

Synthesis of Phenylhydrazone Schiff Base Derivative, their Copper(II) Complexes and Antibacterial Activity Investigation

Sileschi Abera Ayele



Thesis Submitted to the Department of Applied Chemistry School of Applied Natural Science in Partial Fulfillment of the Requirements for Master of Science Degree in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama Science and Technology University

June, 2018

Adama, Ethiopia

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DEDICATION

This thesis is dedicated to my mother **Bekelech Abate**, my father **Abera Ayele**, and all my family for nursing me with affection and love for their dedicated partnership in the success of my life.

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All thanks and honors are to Almighty GOD, who is the entire foundation of all kinds of knowledge and wisdom endowed to mankind. His blessing that prospers my thoughts and fulfilled my desires and my humble efforts in the study and write up of the thesis.

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List of Abbreviations

^{13}C NMR	Carbon thirteen nuclear magnetic resonance
FDA	Food and Drug Administration
DNA	Deoxyribonucleic acid
DMSO	Dimethylsulfoxide
^1H NMR	Proton nuclear magnetic resonance
HIV	Human immunodeficiency virus
IR	Infrared radiation
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
MTB	Mycobacteria tuberculosis
MIC	Minimum inhibitory concentration
SB	Schiff bases
SAR	Structure activity relationship
THF	Tetrahydrofuran
TLC	Tin layer chromatography
TMS	Tetramethylsilane
UV-Vis	Ultraviolet-visible
ZOI	Zone of inhibition

Abstract

*In present study hydrazone of phenylhydrazine, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde and their respective benzenesulfanohydrazones and Cu(II) complexes were synthesized. All the ligands were characterized by IR, ^1H NMR, and ^{13}C NMR. The Cu(II) complexes were also characterized by elemental analysis and IR. The IR spectra of ligand and its metal complexes imply that the Schiff base ligand behaves as basic bidentate ligand coordination through the azomethine nitrogen atom and nitrogen atom. In-vitro antibacterial activity was carried out for all the synthesized compounds by using disc diffusion method against *K.pneumoniae*, *E.coli*, *P.aeruginosa*, and *S. aureus*. Cu(II) complexes of compound **34** showed better activity (15mm zone of inhibition) against *K.pneumoniae* compared to the standard ceftriaxone (11 mm zone of inhibition) whereas the remaining compounds showed less activity compared to the standard. The Cu(II) complexes exerted excellent antibacterial activities than the ligands which might be due to increase in lipophilicity of the compound.*

Key words: antibacterial activity, benzenesulfanohydrazones, complexes, hydrazone

1. Introduction

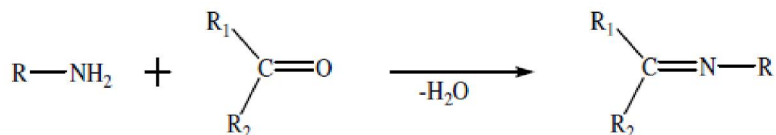
Transition metal complexes are cationic, neutral or anionic species in which a transition metal is coordinated by ligands [1]. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases. This activity of transition metals has started the development of metal based drugs with promising pharmacological application and may offer unique therapeutic opportunities [2].

Medicinal inorganic chemistry can exploit the unique properties of metal ions for the design of new drugs. This has, for instance, led to the clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin [3, 4]. The resounding therapeutic success of cisplatin and its analogues has triggered tremendous effort in search of alternative metal-based chemotherapeutic agents in the past few decades [5]. Studies indicated that metal centers other than platinum might open up new avenues in the development of clinically useful drug [6]. Recent research and discoveries revealed that new alternative metal-based chemotherapeutic agents are drugs with enhanced activity, selectivity, bioavailability and fewer side-effects than conventional drugs to treat current diseases. In addition, Sadler stated that “the ligands not only control the reactivity of the metal but also play critical roles in determining the nature of interactions involved in the recognition of biological target sites such as deoxyribonucleic acid (DNA), enzymes and protein receptors” [7].

Schiff base metal complexes have played a prominent role in the development of coordination chemistry. This area of research has a wide spectrum, ranging from synthesis to application in many diverse fields. Schiff bases (SB), the condensation products of carbonyl compounds and primary amines were first described by Hugo Schiff (1864). The Schiff base compounds containing the azomethine group having the general formula $RHC=N-R'$, where R and R' =Alkyl, cyclo alkyl, aryl, or heterocyclic groups may be differently substituted (scheme 1) as Imines or azomethine or anils where Schiff base or imine are compound containing azomethine group-($HC=N$)-, which forms when (O) atom of aldehyde or ketone has been replaced by (N) atom of primary amine in condensation reaction between primary amine with carbonyls compounds. Schiff bases show considerable chemical and biological importance due to the lone pair of electrons in sp^2 hybridized orbital of nitrogen atom of

azomethines [8], not just that but the activities increase when the Schiff base ligand involve heterocyclic molecules beside in azomethine so Schiff bases are excellent chelating agents. Synthesis of Schiff base from aromatic aldehyde is more facile due to conjugation system than aliphatic aldehyde which readily gets polymerized [9].

The Schiff bases are considered as ‘privileged ligands’ because of their capability to stabilize different metals in various oxidation states and their metal chelates are widely studied owing to the synthetic flexibility, sensitivity and selectivity toward various metal ions [10]. The flexibility and bioactivity of nitrogen and sulfur containing Schiff bases have also been associated with the presence of both imino (-N=CH-) and thioamino (-(C=S)- NH-) moieties in their structures. Coordination of such compounds with metal ions often enhances their activities [11, 12]. The low cost as well as the relatively easy preparation of Schiff base derivatives also provide a major attraction in creating novel leads that can be synthesized in a practical and step-economical fashion. Schiff base has variety of application specially biological system such as antibacterial, antifungal, anticancer, antitumor and etc. They are also used in organic synthesis, medicine and pharmacy, dyes for solar collector, solar filters, catalyst, anti-corrosion agents [13, 14].



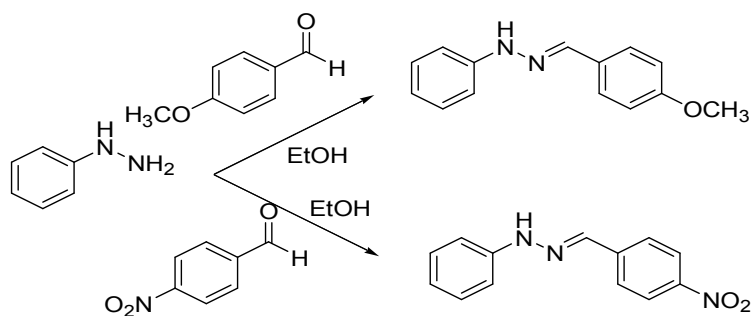
Scheme 1: Formation of Schiff base by condensation reaction (R group may be various substitute)

Schiff base hydrazones play an important role in inorganic chemistry, as they easily form stable complexes with most transition metal ions. Hydrazones are an important class of compounds that contain azomethine bond and are formed by reaction of aldehydes or ketones with hydrazines (N₂H₄ or R-NH-NH₂). Similar to Schiff bases, hydrazones are versatile compounds with vast synthetic utility and flexibility. Over the years, many hydrazone derivatives have exhibited several interesting biological properties such as antimicrobial, antiviral, anti-inflammatory, antiplatelet, antimalarial, anticancer [15-17].

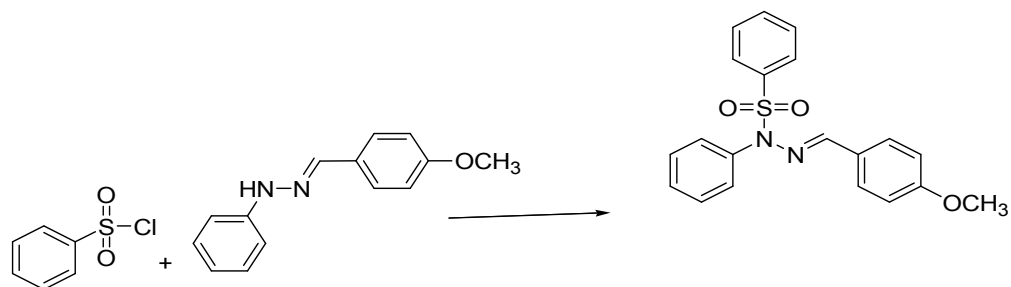
Both, nitrogen atoms of hydrazones are nucleophilic, however, the amino group nitrogen is more reactive. The azomethine carbon atom has both electrophilic as well as nucleophilic

character, consequently hydrazones have been known to react with both nucleophilic and electrophilic reagents. This unique property renders synthetic utility to hydrazones, particularly in the synthesis of heterocyclic systems, for example in Fischer synthesis of indoles and synthesis of azetidines [18]. Introduction of different functional groups adjacent to the hydrazone linkage further expands the scope of these compounds as chemical reagents [19]. Hydrazones are also used as ligands to coordinate a variety of metal ions, the resulting metal complexes have also exhibited a broad spectrum of biological activities such as antitubercular activity against *Mycobacterium tuberculosis* [20], antimicrobial, cytotoxic [21-22], and anticancer activity [23].

The synthesis of new hydrazones Schiff base and their metal complexes has got more attention of researchers for their previous success in the field of pharmaceutical sciences and medicinal chemistry. In this project phenylhydrazone derivative were synthesized using phenylhydrazine and 4-methoxybenzaldehyde and 4-nitrobenzaldehyde (scheme 2 and 3) followed by synthesis of their respective Cu(II) complexes.



Scheme 2: Reaction of phenylhydrazine with benzaldehyde



Scheme 3: Reaction of phenylhydrazone with Benzenesulfonyl chloride

1.2. Statement of the problems

Microorganisms; diseases-causing types, have been able to develop resistance in order to fight back man's hostility which sought to deprive them of their territory using antimicrobial agent. The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatments has been noted as the main cause of this problem [24]. The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need [25]. To the best of our knowledge there is no scientific prior report on the synthesis, characterization and biological activities of benzenesulfanohydrazones derivative of phenylhydrazine and 4-methoxybenzaldehyde/4-nitrobenzaldehyde and their Cu(II) complexes. Taking into account the above facts, the synthesis and antibacterial activities of phenylhydrazones Schiff bases, derivative and their Cu(II) complexes derived from phenylhydrazine and 4-methoxybenzaldehyde/4-nitrobenzaldehyde was conducted.

1.3. Significance of the study

In recent years Schiff base and their metal complexes have attained much attraction because of their extensive biological activities [26]. Understanding the structure of some transition metal complexes was useful in pharmaceutical and industrial applications. This study focuses on the synthesis, and antimicrobial study of phenylhydrazone Schiff bases, derivative and their Cu(II) complexes. Therefore, this study is expected to provide important information with regard to structure, mode of synthesis, and antimicrobial activity of copper(II) complexes. This research could also provide helpful scientific information for researchers who are interested to work on similar area.

1.4. Objectives

1.4.1. General objective

The overall objective of this project is to synthesize and conduct antibacterial activity of phenylhydrazone Schiff base, derivative and their Cu(II) complexes.

1.4.2. Specific objectives

- ❖ To synthesize phenylhydrazone Schiff base and derivatives
- ❖ To propose the structures of phenylhydrazone Schiff base derivatives using melting point and spectroscopic techniques such as IR, ^1H , and ^{13}C NMR.
- ❖ To synthesize phenylhydrazone Schiff base derivative Cu(II) complexes
- ❖ To propose the structures of phenylhydrazone Schiff base derivative Cu(II) complexes using melting point, IR and elemental analysis.
- ❖ To study the antibacterial activity of the synthesized phenylhydrazone Schiff base derivative and Cu(II) complexes.

2. LITERATURE REVIEW

2.1. Schiff bases and metal complexes

Schiff base ligands formed from hydrazines are a class of particularly important schiff bases (hydrazone) which have been of immense interest owing to their potentially beneficial pharmacological properties and their wide variety of bonding modes and stereochemistry. Day by day, the chemistry of carbon–nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused *N*-heterocycles. Hydrazones containing azomethine –NHN=CH protons constitute an important class of compounds for new drug development [27]. Hydrazones easily form an interesting series of ligands whose properties can be modified by introducing different organic substituents to form stable complexes with a wide variety of metal ions.

Hydrazide–hydrazones constitute a class of organic compounds, which attracts the attention of medicinal chemists due to the fact that they contain azomethine group ($R_1-NH-N=CH-R_2$) (Fig. 1) connected with carbonyl group, which is responsible for their different pharmaceutical applications and makes possible the synthesis of different heterocyclic scaffolds [28].

Many schiff base ligands have been obtained by the condensation of an aldehyde or a ketone with aromatic or aliphatic hydrazine. Fig. 2a and 2b illustrates the different carbonyl compounds that have been used as precursors for the ligands preparation with condensation at position R_2 . The resulting schiff bases and their respective metal complexes of these selected examples of alkyl, aryl and heteroatomic carbonyl compounds are biologically active. The ligands can be further classified as mono and bis which could result in open chain and macroacyclic metal complexes upon complexation.

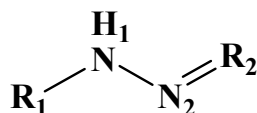


Figure 1. General Structure of hydrazone

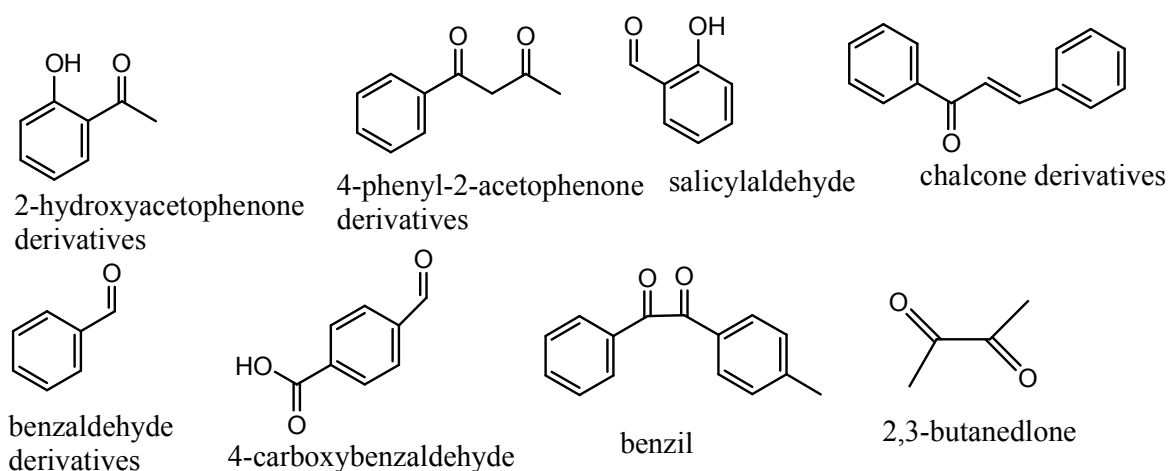


Figure 2a. Examples of different alkyl and aryl series Carbonyl compounds that have been used for the preparation of hydrazone ligands

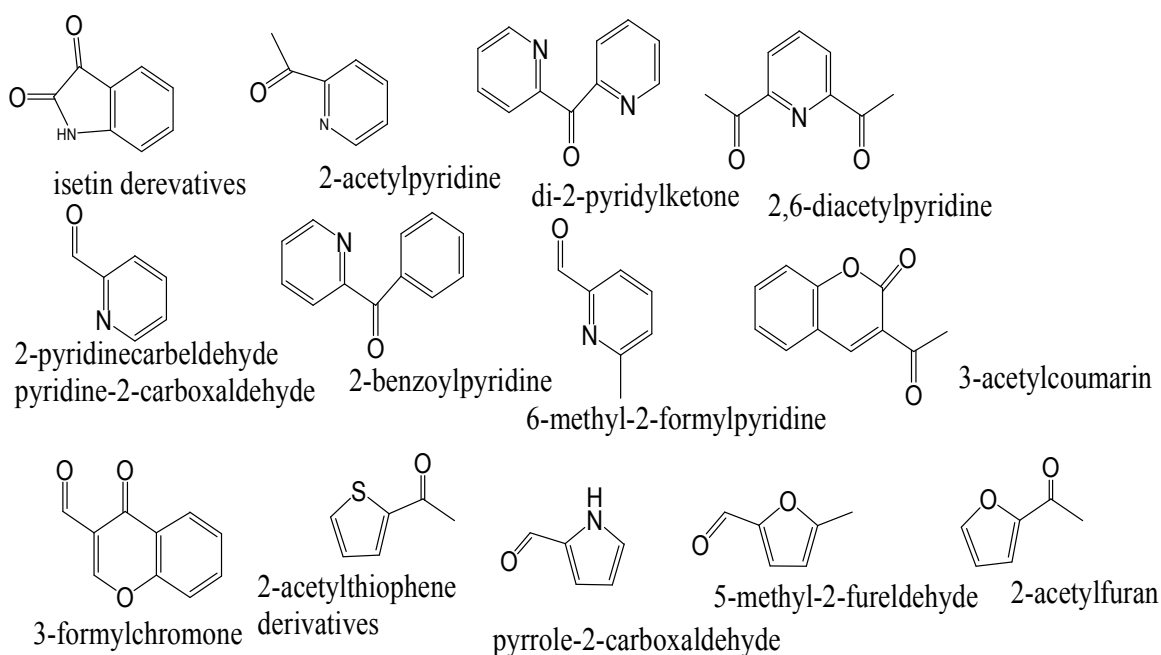


Figure 2b. Examples of different hetero atomic series carbonyl compounds that have been used for the preparation of hydrazone ligands

In recent years, a lot of biologically important hydrazone-hydrazone derivatives with a number of functional groups have been synthesized from many different carbonyl compounds. They were found to possess anticancer [28], anti-inflammatory [27] and antiviral activities [28]. Among the biological properties of this class of compounds, the antimicrobial activity is the most frequently encountered one in scientific literature. Additionally, widely used chemotherapeutic agents such as nitrofurazone **1** [29], furazolidone **2** [30] and nitrofurantoin **3** [31] are known to contain typical hydrazone-hydrazone moiety or hydrazonehydrazone moiety, in which the carbonyl group and nitrogen atom are included in the 1,3-oxazolidine-2-one or imidazolidine-2,4-dione ring (Fig. 3).

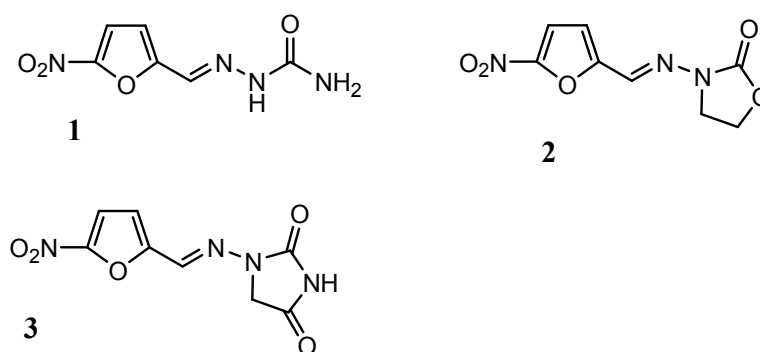


Figure 3. Chemical structures of medicines containing hydrazone moiety:

2.2. Biological activity of hydrazone

Hydrazone derivatives are of great interest particularly in the development of novel therapeutic compounds because of their vast spectrum of biological activity. Many researchers have synthesized these compounds as target structures and have evaluated their biological activities. A compilation of literature reports focusing on the significant bioactivities of a variety of hydrazone derivatives and their metal complexes carried out during the past several decades will be presented herein.

2.2.1. Hydrazones as antibacterial agent

A new series of disulfonamides were synthesized and assayed as antimicrobial agents against *S. aureus*, *B. cereus*, and *E. coli* using disk diffusion method by Alyar *et al.* [32]. The SAR studies of antimicrobial activities indicated that synthesized compounds were the most effective against Gram-negative *E. coli*. The trends of antimicrobial studies showed that

decreasing the length of the carbon chain between NH groups improved the activity. Further, the results showed compound **4** was the most effective compound against *E. coli* with MIC value of 3.72 µg/mL.

Ethane sulfonic acid hydrazide derivatives viz 5-methylsalicylaldehyde- ethane sulfonyl hydrazone, 5-methyl-2 hydroxyacetophenone ethane sulfonyl hydrazone and their Ni(II), Co(II) complexes have been synthesized and tested *in vitro* for antibacterial activity against Gram-positive bacteria; *S. aureus*, *B. subtilis*, *B. magaterium* and Gram-negative bacteria; *Salmonella enteritidis*, *E. coli* using the microdilution broth method by Ozdemir *et al.* [33]. Synthesized compounds were also screened for carbonic anhydrase II activity. Results showed that Ni (II) complexes have more inhibition effect than Co(II) complexes e.g. compound **5** against CA II and tested bacterial strains.

Ozdemir *et al.* [34] synthesized methanesulfonic acid hydrazide derivatives: methylsalicylaldehydemethanesulfonyl hydrazone, 5-methyl-2-hydroxyacetophenonemethane sulfonylhydrazone and their Ni(II), Co(II) complexes. The antibacterial activities of synthesized compounds were studied against Gram-positive bacteria: *S. aureus*, *B. subtilis*, and *B. magaterium*; and Gramnegative bacteria: *S. enteritidis*, and *E. coli* by using the microdilution broth method. The biological activity screening showed that the free ligand **6** have more activity than complexes **7** against the tested bacterial strains.

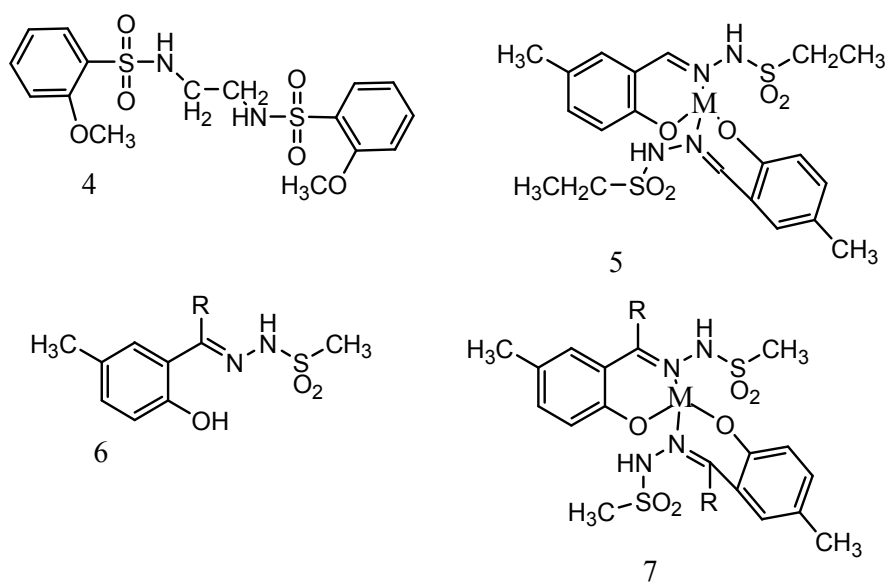
Morjan *et al.* [35] synthesized novel hydrazide– hydrazones of nicotinic acid by the condensation reaction of nicotinic acid hydrazide with various ketones (Fig. 4). The synthesized derivatives were screened *invitro* for antibacterial activity against Gram-positive and Gram negative bacteria. The results of antimicrobial assay revealed that the synthesized compounds had interesting antibacterial activity against Gram-negative strains. Especially compounds **8** and **9** showed very strong activity against *P. aeruginosa* (MIC = 0.22 and 0.19µg/mL, respectively). The activity of these compounds against *K. pneumoniae* was also high (**8**: MIC = 3.12µg/mL, **9**: MIC = 14.00µg/mL). The inhibitory potency against Gram-positive bacteria *Streptococcus pneumoniae* (**8**: MIC = 3.12µg/mL) and against *S. aureus* (**9**: MIC = 7.03µg/mL).

In the research conducted by Rambabu *et al.* [36] new anacardic acid hydrazone derivatives were synthesized and subjected to antibacterial screening. The obtained compounds were

tested against two Gram-negative (*P. aeruginosa* and *E. coli*) and two Gram-positive (*S. aureus* and *S. pyogenes*) bacterial strains (Fig. 4). The antibacterial activity was assessed on the basis of the measurement of the zone of inhibition growth. Antimicrobial assays revealed that three of the obtained derivatives **10**, **11** and **12** had better antibacterial activity (ZOI = 20–24mm) than the activity of ampicillin, because their zones of inhibition were larger than the control antibiotic (ZOI = 18–20mm).

Novel 1,2,3-triazole carbohydrazide derivatives synthesized by Sreedhar *et al.* [37] were tested against four bacterial strains (Fig. 4). The measured zones of growth inhibition for the obtained compounds were similar to those of ciprofoxacin used as control. In the case of compound **114**, its zone of inhibition against *S. aureus* MTCC 96 (ZOI = 23mm) was larger than for the reference compound (ZOI = 22mm). A similar situation appeared for compounds **13** and **16**, their zones of inhibition (ZOI = 23mm) were larger than for ciprofloxacin (ZOI = 22 mm) against *S. pyogenes*.

Dommati *et al.* Synthesized novel benzohydrazide derivatives and evaluated them for in vitro antibacterial activity (Fig. 4). The highest activity, but weaker than control streptomycin, was shown by compounds **17** and **18** against Gram-negative bacteria: *E. coli* MTCC 2692 and *P. aeruginosa* MTCC 2453 (ZOI = 5–13 mm), and Gram positive bacteria: *S. aureus* MTCC 902 and *B. subtilis* MTCC 441 (ZOI = 18–21mm) [38].



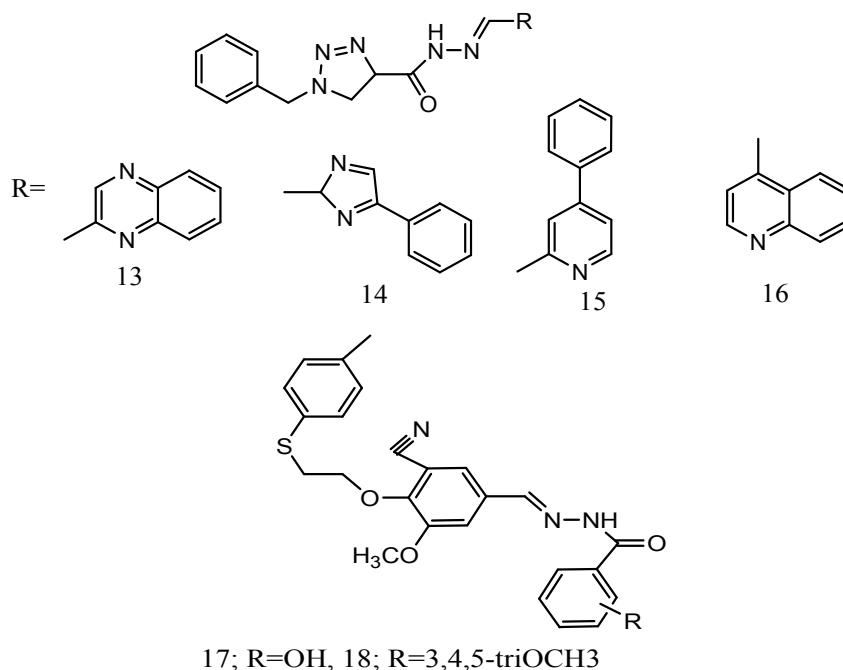


Figure 4. Structure of hydrazones reported as antimicrobial agent

2.2.2. Hydrazones as anti-tuberculosis agent

A new series of 4-pyrrol-1-yl benzoic acid hydrazide analogs, 5-substituted-2-thiol-1,3,4-oxadiazoles, 5-substituted-4-amino-1,2, 4-triazolin-3-thione and 2,5-dimethyl pyrroles have been synthesized by Joshi *et al.* and evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by broth dilution assay method. The examination of the antimycobacterial activity results indicated that compound 5-(4-pyrrol-1-yl-phenyl)-[1,3,4]oxadiazole-2-thiol **19**, showed better activity (MIC = 16.0 μ g/mL) against *M. tuberculosis* H37Rv as compared to other synthesized derivatives [39].

Hunoor *et al.* [40] synthesized Co(II), Ni(II), Cu(II) and Zn(II) complexes with a new heterocyclic Schiff base derived by condensation of isonicotinoylhydrazide and 3-acetylcoumarin. The synthesized compounds were subjected to anti-TB activity and MIC was determined against *M. tuberculosis* H37Rv. The results of antimycobacterial activity showed that none of the tested compounds have the inhibition more than standard. The activity of isoniazid is considerably reduced on coupling with coumarin moiety whereas the activity of synthesized compound has enhanced two fold on complexation with Co(II) and Ni(II) metal ions.

Eswaran *et al.* [41] synthesized hydrazone derivatives by the condensation of 4-hydrazinyl-2,8-bis(trifluoromethyl) quinolone with aliphatic and (hetero) aromatic aldehydes in presence of catalytic amount of acetic acid. The synthesized compounds were evaluated for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv and MDR-TB. (*E*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4-methyl-1*H*-imidazol-5-yl)methylene) hydrazine **20**, and (*E*)-2-((1*H*-imidazol-4-yl)methylene)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)hydrazine **21** were found to be very potent inhibitors, being able to inhibit 99% growth of *M. tuberculosis* at a concentration of 3.12 µg/mL.

Kumar *et al.* [42] synthesized a series of benzoic acid hydrazones and its nicotinoyl derivatives and evaluated for their antitubercular activity towards a strain of *M. tuberculosis*. The *in vitro* antitubercular activity of synthesized compounds against MTB was carried out in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method. The antitubercular activity results indicated that nicotinic acid *N*-(3,5-dinitrobenzoyl)-*N'*-(4-methoxybenzylidene)- hydrazide **22** was the most potent among the synthesized compounds with MIC of 3.5×10^{-3} µM. In general structure activity relationship (SAR) studies of antimycobacterial compounds showed the importance of OCH₃, NO₂, halo groups and nitrogen containing cyclic compounds in enhancing the antimycobacterial activity of aforementioned compounds.

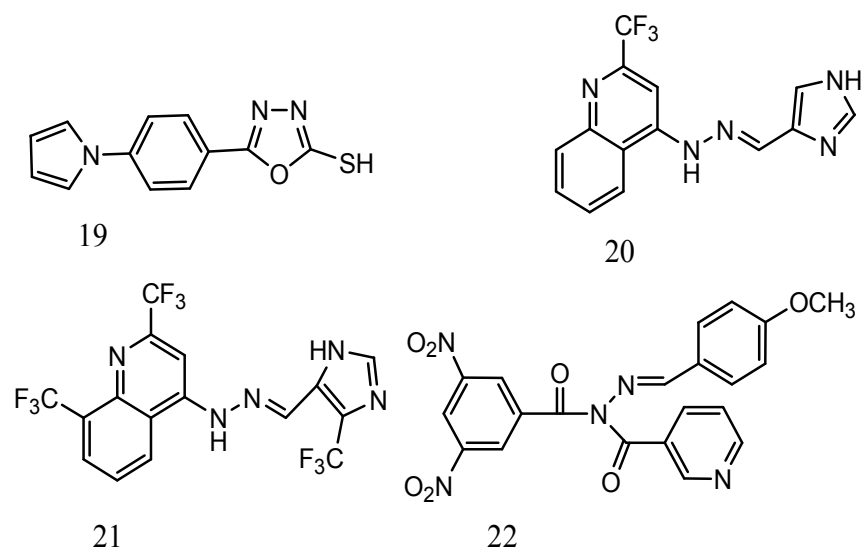


Figure 5. Structure of hydrazones reported as anti-tuberculosis agents

2.2.3. Hydrazones as antifungal agent

Novel heterobicyclic methylthiadiazole hydrazones synthesized by Kodisundaram *et al.* [43] were also tested for antifungal activity against *Aspergillus spp.* and *C. albicans*. The activity of compounds **23** and **24** against *A. flavus* and *A. niger* was two times higher (MIC = 12.5 $\mu\text{g/mL}$) than the antifungal activity of fluconazole (MIC = 25 $\mu\text{g/mL}$). The MICs against *C. albicans* for synthesized compounds were also two times higher (MIC = 6.25 $\mu\text{g/mL}$) than the MIC value of fluconazole (12.5 $\mu\text{g/mL}$).

In the case of hydrazide-hydrazones of 4-nitrobenzoic acid, the antifungal activity against the above mentioned fungi strains was even better (Fig. 7). The MIC₈₀ parameters for compounds **25** and **26** were 0.5 $\mu\text{g/mL}$ against *C. albicans*, whereas for compound **27** it was even 0.125 $\mu\text{g/mL}$ against *C. glabrata*. The MIC₈₀ value for compound **25** against *C. glabrata* was also very low (MIC₈₀ = 0.5 $\mu\text{g/mL}$) [44].

New anacardic acid hydrazone derivatives were subjected to in vitro antifungal assays against *C. albicans* and *A. niger* [36]. Three of the synthesized compounds **10**, **11** and **12** showed good to moderate antifungal activity (ZOI = 11–20mm) based on the measurement of the zone of inhibition growth in comparison with the gryseofulvin used as control (ZOI = 24–28mm) [36].

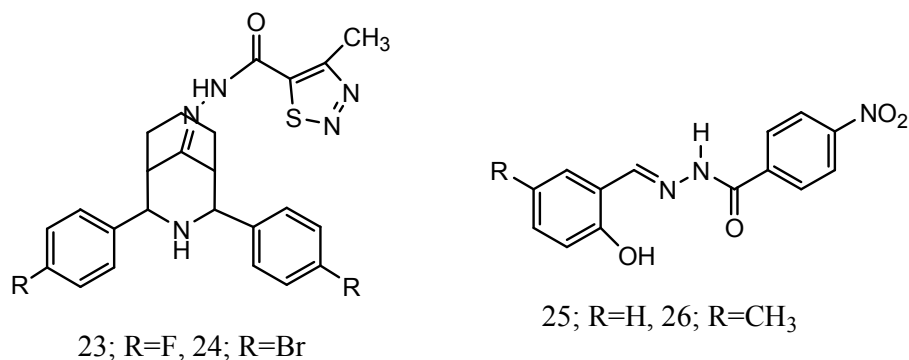


Figure 6. Structure of hydrazones reported as Antifungal agents

2.2.4. Hydrazones as antiviral agent

Dawood *et al.* [45] synthesized fluorinated hydrazone, bishydrazone and pyrazole derivatives containing pyrazole- and isoxazole-based heterocycles and screened their antiviral activity

against *Herpes simplex* type-1 (HSV-1). The results of antiviral activity indicated that compound **27** reduced the number of viral plaques of HSV-1 by 69%.

Fathalla *et al.* [46] synthesized different substituted hydrazinothiazolyl- 2-thiouracil-5-sulphonamide (*p*-phenyl) derivatives **28** by the condensation of 4-oxo-2-thioxo-1, 2,3,4-tetrahydropyrimidine-5-sulfonic acid [4-(2-hydrazino-thiazol-4-yl)-phenyl]-amide with different aldehydes and thiosemicarbazones and tested them against a DNA baculovirus. The preliminary antiviral activity revealed that all compounds showed moderate activity as compared to standard and reduction in plaques from 63-75%.

A series of novel *N'*-arylidene-2-[1-(naphthalen-1-yl)-1*H*-tetrazol- 5-ylthio] acetohydrazides was synthesized and evaluated, as non-nucleoside reverse transcriptase inhibitors (NNRTIs), for their *invitro* HIV-1 and HIV-2 activity using the IIB strain and ROD strain, respectively. The activity was monitored by the inhibition of the virus induced cytopathic effect in the human T-lymphocyte (MT-4) cells. Compounds **29** and **30** showed EC₅₀ of 29.62 μM (CC₅₀ of 169.24 ± 23.83 μM) and 31.62 μM (CC₅₀ > 309.06 μM), and resulting in selectivity index of 6 and > 9, respectively. However, all newly synthesized derivatives were not active against HIV-2 replication [47].

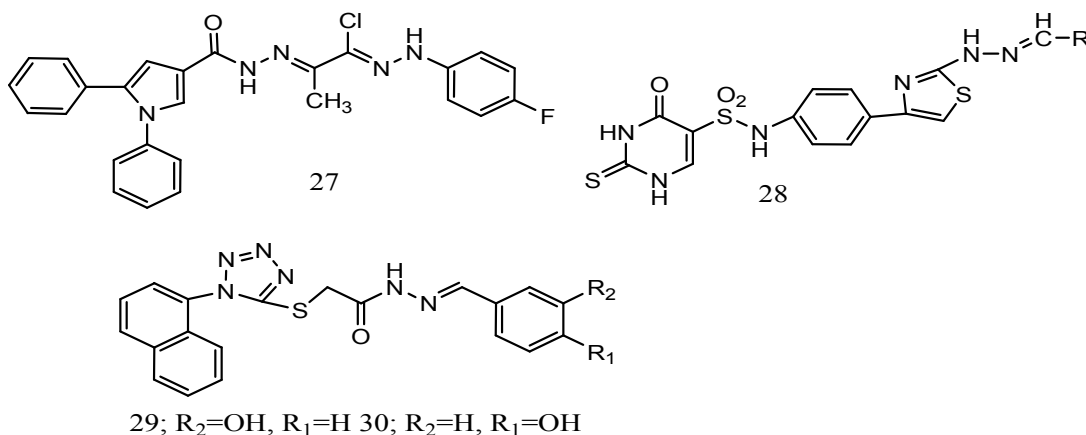


Figure 7. Structure of hydrazones reported as antiviral agents

2.2.5. Hydrazones as anticancer agent

A series of 3- and 5-methyl thiophene-2-carboxaldehyde heterocyclic hydrazones were synthesized and submitted to an investigation of their anticancer activity. The most active compound of the series was *N*-(4,8-dimethyl-quinolin-2-yl)-*N'*-(5-methylthiophen- 2-

ylmethylene)-hydrazine **31**, which exhibited tumour growth inhibition activity against tested cell lines and displayed GI₅₀ values between 1.63μM and 26.5μM [48].

Dolzhenko *et al.* synthesized fluorinated 7-aryl-2-pyridyl-6,7-dihydro [1,2,4]triazolo [1,5-*a*][1,3,5]triazin-5-amines from isonicotinic hydrazides. The antiproliferative activity of the synthesized compounds was evaluated against breast, colon and lung cancer cell lines. The highest antiproliferative activity in the series was found for 2-(pyridine-3-yl)-7-(4-trifluoromethylphenyl)- 6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5] triazin-5-amine **32** [49].

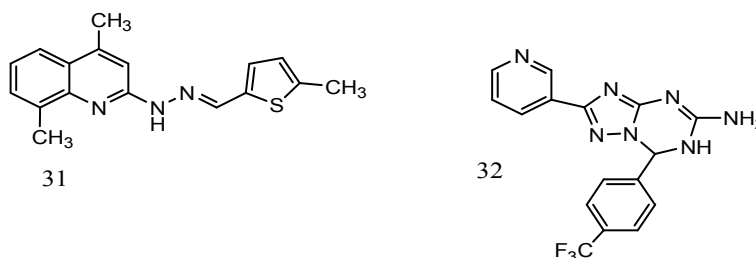


Figure 8. Structure of hydrazones reported as anticancer agents

2.3. The Chemistry of Copper (II)

Copper(II) forms stable complexes with nitrogen and oxygen donor ligands. Cu(II) complexes of nitrogen ligands are generally more stable than Cu(I) complexes. The most common coordination number of Cu(II) are 4, 5 and 6 but regular geometry are rare, and the distinction between square-planar and distorted octahedral coordination is not easily made. Mixed O and N donor ligands such as Schiff bases are of interest in that they provide examples of square-planar coordination and square-pyramidal coordination by dimerization [50, 51]. It has a biological role in sustaining life. Copper has antimicrobial property. Copper complexes have been given much attention in coordination chemistry, anion selectivity and their biological significance. Copper in trace quantities is required by all living organisms to maintain proper cellular functions. A great majority of Cu(II) compounds are blue or green in color because of a single broad absorption band in the region of 600-900nm which is due to spin allowed transition 2T_{2g}, 2E_g. Considerable distortion in octahedral symmetry is observed in Cu(II) complexes [52].

3. MATERIALS AND METHOD

3.1. Experimental site

Synthesis of Schiff bases ligands and complexes and melting point determination were done in Adama Science and Technology University. Elemental analysis and Characterization (IR, HNMR and ^{13}C NMR) of hydrazone Schiff bases, derivative and complexes were done in Addis Ababa University. Antimicrobial assay of the synthesized compound were done in Oromia Public Health Research Capacity Building and Quality Assurance Laboratory.

3.2. Chemicals and reagents

The chemicals and reagents that were used in this investigation are: silica gel, phenylhydrazine, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde, benzenesulphonylchloride, anhydrous $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$. Solvents such as ethanol, methanol, chloroform, dimethylsulfoxide (DMSO), distilled water, glacial acetic acid, and tetramethylsilane (TMS) were used. All the chemicals and reagents utilized in this work were analytical grade and are of pure grade Aldrich.

3.3. Instrumentation

Melting or decomposition point was determined by Stuart SMP3 digital melting point apparatus. IR spectra were recorded using Perkin-Elmer FT-IR spectrophotometer in the range of $4000\text{--}450\text{ cm}^{-1}$. ^1H and ^{13}C NMR was recorded using Bruker Avance NMR spectrometer (400 MHz) using $\text{DMSO-}d_6$ as solvent and tetramethylsilane (TMS) as an internal standard. Elemental analysis of CHNS was done by using Exeter Analytical CE 440 elemental analyzer. The purity of the phenylhydrazone Schiff base derivative and its Cu(II) complexes were checked by silica Gel-G Pellets by thin layer chromatography (TLC) with layer thickness of 0.3 mm. TLC visualization was realized by UV-light.

3.4. Synthesis of hydrazone Schiff base

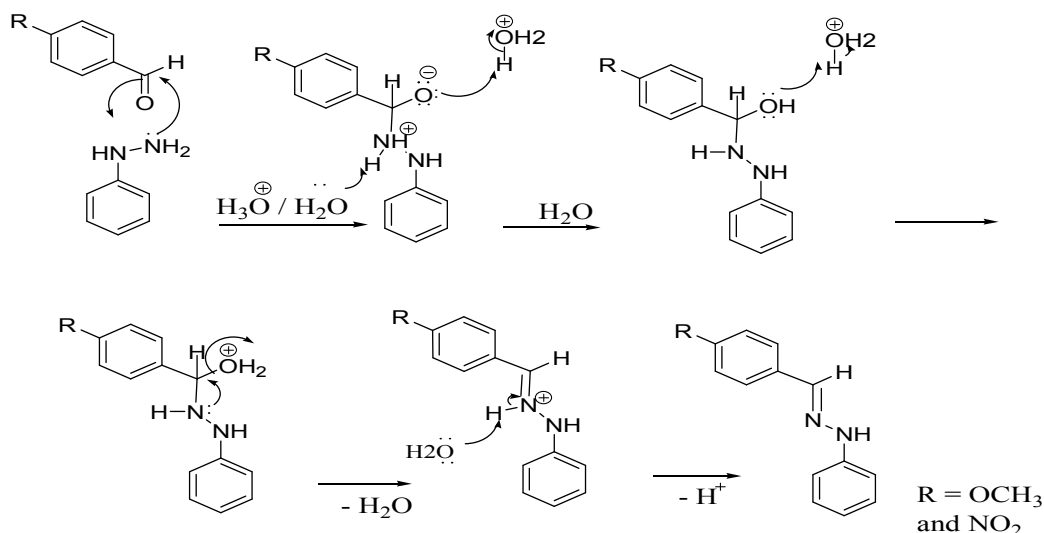
3.4.1 General synthesis procedure

Equimolar quantities (0.984mL, 0.01 moles) of phenylhydrazine and the corresponding aldehyde 4-methoxybenzaldehyde (1.21mL, 0.01 moles) / 4-nitrobenzaldehyde (1.51g, 0.01 mole) were dissolved in 40mL ethanol. Glacial acetic acid (2mL) were added and refluxed

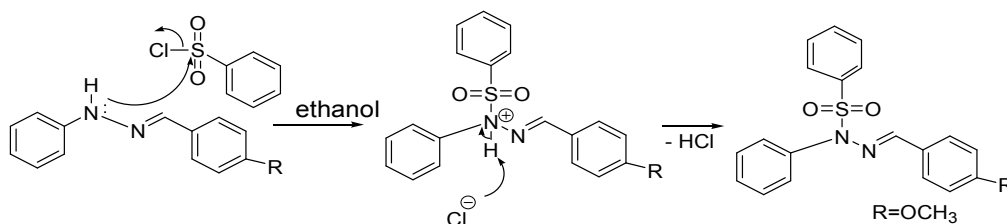
for about 6hr at 60°C. The reaction progress was monitored by TLC. The product was poured on crushed ice. A precipitate was obtained. This precipitate was filtered and washed with ethanol and dried in vacuum desiccators overnight and recrystallized by ethanol. The purity was checked by TLC.

3.4.2 Synthesis of phenylhydrazone Schiff base derivative

The phenylhydrazone Schiff bases (0.01 moles) was dissolved in 40 mL of ethanol and equimolar benzenesulphonylchloride, were added. The mixture was refluxed for 6 hours. The completion of reaction was monitored by single spot TLC on silica gel G plates using ethyl acetate: n-hexane (3:7) as mobile phase. After the completion of reaction the mixture was poured in ice cold water and the product obtained was filtered, washed with ice cold water and dried at room temperature. The crude product was recrystallized using ethanol and placed in desiccators to obtain solid. The purity was checked by TLC (ethyl acetate: n-hexane (3:7)).



Scheme 4. Reaction mechanism for synthesis of phenyl hydrazone



Scheme 5. Reaction mechanism for the synthesis of hydrazone derivative.

3.4.3. Synthesis of Copper (II) complexes compound 34

Cu(II) complexes of hydrazone derivative Schiff base ligand (compound **34**) was prepared by mixing 0.2M of hydrazone Schiff base with 40 mL ethanolic solution of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ keeping ligand-metal ratio 2:1. Glacial acetic acid (2 drop) was added. The result mixture was refluxed for 8 hours. The P^{H} of the solution was monitored. The reaction progress was monitored by TLC. A light brown solution obtained was cooled, the precipitate was filtered and washed by 50 mL ethanol three times to purify and removed the excess of ligand. Finally, the precipitate was dried in desiccators overnight and recrystallized from ethanol. The purity was checked by TLC (ethyl acetate: n-hexane (3:7). Light brown solid was obtained, yield 75%, m.p 139-141°C.

3.4.4. Synthesis of Copper (II) complexes of compound 35

Cu complexes of hydrazone derivative Schiff base ligand were prepared by mixing 0.2 M of hydrazone derivative Schiff base (compound **35**) with 40 mL ethanolic solution of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ keeping ligand-metal ratio 2:1. Glacial acetic acid (2 drop) was added. The result mixture was refluxed for 8 hours. The P^{H} of the solution was monitored. The reaction progress was monitored by TLC. A brown solution obtained were cooled, the precipitate were filtered and washed with ethanol many times to purify and removed the excess of ligand. Finally, the precipitate was dried in desiccators overnight and recrystallized from ethanol. The purity was checked by TLC (ethyl acetate: n-hexane (3:7). brown solid was obtained, yield 70%, m.p 190-192°C.

3.5. Spectral data of synthesized compounds

Compound **33** was obtained as white crystal, 82% yield, mp: 113-114.5, R_f : 0.54 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν : 3483 (N–H), 3300 ($\text{Csp}^2\text{--H}$), 2944 ($\text{Csp}^3\text{--H}$), 1597 (N=C), 1585 (C=C), 1255 (C–O). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz) δ_{H} : 3.80 (s, 3H, OCH₃), 6.73 (m, 1H), 6.95 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.28 Hz, 2H), 7.22 (m, 2H), 7.60 (d, J = 8.8 Hz), 7.83 (s, 1H, N=CH), 10.15 (s, 1H, N-H). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 400 MHz) δ_{C} : 55.6 (OCH₃), 112.3 (CH), 114.6 (CH), 118.8 (CH), 127.5 (CH), 128.9 (C), 129.5 (CH), 137.0 (CH, N=C), 146.0 (C), 159.8 (C).

Compound **34** was obtained as red crystal, 83% yield, mp: 145-146, Rf: 0.48 (hexane: EtOAc, 7:3). IR (cm^{-1}) v: 3500 (N-H), 3305 (CH=N); 1600 (N=C), 1330 (NO_2). ^1H -NMR ($\text{DMSO-}d_6$, 400 MHz) δ_{H} : 6.85 (m, 1H), 7.15 (d, J = 8 Hz, 2H), 7.25 (m, 2H), 7.79 (d, 2H), 7.90 (s, 1H, N=CH), 8.18 (d, J = 8 Hz, 2H), 10.85 (s, 1H, N-H). ^{13}C -NMR ($\text{DMSO-}d_6$, 400 MHz) δ_{C} : 113.0 (CH), 120.4 (CH), 124.4 (CH), 126.4 (CH), 129.6(CH), 134.0 (CH, N=C), 143.0 (C), 144.9 (C), 146.4 (C).

Compound **35** was obtained as gray solid, 80% yield, mp: 213-215°C, Rf: 0.43 (hexane:EtOAc, 7:3). IR (cm^{-1}) 3347 (CH=N), 1614 (N=C), 1585 (C=C), 1510 and 1256 (SO_2). ^1H -NMR ($\text{DMSO-}d_6$, 400 MHz) δ_{H} : 3.83 (s, 3H, OCH_3), 7.83 (s, 1H, N=CH), and aromatic proton from 6.84-7.55 ppm. ^{13}C -NMR ($\text{DMSO-}d_6$, 400 MHz) δ_{C} : 55.4 (OCH_3), 112.2 (CH), 113.9(CH), 114.6 (CH), 125.9 (CH), 127.4 (CH), 128.1 (CH), 129.0 (C), 130.0 (CH), 130.3 (CH), 136.8 (CH, N=C), 137.6 (C), 144.2 (C) and 159.7 (C).

3.7. Antibacterial activity

The *in vitro* antibacterial screening was done against the bacterial species *E. coli*, *K.pneumonia*, *P.aeruginosa* and *S. aureus*.. The bacterial cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37°C. Inoculated medium containing 24hr grown culture were added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. This solution is poured (25mL in each dish) into Petri dishes and then allowed to attain room temperature. Wells (6 mm in diameter) were punched carefully using a sterile cork borer and were filled with test solution 20 mg/mL. The plate was allowed to stand for an hour in order to facilitate the diffusion of the drug solutions, then the plates were incubated at 37°C for 24hr for bacteria and the diameter of the zone of inhibition were measured [54]. The results will be compared with those of standard drug ceftriaxone for bacterial of the same concentration as that of the test compounds under identical conditions.

4. Result and Discussion

4.1. Characterization of the ligand

Compound **33** was obtained as a white crystals with melting point of 113-114°C. Its IR spectrum (Appendix 1) showed two characteristic absorptions at 3483 and 3316 cm^{-1} assignable to $\nu(\text{N-H})$ and $\nu(\text{CH=N})$, respectively. Strong band of azomethine group (C=N) occurred at 1597 cm^{-1} . The ^1H NMR spectrum (Appendix 2) showed peaks at δ_{H} 10.15 and 7.83 attributed to N-H and N=CH moiety, respectively, with integration values corresponding to one proton for each. A singlet peak appeared at δ_{H} 3.80 (3H) belongs to methoxy group (OCH_3). The presence of 1,4-substituted benzene ring with AA'BB' spin system was observed at δ_{H} 7.60 (2H, H-2',6') and 6.95 (2H, H-3',5') whereas the presence of monosubstituted benzene ring was observed at δ_{H} 7.22 (2H, H-3,5), 7.07 (2H, H-2,6) and 6.74 (1H, H-4).

The ^{13}C NMR spectrum (Appendix 3) showed signals at δ_{C} 137.02 and δ_{C} 159.77 attributed to azomethine carbon (C=N) and sp^2 oxygenated quaternary carbon, respectively. The presence of eight aromatic peaks were observed between δ_{C} 112.3-159.8 of which three of them are quaternary at δ_{C} 146.0, 159.8, and 128.9. The former suggests sp^2 quaternary carbon connected to nitrogen whereas the middle one suggests sp^2 quaternary carbon connected to oxygen. In agreement with the ^1H NMR, the presence of methoxy group was confirmed at δ_{C} 55.6. Thus, based on the above spectral data the following structure was proposed which is in agreement with the synthesis protocol where the first moiety (monosubstituted phenyl ring) and the second moiety (disubstituted phenyl ring) are from the hydrazine and 4-methoxybenzaldehyde counterpart, respectively confirming the formation of hydrazone Schiff base..

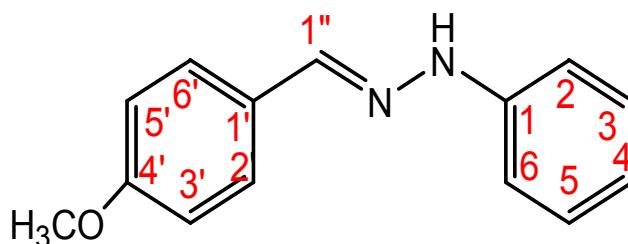


Figure 9. The proposed structure of the synthesized compound **33**

Table 1. ^1H NMR, ^{13}C NMR and DEPT-135 spectra data of compound **33** ($\text{DMSO}-d_6$)

Position	^{13}C NMR (δ ,ppm)	^{13}C NMR(DEPT-135) (δ ,ppm)	^1H NMR (δ ,ppm)
1	146.0	-	-
2, 6	114.6	114.6	7.07
3, 5	127.5	127.5	7.22
4	118.8	118.8	6.74
1''	137.1	137.1	7.84
1'	128.9		-
2', 6'	129.5	129.5	7.95
3', 5'	112.3	112.3	6.76
4'	159.8	-	-
4'-OCH ₃	55.6	55.62	3.80
N-H	-		10.15

Compound **34** was obtained as a red crystals with melting point of 145-147°C. Its IR spectrum (Appendix 5) showed two characteristic absorptions at 3500 and 3305 cm^{-1} assignable to $\nu(\text{N-H})$ and $\nu(\text{CH=N})$, respectively. The strong band observed at 1600 cm^{-1} and 1330 cm^{-1} suggests the presence of azomethine group (C=N) and nitro functional groups respectively. The ^1H NMR spectrum (Appendix 6) showed peaks at δ_{H} 10.85 and 7.88 attributed to N-H and N=CH group protons, respectively, with integration values corresponding to one proton for each one. The presence of 1,4-substituted benzene ring with AA'BB' spin system was observed at δ_{H} 8.18 (2H, H-3',5') and 7.15 (2H, H-2',6') whereas the presence of monosubstituted benzene ring was observed at δ_{H} 7.79 (2H, H-2,6), 7.25 (2H, H-3,5) and 6.85 (1H, H-4).

The ^{13}C NMR spectrum (Appendix 7) showed signal at δ_{C} 134 attributed to azomethine carbon (C=N). The presence of eight aromatic peaks were observed between δ_{C} 113.0-146.4 of which three of them are quaternary with δ_{C} 146.4, 144.4 and 143.0. The former suggests sp^2 quaternary carbon connected to nitrogen (C-1)) whereas the middle one suggests sp^2 quaternary carbon connected to nitro group (C- NO_2). Thus, based on the above spectral data the following structure was proposed which is in agreement with the synthesis protocol where the first moiety (monosubstituted phenyl ring) and the second moiety (disubstituted phenyl ring) are from the hydrazine and 4-nitrobenzaldehyde counterpart, respectively confirming the formation of hydrazone Schiff base.

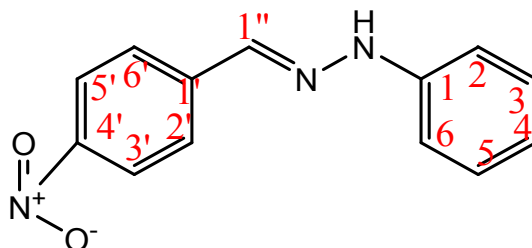


Figure 10. The proposed structure of the synthesized compound **34**

Table 2. ^1H NMR, ^{13}C NMR and DEPT-135 spectra data of compound **34** ($\text{DMSO}-d_6$).

Position	^{13}C NMR (δ ,ppm)	^{13}C NMR (DEPT-135) (δ ,ppm)	^1H NMR (δ ,ppm)
1	146.4	-	-
2, 6	113.0	113.0	7.25
3, 5	126.3	126.3	7.79
4	120.4	120.4	6.85
1''	134.0	134.0	7.85
1'	143.0	-	-
2', 6'	129.6	129.6	7.15
3', 5'	124.4	124.4	8.18
4'	144.4	-	-
N-H	-	-	10.85

Compound **35** was obtained as gray solid with melting point of 213-215°C. Its IR spectrum (Appendix 9) showed characteristic absorptions at 3347 assignable to $\nu(\text{CH}=\text{N})$. Strong bands observed at 1614 cm^{-1} , 1510 cm^{-1} and 1256 cm^{-1} suggest the presence of azomethine group ($\text{C}=\text{N}$), and two ($\text{S}=\text{O}$) respectively. The IR bands of the hydrazone derivative were compared with those of compound 1. The two bands at 1500 cm^{-1} and 1256 cm^{-1} is due to the presence of benzenesulphonyl. The ^1H NMR spectrum (Appendix 10) showed a singlet peak at δ_{H} 7.85 attributed to $\text{N}=\text{CH}$ group, with integration values corresponding to one proton. A singlet peak appeared at δ_{H} 3.80 (3H) belongs to methoxy group (OCH_3).

The ^{13}C NMR spectrum (Appendix 11) showed signals at δ_{C} 136.82 ($\text{C}-1''''$) and δ_{C} 159.7 attributed to azomethine carbon ($\text{C}=\text{N}$) and sp^2 oxygentaed quaternary carbon ($\text{C}-4'$), respectively. The presence of 12 aromatic peaks were observed between δ_{C} 112.2-159.7 of which four of them are quaternary with δ_{C} 144.2, 159.7, 137.6 and 129.0. The first suggests sp^2 quaternary carbon connected to nitrogen ($\text{C}-1$), the second one suggests sp^2 quaternary carbon connected to oxygen ($\text{C}-4'$), third one suggests sp^2 quaternary carbon connected to sulfur ($\text{C}-1''$) whereas the fourth one attributed to $\text{C}-1'$. In agreement with the ^1H NMR, the

presence of methoxy group was confirmed at δ_C 55.6. Thus, based on the above spectral data the structure shown below was proposed which is in agreement with the synthesis protocol where the first moiety (diarylhydrazones) and the second moiety (monosubstituted phenyl ring) are from compound **33** and benzenesulphonylchloride counterpart, respectively confirming the formation of benzenesulfanohydrazones derivative.

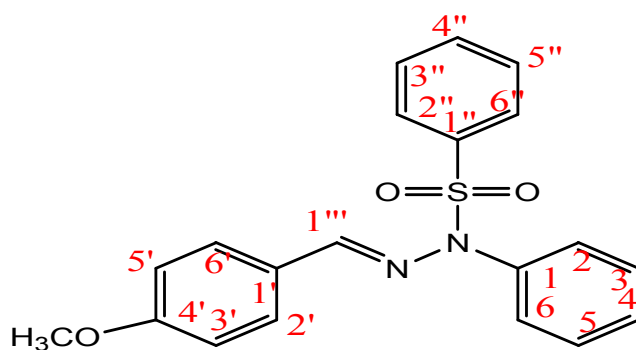


Figure 11. The proposed structure of the synthesized compound **35**

Table 3. ^1H NMR, ^{13}C NMR and DEPT-135 spectra data of compound **35** ($\text{DMSO}-d_6$).

Position	^{13}C NMR (δ ,ppm)	^{13}C NMR (δ ,ppm) (DEPT- 135)	^1H NMR (δ ,ppm)
1	144.2	-	
2, 6	113.9	113.9	6.63
3, 5	127.4	127.4	6.96
4	125.9	-	6.95
1'''	136.8	136..8	7.85 (s)
1'	129.0	-	-
2', 6'	130.3	130.3	7.04
3', 5'	112.2	112.2	6.54
4'	159.7	-	-
4'-OCH3	55.6	55.6	3.80 (s)
1''	137.6	-	-
2'', 6''	114.6	114.6	7.55
3'', 5''	130.0	130.0	6.93
4''	128.1	128.1	7.05

4.2. Characterization of the complexes

Compound **36**, Cu(II) complex of compound **34**, was obtained as a light brown solid crystals with melting point of 140-141°C. The elemental analysis result of Cu(II) complexes (table 4) is in good agreement with the theoretical values. The infrared spectra (appendix 13) showed sharp absorption at 1616 cm^{-1} attributed to azomethine groups (C=N). The sharp absorption at 3013 cm^{-1} was assigned to H-C=N band of the azomethine group. The imine bond C=N stretching shifted to higher value in the spectra of the complexes than the ligand indicating the coordination of azomethine nitrogen to the metal ions [55]. This was further supported by the appearance of a new band in the region 606 cm^{-1} due to metal ligand M-N bonding. The presence of coordination and/or lattice water molecules in the binary complexes is indicated by a broad band within the range $3350\text{--}3550\text{ cm}^{-1}$ due to $\nu(\text{OH})$ vibrational modes of the water molecules [56]. Based on the elemental analysis, IR spectral data and literature indications octahedral geometry was suggested in which abidentate ligand coordination through the azomethine nitrogen atom, nitrogen atom, the fifth and sixth coordination sites being occupied OAc as shown below which is in agreement with the synthesis protocol where ligand (diarylhydrazone) and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ undergo reaction confirming the formation of Cu(II)hydrazone complex.

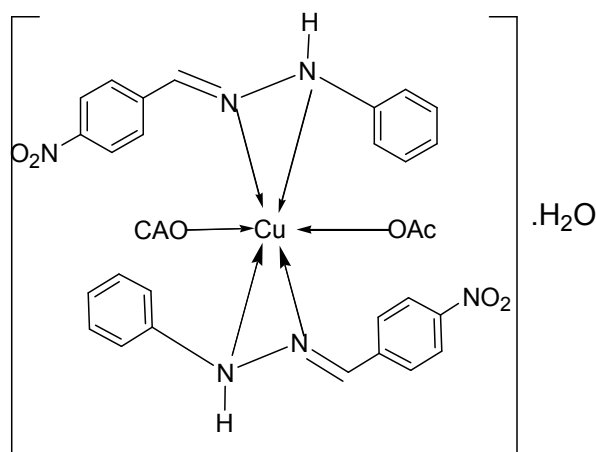


Figure 12. The proposed structure of compound **36**

Compound **37**, Cu (II) complex of compound **35**, was obtained as a dark brown crystals with melting point of 190-192°C. The elemental analysis result of Cu(II) complexes (table 4) is in good agreement with the theoretical values. The infrared spectra (appendix 14) showed strong absorption band at 1611 cm^{-1} assigned to the vibrations of azomethine groups (C=N). The imine bond C=N stretching shifted to lower value in the spectra of the complexes than the ligand indicating the coordination of azomethine nitrogen to the metal ions [55]. This was further supported by the appearance of a new band in the region 652 cm^{-1} in the complexes due to metal ligand M-N bonding. The presence of coordination and/or lattice water molecules in the binary complexes is indicated by a broad band within the range 3350–3550 cm^{-1} due to $\nu(\text{OH})$ vibrational modes of the water molecules [56]. Based on the elemental analysis, IR spectral data and literature indications octahedral geometry was suggested in which bidentate ligand coordination through the azomethine nitrogen atom, nitrogen atom, the fifth and sixth coordination sites being occupied OAC as shown below which is in agreement with the synthesis protocol where the ligand (hydrazone derivative Schiff base) and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ are undergo reaction and confirming the formation of Cu(II)hydrazone derivative Schiff base complex.

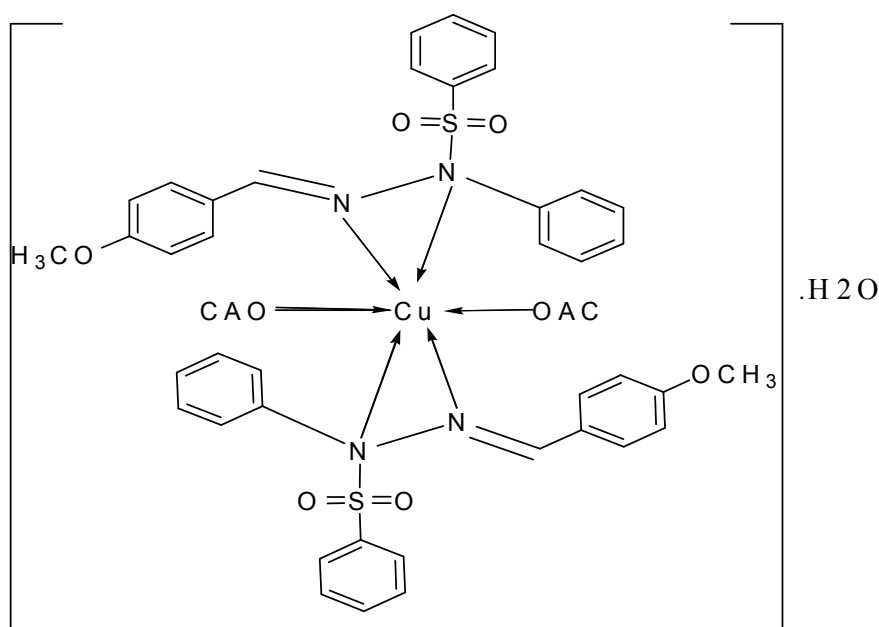


Figure 13. The proposed structure of compound **37**

Table 4. Elemental analysis results for copper complexes.

Complex	Molecular weight(g/mlo)	%C		%N		%H	
		Calc.	Found	Calc.	Found	Calc.	Found
36	681	52.86	52.42	12.33	12.12	3.23	3.14
37	931	56.71	52.21	6.01	5.82	4.73	4.68

4.3. Antibacterial activity results of ligands and Cu(II) complexes

The synthesized hydrazone, hydrazone derivative schiff base and Cu(II) complexes are tested for their antibacterial activity *in-vitro* against *S.aureus*, *E.coli*, *K.pneumonia* and *P.aeruginos*. Ceftriaxone was used as standard antibacterial drug. The zone of inhibition values indicates that all the compounds exhibited a varied range 6-15 mm of antibacterial activity (table 5) against all the tested bacterial strains. Compound 33 showed moderate activity against *E.coli* and *P.aeruginos*. Compounds 34 and 35 showed no activity against all the tested bacterial strains. Compound 37 the Cu(II) complex of 35 have moderate activity than the ligand 35 against *S.aureus* and no activity against *E.coli*, *P.aeruginos* and *K.pneumonia*. Compound 36 the Cu(II) complex of Compound 34 showed moderate activity against *E.coli* (10mm zone of inhibition) and but better activity against *K.pneumoniae* (15mm zone of inhibition) compared to the positive control (11mm zone of inhibition). Results of the antibacterial activity show that the Cu(II) complexes exerted excellent antibacterial activities than the ligand which might be due to increase in lipophilicity of the compound.

Table 5. *In-vitro* antibacterial activity values of the synthesized hydrazone schiff base and Cu(II) complexes.

Compound	<i>S.aureus</i> (Z.OI mm)	<i>E.coli</i> (ZOI mm)	<i>K.pneumonia</i> (ZOI mm)	<i>P.aeruginosa</i> (ZOI mm)
Compound 33	6	8	6	7
Compound 34	6	6	6	6
Compound 35	6	6	6	6
Compound 36	9	10	15	6
Compound 37	8	6	6	6
+Ve. Control	24	23	11	14
-Ve. Control	6	6	6	6

5. CONCLUSION and RECOMMENDATIONS

In present study hydrazone derivatives of phenylhydrazine and 4-methoxybenzaldehyde and 4-nitrobenzaldehyde and their respective benzenesulfanohydrazones and Cu(II) complexes were successfully synthesized. The synthesized ligand characterized by FT-IR, ^1H NMR, ^{13}C NMR, and the complex were characterized by FT-IR and Elemental analyzer, The IR spectra of Schiff base and its metal complexes imply that the Schiff base ligand behaves as basic bidentate ligand coordination through the Azomethine nitrogen atom and nitrogen atom. The fifth and sixth coordination sites being occupied OAC. The elemental analysis result of the both Cu(II) complexes are in good agreement with the calculated. Aanti-bacterial activity of the synthesized compounds was examined. Of all synthesized compounds, Cu(II) complexes of compound 33 showed better activity against *S K.pneumoniae* and moderate activity against *E. coli* compared to positive control Ceftriaxone. Results of the antibacterial activity show that the Cu(II) complex of ligand having electron withdrawing substituents (nitro substituted) exerted excellent antibacterial activities which might be due to increase in lipophilicity of the compound.

These observations promote further research work with structure-activity relationships studies to have a better understanding of structural and chemical parameters required to develop potent compounds. Additional research efforts are also recommended to determine the concentration and target of the compounds and application of computational analysis like docking to have a deeper understanding of the molecular interaction.

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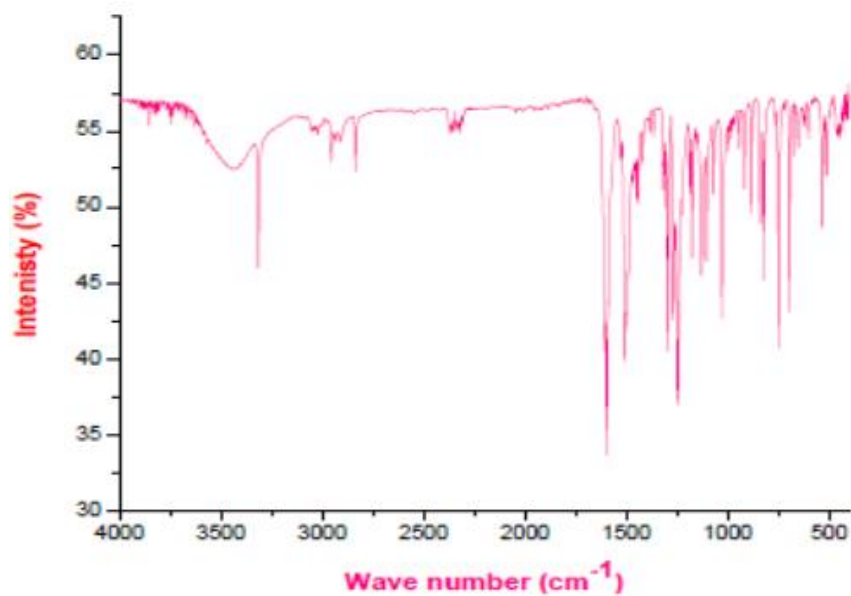
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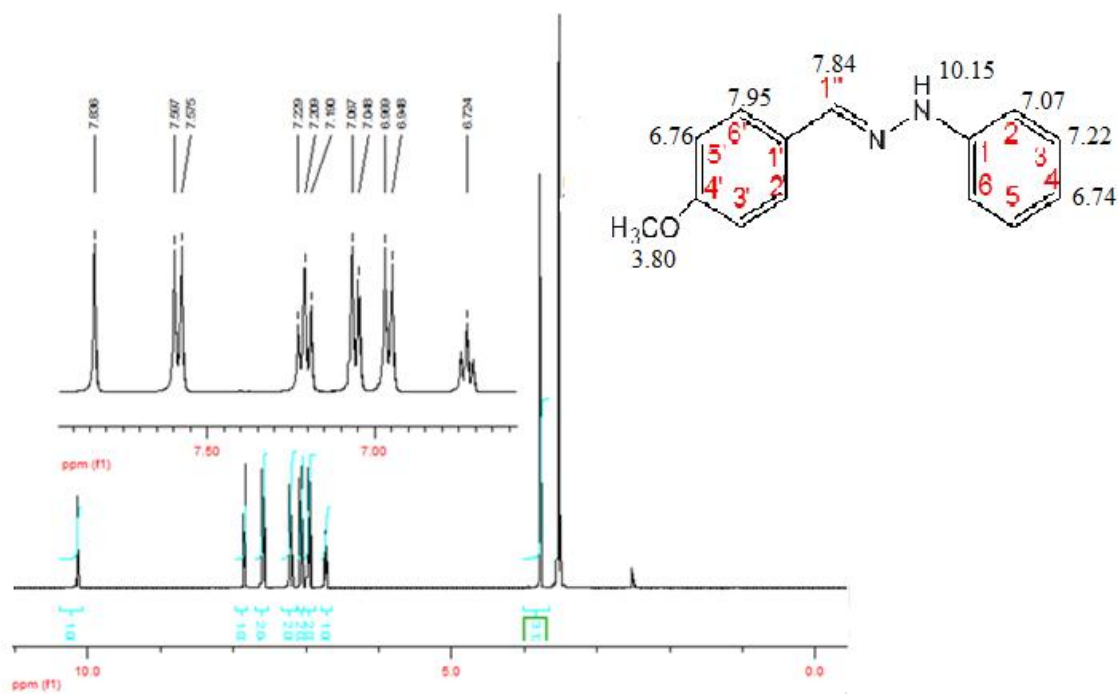
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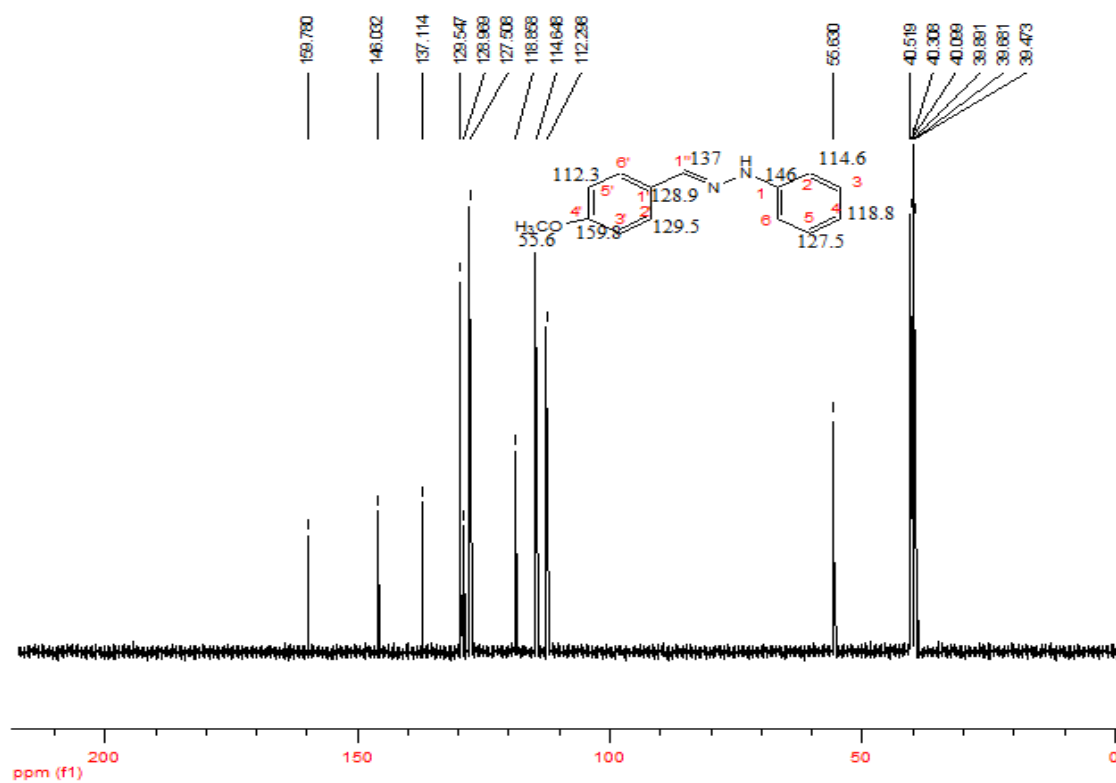
7. APPENDICES



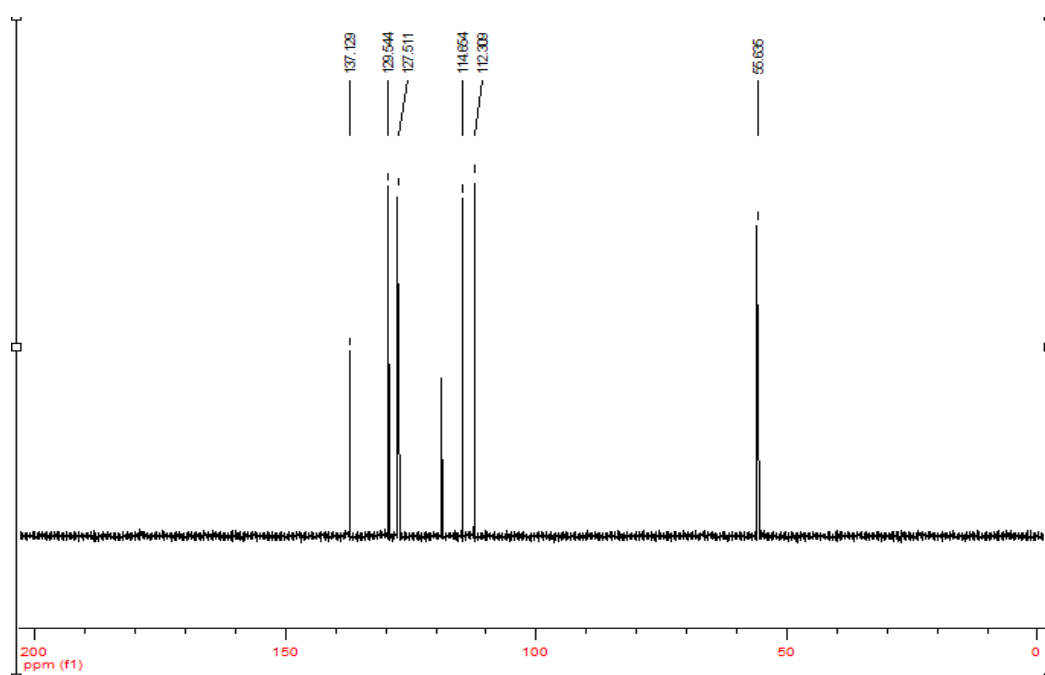
Appendix 1: IR spectrum of compound 33



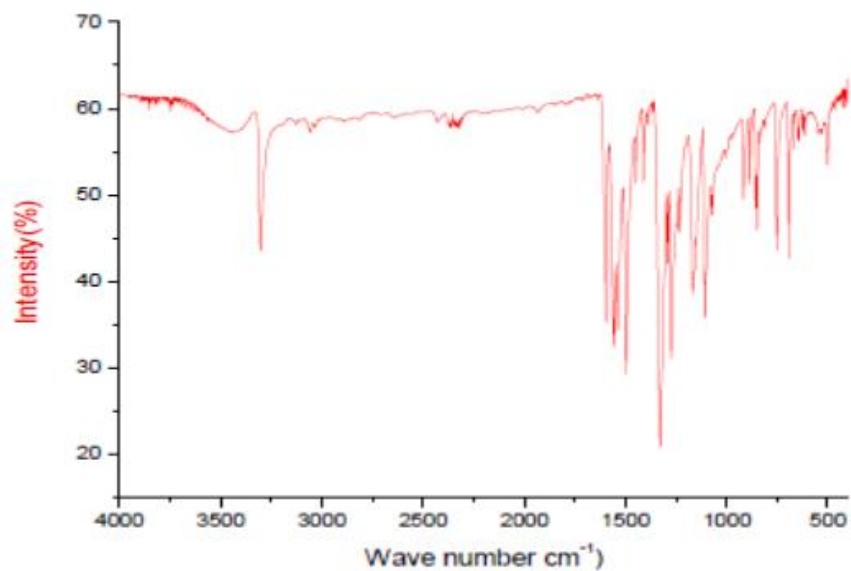
Appendix 2: ^1H NMR of spectrum compound 33



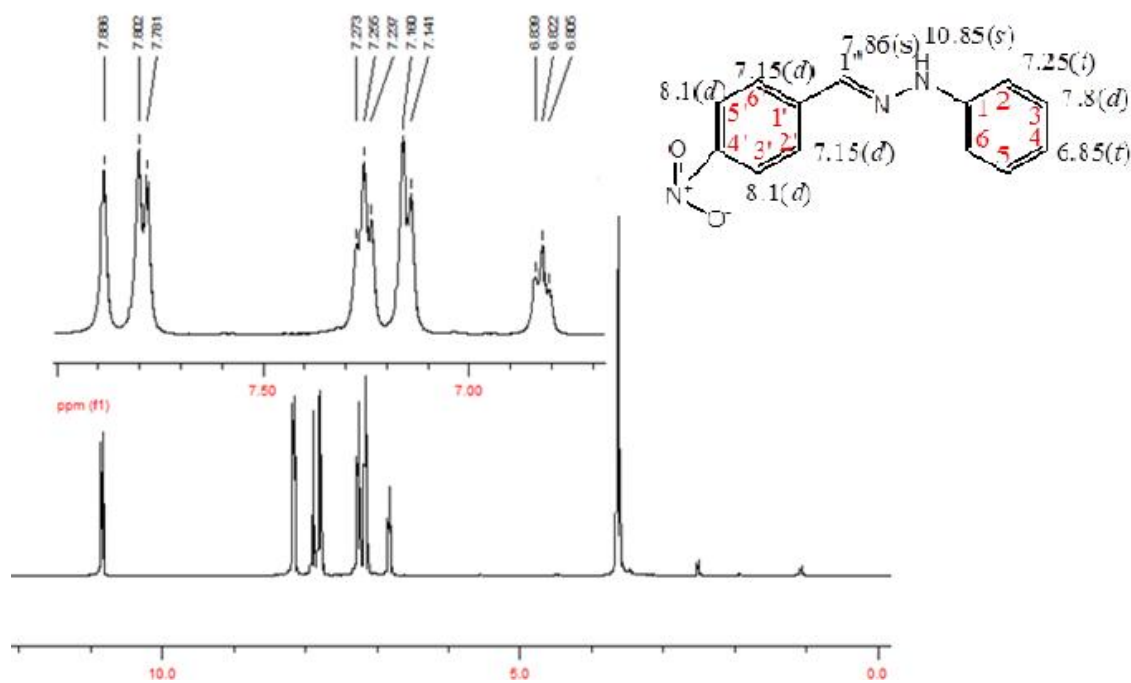
Appendix 3: ¹³C NMR spectrum of compound **33**



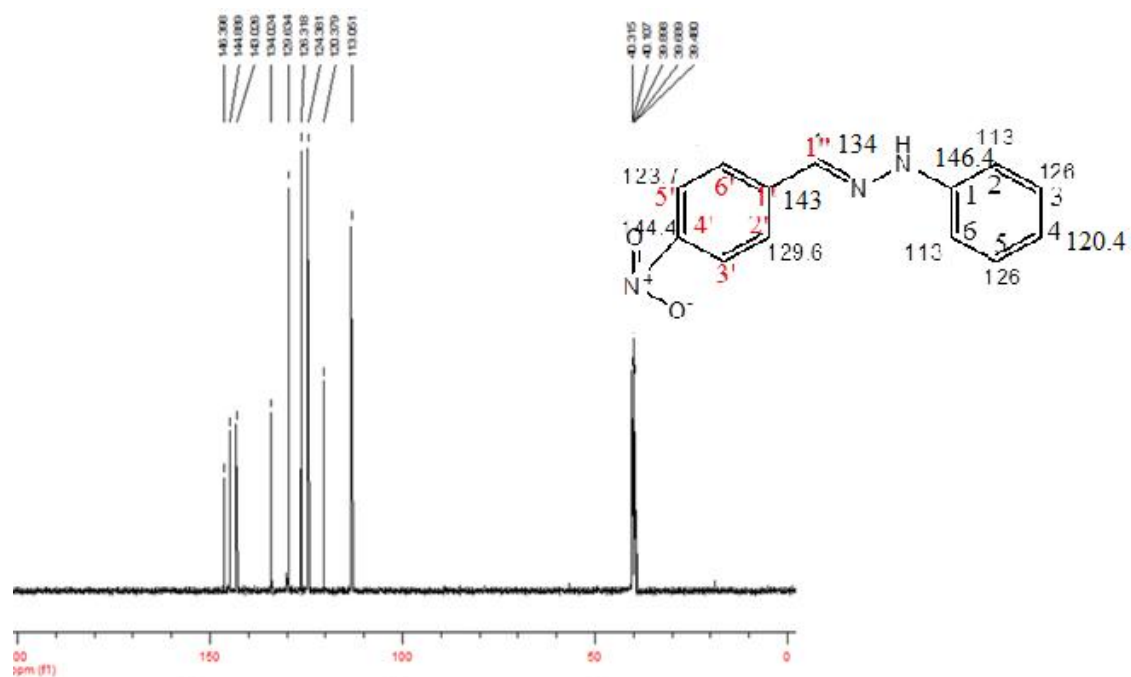
Appendix 4: DEPT-135 spectrum of compound **33**



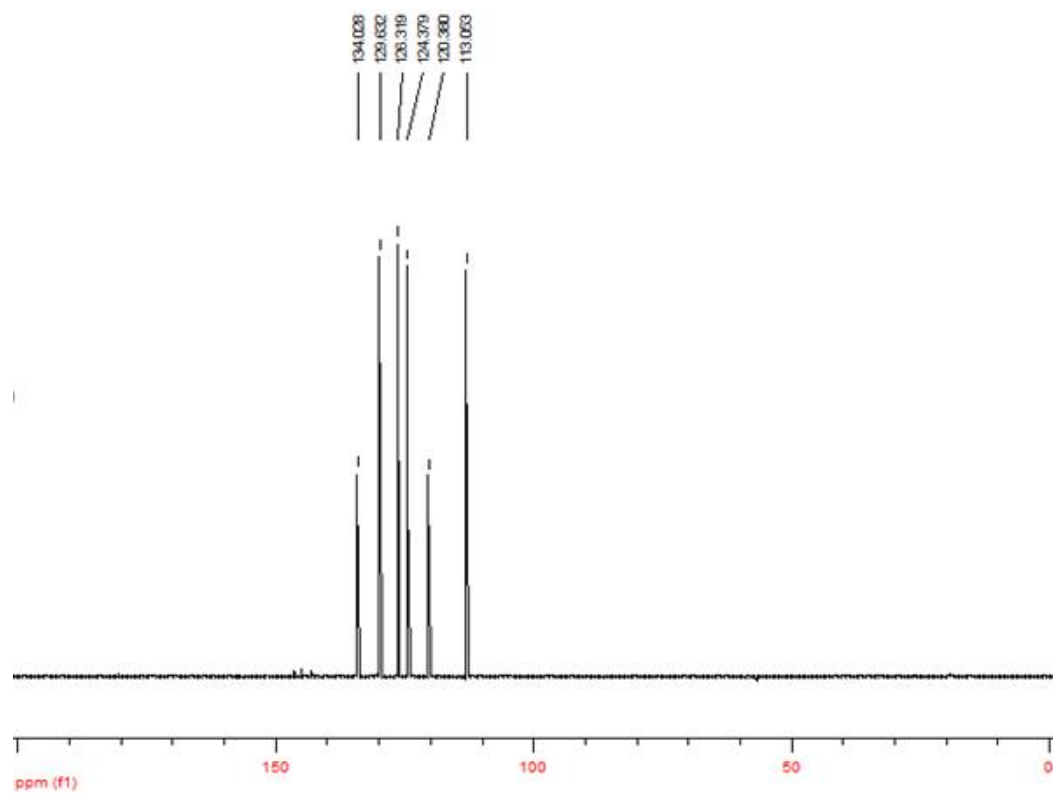
Appendix 5: IR spectrum of compound 34



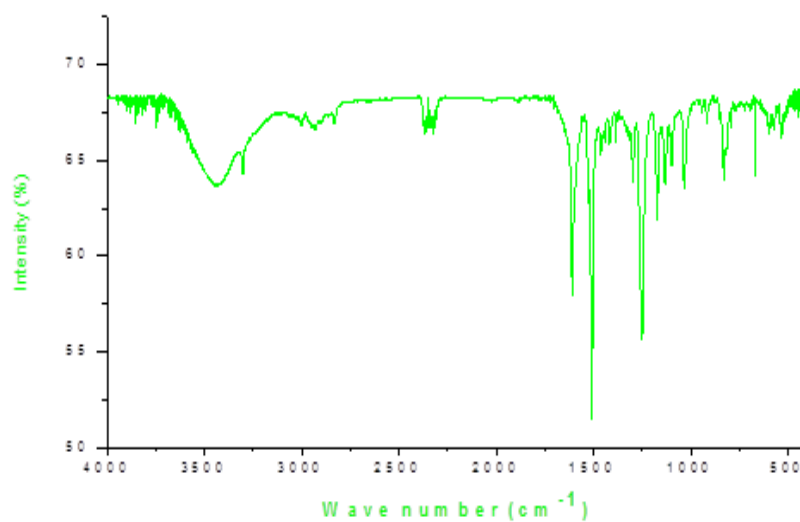
Appendix 6: ^1H NMR spectrum of compound 34



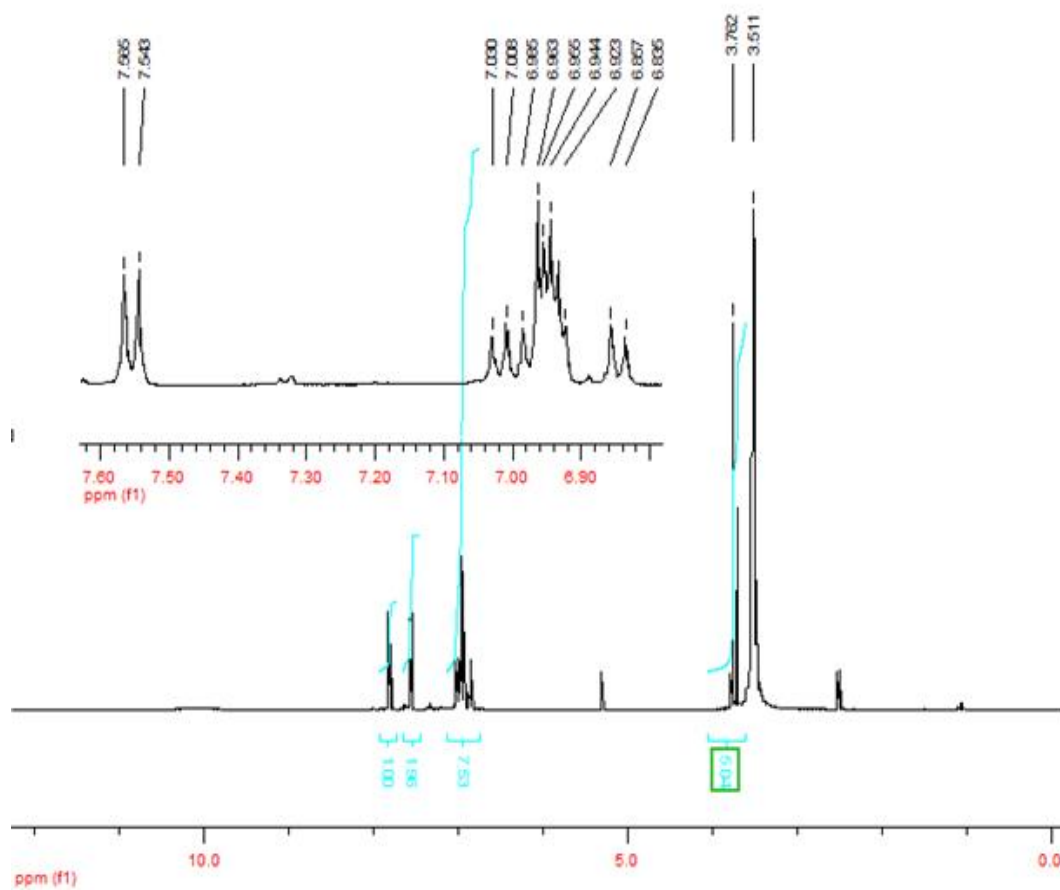
Appendix 7: ^{13}C NMR spectrum of compound **34**



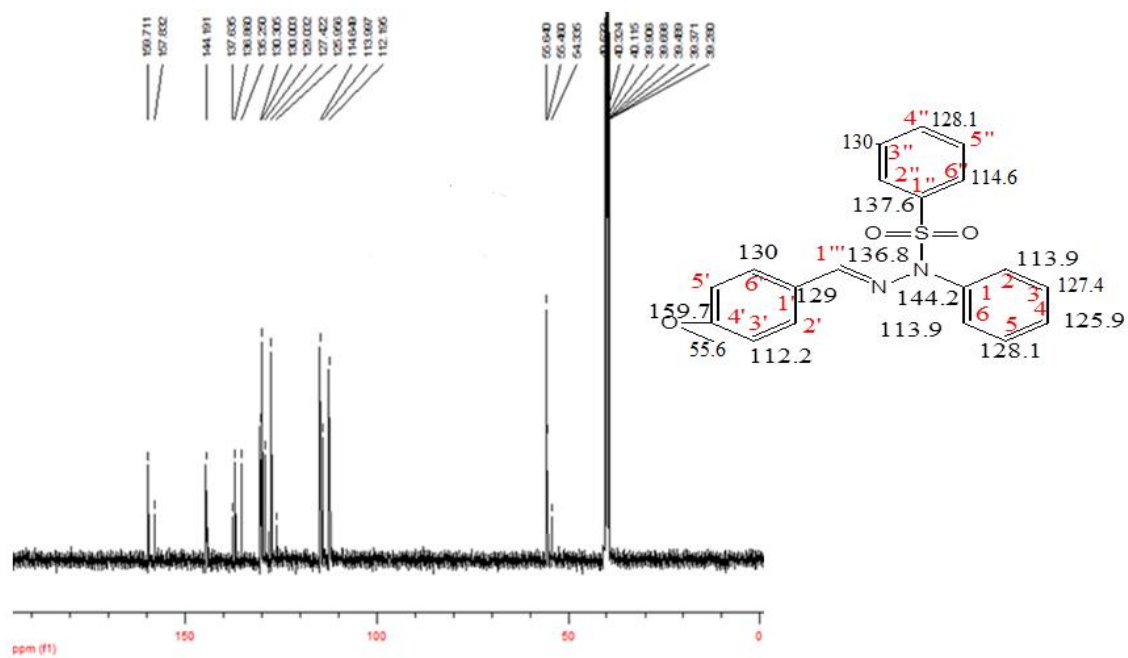
Appendix 8: DEPT-135 spectrum of compound **34**



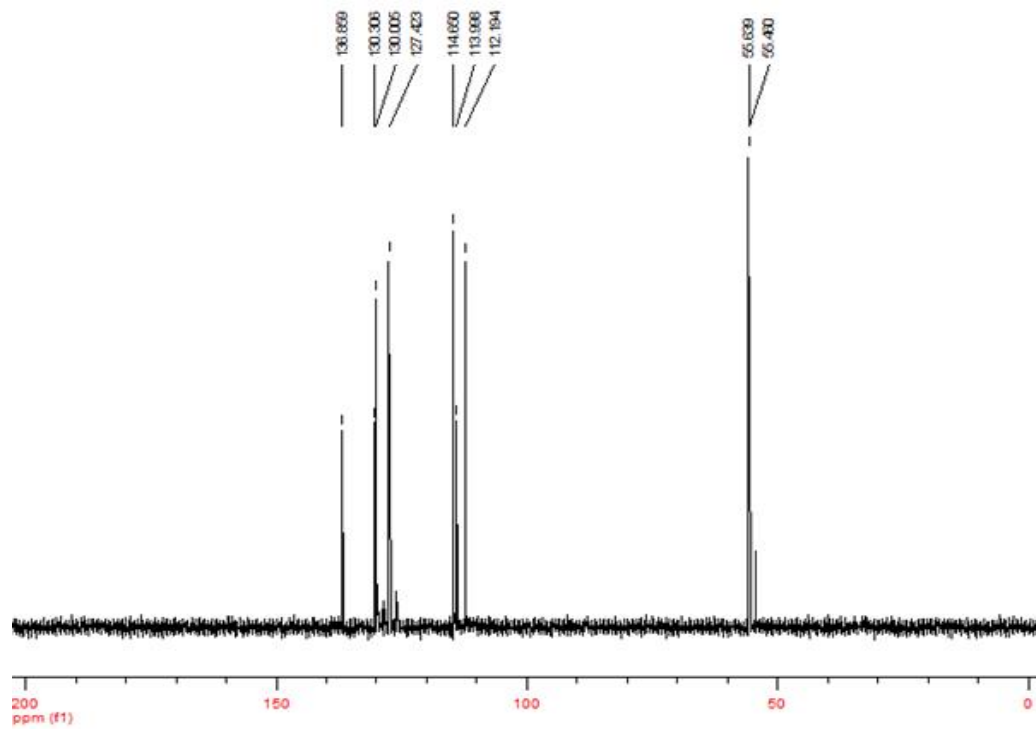
Appendix 9: IR spectrum of compound **35**



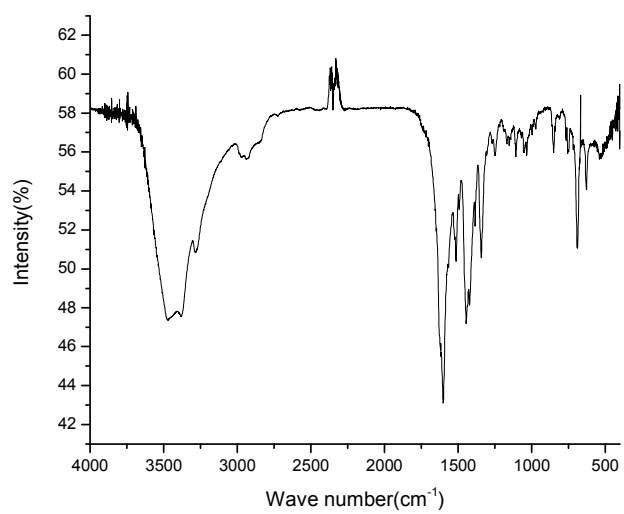
Appendix 10: ¹H NMR spectrum of compound **35**



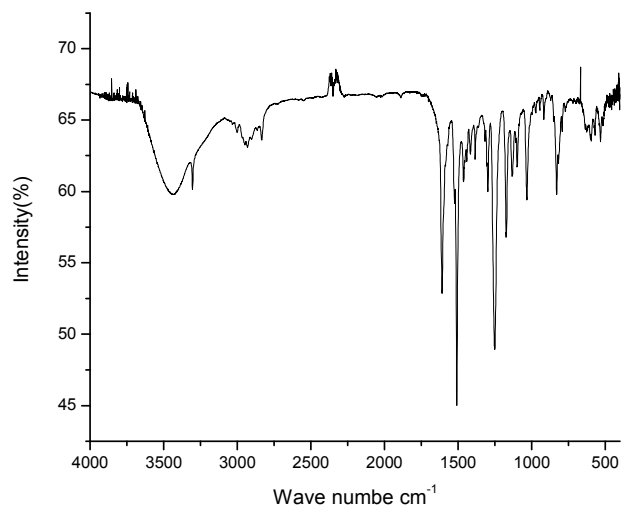
Appendix 11: ¹³C NMR spectrum of compound **35**



Appendix 12: DEPT-135 spectrum of compound **35**



Appendix 13: IR spectrum of compound **36**



Appendix 14: IR spectrum of compound **37**