UNIVERSITY OF KWAZULU-NATAL

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVEL 2,4-DIHYDRO-3*H*-PYRAZOL-3-ONE HYBRIDS: A NEW CLASS OF ANTIBIOTICS

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PREFACE

The experimental work described in this thesis was conducted at the Medicinal Chemistry laboratory at the School of Pharmaceutical Sciences, University of KwaZulu- Natal, Westville, South Africa, and the anti-microbial study at Inkosi Albert Luthuli Hospital, Durban, South Africa from April 2013 to December 2018, under the supervision of Dr. Rajshekhar Karpoormath.

This work has not been submitted in any form for any degree or diploma to any tertiary institution, where use has been made of the work of others, it is duly acknowledged in the text.

Kavita Jain  ….....Date 15/08/2019

As the candidates supervisor I agree to the submission of the thesis:

Prof. Rajshekhar Karpoormath…… Date… 15/08/2019

DECLARATION

I, **Kavita Jain**, declare that

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PUBLICATIONS

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1. Jain, K., Cherukupalli, S., Sayyad, N., Singh, S., Aleti, R.R., Karpoormath, R., 2018. Pyrazolone: An emerging scaffold for design and synthesis of diverse pharmaceutically active derivatives, Bio-organic Chemistry.

Other publications

1. Novel series of phenylalanine analogs endowed with promising anti-inflammatory activity: synthesis, pharmacological evaluation, and computational insights. By Palkar, Mahesh B.; Praveen, D. M.; Ronad, Pradeepkumar M.; Viswanathswamy, A. H. M.; Rane, Rajesh A.; Patel, Harun M.; Shaikh, Mohammad Hanif S.; Hampannavar, Girish A.; **Jain, Kavita S.**; Karpoormath, Rajshekhar from Medicinal Chemistry Research (2014),
2. An Insight into Purine, Tyrosine and Tryptophan Derived Marine Antineoplastic Alkaloids By Palkar Mahesh B; Rane Rajesh A; Thapliyal Neeta; Shaikh Mohammad Hanif S; Alwan Wesam S; **Jain Kavita S**; Karunanidhi Sivanandhan; Patel Harun M; Hampannavar Girish A; Karpoormath Rajshekhar From Anti-cancer agents in medicinal chemistry (2015)
3. Current Perspective of Natural Alkaloid Carbazole and its Derivatives as Antitumor Agents By Shaikh Mahamad Hanif S; Thapliyal Neeta; Rane Rajesh A; Palkar Mahesh B; Faya A M; Patel Harun M; Alwan Wesam S; **Jain Kavita**; Hampannavar Girish A; Karpoormath Rajshekhar. From Anti-cancer agents in medicinal chemistry (2015)
4. A Recent Perspective on Discovery and Development of Diverse Therapeutic Agents Inspired from Isatin Alkaloids. Rajesh A. Rane; Sivanandhan Karunanidhi; Kavita Jain; Mohammad Hanif Shaikh; Girish Hampannavar, Karpoormath, R.; Current Topics in Medicinal Chemistry **2016**, *16* (11), 1262-1289.

Signed: [redacted] Date: 15/08/2019

Dedicated

To

My Husband, Son, and Daughter for

encouragement

My parents for their unconditional love

and my In-laws for support

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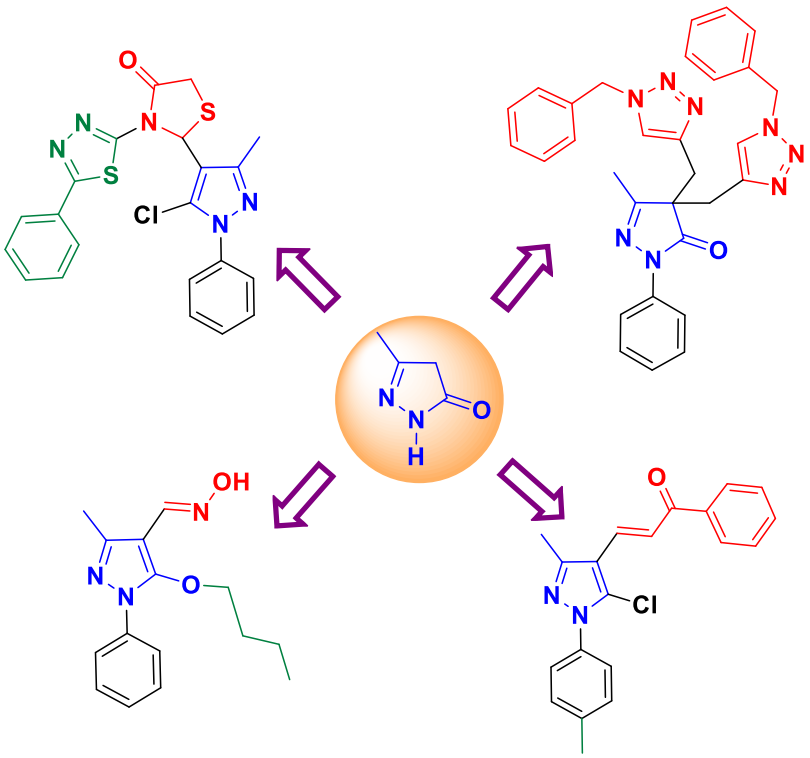
List of abbreviations

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
3D-QSAR	Three Dimensional Quantitative Structure Activity Relationships
3D-QSPR	Three Dimensional Quantitative Structure Property Relationships
AcOH/HOAc	Acetic Acid
AIDS	Acquired Immune Deficiency Syndrome
$CDCl_3$	Deuterated Chloroform
CH_3I	Methyl Iodide
CH_3OH	Methanol
$CHCl_3$	Chloroform
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO-d_6	Deuterated Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
ED_{50}	Effective Dose 50
EIMS	Electron Ionization Mass Spectroscopy
ESI	Electrospray Ionization
Et_3N	Triethylamine
EtOAc	Ethyl Acetate
e.g.	For Example
etc.	Et Cetera
FTIR	Fourier Transform Infrared Spectroscopy
FDA	Food and Drug Administration
HCl	Hydrochloric Acid
HIV	Human Immunodeficiency Virus
HRMS	High-Resolution Mass Spectrometry
IC_{50}	The Half Maximal Inhibitory Concentration
K_2CO_3	Potassium Carbonate
MABA	Microplate Alamar Blue Assay
MBC	Minimum Bactericidal Concentration
MDR	Multidrug-Resistant

MHB	Muller-Hinton Broth
MIC	Minimum Inhibitory Concentration
MOPS	3-(*N*-Morpholino) Propanesulfonic Acid
Mp	Melting Point
Mtb	*Mycobacterium Tuberculosis*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NaOH	Sodium Hydroxide
ND	Not Determined
OD	Optical Density
OHP	Hydroxyproline
PDB	Protein Data Bank
PDGF	Platelet-Derived Growth Factor
$POCl_3$	Phosphoryl Chloride
ppm	Parts Per Million
QSAR	Quantitative Structure–Activity Relationship
RFU	Relative Fluorescence Unit
RIF	Rifampicin
RLU	Relative Luminescent Units
ROS	Reactive Oxygen Species
RT	Room Temperature
SAR	Structure Activity Relationships
STDs	Sexually Transmitted Diseases
SPECT	Single Photon Emission Computed Tomography
TB	Tuberculosis
TBE	Tryphan Blue Exclusion
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
U.K.	United Kingdom
US-FDA	The Food and Drug Administration of the United States
U.S.	United States
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

The rise in multidrug resistance (MDR) pathogenic microbes has emerged as a critical global health burden. To address this problem, the scientific community and Pharmaceutical industries worldwide are focused in developing newer, safer and cost-effective antimicrobial agents. Design and development of potential antimicrobial agents has been one of the focus areas of the Synthetic and Medicinal Chemistry Research Group (SMCRG), UKZN. In continuation of the ongoing research in SMCRG and the need to discover newer antimicrobials, I envisaged to synthesize novel antimicrobial agents containing 2,4-Dihydro-3H-pyrazol-3-one (pyrazolone) as a core pharmacophoric moiety. Pyrazolones are versatile heterocyclic building blocks and is a core scaffold in several marketed drugs such as for analgesic and antipyretic (phenazone, metamizole, propyphenazon, and remifenazone); neuroprotective agent (edaravon); antispasmodic (dipyron); anti-inflammatory agents (famprofazone, phenylbutazone, and remifenazone) and more recently there have been several reports on the pyrazolone analogs as potential antimicrobial agents. In this research work I have synthesized a series of potential pyrazolone hybrids containing substituted 1,3,4-thiadiazole, thiazolidinone, triazole, oximes, and chalcones as antimicrobial agents (Figure 1). The work in this thesis is divided into 7 chapters:



Keywords: Pyrazole-thiadiazole Hybrids; Pyrazoline-oxime; Pyrazoline-chalcone; Molecular hybrid; Antimicrobial agent.

CHAPTER 1. INTRODUCTION

1. Back ground

By 2015, the global life expectancy had improved by 7% (5 years) as compared to 2000. It was observed that females lived up to 73.8 years and males about 69.1 years averaging to 71.4 years. These statistics vary, depending on the income of the country. Low income countries had an average of 50 years while high income countries had 83 years [1-2]. Despite at the verge of recorded improved health conditions globally with the development of new therapies, disease adversity is still the talk of the day. Health calamities have crept into our society posing great threats such as cancer, heart disease, bacterial infections, or some other disorders, which are accounting for increased mortalities yearly. Numerous efforts are being employed to counter these epidemics. The major causes of the diseases in our modern society are the microbial agents such as bacteria and fungi. The risk of microbial infections is increasing rapidly and is one of the major causes of death worldwide [3]. World Health Organization (WHO) estimated that microbial infections accounts for 45% of deaths in Africa and 48% of premature deaths in the rest of the world [4]. Notably, the United Kingdom government carried out global analysis on the consequence of development of an antimicrobial resistance and suggested if the strategies are not laid out to help reverse the deadly trend, it is going to pose a serious health care concern with huge economic impact. The mortality review due to antimicrobial resistance reported about 50,000 losses of lives yearly in the USA and Europe alone. Globally the deaths amount to at least 700,000 every year due to multidrug-resistant (MDR) bacterial infections especially the methicillin-resistant *Staphylococcus aureus* (MRSA) [5].

1.1. Rationale of the study (*Problem identification)*

The rapid increase of MDR bacteria has made microbial infections become a global health problem with great social, economic and ethical consequences. The United States Food and Drug Administration (FDA) has approved 7 main classes of antibiotics for the microbial treatment [6].

1. Penicillins: such as benzyle penicillin and amoxicillin®
2. Cephalosporins: such as cephalexin (Keflex®)
3. Macrolides: such as erythromycin (E-Mycin®), clarithromycin (Biaxin®), and azithromycin (Zithromax®)
4. Sulfonamides: such as co-trimoxazole (Bactrim®) and trimethoprim (Proloprim®)
5. Tetracyclines: such as tetracycline (Sumycin®, Panmycin®) and doxycycline

(Vibramycin®)

6. Aminoglycosides: such as gentamicin (Garamycin®) and tobramycin (Tobrex®)
7. Fluoroquinolones: such as ciprofloxacin (Cipro®), levofloxacin (Levaquin®), and ofloxacin (Floxin®)

Notably, fluoroquinolones was the last class of antibiotics discovered for treatment of bacterial infections in 1987 [7]. Since then no new class of antibiotics has been developed. This void in discovery has modelled a significant therapeutic consequence in treatment of the infections caused by MDR bacteria. Researchers globally are working tirelessly to develop antibiotic to fight these atrocious infections. Currently FDA has reinstated the modified vision for already existing class of drugs for treatment of antimicrobial resistance. The drugs approved by FDA with their respective timelines are as follows; Ceftobiprole (Zevtera, Mabelio**),** a new cephalosporin derivative; 2010, Dalbavancin (Dalvance, Xydalba) Aminoglycosides derivative; May 2014, Oritavancin (Orbactive**)** Aminoglycosides derivative ; August 2014, Tedizolid (Sivextro) Oxazolidinones derivative (third antibiotic);2014, Ceftolozane-tazobactam (Zerbaxa) Cephalosporins combined with β-lactamase; December 2014 and Ceftazidime- avibactam (Avycaz), Cephalosporins derivative; February 2015 [8]. Regardless of drugs that were reinstated, developments of resistance to antimicrobial agents are growing at an alarming rate. There is an enormous requisite to develop new antibacterial pharmacophore to fight against boundless MDR microbes.

1.2. Aim and objectives of the study

Aim

To develop more effective and safer medicinally privileged 2,4-Dihydro-3*H*-pyrazol-3-one (pyrazolone) scaffold based potential and novel molecules to fight against drug resistance to antimicrobial agents

Objectives

1. How modifications of using bioisosteric replacement will affect inhibitory activity against microbial agents.
2. Biological evaluation of the novel synthesized compounds against different bacteria and fungi.
3. How the proposed compounds will affect the microbes biologically and physiologically at molecular level?
4. Optimization and expansion of obtained knowledge in the field of antimicrobial research of novel pyrazolone based compounds.
5. Structural elucidation of the novel compounds by Mass Spectrometry (MS),

Infrared (IR), 1D and 2D Nuclear Magnetic Resonance (NMR) techniques and obtain crystal structures of the synthesized compounds for single X-Ray crystallographic analysis.

1.3. Methodology

Synthetic approaches of substituted 2,4-Dihydro-3*H*-pyrazol-3-one are of special interest. This gives us an opportunity to design different novel pyrazolone chemical entities for antimicrobial based structure activity relationship (SAR) studies through the diversity oriented synthesis.

1.3.1. Synthetic strategy

The outline below highlights the methods used to prepare the main pyrazolone hybrids of the research project. The compounds were synthesised via pyrazolone, thiadiazole, triazole, oximes and chalcones using carboxylated pyrazolone as intermediate. The preparatory methods were as follows

- *Thiadiazole* by condensation and cyclization of thiosemicarbazide with derivative of benzoic acid or benzaldehyde.
- *Formylpyrazolone* by carboxylation of pyrazolone using Vilsmeier–Haack reaction.
- *Thiazolidinone* by cycloaddition of pyrazole and thiadiazole by thioglycolic acid.
- *Propynyl pyrazolone by r*eaction of propargyl bromide over 2,4-Dihydro-3*H*-pyrazol-3- one.
- *Triazole* by addition of benzyl azide over propynyl pyrazolone by click chemistry.
- *Alkoxy formyl pyrazole* by substitution of a ketonic group of formyl pyrazolone by alkoxy group with different aliphatic alcohols.
- *Oximes* by addition of hydroxyl amine on alkoxy formyl pyrazole.
- Chalcone by aldol condensation of alkoxy formyl pyrazole and acetophenone.

1.3.2. Susceptibility Testing

The synthesised compounds were screened for their *in vitro* antimicrobial activity by Minimum Inhibitory Concentration (MIC) assay method.

1.4. Novelty and significance of study

Pyrazolone and its derivatives occupy a very significant position in medicinal chemistry. These compounds have exhibited antimicrobial [9], anticancer [10], anti-tubercular [11], anti- inflammatory [12], antidepressant [13], antihypoglycemic [14], nticonvulsant [15], anti-cardiovascular disease [16], antipyretic [17], antihelmintic [18], herbicidal [19] and antioxidant properties [17]. They have also demonstrated great importance in dye industries, as well as ligands in metallic catalysis [20]. Pyrazolone ring makes up the core

moiety in a number of leading marketed drugs such as Edaravon, Phenazone [21], Metamizole [22] etc. and it is an integral part of biologically active natural products such as nostocine from Nostoc *spongiaeforme*, Fluviols (A–E) from *Pseudomonas fluoresences* [23]. Equally, they have also demonstrated to be an effective and versatile pharmacophore in the pharmaceutical industry. Notably pyrazolone is a validated pharmacophore that can serve as a promising potential candidate in the development of antimicrobial drugs replacing the current regime which is characterised by long dose regimen, high-priced and ineffectiveness due to MDR. The existing antimicrobial resistance (AMR) drug incidence has necessitated an urgent need to develop potential antimicrobial drugs, which are cost effective, more potent, and of short term treatment. Pyrazolone would be a promising scaffold in the development of antimicrobial agents because of its diverse character, high potency, low cost, easy availability, simple handling and well- established toxicity profile.

2.0. Thesis introduction

2.1. Microbes

Microbes are microscopic organisms distributed all over the surface of earth. They are found in all forms of ecosystem such as biosphere, lithosphere and atmosphere. These minute organisms have different sizes and characteristics and are grouped into five main categories; viruses, algae, protozoa, fungi and bacteria [24].

2.1.1. Viruses

A virus is a minute infectious agent. It can only replicate inside the living cells of other organisms. Viruses are capable of infecting all forms of organisms [25]. Viruses have exhibited both beneficial and harmful implication on human being. Beneficial virus have been useful in the study of genetics, virotherapy, material science and nanotechnology and development of biological weapons for defence purposes, whereas harmful viruses have caused common human illnesses such as the influenza, common cold, chickenpox, and cold sores. Viruses are also known to be on the pinnacle of heinous diseases such as Ebola, acquired immune deficiency syndrome (AIDS), avian influenza, and severe acute respiratory syndrome (SARS) [26-27].

2.1.2. Algae

Algae are eukaryotic, chlorophyll containing organisms. The size of algae ranges from microscopic and unicellular to multicellular. The categorization of the algae is based on their colour and are grouped into three categories; Red algae (Rhodophyta), brown algae (Ochrophyta) and green algae (Chlorophyta). Algae has no harmful effects rather they have been known to confer great benefits to health of human by reducing risk of cancer and obesity. Anti-inflammatory, antioxidant and reduction of cholesterol levels in the body are

some of the therapeutic benefits that have been reported [28].

2.1.3. Protozoa

Protozoa are unicellular eukaryotic microorganisms. Notably some protozoa exhibit usefulness to humans in maintaining an ecological balance by hampering increase in bacterial populations. Nevertheless, many protozoa have been known to cause illnesses in animals and humans. For instance, the plasmodium species cause malaria, which is currently one of major remerging health concern in epidemic areas infecting millions. Other diseases caused by protozoa include: amoebiasis, diarrhoea, trypanosomiasis, leishmaniasis, tricho moniasis, toxoplasmosis, balantidial dysentery [29].

2.1.4. Fungi

Fungi are unicellular or multicellular, or syncytial spore-producing eukaryotic organisms that have a nucleus and a cell wall built with chitin [30]. Like other microbes, fungi are beneficial to humankind. Many species of fungi are used in pharmaceutical industry to manufacture drugs such as antibiotics, vitamins supplements, anti-cancer and cholesterol-lowering drugs. Edible fungi such as straw, oyster, shiitakes, truffles, milk and black trumpets mushroom are consumed worldwide and making mushroom farming and processing industry one of the largest food industries in the world [31]. Although some fungi have exhibited tremendous benefits to human, other species are pathogenic to plants, animals and human. Certain fungi infections are difficult to cure, because some species can survive in very severe environments and re-infecting the organisms. Athlete's foot, yeast infection jock itch, ringworm, sporotrichosis, mucormycosis, et-cetera are some of the common fungal infections [32].

2.1.5. Bacteria

Bacteria are microscopic, single-celled prokaryotic-organisms (**Figure 1**) that are found almost ubiquitously. Fossil evidence has revealed that bacteria are direct descendants of the first organism that lived on earth traced back as far as 3.5 million years ago. A gram of soil can typically contain about 40 million and a millilitre of fresh water about one million bacterial cells. There are approximately 5×10^{30} bacteria on earth [33]. The size of bacterial cell is about one-tenth the size of eukaryotic cell and is typically between 0.5 – 5.0 micrometres in length.

Classification and reproduction of bacteria

One of the basic criteria that have been used to classify bacteria is their shape. Notably bacteria only poses three basic shapes namely rod-shaped (bacilli), spherical- shaped (cocci), or helical-shaped (spirals) [34] (**Figure 1**). Structural features such as cell wall are significant in distinguishing the bacteria. The bacteria that have a thick cell wall and able to

retain a violet gram stain are referred as gram-positive and gram-negative if they have thinner cell wall and are thus unable to retain gram stain but instead retain a counter stain which is red in colour. Gram-positive bacteria are on the pinnacle of drug impenetrable thick cell wall [35]. Bacterial cells are capable of self-reproduction by binary fission. To achieve these asexual reproduction, they require plenty of food, favourable temperature and moisture, very vital condition for growth and spreading [36]. **Figure 1** shows the bacterial cell anatomy and shapes.

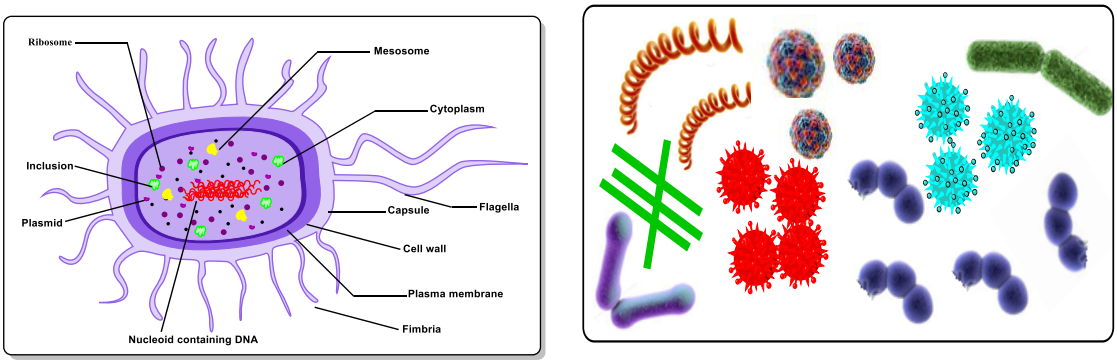


Figure 1. Bacteria cell anatomy and basic shapes of bacteria. [36]

Advantages of bacteria

Although a negative connotation is attached to the word, bacteria encompasses a significant role in certain important biological functions both in living organisms and the atmosphere. For example, some bacteria play very significant role in protein synthesis in leguminous plants, some helps in the digestion in humans and other animals and fabricate vitamins. Additionally, they also play an important role in maintaining balance between organic and inorganic compounds in an ecology system. They are also known to aid the immune systems to fight harmful pathogens in organisms including humans [37].

Disease caused by bacteria

Notably, only few strains of bacteria are responsible for causing infections in organisms including human. A bacterial infection is a rapid propagation of a harmful strain of bacteria in the any area of the body [38]. Some of the diseases caused by bacteria includes leprosy, meningitis, cholera, pneumonia, diphtheria, tuberculosis, whooping cough and food poisoning. Bacterial skin infections such as cellulites, folliculate, impetigo, boils**,** etc. are usually caused by gram-positive strains of *Staphylococcus* and *Streptococcus* [39]. Bacteria are the major cause of food poisoning found mainly in raw or undercooked meat (particularly poultry), unpasteurised milk, untreated water, unhygienically prepared or handled food and contaminated food. The common strains of bacteria that cause poisoning are *Escherichia coli*, *Campylobacter jejuni, Clostridium botulinum***,** *Listeria monocytogenes, Salmonella, Vibro cholera* etc. [40]

Becteria are known to be responsible for sexually transmitted diseases (STDs) such as gonorrhea, syphilis, vaginosis and trichomoniasis, that may cause damage to the reproductive organs [41]. Bacterial meningitis [42], respiratory tract infection [43], otitis media (infection of middle ear) [44] urinary tract infection (UTI) [45], etc. are other types of bacterial infections.

2.2 Antimicrobial agent

Antimicrobial is a Greek word divided into three parts; anti (against), mikros (little) and bios (life). In simple terms antimicrobial can be defined as any chemical entity or substance that can hamper the life or cause death of microorganisms. The antimicrobial agents can be classified in two ways. The first class is based on the microorganisms they work on. For instance, agents that act upon bacteria are called antibacterial, fungai; antifungal, protozoa; antiprotozoal etc. The second class is based on the mode of action. For example the agents that used for totally exterminate microbes are called microbicidal and those that just slow down or prevent reproduction and spreading are called biostatic [46].

Medically, antimicrobials are classified as antibiotics, antiseptics and disinfectants. Antibiotics are used for treatment inside the host and can be administered orally or via injections. Examples of antibiotic drugs are penicillin and its derivative and linezolid [47]. Antiseptics are used to prevent infections on external surfaces of living tissues e.g. wounds i.e. hydrogen peroxide and boric acid [48]. Disinfectants are used on the surface of non-living objects to sterilize and prevent infections.

From their inception, antimicrobials are the only efficient means of treatment to contain and control contagious diseases which are the foremost cause of morbidity and mortality globally. However, inapt and extensive use or misuse of antibiotics has given rise to the development of resistant strains. Antibiotic resistance is very serious incidence in the health section. A tenacity of problem has become a nightmare for chemist and scientist globally. This serves as message to the scientific community to develop new antimicrobial with high efficacy.

2.2.1 History of antimicrobials

The development of antimicrobial agents is divided in three phases (epoch). The first epoch began in 1619 where they tremendously relied on alkaloids for treatment. Treatment for diseases such as malaria was done using cinchona bark extract. In places like South America roots from Ipecacuanha were used to treat dysentery which proved to be effective during that time. Alkaloids, quinine and emetine were the only available therapeutic agents for treatment at the time [49].

1909 ushered in the second phase, a synthetic era the first compound to be synthesised and used as an antibacterial was Salvarsan by Paul Ehrlich. Salvarsan was used as an antibacterial against

the bacteria that cause syphilis. The handling and administration of Salvarsan was cumbersome, and this could be the reason why it could have exhibited levels of toxicity in the human body [50]. In 1932, Klarer and Mietzsch changed the face of medicinal science by suggestion that antibiotics can selectively be toxic to bacteria and not the human body by discovering a potent antibacterial candidate called prontosil [51]. Further exploration of prontosil was carried out and Domagk discovered that the drug would effectively be used for hemolytic *Streptococcus i*nfections [52]. It was also established by, Colebrook and Kenny, in 1935, that drug portrayed comparable effectiveness in puerperal fever. In 1935 Trefouel proposed that the activity of the prontosil could be attributed to the release of *p*-aminobenzene sulphonamide and the notion was validated by Fuller in 1937 [52]. Sulphanilamide moiety was screened *in vitro* and it showed inhibitory activity against streptococci. This discovery led to further development of new sulphonamides derivatives. In 1938, sulphapyridine was developed, the first drug that could effectively treat pneumococcal pneumonia [53]. Furthermore, Sulfathiazole and Sulfadiazine were developed. The drugs exhibited low side effects of nausea and cyanosis as compared to the former drugs yet the retained and exhibited an enhanced antibacterial activity. To-date, further research is still underway with intent to develop safer and more effective potent antibiotic agent.

The third phase is referred to an antibiotic epoch. This is an era that used intermicrobic antagonism, where the genes of bacterial origin; living culture or extracts could be used to generate agents with therapeutic benefits like antibacterial. One of the first innovators of this phenomenon was Pasteur in the 1870s. Pycocyanase was one of the renowned products of intermicrobic antagonism. Further investigation led to extraction from species of penicillium and Aspergillus, however antibiotic obtained were not quantifiable for therapeutic use. To find a specific organism that was potent against pyogenic cocci, Dubos in 1939, discovered tyrothrcycin from Bacillus breve [54]. Tyrothrcycin showed very good activity *in vivo* studies in mice however it was toxic to human. This resulted in solely focusing on investigating antibiotics that were naturally produced. The team responsible for the investigation was Howard W. Florey and his colleagues. Amongst the substance that was listed to work on was penicillin. Intermicrobic antagonism research was carried out under umbrella called “antibiotic” and the word was coined by Waksman in 1942 [55]. The problem with the definition was, it was restricted to substance formed by microorganisms which are antagonistic to the growth or proliferation of other microorganisms when in high dilution. However, drawn as back to 1889, J. A. Vuillemin, had termed this research as antibiosis, a scope that covered wide spectrum in general. The current epoch of antibiotic can be traced back as far as 1940s, when a solid profile of the properties of the extracts obtained from *Penicillium notatum* was established, the work

accredited to Sir Alexander Fleming in 1929 [56]. A fuller report conducted by Abraham et al provided solid evidence of the successful clinical trials. It was until a few years later, that penicillin was purified, and its structure fully characterized, and massive production accomplished. This saw a substantial decrease in mortality by bacterial infections [57]. The discovery of these antibiotics came in handy because in commencement of 1940s, reported cases of the resistance of sulphonamide on *gonococci*, *pneumococci*, and hemolytic *streptococci* was widely spreading. Likewise, it would have been a set back to the earlier situation of 1935 where bacterial diseases were prevalent and pathogenic. Today all antibiotics in clinical use are products of microorganisms.

The instigation of further extensive research in the development of antimicrobial was prompted by the discovery of salvarsan, prontosil, and penicillin. Afterwards, numerous scaffolds of potential antimicrobial activity such as chloramphenicol, tetracyclin, erythromycin, streptomycin etc. have been developed. Hence forth research epoch in field of antibiotic development has continued and there has been an ongoing "race" between scientists in discovery of new drugs [58]**.** A summarized historical timeline of the development of antimicrobial agents is shown in **Table 1** and structures of main class of antimicrobials are in **Figure 2.**

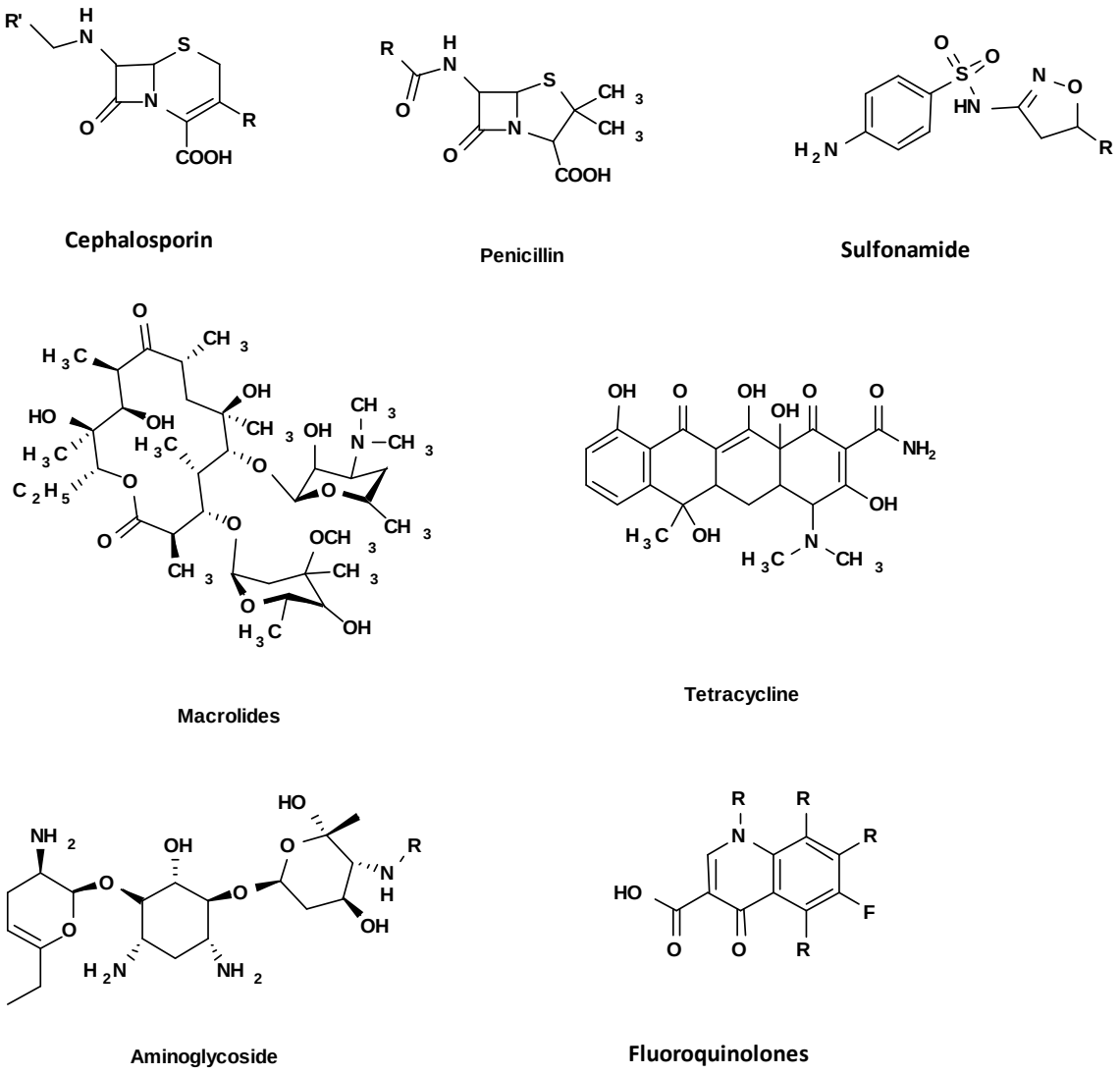


Figure 2. Structures of main class of antimicrobials

Table 1: Dates of discovery of distinct antimicrobial drugs [59].

S. No.	Year of discovery	Name of drug
1	1928	Penicillin
2	1932	Sulfonamide
3	1943	Streptomycin
4	1945	Bacitracin
5	1946	Nitrofurans
6	1947	Chloramphenicol, Polymyxin
7	1948	Chlortetracycline, cephalosporin
8	1950	Pleuromutilin
9	1952	Erythromycin, Isoniazid
10	1954	Vancomycin, Streptogramin
11	1955	Cycloserine
12	1956	Novobiocin
13	1957	Rifamycin
14	1959	Metronidazole
15	1961	Nalidixic acid, Trimethoprim, Lincomycin, Fusidic acid
16	1969	Fosfomycin
17	1971	Mupirocin
18	1976	Carbapenem
19	1977	Oxazolidinone
20	1981	Monobactam
21	1987	Daptomycin

2.2.2 Mechanism of action

The mode of action of antimicrobial drugs is classified into five main categories. The action vary from drug to drug depending on the structure of the organism being targeted as shown in **Figure 3** [60-61].

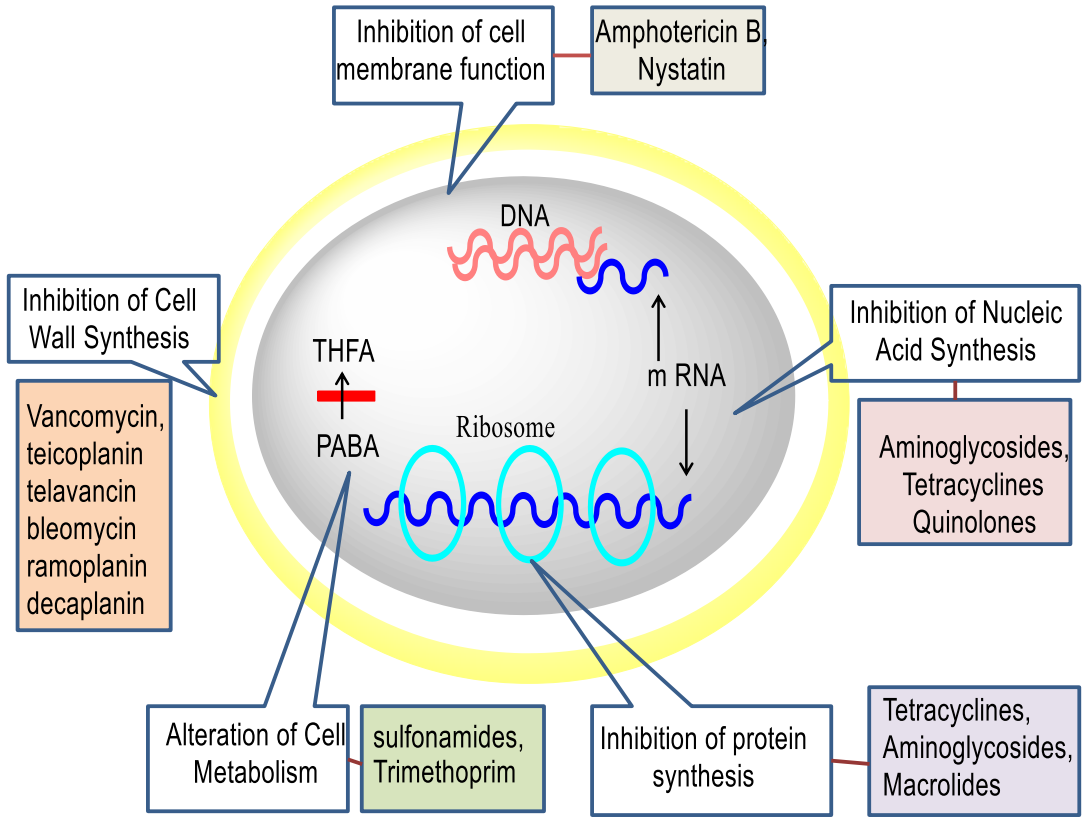


Figure 3. Mechanism of action of antimicrobials [60].

2.2.2.1 *Inhibition of cell wall synthesis*

The antimicrobial activity is achieved by specifically hampering the synthesis of the cell wall of the microbes. The cell wall structure of microbes such as bacteria has deposit of peptidoglycan layer integrated into it. This is a very important part of the cell as it is the outermost and principal section of the wall. They are two classes of antibiotics that work by inhibiting the synthesis of the cell wall. The first class is β-Lactam antibiotics which includes drugs such penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. [61]. These drugs achieve their mode of action by impeding the formation of the peptidoglycan layer of bacterial cell walls consequently killing bacterial. The second class is referred to as glycopeptides that include drugs such as vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin [60]. The mode of action of these antibiotics is inhibition of cell wall biosynthesis in vulnerable microbes by impeding peptidoglycan formation. The drugs do not affect the human cell because it does not have a cell wall. The

inhibitory activities are selective to pathogens and do not cause any harm to the human cells [60].

2.2.2.2 Inhibition of nucleic acid synthesis

Certain antimicrobial agents aim at disrupting the transcription of RNA which makes a template which is later used for proteins synthesis. The transcription process involves initiation, elongation and termination and the antimicrobial agents can interfere with any step in the process [61]. For example, drugs such as rifampicin targets deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase that initiate the transcription of RNA hereby impeding the process. Other antimicrobial agents inhibit the replication of DNA like RNA transcription, the process also proceeds via initiation, elongation and termination. The antimicrobial agents can inhibit any the step in the process. For example, quinolones impede DNA synthesis by inhibiting the twining of DNA strands. Few drugs bind with subunits of RNA and inhibit nucleic acid synthesis. Example; aminoglycosides, tetracyclines and quinolones [61].

2.2.2.3 Inhibition of cell membrane function

The interior of the cell wall is made of the plasma membrane or cell membrane which consists of lipid, protein, and lipoprotein. The cell membrane is responsible for keeping the interior of cell and outside environment separate. The function of the cell membranes is to permeably regulate the transfer of materials between the cell and surrounding. The other functions include cell adhesion, ion conductivity, and cell signaling. Cell membranes serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton. The lyses of the plasma membrane will lead to exposure of the cell metabolic processes making the microbes susceptible and causing the loss of polarization and its integrity. Vital biological processes of the cell such as protein, DNA and RNA synthesises would be exposed and inhibited easily causing subsequent killing of the bacteria [62]. For example, lipopeptide antibiotic such as daptomycin, would bind to the cell membrane causing it to lose polarization disrupting the membrane's integrity resulting in impeding cell process such as protein, RNA and DNA formation consequently killing the microbes. Other antibiotics such as polymyxins interact with cell phospholipids in the membrane disrupting structure resulting into the death of bacterial cell [62]. Example; amphotericin B, nystatin.

2.2.2.4 ***Alteration of cell metabolism***

Antimetabolites are substances that exhibit structural similarity with the metabolites they inhibit. The Antimetabolites will normally pose a toxic repercussion stalling the growth or dividing of the cell. Antimetabolites have equally served as antibiotics and are classified into three main categories; antifolates, pyrimidine derivative and purine derivative. Antifolates

interfere with folate biosynthesis disrupting formation of DNA and RNA. For instance, methotrexate a folic acid derivative mimics the structure of folic acid and targets the dihydrofolate reductase, inhibiting its enzymatic activity and thus halting the production of tetrahydrofolate. A deficit in tetrahydrofolate hinders the formation of DNA, RNA and proteins. Tetrahydrofolate is an integral part in the production of purine and pyrimidine and basic units in DNA and RNA. The second class of antimetabolite are pyrimidine derivatives which exhibit structural similarity with metabolic pyrimidines a very essential base of cytosine (C), thymine (T), and uracil (U) nucleic acid. The pyrimidine derivatives interrupt formation of metabolites inhibiting the production of DNA and RNA. Similarly, the third class of antimetabolites are purine derivatives which exhibit structural similarity metabolic purine an integral base of adenine and guanine nucleic acids. Like the pyrimidine antimetabolites, the purine antimetabolite interrupts the production of DNA, RNA and proteins. For example, azathioprine is used to suppress organ rejection after a transplant which is achieved preventing DNA synthesis in lymphocytes [63].

2.2.2.5. *Inhibition of protein synthesis*

The inhibitory activity is achieved by impeding the synthesis of proteins operating at Ribosome level. The Ribosomes of the prokaryotic and eukaryotic cell differ significantly in size, sequence, structure and protein to RNA ratio. This is very vital phenomenon as bacterial ribosome can selectively be inhibited and not their human counterpart. Generally, like all other biological processes, the prokaryotic mRNA translation into proteins proceeds via initiation, elongation (including aminoacyl tRNA entry, correcting, peptidyl transmission, and ribosomal translocation) and termination. The mode of action of antibacterial agents would target any of the biological stages highlighted. Although the mechanism is not well established, it is believed that at early stage drugs such as rifampicin inhibits prokaryotic DNA transcription into mRNA by inhibiting DNA-dependent RNA polymerase by binding its beta-subunit [64] while Linezolid would attack the instigation stage, mainly impeding the synthesizing of the initiation complex [64]. Drugs such as tetracycline and its derivatives would stall the A site on the ribosome, stalling aminoacyl tRNA binding access [64]. Aminoglycosides disrupt the speeding up the of error in formation and instigating untimely termination [64]. Chloramphenicol stall the peptidyl transmission stage of elongation on the 50S ribosomal subunit exhibited both bacteria and mitochondria. Drugs such as clindamycin, aminoglycosides and macrolides, have exhibited characteristic of impeding of ribosomal translocation [64].

2.3. Antimicrobial resistance

Like in many other classes of drugs, the major problem in antimicrobial drugs is development of resistance. Antimicrobial resistance occurs when the microbes can no longer respond to drugs perpetuating the infections. Resistance in bacteria may occur naturally. Certain bacteria may acquire resistance in two ways; 1) genetic mutation 2) transferable from other resistance strains. The major cause of resistance is the excessive or inapt use of antibiotics in humans, animals and agriculture. The over exposure of antibiotics has expedited development of more superbugs and treatment of the bacterial infections is continually becoming a nightmare. Reduction of the efficacy of antibiotics has therapeutic consequences, resulting into routine surgeries such as joint replacements, gut operations and caesarean being carried under a very high risk including death [65]. The **Figure 4** reveals a bacterial overview in the development of resistance.

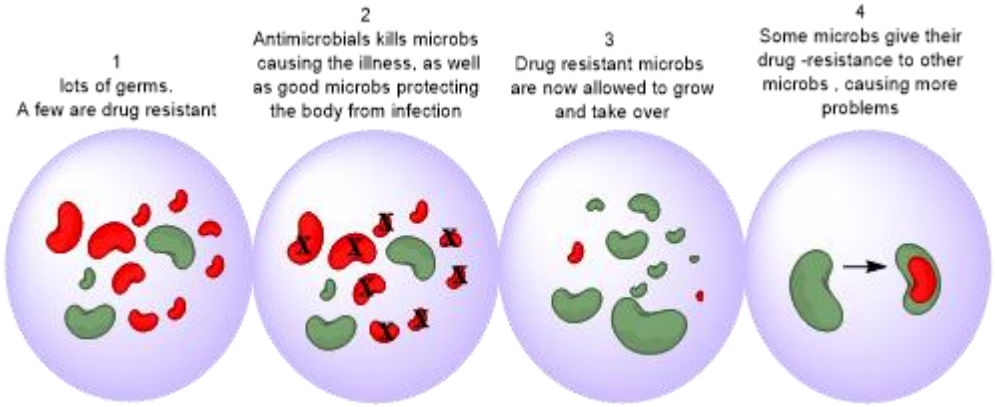


Figure 4. Development of antibacterial resistance via microbes [65].

2.3.1. Historical background of antimicrobial resistance

Numerous studies have been conducted to reveal the historical background of the resistance to antimicrobial. Phylogenetic reconstruction was one of the methods that were employed to understand characteristics speciation of the microbes. The studies revealed that the antimicrobial resistance genes existed naturally way before the era of antibiotics. The structural phylogenic analysis revealed that serine and metallo-β-lactamases, an enzyme accounted for resistance of antibiotics in bacteria emerged about two billion years ago [59]. The bacterial plasmids containing serine β lactamase have existed for millions of years. Studies have further revealed that *Klebsiella oxytoca* and β-lactamases which exhibit close resemblance in phylogeny of housekeeping genes contains genes that evolved millions of years ago in their host. Additional study of clones of β-lactamase obtained from cold seep indicated that the enzyme existed over ten thousands years ago. Based on these facts it can confidently be concluded that the evolution of enzyme is ancient rather than an emergence of modern antibiotic era [59].

The resistance of antibiotic in the modern era observed clinically emerges from the over use of antibiotics. In the late 1930s, cases of resistance from the first effective antimicrobial drugs, sulfonamide derivative, was reported against the *Streptococcus pyogenes* [66]. In 1940s after penicillin was commercially introduced as antibiotic, it was available over the counter before the prescriptions were obligatory. Moreover, the team working on penicillin has identified bacterial penicillinase (β-lactamase), way before therapeutic use of penicillin [66]. During the same time, the London hospital started facing challenges using penicillin as antibiotic due to resistance observed in *Staphylococcus aureus* strain. No sooner had the antibiotic introduced, than the resistant strains emerged. A similar pattern was observed with other resistant strains. The trace of resistance in *Streptomycin,* was observed shortly after *Mycobacterium tuberculosis* drug was introduced [59]. In the late 1950s, multidrug resistance was observed in bacteria such as *Escherichia coli.* Additionally, a series of resistance was found in several antimicrobials such as ampicillin, chloramphenicol and tetracycline. Antimicrobial resistance has consequences because antibacterial drugs have become less effective and accelerating the global health concern that is rapidly outpacing the available treatment options. The emergence and spread of bacterial resistance represent a severe global problem. Antibiotic-resistant bacterial infections are potentially very dangerous and increase the risk of death. In recent decades, problems of multidrug-resistant (MDR) microorganisms have reached an alarming stage [67].

A review on antimicrobial resistance reported that 50,000 lives die annually in U.S. and Europe only. And the rest of the world accounted for 700,000 deaths annually due to drug-resistant illnesses. The countries that are at a high risk are low and middle-income countries. A new report on the mortality and morbidity due to antibiotic-resistant superbugs has warned that if a major action is not taken against the deadly trend, by 2050 one person may die every three seconds and an average 10 million people annually worldwide [68-69] (**Figure 5**).

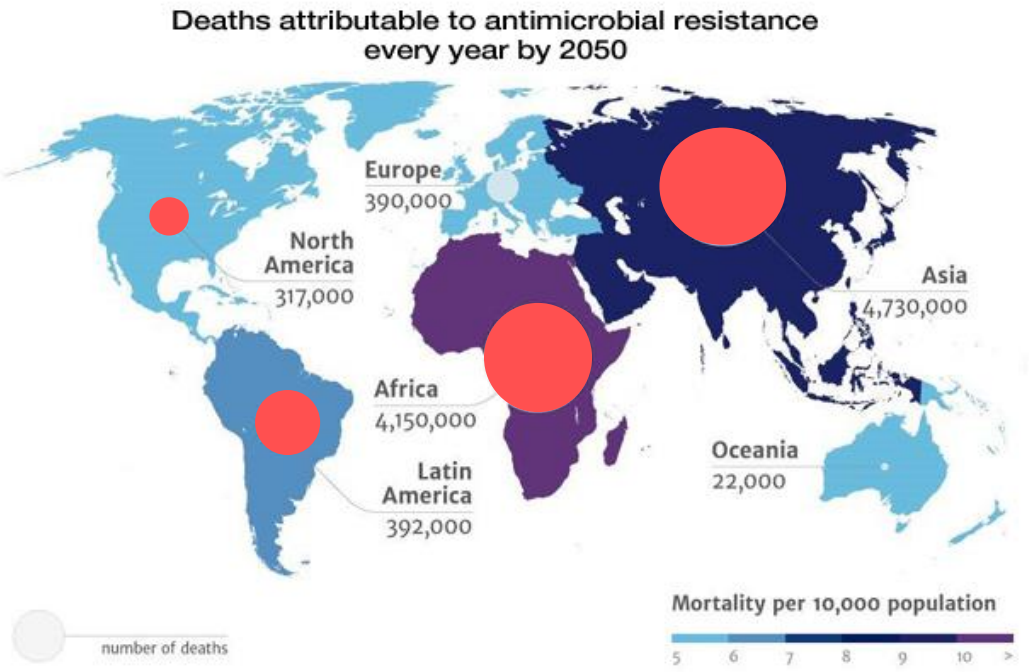


Figure 5. Schematic distribution of death attribute due to MDR by 2050 [70].

2.3.2. Mechanism of antimicrobial resistance

Microorganism can acclimatize to the antimicrobials environment as they strive for survival owing to development of resistant strains. After treatment of antibiotic surviving bacteria may develop resistance, multiply and replenish the bacteria killed. Exposure to antibiotics provides a leeway for bacteria to develop resistance [66,70]. Some bacteria can become resistant by acquisition of genes from resistance strains via a mating process such as conjugation. Virus also may be playing a vital role in transferring of resistance. The virus may carry resistance coded genes on its head and inject the traits in the bacteria they attack [70]. Not only can the bacteria development resistance toward a single drug, they may also exhibit multidrug resistance. The mechanism of resistance of antimicrobial agents is accomplished in four basic ways (1) Enzymatic drug deactivation (2) Altered drug targets [71], (3) Alteration of metabolic pathway (4) Modification in the permeability to the drug [72] (**Figure 6).**

2.3.2.1. ***Enzymatic drug deactivation:*** In this type of the drug resistance, the microbes renders the antibiotic agent disabled by altering its' potent structure. For example, β-lactamases of *Staphylococci* deactivate penicillin (β-lactam) by breaking the amide bond in the β-lactam ring [71].

2.3.2.2. ***Altered drug targets:*** Certain bacteria have avoided attack by antimicrobial agents owing to restructuring or remodelling their outer structure altering the anchoring targets of the antibiotics. An example of such enzyme is enterococci against vancomycin [71].

2.3.2.3. ***Alteration of metabolic pathway:*** Certain micro-organisms attain resistance by developing new metabolic pathway to dodge the initial antimicrobial inhibitory target from the previous pathway. This type of action is observed in resistance of many gram-positive and gram-negative bacteria to macrolides, lincosamides, and streptogramin B; deactivation of target site by methylation of ribosome [72].

2.3.2.4 ***Modification in the permeability to the drug:*** In this mode of action, the microorganism prevents antimicrobial drug from entering through the cytoplasmic membrane by decreasing the permeability of cell membrane. For example, the resistance of *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* against imipenem. Resistance can also by accomplished by developing an efflux which is used to pump out the antibiotics. For example, resistance of *E. coli* against tetracycline [72]. Numerous modes of action of antimicrobials and the diverse mechanisms of resistance exhibited by the microbe are shown in **Figure 6.**

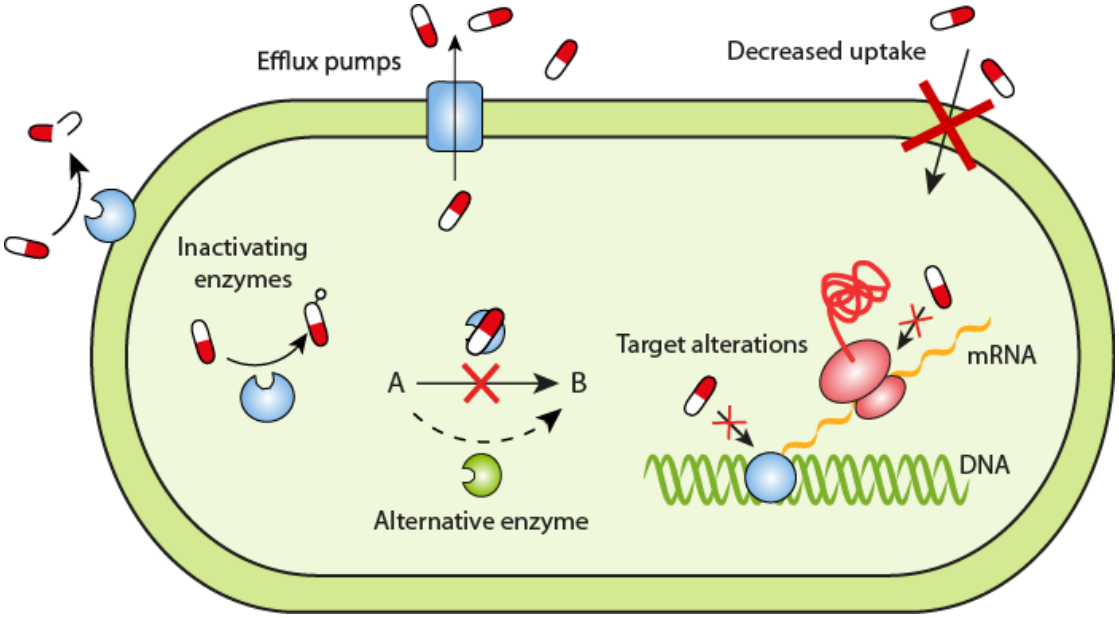


Figure 6. Mechanism of antimicrobial drug resistance [72].

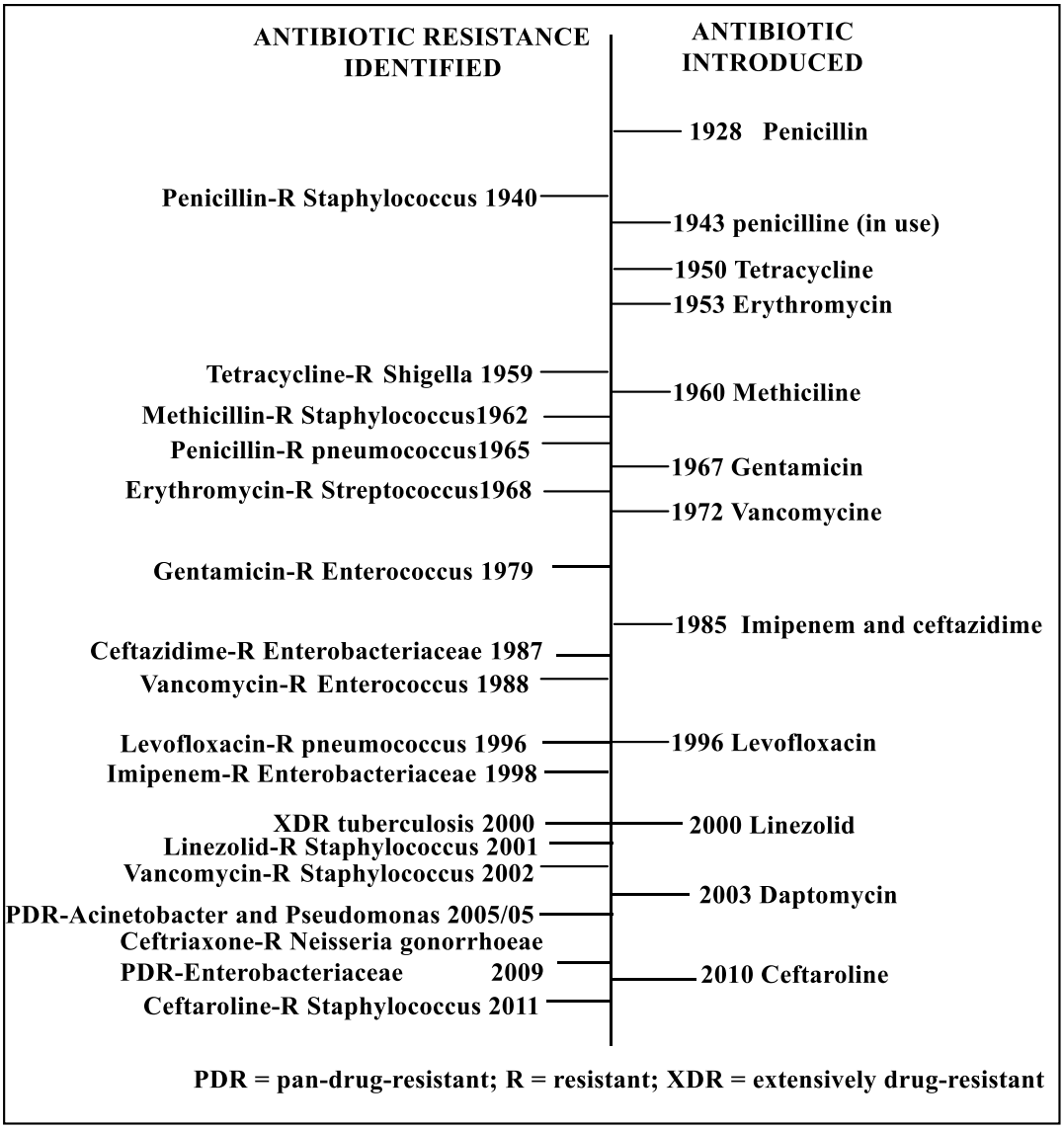


Figure 7. Developing Antibiotic Resistance: A Timeline of Key Events. Dates are based upon early reports of resistance in the literature [73].

2.4. The need: urgent requirement for new and potent antimicrobial agents

From the last several years alarming articles are communicated both on media and from scientific community on the inability of the available antimicrobial drugs for treatment of patients infected with multidrug-resistant bacteria (MDR). Global increase in drug resistance and steady decline in drug discovery of MDR is responsible for high mortality and morbidity. Increase in antibiotic resistance causes financial loss to the global output.

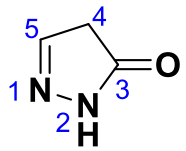
We live in the regime where many antibiotics are no longer effective against microbial infections including the simple ones. Such infections often result in an increased number of hospitalizations, more treatment failures and the persistence of drug-resistant pathogens. One

of the deadly superbug is Methicillin-resistant *Staphylococcus aureus* (MRSA). It is the cause of about 360 000 hospital cases and 19 000 deaths yearly. Other strains such as gonorrhoea which was initially treatable by penicillin in the 1970s, but no longer respond to ceftriaxone (ceftriaxone is not orally administered, it is only through parental routes), a third generation oral cephalosporins due to resistance. Gram-negative infections are becoming untreatable due to resistance elements including extended spectrum β-lactamases and *Klebsiella pneumonia* carbapenemase produced by *Enterobacteriaciae*. 45% of deaths in Africa is due to MDR. If we fail to discover new strong antimicrobial agent consequently 10 million deaths per year are estimated by 2050 due to drug resistance. As mentioned above, multidrug resistance is emerging as a gigantic global health issue for human race. To eradicate these problem huge efforts are required to develop new class of effective antimicrobial agents [74-75].

2.5. Pyrazolone

2.5.1. *Introduction, structure and tautamerism of 2,4-Dihydro-3H-pyrazol-3-one*

Heterocyclic compounds have received great deal of attention by medicinal chemists. These compounds have shown wide therapeutic application and have been at the centre of various studies for development of new drugs. Almost 90% of the marketed drugs contain heterocyclic scaffolds. Azo heterocyclic compounds have been used widely in dye industry, chelating agent in co-ordination chemistry, agrochemical products, photographic couplers and in drug discovery [76–78]. Azo heterocycles contain two nitrogen atoms (R−N=N−R) or diimide (HN=NH) functional group. Examples of Azo heterocyclic compounds that have exhibited biological activity include imidazole, thiadiazole, piperazine, triazine, triazole, oxadiazole, pyrazole and pyrazolone [79]. 2,4-Dihydro-3*H*-pyrazol-3-one is an emerging scaffold gaining attention for its diverse pharmacopial behaviour. It is a 5-membered azo heterocyclic ring containing two adjacent nitrogen atoms on position 1 and 2 linked with single bond. Position 3 has incorporated by ketonic group, it can be viewed as a five membered lactam ring having ketonic group adjacent to nitrogen and position 4 has an active methylene group, which makes it nucleophilic in nature. Replacement of hydrogen atom of CH on position 5 by alkyl or any other group stabilized the molecule. Incorporation of aryl or alkyl group on nitrogen (NH) of position 2 enhances stability of pyrazolone (**Figure 8**). Pyrazolone has a planar conjugated ring structure with six delocalized π-electrons. The aromatic nature arises from the four π electrons and the unshared pair of electrons on the NH nitrogen [80-81].



2,4-dihydro-3*H*-pyrazol-3-one

Figure 8. Basic structure of pyrazolone.

Pyrazolone molecules exist in three isomeric forms i.e. 3-pyrazolone (ketonic), 4-pyrazolone (hydroxyl), 5-pyrazolone (imine) (**Figure 9**). These isomers can interconvert via lactam–lactim and imine–enamine tautomerism [82].

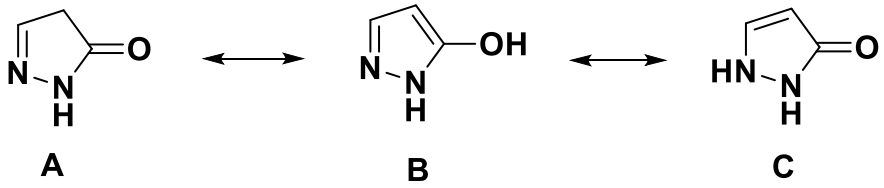


Figure 9. Isomerism in pyrazolone.

Pyrazolone and its derivatives, hold a key position in medicinal and pesticide chemistry for their diverse biological activities. This scaffold has been used as a building block in organic synthesis for designing pharmaceutical and agro chemicals. They have exhibited great importance in dye industries, and ligands for metal catalysis. They have been shown to exhibit antimicrobial [83], analgesic [84], anticancer [85], anti-tubercular [86], anti-inflammatory [87], antidepressant, anticonvulsant [88], antihypoglycemic [89], anti-cardiovascular disease [16], antipyretic, antihelmintic, herbicidal and antioxidant properties [17]. The pyrazolone ring is present as the core moiety in a variety of leading marketed drugs such as edaravon, phenazone, metamizole, dipyron, propyphenazon, novalgin, phenylbutazone, remifenazone, famprofazone, aminopyrine, oxyphenbutazone etc. [21]. The 2,4-Dihydro-3*H*-pyrazol-3-one unit is an integral part of biologically active natural products such as nostocine from *Nostoc spongiaeforme*, and Fluviols (A–E) from *Pseudomonas fluoresences* (**Figure 10**) [23,90-91].

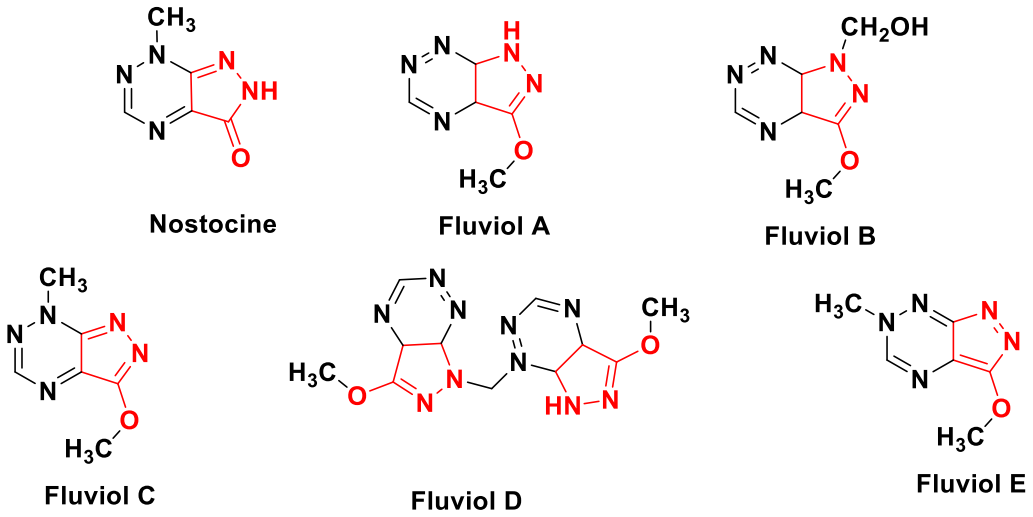


Figure 10. Naturally occurring pyrazolone compounds.

2.5.2. History of pyrazolone

In the beginning of the 19th century medical practitioners were entirely dependent on extracts from plant natural products. Due to inadequate drug supply chemical industry and chemists at the universities in Europe began to focus on the synthetic production of drugs. Till 1875 only natural drug quinine was available for treatment of infections such as pain and fever [92]. In 1884 at Erlangen, Ludwig Knorr and Wilhelm Filehne while searching for a synthetic alternative for quinine, they discovered new drug called antipyrine also known as phenazone [93]. This was accomplished by the reaction of phenyl hydrazine and acetate–esters. Antipyrine is a non-steroidal anti-inflammatory drug which has analgesic, antipyretic and anti-rheumatic activity. This discovery brought an impressive change in medical practice [93]. A new advance in this field occurred in 1949 when H. Stenzl synthesized phenylbutazone. About 50% of the worldwide market of the antipyretic analgesics drugs contains antipyrine as a main scaffold. These drugs include aspirin and its motifis; acetaminophen and propyphenazone, as well as dipyrone [92]. The initial structure that Knorr targeted for antipyrine; was fused rings like quinoline with International Union of Pure and Applied Chemistry (IUPAC) name 1,1a-Dimethyl-1a,2-dihydrodiazirino[1,3-a]quinolin-3(1*H*)-one. However the structure that was obtained was pyrazolyl pentagonal ring with IUPAC name 1,5-Dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (**Figure 11**) [92-93].

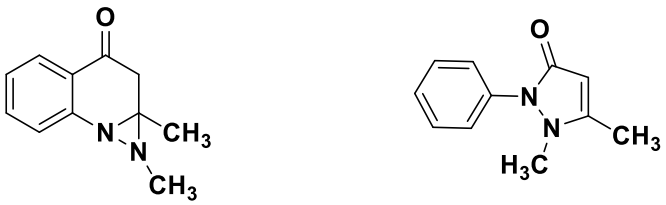


Figure 11. Structure of antipyrine targeted by Knorr and actual structure.

2.5.3 Synthesis of Pyrazolone (2,4-Dihydro-3H-pyrazol-3-one)

Normally pyrazol-3-ones have been synthesized from an open chain precursor. 2,4-Dihydro-3*H*-pyrazol-3-one moiety is generally prepared from β-keto esters with hydrazine hydrate. The cyclo condensation of simple β keto esters and their derivatives with hydrazine hydrate or mono substituted hydrazine can easily be accomplished by conventional method. Various derivatives of prazolone-3-one have been synthesised using different reaction conditions (**Figure 12**). The following is a list of reagents and solvents that have been used for synthesis of prazolone; methanol [94], acetic acid [95], ethanol [96], glycerol [97], methanol with catalytic hydrochloric acid [98], toluene [99]. The reaction can be carried out both conventionally or in the microwave [100].

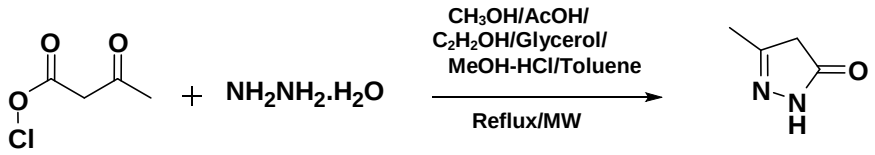


Figure 12. Synthesis of pyrazolone.

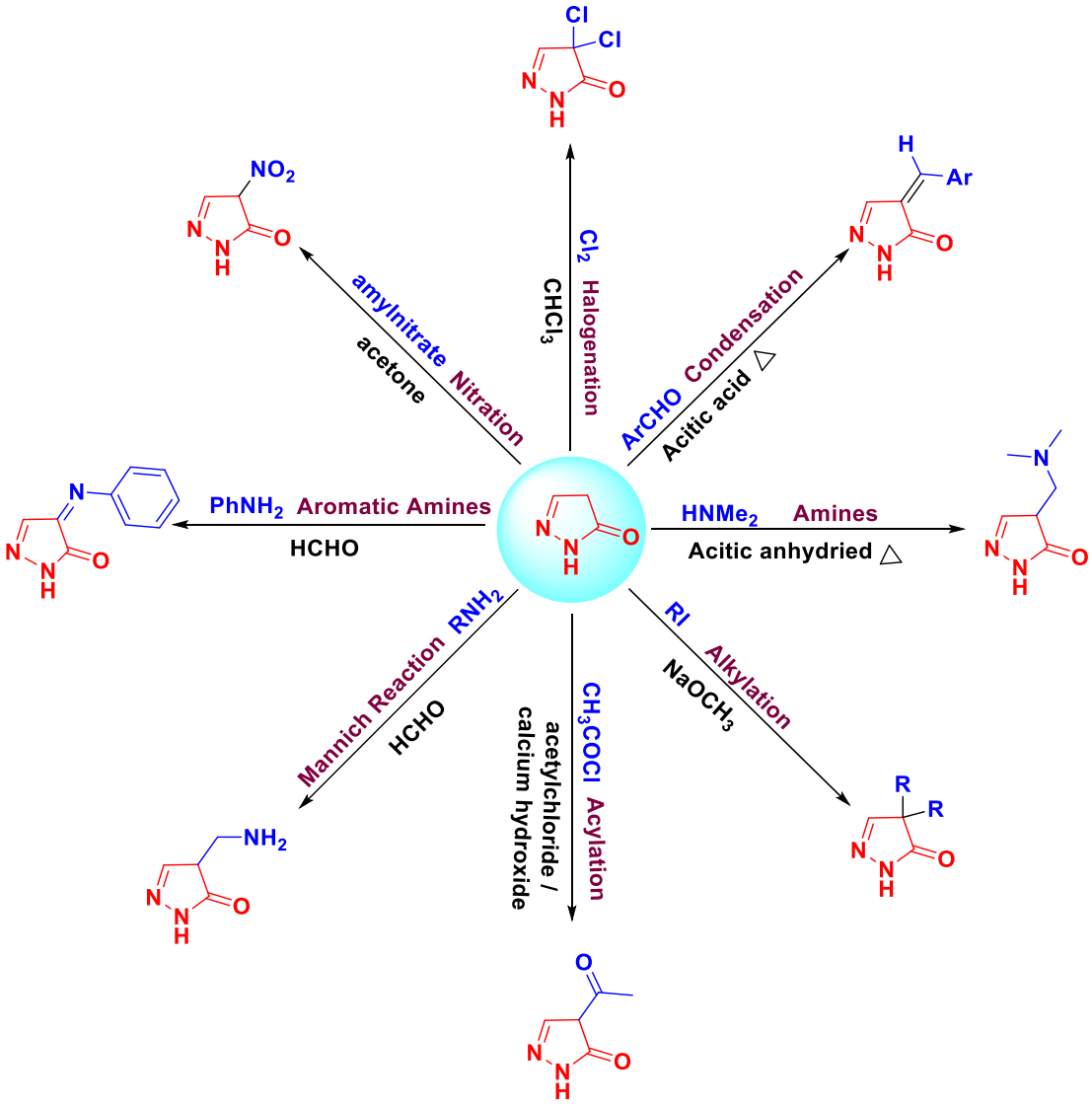


Figure 13. Reactions of pyrazolone.

2.5.4 Reactions of 2,4-Dihydro-3H-pyrazol-3-one

The methylene group at position 4 on pyrazolone is reactive. The hydrogen on the methylene is susceptible to nucleophilic substitution reaction. The reactions pyrazolone undergoes include condensation [12], alkylation [101], acylation [102], halogenation [103], nitration [104] and mannich reaction [105] as highlighted in (**Figure 13**).

2.5.5 Applications of 2,4-Dihydro-3H-pyrazol-3-one

Pyrazol-3-ones are very versatile pharmacophores and are very important both as products and intermediates in chemical analysis, dyes, biological, agrochemicals, photography and pharmaceutical chemistry [106].

2.5.5.1. ***Analytical uses:*** Pyrazol-3-ones find extensive use as analytical reagents as reported in many journals and patents. A few pyrazol-3-derivatives have been used to extract and separate

different metal ions [107],While other derivatives have been used to quantitatively and qualitatively determine phenol [108], cyanides, and ammonia [109], and as photographic sensitizers [110] in dyes as analytical reagents [111].

2.5.5.2. ***Agrochemical uses:*** The compounds with a pyrazol-3-ones scaffold have found wide applications in agriculture and have been used as fungicides [112], herbicides [113] and insecticides [114].

2.5.5.3. ***Dyes and pigments:*** Pyrazole-3-one dye are found to be good precursors for cotton, wool, silk and polyester fabrics [115]. They are also notably useful in leather and wool dyeing (azo dyes). The pigments and dyes of pyrazolones are also used to produce synthetic fibres and plastics. All types of dye have been known to contain pyrazolones [116].

2.5.5.4. ***Photographic coupling agent:*** Pyrazolone has been used as colour coupling agents in photography. Their coupler has exhibited good light absorption characteristics and stability proving to be good for the development of colours in photography [117].

2.5.5.5. ***Ligands:*** Pyrazolone and its derivatives have been studied widely as ligands in coordination chemistry. They form complexes that are stable and displayed promising catalytic [20] and biological activities. They also have wide application as complexing agents in the photometric determination of metal ions. They have portrayed good coordination behaviour which is auspicious in the study of pyrazolones complexation.

2.5.5.6. ***Pharmaceutical applications*****:** Pyrazol-3-ones have displayed a wide range of biological activities such as anti-inflammatory [12], antifungal [9] antitumor [118], analgesic [119], anti-cancer [10], anti-tubercular [11], antidiabetic [120] antimicrobial [121], Chemokine receptor 3 (CCR3) antagonists [122], anti hyperglycemic [123], Kinase insert domain receptor (KDR kinase) inhibitors [124], anxiolytic [125], antibacterial [126], cytokine synthesis inhibitors [127], inhibitors of UDP-N-acetylenol pyruvyl glucose amine reductase [128], inhibitors of bacterial cell wall biosynthesis [129], orally bio available inhibitors of p38 kinase [130], inhibitors of CD80 useful in immune modulation therapy [131], MDR modulators [132] and cardio vascular agents [133] (**Figure 14**).

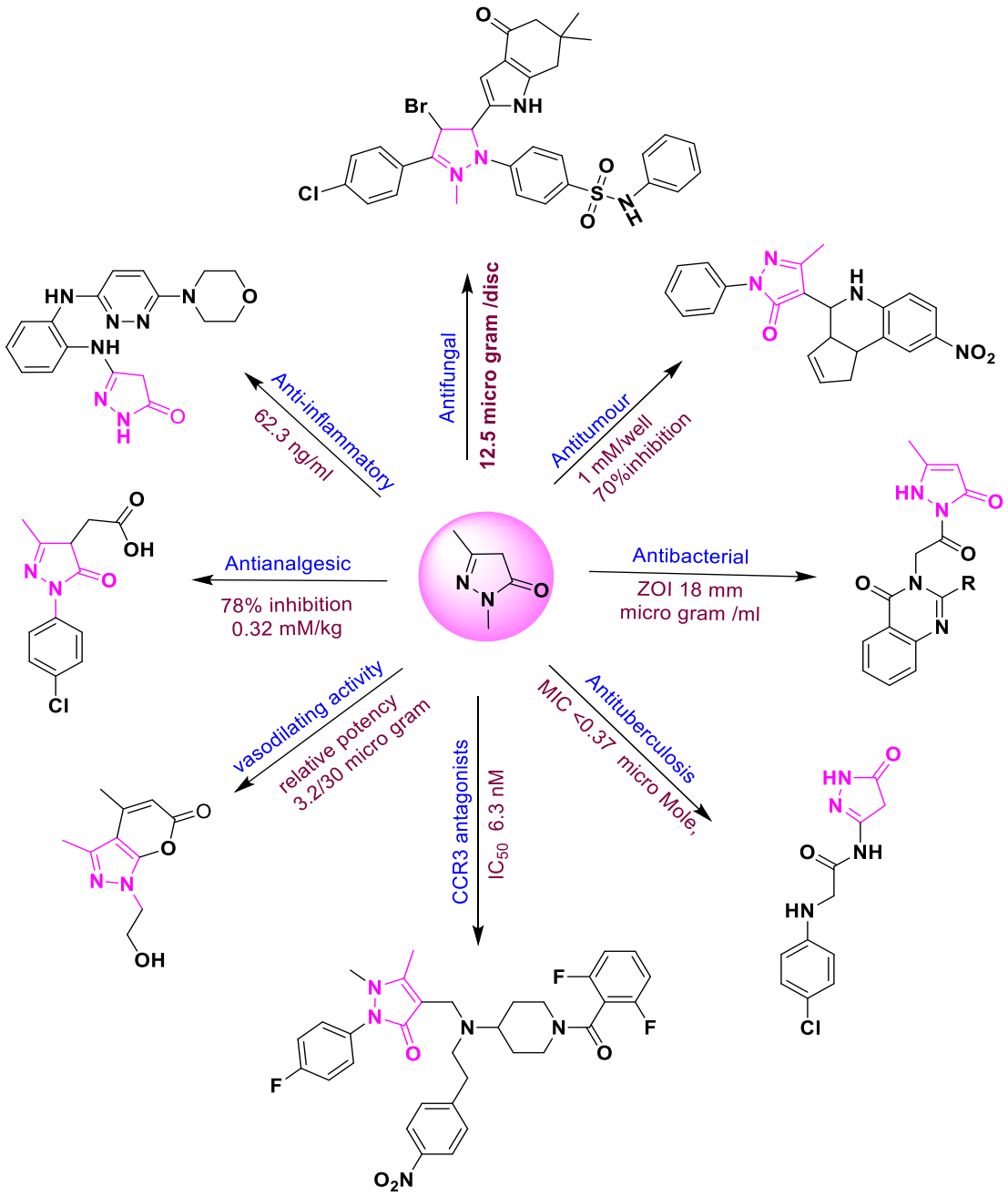


Figure 14. Pharmaceutical applications of pyrazolone.

2.5.6. 2,4-Dihydro-3H-pyrazol-3-one as antimicrobial agent

Below mentioned structure (**Figure 15**) and values of MIC/ **ZOI (**Zone of inhibition) (revealed that derivatives of 2,4-Dihydro-3*H*-pyrazol-3-one are potent antimicrobial agents.

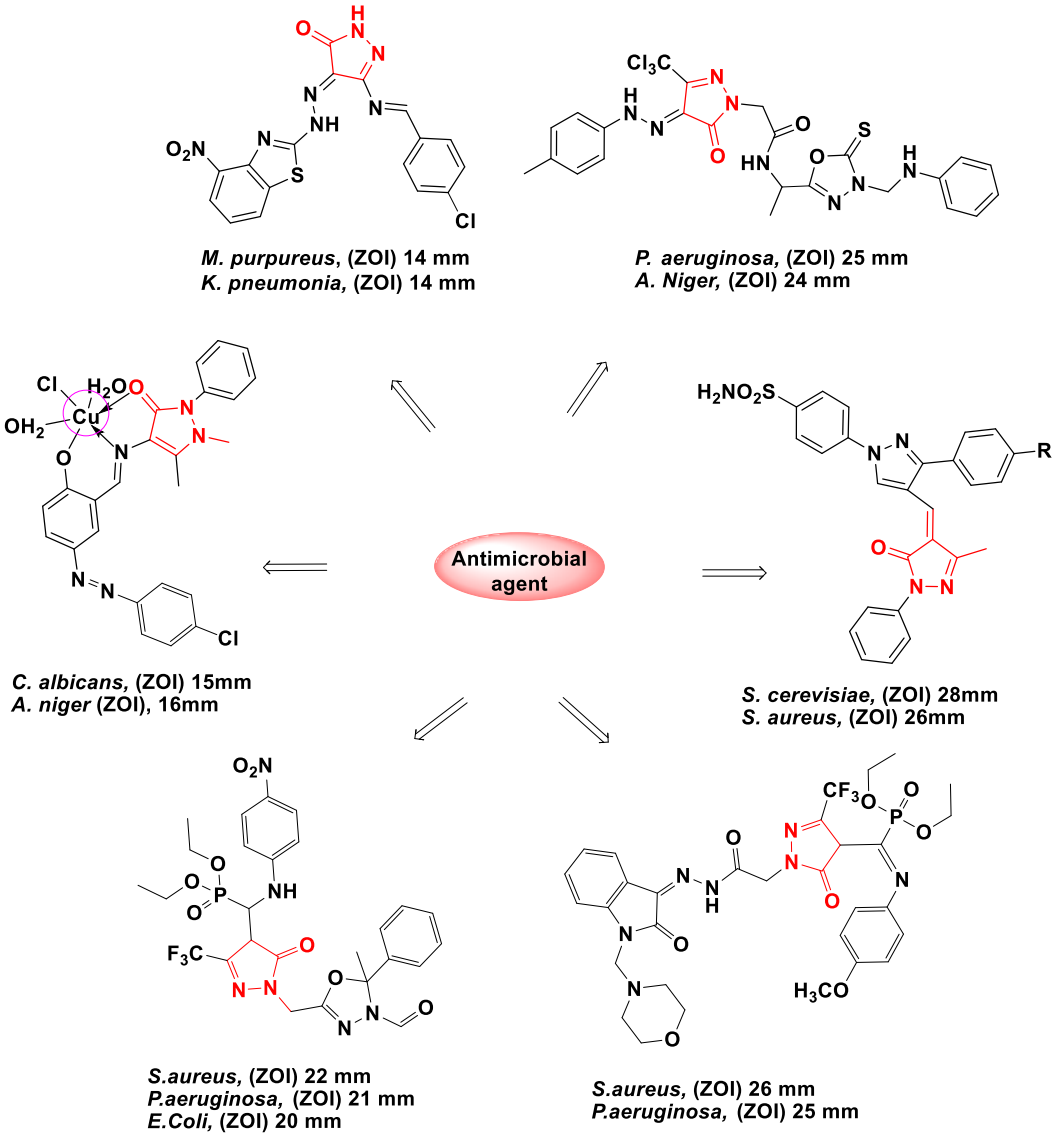


Figure 15. Pyrazolone derivatives as potent antimicrobial agents.

ZOI-Zone of inhibition

2.6. Synthetic strategies being used to overcome the MDR

2.6.1 Hybridization

As the multidrug antimicrobial resistance has become an alarming threat for community, scientists need to find diverse approach to develop effective antimicrobial drug. Molecular hybridization is the technique which may optimize this emerging issue, by developing multifunctional hybrids that have the capacity to deal with more than one biological target associated with these diseases. Hybridization is a strategy of rational design of new ligands or prototype based on the identification of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which through the fusion of these sub units, lead to the design of new hybrid architecture that

maintain characteristic of original pharmacophore [134]. Pyrazolone hybridized with different scaffold proved to be strong antimicrobial drug e.g. pyrazolone- thiadiazole [135], pyrazolone-oxadiazole [136], pyrazolone-sulphonyl phosphates [137], pyrazolone-triazole [138], pyrazolone – morpholine [139] pyrazolone- indole [140] etc. Based on this new synthetic strategy we plan to develop novel hybrid pharmacophore of with pyrazolone scaffold.

2.7. Need for new pyrazolone based drugs

The current marketed antimicrobial drugs are becoming less potent or ineffective due to rise in bacterial resistance, in addition to long dose regimen, cost and side effects. This necessitates the discovery of new antimicrobial drugs, which should be cost effective, more potent, and be able to eradicate the bacteria in short period of time. Compounds with a 2,4-Dihydro-3*H*-pyrazol-3-one moiety have demonstrated a wide spectrum of high potent biological activity including antimicrobial activity (**Figure 15)**. 2,4-Dihydro-3*H*-pyrazol-3-one derivatives have proven to be very effective and versatile pharmacophores. The diverse character, high potency, low cost, easy availability, simple handling and cost-effective nature of pyrazolone prompted us to work and develop new potent antimicrobial agent using 2,4-Dihydro-3*H*-pyrazol-3-one as main pharmacophore.

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CHAPTER 2. MANUSCRIPT 1

After the introduction chapter, a full review paper on the pyrazolone and its derivatives has been communicated titled **"Pyrazolone: An emerging scaffold for design and synthesis of diverse pharmaceutically active derivatives"**.The paper summarizes about the pyrazolone and its design & synthesis along with its biological activities. This paper was prepared and submitted following the guidelines of the journal "Bio-organic Chemistry**".**

PYRAZOLONE: AN EMERGING SCAFFOLD FOR DESIGN AND SYNTHESIS OF DIVERSE PHARMACEUTICALLY ACTIVE DERIVATIVES

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Abbreviations

- 226D: DNA structure
- 5-FU:5-Fluorouracil
- A. *fumigatus*:*Aspergillus fumigatus*
- A. parasiticus:*Aspergillus parasiticus*
- *A. niger*: *Aspergillus niger*
- A549: Adenocarcinomic human alveolar basal epithelial cells
- ABTS:2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
- ALCL:Anaplastic large cell lymphoma
- ALK:Anaplastic lymphoma kinase
- AT rich: Adenine and thymine residues
- ATCC 27294: *Mycobacterium tuberculosis* strain
- AUC:Area under the curve
- *B. subtilis*: *Bacillus subtilis*
- *B. cereus*: *Bacillus cereus*
- BT474: Human breast cancer cell lines
- *C. albicans*: *Candida albicans*
- C. *lunata: Cochliobolus lunatus*
- C646:Histone acetyltransferase p300 inhibitor
- Cat.:Catalyst
- CC_{50}: 50% Cytotoxicity concentration
- Cdk1: Cyclin-dependentkinase 1
- CK2: Calvin klein
- CL: Clearance rates
- c-Met:Tyrosine-protein kinase Met
- COX: Cyclooxygenase
- CT-DNA : Circulating tumor DNA
- Cyclin B1: Human protein encoded by the CCNB1 gene.
- DCM: Dichloro methane
- DLD-1:*Dukes' Type C, Colorectal Adenocarcinoma*
- DMF: Dimethyl formamide
- DMSO: Dimethyl sulphoxide
- DNA: Deoxyribonucleic acid

- *E. coli*: *Escherichia coli*
- EAC: Ehrlich ascites carcinoma
- ECA: Endothelial cell mitogenesis assay
- Et_2NH: Diethyl amine
- Et_3N: Triethyl amine
- EtOH: Ethanol
- F. *solani*:*Fusarium solani*
- FDA: Food and Drug Administration
- GC rich: Guanine-cytosine rich
- $H_{37}Rv$: *Mycobacterium tuberculosis* strain
- H460: Human lung carcinoma cells
- H9C2: Cell line derived from rat cardiac tissue
- HCT116: Human colon cancer cells
- HCT15: Homo sapiens colon duke-15
- HCvEpCs: Human Cervical Epithelial primery Cells
- HEK293T: Human embryonic kidney cell
- Hela: Cervical cancer cells (Henrietta Lacks)
- HEp-2: Homo sapiens HeLa contamination carcinoma
- HepG2: Liver hepato cellular carcinoma.
- HOPh: Phenol
- HT29: Human colon adenocarcinoma cell line
- HUVEC: Human umbilical vein endothelial cells
- IC_{50}: Half maximal inhibitory concentration
- IGF1R: Insulin-like growth factor 1 receptor
- IL6:Interleukin 6
- IQCI: Interqual Clinical Instructor
- iPK: Pharmacokinetics
- *K. pneumoniae*: *Klebsiella pneumoniae*
- Kapp: Apparent binding constant
- Kb- Binding constants
- Kbin: Binding constant of DNA with the number of binding sites
- KDR: Kinase insert domain receptor
- K_i: Inhibition constant

- Ksv: Quenching constant
- *M. luteus: Micrococcus luteus*
- M: Mole
- M^{-1}: Per mole
- MCF7:Michigan Cancer Foundation-7
- MeOH: Methanol
- MIA PaCa-2: Pancreas ductal adenocarcinoma cell lines
- MIC: Minimum inhibitory concentration
- Min: Minute
- MKN45: Human gastric carcinoma cell lines
- mol%:Mole percentage
- MTT:3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
- MW: Microwave
- NSAID: Nonsteroidal anti-inflammatory drug
- °C: Degree centigrade
- *P. aeruginosa*: *Pseudomonas aeruginosa*
- *P. fluorescens: Pseudomonas fluorescens*
- p300 HAT:Histone acetyltransferases
- P388: Leukemia cells sensitive
- PAILuc:Plasminogen activator inhibitor-1
- PC3:Human prostate cancer cell line
- PDB ID: 1T8I:Human DNA topoisomerase I
- PKC: Protein kinase C
- *R. bataicola:Rhizoctoniabataicola*
- RBBP9:Retinoblastoma binding protein 9
- rt: Room temperature
- RT112:Bladder tumor cell lines
- S. *albus*, *Staphylococcus albus*,
- S. *cerevisiae*: *Saccharomyces cerevisiae*
- S. *pyogenes: Streptococcus pyogene*
- S. *sonnie*: *Shigella sonnie*
- S. *typhi: Salmonella typhi*
- *S. aureus*: *Staphylococcus aureus*

- SAR: Structure activity relationship
- SD: Standard deviation
- SKMEL-2: Melanoma cell lines
- SKOV-3: Human ovarian carcinoma cell line
- SW620: Human caucasian colon adenocarcinoma
- $T_{1/2}$: Terminal half-life
- T24: Urinary bladder carcinoma cells
- TEA: Tri ethyl amine
- TGFβR1: Transforming growth factor beta receptor I
- THF: Tetrahydrofuran
- TNF-α: Tumor necrosis factor alpha
- Trk A: Tropomyosin receptor kinase A
- U87MG: Human primary glioblastoma cell line
- *V. cholerae: Vibrio cholerae*
- Vd: Venereal diseases
- VEGFR: Vascular endothelial growth factor receptor
- W: Watt
- XF 498: Cellosaurus glioblastoma cell line
- ZOI: Zone of inhibition
- μM: Micro mole
- μmL: Micro millilitre

Abstract

2,4-Dihydro-3*H*-pyrazol-3-one is considered as a biologically important heterocyclic scaffold that is known to possess wide range of biological activities. Successful introduction of phenazone, metamizole, propyphenazonremifenazone as analgesic antipyretic agents; edaravon as neuroprotective agent; dipyron as antispasmodic; and famprofazone, phenylbutazone, remifenazone asanti-inflammatory agentsin market proved the pharmacutical potential of 2,4-dihydro-3*H*-pyrazol-3-one moiety. Here we report a review describing recent perspective on the diversely substituted 2,4-Dihydro-3*H*-pyrazol-3-one that possess diversity of various biological applications. To the best of our knowledge there is no review reported on this scaffold thus, this review unveils the synthetic strategies and pharmacological activities of differently substituted 2,4-Dihydro-3*H*-pyrazol-3-one with special emphasis on structure activity relationships. It is an effort to pave the way for further development in this promising area of research.

Keywords: Pyrazolone, Synthetic strategies, Anticancer activity, Antibacterial activity, Anti-inflammatory activity.

1. Introduction

The heterocyclic compounds hold a large area in medicinal chemistry and have been widely explored for their applications as therapeutic agents[1], chelating agents in coordination chemistry and agrochemical products, photographic couplers, synthetic scaffold in medicinal and combinatorial chemistry [2]. Almost 90% of the drugs currently marketed are heterocyclic compounds[3].Among all heterocyclic compounds, aza-heterocycles are vital moieties for therapeutic/pharmacological activity [4]. Incorporation of suitable heterocyclic scaffolds enhances the biological potency as azo-heterocycles, thiazole, thiadiazole, triazole, arylazothiophene, pyridine, piperazine, etc. are playing very essential role in medicinal chemistry[5]. 2,4-Dihydro-3*H*-pyrazol-3-one is one such essential drug-like nucleus bearing gigantic biological applicability.2,4-Dihydro-3*H*-pyrazol-3-one is a five member heterocyclic lactam ring which contains two nitrogen atoms adjacent to each other and one ketonotic group on third position (**Figure 1**)[6]. Antipyrine was the first pyrazolone derivative used clinically as nonsteroidal anti-inflammatory and analgesic agent [7]. Pyrazolone derived drugs have displayed momentous biological properties such as anti-inflammatory[8], antibacterial[9], analgesic[10], anti-hyperglycaemic [11], antidepressant and anticonvulsant [12], anticancer [13] and free radical scavenger[14].

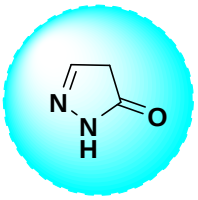
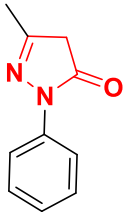
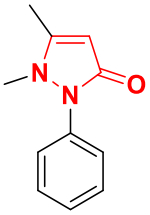
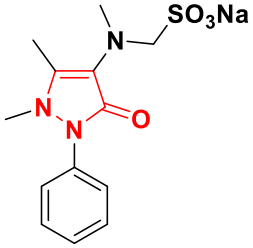
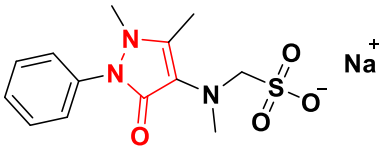
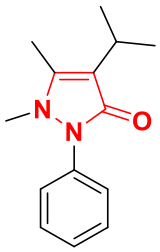
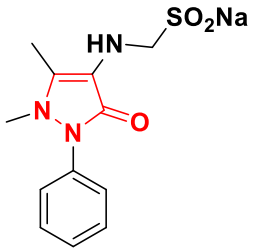
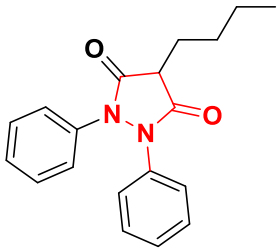
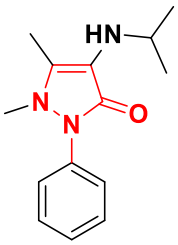
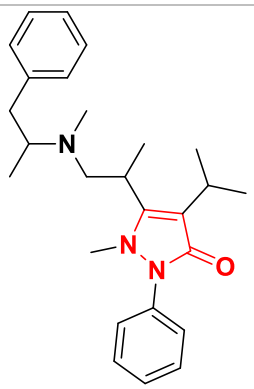
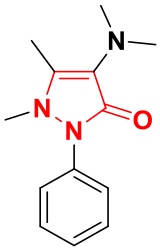
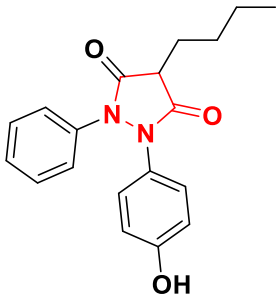


Figure 1. General structure of 2,4-dihydro-3*H*-pyrazol-3-one.

Apart from the numerous biological applications, pyrazolone scaffold is a key pharmacophore in some of the well-known FDA approved pharmaceutical drugs such as metamizole and remifenazone [15] (**Table -1**). However, there is plenty of room for the medicinal chemists to explore this privileged scaffold for developing potential drug-like candidates. Above information proved that five membered pyrazolone derived drugs playing a significant role in therapeutic stand. Inspired by these observations, in this review we summarize and represent the latest progress (2005-2016) on synthetic strategies and biological properties of pyrazolone derivatives, along with special emphasis on SAR of above-mentioned derivatives.

Table 1. Marketed drugs containing pyrazolone nucleus.

Name of the Drug	Structure	Approved activity
Edaravon		Nootropic, neuroprotective agent
Phenazone		Analgesic, antipyretic
Metamizole		Analgesic, antipyretic
Dipyron		Antispasmodic, antipyretic, anti-inflammatory
Propyphenazon		Analgesic, antipyretic
Novalgin		Analgesic

Phenylbutazone		Anti-inflammatory
Remifenazone		Analgesic, anti-inflammatory, and antipyretic
Famprofazone		Analgesic, anti-inflammatory and antipyretic
Aminopyrine		Analgesic, anti-inflammatory and antipyretic
Oxyphenbutazone		Anti-inflammatory

2. Synthetic methods for pyrazolone derivatives

Synthesis of first pyrazolone clinical drug (antipyrine) was done by Ludwig Knorr in 1887 [2]. Since then thousands of derivatives have been synthesized from pyrazolone moiety as intermediate by applying numerous synthetic strategies and analysed for their pharmacological properties. The various strategies for synthesis of pyrazolone derivatives have been presented in **Figure 2**.

Park and co-workers carried out Vilsmeier-Haack chloro formylation and Williamson reaction of **i** with suitable nucleophilic reagents to achieve pyrazole oxime ethers (**A1**) as final compounds (scheme-1)[16]. Tripathy et al. attempted the condensation reaction between **ii** and 7-chloro-1-methyl-1*H*-indole-3-carbaldehyde to afford the desired product (**A2**)in presence of piperidine as a base in ethanol under reflux conditions (scheme-2)[17]. Tripathy and co-workers accomplished the desired product (**A3**) by allowing the reaction between **iii** and 4-ethoxy-1-methyl-1*H*-indole-3-carbaldehyde by using piperidine as a base in ethanol (scheme-3)[18]. Marchetti et al. obtained molecular complex named as [Ru(cymene(Q)Cl] (**A4**) via treating **iv** with dichloro(*p*-cymene) ruthium(II) in presence of sodium methoxide as a strong base in methanol (scheme-4)[19]. Castagnolo and co-workers have reported the synthesis of final compound **A5** by nucleophilic substitution reaction of **v** with variously substituted amines in DMF under microwave irradiation conditions (scheme-5)[20]. Guckian et al. attempted C-H activation reaction of **vi** with bromobenzene to attain **A6** in DMF containing catalytic amount of lead acetate and caesium acetate (scheme-6)[21]. Santos and co-workers reported oxidation of **vii** (antipyrine) followed by demethylation to achieve **A7** (scheme-7)[22]. Patel and co-workers reported nucleophilic substitution reaction of **viii** with4-methylbenzenesulfonyl chloride to attain **A8** intriethylamine (scheme-8)[23]. Burja and co-workers have treated **ix** with methanolic solution of NaOH to attain **A9** in dichloromethane under stirring conditions at room temperature (scheme-9)[24]. Bowers and co-workers have treated **x** with 5-(4,5-dimethyl-2-nitrophenyl)tetrahydrofuran-2-carbaldehyde to afford **A10** in presence of diethylamine in ethanol under reflux conditions (scheme-10)[25].

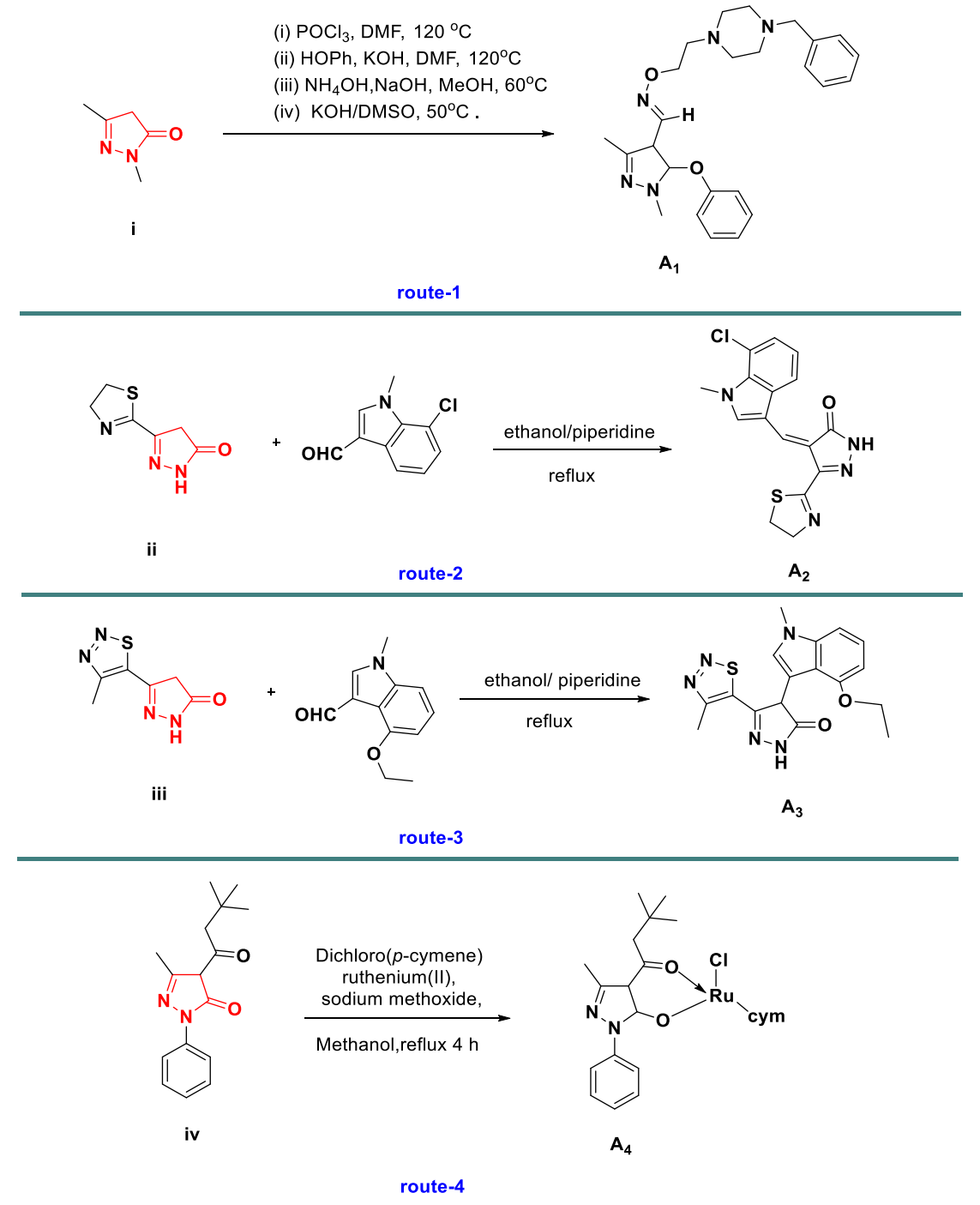


Figure 2. Synthetic strategies of substituted pyrazolone derivatives.

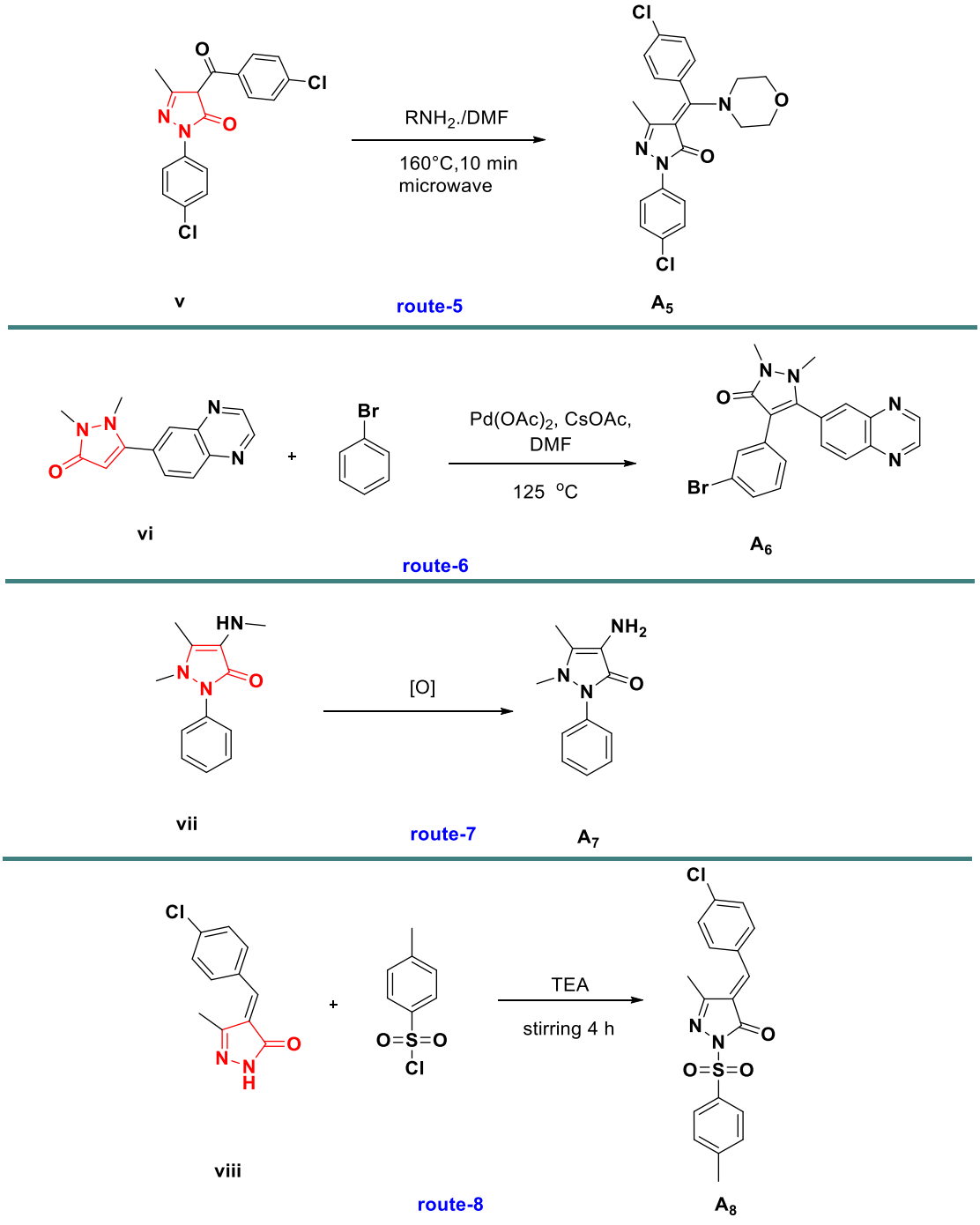
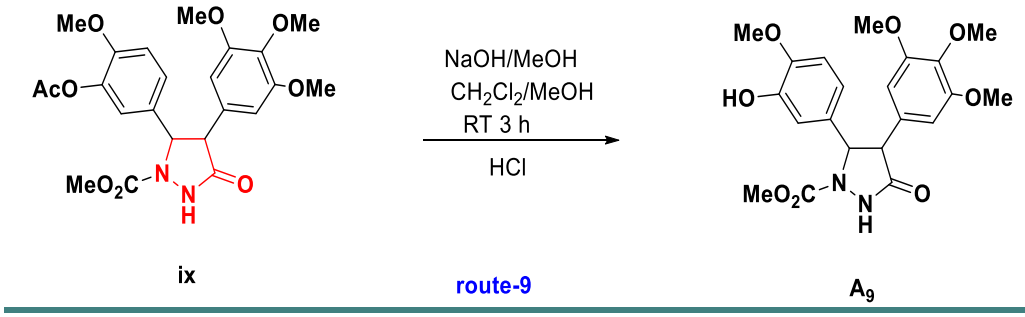
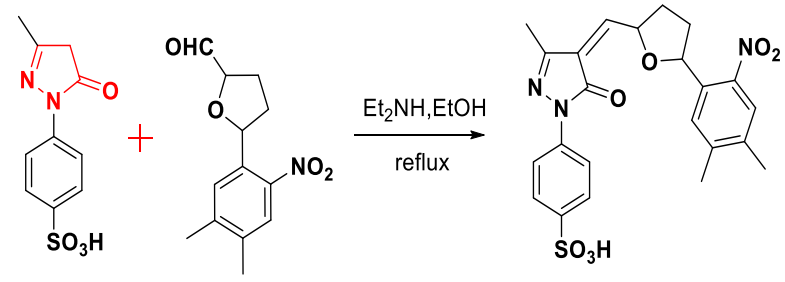
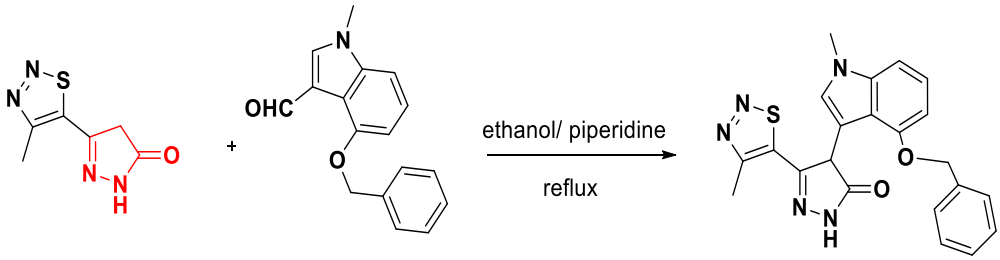


Figure 2.(continued) Synthetic strategies of substituted pyrazolone derivatives.







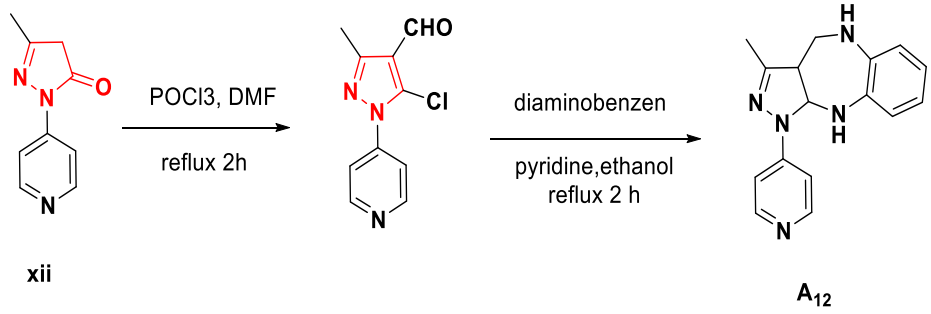


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

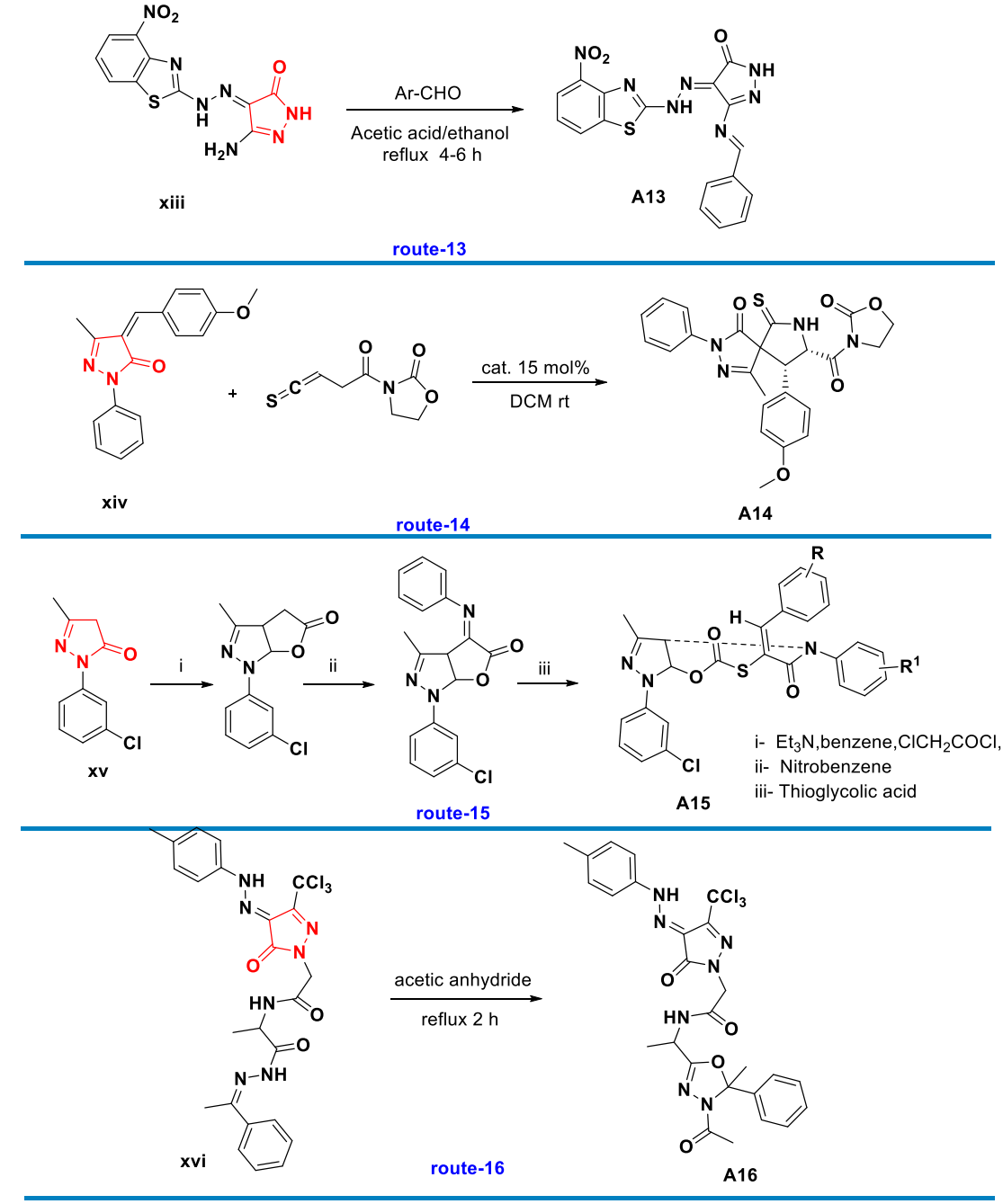


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

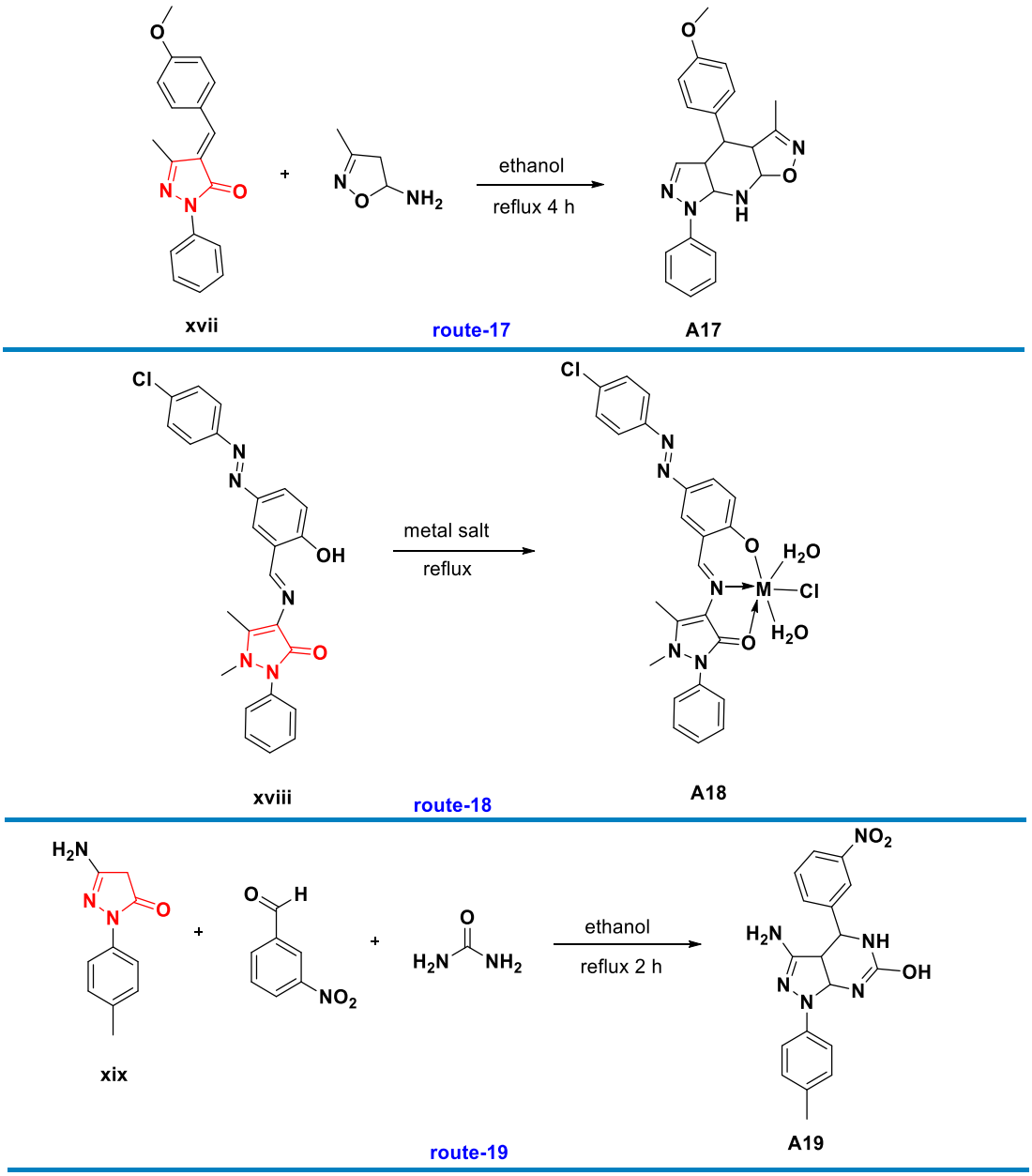


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Tripathy and co-workers have treated **xi** with 4-(benzyloxy)-1-methyl-1*H*-indole-3-carbaldehyde to afford **A11** in presence of catalytic amount of piperidine in ethanol under reflux conditions (scheme-11)[26]. El-baih and co-workers attempted condensation reaction of 5-chloro-3-methyl-1-(pyridin-4-yl)-1*H*-pyrazole-4-carbaldehyde (**xii**) by treating with diaminobenzene to achieve **A12** in presence of catalytic amount of pyridine as base in ethanol (scheme-12)[27]. Sivakumar et al. accomplished the desired product **A13** by reacting **xiii** with benzaldehyde under acidic conditions (scheme-13)[28]. Liu and co-workers reported the cyclization reaction of **xiv** with 3-(4-thioxobut-3-enoyl) oxazolidin-2-one to

attain the desired product **A14** under stirred conditions (scheme-14)[29]. Patel and co-workers have accomplished the desired Spiro product **A15** by treating **xv** with different heterocyclic molecules (scheme-15)[30]. Suresh Kumar et al. have reported a novel pyrazolone compound **A16** by the cyclization reaction of **xvi** with acetic anhydride under reflux conditions (scheme-16)[31]. Hamama and co-workers carried out the cyclization reaction of **xvii** with 3-methyl-4,5-dihydroisoxazol-5-amine to attain **A17** in ethanol under reflux conditions (scheme-17)[32]. Anitha and co-workers reported synthesis of tridentate transition metal complex **A18** by using azo Schiff-base ligand named as (4-(((*E*)-5-((*E*)-(4-chlorophenyl)diazenyl)-2-hydroxybenzylidene) amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (**xviii**) in ethanoic solution (scheme-18)[33]. Chaudhari and co-workers reported cyclo-condensation reaction of **xix** with3-nitrobenzaldehyde and urea to attain the desired product **A19** in ethanol under reflux conditions (scheme-19)[34]. Ravindranath et al. synthesized the target molecule **A20** by allowing the reaction between **xx** and acetic anhydride (scheme-20)[35].

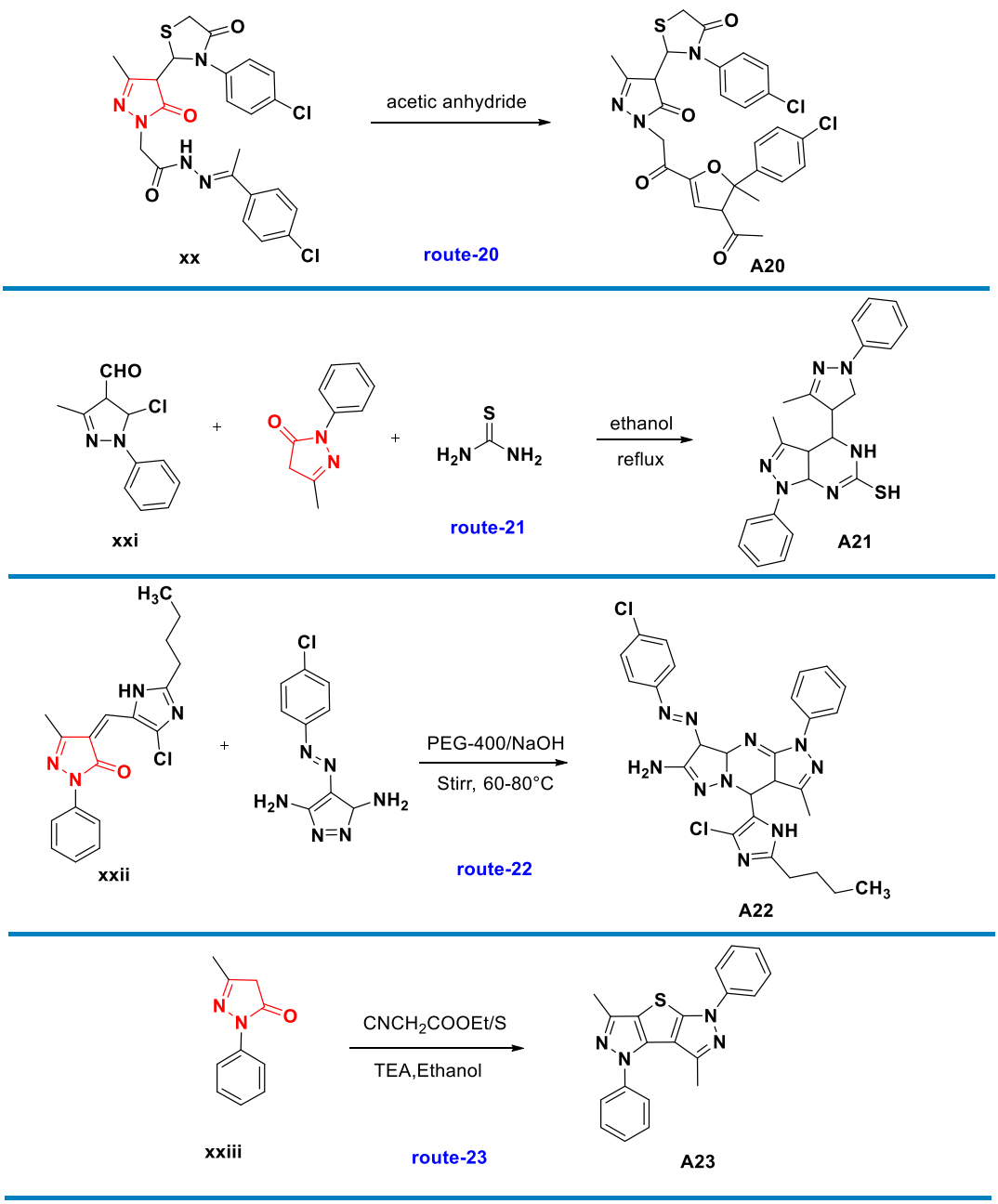


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

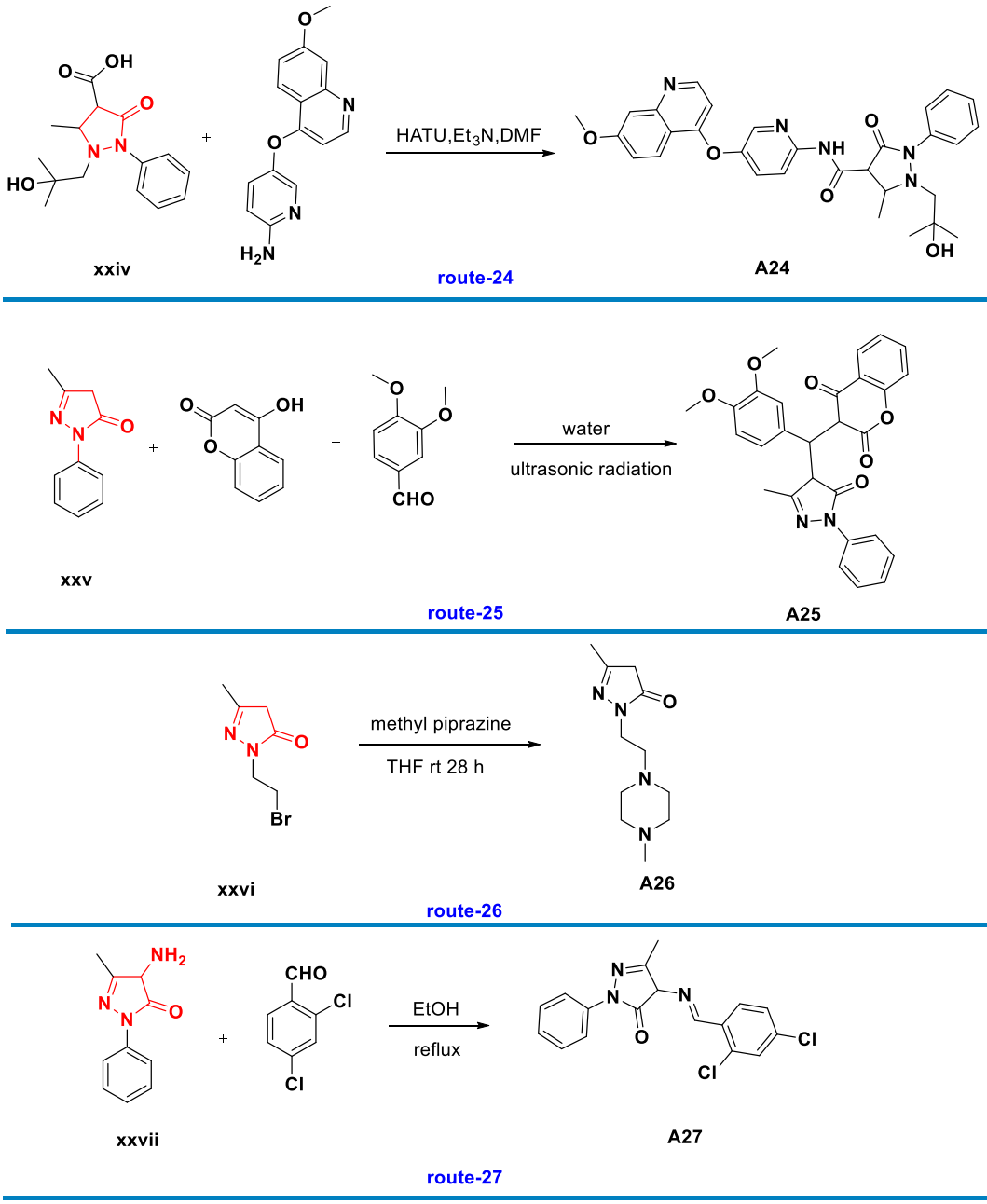


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

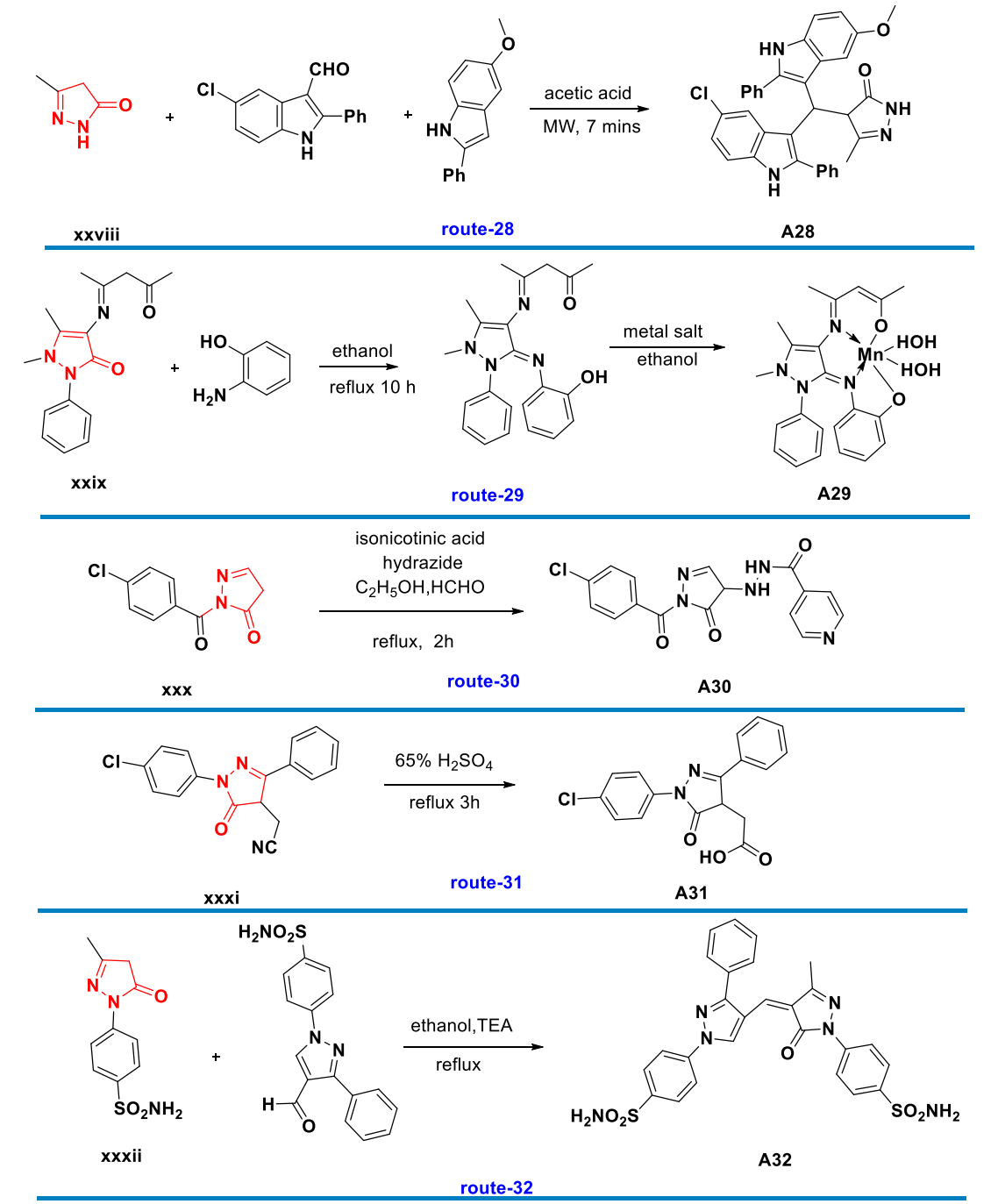


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Chaudhary and co-workers have performed one pot synthesis of **A21** by cyclization of **xxi** with 1-aryl-3-methyl-1*H*-pyrazol-5(4*H*)-one in ethanol under reflux conditions (scheme-21)[36]. Chobe et al. accomplished the cyclization reaction of **xxii** with(*E*)-4-((4-chlorophenyl)diazenyl)-3*H*-pyrazole-3,5-diamineto attain **A22** in NaOH solution containing catalytic amount of PEG-400 (scheme-22)[37]. Hamama and co-workers carried out cyclization reaction of **xxiii** with

ethylcyanoacetate and sulphur to achieve the desired product **A23** in presence of triethylamine in ethanol (scheme-23)[38]. Liu and co-workers developed an efficient coupling reaction between **xxiv** and 5-((7-methoxyquinolin-4-yl)oxy)pyridin-2-amine to afford **A24**in dimethylformmide solvent containing HATU as coupling agent (scheme-24)[39]. Liang and co-workers reported ultrasonic one-pot green synthesis of **A25** by allowing the reaction between **xxv,** 4-hydroxy-2H-chromen-2-one and 3,4-dimethoxybenzaldehyde in water (scheme-25)[40]. Antre and co-workers attempted the nucleophilic substitution reaction of **xxvi** with methyl piperazine to attain **A26** in tetrahydrofuran at room temperature (scheme-26)[41]. Sigroha and co-workers obtained the final compound **A27** by condensation reaction of **xxvii** with 2,4-dichlorobenzaldehyde in ethanol under reflux conditions (scheme-27)[42]. Sasidhar et al. offered three component one-pot synthesis of desired product **A28** employing michael addition of **xxviii** with5-chloro-2-phenyl-1*H*-indole-3-carbaldehyde, 5-methoxy-2-phenyl-1*H*-indole in acidic conditions (scheme-28)[43]. Saxena and co-workers reported the synthesis of tetra dentate ligand **A29** ((*E*)-4-(((*Z*)-3-((2-hydroxyphenyl)imino)-1,5-dimethyl-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino)pentan-2-one by allowing the reaction between **xxix** 2-aminophenol in methanol containing different metal salts (e.g. Ti (III), V (III), Mn (III), Ru (III), MoO (V), MoO2 (VI)) (scheme-29)[44]. Buvana and co-workers have treated **xxx** with formaldehyde and isonicotinic acid hydrazide to attain the desired product **A30** in ethanol (scheme-30)[45].

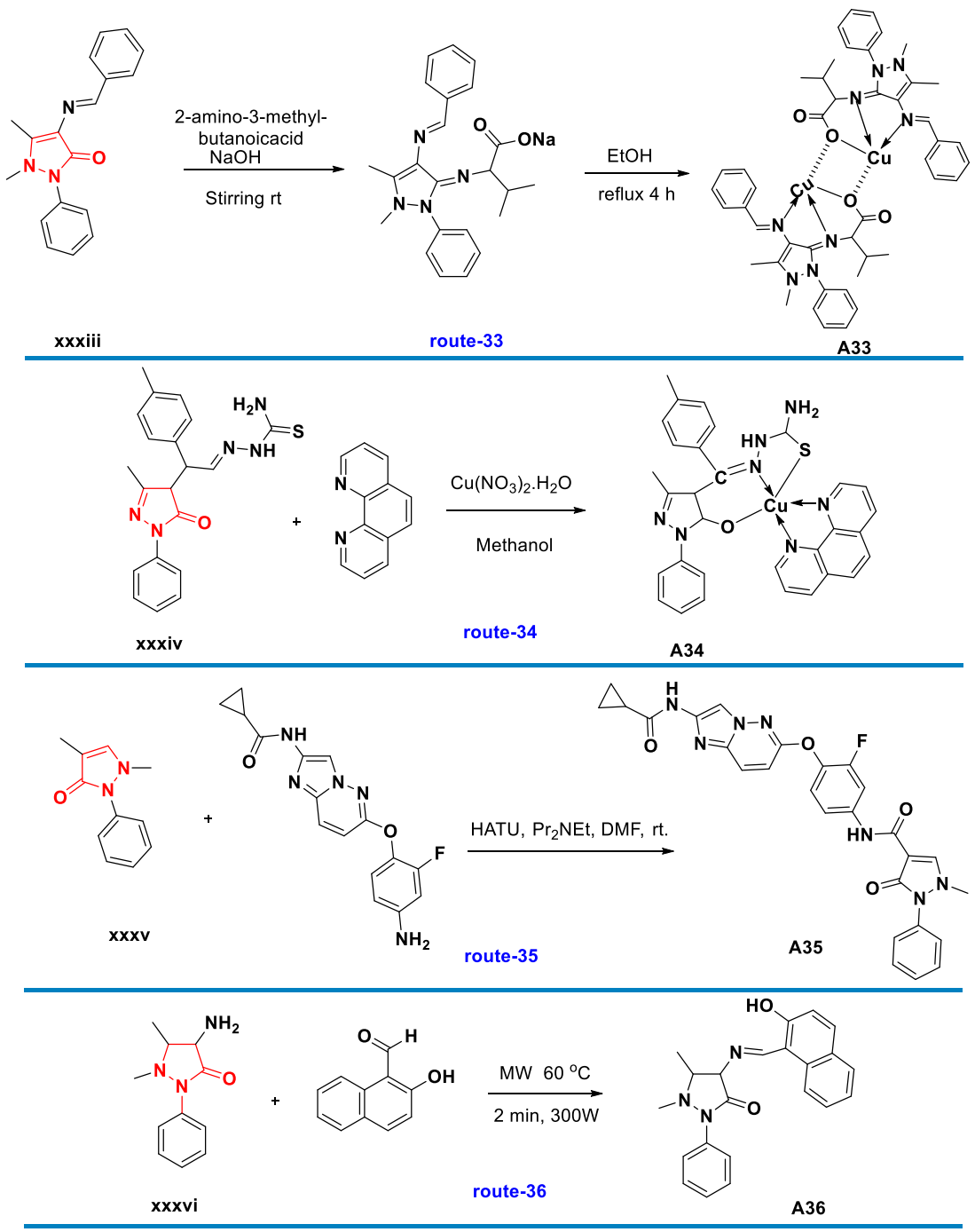


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

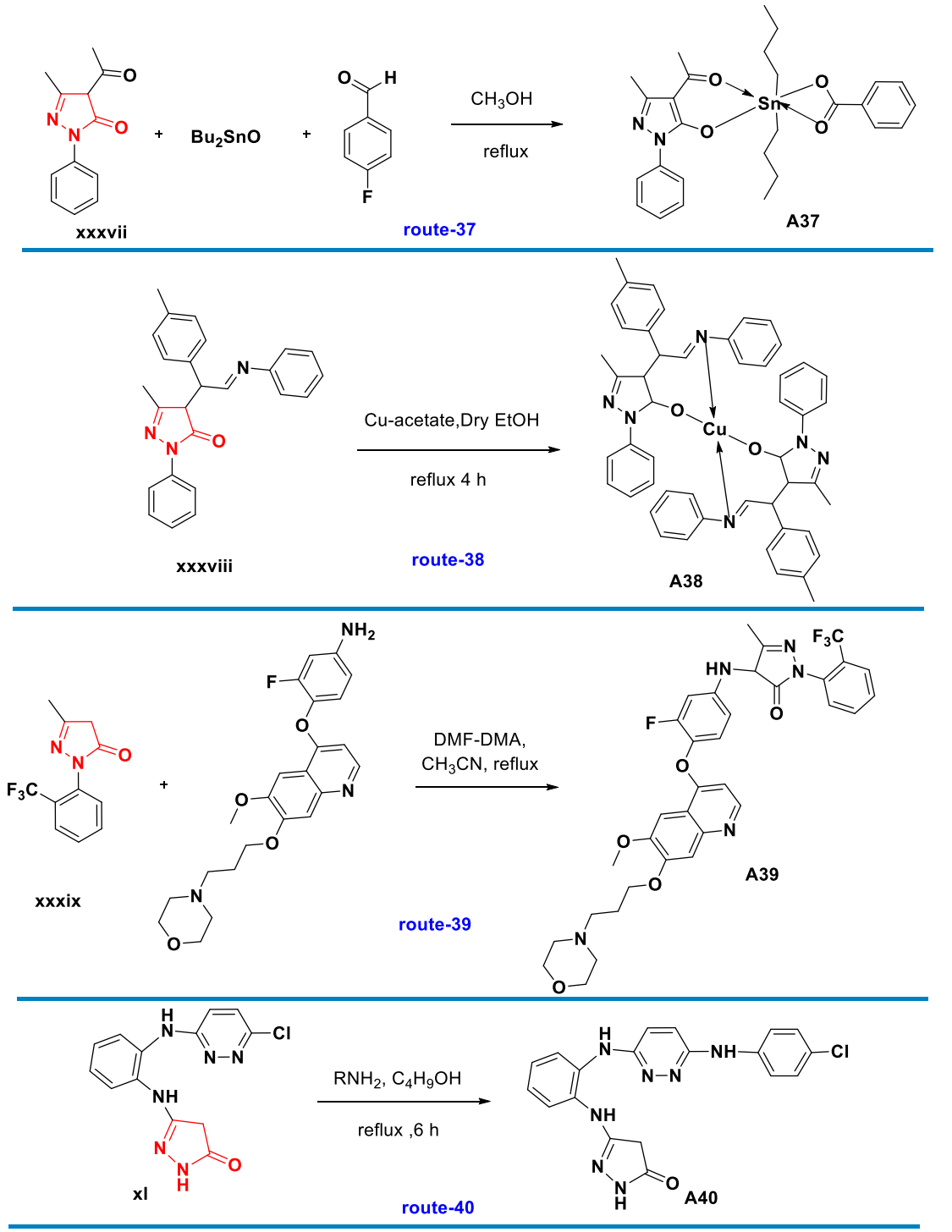


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Ragab and co-workers accomplished the desired product **A31** by allowing oxidation of **xxxi** with65% sulphuric acid under reflux conditions (scheme-31)[46]. Khloya et al. introduced Knovenagel condensation of **xxxii** with4-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide to attain **A32** in ethanol (scheme-32)[47]. Raman and co-workers

attempted the reaction between **xxxiii** and metal salt in ethanol to attain the desired product **A33** (scheme-33)[48]. Vyas and co-workers accomplished the target product **A34** by allowing the reaction of **xxxiv** with 1,10-phenanthroline in methanol containing catalytic amount of $Cu(NO_3)_2.3H_2O$ (scheme-34)[49]. Matsumoto et al. reported the coupling reaction of **xxxv** with*N*-(6-(4-amino-2-fluorophenoxy)imidazo[1,2-*b*]pyridazin-2-yl)cyclopropanecarboxamide to afford **A35** in dimethylformamide comprising small catalytic quantity of HATU as coupling reagent (scheme-35)[50]. Bensaber and co-workers obtained the desired product **A36**, by nucleophilic reaction of **xxxvi** withhydroxy-1-naphthaldehyde under microwave irradiation conditions (scheme-36)[51]. Zhao and co-workers successfully carried out the condensation reaction of **xxxvii** with4-fluorobenzaldehyde, and dibutyl stannous oxide (Bu_2SnO) to attain **A37** in methanol(scheme-37) [52]. Vyas and co-workers attempted and achieved the reaction between **xxxviii** and copper acetate in ethanol to afford **A38** (scheme-38)[53]. Zhou et al. obtained the target product **A39** via treating **xxxix** with3-fluoro-4-((6-methoxy-7-(3-morpholinopropoxy)quinolin-4-yl)oxy)aniline in DMF-DME (scheme-39)[54]. Khalil and co-workers reported nucleophilic substitution reaction of **xl** with aryl amines to afford **A40** in butanol (scheme-40)[15].

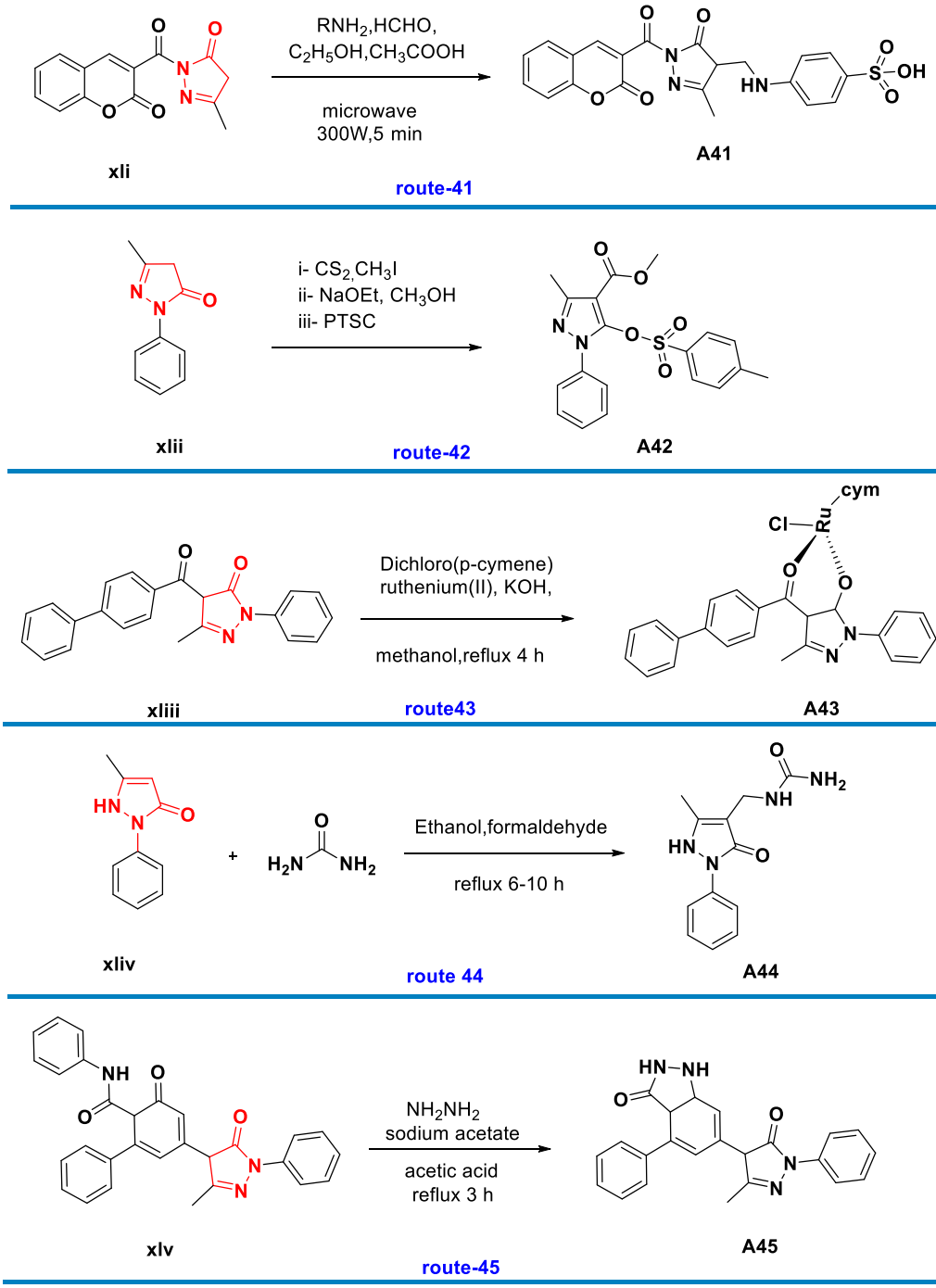


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

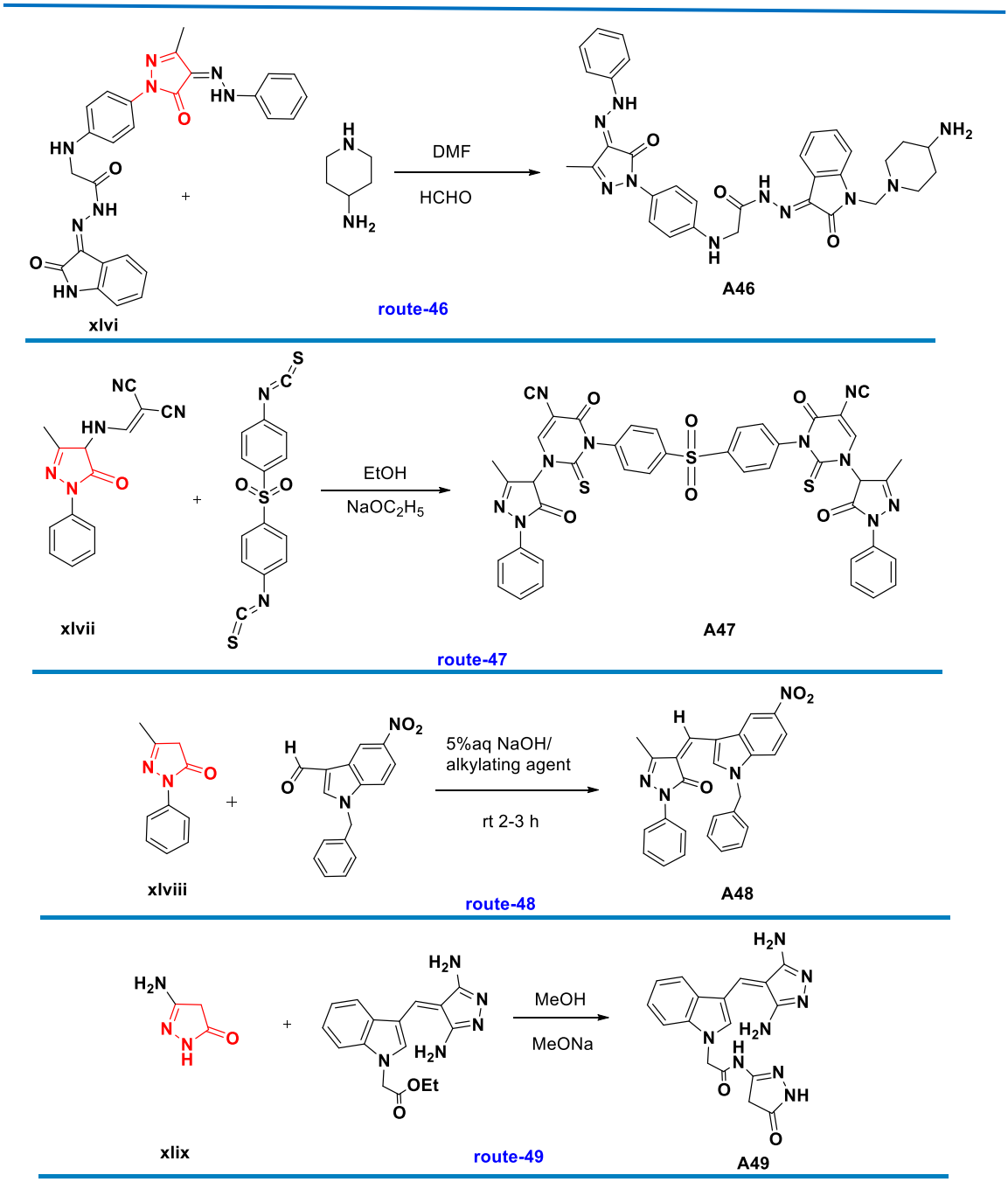


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Sivakumar and co-workers have accomplished the desired product **A41**by treating **xli** with primary amine and formaldehyde in presence of acetic acid in ethanol under microwave irradiation conditions (scheme-41)[8]. Tewari and co-workers acquired the final compound **A42** by treating **xlii** with carbon disulphide and sodium ethoxide in methanol (scheme-42)[55]. Pettinari et al. blended **xliii** and dichloro(*p*-cymene)ruthenium (II), KOH in methanol to give **A43** (scheme-43)[56]. Dube and co-workers offered synthesis of **A44**by employing reaction of **xliv**

with urea and formaldehyde (scheme-44)[57]. Abbady and co-workers reported cyclo-condensation reaction of **xlv** with hydrazine hydrate to afford **A45**in acetic acid containing catalytic amount of sodium acetate (scheme-45)[58]. Kanchana and co-workers carried out the Mannich reaction of **xlvi** with piperidin-4-amine and formaldehyde to attain **A46**in dimethylformamide (scheme-46)[59]. Ghorab and co-workers attempted the reaction between **xlvii** and4,4-sulfonylbis(isothiocyanatobenzene) to achieve **A47** in presence of sodium ethoxide in ethanol (scheme-47)[60]. Indrasena and co-workers have reported the nucleophilic substitution reaction of **xlviii** with 1-benzyl-5-nitro-1*H*-indole-3-carbaldehyde to attain the desired product **A48** in aqueous sodium hydroxide (scheme-48)[61]. Mandour and co-workers reported the synthesis of final compound **A49** by allowing the reaction between **xlix** and ethyl 2-(3-((3,5-diamino-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)acetate in methanol (scheme-49)[62]. Rani and co-workers obtained the desired product **A50** by treating **1** with diethyl phosphonate in anhydrous toluene under reflux conditions (scheme-50)[63].

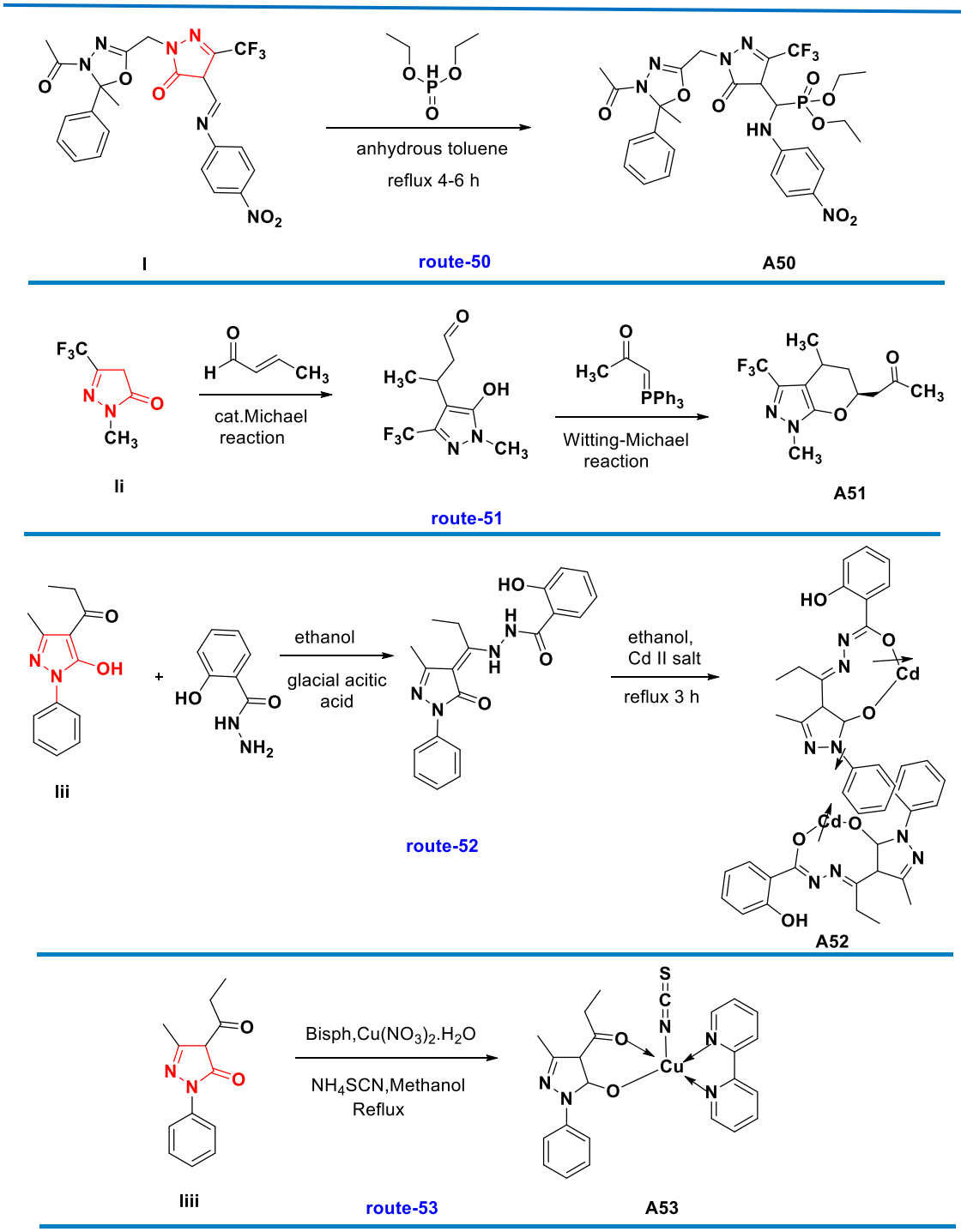


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

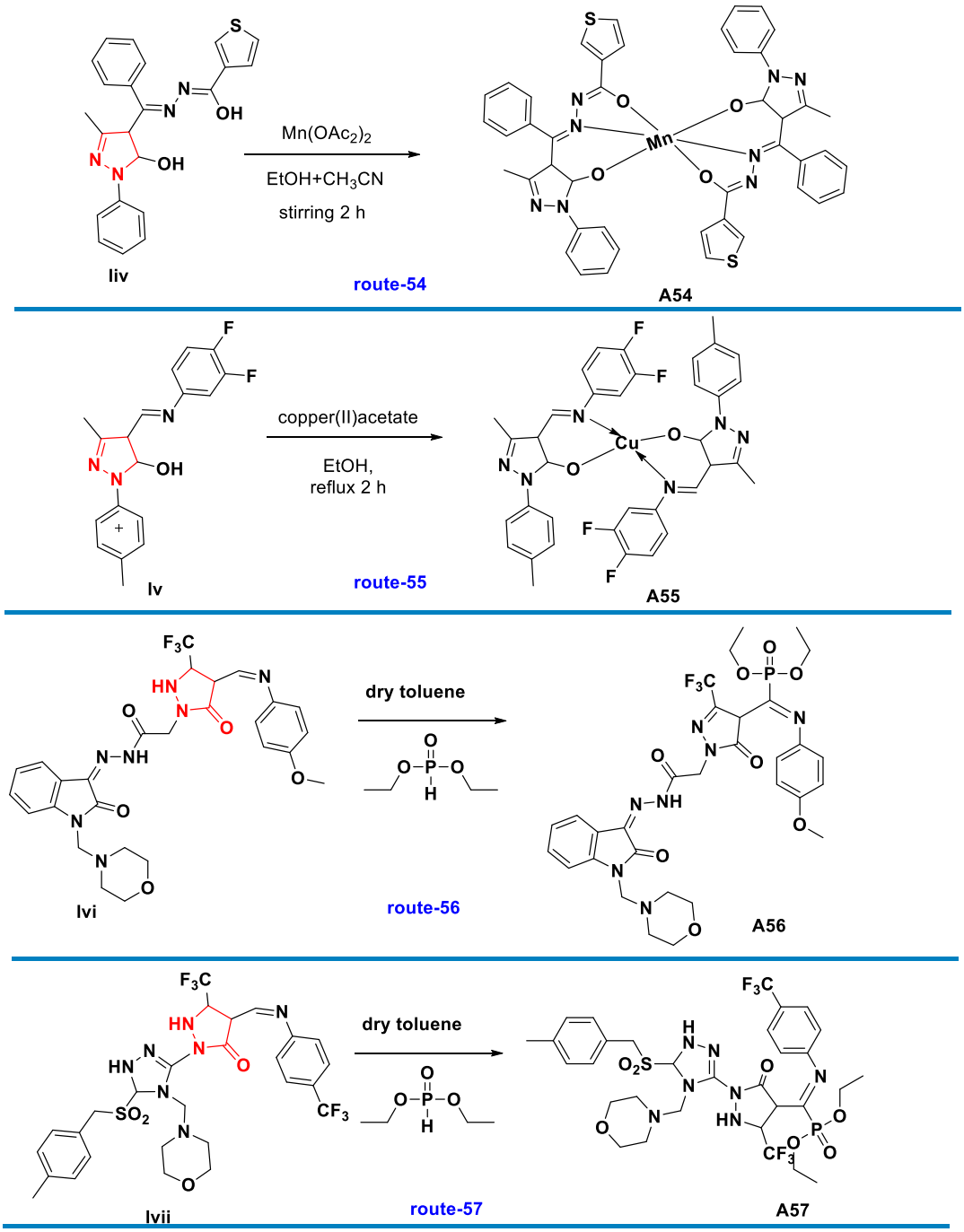


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Zhang and co-workers reported Witting-Michael reaction of **li** with 1-(triphenyl-l5-phosphanylidene) propan-2-one (*E*)-but-2-enal to afford **Ali**as final compound (scheme-51)[13]. Zhao et al. attained the desired product **A52** by allowing the reaction between **lii** and(*Z*)-2-hydroxy-*N*-(1-(3-methyl-5-oxo-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)propyl) benzohydrazide and Cd(II) salt in ethanol containing glacial acetic acid (scheme-52)[64]. Vyas

and co-workers successfully carried out the reaction of **liii** with 2,2-bipyridyl, NH_4NCS and $Cu(NO_3)_2.3H_2O$ to achieve **A53** in ethanol and acetonitrile under reflux conditions (scheme-53)[65]. Li and co-workers accomplished the desired product **A54** by stirring **liv** with $Mn(OAc_2)_2$ in ethanol and acetonitrile (scheme-54)[66]. Joseph and co-workers attempted the reaction between **lv** and copper (II) acetate to afford **A55** under reflux conditions in ethanol (scheme-55)[9]. Subbareddy et al. obtained the final compound **A56** by allowing the reaction between **lvi** and diethyl phosphonate in dry toluene (scheme-56)[63]a. Subbareddy and co-workers attempted and achieved the reaction between **lvii** and diethyl phosphonate to attain the desired product **A57** in dry toluene (scheme-57)[67]b. Yan and co-workers reported nucleophilic substitution reaction of **lviii** with 5-aminoisophthalic acid to achieve **A58** in ethanol under reflux conditions (scheme-58)[68]. Babur and co-workers obtained final compound **A59** via treating **lix** with*N*-methyl-2-pyrrole carboxaldehyde in ethanol containing catalytic amount of piperidine (scheme-59)[69]. Yi and co-workers reported cyclization reaction of **lx** with hydroxylamine hydrochloride to afford **A60** in presence of potassium carbonate as base (scheme-60)[70].

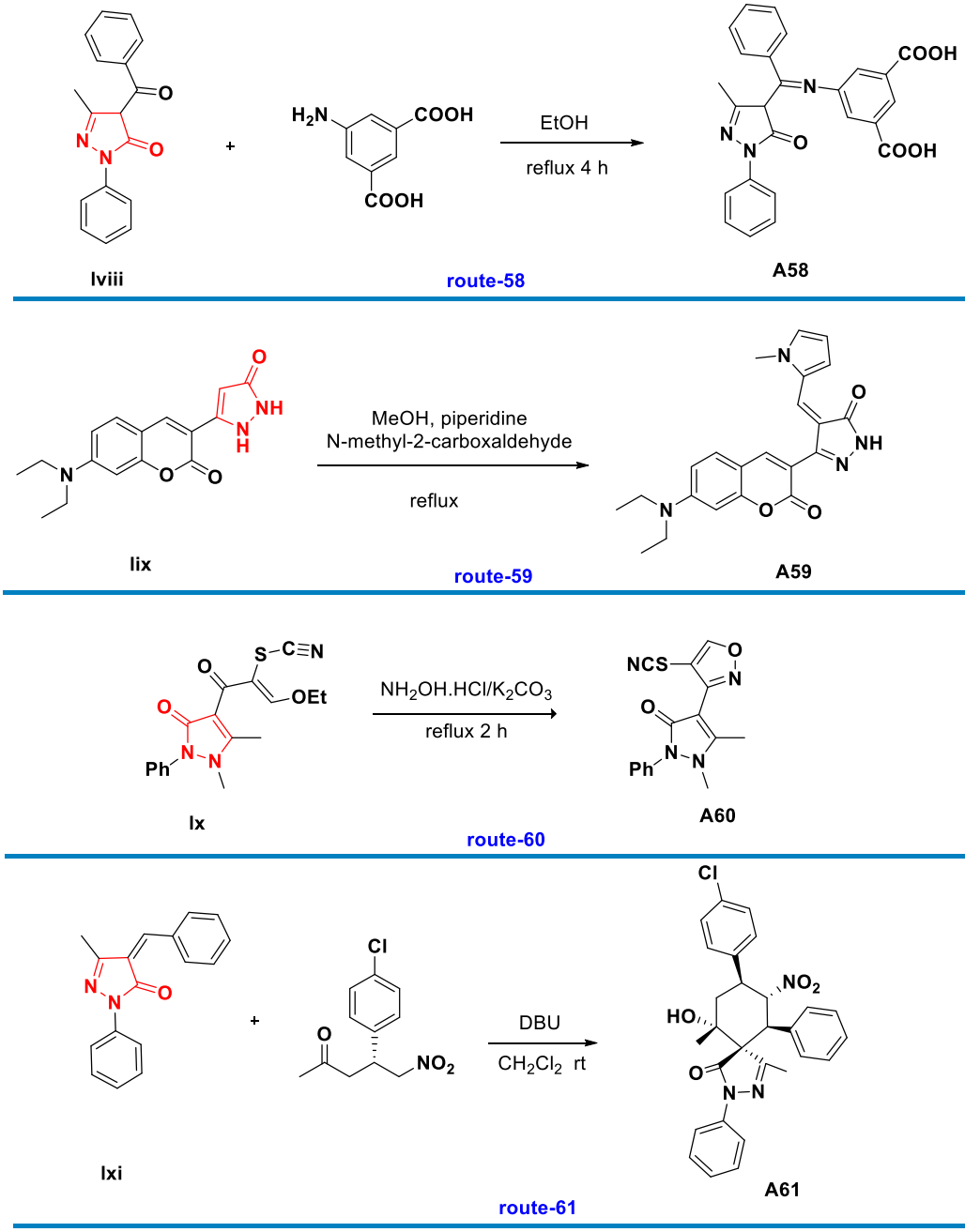


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

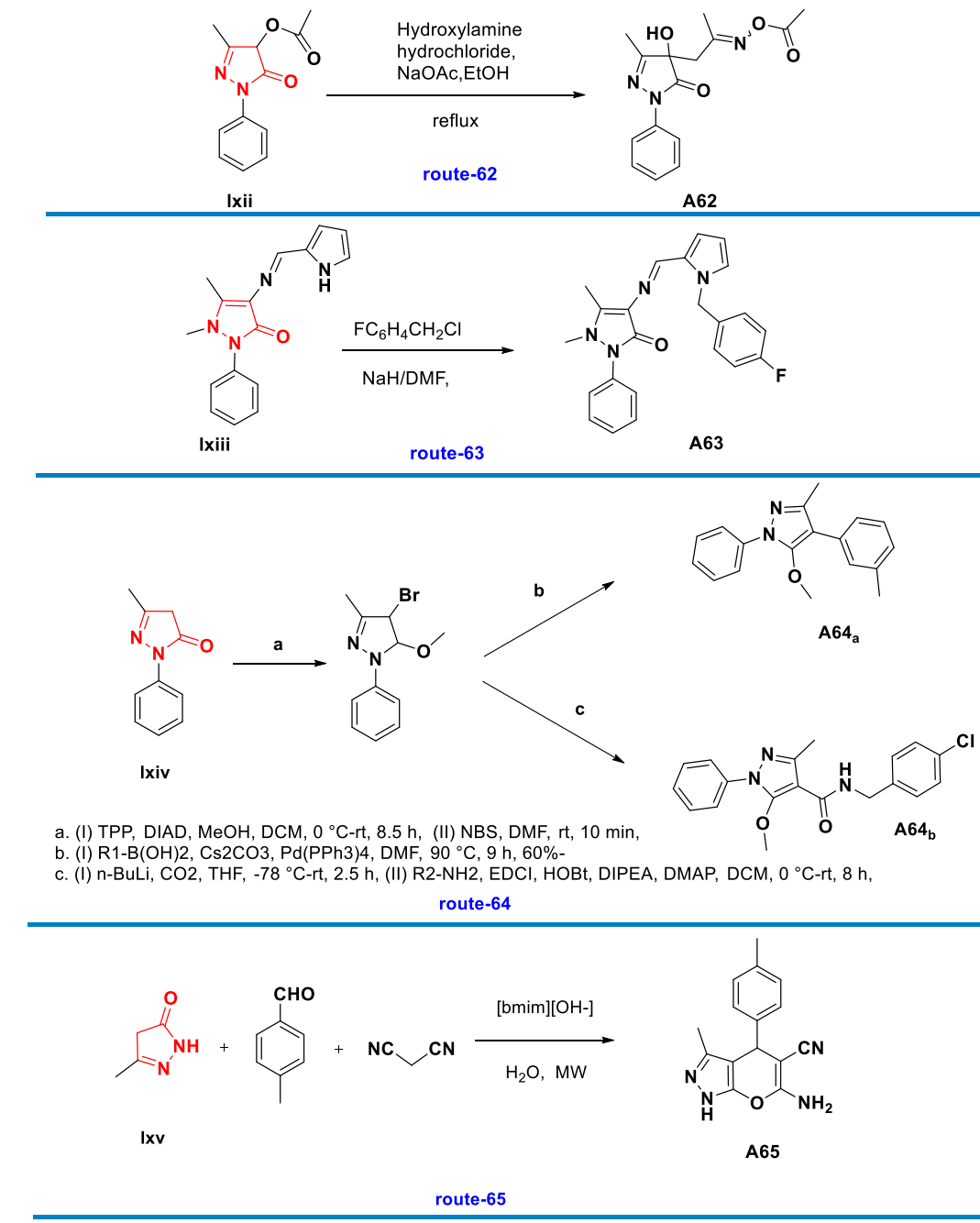


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

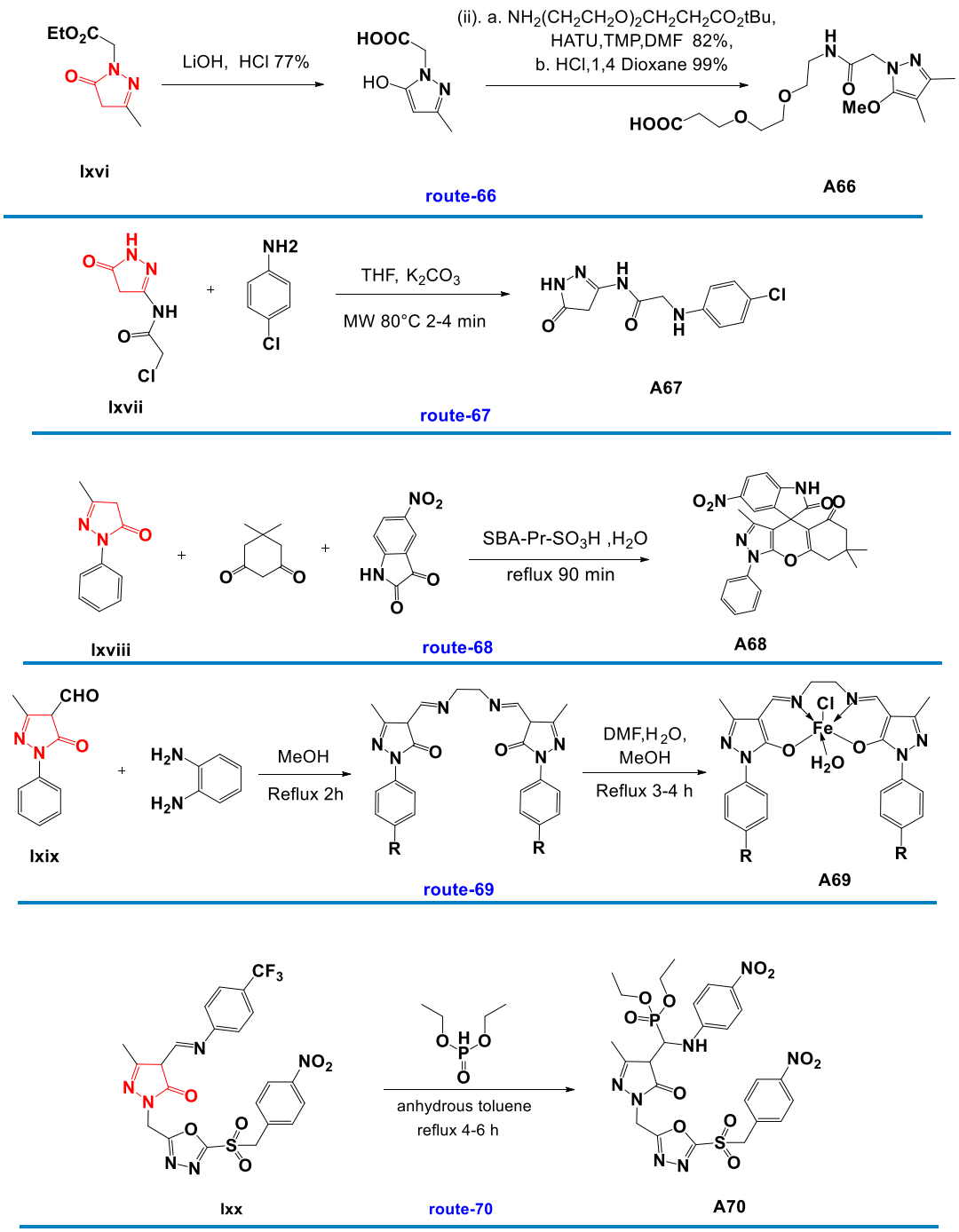


Figure 2. (continued) Synthetic strategies of substituted pyrazolone derivatives.

Zhou and co-workers attempted aldol condensation of **lxi** with (R)-4-(4-chlorophenyl)-5-nitropentan-2-one to afford **A61** in dichloromethane containing catalytic quantity of 1,8-Diazabicyclo[5.4.0]undec-7-ene at room temperature (scheme-61)[71]. Masumoto and co-workers acquired **A62** by treating 3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl acetate

with hydroxylamine hydrochloride (**lxii**), sodium acetate in ethanol under reflux conditions (scheme-62)[72]. Mochona et al. obtained final compound **A63** by allowing the reaction between (*E*)-4-(((1*H*-pyrrol-2-yl)methylene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (**lxiii**) and 1-(chloromethyl)-4-fluoro benzene in DMF under reflux conditions (scheme-63)[73]. Polkam and co-workers accomplished the desired product **A64$_a$** via treating 4-bromo-5-methoxy-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazole (**lxiii**) with 4-methylphenylboronic acid ,cesium carbonate and tri phenyl palladium phosphate, and **A64$_b$**acquired by reacting with 4-chlorobenzylamine, EDCI, HOBt, DIPEA, DMAP at 0 °C to room temperature (scheme-64)[74]. Aliabadi et al. reported one-pot synthesis of **A65** by reacting **lxv** with 4-methyl benzaldehyde and malononitrile in water having catalytic amount of 1-butyl-3-methylimidazolium hydroxide under microwave irradiation conditions (scheme-65)[75]. Kudirka and co-workers have synthesized the target molecule **A66** through Knoevenagel condensation of 2-(5-hydroxy-3-methyl-1*H*-pyrazol-1-yl)acetic acid (**lxvi**) with *tert*-butyl 3-(2-(2-aminoethoxy)ethoxy)propanoate by using HATU, TMP as coupling reagents in DMF (scheme-66)[76]. Krishnasamy and co-workers reported nucleophilic substitution reaction of **lxvii** with 4-chloro aniline to afford **A67** in tetrahydrofuran under microwave irradiation conditions (scheme-67)[77]. Ziarani et al. have reported one-pot synthesis of **A68** by reacting **lxviii** with isatin, dimedone and SBA-Pr-SO_3H in water (scheme-68)[78]. Suratil and co-workers have accomplished the desired product **A69** by reacting **lxix** with o-phenylenediamine and FeCl3·6H2O in DMF under reflux conditions (scheme-69)[79]. Rani and co-workers reported Kabachink-Fields reaction of **lxx** withdiethyl phosphonate to afford **A70** in anhydrous toluene and diethyl phosphonate (scheme-70)[80].

3. Pharmacological activity

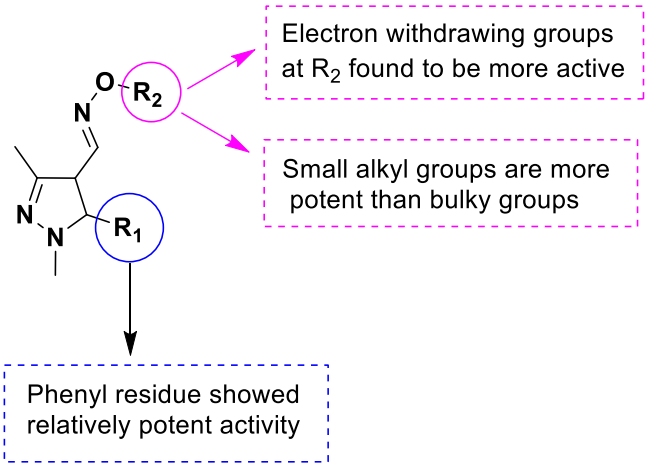
An extensive overview of thepyrazolone moietyhas shownthat 2,4-Dihydro-3*H*-pyrazol-3-one exhibit potential pharmacophore properties against several life-threatening diseases such as cancer, inflammation, bacterial and fungal infections, tuberculosis, etc. The following discussions demonstrate the medicinal properties of 2,4-dihydro-3*H*-pyrazol-3-oneagainst various biological targets. It is quite evident that variations of the functional groups on this scaffold bring diverse biological activities.

3.1. Anticancer activity

Cancer is a multifaceted disease caused by abnormal growth of cells. According to the tissue or organ where it appears, more than 100 types of cancers are existing. A few cancer cells do not have the ability to spread from one part to the other but, most of the cancer cells spread easily from one to another tissue or organ. Most of the cancers caused by tobacco, alcohol, physical inactivity, infectious agents and hereditary [81]. It is a second leading cause of the death

worldwide and affecting every region. Despite the discovery of large number of anticancer drugs, there is a great need for more effective and potent anticancer therapy to improve the current cancer treatment worldwide.

In 2005**,** Park et al. prepared a series of pyrazole oxime ether derivatives and evaluated for their anticancer activity against a panel of cancer cell lines. Among the series, compounds **1** and **2** exhibited potent activity against four cell lines and compounds **3** and **4** having 5-phenoxypyrazole moiety displayed potent antitumor activity against XF 498 and HCT115cell lines with an IC_{50} values 0.02 and 0.01 µM respectively. A brief SAR Study revealed that electron withdrawing groups at oxime position increased the activity and also small alkyl groups were more tolerated than bulkier groups in combination with phenyl moiety at the C-3 position of the parent nucleus as presented in **Figure 3**[16].

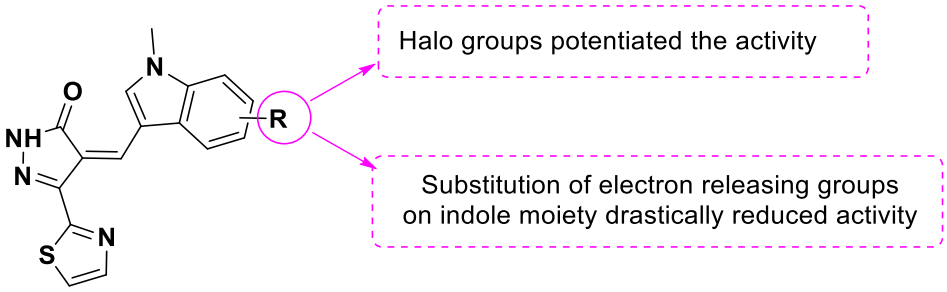


C. No.	R_1	R_2	IC_{50}(µM)							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	OPh	CH_2Ph-4-Me	8.4	38.2	17.9	29.1	NA	NA	NA	NA
2	OPh	CH_2Ph-4-CF_3	5.8	30.5	11.0	34.7	NA	NA	NA	NA
3	OPh	CH_2Ph-4-$C(CH_3)_3$	NA	NA	NA	0.12	0.21	13.27	0.04	0.02
4	OPh-4-Cl	CH_2Ph-4-$C(CH_3)_3$	NA	NA	NA	0.10	0.28	18.78	0.02	0.01

1: HepG2; 2: MCF7; 3: MKN45; 4: A549; 5: SKOV-3; 6: SKMEL-2; 7: XF498; 8: HCT115 and NA: no activity.

Figure3. Antitumor activity and cytotoxicity of 5-phenoxypyrazole compounds.

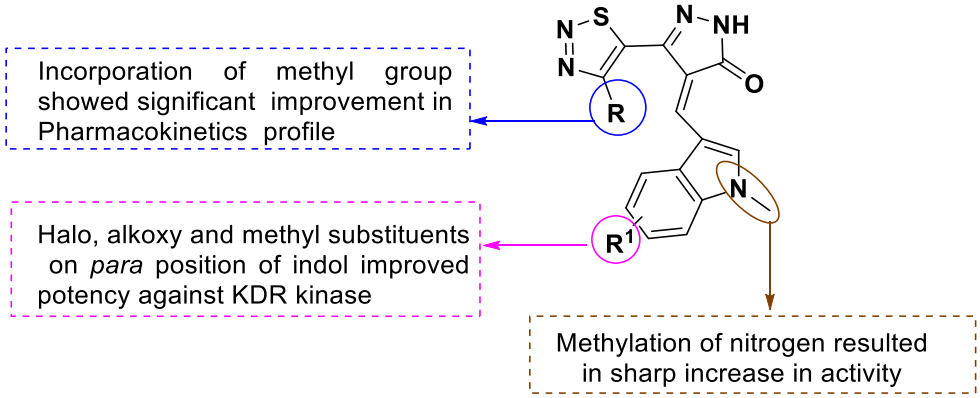
In 2006, Tripathy et al. synthesized a series of indolyl-pyrazolone derivatives and evaluated their pharmacological activity as VEGFR inhibitors. From the tested series, compound **5** and **6** having bromo and chloro substitution on indole moiety exhibited significant inhibition against VEGFR-2 in a nano molar range with an IC_{50} values 6 µM and 9 µM. Further from SAR, it was observed that presence of halo groups (Cl, Br and F) on indole moiety was crucial for significant activity as described in **Figure 4**[17].



C. No	R	VEGFR-2 IC_{50} (nM)
5	4-Br	6
6	4-Cl	9

Figure 4.VEGFR-2 activity of pyrazolone-indole derivatives.

In 2007**,** Tripathy et al. described the synthesis of pyrazolone-based compounds by the condensation of pyrazolone, indole and thiadiazole. All the synthesized compounds were biologically tested against VEGFR-2/KDR kinase inhibition. Among the tested series, compounds **7** and **8**exhibited potent activity profile against KDR kinase. In addition, *in vitro* studies were carried out for highly active compound **7**in a rat model and showed improved pharmacokinetic profile. From the SAR, it was noticed that the presence of halo, alkoxy and alkyl substituents on indole moiety was essential for activity. Also observed that methylation of indole nitrogen and incorporation of methyl group on thiadiazole moiety resulted in increased pharmacokinetic profile in rat model as depicted in **Figure 5**[18].

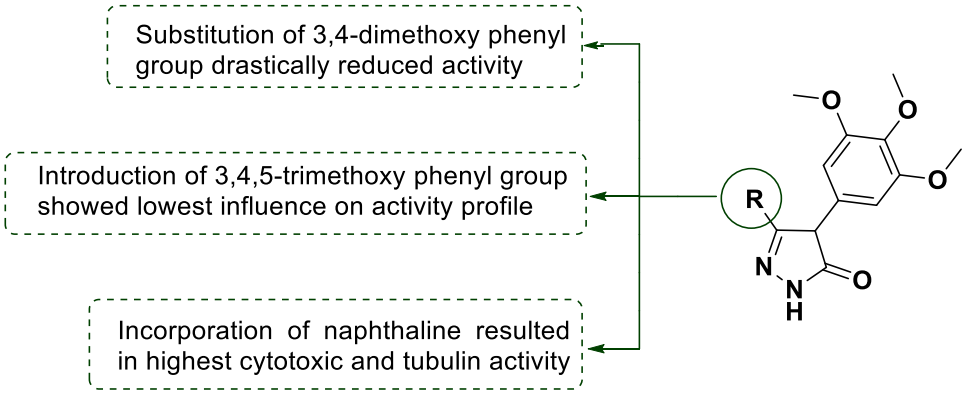


C.No.	R	R^1	KDR kinase IC_{50}(nM)	Pharmacokinetics in rat			
				AUC (ng*h/mL)	T1/2 (h)	Vd (L/kg)	CL (mL/min/kg)
7	CH_3	4-OCH_3	19	192	0.3	2.2	87
8	H	4-Br, 5-OCH_3	6	NA	NA	NA	NA

NA: No activity

Figure 5. 1,2,3-Thiadiazole substituted pyrazolones as potent KDR/VEGFR-2 kinase inhibitors.

In 2010**,** Burja et al. reported the synthesis of pyrazolone-fused combretastatins and their precursors as anti-proliferative and anti-tubulin agents. All the synthesized compounds were evaluated for cytotoxic, tubulin polymerization inhibitory activity and anti-proliferative activity against various cancer cell lines. From the series, compounds **9**, **10**, **11** and **12** displayed significant activity profile, in particular compound **12** having naphthalene on pyrazolone motif showed highest cytotoxic activity (IC_{50} = 0.048 ± 0.001 μM) and tubulin inhibitory activity with 97%. A brief SAR study indicated that, presence of naphthalene group on pyrazolone motif was responsible for potent activity than 3,4-dimethoxy or 3,4,5-trymethoxy phenyl groups as represented in **Figure 6**[82].

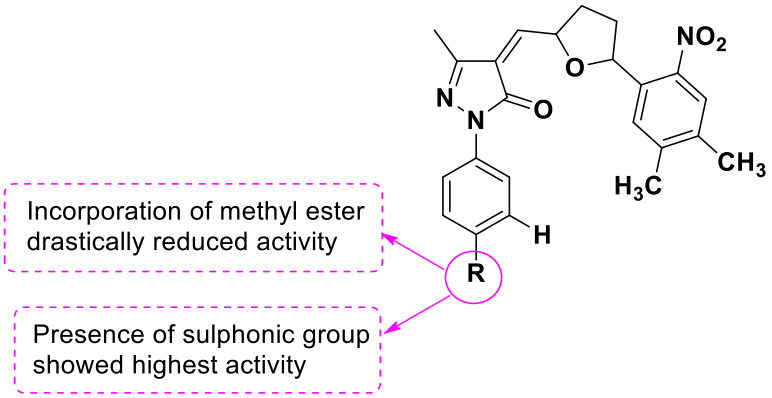


C.No.	R	Cytotoxicity		Antiproliferative activity (IC_{50}, lM ± SD)						
		IC_{50} (µ/M)	% TPI	HeLa	HEp-2	CK2	RT-112	T-24	MIA PaCa-2	SW620
9	3-hydroxyl,4-methoxy Ph	0.176	99	0.176 ±0.011	1.142 ± 0.138	0.709 ± 0.120	2.083 ± 0.074	0.619 ± 0.075	0.462 ± 0.078	0.543 ± 0.175
10	3-floro,4-methoxy Ph	0.158	100	0.158 ± 0.024	1.179 ± 0.236	0.684 ±0.238	3.319 ± 1.932	1.154 ± 0.589	0.556 ± 0.059	0.487 ± 0.188
11	4-methoxy Ph	0.152	100	0.152 ± 0.020	0.529 ± 0.081	0.615 ±0.277	1.888 ± 0.116	1.156 ±0.662	0.575 ± 0.025	0.455 ± 0.176
12	Naphthalene	0.114	98	NA	NA	NA	NA	NA	NA	NA

NA: No activity

Figure 6. Anti-proliferative and tubulin polymerization inhibitory activity of pyrazolone-fused combretastatins.

In 2010, Bowersand co-workers reported the synthesis and molecular docking of polyaromatic pyrazolone analogues by virtual ligand screening of commercially available histone acetyltransferase inhibitor C646. All the synthesized compounds were biologically evaluated for p300 HAT inhibition and cellular histone acetylation on melanoma and lung cancer lines along with reference compound C646. Among the series, compound **13** displayed prominent activity. **Figure 7**. concisely presents the SAR studies and p300 HAT inhibition for the potent compound **13** and reference molecule C646[25].

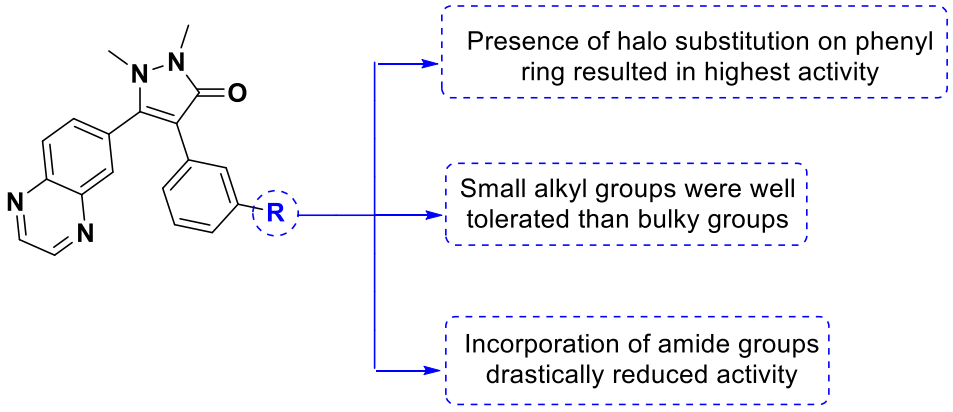


C.No.	R	p300 HAT Inhibition relative to C646 IC_{50} (µM)
13	SO_3H	0.8

C646	CO_3H	1

Figure7. Anti poliferative activity of polyaromatic pyrazolones.

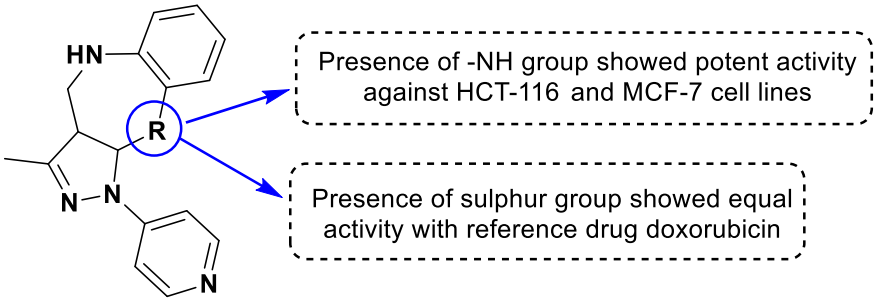
Kevin Guckian et al. in 2010 described synthesis, biological properties and molecular docking studies of novel pyrazolone derivatives containing various meta-substituted phenyl groups. All the synthesized compounds were biologically tested against TGF*β*R1 kinase enzyme. Among the tested series, compounds**14**, **15** and **16** with halo substitution on phenyl ring (4-(3-bromophenyl)-1,2-dimethyl-5-(quinoxalin-6-yl)-1,2-dihydro-3*H*-pyrazol-3-one) exhibited potent TGF*β*R1 kinase activity. Further, from molecular docking studies, it was observed that carbonyl group of pyrazolone interacted with Lys 232. A brief SAR study revealed that presence of electron withdrawing groups on *meta* position of phenyl ring were essential for kinase activity than electron releasing groups as depicted in **Figure 8**[83].



C.No.	R	TGFbRI kinase activity	
		K_i (µM)	PAI-Luc IC_{50} (µM)
14	-Br	0.012	0.22
15	F	0.078	0.99
16	Cl	0.019	1.9

Figure 8. TGFβR1 kinase inhibition of pyridyl and hetero aromatic pyrazolone moieties.

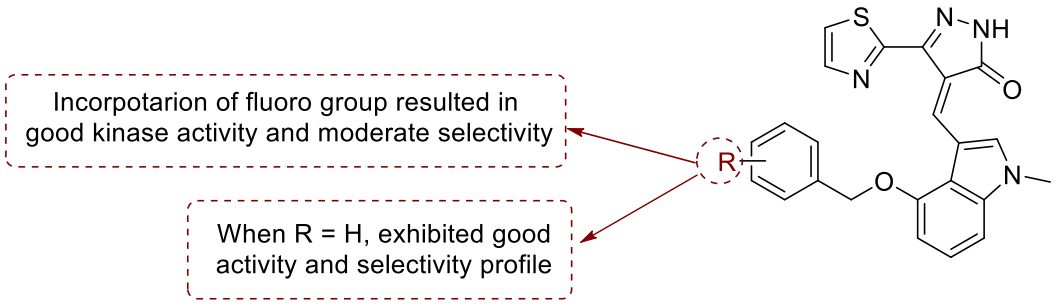
El-Baih and co-workers in 2011 described the synthesis and biological activity of tetrahydrobenzo pyrazolo diazepineand benzo pyrazolo thiazepine derivatives as anti-cytotoxic agents. Most of the tested compounds exhibited good activity profile against MCF-7, HEPG-2 and HCT-116 cell lines. In particular, compounds **17** and **18** displayed best activity results as compared to reference drug doxorubicin. A brief SAR study revealed the importance of functional groups around the scaffold as depicted in **Figure 9**[27].



C.No	R	Antitumor activity Cytotoxicity IC_{50} (µ/mL)		
		HEPG-2	HCT-116	MCF-7
17	NH	11.20	2.82	3.12
18	S	17.30	10.70	2.97
D**oxorubicin**		5.50	3.74	2.97

Figure 9. Antitumor activity of tetrahydrobenzo pyrazolo diazepine and tetrahydrobenzo pyrazolo thiazepine derivatives.

In 2011, Tripathy et al. described the synthesis of benzyloxy/thiadiazole pyrazolone compounds as ALK kinase inhibitors. Among the series, compounds **19** wasfound to have enhanced potency for ALKand selectivity for VEGFR2 kinases. In addition, compound **19** showed broad kinome selectivity and liver microsome stability along with reasonable pharmacokinetic results in rat model. A brief SAR study depicted in **Figure 10** indicated the presence of thiazole moiety towards potent activity [26].

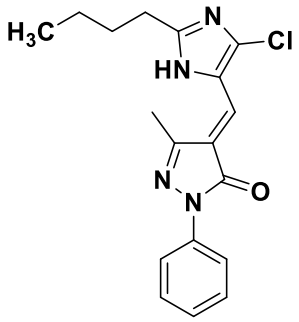


C.No.	R	ALK IC_{50} (nM)	Karpas-299 cell IC_{50}(nM)
19	Thiazole	46	300

Figure10. Anticancer activity of benzyloxy pyrazolone derivatives.

In 2012, Chobe et al. synthesized a series of pyrazolone derivatives and studied the interaction with calf thymus DNA by using thermal denaturation, electronic spectra and viscosity

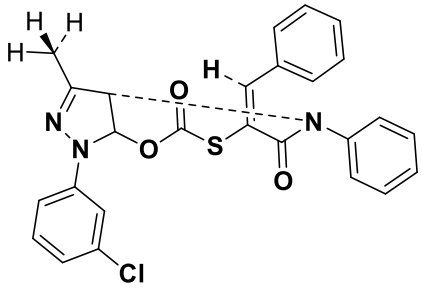
measurement. Further, molecular docking studies revealed that the preferred mode of compound **20** binded with double helical DNA as well as preferable DNA groove. **Figure 11** represents the docking data of synthesized compound [84].



C.No.	Gliding docking score(Kcal/mol)		
	226D (AT rich)	**IQCI (GC rich)**	**2KOV(mixed)**
20	-7.644	-7.00 (DG-6,DC-15)	-5.830 (DC-18, DG-7 and DG-7,B)

Figure 11. Molecular interaction data of pyrazolone derivative with calf thymus DNA

Patel et al in 2012 described the synthesis of spiro pyrazoline compounds by incorporating a fused furan ring containing reactive methylene group. All the synthesized compounds were tested against a panel of 60 cancer cell lines. Among all, compound **21** exhibited potent activity against renal cancer cell line. **Figure 12** represents the percentage growth inhibition of Renal cancer cell line of compound **21** [30].

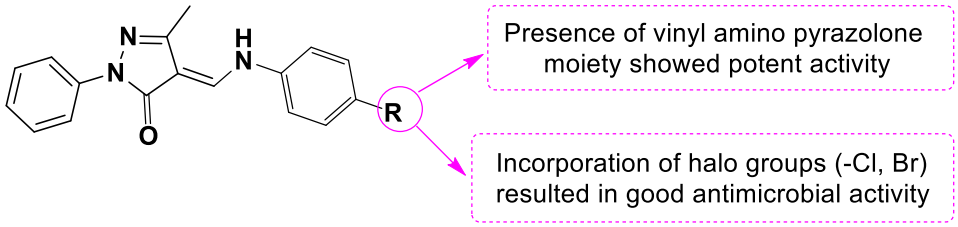


C. No	60 cell line assay in one dose at 10-5 concentration			
	Mean growth %	Range of growth %	Most sensitive cell line	Growth of most sensitive cell line %
21	104.54	-17.68 to 44.00	Renal cancer	-17.68

Figure 12. Anticancer activity of spiro-pyrazoline compounds containing fused furan ring.

In 2012, Hamama and co-workers reported the synthesis and biological evaluation of some novel isolated or fused heterocyclic compounds consisting pyrazolone moiety. All the synthesized compounds were tested for antimicrobial and anti-tubercular activity. Among all, compounds **22**

and **23** exhibited commendable antibacterial activity as compared to the standard drug ampicillin and also found that these compounds have strong degradative effect on DNA. From SAR study, it was revealed that substitution of functional groups on pyrazolone moiety are necessary for enhanced activity as presented in **Figure 13**[38].



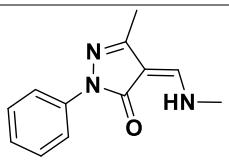
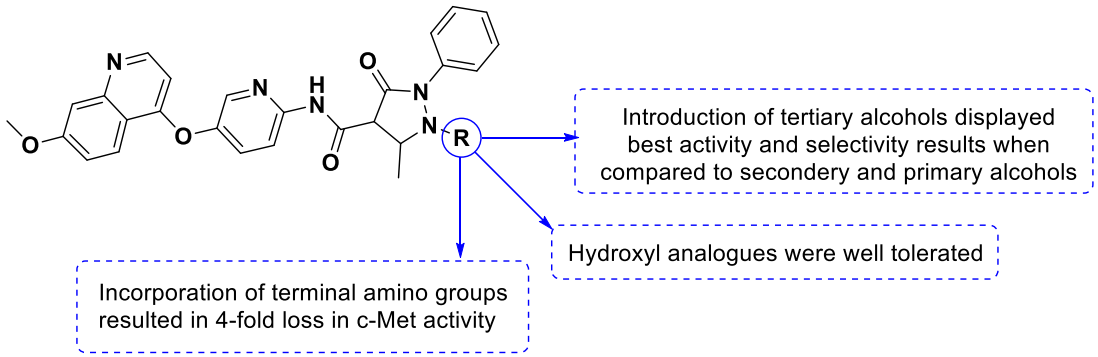
C.No.	R	Degradative effect on DNA	Inhibition zone (mm)	
			Gram positive bacteria *B. subtilis*	Gram negative bacteria *E.coli*
22	Cl	Strong	22	27
23		Strong	20	24

Figure 13. DNA degradative effect and antibacterial activity of pyrazolone containing compounds.

Liu et al. in 2012, reported the synthesis and pharmacological evaluation of pyrazolone derivatives as effective therapeutic agents for c-Met-dependent tumors. From SAR, it was noticed that the presence of tertiary alcohols on pyrazolone moiety are essential for both selectivity and activity. From the tested series, compound **24** displayed potent inhibitory activity and selectivity profiles against c-Met and VEGFR-2 kinases as displayed in **Figure 14**[39].

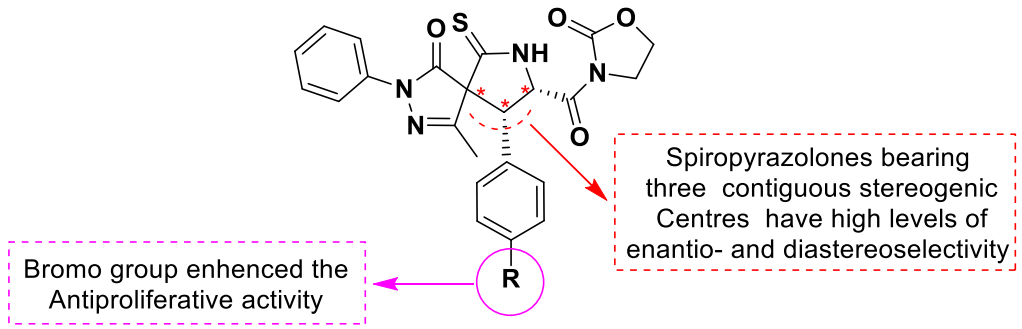


C. No	R	Inhibitory concentration (nM)			
		c-Met	VEGFR-2	IGF-1R	PC-3

		K_i	K_i	Fold	K_i	Fold	IC_{50}
24	2-methylpropan-2-ol	1.0	2430	2422	2150	2144	60.1

Figure 14. Pyrazolone compounds as effective c-Met tumor agents.

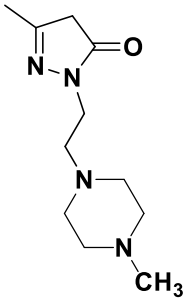
In 2012, Luping Liu et al. have reported library of highly optically active spiropyrazolones. The compounds were evaluated for their cytotoxicity *in vitro* towards human T-cell leukaemia cell line (Jurkat), human cervical cancer cell line (Hela), and human bladder cancer cell line (5637). MTT assay demonstrate that all compounds showed strong antiproliferative activity. Compound **27,**3-((8S,9R)-9-(4-bromophenyl)-1-methyl-4-oxo-3-phenyl-6-thioxo-2,3,7-triazaspiro[4.4]non-1-ene-8-carbonyl)oxazolidin-2-oneexhibited highest antiproliferative activity. The SAR study reveals that spiropyrazolones bearingthree contiguous stereogenic centres have high levels of enantio and diastereoselectivity. Along with these availability of bromo group on phenyl ring of pyrrolidine-2-thione enhanced the potency **Figure 15**[29].



C.No.	**R**	***Invitro* anticancer activity IC_{50} (µM)**		
		Hela	**5637**	**Jurkat**
27	4-Br	38.2 ±4.5	40.7 ±5.1	42.3 ±4.3

Figure 15. *Invitro* anticancer activity of spiropyrazolones.

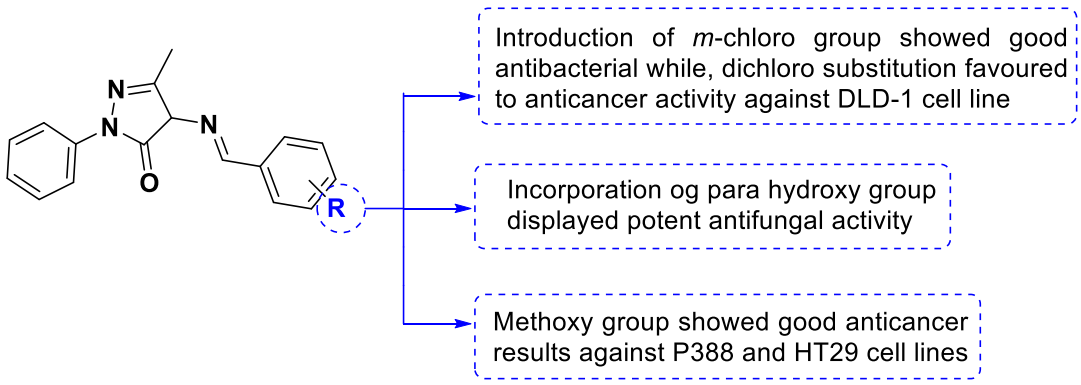
In 2011, Antre and co-workers described the synthesis and biological evaluation of novel pyrazolones as antitumor and antimicrobial agents. All the synthesized compounds were screened against various cell lines and different microbial strains. Among the tested series, compound **25** displayed highest antitumor activity (**Figure 16**) against EAC cells in comparison with 5-fluorouracil as a standard drug [41].



C.No.	Anticancer activity % cytotoxicity	Antibacterial Activity 20 µg/ml			
	EAC cells 150 µg/ml	*E.coli*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
25	47.1	13	14	14	**14**

Figure16. *In-vitro* anticancer and antibacterial activity of substituted pyrazolones.

In 2012, Sigroha and co-workers introduced a series of novel substituted benzylidene-amino dihydropyrazoles and were biologically evaluated for in vitro anticancer and antimicrobial activities. SAR studies indicated that substitution of chloro and hydroxy groups on phenyl ring are responsible for higher activity as depicted in **Figure 17**. From the tested series, compounds **26**, **27** and **28** with *m*-chloro, *p*-hydroxy and methoxy substituents on phenyl ring exhibited prominent activity results [42].

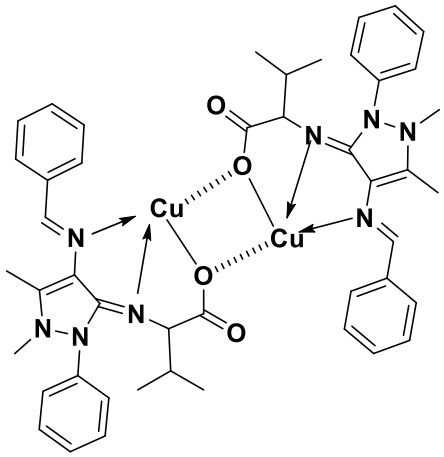


C.No.	R	Antimicrobial activity (pMIC in µM/ml)	Cytotoxicity against human colon and murine leukemia cancer cell lines IC_{50} (µM/ml)

		S. aureus	*B. subtilis*	*E. coli*	*C. Albicans*	*A. niger*	P388	DLD-1	HCT116	HT29
26	3-Cl	1.72	1.72	2.02	2.02	1.42	0.26	-0.14	0.04	0.57
27	4-OH	1.39	1.39	1.39	1.39	1.69	0.27	-0.19	0.19	0.41
28	2-OCH_3	1.41	1.41	1.41	1.41	1.41	0.64	0.03	0.49	1.15

Figure 17. Cytotoxicity and antimicrobial activity of benzylidene-amino dihydropyrazolones.

Raman and co-workers in 2013, described the synthesis of copper (II) and zinc (II) complexes derived from amino acid based pyrazolone derivatives. All synthesized compounds were tested for their binding study with calf thymus DNA by spectroscopic and viscosity methods and oxidative DNA cleavage study with supercoiled pUC19 DNA was carried by using gel electrophoresis. In addition, these compounds were tested against antimicrobial activity and the results of active compound **29**are represented in **Figure 18**. SAR studies indicated that the Schiff base complexes are more favourable to antimicrobial activity than non-schiff base complexes [48].

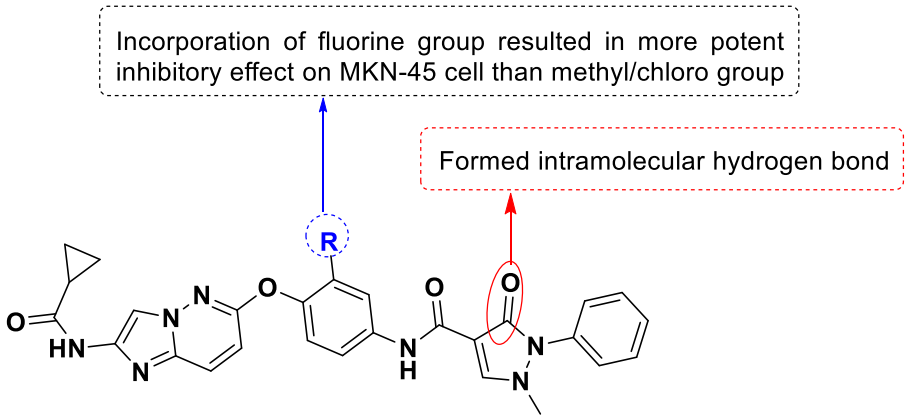


C.No.	**Cytotoxicity EAC cell line**	**Minimum inhibitory concentration(MIC) (×10⁴µM)**							
	GI_{50} (µg/mL)	***S. aureus***	***B. subtilis***	***E. coli***	***K. pneumoniae***	***A. niger***	***F. solani***	***C. lunata***	***R. bataicola***

29	111.31	1.1	1.3	7.0	7.9	1.1	1.4	1.3	1.8

Figure18. Cytotoxic results of metal complex based pyrazolones.

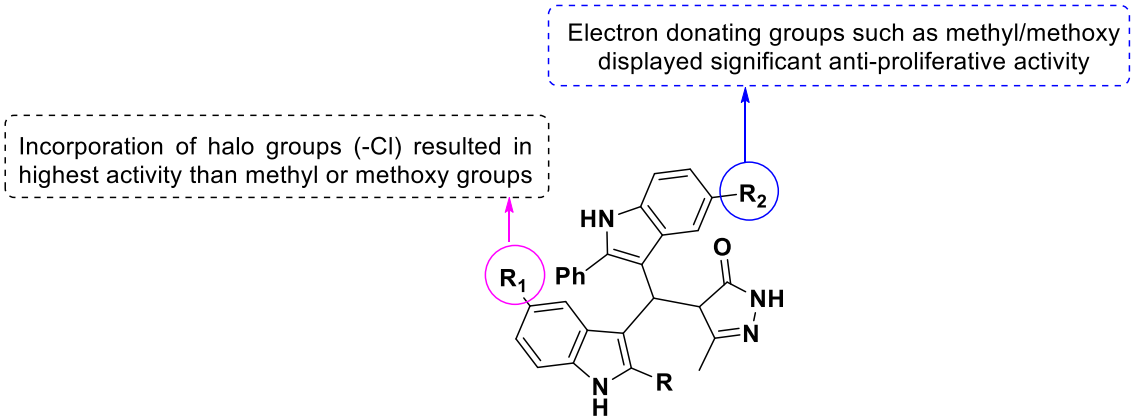
In 2013, Matsumoto and co-workers reported synthesis and pharmacological evaluation of novel pyrazolone and imidazo-pyridazine derivatives as c-mesenchymal epithelial transition factor (c-Met) and vascular endothelial growth factor receptor 2 (VEGFR2) kinase inhibitors. SAR studies presented the significance of carbonyl group on pyrazole moiety for favorable activity as depicted in **Figure 19**. Among all, compounds **30** and **31** having pyrazolone motif displayed significant activity profile against all tested kinase enzymes [50].



C.No.	R	IC_{50} (µ/mL)			
		c-Met	**VEGFR2**	**MKN45**	**HUVEC**
30	H	3.2 (2.8-3.6)	7.1 (3.8-13)	4000 (2100-800)	210 (170-260)
31	F	2.3 (2.1–2.6)	5.6 (5.0-3)	880 (410-1900)	170 (120-240)

Figure19. Anticancer and anti-proliferative activity of pyrazolone derivatives.

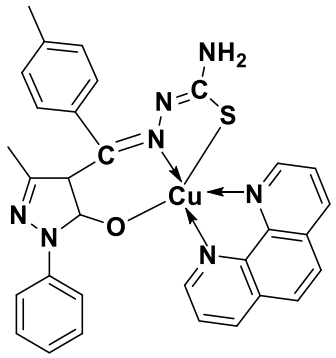
In 2013, Sasidhar and co-workers reported one pot multi-component synthesis of pyrazolones. All the synthesized compounds were screened for *in vitro* cytotoxic and DNA cleavage studies. A brief SAR study revealed that presence of electron donating groups at R-2 position are necessary for potent activity. From the tested series, compounds **32**, **33** and **34** displayed significant cytotoxic activity as compared to standard drug doxorubicin(**Figure 20**)[43].



C.No.	R	R1	R2	*In vitro* cytotoxicity IC_{50} (µM)		
				A-549	HEp-2	HeLa
32	Ph	Me	OMe	1.80	3.50	NA
33	Ph	Cl	OMe	1.10	6.20	1.20
34	H	H	Me	1.30	3.20	2.40

Figure 20. *In vitro* cytotoxic activity of variously substituted pyrazolones.

In 2013, Vyas and co-workers described the synthesis of ternary copper (II) complex derived from a pyrazolone based thiosemicarbazone and 1,10-phenanthroline. All the synthesized compounds were biologically evaluated for anticancer activity (A549 cell line). Among the series, compound **35** showed potent activity against A549 cell line as depicted in **Figure 21**[49].

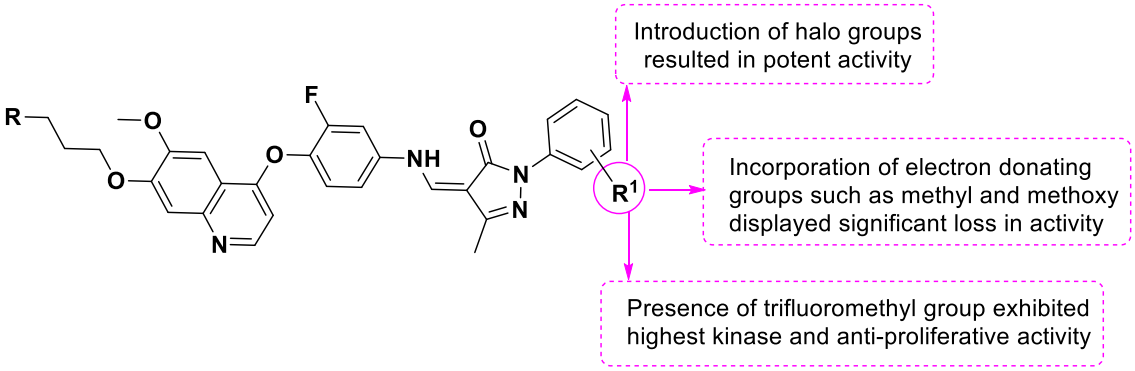


C. No	A549 cell line IC_{50} (µM)
35	0.12

Figure 21. Effect of Cu complex derived pyrazolones on cancer activity

In 2014, Zhou and co-workers reported the synthesis of 6,7-disubstituted 4-phenoxyquinoline derivatives bearing pyrazolone moiety. All synthesized molecules were tested for *in vitro* c-Met

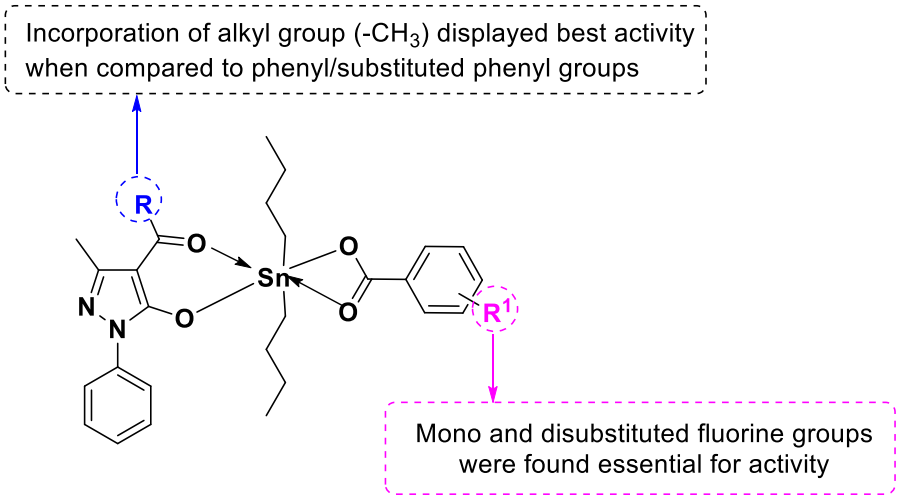
kinase inhibition and for anti-proliferative activity against HT-29, H460, A549, MKN-45, and U87MG cancer cell lines. SAR studies indicated that compounds with halogen, especially with trifluoromethyl group at 2nd position of the phenyl ring are more effective towards potent activity. Among the tested series, compound **36** promising kinase inhibitory activity and commendable anti-proliferative activity as illustrated in **Figure 22**[54].



C.No.	R	R^1	c-Met	Anti-proliferative activity IC_{50} (µM)				
			IC_{50} (nM)	HT-29	H460	A549	MKN-45	U87MG
36	Morpholin	CF_3	2.20	0.14 lM	0.18 lM	0.09 lM	0.03 lM	1.06 lM

Figure22. c-Met kinase and anti-proliferative activity of novel pyrazolones.

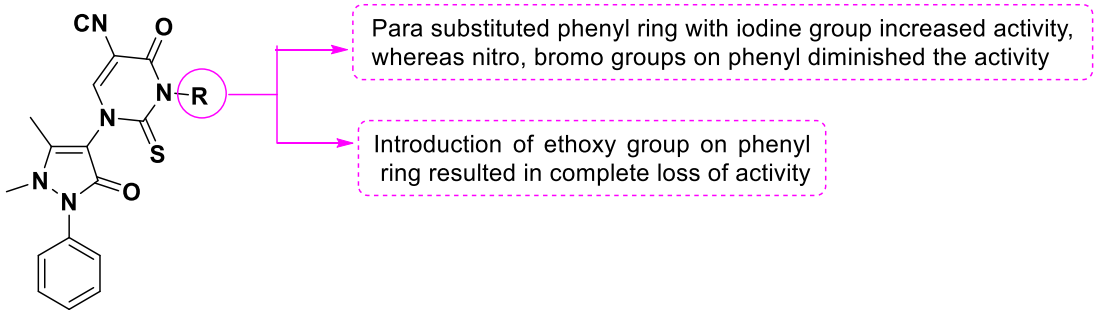
In 2014, Zhao and co-workers reported the synthesis and anticancer evaluation of novel mixed ligand di-n-butyltin(IV) complexes bearing substituted 4-acyl-5-pyrazolone and fluorinated benzoin acid. All synthesized compounds were tested against KB and Hela cell lines and human cervical epithelial cells (HCvEpCs). A brief SAR study indicated the significance of alkyl and aryl functional groups at both R and R^1 positions as represented in **Figure 23**. From the series, compound **37** exhibited potent activity when compared to the standard drug cisplatin[52].



C.No.	R	R^1	IC_{50} (µM)		
			KB	Hela	HCvEpCs
37	CH3	4-F	0.35	0.05	0.26

Figure23. Pyrazolones and their effect on cancer cell lines**.**

In 2014, Ghorab and co-workers reported synthesis of novel pyrazolone derivatives by reacting 4-aminoantipyrine as anti-breast cancer agents. Most of the synthesized compounds showed good results and compound **38** having 4-I-Phgroup exhibited most prominent activity with an IC_{50} value of 30.68µM than the standard drug doxorubicin (IC_{50} = 71.8µM). SAR study on this series has been presented in **Figure 24**[60].

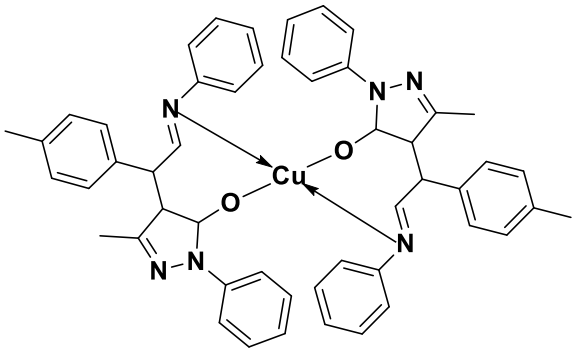


C.No.	R	Human breast cancer cell line IC_{50} (µM)
38	C_6H_4-I	30.68

Figure24. Anticancer activity of isocyano-dihydropyrimidin pyrazolones.

In 2014, Pettinari et al. reported synthesis and *in vitro* anti-proliferative studies of ruthenium (II) arene complexes with 4-(biphenyl-4-carbonyl)-3-methyl-1-phenyl-5-pyrazolonate ligand, and

related 1,3,5-triaza-7-phosphaadamantane (PTA) derivatives. Of the screened compounds, **39**-**41** exhibited significant activity against three human cancer cell lines. A brief SAR study indicated that compounds with hexamethylbenzene ruthenium complex displayed prominent activity than other metal complexes as depicted in **Figure 25**[56].

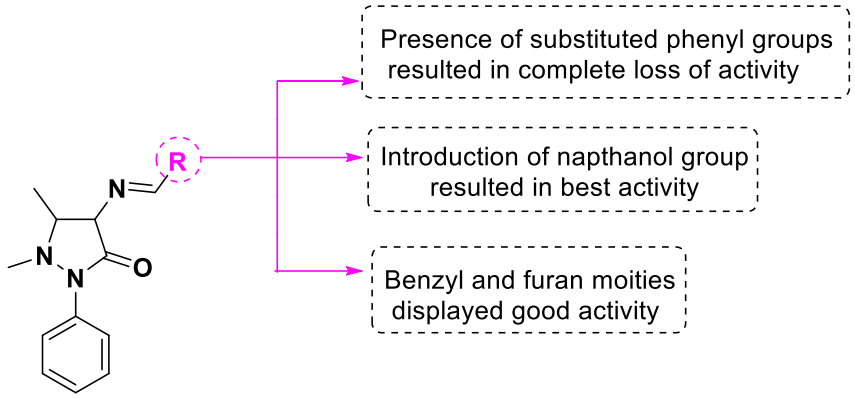


C.No.	R^1	R	Cytotoxicity IC_{50} (µM)			
			HeLa	**MCF-7**	**HepG2**	**HCT-116**
39	HMB	Cl	13 ±3	26± 4	28± 2	30 ± 6
40	HMB	CH_3OH	25 ±3	24± 2	32± 6	34 ± 3
41	HMB	PTA	14 ±2	13± 1	27± 6	9± 3

PTA: phosphine 1,3,5-triaza-7-phosphaadamantane; HMB: Hexamethylbenzene.

Figure25. Cytotoxic results of ruthenium (II) arene complexe with pyrazolone ligand.

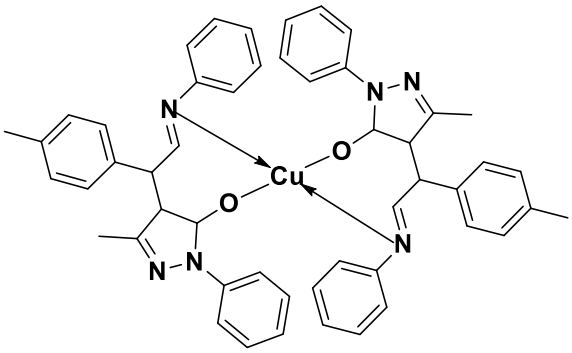
In 2014, Bensaber and co-workers described synthesis of novel pyrazol-3-one derivatives. All synthesized compounds were docked into active site of thymidine phosphorylase were biologically tested *in vitro* on calf thymus DNA to study the interaction with DNA using spectrophotometer and also tested on thymidine phosphorylase enzyme and human cancer cell lines (A549 & MCF-7). Of the evaluated series, compound **42** showed prominent phosphorylase and breast carcinoma cell line activity. **Figure 26** represents the structures, inhibition of active compound along with SAR study [51].



C.No.	R	Inhibition of thymidine phosphorylase IC_{50} (µM)
42	Naphthanol	22

Figure 26. Thymidine phosphorylase inhibitory results of Schiff's base pyrazolones.

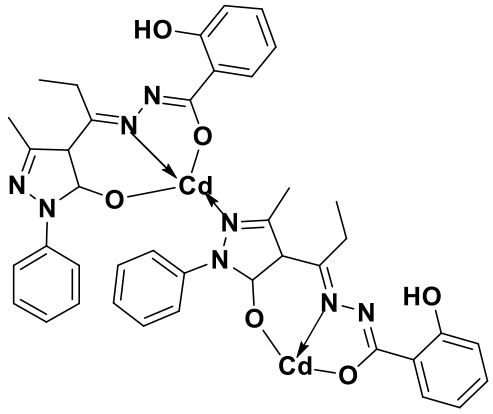
In 2014, Komal and co-workers reported synthesis of pyrazolone based binary and ternary Cu (II) complexes to study the DNA binding, protein binding and anticancer activity on A549 lung carcinoma cell line. SAR study revealed that presence of hydrophobic moiety is responsible for significant binding. From the tested series, compound **43**showed good binding interactions with DNA and protein as displayed in **Figure 27**[53].



C.No.	R	DNA binding parameters (M^{-1})				Protein binding parameters		
		Kb	Ksv	kq	Kapp	Ksv M^{-1}	Kbin M^{-1}	n
43	[Cu(TPMP-BA)2]	33.2 x 10^{-4}	8.0 x 10^{3}	8.0 x 10^{11}	4.95 x 10^{11}	3 0.23 x 10^{6}	8.81 x 10^{6}	1.04 2

Figure 27. Calf thymus DNA and Protein binding parameters of pyrazolone ternary Cu(II) complexes.

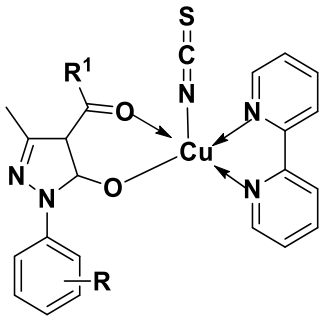
In 2015, Zhao and co-workers synthesized pyrazolone based cadmium complex (Cd–PMPP-SAL) to evaluate induced apoptosis in human esophageal cells via reactive oxygen species generation and caspase-dependent mitochondria-mediated pathway. From the tested series, compound **44** exhibited prominent activity against esophageal cell line. From the results, it was noted that induced apoptosis might be mediated by the increased production of reactive oxygen species. **Figure 28** represents the complex structure and activity data [64].



C. No.	Eca 109 IC_{50}(μM)
44	29.86

Figure 28. Human esophageal cell line activity of pyrazolone based cadmium complex (Cd–PMPP-SAL).

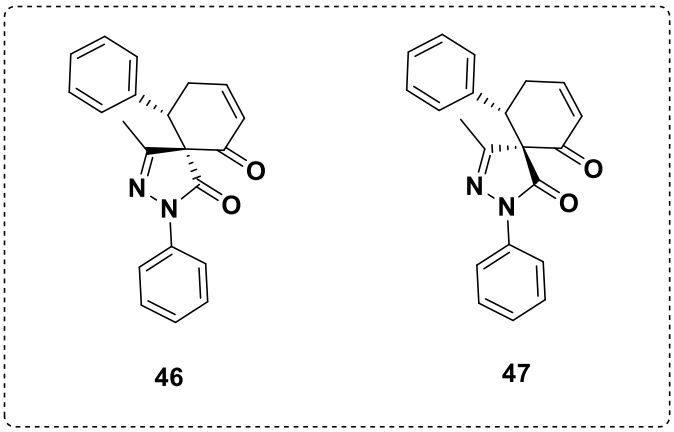
In 2015, Vyas et al. synthesized and characterized a series of new copper complexes bearing pyrazolone moiety. These compounds were biologically tested against A549 and H9C2 (rat cardio myocytes) cell lines. From the tested series, compound **45** displayed significant activity as displayed in **Figure 29**[65].



C.No.	R	R^1	%Mortality at 0.30 (μM)	
			A549	H9C2
45	H	4-CH_3-Ph	85	10

Figure 29. *In vitro* cytotoxic activities of pyrazolone copper complexes against A549.

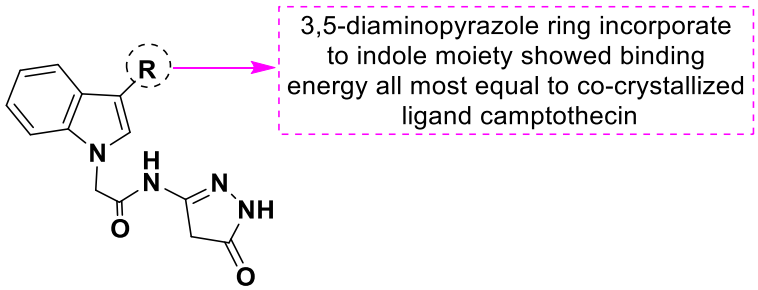
In 2015, Zhang and co-workers introduced a series of novel pyrazolone derivatives and evaluated their biological activity against A549, MDA-MB-231 and HCT-116 cell lines as antitumor agents. From the tested series, compounds **46** and **47** indicated prominent activity against all cell lines. The structures of active compounds and results are depicted in **Figure 30**[13].



C. No	Antitumor activity IC_{50} (µM)		
	MDA-MB-231	**A549**	**HCT116**
46	5.8	25.4	5.3
47	5.5	8.5	4.4

Figure 30. *In vitro* antitumor activities of enantio-enriched pyrazole-derived compounds.

In 2015, Mandour and co-workers reported the synthesis of 1,3-diheterocycle indolyl derivatives bearing pyrazolone moiety as antitumor agents. All the synthesized compounds were biologically tested against A-549, MCF7, HCT-116 and HEPG2 cancer cell lines, also reported to inhibit TopoI enzyme theoretically via molecular docking study. From the series, compounds **48-51** showed potent activity against all cell lines as represented in **Figure 31**. A brief SAR study around the scaffold indicated that 3,5-diaminopyrazole ring incorporated to an indole moiety showed a binding energy almost equal to co-crystallized ligand camptothecin [62].



C.No.	R	Inhibition growth (%) (mean±SEM)

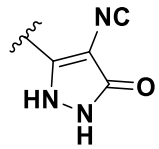
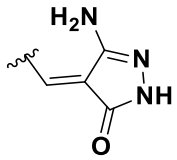
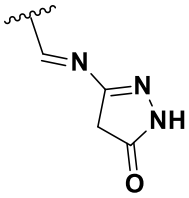
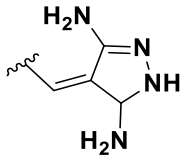
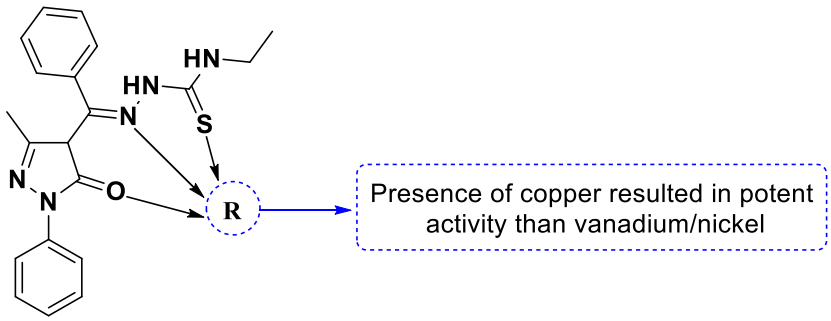
		A549	HEPG2	HCT116	MCF7
48		100±0.00	100±0.00	15.9±1.20	65.9±1.20
49		100±0.00	29.8±1.60	27.6±1.66	35.8±2.60
50		42.3±1.20	100±0.00	100±0.00	22.5±3.50
51		100±0.00	26.9±1.30	16.9±1.30	100±0.00

Figure 31. Anti-proliferative activity and structures of active compounds.

In 2015, Pahontu and co-workers reported the synthesis of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-ethyl-thiosemicarbazone and its copper (II), vanadium(V) and nickel(II) complexes. All synthesized metal complexes were biologically evaluated as anti-proliferative agents by testing against HL-60 cell line. Among the tested series, compounds (**52-55**) with copper complex exhibited potent activity. A brief SAR indicated that presence of copper is essential for potent activity than vanadium/nickel as illustrated in **Figure 32**[85].



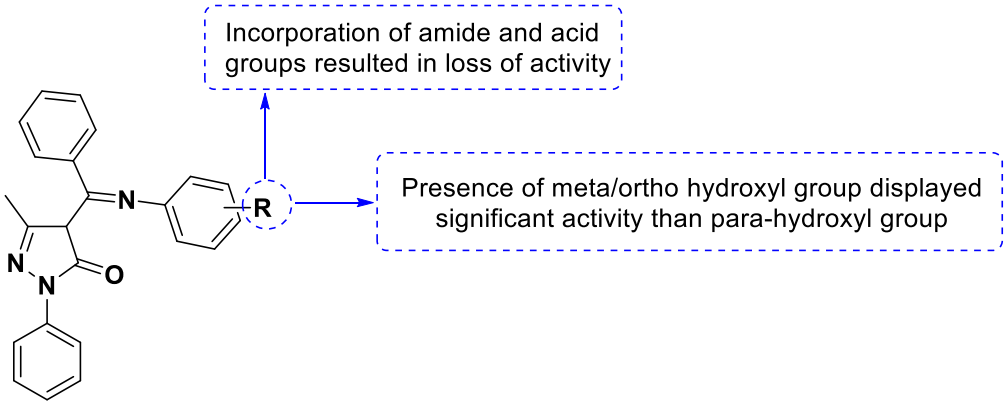
C.No.	R	Inhibition of cell proliferation (%)¥ HL-60 cell line		
		10 µM	1 µM	0.1 µM
52	[Cu(L)(Cl)]-C2H5OH	98.9	41.3	2.0
53	$[Cu(L)_2]$-H2O	99.9	96.0	5.0

54	[Cu(L)(Br)]-H2O	98.8	35.5	0
55	[Cu(L)(NO3)]-2CH3CH2OH	96.8	45.8	4.0

¥: SEM < _4% of a single experiment in triplicate.

Figure 32. Anti-proliferative activity of pyrazolones with copper ligand complexes.

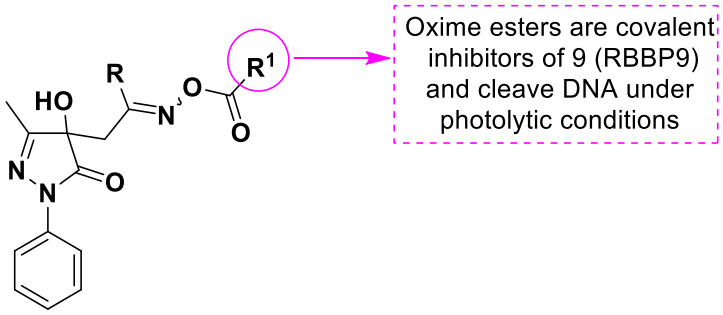
In 2015, Yan and co-workers introduced a series of pyrazolone-enamines as proteasome inhibitors as well as anti-proliferative agents. All the synthesized compounds were evaluated for their ability to inhibit the proliferation of human liver cancer cell line HepG2. From the series, compound **56** with ortho or meta hydroxyl group showed prominent proteasomal and cell line activity. From SAR, it was noted that incorporation of ortho/meta hydroxyl group on imine phenyl group resulted in best activity as depicted in **Figure 33**[68].



C.No.	R	IC_{50} (µM)	
		Proteasomal CT-like	**HepG2**
56	3-OH-4-CH_3	9.8	25

Figure 33. Anti-proliferative activity of pyrazolone-enamine derivatives.

In 2016, Masumoto et al. reported synthesis and DNA cleavage activity of novel functionalized pyrazol-3-ones bearing oxime ester. All synthesized molecules were pharmacologically evaluated by using various DNA types (ccc- and oc-). From the tested series, compounds **57** and **58** displayed significant activity profile. SAR studies revealed that compounds with Cu^{2+} showed good effect on activity as presented in **Figure 34**[72].

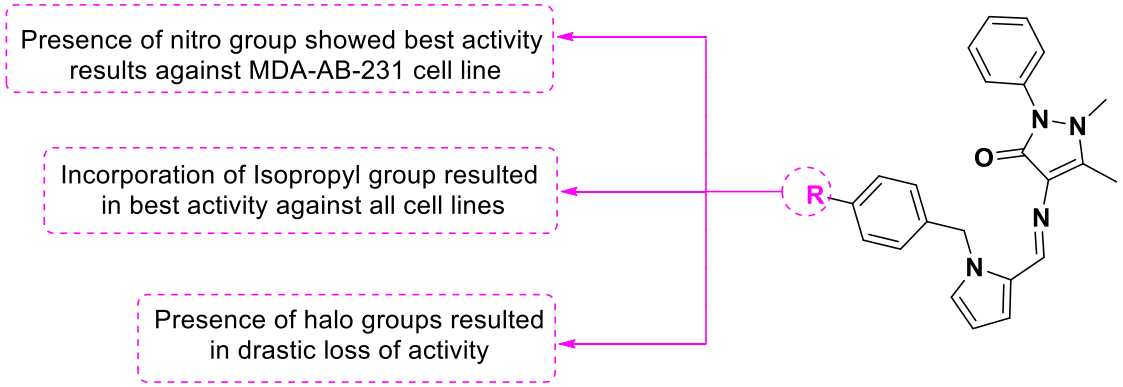


C.No.	R	R^1	DNA type	DNA cleavage activity (%)	
				[a]Without Cu^{2+}	[b]With Cu^{2+}
57	Me	4-NO2-C6H4	ccc- oc-	100 00	87 13
58	Ph	Me	ccc- oc-	100 00	61 39

a,b: Incubation for 3 h.

Figure 34. DNA cleavage activity of pyrazolone oxime esters.

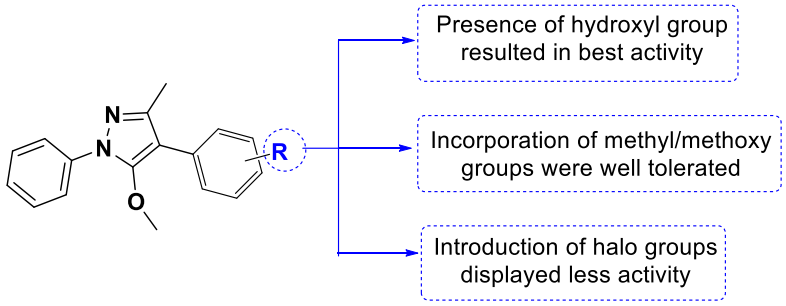
In 2016, Mochona and co-workers reported the synthesis and cytotoxic evaluation of pyrroles bearing benzimidazole or pyrazolone or 1,3,4-oxadiazole moieties. All compounds were tested against MDA-AB-231, BT-474 and lshikawa human cancer cell lines. Most of the compounds showed prominent activity results and in particular, compound **59** ((E)-4-(((1-(4-isopropylbenzyl)-1*H*-pyrrol-2-yl)methylene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one) displayed potent activity against all cell lines. Structure of most active compound, activity data and SAR are presented in **Figure 35**[73].



C.No.	R	Cytotoxicity IC_{50} (µM)		
		MDA-AB-231	Ishikawa	BT-474
59	4-Isopropyl	53.26	89.07	77.7

Figure35. SAR and cytotoxic activity of pyrroles bearing pyrazolone moiety.

In 2016, Polkam and co-workers introduced a series of edaravone derivatives to evaluate their molecular properties, anticancer and antioxidant activity. All the synthesized compounds were tested against PC-3, A549 and HEK293T cell lines and showed potent activity results. From the series, compounds **60** and **61** exhibited the best activity in micro molar range. A brief SAR study indicated that, substitution of halo groups on phenyl ring is essential for potent activity as depicted in **Figure 36**[74].



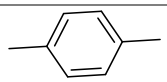
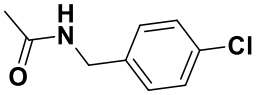
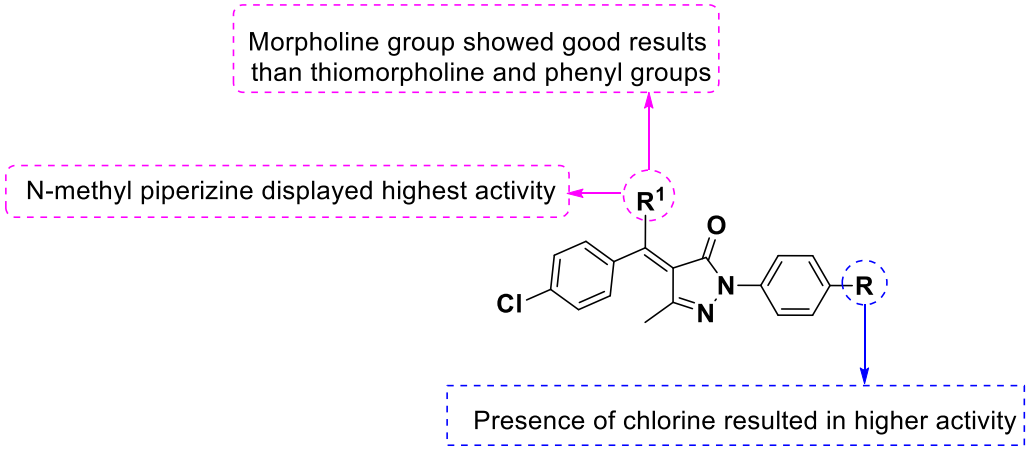
C.No.	R	antioxidant activity IC_{50} (µM)	*In vitro* anticancer activity Cytotoxicity % of inhibition at 25µM		
			PC3	A549	HEK293T
60		26.40 ± 3.63	59.26	85.87	70.45
61		16.07 ± 3.81	45.80	87.02	49.37

Figure 36. Anticancer and antioxidant activities of edaravone derivatives.

3.2. Anti-tubercular activity

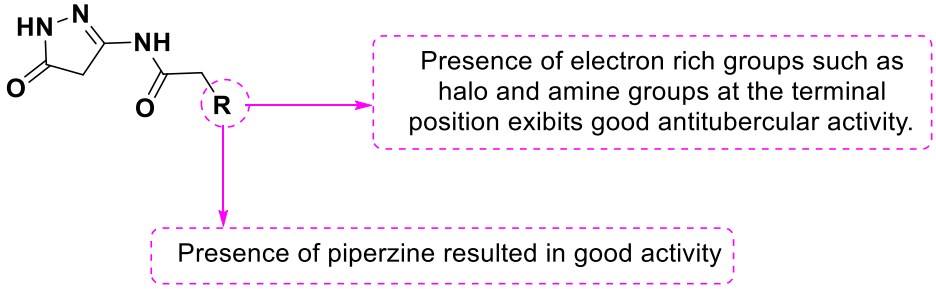
Tuberculosis (TB) is a chronic necrotizing airborne bacterial infection caused by *Mycobacterium tuberculosis* (MTB). Along with *Mtb* there are other bacterial agents which have the capability to cause tuberculosis such as *M. bovis*, *M. africanum*, *M. canetti*, and *M. Microti* [86].However, their percentage of initiating the disease is very low. TB is one of the leading causes of death from infectious disease worldwide [87] thus, development of new drugs and vaccines are essential to eradicating this epidemic.In 2009, Castagnolo and co-workers reported the synthesis, biological evaluation and SAR studies of novel pyrazolone analogues as potential mycobacterium tuberculosis (MTB) inhibitors. Form the tested series, compounds **62** and **63** exhibited prominent activity against *M. tuberculosis* H37Rv (ATCC 27294). A brief SAR study indicated that presence of halogen groups on *N*-phenyl ring is responsible for higher activity as displayed in **Figure 37**[20].



C.No.	R	R^1	M. tuberculosis H37Rv ATCC 27294
			MIC (μg mL^{-1}) M. tuberculosis
62	Cl	N-Me-piperazine	4
63	Cl	Morpholine	4

Figure 37. Pyrazolonesas MTb inhibitors.

In 2016, Krishnasamy and co-workers reported synthesis, characterization and *in vitro* anti-tubercular evaluation of pyrazolone hybrids against *Mycobacterium tuberculosis*. From the series, two compounds (**64** and **65**) exhibited significant activity results than the standard drugs pyrazinamide, ciprofloxacin and streptomycin. A molecular docking study was carried out and revealed that compounds without a double bond in the imine side chain and hydrophobic clashes at the pyrazolone end are crucial for good space in the binding receptacle and for imparting flexibility. A brief SAR study suggested that the presence of electron rich group like Cl, NH_2, etc. at the terminal position of the molecule enhances the anti-tubercular activity as depicted in **Figure 38**[77].



C.No.	R	MTB MIC			
		IC_{50} (mg/mL)	IC_{50} (mM)	Cytotoxicity CC_{50} (mM)	Selectivity index (SI)
64	4-Chloroaniline	<0.1	<0.37	>234.34	633.35

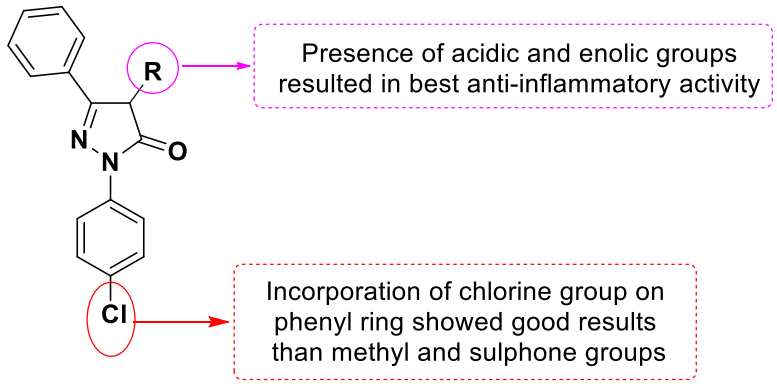
65	Piperazine	<0.1	<0.44	>277.53	630.75
pyrazinamide		1.6	12.99	>507.66	39.08
ciprofloxacin		1.6	4.82	>188.62	39.13
streptomycin		3.12	5.36	>107.47	20.05

Figure 38. *In vitro* antitubercular activity of hybrid pyrazolone against mycobacterium tuberculosis.

3.3. Anti-inflammatory activity

Inflammation happens when the immune system fights against something that may turn out to be harmful. Inflammation is body's immune response to stimuli. The body defend itself against external attackers, such as bacteria, fungi and viruses. Redness, swelling, pain, fever, loss of function and exhaustion are the symptoms of inflammation. Acute inflammation is short-term inflammation and it effects subside after a few days. For example; sore throat, tonsillitis, appendicitis etc. On the other hand, chronic inflammation is long-term inflammation and its effects last for years and osteoarthritis, ulcerative colitis, psoriasis etc. falls under this category. Drugs used for treatment of inflammation are called anti-inflammatory agents. Antipyrine is the first synthetic non-steroidal anti-inflammatory drug (NSAID). As there is anenormous demand of anti-inflammatory agents in the market because traditional drugs have various side effects. To overcome this problem new research is going on[88] to find out novel and low side effect causing drugs.

Fatma Abdel-Fattah Ragab et al. in 2013 synthesized a library of4-chlorophenyl pyrazolone derivatives and were screened for their anti-inflammatory and analgesic activities. In addition, they also carried out their ulcerogenic liability. From the test results, compounds **66** and **67** with acidic centre and enolic group showed higher anti-inflammatory activity than the phenyl butazone reference drug. Compound **67** was also found to be a potent analgesic agent. The results of the observed SAR studies are depicted in **Figure 39**[46].



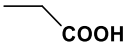
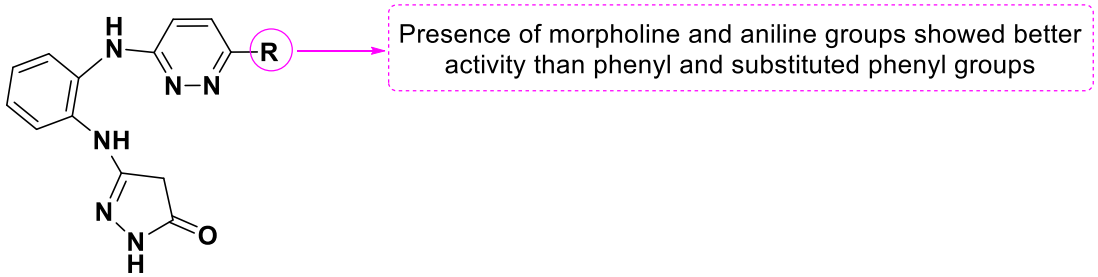
C.No.	R	Anti-inflammatory effect on Carrageenan-Induced Edema of the Hind Paw in Rats (*n*=5)			
		Edema (mm) ±S.E.M		% Inhibition	
		2 h	3 h	2 h	3 h
66		0.480±06	0.48±0.07	85.20	86.67
67		0.75±0.05	0.61±0.13	76.85	82.90

Figure 39. Anti-inflammatory effect of pyrazolone derivatives.

In 2014, Khaliland co-workers reported a novel series of pyrazolone-pyridazine conjugates and were biologically screened for their anti-inflammatory and analgesic properties. Furthermore, few selected compounds were evaluated for their ability to inhibit the production of certain inflammatory cytokines such as TNF-α and IL-6 in serum samples. Compounds**68** and **69**were found very potent, and compound **68** exhibited the highest activity with IC_{50} values of 2.86 μM for COX-1 and 0.39 μM for COX-2.Moreover, it demonstrated the highest selectivity index (SI) of 7.36. TheSAR study revealed thataliphatic amines like morpholine etc. are necessary for best activity as presented in **Figure 40**[15].

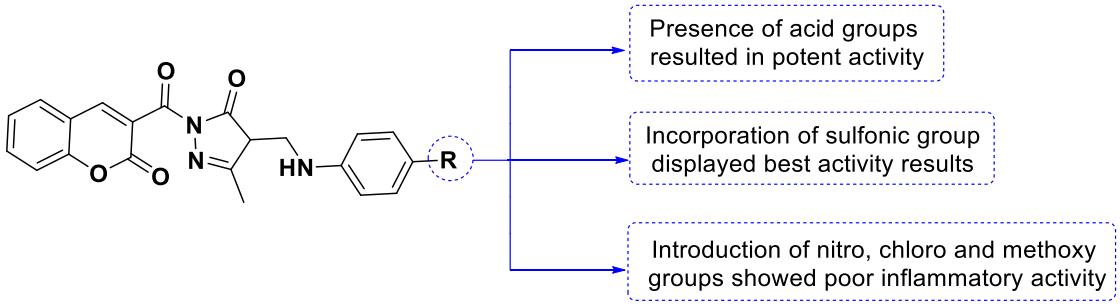


Comp	R	Anti-inflammatory and analgesic activity			
		Analgesic activity %	Anti-inflammatory activity %	TNF-α (ng/ml)	IL-6 (ng/ml)
68	morpholine	63.7 ±5.4	62.3 ±5.8	7.2 ±0.43	11.8± 0.87
69	4-Cl-aniline	68.3 ± 5.3	35.4 ± 2.8	7.12 ± 0.57	9.76 ± 0.58

Figure 40. Anti-inflammatory and analgesic activity of pyrazolone-pyridazine conjugates.

In 2014, Sivakumaret al. reported synthesis of pyrazolone Mannich bases as anti-inflammatory, analgesic and antioxidant agents. From the series, analogues **70-72**exhibited good potency and

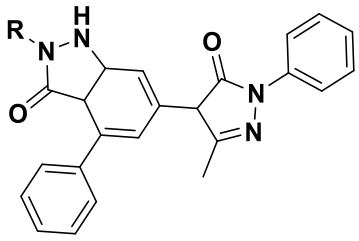
from the SAR study, it was observed that sulfonic group at *para*- or *meta*- positions showed best anti-inflammatory activity as displayed in **Figure 41**[8].



C.No.	R	Anti-inflammatory, ulceration and DPPH radical scavenging activity					
		Anti-inflammatory activity				Ratio of ulceration	Anti-oxidant activity IC_{50} (µg/µl)
		Oedema thickness (mm) ± SEM 4 h	% inhibition after 4 h	Oedema thickness (mm) ± SEM 6 h	% inhibition after 6 h		
70	4-COOH	1.260 ± 0.172	63.63	1.081 ± 0.257	72.61	1/8	155
71	3-SO_3H	1.116 ± 0.1470	67.79	0.793 ± 0.153	79.91	2/8	125
72	4-SO_3H	1.079 ± 0.154	68.86	0.873 ± 0.118	77.88	0/8	120

Figure 41. Anti-inflammatory and anti-oxidant activity of Mannich base pyrazolone derivatives.

Abbadyand co-workersin 2015 described the cyclo-condensation reactions of phenyl-pyrazol-one with active methylene reagents. All synthesized compounds were tested for anti-inflammatory activity and compound **73** showed the highest activity on carrageenan-induced paw edema in rats and the results are displayed in **Figure 42**[58].



73

C.No.	R	Anti-inflammatory activity of compounds on carrageenan induced paw edema in rats (% inhibition ± SEM)
		Time

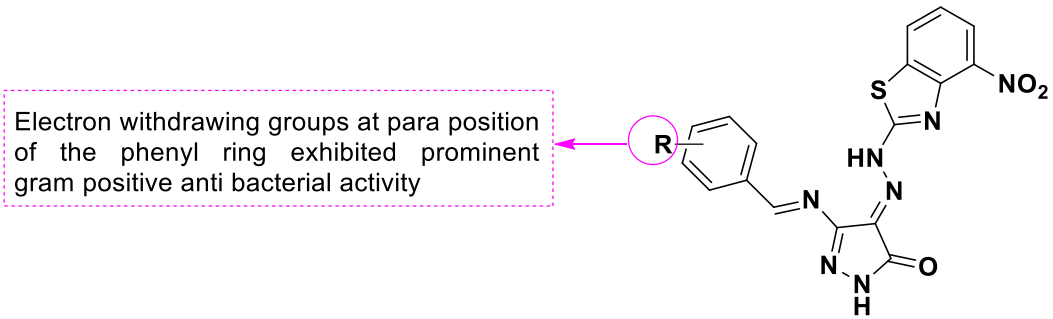
		0.5 h	1h	2h	3h	4h	5h
73	Ph	09.1 ± 0.09	12.4 ± 0.15	19.3 ± 0.05	30.5 ± 0.11	46.3 ± 0.03	56.2 ± 0.11

Figure 42. Anti-inflammatory activity of phenyl-pyrazolone compounds.

3.4. Antimicrobial agents

Antimicrobial agents are drugs that killpathogens or inhibit their growth. Disinfectants, antiseptics, antibiotics, antibacterial, antifungal, antiviral, antiparasitics are antimicrobial drugs. Salvarsan was the first synthetic antimicrobial drug. From the discovery to till date, antimicrobial agents are the only efficient sources of treatment to control infectious diseases. However due to over use, prolong exposer and incorrect dosing of drugs, antimicrobial resistance has developed. Antimicrobial resistance has become alarming threat for mankind. *Streptococcus pyogenes, Neisseria gonorrhoeae, Mycobacterium tuberculosis, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Clostridium difficile, Pseudomonas aeruginosa, Burkholderia cepacia,* and *Staphylococcus aureus* are few examples of common multi drug resistance bacteria. Outstanding research is going on antimicrobials to overcome this alarming issue by developing efficient new antimicrobial agents[89].

In 2012, Sivakumar et al**.** synthesised a series of Schiff's bases of 5-amino-4-[2-(4-nitro-1,3-benzothiazol-2 yl)hydrazinylidene]2,4-Dihydro-3*H*-pyrazol-3-one derivatives and screened for their *in vitro* antimicrobial activity. Compounds **74-76** were found to be highly active among the synthesized compounds. SAR study revealed that electron donating groups at the *para* position of the phenyl ring imparted significant gram-positive antibacterial activity as shown in **Figure 43**[28].



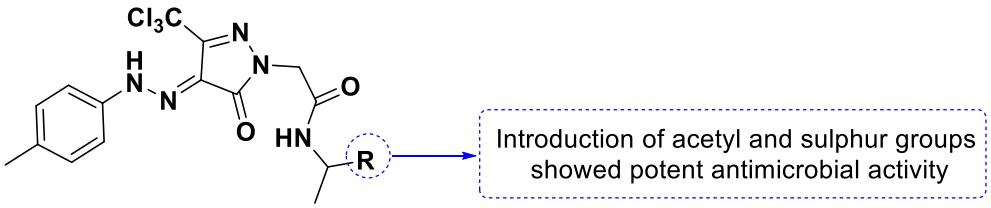
C. No.	R	Antifungal activity ZOI in mm 5 µg/disc									Antibacterial activity ZOI in mm250 µg/disc								
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
74	4-Cl	11	14	13	13	10	11	10	09	10	09	13	11	13	11	10	18	13	14
75	2,4-OH	09	14	10	11	08	09	09	10	10	10	11	10	10	08	09	13	13	13

76	3,4-OH	11	09	13	13	08	09	09	09	11	09	11	10	08	08	08	13	13	11

Fungal strains: *a -Candida albicans; b -Monascus purpureus; c- Aspergillus niger; d-Trichophyton rubrum; e-Aspergillus fumigatus; f-Aspergillus parasiticus.* Bacterial strains: *g-Micrococcus luteus; h-Staphylococcus aureus; i-Bacillus subtilis; j-Corynebacterium; k-Bacillus lentus; l-Staphylococcus albus; m-Escherichia coli; n-Pseudomonas aeruginosa; o-Rhodospirillum rubrum; p-Vibrio cholerae; q-Salmonella paratyphi; r-Klebsiella pneumoniae.*

Figure 43. Antibacterial activity of benzothiazole hydrazinylidene pyrazolone Schiff's bases.

In 2012, Suresh Kumar et al**.** reported a novel series of substituted phenyl hydrazono pyrazolyle derivatives as anti-microbial agents. All the synthesized compounds were screened against gram-positive, gram-negative and fungal activities. Compounds **77**, **78** and **79** displayed potent activity among the tested compounds. SAR studies clearly indicated that the presence of an acetyl and sulphur groups increases the antibacterial activity as depicted in **Figure 44**[31].



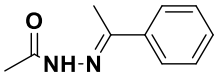
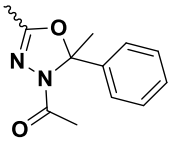
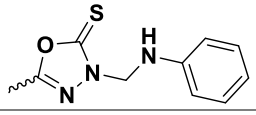
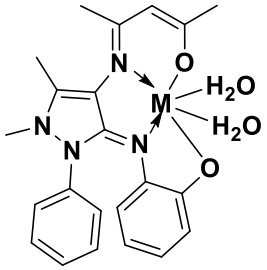
C.No.	R	Antifungal activity10mg/cup ZOI (mm)		Antibacterial activity (MIC, µg ml-1)			
		C. albicans	*A. niger*	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogene*
77		20	19	21	22	22	20
78		19	24	20	19	23	23
79		28	24	19	25	19	19

Figure 44. The antibacterial and antifungal activity of substituted phenyl hydrazono pyrazolyle derivatives.

In 2012,Anitha et al. described the synthesis of tridentate transition metal complexes (VO(II), Co(II), Ni(II), Cu(II) and Zn(II)) of azo schiff-base ligands derived from 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one and (4-chlorophenyl)diazenyl)-2-hydroxy benzaldehyde. The synthesized compounds and metal complexes were biologically tested against bacterial and fungal species.

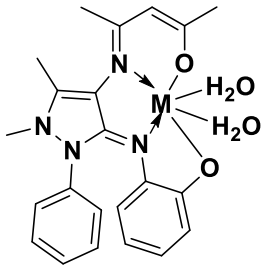
Among all tested series, Compound **80** displayed promising activity results as presented in **Figure 45**[33].



C.No.	R	Antibacterial activity ZOI (mm)							
		S. aureus	***S. typhi***	***E. coli***	***B. subtilis***	***S. sonnie***	***C. albicans***	***A. niger***	***R. bataicola***
80	Cu(II)	14	11	R	13	9	15	16	10

Figure 45. Antimicrobial activity of tridentate transition metal pyrazolone complexes.

In 2013, Saxena and co-workers reported the synthesis and biological evaluation of transition and inner transition metal complexes of ligands derived from 1-phenyl-2,3-dimethyl-4-(4-iminopentan-2-one)pyrazole-5-one and 2-aminophenol.All the tested compounds exhibited antimicrobial activities against *S. aureus and B. subtilis* and compounds **81** and **82** exhibited excellent antimicrobial activity as depicted in **Figure 46**[44].

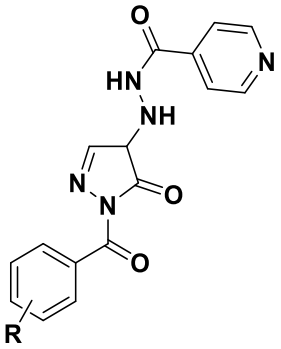


C. No	M	Antibacterial activity 4 µg/ml ZOI (mm)	
		S. aureus	***B. subtilis***
81	MoO	19-23	19-23
82	Mn	15-18	19-23

Figure 46. Antibacterial activity of tetra dentate Schiff's base metal complexes.

In 2013**,** Buvana et al. prepared pyrazol-pyridine-carbohydrazide derivatives and evaluated their biological activity against *B. subtilis* and *E.coli*. Most of the synthesized compounds exhibited

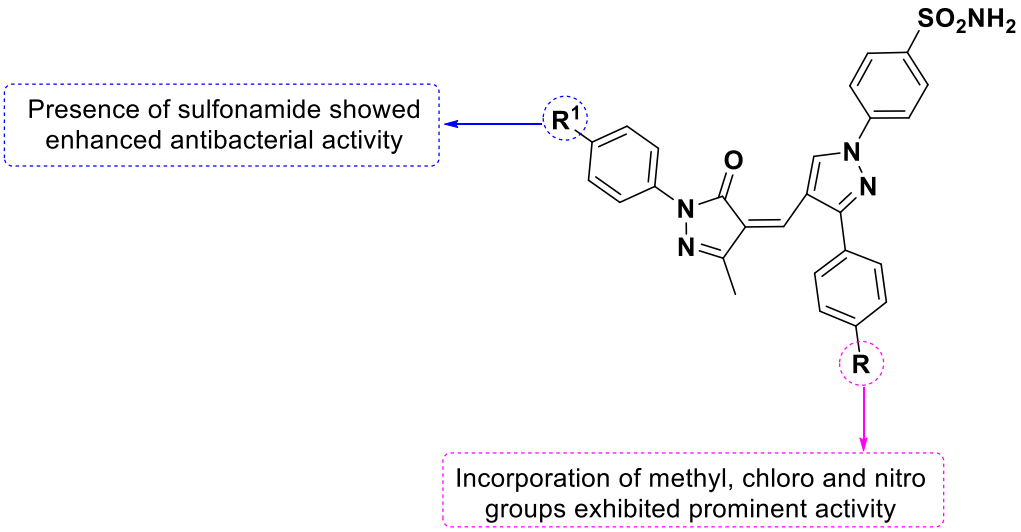
significant activity, especially compound **83** displayed best activity results as illustrated in **Figure 47**[45].



C.No.	R	Antibacterial activity (100 µg) ZOI (mm)	
		S. subtilis	*E.coli*
83	4-Cl	13	12

Figure 47. Antibacterial activity of pyrazolone derivatives.

In 2013, Khloya et al. synthesized novel 4-arylidene pyrazole analogues bearing pyrazolone moiety through Knovenagel condensation. All the synthesized derivatives were screened for their i*nvitro* antimicrobial activity against gram-positive (*Bacillus subtilis and Staphylococcus aureus*), gram-negative bacteria (*Pseudomonas fluorescens and Escherichia coli*) as well as fungal strains (*Candida albicans and Saccharomyces cerevisiae*). Compounds **84-87** showed potent activity. SAR study showed that substitution on the phenyl ring placed at the *para*-position of the pyrazole moiety has an enhanced antibacterial activity as compared to the *meta*- position **Figure 48**[47].

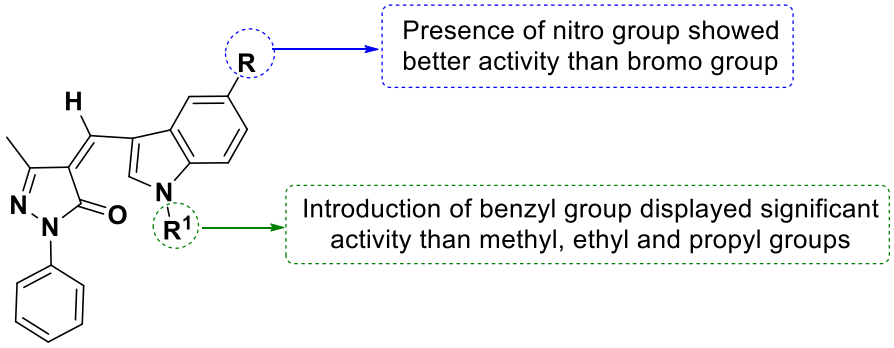


		ZOI (mm)	
		Antifungal activity	Antibacterial activity
R	R^1		

C.No.			*C. albicans*	*S. cerevisiae*	*B. subtilis*	*S. aureus*	*P. fluorescens*	*E. coli*
84	H	H	12 ± 0.43 (400)	28 ± 0.15 (0.04)	28 ± 0.00 (0.04)	26 ± 0.30 (0.4)	14 ± 0.08 (400)	14 ± 0.46 (400)
85	H	SO_2NH_2	12 ± 0.32 (400)	16 ± 0.00 (40)	28 ± 0.09 (0.04)	30 ± 0.15 (0.04)	-	20 ± 0.32 (4)
86	CH_3	SO_2NH_2	12 ± 0.33 (400)	28 ± 0.28 (0.04)	16 ± 0.32 (40)	25 ± 0.42 (0.4)	16 ± 0.12 (40)	14 ± 0.50 (400)
87	Cl	SO_2NH_2	13 ± 0.37 (400)	22 ± 0.44 (4.0)	14 ± 0.00 (400)	16 ± 0.00 (40)	25 ± 0.13 (0.4)	18 ± 0.23 (40)

Figure 48. Antibacterial and antifungal activities of arylidene pyrazole analogues bearing pyrazolone moiety.

In 2014 Indrasena et al. designeda series of indolylidine pyrazolones as potent antimicrobial agents.All the synthesized compounds were tested against *staphylococcusaureus* and potent results of compounds **88** and **89** are presented in **Figure 49**. SAR study demonstrated that presence of nitro group on indole moiety is necessary for significant activity [61].

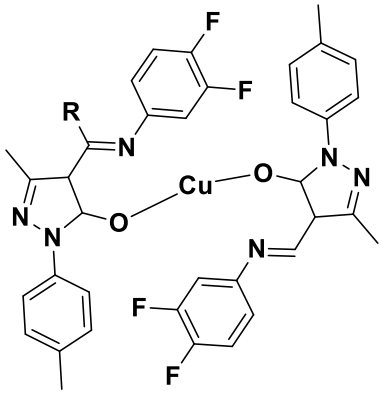


C.No.	R	R^1	Anti-bacterial activity *S. aureus* (MIC µM)
88	H	C_2H_5	25
89	NO_2	CH_2Ph	25

Figure 49. Anti-bacterial activity of indolylidine pyrazolone.

In 2015 Joseph and co-workers reported synthesis and biological evaluation of Schiff base Copper (II) complexes ([Cu(PTPMP-ME)2] and [Cu(PTPMP-F)2])bearing 4-formylpyrazolone..The metal complexes were tested for antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli* and

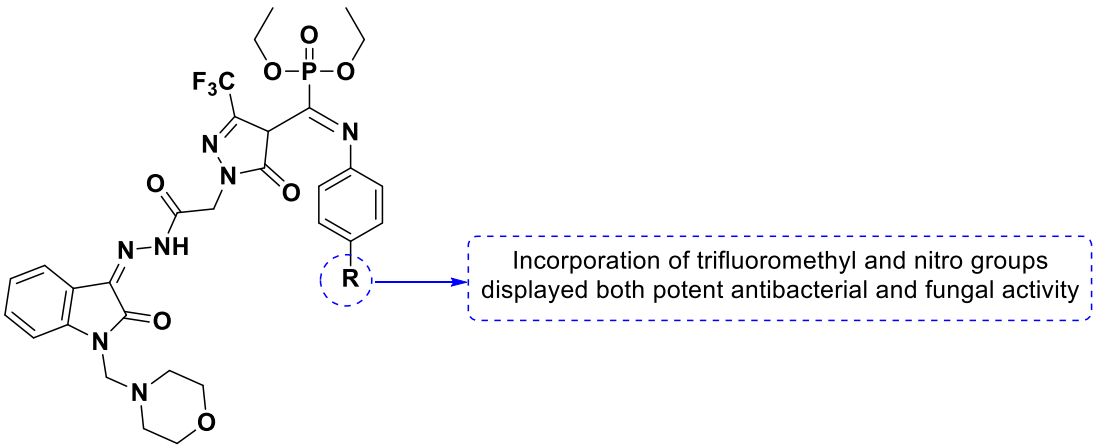
Pseudomonas aeruginosa). From the tested series, complex **90** and **91** exhibited the highest activity and results are presented in **Figure 50** [9].



C.No.	R	Antibacterial activity ZOI (mm) (µg/ml)			
		S.aureus	***B. Subtilis***	***E.Coli***	***P.aeruginosa***
90	[Cu(PTPMP-ME)2]	9	6	9	4
91	[Cu(PTPMP-F)2]	9	9	4	7

Figure 50. Antibacterial activity of copper (II) complexs bearing pyrazolone moiety.

In 2015, Rani and co-workers reported the synthesis and pharmacological evaluation of organo phosphorous pyrazole-5-one derivatives as antibacterial agents. From the tested series, compounds with trifluoromethyl and hydrogen (**92** and **93**) groups on phenyl ring showed potent antibacterial activity as well as compound (**94**) with methoxy group showed potent antifungal activity as presented in **Figure 51**. A brief SAR study illustrated that methoxy and trifluoro methyl on phenyl ring are essential for both antifungal and bacterial activity. [67].

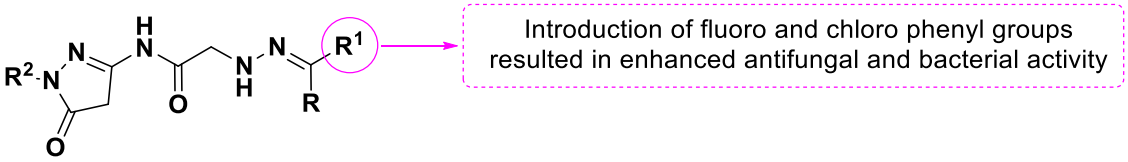


	ZOI (mm) 250(µg/ml)

C.No.	R	Antifungal activity		Antibacterial activity			
		A. niger	*C.albicans*	*S.aureus*	*B.Cerus*	*E.Coli*	*P.aeruginosa*
92	CF_3	10	12	26	22	22	25
93	NO_2	19	17	23	17	20	21
94	H	24	18	20	21	16	13

Figure 51. Antifungal and antibacterial activity of pyrazolone phosphonates.

In 2016, Krishnasamy et al. reported the synthesis, molecular docking and *in vitro* pharmacological activity of hybrid pyrazolones as potent antimicrobial agents. Compounds **94-97** bearing electron-withdrawing groups on phenyl ring displayed prominent activity. A brief SAR indicated that presence of fluoro group on phenyl ring especially favoured for antifungal activity as depicted in **Figure 52**[77].

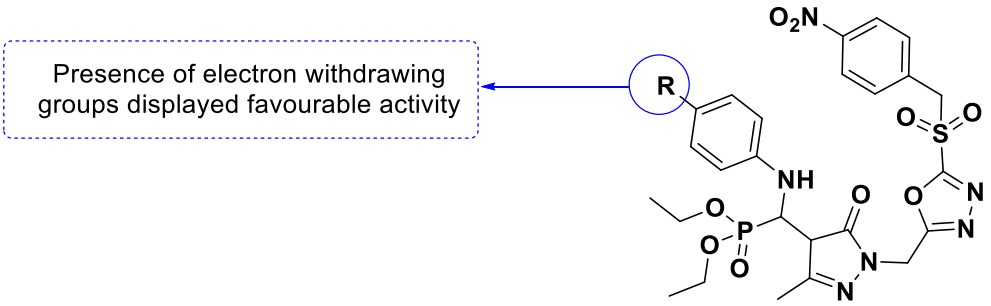


C.No.	R	R^1	R^2	MIC (mg/mL)								
				1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
94	–H	4-ClC_6H_4	–H	25.0	50.0	12.5	50.0	6.25	12.5	3.12	3.12	50.0
95	–H	4-ClC_6H_4	–C_6H_5	12.5	12.5	12.5	12.5	12.5	12.5	6.25	6.25	6.25
96	–CH_3	4 -ClC_6H_4	–C_6H_5	25.0	6.25	25.0	6.25	12.5	25.0	3.12	12.5	6.25
97	–CH_3	4-FC_6H_4	–C_6H_5	50.0	6.25	6.25	6.25	6.25	6.25	12.5	12.5	12.5

1: *M. luteus*; 1: *S. albus*; 3: *S. aureus*; 4: *E. coli*; 5: *K. pneumonia*; 6: *V. cholera*; 7: *A. Fumigatus*; 8: *A. Parasiticus*; 9: *C. Albicans*.

Figure 52. *In vitro* antimicrobial activity of hybrid pyrazolone derivatives.

In 2016, Rani and co-workers reported synthesis and biological properties of novel α aminophosphonate pyrazolones as antimicrobial agents. From the series, compounds **98** and **99** exhibited best activity results as presented in **Figure 53**. SAR study indicated that presence of nitro and chloro groups are essential for good activity [80].

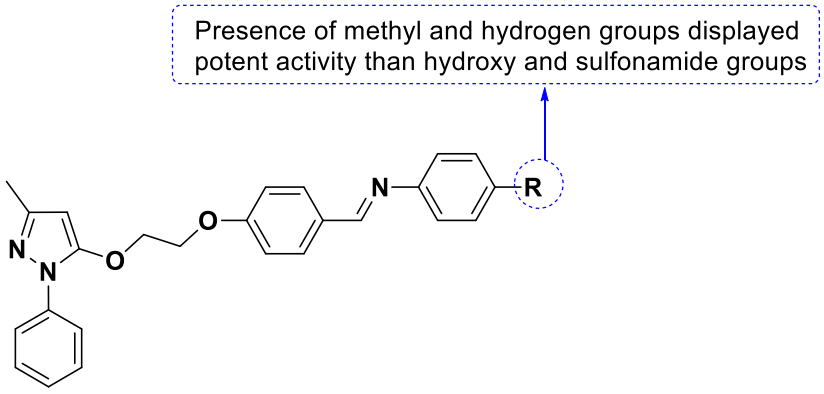


C.No.	R	ZOI (mm) 250(µg/ml)					
		Antibacterial activity				Antifungal activity	
		S.aureus	*B.Cereus*	*E.Coli*	*P. aeruginosa*	*A. niger*	*C.albicans*
8	Cl	16	14	14	15	12	14
99	NO_2	18	16	16	17	14	16

Figure 53. A brief SAR and antimicrobial activity of α-aminophosphonate pyrazolone derivatives.

3.5. Miscellaneous activities

In 2014, Tewari and co-workers reported the synthesis of novel pyrazolone derivatives and evaluated *in vivo* for their anti-inflammatory activity in carrageenan-induced rat paw edema model. Among the series, compounds **100** and **101** displayed equivalent anti-inflammatory activity with nimesulide as a standard drug. A brief SAR indicated that presence of hydrogen and methyl groups are responsible for potent activity as displayed in **Figure 54**[55].

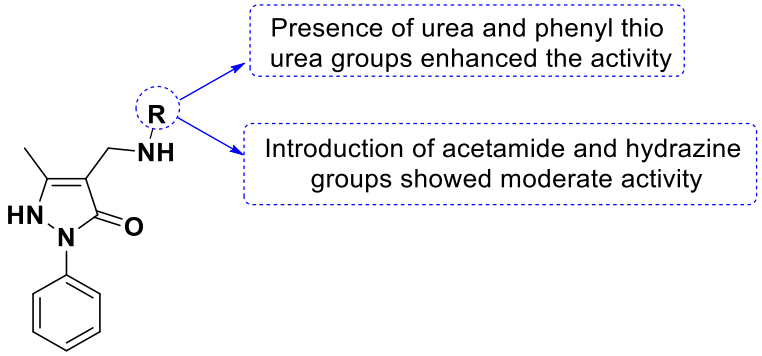


C.No.	R	Cox-2/Cox-1 ratio of pyrazole derivatives.		
		Cox-1 IC_{50} (µM)	Cox-2 IC_{50} (µM)	SI
100	CH_3	32.5	14.3	0.4400
101	-H	32.9	16.8	0.5100

Figure 54. SAR andCOX-1/COX-2 enzyme inhibition activities of pyrazolone derivatives.

In 2015, Dube et al**.** reported the synthesis, molecular docking and biological evaluation of substituted 5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one derivatives as potent COX-2 inhibitors and

further investigated *in vivo* anti-inflammatory activities using carrageenan-induced rat paw edema model. From the series, compounds **102-104** exhibited the potent COX-2 inhibitory activity. A brief SAR study presented in **Figure 55**[65].



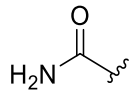
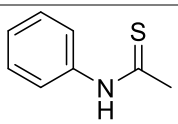
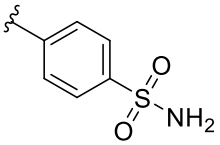
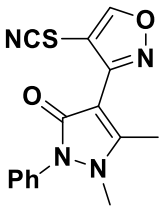
C.No.	R	*In vitro* COX-1/COX-2 enzyme inhibition		
		Cox-1 IC_{50} (µM)	Cox-2 IC_{50} (µM)	SI
102		>100	2.30	>43.48
103		80.00	0.20	400.0
104		>100	1.00	>100.0

Figure 55. SAR and COX inhibitory activities of amino substituted pyrazolones.

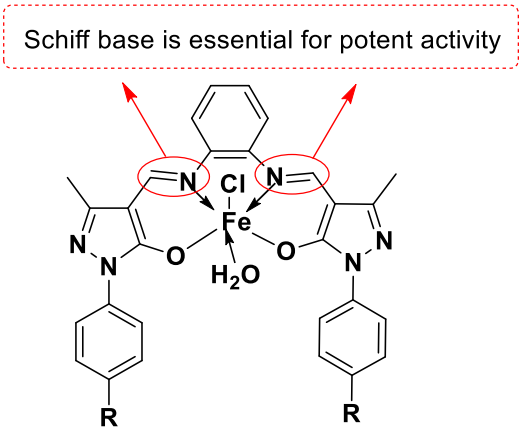
In 2016, Yi and co-workers reported the synthesis of thiocyanato isoxazolyl-pyrazolone derivatives derived from 4-(2-chloroacetyl)-1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one. All the synthesized compounds were screened for aromatase enzyme inhibition. From the series, compound 105 showed best activity results as displayed in **Figure 56**[70].



C.No.	Aromatase inhibition activity IC_{50} (µM)
105	0.0023± 0.0002

Figure 56. Aromatase inhibitory activity of thiocyanato isoxazolyl-pyrazolone.

In 2016, Surati et al. described the synthesis of novel pyrazolone Schiff base tetra dentate iron (III) coordination compounds as antioxidant agents. From the series compound **106** showed potent antioxidant activity and a brief SAR study presented in **Figure 57**[79].



C.No.	R	Scavenging activity (%)
106	CH_3	69

Figure 57. Antioxidant activity of pyrazolone Schiff base tetra dentate iron complex.

4. Conclusion

Literature survey revealed that pyrazolone is a privileged scaffold in medicinal chemistry with diverse pharmacological activities. In this review, we initially discussed various strategies employed for the syntheses of numerous substituted 2,4-dihydro-3*H*-pyrazol-3-ones derived from pyrazolone as a starting material through multi-component reactions (MCRs), conventional heating and/or microwave-assisted organic reactions (MAORs). Moreover, these synthetic protocols provide pharmaceutical chemists an easy access to new libraries of 2,4-Dihydro-3*H*-pyrazol-3-one for a high throughput screening (HTS) analysis. Clinically administered drugs such as edaravon, phenazone, metamizole, dipyron, propyphenazon, metamizole sodium (novalgin),phenylbutazone, remifenazone, famprofazone, aminopyrine, oxyphenbutazone bearing2,4-Dihydro-3*H*-pyrazol-3-one nucleus have been effectively used to date for therapy of numerous ailments. Medicinal attributes of 2,4-Dihydro-3*H*-pyrazol-3-one have been extensively studied for different biological activities such as anticancer, antimicrobials, antidepressant and other applications. A number of researchers have analysed the SAR of 2,4-Dihydro-3*H*-pyrazol-3-one and deduced the bioactive structures in a quantitative manner. The N-2 position of 2,4-Dihydro-3*H*-pyrazol-3-one accomplished favourable tubercular activity, whereas modifications on C-3, C-4, positions as well as metal complexes of pyrazolone attributed towards different anticancer targets. A wide range of pharmaceutical properties displayed by this privileged scaffold

will definitely serve the purpose for developing effective potent chemotherapeutics. This review aimed to provide an extensive information to the scientific community to design novel, target selective, optimized and varied pyrazolone analogues for the treatment of multifactorial diseases.

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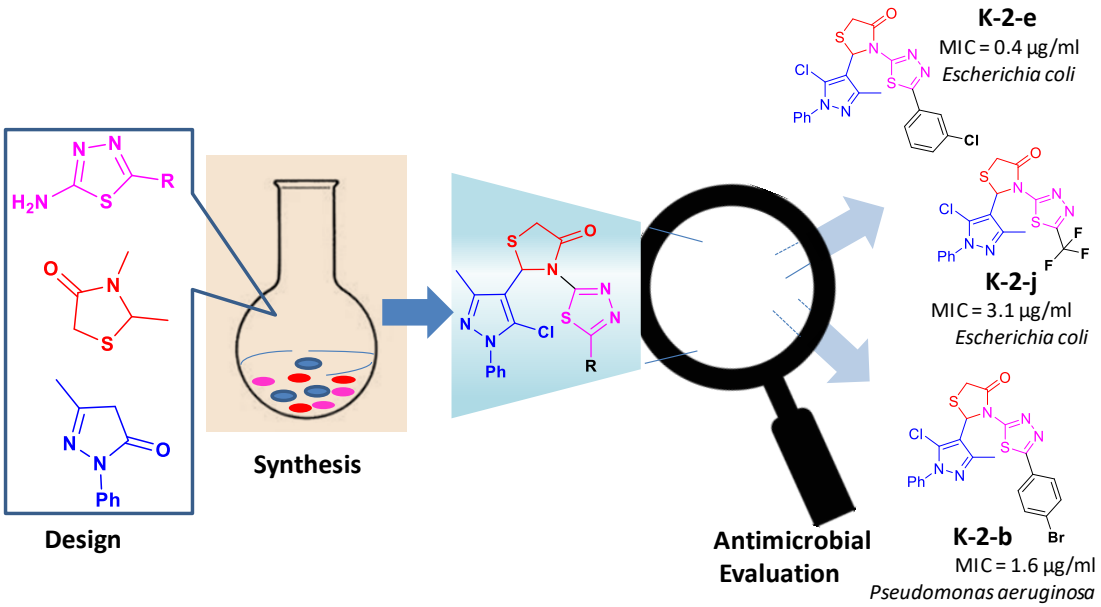
CHAPTER 3. MANUSCRIPT 2

After the review chapter, manuscript-2 based on synthesis and antimicrobial activity of pyrazolone and thiadiazole hybrid derivatives has been prepared titled **"Synthesis of novel pyrazole-thiadiazole hybrids linked by 4-thiazolidinone as antimicrobial agents".** In this paper we report a series of hybrids consisting of three medicinally important moieties namely pyrazole, 1,3,4-thiadiazole and 4-thiazolidinones and their antimicrobial activities. This paper was prepared by following the guidelines of the journal "European Journal of Medicinal Chemistry".

SYNTHESIS OF NOVEL PYRAZOL-THIADIAZOLE HYBRIDS LINKED BY 4-THIAZOLIDINONE AS ANTIMICROBIAL AGENTS



Concept of molecular hybridization with an aim to develop hybrids containing all the three medicinally known scaffolds, pyrazole, 4-thiazolidinone and thiadiazole as potential novel antimicrobial agents.

Abstract

In recent years molecular hybridization technique has been extensively used to develop potential pharmaceutical agents including antimicrobial leads. Employing this technique, we herein report a series of 13 hybrids consisting all the three medicinally important moieties such as pyrazole, and 1,3,4-thiadiazole linked by 4-thiazolidinones. The structures of newly synthesized compounds were characterized and further confirmed from ^{1}H NMR, ^{13}C NMR, HRMS and FT-IR analytical data. All the synthesized molecular hybrid compounds were evaluated for their antimicrobial activity against two gram-positive, four gram-negative and two fungal strains. It was evident from the antimicrobial screening results that hybrids **k-2-e** and **k-2-j** displayed promising antimicrobial activity with MIC 0.4 µg/ml and 3.1 µg/ml respectively against *Escherichia coli* whereas **k-2-b** showed MIC 1.6 µg/ml against *Pseudomonas aeruginosa*. A brief SAR study indicated that hybrids with electrophilic substituents such as halogens displayed highest antimicrobial activity. This work could provide impetus for development of antimicrobial agents based on the pyrazolone-thiazolidinones-thiadiazole

Keywords: Molecular hybrid, Antimicrobial agents, Pyrazole, 1,3,4-Thiadiazole, thiazolidinone.

1. Introduction

Global incidences indicating ever increasing progression rate of drug resistant and multidrug resistant (MDR) as well as extremely drug resistant (XDR) strains of microorganisms is well-recognized to be a serious health burden on the society [1]. Few main factors behind the fast development and spread of resistance traits could be attributed to the escalating time, costs in controlling bacterial diseases and its spread across continents [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) have phenomenal ability to develop drug resistance mechanism against multiple antibiotic classes they have become resistant to penicillin, methicillin, tetracycline and erythromycin; *Escherichia coli* (*E. coli)*, are resistant to penicillin, cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole etc. [3-4]. The increase in emergence of MDR in common pathogens has further aggravated the existing problem to diagnose and treat new infections in humans. Thus, there is an urgent need to develop potent, cost effective, and safer new class of novel antimicrobial agents active against resistant microbial strains [5].

With our focus in finding solution to the existing MDR microbes, there is an urgent need to explore and introduce some new modified heterocyclic scaffolds. Heterocycles are a great source of building blocks and occur in several natural as well as synthetic drug candidates, in particular, the azo heterocycles. One such heterocyclic scaffold is the pyrazolone ring system present in many natural and synthetic compounds and is known to exhibit wide spectrum of biological activities displaying appreciable antibacterial [6], antifungal [7], anti-tuberculosis [8], anticancer [9], anti-inflammatory [10] and COX-2 inhibition [11]. In recent decades, there have been increasing reports on the application of pyrazolone derivatives as a potential antimicrobial agent has shifted the focus of the medicinal chemists to further exploit heterocyclic scaffold. Similarly, thiadiazoles are also of the pharmaceutically important class of heterocyclic scaffold. Specifically, 1,3,4-thiadiazole moiety has been reported to display a diverse biological activity profile including antibacterial [12], antioxidant [13], anti-inflammatory [14], anticancer [15], antidepressant [16], antimalarial [17], antidiabetic [18], antitubercular [19] and antifungal [20] activities. Additionally, the Sulphur atom of 1,3,4-thiadiazole contributes to improved biological activity e.g. liposolubility and mesoionic nature of sulphur enhances the tissue permeability. 4-Thiazolidinone derivatives have a wide spectrum of pharmacological applications and several molecules containing this moiety are patented and are at different stages of research and clinical trials for antimicrobial [21], antihepatitis [22], anti-HIV [23], antiviral [24] and antifungal [25] activities.

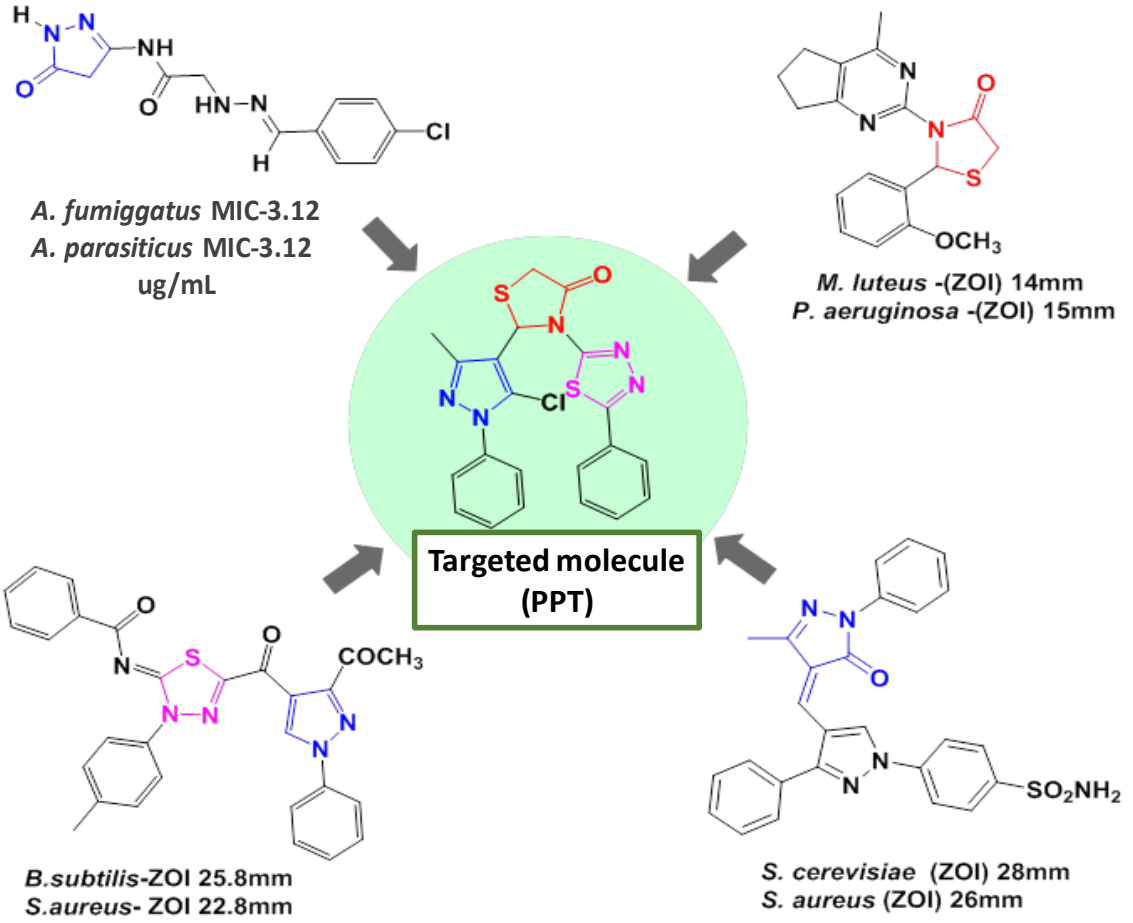


Figure 1. Designing of targeted molecular hybrid pyrazole motif linked to 1,3,4-thiadiazole via 4-thiazolidinone linker pyrazolone-thiazolidinones-thiadiazole (PTT).

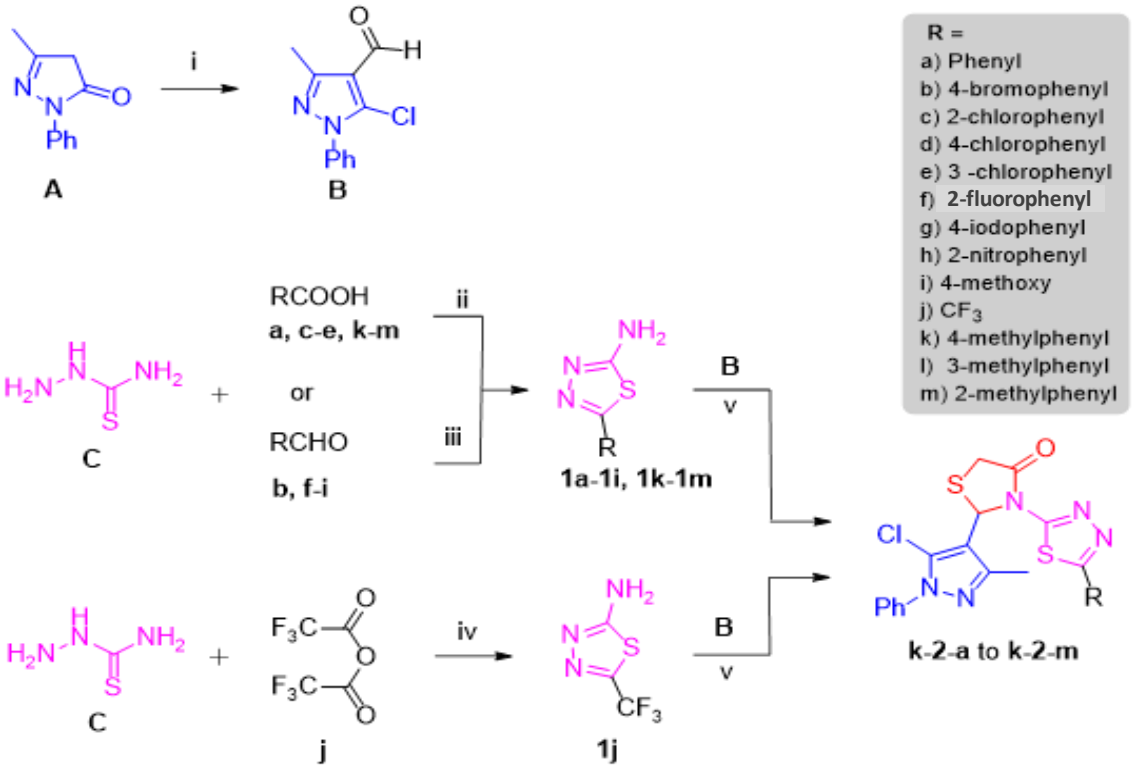
Keeping in mind the limitations associated with current marketed drugs as well as the emergence of MDR strains of microbes, we envisaged developing a new class antimicrobial drugs by molecular hybridization. Molecular hybridization is an important drug discovery technique to conjugate/fuse two or more bioactive scaffolds to generate potential pharmacophore units *via* a covalent linker. In this molecular hybridization technique structural modifications play a key role to obtain multiple-ligands/compounds with good pharmacokinetic profile. It also eliminates the need of concomitant administration of two different drugs [26]. The selection of the two motifs in a hybrid is usually based on their observed or anticipated synergistic or additive pharmacological activities. In the context of attempting to avoid antimicrobial drug resistance (MDR), hybridization is an attractive strategy, particularly when

the pharmacophores/ active molecules are conjugated to possess independent mode of action [27].

Pyrazolone, thiadiazole and thiazolidinone containing compounds have been reported to possess excellent antimicrobial properties (**Figure 1**) [28–31]. Thus by applying molecular hybridization approach we envisaged to design and synthesize novel hybrids consisting of Pyrazole motif linked to 1,3,4-thiadiazole *via* 4-thiazolidinone linker pyrazolone-thiazolidinones-thiadiazole (PTT) as shown in **Figure 1**. This strategy was facilitated by robust and experimentally simple cycloaddition of thioglycolic acid over pyrazole and 1,3,4-thiadiazole (**Scheme 1**).

2. Results and Discussion

Titled compounds **(k-2-a** to **k-2-m)** were synthesized by condensation of **B** (3-methyl-1-phenyl-1*H*- pyrazol-4-carbaldehyde) with **1a-1m** (2-amino-5-R-phenyl-1,3,4-thiadiazole) in a mixture of toluene and thioglycolic acid under reflux using Dean Stark apparatus/microwave conditions as reported by Cunico et.al. (2007) [32]. Starting material **B** was prepared by formylation of **A** (3-methyl-1-phenyl-1*H*- pyrazol-5(4*H*)-one) by Vilsmeier-Haack reaction as reported in the literature [33]. Whereas, compound **1a-1m** were synthesized by refluxing thiosemicarbazide **(C)** with various substituted benzoic acids/ benzaldehydes/ acetic anhydride (**a-m)** using $POCl_3/FeCl_3$, citric acid and sodium citrate under reflux conditions as reported previously [34–38]. The structures of the synthesized compounds were confirmed by IR, NMR and mass spectrometry (MS) techniques. The ^{1}H NMR spectrums of all the compounds revealed a distinct peak resonating at 2.33 - 2.89 ppm, which was assigned to the methyl group of the pyrazole ring. The aromatic protons were observed between 7.21 - 7.91 ppm.



Scheme 1. Synthetic scheme for target (PTT) molecules. Reagents and reaction conditions: (i) DMF, $POCl_3$, reflux; (ii) $POCl_3$, H_2O; (iii) $FeCl_3$, sodium citrate, citric acid, H_2O; (iv) reflux; (v) toluene, thioglycolic acid.

While the characteristic methylene (CH_2) proton peak of thiazolidinone appeared between 3.83 - 4.73 ppm, these non-identical methylene protons displayed characteristic coupling constant i.e. for geminal coupling J = 16.5 - 16.7 Hz (**Figure 2**). In ^{13}C NMR of all the titled compounds, the distinct methyl (CH_3) peak of the pyrazole ring appeared at 12.84 - 12.98 ppm and the methylene carbon (CH_2) peak of thiazolidinone was observed around 33.49 - 33.93 ppm, presenting the disappearance of aldehyde (CHO) peak in ^1H NMR at 9.9 ppm and amine (NH_2) peak at 7.2 - 7.4 ppm, thus confirming the formation of the thiazolidinone (**Figure 2**). The IR spectrums of all the final compounds exhibited a characteristic Cl (chlorine of pyrazole ring) band around 669.46 - 728.72 cm^{-1} and thiazolidinone carbonyl (C=O) was observed at 1689.47 - 1711.07 cm^{-1}. All synthesized compounds demonstrated the characteristic molecular ion peak $[M+H]^+$ corresponding to the molecular weight of the desired compounds. The obtained characterization data were in agreement with the structures of the desired final compounds.

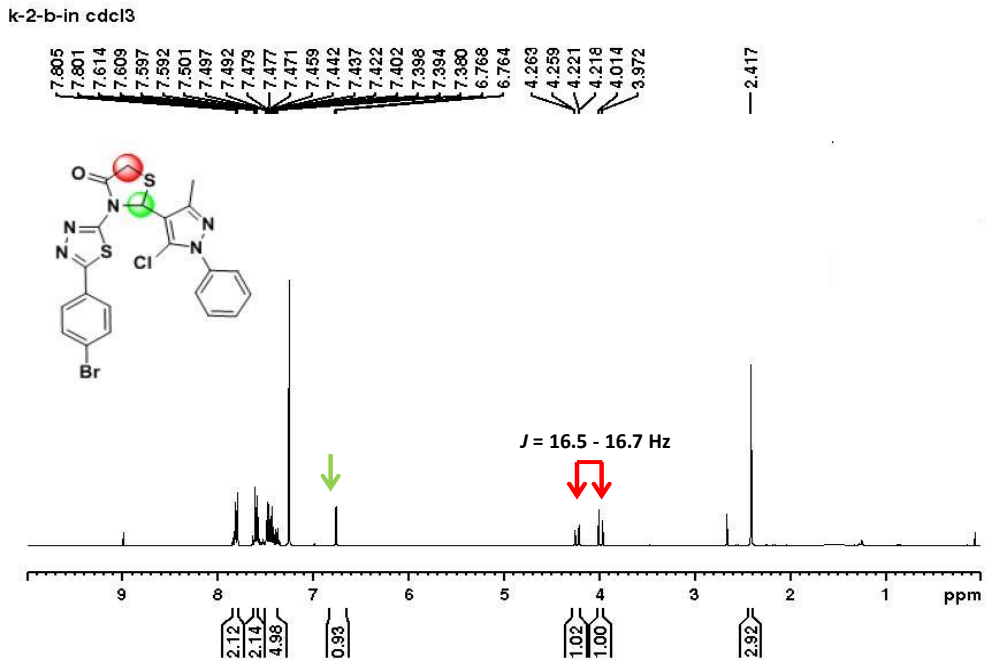


Figure 2. [1]H NMR of compound **k-2-b**.

2.1 Biological evaluations

2.1.1 Microorganism used: Standard cultures of two gram-positive [MRSA- ATCC BAA-1683 *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus*) and SA- ATCC 25923 *Staphylococcus aureus*], four gram-negative [ST-*Salmonella typhimurium*, PA-ATCC 27853 *Pseudomonas aeruginosa*, EC-ATCC 25922 *Escherichia coli*, KP- ATCC 31488 *Klebsiella pneumoniae*] and two fungal species [*Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC66031] were used for the antibacterial and antifungal activity respectively. Culturing and sub-culturing (one day prior to testing) of these microorganisms were carried out at the Discipline of Pharmaceutical Sciences, College of Health Sciences, UKZN, Durban, South Africa.

2.1.2 In vitro evaluation of antibacterial activity: Preliminary antibacterial screening of the synthesized compounds were carried out against panel of bacteria The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37 ºC in a shaking incubator (100 rpm). The bacterial concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton (MHA) agar plates (Biolab, South Africa) were lawn inoculated with the prepared bacterial suspensions using a sterile throat swab and 10 µl of the solution of synthesized compounds (1 mg/ml DMSO) spotted on the MHA plates. The plates were incubated at 37 ºC for 18 h and after incubations the plates were read to determine antibacterial activity which was denoted by clear zones in the area where the solutions were

spotted. Based on the preliminary screening results the minimum inhibitory concentrations (MICs) were determined. The solution of synthesized compounds was serially diluted 2-fold with DMSO ranging from 0.006 to 100 µg/ml and 10 µl of each concentration was spotted on the lawn inoculated MHA plates and incubated at 37 ºC for 24 h. Ciprofloxacin hydrochloride was used as a standard drug.

2.1.3 In vitro evaluation of antifungal activity: The synthesized hybrid of pyrazole-thiadiazole with linker thiazolidinone were further assessed for antifungal activity against the two fungal species using amphotericin B as a reference standard drug by following earlier reported MIC assay method [38-40].

Table 1. Antimicrobial activity of pyrazolone-thiazolidinones-thiadiazole (PTT).

MIC µg/ml								
Compounds	**ST**	**PA**	**EC**	**KP**	**MRSA**	**SA**	**CA**	**CN**
k-2-a	200	100	6.25	na	na	na	>200	>200
k-2-b	200	**1.6**	25	na	na	na	>200	>200
k-2-c	200	25	12.5	na	na	na	>200	>200
k-2-d	>200	50	12.5	na	na	na	>200	>200
k-2-e	>200	12.5	**0.4**	na	na	na	>200	>200
k-2-f	>200	>200	100	200	na	na	>200	>200
k-2-g	na	na	na	na	na	na	>200	>200
k-2-h	>200	100	12.5	na	na	na	>200	>200
k-2-i	>200	25	25	na	na	na	>200	>200
k-2-j	>200	12.5	**3.12**	6.25	na	na	>200	>200
k-2-k	>200	6.25	6.25	12.5	na	na	>200	>200
k-2-l	>200	50	3.12	na	na	na	>200	>200
k-2-m	>200	12.5	12.5	na	na	na	>200	>200
Ciprofloxacin HCl	0.4	0.8	0.1	1.6	25	25	-	-
Amphotericin B	-	-	-	-	-	-	0.39	1.5

ST-*Salmonella typhimurium*, **PA**-*Pseudomonas aeruginosa*, **EC**-*Escherichia coli*, **KP**-*Klebsiella pneumoniae*, **MRSA**-Methicillin-resistant *Staphylococcus aureus*, **SA**-*Staphylococcus aureus*, **CA**-*Candida albicans*, **CN**-*C. neoformans*. * na - not active.

2.2 Discussion

The results (MIC values) of *in vitro* antibacterial and antifungal screening of the tested compounds are summarized in **Table 1**. A systematic analysis of the data as depicted in **Table 1** revealed that all compounds showed moderate inhibition against *Salmonella typhimurium*, *Candida albicans* and *C. Neoformans*. Compound **k-2-b** presented highest inhibition at MIC 1.6 µg/ml followed by **k-2-k** at MIC 6.25 µg/ml, while **k-2-e, k-2-j, k-2-m** displayed moderate activity at 12.5 µg/ml for *Pseudomonas aeruginosa.* Against *Escherichia coli* compound **k-2-e** was highly active at MIC **0.4** µg/ml, whereas **k-2-j** and **k-2-l** were most active at MIC 3.12 µg/ml followed by **k-2-a** and **k-2-k** at MIC 6.25 µg/ml, while hybrids **k-2-c, k-2-d, k-2-h, k-2-m** indicated good inhibition at MIC 12.5 µg/ml. Only hybrids **k-2-j** and **k-2-m** displayed best

activity at MIC 6.25 and 12.5 µg/ml against *Klebsiella pneumonia* respectively. Among the tested hybrids from this series, **k-2-e**, **k-2-b** and **k-2-j** were best active hybrids (**Figure 3**). A brief structure activity relationship (SAR) revealed that hybrids with 4-bromo phenyl, 3-chloro phenyl and trifluoromethyl groups on the thiadiazole ring exhibited best activity against gram-negative bacteria, which could be due to the electrophilic nature of the substituents on the thiadiazole ring responsible for high potency of these hybrids. The antimicrobial activity data for these hybrids is presented in **Table 1.**

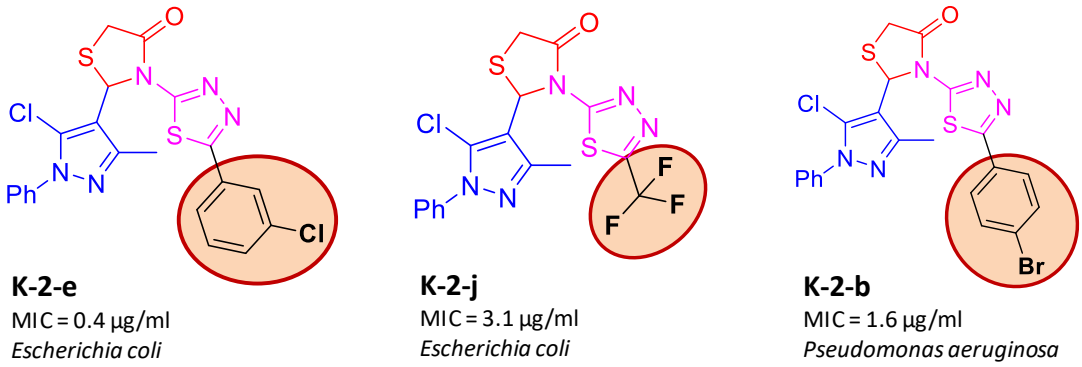


Figure 3. Chemical structures of potent PTT hybrids with antibacterial activity.

3. Experimental

3.1 Materials and methods

All the chemicals and reagents used in this research work were bought directly from Sigma Aldrich and Merck Millipore, South Africa. All the solvents, except those of laboratory reagent grade, were dried and purified when necessary according to previously reported methods. The progress of the reactions and the purity of the compounds were monitored by Thin Layer Chromatography (TLC) on a pre-coated silica gel plates procured from Merck (Pty) Ltd. The melting points of the synthesized compounds were determined using a Thermo Fisher Scientific (IA9000, UK) digital melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker Alpha FT-IR spectrometer (Billerica, MA, USA) using the ATR technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany) spectrometers using $CDCl_3$ and DMSO-d_6. The chemical shifts are reported in δ ppm units with respect to trimethylsilane (TMS) as an internal standard. HRMS spectra were recorded on an autospec mass spectrometer with electron impact at 70 eV.

3.2 Synthesis

3.2.1. ***The general method of preparation of 5-chloro -3-methyl-1-phenyl-1H- pyrazol-4-carbaldehyde (B) by Vilsmeier-Haack reaction*****:** To a stirred mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**A**) (0.02 mole, 1 equiv) and dimethylformamide (0.02 mole, 1 equiv) at 0 °C, $POCl_3$ (0.024 mole, 1.2 equiv) was added dropwise. The reaction mixture was brought to room temperature and then refluxed for 3 h. After completing the reaction, the warm reaction mixture was poured into ice water. The precipitate formed was separated by filtration and washed with cold water. The dried precipitate was recrystallized in ethanol.

3.2.2. ***Synthesis of 2-amino-5-phenyl(R)-1,3,4-thiadizole using carboxalic acid*** **(1a,1c-1e,1k-1m)**: Substituted phenyl carboxylic acid (**a**, **c-e**, **k-m)** (0.01 mole, 1 equiv) and thiosemicarbazide (**C)** (0.01 mole, 1 equiv.) were stirred at 0 °C for 10 min, followed by slow dropwise addition of phosphorus oxychloride (0.03 mole, 3 equiv). The reaction mixture was brought to room temperature and then refluxed for 1 h. Ice cold water (20 ml) was added to the reaction mixture and further refluxed for 3 h. After completion of reaction as indicated by TLC, the mixture was basified by NH_4OH (pH 7 – 8).The formed solid was filtered and recrystallized in ethanol.

3.2.3. ***Preparation of 2-amino -5-phenyl(R)-1,3,4 thiadiazole using carboxyaldehyde*** **(1b, 1f-1i);** To the mixture of thiosemicarbazide (**C**) (0.01 mole, 1 equiv) in warm water (15 ml), an aromatic aldehyde (**b, f - i)** (0.01 mole, 1 equiv) dissolved in warm ethanol was added and stirred at 0 °C for 1 h. The precipitated compound was filtered and dried. The resulting dry solid (1 equiv) was taken in warm water and $FeCl_3$ (3 equiv) solution and warm alcohol was added dropwise followed by reflux for 1 h. After completion of reaction, the reaction mixture was filtered hot. Sodium citrate (1 equiv) and citric acid (2.2 equiv) were added to the mixture and basified with NH_4OH (pH 7 – 8). The resulting solid was filtered and recrystallized in ethanol [34].

3.2.4. ***Synthesis of 5-(trifluoromethyl)-1,3,4-thiadiazol-2-amine*** **(1j):** A mixture of trifluoro acetic anhydride **j** (0.01 mole, 1 equiv), and thiosemicarbazide (**C)** (0.01 mole, 1 equiv), was heated at 40 °C on a water bath for 1 h. The reaction mixture was cooled, diluted with water and basified with NH_4OH (pH 7 – 8) the crystalline precipitate was recrystallized in ethanol to give white crystals.

3.2.5. ***Synthesis of 2-(5-chloro-3-methyl-1-phenyl-1Hpyrazol-4-yl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-one*** **(k-2-a to k-2-m)** ***using conventional /microwave method*****:** 2-amino-5-phenyl(R)-1,3,4 thiadiazole (0.001 mole, 1 equiv) (**1a-1m)** was dissolved in toluene and stirred with 3-methyl-1-phenyl-1*H*- Pyrazol-4-carbaldehyde (**B**, 0.0012 mole, 1.2 equiv) followed by the addition of thioglycolic acid (0.003 mole, 3 equiv) and refluxed for 36 - 40 h by using Dean stark apparatus/microwave at 160 ºC - 30 min x 3 - 4, power - 150 W and pressure - 150 psi. The reaction was monitored by TLC (mobile phase: 2/3, ethyl acetate: hexane) and after complete

cyclo-condensation, crude organic compound was extracted in chloroform by washing with sodium bicarbonate, water, and brine solution respectively and dried over anhydrous sodium sulphate then solvent removed under reduced pressure to get crude compound. Purification was performed by column chromatography on silica gel using hexane and ethyl acetate as eluents.

3.3 Experimental data

Note: We have taken HRMS of selected compounds to support NMR results, remaining all compounds are characterized by NMR and IR only.

3.3.1. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one **(k-2-a):** Yellow solid; Yield**:** 45%; mp: 143 - 145°C; IR (ATR, ν_{max}, cm^{-1}): 685.81 (C-Cl), 1670.38 (C=O), 2773.22 (C-H, CH_2), 2956.64 (C-H, CH_3), 3059.69 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (s, 3H), 3.96 - 4.00 (d, J = 16.5 Hz 1H), 4.21 - 4.25 (d, J = 16.5 Hz, 1H), 6.77 (s, 1H), 7.35 - 7.50 (m, 8H), 7.92 - 7.94 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.92, 33.63, 55.36, 115.82, 125.07, 127.05, 127.45, 128.38, 128.99, 129.03, 129.18, 130.37, 130.94, 137.70, 147.96, 156.67, 164.60, 169.93 ppm;

3.3.2. 3-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-2-(5-chloro-3-methyl-1-phenyl-H-pyrazol-4-yl)thiazolidin-4-one **(k-2-b):** Yellow solid; Yield**:** 40%; mp: 203 - 206 °C; IR (ATR, ν_{max},cm^{-1}): 560.92 (Ar-Br), 691.37 (C-Cl), 1706.10 (C=O), 2917.48 (C-H, CH_2) , 2961.75 (C-H, CH_3), 3052.31 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$): δ = 2.41 (s, 3H), 3.97 - 4.01 (d, J = 16.7 Hz, 1H), 4.21 - 4.26 (d, J = 16.7 Hz, 1H), 6.76 (s, 1H), 7.38 - 7.55 (m, 5H), 7.59 - 7.61 (d, J = 8.4 Hz, 2H), 7.80-7.82 (d, J = 8.6 Hz, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.92, 33.62, 55.36, 115.72, 125.05, 125.21, 125.37, 128.41, 128.77, 129.00, 132.43, 137.68,147.96, 156.78, 163.44, 169.97 ppm; HRMS (ESI^+) *m/z* for $C_{21}H_{15}BrClN_5OS_2 + Na^+$: calcd, 553.9488; found, 553.9488.

3.3.3. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-c):** Yellow solid; Yield**:** 52%; mp: 161 - 163 °C; IR (ATR, ν_{max},cm^{-1}): 691.99 (C-Cl), 1704.67 (C=O), 2915.36 (C-H, CH_2) , 2961.32 (C-H, CH_3), 3053.85 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.44 (s, 3H), 3.98 - 4.02 (d, J = 16.4 Hz, 1H), 4.23 - 4.28 (d, J = 16.4 Hz, 1H) , 6.80 (s, 1H), 7.38 - 7.54 (m, 8H), 8.24 - 8.27 (m, 1H) ppm; ^{13}C NMR **(**100 MHz, $CDCl_3$) δ = 12.95, 33.55 , 55.28, 115.85, 125.07, 127.28, 128.38, 128.99, 130.56, 130.96, 131.45, 132.33, 137.72, 147.98, 158.56, 160.52, 169.99 ppm.

3.3.4. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-d):** Yellow solid; Yield**:** 55%; mp: 195 - 197 °C; IR (ATR, ν_{max},cm^{-1}): 728.83 (C-Cl), 1689.55 (C=O), 2932.05 (C-H, CH_2), 2974.53 (C-H, CH_3), 3080.10 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.37 (s, 3H), 3.93 - 3.97 (d, J = 16. 5 Hz, 1H), 4.17 -

4.22 (d, *J* = 16.5 Hz, 1H), 6.72 (s, 1H), 7.33 - 7.46 (m, 7H), 7.82 - 7.84 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.91, 33.61, 55.36, 115.72, 125.05, 128.41, 128.59, 129.00, 129.48, 137.04, 137.68, 147.96, 156.77, 163.36, 169.97 ppm.

3.3.5. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-e):** Yellow solid; Yield: 52%; mp: 169 - 171°C; IR (ATR, ν_{max},cm^{-1}): 678.85 (C-Cl), 1693.24 (C=O), 2930.27 (C-H, CH_2), 2954.40 (C-H, CH_3), 3041.63 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H), 3.99 - 4.03 (d, *J* = 16.5 Hz, 1H), 4.23 - 4.28 (d, *J* = 16.5 Hz, 1H), 6.78 (s, 1H), 7.39 - 7.51 (m, 7H), 7.80 - 7.82 (m, 1H), 7.99 - 7.99 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.91, 30.93, 55.30, 115.68, 125.06, 125.55, 127.33, 128.42, 129.00, 130.43, 130.87, 131.73, 135.27, 137.68, 147.98, 156.94, 163.06, 169.99 ppm.

3.3.6. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-f):** Yellow solid; Yield: 49%; mp: 152 - 154 °C; IR (ATR, ν_{max},cm^{-1}): 696.87 (C-Cl), 1525.57 (Ar-NO_2), 1687.77 (C=O), 2922.60 (C-H, CH_2) , 2950.94 (C-H, CH_3), 3065.68 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (s, 3H), 3.97 - 4.01 (d, *J* = 16.5 Hz, 1H), 4.22 - 4.26 (d, *J* = 16.5 Hz, 1H), 6.76 (s, 1H), 7.38 - 7.51 (m, 5H), 7.663 - 7.71 (m, 3H), 8.00 - 8.02 (d, *J* = 7.68 Hz, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.93, 33.54, 55.42, 115.61, 124.91, 125.11, 128.42, 129.01, 131.34, 132.37, 132.91, 137.70, 148.04, 158.39, 159.31, 170.14 ppm.

3.3.7. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(4-iodophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-g)**: Yellow solid; Yield: 42%; mp: 225 - 127 °C; IR (ATR, ν_{max},cm^{-1}): 497.46 (Ar-I), 692.09 (C-Cl), 1711.07 (C=O), 2916.60 (C-H, CH_2) , 2974.40 (C-H, CH_3), 3051.23 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.39 (s, 3H), 3.95 - 3.99 (d, *J* = 16.5 Hz, 1H), 4.19 - 4.24 (d, *J* = 16.5 Hz, 1H), 6.74 (s, 1H), 7.35 - 7.47 (m, 5H), 7.63 - 7.65 (m, 2H), 7.78 - 7.80 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.91, 33.61, 55.36, 97.33, 115.72, 125.05, 128.41, 128.78, 129.00, 137.68, 138.37, 147.97, 156.77, 163.61, 169.97 ppm.

3.3.8. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-h)**: Yellow solid; Yield: 45 %; mp: 197 - 199 °C; IR (ATR, ν_{max},cm^{-1}): 693.09 (C-Cl), 1357.56 (Ar-F), 1690.39 (C=O), 2927.41 (C-H, CH_2) , 2955.72 (C-H, CH_3), 3065.60 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.89 (s, 3H), 4.44 - 4.48 (d, *J* = 16.5 Hz, 1H), 4.69 - 4.73 (d, *J* = 16.5 Hz, 1H), 7.66 - 7.97 (m, 8H), 7.78 - 7.81 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.93, 33.54, 55.31, 115.86, 116.10, 125.05, 128.98, 132.29, 132.38, 137.71, 147.94, 157.36, 157.44, 158.49, 158.50, 160.68, 169.99 ppm.

3.3.9. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-i)**: Yellow solid; Yield: 48 %; mp: 168 - 170 °C; IR (ATR, ν_{max},cm^{-1}): 691.99 (C-Cl), 1689.47 (C=O), 2838.75 (C-H, CH_2) , 2936.91 (C-H, CH_3), 3004.62

(C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.39 (s, 3H), 3.83 (s, 3H), 3.93 - 3.97 (d, *J* = 16.5 Hz, 1H), 4.18 - 4.22 (d, *J* = 16.5 Hz, 1H), 6.74 (s, 1H), 6.93 - 6.95 (m, 2H), 7.35 - 7.47 (m, 5H), 7.84 - 7.86 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.92, 33.64, 55.34, 55.45, 114.57, 115.89, 122.69, 125.07, 128.36, 128.97, 137.72, 147.95, 156.06, 161.79, 164.40, 169.83 ppm; HRMS (ESI^+) *m/z* for $C_{22}H_{18}ClN_5O_2S_2 + Na^+$: calcd, 506.0488 ; found, 506.0488.

3.3.10. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-j)**: Yellow solid; Yield: 30 %; mp: 111 - 113 °C; IR (ATR, ν_{max},cm^{-1}): 696.72 (C-Cl), 1143.82 (F), 1695.77 (C=O), 2931.92 (C-H, CH_2), 2973.86 (C-H, CH_3), 3057.99 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H), 4.01 - 4.06 (d, *J* = 16.7 Hz, 1H), 4.27 - 4.31 (d, *J* = 16.7 Hz, 1H), 6.74 (s, 1H), 7.39 - 7.51 (m, 5H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.84, 33.49, 55.39, 115.01, 125.05, 125.65, 128.55, 129.05, 137.57, 148.14, 159.12, 170.38 ppm.

3.3.11. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-p-tolyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-k):** Yellow solid; Yield: 57 %; mp: 151 - 153 °C; IR (ATR, ν_{max}, cm^{-1}): 690.47 (C-Cl), 1690.15 (C=O), 2939.28 (C-H, CH_2), 2979.54 (C-H, CH_3), 3015.97 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.39 (d, *J* = 7.76 Hz, 6H), 3.95 - 3.99 (d, *J* = 16.5 Hz, 1H), 4.20 - 4.24 (d, *J* = 16.5 Hz, 1H), 6.71 - 6.76 (d, *J* = 16.489 Hz, 1H), 7.35 - 7.53 (m, 6H), 7.78 - 7.83 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.90, 21.4, 33.64, 55.34, 115.87, 125.08, 127.30, 127.36, 128.37, 128.98, 129.70, 129.81, 129.86, 137.71, 141.39, 147.97, 156.37, 164.73, 169.88 ppm.

3.3.12. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-m-tolyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-l):** Yellow solid; Yield: 58 %; mp: 162 - 164 °C; IR (ATR, ν_{max},cm^{-1}): 677.68 (C-Cl), 1688.97 (C=O), 2927.05 (C-H, CH_2), 2955.33 (C-H, CH_3), 3036.17 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (s, 3H), 2.34 (s, 3H), 3.89 - 3.93 (d, *J* = 16.5 Hz, 1H), 4.13 - 4.18 (d, *J* = 16.5 Hz, 1H), 6.69 - 6.70 (s, 1H), 7.18 - 7.42 (m, 7H), 7.63 - 7.65 (d, *J* = 7.604 Hz, 1H), 7.70 (s, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.92, 21.31, 33.64, 55.33, 115.84, 124.65, 125.07, 127.99, 128.37, 128.93, 129.06, 129.93, 131.74, 137.71, 139.03, 147.98, 156.56, 164.79, 169.90 ppm.

3.3.13. 2-(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(5-o-tolyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **(k-2-m):** Yellow solid; Yield: 54 %; mp: 158 - 160 °C; IR (ATR, ν_{max},cm^{-1}): 669.46 (C-Cl), 1694.87 (C=O), 2919.62 (C-H, CH_2), 2959.62 (C-H, CH_3), 3062.14 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.41 (s, 3H), 2.57 (s, 3H), 3.95 - 3.99 (d, *J* = 16.5 Hz, 1H), 4.20 - 4.25 (d, *J* = 16.5 Hz, 1H), 6.79 (s, 1H), 7.27 - 7.51 (m, 8H), 7.66 - 7.68 (d, *J* = 7.72 Hz, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 12.98, 21.58, 33.56, 55.37, 115.89, 125.06, 126.26, 128.38,

129.09, 129.10, 130.29, 130.52, 131.55, 137.16, 137.73, 147.93, 157.28, 164.04, 170.00 ppm; HRMS (ESI^+) *m/z* for $C_{22}H_{18}ClN_5OS_2 + Na^+$: calcd, 490.0539; found, 490.0539.

4. Conclusion

In summary, conjugation of suitable active subunits through the molecular hybridization enabled us to create new chemical entities with potential antimicrobial activity. These novel series of hybrids containing pyrazole and 1,3,4-thiadiazole linked by 4-thiazolidinone were prepared by efficient, mild and simple one-pot synthetic protocol. All the synthesized hybrids were evaluated against a panel of gram-positive and gram-negative bacterial, and fungal strains.

The antimicrobial activity data obtained suggested that most of the synthesized hybrids were selectively active against gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*. Compounds **k-2-e, k-2-b** and **k-2-j** exhibiting best activity. However, no or little activity was observed for these compounds against *Salmonella typhimurium*, *Staphylococcus aureus* and MRSA as well as fungal strains. In conclusion the novel series of Pyrazolone-Thiadiazol-Thiazolidinone (PTT) hybrid molecules presented an excellent antimicrobial activity against certain pathogenic strains and can further be developed into new class of antimicrobials.

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6. Conflict of interest

Authors hereby declare that there are no financial/commercial conflicts of interest.

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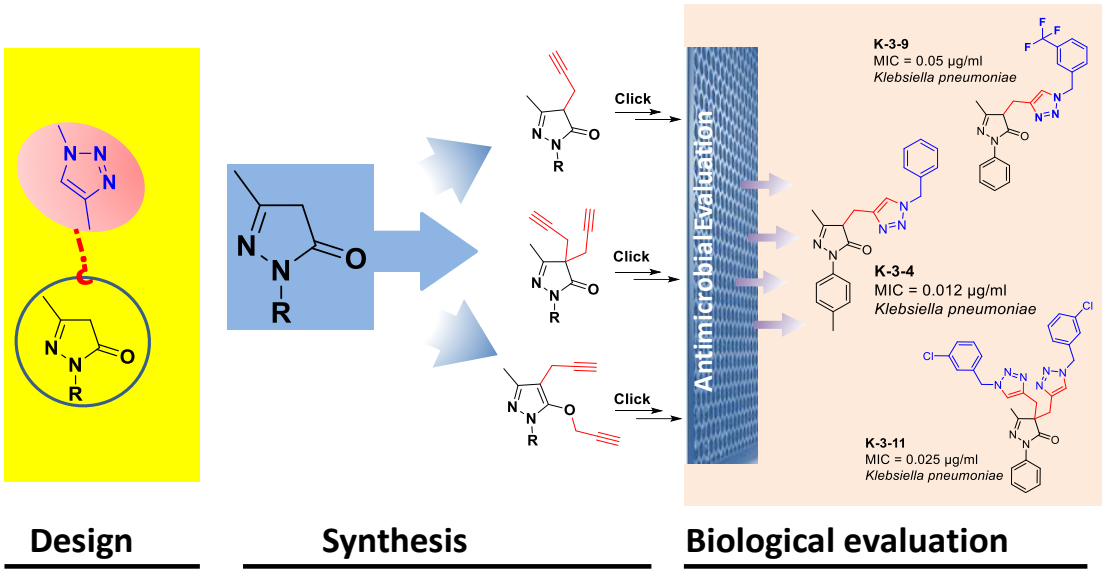
CHAPTER 4. MANUSCRIPT 3

The manuscript-3 entitled "**A powerful divergent one-pot synthesis of three distinct novel molecular architectures: pyrazolone–triazole and their antimicrobial evaluation"** is prepared according to the guidelines of the journal "European Journal of Medicinal Chemistry". In here we report the synthesis of novel hybrids consisting of pyrazolone and 1,2,3 triazole moieties in a single molecular framework and their antibacterial activities.

A POWERFUL DIVERGENT ONE-POT SYNTHESIS OF THREE DISTINCT NOVEL MOLECULAR ARCHITECTURES: PYRAZOLONE–TRIAZOLES AND THEIR ANTIMICROBIAL EVALUATION



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Abstract

The present research work describes, a novel approach to the synthesis of 22 novel hybrids consisting of pyrazolone and 1,2,3 triazole moieties in a single molecular framework. Synthesis of targeted new molecular hybrids (substituted pyrazolone-triazoles) was achieved by a powerful one-pot divergent approach, from readily available propargyl bromide and various substituted benzyl azides resulting in twenty-two new molecular hybrids. All 22 hybrid molecules were characterized and further evaluated for *in-vitro* antibacterial activity against a panel of gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683) and gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium*). In addition, these hybrids were also evaluated against two fungal strains (*Candida albicans* ATCC 90028 and *Cryptococcus. neoformans* ATCC 6603). ^{1}H NMR, ^{13}C NMR, HRMS and FT-IR spectral data confirmed the chemical structures of these hybrid molecules. The minimum inhibitory concentrations of the synthesized compounds exhibited moderate to high potency against the gram-negative bacteria. Among these synthesized compounds, **k-3-4** (MIC 0.012 μg/ml)**, k-3-9** (MIC 0.050 μg/ml), and **k-3-11** (MIC 0.025 μg/ml) exhibited most potent in *vitro* antimicrobial activity against *Klebsiella pneumoniae.* Results of the Structure activity relationships (SAR) study of synthesized hybrid molecules opens up a new door for the positive impact of pyrazolone-triazole hybrid in antimicrobial drug discovery hence widening the scope of the pyrazolone scaffold in the development of potent new generation antimicrobial agents.

Keywords: Pyrazole, Triazole, Pyrazolone-traizole hybrid, Click reaction, Antimicrobial**.**

1. Introduction

The significant rise in resistant strains of pathogenic microorganisms has become a major threat to human kind. In addition, multidrug resistant bacteria's such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Haemophilus* and several other species of bacteria have become increasingly hostile to the current drug regimen, complicating the treatment and increasing the global burden. According to World Health Organization (WHO) report (2016), microbial infections account for 3.09 million deaths (35.1%) per annum in Africa alone [1]. It is a well known fact that microbes reproduce at fast rate multiplying every few hours and during replication, mutation arise helping some of the microbes to survive exposure to antimicrobials. These drug resistant bacteria may then transfer a copy of their DNA to other non-resistant bacteria, making the successive generations resistant to antibiotics. Once the resistance is developed the drug becames ineffective further increasing the rate of morbidity and mortality [2]. Some of the major factors responsible for driving antimicrobial resistance are over use and misuse of antibiotics, long treatment regimens, environmental contamination by waste products arising from antibiotic manufacture and disposal, spread of hospital and other health care acquired resistant bacteria, sub-optimal rapid diagnostics and preventative medicines/vaccination, incorrect dosing of antibiotics in humans, global spread of resistant strains by humans travel, mass drug administration and lastly too few new drugs are being introduced or developed to replace the ineffective drugs [3–5].

To counter the drug resistance in bacteria there is an urgent need to create public awareness for the safe and proper use of antibiotics as well as to develop broad-spectrum potent new drugs to bridge the increasing gap between ineffective and effective antibiotics. Recent trends in new drug discovery show that the researchers are employing molecular hybridization strategy for developing new and safe drugs. Molecular hybridization is a modern concept where two or more active pharmacophores are conjugated to develop a new hybrid molecule with enhanced affinity, efficacy and safety as compared to the parent drugs [6].

Pyrazolone is one such scaffold displaying broad spectrum of pharmacological applications and has emerged as an important building block for the development of new potent drug like compounds. It is a simple five membered lactam ring containing two nitrogen atom at adjacent position and is a derivative of pyrazole that has additional keto group at 5th position (**Figure 1)**. Pyrazolone is a core structure/backbone in numerous marketed drugs like famprofazone and dipyron (**Figure 2)** [4]. Some substituted pyrazoline derivatives have been reported to display anticancer [7], anti-inflammatory [8], antimicrobial [9], antioxidant [10], antifungal [11], anti-tubercular [12] and insecticidal [13] and oral hypoglycaemic activity. Due to its diverse

pharmacological properties, the chemistry of pyrazolones is gaining attention and there have been numerous synthetic methodologies reported recently [14]. These reports inspired us to synthesize new class of hybrid compounds containing pyrazolone as one of the core unit.

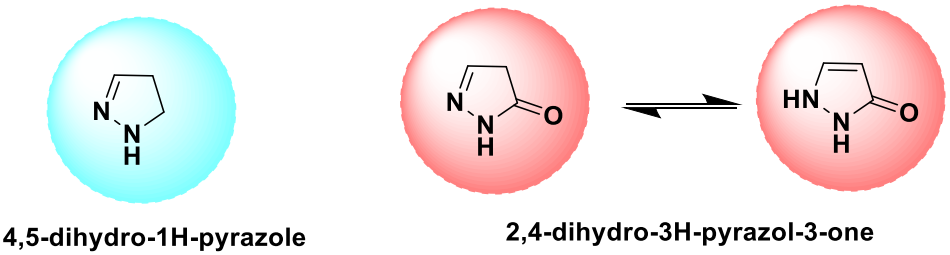


Figure 1. Basic structure of pyrazolone.

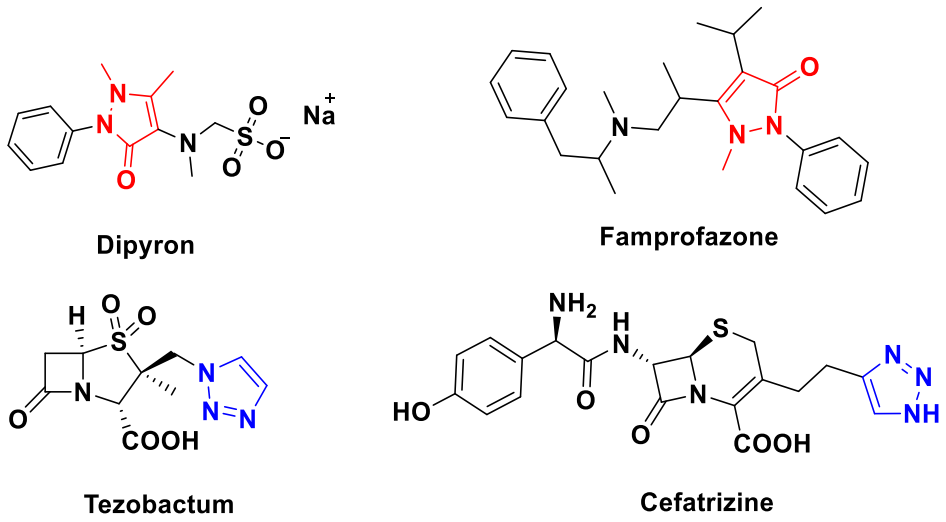


Figure 2. Marketed drugs containing pyrazolone and triazole moieties.

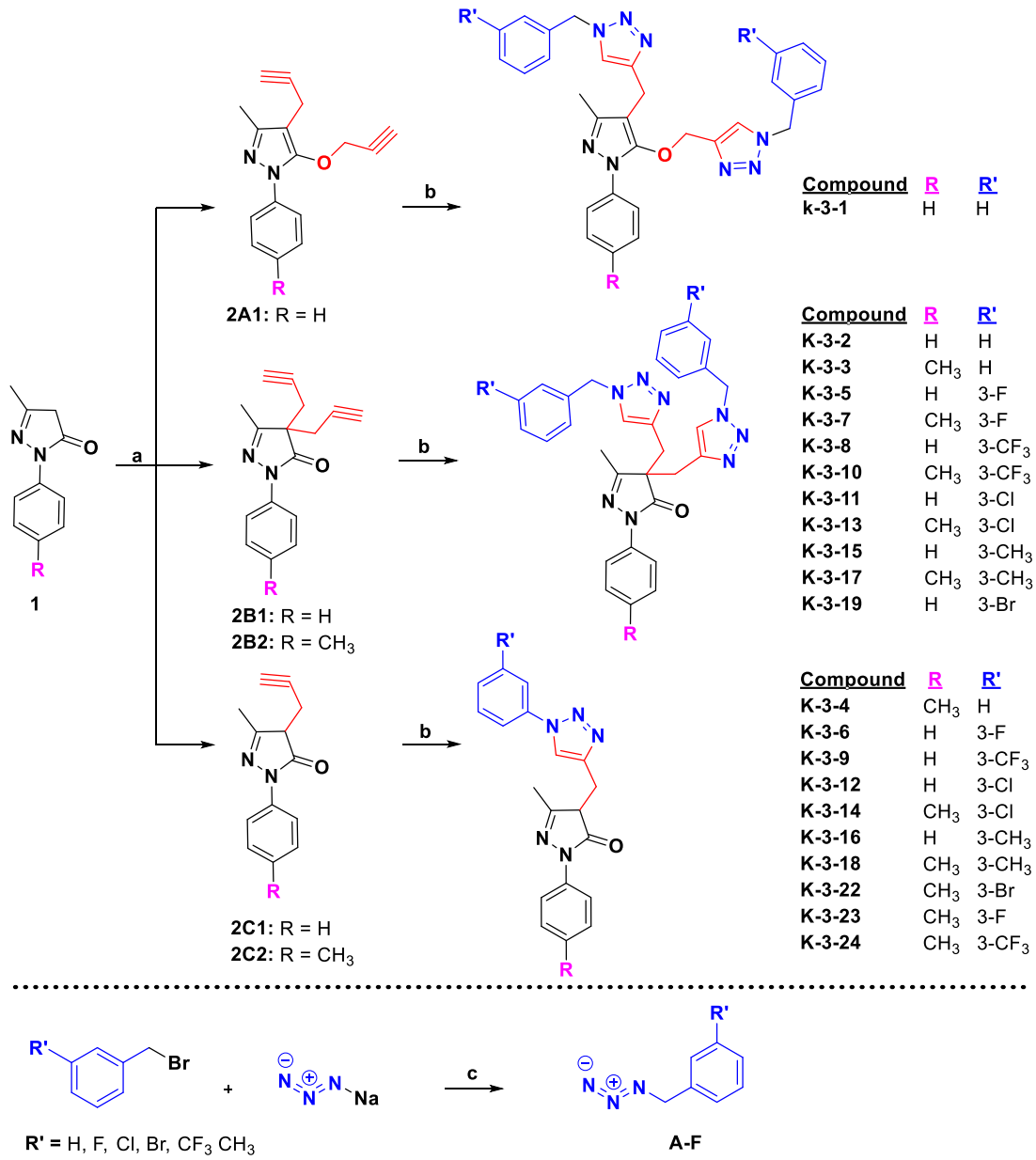
Azides are also considered as very important class of heterocyclic compounds with useful synthetic applications in preparation of 1,2,3-triazoles via 1,3-dipolar cycloaddition with substituted alkynes [15]. Triazoles are known to possess a wide range of pharmacological activities such as antifungal [16], anticancer [17], anti-inflammatory, antinociceptive and ulcerogenic activities [18], antitubercular [19], antileishmanial [20], antimicrobial [21], antiviral [22], and antibacterial activity [23]. Azobactum and cefatrizine (**Figure 2**) are two well-known potential antibacterial marketed drugs based on 1,2,3- triazole. Hence it was thought worthwhile to synthesize some new pyrazolone-triazole hybrids by using well known click reaction strategy.

The present work deals with the synthesis of the title compounds, followed by their antimicrobial screening.

2. Results and Discussion

Heterocycles containing 1,2,3-triazole moiety are considered significant molecules in medicinal chemistry due to their important biological activities. The 1,2,3-trizole nucleus has high aromatic stability to hydrolysis, oxidation and reduction in acidic and basic environment. Further, it has high dipole moment which improves its binding affinity for biological targets through hydrogen bonding and improves their solubility [24].

The final hybrid compounds **k-3-1** to **k-3-24** were synthesized by well-known and widely used click chemistry approach. Reaction proceeded by 1,3 cycloaddition between alkyne and azide to produce 1,2,3 triazoles. Specifically, by stirring pyrazolone alkyne intermediate (**2A, 2B, 2C** as shown in **Scheme 1)** with different substituted benzyl azide (**A-F**) in presence of ascorbic acid and $CuSO_4 \cdot 5H_2O$ in a solution of methanol and water as per reported protocol [25]. One-pot quantitative preparation of three intermediate (**2A**, **2B** and **2C)** were performed by stirring starting material **1** (5-methyl-2-(*p*-tolyl/phenyl)-2,4-dihydro-3*H*-pyrazol-3-one) with dry potassium carbonate in dry DMF followed by dropwise addition of propargyl bromide. Purification of all three intermediates was achieved by silica gel column chromatography in ethyl acetate and hexane as solvent. Whereas, substituted benzyl azide (**A-F**) were synthesized by stirring substituted benzyl bromide and sodium azide in mixture of acetone and water as per previously reported standard protocol [25]. Structures of all synthesized compounds (intermediates and finals) were confirmed by analytical techniques including IR, NMR (^{1}H, ^{13}C and 2D) and HRMS.



Scheme 1. Synthetic scheme for preparation of pyrazolone-triazole hybrid. reagents and reaction conditions: (a) propargyl bromide, K_2CO_3, DMF, room temperature 10 - 15 h; (b) $CuSO_4$, ascorbic acid, benzyl azide, methanol and water (9/1, v/v), room temperature for 2 h; (c) acetone/water (3:1, v/v) room temperature, 3 h.

As per one-pot synthesis of three intermediates is concerned, during alkynylation of compound **1** by varying the equivalents of base and propargyl bromide we have successfully synthesized three compounds (intermediate) in one step i.e. one mono (2C) and two di alkynylation (2A, 2B) products. To confirm the derived products ^{1}H and 2D (^{1}H-C HMBC and ^{1}H-C HSQC) NMR experiments were conducted (**Figure 3)**. From the ^{1}H and 2D NMR data it was observed

that di alkynylation products were regioisomers. As shown in **Figure 3-A**, cross peak in HMBC and HSQC spectra between 6H with C3, C4 and C5, and 9H with C5 are the characteristic ^{1}H-^{13}C correlation. Decrease in chemical shift of C5 from 172.8 to 153.6 ppm indicated the shielding of C5 by substitution reaction; these findings are in support of structure **2A1**. Whereas, in support of structural isomer **2B1**, methylene (CH_2) and methine (CH) proton from two propargyl substrate are identical and HMBC and HSQC experiments showed cross peak between 6H with 3C, 4C and 5C (**Figure 3-B**). Finally, HRMS results confirm that above predicted structures (by NMR) **2A1** and **2B1** were regioisomers.

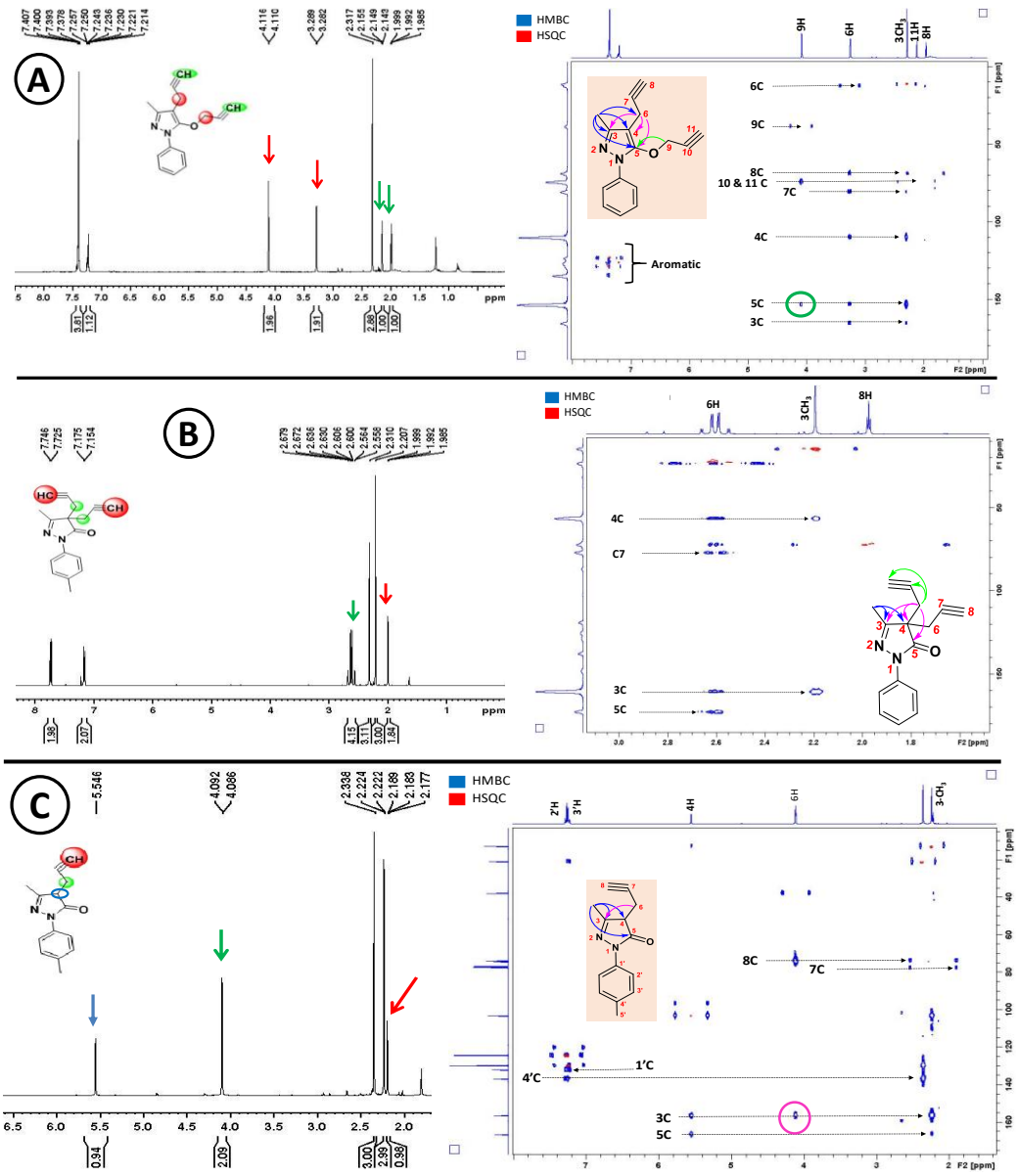


Figure 3. NMR analysis of intermediate compounds: (A): ^{1}H, HMBC and HSQC for 2A1; (B): ^{1}H, HMBC and HSQC for 2B1; (C): 1H, HMBC and HSQC for 2C1.

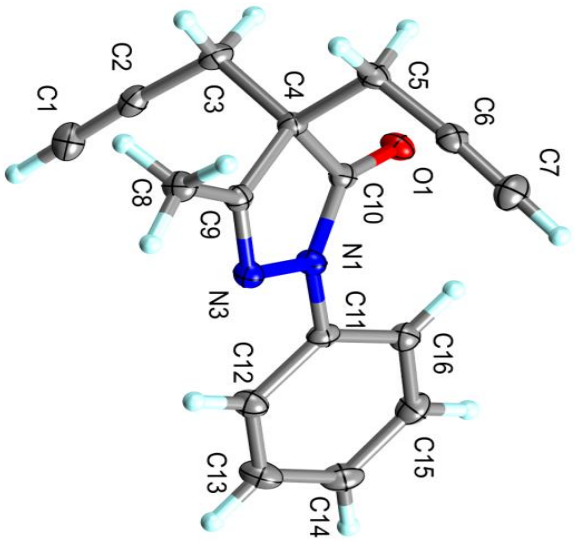


Figure 4. X-ray structure of compound 2B1.

The ^{1}H NMR spectrums of all the final compounds reveal a distinct peak resonating at 2.11 - 2.59 ppm, which was assigned to the methyl group of the pyrazole ring (**Figure 5**). The aromatic protons were observed between 6.88 - 7.77 ppm. The characteristic methylene (CH_2) linker of pyrazolone-triazole proton peak appeared between 3.20 - 4.74 ppm. Whereas, methylene linker between the triazole and phenyl rings displayed between 5.22 - 5.48 ppm. In ^{13}C NMR of all the targeted compounds the distinct methyl (CH_3) peak of the pyrazole ring appeared at 14.12 - 14.53 ppm and the methylene carbon (CH_2) peak of pyrazolone-triazole linker was observed around 20.01 - 30.84 ppm. Whereas methylene carbon (CH_2) peak of triazole and phenyl linker resonated at 53.36 - 54.74 ppm, pyrazolone ketonic group (C5) appeared at 156.01 - 174.75 ppm. Aromatic carbons were observed between 122.01 - 137.54 ppm. Thus above results confirm the formation of the pyrazole-triazole hybrid. The IR spectra of all the final compounds exhibited a characteristic carbonyl band around 1659.45 - 1738.13 cm^{-1}. Synthesized final compounds demonstrated the characteristic molecular ion peak $[M+H]^{+}$ corresponding to the molecular weight of the desired compounds.

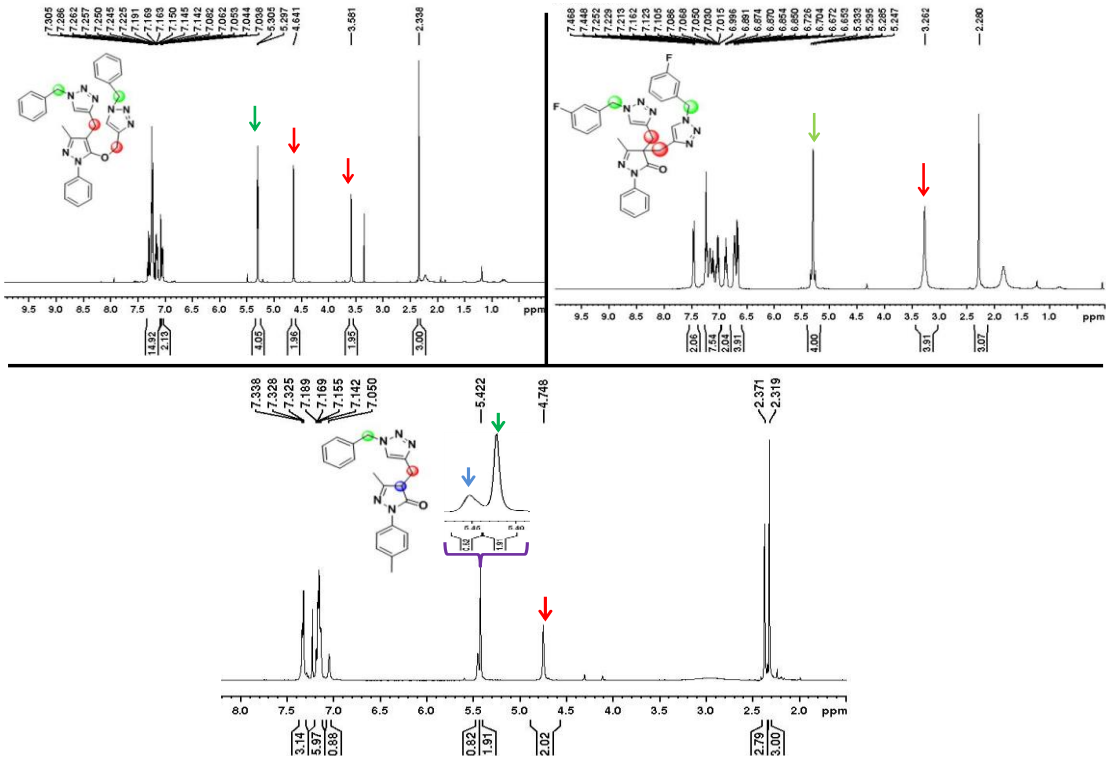


Figure 5. The ^{1}*H NMR of final click products.*

2.1 Biological evaluation

2.1.1 Microorganism used: Standard cultures of two gram-positive bacteria [MRSA- ATCC BAA-1683 *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus*) and SA- ATCC 25923 *Staphylococcus aureus*], four gram-negative bacteria [ST-*Salmonella typhimurium*, PA-ATCC 27853 *Pseudomonas aeruginosa*, EC-ATCC 25922 *Escherichia coli*, KP-ATCC 31488 *Klebsiella pneumoniae*] and two fungal species [*Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC66031] were used for the antibacterial and antifungal activity respectively. Culturing and sub culturing (one day prior to testing) of these microorganisms was carried out in Discipline of Pharmaceutical Sciences, College of Health Sciences, UKZN, Durban, South Africa.

Table 1. Antimicrobial activity of pyrazolone-triazole hybrid molecules.

	MIC µg/ml							
Compounds	**ST**	**PA**	**EC**	**KP**	**MRSA**	**SA**	**CA**	**CN**
k-3-1	na	na	na	1.6	na	>200	>200	>200
k-3-2	12.5	>200	**0.4**	1.6	na	>200	>200	>200
k-3-3	**0.4**	200	200	1.6	>200	>200	>200	>200
k-3-4	na	na	>200	**0.012**	na	200	>200	>200
k-3-5	na	200	**0.8**	1.6	na	200	>200	>200
k-3-6	na	na	na	**0.8**	>200	>200	>200	>200
k-3-7	3.12	200	50	50	>200	na	>200	>200
k-3-8	**0.2**	200	12.5	50	na	na	>200	>200
k-3-9	100	>200	>200	**0.05**	na	na	>200	>200
k-3-10	>200	>200	na	1.6	na	25	>200	>200
k-3-11	**0.8**	**0.2**	na	**0.025**	na	na	>200	>200
k-3-12	1.6	na	100	25	na	na	>200	>200
k-3-13	na	na	**0.8**	**0.8**	na	na	>200	>200
k-3-14	3.12	200	100	1.6	na	na	>200	>200
k-3-15	100	200	**0.4**	12.5	na	na	>200	>200
k-3-16	na	50	**0.8**	**0.4**	na	na	>200	>200
k-3-17	>200	na	6.25	**0.4**	na	na	>200	>200
k-3-18	na	na	**0.4**	**0.8**	na	na	>200	>200
k-3-19	na	na	na	>200	na	na	>200	>200
k-3-22	25	12.5	6.25	50	12.5	na	>200	>200
k-3-23	6.25	**0.4**	6.25	**0.4**	200	200	>200	>200
k-3-24	1.6	50	100	100	na	na	>200	>200
Ciprofloxacin HCl	0.4	0.8	0.1	1.6	25	25	-	-
Amphotericine B	-	-	-	-	-	-	0.39	1.5

ST-*Salmonella typhimurium*, **PA**-*Pseudomonas aeruginosa*, **EC**-*Escherichia coli*, **KP**-*Klebsiella pneumoniae*, **MRSA**-*Staphylococcus aureus* Rosenbach (Methicillin-resistant *S. aureus*), **SA**-*Staphylococcus aureus*, **CA**-*Candida albicans*, **CN**-*C. Neoformans*

2.1.2 In vitro antibacterial analysis: Preliminary antibacterial screening of the synthesized compounds were carried out against panel of bacteria. The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37 ºC in a shaking incubator (100 rpm). The bacterial concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton Agar (MHA) plates (Biolab, South Africa) were lawn inoculated with the prepared bacterial suspensions using a sterile throat swab and 10 µl of the solution of synthesized compound (1 mg/ml DMSO) were spotted onto the MHA plates. The plates were incubated at 37 ºC for 18 h and after incubations the plates were read to determine antibacterial activity which was denoted by clear zones in the area where the solution were spotted. Based on the preliminary screening results the minimum inhibitory concentration (MIC) were determined. The solution of synthesized compounds was serially diluted 2-fold with DMSO ranging from 0.006 - 100 µg/ml) and 10 µl of each concentration was spotted on the lawn inoculated MHA plates and incubated at 37 ºC for 24 h. Ciprofloxacin hydrochloride was used as standard drug.

2.1.3 In vitro evaluation of antifungal activity: The synthesized hybrid pyrazolone-triazole molecule were evaluated for antifungal activities against the two fungal species using amphotericin B as a reference standard drug following earlier reported MIC assay method [26–28].

2.2 Discussion

The results of the *in vitro* antibacterial and antifungal screening (MIC Values) of the tested compounds are summarized in **Table 1**. A systematic analysis of the data as depicted in **Table 1** revealed that all compounds had moderate inhibition for *Candida albicans* and *C. neoformans.* For *Salmonella typhimurium* Compound **k-3-8, k-3-3** and **k-3-11 are highly potent with MIC 0.2 µg/ml** (two folds higher than standard drug), 0.4 µg/ml (equal to standard drug), and 0.8 µg/ml respectively. For *Pseudomonas aeruginosa* compound **k-3-11** showed **very high potency with MIC 0.2 µg/ml** (4 folds higher than standard drug) and compound **k-3-23** displayed potency 0.4 µg/ml (two folds higher than standard drug) while the rest of the compounds exhibited low to moderate inhibition. For *Escherichia coli* compound **k-3-2, k-3-15** and **k-3-18** were active with potency 0.4 µg/ml, whereas compound **k-3-5, k-3-13** and **k-3-16** had MIC values 0.8 µg/ml. For *Klebsiella pneumonia* all compounds exhibited very high activity. Especially, compound **k-3-4** showed extremely high potency with MIC 0.012 µg/ml (130 folds higher than standard drug), and **k-3-11** and **k-3-9** showed very high potency with MIC 0.025 µg/ml and 0.05 µg/ml respectively (65-30 folds higher than standard drug). For methicillin resistant *Staphylococcus aureus*, MRSA compound **k-3-22** showed MIC 12.5 µg/ml (two folds higher than standard drug). For

Staphylococcus aureus compound **k-3-10** exhibited MIC 25 µg/ml (equal to standard drug). Compound**, k-3-3, k-3-4, k-3-8, k-3-9, k-3-10, k-3-11, k-3-22,** and **k-3-23** showed high efficacy indicating that CF_3, Br, Cl, and F **(**electro negative group) attached to the phenyl ring of triazole is responsible for high potency of the compounds**.**

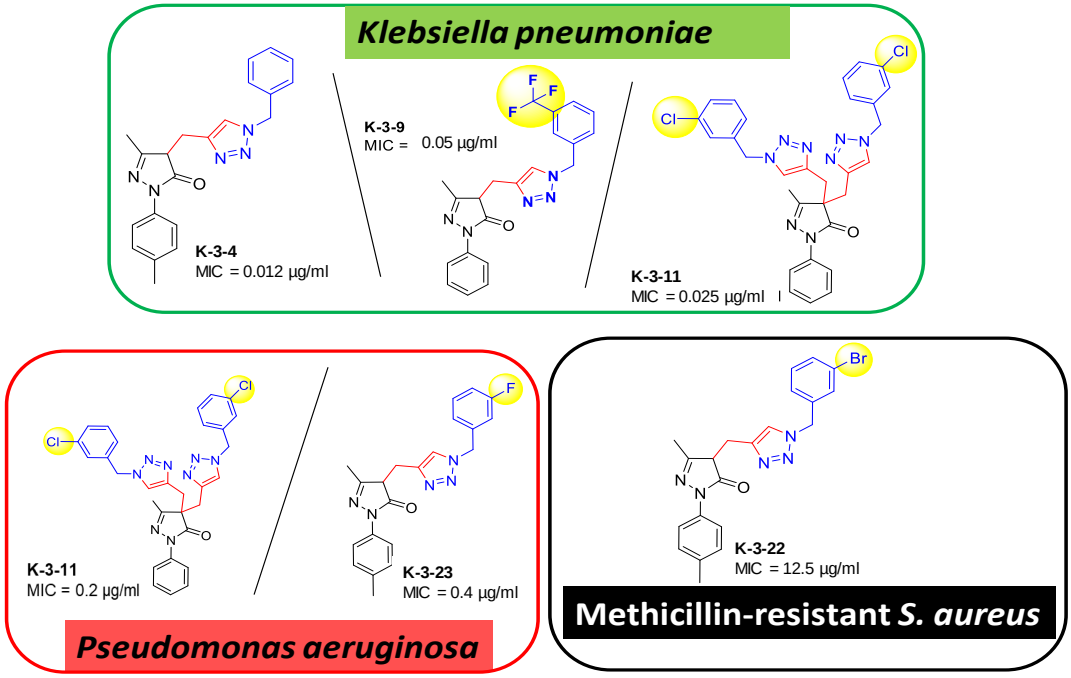


Figure 6. Pyrazolone-triazole hybrids with potent antibacterial activity.

3. Experimental

3.1. Materials and Method

All the chemicals and reagents were commercially procured directly from Sigma Aldrich and Merck Millipore South Africa. All the solvents, except those of laboratory reagent grade, were dried and purified when necessary according to previously reported methods. The progress of the reactions and the purity of the compounds were monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel plates procured from Merck (Pty) Ltd. The melting points of the synthesized compounds were determined using a Thermo Fisher Scientific (IA9000, UK) digital melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker Alpha FT-IR spectrometer (Billerica, MA, USA) using the ATR technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany) spectrometers using $CDCl_3$ and DMSO-d_6. The chemical shifts are reported in δ ppm units with respect to trimethylsilane (TMS) as an internal standard. HRMS spectra were recorded on an Autospec mass spectrometer with electron impact at 70 eV.

3.2 Synthesis

3.2.1. *Preparation of 5-methyl-2-phenyl/p-tolyl-4,4-di(prop-2-yn-1-yl)-2,4-dihydro-3H-pyrazol-3-one (2A1, 2B1, 2C1, 2B2 and 2C2):*

Mixture of 5-Methyl-2-(*p*-tolyl/phenyl)-2,4-dihydro-3*H*-pyrazol-3-one (0.58 mmol, 1equiv), dry potassium carbonate (1.75 mmol, 3 equiv) and dry DMF (10-15 ml) in dry and inert atmosphere was stirred at room temperature for 30 min. Followed by dropwise addition of propargyl bromide (0.74 mmol, 1.3 equiv) with continuous stirring at room temperature for 10-15 h. Reaction was monitored by TLC and crude product purified by column chromatography to separate compounds (**2A**, **2B** and **2C**) at different RF (ratio of the distance moved by the solute) by column chromatography on silica gel using hexane/ethyl acetate as eluent.

3.2.*2. Preparation of substituted benzyl azides (A-F):*

Substituted benzyl bromide (1 equiv, 5.85 mmol) was added dropwise to a solution of sodium azide (2 equiv, 11.7 mmol) in 24 ml acetone/water ((3:1, v/v) and the resulting mixture stirred at room temperature for 3 h. The reaction was diluted with water (20 ml) and extracted with ethyl acetate (3 x 30 ml). The combined organic layers were washed with brine (2 x 20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the desired intermediate compound **(A - F)** as crude colorless oil.

3.2.3. ***Preparation of 4-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one*** **(k-3-1 to k-3-24**):

5-methyl-2-phenyl/p-tolyl-4,4-di(prop-2-yn-1-yl)-2,4-dihydro-3*H*-pyrazol-3-one **(*2A1, 2B1, 2C1, 2B2* and *2C2*)** (0.339 mmol, 1equiv), was stirred with ascorbic acid (40 mol%, 0.150 mmol, 0.4 equiv) and $CuSO_4 \cdot 5H_2O$ (20 mol%, 0.0677 mmol, 0.2 equiv) in a solution of methanol and water (9/1, v/v) for 30 min at room temperature. Benzyl azide was added dropwise and the resulting mixture stirred at room temperature for 2 h to give a colorless precipitate. The precipitate was filtered and washed with ice cold water; resulting pure solid **(k-3-1** to **k-3-24**) is yellowish white in color.

3.3 Experimental data

Note: We have taken HRMS of selected compounds to support NMR results, remaining all compounds are characterized by NMR and IR only.

3.3.1. 5-Methyl-2-phenyl-4,4-di(prop-2-yn-1-yl)-2,4-Dihydro-3H-pyrazol-3-one **(2B1):** Yellowish white solid; Yield**:** 30%; mp: 90 - 92 °C; IR (ATR, ν_{max},cm^{-1}): 1705.71 (C=O), 2930.58 (C-H, CH_3), 3031.72 (C-H, Ar); ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.84 (d, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 1.0, 7.4 Hz, 1H), 2.60 (m, 4H, 6-H), 2.19 (s, 3H, $3CH_3$), 1.97 (t, *J* = 2.7 Hz, 2H, 8-H) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$, 25°C): δ = 172.8 (C5), 161.0 (C3), 77.3 (C7), 72.2 (C8), 56.6 (C4), 23.6 (C6), 14.6 (CH_3) ppm; HRMS (EI) *m/z* calculated for $C_{16}H_{14}N_2O$ $[M+H]^+$: 250.30; found 250.01.

3.3.2. 3-Methyl-1-phenyl-4-(prop-2-yn-1-yl)-5-(prop-2-yn-1-yloxy)-1H-pyrazole **(2A1):** Yellowish white solid; Yield**:** 20%; mp: 93 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.39 – 7.34 (m, 4H), 7.20 (m, 1H), 4.08 (d, *J* = 2.3 Hz, 2H, 9-H), 3.25 (d, *J* = 2.6 Hz, 2H, 6-H), 2.28 (s, 3H, $3CH_3$), 2.11 (t, *J* = 2.3 Hz, 1H, 11-H) 1.96 (t, *J* = 2.7 Hz, 1H, 8-H) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$, 25 °C): δ = 165.5 (C3), 153.6 (C5), 110.2 (C4), 80.9 (C7), 74.2 (C11), 74.1 (C10), 69.0 (C8), 38.5 (C9), 12.3 (C6), 11.16 (CH_3), ppm.; HRMS (EI) *m/z* calculated for $C_{16}H_{14}N_2O$ $[M+H]^+$: 250.30; found 250.01.

3.3.3. 5-Methyl-2-phenyl-4-(prop-2-yn-1-yl)-2,4-Dihydro-3H-pyrazol-3-one **(2C1):** Yellowish white solid; Yield**:** 25%; mp: 141 - 143 °C; IR (ATR, ν_{max},cm^{-1}): 1705.71(C=O), 2930.58 (C-H, CH_3), 3031.72 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 °C): δ = 2.18 (t, *J* = 2.22 Hz, 1H), 2.22 (s, 3H), 2.30 (s, 1H), 3.23 - 3.27 (d, *J* = 2.37 Hz, 1H) 5.58 (s, 1H), 7.35 - 7.43 (m, 5H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.22, 12.90, 38.10, 74.23, 103.28, 123.54, 124.23, 126.53, 126.98, 129.32, 166.48 ppm.

3.3.4. 5-Methyl-4,4-di(prop-2-yn-1-yl)-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one **(2B2):** Yellowish white solid; Yield**:** 30%; mp: 87 °C; IR (ATR, ν_{max},cm^{-1}): 1705.71 (C=O), 2930.58 (C-

H, CH_3), 3031.72 (C-H, Ar), ^{1}H NMR (400 MHz, $CDCl_3$): δ =1.99 (t, *J* = 2.70 Hz, 2H), 2.21 (s, 3H), 2.31 (s, 3H), 2.56 - 2.68 (m, 4H), 7.15 (d, *J* = 8.08 Hz, 2H),7.73 (d, *J* = 8.45 Hz, 2H), ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.55, 20.96, 23.28, 56.21, 72.04, 119.12, 129.35, 134.99, 135.30, 160.45, 172.66 ppm.

3.3.5. **5-*Methyl-4-(prop-2-yn-1-yl)-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one* (2C2):** White solid; Yield**:** 25%; mp:144 - 146 °C ;IR (ATR, ν_{max},cm^{-1}): 1705.71 (C=O), 2930.58 (C-H, CH_3), 3031.72 (C-H, Ar); ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.27 (d, *J* = 8.5 Hz, 2H 2'-H), 7.24 (d, *J* = 8.5 Hz, 2H 3'-H), 5.55 (d, *J* = 1.0 Hz, 1H 4-H), 4.11 (d, *J* = 2.5 Hz, 2H 6-H), 2.35 (s, 3H 5'H), 2.23 (d, *J* = 1.0 Hz, 3H 3-CH_3), 2.22(d, *J* = 2.3Hz, 1H 8-H), ppm. ^{13}C-NMR (400 MHz, $CDCl_3$, 25°C): δ = 166.68 (C5), 156.64 (C3), 136.90(C4'), 137.2(C4'), 132.19 (C1'), 129.87 (C3'), 124.43 (C2'), 103.26(C4), 74.47 (C7), 74.06 (C8), 37.93 (C6), 21.06 (Aromatic CH_3), 12.84 (3-CH_3) ppm.

3.3.6. ***1-Benzyl-4-((5-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)-1H-1,2,3-triazole* (k-3-1):** Yellowish white semi solid; Yield**:** 40%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 °C): δ = 2.48 (s, 3H) 3.72 (s, 2H), 4.79 (s, 2H), 5.44 (d, *J* = 2.98 Hz, 4H), 7.18 - 7.22(m, 2H), 7.27 - 7.47 (m, 15H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.85, 19.10, 43.50, 53.99, 111.39, 121.97, 122.70, 123.22, 126.34, 127.78, 128.00, 128.37, 128.49, 128.73, 128.95, 129.11, 129.37, 134.53, 134.95, 134.96, 139.93, 154.39, 166.16 ppm; HRMS (ESI^+) *m/z* for $C_{30}H_{28}N_8O^+Na^+$: calcd, 539.2285; found 539.2285.

3.3.7. ***4,4-Bis((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one* (k-3-2):** Yellowish white ; solid; Yield**:** 60%; mp: 137 - 139 °C; IR (ATR, ν_{max},cm^{-1}): 1705.71 (C=O), 2930.58 (C-H, CH_3), 3031.72 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 °C): δ = 2.25 (s, 3H) 3.20 (s, 4H), 5.22 - 5.31 (t, *J* = 17.50 Hz, 4H), 6.88 (d, *J* = 7.50 Hz, 4H) 7.03-7.25 (m, 11H) 7.43 (d, *J* = 7.77 Hz, 4H); ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.61, 30.92, 54.05, 60.39, 118.73, 125.04, 127.60, 128.54, 128.83, 128.95, 134.23, 137.36, 162.47, 174.36 ppm; HRMS (ESI^+) *m/z* for $C_{30}H_{28}N_8O^+Na^+$: calcd, 539.2285; found 539.2285.

3.3.8. ***4,4-Bis((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one* (k-3-3):** Light brown ; solid; Yield**:** 48%; mp: 155 - 156 °C; IR (ATR, ν_{max},cm^{-1}): 1700.44 (C=O), 2928.87 (C-H, CH_3), 3033.06 (C-H, Ar; ^{1}H NMR (400 MHz,$CDCl_3$, 25 °C): δ = 1.22 (s, 1H), 2.32 (s, 6H), 3.23 (s, 3H), 5.31 (s, 4H), 6.93 - 7.34 (m, 16H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 20.96, 30.81, 54.47, 118.81, 127.72, 128.57, 128.96, 129.31, 134.19, 134.73, 134.93, 162.37 ppm.

***3.3.9. 4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one* (k-3-4):** Brownish white; solid; Yield**:** 55%; mp:132-134 °C; IR (ATR, ν_{max},cm^{-1}): 1701.25 (C=O), 2920.05 (C-H, CH_3), 2952.81 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$,): δ = 2.31 (s, 3H),

2.37 (s, 3H), 4.74 (s, 2H), 5.42 (s, 2H), 7.05 (s, 1H), 7.14 - 7.18 (m, 6H), 7.32 - 7.33 (m, 3H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$,): δ = 13.29, 21.06, 42.78, 54.12, 101.09, 122.33, 124.24, 127.88, 128.81, 129.14, 132.02, 134.37, 137.10, 140.29, 156.74, 166.63 ppm; HRMS (ESI^+) *m/z* for $C_{21}H_{21}N_5O^+ Na^+$: calcd, 382.1646; found 382.1646.

3.3.10. 4,4-Bis((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **(k-3-5):** White solid; Yield**:** 57%; mp:129 - 131 °C; IR (ATR, ν_{max},cm^{-1}): 1347.39 (C-F), 1701.60 (C=O), 2928.79 (C-H, CH_3), 2956.84 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ºC): δ = 2.28 (s, 3H), 3.26 (s, 4H), 5.28-5.33 (m, 4H), 6.65 - 6.72 (m, 4H), 6.84 - 6.89 (m, 2H), 6.99-7.21 (m, 7H), 7.45 (d, *J* = 7.90 Hz, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 14.57, 30.88, 53.38, 60.35, 114.57, 114.80, 115.52, 115.72, 118.58, 123.05, 123.08, 125.14,128.83, 130.62, 130.70, 136.58, 136.66, 137.24, 161.57, 162.43, 164.04, 174.34 ppm.

3.3.11. 4-((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-Dihydro-3H-pyrazol-3-one **(k-3-6):** Brownish white solid; Yield**:** 55%; mp:133 - 135 °C; IR (ATR, ν_{max},cm^{-1}): 1311.46 (C-F), 1717.52 (C=O), 2922.85 (C-H, CH_3), 2954.50 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ºC): δ = 0.78 - 0.86 (m, 1H), 1.21 (s, 3H), 2.50 (d, *J* = 10.93 Hz, 1H), 4.85 (s, 1H), 5.24 - 5.54 (m, 2H), 6.89 - 7.39 (m, 10H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 14.20, 21.05, 60.40, 123.62, 124.38, 126.35, 126.76, 127.40, 128.14, 128.32, 128.91, 128.97, 129.08, 129.42, 129.58, 130.31, 130.47, 134.80, 136.43, 171.17 ppm.

3.3.12. 4,4-Bis((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one **(k-3-7):** White solid; Yield**:** 65%; mp:150 - 152°C; IR (ATR, ν_{max},cm^{-1}): 1335.27 (C-F), 1697.97 (C=O), 2922.76 (C-H, CH_3), 2955.05 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 2.27 (s, 3H), 2.30 (s, 3H), 3.25 (s, 4H), 5.29 - 5.36 (m, 4H), 6.69 - 7.32 (m, 14H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 14.55, 20.92, 30.86, 53.48, 114.62, 114.84, 115.47,115.68, 118.63, 123.16, 129.31, 130.61, 130.69, 134.83, 136.60, 136.67, 161.56, 162.30, 164.04 ppm.

3.3.13. 5-Methyl-2-phenyl-4,4-bis((1-(3-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-8):** White solid; Yield**:** 50%; mp:147 - 149 °C; IR (ATR, ν_{max},cm^{-1}): 1326.80 (C-F), 1702.04 (C=O), 2918.37 (C-H, CH_3), 3073.19 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ºC): δ = 2.30 (s, 3H), 3.28 (s, 4H), 5.34 - 5.42 (m, 4H), 6.99 – 7.01(d, *J* = 7.95 Hz, 2H), 7.10- 7.15(q, 3H), 7.21 - 7.26 (m, 4H), 7.37 (s, 2H) 7.47 (t, *J* = 8.47 Hz, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 14.53, 30.85, 53.35, 60.34, 118.41, 122.28, 124.29, 124.33, 124.37, 124.40, 124.98, 125.08, 125.38, 125.42, 125.45, 125.49, 128.82, 129.62, 130.62, 131.07, 131.38, 135.42, 137.28, 162.43, 174.32 ppm.

3.3.14. 5-Methyl-2-phenyl-4-((1-(3-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-9):** Brownish white solid; Yield**:** 60%; mp:149 - 151 °C; IR

(ATR, ν_{max},cm^{-1}): 1365.73 (C-F), 1659.45 (C=O), 2996.18 (C-H, CH_3), 3090.62 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.40 (s, 3H), 4.81 (s, 2H), 5.51 (s, 2H), 7.27 - 7.62 (m, 10H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 13.43, 43.31, 53.92, 102.10, 122.30, 123.86, 124.56, 125.01, 125.66, 125.70, 126.82, 129.45, 129.80, 131.18, 131.32, 131.65, 134.80, 135.43 ppm.

3.3.15. 5-Methyl-2-(p-tolyl)-4,4-bis((1-(3-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2,4-dihydro-3H-pyrazol-3-one **(k-3-10):** White solid; Yield**:** 58%; mp: 132 - 133 °C; IR (ATR, ν_{max},cm^{-1}): 1352.24 (C-F), 1693.20 (C=O), 2930.22 (C-H, CH_3), 2964.54 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 °C): δ = 2.22 - 2.25 (d, *J* = 11.71 Hz , 6H), 3.21 (s, 4H), 5.28 - 5.36 (m, 4H), 6.94 - 7.40 (m, 15H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.51, 20.84, 30.83, 53.35, 60.25, 118.47, 122.28, 124.30, 124.30, 124.38, 124.99, 125.37, 125.40, 129.28, 129.62,130.66, 131.08, 131.40, 134.80, 134.82, 135.43, 162.29, 174.08 ppm.

3.3.16. 4,4-Bis((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **(k-3-11):** White solid; Yield**:** 55%; mp:148 - 150 °C; IR (ATR, ν_{max},cm^{-1}): 743.81 (C-Cl), 1698.05 (C=O), 2930.30 (C-H, CH_3), 2989.31 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.46 (s, 3H), 3.44 (s, 4H), 5.45 (s, 4H), 6.91 (d, *J* = 7.50 Hz, 2H), 7.14 (t, *J* = 7.99 Hz, 2H), 7.27 - 7.43 (m, 8H),7.64 (d, *J* = 8.28 Hz, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.59, 30.84, 53.35, 60.28, 118.60, 125.16, 125.57, 127.79, 128.85, 130.32, 134.80, 136.20, 137.26, 162.40, 174.32 ppm.

3.3.17. 4-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-Dihydro-3H-pyrazol-3-one **(k-3-12):** Brownish white semi solid; Yield**:** 62%; IR (ATR, ν_{max},cm^{-1}): 754.14 (C-Cl), 1648.77 (C=O), 2955.06 (C-H, CH_3), 3063.48 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.32(s, 3H) 2.10 (s, 3H), 4.17 (s, 2H), 5.50 (s, 2H), 7.07 - 7.49 (m, 10H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.20, 21.05, 60.40, 123.62, 124.38, 126.35, 126.76, 127.40, 128.14, 128.32, 128.91, 128.97, 129.08, 129.42, 129.58, 130.31, 130.47, 134.80, 136.43, 171.17 ppm.

3.3.18. 4,4-Bis((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one **(k-3-13):** White solid; Yield**:** 55%; mp: 145 – 147 °C; IR (ATR, ν_{max},cm^{-1}): 744.39 (C-Cl), 1696.39 (C=O), 2922.03 (C-H, CH_3), 2989.74 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.30 (s, 6H), 2.64 (d, *J* = 19.83Hz, 1H), 3.25 (s, 3H), 5.27 (s, 4H), 6.74 (d, *J* = 7.89 Hz, 2H), 6.96 - 7.16 (m, 8H) 7.29 (d, *J* = 7.89 Hz, 2H), 7.49 - 7.74 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.56, 20.96, 30.82, 53.57, 72.05, 72.17, 118.64, 118.89, 119.12, 125.65, 127.83, 128.79, 129.34, 130.30, 134.79, 134.81, 136.21, 161.48, 162.29, 174.10.

3.3.19. 4-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-14):** White solid; Yield**:** 60%; mp: 125 - 127°C; IR (ATR, ν_{max},cm^{-1}):

782.60 (C-Cl), 1660.05 (C=O), 3071.96 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.28 (s, 3H) 2.37 (s, 3H), 4.69 (s, 2H), 5.34 (s, 2H), 6.96 - 6.98 (d, *J* = 7.20 Hz, 1H), 7.08 - 7.19 (m, 2H), 7.21 (s, 4H), 7.23 (s, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 13.29, 21.07, 53.37,101.22, 122.44, 124.19, 125.90, 127.87, 129.00, 130.08, 130.46, 132.03, 134.99, 136.37, 137.17, 140.49, 156.84, 166.70 ppm.

3.3.20. 5-Methyl-4,4-bis((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **(k-3-15):** White solid; Yield**:** 55%; mp: 131 - 133°C; IR (ATR, ν_{max},cm^{-1}): 1701.25 (C=O), 2921.91 (C-H, CH_3), 3078.67 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.23 (s, 9H), 2.62 - 2.70 (d, *J* = 13.2 Hz, 1H) 3.29 (s, 3H), 5.31 (s, 4H), 6.77 - 7.68 (m, 15H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.58, 21.24, 30.78, 54.74, 72.12, 118.92, 118.99, 119.06, 124.87, 125.14, 128.61, 128.78, 128.87, 129.45, 134.14, 137.30, 138.77, 162.53 ppm.

3.3.21. 5-Methyl-4-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2-phenyl-2,4-Dihydro-3H-pyrazol-3-one **(k-3-16):** White solid; Yield**:** 53%; mp: 123 - 125 °C; IR (ATR, ν_{max},cm^{-1}): 1701.55 (C=O), 2919.25 (C-H, CH_3), 3063.50 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.34 (s, 3H), 2.42 (s, 3H), 4.78 (s, 2H), 5.42 (s, 2H), 6.99 - 7.17(m, 2H), 7.24 - 7.30 (m, 1H), 7.35 - 7.43 (m, 7H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 13.41, 21.33, 42.99, 54.31, 101.49, 123.49, 124.94, 125.32, 126.53,126.89, 128.57, 128.79, 128.99, 129.35, 129.44, 129.52, 129.59, 134.22, 134.73, 139.04, 157.37 ppm.

3.3.22. 5-Methyl-4,4-bis((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(p-tolyl)-2,4-dihydro-3H-pyrazol-3-one **(k-3-17):** White solid; Yield**:** 55%; mp: 147 – 149 °C; IR (ATR, ν_{max},cm^{-1}): 1700.03 (C=O), 2919.82 (C-H, CH_3), 3024.31 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.18 (s, 6H), 2.30 (s, 3H), 2.64 - 2 65 (m, 1H), 3.22 (s, 3H), 5.26 (s, 4H), 6.73 (s, 2H), 6.82 (s, 2H), 7.01 (t, *J* = 7.2 Hz, 7H) 7.26 - 7.28 (d, *J* = 7.2 Hz, 2H), 7.48 - 7.74 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 14.55, 21.21, 30.86, 54.45, 119.00, 124.84, 128.56, 128.87, 129.27, 129.31,129.36, 134.19, 134.77, 134.86, 138.77, 162.34, 174.20 ppm.

3.3.23 5-Methyl-4-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-18):** White solid; Yield**:** 53%; mp:127 - 129 °C; IR (ATR, ν_{max},cm^{-1}): 1702.16(C=O), 2919.51 (C-H, CH_3), 3059.69 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.29 (s, 3H), 2.31 (s, 3H), 2.34 (s, 3H), 4.70 (s, 2H), 5.36 (s, 3H), 6.93 (d, *J* = 6.61 Hz, 2H), 7.03 (s, 1H), 7.10 - 7.20 (m, 6H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ = 13.30, 21.32, 42.80, 54.16, 101.16, 122.40, 124.22, 124.91, 125.27, 128.55, 128.72, 128.94, 129.02, 129.56, 130.03, 132.07, 134.27, 137.06, 139.02, 156.78, 166.68 ppm.

3.3.24 4,4-Bis((1-(3-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **(k-3-19):** White solid; Yield**:** 62%; mp: 150 - 152 °C; IR (ATR,

ν_{max},cm^{-1}): 689.45 (C-Br), 1704.20 (C=O), 2921.09 (C-H, CH_3), 3082.25 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.33 (s, 3H), 3.24 (s, 4H), 5.17 - 5.34 (m, 4H), 6.74 (d, *J* = 8.02 Hz, 4H), 7.13 - 7.48 (m, 11H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 14.59, 30.83, 53.36, 60.63, 118.31, 122.69, 125.33, 128.92, 129.05, 131.99, 132.12, 133.21, 137.15, 162.48, 174.26 ppm.

3.3.25 4-((1-(3-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-22):** White solid; Yield**:** 53%; mp: 131 - 133°C; IR (ATR, ν_{max},cm^{-1}): 675.75 (C-Br), 1738.13 (C=O), 2999.86 (C-H, CH_3), 3107.70 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 2. 31 (d, *J* = 13.53 Hz, 6H), 4.70 (s, 2H), 5.36 (d, *J* = 26.02 Hz, 3H), 6.98 - 7.05 (m, 3H) 7.149 (s, 4H), 7.41 (d, *J* = 7.88 Hz, 2H), ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 13.31, 21.09, 42.75, 53.48, 100.92, 122.40, 123.02, 124.34, 129.56, 130.13, 131.79, 132.32, `133.37, 137.42, 156.53 ppm.

3.3.26. 4-((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-2-(p-tolyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-23):** White solid; Yield**:** 53%; mp: 130 - 132°C; IR (ATR, ν_{max},cm^{-1}): 1254.12 (C-F), 1737.23 (C=O), 2924.74 (C-H, CH_3), 3092.69 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ 2.37 (d, *J* = 14.29 Hz, 6H), 4.70 (s, 2H), 5.42 (d, *J* = 15.23 Hz, 2H), 6.85 (d, *J* = 9.30 Hz, 1H), 6.95 (d, *J* = 7.87Hz, 1H), 7.01 - 7.03 (m, 1H) 7.04 - 7.27 (m, 5H), 7.30 - 7.36 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 13.29, 21.06, 42.81, 53.45, 101.19,114.70, 114.92, 115.70, 122.49, 123.35, 123.38, 124.21, 124.65, 129.94, 130.08, 130.78, 130.86, 132.02, 136,76, 136.83, 137.18, 156.80, 161.73, 164.19, 166.69 ppm.

3.3.27. 5-Methyl-2-(p-tolyl)-4-((1-(3-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2,4-Dihydro-3H-pyrazol-3-one **(k-3-24):** White solid; Yield**:** 53%; mp: 127 – 129 °C; IR (ATR, ν_{max},cm^{-1}): 1738.07 (C=O), 2952.34 (C-H, CH_3), 3029.47 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 2.36 - 2.39 (d, *J* = 12.41 Hz, 6H), 4.78 (s, 2H), 5.52 (s, 2H), 7.23 (t, *J* = 17.68 Hz, 5H), 7.36 (d, *J* = 7.45 Hz, 1H), 7.44 (s, 1H) 7.51 (t, *J* = 8.38 Hz, 1H), 7.62 (d, *J* = 7.45 Hz, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$, 25 ºC): δ = 13.28, 21.04, 42.83, 53.48, 101.34, 122.29, 122.45, 124.14, 124.47, 124.51, 124.69, 125.00, 125.67, 125.71, 125.74, 129.81, 129.95, 130.09, 131.09, 131.36, 131.69, 132.03, 135.48, 137.20, 140.62, 156.92, 166.78 ppm.

4. Conclusion

Antimicrobial resistance has emerged as a major global health concern and requires concreted attention including discovery of new efficacious antimicrobial drug.

In this study a novel series of 22 hybrid molecules based on bioactive pyrazolone and triazole scaffold were designed and synthesized. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectra. Screening results revealed moderate to high potency of synthesized abducts.

In the *in vitro* antimicrobial evaluation some of the target compounds exhibited good bioactivities against the tested bacterial strains, comparable or even superior to reference drugs. Particularly interacting compounds**, k-3-4, k-3-9,** and **k-3-11,** showed very high antibacterial activity. The encouraging results observed from this study motivated us to further develop the structural features of compound in order to obtain potential drug candidates. Currently, work is in progress to develop a pharmocophoric model based on these compounds to develop new potent antimicrobial agents

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6. Conflict of interest

Authors hereby declare that there are no financial/commercial conflicts of interest.

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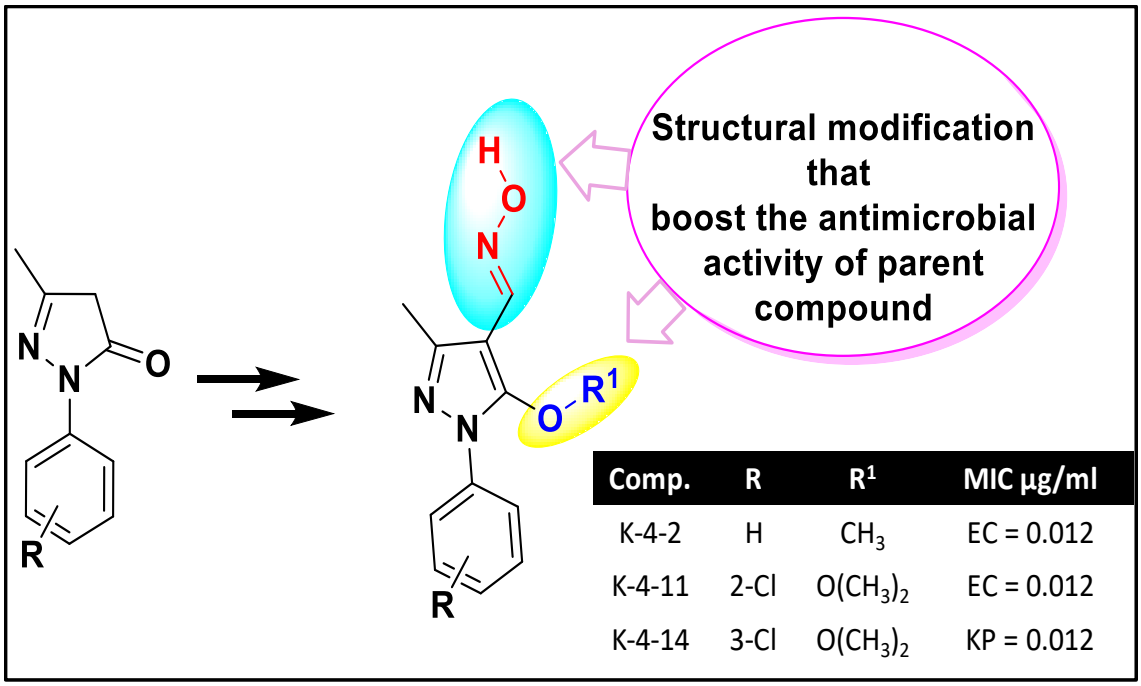
CHAPTER 5. MANUSCRIPT 4

After the manuscript-3, manuscript-4 based on design, synthesis and antimicrobial activity of pyrazolone and oxime derivatives has been prepared titled **"Pyrazoline-oxime prodrugs as potential antimicrobial agents: design, synthesis and antimicrobial evaluation"**.The paper summarizes the design synthesis and antimicrobial activities of pyrazoline-oxime derivatives. This paper was prepared following the guidelines of the journal "European Journal of Medicinal Chemistry**".**

PYRAZOLINE-OXIME PRODRUGS AS POTENTIAL ANTIMICROBIAL AGENTS: DESIGN, SYNTHESIS AND ANTIMICROBIAL EVALUATION



Comp.	R	R^1	MIC µg/ml
K-4-2	H	CH_3	EC = 0.012
K-4-11	2-Cl	$O(CH_3)_2$	EC = 0.012
K-4-14	3-Cl	$O(CH_3)_2$	KP = 0.012

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Abstract

A series of novel pyrazoline derivatives was synthesized by incorporating oxime as a bioactive functional group. These novel compounds were synthesized by employing both conventional and microwave techniques and the structures of all the new compounds confirmed by ^{1}H NMR, ^{13}C NMR, HRMS and FT-IR spectroscopic techniques. All the compounds were screened for their *in vitro* antimicrobial activity against a panel of gram-positive and gram-negative bacteria as well as fungal strains. Antibacterial activity evaluation revealed that almost all the compounds exhibited significant activity, while k-**4-2, k-4-11** and **k-4-14** were highly potent pyrazolone-oxime derivatives with MIC 0.012 µg/ml against *Escherichia coli* and *Klebsiella pneumonia,* and thus proving itself as promising novel drug.

Keywords: Prodrug, Pyrazole, Oximes, Antimicrobial.

1 Introduction

Antibiotics have always been considered to be one of the wonder discoveries of the 20th century. In the last two decades, the morbidity and mortality due to microbial (bacteria, viruses, fungi) infections is on the rise especially in the developing countries and is comparable to deaths caused by tuberculosis and malaria[1]. One of the main reasons for the rise in deaths due to microbial infections can be attributed to constant rise of the microbial resistance to the antibiotics. Further, the microbial resistance may be due to over use / misuse of antibiotics both in humans and animals, long treatment regimens, environmental contamination by waste products arising from antibiotic manufacture and disposal, spread of hospital and other health care acquired resistant bacteria, sub-optimal rapid diagnostics and preventative medicines/vaccination, incorrect dosing of antibiotics in humans, global spread of resistant strains by humans travel, mass drug administration and lastly too few new drugs are being introduced or developed to replace the ineffective drugs[2–5].Furthermore, the primary organisms responsible for invasive microbial infections (*e.g. A. niger, S. aureus, B. subtilis, E. Coli, P. aeruginosa, M. tuberculosis,C. cryptococcus, Aspergillus* species etc.) have developed extreme resistance. In the specific case different kind of microbes contribute to emergence and dissemination of resistance against certain class of antimicrobial agents, complicating patient's health condition and treatment strategy. In addition, a matter of grave concern is the limited availability of effective antimicrobial drugs, narrow therapeutic spectrum, drug resistance, high toxicity and low bioavailability[6].Thus there is an urgent need to discover and develop novel antimicrobial agents.

Prodrug strategy is one of the extensively used strategies in drug discovery and drug development, and until 2009 almost 15% of the most selling drugs were prodrugs, which is constantly rising[7]. This strategy improves the physicochemical, biopharmaceutical or pharmacokinetic properties forthe potent parent drug, thus reducing toxicity, dosage and contributing to the decrease in resistance. We herein report a novel series of pyrazolone-oxime derivatives as prodrugs with synthesis and their biological evaluation.

Currently more than 95% of the marketed drugs consist of heterocyclic scaffold as a core moiety. Heterocycles offer a high degree of structural diversity and present in a variety of drugs, vitamins, natural products, biomolecules and biologically active compounds including antibacterial, antifungal, antiviral, anti-inflammatory, and antitumor drugs. Pyrazolone is one such five membered heterocyclic lactam ring with two nitrogen atoms adjacent to each other and one ketonic group on third position. Pyrazolone and its derivatives have been reported with a wide range of pharmacological potential i.e. pesticides, insecticides[8], fungicides[9] and herbicides[10]. As medicines they are broadly reported for anti-bacterial[11], antitubercular[12],

anti-cancer, free radical scavenger agents[13], analgesic, anti-inflammatory[14] and selective enzyme inhibitory activities[15].

The discovery of antihypertensive and anti-alopecia agent minoxidil by Upjohn in the early 1960s, introduced heterocyclic N-oxides in the mainstream drug development, with its applications in a growing number of anti-bacterial, anti-viral (anti-HIV), anti-cancer, anti-protozoal and anti-fungal agents[16]. In addition to pharmaceutical applications, a number of heterocyclic N-oxides are present in agrochemicals and cosmetics as well as N-oxide or aldoxime or oximes act as a versatile ligand[17].Furthermore, oximes are also used as antidotes for nerve agents[18], metabolic activation**,** allergenic activity[19], antiparasitic drugs[20]**,** fungicides, herbicide s[21]etc..

Recently, pyrazole oxime derivatives have attracted considerable attention in chemical and medicinal research because of their diverse bioactivities as well as simple and convenient synthesis. They are widely used as fungicide [22], insecticide [23], acaricide [24], antitumor [25] antiviral [26] and anti-bacterial agents [27].

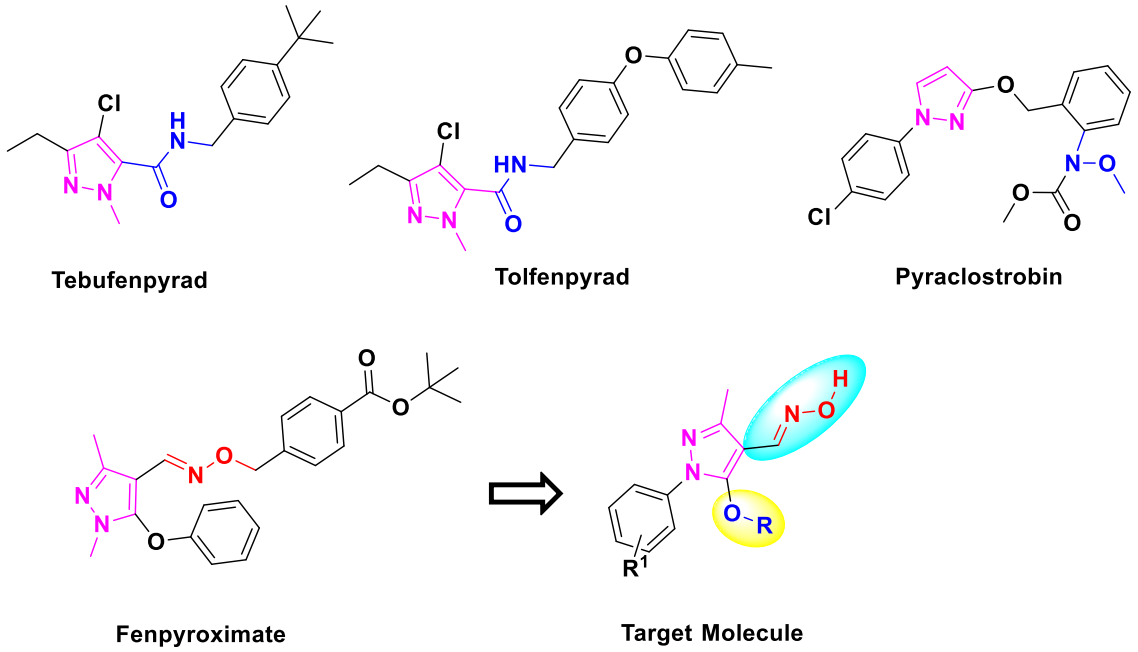


Figure 1. Pyrazolone and pyrazolone-oxime containing marketed drugs.

Tebufenpyrad and Tolfenpyrad are commercially used insecticides. Tebufenpyrad, tolfenpyrad[23], penthiopyrad and pyraclostrobin have been reported for potential antifungal activity[26], and are structurally embedded with a pyrazole-5-carboxamide **(Figure1).** Similarly, fenpyroximate is another derivative containing unique pyrazole oxime scaffold [24] and has been widely used in protecting various crops due to its high efficiency against agricultural mites [28]. Pyrazolo-oximes are being used as antiviral [26], acaricidal, insecticidal and fungicidal

activities[24], and mitochondrial respiration inhibitors[23]. Further, pyrazole oximes have novel mode of action, broad spectrum activity, low toxicity toward mammalian cells, and have favorable profiles to humans. These findings prompted us to design and synthesize novel series of alkoxy pyrazole oxime derivatives as potential antimicrobial agents.

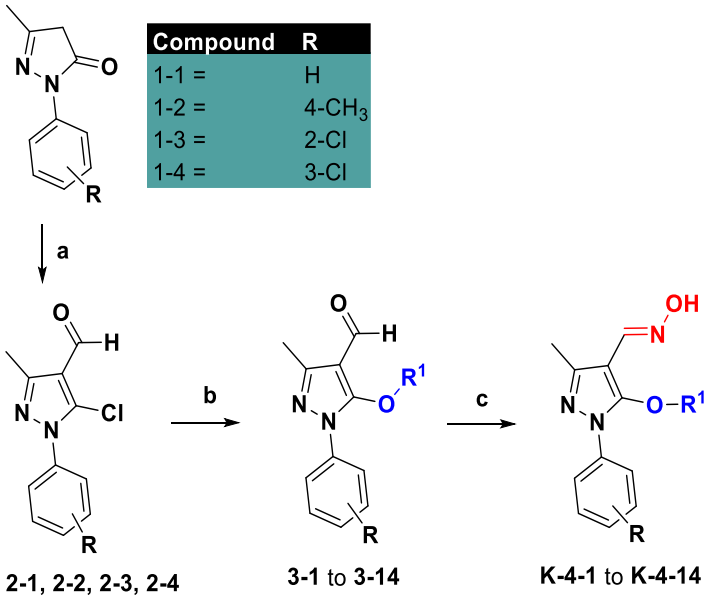
2. Results and Discussion

The oxime motif has been successfully employed in a number of recent drug development projects. This research paper is directed toward the development of potent antimicrobial agents using prodrug approach; the process of development includes the discovery, synthesis, and evaluation on the basis of biological activities of Pyrazolone-oxime compounds and related emerging bioactive compounds.

As shown in **Scheme 1**, titled compounds **K-4-1** to **K-4-14** were synthesized by refluxing of aldehyde intermediate **(3-1** to **3-14)** with **hydroxylamine Hydrochloride (**$NH_2OH{\cdot}HCl$) using strong inorganic base (cesium carbonate) in a mixture of solvent DMSO/H_2O (3:1, v/v). As reported by D. Prajapati (2007) [29] Vilsmeier-Haack formylation reaction was carried out on different substituted starting material, **(1-1, 1-2, 1-3** and **1-4)** to get carbaldehyde intermediate (**2-1, 2-2, 2-3** and **2-4**). Furthermore, catalytic alkylation of carbaldehyde into alkoxy carbaldehyde by refluxing compounds **2-1, 2-2, 2-3** and **2-4** in various alcohol (methanol/ethanol/2-propanol/*n*-butanol) using cesium carbonate as base and copper catalyst ($Cu(OAc)_2$ OR CuI) for 15 h by conventional heating (or microwave10+10 min at 70 °C, power - 150 W, pressure - 150 psi).Structures of the synthesized compound were confirmed by IR, NMR and HRMS spectroscopic techniques.

The ^{1}H NMR spectrums of all the intermediate and final compounds revealed a distinct peak resonating at 2.31-2.59 ppm, which was assigned to the methyl group of the pyrazole ring. The aromatic protons were observed at 7.17-7.77 ppm. Intermediates displayed characteristic aldehyde proton (CHO) peaks at 9.80-9.89 ppm as shown in **Figure 2-A**, while methoxy (CH_3) at 4.12-4.22, ethoxy (CH_3 and CH_2) at 1.31-1.34 and 4.13-4.36 ppm respectively. But *iso*-propoxy (CH_3-CH-CH_3) at 1.22 ppm for CH_3 and 4.75ppm for CH proton. As shown in **Figure 2-B**, for all the title compounds characteristic aldehyde peak of the intermediate compounds at 9.80-9.89 was no observed; whereas new proton peak of oxime appeared between 8.01-8.28 ppm and hydroxyl (N-OH) proton peak of oxime appears between 9.20-9.45 ppm. In ^{13}C NMR of all the final targeted compounds the distinct methyl (CH_3) peak of the pyrazole ring appeared at 13.64 - 14.87 ppm and the oxime (CH) peak was observed around 148-149 ppm.Whereas, ^{13}C NMR of all intermediates display aldehyde carbon peak at 182.00-182.92 ppm, methoxy at 63.14 ppm, isopropoxy CH_3 at 21.07 and CH at 80.22 ppm, similarly for butoxy CH_3 at 13.55 and CH_2 at

18.77, 31.06, 76.58 ppm respectively. From these interpretations of spectral data, we have confirmed the formation of oxime and intermediates. The 2D NMR (HMBC and HSQC) were performed to confirm the final structure and the exact position of the substitution. **Figure 2-C** is the overlap of HSQC with HMBC which clearly displays the cross coupling of C6 with C3, C4 and C5 (Pink arrow); and $3CH_3$ shows cross peak with C4. These finding clearly indicate the position oxime. Similarly, cross of C9 with C5 (Green arrow) confirm the alkoxy substitution happen at position five of pyrazolone ring. Further, the IR spectra of all the final compounds exhibited a characteristic (C-O) of methoxy/ ethoxy /propoxy/ butoxy band around 1066.19-1139.02 cm^{-1} whereas (C=N) of oxime at 2221.83-2220.89 cm^{-1} and hydroxyl (OH) was observed at 3167.40-3381.40 cm^{-1}. In HRMS, final compounds demonstrated (**Figure 2-D**) the characteristic molecular ion peak $[M+H]^+$ corresponding to the molecular weight of the desired compounds.The structures of the desired compound ***K-4-2*** and ***K-4-3*** were confirmed by single X-ray crystal diffraction analysis (**Figure 3**).



Compound	R
1-1 =	H
1-2 =	4-CH_3
1-3 =	2-Cl
1-4 =	3-Cl

Compound	R	R^1
K-4-1	H	CH_2CH_3
K-4-2	H	CH_3
K-4-3	H	$(CH2)_3CH3$
k-4-4	H	CH $(CH3)_2$
K-4-6	4-CH_3	CH_3
K-4-7	4-CH_3	CH_2CH_3
K-4-8	4-CH_3	CH $(CH3)_2$
K-4-9	2-Cl	CH_3
K-4-10	2-Cl	CH_2CH_3
K-4-11	2-Cl	CH $(CH3)_2$
K-4-12	3-Cl	CH_3
K-4-13	3-Cl	CH_2CH_3
K-4-14	3-Cl	CH $(CH3)_2$

Scheme1. Synthetic Scheme of target molecules. Reagents and conditions: (a) $POCl_3$, DMF, 0 ^{0}C, RT and reflux 3 h; (b) ethanol, CS_2CO_3, $Cu(OAc)_2$, microwave 20 min/conventional 15 h; (c) NH_2.OH.HCl, Cs_2CO_3, DMSO/H_2O (3:1, v/v), 100 ^{0}C 5-7 h.

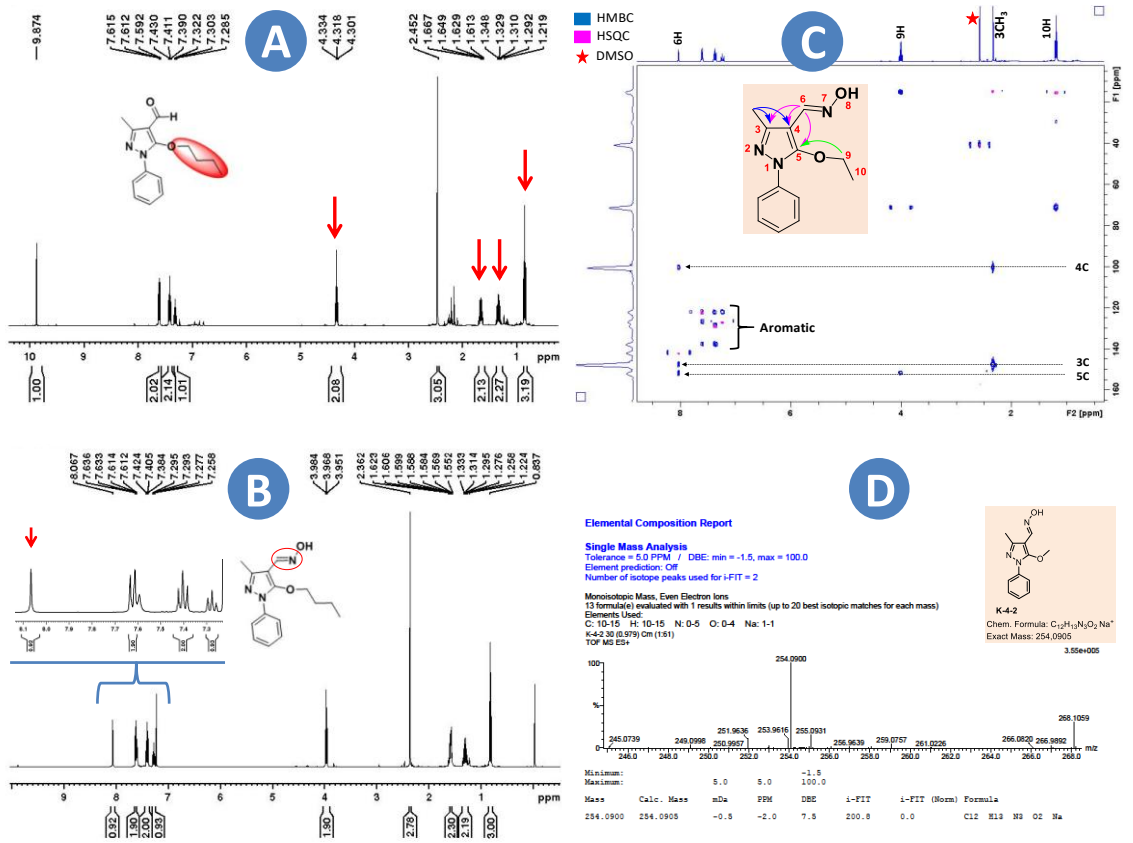


Figure 2: Spectral studies of intermediate and final compound: A:^{1}H NMR of intermediate 3-3;B:^{1}H NMR of final compound K-4-3;C: 2D (HSQC and HMBC) of final compound K-4-1;D: HRMS of final compound K-4-2.

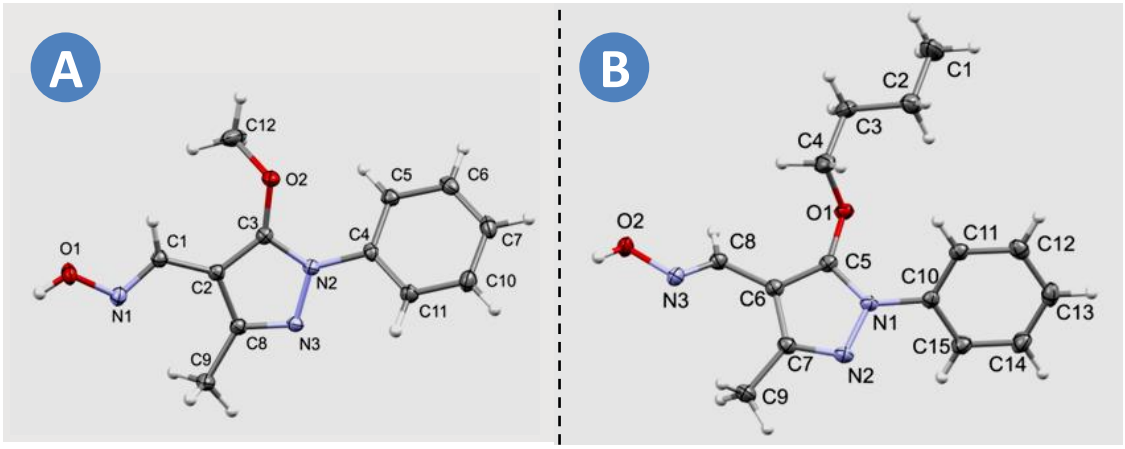


Figure 3 Crystal structure of compound K-4-2 (A) and K-4-3 (B).

2.1 Biological evaluation

2.1.1 Microorganism used: Standard cultures of two gram-positive [MRSA- ATCC BAA-1683 *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus*) and SA- ATCC

25923*Staphylococcus aureus*], four Gram-negative [ST-*Salmonella typhimurium,* PA-ATCC 27853 *Pseudomonas aeruginosa*, EC-ATCC 25922 *Escherichia coli*, KP- ATCC 31488 *Klebsiella pneumoniae*], and two fungal species [*Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC66031] were used for the antibacterial and antifungal activity respectively. Culturing and sub culturing (one day prior to testing) of these microorganisms was carried under supervision of Dr. Chunderika at the discipline of Pharmaceutical Sciences, College of Health Sciences, UKZN, Durban, South Africa.

Table 1. Antimicrobial and antifungal activity of pyrazolone-oxime prodrug molecules.

Compound	**Diameter of zone inhibition (MIC µg/ml)**							
	Gram- negative bacteria				**Gram- positive bacteria**		**Fungal strains**	
	ST	PA	EC	**KP**	**MRSA**	**SA**	**CA**	**CN**
k-4-1	>200	1.6	1.6	50	na	na	>200	>200
K-4-2	0.2	0.8	0.012	0.8	na	na	>200	>200
K-4-3	0.4	>200	200	0.8	na	na	>200	>200
K-4-4	50	0.2	0.4	>200	na	na	>200	>200
K-4-6	200	>200	na	0.8	na	na	>200	>200
K-4-7	0.2	0.05	na	>200	na	100	>200	>200
K-4-8	200	50	>200	0.4	na	na	>200	>200
K-4-9	na	na	na	na	na	na	>200	>200
K-4-10	0.2	na	na	na	na	na	>200	>200
K-4-11	0.2	na	0.012	0.4	na	na	>200	>200
K-4-12	12.5	na	0.2	0.2	na	200	>200	>200
K-4-13	0.2	na	50	0.8	na	>200	50	>200
K-4-14	50	na	0.2	0.012	3.12	na	>200	>200
Ciprofloxacin HCl	0.4	0.8	0.1	1.6	25	25	-	-
Amphotericine	-	-	-	-	-	-	0.39	1.5

Positive control (standard): Ciprofloxacin HCl; **ST**-*Salmonella typhimurium,* **PA**-*Pseudomonas aeruginosa*, **EC**-*Escherichia coli*, **KP**-*Klebsiella pneumoniae*, **MRSA**-*Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus,*), **SA**-*Staphylococcus aureus,* **CA**-*Candida albicans*, **CN**-*C. neoformans;* *na –not active

2.1.2In vitro antibacterial analysis: Preliminary antibacterial screening of the synthesized compounds were carried out against panel of bacteria. The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37 ºC in a shaking incubator (100 rpm). The bacterial concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton Agar (MHA) plates (Biolab, South Africa)

were lawn inoculated with the prepared bacterial suspensions using a sterile throat swab and 10ul of the solution of synthesized compound (1mg/ml DMSO) were spotted onto the MHA plates. The plates were incubated at 37 ºC for 18 h and after incubations the plates were read to determine antibacterial activity which was denoted by clear zones in the area where the solution were spotted. Based on the preliminary screening results the Minimum Inhibitory Concentrations (MICs) were determined. The solution of synthesized compounds was serially diluted 2-fold with DMSO ranging from 0.006-100 µg/ml and 10µl of each concentration was spotted on the lawn inoculated MHA plates and incubated at 37 ºC for 24 h. Ciprofloxacin hydrochloride used as standard drug.

2.1.3In vitro evaluation of antifungal activity: The synthesized pyrazoline-oximes molecular hybrids were evaluated for antifungal activity against two fungal species using amphotericin B as a reference standard drug by following earlier reported MIC assay method [30–32].

2.2 Discussion

The results of *in vitro* antibacterial and antifungal screening (MIC values) of the tested compounds are summarized in **Table 1**. A systematic analysis of the data as depicted in table revealed that all compounds showed low to moderate inhibition for *Staphylococcus aureus, Candida albicans and C. neoformans.* For *Salmonella typhimurium* compounds **K-4-2, K-4-7, K-4-10, K-4-11** and **K-4-13** exhibited high potency at **MIC 0.2 µg/ml** which is two folds higher than standard drug Ciprofloxacin HCl. As well as compounds **K-4-3** showed MIC 0.4µg/ml which is equal to standard drug. For *Pseudomonas aeruginosa* compound **K-4-7** showed very high potency with **MIC 0.05µg/ml** which is 16 folds higher than standard drug and compound **K-4-4** displayed **MIC 0.2 µg/ml** which is 4 folds higher than standard drug. For *Escherichia coli* all compounds have very high potency especially compounds **K-4-2** and **K-4-11** that exhibited extremely high potency with **MIC 0.012µg/ml which is 10 folds** higher than standard drug. Compound **k-4-12** and **k-4-14** exhibited good inhibition with MIC 0.2 µg/ml. For *Klebsiella pneumonia* **k-4-14** exceptionally high potency with **MIC 0.012µg/ml which is 130 folds** higher than standard drug ciprofloxacin HCl, compound **k-4-12** is active with MIC0.2µg/ml 8 folds higher than standard drug, compound **k-4-8** is 4 folds higher than standard drug with MIC 0.4 µg/ml and compound **k-4-2, k-4-3, k-4-6**, and **k-4-13** are 2 folds active as compare to standard drug with MIC 0.8 µg/ml. For *Staphylococcus aureus* Rosenbach (MRSA) compound **k-4-14** showed potency with **MIC 3.12 µg/ml which is 8 folds** higher than standard drug.

Overall, the screening results revealed that compound **k-4-2, k-4-11** and **k-4-14** are highly potent oxime derivative of pyrazolone. These results revealed that isopropoxy group link on Pyrazolone

ring as well as chloro group present on phenyl ring makes it effective inhibitor for gram-negative bacteria. Whereas for gram-negative bacteria, especially for *Klebsiella pneumonia* combine effect of *iso*propoxy and aromatic *meta*-chloro group drastically improved the activity.

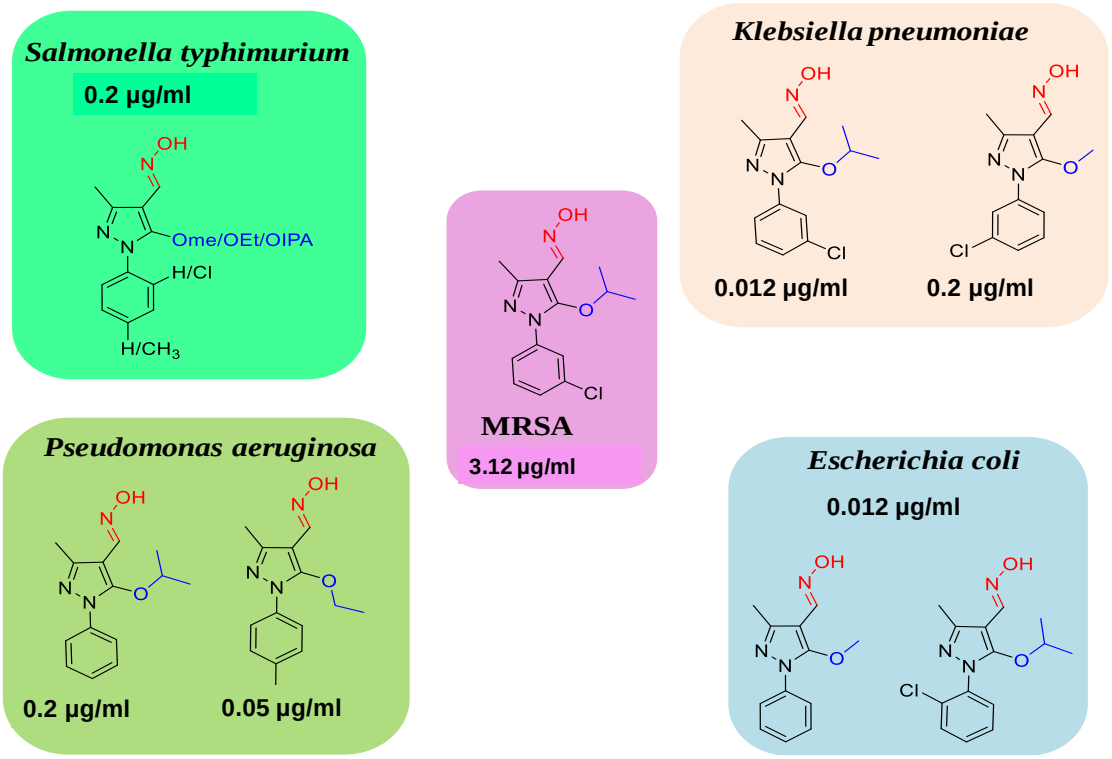


Figure 4. Pyrazoline-oxime hybrids those who shows potent antibacterial activity.

3. Experimental

3.1 Materials and Method

All the chemicals and reagents were purchased from Sigma Aldrich and Merck Millipore South Africa. All the solvents, except those of laboratory-reagent grade, were dried and purified when necessary according to previously published methods. The progress of the reactions and the purity of the compounds were monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel plates procured from Merck (Pty) Ltd. The melting points of the synthesized compounds were determined using a Thermo Fisher Scientific (IA9000, UK) digital melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker Alpha FT-IR spectrometer (Billerica, MA, USA) using the ATR technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany) spectrometers using $CDCl_3$ and DMSO-d_6. The chemical shifts are reported in δ ppm units with respect to trimethylsilane (TMS) as an internal standard. HRMS spectra were recorded on an Autospec mass spectrometer with electron impact at 70 eV.

3.2 Synthesis

3.2.1. ***Preparation of Pyrazoline carbaldehyde (2-1, 2-2, 2-3 and 2-4) by Vilsmeier-Haack reaction***: Vilsmeier-Haack reagent was freshly prepared by careful addition of $POCl_3$ (0.42 mole, 7 equiv.) to dimethylformamide (0.18 mole, 3 equiv.) at 0 °C with constant stirring. To this reaction mixture (maintained at 0 °C) was added different substituted pyrazolone derivatives (**1-1, 1-2, 1-3** and **1-4**) (0.06 mole, 1 equiv.) and stirred initially for 30 min and later slowly brought to RT and stirred for 1 h. The reaction temperature was then raised to 80 °C and stirred for another 3 h. After cooling to RT, the reaction mixture was poured into ice water and the resultant precipitate separated by filtration and washed with cold water. Obtained residue was further recrystallized from ethanol to afford the desired **(2-1, 2-2, 2-3** and **2-4**) compound as off white crystalline solid (Yield = 75 %).

3.2.2. ***Preparation of 5-alkoxy pyrazole-4-carbaldehyde (3-1 to 3-14):*** To the mixture of pyrazole-4-carbaldehyde (1-1, 1-2, 1-3 and 1-4) (0.020 mole, 1equiv.), cesium carbonate (0.03 mole, 1.5 equiv.) and copper (II) acetate (0.005 mole, 0.25 equiv.) appropriate alcohol (methanol/ethanol/IPA/*n*-butanol) was added and refluxed for 15 h /microwave at 70 °C, 10+10 min., (Power-150 W, pressure- 150 psi). Reaction progress was monitored by TLC (5/95, ethyl acetate/hexane), after completion of reaction the formed solid was filtered off and purified by column chromatography on silica gel using hexane and ethyl acetate as eluent to get(**3-1** to **3-14**).

3.2.3.*Preparation pyrazole-4-carbaldehyde-5- oxime (K-4-1 to K-4-14):* Mixture of 5-alkoxy pyrazole-4-carbaldehyde (**3-1** to **3-14**) (0.5 mmol, 1 equiv.), $NH_2OH{\cdot}HCl$ (0.6 mmol, 1.2 equiv.) and cesium carbonate (0.6 mmol, 1.2 equiv.) in 2 ml DMSO/H_2O (3/1, v/v) were stirred at 100 ^{0}C for 5–7 h. Reaction progress was monitored by TLC (ethyl acetate/hexane). After completion, the reaction mixture was cooled to RT and treated with water (1 ml). The resulting mixture was extracted with ethyl acetate (3 x 25 ml),combined organic layer dried over sodium sulphate (Na_2SO_4) and solvent evaporated gave a residue that was purified by column chromatography (Aluminium oxide, pH = 7) using ethyl acetate and hexane as solvent to get desired compound (**k-4-1** to **k-4-14**).

3.3 Experimental data

Note: We have taken HRMS of selected compounds to support NMR results; the remaining all compounds were characterized by NMR and IR only.

***3.3.1: 5-Ethoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*(3-1):**Brown, semi-solid; Yield = 40%;^{1}H-NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 1.31 (t, *J* = 7.09 Hz, 3H), 2.45 (s,3H), 4.36-4.42 (q,2H), 7.31 (t, *J* = 7.43 Hz, 1H), 7.41 (t, *J* = 7.80 Hz, 1H), 7.61 (dd, *J* = 7.03,1.09 Hz, 2H), 9.86 (s, 1H) ppm.

***3.3.2: 5-Methoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde* (3-2):** Light brown; semi-solid; Yield =40%; ^{1}H-NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.45 (s, 3H), 4.13 (s, 3H), 7.31 (t, *J* = 7.44 Hz, 1H), 7.42 (t, *J* =7.93 Hz, 2H), 7.59 (dd, *J* = 7.28,1.09 Hz, 2H), 9.89 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$,25 ^{0}C): δ = 13.89, 63.14, 107.46, 123.11, 127.72, 129.08, 137.25, 151.36, 182.92 ppm.

***3.3.3: 5-Butoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*(3-3):** Brown; semi-solid; Yield = 30%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 0.83(t, *J* =7.47 Hz, 3H),1.29-1.34(q, 2H), 1.61-1.66(q, 2H), 2.45 (s, 3H), 4.31 (t, *J* = 6.51 Hz, 2H), 7.30 (t, *J* = 7.18 Hz, 1H), 7.41 (t, *J* =7.66 Hz, 2H), 7.60 (d, *J* = 7.69, Hz, 2H), 9.87 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 13.55, 13.98, 18.77, 31.06, 76.58, 107.79, 123.14, 127.62, 129.00, 137.40, 151.17, 155.79, 182.98 ppm.

***7.2.4: 5-Isopropoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*(3-4):** Brown; semi-solid; Yield =32%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.22 (d, *J* = 6.44 Hz, 6H),2.45 (s, 3H), 4.74-4.79 (q, 1H), 7.30 (t, *J* = 7.29 Hz, 1H), 7.41 (t, *J* =7.70 Hz, 2H), 7.61 (d, *J* = 7.70 Hz, 2H), 9.82 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 13.89, 21.07, 22.23, 80.22, 108.61, 123.15, 129.57, 135.13, 137.58, 150.87, 154.78, 183.22 ppm.

***3.3.5: 5-Methoxy-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carbaldehyde*(3-6):** Brown; semi-solid; Yield = 28%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.37 (s, 3H), 2.47 (s, 3H), 4.13 (s,3H), 7.23 (d, *J*= 7.89Hz, 2H), 7.47(d, *J*=8.43Hz, 2H), 9.90 (s,1H) ppm

***3.3.6: 5-Iisopropoxy-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carbaldehyde*(3-8):**Dark brown; Semi-solid; Yield = 35%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.26 (d, *J*= 6.21Hz, 6H), 2.39 (s,3H), 2.50 (s, 3H), 4.78-4.84 (m,1H) 7.25 (d, *J*= 7.91Hz, 2H), 7.54(d, *J*= 8.48Hz, 2H), 9.86 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 13.89, 21.07, 22.23, 80.22, 108.61, 123.15, 129.57, 135.13, 137.58, 150.87, 154.78, 183.22 ppm.

***3.3.7: 1-(2-Chlorophenyl)-5-methoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-9):** Brown; Semi-solid; Yield = 30%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.47 (s, 3H), 4.15 (s, 3H), 7.36-7.41 (m,3H), 7.50 (d, *J*= 7.14Hz, 2H), 9.92 (s,1H) ppm

***3.3.8: 1-(2-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-10):** Dark brown; Semi-solid; Yield = 33%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.22 (t, *J* = 7.18 Hz, 3H), 2.44 (s, 3H), 4.39-4.44 (q,2H), 7.33 -7.39 (m, 3H), 7.48 (d, *J* = 7.14 Hz, 1H), 9.87 (s, 1H) ppm.

***3.3.9: 1-(2-Chlorophenyl)-5-isopropoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-11):** Brown; Semi-solid; Yield = 35%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C):δ = 1.17 (d, *J*= 6.09Hz 6H), 2.46 (s,3H), 4.78-4.84(m,1H) 7.34-7.40 (m,3H), 7.50 (d, *J*= 7.57Hz 1H), 9.83 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.06, 22.25, 29.65, 29.69, 80.09, 107.20, 127.46, 129.46, 130.41, 130.73, 132.19, 134.86, 151.65, 156.05, 183.18, ppm.

***3.3.10: 1-(3-Chlorophenyl)-5-methoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-12):** White; Solid; Yield =45%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.45 (s,3H), 4.19 (s, 3H), 7.28-7.37 (m,2H), 7.54 (d, *J*= 7.49Hz, 1H), 7.67 (s, 1H), 9.91 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 13.92, 63.37, 120.81, 123.04, 127.63, 130.07, 134.81, 138.29, 151.80, 182.82, ppm.

***3.3.11: 1-(3-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-13):** Blakish brown; Semi-solid; Yield = 50%; ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.34 (t, *J* = 7.56 Hz, 3H), 2.44 (s, 3H), 4.42-4.47 (q, 2H), 7.26-7.35 (m,2H), 7.56 (d, *J*= 8.02Hz, 1H), 7.69 (s, 1H) 7.76 (s, 1H), 9.80 (s,1H), ppm.

***3.3.12:1-(3-Chlorophenyl)-5-isopropoxy-3-methyl-1H-pyrazole-4-carbaldehyde*(3-14):**Brown; Semi-solid; Yield = 40%;^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.21 (d, *J*= 6.02Hz, 6H), 2.40 (s, 3H), 4.80-4.85 (m,1H), 7.21-7.31(m,2H), 7.53 (d, *J*= 7.85Hz, 1H), 7.67 (s, 1H), 9.79 (s,1H) ppm.

***3.3.13: (E)-5-Ethoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde oxime* (k-4-1):** Crimsons white solid; Yield = 45%; mp = 102-105 ^{0}C; IR (ATR, ν_{max},cm^{-1}): 1105.73 (C-O, OC_2H_5), 2221.83 (C=N), 2923.99 (C-H, CH_3), 3063.41 (C-H, Ar), 3172.88(OH); ^{1}H-NMR (400 MHz, $CDCl_3$, 25^{0}C): δ = 8.02 (s, 1H, 6-H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.36 (t, *J* = 7.7 Hz, 2H), 7.23 (t, *J* = 7.5 Hz, 1H), 3.99 (q, *J* = 7.0 Hz, 2H, 9-H), 2.32 (s, 3H, 3-CH_3), 1.18 (t, *J* = 7.0 Hz, 3H).ppm; ^{13}C-NMR (400 MHz, $CDCl_3$, 25^{0}C): δ = 152.08 (C5), 148.0 (C3),142.36 (C6), 100.6 (C4), 71.5 (C9), 15.00 (C10) , 14.56 (CH_3), ppm.

***3.3.14: (E)-5-Methoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde oxime* (k-4-2):** Yellowish white solid; Yield = 40%; mp = 118–121^{0}C; IR (ATR, ν_{max},cm^{-1}): 1067.40 (C-O, OCH_3), 2921.97 (C-H, CH_3), 2986.37 (C-H, Ar), 3177.41(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.31 (s,3H), 3,79 (s, 3H), 7.21-7.24 (m,1H), 7.30-7.38 (t, *J* = 8.07 Hz, 2H), 7.56-7.58 (dd, *J* = 7.43, 1.27 Hz, 2H), 8.04 (s,1H), 9.35 (s, 1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.44, 29.68, 40.73, 42.63,62.07,100.16, 122.37, 126.96, 129.06, 137.91,147.71, 148.03, 152.87 ppm; HRMS (ESI^{+}) *m/z* for $C_{12}H_{13}N_3O_2$+ Na^{+}: calcd: 254.0900,found: 254.0905.

***3.3.15: (E)-5-Butoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-3):**
Light brown solid; Yield = 25%; mp = 107-110 ^{0}C; IR (ATR, ν_{max}, cm^{-1}): 1070.25 (C-O, OC_4H_9), 2220.89 (C=N), 2926.22 (C-H, CH_3), 2968.69 (C-H, Ar), 3172.22(OH); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ =1.26 (s,3H), 1.51-1.56 (m, 4H), 2.32 (s, 3H), 3.91-3.95 (m,2H) 7.22-7.24

(m,1H), 7.33-7.37 (m,2H), 7.57-7.59 (m,2H), 8.02 (s,1H); ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ =13.64, 14.23, 31.64, 100.36, 122.70, 125.83, 126.07, 127.08, 128.95, 129.62, 148.02, 152.61, 171.24 ppm.

***3.3.16: (E)-5-Isopropoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-4):** White; solid; Yield = 25%; mp = 110–113^{0}C; IR (ATR, ν_{max},cm^{-1}): 1071.23 (C-O, OC_3H_7), 2924.18 (C-H, CH_3), 3059.43 (C-H, Ar), 3167.99(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ. = 0.81 (t, *J* = 7.47 Hz, 3H), 1.22-1.33(m, 2H), 1.55- 1.62 (m, 2H), 2.36 (s,3H), 3.96 (t, *J* = 6.85 Hz, 3H), 7.25-7.29 (m, 1H), 7.38-7.42 (m, 2H), 7.59-7.63 (m, 2H) 8.06 (s,1H), ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.59, 22.10, 40.83, 79.07, 122.87, 124.93, 127.07, 128.46, 129.00, 137.74, 142.21, 148.89 ppm; HRMS (ESI^+) *m/z* for $C_{14}H_{17}N_3O_2$+ Na^+ calcd:282.1218.found: 282.1209.

***3.3.17. (E)-5-Methoxy-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carbaldehyde oxime*(k-4-6):**Whitesolid; Yield = 52%; mp = 106–109^{0}C; IR (ATR, ν_{max},cm^{-1}): 1066.19 (C-O, OCH_3), 2922.86 (C-H, CH_3), 2968.26 (C-H, Ar), 3198.23(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.35 (s,6H), 3.83 (s,3H), 7.21 (d, *J*=9.67Hz, 3H), 7.48(d, *J*=8.29Hz, 1H), 8.28 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 13.89, 14.27, 21.07, 62.04, 63.08, 76.71, 122.52, 122.62, 123.12, 129.70, 129.83, 134.72, 135.30, 137.17, 137.85, 147.89. ppm.

***3.3.18: (E)-5-Ethoxy-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carbaldehyde oxime*(k-4-7):** Light brown solid; Yield = 45%; mp = 108–111^{0}C; IR (ATR, ν_{max},cm^{-1}): 1070.62 (C-O, OC_2H_5), 2920.34 (C-H, CH_3), 2974.80 (C-H, Ar), 3173.09(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.22 (t, *J* = 7.34Hz, 3H), 2.35 (d, *J* = 5.03Hz, 1H), 3.99-4.05 (q,2H), 7.21 (t, *J* = 7.80Hz, 1H), 7.49 (d, *J* = 8.13Hz, 1H), 8.05 (s,1H) ppm; ^{13}CNMR (100 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.52, 15.12,21.06, 71.31, 100.40,122.47, 129.61, 135.58, 136.92, 142.52, 147.68, 152.08 ppm.

***3.3.19: (E)-5-Isopropoxy-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carbaldehyde oxime*(k-4-8):**Light brown solid; Yield = 50%; mp = 109–112^{0}C; IR (ATR, ν_{max},cm^{-1}): 1139.02 (C-O, OC_3H_7), 2923.54 (C-H, CH_3), 3017.95 (C-H, Ar), 3368.88 (OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.27 (d, *J* = 6.04Hz, 6H), 2.35(d, *J* = 5.89Hz 6H), 4.22-4.29 (m,1H), 7.18 (d, *J* = 8.62Hz, 2H), 7.48 (d, *J* = 8.62Hz, 2H), 8.01 (s,1H)ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 23.16, 23.19, 28.84, 32.35, 32.44, 41.59, 47.49, 60.45, 117.41, 120.31, 160.74, 160.76, 165.28, 165.64, 172.61, 178.86 ppm.

***3.3.20: (E)-1-(2-Chlorophenyl)-5-methoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-9):** Light brown solid; Yield =40%; mp = 137–140^{0}C; IR (ATR, ν_{max},cm^{-1}): 762.26 (C-Cl), 1083.41 (C-O, OCH_3), 2923.63 (C-H, CH_3), 2992.33 (C-H, Ar), 3381.40(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.33 (s, 3H), 3.84 (s, 3H), 7.30-7.40 (m,4H), 7.47-7.49 (m,1H), 8.10

(s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.53, 40.84, 42.62, 61.99, 98.17, 127.51, 129.61, 130.26, 130.29, 130.46, 142.17, 148.75, 154.02 ppm.

***3.3.21: (E)-1-(2-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime* (k-4-10):**Brown solid; Yield = 45%; mp = 101–104^{0}C; IR (ATR, ν_{max},cm^{-1}): 764.45(C-Cl), 1094.19(C-O, OC_2H_5), 2873.80 (C-H, CH_3), 2967.22 (C-H, Ar), 3167.40(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.14 (t, *J* = 7.14Hz, 3H), 2.35 (s, 3H), 4.02-4.08 (q,2H) 7.32-7.38 (m,3H), 7.47-7.49(m,1H), 8.07 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.68, 15.11, 40.69, 71.19, 98.96, 127.44, 129.60, 130.28, `130.38, 135.37, 142.22, 148.55, 153.28 ppm; HRMS (ESI^+) *m/z* for $C_{13}H_{14}ClN_3O_2$+Na^+ calcd:302.0672,found: 302.0662.

***3.3.22: (E)-1-(2-Chlorophenyl)-5-isopropoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-11):** Light brown solid; Yield =48%; mp =112–115^{0}C; IR (ATR, ν_{max},cm^{-1}): 766.46(C-Cl), 1089.58 (C-O, OC_3H_7), 2925.67 (C-H, CH_3), 2976.95 (C-H, Ar), 3216.03(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.13 (d, *J* = 6.09Hz, 6H), 2.42 (s, 3H), 4.25-4.31 (m, 1H), 7.37-7.44 (m, 4H), 7.52-7.55 (m, 1H), 8.08 (s, 1H) ppm ; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.87, 22.16, 40.84, 42.68, 54.44, 100.00, 127.33, 127.47, 127.52, 129.46, 129.57, 130.24, 130.29, 130.41, 130.88, 132.18, 135.51, 142.72, 148.40, 152.58 ppm.

***3.3.23: (E)-1-(3-Chlorophenyl)-5-methoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-12):** Light brownsolid; Yield = 45%; mp = 98–101^{0}C; IR (ATR, ν_{max},cm^{-1}): 781.07(C-Cl), 1067.09 (C-O, OCH_3), 2920.78 (C-H, CH_3), 3057.52 (C-H, Ar), 3251.39(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.33 (s,3H), 3.88 (s, 3H), 7.23 (d, *J* = 6.45Hz, 2H), 7.32 (t, *J* = 8.06Hz, 1H), 7.55 (d, *J* = 8.06Hz, 6H), 7.68 (s, 1H), 8.08 (s, 1H)ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.39, 40.75, 40.79, 42.67, 62.24,100.23, 120.00, 122.24,126.89, 129.93, 130.11, 134.80, 138.92, 141.96, 148.65, 153.05 ppm.

***3.3.24: (E)-1-(3-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-13):**Brown solid; Yield = 50%; mp = 97–100^{0}C; IR (ATR, ν_{max},cm^{-1}): 780.71(C-Cl), 1097.19 (C-O, OC_2H_5), 2925.12 (C-H, CH_3), 2960.06 (C-H, Ar), 3217.60(OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.21-1.29 (m,3H) 2.50 (s,3H), 4.06-4.11 (m,2H), 7.22 (d, *J* = 4.77Hz, 1H), 7.31 (t, *J* = 9.72Hz, 1H), 7.57 (d, *J* = 8.22Hz, 1H), 7.71 (s, 1H), 8.04 (s,1H) ppm ; ^{13}CNMR (400 MHz, $CDCl_3$): δ = 14.57, 15.15, 15.25, 40.83, 71.66,100.92, 120.02, 120.27, 120.77, 122.25, 123.02, 126.77, 127.55, 130.04, 134.74, 139.11, 142.11, 148.52, 152.21, ppm.

***3.3.25: (E)-1-(3-Chlorophenyl)-5-isopropoxy-3-methyl-1H-pyrazole-4-carbaldehyde oxime*(k-4-14):**Dark brown solid; Yield = 40%; mp = 98–101^{0}C; IR (ATR, ν_{max},cm^{-1}): 783.56(C-Cl), 1094.86 (C-O, OC_3H_7), 2923.37 (C-H, CH_3), 2976.11 (C-H, Ar), 3229.86(OH); ^{1}H NMR (400 MHz,$CDCl_3$): δ = 1.21 (d, *J* = 6.66Hz, 6H), 2.40 (s, 3H), 4.34-4.40 (m, 1H), 7.26 (d, *J* = 7.81Hz, 1H), 7.36 (t, *J* = 8.24Hz, 1H), 7.62 (d, *J* = 8.24Hz, 1H), 7.76 (s, 1H), 8.05 (s,1H) ppm; ^{13}CNMR

(400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.73, 22.12, 40.75, 79.40, 101.90, 120.38, 122.55, 126.79, 129.96, 134.65, 139.27, 142.21,148.42, 151.34 ppm.

4. Conclusion

In this study we have described a novel series of 13 pyrazolo-oxime hybrid molecules. The titled compounds were prepared by green chemistry, i.e. microwave synthesis, which is an efficient, mild and simple synthetic protocol. The synthesized adducts were screened for antimicrobial activity against two gram-positive, four gram-negative bacteria and against two fungal species. In particular, compounds **k-4-2, k-4-11** and **k-4-14** were the most promising and highly potent oxime derivatives with a MIC 0.012 µg/ml when compared to ciprofloxacin HCl. These compounds showed good antimicrobial activity and can be identified as the most promising agent against a number of infectious diseases caused by pathogenic microorganisms. Further studies are required to evaluate the mechanism of action for the antimicrobial activity of synthesised pyrazolo-oxime hybrid compounds.

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6. Conflict of interest

Authors hereby declare that there are no financial/commercial conflicts of interest.

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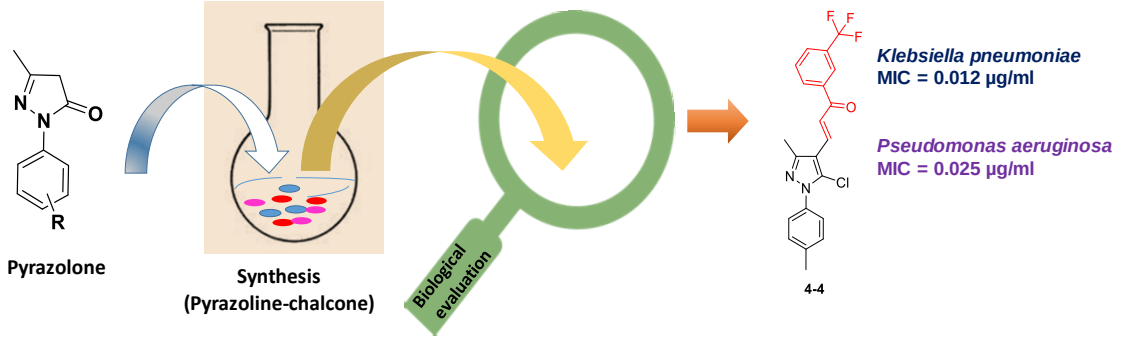
CHAPTER 6. MANUSCRIPT 5

The manuscript-5 is based on design, synthesis and antimicrobial activity of pyrazolone and chalcone derivatives titled **"Pyrazoline-chalcone as a potential antimicrobial agent: design, synthesis and antimicrobial evaluation"**. The paper summarizes the synthesis and antimicrobial activities of pyrazoline-chalcone derivatives. This paper was prepared by following the guidelines of the journal "European Journal of Medicinal Chemistry".

PYRAZOLINE-CHALCONE AS A POTENTIAL ANTIMICROBIAL AGENT: DESIGN, SYNTHESIS AND ANTIMICROBIAL EVALUATION



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Abstract

As part of our ongoing efforts to develop new or modified potent antimicrobial agents, we herein report the synthesis of a new class of structurally novel pyrazoline-chalcone derivatives. Condensation of various substituted 4-carbaldehyde pyrazole and substituted acetophenone gave expected pyrazole-chalcone derivatives that yield a new class of compounds with interesting antimicrobial properties. Evaluation of antibacterial activity by MIC assay method showed that almost all the compounds exhibited significant activity. Compound **4-4** (MIC- 0.012 µg/ml for *Pseudomonas aeruginosa* and 0.025 µg/ml for *Klebsiella pneumoniae)*, **4-13** (MIC 0.2 µg/ml for *Pseudomonas aeruginosa*) and **4-5** (MIC 0.05 µg/ml for *Klebsiella pneumoniae*, 0.2 µg/ml *Pseudomonas aeruginosa*, and *Escherichia coli*) were found to be highly potent.

Keywords: Antimicrobial, Pyrazole, Chalcones, Molecular hybrid.

1. Introduction

Penicillin was the first antibiotic discovered in 1928 by Alexander Fleming and in 1942 was introduced in clinical use[1]. Subsequently in the years that followed several antimicrobials were discovered such as gramicidin S (1942) the first peptide antibiotic, streptomycin (1944) the first amino glycoside, chlortetracycline (1948), the first tetracycline and vancomycin (1955) the first glycopeptides antibiotics [2]. These drugs played a vital role in the fight against infectious diseases caused by bacteria and other microbes. However, in the last few decades resistance to a number of antimicrobial agents such as β-lactam antibiotics, macrolides, quinolones, and vancomycin[3] was observed and several clinically major species of pathogenic bacteria were isolated, which has posed new challenges for the diagnosis and treatment, becoming a global health issue[4]. In addition the emergence of multidrug-resistant (MDR) microorganisms have reached an alarming level in recent years[5]. MDR bacteria cause community acquired infections, especially Methicillin-resistant *S. aureus* (MRSA) is the most prevalent causes of morbidity and mortality [6]. With the emergence of new microbial strains resistant to many conventional available antibiotics, there is growing interest/need for the discovery of new or potential antibacterial agents [1]. From literature reports and looking at the structure and activity relationships, it is considered that certain small heterocyclic molecules such as pyridine, furan, thiophene, pyran, oxazole, indole etc.[7] act as highly functionalized scaffolds and are known pharmacophores in number of biologically active and pharmaceutically marketed drugs.

Heterocycles are the building blocks for most of the pharmaceuticals and their importance in recent years has increased for their known broad spectrum of pharmacological applications. Azo-heterocycles such as thiazole, thiadiazole, triazole, aryl azothiophene, pyridine, piperazine, pyrazolone etc.[8] have gained more importance in recent years for developing new pharmaceutical drugs. In this research work, we have mainly focused on the pyrazolone and pyrazolone induced heterocycles. The first pyrazolone molecule was reported by Knorr in 1883 [9]. Antipyrine was the first pyrazolone derivative for clinical use and was synthesized in 1884[10] and was used as antipyretic and antiarthritic agent. Many other nonsteroidal anti-inflammatory drugs (NSAIDs) like phenylbutazone, oxyphenbutazone, aminophenazone, propyphenazone, contain pyrazolone as their basic nucleus[11]. Thus, pyrazolone scaffold has gained increasing attention due to its diverse pharmacological properties such as cytotoxic, antitumor[12], anti-inflammatory[13], antimicrobial[14], antioxidant[15], antifungal[16], anti-tubercular[17] insecticidal agents[18]. Pyrazolones are believed to be involved in various biochemical and physiological reactions and widely used as analgesic, antipyretic and anti-inflammatory drugs and thus scientific research programs are in progress across the globe to

improve synthetic techniques to prepare numerous pyrazolone derivatives and investigate their pharmaceutical applications[9]. Pyrazole is a five membered heterocycle compound also known as 1,2 diazole. It posseses wide range of biological activities such as antimicrobial, antifungal, antibacterial and antioxidant.

Chalcones are compounds with simple chemistry that offer easy synthetic access to various substituted derivatives. Chalcones can be easily synthesized by condensation of aromatic aldehyde and acetophenone under basic conditions giving *α, β*-unsaturated ketones[19]. Being essential constituents of various natural products, chalcones are regarded as important synthetic precursors. Compounds with the chalcones backbone have been reported for various biological activities, including anti-inflammatory, antimicrobial, antifungal, antioxidant and anticancer[20]. Various substituted (fluoro, methoxylated, hydroxylated), natural and synthetic chalcones have already been reported for their possible role as significant anti-inflammatory, antioxidant and antimicrobial activities[19, 21-22].

Literature survey and its analysis targeting the pyrazole-chalcone pharmacophore revealed that they have good biological spectrum such as anti-inflammatory[23], antimicrobial[24], anticancer[25], antibacterial and antifungal[26] (**Figure 1.**) etc..

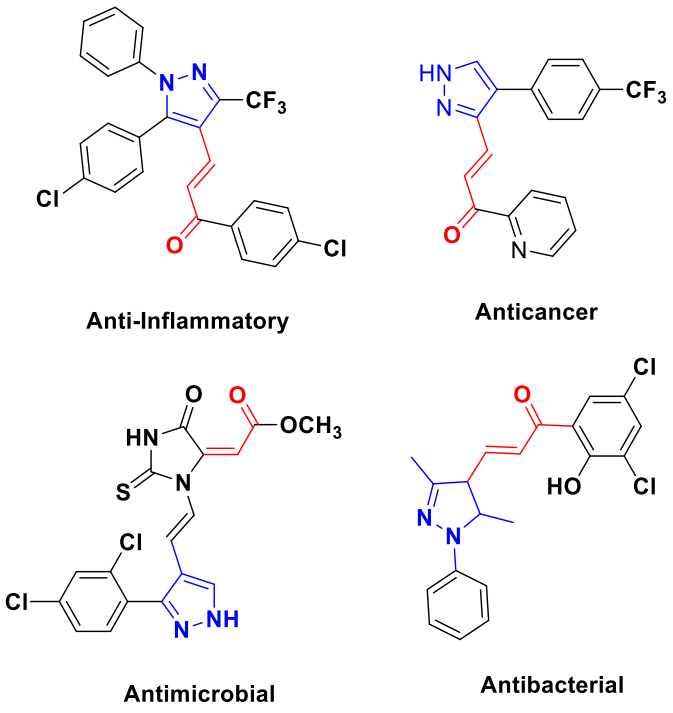


Figure 1. Biological spectrum of pyrazole chalcones.

On the basis of these, we have designed and hypothesized that combination of both the active scaffolds in one framework as hybrids of pyrazole and chalcone may have synergistic effect and improve the antimicrobial activity that could overcome several limitations associated with current marketed antimicrobial agents. These predictions encouraged us to synthesize a novel series of hybrid compounds of substituted pyrazole and chalcones as potential antimicrobial agents.

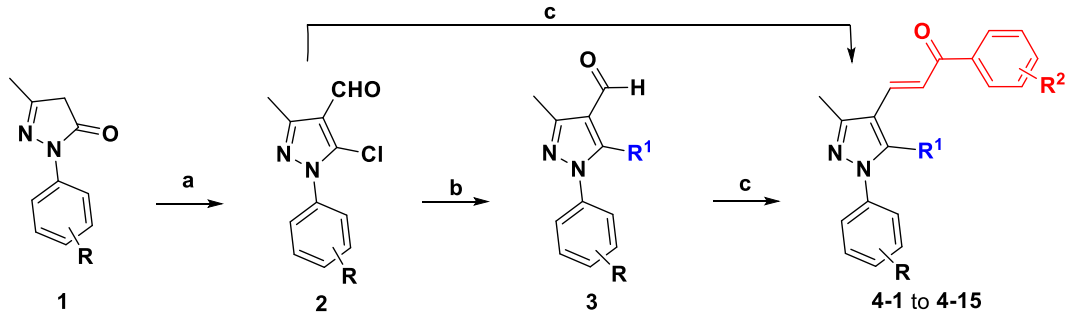
2. Results and Discussion

The proposed target molecules were synthesized quantitatively and qualitatively by using simple, rapid, economical, and green chemistry techniques. Designed synthetic scheme for the pyrazoline-chalcone is as show in **Scheme 1**, derivatization of final molecules were carried out from the readily available starting material.

Accordingly, all final compounds (**4-1** to **4-15**) were synthesized by stirring intermediate **2** or **3** in ethanol with different substituted acetophenones and ethanolic potassium hydroxide according to the reported method by K.S. Girisha *et al*. (2010) [27]. Pyrazoline carbaldehyde i.e. intermediates **2** were prepared by Vilsmeier-Haack formylation reaction, using synthetic method reported by Prajapati *et al.*(2007) [28]. Intermediates **2** were further derivatized to give final chalcone compounds **4-1** to **4-10** from readily available substituted acetophenone in presence of inorganic base. Furthermore, possibility of more Pyrazolone-chalcone derivatization executed by synthesizing alkoxy derivative of pyrazoline-carbaldehyde (**3**). In detail, carbaldehyde **2** were refluxed in presence of copper (II) acetate catalyst and strong inorganic base cesium carbonate in presence of solvent (ethanol);then, intermediate **3** were converted to pyrazoline-chalcone hybrid molecules **4-11** to **4-15** as per above described procedure.

Structures of the synthesized final compounds were confirmed by IR, NMR (^{1}H and 2D) and HRMS spectroscopic techniques. The ^{1}H NMR spectrums of all the compounds revealed a distinct peak resonating at 2.31 - 2.59 ppm, which was assigned to the 3-methyl group of the pyrazole ring. Characteristic aldehyde peak from intermediate (**2**/**3**) which appears at 9.80 - 9.87 ppm was absent in all the final compounds (**Figure 2**). The aromatic protons were observed between 7.17 - 7.77 ppm; while the characteristic ethylene (CH=CH) proton peak of chalcones appeared between 6.82 – 7.60 ppm and ~ 7.70-7.88 ppm which shows coupling constant (J = 15.92 Hz) (**Figure 3**) are in favor of chalcone protons as well as *trans* germinal coupling between these two protons. Electronic effect caused by substitutions on aromatic ring of acetophenone responsible for slight variation (6.82 ppm to7.60 ppm) in chemical shift of one chalcone proton. In **Figure 3** overlapping of HMBC and HSQC shows cross peak of C6 with carbon C3, C4, C5, C7 and C8, these interactions of C6 with other neighbor carbons are the significant finding in favor of final compound characterization. Similarly, ^{13}C data such as distinct methyl (CH_3) peak of the pyrazole

ring appeared at 14.12 - 14.53 ppm and the ethylene carbon (CH=CH) peak of chalcones were observed around 127.01 - 129.45 and 138 - 149 ppm. IR spectra of all the final compounds exhibited a characteristic chlorine (Cl) band around 669.46-728.72 cm^{-1}.Whereas, chalcones (CH=CH) at 1572.47-1640.25 $cm^{-1}$and carbonyl (C=O) was observed at 1636.47-1676.45 cm^{-1}. HRMS characterization for few sample compounds were performed and they demonstrated the characteristic molecular ion peak $[M+H]^+$ corresponding to the molecular weight of the desired compounds. Thus, all above ^1H, ^{13}C, 2D NMR, IR, HRMS characterization data confirm the formation of the Pyrazoline-chalcone molecular hybrids.



Compound	R	R^1	R^2	Compound	R	R^1	R^2
4-1	4-CH_3	Cl	H	**4-9**	3-Cl	Cl	2-OH, 5-Cl
4-2	4-CH_3	Cl	4-CH_3	**4-10**	3-Cl	Cl	3,4-$(OCH_3)_2$
4-3	4-CH_3	Cl	4-Br	**4-11**	4-CH_3	OCH_2CH_3	4-CH_3
4-4	4-CH_3	Cl	4-CF_3	**4-12**	3-Cl	OCH_2CH_3	4-Br
4-5	4-CH_3	Cl	2,5-$(OCH_3)_2$	**4-13**	3-Cl	OCH_2CH_3	2-OH, 5-Cl
4-6	4-CH_3	Cl	3,4-$(OCH_3)_2$	**4-14**	3-Cl	OCH_2CH_3	2-OH, 5-F
4-7	3-Cl	Cl	4-Br	**4-15**	3-Cl	OCH_2CH_3	2-OH, 5-OCH_3
4-8	3-Cl	Cl	4-NH_2				

Scheme 1. Synthetic Scheme of target molecules. Reagents and conditions: (a) $POCl_3$, DMF, 0 ^0C, RT and reflux 3 h; (b) KOH, ethanol, acetophenone, RT; (c) ethanol, CS_2CO_3, $Cu(OAc)_2$, Microwave, 20 min/conventional 15 h.

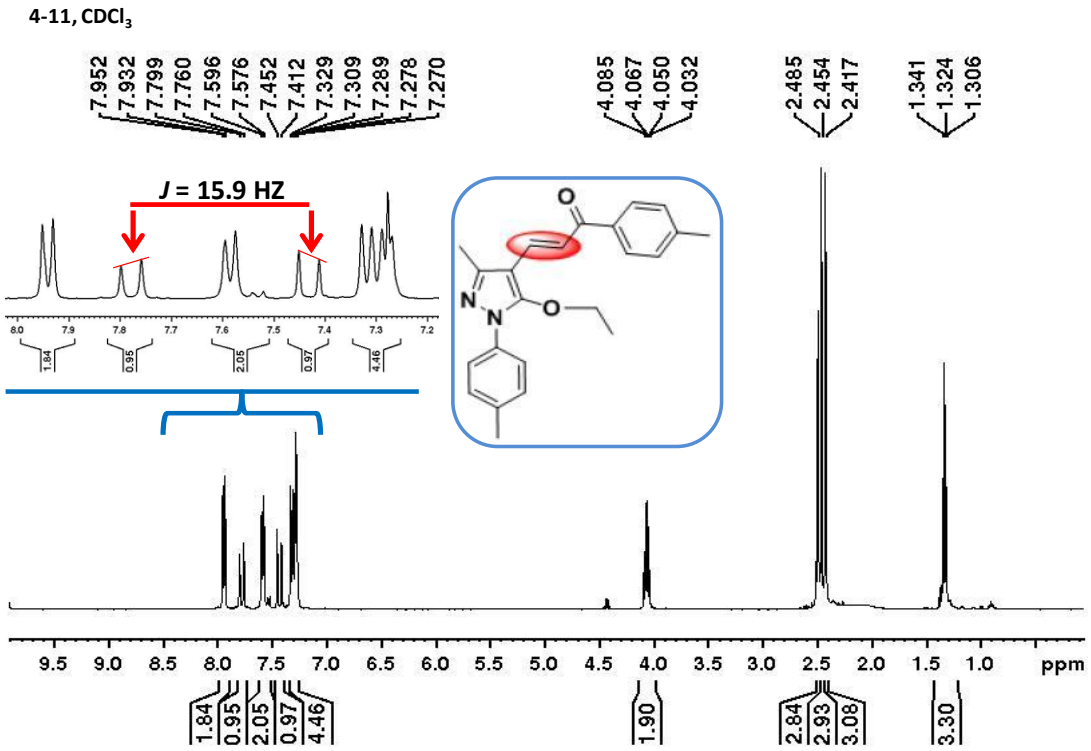


Figure 2.[1]H NMR of pyrazole-chalcone compound **4-11**.

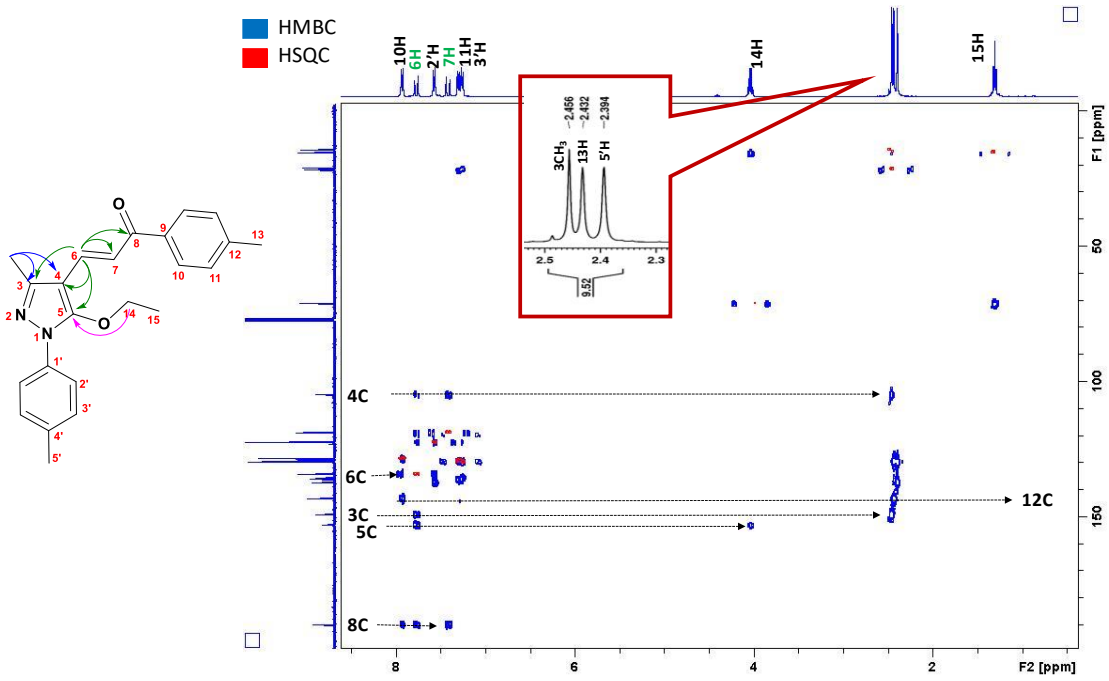


Figure 3. 2D NMR of pyrazole-chalcone compound **4-11**.

2.1 Biological evaluation

2.1.1 Microorganism used: Standard cultures of two gram-positive bacteria [MRSA- ATCC BAA-1683 *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus*) and SA- ATCC 25923 *Staphylococcus aureus*], four gram-negative bacteria [ST-*Salmonella typhimurium,* PA-ATCC 27853 *Pseudomonas aeruginosa*, EC-ATCC 25922 *Escherichia coli*, KP- ATCC 31488 *Klebsiella pneumoniae*] and two fungal strains [*Candida albicans* ATCC90028 and *Cryptococcus neoformans* ATCC66031] were used for the antibacterial and antifungal activity respectively. Culturing and sub culturing (one day prior to testing) of these microorganisms was carried under supervision of Dr. Chunderika at the discipline of Pharmaceutical Sciences, College of Health Sciences, UKZN, Durban, South Africa.

Table 1. Antimicrobial and antifungal activity of pyrazolone-chalcone molecular hybrids.

Compound	**Diameter of zone inhibition (MIC µg/ml)**							
	Gram- negative bacteria				**Gram-positive bacteria**		**Fungal trains**	
	ST	**PA**	**EC**	**KP**	**MRSA**	**SA**	**CA**	**CN**
4-1	0.2	na	>200	na	na	na	>200	>200
4-11	na	na	0.1	1.6	na	50	>200	>200
4-4	12.5	0.012	200	0.025	na	25	>200	>200
4-13	0.4	0.2	200	0.4	na	25	>200	>200
4-5	0.4	0.2	0.2	0.05	na	200	>200	>200
4-7	6.25	1.6	na	na	6.25	na	>200	>200
4-14	na	>200	>200	>200	na	na	>200	>200
4-15	200	100	200	>200	na	na	>200	>200
4-10	6.25	0.8	na	na	6.25	na	>200	>200
4-8	6.25	na	na	na	na	6.25	>200	>200
4-6	6.25	6.25	na	na	na	na	>200	>200
4-3	12.5	0.8	na	6.25	25	6.25	>200	>200
4-12	6.25	na	0.8	3.125	6.25	na	>200	>200

4-2	12.5	na	100	100	200	na	>200	>200
4-9	12.5	na	1.6	6.25	na	0.4	>200	>200
Ciprofloxacin HCl	0.4	0.8	0.1	1.6	25	25	-	-
Amphotericine B	-	-	-	-	-	-	0.39	1.5

Positive control (standard): Ciprofloxacin HCl; **ST**-*Salmonella typhimurium,* **PA**-*Pseudomonas aeruginosa*, **EC**-*Escherichia coli*, **KP**-*Klebsiella pneumoniae*, **MRSA**-*Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus,*), **SA**-*Staphylococcus aureus*, **CA**-*Candida albicans*, **CN**-*C. neoformans;* *na –not active

2.1.2 In vitro antibacterial analysis: Preliminary antibacterial screening of the synthesized compounds were carried out against penal of bacteria. The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37 ºC in a shaking incubator (100 rpm). The bacterial concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton Agar plates (MHA) (Biolab, South Africa) were lawn inoculated with the prepared bacterial suspensions using a sterile throat swab and 10 µl of the solution of synthesized compound (1 mg/ml DMSO) were spotted onto the MHA plates. The plates were incubated at 37 ºC for 18 h and after incubations the plates were read to determine antibacterial activity which was denoted by clear zones in the area where the solution were spotted. Based on the preliminary screening results the minimum inhibitory concentrations (MICs) were determined. The solution of synthesized compounds was serially diluted 2-fold with DMSO ranging from 0.006 - 100 µg/ml) and 10 µl of each concentration was spotted on the lawn inoculated MHA plates and incubated at 37 ºC for 24 h. Ciprofloxacin hydrochloride was used as standard drug.

2.1.3 In vitro evaluation of antifungal activity: The synthesized hybrid of Pyrazoline-chalcone were evaluated for antifungal activity against the two fungal species using Amphotericin B as a reference standard drug by following earlier reported MIC assay method [29–31].

2.2 Discussion

The results of *in vitro* antibacterial and antifungal screening (MIC values) of the tested compounds are summarized in **Table 1**. A systematic analysis of the data as depicted in **Table1** revealed that all compounds displayed little or no inhibitory activity against the fungal strains, *Candida albicans and C. neoformans*. However, against *Salmonella typhimurium*, compound **4-1** presented highest inhibition (MIC 0.2 µg/ml) among all synthesized compounds which was **2 folds higher than the standard drug,** and compound **4-5** and **4-13** were equipotent to standard drug (MIC 0.4 µg/ml). For *Pseudomonas aeruginosa* compound **4-4** exhibited highest inhibition (**MIC 0.012µg /ml**) which is **50 folds higher than standard drug ciprofloxacin HCl**, **4-5** and **4-13** displayed 4 folds higher inhibition (MIC 0.2 µg/ml) than standard drug, **4-3** and **4-10** are equipotent (MIC 0.8 µg/ml) to standard drug. For *Escherichia coli* **4-11** displayed highest inhibition (MIC 0.1 µg/ml) equal to the standard drug, whereas compound **4-5** showed good potency (MIC 0.2 µg/ml). For *Klebsiella pneumonia* compound **4-4** showed best inhibition (MIC 0.025 µg/ml) which was almost **80 folds higher than standard drug.** Similarly compound **4-5** (MIC 0.05 µg/ml) was 32 folds higher and compound **4-13** exhibited 4 folds higher (MIC 0.4 µg/ml) inhibitory activity than standard drug. In case of gram-positive bacteria; for *Staphylococcus aureus* Rosenbach (MRSA) compound **4-7, 4-10** and **4-12** showed highest inhibition (MIC 6.25 µg/ml) which was 4 folds higher than standard drug and **4-3** was equipotent to standard drug. For *Staphylococcus aureus* compound **4-9** indicated significantly high inhibition (MIC 0.4 µg/ml) **almost 62 folds higher than standard drug**, followed by compounds **4-3** and **4-8** (MIC 6.25 µg/ml) with 4 folds higher activity than standard drug, while compounds **4-4** and **4-13** were equipotent as compared to the standard drug. Among the tested series of compounds, **4-4, 4-5** and **4-13** were best active against the gram-negative bacteria. Structure activity relationship (SAR) revealed that CF_3, F, Cl and OCH_3 groups attached to phenyl ring of chalcones have exhibited high potency. For gram-positive bacteria, compound **4-9** displayed best inhibition activity, followed by compounds **4-3**, **4-7**, **4-8**, **4-10** and **4-12** indicating significant activity. From these antibacterial screening data, it was observed that halogen and methoxy substitution on aromatic chalcones enhanced the antibacterial activity of the designed pyrazoline-chalcone hybrids.

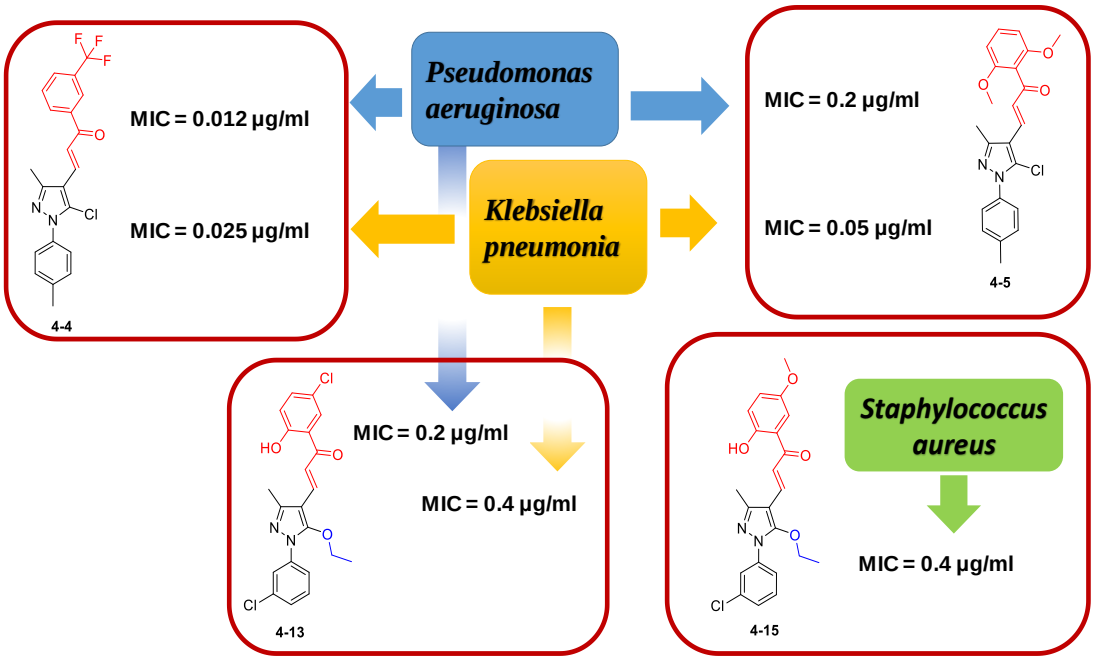


Figure 4. Pyrazoline-chalcone molecular hybrids with potent antibacterial activity.

3. Experimental

3.1. Materials and Method

All the chemicals and reagents used in this research work were purchased from Sigma Aldrich and Merck Millipore South Africa. All the solvents, except those of laboratory-reagent grade, were dried and purified when necessary according to previously published methods. The progress of the reactions and the purity of the compounds were monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel plates procured from Merck (Pty) Ltd. The melting points of the synthesized compounds were determined using a Thermo Fisher Scientific (IA9000, UK) digital melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker Alpha FT-IR spectrometer (Billerica, MA, USA) using the ATR technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany) spectrometers using $CDCl_3$ and DMSO-d_6. The chemical shifts are reported in δ ppm units with respect to trimethylsilane as an internal standard. HRMS spectra were recorded on an Autospec mass spectrometer with electron impact at 70 eV.

3.2 Synthesis

3.*2.1 Preparation of Pyrazoline carbaldehyde (2) by Vilsmeier-Haack reaction:* Vilsmeier-Haack reagent was freshly prepared by careful addition of $POCl_3$ (0.42 mole, 7 equiv.) to dimethylformamide (0.18 mole, 3 equiv.) at 0 °C with constant stirring. To this reaction mixture (maintained at 0 °C) compound **1**(0.06 mole, 1 equiv.) was added and stirred initially for 30 min and later slowly brought to RT and stirred for 1 h. The reaction temperature was raised to 80 °C and stirred for additional 3 h. After cooling to RT, the reaction mixture was poured into ice water and the formed precipitate separated by filtration and washed with cold water. The obtained residue was further recrystallized from ethanol to afford the desired compound (**2**) as off white crystalline solid (Yield: 75 %).

3.2.2. Preparation of 5-alkoxy pyrazole-4-carbaldehyde (3): To the mixture of pyrazoline-carbaldehyde **2** (0.020 mole, 1 equiv.), cesium carbonate (0.03 mole, 1.5 equiv.) and copper (II) acetate (0.005 mole, 0.25 equiv.), ethanol was added and refluxed for 15 h / microwave at 70 °C, 10 + 10 min. (power - 150 W, pressure - 150 psi.). Reaction progress was monitored by TLC (5/95, ethyl acetate/hexane). After completion of reaction, the formed solid was filtered off and purified by column chromatography on silica gel using hexane and ethyl acetate as eluent to get compound **3**.

3.2.3 Preparation of pyrazoline-chalcone derivatives (4-1 to 4-15): Starting material **2** or **3** (0.1 mole, 1 equiv.) was dissolved in 20 ml of ethanol. To this solution substituted acetophenones (0.1 mole, 1 equiv.) were added and the reaction mixture was cooled to 0 ^oC. To the cold reaction mixture ethanolic potassium hydroxide (0.1 mole, 1 equiv.) was added slowly by maintaining the temperature at 0 - 5 ^oC. Continued stirring at RT for 5 - 10 h under TLC monitoring. On completion of reaction, the reaction mixture was poured over crushed ice. The separated solid was filtered, washed with cold water and recrystallized in ethanol to get desired compound **4-1** to **4-15**.

3.3 Experimental data

Note: We have taken HRMS of selected compounds to support NMR results, remaining all compounds were characterized by NMR and IR only.

3.3.1. (E)-3-(5-Chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-one **(4-1):** White solid; Yield = 66%; mp = 122-124°C; IR (ATR, ν_{max},cm^{-1}): 780.36 (C-Cl), 1602.72 (C=C), 1656.07 (C=O), 2922.20 (C-H, CH_3), 3021.36 (C-H, Ar); ^1H NMR (400 MHz, $CDCl_3$, 25 ^0C): δ = 2.39 (s, 3H), 2.49 (s, 3H); 7.27 (d, *J* = 8.16 Hz, 2H) 7.39 (d, *J* = 8.14Hz, 2H), 7.46 - 7.50 (m, 2H), 7.53 - 7.57 (m, 2H) 7.75 (d, *J* = 16.00 Hz, 1H), 7.98 (d, *J* = 7.92 Hz, 2H) ppm; ^{13}CNMR

(400 MHz, $CDCl_3$, 25 ^0C) δ = 14.25, 21.21, 114.15, 120.60, 124.91, 128.42, 128.65, 128.75, 129.73, 132.72, 133.62, 135.20, 138.36, 138.87, 149.94, 190.26; HRMS (ESI^+) *m/z* for $C_{20}H_{17}ClN_2O+ Na^+$: calcd. 359.0927 found, 359.0924.

3.3.2. (E)-3-(5-Chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-(p-tolyl)prop-2-en-1-one **(4-2)**: Light yellow solid; Yield =60%; mp = 138 – 140^{0}C; IR (ATR, ν_{max},cm^{-1}): 733.38 (C-Cl), 1606.87 (C=C), 1657.36 (C=O), 2918.78 (C-H, CH_3), 3036.51 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.40 (d, *J* = 4.83 Hz, 6H), 2.49 (s, 3H), 7.27 (dd, *J* = 8.27 and 3.14 Hz, 4H), 7.39 (d, *J* = 8.37 Hz, 2H), 7.50 (d, *J*=16.12Hz, 1H), 7.73 (d, *J* =15.96 Hz, 1H), 7.89 (d, *J* =8.08 Hz, 2H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.24, 21.20, 21.68, 114.20, 120.69, 124.90, 128.56, 129.34, 129.71, 133.16, 135.23, 135.76, 138.82, 143.54, 149.88, 189.76 ppm.

3.3.3. (E)-1-(4-Bromophenyl)-3-(5-chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)prop-2-en-1-one**(4-3):** White solid; Yield = 60%; mp = 149 – 151^{0}C; IR (ATR, ν_{max},cm^{-1}): 651.79(C-Br), 733.31(C-Cl), 1590.44 (C=C), 1662.31 (C=O), 2916.92 (C-H, CH_3), 3049.63 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.39 (s, 3H), 2.48 (s, 3H), 7.26 (d, *J* =7.89 Hz, 2H), 7.39 (d, *J* = 8.34 Hz, 2H), 7.44 (d, *J* = 15.74Hz, 1H), 7.61 (d, *J* = 8.60 Hz, 2H), 7.74 (d, *J* = 15.99 Hz, 1H), 7.84 (d, *J* = 4.49 Hz, 2H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.22, 21.21, 114.03, 119.90, 124.89, 127.79, 128.87, 129.74, 129.94, 131.94, 134.19, 135.14, 137.07, 138.94, 150.01, 189.12 ppm.

3.3.4.(E)-3-(5-Chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-(3(trifluoromethyl)phenyl)prop-2-en-1-one **(4-4):**White solid; Yield = 60%; mp = 120 – 122^{0}C; IR (ATR, ν_{max},cm^{-1}): 691.45 (C-Cl), 1610.33 (C=C), 1658.96 (C=O), 2952.08 (C-H, CH_3), 3046.03 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.39 (s, 3H), 2.49 (s, 3H); 7.27 (d, *J* = 8.77 Hz, 2H), 7.39 (d, *J* = 7.68 Hz, 2H), 7.46 (d, *J* = 15.45 Hz, 1H), 7.56 - 7.64 (m, 1H), 7.76 - 7.81 (m, 1H), 8.11 - 8.22 (m, 2H), ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.25**,** 21.21, 113.97, 119.70, 124.68, 125.23, 125.27, 129.08, 129.28, 129.76, 131.11, 131.44, 131.48, 134.87, 135.11, 138.92, 139.00, 150.10, 188.92ppm.

3.3.5. (E)-3-(5-Chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-(2,6-dimethoxyphenyl)prop-2-en-1-one **(4-5):** Light brown solid; Yield = 73%; mp = 113 - 115 ^{0}C; IR (ATR, ν_{max},cm^{-1}): 774.19 (C-Cl), 1101.46 (C-O, OCH_3), 1624.01 (C=C), 1641.02 (C=O), 2922.20 (C-H, CH_3), 3003.96 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.38 (d, *J* = 8.16Hz, 6H), 3.72 (s, 6H); 6.54 (d, *J* = 8.45Hz, 2H), 6.85 (d, *J* = 16.08Hz, 1H), 7.19 - 7.31 (m, 6H), ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.39, 21.19, 55.97, 104.16, 118.49, 124.84, 127.48, 129.69, 130.86, 133.75, 135.21, 138.78, 184.78 ppm.

***3.3.6. (E)-3-(5-Chloro-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one* (4-6):** White solid; Yield = 60%; mp = 154 – 156^{0}C; IR (ATR, ν_{max},cm^{-1}): 700.27 (C-Cl), 1152.32 (C-O, OCH_3), 1597.56 (C=C), 1651.32 (C=O), 2915.90 (C-H, CH_3), 3013.37 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.38 (s, 3H), 2.48 (s, 3H), 3.93 (d, *J* = 3.37 Hz, 6H) 6.90 (d, *J* =8.21Hz, 1H), 7.26 (d, *J* = 8.00 Hz, 2H), 7.38 (d, *J* = 8.00 Hz, 2H), 7.52 (d, *J* = 16.00 Hz, 1H), 7.61 (d, *J* = 10.00 Hz, 2H), 7.72 (d, *J* = 16.00 Hz, 1H)), ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.21, 21.20, 56.03, 56.1, 110.02, 110.08, 114.23, 120.43, 122.81, 124.90, 128.46, 129.71, 131.45, 132.74, 135.23, 138.81, 149.26, 149.82, 153.20, 188.46 ppm.

***3.3.7. (E)-1-(4-Bromophenyl)-3-(5-chloro-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)prop-2-en-1-one* (4-7):** White solid; Yield = 73%; mp = 163 – 165^{0}C; IR (ATR, ν_{max},cm^{-1}): 683.46 (C-Br), 778.05 (C-Cl), 1584.25 (C=C), 1665.48 (C=O), 2981.85 (C-H, CH_3), 3089.34 (C-H, Ar); ^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.49 (s, 3H), 7.39-7.47 (m, 4H), 7.58 (d, *J* = 1.51 Hz, 1H), 7.62 (d, *J* = 8.50Hz, 2H), 7.73 (d, *J* = 16.01Hz, 1H), 7.84 (dd *J* = 8.66 and 1.81Hz, 2H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.24, 114.73, 120.54, 122.91, 125.16, 128.82, 129.94, 130.13, 131.98, 133.68, 138.54, 150.55, 189.00. ppm.

***3.3.8. (E)-1-(3-Aminophenyl)-3-(5-chloro-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)prop-2-en-1-one* (4-8):** Yellow solid; Yield = 60%; mp = 122 – 124^{0}C; IR (ATR, ν_{max},cm^{-1}): 779.92 (C-Cl), 1579.68 (C=C), 1657.56 (C=O), 2921.66 (C-H, CH_3), 3066.57 (C-H, Ar), 3352.22 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.48 (s, 3H), 3.81 (s, 2H), 6.86 (dd, *J* = 6.12 and 1.92Hz, 1H),7.25-7.29 (m, 2H), 7.34 (d, *J* = 7.68Hz, 1H), 7.37-7.40 (m, 2H), 7.44-7.48 (m, 2H), 7.58 (s, 1H), 7.69 (d, *J* = 15.78 Hz, 1H), ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.39, 114.49, 118.92, 119.61, 121.62, 123.02, 125.27, 128.83, 129.60, 130.21, 132.90, 190.37 ppm.

3.3.9.(E)-3-(5-Chloro-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-(5-chloro-2-hydroxyphenyl)prop-2-en-1-one (4-9): Light yellow solid; Yield = 60%; mp = 129 - 131^{0}C; IR (ATR, ν_{max},cm^{-1}): 745.54 (C-Cl), 1572.47 (C=C), 1636.50 (C=O), 2921.68 (C-H, CH_3), 3077.66 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 2.52 (s, 3H), 6.96 (d, *J* = 8.89 Hz, 1H), 7.40 - 7.53 (m, 5H), 7.59 (m, 1H), 7.74 (d, *J* = 2.30 Hz, 1H), 7.84 (d, *J* = 15.62 Hz, 1H) 12.73 (s, 1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.25, 114.63, 118.39, 120.30, 120.56, 122.92, 123.54, 125.18, 128.60, 128.96, 129.27, 130.17, 134.86, 134.97, 136.16, 138.43, 150.81, 162.10, 192.58 ppm.

***3.3.10.(E)-3-(5-Chloro-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one* (4-10):** White solid; Yield = 60%; mp = 166 – 168^{0}C; IR (ATR, ν_{max},cm^{-1}): 763.78 (C-Cl), 1021.17(C-O, OCH_3), 1592.01 (C=C), 1658.74 (C=O), 2965.93

(C-H, CH_3), 3066.30 (C-H, Ar);^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 2.49 (s, 3H), 3.93 (d, *J* = 2.38Hz, 6H), 6.91 (d, *J*=8.18Hz, 1H),7.36 - 7.47 (m, 3H), 7.53 (d, *J*=15.89Hz, 1H), 7.58 - 7.63 (m, 3H), 7.70 (d, *J* = 15.95 Hz, 1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.24, 56.05, 56.12, 110.03, 110.81, 114.96, 121.07, 122.86, 122.91, 125.15, 128.36, 128.71, 130.11, 131.33, 132.23, 134.87, 138.64, 149.31, 150.40, 153.31, 188.32 ppm; HRMS (ESI^+) *m/z* for $C_{21}H_{18}Cl_2N_2O_3 + Na^+$: calcd. 435.0582 found, 435.0592

***3.3.11. (E)-3-(5-Ethoxy-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-1-(p-tolyl)prop-2-en-1-one* (4-11):** Yellow solid; Yield = 68%; mp = 117–119^{0}C; IR (ATR, ν_{max},cm^{-1}): 1102.12 (C-O, OC_2H_5), 1607.27 (C=C), 1656.86 (C=O), 2917.56 (C-H, CH_3), 3032.60 (C-H, Ar); ^{1}H-NMR (400 MHz, $CDCl_3$, 25^{o}C): δ = 7.92 (d, *J* = 8.1 Hz, 2H 10-H), 7.76 (d, *J* = 15.6 Hz, 1H, 6-H), 7.56 (d, *J* = 8.4 Hz, 2H, 2'-H), 7.41 (d, *J* = 15.7 Hz, 1H 7-H), 7.29 (d, *J* = 8.03 Hz, 2H, 11-H), 7.25 (d, *J* = 8.0 Hz, 2H, 3-H), 4.03 (q, *J* = 7.0 Hz, 2H, 14-H),2.45 (s, 3H, 3-CH_3), 2.43 (s, 3H, 13H), 2.39 (s, 3H, 5'-H), 1.30 (t, *J* = 7.0 Hz, 3H, 15-H) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$, 25^{o}C): δ = 190.0 (C8), 153.1 (C5), 149.1 (C3), 143.3 (C12), 137.2(C4'), 136.2 (C9), 135.6 (C1'), 134.2 (C6), 129.8 (C3'), 129.3 (C11), 128.5 (C10), 122.4 (C2'), 118.8 (C7), 104.9 (C4), 71.1 (C14), 21.7 (C13), 21.1(C5'), 15.5 (C15), 14.4 (3CH_3)ppm.

3.3.12.(E)-1-(4-Bromophenyl)-3-(1-(3-chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazol-4-yl)prop-2-en-1-one (4-12): Yellow solid; Yield = 60%; mp: 119 - 121^{0}C; IR (ATR, ν_{max},cm^{-1}): 605.78 (C-Br), 778.52 (C-Cl), 1586.52 (C=C), 1676.78 (C=O), 2923.06 (C-H, CH_3), 3037.28 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.31 (t, *J* =7.01 Hz, 3H), 2.42 (s, 3H), 4.04-4.09 (q, 2H), 7.22-7.38 (m, 4H), 7.61 (d, *J* = 8.48 Hz, 3H), 7.70-7.76 (m, 1H), 7.84 (d, *J* = 8.51Hz, 2H), 7.61 (d, *J* =8.60 Hz, 2H), 7.74 (d, *J* = 15.99 Hz, 1H), 7.84 (d, *J* = 4.49 Hz 2H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.40, 15.43, 71.64, 104.64, 105.19, 118.90, 119.90, 120.00, 122.20, 127.20, 127.28, 127.64, 129.82, 130.20, 130.28, 131.92, 134.39, 134.63, 134.95, 137.27, 138.91, 149.70, 152.39, 189.07 ppm.

***3.3.13. (E)-1-(5-Chloro-2-hydroxyphenyl)-3-(1-(3-chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazol-4-yl)prop-2-en-1-one* (4-13):** Orange solid; Yield = 70; mp = 136–138^{0}C; IR (ATR, ν_{max}, cm^{-1}): 776.79 (C-Cl), 1161.08 (C-O, OC_2H_5), 1640.15 (C=C), 1680.31 (C=O), 2915.27 (C-H, CH_3), 3081.61 (C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.35 (t, *J* = 7.00 Hz, 3H), 2.44 (s, 3H), 4.06-4.11 (m, 2H) 6.94-6.97 (m, 1H), 7.17-7.22 (m, 1H), 7.28 (d, *J*=8.07Hz, 1H), 7.35-7.39 (m, 2H),7.46 (dd, *J*=9.12, 3.13Hz,1H), 7.62 (d, *J* = 7.98Hz, 1H),7.76 (s, 1H), 7.83 (d, *J* = 15.36Hz, 1H), 12.69 (s,1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C): δ = 14.37, 15.47, 71.77, 114.03, 114.26, 116.64, 119.60, 119.66, 119.90, 120.06, 122.35, 123.43, 123.66, 127.40, 130.26, 135.03, 138.81, 153.67, 156.04, 159.73, 192.70 ppm.

***3.3.14. (E)-3-(1-(3-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazol-4-yl)-1-(5-fluoro-2-hydroxyphenyl)prop-2-en-1-one* (4-14):** Orange solid; Yield = 66%; mp =118–120^{0}C; IR (ATR, ν_{max},cm^{-1}): 775.48 (C-Cl), 1058.08 (C-O, OC_2H_5), 1244.46(C-F), 1593.12 (C=C), 1676.51 (C=O), 2928.96 (C-H, CH_3), 3078.37(C-H, Ar); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.32-1.36(m,3H), 2.43(s,3H), 4.05-4.10 (q,2H),6.93-6.97 (m,1H),7.16-7.22(m,1H), 7.28 (d, *J*=7.86Hz, 1H), 7.32-7.38 (m,2H), 7.45 (dd, *J* = 9.17 and 2.78 Hz,1H), 7.59 (dd, *J* = 14.28 and 7.29 Hz, 1H), 7.70-7.85 (m,2H) 12.68 (s, 1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.35, 15.49, 71.80, 114.27, 116.62, 119.66, 119.82, 119.89, 120.73, 122.36, 122.99, 123.42, 123.66, 127.40, 127.52, 130.04, 130.21, 130.26, 134.78, 135.02, 135.71, 138.79, 153.67, 156.04, 159.73, 192.69 ppm.

***3.3.15. (E)-3-(1-(3-Chlorophenyl)-5-ethoxy-3-methyl-1H-pyrazol-4-yl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one*(4-15)**: Brown solid; Yield = 60%; mp = 110–112^{0}C; IR (ATR, ν_{max},cm^{-1}): 774.69 (C-Cl), 1057.98 (C-O, OC_2H_5), 1591.97 (C=C), 1637.85 (C=O), 2931.46 (C-H, CH_3), 3076.45 (C-H, Ar), 3396.13(OH, C_6H_5OH); ^{1}H NMR (400 MHz,$CDCl_3$, 25 ^{0}C): δ = 1.34 (t, *J* = 7.05 Hz, 3H), 2.43 (s,3H), 3.80 (s,3H) 4.05-4.10 (q, 2H),6.94 (d, *J*=9.60Hz, 1H),7.10 (dd, *J*= 6.09 and 2.96Hz, 1H), 7.27-7.29 (m, 2H), 7.36 (t, *J* = 8.16 Hz,1H), 7.44 (d, *J* = 15.43Hz, 1H), 7.62 (d, *J* = 8.09Hz, 1H), 7.76-7.82 (m, 2H), 12.51 (s, 1H) ppm; ^{13}CNMR (400 MHz, $CDCl_3$, 25 ^{0}C):δ = 14.26, 15.50, 56.01, 71.62, 105.30, 112.51, 117.31, 119.31, 119.71, 120.05, 122.32, 127.30, 130.24, 134.94, 138.85, 149.88, 151.69, 153.50, 157.93, 193.24 ppm. HRMS (ESI$^+$) *m/z* for $C_{22}H_{21}ClN_2O_4$+ Na^+: calcd.435.1088 found, 435.1073.

4. Conclusion

In conclusion, we have synthesized 15 new analogues of structurally novel pyrazole-chalcone derivatives by condensation of various substituted 4-carbaldehyde pyrazole with substituted acetophenones. All the synthesized compounds showed interesting antimicrobial properties, with compounds **4-4, 4-5** and **4-13** displaying highly potent (MIC- 0.012, 0.2 and 0.05 µg/ml) inhibitory activity against various bacterial species. Structure activity relationship revealed that electro-negative link on the phenyl ring of chalcones groups displayed high potency against bacteria. Results of antimicrobial activity are inspiring and the lead candidates can be further exploited to improve their antimicrobial potential.

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6. Conflict of interest

Authors hereby declare that there are no financial/commercial conflicts of interest.

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CHAPTER 7: SYNTHESIS CHAPTER

1. Conclusion

The latest report of the WHO on antimicrobial resistance and its surveillance is alarming: resistance to pathogenic bacteria has reached very high levels in many parts of the world; none or few of the available treatment options remain effective for common infectious disease. This severe global problem is the consequence of the widespread use and misuse of antibiotics in medicine, agriculture, aquaculture, horticulture and other human activities. With the large-scale production of antibiotics and a constant rise in antibiotic consumption, we exert a strong selective pressure towards bacteria to evolve resistance in various ecosystems, and today many of the pathogenic microorganisms found in the clinics are resistant to multiple antibiotics. The escalating number of resistant pathogens poses major problems on the treatment of patients with hospital or community-acquired multi-drug resistant infections. The emergence of various drug resistant strains poses a serious threat and requires an immediate action at this juncture. Proper clinical measures such as early detection of the strains, improved patient compliance and development of novel and effective lead molecules will be crucial in controlling microbial infections.

The aim of this entire study was to identify a novel and effective antimicrobial agent that were inspired from pyrazolone. Pyrazolone (also known as 2,4-Dihydro-3H-pyrazol-3-one) was fused with various aromatic/heterocyclic moieties of pharmaceutical importance using molecular hybridization technique to yield novel pyrazolone hybrid compounds. Thus, 63 novel pyrazolone hybrids were synthesized and well characterized by thin layer chromatography (TLC), infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR), and high-resolution mass spectrometry (HR-MS). All the title compounds were screened for their antimicrobial properties. Results obtained from biological evaluation suggested that the synthesized compounds were highly active as potential antimicrobial agents. Hence, the research work highlights the potential applications of novel pyrazolone hybrid compounds as a potential lead for antimicrobial agents.

In chapter 2, we have performed comprehensive literature assessment of 2,4-Dihydro-3H-pyrazol-3-one derivatives for their broad spectrum of biological activities. The literature review revealed that pyrazolone is a versatile scaffold studied extensively in building up of diverse chemical libraries. These libraries of compounds were assessed for their immense pharmacological properties such as antimicrobial, antitubercular, anticancer, anti-inflammatory, and antioxidant scavenging activity, aromatase inhibition, as well as COX-1and COX-2 inhibiting activity. Thereby confirming for the pharmaceutical importance of pyrazolone and its derivatives. The inspiring research finding from the broad literature search encouraged us to choose 2,4-

Dihydro-3*H*-pyrazol-3-one for further amendment as antimicrobial agents. This significant literature analysis has emerged as a review article communicated to Bio-organic Chemistry.

In chapter 3, wesynthesized novel pyrazole-1,3,4-thiadiazole scafold as a potent antimicrobial agent via 4-thiazolidinone linker using molecular hybridization approach. The designed schemes produced the desired compounds in moderate yields (50-60%). The title compounds (**k-2-a** to **k-2-m**) were obtained by treating various derivatives of 2-amino-5-phenyl-1,3,4 thiadiazole (**1a-1m)** in toluene and thioglycolic acid with 3-methyl-1-phenyl-1*H*- Pyrazol-4-carbaldehyde, depicted in Figure 2 (Chapter 3). Structures of all the synthesized compounds were characterized based on spectral data such as IR, ^{1}H NMR, ^{13}C NMR, and HR-MS. Antimicrobial activity studies were carried out at Inkosi Albert Luthuli Central Hospital, Durban, South Africa and Dr. Chunderika Mocktar of Discipline of Pharmaceutical Science, School of Health Science, UKZN. The tested compounds **k-2-e** and **k-2-j** exhibited MIC 0.4 µg/ml and 3.125 µg/ml respectively against *Escherichia coli* whereas **k-2-b** showed MIC 1.6 µg/ml against *Ps-Aeruginosa*. Whereas other compounds shown moderate potency for all strains. From the obtained results, it is proposed that pyrazole-1,3,4-thiadiazole hybrids linked via with 4-thiazolidinone can be effective lead compounds towards developing potent antimicrobial agents.

Based on the encouraging results obtained from chapter 3, we further evaluated the antimicrobial potential of pyrazolone derivatives by a powerful divergent approach to discover three distinct molecular architectures; Pyrazolone-triazole. Titled compounds (**k-3-1** to **k-3-24**) were synthesized by stirring5-methyl-2-(phenyl/*p*-tolyl)-4,4-di(prop-2-yn-1-yl)-2,4-Dihydro-3H-pyrazol-3-one (**PrBr 1a,b,c** and **PrBr 2a,b**) with benzyl azide, ascorbic acid and $CuSO_4 \cdot 5H_2O$ in methanol and water. Starting material (**PrBr 1a,b,c** and **PrBr 2a,b**) was prepared by stirring 5-methyl-2-(*p*-tolyl/phenyl)-2,4-Dihydro-3H-pyrazol-3-one with propargyl bromide in DMF; whereas substituted benzyl azide (**A-F**) were synthesised by stirring substituted benzyl bromide and sodium azide in acetone and water, depicted in **Figure 3** (Chapter 4). Structures of all the synthesized compounds were characterized based on spectral data such as IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Antimicrobial activity studies were carried out at Inkosi Albert Luthuli Central Hospital, Durban, South Africa and Dr. Chunderika Mocktar of Discipline of Pharmaceutical Sciences, School of Health Sciences, UKZN, antimicrobial investigation of these pyrazolone-triazole derivatives resulted in three lead molecules **k-3-4** (MIC 0.012 µg/ml), **k-3-9** (MIC 0.05 µg/ml), and **k-3-11** (MIC 0.025 µg/ml) with very high efficacy against *Klebsiella pneumoniae.* Further, all other final compounds displayed moderate to very high antibacterial activity. Displayed results revealed that pyrazolone-triazole are highly potent against gram-negative bacteria. The promising antibacterial findings encouraged us to further optimize the pyrazole derivatives to yield potent antimicrobial agents.

In the chapter 5, various pyrazolone-oximes (**k-4-1** to **k-4-14**) were synthesized and evaluated for their antimicrobial properties. The pyrazolone-oximes were synthesized by refluxing of 5-(ethoxy/methoxy/isopropoxy)-3-methyl-1-(phenyl/p-tolyl/2-chloro-phenyl/3-chloro-phenyl)-1*H*-pyrazole-4-carbaldehyde **(k-4-1a** to **k-4-14a)** with $NH_2OH{\cdot}HCl$ and Cs_2CO_3 in DMSO-H_2O. Starting material, namely 5-chloro-1-(phenyl /p-tolyl/2-chloro-phenyl/3-chloro-phenyl)-3-methyl-1*H*-pyrazole-4-carbaldehyde (**1b**, **5b**, **9b**, **12b**) was prepared by formylating 3-methyl-1-(phenyl/*p*-tolyl/2-chloro-phenyl/ 3-chloro-phenyl)-1*H*-pyrazol-5(4*H*)-one **(1c, 5c, 9c, 12c).** Whereas, 5-(ethoxy/methoxy/isopropoxy)-3-methyl-1-(phenyl/*p*-tolyl/2-chloro-phenyl/3-chloro-phenyl)-1*H*-pyrazole-4-carbaldehyde **(k-4-1a** to **k-4-14a)** were synthesized by refluxing 5-chloro-1-(*p*-tolyl/2-chloro-phenyl/3-chloro-phenyl)-3-methyl-1*H*-pyrazole-4-carbaldehyde **(1b**, **5b**, **9b**, **12b)** in methanol/ethanol/2-propanol/*n*-butanol with cesium carbonate and copper acetate, depicted in Figure 2 (chapter 5). Structures of all the synthesized compounds were characterized based on spectral data such as IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Antimicrobial activity studies were carried out at Inkosi Albert Luthuli Central Hospital, Durban, South Africa and Dr. Chunderika Mocktar of Discipline of Pharmaceutical Sciences, School of Health Sciences, UKZN. Evaluation of antibacterial activity showed that almost all the compounds exhibited potent activity, compound **k-4-2**, **k-4-11** and **k-4-14** showed excellent potency with MIC 0.012 µg/ml against *Escherichia coli*, and *Klebsiella pneumoniae* and thus it could be proved promising novel drug.

After observing antimicrobial potential of pyrazolone-oxime we further developed new series of pyrazolone-chalcone. Chalcone derivatives were synthesized by stirring starting material 5-(chloro/ethoxy)-3-methyl-1-(*p*-tolyl/3-chloro-phenyl)-1*H*-pyrazole-4-carbaldehyde **(A-D)** in ethanol with substituted acetophenones. Starting material, namely 5-chloro-1- (*p*-tolyl/3-chloro-phenyl)-3-methyl-1*H*-pyrazole-4-carbaldehyde **(A, B)** was prepared by formylating 3-methyl-1-(*p*-tolyl/3-chloro-phenyl)-1*H*-pyrazol-5(4*H*)-one **(I, II).** Whereas, 5-(ethoxy)-3-methyl-1-(*p*-tolyl/3-chloro-phenyl)-1*H*-pyrazole-4-carbaldehyde (**C** and **D**) were synthesized by refluxing 5-chloro-1-(*p*-tolyl/ 3-chloro-phenyl)-3-methyl-1*H*-pyrazole-4-carbaldehyde **(A, B)** in ethanol depicted in Figure 2 (Chapter 6). Structures of all the synthesized compounds were characterized based on spectral data such as IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Antimicrobial activity studies were carried out at Inkosi Albert Luthuli Central Hospital, Durban, South Africa and Dr. Chunderika Mocktar of Discipline of Pharmaceutical Sciences, School of Health Sciences, UKZN. Evaluation of antibacterial activity showed that almost all the compounds exhibited excellent potency. Compound **k-5-3** (MIC 0.012 µg/ml for *Pseudomonas aeruginosa* and 0.025 µg/ml for *Klebsiella pneumonia)*, **k-5-4 (**MIC 0.2 µg/ml for *Pseudomonas aeruginosa*) and **k-5-**

5 (MIC 0.05 µg/ml for *Klebsiella pneumonia* 0.2 µg/ml Pseudomonas *aeruginosa*, and *Escherichia coli*) found to be highly potent.

Hence, overall study suggested the medicinal/pharmaceutical importance of hybridized pyrazolone derivatives in the discovery of potent antimicrobial, particularly antibacterial agents.

2. Future works

In this research work, all the series of compounds have resulted in encouraging results, the generated antimicrobial activity data can be effectively used in developing further lead compounds through three dimensional quantitative structure activity relationship (3D-QSAR) models. 3D-QSAR is one of the promising *in silico* methods in the identification of lead molecules and widely employed in pharmaceutical companies. It also assists in the optimization of the 3D properties of a lead molecule in the new drug discovery programme to exert better pharmacological activity profiles. In-house ligands will be screened against the validated 3D-QSAR model to predict the IC_{50} values of un-synthesized compounds. Ligands with potent predicted IC_{50} values will be considered as leads. Thus, the generated potential leads can further be optimized structurally to improve the pharmaceutical properties such as solubility, cell permeability and the efficiency profiles through the development of 3D Quantitative Structure-Property Relationship (3D-QSPR) models.

The lead compounds identified by the above mentioned computer-aided ligand based drug design techniques will be synthesized and assessed for their antimicrobial properties. In order to deduce the binding mode and crucial interactions of the ligands with the target proteins (enzymes/receptor), molecular docking (structure-based drug design) studies will be performed. For this task, suitable protein targets will be searched in protein data bank (PDB). Moreover, in depth mechanistic studies will be conducted to deduce mode of action of compounds with biological systems.

From the results obtained from the pyrazolone derivatives, it is clear that all the pyrazolone-inspired novel hybrids displayed a range of excellent to good antibacterial activity. These motivate us to optimize further by hybridizing the novel pyrazolone with antibacterial moieties extensively. To carry out this proposed task, ligand (3D-QSAR and pharmacophore modelling) and structure-based drug design techniques (molecular docking and molecular dynamics) will be employed.

Thus, the research work will be extended immensely through an effective computer-aided drug design approaches and contribute extensively to the advancement in the research area of developing novel class of pyrazolone-based antimicrobial compounds.

Appendix-1

1.1 CHAPTER 3

Title: Synthesis of Novel Pyrazole-1,3,4-Thiadiazole Hybrids Linked by 4-Thiazolidinone as a Antimicrobial Agent

Authors name: Kavita Jain[a], Nisar Syyad and Rajshekhar Karpoormath[a,*]

Affiliations: [a] *Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa.*

To receive all correspondence: Rajshekhar Karpoormath: Tel: +27 (0) 312607179, *E-mail:* karpoormath@ukzn.ac.za

Supplementary data:

Spectral Information:

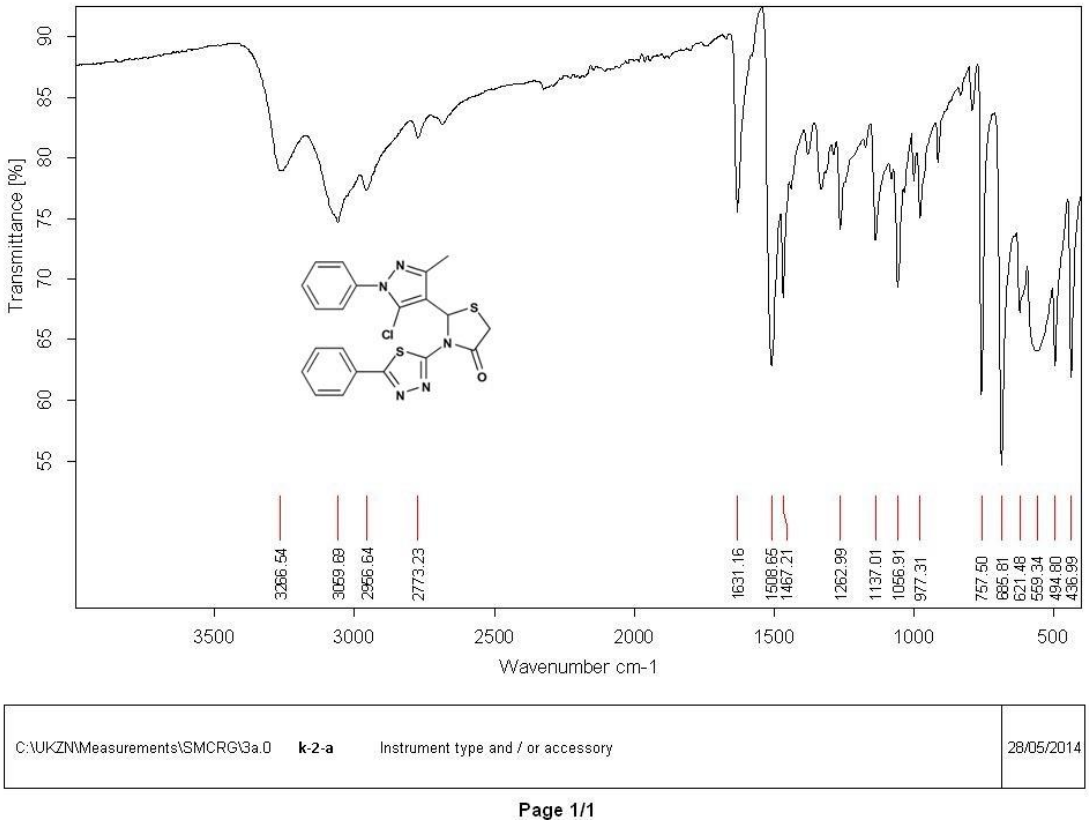


Figure 1. IR spectrum of compound k-2-a

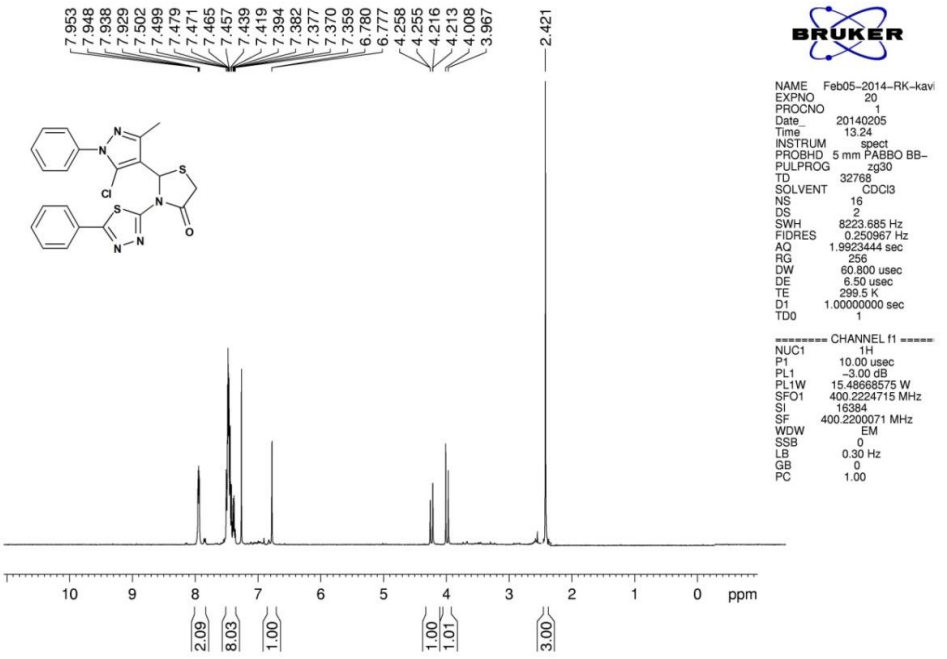


Figure 2. ^{1}H NMR of compound k-2-a

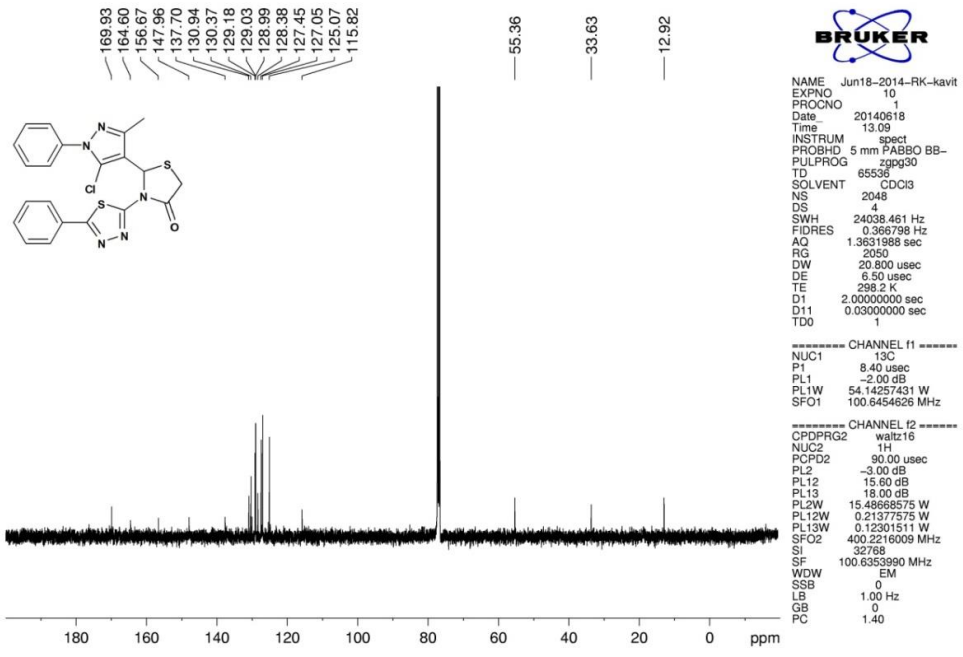


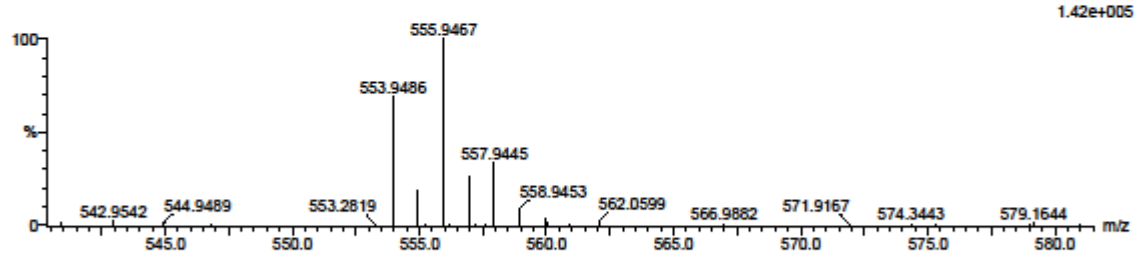
figure 3. ^{13}C NMR of compound k-2-a

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

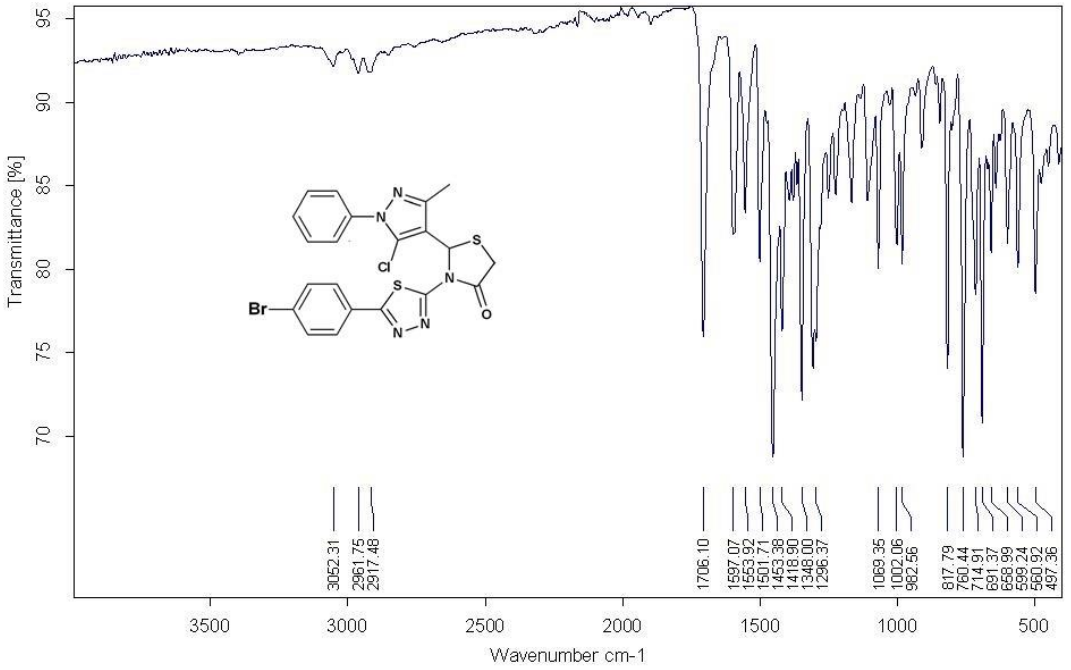
Monoisotopic Mass, Even Electron Ions
473 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-4 Na: 0-1 S: 0-2 Cl: 0-1 Br: 0-1

Kav-13b 5 (0.135) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
553.9486	553.9488	-0.2	-0.4	15.5	22.6	0.0	C21 H15 N5 O Na S2 Cl Br

Figure 4 . HRMS of compound k-2-b



C:\UKZN\Measurements\SMCRG\3b.0 k-2-b Instrument type and / or accessory 28/05/2014

Page 1/1

Figure 5. IR spectrum of compound k-2-b

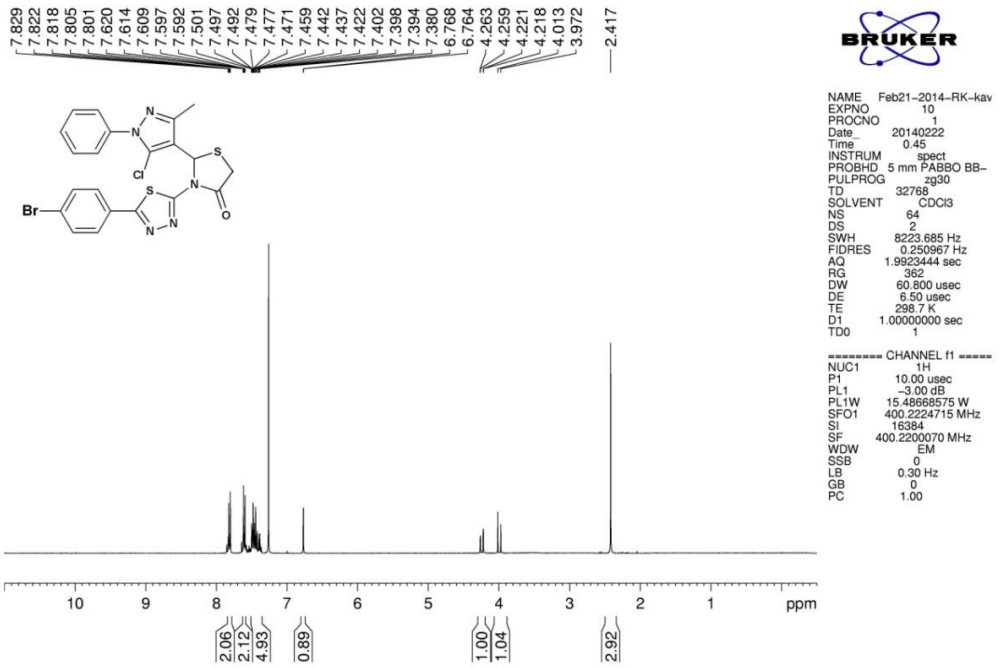


Figure 6. ^{1}H NMR of compound k-2-b

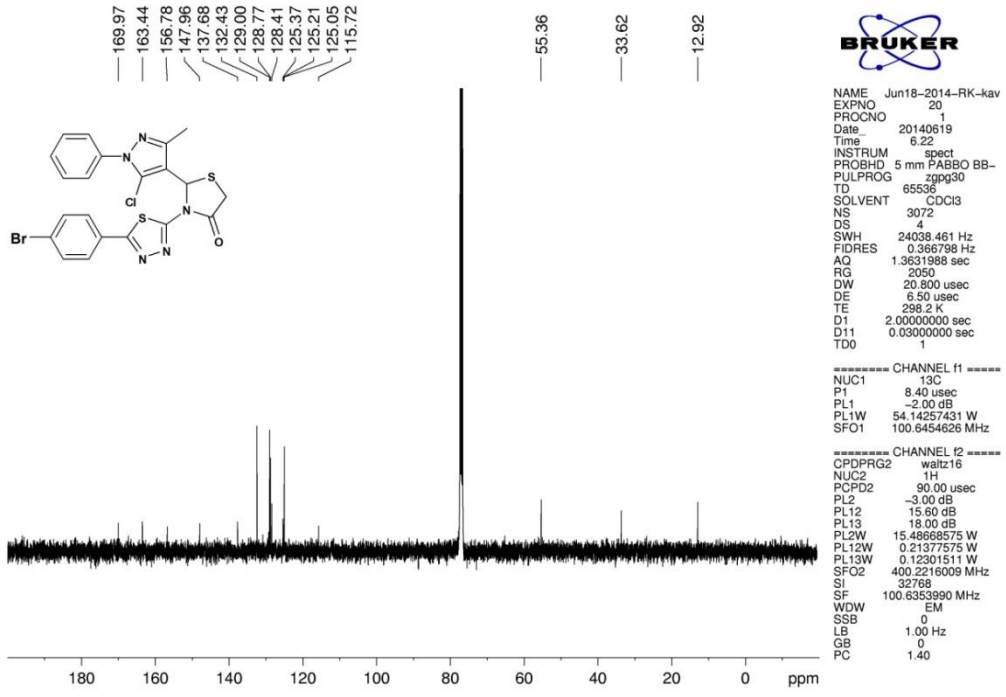


Figure 7. ^{13}C NMR of compound k-2-b

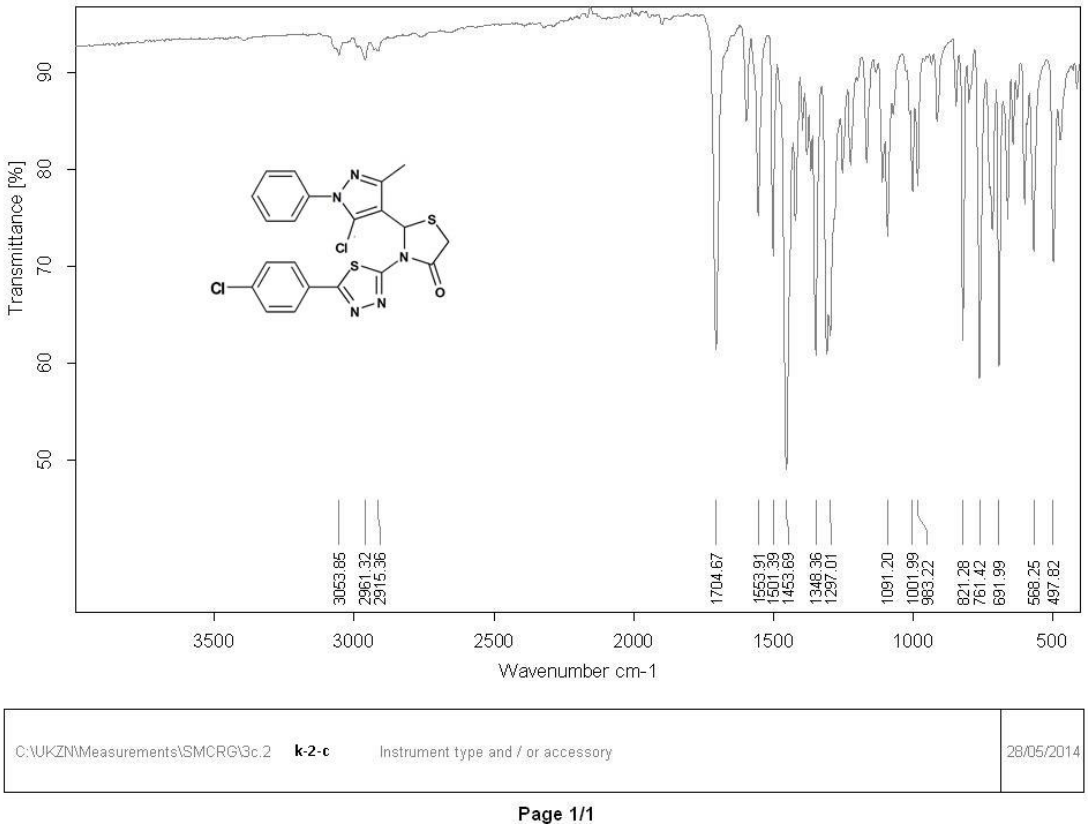


Figure 8. IR spectrum of compound k-2-c

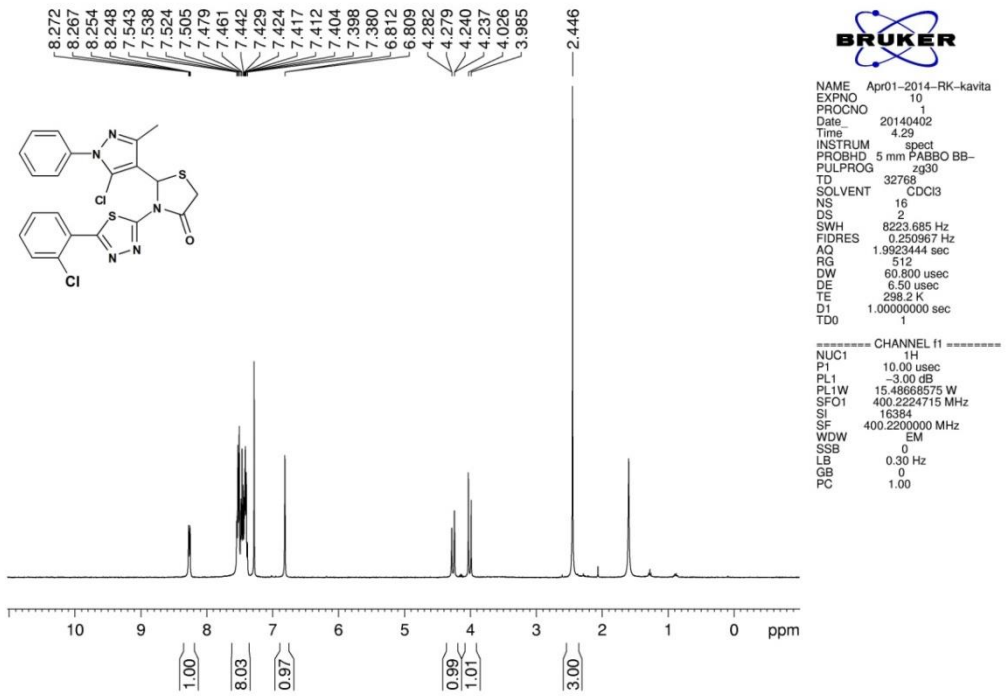


Figure 9.[1]H NMR of compound k-2-c

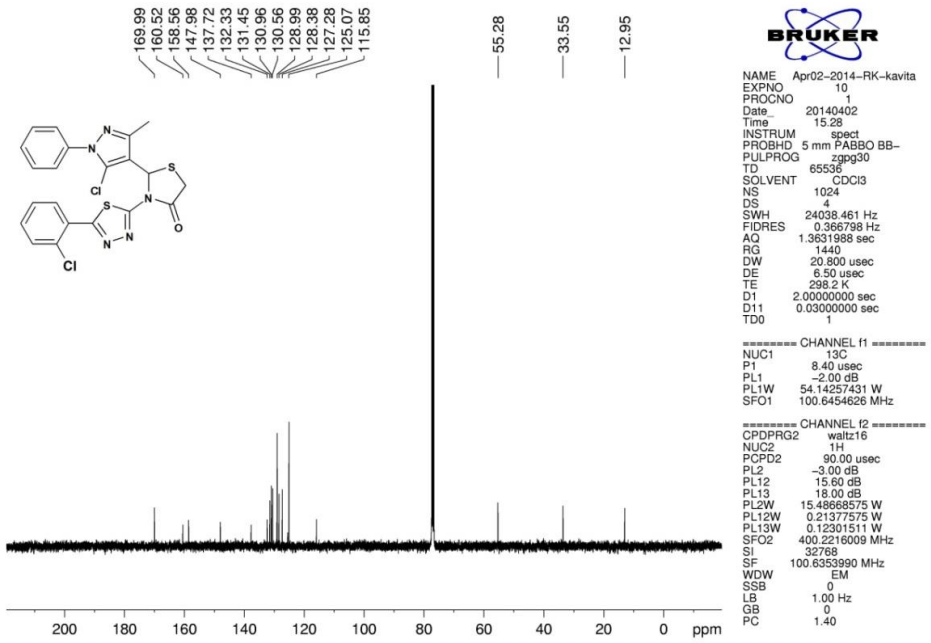


Figure 10. ^{13}C NMR of compound k-2-c

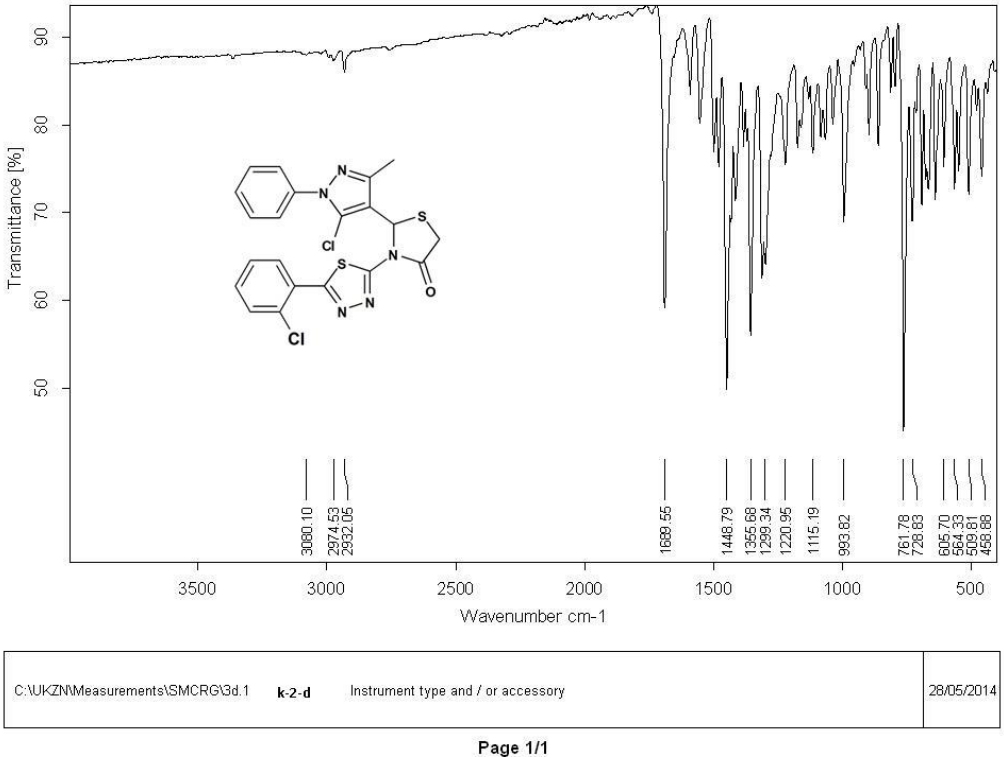


Figure 11. IR spectrum of compound k-2-d

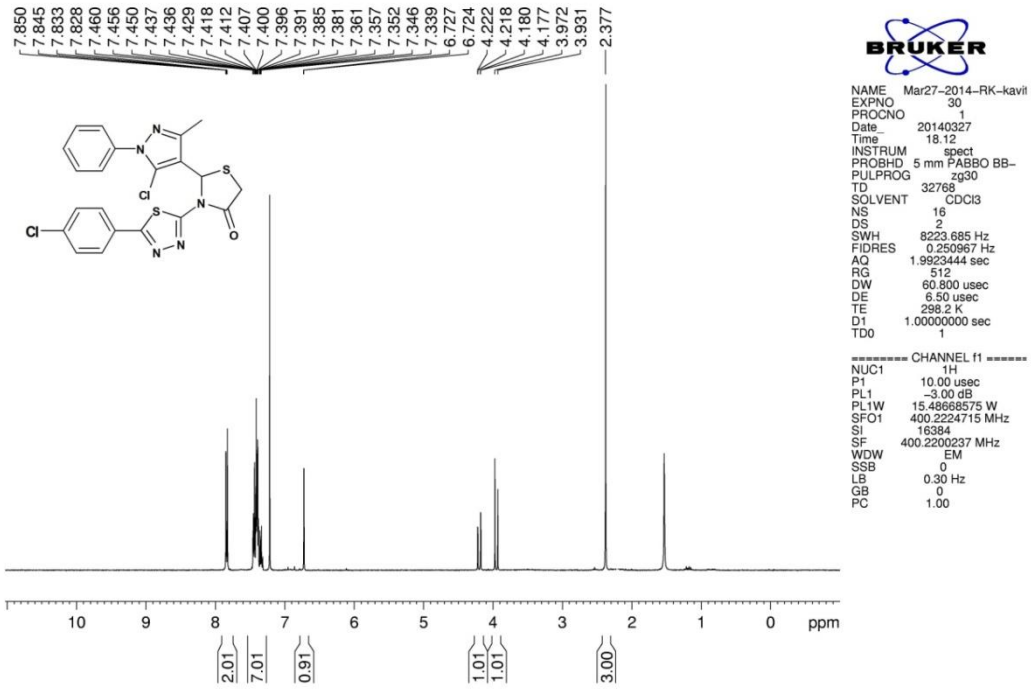


Figure 12. ^{1}H NMR of compound k-2-d

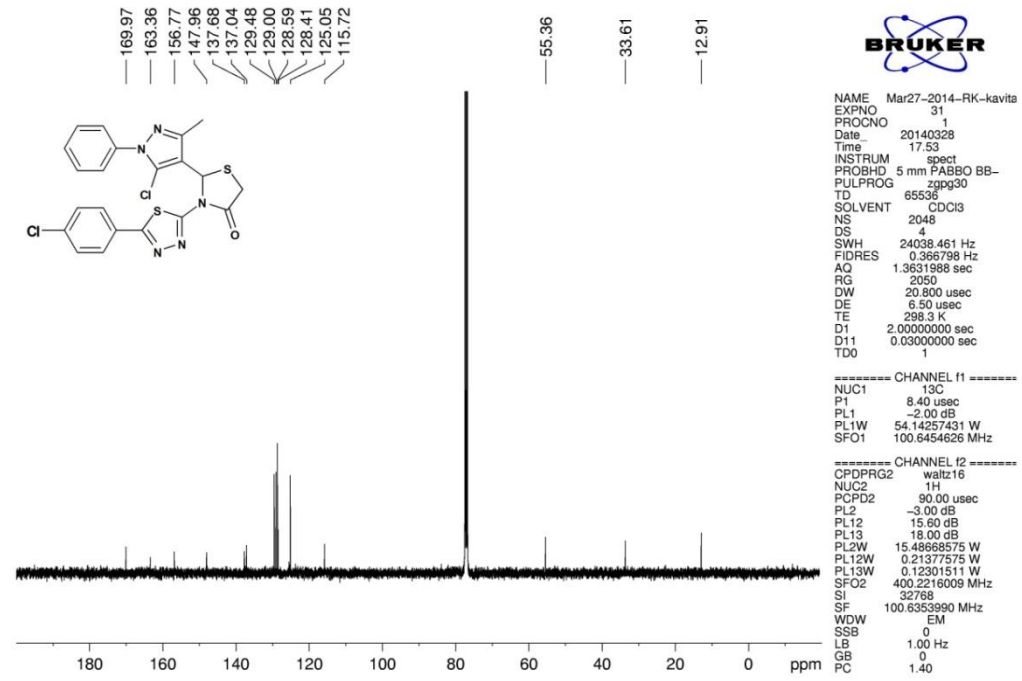


Figure 13. ^{13}C NMR of compound k-2-d

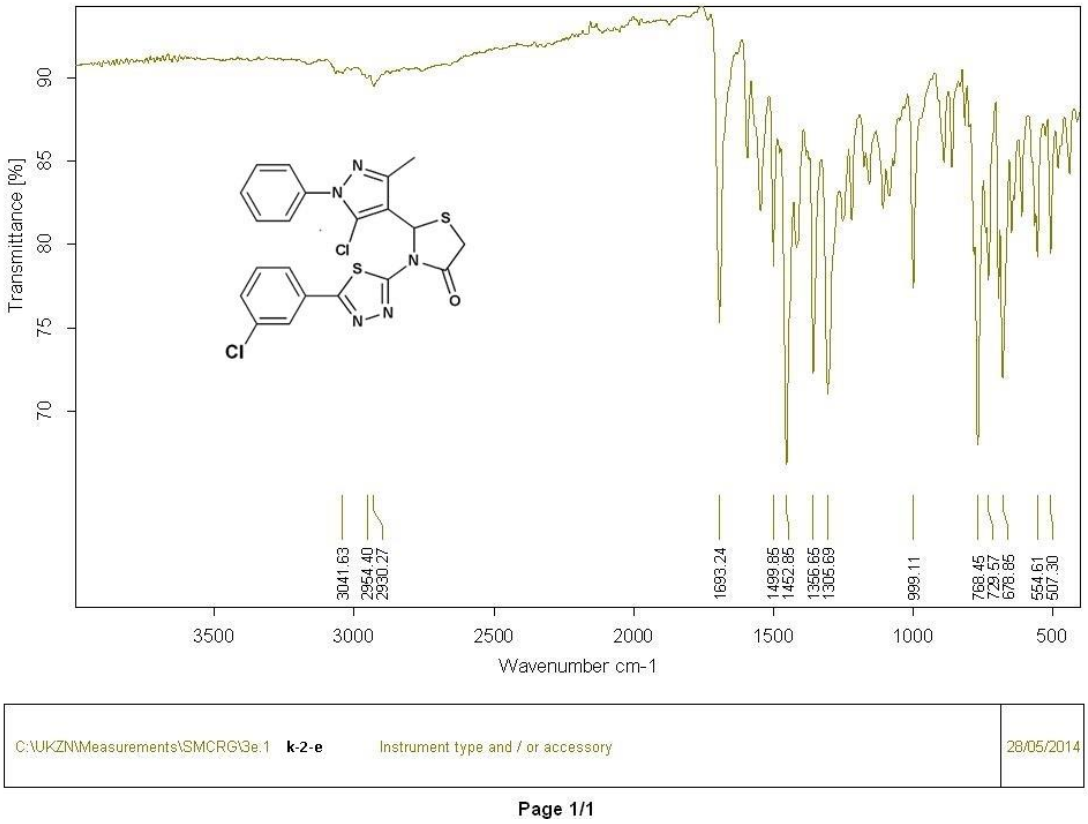


Figure 14. IR spectrum of compound k-2-e

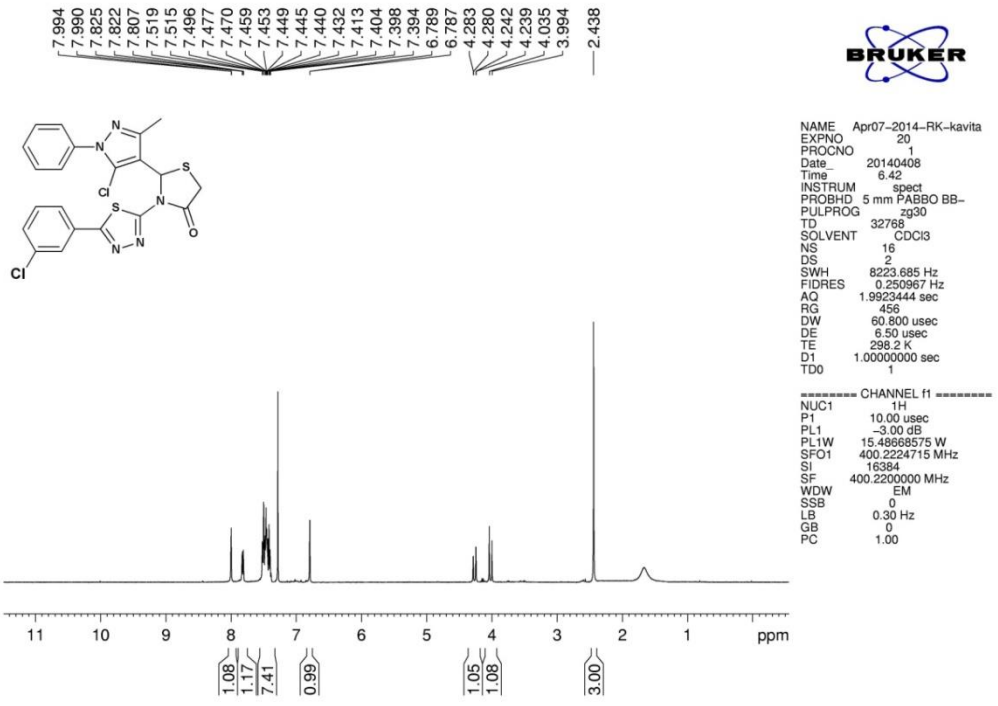


Figure 15. ^{1}H NMR of compound k-2-e

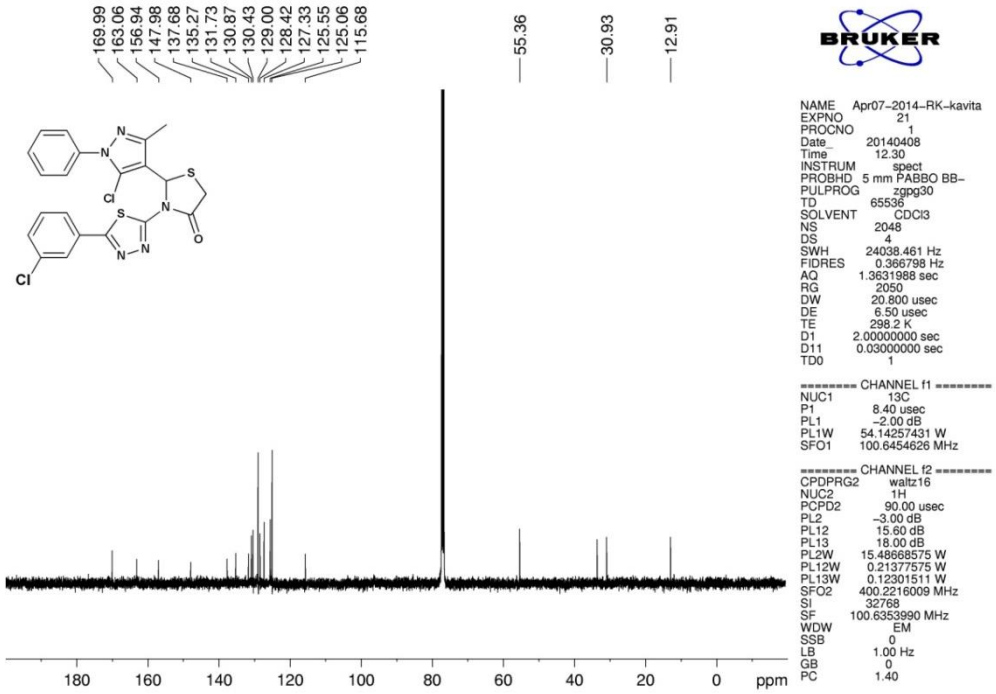


Figure 16. ^{13}C NMR of compound k-2-e

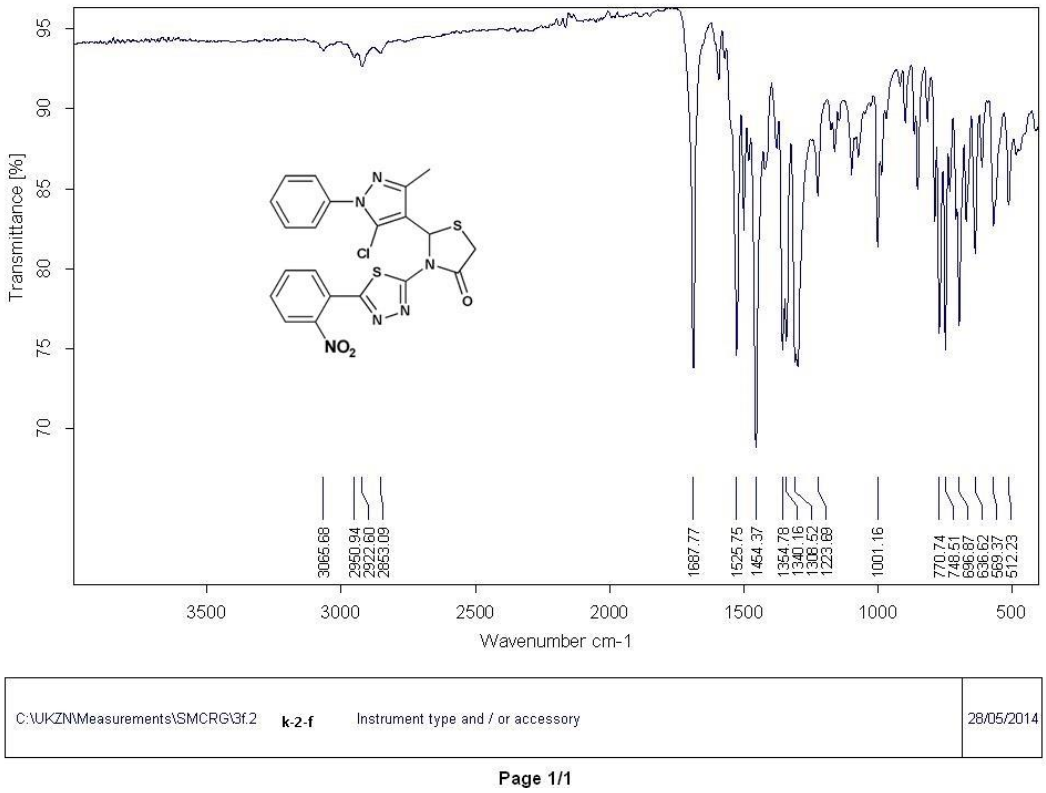


Figure 17. IR spectrum of compound k-2-f

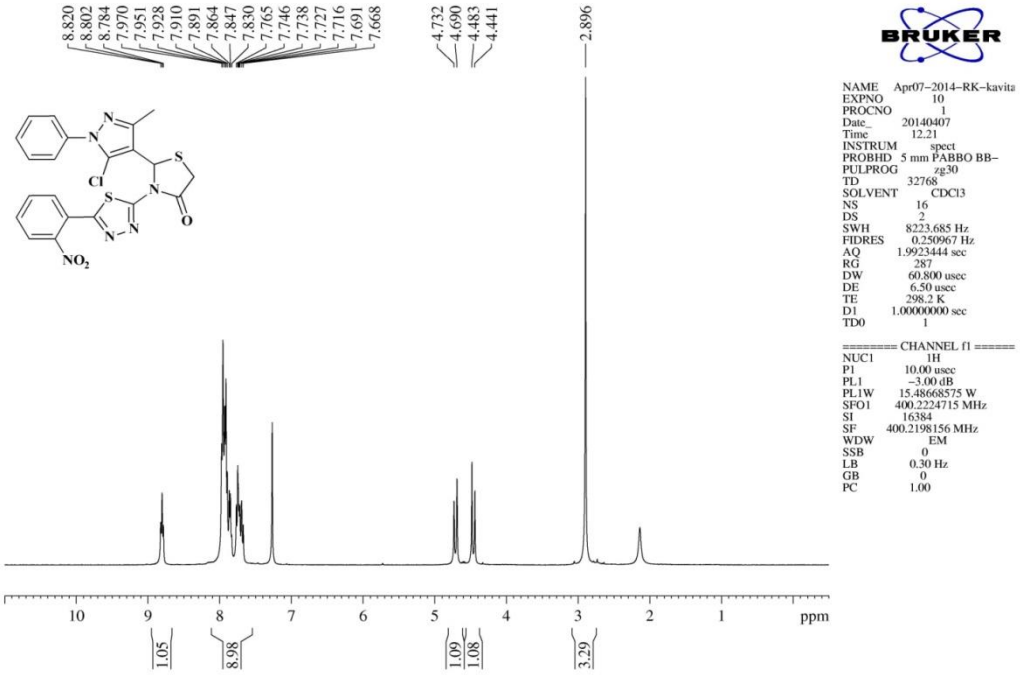


Figure 18. ^{1}H NMR of compound k-2-f

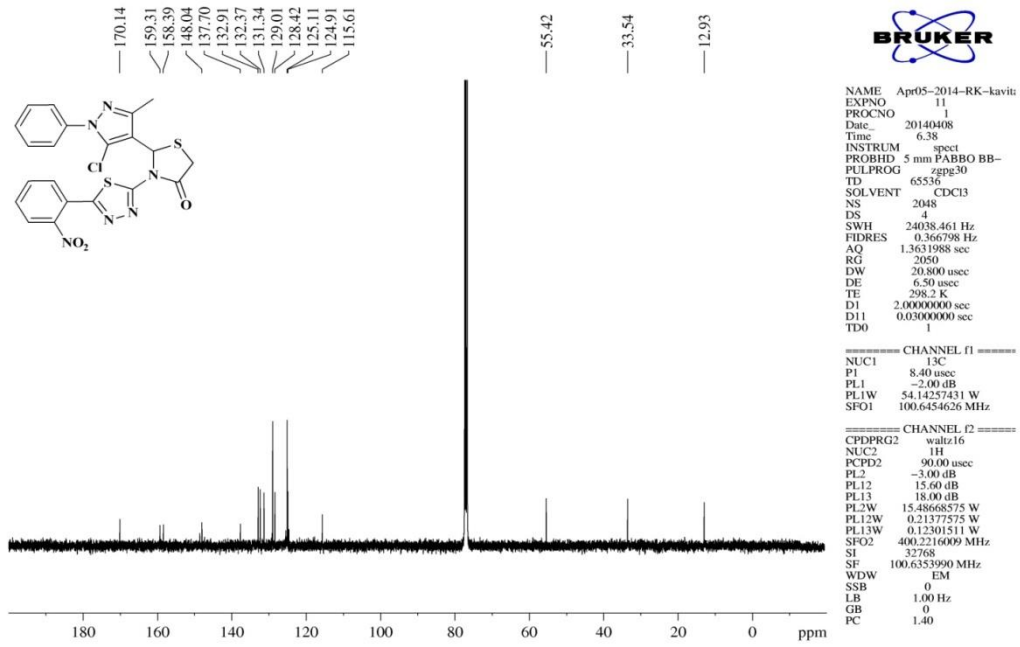


Figure 19. ^{13}C NMR of compound k-2-f

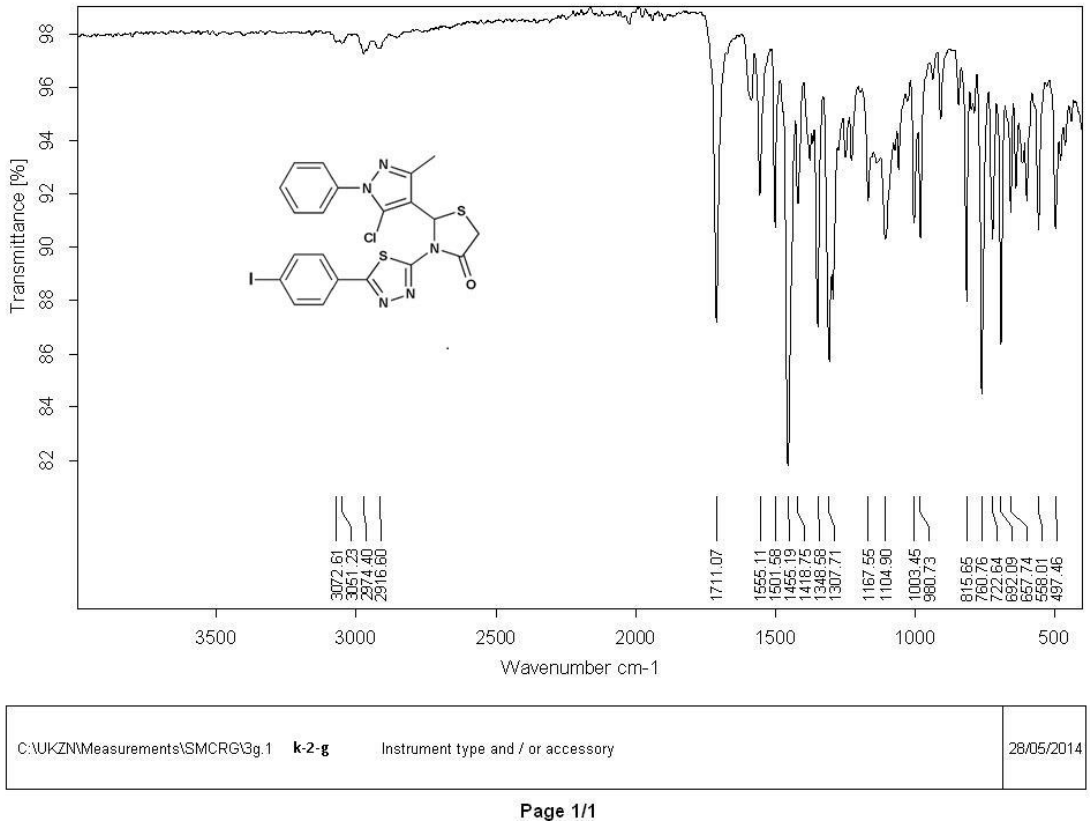


Figure 20. IR spectrum of compound k-2-g

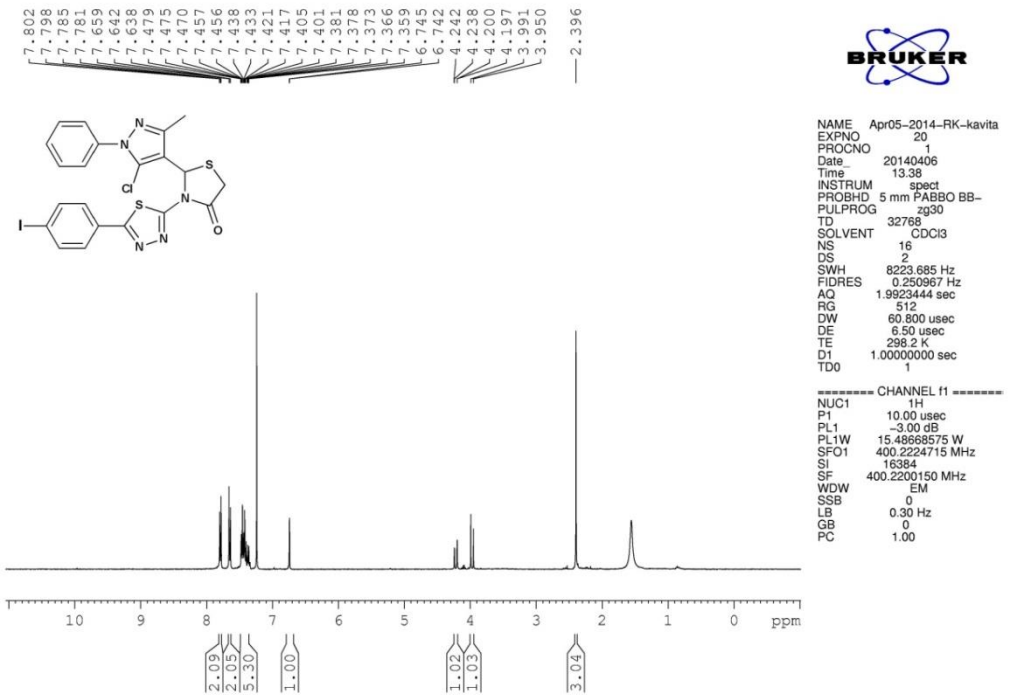


Figure 21.[1]H NMR of compound k-2-g

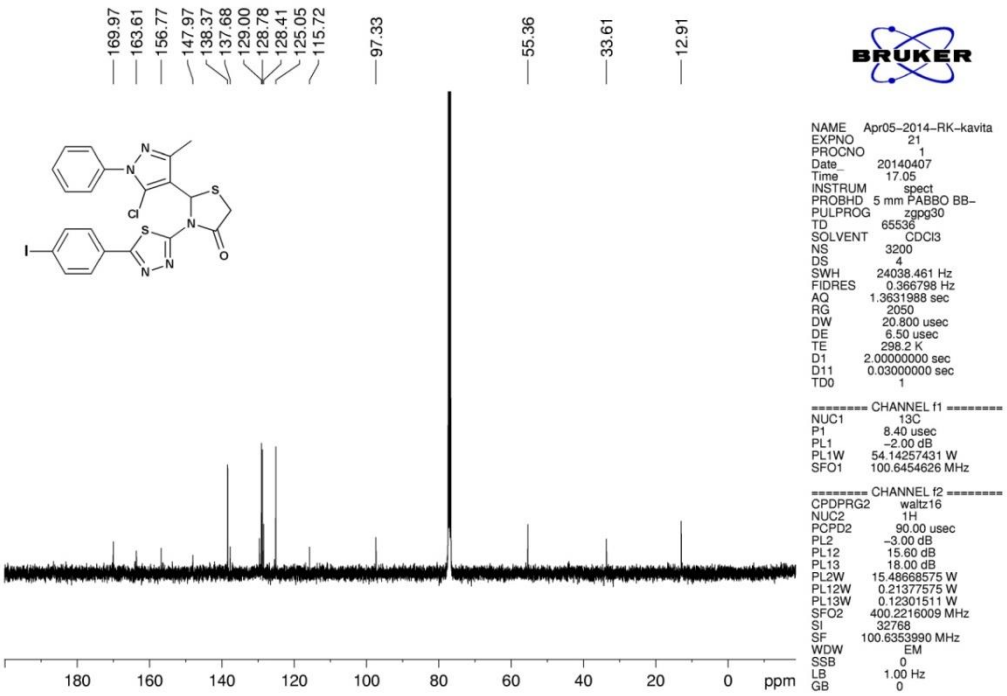


Figure 22. ^{13}C NMR of compound k-2-g

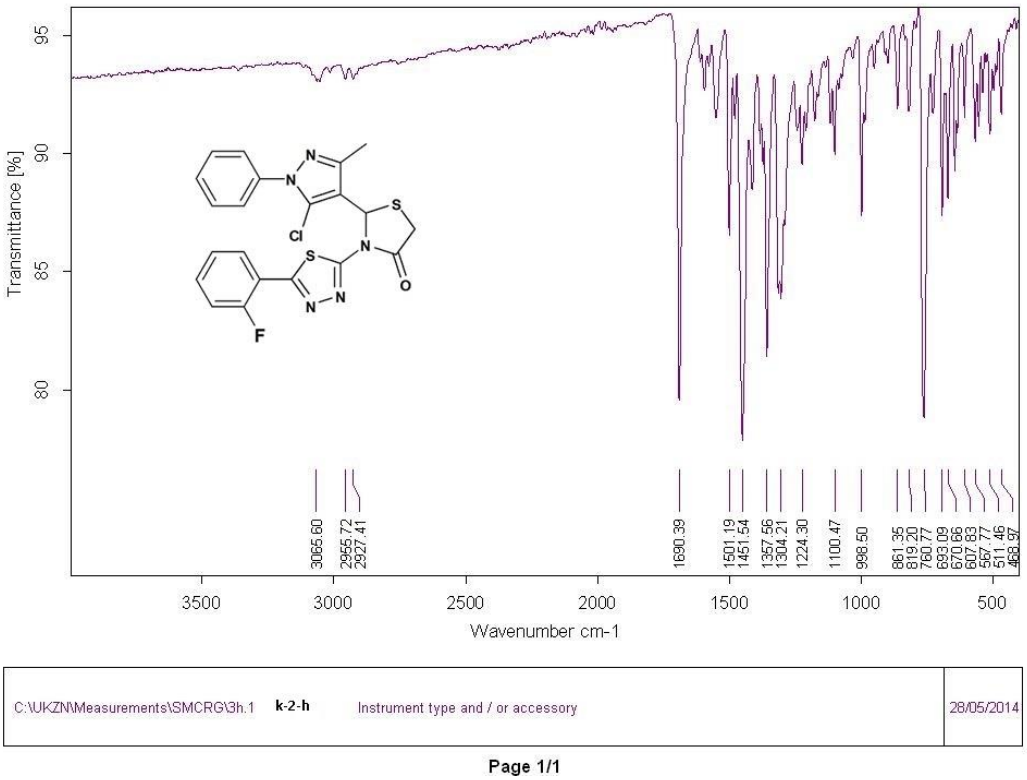


Figure 23. IR spectrum of compound k-2-h

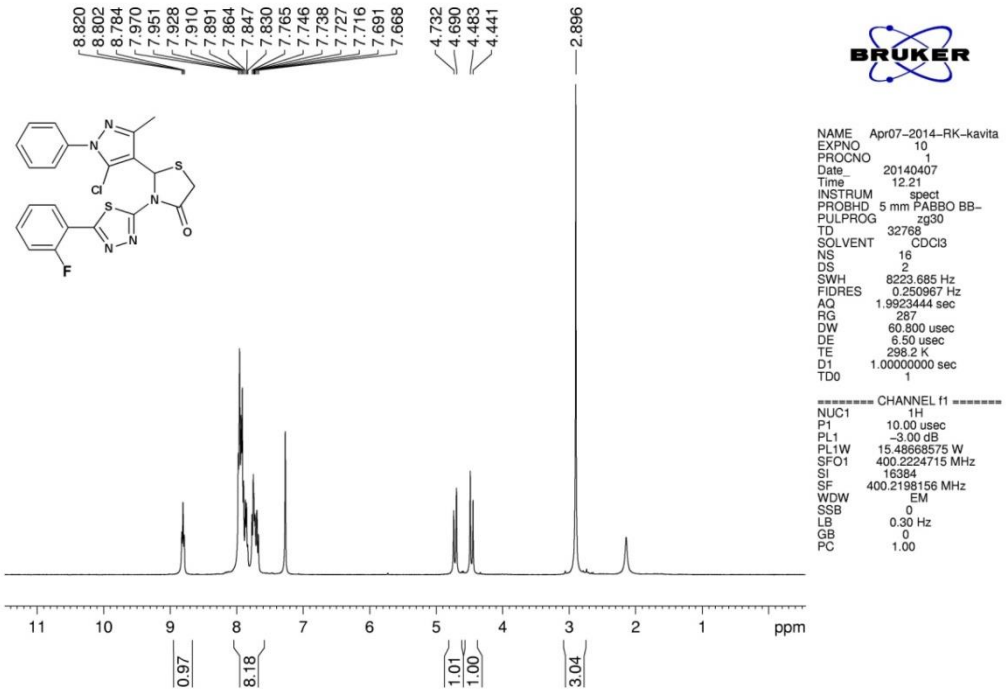


Figure 24. ^{1}H NMR of compound k-2-h

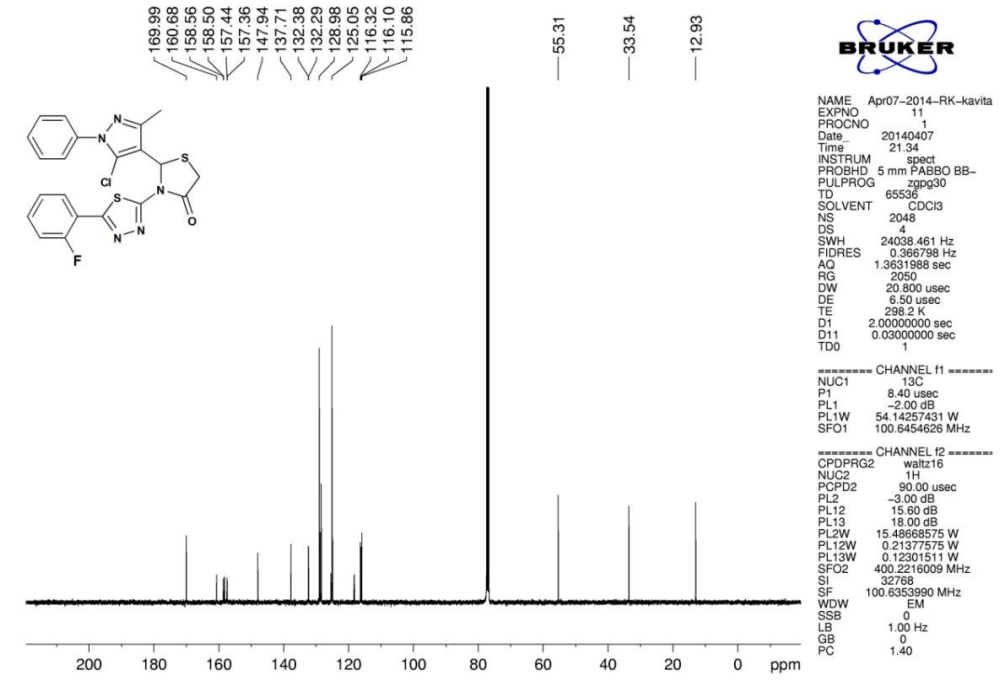
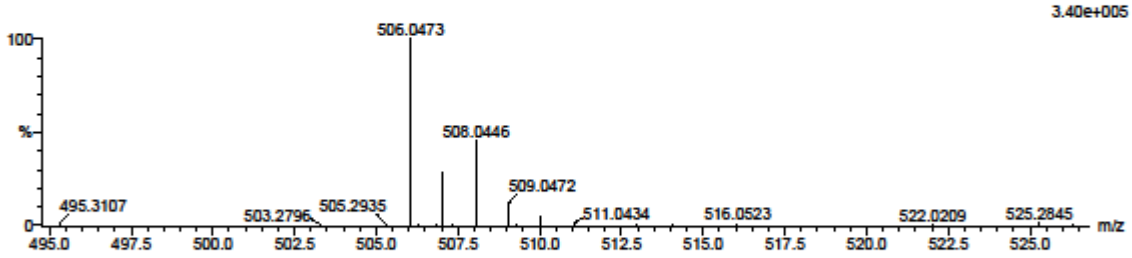


Figure 25. ^{13}C NMR of compound k-2-h

Elemental Composition Report **Page 1**

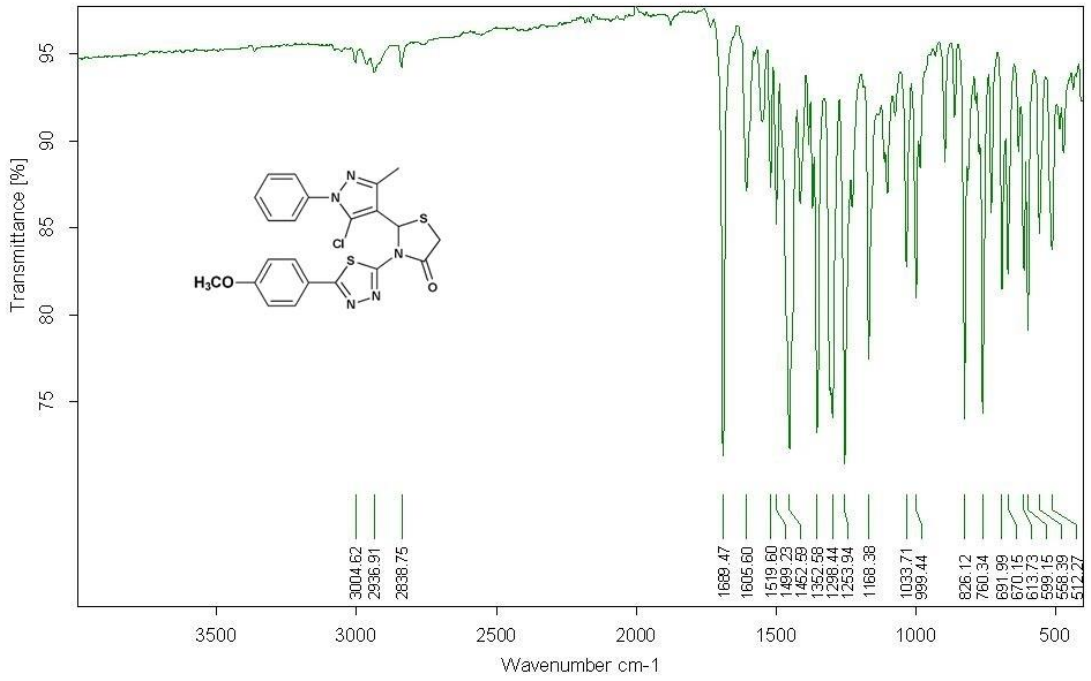
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
216 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-4 Na: 0-1 S: 0-2 Cl: 0-1
Kav-13l 42 (1.384) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
506.0473	506.0488	-1.5	-3.0	15.5	56.9	0.0	C22 H18 N5 O2 Na S2 Cl

Figure 26. HRMS of compound k-2-i



C:\UKZN\Measurements\SMCRG\3i.2 k-2-i Instrument type and / or accessory 28/05/2014

Page 1/1

Figure 27. IR spectrum of compound k-2-i

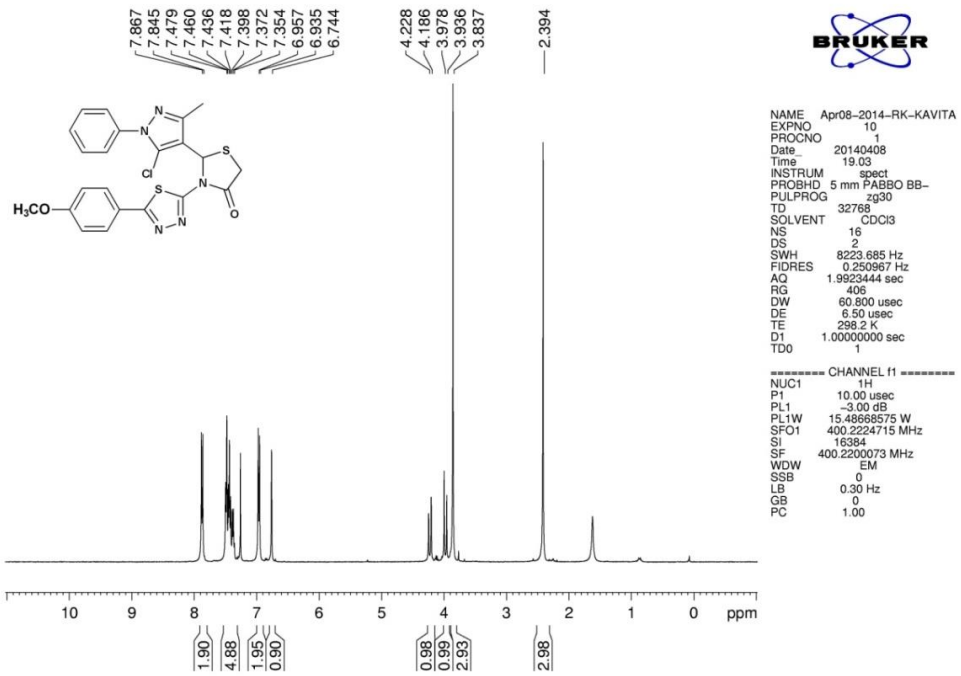


Figure 28. ^{1}H NMR of compound k-2-i

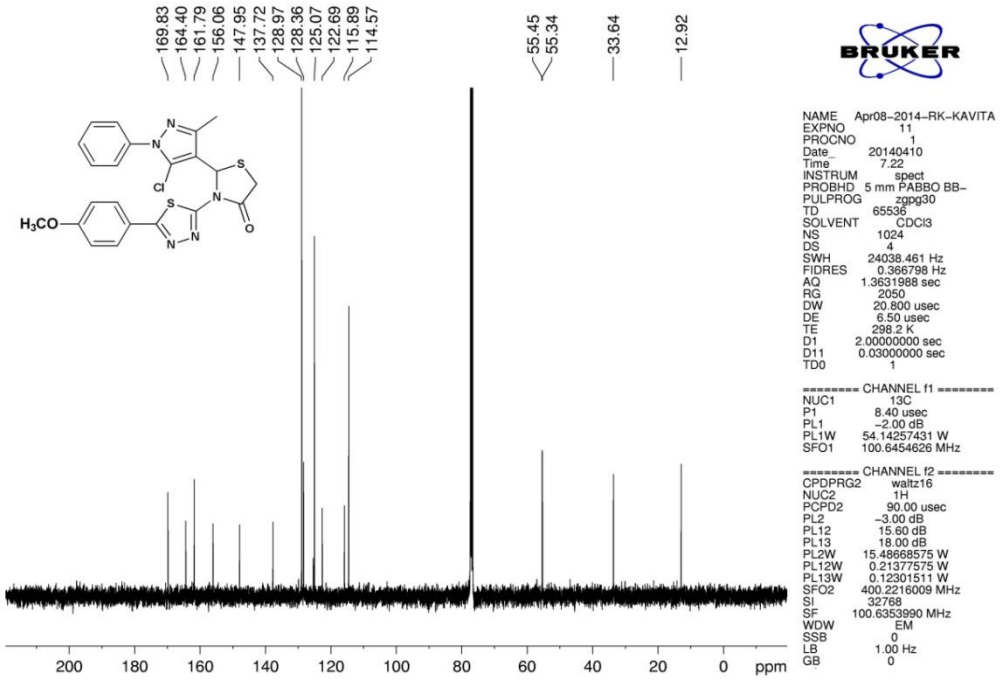


Figure 29. ^{13}C NMR of compound k-2-i

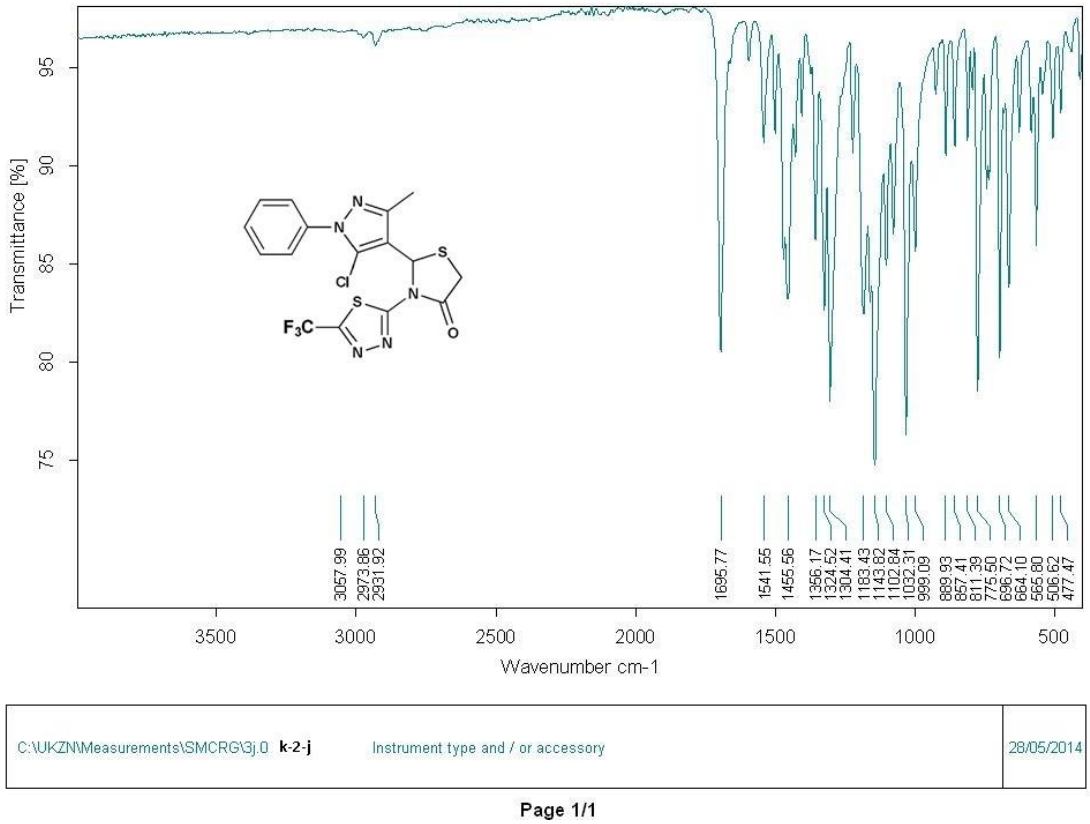


Figure 30. IR spectrum of compound k-2-j

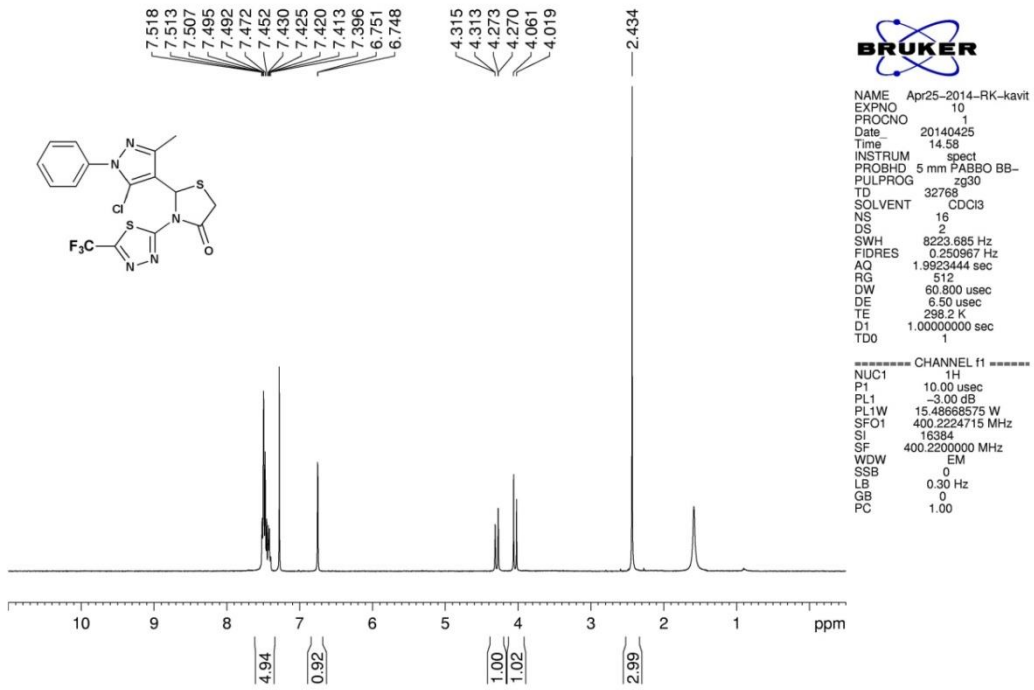


Figure 31. H NMR of compound k-2-j

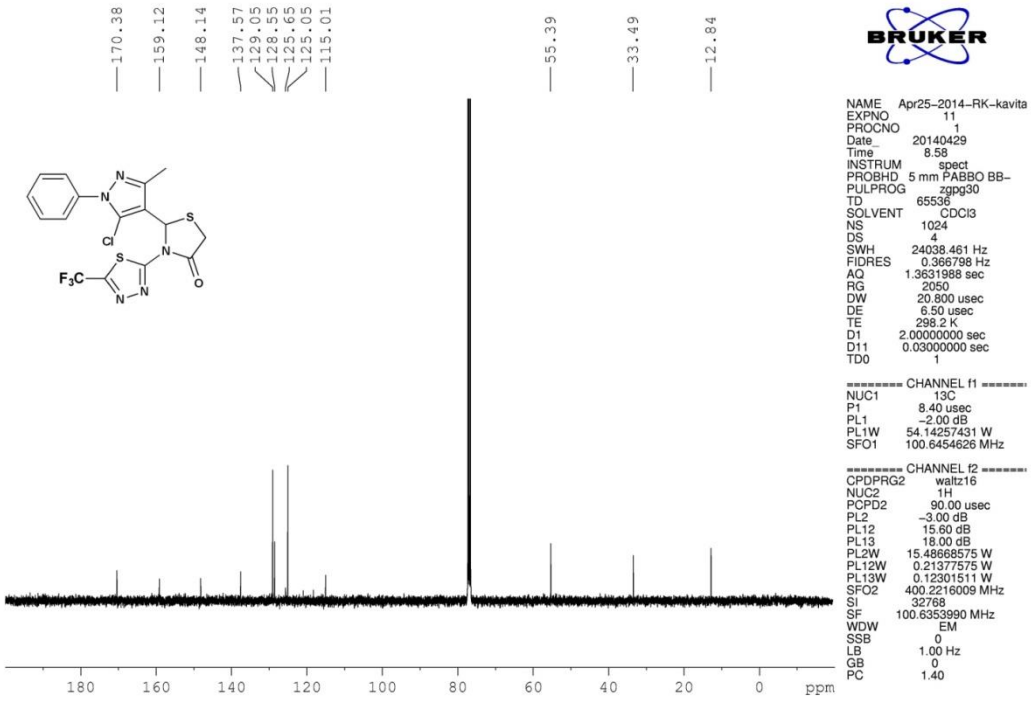


Figure 32. ^{13}C NMR of compound k-2-j

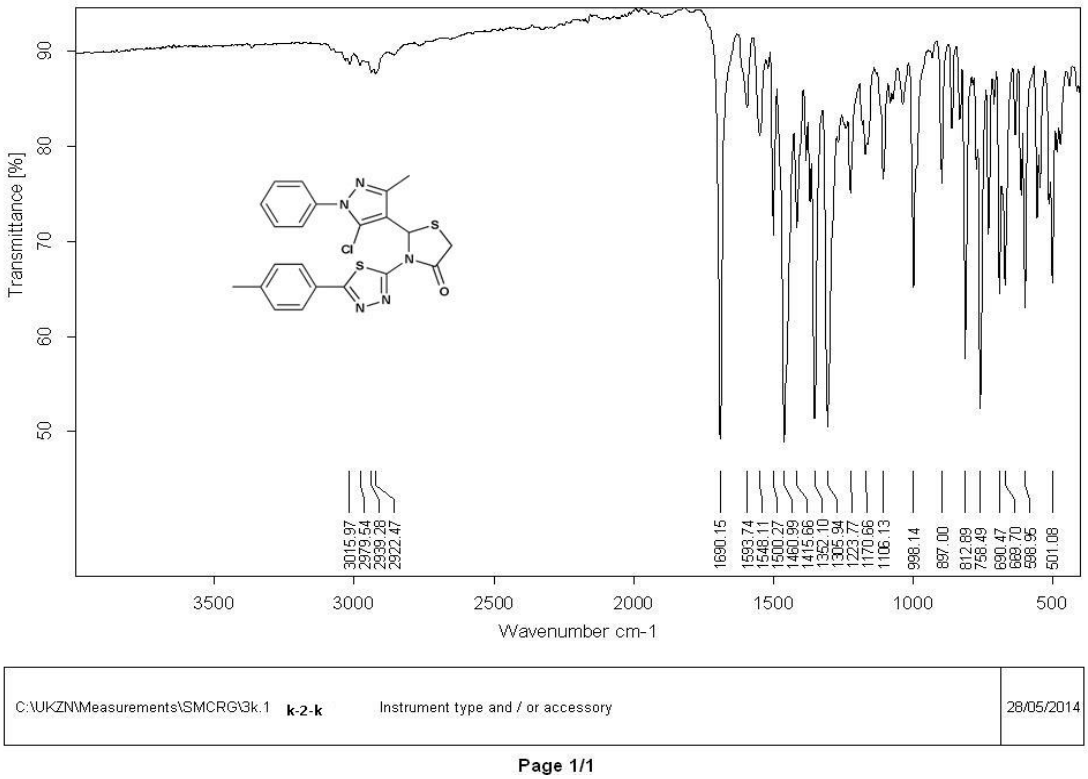


Figure 33. IR spectrum of compound k-2-k

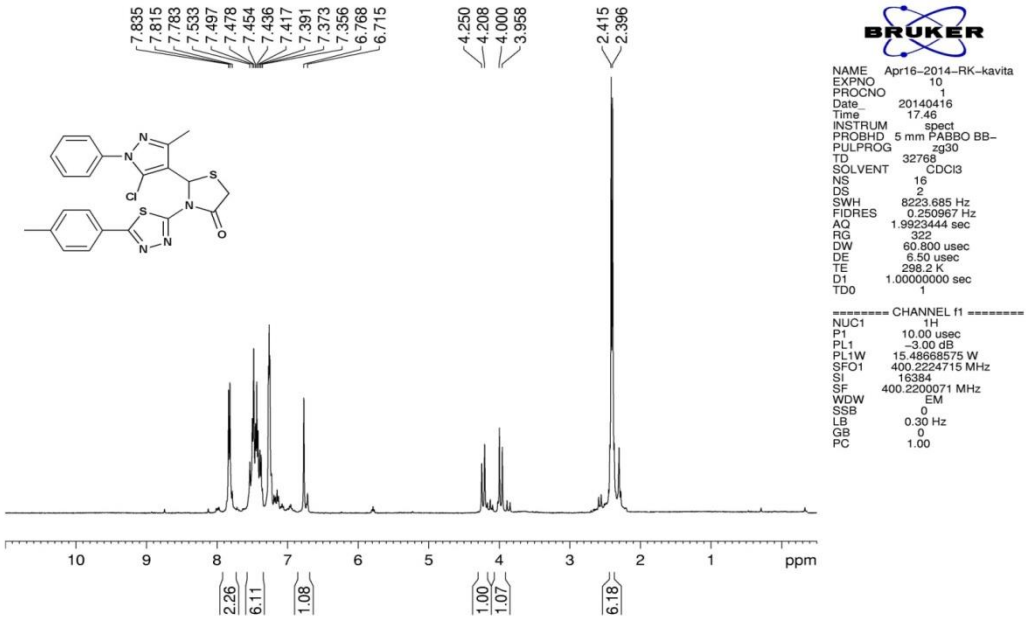


Figure 34. ^{1}H NMR of compound k-2-k

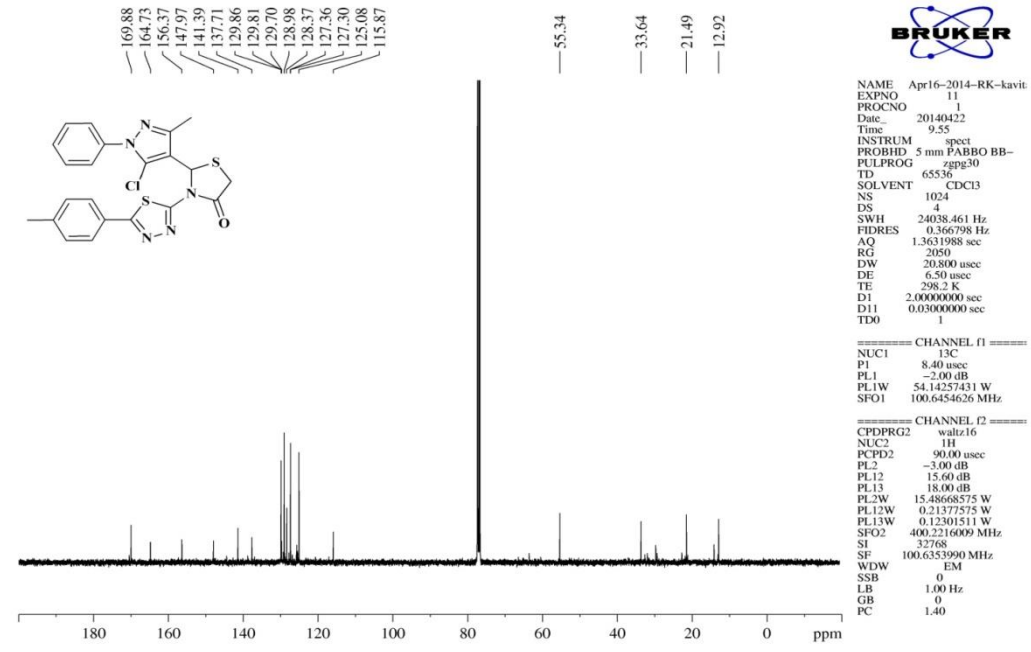


Figure 35. ^{13}C NMR of compound k-2-k

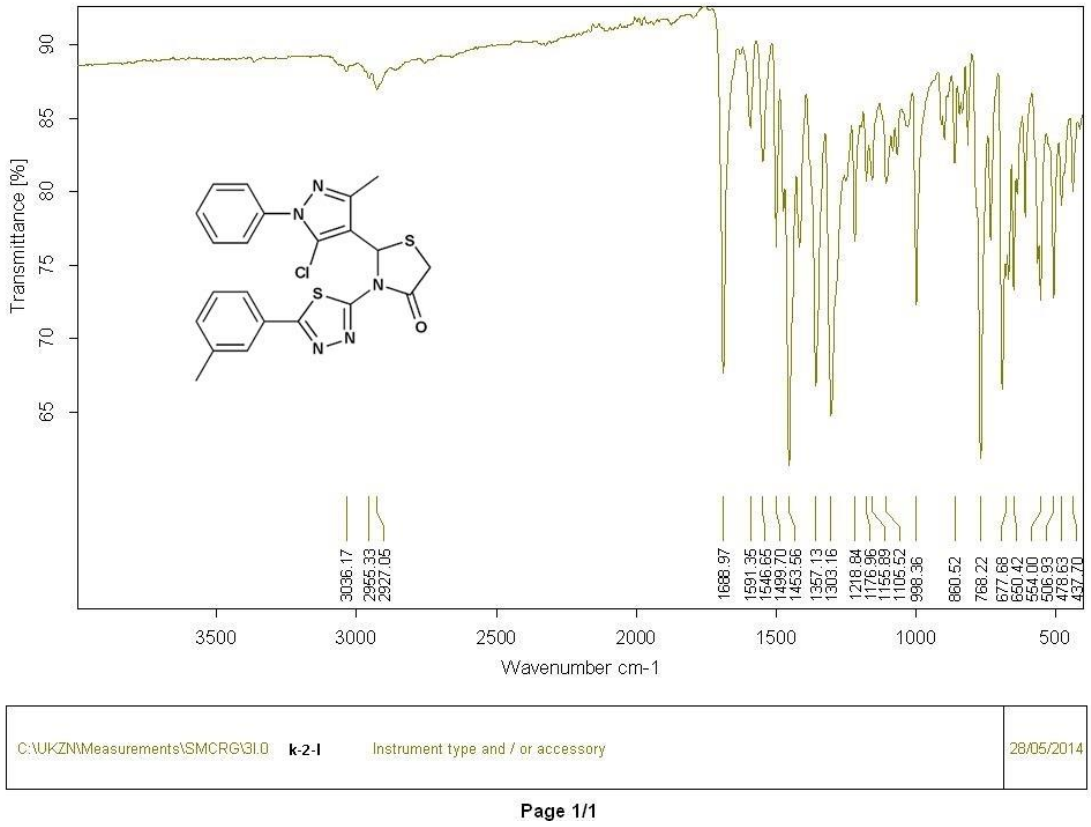


Figure 36. IR spectrum of compound k-2-l

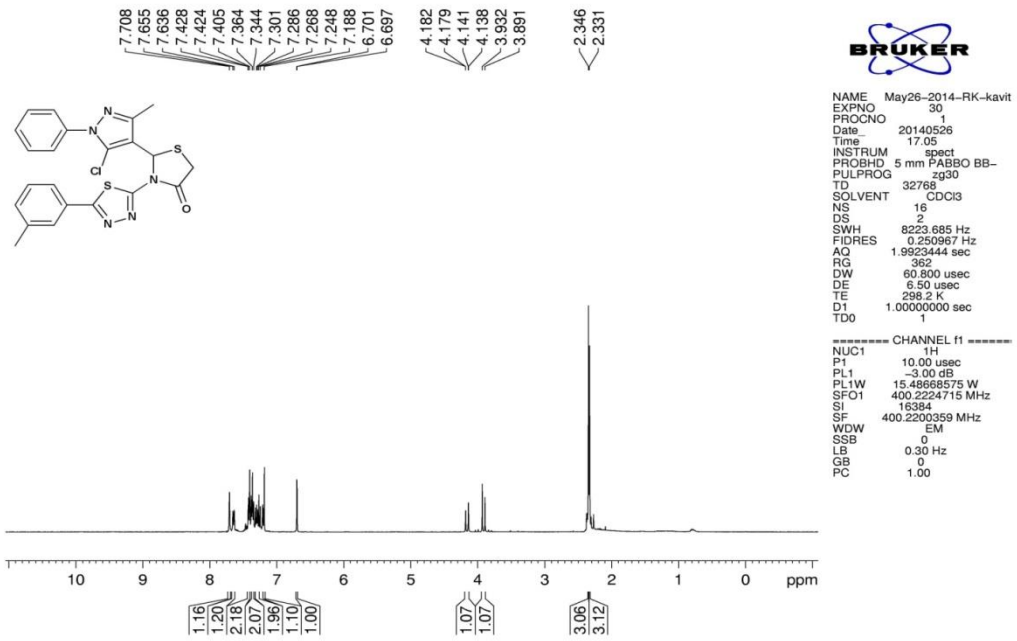


Figure 37. ^{1}H NMR of compound k-2-l

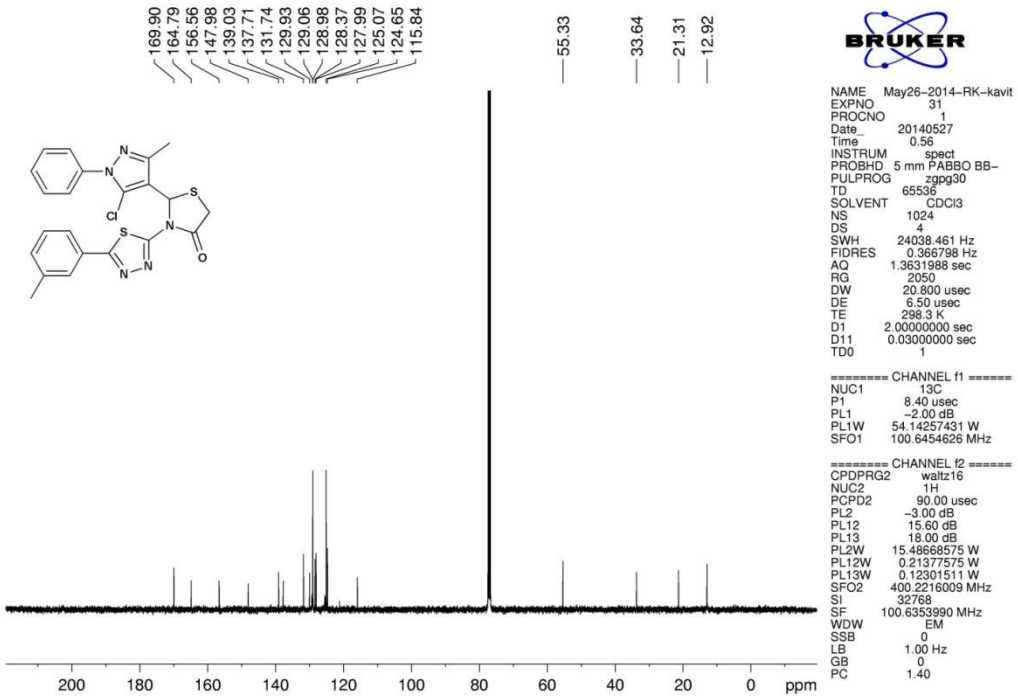


Figure 38. ^{13}C NMR of compound k-2-l

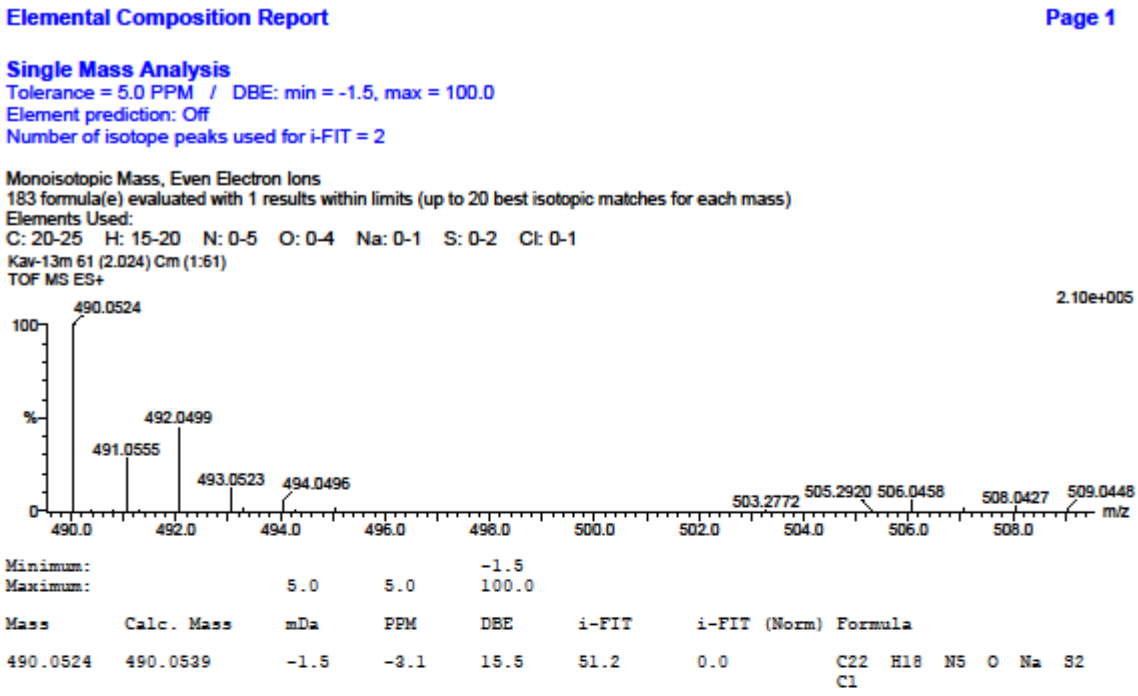


Figure 39. HRMS of compound k-2-m

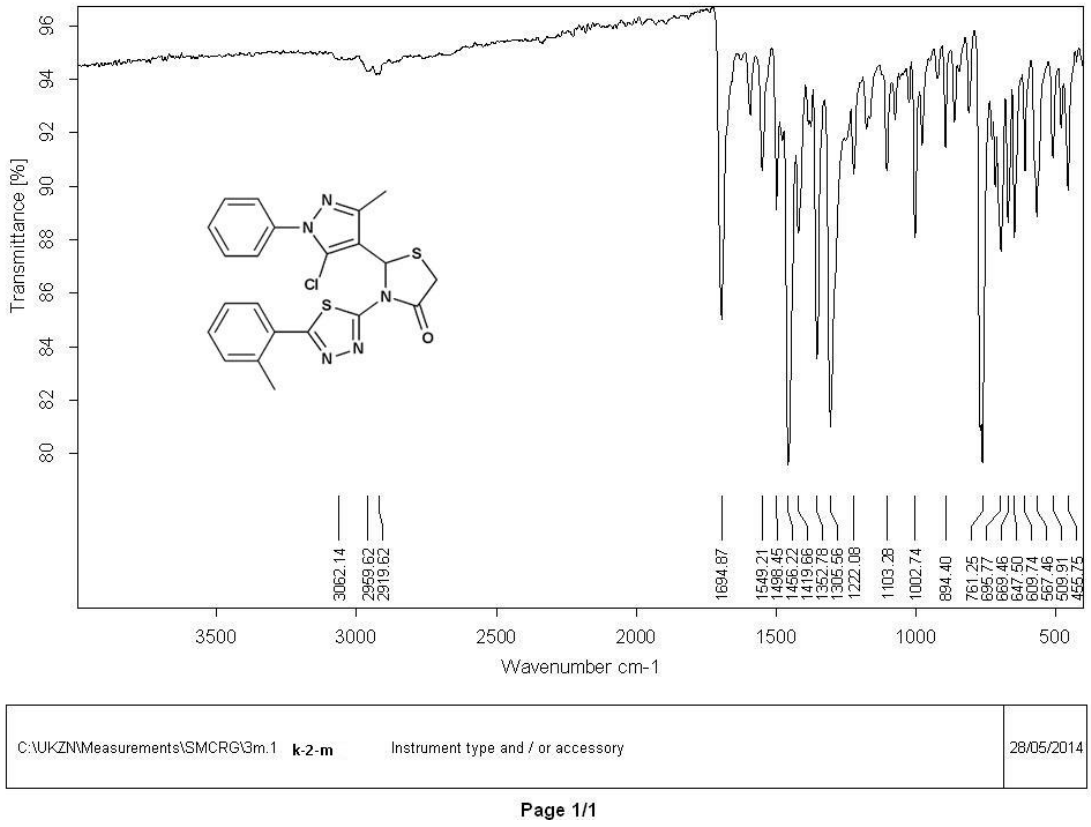


Figure 40. IR spectrum of compound k-2-m

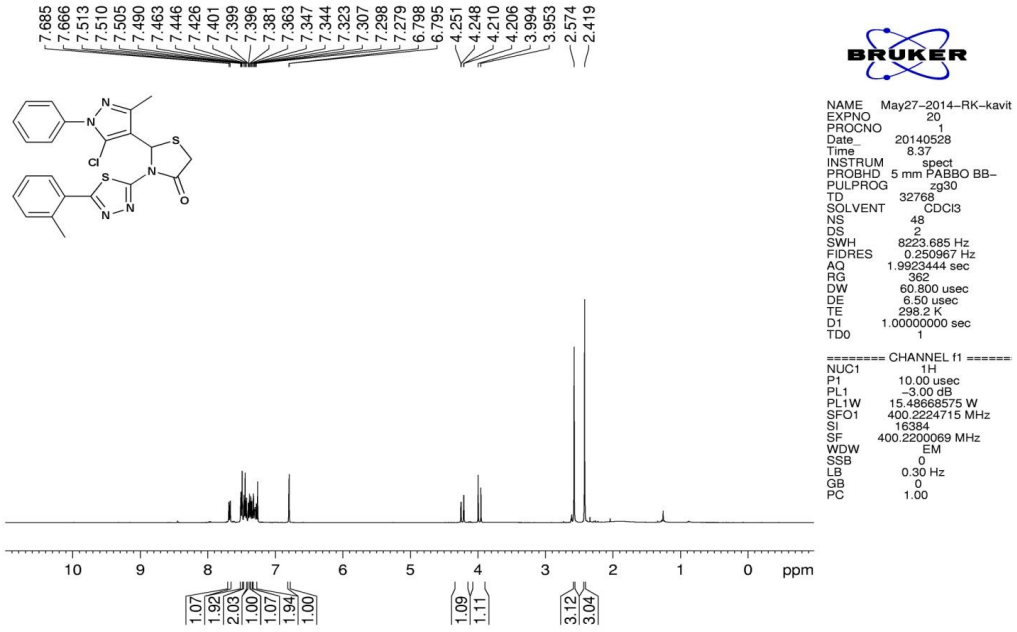


Figure 41. ^{1}H NMR of compound k-2-m

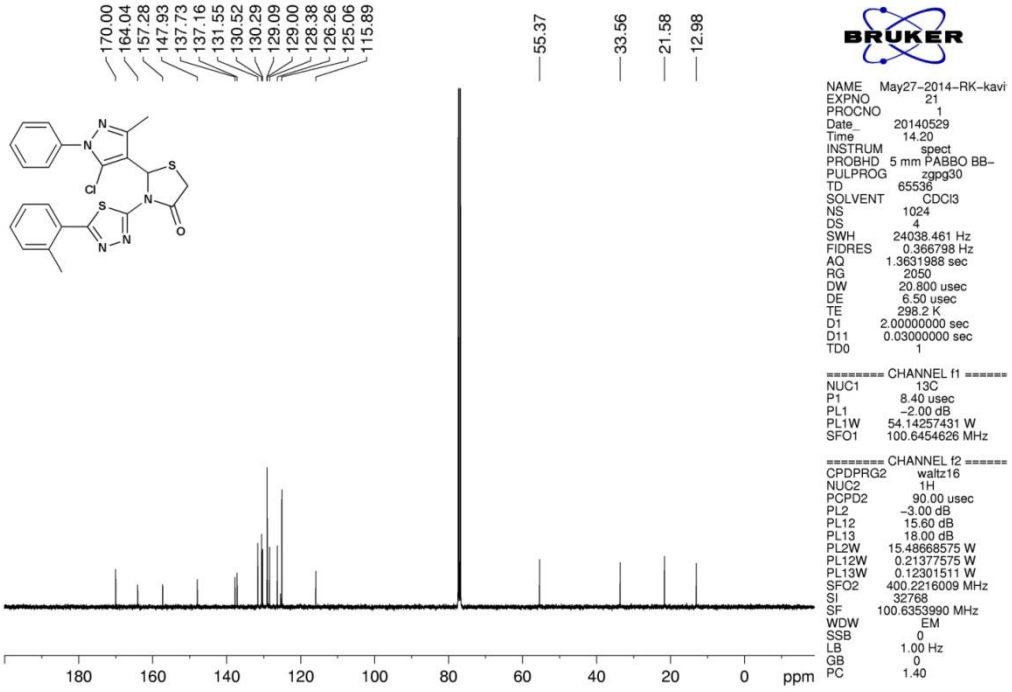


Figure 42. ^{13}C NMR of compound k-2-m

1.2 CHAPTER 4

Tital: A powerful divergent approach to discover three distinct molecular architectures in one blow: Pyrazolone–triazole and their antimicrobial evaluation

Authors name: Kavita Jain[a], Nisar Syyad and Rajshekhar Karpoormath[a,*]

Affiliations: [a] *Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa.*

To receive all correspondence: Rajshekhar Karpoormath: Tel: +27 (0) 312607179,

E-mail: **karpoormath@ukzn.ac.za**

Supplementary data:

Spectral Information:

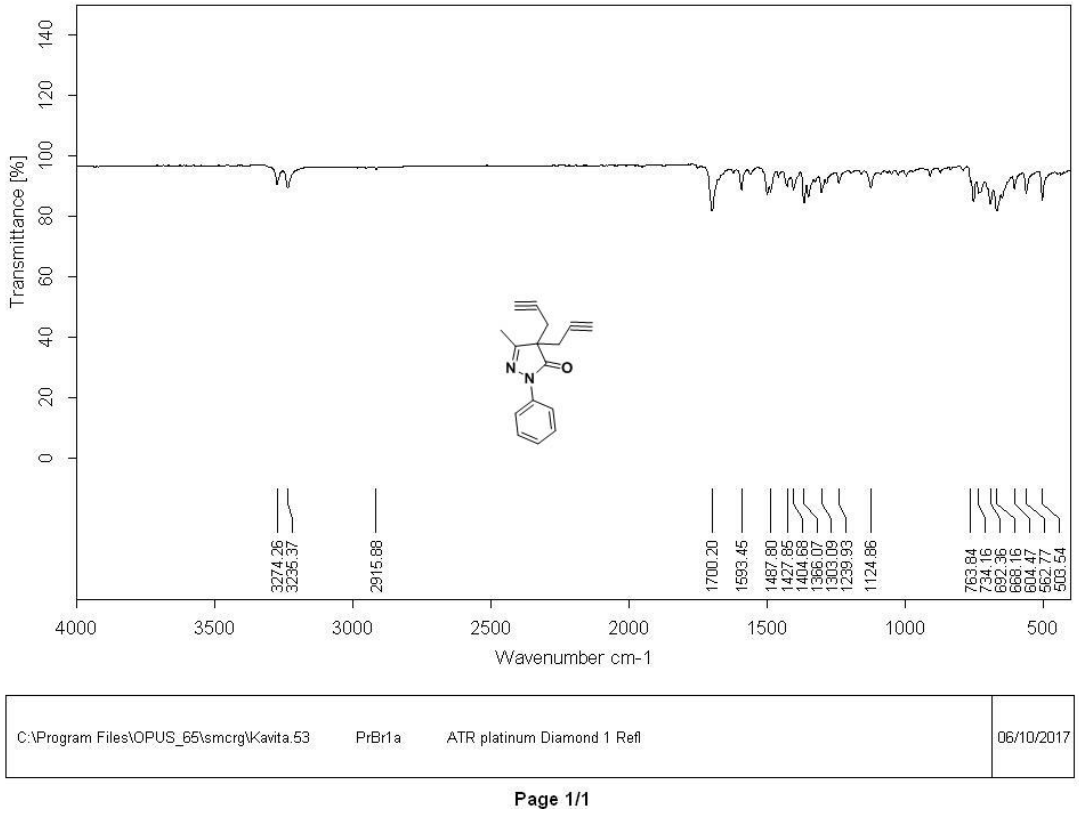


Figure 1. IR spectrum of compound 2B1

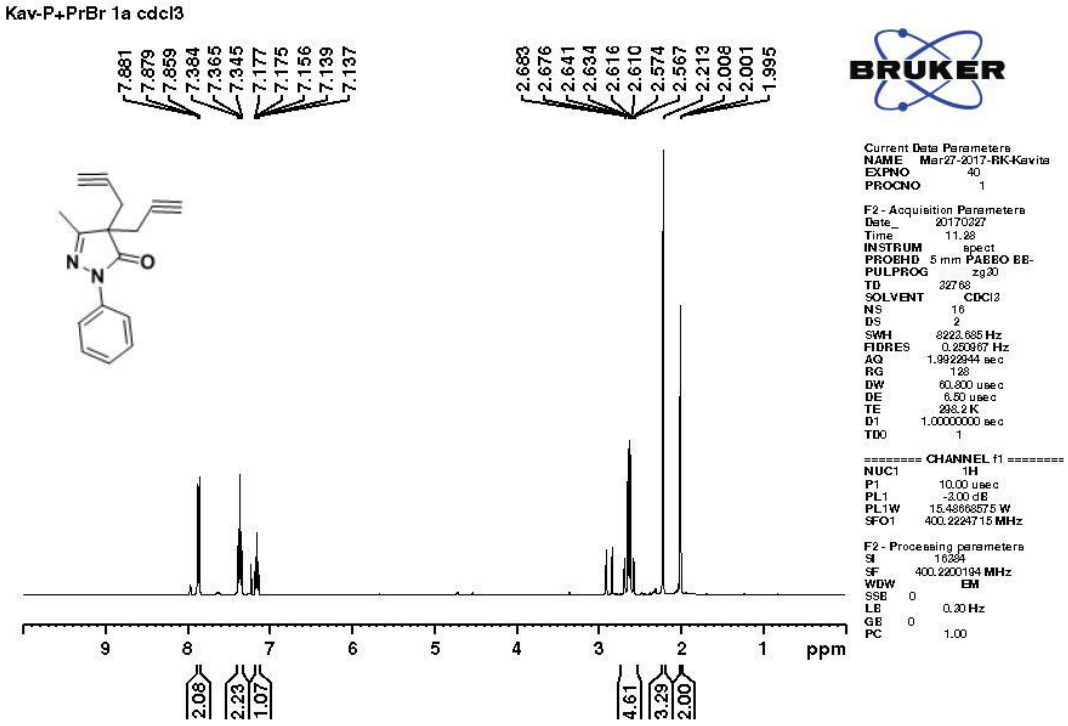


Figure 2. [1]H NMR of compound 2B1

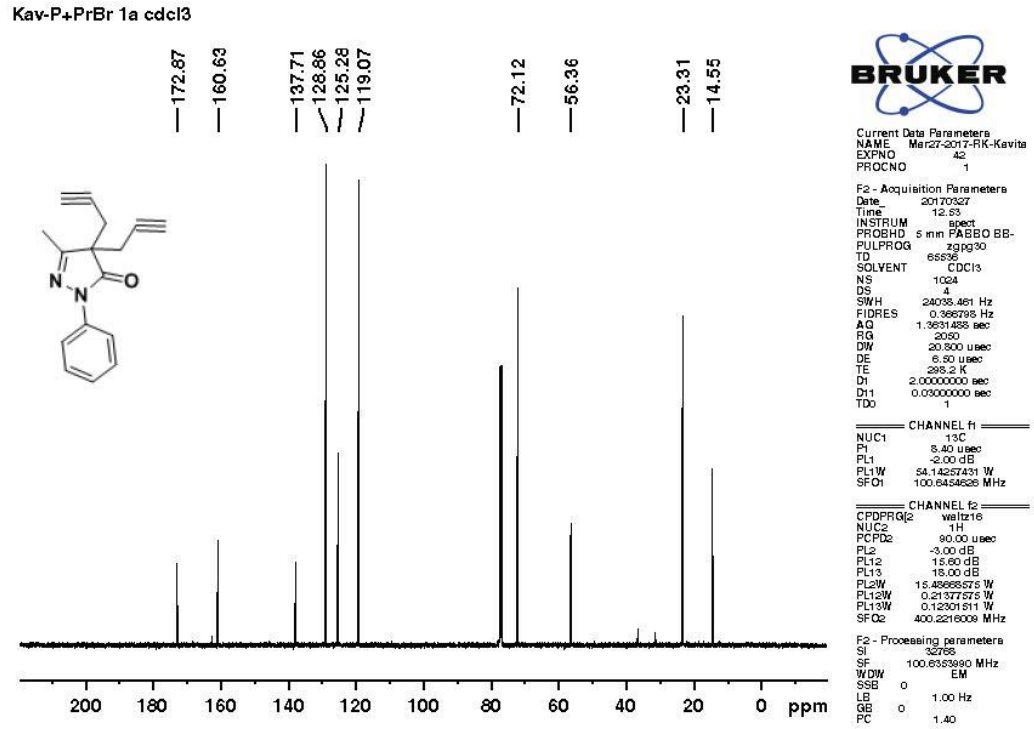


Figure 3. [13]C NMR of Compound 2B1

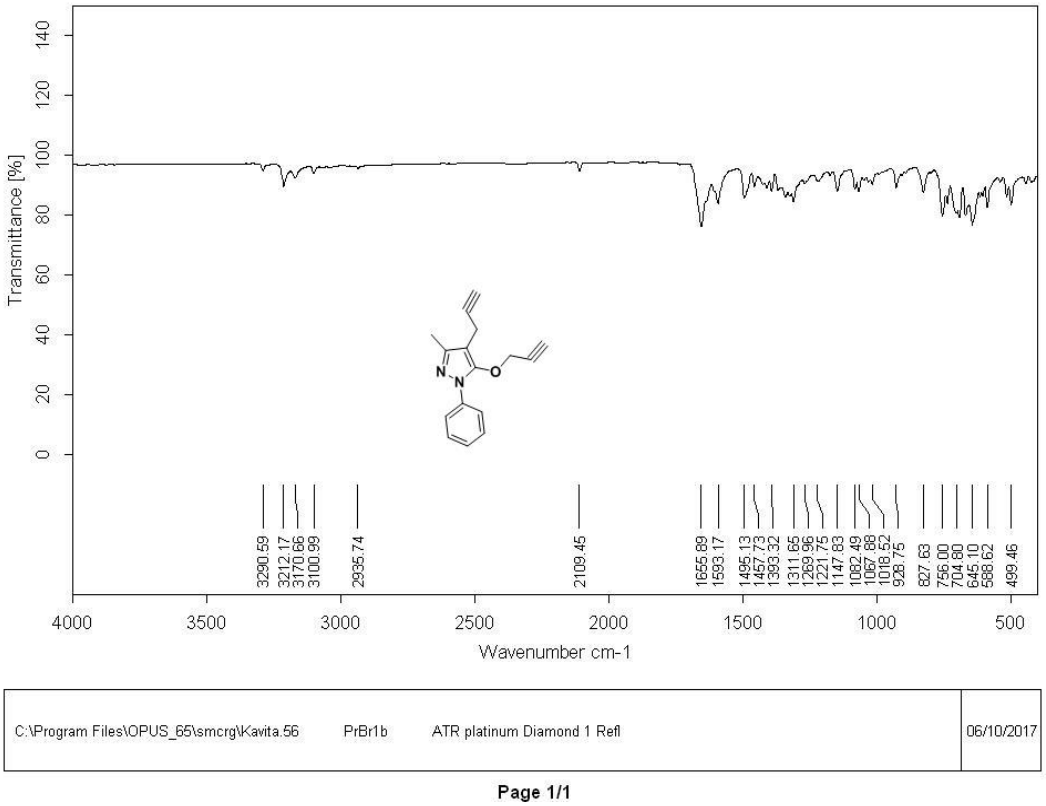


Figure 4. IR spectrum of compound 2A1

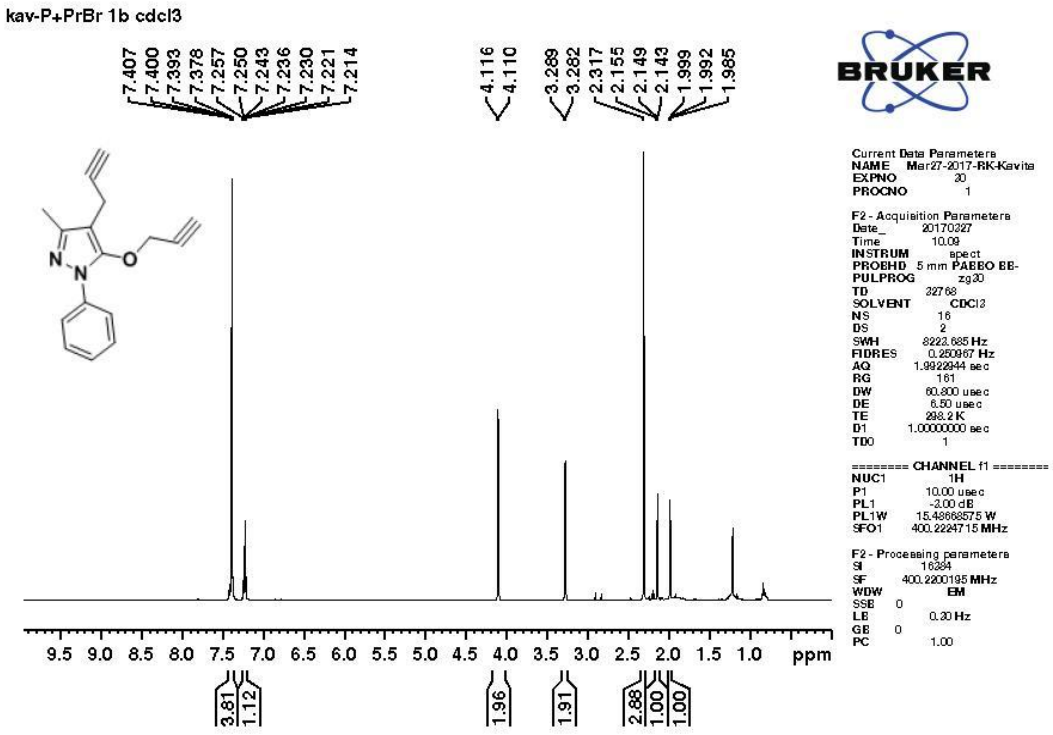


Figure 5. ^{1}H NMR of compound 2A1

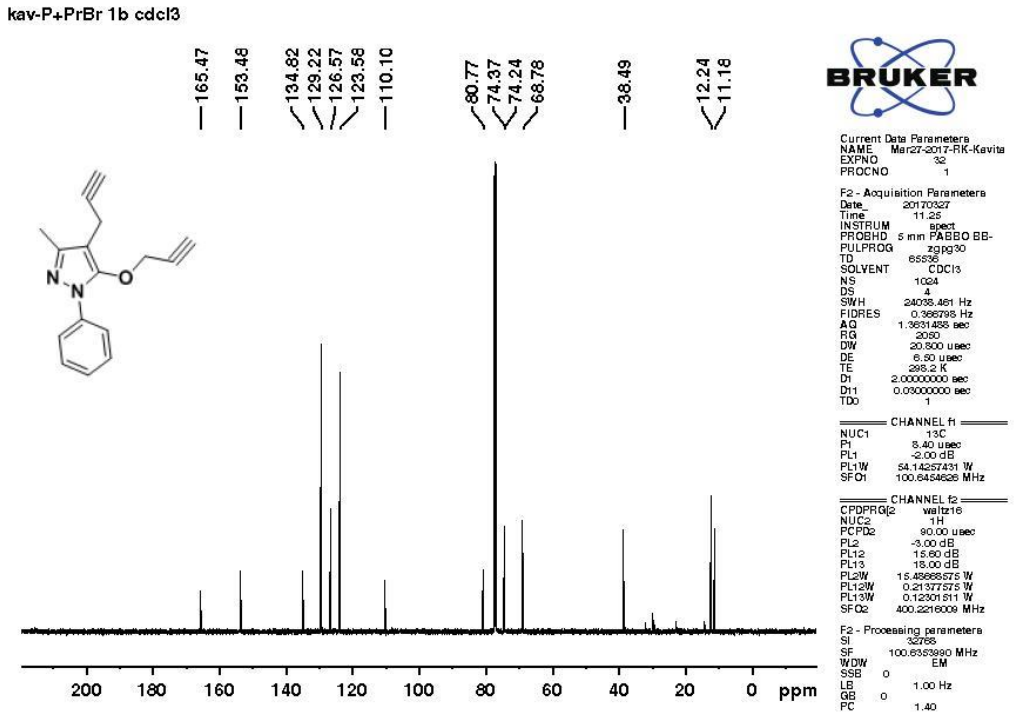


Figure 6. ^{13}C NMR of compound 2A1

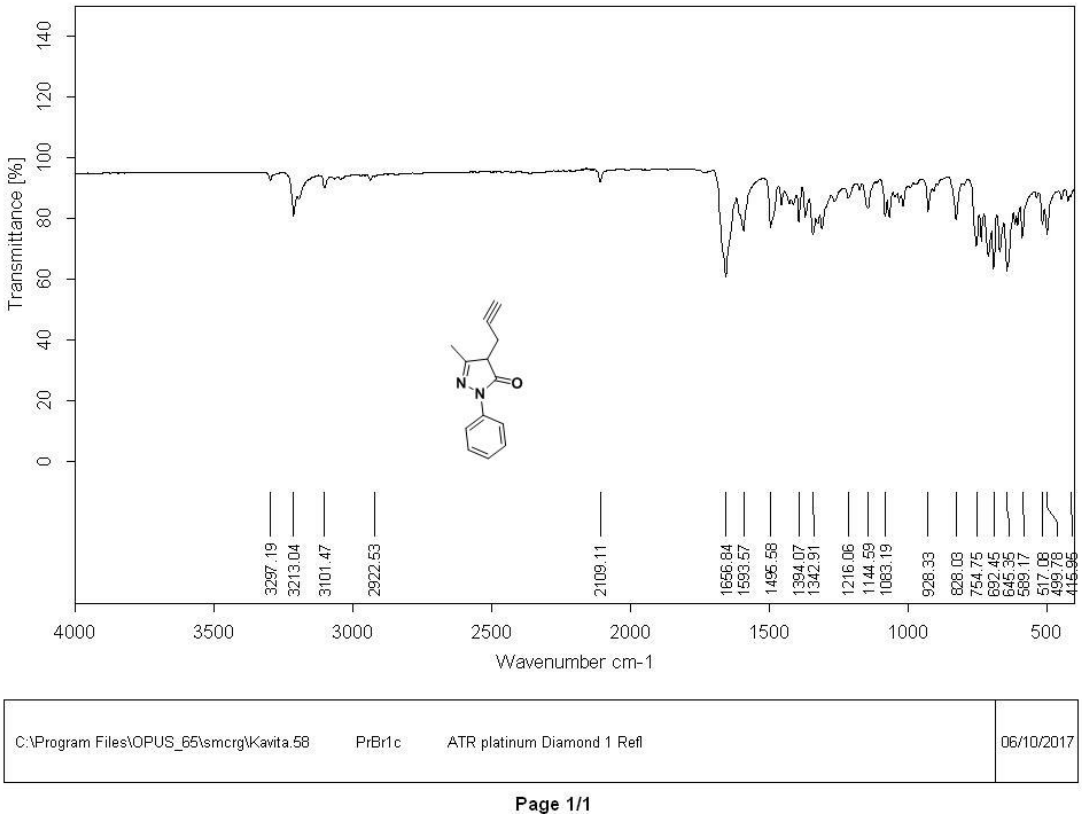


Figure 7. IR spectrum of compound 2C1

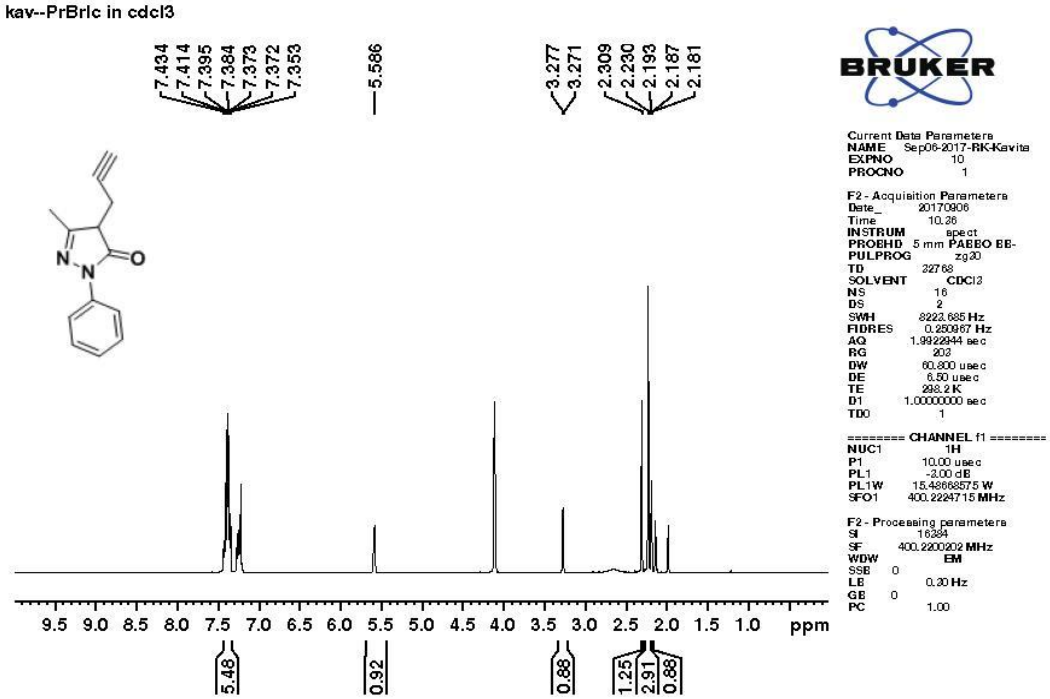


Figure 8. ^{1}H NMR of compound 2C1

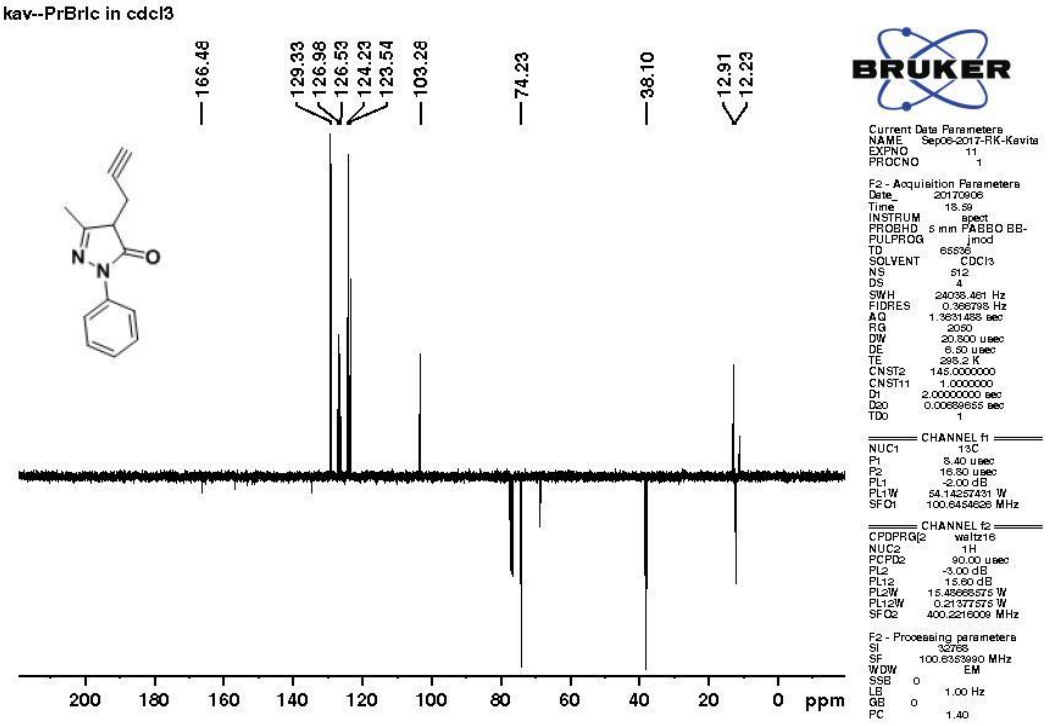


Figure 9. ^{13}C NMR of compound 2C1

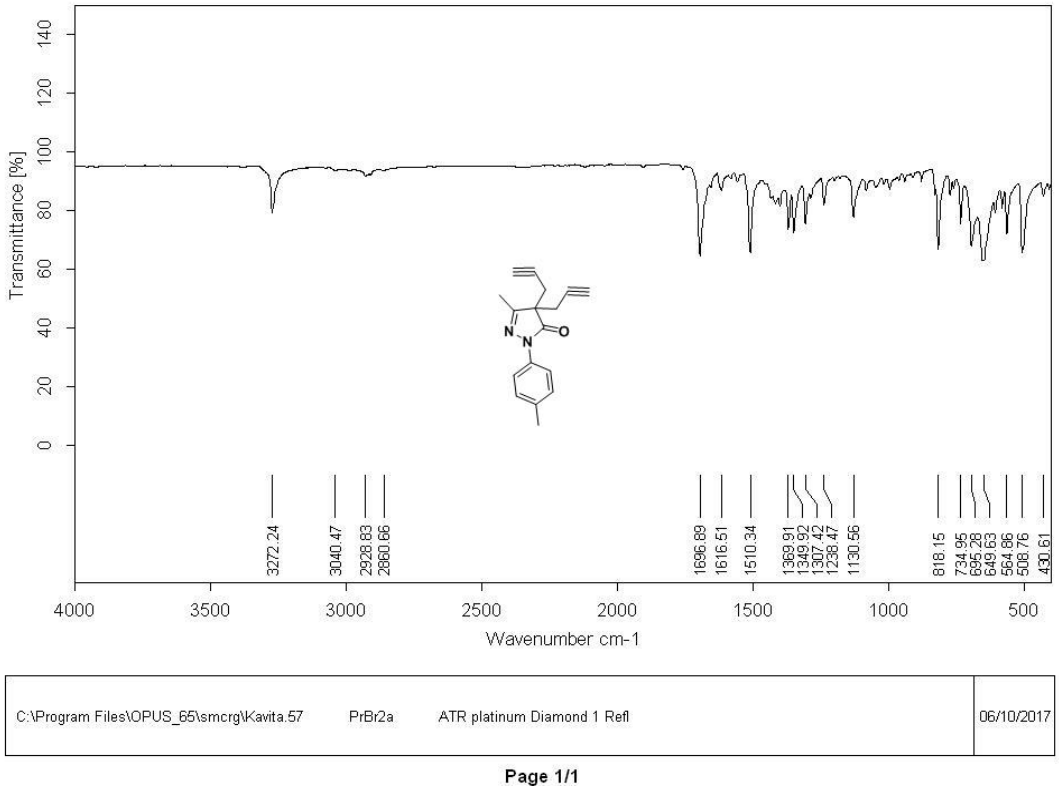


Figure 10. IR spectrum of compound 2B2

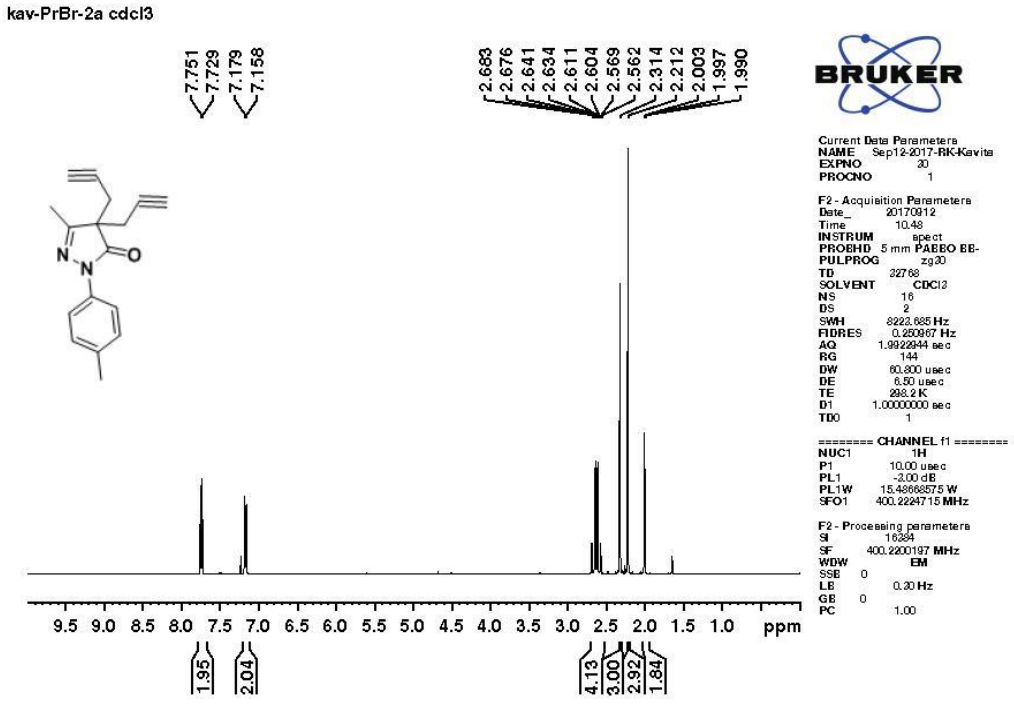


Figure 11. ^{1}H NMR of compound 2B2

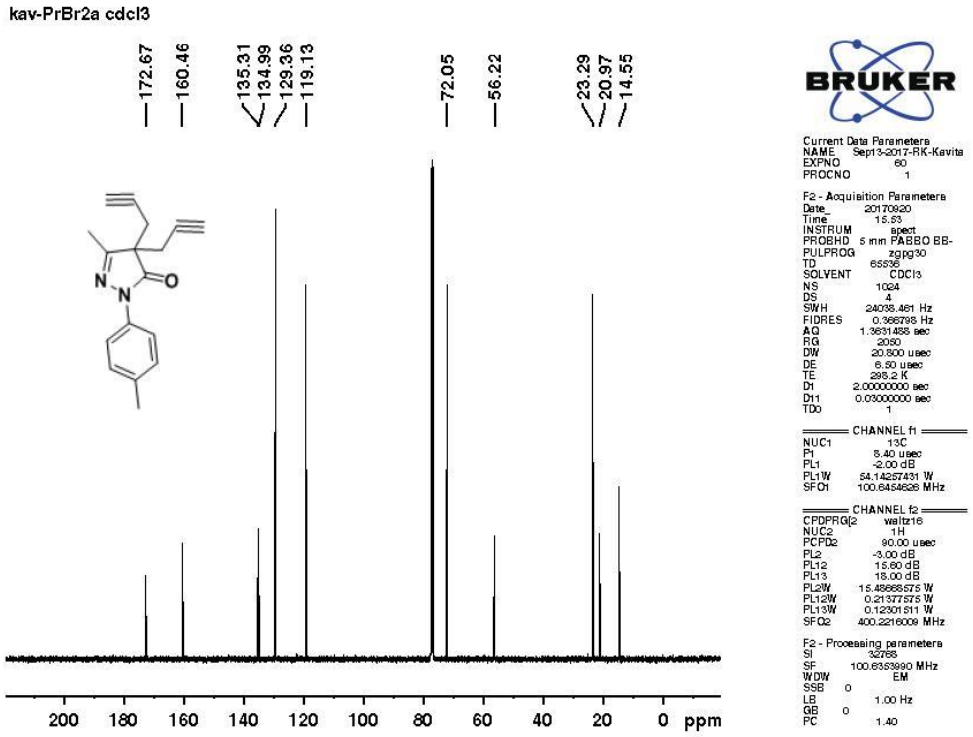


Figure 12. ^{13}C NMR of compound 2B2

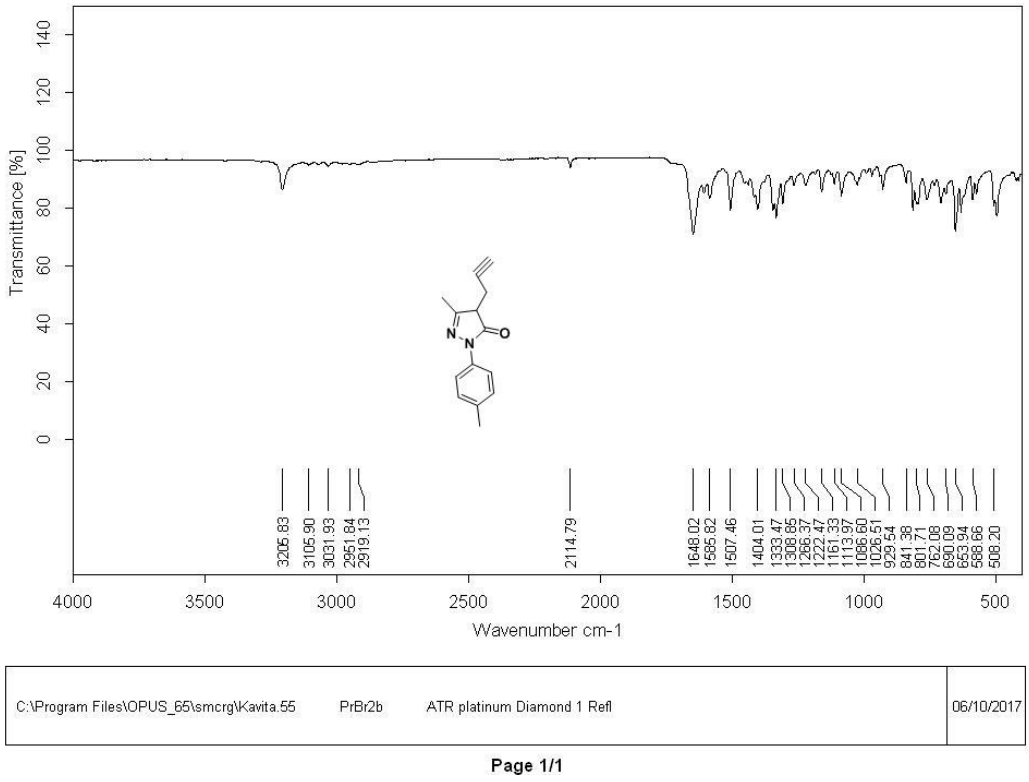


Figure 13. IR spectrum of Compound 2C2

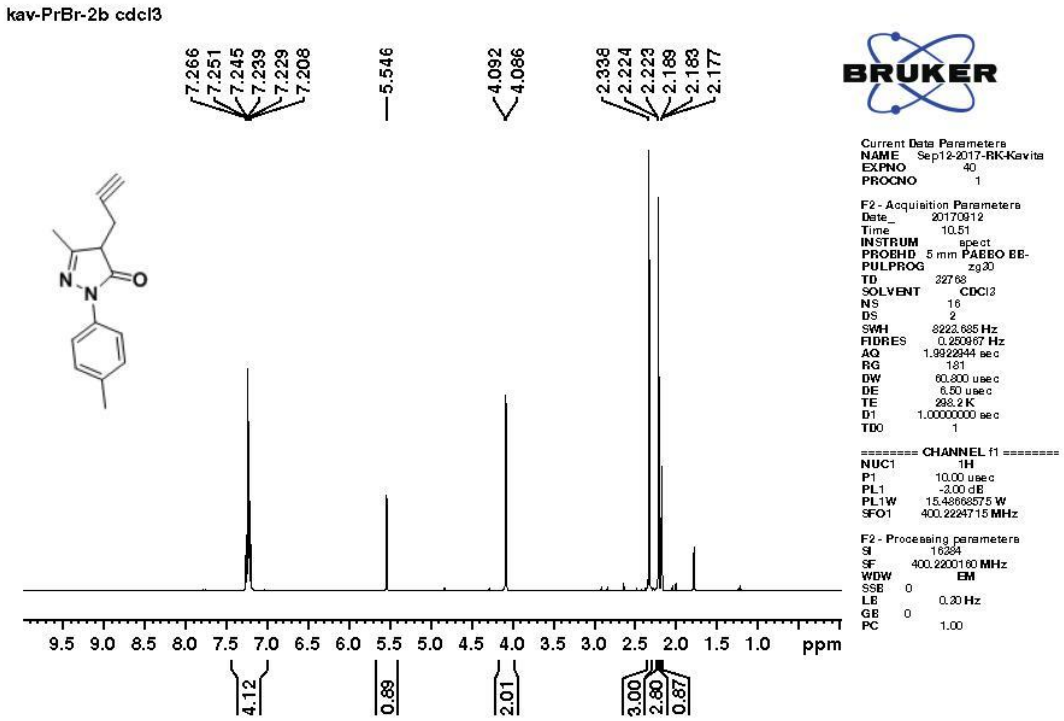


Figure 14. ^{1}H NMR of Compound 2C2

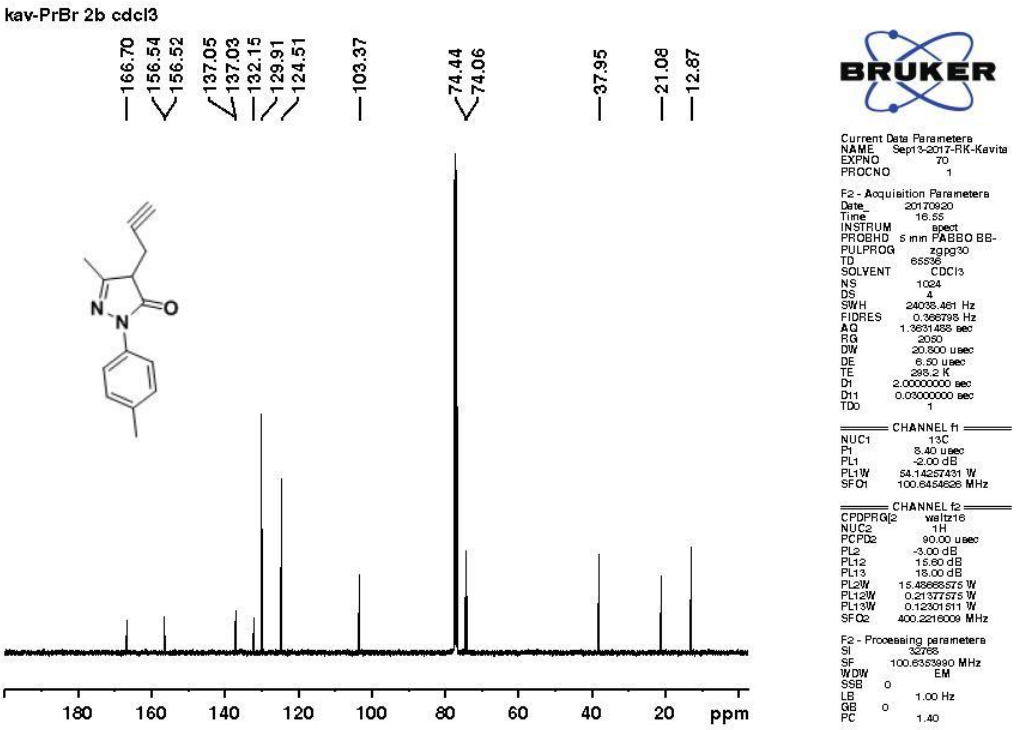
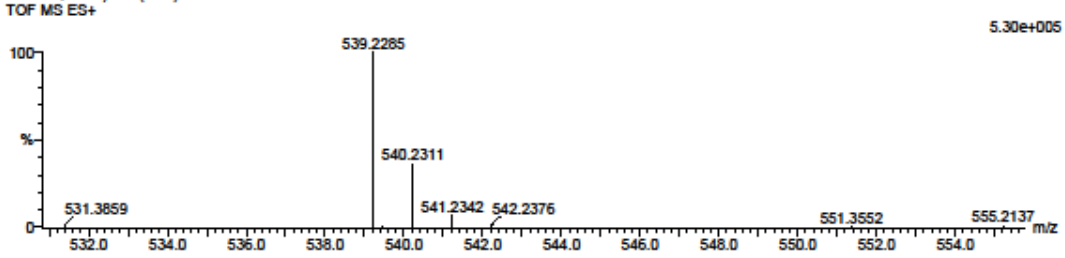


Figure 15. ^{13}C NMR of compound 2C2

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 30-35 H: 25-30 N: 5-10 O: 0-4 Na: 1-1
K-3-1 2 (0.034) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
539.2285	539.2284	0.1	0.2	20.5	28.1	0.0	C30 H28 N8 O Na

Figure 16. HRMS of compound k-3-1

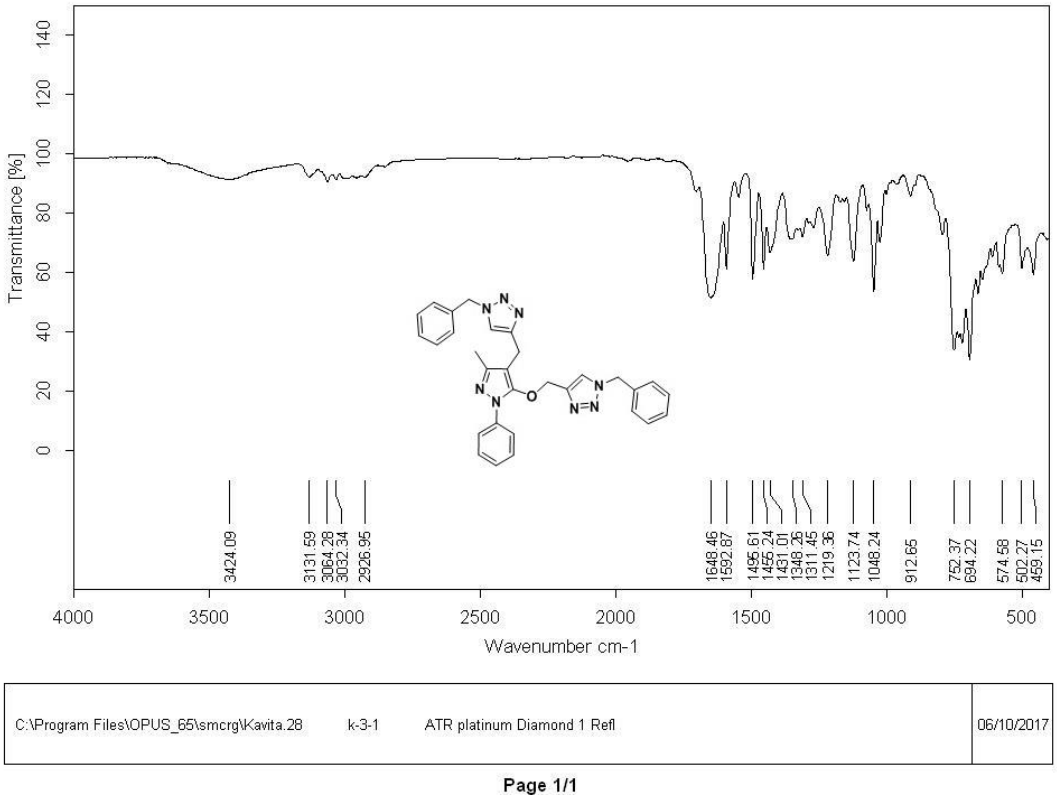


Figure 17. IR spectrum of compound k-3-1

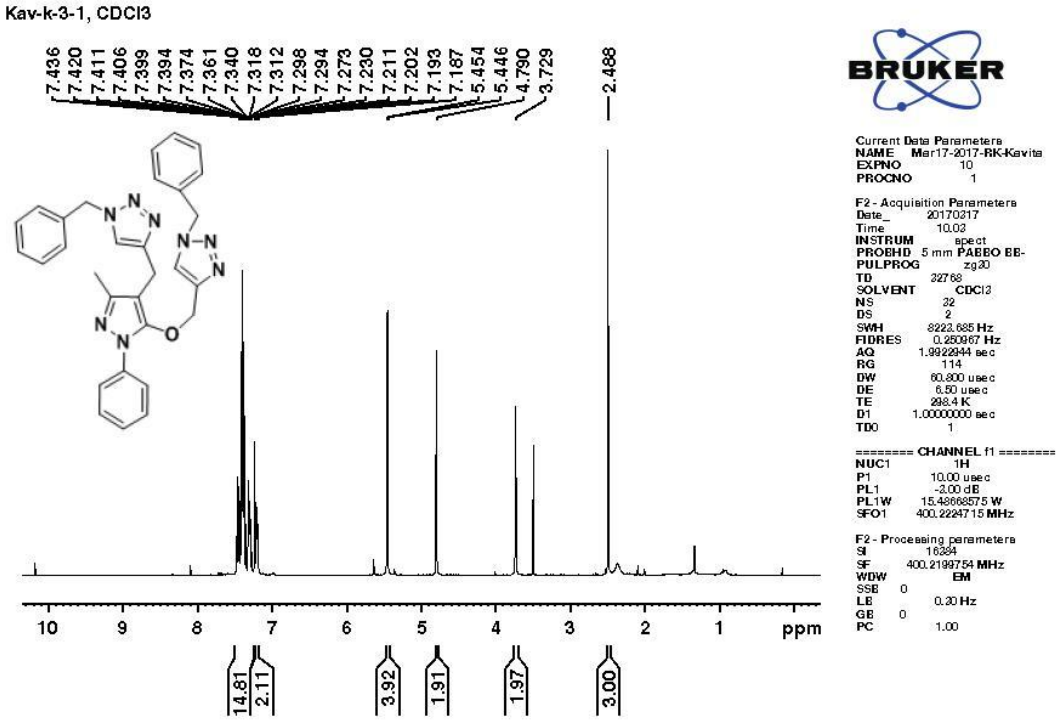


Figure 18. ^{1}H NMR of Compound k-3-1

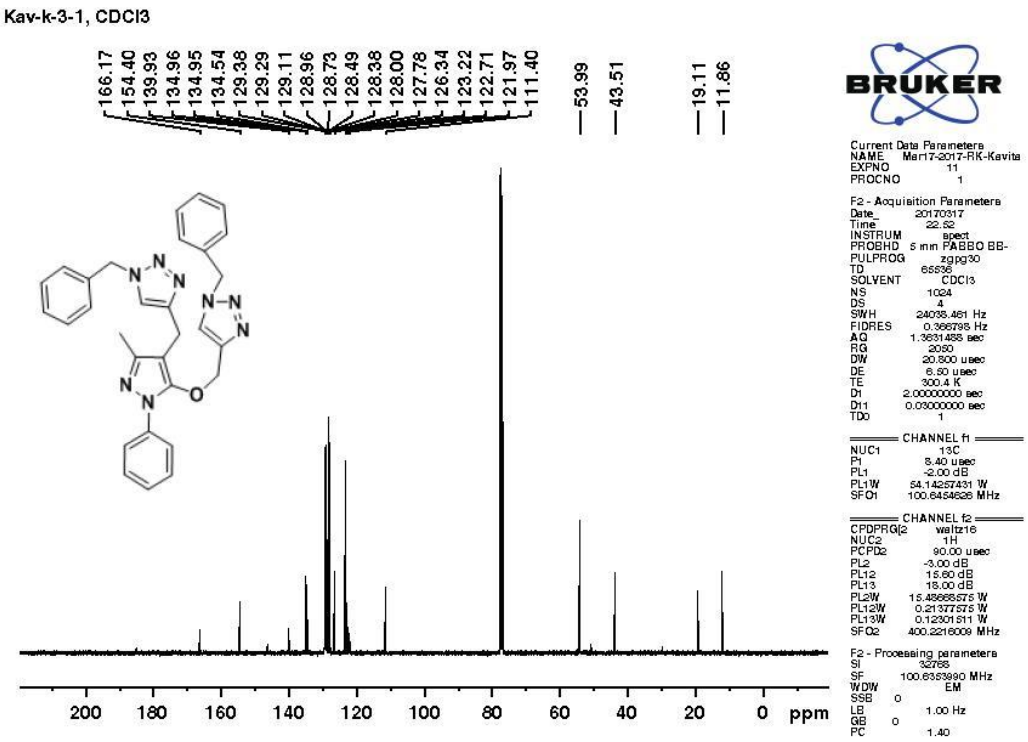
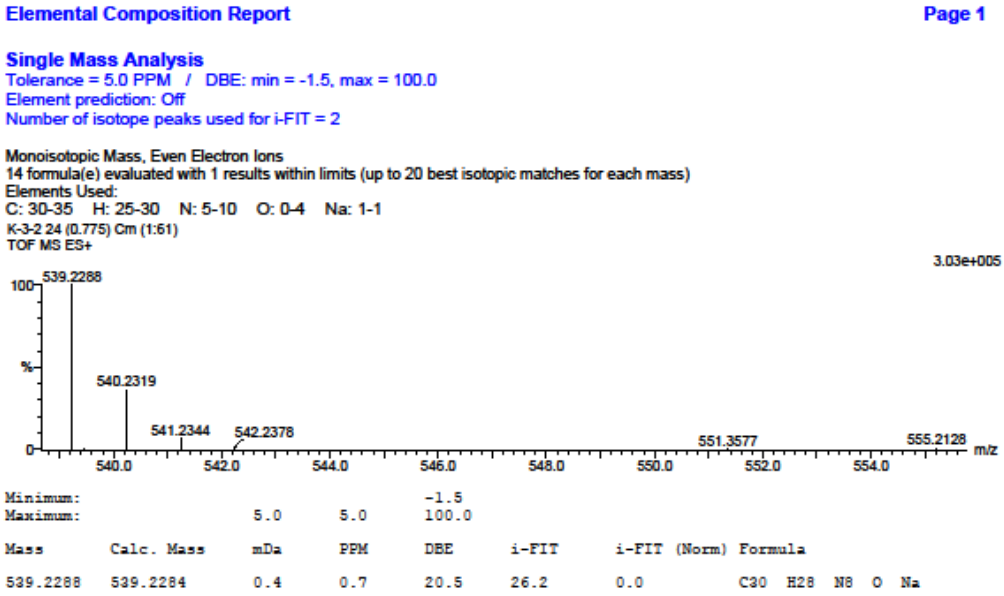


Figure 19. ^{13}C NMR of compound k-3-1



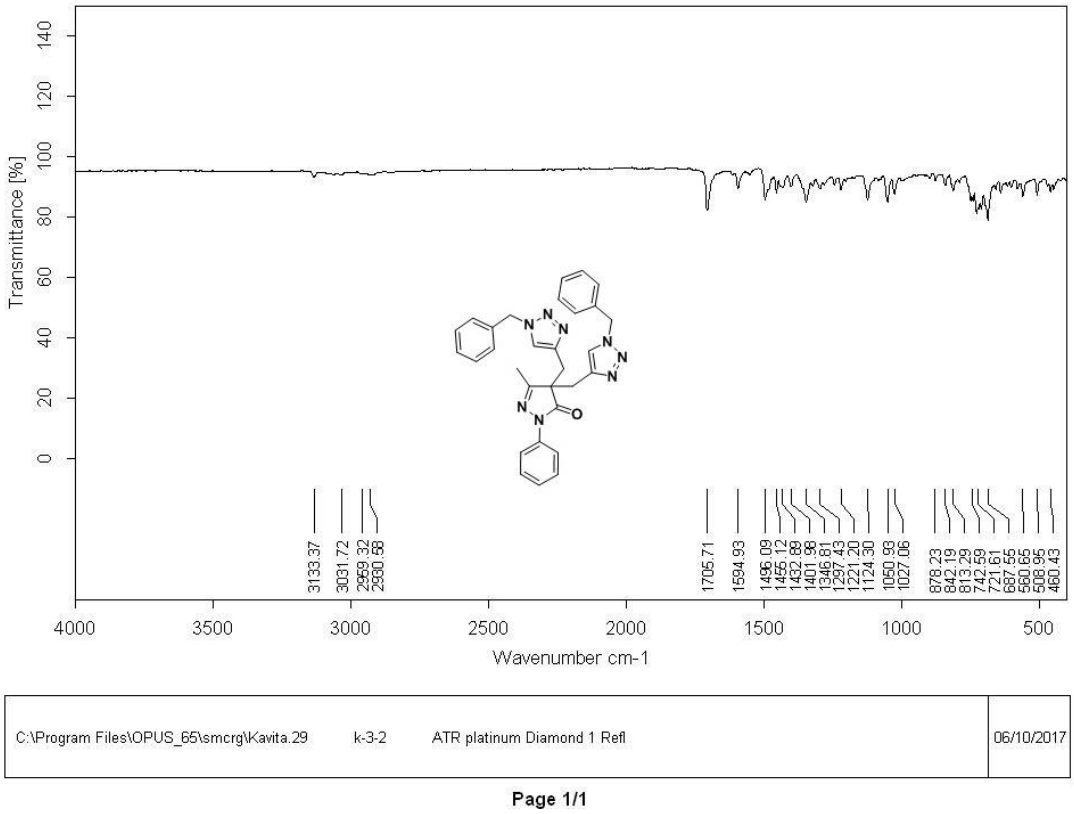
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 30-35 H: 25-30 N: 5-10 O: 0-4 Na: 1-1
K-3-2 24 (0.775) Cm (1:61)
TOF MS ES+

Minimum:				-1.5						
Maximum:		5.0	5.0	100.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
539.2288	539.2284	0.4	0.7	20.5	26.2	0.0	C30	H28	N8	O Na

Figure 20. HRMS of compound k-3-2



C:\Program Files\OPUS_65\smcrg\Kavita.29 k-3-2 ATR platinum Diamond 1 Refl 06/10/2017

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Figure 21. IR spectrum of compound k-3-2

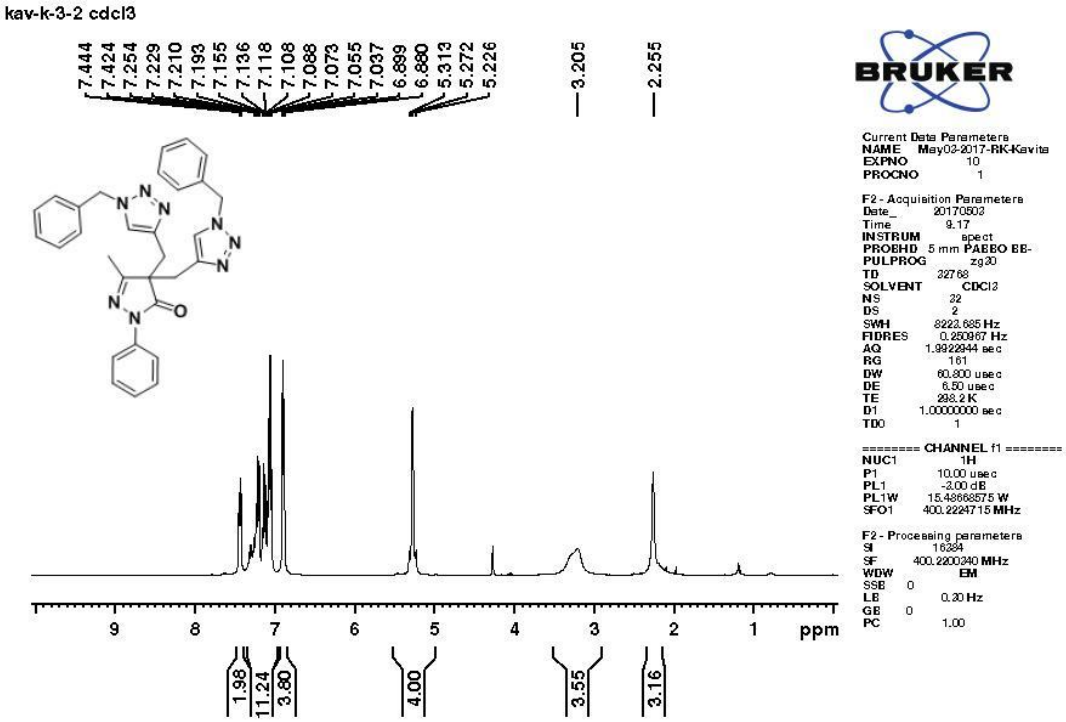


Figure 22. ^{1}H NMR of compound k-3-2

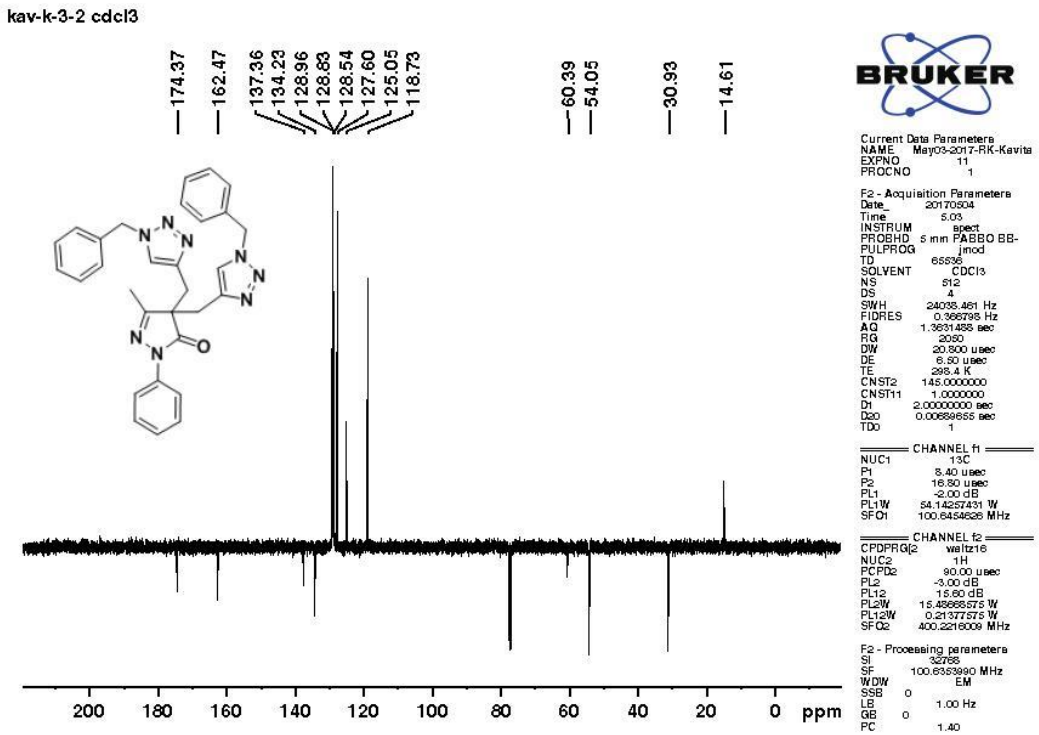


Figure 23. ^{13}C NMR of compound k-3-2

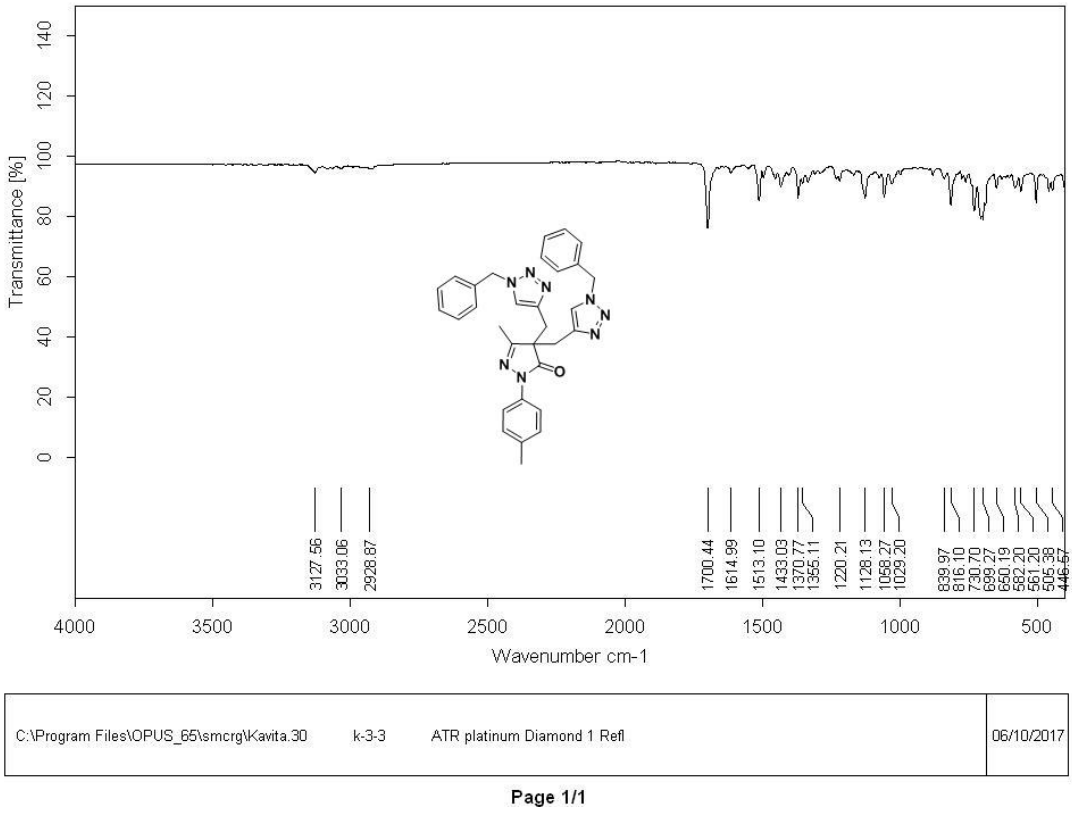


Figure 24. IR spectrum of compound k-3-3

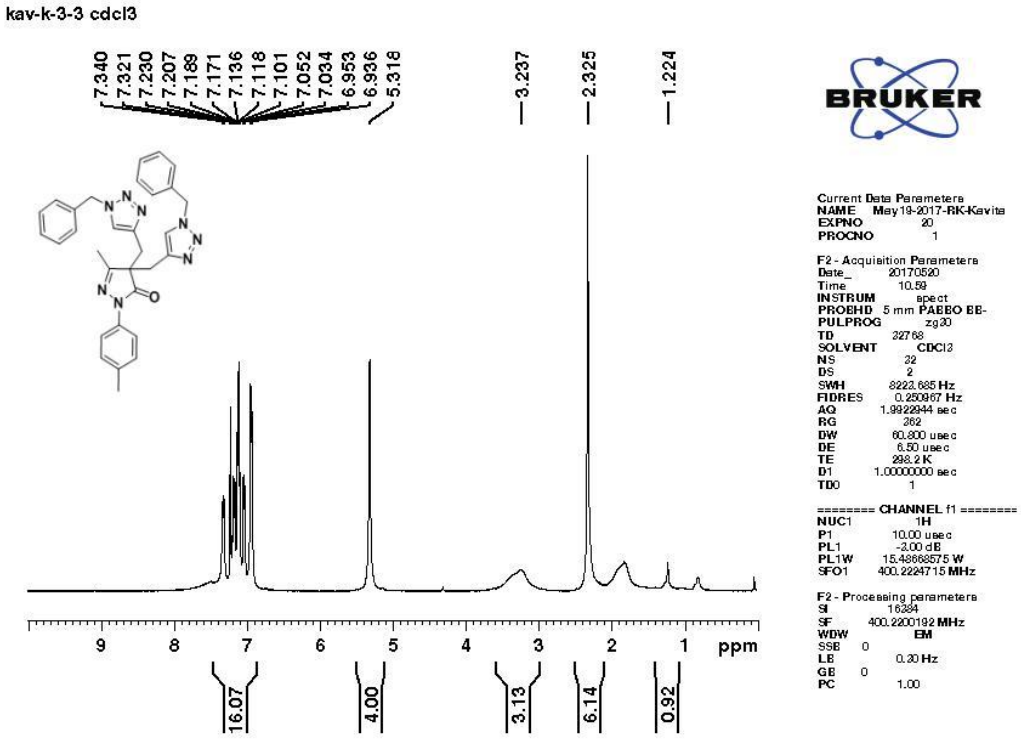


Figure 25. ^{1}H NMR of compound k-3-3

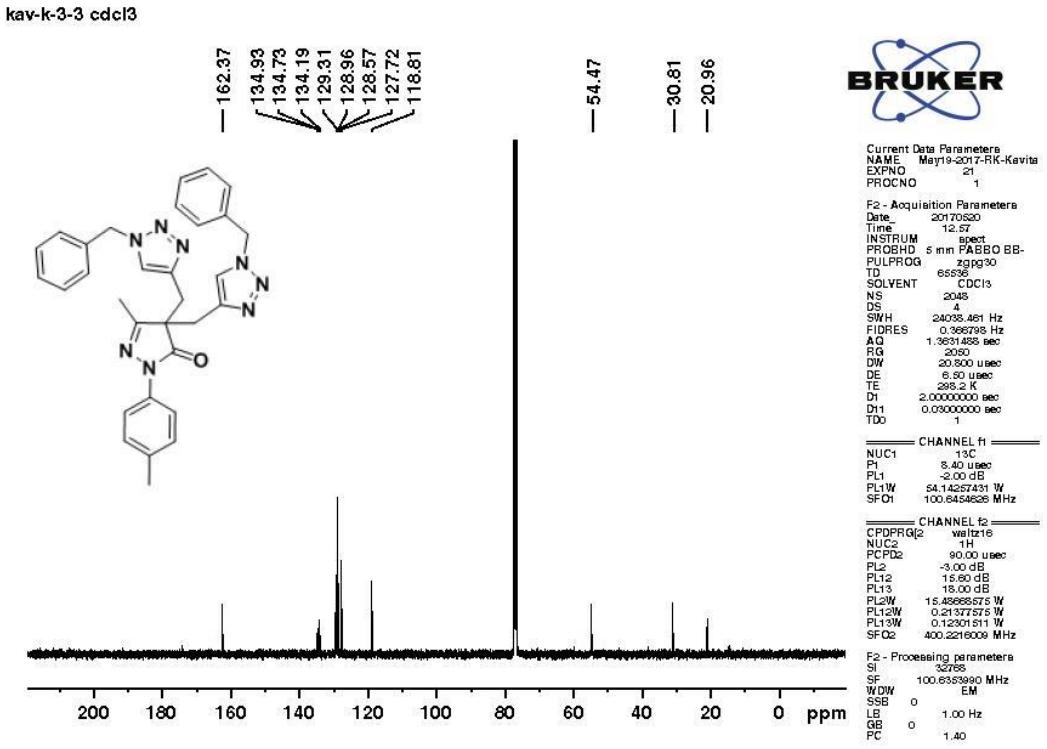


Figure 26. ^{13}C NMR of compound k-3-3

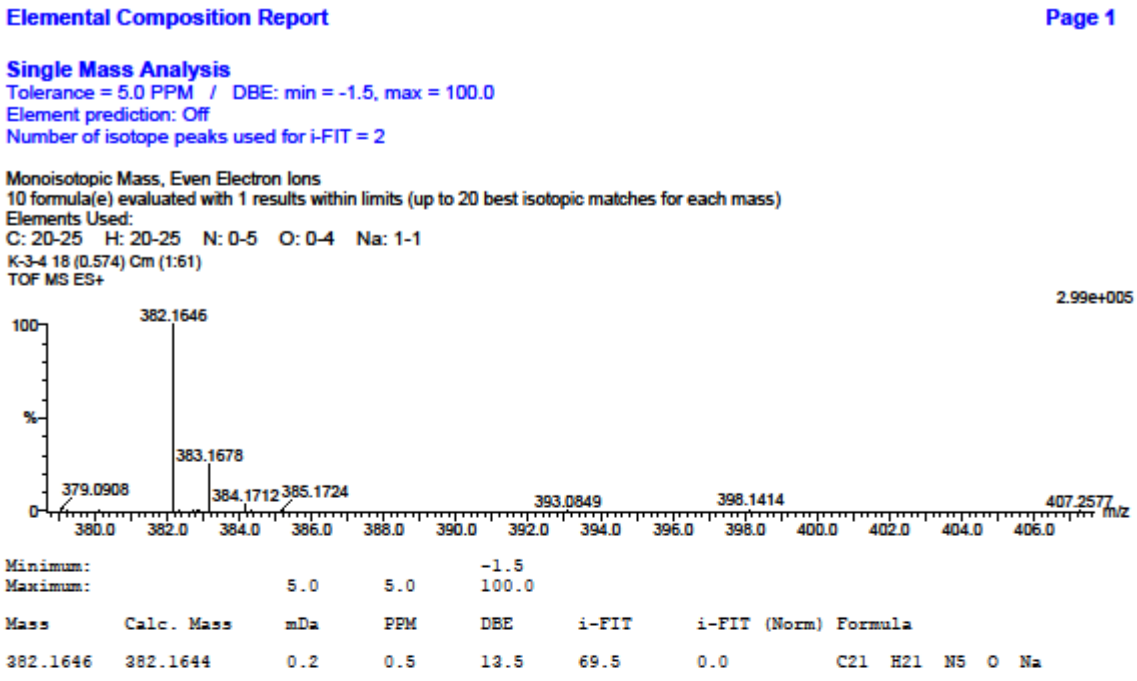


Figure 27. HRMS of compound k-3-4

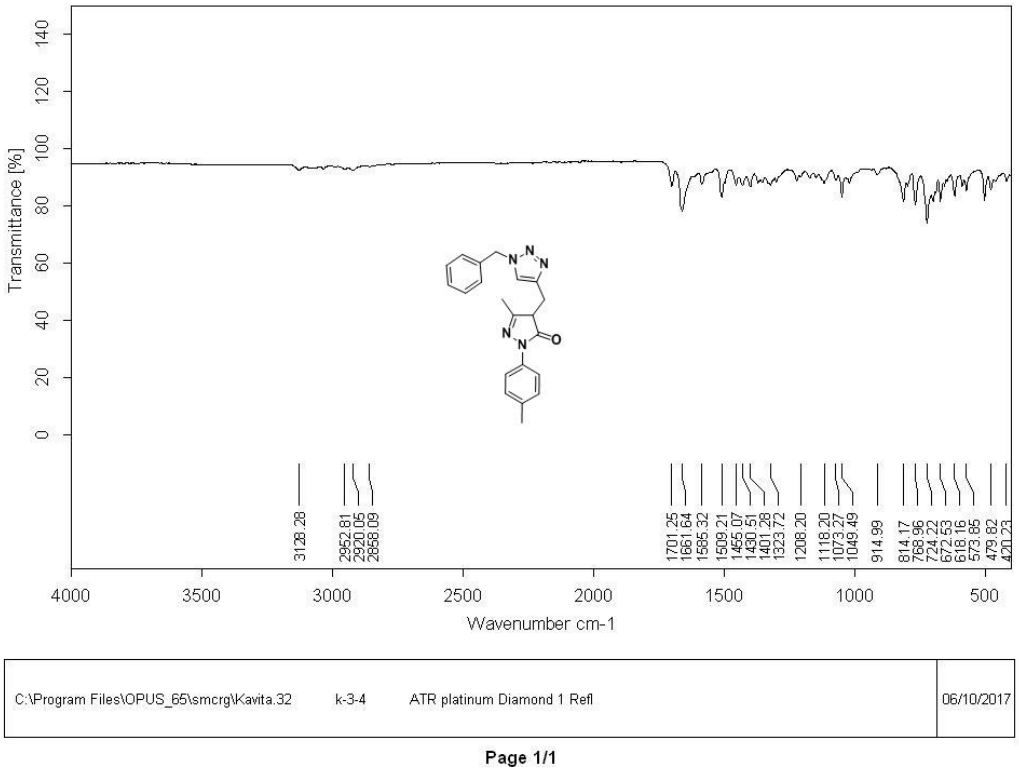


Figure 28. IR spectrum of compound k-3-4

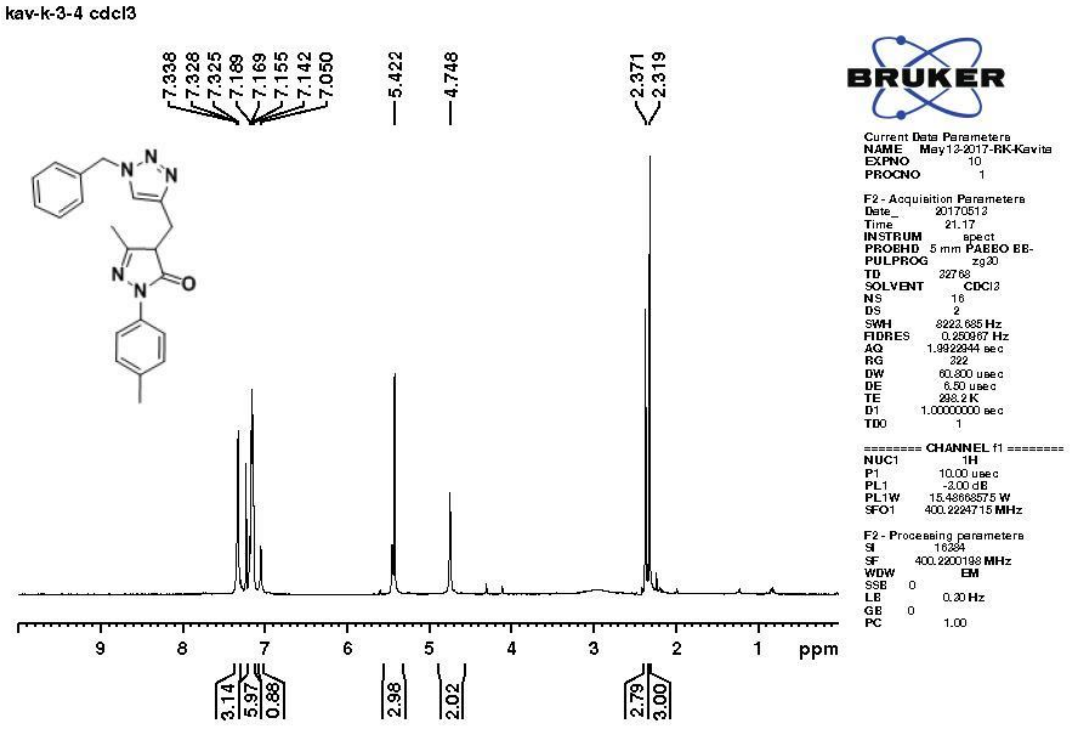


Figure 29. ^{1}H NMR of compound k-3-4

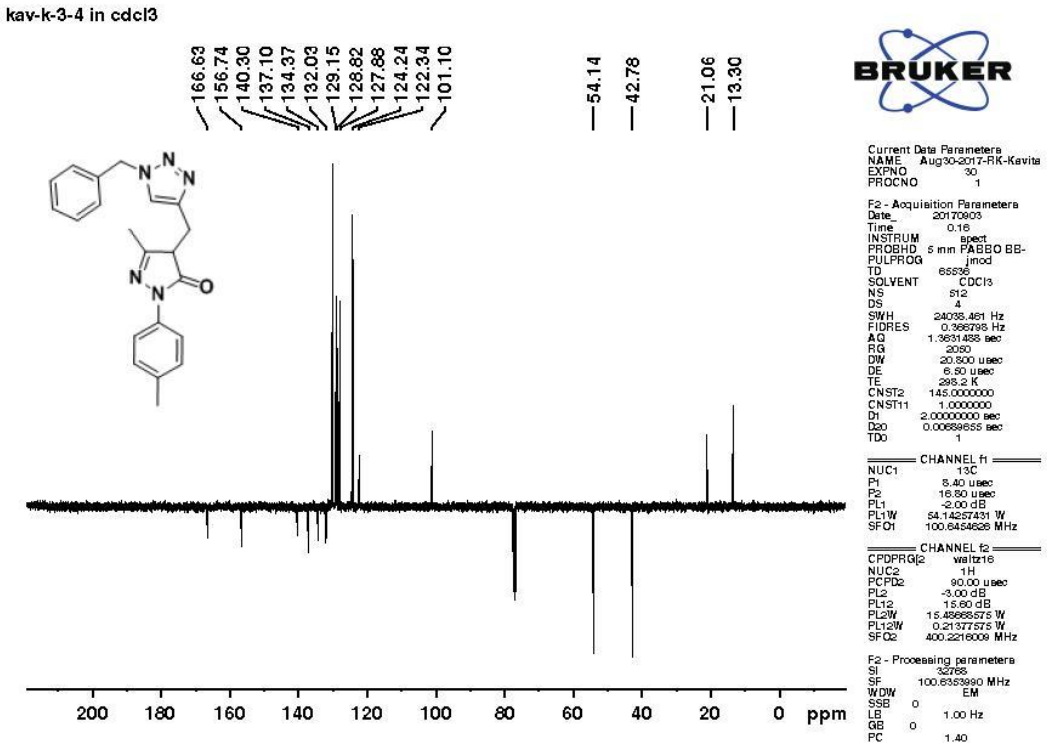


Figure 30. ^{13}C NMR of compound k-3-4

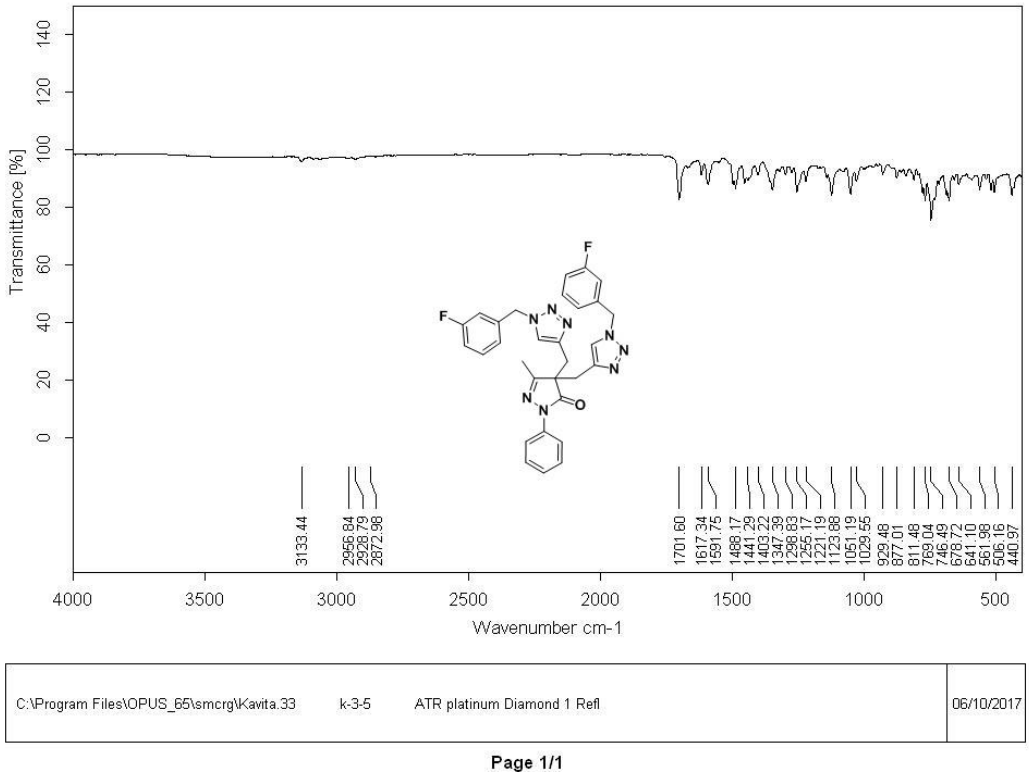


Figure 31. IR spectrum of compound k-3-5

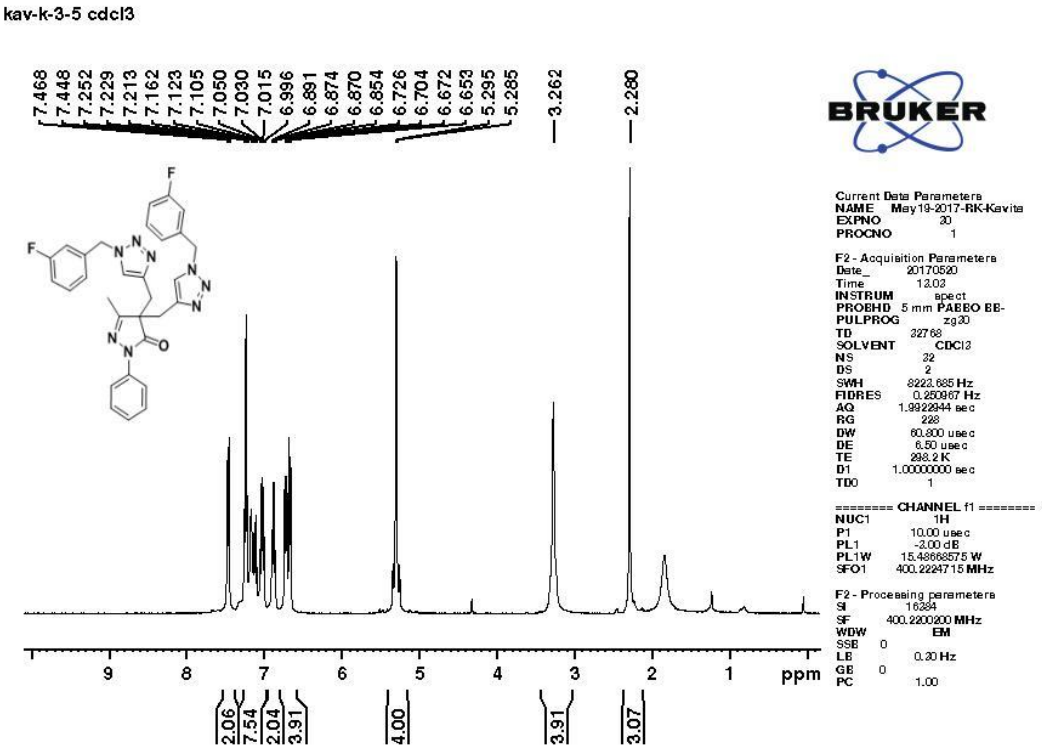


Figure 32. ^{1}H NMR of compound k-3-5

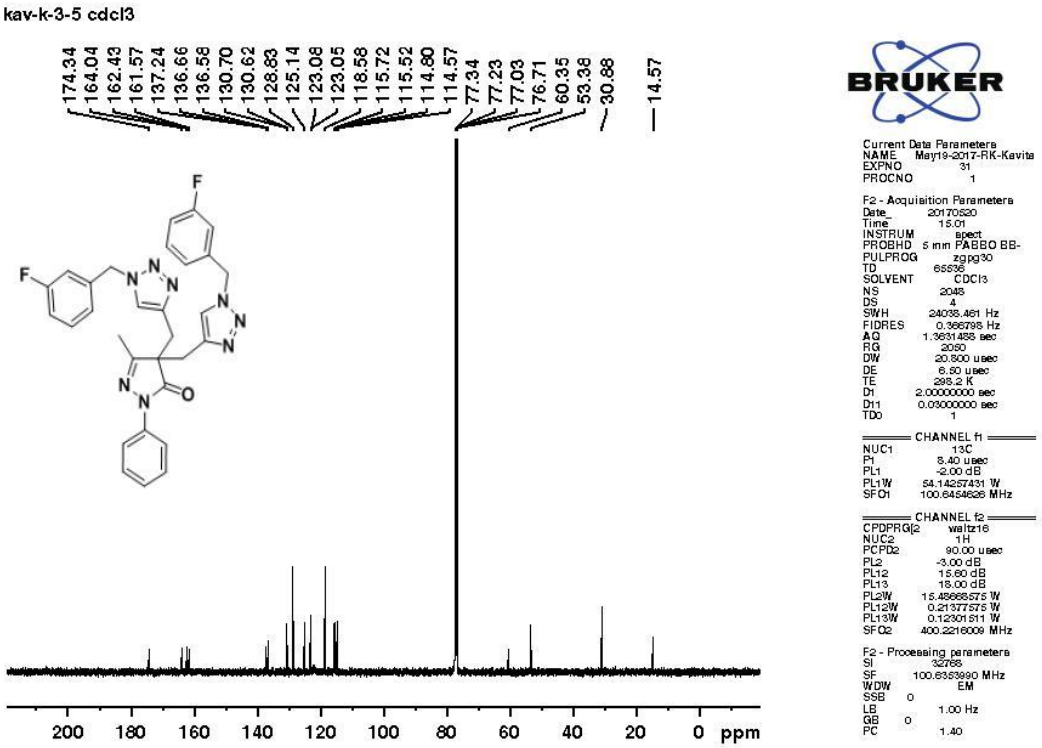


Figure 33.^{13}C NMR of compound k-3-5

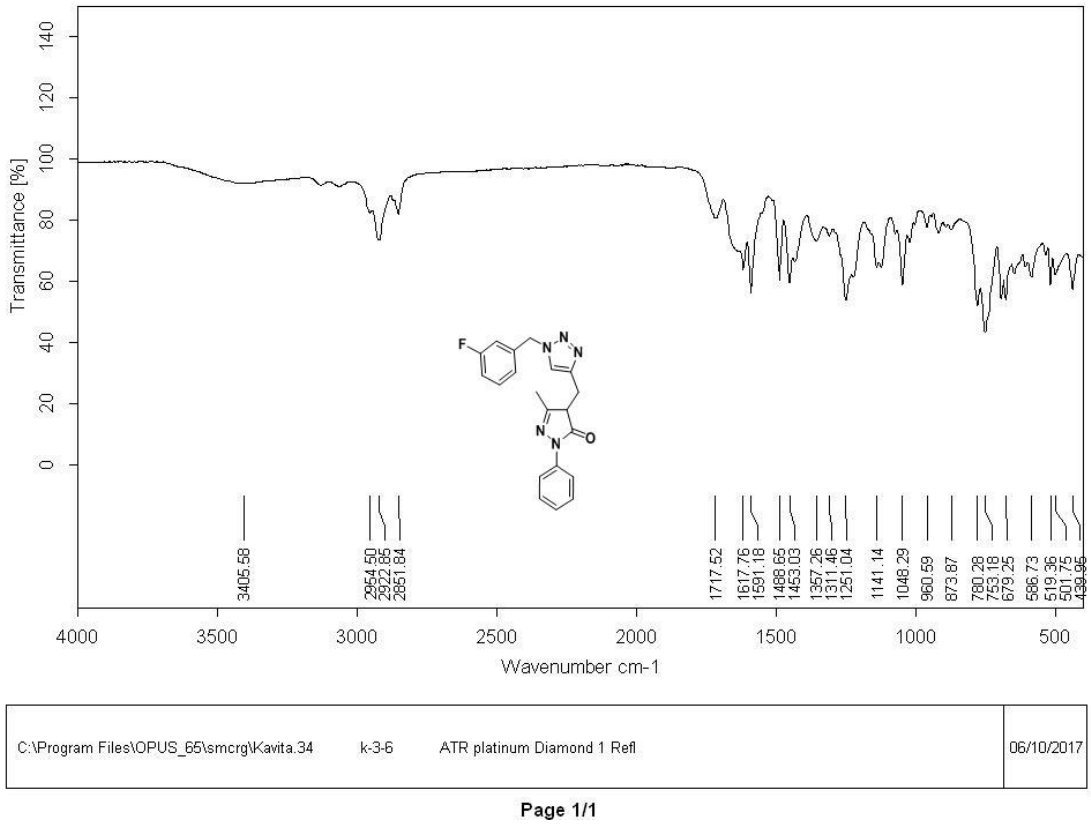


Figure 34. IR spectrum of compound k-3-6

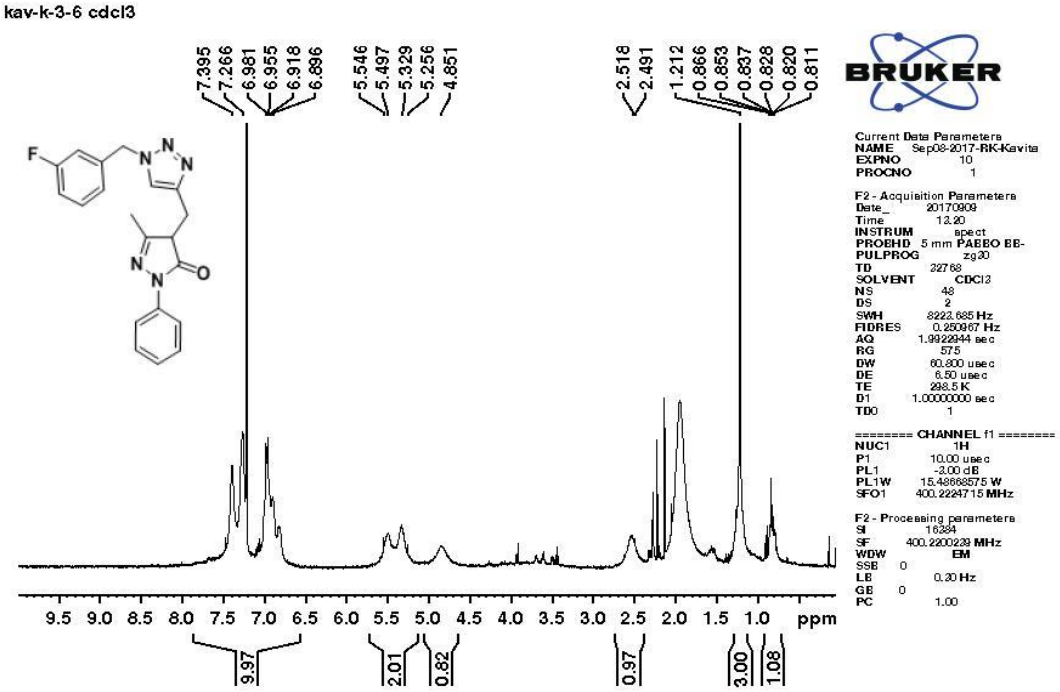


Figure 35. ^{1}H NMR of compound k-3-6

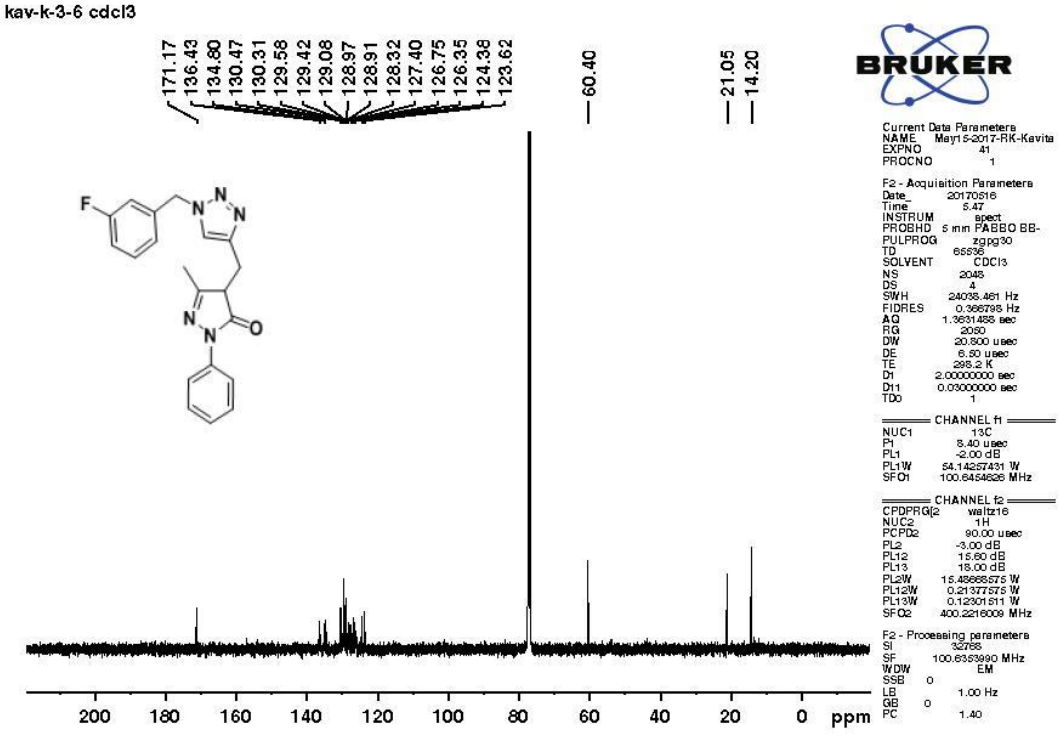


Figure 36. ^{13}C NMR of compound k-3-6

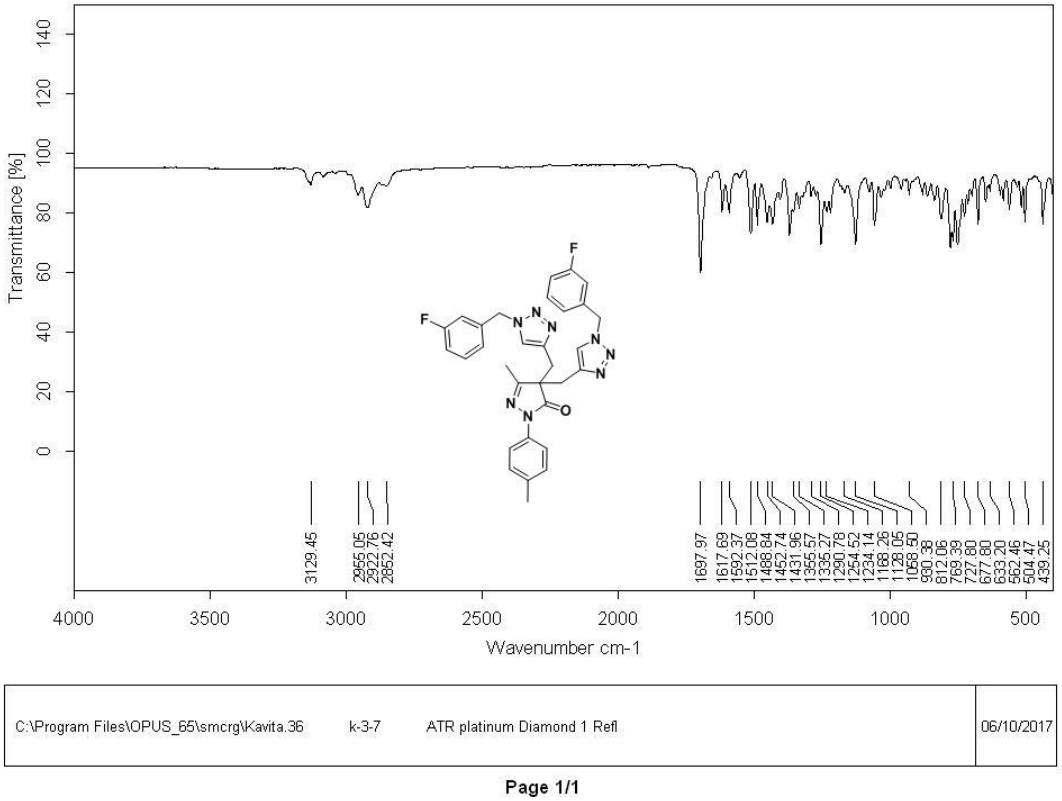


Figure 37. IR spectrum of compound k-3-7

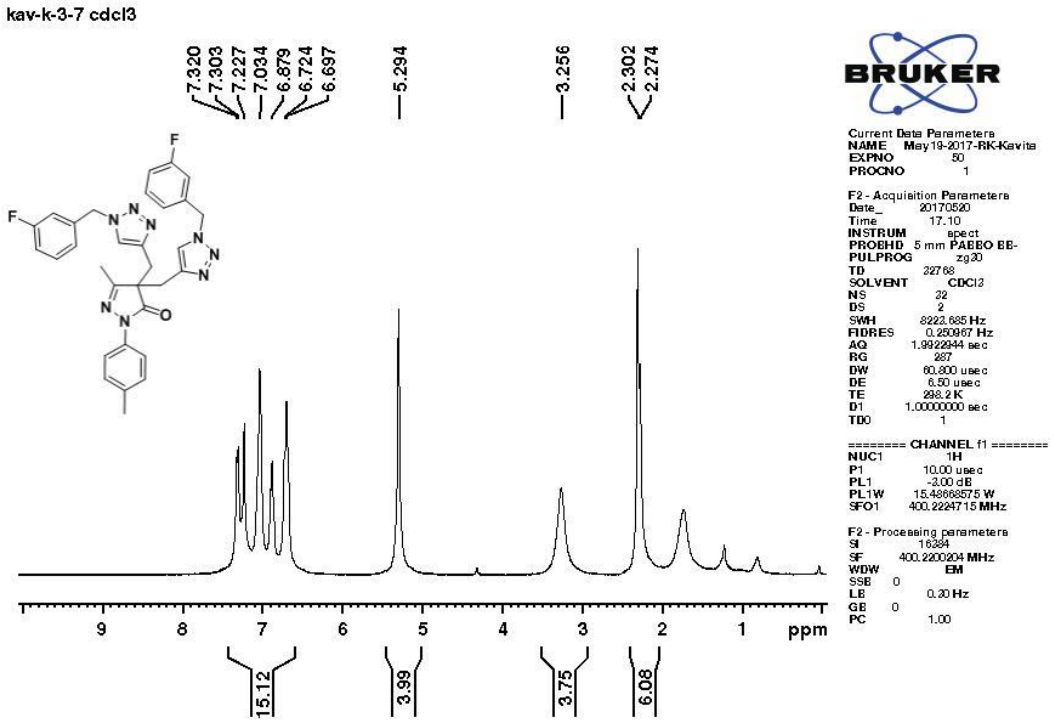


Figure 38. ^{1}H NMR of compound k-3-7

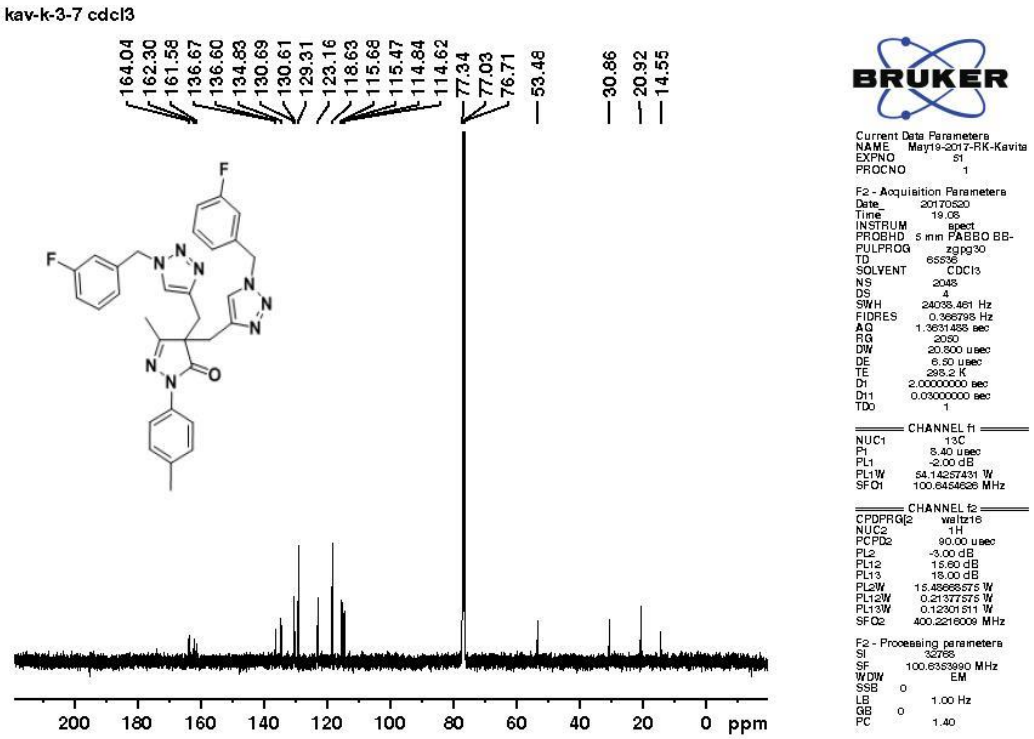


Figure 39. ^{13}C NMR of compound k-3-7

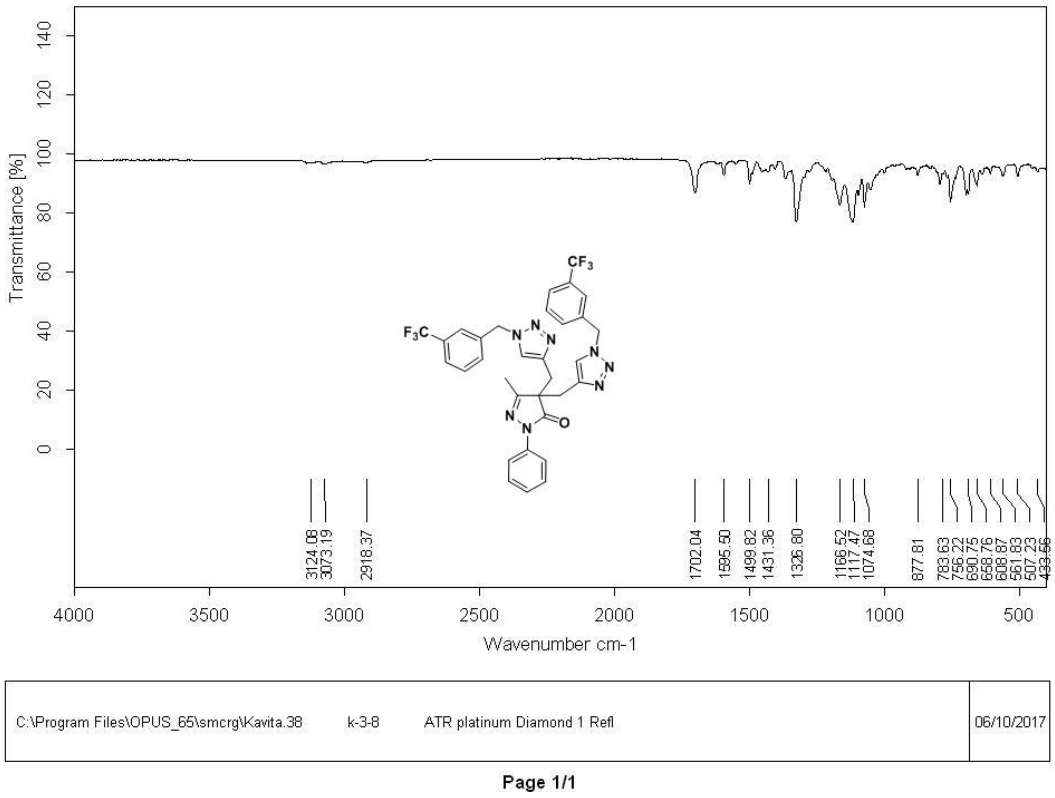


Figure 40. IR spectrum of compound k-3-8

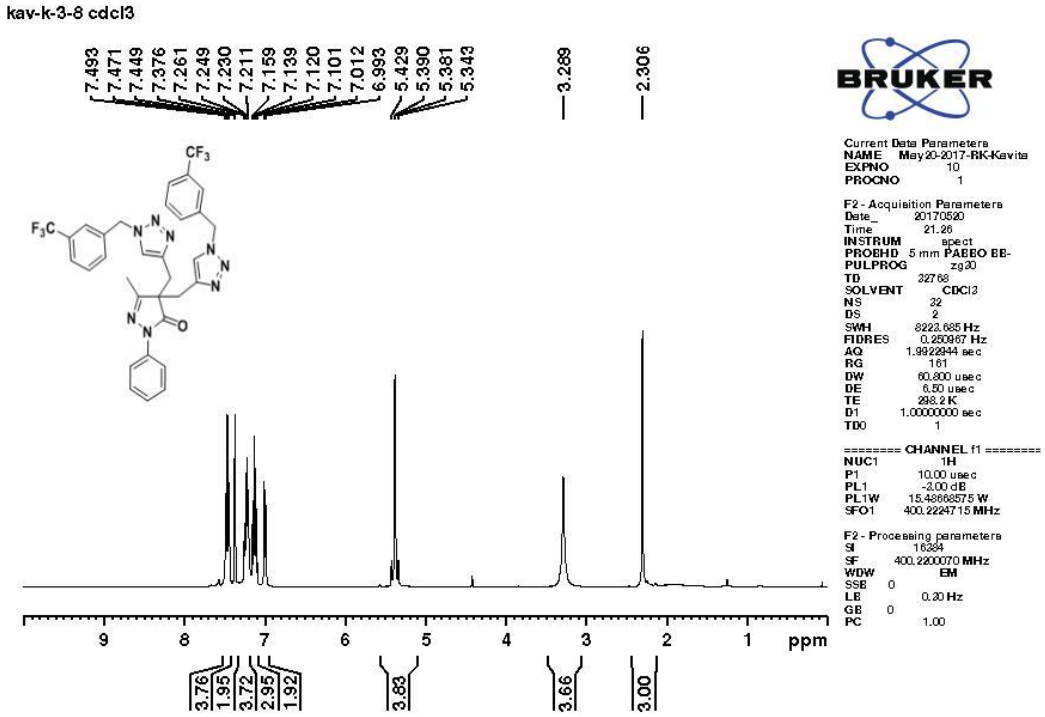


Figure 41. ^{1}H NMR of compound k-3-8

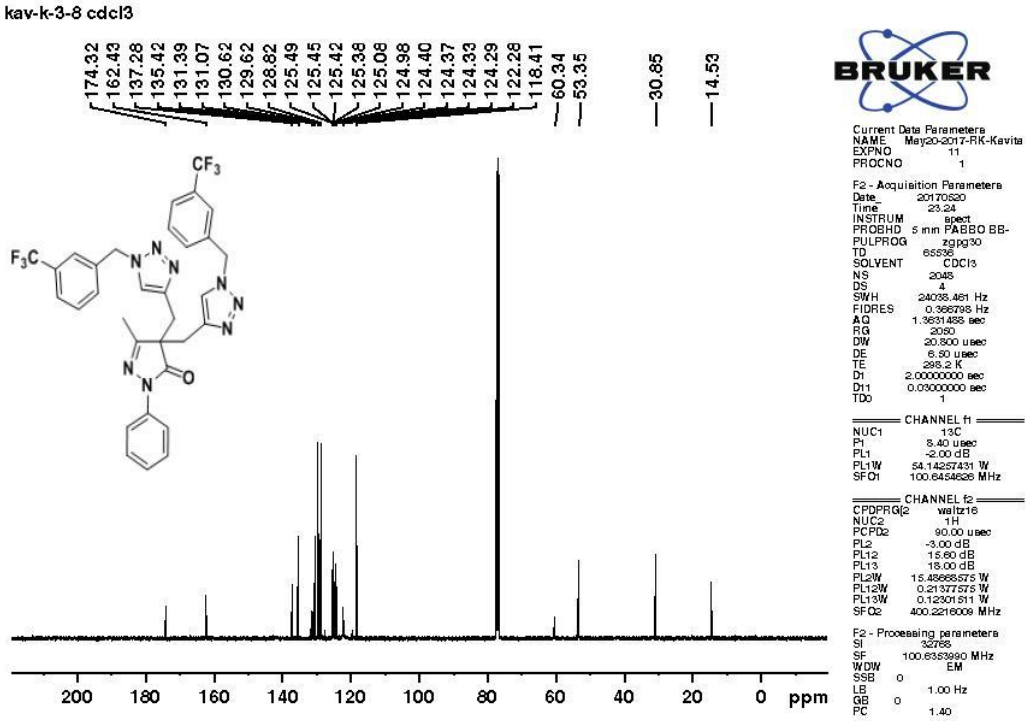


Figure 42. ^{13}C NMR of compound k-3-8

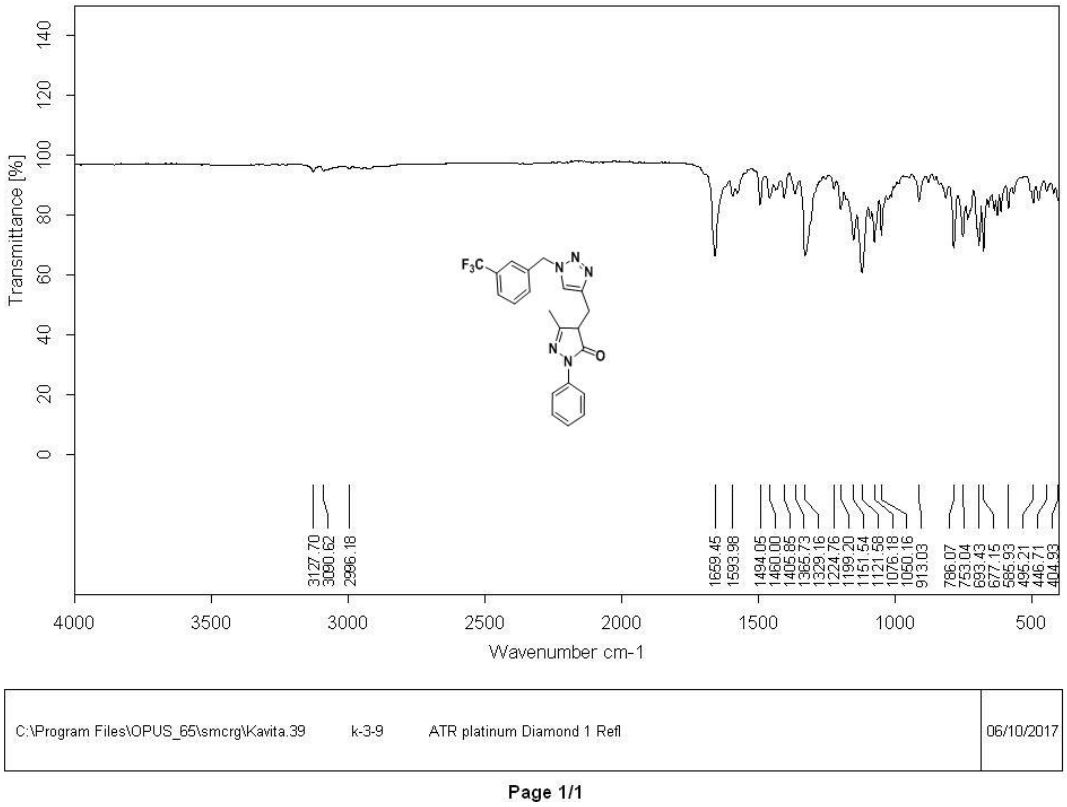


Figure 43. IR spectrum of compound k-3-9

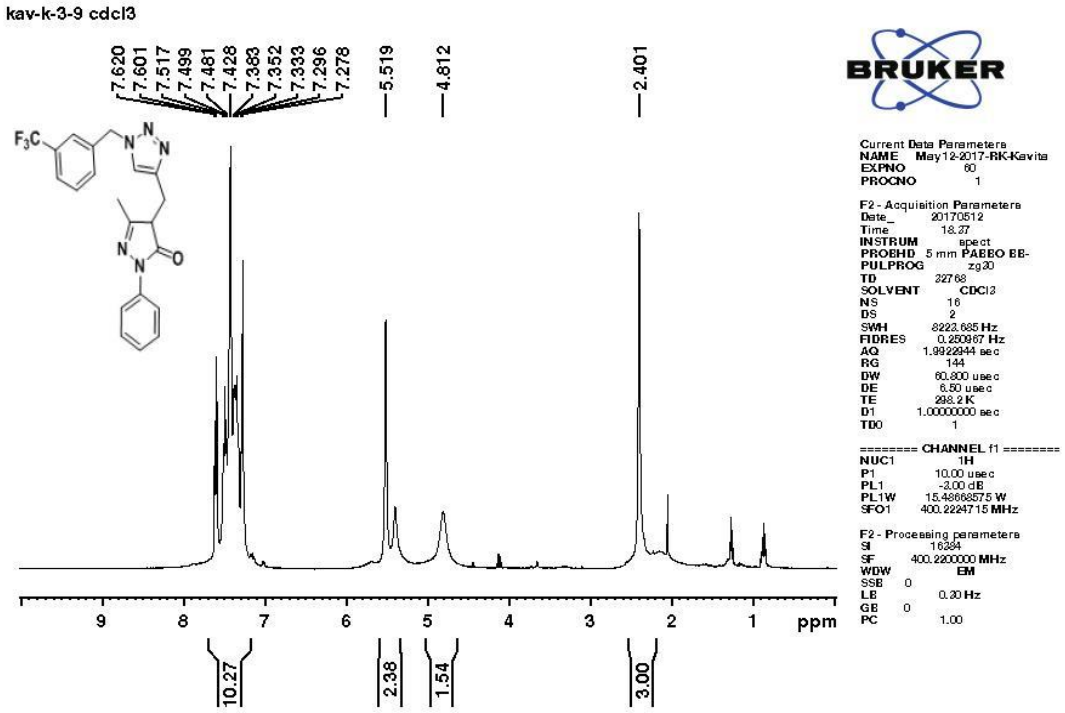


Figure 44. ^{1}H NMR of compound k-3-9

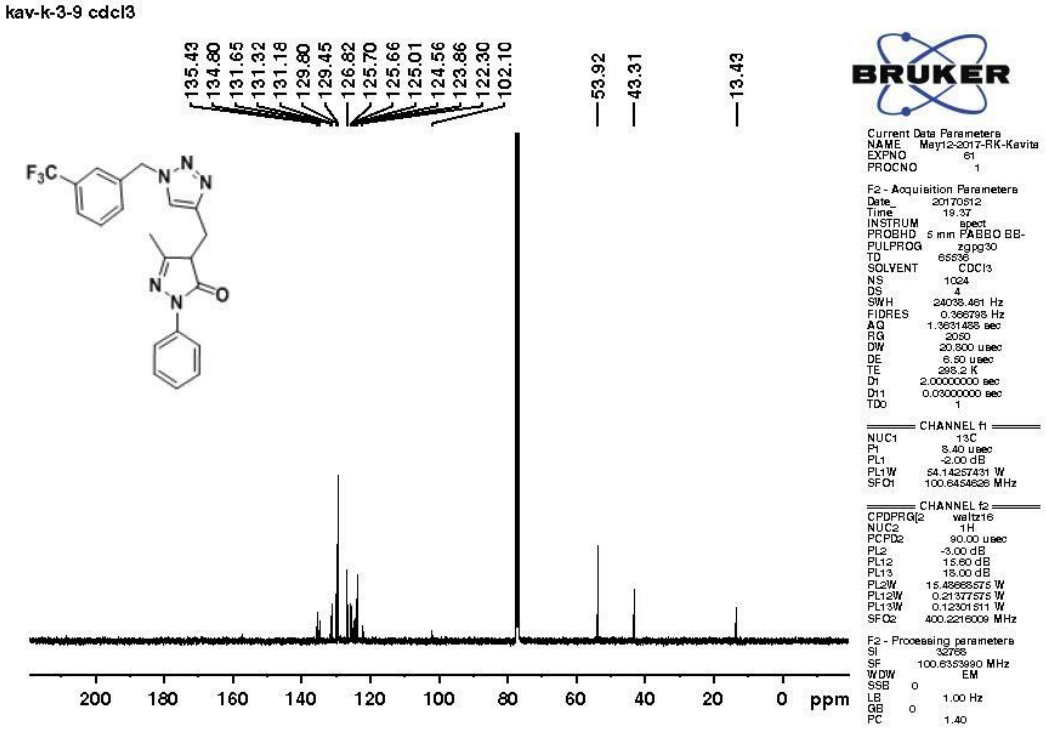


Figure 45. ^{13}C NMR of compound k-3-9

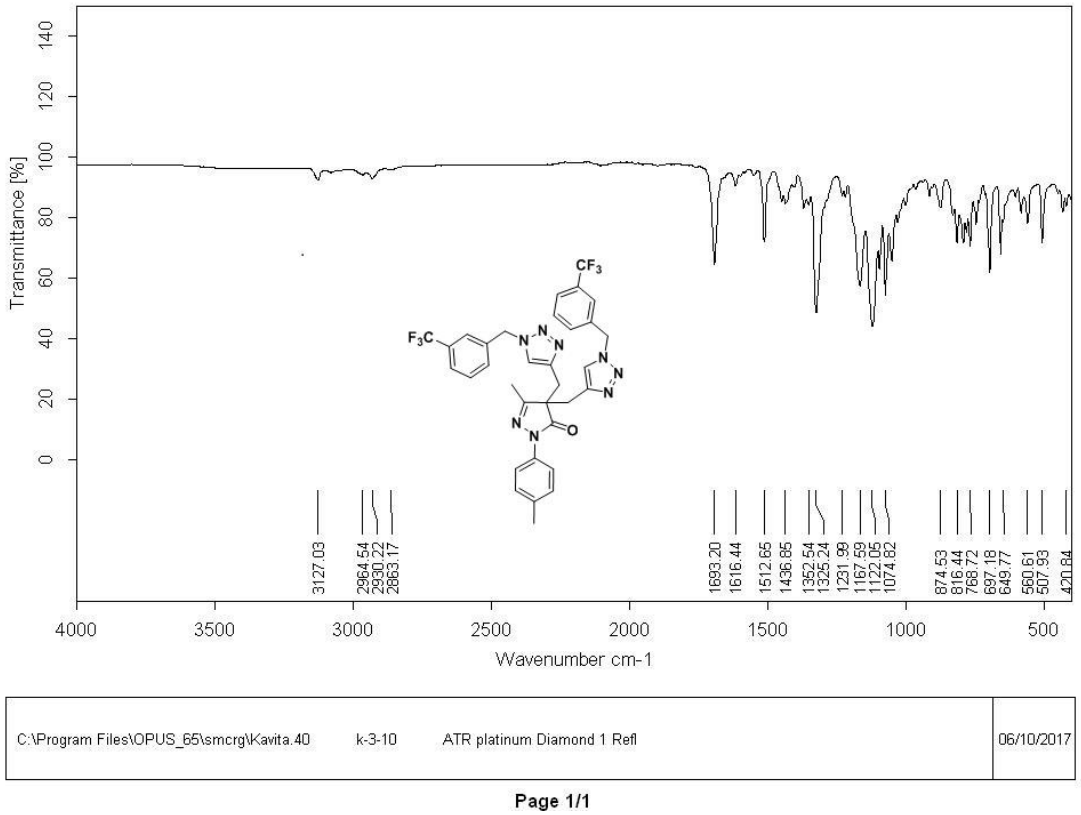


Figure 46. IR spectrum of compound k-3-10

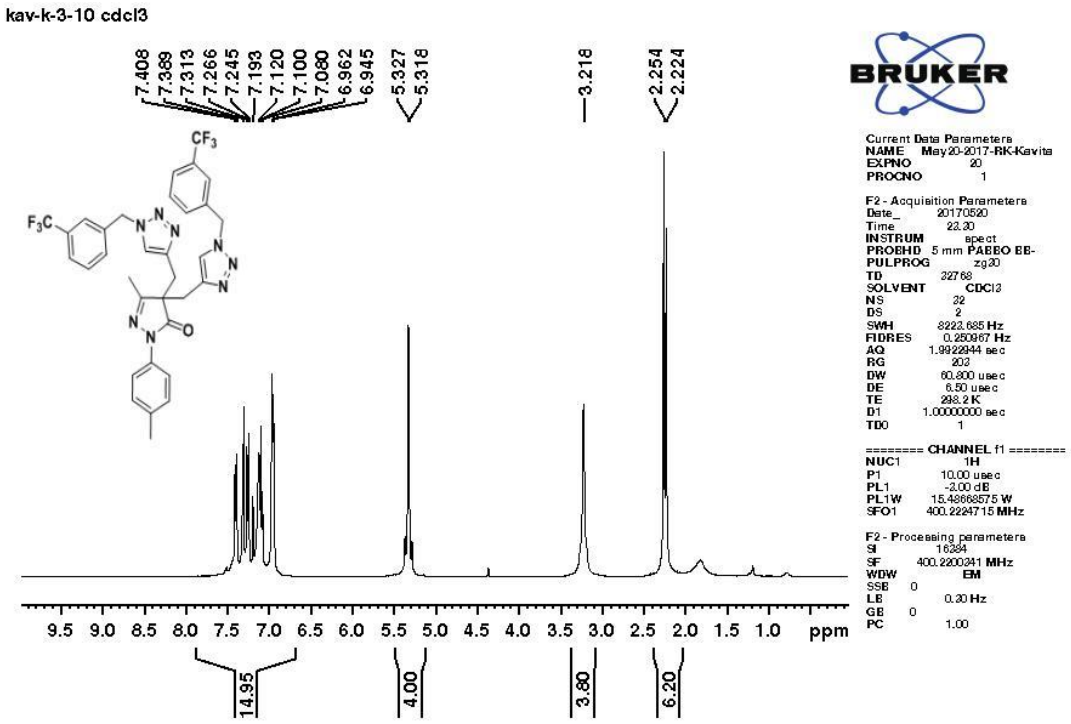


Figure 47.[1]H NMR of compound k-3-10

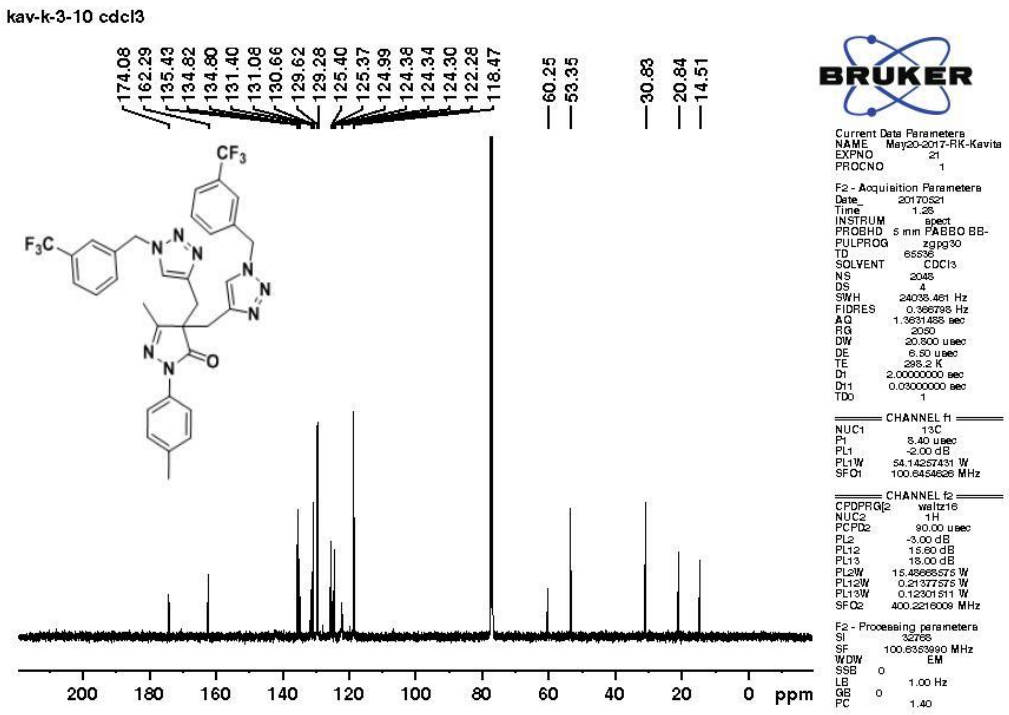


Figure 48. ^{13}C NMR of compound k-3-10

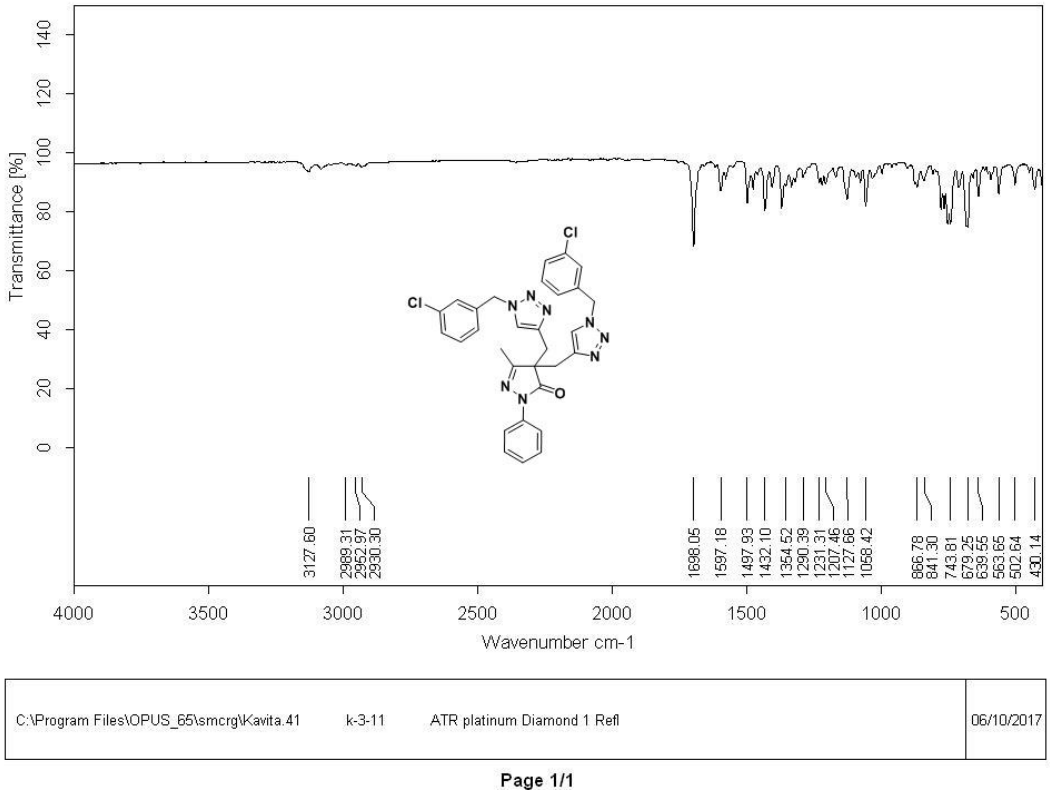


Figure 49. IR spectrum of compound k-3-11

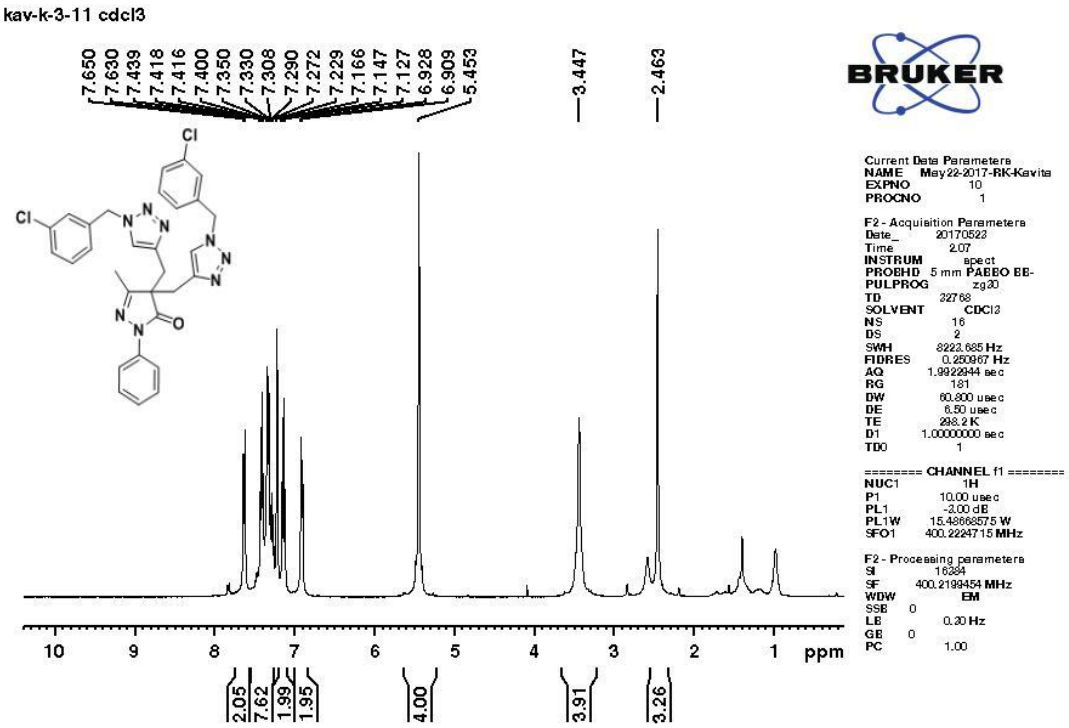


Figure 50.[1]H NMR of Compound k-3-11

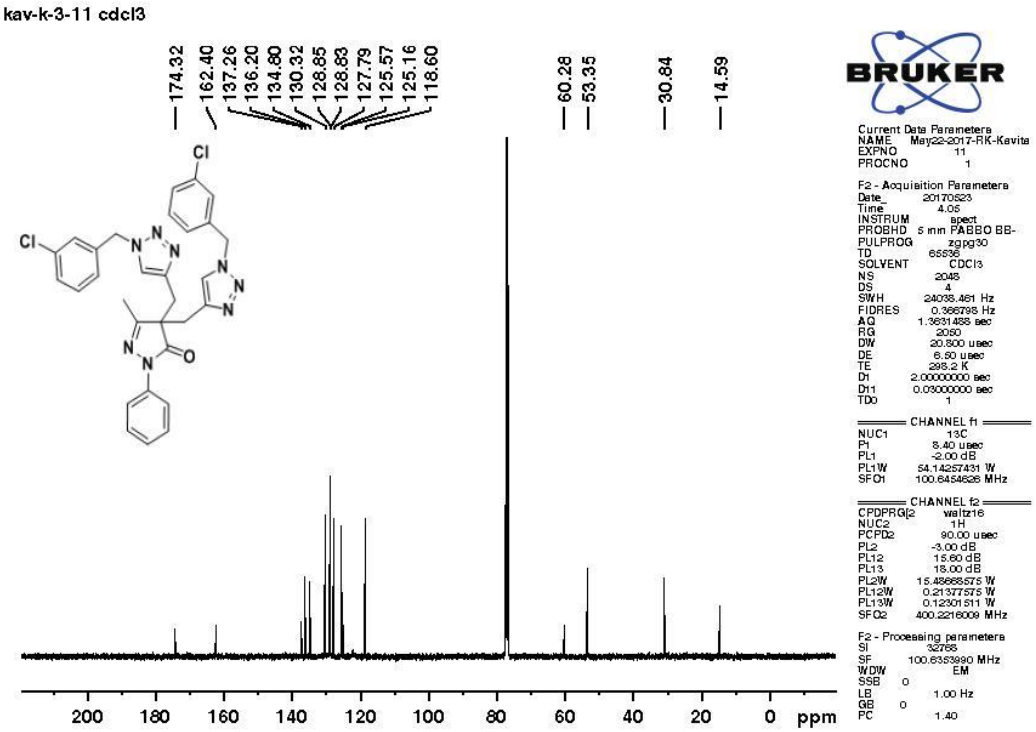


Figure 51.[13]C NMR of compound k-3-11

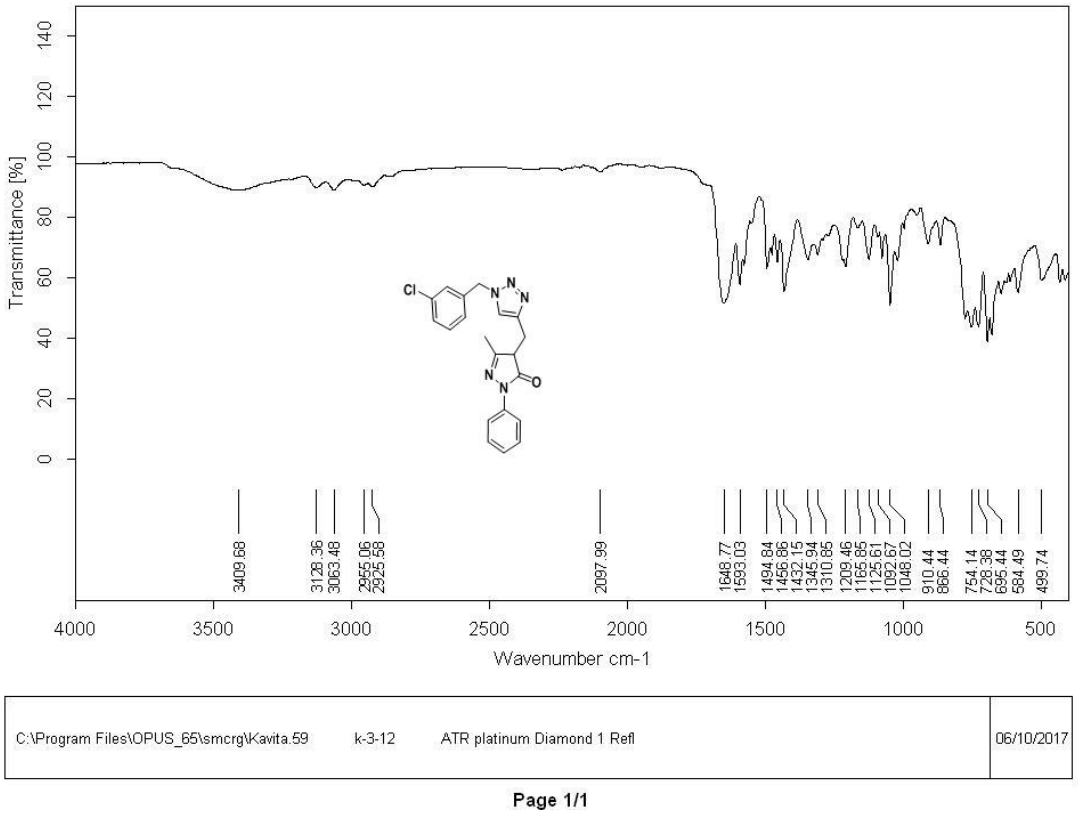


Figure 52. IR spectrum of compound k-3-12

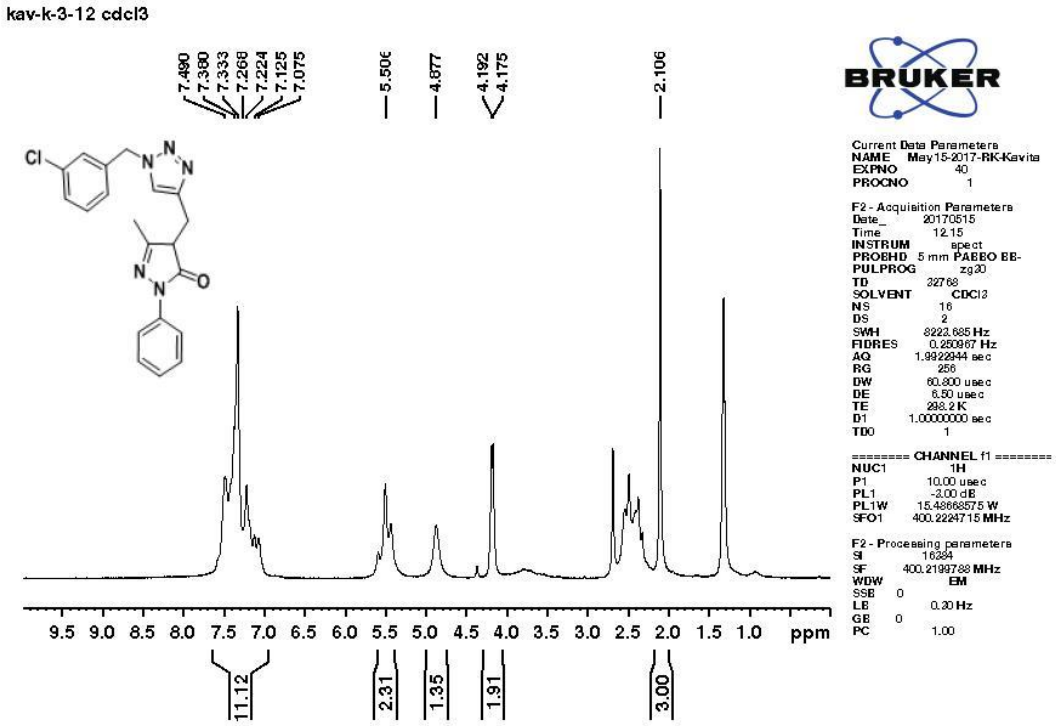


Figure 53.[1]H NMR of compound k-3-12

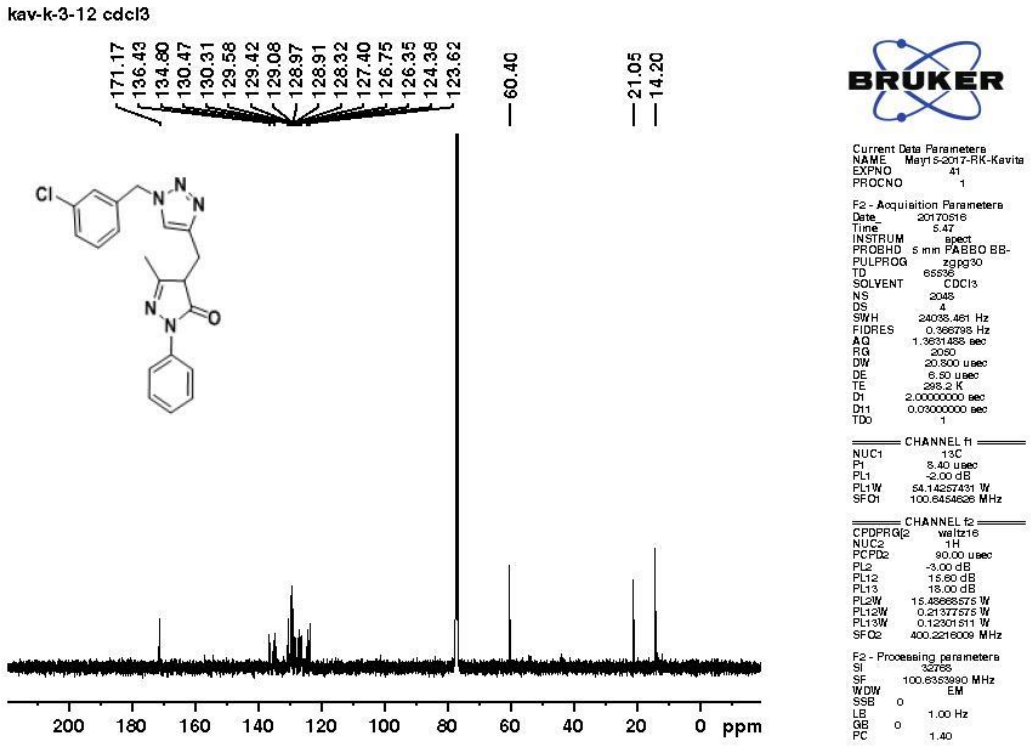


Figure 54. ^{13}C NMR of compound k-3-12

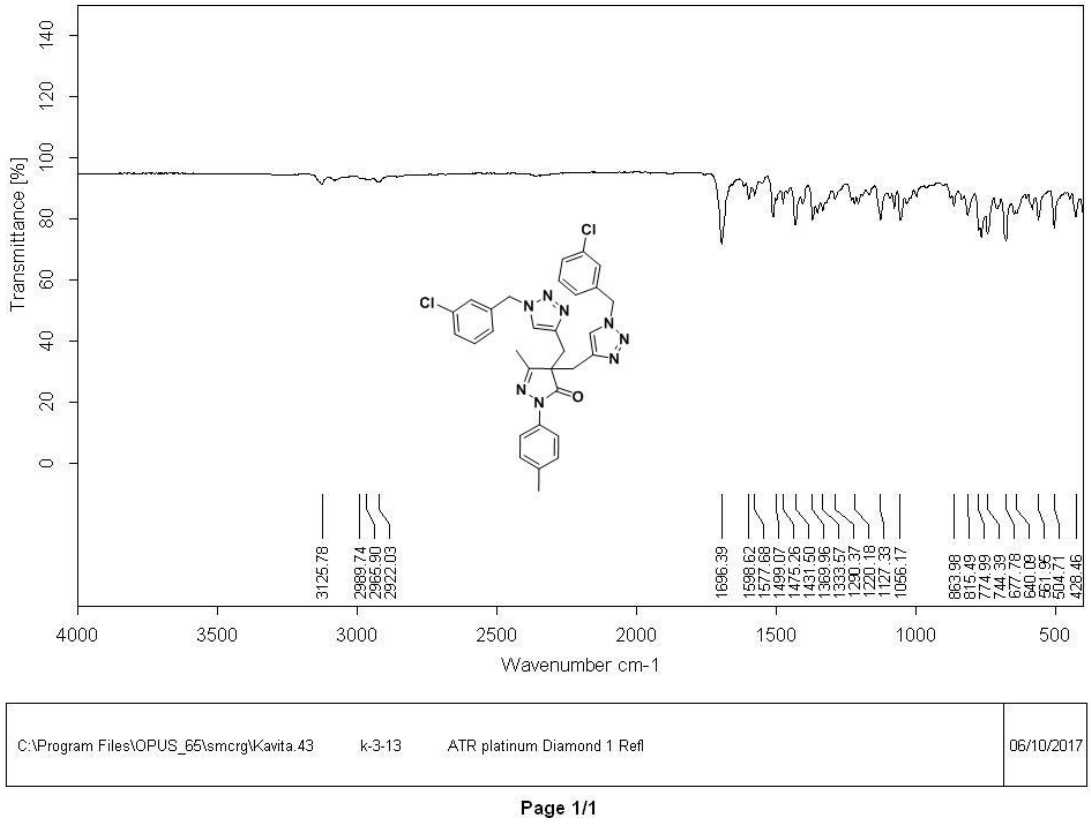


Figure 55. IR spectrum of compound k-3-13

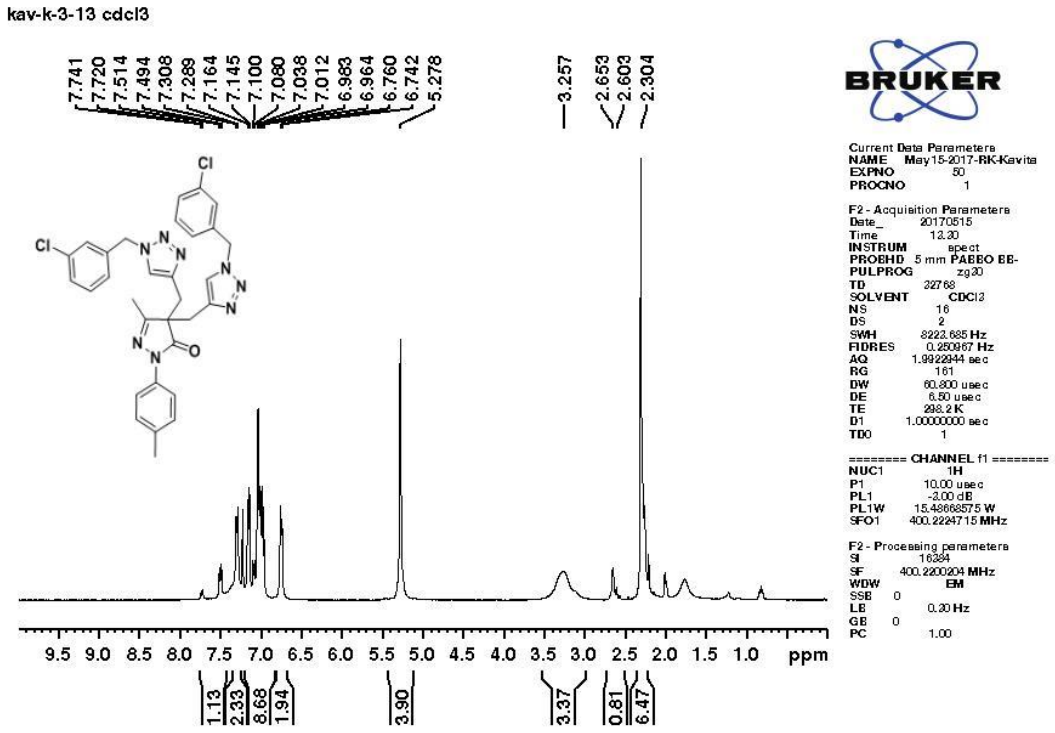


Figure 56.[1]H NMR of compound k-3-13

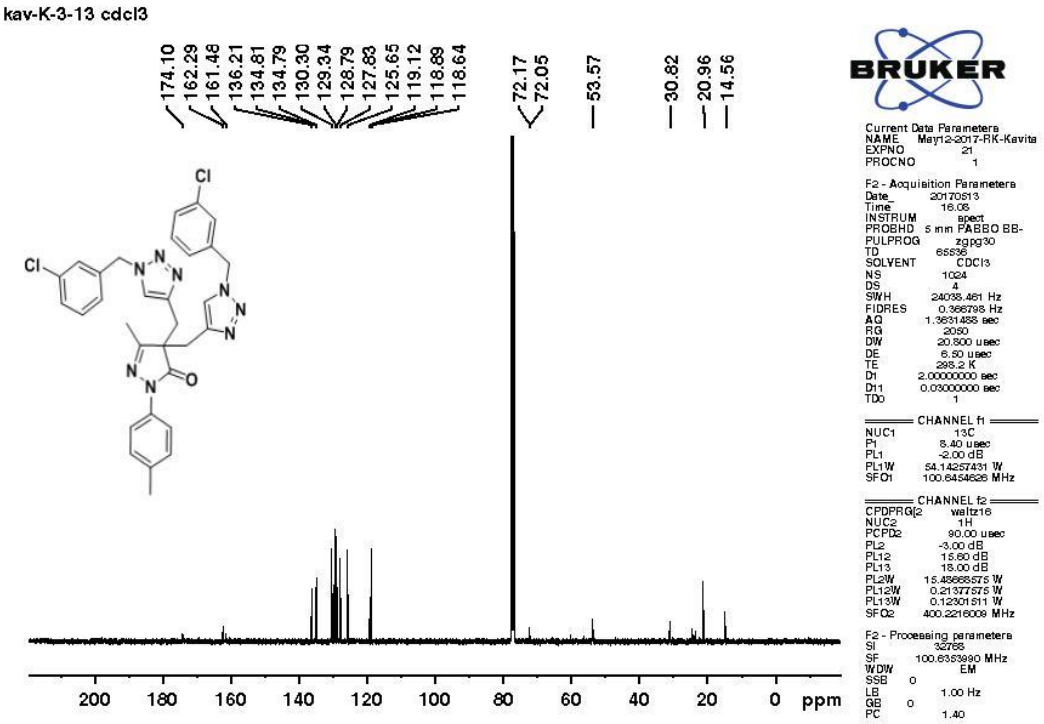


Figure 57.[13]C NMR of compound k-3-13

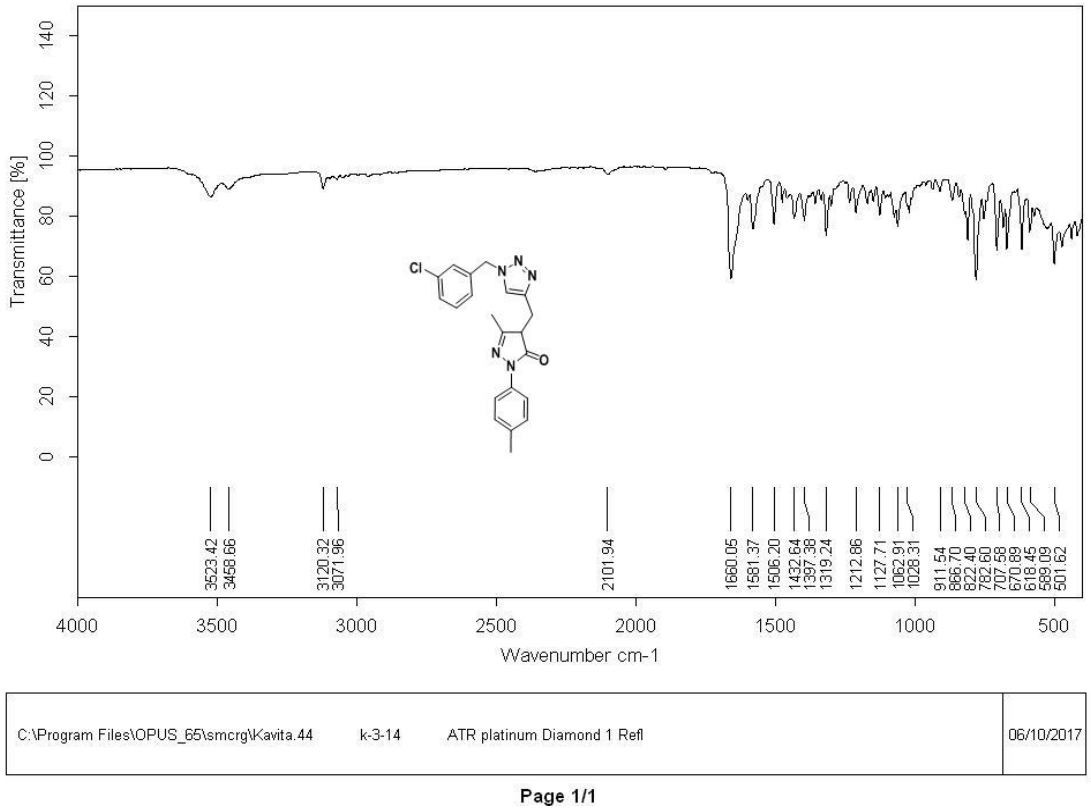


Figure 58. IR spectrum of compound k-3-14

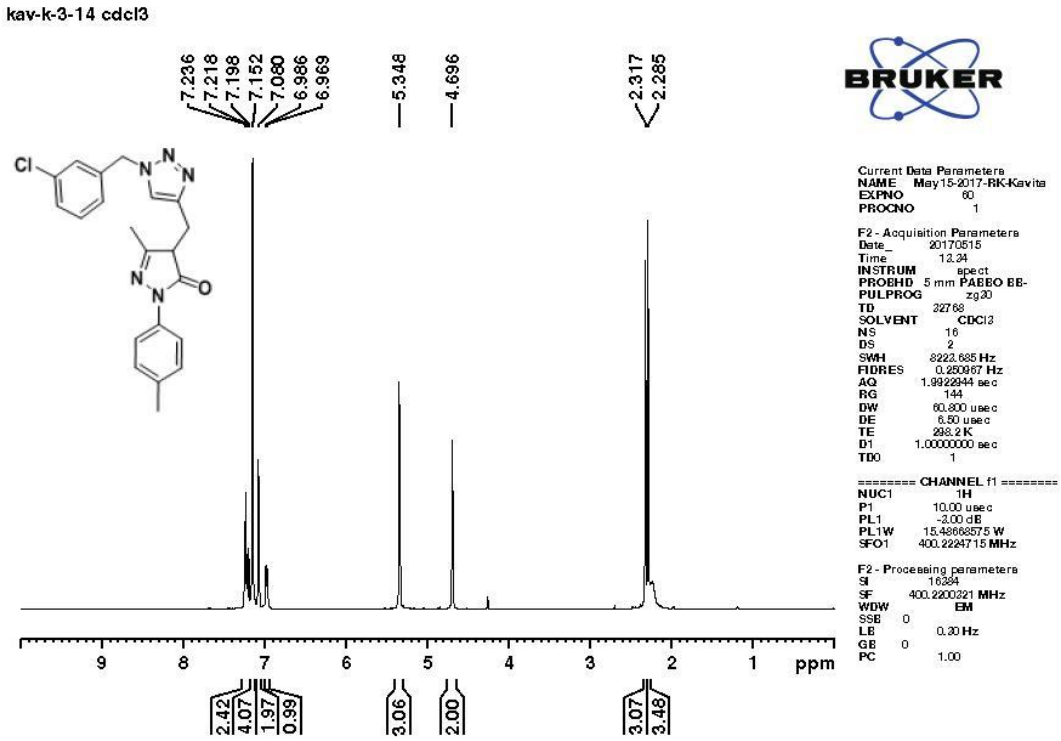


Figure 59.[1]H NMR of compound k-3-14

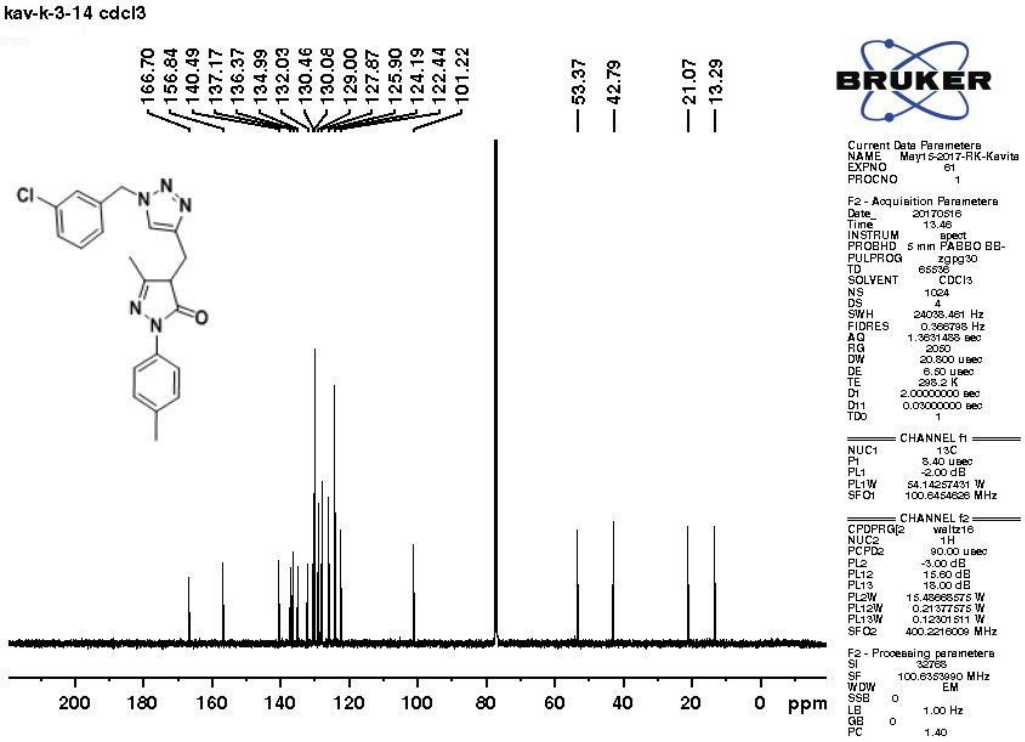


Figure 60.^{13}C NMR of compound k-3-14

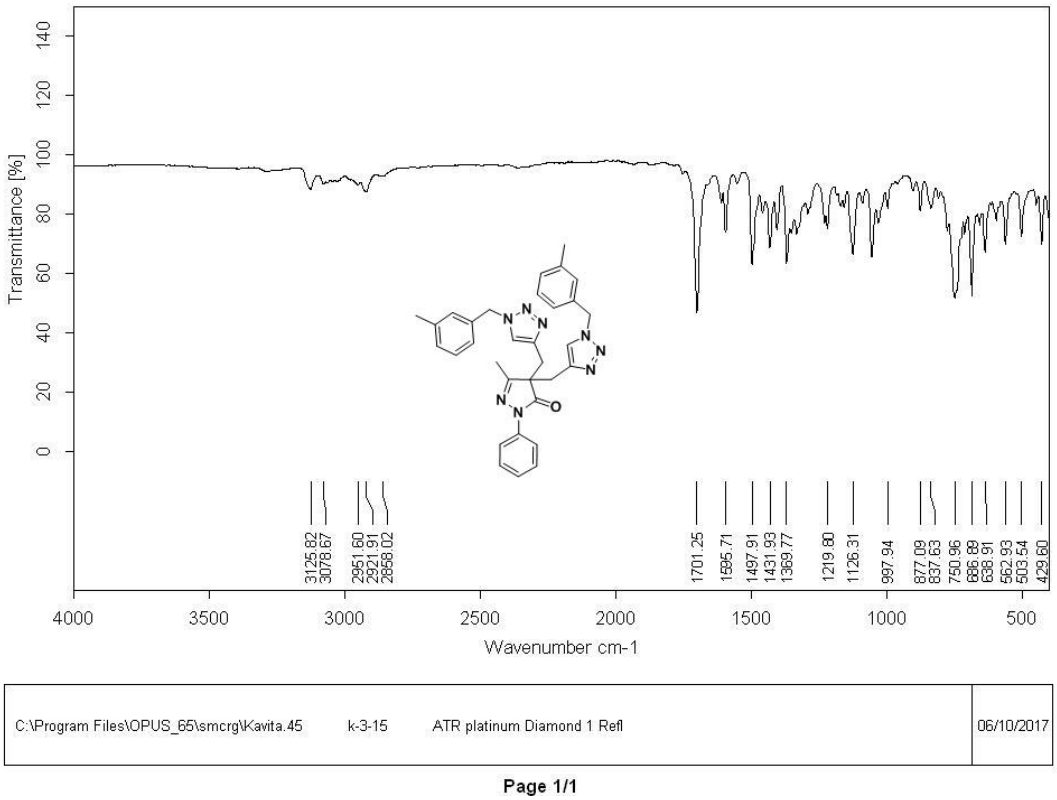


Figure 61. IR spectrum of compound k-3-15

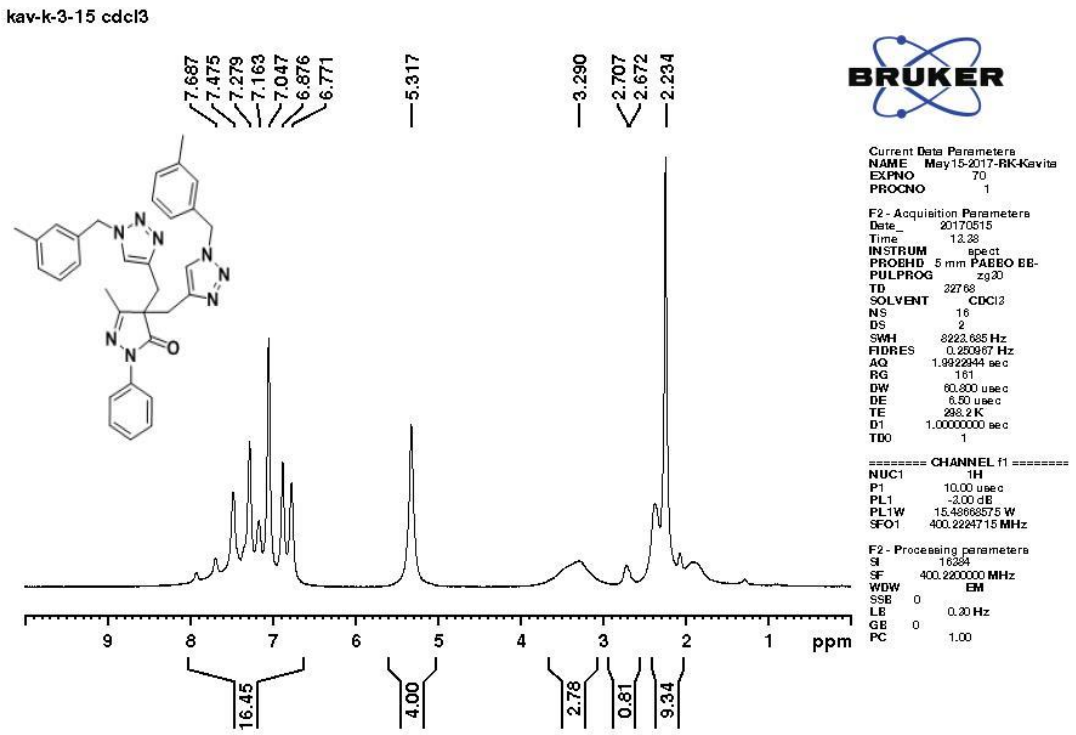


Figure 62.[1]H NMR of compound k-3-15

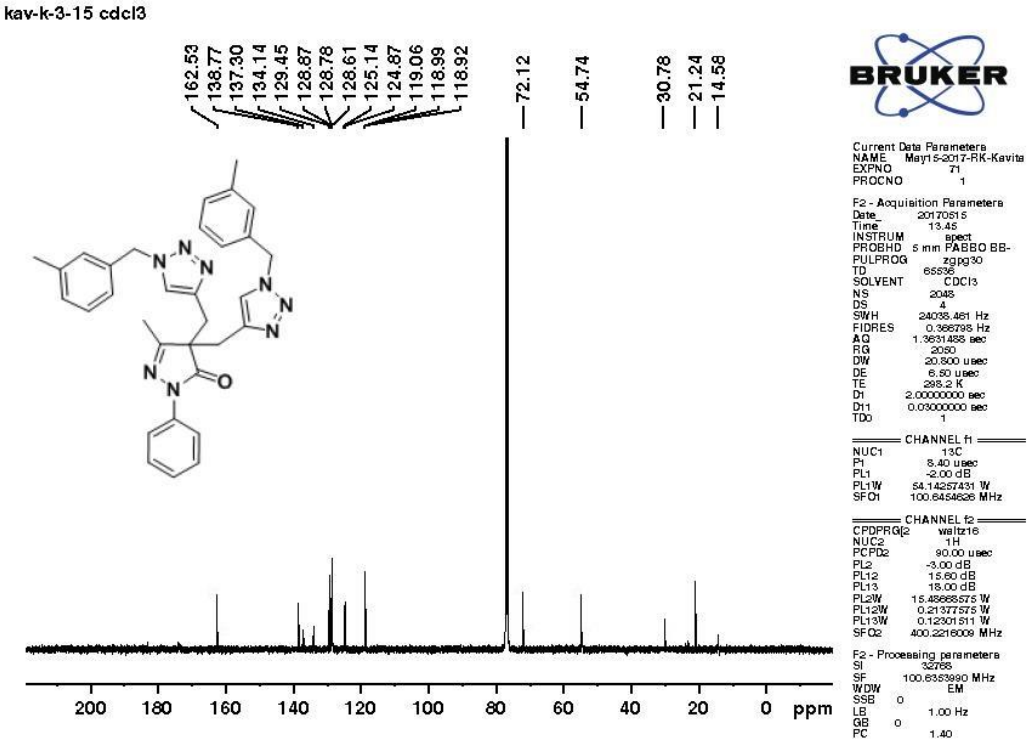


Figure 63.[13]C NMR of Compound k-3-15

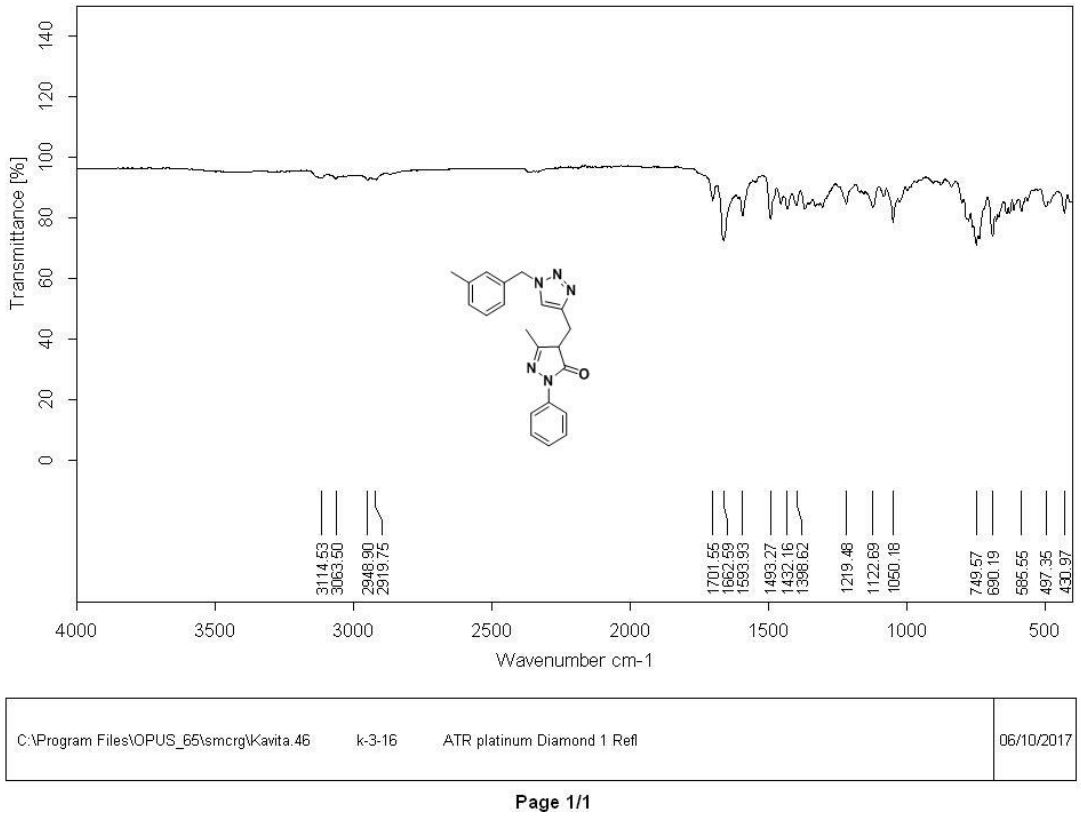


Figure 64. IR spectrum of compound k-3-16

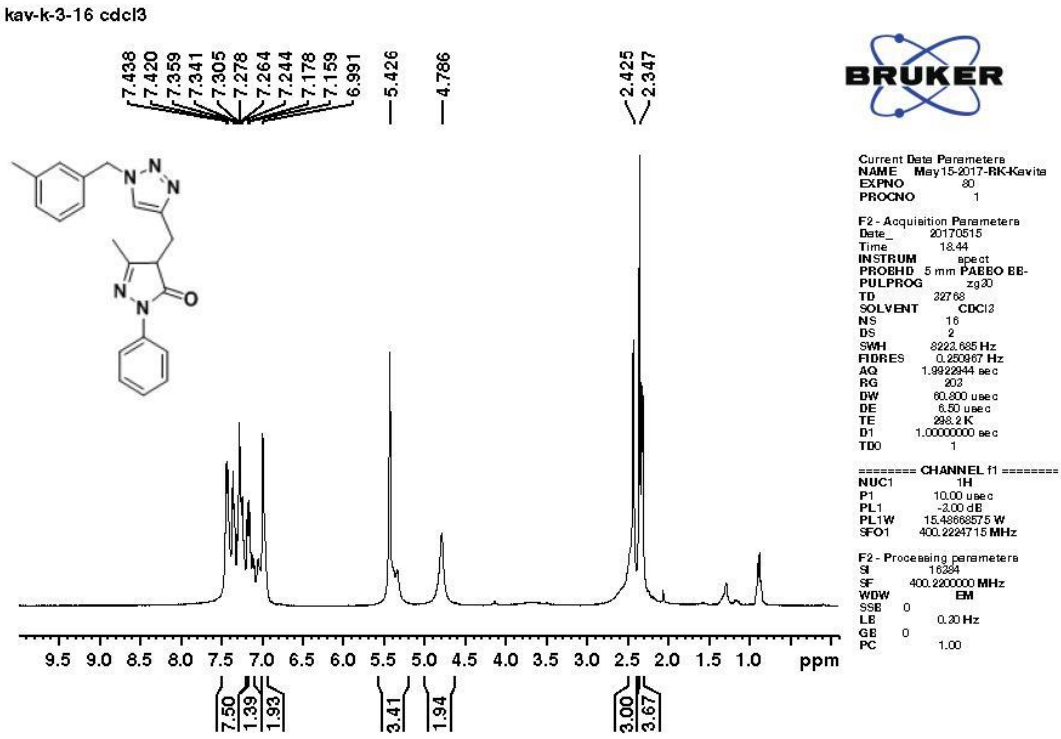


Figure 65. ^{1}H NMR of compound k-3-16

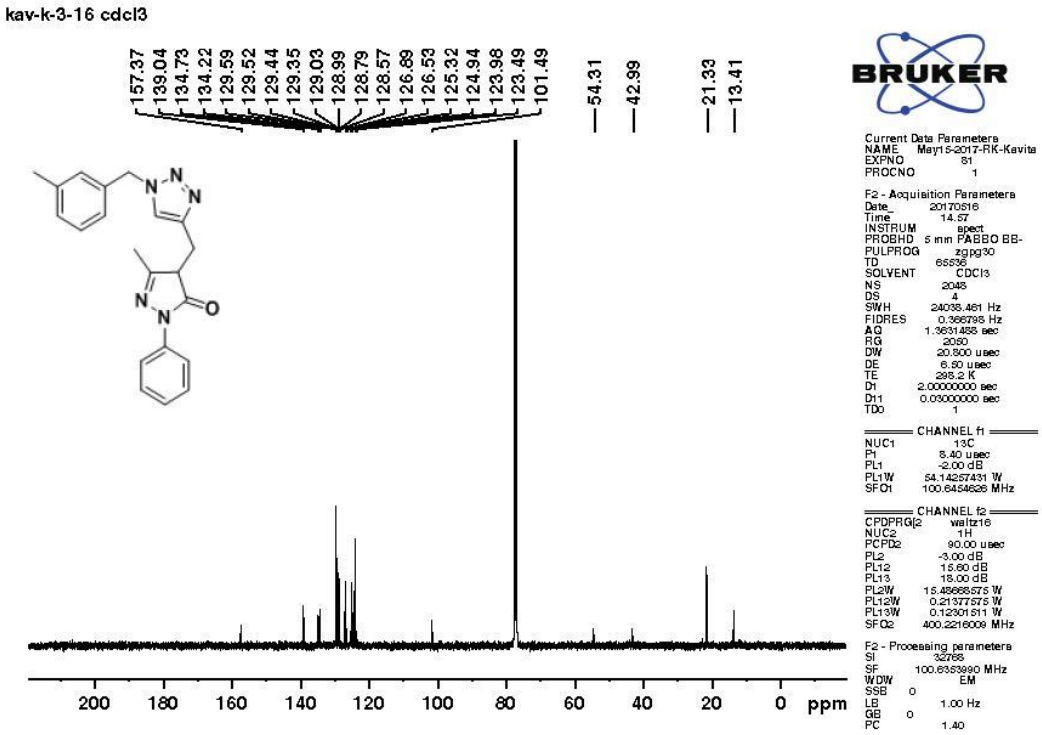


Figure 66. ^{13}C NMR of compound k-3-16

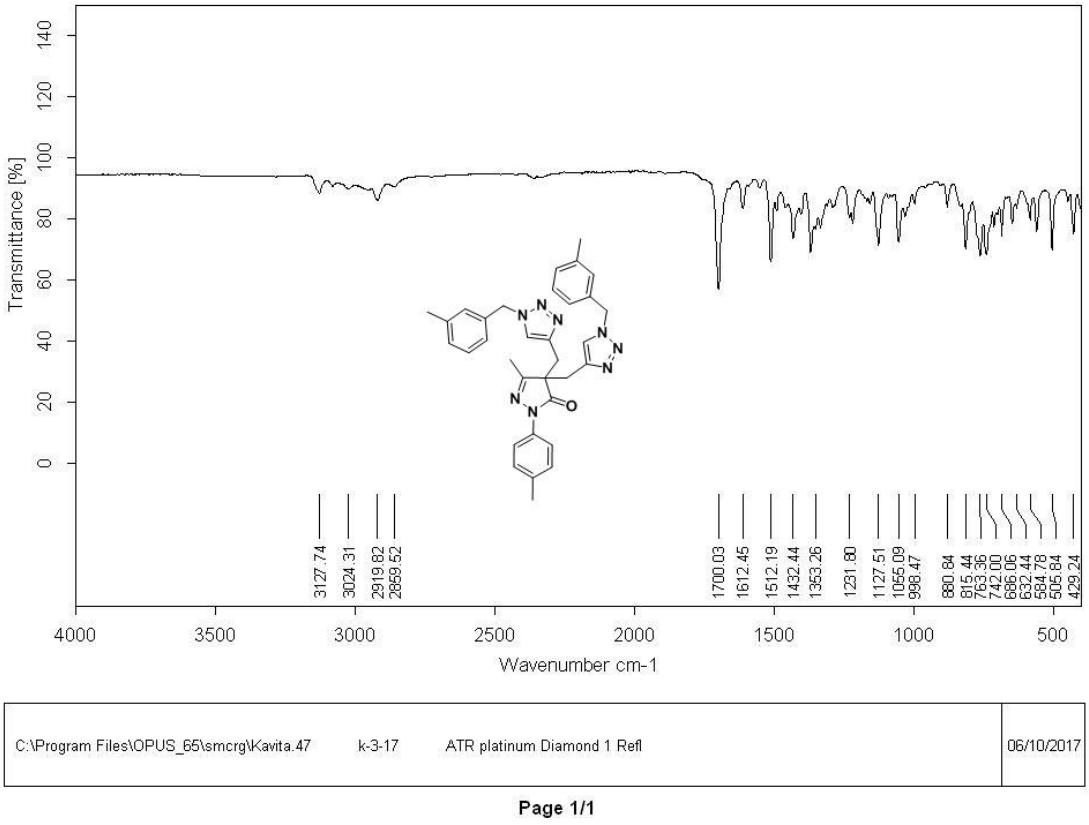


Figure 67. IR spectrum of compound k-3-17

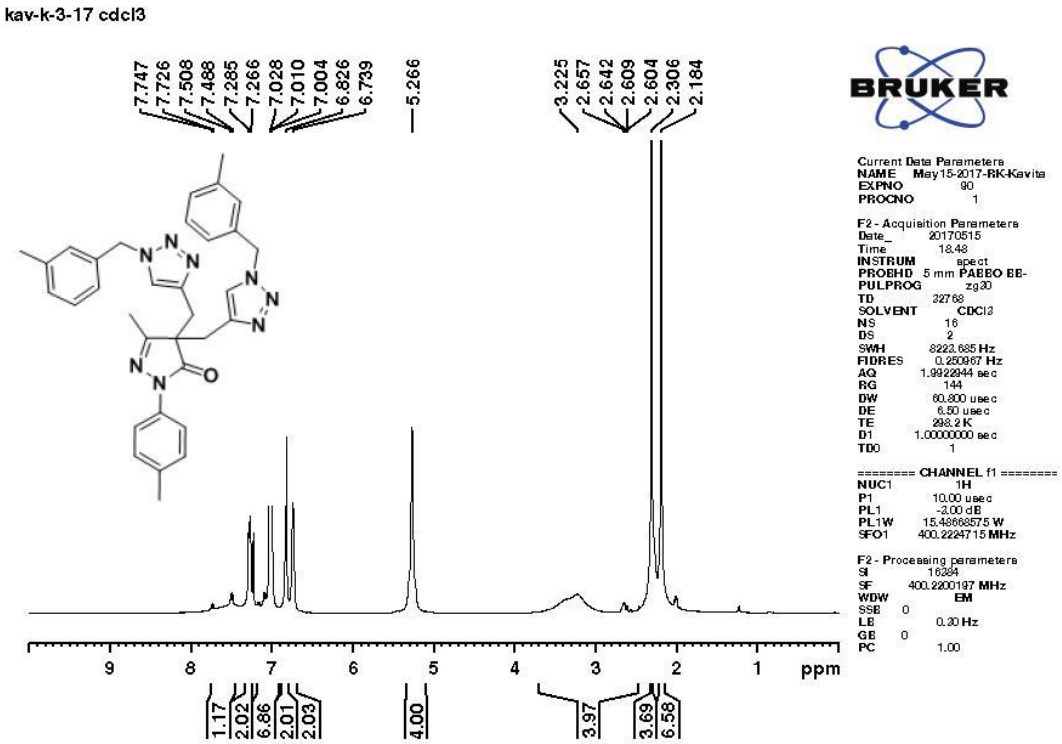


Figure 68. ^{1}H NMRof compound k-3-17

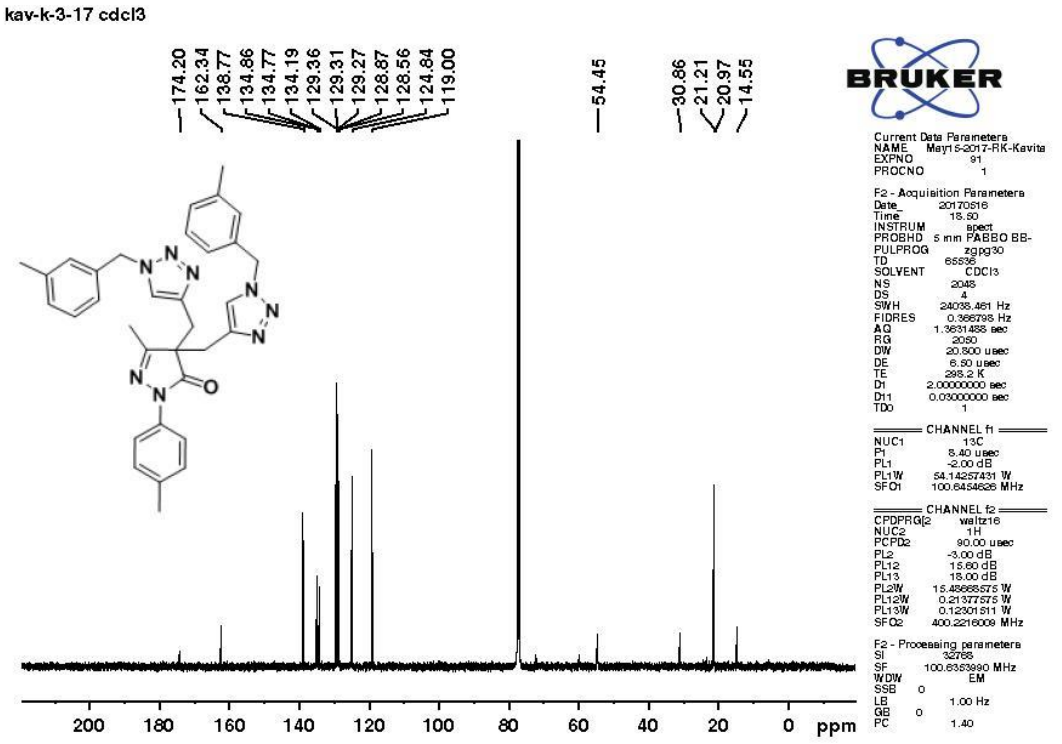


Figure 69. ^{13}C NMR of compound k-3-17

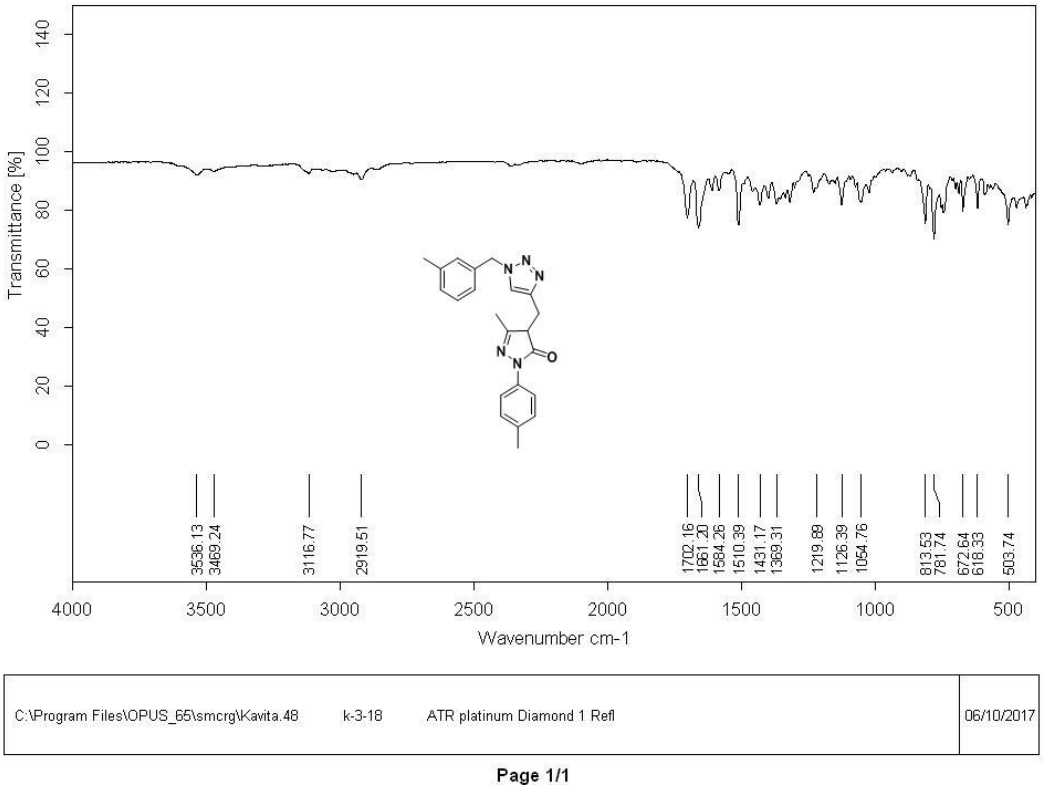


Figure 70. IR spectrum of compound k-3-18

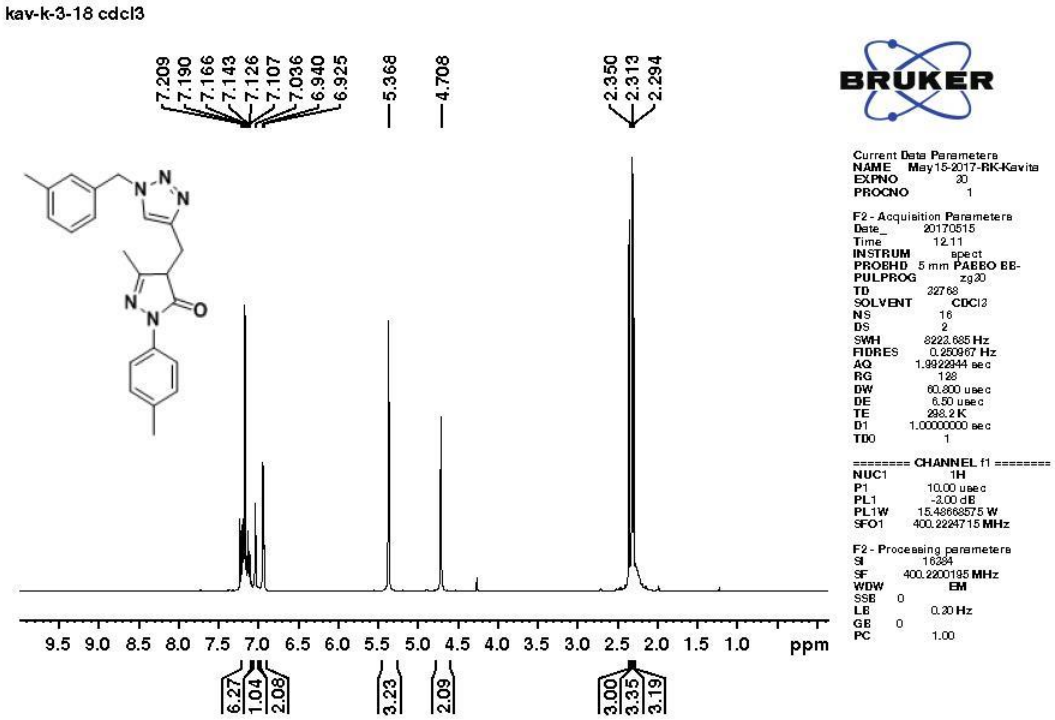


Figure 71.[1]H NMR of compound k-3-18

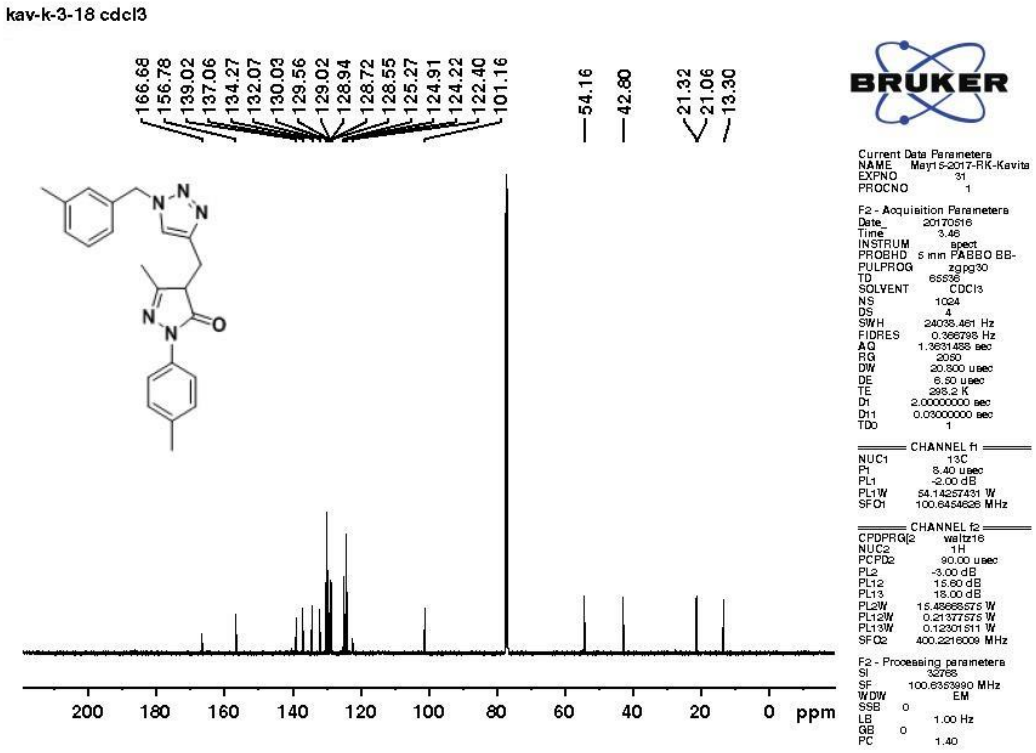


Figure 72. ^{13}C NMR of compound k-3-18

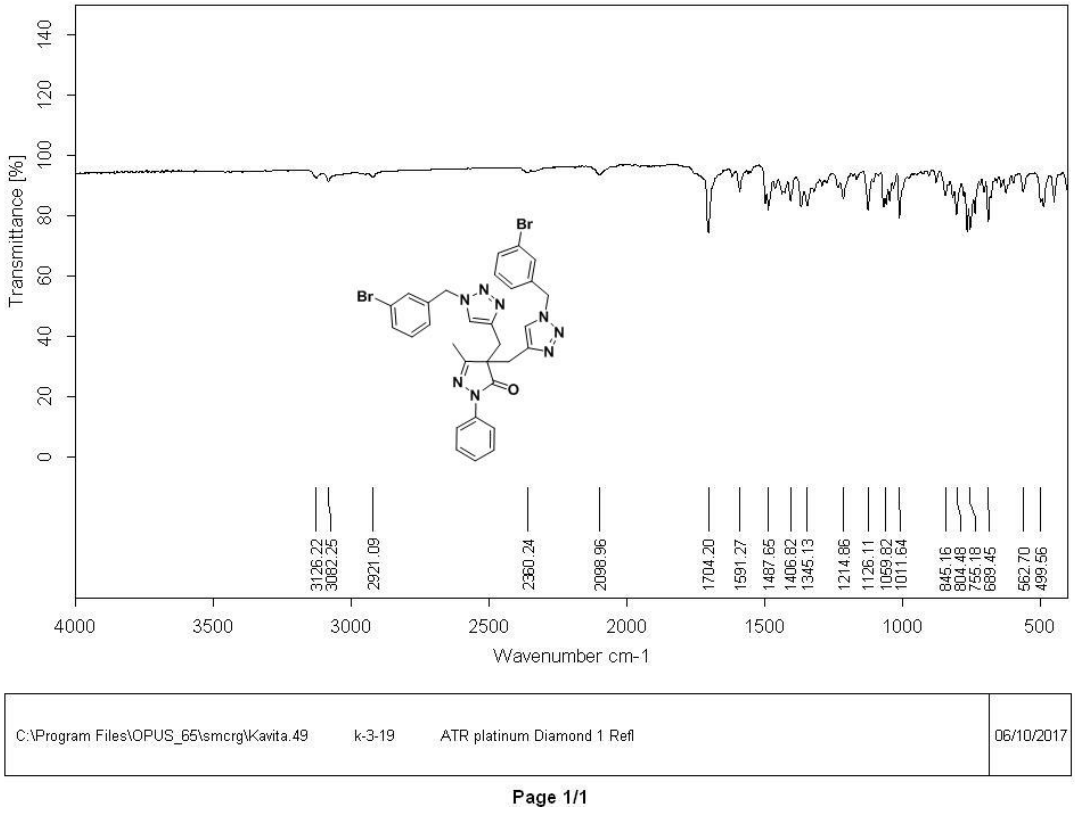


Figure 73. IR spectrum of compound k-3-19

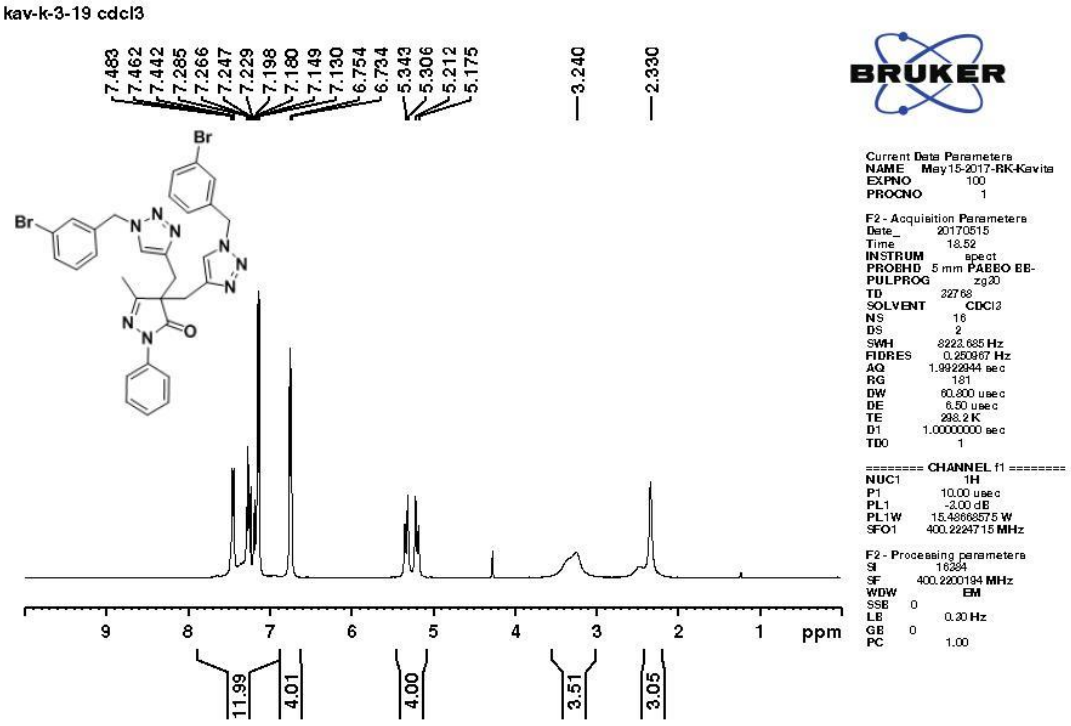


Figure 74.[1]H NMR of compound k-3-19

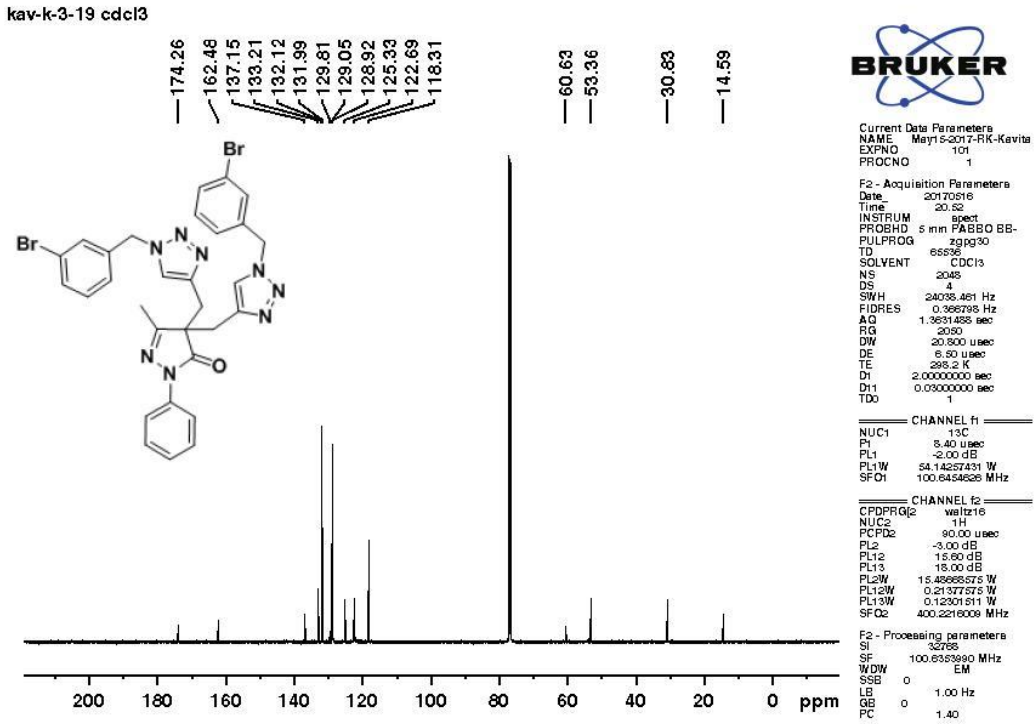


Figure 75.[13]C NMR of compound k-3-19

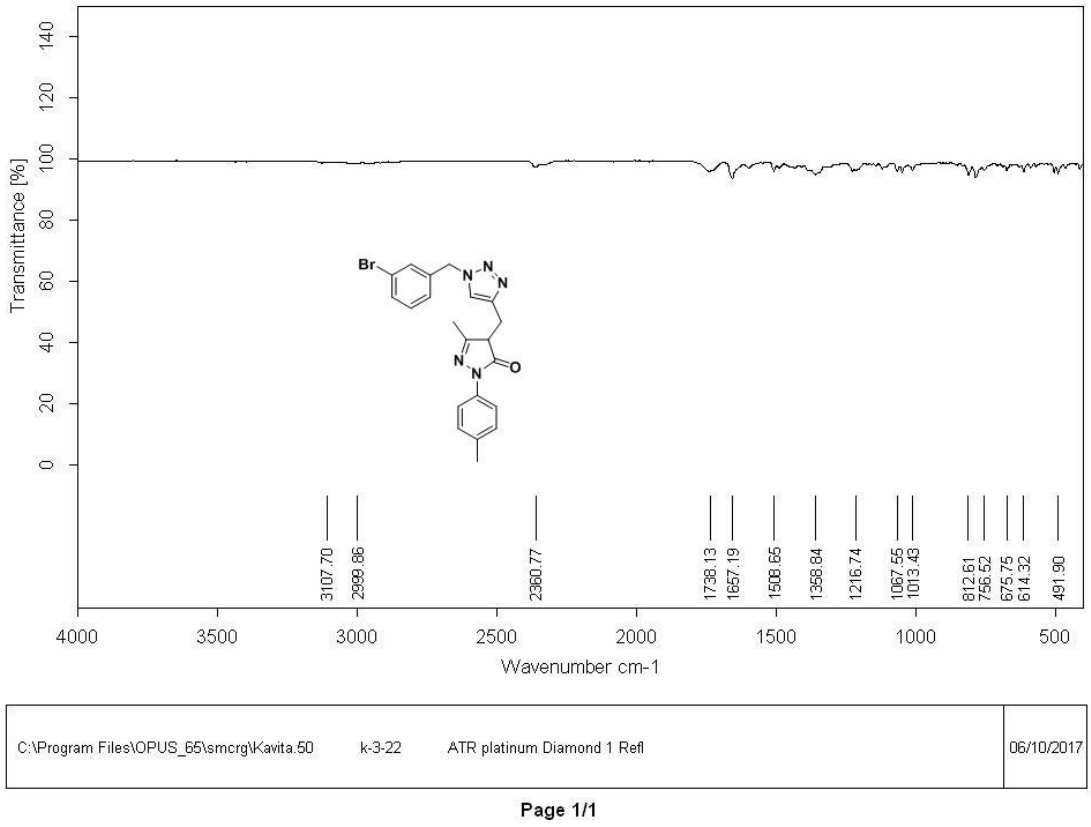


Figure 76. IR spectrum of compound k-3-22

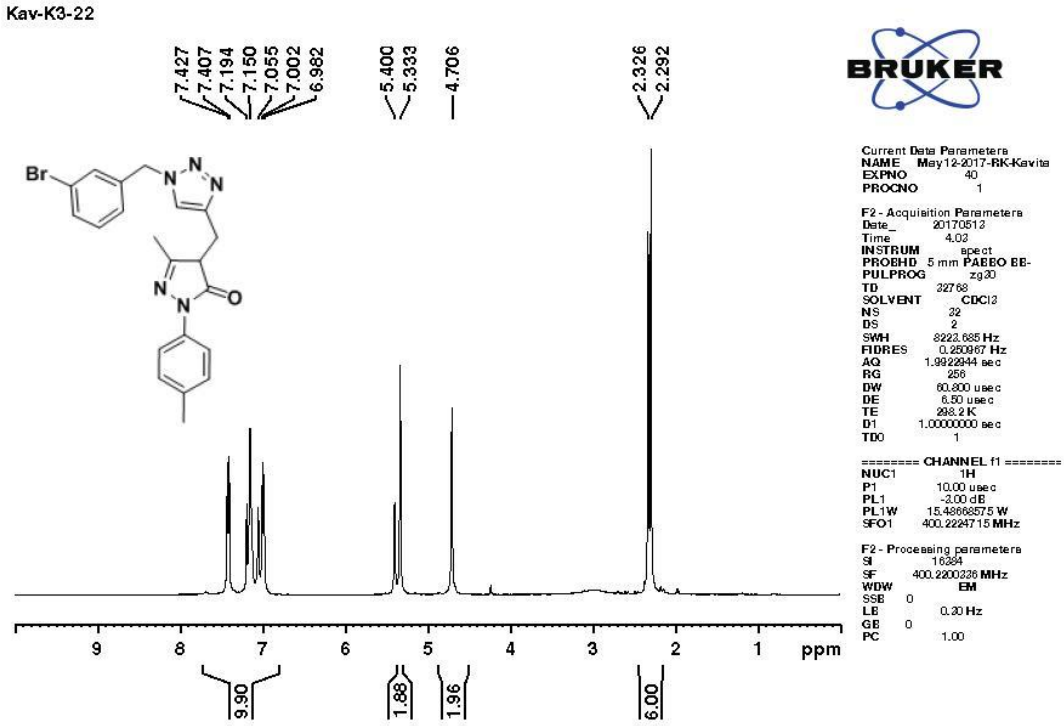


Figure 77.[1]H NMR of compound k-3-22

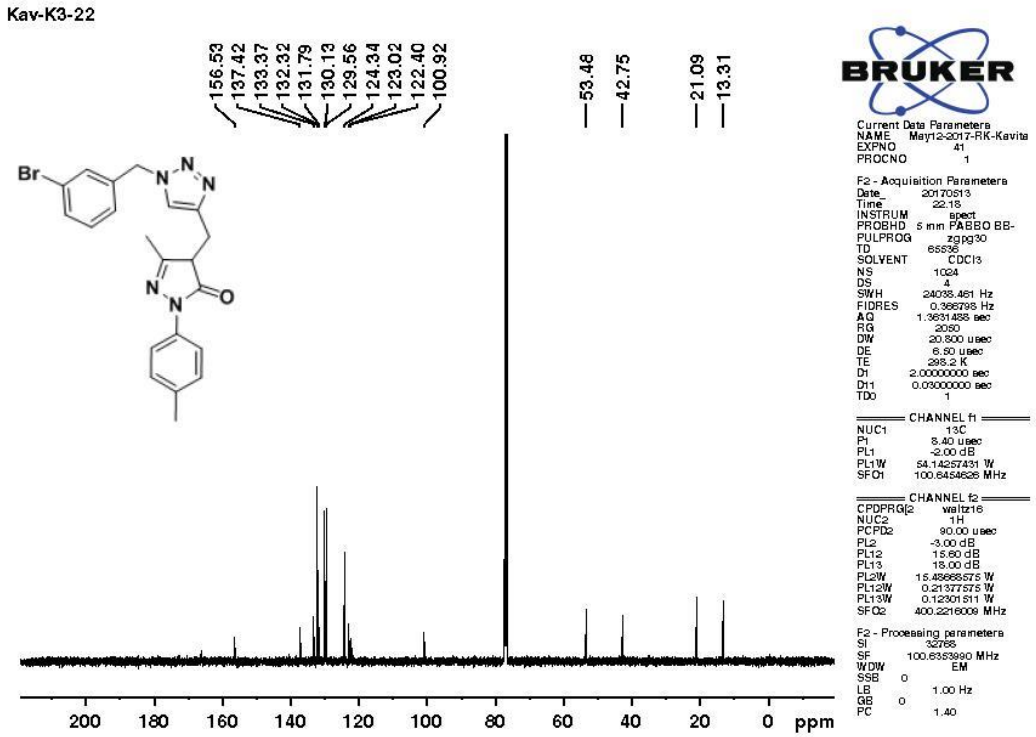


Figure 78.^{13}C NMR of compound k-3-22

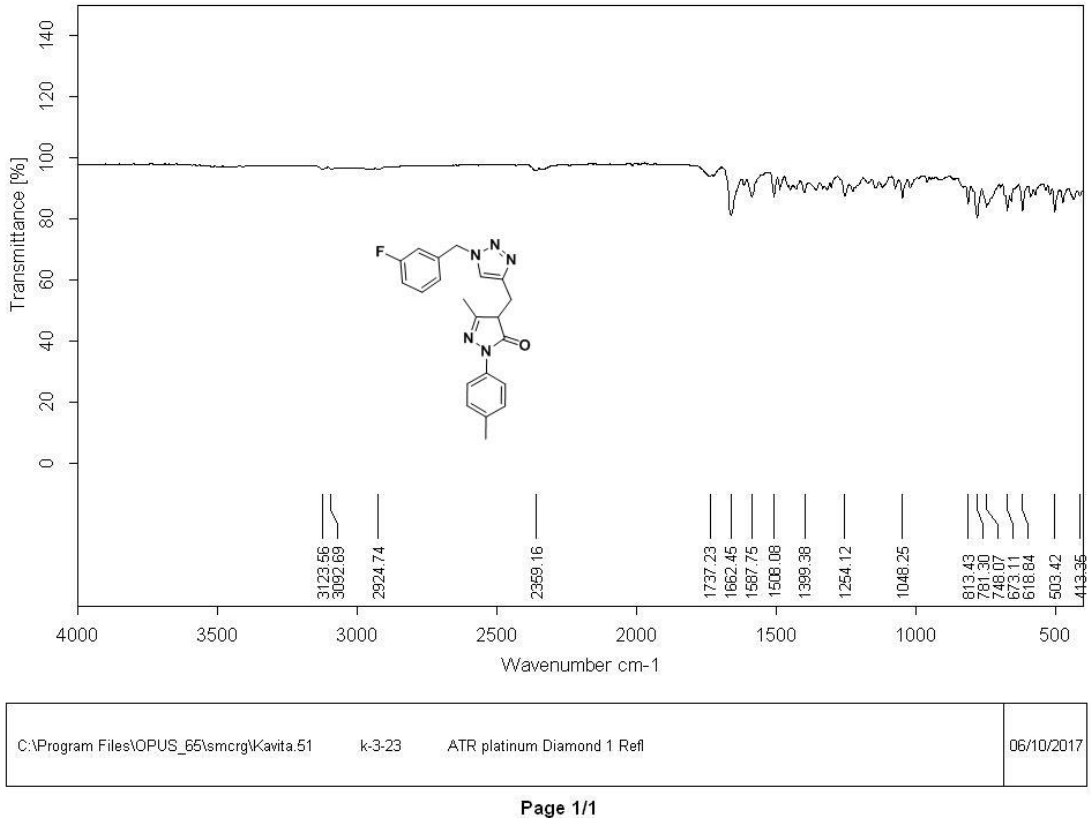


Figure 79. IR spectrum of compound k-3-23

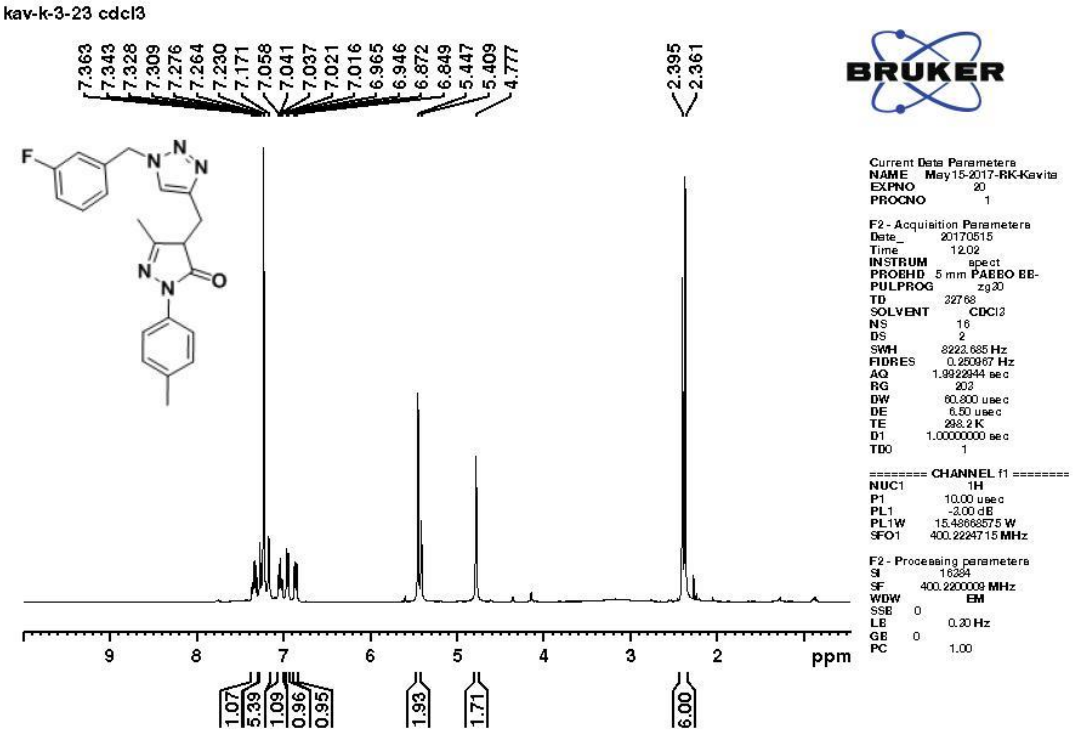


Figure 80. ^{1}H NMR of compound k-3-23

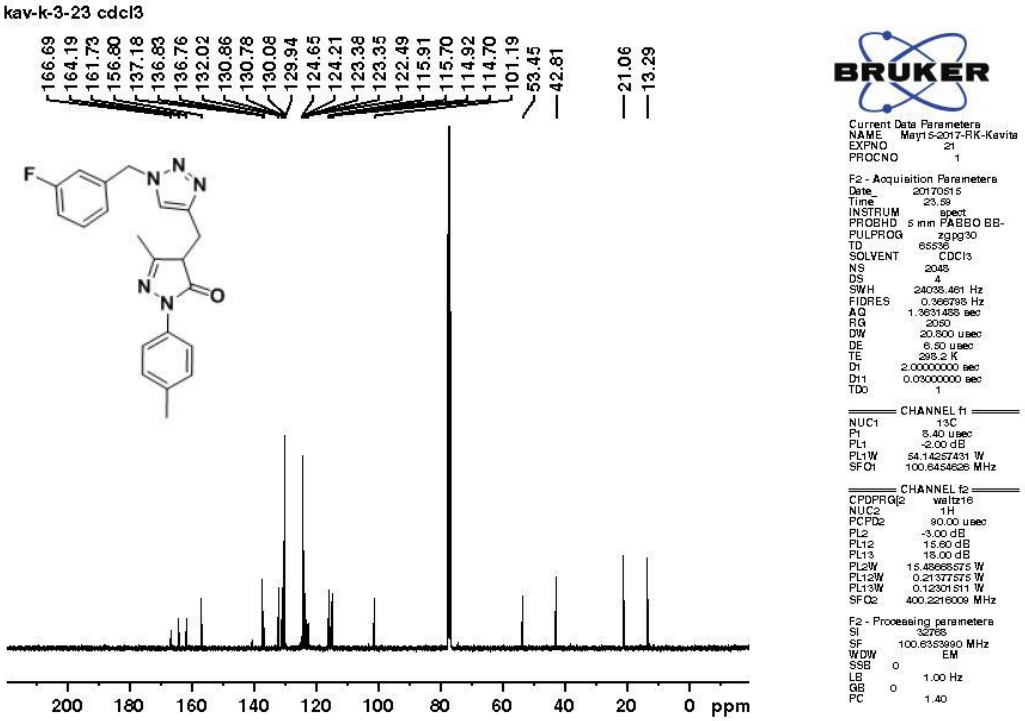


Figure 81.^{13}C NMR of compound k-3-23

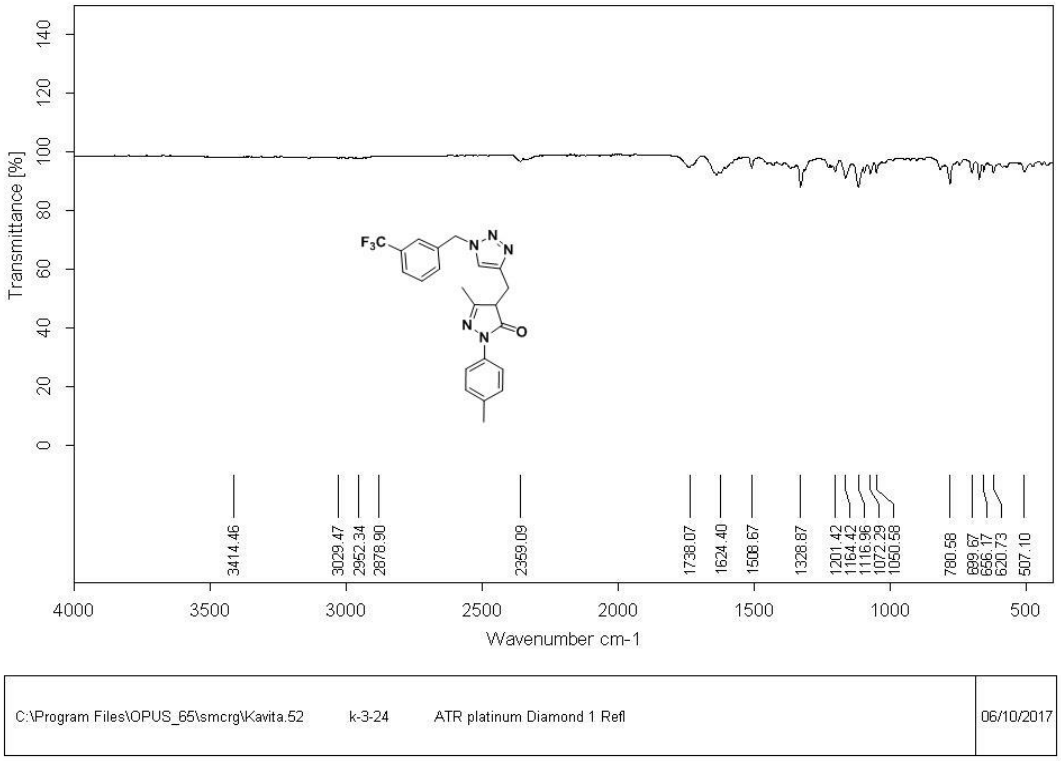


Figure 82. IR spectrum of compound k-3-24

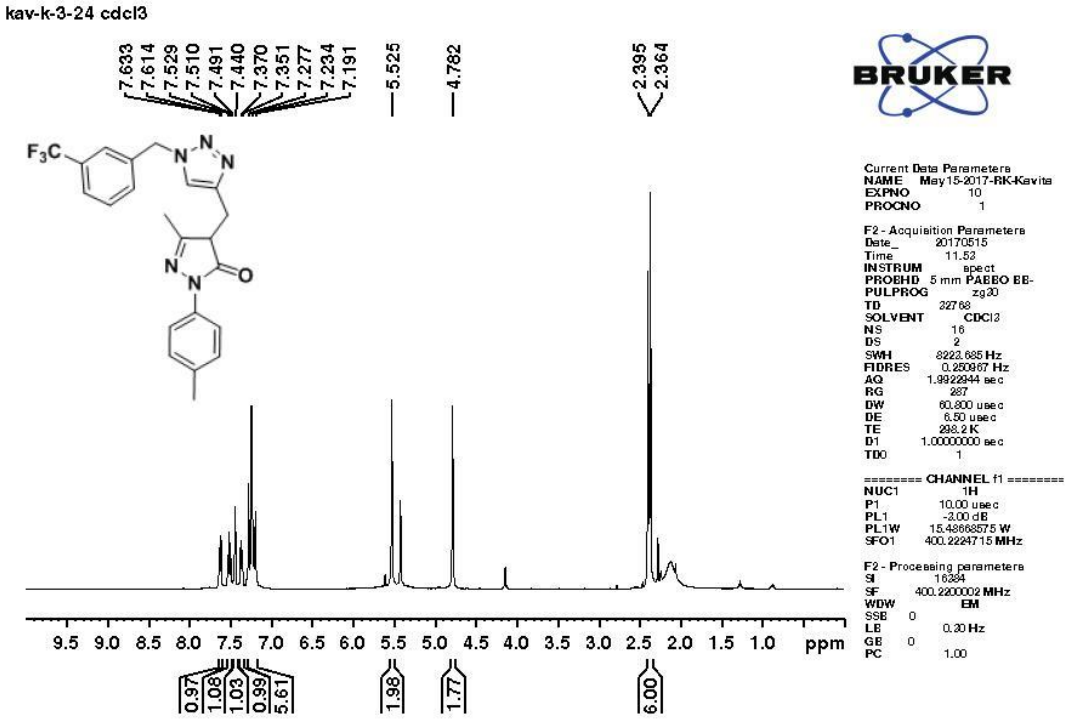


Figure 83.[1]H NMR of compound k-3-24

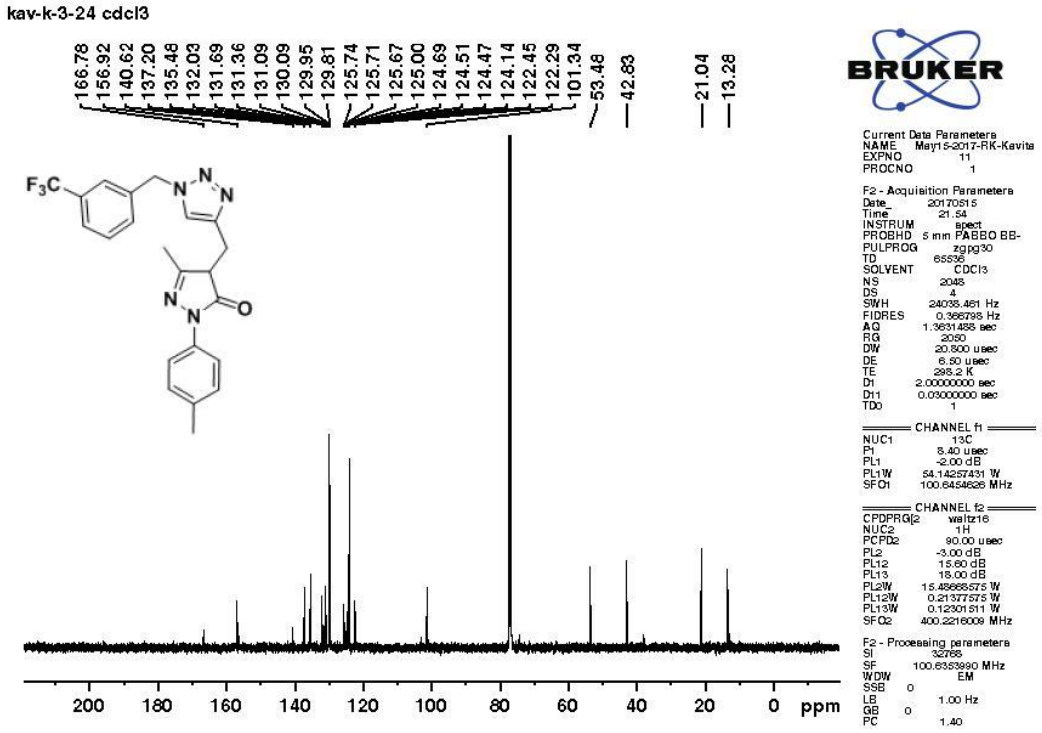


Figure 84. ^{13}C NMR of compound k-3-24

1.3 CHAPTER 5

Title: Synthesis and antimicrobial evaluation of pyrazoline Incorporated to known bioactive structure oxime

Authors name: Kavita Jain[a], Nisar Syyad and Rajshekhar Karpoormath[a,*]

Affiliations: [a] *Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa.*

To receive all correspondence: Rajshekhar Karpoormath: Tel: +27 (0) 312607179, *E-mail:* karpoormath@ukzn.ac.za

Supplementary data:

Spectral Information:

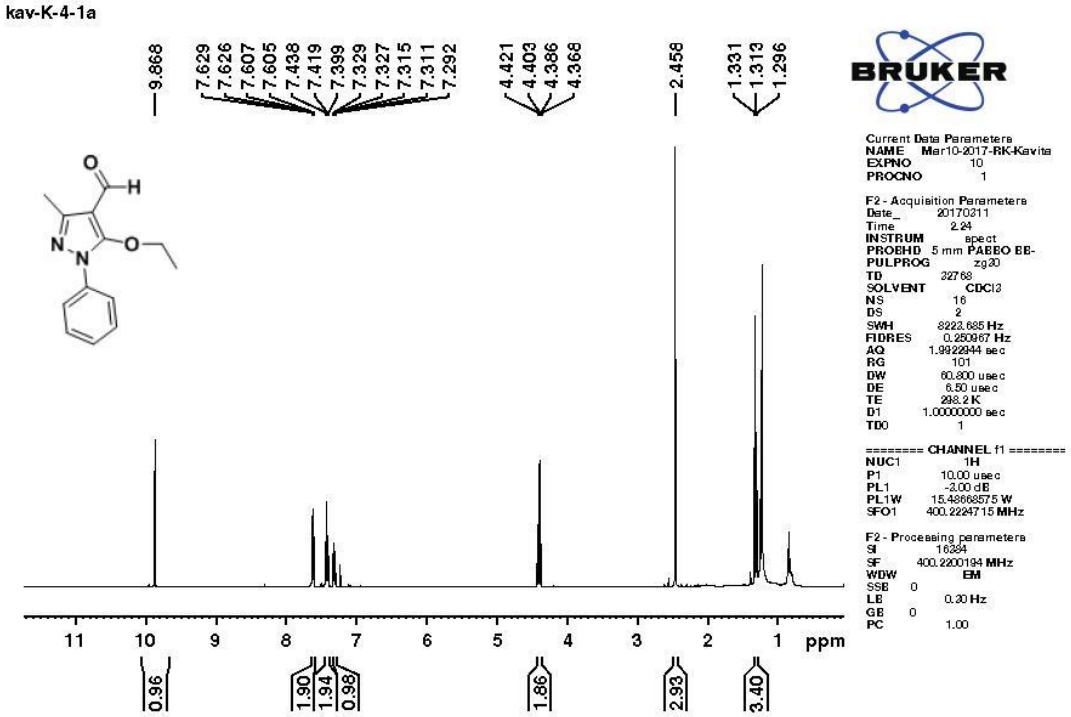


Figure 1. [1]H NMR of compound 3-1

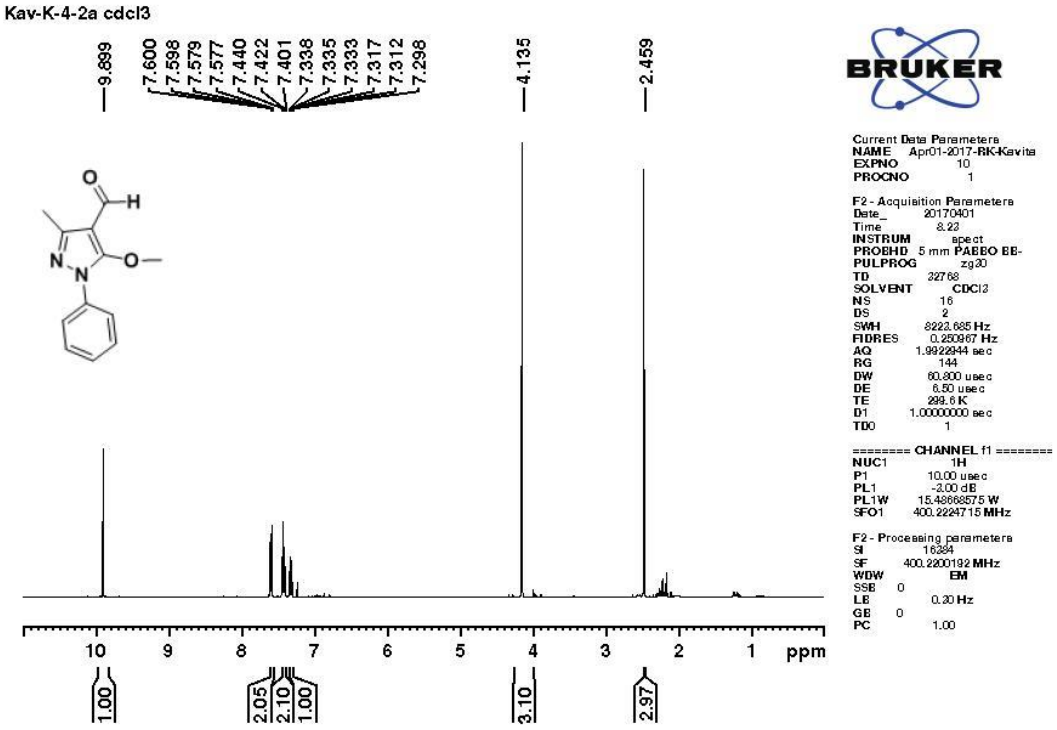


Figure 2.[1]H NMR of compound 3-2

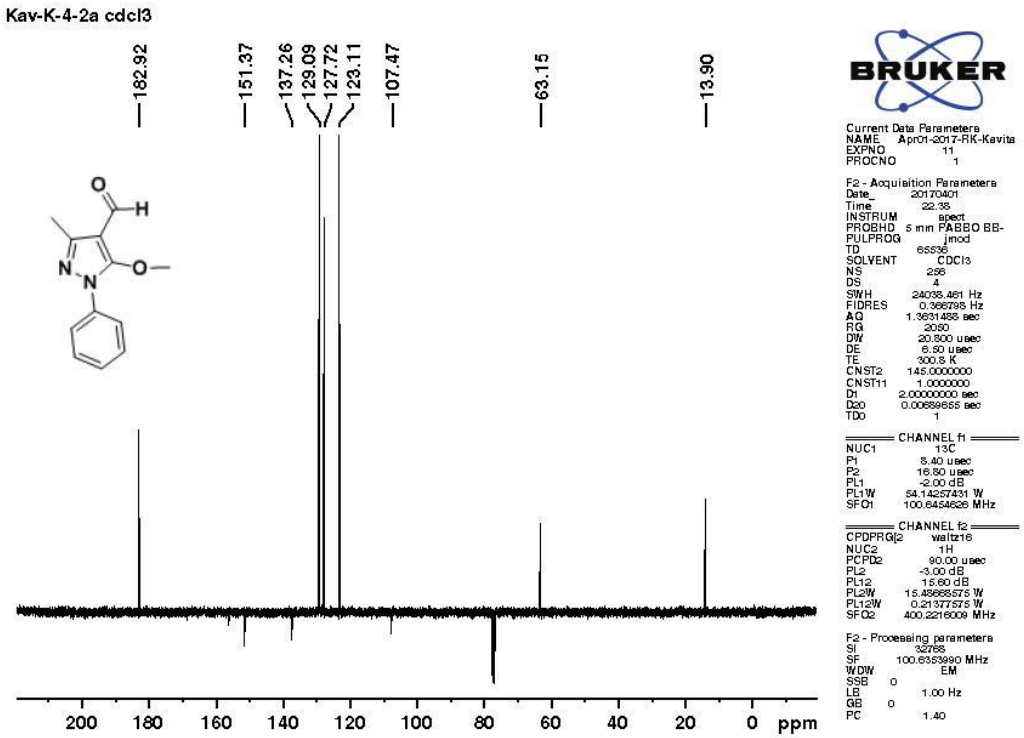


Figure 3.[13]C NMR of compound 3-2

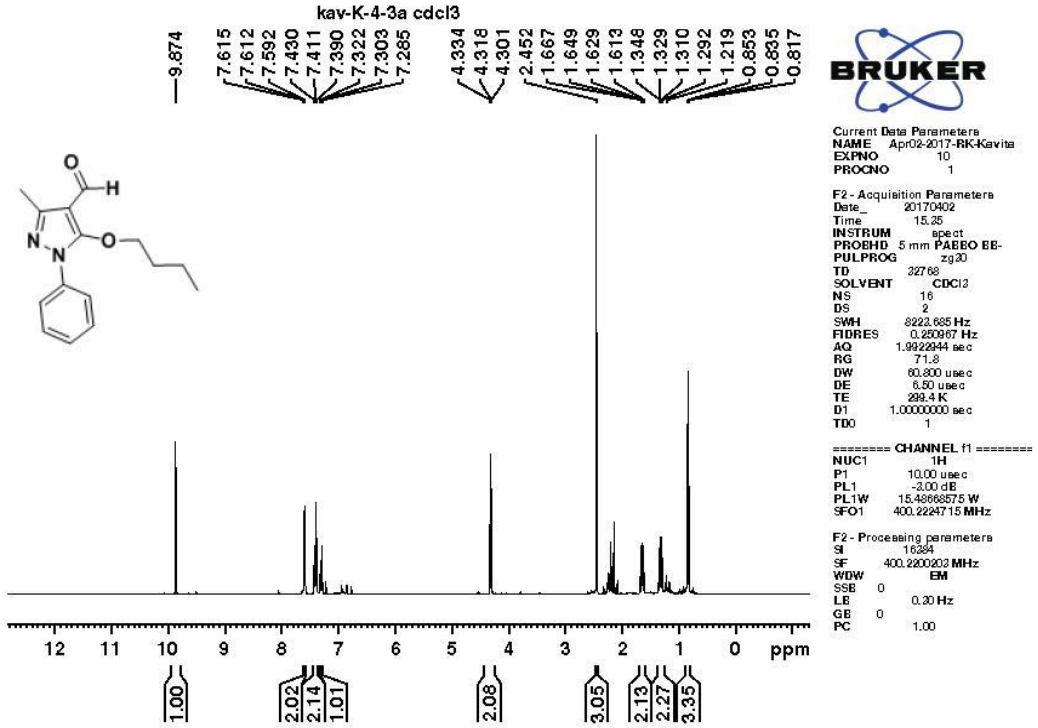


Figure 4.[1]H NMR of compound 3-3

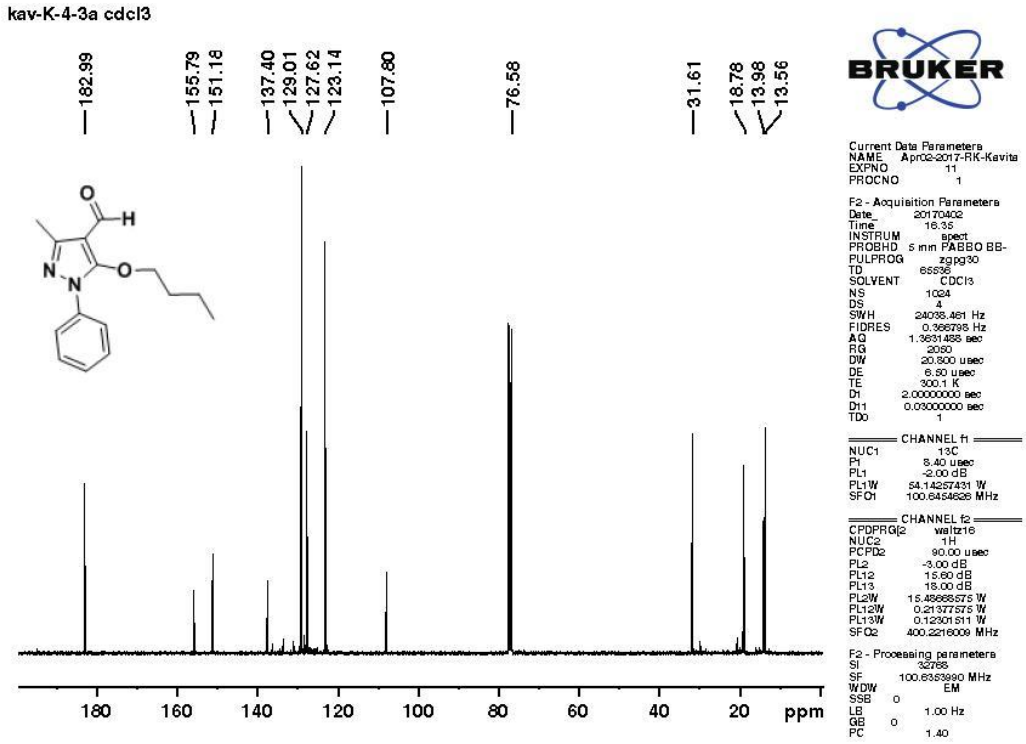


Figure 5. [13]C NMR of compound 3-3

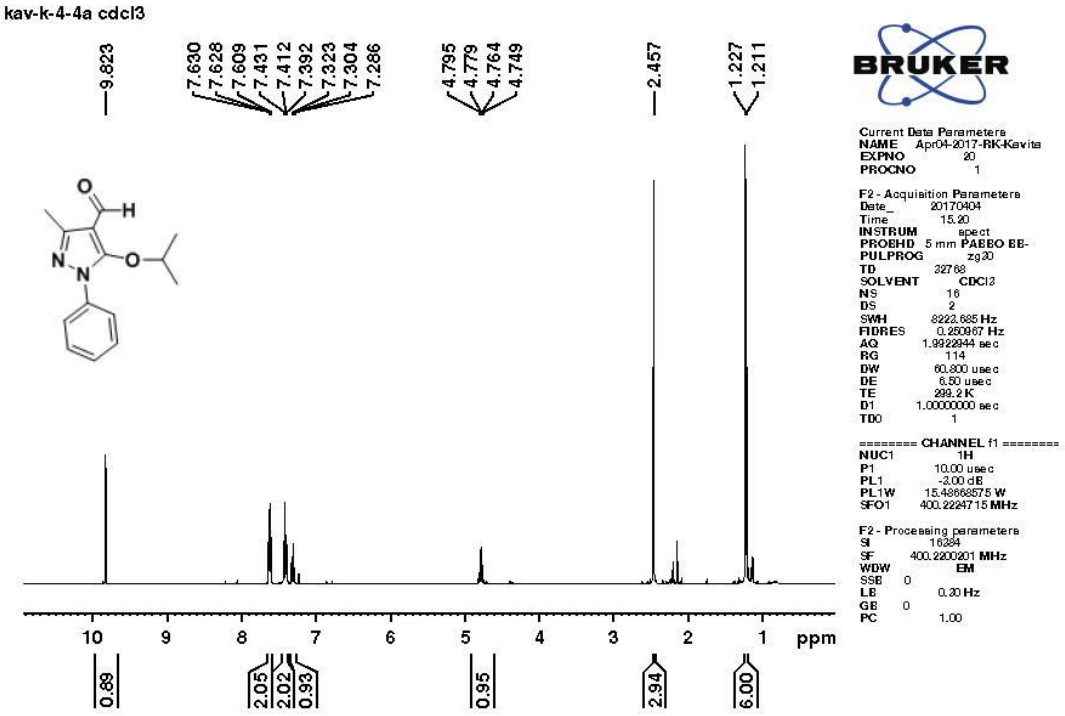


Figure 6. ^{1}H NMR of compound 3-4

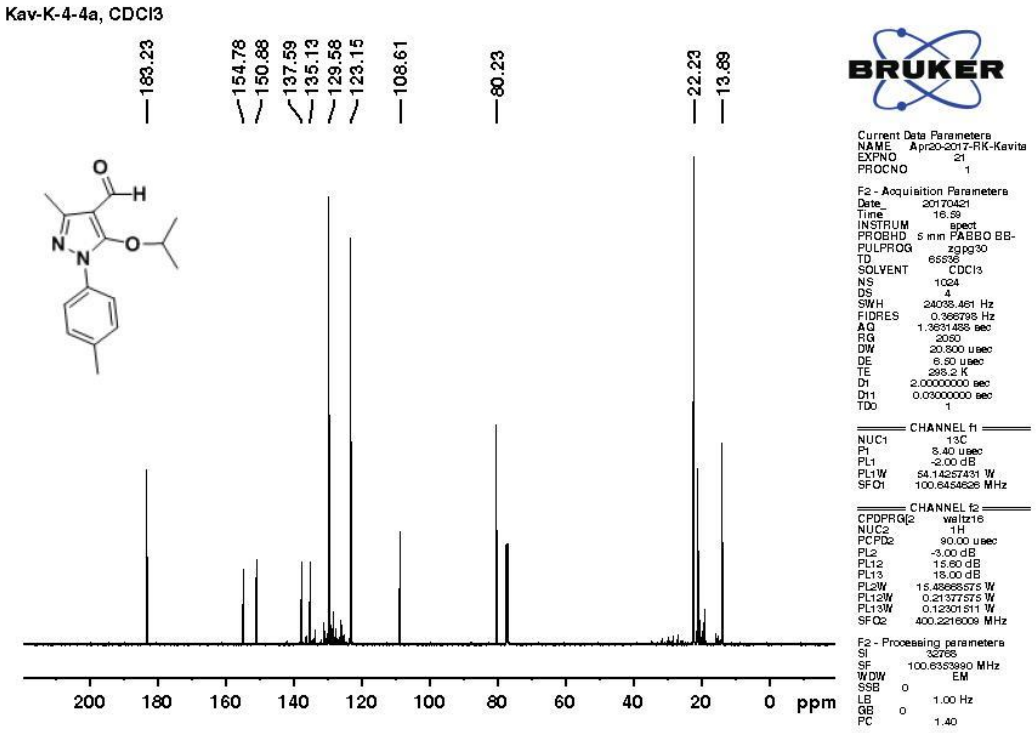


Figure 7. ^{13}C NMR of compound 3-4

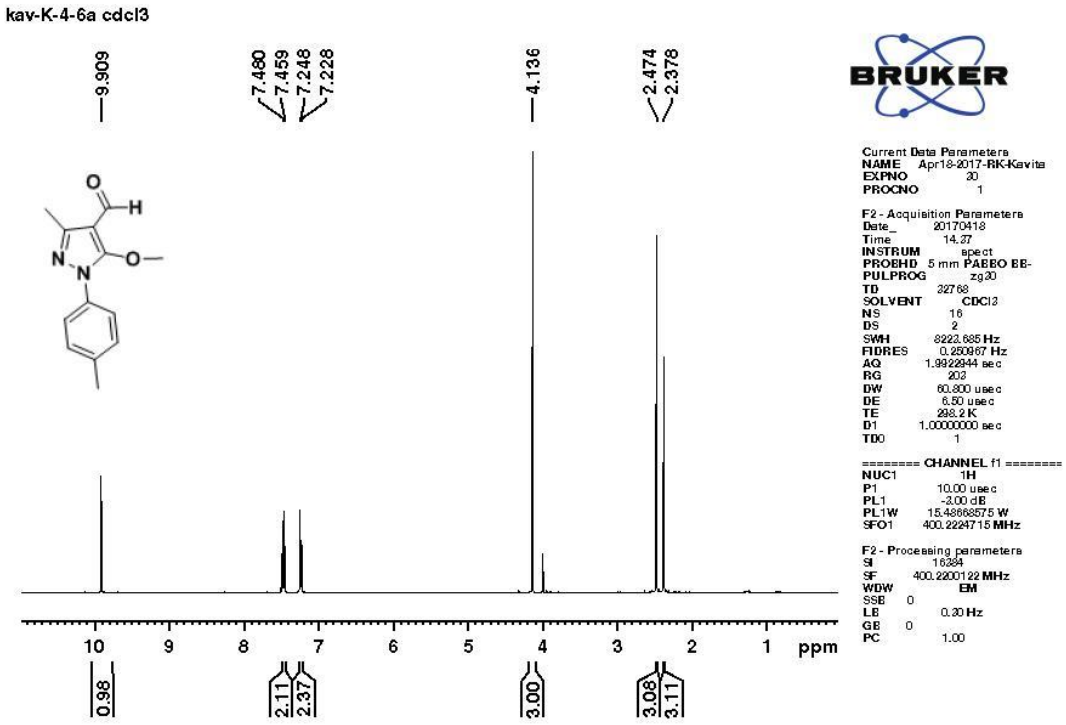


Figure 8.[1]H NMR of compound 3-6

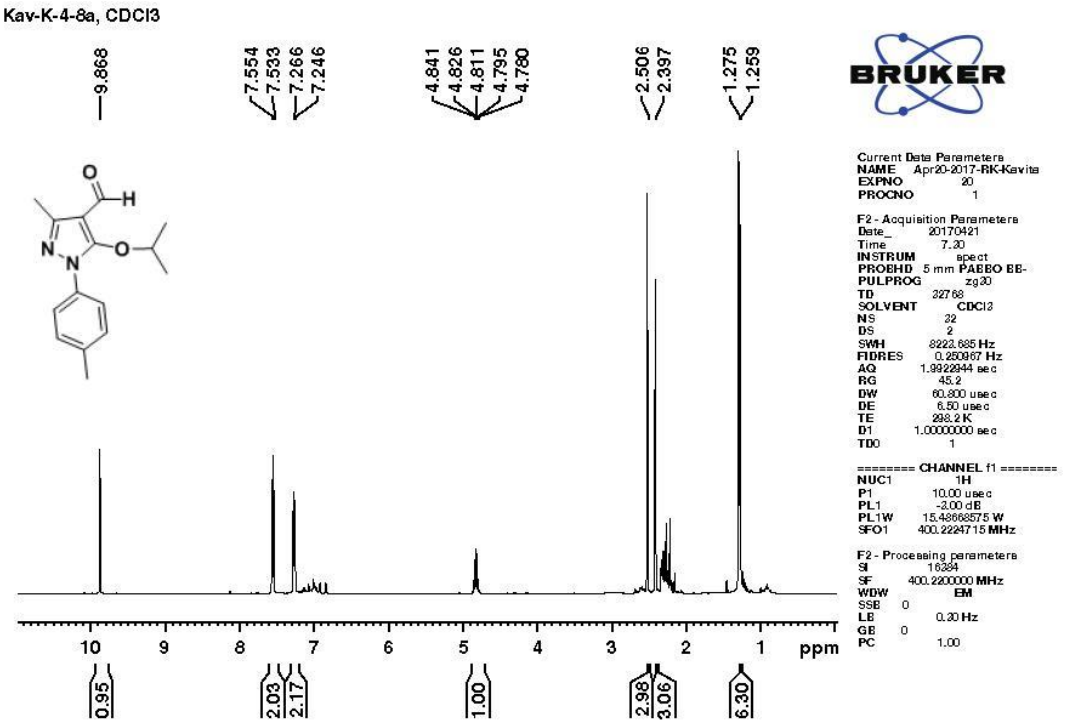


Figure 9.[1]H NMR of compound 3-8

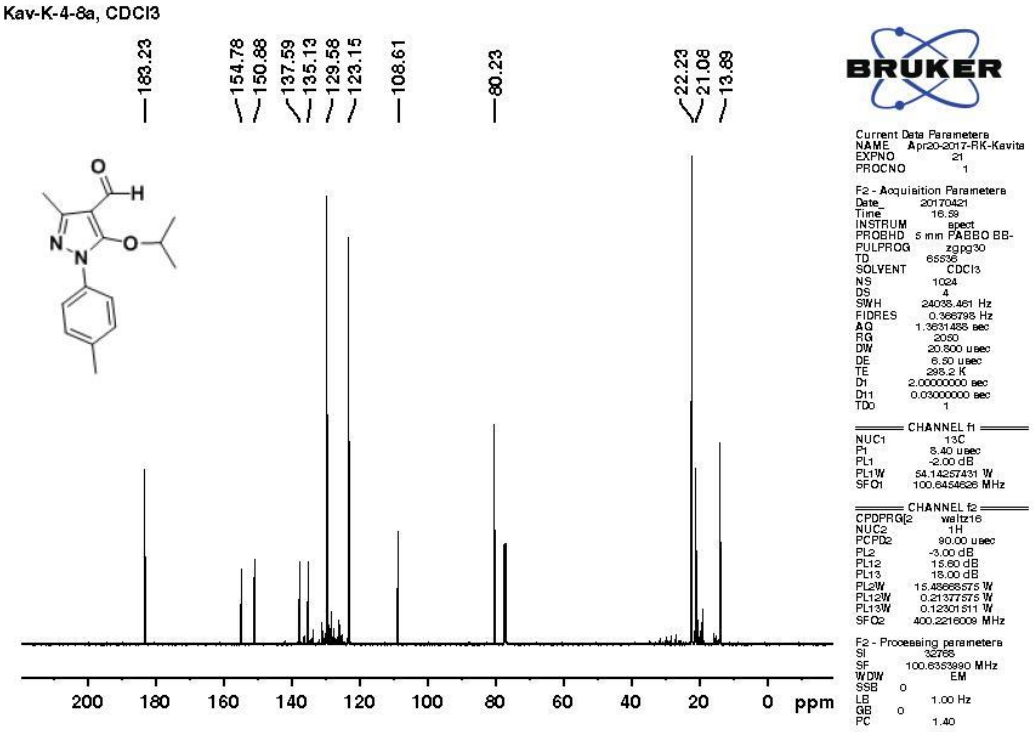


Figure 10. ^{13}C NMR of compound 3-8

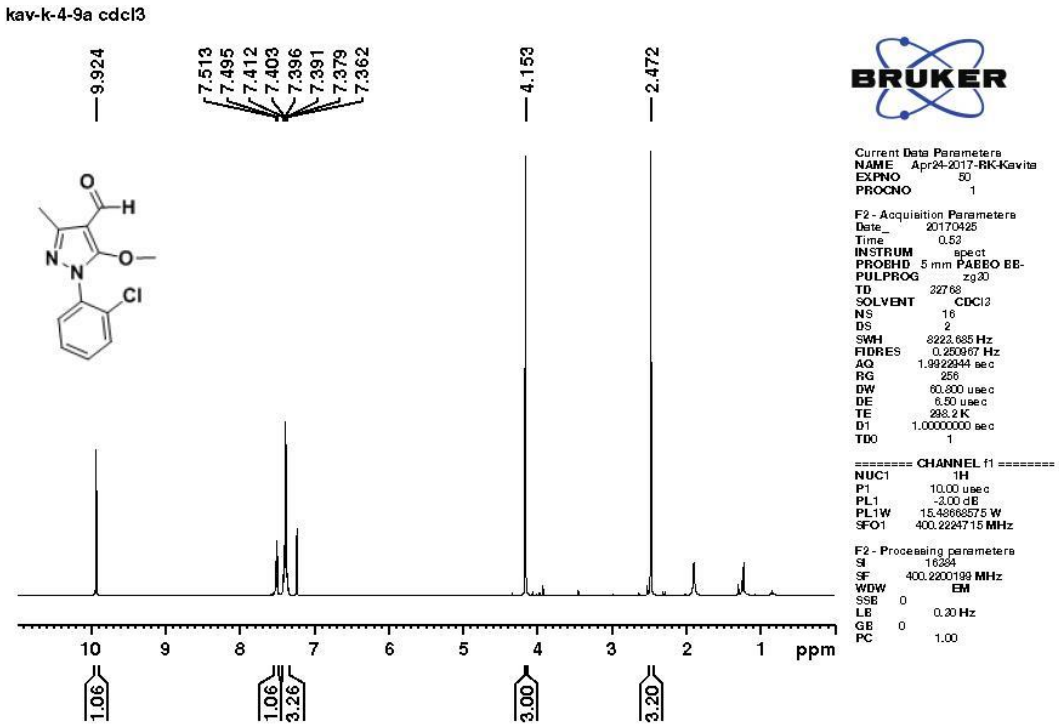


Figure 11. ^{1}H NMR of compound 3-9

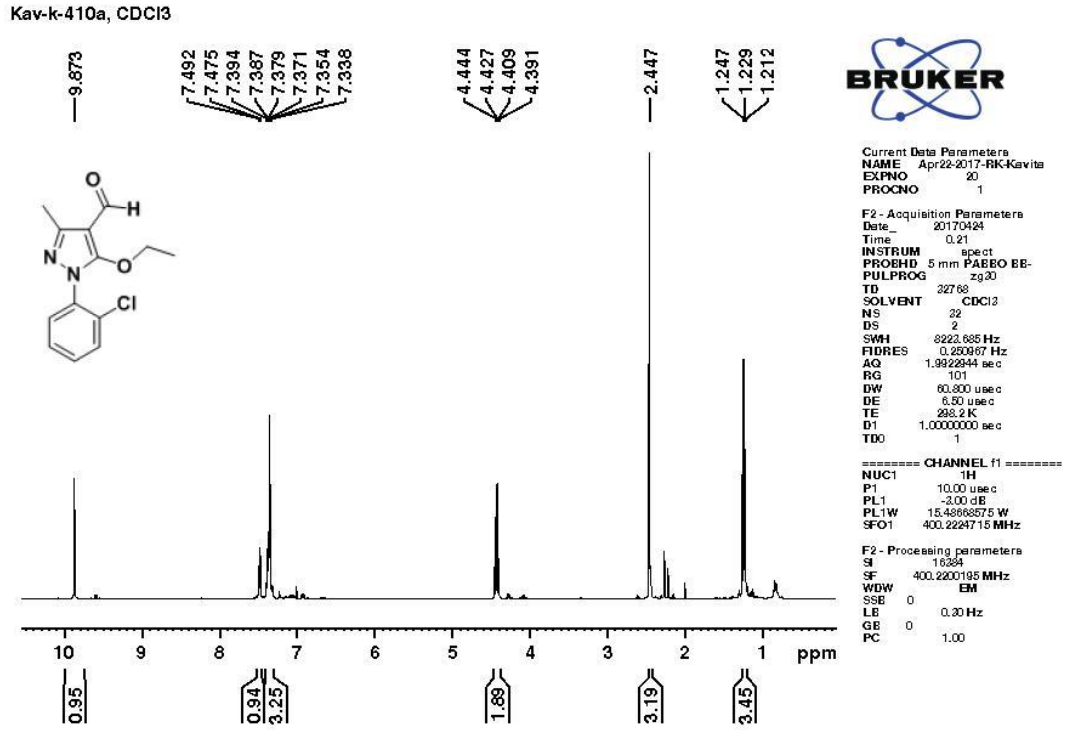


Figure 12. ^{1}H NMR of compound 3-10

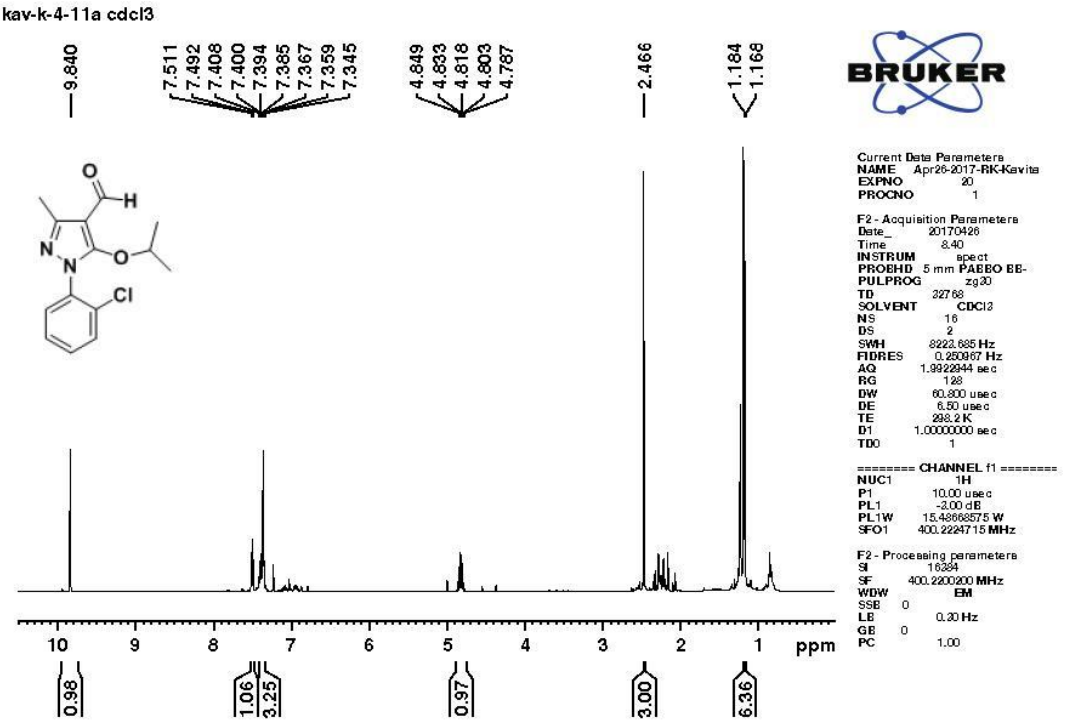


Figure 13. ^{1}H NMR of compound 3-11

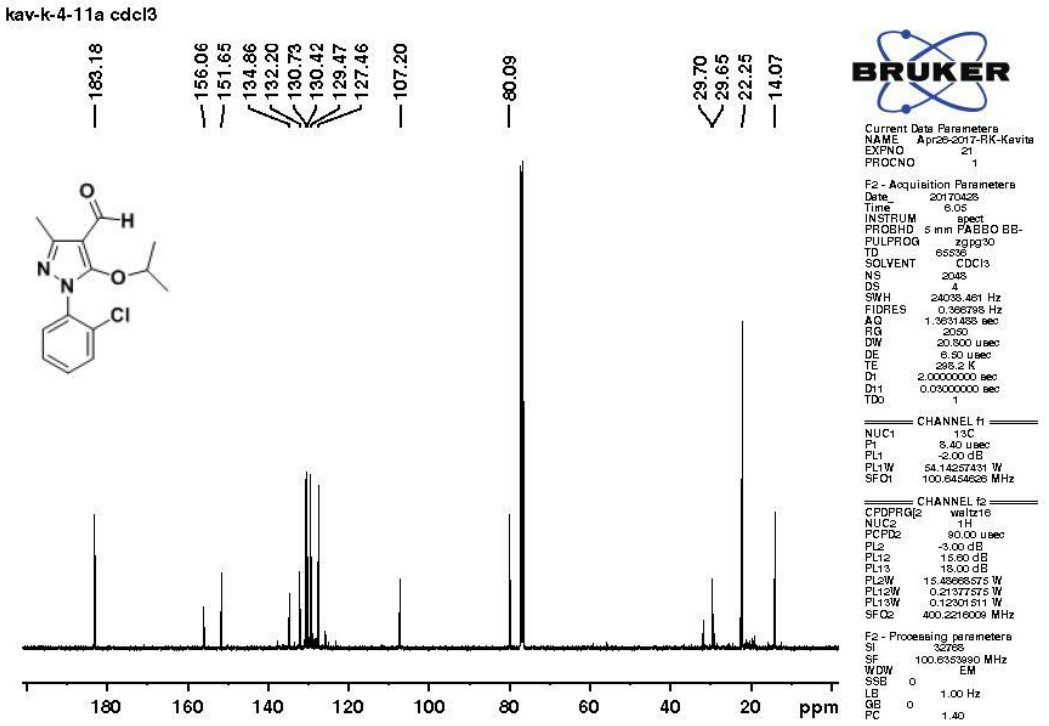


Figure 14. ^{13}C NMR of compound 3-11

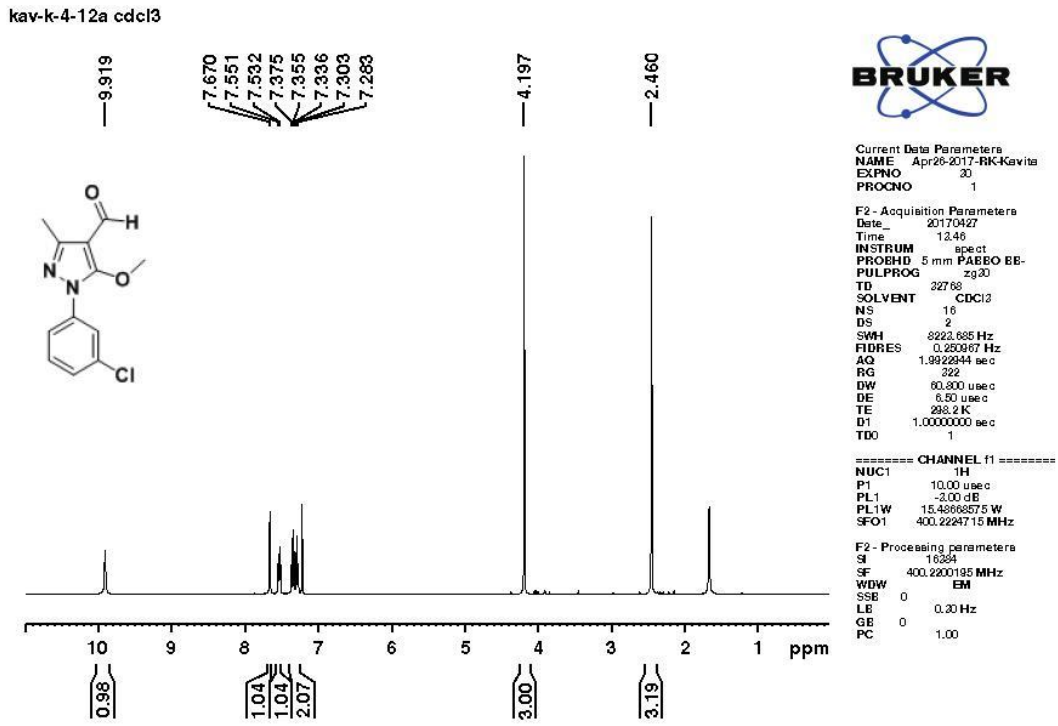


Figure 15. ^{1}H NMR of compound 3-12

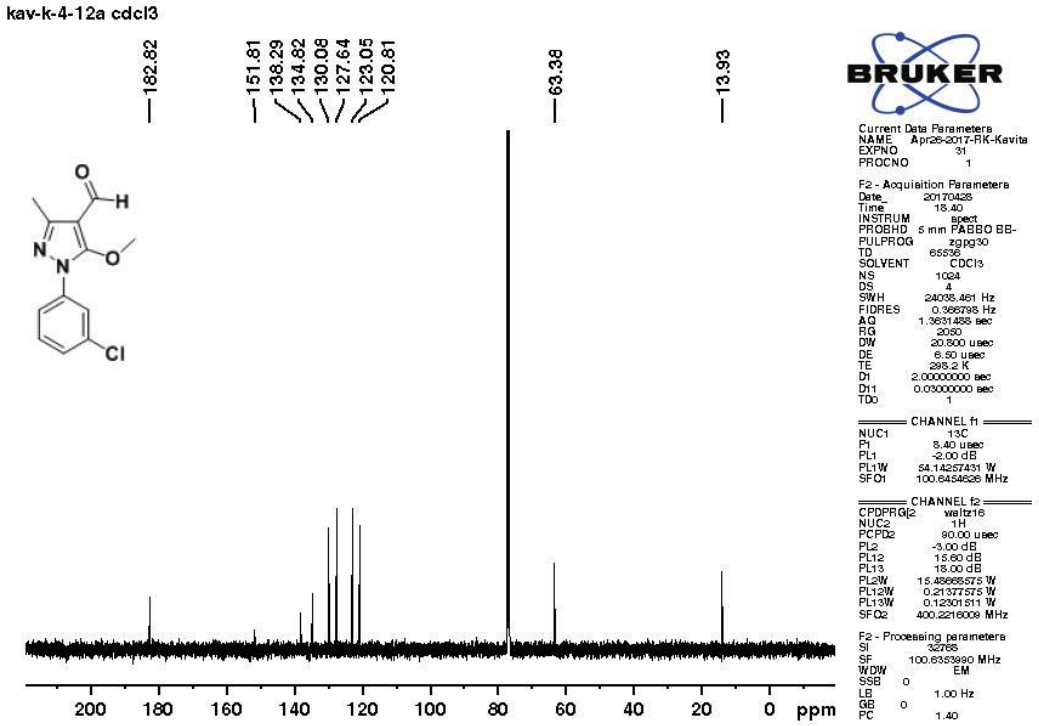


Figure 16. ^{13}C NMR of compound 3-12

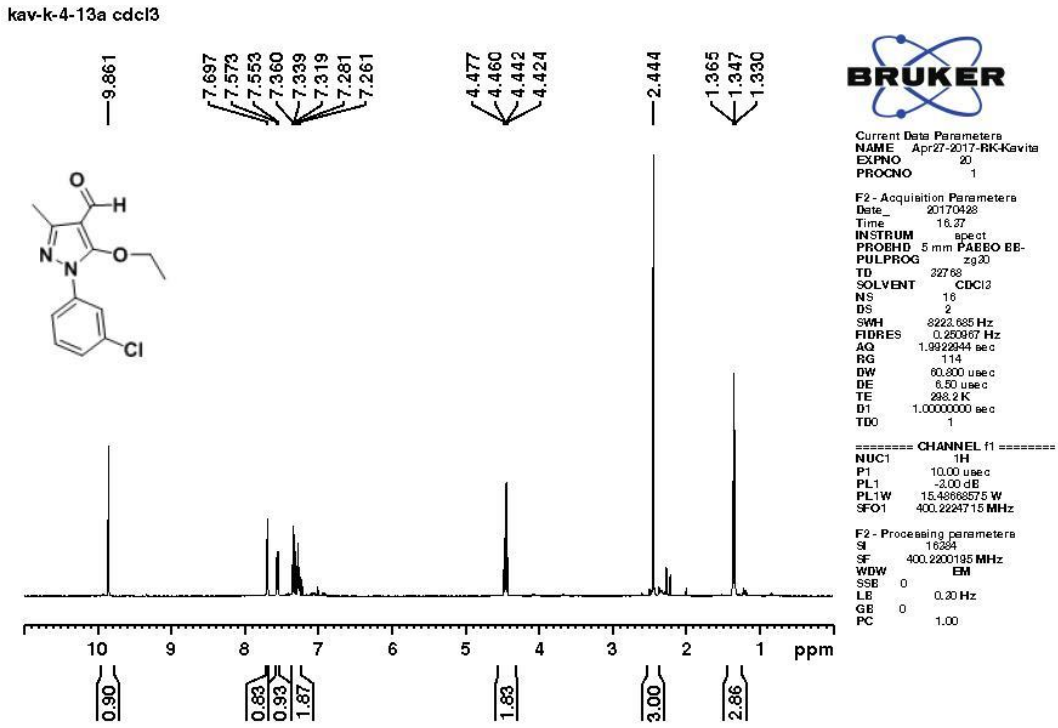


Figure 17. ^{1}H NMR of compound 3-13

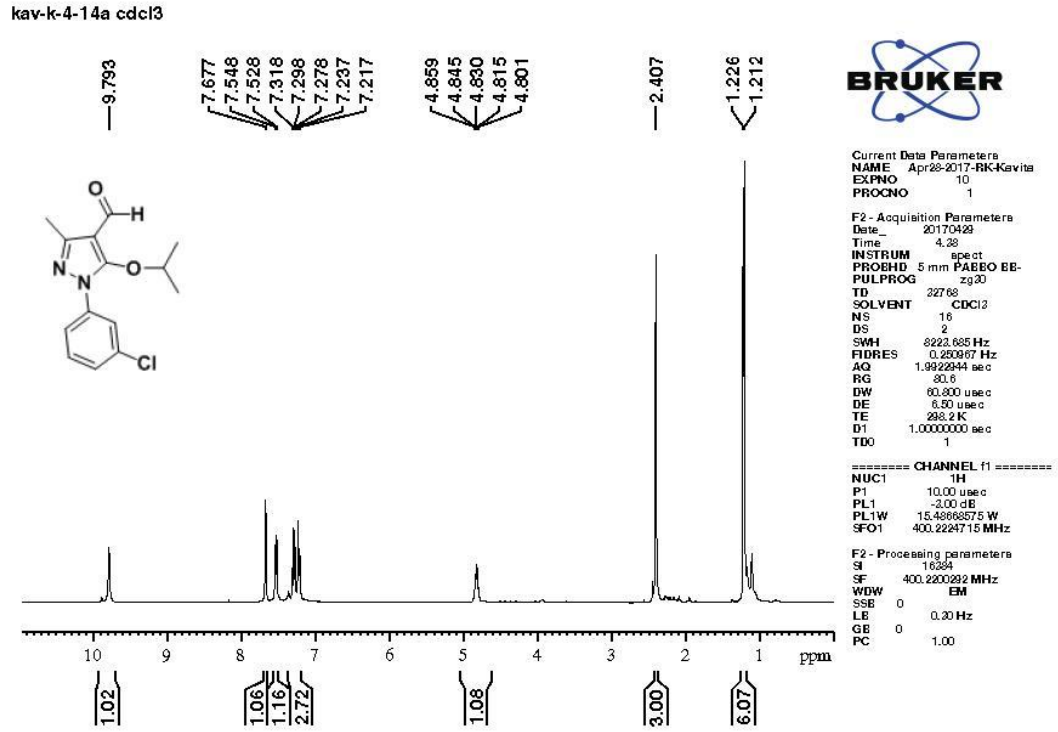


Figure 18. ^{1}H NMR of compound 3-14

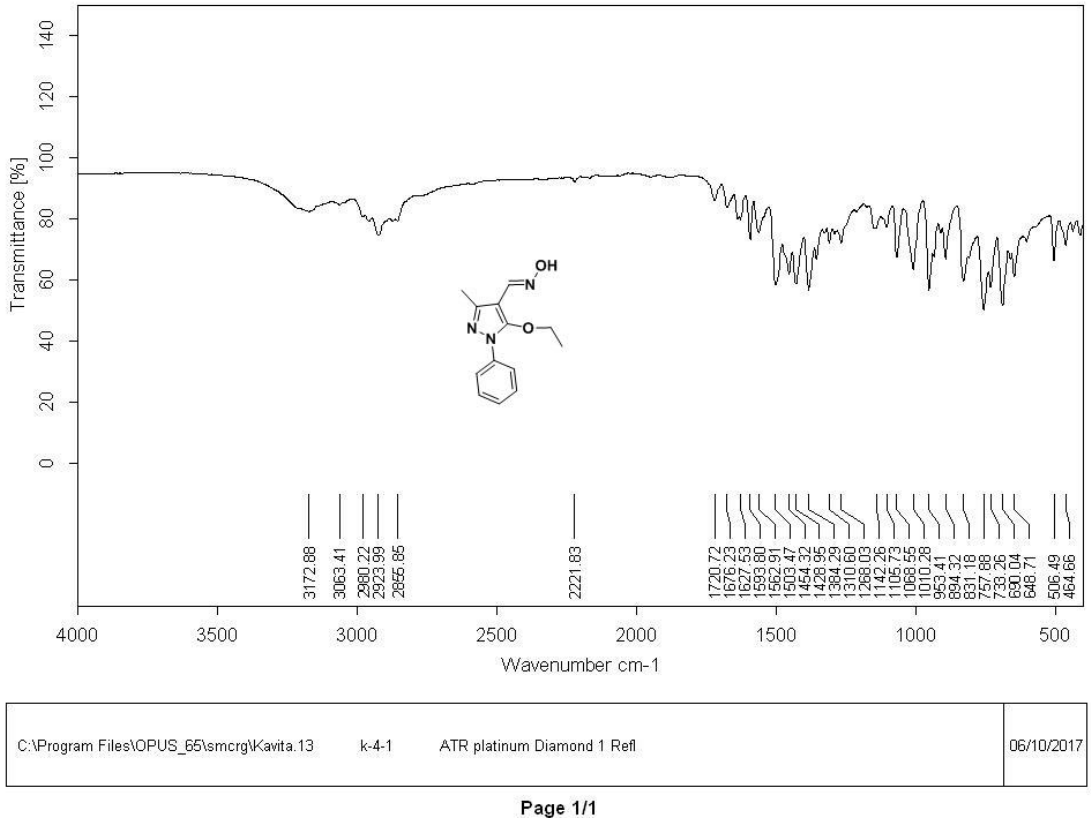


Figure 19.IR spectrum of compound k-4-1

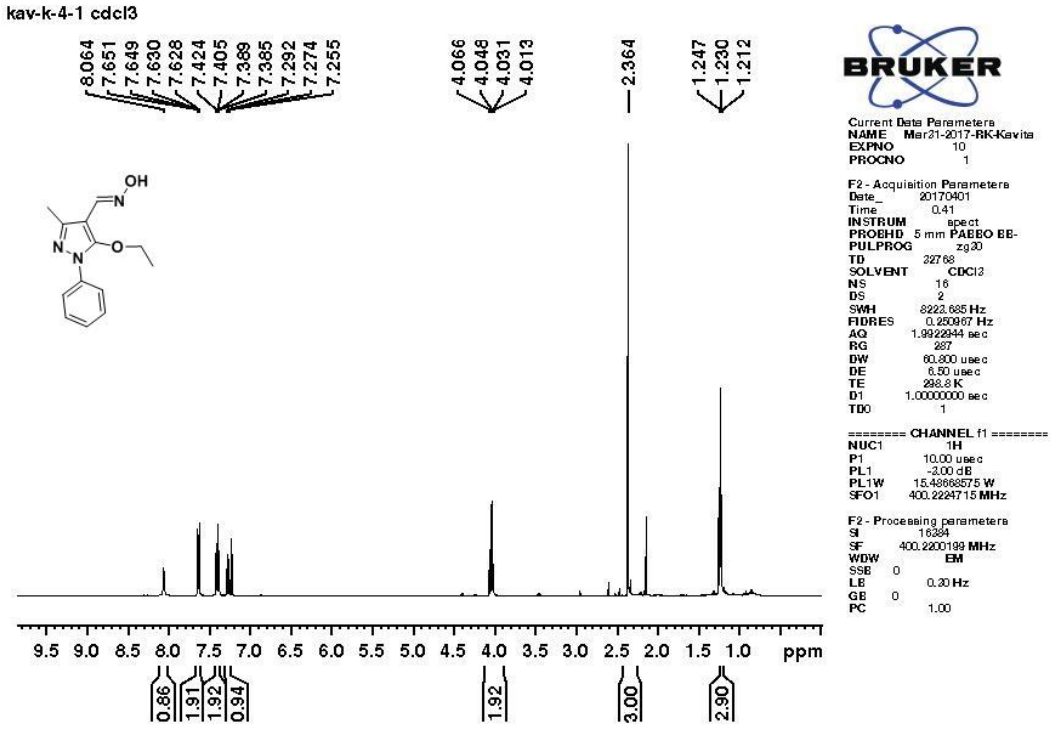


Figure 20.[1]H NMR of compound k-4-1

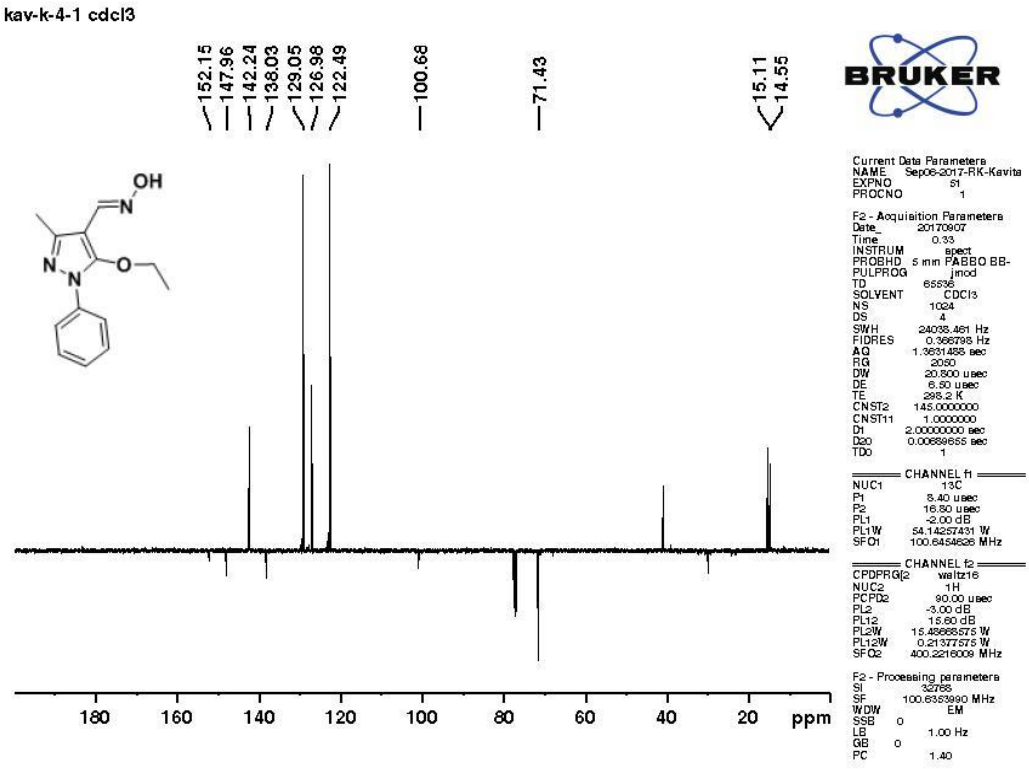
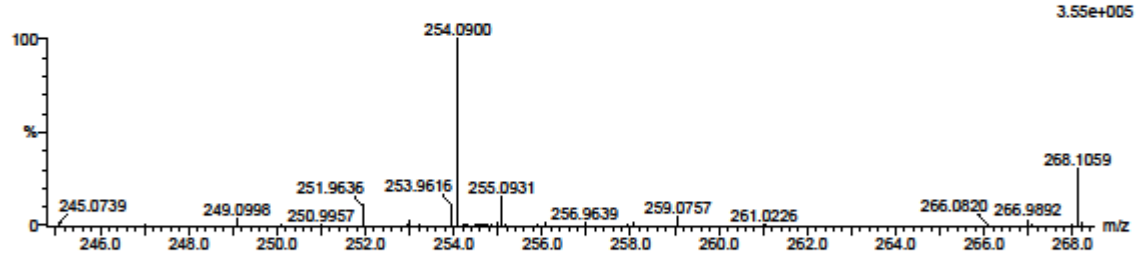


Figure 21.[13]C NMR of compound k-4-1

Elemental Composition Report **Page 1**

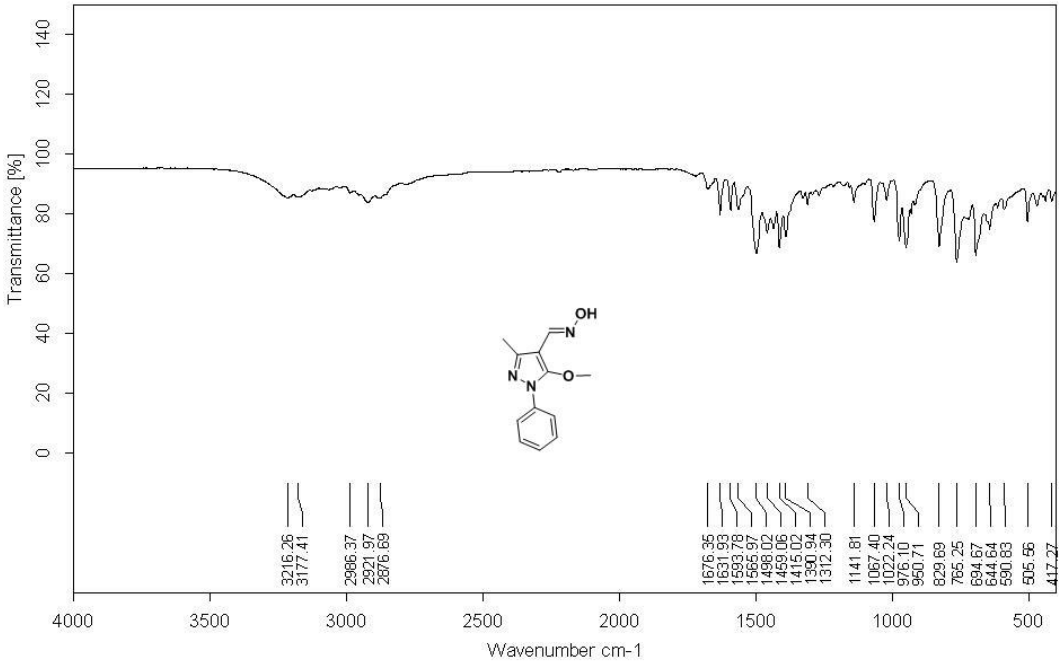
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
13 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 0-5 O: 0-4 Na: 1-1
K-4-2 30 (0.979) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
254.0900	254.0905	-0.5	-2.0	7.5	200.8	0.0	C12 H13 N3 O2 Na

Figure 22. HRMS of compound k-4-2



C:\Program Files\OPUS_65\smcrg\Kavita.15 k-4-2 ATR platinum Diamond 1 Refl 06/10/2017

Page 1/1

Figure 23. IR spectrum of compound k-4-2

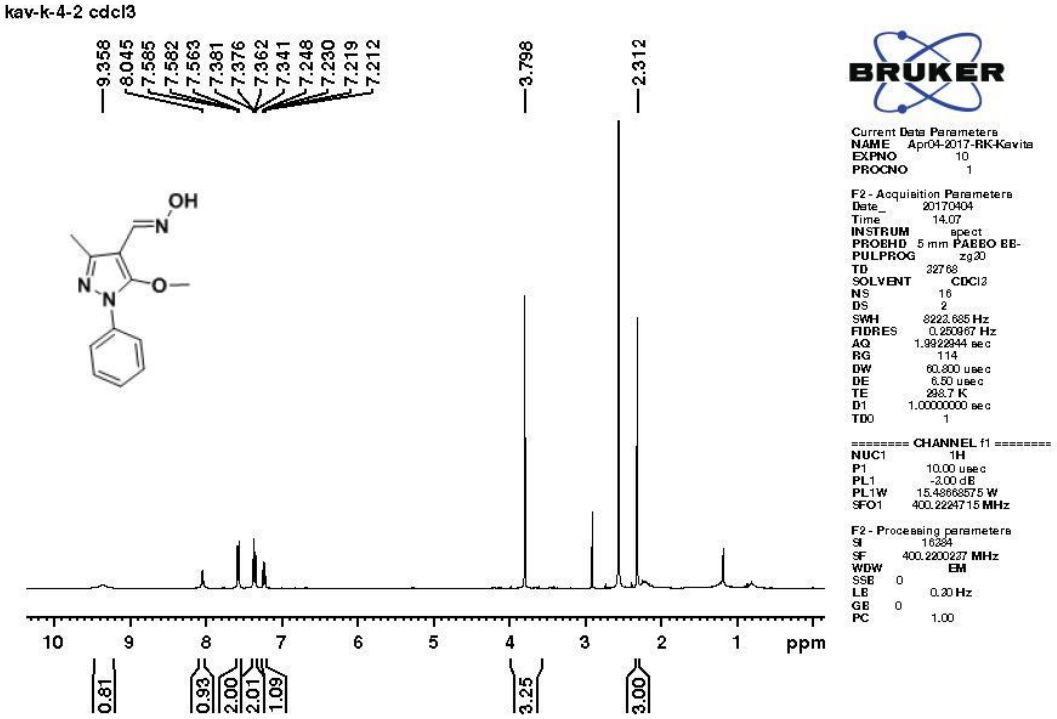


Figure 24. ^{1}H NMR of compound k-4-2

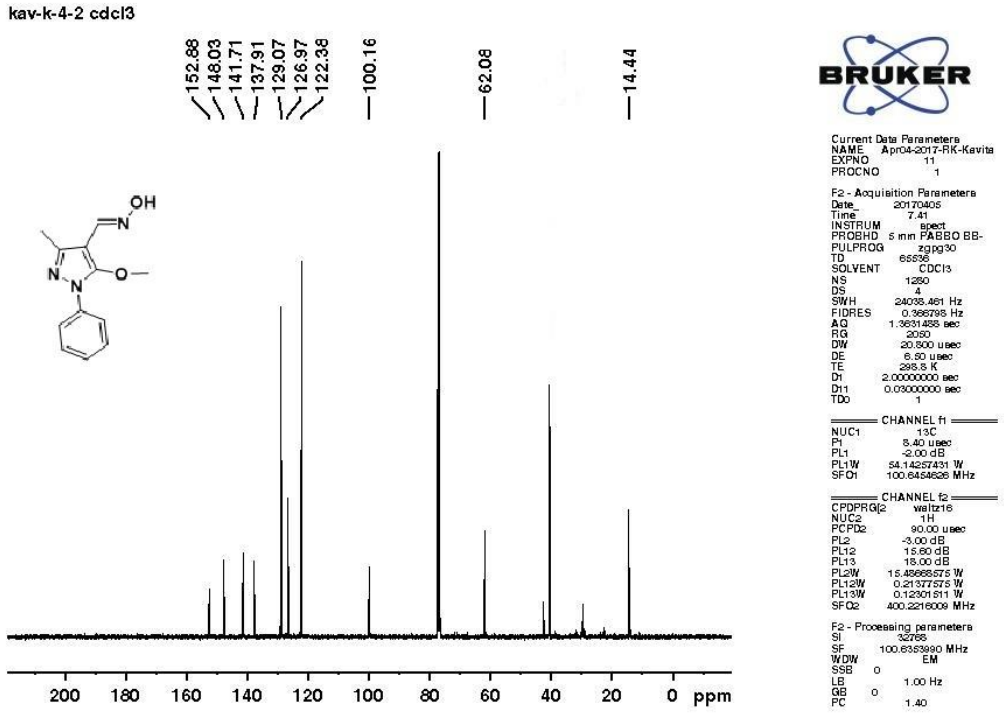


Figure 25.^{13}C NMR of compound k-4-2

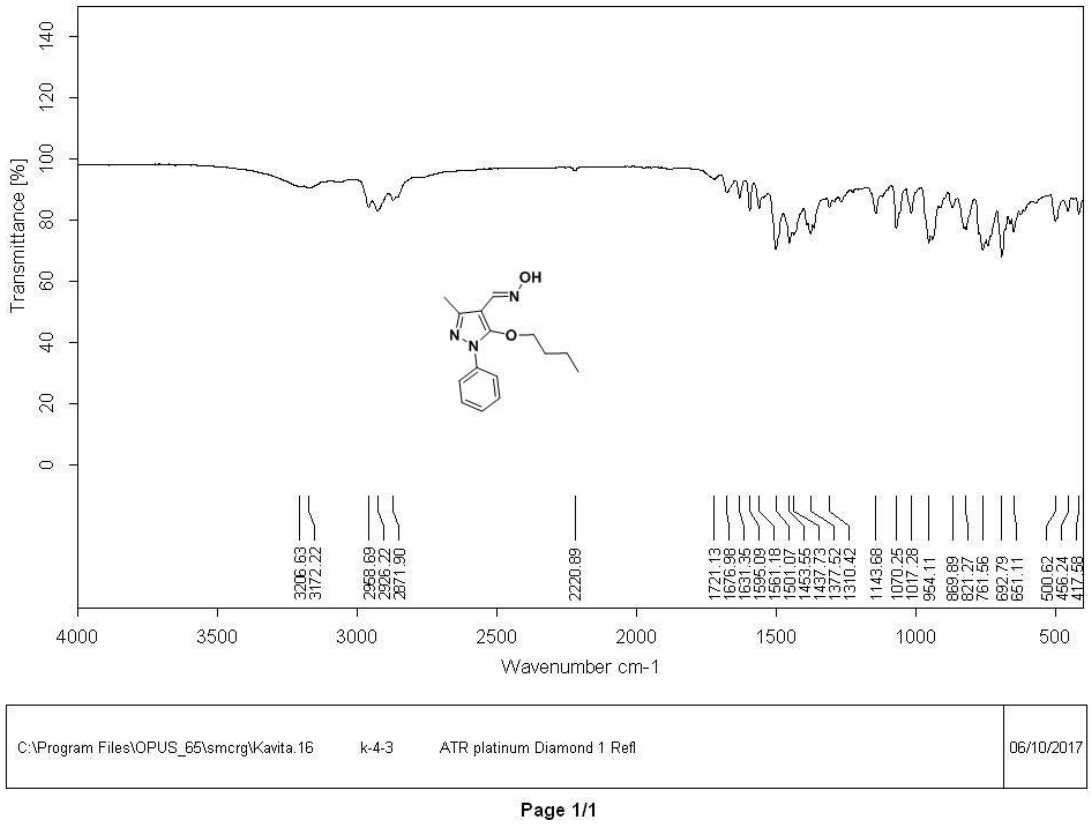


Figure 26. IR spectrum of compound k-4-3

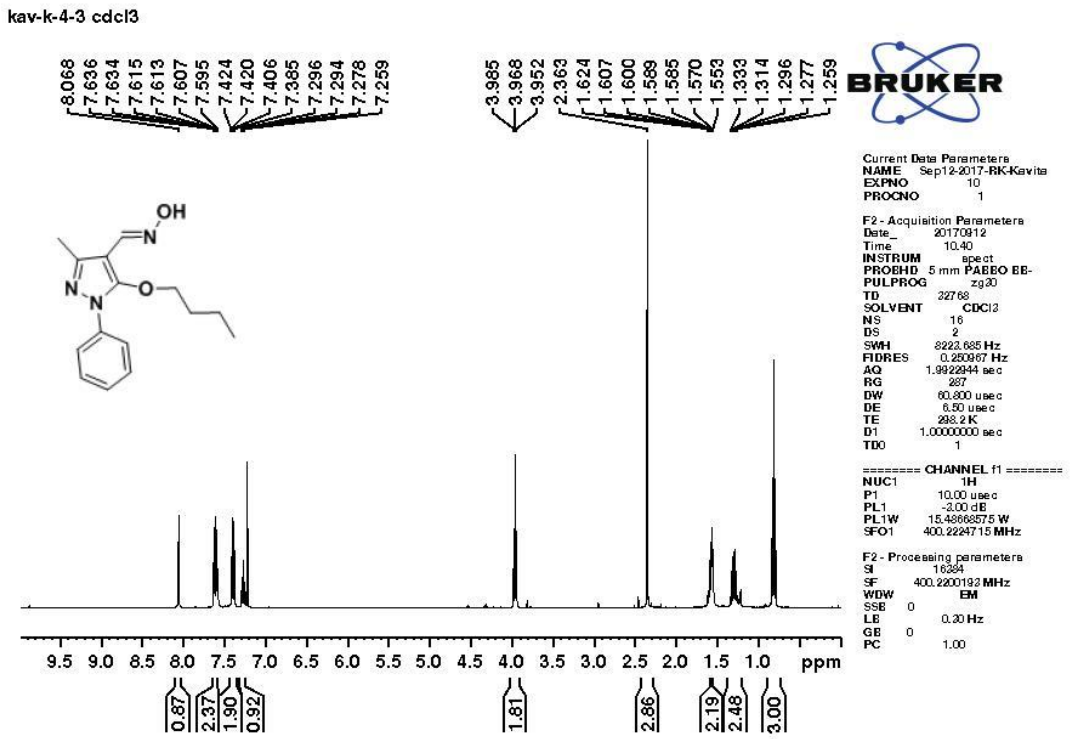


Figure 27. ^{1}H NMR of compound k-4-3

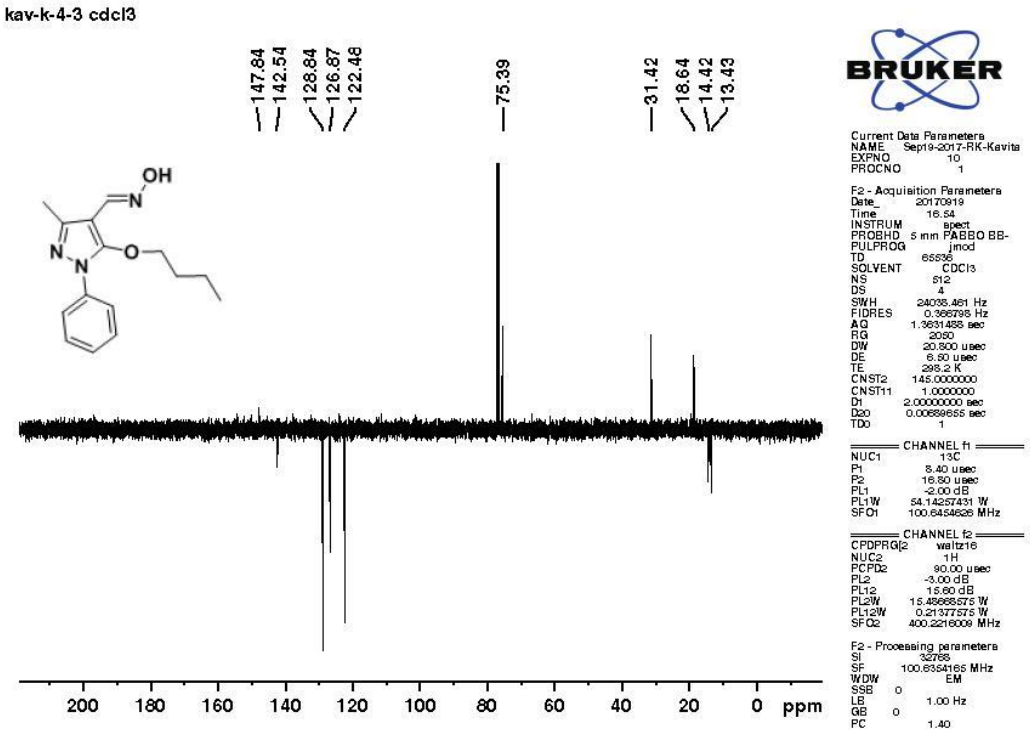


Figure 28. ^{13}C NMR of cmpound k-4-3

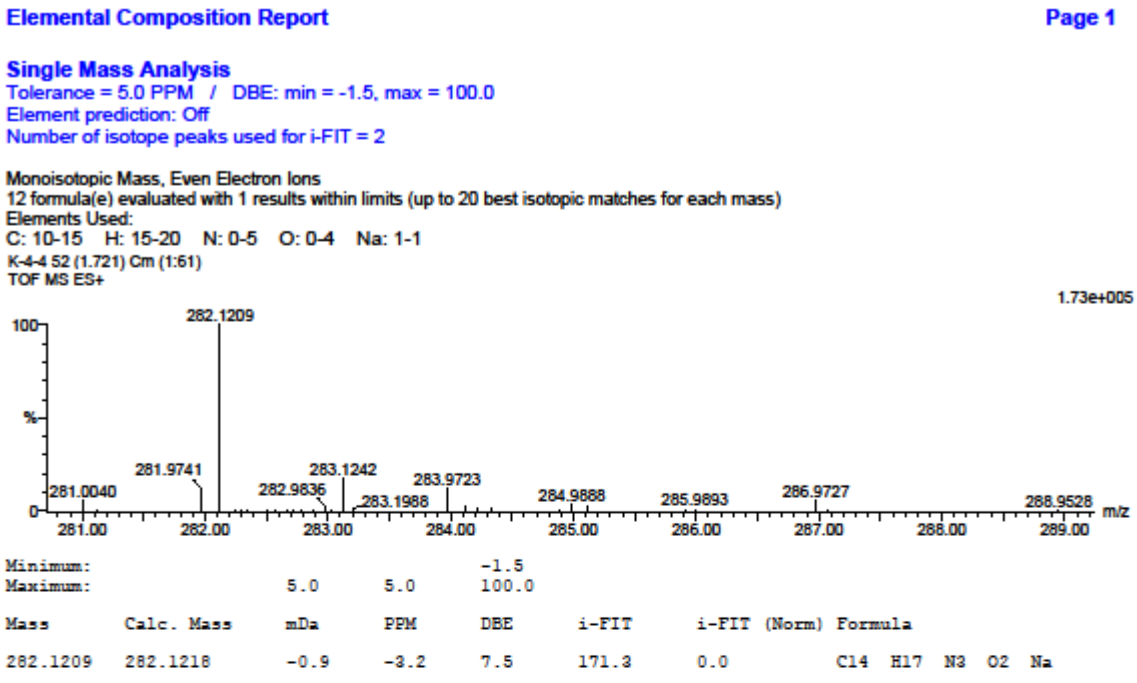


Figure 29. HRMS of compound k-4-4

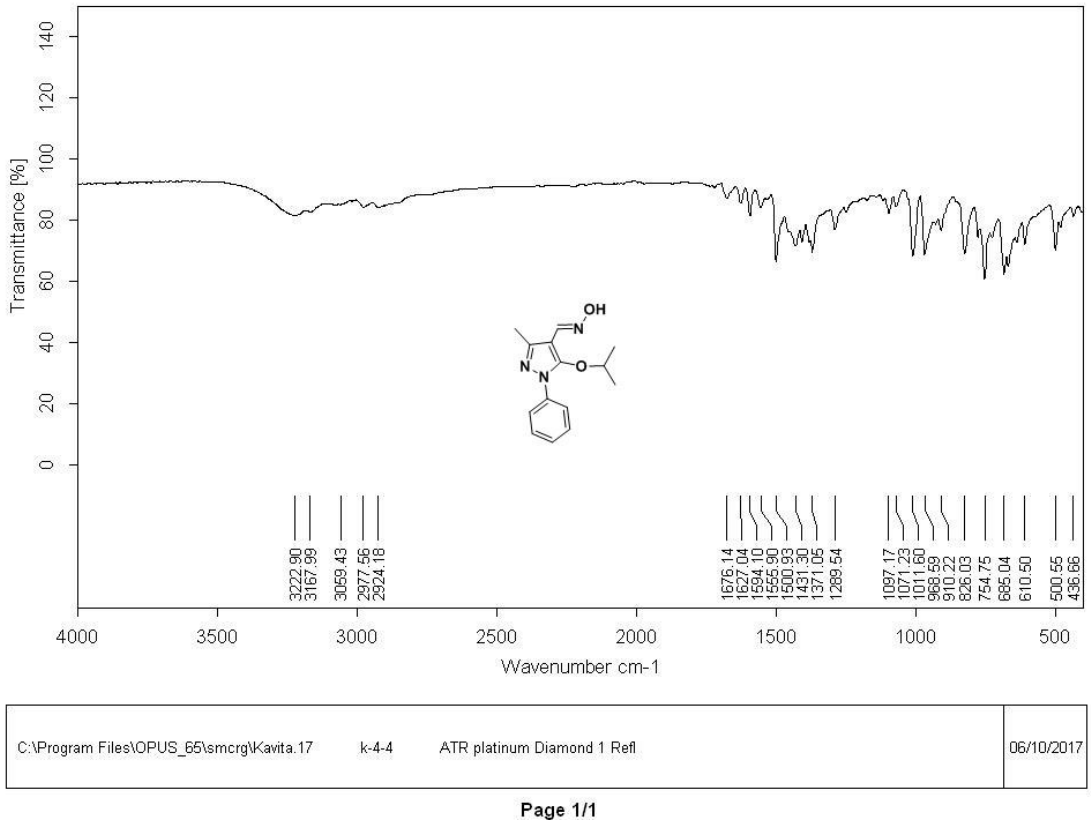


Figure 30. IR spectrum of compound k-4-4

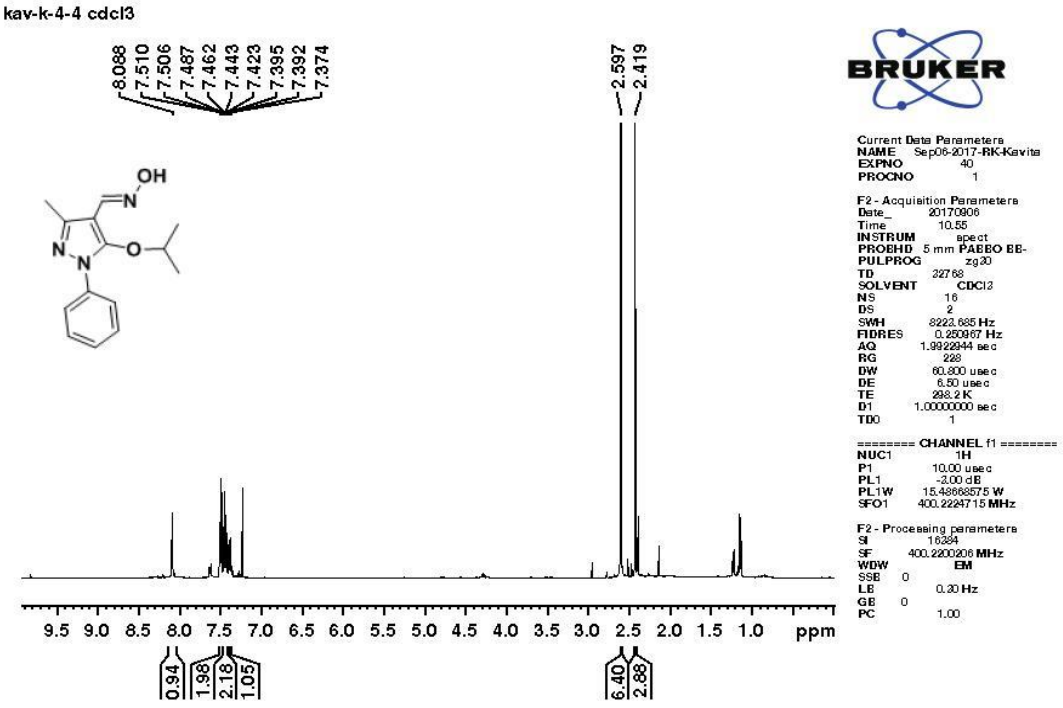


Figure 31. ^{1}H NMR of compound k-4-4

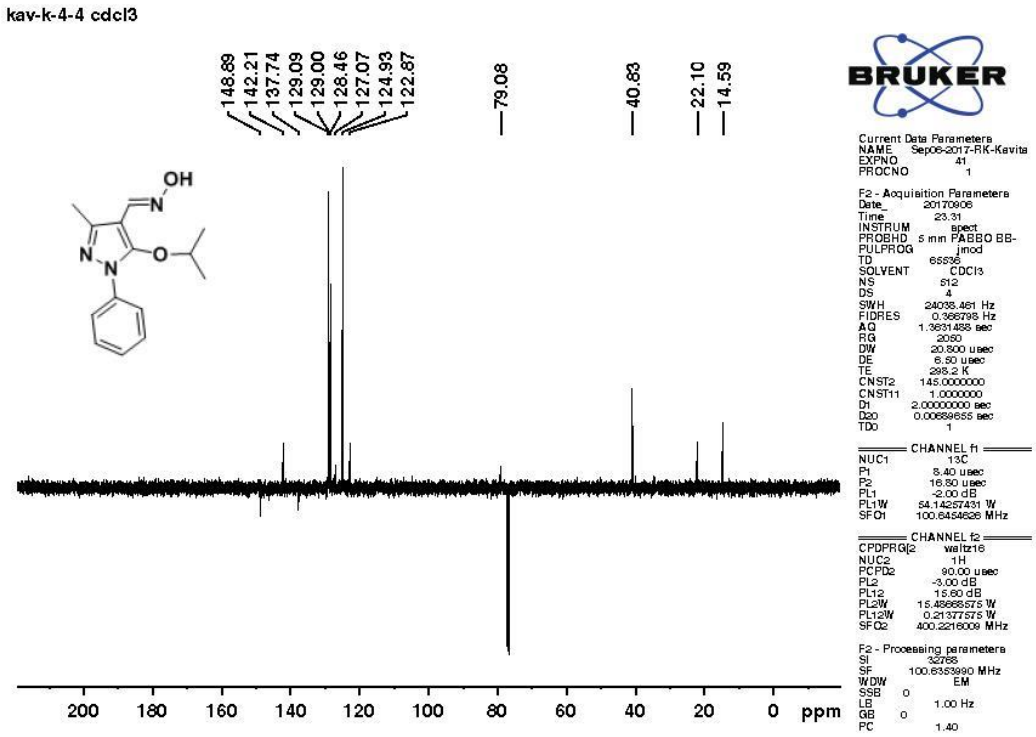


Figure 32. ^{13}C NMR of compound k-4-4

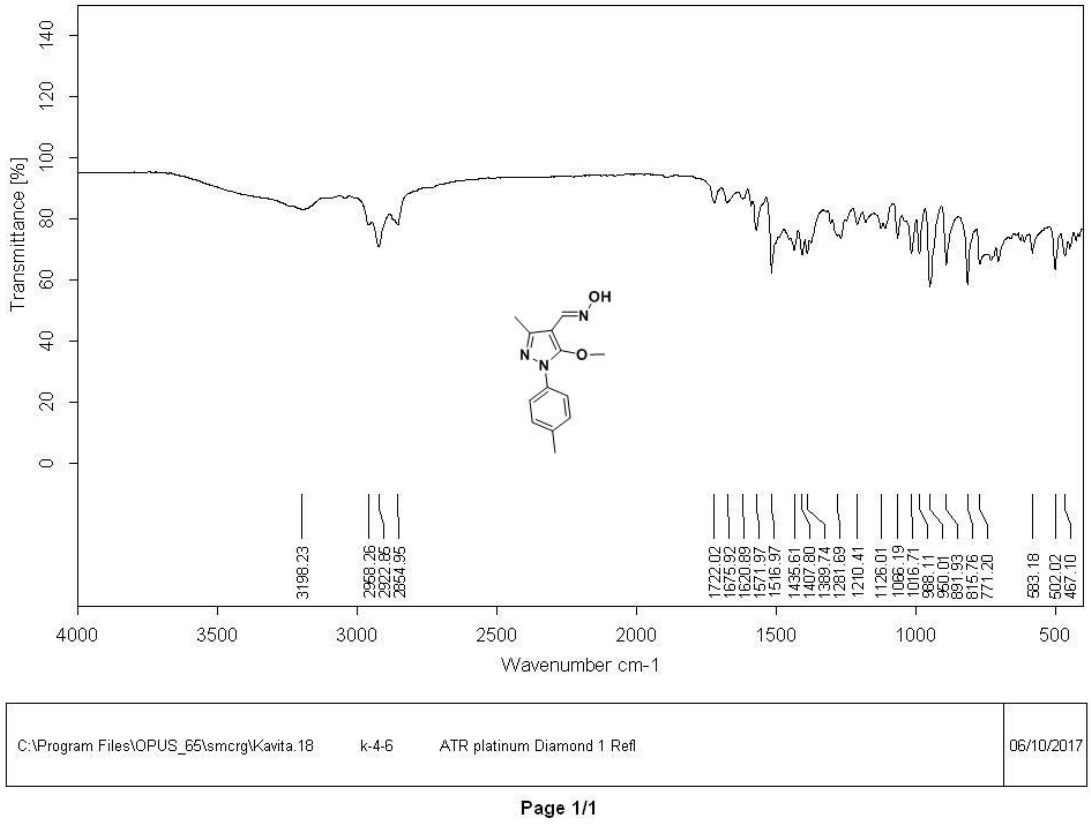


Figure 33. IR spectrum of compound k-4-6

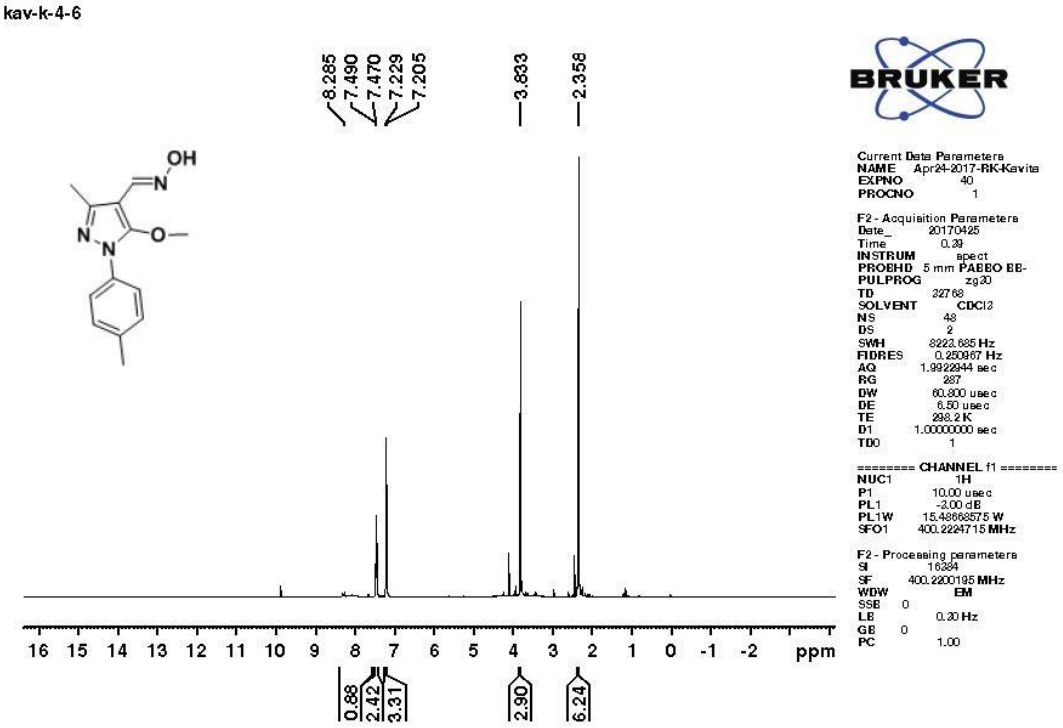


Figure 34. ^{1}H NMR of compound k-4-6

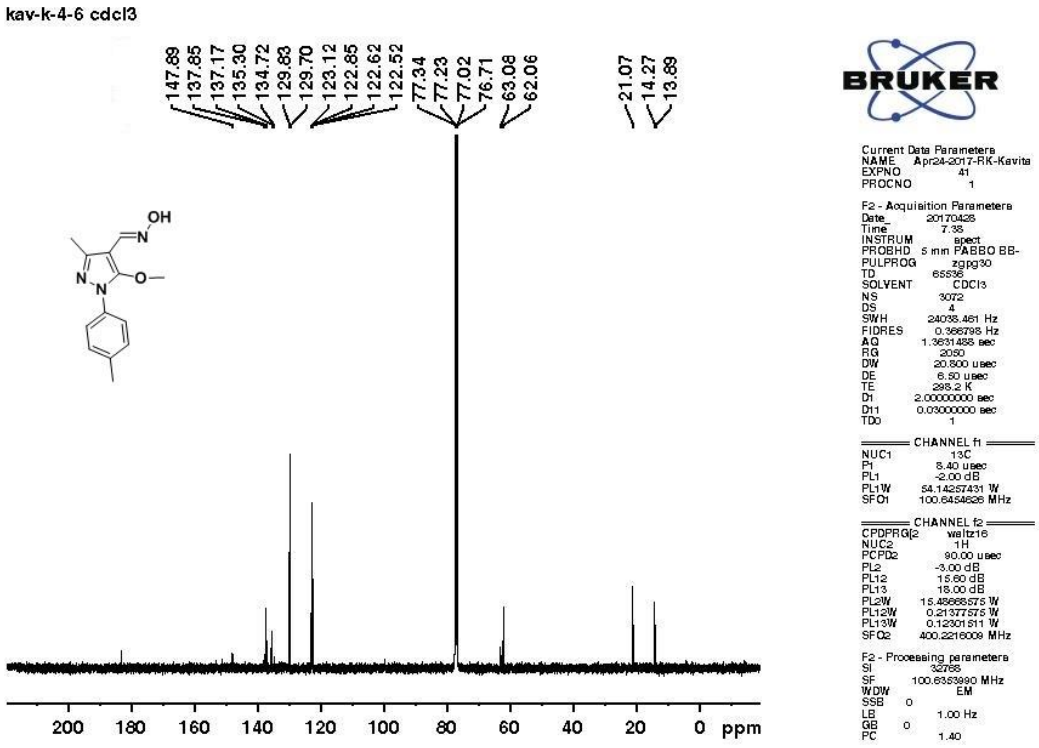


Figure 35. ^{13}C NMR of compound k-4-6

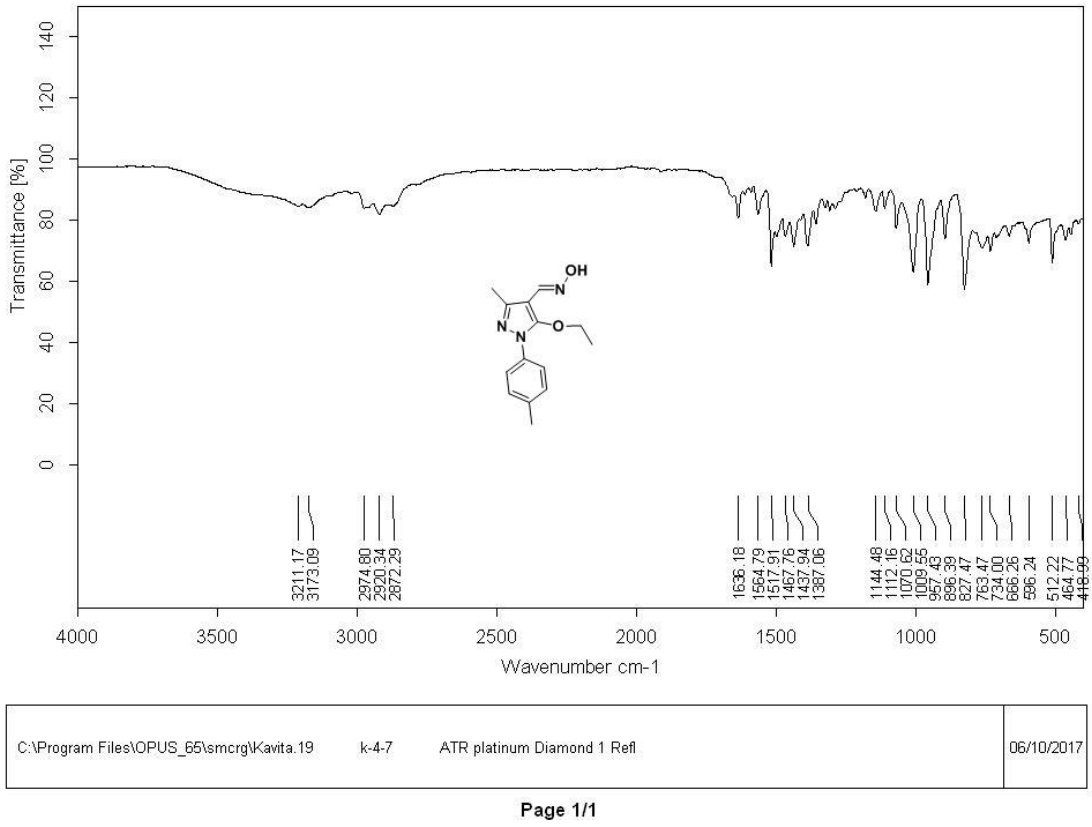


Figure 36. IR spectrum of compound k-4-7

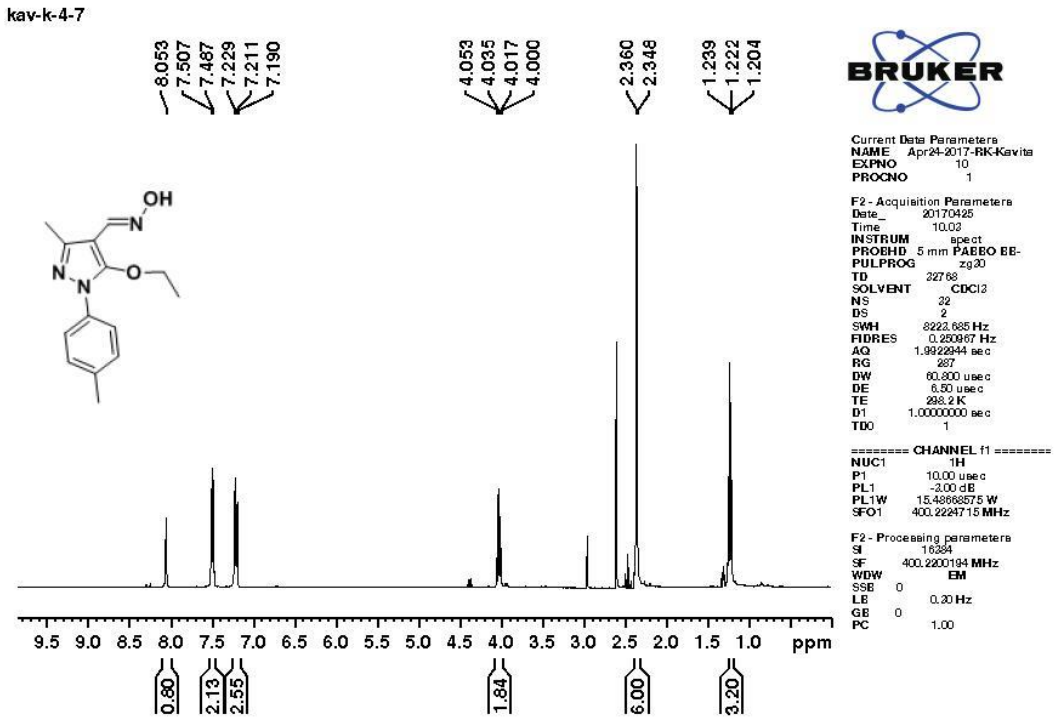


Figure 37. ^{1}H NMR of compound k-4-7

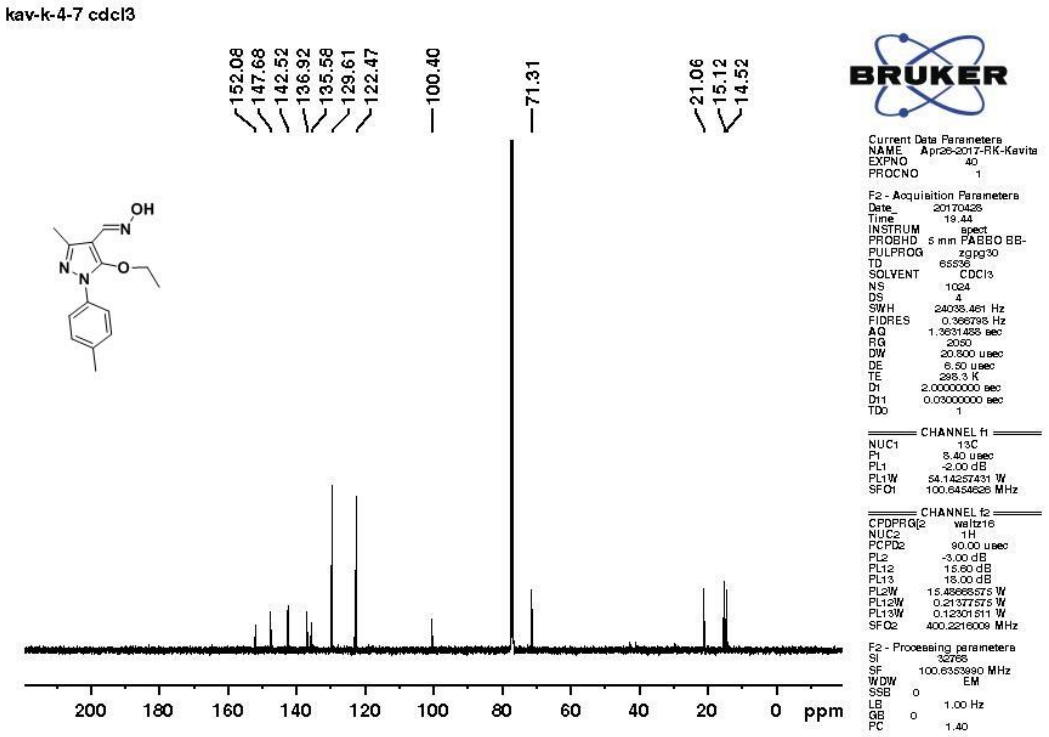


Figure 38. ^{13}C NMR of compound k-4-7

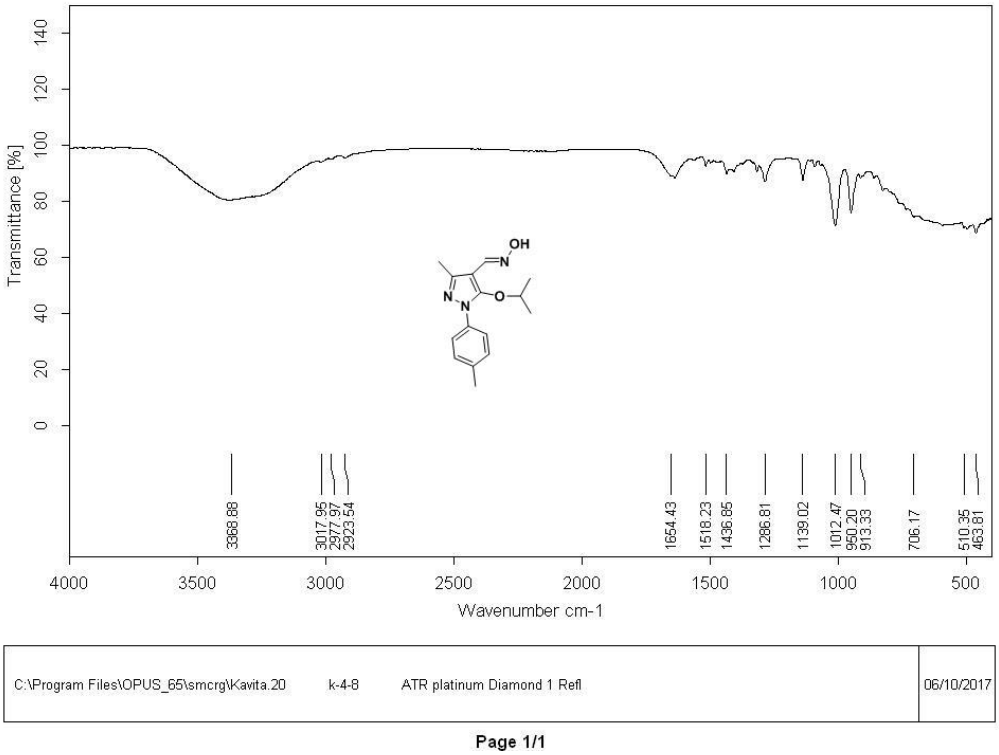


Figure 39. IR spectrum of compound k-4-8

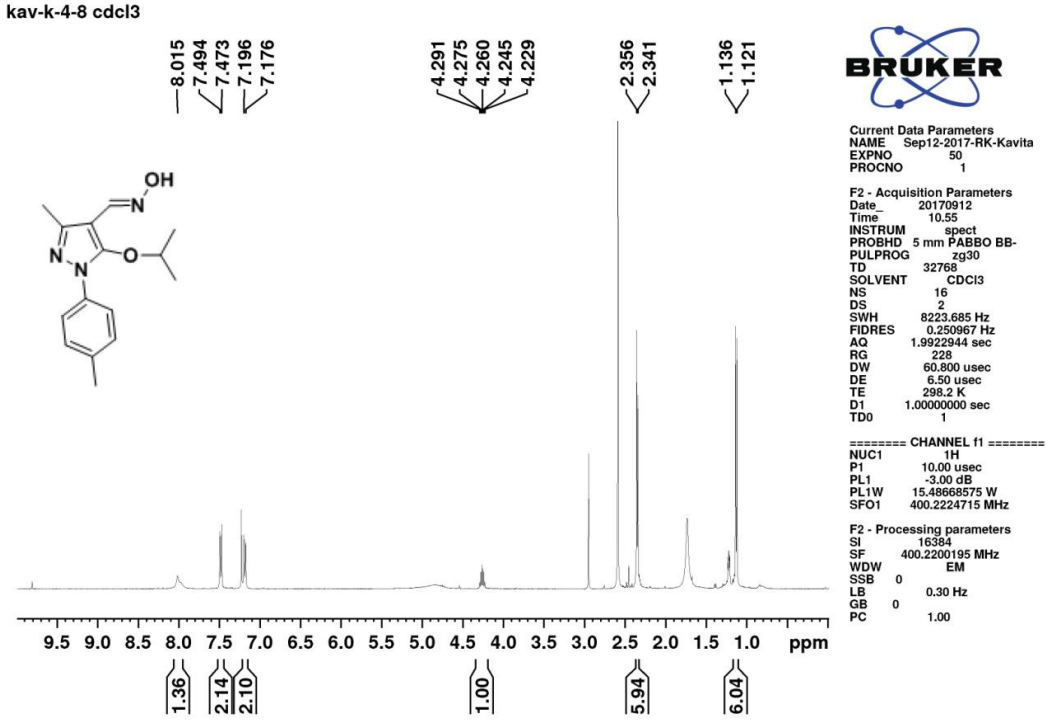


Figure 40. ^{1}H NMR of compound k-4-8

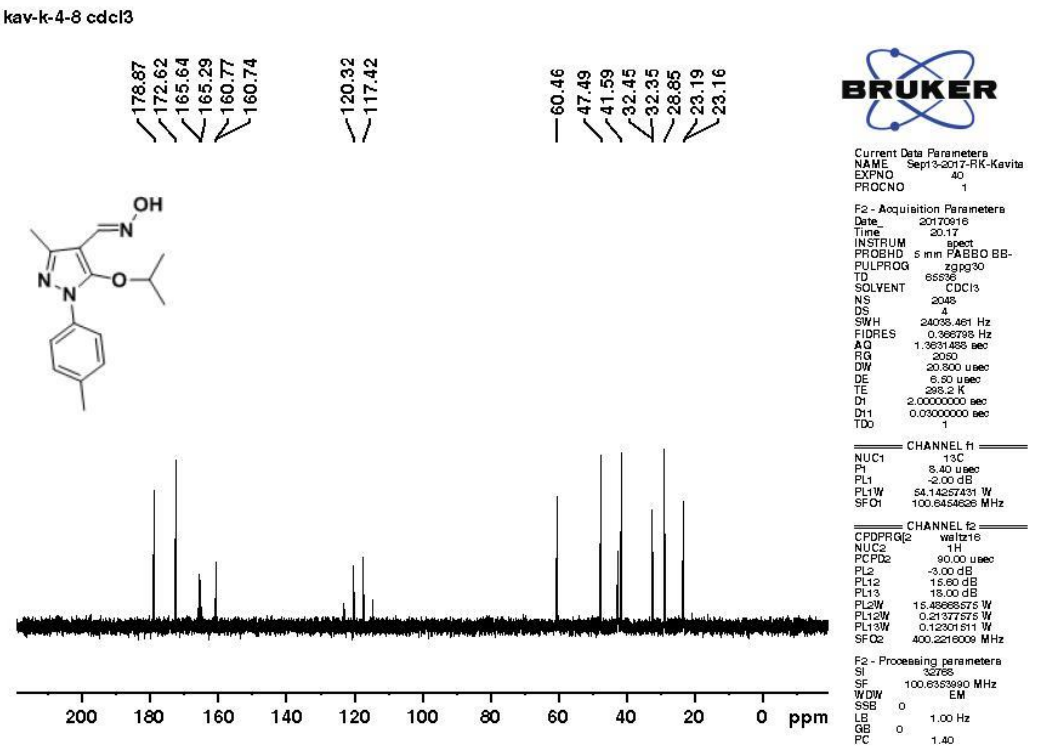


Figure 41. ^{13}C NMR of compound k-4-8

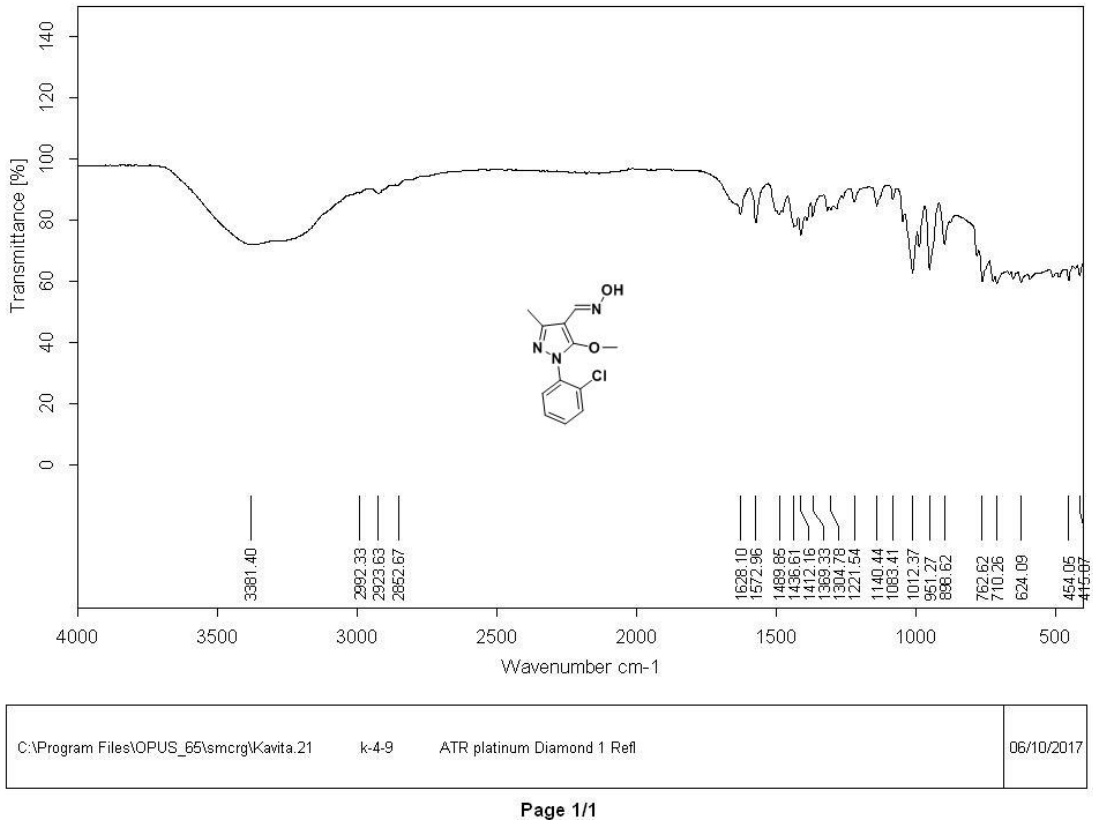


Figure 42. IR spectrum of compound k-4-9

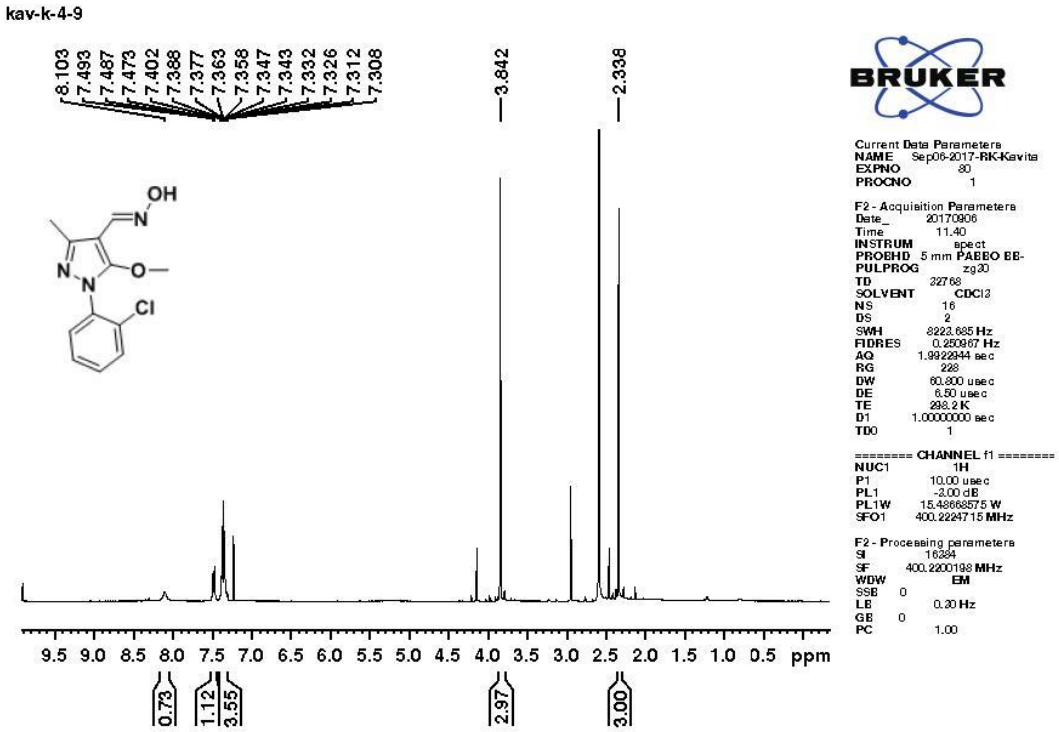


Figure 43. ^{1}H NMR of compound k-4-9

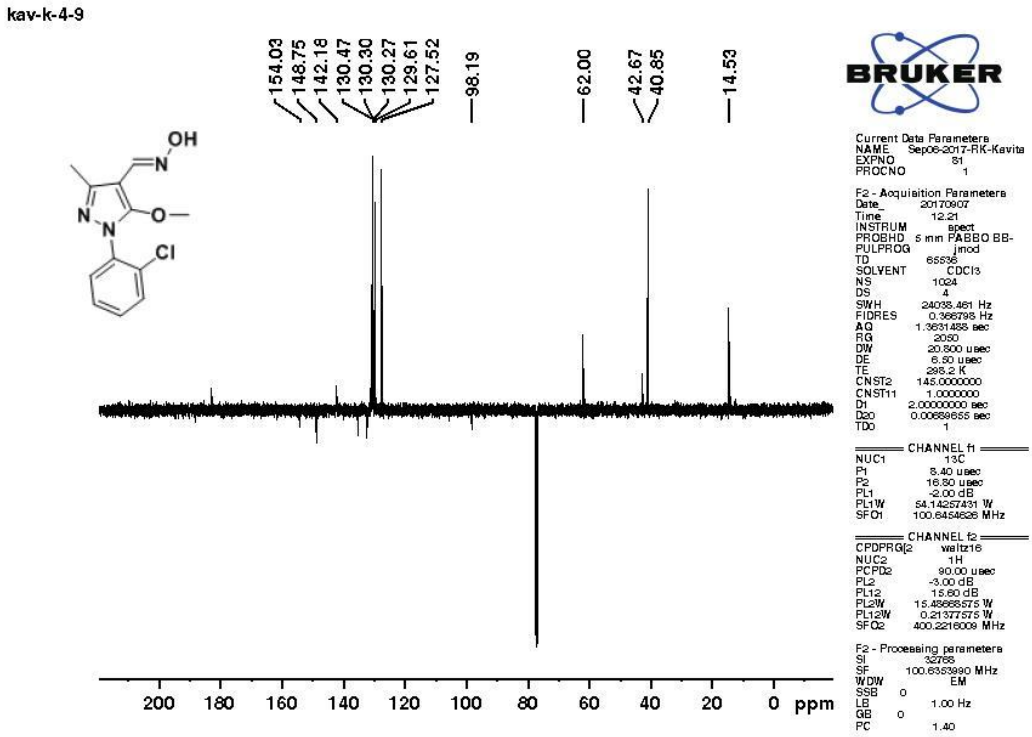


Figure 44. ^{13}C NMR of compound k-4-9

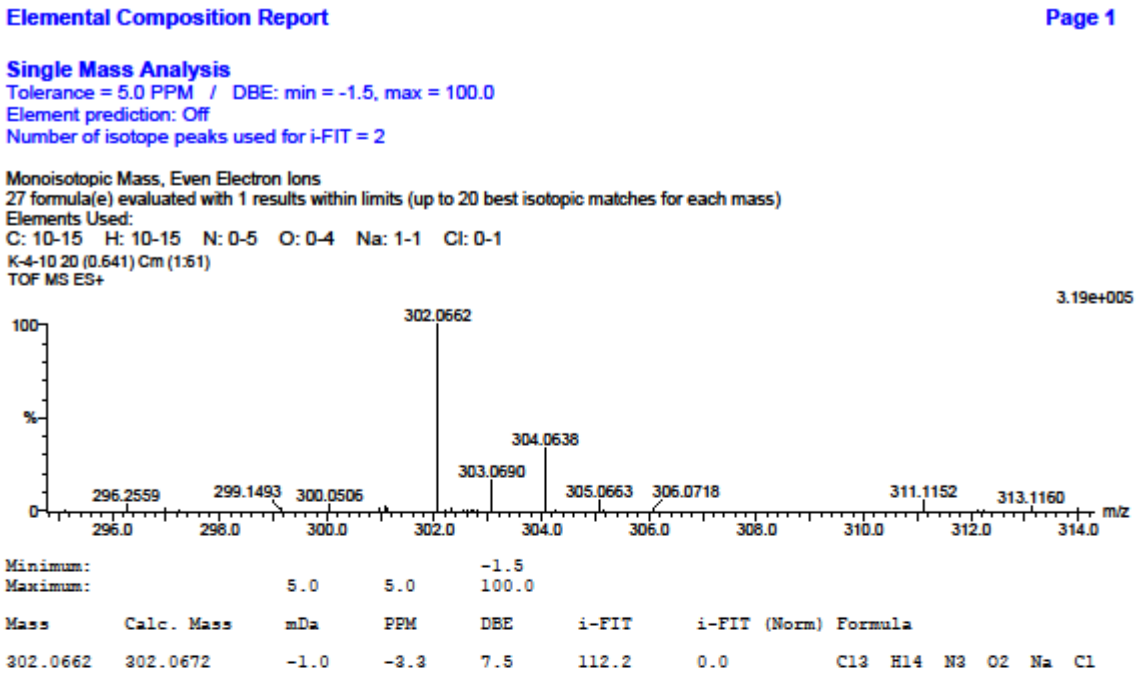


Figure 45. HRMS of compound k-4-10

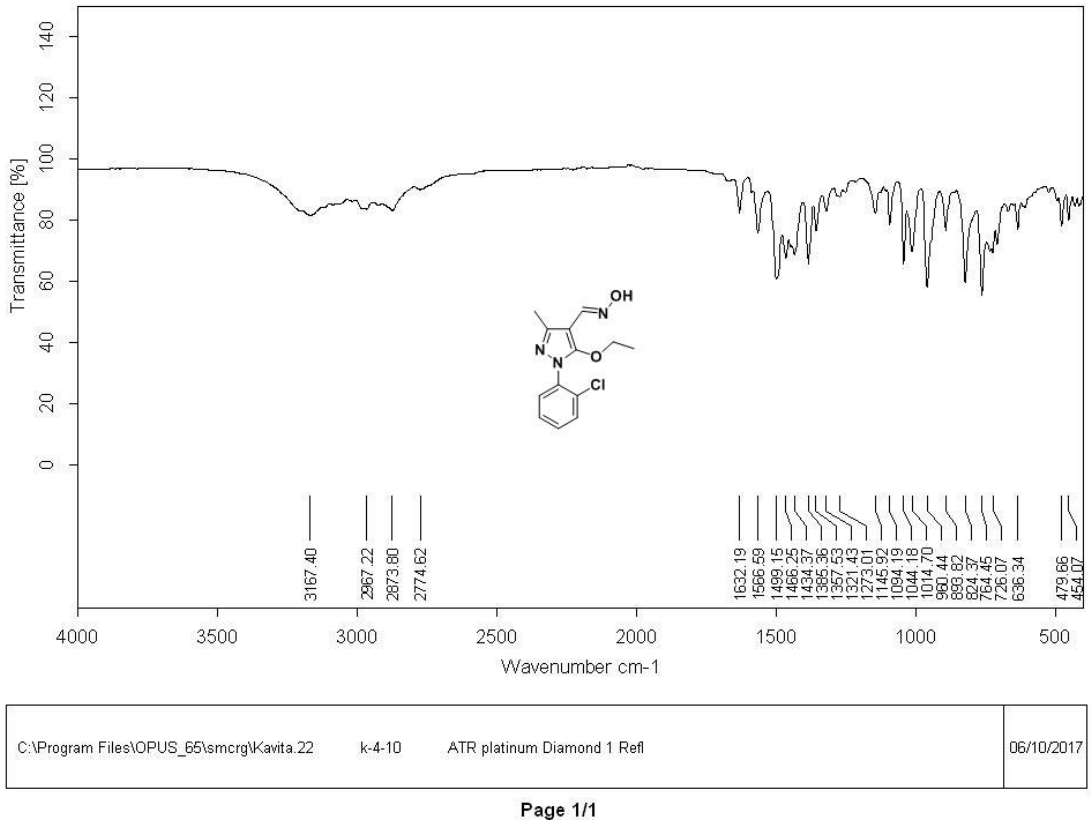


Figure 46. IR spectrum of compound k-4-10

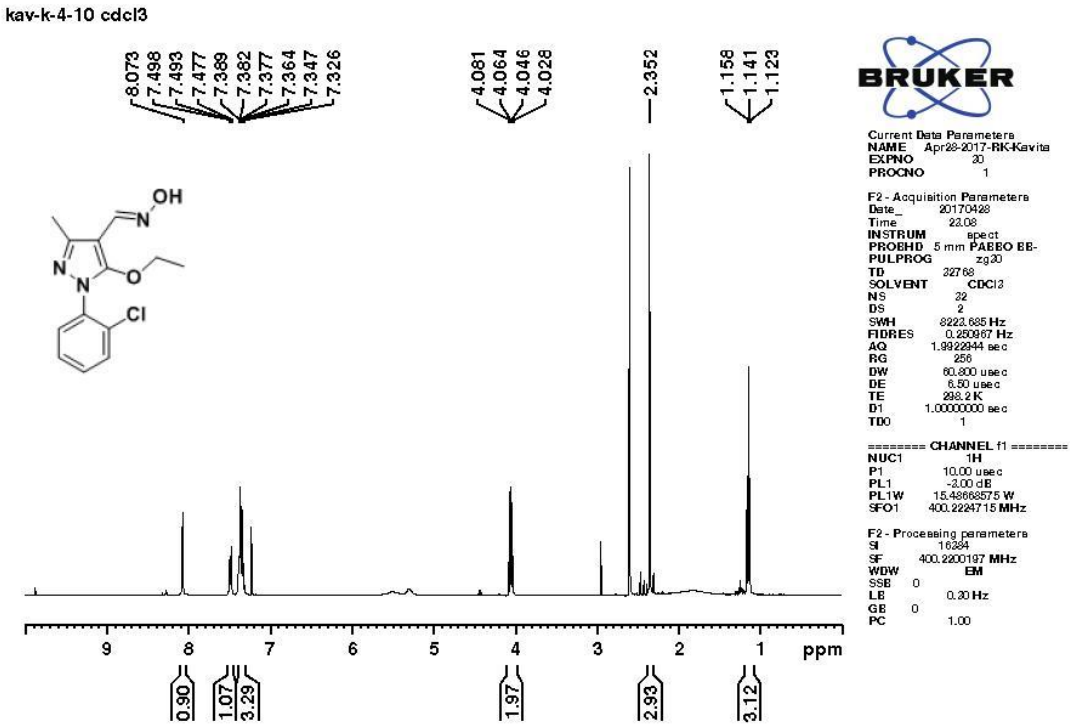


Figure 47. ^{1}H NMR of compound k-4-10

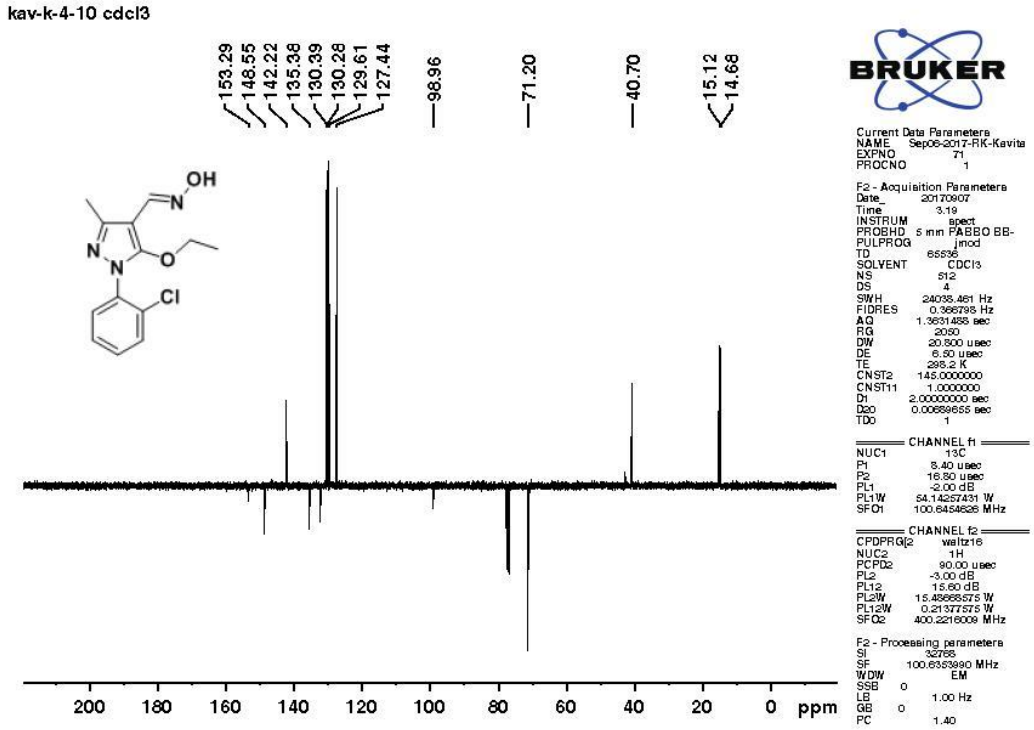


Figure 48. ^{13}C NMR of compound k-4-10

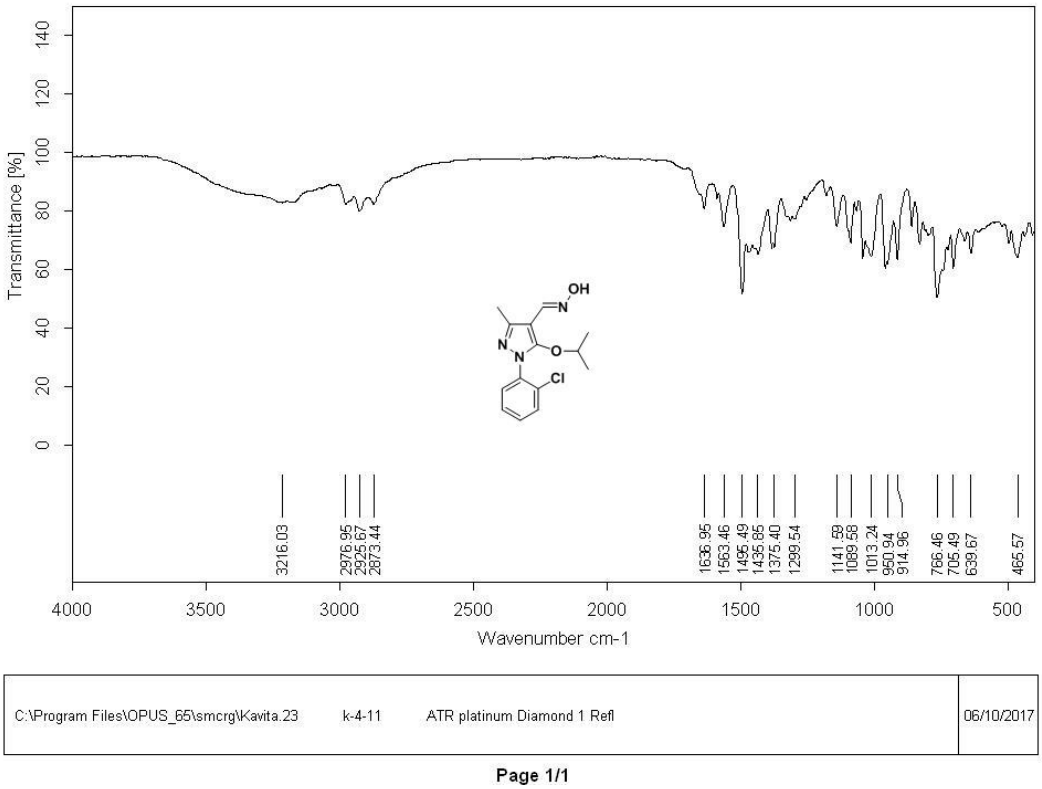


Figure 49. IR spectrum of compound k-4-11

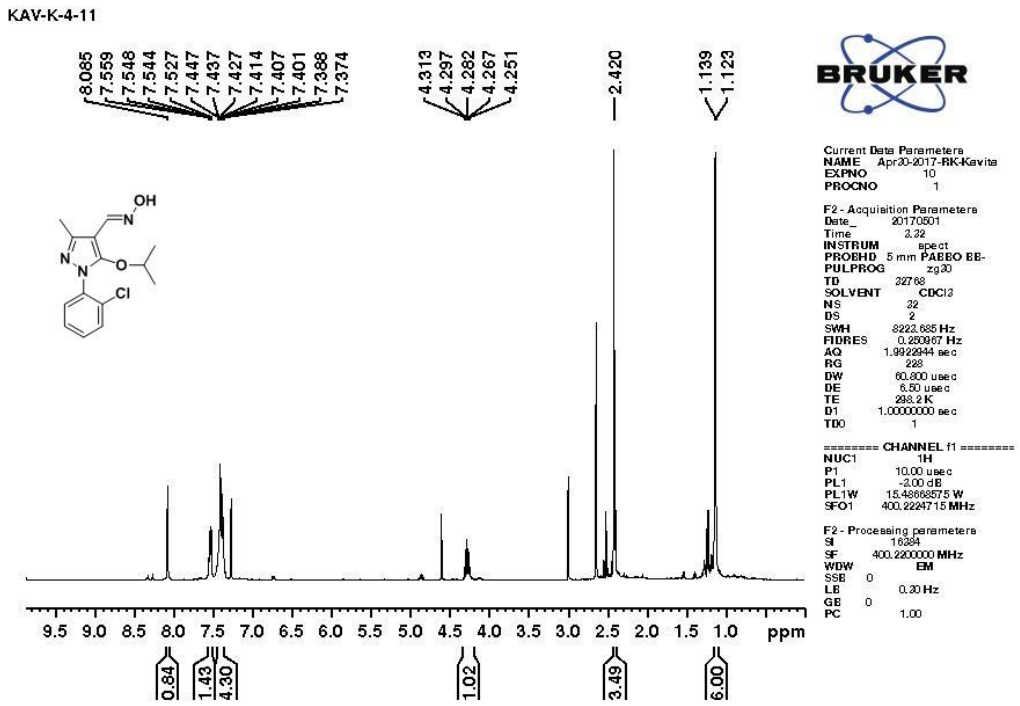


Figure 50. ^{1}H NMR of compound k-4-11

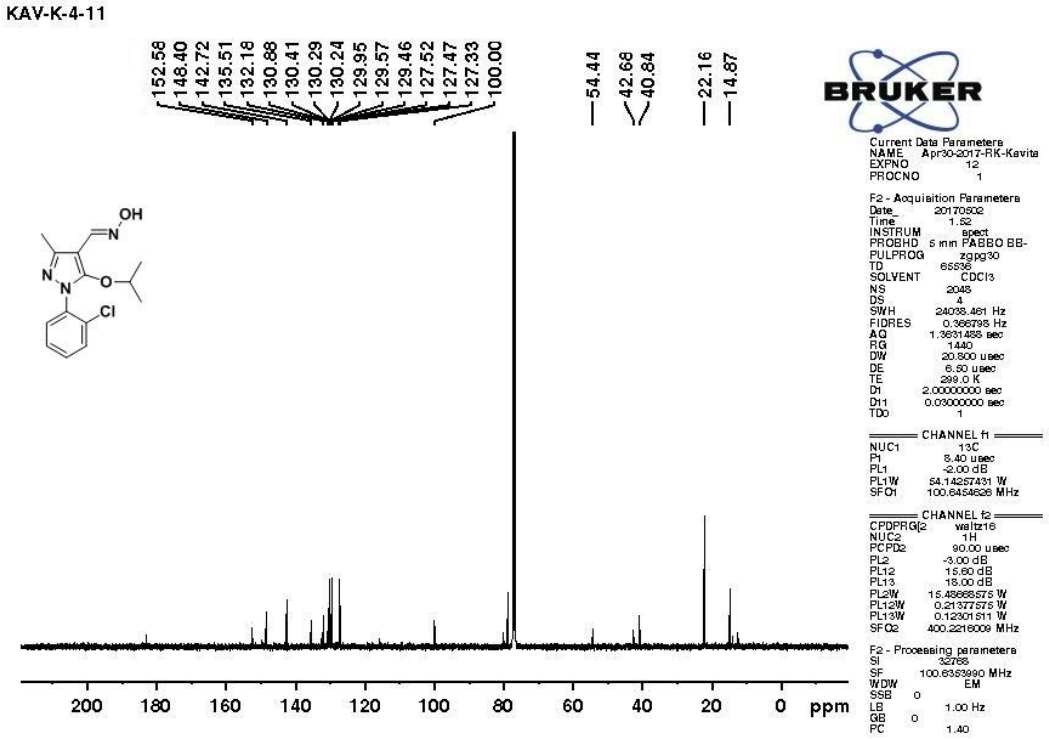


Figure 51. ^{13}C NMR of compound k-4-11

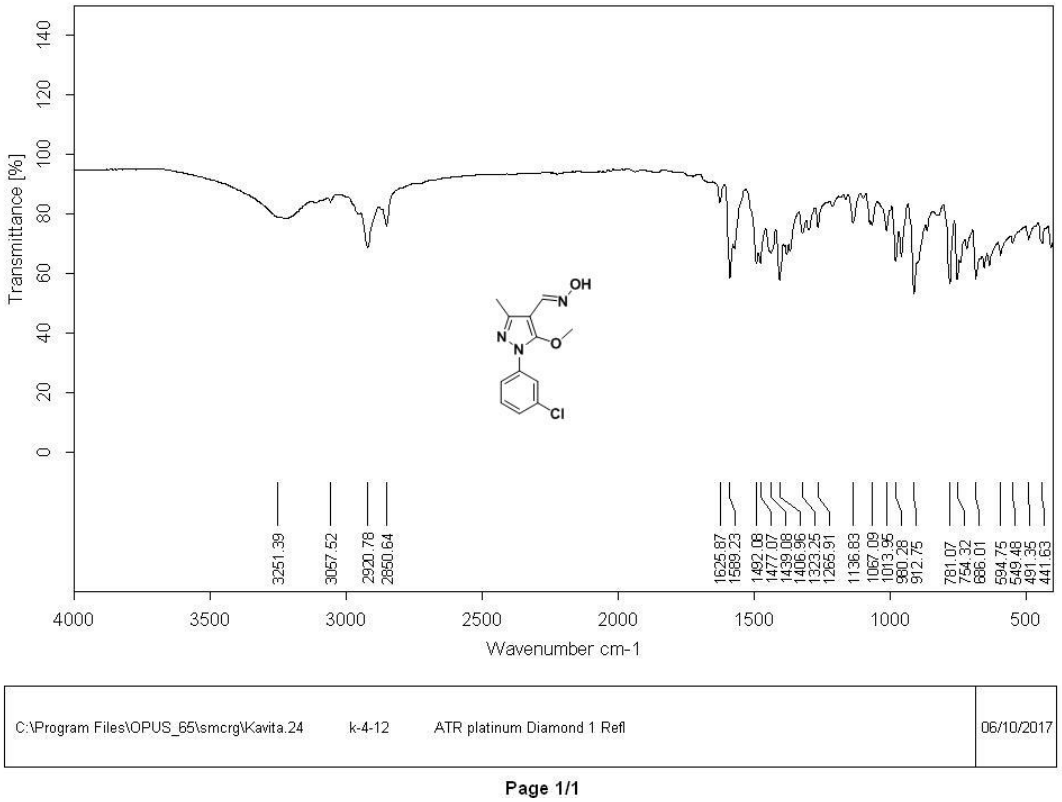


Figure 52. IR spectrum of compound k-4-12

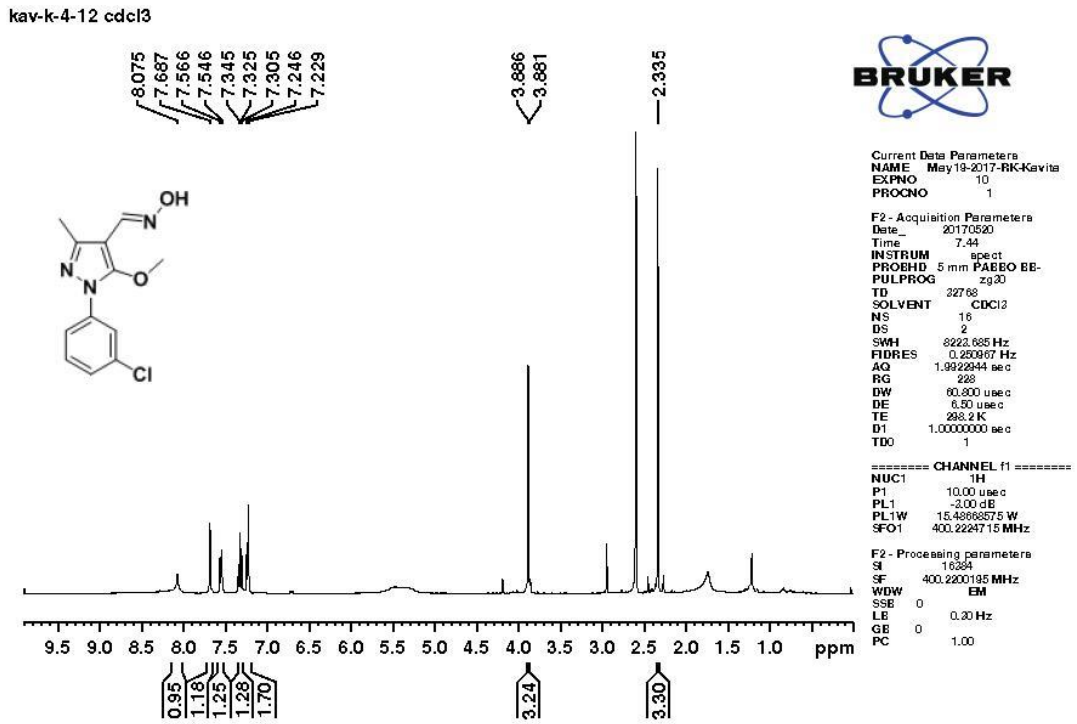


Figure 53. ^{1}H NMR of compound k-4-12

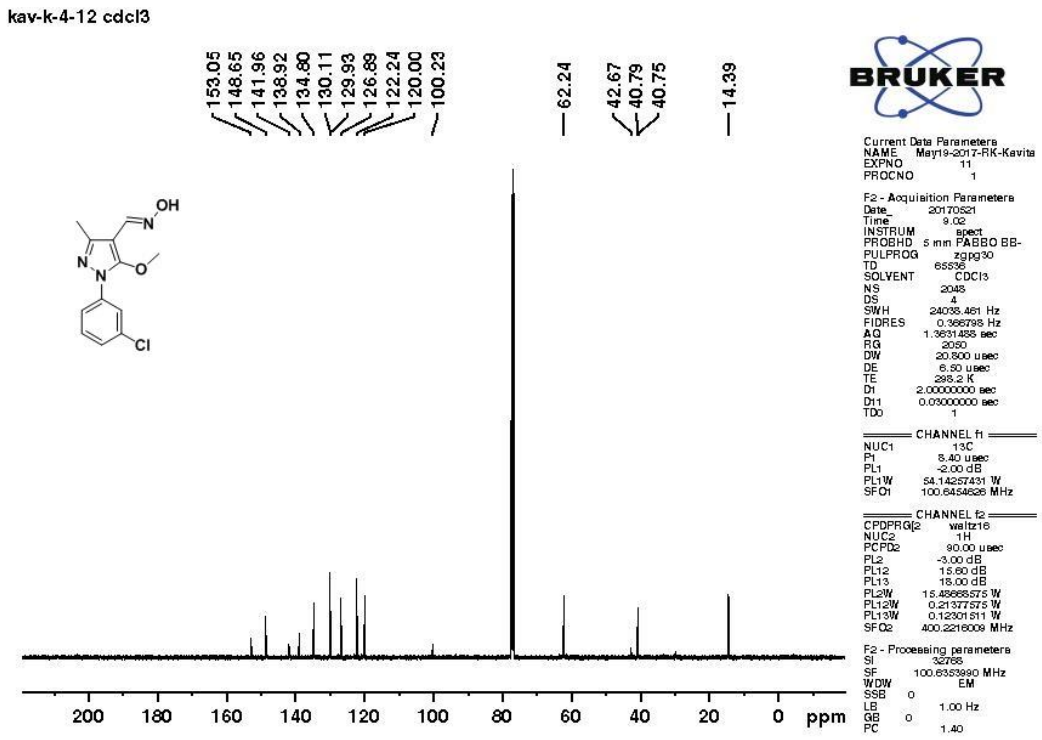


Figure 54. ^{13}C NMR of compound k-4-12

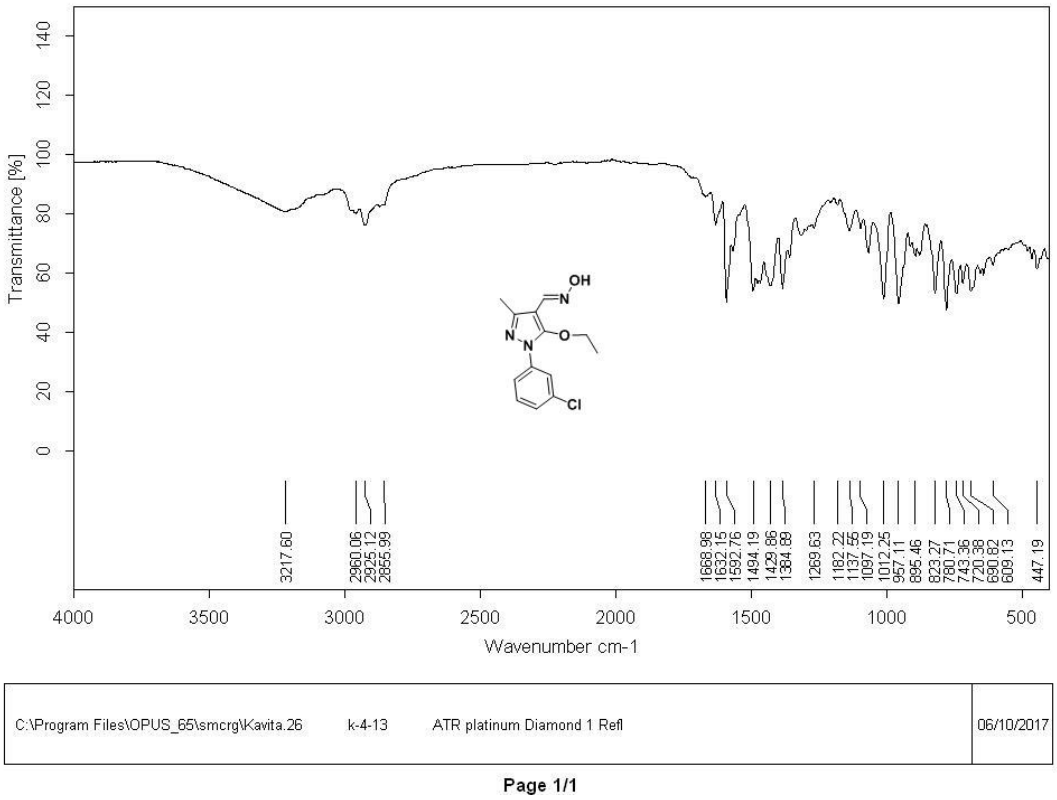


Figure 55. IR spectrum of compound k-4-13

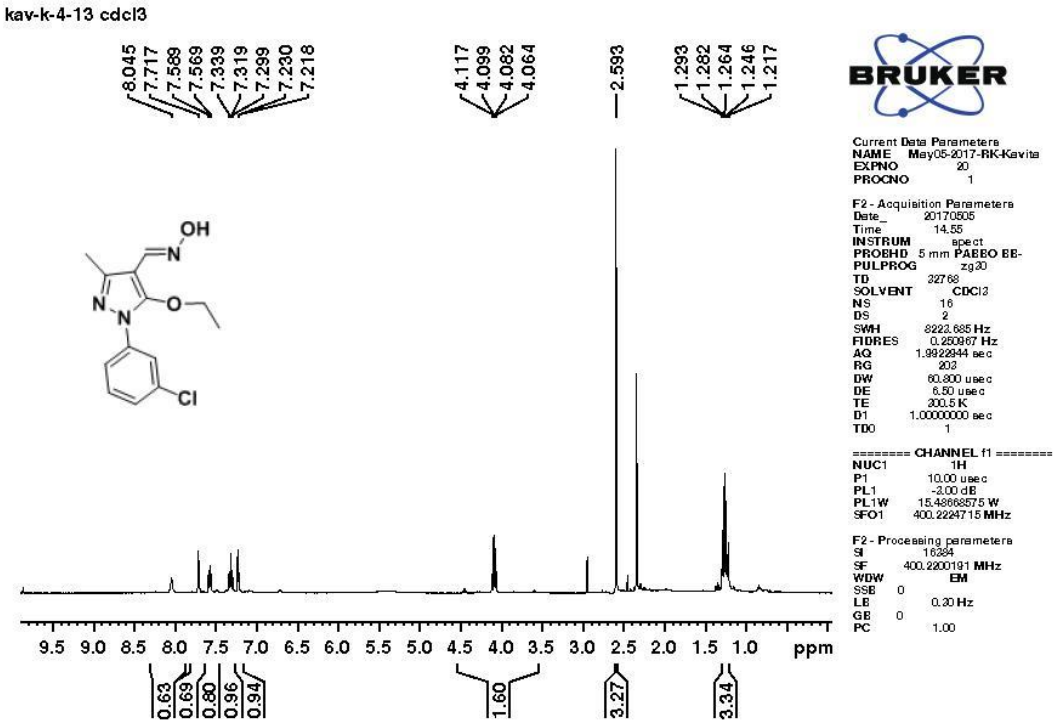


Figure 56. ^{1}H NMR of compound k-4-13

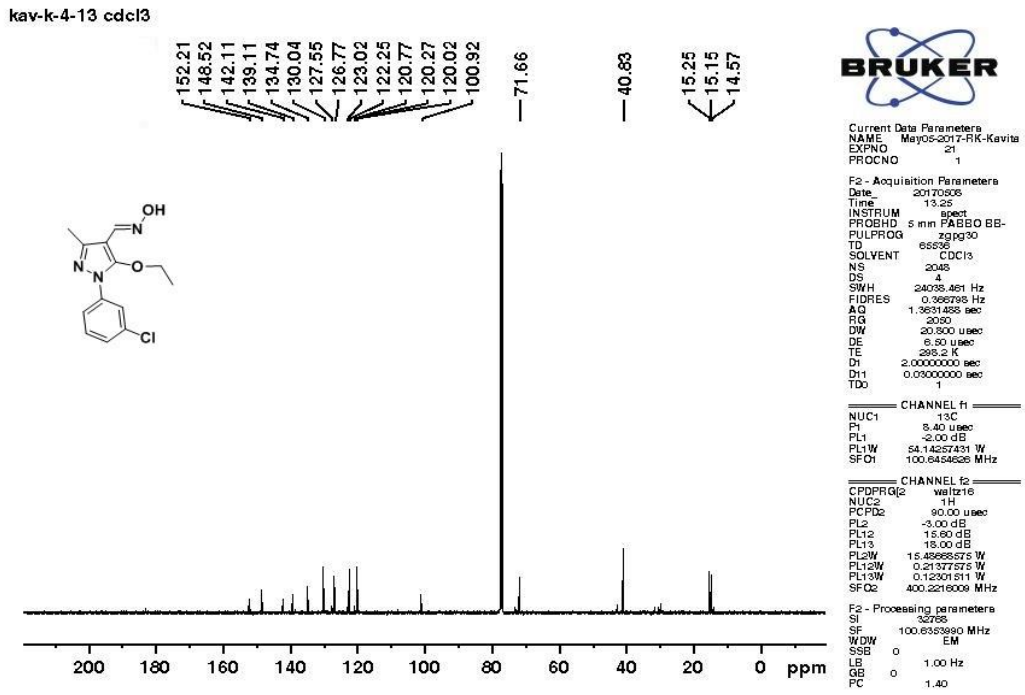


Figure 57. ^{13}C NMR of compound k-4-13

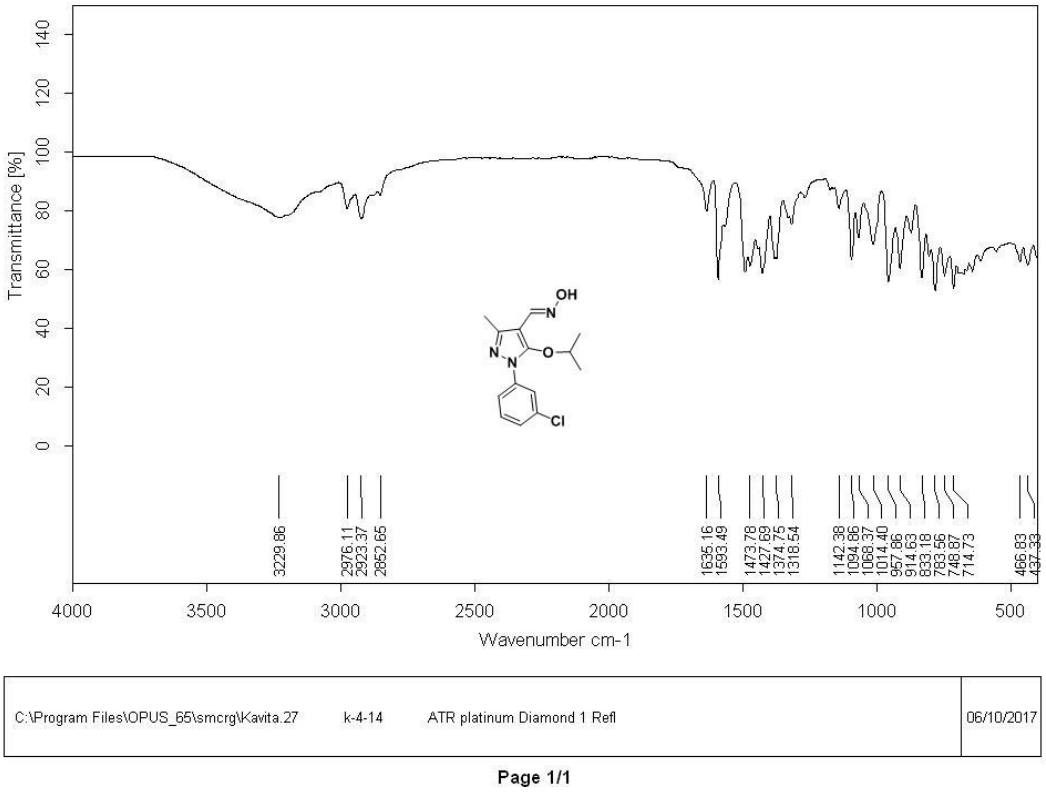


Figure 58. IR spectrum of compound k-4-14

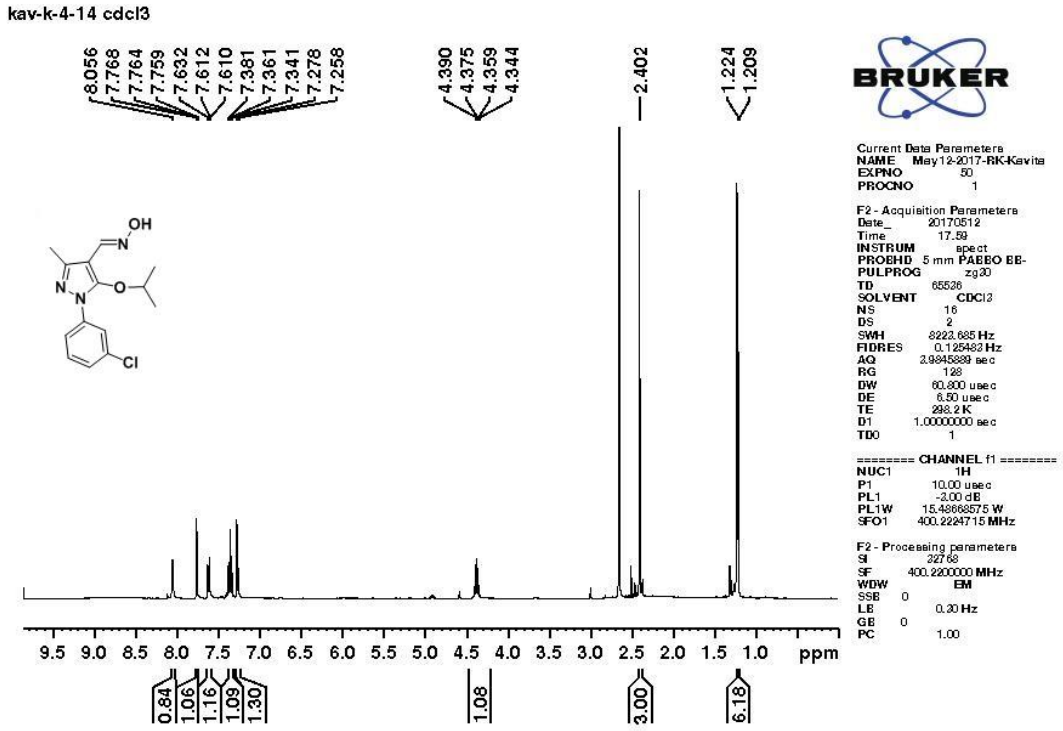


Figure 59. ^{1}H NMR of compound k-4-14

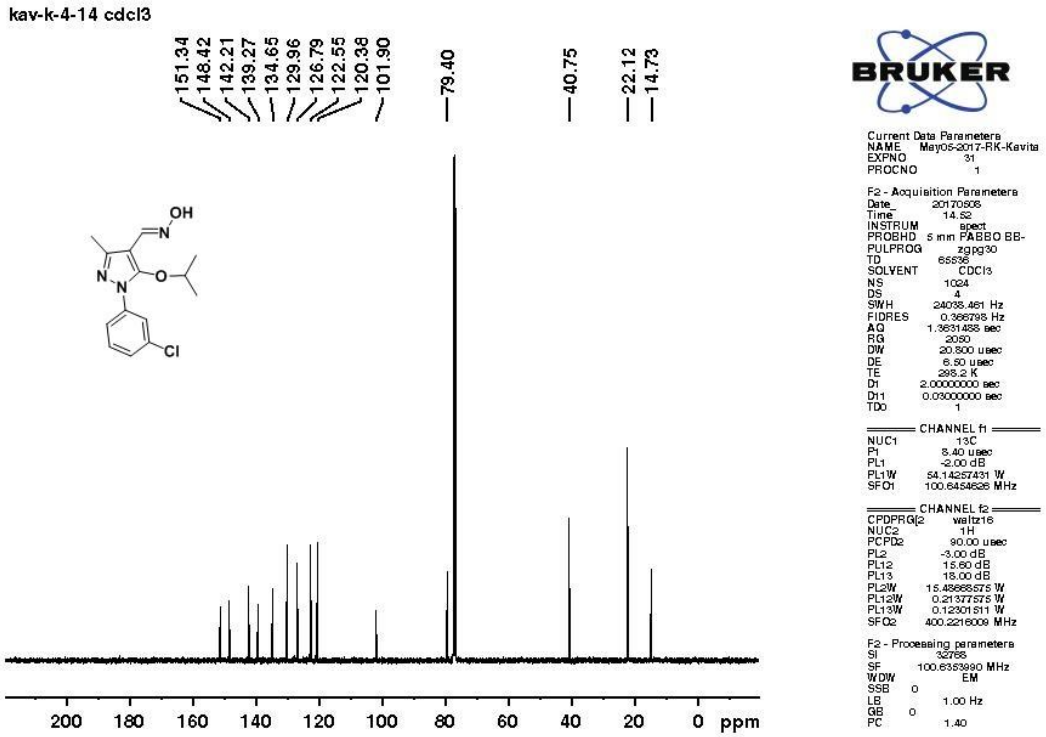


Figure 60. ^{13}C NMR of compound k-4-14

1.4 CHAPTER 6

Title: Preparation and antimicrobial activity of novel pyrazole-chalcones derivatives

Authors name: Kavita Jain[a], Nisar Syyad and Rajshekhar Karpoormath[a,*]

Affiliations: [a] *Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa.*

To receive all correspondence: Rajshekhar Karpoormath: Tel: +27 (0) 312607179, *E-mail:* karpoormath@ukzn.ac.za

Supplementary data:

Spectral Information:

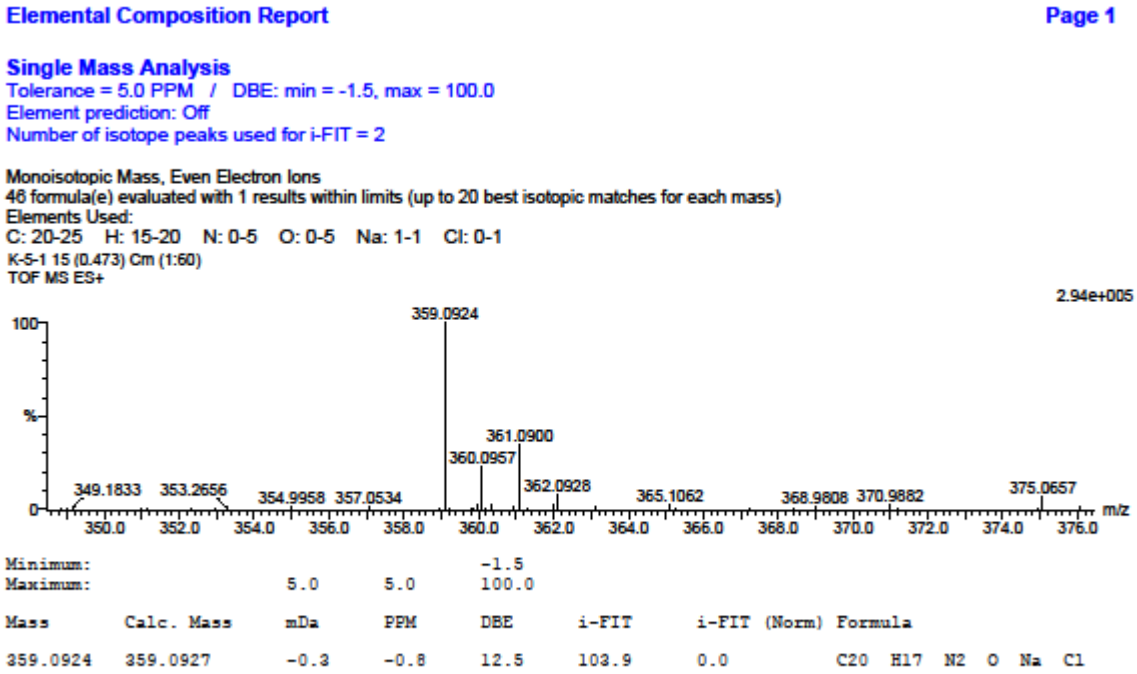


Figure 1. HRMS of compound 4-1

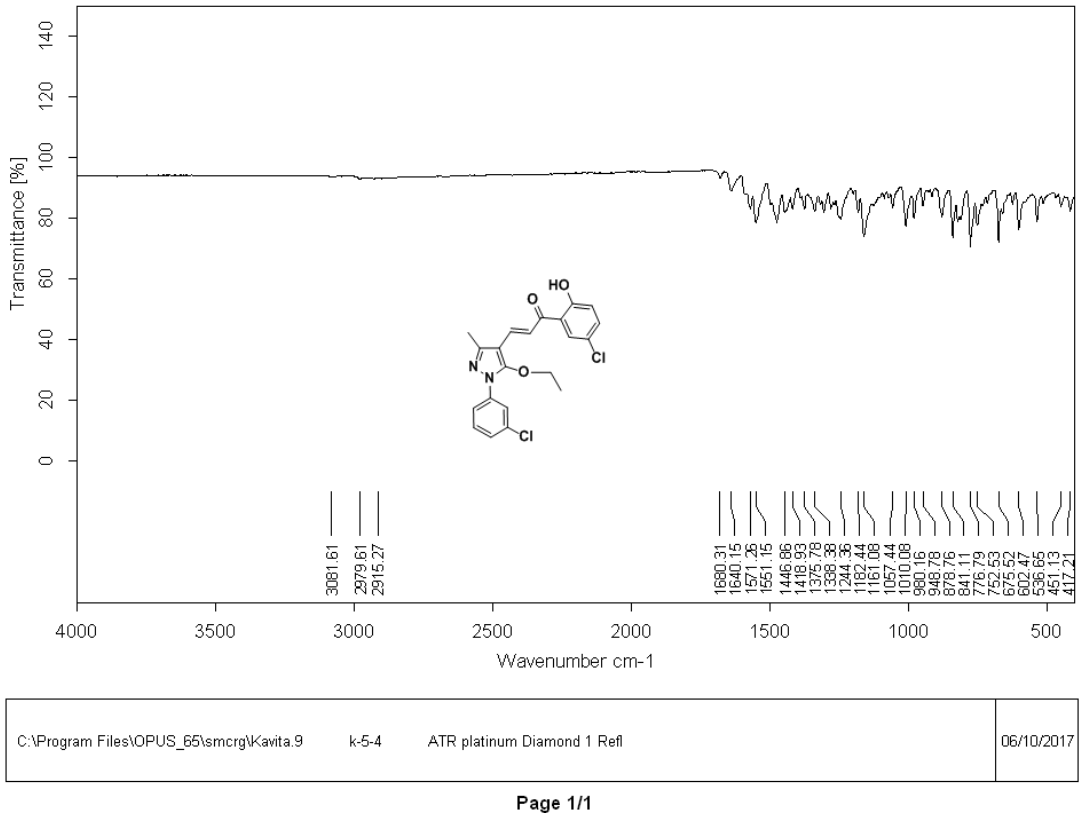


Figure 2.IR spectrum of compound 4-1

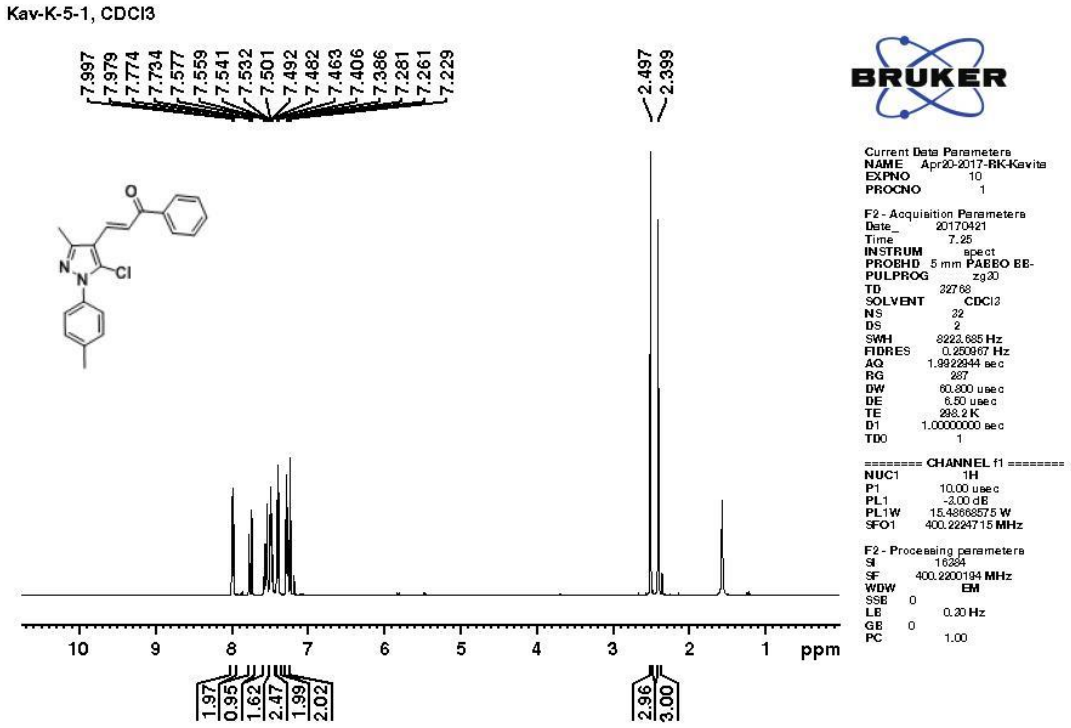


Figure 3.[1]H NMR of compound 4-1

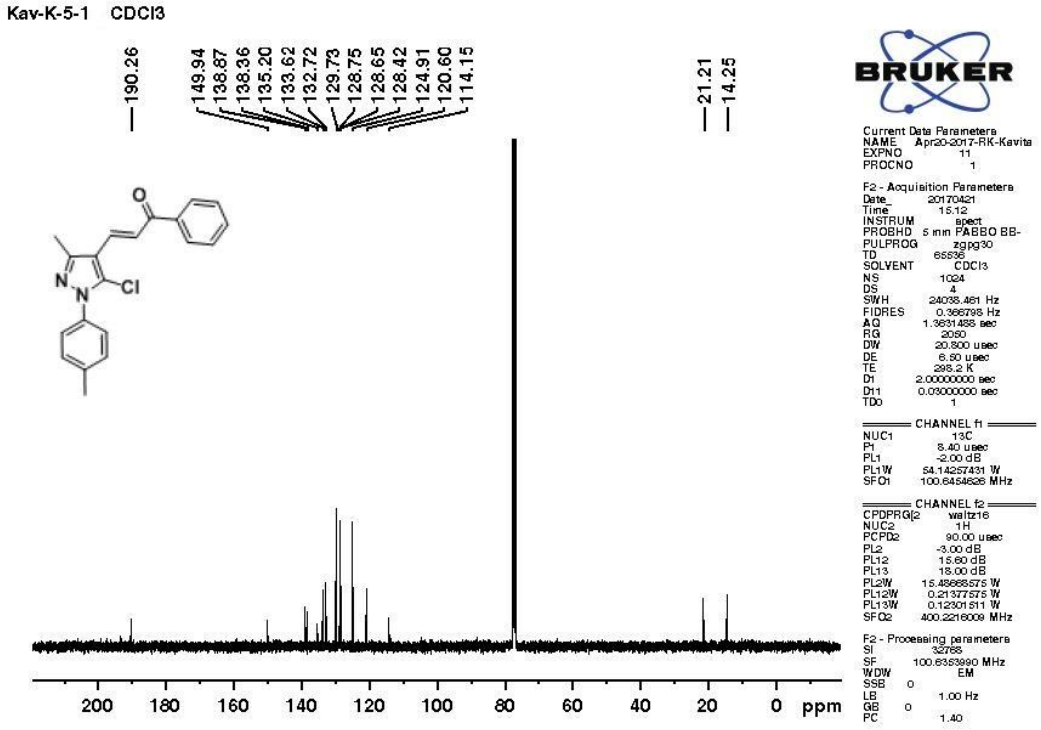


Figure 4.^{13}C NMR of compound 4-1

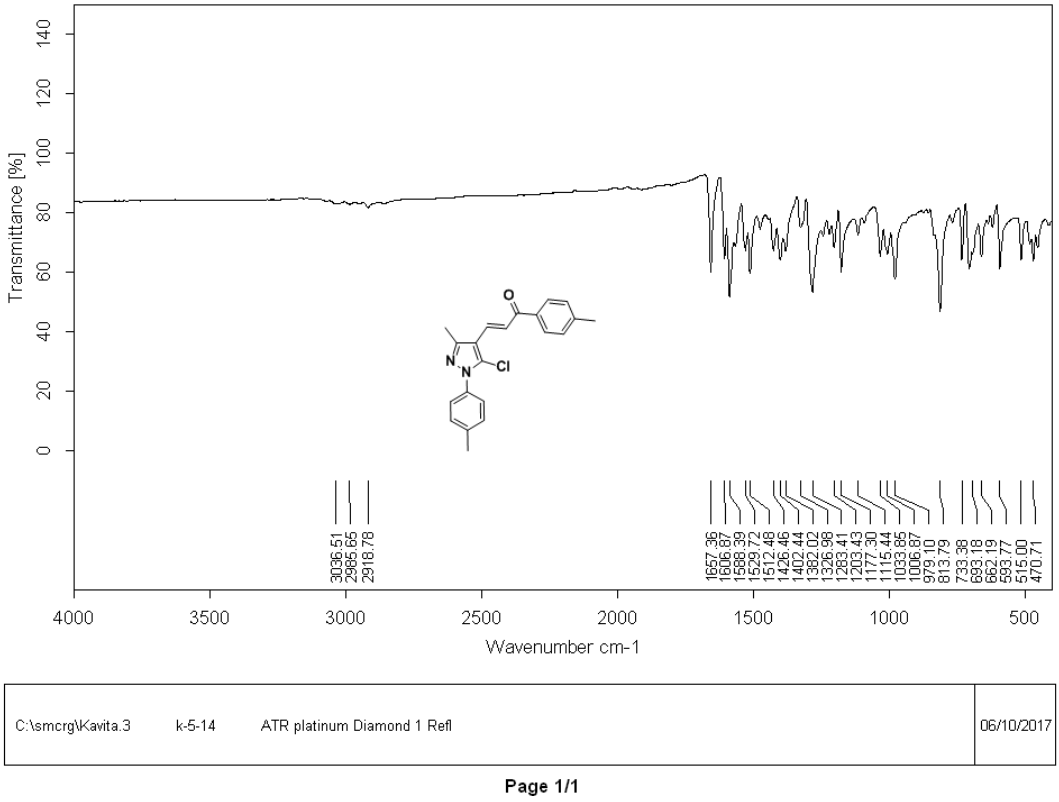


Figure 5. IR spectrum of compound 4-2

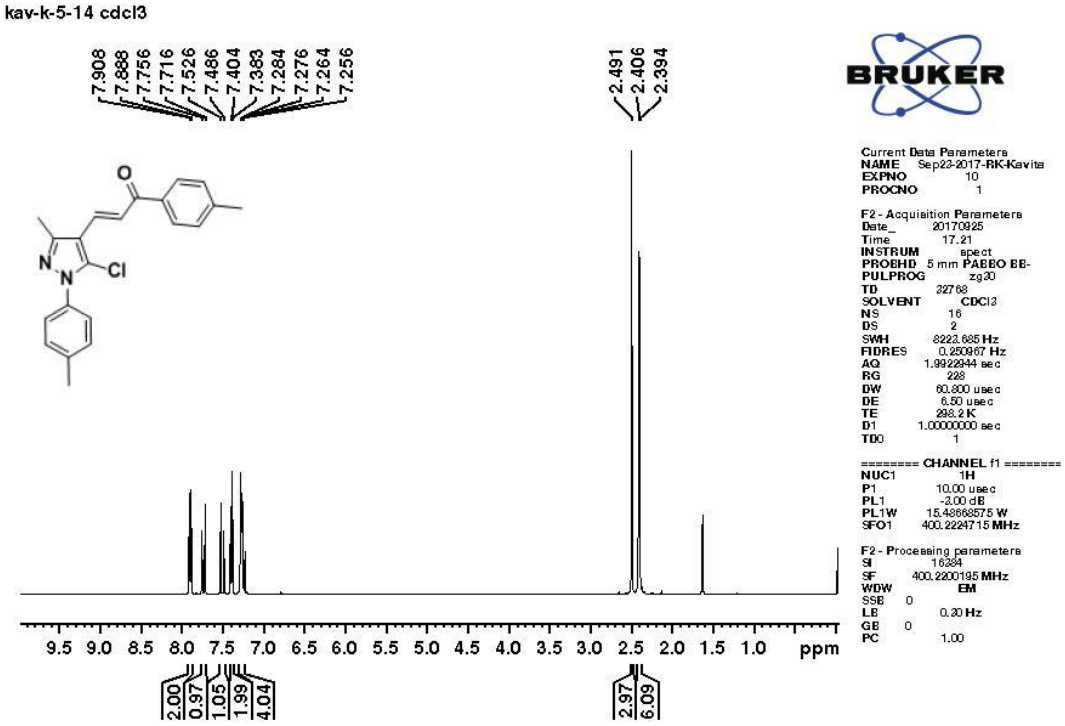


Figure 6. ^{1}H NMR of compound 4-2

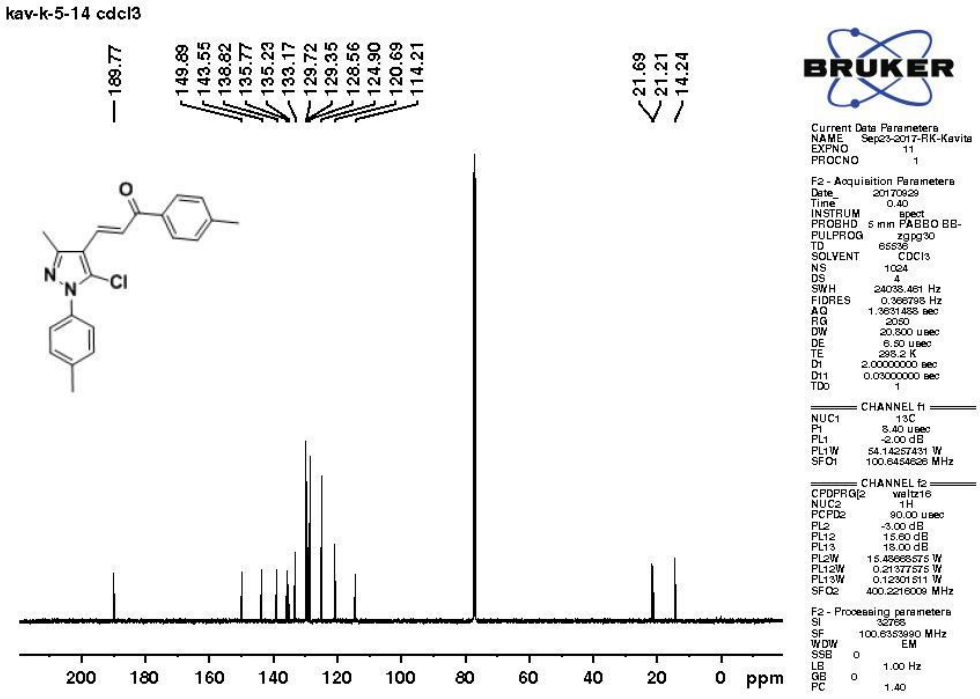


Figure 7. ^{13}C NMR of compound 4-2

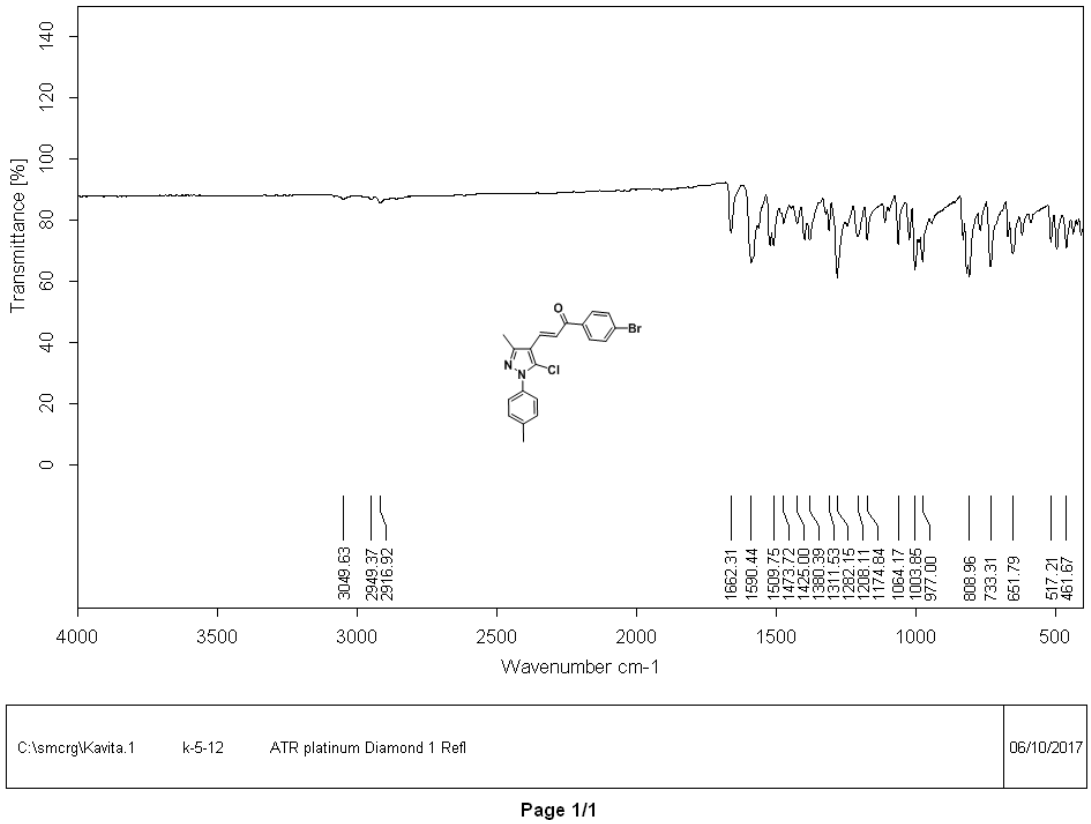


Figure 8. IR spectrum of compound 4-3

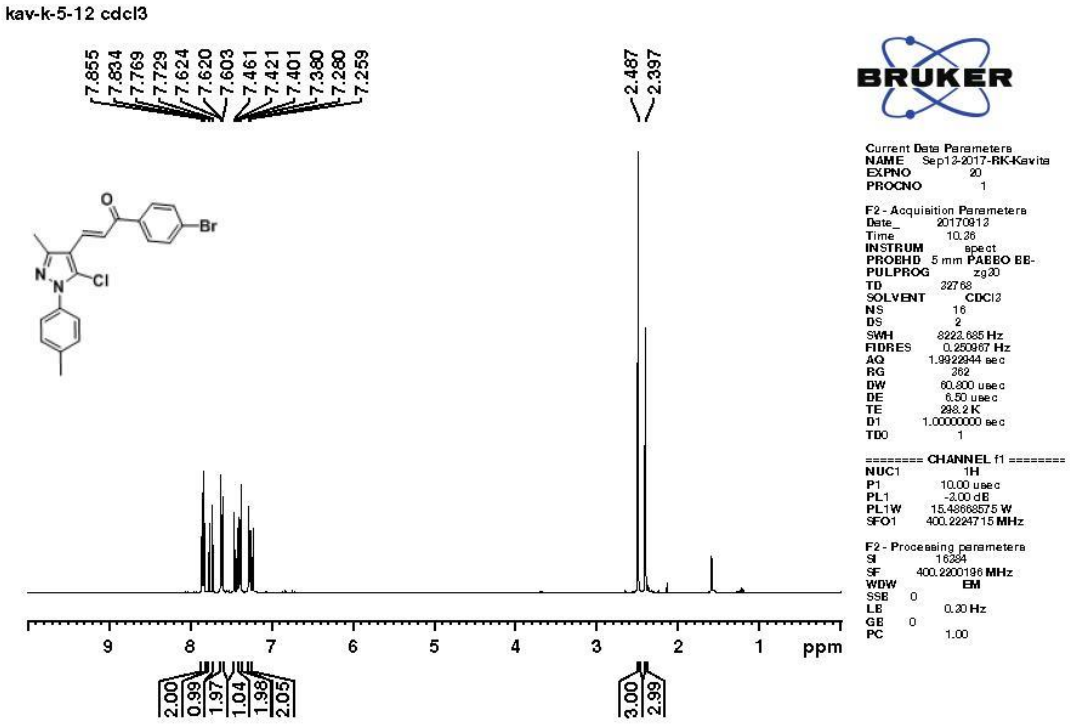


Figure 9. ^{1}H NMR of compound 4-3

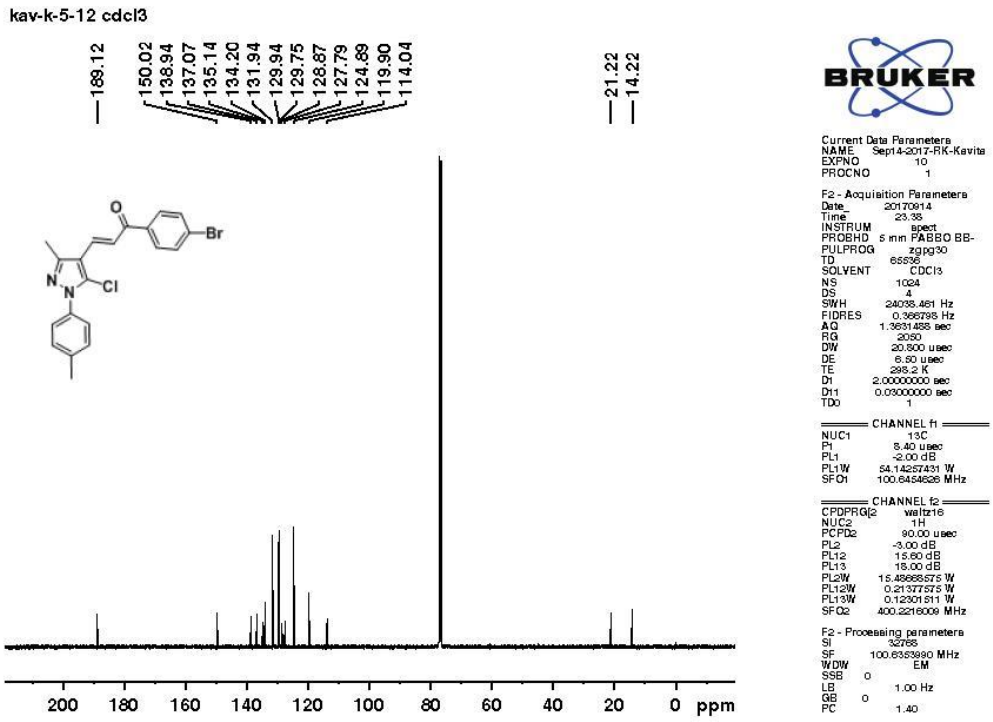


Figure 10. ^{13}C NMR of compound 4-3

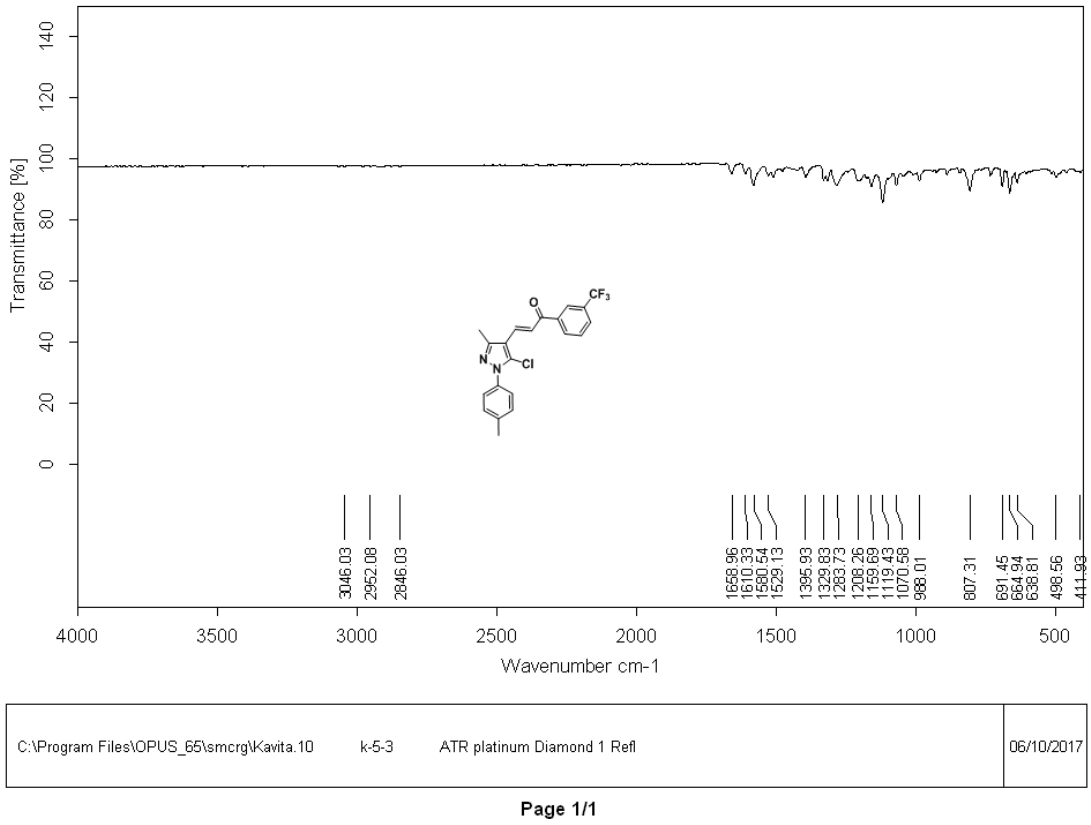


Figure 11. IR spectrum of compound 4-4

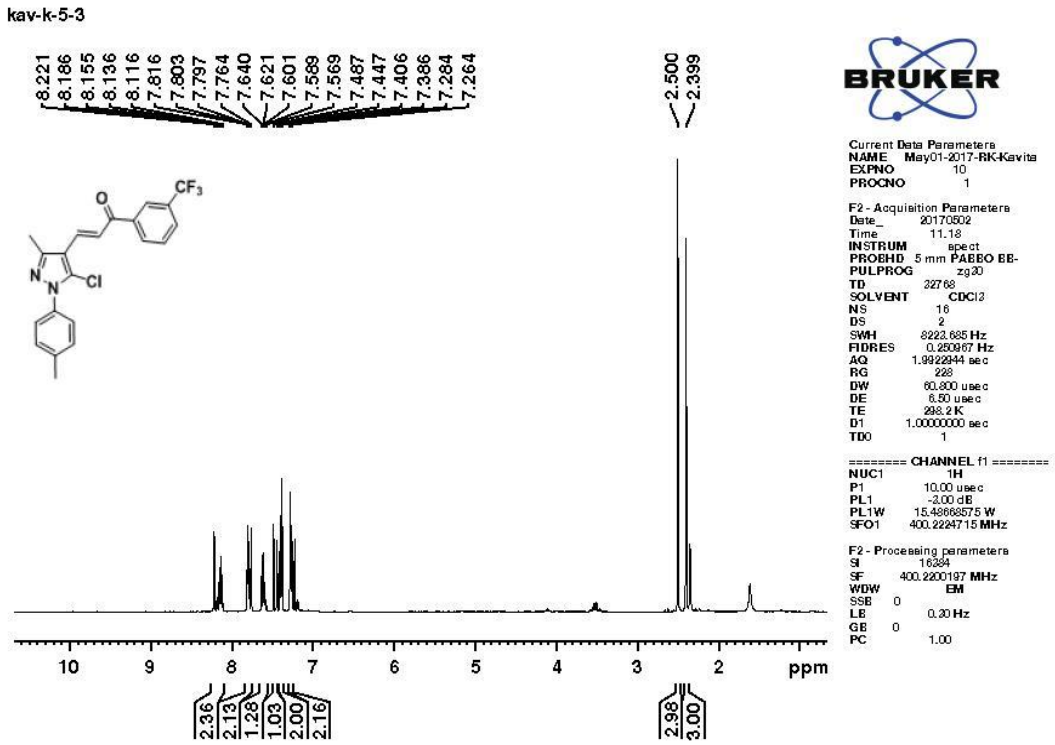


Figure 12.[1]H NMR of compound 4-4

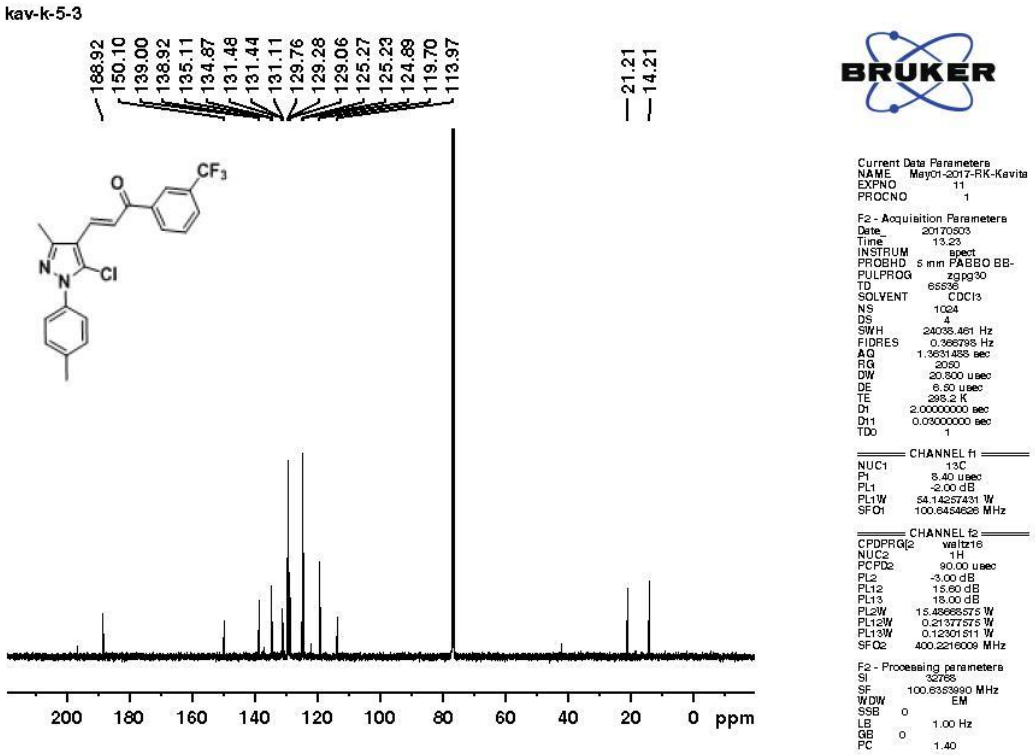


Figure 13.[13]C NMR of compound 4-4

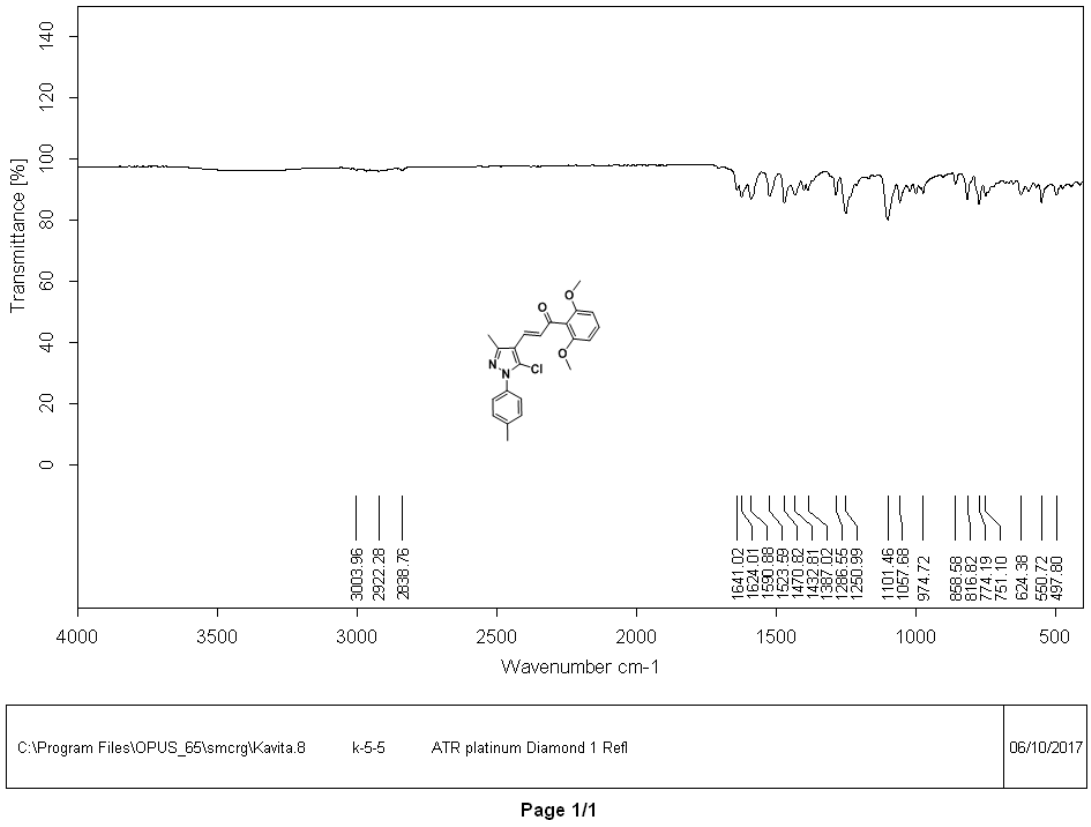


Figure 14. IR spectrum of compound 4-5

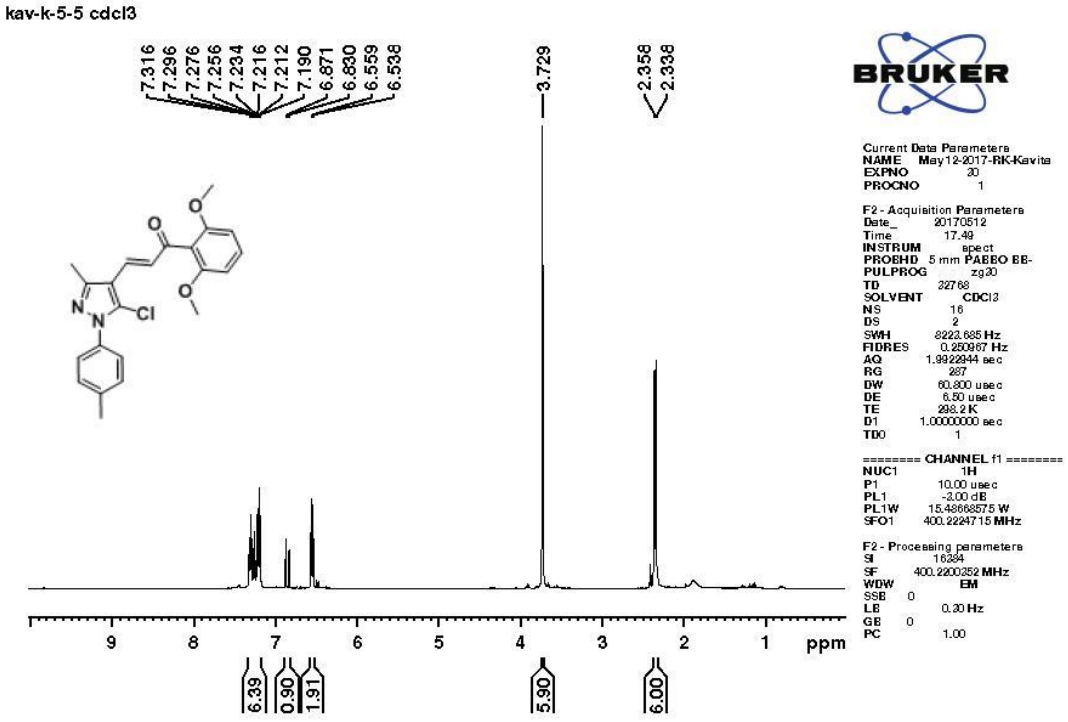


Figure 15. ^{1}H NMR of compound 4-5

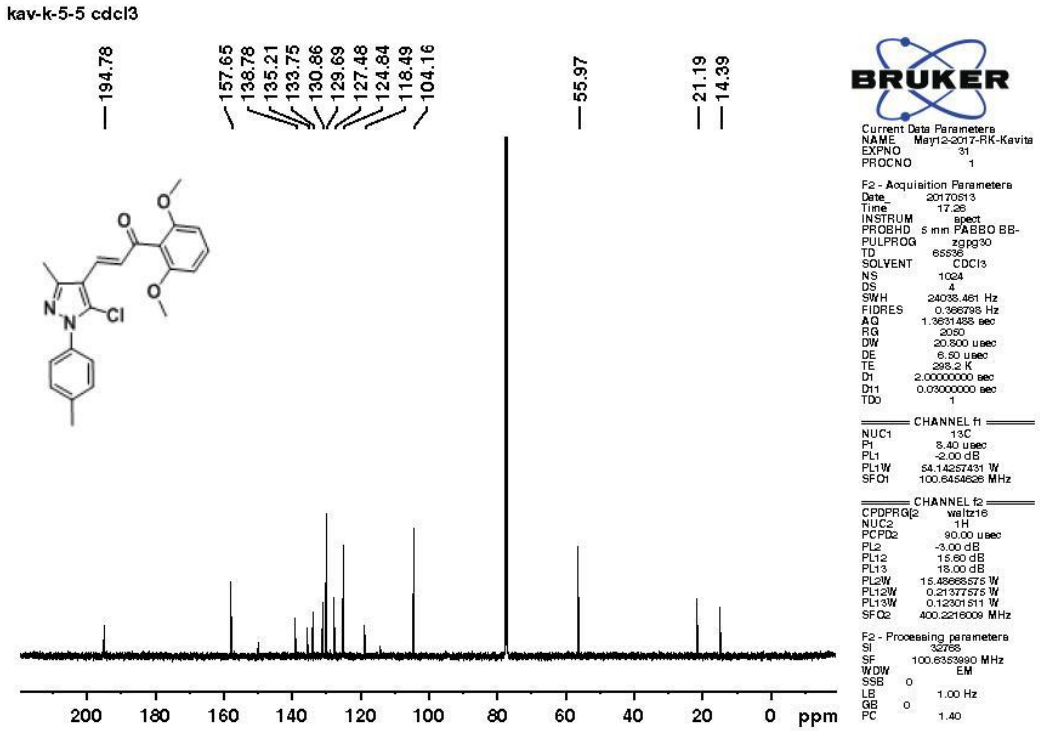


Figure 16. ^{13}C NMR of compound 4-5

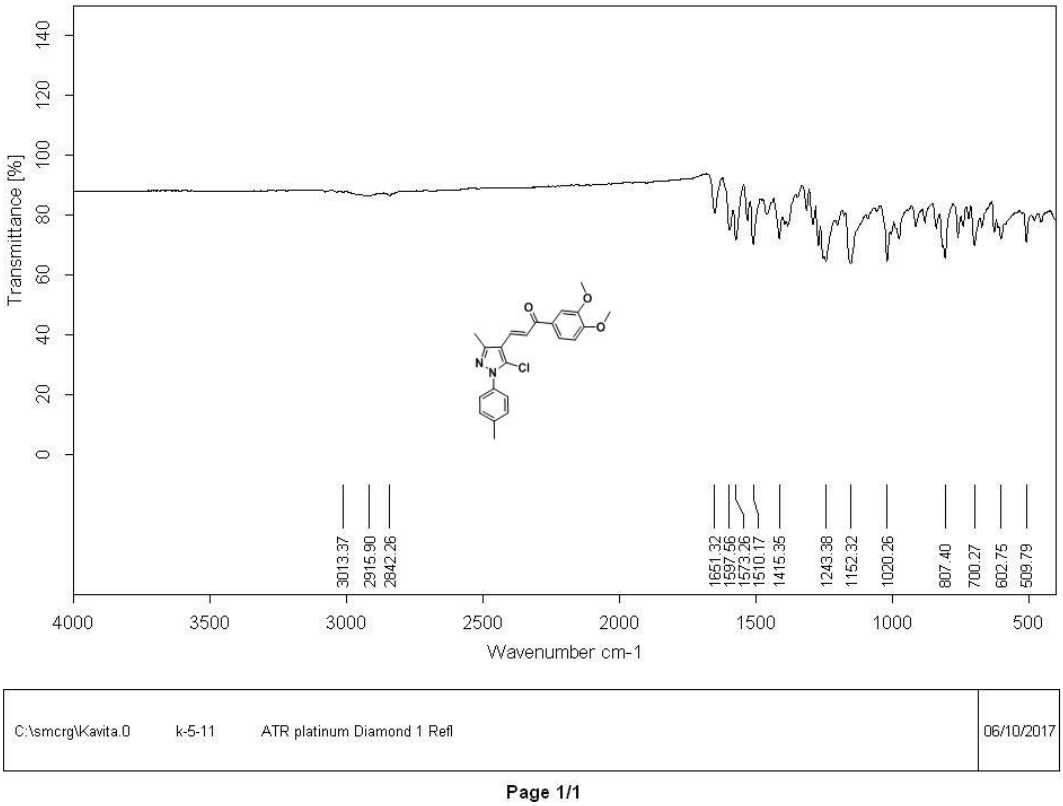


Figure 17. IR spectrum of compound 4-6

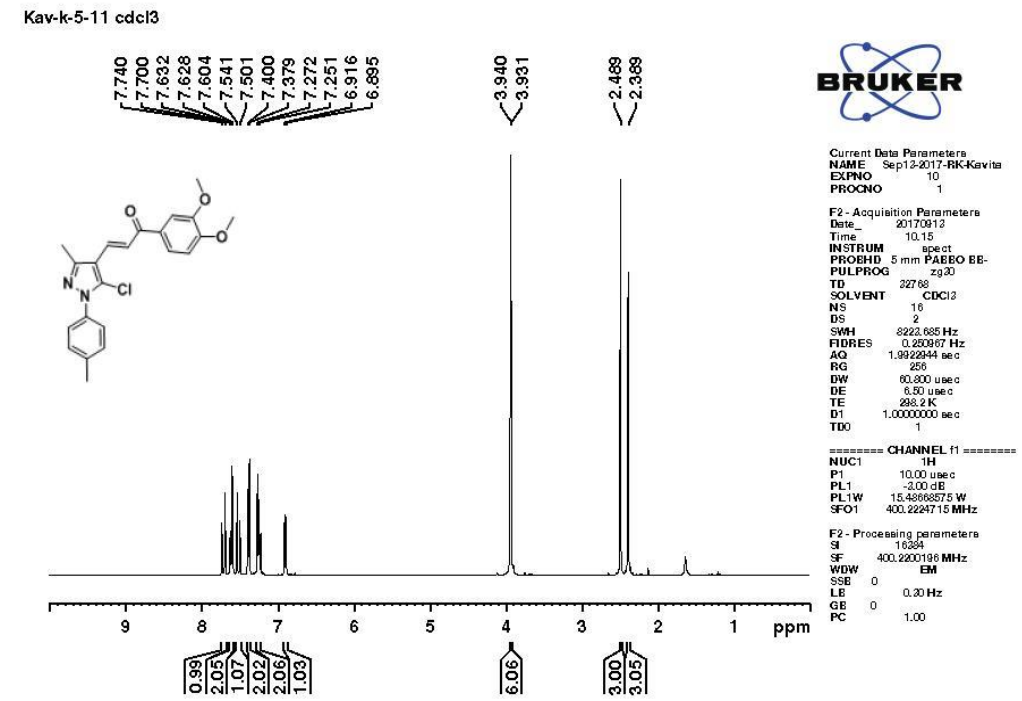


Figure 18. ^{1}H NMR of compound 4-6

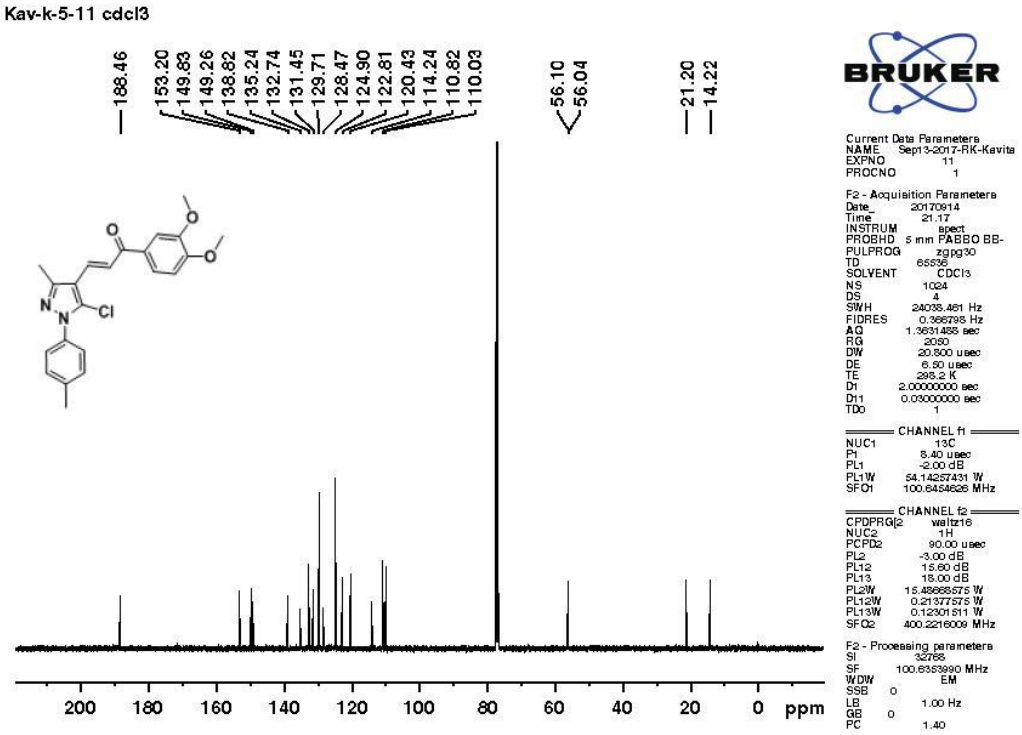


Figure 19. ^{13}C NMR of compound 4-6

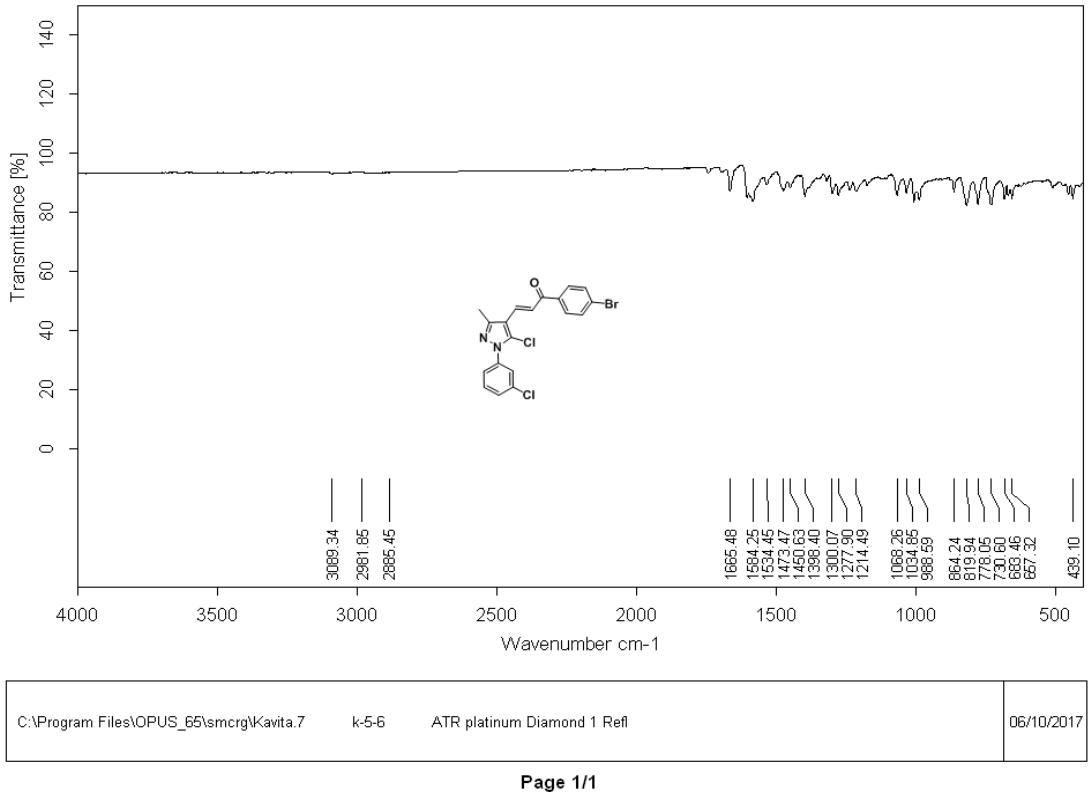


Figure 20. IR spectrum of compound 4-7

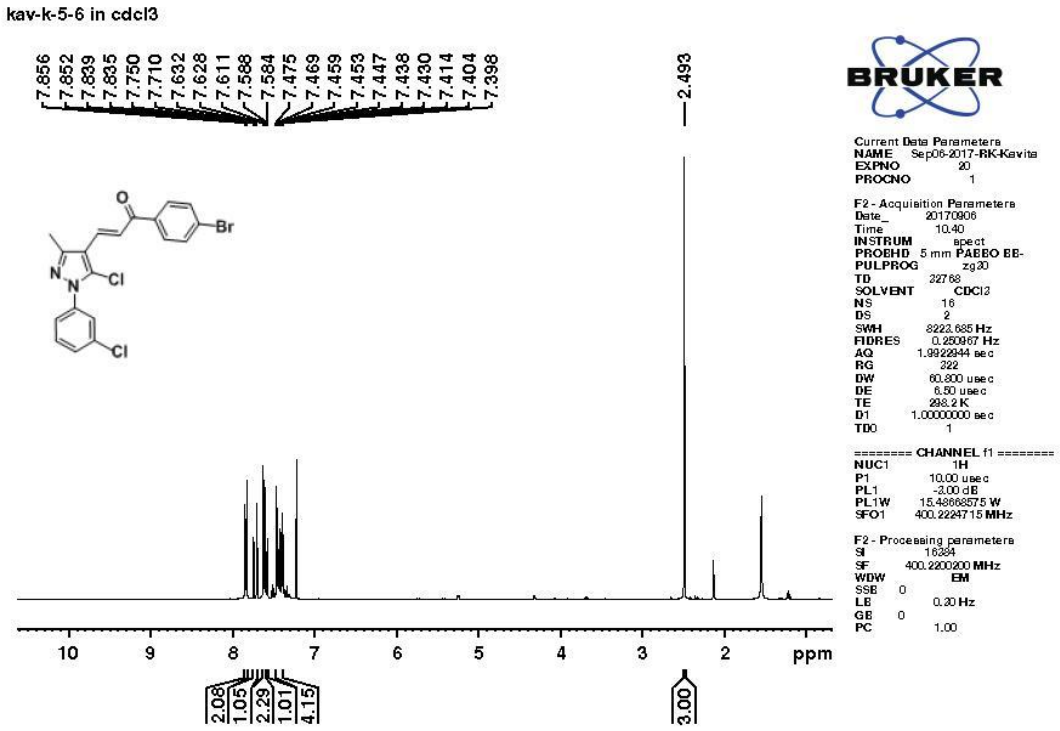


Figure 21. ^{1}H NMR of compound 4-7

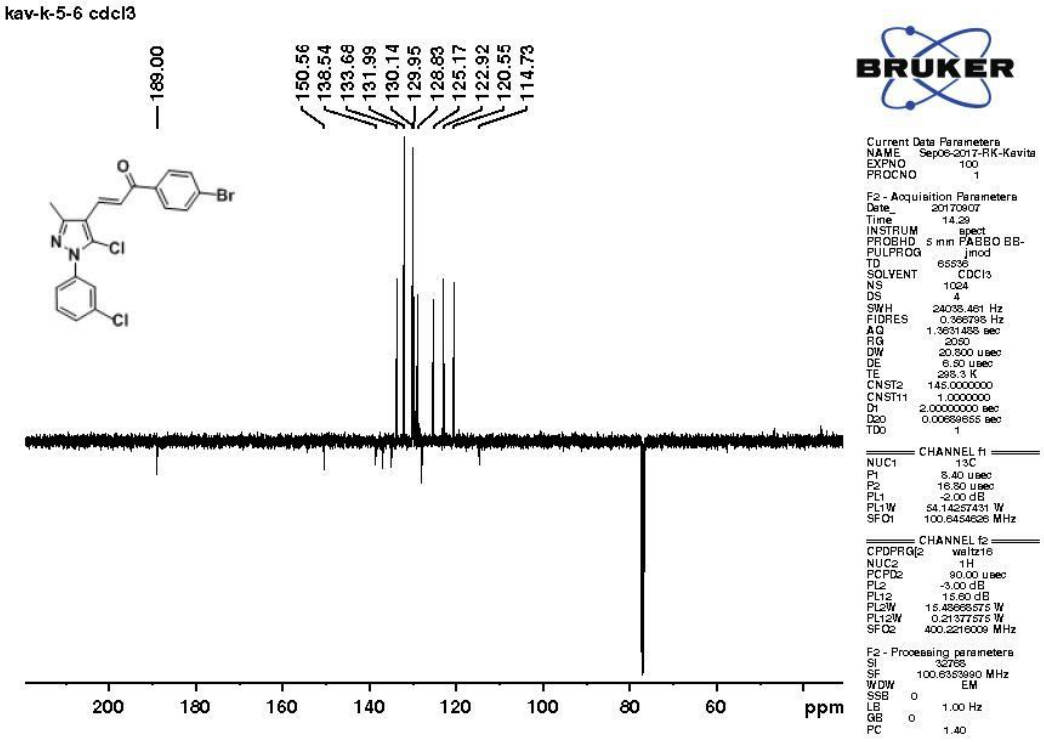


Figure 22. ^{13}C NMR of compound 4-7

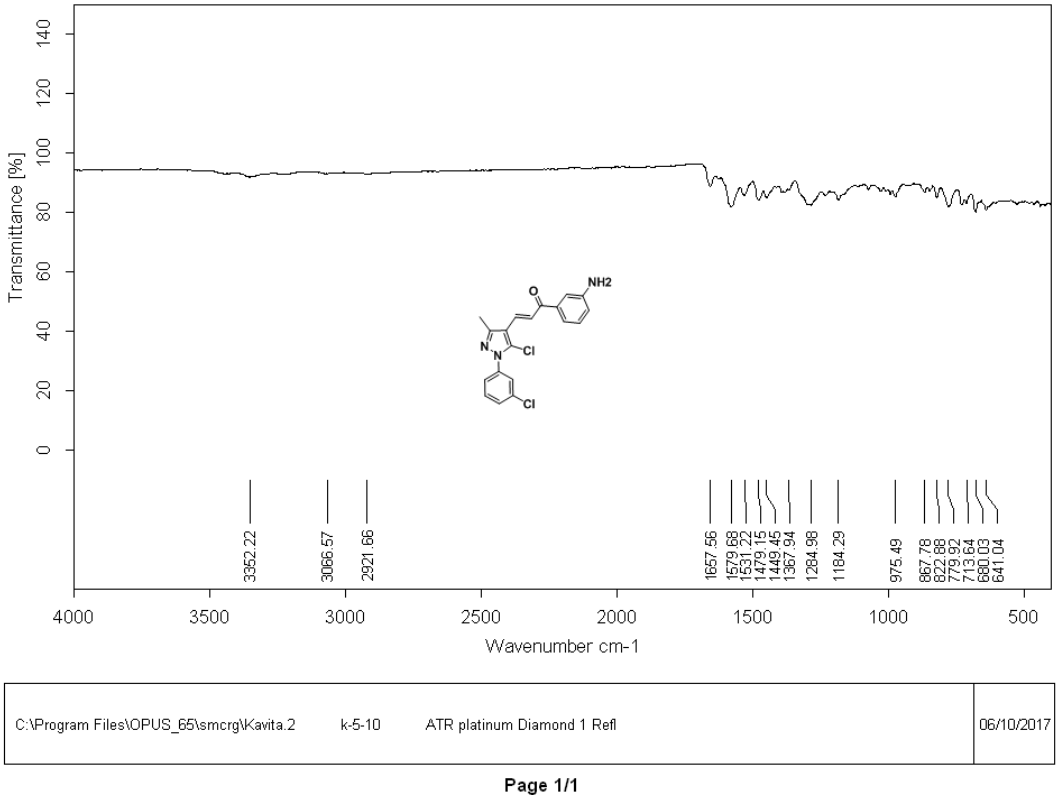


Figure 23. IR spectrum of compound 4-8

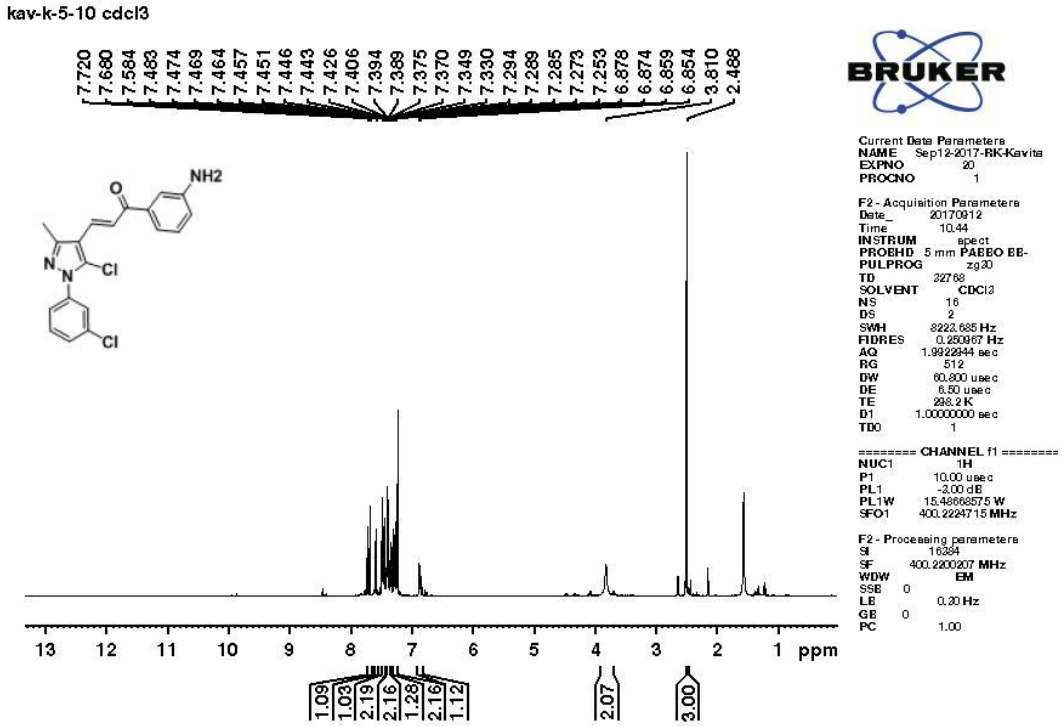


Figure 24. ^{1}H NMR of compound 4-8

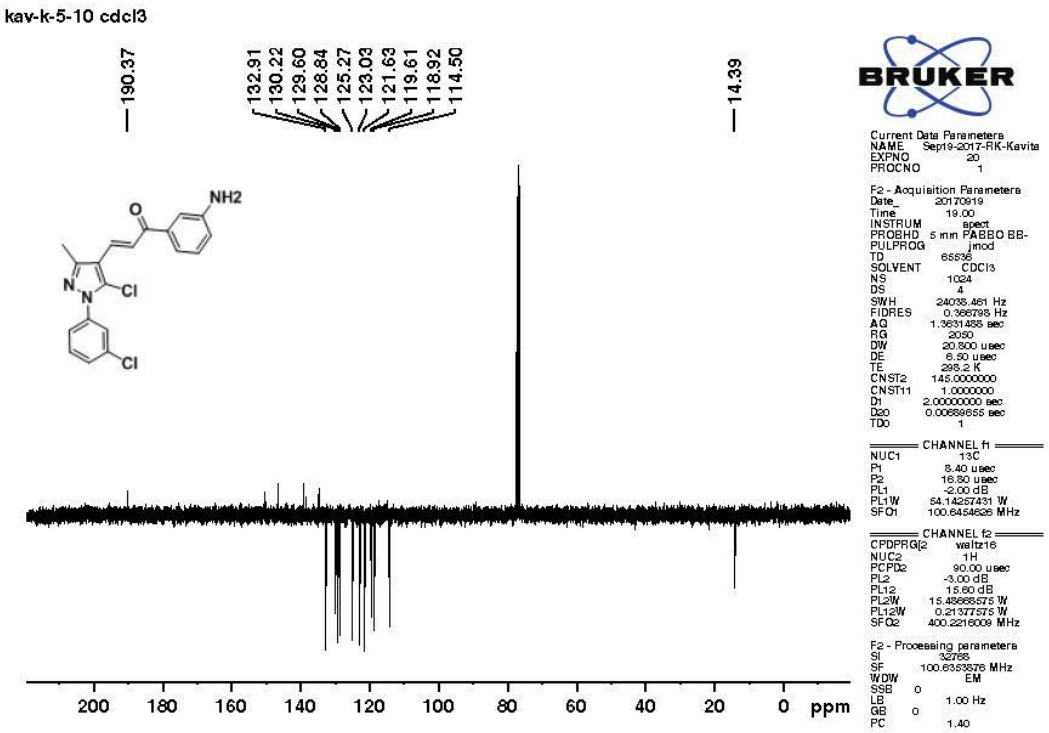


Figure 25. ^{13}C NMR of compound 4-8

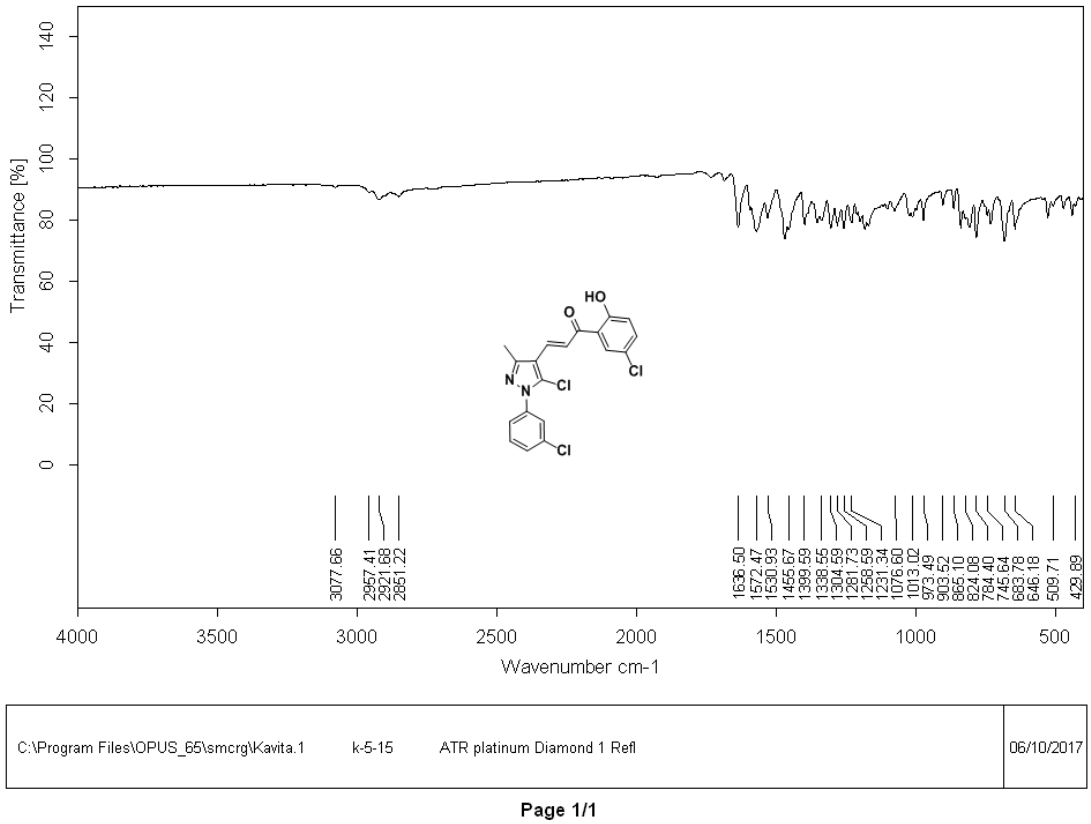


Figure 26. IR spectrum of compound 4-9

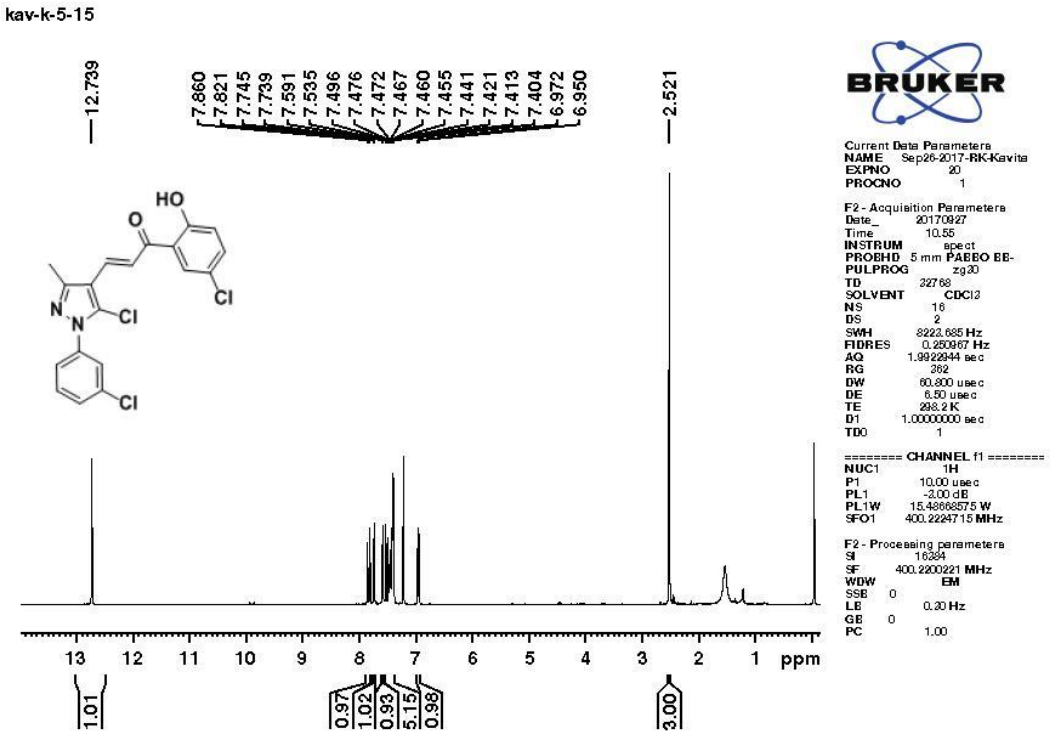


Figure 27. ^{1}H NMR of compound 4-9

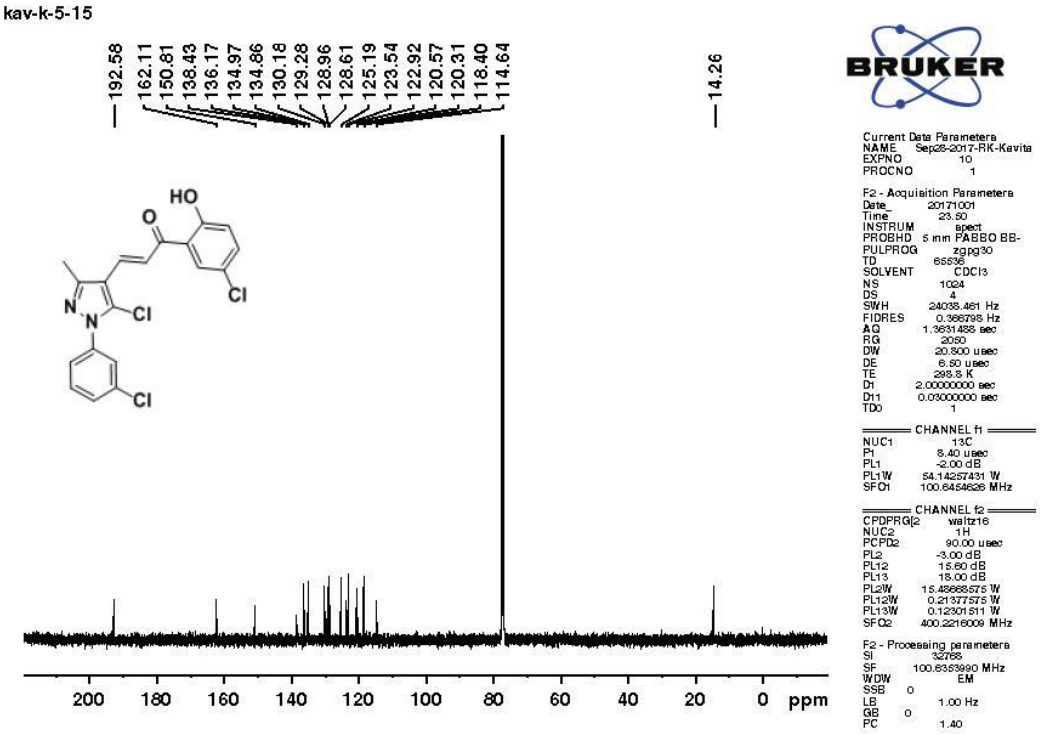


Figure 28. ^{13}C NMR of compound 4-9

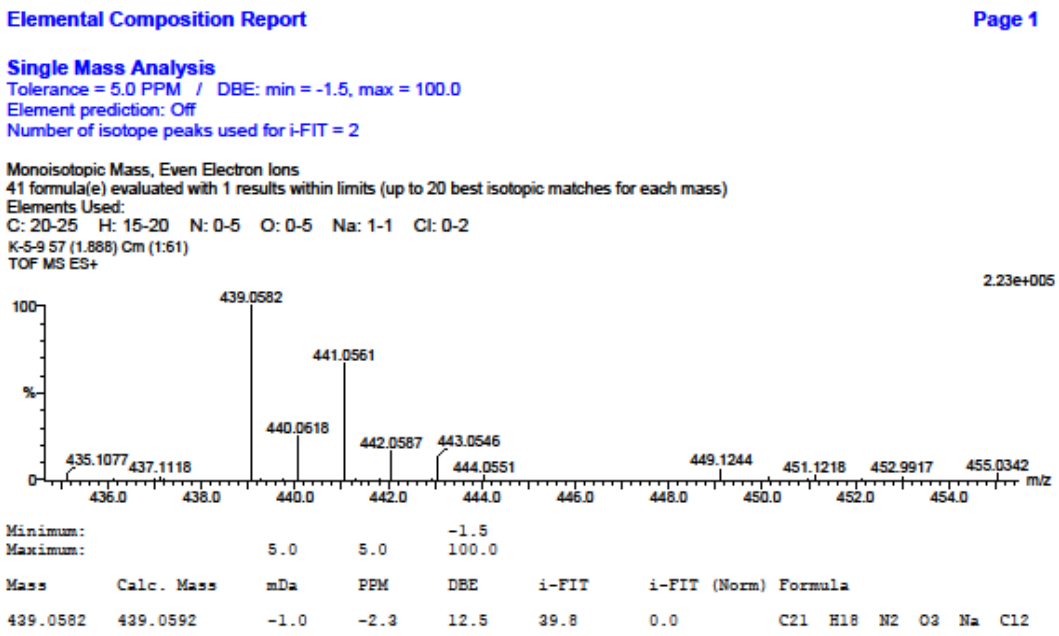


Figure 29. HRMS of compound 4-10

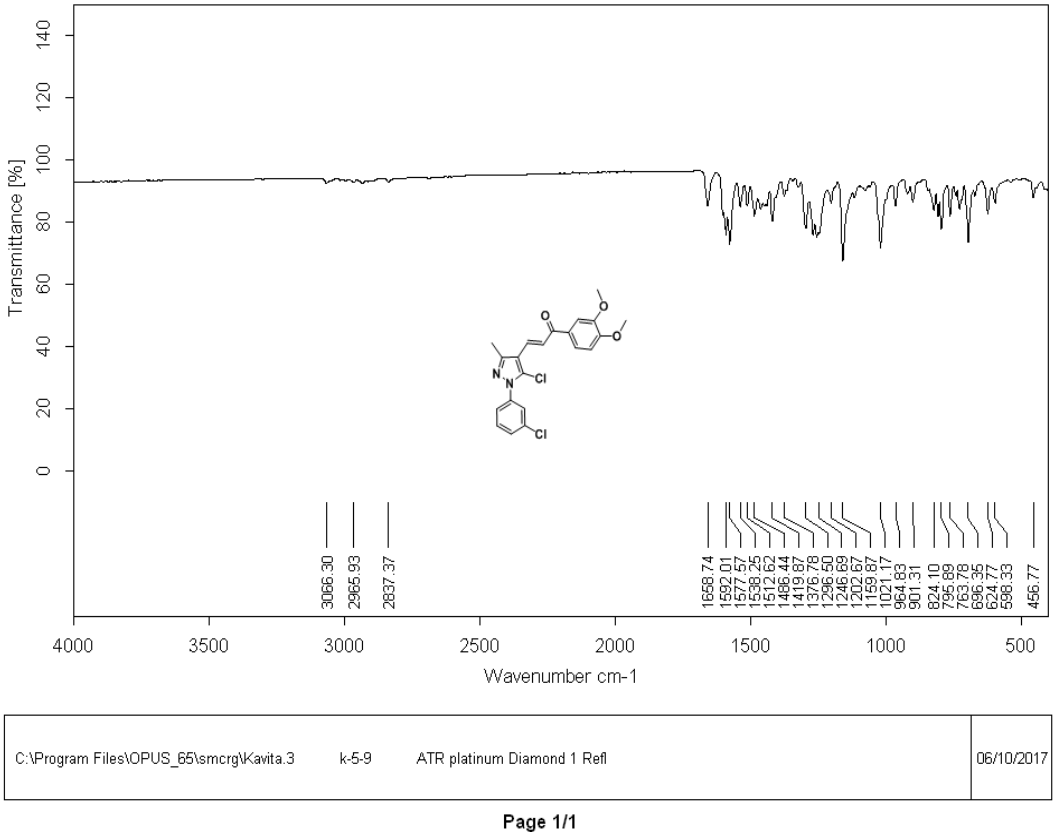


Figure 30. IR spectrum of compound 4-10

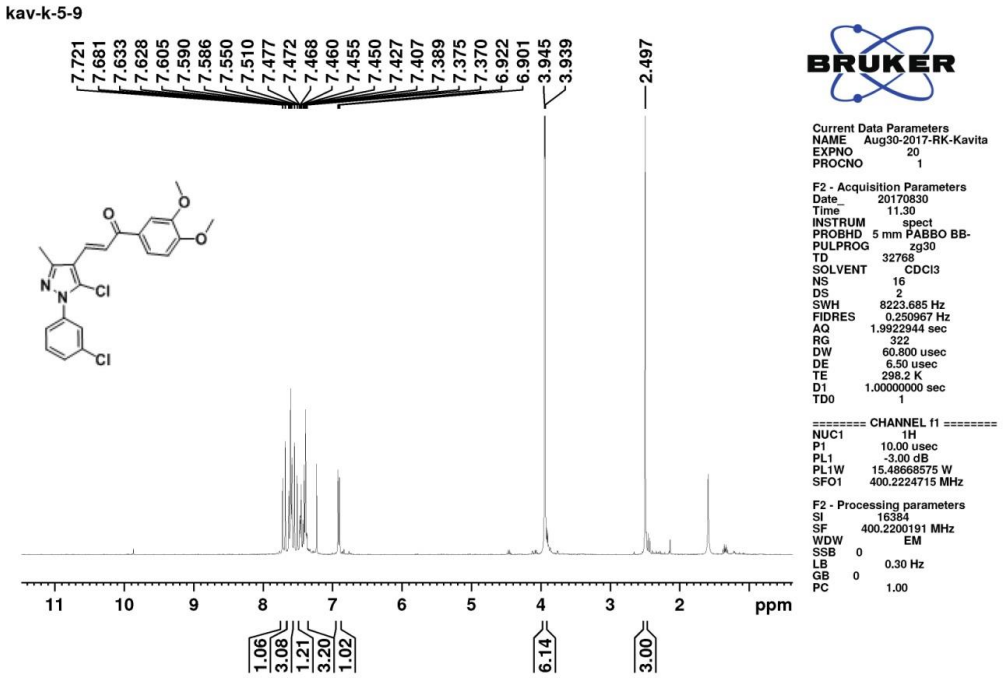


Figure 31. ^{1}H NMR of compound 4-10

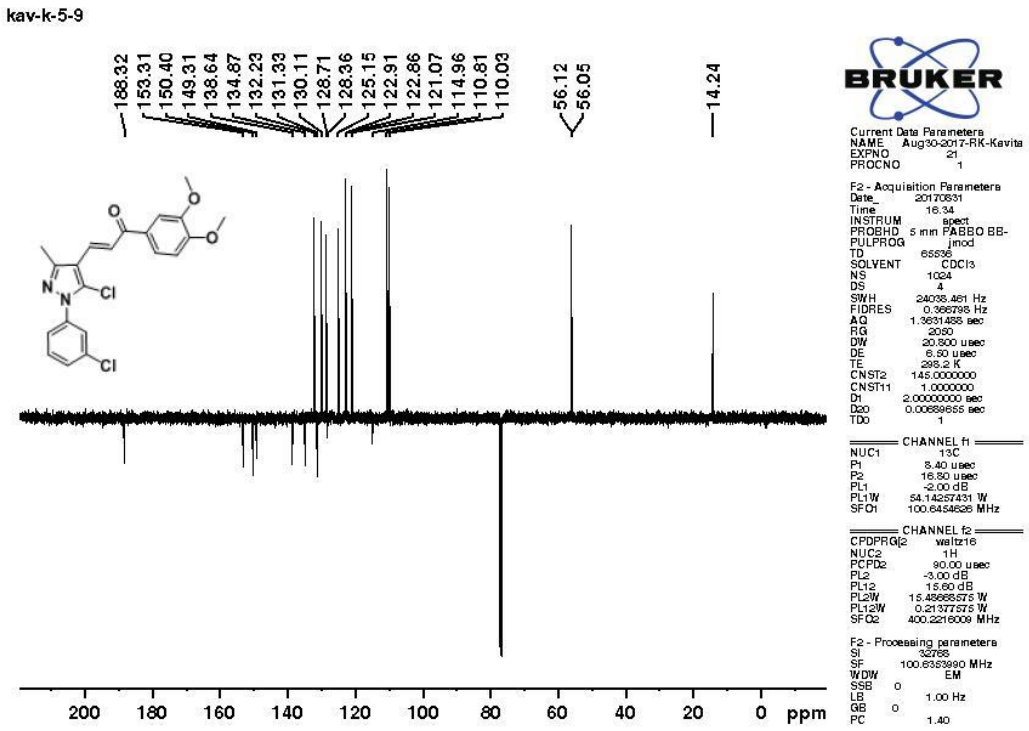


Figure 32. ^{13}C NMR of compound 4-10

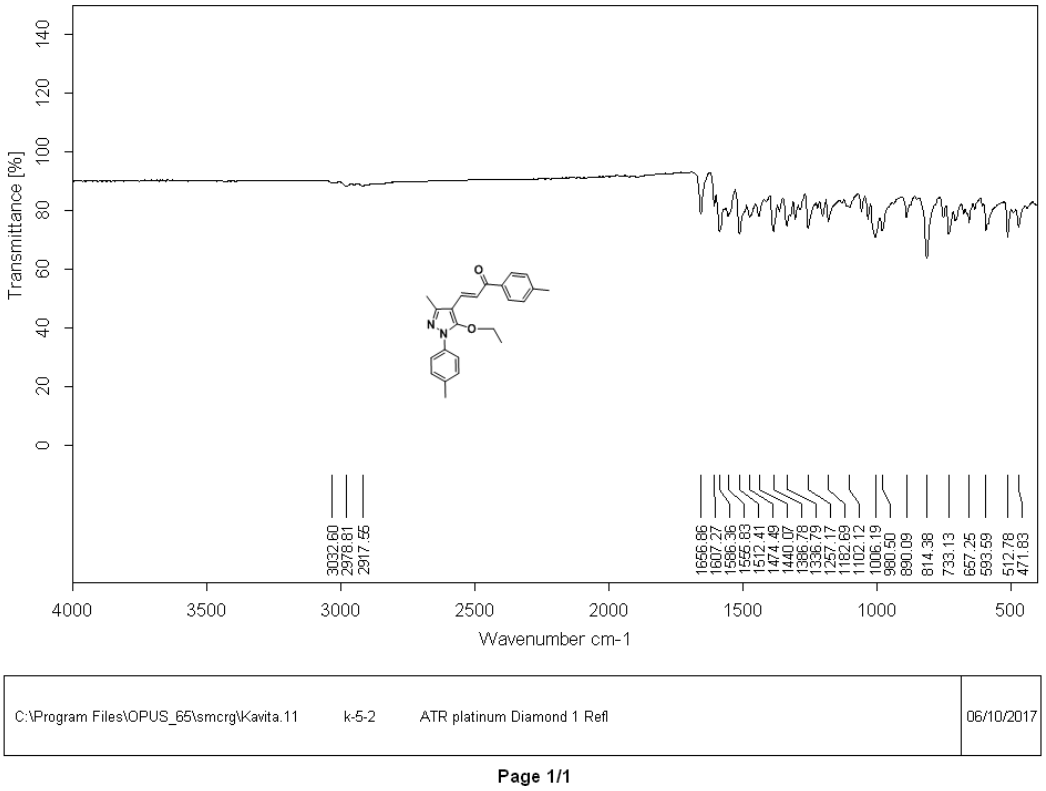


Figure 33.IR spectrum of compound 4-11

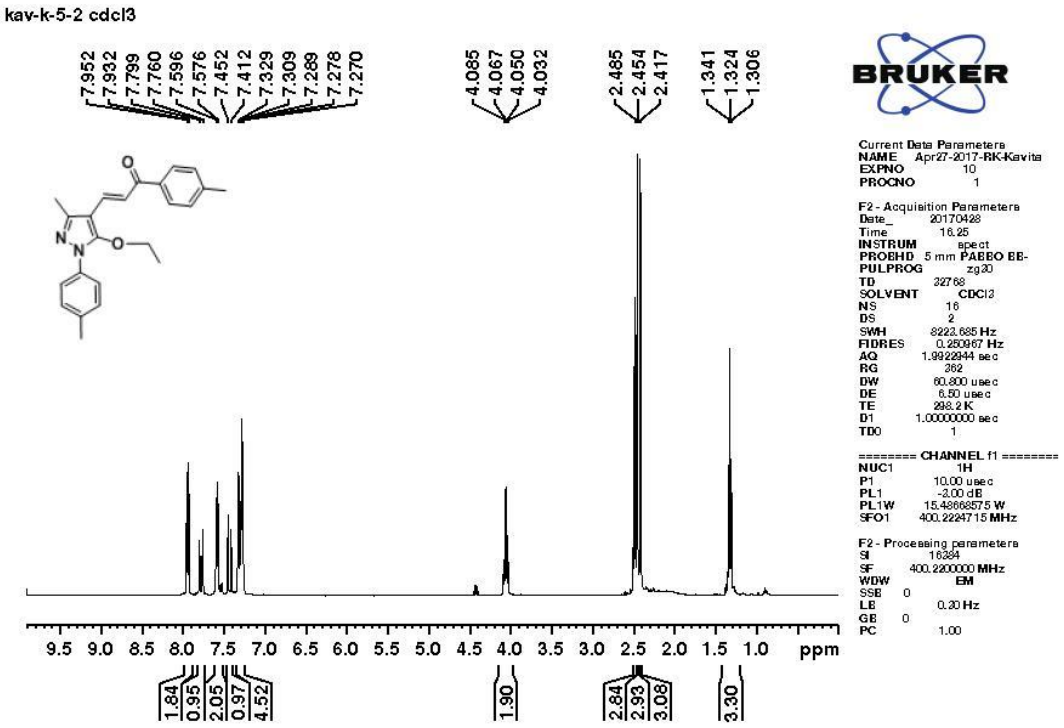


Figure 34.[1]H NMR of compound 4-11

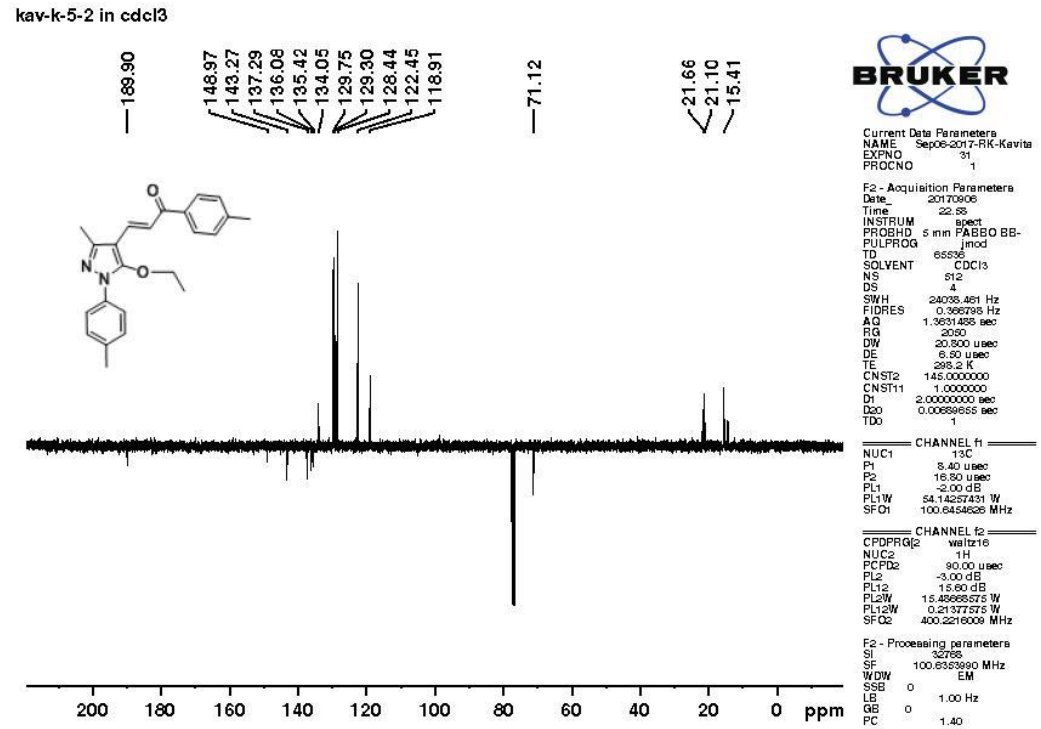


Figure 35.[13]C NMR of compound 4-11

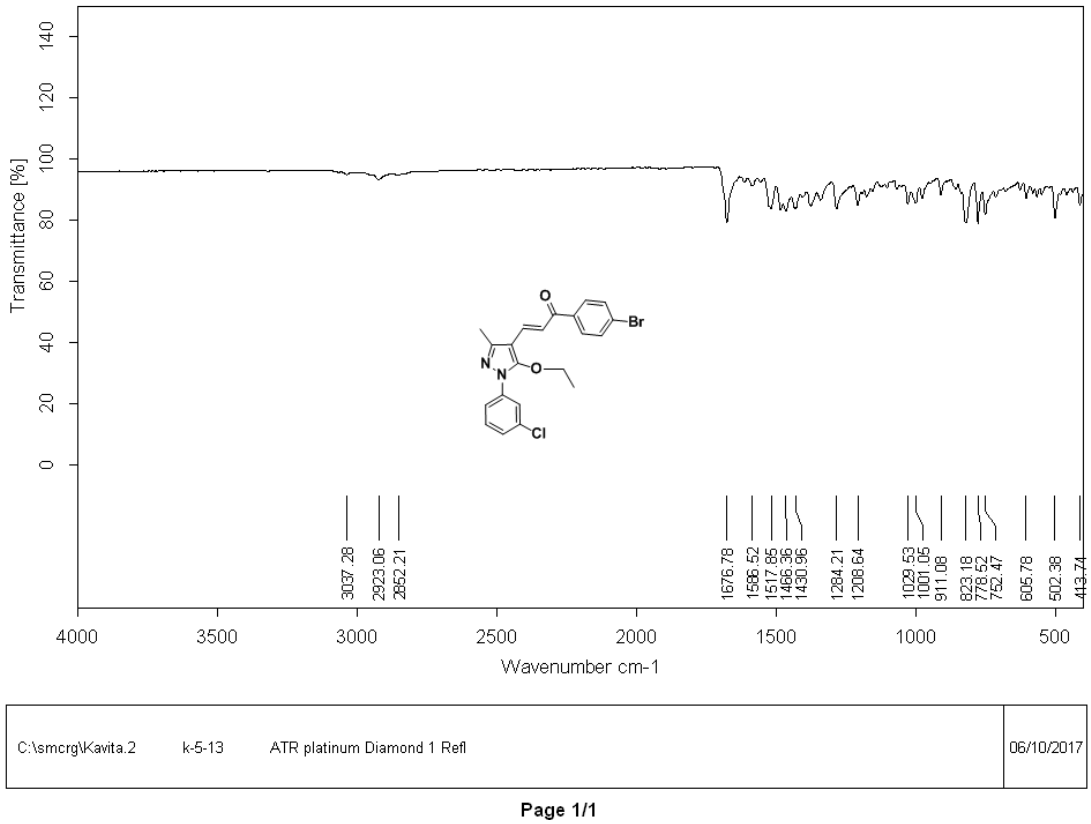


Figure 36. IR spectrum of compound 4-12

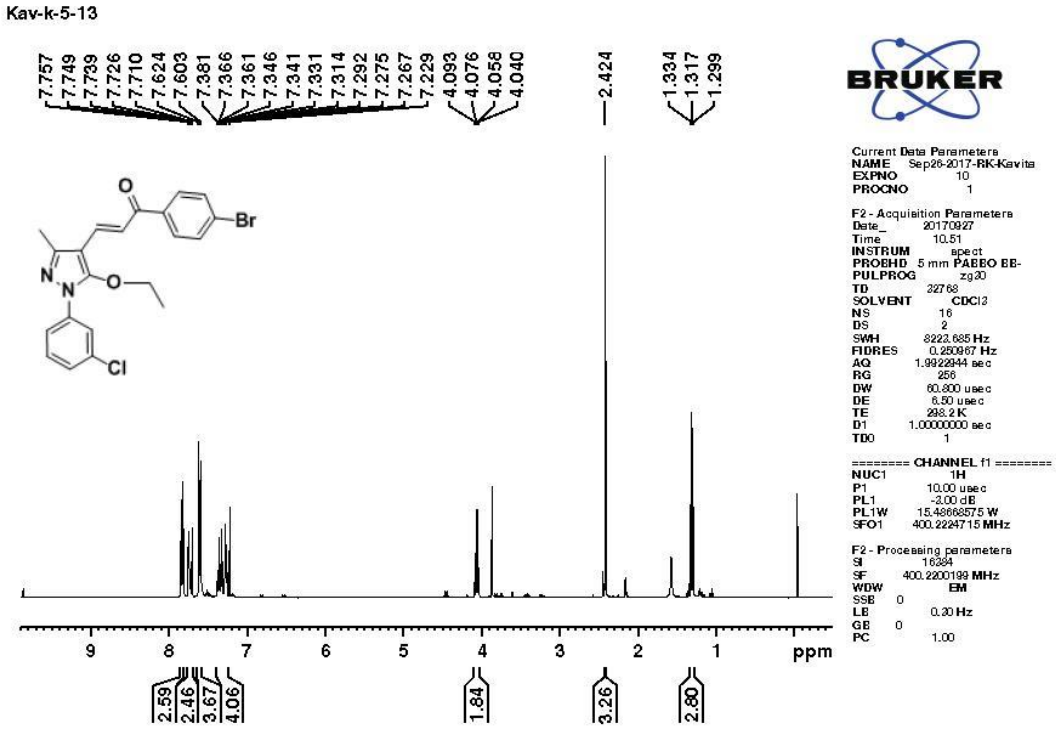


Figure 37. ^{1}H NMR of compound 4-12

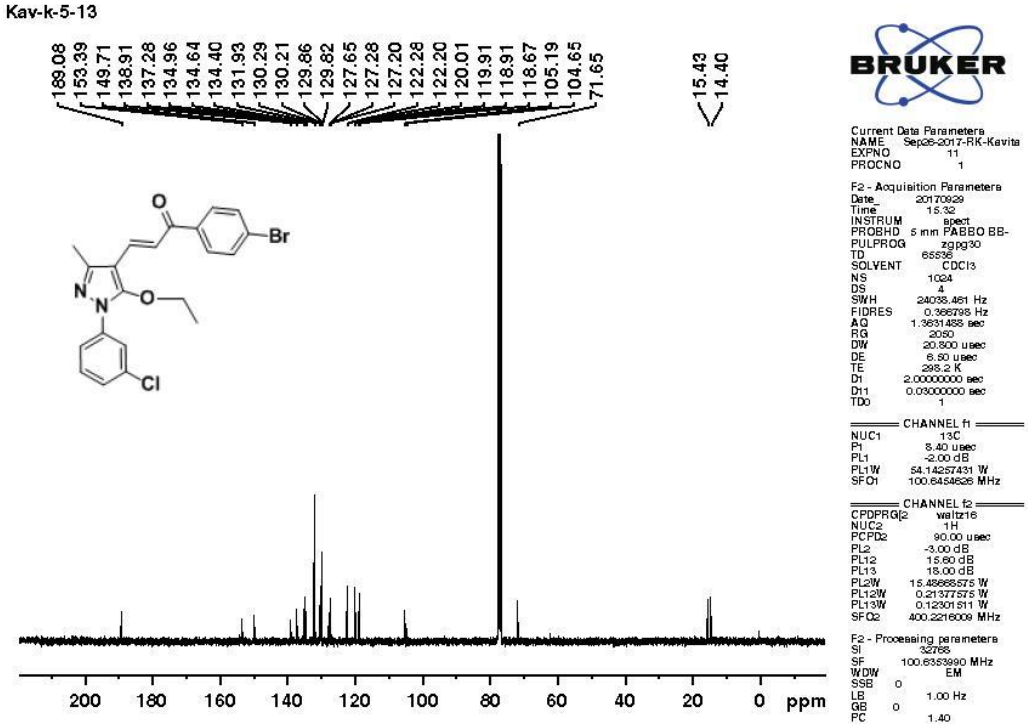


Figure 38. ^{13}C NMR of compound 4-12

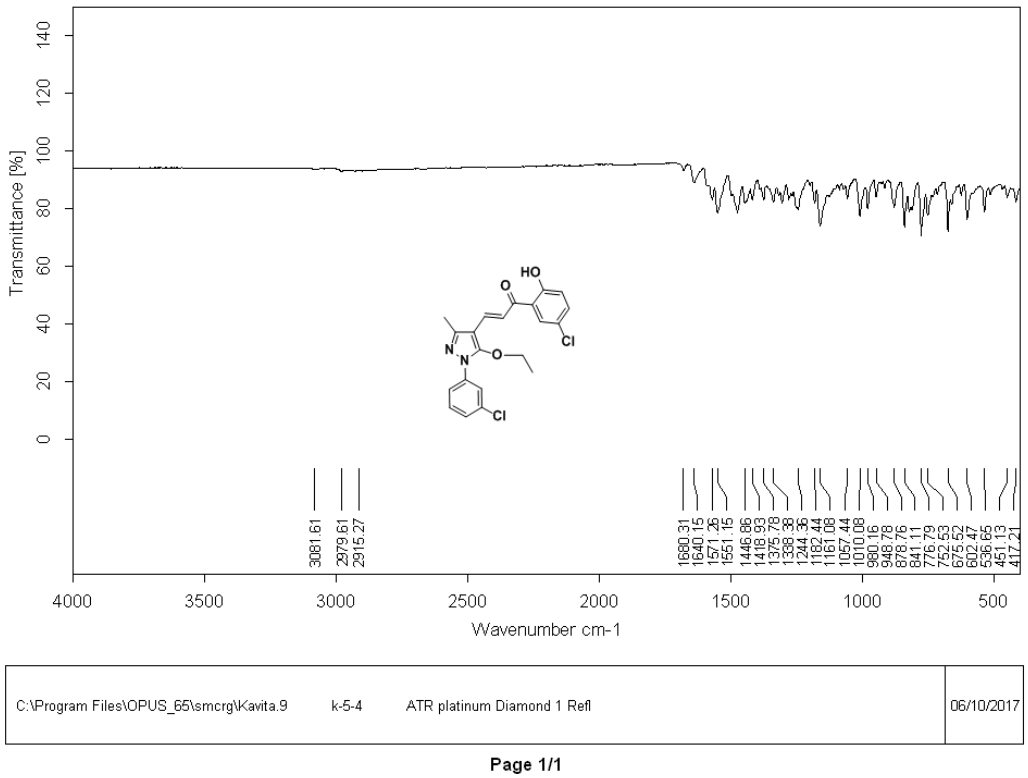


Figure 39.IR spectrum of compound 4-13

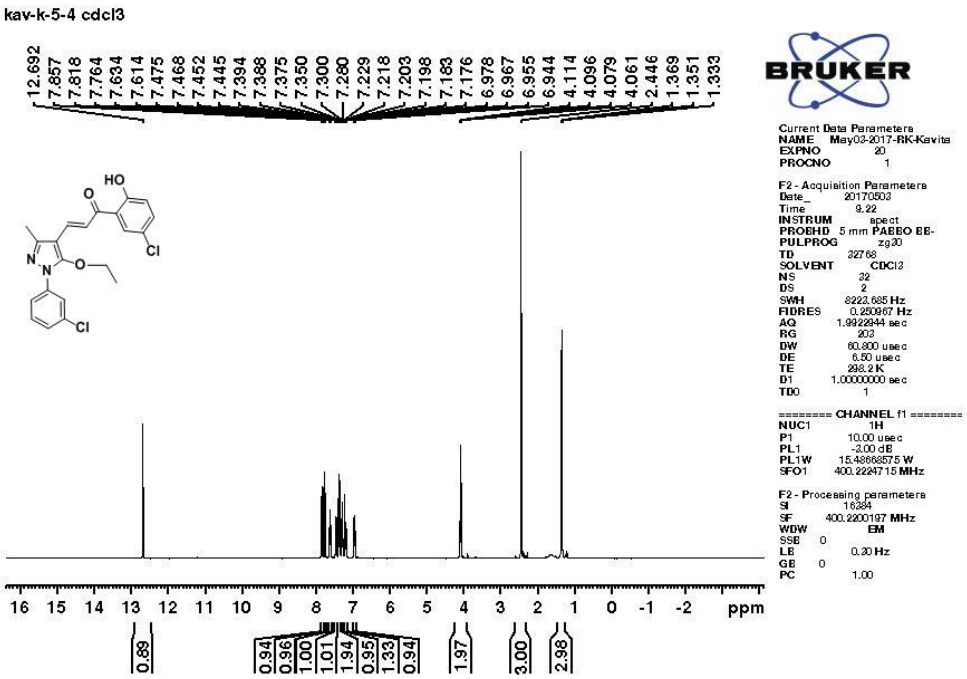


Figure 40. ^{1}H NMR of compound 4-13

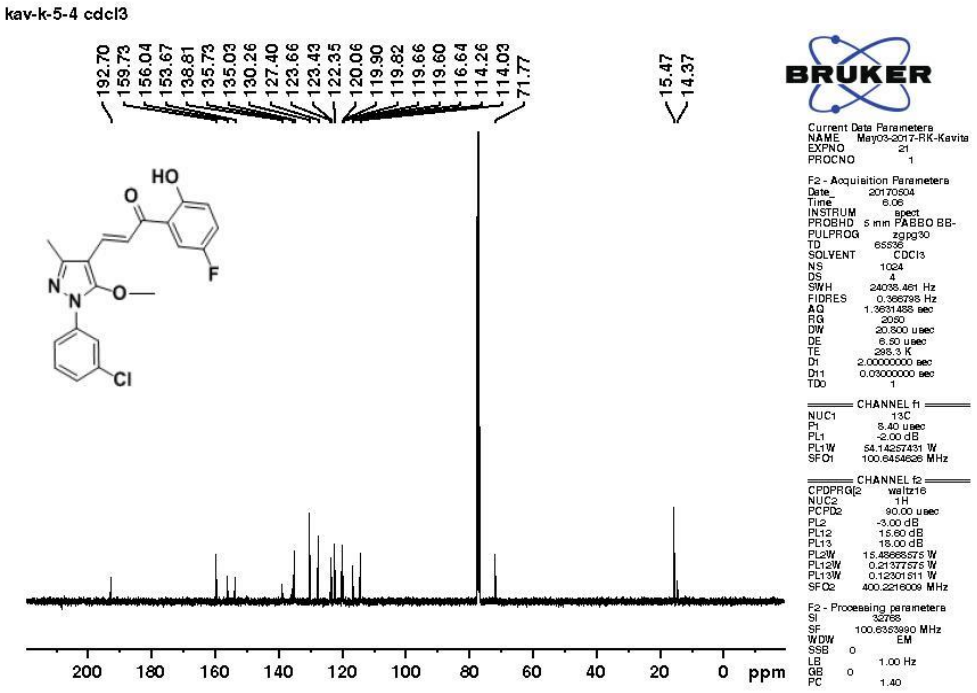


Figure 41. ^{13}C NMR of compound 4-13

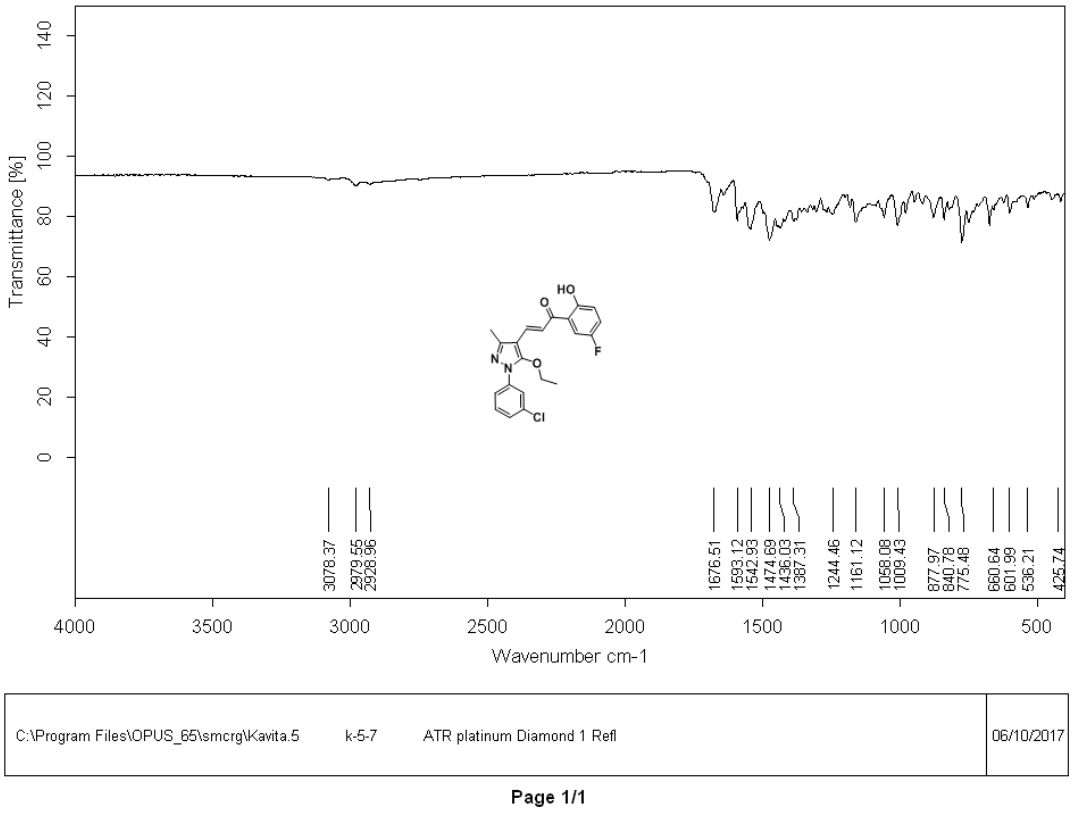


Figure 42. IR spectrum of compound 4-14

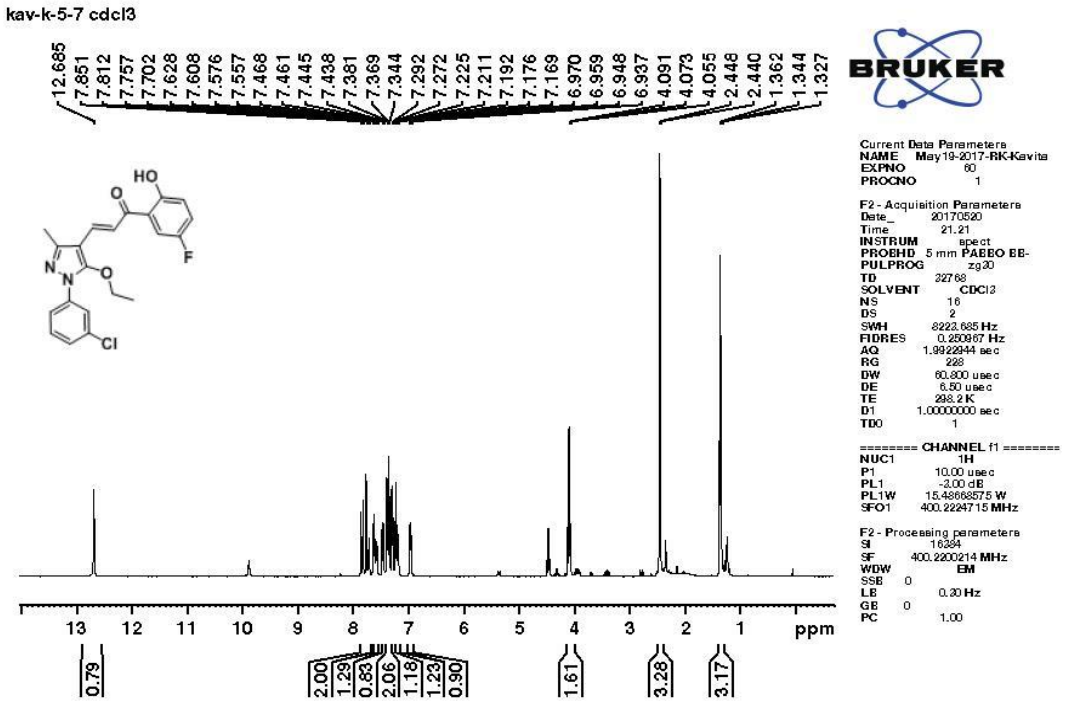


Figure 43. ^{1}H NMR of compound 4-14

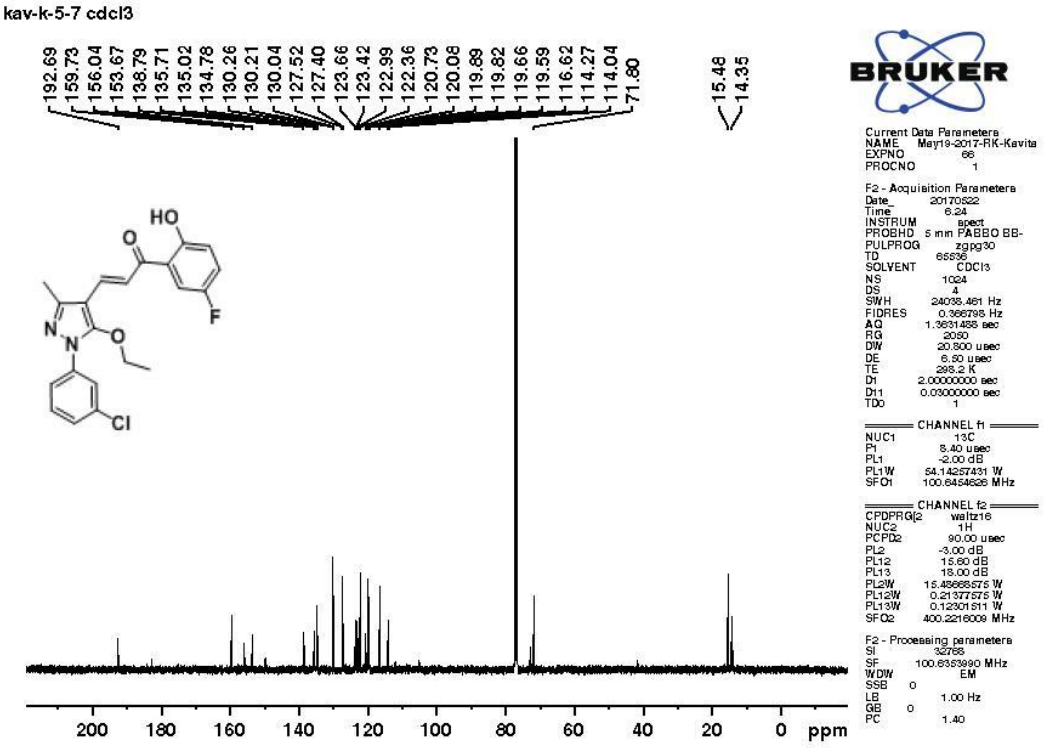


Figure 44. ^{13}C NMR of compound 4-14

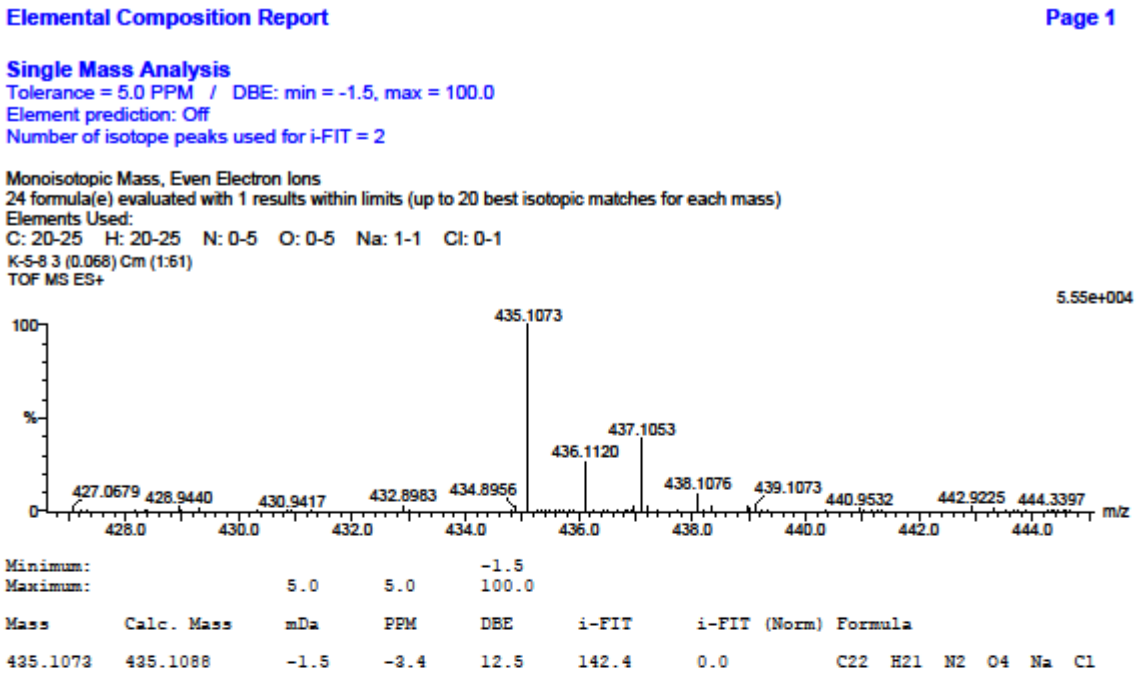


Figure 45. HRMS of compound 4-15

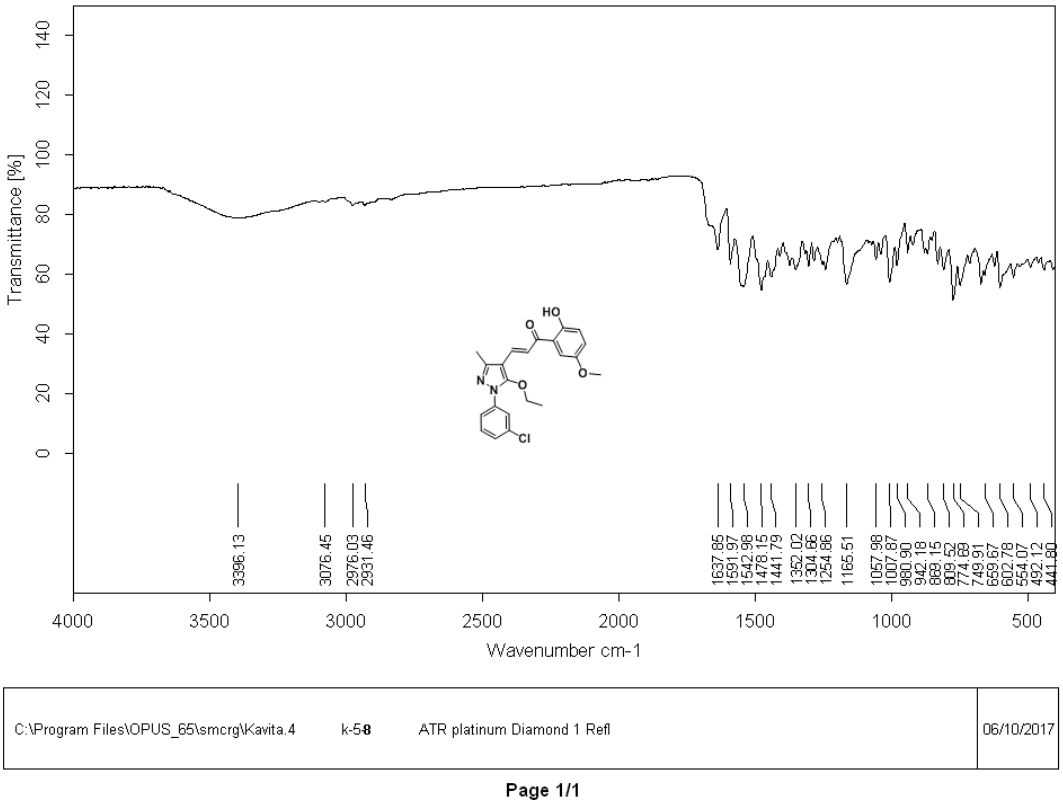


Figure 46. IR spectrum of compound 4-15

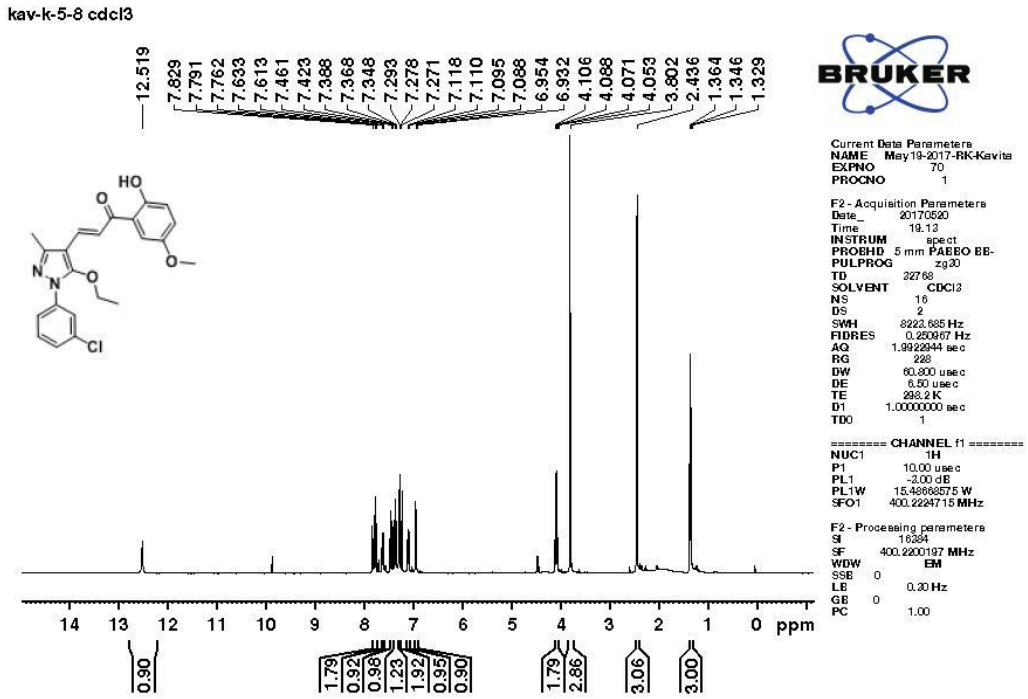


Figure 47. ^{1}H NMR of compound 4-15

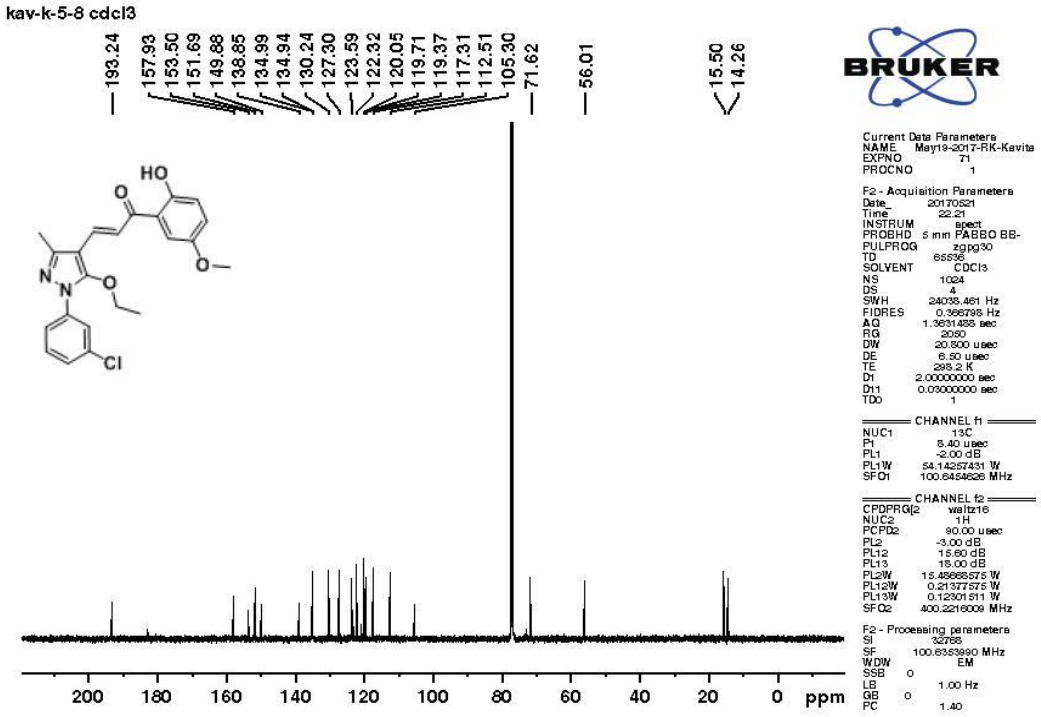


Figure 48. ^{13}C NMR of compound 4-15