SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SCHIFF BASES OF 4-AMINO-5-(2-CHLOROPHENYL)-*4H*-1,2,4-TRIAZOLE-3(*2H*)-THIONE

A DISSERTATION SUBMITTED FOR
THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
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BOARD EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled **"Synthesis, Characterization and Evaluation of Biological Activities of 4-Amino-5-(2-Chlorophenyl)-4*H*-1,2,4-Triazole-3(2*H*)-Thione Derivatives''** prepared by Govinda Pathak under the supervision of associate professor Dr. Bhushan Shakya, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, is hereby submitted for partial fulfillment of Master of Science(M.Sc.)Degree in Chemistry. This Dissertation has not been submitted in any other university or institution previously for the award of degree.

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LETTER OF RECOMMENDATION

This is to recommend that the dissertation work entitled **"Synthesis, Characterization and Biological Screening of Schiff Bases 4-Amino-5-(2-Chlorophenyl)-4*H*-1,2,4-Triazole-3(*2H*)-Thione Derivatives''** has been carried out by GOVINDA PATHAK as a partial fulfillment for the requirement of M.Sc. degree in chemistry under my supervision.

To the best of my knowledge, this work has not been submitted for any other degree in this institute.

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DECLARATION

I, Govinda Pathak, hereby declare that the work presented here is genuine work done originally by me and has not been published or submitted elsewhere for the requirement of degree program. Any literature, data or work done by others and cited in this dissertation has been given due acknowledgement and listed in the reference section.

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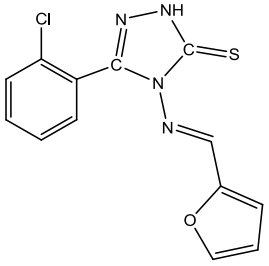
ABSTRACT

Life, like ours, is totally dependent on the heterocyclic compounds. It takes birth with purine / pyrimidine bases, nourishes on carbohydrates and in case of disease, is cured from medicines, many of which are heterocyclic in nature. The chemistry of 1,2,4 triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. The heterocyclic compound 1,2,4 triazole moiety has been incorporated into a wide variety of therapeutically interesting drug candidates including antibacterial, antifungal, analgesics and anti-inflammatory, antiviral, sedatives, anxiolytics, anti-convulsants, antimigraine, antihistaminic and other activities.

A series of intermediates were synthesized by reaction 2-chlorobenzohydrazide with carbon disulphide and potassium hydroxide followed by cyclization using hydrazine hydrate. The structure of new triazoles derivatives were confirmed by spectroscopic techniques like UV, IR, ^{1}H-NMR and ^{13}C-NMR. The synthesized compounds were screened for their antimicrobial activities and were found moderate activities against a variety of microorganism.



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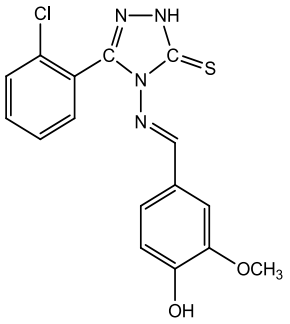

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Keywords:

1,2,4-triazole, Schiff's base, Thione derivatives, Synthesis, Application, Antimicrobial activities.

LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
br. s.	Broad singlet (NMR)
C. albicans	*Candida albicans*
cm	Centimeter
dd	Doublet of doublets(NMR)
DMSO	Dimethyl sulphoxide
FT-IR	Fourier Transformer Infrared
g	Gram
IR	Infrared
J	Spin-spin coupling constant (NMR)
m	Multiplet (NMR), Medium Intensity (IR)
M	Moles per litre
m. p.	Melting Point
mg	Milligram
MHA	Muller Hinton Agar
MHz	Mega Hertz (NMR)
NA	Nutrient agar
PC	Positive control
PDA	Potato Dextrose agar
s	Singlet (NMR), Strong intensity (IR)
S. aureus	*Staphylocous aureus*
S. typhi	*Salmonella typhi*
TMS	Tetramethylsiliane
w	Weak intensity (IR)
ZOI	Zone of inhibition
δ	Chemical Shift (NMR), bending vibration (IR)
μL	Microlitre

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CHAPTER 1

1. INTRODUCTION

Heterocyclic compounds are organic compounds containing at least one element other than carbon, such as sulphur, oxygen or nitrogen within a ring structure. Among these heterocyclic compounds *N*-heterocycles were found to have good biological activity. In the past few decades, the synthesis of these heterocyclic compounds has been a subject of great interest because of their wide applicability. Heterocyclic compounds are widely distributed in nature. By virtue of their therapeutic properties, they could be employed in the treatment of infectious diseases. Many heterocyclic compounds synthesized in laboratories have been successfully used as clinical agents.

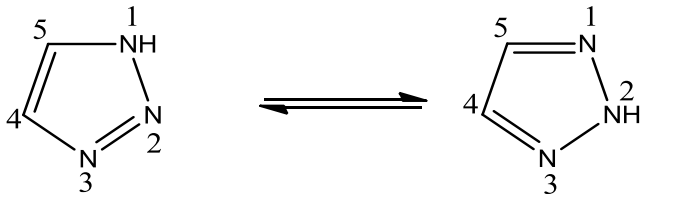
Today research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The success of imidazole as an important moiety of number of medicinal agents led to introduction of new heterocyclic compound the triazoles[1].Triazoles are heterocyclic organic compounds having a five membered ring molecular structure containing three nitrogen atom. Triazoles are two of types 1,2,4 triazoles and 1,2,3 triazoles. The basic heterocyclic rings present in the various medicinal agents are 1,2,3-triazole and 1,2,4-triazole. A large volume of research has been carried out on triazole and their derivatives, which has proved the pharmacological importance of this heterocyclic nucleus.

It has been found that 1,2,4 triazoles have more biological potent than 1,2,3 triazoles [2]. Triazole derivatives have drawn huge attention to chemists due to their broad diversity of activities, low toxicity and high-quality pharmacokinetic and pharmacodynamic outlines [3].

1.1 Chemistry of triazoles

1.1.1 Chemistry of 1, 2, 3 triazole

1,2,3-Triazoles have two tautomeric forms, 1*H*-1,2,3-triazole (fig.1.1a) and 2*H*-1,2,3-triazole (fig.1.1b).

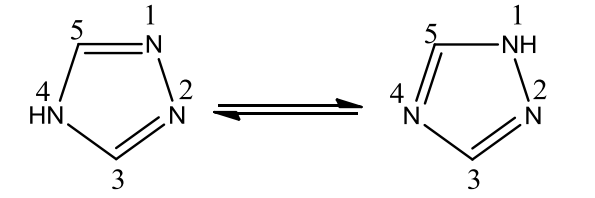


(a) **(b)**

Figure1.1: (a) 1*H*-1, 2,3-triazoles and (b) 2*H*-1, 2, 3-triazoles

1.1.2 Chemistry of 1, 2, 4 triazoles

1,2,4-Triazoles exhibit two tautomeric forms namely [4*H*]-1,2,4triazoles (fig1.2a) and [1*H*]-1,2,4-triazoles (fig.1.2b).



(a) **(b)**

Figure1.2: (a) 4*H*-1, 2,4-triazoles and (b) 1*H*-1, 2, 4-triazoles

The higher stability for 1-*H* isomer (fig.1.2b) indicated by x-rays studies, basicity measurements, dipole moment studies, NMR-spectra and theoretical methods

Triazole is a crystalline solid, white to pale yellow in colour, weakly basic compound, with characteristic odour. It is soluble in water and alcohol and have melting point (mp) 120 °C and boiling point (bp) at 260 °C.

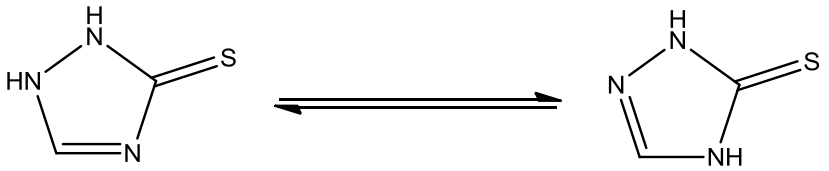
1.1.3 Amphoteric nature

1,2,4-Triazoles are amphoteric in nature, forming salts with acids as well as bases.

1.2 Schiff bases

Schiff bases are condensation products of primary amines with carbonyl compounds gaining importance day by day in present scenario. Schiff bases are the compounds

carrying imine or azomethine (–C=N–) functional group. The thione-thiol tautomerism and intermolecular double bond is due to proton transfer in reaction, probably due to influence of concentration, temperature, and irradiation with indirect sunlight. The thione 1,2,4 triazole have two toutomerism as show in (fig1.3)



1*H*-1,2,4-triazole-3(2*H*)-thione 1*H*-1,2,4-triazole-5(4*H*)-thione

Figure 1.3: (a) 1*H-1*,2,4-triazole-3(*2H*)-thione and (b) 1*H-1*,2,4-triazole-5(*4H*)-thione

CHAPTER 2

2. OBJECTIVES

1. To synthesize some derivatives of 1,2,4 triazole starting from *o*-chlorobenzoic acid.

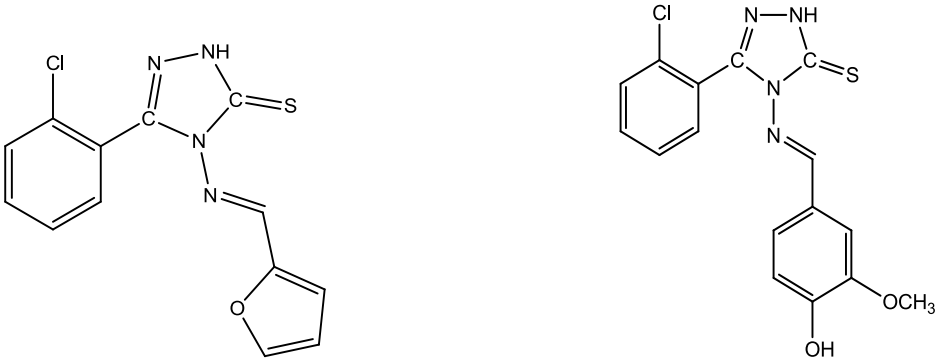


Figure 2.1: Structure of targeted molecules

2. To characterize the synthesized compound by using spectroscopic techniques.

3. To evaluate the antimicrobial activities of synthesized compounds.

CHAPTER 3

3. LITERATURE REVIEW

3.1 Synthesis of Triazoles

The early methods of preparation of 1,2,4-triazoles were simple and low yields were obtained but they made the nucleus available for study within a year of the original discovery by Baldin [1]. These have now been replaced by later modifications and by more efficient methods.

Kurzer *et al*. described the formation of 3-hydroxy-1,2,4- triazoline-5-thione (Fig.3.1) from 1, 4-diethoxycarbonylthiosemicarbazide under alkaline conditions [4].

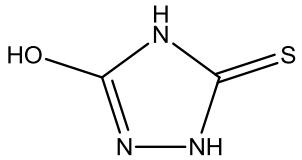


Figure 3.1: 3-hydroxy-1, 2, 4- triazoline-5-thione

Yeung *et al.* described an efficient one-step procedure, the synthesis of 3,5-disubstituted 1,2,4-triazoles by base catalyzed condensation of nitriles and hydrazide under microwave irradiation [5]. The formation of 1,2,4-triazole was first observed by Pinner [6] during the synthesis of amidrazones from an imidate and hydrazine. More ever, the synthesis of 1,2,4-triazoles by condensation between hydrazine's or mono substituted hydrazine and diacylamines in the presence of weak acid is known as the Einhorn–Brunner reaction [7].

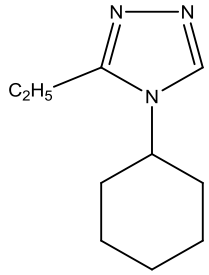
3.2 Biological Importance of Triazoles

Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as antibacterial [8,10], antifungal [11,12], antiviral [13], antimalarial [14,15], antituberculosis [16,17,18], anticonvulsant [19], anticancer [20], antioxidant [21], antidepressant [22], anti-inflammatory [23] etc.

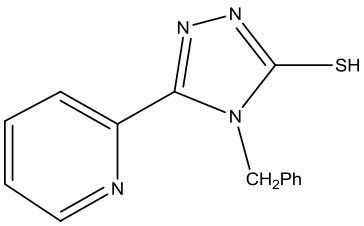
In recent years, the synthesis of these heterocyclic compounds has received considerable attention [24-29]. This wide range of applications has been covered by large number of paper in literature, many in the form of patents. There are various

known drugs in market containing the triazole moiety likes voriconazole, triazolam, fluconazole, itraconazole, furacyclin, alprazolam, etizolam etc.

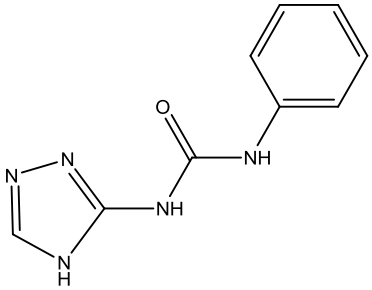
Some other important 1,2,4-triazoles along with their applications are as follows



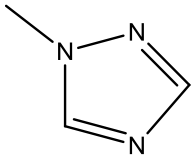
Anti convulsants [30-31]



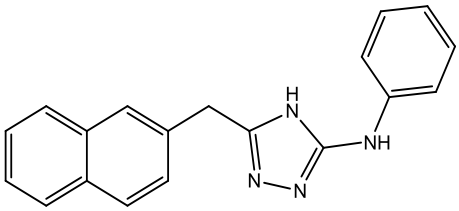
Dopamine-ß-hydroxylase[32]



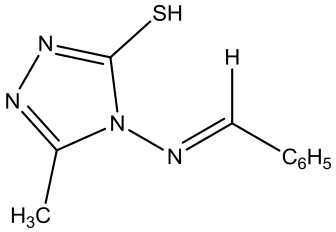
Antihypertensive activity [33]



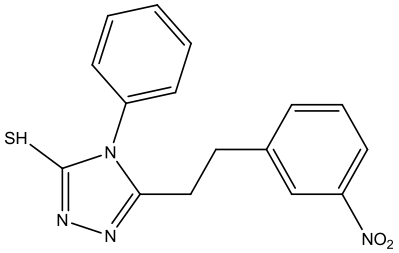
Insecticides [33]



Anti-inflammatory [34]



Anti-microbial [35]



Anti-amoebic [36]

Figure 3.2: Some medicinally important 1,2,4 triazoles derivatives

3.2.1 Antibacterial Activities:

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. Thus, by killing or reducing the metabolic activity of

bacteria, their pathogenic effect in the biological environments will be minimized. These agents interfere with the growth and reproduction of causative organisms like bacteria, fungi, parasites, virus etc. Since, discovery of antimicrobial agents have substantially reduced the threat posses by infectious diseases. Many drugs containing triazole nucleus are reported as antibacterial agent.

Kumar *et al.* reported the synthesis of 4-amino5-aryl-1,2,4-triazoles (Fig.3.3a) and screened for their antibacterial activity [37].

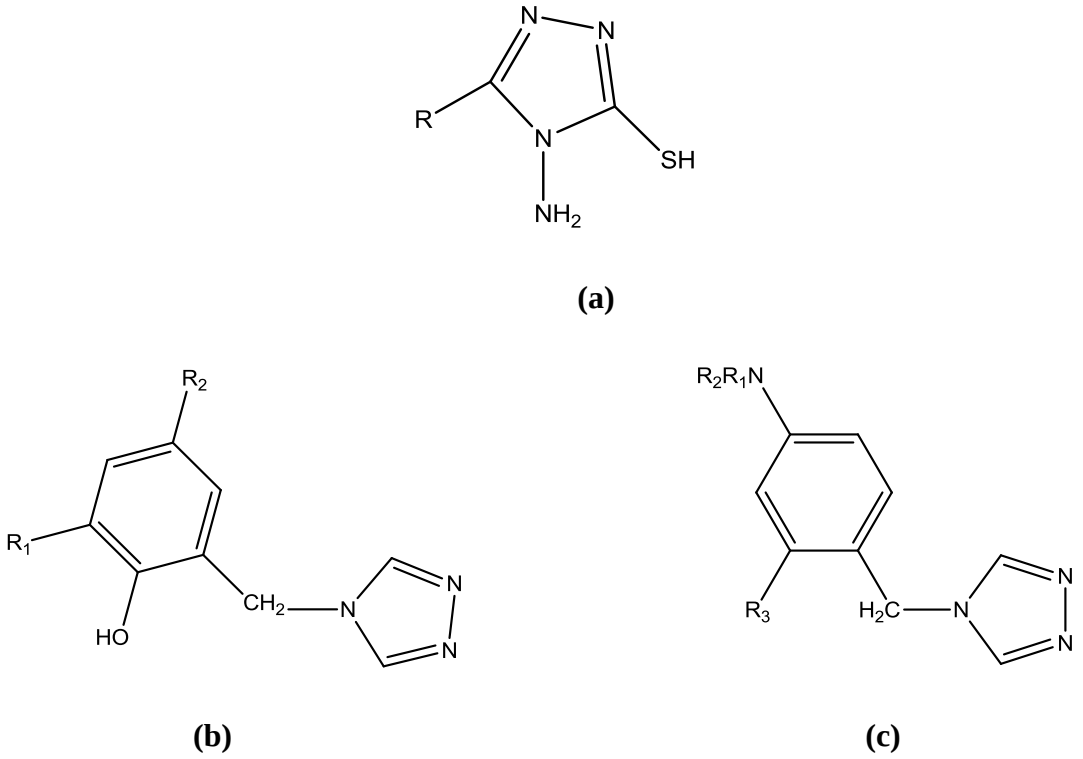


Figure 3.3: Some potent antimicrobial triazole derivatives

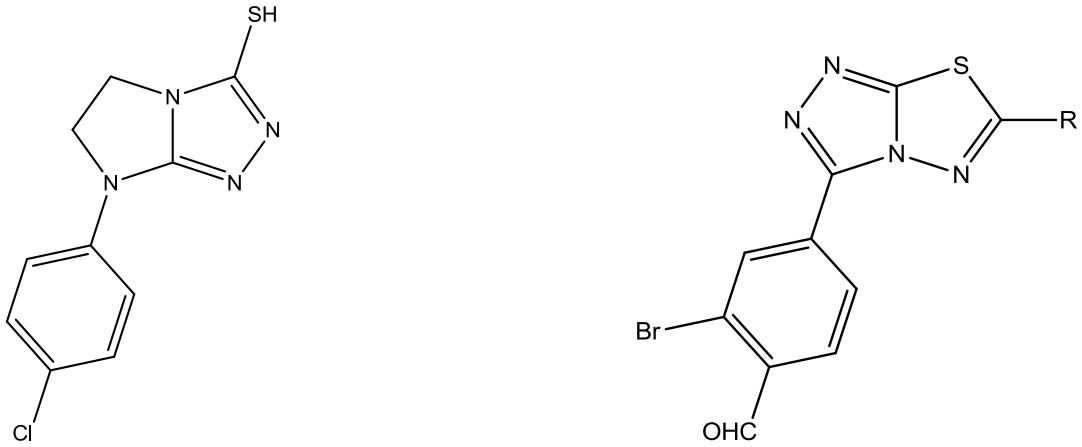
Patel *et al.* reported the synthesis and antibacterial activities of some substituted-[1,2,4]-triazolo[3,4-*b*][1,3,4]-thiadiazoles (Fig.3.3b and 3.3c) They observed that compounds carrying mercapto and hydroxy groups exhibited good antibacterial activity [38].

3.2.2 Antifungal activity

The class of drugs which has potent against fungal infection in human body with minimum toxicity is antifungal drugs. The antifungal agent selectively eliminates fungal pathogens from host cell. Some most common antifungal drugs are Itraconazole[39], Fluconazole[40], Voriconazole[41] and Posaconazole[42].

Siddiqui *et al*. reported the synthesis of a series of 3-(*p*-substituted aniline ethyl)-4-(*p*-substituted phenyl)-5-thioxo-1,2,4-triazoles and investigated as active antifungal agents [43].

Krzysztof Sztanke *et al*. synthesized series 3-(un)substituted-7-aryl-5H-6,7-dihydroimidazo[2,1c][1,2,4]triazoles compounds and its derivatives(Fig3.4a) which are screened for antimicrobial and antifungal activities. 7-(4-Chlorophenyl)-5H-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole-3-thiol showed the superior antifungal activity as compared to Miconazole [44].



(a) **(b)**

Figure3.4: Some antifungal triazoles nucleus derivatives

In search of new antifungal agents Holla *et al*. synthesized some triazolothiadiazoles (Fig.3.4b) These compounds showed excellent antifungal activity against *T. paradoxa*, a fungus that is reported to cause stem bleeding disease in coconut plants [45].

3.2.3 Analgesic Activities

Kumar *et al.* synthesized derivatives such as 5-[(Biphenyl-4-yloxy)methyl]-4fluorophenyl-3-mercapto-(4*H*)-1,2,4-triazole (Fig.3.5) and screened for the analgesic activity which show wide potency against allergy.[46]

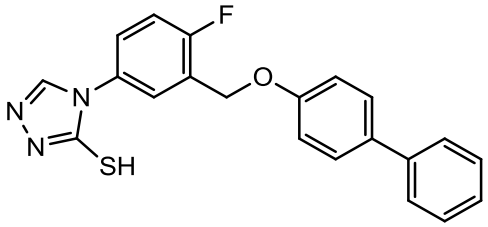


Figure 3.5: A drug having analgesic activity

3.2.4 Anti inflammatory Activities

The property of drugs used to treatment or reduces inflammation or swelling is called anti inflammatory drugs. Inflammation can be both good and bad. It helps our body defend itself from infection and injury. On the other hand, chronic inflammation can lead to weight gain and disease. So it is necessary to develop new drugs having effective anti-inflammatory activities with minimum toxicity. Inflammation reflects the response of organisms to various *stimuli* and is related to many disorders such as arthritis, asthma, and psoriasis, which require prolonged or repeated treatment.

The anti-inflammatory activities has been evaluated for [4-Amino-5disubsituted-4-*H*-1,2,4-triazole-3-yl)thiol] alkanoic acid derivatives synthesized by Sung *et al* [47] (Fig.3.6).

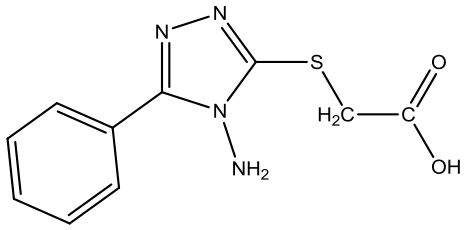
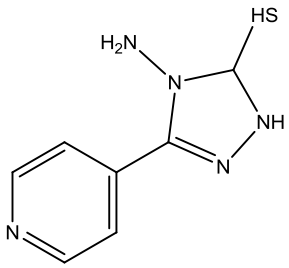


Figure3.6: A potent antitubercular triazole derivatives

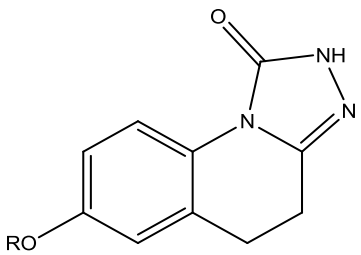
3.2.5 Anticonvulsant Activities

Anticonvulsants are the group of drugs used to prevent or reduce the severity of epileptic fits or other convulsions. There is a continuing demand for new anticonvulsant agents as it has not been possible to control every kind of seizure with the currently available antiepileptic drugs. Many triazole derivatives are reported to possess pronounced anticonvulsant activities.

Jin *et al*. reported the synthesis of 7-alkoxy-4,5dihydro[1,2,4]-triazolo [4,3-a]quinoline1(2*H*)-ones (Fig.3.7) and investigated for anticonvulsant activity and neurotoxicity[48].



(a)



(b)

Figure3.7: Some reported anticonvulsant drug with triazole nucleus

Pandeya *et al.* synthesized various Schiff bases such as N-[4-(4′-chlorophenyl-thiazol-2-yl] semicarbazides and 3-(4′-pyridyl)-4-amino-5-mercapto-4(*H*)-1,2,4-triazoles (Fig.3.7b). The compounds were evaluated for anticonvulsant and neurotoxic properties [49].

3.2.6 Anticancer Activities

Cancer is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body *via* lymph or blood).The class of any drugs that is effective in the treatment of cancerous disease is called anticancer drugs or agents. These drugs inhibit abnormal growth of drugs. Several classes of drugs may be used in cancer treatment, depending on the nature of organ involved.

Baviskar *et al*. synthesized clubbed triazolyl indeno [1, 2*c*]isoquinolines (Fig.3.8a and 3.8b) as anticancer agent [50]. It show potent against many carcinogenic agent.

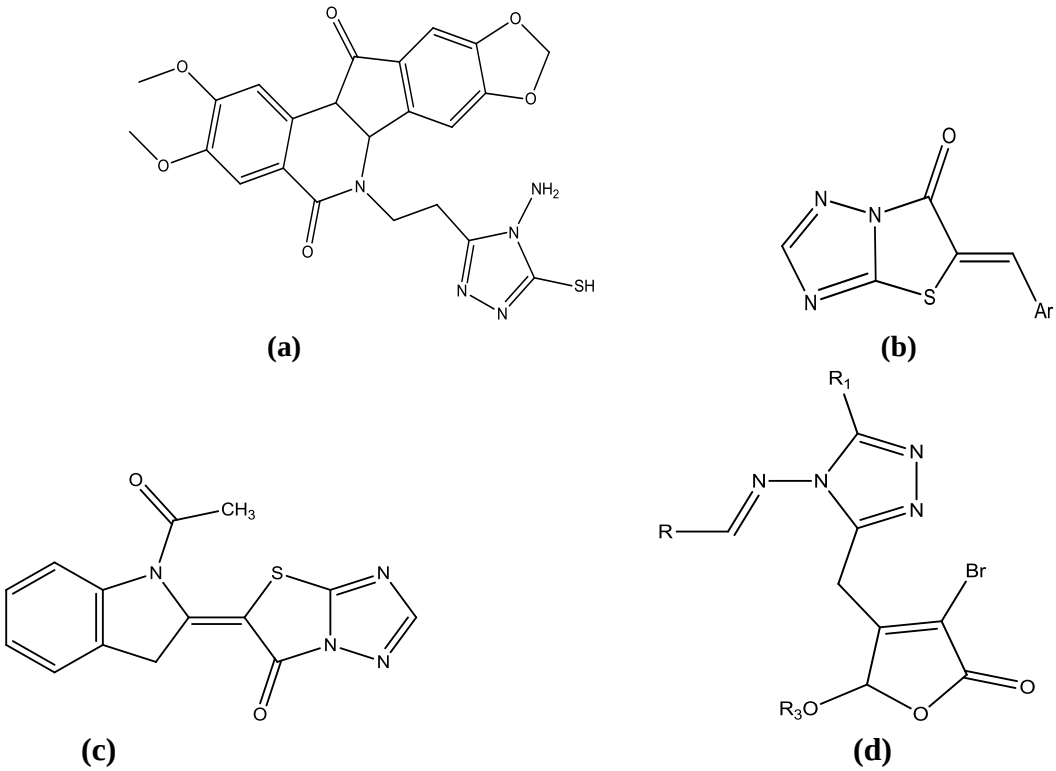


Figure3.8: Some potent anticancer drugs

Synthesis and anticancer activity evaluation on the panel of human cell lines of some new(5-substituted-thiazolo-[3,2-*b*]-[1,2,4]-triazol-6-ones)(Fig.3.8c)have,been described by Roman *et. al* [51].

Li *et al* reported the synthesis of a new series of hybrid 1,2,4triazole Schiff bases bearing γ-substituted butenolide (Fig3.8d).The synthesized compounds were evaluated for their in vitro anticancer activities against cervical cancer cell lines [52].

3.2.7 Anti tubercular Activities

Chemical substances obtained from various microorganism species that can alone or in combination with other agent, can be use for treating various form of tuberculosis are called Anti tubercular drugs. Though it is quite difficult to develop new anti tubercular drugs in laboratories some of scientist synthesized drugs having triazole skeleton.
The antitubercular activities has been also evaluated by Kumar *et al* for 2-substituted-5[isopropylthiazole] clubbed 1,2,4-triazole (Fig.3.9)[53].

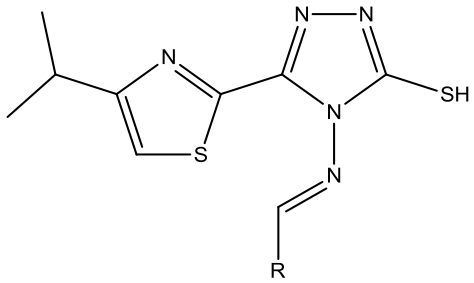


Figure3.9: Antitubercular triazole derivatives

3.2.8 Antioxidant Activity

Antioxidants are those substances that can prevent or slow damage to cells caused by free radicals. The unstable molecules may cause several side effects which can cause several health problems. Antioxidants are of two types. If body itself can produce some antioxidant then it is called Endogenous and if it come from out sides called exogenous antioxidant. Antioxidant can protect against cell damage from free radicals. Some of triazole derivatives are use as antioxidant.
Suresh *et al.* reported the synthesis of 8-chloro-1,4-substituted (1,2,4[4,3- *a*] quinoxalines) (Fig.3.10) and evaluated for antioxidant and antimicrobial activity [54].

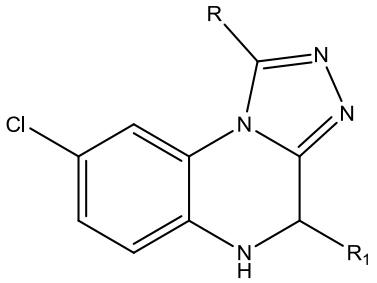


Figure 3.10: 1, 2, 4-triazole derivatives with effective antioxidant property

3.2.9 Antihypertensive Activities

Hypertension, also referred to as high blood pressure is a medical condition in which blood pressure is chronically elevated. Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm and is a leading cause of chronic renal failure.

Akbarzadeh *et al*. synthesised a series of new 5-substituted analogues of 4*H*-3-(2-phenoxy) phenyl-1,2,4-triazole and its chlorinated derivatives(Fig.3.11)[55].

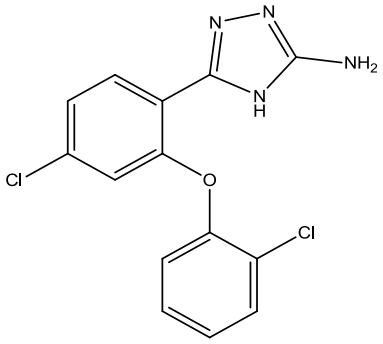
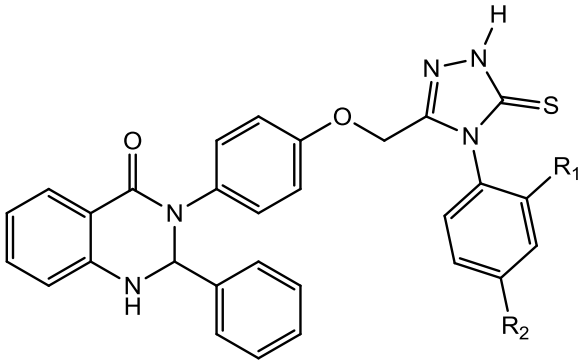


Figure3.11: A potent antihypertensive drug belonging to triazole family

3.3.0 Antimalarial Activities

Malaria is mosquito bite born disease that affects humans and other animal. It is caused by biting of female anopheles mosquito. Due to lack of sufficient anti malarial drugs many people in developing country lose their life day by day because of dangerous infectious disease malaria. So drugs having potent anti malarial activities have been explored [56].

Havaldar *et al*. synthesized a series of derivatives such as 3-[4-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-[1,2,4]-triazol-3-ylmethoxy)-phenyl]-2-phenyl-3*H*-quinazolin-4-ones (Fig:3.3.10) and screened for anti-malarial activity.[57]



Compounds	R1	R2
13a	H	H
13b	H	F
13c	H	NO_2
13d	F	F

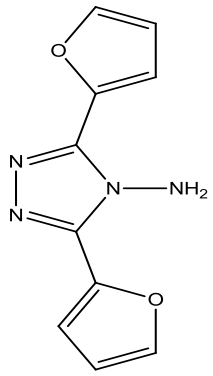
Figure 3.12: Some reported antimalarial drugs with triazole nucleus

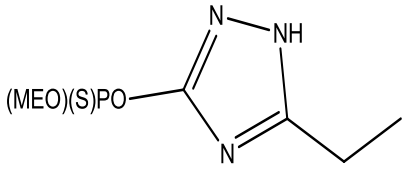
3.3 Other Application of triazoles

3.3.1 Agricultural Application

Azoles derivatives are widely used in plant protection such as pesticides (fig3.13a). It has been proved that triazoles derivatives containing fertilizer high crop tolerate and low level toxicity to mammals.

Some triazole derivatives (Fig3.13b) are used as herbicides to control the growth of weeds has been developed [58].





(a) **(b)**

Herbicidal Pesticidal

Figure 3.13: Some potent herbicidal and Pesticidal triazoles

3.3.2 Industrial application

3.3.2.1 Textile industry

In textile industry triazoles derivatives have many applications for e.g, sodium salt of a sulphonated triazole derivative possesses good detergent action and *N*-benzylated aminotriazoles (Fig3.14)have useful properties in inhibiting the acid fading of dyestuff [59].

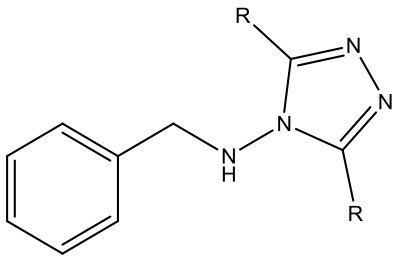


Figure 3.14: A industrially important drugs

3.3.2.2 Cotton industry

In the cotton industry, 3-amino-1,2,4-triazole under its trade name Amizol, has been used as a commercial defoliant for a number of years [60].

3.3.2.3 Chemical industry Some selected triazole has been used as light emitting diodes (Electroluminescent devices) [61-62]. Some triazole systems have extensive use in the separation of silver from other metal cations in liquid membrane systems [63]. These compounds have also been reported as inhibitors of corrosion of copper, brass, aluminum and steel in marine environment [64] and inhibit fog formation in photographic emulsions [65], plant growth inhibitors [66] and herbicides [67].

CHAPTER 4

4. MATERIALS AND METHOD

4.1 Materials

Orthochorobenzoic acid was purchased from local dealer of chemicals and equipments. Other chemicals like hydrazine hydrate, ethanol, carbon disulphide, methanol, potassium hydroxide, vanillin and cinnamaldehyde were purchased from local market of various companies.

4.2 Method

The melting point of synthesized compounds was determined and then characterized by spectroscopic techniques *viz*. UV-Visible, IR and NMR.

4.2.1 Melting point determination

Melting point of the compounds was determined by using optics technology melting point apparatus and is uncorrected.

4.2.1 Ultraviolet Spectroscopy (UV)

UV spectroscopy of these compounds was measured at range of 350nm to 750nm in double beam Spectrophotometer from Labtronics (Model LT-2002) using DMSO solvent, at Amrit Science Campus Department of Chemistry, Tribhuvan University Kathmandu.

4.2.1 Infrared Spectroscopy (IR)

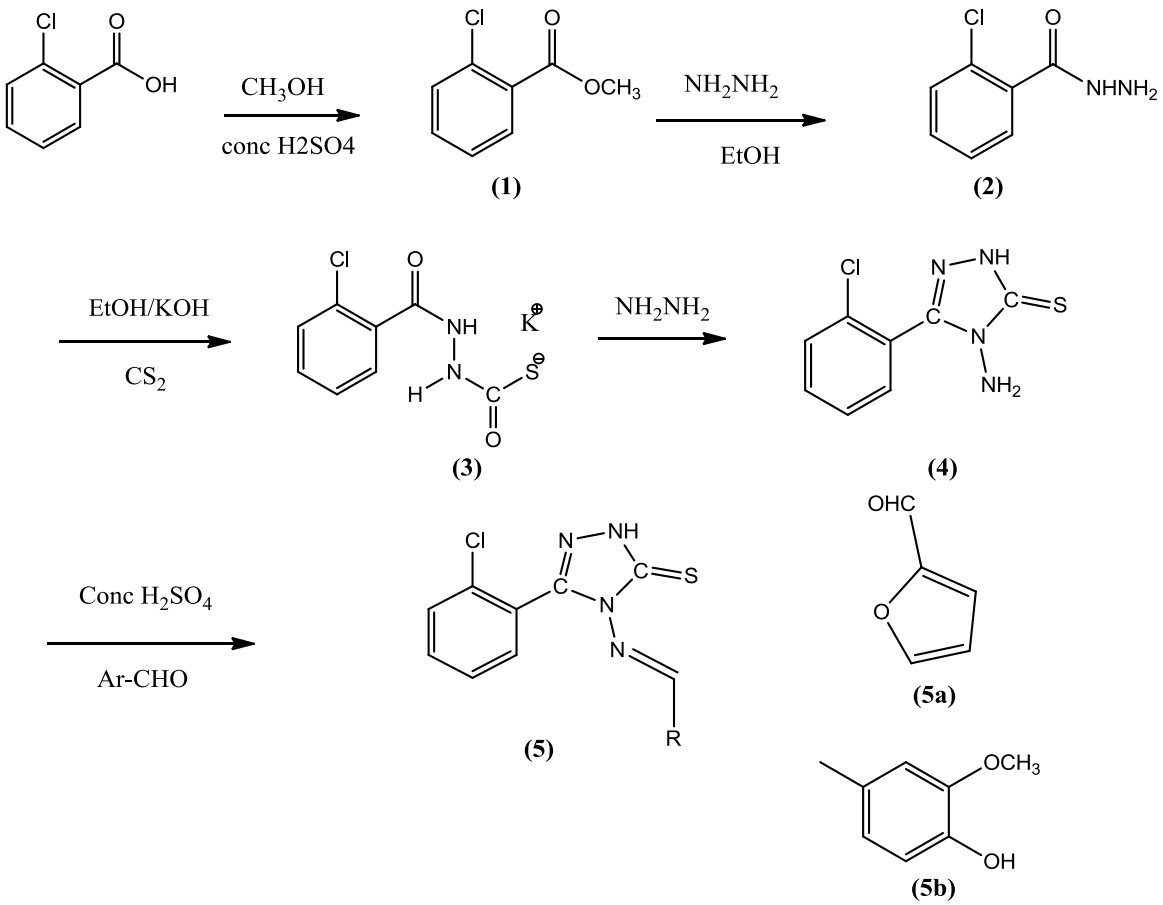
Infrared spectra of the compound were measured in the range of 4000-400cm^{-1} on Spectrum GX Fourier transform infrared (FT-IR) spectroscopy instrument (Perkin Elmer, USA) at Chonbuk National University, South Korea.

4.2.2 Nuclear Magnetic Resonance (NMR)

^{1}H-NMR and ^{13}C-NMR spectroscopy was used for analysis of synthesized Schiff's bases.NMR spectra were recorded at room temperature on Varian VNMRS 400MHZ NMR spectrometer at Sogang University, South Korea using TMS as an internal standard. The signal are described as singlet (s), doublet (d), triplet (t), doublet of doublet (dd) and multiplet (m).

4.2.3 Synthesis of Schiff Bases of 1,2,4-Triazoles Derivatives

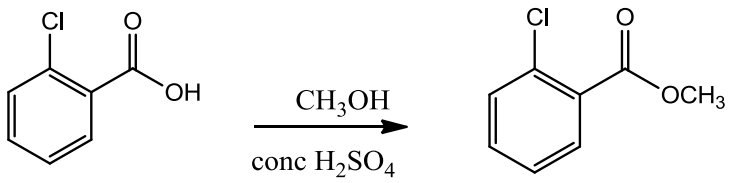
The reaction involved for the synthesis of desired product is shown in scheme 3.1



Scheme 4.1 General scheme for synthesis of Schiff bases of triazoles thione

4.3.1 Method for synthesis of methyl ester (1):

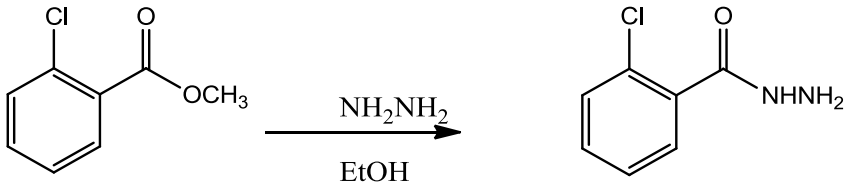
To a solution of orthochorobenzoic acid (15.45 g, 0.1mol) in anhydrous methanol (22 mL), 1mL *conc.* sulphuric acid was added slowly at ice cold condition and the reaction mixture was allowed to reflux with constant stirring for 5 h. The excess solvent was evaporated on water bath till the total volume of the solution was reduced to half. Then the reaction mixture was cooled and content was transferred into a separation funnel containing 50 mL of distilled water. The synthesized ester was extracted several times with 5 mL of distilled water. The synthesized ester was organic layer was washed with 20% (w/v) solution of sodium bicarbonate to remove any unreacted acid. Then, it was dried over anhydrous magnesium sulphate and filtered through filter paper directly into receiver. The yield of synthesized ester was recorded.



Yield (76%), (12.95 g 0.076 mol), Colorless liquid.

4.3.2 Method for synthesis of acid hydrazide (2):

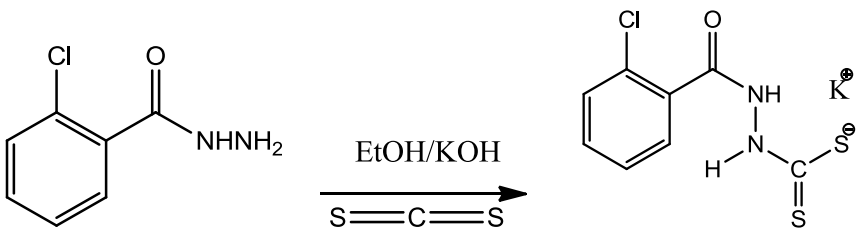
To the solution of methyl ester of orthochorobenzoic acid (5.11 g, 0.03 mol) in 60 mL absolute ethanol, 2.25 g (0.045 mol) of 99.9% hydrazine hydrate was added in small portions at a time with constant stirring. The reaction mixture was refluxed for 3-6 h. Excess solvent was evaporated on water bath (till the total volume to the solution was reduced to half) and was cooled, whereby crystalline solid separated out. The solid was filtered, washed with cold and recrystallized with cold ethanol. Then the yield and melting point of hydrazide were recorded.



Yield (72%), (3.672 g, 0.0216 mol)

4.3.3 Method for synthesis potassium 2-(2-chorobenzoyl) Dithiocarbathioate (3):

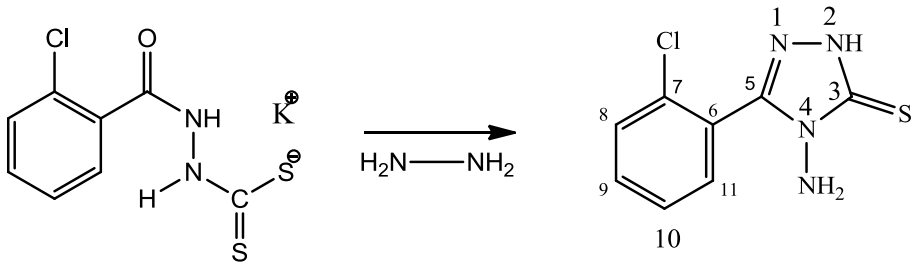
0.84 g (0.015 mol) potassium hydroxide was dissolve (in absolute ethanol (20 mL), 1.70 g (0.01 mol) of acid hydrazide was added at ice cold condition. Then in this mixture, (1.14 g) carbon disulphide was added drop wise with constant stirring at room temperature (should not exceed 30°C). The reaction mixture was stirred overnight (16 h) at room temperature in a magnetic stirrer. 20 mL of anhydrous diethyl ether was added to the reaction in mixture. The solid product of potassium dithiocarbazinate was by filtered. Then, obtained precipitate washed several times with anhydrous diethyl ether (5 mL).



Yield, (58%), (2.85 g, 0.0116 mol)

4.3.3 Method for synthesis of 4-amino-3-(2-chlorophenyl)-4*H*-1, 2, 4-Triazole-3(*2H*)-Thione (4):

A suspension of potassium dithiocarbazinate (2.46 g, 0.01 mol) in water (10 mL) and hydrazine hydrate (1 g, 0.02 mol)was refluxed for 3-5 hr in magnetic stirrer till the colour of the reaction mixture chanced to green and evolution of hydrogen sulphide is ceased. The reaction mixture was cooled to room temperature and diluted with cold water(100 mL) containing some crushed ice. On acidification with concentrated hydrochloric acid, the corresponding triazole was precipitated which was filtered, washed with cold water and recrystallized with ethanol. The yield and melting point of synthesized Thione were recorded.



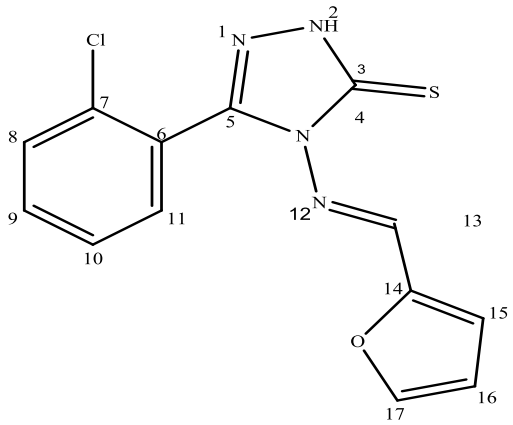
Yield (68%), (1.35g, 0.006 mol); m.p. 176 °C, IR ν_{max}(selected bands)cm^{-1} 3287 (s), 3066(m), 1566(w), 1608(s), 1464(s), 1281(m), 1033(m), 767(s), 723(s)^{1}H NMR (400MHz, DMSO-d_6) δ = 7.918 (1H, dd, *J* = 8.0, 1.2 Hz, H-8), 7.714 (1H, d, *J* = 8.0 Hz, H-11), 7.647 (1H, td, *J* = 7.2, 1.6 Hz, H-9), 7.560 (1H, t, *J* = 7.6 Hz, H-10). ^{13}C NMR (100MHz, DMSO-d_6) δ = 179.1 (C-3), 149.4 (C-5), 132.4 (C-11), 131.9 (C-7), 130.4 (C-8), 130.2 (C-9), 125.2 (C-10), 123.6 (C-6).

4.3.4 Method for synthesis Schiff base

4.3.4.1 Synthesis of 5-(2-chlorophenyl)-4-(furan-2-ylmethyleneamino)-4*H*-1,2,4-triazole-3(2*H*)-thione (5a):

To a solution of triazole (0.45g 002 mol) in anhydrous ethanol (5 mL), furfuraldehyde (1.92 g, 0.02 mol), was added a small amount at a time with constant stirring. To this concentrated sulphuric acid (5 drops) was added and the reaction mixture was refluxed

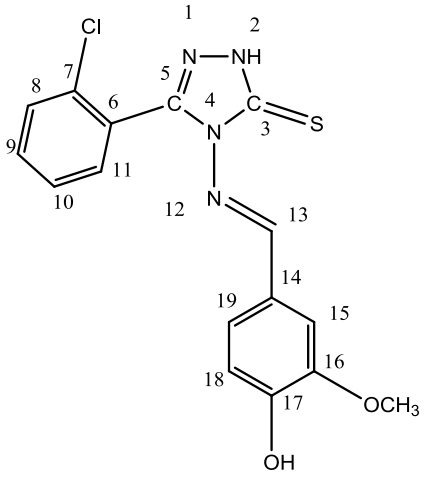
for 5 hours. On cooling the reaction mixture at a room temperature, 5-(2-chlorophenyl)-4-((furan-2-ylmethylene) amino) 4*H*-1,2,4-triazole-3(2*H*)-thione.was precipitated. The precipitated solid was filtered under suction , washed with cold and recrystallized with ethanol. The yield and melting point were recorded.



Yield (65%), (3.95g, 0.013 mol) mp.196 °C, IR spectrum(cm^{-1}): 3095 (s), 1615 (s), 1573 (m), 1495 (s), 1476 (s), 1290 (s), 1045 (m), 840 (m), 760 (s) ^{1}H NMR (400MHz, DMSO-d_6) δ = 9.689 (1H, s, H-13), 7.972 (1H, br. s, H-8), 7.677 - 7.59 (4H, m, H-17, H-11, H-9 and HN-), 7.156 (1H, td, *J* = 7.6, 2.0 Hz, H-10), 7.305 (1H, d., H-15), 6.728 (1H, dd. *J* = 3.2, 1.6 Hz, H-16). ^{13}C NMR (100MHz, DMSO-d_6) δ = 179.9 (C-3), 149.0 (C-14), 148.3 (C-5), 145.3 (C-17), 134.2 (C-13), 132.6 (C-11), 132.3 (C-7), 131.9 (C-9), 130.1 (C-8), 128.0 (C-10), 122.6 (C-6), 117.4 (C-15), 111.5 (C-16).

4.3.4.2 Method for synthesis of 5-(2-chlorophenyl)-4-((4-hydroxy-3-methoxybenzylidene) amino)-4*H*-1, 2, 4-triazole-3(2*H*)-thione (5b):

To a solution of triazole (0.45 g, 0.002 mol) in anhydrous ethanol, vanillin 0.304 g, 0.002 mol) was added a small amount at once with constant stirring. Then concentrated sulphuric acid, (5 drops) was added and the reaction mixture was refluxed for 5hr.On Cooling the reaction mixture at room temperature, 5-(2-chlorophenyl)-4-((4-hydroxy-3-methoxybenzylidene)amino)-4*H*-1,2,4-triazole-3(2*H*)-thione was precipitated. The precipitated solid was filtered under suction, washed with cold ethanol. The yield and melting point were recorded.



(Yield (60%), (0.432 g, 0.012 mol) mp.187 °C IR (cm^{-1}) 3363 (m), 3062 (m), 1666 (m), 1597 (s), 1512 (s), 1463 (s), 1265(s), 1296 (s), 1261 (s), 1261 (s), 1026 (s), 817 (m), 717 (m) ^{1}H NMR (400MHz, DMSO-d_6) δ = 10.145 (1H, br. s., HO-), 9.731 (1H, br. s., HN-), 7.828 (1H, dd, *J* = 8.4, 2.0 Hz, H-8), 7.786 (1H, d, *J* = 8.4, Hz, H-15),7.746 (1H, dd, *J* = 8.8, 2.0 Hz, H-11), 7.626 (1H, m, H-9), 7.530 (1H, m, H-10), 7.438 (1H, dd, *J* = 8.4, 2.0 Hz, H-19), 6.918 (1H, d, *J* = 11.6, H-18), 3.833 (3H, br. s., C$\underline{H}_3$); ^{13}C NMR (100MHz, DMSO-d_6) δ = 178.9 (C-3), 155.0 (C-13), 152.2 (C-17), 150.7 (C-16), 148.0 (C-5), 137.3 (C-11), 134.0 (C-7), 132.4 (C-9), 131.1 (C-14), 130.9 (C-8), 125.4 (C-10), 123.5 (C-6), 122.9 (C-19), 117.6 (C-18), 113.4 (C-15), 46.0 (C$\underline{H}_3$).

CHAPTER 5

5. RESULTS AND DISCUSSION:

Yield of all synthesized compound were obtained good (Table 5.1) and structure of newly synthesized compound were confirmed by UV, IR, ^{1}H-NMR and ^{13}C-NMR.

5.1 General Characterististics of the Synthesized Compound

Table 5.1: Physical Characteristics of synthesized compounds

Compound	State	Molecular formula	Molecular Weight	Colour	Yield (%)	M.P. (°C)
1	Crystalline solid	$C_7H_5ClO_2$	156.57	White		142
2	Liquid	$C_8H_7ClO_2$	170.59	Colorless	76	
3	Crystalline solid	$C_7H_7ClN_2O$	170.60	colorless White	72	162
4	Crystalline	$C_8H_6ClKN_2O_2S$	268.76	White	58	210
5	Amorphous solid	$C_8H_7ClN_4S$	226.69	Light yellow	60	176
5a	Amorphous solid	$C_{13}H_9ClN_4OS$	304.75	Pale yellow	65	192-198
5b	Amorphous solid	$C_{16}H_{13}ClN_4O_2S$	360.82	Light green	60	182-188

5.2 Spectral analysis:

Ultraviolet, infrared and nuclear magnetic resonance spectroscopic studies are very informative about the structure of 1, 2, 4-triazoles and their derivatives.

5.2.1 UV Spectroscopy

Table 5.2: Diagnostic peak obtained (λ_{max}) in UV spectroscopy

Compound	λ_{max}	Inference
4	302	$\pi \rightarrow \pi^*$
	308	$\pi \rightarrow \pi^*$
	321	$n \rightarrow \pi^*$
5a	302	$\pi \rightarrow \pi^*$
	308	$\pi \rightarrow \pi^*$
	344	$n \rightarrow \pi^*$
5b	302	$\pi \rightarrow \pi^*$
	308	$\pi \rightarrow \pi^*$
	344	$n \rightarrow \pi^*$

The absorption spectra of the compound **4** and its Schiff bases (**5a** and **5b**) exhibited three absorption bands as shown in table 5.2. The first two bands around 302 and 308

nm corresponds to $\pi \rightarrow \pi^*$ transitions due to C=C and C=N groups. The third band corresponds to $n \rightarrow \pi^*$ transition due to C=S.

5.2.2 IR Spectra

Table5.3 Diagnostic Bands (Cm^{-1}) in IR Spectra of synthesized compounds

Compound → Group ↓	4	5a	5b
ν(ArO-H)	-	-	3363 (m)
ν(NH_2)	3287 (s)	-	-
ν(C=CH)	3066 (m)	3095 (s)	3062 (m)
δ(NH_2)	1566 (w)	-	-
ν(C=N)	1608 (s)	1615 (s)	1666 (m)
ν(C=C)	1484 (s)	1573 (m) 1495 (s)	1597 (s) 1512 (s)
ν(N-C=S)	1464 (s) 1281 (m)	1476 (s) 1290 (s)	1463 (s) 1265(s)
ν(Ar-OCH_3)	-	-	1296 (s)
δ(Ar-OH)	-	-	1261 (s)
δ(=C-H)	1033 (m)	1045 (m)	1026 (s)
ν(C-Cl)	767 (s)	840 (m)	817 (m)
ρ(Ar-H)	723 (s)	760 (s)	717 (m)

In IR Spectrum, the analysis of compound **(4a)** gives different absorption bands. The strong absorption band at 3287 cm^{-1} is due to stretching vibration of NH_2.The medium absorption band at 3066cm^{-1} is due to (C=CH). The strong absorption region at 1608 cm^{-1},1484 cm^{-1} are due to stretching vibrations of (C=N) and (C=C) respectivily while weak absorption at 1566 cm^{-1} and absorption at 767cm^{-1} are due to bending vibration of (NH_2) and (C-Cl) respectively. The strong and medium absorption band at 1464 cm^{-1} and 1281cm^{-1} due to stretching vibration of (N-C=S). Similarly weak absorption at 723cm^{-1} is due to out of plane bending vibration of (Ar-H) respectively.

In I.R spectrum of compound **(5a)** strong absorption at 3095cm^{-1} and 1615cm^{-1} due to stretching vibration of absorption (C=CH) and (C=N) respectively. The medium absorption and strong absorption at (1573 and 1495) cm^{-1} are due to absorption vibration(C=C). The stretching vibration of (N-C=S) exhibit at (1476 and 1290) cm^{-1}.The medium absorption at 1045 $cm^{-1}$and 760 cm^{-1} are due to bending vibration of(=C-H) and (C-Cl) respectively. The strong absorption region at 760 cm^{-1} is due to bending vibration of (Ar-H).The absence of medium intensity bands in region (3500-3200) cm^{-1} in the IR spectra of compound **(5a)** confirmed the formation of the schiff base.

In IR spectrum of **(5b)** Strong absorption at 3363 cm^{-1} correspond to (Ar-OH).The aromatic ring breathing vibration (C=C) along with (C=N) due to stretching vibration of imine vibration were observed at (3062 to 1662) cm^{-1}. The two absorption band at 1597 cm^{-1} and 1512 cm^{-1} is due to stretching vibration (C=C).The strong adsorption at 1463cm^{-1}-1265cm^{-1} and 1296 cm^{-1} is due to stretching vibration of (N-C=S) and (Ar-OCH_3) whereas bending vibration of (Ar-OH), (C-Cl) and (Ar-H) exhibit medium intesity bands at 1261cm^{-1} , 817cm^{-1} and 717cm^{-1}respectively. The (N-H) absorption bands in the region 3500-3200 cm^{-1} was absent indicating the formation of the Schiff base **(5b).**

5.2.3 ^{1}H-NMR Spectra

Table 5.4: ^{1}H-NMR Spectral Assignments (ppm) of synthesized Compound (400 MHz, DMSO-*d_6)*

Compound Proton	4	5a	5b
8	7.919	7.972	7.828
9	7.647	7.677-7.59	7.626
10	7.560	7.15	7.530
11	7.714	7.677-7.59	7.746
12	9.68		
15	7.30	7.786	
16	6.72	6	
17		7.92	
18		7.677-7.59	6.918 7.438
HN		7.677 - 7.59	9.731
HO			10.145
CH_3			3.833

The ^{1}H-NMR spectra of synthesized compounds were consistent with the analogous triazole thione Schiff's bases. In Compound **(4)** the observed peak at 7.919 and 7.647 ppm correspond to proton of phenyl ring. Similarly, low frequency peak at 7.560 and 7.714 ppm peak are corresponding to phenyl ring proton ^{1}H (11).

The H-NMR spectra of (**5a)** revealed diagnostic H-N proton signal at 7.677-7.59ppm.The doublet at 9.68 ppm was attributed to ^{1}H (13) with broad signal. The signal at 7.30, 6.72 and 7.92 ppm correspond to proton of five membered ring of triazole. The signal at region 7.15 ppm detected for H (10) of phenyl ring.

In the spectra of **(5b)** , the diagnostic signal of H-O proton observed as abroad singlet at 10.145 ppm, while the peak of 9.731 ppm correspond to N-H proton. The diagnostic peak at 7.828, 7.626 and 7.530 ppm correspond to skeleton phenyl ring. The peak at

region 7.786 H (15) correspond to phenyl ring of vanillin ring. The low frequency broad singlet at 3.833 ppm was observed for CH_3 group.

5.2.4 C-NMR Spectra

Table 5.5: ^{13}C-NMR Spectral Assignments (ppm) of synthesized Compound (100 MHz, DMSO-d_6)

Compound Carbon	4	5a	5b
3	179.1	179.9	178.9
5	149.4	148.3	148.0
6	123.6	122.6	123.5
7	139.9	132.3	134.0
8	130.4	130.1	130.9
9	130.2	131.9	132.4
10	125.2	128	125.4
11	132.4	132.6	137.3
13		134.2	155.0
14		149.0	131.1
15		117.4	113.4
16		111.5	150.7
17		145.3	152.2
18			117.6
19			122.9
$C\underline{H}_3$			46.0

The appearance of peak δ=179.1 and 149.4 ppm indicates the C=N carbon of triazole. The peak at 123.6 attributed to C (6).The peak for other aromatic carbon appeared in region 139.9 to 132.4 ppm.

^{13}C-NMR spectrum of Schiff's base, **5a** exhibit signal at 145.3 to 179.9 ppm correspond to aromatic carbons. The aliphatic carbon (13) resonance is assigned at134.2 ppm.

In ^{13}C-NMR spectrum of Schiff's bases **5b** the signal range in the range 123.5-137.3 ppm is assigned to aromatic carbons. The resonance at 46.0 ppm is due to aliphatic carbon CH_3.The signal at 131.1 to 122.9 ppm is due to carbon of vanillin group.

5.3 ANTIMICROBIAL EVALUATION:

5.3.1 Antibacterial activity:

The Schiff's base of triazoles **5a** and **5b** were studied for antibacterial activity against various bacterial strains namely *Staphylococcus aures, Escherichia coli, klebsiella spp,* using Ciprofloxacin as standard. The entire compound sample show moderate activity against *S.aures.* Compound **(5b)** exhibit more potent than compound **(5a)**.Against *E. coli* Compound(5a) was found to inactive at 5% concentration while moderate activity with ZOI of 13 mm and 15 mm at concentration at 3% and 1% respectively. Similarly compound **(5b**) found more potent aginst *E.coli with* ZOI of4mm, 16 mm, and 14 mm at concentration 5%, 3% and 1% respectively. Similarly compound **(5b)** exhibited more effective against *Klebsiella spp* while compound (**5a)** found moderate activity and result are summarized in Table 5.6

Table 5.6: Antibacterial activity of Synthesized Schiff's base

Bacterial strain	**Diameter of zone of inhibition**						
	Compound 5a			Compound 5b			Ciprofloxacin
	5%	3%	1%	5%	3%	1%	3%
Staphylococcus aureus	5	7	9	9	6	8	23
Escherichia coli	-	13	15	4	16	14	30
Klebsiella spp	-	7	10	6	7	6	21

By analysis of above data, it has been observed that compound (**5b**) display more potent against *Staphylococcus aures, Escherichia coli, klebsiella spp,* at different concentration while compound (**5a**) shows moderate activity. All the test is performed using Ciprofloxacin as standard.

5.3.2 Antifungal activity: In case of antifungal activity test of synthesized Schiff's bases (**5a)** and (**5b)** exhibit moderate activity at concentration 5%, 3% and 1% against *Candida albicans* (yeast).The activity of Schiff's base **(5b)** exhibited comparatively good against C.*albicans* with (ZOI) 14 mm, 9 mm, 6 mm at concentration 5%, 3% and 1% while compound **(5a)** show moderate activity as shown in Table5.7

Table5.7: Antifungal activity of Synthesized Schiff bases

Fungal Strain	Diameter of zone of inhibition (mm)						
	Compound (5a)			**Compound (5b)**			**Ketoconazole**
	1%	3%	5%	1%	3%	5%	3%
Candida albicans (yeast)	4	5	3	6	9	14	14

From analysis of above data, it show that compound (**5b**) exhibit more potent against *Candida albicans* than compound (**5a**) at different concentration using Ketoconazole as standard.

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Triazoles have attracted considerable attention in the field of medicine due to their unique structures and properties. Triazoles derivatives possess great importance in the medicinal chemistry and as promising medical agents for the scientist working over this field. Out of 1,2,3 triazole and 1,2,4 triazoles, 1,2,4 triazoles are very important in various sectors like pharmacological field as antifungal, antibacterial, anti convulsants, antioxidant, antiviral, anti-flammatory, anti-tubercular and anti malarial agents. The synthesis of the compounds gives ideas about large pharmaceutical application. The multipurpose synthetic applicability and biological activity of these heterocyclic compounds will facilitate the medicinal chemists to plan, design and implement new approaches towards the discovery of novel drugs.

The present work synthesis of Schiff base of 1, 2, 4-triazole derivatives viz 3-(2-chlorophenyl)-4-((furan-2-ylmethylene)amino)-4*H*-1,2,4-triazole-3(2*H*)-thione and 5-(2-chlorophenyl)-4-((4-hydroxy-3-methoxybenzylidene)-amino)-4*H*-1,2,4-triazole-3(2*H*)-thione. All the synthesized compounds are thermally stable. The formation and characterization of Schiff bases was characterized by UV, IR, ^{1}H- NMR and ^{13}C-NMR spectroscopy.

6.2 LIMITATION OF RESEARCH

Even each and every step was done in great care, still there were some limitation and shortcomings. The crystals were formed in each and every step were not analyzed by x-ray crystallography. The structures can be further confirmed by mass spectrometry, but it was not available in laboratory.

6.3 RECOMMEDATION FOR FURTHER WORK

Well over 20,000 triazoles are known but practical application has been very few until recently. However more derivatives can be synthesized by using variety of aldehydes. Due to lack of budget and time further significant work could not be accomplished.

More investigations must be carried out to evaluate more activities of triazoles for many diseases whose treatment are difficult in the medical science.

Besides this, some triazoles and their complexes exhibit other biological as well as agricultural activities which can also be explored in further research.

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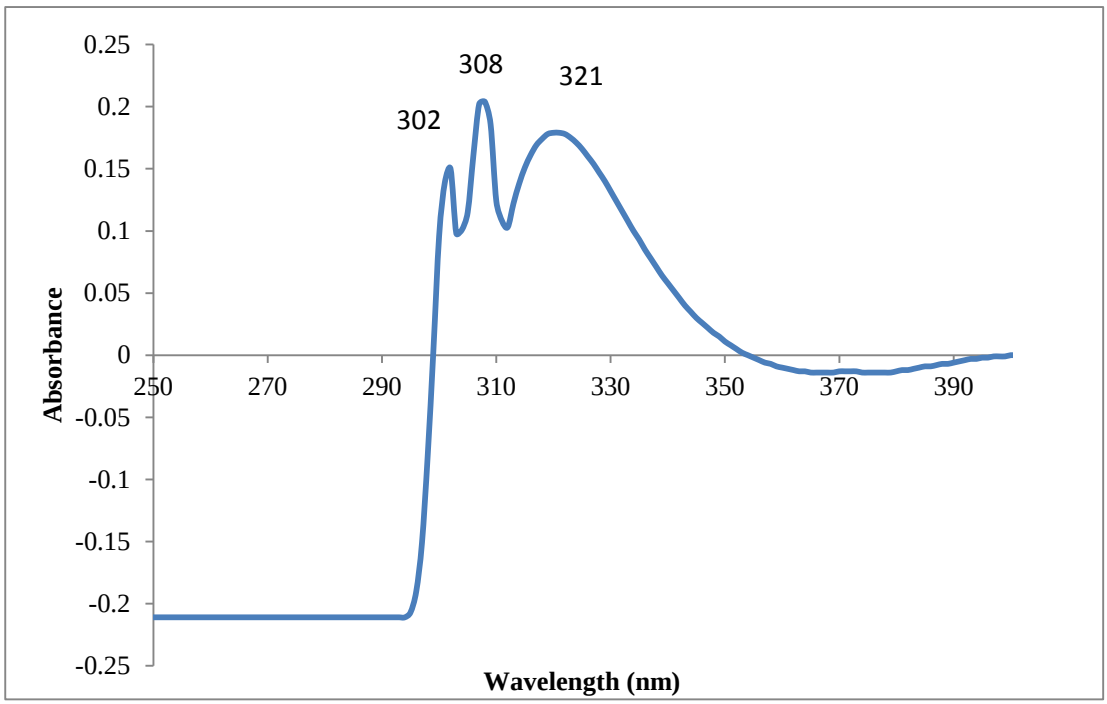
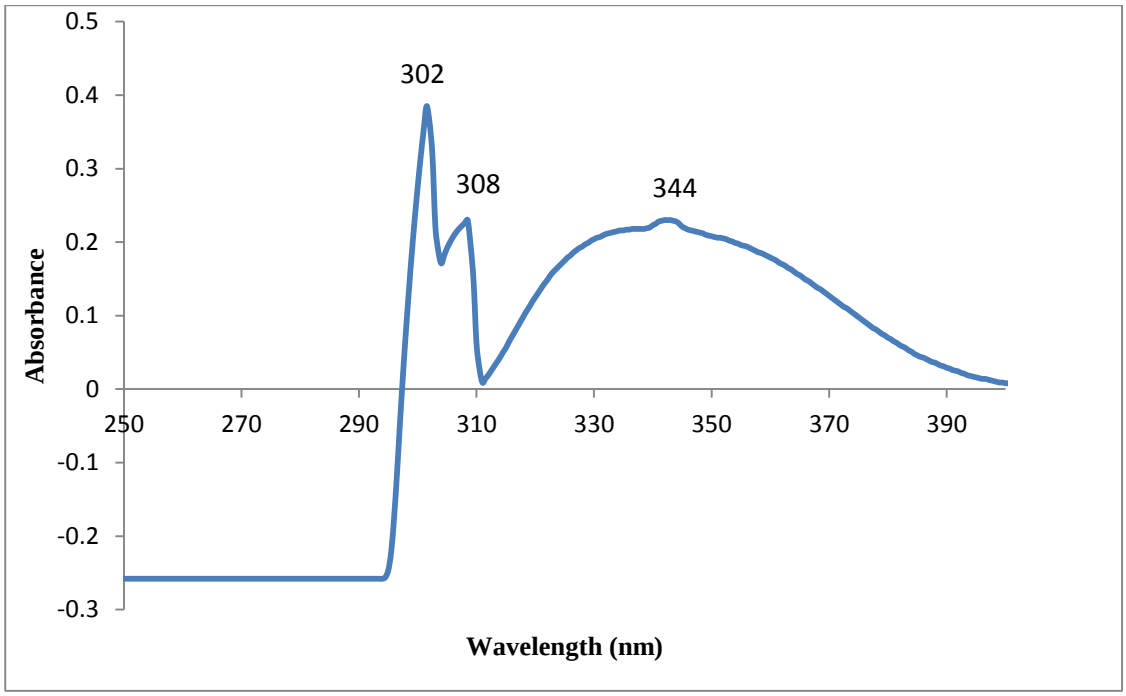
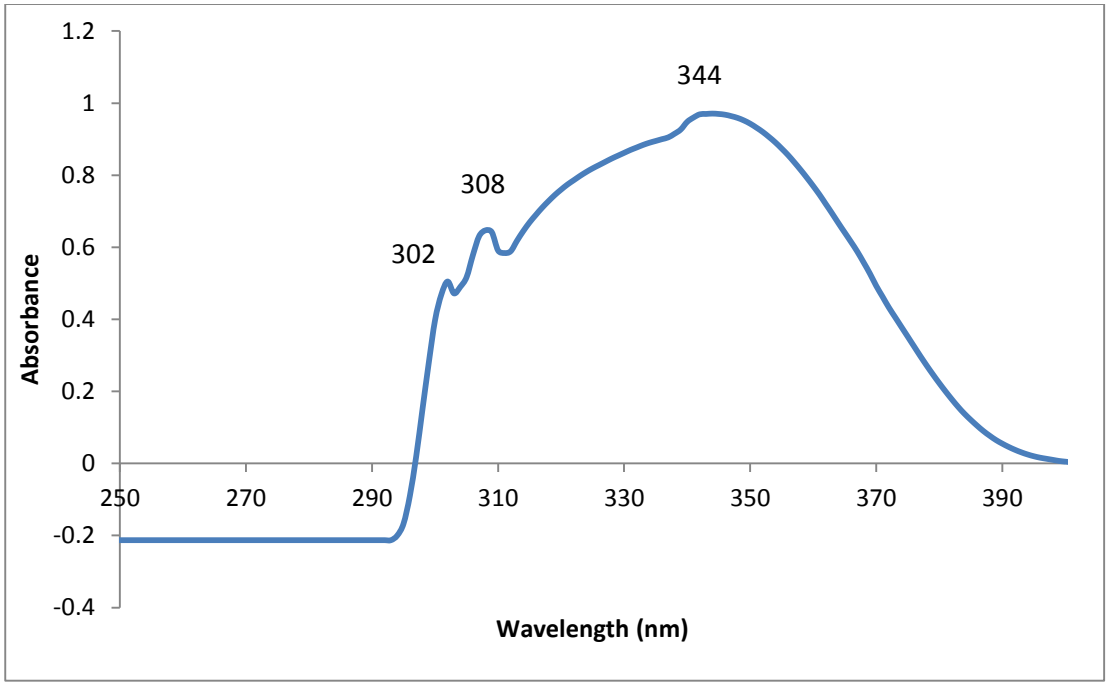
APPENDIX



Appendix A1: UV Spectrum of compound 4

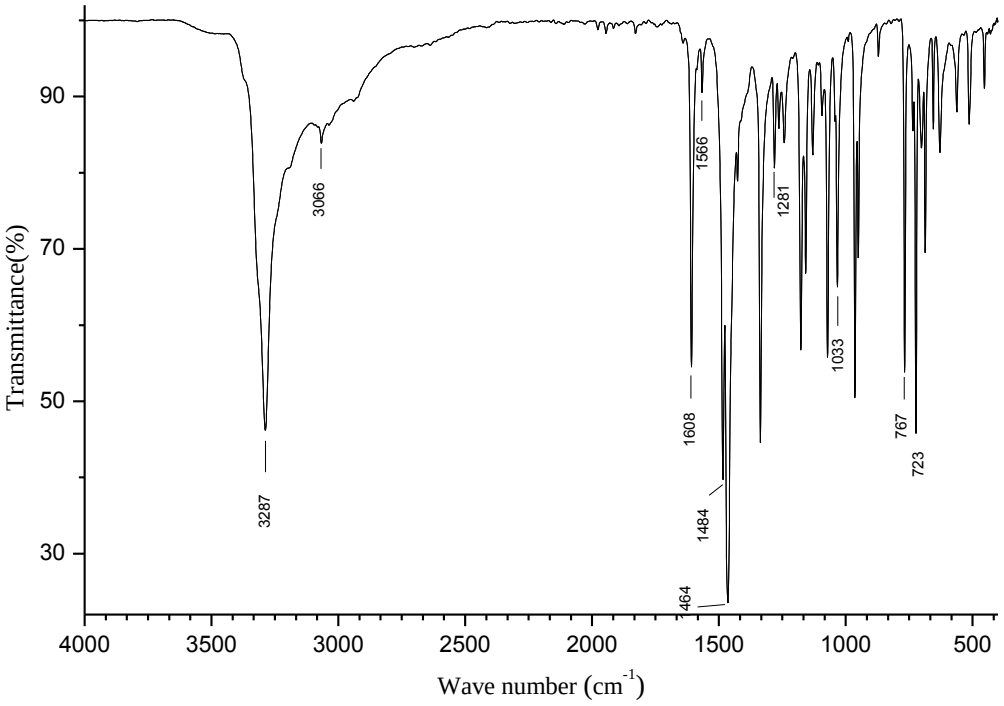


Appendix A2: UV Spectrum of compound 5a

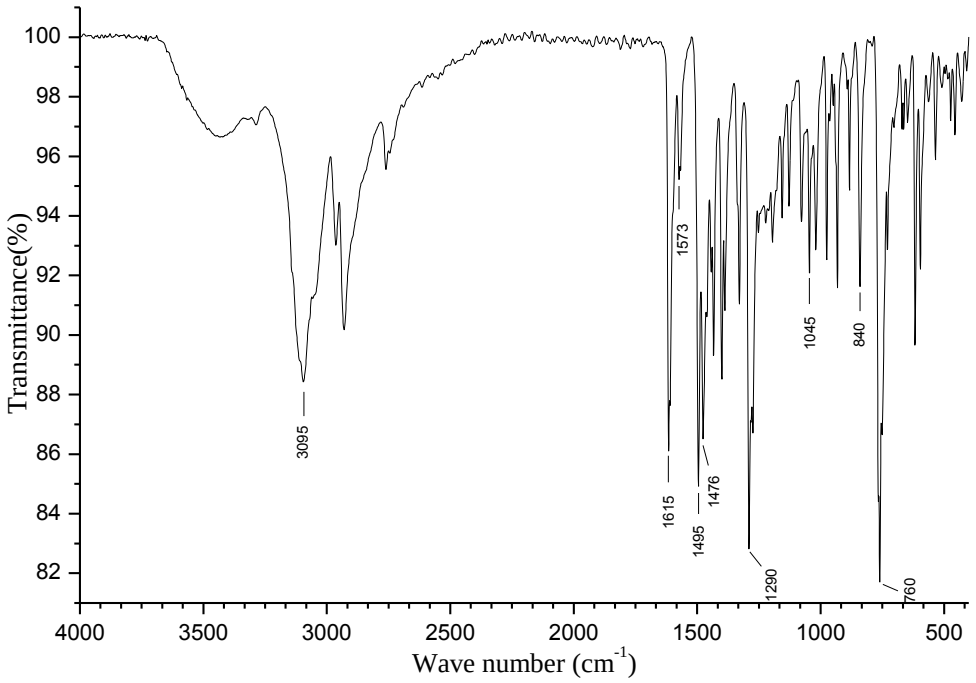


AppendixA3: UV spectrum of compound 5b

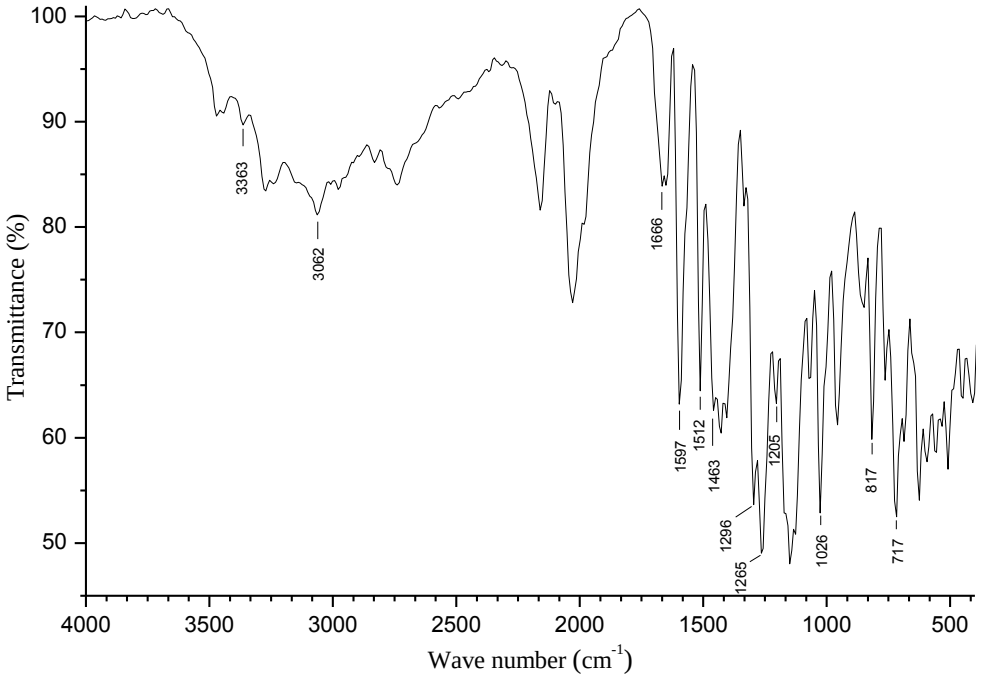
IR spectra of synthesized compounds



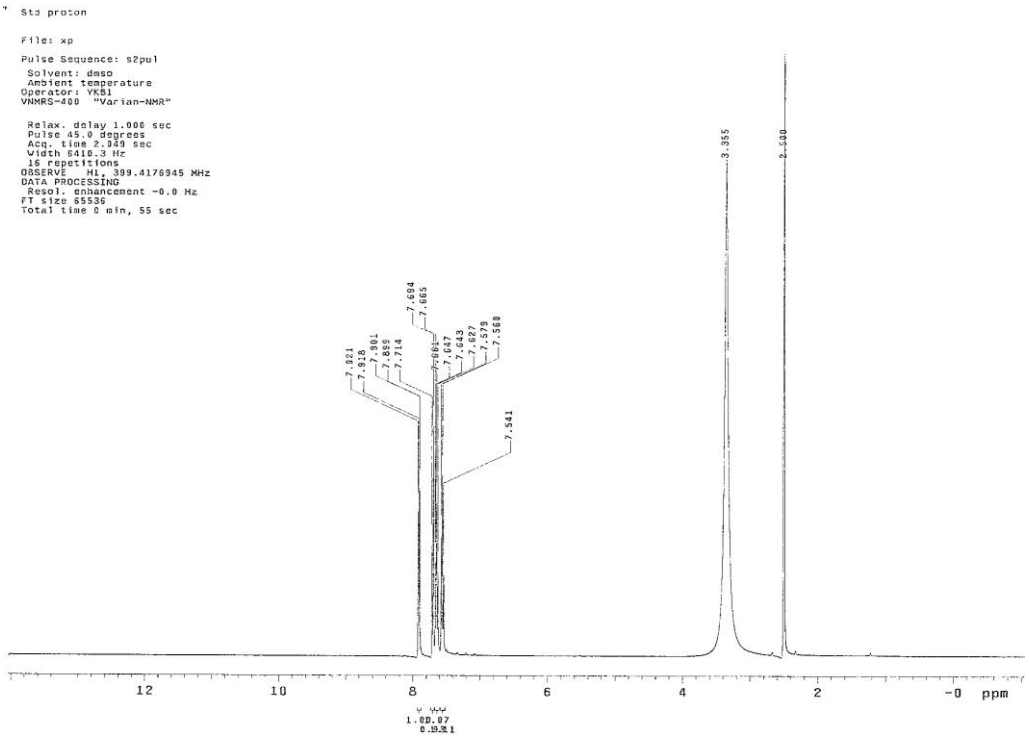
Appendix B1: IR Spectrum of compound **4**



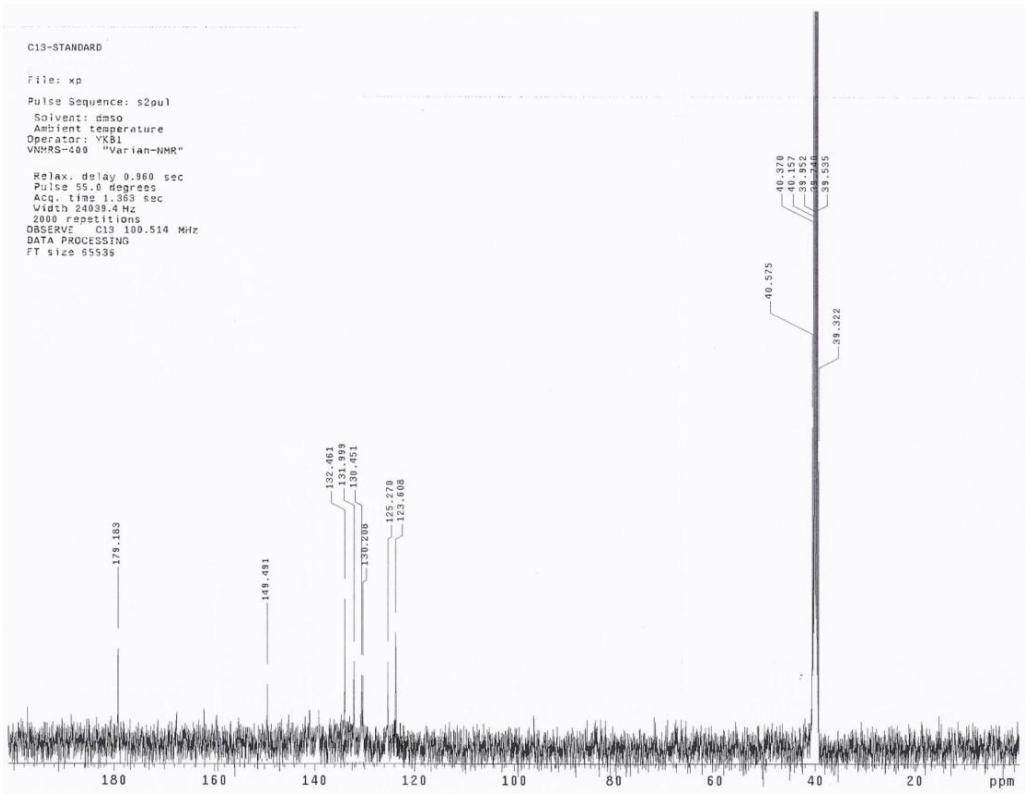
Appendix B2: IR Spectrum of compound **5a**



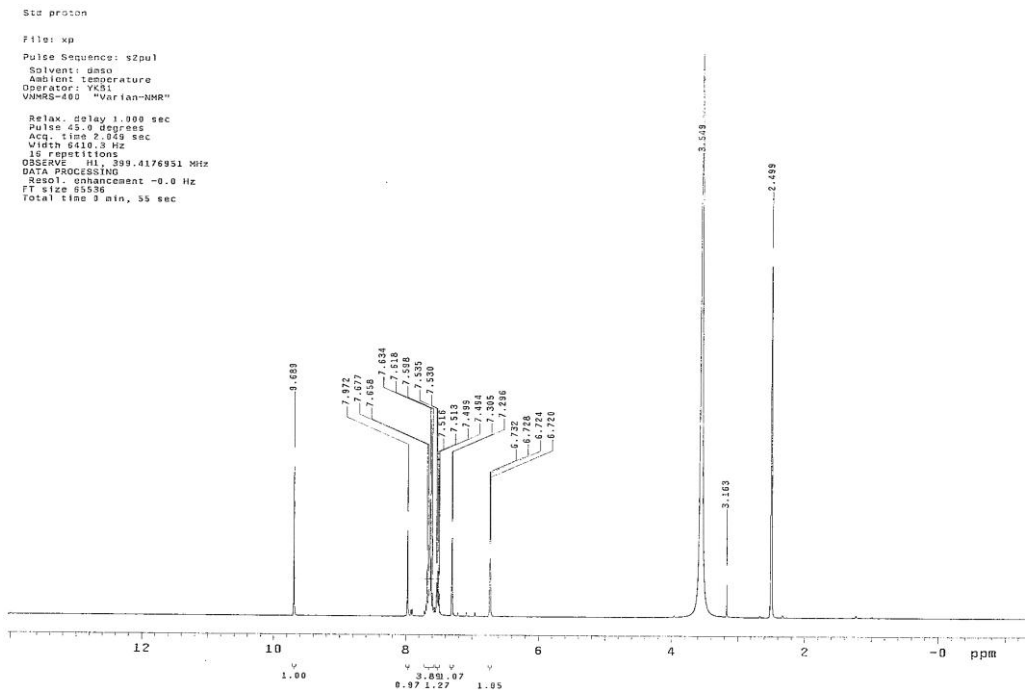
Appendix B3: IR Spectrum of compound **5b**



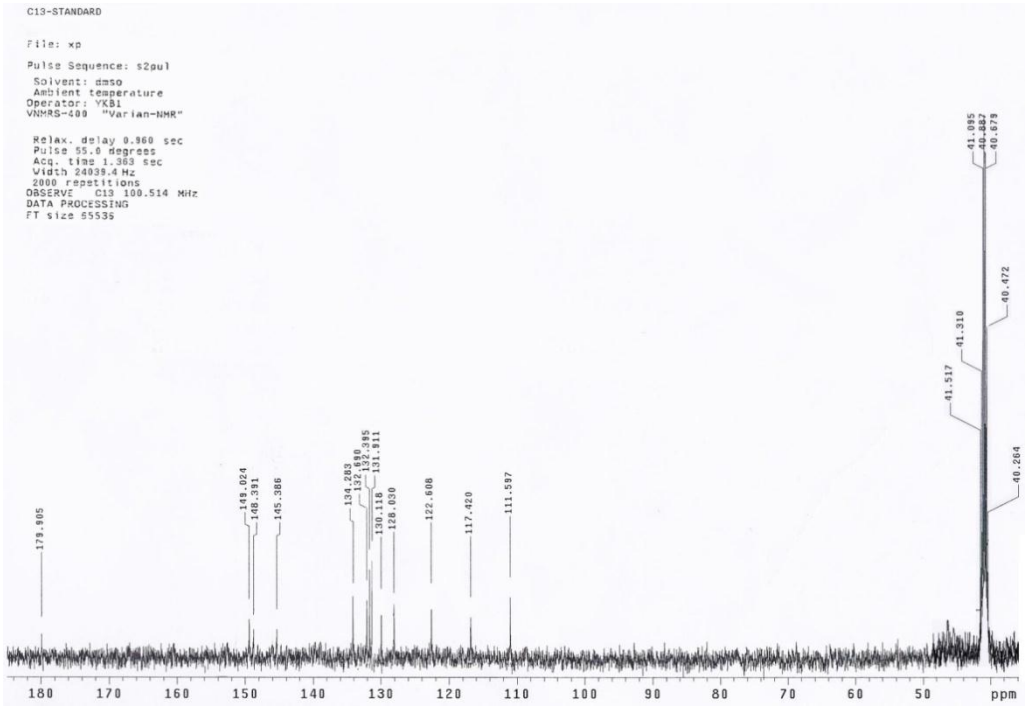
Appendix C1: [1]H-NMR Spectrum of compound 4



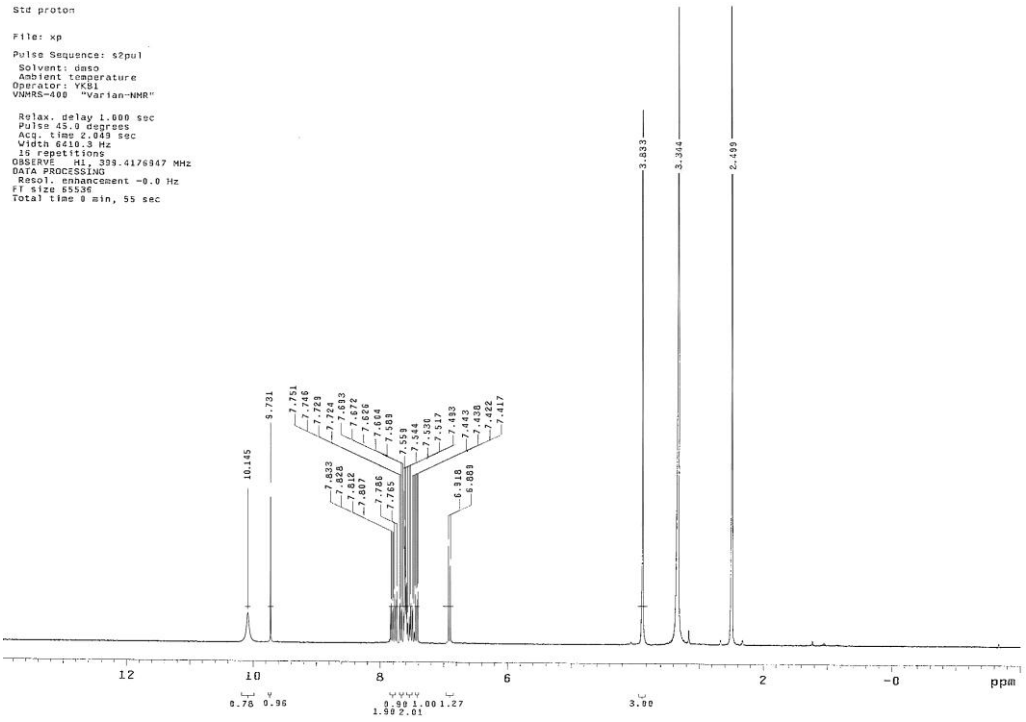
Appendix C2: [13]C-NMR Spectrum of compound 4



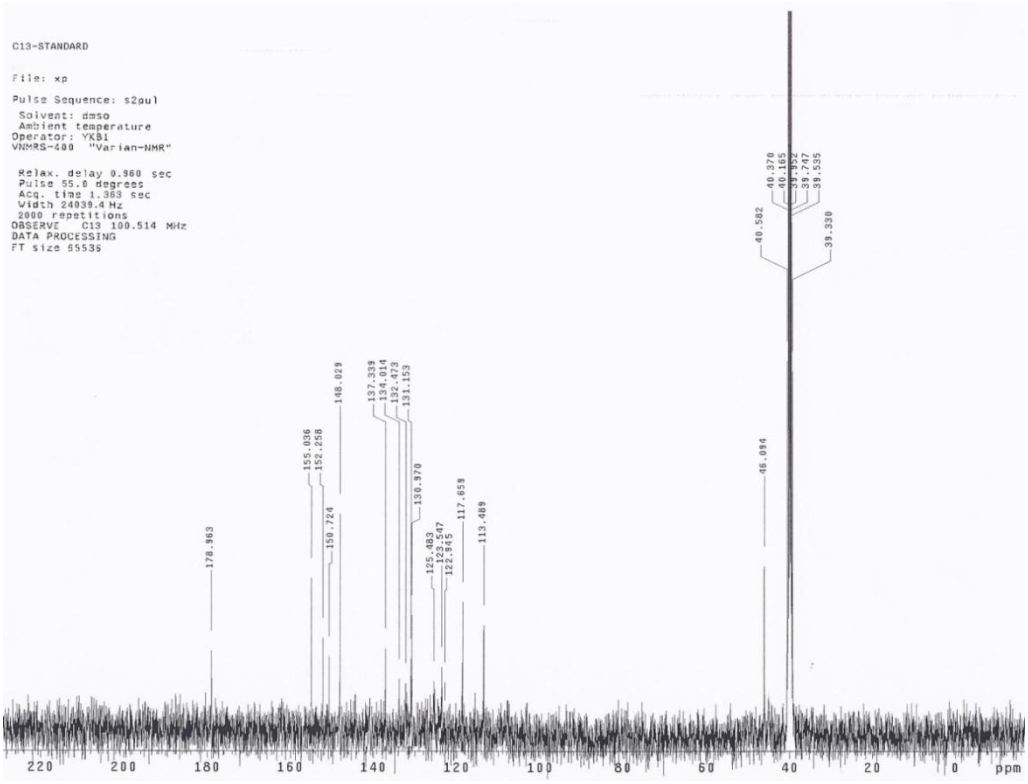
Appendix C3: ^{1}H-NMR Spectrum of compound **5a**



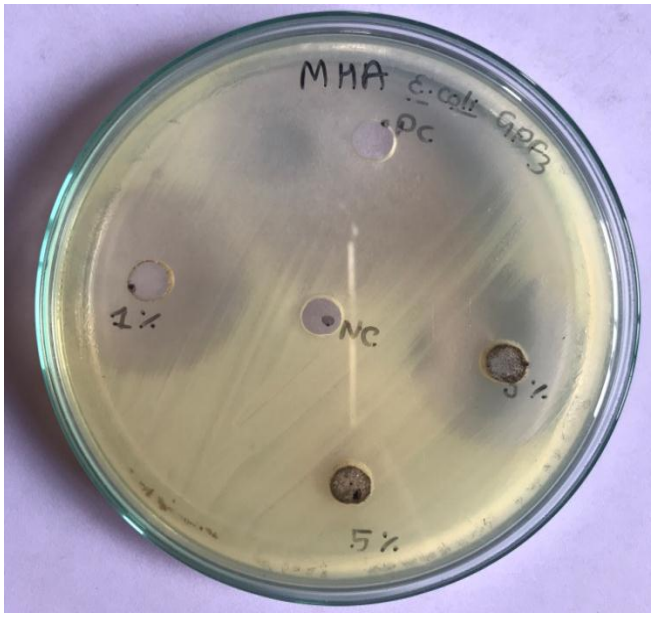
Appendix C4:^{13}C-NMR Spectrum of compound **5a**



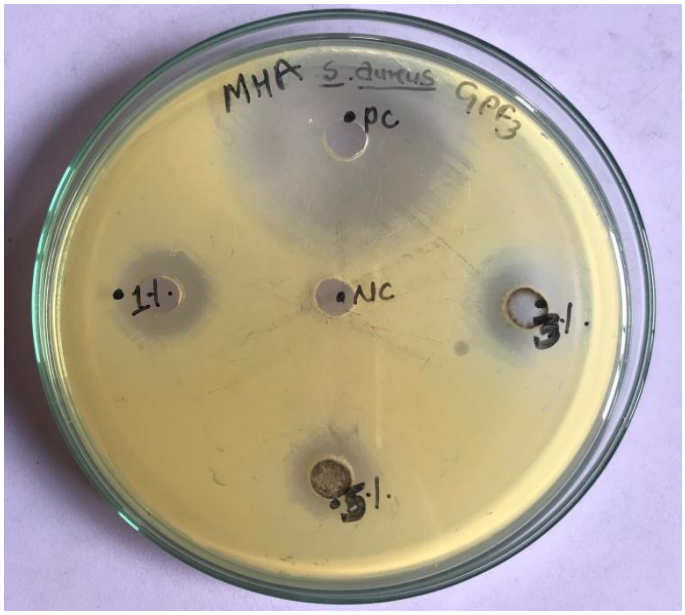
Appendix C5:[1]H-NMR Spectrum of compound **5b**



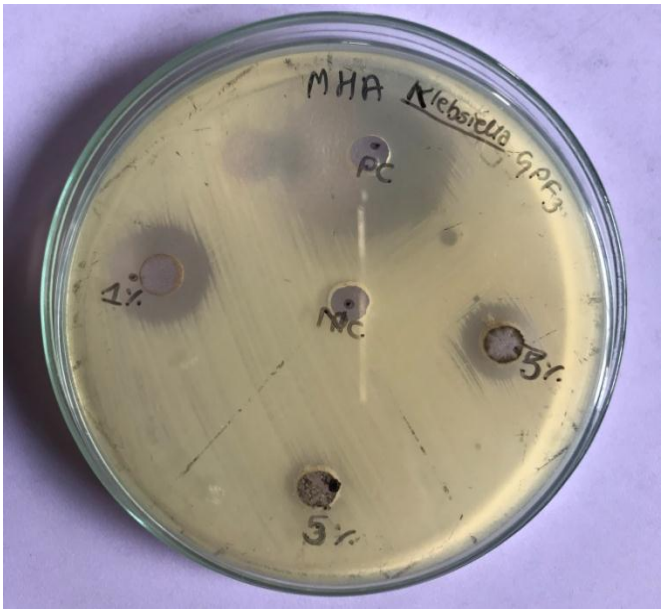
Appendix C6:[13]C-NMR Spectrum of compound **5b**



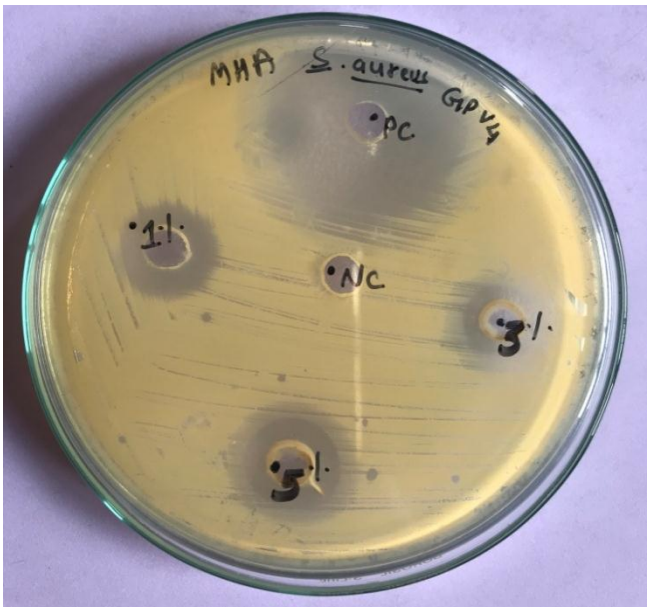
Appendix D1: Antibacterial activity of compound **5a** against *E.coli*



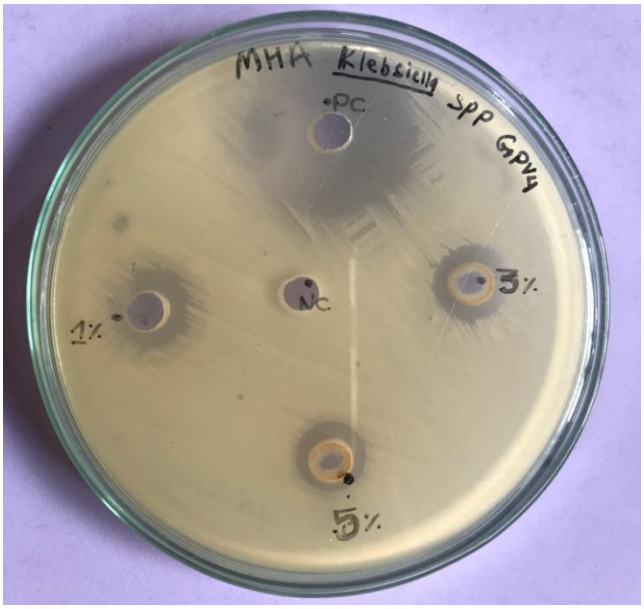
AppendixD2: Antibacterial activity of compound **5a** against *Staphylococcus aureus*



AppendixD3: Antibacterial activity of compound **5a** against *Klebsiclla spp*



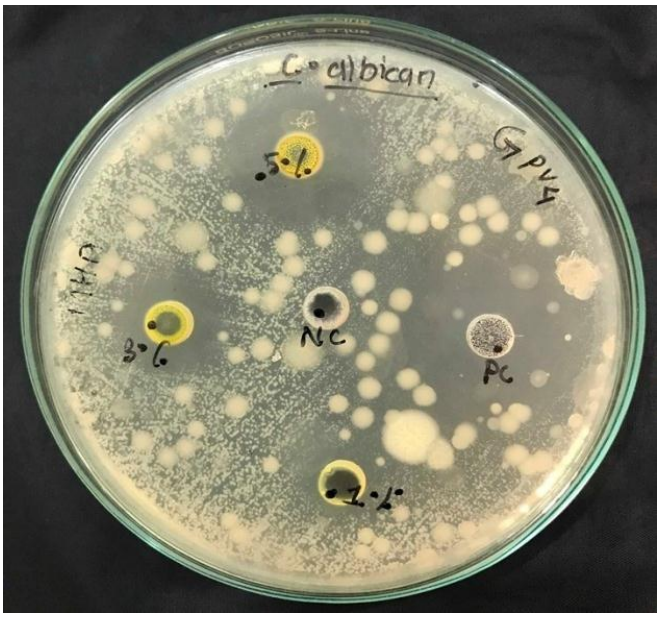
AppendixD4: Antibacterial activity of compound **5b** against *Staphylococcus aureus*



AppendixD5: Antibacterial activity of compound **5b** against *Klebsiclla spp*

AppendixD6: Antibacterial activity of compound **5b** against *E.coli*

AppendixD7: Antifungal activity of compound **5a** against *Candida albicans*



AppendixD8: Antifungal activity of compound **5b** against *Candida albicans*