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Chemistry Department

Synthesis of Some Heterocyclic Compounds at Position Six of 2-Methyl Phenol and Evaluation of their Antioxidant Properties Besides to Antibacterial Activities for Some of them

A Thesis

Submitted to the Council of the College of Education for Pure Science (Ibn-Al-Haitham) University of Baghdad In Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

By

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Dedication

To

MEMORY OF MY FATHER

ABED SAOUD

with love

Acknowledgment

Above all else, I want to express my great thanks to Allah for the uncountable gifts and for helping me to present this thesis.

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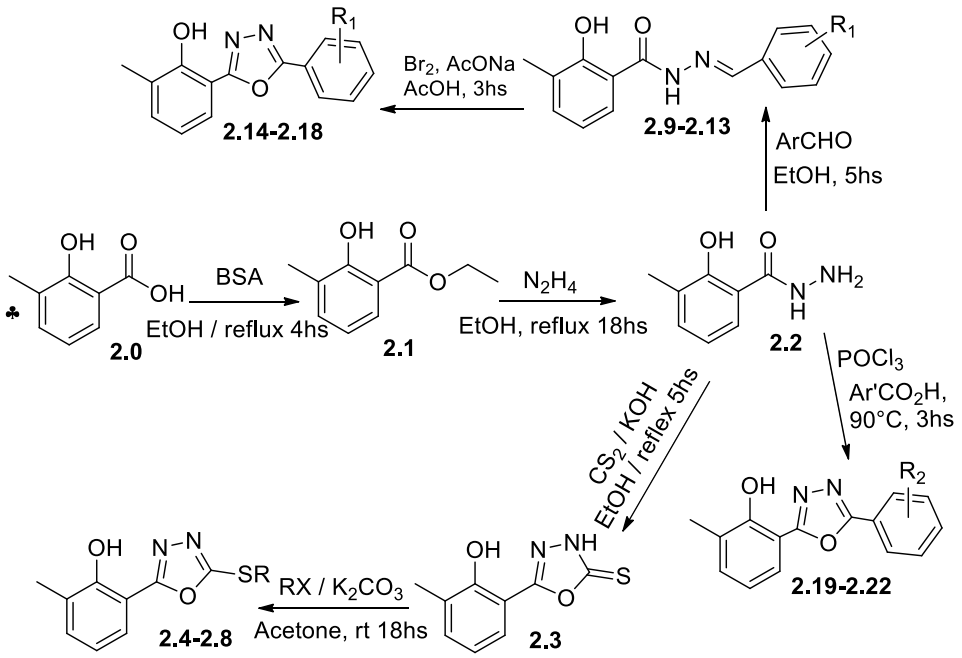
Shaimaa

Abstract

Antioxidant compounds are one of the famous compounds in therapeutic and industrial fields. The revolution of science development requires new materials attend to this development. New thirty nine compounds were synthesized attached with 2-methylphenol at position six to enhance the antioxidant ability of these derivatives by increasing the steric hindrance around the hydroxyl group of the 2-methylphenol as well increase the resonance. The 1,3,4-oxadiazole-5-thione (coded as **2.3**) was formed at position six of the 2-methylphenol and five thio derivatives (**2.4-2.8**). The antioxidant activity screened by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays utilized three 2-methylphenol, BHT and ascorbic acid as references. Compound **2.3** exhibited higher antioxidant than its derivatives in both assays. New nine 1,3,4-oxadiazole-5-aryl derivatives were formed at the same position utilized two methods. The first five compounds (**2.14-2.18**) were synthesized from corresponding hydrazones (**2.9-2.13**). The rest four (**2.19-2.22**) compounds synthesized from reaction of the acid hydrazide of 3-methyl-2-hydroxybenzoic acid with aryl carboxylic acid in the presence of phosphorus oxychloride ($POCl_3$) as dehydrating agent. The antioxidant capacity was screened by the same two assays. Compound **2.12** showed higher antioxidant than other hydrazones. The antioxidant ability oxadiazoles showed antioxidant ability less than their corresponding hydrazones. The antioxidant ability of the oxadiazoles formed by second methods also investigated by DPPH and FRAP. Generally, the antioxidant ability of all oxadiazoles depends on the type substituted group and their steric hindrance, e.g. aryl with 3,5-di-*tert*-butyl-4-hydroxy.showed higher antioxidant ability than others oxadiazoles Oxadiazole-5-amine (**2.23**) as well formed at position six of the 2-methylphenol besides two Schiff bases derivatives (**2.24-2.25**). Compounds (**2.26**) and (**2.27**) were synthesized from Schiff bases reduction by sodium borohydrate. Compounds (**2.28-**

2.29) were synthesized from the reaction of the oxadiazole-5-amine with two carboxylic acid in the presence of 1,1'-carbonyldiimidazole (CDI) as coupling agent. Compound (**2.24**) recorded antioxidant ability higher than 2-methyl phenol and BHT in both assays. The antioxidant ability raised up with the Schiff bases derivatives. However, compound (**2.26**) exhibited antioxidant less than the corresponding Schiff bases as well compound (**2.27**) showed antioxidant ability less than the Schiff bases. Furthermore, 1,3,4-thiadiazole-5-amine (**2.30**) was formed at the same position. Three derivatives were synthesized (**2.31-2.33**). The first derivative was Schiff base and the second one was produced from reduction of the Schiff bases, while the third one from reaction the amine group with 3,5-di-*tert*-butyl-4-hydroxybenzoic acid in the presence of CDI. The antioxidant ability of this group was too closed to the oxadiazole-5-amine and its derivative. This result refers to the type of the ring e.g. oxadiazole or thiadiazole does not have impact on the antioxidant but the steric hindrance, resonance and type of substituted group (electron donating group (EDG) or electron with drawing group (EWG)) possess the major impact on increasing the antioxidant Three hydrazinecarbothioamide derivatives (**2.34-2.36**) were synthesized from the reaction of the acid hydrazide with aryl *iso*thiocyanate. These compounds synthesized to be substrate for synthesis of the 4-aryl-1,2,4-triazol-3-yl-5-thione ring (**2.37-2.39**). The hydrazinecarbothioamide showed significant antioxidant in both assays and it was slightly less than ascorbic acid. Furthermore, the results exhibited that the type of the substituted group of the aryl *iso*thiocyanate did not affect on the antioxidant ability. The corresponding 1.2.4-triazoles-4-amino displayed antioxidant ability less than their hydrazinecarbothioamide, however their antioxidant ability was higher than BHT. Finally, these compounds characterized with m.p, IR, 1D NMR and some compounds characterized by 2D NMR. Most of the compounds were identified by mass spectroscopy. The following Scheme demonstrated all research work.

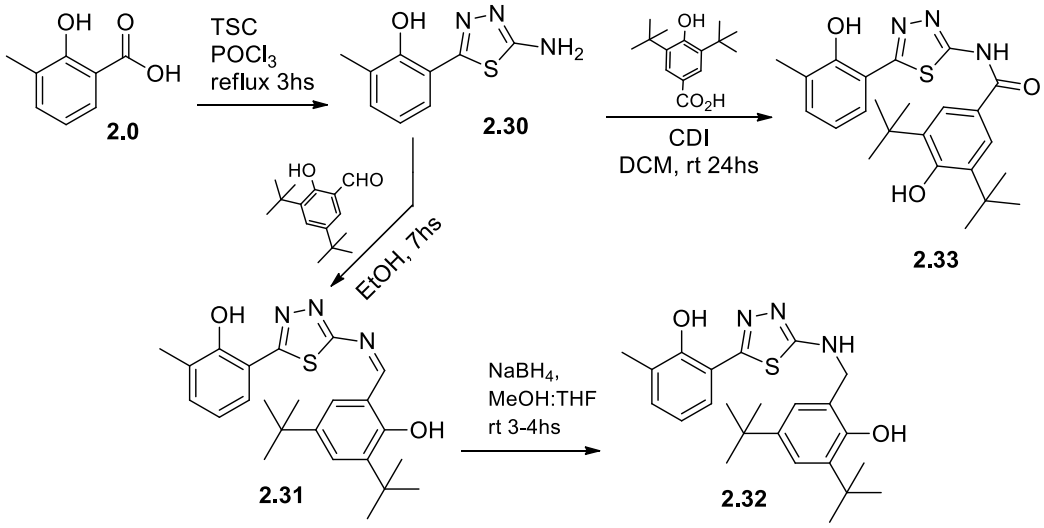


R=Et (**2.4**), benzoimidazol (**2.5**), 4-Brbenzoyl (**2.6**), ethoxyformyl (**2.7**), benzyl (**2.8**).

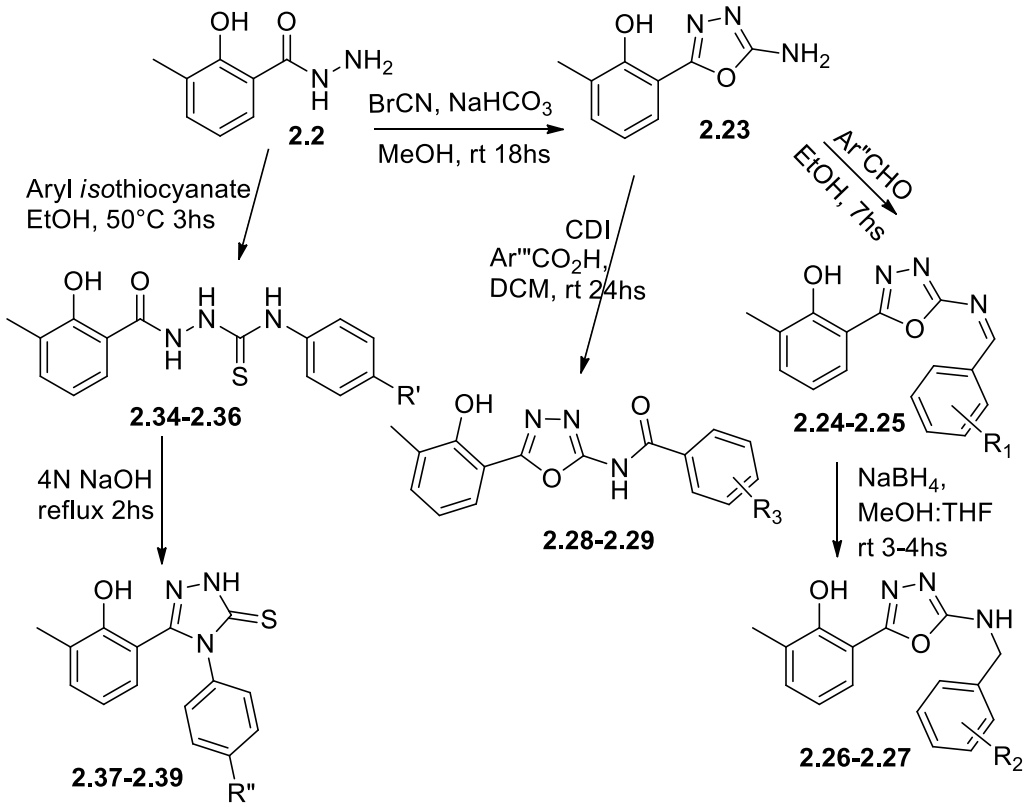
R_1=4-Me (**2.9**), 4-OH (**2.10**), 4-OH-3-OMe (**2.11**), 4-OH-3,5-di-OMe (**2.12**), 2-OH-3,5-di-*tert*-butyl (**2.13**).

R_2= 4-Cl (**2.19**), 4-OMe (**2.20**), 4-OH-3,5-di-*tert*-butyl (**2.21**), 2-OH-3-Me (**2.22**).

Scheme (i):-Synthesized compounds (**2.1-2.22**)



Scheme (ii):-Synthesized compounds (**2.30-2.33**)



R_1=4-OH-3,5-di-OMe (**2.24**), 2-OH-3,5-di-*tert*-butyl (**2.25**).
R_2=4-OH-3,5-di-OMe (**2.26**), 2-OH-3,5-di-*tert*-butyl (**2.27**).
R_3=4-OMe (**2.28**), 4-OH-3,5-di-*tert*-butyl (**2.29**).
R'=Me (**2.34**), Cl (**2.35**), OMe (**2.36**).
R"=Me (**2.37**), Cl (**2.358**), OMe (**2.39**).

Scheme (iii):-Synthesized compounds (**2.23-2.27**) and (**2.34-2.39**)

Table of contents

List of Figures

List of Schemes

List of Tables

List of Abbreviation

ACAT	acetyl-coenzyme A acetyltransferases
APT	Attached Proton Test
BHA	butylated hydroxyl anisole
BHT	butylated hydroxyl toluene
BMT	Bone marrow transplantation
bs	Broad singlet
BSA	Benzene sulfonic acid
˚C	Celsius
cm^{-1}	Reciprocal centimeters
CO_2	Carbon dioxide
COX-2	Cyclooxygenase-2
d	doublet
DBU	1,8-DiazaBicyclo[5.4.0]Undec-7-ene
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	Deuterated dimethyl sulfoxide-d_6
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-1-picrylhydrazyl
EIMs	Electron ionization mass spectra
FAG	Glucuronide of Ferulic Acid
FRAP	ferric reducing antioxidant power
FTIR	Fourier Transform Infrared (
g	gram
HAT	H-atom transfer
HIV	human immunodeficiency virus
HMBC	Heteronuclear Multiple Bond Correlation
HMPA	Hexamethylphosphoramide
hs	hours
IC_{50}	Half maximal inhibitory concentration
J	Coupling constant
LTB4	Leukotriene B4
m.p.	Melting point
MHz	megahertz
ml	Milliliter
mol	mole
MW	Microwave
NMP	N-Methyl-2-pyrrolidone

NMR	Nuclear magnetic resonance
NSAIDs	non-steroidal anti-inflammatory drugs
PG	propyl gallate
PPA	Polyphosphoric Acid
ppm	Part per million
PTSC	Para Toluenesulfonyl Chloride
q	Quartet
RNS	reactive nitrogen species
ROS	reactive oxygen species
rt	Room temperature
s	Singlet
SET	single electron transfer
SOD	enzyme superoxide dismutase
T	Triplet
TBHP	*tert*-Butyl hydroperoxide
TBHQ	*tert*-butyl hydroquinone
TLC	thin layer chromatography
TMS	Tetramethylsilane
TOH	Tocopherols
TPTZ	2,4,6-tripyridyl-*s*-triazine
TSC	Thiosemicarbazide
UV	Ultraviolet

Chapter 1 : Introduction

1.1 Introduction.

It was about more than seventy years, the researchers well connected between the effect of oxidative stress and reactive oxygen species (ROS) with etiology and progression of essential human degenerative diseases. This extensive knowledge focuses on the essential role that antioxidant plays to prevent a large number of diseases. Last few decades plentiful evidence that the antioxidant material in fruit and vegetable possesses prophylactic medicine of various diseases such as cancer, heart diseases, diabetes and cataracts. Furthermore, quite number of drugs own antioxidant properties such as anti-cancer anti necrotic, anti-inflammatory, neuroprotective, digestive and hepatoprotective, likewise those antioxidant properties were the part of their activity. On the other hand, the free radical scavenging substrate is exceedingly used in food and pharmaceutical industries to prohibit industrial material from oxidation and damage via exposition to light, heat, and oxygen[1]. A result to these benefits the material possesses antioxidant properties considered as important material. Natural and synthetic phenolic antioxidants are one of the important antioxidant species including polyphenolic antioxidant, flavonoids antioxidant, tocopherols and hindered phenolic antioxidant. A large number of these antioxidant species are well known with their therapeutic properties.

1.2 Free Radicals and Antioxidant.

Free radical can be defined as a molecule or atom possesses unpaired electron usually it symbolized as radical dot (˙) and they are unstable highly and reactive, comparison with similar ions. distinctly that the free radicals engage in many biological processes.[2]. The subsistence of enzyme superoxide dismutase (SOD) in living systems, involving humans, the effects of free radicals are regarded as very normal. for example,

plainly hinted on the involving of the superoxide radical, ($^{\bullet}O_2$, $^{\bullet}O$, $^{\bullet}OOH$ and $^{\bullet}OH$).[3], otherwise, during aerobic respiration the reactive oxygen species (ROS) can be produced.[4] moreover, hydrogen peroxide radical, hypochlorite radical, nitric radical, and different lipid peroxide are contributed to be one of the most important radical species in biological functions.[5]. The reactive oxygen species (ROS) and other free radical species are efficient to react either directly or indirectly, to damage all biomolecules, like proteins, lipids, DNA, and carbohydrates producing serious cell damage.[6] This damage is one of the main reasons to cause major diseases such as inflammatory disease,[7] cancers disease,[8] degenerative disease [9] and chronic diseases.[10] Figure 1.1 summarized the clinical effect of ROS[11].

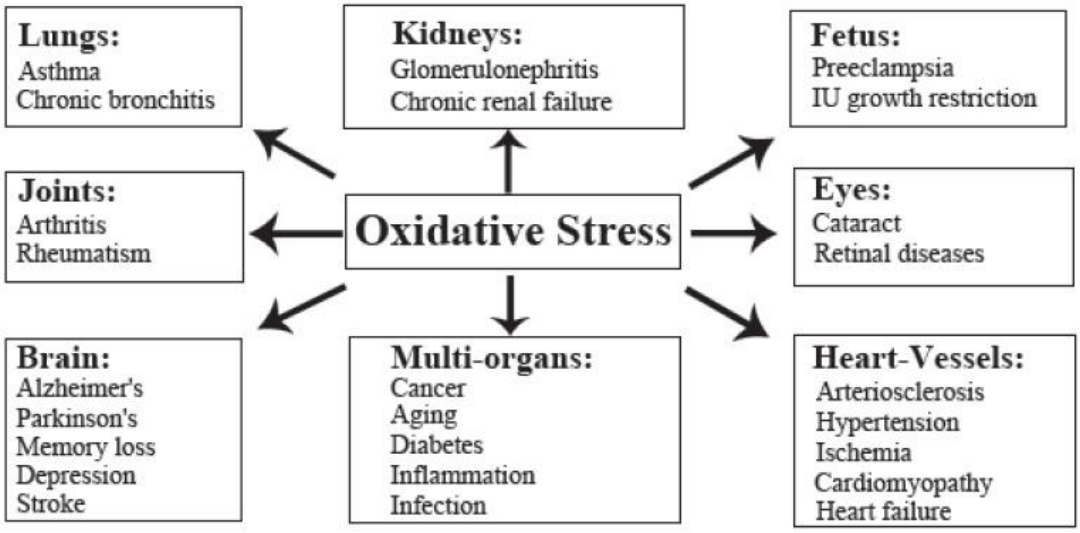
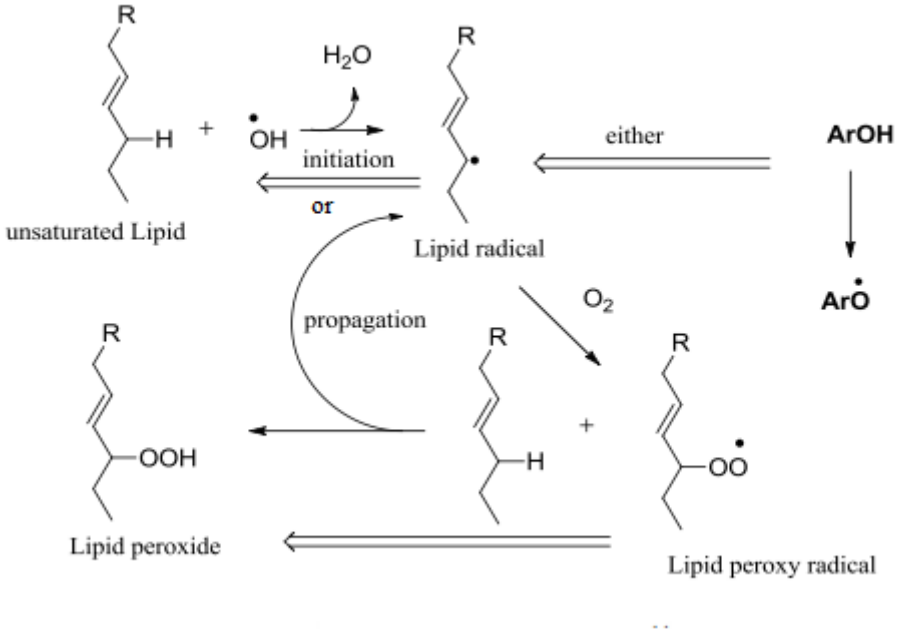


Figure 1.1:-Summarized clinical effect of ROS.

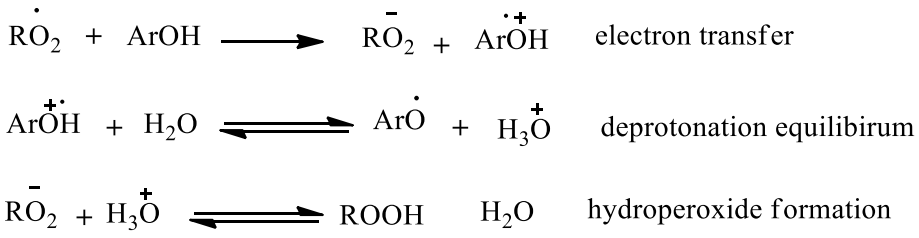
Broadly, free radical scavenging (antioxidants) can be elucidated as each substance or material (at low concentration) possesses ability to delay oxidation or prevent free radical effect.[12]. This qualification inspires many researchers to focus on the importance of these materials in therapeutic fields and in industrial fields. The antioxidant owns two pathways to prevent the oxidation effect. The first one is by H-atom

transfer (HAT) which prevents peroxidation of lipid as depicted in Scheme 1.1.



Scheme 1.1:-HAT mechanism of lipid peroxidation pathway.[13]

In this mechanism, the phenol donating hydrogen atom and terminate the reactive free radical by two possible pathways, the first pathway, by terminate formation of free radical instance (before starting the initiation stage), while the second pathway depends on terminate the free radical after initiation reaction. In both pathways, the phenol should be converted to stable free radical[14]. In another word, it does not react and generats new free radical. While, the second pathway, it terminates the free radical by single electron transfer (SET).[14] as shown in Scheme 1.2.



Scheme 1.2:-Mechanism of single electron transfer.

In this mechanism, when antioxidant transfers a single electron to assistance in the reduction of target compounds and converted to radical-cationic antioxidant compound then deprotonated through interaction with water. However increase the life of radical-cationic can increase the radical propagation reaction then decrease the antioxidant capacity[15]. In spite of, many antioxidant compounds differentiate follow either HAT or SET mechanism, many researchers confirmed that both mechanisms could be accorded at the same time[15-17].

1.3 Source of antioxidants.

Natural and synthetic are the main sources of the antioxidant compounds. The fruits, vegetables, nuts, seeds, leaves, roots and barks[18] which are the familiar natural antioxidant .

1.3.1 Natural phenolic antioxidant.

Substrates dwell in plants or their extracts possess antioxidant capacity can be classified as natural antioxidant. Phenolic compounds are the famous class in natural antioxidants such as poly phenolic compounds flavonoids, cinnamic acid derivative, coumarins and tocopherols. Many researchers assert that there is akin relationship between reduce health diseases and diet habit. Even though, herbal remedy and plants were known in most old nations. These days, there are many scientific researchers who emphasized that the vegetables, herbalists and fruits can be significant therapy for instance, anticancer[19, 20], analgesic[21, 22], anti-rheumatic[23] and anti-inflammatory[24]. Furthermore, phenolic compounds in plants are well known for their ability to reduce risk of several diseases like, cardiovascular[25], diabetes[26-28], cancer[29], hypertension[30] and inflammatory processes[31]. In vegetables and fruits, vitamins A, E, C, carotenes, dietary fiber and phenolic compounds which are recognized as good antioxidant in one of the main constituents responsible for these protective effects beside metals such as potassium,

zinc and selenium[32-35]. Besides to the antioxidant ability of the natural phenolic compounds they can own antimutagens and metal chelators[36]. Although, vegetables and fruits are responsible to reduce the risk of developing disease. the results are sometimes incompatible[37]. It has been reported that some phenolic compounds considered harmful. when engorging large amounts[38]. Representation of proteins from complexes with polysaccharides by lipid metabolism is one of the commonly side effect of phenolic compounds[39]. There are three species of antioxidants in the human bodies .The first one, is achieved by enzymes to adjust initial free radical production[40]. Usually in respiration process the hydrogen peroxide, hydroxyl radical and superoxide are generated as subproduct and the glutathione peroxidase as antioxidant aid to reduce the effect of ROS[41]. The α-tocopherol, ascorbate and others antioxidants, which are in vegetables and fruits can be provided as antioxidant can play pivotal role to break the chain reaction of the free radicals, either by H–atom transfer or single electron transfer. Figure 1.2 demonstrated some important phenols in plants and fruits[42].

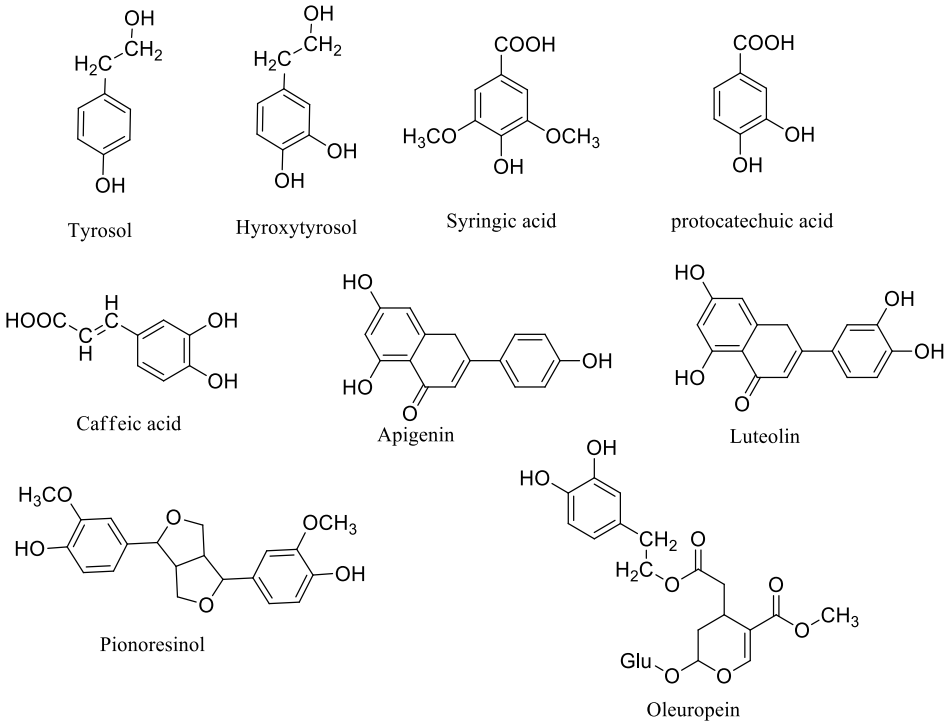


Figure 1.2:-Some natural phenolic compounds in plants and fruits.

1.3.2 Synthetic phenolic antioxidant.

Drugs, oil, food, pigments, rubber and polymers required antioxidant compounds as stabilizer to prevent oxidative stress. Furthermore, the therapeutic effect of these compounds highlighted the interest to develop and synthesize new phenolic derivatives. In industrial field such as feeding industrial, confectionery and edible oil industry, butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), and *tert*-butyl hydroquinone (TBHQ) propyl gallate (PG) are usually utilized in this field. Rubbers and polyolefin industrials usually use one of the most common antioxidants ,which is known as AO-60 [43, 44] as Figure 1.3 illustrated its structure.

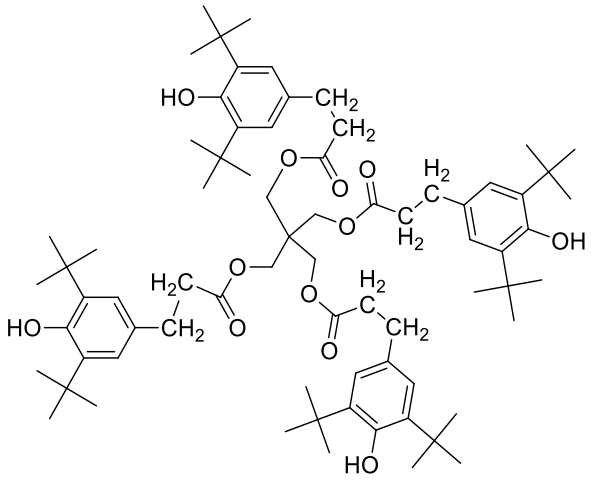
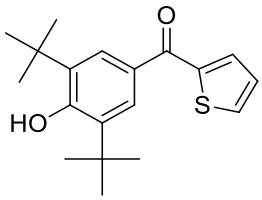


Figure 1.3:-AO-60 antioxidant compound.

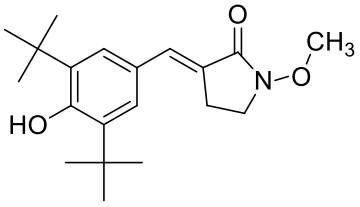
In therapeutic field, hindered phenols that content heterocyclic reveled significant biological activity as well, some of these compounds utilized as medicinal. For example, R-830 (Figure 1.4) was affirmed as potent inhibitor of guinea pig lung lipoxygenases and cyclooxygenase moreover, known as significantly inhibited LTB4 production by human neutrophils with the calcium ionophore A23187. This compound was known as efficient anti-inflammatory drug for animal like, carrageenan-induced[45].



R-830

Figure 1.4:-R-830 as anti-inflammatory drug.

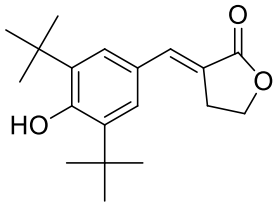
The *N*-methoxy-3-(3,5-di-*tert*-butyl-4-hydroxyben-zylidene)-2-pyrro lidone (E-5110) known as nonsteroidal anti-inflammatory agent (Figure 1.5) and it can inhibite prostaglandin E2 (PGE2) generation and it is able to superoxide generation by human (PMN) [46, 47], As well, it possesses analgesic profile likewise indomethacin, however it revealed more less ulcerogenic than indomethacin and piroxicam[48].



E-5110

Figure 1.5:-Structure of E-5110.

Compound KME-4 (Figure 1.6) exhibited significant analgesic capacity[49], as well possess an ability to reduce the radiographic bone damage scores[50].



KME-4

Figure 1.6:-KME-4 as analgesic capacity.

In addition to the antioxidant ability, Ziakas *et al*.[51] synthesized hindered phenol that contains heterocyclic which possesses significant anti-inflammatory properties as shown in Figure 1.7.

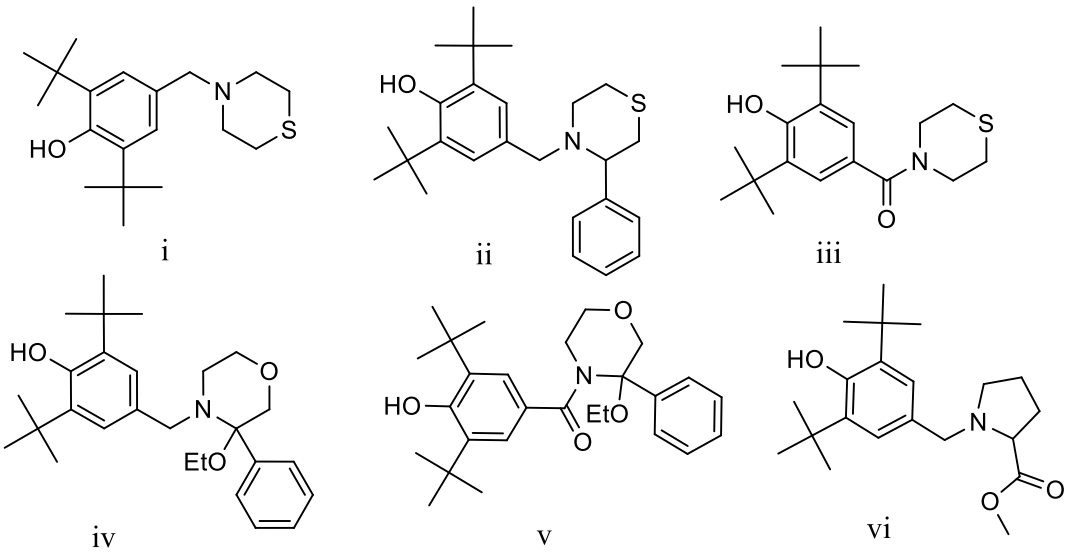


Figure 1.7:-Some hindered phenol containing heterocyclic has anti-inflammatory diseases.

Thiadiazole and thiazolenon containing hindered phenol such as PD 164387 and PD 138387 (Figure 1.8) exhibited selective cyclooxygenase-2 (COX-2) inhibitions. [52].

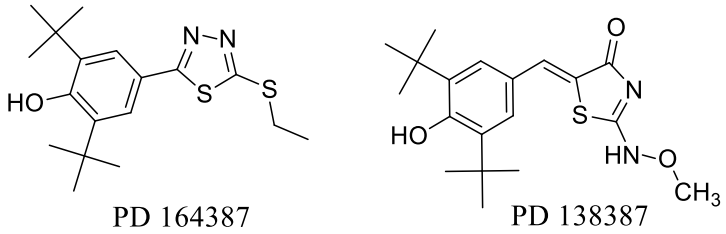


Figure 1.8:-Structure of PD 164387 and PD 138387.

BO-653 and BOB known as drug for atherosclerosis besides its significant antioxidant capacity[53]. Figure 1.9 demonstrated the chemical structure of BO-653 and BOB.

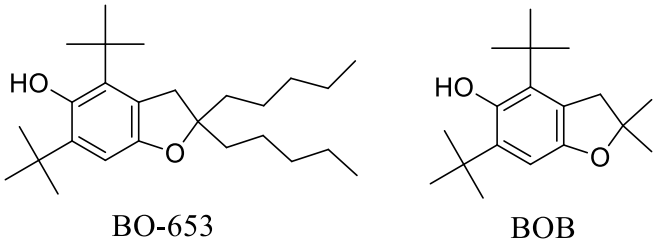
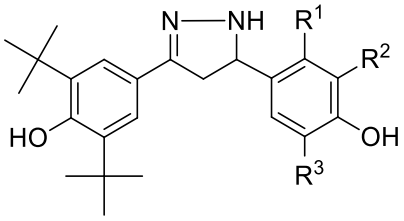


Figure 1.9:-Structure of BO-653 and BOB as drug for atherosclerosis.

Jeong *et al*.[54, 55] synthesized a series of 3,5-bis substituted phenol–pyrazole and investigated their effect as ACAT inhibitors as demonstrated in Figure 1.10



a R^1=H,R^2=R^3=t-Bu b R^1=H,R^2=R^3=i-Pr
c R^1=H,R^2=R^3=Me d R^1=R^2=R^3=Me
e R^1=H,R^2=R^3=F f R^1=H,R^2=R^3=Ph
g R^1=R^2=R^3=OMe h R^1=R^2=R^3=NO_2
i R^1=H,R^2=R^3=H

Figure 1.10:-2,6-di-*tert*-butylphenol derivatives.

Darbufelone (Figure 1.11) known as anti-inflammatory drug as well founded to encourage growth inhibition of lung cancer cells both in *vitro* and in *vivo*[56].

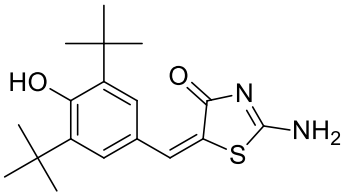


Figure 1.11:-Structure of Darbufelone.

Rangaswamy *et al*.[57] synthesized benzofuran with hetrocyclic attached multi phenol (Figure 1.12) exhibited significant antioxidant.

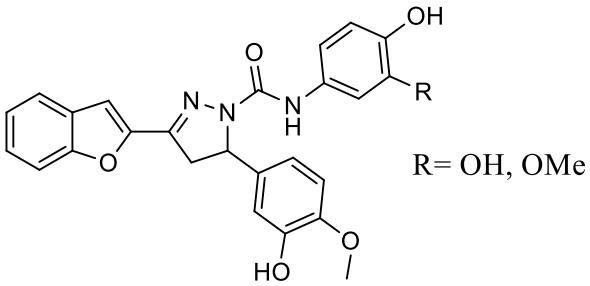


Figure 1.12:-Struture containing pyrazole ring.

Piazzon *et al*.[58] reported that the new synthesized of acyl Glucuronide of Ferulic Acid (FAG) and caffeic acid-3’-O-glucuronide (Figure 1.13) exhibited extraordernary antioxidant properties.

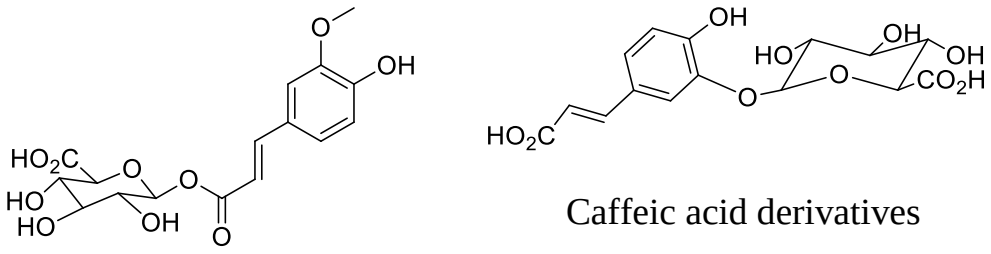
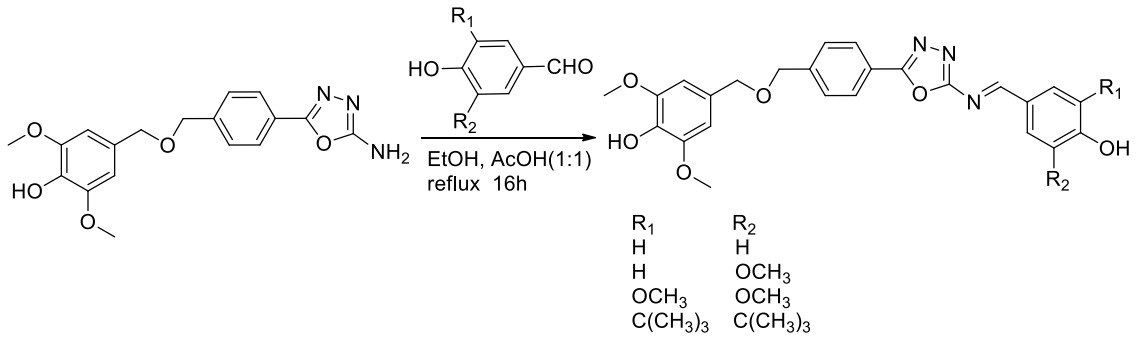


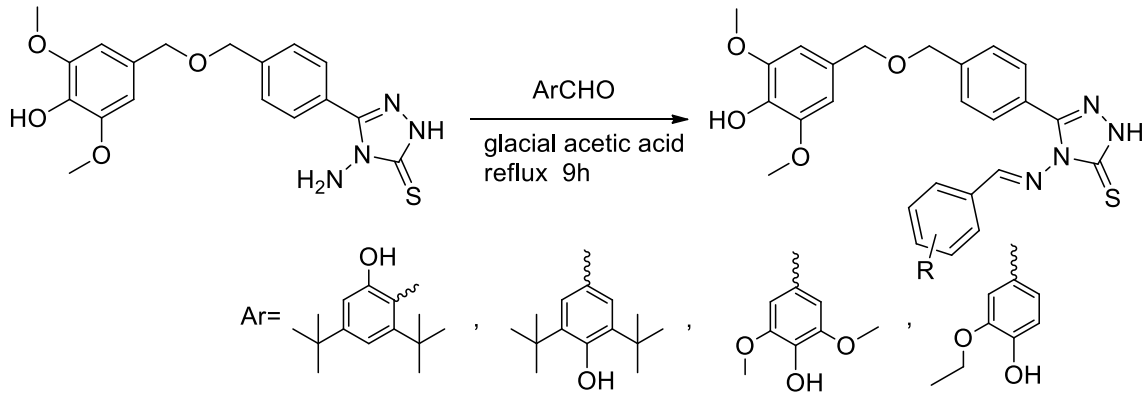
Figure 1.13:-Structure of Ferulic Acid (FAG) and caffeic acid-3'-O-glucuronide.

1n 2014 K.F.Ali[59] synthesized 1,3,4-oxadiazole amine attached with 4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzene-1-yl and their Schiff basses. Moreover, he reported that the Schiff basses that include hindrance phenol and *ortho* substituted phenol exhibited antioxidant activity higher than substituted phenol as shown in Scheme 1.3.



Scheme 1.3:-Synthetic route of target compounds.

Furthermore, in 2016 the 4-amine-1,2,4-triazole-5-thione have been synthesized attached same phenolic group and as well it was found that the antioxidant ability depends on the number of substituted groups and the type of these substituted[60] as depicted in Scheme 1.4.

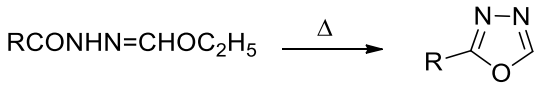


Scheme 1.4:-Synthesized roule of multi phenol.

1.4 1.3.4-Oxadiazole and its biological activity.

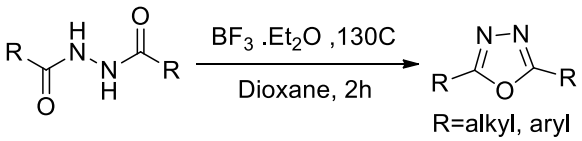
In 1955, Ainsworth.C. and Müller *et al*. at separated publications, they reported first methods for synthesizing 1,3,4-oxadiazole[61, 62]. Since that time synthesis of oxadiazole ring attains big attention. Large number methods, have been reported as well a large number of oxadiazole derivatives has been reported. In this section, some of these methods will be presented. 2,5-di substituted 1,3,4-oxadiazole gain wide interest among all oxadiazole derivatives there at least more than twenty different methods with catalyst for synthesizing new 2,5-di substituted 1,3,4-oxadiazol derivatives. In general, most theses methods depend on condensation reaction either of carboxylic acid with acid hydrazide or from cyclization reaction of 1,2-diacyl or aryl hydrazine using dehydrating agent. Cyclization the hydrazones usually using oxidation agent. For that, we will demonstrated the most important methods for synthesized 1,3,4-oxadiazole ring.

a) Heating the 1-Acyl-2-ethoxymethylene hydrazines at atmospheric pressure afforded alkyl 1,3,5-oxadiazole[63] as shown in Scheme 1.5.



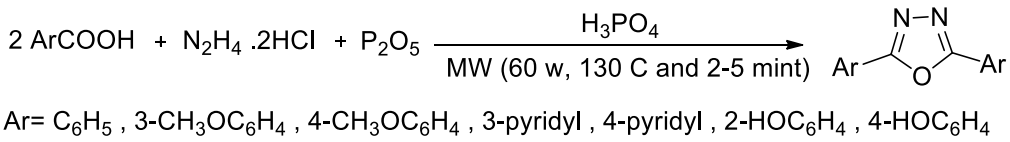
Scheme 1.5:-Synthesis of oxadiazole by heat.

b) Using $BF_3.Et_2O$ as dehydration agent to cyclize 1,2-diacyl or 1,2-diaroyl hydrazines[64] as shown in Scheme 1.6.



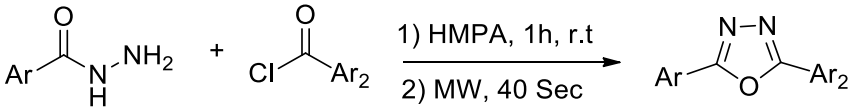
Scheme 1.6:-Synthesis of oxadiazole by using $BF_3.Et_2O$.

c) Fouad Bentiss *et al.* utilized phosphorus pentoxide and phosphoric acid as dehydration and microwave to cyclize two equivalents of aromatic carboxylic acid andwith hydrazine dihydrochloride to afford corresponded symmetrical 2,5-di aryl 1,3,4-oxadiazole with efficient yields[65] as shown in Scheme 1.7.



Scheme 1.7:-Synthesis of oxadiazole by utilized phosphoric acid H_3PO_4.

d) Sabir H *et al.* they reported synthezies asymmetrical 2,5-di aryl 1,3,4-oxadiazole in less than one mint under microwave heating using hexamethylphosphoramide (HMPA) as a solvent[66] as depicted in Scheme 1.8.

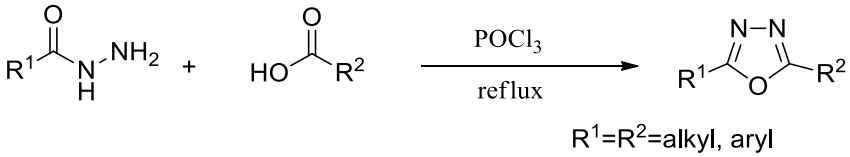


Scheme 1.8:-Synthesis of oxadiazole by using hexamethylphosphoramide.

e) Treatment symmetrical and asymmetrical 1,2-diarylhydrazines with zirconium (IV) chloride (10% mole) as cyclodehydration agent[67] as shown in Scheme 1.9.

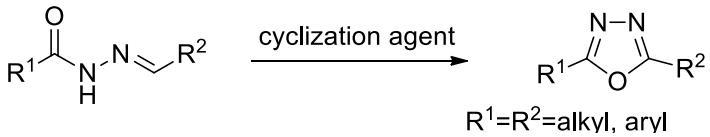
Scheme 1.9:-Synthesis of oxadiazole by zirconium (IV) chloride.

f) Synthesis 2,5-disubstituted 1,3,4-oxadiazole by using Phosphorus oxychloride ($POCl_3$) as dehydration agent [68]. This method is considered widely popular and easier method as shown in Scheme 1.10.



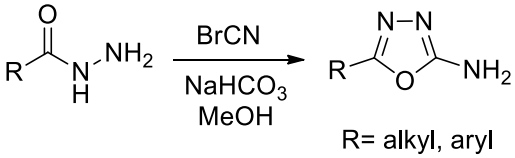
Scheme 1.10:-Synthesis of oxadiazole by using Phosphorus oxychloride.

g) Hydrazone cyclization to di substitute oxadiazole is widely used by some cyclization agent such as bis (trifluoroacetoxy)[69], *N*-chlorosuccinimide/1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [70], chloramine-T[71], potassium permanganate on the surface of SiO_2 with acetone or water under microwave irradiation[72] and bromine as oxidative agent in glacial acetic acid, in the presence of anhydrous sodium acetate[73-76], as depicted in general Scheme 1.11.



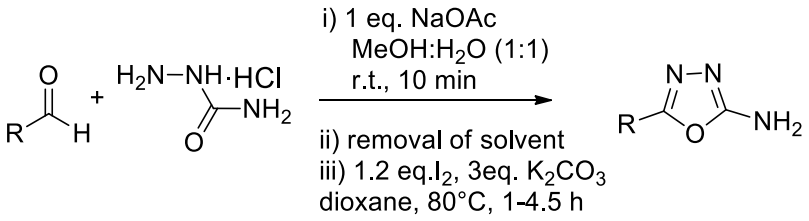
Scheme 1.11:-Synthesis of oxadiazole by using cyclization agent.

h) Alkyl and aryl of 1,3,4-oxadiazole-2-amine synthesized from cyclization of acid hydrazide in the presence of cyanogen bromide[77] as displayed below Scheme 1.12.



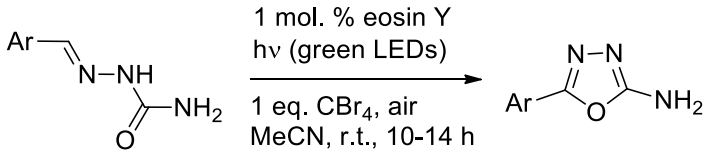
Scheme 1.12:-Synthesis of oxadiazole by using cyanogen bromide.

i) Direct reaction between aryl, alkyl and vinyl aldehyde with semicarbazide in basic medium to synthesize 1,3,4-oxadiazole-2-amine as demonstrated[78], in Scheme 1.13.



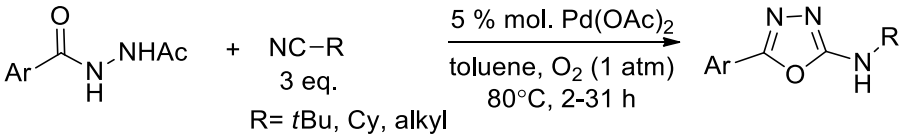
Scheme 1.13:-Synthesis of oxadiazole by using basic medium.

j) Aryl hydrazone was converted to oxadiazole amine in the presence of 1 mol eosine Y under UV light[79], displayed in Scheme 1.14.



Scheme 1.14:-Synthesis of oxadiazole by using eosine Y.

k) N-substituted oxadiazole amine was synthesized from reaction of N-acyl acid hydrazide with alkyl isocyanate in presence of palladium acetate[80], as depicted in Scheme 1.15.



Scheme 1.15:-Synthesis of oxadiazole by using palladium acetate.

This variety of synthesized methods reflected the importance of the oxadiazole derivatives especially their biological activity. The oxadiazole-2-thion showed various biological activities for instance. Thymidine phosphorylase inhibition[81], antibacterial[82, 83], anti-inflammatory, ulcerogenic liability and analgesic activities[84] Furthermore, these derivatives show meditative to significant antioxidant ability[85, 86]. As well as 2,5-di substituted 1,3,4-oxadiazole exhibited wide biological ability for instance, anticancer[87, 88], antibacterial[89], anti-inflammatory[90, 91], antifungal[92], anti-HIV[93], analgesic[84] and antioxidant[94, 95]. In addition to that, the 1,3,4-oxadiazole amine and their derivatives also had grate attention for their diverse biological activity.

For instance Samir Bondock *et al.*[96] reported the synthesis of new oxadiazole amine derivatives and investigated their antitumor activity. Two of these compounds (Figure 1.14) showed considerable antitumor activity.

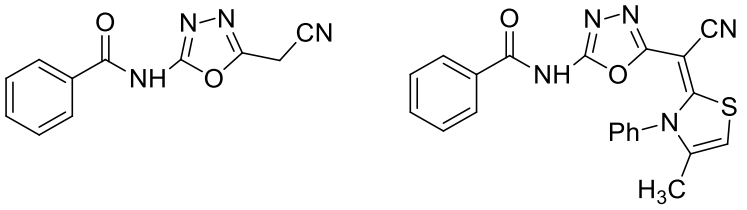


Figure 1.14:-Some oxadiazole have antitumor activity.

The phenolic oxadiazole showed intensive interest in biological activity besides to antioxidant ability for example, Mullincan *et al.*[97] synthesized oxadiazole containing hindrance phenol as shown in Figure (1.15). These compounds exhibited significant antioxidant. Furthermore they study their oral activity as ulcerogenic anti-inflammatory compounds. Treatment with arachidonic acid as non-steroidal anti-inflammatory drugs (NSAIDs). These drugs have been quite used for the treatment of inflammatory diseases. although, they have been implicated to reign most side effects such as dyspepsia, gastric ulceration, and nephrotoxicity[98].

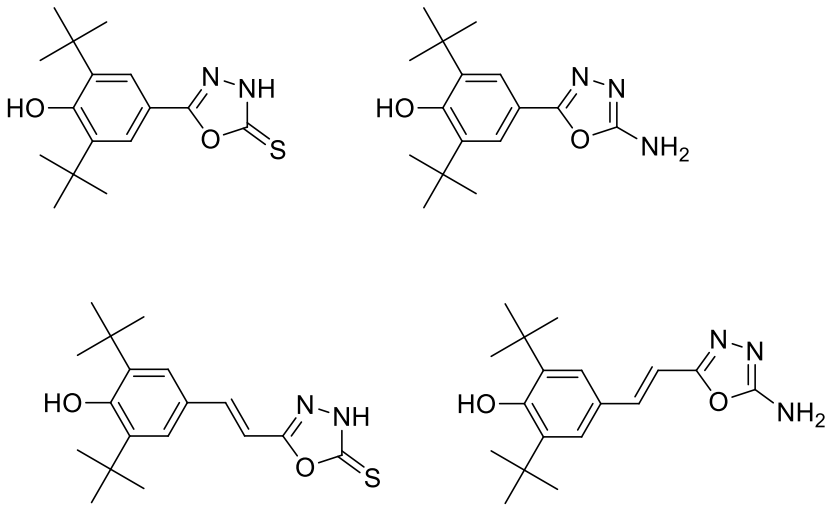


Figure 1.15:-Structure of some hindered phenol derivatives.

In 2016 R.M Shakir[99] reported the synthesis of new 5-aryl-1,3,4-oxadiazole containing 2,6-dimethoxyphenol. These compounds (Figure

1.16) showed significant antioxidant capacity as well reported that the type and position of substituted group in aryl play major role to enhance or reduce the antioxidant capacity.

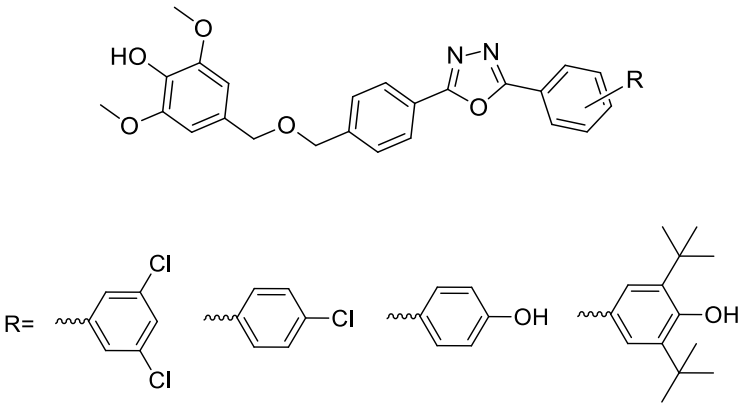


Figure 1.16:-Structure of oxadiaole derivatives containing 2,6-dimethoxyphenol.

In addition to the biological activity, the 1,3,4-oxadiazole derivatives showed interested physical properties such as electrochemical properties[100, 101], electro-optical properties[102] and luminescence property[103, 104] and photophysical properties[105].

According to these biological activities including the antioxidant ability, in this research, 1,3,4-oxadiazole-5-thion **(2.3)** and their thio alkyl **(2.4-2.8)** besides to 5-aryle-1,3,4-oxadiazole. Furthermore, 1,3,4-oxadiazole-5-amine **(2.23)** and their derivatives **(2.24-2.29)** have been formed at position six of 2-methyl phenol to increase their hindrance around the phenolic hydroxyl and participate to stabilize the free radical of these compounds after donating hydrogen radical and evaluated their antioxidant ability by DPPH and FRAP

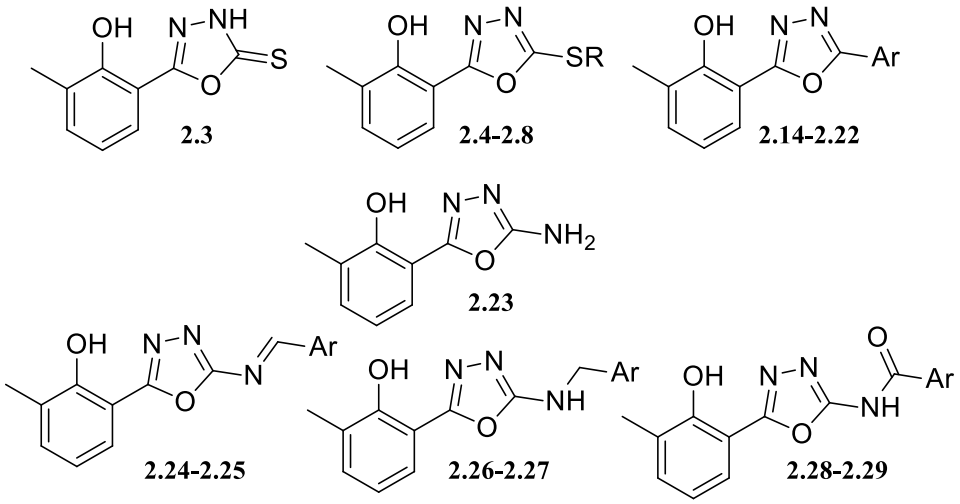


Figure 1.17:-Structure of compounds synthesized in this research

1.5 Hydrazones and their biological activity.

Hydrazones considered the best intermediate compounds for synthesis of 1,3,4-oxadiazole ring which has a wide range of biological activity such as analgesic[106], anti-inflammatory[107], antibacterial[108], anticancer[109] and antioxidant[110].

Salicyl hydrazones (Figure 1.18) exhibited significant antioxidant capacity as well showed ability to increase plasma hydrolysis.[111]

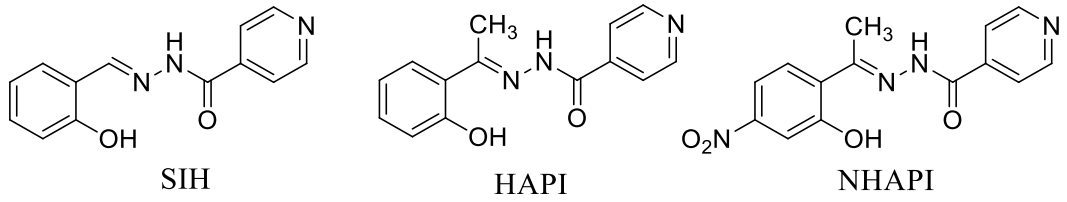
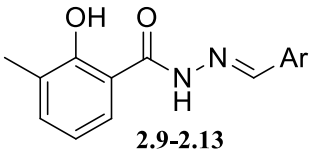


Figure 1.18:-Structures of compounds SIH, HAPI and NHAPI.

Recently, many researchers focus on intensive ability of hydrazons to be as anti- tuberculosis [112-114].

According to these wide biological activities this research, presented synthesized new hydrazones as depicted Figure1.19.



2.9-2.13

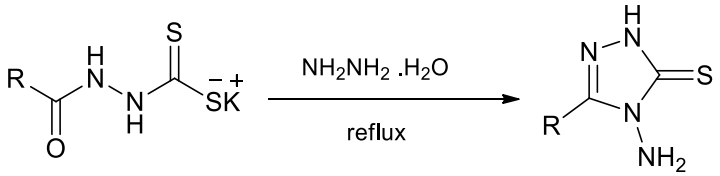
Ar= 4-MeC_6H_4, 4-OHC_6H_4, 3-MeO-4-OHC_6H_3
3,5-diMeO-4-OHC_6H_2, 3,5-di-*tert*-butylC_6H_2

Figure 1.19:- Structure of hydrazone compounds synthesized in this research.

1.6 1,2,4-triazole and their biological activity

Besides to the oxadiazole, 1,2,4-triazole derivatives draw attention for their variety applications. For that, many methods have been reported to synthesize 1,2,4-triazole derivatives.

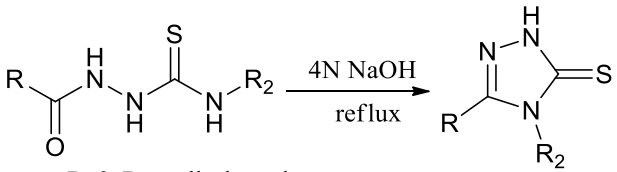
a) The derivatives of 4-amino-1,2,4-triazole-5-thione synthesized from reaction of potassium salt of hydrazinecarbodithioate with hydrazine hydrate[115, 116] as displayed in Scheme 1.16.



R= alkyl, aryl

Scheme 1.16:-Synthesis of 1,2,4-triazole in method a.

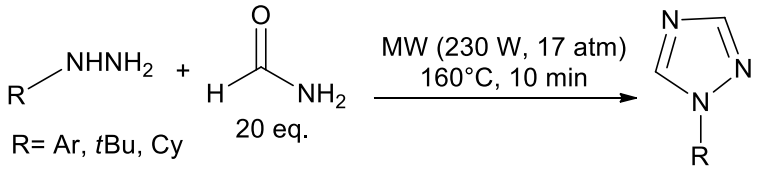
b) 3-aryl or alkyl -1,2,4-triazole-5-thione synthesized widely from refluxing the corresponding hydrazine carbothioamide in 4N sodium hydroxide[117, 118] as demonstrated in Scheme 1.17



R & R_2= alkyl, aryl

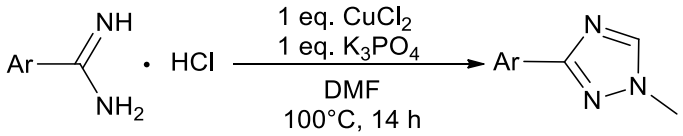
Scheme 1.17:-Synthesis of 1,2,4-triazole in method b.

c) Recently, G. M. Shelke *et al.*[119] reported the synthesis of substituted 1,2,4-triazoles by simple, efficient, and method from hydrazines and formamide under microwave irradiation without any catalyst



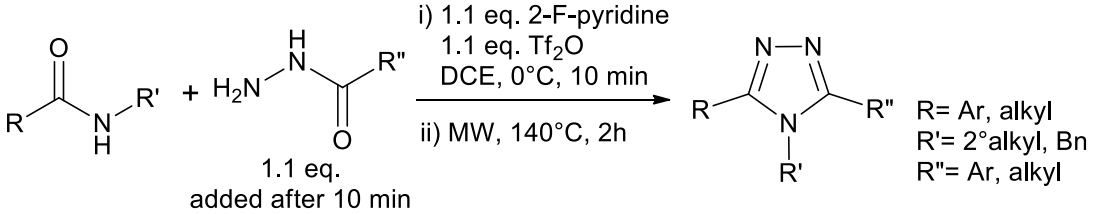
Scheme 1.18:- Synthesis of 1,2,4-triazole without any catalyst.

d) In 2015 H. Huang *et al.*[120] reported new catalytic oxidant system involving copper catalyst, K_3PO_4 as the base, and air as oxidant which enables an efficient synthesis of 2,4,6-trisubstituted, 2,6-disubstituted 1,3,5-triazines and 1,3-disubstituted 1,2,4-triazoles from amidines with trialkylamines, and DMF, as shown in Scheme 1.19.



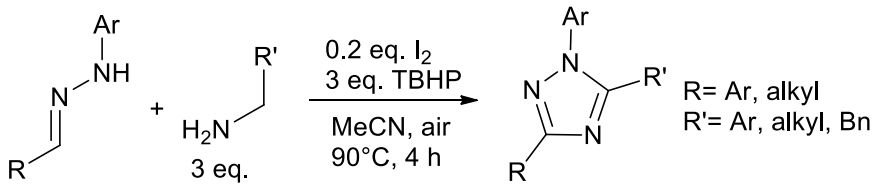
Scheme 1.19:-Synthesis of 1,2,4-triazoles by using copper catalyst.

e) As well, in 2015, W. S. Bechara *et al.*[121] reported the synthesis of 3,4,5-trisubstituted 1,2,4-triazoles by one pot from reaction of secondary amides and hydrazides using triflic anhydride as demonstrated in Scheme 1.20.



Scheme 1.20:-Synthesis of 1,2,4-triazoles by using Triflic anhydride.

f) In 2016, Z. Chen *et al.*[122] synthesized 1,3,5-trisubstituted 1,2,4-triazoles from reaction aliphatic amines with hydrazone under oxidative condition by iodine as displayed in Scheme 1.21.



Scheme 1.21:- Synthesis of 1,2,4-triazoles by using iodine.

The triazoles derivatives showed interested biological activity. For instance , antiviral[123, 124], inhibitors of methionine aminopeptidase-2[125], anhydrase inhibitors[126], anti-cancer[127, 128], anti-inflammatory[129, 130], inhibitors of the HIV-1[131], antibacterial[117], antifungal[132, 133], analgesic [134] and antioxidant[135].

According to these biological activities in this research, synthesized triazoles derivatives at position six of 2-methyl phenol to enhance the antioxidant ability, as depicted in this Figure 1.20.

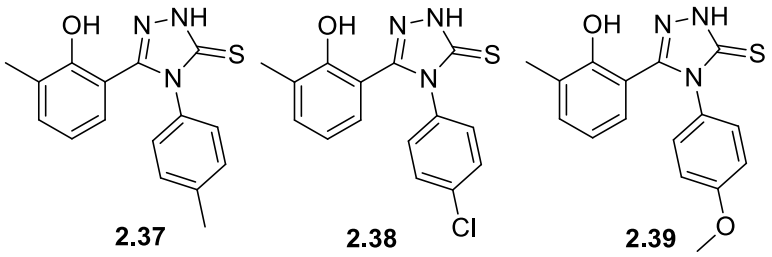
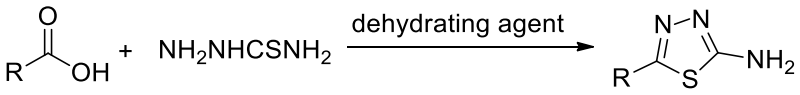


Figure 1.20:-Synthesized 1,2,4-triazole derivatives compoundsin this research.

1.7 1,3,4-thiadiazole and their biological activity.

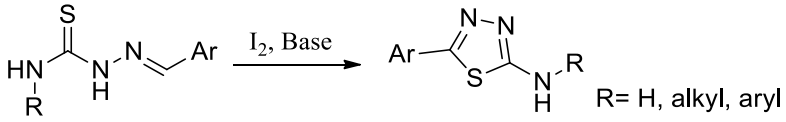
Besides to the 1,3,4-oxadiazole and 1,2,4-triazole, the 1,3,4-thiadiazole derivatives had been received intensive attention due their various biological activates. This various biological impact of the researcher to creat new methods for synthesizing thiadiazole derivatives.

a) The popular method for synthesized 1,3,4-thiadiazole-2-amine from reaction aliphatic and aromatic carboxylic acid with thiosemicarbazide in the presence of dehydration agent such as, H_2SO_4, P_2O_4, PPA and $POCl_3$[136-139] as demonstrated in general Scheme 1.22.



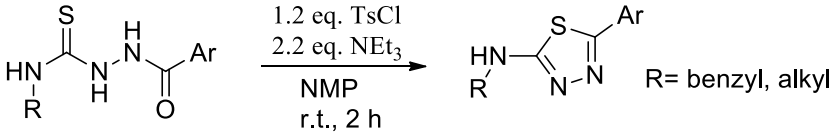
Scheme 1.22:-Synthesis of thiadiazole using dehydrating agent.

b) Cyclization of the thiosemecarbazide hydrazone in the presence of iodine as oxidative agent have been used to synthesize N-substituted 1,3,4-thiadiazole-2-amine[78, 140] as displayed in Scheme 1.23.



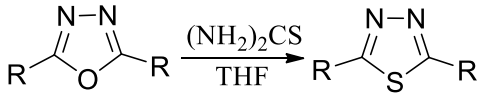
Scheme 1.23:-Synthesis of thiadiazole by using oxidative agent.

c) In 2013 S.J. Yang *et al.*[141] reported that *p*-TsCl, triethylamine in *N*-methyl-2-pyrrolidone (NMP) an efficient agent for cyclization of thiosemi- carbazide intermediate to the corresponding 1,3,4-thiadiazoles-2-amine as displayed in Scheme 1.24.



Scheme 1.24:-Synthesis of thiadiazole using TsCl agent.

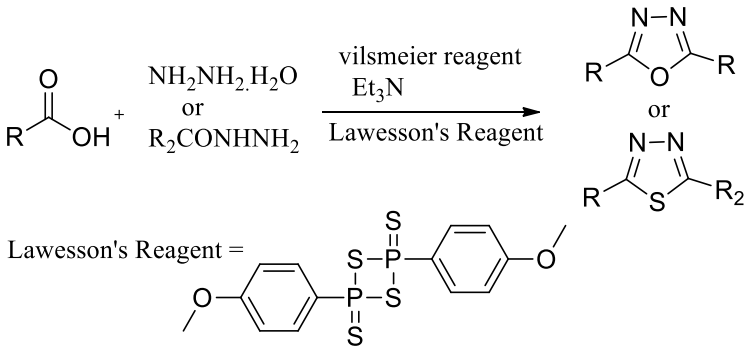
d) V. Padmavathi *et al.*[142, 143] synthesized the 1,3,4-thiadiazole ring from converted corresponding 1,3,4-oxadiazole ring utilized thiourea in THF as depicted in Scheme 1.25.



Scheme 1.25:-Synthesis of thiadiazole using thiourea agent.

e) In 2017, Maaroof Zarei [144] reported novel method for synthesise 1,3,4-thiadiazole utilized vilsmeier reagent, triethylamine and Lawesson's

reagent from reaction carboxylic acid and hydrazine or acid hydrazide as shown in Scheme 1.26.



Scheme 1.26:- Synthesis of thiadiazole utilized vilsmeier reagent.

A large numbers of thiadiazole derivatives have been synthesized and investigated their biological activity. Most of these compounds revealed various pharmacological properties for instance, anti-leishmanial activity[145, 146] antimicrobial[147] anti-inflammatory[148, 149], analgesic activities[149, 150], anticancer[151, 152] and antioxidant[138, 153]. Furthermore, it has been reported that some thiadiazole derivatives exhibited considerable electrochemical properties[154] as well showed optical properties[155].

Due to these interesting biological activities including the antioxidant ability in this research 1,3,4-thiadiazole-5-amine **(2.30)** and their derivatives **(2.31-2.33)** have been synthesized at position six of 2-methyl phenol to enhance the hindrance around the phenolic hydroxyl and share with stabilizing the free radical of these compounds after donating hydrogen radical by resonance, as well, their antioxidant ability have been evaluated by DPPH and FRAP.

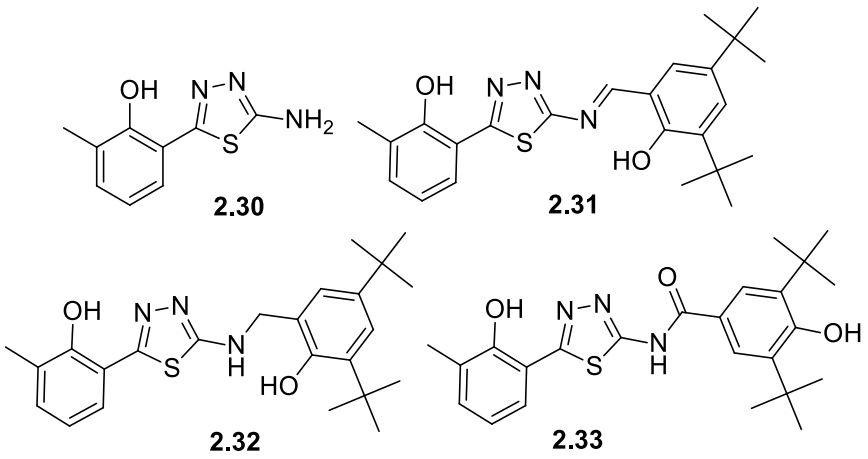


Figure 1.21:-There synthesized 1,3,4-thiadiazole and their derivatives

Chapter 2 : Experiment details

2.0 General Chemistry

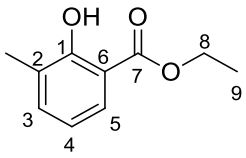
The chemicals used for synthesis were purchased from Sigma-Aldrich, GCC and Merck. Melting point was determined by open capillary tube method using OMEGA MPS10, apparatus and is uncorrected. Purities of compounds were checked with a thin layer chromatography (Silica gel TLC) plates brand Merck, and the spots were visualized with UV lights and iodine vapors. The IR spectrums were obtained with Perkin Elmer 400 Fourier Transform Infrared (FTIR) Spectrometer. NMR spectra were recorded on Bruker AVN 400 (University of Malaya, Malaysia) and 300 MHz (AL-Bayt University, Jordan). DMSO-d_6 was used as a solvent with TMS as internal standard, measurements were the mass spectra were recorded using Shimadzu GCMS-QP2010Ultra (Al-Mustansiriyah University, College of Science, Department of Chemistry), UV spectroscopy Power Wave X340,BIO-TEK instrument INC was used to record the FRAP Assay and DPPH assay (as antioxidant assay).

2.0.1 List of Chemicals

Name of material	Name of company	Purity
1,1'-carbonyldiimidazole (CDI)	SIGMA-ALDRICH	97%
2-(chloromethyl)-1*H*-benzimidazole($C_8H_7ClN_2$)	SIGMA-ALDRICH	97%
2-hydroxy-3,5-di-*tert*-butylbenzaldehyde($C_{15}H_{22}O_2$)	SIGMA-ALDRICH	99%
2-hydroxy-3-methylbenzoic acid($C_8H_8O_3$)	SIGMA-ALDRICH	97%
3,5-di-tert-butyl-4-hydroxy benzoic acid($C_{15}H_{22}O_3$)	SIGMA-ALDRICH	98%
4-bromophencylbromide	TCI	99%
4-chloro benzoic acid($C_7H_5ClO_2$)	RIEDEL-DEHAEN	99%
4-chloro isothiocyanate($C_7H_4ClN_2S$)	SIGMA-ALDRICH	99%
4-hydroxy-3,5-dimethoxybenzaldehyde($C_9H_{10}O_4$)	SIGMA-ALDRICH	98%
4-hydroxy-3-methoxybenzaldehyde($C_8H_8O_3$)	SIGMA-ALDRICH	98%
4-hydroxybenzaldehyde(C_7H_6O)	SIGMA-ALDRICH	98%
4-methoxy benzoic acid($C_8H_8O_3$)	SIGMA-ALDRICH	97%
4-methoxy isothiocyanate(C_8H_7NOS)	SIGMA-ALDRICH	98%
4-methyl isothiocyanate(C_8H_7NS)	SIGMA-ALDRICH	98%
4-methylbenzaldehyde(C_8H_8O)	TCI	98%
Acetonitrile(C_2H_3N)	HIMEDIA	99.7%
Benzene sulfonic acid($C_6H_6O_3S$)	SIGMA-ALDRICH	98%
Benzyl bromide(C_7H_7Br)	SIGMA-ALDRICH	98%
Bromine(Br_2)	SIGMA-ALDRICH	99.5%
Carbon disulfide(CS_2)	CDH	99.5%
Chloroform($CHCl_3$)	Pubchem	99%
Cyanogen bromide(BrCN)	SIGMA-ALDRIG	99%
Dichloro methane DCM	GCC	99%
Diethyl ether($C_4H_{10}O$)	HIMEDIA	99%
Dimethyl form amide(DMF)	SCR	99.5%
Ethanol(EtOH)	BDH	99.9%
Ethyl acetate($C_4H_8O_2$)	RIEDEL-DEHAEN	99.5
Ethyl bromide(C_2H_5Br)	SIGMA-ALDRICH	98%
Ethyl bromoacetate($C_4H_7BrO_2$)	FLUKA	97%
Glacial acetic acid(GAA)	GCC	99,6%
Hexane(C_6H_{12})	SIGMA-ALDRICH	95%

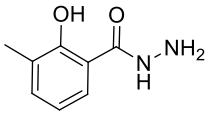
Hydrazine hydrate (N_2H_4)	CDH	80%
Hydrazine hydrochloride(N_2H_4.HCl)	BDH	98.5%
Hydrochloric acid(HCl)	GCC	35.4%
Magnesium sulfate($MgSO_4$)	ROMIL	97%
Methanol(MeOH)	GCC	99.8%
Phosphorus oxychloride($POCl_3$)	MERCK	99%
Potassium carbonate(K_2CO_3)	SIGMA-ALDRICH	99.99%
Potassium hydroxide(KOH)	ROMIL	98%
Sodium acetate($C_2H_3NaO_2$)	ROMIL	98%
Sodium borohydride($NaBH_4$)	SIGMA-ALDRICH	98%
Sodium hydrogen carbonate($NaHCO_3$)	ROMIL	98%
Sodium hydroxide(NaOH)	ROMIL	98%
Sodium sulfate(Na_2SO_4)	Reagent World	99%
Tetra hydrofuran (THF)	SCR	99%
Thiocarbonyldihydrazide(CH_6N_4S)	SIGMA-ALDRICH	98%
Thiosemicarbazide(CH_5N_3S)	SIGMA-ALDRICH	98%

2.1 Synthesis of Ethyl-2-hydroxy-3-methylbenzoate 2.1



Benzenesulfonic acid (15.8 g, 0.1 mol) was added to a solution of 2-hydroxy-3-methylbenzoic (18 g, 0.1 mol) in 45 ml of absolute ethanol. The mixture was stirred and heated under reflux for 4 hs. Upon cooling, the excess of ethanol was removed under reduced pressure. The crude was extracted by ethyl acetate (25 ml× 2).The combined organic layer washed with saturated sodium hydrogen carbonate and then by water. After that, the organic layer dried with anhydrous magnesium sulfate and was evaporated under reduced pressure to afford light brown liquid R_f =0.64 (hexane: ethyl acetate) 3:1. Yield 17.82 g, 83.6%; IR (KBr, υ_{max}/ cm^{-1}); 3141 (OH_{phenol}), 3040 ($CH_{aromatic}$) 2981,2931 ($CH_{aliphatic}$), 1670 (C=O), 1614,1462 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 1.3 (t, 3H, CH_3), 2.15 (s, 3H, CH_3), 4.3 (q, 2H, CH_2), 6.8 (t, 1H, *J* 7.4, H_4), 7.27 (d, 1H, *J* 7.3, H_3), 7.71 (d, 1H, *J* 7.9, H_5), 11.89 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ, ppm; 13.8 (1C, C_9, CH_3), 15.2 (1C, CH_3), 61.5 (1C, C_8, CH_2), 112.1 (1C, C_6), 124.3 (1C, C_4), 126.2 (1C, C_2), 128.8 (1C, C_5), 136.5 (1C, C_3), 159.4 (1C, C_1), 164.5 (1C, $C_{7(C=O)}$).

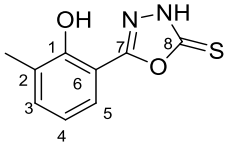
2.2 Synthesis of 2-hydroxy-3-methylbenzohydrazide 2.2



Ethyl 2-hydroxy-3-methylbenzoate (15 g, 0.08 mol) was dissolved in 50 ml of ethanol and stirred with heating to 60 ˚C for 15 minutes, then excess (about 20 ml) of hydrazine hydrate 80% was added with small portions. After completing the addition, the mixture was left under reflux for 18 hs. On cooling, a precipitate was filtered, washed with cold water

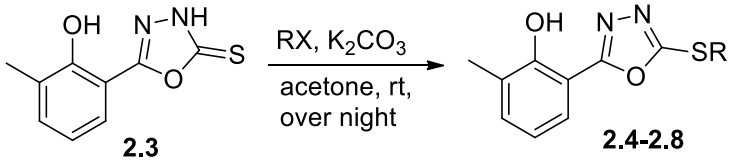
and recrystallized from aqueous ethanol to afford white precipitate 11.9 g, 86% yield, m.p. (191-193)°C. IR (KBr, υ_{max}/ cm^{-1}); 3323, 3267, 3136 (NH_2, NH and OH_{phenol}), 3049, 3014 ($CH_{aromatic}$) 2972, 2765 ($CH_{aliphatic}$), 1650 (C=O), 1583, 1433 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.22 (s, 3H, CH_3), 4.69 (bs, 2H, NH_2), 6.95 (t, 1H, *J* 7.4, H_4), 7.36 (d, 1H, *J* 7.34, H_5), 7.44 (d, 1H, *J* 7.82, H_3), 9.41 (bs, 1H, NH).

2.3 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.3



To a solution of hydrazide (1.66 g, 10 mmol) and excess of carbon disulfide (2.5-3 mL) in absolute ethanol, potassium hydroxide (0.62 g, 11 mmol) was added in one portion at ambient temperatures. The mixture was stirred and refluxed for 5 hs. After that, the solvent was removed under vacuum. Distilled water (25 mL) was added to the residue and stirred for another 15 minutes. It was filtered and the filtrate acidified with 5% hydrochloric acid and finally re-filtered. The white precipitate was washed with water, recrystallized from ethanol to afford white crystal. Yield 1.83 g, (88 %); m.p. (212-213) °C; IR (KBr, υ_{max}/ cm^{-1}); 3365 (NH), 3066 (OH_{phenol}), 2931, 2765 ($CH_{aliphatic}$), 2560-2463 (SH), 1606 (C=N), 1579, 1469 (C=C), 1122 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.21 (s, 3H, CH_3), 6.92 (t, 1H, *J* 7.2, H_4), 7.34 (d, 1H, *J* 7.3, H_3), 7.45 (d, 1H, *J* 7.8, H_5), 9.16 (bs, 1H, NH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.51 (1C, CH_3), 109.65 (1C, C_6), 120.51 (1C, C_4), 126.35 (1C, C_5), 127.04 (1C, C_2), 135.11 (1C, C_3), 154.30 (1C, C_1), 160.59 (1C, $C_{7(C=N)}$), 177.24 (1C, $C_{8(C=S)}$).

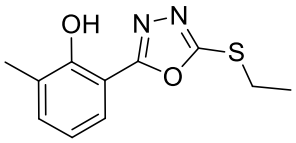
2.4 General Alkylation Method of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol (2.4-2.7)



2.3 2.4-2.8

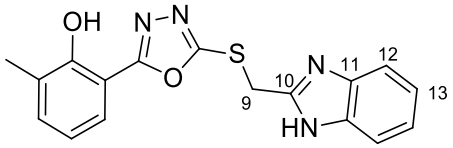
Alkyl halide (1 mmol) was added in small portions to a stirred suspension of 1,3,4-oxadiazole (0.21 g, 1 mmol) in dry acetone and anhydrous potassium carbonate(0.13 g, 1 mmol). The mixture was left to stand overnight with stirring at ambient temperature. The solvent was evaporated and the residue extracted with 25 mL chloroform. The combined organic layer dried under anhydrous magnesium sulfate and recrystallized from suitable solvent.

2.4.1 6-(5-(ethylthio)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.4



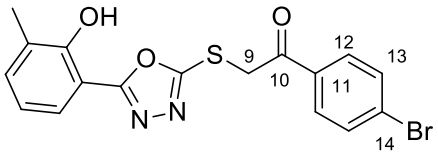
Recrystallized of the crude product from ethanol to give white crystal. Yeild 0.197 g 83%; m.p. (74-76 ^{o}C); IR (KBr, υ_{max}/ cm^{-1}); 3172 (OH_{phenol}), 3030 ($CH_{aromatic}$), 2964, 2864 ($CH_{aliphatic}$), 1610 (C=N), 1556, 1425 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm; 1.44 (t, 3H, *J* 7.3, $CH_2\underline{CH_3}$), 2.25 (s, 3H,CH_3), 3.34 (m, 2H, SCH_2), 6.96 (d, 1H, *J* 7.2, H_3), 7.45 (m, 2H, H_4, H_5), 9.89 (s, 1H, OH) ; ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 15.30 (1C, CH_2 $\underline{C}H_3$), 16.20 (1C, CH_3), 27.25 (1C, CH_2), 108.25 (1C, C_6), 120.34 (1C, C_4), 125.21 (1C, C_5), 126.44 (1C, C_2), 134.95 (1C, C_3), 154.78 (1C, C_1), 163.73 (1C, C_8), 165.28 (1C, C_7); EIMs, m/z= 236 [$M^{\bullet+}$] 100%, (Calc. for $C_{11}H_{12}N_2O_2S$, 236.06).

2.4.2 6-(5-(((benzoimidazol-2-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.5



The solid crude was recrystallized from (1:1) ethyl acetate: methanol to afford light brownish crystal. Yeild 0.26 g, (77%); m.p.(173-175)˚C ; IR (KBr, υ_{max}/ cm^{-1}); 3174 (NH), 3080 (OH $_{phenol}$), 3024 ($CH_{aromatic}$), 2989, 2924 ($CH_{aliphatic}$), 1618 (C=N), 1556, 1439 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.23 (s, 3H, CH_3), 4.80 (s, 2H, SCH_2), 6.93 (t, 1H, *J* 7.46, H_4), 7.18 (m, 2H, H_{13}), 7.37 (d, 1H, *J* 7.1, H_5), 7.52 (m, 3H, H_3, H_{12}), 9.85 (s, 1H, OH), 12.67 (bs, 1H, NH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.17 (1C, CH_3), 30.16 (1C, CH_2), 108.11 (1C, C_6), 120.42 (1C, C_4), 122.61 (4C, C_{12}, C_{13}), 125.37 (1C, C_5), 126.54 (1C, C_2), 135.16 (1C, C_3), 149.85 (3C, C_{10}, C_{11}), 154.74 (1C, C_1), 162.69 (1C, C_8), 165.67 (1C, C_7); EIMs, m/z= 338 [$M^{\bullet+}$] 75%, (Calc. for $C_{17}H_{14}N_4O_2S$, 338.08),.base peak= 131 [$BIMCH_2^+$] 100%.

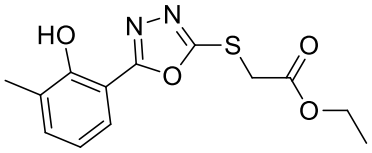
2.4.3. 6-(5-((4-bromobenzoyl)methylthio)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.6



Recrystallized of the crude product from acetonitrile to give white precipitate. Yield 0.35 g, 86%, m.p. (164-166)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3192 (OH_{phenol}), 3033 ($CH_{aromatic}$), 2960, 2924 ($CH_{aliphatic}$), 1678 (C=O), 1614 (C=N), 1591, 1477 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.25 (s, 3H, CH_3), 5.16 (s, 2H, SCH_2), 6.95 (t, 1H, *J* 7.3, H_4), 7.39 (d, 1H, *J* 7.0, H_3), 7.55 (d, 1H, *J* 7.3, H_5), 7.81 (d, 2H, *J* 8.1, H_{13}), 8.02 (d, *J* 8.3, 2H,

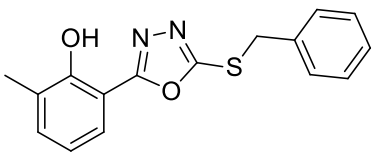
H_{12}), 9.81 (s, 1H, OH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.19 (1C, CH_3), 40.83 (1C, CH_2), 108.13 (1C, C_6), 120 (1C, C_4), 125.33 (1C, C_5), 126.55 (1C, C_2), 128.73 (1C, C_{14}), 130.94 (2C, C_{12}), 132.50 (2C, C_{13}), 134.44 (1C, C_{11}), 135.16 (1C, C_3), 154.73 (1C, C_1), 163.20 (1C, $C_{8(C=N)}$), 165.38 (1C, $C_{7(C=N)}$), 192.44 (1C, $C_{10(C=O)}$); EIMs, m/z= 404 [$M^{\bullet+}$] 75%, (Calc. for $C_{17}H_{13}BrN_2O_3S$, 403.98),.base peak= 207 [5-(2-hydroxy-3-methylphenyl)-1,3,4-oxadiazol-2-thio radical] 100%.

2.4.4 6-(5-((ethoxyformyl)methylthio)-1,3,4-oxadiazol-2-yl)-2-methyl phenol 2.7



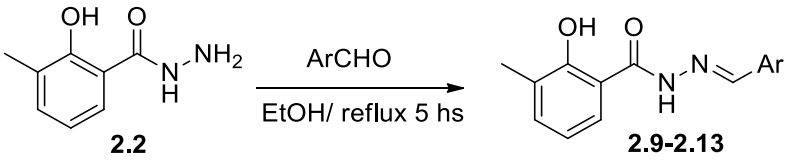
Recrystallized of the crude product from chloroform to give pale yellow precipitate. Yield 0.22 g , 74%, m.p.(71-73) ˚C, IR (KBr, U_{max}/ cm^{-1}); 3207 (OH_{phenol}), 3037 ($CH_{aromatic}$), 2983, 2931 ($CH_{aliphatic}$), 1728 (C=O), 1603 (C=N), 1556, 1481 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.20 (t, 3H, *J* 6.96, H_{12}), 2.26 (s, 3H, CH_3), 4.16 (q, 2H, *J* 7.16, H_{11}), 4.32 (s, 2H, SCH_2, H_9), 6.99 (t, 1H, *J* 7.72, H_4), 7.41 (d, 1H, *J* 7.35, H_3), 7.59 (d, 1H, *J* 7.2, H_5), 9.82 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 13.93 (1C, C_{12}), 15.74 (1C, CH_3), 33.87 (1C, C_9), 61.63 (1C, C_{11}), 107.72 (1C, C_6), 119.95 (1C, C_4), 124.87 (1C, C_5), 126.08 (1C, C_2), 134.65 (1C, C_3), 154.28 (1C, C_1), 162.44 (1C, $C_{8(C=N)}$), 164.94 (1C, $C_{7(C=N)}$), 167.62 (1C, $C_{10(C=O)}$); EIMs, m/z= 294 [$M^{\bullet+}$] 100%, (Calc. for $C_{13}H_{14}N_2O_4S$, 294.07).

2.4.5 6-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.8



The desired product was purified by flash column chromatography using hexane: ethyl acetate (6:1) as eluent to give white precipitate. Yield 0.2 g, 67%, m.p. (100-103) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3153 (OH_{phenol}), 3041 ($CH_{aromatic}$), 2924, 2860 ($CH_{aliphatic}$), 1606 (C=N), 1556, 1414 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.26 (s, 3H, CH_3), 4.60 (s, 2H, SCH_2), 6.97 (td, 1H, *J* 7.6, 3.2, H_{13}), 7.29-7.51 (m, 6H, H_3, H_4, H_{11}, H_{12}), 7.58 (d, 1H, *J* 7.9, H_5), 9.85 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.71 (1C, CH_3), 35.92 (1C, C_9), 107.68 (1C, C_6), 119.91 (1C, C_4), 124.81 (1C, C_5), 125.99 (1C, C_2), 127.79 (1C, C_{13}), 128.57 (2C, C_{11}), 129.03 (2C, C_{12}),134.57 (1C, C_3), 156.42 (1C, C_{10}), 154.31 (1C, C_1), 162.74 (1C, $C_{7(C=N)}$), 164.96 (1C, $C_{8(C=N)}$); EIMs, m/z= 298 [$M^{\bullet+}$] 100%, (Calc. for $C_{16}H_{14}N_2O_2S$, 298.08).

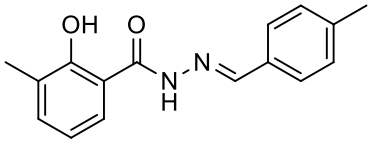
2.5 General Synthesis of N'(substituted benzylidene)2-hydroxy-3-methylbenzohydrazide (hydrazones) (2.9-2.13)



2.2 **2.9-2.13**

To a warm stirred solution of arylaldehyde (3 mmol) in 20 mL absolute ethanol, 2-hydroxy-3-methylbenzohydrazide (0.48 g, 3 mmol) was added in small portions, and refluxed for 5 hs, upon cooling, the mixture was stored overnight in a refrigerator at 5°C. The precipitate was washed with cold ethanol and recrystallized from suitable solvent.

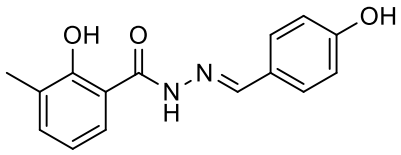
2.5.1 N'(4-methylbenzylidene)2-hydroxy-3-methylbenzohydrazide 2.9



The crude precipitate recrystallized from ethanol to afford white crystals. Yield 0.52 g, 67 %, m.p. (173-174)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3240

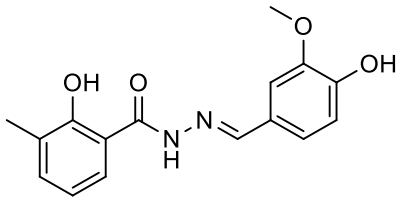
(NH and OH_{phenol}), 3087-3028 ($CH_{aromatic}$), 2954, 2910 ($CH_{aliphatic}$), 1603 ($C=O_{amide}$) and (C=N), 1572, 1446 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.19 (s, 3H, C_2-CH_3), 2.36 (s, 3H, C_{12}-CH_3), 6.87(t, 1H, *J* 7.6, H_4), 7.3 (d, 2H, *J* 7.9, H_{11}), 7.37 (d, 1H, *J* 7.3, H_3), 7.65(d,2H, *J* 7.9, H_{10}), 7.81 (d, 1H, *J* 7.9, H_5), 8.48 (s, 1H, $H_{8(HC=N)}$), 11.99 (s, 1H, NH), 12.67 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.46 (1C, C_2-CH_3), 21.02 (1C, C_{12}-CH_3), 112.73 (1C, C_6), 117.96 (1C, C_4), 124.69 (1C, C_5), 126.17 (1C, C_2), 127.27 (2C, C_{10}), 129.48 (2C, C_{11}), 131.21 (1C, C_9), 135.01 (1C, C_3), 140.33 (1C, C_{12}), 149.44 (1C, $C_{8(C=N)}$), 159.43 (1C, C_1), 166.61 (1C, $C_{7(C=O)}$); EIMs, m/z= 268 [$M^{\bullet+}$] 77%, (Calc. for $C_{16}H_{16}N_2O_2$, 268.12),.base peak= 134 $[C_8H_6O_2]^{\bullet+}$ 100%.

2.5.2 N'(4-hydroxybenzylidene)2-hydroxy-3-methylbenzo hydrazide 2.10



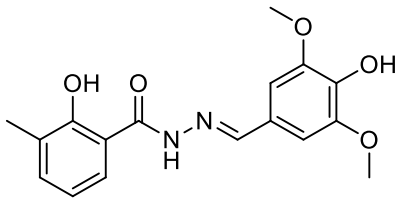
Recrystallized of the crude product from methanole to give white precipitate. Yield 0.6 g, 78%, m.p. (240-242) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3278 NH, 3205 (OH_{phenol}), 3080-3020 ($CH_{aromatic}$), 2985, 2912 ($CH_{aliphatic}$), 1603 (C=O) and (C=N), 1554, 1435 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.19 (s, 3H, CH_3), 6.86 (m, 3H, H_4, H_{11}), 7.36 (d,1H, *J* 6, H_3), 7.6 (d, 2H, *J* 8.3, H_{10}), 7.8 (d,1H, *J* 2.24 , H_5) 8.41 (bs,1H, $H_{8(HC=N)}$), 10.02 (bs, 1H, C_{12}- OH), 11.86 (bs, 1H, NH), 12.8 (bs, 1H, C_1- OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.47 (1C, CH_3), 112.76 (1C, C_6), 115.75 (2C, C_{11}), 117.89 (1C, C_4), 124.54 (1C, C_5), 124.88 (1C, C_2), 126.15 (1C, C_9), 129.12 (2C, C_{10}), 134.87 (1C, C_3), 149.73 (1C, $C_{8(C=N)}$), 159.46 (1C, C1), 159.73 (1C, C_{12}), 166.38 (1C, $C_{7(C=O)}$); EIMs, m/z= 270 [$M^{\bullet+}$] 44%, (Calc. for $C_{15}H_{14}N_2O_3$, 270.10),.base peak= 135 [(2-hydroxy-3-methylbenzylidyne)-oxonium] 100%.

2.5.3 N'(4-hydroxy-3-methoxybenzylidene)2-hydroxy-3-methylbenzo-hydrazide 2.11



Recrystallized of the crude product from methanol to give pale yellow precipitate. Yield 0.72 g, 83%, m.p.(203-206) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3311 NH, 3194 (OH_{phenol}), 3060 ($CH_{aromatic}$), 2962, 2856 ($CH_{aliphatic}$), 1633 (C=O), 1603 (C=N), 1550, 1431 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.23 (s, 3H, CH_3), 3.88 (s, 3H, O-CH_3), 6.89 (m, 2H , H_4, H_{13}), 7.16 (d, 1H, *J* 8, H_{14}), 7.39 (d, 2H, *J* 1.2, H_{10}, H_3), 7.83 (d, 1H, *J* 7.9, H_5), 8.44 (s, 1H, $H_{8(HC=N)}$), 9.66 (s, 1H, $OH_{Vanillin}$), 11.90 (s, 1H, NH), 12.80 (s, 1H, OH_{phenol}); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 15.96 (1C, CH_3), 56.03 (1C, O-CH_3), 109.47 (1C, C_{10}), 113.31 (1C, C_6), 115.94 (1C, C_{13}), 118.39 (1C, C_{14}), 123.11 (1C, C_4), 125.12 (1C, C_5), 125.81 (1C, C_9), 126.66 (1C, C_2), 135.38 (1C, C_3), 148.57 (1C, C_{11}), 149.86 (1C, C_{12}), 150.45 (1C, $C_{8(C=N)}$), 159.96 (1C, C_1), 166.89 (1C, $C_{7(C=O)}$);. EIMs, m/z= 300 [$M^{\bullet+}$] 70%, (Calc. for $C_{16}H_{16}N_2O_4$, 300.11),.base peak= 135 [(2-hydroxy-3-methylbenzylidyne)-oxonium] 100%.

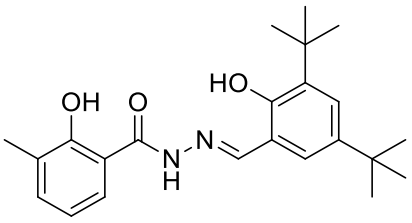
2.5.4 N'(4-hydroxy-3,5-dimethoxybenzylidene)2-hydroxy-3-methyl-benzohydrazide 2.12



Recrystallized of the crude product from methanole to give pale browne precipitate. Yield 0.77 g, 81%, m.p.(210-213) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3317 (NH and OH_{phenol}), 3086-3022 ($CH_{aromatic}$), 2951, 2844

($CH_{aliphatic}$), 1630 (C=O) and (C=N), 1587, 1425 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.2 (s, 3H, CH_3), 3.83 (s, 6H, 2×OCH_3), 6.87 (t, 1H, *J* 7.6, H_4), 7.02 (s, 2H, H_{10}), 7.37 (d, 1H, *J* 7.3, H_3), 7.8 (s, 1H, *J* 7.6, H_5), 8.39 (s, 1H, $H_{8(HC=N)}$), 9.01 (s, 1H, C_{12}-OH), 11.93 (s, 1H, NH), 12.74 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.48 (1C, CH_3), 56.02 (2C, 2×O-CH_3), 104.83 (2C, C_{10}), 112.82 (1C, C_6), 117.92 (1C, C_4), 124.11 (1C, C_9), 124.67 (1C, C_5), 126.16 (1C,C_2), 134.91 (1C, C_3), 138.29 (1C, C_{12}), 148.12 (1C, $C_{8(C=N)}$), 150.02 (2C, C_{11}), 159.42 (1C, C_1), 166.39 (1C, $C_{7(C=O)}$); EIMs, m/z= 330 [$M^{\bullet+}$] 82%, (Calc. for $C_{17}H_{18}N_2O_5$, 330.12),.base peak= 135 [(2-hydroxy-3-methylbenzylidyne)-oxonium] 100%, 196 [4-(hydrazonomethyl)-2,6-dimethoxyphenol] 100%.

2.5.5 N'(2-hydroxy-3,5-di-*tert*-butylbenzylidene)2-hydroxy-3-methyl-benzohydrazide 2.13

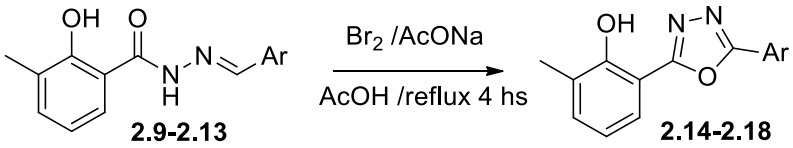


Recrystallized of the crude product from ethanol and water to give white precipitate. Yield 0.85 g, 77%, m.p.(205-208) ˚C, IR (KBr, U_{max}/ cm^{-1}); 3217 NH, 3174 (OH_{phenol}), 3049 ($CH_{aromatic}$), 2962, 2864 ($CH_{aliphatic}$), 1633 (C=O) and (C=N), 1587, 1435 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.30-1.42 (s, 18H, CH_3), 2.21 (s, 3H, CH_3), 6.91 (m, 1H, H_4), 7.26 (d, 1H, *J* 2.3, H_{12}), 7.34 (d,1H, *J* 2.1, H_{14}), 7.41 (d,1H , *J* 6.8, H_3), 7.8 (d, 1H, *J* 7.9, H_5), 8.65 (s, 1H, $H_{8(HC=N)}$), 12.23 (bs, 1H, NH), 12.43 (bs, 2H, 2OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.57 (1C, CH_3), 29.25 (3C, C_{16}, CH_3), 31.25 (3C, C_{18}, CH_3), 33.84 (1C, C_{17}), 34.62 (1C, C_{15}), 116.99 (1C, C_6), 117.74 (1C, C_9), 119.12 (1C, C_4), 120.30 (1C, C_{14}), 124.97 (1C, C_{12}), 125.65 (1C, C_5), 125.81 (1C, C_2), 126.19 (1C, C_3), 135.64 (1C, C_{11}), 140.38 (1C, C_{13}), 152.11 (1C, $C_{8(C=N)}$), 154.83 (1C, C_{10}),

159.62 (1C, C_1), 166.48 (1C, $C_{7(C=O)}$); EIMs, m/z= 382 [$M^{\bullet+}$] 100%, (Calc. for $C_{23}H_{30}N_2O_3$, 382.23).

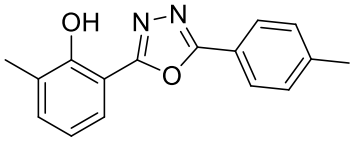
2.6 General synthesis of 6-(5-(Aryl)-1,3,4-oxadiazol-2-yl)-2-methyl-phenol (2.14-2.18)

Method A



To a suspension of N'(substituted benzylidene)2- hydroxy-3-methylbenzohydrazide (1 mmol) in 5 mL glacial acetic acid and (2 mmol) of anhydrous sodium acetate, bromine solution (1 mmol) in 3 mL glacial acetic acid was added dropwise at ambient temperatures. The mixture was refluxed for 4 hs. After cooling the mixture was poured into 100 mL ice water and stirred for another 30 minutes. The precipitate was collected, washed with water and dried. The crude product was purified either by flash column chromatography using suitable eluent or by recrystallization from suitable solvent.

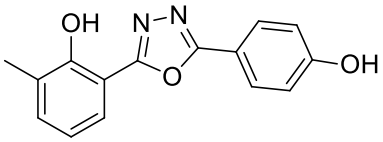
2.6.1 6-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.14



The product was purified by flash column chromatography using hexane : ethyl acetate (3:1) as eluent to give off-white precipitate. Yield 0.18 gm, 73%, m.p.(158-160) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3128 (OH_{phenol}), 3026 ($CH_{aromatic}$), 2962, 2848 ($CH_{aliphatic}$), 1610 (C=N), 1543, 1417 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.03 (s, 3H, C_2-CH_3), 2.2 (s, 3H, C_{12}-CH_3), 6.7 (t, 1H, *J* 7.6, H_4), 7.13 (d, 2H, *J* 7.9, H_{11}), 7.21 (d, 1H, *J* 7.3, H_3), 7.49 (d, 2H, *J* 7.9, H_{10}), 7.64 (d,1H, *J* 7.9, H_5), 11.83 (s, 1H, OH); ^{13}C

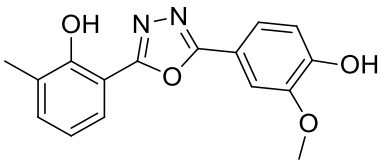
NMR (75 MHz, DMSO-d_6) δ, ppm; 15.26 (1C, C_2-CH_3), 20.83 (1C, C_{12}-CH_3), 112.53 (1C, C_6), 117.76 (1C, C_4), 124.50 (1C, C_5), 125.96 (1C, C_2), 127.06 (2C, C_{11}), 129.28 (2C, C_{10}), 131.00 (1C, C_{12}), 134.81 (1C, C_3), 140.13 (1C, C_9), 159.23 (1C, C_1), 163.59 (1C, $C_{7(C=N)}$), 165.39 (1C, $C_{8(C=N)}$); EIMs, m/z= 266 [$M^{\bullet+}$] 12%, (Calc. for $C_{16}H_{14}N_2O_2$, 266.11),.base peak= 106 [$C_7H_6O]^{\bullet+}$ 100.

2.6.2 6-(5-(4-hydroxylphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.15



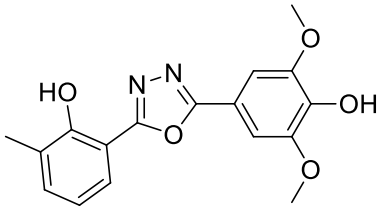
Recrystallized of the crude product from methanole to give pale beige precipitate. Yield 0.2 g, 75%, m.p.(224-227) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3464 (OH), 3150 (OH_{phenol}), 3078 ($CH_{aromatic}$), 2978, 2924 ($CH_{aliphatic}$), 1622 (C=N), 1599, 1402 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.18 (s, 3H, CH_3), 6.85 (m, 3H, H_4, 2×H_{11}), 7.35 (d, 1H, *J* 6, H_3), 7.59 (d, 2H, *J* 8.3, H_{10}), 7.79 (d,1H , *J* 6.6, H_5), 11.85 (s, 1H, C_{12}-OH), 12.79 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.50 (1C, CH_3), 112.80 (1C, C_6), 115.78 (2C, C_{10}), 117.92 (2C, C_{11}), 124.63 (1C, C_4), 124.91 (1C, C_5), 126.18 (1C, C_2), 129.15 (1C, C_9), 134.91 (1C, C_3), 159.50 (1C, C_1), 159.78 (1C, C_{12}), 164.34 (1C, $C_{7(C=N)}$), 165.07 (1C, $C_{8(C=N)}$); EIMs, m/z= 268 [$M^{\bullet+}$] 100%, (Calc. for $C_{15}H_{12}N_2O_3$, 268.08).

2.6.3 6-(5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.16



The product was purified by flash column chromatography using hexane :ethyl acetate (4:1) as eluent to afford pale yellow precipitate. Yield 0.23 g, 78%, m.p. (110-115) ˚C, IR (KBr, υ_{max}/ cm^{-1}) 3282 ($OH_{vanillin}$), 3120 (OH_{phenol}), 3012 ($CH_{aromatic}$), 2931, 2850 ($CH_{aliphatic}$), 1674 (C=N), 1591, 1425(C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.07 (s, 3H, CH_3), 3.72 (s, 3H, O-CH_3), 6.73 (m, 2H, H_4, H_{10}), 7 (d, *J* 8.07, 1H, H_3), 7.23 (m, 2H, H_5, H_{13}), 7.67 (d, *J* 8.07, 1H, H_{14}), 11.74 (s,1H, C_{12}-OH), 12.64 (s, 1H, C_1-OH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 15.96 (1C, CH_3), 56.03 (1C, O-CH_3), 109.47 (1C, C_{10}), 113.31 (1C, C_6), 115.93 (1C, C_{13}), 118.40 (1C, C_{14}), 123.09 (1C, C_4), 125.12 (1C, C_5), 125.80 (1C, C_9), 126.66 (1C, C_2), 135.38 (1C, C_3), 148.57 (1C, C_{11}), 149.85 (1C, C_{12}), 159.95 (1C, C_1), 161.68 (1C, $C_{7(C=N)}$), 166.77 (1C, $C_{8(C=N)}$); EIMs, m/z= 298 [$M^{\bullet}$+] 10%, (Calc. for $C_{16}H_{16}N_2O_4$, 298.10),.base peak= 230 [3'-methoxy-3-methyl-[1,1'-biphenyl]-2,4'-diol]$^{+}$ 100%.

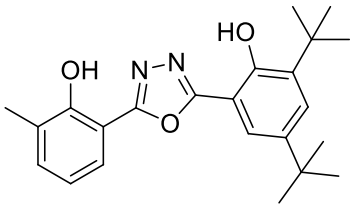
2.6.4 6-(5-(4-hydroxy-3,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.17



Recrystallized of the crude product from methanole to give light beige precipitate. Yield 0.27 g, 84%, m.p.(122-125) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3510 (OH), 3242 (OH_{phenol}), 3091-3008 ($CH_{aromatic}$), 2970, 2864 ($CH_{aliphatic}$), 1630 (C=N), 1591, 1423 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.05 (s, 3H, CH_3), 3.69 (s, 6H, 2×OCH_3), 6.72 (t, 1H, *J* 7.63, H_4), 6.87 (s, 2H, H_{10}), 7.22 (d, 1H, *J* 7.35, H_3), 7.65 (d, 1H, *J* 7.91, H_5), 11.78 (s, 1H, C_{12}-OH), 12.60 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.52 (1C, CH_3), 56.06 (2C, O-CH_3), 104.87 (2C, C_{10}), 112.86 (1C, C_6), 117.96 (1C, C_4), 124.15 (1C, C_9), 124.71 (1C, C_5), 126.20 (1C, C_2), 134.95

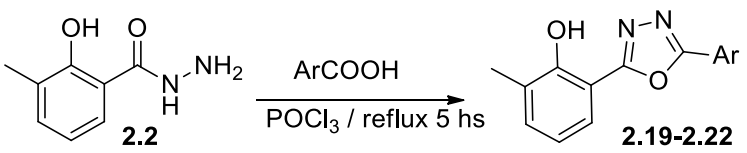
(1C, C_3), 138.33 (1C, C_{12}), 150.06 (2C, C_{11}), 159.46 (1C, C_1), 163.30 (1C, $C_{7(C=N)}$), 165.65 (1C, $C_{8(C=N)}$); EIMs, m/z= 328 [$M^{\bullet+}$] 100%, (Calc. for $C_{17}H_{16}N_2O_5$, 328.11).

2.6.5 6-(5-(2-hydroxy-3,5-di-*tert*-butylphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.18



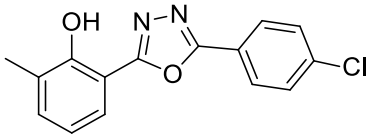
The product was purified by flash column chromatography using hexane :ethyl acetate (8:1) as eluent to afford white precipitate. Yield 0.27 g, 72%, m.p.(225-227) ˚C, IR (KBr, υ_{max}/ cm^{-1}) 3192 (OH) & (OH_{phenol}), 3020 ($CH_{aromatic}$), 2956, 2871 ($CH_{aliphatic}$), 1610 (C=N), 1543, 1433(C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.26-1.38 (s, 18H, 6×CH_3), 2.17 (s, 3H, CH_3), 6.86 (t, *J* 6.9, 1H, H4), 7.29 (m, 3H, H_3, H_{12}, H_{14}), 7.75 (d,1H, *J* 7.9, H_5), 12.18 (s, 1H, C_{10}-OH), 12.38 (s, 1H, C_1-OH); ^{13}C APT (75 MHz, DMSO-d_6) δ, ppm; 15.45 (1C, CH_3), 29.12 (3C, C_{16}, CH_3), 31.13 (3C, C_{18}, CH_3), 33.72 (1C, C_{17}), 34.50 (1C, C_{15}), 116.88 (1C, C_6), 117.61 (1C, C_9), 119.00 (1C, C_4), 120.17 (1C, C_{14}), 124.84 (1C, C_{12}), 125.53 (1C, C_5), 125.69 (1C, C_2), 126.07 (1C, C_3), 135.64 (1C, C_{11}), 140.26 (1C, C_{13}), 154.71 (1C, C_{10}), 159.49 (1C, C_1), 162.55 (1C, $C_{7(C=N)}$), 165.63 (1C, $C_{8(C=N)}$); EIMs, m/z= 379 [M^+] 2%, (Calc. for $C_{23}H_{28}N_2O_3$, 380.21), base peak= 364 [$C_{22}H_{24}N_2O_3$]$^{\bullet+}$ 100%.

Method B



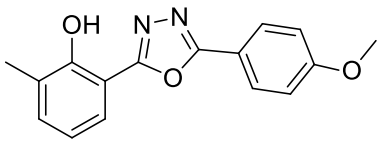
Excess of phosphorusoxy chloride (5 mL) was added dropwise, at room temperature to a mixture of 2-hydroxy-3-methylbenzohydrazide (0.24 g, 1.4 mmol) and substituted carboxylic acid (1.4 mmol) in a 250 mL round bottom flask. The mixture was heated up to 80-90 °C and stirred for 5 hs. Upon cooling, 100 mL of crushed ice was poured into the mixture and stirred for 15 minutes. PH of the mixture was as adjusted to 7-8 by adding a solution of sodium bicarbonate. The precipitate was filtered, washed with distilled water and dried. The desired product was purified by recrystallization from appropriate solvent or by flash column chromatography.

2.6.6 6-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.19



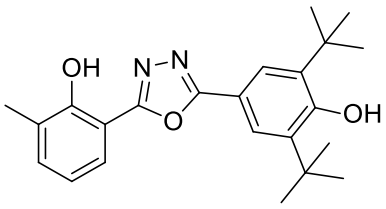
The desired product was purified by flash column chromatography using hexane :ethyl acetate (8:1) as eluent to give white precipitate. Yield 0.33 g, 79%, m.p.(171-173) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3207 (OH_{phenol}), 3086-3033 ($CH_{aromatic}$), 2972, 2852 ($CH_{aliphatic}$), 1610 (C=N), 1539, 1406 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.2 (s, 3H, CH_3), 6.93 (t, *J* 8.77, 1H, H_4), 7.11 (d, *J* 9.01, 2H, H_{11}), 7.33 (d, *J* 8.78, 1H, H_3), 7.73 (d, *J* 8.56, 1H, H_5), 8.00 (d, *J* 9.1, 2H, H_{10}), 10.1 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.72 (1C, CH_3), 107.77 (1C, C_6), 119.91 (1C, C_4), 121.84 (1C, C_9), 125.13 (1C, C_5), 126.00 (1C, C_2), 128.59 (2C, C_{10}), 129.62 (2C, C_{11}), 134.72 (1C, C_3), 137.00 (1C, C_{12}), 154.71 (1C, C_1), 162.16 (1C, $C_{7(C=N)}$), 164.11 (1C, $C_{8(C=N)}$); EIMs, m/z= 286 [$M^{\bullet+}$] 100%, (Calc. for $C_{15}H_{11}ClN_2O_2$, 286.05);

2.6.7 2-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-6-methylphenol 2.20



Recrystallized of the crude product from ethanol and water to give pale pink precipitate. Yield 0.29 g, 71%, m.p.(161-164) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3421 (OH_{phenol}), 3097 ($CH_{aromatic}$), 2987, 2852 ($CH_{aliphatic}$), 1610 (C=N), 1543, 1429 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.28 (s, 3H, CH_3), 3.87 (s, 3H, O-CH_3), 7.02 (t, *J* 7.6, 1H, H_4), 7.19 (d, *J* 9, 2H, H_{10}), 7.42 (d, *J* 7.3, 1H, H_3), 7.82 (d, *J* 7.9, 1H, H_5), 8.09 (d, *J* 8.9, 2H, H_{11}), 10.18 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.70(1C, CH_3), 55.56 (1C, O-CH_3), 107.82 (1C, C_6), 114.92 (2C, C_{11}), 115.16 (2C, C_{10}), 119.85 (1C, C_4), 124.87 (1C, C_5), 125.88 (1C, C_2), 128.71 (1C, C_9), 134.47 (1C, C_3), 154.63 (1C, C_1), 162.31 (1C, $C_{7(C=N)}$), 162.82 (1C, $C_{8(C=N)}$), 163.54 (1C, C_{12}); EIMs, m/z= 282 [$M^{\bullet+}$] 100%, (Calc. for $C_{16}H_{14}N_2O_3$, 282.10)

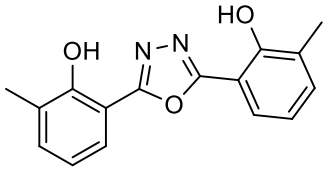
2.6.8 6-(5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.21



The desired product was purified by flash column chromatography using hexane :ethyl acetate (10:1) as eluent to give orangish yellow precipitate. Yield 0.4 g, 73%, m.p.(165-168) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3610 ($OH_{di\text{-}tert\text{-}butyl}$), 3176 ($OH_{phenol}$), 3097 ($CH_{aromatic}$), 2962, 2871 strong ($CH_{aliphatic}$), 1610 (C=N), 1550, 1417 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.45 (s, 18H, CH_3), 2.27 (s, 3H, CH_3), 5.68 (s, 1H, OH), 7.01 (t,

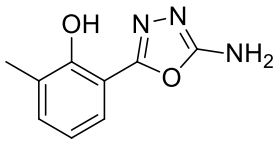
1H, *J* 7.6, H_4), 7.4 (d, 1H, *J* 7.2, H_3), 7.78 (d, 1H, *J* 7.7, H_5), 7.87 (s, 2H, H_{10}), 10.24 (s, 1H, OH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ, ppm; 15.69 (1C, CH_3), 29.92 (6C, C_{14}, CH_3), 34.59 (2C, C_{13}), 107.78 (1C, C_6), 114.09 (1C, C_9), 119.85 (1C, C_4), 123.60 (1C, C_5), 124.77 (2C, C_{10}), 125.80 (1C, C_2), 134.37 (1C, C_3), 139.65 (2C, C_{11}), 154.59 (1C, C_1), 157.79 (1C, C_{12}), 163.41 (1C, $C_{7(C=N)}$), 163.55 (1C, $C_{8(C=N)}$); EIMs, m/z= 379 [M^+] 100%, (Calc. for $C_{23}H_{28}N_2O_3$, 380.21).

2.6.9 6,6'-(1,3,4-oxadiazole-2,5-diyl)bis(2-methylphenol) 2.22



Recrystallized of the crude product from ethanol and water to give pale yellow precipitate. Yield 0.24 g, 61%, m.p.(280 dec.) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3475 & 3222 (OH_{phenol}), 3022 ($CH_{aromatic}$), 2962, 2848 ($CH_{aliphatic}$), 1614 (C=N), 1547, 1481 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.24 (s, 6H, $2CH_3$), 6.95 (t, 2H, *J* 7.6, H_4), 7.37 (d, 2H, *J* 7.6, H_3), 7.47 (d, 2H, *J* 8.0, H_5), 9.85 (s, 2H, 2×OH); ^{13}CAPT (100 MHz, DMSO-d_6) δ, ppm; 15.54 (2C, 2×CH_3), 108.67 (2C, C_6), 119.54 (2C, C_4), 125.38 (2C, C_5), 126.06 (2C, C_2),134.14 (2C, C_3), 153.33 (2C, C_1), 159.62 (2C, $C_{7(C=N)}$); EIMs, m/z= 282 [$M^{\bullet+}$] 100%, (Calc. for $C_{16}H_{14}N_2O_3$, 282.10).

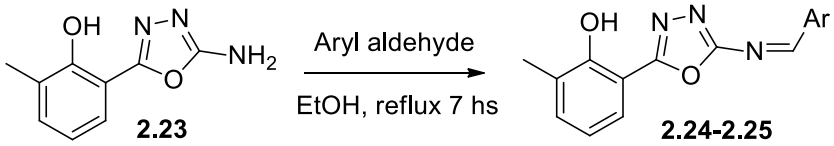
2.7 Synthesis of 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.23



To a stirred mixture of 2-hydroxy-3-methylbenzohydrazide (1.49 g, 9 mmol) and sodium hydrogen carbonate (0.756 g, 9 mmol) in methanol, cyanogen bromide (0.954 g, 9 mmol) was added small portion. The mixture

was left with stirring overnight. The resulting precipitate was filtrated and washed with cold water. The crude precipitate was recrystallized from ethanol to afford white crystals. Yield 1.479 g, 87%, m.p. (216-218)°C, IR (KBr, υ_{max}/ cm^{-1}); 3384,3334 NH_2, 3255-3124 (OH_{phenol}), 2979, 2794 ($C\text{-}H_{aliphatic}$), 1618 (C=N), 1591, 1421 (C=C); ^1H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.23 (s, 3H, CH_3), 6.93 (t, 1H, *J* 7.21, H_4),7.27 (m, 1H, H_3), 7.41(d, 1H, *J* 7.46, H_5), 7.50 (s, 2H, NH_2), 10.25 (bs, 1H, OH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.07 (1C, CH_3), 108.50 (1C, C_6), 120.09 (1C, C_4), 123.40 (1C, C_5), 125.87 (1C, C_2), 133.46 (1C, C_3), 154.33 (1C, C_1), 158.27 (1C, C_7), 163.32 (1C, C_8).

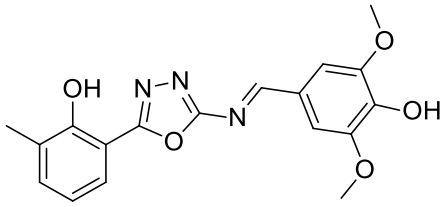
2.7.1 General synthesis of 6-(5-(substitutedbenzylideneamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol (2.24-2.25)



2.23 **2.24-2.25**

To a warm stirred solution of arylaldehyde (1 mmol) in 20 mL absolute ethanol, 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol (0.2 g, 1 mmol) was added in small portions, and refluxed for 7 hs, upon cooling; the mixture was stored overnight in a refrigerator at 5°C. The precipitate was washed with cold ethanol and recrystallized from suitable solvent.

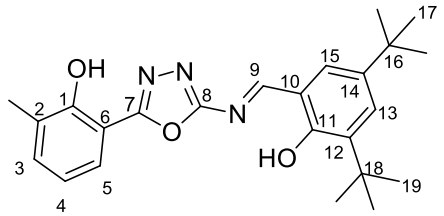
2.7.1.1Synthesis of 6-(5-(3,5-dimethoxy-4-hydroxybenzylidene amino)-1,3,4-oxadi-azol-2-yl)-2-methylphenol 2.24



Recrystallized of the crude product from methanol to give yellow precipitate. Yield 0.29 g, 78%, m.p.(218-220) ˚C, IR (KBr, υ_{max}/ cm^{-1});

3182 (OH_{phenol}) & ($OH_{3,5\text{-}dimethoxy}$), 3008 ($CH_{aromatic}$), 2958, 2844 ($CH_{aliphatic}$), 1649 ($C=N_{exo}$), 1610 ($C=N_{endo}$), 1587, 1469 (C=C); ^{1}H NMR (300MHz, DMSO-d_6) δ, ppm; 2.28 (s, 3H, CH_3), 3.88 (s, 6H, 2×OCH_3), 7.01 (t, 1H, *J* 7.6, H_4), 7.44 (m, 3H, H_3, $2H_{11}$), 7.70 (d, 1H, *J* 7.6, H_5), 8.51 (s, 1H, CH=N, H_9), 9.19 (s, 1H, C_{13}-OH), 9.78 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.65 (1C, CH_3), 56.07 (2C, 2×OCH_3), 108.12 (2C, C_{11}), 119.83 (1C, C_6), 124.49 (2C, C_4, C_5), 125.91 (1C, C_2), 134.37 (1C, C_3), 142.61 (1C, C_{10}), 148.19 (2C, C_{12}), 154.79 (1C, C_{13}), 162.57 (1C, C_1), 165.37 (1C, C_9), 165.89 (1C, C_7), 169.70 (1C, C_8); EIMs, m/z= 355 [$M^{\bullet+}$] 24%, (Calc. for $C_{18}H_{17}N_3O_5$, 355.12),.base peak= 135 [(2-hydroxy-3-methylbenzylidyne)-oxonium] 100%.

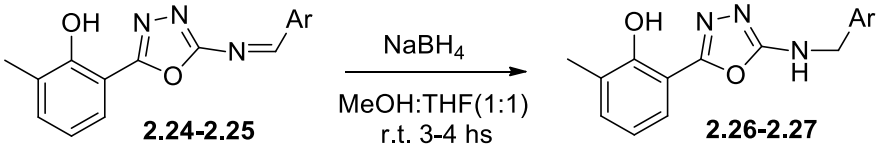
2.7.1.26-(5-(3,5-di-*tert*-butyl-2-hydroxylbenzylideneamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.25



The desired product was washed with water and purified by ethanol to give pale yellow precipitate. Yield 0.32 g, 76%, m.p.(204-206) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3384 ($OH_{di\text{-}tert\text{-}butyl}$), 3120 ($OH_{phenol}$), 3035 ($CH_{aromatic}$), 2951, 2875 ($CH_{aliphatic}$), 1666 ($C=N_{exo}$), 1603 ($C=N_{endo}$), 1560, 1417 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.22-1.31 (s, 18H, 6×CH_3), 2.17 (s, 3H, CH_3), 6.72-7.55 (m, 5H, H_3, H_4, H_5, H_{13}, H_{15}), 8.62 (s, 1H, $H_{9(CH=N)}$) 9.91 (s, 1H, C_{11}-OH), 11.63 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.60 (1C, CH_3), 29.08 (3C, C_{17}, $C(\underline{CH_3})_3$), 31.03 (3C, C_{19}, $C(\underline{CH_3})_3$), 33.97 (1C, C_{18}, $\underline{C}(CH_3)_3$), 34.53 (1C, C_{16}, $\underline{C}(CH_3)_3$), 108.06 (1C, C_6), 119.53 (1C, C_4), 120.10 (1C, C_{10}), 122.84 (1C, C_5), 128.47 (2C, C_2, C_{13}), 132.88 (1C, C_3), 136.44 (1C, C_{12}), 141.33 (1C, C_{14}), 149.81 (1C, C_{15}),

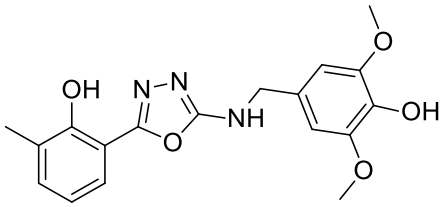
153.89 (1C, C_{11}), 157.81 (1C, C_1), 162.74 (1C, C_9), 164.96 (1C, C_7), 165.08 (1C, C_8).

2.7.2 General synthesis of 6-(5-(substitutedbenzylamino)-1,3,4-oxa diazol-2-yl)-2-methylphenol (2.26-2.27)



Sodium borohydride (0.53 mmol) was added pinch wise to a stirred solution of Schiff base (0.2 g, 0.53 mmol) in methanol: THF (1:1), in period of 20 minute at ambient temperature. After that, it allowed stirring for further 3-4 hs. The excess of solvent was reduced to half by evaporation under reduced pressure. Then 100 mL of crushed ice was added to the remaining solution and was left stirring for 30 mints. The precipitated was collected by filtration and crystalized by suitable solvent.

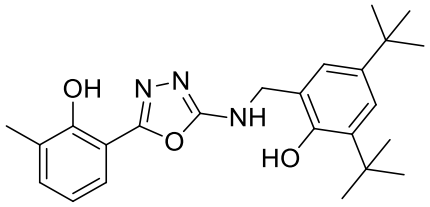
2.7.2.16-(5-(3,5-dimethoxy-4-hydroxylbenzylamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.26



Recrystallized of the crude product from methanol to give yellow precipitate. Yield 0.135 g, 71%, m.p.(100-103) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3342 (NH), 3221 (OH_{phenol}) & ($OH_{3,5\text{-dimethoxy}}$), 3010 ($CH_{aromatic}$), 2927, 2852 ($CH_{aliphatic}$), 1606 ($C{=}N_{endo}$), 1559, 1431 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.31 (s, 3H, CH_3), 3.91 (s, 8H, CH_2, 2×OCH_3), 7.04 (t, *J* 6.78, 1H, H_4), 7.38 (m, 3H, H_3, 2×H_{11}), 7.72 (d, *J* 7.32, 1H, H_5), 8.21 (s, 1H, NH), 9.22 (s, 1H, C_{13}-OH), 9.81 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.18 (1C,

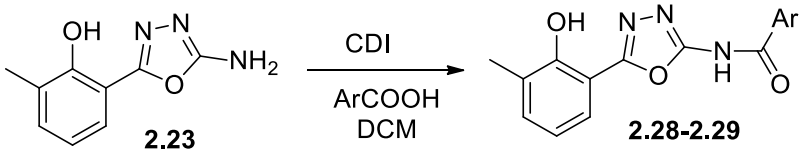
CH_3), 55.98 (2C, 2×OCH_3), 59.95 (1C, CH_2), 109.21 (2C, C_{11}), 114.41 (1C, C_6), 122.69 (1C, C_4), 123.94 (1C, C_5), 126.70 (1C, C_2), 129.34 (1C, C_3), 136.89 (1C, C_{10}), 137.04 (2C, C_{12}), 148.13 (1C, C_{13}), 150.47 (1C, C_1), 158.66 (1C, $C_{8(C=N)}$), 165.27 (1C, $C_{7(C=N)}$); EIMs, m/z= 357 [$M^{\bullet+}$] 24%, (Calc. for $C_{18}H_{19}N_3O_5$, 357.13),.base peak= 167 [(4-hydroxy-3,5-dimethoxyphenyl)methylium] 100%.

2.7.2.26-(5-(3,5-di-*tert*-butyl-2-hydroxylbenzylamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.27



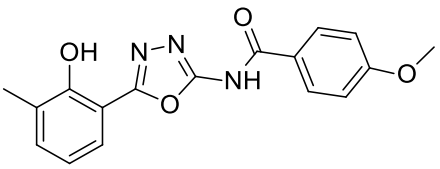
Recrystallize of the crude product from ethanol to give pale yellow precipitate. Yield 0.134 g, 67%, m.p.(195-198) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3384 ($OH_{di\text{-}tert\text{-}butyl}$), 3339 (NH), 3251-3113 (OH_{phenol}), 3020 ($CH_{aromatic}$), 2958, 2860 ($CH_{aliphatic}$), 1618 (C=N), 1595, 1425 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.22-1.29 (s, 18H, 2×C($\underline{CH_3}$)$_3$), 2.24 (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 6.65 (s, 1H, NH), 6.89-6.96 (m, 1H, H_4), 7.29 (d, *J* 7.16, 1H, H_3), 7.40 (d, *J* 7.72, 1H, H_5), 7.52 (s, 2H, H_{13}, H_{15}), 9.76 (s, 1H, C_{11}-OH), 10.29 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.61 (1C, CH_3), 29.57 (3C, C_{17}, C($\underline{CH_3}$)$_3$), 31.76 (3C, C_{19}, C($\underline{CH_3}$)$_3$), 33.53 (1C, C_{18}, $\underline{C}(CH_3)_3$), 34.36 (1C, C_{16}, $\underline{C}(CH_3)_3$), 62.11 (1C, C_9, CH_2), 108.06 (1C, C_6), 118.72 (1C, C_4), 119.55 (1C, C_{15}), 122.84 (1C, C_{13}), 124.54 (1C, C_5), 125.35 (1C, C_{10}), 125.69 (1C, C_2), 132.89 (1C, C_3), 134.02 (1C, C_{12}), 135.67 (1C, C_{14}), 153.88 (1C, C_{11}), 154.01 (1C, C_1), 157.72 (1C, $C_{8(C=N)}$), 162.82 (1C, $C_{7(C=N)}$).

2.7.3 General synthesis of 6-(5-(substitutedbenzamido)-1,3,4-oxa diazol-2-yl) 2-methyl phenol (2.28-2.29)



1,1'-Carbonyldiimidazole (CDI) (0.558 g, 3 mmol) was added in one portion to stirred solution of aryl carboxylic acid (1.15 mmol) in DCM (120 mL) at ambient temperature. The homogenous mixture converted to white milky then to clear yellow, attended with evolved CO_2 gas. The clear mixture was stirring for (30-45) mints, until no more evolving of CO_2 gas. 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol (0.22 g, 1.15 mmol) was added in one portion. The solution was turned to cloudy white. The reaction mixture was stirred for 6 hs. The reaction mixture was then quenched with 50 mL of 1M hydrochloric acid and extracted by dichloromethane (2×20 mL). The combine organic layer was washed with distilled water and then with brine. The organic layer was dried under sodium sulfate and evaporated under reduced pressure. The resulting product was purified by column chromatography using suitable eluent.

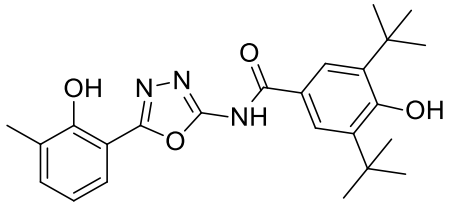
2.7.3.16-(5-(4-methoxybenzamido)-1,3,4-oxadiazol-2-yl)-2-methylphe-nol 2.28



The desired product was purified by flash column chromatography using hexane :ethyl acetate (3:1) as eluent to give white precipitate. Yield 0.22 g, 75%, m.p.(226-227) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3323 (NH), 3114 (OH_{phenol}), 3012 ($CH_{aromatic}$), 2920, 2765 ($CH_{aliphatic}$), 1666 ($C=O_{amid}$), 1622 ($C=N_{endo}$), 1591, 1419 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.18

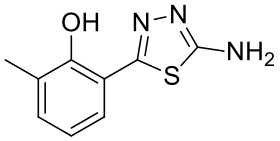
(s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 6.81 (t, *J* 7.64, 1H, H_4), 7.12 (d, *J* 8.07, 2H, H_{12}), 7.28 (d, *J* 5.87, 2H, H_{11}), 7.35 (d, *J* 7.21, 1H, H_3), 7.74 (d, *J* 7.58, 1H, H_5), 9.19 (s, 1H, OH), 9.50 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.60 (1C, CH_3), 55.24 (1C, OCH_3), 108.06 (1C, C_6), 115.77 (2C, C_{12}), 119.53 (1C, C_4), 122.84 (1C, C_5), 125.34 (1C, C_{10}), 126.37 (1C, C_2), 128.30 (2C, C_{11}), 132.87 (1C, C_3), 153.88 (1C, C_{13}), 157.73 (1C, C_1), 162.83 (1C, C_7), 165.71 (1C, C_8), 166.61 (1C, $C_{9(C=O)}$).

2.7.3.26-(5-(3,5-di-*tert*-butyl-4-hydroxybenzamido)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.29



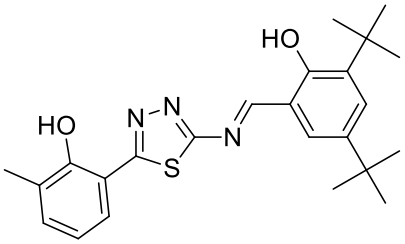
The desired product was purified by flash column chromatography using hexane :ethyl acetate (6:1) as eluent to give pale yellow precipitate. Yield 0.33 g, 69%, m.p.(139-141) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3581 ($OH_{di\text{-}tert\text{-}butyl}$), 3389 (NH), 3124 ($OH_{phenol}$), 3020 ($CH_{aromatic}$), 2962, 2881 ($CH_{aliphatic}$), 1697 (C=O), 1606 (C=N), 1556, 1427 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.42 (s, 18H, 2×C($\underline{CH_3}$)$_3$), 2.19 (s, 3H, CH_3), 5.57 (s, 1H, $OH_{di\text{-}tert\text{-}butyl}$), 6.89 (t, *J* 6, 1H, H_4), 7.32 (d, *J* 6, 1H, H_3), 7.43 (d, *J* 7.68, 1H, H_5), 7.79 (s, 2H, H_{11}), 9.69 (bs, 1H, OH), 9.84 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 14.30 (1C, CH_3), 29.95 (6C, C_{15}, 2×C($\underline{CH_3}$)$_3$), 34.43 (2C, C_{14}, $\underline{C}(CH_3)_3$), 108.79 (1C, C_6), 114.05 (1C, C_{10}), 120.74 (1C, C_4), 122.87 (1C, C_5), 126.01 (2C, C_{11}), 127.31 (1C, C_2), 133.14 (1C, C_3), 138.30 (2C, C_{12}), 155.94 (1C, C_{13}), 158.53 (1C, C_1), 161.70 (1C, $C_{7(C=N)}$), 164.39 (1C, $C_{8(C=N)}$), 166.13 (1C, $C_{9(C=O)}$).

2.8 Synthesis 6-(5-amino-1,3,4-thiadiazol-2-yl)-2-methylphenol 2.30



A mixture of 2-hydroxy-3-methylbenzoic (1.8 g, 12 mmol) and thiosemicarbazide (1.09 g, 12 mmol) with excess concentrated Phosphorus oxychloride was refluxed for 1h. Then cooled to ambient temperature, After that 10 mL of distilled water was added carefully and the mixture was refluxed for further two hours. The mixture was poured into crashed ice the PH was adjusted to 6-7 with cold sodium carbonate solution. The product was isolated by filtration and crystallized from ethanol to give greenish yellow precipitate. Yield 1.49 g, 61%, m.p.(179-181) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3346-3290 (NH_2), 3168 (OH_{phenol}), 3072 ($CH_{aromatic}$), 2958, 2858 ($CH_{aliphatic}$), 1626 (C=N), 1508, 1419 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.23 (s, 3H, CH_3), 6.85 (t, *J* 7.6, 1H, H_4), 7.2 (d, *J* 6.9, 1H, H_3), 7.34 (d, *J* 6.9, 1H, H_5), 7.56 (bs, 2H, NH_2), 11.34 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.30 (1C, CH_3), 115.22 (1C, C_6), 119.96 (1C, C_4), 125.89 (1C, C_5), 126.50 (1C, C_2), 132.18 (1C, C_3), 154.08 (1C, C_1), 158.97 (1C, C_7), 167.98 (1C, C_8).

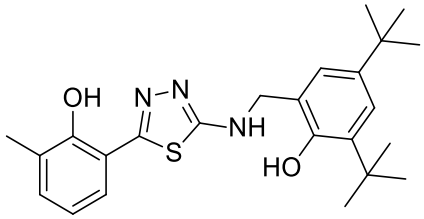
2.8.1 Synthesis of 6-(5-(3,5-di-*tert*-butyl-2-hydroxylbenzylidene-amino)-1,3,4-thiadiazol-2-yl)-2-methylphenol 2.31



To a warm stirred solution of 3,5-di-*tert*-butyl-2-hydroxy benzaldehyde (2 mmol) in 20 mL absolute ethanol, 6-(5-amino-1,3,4-thiadiazol-2-yl)-2-methylphenol (0.41 g, 2 mmol) was added in small

portions, and refluxed for 7 hs, upon cooling; the mixture was stored overnight in a refrigerator at 5°C. The precipitate was washed with cold ethanol and recrystallized from methanole to give yellow precipitate. Yield 0.56 g, 67%, m.p.(219-221) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3168 (OH_{phenol}) & ($OH_{3,5\text{-di-}tert\text{-butyl}}$), 3059 ($CH_{aromatic}$), 2954, 2871 ($CH_{aliphatic}$), 1647 (C=N), 1535, 1460 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.30 (s, 9H, H_{19}, C($\underline{CH}_3$)$_3$), 1.39 (s, 9H, H_{17}, C($\underline{CH}_3$)$_3$), 2.25 (s, 3H, CH_3), 6.90 (t, *J* 7.6, 1H, H_4), 7.25 (d, *J* 7.1, 1H, H_3), 7.53 (d, *J* 8.4, 1H, H_5), 7.57 (d, *J* 2.3, 1H, H_{13}), 7.64 (d, *J* 2.3, H_{15}), 8.39 (bs, 1H, $H_{9(CH=N)}$), 9.98 (s, 1H, C_{11}-OH), 11.70 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.82 (1C, CH_3), 29.31 (3C, C_{17}, C($\underline{CH}_3$)$_3$), 31.26 (3C, C_{19}, C($\underline{CH}_3$)$_3$), 34.19 (1C, C_{18}, $\underline{C}(CH_3)_3$), 34.75 (1C, C_{16}, $\underline{C}(CH_3)_3$), 108.29 (1C, C_{10}), 113.93 (1C, C_6), 119.76 (1C, C_4), 120.33 (1C, C_{13}), 123.06 (1C, C_{15}), 128.69 (1C, C_2), 131.35 (1C, C_5), 133.11 (1C, C_3), 136.66 (1C, C_{12}), 141.56 (1C, C_{14}), 154.12 (1C, C_{11}), 158.04 (1C, C_1), 161.17 (1C, C_9), 163.06 (1C, C_7), 165.09 (1C, C_8); EIMs, m/z= 423 [$M^{\bullet+}$] 10%, (Calc. for $C_{24}H_{29}N_3O_2S$, 423.20),.base peak= 207 [2-(5-amino-1,3,4-thiadiazol-2-yl)-6-methylphenol]$^{\bullet+}$ 100%.

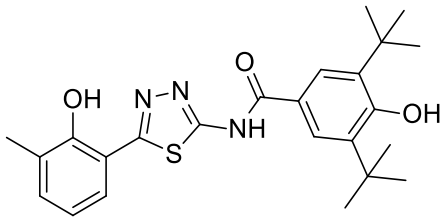
2.8.2 Synthesis of 2-(5-(3,5-di-*tert*-butyl-2-hydroxylbenzylamino)-1,3,4-thiadiazol-2-yl)-6-methylphenol 2.32



A Schiff base **2.31** (0.3 g, 0.7 mmol) was dissolved in methanol and THF (1:1), the sodium borohydride (0.026 g, 0.7 mmol) was added pinch wise during 20 minute of solid to a solution of the Schiff base at 0˚C, then immediately change in the colour of Schiff base was observed. The mixture was stirred at room temperature for 3-4 hs. The excess of solvent was

removed by evaporation. The residue was washed with cold water and crystallized from ethanol to give pale yellow precipitate. Yield 0.18 g, 63%, m.p.(158-161) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3384 (NH), 3334-3256 ($OH_{3,5\text{-di-}tert\text{-butyl}}$), 3176 ($OH_{phenol}$), 3097 ($CH_{aromatic}$), 2954, 2864 ($CH_{aliphatic}$), 1614 (C=N), 1547, 1473 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.24-1.31 (s, 18H, 2×C($\underline{CH_3}$)$_3$), 2.26 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 6.67 (s, 1H, NH), 6.94 (t, *J* 7.6, 1H, H_4), 7.31 (d, *J* 6.9, 1H, H_3), 7.42 (d, *J* 7.2, 1H, H_5), 7.54 (m, 2H, H_{13}, H_{15}), 9.47 (s, 1H, C_{11}-OH), 10.31 (s, 1H, C_1-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.20 (1C, CH_3), 30.16 (3C, C_{17}, C($\underline{CH_3}$)$_3$), 32.35 (3C, C_{19}, C($\underline{CH_3}$)$_3$), 34.12 (1C, C_{18}, $\underline{C}(CH_3)_3$), 34.95 (1C, C_{16}, $\underline{C}(CH_3)_3$), 62.70 (1C, CH_2), 108.65 (1C, C_{10}), 119.31 (1C, C_6), 119.33 (1C, C_4), 120.14 (1C, C_{13}), 123.43 (1C, C_{15}), 125.94 (1C, C_2), 126.28 (1C, C_5), 133.48 (1C, C_3), 134.61 (1C, C_{12}), 136.26 (1C, C_{14}), 154.47 (1C, C_{11}), 154.60 (1C, C_1), 158.31 (1C, C_8), 163.41 (1C, C_7); EIMs, m/z= 424 [M^+] 100%, (Calc. for $C_{24}H_{31}N_3O_2S$, 425.21).

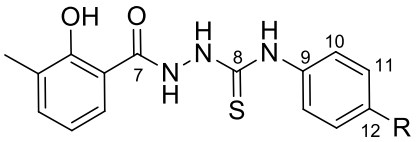
2.8.3 6-(5-(3,5-di-*tert*-butyl-4-hydroxbenzamido)-1,3,4-thiadiazol-2-yl)-2-methyl phenol 2.33



To a 500 mL round bottom flask equipped with stir bar was added 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (0.48 g, 2 mmol) and DCM (120 Ml) to this stirred heterogeneous solution was added CDI (0.32 g, 2 mmol) in one portion, turning the solution from milky white to a clear yellow and resulting in the evolution of CO_2 gas. The now yellow solution was allowed to stir for 45 minutes. At this time, 6-(5-amino-1,3,4-Thiadiazole-2-yl)-2-methylphenol (0.4 g, 2 mmol) was added all at once turning the solution a cloudy white. The reaction mixture was stirred for 6 hs. the

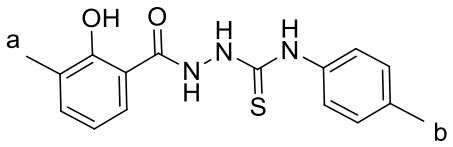
reaction mixture was then quenched with 50 mL of 1M hydrochloric acid, and extracted by dichloromethane (2×20 mL). The combine organic layer washed with distilled water and then with brine. The organic layer was dried under sodium sulfate and evaporated under reduced pressure.The desired product was purified by flash column chromatography using hexane :ethyl acetate (9:1) as eluent to give pale yellow precipitate. Yield 0.55 g, 65%, m.p. (syrup) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3626 ($OH_{3,5\text{-di-}tert\text{-butyl}}$), 3408 (NH), 3128 ($OH_{phenol}$), 3020 ($CH_{aromatic}$), 2954, 2856 ($CH_{aliphatic}$), 1687 (C=O), 1637 (C=N), 1595, 1435 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.51 (s, 18H, 2×C($\underline{CH_3}$)$_3$), 2.23 (s, 3H, CH_3), 5.68 (s, 1H, C_{13}-OH), 6.85 (t, *J* 7.8, 1H, H_4), 7.20 (d, *J* 7.8, 1H, H_3), 7.34 (d, *J* 7.6, 1H, H_5), 7.56 (s, 2H, H_{11}), 11.34 (s, 1H, C_1-OH), 13.23 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.83 (1C, CH_3), 29.97 (6C, C_{15}, 2×C($\underline{CH_3}$)$_3$), 34.37 (2C, C_{14}, $\underline{C}(CH_3)_3$), 117.62 (1C, C_6), 119.57 (1C, C_{10}), 121.47 (1C, C_4), 124.09 (1C, C_5), 126.20 (2C, C_{11}), 127.54 (1C, C_2), 131.94 (1C, C_3), 138.11 (2C, C_{12}), 154.35 (1C, C_{13}), 158.20 (1C, C_1), 161.58 (1C, $C_{7(C=N)}$), 163.91 (1C, $C_{8(C=N)}$), 167.75 (1C, $C_{9(C=O)}$).

2.9 General synthesis of 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl)-hydrazinecarbothioamide (2.34-2.36)



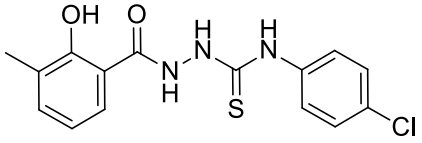
Aryl *iso*thiocyanate (1 mmol) was added dropwise to a stirred solution of 2-hydroxy-3-methylbenzohydrazide (0.16 g, 1 mmol) in 15 mL absolute ethanol and the mixture was heated to 50 °C for 3 hs. The solid mass was collected after cooling, washed with cold absolute ethanol and then dried under reduced pressure and recrystallized from suitable solvent

2.9.1 2-(3-methyl-2-hydroxybenzoyl)-N-(4-methylphenyl)-hydrazine-carbothioamide 2.34



Recrystallize of the crude product from aqueous ethanol to afford white crystal. Yield 0.24 g, 81%, m.p. (177-179) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3365-3244 (CO<u>NH</u>, CS<u>NH</u>, Ph<u>NH</u>CS), 3165 (OH_{phenol}), 3030 ($CH_{aromatic}$), 2966, 2860 ($CH_{aliphatic}$), 1647 (C=O), 1605, 1531 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.19 (s, 3H, $CH_{3(a)}$), 2.29 (s, 3H, $CH_{3(b)}$), 6.83 (t, *J* 7.6, 1H, H_4), 7.14 (d, *J* 8.1, 2H, H_{11}), 7.33 (m, 3H, H_3, H_{10}), 7.76 (d, 1H, *J* 7.6, 1H, H_5), 9.72 (s, 1H, CONH), 9.86 (bs, 1H, ph<u>NH</u>CS), 10.86 (s, 1H, CS<u>NH</u>NH),12.46 (s, 1H, OH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 15.95 (1C, CH_3), 21.02 (1C, CH_3), 113.14 (1C, C_6), 118.37 (1C, C_4), 125.85 (1C, C_5), 126.39 (1C, C_2), 128.97 (2C, C_{11}), 130.82 (2C, C_{10}), 134.76 (1C, C_{12}), 135.51 (1C, C_3), 137.00 (1C, C_9), 159.61 (1C, C_1), 164.22 (1C, $C_{7(C=O)}$), 181.01 (1C, $C_{8(C=S)}$).

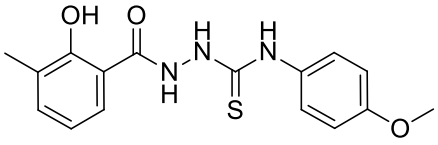
2.9.2 2-(3-methyl-2-hydroxybenzoyl)-N-(4-chlorophenyl)-hydrazine carbothioamide 2.35



The crude material recrystallized from methanol, to afford white crystal. Yield 0.25 g, 79%, m.p. (188-190)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3460-3275 (CO<u>NH</u>, CS<u>NH</u>, Ph<u>NH</u>CS), 3222 (OH_{phenol}), 3074 ($CH_{aromatic}$), 2962, 2860 ($CH_{aliphatic}$), 1649 (C=O), 1603, 1410 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.19 (s, 3H, CH_3), 6.84 (t, *J* 7.7, 1H, H_4), 7.40 (m, 5H, H_3, 2×H_{10}, 2×H_{11}), 7.76 (d, *J* 7.8, 1H, H_5), 9.90 (s, 1H, CONH), 9.96 (bs, 1H, Ph<u>NH</u>CS), 10.91 (s, 1H , CS<u>NH</u>NH), 12.43 (s, 1H, OH); ^{13}C APT (100

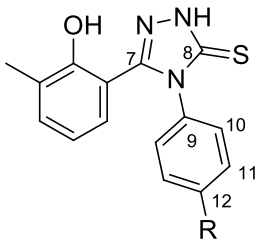
MHz, DMSO-d_6) δ, ppm; 15.95 (1C, CH_3), 113.06 (1C, C_6), 118.42 (1C, C_4), 125.82 (1C, C_5), 126.46 (1C, C_2), 128.39 (2C, C_{10}), 128.46 (2C, C_{11}), 135.59 (1C, C_3), 138.60 (2C, C_9, C_{12}), 159.63 (1C, C_1), 170.66 (1C, $C_{7(C=O)}$), 181.57 (1C, $C_{8(C=S)}$).

2.9.3 2-(3-methyl-2-hydroxybenzoyl)-N-(4-methoxyphenyl)-hydrazine carbothioamide 2.36



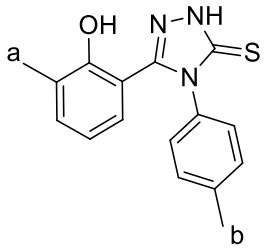
Recrystallized from ethanol, to afford white crystal. Yield 0.26 g, 82%, m.p. (176-178) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3448-3244 (CO<u>NH</u>, CS<u>NH</u>, Ph<u>NH</u>CS), 3159 (OH_{phenol}), 3020 ($CH_{aromatic}$), 2958, 2843 ($CH_{aliphatic}$), 1647 (C=O), 1603, 1520 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.18 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.82 (t, *J* 7.72,1H, H_4), 6.90 (d, *J* 8.85, 2H, H_{10}), 7.27 (d, *J* 8.85, 2H, H_{11}), 7.36 (d, *J* 6.97, 1H, H_3), 7.75 (d, *J* 7.91, 1H, H_5), 9.69 (s, 1H, CO<u>NH</u>), 9.82 (s, 1H, Ph<u>NH</u>CS), 10.86 (s, 1H , CS<u>NH</u>NH), 12.49 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.44 (1C, CH_3), 55.16 (1C, OCH_3), 112.61 (1C, C_6), 113.22 (2C, C_{11}), 117.84 (1C, C_4), 125.33 (1C, C_5), 125.87 (1C, C_2), 127.29 (2C, C_{10}), 131.90 (1C, C_9), 134.99 (1C, C_3), 156.76 (1C, C_1), 159.11 (1C, C_{12}), 170.15 (1C, $C_{7(C=O)}$), 181.46 (1C, $C_{8(C=S)}$); EIMs, m/z= 331 [$M^{\bullet+}$] 6%, (Calc. for $C_{16}H_{17}N_3O_3S$, 331.10),.base peak= 135 [(2-hydroxy-3-methylbenzylidyne)-oxonium] 100%.

2.10 General Synthesis of 2-methyl-6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione)phenol (2.37 -2.39)



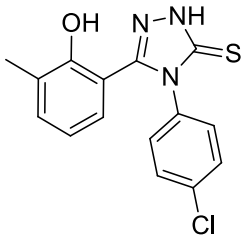
A suspension of 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl)-hydrazinecarbothioamide (0.2 g, 0.6 mmol) in 10 ml sodium hydroxide solution (4N) was refluxed for 2 hs. After cooling to room temperature, the mixture was filtered, and the filtrate was poured into 50 ml ice water. The pH was adjusted to pH 5 using 10% hydrochloric acid. The solid was separated, collected and washed with cold water, dried and purified by column chromatograph or recrystallized with suitable solvent.

2.10.1 6-(4-(4-methylphenyl)-1,2,4-triazol-3-yl-5-thione)-2-methylphenol 2.37



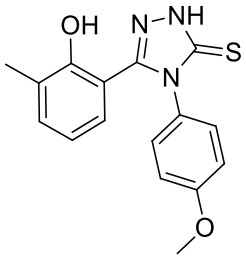
The crude material was recrystallized from methanol, to afford white crystal. Yield 0.15 g, 84%, m.p. (269-271)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3089 (OH_{phenol}), 3014 ($CH_{aromatic}$), 2927, 2765 ($CH_{aliphatic}$), 1603 (C=N), 1508, 1410 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.09 (s, 3H, $CH_{3(a)}$), 2.28 (s, 3H, $CH_{3(b)}$), 6.68 (t, *J* 7.5, 1H, H_4), 6.97 (d, *J* 7.5, 1H, H_5), 7.15 (m, 5H, H_3, 2×H_{10}, 2×H_{11}), 9.13 (s, 1H, OH), 13.99 (s, 1H, NH); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.77 (1C, C_2-CH_3), 21.16 (1C, C_{12}-CH_3), 113.75 (1C, C_6), 119.31 (1C, C_4), 125.9 (1C, C_2), 128.34 (2C, C_{10}), 129.15 (1C, C_5), 129.73 (2C, C_{11}), 132.27 (1C, C_{12}), 133.47 (1C, C_3), 138.83 (1C, C_9), 149.87 (1C, $C_{7(C=N)}$), 154.39 (1C, C_1), 168.32 (1C, $C_{8(C=S)}$).

2.10.2 6-(4-(4-chlorophenyl)-1,2,4-triazol-3-yl-5-thione)-2-methyl phenol 2.38



The crude material was recrystallized from ethanol to afford white crystal. Yield 0.15 g, 80%, m.p. (184-187)˚C, IR (KBr, U_{max}/ cm^{-1}); 3452-3290 (NH), 3230-3149 (OH_{phenol}), 3025 ($CH_{aromatic}$), 2931, 2765 ($CH_{aliphatic}$), 1610 (C=N), 1589, 1489 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm; 2.18 (s, 3H, CH_3), 6.83 (t, *J* 7.6, 1H, H_4), 7.03 (d, *J* 7.6,1H, H_3), 7.35 (m, 4H, 2×H_{10}, 2×H_{11}), 7.74 (d, *J* 7.3,1H, H_5), 9.18 (s, 1H, OH), 14.09 (s, 1H, NH)); ^{13}C APT (100 MHz, DMSO-d_6) δ, ppm; 16.72 (1C, CH_3), 113.55 (1C, C_6), 119.57 (1C, C_5), 126.50 (1C, C_2), 129.31 (2C, C_{10}), 130.43 (1C, C_4), 133.65 (1C, C_{12}), 133.76 (1C, C_3), 135.65 (2C, C_{11}), 138.51 (1C, C_9), 149.86 (1C, $C_{7(C=N)}$), 154.20 (1C, C_1), 168.13 (1C, $C_{8(C=S)}$).

2.10.36-(4-(4-methoxyphenyl)-1,2,4-triazol-3-yl-5-thione)-2-methyl-phenol 2.39



The desired product was purified by flash column chromatography using hexane:ethyl acetate (3:1) as eluent to give light brownish precipitate. Yield 0.14 g, 77%, m.p. (271-272)˚C,%, IR (KBr, U_{max}/ cm^{-1}); 3082 (OH_{phenol}), 3035 ($CH_{aromatic}$), 2927, 2773 ($CH_{aliphatic}$), 1606 (C=N), 1508, 1406 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.09 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.68 (td, *J* 7.54, 2.64, 1H, H_4), 6.94 (m, 3H, H_5,

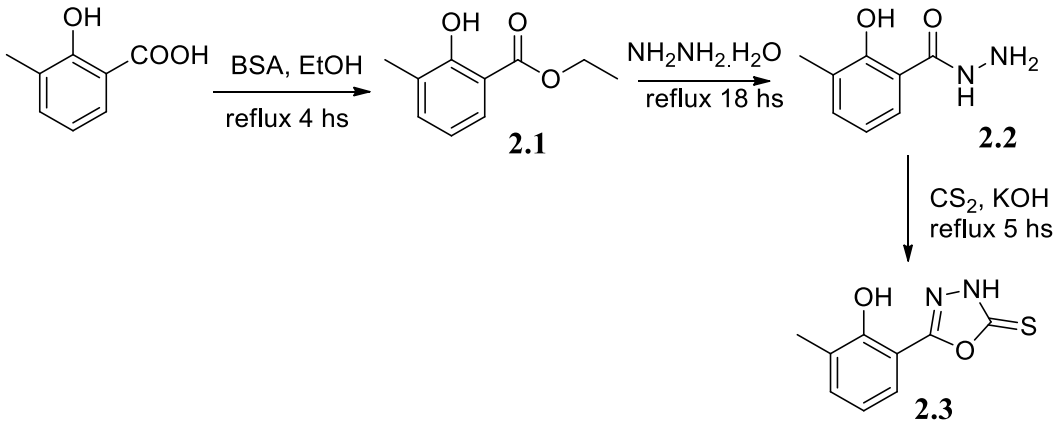
2×H_{10}), 7.13 (d, *J* 7.35, 1H, H_3), 7.21 (dd, *J* 8.85, 2.64, 2H, H_{11}), 9.13 (s, 1H, OH), 13.98 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.72 (1C, CH_3), 55.24 (1C, OCH_3), 113.22 (1C, C_6), 113.87 (2C, C_{11}), 118.79 (1C, C_4), 125.40 (1C, C_5), 126.92 (1C, C_2), 128.65 (1C, C_9), 129.33 (2C, C_{10}), 132.93 (1C, C_3), 149.51 (1C, $C_{7(C=N)}$), 153.88 (1C, C_1), 159.11 (1C, C_{12}), 167.97 (1C, $C_{8(C=S)}$); EIMs, m/z= 313 [$M^{\bullet+}$] 100%, (Calc. for $C_{16}H_{15}N_3O_2S$, 313.09).

Chapter 3 : Results and discussion

3.1 Formation oxadiazole ring

3.1.1 Synthesis of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol and their alkyl derivative's

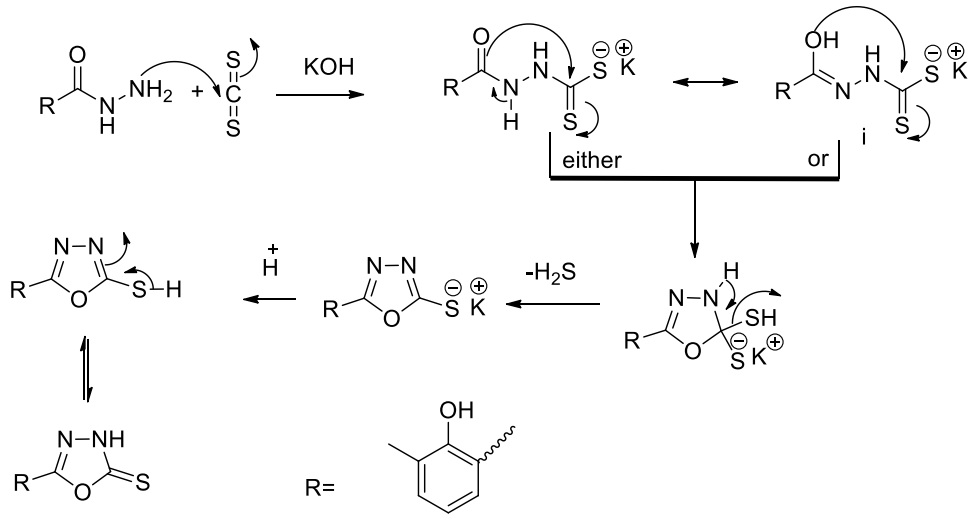
The 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol was synthesised in three steps. The first step was started by converting the 2-hydroxy-3-methylbenzoic to their corresponding ethyl ester **2.1**. The esterification was carried out by reacting the targeted acid with absolute ethanol in presence of benzenesulfonic acid (BSA) as a catalyst. The second step was synthesize of the acid hydrazide **2.2** from the corresponding ester by using normal hydrazination procedure The hydrazide can also be considered as a good substance to form many different types of heterocyclic at position six. This will increase steric hindrance, and also could increase the antioxidant activity. The hydrazide **2.2** obtained in good yield. The last step was achieved from reacting the hydrazide with carbon disulfide in the presence of potassium hydroxide to give high yield of the desired product. The outline of these three steps as described in Scheme 3.1



Scheme 3.1:-Synthesis of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol

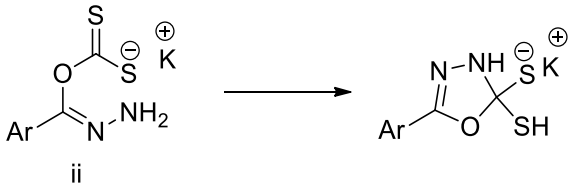
The synthesis of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol. **2.3** was characterized by IR, ^{1}H NMR and ^{13}C APT spectra. The collected data

was in agreement with the proposed structure. The suggested mechanism could take place as described in Scheme 3.2.



Scheme 3.2:-Suggest mechanism of synthesis 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.3.

This mechanism was quite similar to Ainsworth *et al*.[156] and Young *et al*.[157] when they emphasized that the formation of the intermediate (i) is more acceptable than formation of the "xanthate-type".



Scheme 3.3:-Synthesis oxadiazole from xanthate type.

As well, the cyclization of 4-amino-1,2,4-triazol takes place through the potassium salt of hydrazine carbodithioate salt (which was isolated in solid form by reacting the acid hydrazide with CS_2 in the presence of KOH at room temperature). This could complement the suggested intermediate where it will go from intermediate (i) and not from (ii).

Furthermore, in our proposed mechanism, we put two possibilities of cyclization of the intermediate (i). Firstly, it could undergo through keto form of hydrazide carbonyl. However, it is more acceptable if it is done in

step (i), where we convert it to Enol and attached simultaneously. The second possibility is that the cyclization will form the Enol and then the hydroxyl group will attach to the thiocarbonyl. Both are possible, but the cyclization from enol is the preferred explanation. Aqueous alkaline solution in ethanol could enhance the stability of Enol form than the keto form during the intramolecular hydrogen bonding and thus the formation of seven membered ring for the intermediate as depicted in Figure 3.1.

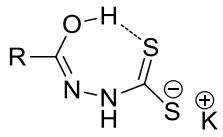
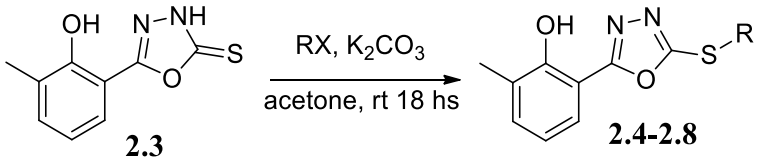


Figure 3.1:-Intramolecular hydrogen bonding could stabilize the enol form.

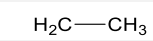
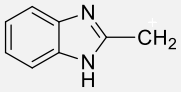
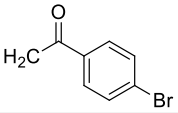
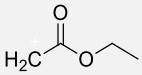
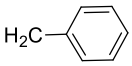
Newly five derivatives of 6-(5-(alkaylthio)-1,3,4-oxadiazol-2-yl)-2-methylphenol (**2.4-2.8**) were synthesized from alkylation reaction of **2.3** in acetone. Five alkyl halides were used in the presence of anhydrous potassium carbonate as a base at room temperature as depicted in Scheme 3.4



Scheme 3.4:-Alkylation of 2.3.

The alkylation of the mentioned oxadiazole was an S_N2 mechanism. The difference between the reactivity of hydroxyl of phenol and thiol group enabled us to successfully force a selective alkylation on thiol to get thioalkyl. Furthermore, this could indicate that the phenolic hydroxide becomes more hindrances due to the ring formation on position six. No alkylation has been observed on hydroxyl group at room temperature. Table 3.1 summarized some properties of the synthesized compounds.

Table 3.1:-Physical properties of the synthesized compounds

No.	R	Yield %	m.p. °C	M.F	EIMs Calc. $M^{\bullet+}$	EIMs Found $M^{\bullet+}$
2.3	-	88	212-213	$C_9H_8N_2O_2S$	208.03	208
2.4		83	74-76	$C_{11}H_{12}N_2O_2S$	236.06	236
2.5		77	173-175	$C_{17}H_{14}N_4O_2S$	338.08	338
2.6		86	164-166	$C_{17}H_{13}BrN_2O_3S$	403.98	403
2.7		74	71-73	$C_{13}H_{14}N_2O_4S$	294.07	294
2.8		67	100-103	$C_{16}H_{14}N_2O_2S$	298.08	298

3.1.1.1 Characterizations of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methyl phenol and their derivative's

As mentioned earlier the 2-hydroxy-3-methylbenzoic acid was converted to the corresponding ethyl ester. **2.1** which was characterized by IR, ^{1}H NMR and ^{13}C (APT) The ester converted to the corresponding hydrazide **2.2** and this hydrazide was characterized by the same methods (see the Appendix A). The 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylpheno **2.3** was identified from its IR spectrum through the disappearance of some peaks. This indicated that the cyclization between the acid hydrazide with CS_2 was successful. The peaks of NH_2 and C=O disappeared and considered as an initial indicator that the cyclization have proceeded. The C=S bond has been observed at 1122 cm^{-1} and the C=N at 1606 cm^{-1}, as well the peak for NH was observed at 3365 cm^{-1}, while the OH band was located at 3066. In ^{1}H NMR spectrum, show disappeared of NH_2 band of hydrazide, while the NH peak was observed at 9.16 ppm. Furthermore, the oxadiazole-5-thione was known with these tautomer structures [158, 159] as shown in Figure 3.2 below:

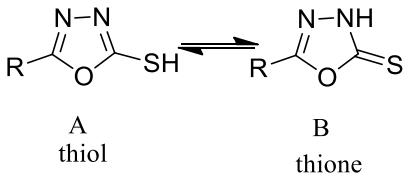


Figure 3.2:-Tautomerism structure of oxadiazol (thiol-thione).

In solution, both of thiol-thione structures might be existent. However, based on the ^{1}H-NMR data, it is clear that it exists as the thione form (B) in DMSO-d_6. We observed the appearance of NH proton and there was no signal for the thiol proton. This suggestion was further strengthened with ^{13}C APT. The ^{13}C APT, in Figure 3.3 displayed the peaks at 177.24 ppm and are more compatible with thione form (B) than thiol form (A). In the solid state, the form A could be the more stable and this corresponds to the IR spectrum and is in agreement with literatures.[159-161].

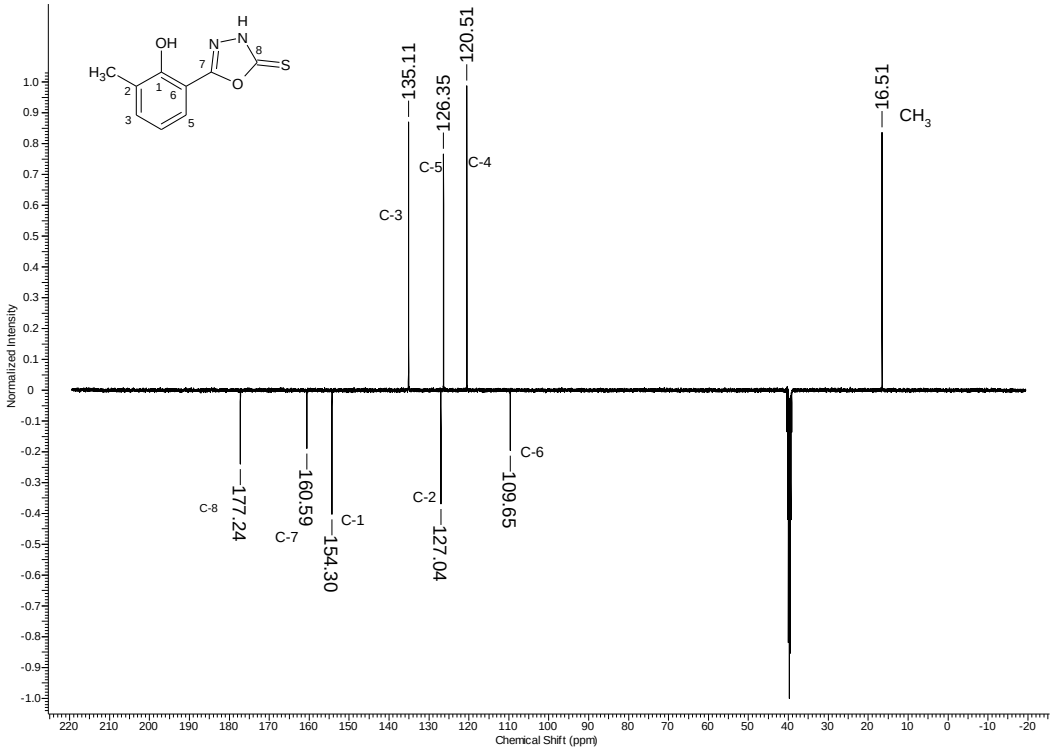


Figure 3.3:-^{13}C APT (400 MHz, DMSO-d_6) of compound 2.3

Likewise, the rest signals of ^{1}H NMR consented with proposed structure. A singlet for three protons of methyl group (CH_3) belonged to 2-methylphenol ring was appeared at 2.21 ppm. As well, the aromatic proton

is appeare as triplet at 6.92 ppm with coupling constants *J* 7.2 Hz for H_4. While the signals for H_3 and H_5 appeared as doublet at 7.34 ppm and 7.45 ppm with *J* 7.3 Hz and *J* 7.8 Hz respectively. The broad singlet signal appeared at 9.16 ppm for NH. The ^{13}C APT spectrum also displays five negative peaks (pointing downwards) belonged to five quaternary carbons C_1, C_2, C_6, C_7, C_8 at 154.30, 127.04, 109.65, 160.59, 177.24 respectively. In addition to that, the CH_3 and CH were located as positive peak for CH_3, C_3, C_4, C_5 at 16.51, 135.11, 120.51, 126.35 respectively as shown earlier in Figure 3.3.

The five thioalkyl derivatives **2.4-2.8** were characterized by the same methods. Generally, IR spectrum recorded the disappearance of NH and C=S bands. The band of C=N was appeared at the range 1618-1603 cm^{-1} was the first evidence for success of alkylation process. Furthermore, IR spectra demonstrated the appearance of NH benzimidazole for compound **2.5** at 3174 cm^{-1}, also the carbonyl group (C=O) for compound **2.6** and **2.7** at 1678 cm^{-1}, 1728 cm^{-1} respectively. The OH of phenol appeared as a medium band at (3207-3080) cm^{-1} due to the non-hydrogen bonding for the hindered phenol[162]. However, earlier work has shown that the OH group of a highly hindered phenolic was capable to form of hydrogen bonding [136] but only too small extent and was insignificant. Thus, the OH group appeared as a medium and not abroad. The IR spectra for compounds **2.4-2.8** depicted strong absorptions at (3041-3024) cm^{-1}, (2989-2738) cm^{-1}, (1618-1603) cm^{-1}, (1591-1425) cm^{-1} attributed to (C-H) aromatic, (C-H) aliphatic, (C=N) and (C=C) aromatic, respectively. The ^1H NMR spectra of compounds **2.4-2.8** showed new signal besides the methyl phenol belonged to the thioalkyl group. The interesting singlet signal of SCH_2 was appeared at δ (4.80, 5.16) ppm for compounds **2.5**, **2.6** respectively. Moreover, the signal of compound **2.4** was appeared as a multiplet signal at δ 3.34 ppm for CH_2 of ethyl group and not quartate as expected, due to interfere with

H_2O peak of the solvent (DMSO-d_6) .Although, the appearance of CH_3 as triplet at δ 1.44 ppm with *J* 7.3 indicated that the neighboring group is CH_2. The ^{1}H NMR spectrum of **2.6**, which is taken as example, showed new two doublet peaks each one for two protons at 8.0 for H_{12}, at 7.81 for H_{13}, with *J* 8.3 Hz and 8.1 Hz as depicted in Figure 3.4.

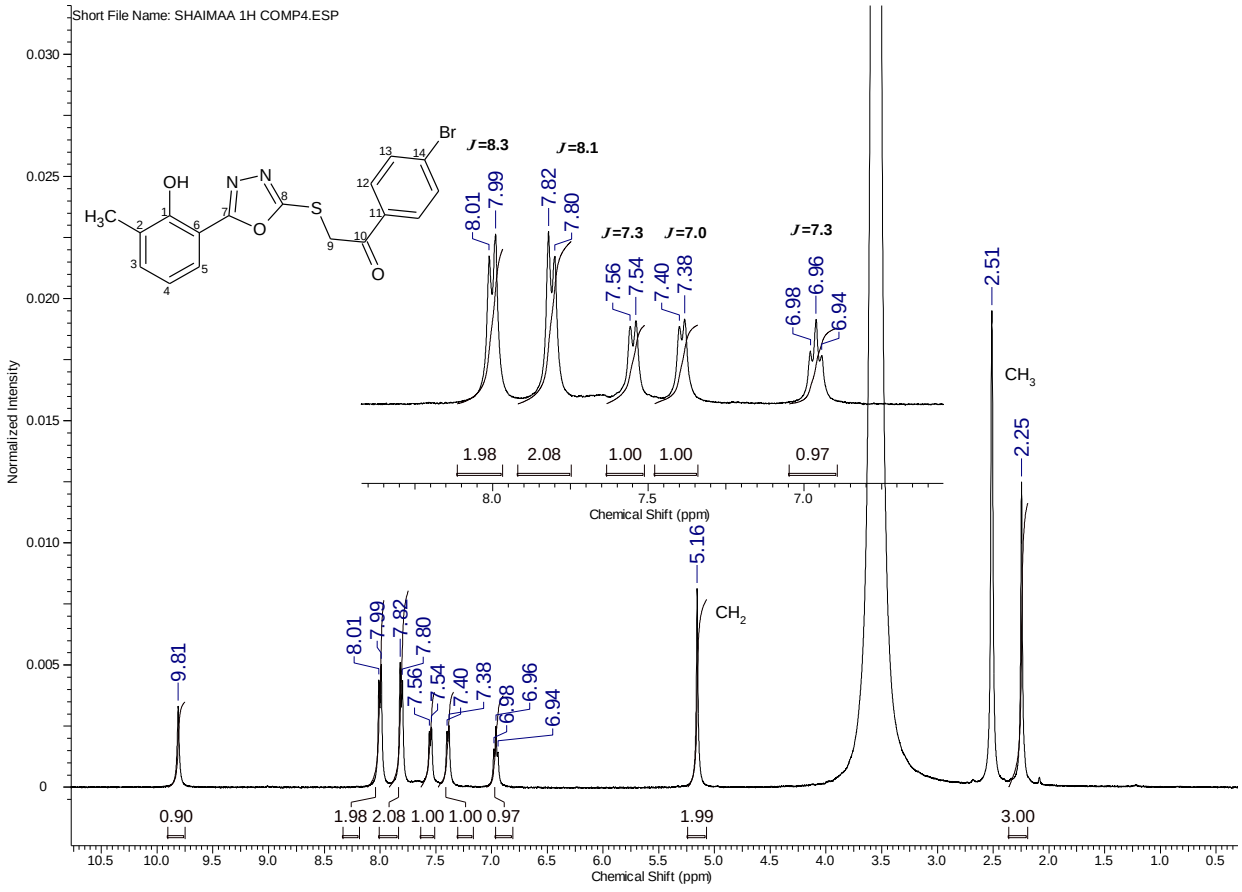


Figure 3.4:-^{1}H NMR (400MHz, DMSO-d6) of compound 2.6

The ^{1}H NMR spectrum of compound **2.5** showed all expected protons for benzimidazole group as multiplet signal at δ 7.18 ppm with integral equal two protons for H_{13}, as well the multiplet peaks for three protons at δ 7.52 ppm refer to H_5 and H_{12}. The ^{1}H NMR spectrum of compound **2.7** displayed the protons of ethyl ester group as triplet for three protons at 1.20 ppm with *J* equals 6.96 Hz and quartet for two protons at 4.17 ppm with *J* equals 7.16 Hz. The two protons of SCH_2 group were assigned as singlet at 4.32 ppm. The rest protons of 2-methylphenol were located at their expected locations (see Appendix B). The spectrum of thiobenzyl **2.8**

exhibited eight aromatic protons besides to two protons of SCH_2 group were located as singlet at 4.60 ppm.

The ^{13}C APT spectra of these compounds confirmed the proposed structures of thioalkyl derivatives. All carbons peaks of methylphenol were appeared in expected position (see Appendix B). APT spectra displayed significant negative peaks appeared at (27.25-40.83) ppm for SCH_2 group which is in agreement with the SCH_2 peak at δ (3.34-5.16) ppm in $^{1}HNMR$ spectra. Furthermore, the ^{13}C spectra showed all negative and positive peaks for alkyl group. The spectrum of compound **2.6** will be taken as example. Six peaks of the positive region appeared at 16.19, 120.45, 125.33, 130.94, 132.50, 135.16 belonged to CH_3, C_4, C_5, C_{12}, C_{13}, C_3 respectively. While the negative region peaks appeared at 40.83, 108.13, 126.55, 128.73, 134.44, 154.73, 163.20, 165.38, 192.44 for CH_2, C_6, C_2, C_{14}, C_{11}, C_1, C_8, C_7, C_{10} respectively as depicted in Figure 3.5.

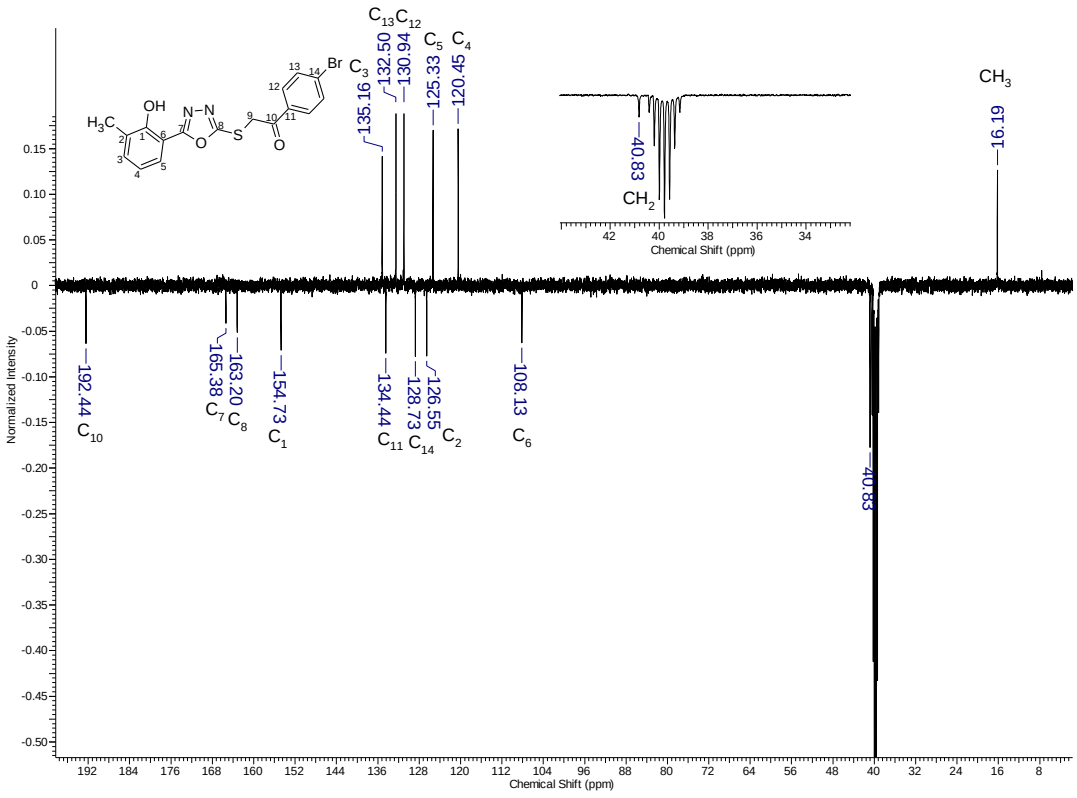


Figure 3.5:-^{13}C APT (400 MHz, DMSO-d_6) of compound 2.6

The spectrum of compound **2.5** which, is content benzimidazole group showed the merge of two peaks. The signal of C_{10} and C_{11} appeared at 149.85 and C_{12}, C_{13} at 122.61, which could be attributed to less sensitivity of APT spectra when compared with normal ^{13}C NMR or DEPT[163]. Furthermore, the expansion region of these peaks showed these two peaks which are not sharp as depicted in Figure 3.6.

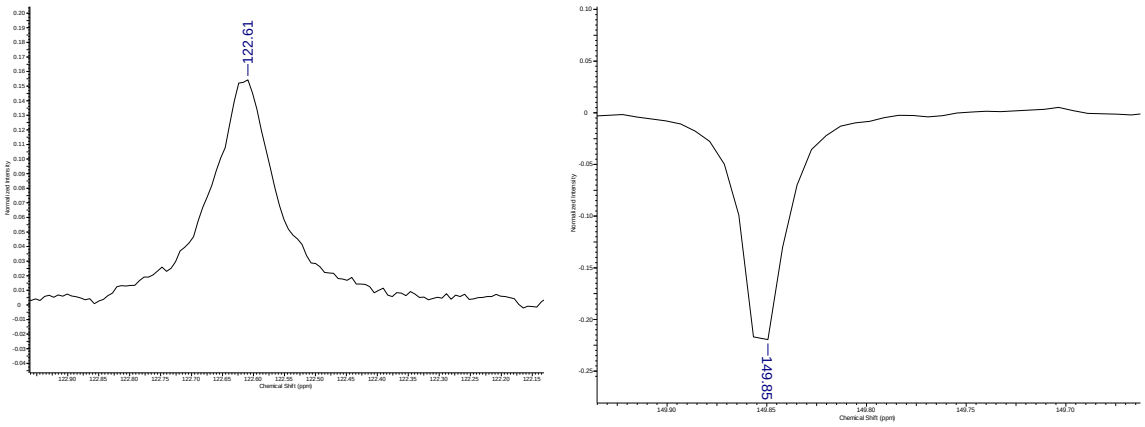


Figure 3.6:-Expansion region of 13C APT NMR for two carbons of compound 2.5.

While the single peak for one carbon appeared as sharp. Figure 3.7 demonstrated signal peak for C_1 as example. From that, we assume that the peaks of C_{10} and C_{11} are merged due to less sensitivity of the APT as well for C_{12} and C_{13}.

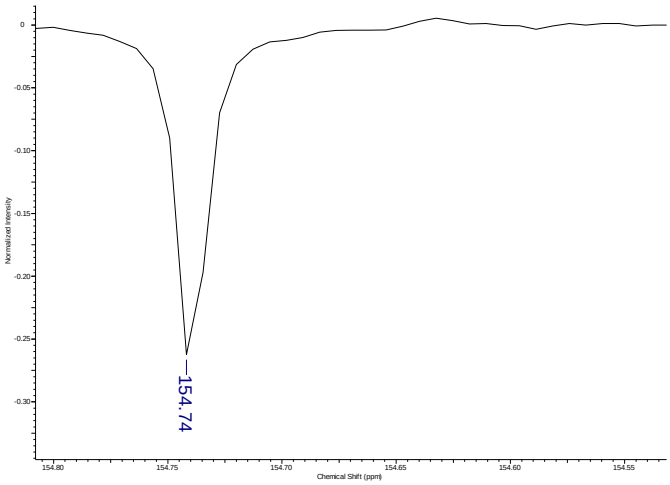
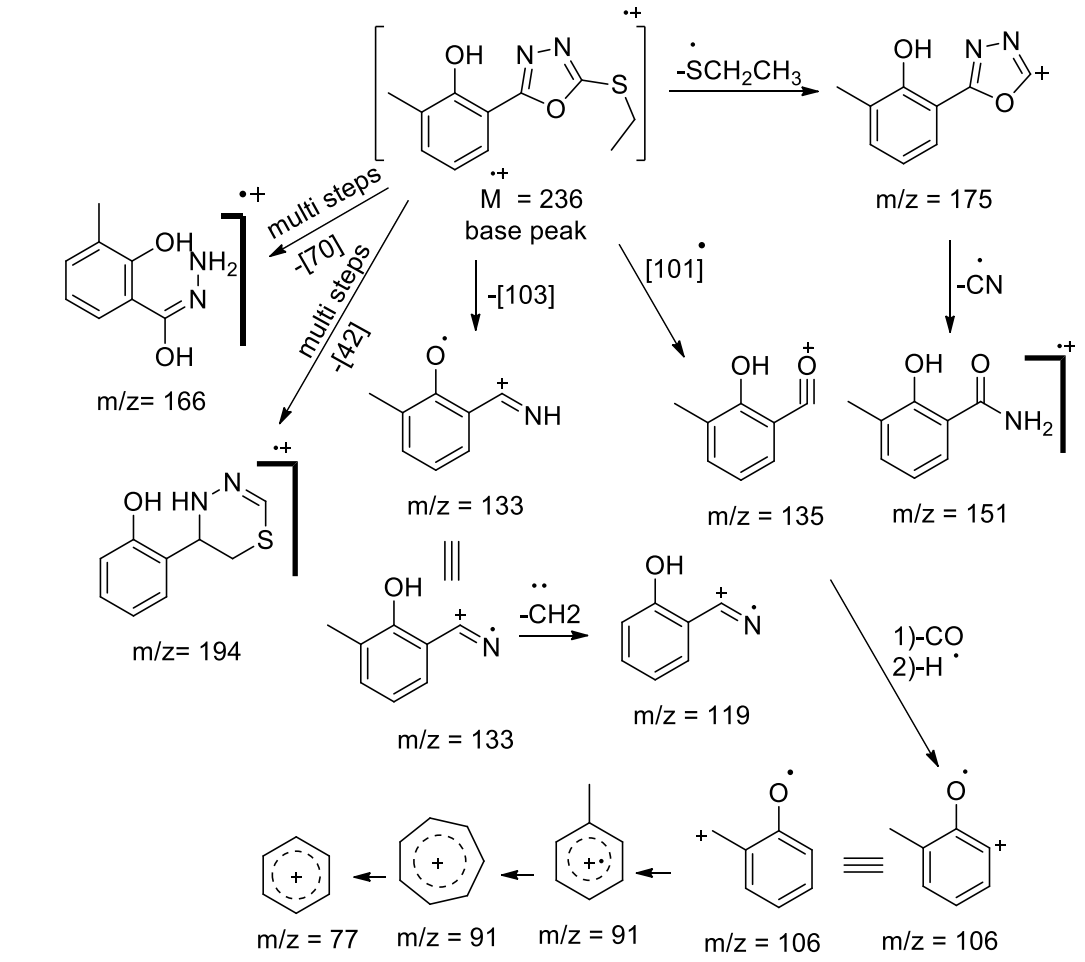


Figure 3.7:-Expansion region for single carbon at ^{13}C APT

These results are in agreement with the ^{1}HNMR and also with the EIMs spectrum. The EIMs results confirm the proposed structure by matching the theoretical calculated mass with the experimental mass ($M^{\cdot+}$) as well the fragmentations in EIMs confirmed their proposed structure.

Besides the ^{1}H and ^{13}C NMR the structure of 1,3,4-oxadiazole-5-thione **2.3** and their derivatives (**2.4-2.8**) were confirmed by EIMs. EIMs gives the molecular ion $M^{\cdot+}$ peak and the base peak (100%). The Mass spectra showed the molecular ions ($M^{\cdot+}$) of the synthesized compounds (**2.4-2.8**). The base peak showed the same value of the molecular ion as in compounds **2.4, 2.7** and **2.8**. The base peak of compound **2.5** was appeared as 2-methyl benzimidazole carbocation [$BIMCH_2^+$], while it appeared for compound **2.6** as 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol carbocation. Compound **2.4** and compound **2.5** have been taken as examples to illustrate their EIMs fragmentations, Scheme 3.5 depicted the fragmentation pattern of compound **2.4**



Scheme 3.5:-Fragmentation of EIMs of compound 2.4

These compounds followed the most EIMs fragmentation pattern of the 1,3,4- oxadiazole ring, which were reported in some literatures. [164]

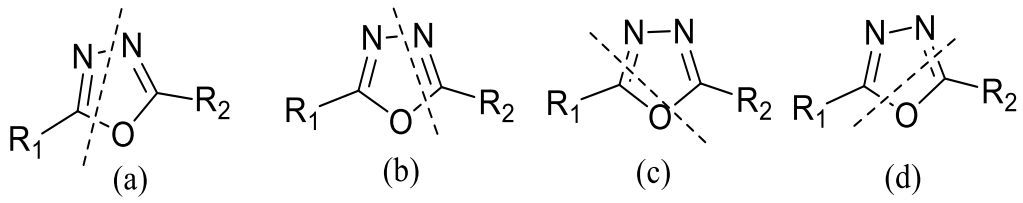
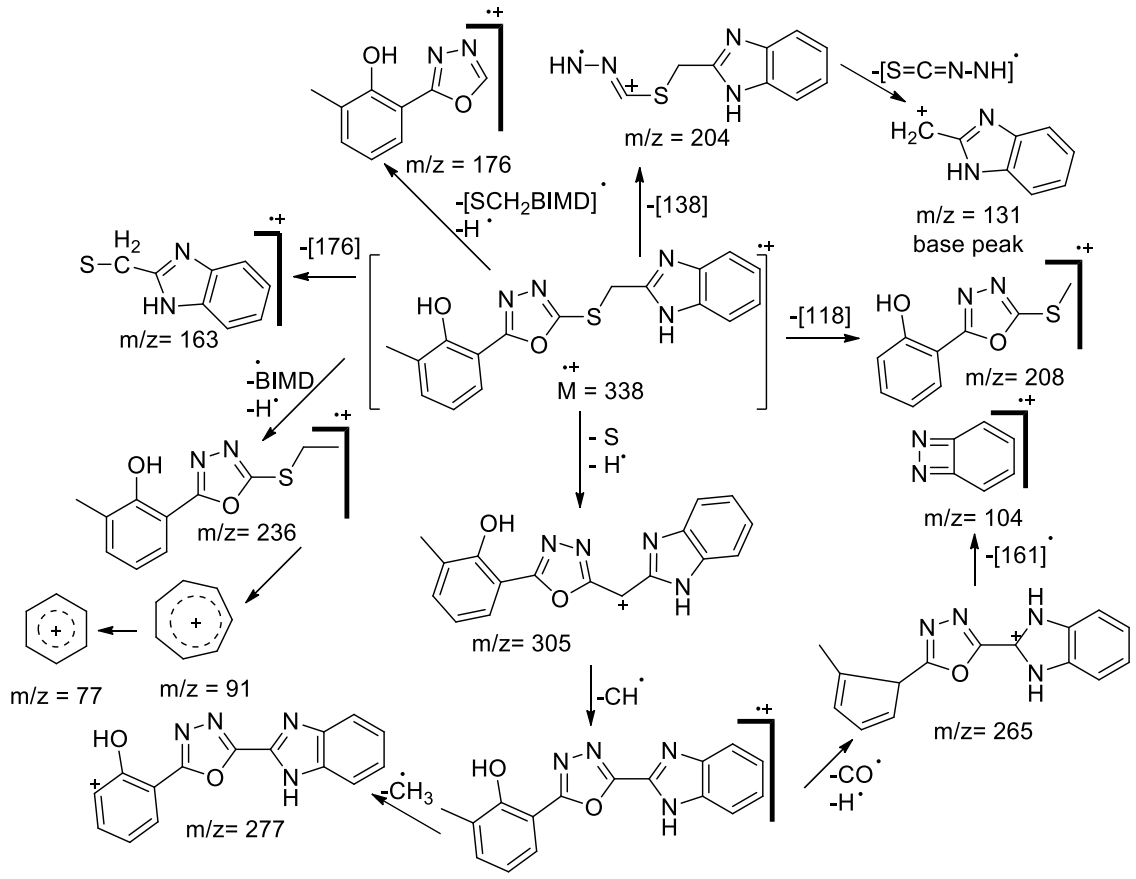


Figure 3.8:-Pathway of oxadiazole fragmentations.[164]

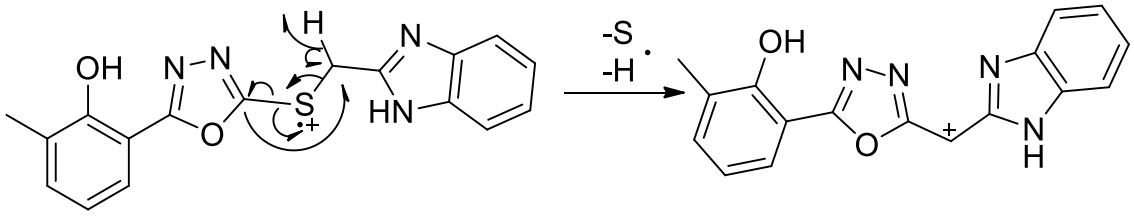
The fragments of title compound express the part of the proposed structure. The fragmentation pattern of compound **2.5** was depicted at Scheme 3.6



Scheme 3.6:-Fragmentation of EIMs of compound 2.5

The mass spectrum displayed the molecular ion $M^{\bullet+}$= 338 and the base peak at m/z= 131 as carbocation of 2-methyl-benzimidazole. The rest fragments express the part of the proposed structure.

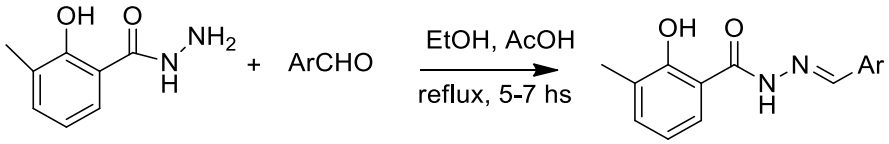
The interested fragment was located at m/z= 305 which was generated from losing sulphur atom then hydrogen radical with rearrangement. The proposed pathway for this fragmentation was depicted in Scheme 3.7.



Scheme 3.7:-Mechanism losing sulphur atom and hydrogen radical.

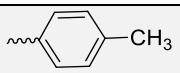
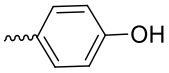
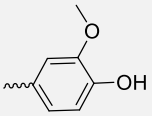
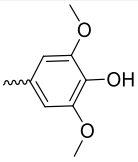
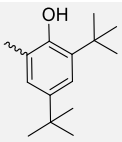
3.1.2 Synthesis of N'(substituted benzylidene) 2- hydroxy-3-methylben zohydrazide (hydrazones) 2.9-2.13

These hydrazones were synthesized as an intermediate compounds to synthesize 6-(5-(Aryl)-1,3,4-oxadiazol-2-yl)-2-methylphenol **2.14-2.18**. Five hydrazone were synthesized from the reaction of the acid hydrazide **2.2** with appropriate substituted benzaldehyde to produce the corresponding hydrazones with significant yield, as shown in Scheme 3.8 and Table 3.2.



Scheme 3.8:-Synthesis of the hydrazones.

Table 3.2:-Aryl group and some selected properties of compounds.

No.	Ar	Yield %	m.p. ˚C	M.F	EIMs Calc. $M^{\bullet+}$	EIMs Found $M^{\bullet+}$
2.9		67	173-174	$C_{16}H_{16}N_2O_2$	268.12	268
2.10		78	240-242	$C_{15}H_{14}N_2O_3$	270.10	270
2.11		83	203-206	$C_{16}H_{16}N_2O_4$	300.11	300
2.12		81	210-213	$C_{17}H_{18}N_2O_5$	330.12	330
2.13		77	205-208	$C_{23}H_{30}N_2O_3$	382.23	382

3.1.2.1 Characterization of N'(substituted benzylidene) 2- hydroxy-3-methylbenzohydrazide (hydrazones) 2.9-2.13

The newly hydrazones were characterized by IR, 1D NMR and EIMS. 2D NMR was used to confirm compound **2.11** for further characterization. The IR spectra exhibited new signal of the imine group (C=N) at (1633-1603) cm^{-1}. The Carbonyl group of hydrazone was displayed at (1633-1630) cm^{-1}. The ^{1}H-NMR of these compounds showed disappearance of the NH_2 peak at 4.69 ppm. Moreover, new peaks appeared such as CH=N at (8.39-8.65) ppm. The protons peaks from aromatic aldehyde part were appeared at their expected area and the OH signal was appeared at range (12.43-12.80) ppm, as well, the NH signal was appeared at (11.86-12.23) ppm. The NMR spectra exhibited the substituted group of aldehyde. The three protons of methyl group for compound **2.9** appeared as singlet at 2.36 ppm as shown in Figure 3.9.

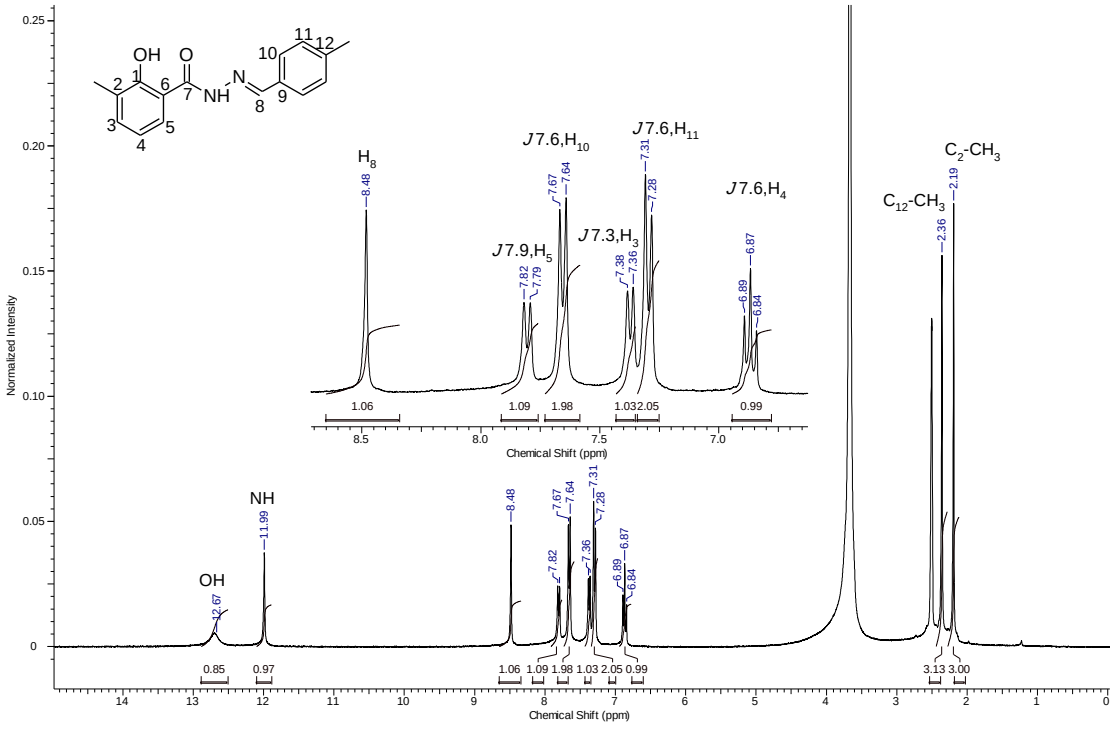


Figure 3.9:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.9.

The *para* hydroxyl group of compound **2.10** appeared as broad singlet signal at 10.02 ppm, the methoxy group for compounds **2.11** and **2.12**

appeared at 3.42 ppm and 3.83 ppm integrating for three and six protons respectively. The di-*tert*-butyl group for compound **2.13** appeared as two singlets with nine protons integrating for each peak.

The ^{13}C NMR, ^{13}C APT spectra showed new carbons of aromatic substituted aldehyde besides the interesting peak were assigned to HC=N at (148.12-152.11) ppm. Moreover, the ^{13}C NMR assigned the substituted group at the aromatic aldehyde. The methyl group of compound **2.9** appeared at 15.46, 21.02 ppm respectively as shown in Figure 3.10.

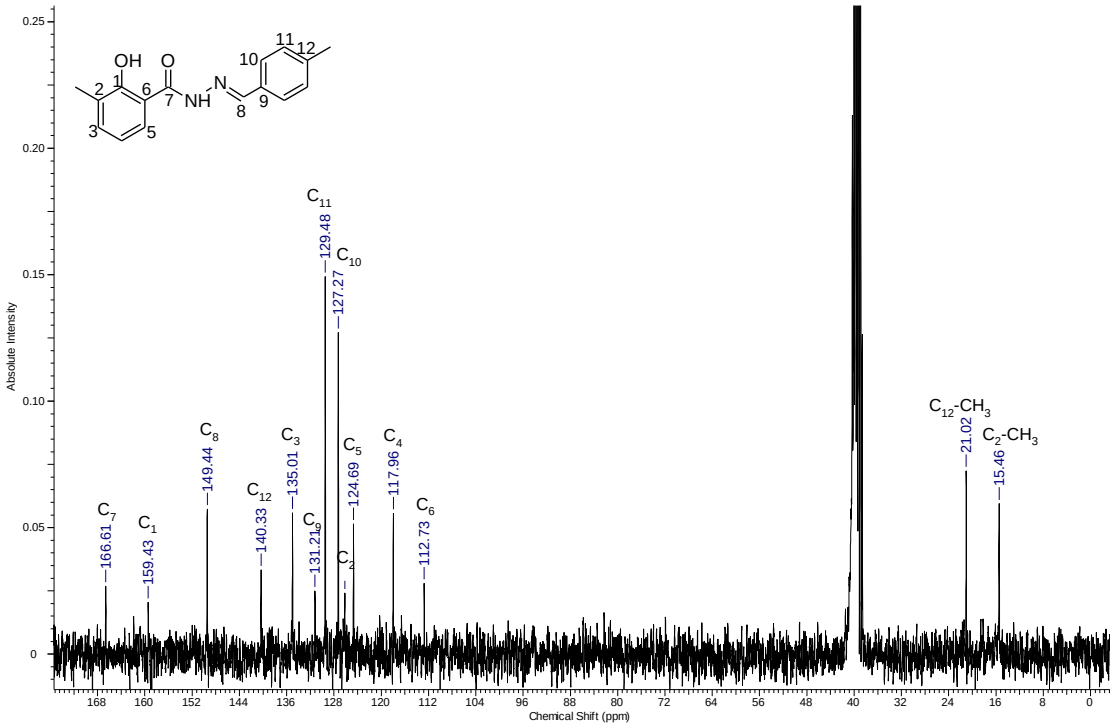


Figure 3.10:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.9.

The carbon attached with *para* hydroxyl group of compound **2.10** appeared at 159.73 ppm, the methoxy group of compound **2.11** and **2.12** appeared at 56.03 ppm and 56.02 ppm respectively. The carbons of two di-*tert*- butyl group of compound **2.13** appeared at 29.25 ppm and 31.25 ppm, respectively.

The HMBC spectrum was used to confirm the structure of compound **2.11** to solve any confusing with ^{1}H and ^{13}C NMR. HMBC spectrum

disclosed the correlation for long distance coupling J_3 and weak for J_2. The HMBC spectrum exhibited three correlations at 12.77-113.44 ppm, 12.77-127.19 ppm and 12.77-159.89 ppm as displayed in Figure 3.11.

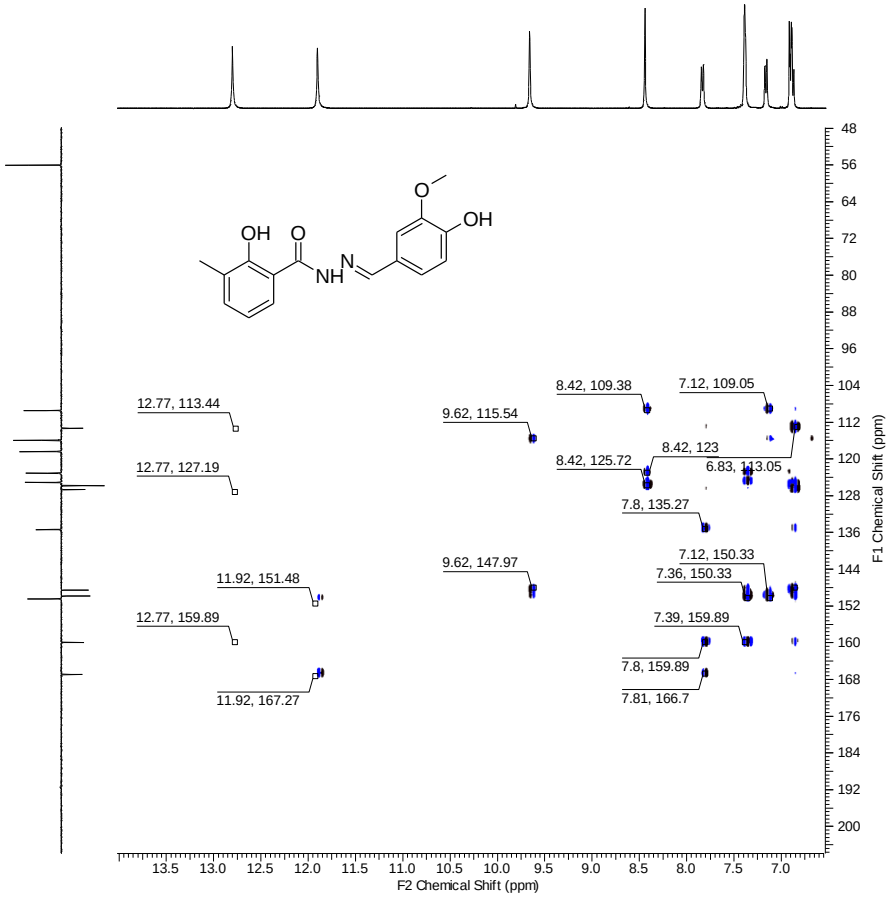


Figure 3.11:-HMBC Spectrum, expansion aria of compound 2.11.

These correlations disclosed the relations between the OH of 2-methylphenol with C_6 and C_2 as J_3, while the correlation with C_1 exhibited as J_2.Although, the protons at 11.92 ppm and 9.62 ppm displayed just two correlations, However the HMBC allowed to distinguish between the NH of hydrazone and the OH of 3-methoxyphenol. The peak at 11.92 ppm showed correlation with C_8 (C=N) at 11.92-151.48 ppm as J_3.However, the correlation with C_7 (C=O) appeared as J_2. These correlations indicated that the proton at 9.62 ppm belongs to hydroxyl group of 3-methoxyphenol. The two characteristic correlations at 9.62-115.54 ppm and 9.62-147.97

ppm indicated the correlation between the proton of OH group and C_{13} as well with C_{11}. The most important correlation was depicted in Figure 3.12.

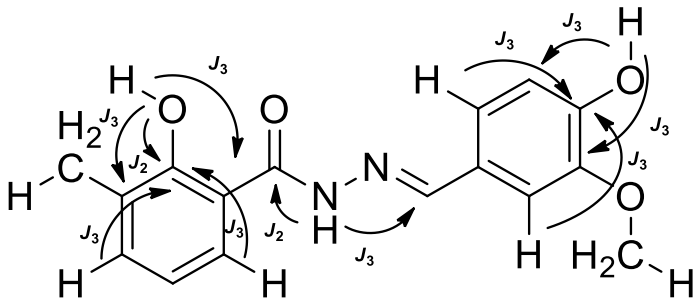
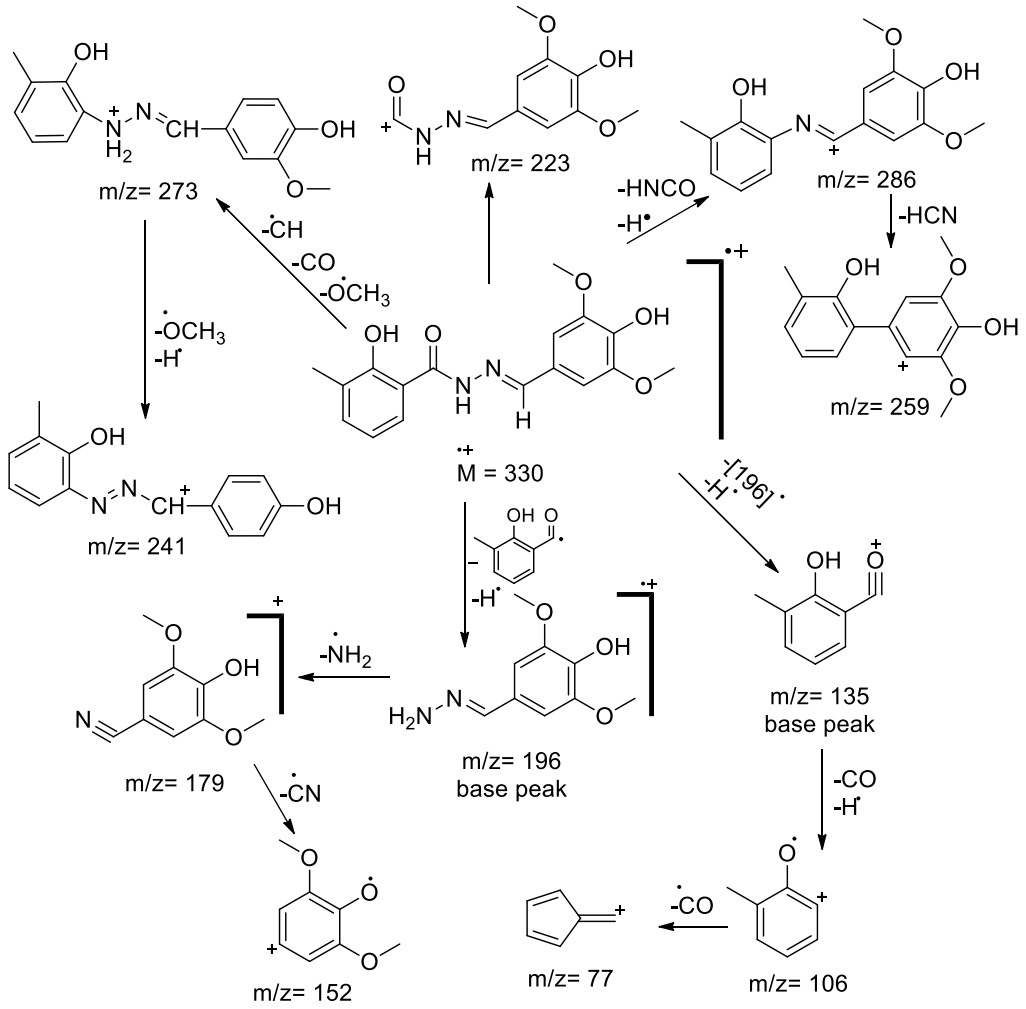


Figure 3.12:- Selected HMBC correlations of compound 2.11.

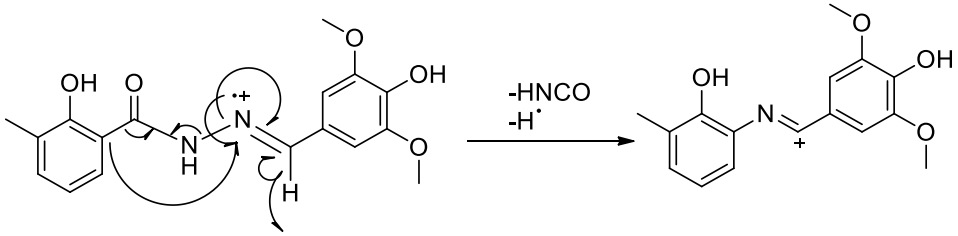
Furthermore, the HMBC spectrum allowed distinguishing between C_1 and C_{12}. The C_1 at 159.89 ppm showed correlations with OH of 2-methylphenol as mentioned earlier besides the correlation with H_3 at 7.39-159.89 ppm and correlation with H_5 at 7.8-159.89 ppm as J_3. The C_{12} exhibited correlation with H_{10} at 7.36-150.33 ppm and with H_{14} at 7.12-150.33 ppm as J_3. As demonstrated in HMBC, key correlations. Furthermore, the structures of these compounds were characterized from their EIMs spectra. The mass spectra confirmed the molecular weight of these compounds from their molecular ion, base peak and their fragmentations. Compounds **2.12** and **2.13** have been taken as examples. The EIMs spectra of hydrazone (**2.9-2.13**) confirm the proposed structure by exhibiting the molecular ion peak as well the base peak, moreover all the fragments matched the part of the structure. Compound **2.13** showed the value of molecular ion is the same as the value of base peak. The rest of compounds showed the base peak as 2-hydroxy-3-methylbenzylidyne)oxonium m/z= 135, while compound **2.9** showed the radical cation of $[C_8H_6O_2]^{\bullet+}$ at m/z= 134. The interested fragmentation spectrum of compound **2.12** showed two peaks with 100% intensity as two base peaks. The first base peak was at m/z= 135 which can be a source of resulting peaks at m/z= 106 and m/z= 77. The second base peak was at m/z= 196, which were source of fragment m/z= 179 and m/z= 152. The

fragmentation of compounds **2.12** and **2.13** was taken as example to demonstrate the pattern of fragmentation as shown in Scheme 3.9 and 3.12.



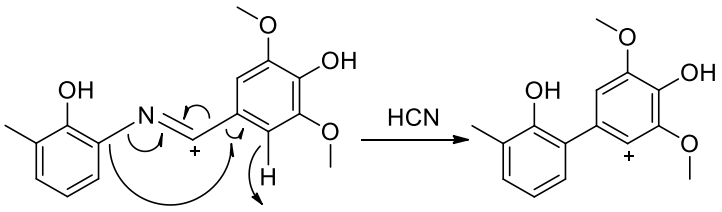
Scheme 3.9:-Fragmentation of EIMs of compound 2.12.

The spectra of compound **2.12** showed interested fragmentation with rearrangement, by losing HNCO as shown below



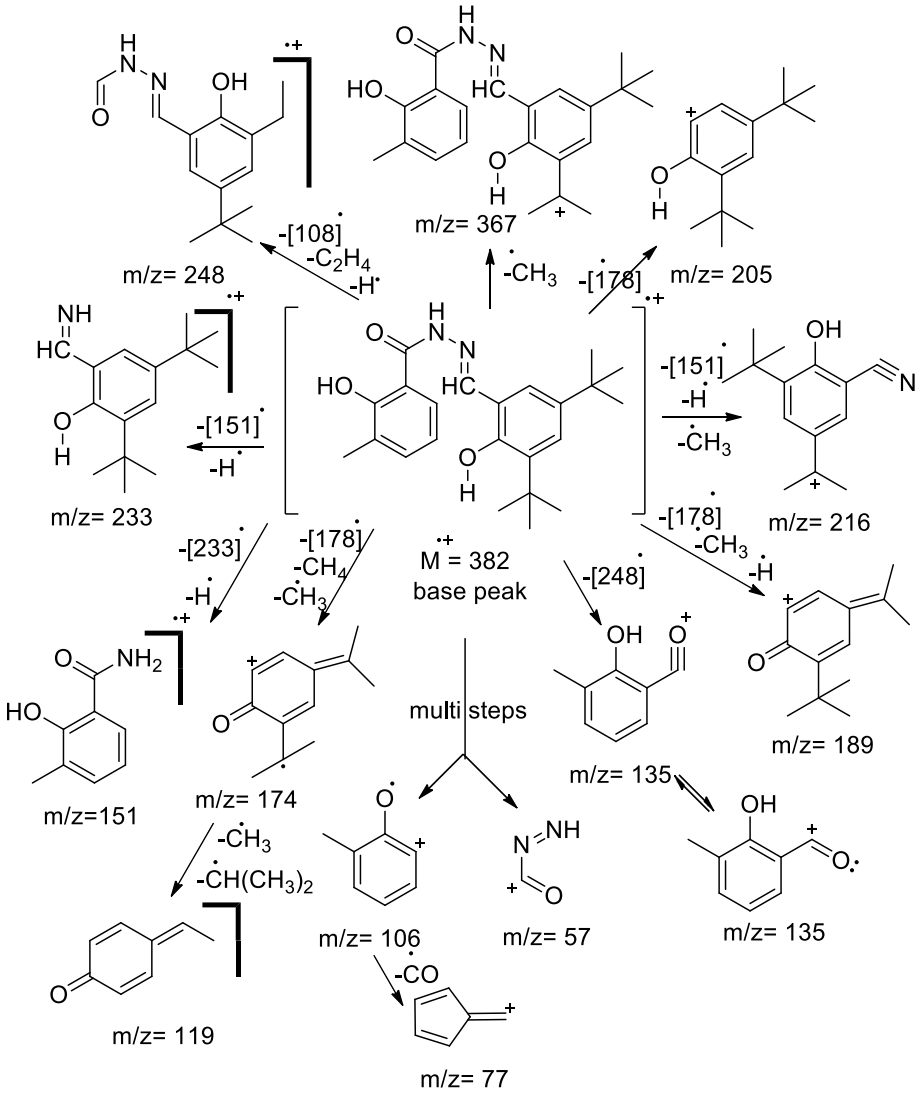
Scheme 3.10:-Proposed pathway of losing HNCO from the hydrazone.

The m/z= 259 confirms the existence m/z= 286 by losing HCN as shown in Scheme 3.11.



Scheme 3.11:- Proposed pathway of losing HCN.

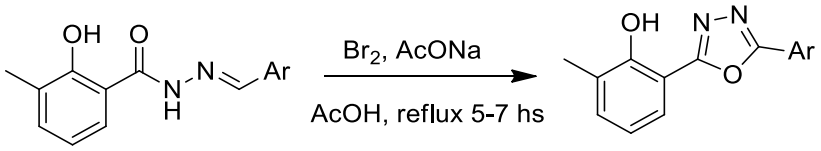
Besides to molecular ion of compound **2.13** which is appeared as base peak exhibited the di-*tert*-butyl phenol cation at m/z= 205 as well the carbocation m/z= 233 which is apart of the hydrazones structure. Scheme 3.12 demonstrated the pattern of these fragmentations.



Scheme 3.12:- Fragmentation of EIMs of compound 2.13.

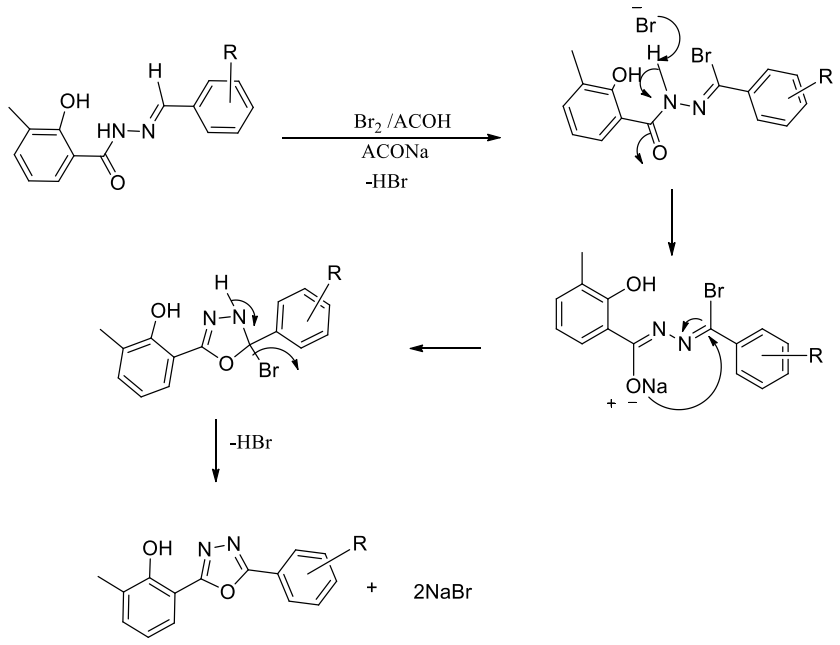
3.1.3 Synthesis of 6-(5-(aryl)-1,3,4-oxadiazol-2-yl)-2-methylphenol (2.14-2.22).

Nine of newly 1,3,4-oxadiazole were synthesized by two methods. The first method was carried out by cyclization of the hydrazones in the presence of bromine and sodium acetate in glacial acetic acid.as depicted in Scheme 3.13



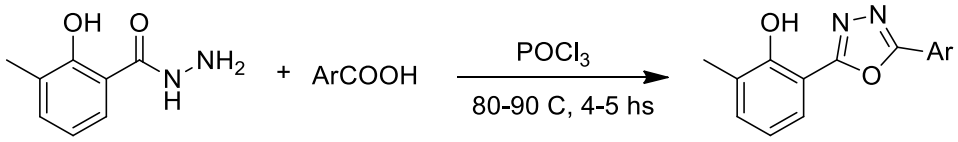
Scheme 3.13:-Synthesis oxadiazole using Br_2 as oxidizing agent

The proposed mechanism for this cyclization was carried out in two steps. The first step was bromination of the imines group in hydrazone. The bromination of arylidene aryl hydrazones have been comprehensively investigated by Chattaway and Walker [165] and many researchers reported similar investigation in the literatures.[166, 167] The second step is the cyclization, where sodium acetate plays an important role. Scheme 3.14 demonstrated the proposed mechanism.



Scheme 3.14:-Proposed mechanism of cyclization of the hydrazone.

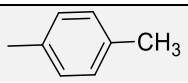
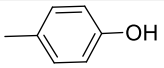
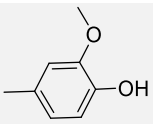
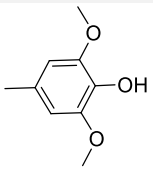
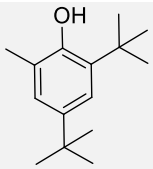
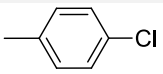
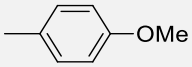
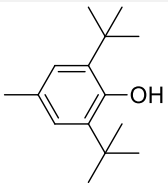
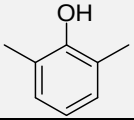
The second method was from cyclization of the acid hydrazid **2.2** with the appropriate aryl carboxylic in the presence of $POCl_3$ as displayed in Scheme 3.15.



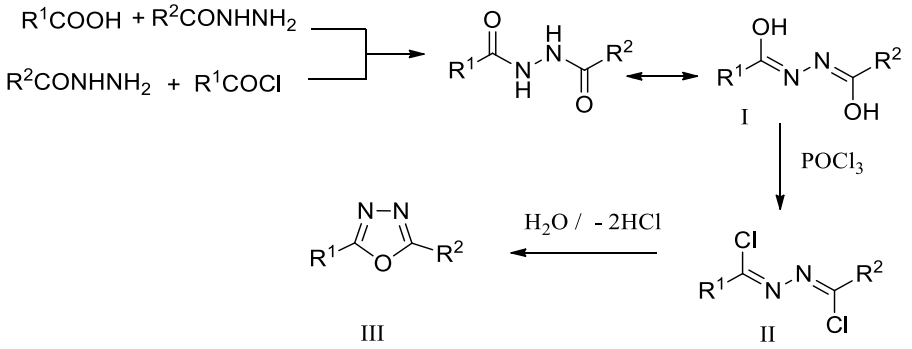
Scheme 3.15:-Synthesis oxadiazole in presence of $POCl_3$ as dehydrating agent.

The aryl and some selective physical properties of the 6-(5-Aryl-1,3,4-oxadiazol-2-yl)-2-methylphenol were tabulated in Table 3.3

Table 3.3:-Experimental data of the synthesized oxadiazole.

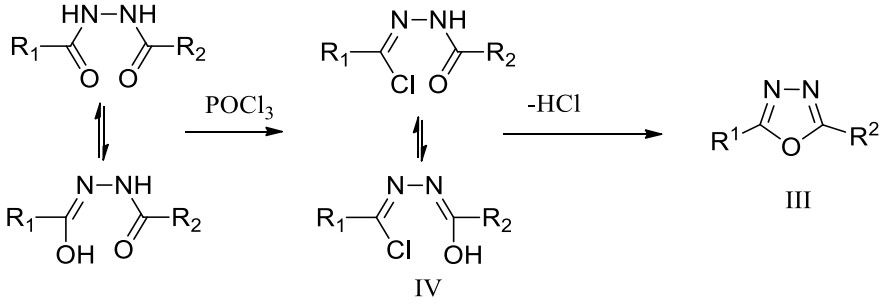
No.	Ar	Yield %	m.p. ˚C	M.F	EIMs Calc. $M^{\cdot+}$	EIMs Found
2.14		73	158-160	$C_{16}H_{14}N_2O_2$	266.11	266
2.15		75	224-227	$C_{15}H_{12}N_2O_3$	268.08	268
2.16		78	110-115	$C_{16}H_{14}N_2O_4$	298.10	298
2.17		84	122-125	$C_{17}H_{16}N_2O_5$	328.11	328
2.18		72	225-227	$C_{23}H_{28}N_2O_3$	380.21	379
2.19		79	171-173	$C_{15}H_{11}ClN_2O_2$	286.05	286
2.20		71	161-164	$C_{16}H_{14}N_2O_3$	282.10	282
2.21		73	165-168	$C_{23}H_{28}N_2O_3$	380.21	379
2.22		61	280 dec.	$C_{16}H_{14}N_2O_3$	282.10	282

The mechanism of cyclization by carboxylic acid and hydrazide with diacyl or aryl hydrazine in presence of phosphorus oxychloride to form 1,3,4-oxadiazole ring passed by several suggestions. Before 1969, it was assumed to take place by the formation of the intermediates II, α,α'-dichloroazines,[168, 169] as shown in Scheme 3.16.



Scheme 3.16:-The suggested mechanism of formation oxadiazole through formation of α,α'-dichloroazines.

In 1969 Levin *et al*. [170] refuted this mechanism. They found that α,α'-dichloroazines (synthesized from a different method) was dissolved in distilled water, it did not give the corresponding oxadiazole. For that, they proposed a different mechanism , which is still in use until today.[171] Scheme 3.17 demonstrates Levin *et al* proposed mechanism.

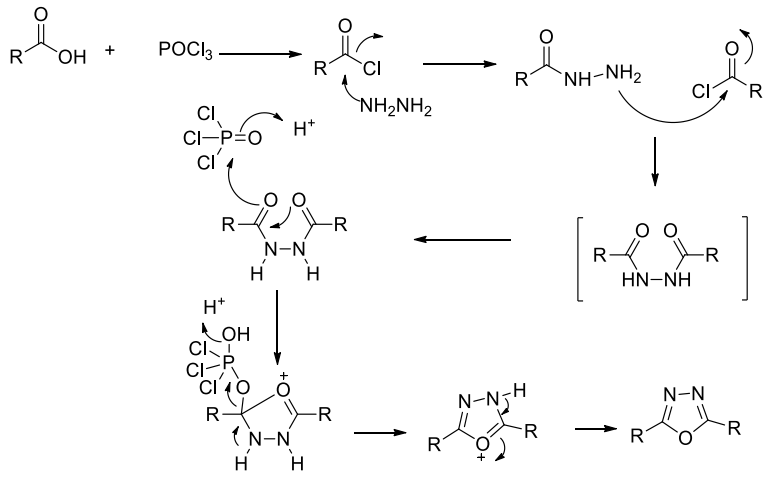


Scheme 3.17:-Levin *et al* suggested mechanism of reaction $POCl_3$.

However, this mechanism has some weak points as mentioned above, cyclization of the carboxylic acid with hydrazide or diacyl, aryl hydrazine to form the oxadiazole usually employed dehydrating agents. The $POCl_3$ is known as dehydrating agent rather than a chlorinating agent, [172, 173].

Furthermore, this cyclization could be occurred in the presence of an alternative dehydrating agent such as concentrated H_2SO_4 or PPA, P_2O_5 and trifluroacetic acid.[136] This fact elucidates that the intermediate α-chloro-alkylideneacyl hydrazine is not a requirement to complete the cyclization. Moreover, the behaviour of $POCl_3$ in most reactions is different from the other chlorinating agents. For example, thionyl chloride could react with alcohols to obtain alkyl chloride, while $POCl_3$ gives an alkene in elimination reaction (E_2) [173]. $SOCl_2$ or PCl_3 could react with carboxylic acid to form acid chloride, while $POCl_3$ will not produce the acid chloride and only the sodium salt of carboxylic acid can form acyl chloride.[174]. In addition to, the dehydration of amides to nitriles in the presence of $POCl_3$ is well known in the literature and it is not included in the chlorination.[175].

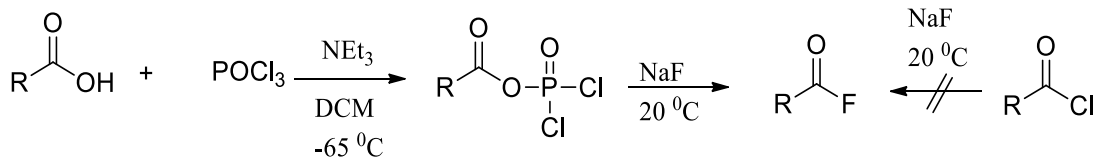
Bentiss and Lagrenée[176] synthesized symmetrical 2,5-disubstituted 1,3,4-oxadiazole by reacting carboxylic acid with hydrazine dihydrochloride. They proposed a different mechanism as shown in Scheme 3.18.



Scheme 3.18:-Proposed mechanism for 1,3,4-oxadiazole formation[176].

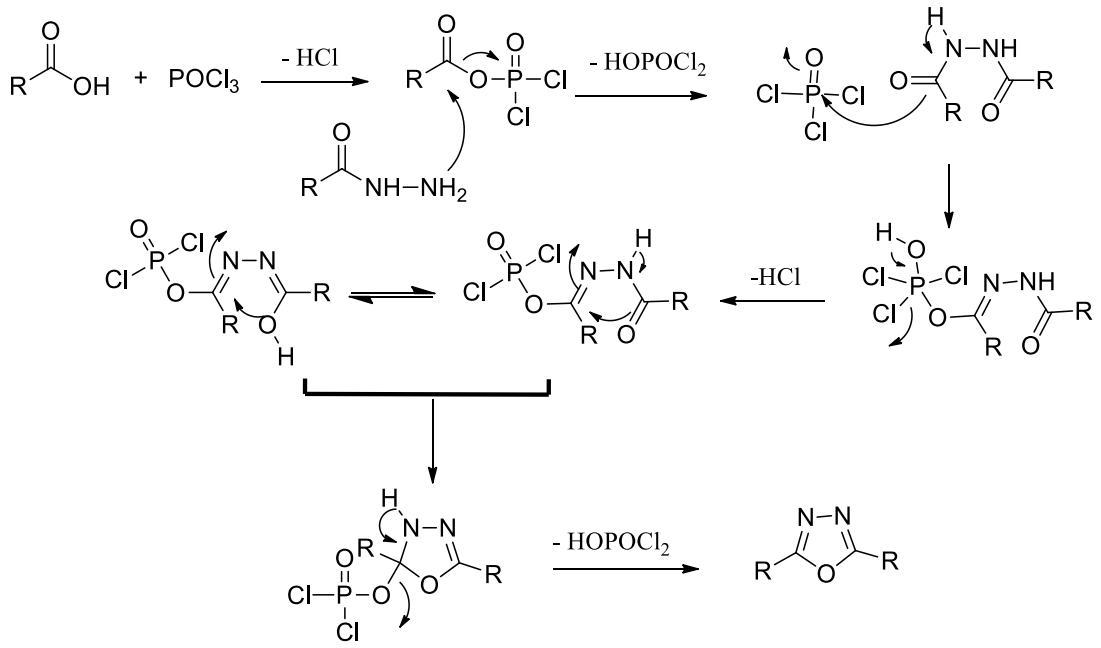
Even, this proposed mechanism did not contain the α-chloro-alkylideneacyl hydrazine. However, the reaction of carboxylic acid with $POCl_3$ did not lead to acetyl chloride as mentioned earlier. In 1978,

Effenberger *et al*.[177] were able to isolate the intermediates of dichlorophosphates .They observed that its intermediate can react with sodium fluoride to give acid fluoride, while, reacting acid chloride with NaF did not lead to afford the corresponding acid fluoride as demonstrated in Scheme 3.19.



Scheme 3.19:-Reaction of $POCl_3$ with carboxylic acid.

According to these observations, a new mechanism had proposed as demonstrated in Scheme 3.20.



Scheme 3.20:-New suggested mechanism of cyclization in the presence of $POCl_3$.

From our observations, the 1,3,4-oxadiazole did not form when one equivalent of $POCl_3$ was used. This reaction needs at least two equivalents of $POCl_3$ to afford the oxadiazole ring. Furthermore, an excess of $POCl_3$ (four or more equivalents) did not affect the yield. Even though, we agree with Bentiss and Lagrenéeinone, that dichlorophosphate ion ($^-OPOCl_2$) will be better leaving group than chloride ion Cl^-., we assume that the cleavage

of $^{-}OPOCl_2$ from carbon sp^3 was more favourable than sp^2; which is known to be inefficient when compared to a leaving group on sp^3 hybridized carbon.[178]

3.1.3.1 Characterization of 6-(5-Aryl-1,3,4-oxadiazol-2-yl)-2-methyl phenol 2.14-2.22

The newly compounds were characterized by IR, NMR and mass spectroscopy. The IR spectra showed the OH group at (3471-3120) cm^{-1}, ($CH_{aliphatic}$) at (2987-2746) cm^{-1}, ($CH_{aromatic}$) at (3097-3008) cm^{-1} and C=N at (1622-1610) cm^{-1}. The ^1H NMR spectra exhibited that the HC=N and the NH peaks were disappeared after cyclization of the hydrazones. The NMR spectra of compounds, which synthesized from cyclization the hydrazide with the carboxylic acid exhibited disappearing of the NH, NH_2 and OH of carboxylic acid. Furthermore, the protons of the 2-methylphenol group appeared at their expected area. The ^1H NMR spectra displayed the methyl group at (2.03-2.28) ppm, integrating for three protons, while in compound **2.22** appeared at 2.24 ppm integrating for six protons. As well the protons of 5-ary group of the 1,3,4-oxadiazoe appeared in their expected region. The *para*-methyl group of compound **2.14** appeared at 2.2 ppm as singlet peak as depicted in Figure 3.13.

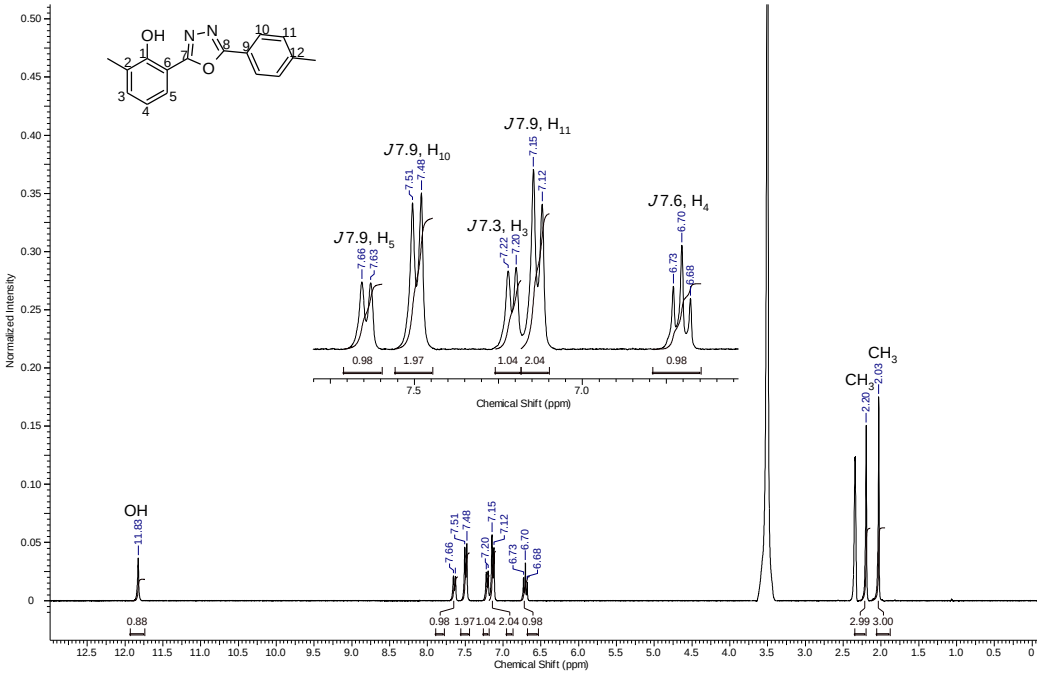


Figure 3.13:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.14

The *para* hydroxyl of compound **2.15** appeared at 12.79 ppm, ^{1}H NMR of compound **2.16** showed singlet at 3.72 ppm, integrating for three protons disclosed to 3-methoxyl. The multiplet at 6.73 ppm, integrating for two protons disclosed the H_4 and H_{10}. The H_{13} and H_{14} appeared at 7.23 ppm and 7.67 ppm respectively, as shown in Figure 3.14.

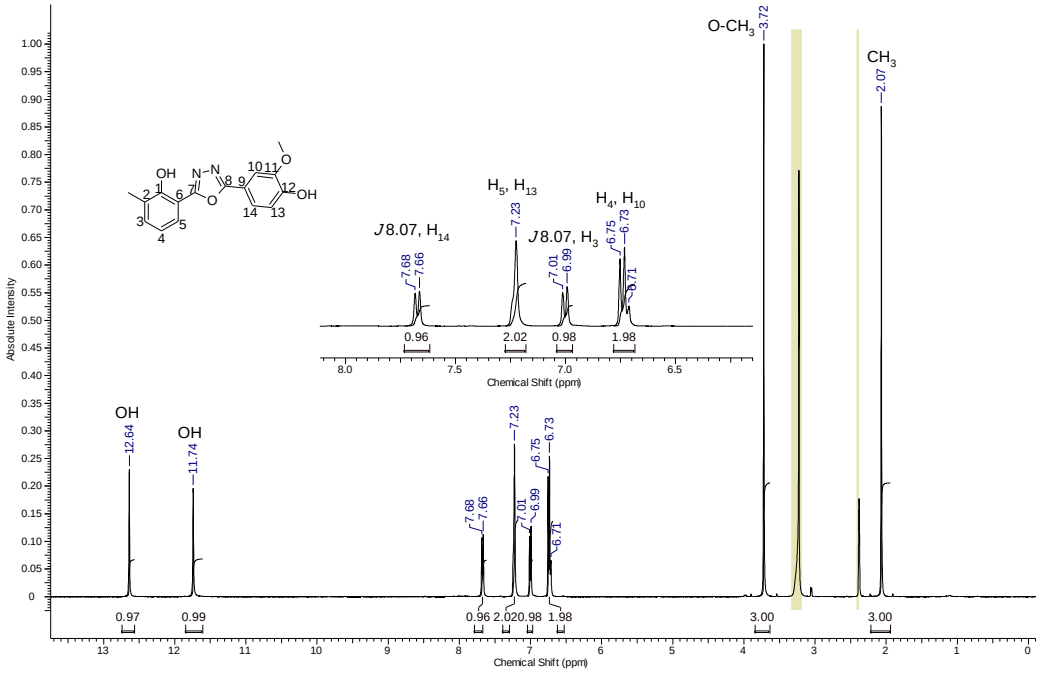


Figure 3.14:-^{1}H NMR (400MHz, DMSO-d_6) of compound 2.16

Compound **2.17** exhibited singlet peak at 3.69 ppm for two methoxy groups. compound **2.21** showed singlet at 1.45 ppm for two *tert*-butyl groups integrating for 18 H, while the two *tert*-butyl groups for compound **2.18** exhibited at two singlet 1.26 ppm and 1.38 ppm. For more details, see Appendix B.

The ^{13}C NMR spectra displayed two characteristic peaks at 161.68-164.34 ppm and 162.28-165.65 ppm belonged to C_7 and C_8 of the oxadiazole ring. The methyl of the 2-methylphenol of compound **2.14** appeared at 15.26 ppm while the 4-methyl appeared at 20.83 ppm. The carbon bearing the *para* hydroxyl group of compound **2.15** was appeared at 159.78 ppm. The spectrum of compound **2.16** show two singlets at 56.03 ppm and 56.06 ppm attributed for two methoxy groups of compound **2.17**. The six methyl of 3,5-di-*tert*-butyl group of compound **2.21** appeared as single peak at 29.92 ppm (Figure 3.15) whereas the six methyl of 2,4-di-*tert*-butyl of compound **2.18** appeared as two single peaks at 29.12 ppm and 31.13 ppm.

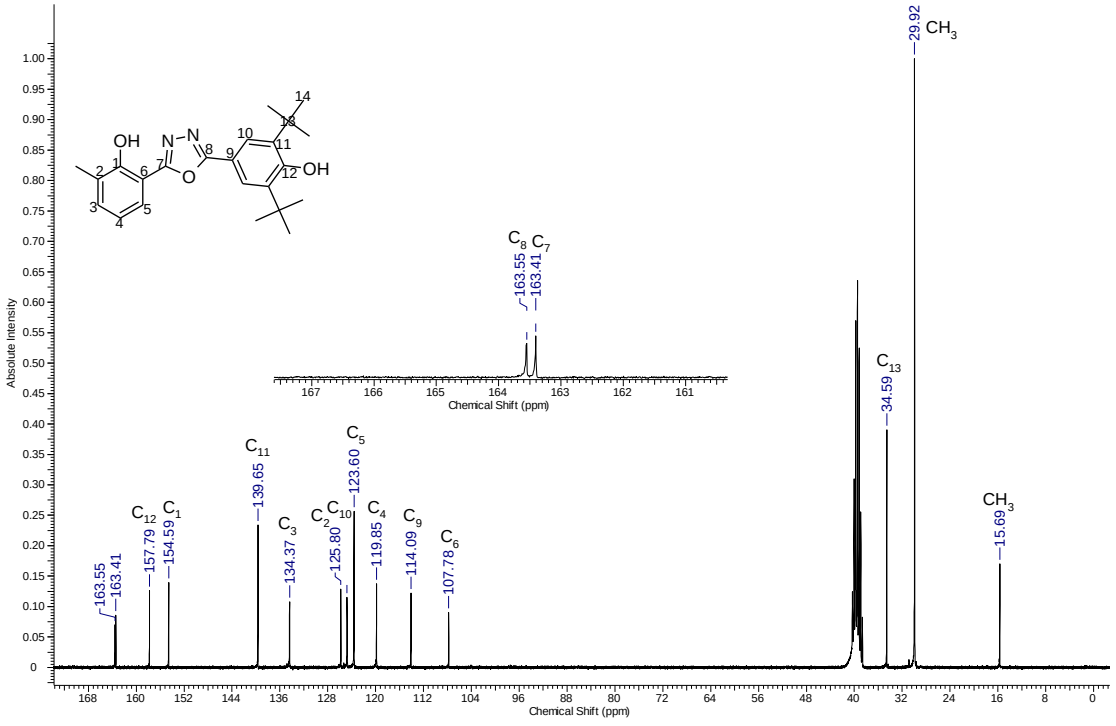
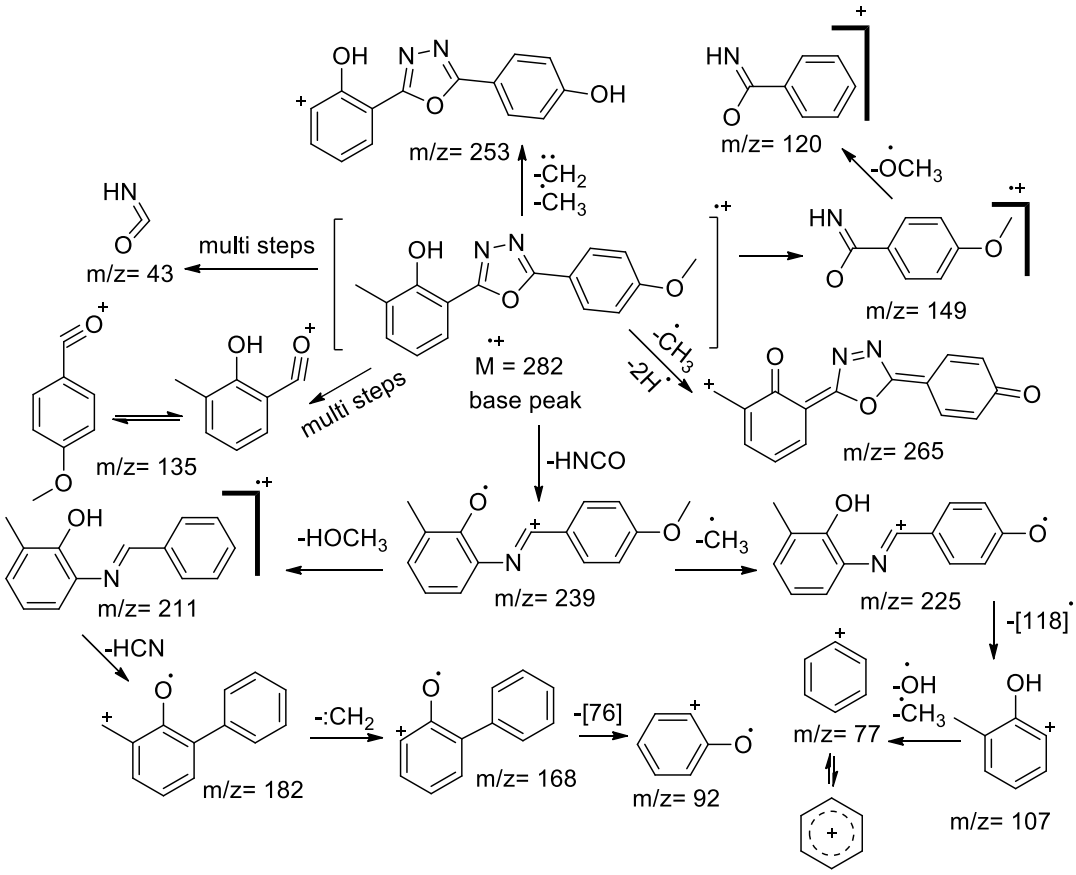


Figure 3.15:- **^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.21**.

The values molecular ion, base peak and their fragmentation confirmed the structures of the newly synthesized oxadiazole by mass spectroscopy.

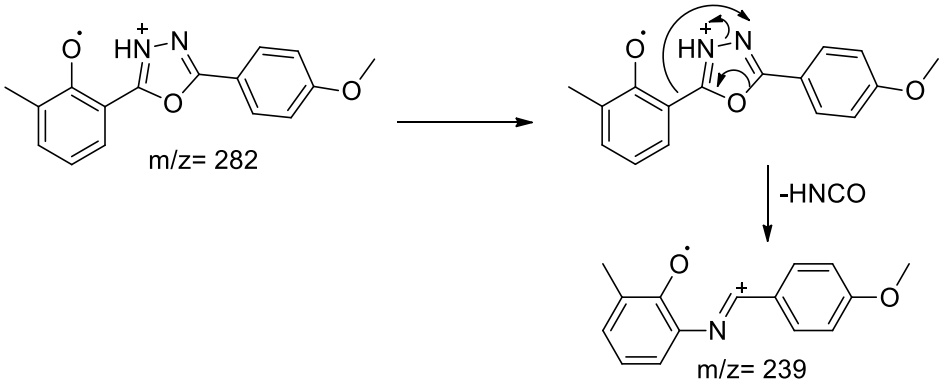
The EIMs spectra confirm the molecular ion of the synthesized compounds as well showed the base peak. All fragments express the part of the structure (Appendix C). Compounds **2.18** and **2.21** showed the molecular ion as carbocation (M^+) while the rest compounds the molecular ions appeared as radical cation ($M^{\bullet+}$**)**. The fragmentations of compound **2.20** and **2.21** have been taken as an example. Scheme 3.21 demonstrated the fragmentation of compound **2.20**.



Scheme 3.21:-Fragmentation of EIMs of compound 2.20.

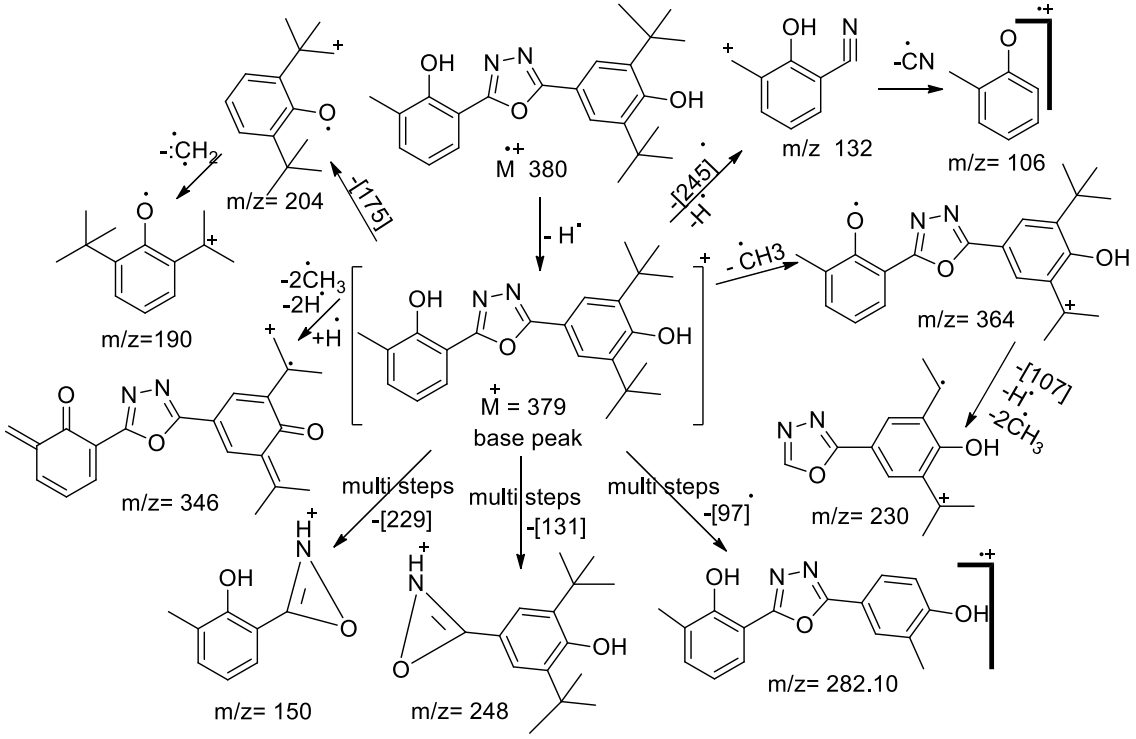
Compound **2.20** exhibited interested fragmentation by losing isocyanic acid from molecular ion. Frański, R *et al*.[164, 179, 180] reported losing isocyanic acid from M+1 of the 1,3,4-oxadiazole, while R M. Shakir

[95] reported losing isocyanic acid from the molecular ion. In our case, the losing isocyanic acid was found losing from molecular ion itself. The proposed mechanism of losing HNCO is demonstrated in Scheme 3.22.



Scheme 3.22:-Mechanism of losing HNCO from oxadiazole derivative.

As well as, compound **2.21** was taken as example and its fragmentation patron is demonstrated at scheme 3.23.

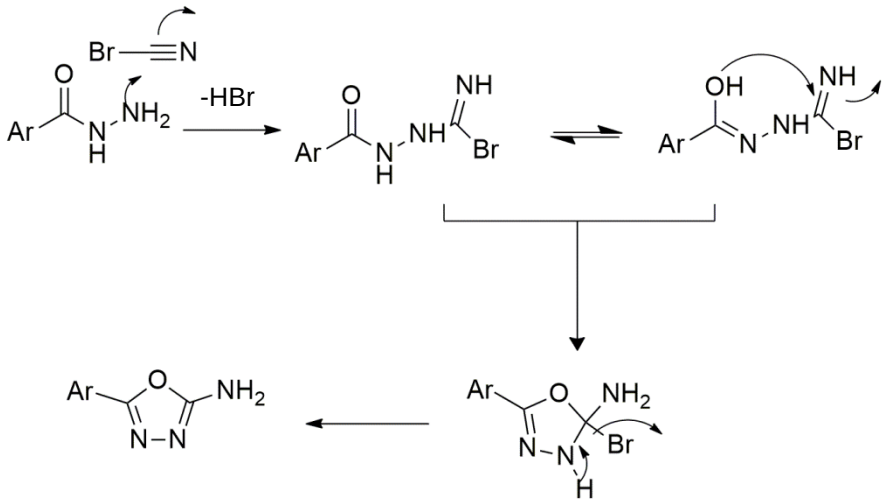


Scheme 3.23:-Fragmentation of EIMs of compound 2.21.

The two fragmentations at m/z= 150 demonstrated the 2-methylphenol attached fragment of oxadiazole while m/z= 248 demonstrated the 2,6-di-*tert*-butylphenol attached fragment of oxadiazole.

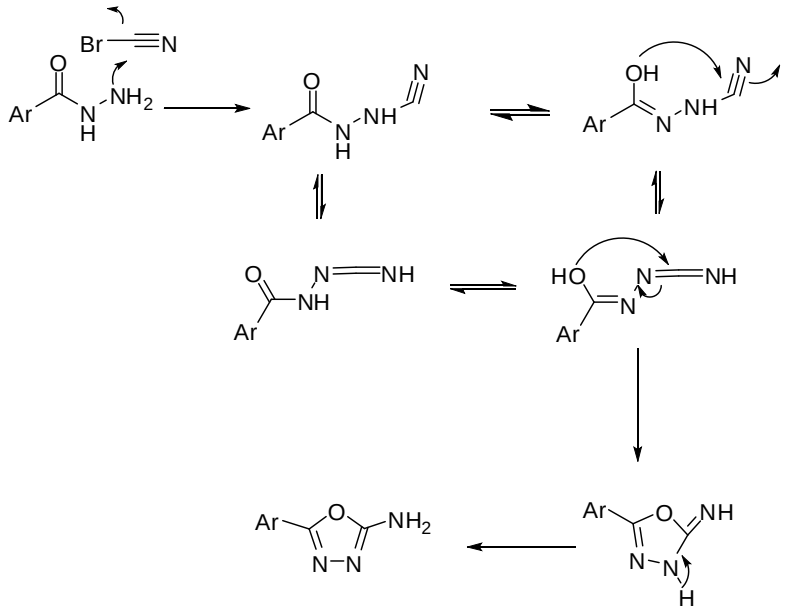
3.1.4 Synthesis of 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol.

6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol **2.23** was synthesized by reaction of the methanolic solution of 2-hydroxy-3-methylbenzohydrazide **2.2** with cyanogen bromide in the presence of sodium hydrogen carbonate as scavengers at ambient temperature. We proposed two mechanisms for the formation of this ring. Scheme 3.24 demonstrated the first proposed mechanism.



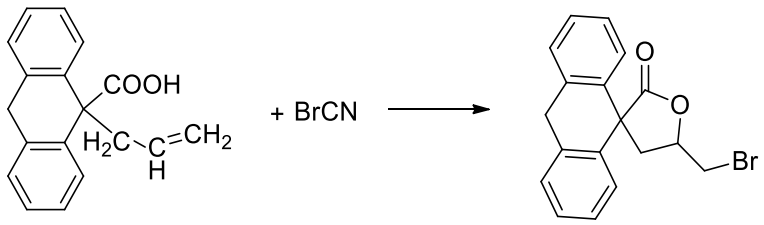
Scheme 3.24:-Suggested mechanism of formation 5-amino-1,3,4-oxadiazole.

The bromine was known as a good leaving group but complication arises when it tries to leave directly from a carbon with two π bonds. For that in this proposed mechanism we assume that the cleavage of bromine from carbon Sp^3 was more acceptable than sp^2; which is known to be inefficient when compared to a leaving group on sp^3 hybridized carbon [178]. Even though, no literatures have been reported about this mechanism yet. As such, more studies are required to confirm this proposed mechanism. The second mechanism was shown in Scheme 3.25.



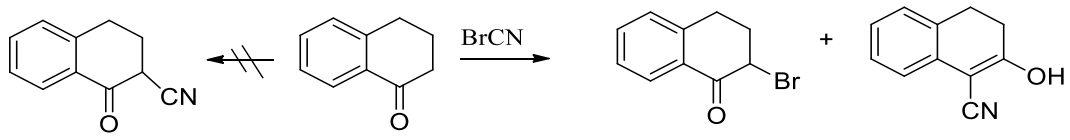
Scheme 3.25:-The second suggested mechanism of synthesis 5-amino-1,3,4-oxadiazole.

In peptide bond cleavage, Gross and Witkop[181] suggested that the nucleophile would attack the cyanide group and consequently the bromine cation will be expelled. Shaw and Adams.[182] reported the same observation. Vinod [183] synthesized cyanamide and dicyanamides by reacting BrCN with primary and secondary amines. However, other researchers reported different behaviours for reaction involving cyanogen bromide. In 1953, Arnold *et al.*[184] reported that a double bond in the presence of BrCN will involve in iodination reaction and cyclization reaction as illustrated in the following Scheme 3.26.



Scheme 3.26:-Different behaviors for reaction cyanogen bromide.

Whereas, Parfitt[185] reported that the reaction of BrCN with 1-tetralone gave two products and that did not include the addition of cyanide ion and bromide cation as leaving group (Scheme 3.27)



Scheme 3.27:-Reaction of cyanogen bromide.

Finally, Kandeel *et al*.[186] reported the cyclization of cyanogen bromide could not include a direct addition CN ion. For these observations, and the difference in behavior of BrCN interactions, two mechanisms were proposed for the formation of 5-aminooxadiazole ring

3.1.4.1 Characterization of 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.23.

The structure of compound **2.23** confirmed from their IR, ^{1}H, ^{13}C NMR and EIMs spectra. The IR spectrum showed the first evidence for success of the cyclization by disappearance of two bands for C=O at 1650 cm^{-1} and NH at 3267 cm^{-1} for the hydrazide **2.2**, Also from raising new band of C=N at 1610 cm^{-1}. The NH_2 and OH peaks appeared at (3384-3124) cm^{-1}. Beside the C=C band at (1591-1473) cm^{-1}.

The ^{1}H NMR spectrum also was in agreement with IR results. The protons of methyl group for methyl phenol group appeared at 2.23 ppm as singlet peak. The aromatic proton appeared at 6.93 for H_4 as triplet with *J* 7.21, H_3 at 7.27 ppm as multiplet, and H_5 at δ 7.41 ppm with *J* 7.46 as doublet. The interesting peak of NH_2 appeared at 7.50 ppm with integral equal two protons. ^{13}C APT spectrum of compound **2.23** showed for positive peaks at 16.07, 120.09, 123.40, and 133.46 for CH_3, C_4, C_5, and C_3 respectively. Besides, the positive peaks of the APT displayed the negative

peaks at 108.50, 125.87, 154.33, 158.27 and 163.32 for C_6, C_2, C_1, C_7 and C_8 respectively as depicted in Figure 3.16.

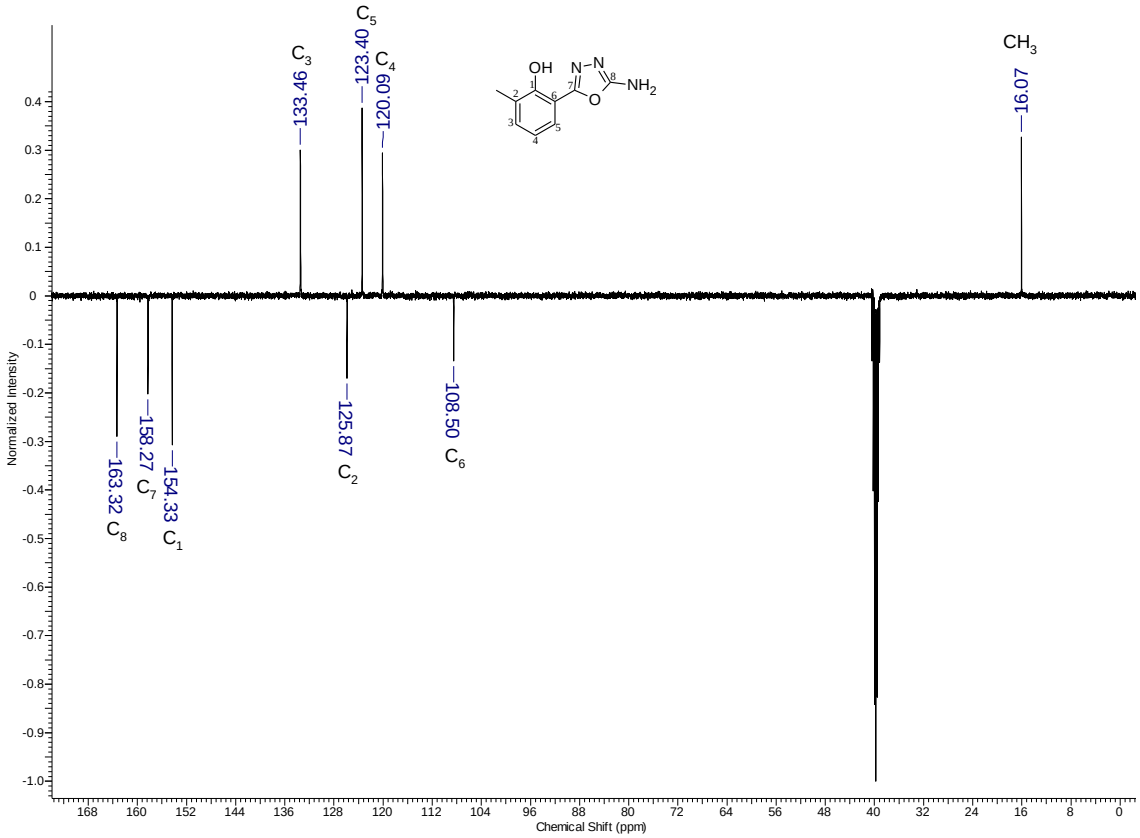
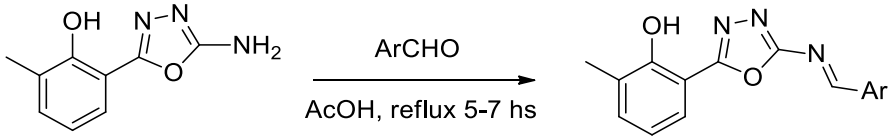


Figure 3.16:- ^{13}C APT (300 MHz, DMSO-d_6) of compound 2.23

3.1.5 Synthesis and characterization of 6-(5-(substitutedbenzyl ideneamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol (2.24-2.25).

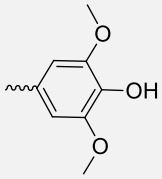
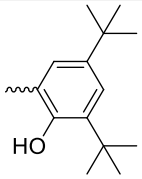
Two newly Schiff bases of 6-(5-amino-1,3,4-oxadiazol-2-yl)-2-methylphenol were synthesized from reaction compound **2.23** with 3,5-dimethoxy-4-hydroxy benzaldehyde (syringaldehyde) and 3,5-di-*tert*-butyl-2-hydroxy benzaldehyde in acetic acid as shown in Scheme 3.28.



Scheme 3.28:-Synthesis of 6-(5-(substitutedbenzylideneamino)-1,3,4-oxadiazol-2-yl)-2-methylphenol.

The products were purified by crystallization to afford good yield the Aryl group and some physical properties were tabulated it Table 3.4.

Table 3.4:-Physical properties of Schiff bases

No	Ar	Yield %	M.P. ºC	MF	EIMs Cal.	EIMs Exp.
2.24		78	218-220	$C_{18}H_{17}N_3O_5$	355.12	355
2.25		76	204-206	$C_{24}H_{29}N_3O_3$	407.50	407

These compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectra besides to EIMs. The IR spectra showed disappearance of the NH_2 at 3384 cm^{-1} and appearance of new peak at 1649 cm^{-1} for compound **2.24** and 1666 cm^{-1} for compound **2.25** which attributed to C=N exo imine [187], while the endo imine appeared at 1610 cm^{-1} and 1603 cm^{-1} for compounds **2.24** and **2.25** respectively. The ^{1}H NMR of compound **2.24** exhibited the three protons of 2-methyl phenol group at their expected area. Furthermore, the NMR spectrum exhibited the protons of the imine group (HC=N) at 8.51ppm. The six protons of two methoxy groups were appeared at 3.88 ppm and the two protons of H_{11} appeared as multiplet due to over lab with proton H_3, the C_1-OH and C_{13}-OH were appeared at 9.78 ppm and 9.19 ppm, as depicted in Figure 3.17.

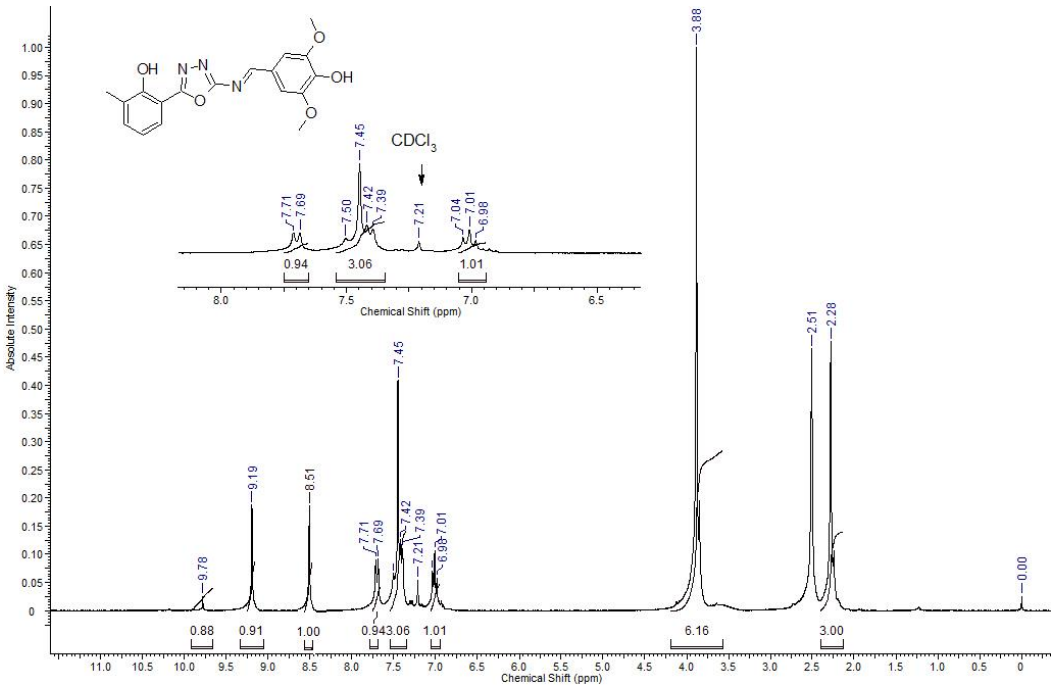


Figure 3.17:-^{1}H NMR (300MHz, DMSO-d_6 and $CDCl_3$) of compound 2.24.

The NMR spectrum of compound **2.25** showed the eighteen protons for two di-*tert*-butyl group at 1.22 ppm and 1.31 ppm as two singlet peaks, the methyl group for 2-methyl phenol appeared at 2.17 ppm. The five aromatic protons were appeared as multiplet peaks. The proton of imine group was appeared as singlet peak at 8.62 ppm. The two hydroxyl groups appeared at 9.91 ppm and 11.63 ppm for C_{11}-OH and C_1-OH group respectively as depicted in Figure 3.18.

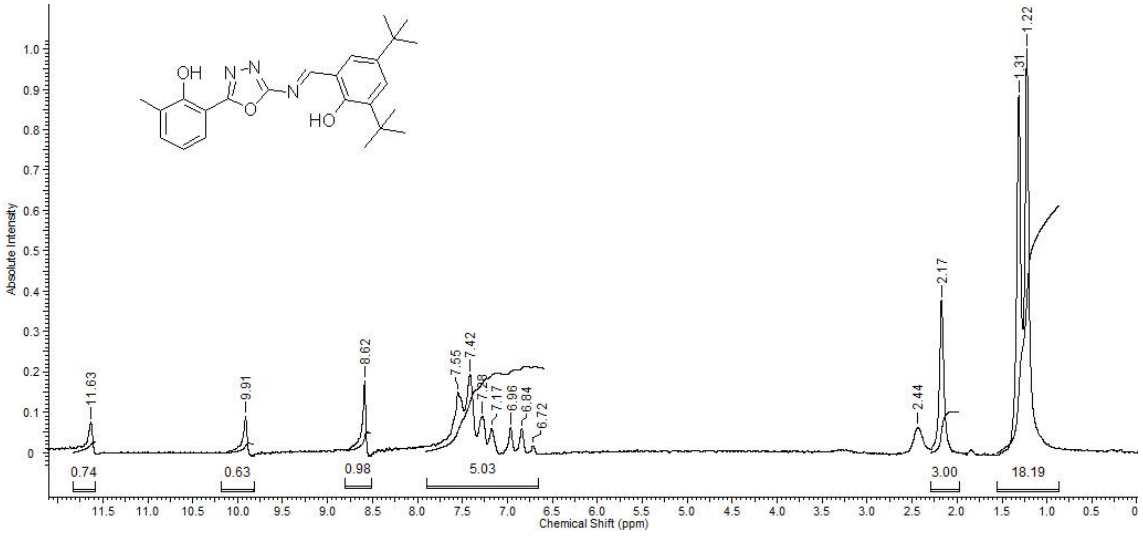


Figure 3.18:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.25.

The ^{13}C NMR spectra of these compounds displayed all expected carbon such as the methyl group at 15.65 ppm and 15.60 ppm for compounds **2.24** and **2.25** respectively. The two methoxy groups for compound **2.24** were appeared at 56.07 ppm. The aromatic carbons attached with OCH_3 appeared as one signal at 148.19 ppm and the aromatic carbon attached with hydroxyl group appeared at 154.79 ppm. The two C=N for the oxadiazole ring appeared at 165.89 ppm for C_7 and 169.70 ppm for C_8, while the carbon of HC=N group appeared at 165.37 ppm (see the appendix B).

The spectrum of compound **2.25** displayed six methyl group for two of the di-*tert*-butyl group at 29.08 ppm and 31.03 ppm while, the quaternary carbons for this group (di-*tert*-butyl) appeared at 33.97 ppm and 34.53 ppm, moreover the carbons attached the *tert*-butyl group appeared at 136.44 ppm and 141.33 ppm, as depicted in Figure 3.19.

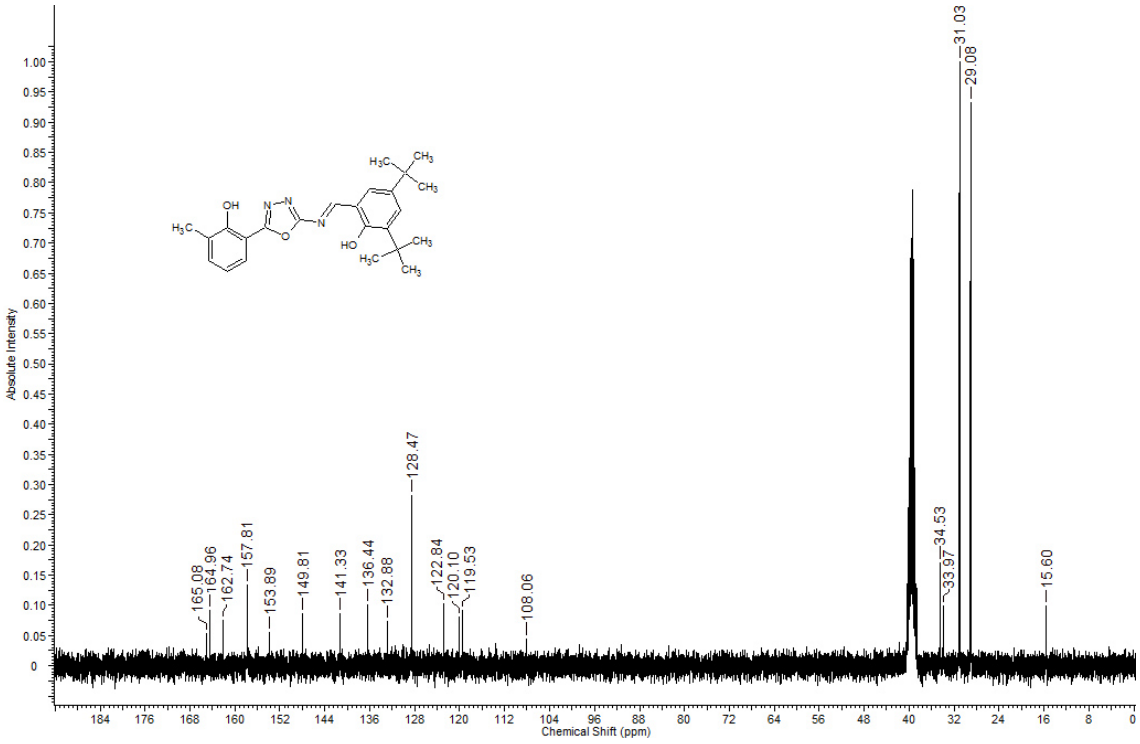
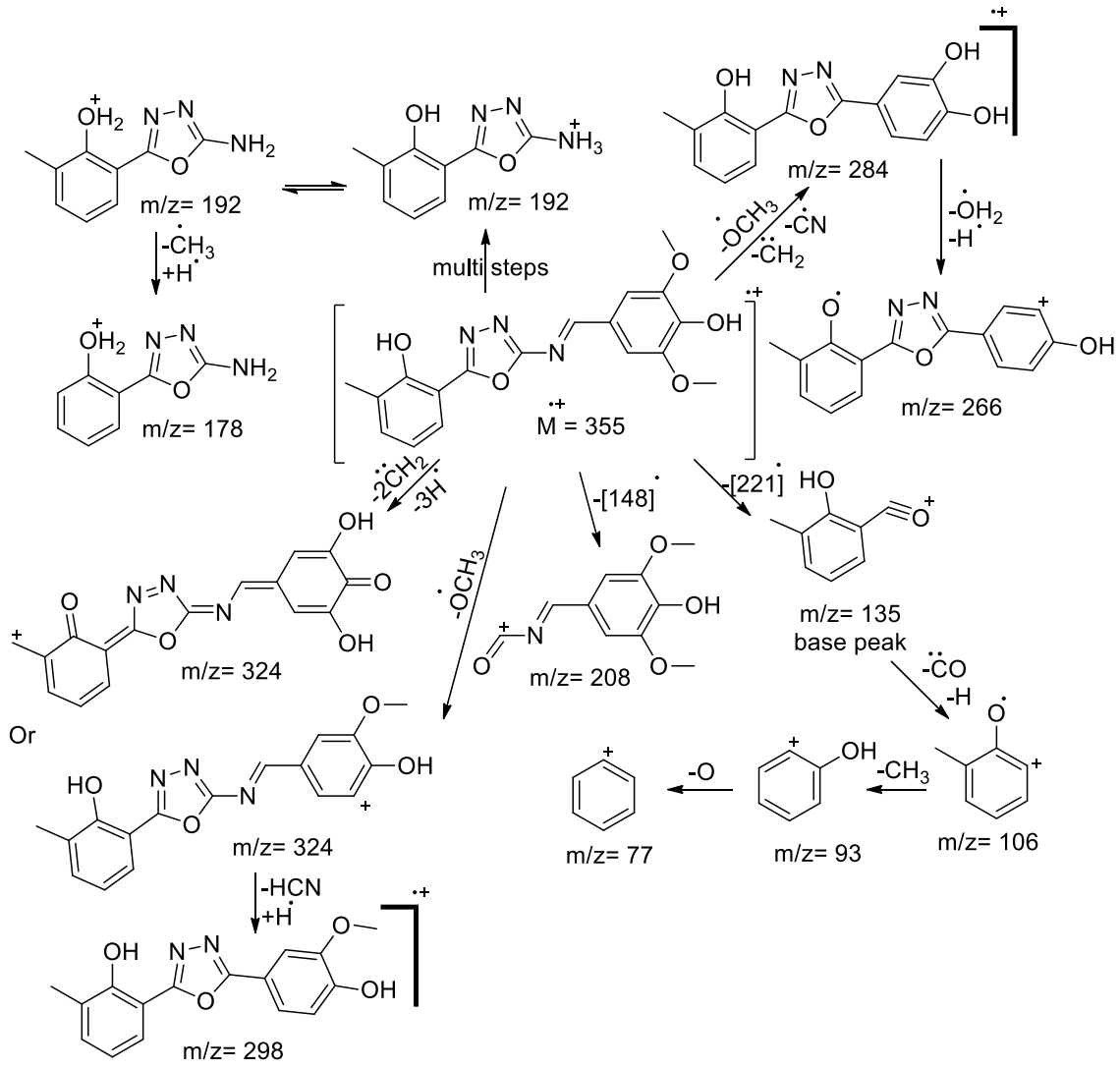


Figure 3.19:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.25.

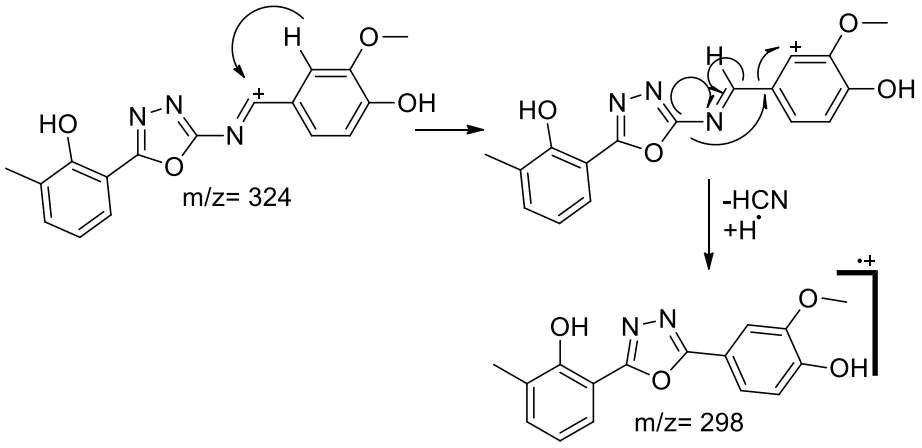
Besides to the IR, 1D NMR the mass spectroscopy confirms the structure of the oxadiazole amine derivatives. For instance, the EIMs for

compound **2.24** showed the molecular ion at $M^{\bullet+}$= 355 and the base peak at m/z= 135. All the rest fragments confirm the structure as displayed in Scheme 3.29.



Scheme 3.29:-Fragmentation of EIMs of compound 2.24.

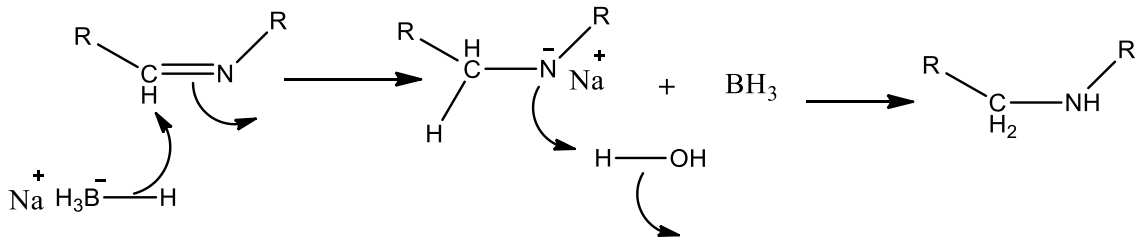
Losing HCN from carbocation m/z= 324 and convert to radical cation m/z= 298 one of the most interested fragments in this compound. The proposed mechanism of this fragment is demonstrated in Scheme 3.30.



Scheme 3.30:-Proposed mechanism of losing HCN from the oxadiazoe amine derivatives.

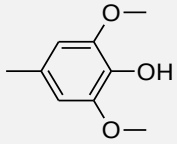
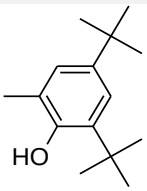
3.1.6 Synthesis and characterization of 6-(5-(substitutedbenzyl amino)-1,3,4-oxadiazol-2-yl)-2-methylphenol 2.26-2.27

Two newly compounds were synthesized from reduction of compound **2.24** and **2.25** by using excess of sodium borohydride ($NaBH_4$) in 1:1 methanol : THF. The aryl group and some physical properties were tabulated in Table 3.5.The general mechanism was demonstrated in Scheme 3.31.



Scheme 3.31:-The general mechanism for a $NaBH_4$ reduction.

Table 3.5:-Physical properties of reduction compounds.

No	Ar	Yield %	M.P. ºC	MF	EIMs Calc.	EIMs Exp.
2.26		71	100-103	$C_{18}H_{19}N_3O_5$	357.13	357
2.27		67	195-198	$C_{24}H_{31}N_3O_3$	409.52	409

The newly compounds were characterized from their IR, ^{1}H NMR, ^{13}C NMR spectra and EIMs. The IR spectra showed new peak attributed to NH at 3342 cm^{-1} and 3339 cm^{-1} for compounds **2.26** and **2.27** respectively. The hydroxyl group of phenol appeared at range (3221-3113) cm^{-1}. The exo imine group at 1649 cm^{-1} and 1666 cm^{-1} for compounds **2.24** and **2.25** were disappeared while; the endo imine appeared at range (1618-1606) cm^{-1}. The ^{1}H NMR spectrum for compound **2.26** exhibited the six protons of two methoxy group and the two protons of $NC\underline{H}_2$ appeared at 3.87ppm, 3.91 ppm respectively, integration for eight protons. The aromatic protons appeared at 7.04 ppm as H_4 with *J* 6.78 Hz , also the multiplat peaks at 7.38 ppm for three protons H_3, $2H_{11}$ and 7.72 ppm as doublet signal with *J* 7.32 Hz of H_5. The NH and two OH groups were exhibited at 8.21 ppm, 9.22 ppm and 9.81 ppm, As depicted in Figure 3.20.

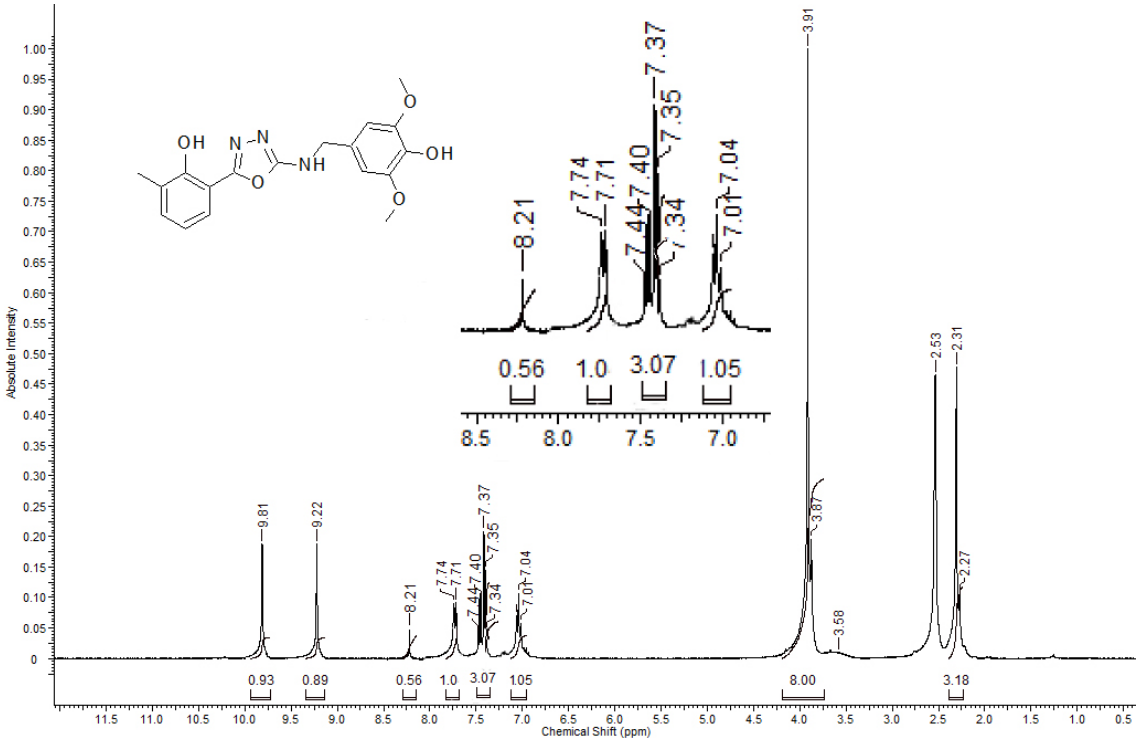


Figure 3.20:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.26

The methyl group appeared as singlet peak at 2.31 ppm with small shoulder at 2.27 ppm. This could be attributed to the formation of two geometrical isomer, which could be stabilized by hydrogen bonding as displayed in Figure 3.21

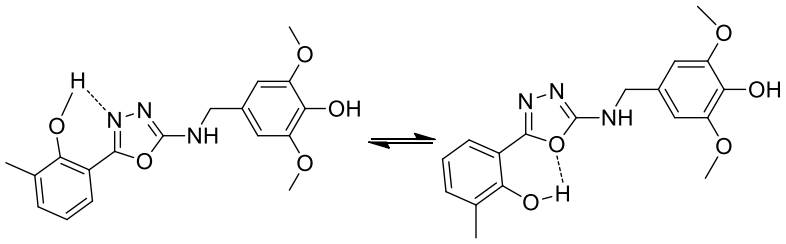


Figure 3.21:-Intramolecular hydrogen bond of compound 2.26.

The ^{1}H NMR spectrum of compound **2.27** showed two singlets peaks at 1.22 ppm and 1.29 ppm with nine protons for each peak. The two protons of NC$\underline{H_2}$ group appeared at 4.51 ppm . The five aromatic protons appeared at (6.89-6.96) ppm as H_4, 7.29 ppm as H_3, 7.40 ppm as H_5 and singlet signal at 7.52 ppm with integration for two proton as H_{13} and H_{15}.

The proton NH assigned at 6.65 ppm and the two protons of OH appeared at 9.76 ppm and 10.29 ppm respectively.

The ^{13}C NMR spectra of compounds **2.26** and **2.27** showed two clearly evidence of successful of reduction imine group by disappearing the HC=N peak at 165.37 ppm and 162.74 ppm respectively as well, rising new signal at range (59.95-62.11) ppm attributed to one carbon for $N\underline{C}H_2$ group. The two carbons of OMe for compound **2.26** appeared at 55.98 ppm while the two di-*tert*-butly groups for compound **2.27** appeared at 29.57 ppm and 31.76 ppm. The aromatic carbons appeared at their expected area as shown with ^{13}C NMR of compound **2.27**, Figure 3.22.

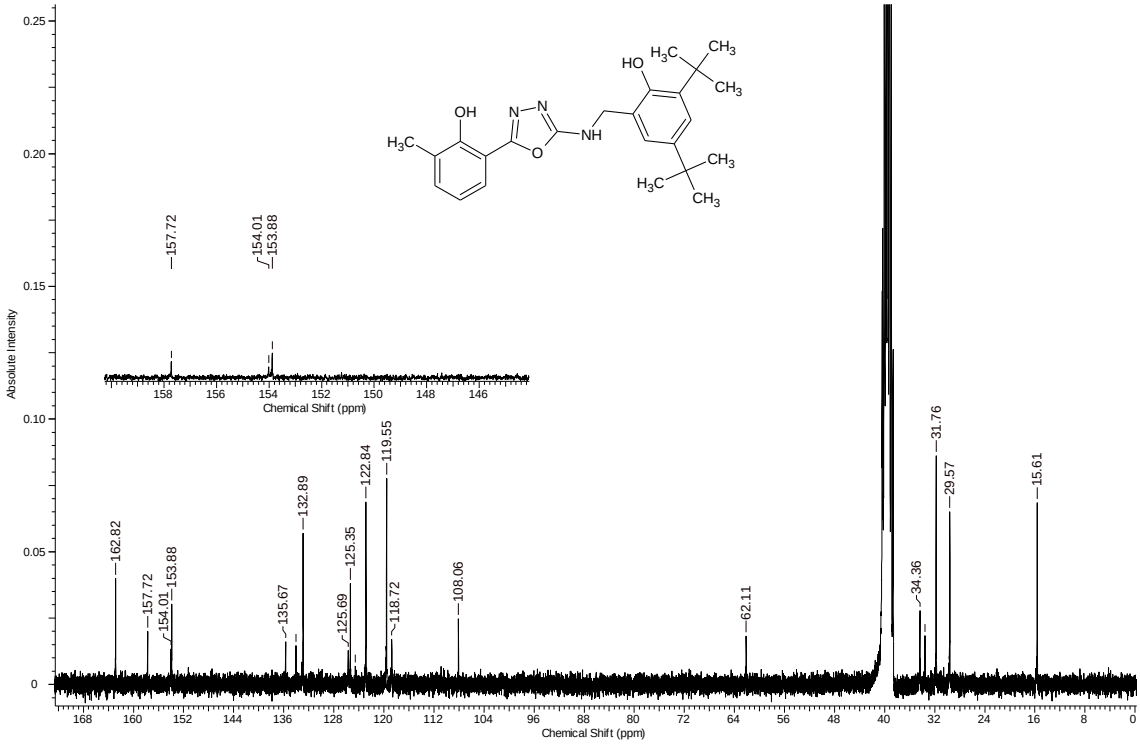
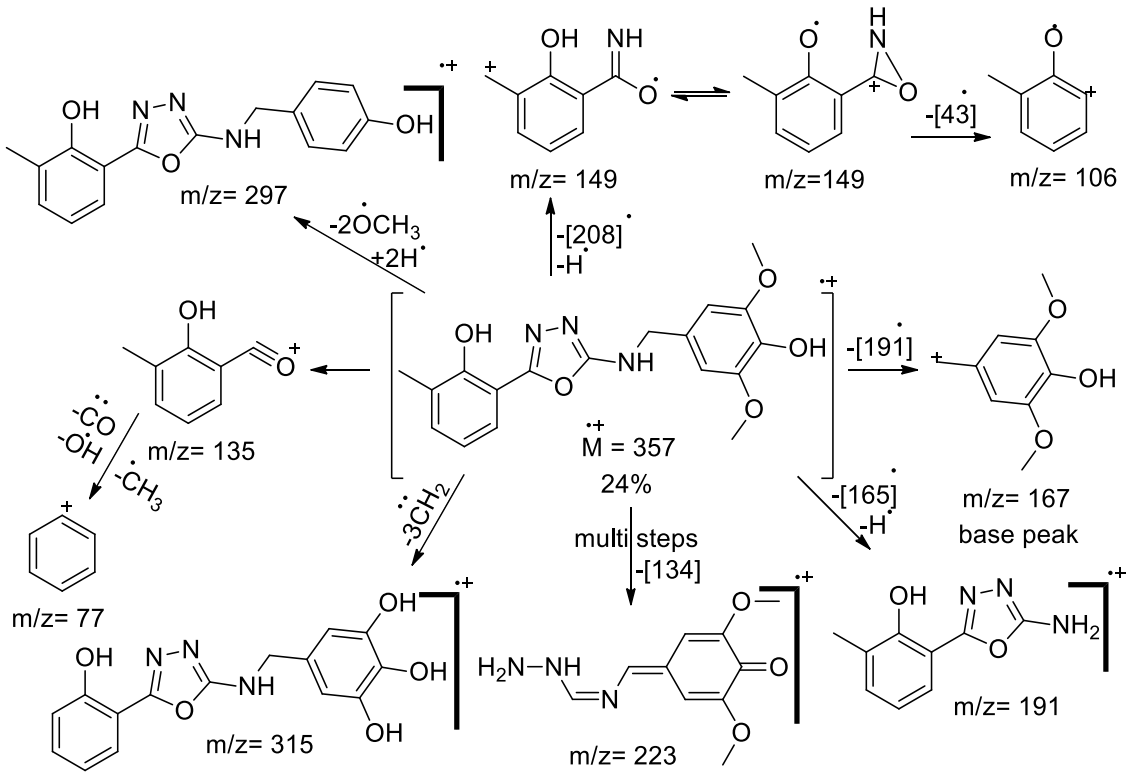


Figure 3.22:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.27.

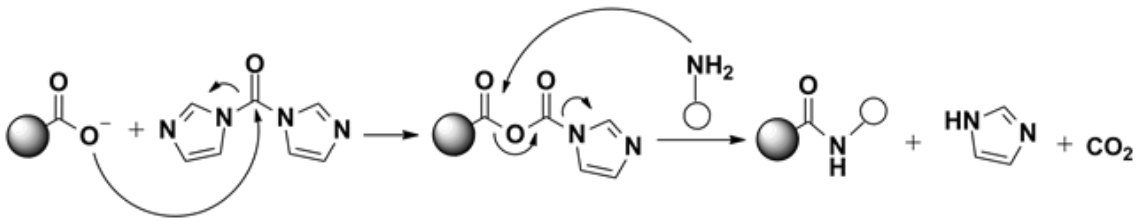
The mass spectrum of compound **2.26** which synthesized by reducing the imine group for compound **2.24** also confirm the molecular structure by exhibiting the molecular ion at $M^{\bullet+}$= 357. The base peak of these fragmentation was located at m/z= 167. Moreover, all the structures of fragments correspond to a part of this compound as shown in Scheme 3.32.



Scheme 3.32:-Fragmentation of EIMs of compound 2.26.

3.1.7 Synthesis and characterization of 6-(5-(substituted benzoamido)-1,3,4-oxadiazole-2-yl)-2-methylphenol

The direct reaction between carboxylic acid with amine is well known in organic chemistry, it could not give the corresponding amide. However, this reaction can give the amide in the presence of coupling reagent such as 1,1'-carbonyldiimidazole (CDI) and N,N'-dicyclohexylcarbodiimide (DCC). These compounds were synthesized from reaction of oxadiazole amine **2.23** with aryl carboxylic acid in the presence of (CDI) as coupling reagent. The mechanism of this reaction is well-known in literature [188] as described in this Scheme 3.33.



Scheme 3.33:- CDI coupling mechanism

The two newly compounds **2.28** and **2.29** were characterized by IR, 1D NMR and EIMs spectra. The IR spectrum showed interesting peak at 1697-1666 cm^{-1} assigned to carbonyl group of amide as well the C=N were assigned at 1622 cm^{-1} and 1606 cm^{-1} for compounds **2.28** and **2.29** respectively. Moreover, NH group appeared in both compounds at 3323 $cm^{-1}$and 3389 cm^{-1}. All these peaks are considered good evidences for successful reaction. The ^{1}H NMR recorded disappearing of the NH_2 at 7.50 ppm from resulting compounds moreover, the new peak assigned to the NH appeared at 9.50 ppm for compound **2.28** and at 9.84 ppm for compound **2.29**. The ^{1}H NMR of compound **2.28** showed the three protons of methoxy group at 3.73 ppm besides to the singlet peak for three protons of methyl group and the aromatic protons which appeared at their expected area. The two di-*tert*-butyl groups of compound **2.29** assigned at 1.42 ppm as one singlet, integration for eighteen protons. The rest protons appeared at their expected area. Figure 3.23 demonstrated the ^{1}H NMR spectrum of compound **2.29**.

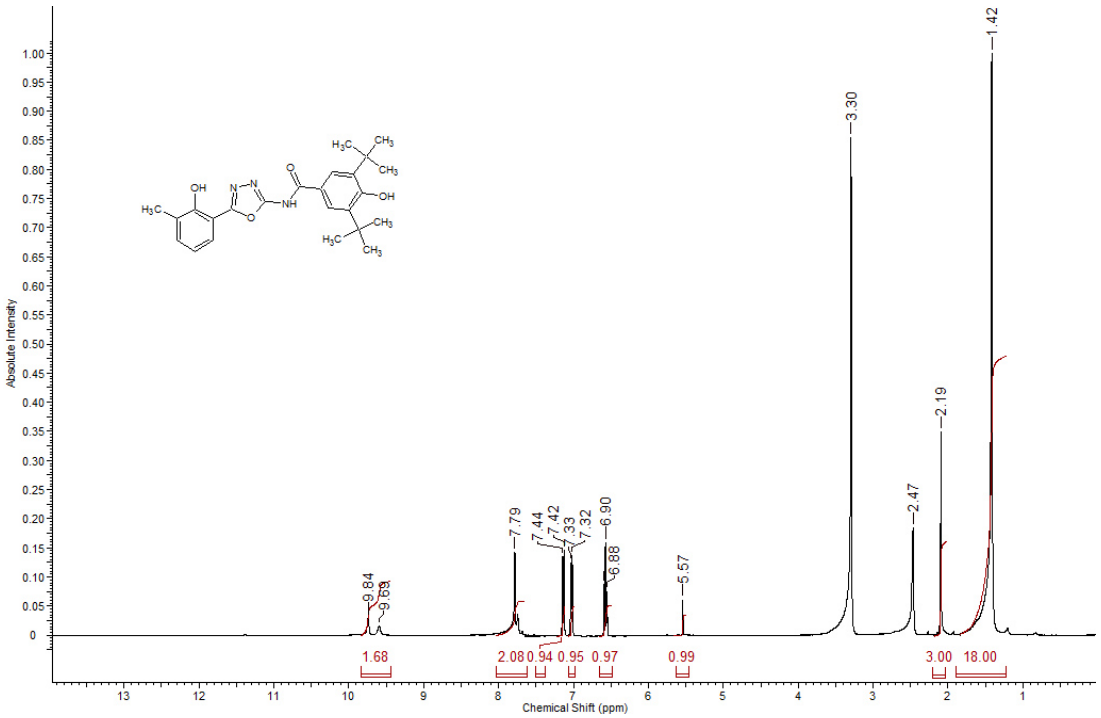


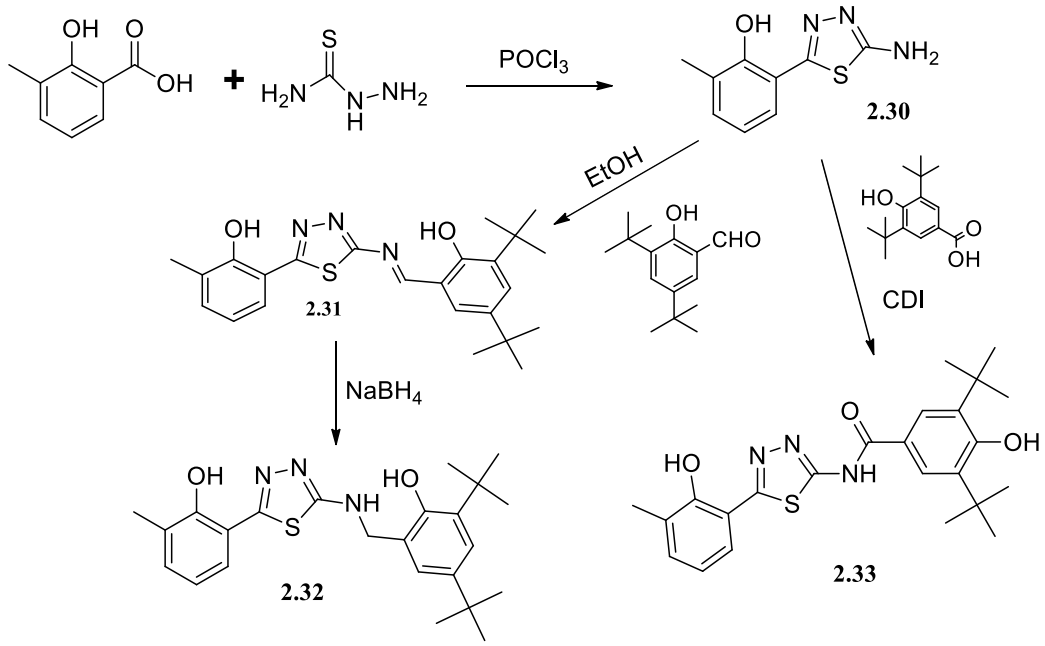
Figure 3.23:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.29.

The ^{13}C NMR of new synthesized compounds showed the carbonyl of amide at 166.61 ppm and 166.13 ppm for compound **2.28** and **2.29** respectively. The spectrum of compound **2.28** showed the 2-methyl group of phenol at 15.60 ppm, while the 4-methoxy (from carboxylic acid part) appeared at 55.24 ppm. The CH aromatic carbon appeared at 115.77 ppm, 119.53 ppm, 122.84 ppm, 128.30 ppm and 132.87 ppm, for C_{12}, C_4, C_5, C_{11}, C_3 and C_6, C_{10}, C_2, C_{13}, C_1, C_7, C_8.the quaternary carbon appeared at 108.06 ppm,125.34 ppm, 126.37 ppm, 153.88 ppm, 157.73 ppm, 162.83 ppm and 165.71 ppm respectively. The ^{13}C NMR of compound **2.29** exhibited the six-methyl of two symmetrical di-*tert*-butyl groups as singlet peak at 29.95 ppm and their quaternary appeared at 34.43 ppm the methylgroup of 2-methyl phenol appeared at 14.30 ppm. The rest peaks which are assigned to CH, C quaternary included the two C=N and the C=O were appeared at the expected area.(see Appendix B).

3.2 Formation of Thiadiazole Ring.

3.2.1 Synthesis of 6-(5-amino-1,3,4-thiadiazol-2-yl)-2-methylphenol 2.30 and their derivatives (2.31-2.33)

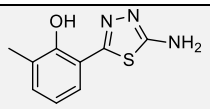
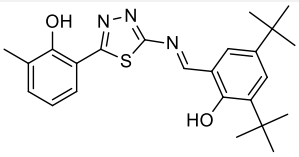
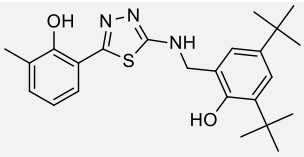
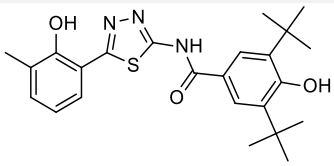
Thiadiazole ring was formed at position six by reaction of 2-hydroxy-3-methylbenzoic with thiosemicarbazide in the presence of $POCl_3$ as dehydrating agent. The mechanism of this reaction could take place as the same suggested mechanism in section 3.1.3. Three newly derivatives were synthesised. The first derivative was compound **2.31**, which is formed from reaction of compound **2.30** with 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde in presence of ethanol as depicted in Scheme 3.34.



Scheme 3.34:-Pathway for synthesis of 5-amino-1,3,4-thiadiazole and their derivatives.

The second derivative is compound **2.32** which synthesized from reduction of compound **2.31** by sodium borohydrate in methanol and tetra hydro furan as a solvent. The derivative **2.33** was synthesized from reaction of the 6-(5-amino-1,3,4-thiadiazol-2-yl)-2-methylphenol **2.30** with 3,5-di-*tert*-butyl-4-hydroxybenzoic acid in the presence of 1,1'-Carbonyldi imidazole (CDI) as coupling reagent as in section 3.1.7. The selective physical properties of these compounds were tabulated in Table 3.6.

Table 3.6:-Physical properties of 5-amino-1,3,4-thiadiazol and their derivatives.

No.	Structure	Yield %	M.P. ºC	MF	EIMs Calc.	EIMs Exp.
2.30		61	179-181	$C_9H_9N_3OS$	207.25	207
2.31		67	219-221	$C_{24}H_{29}N_3O_2S$	423.57	423
2.32		63	158-161	$C_{24}H_{31}N_3O_2S$	425.21	424
2.33		65	syrup	$C_{24}H_{29}N_3O_3S$	439.57	439

3.2.1.1 Characterization of 6-(5-amino-1,3,4-thiadiazol-2-yl)-2-methyl phenol and their derivatives 2.30-2.33.

The thiadiazole and their derivative were charectrized by IR, 1D NMR and EIMs spectra. The IR spectrum of compound **2.30** displayed the NH_2 band at (3346, 3290) cm^{-1} and OH at 3168 cm^{-1} besides to the CH aromatic and aliphatic at 3072 cm^{-1} and (2958, 2858) cm^{-1}. C=N of the thiadiazole ring appeared at 1626 cm^{-1}. The ^1H NMR of this compound showed new broad peak at 7.56 ppm attributed to the NH_2. The three aromatic protons of the 2-methyl phenol ring appeared as triplet, doublet and doublet at 6.85 ppm, 7.20 ppm and 7.34 ppm with coupling constant equal to 7.6 Hz, 6.9 Hz and 6.9 Hz attributed to H_4, H_3 and H_5 respectively. The three protons of methyl group appeared at 2.23 ppm as demonstrated in Figure 3.24.

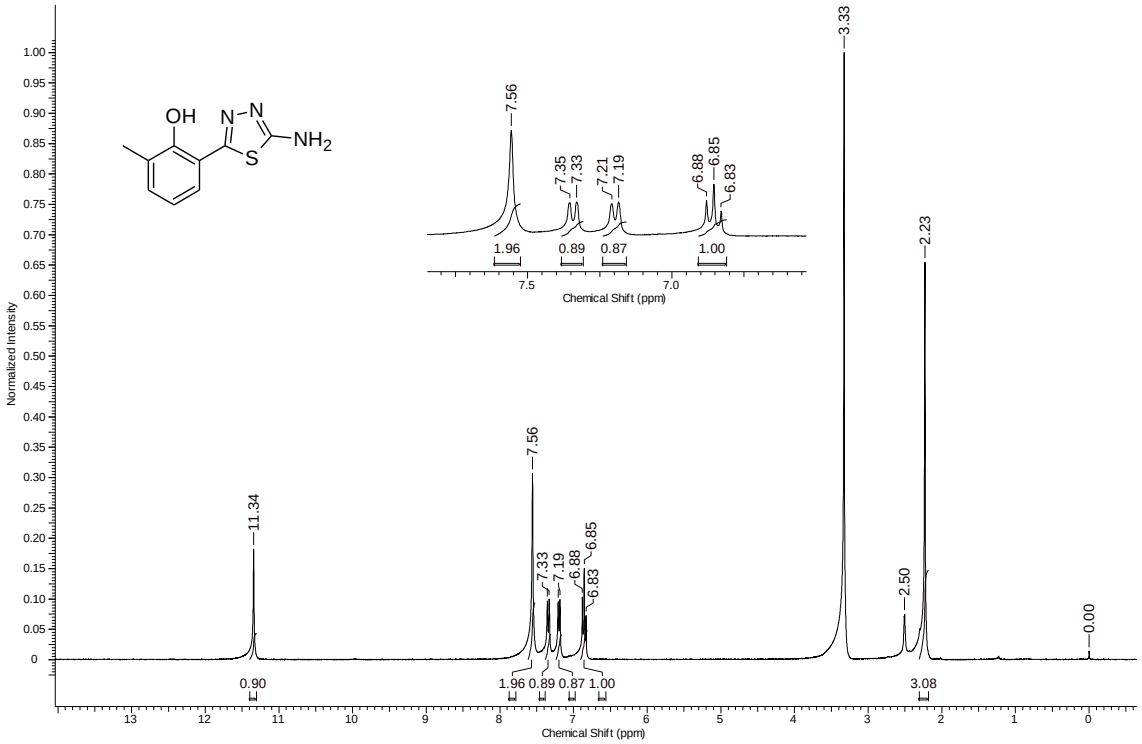


Figure 3.24:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.30.

The ^{13}C NMR of compound **2.30** showed in addition to the carbons of 2-methyl phenol two new peaks at 158.97 ppm and 167.98 ppm assigned to two C=N of the thiadiazole ring, as demonstrated in Figure 3.25.

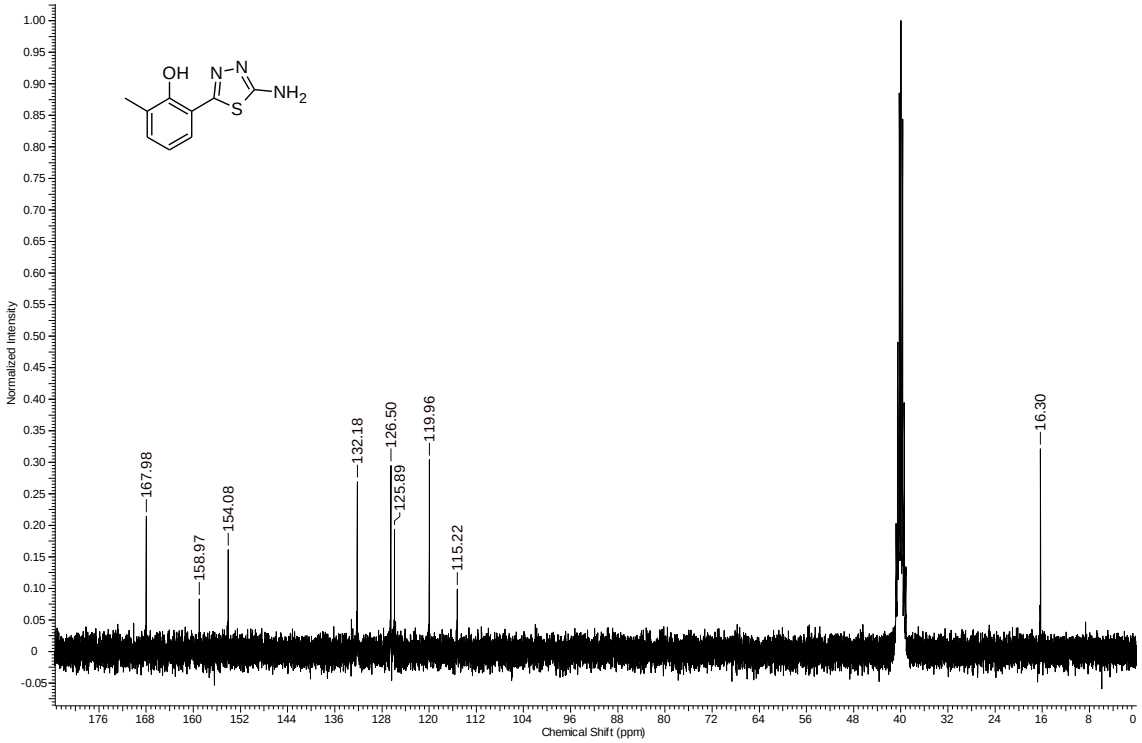


Figure 3.25:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.30.

Disappearance of the NH_2 from the IR spectrum of compound **2.30** was the initial evidence for successful formation of the new Schiff bases **2.31**. Furthermore, the peak at 1626 cm^{-1} which is assigned to the formation of the imine group was appeared at 1647 cm^{-1}. The Schiff bases of amino oxadiazole and amino thiadiazole with existence of 2-hydroxyl group could be attributed to cause shifting to large wave number due to the formation of enol-keto form in addition to exo-endo form [189] as depicted in Figure 3.26.

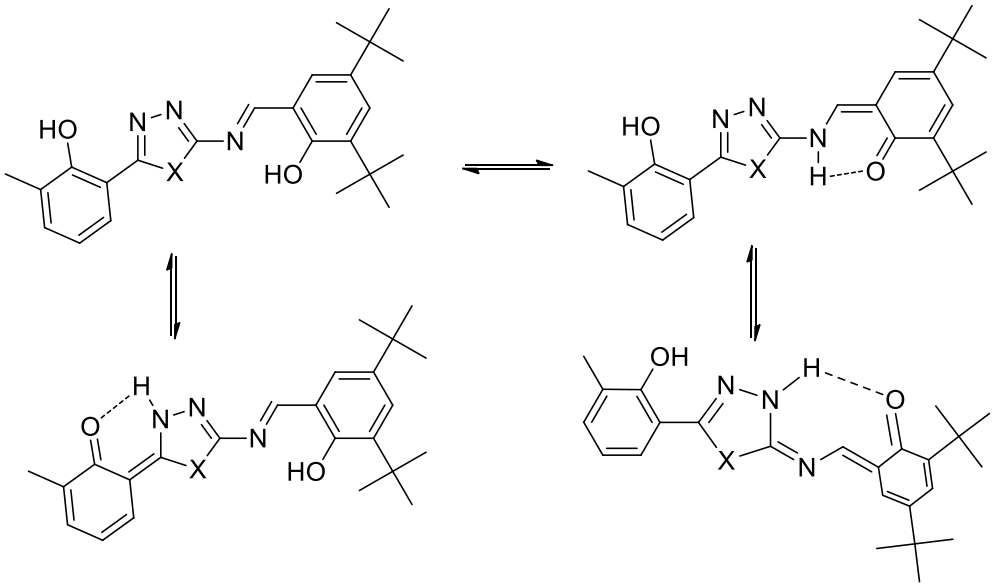


Figure 3.26:-Possible formation Keto-enol and endo –exo toutomerism in Schiff base

The ^1H NMR of compound **2.31** showed the peak of CH=N at 8.39 ppm, while the two OH group appeared at 9.98 ppm and 11.70 ppm. The H_{13} and H_{15} appeared as doublet for one proton at 7.57and 7.65 ppm with *J* 2.23 Hz due to 1,3-splitting [190] as well showed all expected protons. The ^1H NMR of **2.32** recorded two protons of CH_2 group at 3.80 ppm, which are significant identification for the success of the reduction besides to the NH at 6.67 ppm. The protons of two hydroxyl groups and the aromatic besides to the methyl group appeared at their expected regions as shown in Figure 3.27.

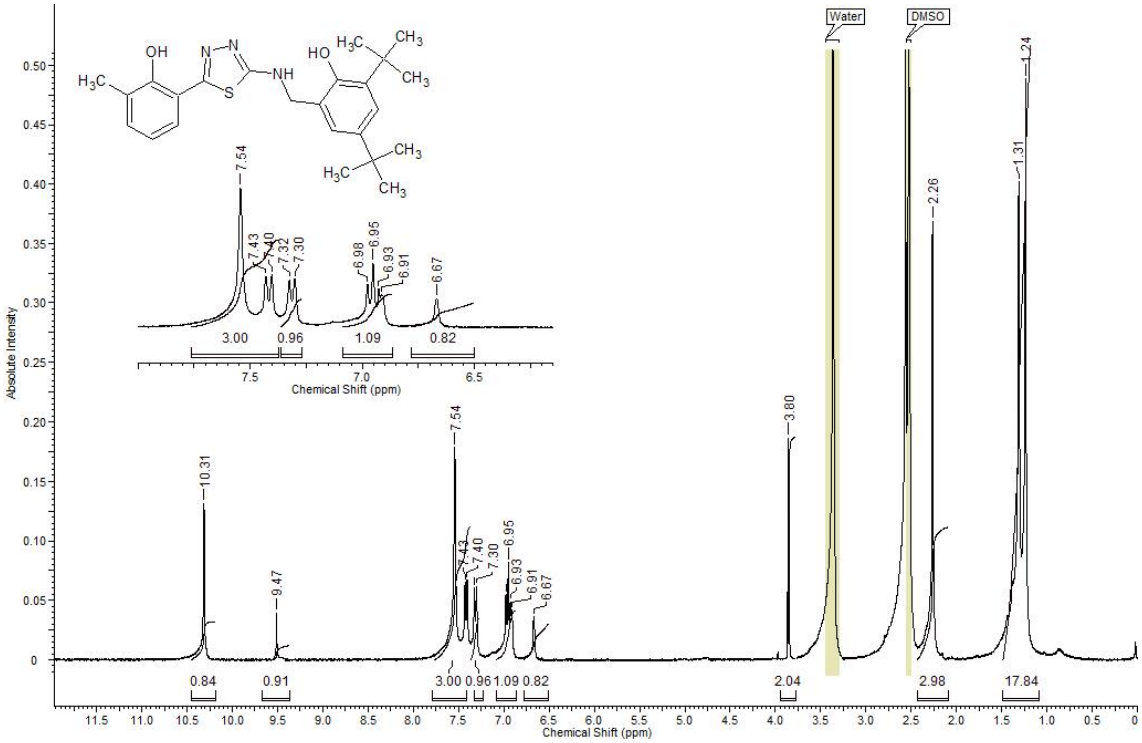


Figure 3.27:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.32

The ^{13}C NMR of this compound exhibited all expected carbons as depicted in Figure 3.28. Furthermore, the carbon at 62.70 ppm which indicated CH_2 compatible with peak at 3.80 ppm at the ^{1}H NMR spectrum.

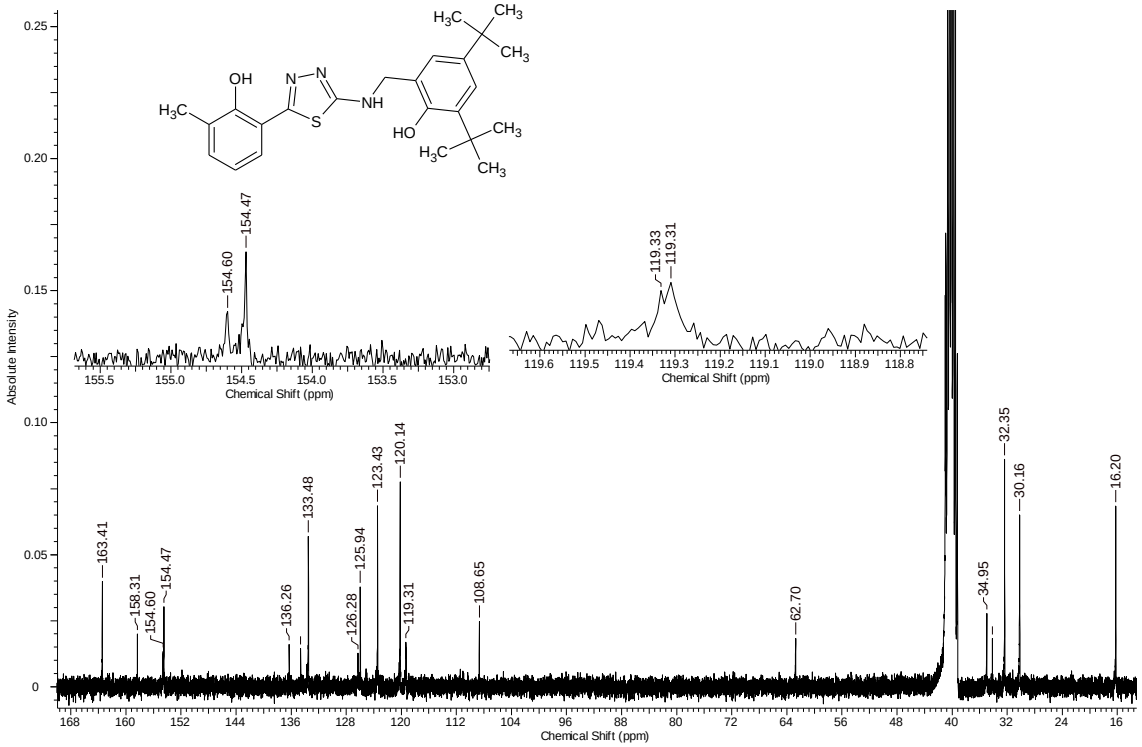


Figure 3.28:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.32.

The FTIR spectrum of compound **2.33** showed interested carbonyl of amide at 1687 cm^{-1} besides to the strong band at 3626 cm^{-1} for sterical hindered phenol. While, the hydroxyl of 2-methylphenol located at 3128 cm^{-1} as possible formation hydrogen bonding and the peak of stretching NH appeared at 3408 cm^{-1}. Furthermore, strong absorptions at 2954-2856 cm^{-1} refers to existence of di-*tert*-butyl group. The aromatic C-H as well located at 3020 cm^{-1}.

The ^{1}H NMR spectrum of compound **2.33**confirms the proposed structure by showing all expected protons for the 2-methylphenol group and 3,5-di-*tert*-butylphenol group besides to the proton of NH amide. The 3,5-di-*tert*-butylphenol group displayed the symmetrical of two groups of di-*tert*-butyl appeared at 1.51 ppm with integration for eighteen protons. In addition to, the two protons of C-H aromatic (H_{11}) were assigned as singlet peak at 7.56 ppm. The OH group of hindered phenol assigned in high filed at 5.68 ppm due to incapable formation hydrogen bonding [191]. The seven protons of 2-methylphenol assigned as singlet at 2.23 ppm for three protons for methyl group and the three aromatic protons appeared as triplet at 6.85 ppm with *J* 7.8 Hz for H_4, doublet at 7.20 ppm with *J* 7.8 Hz for H_3 and doublet at 7.34 ppm with *J* 7.6 Hz for H_5. Finally, the proton of NH amide assigned at 13.23 ppm as singlet peak, as displayed in Figure 3.29.

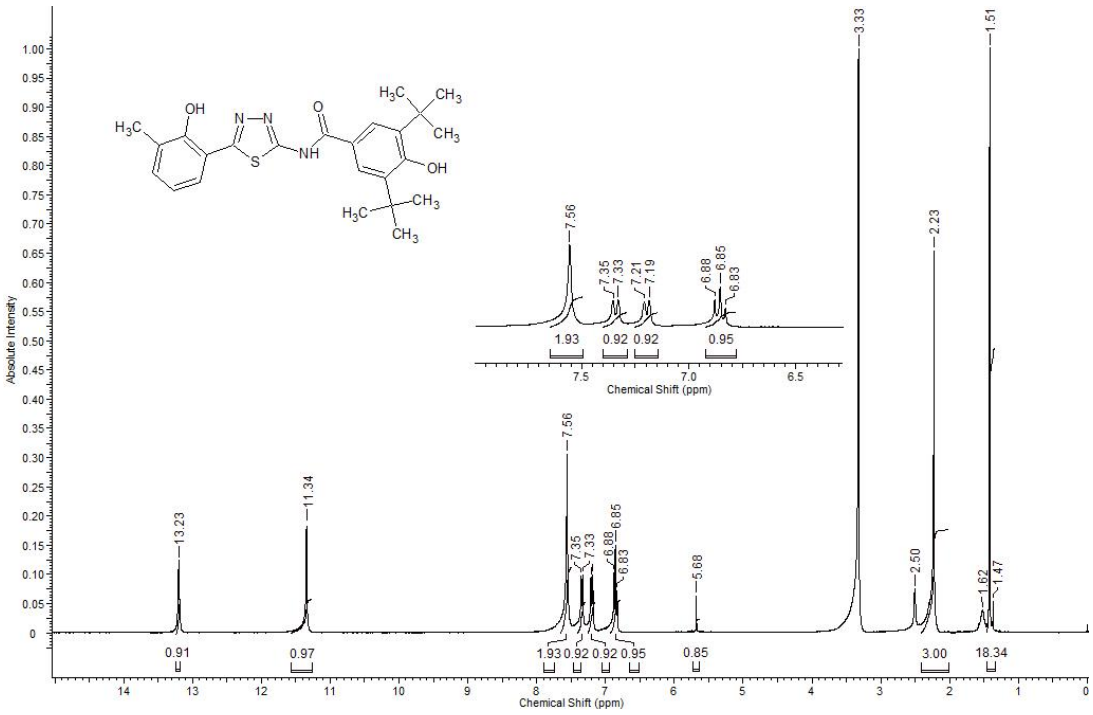


Figure 3.29:-^{1}H NMR (300MHz, DMSO-d_6) of compound 2.33.

The ^{13}C NMR spectrum showed the all carbons of 2-methylphenol group besides to the 3,5-di-*tert*-butyl-4-hydroxyphenol. In addition to, two carbons (C=N) of thiadiazole ring. The interested peak of the carbonyl amide was located at 167.75 ppm as depicted in Figure 3.30.

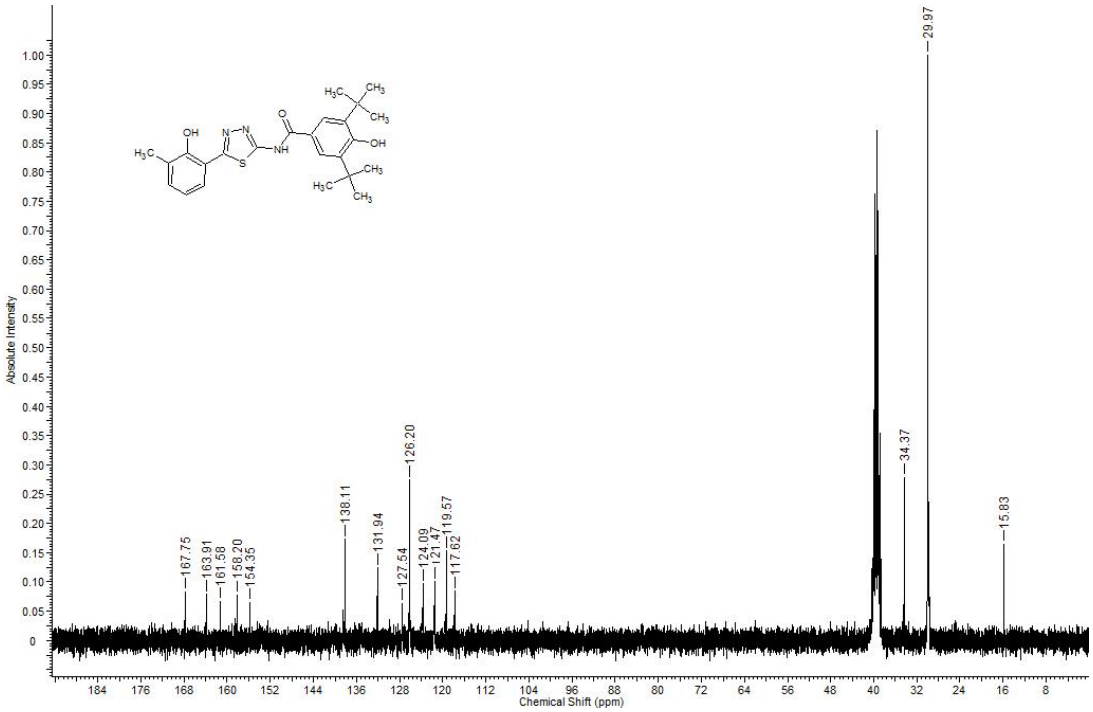
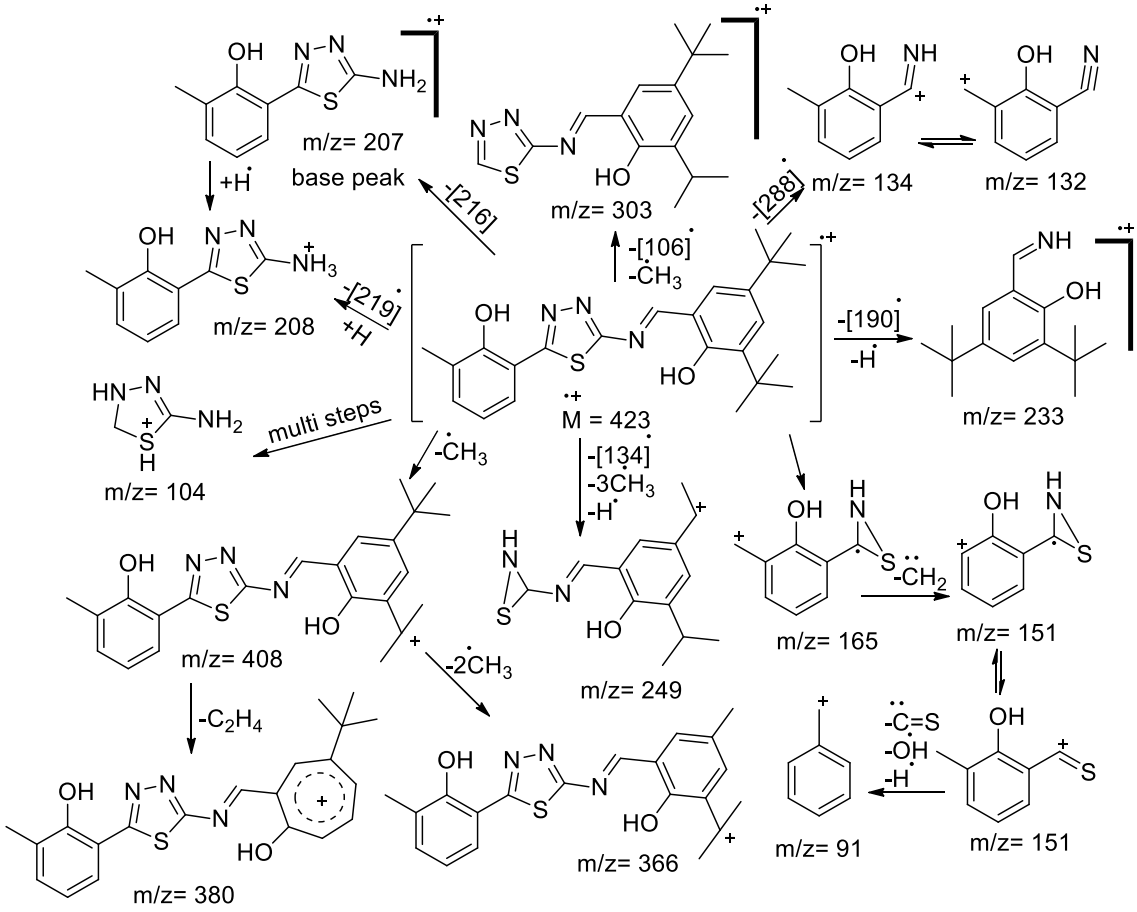


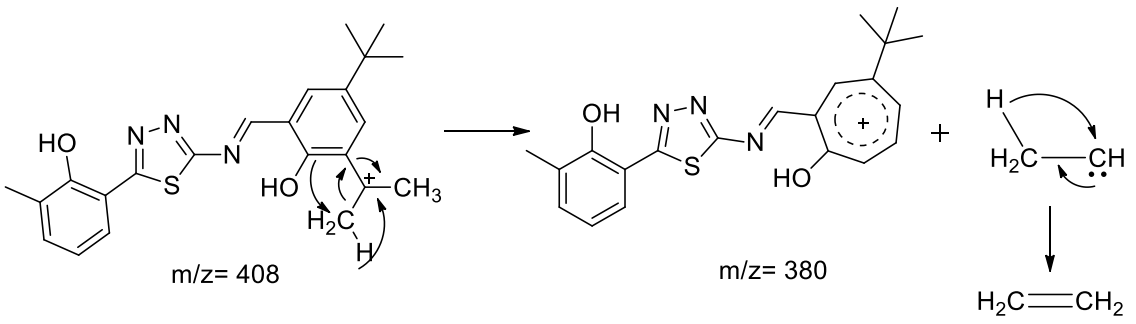
Figure 3.30:-^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.33.

The structure of thiadiazole amine and their derivatives had been characterized by EIMs spectra and these spectra confirm the proposed structure of these compounds. The spectra of compound **2.31** and **2.32** have been taken as example to illustrate the patron of fragment of these compounds. The molecular ion of this compound located at $M^{\bullet+}$= 423 as radical cation. The interested peak m/z= 207 of base peak corresponds to thiadiazole amine carbocation radical as well the fragment m/z= 233 which corresponded to 2,4-di-*tert*-butyl phenol with imine group as radical cation as depicted in Scheme 3.35.



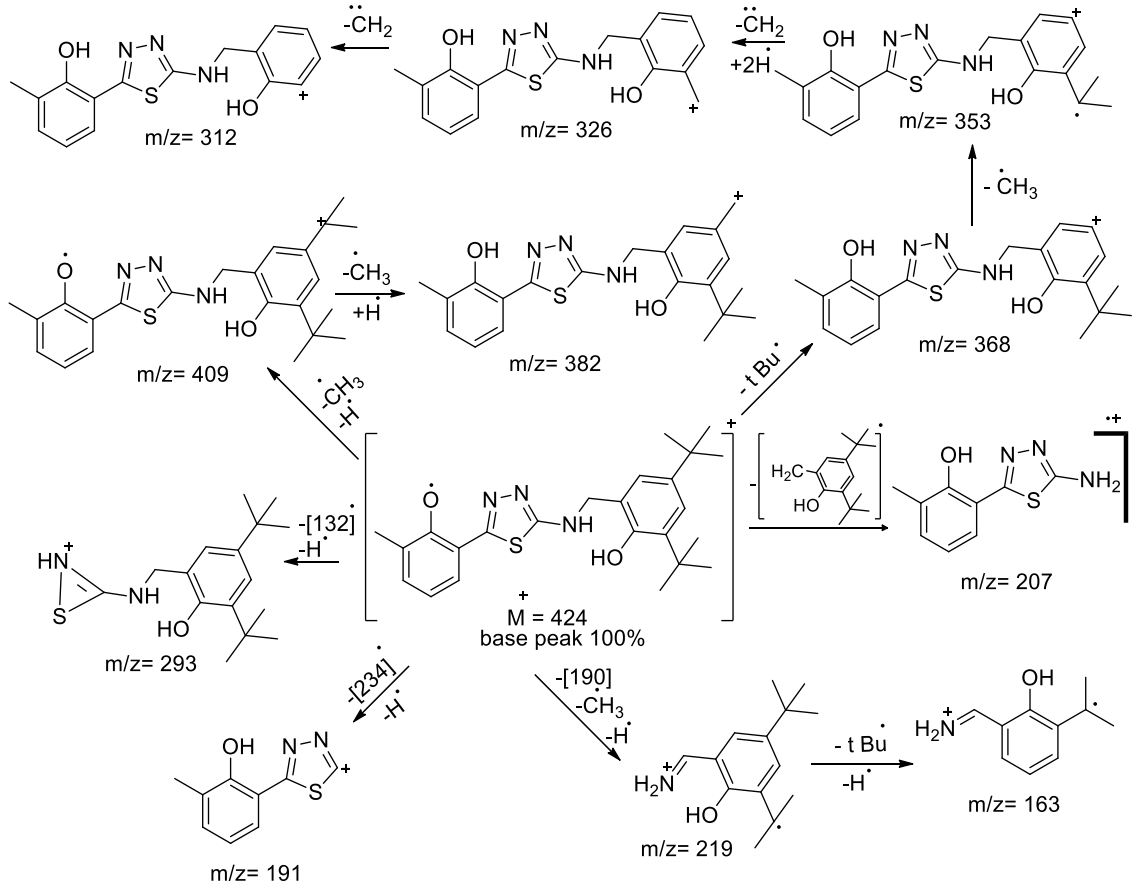
Scheme 3.35:-Fragmentation of EIMs of compound 2.31.

The molecular ion 423 after losing methyl di-*tert*-butyl phenol group generated the carbocation m/z= 408. An interested fragment was located from convert fragment 408 to 380 by losing molecules of ethylene accompanied by rearrangement as shown in Scheme 3.36.



Scheme 3.36:-Proposed mechanism of losing ethylene.

The mass spectrum of compound **2.32** was confirmed by the molecular ion which is also the base peak. As depicted in Scheme 3.37.

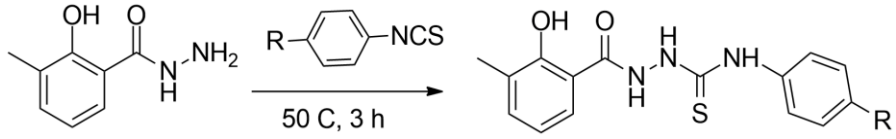


Scheme 3.37:-Fragmentation of EIMs of compound 2.32.

3.3 Formation 1,2,4-Triazole ring

3.3.1 Synthesis of 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl) hydrazinecarbothioamide 2.34-2.36

The 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl) hydrazinecarbothioamide (**2.34-2.36**) were synthesized to be intermediate compounds for synthesis 1,2,4-triazole ring. The compounds (**2.34-2.36**) synthesized from the reaction of hydrazide **2.2** with three different substituted phenyl *iso*thiocynate in ethanol, at 50 ºC for 3 hs. Recrystallized from suitable solvent and characterized by IR, ^{1}H NMR, ^{13}C NMR and EIMs. 2D NMR also used to clarify the ^{1}H NMR spectrum of compounds **2.34** and **2.35** Scheme 3.38 demonstrated the general pathway of synthesis of these compounds.



Scheme 3.38:-General pathway of synthesized 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl) hydrazinecarbothioamide 2.34-2.36.

The substituted group and some selected physical properties were presented in Table 3.7.

Table 3.7:- Physical properties of compounds (2.34-2.36).

No. of Comp.	R	Yeild	m.p. ˚C	MF	EIMs Cal.	EIMs Exp.
2.34	Me	81	177-179	$C_{16}H_{17}N_3O_2S$	315.39	315
2.35	Cl	79	188-190	$C_{15}H_{14}ClN_3O_2S$	335.80	335
2.36	OMe	82	176-178	$C_{16}H_{17}N_3O_3S$	331.38	331

3.3.1.1 Characterizations of 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl) hydrazine carbothioamide (2.34-2.36).

Three compounds (**2.34-2.36**) were synthesized to be used as starting material for the formation of 1,2,4 triazole ring at position six. These compounds were characterized by; IR, 1D NMR, 2D NMR and EIMs spectra. The IR spectrum indicated the presence of OH in phenol and aliphatic C-H, C=O group and some new peaks, e.g. C=S at (1271-1244) cm^{-1}. Besides the protons of methyl phenol the ^{1}H NMR spectra exhibited the aryl *iso*thiocyanate peaks in aromatic ring corresponded to four protons for *para* substituted phenyl *iso*thiocyanate. The protons of substituted group also appeared at the expected range, e.g. *para*-CH_3 in compound **2.34** was appeared at 2.29 ppm and *para*-OCH_3 group of compound **2.36** at 3.75 ppm. In this structure, there are four peaks located in low magnetic field (>9.00 ppm) two of them appeared as merged peak at (9.72-9.86) ppm assigned to two NH group. The peak at lowest magnetic field attributed to proton of OH group, which is confirmed by HMBC. The next lower magnetic field attributed to proton of NH in CSNHNH group. Figure 3.31, exhibited the ^{1}H NMR of compound **2.34** as example.

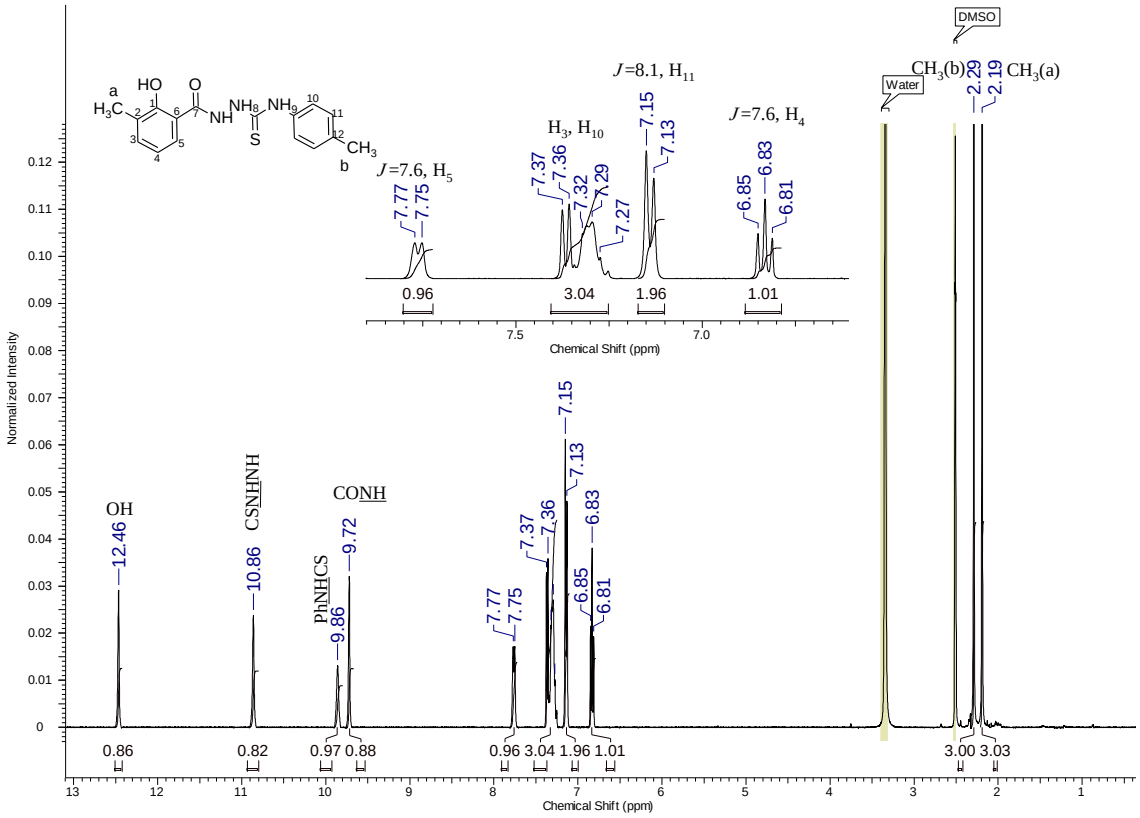


Figure 3.31:-^{1}H NMR (400MHz, DMSO-d_6) of compound 2.34.

The aromatic protons of 2-methylphenol were located at 6.83 ppm as triplet with *J* 7.6 Hz for H_4 and 7.76 ppm as doublet with *J* 7.6 Hz for H_5 while, the H_3 appeared as multiple due to over lab with $2H_{10}$ of the 4-methylphenol group. The protons of two methyl groups (2-methyl and 4-methyl) were assigned at 2.19 ppm and 2.29 ppm with integration equal to three protons for each one. This result is confirmed by HMBC

As earlier mentioned the ^{13}C APT spectra considered less sensitive than normal ^{13}C NMR. However, ^{13}C APT spectra were in agreement with the IR and ^{1}H NMR spectra. In spite of some carbons, signals of aryl *iso*thiocyanate are emerged as merged signal. As well the C=O and C=S were not quite significant however, the HMBC spectra confirmed their existence.

The HMBC spectrum is quite beneficial to distinguish between four peaks at the lower field (12.46, 10.86, 9.86, 9.72), which refer to three

different NH and one OH of phenol. Furthermore, to confirm the merge carbons at the aromatic range and the existence of C=O, C=S. The expanded reign of the HMBC spectrum of compound **2.34,** was displayed in Figure 3.32

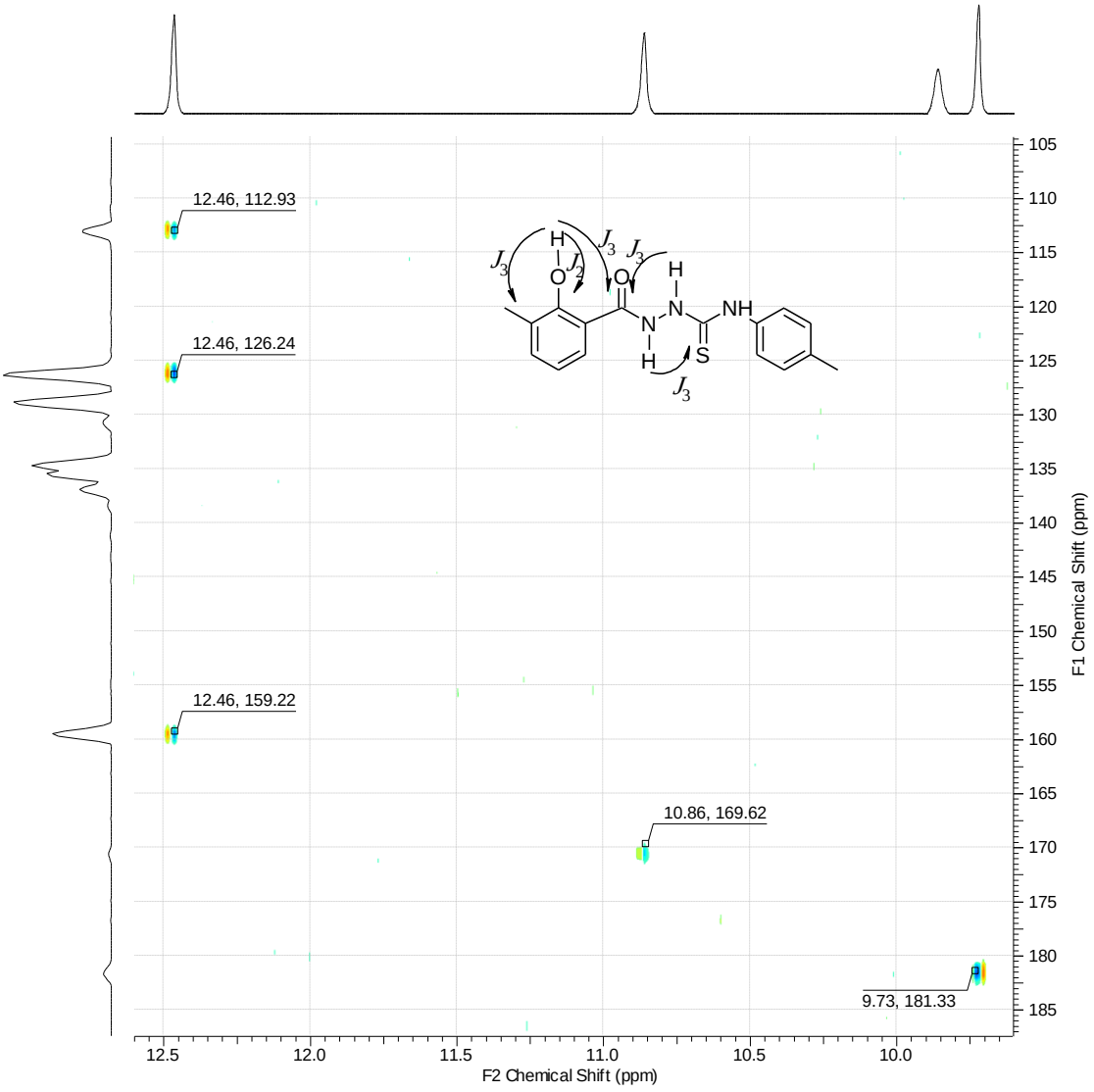


Figure 3.32:- HMBC expansion of compound 2.34.

The HMBC spectrum disclosed the correlation for long distance coupling J_3 and weak for J_2. From this correlation the HMBC spectrum of compound **2.34** illustrated that the last proton at 12.46 ppm belonging to the hydroxyl group of phenol. This proton exhibited three correlations with three different carbons. The first correlation appeared between the proton at

12.46 ppm with C_6 (112.93) ppm as J_3 coupling and it is not enough to distinguish between the proton of OH and the protons of CONH. However, the second correlation with C_2 (126.24) ppm was also as J_3 coupling and the third correlation with C_1 (159.22) ppm as J_2. All these correlations were enough to determine that the last proton belonged to OH. The proton at 10.85 ppm exhibited one correlation with C=O (169.62) ppm as J_3 coupling while, the proton at 9.72 ppm exhibited correlation with C=S (181.33) ppm also as J_3 coupling. As result of these two correlations, the proton at 10.85 ppm belongs to NHCS and the proton at 9.73 ppm belongs to CONH. Even though, there is no correlation appeared with proton at 9.86 ppm, however that indicated this proton belonging to ArNHCS.

Furthermore HMBC spectrum can illustrate the other correlations between (H, H), (H, C) and (C, C) to determine the structure of compound **2.34**, such as illustrated between H_3 and H_5 besides the two CH_3 group in this structure. The doublet signal at 7.76 ppm with *J* 7.6 Hz for one proton display correlation with C_3, C_1 and C=O. these correlations referred to proton H_5. as depicted in expansion reign Figure 3.33

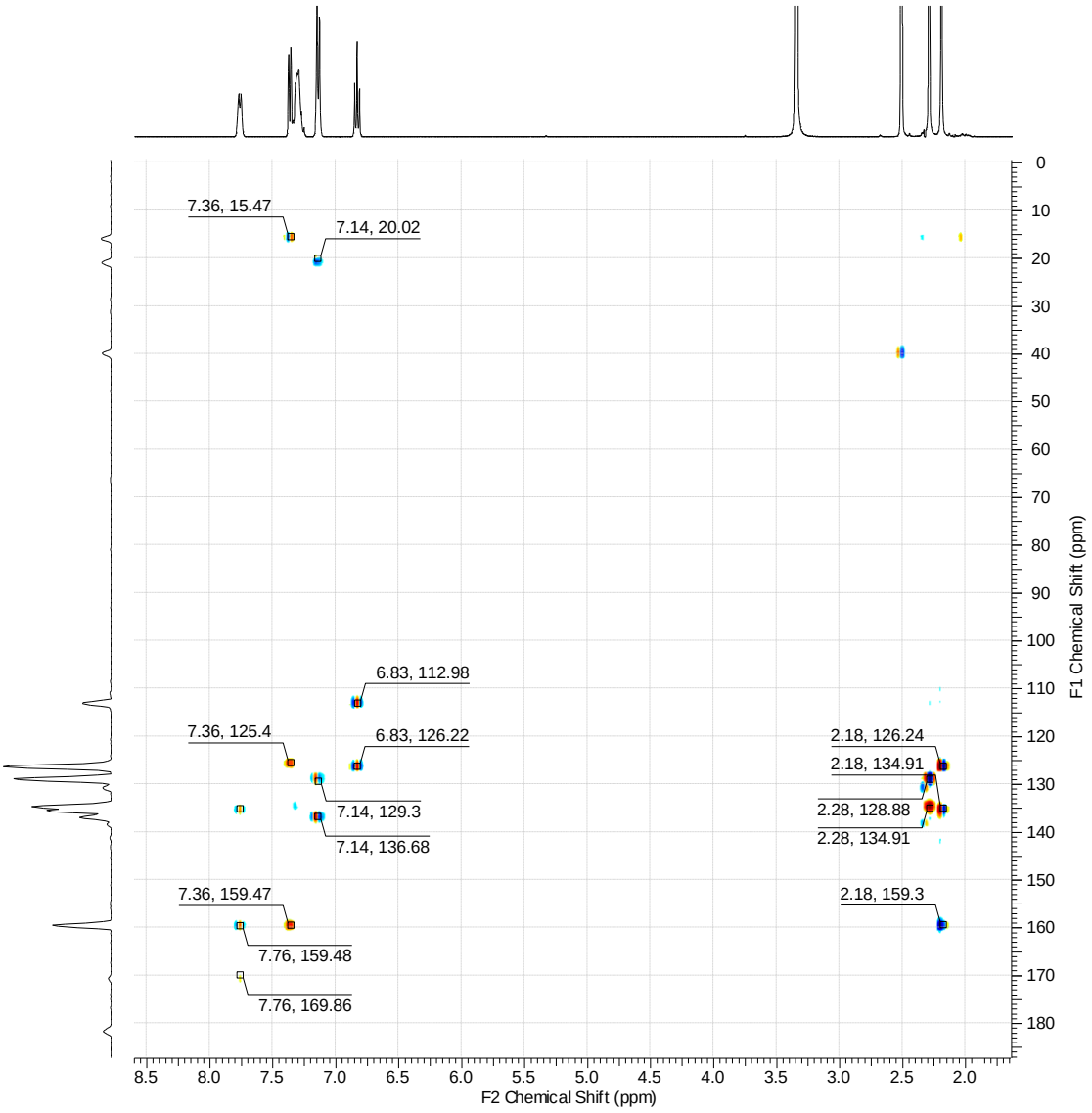


Figure 3.33:-HMBC expansion region of compound 2.34.

The proton at 2.18 ppm displayed correlation with C_2 (126.24 ppm) as J_2 coupling, C_3 (134.91 ppm) as J_3 coupling and C_1 (159.3 ppm) as J_3 these correlations indicated that proton belonged to methyl group at phenol ring. While, the proton at 2.28 ppm displayed correlation with C_{12} as J_2 coupling and C_{11} as J_3 coupling. As a result, the proton at 2.28 ppm belonged to *para* methyl group of *iso*thiocyanete part. As well, the HMBC spectrum had another benefit by illustrating between H_{10} and H_{11} and between two carbons of methyl group. The doublet signal at 7.14 ppm with J 8.1 Hz for two protons showed correlation with carbon at C_{10} as J_2 and C_9 as J_3. This

correlation indicated that this proton is H_{11}, which also had correlation with carbon at 20.02 as J_3. That correlation main, this carbon corresponded to carbon of *para* methyl group of *iso*thiocyanete part. The HMBC spectrum of compound **2.35** disclosed same results for the OH group and three NH. As well the correlation (C_9-H_{11}) as J_3 and (C_{12}-H_{10}) as J_3 besides to (C_9-H_{10}) and (C_{12}-H_{11}) as J_2 located at (7.4-138) which confirmed the merge between C_9 and C_{12} at ^{13}C ATP spectrum For more details please see Appendix B.

The ^{13}C NMR spectrum of compound **2.36** showed the carbon of methyl group at 15.44 ppm while, the carbon of methoxy group is located at 55.16 ppm. The aromatic C-H carbon and quaternary carbon are assigned at their expected location. The carbons of carbonyl and thiocarbonyl were appeared at 170.15 ppm and 181.46 ppm respectively. As shown in Figure 3.34

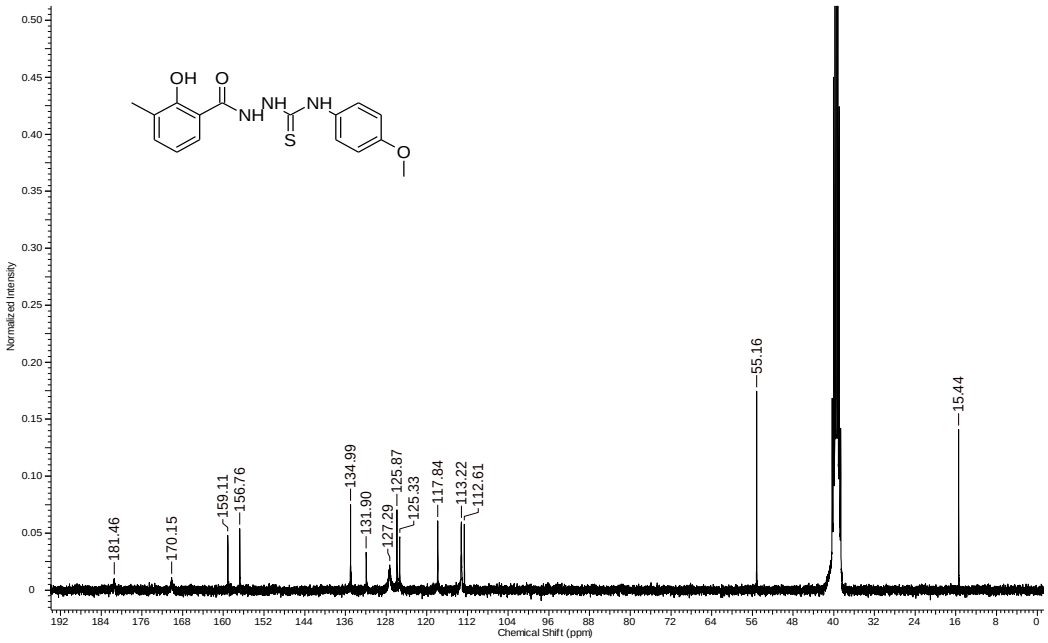


Figure 3.34:- ^{13}C NMR (300 MHz, DMSO-d_6) of compound 2.36.

The fragments of EIMs spectra of N-(aryl) hydrazinecarbothioamide derivatives of 2-methyl phenol (**2.34-2.36**) harmonized with IR, 1D NMR and 2D NMR and confirm the proposed structure. For instance, the

spectrum of compound **2.36** exhibited the molecular ion at $M^{\bullet+}$= 331 As well the base peak was m/z= 135 which is represented to (2-hydroxy-3-methylbenzylidyne)oxonium which is corresponding to (2-hydroxy-3-methylphenyl)(oxo)methylium as shown in Figure 3.35.

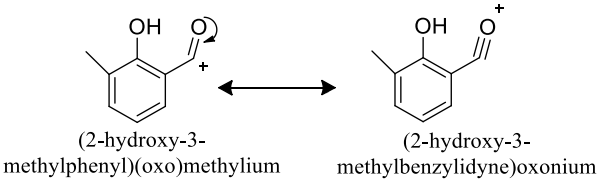
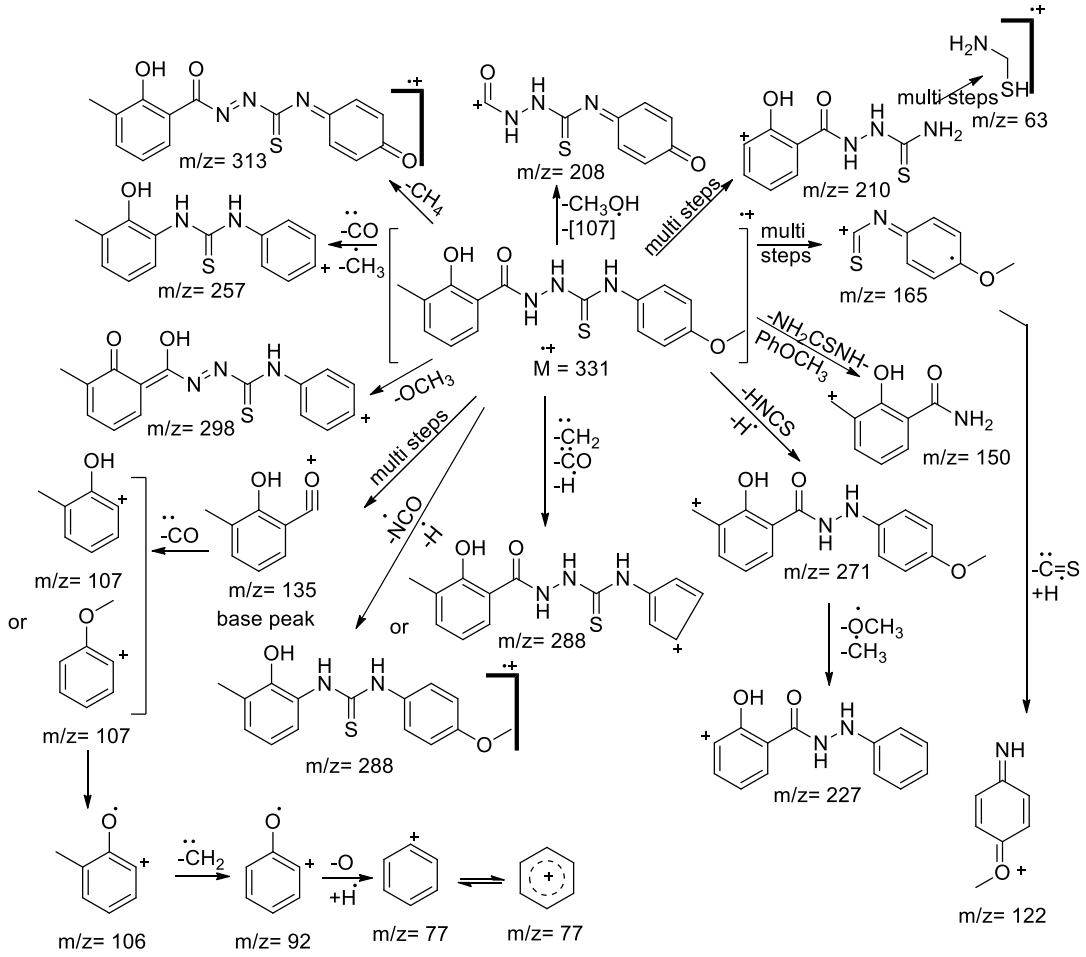


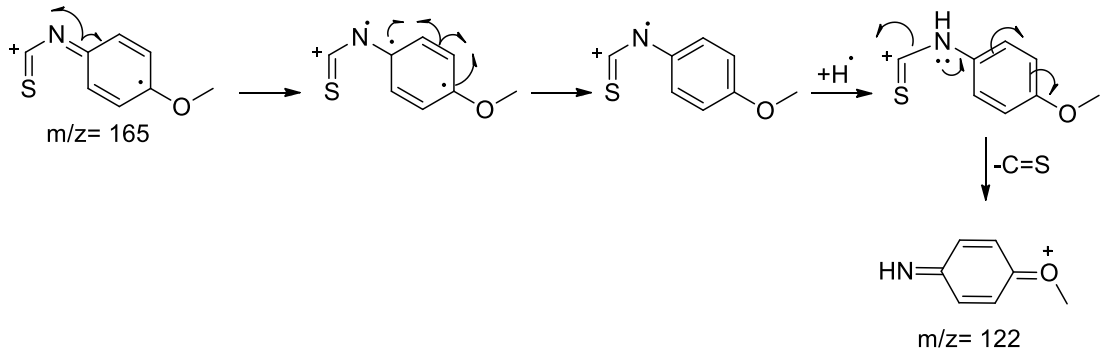
Figure 3.35:-Proposed structure of base peak at m/=135.

This fragment has been located as base peak for compounds **2.10**, **2.11**, **2.12**, **2.24** and **2.36**. Moreover, it is located for all synthesized compounds as identifier fragment. The pattren of mass fragmentations for compound **2.36** was demonstrated at Scheme 3.39.



Scheme 3.39:-Fragmentation of EIMs of compound 2.36.

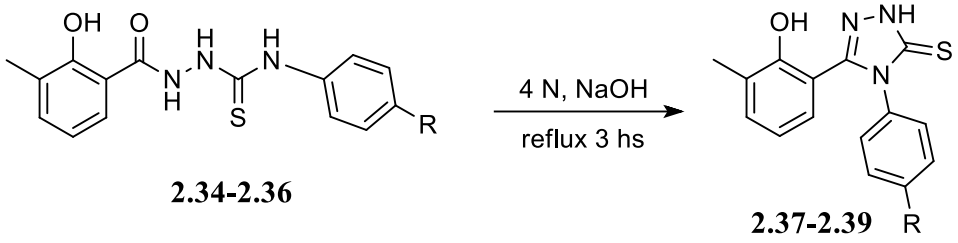
An interested fragment was located from convert fragment m/z=165 to m/z= 122 by losing molecule of :C=S accompanied by rearrangement as proposed mechanism shown in Scheme 3.40.



Scheme 3.40:-Proposed mechanism of losing C=S and rearrangement.

3.3.2 Synthesis of 6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione) 2-methyl phenol (2.37-2.39).

The 2-methyl-6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione)phenol (**2.37-2.39**) were synthesized by treating the 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl) hydrazinecarbothioamide (**2.34-2.36**) with 4N sodium hydroxide solution and refluxed for 3 hs to give the target compound. The reaction path way demonstrated in Scheme 3.41



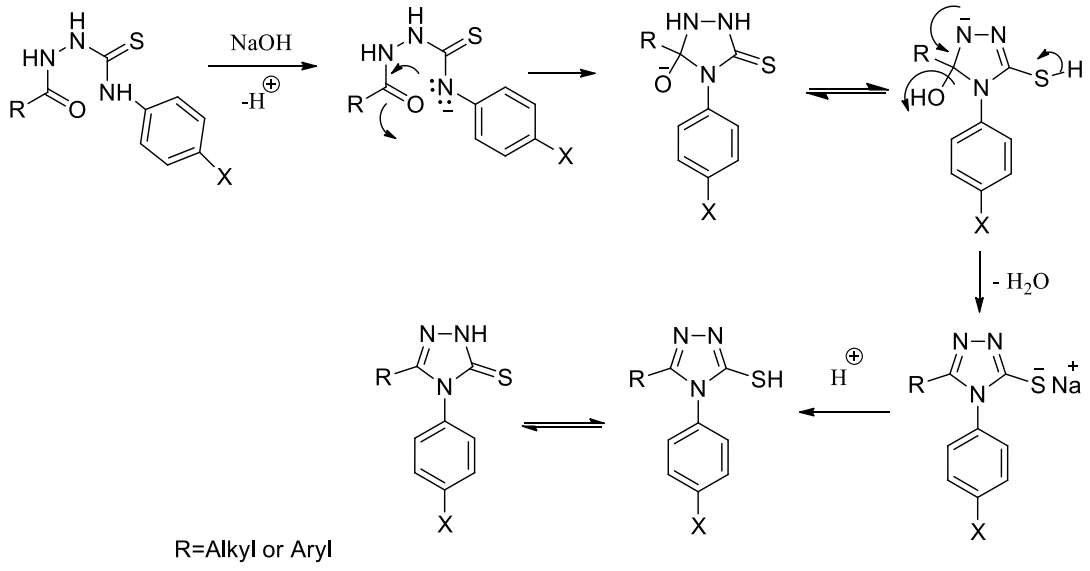
Scheme 3.41:- Synthesis of 6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione) 2-methylphenol (2.37-2.39).

The yield, melting point, molecular formula, and EIMs were tabulated in Table 3.8.

Table 3.8:- Physical properties of compounds (2.37-2.39).

No. of Comp.	R	Yeild	m.p. °C	MF	EIMs Calc.	EIMs Exp.
2.37	Me	84	269-271	$C_{16}H_{17}N_3O_2S$	315.39	315
2.38	Cl	80	184-187	$C_{15}H_{14}ClN_3O_2S$	335.80	335
2.39	OMe	77	271-272	$C_{16}H_{17}N_3O_3S$	331.38	331

The suggested mechanism of formation the 1,2,4-triazole is described in Scheme 3.42[118].



Scheme 3.42:-Proposed mechanism of formation triazol-5-thione.

3.3.2.1 Charactrizations of 6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione) 2-methylphenol (2.37-2.39).

The target compounds were characterized from their IR, 1D NMR and EIMs spectra. 2D NMR (HMBC) was used with compound **2.37** for further characterization. The IR spectra showed the disappearance of the carbonyl group and appearance of new peak at range (1610-1603) cm^{-1} corresponded

to C=N. As well, the IR spectra exhibited the peak of OH of phenol for compounds **2.38** at 3149 cm^{-1}. The low wave number value for the phenolic hydroxyl group of compounds **2.37** and **2.39** might be attributed to intra hydrogen bonding which causes shift towards lower wave numbers [192-194]. The intra hydrogen bonding between the OH of phenol as *ortho* substituted widely known in literature, which can be summarized in Table 3.9[194].

Table 3.9:-Observed wavenumbers (cm^{-1}) for υ(OH) and γ(OH) vibrations in phenol and a number of 2-hydroxybenzoyl compounds with intra molecular hydrogen bonding.

Compound	υ**(OH)**	γ**(OH)**
Phenol	3655	322
Methyl salicylate	3258	714
Salicylaldehyde	3190	714
2-Hydroxyacetophenone	3100	787
Salicylamide	3070	807
Sodium salicylate	2910-1900	984[c]

This intra molecule hydrogen bonding could be overlapping with NH and with the weaker stretching of C-H aromatic at which assigned at (3082-3089) cm^{-1}. The IR spectra exhibited the $CH_{aliphatic}$ at (2931-2765) cm^{-1}. The C=N of 1,2,4-triazole ring displayed at (1603-1615) cm^{-1} and the C=S at (1232-1248[195]) cm^{-1}.

The ^{1}H NMR for these compounds disclose the success of the cyclization through the disappearing of two signal of NH group, moreover through the shifting of signal value of the OH and NH group in 1,2,4-triazole structure. The ^{1}H NMR spectra showed all the protons signal of the phenol ring. The 2-methyl group appeared at (2.09-2.18) ppm and the protons of 4-aryl were located at their expected range. The protons of substituted group such as 4-CH_3 for compound **2.37** and 4-OCH_3 for

compound **2.39** also were located. However, we cannot distinguish between peaks of the OH and NH. For that, HMBC spectrum for compound **2.37** was used as example to distinguish between them. The ^{1}H NMR of compound **2.37** taken as example to illustrate the triazole structure as exhibited in Figure 3.36.

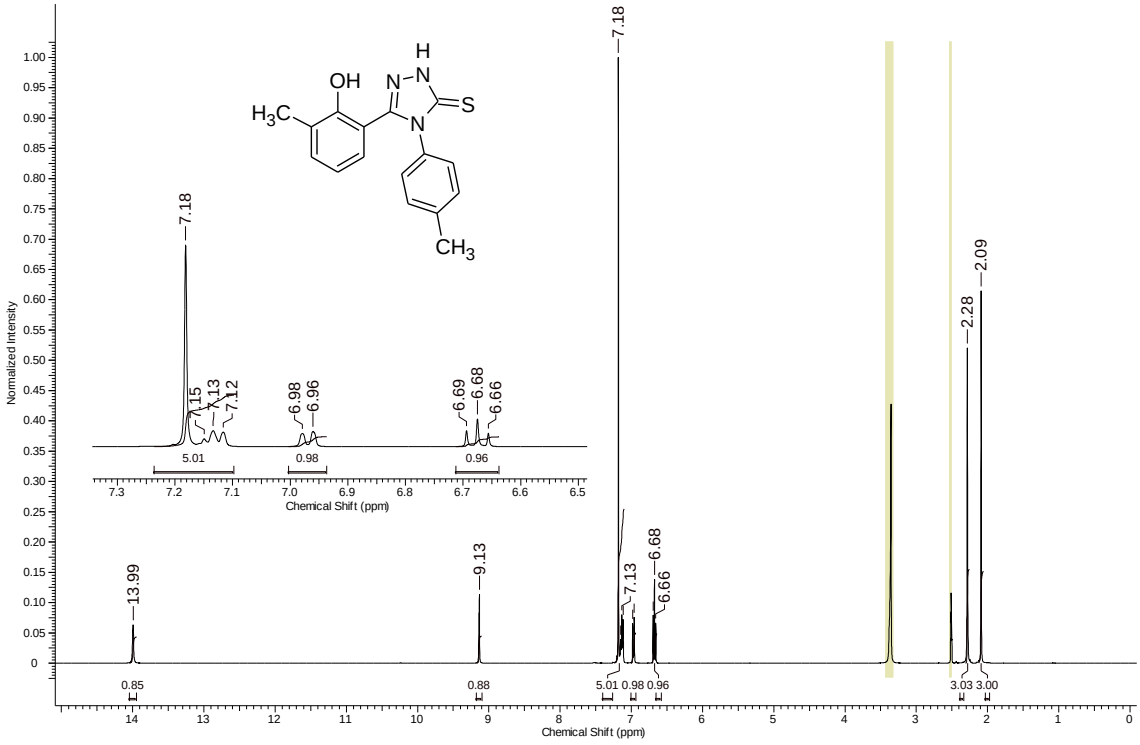


Figure 3.36:-^{1}H NMR (400MHz, DMSO-d_6) of compound 2.37.

Figure 3.36 exhibited the protons of CH_3 group of phenol ring (2-methyl) at 2.09 ppm. While the protons of CH_3 group of aryl (4-methyl) was appeared at 2.28 ppm. The aromatic area displayed as three peaks, triplet signal at 6.68 ppm with *J* 7.5 Hz attributed to H_4, doublet signal at 6.97 ppm with *J* 7.5 Hz attributed to H_3 and multiple signal at 7.15 ppm for five protons attributed to H_5, $2H_{10}$ and $2H_{11}$. The OH group appeared in triazole at higher magnetic field than NH as HMBC spectrum showed. Where as the corresponded spectrum of thiocarbamide showed the opposite (the OH at the lower magnetic field than NH) as mentioned earlier as section 3.3.1.1.

The APT spectra of compounds (**2.37-2.38**) and ^{13}C NMR of compound **2.39** showed all carbons peaks and confirms the structure. The APT spectrum for compound **2.37** exhibited the 2-methyl group and 4-methyl group appeared as positive peak at 16.77 ppm and 21.16 ppm. The HMBC spectrum allowed distinguishing between them.

Five positive peaks were located at (119.31, 128.34, 129.15, 129.73 and 133.47) ppm belonged to C_4, C_{10}, C_5, C_{11} and C_3. Furthermore, seven negative peaks attributed to seven quaternary carbons C_6, C_2, C_{12}, C_9, C_7, C_1 and C_8 respectively, as demonstrated in Figure 3.37.

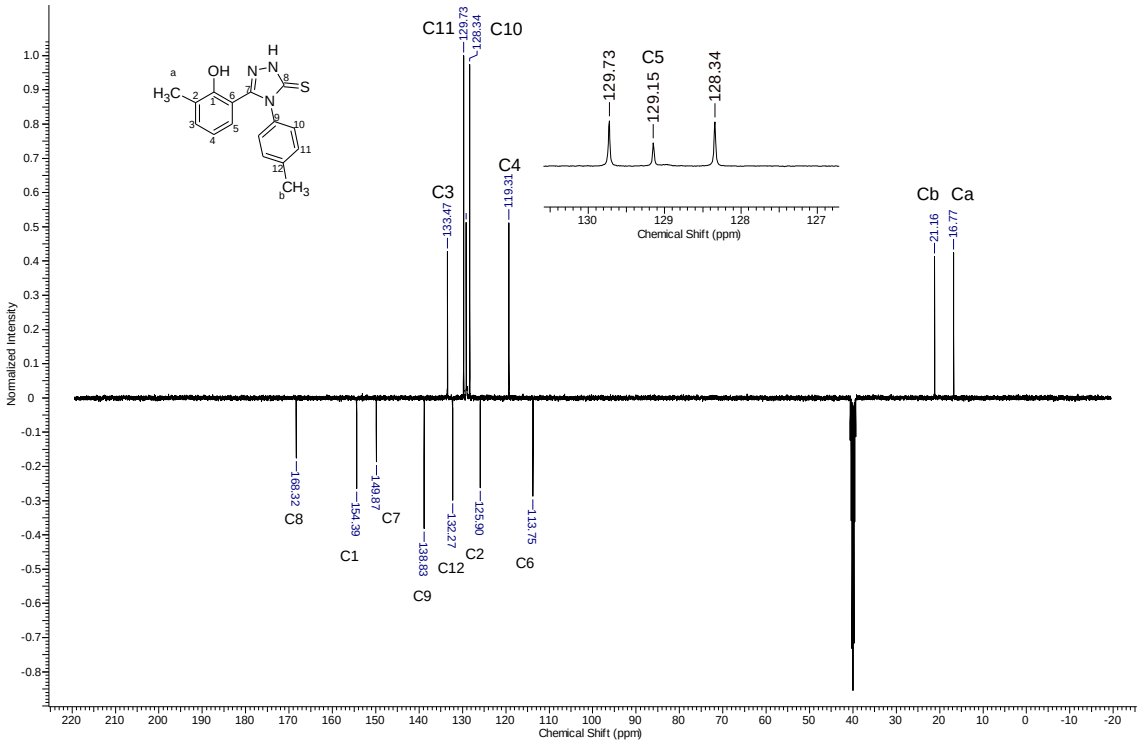


Figure 3.37:- ^{13}C APT (400 MHz, DMSO-d_6) of compound 2.37.

HMBC spectrum was too benefiting to solve any confusing in the characterization of this compound by ^{1}H NMR and ^{13}C NMR. From ^{1}H NMR it was too difficult to distinguish between the peak of OH of phenol and NH at the 1,2,4-triazole ring. The HMBC spectrum showed three correlations (9.14, 113.86) ppm, (9.14, 125.92) ppm and (9.13, 154.3) ppm clearly indicate to the proton of hydroxyl with two J_3 correlations and one

J_2 correlation. Furthermore, the two correlations were located at (13.99, 149.18) ppm and (13.99, 168.19) ppm as J_3 and J_2 respectively. This result definitely leads to assuming the second peak which belongs to NH, as shown in Figure 3.38, for more details see Appendix B.

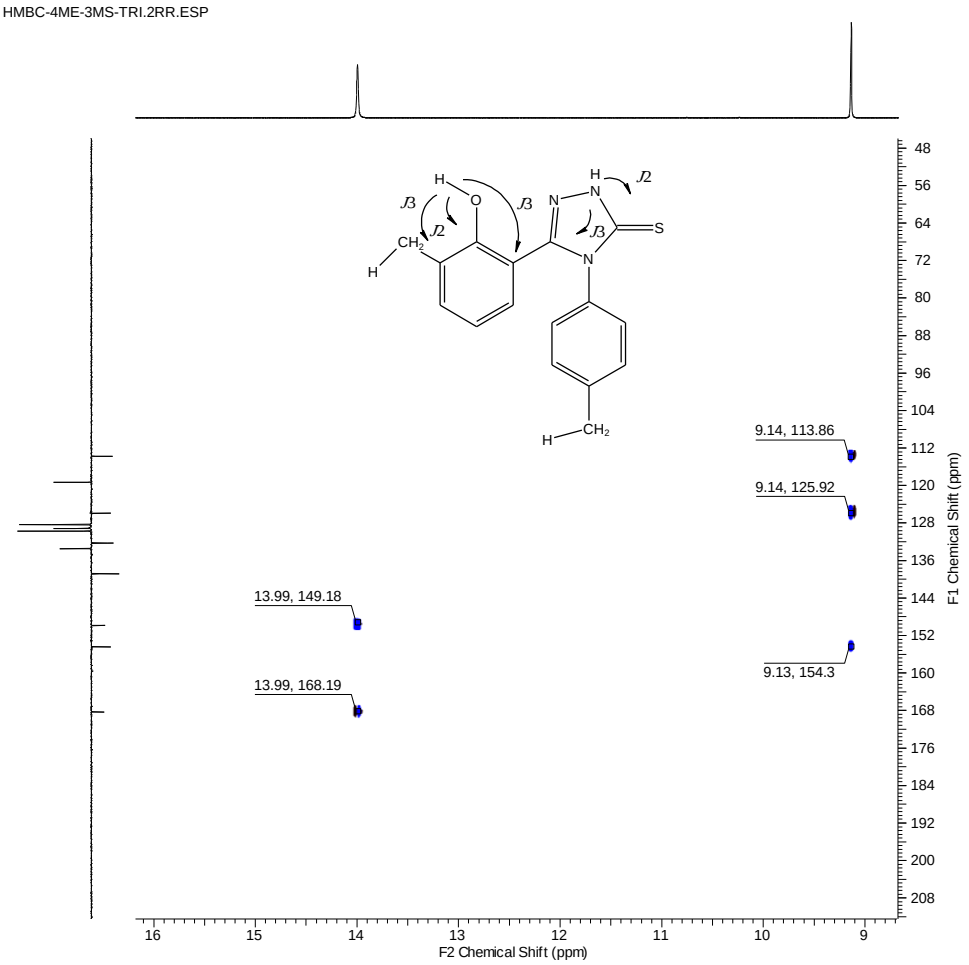


Figure 3.38:-HMBC expansion of compound 2.37.

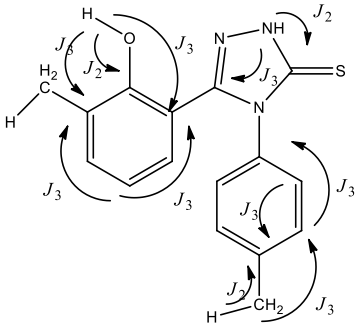
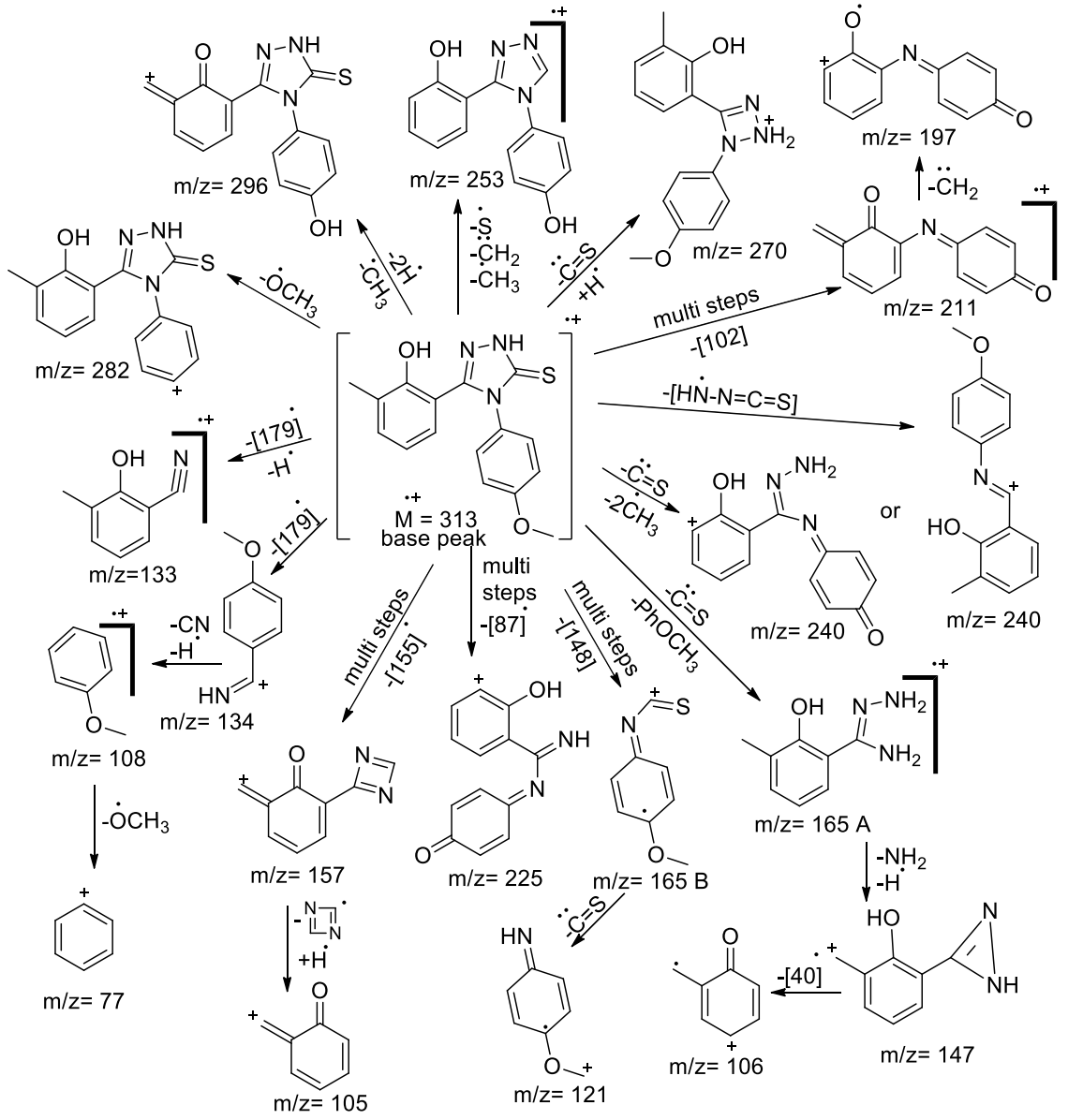


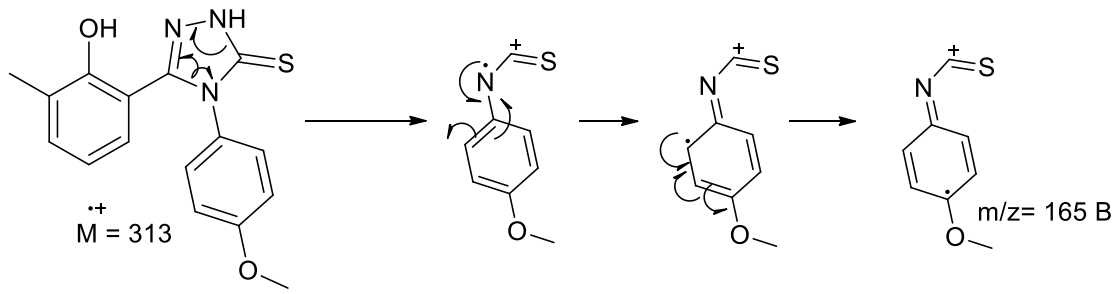
Figure 3.39:-Selected HMBC correlations of compound 2.37.

The mass spectroscopy confirms the structure of the 1,2,4-triazoles. The spectrum showed the molecular ion and the base peak for all compounds. Scheme 3.43 depicted the mass fragment for compound **2.39** as example to the pattern of fragmentations of these 1,2,4-triazoles.



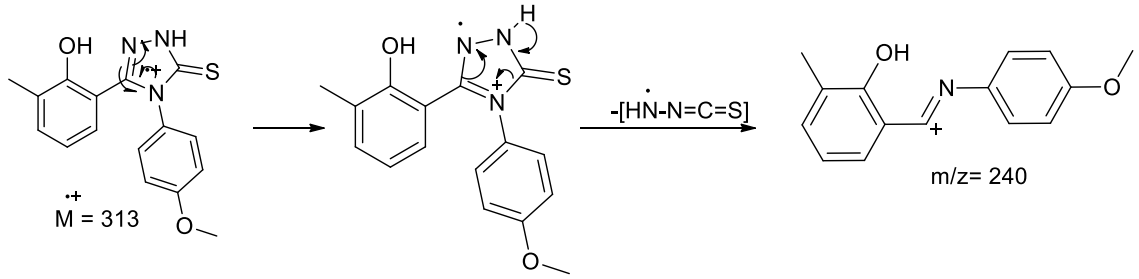
Scheme 3.43:-Fragmentation of EIMs of compound 2.39.

The fragment m/z= 165 could be attributed to another structure as coded m/z= 165 B which can be formed from another pathway as illustrated in Scheme 3.44.



Scheme 3.44:-Pathway to convert fragment from $M^{\bullet+}$=313 to m/z= 165 B.

An interested fragment was located from convert fragment 313 to 240 by losing molecules of $HN^{\bullet}$-N=C=S accompanied by rearrangement as shown in Scheme 3.43.



Scheme 3.45:-Mechanism of losing m/z= $[72]^{\bullet}$.

All fragments are corresponded a part of the target compound either as radical cation or cation.

3.4 Antibacterial Activity

3.4.1 Determination of the antibacterial activity

The antibacterial activities of the following synthesized compounds **(2.3)**, **(2.4-2.8), (2.9-2.13)** and **(2.14-2.22)** were tested against standard typed strains of eight bacteria. They were obtained from the Central service laboratory-college education for pure science-Ibn Al-haitham . These microorganism's strains consist of Gram negative bacteria: *Acinetobacter calcoaceticus* ATCC 23055, *Escherichia coli* ATCC10538, *Pseudomonas aeruginosa* ATCC15442 , *Salmonella typhimurium* ATCC14028. The

Gram positive bacteria were *Bacillus subtilis* ATCC6051, *Enterococcus faecalis*, ATCC 29212*Streptococcus pyogenes* ATCC19615, *Staphylococcus aureus* ATCC 29213. The antibacterial activities were assayed in terms of minimal inhibitory concentrations (MICs) by employing the dilution assays according to the CLSI guidelines [196]. The dimethyl sulfoxide (DMSO) which was used as solvent was included as (negative control) with concentrations between 0.05 to 0.5 mg/mL of synthesized compounds. Amoxicillin and kanamycin in DMSO (commercial antibiotics).in the same concentrations range were taken as a positive control. Bacterial stock cultures were supplied on nutrient agar plates. A loopful of bacterial cultures from the nutrient agar plates were diluted with 100 mL nutrient broth in 250 mL a side arm Erlenmeyer flask and aerobically incubated at 37 °C for overnight with continuous shaking. After incubation, cultures were diluted with fresh media to give an O.D of 600 nm of 0.1volum. Fifty μL of standardized 18 h incubated bacterial culture was introduced into test tubes of 5 mL media (Muelen Hintan broth) followed by the addition of various concentration of the studied compounds. The MIC was estimated as the lowest concentration that inhibits the growth of the bacterial strains. All assays were performed in triplicate and MIC's values were noted in mg/mL.

3.4.2 Antibacterial Activity of Synthesis Compounds 2.3-2.22

The antibacterial activities for synthesized compounds were screened by microbroth dilution assays. Standard strains of Gram-negative and Gram-positive bacteria were used. Amoxicillin and Kanamycin were used as references. Minimum inhibitory concentrations ((MIC) mg/mL) of the synthesized compounds against the test microorganisms were established and the results were tabulated in Table 5. Most of synthesized compounds displayed significant antibacterial activity against gram positive bacteria, while few compounds exhibited significant activities against gram negative bacteria. The results exhibited close relationship between the chemical

structure and reactivity against microorganisms. The 1,3,4-oxadiazole-5-thione **(2.3)** gave significant anti-bacterial activity towered gram positive and negative bacteria. This result shows that the hydroxyl group of phenol and thioamide group are play an important role to enhance the anti-bacterial activity. This reactivity decreased with their thioalkyl derivatives **(2.4-2.8)**. However, compound **(2.5)** exhibited highest activity among the thioalkyl derivative and that could be attributed to the existence of the benzimidazole group. The biological activity of compounds **(2.9-2.13)** clearly demonstrated the relationship between the chemical structure and the anti-bacterial activities. The anti-bacterial activity enhances with the existence of hydroxyl group of phenol in hydrazone as well existence of methoxy group at *ortho* position of the phenol in same structure. The significant anti-bacterial activity against (gram negative and gram positive) appeared with compound **(2.12)** which is possessing two methoxy groups at *ortho* position of the hydroxyl group of phenol. The sequence of anti-bacterial activity was **(2.12>2.11>2.10>2.13>2.9)**. These results clearly reflected the decreasing of methoxy group nearby the phenol reduces the antibacterial activity. For instance, structure of compound **(2.9)** which is possessing *para* methyl instead of *para* hydroxyl exhibited the lowest activity. However, compound **(2.13)** possessing *ortho* hydroxyl group showed low antibacterial activity. This believed to be due to the effect of the steric hindrance of *tert*-butyl group at *ortho* position of phenol which reduced the effect of hydroxyl group and reduce their toxophoric effect.

The 2,5-disubstituted 1,3,4-oxadiazoles **(2.14-2.22)** showed antibacterial activity slightly less than the hydrazones. The minimum inhibitory concentrations of these compounds demonstrated the importance of the effect of increasing the hydroxyl and the effect of methoxy group. Compounds **(2.17)** exhibited higher activity against both gram positive and negative bacteria flowed by **(2.16), (2.15)** and **(2.22)**. Even though, compounds **(2.18)** and **(2.21)** possess high antioxidant activity, while their

antibacterial was lower than compounds **(2.17)**, **(2.16)** and **(2.15)**. These results clearly exhibited that the antibacterial activity depend on the toxophoric of phenol and the hindrances of this phenol constringe the toxophoric effect and the antibacterial effect doesn't correlate with the antioxidant activity of the synthesized compounds.

Table 3.10: **Antibacterial activities of synthesized compounds 2.3-2.22**

NO	Bacteria/MICs (mg/mL)							
	Gram-negative bacteria				*Gram-positive bacteria*			
	Acinetobacter calcoaceticus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Bacillus subtilis***	***Enterococcus faecalis***	***Streptococcus pyogenes***	***Staphylococcus aureus***
2.3	0.3	>0.5	>0.5	0.4	0.05	0.1	0.1	0.15
2.4	nd	nd	nd	nd	>0.5	nd	>0.5	>0.5
2.5	0.35	>0.5	0.35	>0.5	0.15	0.2	0.1	0.05
2.6	>0.5	0.5	>0.5	>0.5	0.25	0.35	0.4	0.5
2.7	nd	>0.5	nd	>0.5	>0.5	>0.5	>0.5	>0.5
2.8	0.5	0.5	>0.5	0.5	0.4	>0.5	0.5	>0.5
2.9	>0.5	>0.5	0.35	>0.5	0.35	0.5	0.3	0.35
2.10	0.35	>0.5	0.4	0.25	0.20	0.35	0.25	0.3
2.11	0.3	0.5	0.2	>0.5	0.15	0.25	0.05	0.2
2.12	0.1	0.05	0.4	0.15	0.05	0.2	0.15	0.1
2.13	0.2	0.3	>0.5	0.15	0.2	0.35	0.25	0.25
2.14	>0.5	>0.5	>0.5	>0.5	0.5	nd	0.5	>0.5
2.15	0.4	0.5	>0.5	0.5	0.35	0.4	0.3	0.5
2.16	0.4	0.35	0.2	>0.5	0.30	02	0.25	0.2
2.17	0.15	0.1	0.4	0.1	0.15	0.05	0.1	0.2
2.18	0.3	>0.5	>0.5	0.5	0.25	0.25	0.3	0.4
2.19	nd	nd	nd	nd	0.45	>0.5	0.4	>0.5
2.20	>0.5	>0.5	>0.5	>0.5	>0.5	0.5	>0.5	>0.5
2.21	>0.5	0.5	>0.5	>0.5	0.3	0.35	0.2	0.25
2.22	0.3	0.5	>0.5	0.25	0.25	0.3	0.3	0.2
Amoxicillin,	0.05	<0.05	nd	<0.05	<0.05	<0.05	0.05	<0.05
Kanamycin	<0.05	<0.05	<0.05	<0.05	<0.05	>0.5	<0.05	<0.05

nd; not detected

Chapter 4 : The Antioxidant Properties of The Synthesized Compounds

4.1 Introduction

Reactive oxygen species (ROS) as well the reactive nitrogen species (RNS) are well known to engage in physiological processes and biochemical reactions. They possess prospect to cause oxidative stress which are leading to harmful oxidative reactions in organisms. These free radical reactions are the leading to irrefutable number of human diseases, such as cardiovascular disease,[197] inflammations,[198], cancer,[199] and brain dysfunction. [200]. Compounds have antioxidants ability that play significant role in prohibition oxidative stress that may cause several degenerative diseases. Plants can be considered the primary sources of antioxidants; the protective effect of plant spices and herbs point out the presence of antioxidative and antimicrobial constituents in their tissues.[201]. In this research, a new method has been presented, which synthesized new derivatives possess five membered ring heterocyclic at position six of 2-methylphenol to increase the steric hindrances and mesomeric effect at the same time. These compounds could be promising drugs at the future due to their antioxidant ability and existence of the five membered ring heterocyclic.

4.2 Structure effect on efficiency of antioxidant

In the structure-activity relationships (SAR) literature reviews emphasized robustly at the relationship between the structure and boost / diminution of the antioxidant efficiency in both hydrogen transfer mechanism and electron transfer mechanism. There are diverse factors that play significant role to improving or reducing antioxidant ability. Therefore, when designing new antioxidant, some important parameters should be taken in consideration. These parameters may enhance or enfeeble the antioxidant capacity

4.2.1 Effect of Steric Hindrance and Alkyl group

In the middle of the last century, hindered phenol has drawn attention of many researchers inasmuch of some coveted properties, such as superior antioxidant. Relationship between increasing steric hindrance by alkyl group antioxidant capacity had intensive attention in many literatures. In 1950, Rosenwald *et al.*[202] endeavoured to reveal the structure factors involved in affecting maximum potency of the alkyl phenol. In his study, we found that the increase of one methyl group on *ortho* position to *para* cresol increased the antioxidant capacity of phenol. In another word, the antioxidant ability of 2,4-dimethylphenol is higher than *ortho* cresol and *para* cresol.as depicted in Figure 4.1.

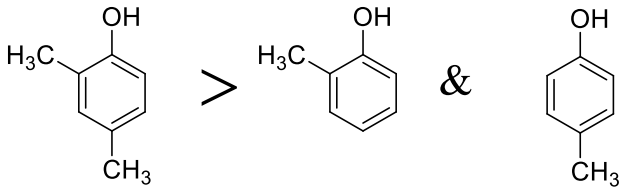


Figure 4.1:-The effect of methyl group on the antioxidant properties.

The same impact has been recorded when *tert*-butyl group was added at *ortho* position. The 2-*tert*-butyl-4-methylphenol exhibited higher antioxidant ability than 2-*tert*-butylphenol and *p*-cresol, as shown in Figure 4.2.

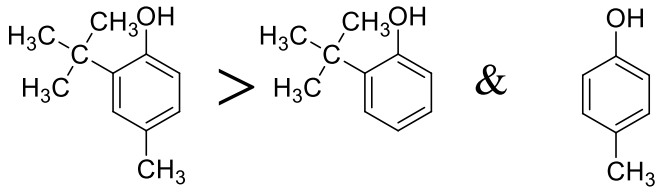


Figure 4.2:-Steric hindrance increases the antioxidant properties.

Furthermore, Rosenwald *et al.* found that expanding the alkyl group at the *para* position does not enhance the antioxidant ability, as demonstrated in Figure 4.3.

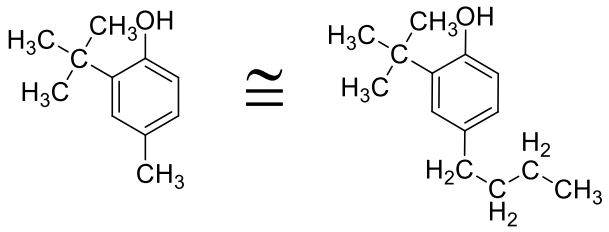


Figure 4.3:-Increasing the size of carbon chain at *para* position does not improve the antioxidant properties.

They elucidated that the *ortho* alkyl phenols are highly efficient than *para* alkyl phenols. Commonly known, increase in to the divaricate of the *ortho* substituent in the monoalkylphenols accretion the antioxidant capacity. They recognised that *the tert*-butyl in *para* position has a destructive effect on the antioxidant capacity. The antioxidant ability of 2,4-di-*tert*-butyl-6-methylphenol is about one half less than , 2,6-di-*tert*-butyl-4-methylphenol as well , the antioxidant ability of the 2,6- dimethyl-4-*tert*-butylphenol is about one eighth less than 2,4-dimethyl-6-*tert*-butylphenol, as demonstrated in Figure 4.4.

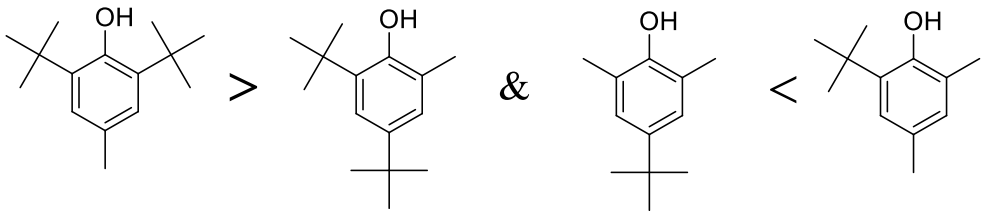
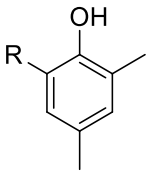


Figure 4.4:-*Tert*-butyl in *para* position decreases the antioxidant properties.

Effect of connect alkyl group at *ortho* position to 2,4-di-methylphenol on antioxidant capacity have been studied by Wasson and Smith[203]. The antioxidant ability estimated in petroleum-based lubricating oil, an efficient antioxidant, which is able to delay oxidation of oil. The effect of substituted group on position six summarized in Table 4.1. Table 4.1 shows the first group substituted on position 6 and life hours of oil without any oxidation.

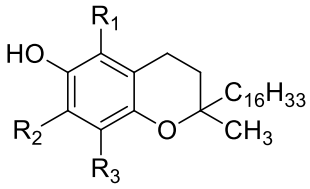
Table 4.1:-Relation of R with antioxidant.



R substituted on position 6	life hours
H	72
Methyl	72
*Iso*propyl	72
sec-butyl	150
tert-butyl	250

These results exhibited that the *tert*-butyl group is preferable in enhancing the antioxidant properties more than other alkyl group in the same position. These studies clearly reflect the effect of the steric hindrance. Moreover, Burton *et al*.[204] investigated the influence of methyl group and there positions on tocopherol as displayed in Table 4.2.

Table 4.2:-Effect of methyl group at tocopherol on antioxidant activities.



Name	R_1	R_2	R_3	K_{inh} ($M^{-1}S^{-1}\times10^{-4}$)
α-Toc	CH_3	CH_3	CH_3	320
BMT	CH_3	CH_3	H	180
β -Toc	CH_3	H	CH_3	130
γ- Toc	H	CH_3	CH_3	140
δ- Toc	H	H	CH_3	44

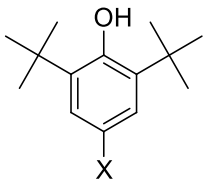
4.2.2 Effect of Bond Dissociation Energy (BDE) of O-H evaluation antioxidant ability

Bond Dissociation Energy (BDE) is one of the fundamental physical guideline, which can be used to estimate the antioxidant ability. In general, when the bond between O-H is weak, it reacts fast with free radicals. In another word, decreasing in BDE value can be considered to increase the antioxidant activity.[205]. Moreover, the electron-donating and electron-withdrawing substituent, steric hindrance and hydrogen bonding of the OH group has leverage on the BDE values.[206-208]. Diverse methods have

been used to achieve useful BDE values, including theoretical calculations using full basis methodology, and locally dense basis sets (LDBS) as described by Wright and Coworkers.[14]. A large number of investigations have been achieved to estimate the effect of substitution as electron-withdrawing groups (EWG) and electron-donating groups (EDG) on O–H BDE. Jovanovic *et al.*[209] reported that BDE value

The BDE value of *para* substituted phenols has been found to be influence with the type of substitutes and it is connected with the Hammett σ^+ values. They developed a photoacoustic method for determining the BDE values of phenols and displayed for the first time that a linear relationship existed between the Hammett σ+ *para*-substituent constant and BDEs. Wayner *et al.*[210] investigated the correlation between experimental ΔBDE for different substituted phenols. The studies proposed that the EWG substituent encourage an increasing of bond dissociation enthalpy value of the O-H bond. Table 4.3 summarized the effect of the EWG and EDG substituent on BDE value.

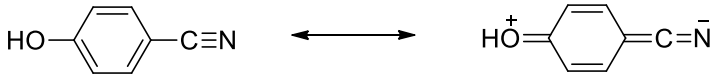
Table 4.3:-Effect the *para* substituted on BDE value [211-213]



Substituent	BDE (kcal/mol)	Substituent	BDE (kcal/mol)
NO_2	84.94	Ph	81.24
COOH	84.27	Me_3C	81.24
CO_2Me	84.1	Me	81.02
CHO	84.23	RS	81.03
CN	84.24	PhCH=CH	78.9
H	82.8	OMe	78.31
Cl	82.41		

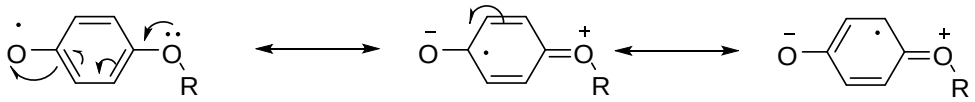
Table 4.3 displayed that the electron-withdrawing group elevates the value of BDE, which is the main decrease of the antioxidant ability. This

could be attributed to stabilization of the phenol by polar structure as shown in Scheme 4.1.



Scheme 4.1:-Stabilization of *p*-cyanophenol.

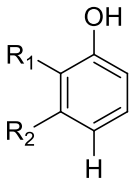
Whereas, the electron donating group decreases the BDE value (increases the antioxidant ability) by stabilization of the phenoxyl radical by mesomeric structures including a positive charge on the substituent Scheme 4.2.[212]



Scheme 4.2:-Mesomeric structure of *p*-alkyloxyphenol.

The effect of *meta* and *ortho* substituent on the BDE value was demonstrated in Table 4.4.

Table 4.4:-Effect *ortho* and *meta* substituted on BDE value.[213]



R1	R2	BDE (kcal/mol)	R1	R2	BDE (kcal/mol)
H	H	87.6	OMe	H	83.7
Me	H	84.1	H	CMe_3	86.6
CMe_3	H	82.8	H	Me	86.7

4.2.3 Effect of position of substitutent.

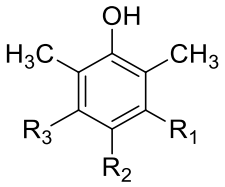
There is a large interaction between all factors, e.g. we could not separate the effect of position and shed factors such as, electronic behaviors of the substituent, EWG or EDG in inductive effect and the intramolecular hydrogen bonding. It is well known that the alkyl group improves the antioxidant ability as in the presented order *o*->*p*->*m*-, mostly when

hydroxyl group flanked by two *ortho*-methyl group or branched alkyl groups such as *iso* propyl, secondary butyl or di *tert*-butyl.

The phenoxy radical stabilized in presence of substituted alkyl by inductive and hyper conjugative effects.

Ortho alkyl groups as well afford steric hindrance and it points out to that the EWG groups reduce the antioxidant capacity at *para* position and vise versa. *para*-methoxy have importance in controlling the antioxidant activities of methoxy phenols due to stereo electronic effects
as demonstrated in Table 4.5[204].

Table 4.5:-Effect of substitution in position 3, 4 and 5.



No.	R_1	R_2	R_3	K_{inh} $M^{-1}S^{-1}\times10^{-4}$
i	H	CH_3	H	8.5
ii	H	OCH_3	H	94
iii	H	OCH_3	CH_3	130
iv	OCH_3	OCH_3	CH_3	39
v	CH_3	CH_3	CH_3	36
vi	CH_3	CH_3	H	11
vii	CH_3	H	CH_3	7.5
viii	H	H	H	2.5

para-methoxy plays a pivotal role of stabilizing a phenoxyl radical by conjugative electron delocalization with the oxygen. Stabilization of methoxy phenols demands the oxygen lone pair at orbital p which can overlap with the semi-occupied orbital (SOMO) of the radical. The capacity of overlap relies on the dihedral angle, θ, between the oxygen lone pair and the SOMO (which is orthogonal to the atoms of the aromatic plane) and the angle θ should be the same as the angle θ' between the $O_1{-}C_2$ bond. This plane is depicted in Figure 4.5. Therefore, the stabilization of the radical will be at a maximum when θ = 0◦ and at minimum when θ =90°.[204, 214]

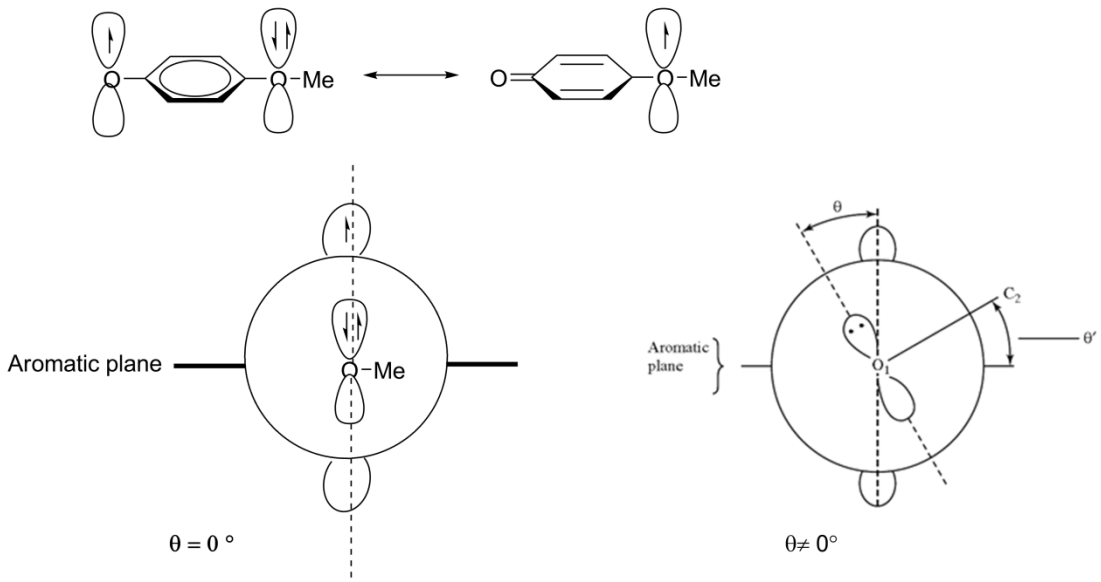


Figure 4.5:-Stereoelectronic effects of heteroatoms on stabilization of free radical.

The antioxidant ability of compound **iv** (39×10^{-4} M^{-1} s^{-1}) is higher than antioxidant of compound **v** (36×10^{-4} M^{-1} s^{-1}) with *p*-methyl as well higher than **vii** (7.5×10^{-4} M^{-1} s^{-1}) without any *p*-substituent, this improvement could be attributed to orthogonal of *para*-methoxyl in **iv** where the activity is inhibited by the withdrawal inductive effect of oxygen. In another hand, it could be attributed to the 'effective' θ for **iv** in solution is less than 90° or, as proposed, the withdrawal inductive effect of an orthogonal methoxy group is outweighed by a remaining of donating mesomeric effect due to a resonance participation from the other lone pair on the oxygen.[204] Furthermore, it has been found that the *p*-SMe is more effective in increasing the antioxidant ability while the sulfur atom inhibited the BDE value.

4.2.4 Effect of intramolecular hydrogen bonding

The intramolecular hydrogen bonding have been reported to reduce antioxidant activity.[215] Generally, the electron donating substitute at position 2,4,6 of phenol can increase the free radical scavenging ability.[215] While Lawandy *et al.*[216] found that the antioxidant ability depends on the position of substituent where *p>o>m* and is attributed to an intramolecular hydrogen bonding.[217] The interest in *ortho*-methoxy

phenols as antioxidants is driven by their frequent occurrence and importance in various natural products including ubiquinols, curcumin, lignin model compounds and others. An *ortho*-methoxy group could offer stabilization of the phenoxyl radical formed by the resonance of the type (Figure 4.6).[218]

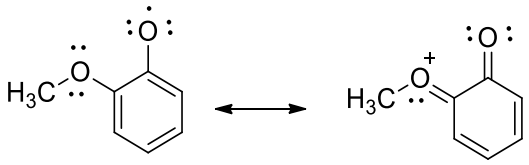


Figure 4.6:-Stabilizing phenoxyl radical by resonance.

The intramolecular hydrogen bonding of *o*-methoxy phenol was demonstrated in Figure 4.7. In a non-polar solvents, less than 0.1% , it exists as a free phenol.[219].

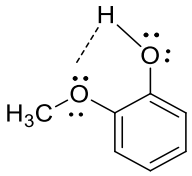


Figure 4.7:-Intramolecular hydrogen bonding of *o*-methoxy phenol.

This intramolecular hydrogen bond is capable to stabilize the parent compound by 4 kcal mol^{-1}. The opposing electronic effect of the methoxy group[14] reduces the efficiency when compared to the *para*-methoxyphenol. The non-linearity of the intramolecular hydrogen bond in the *ortho*-methoxy isomer abandoned the phenolic hydrogen atom available for abstraction.[220] for that, the opposing effects and the activating effect of the *ortho*-methoxy against the stabilizing effect of H-bonding will be capable to reduce the reactivity of the *o*-methoxy isomer compared to the *p*-methoxy. Although, intramolecular hydrogen bonding some time can reduce the antioxidant capacity for *o*-methoxy, the 1,2-dihydroxybenzene (and its derivatives) exhibited extraordinary antioxidant activity when compared with most of *ortho*-methoxyphenols. in nature especially 1,2-

dihydroxybenzene derivatives are excessively existence such as in the flavonoids. This enhancement of the activity could be attributed to boosting stabilization of the semiquinone radical formed from catechol, and of the identically transition state, by powerful hydrogen bonding in resonance canonical structures (a) and (b)[221] as depicted in Figure 4.8.

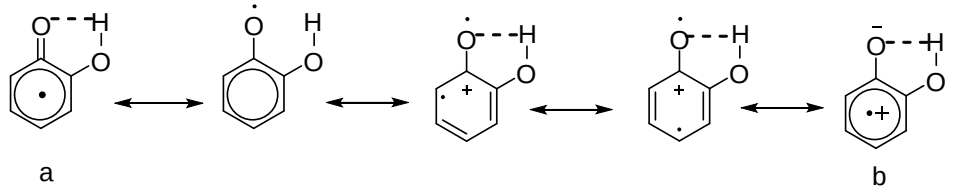


Figure 4.8:-Effect of hydrogen bonding on stability of free radical.

Enhancement stabilization of the 1,2-dihydroxybenzene radical, (by hydrogen bonding) was determined by calculations. The catechol is stabilized by a moderately powerful hydrogen bond of (4 kcal mol^{-1}) whereas, the respective radical has a much powerful hydrogen bond (8 kcal mol^{-1}).[14] As mentioned previously, the electron donating substituent group can enhance the antioxidant activity by boosting the electron density of phenolic oxygen due to the localization of a radical electron of the phenoxyl radical at *para*-position[215]. Many researchers reported that the *meta* position plays a limited role in reducing the antioxidant ability. Kajiyama *et al.*[222] have estimated the antioxidant ability of various groups in *meta* position of phenol such as NH_2, OCH_3 CMe_3, Et and OCH_2Ph. They concluded that the *meta* position does not have any effect for increasing the antioxidant activity.

4.2.5 The solvent effect.

The main exception of alkoxyl radicals from phenols, when abstracted hydrogen attributed to lower the rate of constants in polar (and especially hydrogen-bond-donating) solvents.[223, 224]. This has been due to the participation of the reactive O-H in a hydrogen-bond network, which offers prevention against the offensive of the reactive alkoxyl radical. In other

hand, Litwinienko *et al*.,[225] reported that hydrogen donors with C-H bonds exhibited no noticeable kinetic solvent effects in their reaction with cumyloxyl, [226] peroxyl[227] and 1,1- diphenyl-2-picrylhyrazyl (DPPH) radicals.[228].

From the essential understanding in radical chemistry that only hydrogen abstractions from O-H bonds and not those from C-H bonds, are expected to be slower in polar solvents.[225] in another hand,, Koner *et al*.[229] confirmed that the polar solvent possess effect on a transition state of antioxidant kinetic and also on abstract C-H as shown in Figure 4.9.

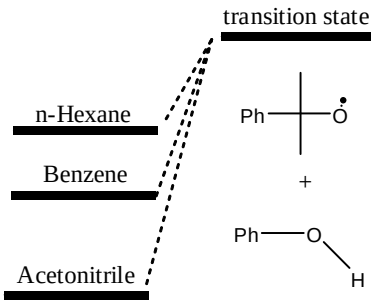


Figure 4.9:-Solvent effect on hydrogen abstraction.

The effect of the solvents on the rate constant of the antioxidants (with the radical species) is dependent on how the solvent interacts with reactants and also on the mechanism of the antioxidant action. Table 4.8 presented an example on the effect of solvent on the rate constant for abstraction hydrogen of O-H phenol with DPPH and α-TOC (TOH).

Table 4.6:-Solvent effect on rate of constant of abstraction O-H.

Solvent	$K\times10^{-3}$ PhOH+DPPH	$K\times10^{-2}$ TOH+DPPH
***n*-pentane**	-	74
***n*-octane**	160	74
carbon tetrachloride	93	36
chlorobenzene	59	27
benzene	31	18
anisole	7.2	14
Acetonitrile	-	4.9
Acetic acid	3.1	6.2
***tert*-butyl alcohol**	2.9	5.7

4.3 Methods for evaluation of antioxidant activity.

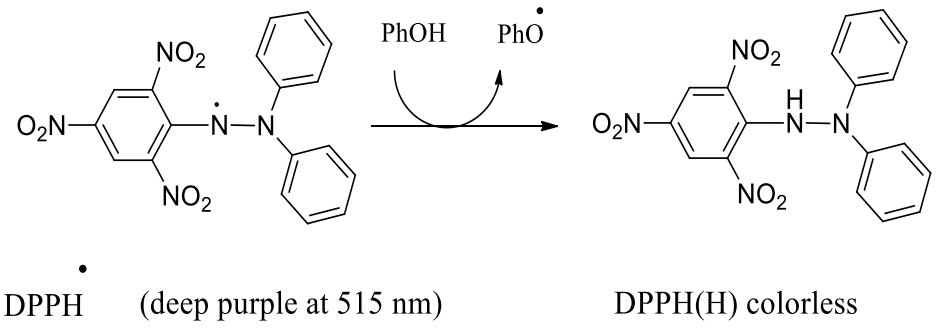
There are many methods that had been utilized to estimate the activity of natural and synthetic antioxidant [230-232] The antioxidant assays were classified into two classes which depend on the antioxidant mechanism, either H-atom transfer reaction (HAT) it is based on electron transfers (ET).[233]. The assays based on HAT for evolution of the antioxidant such as oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), DPPH assay and crocin bleaching assays.

The assays based on ET depend on the reduction of an oxidant accompany with the colour changes. The degree of colour change is corresponded with the concentrations of sample antioxidant. The assays based on ET mechanism contain the total phenols assay by Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP) and “total antioxidant potential” assay using a Cu (II) complex as an oxidant. According to that, it is proposed that the total phenols assay by FCR can be applied to quantify an antioxidant reducing capacity and the ORAC assay can be used to quantify peroxyl

radical scavenging ability.[234]. In this research, it has been focused on two methods in evaluation of the antioxidant activity of the newly synthesized compounds. First method, DPPH assay was used which depends on HAT mechanism and the second method was FRAP assay which depends on SET mechanism.

4.3.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay.

The DPPH is stable and free radicals are commercially available.It was used for determining the antioxidant capacity. The DPPH is a nitrogen cantered radical having maximum absorbance at 515 nm. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical transform to 1,1-diphenyl-2-picryl hydrazine on reacting with hydrogen donating species.[235] In 1954, Braude *et al.*[236] reported that DPPH$^{\bullet}$ undergoes a HAT mechanism with antioxidant as depicted in Scheme 4.3.



Scheme 4.3:-Reaction of DPPH radical in presence of phenol under HAT mechanism.

In 1958, Blois [237] reported that the phenolic compound possess more than one phenolic hydroxy functional group, the ArO$^{\bullet}$ radical which is formed is sufficiently stable enough to undergo to second HAT reaction simultaneous with the second molecule of DPPH. The DPPH$^{\bullet}$ assay has great attention to estimate antioxidant capacity of phenolic compounds in plants, their derived especially that used as food products.[238]. Even

though, several researchers reported that the mechanism reaction of DPPH with phenol undergoes HAT mechanism.[239-241], another researcher proposed that the reaction of DPPH with phenols follow single electron transfer (SET) mechanism as exhibited in Scheme 4.4 .

DPPH•(Violet at 515 nm) + ArOH → DPPH−(Colorless) +[ArOH]•+(SET)

Scheme 4.4:-Reaction of DPPH radical with phenol under SET mechanism.

Furthermore, some researchers proposed that this mechanism is a mix between HAT and SET[242] as well the steric hindrance of an antioxidant compounds control the type of reaction mechanism. [234]. In 2000 Cano *et. al*. reported that interference of the colour of the samples such as in anthocyanines may lead to reduce the antioxidant activity.[243]. The insufficiency of this assay is the bulky antioxidant like BHT protocatechuic acid. Brand-Williams et al reported that the bulky antioxidant reaches the end point after 3hours while the most antioxidant reaches the end point after 2 hours [240]. In spite of this, the method is still widely used to determine antioxidant ability.[244-246]. It has been recognized that the medium of reaction has a significant effect on the % inhibitions of DPPH value.[247-249]. The percent of inhibition of DPPH value can be estimated from the following equation[250]:

$$\%\ \textbf{inhibition of DPPH} = \frac{A_o - A1}{A1} \times 100$$

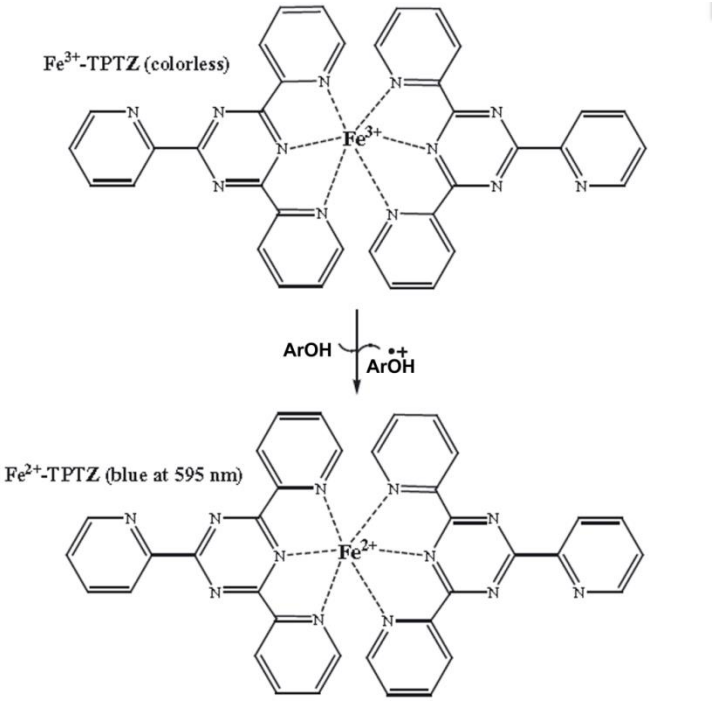
Where A_o is known as the absorbance of a standard excluded any sample while the $A1$ defined as the absorbance of synthesized sample at 515 nm.

4.3.2 Ferric reducing antioxidant power (FRAP) assay

Benzie and Strain[251] constructed a new assay to determine the ferric reducing power of human plasma. This assay depended on ferric reducing antioxidant power (FRAP) of plant extracts and to determine antioxidant in food as well as other synthetic compound.[252]. The Fe^{3+}-

TPTZ (iron[III]-2,4,6-tripyridyl-*s*-triazine) reduced to Fe^{2+}−TPTZ by SET with an antioxidant compound.

The effect of this reaction is changing the colourless solution to a powerful blue colour at λ_{max}= 595 nm, as exhibited in Scheme 4.5.



Scheme 4.5:-Reduction of Fe^{3+}−TPTZ to Fe^{2+}−TPTZ in presence of phenol.[253].

This assay considered interested method for evaluation to the conjugation in phenols as well as the number of hydroxyl constituents.[254]. In other hand, it has been noted that the FRAP value afford different results depending on the time of measurement besides to the reaction medium.[234]. The acid medium is the preferred environment for this assay.

The assay reaction must be carried out in an acidic environment in order to enhance the iron solubility. Even though the acid medium can lower the ionization potentials (IP) of the reactants and reduce the redox potential of the system.[234]. The interference between antioxidant assay and effect of solvent has been reported by Saliha Esin *et al*.[255] and they

showed it takes the following of this order of ORAC > ABTS > DPPH > FRAP.

This result demonstrated that the FRAP assay has less interference with solvent. The following equation.[256] was used to estimate the FRAP value:

$$\textbf{FRAP value} = \frac{0\text{–}4\ min\ \Delta A593nm\ of\ test\ sample}{0\text{–}4\ min\ \Delta A593nm\ of\ standard} \times [standard]\ \ (\mu M) \times Y \times 1000$$

Y is the absorbance of the spectrophotometer.

4.4 Results and Discussion

4.5.1 Antioxidant ability of the synthesized 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol (2.3) and their thioalkyl (2.4-2.8)

The 5-thio-1,3,4-oxadiazole ring successfully formed at position six of the *o*-methyl phenol. As well, their thioalkyl derivatives as demonstrated in Figure 4.10.

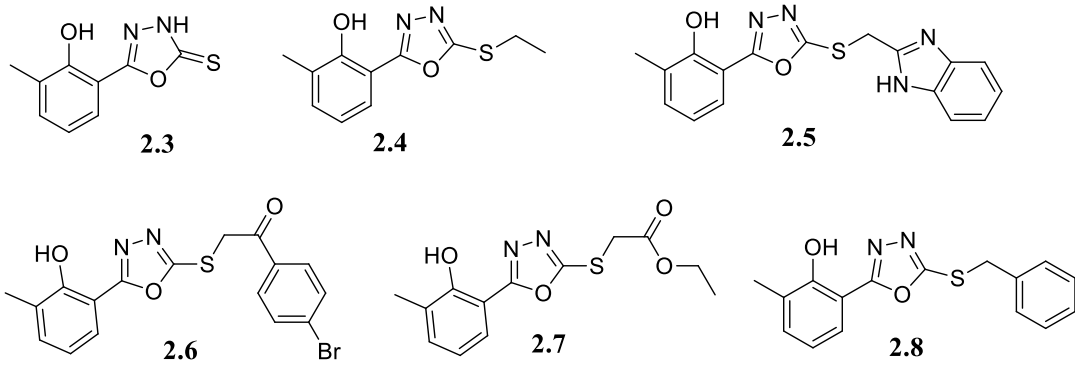
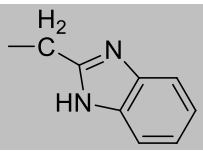
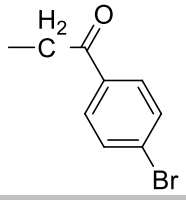
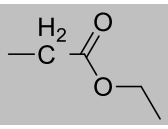
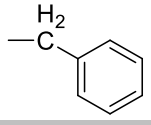


Figure 4.10:-Proposed structure of compound (2.3) and their derivatives (2.4-2.8)

The antioxidant ability of these compounds was tested by DPPH and FRAP assays The radical scavenging power in both assays was compared with three standard antioxidants, 2-methylphenol, butylated hydroxy toluene (BHT) and ascorbic acid. Compound **(2.3)** exhibited highest inhibition percentage in DPPH assay than their alkyls (**2.4-2.8)**. This

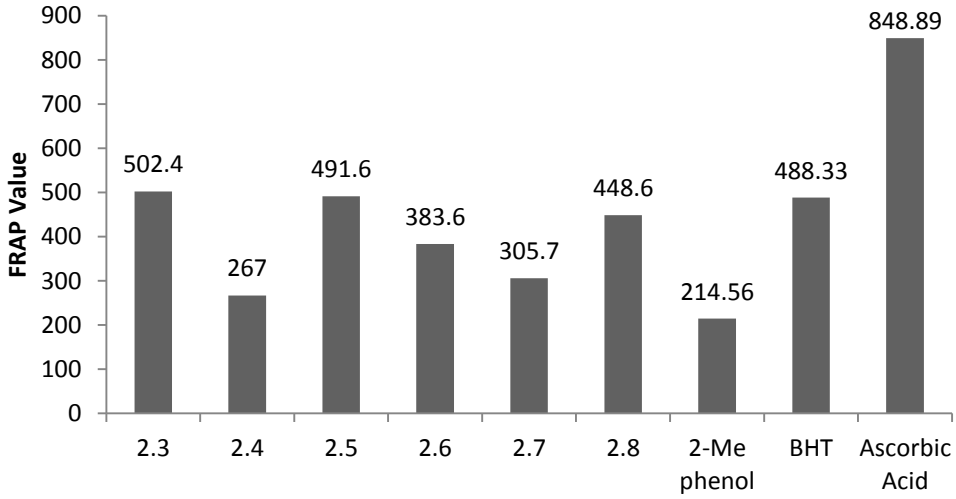
diminution of antioxidant properties of alkyl derivative of compound **(2.3)** could be attributed to the disappearing of the thioamide group which were reported as free radical scavengers.[257] Moreover, the NHCS group is considered as a part of thiourea system which is known as effective antioxidant.[258] The alkyls derivatives of compound **(2.3)** showed inhibition percentage higher than 2-methylphenol (2-Me Phenol). Compounds **(2.3)** and **(2.5)** exhibited DPPH inhibitions slightly higher than BHT, whereas their IC_{50} value was less than BHT, as displayed in Table 4.7.

Table 4.7:-DPPH inhibition and IC_{50} of the 5-thio-1,3,4-oxadiazole and their thioalkyl derivatives

Compound No.	Alkyl Group	DPPH Inhibition % ± SD [a] 100µg/mL	IC_{50} ±SEM [b] (100µg/ml)
2.3	-	**68.21± 0.0243**	**43.101±0.0451**
2.4	Et	**34.16± 0.0513**	**> 100**
2.5		**66.72±0.0129**	**49.03±0.0335**
2.6		**45.07±0.037**	**89.90±0.0172**
2.7		**38.63±0.0662**	**> 100**
2.8		**48.66±0.0212**	**84.08±0.0568**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

[a] (SD) Standard Deviation; [b] (SEM) Standard Error of the Mean and IC_{50}: 50% effective concentration

The FRAP value of compounds **(2.3)** and **(2.4-2.8)** were compatible with DPPH results as displayed in Figure 4.11. The FRAP value of compound **(2.3)** showed the highest antioxidant activity than their alkyl derivatives. The decreasing in FRAP value of compounds **(2.4-2.8)** could be attributed to lose the NHCS group as mentioned earlier. Compound **(2.5)** with thiomethyl benzimidazole exhibited higher antioxidant properties than the other thioalkyl in both assays and that could be attributed to precipitation of NH of banzimidazole in antioxidant properties[259-261]. Assuming that the formation of oxadiazole ring at position six of the 2-methyl phenol could play a pivotal role to enhance the antioxidant ability by increasing the steric hindrance which leads to increase the antioxidant capacity[262].



Standard deviation (SD) value in FRAP was between 0.01–0.16.

Figure 4.11:-FRAP value of compounds (2.3-2.8).

4.5.2 Antioxidant ability of the synthesized hydrazones (2.9-2.13)

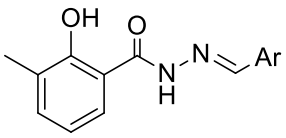


Figure 4.12:-General structure of hydrazones

The hydrazones **(2.9-2.13)** exhibited antioxidant ability in both assays higher than their corresponding oxadiazole **(2.14-2.18)** and that could be

attributed to the generic structure of the newly synthesized hydrazones (**2.9-2.13)**. The structure of these hydrazons includes a recognized free radical scavenger as phenol group (ring A), the NH as secondary amine which considered as alternative secondary antioxidant, the imine group (N=CH) as shown in Figure 4.13 which enable resonance with aromatic (ring B) and their substituted groups such as 4-CH_3 ,4-OH, 3-OMe-4-OH, 3,5-di-OMe-4-OH and 2-OH-3,5-di-*tert*-butyl group which act as electron donating group (EDG) to immerse the radical scavenging activity of phenols[263]. These EDGs enable to lower the bond dissociation enthalpy (BDE) of the phenol (ring B) as well increase the stabilization of the phenoxyl[264].

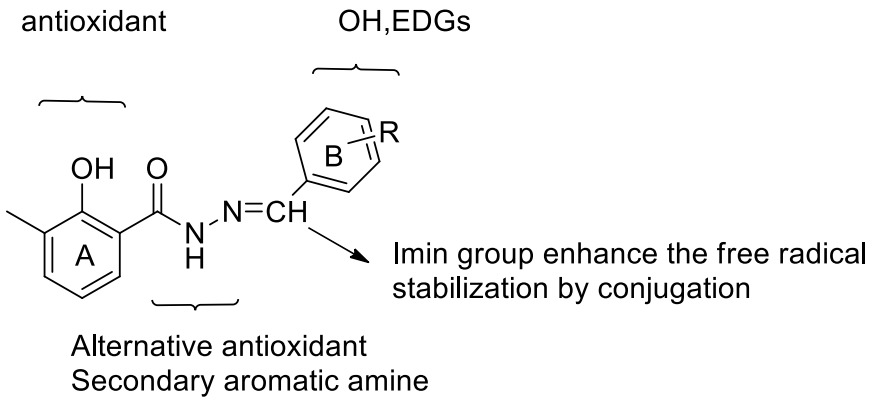
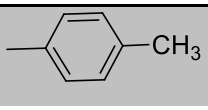
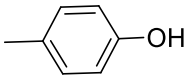
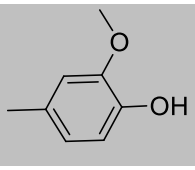
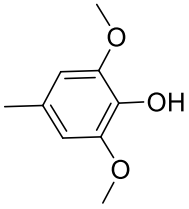
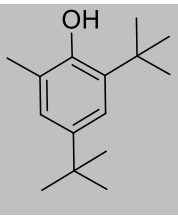


Figure 4.13:- General structure of hydrazones (2.9-2.13).

The DPPH inhibitions percentage and IC_{50} value were tabulated in Table 4.8

Table 4.8:-DPPH inhibitions percentage and IC_{50} value of hydrazones (2.9-2.13).

No.	Ar	DPPH Inhibition % ± SD 100µg/mL	IC_{50} ±SEM (100µg/ml)
2.9		**65.72±0.0183**	**> 100**
2.10		**69.83±0.0311**	**78.32±0.027**
2.11		**74.65±0.0615**	**60.04±0.0198**
2.12		**91.14±0.0187**	**38.09±0.061**
2.13		**87.31±0.030**	**43.15±0.0306**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

Compound **(2.9)** with 4-methyl group exhibited lowest antioxidant ability among the hydrazons however, their DPPH scavenging activity was slightly similar to BHT. Compound **(2.12)** with two substituted methoxy group at the *ortho*-position of phenol displayed inhibition percentage in DPPH assay slightly higher than ascorbic acid, while DPPH scavenging activity of compound **(2.11)** with one substituted methoxy group at *ortho* phenol showed less antioxidant activity than ascorbic acid. Both compounds **(2.12)** and **(2.11)** showed antioxidant activity higher than compound **(2.10)** without any *ortho* substituted of the phenol group as

depicted in (Table 4.8). These results are consistent with the concept that the number of hydroxyl group and the electron donating group enhance the antioxidant capacity[265]. Although compound **(2.13)** has di-*tert*-butyl group flanking the OH, it displayed DPPH inhibition percentage less than compound **(2.12)** and that could be attributed to the presence of the second *tert* butyl group at position *para* which has a detrimental effect on the antioxidant properties[266]. The FRAP value of the hydrazones consistent with the DPPH results Figure 4.14 and it showed the same sequence; 2.12>2.13>2.11>2.10.

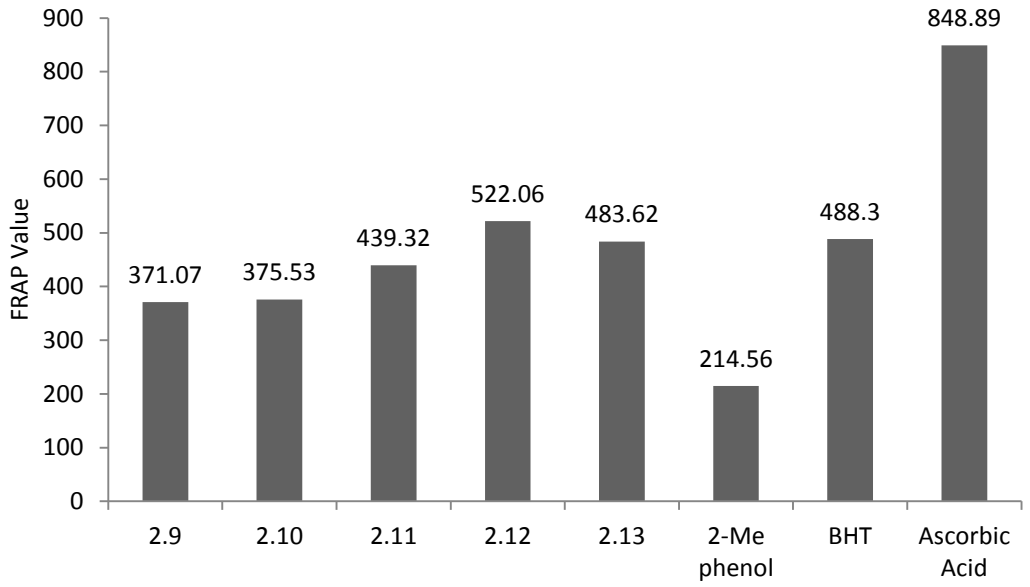
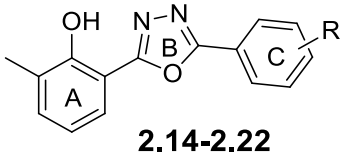


Figure 4.14:-FRAP value of the hydrazones (2.9-2.13).

4.5.3 Antioxidant ability of the synthesized 1,3,4-oxadiazole



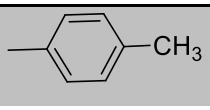
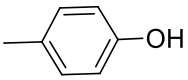
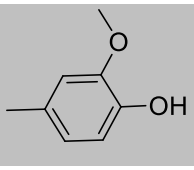
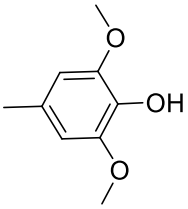
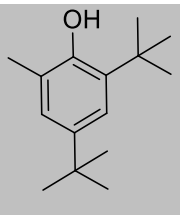
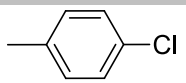
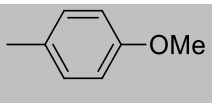
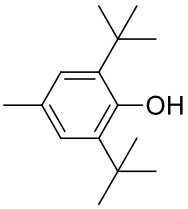
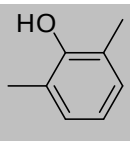
2.14-2.22

Figure 4.15:-General structure of 1,3,4-oxadiazole (2.14-2.22).

The DPPH scavenger activity (Table 4.9) and FRAP values of the 1,3,4-oxadiazoles **(2.14-2.22)** exhibited less activities than their corresponding hydrazone and that could be attributed to evanescence the

CONHN=CHAr group which is involved to enhance the antioxidant activity. However, the oxadiazoles included hydroxyl group at (ring C) which showed antioxidant capacity more than without hydroxyl group. Furthermore, the antioxidant capacity of these oxadiazoles declined with the decrease of the hindrances around the hydroxyl group of phenols. In other word, compound **2.21** with two di-*tert* butyl group around *para* hydroxyl exhibited DPPH inhibition 93.09 % and IC_{50}=31.50 and compound **2.17** with two methoxy group around *para* hydroxyl showed DPPH inhibition 82.14% and IC_{50}=49.04. The antioxidant ability decreases with compound **2.18** , **2.16** and **2.15** respectively. The DPPH inhibitions of oxadizoles without hydroxyl substituted (**2.14**, **2.19** and **2.20**) exhibited antioxidant ability depending on the substituted group and it takes the following order 4-Me **(2.14)**>4-OMe **(2.20)**>4-Cl **(2.19)**. The FRAP value of these oxadiazoles was in agreement with the DPPH inhibition percentage as shown in Figure 4.16.

Table 4.9:-DPPH inhibitions percentage and IC_{50} value of 1,3,4-oxadiazole (2.14-2.22).

No.	Ar	DPPH Inhibition % ± SD 100µg/mL	IC_{50} ±SEM (100µg/ml)
2.14		**59.66±0.029**	**> 100**
2.15		**62.18±0.021**	**81.96±0.022**
2.16		**68.90±0.027**	**80.11±0.014**
2.17		**82.14±0.023**	**49.04±0.018**
2.18		**78.33±0.034**	**53.08±0.012**
2.19		**41.70±0.052**	**> 100**
2.20		**54.72±0.041**	**82.39±0.027**
2.21		**93.09±0.033**	**31.50±0.0189**
2.22		**74.31±0.062**	**57.83±0.0171**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

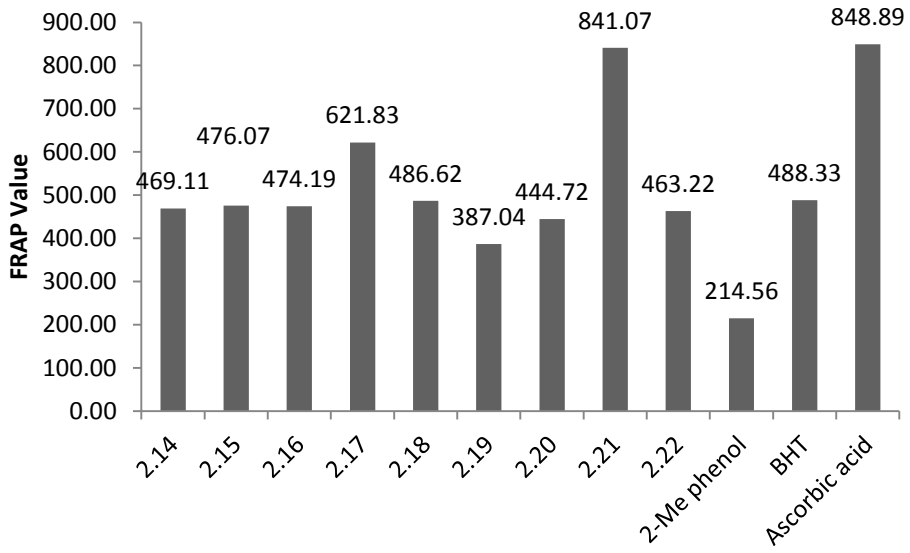
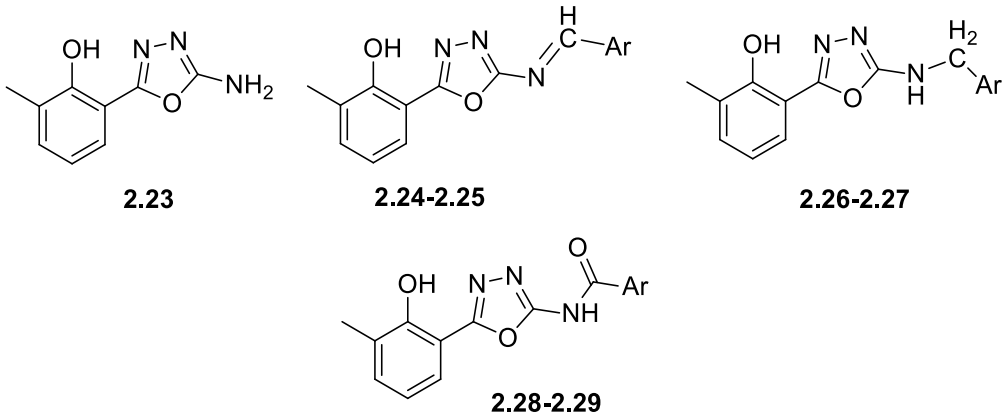


Figure 4.16:-FRAP value of the 1,3,4-oxadiazole (2.14-2.22)

4.5.4 Antioxidant ability of the synthesized 6-(5-amino-1,3,4-oxadiazole-2-yl)-2-methylphenol (2.23) and their derivatives (2.24-2.29)



The antioxidant properties of the oxadiazole amine **2.23** and there derivatives (**2.24-2.29**) showed antioxidant ability higher than 2-methylphenol. The DPPH inhibition showed that all derivatives of compound **2.23** recorded antioxidant activity higher than compound **2.23**, while compound **2.28** recorded antioxidant lower than compound **2.23** as displayed in Table 4.10. The free radical scavenging activity of their Schiff bases derivatives (**2.24-2.25**) showed antioxidant higher than their

reduction product. One word, the compounds **2.26** and **2.27** possess four agents which could enhance the antioxidant ability e.g. two hindered phenol, resonance and secondary amine group are considered as alternative secondary antioxidant[267]. The Schiff bases derivatives (**2.24-2.25**) has CH=N group instead of the NH. The CH=N enables resonance between two adjacent aromatic rings [268] (ring B and ring C). Clearly form these results it can be deduced that the resonance is more affected in the enhancement of the antioxidant ability by increasing the stability of their free radical after donating his proton. Figure 4.17 demonstrated the resonance possibility of the radical of compound **2.24.**

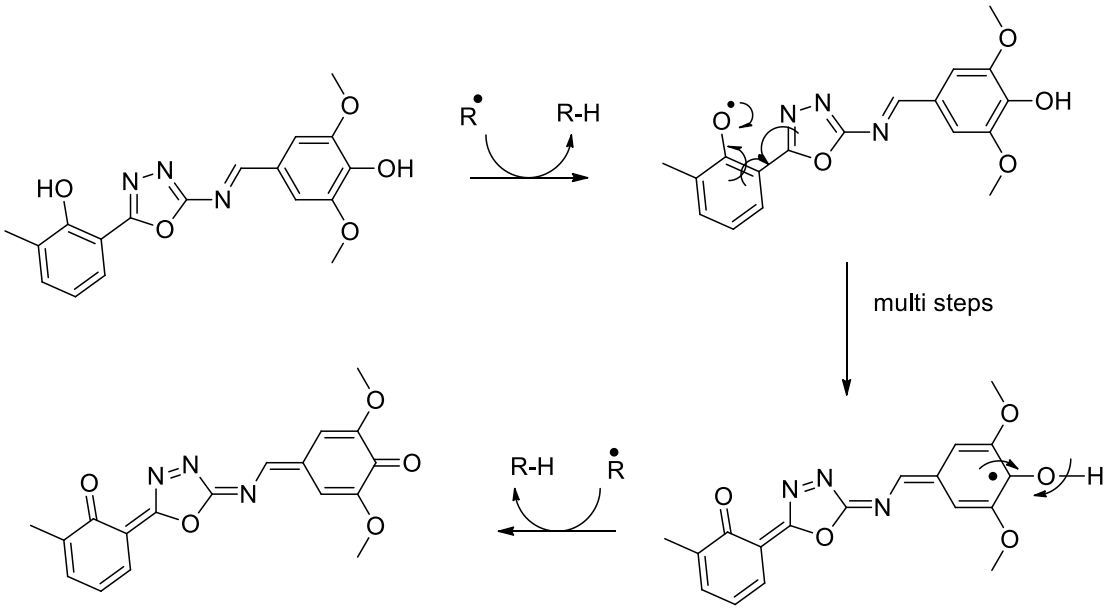
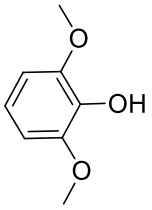
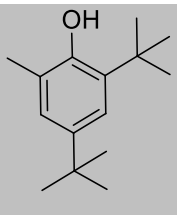
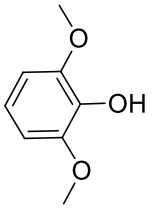
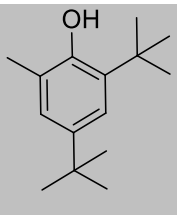
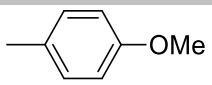
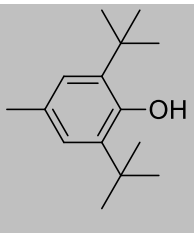


Figure 4.17:-Possible resonance structure of compound 2.24

Otherwise, existence two antioxidant group or more in the same structure sometime lead to possess pro-oxidant properties [269] which can decrease the antioxidant properties[270]. This fact could be responsible for decreasing the antioxidant ability of compound **2.26** and **2.27**. The antioxidant ability of compound **2.26** and **2.27** showed moderate DPPH inhibition, even though compound **2.27** owns 3,5-di-*tert*- butyl phenol in its structure. Obviously, this result clearly indicated that the resonance effect is more efficient than the existence of another radical scavenging group.

Table 4.10:-DPPH inhibitions percentage and IC_{50} value of oxadiazole amine (2.23) and their derivatives (2.24-2.29)

No.	Ar	DPPH Inhibition % ± SD 100µg/mL	IC_{50} ±SEM (100µg/ml)
2.23	-	**63.67±0.024**	**52.08±0.02**
2.24		**89.81±0.011**	**49.11±0.031**
2.25		**81.32±0.016**	**60.95±0.011**
2.26		**72.06±0.038**	**71.15±0.013**
2.27		**70.22±0.017**	**78.38±0.021**
2.28		**56.75±0.0271**	> 100
2.29		**82.46±0.0162**	**58.34±0.031**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

Although compounds **2.24-2.27** exhibited antioxidant ability higher than BHT, the IC_{50} for these compounds was lower than IC_{50} of BHT. The IC_{50} value of compound **2.27** was a bit lowest value than BHT but higher than ascorbic acid. Compound **2.28** showed antioxidant about two times more than 2-Me phenol but less than BHT and its IC_{50} value was undetectable (> 100). Compound **2.29** with 2,6-di-*tert*-butyl phenol exhibited significant antioxidant ability 82.46 with IC_{50} = 58.34.

The FRAP value of these compounds exhibited the same antioxidant ability comparing with the standard antioxidants as depicted in Figure 4.18.

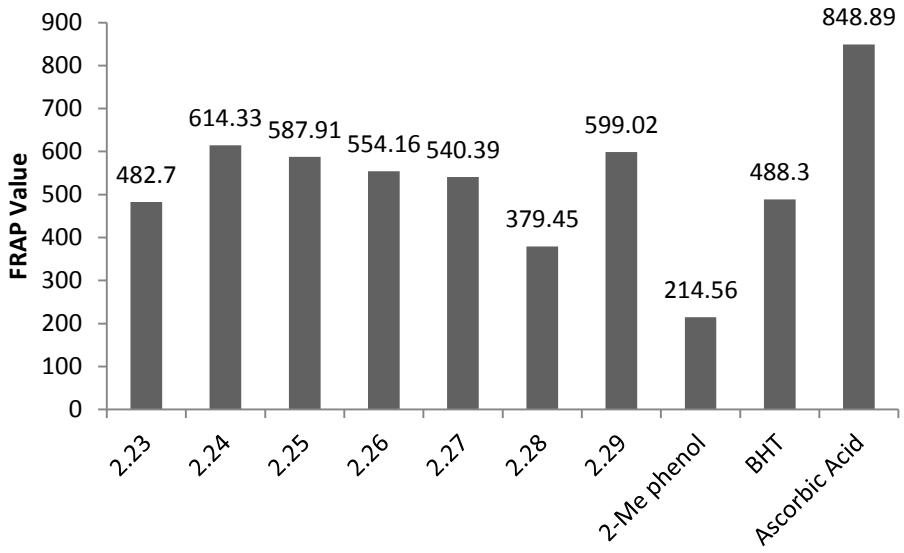
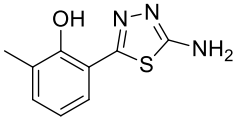
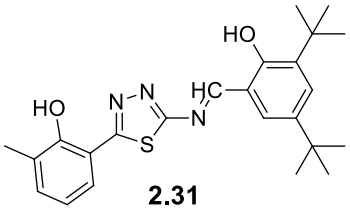


Figure 4.18:- FRAP value of the oxadiazole amine (2.23) and their derivatives (2.24-2.29).

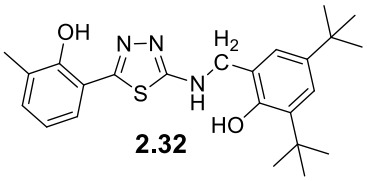
4.5.5 Antioxidant ability of the 6-(5-amino-1,3,4-thiadiazole-2-yl)-2-methylphenol (2.30) and their derivatives (2.31-2.33).



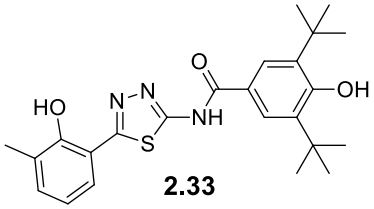
2.30



2.31



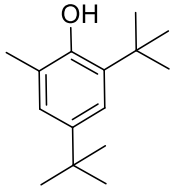
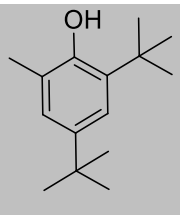
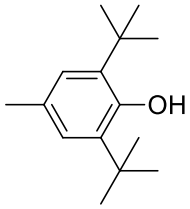
2.32



2.33

The antioxidant properties of these series are too close to antioxidant properties of the oxadiazole amine series and their derivatives (**2.25**, **2.27** and **2.29**). The similarity in DPPH as tabulated in Table 4.11. This similarity could be attributed to the similarity in the general structure. Furthermore, the type of the five membered ring (1,3,4-oxadiazole or 1,3,4-thiadiazole) is not effective in enhancing the antioxidant ability. In other word, the antioxidant ability depends on the steric hindrances caused by the five membered ring and on long chain resonance which the five membered ring participating with it.

Table 4.11:- DPPH inhibitions percentage and IC_{50} value of thiadiazole amine (2.30) and their derivatives (2.31-2.33)

No.	Ar	DPPH Inhibition % ± SD 100µg/mL	IC_{50} ±SEM (100µg/ml)
2.30	-	**65.44±0.02**	**>100**
2.31		**80.45±0.017**	**78.66±0.02**
2.32		**71.17±0.021**	**80.29±0.0141**
2.33		**83.62±0.013**	**67.43±0.017**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

Moreover, the similarity in FRAP value for these series with FRAP value of previous series enhances our assuming that the type of five membered ring does not play an important role in improving the antioxidant ability. Figure 4.19 demonstrates the FRAP value of these series.

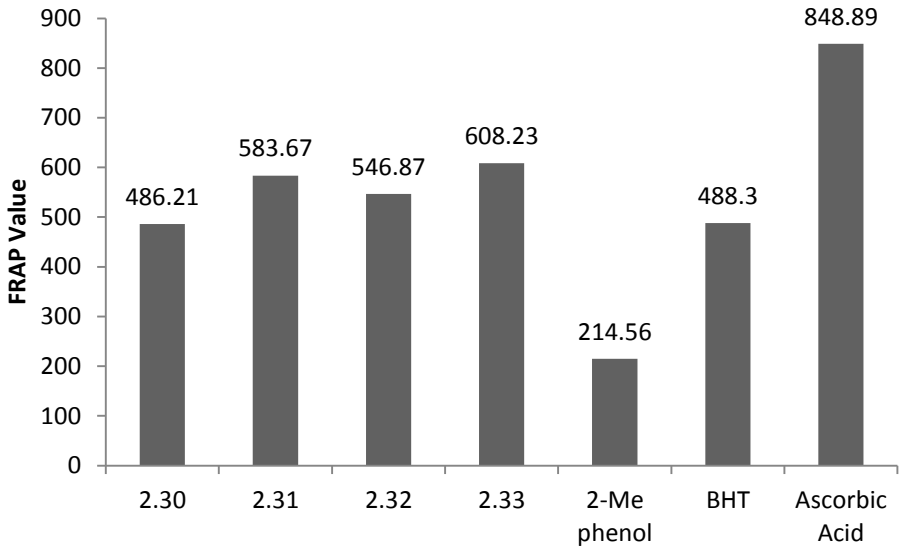
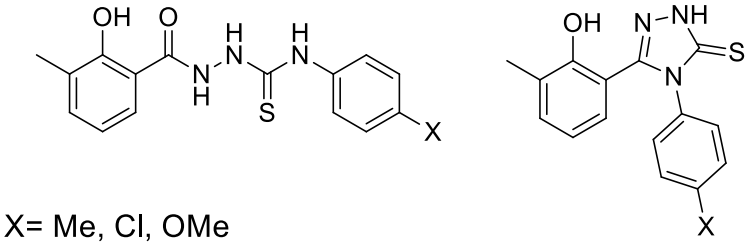


Figure 4.19:-FRAP value of the thiadiazole amine (2.30) and their derivatives (2.31-2.33).

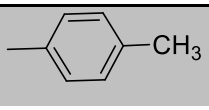
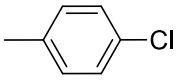
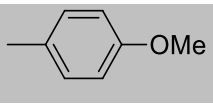
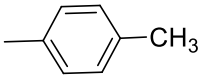
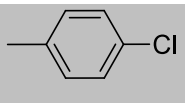
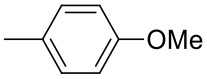
4.5.6 Antioxidant ability of the 2-(3-methyl-2-hydroxybenzoyl)-N-(4-aryl)-hydrazinecarbothioamide (2.34-2.36) and their 2-methyl-6-(4-(aryl)-1,2,4-triazol-3-yl-5-thione)phenol (2.37 -2.39)



X= Me, Cl, OMe

The hydrazinecarbothioamide derivatives (**2.34-2.36**) showed significant DPPH inhibition percentage. All these compounds showed DPPH inhibition higher than BHT and slightly less than ascorbic acid (Table 4.12). Furthermore, the DPPH inhibitions for these compounds are too closed together. This result indicted that the *para* substituted group (EDG or EWG) does not possess any activity to improve the antioxidant ability, the same results have been observed with FRAP value (Figure 4.21).

Table 4.12:-DPPH inhibitions percentage and IC_{50} value of compounds (2.34-2.39)

No.	Ar	DPPH Inhibition % ± SD 100µg/mL	IC_{50} ±SEM (100µg/ml)
2.34		**88.23±0.01**	**32.43±0.0141**
2.35		**87.80±0.013**	**34.25±0.022**
2.36		**88.12±0.021**	**40.21±0.01**
2.37		**72.96±0.01**	**51.77±0.023**
2.38		**73.07±0.017**	**53.16±0.015**
2.39		**72.66±0.023**	**54.37±0.02**
2-Me phenol		**20.15±0.0189**	**> 100**
BHT		**66.03± 0.022**	**79.835±0.015**
Ascorbic acid		**90.65±0.025**	**22.71±0.020**

The corresponding triazole (**2.37-2.39**) exhibited decreasing of antioxidant ability in both DPPH and FRAP assays. The antioxidant ability of these triazoles was too closed together. These result sported previous assumption that the substituent group at the *para* position did not affect the antioxidant ability. The diminution in DPPH inhibitions and FRAP value of the triazoles (**2.37-2.39**) could be elucidated by their structural differences. The structures of hydrazinecarbothio amides (**2.34-2.36**) possess three primary antioxidant groups, while the trizoles (**2.37-2.39**) has just two primary antioxidant group as demonstrated in Figure 4.20.

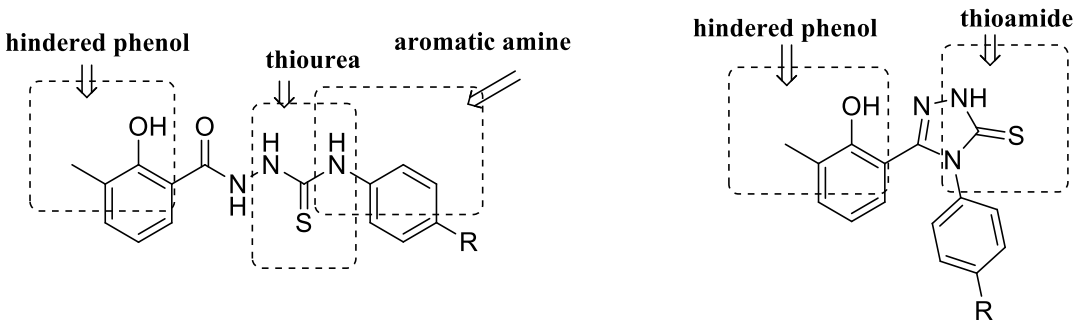


Figure 4.20:-Comparison between hydrazinecarbothio amides (2.34-2.36) structure with triazole derivatives (2.37-2.39).

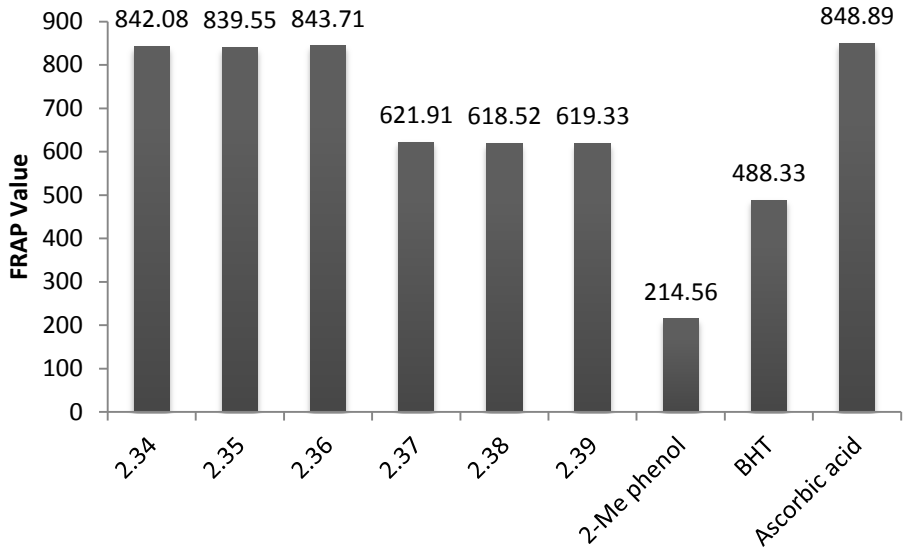


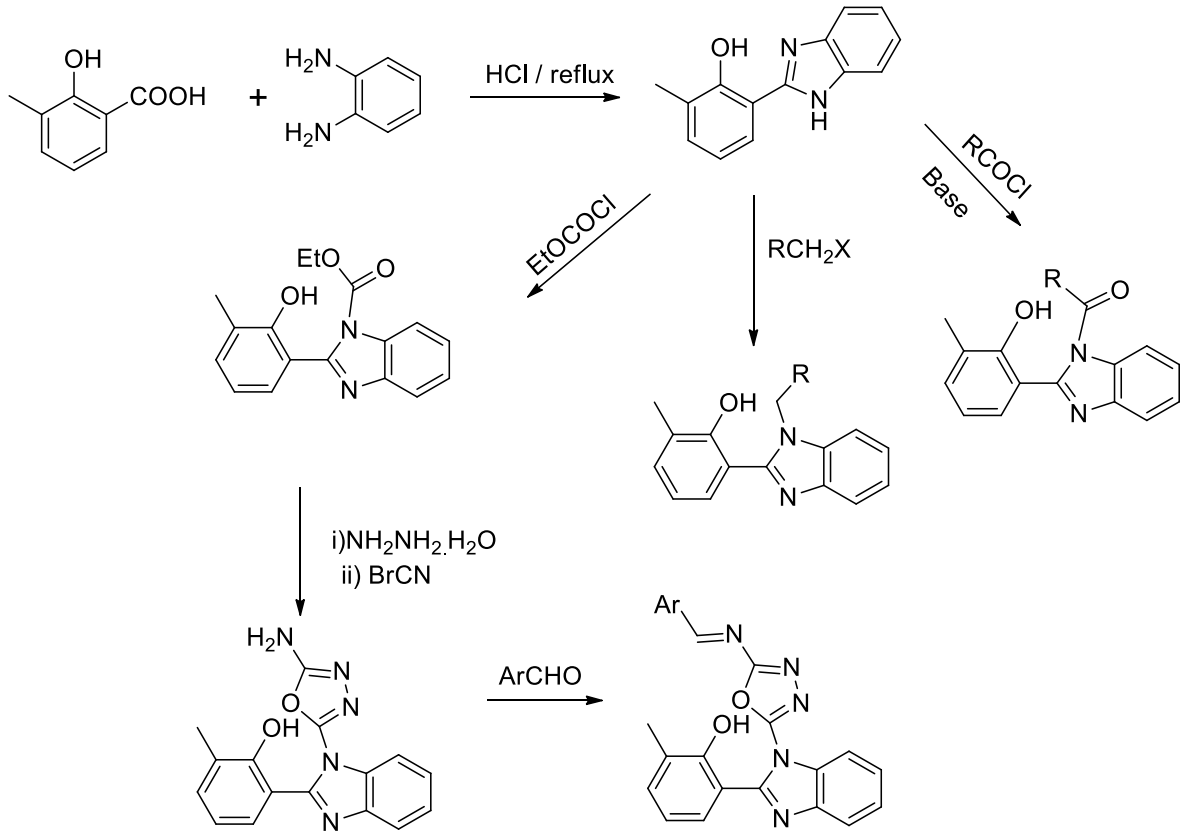
Figure 4.21:- FRAP value of compounds (2.34-2.39).

Conclusion

Newly thirty nine compounds successfully synthesized at position six of the 2-methylphenol to increase their antioxidant ability by increase the sterical hindrance around the hydroxyl of phenol. Moreover, these compounds can enhance the antioxidant ability by stabilized the phenolic free radicals by resonance. 1,3,4-oxadiazole-5-thione (**2.3**) exhibited higher antioxidant ability than it thioalkyl derivatives (**2.4-2.8**), and that could be attributed to existence of thioamide group. However, all thioalkyl derivatives exhibited antioxidant ability in both DPPH and FRAP assays. The hydrazones (**2.9-2.13**) of corresponding acid hydrazide as substrate to synthesized 5-aryl-1,3,4-oxadiazole (**2.14-2.18**) showed DPPH inhibition and FRAP values higher than corresponding 5-aryl-1,3,4-oxadiazole. Generally, the type of substituted group at the 5-aryl for compounds (**2.14-2.22**) and their position dominated at the antioxidant ability, in other word decrease or reduce the antioxidant ability.as well as 1,3,4-oxadiazole-5-amine (**2.23**) was formed as the same position with 2-methylphenol and six derivatives. The antioxidant of the Schiff bases with 2,6-dimethoxyphenol showed higher antioxidant than 1,3,4-oxadiazole-5-amine and other derivatives. as 1,3,4-thiadiazole-5-amine (**2.30**) and their derivatives (**2.31-2.33**) showed same antioxidant behavior. In other word, that displayed the antioxidant ability depended on the sterical hindrances caused by the five membered ring and long chain resonance which the five membered ring participated with it. hydrazinecarbothioamide derivatives (**2.34-2.36**), which are synthesized to be substrate for formation 4-aryl-1,2,4-triazol-3-yl-5-thione **(2.37-2.39)** ring showed significant DPPH inhibition percentage and FRAP values than their corresponding 1,2,4-triazole. This result could be attributed that sharing thiourea derivatiuves and secondary amine as free radical scavenger group.

Future work

According to success our method by enhance the antioxidant ability of sub sterical hindrance phenolic compounds which is already known as weak by formation heterocyclic ring nearby to hydroxyl group our future work will be divided to two pathway. The first pathway study the anti-inflammatory ability for these compounds, where most inti-inflammatory compounds exhibited good antioxidant ability. The second pathway, is to continue formation heterocyclic at same position to increase the hindrance around the phenolic hydroxyl as well increase the long conjugated as depicted in Scheme below.



Appendix A IR Spectra

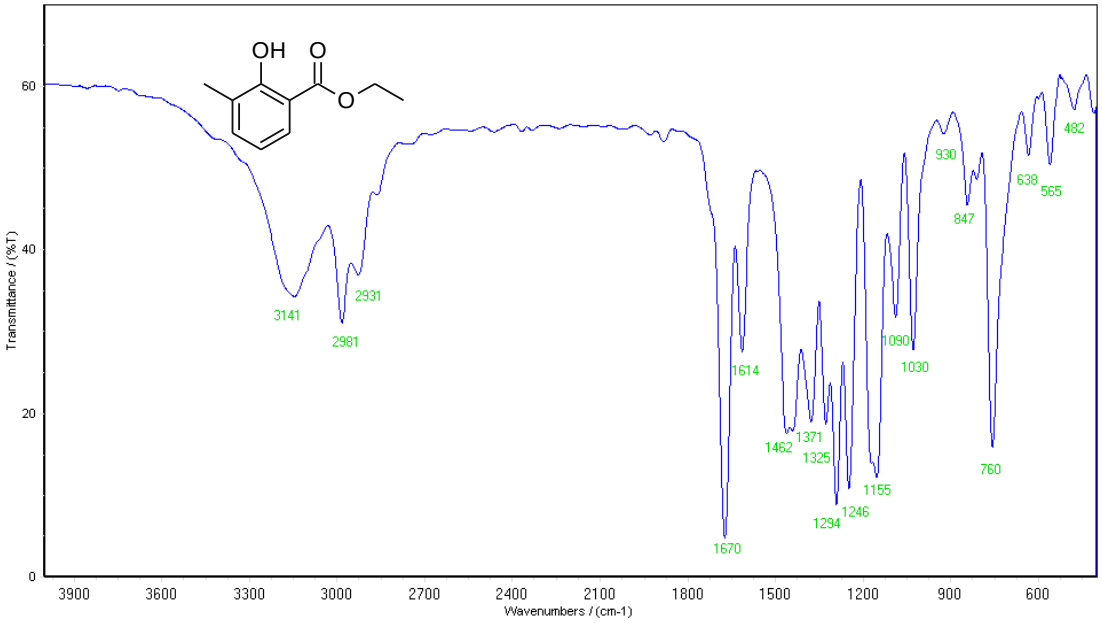


Figure A-1:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.1.**

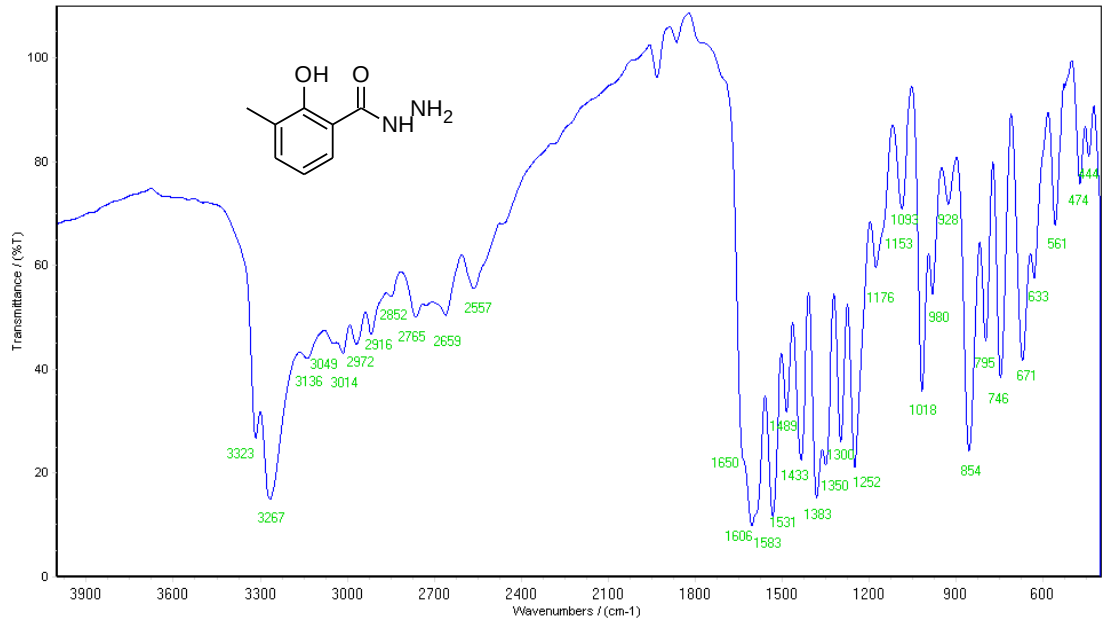


Figure A-2:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.2.**

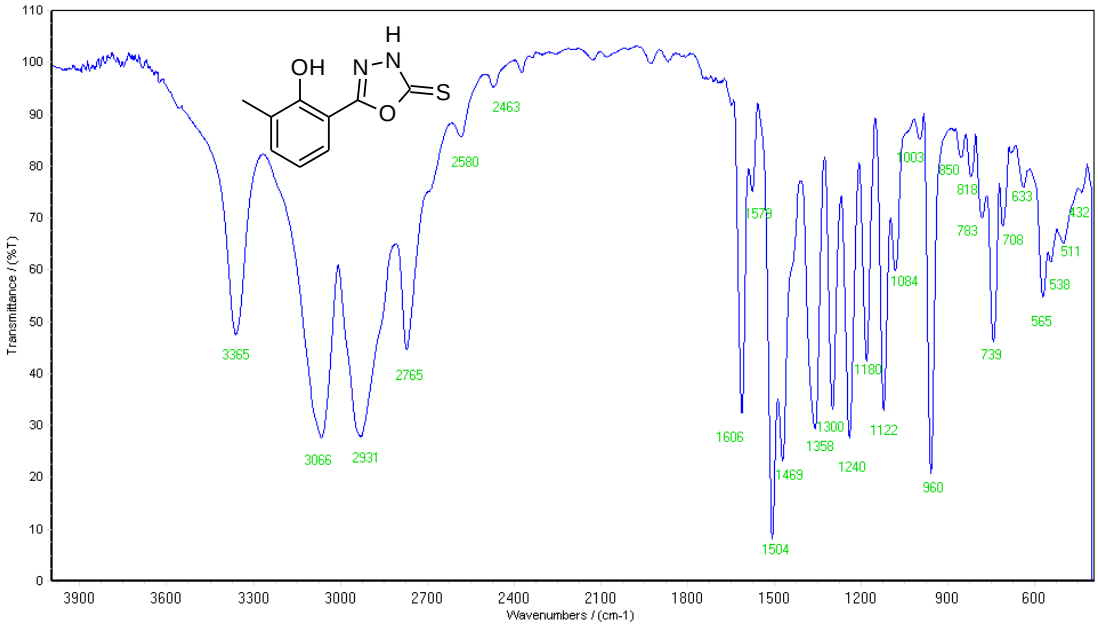


Figure A-3:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.3.**

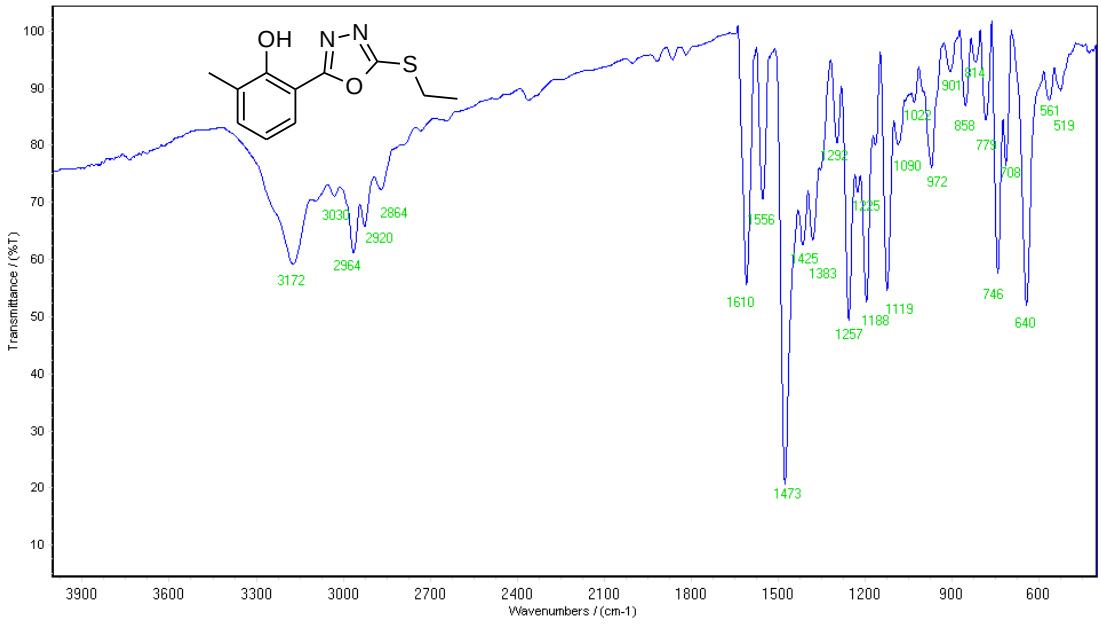


Figure A-4:-FT.IR (KBr, Umax/ cm-1) spectrum of compound **2.4.**

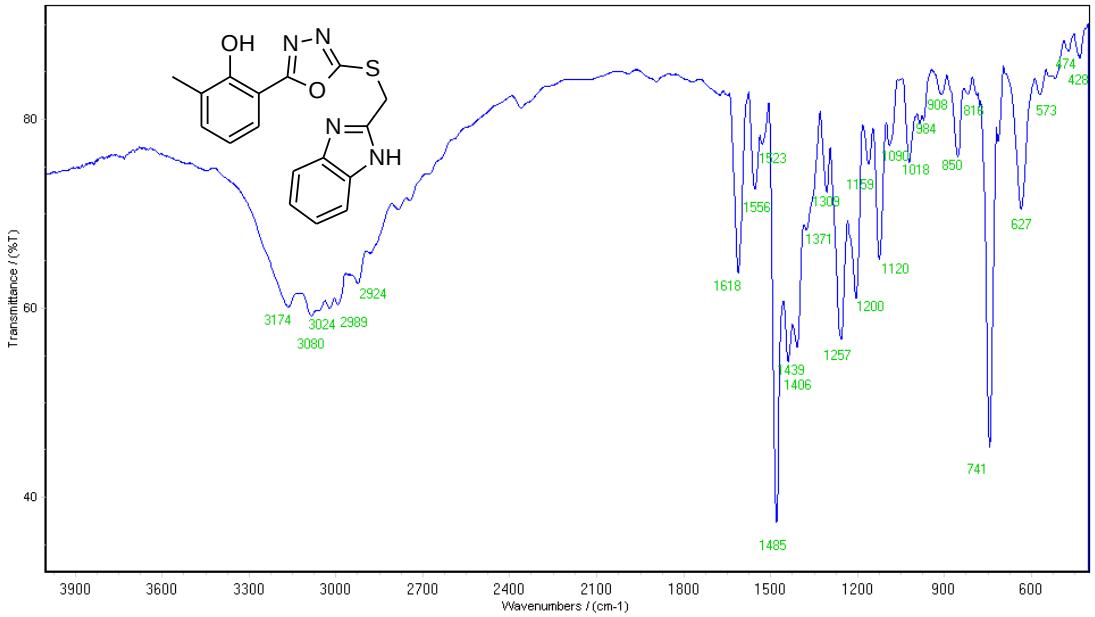


Figure A-5:-FT.IR (KBr, Umax/ cm-1) spectrum of compound **2.5.**

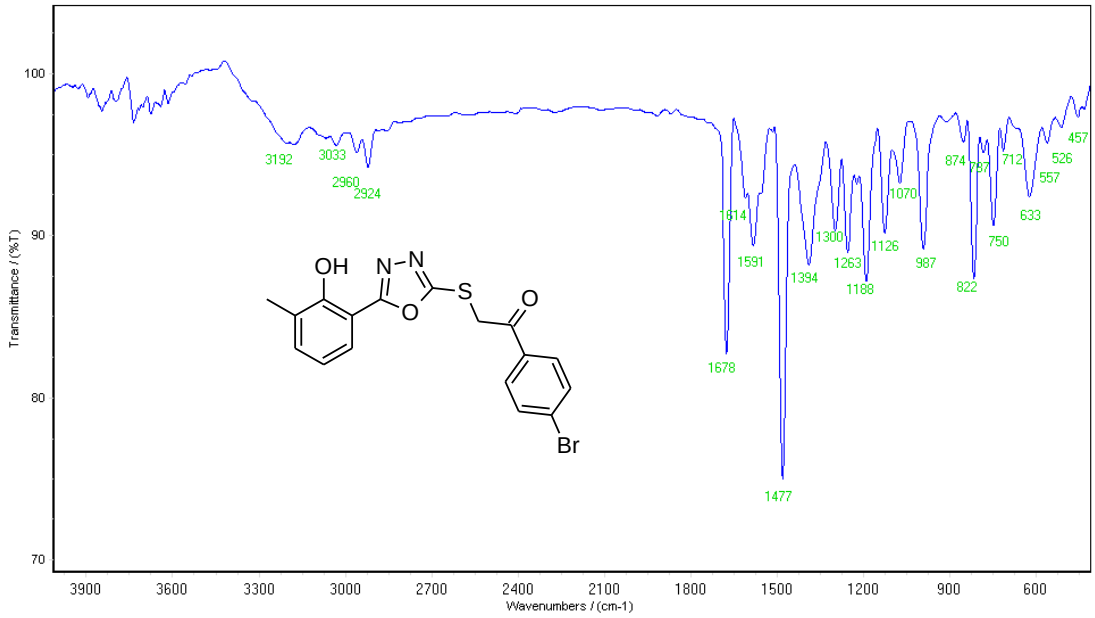


Figure A-6:-FT.IR (KBr, Umax/ cm-1) spectrum of compound **2.6.**

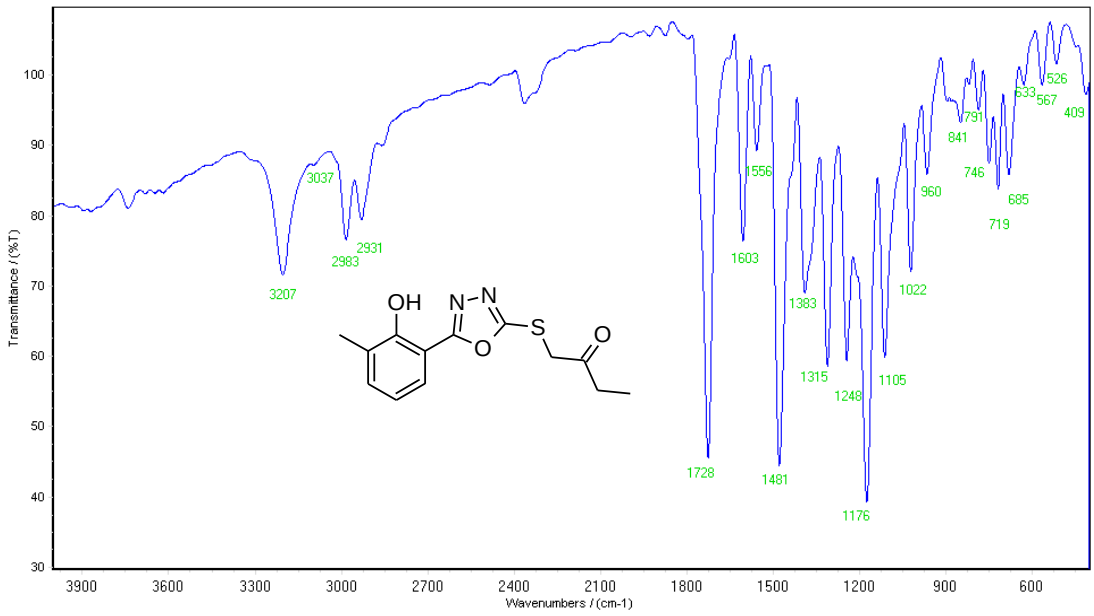


Figure A-7:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.7**.

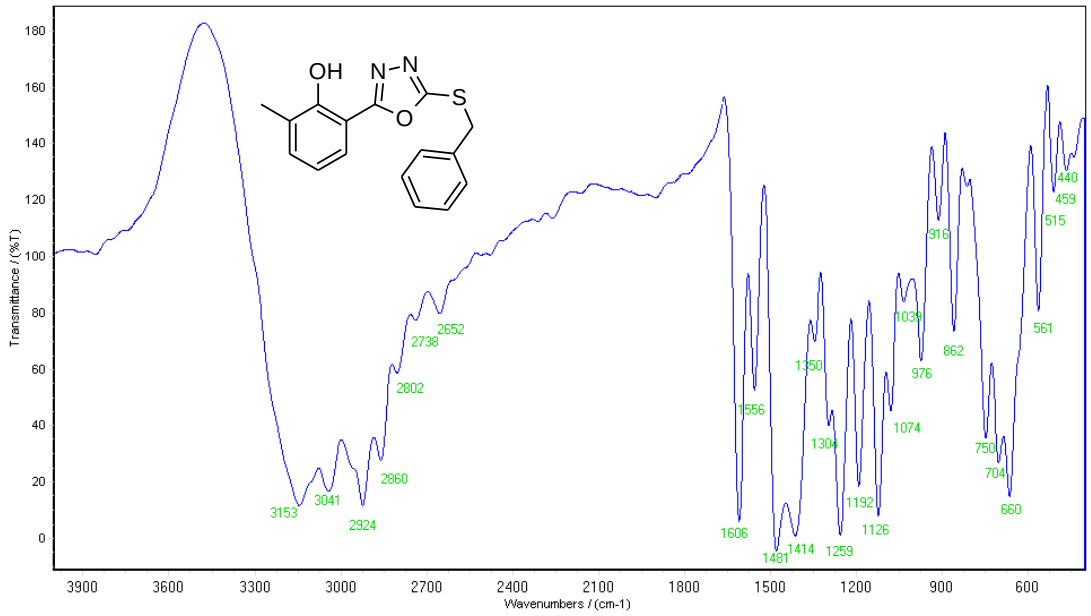


Figure A-8:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.8**.

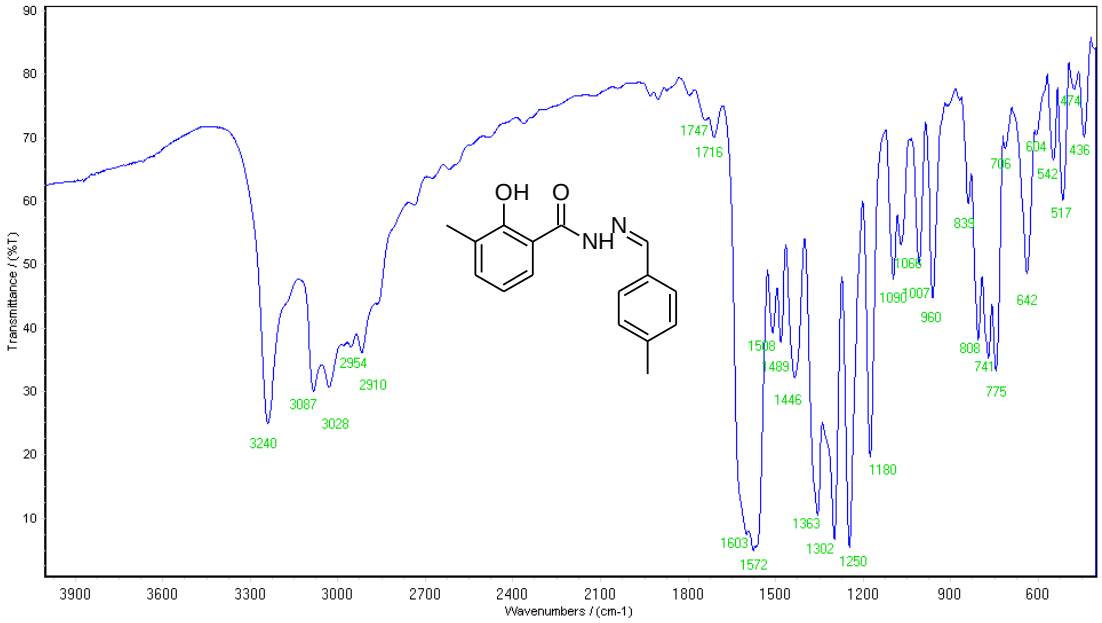


Figure A-9:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.9.**

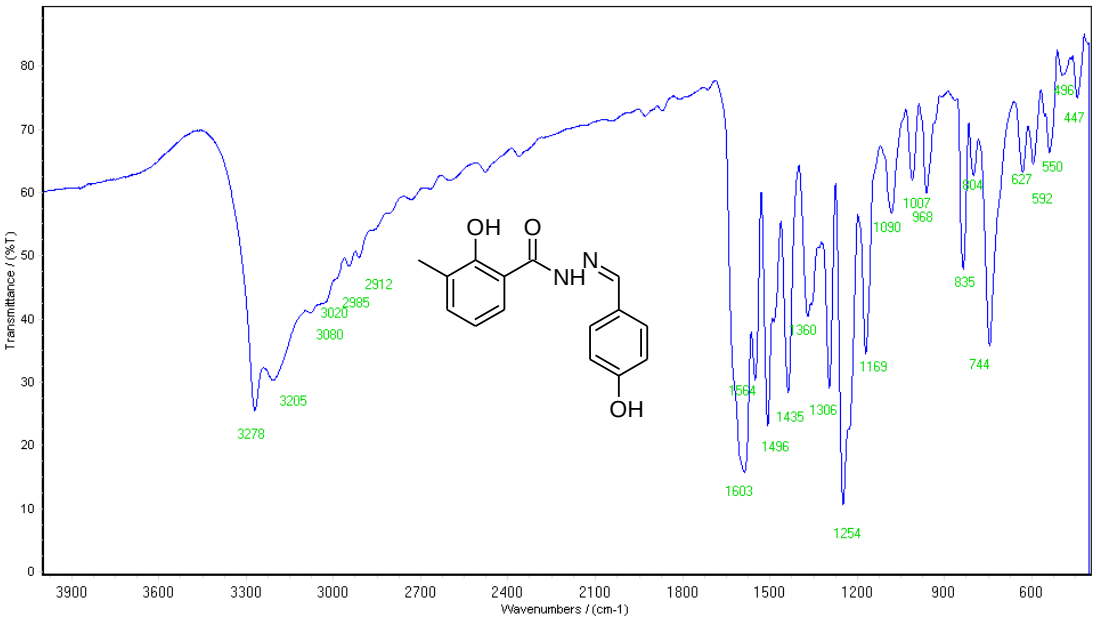


Figure A-10:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.10**.

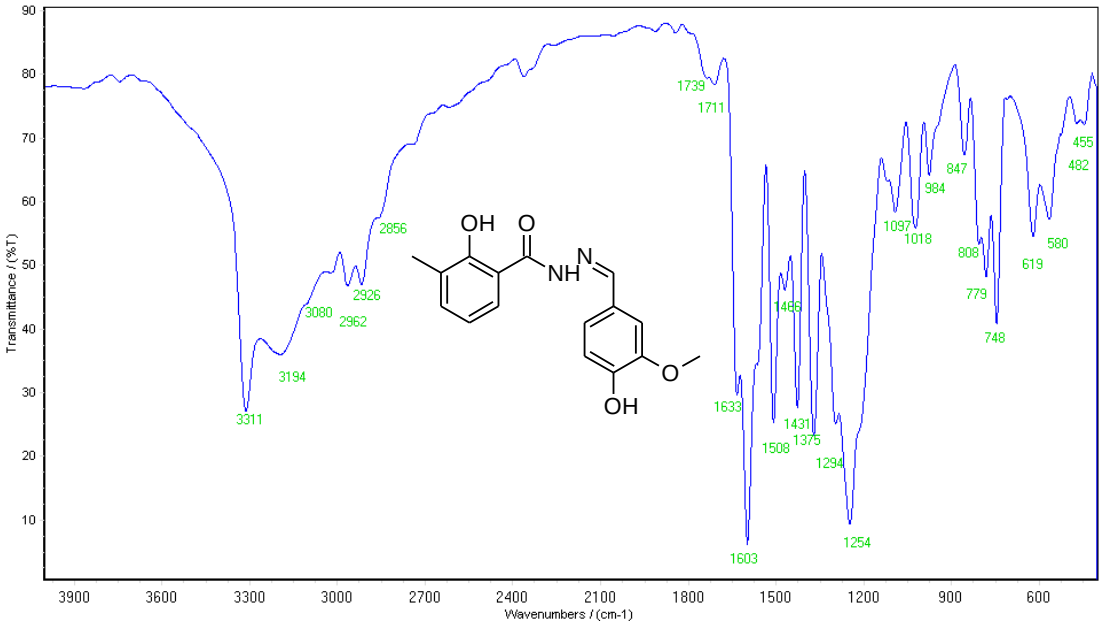


Figure A-11:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.11**.

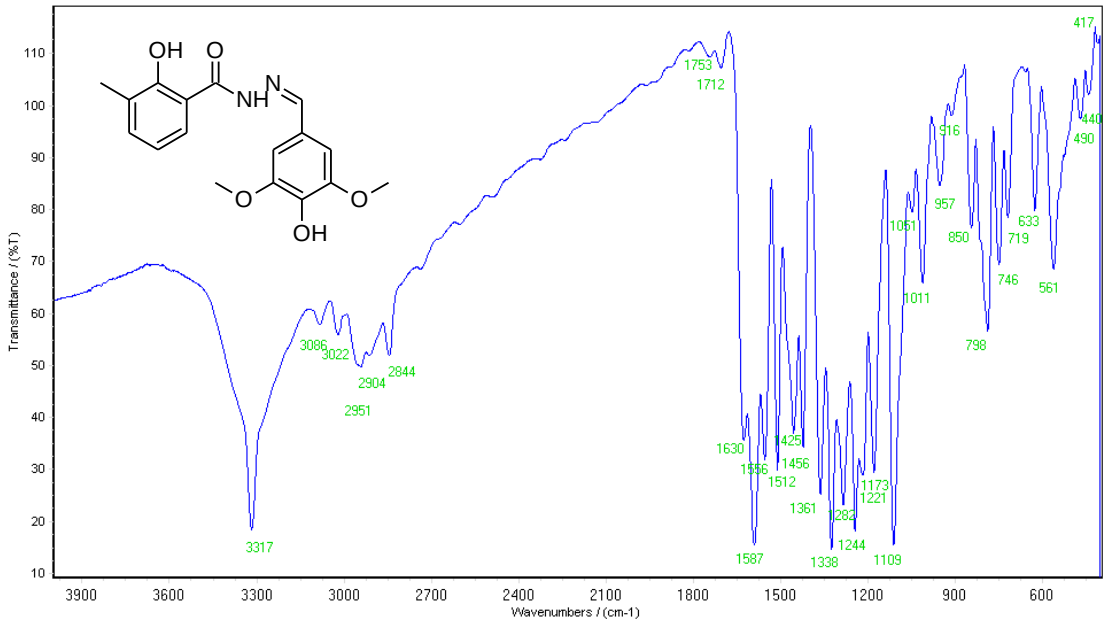


Figure A-12:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.12**.

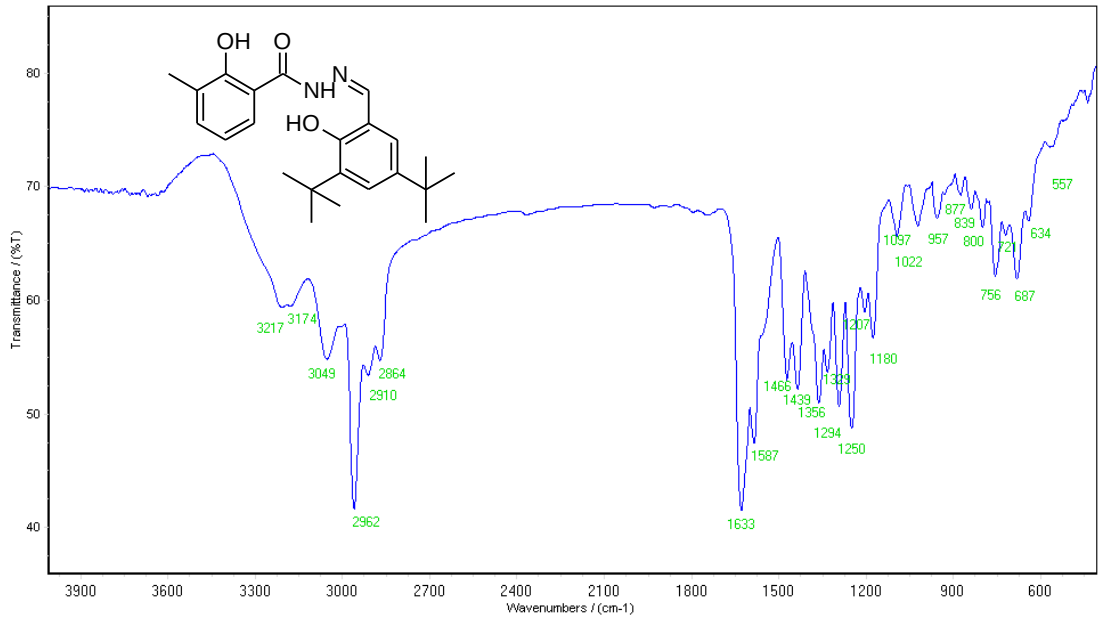


Figure A-13:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.13**.

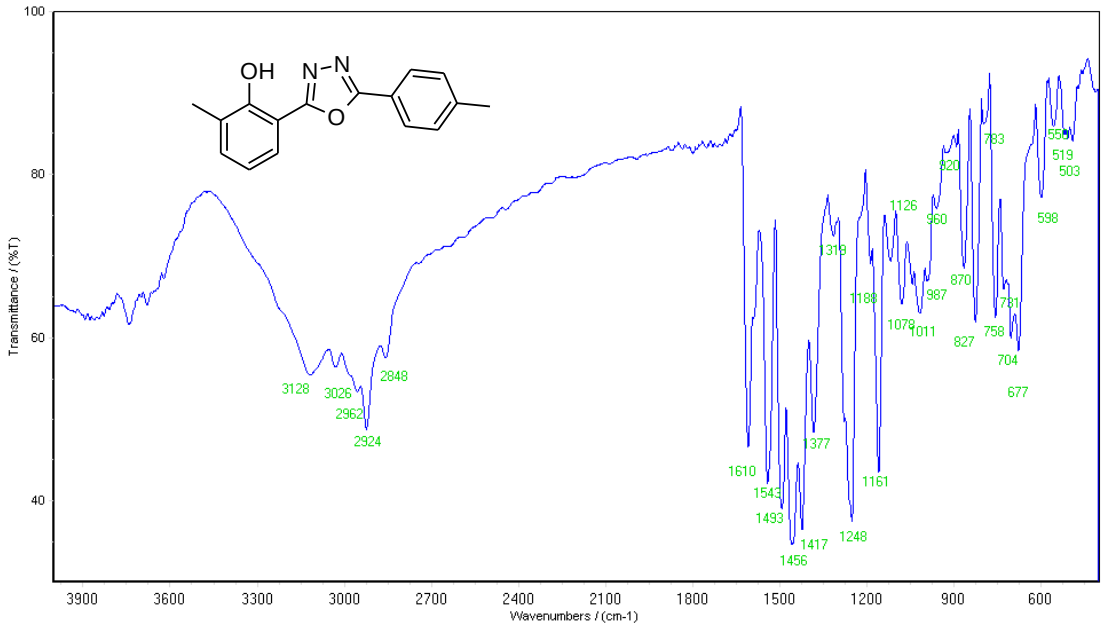


Figure A-14:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.14.**

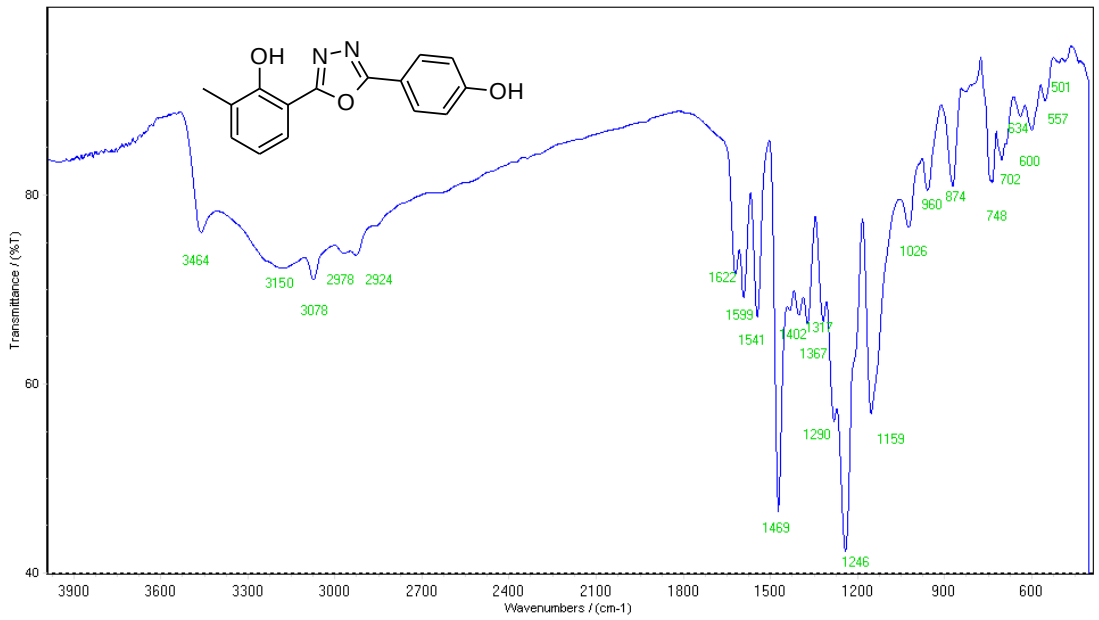


Figure A-15:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.15**.

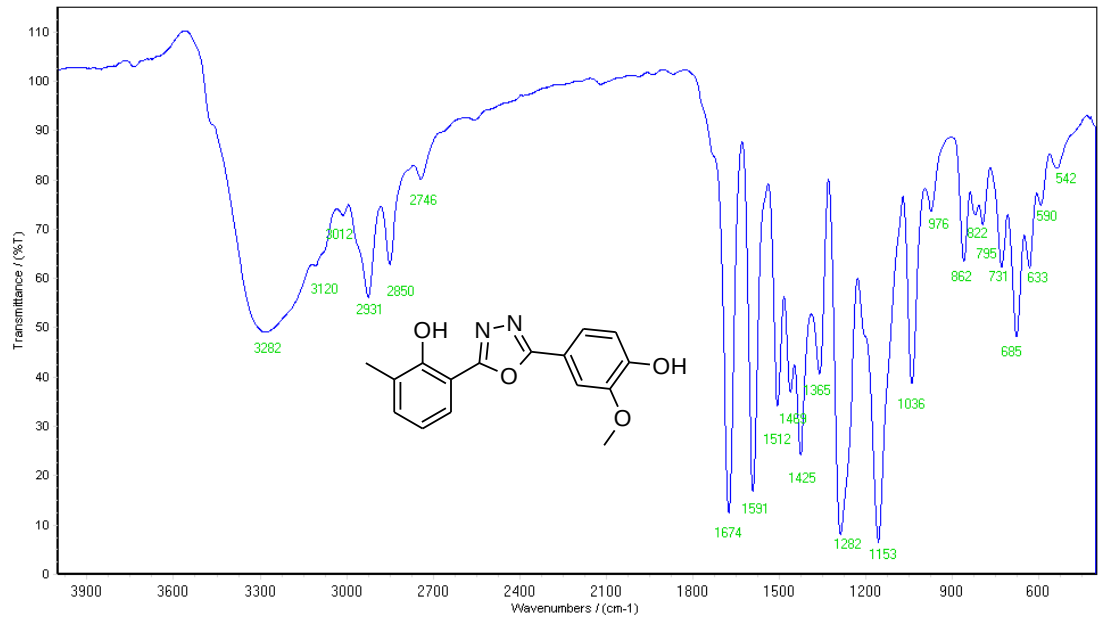


Figure A-16:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.16.**

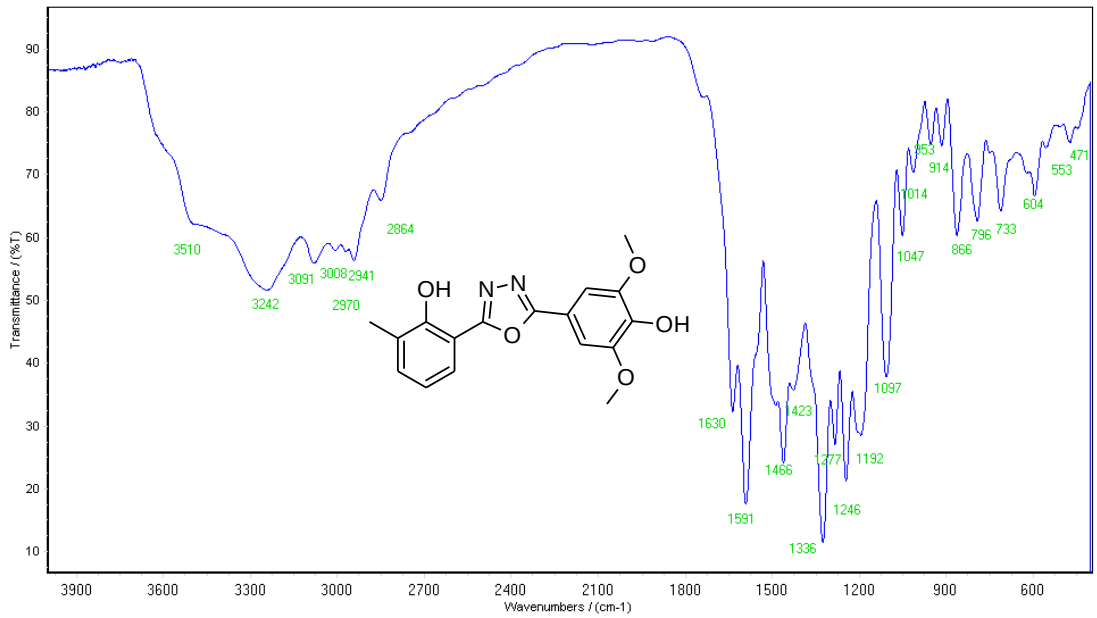


Figure A-17:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.17**.

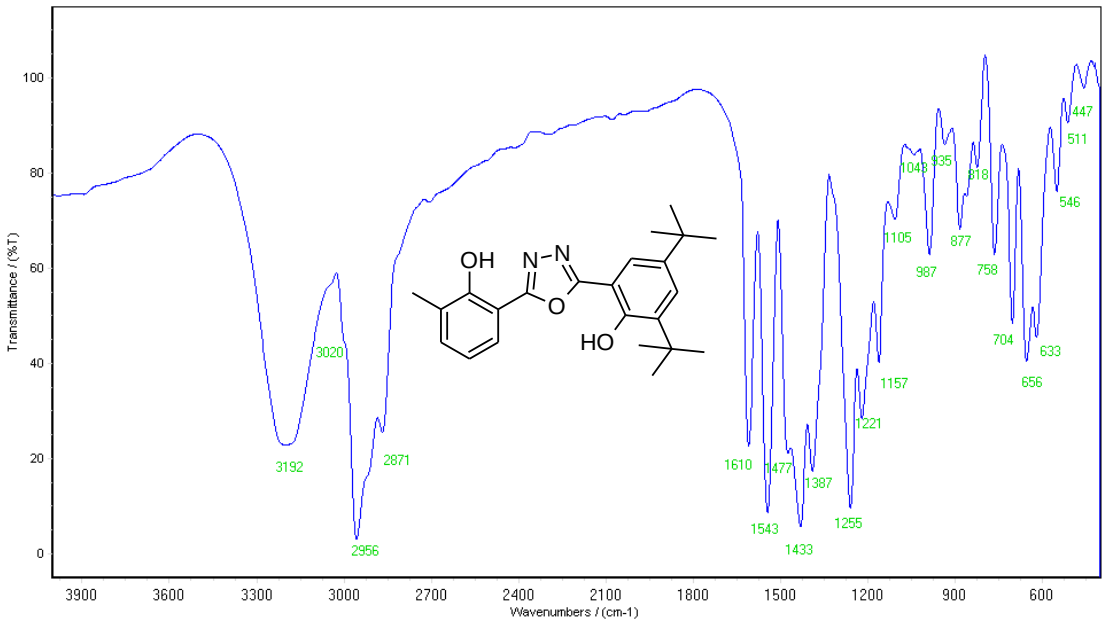


Figure A-18:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.18**.

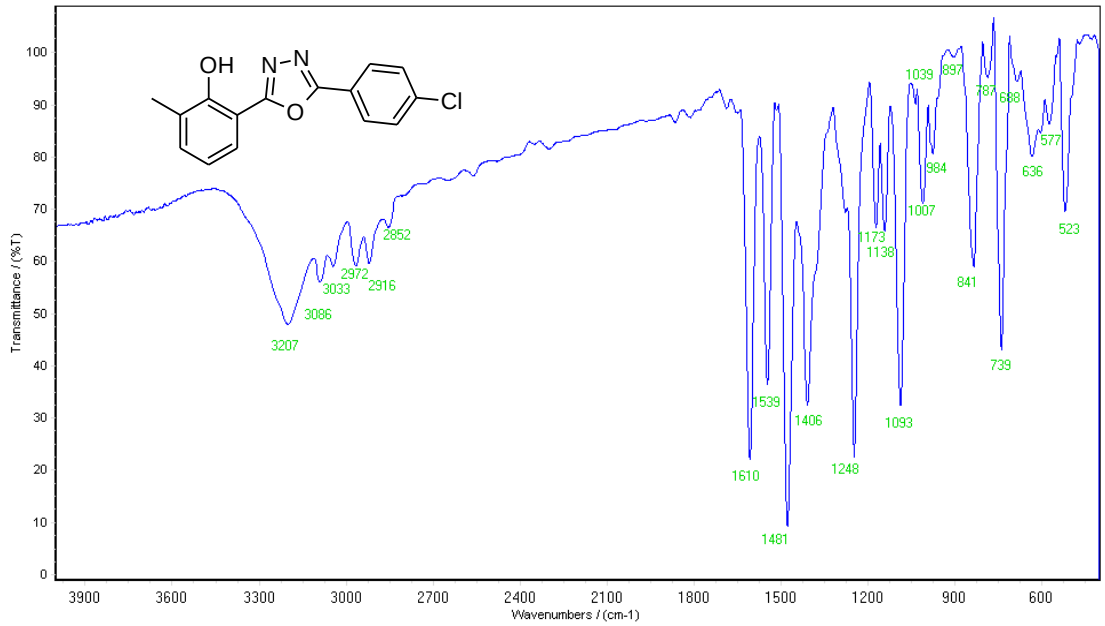


Figure A-19:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.19.**

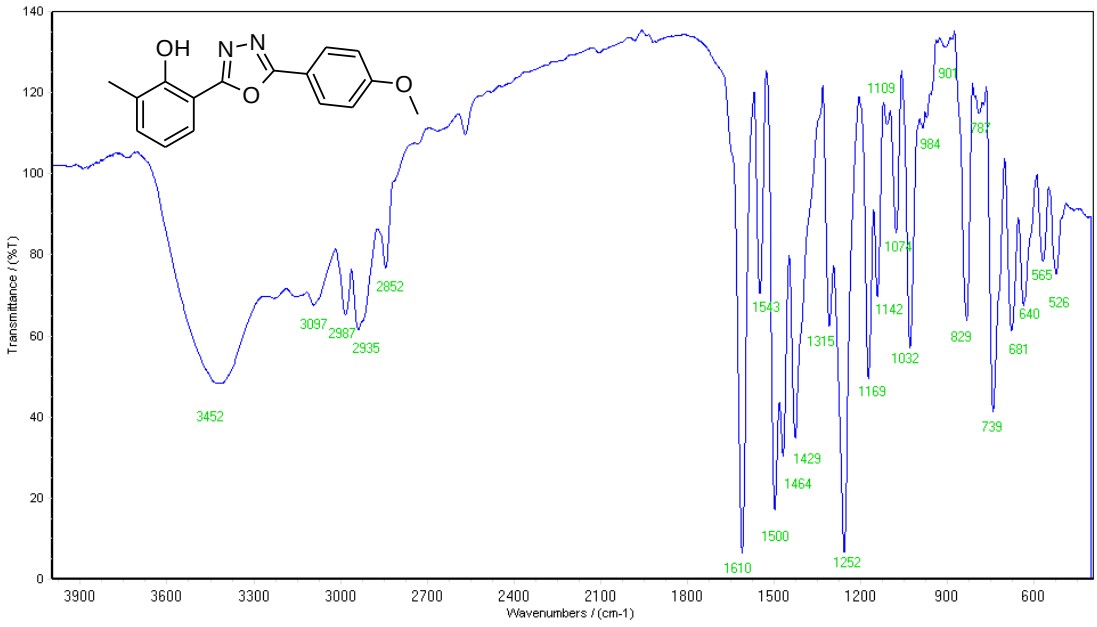


Figure A-20:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.20**.

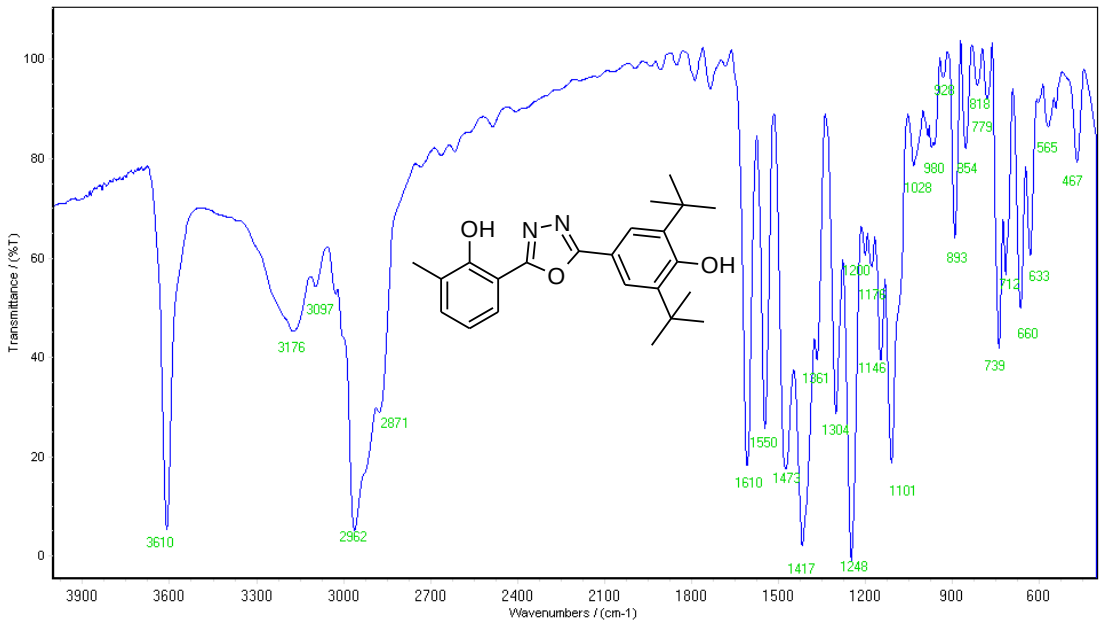


Figure A-21:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.21**.

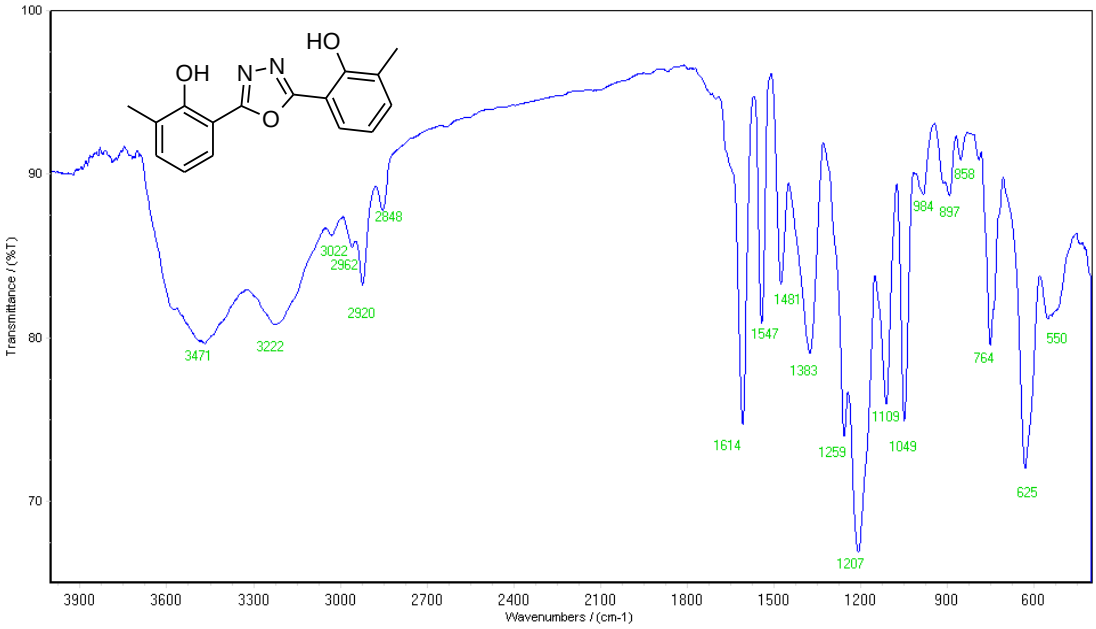


Figure A-22:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.22**.

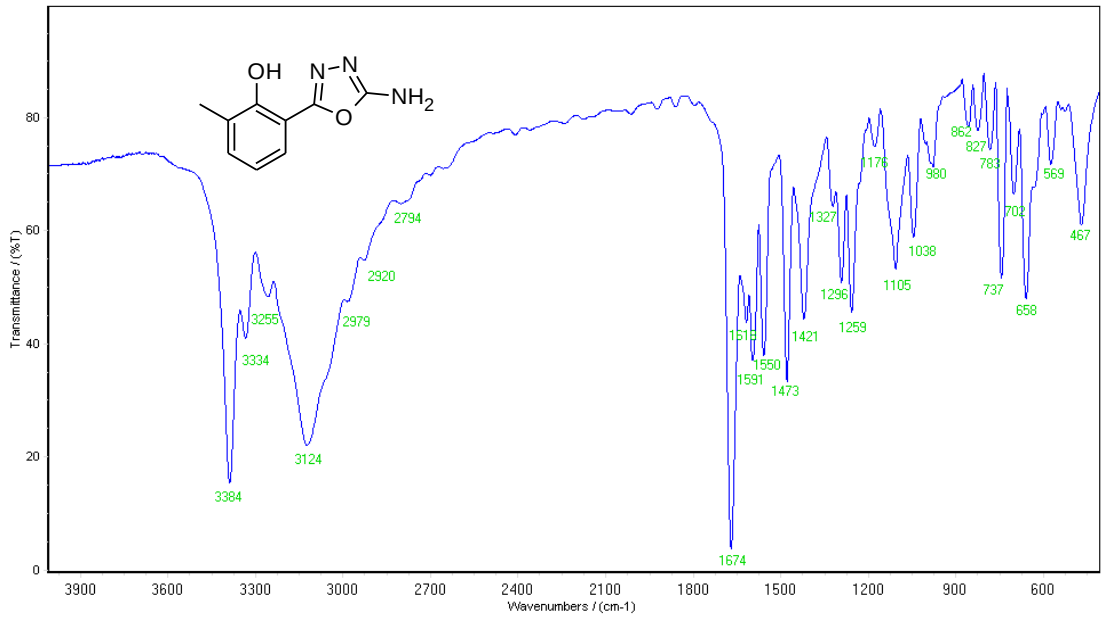


Figure A-23:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.23.**

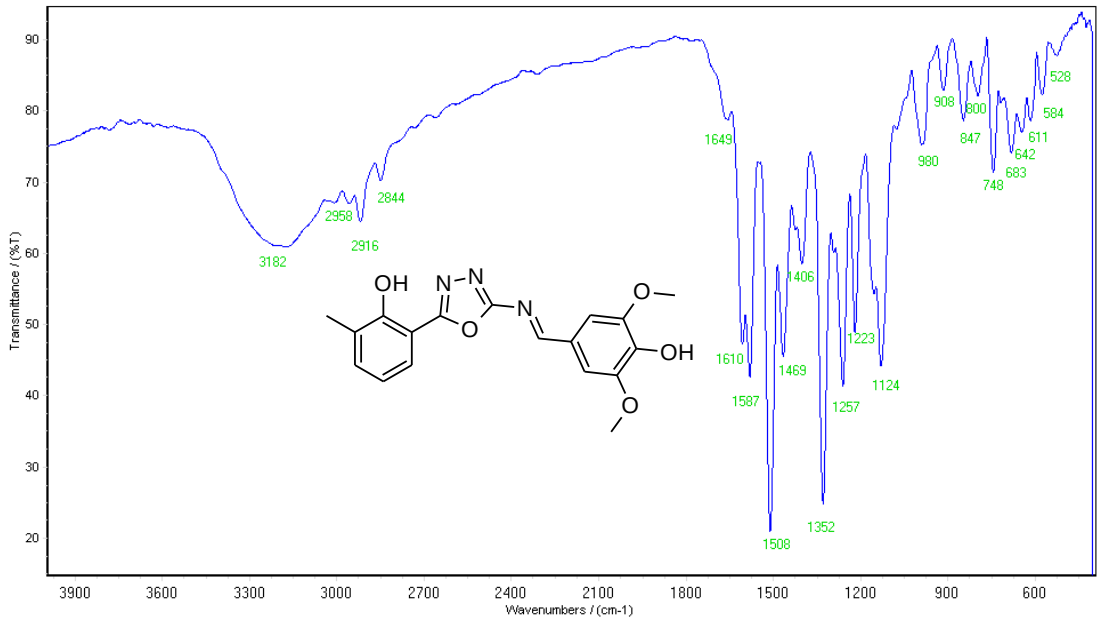


Figure A-24:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.24.**

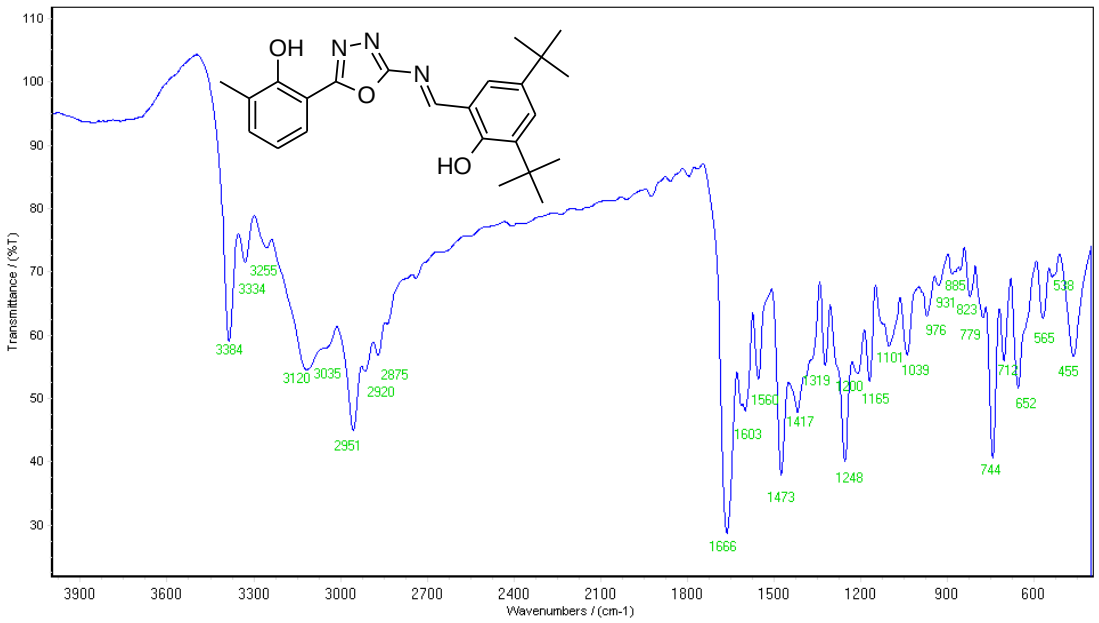


Figure A-25:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.25.**

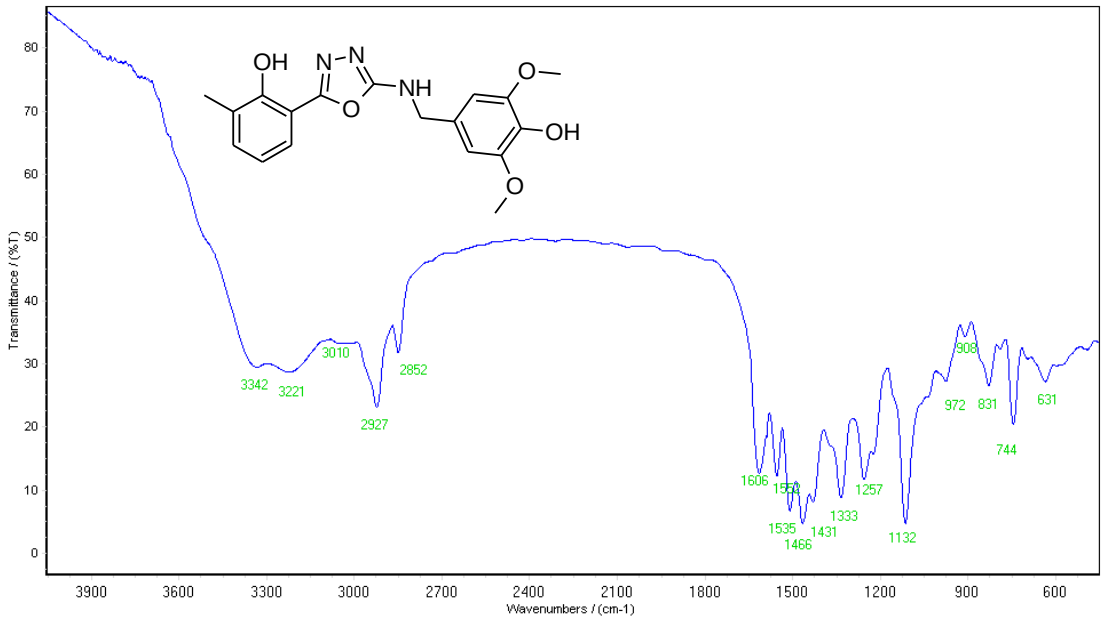


Figure A-26:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.26**.

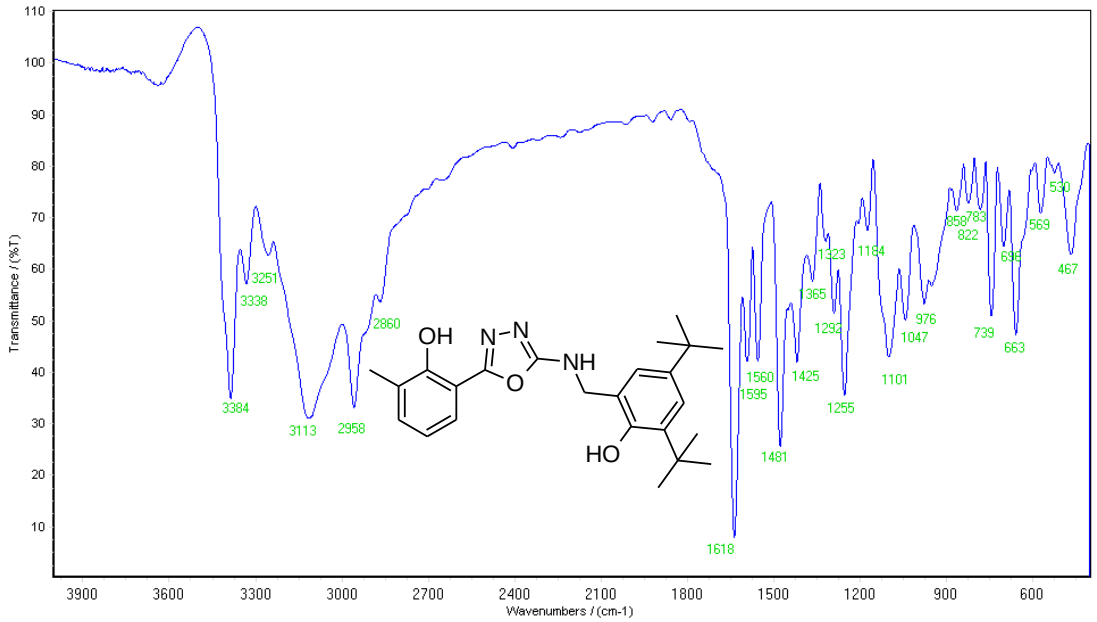


Figure A-27:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.27**.

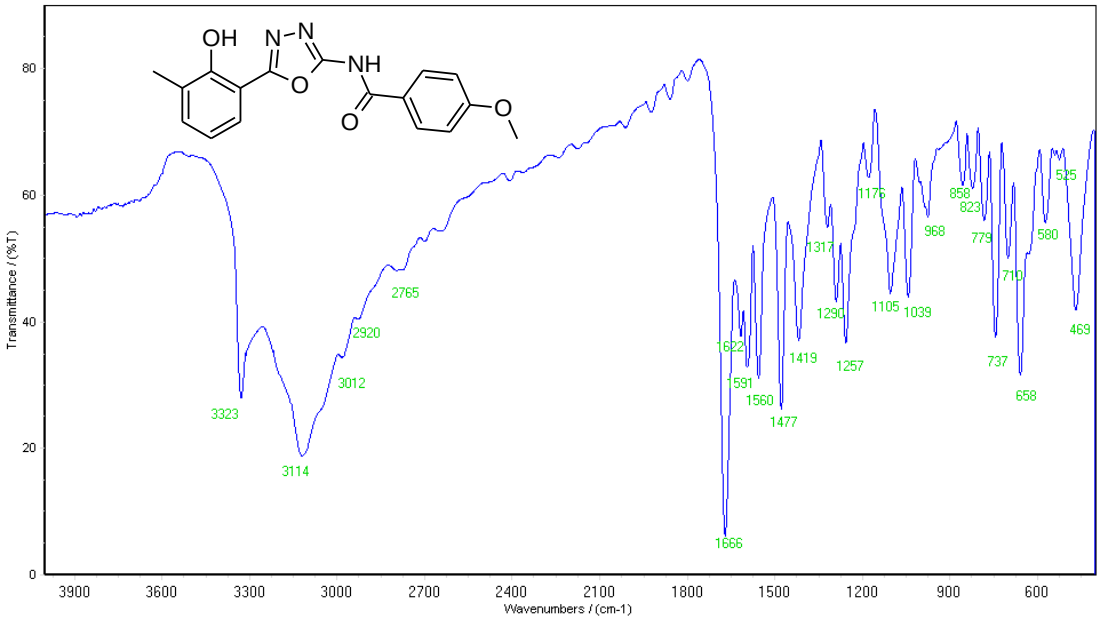


Figure A-28:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.28.**

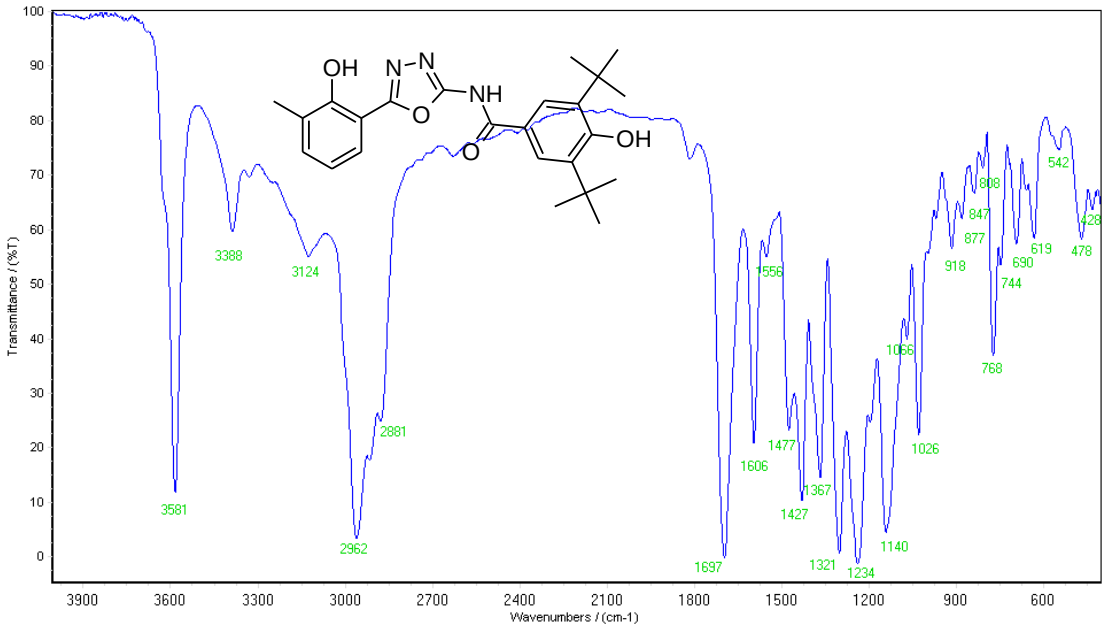


Figure A-29:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.29.**

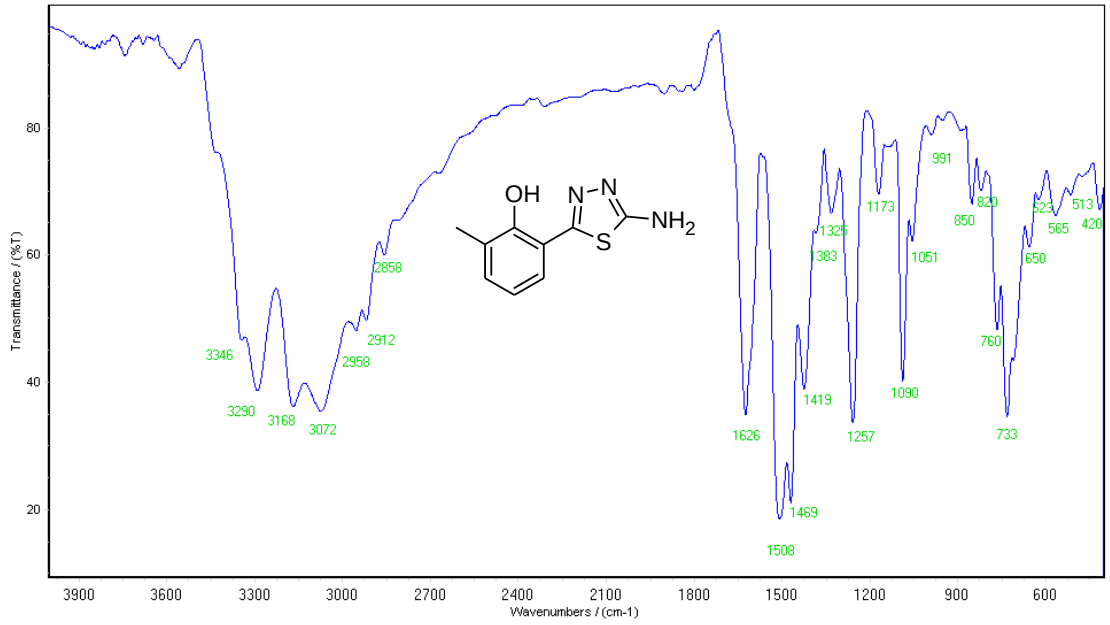


Figure A-30:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.30.**

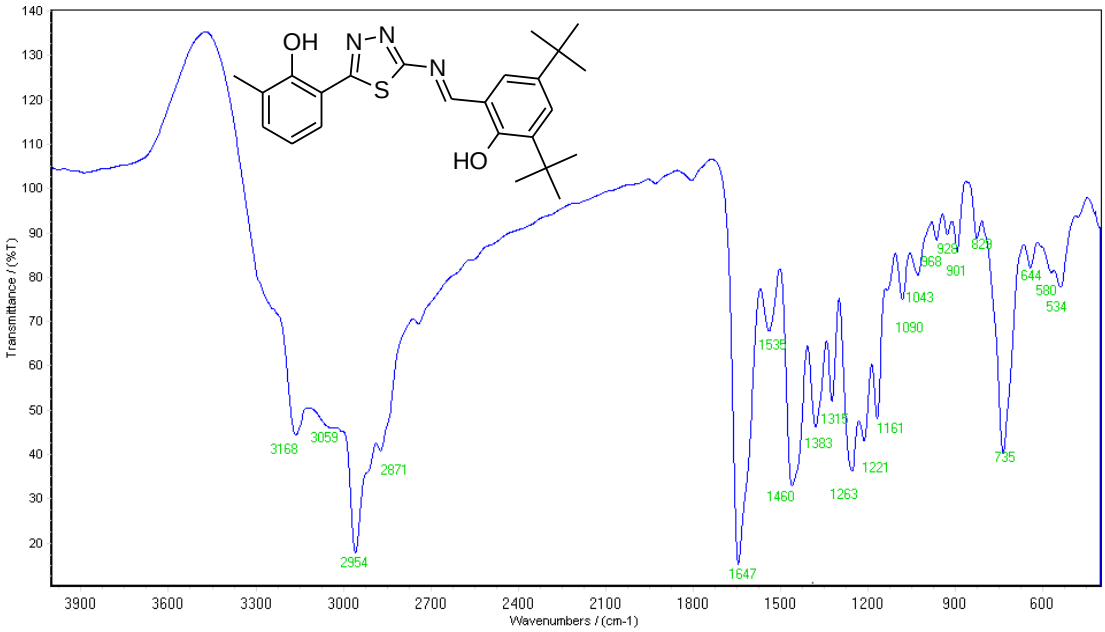


Figure A-31:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.31.**

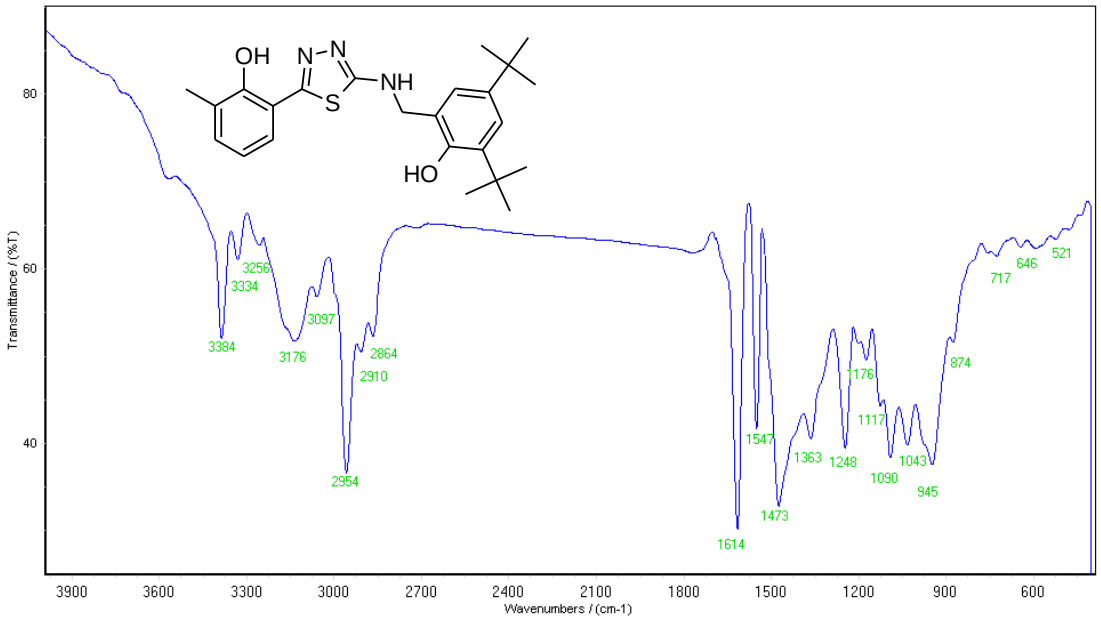


Figure A-32:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.32.**

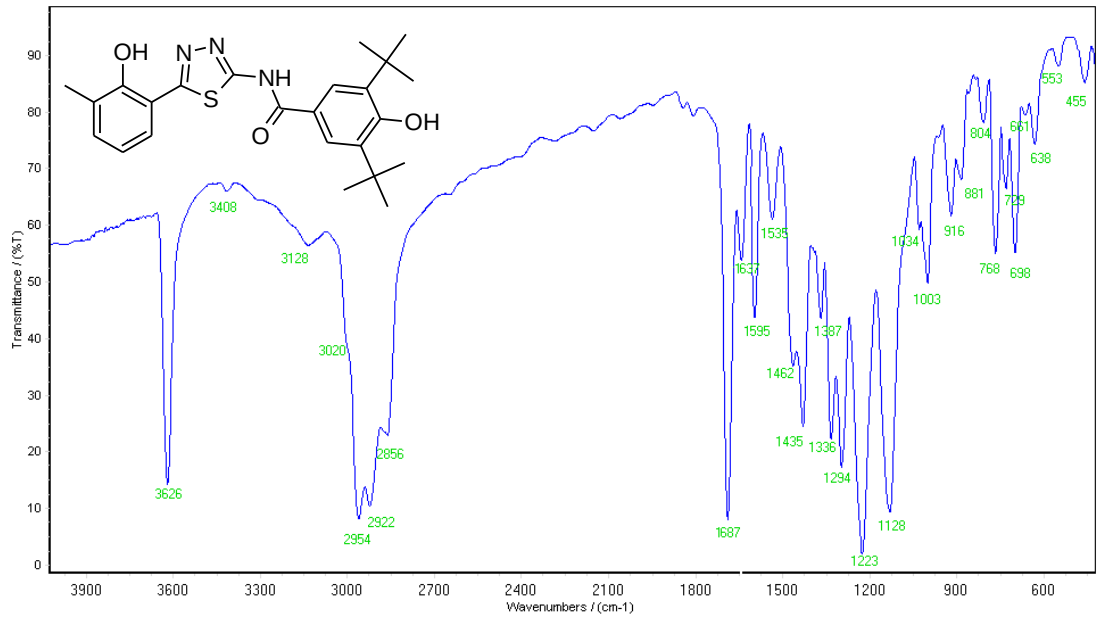


Figure A-33:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.33.**

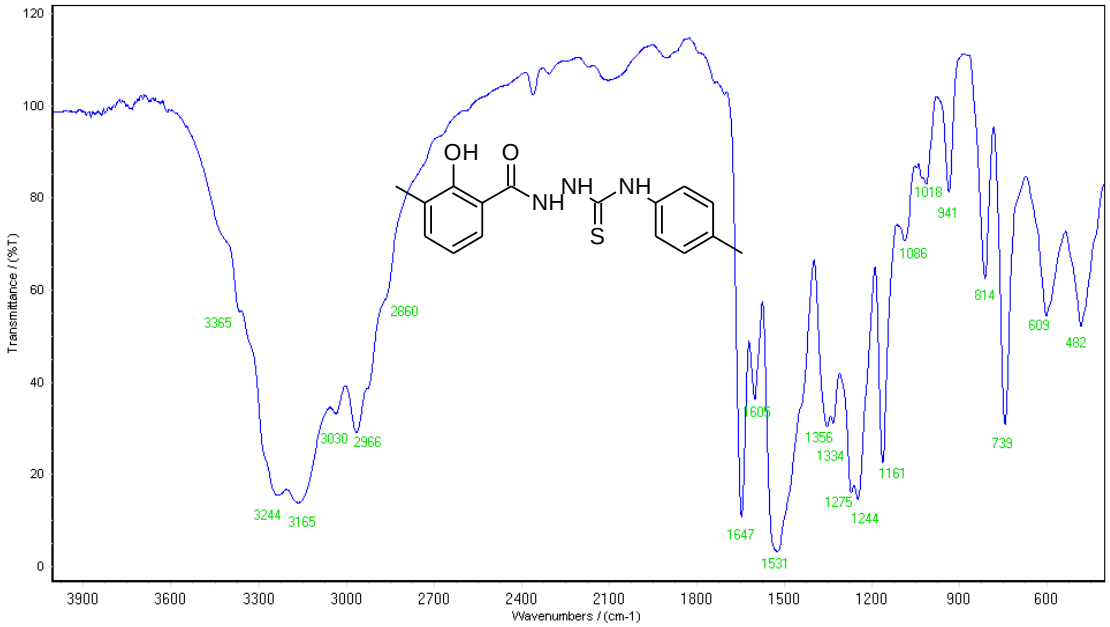


Figure A-34:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.34.**

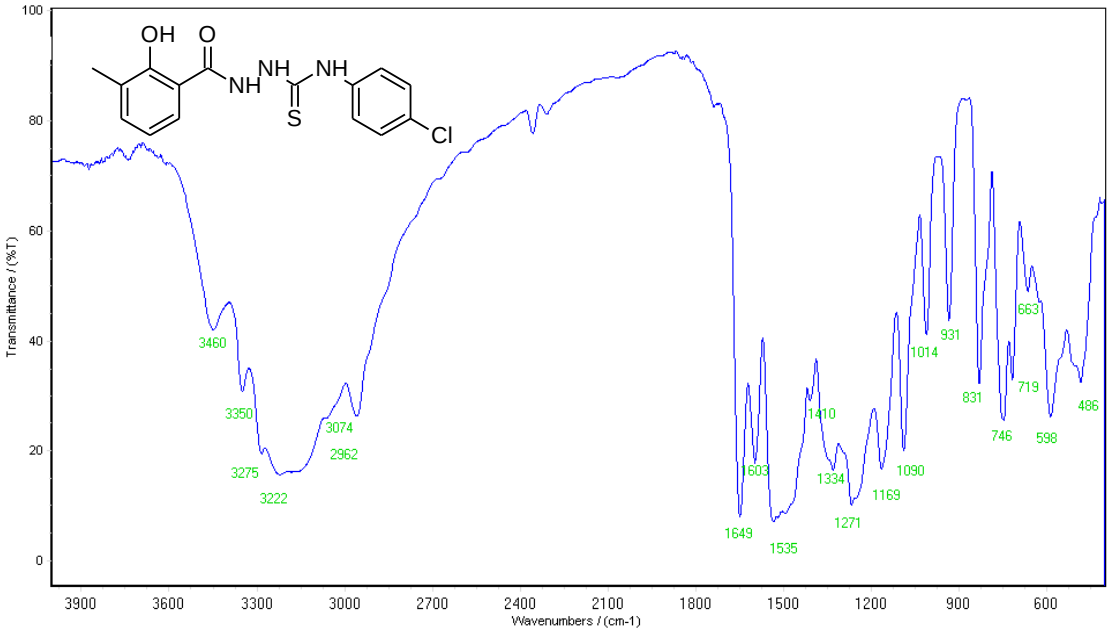


Figure A-35:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.35**.

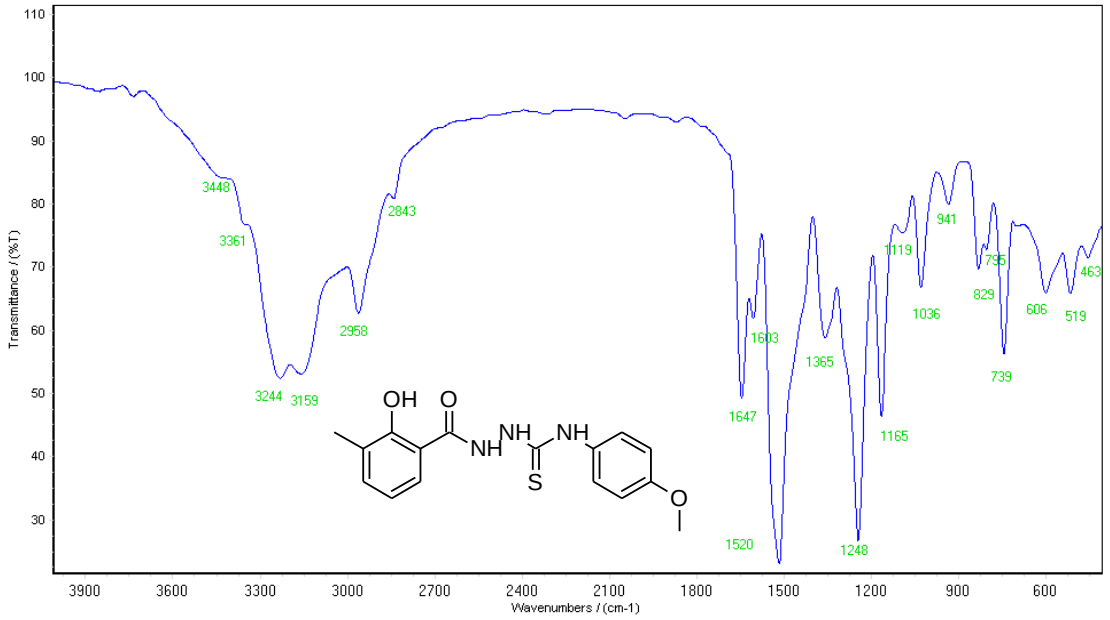


Figure A-36:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.36.**

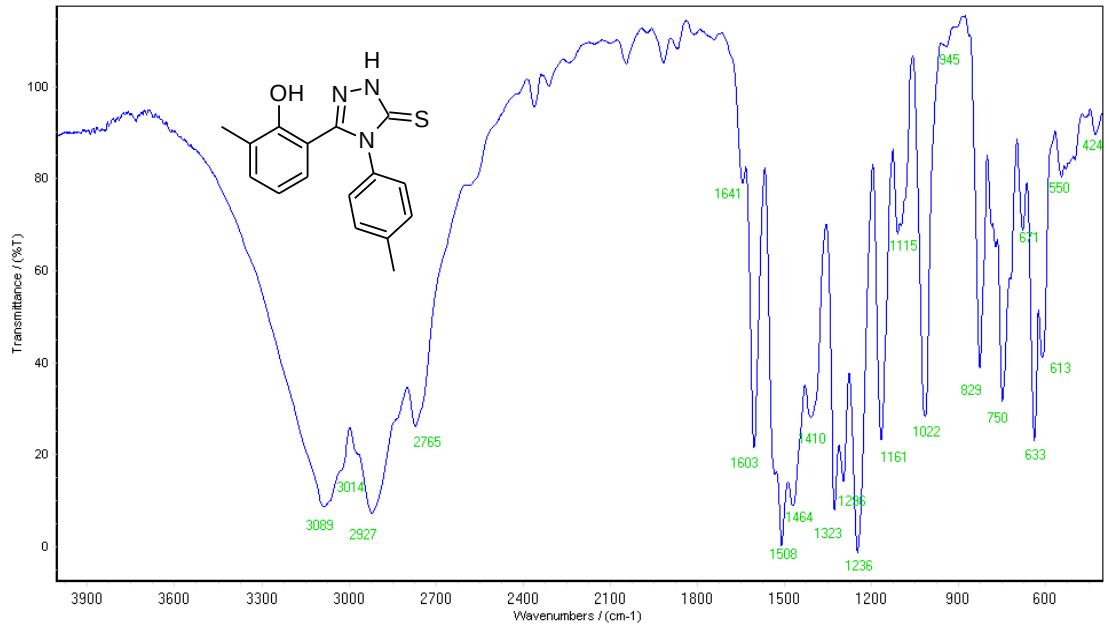


Figure A-37:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.37**.

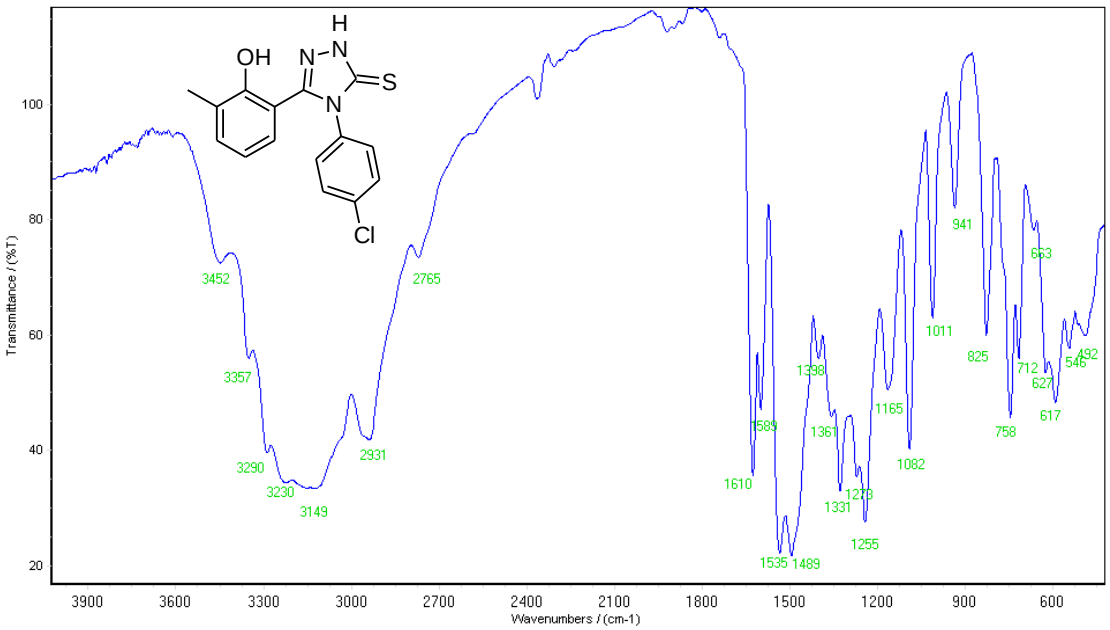


Figure A-38:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.38.**

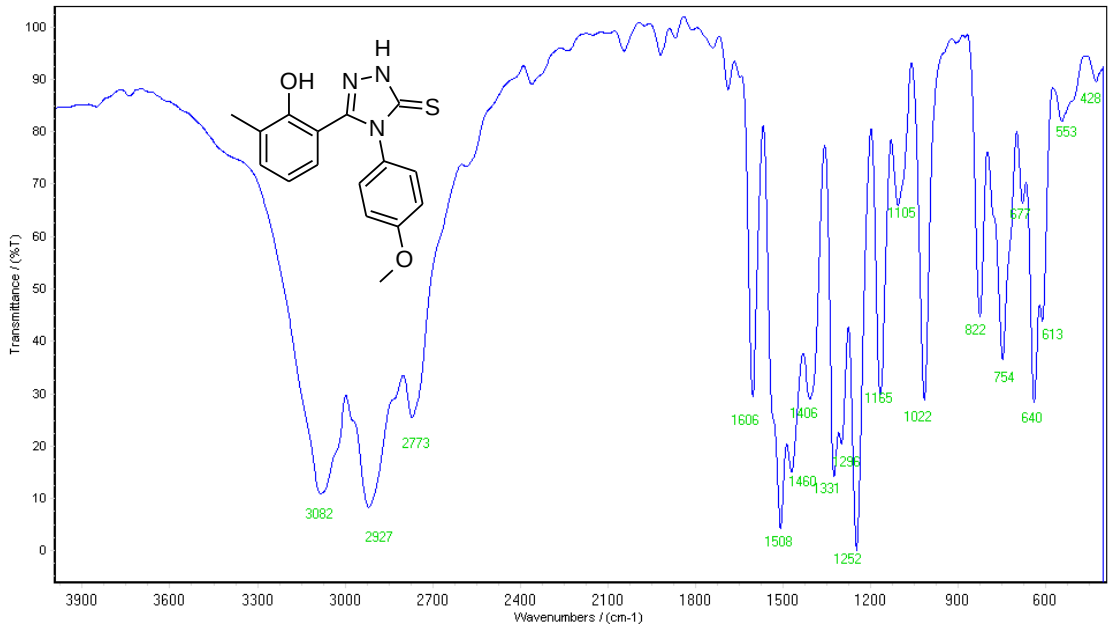


Figure A-39:-FT.IR (KBr, υ_{max}/ cm^{-1}) spectrum of compound **2.39**.

Appendix B NMR Spectra

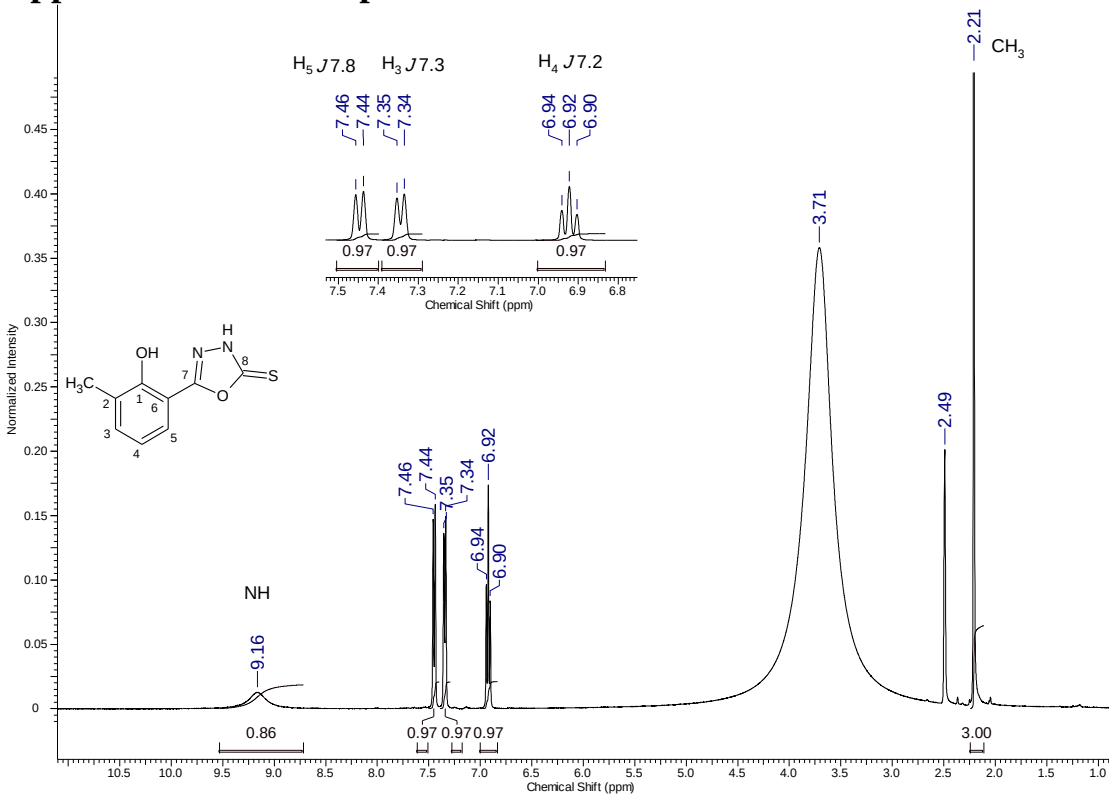


Figure B-1:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.3**

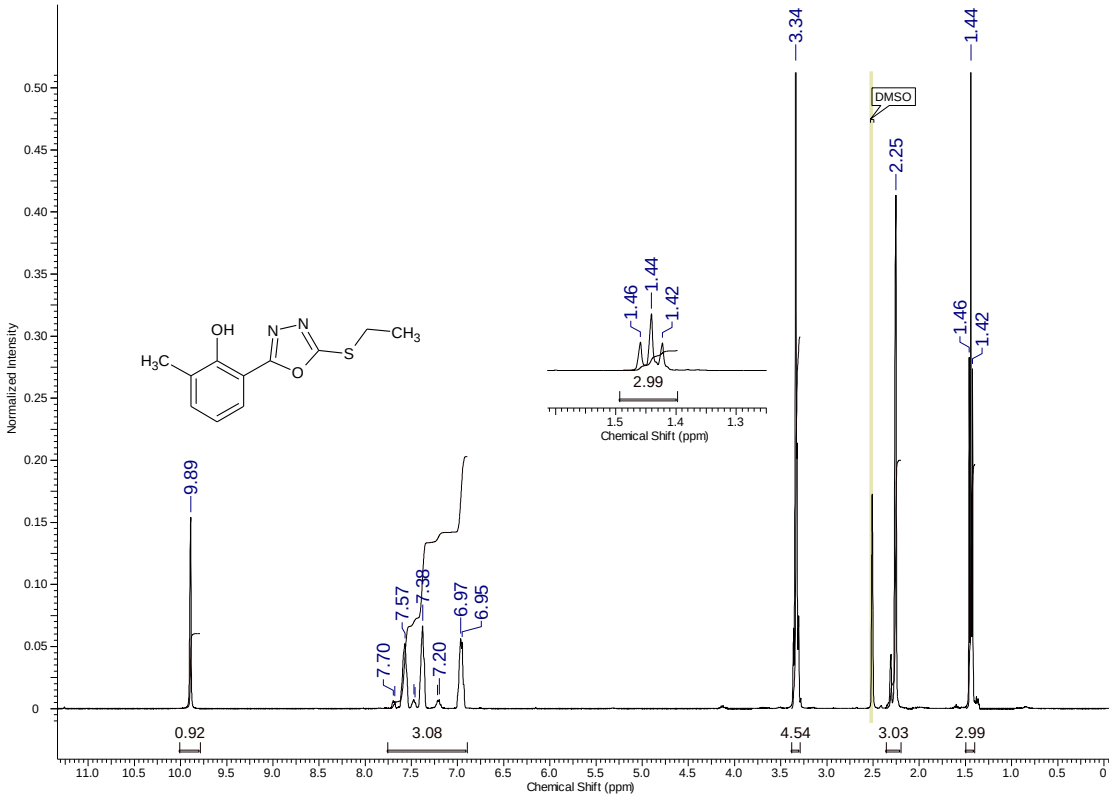


Figure B-2:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.4**

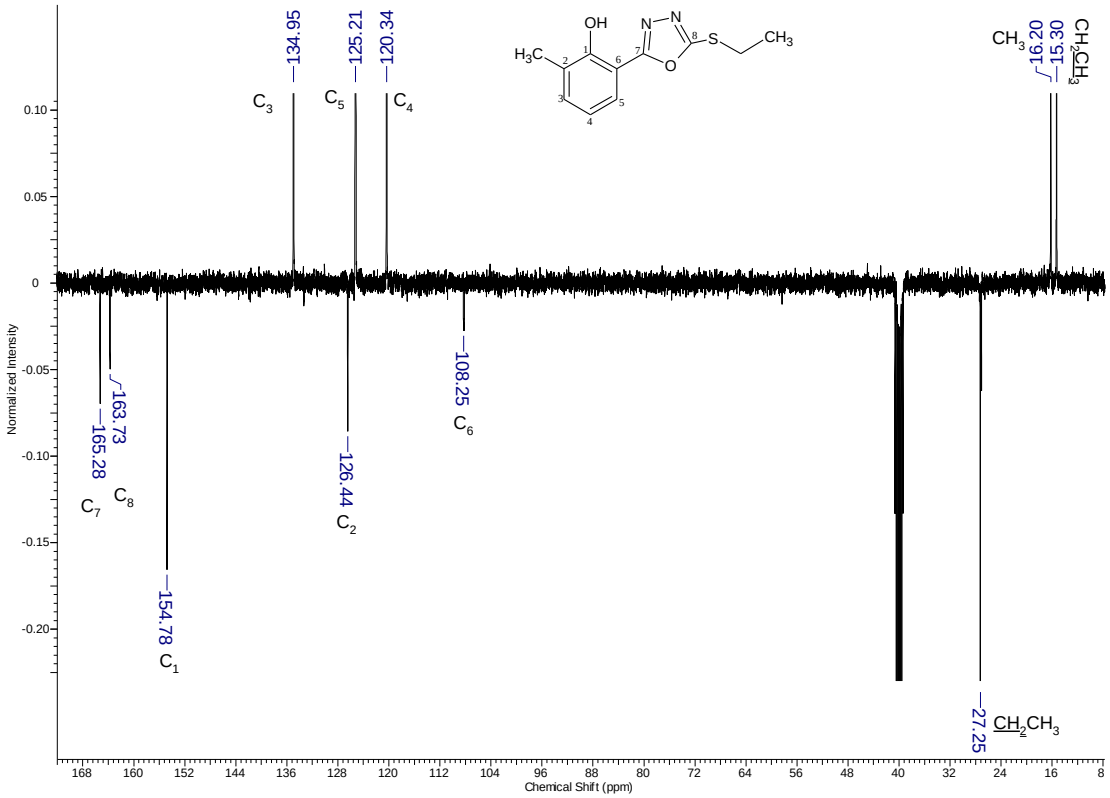


Figure B-3:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.4**

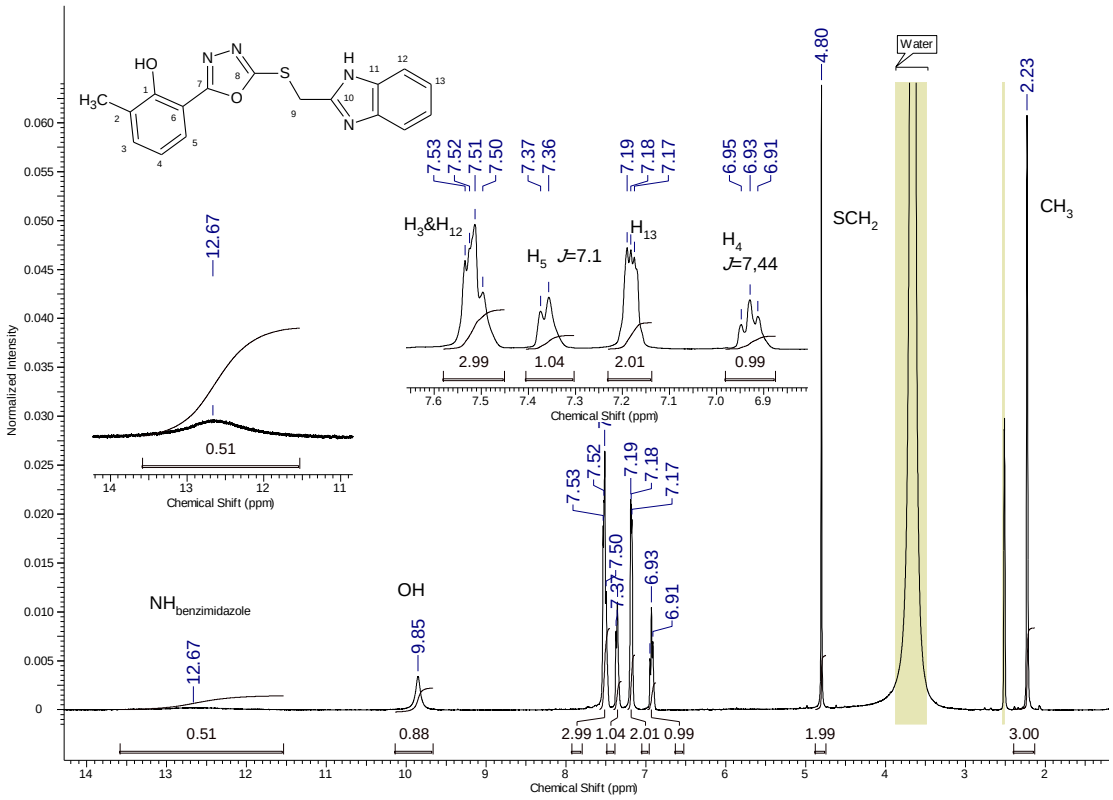


Figure B-4:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.5**

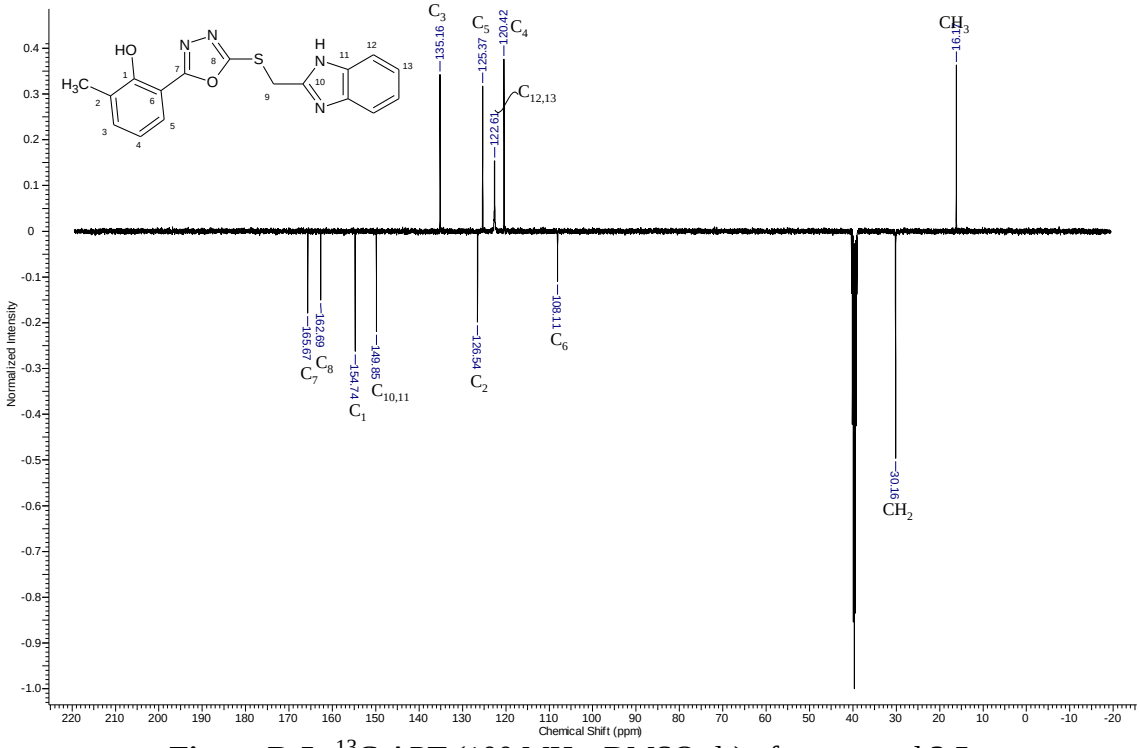


Figure B-5:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.5**

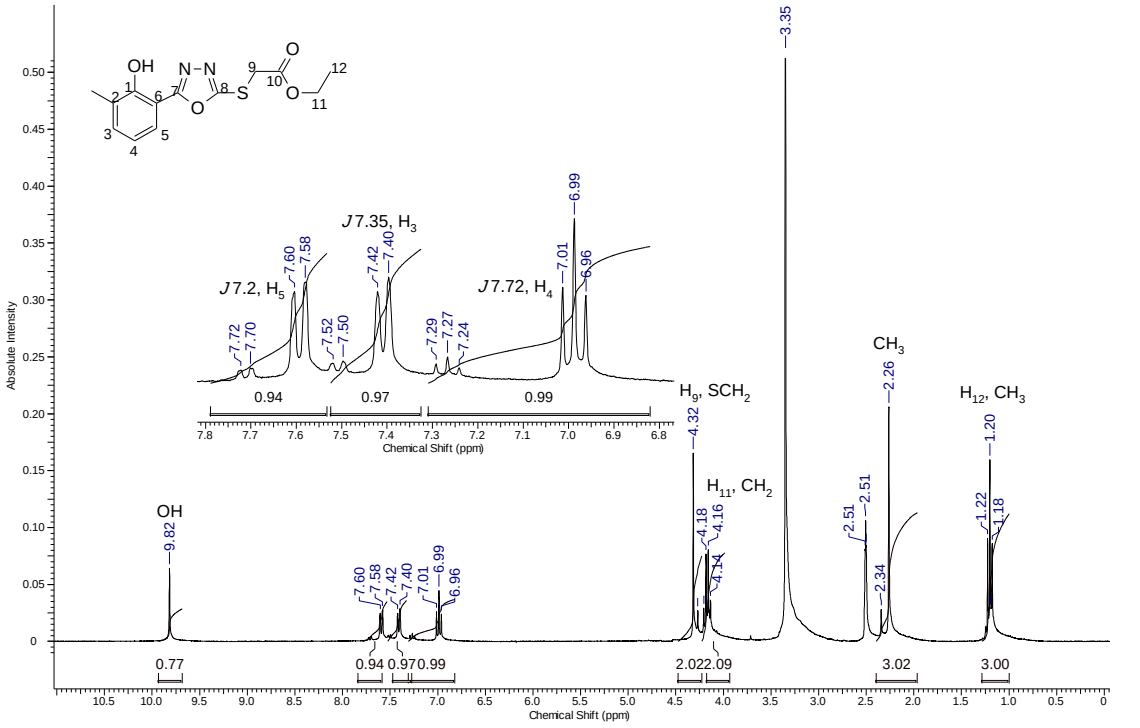


Figure B-6:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.7**

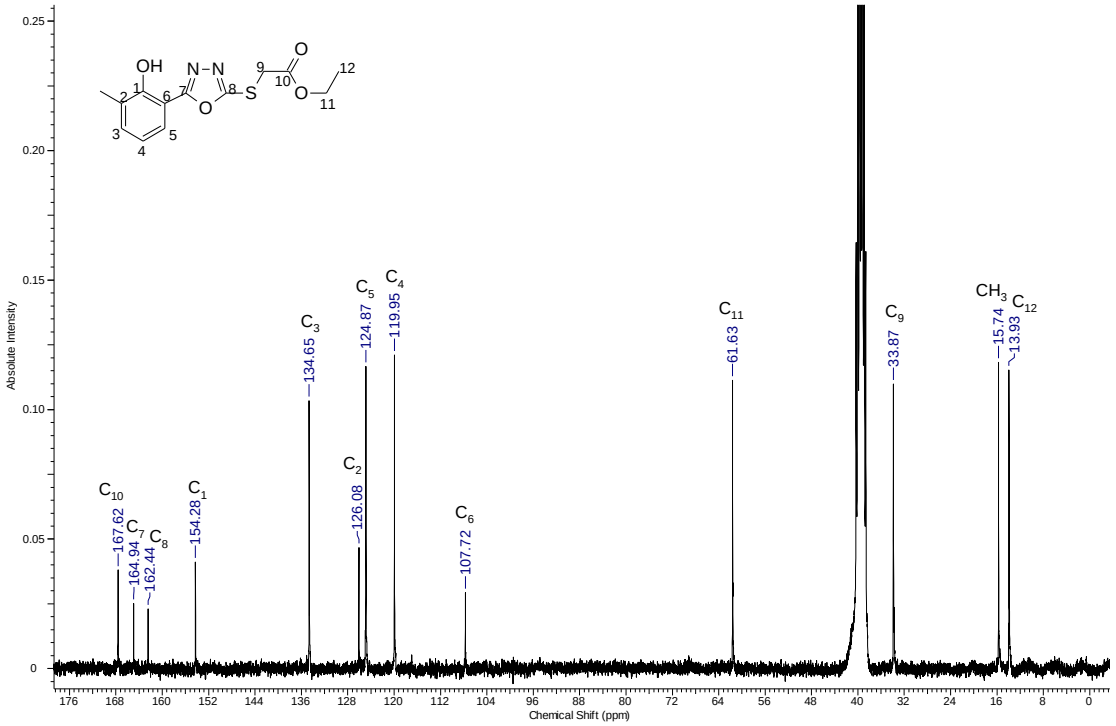


Figure B-7:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.7**

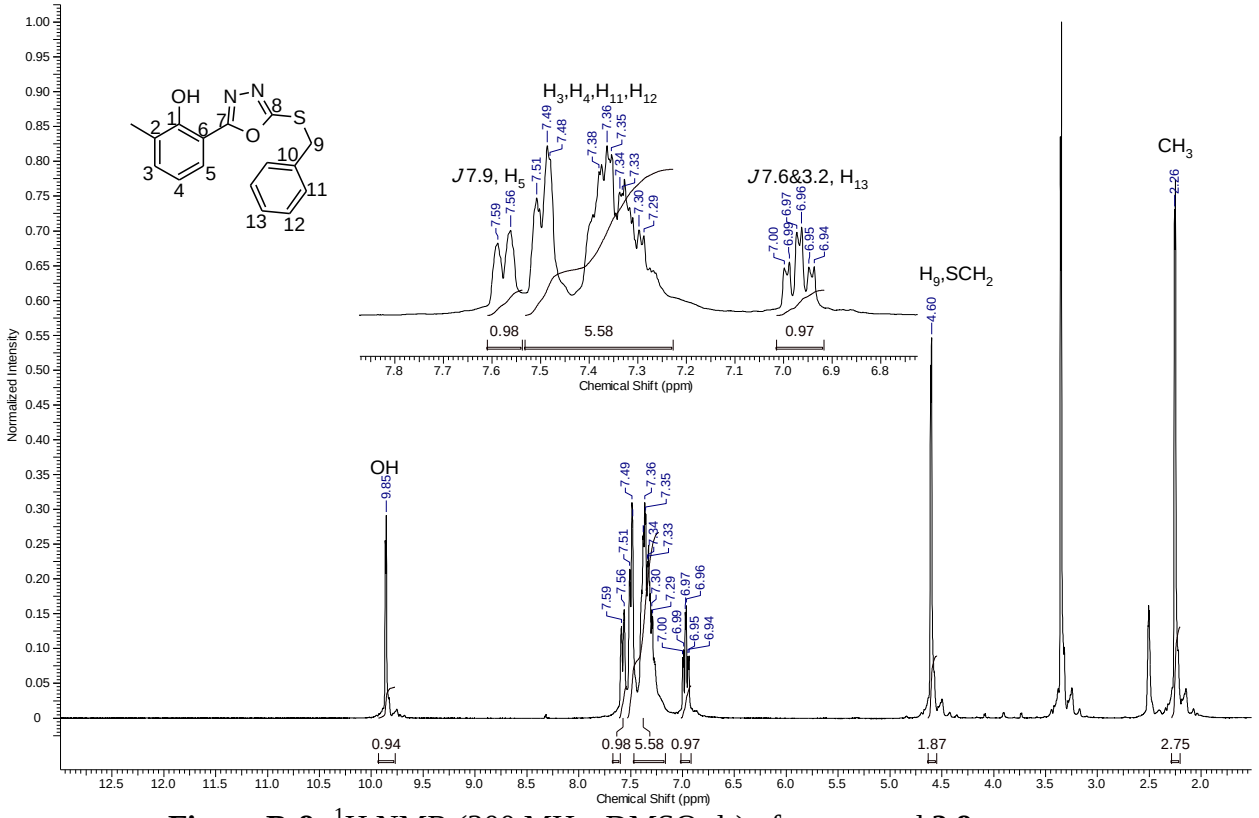


Figure B-8:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.8**

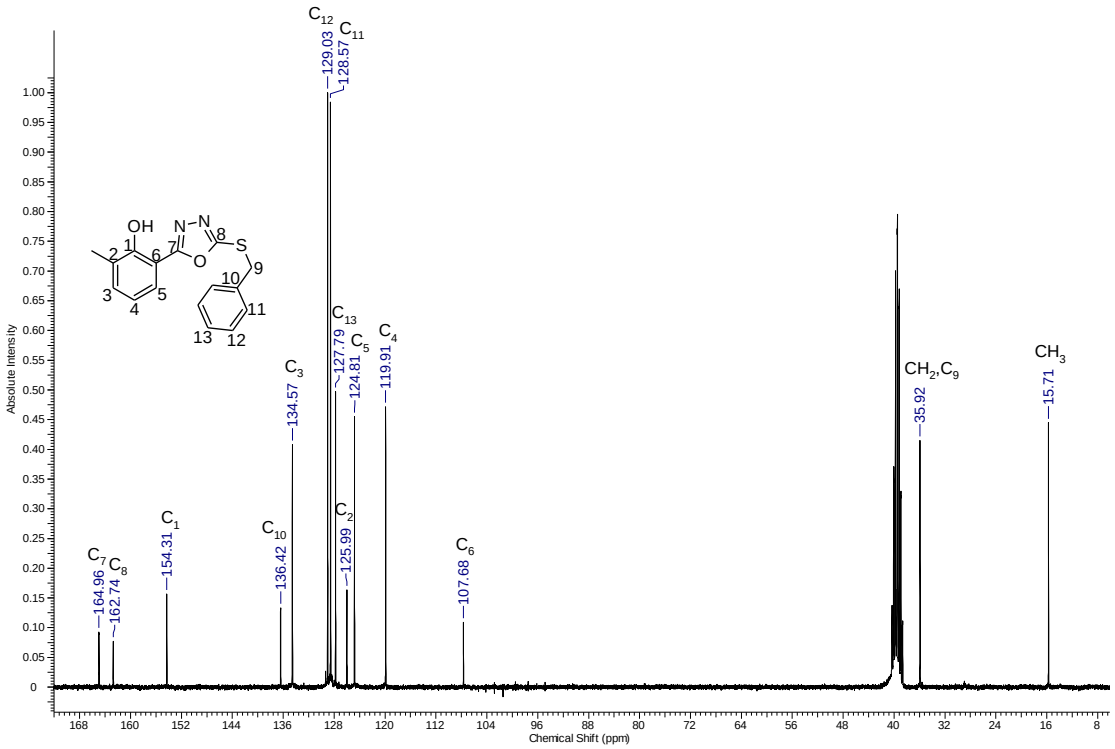


Figure B-9:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.8**

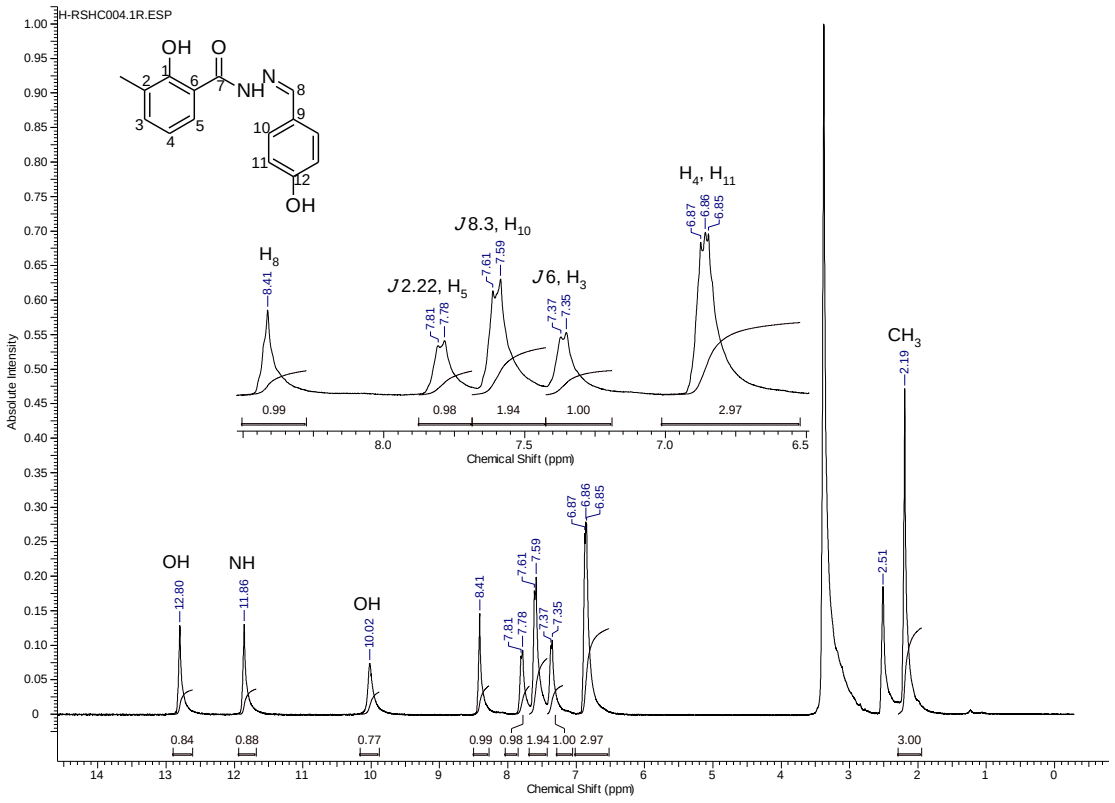


Figure B-10:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.10**

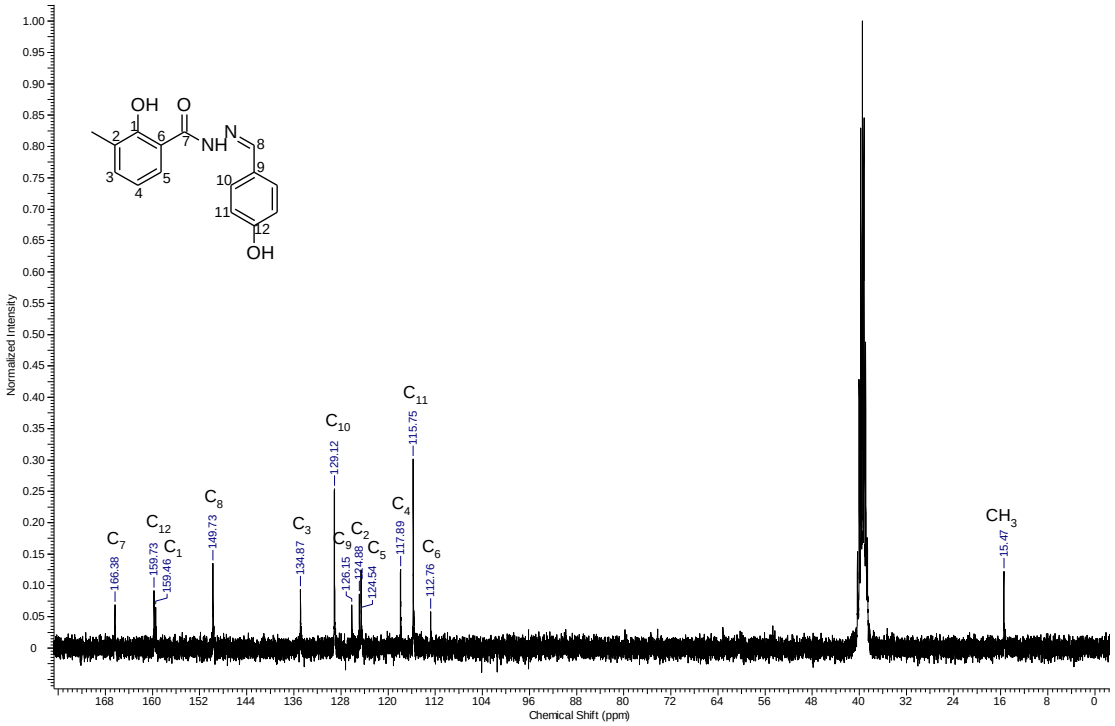


Figure B-11:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.10**

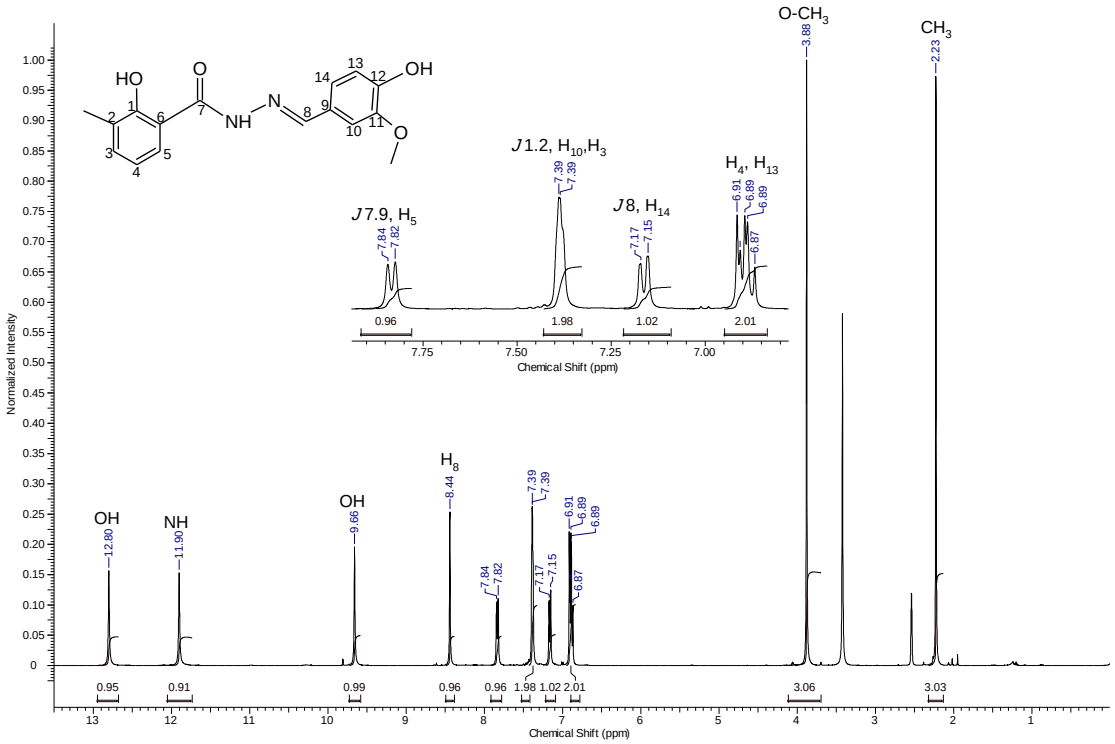


Figure B-12:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.11**

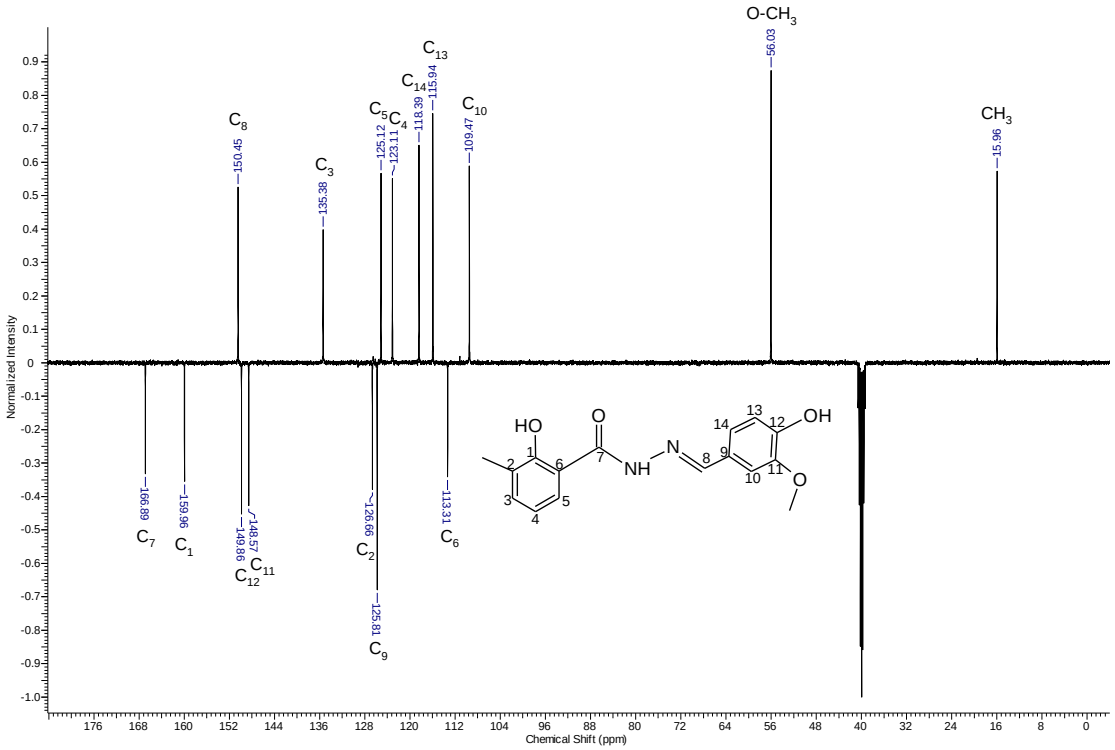


Figure B-13:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.11**

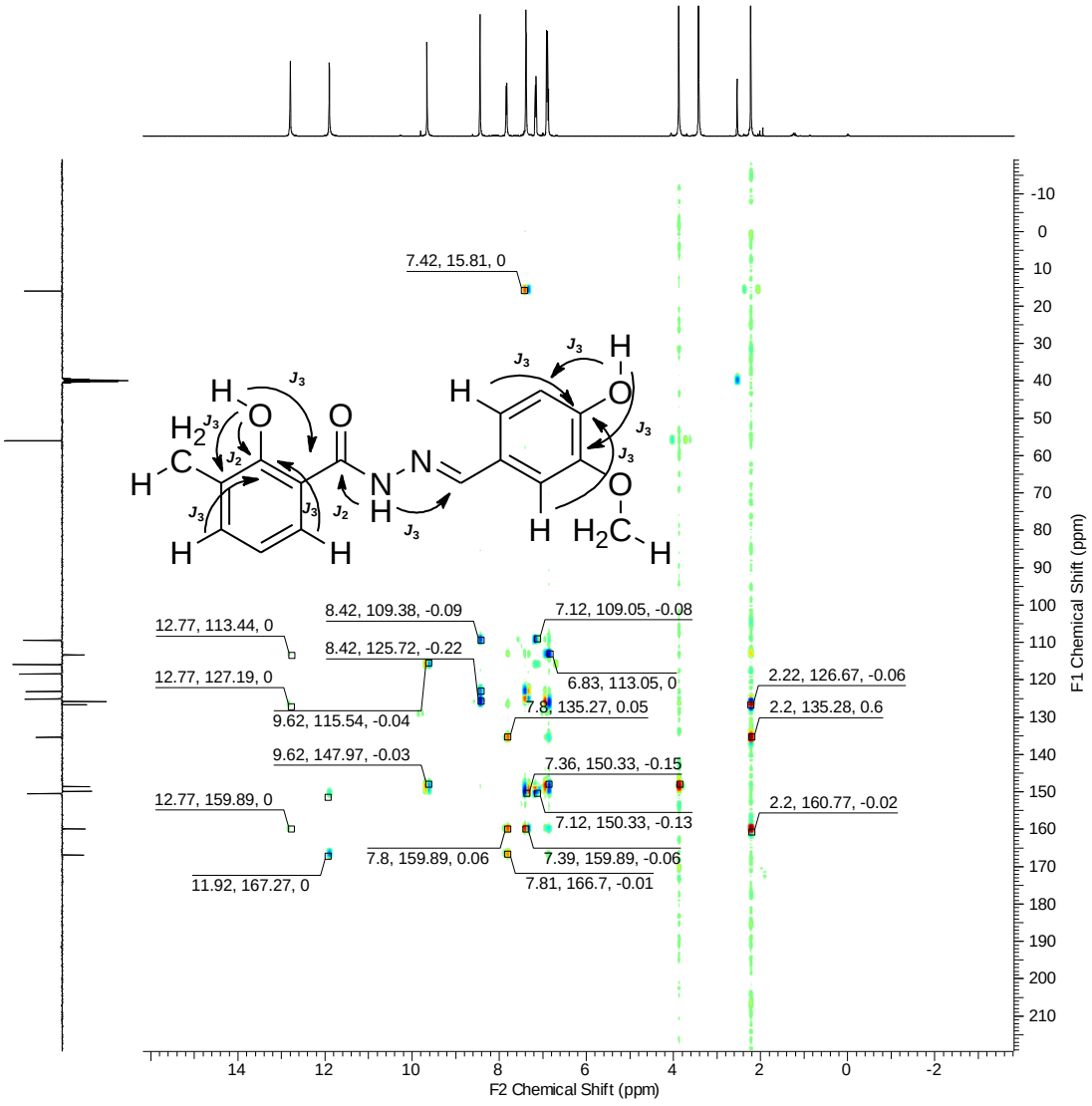


Figure B-14:-HMBC of compound **2.11**

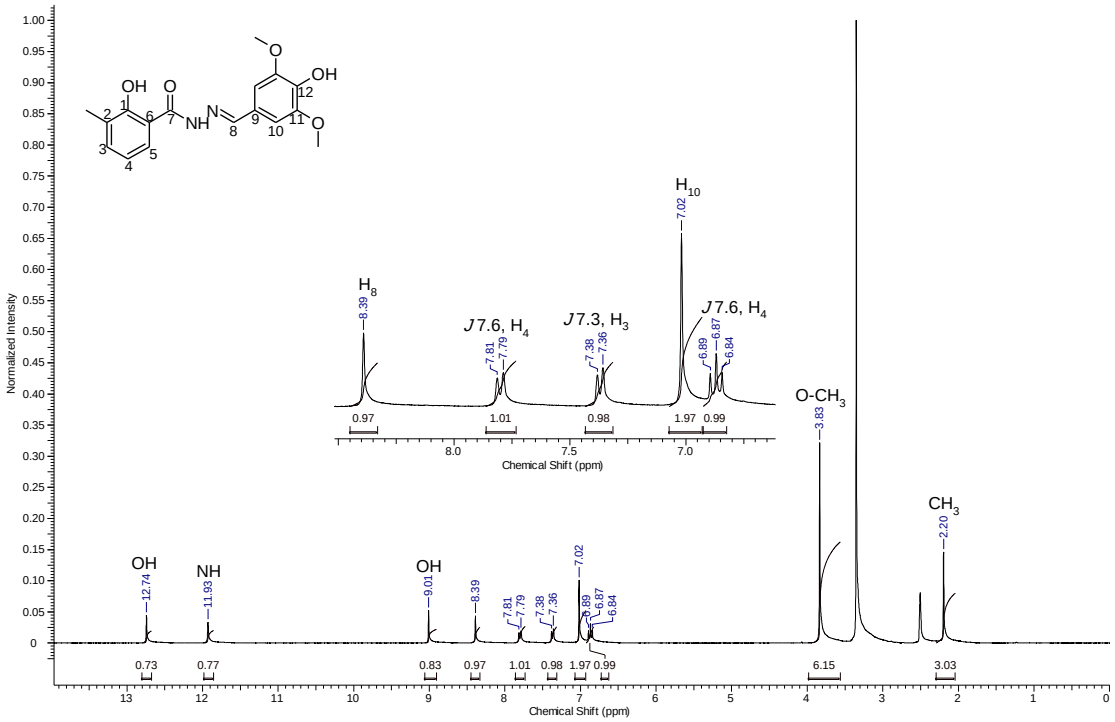


Figure B-15:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.12**

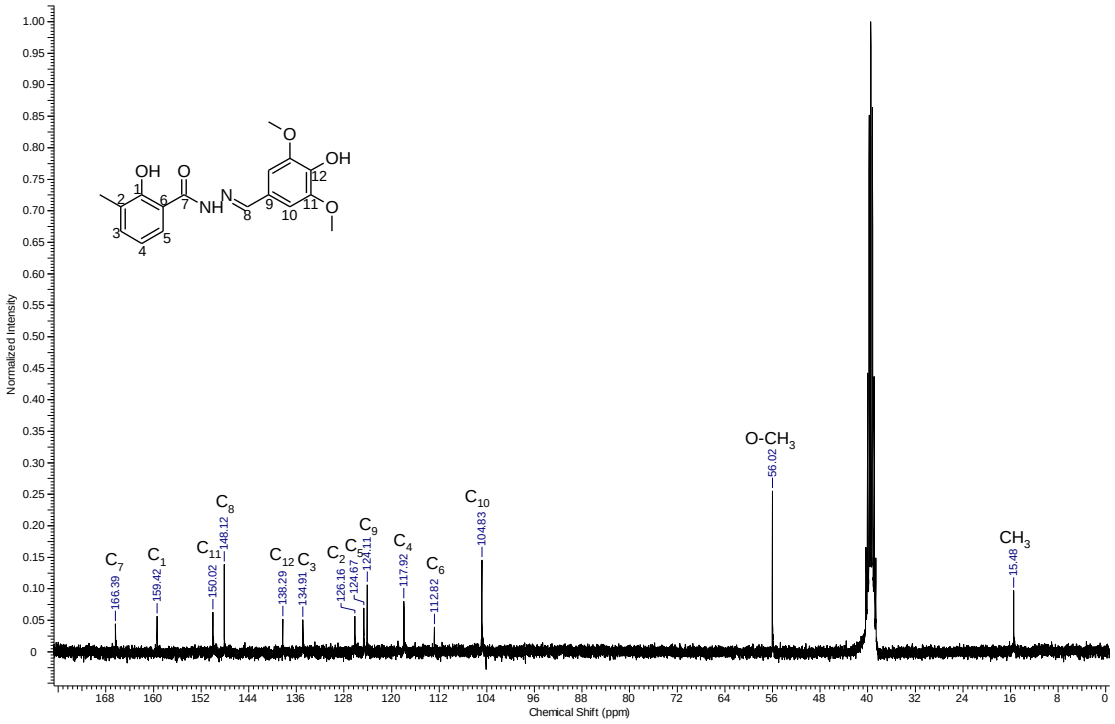


Figure B-16:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.12**

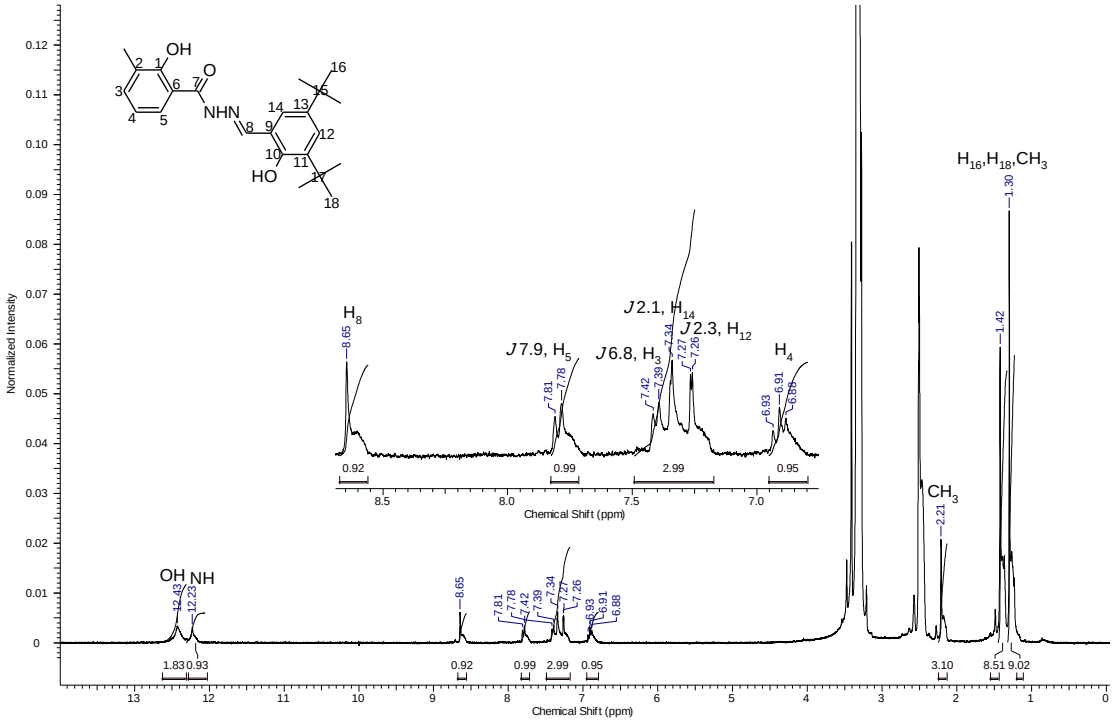


Figure B-17:-^{1}H NMR (300 MHz, DMSO-d_6) of compound 2.13

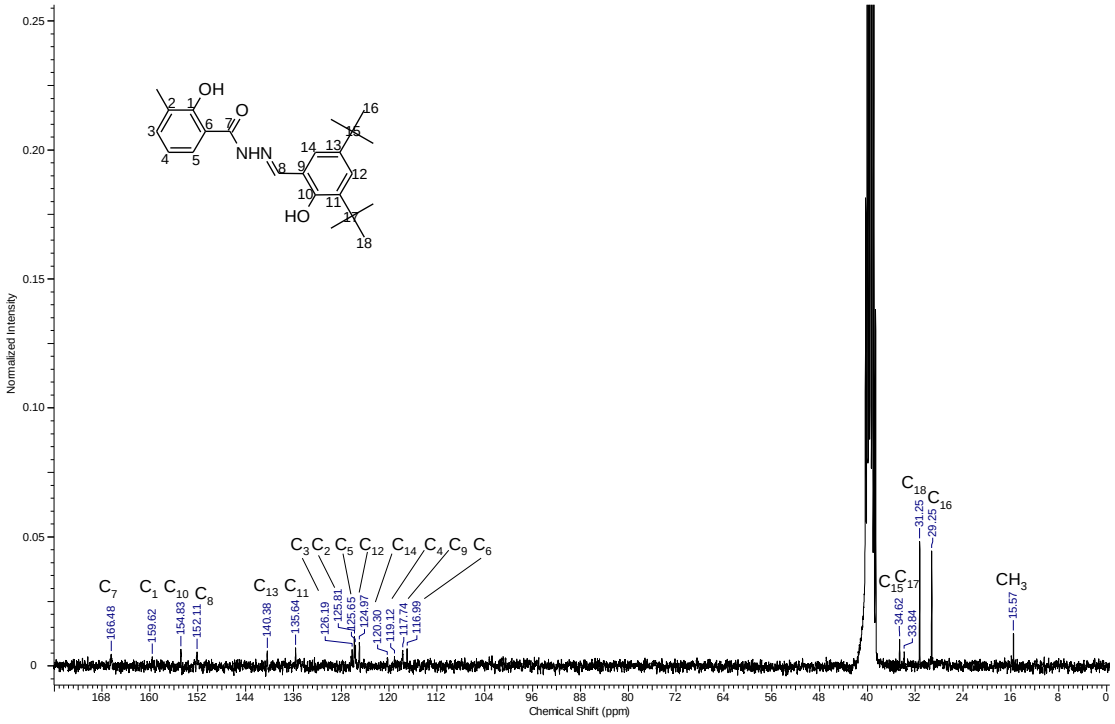


Figure B-18:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.13**

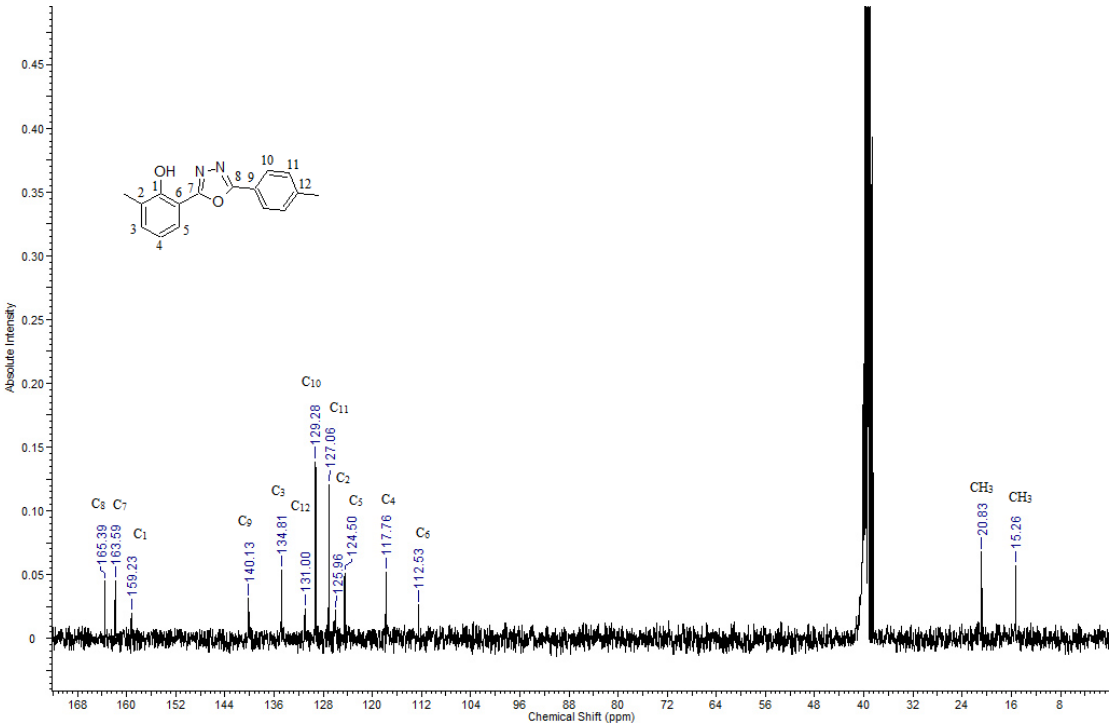


Figure B-19:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.14**

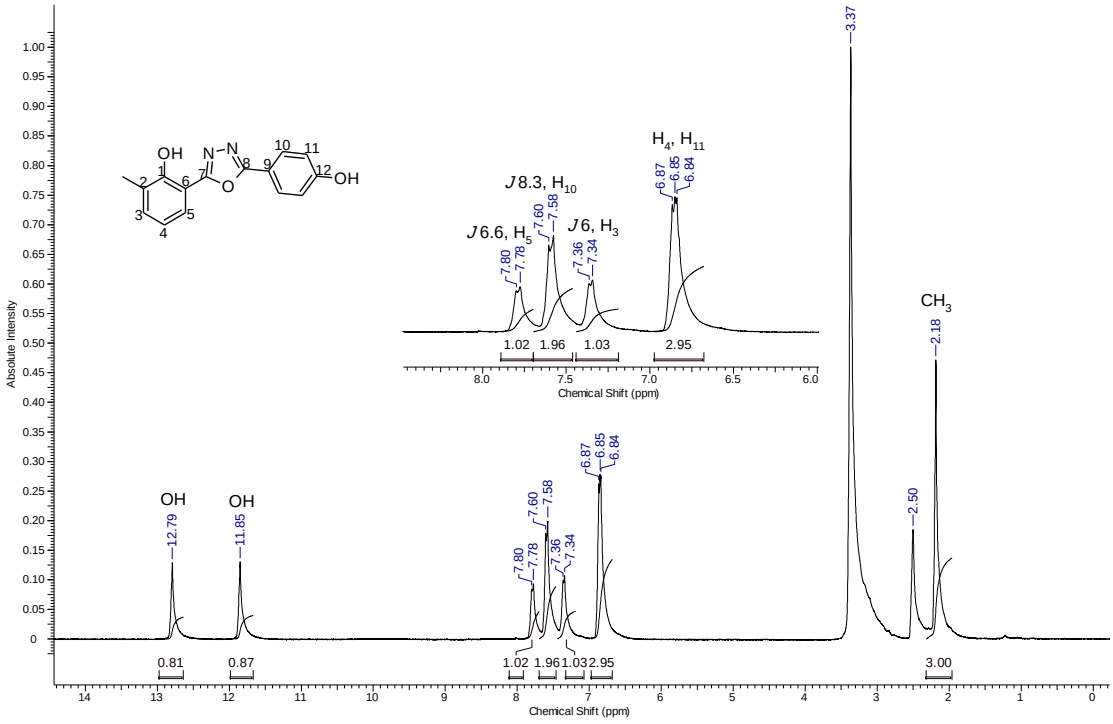


Figure B-20:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.15**

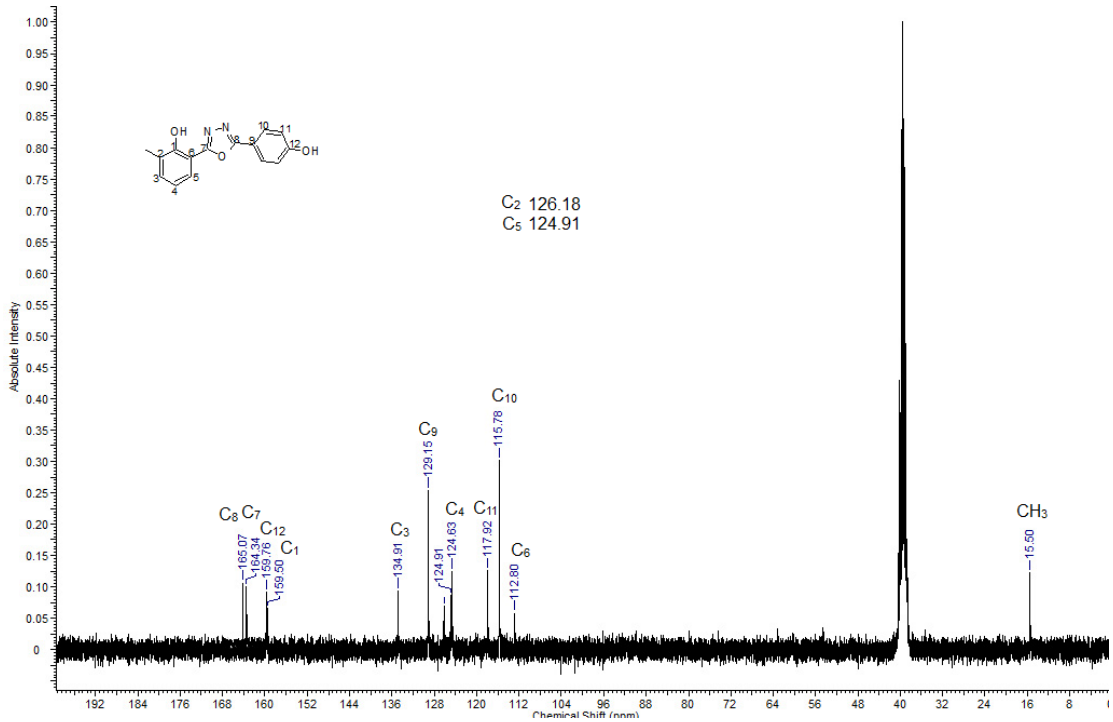


Figure B-21:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.15**

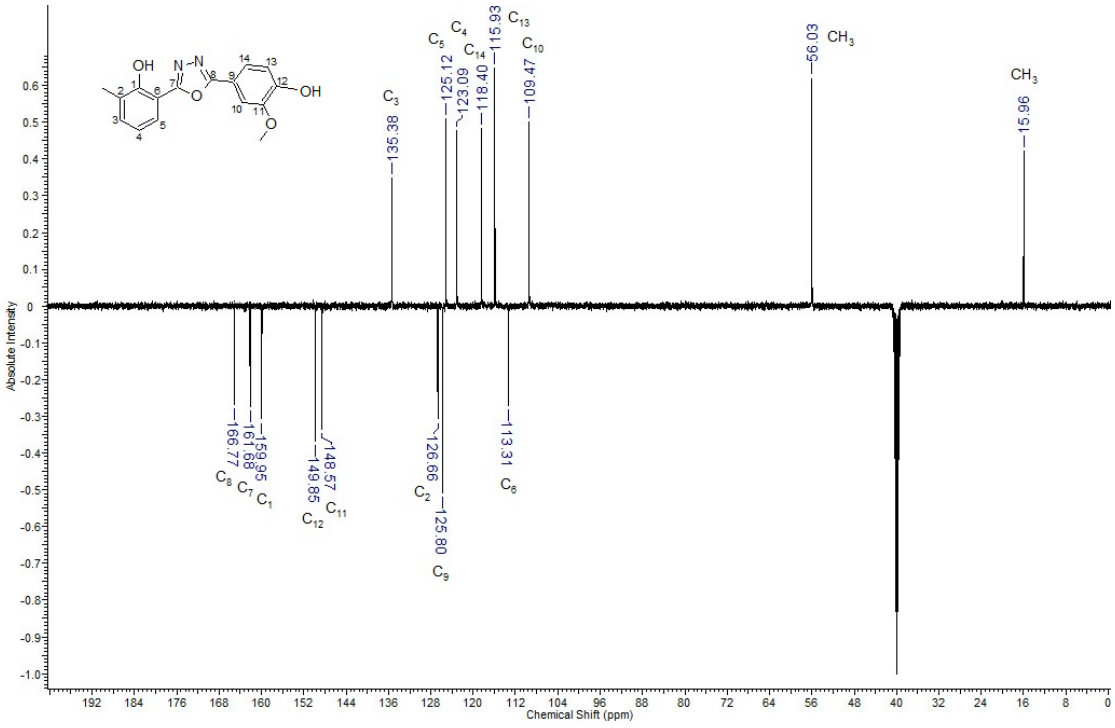


Figure B-22:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.16**

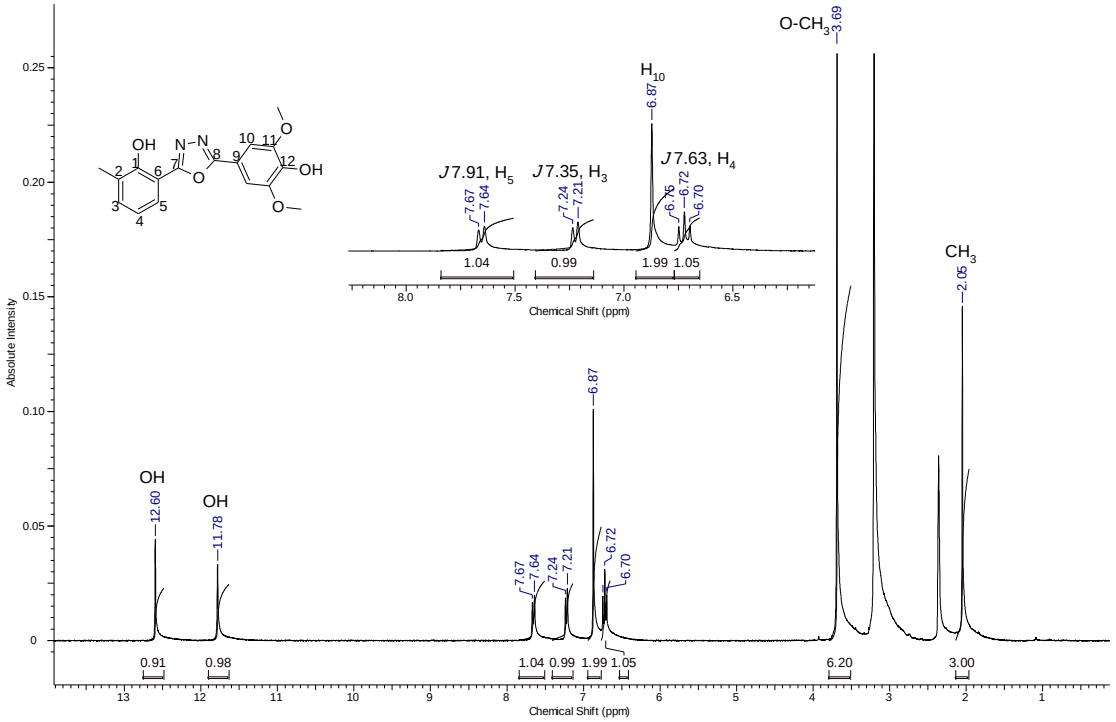


Figure B-23:- (300 MHz, DMSO-d_6) of compound **2.17**

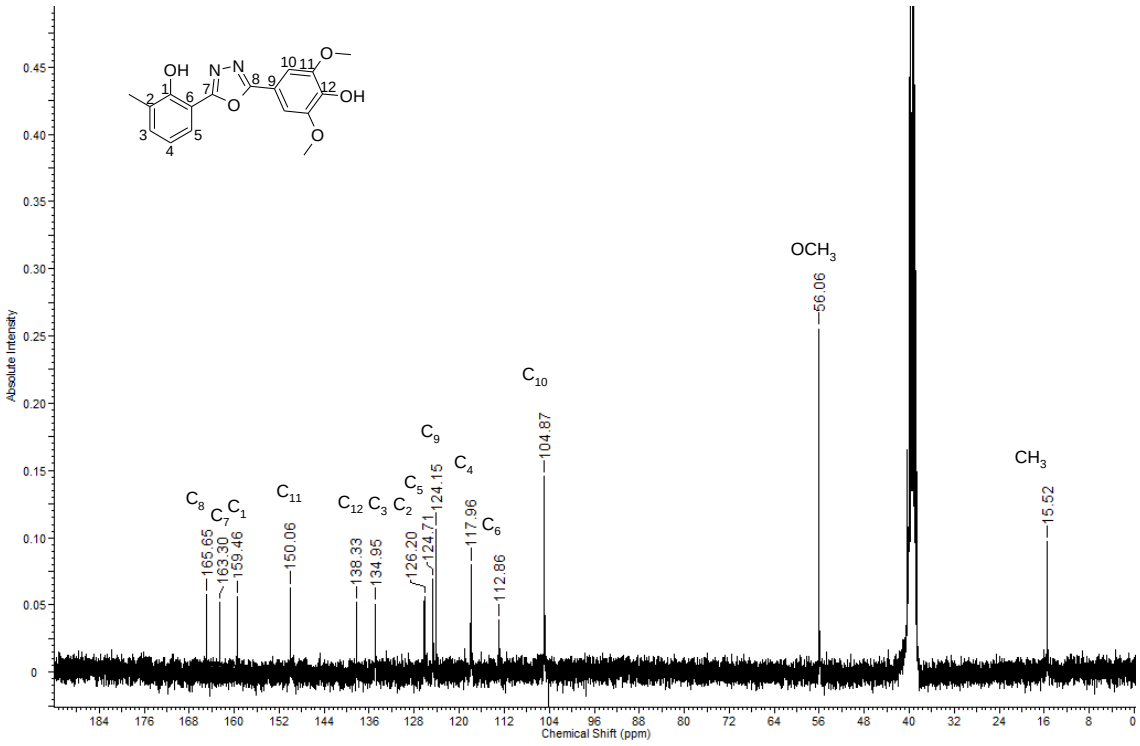


Figure B-24:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.17**

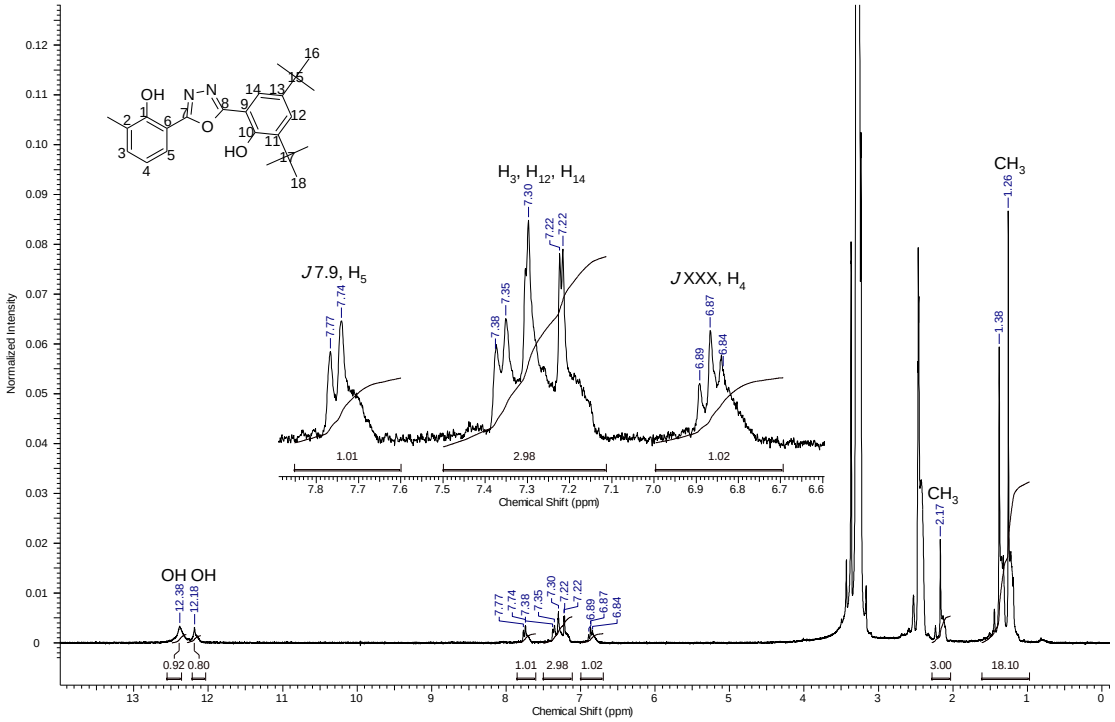


Figure B-25:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.18**

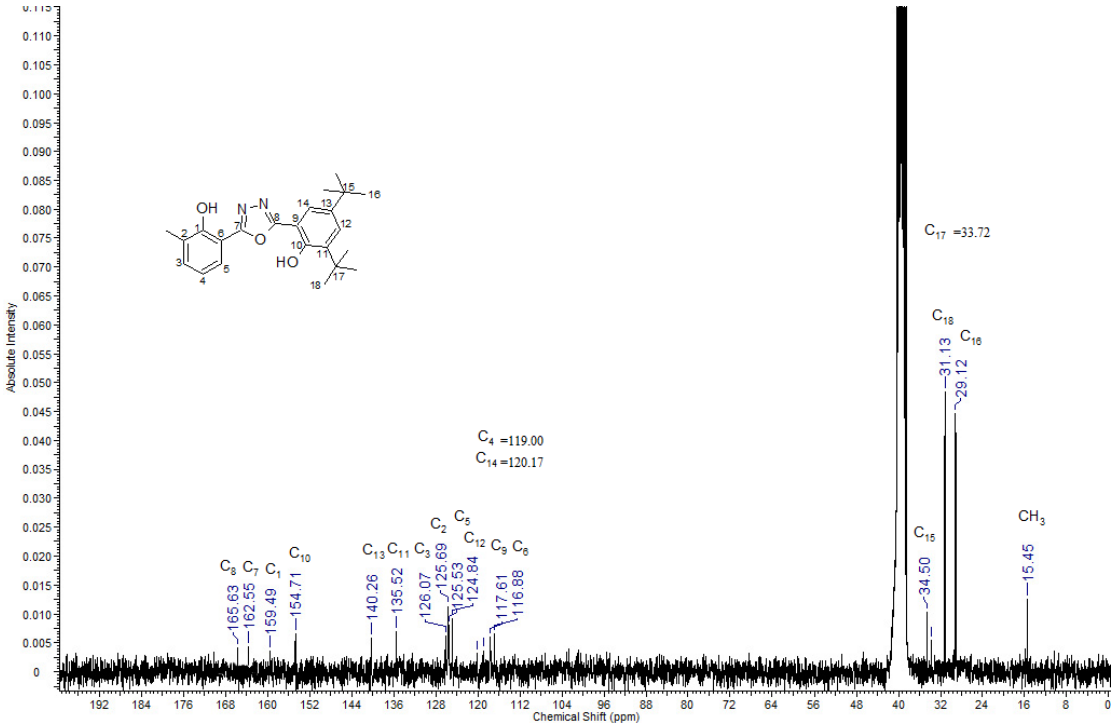


Figure B-26:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.18**

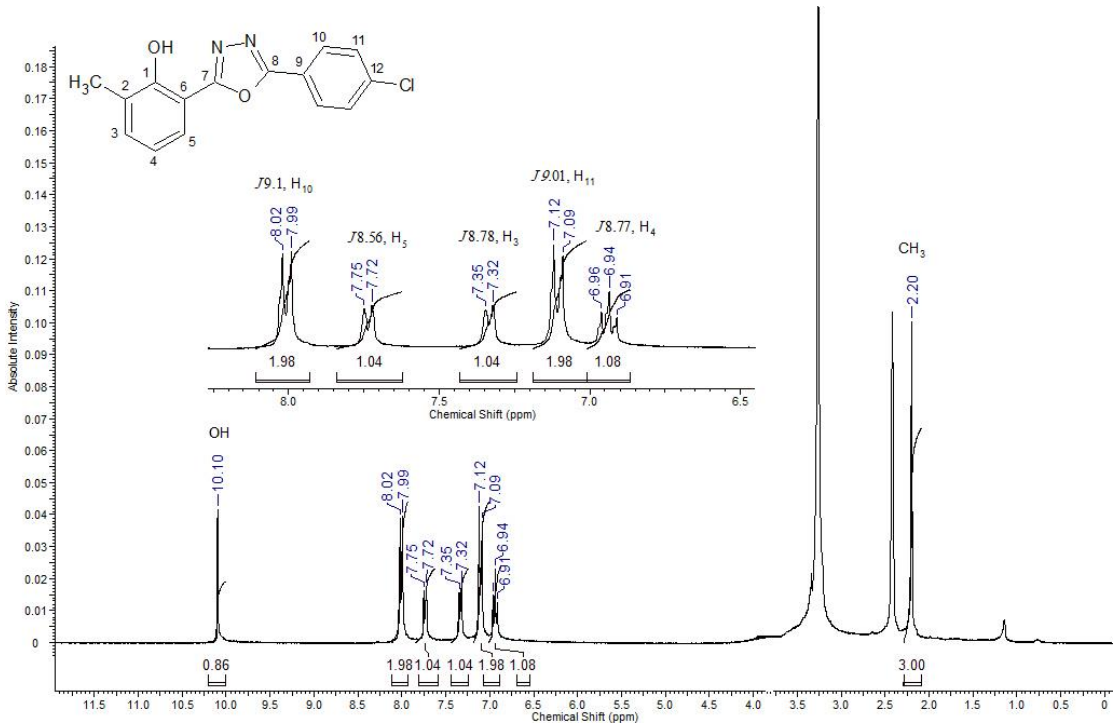


Figure B-27:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.19**

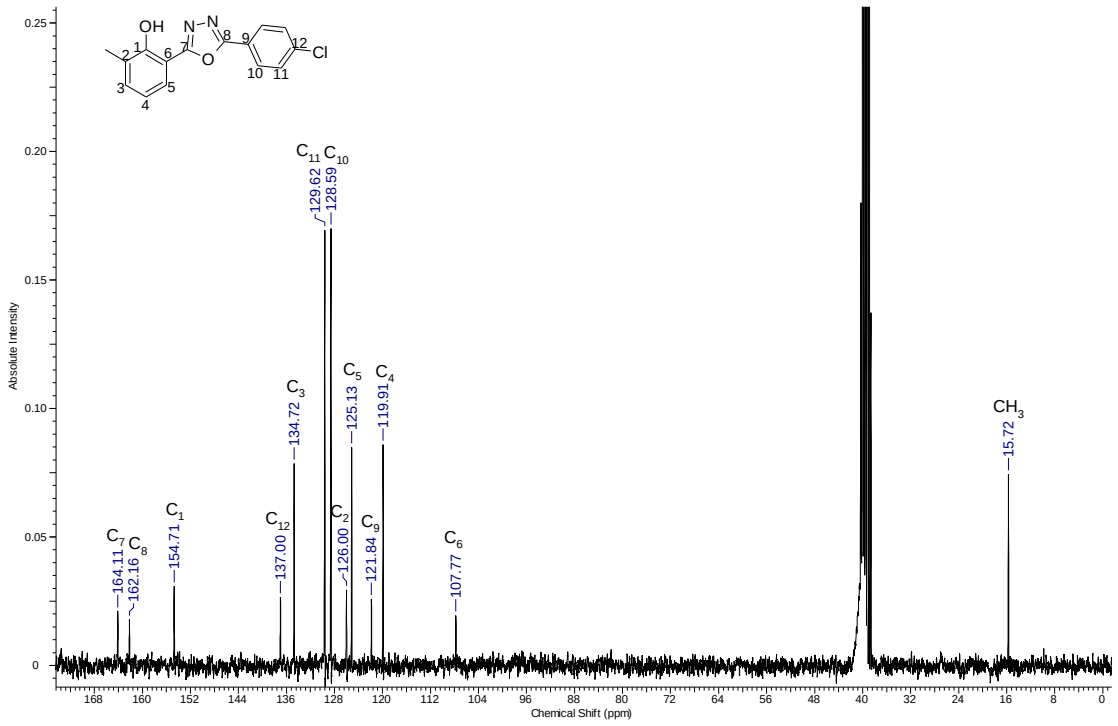


Figure B-28:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.19**

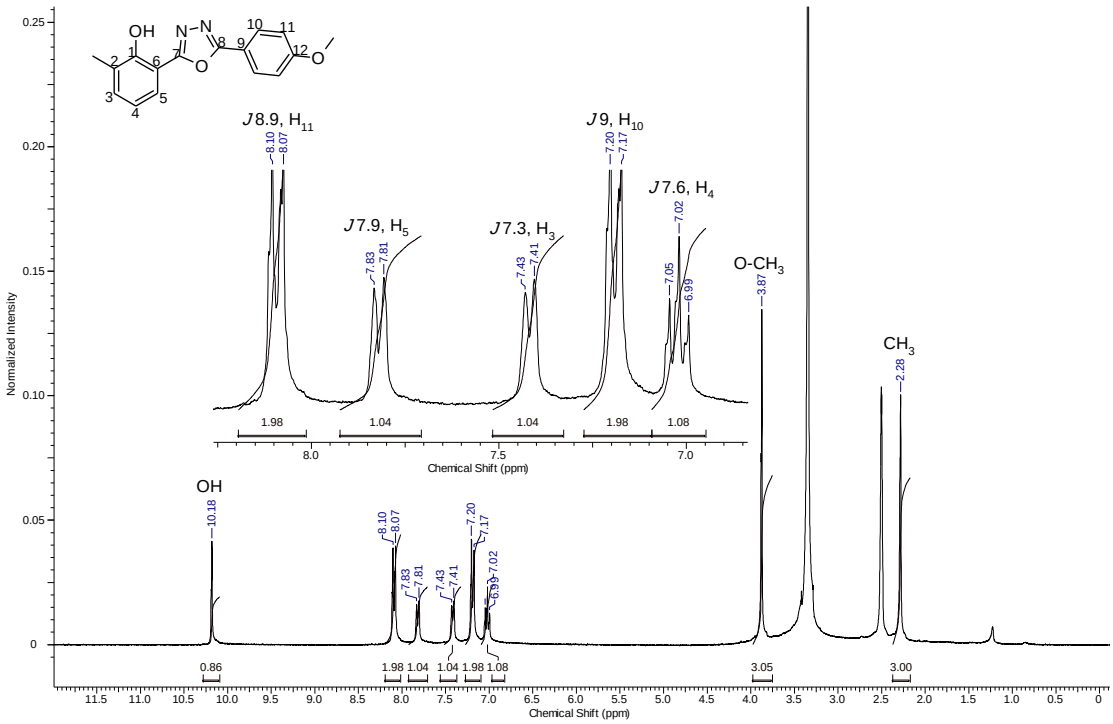


Figure B-29:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.20**

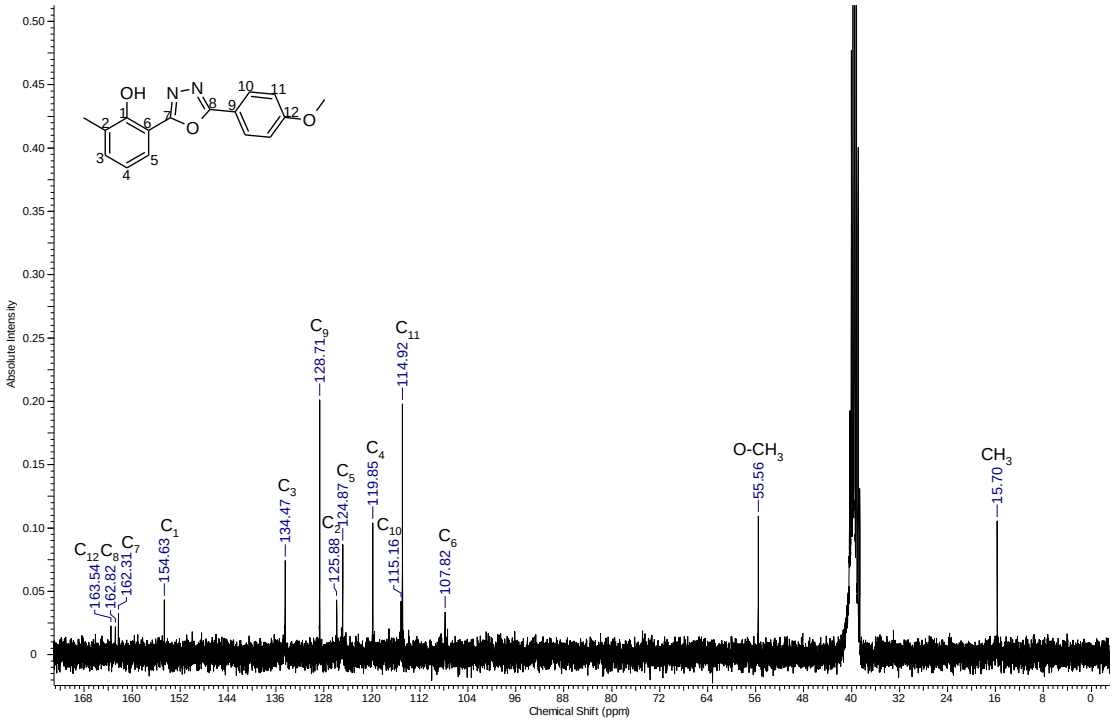


Figure B-30:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.20**

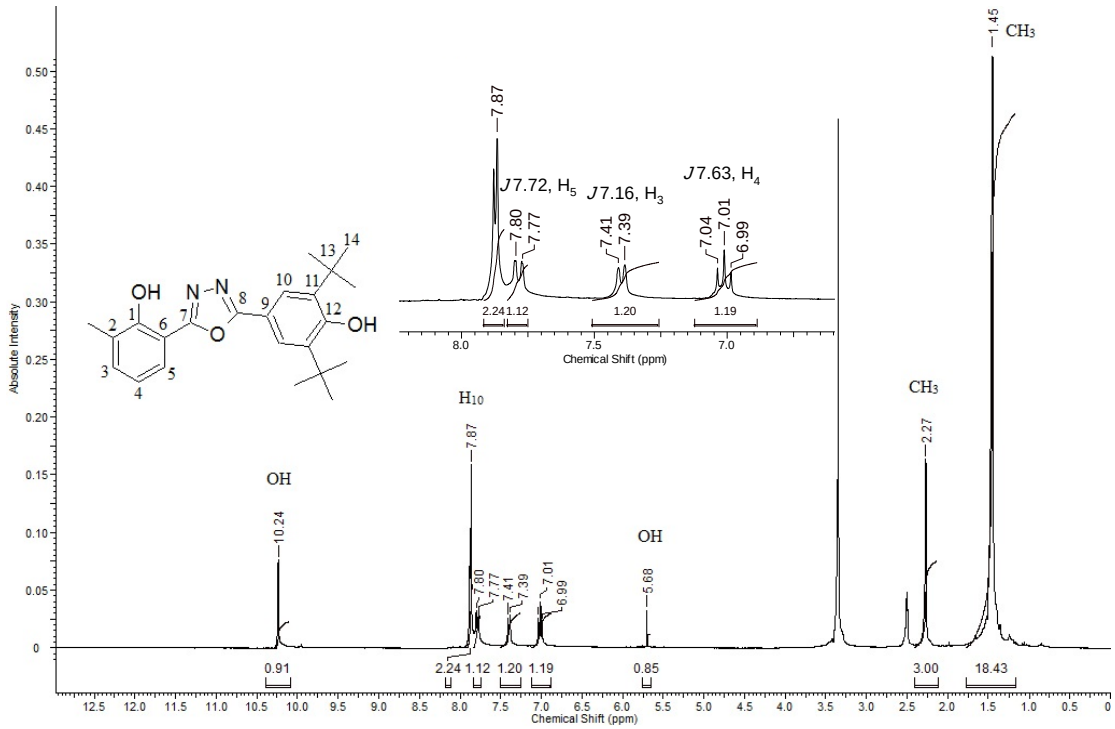


Figure B-31:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.21**

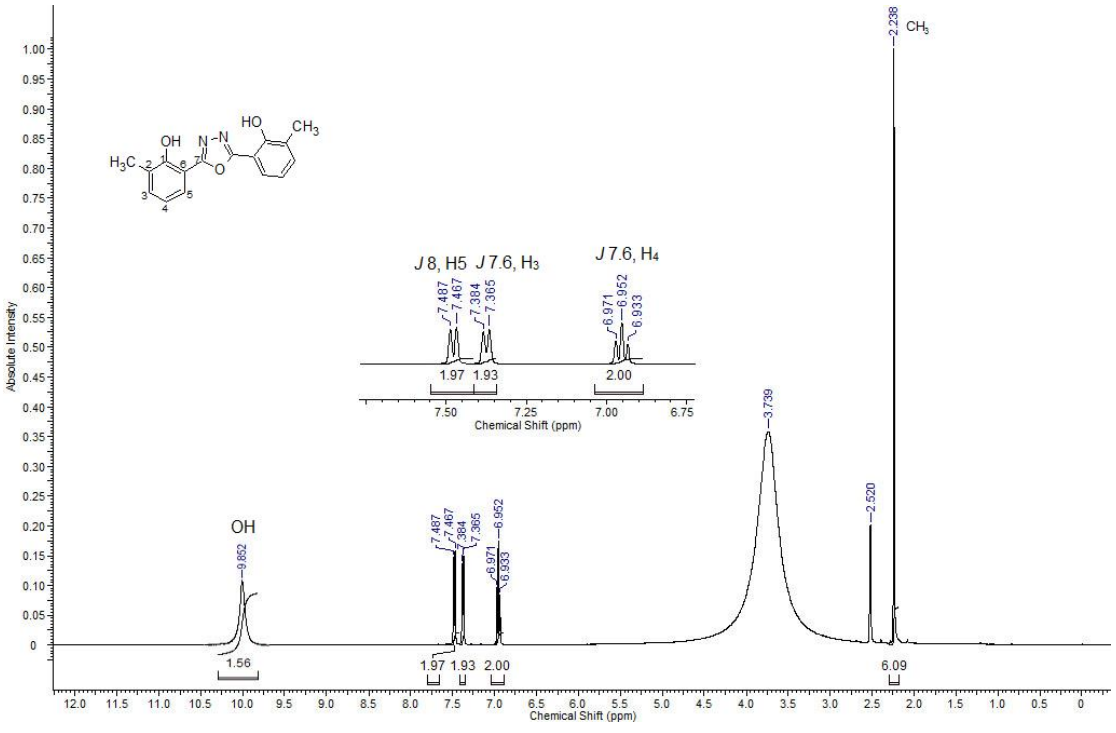


Figure B-32:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.22**

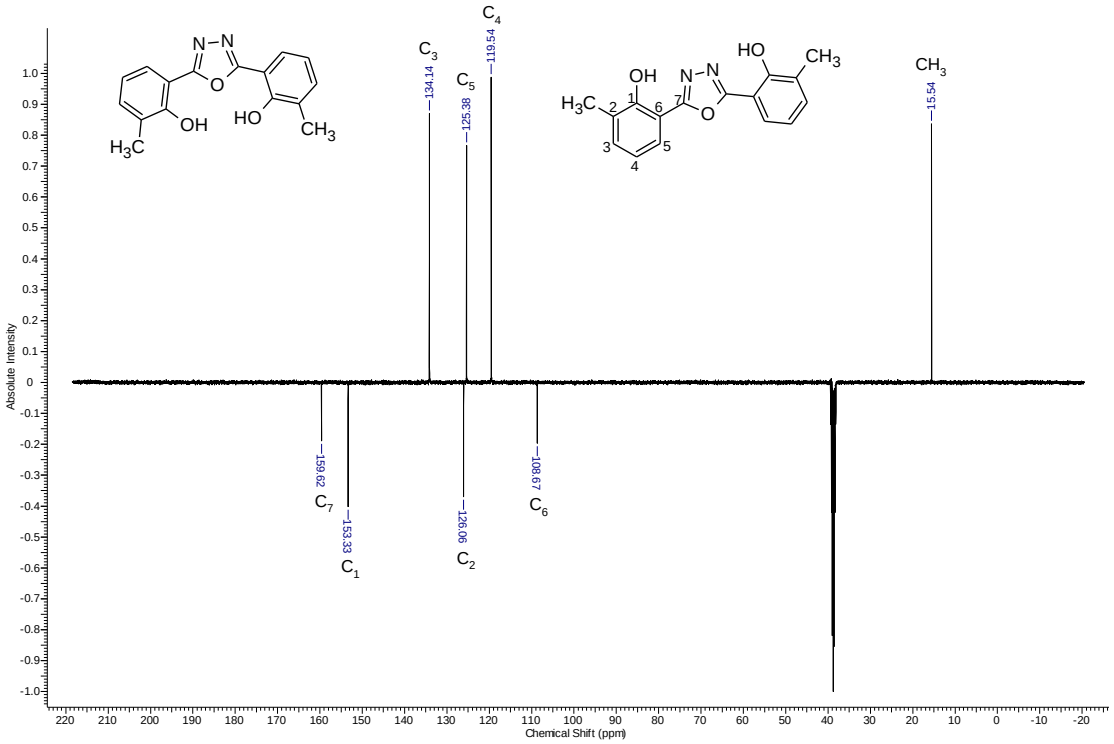


Figure B-33:-^{13}C NMR (100 MHz, DMSO-d_6) of compound **2.22**

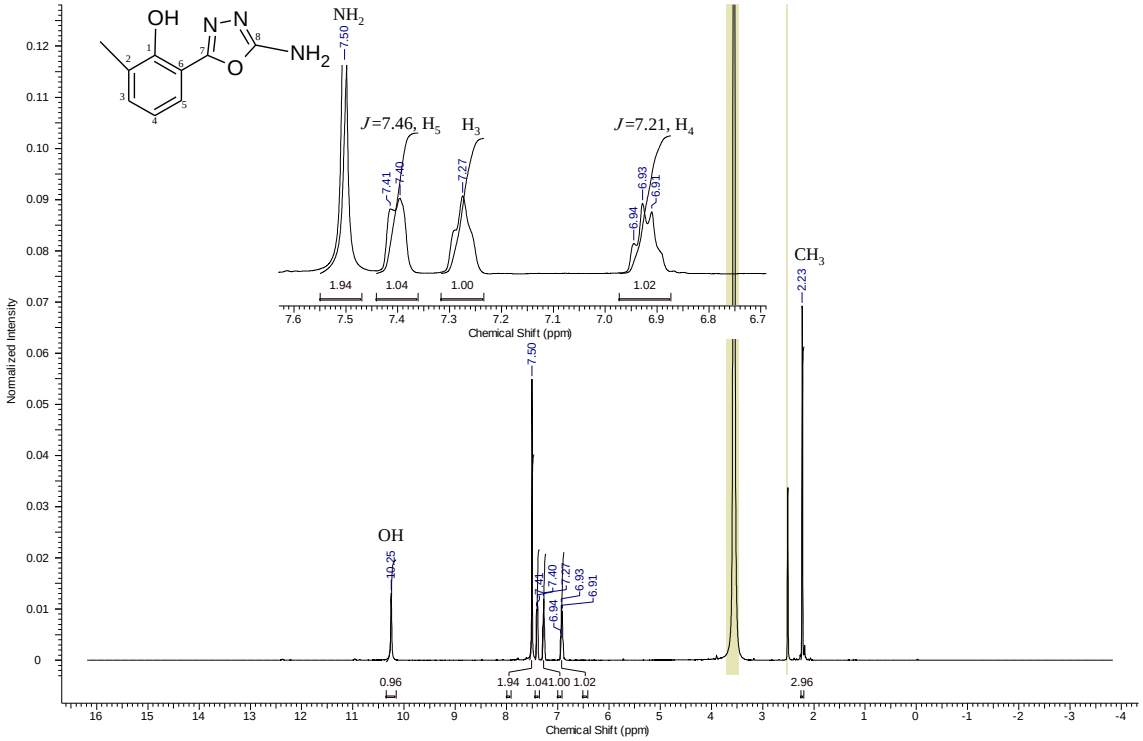


Figure B-34:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.23**

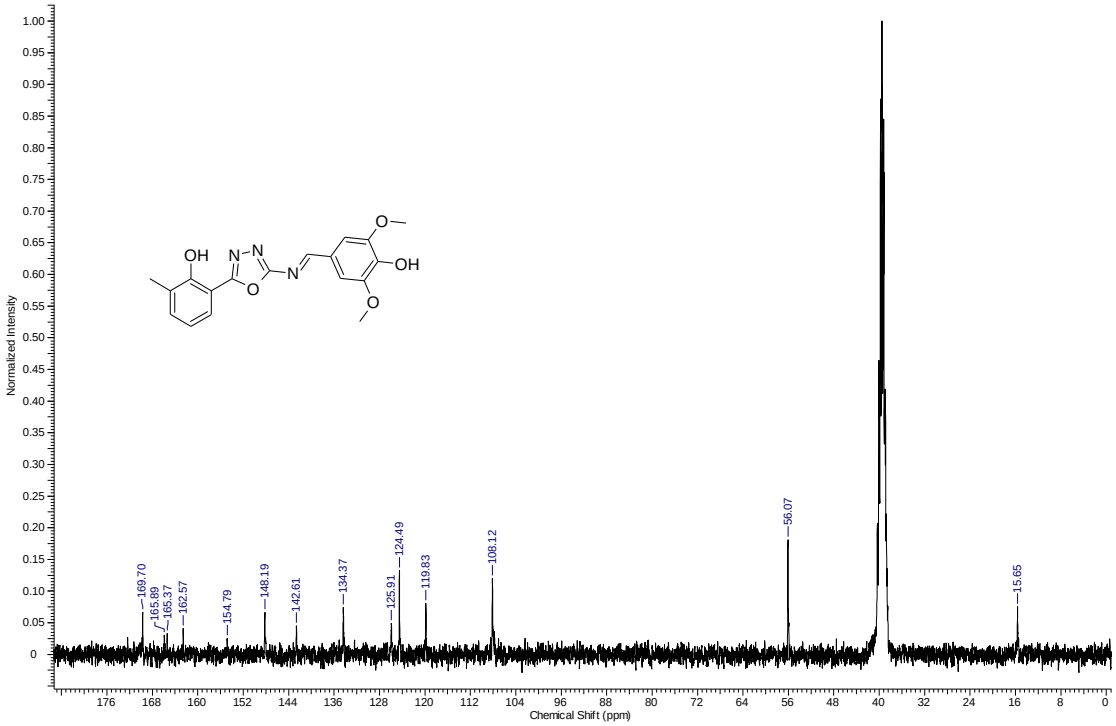


Figure B-35:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.24**

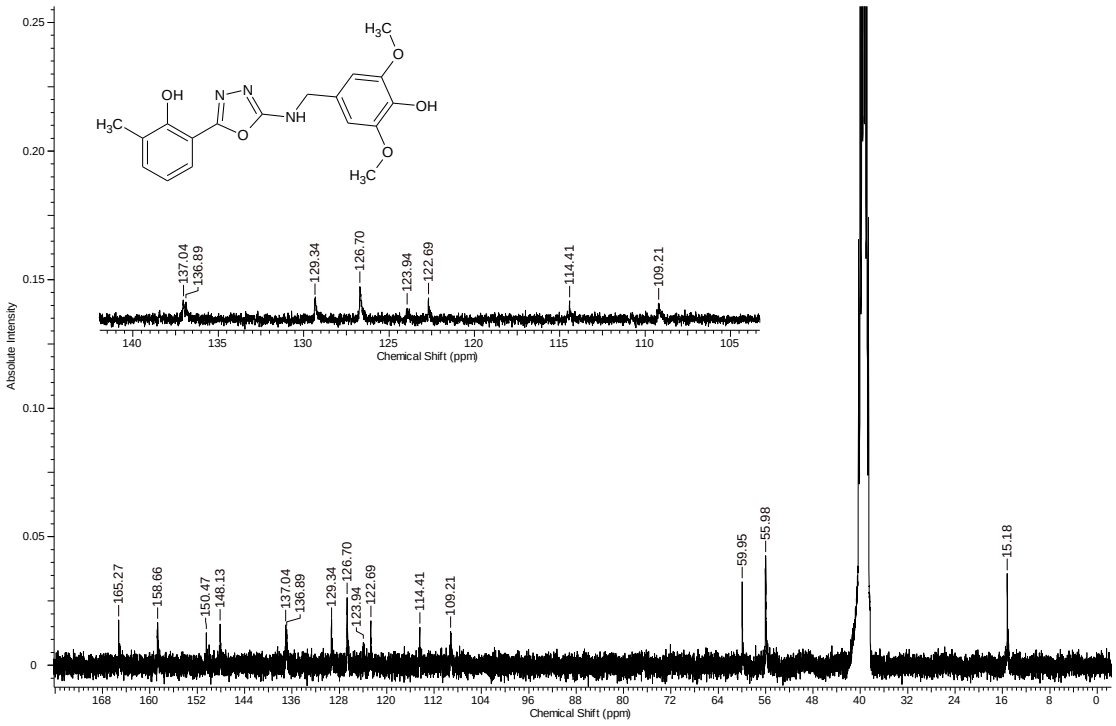


Figure B-36:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.26**

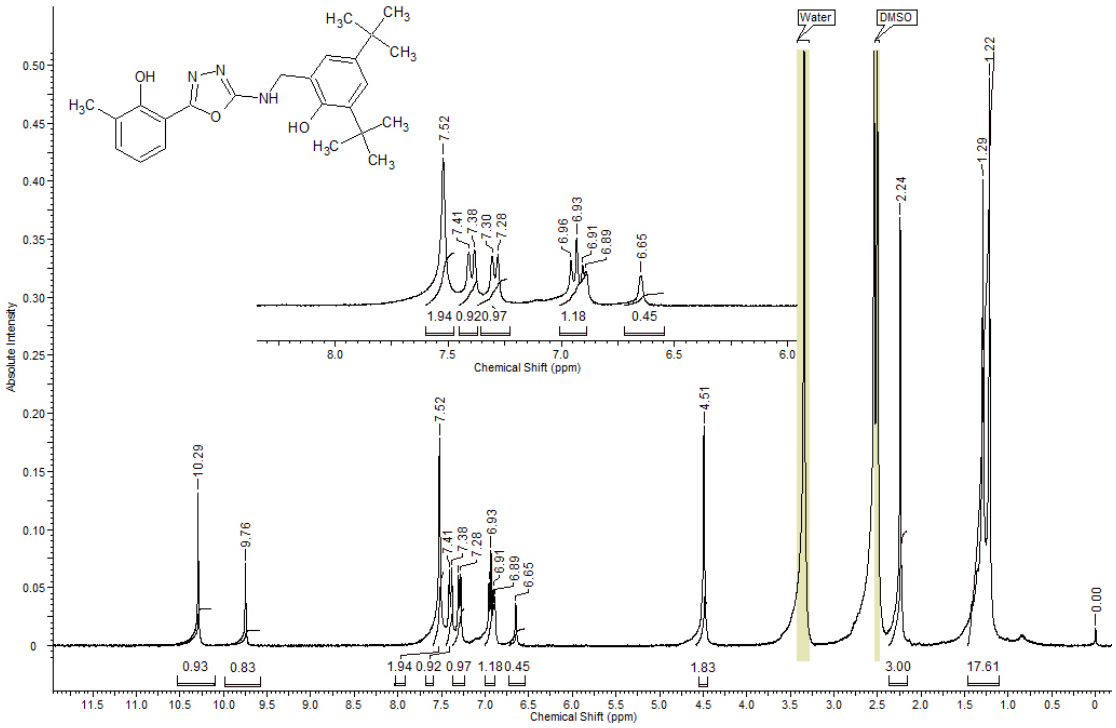


Figure B-37:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.27**

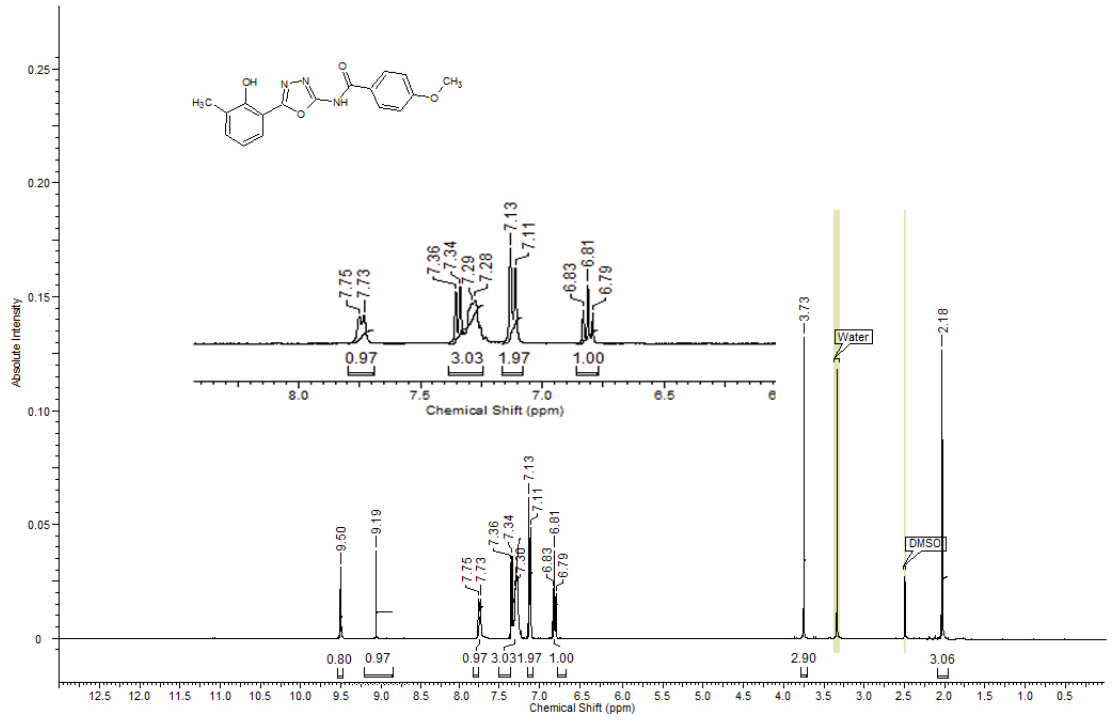


Figure B-38:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.28**

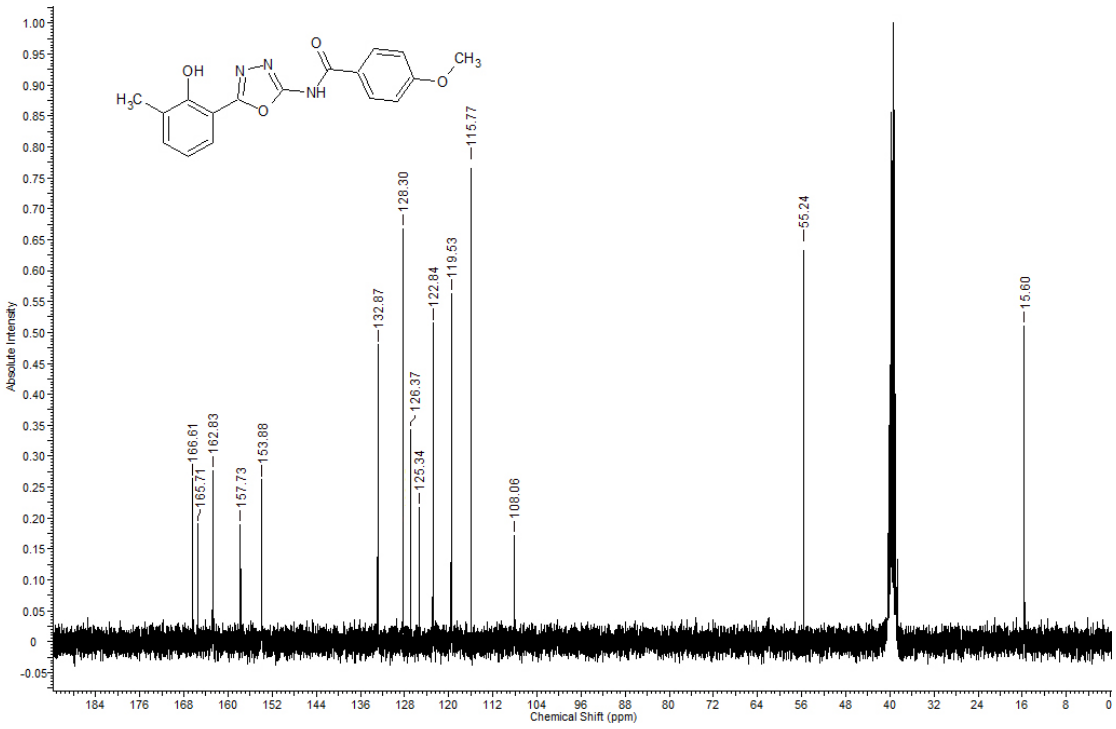


Figure B-39:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.28**

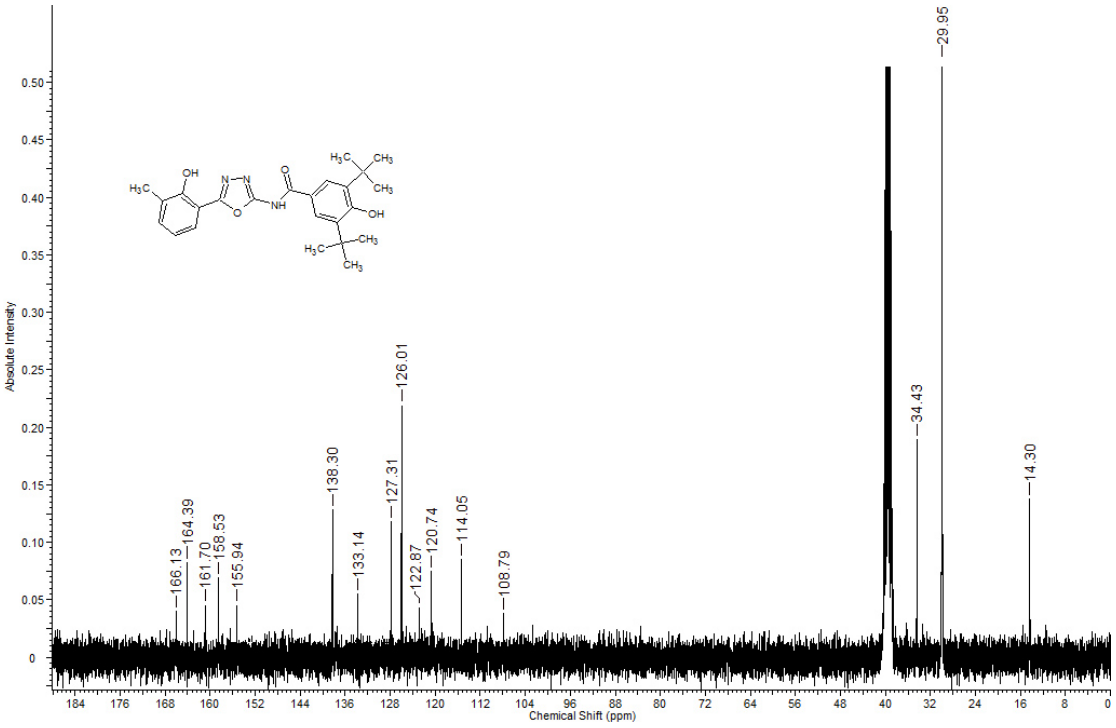


Figure B-40:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.29**

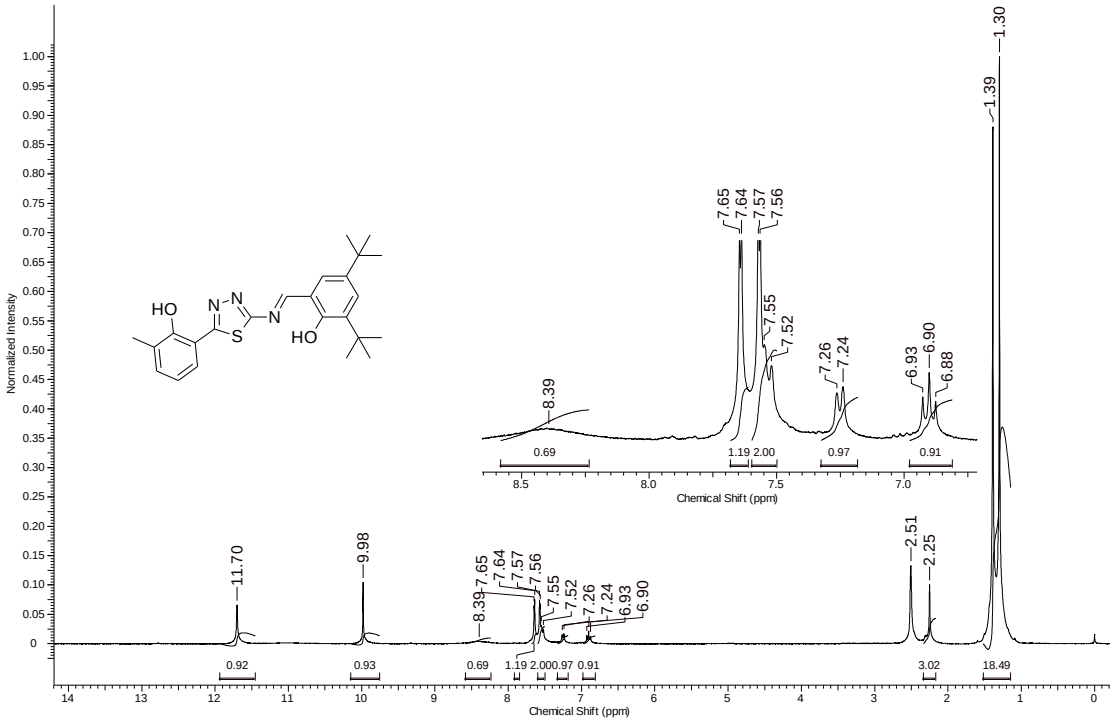


Figure B-41:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.31**

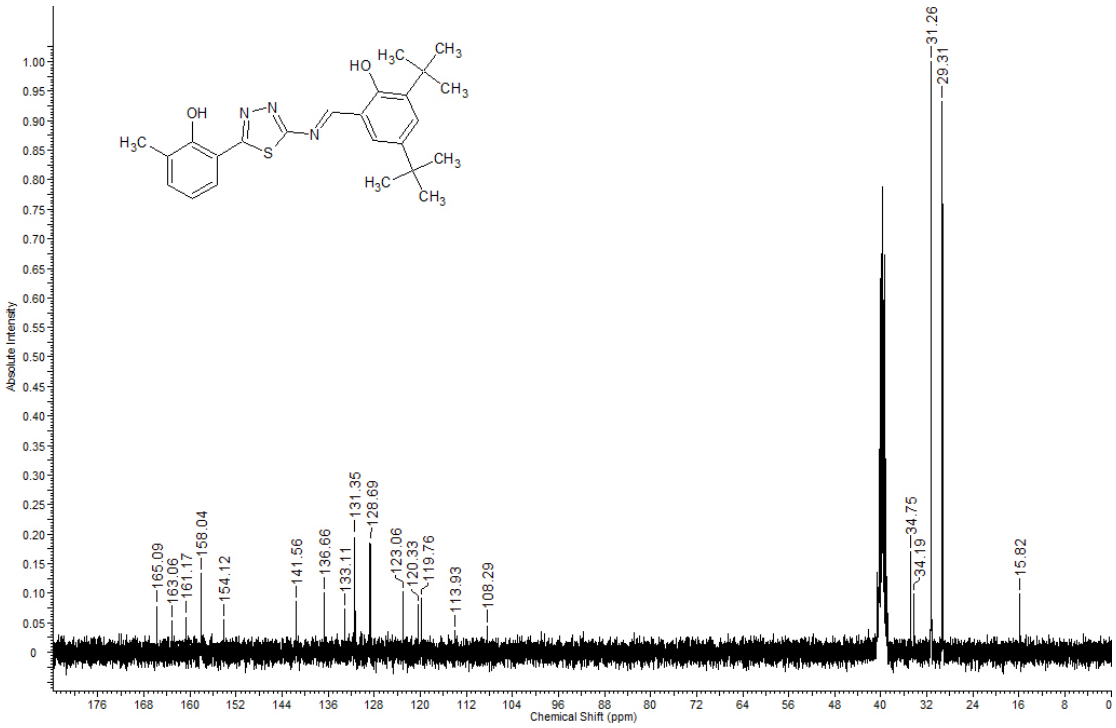


Figure B-42:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.31**

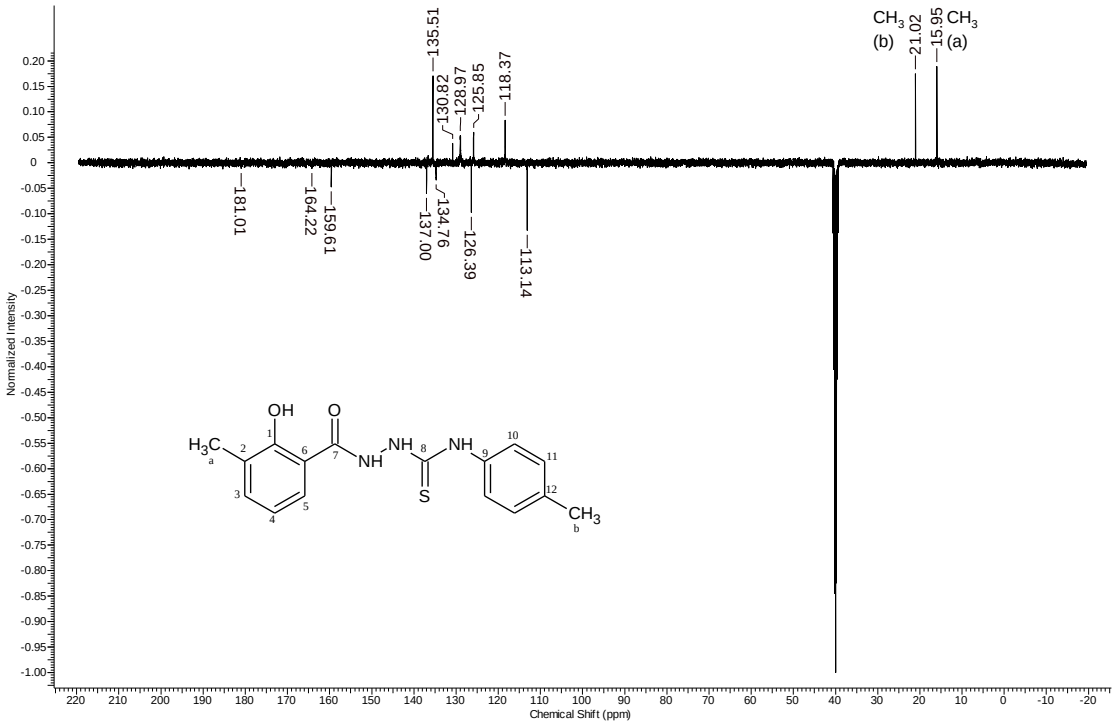


Figure B-43:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2. 34**

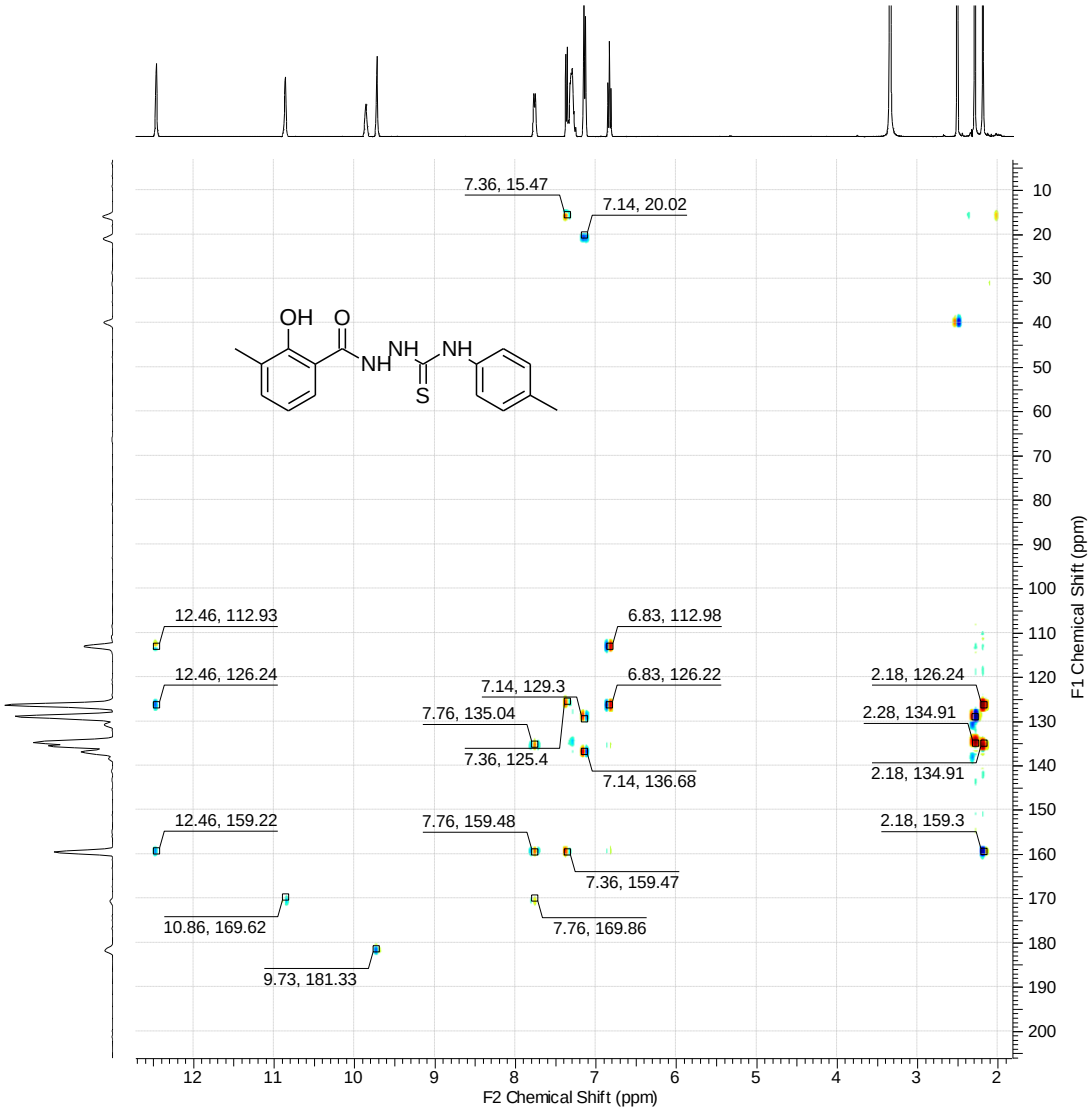


Figure B-44:-HMBC of compound **2. 34**

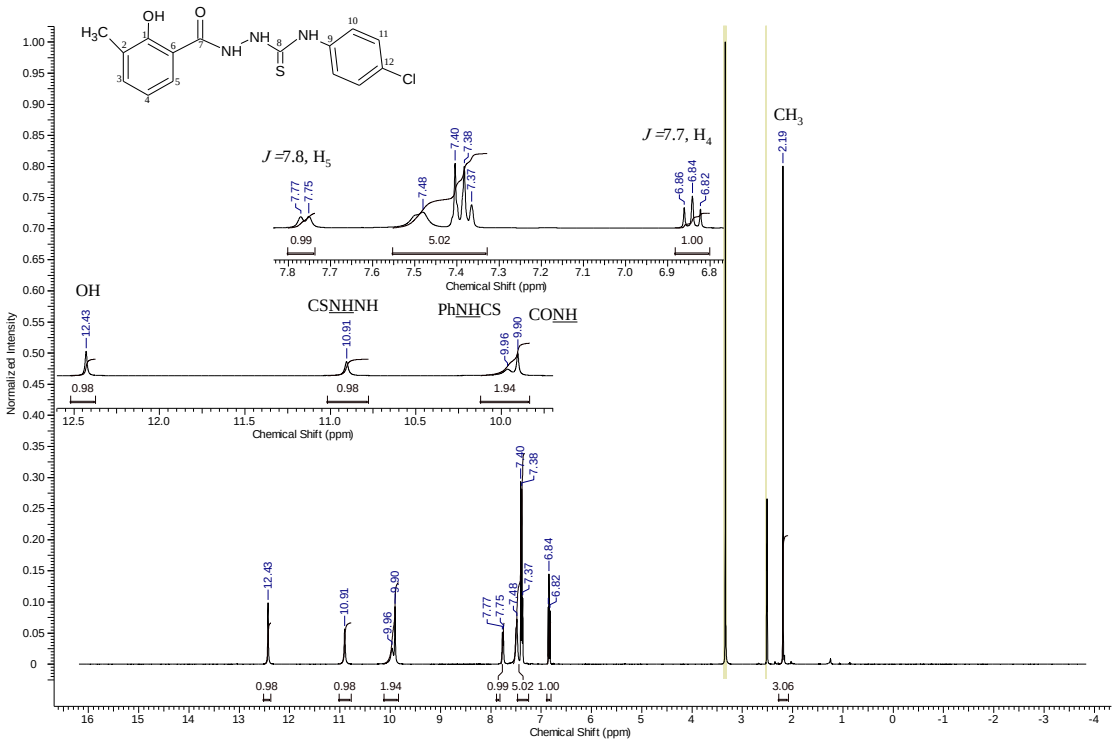


Figure B-45:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.35**

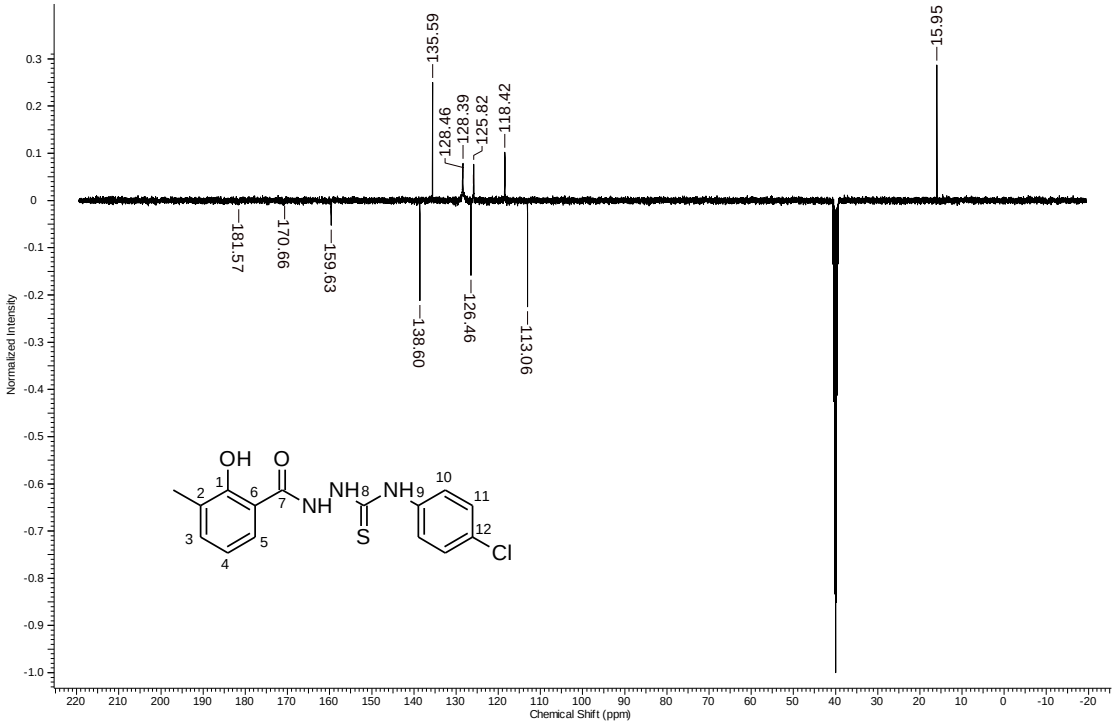


Figure B-46:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.35**

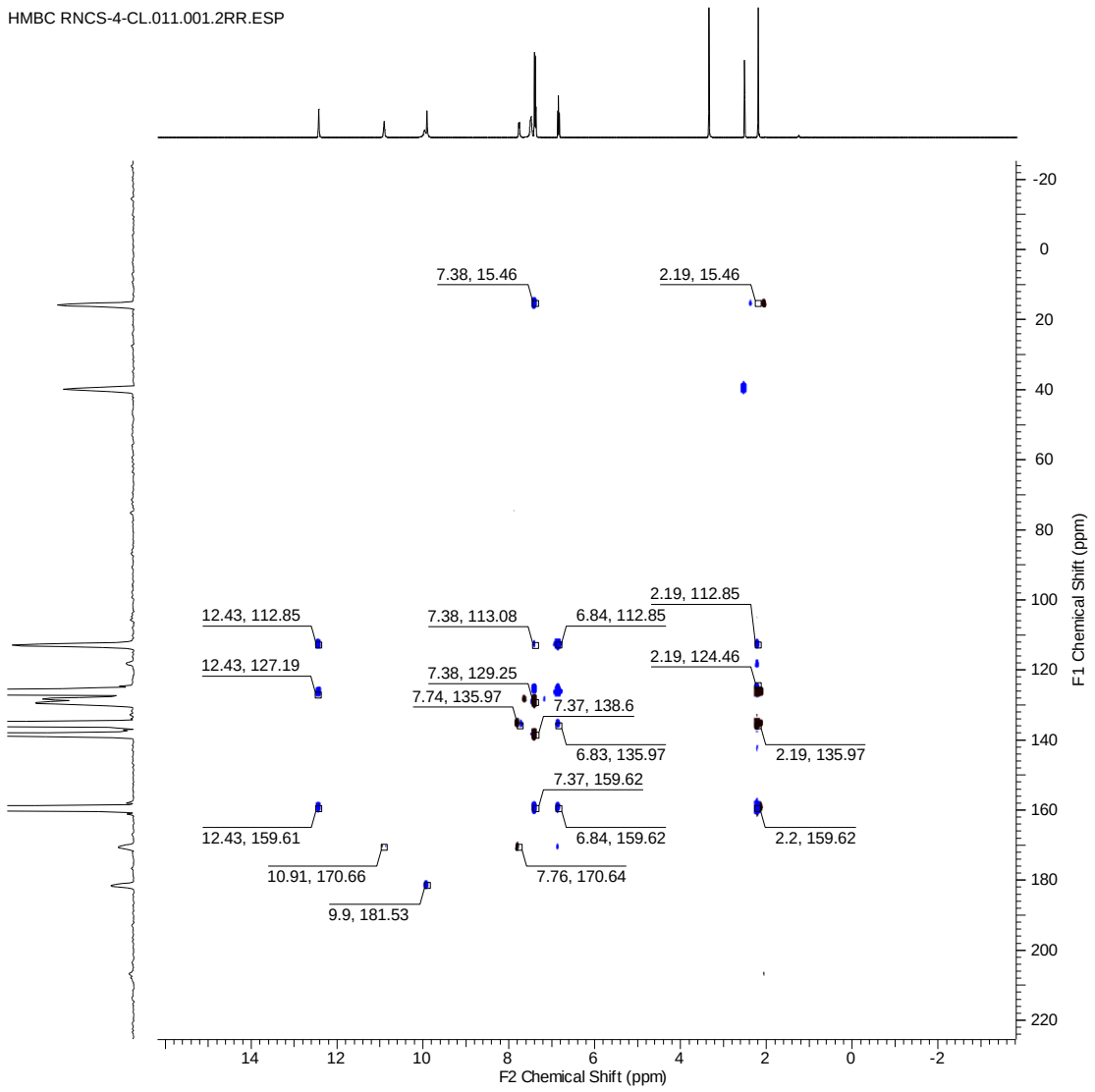


Figure B-47:-HMBC of compound **2.35**

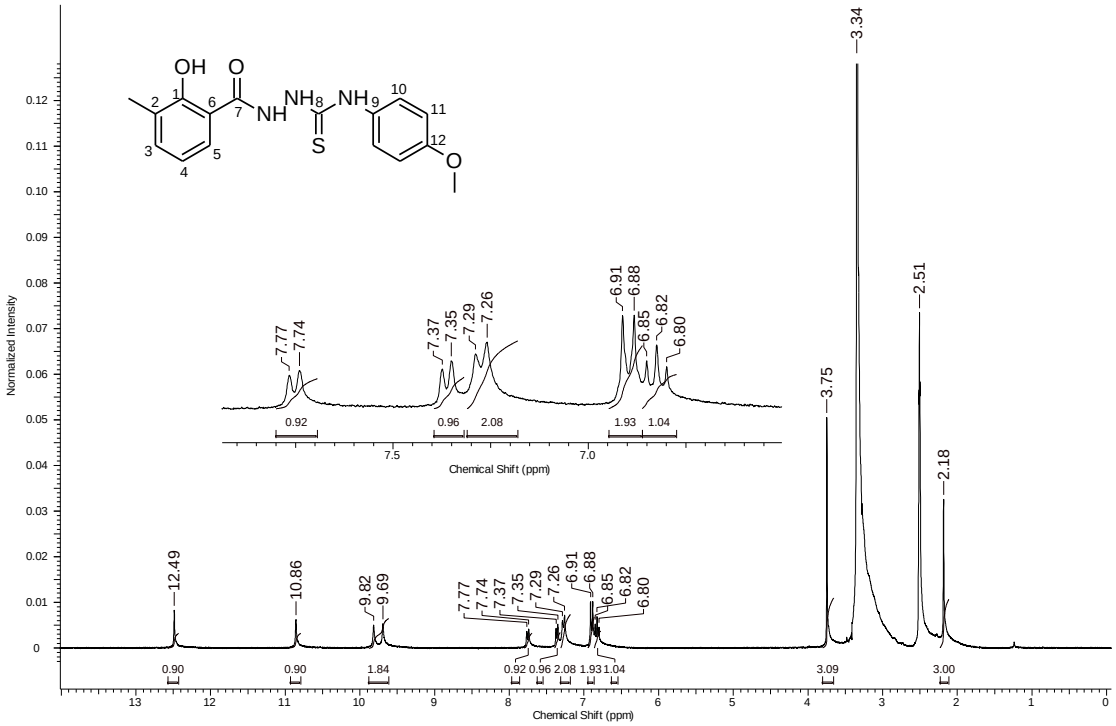


Figure B-48:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.36**

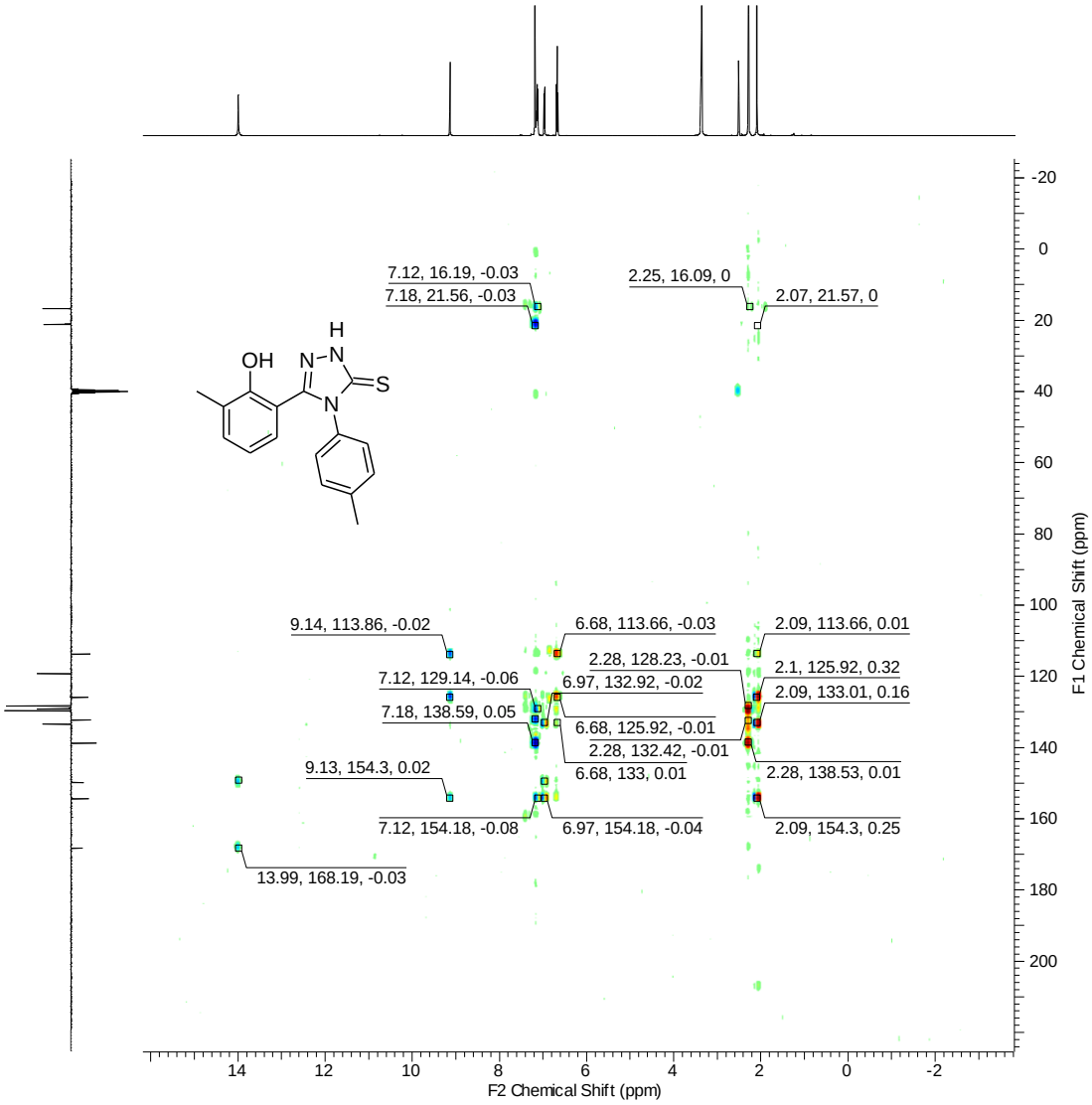


Figure B-49:-HMBC of compound **2.37**

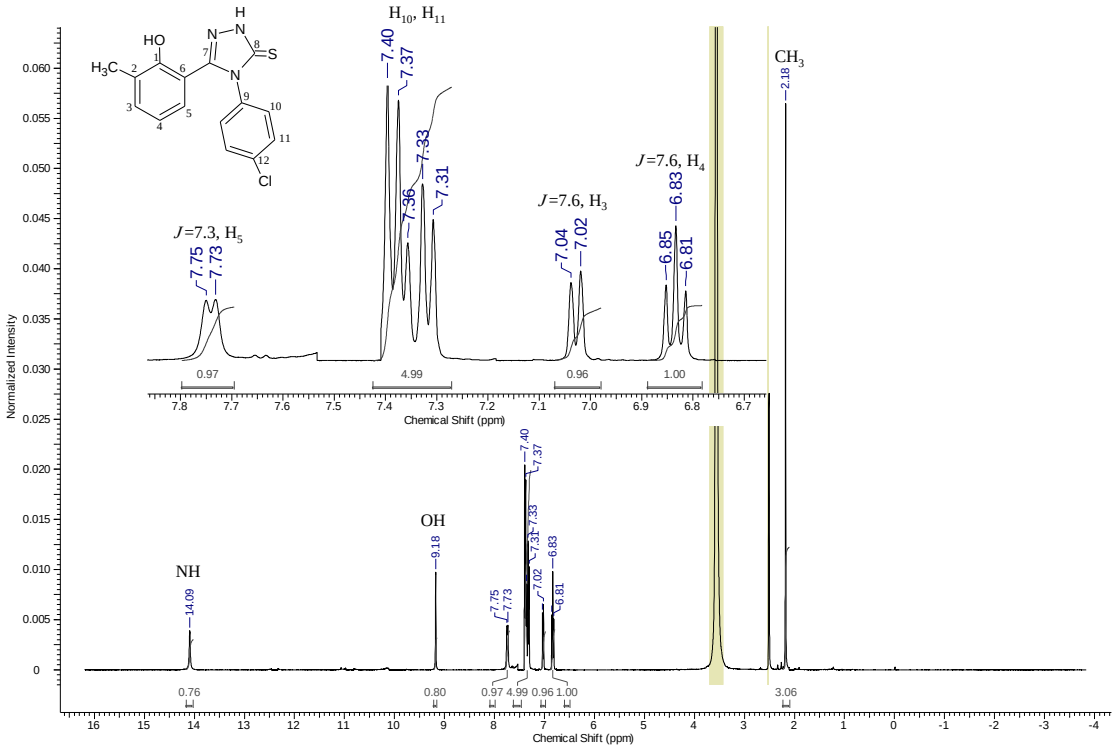


Figure B-50:-^{1}H NMR (400 MHz, DMSO-d_6) of compound **2.38**

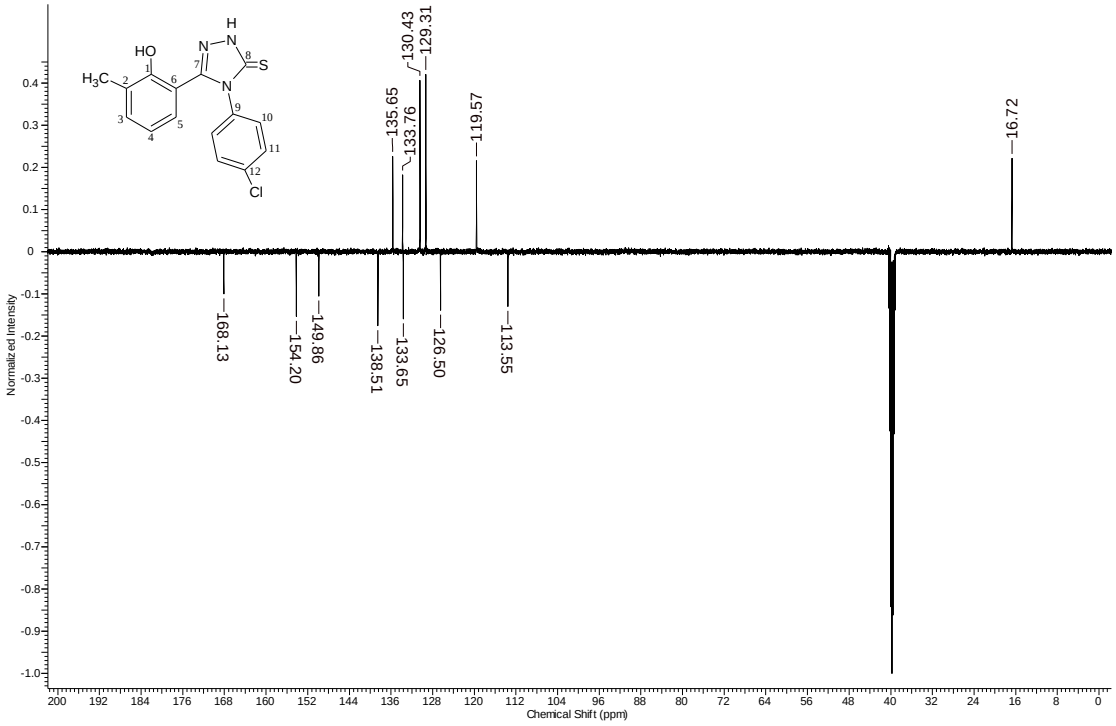


Figure B-51:-^{13}C APT (100 MHz, DMSO-d_6) of compound **2.38**

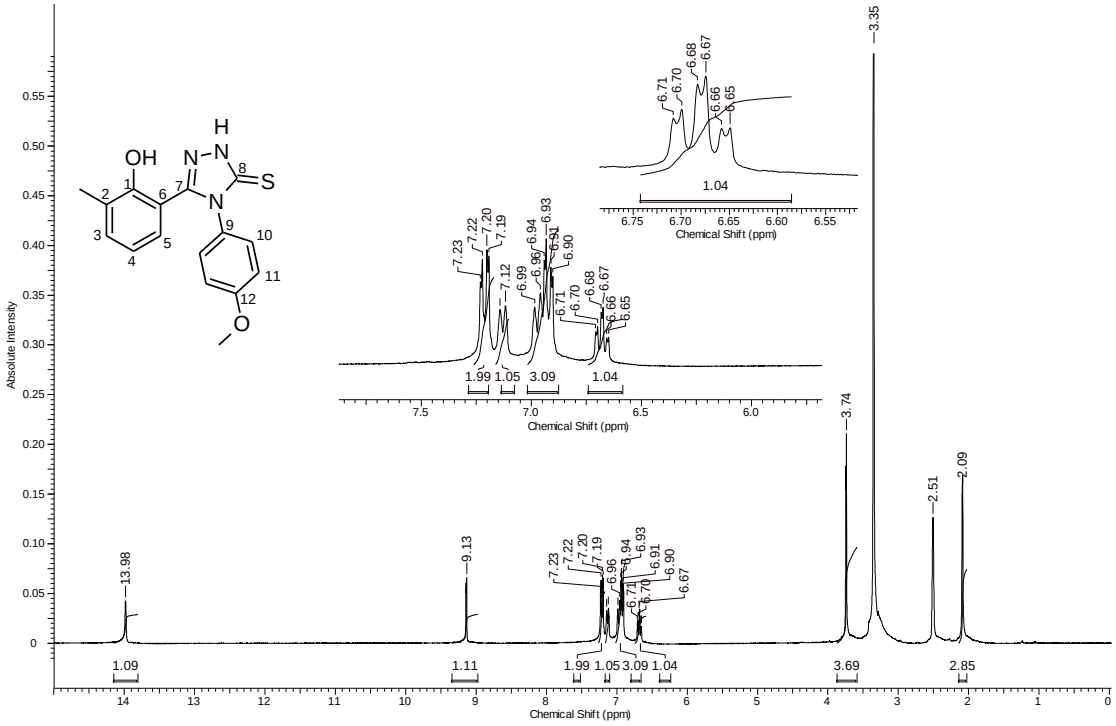


Figure B-52:-^{1}H NMR (300 MHz, DMSO-d_6) of compound **2.39**

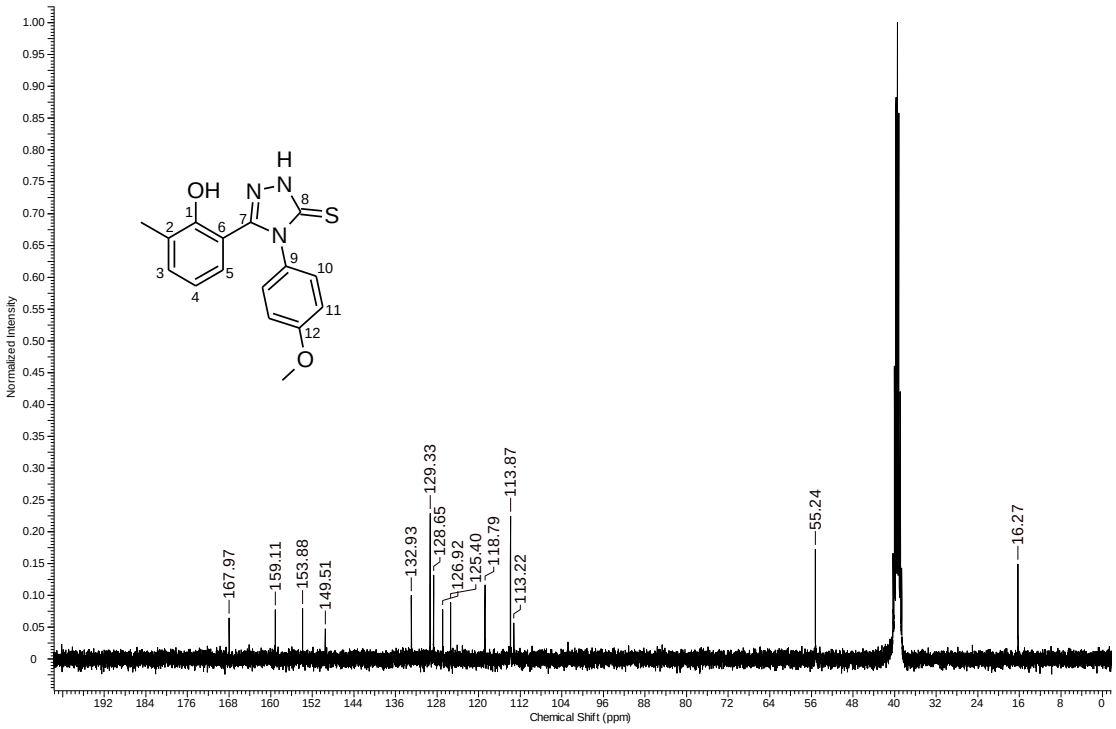


Figure B-53:-^{13}C NMR (75 MHz, DMSO-d_6) of compound **2.39.**

Appendix C:-Selective Mass Spectroscopy.

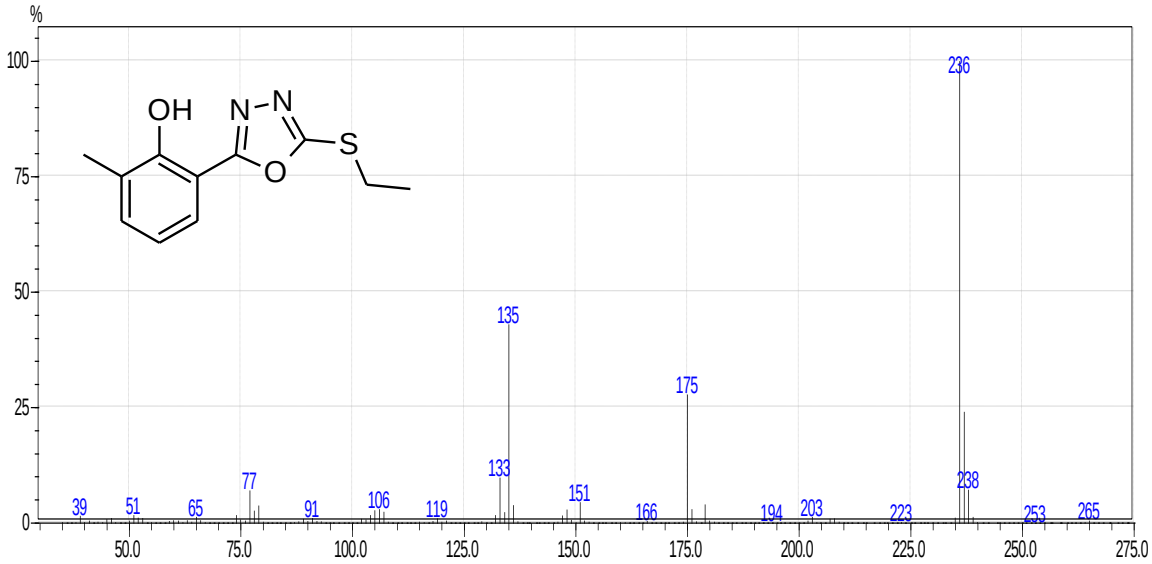


Figure C-1:-EIMs of compound **2.4** ($M^{\bullet+}$= 236).

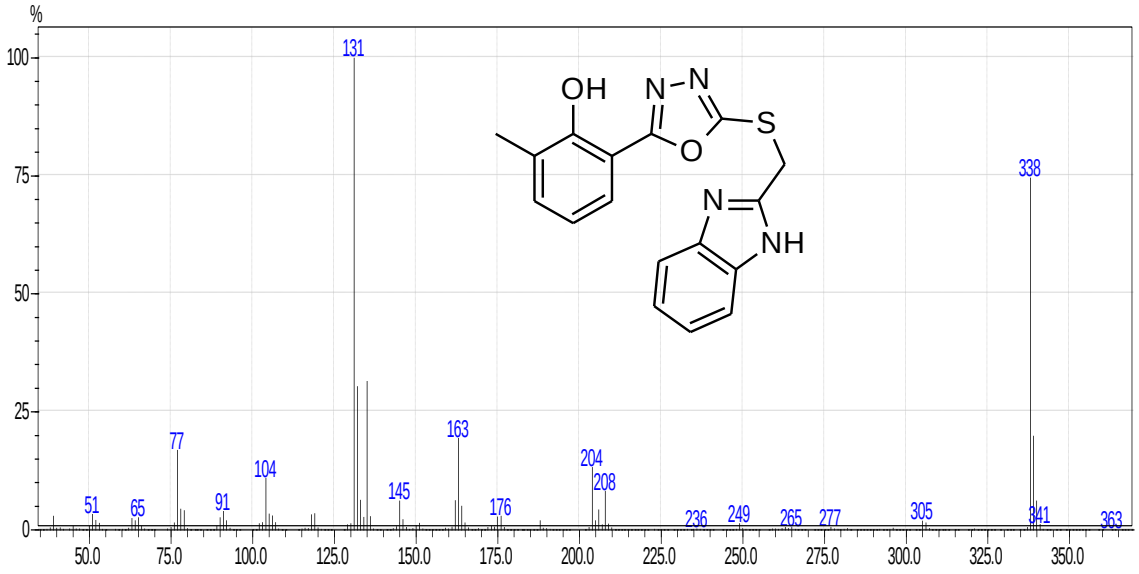


Figure C-2:-EIMs of compound **2.5** ($M^{\bullet+}$= 338).

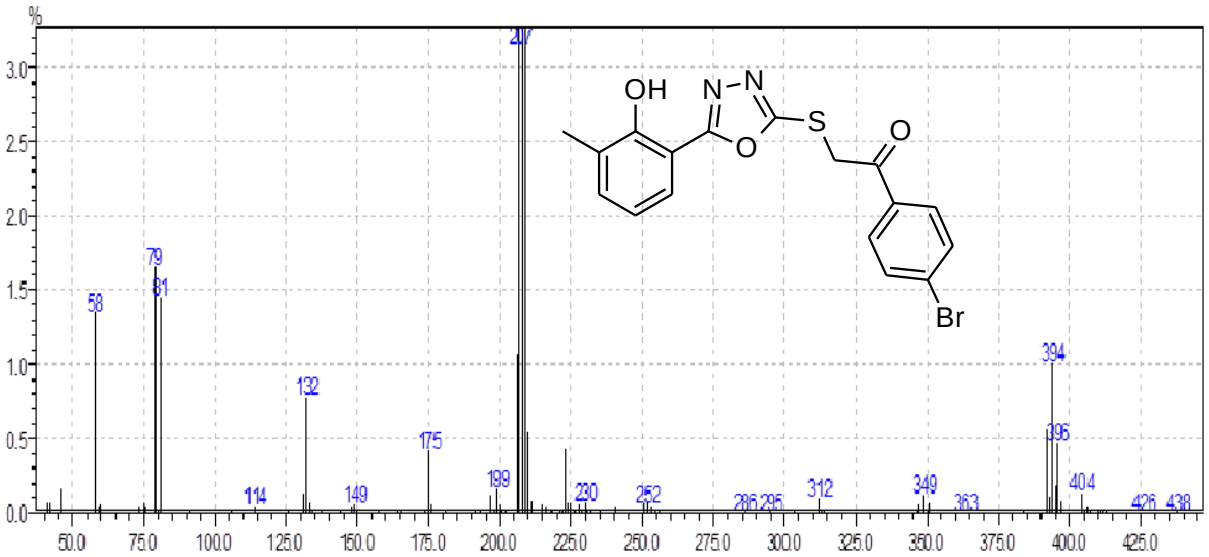


Figure C-3:-EIMs of compound **2.6** ($M^{\bullet+}$= 404).

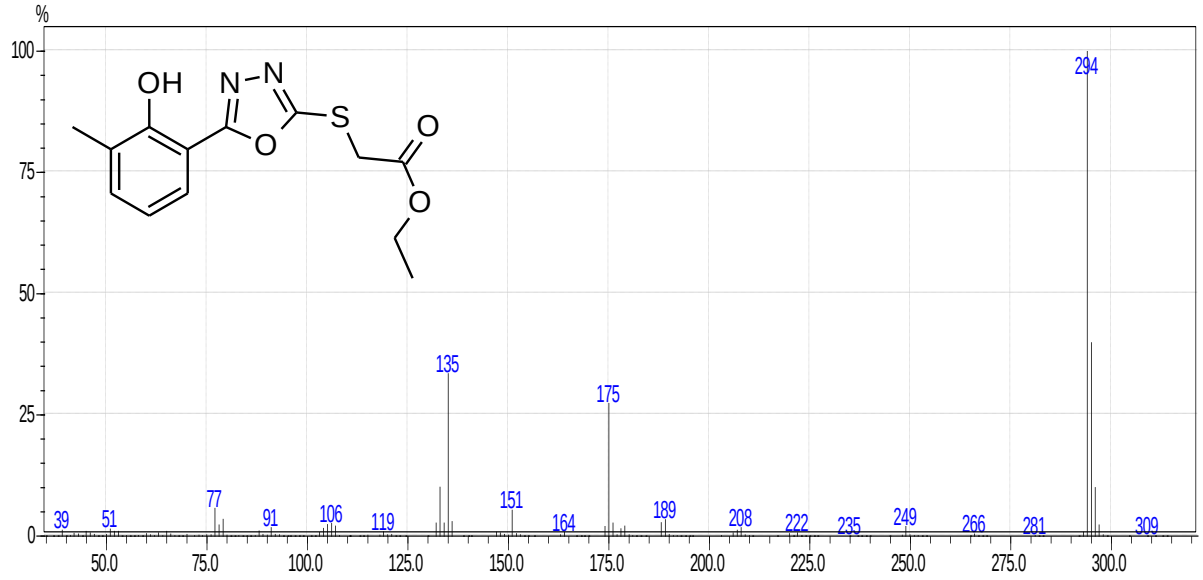


Figure C-4:-EIMs of compound **2.7** ($M^{\bullet+}$= 294).

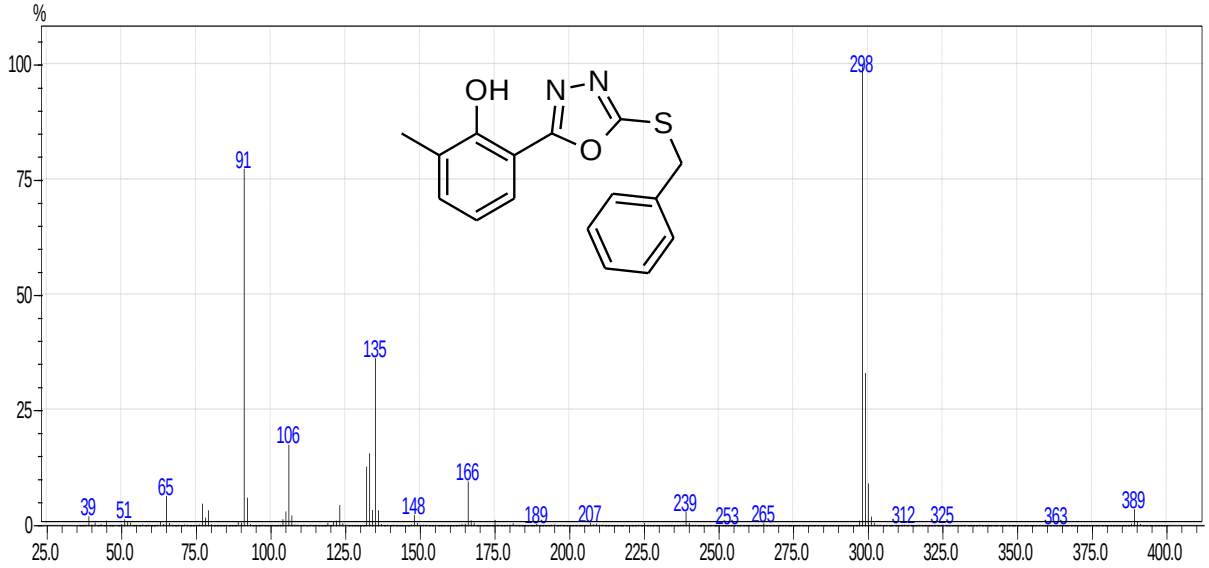


Figure C-5:-EIMs of compound **2.8** ($M^{\bullet+}$= 298).

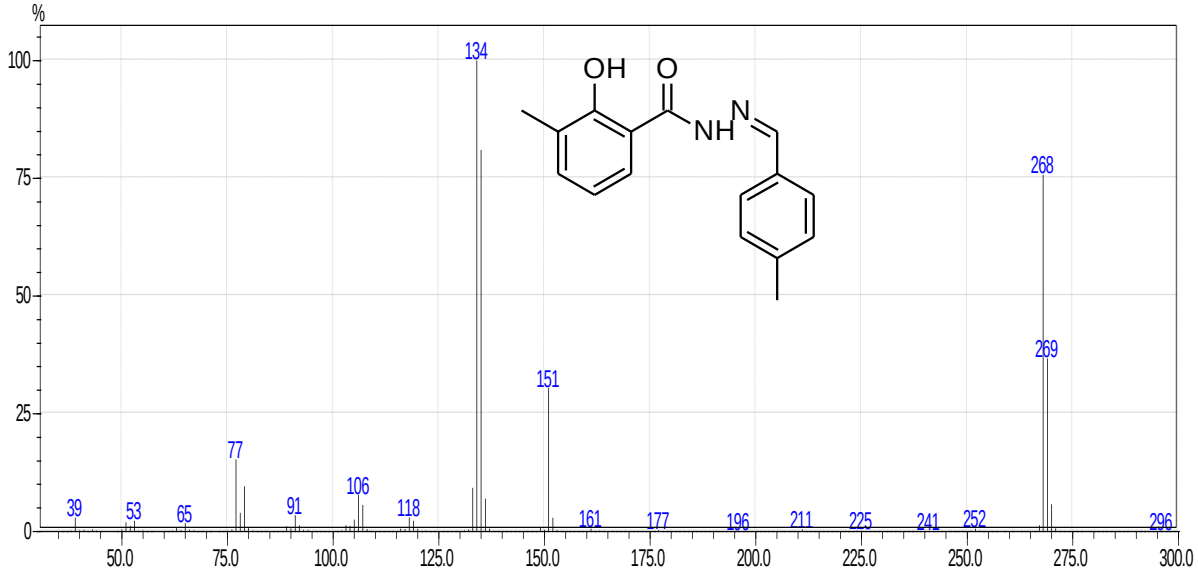


Figure C-6:-EIMs of compound **2.9** ($M^{\bullet+}$= 268).

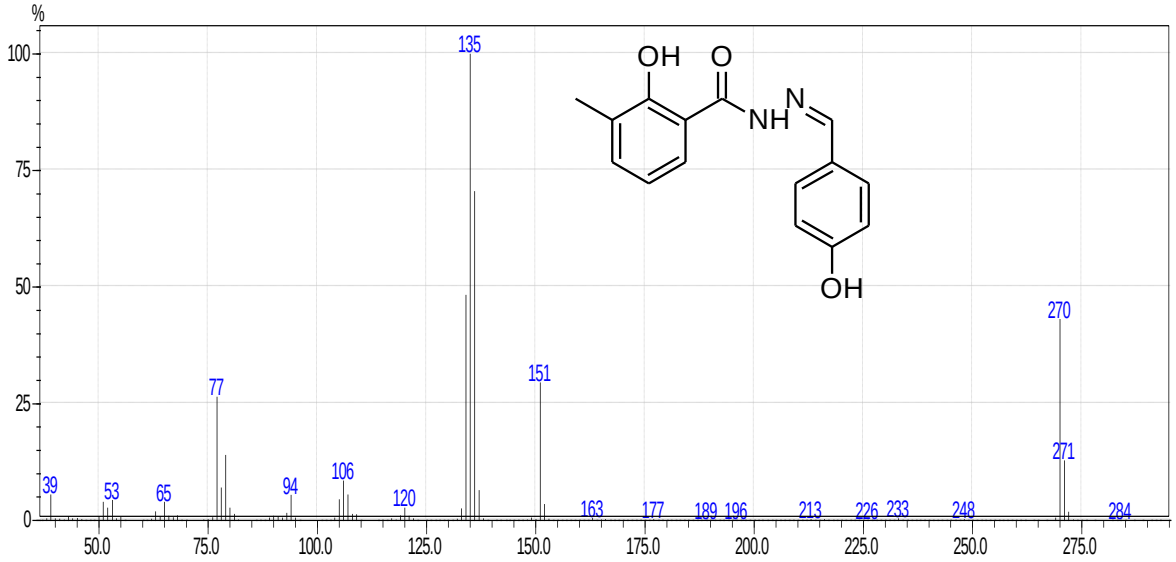


Figure C-7:-EIMs of compound **2.10** ($M^{\bullet+}$= 270).

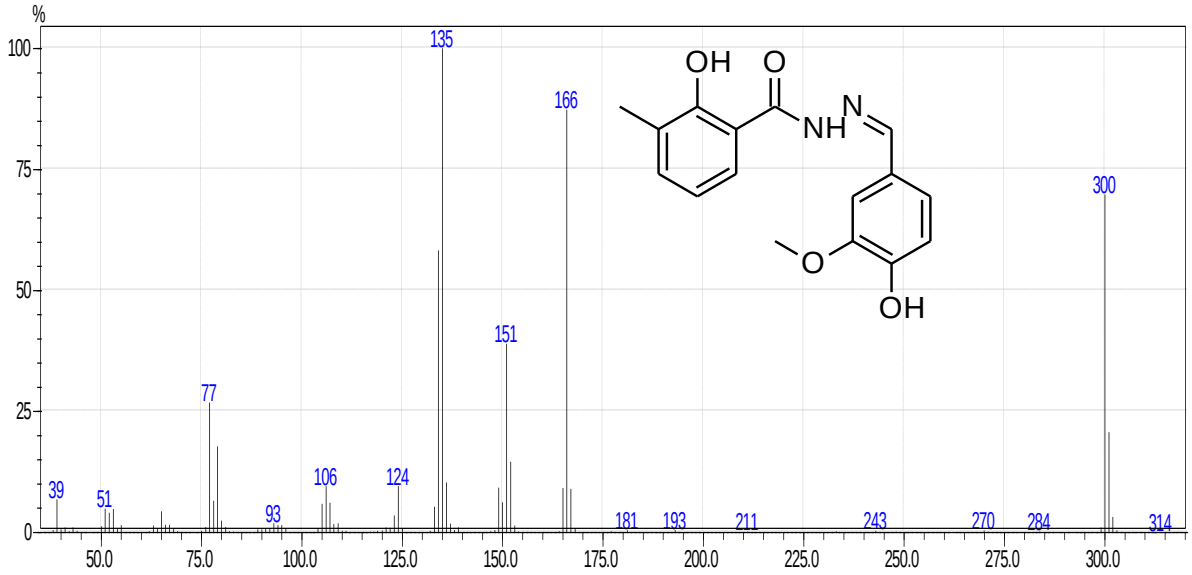


Figure C-8:-EIMs of compound **2.11** ($M^{\bullet+}$= 300).

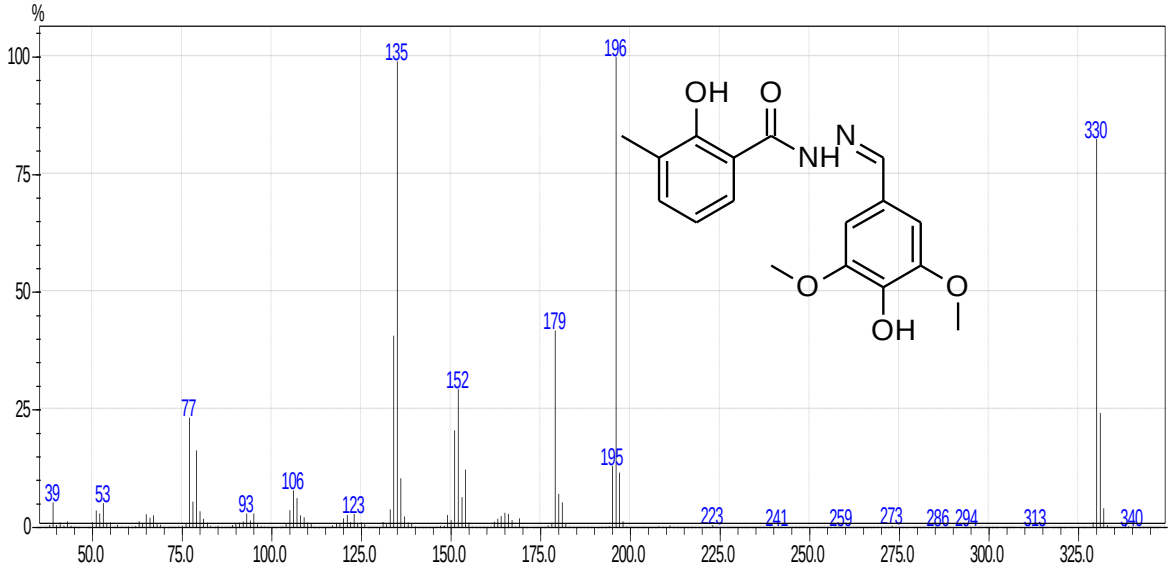


Figure C-9:-EIMs of compound **2.12** ($M^{\bullet+}$= 330).

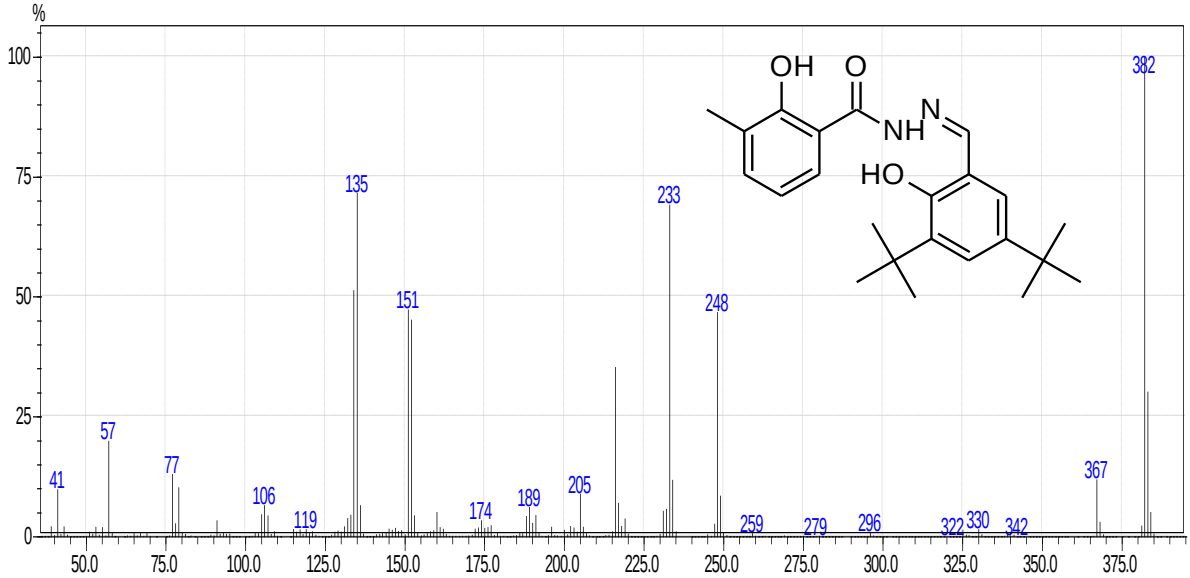


Figure C-10:-EIMs of compound **2.13** ($M^{\bullet+}$= 382).

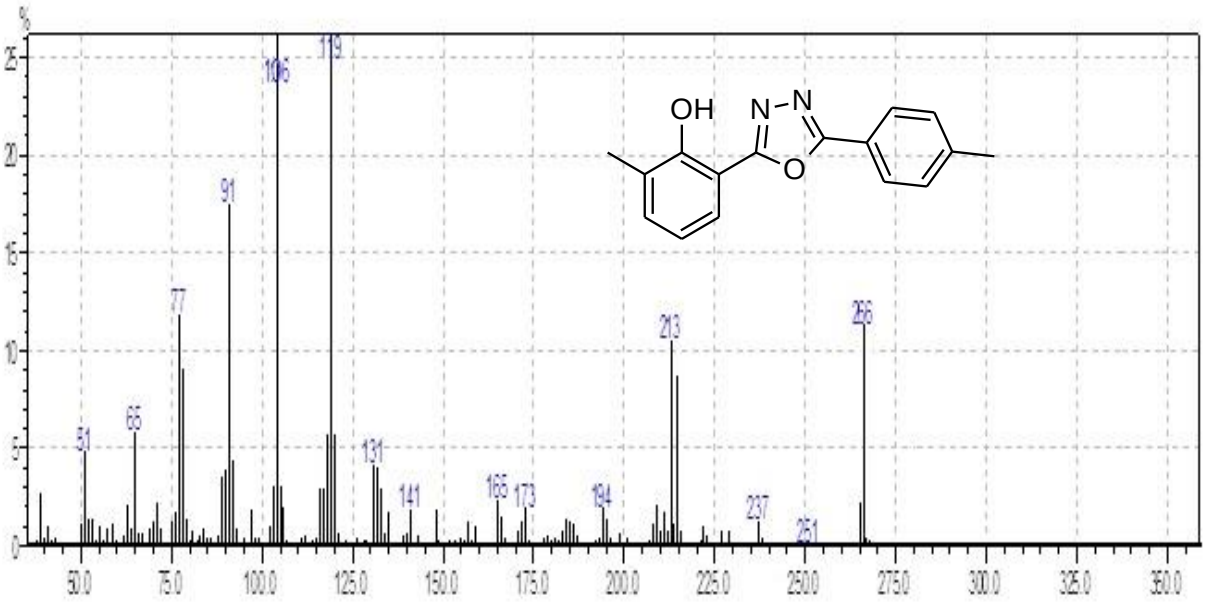


Figure C-11:-EIMs of compound **2.14** ($M^{\bullet+}$= 266).

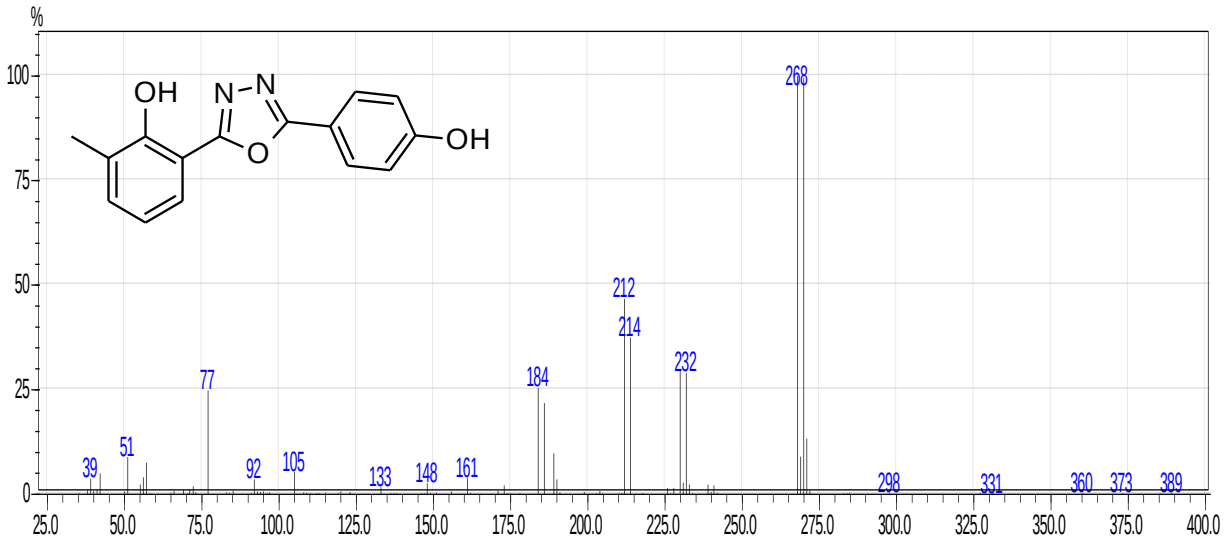


Figure C-13:-EIMs of compound **2.15** ($M^{\bullet+}$= 268).

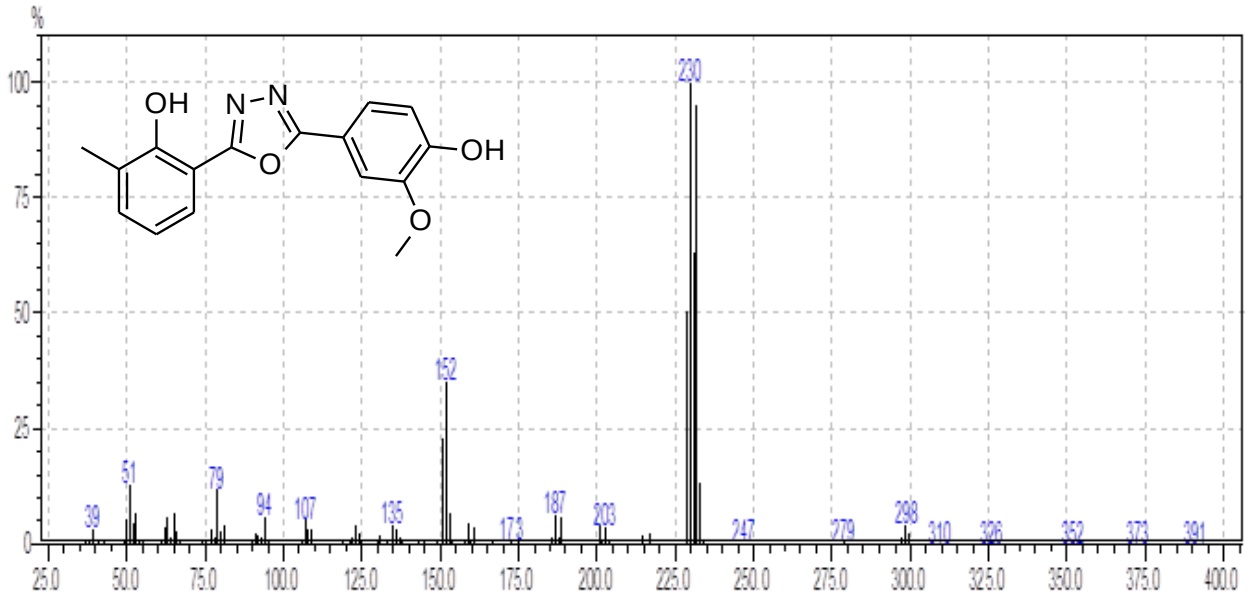


Figure C-14:-EIMs of compound **2.16** ($M^{\bullet+}$= 298).

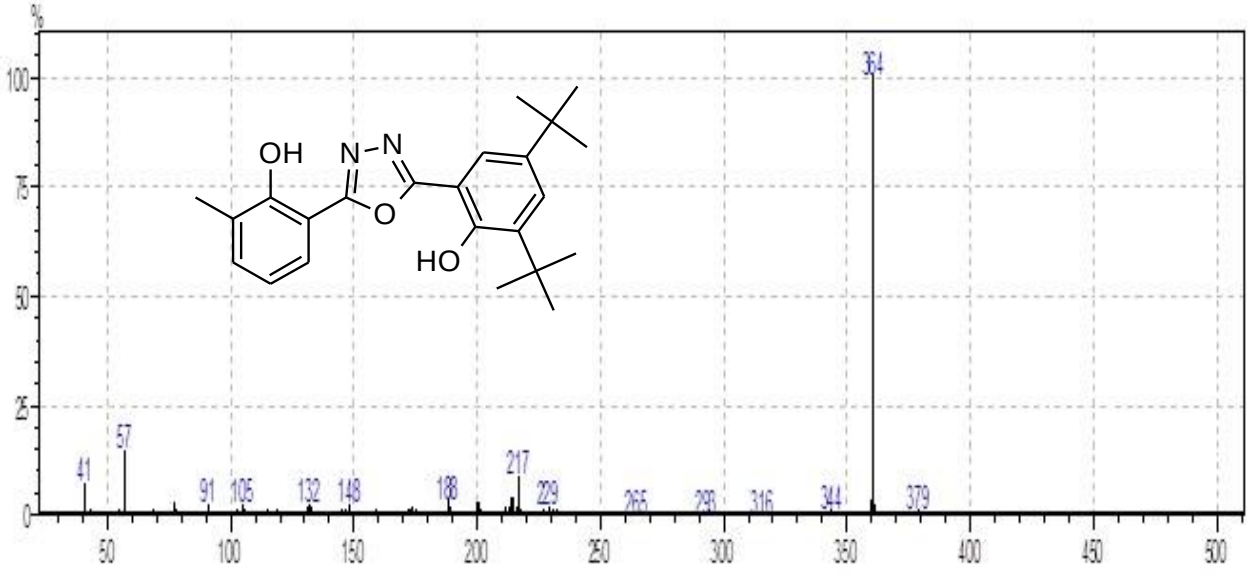


Figure C-15:-EIMs of compound **2.18** (M^{+}= 379).

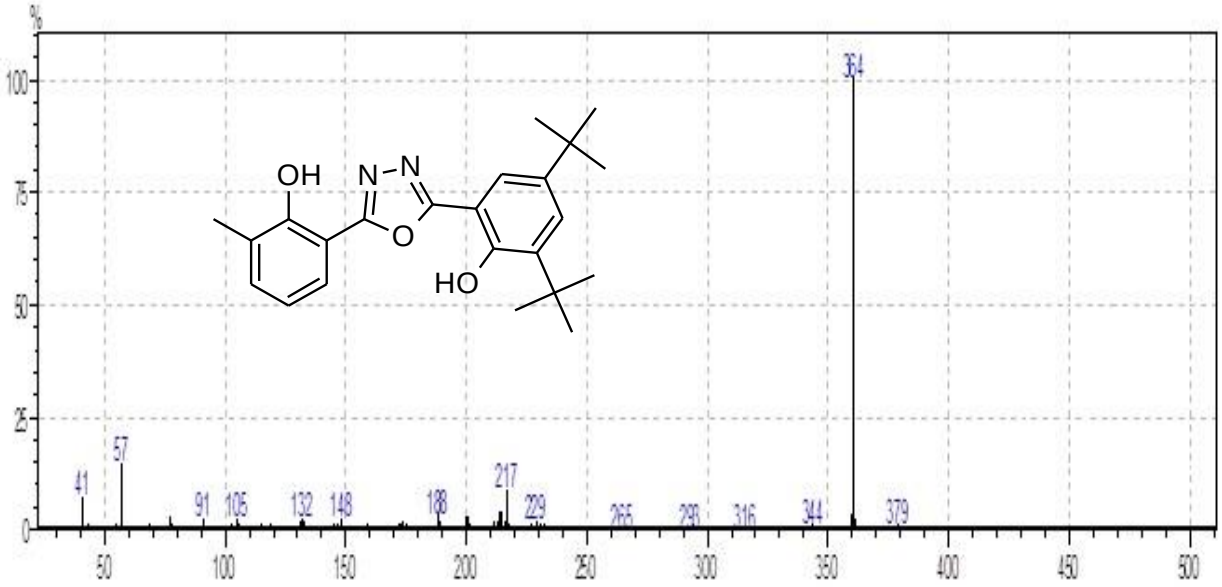


Figure C-16:-Zooming EIMs of compound **2.18** (M^{+}= 379).

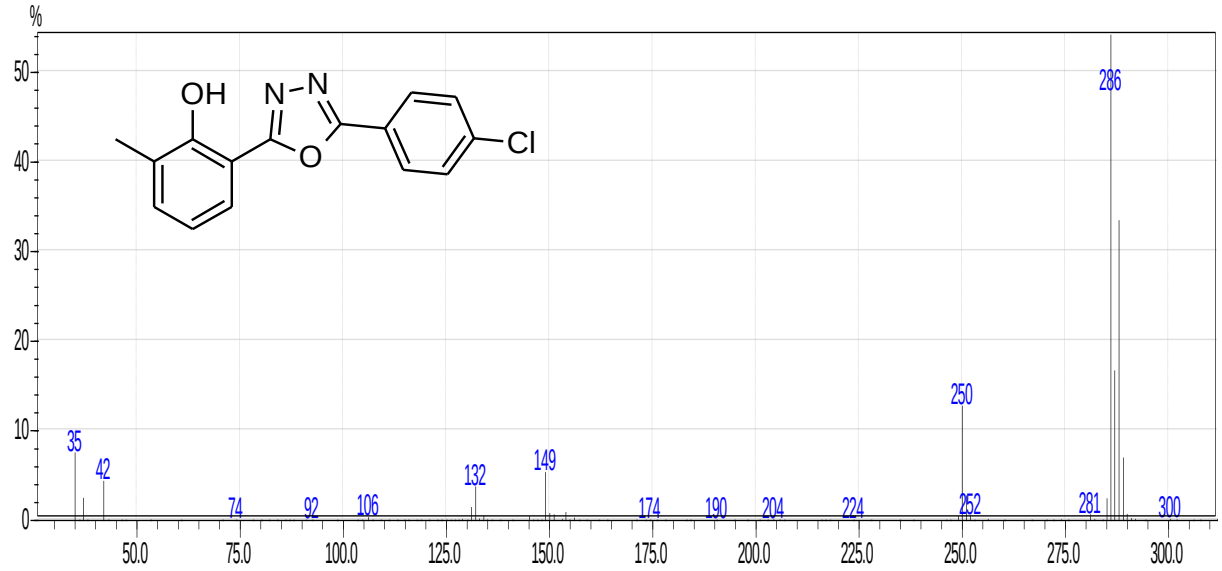


Figure C-17:-EIMs of compound **2.19** ($M^{\bullet+}$= 286).

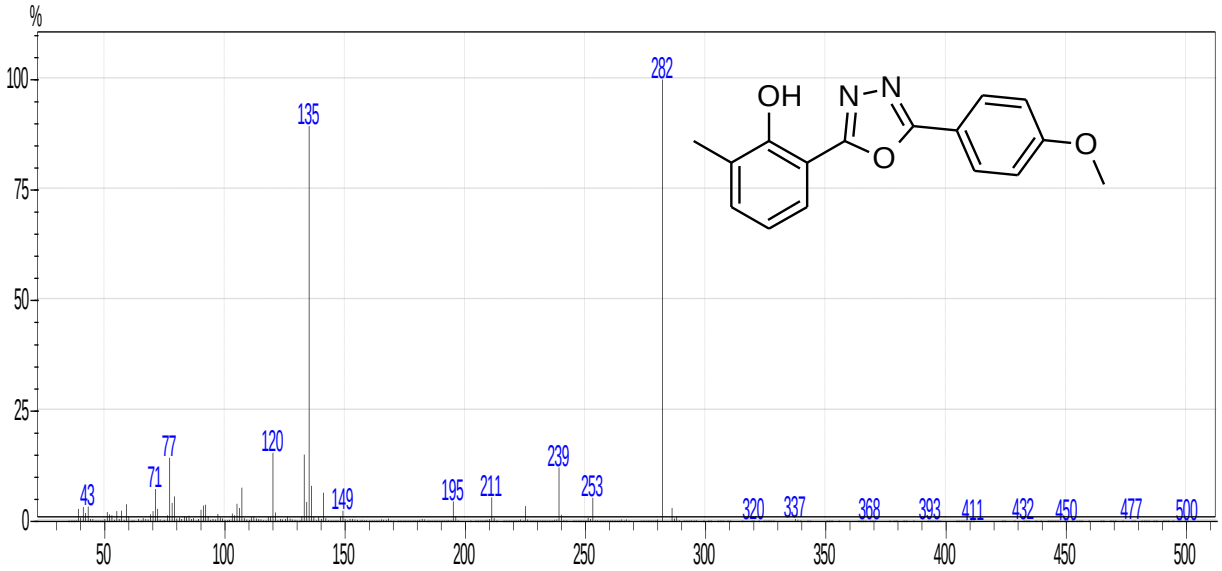


Figure C-18:-EIMs of compound **2.20** ($M^{\bullet+}$= 282).

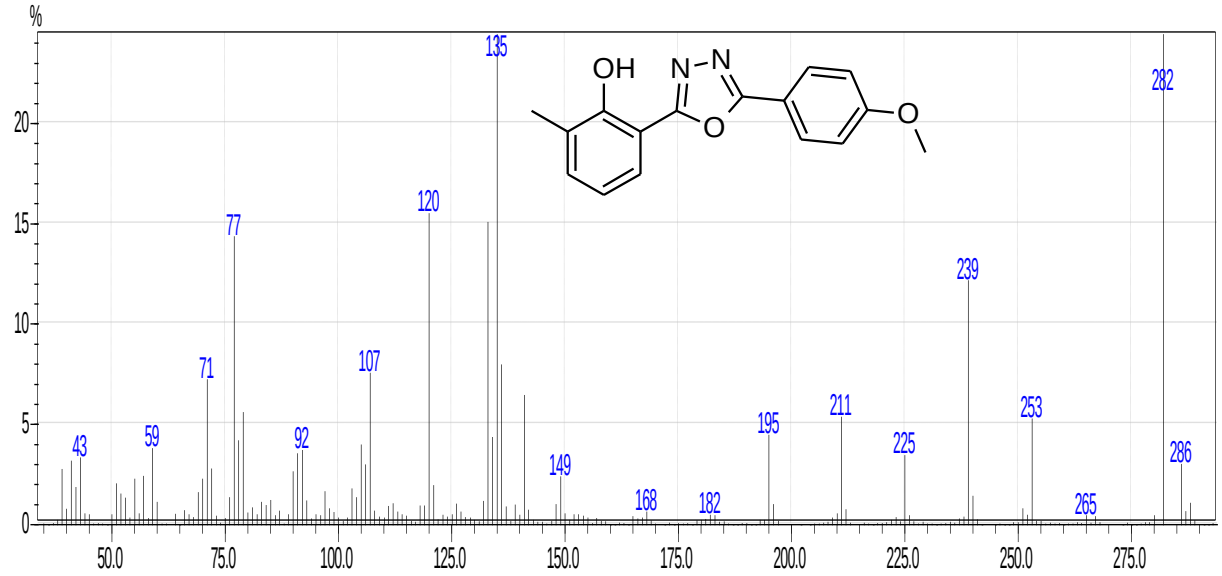


Figure C-19:-Zooming EIMs of compound **2.20** ($M^{\bullet+}$= 282).

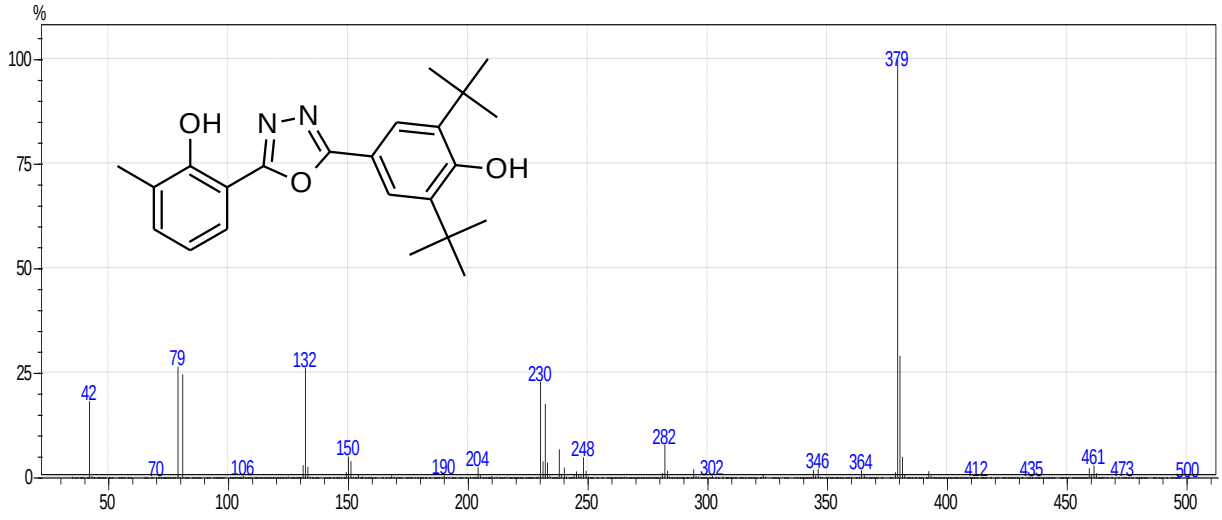


Figure C-20:-EIMs of compound **2.21** (M^{+}= 379).

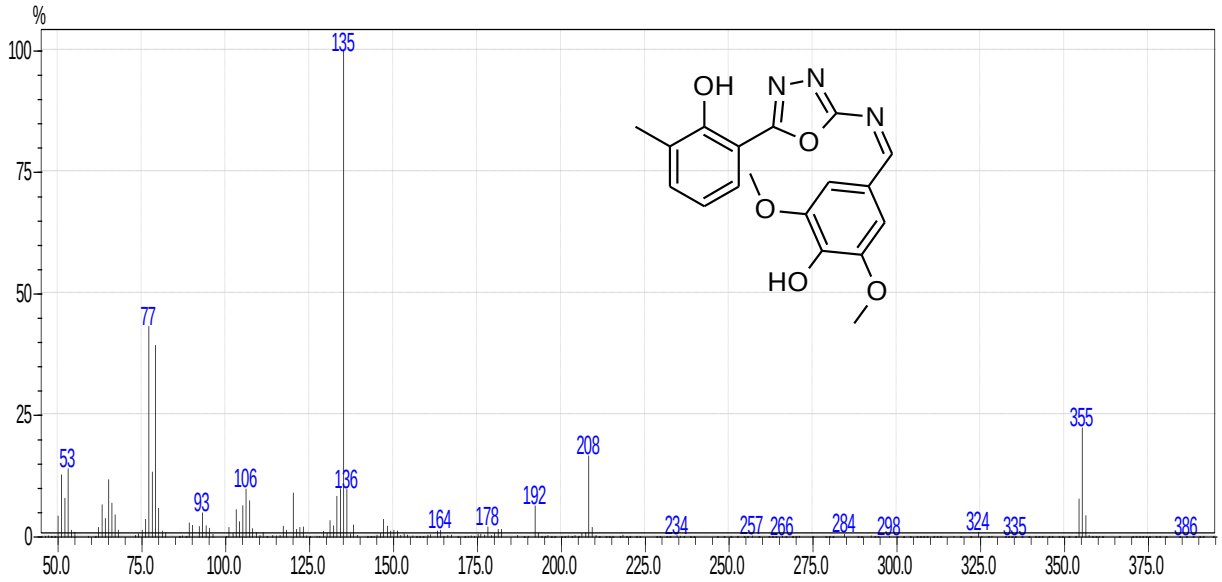


Figure C-21:-EIMs of compound **2.24** ($M^{\bullet+}$= 355).

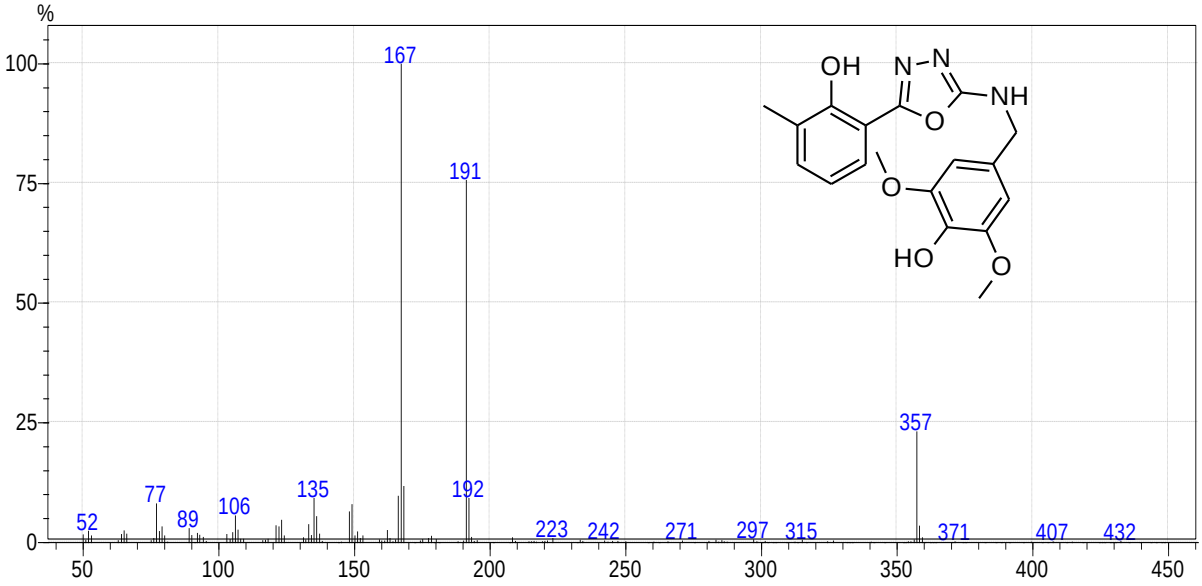


Figure C-22:-EIMs of compound **2.26** ($M^{\bullet+}$= 357).

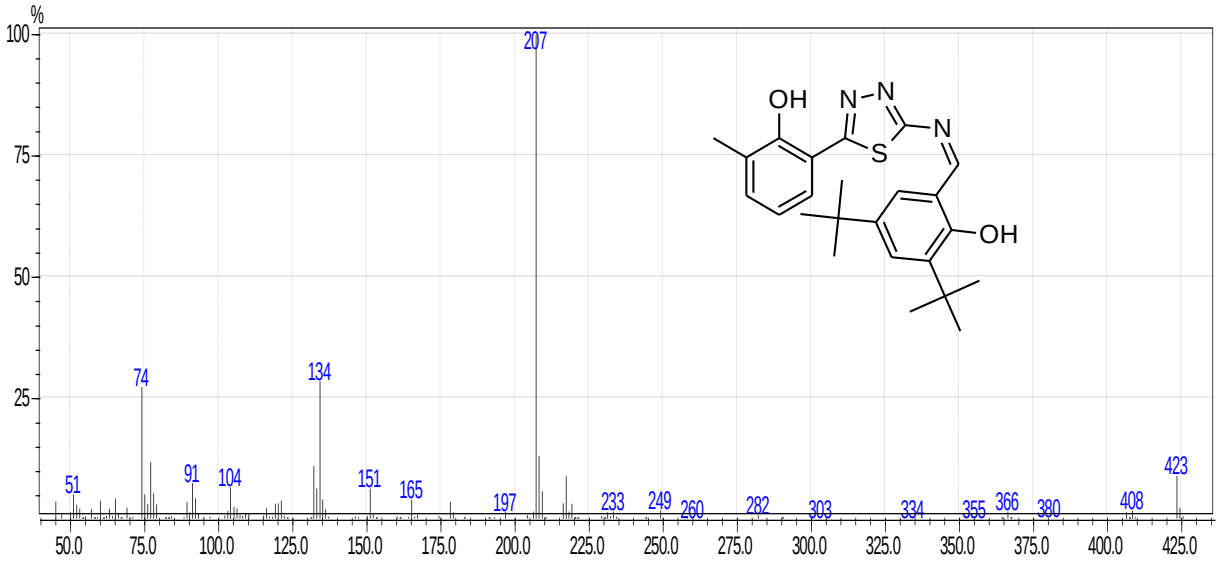


Figure C-23:-EIMs of compound **2.31** ($M^{\bullet+}$= 423).

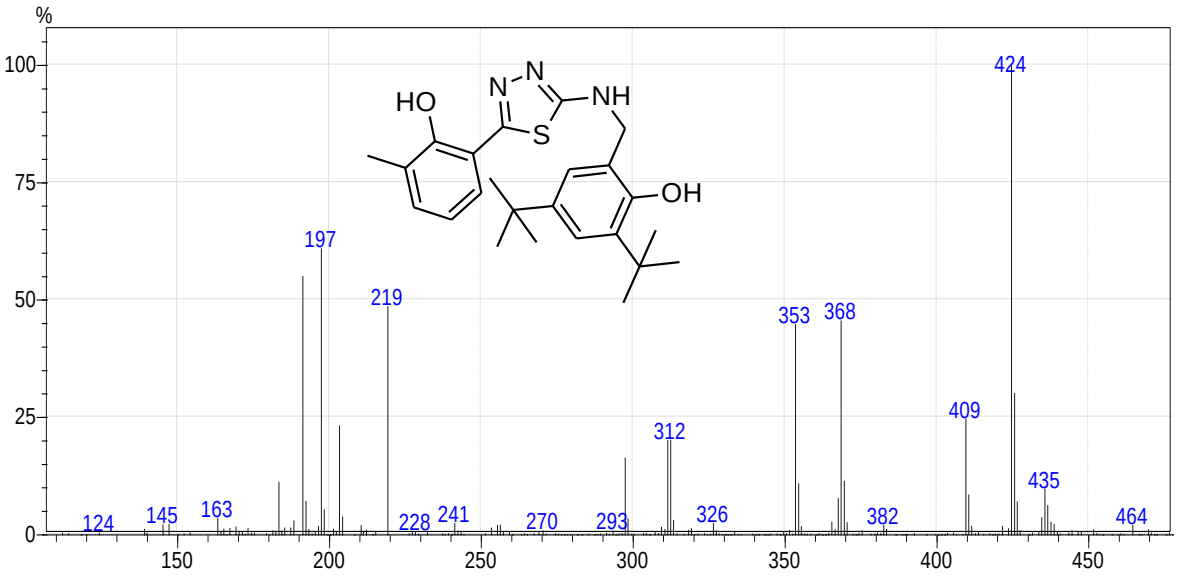


Figure C-24:-EIMs of compound **2.32** (M^{+}= 424).

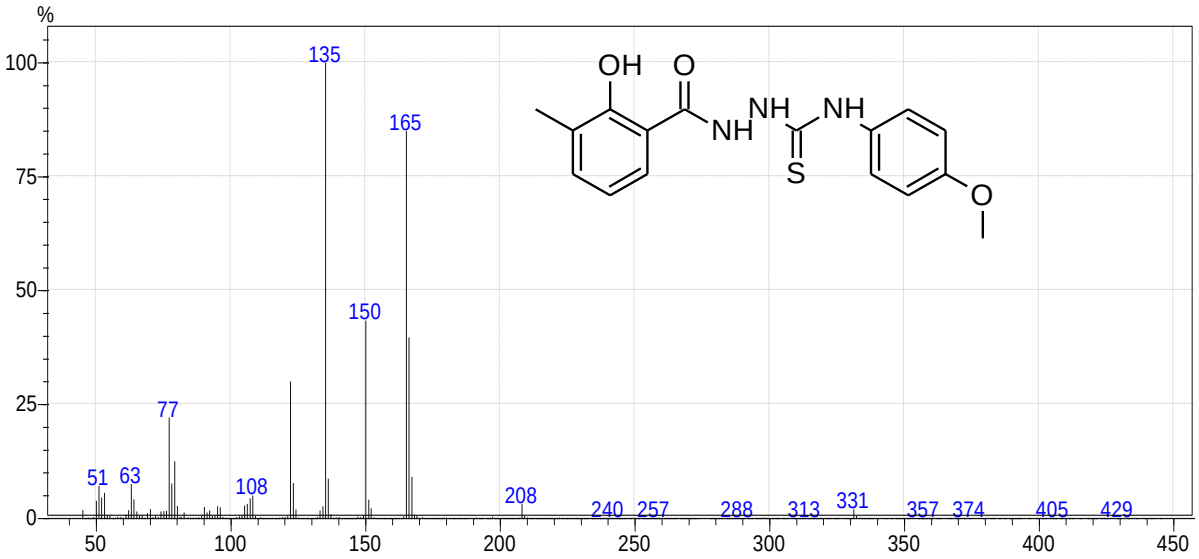


Figure C-25:-EIMs of compound **2.36** ($M^{\bullet+}$= 331).

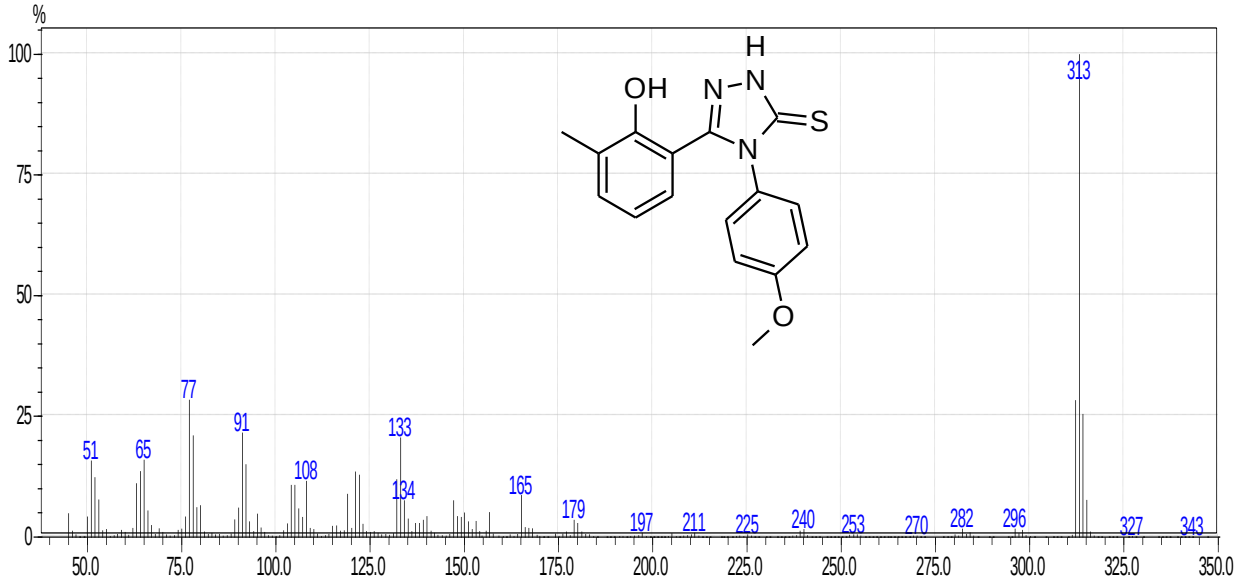


Figure C-26:-EIMs of compound **2.39** ($M^{\bullet+}$= 313).

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