for a variety of infectious diseases that had been plaguing humanity for decades. The first antimicrobial agent in the world was **salvarsan**, an anti-syphilis therapeutic agent which was synthesized by

Ehrlich in 1910. In 1935, sulphonamides were developed by Domagk and other researchers. These drugs were synthetic compounds and had limitations in terms of safety and efficacy. **Penicillin** was discovered in 1928, by Fleming. He found that the growth of *Staphylococcus aureus* was inhibited in a zone surrounding a contaminated blue mold (a fungus from the *Penicillium* genus) in culture dishes, leading to the finding that a microorganism would produce substances that could inhibit the growth of other microorganisms. The antibiotic was named penicillin, and it came into clinical use in the 1940s. In 1944, **streptomycin**, an aminoglycoside antibiotic, was obtained from the soil bacterium *Streptomyces griseus*. Thereafter, **chloramphenicol**, **tetracycline**, **macrolide**, and **glycopeptides** (e.g., vancomycin) were discovered from soil bacteria. The synthesized antimicrobial agent **nalidixic** acid, a quinolone antimicrobial drug, was obtained in 1962[4].

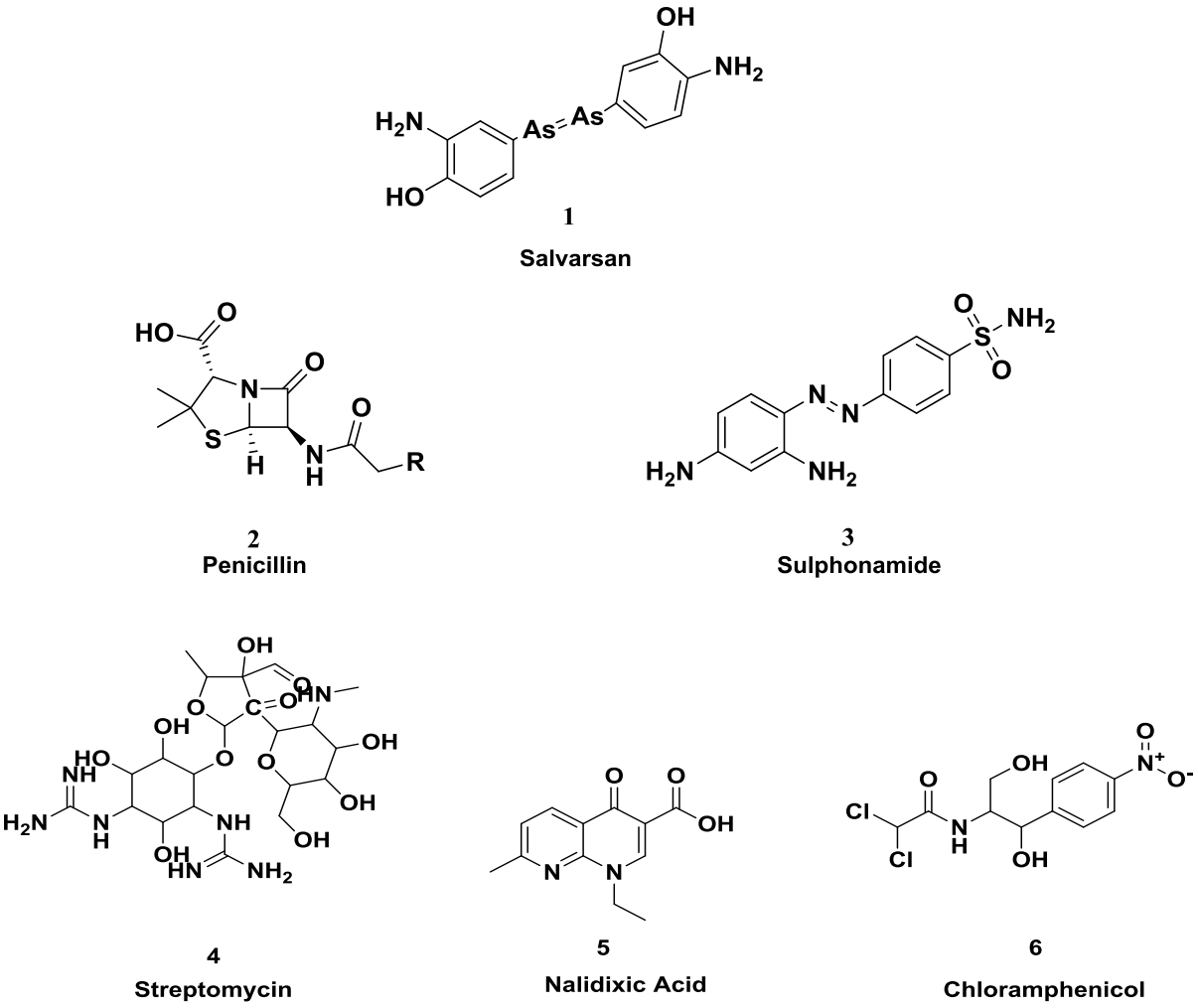


Figure1: Structures of antimicrobial early agents

2.3 Mechanism of action

Antimicrobial agents take advantage of structural differences between animal cells and bacteria (prokaryotes), fungi, or protozoa. The goal is to have highly selective toxicity towards these microbes with minimal and preferably no toxicity in humans[5]. Selective toxicity is a process whereby the applied antimicrobial agent acts against the parasite but not to the host. Selective toxicity could be due to a function of a specific receptor required for drug attachment, or it may depend on inhibition of biochemical pathways essential to the organism for survival but not to the host. The mechanisms are: (1) Inhibition of cell wall synthesis, (2) Inhibition of cell membrane function, (3) Inhibition of protein synthesis, and (4) Inhibition of nucleic acid synthesis.

2.4 Classification on mode of Action:

Inhibition of cell wall synthesis: Penicillins, cephalosporin, vancomycin, bacitracin, cycloserin[6]

- Binding of the drug to specific drug receptor PBPs (Penicillin- binding proteins) on bacteria. There are 3 to 6 PBPs which have different effects and some are enzymes involved in cross-linking reactions.
- After attachment, peptidoglycan synthesis is inhibited as final transpeptidation is blocked followed by inactivation of an inhibitor of autolytic enzyme in the cell wall. The autolytic enzymes are activated which lyses the cell and this process results in bacterial death.
- The Gram-positive and Gram-negative bacteria differ in susceptibility to penicillins or cephalosporins due to the amount of peptidoglycan, presence of and lipids receptors, nature of cross-linking and activity of autolytic enzymes. Gram-positives have a thicker peptidoglycan layer than Gram-negative bacteria.

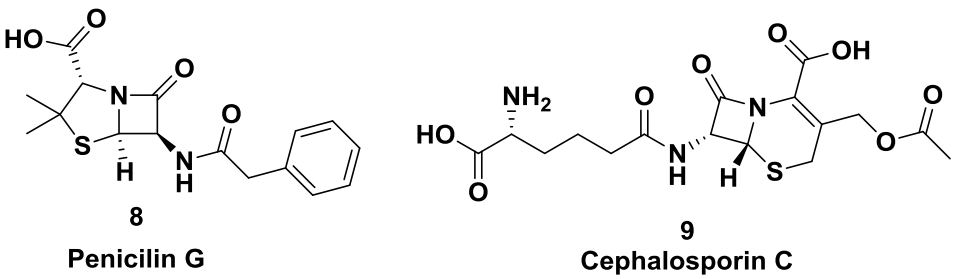
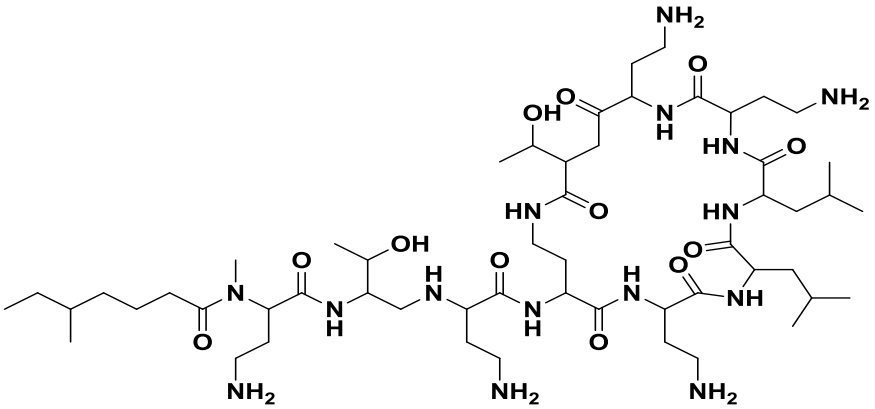


Figure 2: Structures of Penicillin G and cephalosporin C

2.5 Alteration of cell membrane function.

Polymyxin, amphotericin B, imidazole are examples[7].

- These drugs interfere with the functional integrity of the cytoplasmic membrane. Macromolecules and ions escape from the cell causing cell damage or death. Structural difference of the cytoplasmic membrane of bacteria and fungi from that of animal cells allows for selective toxicity



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Colistin (Polymyxin)

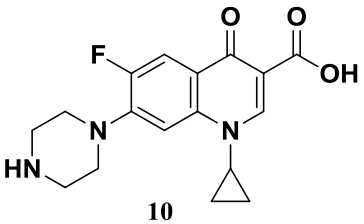
Figure 3: Structure of polymyxin

2.6 **Inhibition of protein synthesis**[8].

- Bacteria have 70S ribosomes, whereas mammalian cells have 80S ribosomes. Subunits of each type of ribosome, their chemical composition, and functional specifities differ. Thus a drug can inhibit protein synthesis in bacterial ribosomes but not in mammalian ribosomes dure to these structural differences
- Drugs that act on 50S ribosomal subunit. Chloramphenicol, erythromycin and chindamycin are examples.
- Drugs that act on 30S ribosomal subunit. Tetracycline and aminoglycosides are examples.

2.7 Inhibition of nucleic acid synthesis[9-11].

- Mechanisms of inhibition include; (a) Inhibition of nucleotide synthesis. Sulphonamides, trimethoprin Sulphonamides compete for the enzyme required for essential metabolite PABA involved in the synthesis of folic acid needed for synthesis of nucleic acid. (b) Inhibition of DNA synthesis. Quinolones block DNA gyrase. (c) Inhibition of mRNA synthesis- Rifampin.



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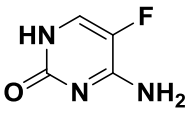
Ciprofloxacin



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Flucytosine

Figure 4: Structures of Ciprofloxacin and Flucytosine

2.8 Antimicrobial (Drug) Resistance[12-16]

The capacity of microorganisms to acquire resistance to antimicrobial agents has surpassed our imagination. In some cases, antimicrobial agents formerly effective are no longer useful. **Antibiotic resistance** is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics. Pathogens resistant to multiple antibiotics are considered *multidrug resistant* (MDR) or, more colloquially, **superbugs.** In the simplest cases, drug-resistant organisms may have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. In the case of some MDR pathogens, resistance to second- and even third-line antibiotics is, thus, sequentially acquired, a case typically illustrated by *Staphylococcus aureus* in some nosocomial settings. Some pathogens, such as *Pseudomonas aeruginosa*, also possess a high level of intrinsic resistance.

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

2.8.1 Drug inactivation or modification: for example, enzymatic deactivation of *penicillin* G in some penicillin-resistant bacteria through the production of β-lactamases.

2.8.2 Alteration of target site: for example, alteration of PBP—the binding target site of penicillins—in MRSA and other penicillin-resistant bacteria

2.8.3 Alteration of metabolic pathway: for example, some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to using preformed folic acid.

2.8.4 Reduced drug accumulation: by decreasing drug permeability or increasing active efflux (pumping out) of the drugs across the cell surface

There are three known mechanisms of fluoroquinolone resistance. Some types of efflux pumps can act to decrease intracellular quinolone concentration. In Gram-negative bacteria, plasmid-mediated resistance genes produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones[17]. Finally, mutations at key sites in DNA gyrase or topoisomerase IV can decrease their binding affinity to quinolones, decreasing the drug's effectiveness. Research has shown the bacterial protein LexA may play a key role in the acquisition of bacterial mutations giving resistance to quinolones and rifampicin. DNA damage induces the SOS gene repressor LexA to undergo autoproteolytic activity. This includes the transcription of genes encoding Pol II, Pol IV, and Pol V, which are three nonessential DNA polymerases that are required for mutation in response to DNA damage[18]

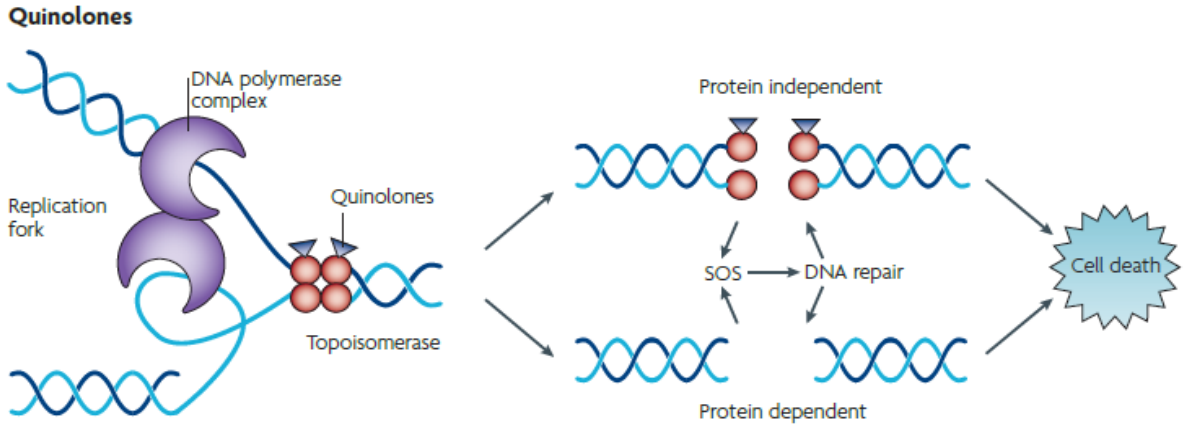


Figure 5: Sight of action and Mechanism of Action[19]

2.9 Tuberculosis

Tuberculosis (TB) is an extremely infectious disease which predominantly affects the lungs but it can also affect other organs. It is the world's second-most fatal infectious agent,

after HIV/AIDS (WHO, 2013). The bacterium that causes TB, *Mycobacterium tuberculosis*, is transmitted by droplets when an infected person coughs or sneezes. The unique clinical characteristics of this dangerous pathogen are a result of its high lipid content. The *M. tuberculosis* complex (MTBC) comprises of four other TB-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti*. *M. africanum* is not prevalent in other parts of the world but it is a significant cause of tuberculosis in many parts of Africa especially in SouthernAfrica[20-22].

In recent years, multi-drug resistant (MDR) tuberculosis as well as extremely drug-resistant (XDR) tuberculosis has evolved, threatening the ability to treat this deadly human pathogen. Hence, it is of great importance that new drugs and treatment paradigms be made readily available to curb this crisis. Historically, one of the most effective therapeutic classes of antibacterials have been the structural β-lactam ring class of antibiotics and are widely used due to their non-toxicity and safety compared with other agents, all of which contain the structural β-lactam ring motif. This class of antibacterial compounds inhibits the bacterial D, D-transpeptidases, which catalyze the final step of peptidoglycan cross-linking which is essential for cell-wall maturation and cell survival[23-25]. Resistance to this class of antibiotics in *M. tuberculosis* arises from β-lactamase (BlaC), which catalyzes the hydrolysis of the β-lactam antibiotic ring[26]. Because of the built-in resistance as a result of the production of the β-lactamase, β-lactams have never been systematically applied with success to the treatment of TB infections. Accordingly, there is a compelling need to develop more effective methods of treatment for tuberculosis, especially for treatment of MDR tuberculosis and XDR tuberculosis[27, 28].

2.10 Anti-TB drugs

First Line Treatment for TB comprises of isoniazid in conjunction with pyridoxal phosphate to avert peripheral neuropathy caused by isoniazid, rifampicin, pyrazinamide and ethambutol for a period of eight weeks followed by the combination treatment with isoniazid and rifampicin alone for a further 16 weeks. The patient is considered to be free of living bacteria after six months. For latent tuberculosis, the standard treatment regimen is six to nine months of isoniazid alone[29-31].

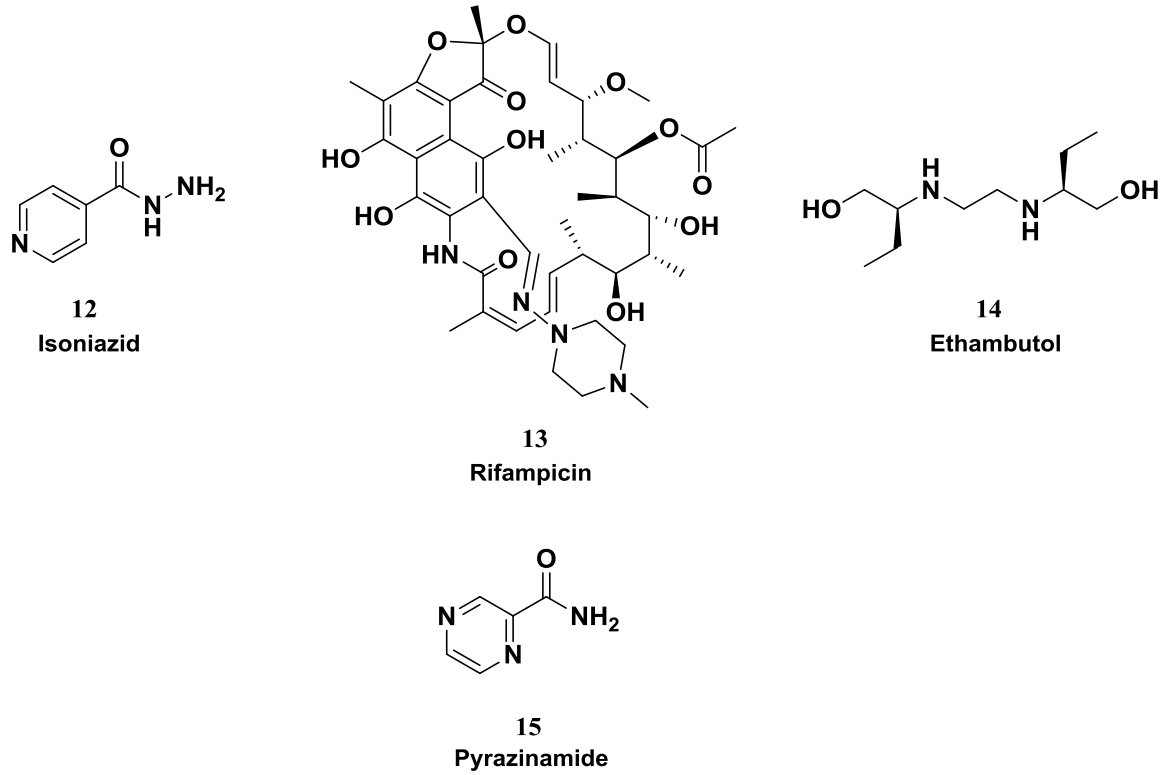


Figure 6: Anti-TB Drugs

2.11 Second Line Treatment of *M.Tuberculosis*

The second line treatment regimen is only used to treat disease that is resistant to first line therapy (i.e., for extensively drug-resistant tuberculosis (XDR-TB) or multidrug-resistant tuberculosis (MDR-TB))[32]. Some of the drugs used for second line treatment include;

- **aminoglycosides** e.g., amikacin (AMK), kanamycin (KM);
- **polypeptides** e.g., capreomycin, viomycin, enviomycin;
- **fluoroquinolones** e.g., ciprofloxacin (CIP), levofloxacin, moxifloxacin (MXF);
- **Thioamides:** ethionamide, prothionamide
- cycloserine
- terizidone

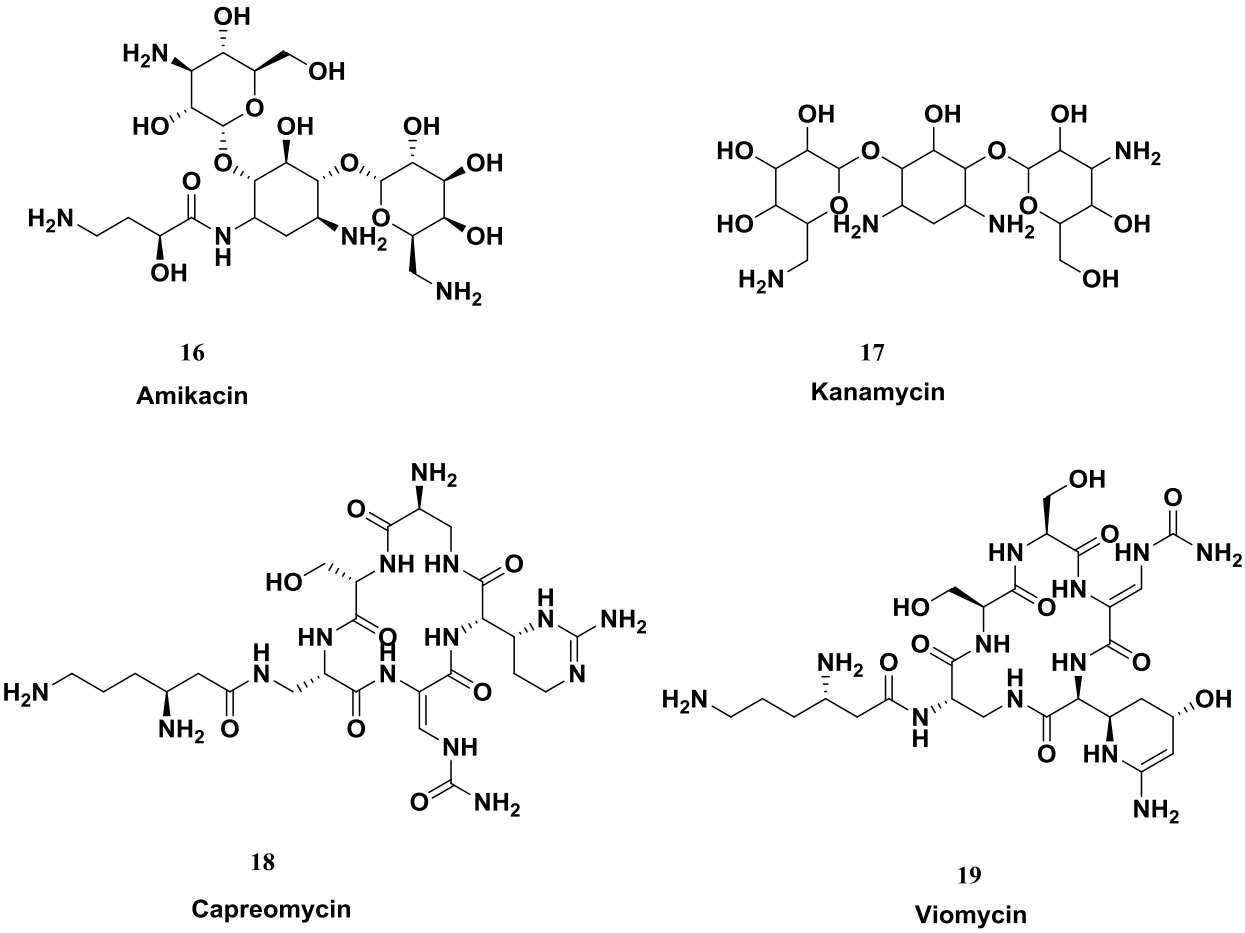


Figure 7: Second Line Treatment of *M.Tuberculosis*

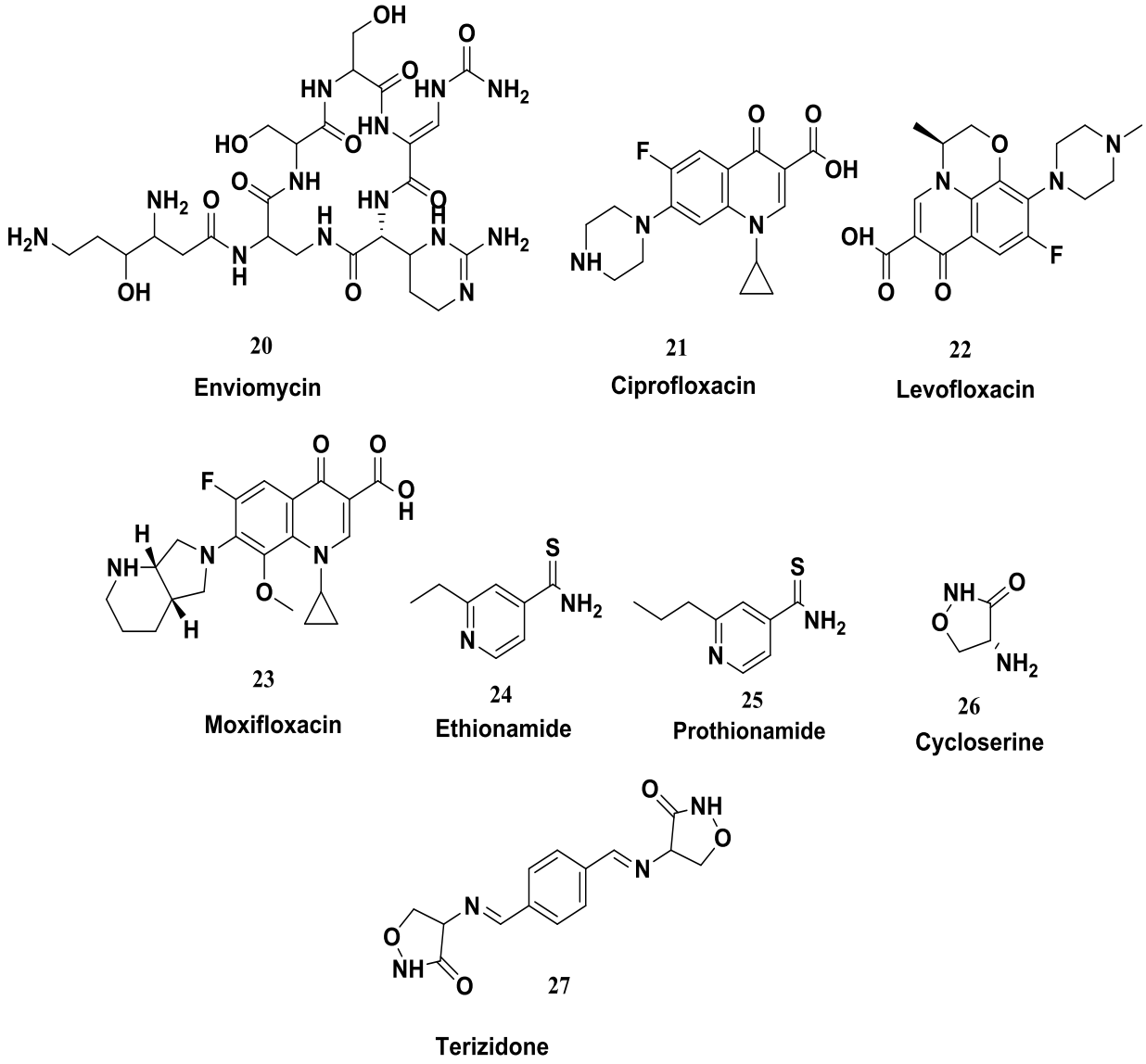


Figure 8: Second Line Treatment of *M.Tuberculosis*

2.12 Third Line Treatment:

Third-line drugs include drugs that may be useful, but have uncertain or unproven effectiveness. These drugs are listed here either because they are not very effective (e.g., clarithromycin) or because their efficacy has not been proven (e.g., linezolid, R207910). Rifabutin is effective, but is not included because for most developing countries, it is impractically expensive[33, 34]

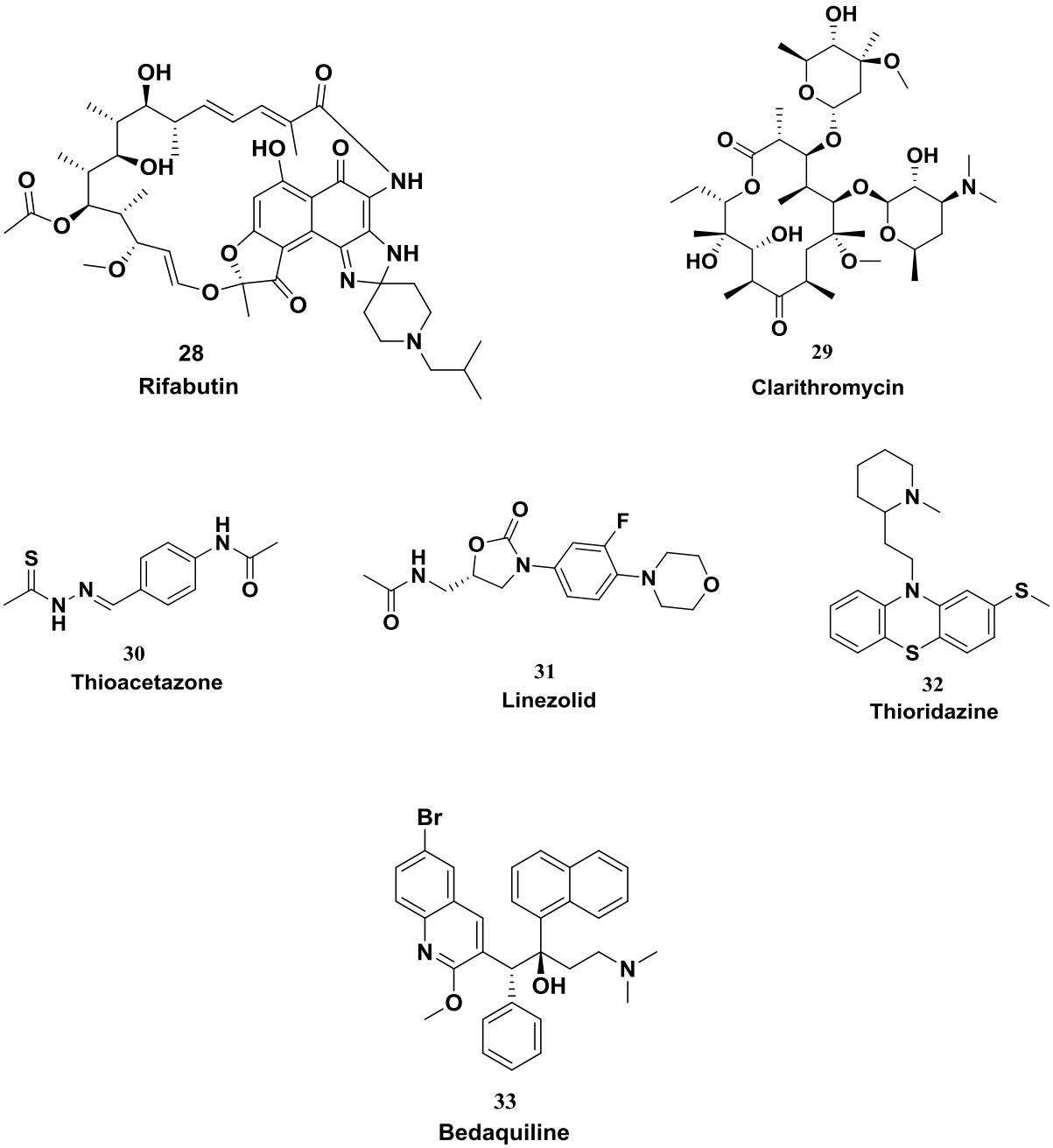


Figure 9: Third-line treatment

2.13 Anti-TB drugs: mechanism of action and resistance

As an Example, Isoniazid (isonicotinic acid hydrazide, INH) has been the most regularly used anti- tuberculosis drug since recognition of its medicinal use in 1952. INH penetrates the host cells and diffuses across the *M. tuberculosis* membrane[35]. INH also requires oxidative activation by the *M. tuberculosis* catalase-peroxidase enzyme KatG and this activation of produces oxygen-derived free radicals (superoxide, hydrogen peroxide, and peroxynitrite) and organic free radicals that hinder the development of mycolic acids of the bacterial cell

wall, causing DNA damage and death of the bacillus[36, 37]. The fluoroquinolones currently used as second-line drugs in TB treatment exert their potent antibacterial activity by trapping DNA gyrase and topoisomerase IV thereby blocking the movement of replication forks and transcription complexes. *M. tuberculosis* however lacks topoisomerase IV, but contains the genes gyrA and gyrB encoding the A and B subunits, respectively, of DNA gyrase[33, 38].

Resistance to INH consists of KatG mutations, which decrease the drug activity and avert the prodrug from being converted into its active metabolite. INH resistance may also arise from mutations in the inhA gene, resulting in reduced affinity of the enzyme for NADH without affecting its enoyl reductase activity[39]. Mutations in inhA also cause resistance to the structurally related second-line drug ethionamide[40]. Fluoroquinolone resistance in *M. tuberculosis* is commonly associated with mutations in the conserved quinolone resistance-determining region (QRDR) of gyrA and gyrB which are involved in the drug-DNA gyrase interaction[41-43].

2.14 Cancer

A Research conducted by WHO revealed that 8.2 Million people succumbed to cancer globally in 2012 and 60% of the world's overall new annual cases occur in Africa, Asia and central and South America[44]. There are more than 100 types of cancers which can affect any part of the body and current drugs as well as measures to halt the propagation and spread of cancer in the humans are not effective because of the on-going mutations of existing cancers and discovery of new cancers which have devastating effects on the human body[45].

Cancer is able to escape the first line of the body's defence mechanism which is the immune system and immunotherapy which involves cancer vaccines, cytokine therapy and administration of monoclonal antibodies to block immune checkpoints is not effective to a greater extent because of mechanisms such as apoptosis and various mutations that occur and continue to propagate cancer throughout the body[46].

Cancer is an extremely complex disease which accounts for 10 million new diagnoses world-wide per annum. Of these, 6.7 million will succumb and at present there are 24.6 million cancer patients living with cancer and hoping to survive[47]. Cancer which is also referred to as malignant neoplasm or malignancy is a collection of diseases which involves abnormal cell growth and it has the ability to invade and spread throughout the body, benign tumours do not spread to other parts of the body. No symptoms are observed during early stages of

development, however symptoms tend to be more visible as the mass continues to grow or ulcerates[48]. The findings that result are very much dependant on the location where the cancer occurs and the type of cancer. Few cancer symptoms are specific while most of them occur in individuals who have other medical conditions.

Developing countries face an increasing burden of cancer as a result of embracing cancer-associated lifestyle choices such as ‘‘westernized’’ diets, smoking and physical inactivity. According to statistics conducted by GLOBOCAN 2008, there is an annual increase of the global burden of cancer and also an increase in the projected number of new cancer cases and deaths. This could be as a result of genetic mutations as well as environmental factors[49].

The most frequently diagnosed cancer has been found to be breast cancer and it is also the prominent cause of death by cancer in females, accounts for 23% of the overall cancer cases and 14% of the cancer deaths. In males however, lung cancer has been found to be the prominent cause of cancer, comprising 17% of the total new cancer cases and 23% of the total cancer deaths. Research in developing countries has shown that the leading cause of cancer in females is breast cancer. This is a radical shift from the previous decade where cervical cancer was the most common cause of death (**Figure 1**) [48, 50].

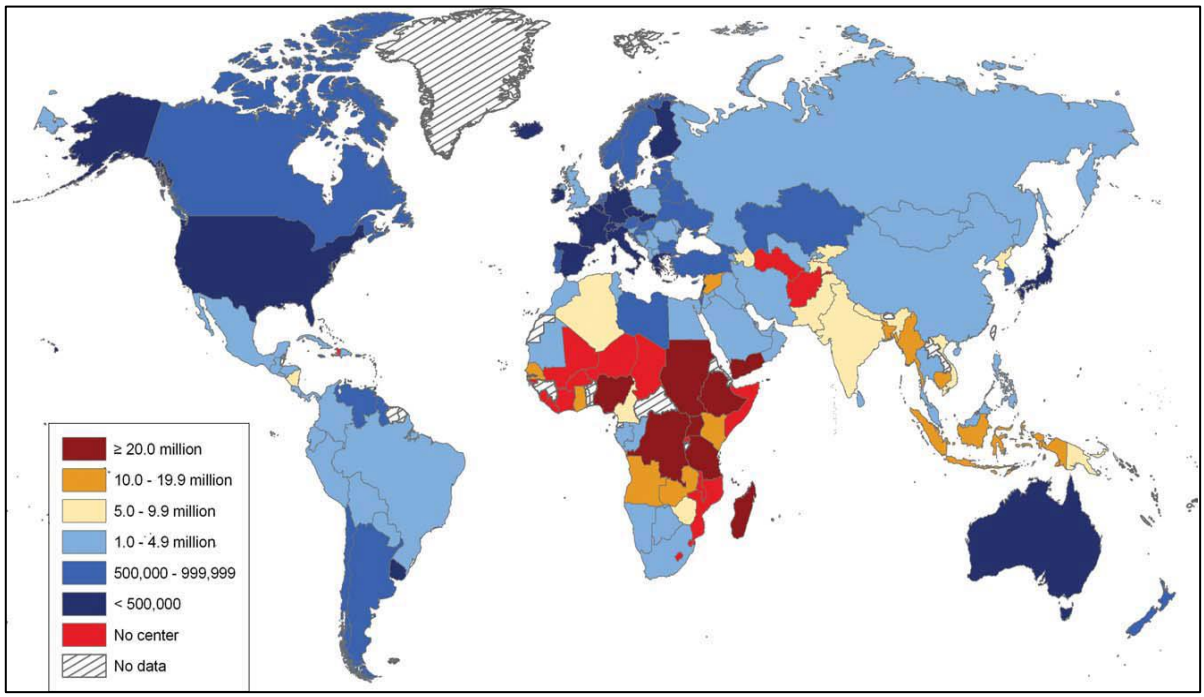


Figure 10: Region-wisenumber of people treated by each radiotherapy centre [50]

2.15 History of Cancer

The record of history reveals that humans and animals have had the burden of cancer inflicted upon them for centuries. The word cancer originated from a Greek word 'karkinos' to describe carcinoma tumours by a physician Hippocrates (460-370 B.C). Some of the initial evidence of cancer was also found amongst fossilized bone tumours, human mummies in ancient Egypt, and ancient manuscripts. Growths were also observed in mummies which are suggestive of osteosarcoma (bone cancer). The oldest description of cancer was discovered in Egypt and goes as far back as 3000 BC[51]. It's named the Edwin Smith Papyrus and is a copy of part of an ancient Egyptian textbook on trauma surgery[52]. It describes 8 cases of tumours or ulcers of the breast that were removed by cauterization with a tool called the fire drill. The writings describe the disease as untreatable[53].

2.16 Causes of Cancer

Cancer is triggered by the abnormal growth of cells in the body. Cancers tend to be different and some types of cancers can be more aggressive than others; however the common characteristic is that all cancer cells grow and divide continuously. Others types of cancer cells have the ability to metastasize, i.e. they move from a local site to other parts of the body through blood circulation or the lymphatic system and they begin to grow and divide, e.g. when a lung cancer cell spread to the liver through blood circulation, the cancer is still regarded as a lung cancer, not a liver cancer. Generally the development of cancer cells is attributed to the damage of DNA in cancer cells, which is not repaired. Damaged DNA can be passed from parent to progeny which accounts for inherited cancers. DNA damage occurs by different mechanisms and influenced by different factors like smoking, UV-rays, carcinogenic chemicals, etc. Apart from inherited genetics the environmental factors play a key role in the development of cancer. There are a number of elements that contribute to the risk of cancer or cancer death and this includes environmental pollutants, radiation, tobacco, diet and obesity, stress, infections and lack of physical activity[54].

2.16.1 Infection

Oncoviruses (Cancer causing viruses) are the usual infectious agents that cause cancer. These include hepatitis B and C viruses (hepatocellular carcinoma), Kaposi's sarcoma herpes virus(Kaposi's sarcoma and primary effusion lymphomas), human papillomavirus(cervical carcinoma),Epstein–Barr virus(B-cell lympho-proliferative disease and nasopharyngeal

carcinoma) and Human T-cell leukaemia virus-1(T-cell leukaemias)[55]. Bacteria and parasites also increase the risk of cancer as seen in *Helicobacter pylori*-induced gastric carcinoma[56]. Parasitic infections strongly associated with cancer include *Schistosoma haematobium* (squamous cell carcinoma of the bladder) and the liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis* (*chalangiocarcinoma*)[57-59].

2.16.2 Genetics

Transformation of a normal cell into a cancerous cell requires alteration of (a) **Oncogenes** – these are genes which promote cell growth and reproduction[60] and (b) **Tumour suppressor genes** – these are genes which inhibit cell division and survival[61].

Malignant transformation can occur through the formation of new oncogenes, the over-expression of normal oncogenes, or by the under-expression and/or disabling of tumour suppressor genes. Genetic changes can occur at different levels and by different mechanisms such as errors in mitosis can result in the gain or loss of an entire chromosome, mutations, which in turn change the nucleotide sequence of genomic DNA while large-scale mutations involve the deletion or gain of a portion of a chromosome[62]. Genomic amplification occurs when a cell gains many copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location[63].

Mutations also occur in a small scale and these include insertions and deletions as well as point mutations which may occur in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product[64]. DNA viruses or retroviruses can disrupt a single gene and this may result from integration of genomic material from a virus, leading to the expression of *viral* oncogenes in the affected cell and its progeny[65].

Mutations often occur during DNA replication. Even though error correction is built into the process and protects the cell against cancer and other replication errors. If substantial amount of error occurs the damaged cell will undergo programmed cell death (apoptosis). If the error correction mechanism fails then the mutations will survive and be passed along to daughter cells[66].

Some of the errors which cause cancer are:

- A mutation in the error-correcting machinery of a cell might result in rapid accumulation of errors and this can be passed from the cell containing the errors to progeny cells[67].
- Mutation in an oncogene can result in rapid reproduction of the mutated cell and the frequency of reproduction will be greater than its normal counterparts[68].
- Loss of a tumour suppressor gene can result due to mutation, disrupting the apoptosis signalling pathway and resulting in the cell becoming 'immortal'[45].
- A mutation in signalling machinery of the cell might send error-causing signals to nearby cells[69].

2.17 Metastasis

Metastasis is the spread of cancer from one part of the body to other locations in the body resulting in metastatic tumours. Most cancer deaths are due to cancers that have metastasized. Metastasis can occur via the blood circulatory system or the lymphatic system or even both[70]. The steps that occur during metastasis are local invasion, intravasation into the blood or lymph circulation through the body, extravasation into the new tissue, proliferation and angiogenesis. The lungs, liver, brain and the bones are the most common places for metastases to occur (**Figure 2**)[48, 71-73].

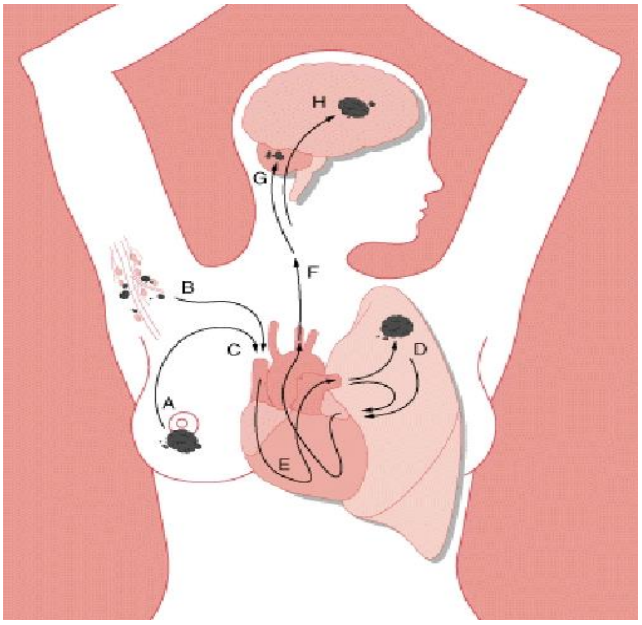


Figure 11: Schematic illustration of the metastatic process[74]

2.18 The Cell cycle

The cell cycle is a sequence of events which take place in a cell prior to replication which produces daughter cells. In eukaryotes the cell cycle involves three periods: interphase, the mitotic (M) phase and cytokinesis. The three periods consists of different stages and are described in **table-1**.

Table 1: Cell cycle phases and description[75]

State	Description	Abbreviation	Description
Quiescent/ Senescent	Gap O	Go	A resting phase: The cell has departed from the cycle and has stopped further division.
Interphase	Gap 1	G1	The G1 checkpoint control mechanism ensures that everything is ready for DNA synthesis.
Interphase	Synthesis	S	Replication of DNA takes place.
Interphase	Gap 2	G2	G2 is a checkpoint control mechanism which ensures that everything is ready to enter mitosis.
Cell division	Mitosis	M	Cell growth is halted at M phase and cellular energy is focused on cell division into two daughter cells. A Metaphase Check point ensures that the cell is ready to undergo complete cell division.

2.19 Cancer and the Cell Cycle

When the cells leave quiescence, they enter the first gap phase (G1) before they enter DNA synthesis (S phase). There are many cell signalling pathways that feed into the cell cycle machinery in G1. Also in G1 all pre-requisites for proper S-phase progression are being checked. A second gap phase (G2) follows before cells enter mitosis (M phase) which is the actual cell division phase (**Figure 3**). The cell cycle is regulated by the activities of protein complexes at the molecular level. The core of each complex comprises a cyclin, the essential regulatory subunit and a cyclin-dependent kinase (CDK), the catalytic subunit. These regulatory units are referred to as checkpoints which are complex signal transduction pathways that regulate the order of events in the cell cycle and integrate the cell cycle

progression with DNA repair[76]. Genetic instability has been shown to emerge as a result of the loss of cell cycle checkpoint control[77]. Consequently, the chance that these unstable cells will progress to cancer is increased. Improper regulation of cell cycle checkpoint control system may lead to independence of growth regulating signals[78]. Abnormal expression of positive regulators such as cyclins or the loss of function of negative regulators such as CKIs can also cause cancer[75].

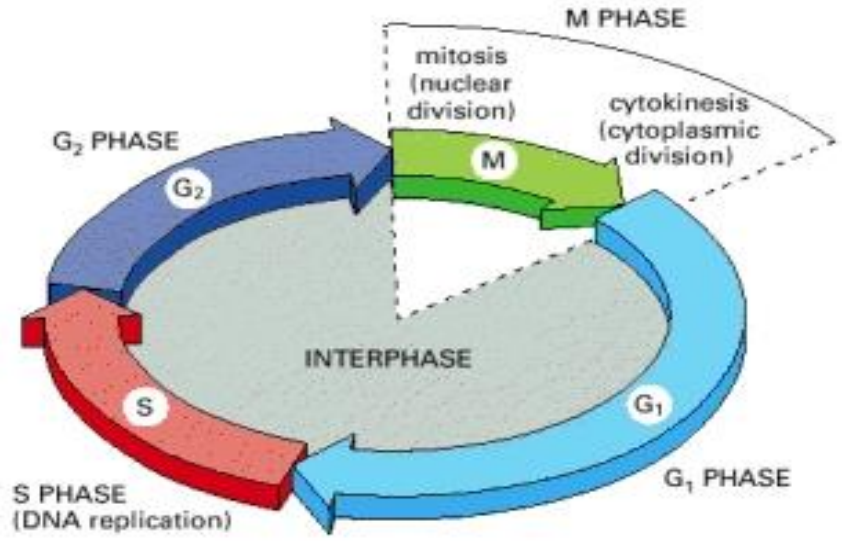


Figure 12: The phases of the cell cycle[79]

2.20 Cell cycle checkpoints and cancer

As previously mentioned, the cell cycle proceeds by an ordered set of events where the latter events depend upon the integrity and accurate completion of early events in the cell cycle. To ensure accuracy of the cell cycle progression, cells are well armed with checkpoints situated throughout all phases of the cell cycle. DNA damage activate DNA damage checkpoints that arrests cell cycle. DNA damage checkpoints are categorized into at least 3 checkpoints according to the cell cycle stages: G1/S (G1) checkpoint, intra-S phase checkpoint, and G2/M checkpoint. When DNA replication is activated by drugs that interfere with DNA synthesis, DNA lesions, or obstacles on DNA the cells activate DNA replication checkpoint that arrests cell cycle at G2/M transition until DNA replication is complete[80]. There are however even

more checkpoints which play a crucial role in the cell cycle such as the spindle checkpoint, which arrests cell cycle at M phase until all chromosomes are aligned on spindle and Morphogenesis checkpoint, which plays an important role in the detection of abnormality in cytoskeleton and arrests cell cycle at G2/M transition(**Figure 4**)[81]. Checkpoint failure often results in mutations and genomic rearrangements giving rise to genetic instability. In cancer cells checkpoints are defective and control mechanisms are usually lost by mutation[82]. Also, carcinogenic viruses such as SV40 produce proteins such as T-antigen, which bypass G1/S control and transform cells by essentially eliminating p53 and pRb[83]. In addition to cell cycle checkpoints, cyclins and CDKs (protein kinases which regulate the cell cycle events such as transcription, mRNA processing) provide novel targets for cancer treatment. Chemical inhibitors of CDKs include Olomoucine. Recently, a novel peptide was synthesized that serves as a docking site for cyclin/CDK2 complexes, inhibiting their action and inducing apoptosis preferentially in tumour cells[84]. Chemotherapy which creates multiple cell cycle arrests by engaging both the G1/S and the G2/M checkpoints was one of the promising approaches against the on-going fight against different types of cancer[85, 86].

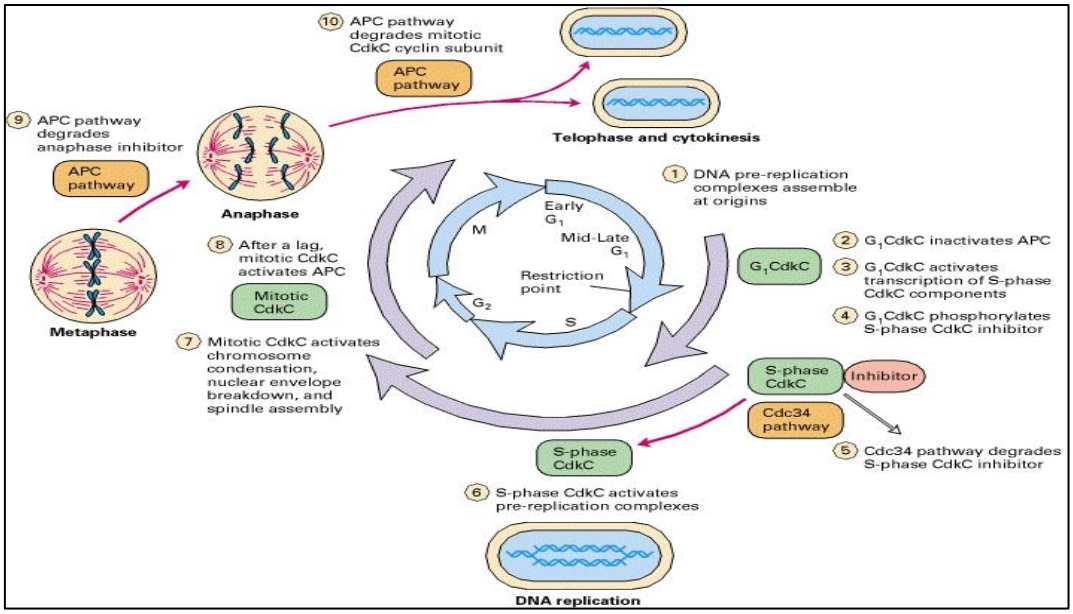


Figure 13: Current model for regulation of the eukaryotic cell cycle[87]

2.21 Chemotherapy

Chemotherapy refers to the treatment of cancer which is mediated by chemical substances, particularly one or a combination of anti-cancer drugs. The administration of chemotherapeutic agents is always with a therapeutic intent or it may be intended to prolong life and reduce symptoms[88]. Chemotherapy is one of the major subdivisions of medical oncology along with targeted therapy and hormonal therapy. Most of the time, chemotherapy is often used in conjunction with other cancer treatments such as radiation therapy, surgery, and/or hyperthermia therapy. Chemotherapeutic agents are cytotoxic therefore also possess the ability to damage cells that divide rapidly under normal circumstances such as cells in the bone marrow and digestive tract. This results in myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss)[89].Selected new anticancer drugs have been developed to be target specific and are not broadly cytotoxic[90].

2.21.1 Targeted therapy

Targeted therapies influence the processes that control division, growth and spread of cancer cells as well as the signals that cause cancer cells to die. Targeted therapies interfere with specific molecular targets which are associated with cancer and act in various mechanisms such as blocking the action of certain enzymes, proteins and other molecules involved in the spread of cancer[91]. The actions of targeted therapeutic drugs are often cytostatic, that is they block tumour cell proliferation[92]. Other kinds of targeted therapies empower the immune system in the killing of cancer cells or they can act on their own to transport toxic substances straight to cancer cells and kill them[91]. Examples of targeted therapies include monoclonal antibodies as well as small organic molecules. Some of small molecules like trastuzumab, sorafenib and sunitinib were approved to treat cancers of breast, liver and pancreas respectively[93].

2.21.2 Growth signal inhibitors

Growth signal inhibitors are hormone-like elements that modulate signals responsible for cell growth and division. Cetuximab, trastuzumab, imatinib and gefitinibare amongst the first targeted therapies that block growth signals[94, 95].

2.21.3 Angiogenesis inhibitors

Angiogenesis refers to the formation of new blood vessels. Agents which inhibit angiogenesis are types of targeted therapy which employs drugs or other elements to arrest the formation of new blood vessels by tumours[96]. Some angiogenesis inhibitors are endogenous in nature

and are the part of the body's internal control mechanism. Others acquired exogenously through pharmaceutical drugs or even diet. The first angiogenesis inhibitor, bevacizumab (Avastin) was approved and discovered in 2004. It is currently used to treat advanced colorectal, kidney and lung cancers[97].

2.22 Types of drugs used to treat cancer

2.22.1 Alkylating agents

These drugs cause damage to DNA to avert the reproduction of cancer and they function throughout the phases of the cell cycle. Alkylating agents are used to treat a wide range of cancers such as leukemia, Hodgkin disease, multiple myeloma, lymphoma and sarcoma, including cancers of the lung, breast, and ovary[98].

Since alkylating agents have the potential to cause DNA damage, they can cause long-term damage to the bone marrow and in some cases this can ultimately lead to acute leukaemia[99].

Nitrogen mustards: Chlorambucil, mechlorethamine, cyclophosphamide (Cytoxan®),ifosfamide, and melphalan[100].

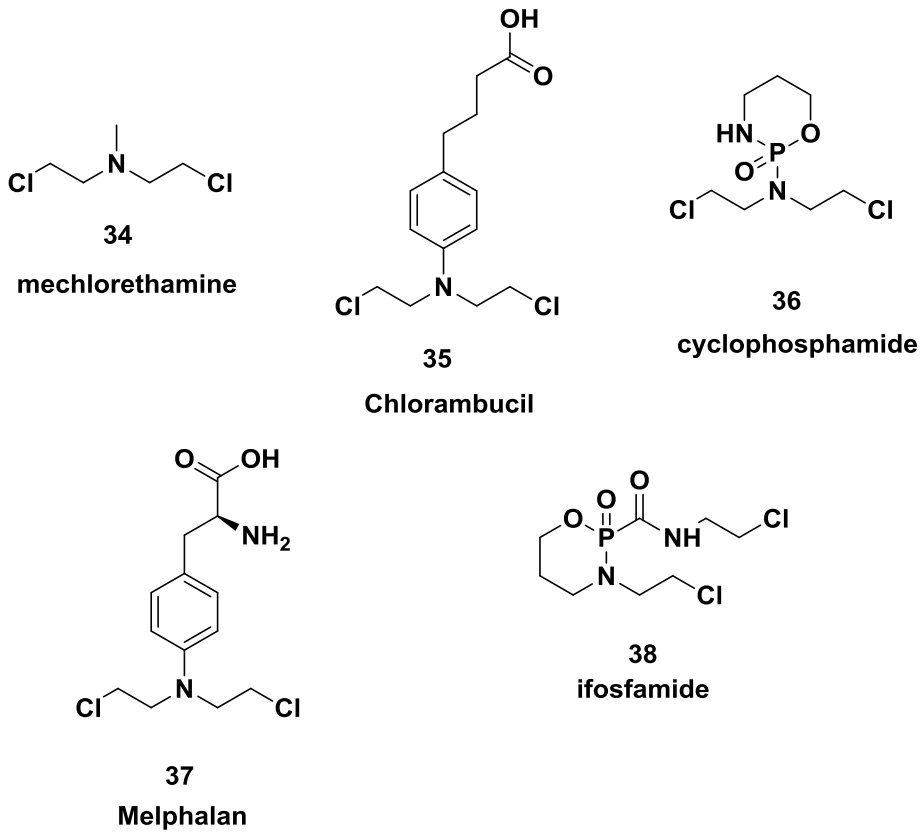
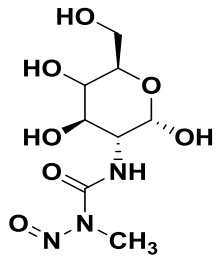
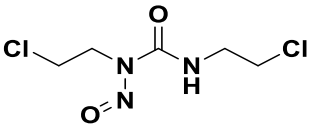


Figure 14: Types of alkylating agents

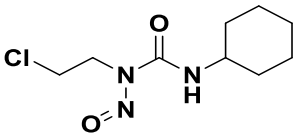
Nitrosoureas: Streptozocin, carmustine (BCNU), and lomustine[101].



38
Streptozotocin



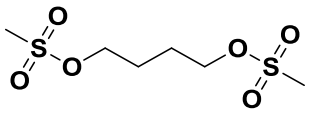
39
Carmustine



40
Lomustine

Figure 15: Various nitrosoureas

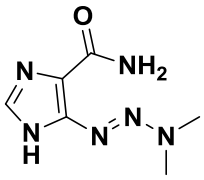
Alkyl sulfonates: busulfan[102]



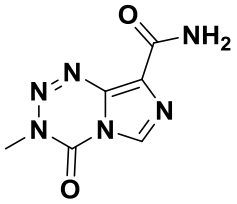
41
Busulfan

Figure 16: Structure of Busalfan

Triazines: Dacarbazine (DTIC) and temozolomide (Temodar®)[103]



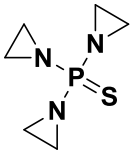
42
decarbazine



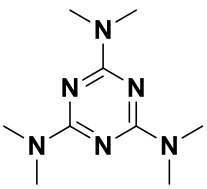
43
temozolomide

Figure 17: Triazines

Ethylenimines: Thiotepa and altretamine (hexamethylmelamine)[104]



44
thiotepa



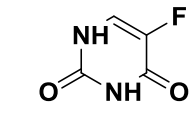
45
Altretamine

Figure 18: Ethylenimines

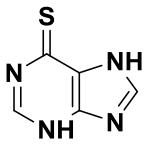
2.22.2 Anti-metabolites

These drugs hamper DNA and RNA growth and development by replacement of the normal building blocks of RNA and DNA. They cause damage to cells during the S phase of the cell cycle and are frequently used to treat leukaemias, ovary, intestinal tract, breast, and other types of cancer[105, 106].

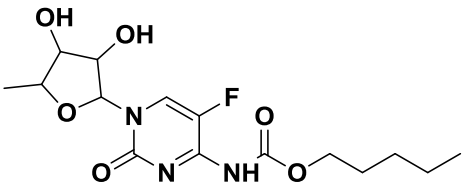
Anti-metabolites bear a resemblance to either nucleosides or nucleobases however they have different chemical groups. These drugs act by either blocking enzymes required for DNA synthesis or becoming fused into DNA or RNA. If the enzymes required for DNA synthesis are blocked, mitosis is prevented and DNA duplication is halted. Also, after mis-incorporation of the molecules into DNA, DNA damage can occur and apoptosis is induced[107].



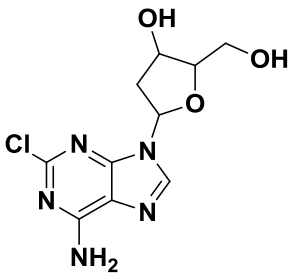
46
5-fluorouracil (5-FU)



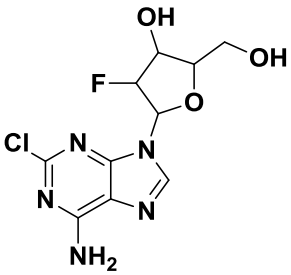
47
6-mercaptopurine (6-MP)



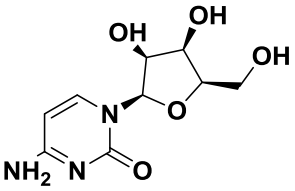
48
Capecitabine (Xeloda®)



49
Cladribine



50
Clofarabine



51
Cytarabine (Ara-C®)

Figure 19: Antimetabolites

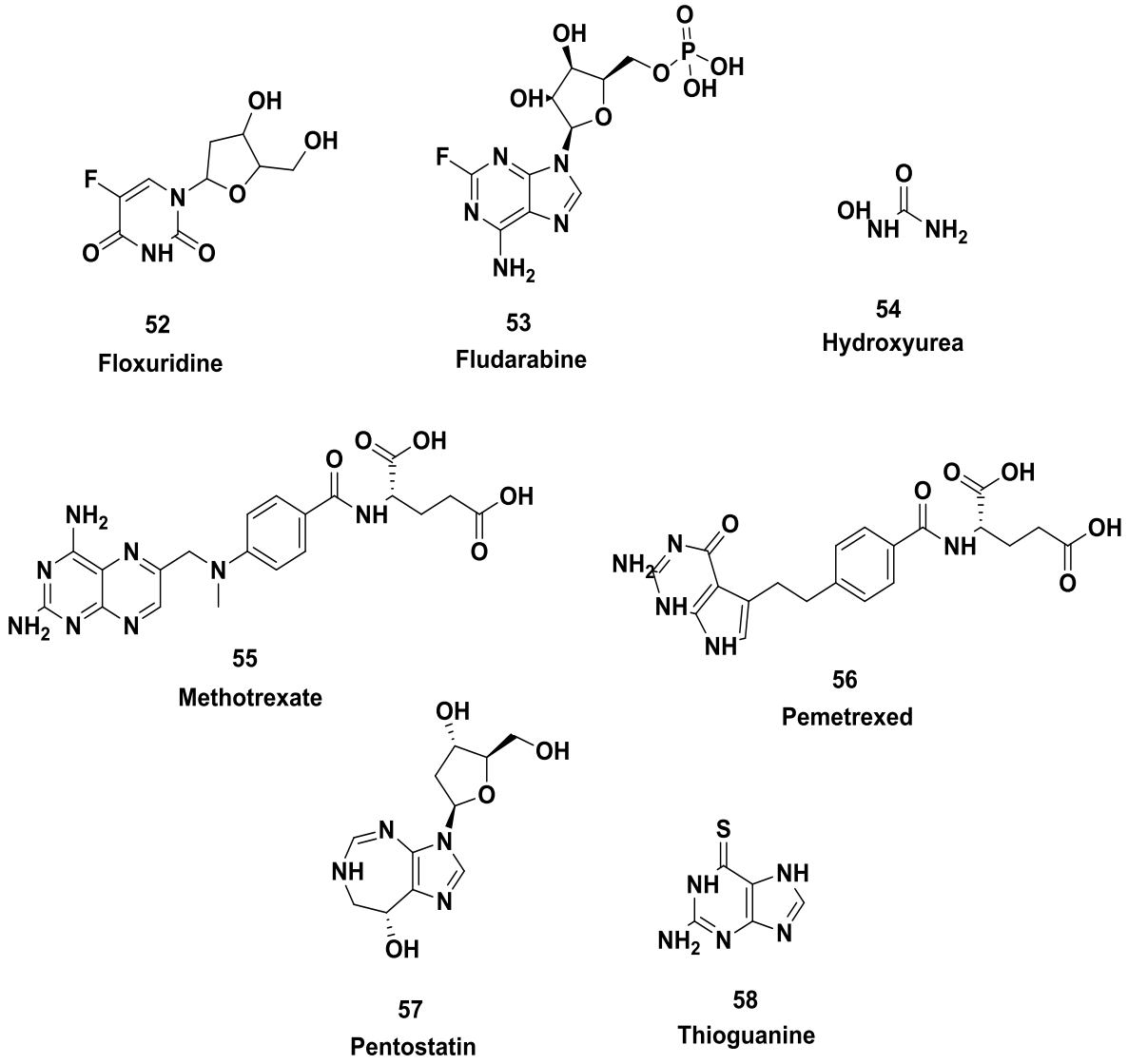


Figure 20: Antimetabolites

2.22.3 Anti-tumour antibiotics

Anthracyclines

These anti-tumour antibiotics cause obstructions in the enzymes which are involved in DNA replication and they work in all phases of the cell cycle, however these drugs can cause permanent damage to the heart if given in high doses[108, 109].

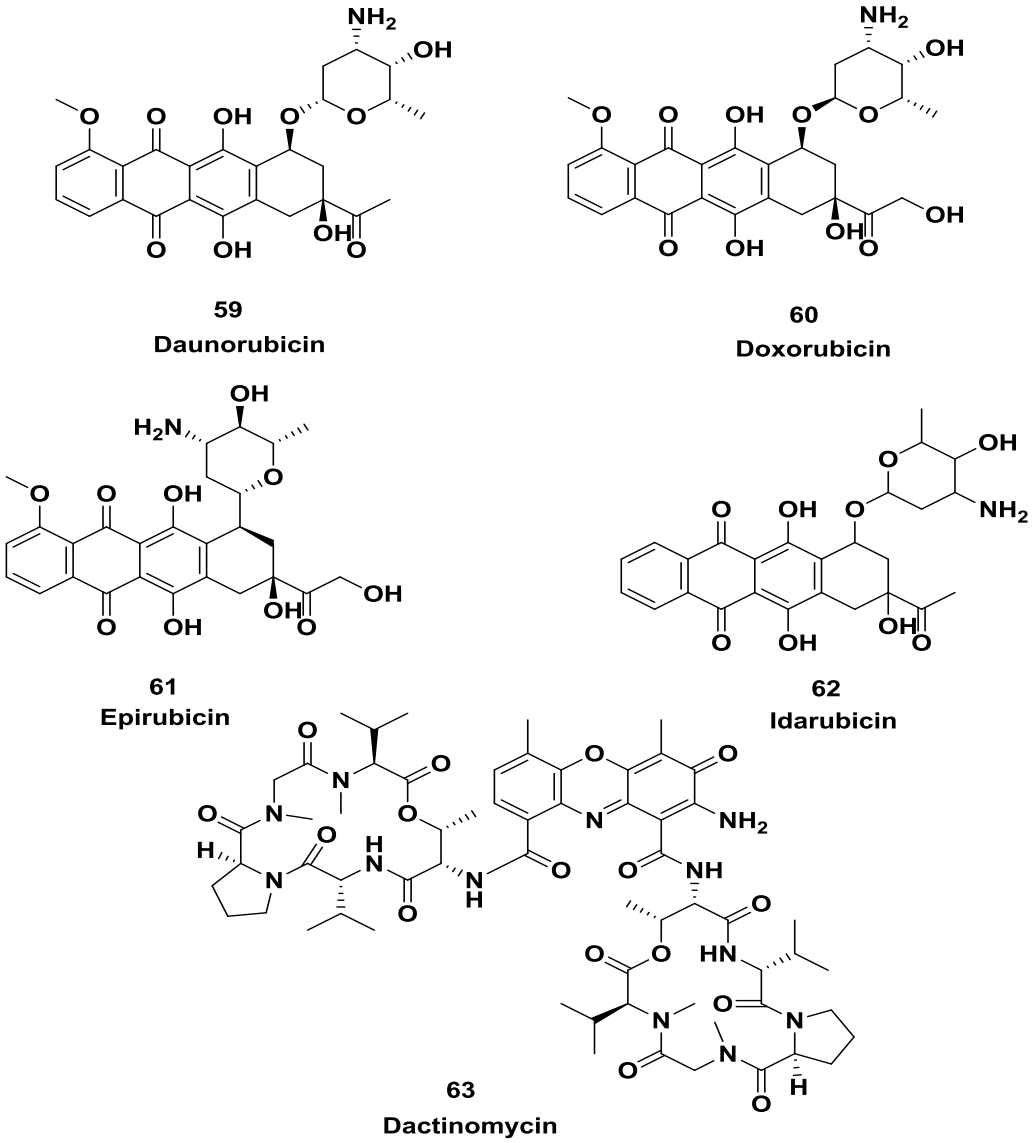


Figure 21: Anthracyclines

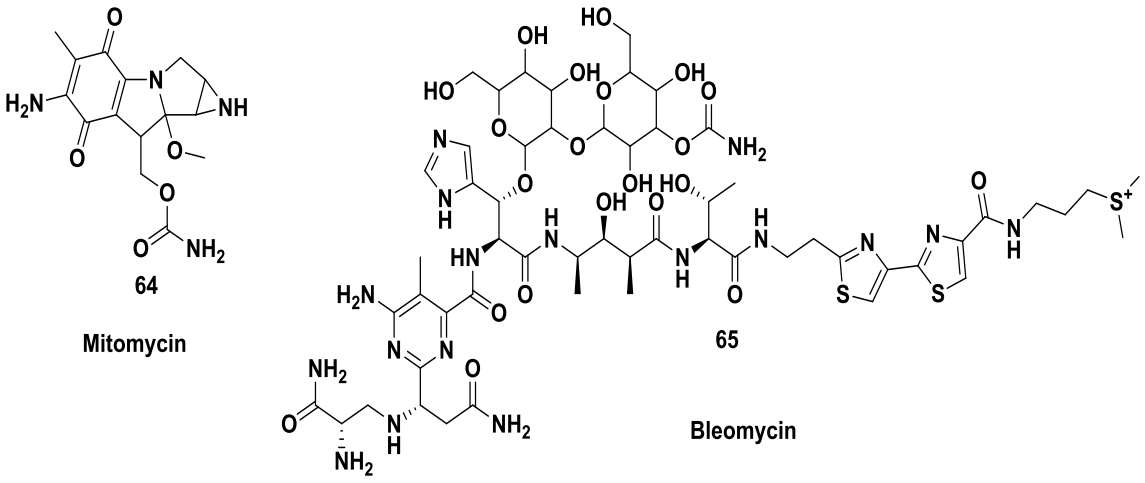
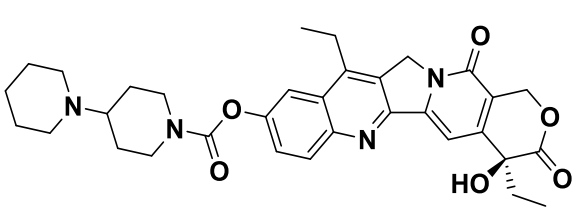


Figure 22: Anthracyclines

2.22.4 Topoisomerase inhibitors

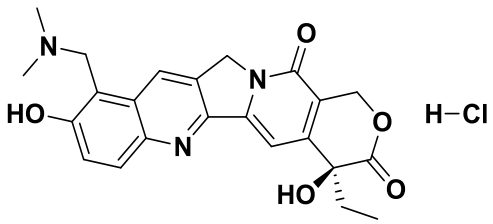
Topoisomerase inhibitors exert their effect by inhibiting topoisomerase I and II. During DNA replication when the DNA double helix is relaxed or transcription, the adjacent unopened DNA supercoils. The topoisomerase enzymes come in to play at this point to remove torsional stress by producing single or double-strand breaks into DNA and this result in the reduction of tension in the DNA strand. This process allows for the normal unwinding of DNA to occur during replication or transcription. Inhibition of topoisomerase I or II interferes with both of these processes[110].

Two of the widely known topoisomerase I inhibitors irinotecan and topotecan are prepared by chemical synthesis from the natural product camptothecin, which is acquired from the Chinese ornamental tree *Camptotheca acuminata*[111, 112].Topoisomerase II inhibitors can be categorized into two groups. These inhibitors cause an increased level of enzymes bound to DNA and inhibits DNA from replicating and being transcribed. They also encourage DNA strand breaks and therefore this can also lead to apoptosis. These Topoisomerase II agents include mitoxantrone doxorubicin etoposide and teniposide. The second group are catalytic inhibitors and these type of drugs act by hindering the action of topoisomerase II and as a result they prevent DNA synthesis and translation[113]. This group of drugs includes aclarubicin, merbarone, and novobiocin.[113]



66

Irinotecan



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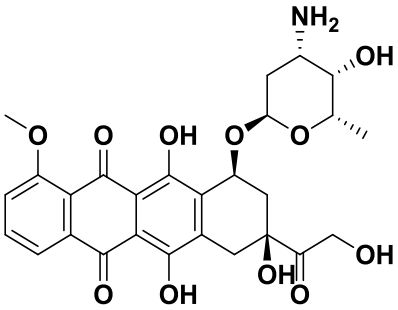
Topotecan



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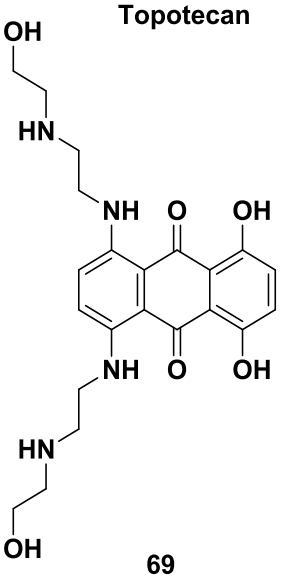
Doxorubicin



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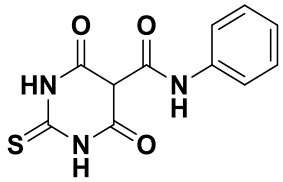
Mitoxantrone



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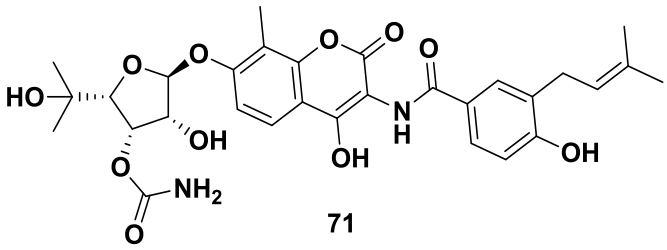
merbarone



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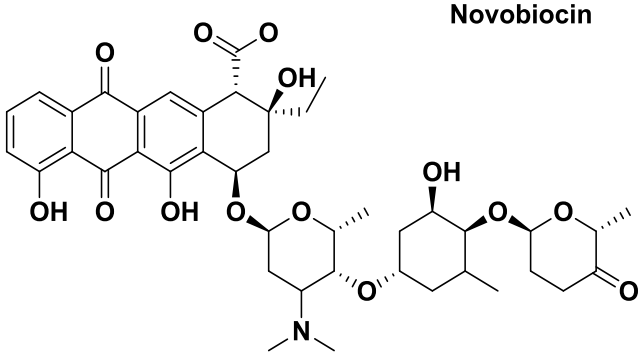
Novobiocin



72

Aclarubicin

Figure 23: Topoisomerase inhibitors

2.23 Examples of Drug targets in cancer

2.23.1 Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

Nuclear factor kappa-light-chain-enhancer of activated B cells(NF-κB)is a transcription factor which is important for immunity and inflammation and it is involved in diverse cellular responses to stimuli such as bacterial or viral antigens, stress, ultraviolet irradiation, cytokines, free radicals, oxidized LDL. The major function of NF-κB is in the regulation of the immune response to infection[114]. Research has shown that incorrect regulation of NF-κB leads to cancer, autoimmune diseases, inflammation, viral infection, septic shock, and improper immune development[115,116].

Activated NF-κB triggers the expression of genes that keep the cell proliferating and protect the cell from conditions that induce apoptosis. Abnormalities in NF-κB results in an increased susceptibility to apoptosis[117]. This occurs because NF-κB regulates anti-apoptotic genes especially theTRAF1andTRAF2 and therefore, checks the activities of the caspase family of enzymes, which are central to most apoptotic processes[118]. In tumour cells, NF-κB is active either due to mutations in genes encoding the NF-κB transcription factors themselves or in genes that control NF-κB activity (such as IκB genes); in addition, some tumour cells secrete factors that cause NF-κB to become active. Blocking NF-κB can cause tumour cells to stop proliferating to die, or to become more sensitive to the action of anti-tumour agents[119]. Thus, NF-κB has become one of the main anti-cancer targets and a subject of choice in cancer research.

Aberrant activation of NF-κB is often detected in several cancers. Furthermore, the suppression of NF-κB restricts the multiplication of cancer cells. NF-κB has been noted to be a major player in the inflammatory response, hence methods of inhibiting NF-κB signalling has potential therapeutic application in cancer and inflammatory diseases[120].

Some of reported drugs that inhibit the signalling of NF-κB include metine, fluorosalan, sunitinib malate, bithionol, narasin, tribromsalan and lestaurtinib. Their mechanism of action of NF-κB signalling inhibition occurs via the inhibition of I-kappa-B-alpha (IκBα) phosphorylation[121]. One of the quinolone drugs known to have significant inhibitory activity against NF-κB is Moxifloxacin which acts by blocking the activation of NF-κB translocation. This drug has also shown to have anti-proliferative and apoptotic effects of Etoposide (VP-16) which is a topoisomerase II inhibitor [122].

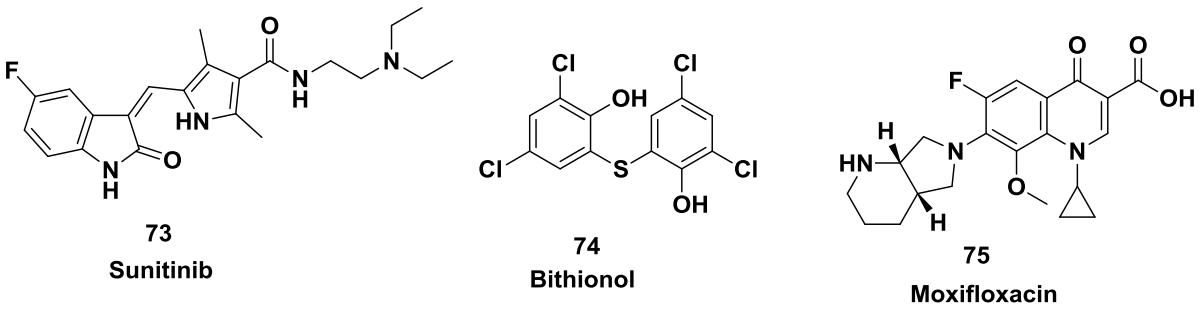


Figure 24: NF-κB inhibitors

2.23.2 DNA gyrase

DNA gyrase (a type 2 topoisomerase) is a vital enzyme found in bacteria which catalyses the ATP-dependent negative super-coiling of double-stranded DNA[123]. DNA gyrase is a major intracellular target for different antibacterial agents. Gyrase is found largely in prokaryotes as well as some eukaryotes also posses it, but the enzymes have different affinities for different molecules and are immensely different in structure. Humans lack this enzyme; therefore this makes gyrase a validated drug target. The two major classes of antibiotics that inhibit gyrase are:

- The quinolones (e.g. nalidixic acid and ciprofloxacin) which act by binding to these enzymes and prevent them from de-catenation of DNA that is replicating[124].
- Amino-coumarins (e.g. novobiocin)- which work by competitive inhibition of energy transduction of DNA gyrase by binding to the ATPase active-site located on the GyrB subunit[125].

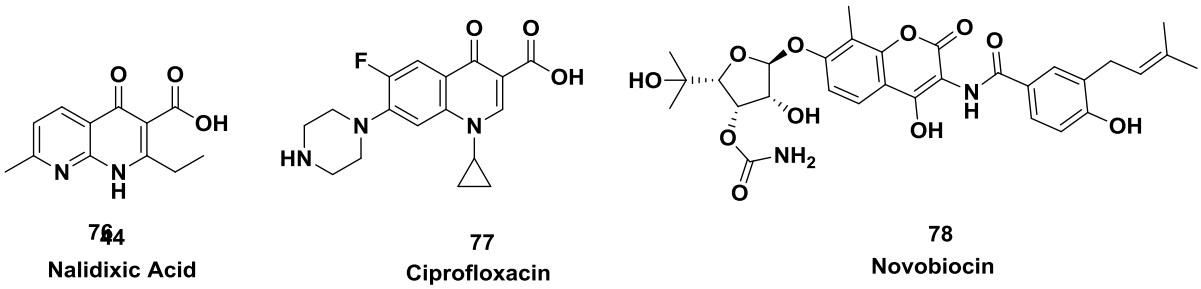


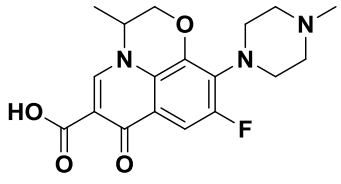
Figure 25: DNA gyrase inhibitors

2.23.3 Telomerase

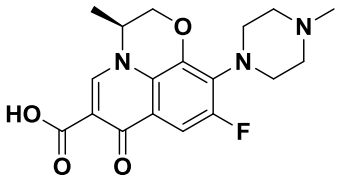
Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the3'end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes[126]. The telomeres region comprises of DNA which is noncoding and also plays

an important role in the prevention of the loss of important DNA from chromosome ends. This result in the loss of only 100–200 nucleotides every time the chromosome is copied and this causes no destruction to the organisms DNA. Telomerase is usually expressed in human cancer cells and its higher expression produces vulnerability of cancer cells. This increased expression renders them distinguishable from normal cells even though normal cells do also possess some active telomerase. If telomerase is present abundantly, it permits cells to keep growing and dividing. This increased expression of telomerase makes it an attractive target for target-based drug design and administration[127-129]

The activity of telomerase is compulsory for the immortality of many cancers and it is inactive in somatic cells. This indicates that inhibition of telomerase can selectively inhibit cancer cell progression with minimal side effects[130]. If telomerase is turned off by a drug in cancer cells, the telomeres will gradually shorten as these cancer cells carry on dividing and ultimately lead to the loss of chromosomes and eventually cell death[131, 132]. Some of the newer quinolones such as ofloxacin and levofloxacin have been found to inhibit telomerase activity in transitional cell carcinoma cell lines by a mechanism which is still unclear[133].



79
Ofloxacin



80
Levofloxacin

Figure 26: Telomerase inhibitors

2.24 Description of quinolones and related analogues

The quinolone group of antibacterial agents has generated substantial clinical interest since their discovery in the early 1960s and they have been widely active against a number of bacterial infections[134]. The 4-pyridone-3-carboxylic acid associated with a 5, 6-fused aromatic ring is the common chemical feature of bactericidal quinolones[134]. In the resulting heterocyclic ring, the 1-, 5-, 6-, 7-, and 8-positions are the main positional targets of chemical variation. Many improvements have been made on the basic quinolone ring which includes the replacement of hydrogen with fluorine at position 6, substitution with a cyclic amine residue at position 7 and addition of new variables at position 1 of the quinolone ring. These chemical improvements have led to an improved efficacy of antibacterial activity such as

improvements of moxifloxacin and garenoxacin[135,136]. The on-going drug resistance continues to limit drug choice; however the introduction and application of the new quinolones continues to give an exciting overview in antimicrobial chemotherapy. Quinolone antibacterial drugs have made a major impact in the field of antimicrobial chemotherapy, however they became a neglected group for some years until the development of the fluoroquinolones in the 1970s and 1980s[137]. The fluoroquinolones have a broad spectrum of activity compared with the earlier compounds. The first generation of quinolones began with the introduction of nalidixic acid in 1962 for treatment of urinary tract infections (UTIs)in humans[138]. Quinolones achieve their antibacterial effect by preventing bacterial DNA from unwinding and duplicating[139]. Most quinolones in clinical use today belong to fluoroquinolones, which have a fluorine atom attached to the central ring system, characteristically at the 6-position or C-7 position[140, 141].

Quinolones are bactericidal and exhibit concentration-dependent killing[142]. The known targets where quinolones act are the bacterial DNA gyrase and topoisomerase IV which are enzymes essential for DNA duplication and transcription[143]. Newer fluoroquinolones (i.e., levofloxacin, sparfloxacin, trovafloxacin and grepafloxacin) are broad-spectrum agents with improved biological activity against many Gram-negative and Gram-positive pathogenic microorganisms[144, 145]. The quinolone pharmacophore has also been found to inhibit NF-κB which is a protein complex that controls transcription of DNA. Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and improper immune development[146-148]. These findings have provided us with a firm basis for further research on such compounds to develop more potent derivatives.

2.25 Natural Sources of Quinolones: Rutaceae Family of Plants

A Rutaceae family consist of just about 160 genera from which many important compounds are synthesized such as limonoids, flavonoids, alkaloids, coumarins and volatile oils[149]. Many kinds of alkaloids have also been isolated from these genera which contain aromatic heterocyclic rings such as: quinoline and acridine quinazolin. The major part of quinoline alkaloids are quinolone derivatives, which are classified into four groups: 2-quinolone, 4-quinolone, 1-methyl-2-quinolone and 1-methyl-4-quinolone frameworks (**Figure-18**)[150, 151].All quinolone derivatives exhibit medicinal value, among which the 2-quinolone derivatives are the focal point in the present thesis. More than 300 quinolone alkaloids bearing the 2-quinolone ring have been isolated[152]. Such naturally occurring quinolone derivatives have attracted much deserved interest and attention due to their isolations;

structural elucidation and synthesis have shown to be of major importance with respect to combating pathogenic organisms. Some of the reported medicinal properties of 2-quinolones derivatives are cytotoxic activity, antitumor activity, antimicrobial activity, antibacterial activity and antimalarial activity[151, 153].

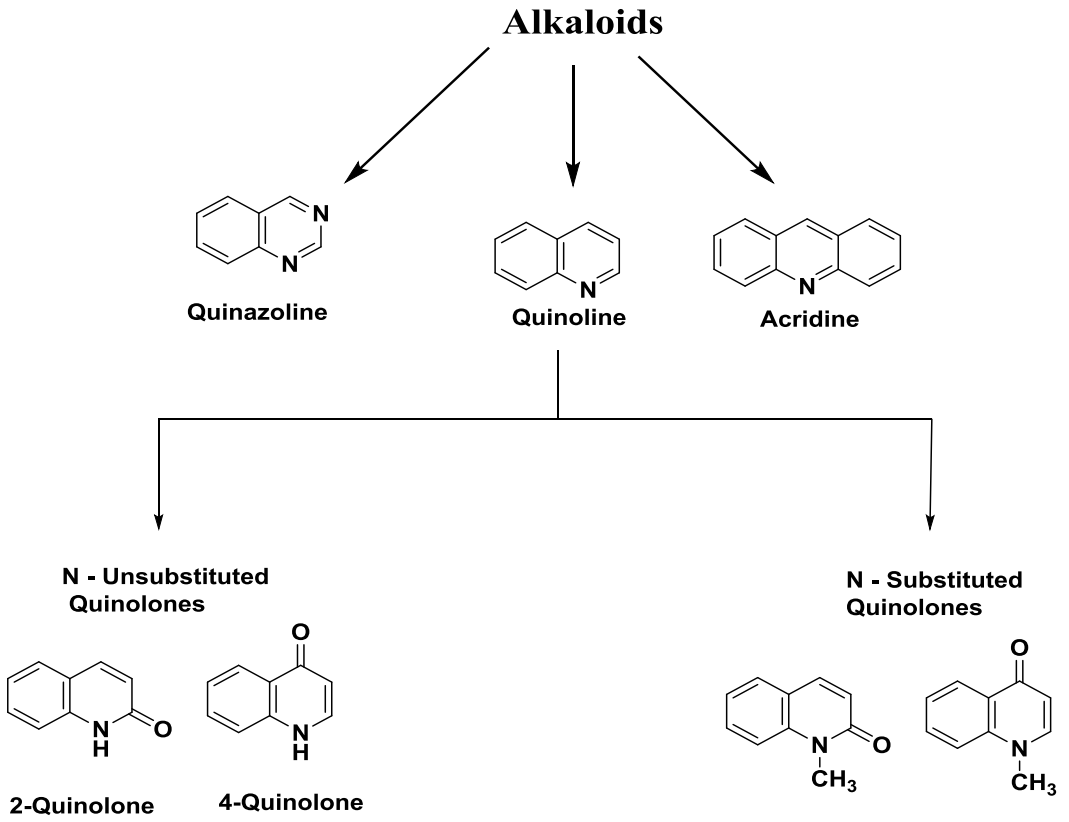


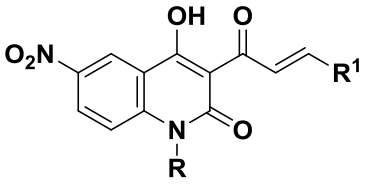
Figure27: Types of quinolones

2.26 Biological activities of 2 Quinolones

Most of 2 quinolones derivatives are said to possess a wide range of biological activities ranging from antibacterial, anticancer and anti-mycobacterial activities. Moreover, substituents at C-7 and C-8 have shown profound biological activity.

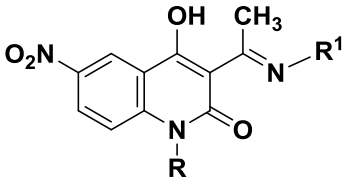
2.26.1 Antimicrobial activity

Abdel-Ghani*et al*., reported the synthesis and antimicrobial activities of new 6-nitro-4-hydroxy-2-quinolone derivatives. These compounds were evaluated against Gram positive & Gram negative bacteria and fungi strains such as *Aspergillus nigar and Aspergillus flavus.* Most of these compounds showed moderate to good activity against all the species of bacteria and fungi[154].



81

a. R = CH_3 , R^1 = C_6H_5
b. R = CH_3 , R^1 = $C_6H_4OCH_3$
c. R = C_2H_5 , R^1 = C_6H_5
d. R = C_2H_5 , R^1 = $C_6H_4OCH_3$



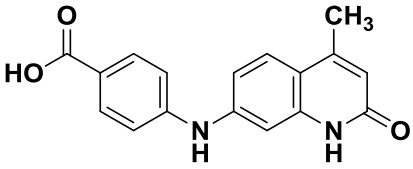
82

a. R = CH_3 , R^1 = $CSNH_2$
b. R = CH_3 , R^1 = $NHCONH_2$
c. R = C_2H_5 , R^1 = $CONH_2$

Figure 28: Antimicrobial activity

2.26.2 Evaluation of novel DNA-gyrase B inhibitors

Shiroya *et al*., reported the *in silico* design, synthesis and evaluation of novel DNA-gyrase B inhibitors. Based on docking simulations and results obtained, series of 2-quinolone analogues were designed, synthesized, characterized and evaluated for their antibacterial activity against *S. aureus* and *E. coli*. Compound-2 showed good antibacterial activity against *S. aureus* and *E*. coli[155].

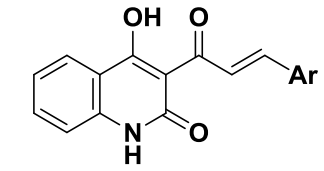


83

Figure 29: Antibacterial activity

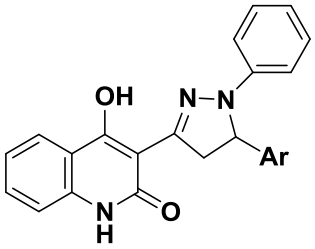
2.26.3 Antimalarial activity

Sarveswari *et al*., 2014 reported the synthesis and evaluation of 4-hydroxy-2(1*H*)-quinolone derived chalcones, pyrazolines as antimicrobial and antimalarial agents. Among the tested compounds **4h** and **5d** were found to have a potent antimalarial activity than the standard drugs and the others are found to show considerable antimalarial activity and moderate antimicrobial activity[156].



84

Ar = Thiophen-2-yl



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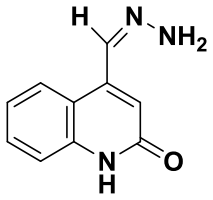
Ar = 4-Methoxyphenyl

Figure 30: Antimalarial activity

2.26.4 Anticancer activity

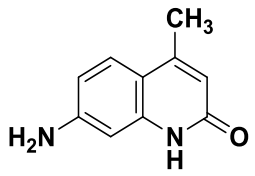
Jayashree *et al*., reported the synthesis, characterisation, antioxidant and anticancer evaluation of novel Schiff bases of 2-quinolones.*In vitro* cytotoxicity on A549 (lung cancer cell line) by MTT assay was employed. The activities of all the test compounds were compared to the standard drug Methotrexate, which showed its cytotoxicity at a concentration of 1µM. Cytotoxicity was checked at 24 hours duration and compound JMB-7 was found to be highly potent than all other synthesized compounds[157].



86

Figure 31: Anticancer activity

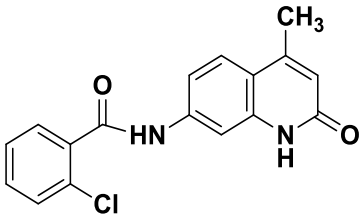
Kumar *et al*., also reported on the cytotoxic capability of 2-quinolone derivatives using *in vitro* and *in vivo* (solid tumour and liquid tumour) models of cancer. *In vitro* cytotoxicity studies were carried out in MCF-7 cells by MTT assay and identified potent compounds[158].



87

JST

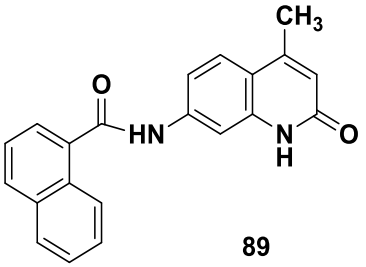
CTC_{50} = 127.46 µm



88

JST2

CTC_{50} = 83.04 µm



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JST13

CTC_{50} = 22.04 µm

Figure 32: Anticancer activity

2.26.5 Anti-inflammatory activity

Suthar *et al*., synthesized novel quinolone substituted thiazolidin-4-ones and evaluated as anti-inflammatory agents. *In vivo* anti-inflammatory activity of quinolone substituted thiazolidin-4-ones on carrageenan-induced rat paw edema model identified compound **6b** as a potent anti-inflammatory agent[159].

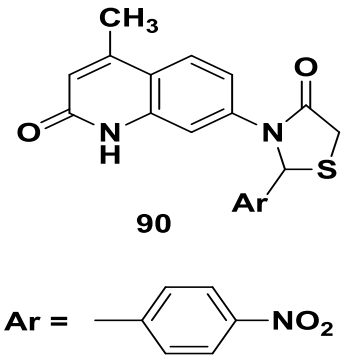

Figure 33: Anti-inflammatory activity

2.26.6 Antifungal activity

Heeb *et al*., reported 4-formyl-1-sulfanyl-2(1*H*)-quinolone produced by some pseudomonads has shown to exhibit strong fungicidal properties[160].

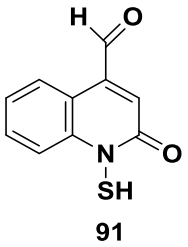

Figure 34: Antifungal activity

2.27 Justification of the research

Quinolones are a class of heterocyclic compounds, commonly occurring in nature as quinolone alkaloids. Compounds having the 2-quinolone moiety are known to have remarkable biological actions including antimalarial, antiviral, and tuberculostatic, antibacterial, antifungal, anticancer, antidiabetic, cardiotonic and bronchodilator activity etc.

Substitution of the 2-quinolone moiety at positions 7 and 8 are critical for potent antimicrobial activity. However structural modifications and substitutions at different positions of the quinolone ring have also showed potential as anticancer, antimalarial and have also portrayed great tuberculostatic effects[161-163]

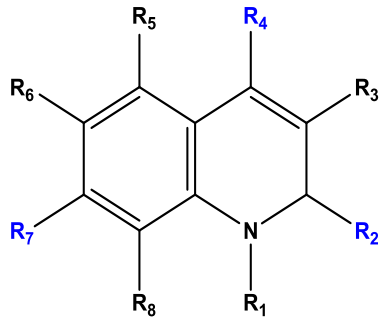

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Figure 35: substitutions at various positions of the quinolone ring

Position 1: This position has a hydrophobic interaction with the major grove of DNA. A cyclopropyl substituent is now regarded to be the most potent variation here as seen in Ciprofloxacin, followed by addition of a 2,4-difluorophenylgroup[164].

Position 2: This site is very close to the site for DNA gyrase binding so it is believed that any added bulky group inhibits access and thus resulting in a lower level of antimicrobial activity. Only a sulphur, incorporated into a small ring has been able to replace hydrogen at the R-2 position[164].

Positions 3 and 4: These two positions are considered very important for binding to cleaved or perturbed DNA, and no useful substitutions have yet been reported. Therefore, the 3-carboxylate and 4-carbonyl groups are regarded as essential substituents for antimicrobial activity[164, 165].

Position 5: Substitution at this location appear to have the ability to alter overall steric configuration of the molecule. reasonably sized additions, such as an amino, hydroxyl, or methyl group can noticeably increase *in vitro* activity against Gram-positive bacteria[166].

Position 6: Substitution with a fluorine molecule here has shown to improve antimicrobial activity compared to the original quinolones, and gave rise to the now widely used and clinically successful fluoroquinolones compounds. Whatever structure is placed at this site,

the substituents at positions 1, 7, and 8 continue to be key determinants of overall biological activity in the compounds under active development[167].

Position 7: This position directly interacts with DNA gyrase, or topoisomerase IV. The optimal substituents are 5- or 6-membered nitrogen heterocycle most commonly, aminopyrrolidines and piperazines. Placement of aminopyrrolidine improves Gram-positive activity, whereas a piperazine enhances potency against Gram-negative bacteria. Alkylation ($-CH_3$) of the 5-membered or 6-membered also enhances activity against Gram-positive bacteria. Specific placement of amino or methyl groups on the position 7 has similar resultant. Increased bulkiness here has shown to confer protection from the efflux exporter proteins of bacteria such as with moxifloxacin and trovafloxacin[168].

Position 8: This position has shown to confer overall molecular steric configuration Therefore, changes made here affects target affinity, probably by altering drug access to the enzyme or DNA binding sites. A free halogen (F or Cl) here may provide improvement in activity against anaerobes. Halogen substituents, as well as a methyl or methoxy also increase the *in vitro* activity against Gram-positive cocci. The R-8–substituted quinolones also exhibit enhanced bacteriostatic and lethal activities against GyrA mutants of both *E. coli* and *Mycobacterium* species. Furthermore in *S. aureus* a substitution here created the most lethal agent for both wild type cells as well as those strains with a pre-existing topoisomerase IV mutation[169].

Quinolone derivatives have been extensively studied documented for decades and have proven to be very effective scaffolds to fight against pathogenic microorganisms Therefore, in view of the above facts and in continuation of our search on biologically active hybrid molecules, we ideated to synthesize novel quinolone substituted urea and thiourea derivatives and their subsequent biological evaluation for anti-cancer, antibacterial and anti-mycobacterial activity. In this research, the major areas of substitution in the quinolone ring are the C-2, C-4 and C-7 positions. It was considered of interest to synthesize some new chemical entities incorporating a methyl group at C-4 and attachment of different isocyanates/ and isothiocyanates to the NH_2 group at C-7 at a single molecular frame work thus forming a urea and thiourea linkage to investigate their biological activities.

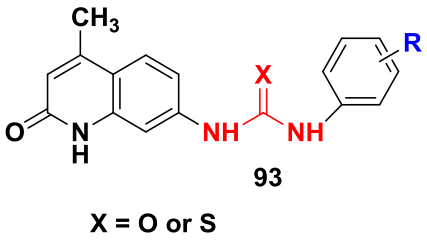
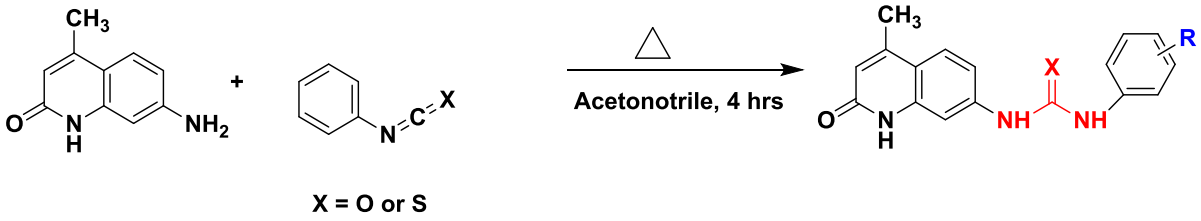


Figure 36: Proposed 2-quinolone substituted urea model

2.28 Detailed Research Aims and Objectives

This research project aims at the design and synthesis of biologically active hybrid molecules consisting of 7-amino-4-methyl 2-quinolone pharmacophore coupled with isocynates and isothiocynates at C-7 thus forming quinolone-urea hybrid molecule.



Scheme 1: The synthetic route to prepare 2 quinolone urea/thiourea hybrid compounds

Detailed objectives

- To synthesize Novel substituted quinolone derivatives comprising of isocyanates and isothiocyanates at C-7 by using conventional Pechman condensation reaction of *m*-phenylene diamine with ethylacetoacetate followed by the formation of urea at C-7 by refluxing the resulted quinolone with various isocynates and isothiocynates under acetonitrile to afford quinolone-phenylurea hybrid compounds.
- Structural elucidation of the synthesized compounds using spectroscopic techniques: ^{1}H NMR, ^{13}CNMR, IR, and LCMS.
- *In vitro* antimicrobial activity of the synthesized novel compounds against cancer cell lines and a wide range of Gram positive and Gram negative bacteria and *Mycobacterium tuberculosis* (H37Rv).

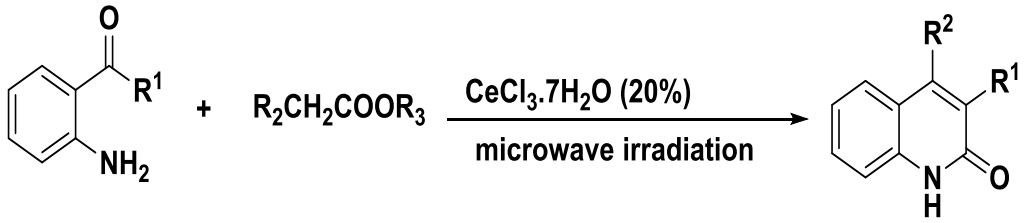
2.29 Significance of 2-Quinolones

2-quinolones are an essential group of compounds which are also isomeric to 4-quinolones and isosteric to coumarins[155]. As stated previously, compounds that have the 2-quinolone moiety are regarded to possess attention-grabbing biological activities which include antimalarial, anticancer, antibacterial, antiviral, cardio-tonic and N-methyl-D-aspartate

receptor inhibitory functions. Quinolones obstruct the DNA breakage–reunion cycle by binding to the subunit A and by blocking the gyrase–DNA complex, whereas the latter act on subunit B[170, 171]. The 2-quinolone moiety is positioned in several alkaloids, particularly in the Rutaceae family, many of which possess remarkable biologic activity such as antihypertensive, cardiotonic, diuretic, anti-inflammatory and bronchodilatory agents[172,161].

2.29.1 Examples of the synthesis of 2-quinolones

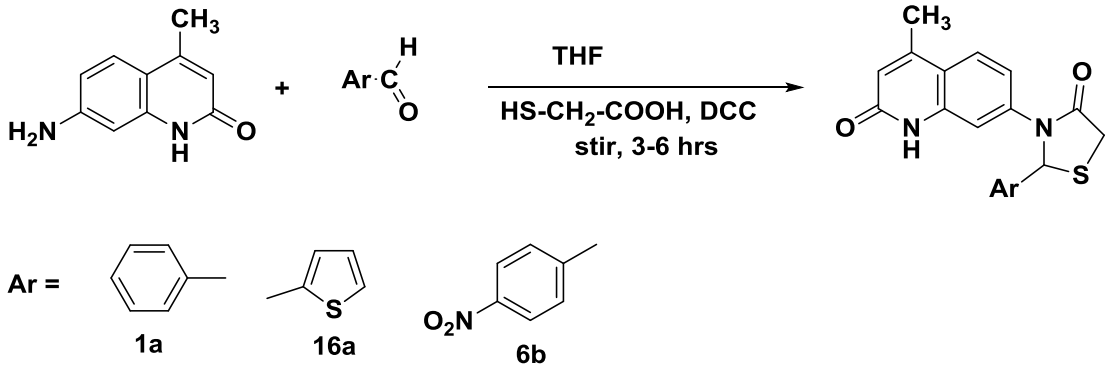
A quick and an efficient process for the preparation of a diversely substituted 2-quinolones involve the reactions of *o*-aminoarylketones with an ester containing a reactive α-methylene moiety in the presence of a catalytic amount of cerium chloride hepta-hydrate under solvent-free conditions. The microwave irradiation method has been reported to give higher yields[173].



Scheme 2: Preparation of a variety of substituted 2-quinolones

2.29.2 Reaction with Thiazolidinones

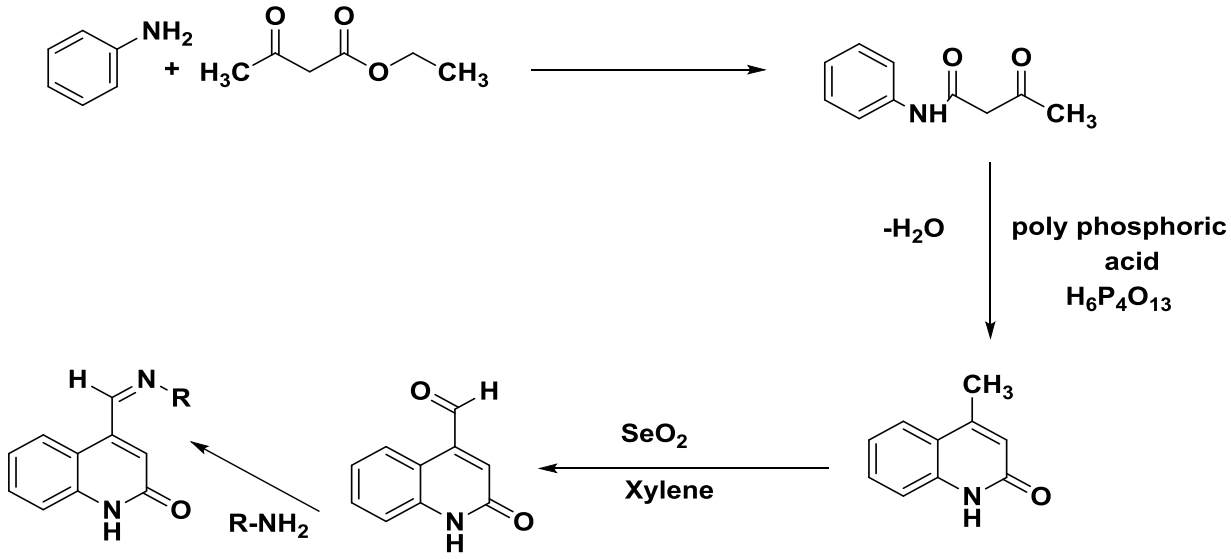
Suthar*et al*[159] reported the reaction of the 7-amino-4-methyl-2-quinolone moiety with thiazolidinone which occurs via the formation of a Schiff base to form quinolone substituted thiazolidin-4-ones. Synthesized compounds were evaluated for anti-inflammatory and anti-cancer activities. Compound **6b** was found to have potent anti-inflammatory activity and two other compounds (**1a** and **16a**) exhibited maximum cytotoxic effect against BT-549, HeLa, COLO-205, and ACHN human cancer cell lines.



Scheme 3: reaction with thiazolidinones

2.29.3 Reaction with Schiff bases

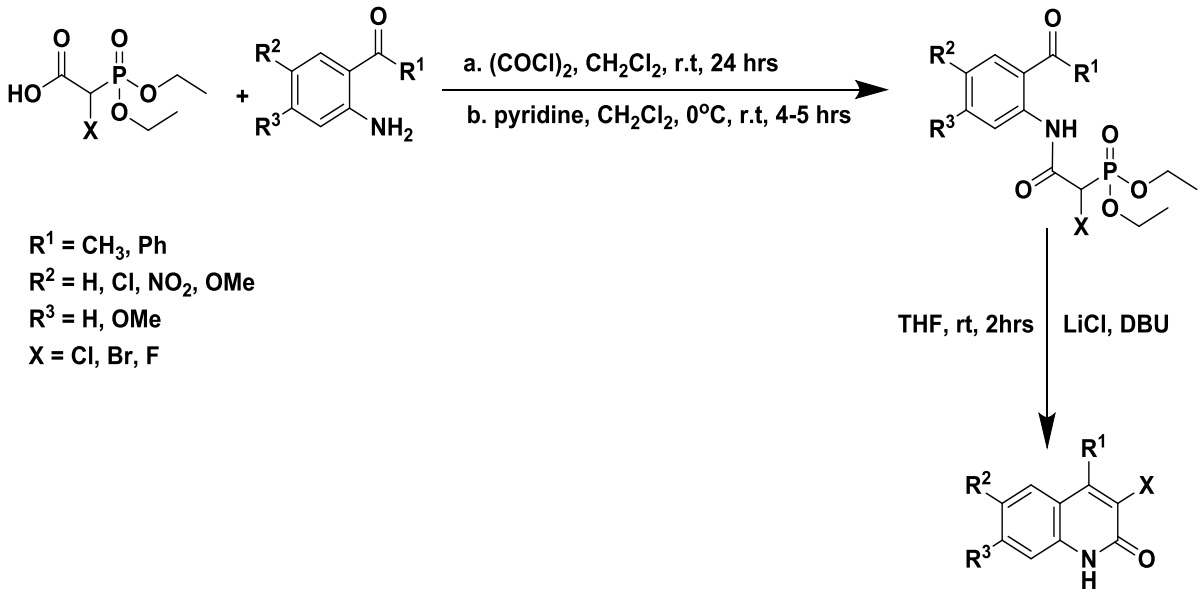
Jayashree *et al*[157] reported the synthesis of novel Schiff bases of 2-quinolone as depicted by the scheme below. All the synthesized test compounds were screened for their *in vitro* anti-proliferative activity against A-549, human lung cancer cell line by MTT assay. The study revealed that Schiff's bases of 2-quinolone ring system can be used for improving the chemical status of the existing structures which have shown some anticancer activity.



Scheme 4: Schiff bases of 2-quinolone

2.29.4 Halogenation Reactions

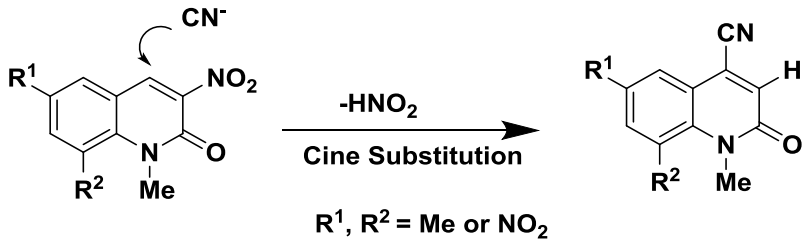
Zhao *et al*[174] reported the halogenation of the 2-quinolone moiety and prepared 4-substituted-3-halo-2-quinolones (halo = F, Cl, Br) by utilizing 2-halo diethyl phosphonoacetic acids (halo = F, Cl, Br) and *o*-aminophenyl ketones as the starting materials. The final compounds were obtained by an intramolecular Horner–Wadsworth–Emmons olefination of halogen-containing N-acyl-*o*-aminophenyl ketones and the transformation process occurred under mild conditions.



Scheme 5: Halogenation of 2-quinolones

2.29.5 Alkylation of Quinolones

Chen *et al*[175] reported on the reactivity of 8-substituted **MeQones**. When 1-methyl-3,6, 8-trinitro-2-quinolone was treated with potassium (or trimethylsilyl) cyanide, cyanation proceeded at the 4-position region-selectively as a result of *cine*-substitution. This reaction is initiated with addition of cyanide species, and the cyano-quinolone is formed by the protonation of the resultant anionic intermediate followed by elimination of nitrous acid. The high reactivity was maintained even when one of the nitro groups on the benzene moiety was replaced by a methyl group, which afforded corresponding *cine*-substituted products upon the treatment with potassium cyanide.



Scheme 6: Alkylation of 2-quinolones

2.29.6 Nucleophilic substitution reactions

Nishiwaki*et al*[176] reported the nucleophilic substitution reaction of 4-chloro-3 nitro-2-quinolone with various nucleophiles and found the position-4 to be highly active to yield4-

functionalized 3-nitroquinolones. By this method, functional groups such as azide, amino, fluoro, alkoxy, alkylthio groups, and malonates are introduced at this position (**Table 2**).

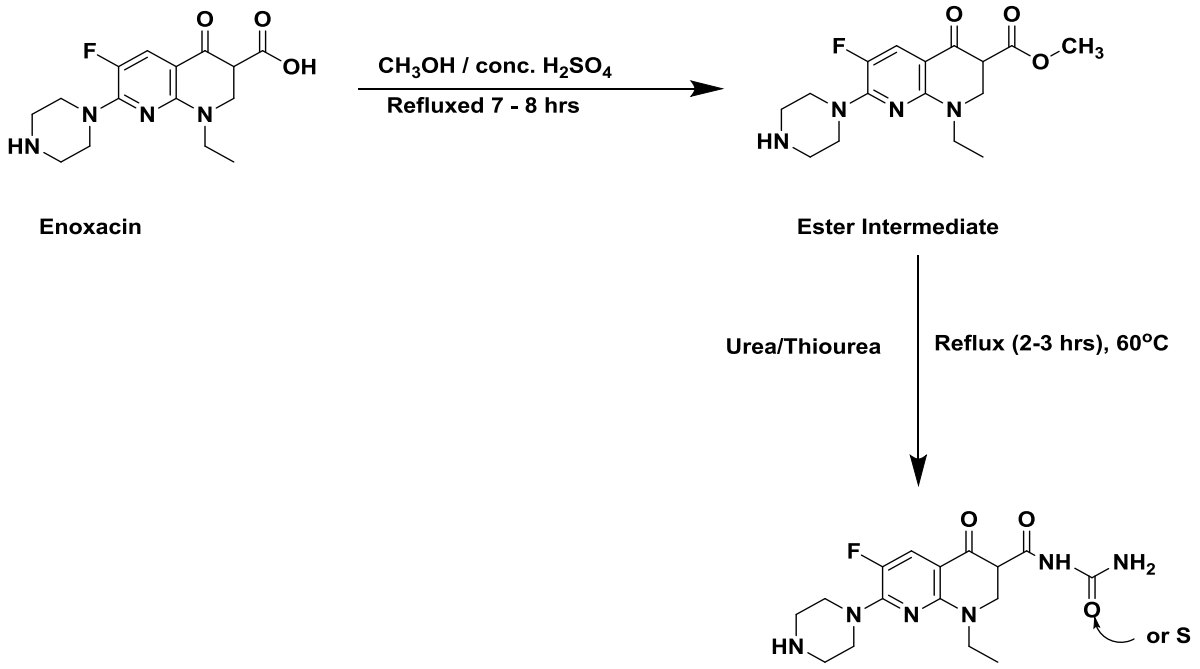
Scheme 7: Nucleophilic substitution reactions of 2-quinolones

Table 2: Nucleophililes substituted on 2 quinolone ring

Nucleophilic reagent	Nu	Yield/%	Nucleophilic reagent	Nu	Yield/%
KF + 18-Crown-6	F	95	NH_3	NH_2	91
MeONa	MeO	85	$PhCH_2NH_2$	$PhCH_2NH$	94
PhOH + K_2CO_3	PhO	93	$PhNH_2$	PhNH	98
EtSH + NEt_3	EtS	95	piperidine	piperidino	96
PhSH + pyridine	PhS	96	$CH_2(COOMe)_2$ + K_2CO_3	$CH(COOMe)_2$	95

2.29.7 Reaction of quinolones with isocyanates and isothiocyanates

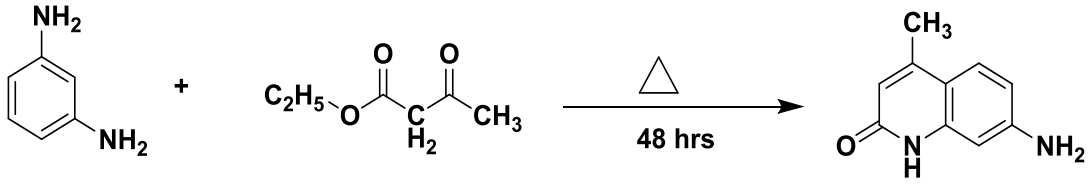
Haroon *et al*[177] reported the synthesis and biological significance of quinolone substituted urea and thiourea derivatives. All synthesized compounds displayed activities closer to that of enoxacin against all the Gram-positive test strains. The ester intermediate (obtained by acid-catalysed esterification of enoxacin with methanol was heated under reflux with either isocyanates or isothiocyanates at 60^{o}Cto afford urea and thiourea derivatives of enoxacin.



Scheme 8: Reaction of isocynates and isothiocyantes with 2-quinolones

2.29.8 Synthesis of 7-amino-4-methyl-2-quinolone:

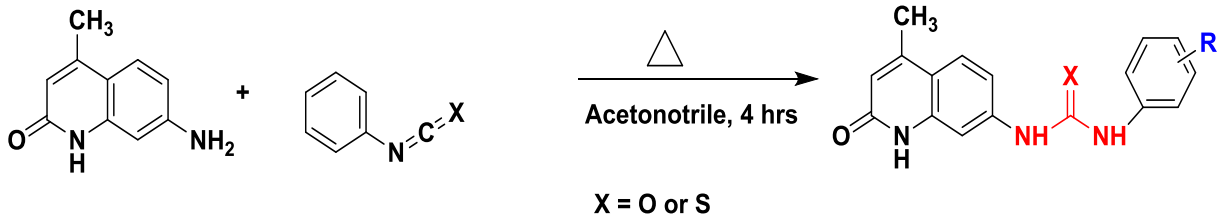
Since the introduction of nalidixic acid in 1962[178],extensive work has been carried out on this moiety and exploited for its medicinal applications such as antibacterial, anti-tubercular, anti-inflammatory, anti-cancer agents, however no work has been reported on it with respect to its derivatisation with different Isocyanates and Isothiocyanates at C-7 position. Synthesis of 7-amino-4-methyl-2-quinoloneoccurred through a solvent free Pechman condensation reaction between *m*-phenylenediamine and the β-keto ester, ethylacetoacetate[159].



Scheme 9: synthesis of 7-amino-4-methyl 2 quinolone

2.29.9 Synthesis of quinolone-phenyl urea

Synthesis of quinolone substituted urea derivatives proceeds by an attachment of isothiocyanates and isocyanates to the NH_2 group at C-7 of the 7-amino-4-methyl-2-quinolone ring[179].



Scheme 10: Synthesis of quinolone urea/thiourea hybrid

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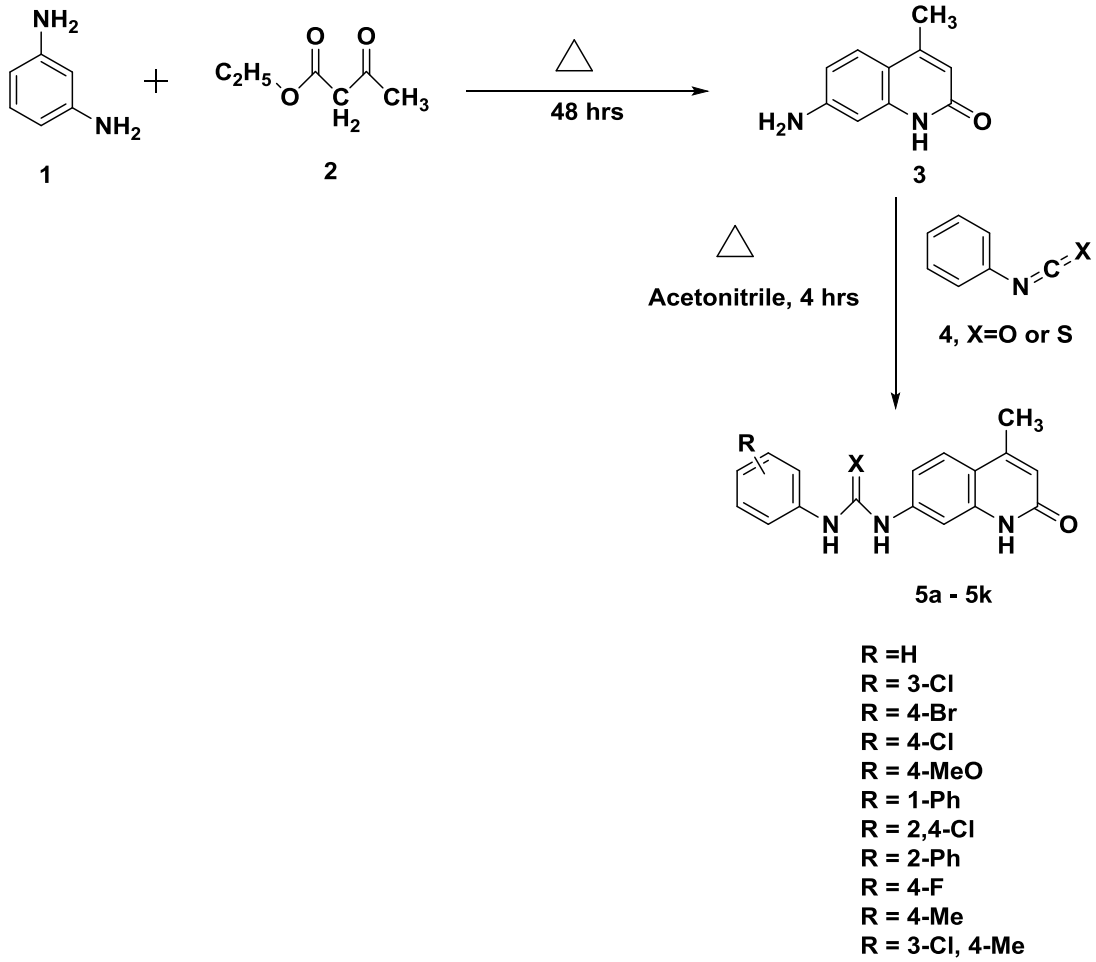
Chapter 3
Experimental

3.1 General Procedure

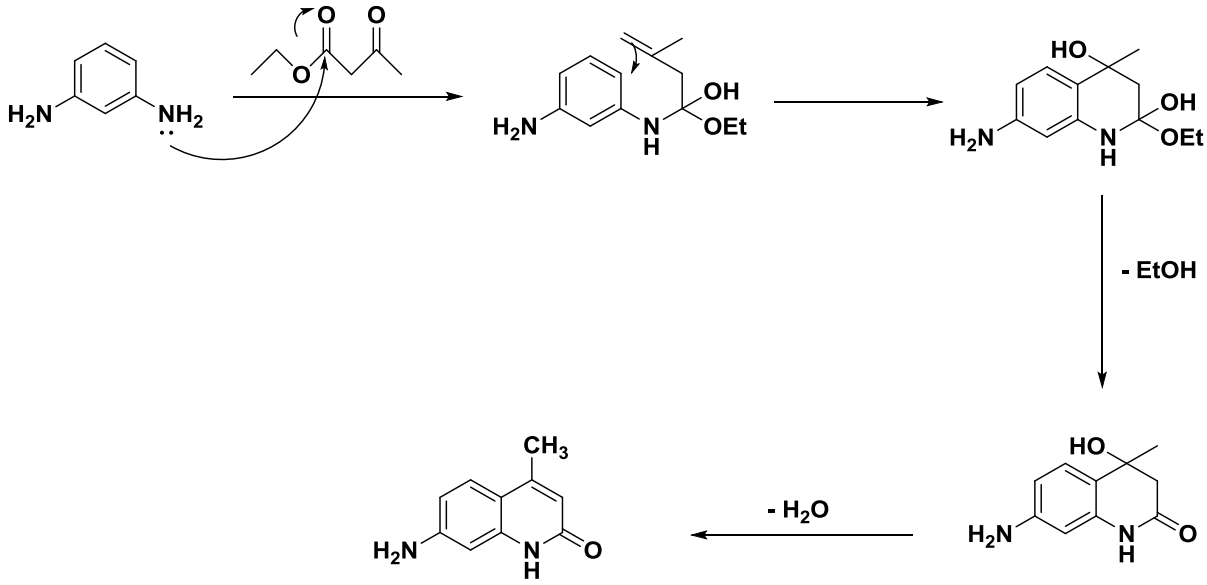
All the synthesized compounds were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopic techniques. The analytical grade (AR) chemicals and reagents were obtained from Merck and Sigma-Aldrich. The progress of the reactions and the purity of the synthesized compounds were monitored by Thin Layer Chromatography on pre-coated silica gel 60 F_{254} (mesh) (E. Merck) and spots were visualized under UV light (long and short wavelength). Compounds were purified by recrystallization technique using acetonitrile. Melting points were recorded in open capillaries using (Electro-thermal 9300) digital melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on (Bruker Avance IV) NMR spectrometer at 400 and 101 MHz, respectively using DMSO-d_6. Perkin Elmer 100 FT-IR spectrophotometer with universal ATR sampling accessory was used to record all IR spectra.



Scheme 11: Synthetic route for the synthesis of novel series of Quinolone-urea/thiourea derivatives

3.2 Synthesis of 7-amino-4-methyl 2 quinolone

7-amino-4-methyl-quinoline-2(1*H*)-one was synthesized by a simple pechman condensation reaction which involves the cyclocondensation of *m*-phenylenediamine and a β-keto ester, like ethylacetoacetate. 10.8g (0.1 mol) of *m*-phenylenediamine and 12.64 ml (0.1 mol) of ethylacetoacetate were taken in a 250 ml round bottom flask (RBF). The reaction mixture was heated under reflux for 48 hours (as monitored by TLC). After completion of the reaction, 200 ml of water was added to the RBF and the contents were heated to the boiling, point of water.The mixture was then filtered and the filtrate chilled in the refrigerator till the formation of precipitate. The precipitate was collected, dried, washed with hexane and recrystallized from ethanol. TLC solvent system: 5% Methanol in DCM. Pure light brown compound was obtained and confirmed by NMR spectroscopy[1, 2].

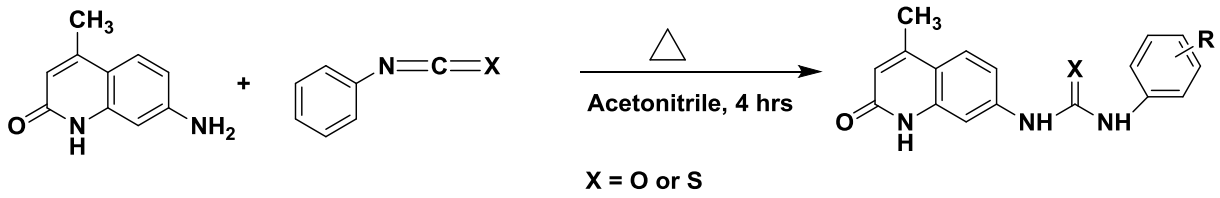


Scheme 12: General mechanism for the synthesis of 7-amino-4methyl-2-quinolone[3]

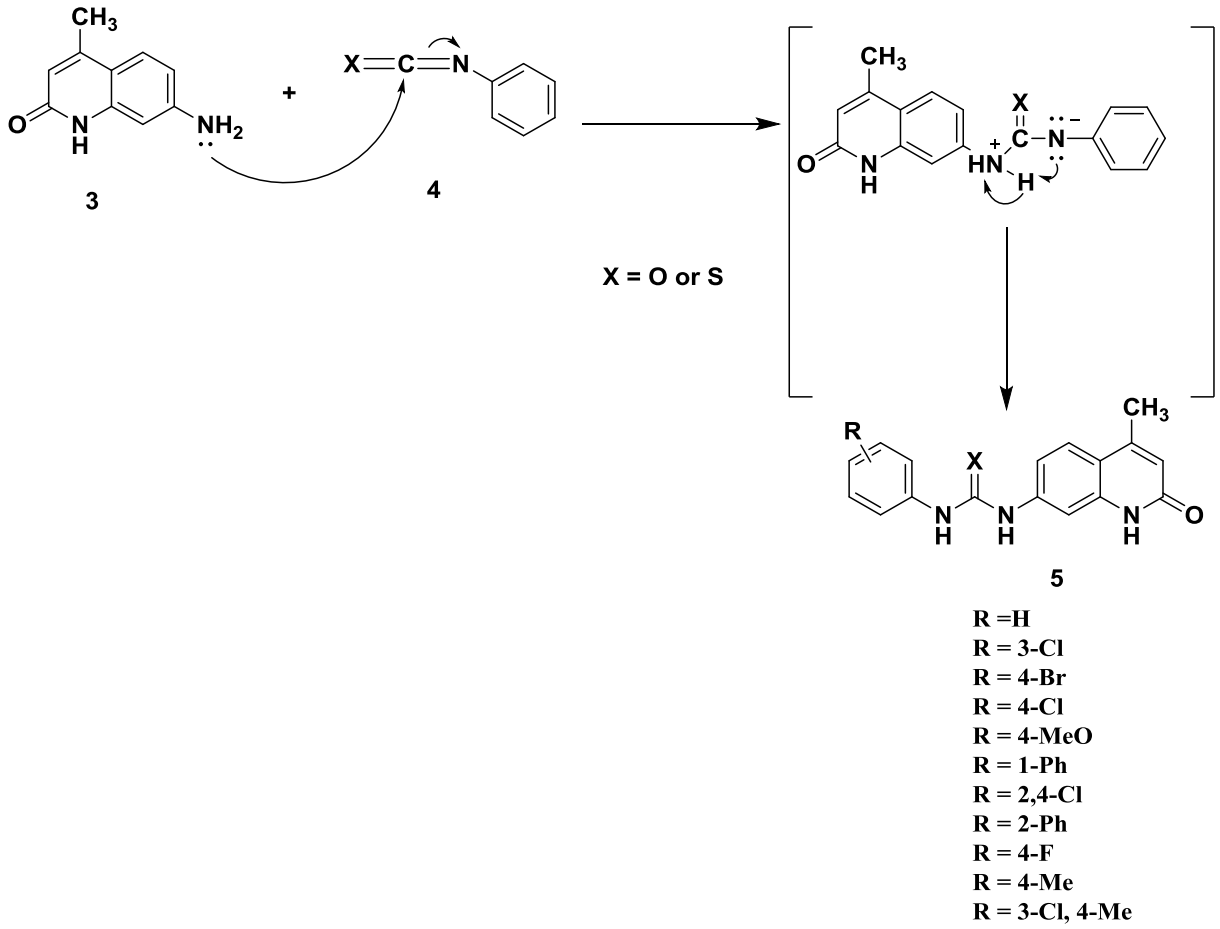
3.3 Synthesis of quinolone – phenylurea/phenylthiourea derivatives

General Procedure:

Quinolone-urea/thiourea derivatives were prepared by adding the relevant phenyl isocyanates/ isothiocyanates(0.2 mol) to the corresponding amino derivative (**7-amino-4-methyl-2-quinolone**) (0.1 mol) in acetonitrile and the reaction was refluxed under argon atmosphere for 4 hours. After removal of the solvent under reduced pressure,the obtained residue was crystallized from acetonitrile to afford the desired derivatives[4].



Scheme 10: Synthesis of quinolone-urea/thiourea derivatives



Scheme13: Mechanism of the synthesis of 1-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-phenylurea

3.4 Anti-cancer activity (PASS Online)

Due to human error as well as precipitation and low solubility of the compounds, the MTT assay was not successfully conducted. PASS (*Prediction of activity spectra for substances*) is an online prediction tool which predicts over 4000 kinds of biological activity, was used to predict the potential activity of the compounds using a template compound[5]. Based on the

results obtained from the PASS online predictive tool, it was deduced that the compounds possess potential anticancer activity; therefore the compounds will be re-evaluated for their anticancer activity using an MTT assay. Once troubleshooting is conducted, the MTT assay will be carried us as follows;

Procedure:

On the first day, 5 ml of complete media will be added to trypsinized cells on a Trypsin one T-25 flask. This mixture will be centrifuged in a sterile 15 ml falcon tube at 500 rpm for 5 min. Media will be removed and the cells were re-suspended to 1.0 ml with complete media. The cells will then be counted and recorded per ml. the cells will be diluted (CV=CV) to 75,000 cells per ml and complete media was used to dilute cells. 100 µl of cells (7500 total cells) were added into each well in the 96 well plates and incubated overnight. On the second day the cells will be treated with compounds **5a-5k** and incubated overnight**.** The final volume was 100 µl per well. On day three 20 µl of 5 mg/ml MTT will added to each well. In one set of wells MTT will be added together with the compound but cells will not be added. This will served as a control. The 96 well plates will then be incubated for 3.5 h at 37^{o}C in the culture hood. After the incubation period is over, the media will be carefully removed so that cells are not disturbed. 150 µl MTT solvent will be added and the plates covered with tinfoil and the cells will then be agitated on an orbital shaker for 15 min. Absorbance will be read at 590 nm with a reference filter of 620 nm. The IC_{50} or the half maximal inhibitory concentration will thus be determined. A lower IC_{50} value will imply a strong inhibitor the and all activities $\geq$ 100 µM will be considered to be inactive for the cancer cell lines and the screening will be performed in triplicate and the average of the triplicate values will be given[6-8].

3.5 *In vitro* antimicrobial activity

Encouraging results obtained from cytotoxicity assay prompted the research to further evaluate the quinolone-urea/thiourea derivatives for their antimicrobial potential against several bacterial strains (gram negative and gram positive) by known MIC assay determination method using resazurin dye[9, 10]. All *In vitro* antimicrobial activity was conducted in collaboration with the Department of Microbiology at Inkosi Albert Luthui Hospital in Durban (RSA).

3.5.1 Microorganisms used

Gram positive microorganism cultures used: *Staphylococcus aureus*(ATCC 25923), *Bacillus Subtilis (*ATCC 6051). Gram negative microorganism cultures used: *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa*(ATCC 27853), *Salmonella typhimurium*(ATCC), *Streptococcus pneumonia* (ATCC 49619). Fungal strains used: *Candida albicans (ATCC 90028), Cryptococcus neoformans (ATCC 66031)*. The microorganism culturing and sub-culturing was performed a day before the commencement of actual testing.

3.5.2 Preparation of medium

22 g of Muller-Hinton Broth containing (Acid Hydrolysate of Casein, Beef Extract and Starch) was dissolved in 1 L of double distilled water (ddH_2O). The pH of this medium was adjusted to 7.4 ± 0.1 and sterilized by autoclave for 15 min at 121°C. The solution was allowed to cool and stored at a temp of 4°C. Sterility check was performed by incubating un-inoculated media in an aerobic incubator at 37 °C for 18-24 h. For antifungal activity, RPMI 1640 medium with L-glutamine and 0.165 M MOPS and without sodium bicarbonate (Lonza) was used.

3.5.3 Preparation of test compounds (stock solution and working standard)

A quantity of 4.000 mg of the synthesized final compoundsand standard drug (Amoxicillin) were dissolved in 1 ml of DMSO to give stock solution (4000 µg/ml). A further, 100 µl of stock solution was diluted with 900 µl of ddH_2O to afford working standard solution at a concentration of 400 µg/ml.

3.5.4 Preparation of inoculum

A colony of microorganisms was suspended in 4.5 ml sterile ddH_2O a day before testing procedures. Inoculates were adjusted to 0.5 McFarland standard (1.5 X 10^8cfu/ml) using a turbidity-meter.

3.5.5 Broth micro-dilution method

In vitro antimicrobial activity of the newly synthesized compounds was evaluated using the broth micro-dilution method. 100 µl of sterile ddH_2O was added to all outer-perimeter wells of a 96-well microtiter plates to minimize evaporation of the medium in the test wells during incubation. To the remaining test wells 100 µl of MHB was added. Two-fold serial dilutions of the test compounds and the standard drug (amoxicillin) were made directly on the microplate using MHB[11].

The compounds were tested at final concentration of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg/ml. 10 µl of the prepared bacterial or fungal inoculum was added to the wells. The microtiter plates were covered and sealed with parafilm and incubated at 37 ± 1 ºC for 24 h. After this, 10 µl of freshly prepared resazurin (0.4 mg/ml) was added to the test wells and incubated further for 5h. MIC was determined as a blue colour in the test well and interpreted as no bacterial growth and a pink colour indicated growth. The MIC was thus defined at the lowest drug concentration that prevented a colour change from blue to pink. This experiment was conducted in duplicate and the average MIC values in µg/ml are recorded.

3.6 Anti-TB activity

The Anti-TB activity of the synthesized final compounds was also carried out on H37Rv strain. This was determined by measuring bacterial growth after 5 d in the presence of test compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of 2%. The highest concentration of compound was 200 µM where compounds were soluble in DMSO at 10 mM. For compounds with limited solubility, the highest concentration was 50X less than the stock concentration e.g. 100 µM for 5 mM DMSO stock, 20 µM for 1 mM DMSO stock. For potent compounds, assays were repeated at lower starting concentrations. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (100 µM rifampicin) and maximum growth (DMSO only), as well as a rifampicin dose response curve. Plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by OD_{590} and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy 4 plate reader. Growth was calculated separately for OD_{590} and RFU[12-14]. The antitubercular assay was carried out in a collaborative effort at NIH, USA.

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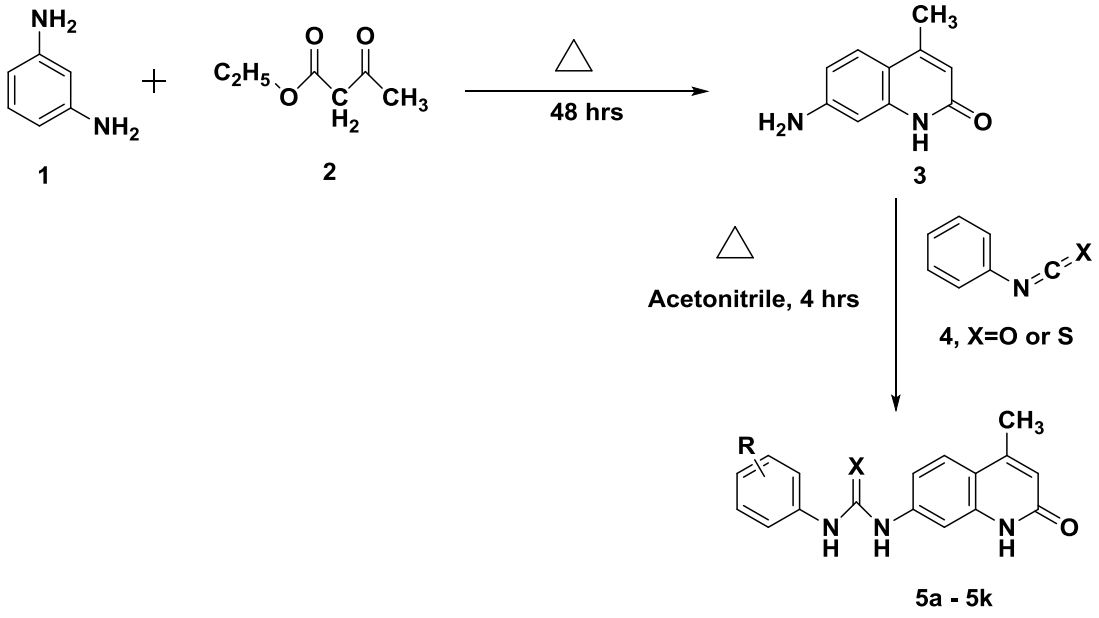
Chapter 4
Results and Discussion

4.1 Chemistry

The research project comprised of 11 novel compounds (**5a-k**) of quinolone substituted urea and thiourea derivatives which have been synthesized by means of pechman condensation reaction of *m*-phenylene diamine **(1)** with ethylacetoacetate **(2)** to give 7-amino-4-methyl-2-quinolone **(3)** followed by nucleophilic attack on the free NH_2 group of the quinolone at C-7 by isocyanates/isothiocyanates **(4)** in an inert atmosphere[1]. The reaction of isocyanates and isothiocyanates with 7-amino-4-methyl-2-quinolone proceeds without the requirement of a catalyst, however in addition to electronic effects, steric hindrances played an important role in the reactivity of the NH_2 of the quinolone ring therefore the reaction needed up to 4 - 5 hours to go to completion.

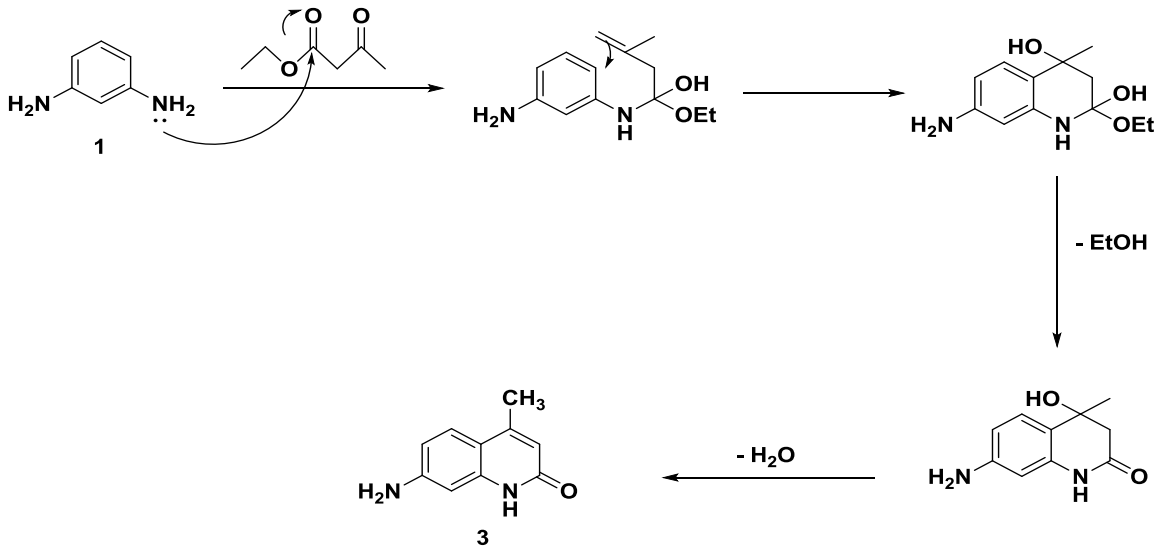


R =H
R = 3-Cl
R = 4-Br
R = 4-Cl
R = 4-MeO
R = 1-Ph
R = 2,4-Cl
R = 2-Ph
R = 4-F
R = 4-Me
R = 3-Cl, 4-Me

Scheme 11: Synthesis of novel quinolone substituted urea/thiourea derivatives

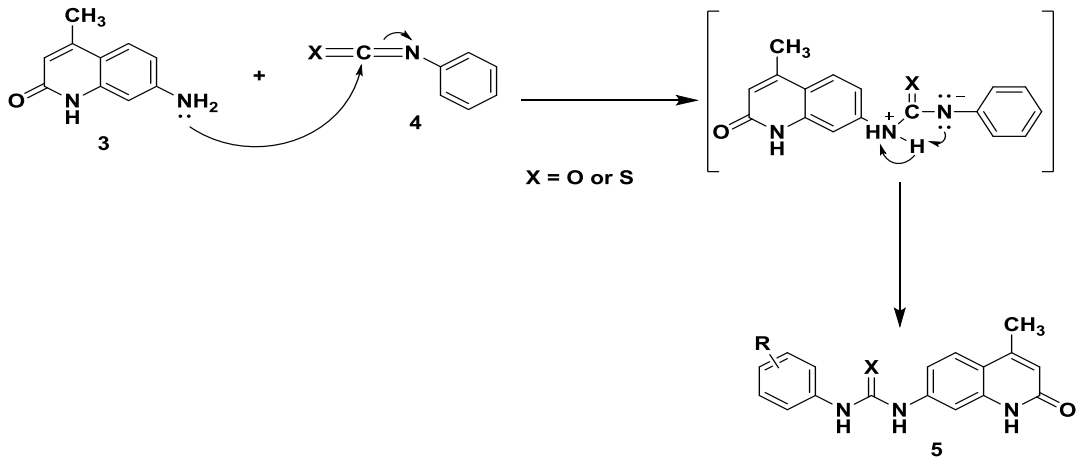
4.1.1 Reaction Mechanisms

The synthesis of the lead compounds (**5a-k**) was accomplished through an efficient synthetic strategy as outlined in the **Scheme-11** and the 7-amino-4-methyl-2-quinolone which served as a starting material **(3)** was synthesized by reacting *m*-phenylene diamine **(1)** with ethylacetoacetate **(2)** by a simple condensation reaction without a catalyst.



Scheme 12: Mechanism for the synthesis of 7-amino-4methyl-2-quinolone[2]

Furthermore various isocyanates and isothiocyanates **(4)** were employed for the synthesis of the target compounds (1-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-phenylurea or thiourea) under argon atmosphere using acetonitrile as a solvent[3].



Scheme 13: Mechanism of the synthesis of 1-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-phenylurea

4.1.2 Physicochemical properties

The synthesized compounds appeared as yellowish, white and slightly cream coloured solids. The percentage (%) yield of the synthesized compounds **(5a-k)** ranged from 50 - 80 %. The melting points for the synthesized compounds were found to be between 218 and 370°C (**Table 3**).

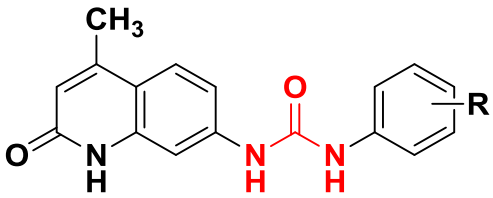


Table 3: Physio-chemical Properties

Comp No	R	% Yield	M.P.[a] (°C)	Appearance	MF	MW
5a	H	50	204-206	Yellow powder	$C_{17}H_{15}N_3OS$	309.39
5b	3-Cl	55	355-357	Yellow powder	$C_{17}H_{14}ClN_3OS$	343.83
5c	4-Br	50	356-358	White powder	$C_{17}H_{14}BrN_3O_2$	372.22
5d	4-Cl	70	218-220	White powder	$C_{17}H_{14}ClN_3OS$	343.83
5e	4-OMe	83	370-372	White powder	$C_{18}H_{17}N_3O_3$	323.35
5f	1-Ph	60	227-229	White powder	$C_{21}H_{17}N_3O_2$	343.39
5g	2,4-Cl	45	228-230	White powder	$C_{17}H_{13}Cl_2N_3O_2$	362.21
5h	2-Ph	55	368-370	White powder	$C_{18}H_{17}N_3O_2$	307.35
5i	4-F	80	285-287	White powder	$C_{16}H_{12}FN_3O_2$	297.29
5j	4-Me	46	340-341	White powder	$C_{18}H_{16}ClN_3O_2$	341.80
5k	3-Cl, 4-Me	48	217-219	White powder	$C_{18}H_{17}N_3O_2$	307.35

[a]M.P: Melting Point

4.1.3 Determination of purity

Purity of the compounds (**5a-k**) was determined by liquid chromatography (LC) coupled to mass spectrometry (MS) with detection by ultraviolet (UV) absorbance and total ion count from MS. The percentage purity ranged between 22 and 100% (**Table 4)**

Table 4: The percentage purity data

Compound	% Purity	Observed molecular mass	Expected molecular mass
5a	22	157.1	293.3
5b	72	342.1	362.2
5c	76	113.1	343.4
5d	68	113.1	323.4
5e	91	157	343.8
5f	88	113.1	315.7
5g	100	113.1	307.4
5h	90	615.3	311.3
5i	11	113.1	307.4
5j	89	420.2	341.8
5k	75	157	343.8

4.1.4 Determination of fluorescence

Fluorescence of compounds (**5a-k**) was also measured at an excitation wavelength of 560 nm and an emission wavelength of 590 nm in a microbiological medium. All compounds displayed no fluorescence.

4.1.5 Spectroscopic Data

Structural elucidation was carried out by means of the following spectroscopic techniques, viz. FT-IR, ^{1}H NMR, ^{13}C NMR and EI-MS) which are depicted in the **Table 5** and the respective spectra for all the compounds were incorporated as appendix.

Table 5. Spectral characterization data of quinolone substituted urea/thiourea derivatives (**5a-k**)

Compound	IR [ATR, ν_{max}, cm^{-1}]	^{1}H NMR [400 MHz, DMSO-*d6*, δ, ppm]	^{13}C NMR [101 MHz, DMSO-*d6*, δ, ppm]	EIMS (*m/z*)
5a	3081.47 (Ar N-H), 3008.88 (NH of urea), 2934.5 (NH of urea), 1641.53 (Ar C=O), 1594.52 (C=O of urea), 1553.66 (Ar CH_3)	11.46 (s, 1H, -NH-), 9.04 (s, 1H, -NH-of urea) , 8.77 (s, 1H, -NH- of urea), 7.70-7.57 (m, 4H, Ar-H), 7.23-7.20 (m, 4H, Ar-H), 6.21 (s, 1H, =CH- of quinolone ring), 2.36 (s, 3H, -CH_3)	160.9, 151.0, 146.5, 140.3, 138.5, 137.4, 130.0, 127.4, 124.2, 117.1, 116.8, 115.9, 113.5, 111.5, 101.9, 17.6	293.11 (M^+)
5b	3083.27 (Ar N-H), 3001.94 (NH of urea), 2944.31 (NH of urea), 1644.33 (Ar C=O), 1590.56 (C=O of urea), 1582.86 (Ar CH_3)	11.54 (s, 1H, -NH-), 10.06 (s, 1H, -NH-of thiourea) , 9.92 (s, 1H, -NH- of thiourea), 7.52-7.49 (m, 4H, Ar-H), 7.36-7.32 (t,*J*= 7.74 Hz, 4H, Ar-H), 6.29 (s, 1H, =CH- of quinolone ring), 2.38 (s, 3H, -CH_3)	179.3, 161.8, 147.5, 141.2 139.3, 139.1, 128.4, 124.8, 124.5, 123.6, 119.3, 117.9, 117.9, 116.2, 116.1, 1084, 108.2, 18.3	309.05 (M^+)
5c	3131.46 (Ar N-H), 3078.88 (NH of urea), 2948.36 (NH of urea), 1644.48 (Ar C=O), 1604.95 (C=O of urea), 1584.28 (Ar CH_3)	11.55 (s, 1H, -NH-), 10.15 (s, 1H, -NH-of urea) , 9.98 (s, 1H, -NH- of urea), 7.65-7.63 (d,*J*=8.68 Hz, 1H, Ar-H), 7.51-7.49 (m, 5H, Ar-H), 7.32-7.30 (m, 1H, Ar-H), 6.29 (s, 1H, =CH- of quinolone ring), 2.38 (s, 3H, -CH_3)	179.3, 161.8, 147.5, 141.0, 139.1, 138.7, 131.1, 125.5, 124.9, 119.4, 117.1, 116.5, 116.2, 108.3, 18.3	371.05 (M^+)
5d	3130.18 (Ar N-H), 3078.56 (NH of urea), 2948.36 (NH of urea), 1644.77 (Ar C=O), 1516.29 (C=O of urea),1398.35 (Ar CH_3)	11.55 (s, 1H, -NH-), 10.14 (s, 1H, -NH-of thiourea) , 9.98 (s, 1H, -NH- of thiourea), 7.65-7.63 (d,*J*=8.68 Hz, 1H, Ar-H), 7.55-7.49 (m, 3H, Ar-H), 7.40-7.38 (m, 2H, Ar-H), 7.33-7.30 (m, 1H, Ar-H), 6.29 (s, 1H, =CH- of quinolone ring), 2.38 (s, 3H, -CH_3)	179.4, 161.8, 147.5, 141.0, 139.1, 138.3, 128.4, 128.2, 125.3, 124.9, 119.4, 117.1, 116.2, 108.3, 18.3	343.76 (M^+)

5e	3304.99 (Ar N-H), 3006.67 (NH of urea), 2898.00(NH of urea), 1695.86 (Ar C=O), 1636.83 (C=O of urea), 1607.18 (Ar CH_3)	11.45 (s, 1H, -NH-), 8.92 (s, 1H, -NH-of urea) , 8.49 (s, 1H, -NH- of urea), 7.59-7.55 (m, 2H, Ar-H), 7.37-7.35 (d,*J*= 8.92 Hz, 2H, Ar-H), 7.22-7.19 (m, 1H, Ar-H), 6.88-6.86 (d,*J*= 8.92 Hz, 2H, Ar-H), 6.20 (s, 1H, =CH- of quinolone ring), 3.71 (s, 3H, Ar-OCH_3), 2.36 (s, 3H, -CH_3)	162.0, 154.6, 152.3, 147.6, 141.8, 139.6, 132.3, 125.3, 120.1, 119.8, 118.1, 114.3, 113.9, 112.5, 102.7, 55.1, 18.3	323.17 (M^+)
5f	3275.42 (Ar N-H), 3052.38 (NH of urea), 2896.50 (NH of urea), 1696.89 (Ar C=O), 1656.78 (C=O of urea), 1637.57 (Ar CH_3)	11.52 (s, 1H, -NH-), 9.39 (s, 1H, -NH-of urea) , 8.81 (s, 1H, -NH- of urea), 8.13-8.11 (d,*J*= 8.32 Hz, 1H, Ar-H), 8.04-8.02 (d,*J*= 7.48 Hz, 1H, Ar-H), 8.95-8.93 (d,*J*= 7.84 Hz, 1H, Ar-H), 7.67-7.47 (m, 6H, Ar-H), 7.33-7.30 (d,J= 8.92 Hz, 1H, Ar-H), 7.22-7.19 (m, 1H, Ar-H), 6.22 (s, 1H, =CH- of quinolone ring),2.38 (s, 3H, -CH_3)	162.0, 152.5, 147.6, 141.6, 139.7, 133.9, 133.6, 128.4, 125.9, 125.9, 125.8, 125.7, 125.4, 123.1, 121.2, 118.2, 117.4, 114.5, 112.5, 102.7, 18.3	343.09 (M^+)
5g	3323.26 (Ar N-H), 3282.81 (NH of urea), 2988.54 (NH of urea), 1711.83 (Ar C=O), 1640.22 (C=O of urea), 1620.48 (Ar CH_3)	11.52 (s, 1H, -NH-), 9.74 (s, 1H, -NH-of urea) , 8.44 (s, 1H, -NH- of urea), 8.22-8.20 (d, *J*= 8.98 Hz, 1H, Ar-H), 7.63-7.61 (d, *J*= 8.58 Hz, 1H, Ar-H), 7.52 (1H, Ar-H), 7.41-7.38 (d, *J*= 9.12 Hz 1H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 6.23 (s,1H, =CH- of quinolone ring), 2.37 (s, 2H, -CH_3)	162.0, 151.7, 147.6, 141.1, 139.6, 134.9, 128.5, 127.6, 126.3, 125.5, 122.7, 122.0, 118.5, 114.8, 112.5, 102.9, 18.3	361.04 (M^+)
5h	3326.25 (Ar N-H), 3152.37 (NH of urea), 2954.46 (NH of urea), 1696.64 (Ar C=O), 1653.70 (C=O of urea), 1632.75 (Ar CH_3)	11.40 (s, 1H, -NH-), 8.91 (s, 1H, -NH-of urea) , 7.55-7.52 (d, *J*= 9.88 Hz, 2H, Ar-H), 7.35-7.29 (m, 4H, Ar-H), 7.26-7.24 (d, *J*= 7.84 Hz, 1H, Ar-H), 7.18-7.15 (d, *J*= 8.86 1H, Ar-H), 6.69 (s, 1H, =CH- of quinolone ring), 6.17 (s, 1H, -NH- of urea), 4.32-4.30 (d, J= 5.76 2H, benzylic-H),), 2.34 (s, 3H, -	162.0, 154.8, 147.6, 142.3, 140.1, 139.7, 128.2, 127.1, 126.7, 125.2, 117.8, 114.0, 112.3, 102.3, 42.7, 18.3	307.13 (M^+)

		CH_3)		
5i	3392.83 (Ar N-H), 3084.77 (NH of urea), 2952.07 (NH of urea), 1644.59 (Ar C=O), 1606.41 (C=O of urea), 1552.61 (Ar CH_3)	11.56 (s, 1H, -NH-), 10.19 (s, 1H, -NH-of urea) , 10.07 (s, 1H, -NH- of urea), 7.66-7.64 (d, *J*= 8.68 Hz, 2H, Ar-H), 7.54-7.00 (m, 3H, Ar-H), 7.35-7.30 (m, 2H, Ar-H), 7.21-7.16 (m, 1H, Ar-H), 6.30 (s, 1H, =CH- of quinolone ring), 2.39 (s, 3H, -CH_3)	179.1, 161.8, 147.6, 141.1, 139.0, 139.0, 126.2, 126.1, 124.8, 119.4, 117.2, 116.3, 116.2, 115.1, 114.9, 108.4, 108.3, 18.3	311.09 (M^+)
5j	3392.83 (Ar N-H), 2958.86 (NH of urea), 2917.21(NH of urea), 1692.89 (Ar C=O), 1643.27 (C=O of urea), 1626.02 (Ar CH_3)	11.45 (s, 1H, -NH-), 8.95 (s, 1H, -NH-of urea) , 8.57 (s, 1H, -NH- of urea), 7.60-7.55 (m, 2H, Ar-H), 7.35-7.32 (d, *J*= 8.53 Hz, 2H, Ar-H), 7.21-7.19 (d, *J*= 8.68 Hz, 1H, Ar-H), 7.10-7.08 (d, *J*= 8.11 2H, Ar-H), 6.20 (s, 1H, =CH- of quinolone ring), 2.36 (s, 3H, -CH_3 of quinolone ring), 2.24 (s, 3H, -CH_3 of benzylic ring)	162.05, 152.20, 147.68, 141.69, 139.66, 136.76, 130.93, 129.18, 125.35, 118.37, 118.16, 114.45, 112.62, 102.79, 20.30, 18.34	307.13 (M^+)
5k	3281.11 (Ar N-H), 3012.55 (NH of urea), 2826.97 (NH of urea), 1695.30 (Ar C=O), 1641.47 (C=O of urea), 1625.74 (Ar CH_3)	11.47 (s, 1H, -NH-), 9.04 (s, 1H, -NH-of urea) , 8.77 (s, 1H, -NH- of urea), 7.70-7.67 (d, *J*= 13.23 Hz , 1H, Ar-H), 7.60-7.56 (m, 2H, Ar-H), 7.25-7.19 (m, 3H, Ar-H),), 6.21 (s, 1H, =CH- of quinolone ring), 2.36 (s, 3H, -CH_3 of quinolone ring), 2.26 (s, 3H, -CH_3 of benzylic ring)	162.0, 152.0, 147.6, 141.4, 139.6, 138.6, 138.5, 133.0, 133.0, 131.1, 131.1, 128.4, 128.3, 125.3, 118.3, 118.2, 117.0, 114.6, 112.6, 103.0, 18.7, 18.3	341.09M^+)

4.1.6 Spectral analysis and elucidation

4.1.6.1 Infra-red spectroscopy

Infra-red spectroscopy served as an analytical tool in the identification of different functional groups in all the synthesized novel compounds. The characteristic bands of all compounds were identified to be the N-H stretches at C-7 of the quinolone ring, the N-H stretches of the urea/thiourea which is linked at C-7and the N-H stretches at C-1 of the quinolone ring which ranged from **2800.67 – 3334.99**. (Quinolone-phenylisothiocyanate)

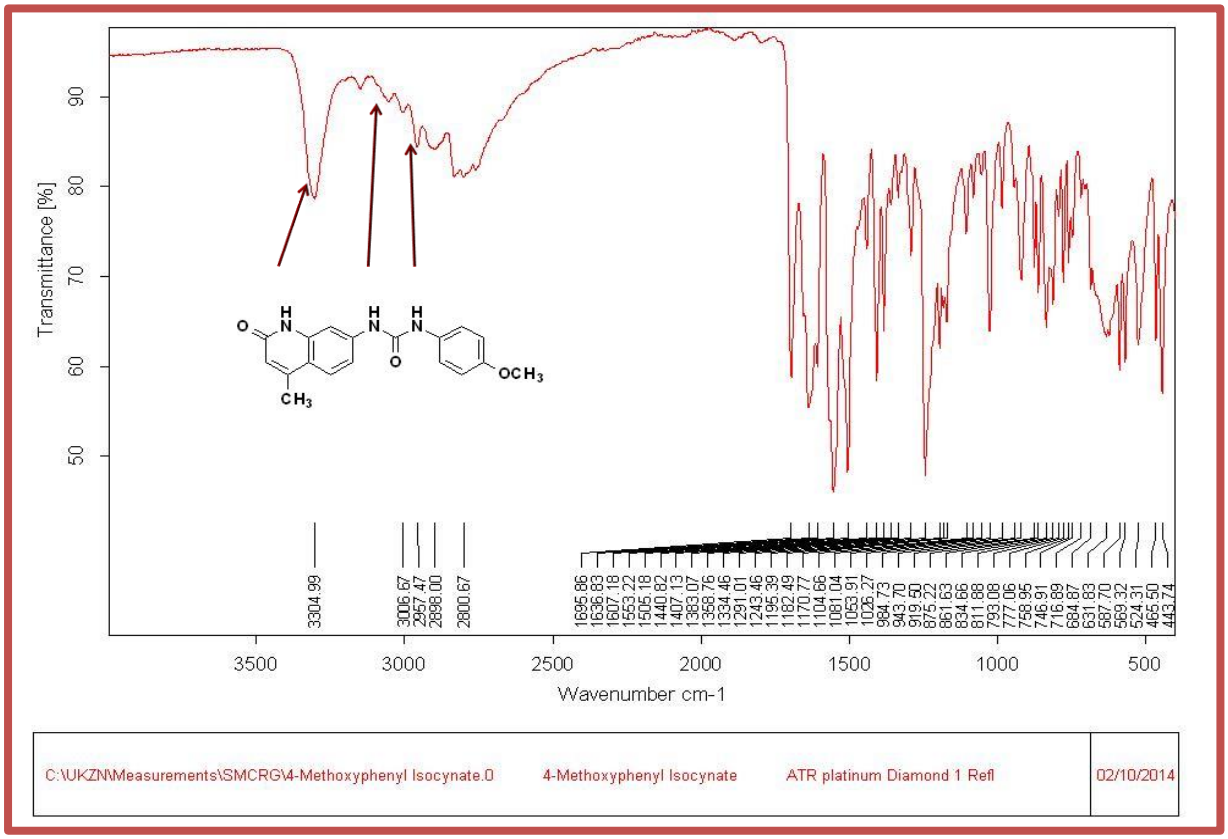


Figure 29: IR spectrum of compound 5e

The IR spectrum of the newly synthesized novel compounds also reflected a consistent prominent peak around 1636-1695 cm^{-1}which indicated the presence of a carbonyl group (C=O) in all the synthesized compounds. Furthermore the IR spectrum reflected peaks around 1500-1553 cm^{-1}which were attributed to the C-C stretch at C4 of the quinolone ring.

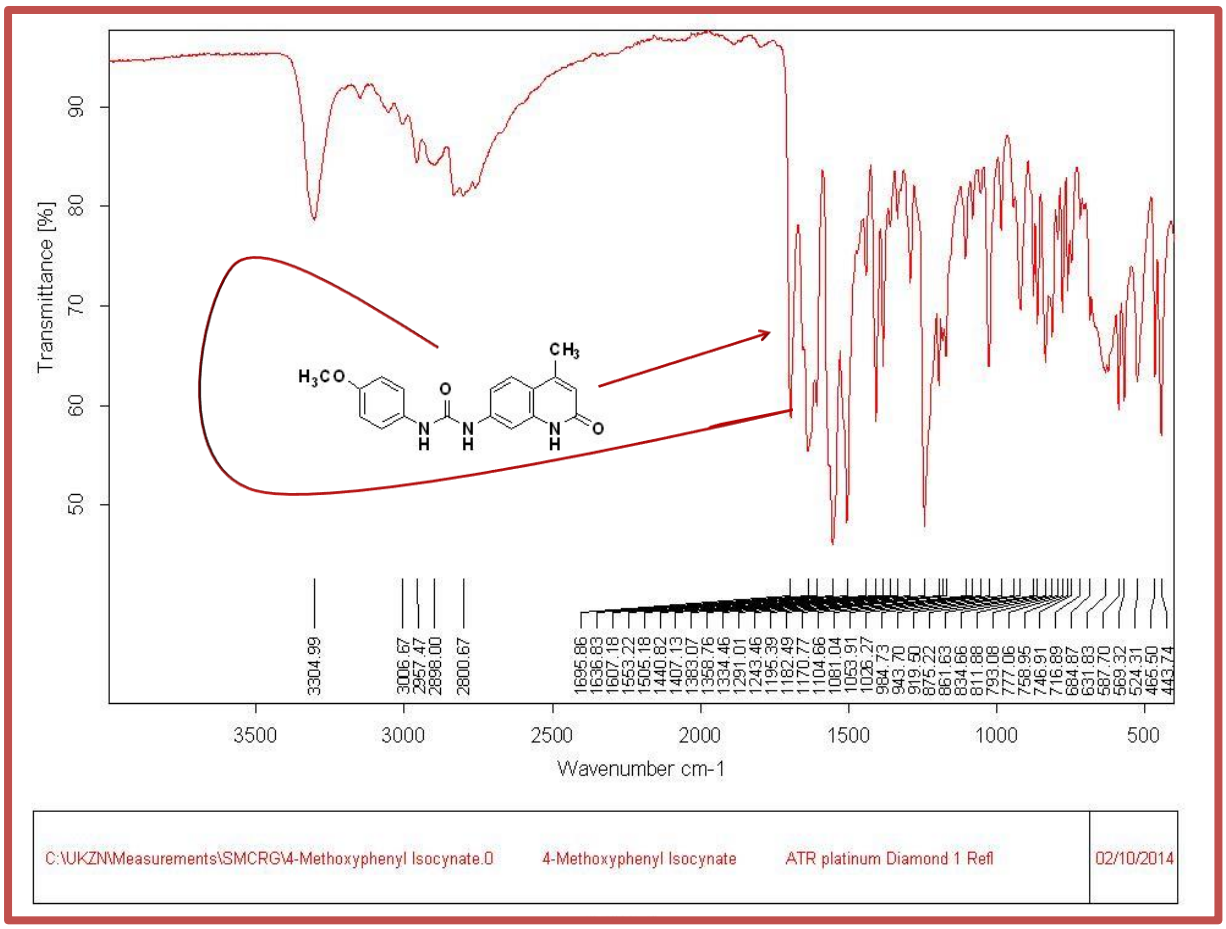


Figure 30: IR spectrum of compound 5e

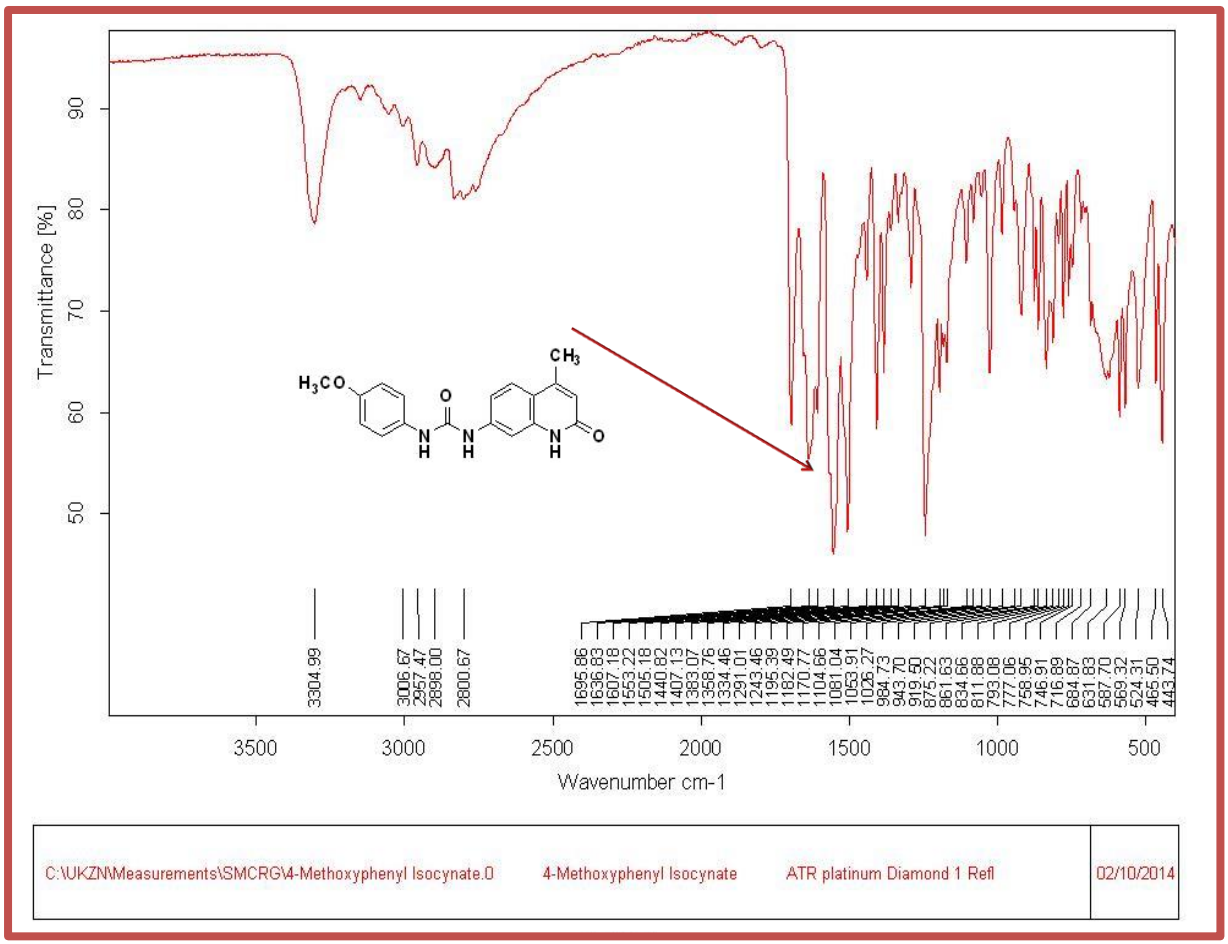


Figure 31: IR spectrum of compound 5e

All synthesized compounds showed resemblance of the characteristic peaks in the IR spectrum which further confirms the presence of the expected functional groups in the newly synthesized compounds.

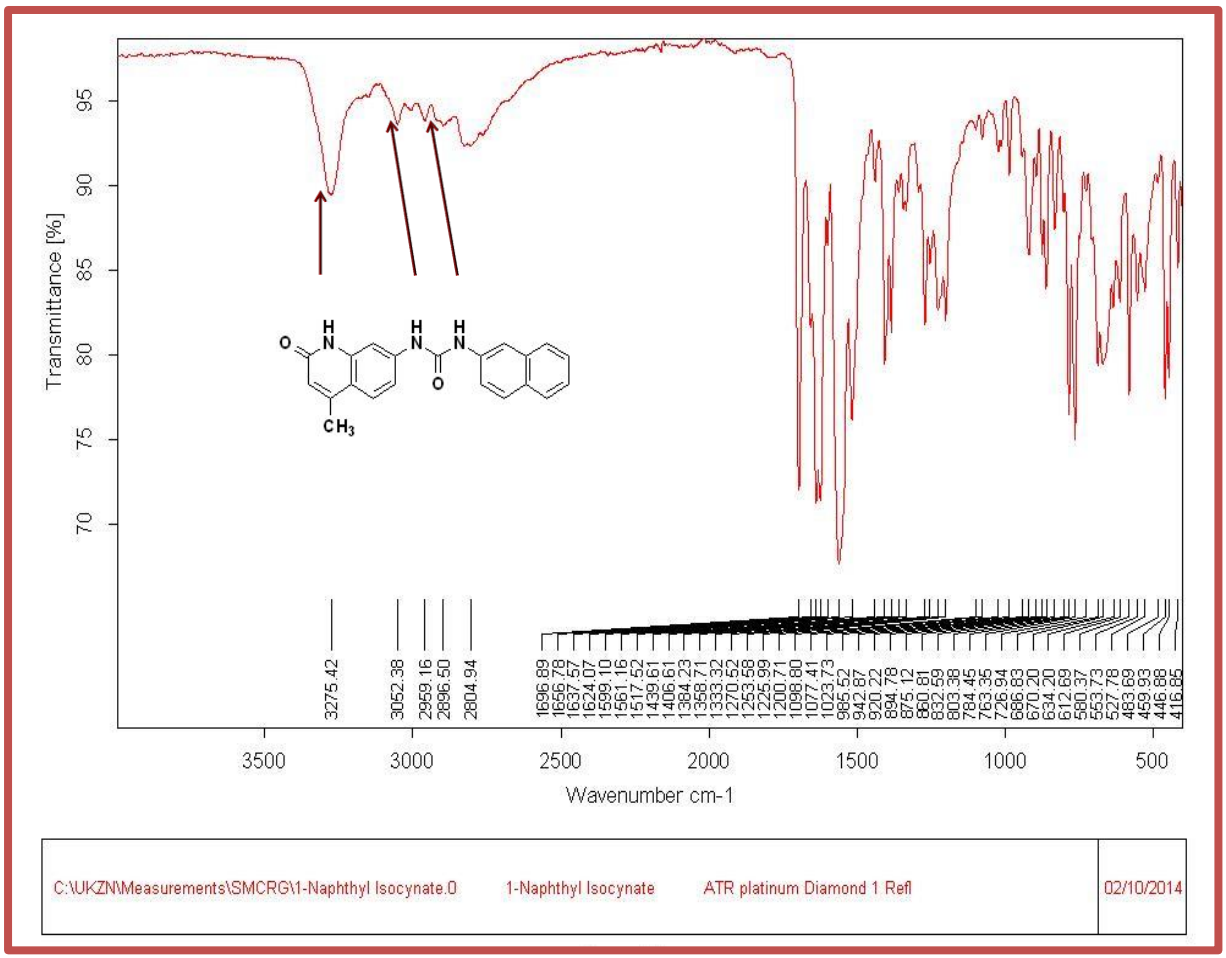


Figure 32: IR spectrum of compound 5f

IR of compound 5f also confirmed the formation of the target compounds by displaying expected IR peaks at the expected wavelength number. The characteristic peaks were observed which confirmed the presence of different functional groups such as NH peaks of the urea linkage as well as NH of the quinolone ring as well as the carbonyl peaks.

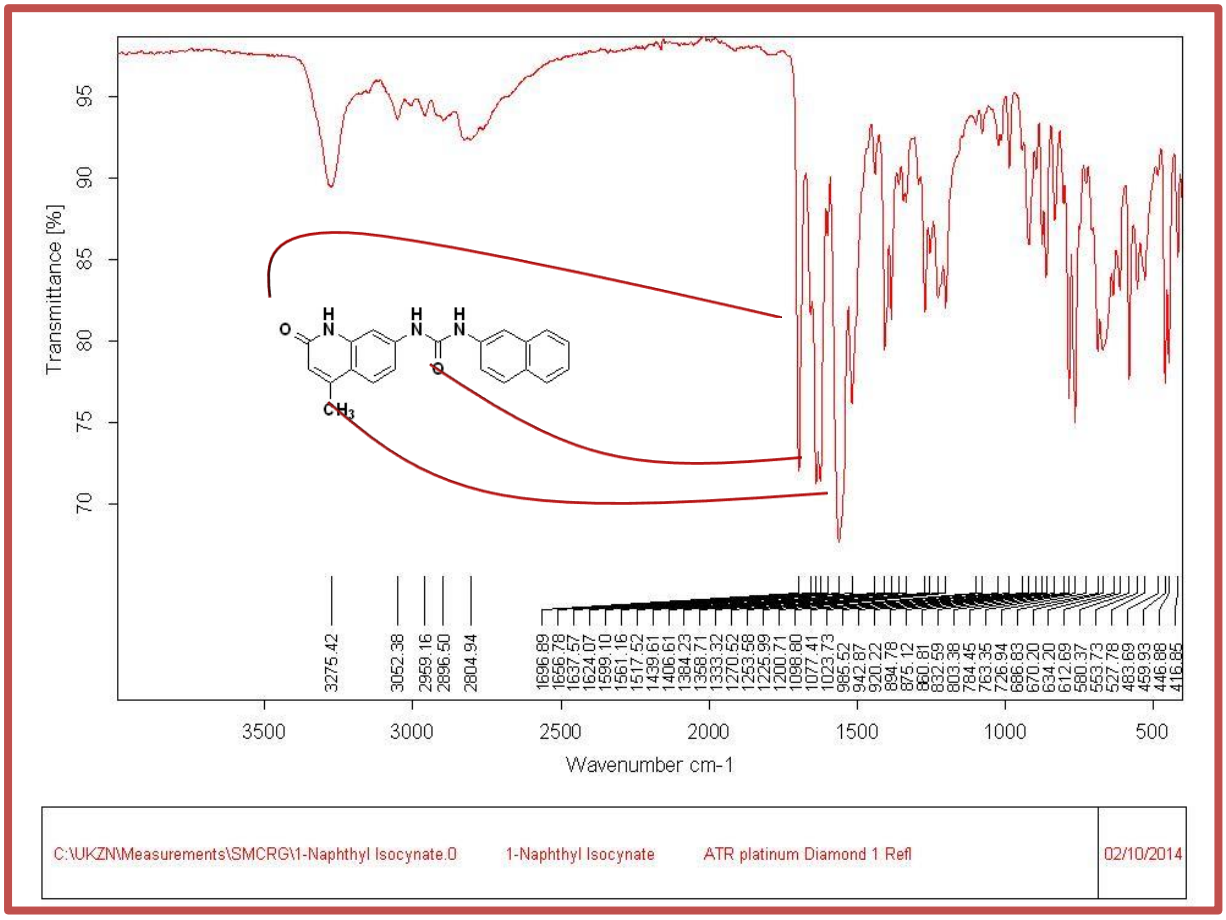


Figure 33: IR spectrum of compound 5f

4.1.6.2 ^{1}H NMR Spectroscopy

Proton NMR experiment was conducted to confirm the structure of the newly synthesized compounds. From the ^{1}H NMR spectra of the final compounds (**5a-k**), the characteristic peak of the amino group of the quinolone ring (NH) was observed at δ 11.45 ppm. This further indicated the integrity or the formation of the quinolone ring. Two distinctive peaks were also observed at δ 8.92 and 8.49 ppm and these prominent peaks were attributed to amino (NH) protons of the urea linkage as depicted in the figure-**34** below.

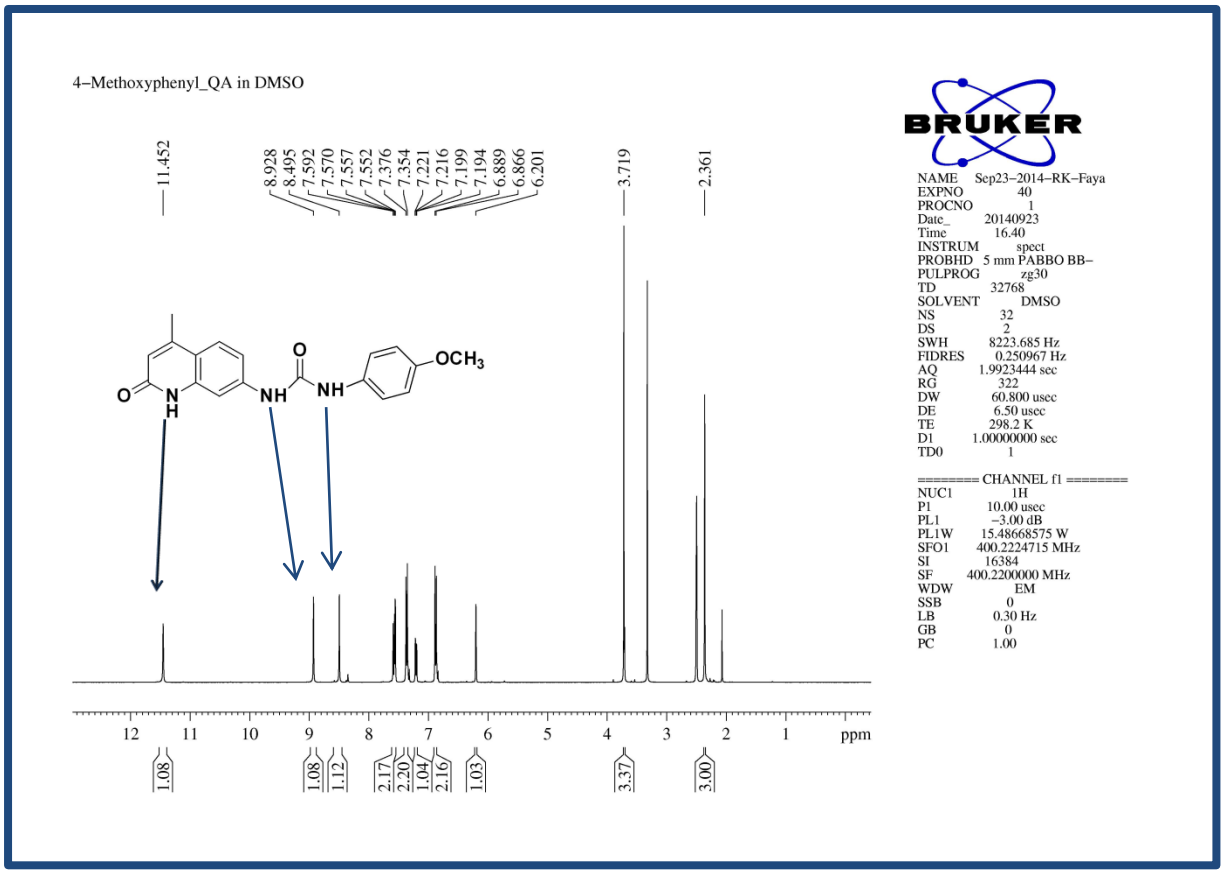


Figure 34: ^{1}H NMR spectrum of compound 5e

Furthermore, the formation of the 7-amino-4-methylquinolin-2(1*H*)-one urea/thiourea hybrid molecules were evident from the^{1}H NMR spectra where the prominent NH peaks resonated at δ 11.45ppm , δ 8.92 ppm and δ 8.49 ppm. These NH peaks confirmed the formation of the urea linkage bridge as well as the presence of the quinolone NH. The expected methyl peak situated at C-4 of the quinolone ring resonated around δ 2.38 ppm and it was also observed to be a singlet peak. C-3 proton of the quinolone ring was observed in the spectrum and signaled at δ 6.22 ppm. In addition, the various aromatic protons of the target compounds appeared in singlets or either doublets and were observed around δ 8.13-7.30 ppm as shown in the figure-**34**. The ^{1}H NMR spectra also indicated a very low percentage of impurity.

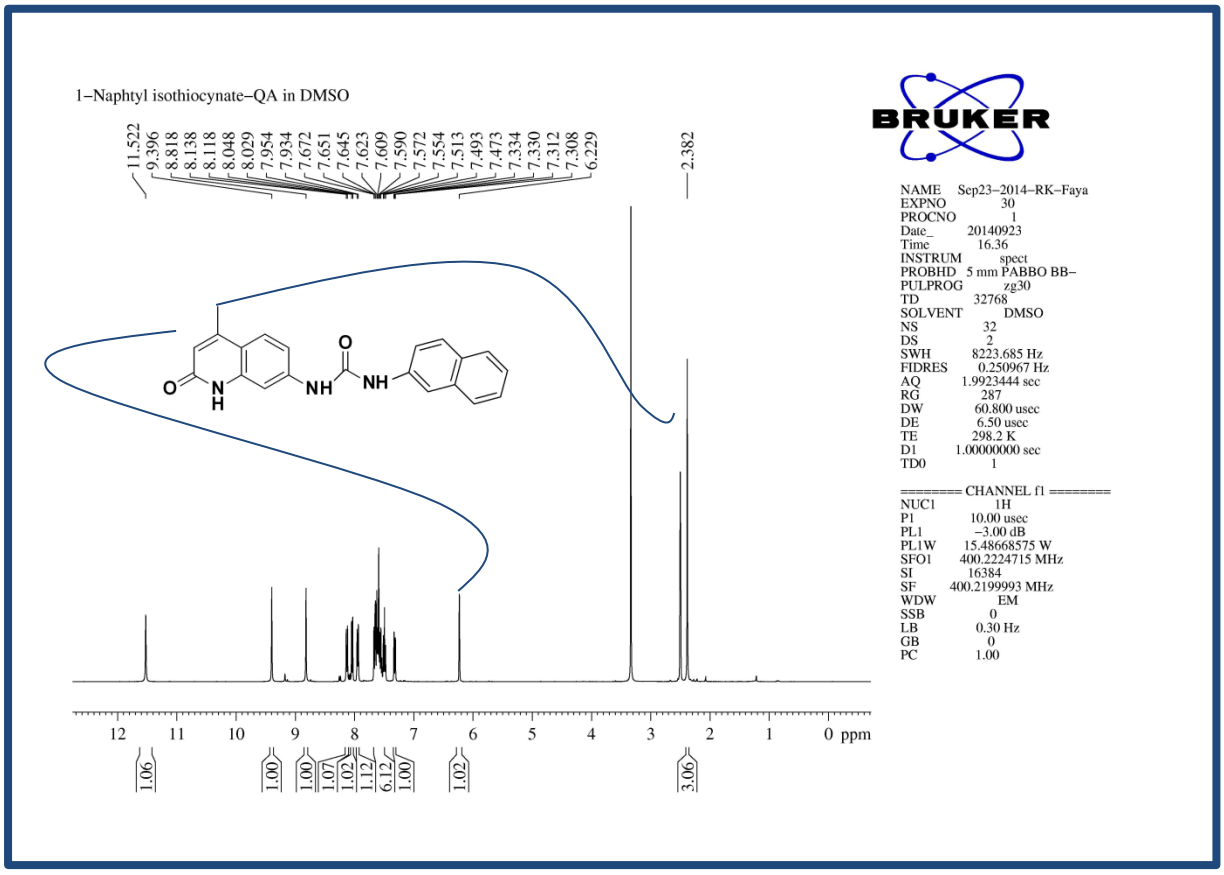


Figure 35: ^{1}H NMR spectrum of compound 5f

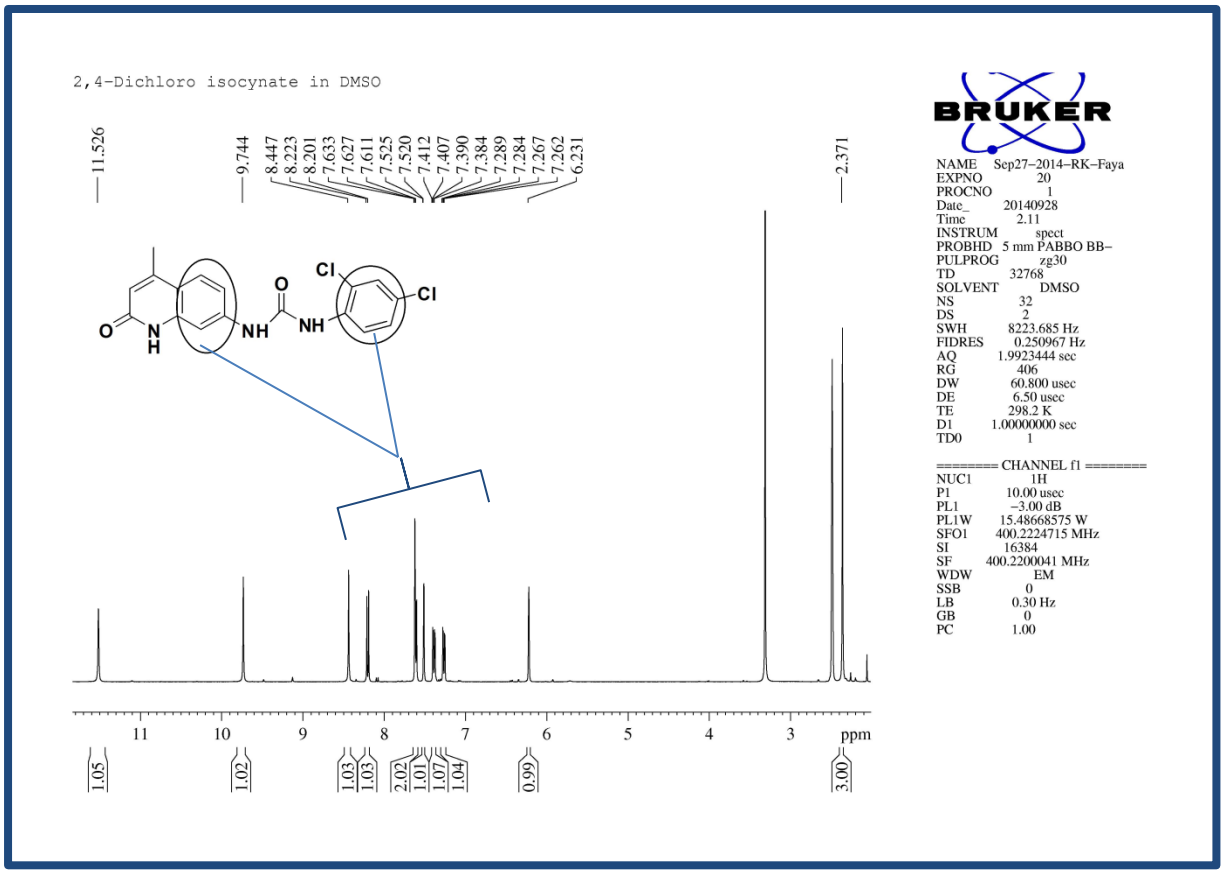


Figure 36: ^{1}H NMR spectrum of compound 5g

4.1.6.3 ^{13}C NMR spectroscopy

^{13}C NMR was also used to evaluate and determine the presence of carbon atoms of the synthesized compounds. Formation of compounds **(5a-k)** was confirmed by the appearance of a distinctive signal at δ 161.6ppmindicated the presence of the carbonyl group (C=O) at C-2 of the quinolone ring. Another carbonyl group of the urea linkage resonated at δ 152.3 ppm confirmed the integrity of the urea linkage. Another characteristic peak resonated at δ 120.7 ppm which indicated the presence of the C-H at C-3 of the quinolone ring and also at δ 19.0 ppm for CH_3 group further indicated the formation of the quinolone moiety. The various aromatic carbons resonated between δ 147.0 – 113.2 ppm as shown in the figure **37**. All of the synthesized compounds were found to contain the expected number of carbon atoms.

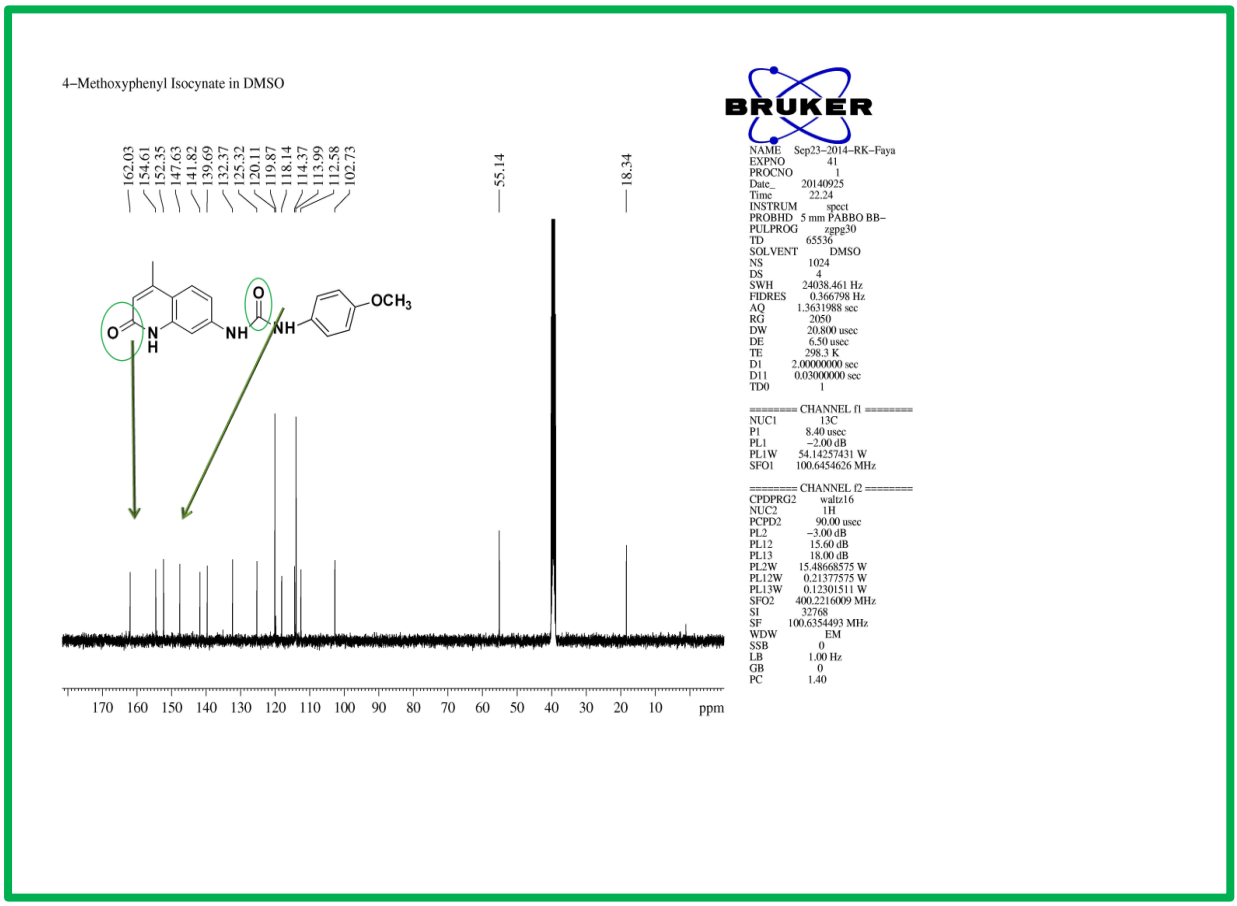


Figure 37: ^{13}C NMR spectrum of compound 5e

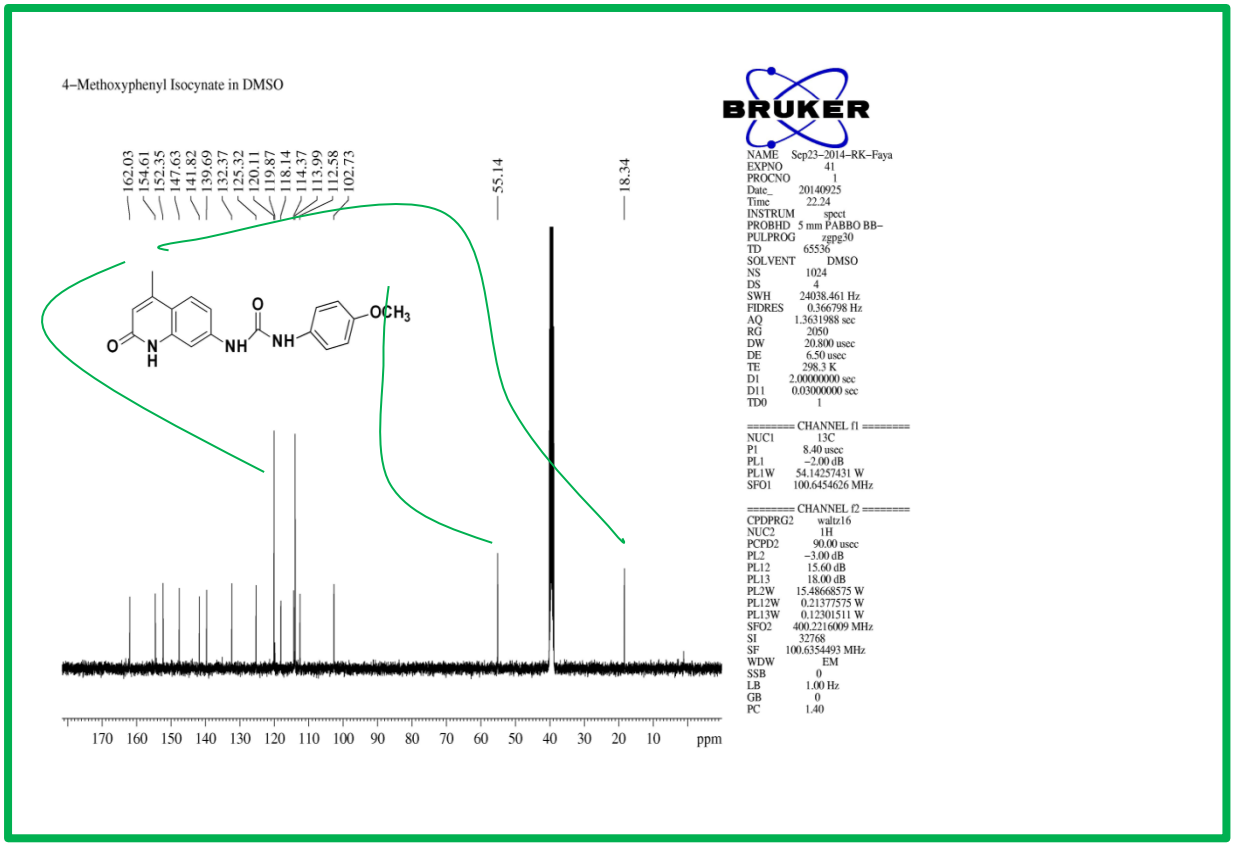


Figure 38: ^{13}C NMR spectrum of compound 5e

4.2 Anticancer activity (Pass-online prediction)

PASS (*Prediction of activity spectra for substances*) is an online prediction tool which predicts over 4000 kinds of biological activity, which includes pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters[1]. PASS has been well accepted by the research community, and actively used in the field of medicinal chemistry, by both academic organizations and pharmaceutical companies[2]. A model scaffold of the novel compounds was used as a template for predictions on the PASS online tool and the results are tabulated in **Table 6**. The results showed varied activities ranging from anti-cancer and anticancer related activities as well as antimicrobial and antitubercular activities.

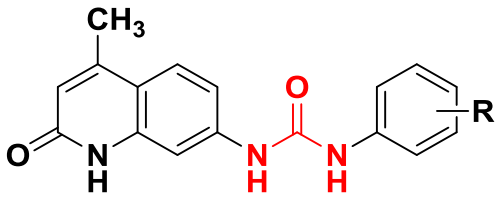


Table 6: Pass-online evaluation of synthesized compounds

Number	Probability to be active (Pa)	Probability to be inactive (Pi)	Activity
1	0.782	0.004	Cytochrome P450 stimulant
2	0.525	0.012	Heat shock protein antagonist
3	0.496	0.004	Tyrosine-protein-kinase receptor FLT3 inhibitor
4	0.494	0.047	Leukopoesis stimulant
5	0.477	0.039	Kinase inhibitor
6	0.400	0.039	Prostate disorders treatment
7	0.376	0.023	Cancer associated disorders treatment
8	0.368	0.020	Antineoplastic alkaloid
9	0.353	0.096	Pancreatic elastase inhibitor
10	0.166	0.005	Bacterial efflux pump inhibitor
11	0.285	0.125	Botulin neurotoxin A light chain inhibitor
12	0.175	0.097	Serine-pyruvate transaminase inhibitor
13	0.122	0.044	Interleukin 2 antagonist
14	0.198	0.122	anticarcinogenic
15	0.195	0.176	antituberculosic

4.2.1 Prediction of activity spectra for substances (PASS)

PASS is freely available online software to predict the potential activity of the novel compounds (Ref). Novel quinolone substituted urea/ thiourea were evaluated by PASS and the results obtained predicted potential anticancer activity for the synthesized compounds. The PASS predicted anticancer activity of the novel compounds were based on their specific inhibitory activity against heat shock protein (HSP), Tyrosine-protein-kinase receptor FLT3 inhibitor, Kinase inhibitor and as antineoplastic alkaloid with probable activity (Pa) values of 0.525, 0.496, 0.477 and 0.368 respectively. These compounds also displayed low Pi (Probable inactive) indexes with all cancer associated hits. Predictions of antitubercular and antimicrobial potential of these compounds were low, which was consistent with our experimental results. The probable mechanism of action of the antimicrobial activity of these compounds was predicted as efflux pump inhibitors with a Pa index of 0.116 (**Table 6**).

The ability of the compound to possess activity could be attributed: 1) Type of scaffold and 2) Pharmacophoric substituents on the scaffold (electron donating/withdrawing, charge on the molecule, hydrophobicity/lipophilicity). The potential activity of the novel compounds towards the cancer cell lines was discussed based on the knowledge of the quinolone moiety and all pharmacophoric features displayed by the 2-quinolone-urea/thiourea conjugates. All hybrids synthesized possessed a wide range of electron donating and electron withdrawing groups which have been studied in ring activation[3]. A short virtual screening procedure was also conducted using one of the novel compounds as a template on zincpharmer database to determine the pharmacophoric features thought to be responsible for activity (**Figure 28**). From the zincpharmer library, 4 compound hits were obtained with similar pharmacophoric features. All the results from the virtual screening protocol revealed that these drug candidates possessed anticancer activity thus we can also deduce that based on the similarity of our novel compounds with those from the virtual screening protocol, we can conclude that our compounds could also possess marked activity towards cancer cell lines. The compound IDs from the 4 hits from Zincpharmer included **Zinc46010622**, **Zinc46010563**, **Zinc46010602**, and **Zinc46010582**. These compounds contain benzoxazol-urea moieties with similar pharmacophoric features with that of our novel 2-quinolone-urea/thiourea derivatives. The cytotoxicity results of these benzoxazol compounds ranged from 1.6 µM with MCF-7, 0.4 µM with PC-3, 0.06 µM with IMR-32 respectively[4]. These results indicate the potential of the novel 2-quinolone-urea/thiourea derivatives in its evaluation against cancer cell lines

based on the comparison of the pharmacophoric features between the virtual screening results and our novel compounds.

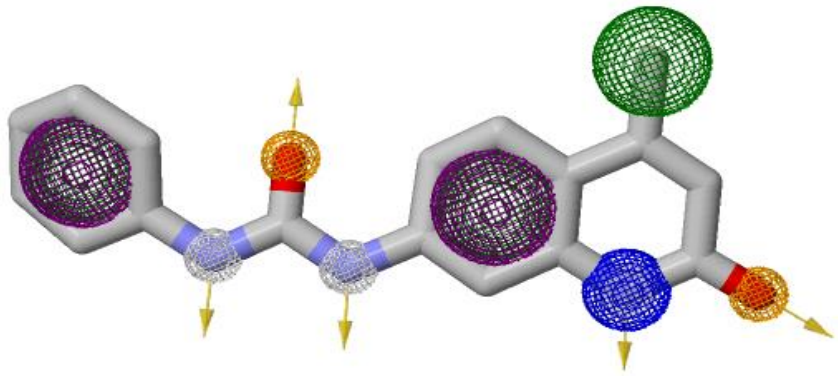


Figure 28: Pharmacophoric features of 2-quinolone-urea/thiourea

***In vitro* anticancer activity**

The novel synthesized compounds were evaluated against a panel of cancer cell lines and our preliminary results displayed promising activity. However, these results were not reproducible, which could be attributed to human error, precipitation and low solubility of the compounds. Based on these factors further troubleshooting was conducted and as a result the compounds are being revaluated for their anticancer activity.

4.2.2 *In vitro* antimicrobial activity

The quinolone-urea/thiourea derivatives were also evaluated for their antimicrobial potential against several bacterial strains (gram negative and gram positive) by known MIC assay determination method using resazurin dye[4,5] (**Table 7**). This study was conducted to determine the activity of the quinolone-urea hybrid compounds on DNA gyrase as well as prokaryotic topoisomerases after observing that these kind of compounds could have a promising impact on eukaryotic topoisomerases.

Microorganism used

Gram positive microorganism cultures used: *Staphylococcus aureus* (ATCC 25923), *Bacillus Subtilis (*ATCC 6051). Gram negative microorganism cultures used: *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC), *Streptococcus pneumonia* (ATCC 49619). Fungal strains used: *Candida albicans (ATCC 90028), Cryptococcus neoformans (ATCC 66031).* The culturing and subculturing of

microorganism were performed a day before the actual testing and the antimicrobial assay was carried out in a collaborative effort at the Department of Microbiology, Inkosi Albert Luthuli hospital, Durban, South Africa.

Table 7 Antimicrobial activity data

Compound	***Staphylococcus aureus* ATCC25923**	***Bacillus subtilis* ATCC6051**	***Escherichia coli* ATCC35218**	***Pseudomonas aeruginosa* ATCC27853**	***Salmonella typhimurium* ATCC**	***Strptococcus pneumoniae* ATCC49691**
5a	>200	>200	25	>200	>200	100
5b	>200	>200	25	>200	>200	100
5c	>200	>200	25	>200	100	100
5d	>200	>200	25	>200	>200	100
5e	>200	>200	25	>200	100	50
5f	>200	>200	12.5	>200	>200	100
5g	>200	>200	25	>200	>200	12.5
5h	>200	>200	25	>200	>200	100
5i	>200	>200	25	>200	>200	100
5j	>200	>200	>200	>200	>200	100
5k	>200	>200	>200	>200	>200	25

The MIC values ranged from 12.5 - >200 µg/mL and all compounds exhibited poor to moderate *in-vitro* activity against the screened bacterial strains. The reference drug Amoxicillin indicated an MIC of **0.39** µg/mL. Compound **5f** and **5g** showed potent activity (MIC = 12.5 µg/mL) against *E.coli* ATCC35218 and *Streptococcus pneumonia* ATCC49691 respectively

4.2.3 Discussion of Antimicrobial Activity

All the synthesized compounds (**5a-5k**) were screened for their *in vitro* antibacterial activity against a panel of microorganisms which comprised of three Gram +ve [*Staphylococcus aureus*ATCC25923, *Bacillus subtilis*ATCC6051, *Strptococcuspneumonia*ATCC49691] and three Gram –ve [*Escherichia coli* ATCC35218, *Pseudomonas aeruginosa*ATCC27853, *Salmonella Typhimurium* ATCC]. The MIC results were moderate as seen from compound **5f** and **5g** which both had activity of 12.5µg/mL against *E.coli and Streptococcus pneumonia*. Other compounds also showed moderate activity which was around 25µg/mL as seen with compounds 5a to 5i for their activity towards *E.coli.* All compounds showed no activity towards other clinical strains (*Staphylococcus aureus, Bacillus subtillus, Pseudomonas earuginosa, Salmonella typhinium*) with activity of >100µg/mL. These results obtained were consistent with the PASS online prediction which also predicted very low antibacterial activity.

Microorganisms have many defense mechanisms at their disposal to combat the action of drugs. A probable mechanism which could be responsible for the low activity of our novel series of quinolone urea/thiourea hybrid compounds could be the action of efflux pumps[6]. Among the various mechanisms involved in bacterial resistance, the balance of membrane permeability which controls inflow and outflow of various molecules plays a key role in the influx and efflux of antibiotics, thereby limiting their intracellular concentration. Efflux pumps have now been shown in both Gram-positive and Gram-negative bacterial pathogens as a major resistance strategy[7]. Efflux transporters such as that of *E.coli* (AcrAB efflux system) confers a 'general resistance mechanism' that can reinforce the effect of other resistance mechanisms such as mutations of the antibiotic targets which will result in the inactivity or elimination of the drug[8]. The impact of efflux pumps on antimicrobial resistance is also due to the genetic elements which code for these pumps and once these genes mutate they are able to survive harsh conditions such as the presence of an antimicrobial agent[9,7].

With the above facts in mind, it is hypothesized that the action of the newly synthesized hybrid compounds could have been halted by the efflux pumps which could have pumped the compounds out of the cells[10].

4.2.4 *Invitro* characterization of anti-mycobacterial activity

The quinolone-urea/thiourea derivatives were further evaluated for their antitubercular activity against $H_{37}R_V$ strain.

Table 8: Antitubercular activity data (MIC in µM) of a series of novel quinolone urea/thiourea derivatives (**5a-k**)

Compound	***Mycobacterium tuberculosis* ($H_{37}Rv$)**
5a	>200
5b	>200
5c	>200
5d	>200
5e	>200
5f	>200
5g	>200
5h	>200
5i	>200
5j	>200
5k	>200
Rifampicin	**0.0067**

4.2.5 Discussion of anti-mycobacterial activity

The Pass online tool predicted very low levels of activity of these newly synthesized compounds towards Tuberculosis with a Pa value of 0.195 and a high Pi value of 0.176. The experimental results (MIC >200µM) obtained were in agreement with PASS predicted low activity of these compounds towards $H_{37}Rv$ strains.

A study conducted by Da Silva et al., 2011 showed that tuberculosis confers resistance by a different number of mechanisms[11]. Firstly the cell wall of tuberculosis contains complex lipids which act as a barrier for drugs to block permeability, secondly tuberculosis consists of enzymes which often confers resistance, thirdly tuberculosis consists of drug efflux pumps which actively pumps out the drug rendering it ineffective and lastly spontaneous mutations within the TB genome gives rise to proteins that make the drug ineffective[12]. With regards to this present research the lack of activity of these novel compounds could be due to target

modification such as mutations in the DNA gyrase and/or topoisomerase IV genes however another probable mechanism of resistance which was also thought to hinder the action of these compounds to act as antimicrobial agents could be due to the effect of efflux pump systems and the inability of the quinolone urea compounds to actively permeate through the mycolic acid of the tuberculosis strain which would limit the potential of the compound to reachthetargetsite[13]

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Chapter 5
Conclusion

5.1 CONCLUSION

The aim of this study was to investigate the effectiveness of eleven newly synthesized derivatives of quinolone-urea/thiourea against a panel of cancer cell lines and to further evaluate the antitubercular and antimicrobial potential of these derivatives.To a great extent, this work has accomplished the aims of the study. Results from this work confirmed the following conclusions:

- The 2-quinolone-urea/thiourea novel compounds are thought to be effective against cancer cell lines based on pharmacophoric comparison with a virtual screen derived compounds with IC_{50} values ranging from 0.06 µM to 1.6 µM.
- The synthesized compounds displayed moderate antimicrobial activities against bacterial clinical isolates with MIC values ranging from 12.5 – 50 µg/mL
- The synthesized compounds however were not able to show any antitubercular activity against the H37Rv strain all MIC values of the eleven derivatives were >200 µM
- Structural elucidation using spectroscopic techniques such as FT-IR and NMR confirmed the formation of the eleven new compounds.

5.2 Recommendation and Future Studies

One of the major challenges faced in this research work was synthesizing more compounds due the fact that certain isocyanates and isothiocyanates were found to be less reactive to 7-amino-4-methyl-2-quinolone ring which could be due to steric hindrances and low reactivity of the amino group attached at C-7 of the quinolone ring. One of the possibilities which could be employed in the formulation of the quinolone-urea/thiourea hybrid compounds can involve thereaction ofother plausible positions on the quinolone ring with isocyanates and isothiocyanates. Comparative analysis between these quinolone pharmacophore[1,2] and their reactivity towards isocyanates and isothiocyanateswould further provide a deeper insight into the mechanisms by which different quinolones react with ureas and thioureas.

Another approach to gain better anticancer and antibacterial activity of the synthesized quinolone derivatives would be solving the solubility concerns of the novel compounds and optimization by introducing halogens such as fluorine at a strategic position of the quinolone ring. The activity of quinolone-urea/thiourea could also be improved by complexation with silver and gold nanoparticles which have been documented by many researchers[3] and found to be effective drug carriers to specific targets and also they confer good electrical

conductivity on the drugs themselves[4]. This could be a valuable model for optimizing the activity of the quinolone-urea/thiourea hybrid molecule.

Future work would thus include:

- Enhancement of drug solubility by employing various techniques such as physical modifications (*Nano-suspension, particle size redusction, co-cyrstalization),* chemical modifications (*Ph change, complexation, salt formation*) and other miscellaneous methods such as *solubilizers, sufactants and novel excipients*.
- Optimization of the quinolone-urea/thiourea by substituting different halogens at different positions of the quinolone ring.
- Employing the activity of nanoparticles as drug carriers during anticancer and antimicrobial evaluation.
- Using synergistic testing possibilities of the synthesized compounds together with nanoparticles by disk diffusion assays and checkerboard assay methodologies.
- Synthesizing a library of 2-quinolone and 4-quinolone compounds and comparing their anticancer potential as well as antimicrobial activity when conjugated with different ureas, thioureas and halogens.

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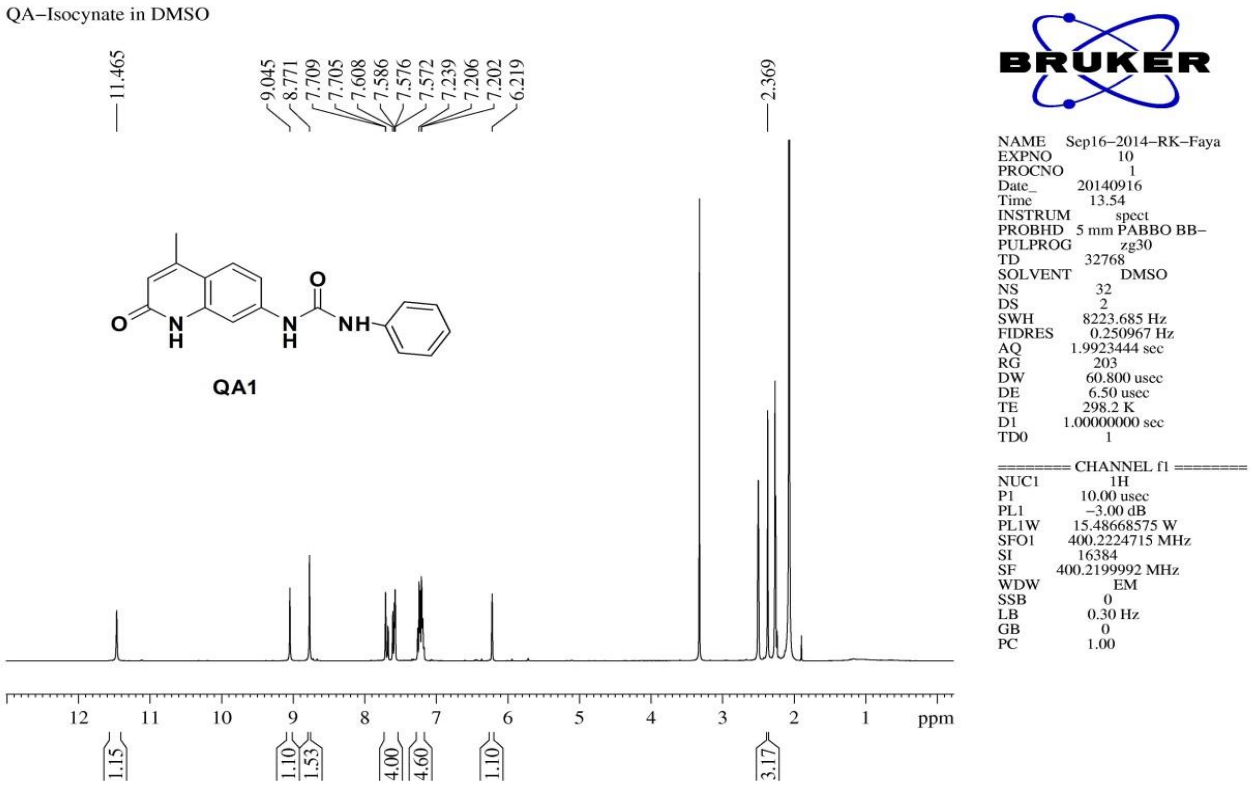
Appendix



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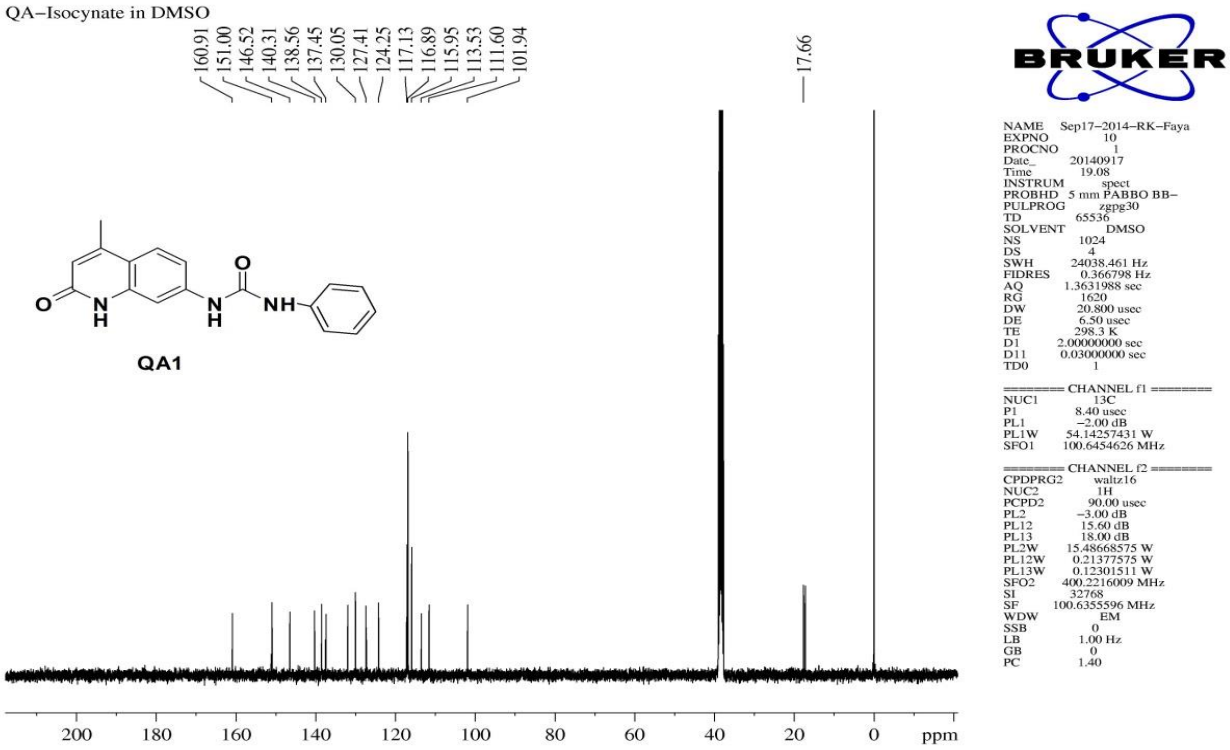
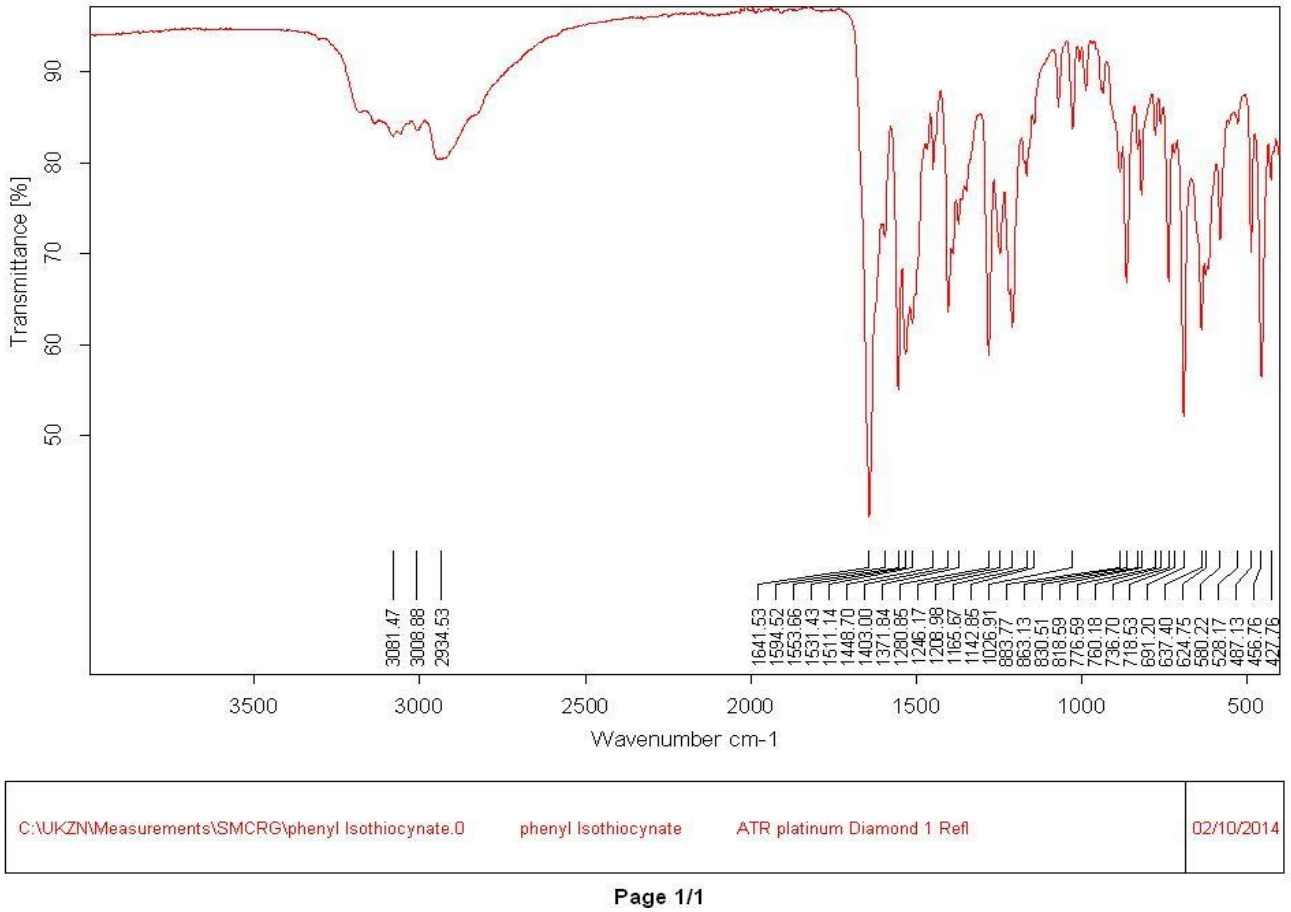
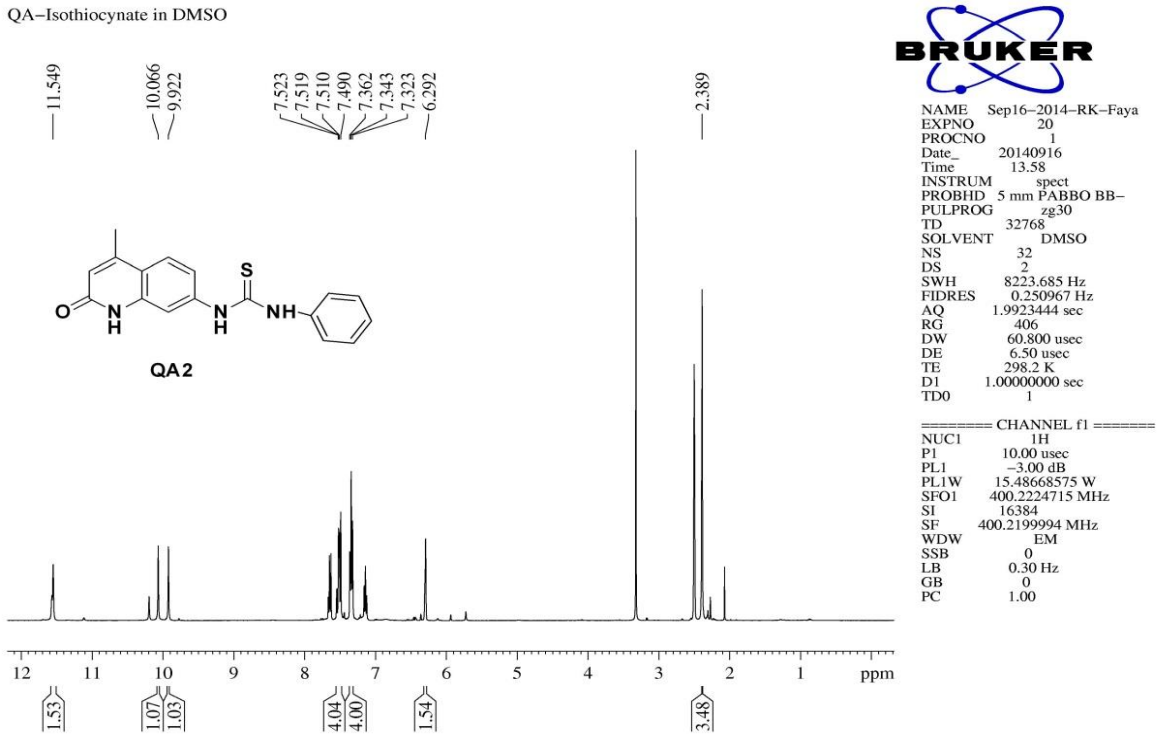


Spectrum 2: ^{13}C NMR spectrum of compound **5a**



Spectrum 3: IR spectrum of compound **5a**



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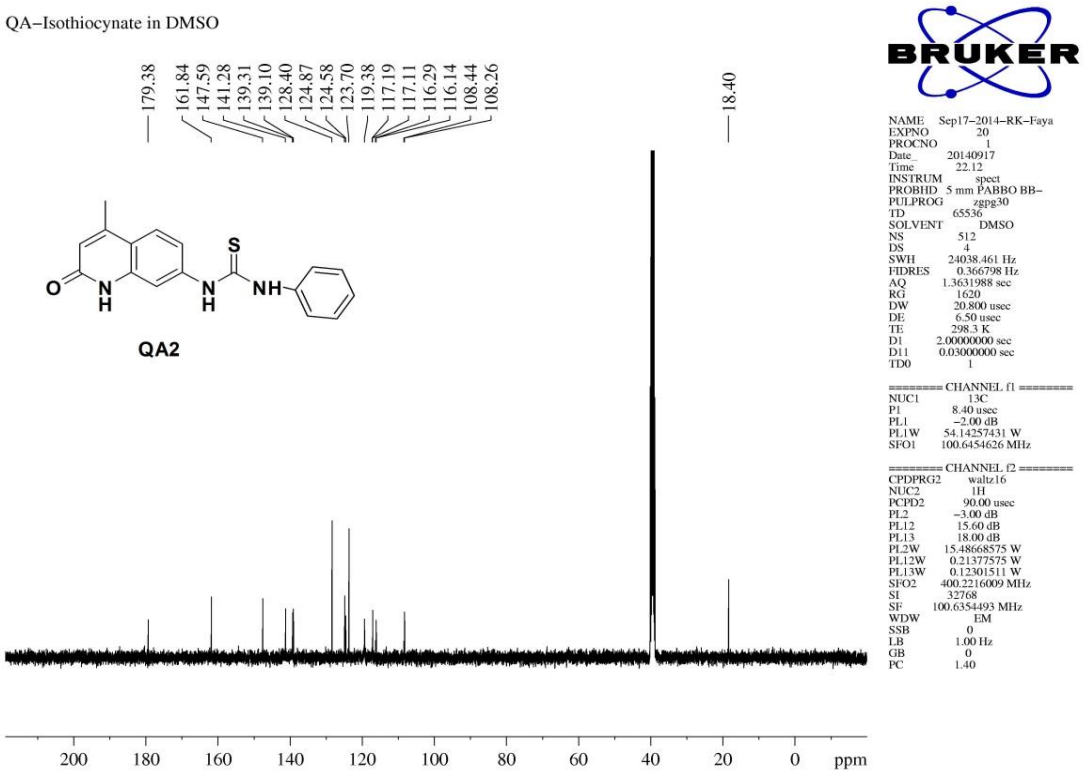
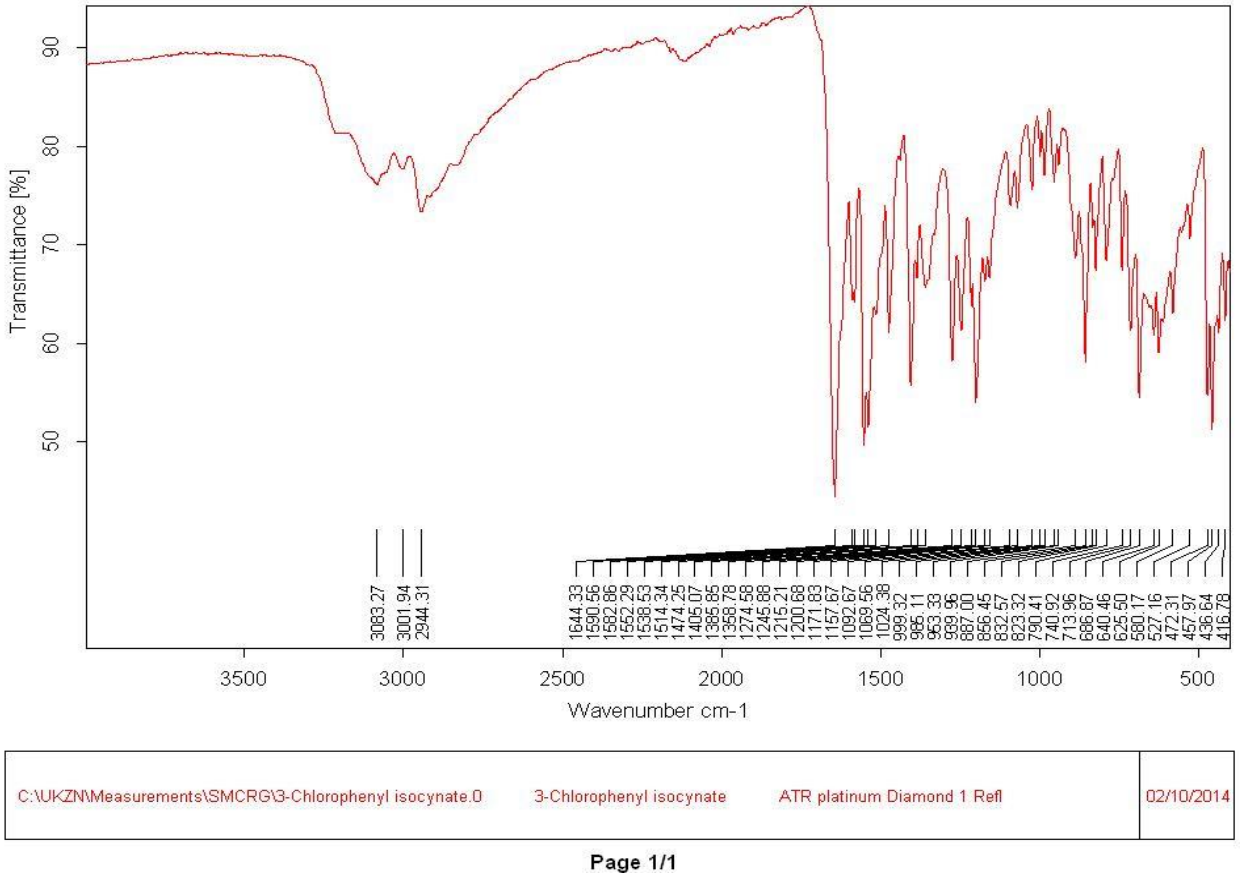
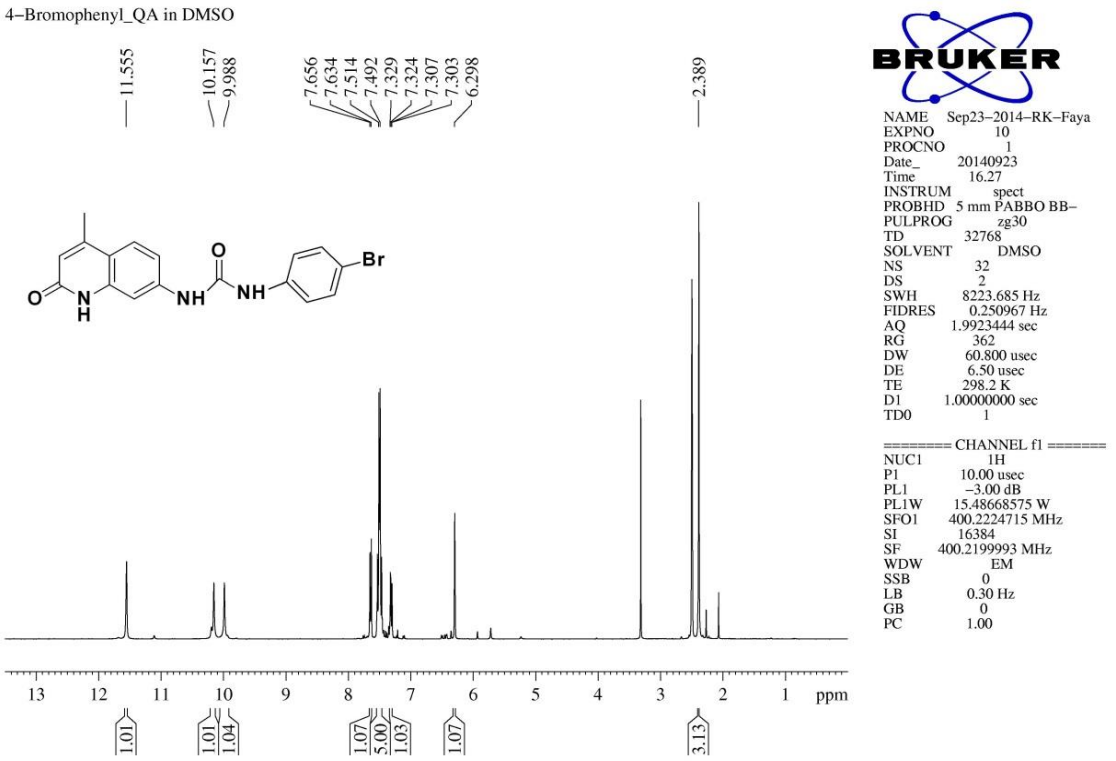


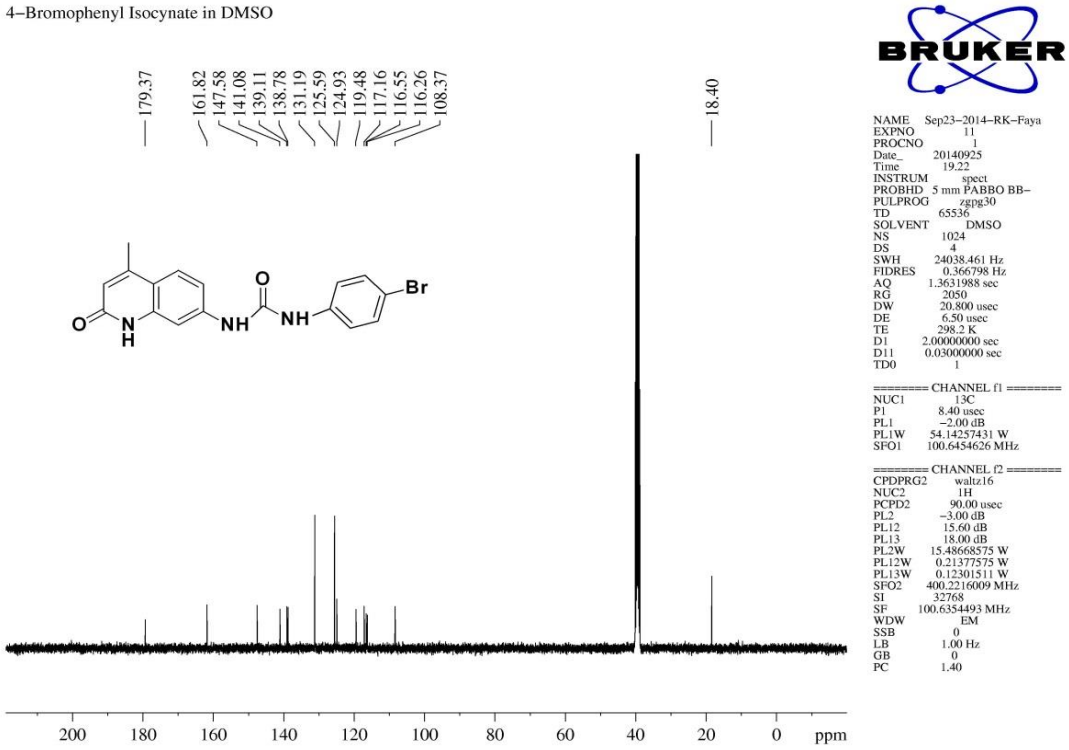
Spectrum 5: ^{13}C NMR spectrum of compound **5b**



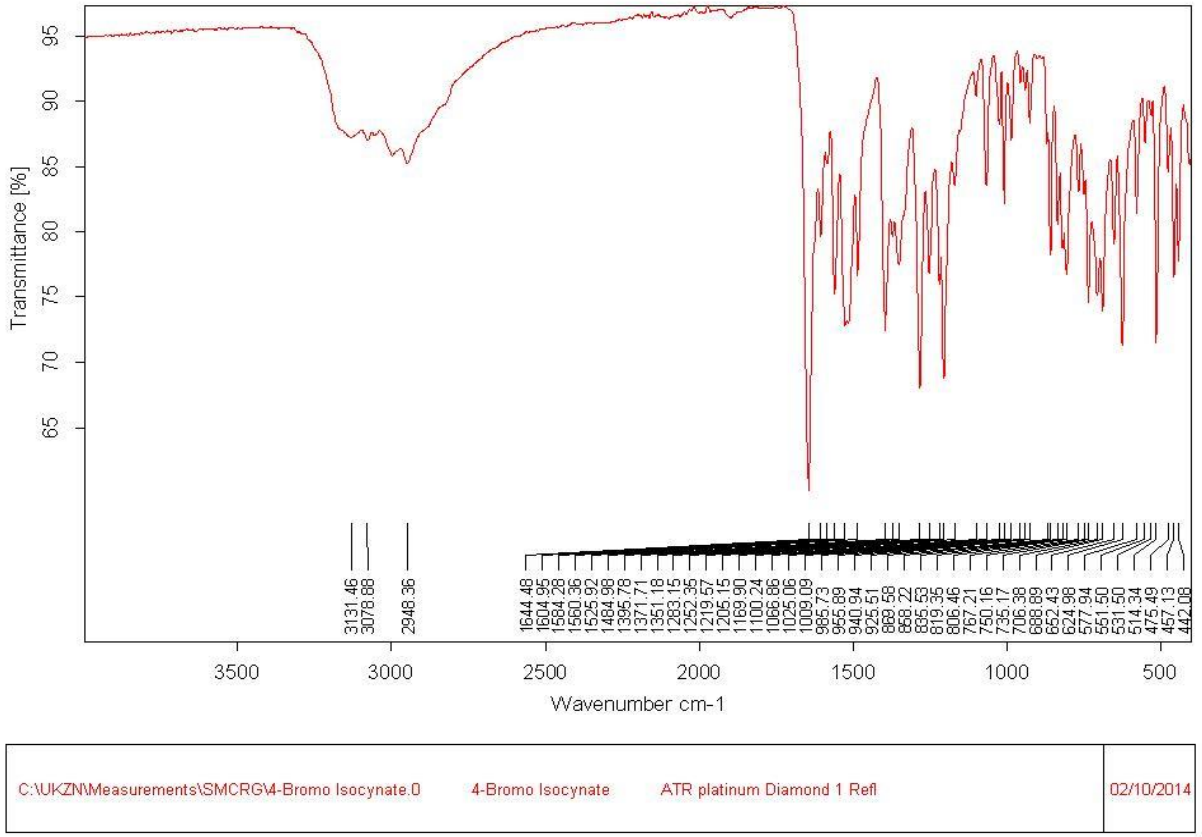
Spectrum 6: IR spectrum of compound **5b**



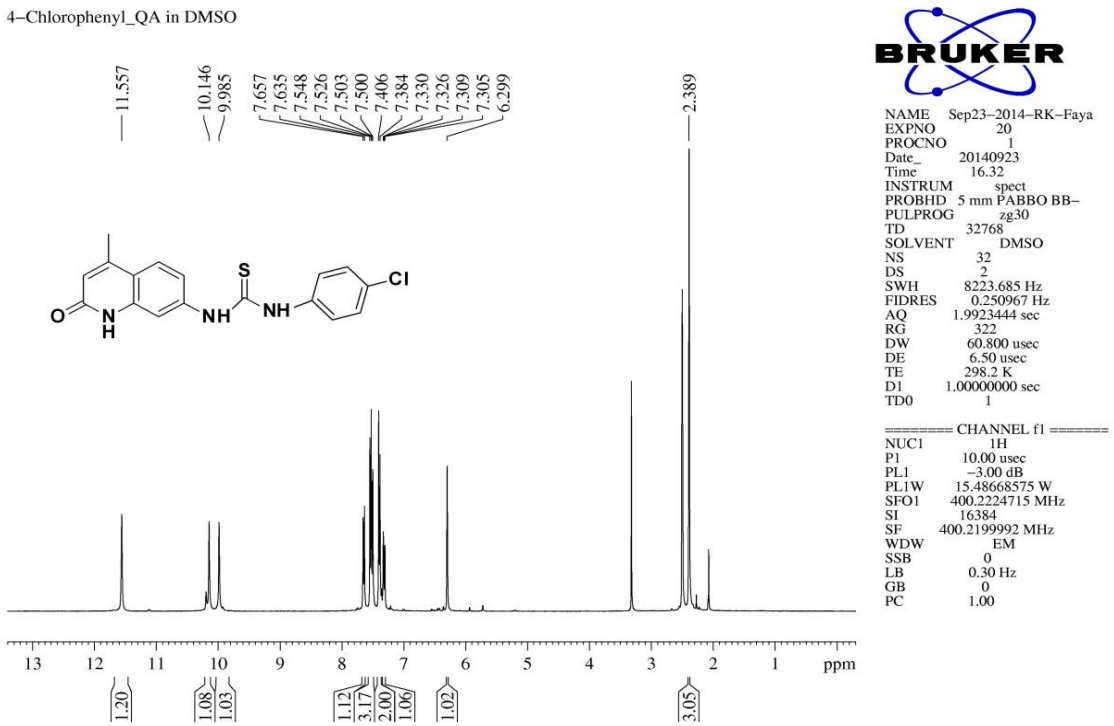
Spectrum 7: ^{1}H NMR spectrum of compound **5c**



Spectrum 8: ^{13}C NMR spectrum of compound **5c**



Spectrum 9: IR spectrum of compound **5c**



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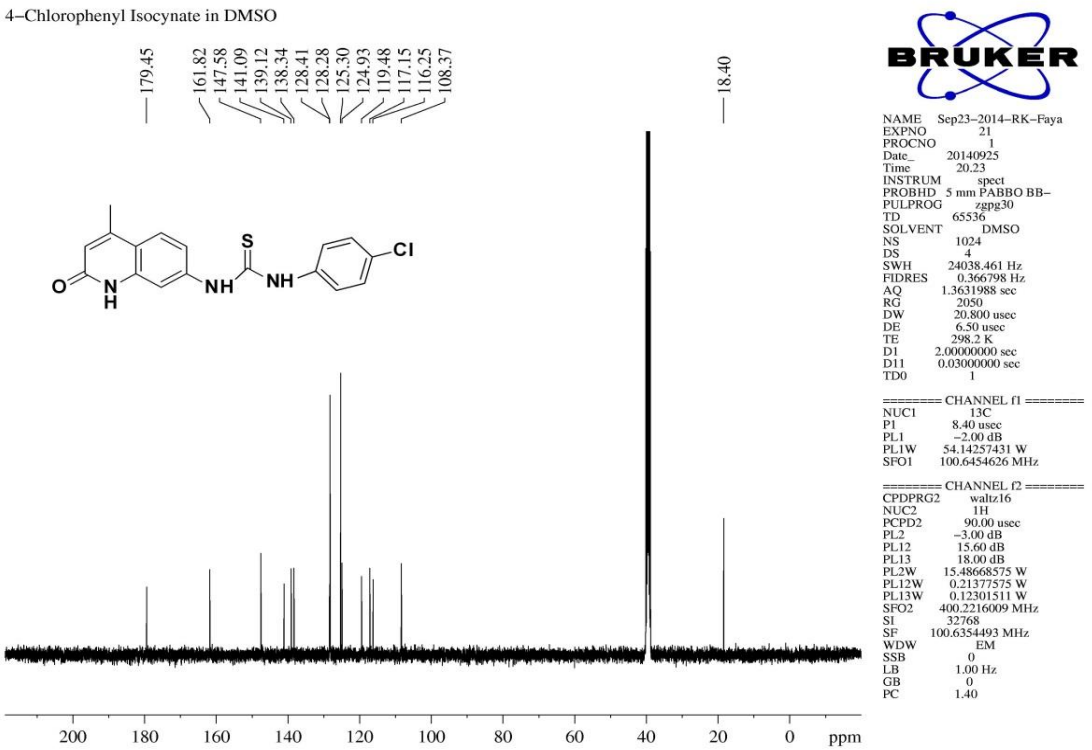
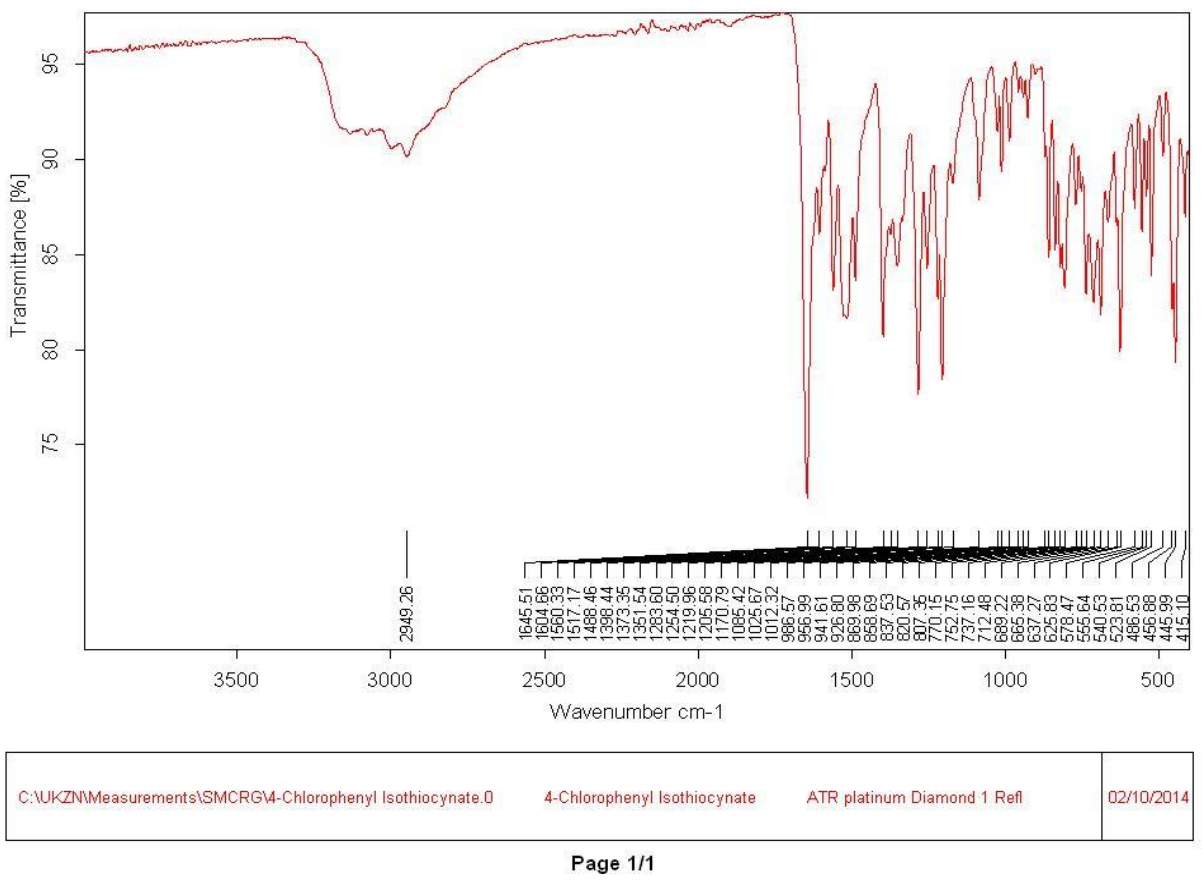
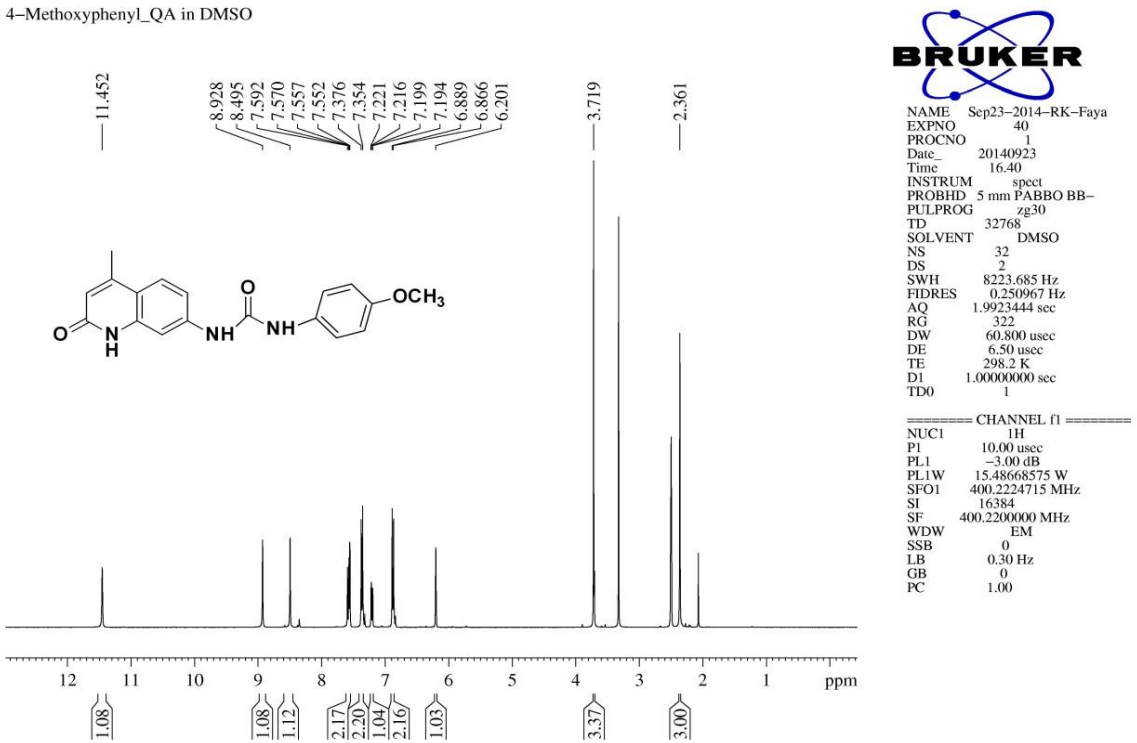


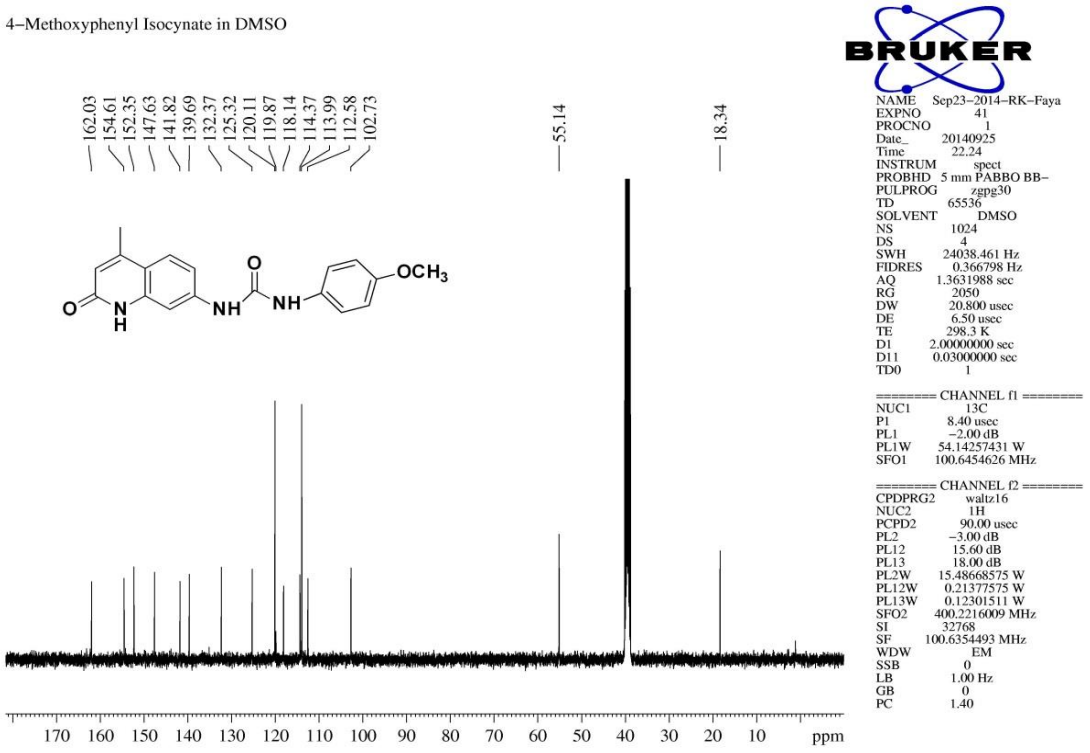
Spectrum 11: ^{13}C NMR spectrum of compound **5d**



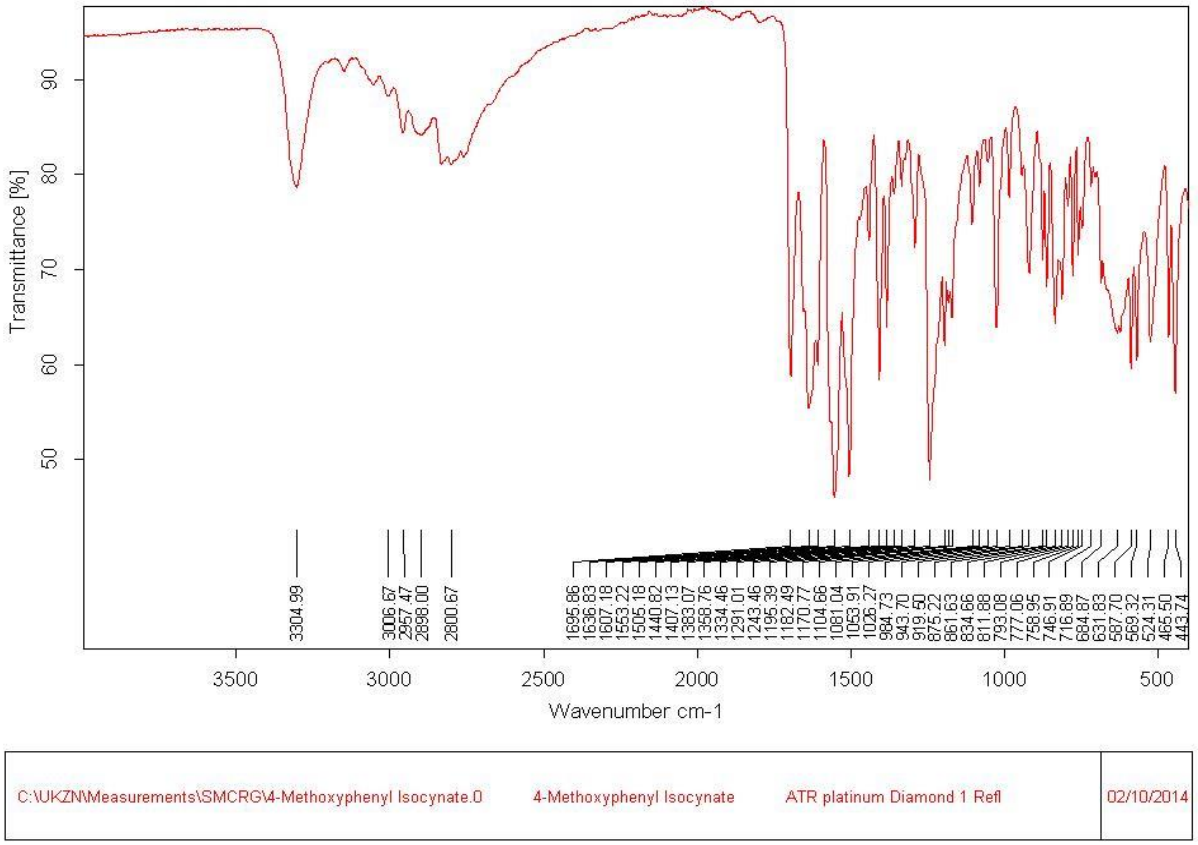
Spectrum 12: IR spectrum of compound **5d**



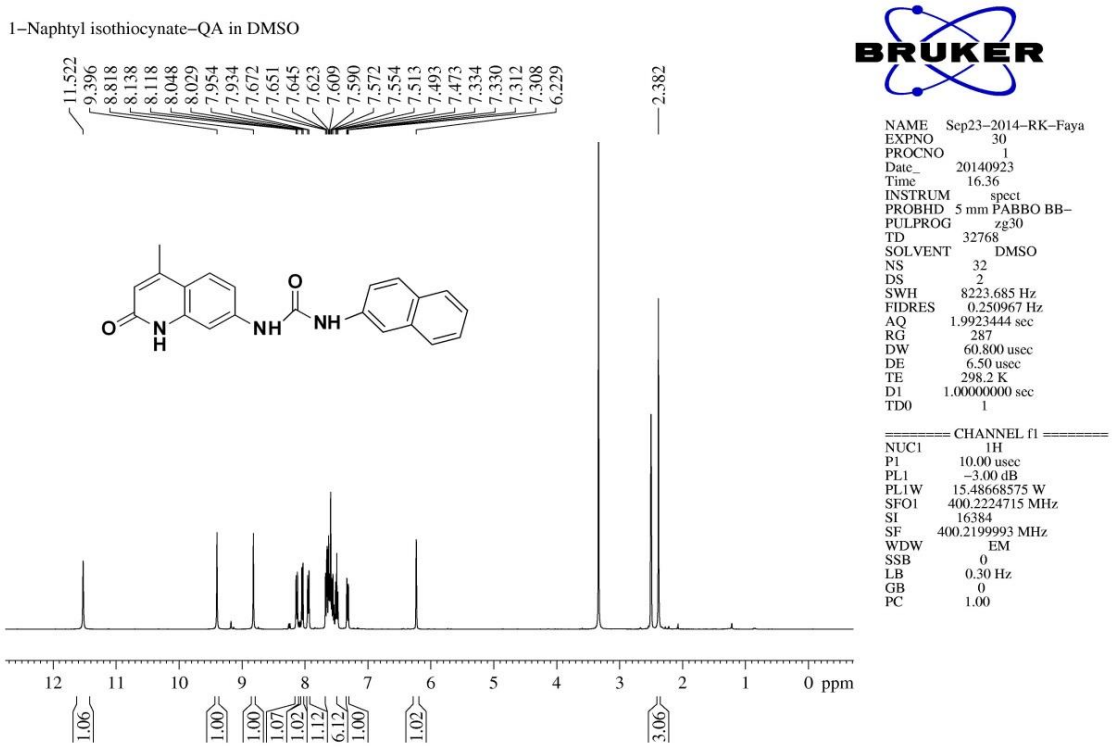
Spectrum 13: ^{1}H NMR spectrum of compound **5e**



Spectrum 14: ^{13}C NMR spectrum of compound **5e**



Spectrum 15: IR spectrum of compound **5e**



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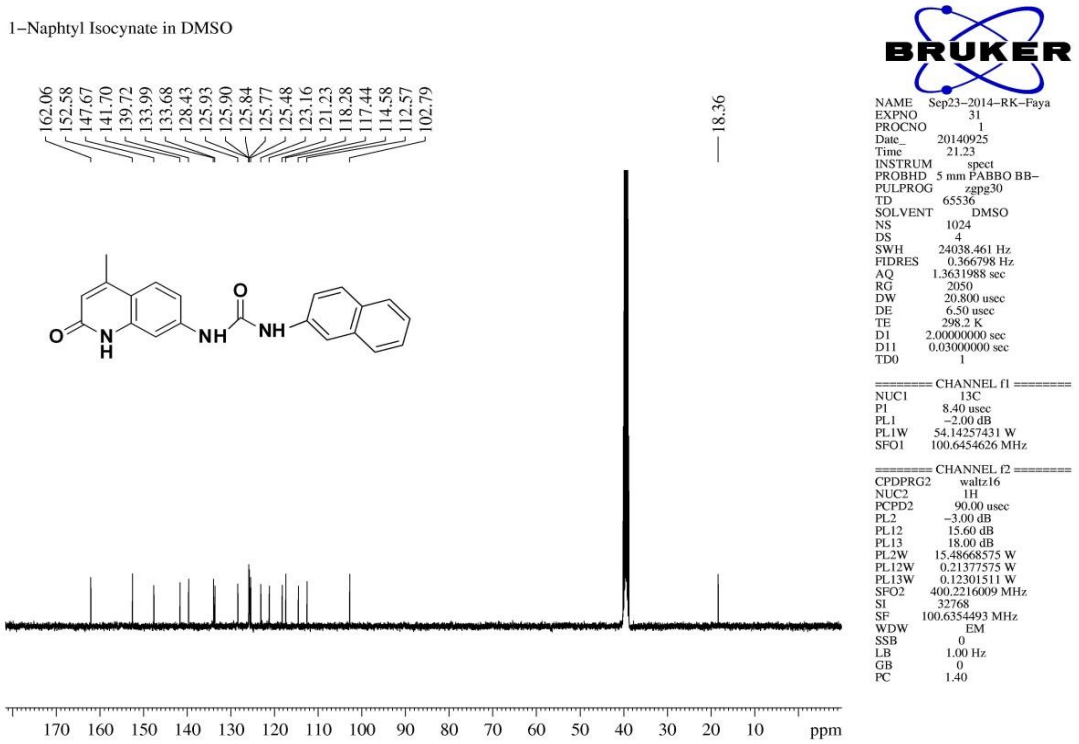
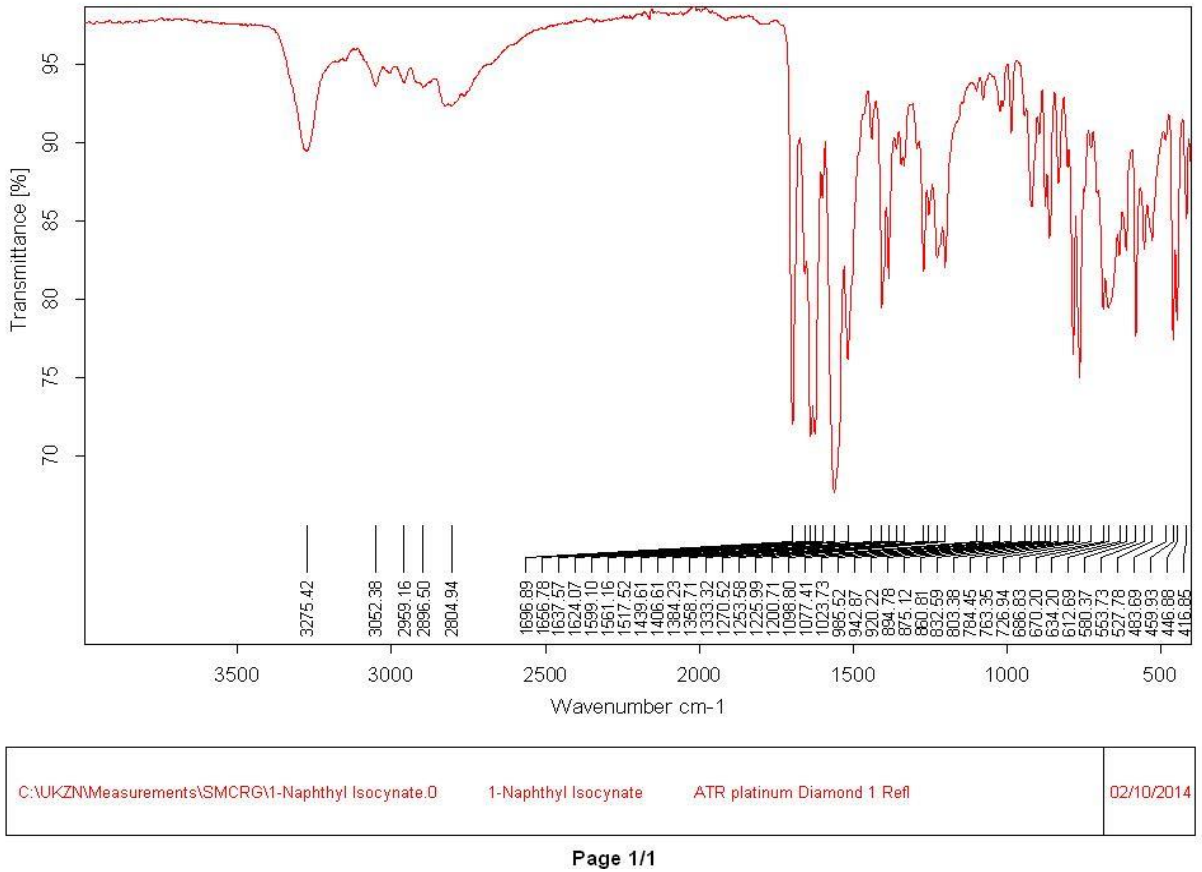
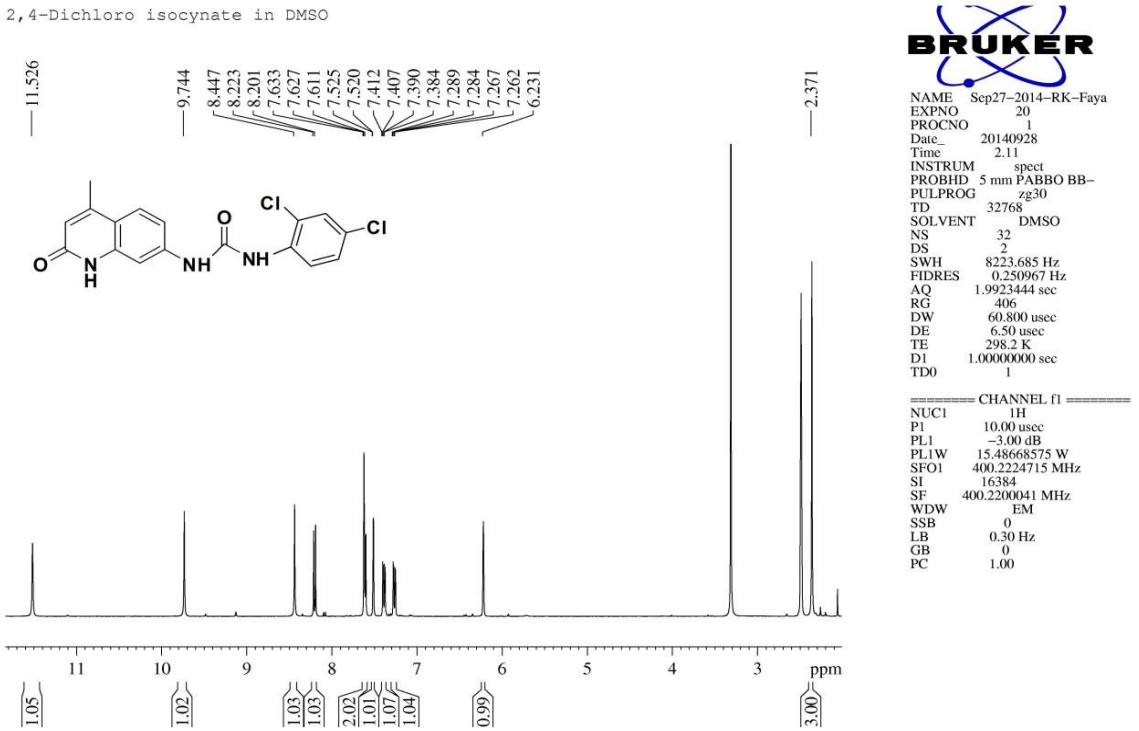


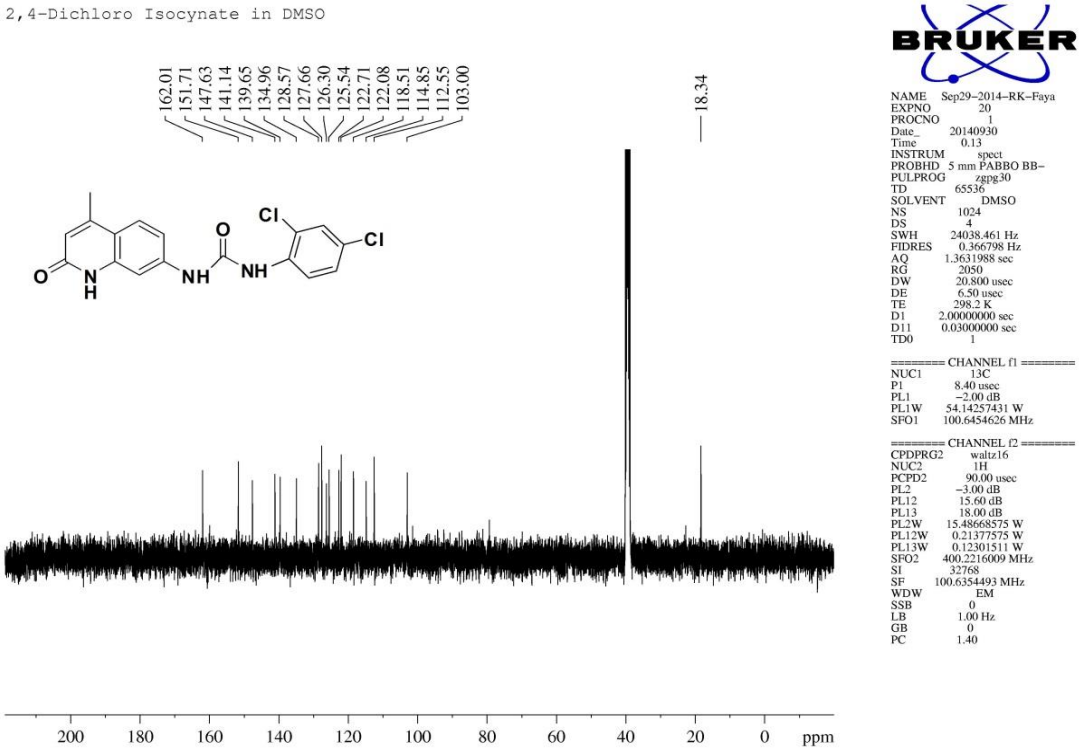
Spectrum 17: ^{13}C NMR spectrum of compound **5f**



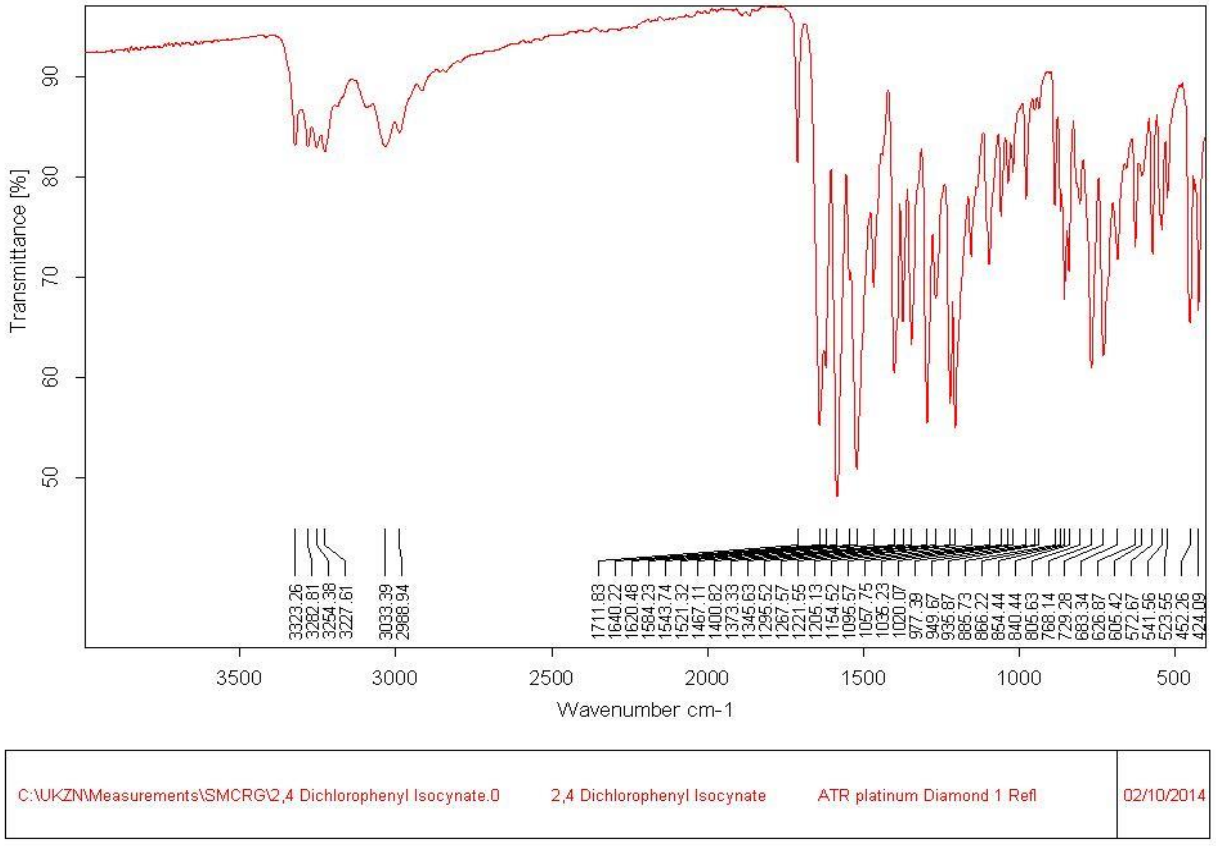
Spectrum 18: IR spectrum of compound **5f**



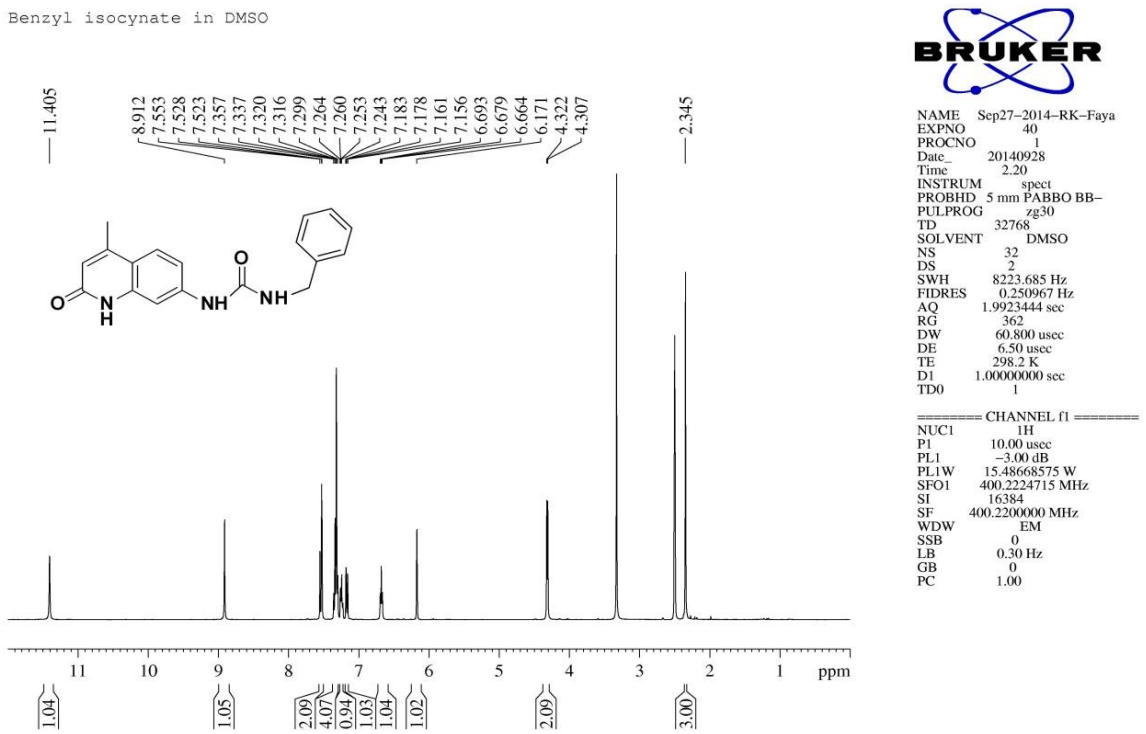
Spectrum 19: ^{1}H NMR spectrum of compound **5g**



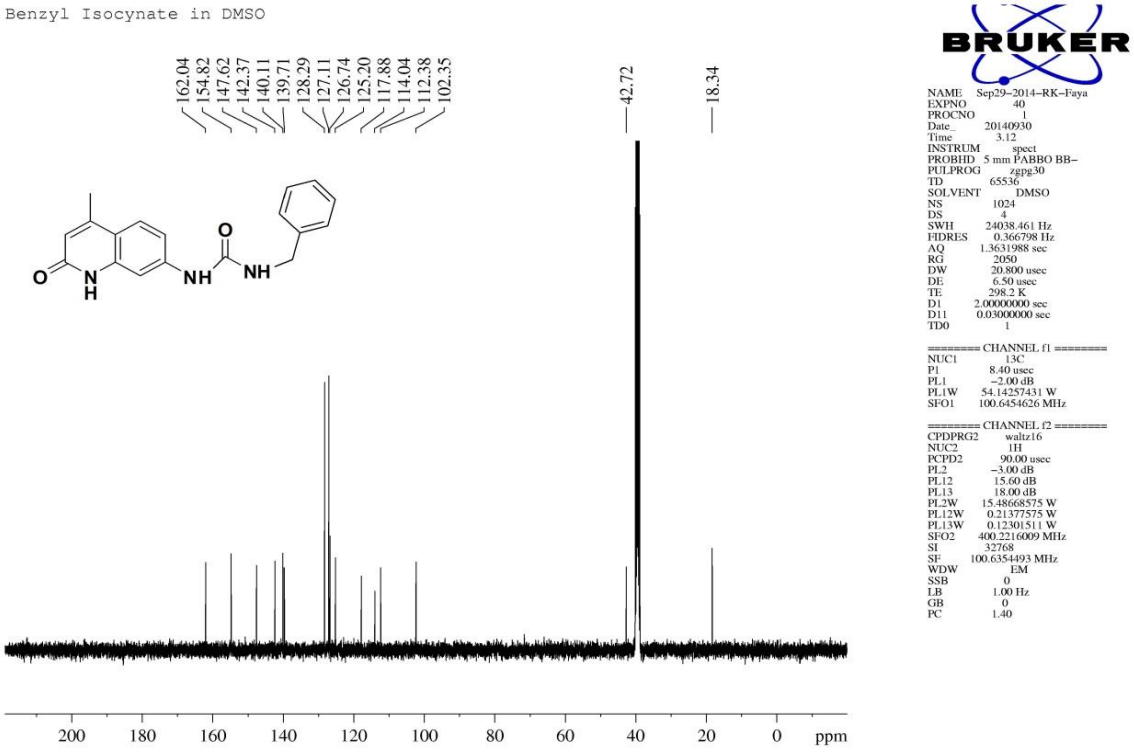
Spectrum 20: ^{13}C NMR spectrum of compound **5g**



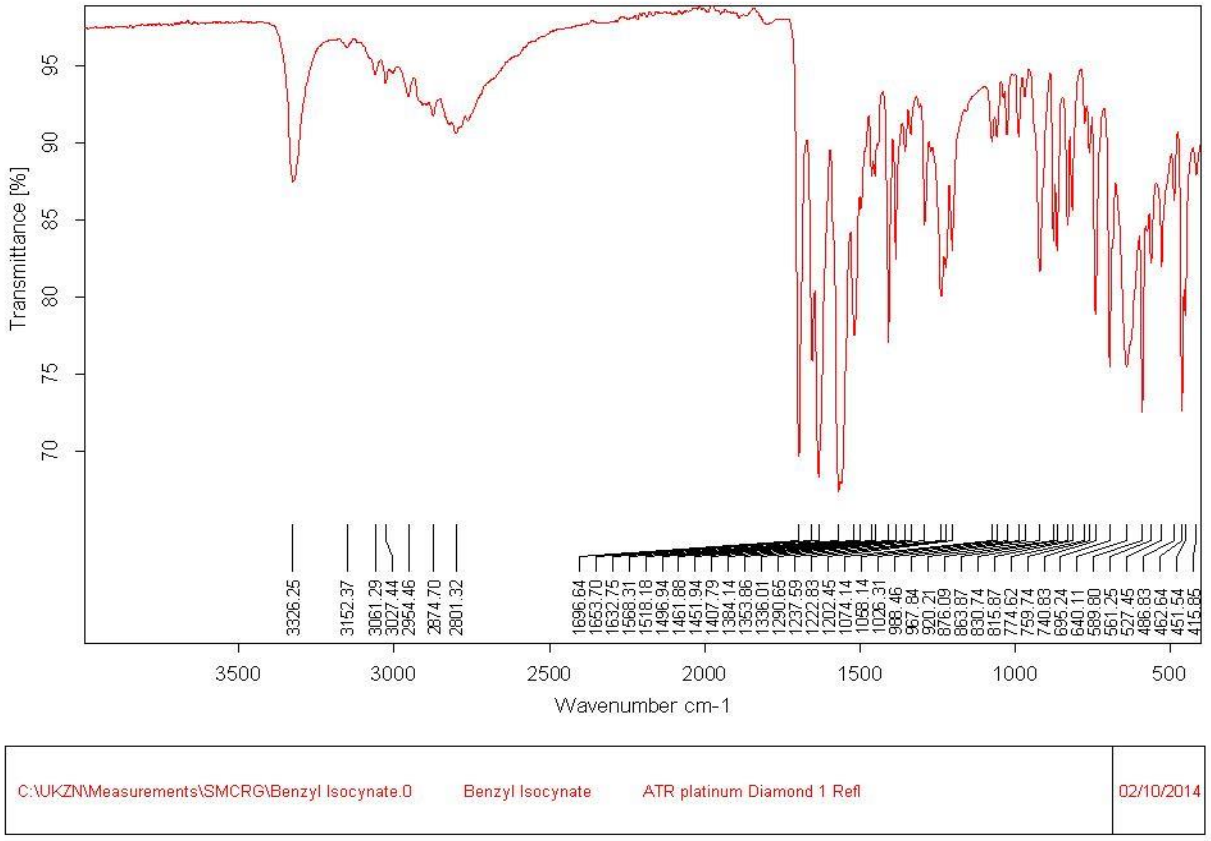
Spectrum 21: IR spectrum of compound **5g**



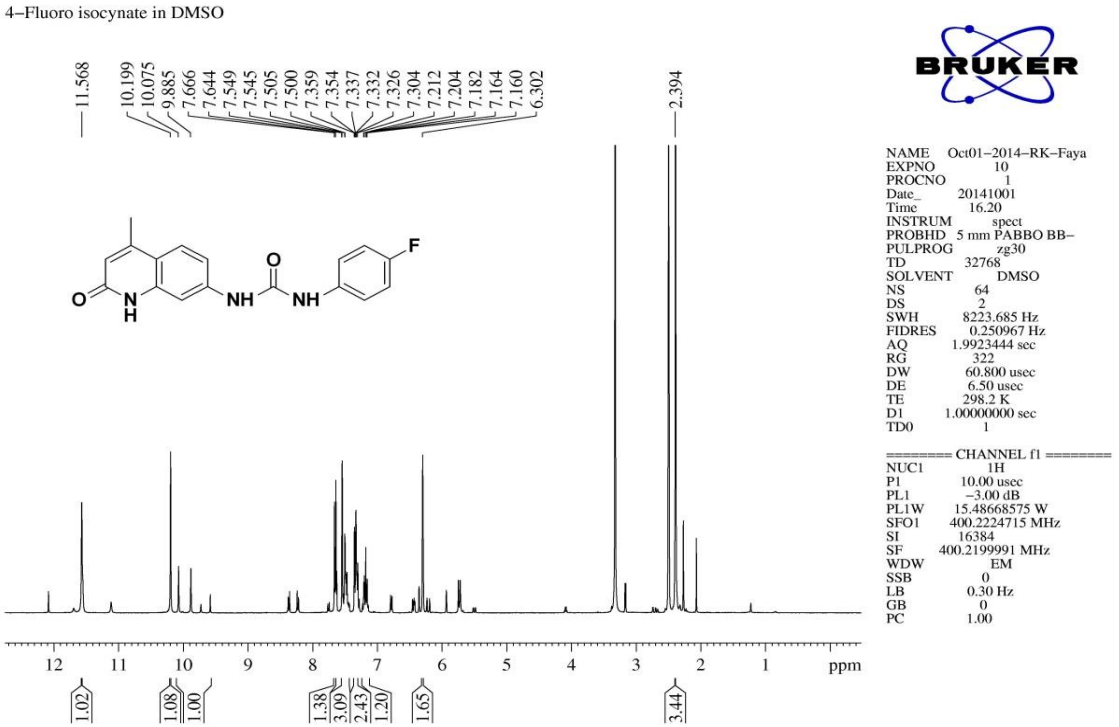
Spectrum 22: ^{1}H NMR spectrum of compound **5h**



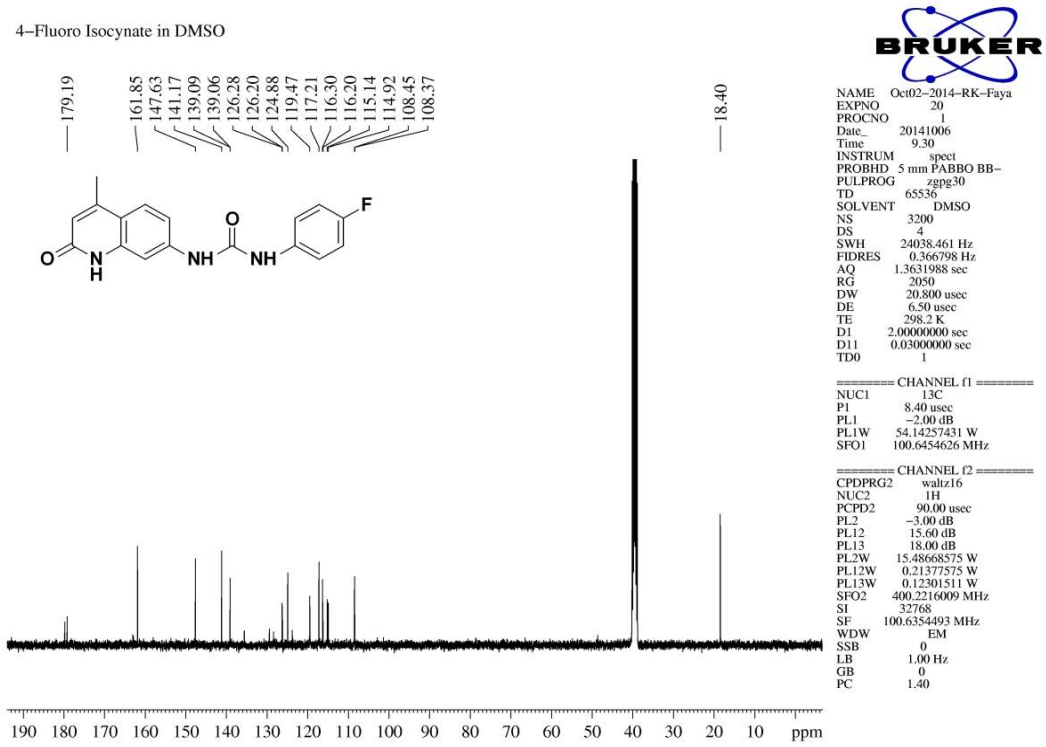
Spectrum 23: ^{13}C NMR spectrum of compound **5h**



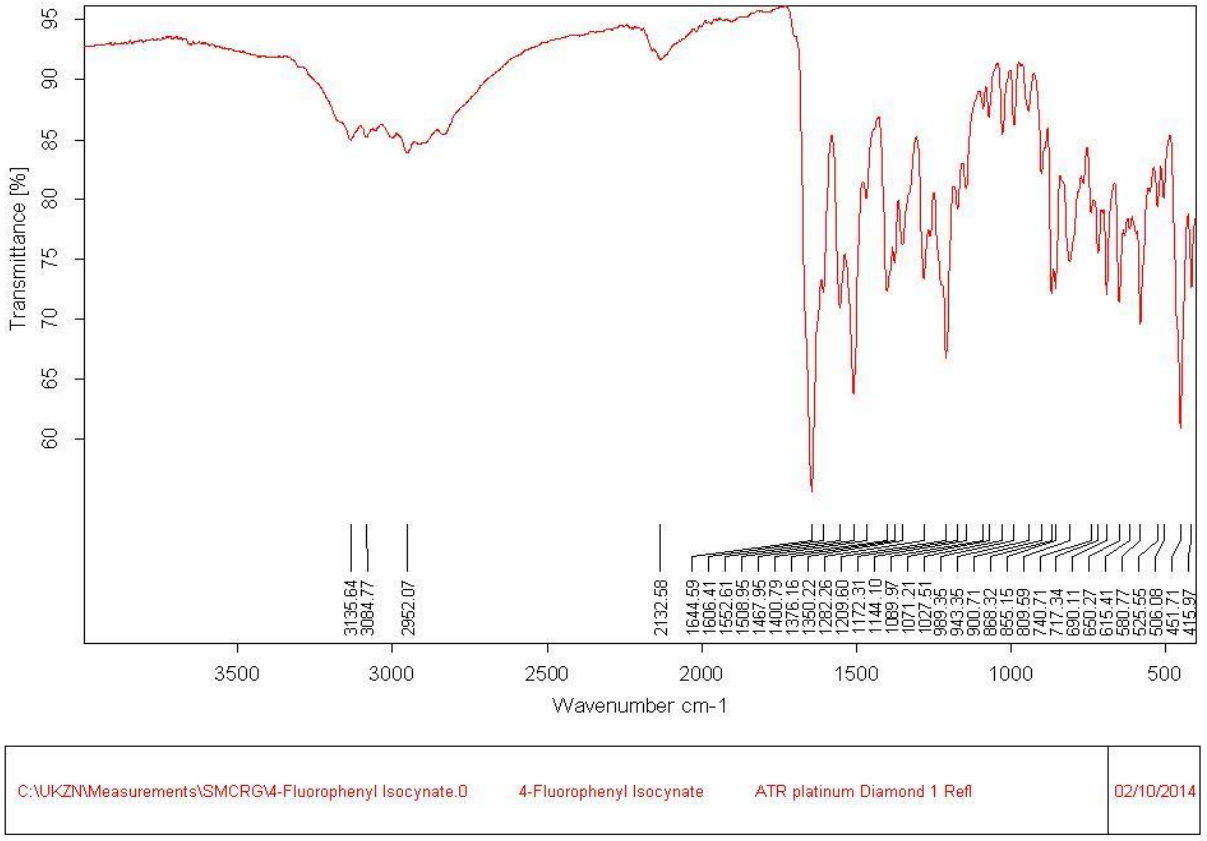
Spectrum 24: IR spectrum of compound **5h**



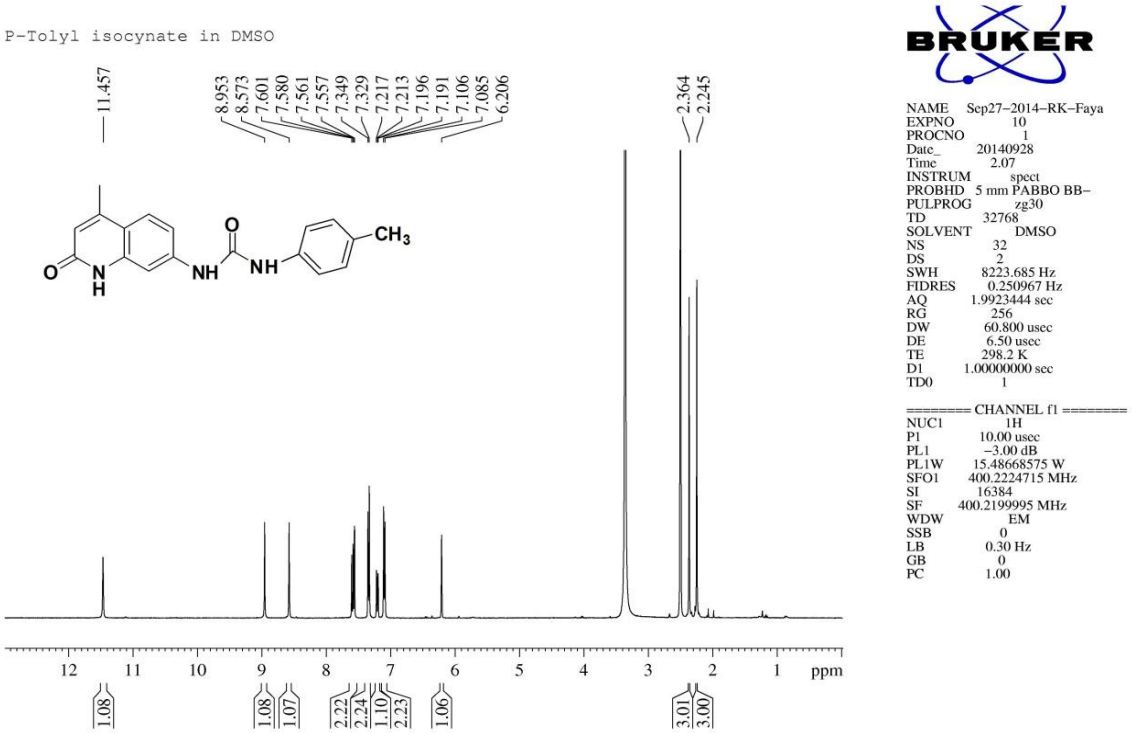
Spectrum 25: ^{1}H NMR spectrum of compound **5i**



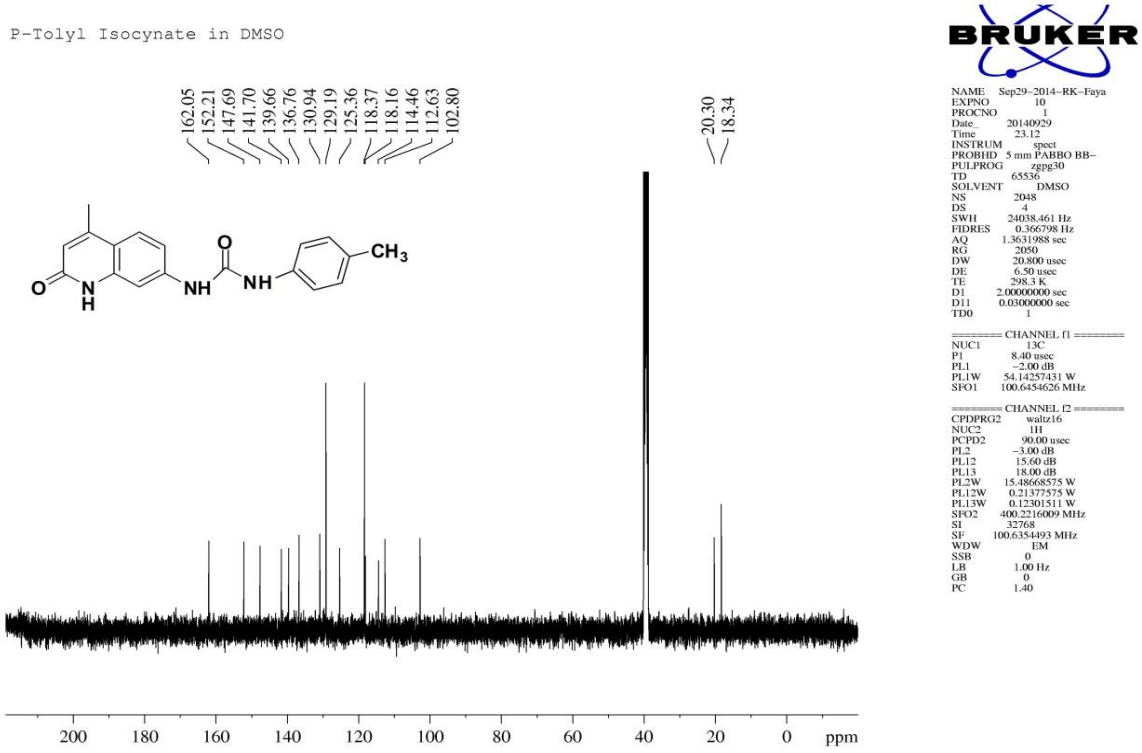
Spectrum 26: ^{13}C NMR spectrum of compound **5i**



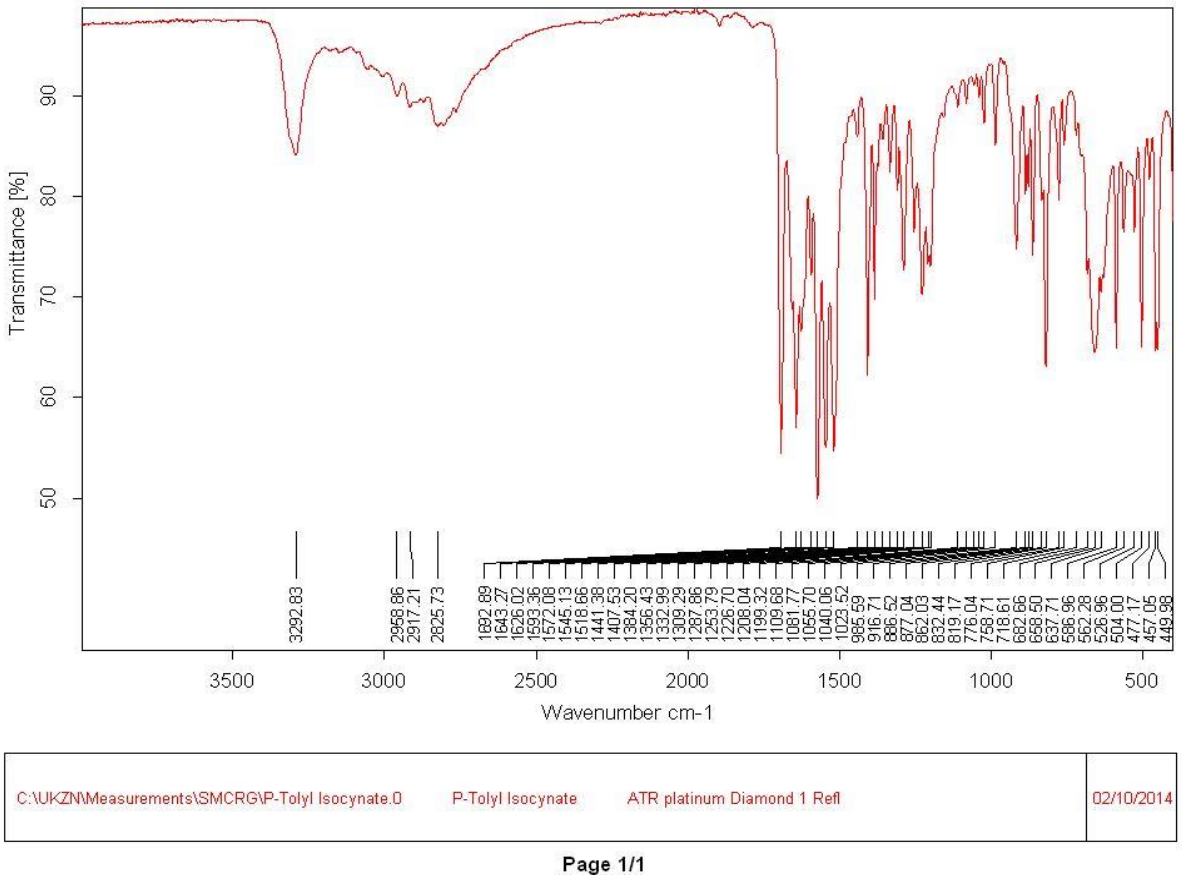
Spectrum 27: IR spectrum of compound **5i**



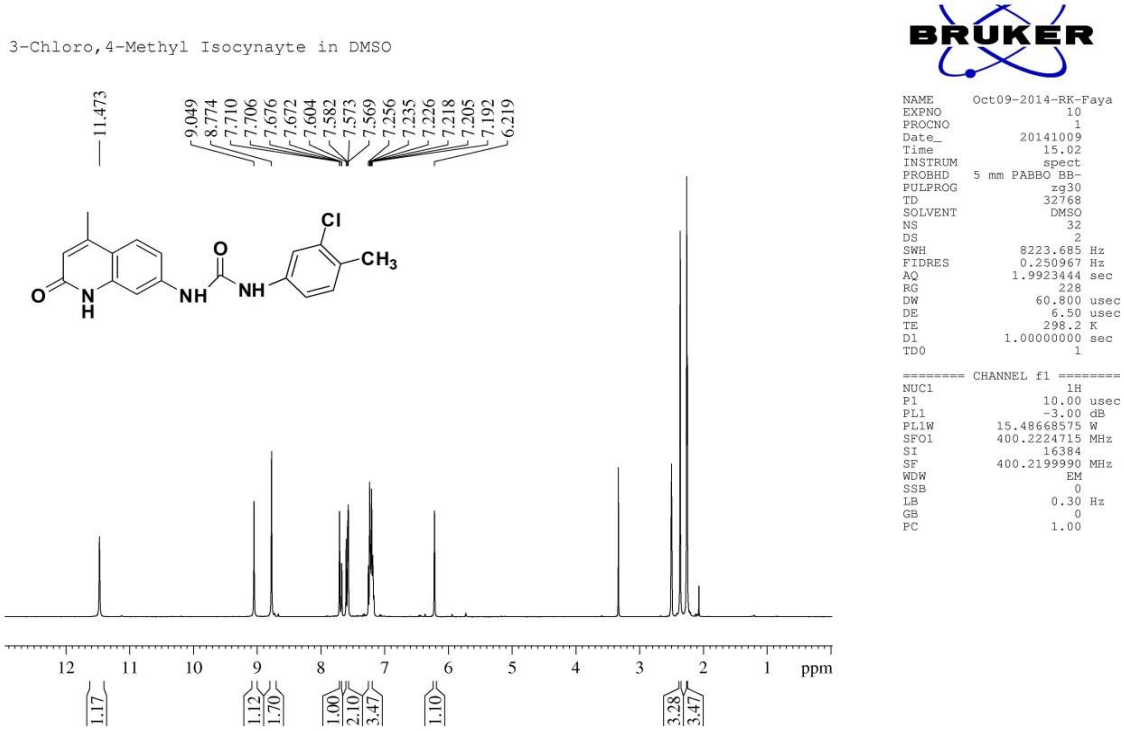
Spectrum 28: ^{1}H NMR spectrum of compound **5j**



Spectrum 29: ^{13}C NMR spectrum of compound **5j**



Spectrum 30: IR spectrum of compound **5j**



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TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH             8223.685 Hz
FIDRES          0.250967 Hz
AQ              1.9923444 sec
RG                   228
DW                60.800 usec
DE                 6.50 usec
TE                298.2 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.00 usec
PL1                -3.00 dB
PL1W        15.48668575 W
SFO1        400.2224715 MHz
SI                 16384
SF          400.2199990 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
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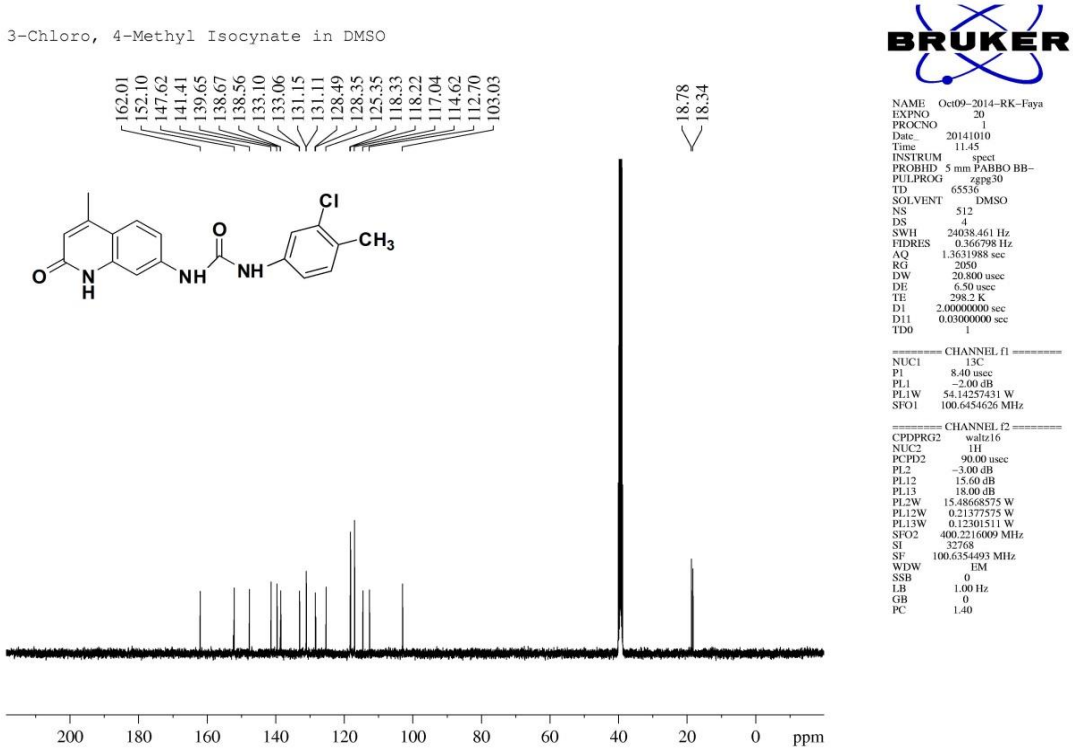
Spectrum 31: ^{1}H NMR spectrum of compound **5k**



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SFO1 100.6454626 MHz

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PL12 15.60 dB
PL13 18.00 dB
PL2W 15.48668575 W
PL12W 0.21377575 W
PL13W 0.12301511 W
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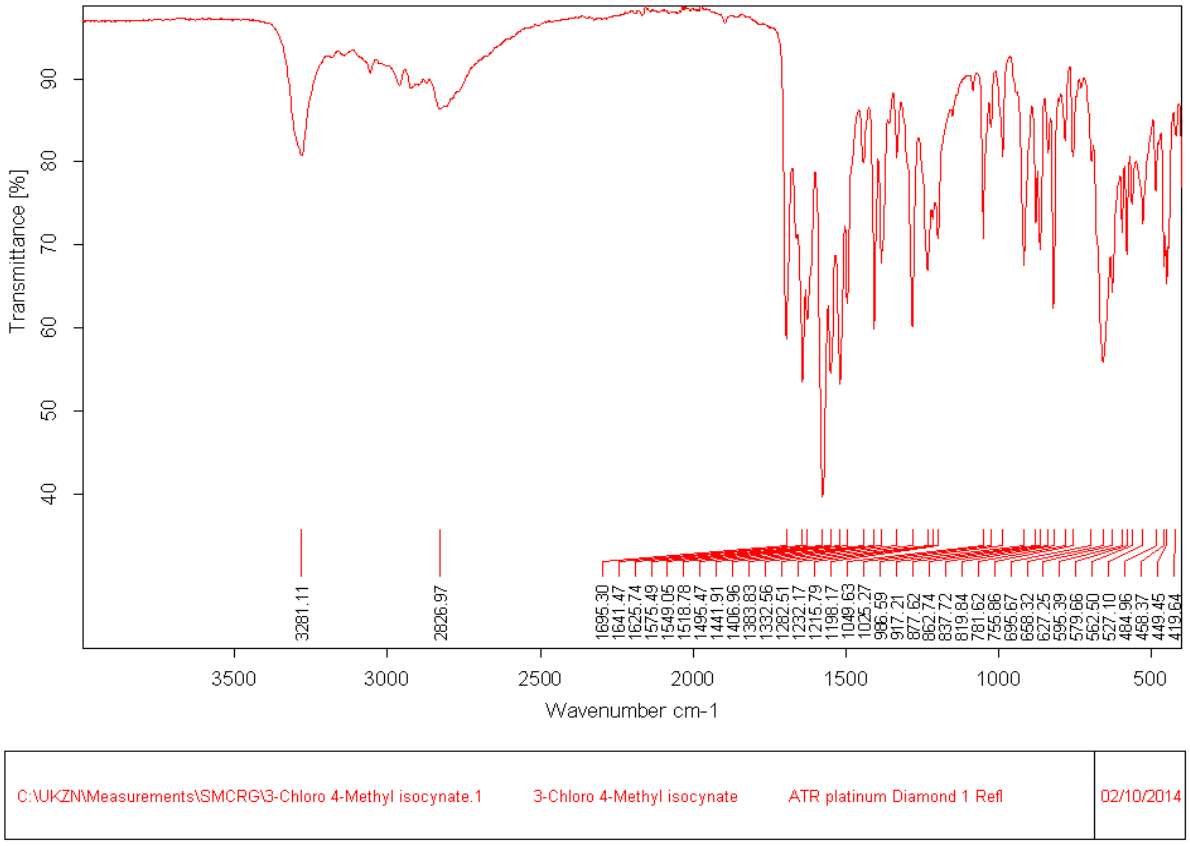
Spectrum 32: ^{13}C NMR spectrum of compound **5k**



Spectrum 33: IR spectrum of compound **5k**