SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF 1,3,4-OXADIAZOLE THIOL DERIVATIVES

A DISSERTATION

SUBMITTED FOR

THE PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

BY

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BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled "**Synthesis, Characterization and Evaluation of Antimicrobial Activity of 1,3,4-oxadiazole Thiol Derivatives**" prepared by **Mr. Madhab Prasad Joshi**, under the supervision of **Associate Professor Dr. Bhushan Shakya**, Department of chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, is hereby submitted for the partial fulfillment of Master of Science (M.Sc.) degree in chemistry. This dissertation has not been submitted to any other university or institution previously for the award of a degree.

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LETTER OF RECOMMENDATION

It is to recommend that this dissertation work entitled "**Synthesis, Characterization and Evaluation of Antimicrobial Activity of 1,3,4-oxadiazole Thiol Derivatives**" has been carried out by **Mr. Madhab Prasad Joshi** as partial fulfillment of the requirement of an M.Sc, Degree in Chemistry under my supervision. To the best of my knowledge, this work has not been submitted elsewhere for any other degree.

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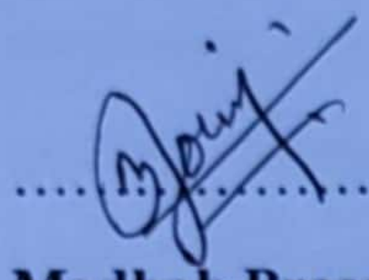
DECLARATION

I, **Madhab Prasad Joshi**, hereby declare that the work presented herein is genuine work done originally by me under the supervision of **Assoc. Prof. Dr. Bhushan Shakya** has not been published or submitted elsewhere for the requirements of a degree program. Any literature or data work done by others and cited in this dissertation has been given due acknowledgment and listed in the reference section.



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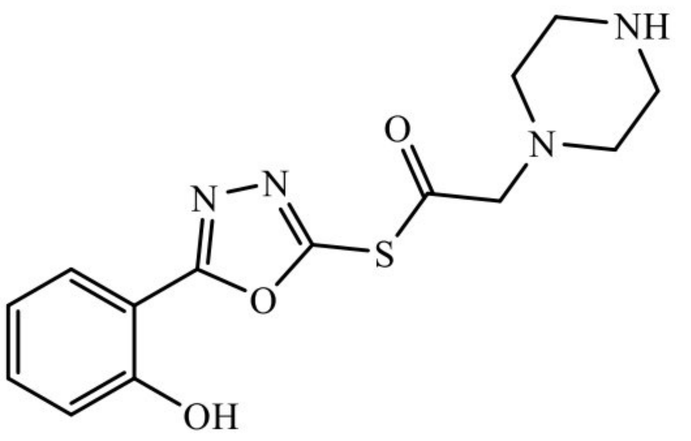
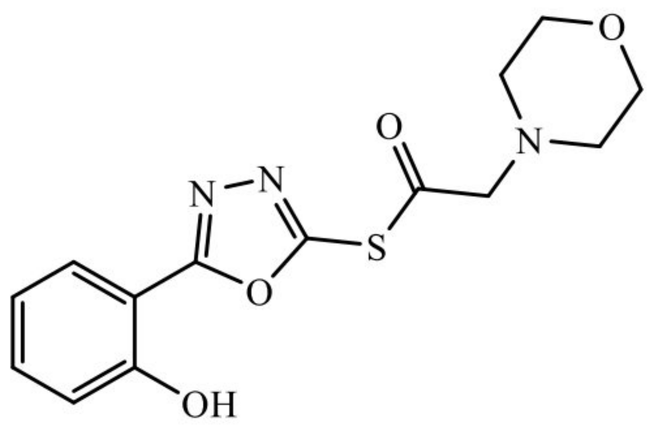
ABSTRACT

With the worldwide development of antimicrobial resistance and the emergence of new types of illness, researchers are compelled to look for novel compounds that would be sensitive to microorganisms. The 1,3,4-oxadiazole scaffold is a significant heterocyclic fragment that is being considered a potential building block for drug discovery. Substituted 1,3,4-oxadiazoles have been shown to have a diverse range of pharmacological activities, including antibacterial, anticancer, antioxidant antiviral, antifungal antitubercular, anti-inflammatory and analgesic properties.

A new class of 1,3,4-oxadiazole thiol derivatives, *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-(piperazin-1-yl)ethanethioate **(4a)** and *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-morpholinoethanethioate **(4b)** were synthesized by using available precursors and conventional synthetic methods. The formation of the newly synthesized compounds was confirmed by the chromatographic method (TLC) and physical properties (melting point and boiling point). The structures of the newly synthesized compounds were assigned by using UV-Visible, FT-IR, and NMR spectroscopic techniques. The synthesized compounds were evaluated for their antibacterial and antifungal activities against several bacterial and fungal strains. Among the two synthesized compounds, compound (**4b)** which is the morpholine-linked derivative exhibited promising antimicrobial activity against most of the tested strains. However, the compound (**4a)** showed moderate activity against some bacterial and fungal strains.



4a



4b

Keywords: 1,3,4-oxadiazole, morpholine, piperazine, antimicrobial activity.

LIST OF ABBREVIATIONS

^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
b.p.	Boiling point
DCC	Dicyclohexyl carbodiimide
DMSO	Dimethyl sulphoxide
EC_{50}	Half maximal effective concentration
FTIR	Fourier-transform infrared
GP	Growth percentage
m.p.	Melting point
MES	Maximal electroshock seizure
MIC	Minimum inhibitory concentration
PC	Positive control
ppm	Parts per million
PTZ	Pentylenetetrazole
SAR	Structure-activity relationship
TLC	Thin layer chromatography
UV	Ultra-violet
ZOI	Zone of inhibition

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CHAPTER 1

1. INTRODUCTION

1.1 Heterocycles in medicinal chemistry

The term "heterocyclic compounds" refers to cyclic organic compounds that contain at least one heteroatom. Nitrogen, oxygen, and sulphur are the three most common heteroatoms, however heterocyclic rings with other heteroatoms are also well-known (Al-Mulla, 2017). A large number of heterocyclic scaffolds can be regarded as privileged structures. Most typically, five- or six-membered rings with heterocycles or other positional combinations of nitrogen, sulphur, and oxygen can be observed. A heterocycle is found in more than 80% of all biologically active chemical entities, according to statistics. This reflects the importance of heterocycles in modern drug design. Utilizing heterocycles allows for the modification of physiologically active agent solubility, lipophilicity, polarity, and hydrogen bonding capacity, which optimizes the absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) characteristics of medications or therapeutic candidates. Therefore, the usage of heterocycles by medicinal chemists is crucial since doing so allows for the expansion of the chemical space that may be used to create drugs and the advancement of more successful drug discovery initiatives (Jampilek, 2019).

Due to the alarming rise in multi-drug resistant bacterial species and the gradual development of bacterial resistance to a wide range of treatments, there is an increasing interest in the development of novel antibacterial drugs. Resistance of microbial pathogens toward antimicrobial agents is a rising and scary global problem these days. Therefore, designing and synthesizing new antimicrobial drugs that can kill or inhibit those resistant pathogens is a major challenge for synthetic chemists. Heterocyclic compounds, which are described by the IUPAC as "cyclic compounds possessing at least two distinct elements as ring members," present themselves as an essential category of organic chemicals with their roots firmly established in organic and medical chemistry (Mustafa, 2018).

1.2 Organic synthesis

Chemical synthesis's branch on organic synthesis is mainly concerned with the deliberate construction of organic molecules. It is the art and science of creating organic molecules, such as those in living things and some synthetic materials, whose main component is carbon.

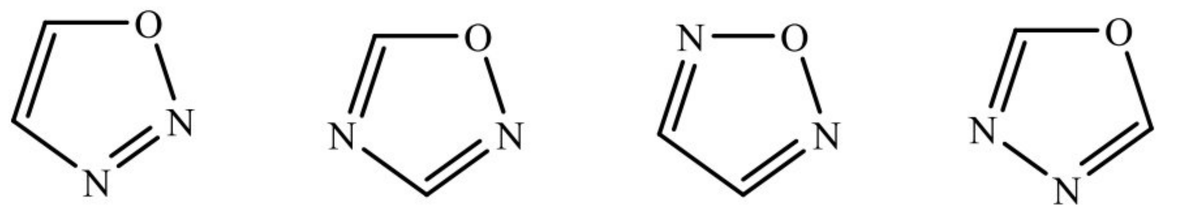
Designing synthetic routes to a molecule is the heart of organic synthesis. The simplest molecular synthesis is one in which the desired target molecule can be produced from a conveniently accessible starting material by a single-step or multiple-step reaction. However, molecular synthesis is not that much straightforward in some cases. To convert a selected starting material to the desired molecule, numerous steps that add, substitute, or remove functional groups, as well as steps that build up the target molecule's carbon atom framework, may be required (Wiebe et al., 2018).

The primary concern of organic synthesis is chemical synthesis and derivatize them with non-trivial carbon connectivities. The design and selection of target molecules are guided by a specific interest in biological phenomena (Zhdankin, 2011).

To create the complex polycyclic scaffolds present in nature, chemical synthesis research primarily focuses on numerous bond-forming stages and the development of novel methodologies and technologies. The development of noble and covalent ligand reactions that are application-oriented and produce various functional derivatives from simple substrates or synthetic intermediates is the main goal of chemical derivatization research. A large number of syntheses for a wide range of compounds have been recorded over the last hundred years. Building the target molecule's carbon framework or skeleton, adding, removing, or changing functional groups in a way that produces the functionality of the target compound, and using selective stereocontrol at all points where centers of stereoisomerism are formed or influenced are the steps that are most frequently used in chemical synthesis (Carruthers et al., 2004).

1.3 Oxadiazole

Oxadiazoles are a group of heterocyclic aromatic chemical compounds of the azole family containing two nitrogen atoms, two carbon atoms, and one oxygen atom. There are four isomers of oxadiazole in which 1,3,4-oxadiazole have enormous importance (Sanchit & Pandeya, 2011).



1,2,3-oxadiazole 1,2,4-oxadiazole 1,2,5-oxadiazole 1,3,4-oxadiazole

Figure 1.1: Isomers of oxadiazole

1.3.1 Physical properties of 1,3,4-oxadiazole

1,3,4-oxadiazole is liquid at room temperature having a boiling point of 150 °C (Ainsworth, 1965).

1.3.2 Chemistry of 1,3,4-oxadiazole

1,3,4-Oxadiazole is considered a weak base because of an extra hetero atom governing the inductive effect (Khalilullah & Ahsan, 2012). When two -CH groups in oxadiazole are replaced by two nitrogens, the resulting oxadiazole ring has less aromaticity and takes the characteristics of a conjugated diene The oxadiazole ring is very resistant to electrophilic substitution because of the low electron density on the carbon atom. The oxadiazole ring makes nucleophilic substitution challenging, but if the substitution involves halogen, the oxadiazoles can undergo nucleophilic substitution by eliminating the halogen atom with nucleophiles. Additionally, because it produces new aliphatic nitrogen-containing compounds and other ring systems, reactions involving the 1,3,4-oxadiazole's ring have attracted a lot of interest in several medicinal chemistry domains. In recent years, there has been an increase in the use of molecular, electronic, and nuclear resonance spectra to clarify the structure of 1,3,4-oxadiazole derivatives (Joule et al., 2020).

1.3.3 Biological importance of 1,3,4-oxadiazole

The three isomers of oxadiazole namely 1,2,5-oxadiazole, 1,2,4-oxadiazole, and 1,3,4-oxadiazole have a wide range of pharmacological importance; they appear in a variety of pharmaceutical products including raltegravir, oxalamine, fasiplon, butalamine, and pleconaril. The fourth isomer of oxadiazole i.e. 1,2,3-oxadiazole is unstable (Kamal et al., 2014).

Oxadiazole gains heavy interest from many research scholars including the invention of novel remedial molecules. It can be found in many physiologically active compounds that have fungicidal, anticancer, antitubercular, and other properties. Due to the presence of the —N=C—O— group in its structure, which tends to improve the hydrophobicity, 1,3,4-oxadiazole is frequently utilized as a pharmacophore in drug design. This might make it easier for the drugs to diffuse across membranes and reach the infection site (Bajaj et al., 2015).

CHAPTER 2

2. OBJECTIVES

2.1 General objective

- Synthesis of 1,3,4-oxadiazole derivatives and evaluation of their antimicrobial activity.

2.2 Specific objectives

- Synthesis of 1,3,4-oxadiazole derivatives from methyl salicylate.
- Characterization of the synthesized compounds by different spectral techniques such as UV, IR, and NMR.
- Assessment of the antimicrobial activity of the synthesized compounds.

CHAPTER 3

3. LITERATURE REVIEW

3.1 Synthesis of 1,3,4-oxadiazole

Gan et al. (2017) synthesized 1,3,4-oxadiazole starting from benzoic acid. Benzoic acid is treated with methanol in an acidic medium to obtain methyl benzoate which was further treated with hydrazine giving acid hydrazide. Finally, acid hydrazide was cyclized in presence of carbon disulphide in ethanol and potassium hydroxide in an acidic medium to obtain phenyl-substituted 1,3,4-oxadiazole.

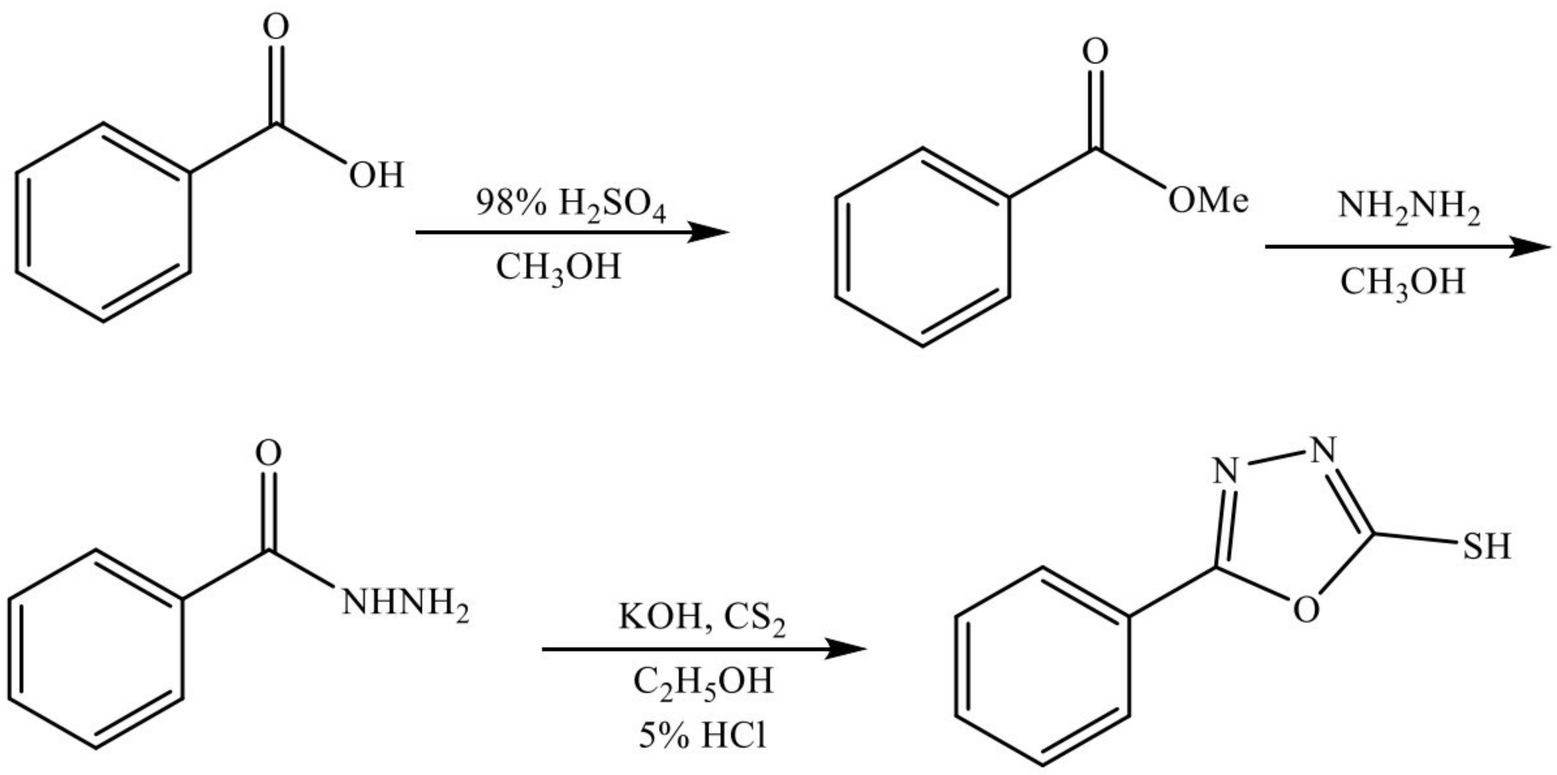


Figure 3.1: Synthesis of phenyl substituted 1,3,4-oxadiazole thiol compound

Abu-Hashem, (2021) synthesized 1,3,4-oxadiazole starting from 4-oxo 4-phenyl butanoic acid through acid hydrazide as an intermediate. The acid hydrazide intermediate was treated with isothiocynic acid in ethanol to give oxophenyl hydrazine carbothioamide which was further reacted with HgO in ethanol for 6h at 160 ℃ to obtain amine substituted 1,3,4-oxadiazole.

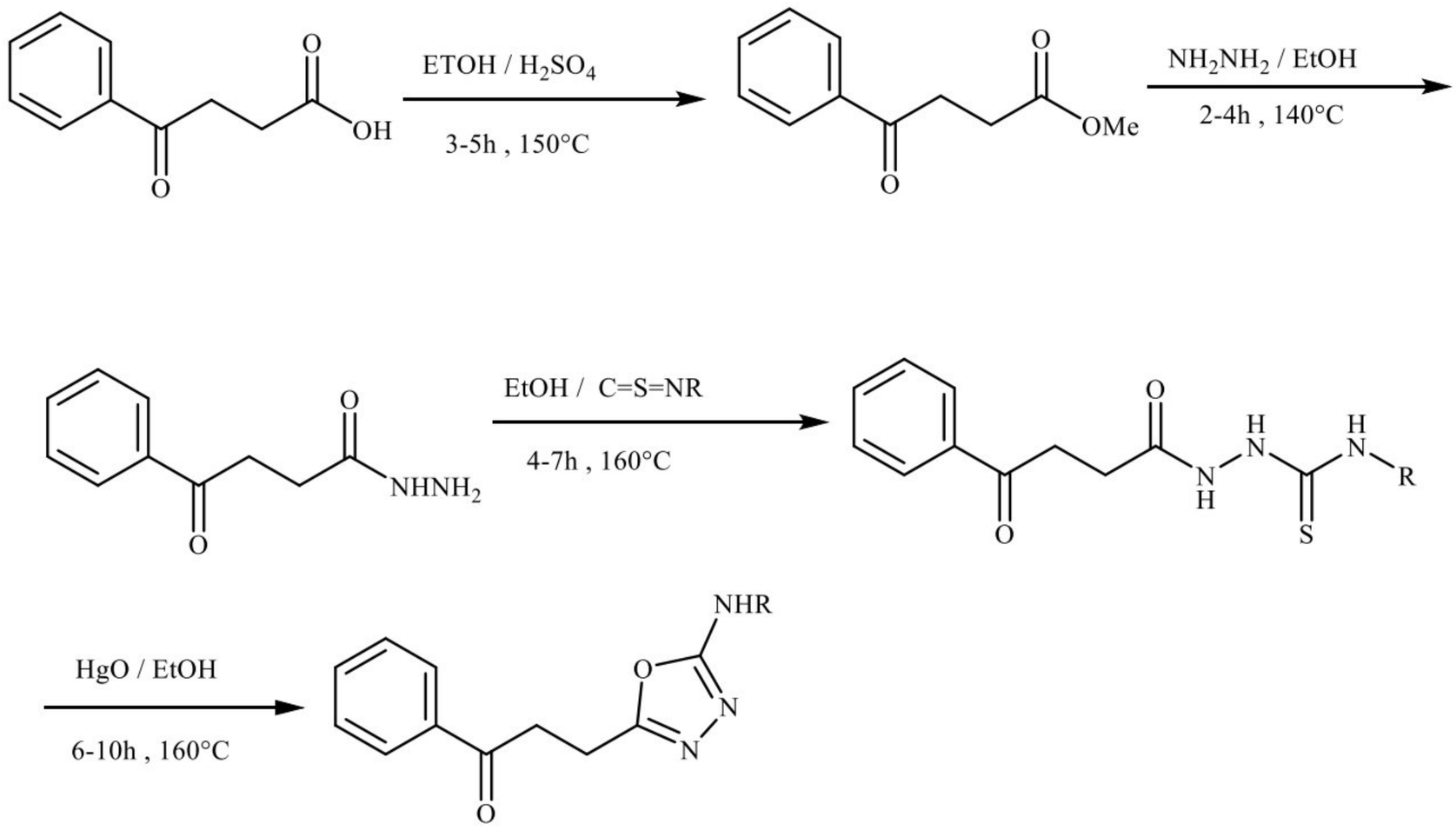


Figure 3.2: Synthesis of amine substituted 1,3,4-oxadiazole derivative

Li et al. (2018) designed and synthesized bisthioether derivatives containing a 1,3,4-oxadiazole moiety and evaluated antibacterial and nematocidal activities. The synthetic route is as follows:

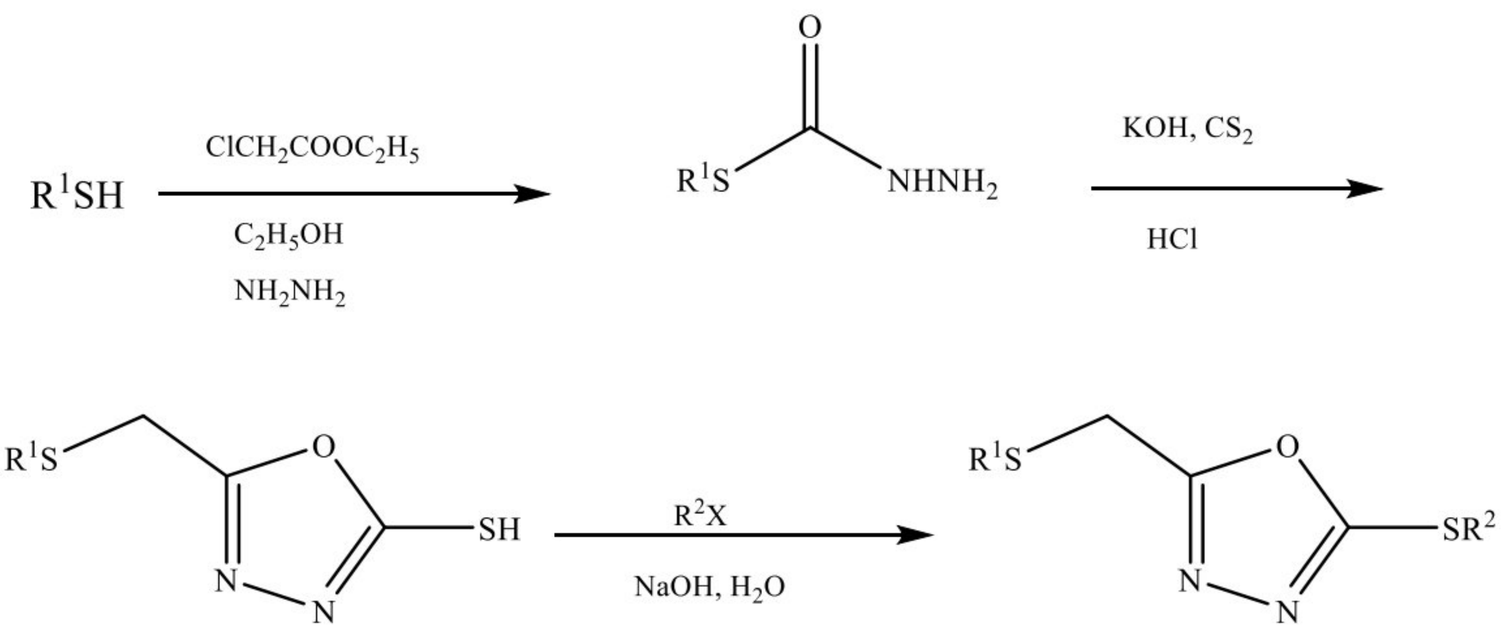


Figure 3.3: Synthesis of bisthioester derivative containing 1,3,4-oxadiazole moiety

Rivera et al. (2006) reported the cyclization of the acylthiosemicarbazide to 5-aryl-2-amino-1,3,4-oxadiazoles using 1,3-dibromo-5,5-dimethylhydantoin as an effective oxidizing agent. The reagents utilized in this procedure, according to them, are cheap and secure to use, which is their main advantage. It is also appropriate for large-scale synthesis in conditions where other oxidizing agents are the use of other oxidizing agents is restricted.

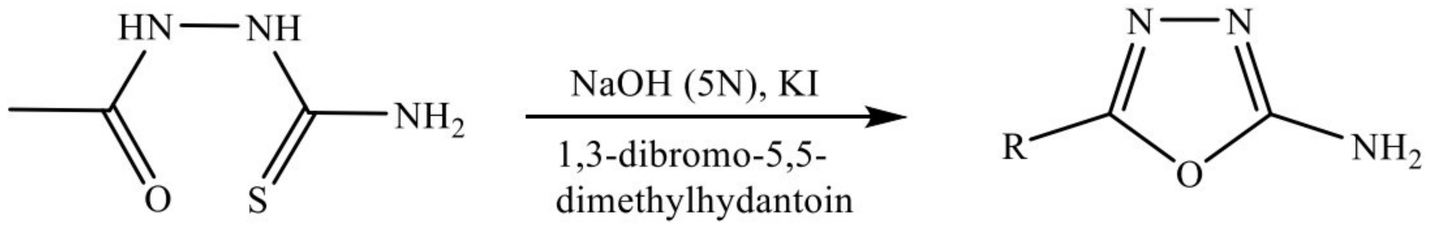


Figure 3.4: Synthesis of 5-aryl-2-amino-1,3,4-oxadiazole

The cyclization reaction of acylthiosemicarbazide with iodine acting as the oxidizing agent is another fascinating method for the design and synthesis of amino-1,3,4-oxadiazole. By heating the naphthalene moiety in ethanol in the presence of iodine and sodium hydroxide, El-Sayed et al. (2012) reported synthesizing the naphthalene derivative of N-phenyl-1,3,4-oxadiazol-2-amine.

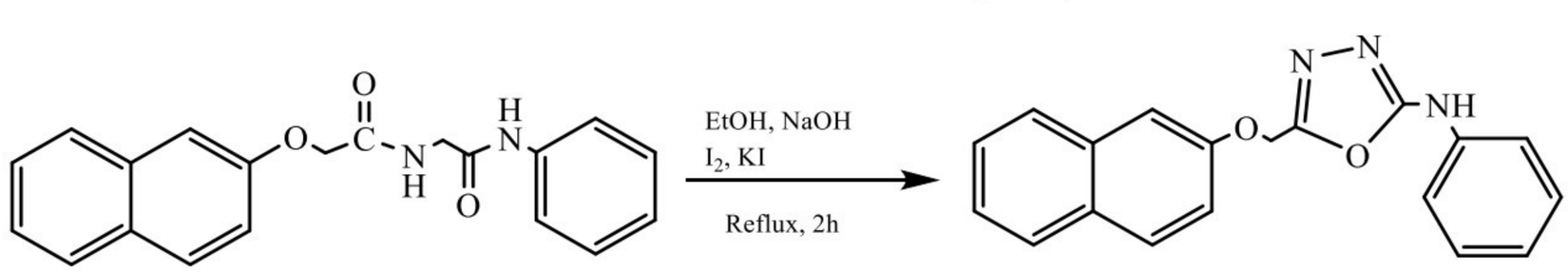


Figure 3.5:Structure of naphthalene derivative of N-phenyl-1,3,4-oxadiazol-2-amine

Jha et al. (2010) disclosed the cyclization of substituted aromatic hydrazides in carbon disulfide and $POCl_3$ and with aromatic acids to produce derivatives of 2,5-disubstituted-1,3,4-oxadiazoles.

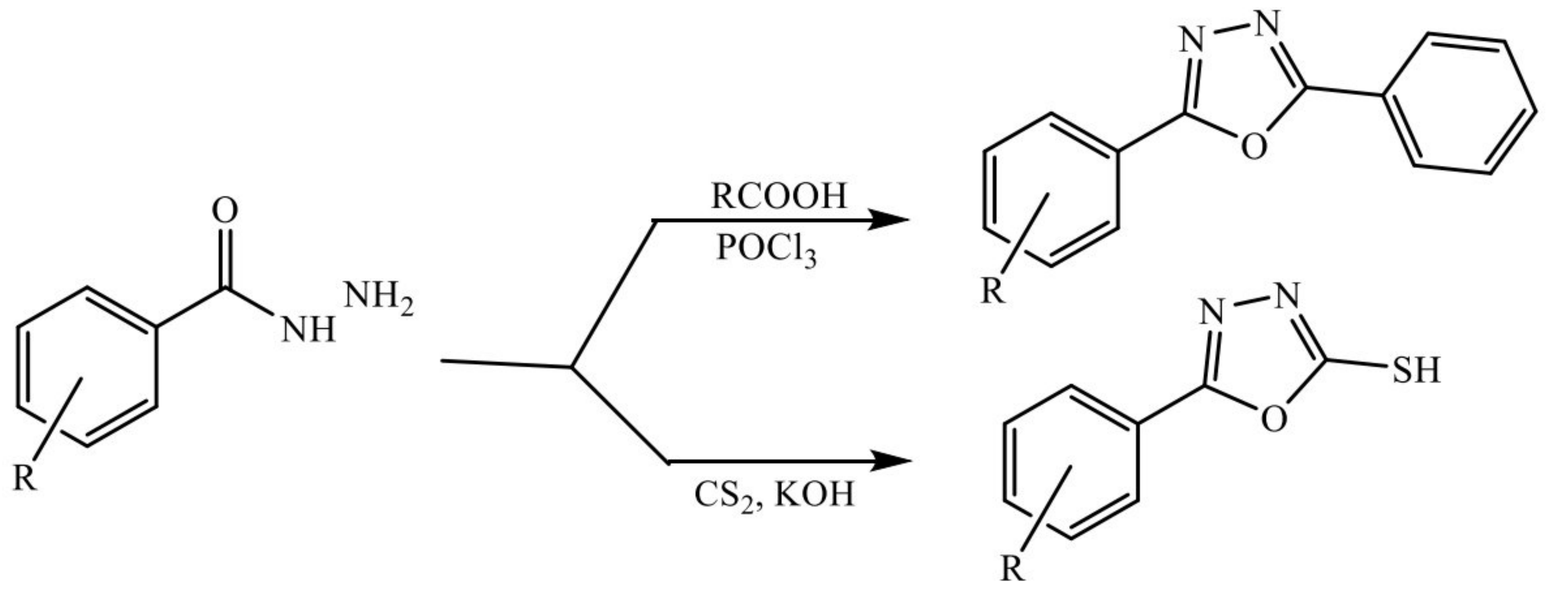


Figure 3.6: Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles

A new route was adopted for the direct annulation of hydrazides with methyl ketone to obtain 1,3,4-oxadiazole (Gao et al., 2015).

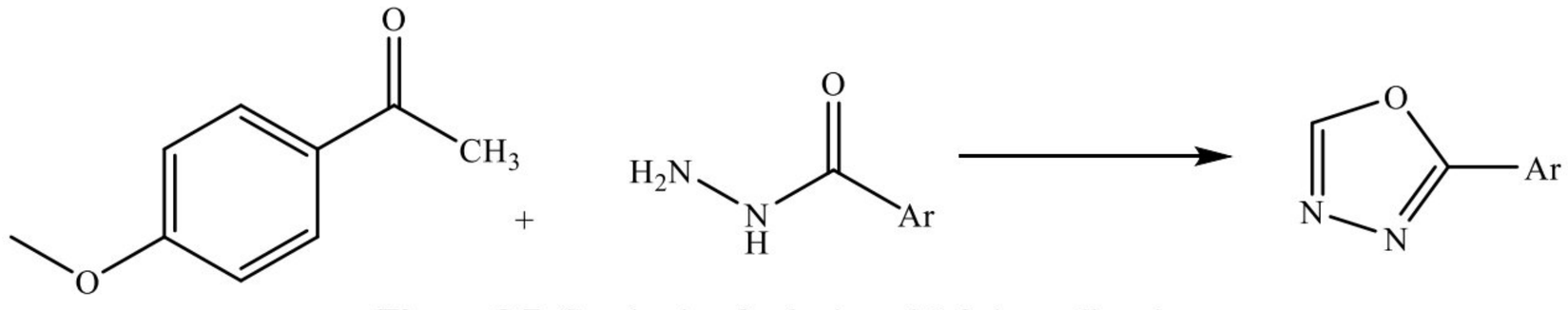


Figure 3.7: Synthesis of substituted 1,3,4-oxadiazoles

Oxadiazoles have been developed with the help of condensation of substituted aryl hydrazones and aldehydes by using iodine in the presence of potassium carbonate in DMSO (Yu et al., 2013).

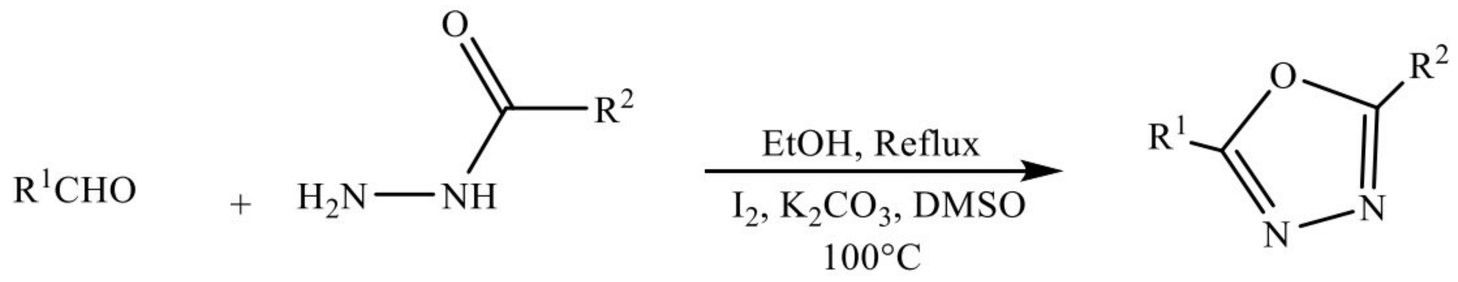


Figure 3.8: Synthesis of 2,5-disubstituted-1,3,4-oxadiazole

The radical-advanced cross-dehydrogenative coupling approach was used to produce 2,5-diaryl-1,3,4-oxadiazoles. Utilizing di-tert-butyl peroxide and dicumyl peroxide, aryl tetrazoles are N-acylated with aromatic aldehydes and then thermally rearranged (L. Wang et al., 2015).

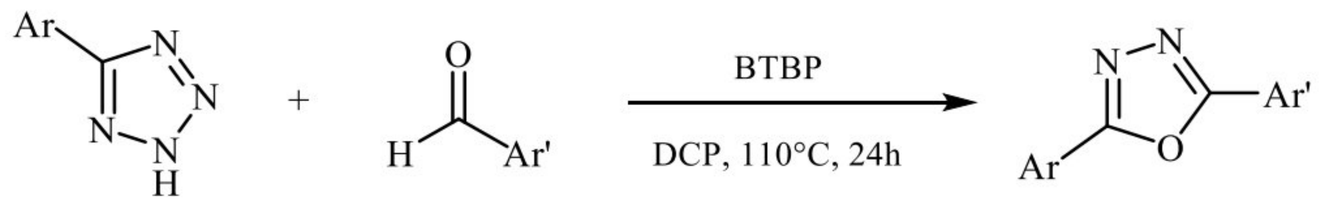


Figure 3.9: Synthesis of 2,5-diaryl 1,3,4-oxadiazole

The oxidative cyclization of semicarbazones by utilizing eosin Y as a catalyst and CBr_4 as a brominating agent to yield substituted amino-1,3,4-oxadiazoles. The reaction also utilized methyl cyanide with noticeable light and oxygen (Kapoorr et al., 2015).

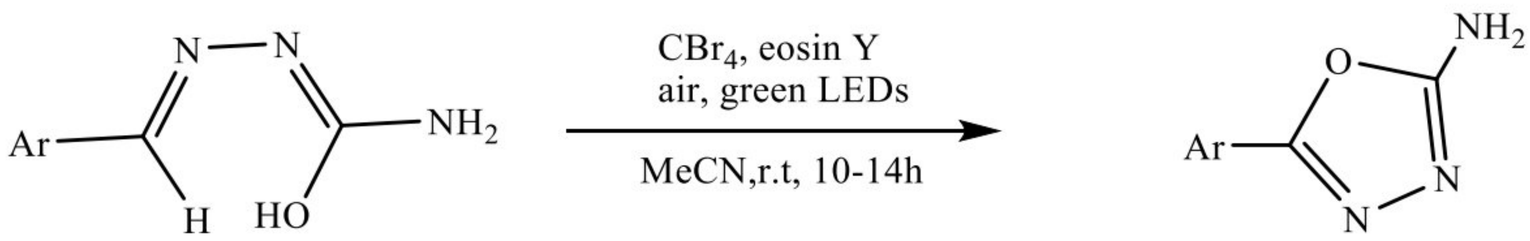


Figure 3.10: Synthesis of 5-substituted 2-amino-1,3,4-oxadiazoles

The oxidative annulation of aryl acid hydrazide by adding alkyl isocyanide into O-H and N-H bond of hydrazide in the presence of palladium acetate and toluene for 2-3h and at about 80 ℃ gave 2-aryl amino-1,3,4-oxadiazole (Fang et al., 2014).

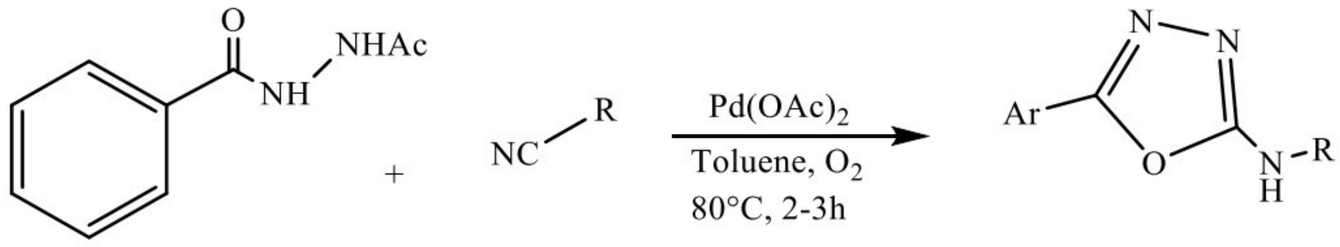


Figure 3.11: Synthesis of 2-aryl amino-1,3,4-oxadiazole.

The oxadiazole heterocycles were formed by the desulphurization reaction using oxone and iodobenzene at room temperature in presence of triethyl amine. The

formation of 1,3,4-oxadiazole derivative by this process was easier, and simple (Patel et al., 2012).

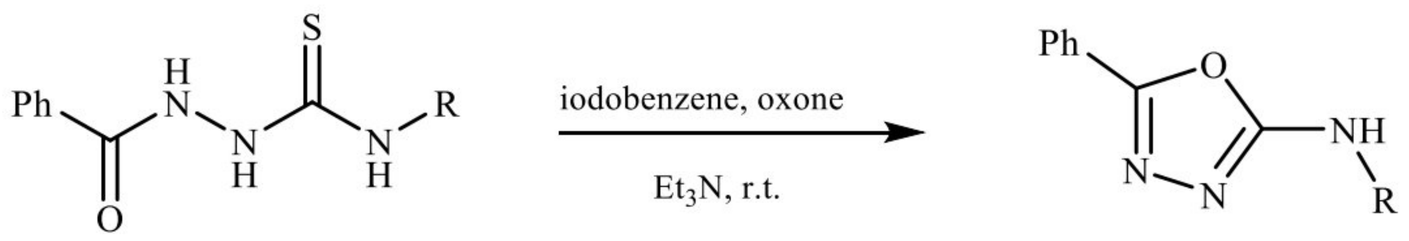


Figure 3.12: Synthesis of amine-substituted aryl-1,3,4-oxadiazole

The treatment of a thiosemicarbazide EDC-HCl in DMSO for 2h at 60 ℃ yields corresponding 2-amino-1,3,4-oxadiazoles (Yang et al., 2013).

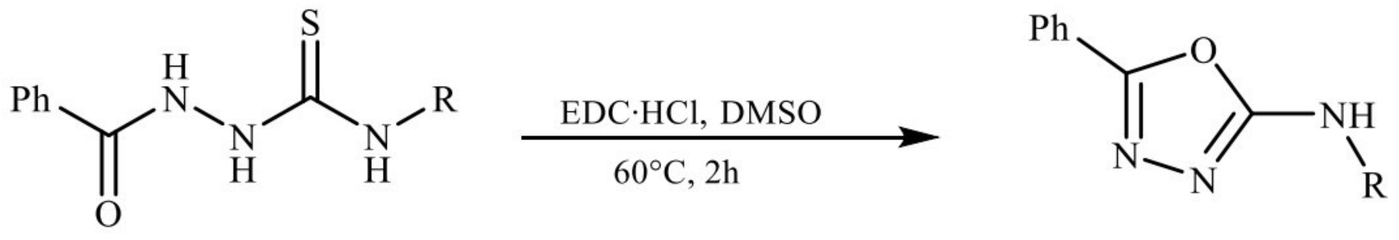


Figure 3.13: Synthesis of phenyl substituted 2-amino-1,3,4-oxadiazole

3.2 Biological activity

3.2.1 Antimicrobial activity

The activity of antibacterial agents against clinical MRSA strains and drug-resistant *S. aureus* strains was assessed. Among the synthesized compounds, the following one showed excellent antibacterial activity against MRSA (MIC: 0.250-1 g/mL) and *S. aureus* (MIC: 2 g/mL). The synthesized compound showed very low cytotoxicity to NRK-52E cells while inhibiting the bacteria and quickly destroying their membranes (Guo et al., 2019).

The method of structural-based molecular docking revealed the findings of phenoxy methyl-phenyl-1,3,4-oxadiazole derivatives. The compounds were synthesized and evaluated for their antiproliferative activities against MDA-MB-453 and MCF-7. Furthermore, the test compounds were predicted for the Lipinski rule of five and pharmacokinetic properties. These research results reveal that the two compounds listed below have greater cytotoxicity, dose-dependent activity, and reduced cell viability (Lakshmithendral et al., 2019).

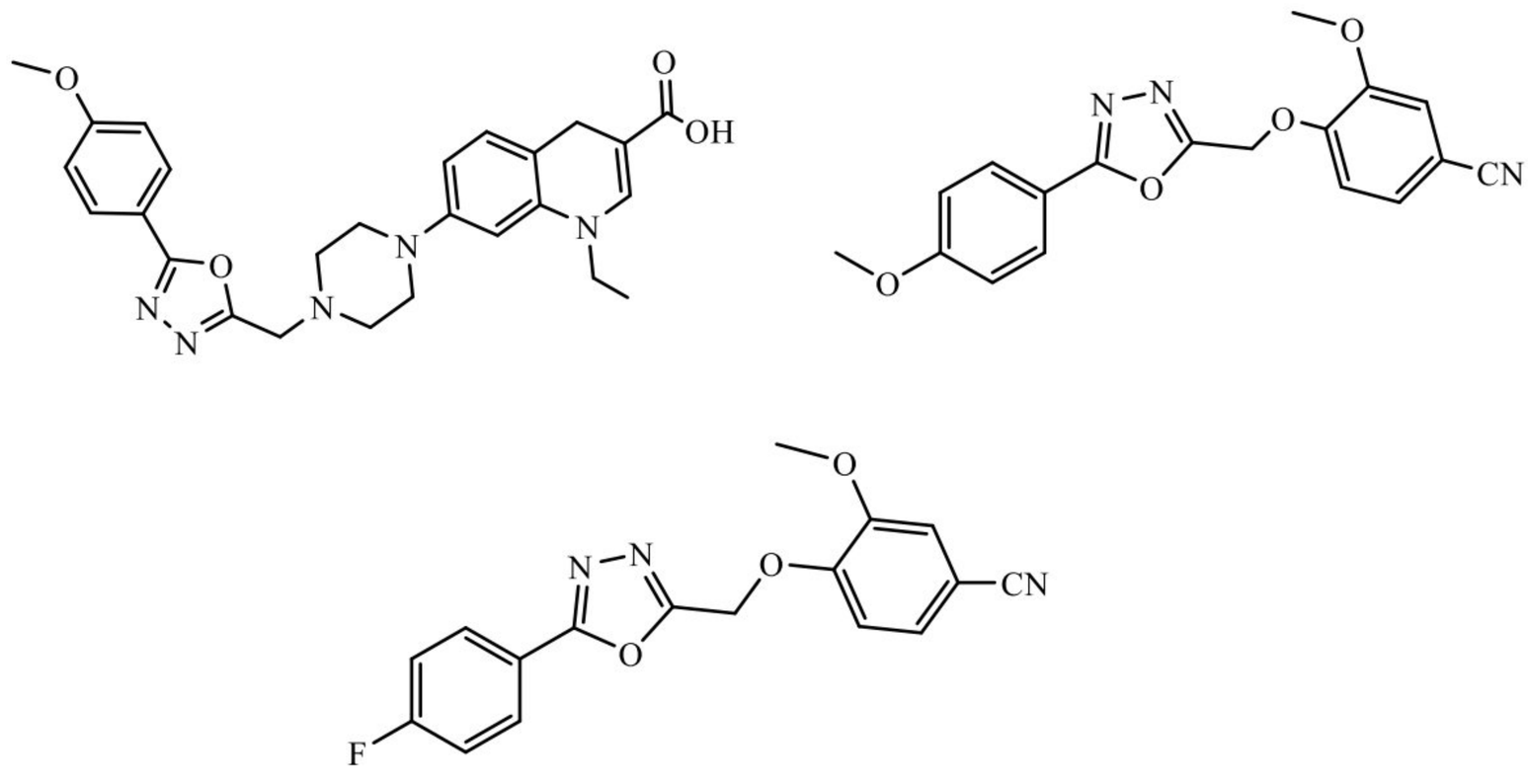


Figure 3.14: Chemical structure of phenoxy methyl-phenyl-1,3,4-oxadiazole derivatives

Some 1,3,4-oxadiazole thioether derivatives were created and tested for antibacterial activity against *X. oryzea PV. Oryza (*Xoo*)* via the *in vitro* turbidimeter test. The majority of these compounds showed good antibacterial activity, according to preliminary bioassay results. The following compound had the best inhibitory effect against Xoo out of the synthesized compounds, with a half-maximum effective concentration (EC_{50}) value of 4.780 µg mL^{-1} (Song et al., 2018).

With the help of an analysis of the 120 derivatives of the lead structure, the structure-activity relationship (SAR) for the recently discovered oxadiazole class of antibiotics was described. *In-silico* docking and scoring against a penicillin-binding protein's crystal structure led to the discovery of this class of antibiotics. They inhibit cell-wall biosynthesis and exhibit promising antibacterial activity against the Gram-positive bacterium, *S. aureus*, including vancomycin-resistant and linezolid-resistant *S. aureus* (VRSA) and methicillin-resistant *S. aureus* (MRSA). Several of them showed long life, a wide volume of distribution, and low clearance, and effective in a mouse model of MRSA infection. This class of medicines shows tremendous potential as a treatment for MRSA infection (Spink et al., 2015).

A series of arylamino-(p-nitrosothiomethyl)-1,3,4-oxadiazoles were created by Vashi et al. (1996) and tested for biological activity against gram-positive and gram-negative bacteria such *S. citrus* and *B. megaterium*, *E. coli*, and *S. typhi*. The majority of the compounds demonstrated effective activity because they contained methyloxy and isopropyl groups.

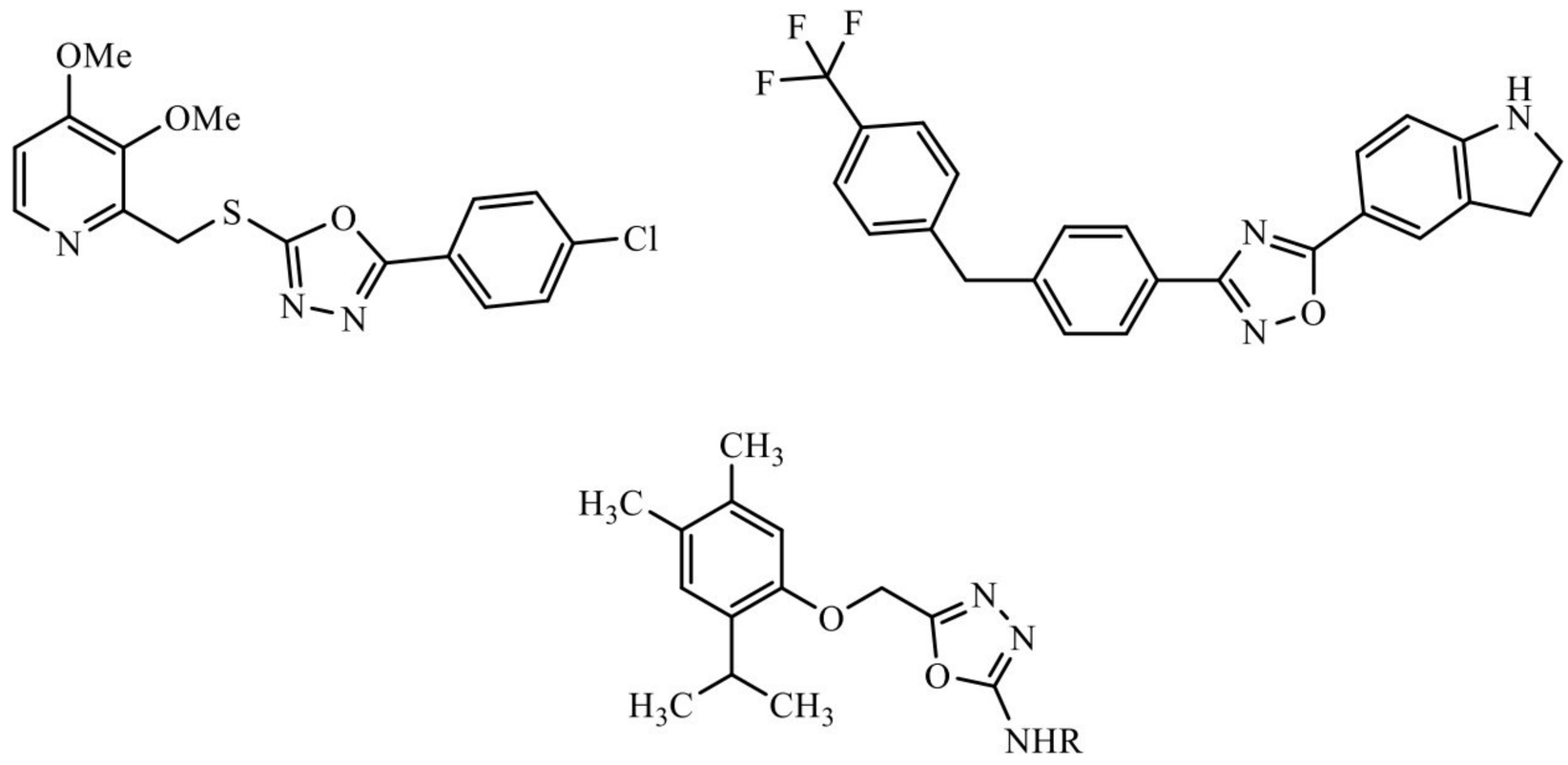
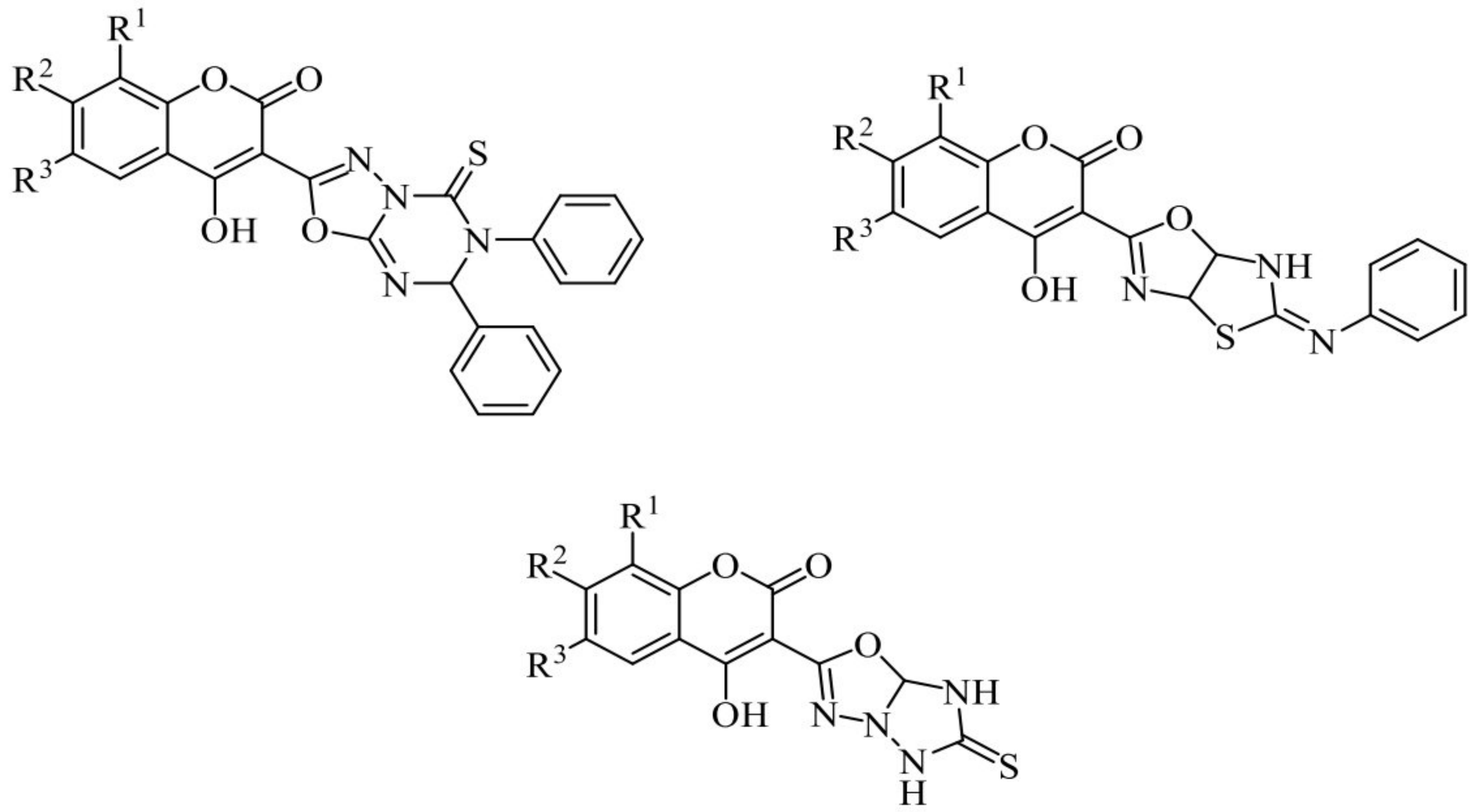


Figure 3.15: Chemical structure of arylamino derivatives of 1,3,4-oxadiazoles

The antibacterial activity against *S. aureus, S. typhi*, and *E. coli* was also investigated for some novel oxadiazole-[1,3,5] thiazolo-1,3,4-oxadiazole and 1,2,4-triazolo1,3,4-oxadiazole derivatives. The MIC values were obtained by tube dilution method using ciprofloxacin as a positive control. The majority of the compounds in this class have been found to have considerable activity (Mulwad & Chaskar, 2006).



R^1, R^2, R^3 = H or CH_3

Figure 3.16: Structures of oxadiazole-triazine, thiazolo-1,3,4-oxadiazole derivatives

The biological activity of a few 1,3,4-oxadiazole coumarin derivatives against various bacterial strains was produced and tested. The vast majority of the synthetic chemicals were discovered to be effective against those strains (Khan & Akhtar, 2003).

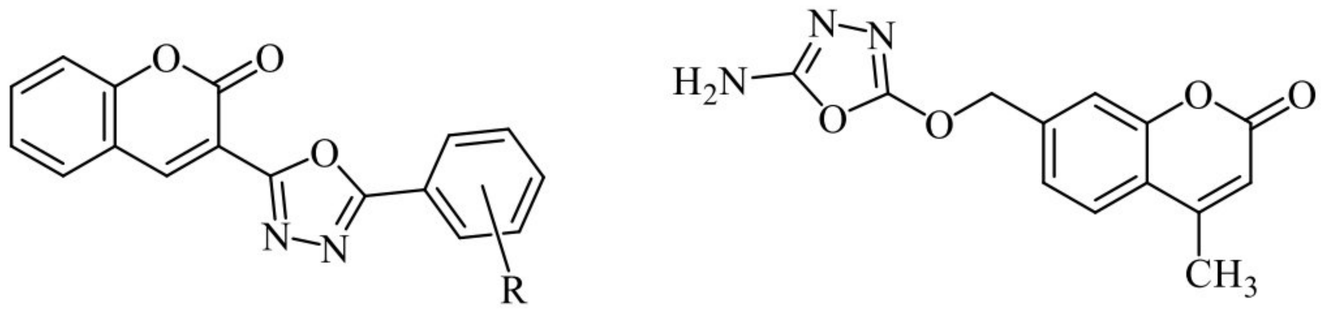


Figure 3.17: Chemical structure of some coumarin derivatives of 1,3,4-oxadiazoles

Different Naphthyloxymethyl-1,3,4-oxadiazole derivatives were created, and their antibacterial effects were tested using the microbroth dilution method on *S. aureus, E. coli, P. aeruginosa, C. albicans, C. krusei*, and *C. parapsilosis*. While compound (a) was only moderately active (64 μg mL^{-1}) against *C. krusei*, compounds (b) and (c) were significantly (32 μg mL^{-1}) active (Khan & Akhtar, 2003).

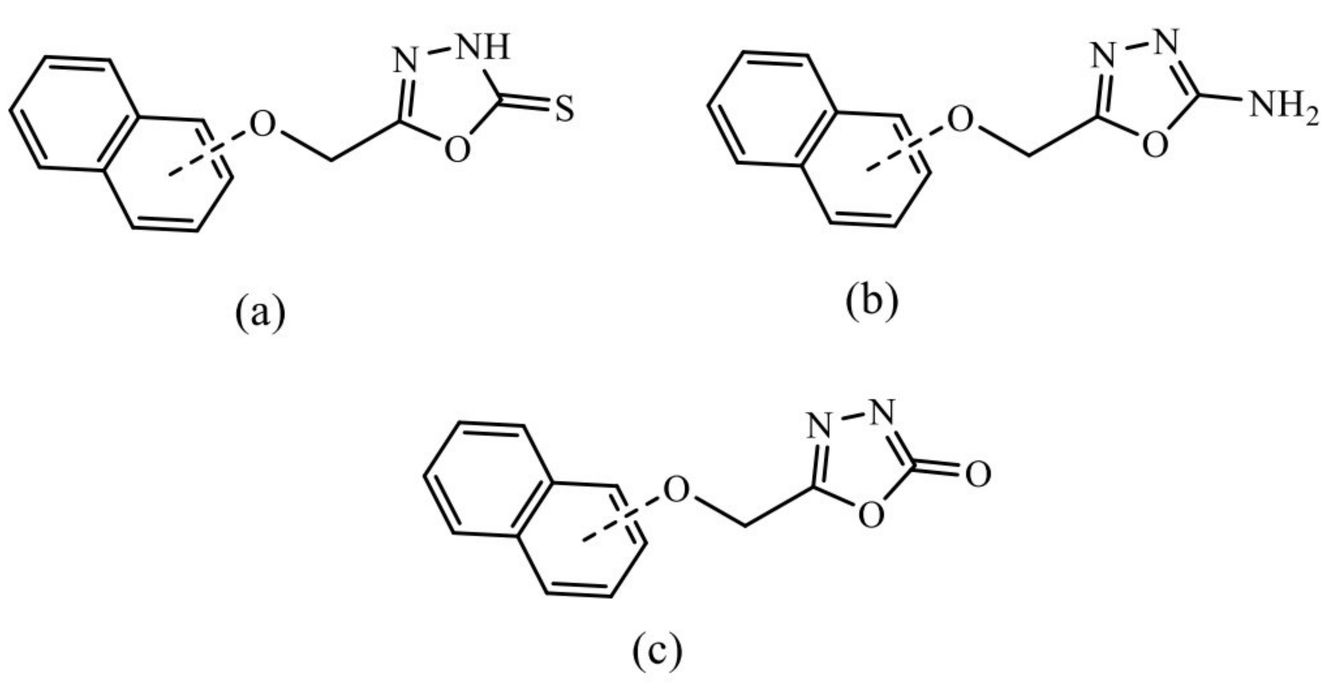


Figure 3.18: Chemical structure of various Naphthyloxymethyl-1,3,4-oxadiazole derivatives

Microwave techniques were to develop and synthesize a variety of 2,5-disubstituted 1,3,4-oxadiazoles. These substances were tested for their *in vitro* antifungal activity against *C. albicans* and *F. oxysporum* using miconazole as a positive control. By contrasting their MIC values with miconazole and fluconazole, the structure-activity relationship (SAR) has been determined. The following substances were discovered to be equally effective as miconazole against tested strains (Sangshetti et al., 2011).

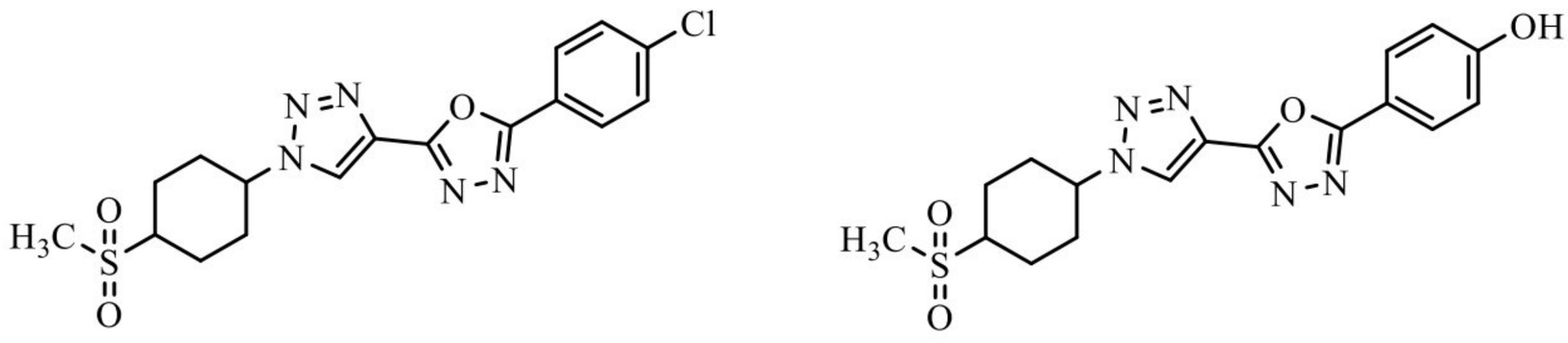


Figure 3.19: Chemical structure of 2,5-disubstituted-1,3,4-oxadiazoles

Numerous aryl-4-hydroxy derivatives with a 1,3,4-oxadiazole moiety were created and tested for their ability to inhibit the growth of *X. oryzae pv. oryzae* (Xoo*), R.*

solanacearum, and *X. axonopodis pv. citri* (Xac). Additionally, the tobacco mosaic virus was shown to be resistant to some therapeutic and protective activities. With half-maximal effective concentration (EC_{50}) values of 8.5 μg mL^{-1} and 7.2 μg mL^{-1}, respectively, further research showed that the first two compounds had the best inhibitory effect against Xoo (Wang et al., 2017).

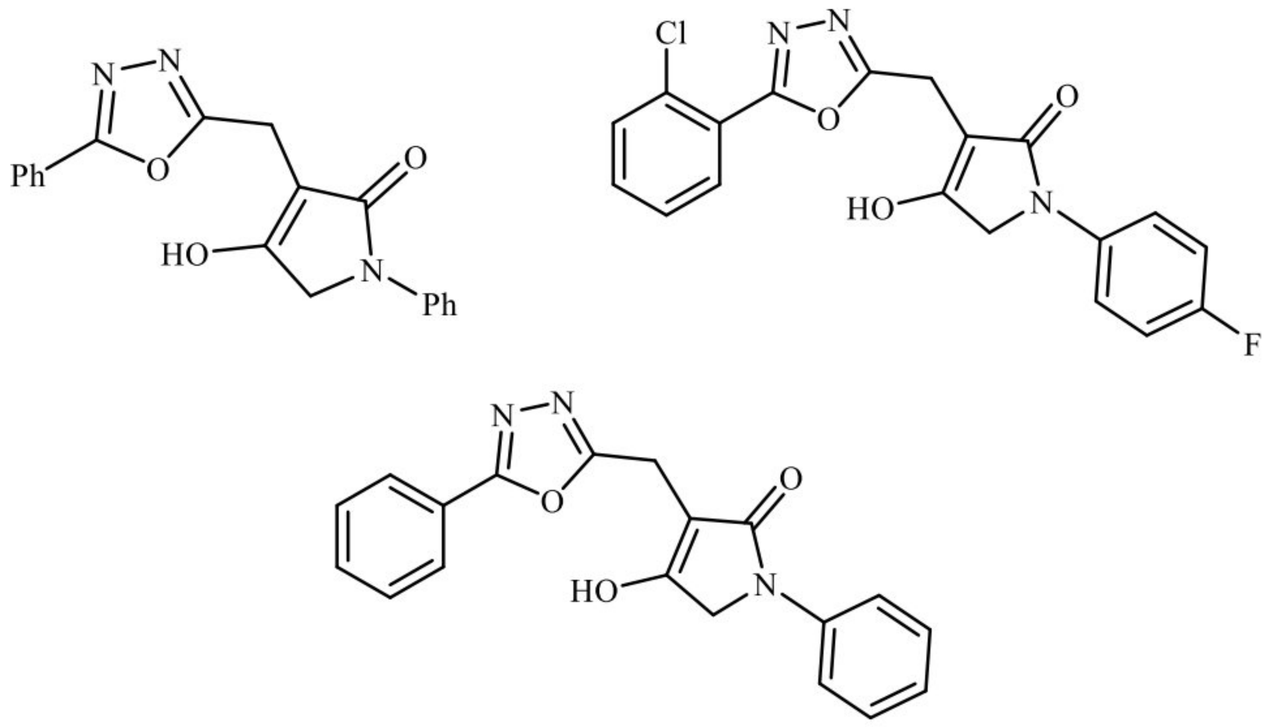
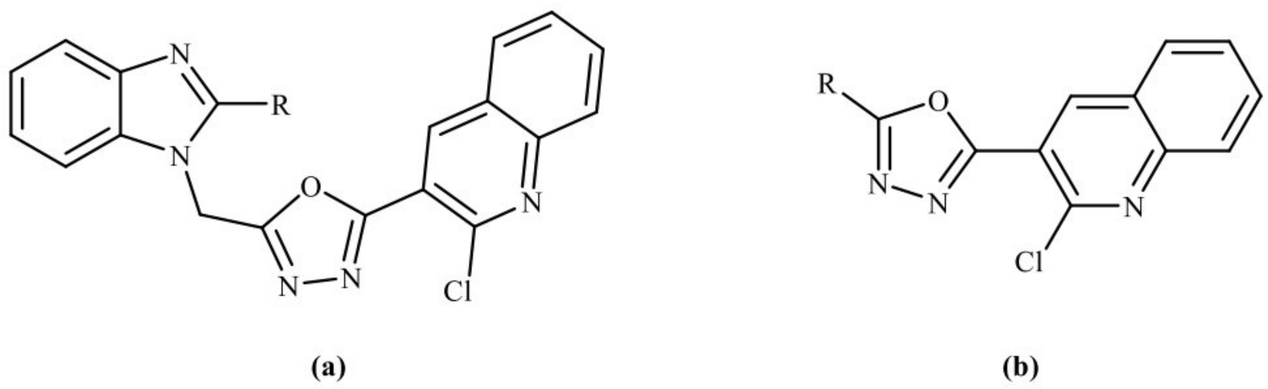


Figure 3.20: Structure of 1-aryl-4-hydroxy bearing 1,3,4-oxadiazole moiety

3.2.2 Anticancer activity

Substituted benzimidazol-1,3,4-oxadiazole quinolone derivatives were synthesized and tested for anticancer activity. 60 cells were screened on various cell lines at a single high dose (10^{-5} M). In an *in vitro* screen on the tested cancer cell lines, compound (b) showed 95.71 growth percent and was highly active on SNB-75 (CNS cancer) and UO-31 (renal cancer) (GP 53.35 and 64.30, respectively), while compound (a) showed 96.85 GP and was highly active on SNB-75 (CNS cancer) (CNS cancer GP 51.28) (Mazumder & Shaharyar, 2015).



(a) **(b)**

Figure 3.21: Chemical structure of chloro derivative of naphthalene substituted 1,3,4-oxadiazoles

The anticancer activity of triphenylpyrazol-phenyl-1,3,4-oxadiazole derivatives was investigated. The compounds (concentration 10^{-4} M) demonstrated potent activity against CCRF-CEM, K-562, MOLT-4, PRMI-8226, and SR in a primary *in vitro* test (leukemia cell lines) (Liszkiewicz et al., 2003).

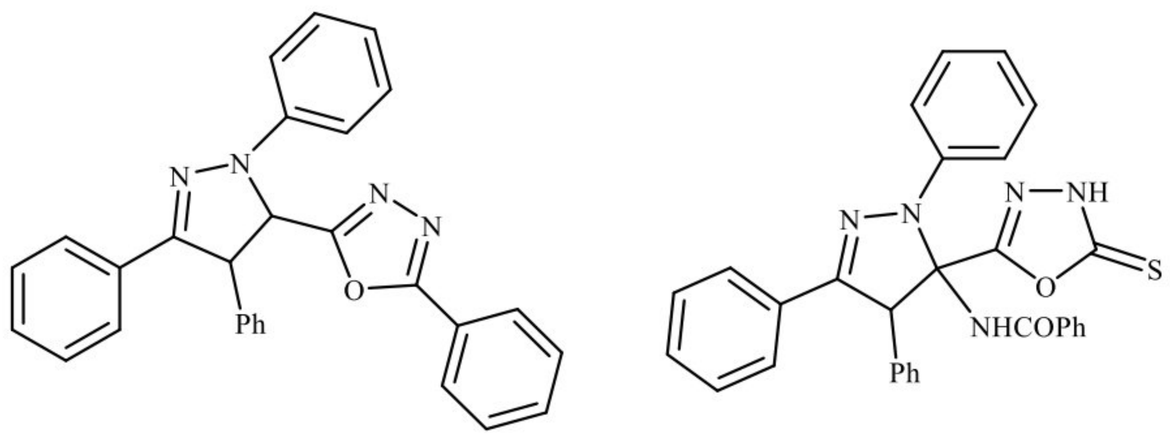


Figure 3.22: Structure of phenyl-substituted oxadiazoles

The synthesis of a series of benzimidazole substituted 1,3,4-oxadiazole. The compounds were tested for anticancer activity *in vitro* on NCI 60 cells at a single high dose (10^{-5} M). Only the second was discovered to be the most active in a breast cancer cell line (Salahuddin et al., 2017).

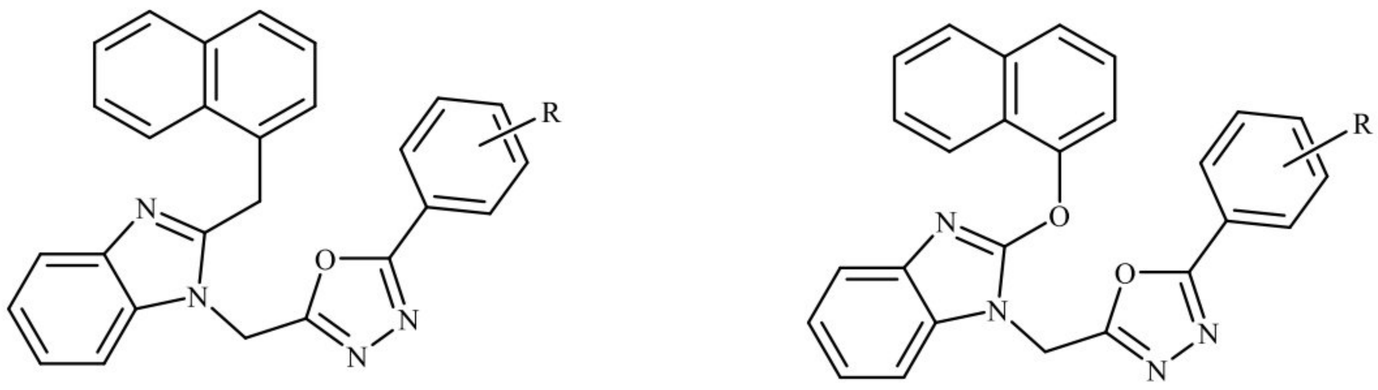


Figure 3.23 Structure of benzimidazole substituted 1,3,4-oxadiazoles

3.2.3 Anti-inflammatory activity

It was discovered that pyridine-substituted 1,3,4-oxadiazole-2-thiol derivatives have anti-inflammatory activity. Using indomethacin as a reference drug, both compounds demonstrated significant inhibition, with 40.70 % and 39.20 % inhibition, respectively (Khan et al., 2004).

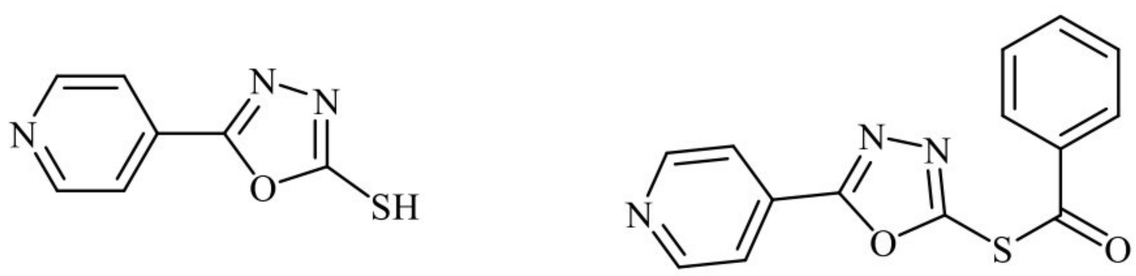


Figure 3.24: Structure of pyridine substituted 1,3,4-oxadiazole-2-thiol derivatives

A new series of 2,5-disubstituted-1,3,4-oxadiazoles was synthesized and tested for anti-inflammatory activity. The following compounds demonstrated the highest activity among the synthesized compounds (Amir et al., 2007).

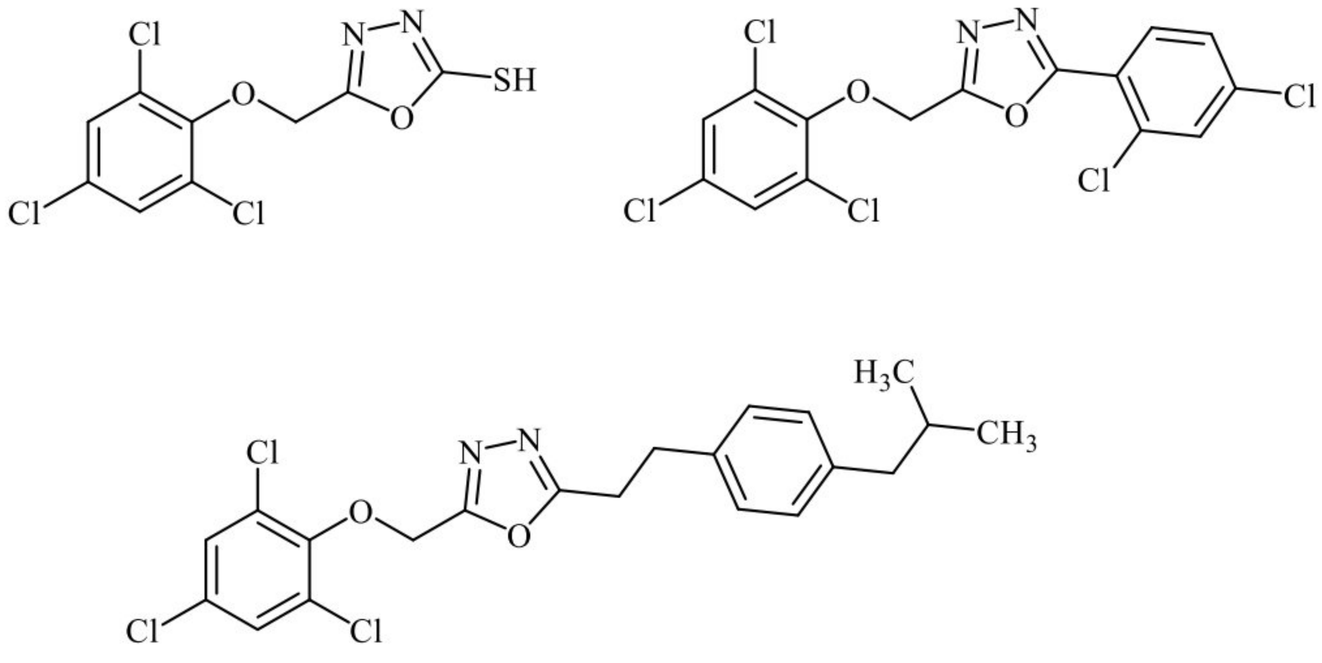


Figure 3.25: Chemical structure of A new series of new 2,5-disubstituted-1,3,4-oxadiazoles

The synthesis of alkyl-substituted 1,3,4-oxadiazole derivatives and assessment of their anti-inflammatory activity was reported by Omar et al. (1996). The promising results have been noticed when R^1 and R^2 are replaced by 3-pyridyl and 4-pyridyl.

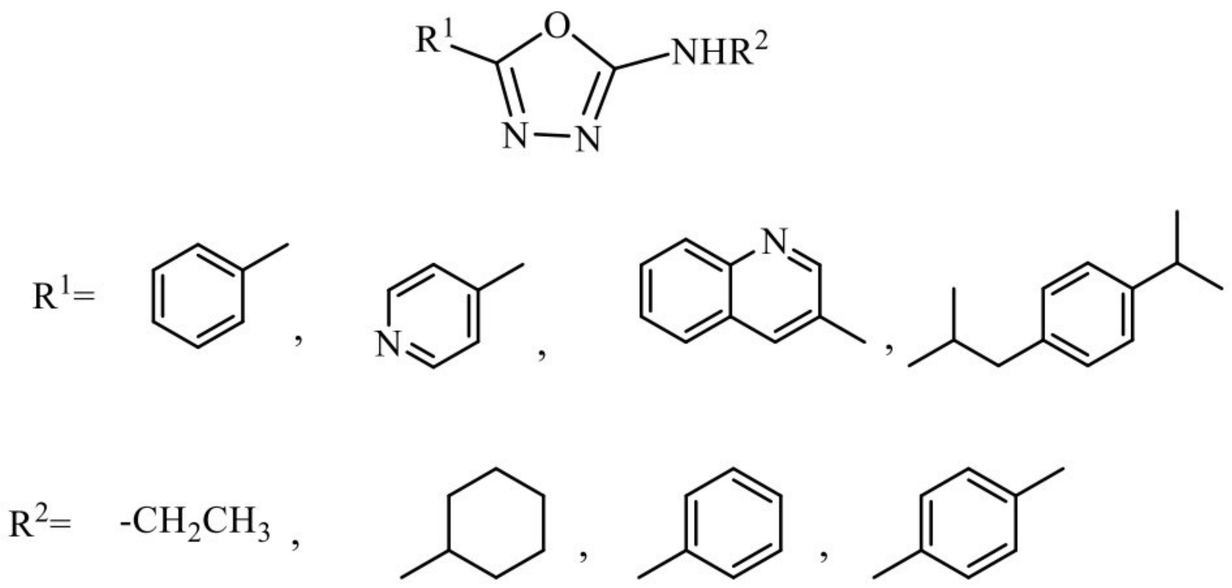


Figure 3.26: Structure of amine substituted 1,3,4-oxadiazole derivatives

3.2.4 Anticonvulsant activity

A couple of arylsulphoamido substituted 1,3,4-oxadiazole derivatives were prepared and checked for their anticonvulsant activity adopting the maximum electroshock seizure (MES) method and subcutaneous pentylenetetrazole (scPTZ) methods (Ladva et al., 1996).

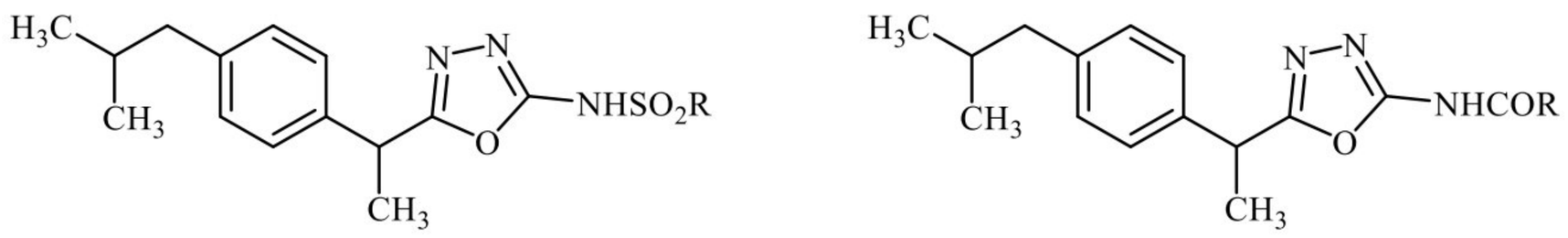


Figure 3.27: Chemical structure of arylsulphoamido substituted 1,3,4-oxadiazole derivatives

3.2.5 Antitubercular activity

Several benzimidazole substituted aryl sulfonamide-1,3,4-oxadiazoles have been designed, synthesized, and tested for antitubercular activity against *M. tuberculosis* (Kagthara et al., 1999).

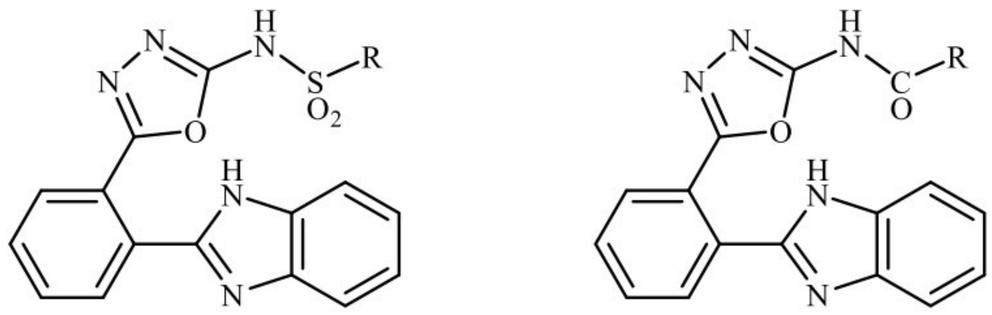


Figure 3.28: Chemical structure benzimidazole substituted aryl sulfonamide-1,3,4-oxadiazoles

3.2.6 Hypoglycemic activity

Hokfelt & Jonsson. (1962) created compounds of arylamino-oxoquinazoline-1,3,4-oxadiazole and examined their hypoglycemic action. Some of the synthetically created substances demonstrated potential hypoglycemic action. The alkyl group present in the compound comprises three to five carbon atoms that were found to have maximum activity.

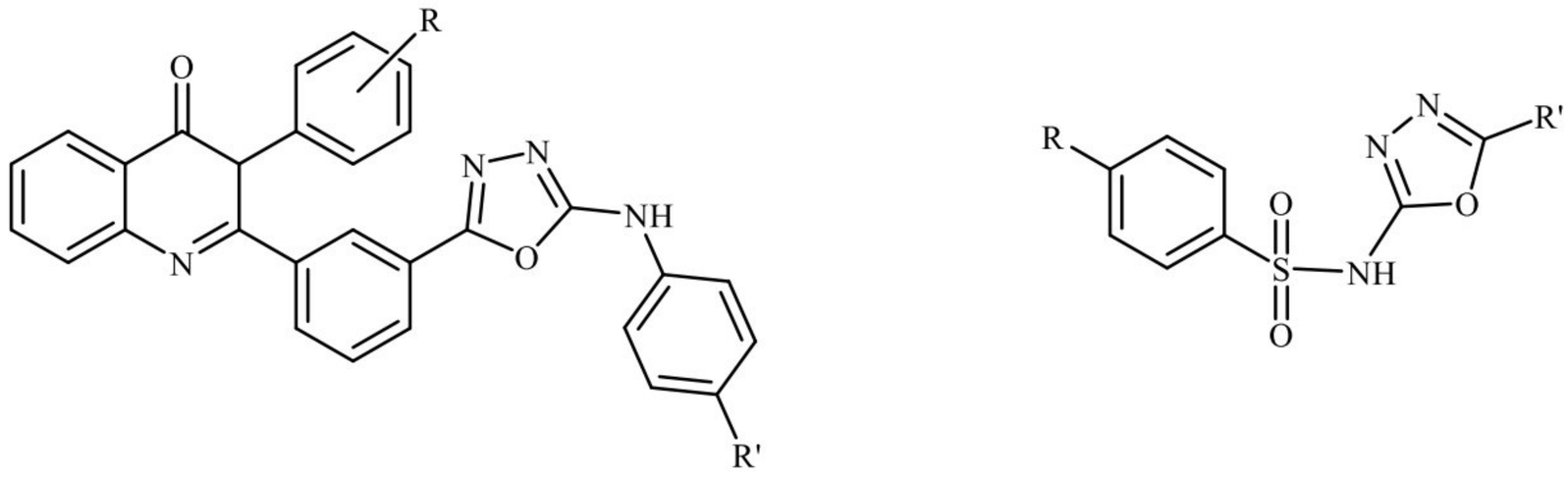


Figure 3.29: Structure of arylamino-oxoquinazoline-1,3,4-oxadiazoles derivatives

CHAPTER 4

4. MATERIALS AND METHODS

4.1 Materials

The precursors for the synthesis of the desired compounds were obtained from the chemistry laboratory of the Amrit campus and some of them were purchased from a commercial source. Sulphuric acid, methyl salicylate, methanol, potassium hydroxide (Fisher Scientific), piperazine, morpholine (Loba Chemie), and carbon disulphide (Merck) were used as received.

4.2 Methods

Thin-layer layer chromatography and melting point analysis were used to evaluate the compounds' purity. The synthesized compounds were characterized using a variety of spectroscopic methods.

4.2.1 Thin layer chromatography

R_f values of the synthesized compounds were calculated from the TLC analysis using silica gel-coated TLC plates. The solvents used for the analysis were *n*-hexane, ethyl acetate, and ethanol. Spots were visualized by using iodine vapors.

4.2.2 Melting point determination

Using a Thiele tube, the melting points of the synthesized compounds were evaluated and compared to the literature data.

4.2.3 UV-Visible spectroscopy

The absorption spectra of the synthesized compounds were captured using a double-beam UV-Visible spectrometer. All of the compounds are dissolved in the proper solvent, diluted to the lowest possible concentrations (1–10 ppm), and then measured for the absorption maximum.

4.2.4 FT-IR spectroscopy

The PerkinElmer Spectrum IR Version 10.6.2 was used to record the IR spectra of the synthesized compound in the Department of Chemistry, Amrit Campus. FT-IR spectra were measured in the range of 4000 cm^{-1} to 400 cm^{-1}.

4.2.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR and ^{13}C-NMR spectra of synthesized compounds were recorded by using deuterated methanol as a solvent at the lab of Jeonbuk National University, South Korea.

4.3 Synthesis

Here is the reaction scheme of the synthesis of targeted compounds.

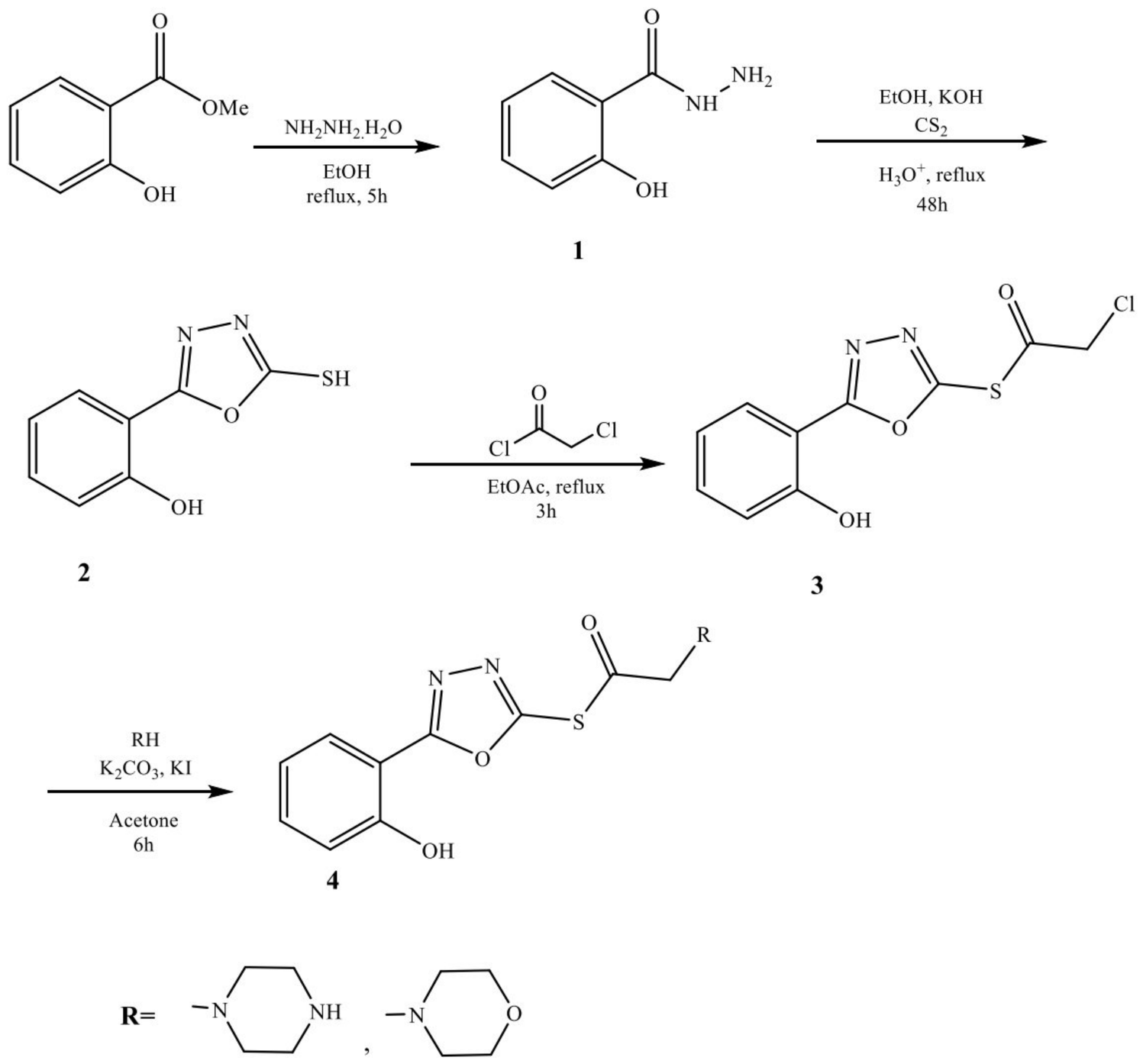


Figure 4.1: Reaction scheme of the synthesis of targeted compounds

Step 1: Synthesis of acid hydrazide (1)

A mixture of 15.200 g (0.1 mol) of methyl salicylate, 7.500 mL (0.15 mol) of hydrazine hydrate, and 50 mL of ethanol was refluxed for 5 hours. The excess solvent was evaporated in a water bath and the reaction mixture was cooled. The solid was washed with cold ethanol and dried. It was then recrystallized from ethanol.

Yield, 83 %, 12.600 g (0.083 mol).

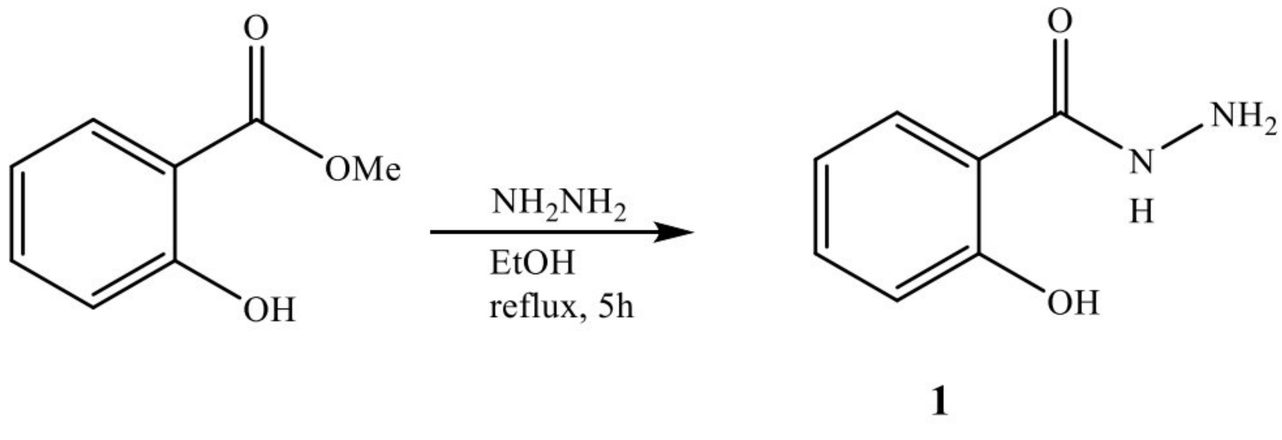
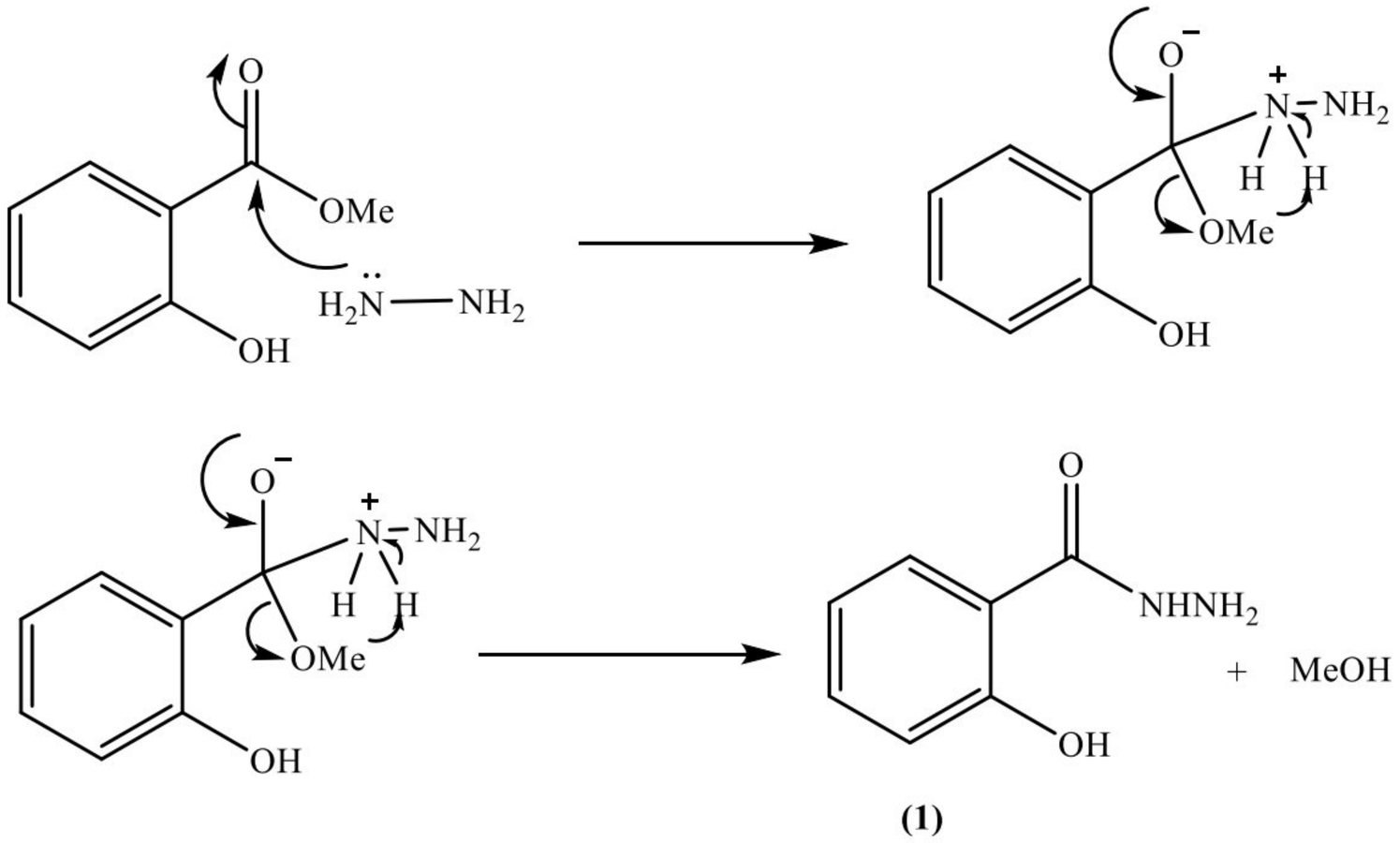


Figure 4.2: Reaction involved in the synthesis of acid hydrazide

Reaction mechanism



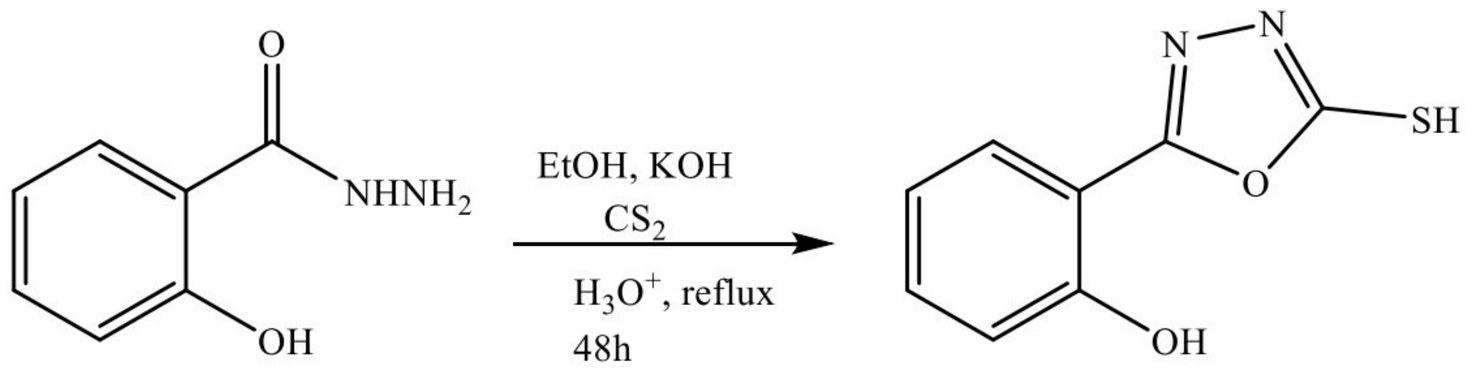
(1)

Figure 4.3: Reaction mechanism of the formation of acid hydrazide **(1)**

Step 2: Synthesis of 2-(5-mercapto-1,3,4-oxadiazol-2-yl)phenol (2)

7.600 g (0.05 mol) acid hydrazide was dissolved in 50 mL ethanol in an RB flask and cooled in an ice bath. 50 mL of CS_2 and 2.800 g (0.05 mol) of KOH in ethanol were added to the reaction mixture. It was stirred for 1 hour using a magnetic stirrer. The reaction mixture was refluxed for 48 hours. When the evolution of H_2S was stopped (it was checked by using lead acetate paper), the excess solvent was evaporated in a water bath. It was cooled on an ice bath and acidified with 4N HCl and the solid mass separated. It was filtered, dried, and recrystallized with ethanol.

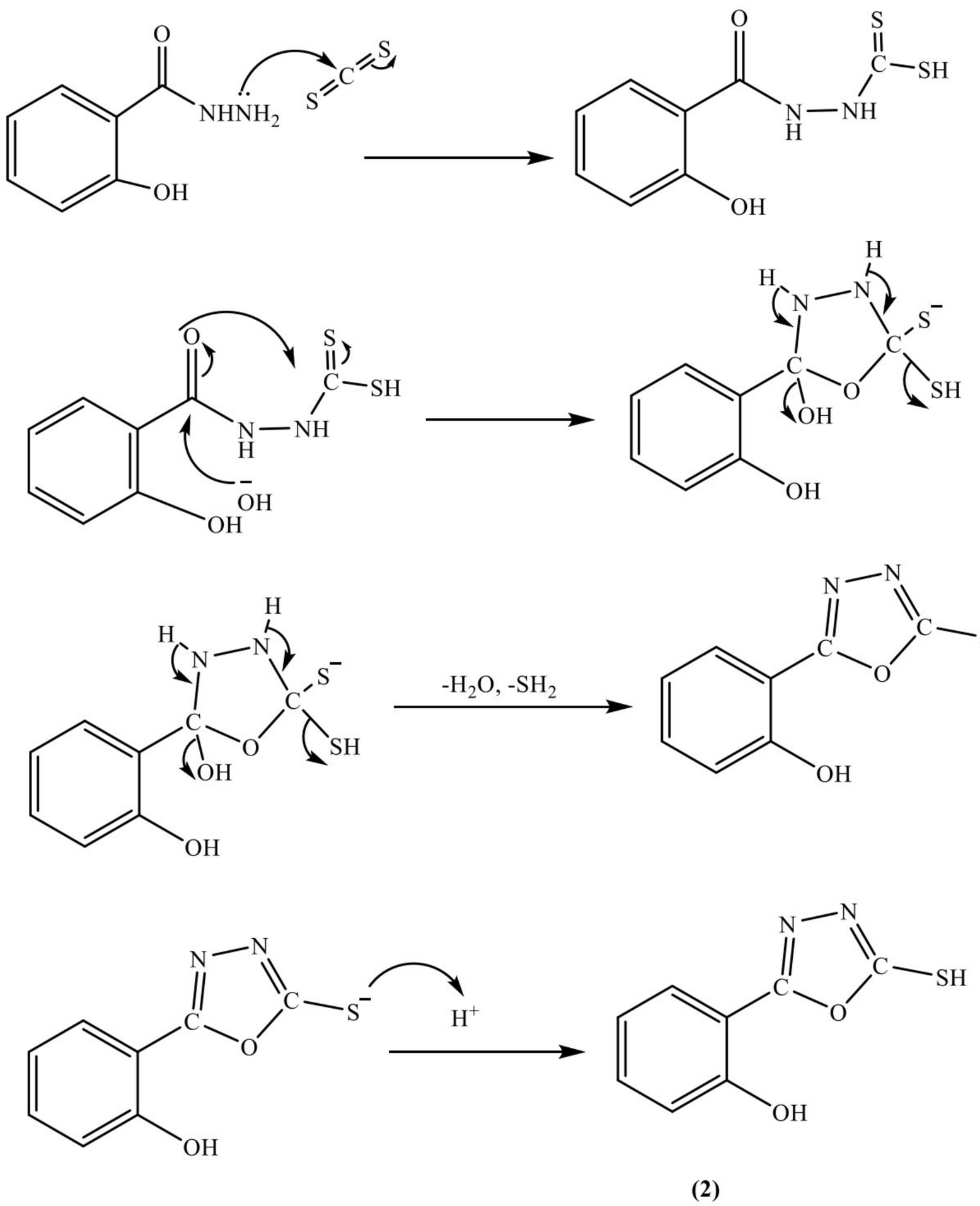
Yield, 74 %, 7.180 g



1 **2**

Figure 4.4: Reaction involved in the synthesis of 2-(5-mercapto-1,3,4-oxadiazol-2-yl)phenol **(2)**

Reaction mechanism



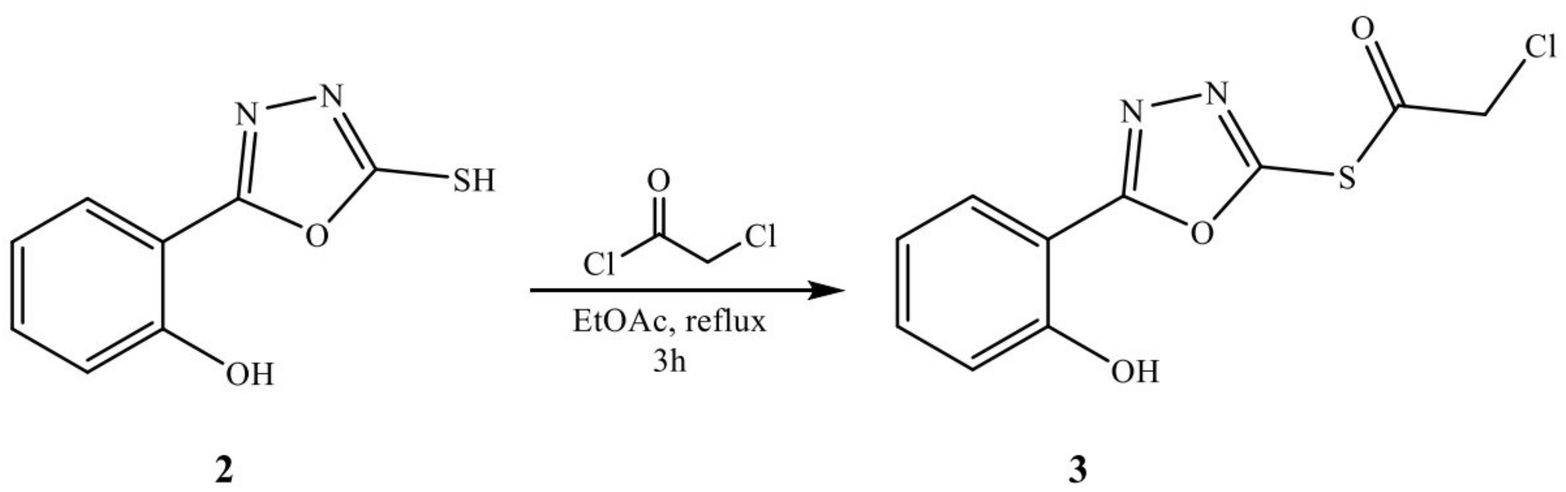
(2)

Figure 4.5: Reaction mechanism for the synthesis of **(2)** from **(1)**

Step 3: Synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-chloroethanethioate (3)

To 5.820 g (0.03 mol) of compound **(2)**, 3.390 mL (0.03 mol) of chloroacetyl chloride and 50 mL of ethyl acetate were added. It was refluxed for 5 hours, and when the solid separated the excess solvent was evaporated in a water bath. It was filtered, dried, and recrystallized from ethanol.

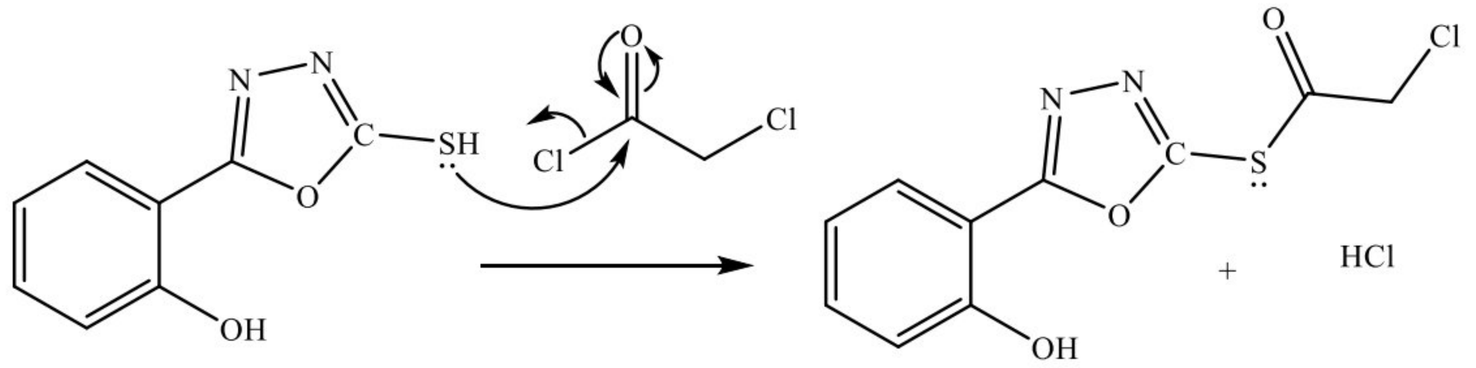
Yield, 78 %, 6.329 g



2 **3**

Figure 4.6: Reaction involved for the synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-chloroethanethioate (3)

Reaction mechanism



(3)

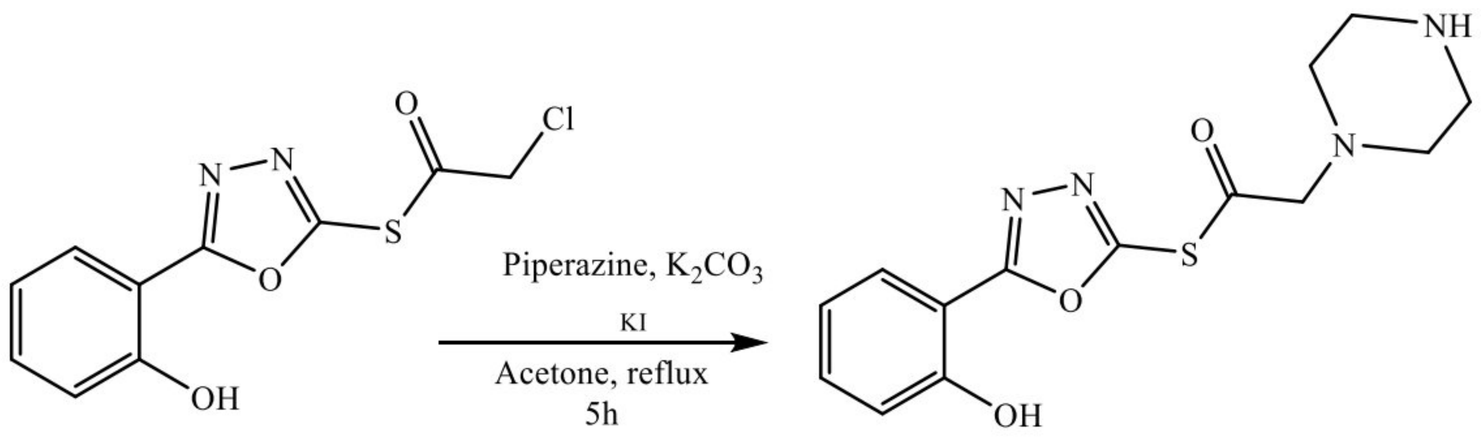
Figure 4.7: Reaction mechanism for the synthesis of **(3)** from **(2)**

Step 4

Synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-(piperazin-1-yl)ethanethioate (4a)

A mixture of compound **3** (2.700 g, 0.01 mol), K_2CO_3 (3.450 g), piperazine (0.860 g), KI (0.28 g), and 50 mL acetone was refluxed. After refluxing for 6 hours the excess solvent was evaporated and the residue was dissolved in methanol and filtered. The filtrate was concentrated, dried, and recrystallized from ethanol.

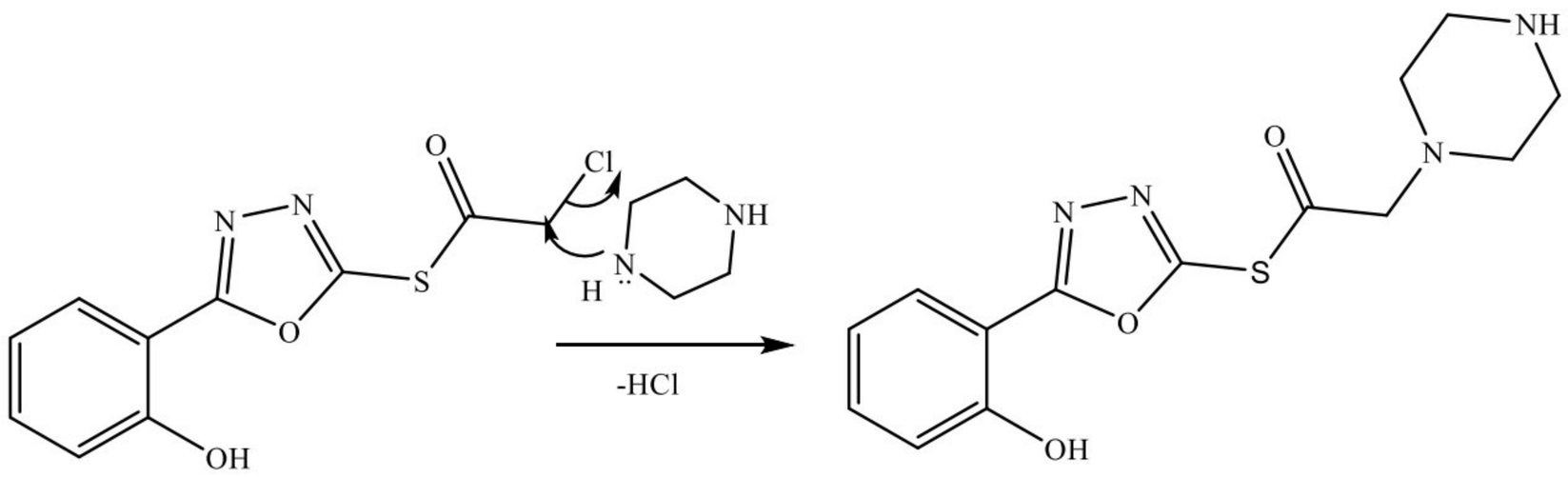
Yield, 58 %, 1.850 g



3 **4a**

Figure 4.8: Reaction involved for the synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-(piperazin-1-yl)ethanethioate **(4a)**

Reaction mechanism



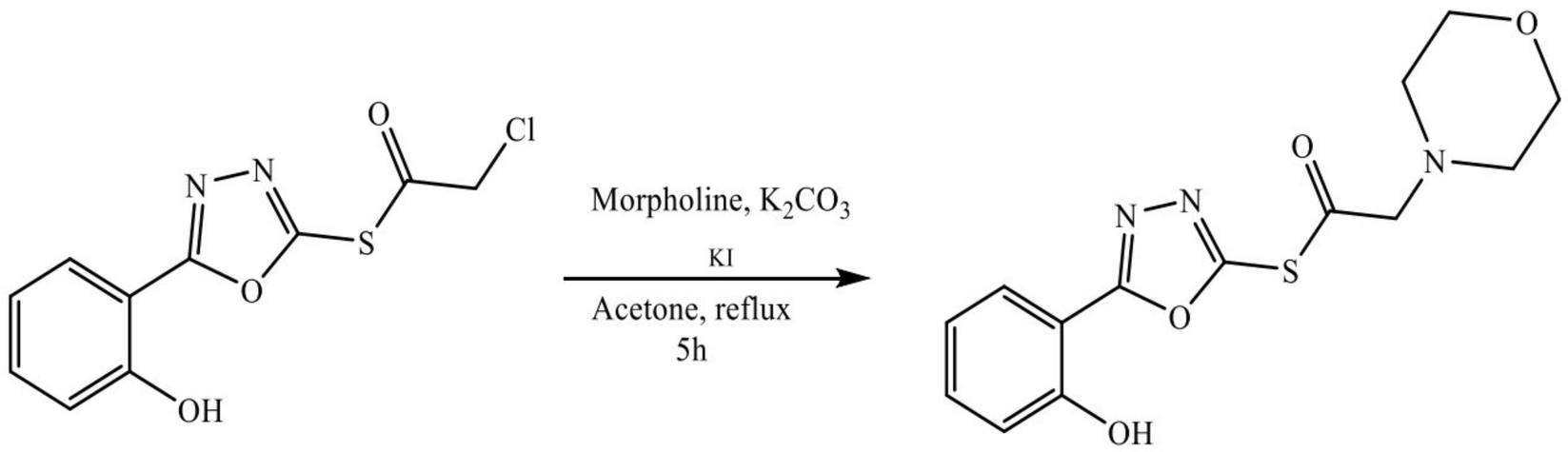
(4a)

Figure 4.9: Reaction mechanism for the synthesis of **(4a)** from **(3)**

Synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-morpholinoethanethioate (4b)

A mixture of compound **3** (2.700 g, 0.01 mol), K_2CO_3 (3.450 g), morpholine (0.870 g), KI (0.280 g), and 50 mL acetone was refluxed. After refluxing for 6 hours the excess solvent was evaporated and the residue was dissolved in methanol and filtered. The filtrate was concentrated, dried, and recrystallized from ethanol (Bettenhausen & Strohriegl, 1996).

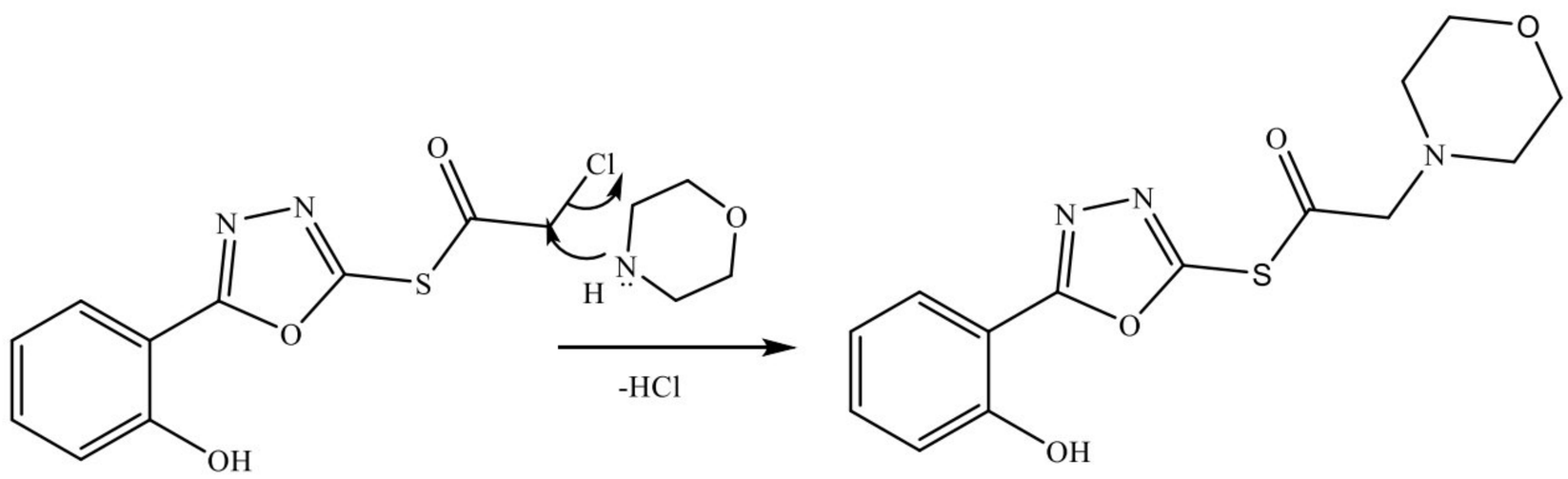
Yield, 51 %, 1.630 g



3 **4b**

Figure 4.10: Reaction involved for the synthesis of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-morpholinoethanethioate **(4b)**

Reaction mechanism



(4b)

Figure 4.11: Reaction mechanism for the synthesis of **(4b)** from **(3)**

4.4 Antimicrobial screening

The antibacterial activity of the newly synthesized compounds was screened against some gram-positive bacteria, gram-negative bacteria, and some fungal strains. It was done by using the agar well diffusion method.

DMSO was used as a solvent and as a positive control amoxicillin was used for the antibacterial activity. Similarly for antifungal screening, clotrimazole was used as a standard (positive control).

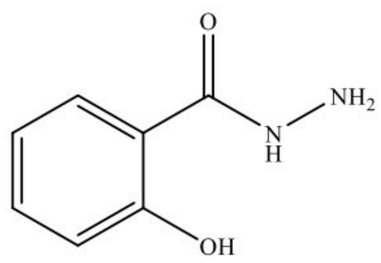
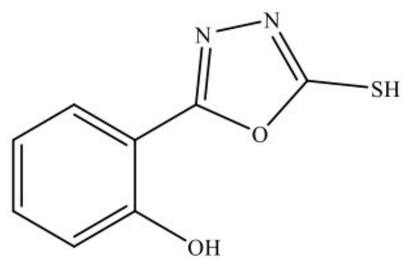
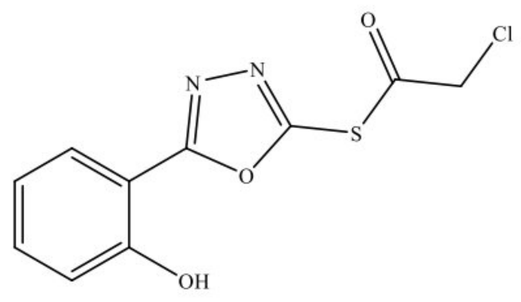
CHAPTER 5

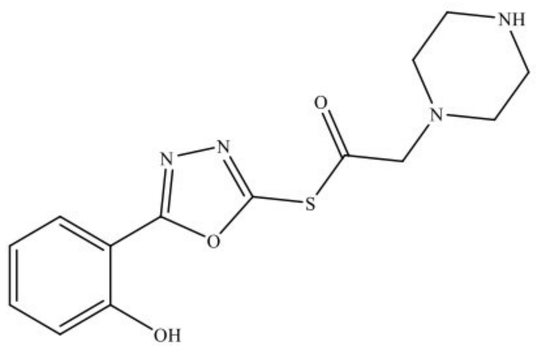
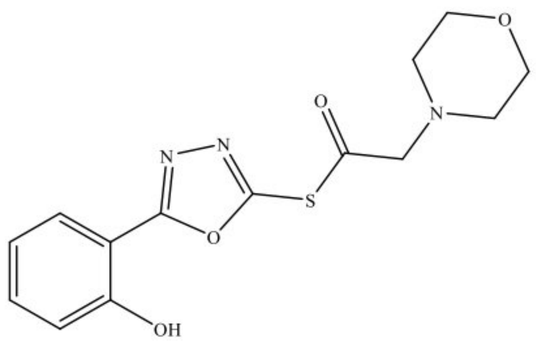
5. RESULTS AND DISCUSSION

5.1 General Discussion

The morpholine and piperazine derivatives of *S*-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) propanethioate were synthesized. Methyl salicylate was taken as the synthetic precursor for the preparation of these compounds. All the synthesized compounds at the successive steps were purified by recrystallization by using appropriate solvents. The purity of all synthesized compounds was checked by TLC analysis and determination of melting point. Final compounds and all the intermediates were synthesized in good yield. The compounds are thermally stable and found to be soluble in DMSO.

Table 5.1 General physical properties of the synthesized compounds

Compd	Structure	Physical appearance	Molecular mass	Yield (%)	M.P. (ºC)	R_f
1		White solid	152.06	83	145	0.65
2		White solid	194.01	74	189	0.52
3		Crystalline white solid	270	78	166	0.75

4a		Greyish white solid	320.09	58	228	0.48
4b		Brownish white solid	321.08	51	219	0.52

5.2 Spectral analysis

The characterization of synthesized compounds was done by using UV-Visible, IR, H-NMR, and C-NMR spectroscopy. All the spectra are found to be consistent with their structure.

5.2.1 UV-Visible spectroscopy

The UV-Visible spectral data of synthesized compounds are presented in table 5.2.

Table 5.2 λ_{max} of the synthesized compounds

Compound	λ_{max} (nm)	Inference
1	304	$\pi \rightarrow \pi^*$
	313	$n \rightarrow \pi^*$
2	304	$\pi \rightarrow \pi^*$
	313	$n \rightarrow \pi^*$
3	305	$\pi \rightarrow \pi^*$
	313	$n \rightarrow \pi^*$
4a	286	$\pi \rightarrow \pi^*$
	309	$n \rightarrow \pi^*$
4b	317	$\pi \rightarrow \pi^*$
	344	$n \rightarrow \pi^*$

In compound (**1**) the band at 304 nm is due to the $\pi \rightarrow \pi^*$ transition of C=C of the aromatic ring. Similarly, the band at 313 nm corresponds to the $n \rightarrow \pi^*$ transition of C=O of the carbonyl group of the acid hydrazide (Förster, 2004).

In the UV-Visible spectra of compounds (**2**) and (**3**), the band at 313 nm corresponds to C=N of the 1,3,4-oxadiazole ring. The peak at 304 and 314 nm are due to C=C of the aromatic ring (Perkampus, 2013).

The peak at 286 nm in compound **(4a)** is due to C=N of the oxadiazole ring and at 309 nm is attributed to C=C of the aromatic ring.

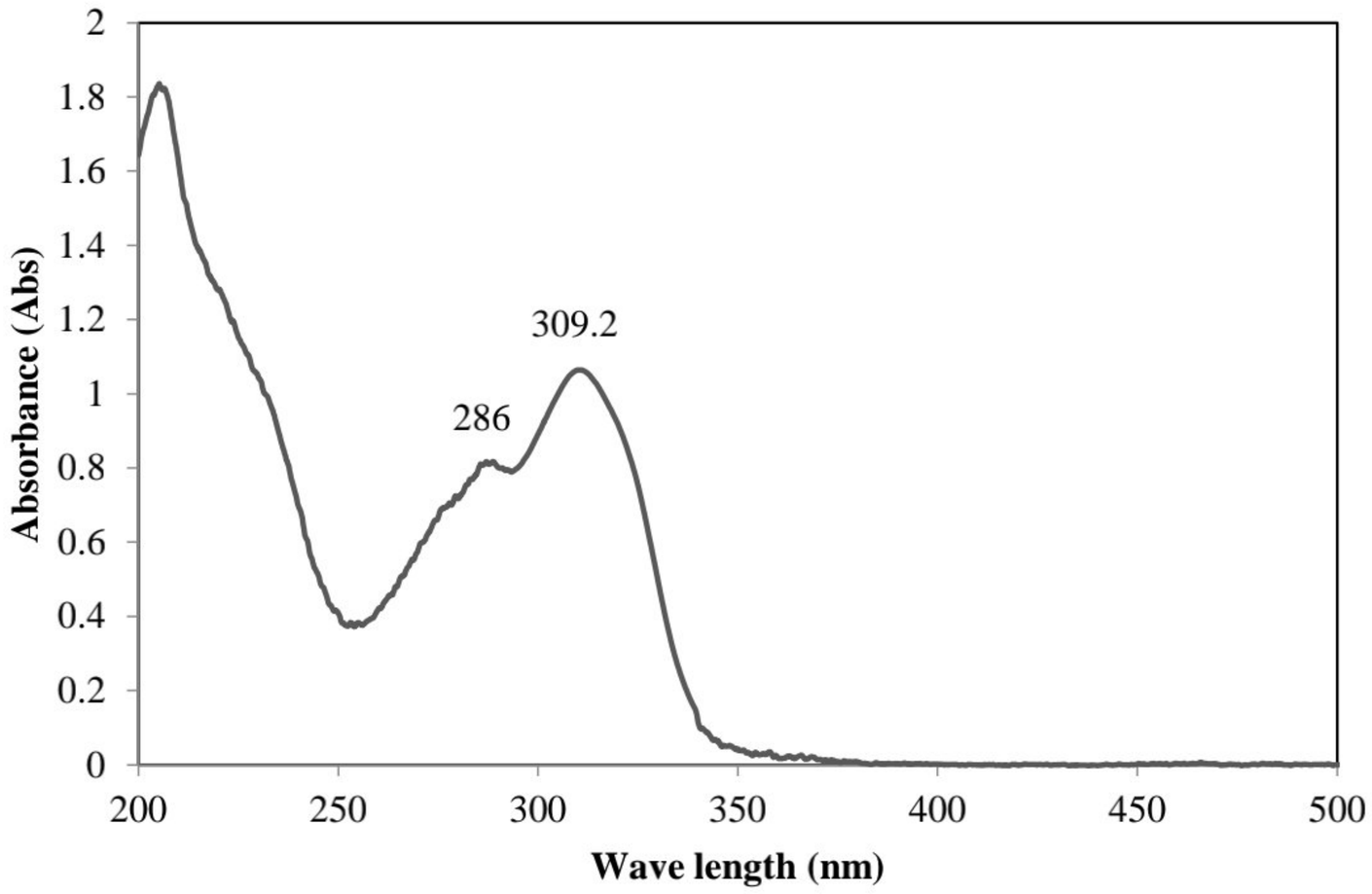


Figure 5.1: UV-Visible spectrum of compound **(4a)**

Similarly, in compound (**4b**) peaks at 344 nm and 317 nm are C=N and C=C of the oxadiazole and aromatic ring respectively (Kostyuchenko et al., 2014).

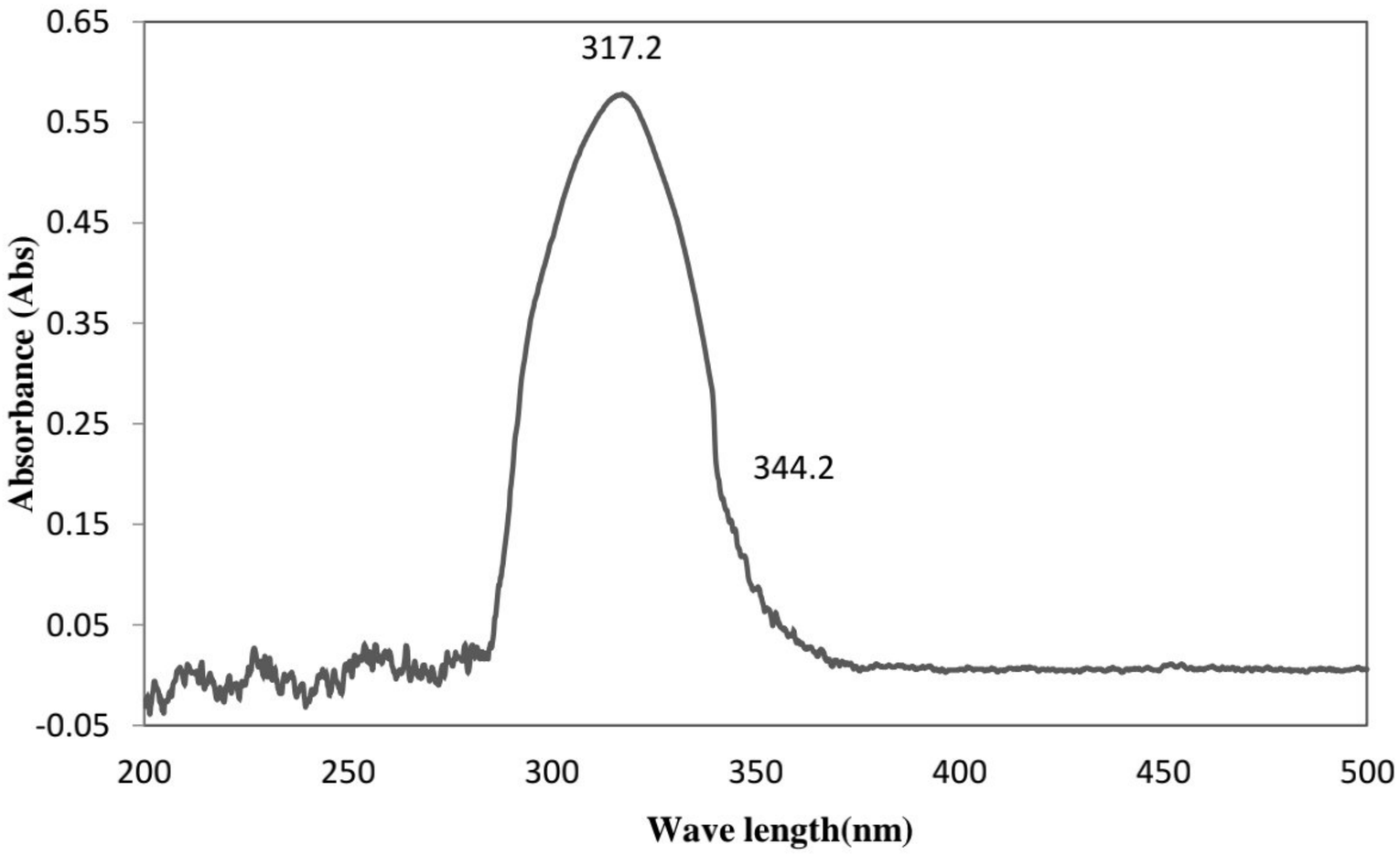


Figure 5.2: UV-Visible spectrum of compound **(4b)**

5.2.2 FTIR spectroscopy

The assignments of IR spectral bands most useful in establishing the structural identity of the synthesized compounds are listed in table 5.3.

In compound (**1**) the two split medium bands at 3323 cm^{-1} and 3260 cm^{-1} are due to N-H stretching of the $–NH_2$ group of acid hydrazide. The medium broad band in the same region is due to the O-H stretching of the hydroxyl group present in the compound. The strong peak at 1646 cm^{-1} is due to the C=O of the compound. The strong absorption band at 1484 cm^{-1} is due to carbon-carbon (C=C) stretching vibrations. The strong absorption band at 759 cm^{-1} is due to out-of-plane bending vibrations of the –C-H group of the aromatic part of the molecule (Silverstein & Bassler, 1962).

Table 5.3 Wavenumbers of the synthesized compounds in FTIR spectra

Group	Bands (cm^{-1})				
	1	2	3	4a	4b
v(NH)	3323	-	-	3420	-
v(Ar-OH)	3260	3345	3388	3350	3246
v(Ar C-H)	3062	3072	3077	2965, 2930	2861
vC-H)	2840	2945	2943	2865	2861
v(S-H)	-	2592	-	-	-
v(C=N)	-	1613	1772	1605	1626
v(C=O)	1646	-	1607	1628	1592
v(Ar C=C)	1484	1515	1570	1474	1424
v(C-N)	-	-	-	1258	
v(C-O)	-	1186	1186	1192	1116
v(C-S)	-	971	971	1030	935
v(Ar-CH)	759	-	-	-	-
v(C-Cl)	-	-	744	-	-

The medium peak at 3345 cm^{-1} is due to the presence of the –OH group in the ring. The absence of two split peaks at 3323 cm^{-1} and 3260 cm^{-1} in compound (**2**), indicates the formation of oxadiazole. The medium absorption band at 1613 cm^{-1} confirms the presence of the –C=N group in the molecule. The strong band at 1186 cm^{-1} indicates that there is a –C-O group in the compound which is the part of oxadiazole ring. The strong peak at 1515 cm^{-1} is due to the –C=C stretching vibration of the ring. The strong absorption band at 971 cm^{-1} confirms that there is a –C-S group in the compound. Moreover, the weak band at 2592 cm^{-1} cited for the –S-H group in the compound (Chandrakantha et al., 2010)

In compound (**3**)**,** the medium peak at 3388 cm^{-1} is due to the -OH group in the compound. The sharp band at 1607 cm^{-1} is due to –C=O indicating the formation of compound (**3**) by reacting compound (**2**) with chloroacetyl chloride. Furthermore, the strong band at 744 cm^{-1} corresponds to –C-Cl stretching vibration again confirming

that there is a formation of compound (**3**). Moreover, the band at 2592 cm^{-1} due to –S-H which was observed in compound (**2**) is not appeared in the spectrum of compound (**3**) revealing that there is a formation of the new compound (Dawood & Gomha, 2015).

In compound (**4a**), NH stretching vibration for the piperazine ring of the molecule was observed between 3500 cm^{-1} and 3200 cm^{-1}, which suggests the formation of the compound (**4a**). The observed vibrations at 2930 cm^{-1} and 2865 cm^{-1} can be attributed to the CH vibration of the piperazine ring which is of a lower vibration as compared to the CH vibration of the phenyl ring. The medium band at 1258 cm^{-1} is attributed to the CN stretching of the piperazine part of the molecule. The strong band at 1628 cm^{-1} is due to the carbonyl C=O stretching vibration. The peak at 1192 cm^{-1} is attributed to the CO single bond stretching vibration of the oxadiazole part of the compound. The strong absorption band at 1474 cm^{-1} is due to carbon-carbon (C=C) stretching vibrations (Şahin et al., 2002).

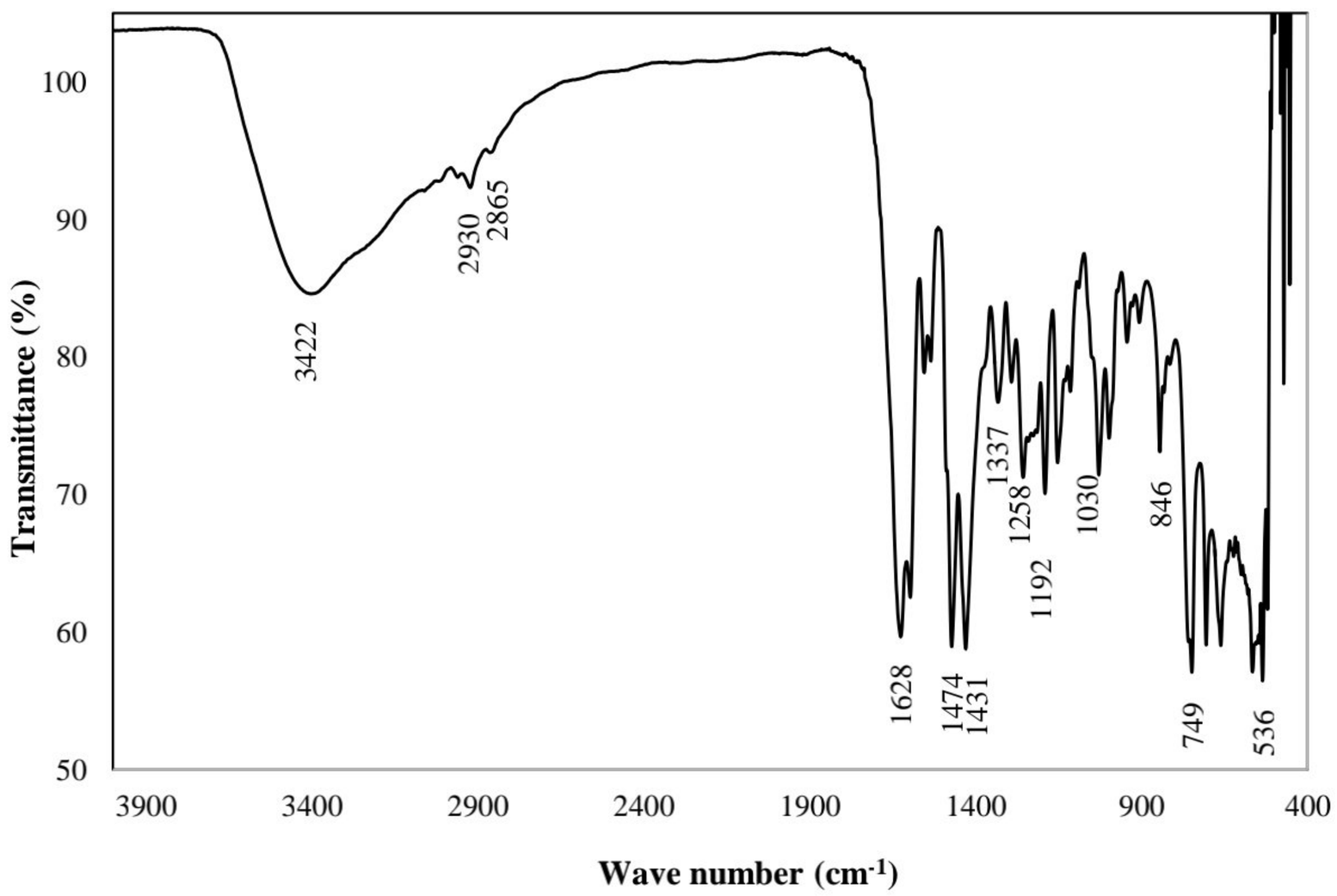


Figure 5.3: FT-IR spectrum of compound (**4a**)

By analyzing the IR spectra of compound (**4b**), the peak at 1116 cm^{-1} is supported by the stretching vibration of the CO single bond present in the morpholine ring of the compound. The broad band at 3246 cm^{-1} is attributed to the OH stretching vibration. Furthermore, the absence of a strong peak at 750 cm^{-1} indicates that there is no C-Cl

group which suggests the formation of the compound **(4b)** by replacing the chlorine atom of the compound **(3)** (Romano et al., 2012).

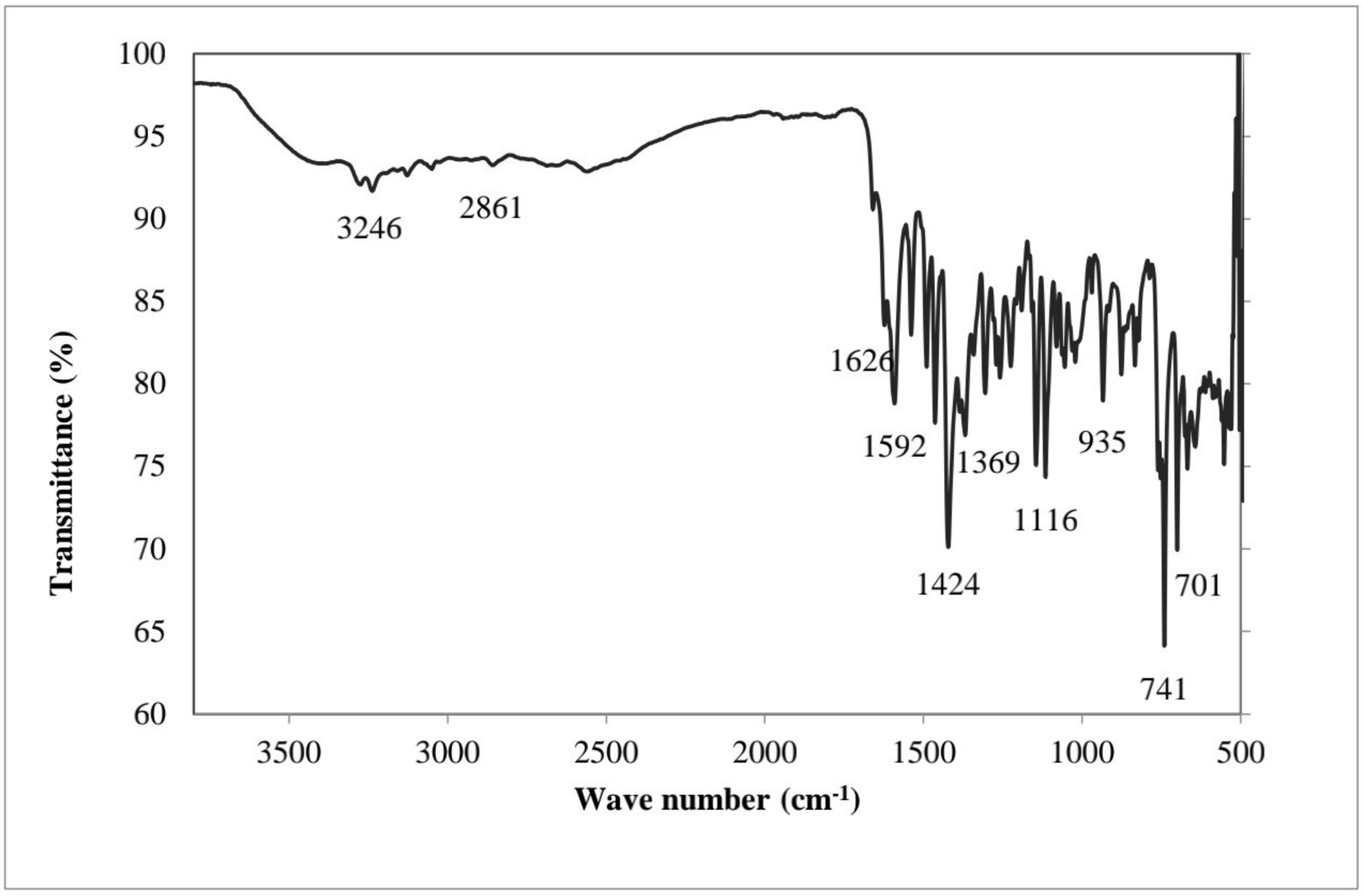


Figure 5.4: FT-IR spectrum of compound **(4b)**

5.2.3 NMR Spectroscopy

5.2.3.1 ^{1}H-NMR Spectroscopy

The proton NMR spectroscopy of compounds (**4a)** and (**4b)** was carried out.

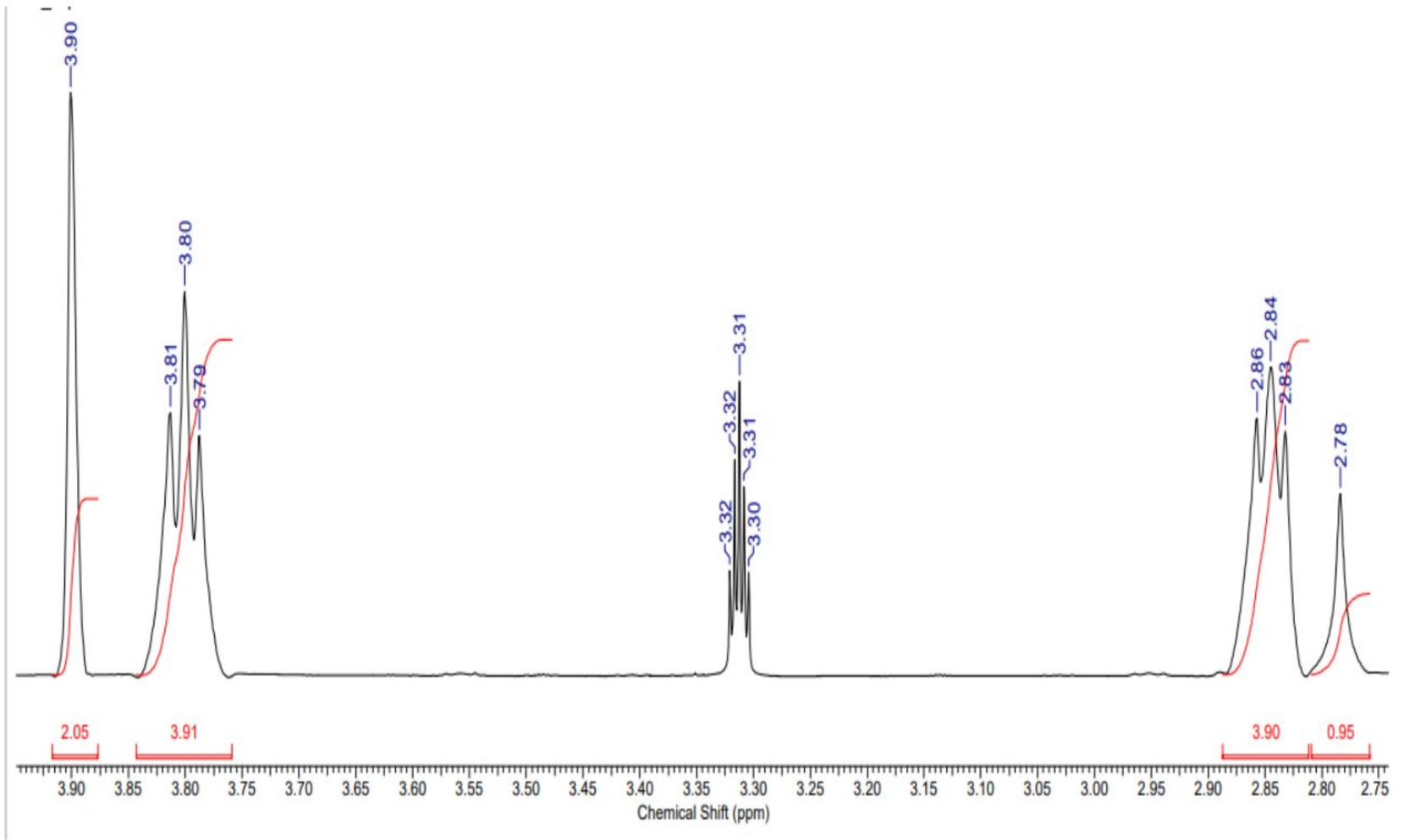
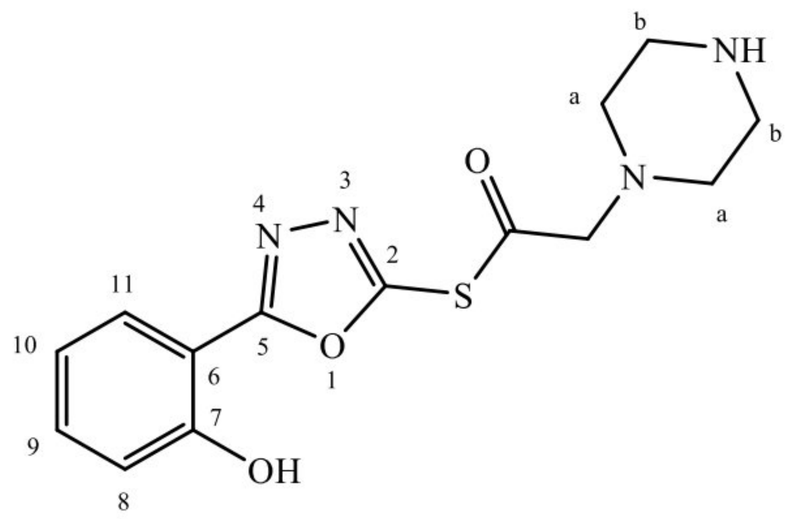


Figure 5.5: ^{1}H-NMR spectrum of compound **(4a)**

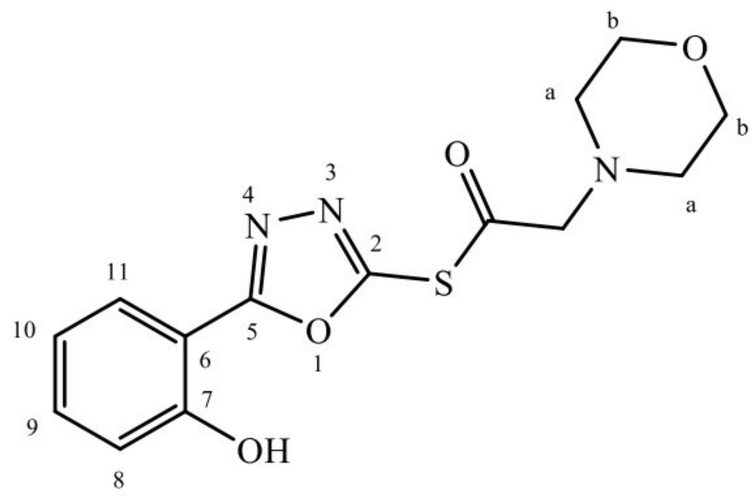
In ^{1}H-NMR spectra of compound (**4a**), the singlet at 8.56 ppm is attributed to the hydrogen of the OH group. The two triplet band at the region of 7.3-7.68 ppm is associated with the protons of the aromatic ring. A singlet at 3.9 ppm is due to the CH_2 group residing next to the carbonyl group of the compound. The quartet at 2.78-2.86 ppm is due to the protons of CH_2 on either side of the NH group of the piperazine ring of the compound. The triplet at 3.31 ppm is due to the other two CH_2 groups of the piperazine ring of the compound (Ameline et al., 2019).

Table 5.4 ^{1}H-NMR spectral assignments (ppm) of synthesized compounds

Proton↓	Chemical Shift (ppm)	
	(4a)	(4b)
OH	8.56 (s, 1H)	8.56 (s, 1H)
NH	2.78 (s, 1H)	
CH_2	3.90 (s, 2H)	4.03 (s, 2H)
8	6.92 (td, J = 7.58, 1.22 Hz,1H)	6.90 (td, J =7.52, 0.86 Hz,1H)
9	7.28-7.37 (m, 1H)	7.31 (ddd, J=8.31, 7.52, 1.71 Hz, 1H)
10	6.98 (dd, 1H, J=8.31, 7.83 Hz)	6.97 (dd, J=8.31, 7.83 Hz, 1H)
11	7.67 (dd, *J* =7.83, 1.47Hz, 1H)	7.66 (dd, *J* =7.83, 1.71. Hz, 1H)
a	2.84 (t, J = 5.14 Hz, 4H)	2.80 (t, J =4.77 Hz, 4H)
b	3.80 (t, J = 5.14 Hz, 4H)	3.65 (t, J = 4.77 Hz, 4H)



(4a)



(4b)

In compound (**4b**), the two triplets at 6.9 and 7.6 ppm are due to the CH_2 group of the aromatic ring of the compound. The strong peak at 8.56 ppm corresponds to the hydrogen of the OH group of the ring. Similarly, the band at 4.03 ppm is due to the CH_2 group residing next to the carbonyl group of the compound. Two triplet peaks at 2.8 and 3.65 ppm is attributed to the four CH_2 group of the morpholine ring present in the compound in which the peak at 3.65 ppm is due to CH_2 groups of either side of the oxygen atom of the morpholine ring (Subramanyam et al., 2018).

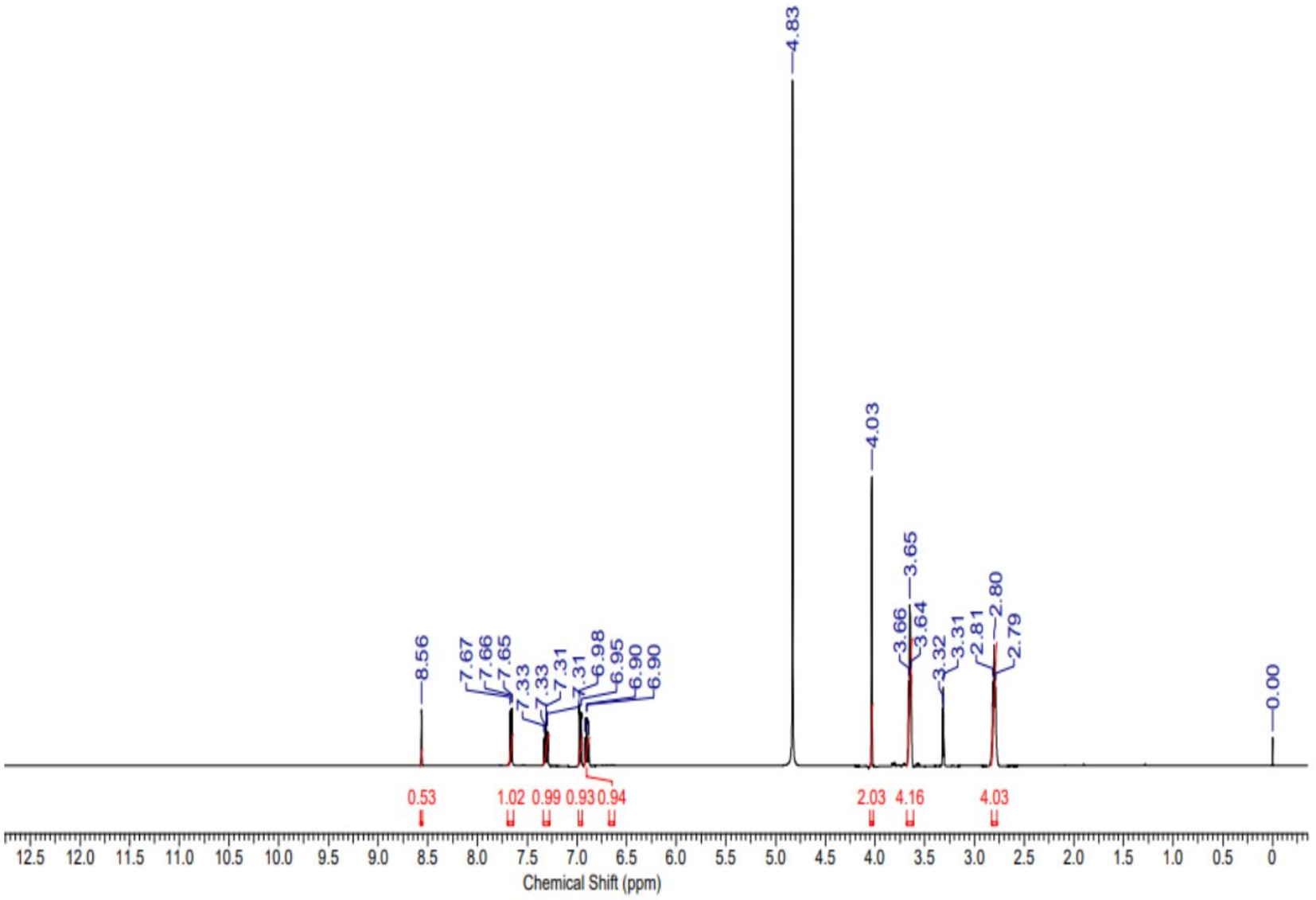


Figure 5.6: ^{1}H-NMR spectrum of compound **(4b)**

5.2.3.2 ^{13}C-NMR Spectroscopy

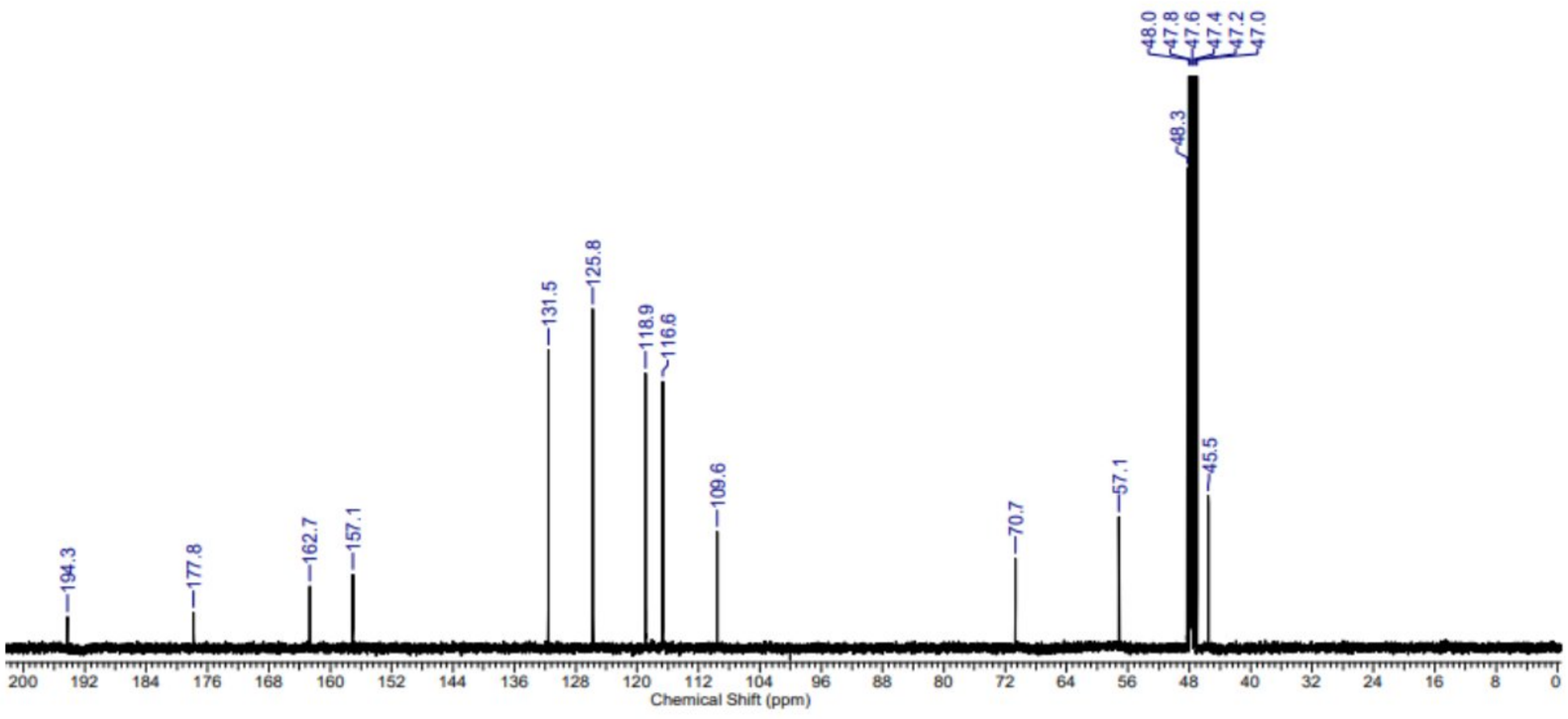


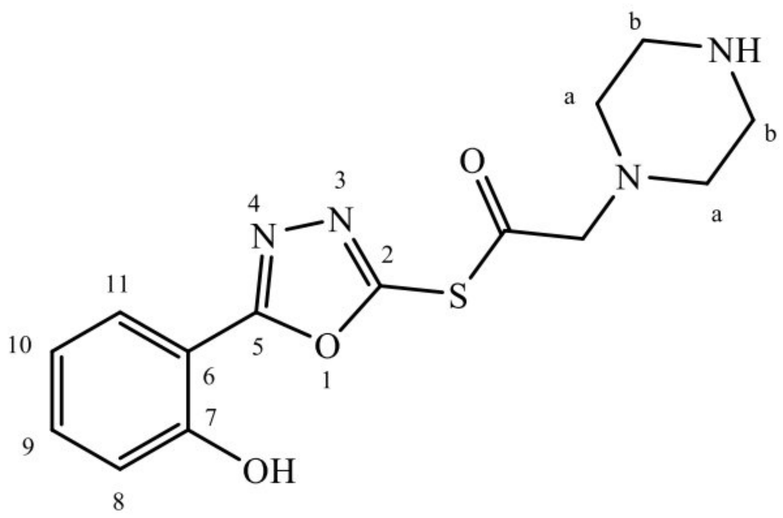
Figure 5.7: ^{13}C-NMR spectrum of compound **(4a)**

The ^{13}C-NMR spectra of the compound (**4a)** showed the peak at 194.3 ppm is due to the carbon connected to the oxygen of the carbonyl group. The peak at 177.8 ppm is

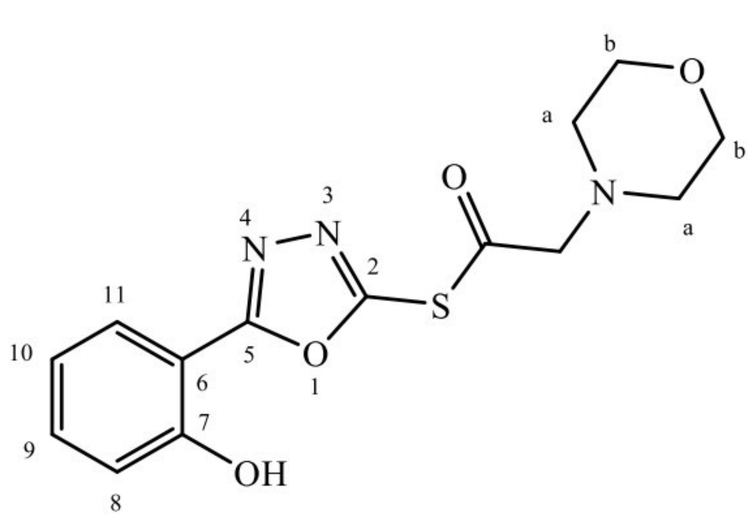
attributed to the carbon of the oxadiazole ring linked to the sulfur atom present in the compound. The peak at 162.7 ppm is due to the carbon of the oxadiazole ring connected to the benzene ring of the compound. The peak at 70.7 ppm corresponds to the carbon atom next to the carbonyl group of the compound. The six peaks from 109.1 ppm to 157.1 ppm on the spectral data reveal that these peaks are due to six different carbon atoms of the benzene ring present in the compound. The peak at 45.5 ppm and 57.1 ppm represents the carbon atoms of the piperazine ring of the compound (Rezki et al., 2015).

Table 5.5 ^{13}C-NMR spectral assignments (ppm) of synthesized compounds

Carbon↓	Chemical Shift (ppm)	
	(4a)	(4b)
C=O	194.3	194.6
CH_2	70.0	67.2
2	177.8	177.7
5	162.7	62.8
6	109.6	109.7
7	157.1	157.6
8	116.6	116.9
9	131.5	131.5
10	118.9	118.6
11	125.8	125.9
a	57.1	55.6
b	45.5	45.4



(4a)



(4b)

The peak corresponding to 45.4 ppm and 55.6 ppm in the compound (**4b**) are due to the carbon atoms of the morpholine ring of the compounds. Similarly, the peak at 194.6 ppm is due to the highly de-shielded carbon at the carbonyl center in the compound. The six peaks ranging from 109.7 ppm to 157.6 ppm in the compound (**4b**) are attributed to the six carbon atoms of the benzene ring of the compound in which the highest, 157.6 ppm is due to the more shielded carbon which connects OH

group among other carbon atoms of the ring. Furthermore, the peak at 177.7 ppm in the ^{13}C spectra of the compound (**4b**) is due to the carbon atom of the oxadiazole ring connected to the sulfur atom of the compound. The peak at 67.2 ppm represents the carbon atom adjacent to the carbonyl carbon of the ring. In addition, the peak at 162.8 ppm is attributed to another carbon atom of the oxadiazole ring of the compound which is linked to the benzene ring of the compound (Chandrakantha et al., 2010).

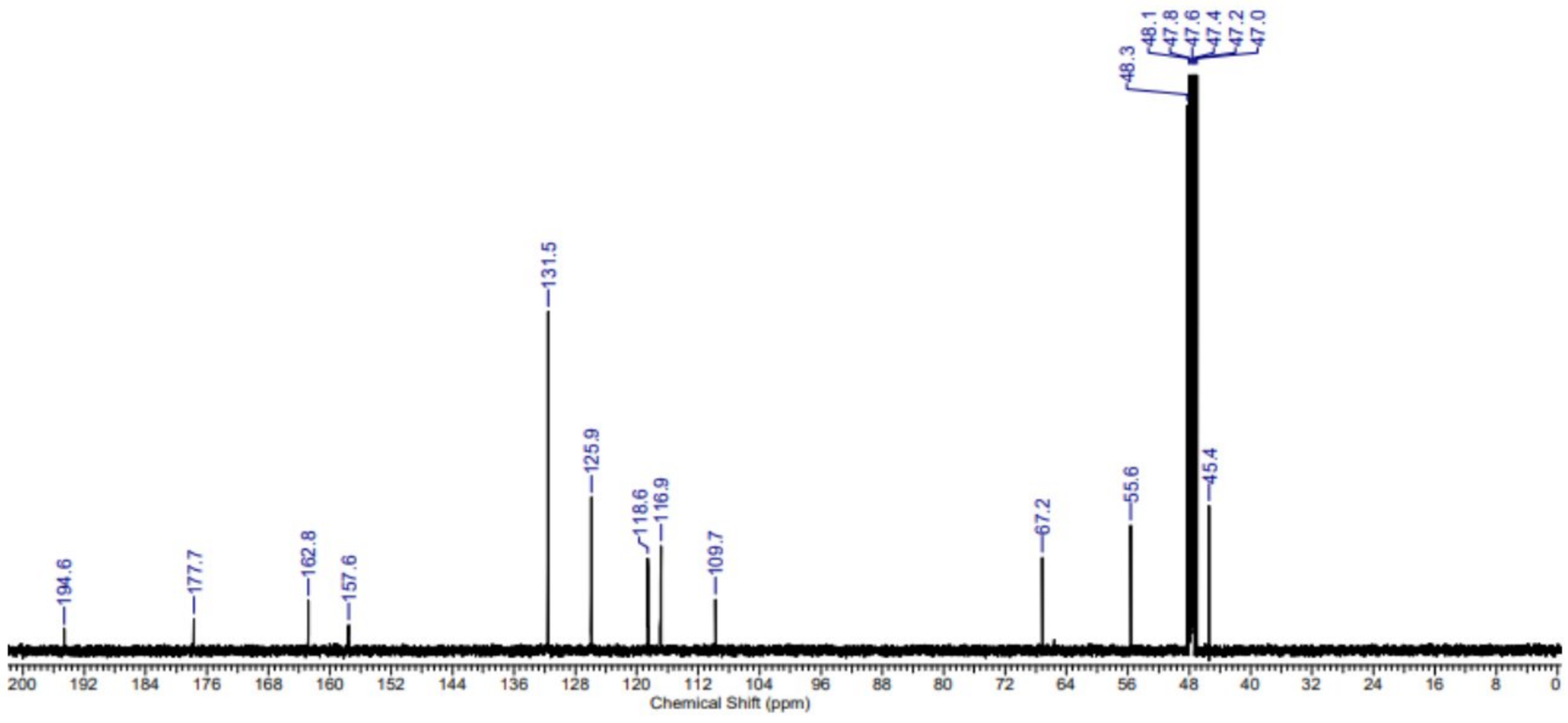


Figure 5.8: ^{13}C-NMR spectrum of compound (**4b**)

5.3 Antimicrobial activity

5.3.1 Antibacterial activity

Table 5.6: Antibacterial screening of the synthesized compounds (**4a)** and (**4b)**

Bacterial strains	Diameter of zone of inhibition (mm)		
	Compound **(4a)**	Compound **(4b)**	Amoxycillin PC
Bacillus subtilis[a]	7.08	15.38	27.62
Enterococcus faecalis[a]	0.00	4.58	31.30
Escherichia coli[b]	4.52	26.76	20.00
Klebsiella pneumoniae[a]	3.76	28.44	0.00
Proteus vulgaris[b]	5.64	14.68	13.60
Pseudomonas aeruginosa[b]	0.00	9.34	0.00
Salmonella enterica subsp. enterica pv Typhi[b]	0.00	4.80	25.42
Staphylococcus aureus[a]	0.00	7.56	40.14
Staphylococcus epidermidis[a]	0.00	6.06	22.04
Shigella dysenteriae[a]	0.00	6.48	28.12

[a]Gram positive bacteria
[b]Gram negative bacteria
The concentration of the test solution used for ZOI determination: 100 $mg.mL^{-1}$

The synthesized compounds exhibited moderate activity against the tested bacterial strains. However, the compound (**4b)** showed very good activity against some bacterial strains. Despite this, compound (**4a)** showed little or no activity against the tested strains. The results are summarized the table 5.6. Compound (**4b)** exhibited more potent activity than positive control against *E. coli*, *K. pneonomiae,* and *P. vulgaris*. The compound **(4a)** was found to be ineffective against *E. faecalis*, *P. aeruginosa, S. typhi, S. aureus, S. epidermis,* and *S. dysenteriae*. However, it showed little activity against some gram-positive strains.

5.3.2 Antifungal activity

Table 5.7: Antifungal screening of the synthesized compounds (**4a)** and (**4b)**

Fungal strains	Diameter of zone of inhibition (mm)		
	Compound **(4a)**	Compound **(4b)**	Clotrimazole PC
Candida albicans	0.00	16.14	18.20
Saccharomyces cerevisiae	0.00	31.20	23.34

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Two new 1,3,4-oxadiazole derivatives S-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-(piperazin-1-yl)ethanethioate **(4a)** and S-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) 2-morpholinoethanethioate **(4b)** were successfully prepared in the lab starting from the methyl salicylate as a synthetic precursor. The purity of the synthesized compound was checked by melting point determination and TLC analysis. The characterization of the synthesized compounds was done by using different spectroscopic techniques (UV, FT-IR, ^{1}H-NMR, and ^{13}C-NMR). By analyzing the spectra obtained by all the above means of characterization techniques all the data are found to be consistent with the synthesized compounds. Analysis reveals that the synthesized compound (**4b)** which is morpholine attached derivative was found to be quite more effective against most of the bacterial strains, especially against gram-positive bacteria. The result is also similar for the antifungal activity in which compound (**4b)** was found to be effective against the tested fungal strains while compound (**4a)** didn't show the activity against both the fungal strains by using the agar well diffusion method. In conclusion, the study reveals that compound **(4b)** which contains a morpholine ring is more potent than compound **(4a)** containing a piperazine ring, and the better activity of **(4b)** is due to the presence of a more electronegative oxygen atom in the compound.

6.2 Recommendation for further work

In recent years the 1,3,4-oxadiazole moieties have drawn attention to synthetic chemists due to their pharmacological importance. The synthesis done in this research can be served as a potential drug for the treatment of various diseases caused by bacteria and fungi. These molecules can also be taken for more investigation and identification of other biological activities and can be used to treat existing and novel diseases whose treatments are challenging. Therefore one can or more specifically the synthetic chemist who wants to work on the 1,3,4-oxadiazole moieties has room for investigation because 1,3,4-oxadiazole moiety containing compounds have been used for the treatment of many pathological conditions and the area of interest of the research to the many synthetic chemists. Various other biological activities such as

analgesic, antitubercular, anticancer, etc. are yet to study which may unlock their new biopotentials.

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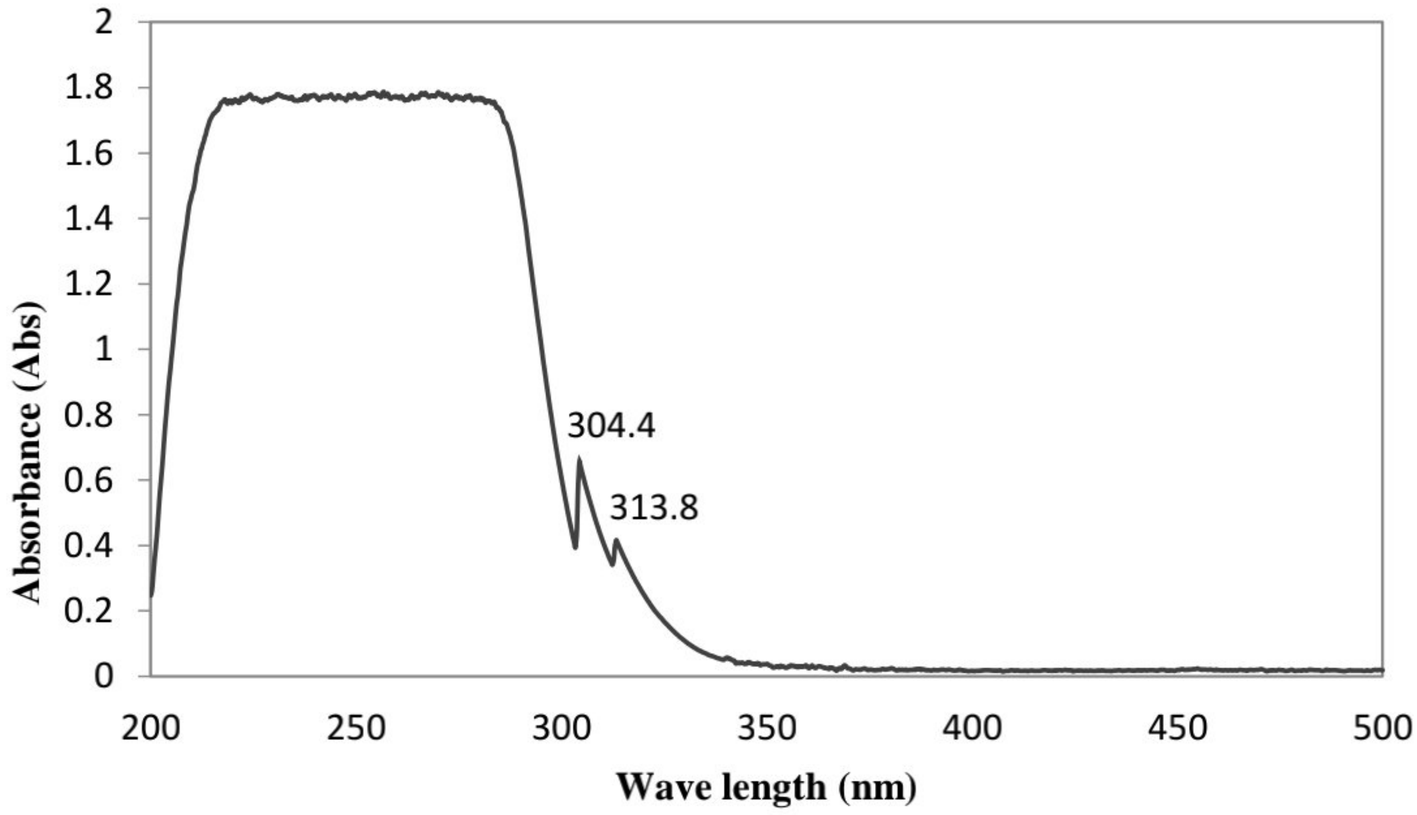
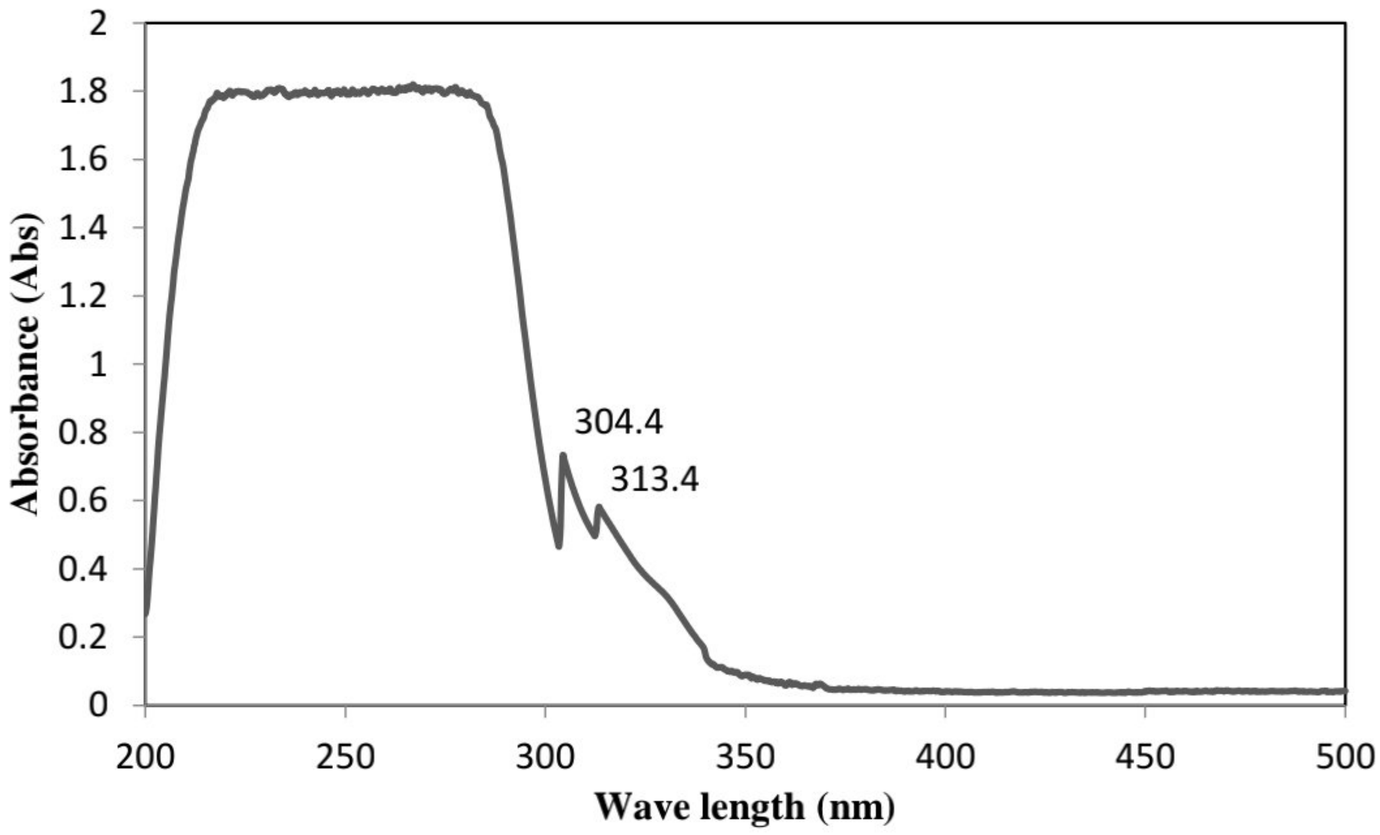
APPENDICES

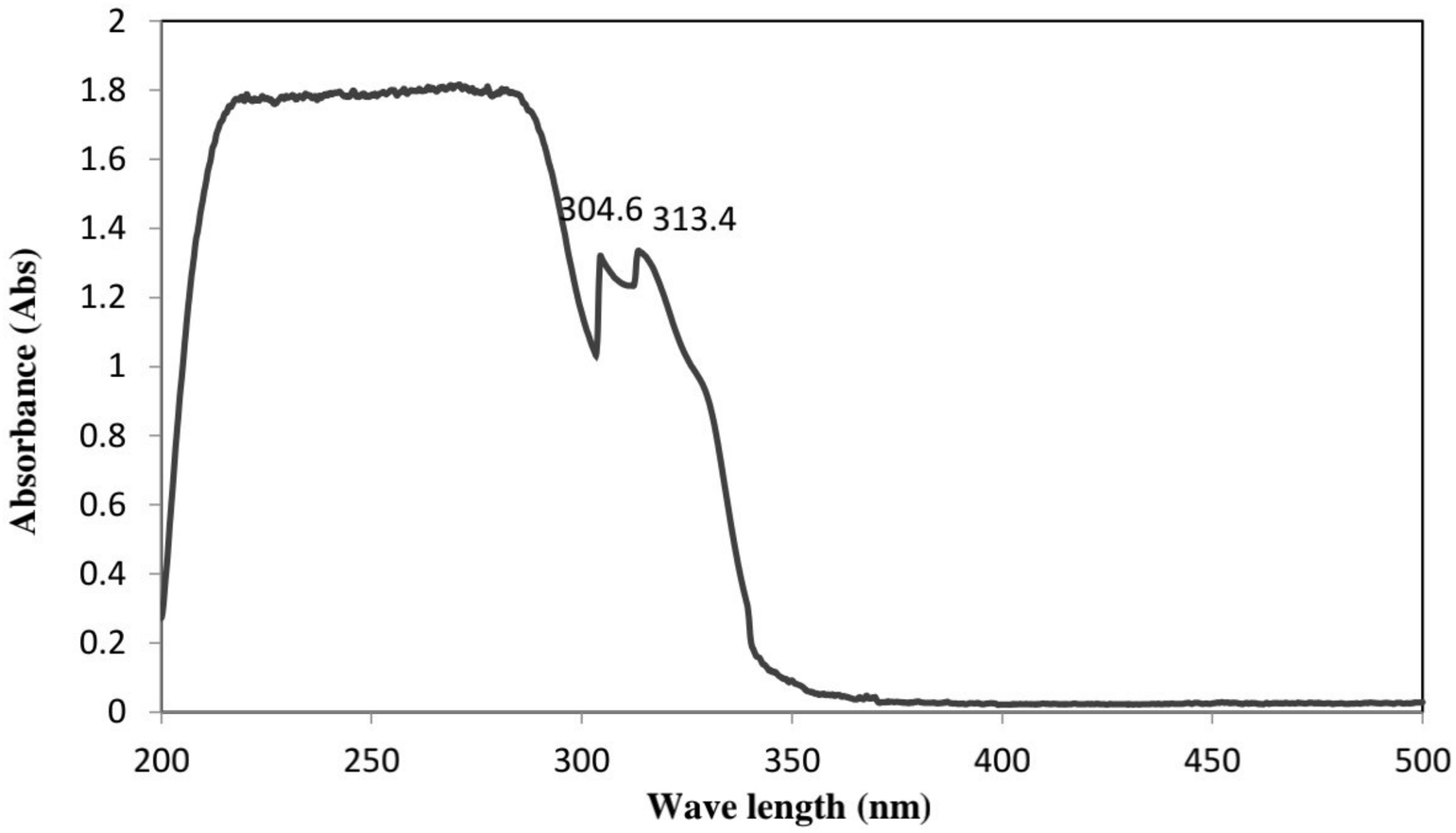
Appendix A: UV-Visible spectra of synthesized compounds



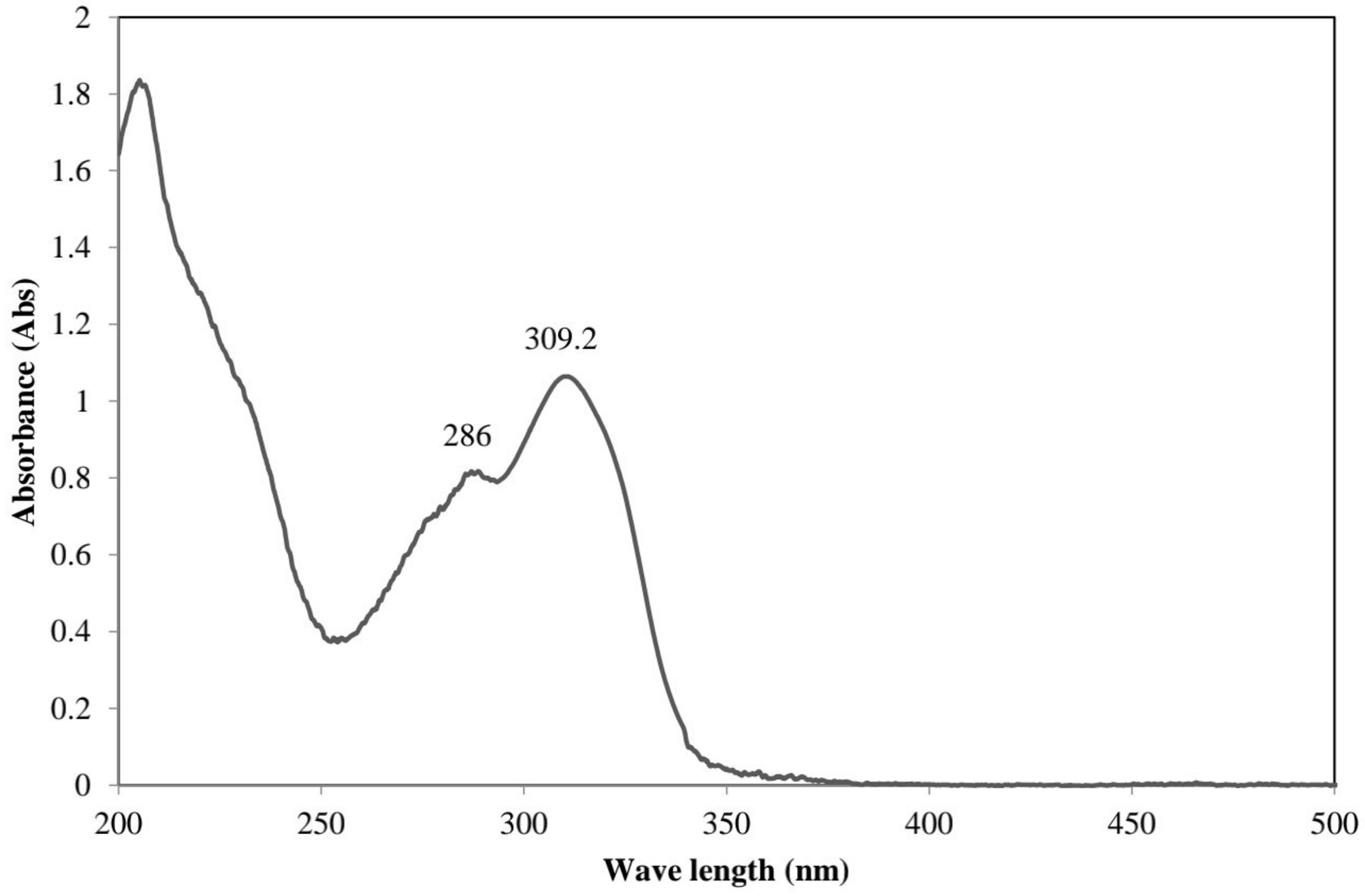
Appendix A1: UV-Visible Spectrum of Compound (**1**)



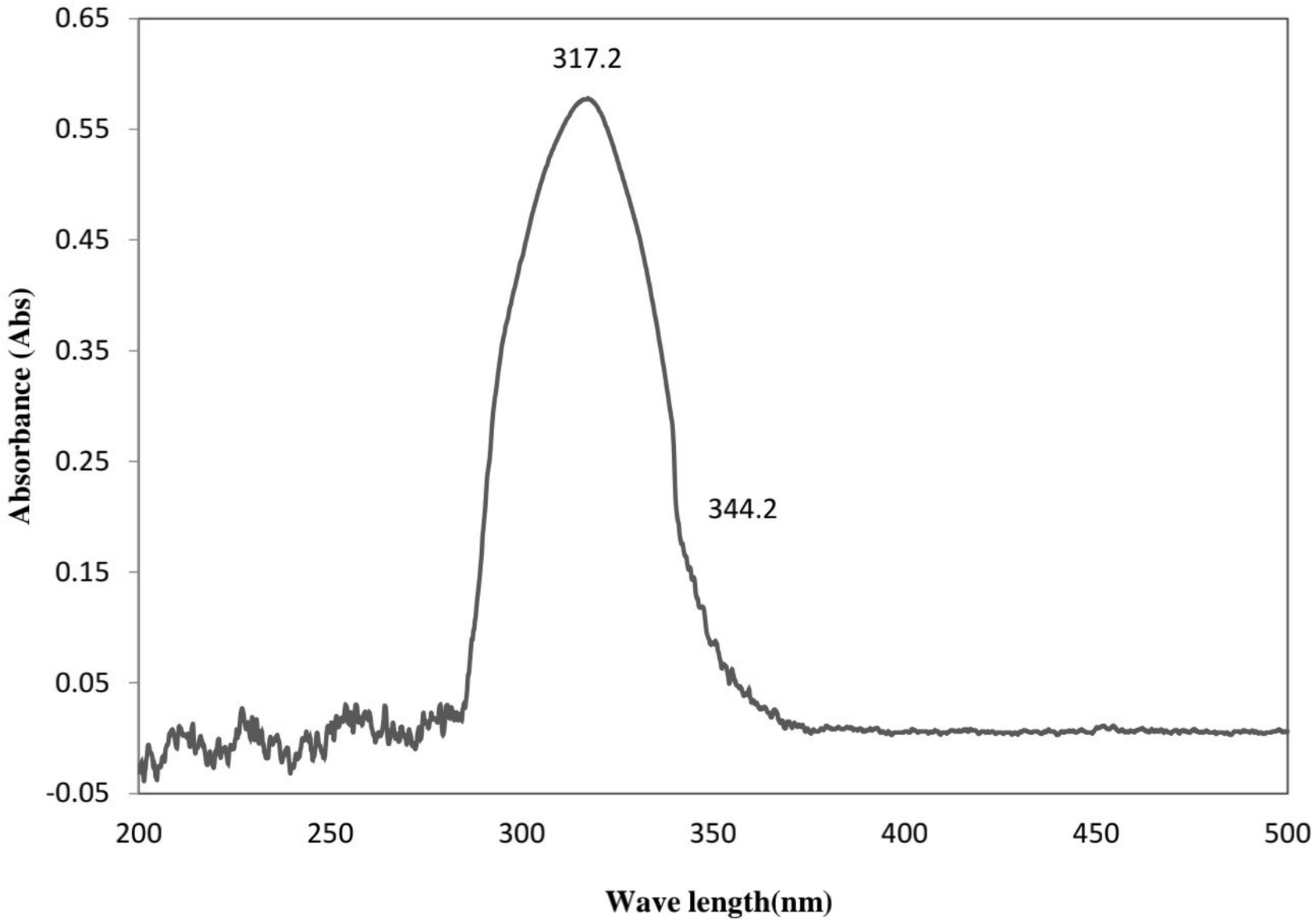
Appendix A2: UV-Visible Spectrum of Compound (**2**)



Appendix A3: UV-Visible Spectrum of Compound (**3**)

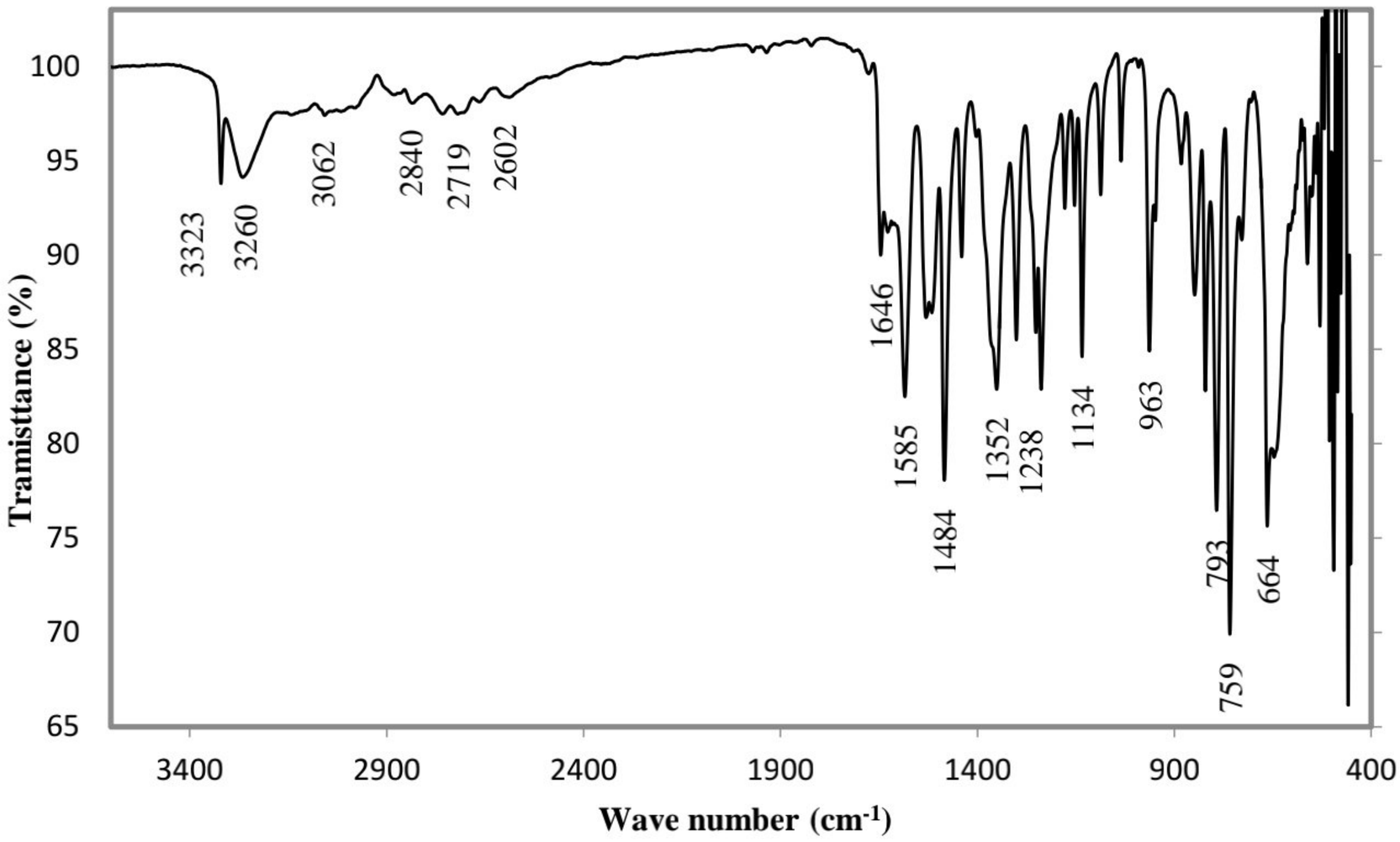


Appendix A4: UV-Visible Spectrum of Compound (**4a)**

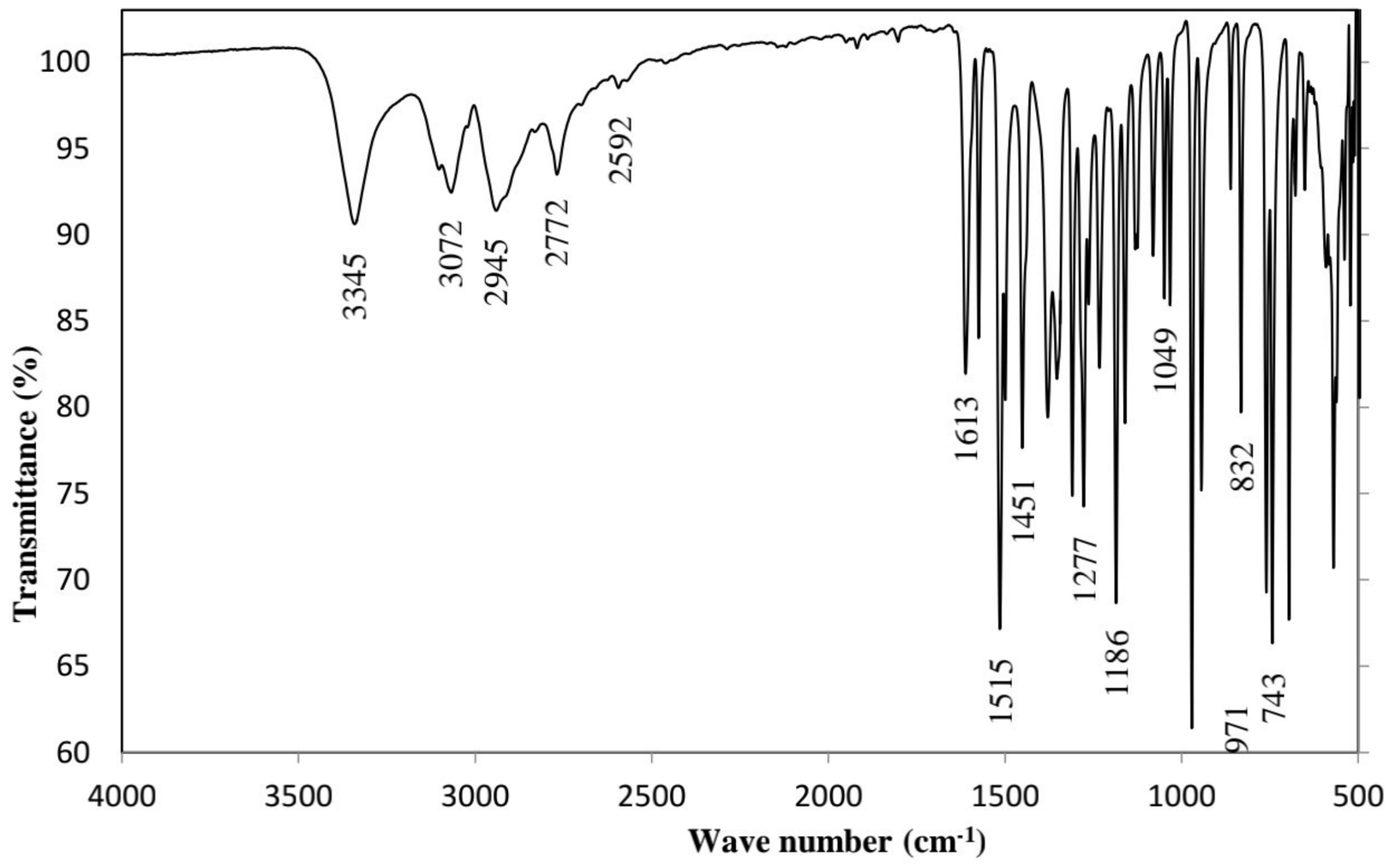


Appendix A5: UV-Visible Spectrum of Compound (**4b)**

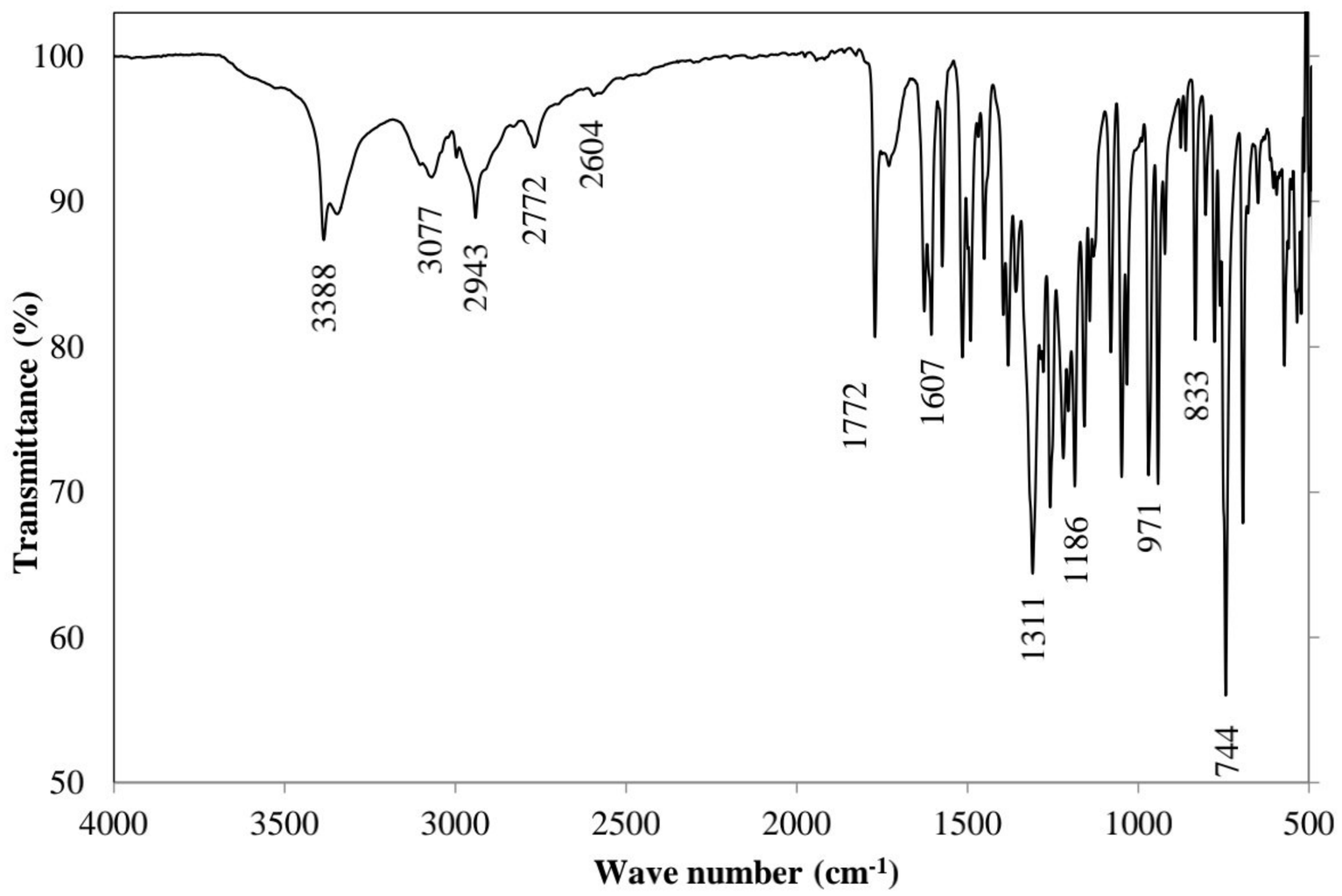
Appendix B: IR spectra of synthesized compounds



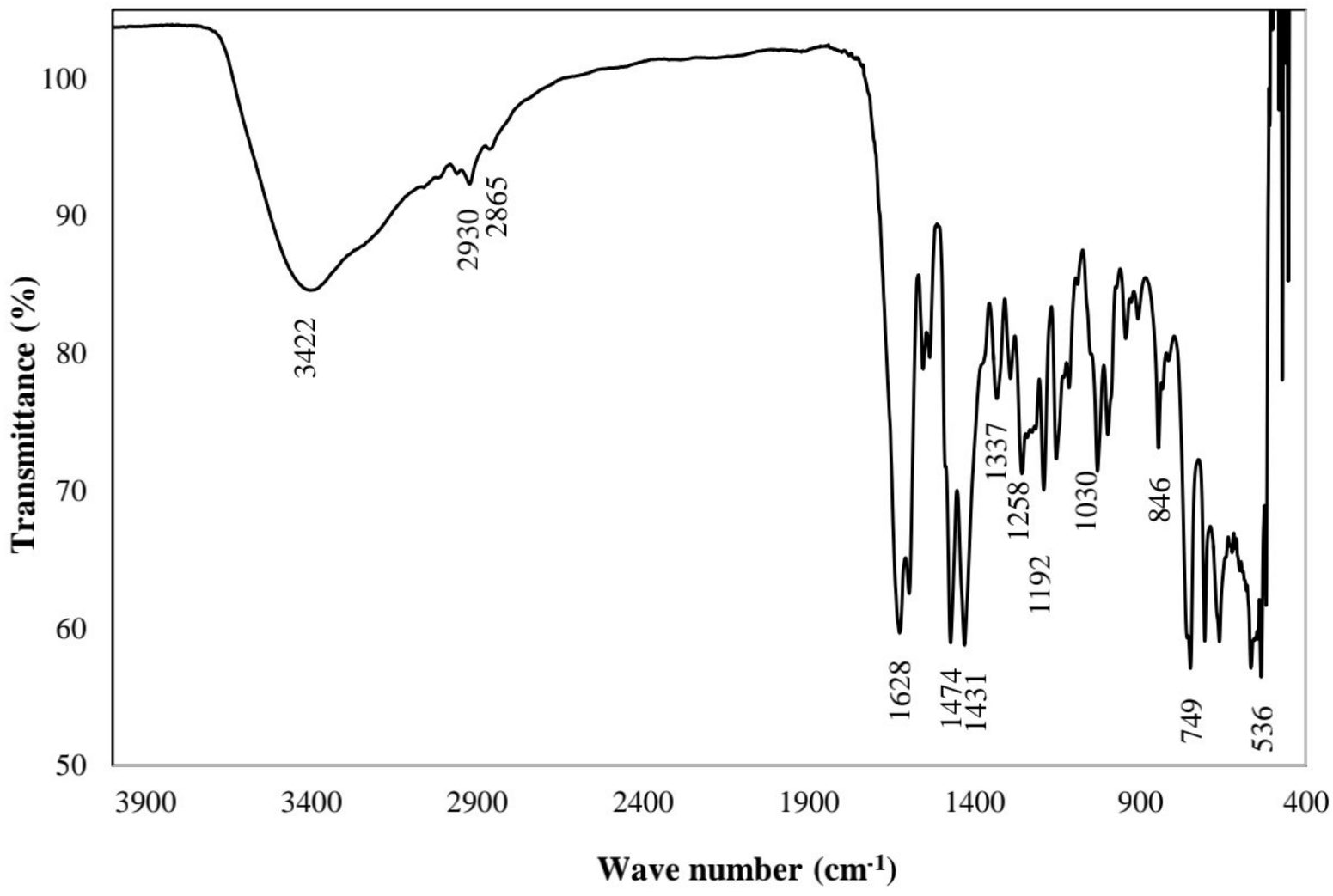
Appendix B1: IR spectrum of Compound (**1**)



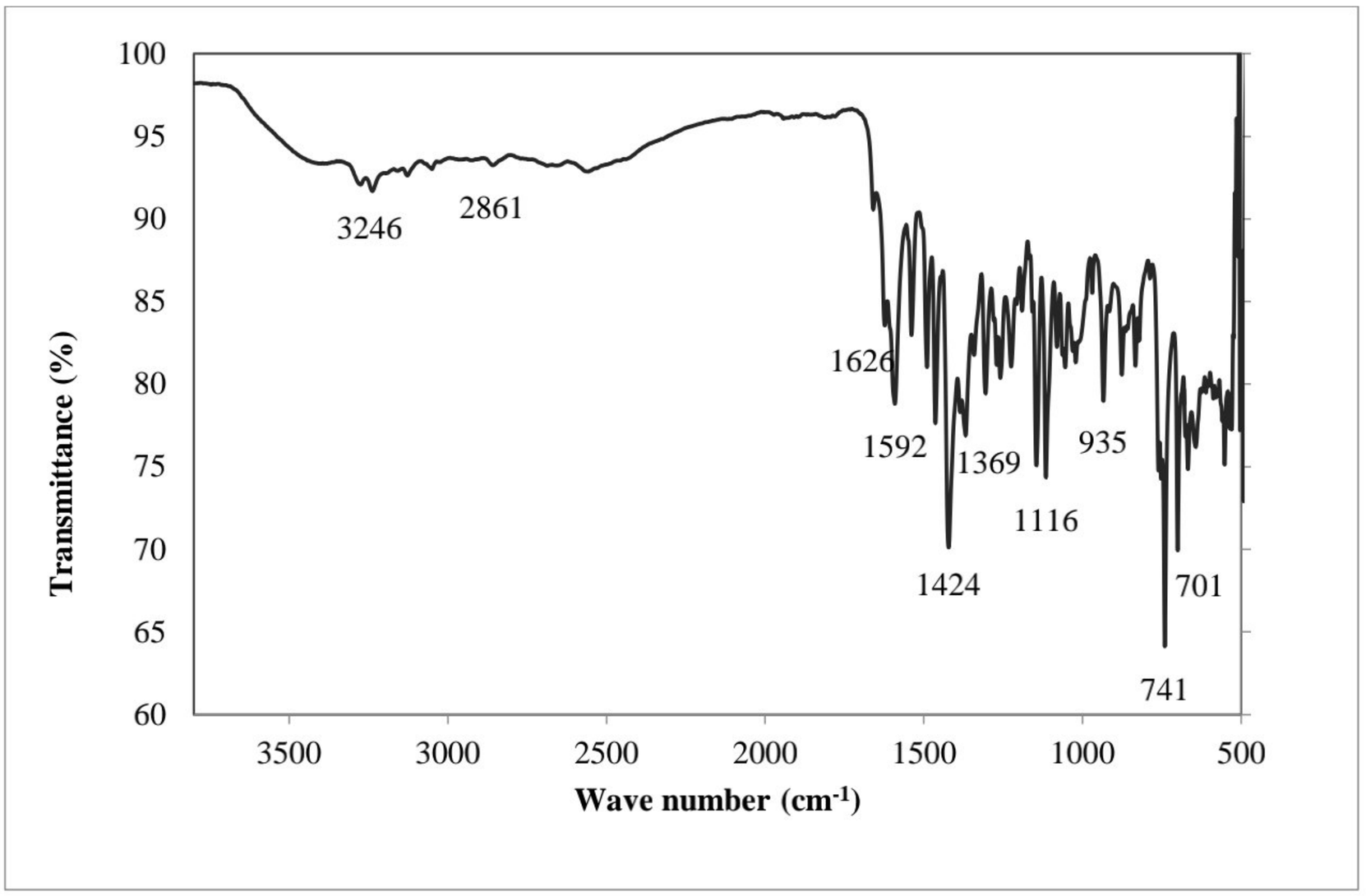
Appendix B2: IR spectrum of Compound (**2**)



Appendix B3: IR spectra of Compound (**3**)

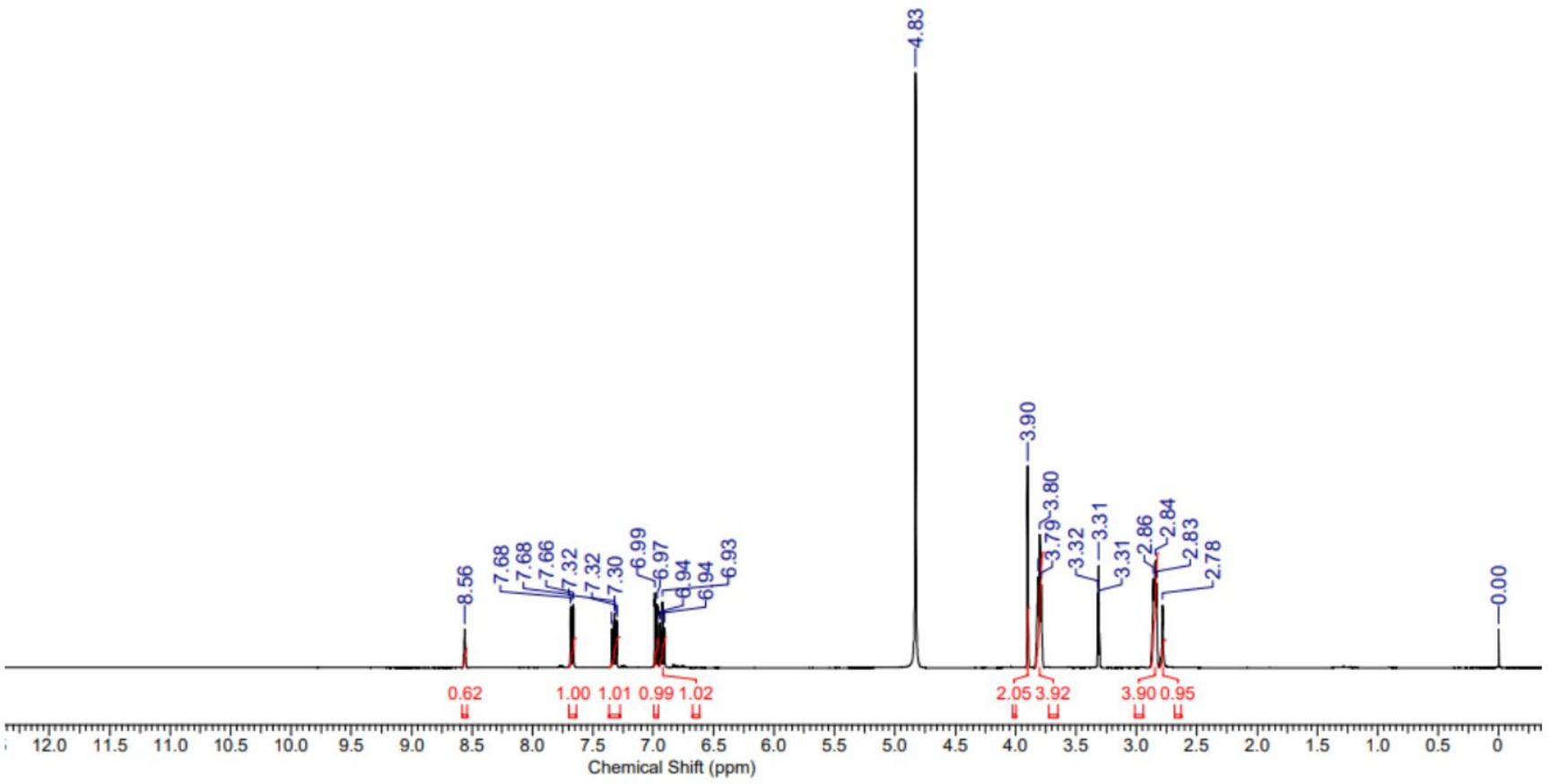


Appendix B4: IR spectrum of Compound (**4a)**

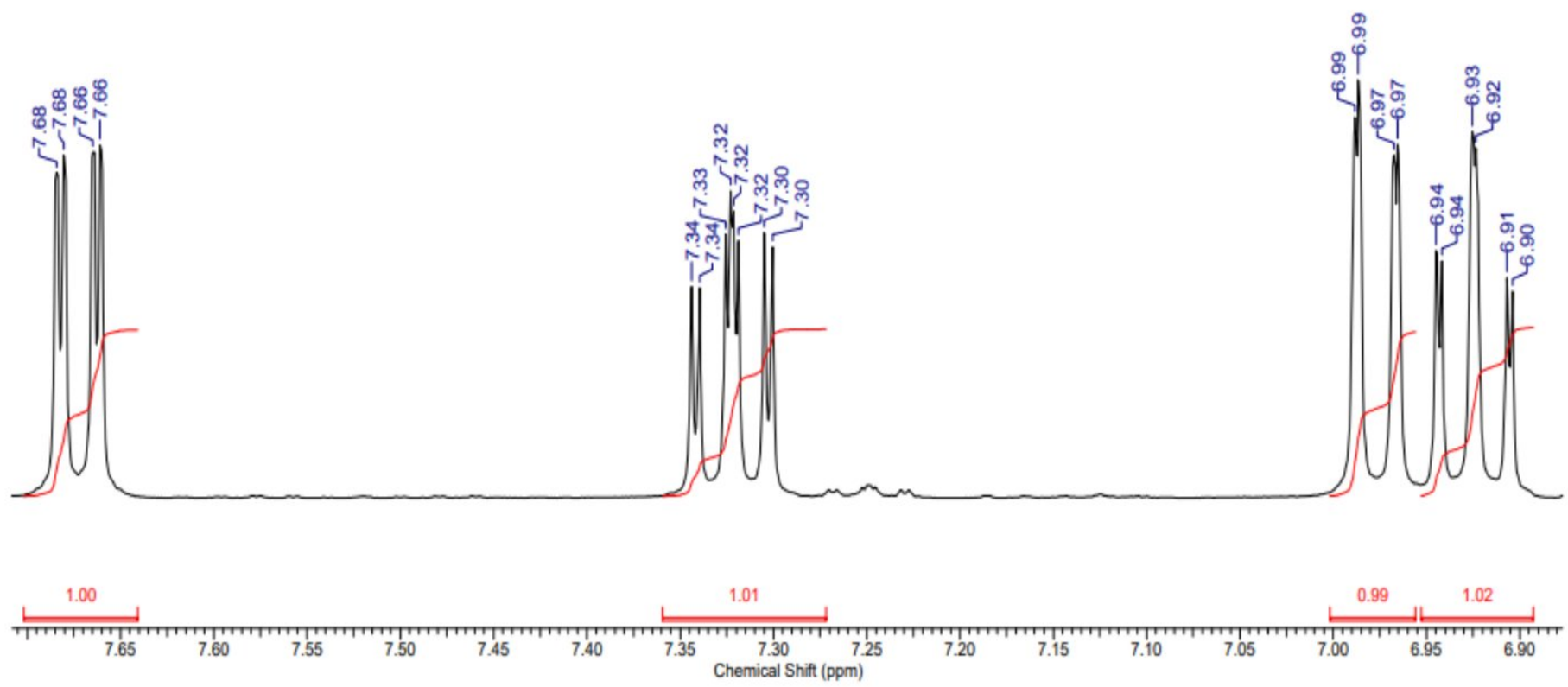


Appendix B5: IR spectrum of Compound (**4b)**

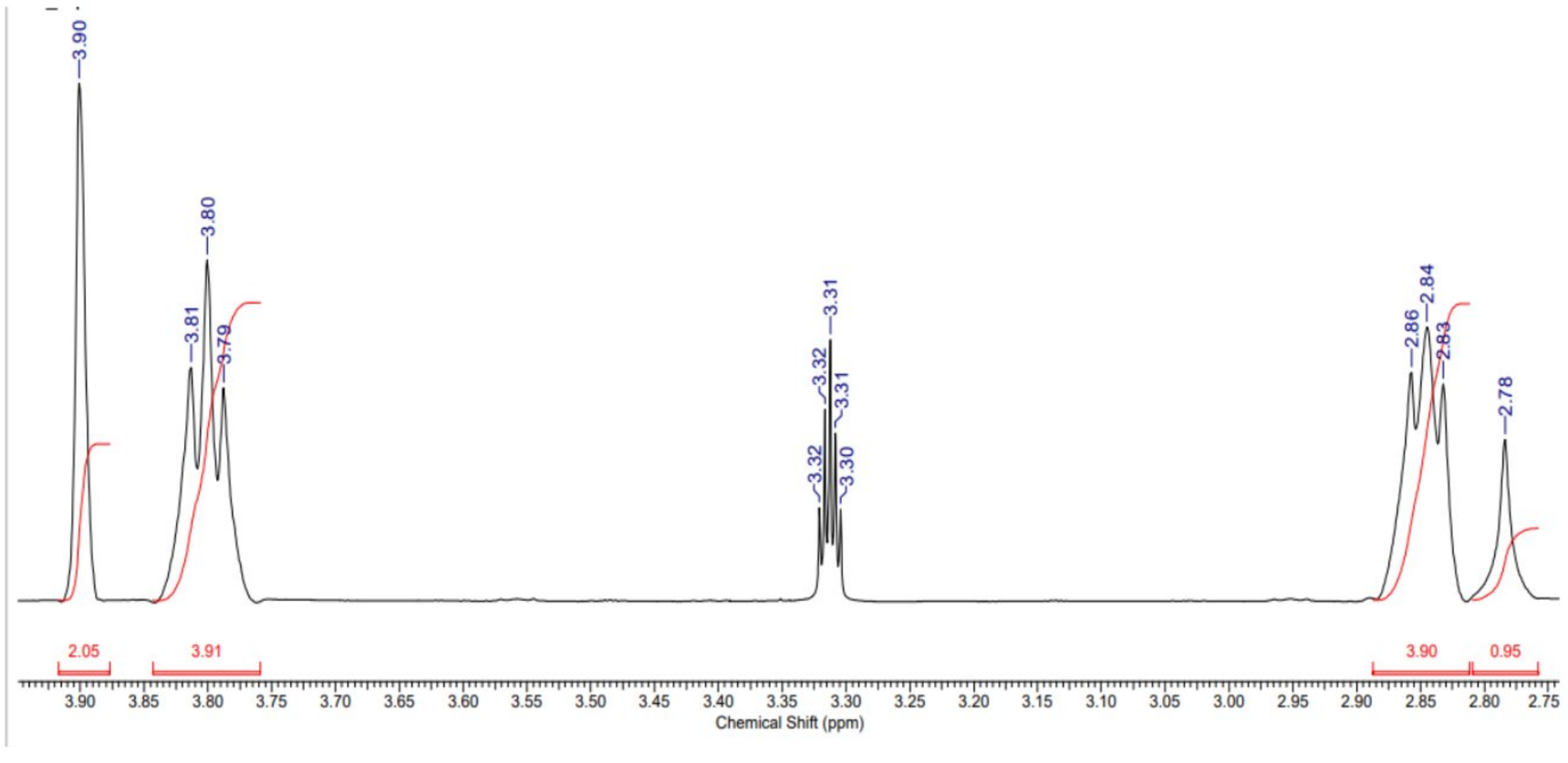
Appendix C: NMR spectra of synthesized compounds



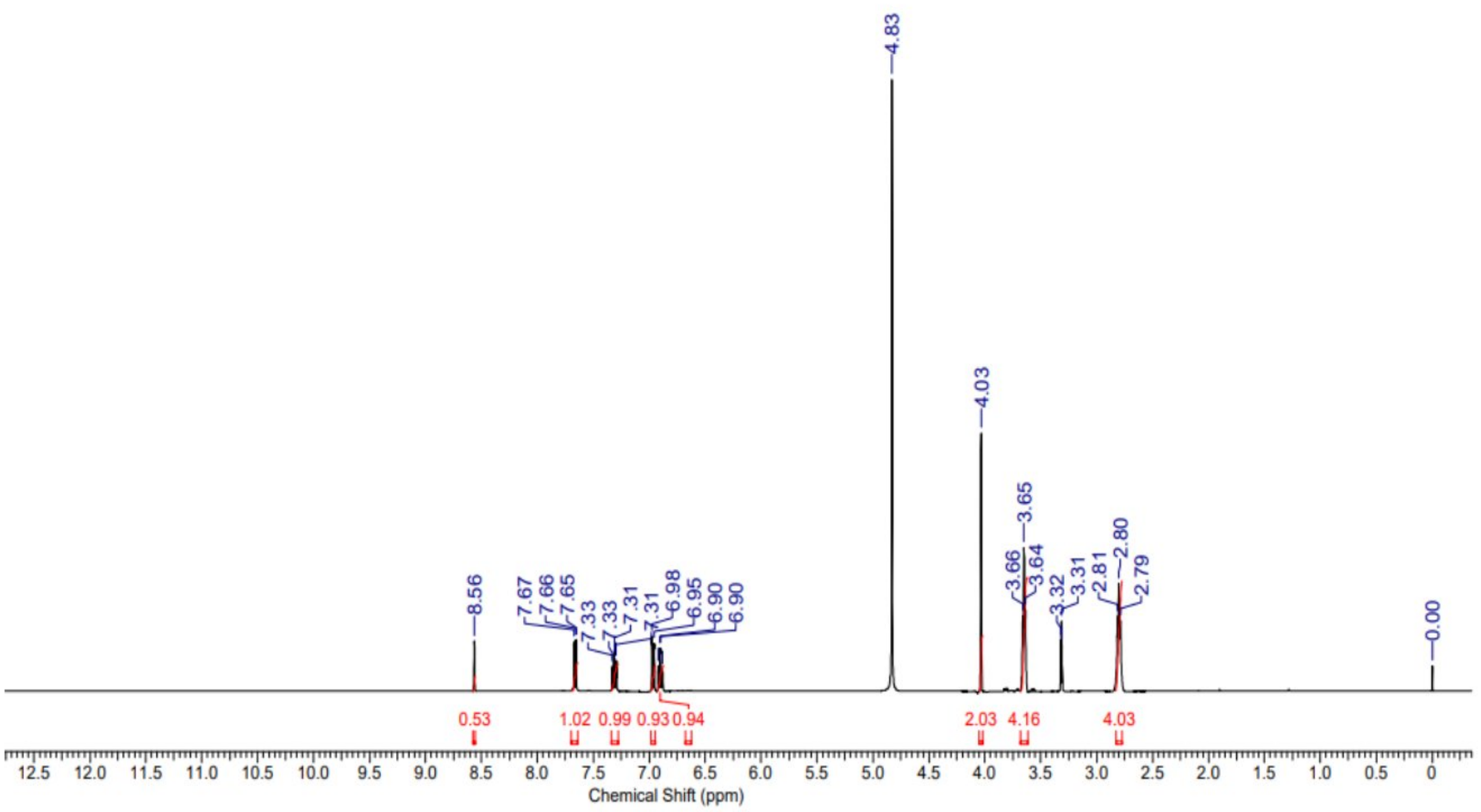
Appendix C1: ^{1}H-NMR spectrum of compound **(4a)**



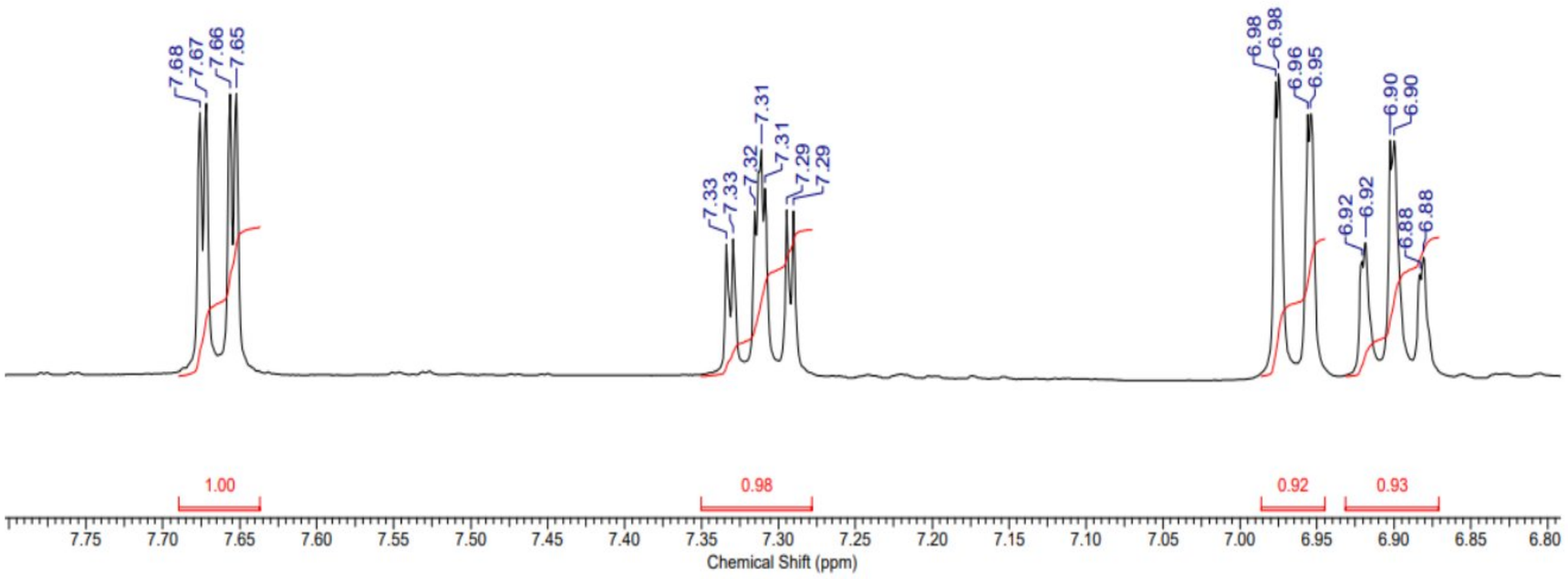
Appendix C2: ^{1}H-NMR spectrum of compound **(4a)** Expanded 1



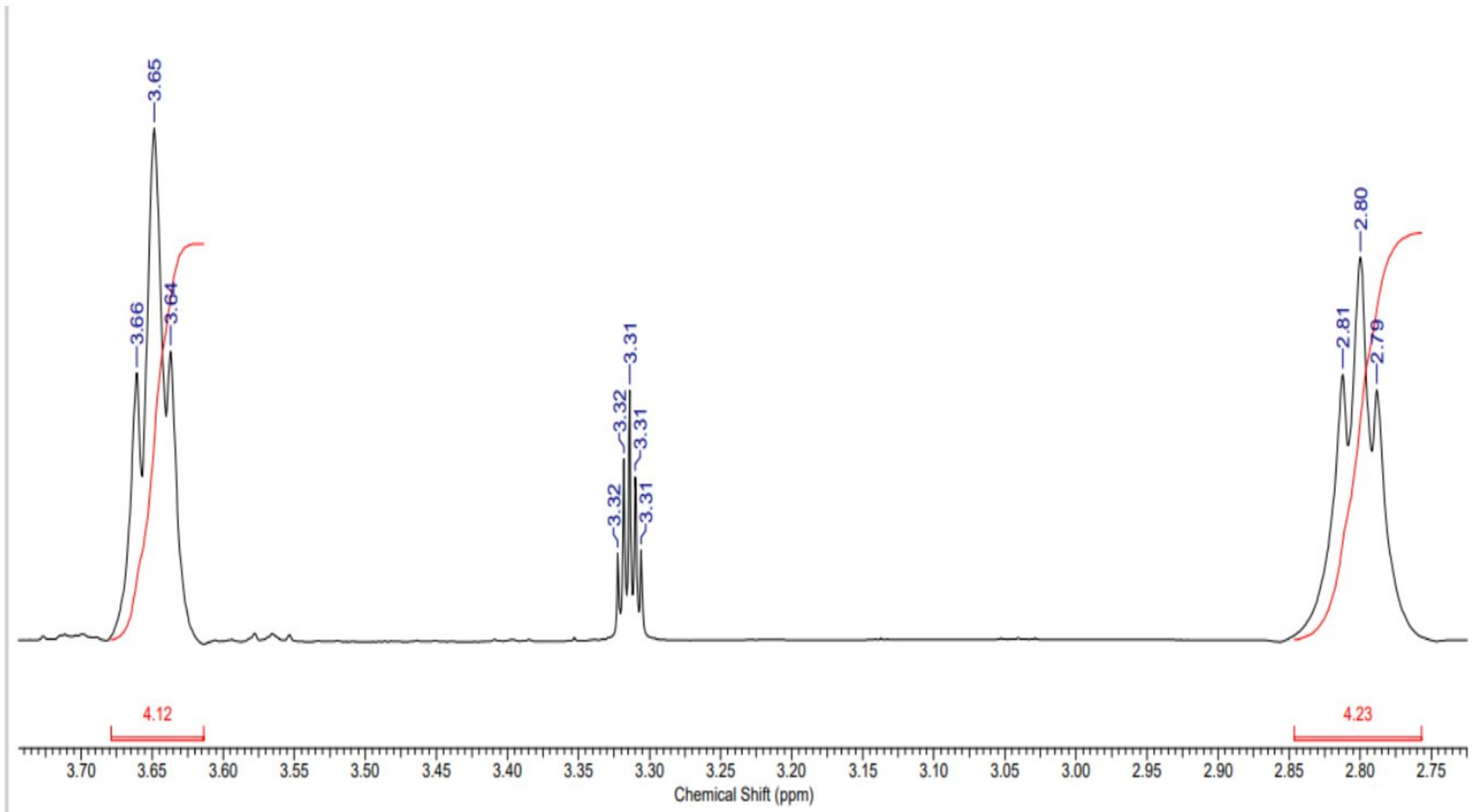
Appendix C3: [1]H-NMR spectrum of compound **(4a)** Expanded 2



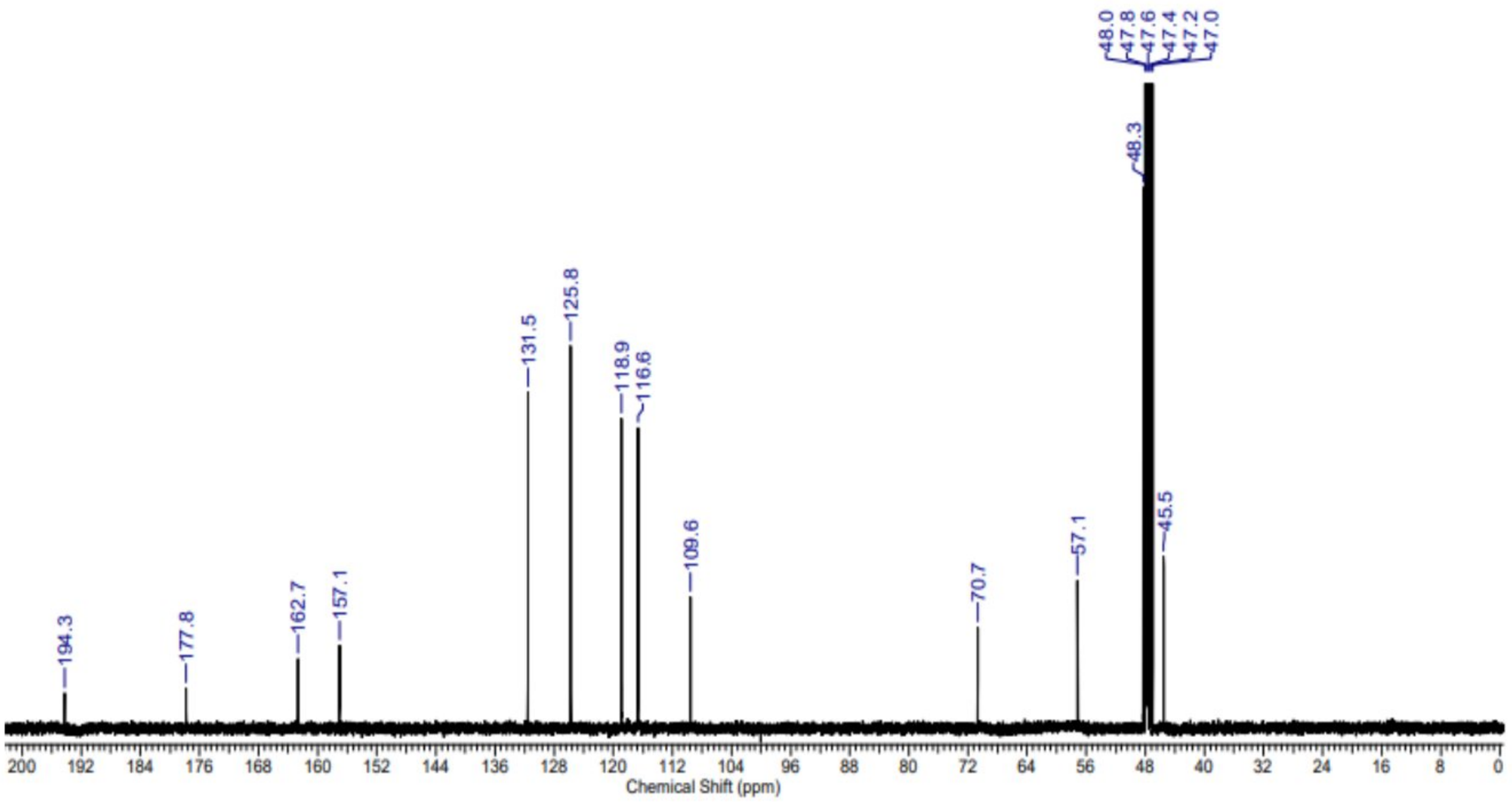
Appendix C4: [1]H-NMR spectrum of compound (**4b)**



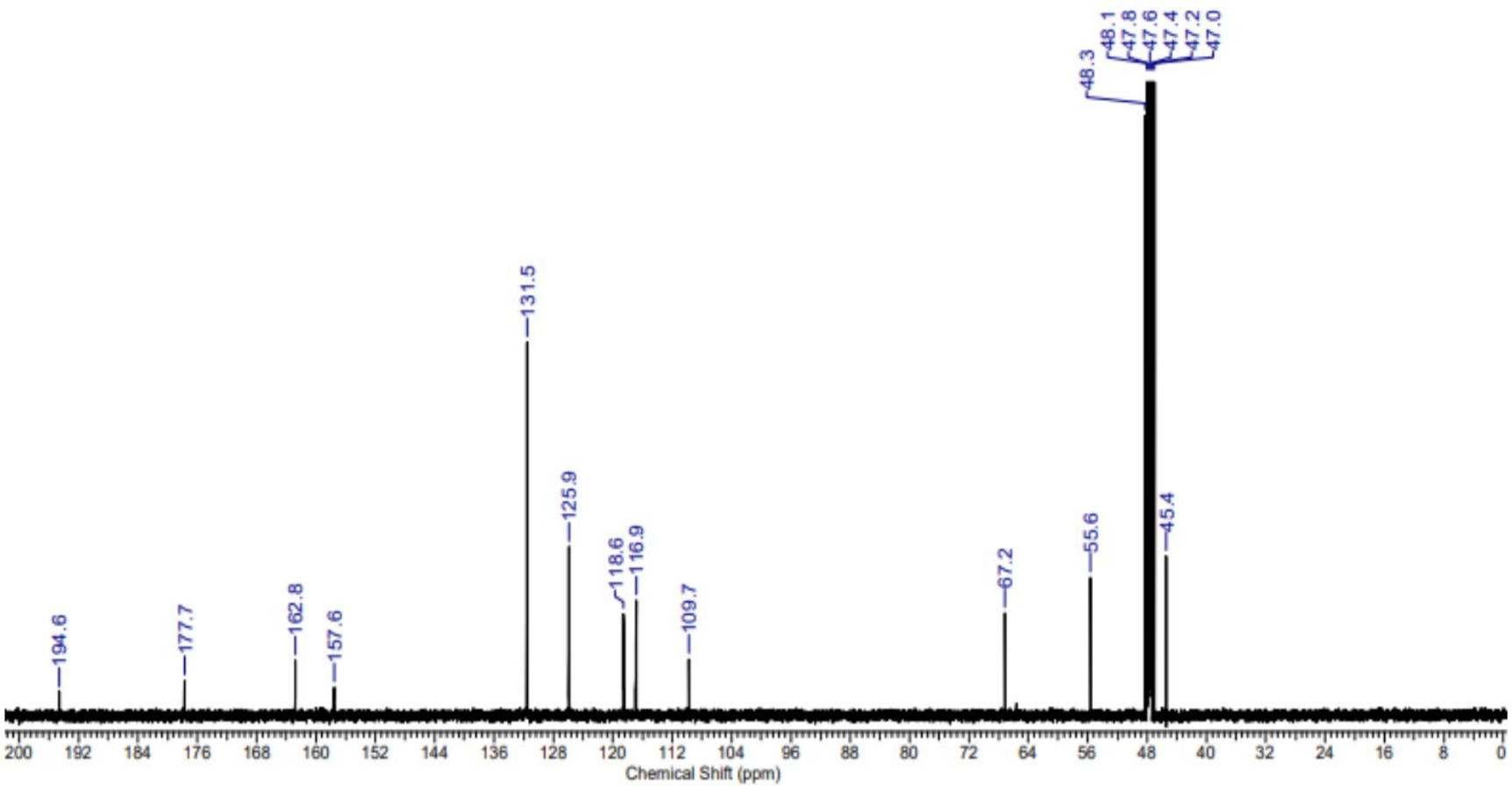
Appendix C5: ^{1}H-NMR spectrum of compound **(4b)** Expanded 1



Appendix C6: ^{1}H-NMR spectrum of compound **(4b)** Expanded 2



Appendix C7: ^{13}C-NMR spectrum of compound **(4a)**



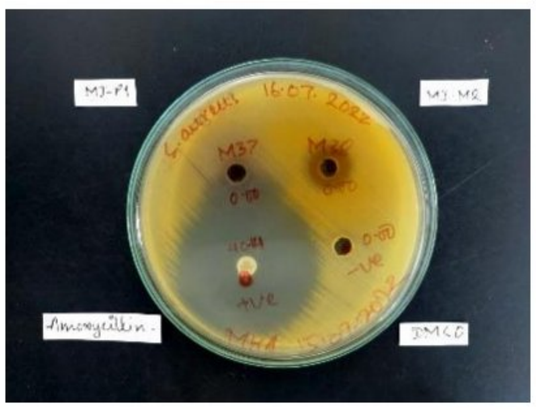
Appendix C8: ^{13}C-NMR spectrum of compound **(4b)**

Appendix D: Antimicrobial activity of synthesized compounds

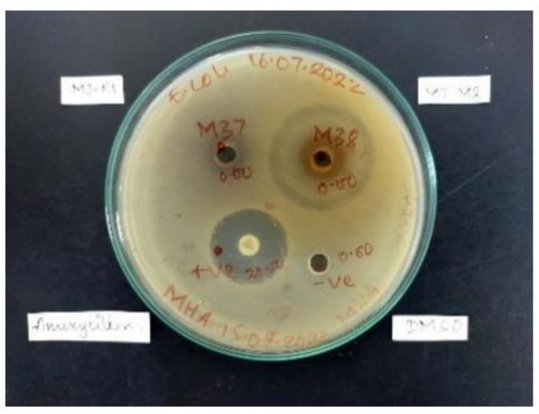
Against *Pseudomonas aeruginosa*

Against *Proteus vulgaris*

Against *Salmonella typhi*

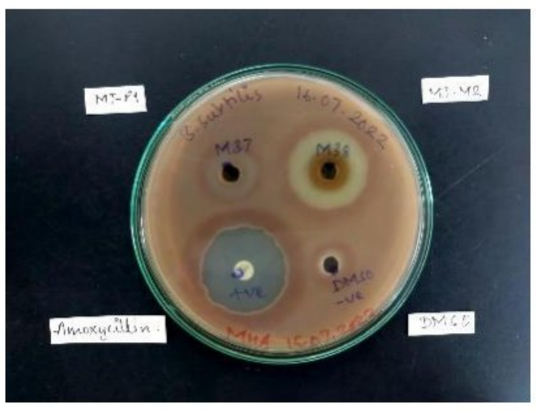


Against *Staphylococcus aureus*



Against *Escherichia coli*

Against *Enterococcus faecalis*



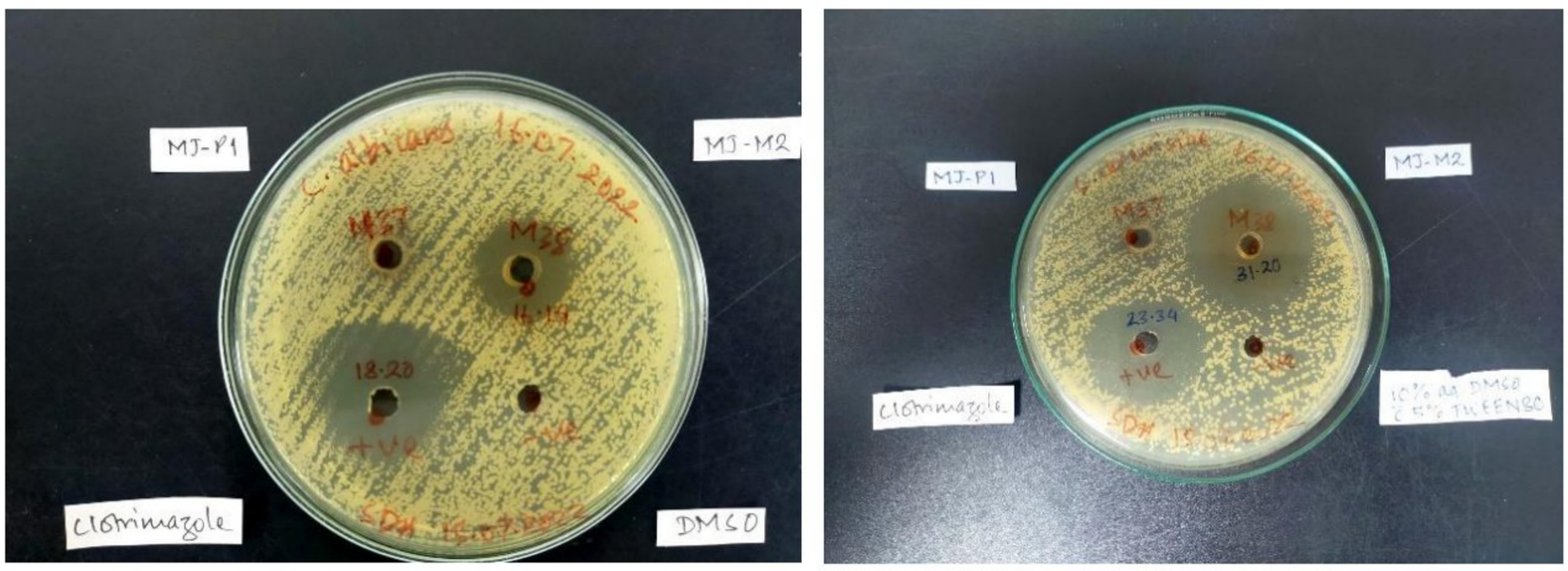
Against *Bacillus subtilis*

Against *Staphylococcus epidermidis*

Against *Shigella dysenteriae*

Against *Klebsiella pneumoniae*

Appendix D1: Antibacterial activity of compound (**4a**) and (**4b**)



Against *Candida albicans*

Against *Saccharomyces cerevisiae*

Appendix D2: Antifungal activity of compound (**4a**) and (**4b**)