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An-Najah National University
Faculty of Graduate Studies

CURCUMIN BASED DIAZOLES AND OXAZOLES WITH POTENTIAL ANTIBACTERIAL ACTIVITIES

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DEDICATION

To my father, my mother, my beloved husband Aws,

And my sons for their inspiration.

And
to all of my friends.

ACKNOWLEDGMENT

Praise and thanks to Allah, for helping and directing me to the right path. Special thanks are due to my direct research supervisor Dr. Othman Hamed, for the opportunity to work with him in his research group. I am deeply grateful to him for his constant presence, his willingness to help at any time and his encouragement throughout this research project. I thank also the second research supervisor Dr. Ayman Hussein, for his assistance and supervision during the course of this work.

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الإقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحت عنوان

Curcumin Based Diazoles and Oxazolles with Potential Antibacterial Activities

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي من نتاج جهدي الخاص باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is my research own work and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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Curcumin Based Diazoles and Oxazoles With Potential Antibacterial Activities

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Abstract

A new series of curcumin based heterocyclic compounds pyrazoles, isoxazoles and others have been prepared via reactions of curcumin with hydrazine derivatives. The reactions were conducted in the presence of acetic acid as catalyst and solvent. In addition to these heterocyles, curcumin based β-iminoalcohol (7) was also prepared. the details of the synthetic procedures are described in the experimental part.

The prepared heterocyclic compounds have been characterized by various spectroscopic techniques such as , FT-IR, ^{13}C and ^{1}H NMR spectroscopy. Some of the compounds were analyzed by elemental analysis and others by LC/MS.

The curcumin based heterocyclic compounds were then evaluated for potential antibacterial activities against four different types of bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Bioactivities against *Proteus mirabilis* and *Pseudomonas aeruginosa*, were negative with all compounds. However some of them showed some activities against *Escherichia coli*.

All compounds were active against *S. aureus*, mainly in the case of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7- diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol), compound no. 4, was the best.

CHAPTER ONE

INTRODUCTION

1.1 Background

Since the old time, mankind has suffered from diseases and infectious bacteria. During the 20th century mankind found a way to put an end (at least partially) to the suffering by the advent of antibacterial agents. Since then, a multitude of antibacterial agents have been developed and used in a clinical setting. However, almost as quickly as the antibacterial agents have been developed resistance to them was also observed.[1] As we entered the 21st century the prospect for "superbugs" which are resistant to all antibacterial agents becoming more of a reality, especially when bacteria becoming capable of invading the whole human body.

Therefore, mankind is in great need to develop a new and innovative antibacterial agent to regain the dominance over the pathogenic bacteria.

Bacteria were first observed by Antonie van Leeuwenhoek in 1676, using a single-lens microscope of his own design[2]. He called them "animalcules" and published his observations in a series of letters to the Royal Society[3,4]. The name *bacterium* was introduced much later, by Christian Gottfried Ehrenberg in 1838[5]. Robert Koch became a pioneer in medical microbiology and worked on cholera, anthrax and tuberculosis. In his research into tuberculosis, Koch finally proved the germ theory, for which

he was awarded a Nobel Prize in 1905[6]. In *Koch's postulates*, he set out criteria to test if an organism is the cause of a disease; these postulates are still used today[7].

1.2 Types of Bacteria

Hans Christian Gram in the late 1800s developed a method known as the double staining method which is considered a valuable tool in the classification of bacteria[8]. This method reveals differences in the cell wall structures of according to which bacteria were classified into two groups.

1. Gram-positive bacteria
2. Gram-negative bacteria

The names originated from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species.[9]

The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids (which are bacterial polysaccharides that are rich in phosphodiester linkages, the main function of teichoic acids is to provide rigidity to the cell-wall by attracting cations such as magnesium and sodium, they serve also as an attachment site for some parasites).

In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopoly saccharides and lipoproteins.

These differences in structure can produce differences in antibiotic susceptibility; for instance, vancomycin can kill only Gram-positive bacteria and is ineffective against Gram-negative pathogens.

Examples on Gram-negative bacteria, are *Haemophilus influenzae* and *Pseudomonas aeruginosa*, and Gram-positive bacteria, are *Bacillus, Clostridium*, *Sporohalobacter*, *Anaerobacter* and *Heliobacterium*.

The bacteria used in this study to evaluate the antibacterial activity of the prepared compounds, are:

1. *Staphylococcus aureus*

It is a facultatively anaerobic, Gram-positive coccus, it has a golden color on the agar plat , *aureus* means "golden" in Latin.

S. aureus was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses[10].

S. aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis[11].

2. *Escherichia coli*

It is Gram-negative, facultative anaerobic and non-sporulating bacilli[12].

E. coli was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885[13], and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria[14].

Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls[15].

3. Proteus mirabilis

It is a Gram-negative, facultatively anaerobic bacterium. It is like E.coli classified as part of the Enterobacteriaceae family of gamma-proteobacteria, *Proteus mirabilis* was discovered in 1885 by the Erlanger pathologist Gustav Hauser[16.]

Proteus can cause wound infections, septicemia and pneumonias, mostly in hospitalized patients [17].

4. Pseudomonas aeruginosa

In 1882, Gessard first discovered *Pseudomonas*, a strictly aerobic, gram-negative rod-shaped bacterium of relatively low virulence[18]. Pseudomonal species have been found in soil, water, plants, and animals; *Pseudomonas*

aeruginosa colonization reportedly occurs in more than 50% of humans, and *P. aeruginosa* is the most common pseudomonal species [19].

It is often causing nosocomial infections. typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections[20,] These organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents.

1.3 Antibacterial Agents

In 1910, Paul Ehrlich developed the first antibiotic, by changing dyes that selectively stained *Treponema pallidum*—the spirochaete that causes syphilis into compounds that selectively kills the pathogen.[21] Ehrlich had been awarded a 1908 Nobel Prize for his work on immunology and pioneered the use of stains to detect and identify bacteria, with his work being the basis of the Gram stain and the Ziehl-Neelsen stain[22].

In 1928 Sir Alexander Fleming developed the first antibacterial agent, penicillin G (Fig 1), since this time the golden age of antibacterial agent and antibiotic agent has started and the bacteria seemed under control.

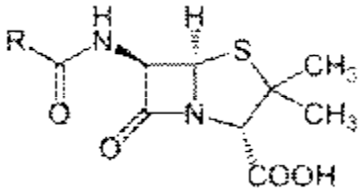
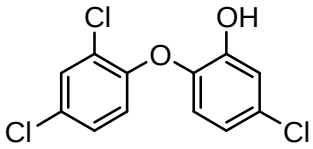


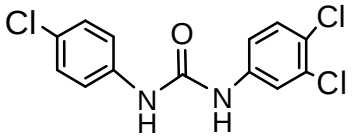
Fig 1.1: Penicillin G

Antibacterial agents are those materials which inhibit, destroy or interfere with the growth and reproduction of pathogenic microorganisms, such as bacteria, fungi, protozoa, and viruses. While antibiotics and antibacterial both attack bacteria, these terms have evolved over the years to mean two different things. Antibacterial unlike antibiotics, are used to disinfect surfaces and eliminate potentially harmful bacteria. For this reason, they have found a lot of applications in house hold products such as soaps, detergents, health and skincare products and household cleaners. Currently, more than 700 type of products are being marketed. Antibacterial agents are now in plastic food storage containers, mattress, and bathrooms and bedrooms products such as pillows, sheets, towels, and slippers.

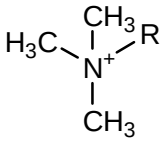
Antibacterial agents may be divided into two categories according to their speed of action and residue production[23]: The first group contains those that act rapidly to destroy bacteria, but quickly vanish by evaporation or breakdown and leave no active residue behind. Examples of this type are alcohols, chlorine, and peroxides. The second group consists of compounds that leave long-acting residues on the surface to be disinfected such as for example triclosan, triclocarban, and benzalkonium chloride (Scheme 1).



Triclosan



triclocarban



R: large alkyl group

quaternary ammonium compounds

scheme 1: Examples on a commercial antibacterial agents.

Among the mostly used antibacterial agents are triclosan and quaternary ammonium compound. Recent survey reported that 76% of liquid soaps from 10 states in the US contained triclosan and approximately 30% of bar soaps contained triclocarban[24]. More recently, triclosan has been bonded into the surface of many different products with which humans come into contact, such as plastic kitchen tools, cutting boards, highchairs, toys, bedding and other fabrics.

Recent studies by various laboratories showed that bacteria can develop resistance against residual antibacterial agent such as triclosan[25].

Resistance to antibacterial reagents has been found where the antibacterial agents are used continuously (as in the hospital, house hold products, and food industry). In another recent study, 7% of *Listeria monocytogenes* strains isolated from the environment and food products showed resistance to quaternary ammonium compounds[1].

For instance bacteria such as *Staphylococcus aureus*, cause food poisoning and a source of hospital infections, have developed resistance to a number of antibiotics but not to vancomycin—yet. When they do develop resistance to vancomycin, "as many suspect is just a matter of time, we will be in trouble," says chemistry professor Dale L. Boger of Scripps Research Institute[26,27].

Bacterial resistance has become a serious issue, hence, new antibacterial strategies become very demanding. One approach is developing new antibacterial strategies based on natural products.

1.4 Natural Products with Antibacterial Activities

"Natural products provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they may provide[28]. According to the World Health Organization (WHO), more than 80% of the world's populations rely on traditional medicine for their primary healthcare needs. This has captured the interest of many researchers to explore local medicinal plants for valuable medicinal traits. Several studies indicate that medicinal plants contain compounds like peptides, long unsaturated chains of fatty

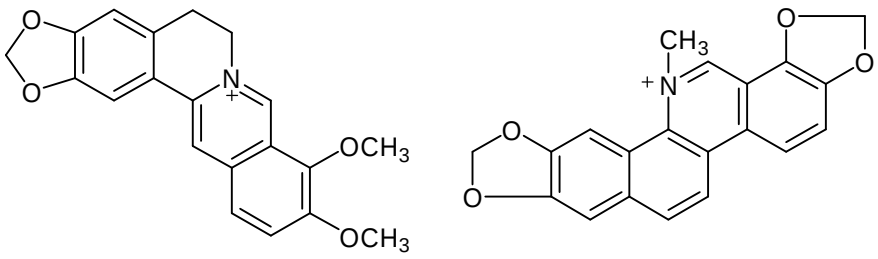
acids, aldehydes, alkaloids, essential oils, phenols. Some of these compounds are significant in therapeutic application against animal pathogens, including bacteria, fungi and viruses"[29,30].

Examples on natural products that exhibit antibacterial activities are:

1.4.1 Alkaloids

Alkaloids are nitrogen containing cyclic compounds that are available as natural products in plants, animals, insects, marine invertebrates and many other microorganisms. Plants produce them for protection from insects. Alkaloids exhibit a variety of bioactivities ranging from medical to cytotoxicity[31].

Alkaloids are classified into several categories based on the chemical structure of their nucleus. Isoquinoline alkaloids are thus based on the isoquinoline nucleus. Examples on isoquinoline alkaloids that show antibacterial activities are berberine and sanguinarine (Fig. 2), both alkaloids are currently used clinically as antimicrobial agents. Berberine and sanguinarine occur in several general of families including the Berberidaceae, Papaveraceae, and Rutaceae, they possess a variety of pharmacological properties including antimicrobial, antileukemic, antiulcerous, gastric antisecretory, and enzyme inhibitory activities.[32]



(a) (b)

berberin sanguinarine

Fig 1.2

1.4.2 Colloidal Silver[33]

Colloidal Silver is called "Natural Antibiotic." It is silver suspended in a distilled water solution.

Colloidal chemistry is the science that converts minerals and metals into micro particles (submicroscopic) to be used by our living cells. These are particles remain suspended without forming an ionic or dissolved solution.

Silver is a powerful, natural, universal antimicrobial agent that kills bacteria, viruses, mold, fungi, and parasites. Colloidal Silver is a relatively tasteless, odorless liquid considered harmless in any concentration for internal and external use. It was approved by the FDA (Food and Drug Administration), and can be used for all kinds of patients, whether children, or pregnant or lactating women. It is known to have very few minor side-effects, and the human body shows no development of any tolerance. Because colloidal silver has strong germicidal action, it has been found to be highly effective in the prevention and remedy of infections and diseases, including AIDS, Staph, and Strep. Sir Malcom Morris reports in The British Medical Journal

(May 12, 1917) that Colloidal Silver is free from drawbacks of other silver preparations and that it has distinctly soothing effect, he recounted that colloidal silver rapidly subdues inflammation and promotes healing.

Jim Powell reported in the March 1978 issue of Science Digest, quoting pioneering silver researcher and biochemist Dr. Harry Margraf of St. Louis, that silver is emerging as "the best all around germ fighter we have."

1.4.3 Oregano Oil[34]

Another potential natural anti-bacterial agent is oil derived from oregano. Recent studies conducted at Georgetown University have shown that carvacrol (Fig 3) which is one of the oregano components, treats bacterial infections very effectively and sometimes better than modern antibiotics. Carvacrol may be an effective remedy to drug-resistant bacteria. The phenolic hydroxyl group of Carvacrol is essential for action against the Food-Borne Pathogen *Bacillus cereus* [35].

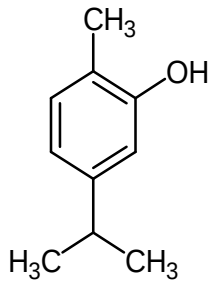


Fig 1.3:Carvacrol

1.4.4 Flavonoids

Flavonoids, also referred to as bioflavonoids, are polyphenol antioxidants found in most plants. They are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants[36].

Flavonoids are widely dispursed throughout plants and are what give the flowers and fruits of many plants their vibrant colors. They occur as aglycones, glycosides and methylated derivatives[37]. They have been reported to possess a variety of biological activities including antiallergic, antidiabetic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic, hepatoprotective, and antioxidant activities[38].

Since these secondary metabolites are synthesized by plants in response to microbial infection, it should not be surprising that they have antimicrobial activities against several microorganisms[39-40]**.** Flavonoids are present in fruits such as blueberries, red beans, cranberries, and blackberries. Many other natural products such as red and yellow fruits and vegetables and some nuts, also contain flavonoids. It is also present in green plants.

Flavonoids are isolated from many medicinal plants, such as *Galium fissurense* Ehrend. & Schönb.-Tem. (Rubiaceae), which are an endemic plant in Turkey[41]. The leaves of these plants have long been used in folk medicine for a variety of purposes, especially as a diuretic, astringent, choleretic and in the treatment of some stomach, gout and epilepsy[41].

Flavonoids also isolated from *Viscum album* L.(Loranthaceae) which is reported to have various biological activities such as hypoglycemic, anti-inflammatory, anti-viral, and analgesic activities[37,41].

Examples on flavonoids that have *in vitro* antimicrobial activity against strains of ***K. pneumoniae,*** are shown in Fig 1.4 [37].

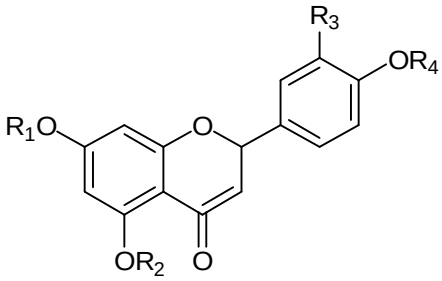


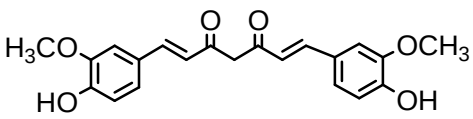
Fig 1.4 :
1- R_1 =R_2 : CH_3, R_3 :H, R_4:-Glc.
2-R_1 =R_2 :H, R_3 : OH, R_4:-Glc.
3- R_1 :-Glc., R_2 =R_3 =R_4 : H.

1.4.5 Curcumin

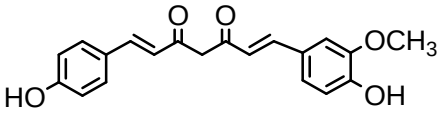
Another example of natural material with some antibacterial activities is curcumin. It is an orange–yellow crystalline powder practically insoluble in water and ether but soluble in ethanol, dimethylsulfoxide, and acetone.

Curcumin was first isolated in 1815 by Vogel [42]; in 1870 it was isolated in crystalline form and identified as (1E,6E) -1,7-bis(4-hydroxy-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione or diferuloylmethane[43]. The feruloylmethane skeleton of curcumin was confirmed in 1910 by the initial work and synthesis by Lampe[44]. Curcumin has a melting point of 183°C; its molecular formula is $C_{21}H_{20}O_6$ and molecular weight 368.37.

Besides curcumin, turmeric contains other chemical constituents known as the curcuminoids (Scheme 2) [44] The curcuminoids impart the characteristic yellow color to turmeric. The major curcuminoids present in turmeric are demethoxycurcumin, bisdemethoxycurcumin, and the recently identified cyclocurcumin[45]. Commercial curcumin contains about 77% curcumin, 17% demethoxycurcumin, and 3% bis-demethoxycurcumin as its major components.

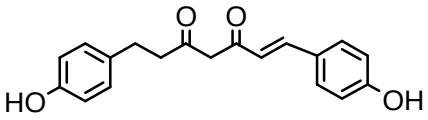


Curcumin I



Curcumin II

Demethoxycurcumin



Curcumin III

Bis-demethoxycurcumin

Scheme 2 :Structures of various curcumins.

Curcumin is the major constituent of the yellow pigments isolated from rhizome of *Curcuma longa* (turmeric). The root of this plant has been used in India as preservative, colorant, flavoring in meals (curry) and as a traditional medicine.

Several studies in recent years have shown that curcumin has antioxidant, , anti-microbial, anti-parasitic, anti-mutagen ,anticancer properties[46,47], and anti-inflammatory[48].

Curcumin acts as a superoxide radical scavenger[49], A recent report describes the H-atom donation from the β-diketone moiety to a lipid alkyl or a lipid peroxyl radical as a potentially more important antioxidant action of curcumin [50].

Curcumin anti-inflammatory activity was attributed to the hydroxyl and phenol groups in the molecule and these groups are also essential for the inhibition of prostaglandins PG synthetase and leucotrienessynthesis(LT)[51.]

Also it was suggested that the anti-inflammatory action is associated with the β-dicarbonylic system, which has conjugated double bonds (dienes). This system seems to be responsible, not only for anti-inflammatory power, but also for antiparasitic activity[52.]

The presence of a diene ketone system provides a lipophilicity to the compounds and thus probably better skin penetration. Structure–activity relationship studies suggest that a hydroxy group at the para-position is most critical for the expression of biological activity[52].

1.5 Heterocyclic Compounds:

Heterocycles occur in a diversity of natural products and are of great importance in a wide variety of applications. Among the heterocycles, the nitrogen-containing five-membered heterocycles are especially important since they have a variety of bioactivities. Five membered heterocyles include pyrroles, pyrazoles, imidazoles, 1,2,3-triazoles, 1,2,4-triazoles, and tetrazoles with one to four nitrogen atoms in the ring (Fig1.5a). Additionally, aromatic nitrogen heterocycles with oxygen as in isoxazoles, oxazoles, 1,3,4-oxadiazoles, and 1,2,4-oxadiazoles (Fig1.5b); or sulfur as isothiazoles and thiazoles (Fig1.5c) are also of great importance in drug synthesis.

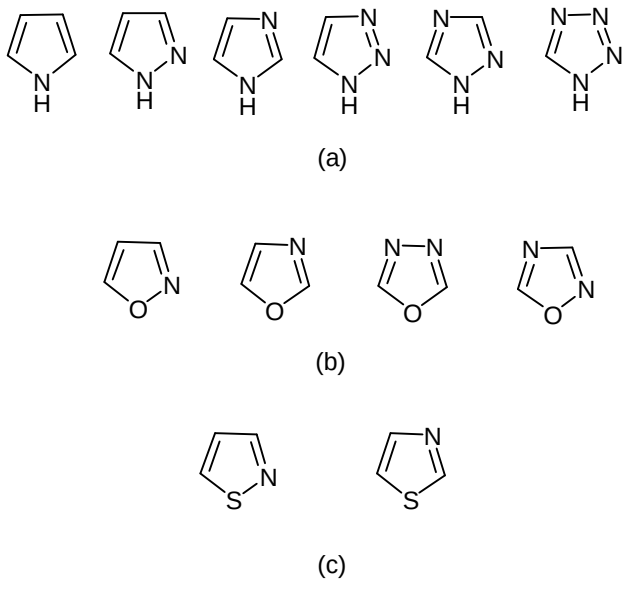


Fig 1.5: Aromatic five – membered heterocycles with nitrogen atoms.

Following are examples on heterocycles that exhibit antibacterial activities are:

1.5.1 2-Pyridones

2- Pyridones (Figure 1.6) are active against Staphylococcus aureus which is resistant to ciprofloxacin, and are active against Streptococcus Pneumonia which is resistant to penicillin. 2-Pyridones are also a DNA gyrase inhibitor[53].

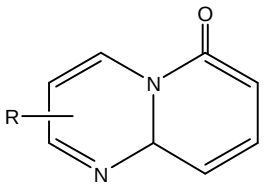
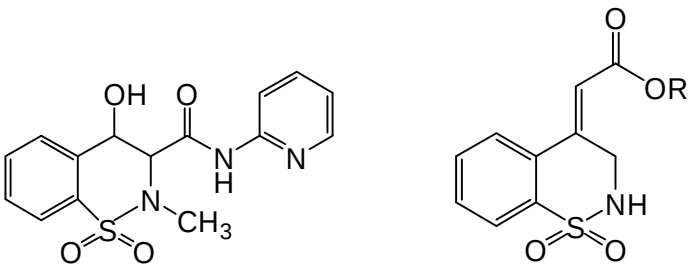


Fig 1.6: 2- Pyridone

1.5.2 Heterocyclic Based Compounds:

Piroxicam (N-(2-pyridyl)-2-methyl-4-hydroxy-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide) is a derivative of 1,2-benzothiazine, it is an anti-inflammatory of the type NSAIDS[54.] The newly synthesized benzothiazine derivatives have shown antipyretic and higher analgesic activities[55], anti-tumor and anticancer activity[56], and Microbicides[57]. Piroxicam was used in healthy chickens as antioxidant[58.]

Certain derivatives of Benzothiazines have shown marginal[59] antibacterial activities against some types of bacteria such as *Neisseria gonorrhoeae, S. aureus* and *Micrococcus luteus*[60,61]. The structure of piroxicam and some of its derivatives are shown in the figure 1.7.



(1) (2) : a, b

a = CH_3

b = C_2H_5

Fig 1.7: (1) Piroxicam, (2) Piroxicam derivatives

1.5.3 Compound 64716

1-Ethyl-4 (1H)-oxo-(1,3)dioxolo(4,5-*g*)cinnoline-3-carboxylic acid is a new synthetic antibacterial agent showed some activity against the gram-negative bacteria[62], Fig 1.8.

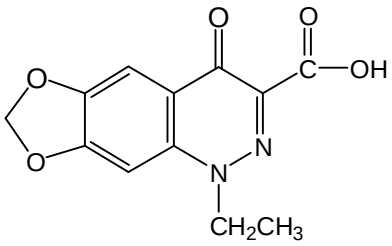


Fig 1.8: 1-ethyl-4 (1H)-oxo-(1,3)dioxolo(4,5-*g*)cinnoline-3-carboxylic acid

1.5.4 Pyrazoles and Isoxazoles Derivatives :

Some new 1*H*-pyrazole-3-carboxylic acid and pyridazinone derivatives, Fig 1.9, were synthesized and evaluated for their antibacterial activities against *Bacillus cereus*, *S. aureus*, *Escherichia coli* and *Pseudomonas putid*, positive results were obtained.[63]

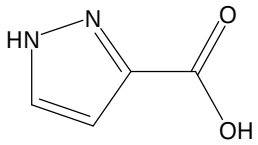
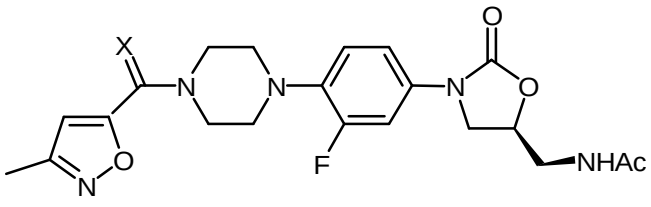


Fig 1.9: 1*H*-pyrazole-3-carboxylic acid

Two series of oxazolidinone derivatives having substituted isoxazoles, Fig (1.10), were also synthesized and tested for antibacterial activities against several Gram-positive strains including the resistant strains of *Staphylococcus* and *Enterococcus*. Some of them showed *in vitro* activities (MIC) comparable or superior to the reference compound vancomycin[64].



1 : x = H_2 R = H, alkyl, alkoxy, aryl,
2 : x = O heterocyclic, halogen

Fig 1.10 : oxazolidinone derivatives with substituted isoxazoles

1.6 AIMS OF THE STUDY

The overall aims of this study are:

1. Design and prepare novel curcumin based heterocyclic diazoles and oxazoles with antibacterial activities.
2. Characterize the prepared heterocyclic compounds by various spectroscopic methods.
3. Assess the antibacterial potency (and spectrum of activity).

As mentioned earlier in the introduction, heterocyclic compounds are very important class of organic compounds. They showed a broad spectrum of bioactive activities ranging from antibacterial to anticancer. For this reason they were chosen for this study.

Curcumin (Scheme 2) was chosen as the base of the heterocyclic derivatives due to several reasons and among these are:

1. curcumin exhibits some antibacterial activities.
2. It is safe even when consumed at a daily dose of 12 g for 3 months as was shown in several studies[65].
3. It has been utilized for centuries in Eastern medicine as a topical treatment for wounds, inflammation, and tumors. It showed several activities as an anti-inflammatory, anti-oxidant, anti-viral, wound healing, hypocholesterolemic effects in diabetic patients. Curcumin has also been shown to suppress carcinogenesis of the skin, liver, lung, colon, stomach and breast[66,67].

4. It has a unique structure, it incorporates several functional groups. The aromatic rings which are methoxylated phenols, the two carbonyl groups form a diketone, the enol of the heptadiene-3,5-diketone , and unsaturated carbonyls.
5. The diketone functional group in curcumin is very useful in organic synthesis it could be converted to several other functional groups, among which are alcohols, imines, and amines.

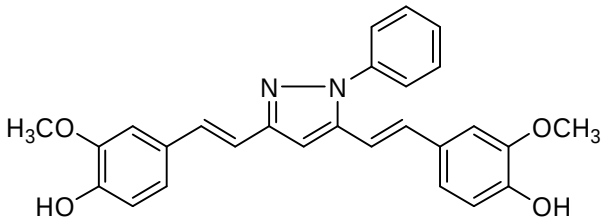
In this study curcumin will be incorporated with hydrazine derivatives to form pyrazoles and isoxazoles. Both pyrazoles and oxazoles are heterocyclic aromatic compounds with large number of medical applications as mentioned earliar in the introduction.

Pyrazoles showed anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities.

Isoxazoles are interesting heterocyclics because of their biological activities[68] e.g. as fungicides[69], herbicides[70], or as nicotinic acetylcholine receptor ligands[71], and as antibacterial agents[72.]

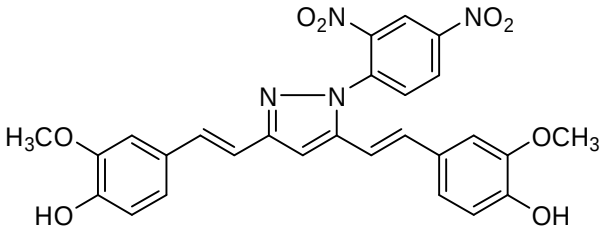
Some of the pyrazoles and isooxazoles, which we intend to prepare and evaluate their antibacterial activities, are summarized in the following scheme.

1.



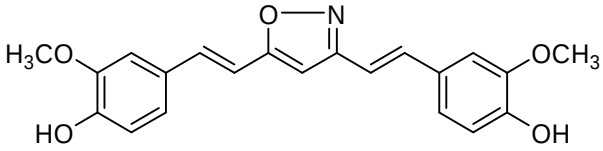
4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

2.



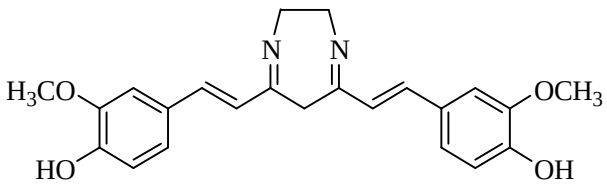
4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

3.

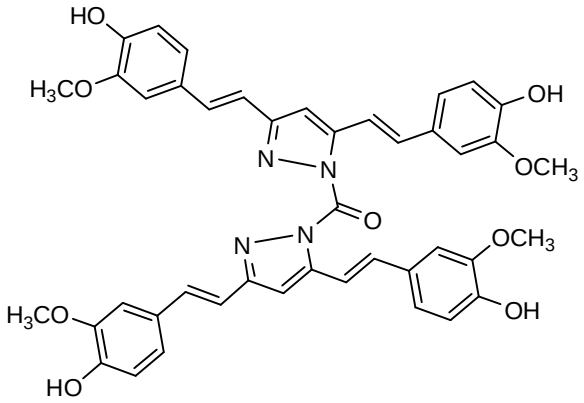


4-((1*E*)-2-(3-(4-hydroxy-3-methoxystyryl)isoxazol-5-yl)vinyl)-2-methoxyphenol

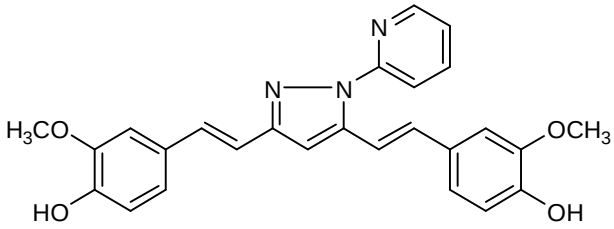
4.



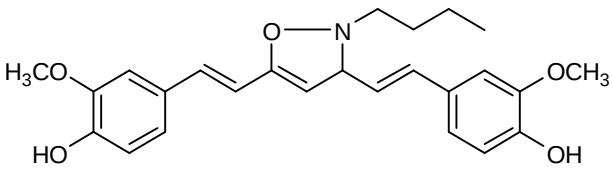
4,4'-((1*E*,1'*E*)-(3,6-dihydro-2*H*-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol)

5. 

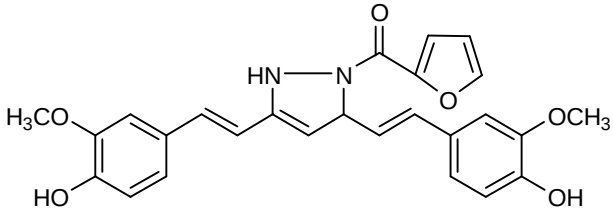
bis(3,5-bis((*E*)-4-hydroxy-3-methoxystyryl)-1*H*-pyrazol-1-yl)methanone

6. 

4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

7. 

4-((1*E*)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol

8. 

1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1*H*-pyrazol-2(3*H*)-yl)ethanone

scheme 3 : Curcumin based Pyrazoles and Isoxazoles to be prepared and evaluated for antibacterial activity.

CHAPTER TWO

EXPERIMENTAL

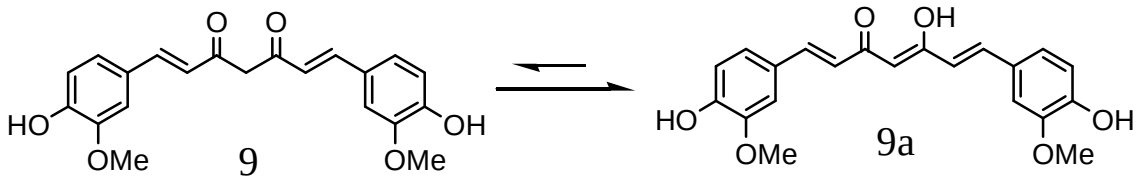
2.1 General Experimental

All chemicals were purchased from Aldrich Chemical Company and used without any further purification unless otherwise specified. All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR spectroscopy, and elemental analysis. Nuclear Magnetic Resonance spectra were recorded on Varian Gemini 2000, 300 MHz instrument. Infrared spectra were recorded on a Shimadzu 820 PC FT-IR spectrometer, samples were run as KBr disk. All ^{1}H NMR experiments were reported in δ units, parts per million (ppm) downfield from tetramethylsilane. All ^{13}C NMR spectra were reported in ppm relative to deuterochloroform (77.0 ppm). Samples purifications were performed using flash chromatography with silica gel (100-200) mesh.

2.2 Extraction of curcuminoid (9) from curmeric poweder.

Curcuma longa powder (20.0 g) was suspended in methanol 300 ml and stirred vigorously for about 24.0 hours at room temperature. The mixture was filtered then methanol was removed under vacuum. The yellow gummy residue containing curcuminoids was subjected to purification by flash chromatography on silica (100-200 mesh). The first fraction was eluted with hexane-ethyl acetate (9:1), then hexane-ethyl acetate (4:6), and then methanol-ethyl acetate (1:9) was used to elute the second fraction. It was found that the Second fraction contains the desired curcumin. Solvent of

the second fraction was removed under reduced pressure using a rotator evaporator. The yellow-orange residue weighed about 3.57 g (11.9%) . The residue was characterized by ^{1}H and ^{13}C NMR and IR spectra to be the desired curcumin **I (**9**)** with small fraction of **II** and **III-** (less than 10%)- scheme 2 in the introduction. ^{1}H NMR (300 MHz) ($CDCl_3$-DMSO) (8:2, d6) δ: 9.45 (broad, 2H, OH), 7.45-7.55 (m, 2H), 7.25 (d, 1H), 7.05 (d, 2H), 6.85 (m, 2H), 6.7 (d, 2H), 5.9 (s, 2H) 3.85 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$-DMSCO 8:2, d6) δ: 184, 149, 148, 140.5, 130, 127, 124, 121, 115, 115.5, 112, 101, 56.



Curcumin

2.3 General Procedure

For preparation of curcumin based heterocyclic compounds (1- 8)

(Scheme 3) :

The general experimental procedure for the preparation of compounds (1-8) was as follows: In a round bottom flask equipped with magnetic stirring bar and a condenser, curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8 ml). To the solution of curcumin in acetic acid was added the desired hydrazine (1.5 mmole) followed by sodium acetate (0.2 g). The

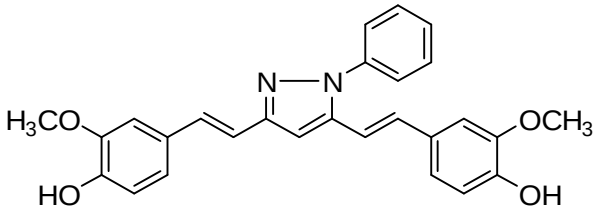
produced solution was refluxed for 12 hrs. The progress of the reaction was monitored by TLC analysis. After complete conversion (4-7 h), the mixture was cooled down to room temperature and then neutralized with ammonium hydroxide. The produced solid was collected by suction filtration and purified by flash chromatography.

2.3.1 Preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and phenyl hydrazine hydrochloride (1.5 mmole, 0.25g) was added to the solution followed by sodium acetate (1.5 mmole, 0.2 g) to neutralize the acid present with phenyl hydrazine. The mixture was refluxed for 4 hrs. Then neutralized with ammonium hydroxide to produce light greenish precipitate which was collected by suction filtration, then purified by flash chromatography (hexane/EtOAc 6:4). The product weight was 0.48 g (71.6 %), m.p. 127-130 ^{o}C. IR (cm^{-1}:1700 cm^{-1}(C=N), 1080 cm^{-1} (C-O ether) of OCH_3, 3100 and 1600cm^{-1} are for =C-H and C=C in aromatic rings).

^{1}H NMR (300 MHz, $CDCl_3$): d 3.90 (s, 6H, OCH3), 6.80 (s, 1H, C4-H), 7.02 (d, 2H, J = 14.8 Hz, C2-H and, C6-H), 7.10 (d, 2H, J = 14.8 Hz, C1-H and C7-H), 7.15–7.32 (m, 5H, Ar-H), 7.65–7.71 (m, 2H, Ar-H), 7.98–8.12 (m, 2H, Ar-H), 8.22–8.26 (m, 2 H, Ar-H), 8.43 (d, 2H, J = 8.2 Hz, Ar-H). ^{13}C NMR(300 MHz, $CDCl_3$): 56.05, 56.09, 101.22, 110.02 110.98, 112.57, 115.97, 116.14, 117.64, 120.67,125.24, 128.17, 128.29, 128.84, 129.02,

129.06, 129.08, 131.22, 133.34, 139.72, 142.82, 147.20, 147.74, 148.24, 148.33, 151.54, 161.00. Anal. Calcd for $C_{27}H_{24}N_2O_4$: C 73.62, H 5.49, N 6.36, O 14.53; found: C 71.03 , H 5.12 , N 6.78, O 17.07 .



4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

2.3.2 Preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H- pyrazol-3-yl)vinyl)-2-methoxyphenol

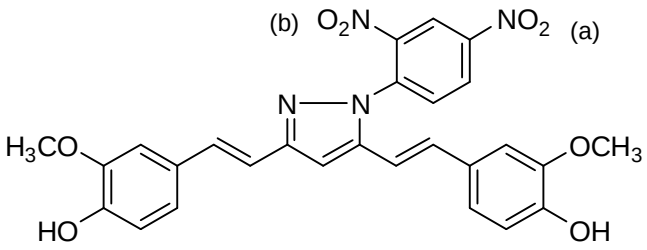
Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and 2,4- dinitrophenyl hydrazine hydrochloride (1.5 mmole, 0.355g) was added to the solution followed by sodium acetate (1.5 mmole, 0.2 g) to neutralize the acid. The solution was refluxed for 7 hrs, Then was neutralized with ammonia solution to produce orange-red precipitate which was collected by suction filtration. Produced solid was purified by flash chromatography (hexane/EtOAc 6:4).

The product weight was 0.6 g (78.9 %), m.p. 92-95 ^{o}C. IR : (1690 cm^{-1} (C=N), 1590cm^{-1} for (C=C aromatic), 1080 cm^{-1} (C-O ether) of OCH_3, 1350 cm^{-1} $–NO_2$ (a) and 1520 for $–NO_2$ (b), 3600 cm^{-1} (O-H of phenol). ^{1}H NMR

(300 MHz, $CDCl_3$): d 3.780 (s, 3H, OCH3), 3.80 (s, 3H, OCH_3), 6.0 (s, 2H, OH), 6.80

(s, 1H, C-4H) 6.83(m, 4H, C1-H, C2-H, C-6-H, and, C7-H), 6.9 (m, 1H, Ar-H), 7.1 (d, 2H, Ar-H), 7.2 (d, 2H, Ar-H), 7.4 (d, 1H, Ar-H), 8.0 (d, 1 H, Ar-H containing NO_2), 8.43 (d, 1H, Ar-H containing NO_2), 8.9 (d, 1H, Ar-H containing NO_2).

^{13}C NMR(300 MHz, CDCl3): 56.05, , 102.07, 110.0, 110.94, 111.28, 111.72, 116.11, 116.89, 121.59, 121.86, 123.63, 126. 83, 127.91, 128.43, 128.92, 130.23, 133.07, 135.18, 135.69, 147.16, 144.89, 145.48, 146.23, 148.23, 148.42, 154.02. Anal. Calcd for $C_{27}H_{22}N_4O_8$: C 61.13, H 4.18, N 10.56, O 24.13 ; found: C 61.59, H 4.68, N 9.85, O 23.88.



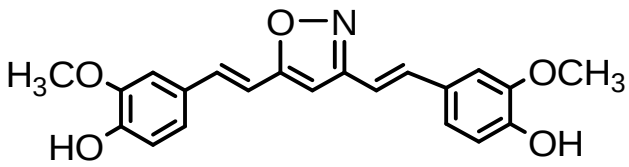
4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

2.3.3 preparation of 4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and hydroxylamine hydrochloride (1.5 mmole, 0.11g) was added to the

solution followed by sodium acetate (1.5 mmole, 0.2 g) to neutralize the acid present with hydroxyl amine. The solution was refluxed for 7 hrs, Then neutralized with ammonium hydroxide to produce dark grey precipitate which was collected by suction filtration. Produced solid was purified by flash chromatography (hexane/EtOAc 6:4). The product weight was about 0.40 g (72 %), m.p. 116-119 ^{o}C; R_f = 0.50 (EtOAc/MeOH 9:1) IR : (3700 cm^{-1} (O-H of phenol group), 1615 cm^{-1} (C=N), 1580 cm^{-1} (C=C) aromatic rings, 1350 cm^{-1} (C-O ether) of OCH_3.

^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR: δ 3.85 (s, 6H, 2OCH_3), 6.65 (s, 1H, C_4-H), 66.84-7.01 (m, 3H), 7.04-7.15 (m, 4H, *Ar-H*), 7.16 (m, 3H), 8 (s, 2H, OH). ^{13}C NMR (300 MHz, $CDCl_3$) : 56.14, 56.18, 98.33,110.56, 110.82, 113.12, 115.98, 116.11, 116.24, 121.8, 122.16, 127.49, 127.83, 129.22, 135.27, 135.98, 148.22, 148.42, 162.7, 168.84. Anal. Calcd for $C_{21}H_{19}NO_5$: C 69.03, H 5.24, N 3.38, O 21.9 ; found: C 68.89, H 5.96, N 4.04, O 21.11.



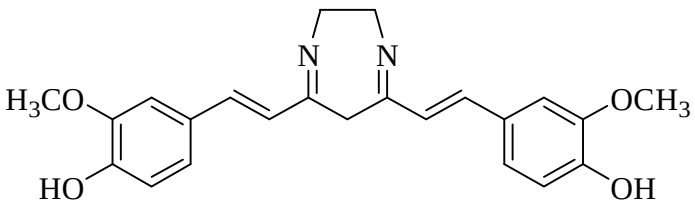
4-((1*E*)-2-(3-(4-hydroxy-3-methoxystyryl)-4,5-dihydroisoxazol-5-yl) vinyl)-2-methoxyphenol

2.3.4 preparation of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol):

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and ethylene diamine (1.5 mmole,0.1 ml) was added to the solution.. The solution was refluxed for 4 hrs, Then neutralized with ammonium hydroxide to produce red precipitate which was collected after evaporation in rotary evaporator under vacuum. The solid product was purified by flash chromatography (hexane/EtOAc 6:4). The product wheight was 0.45g (76.3%), m.p. 65-68 ^{o}C

(the low m.p. could be due to hydration of the material.)

The product was analyzed by LC/MS [M + 1] found: 394.0; IR : (1700 cm^{-1}two (C=N) groups, 1580 cm^{-1} (C=C aromatic), 1080 cm^{-1} (C-O ether), 3700 cm^{-1} (O-H of phenol); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.764 (s, 4H, CH_2CH_2); 3.78 (s, 6H, OCH_3), 5.72 (s, 2H, OH), 6.81-6.85 (m, 3H), 6.91 (d, 2H, J = 15.2 Hz), 7.05 (d, 2H, J = 12.1 Hz), 7.2 (s, 2H), 7.4- 7.6 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) 24.5, 48.3, 56.4, 112.9, 116.3, 120.6, 122.4, 127.8, 129.3, 148.2, 149.3, 165.8.



4,4'-((1*E*,1'*E*)-(3,6-dihydro-2*H*-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol)

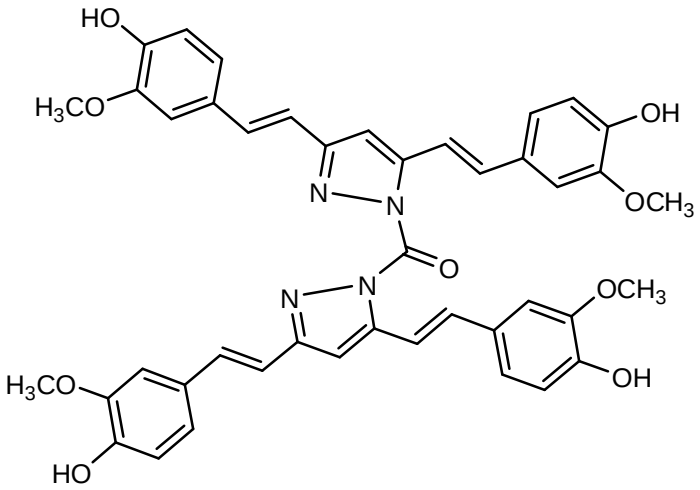
2.3.5 preparation of 3,5-bis(4-hydroxy-3-methoxystyryl)pyrazolidine-1-carbohydrazide

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and carbohydrazide (1.5 mmole,0.135 g) was added to the solution.. The solution was refluxed for 5 hrs, Then neutralized with ammonium hydroxide to produce light brown precipitate which was collected after evaporation in rotary evaporator under vacuum and purified by flash chromatography (hexane/EtOAc 4:6).

The product weight was 0.12g (88.6%), m.p. 151-154 ^{o}C. IR : (1550 cm^{-1} (C=C) aromatic rings, 1600 cm^{-1} (C=O), and 3300 cm^{-1} (O-H phenol).

^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 12H, OCH_3), 6.5-7.4 (m, 22H), 8.8(s, 4H, OH).

^{13}C NMR(300 MHz, DMSO-d_6) 56.07, 100.1, 110.0, 116.08, 120.1, 123.6, 129.8, 130.4, 144.7, 147.3, 147.5, 148.5. Anal. Calcd for $C_{26}H_{23}N_3O_4$: C 68.42, H 5. 7, N 5.94, O 19.09 ; found: C 66.8 , H 5.25, N 6.49, O 21.46.



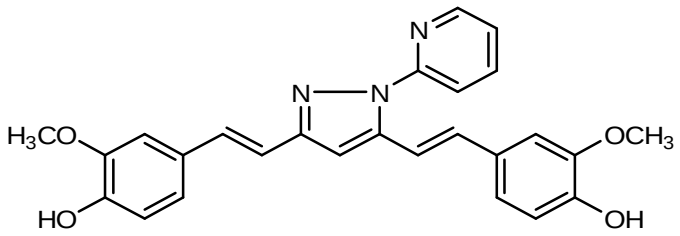
bis(3,5-bis((*E*)-4-hydroxy-3-methoxystyryl)-1*H*-pyrazol-1-yl)methanone

2.3.6 preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and 2-hydrazino pyridine (1.5 mmole,0.164 g) was added to the solution.. The solution was refluxed for 4 hrs, Then neutralized with ammonium hydroxide to produce dark orange precipitate which was collected by suction filtration and purified by flash chromatography (hexane/EtOAc 6:4). The product wheight was 0.6g (89.5%), m.p. 122-125 ^{o}C. IR : (1580 cm^{-1} (C=C) aromatic rings, 1080 cm^{-1} (C-O ether) of OCH_3, 1700 cm^{-1} (C=N), at 3600 cm^{-1} for (O-H) phenol.

^{1}H NMR: δ 3.78 (s, 6H, $2OCH_3$), 6.0 (2H, OH), 6.65 (s, 1H, C_4-H), 6.75 (t, 2H), 6.95 (t, 2H), 7.05 (d, 1H), 7.1 (t, 2H), 7.25 (d, 1H), 7.38 (t, 1H), 7.5 (d, 1H), 7.72 (d, 1H), 7.74 (d, 1H), 8.0 (t, 1H), 8.5 (d, 1H). ^{13}C NMR : 56.17,101.33, 102.45, 110.135, 110.871, 111.79, 115.56, 116.21, 117.50, 120.62, 122.37, 123.68, 126.69, 127.512, 128.85, 132.38, 132.79,141.17,

144.02,, 147.81, 148.41, 150.08, 152, 203, 153.37, 183.64. Anal. Calcd for $C_{26}H_{23}N_3O_4$: C 70.73, H 5.25, N 9.5, O 14.5 ; found: C 69.03, H 5.87, N 8.56, O 16.54.



4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

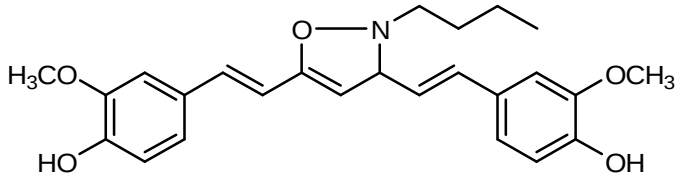
2.3.7 preparation of 4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and n-butyl amine (3mmole,0.25 ml) was added to the solution, The solution

was refluxed for 4 hrs, Then neutralized with ammonium hydroxide to produce dark brown precipitate which was collected after evaporation in rotary evaporator under vacuum and purified by flash chromatography (hexane/EtOAc 6:4).

The product weight was 1g (78%), m.p. 106-109 ^{o}C. IR : (at 1550 cm^{-1} (C=C) aromatic rings, 1080 cm^{-1} (C-O ether), 1360 cm^{-1} for (C-H alkane) of CH_3, 1220 cm^{-1} for (C-N aliphatic amine) and at 3600 cm^{-1} for (O-H) phenol group.

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.79 (m, 4H), 1.1 (t, 2H), 1.3 (t, 3H), 3.78 (s, 6H, OCH_3), 6.62 (s, 1H, C_4-H), 6.84 (m, 4H, C_2-H, C_6-H), 7.1(m, 2H), 7.2 (m, 2H), 7.4 (d, 2H), 7.6(s, 2H, OH). ^{13}C NMR (300 MHz, DMSO): 13.6, 19.2, 22.3, 46.8, 56.4, 101.2, 111.9, 116.1, 121.5, 123.8, 125.3, 137.2, 141.2, 147.9, 150.8, 174.6, 183.2. Anal. Calcd for $C_{25}H_{29}NO_5$: C 70.9, H 6.9, N 3.31, O 18.89; found: C 70.18, H 7.31, N 3.68, O 18.83.



4-((1*E*)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol

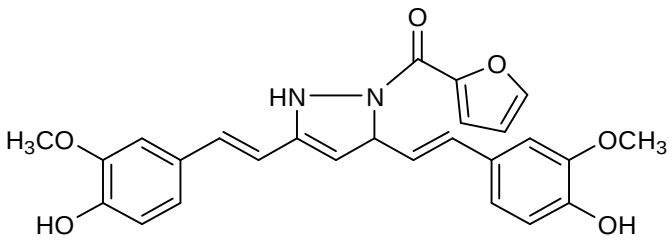
2.3.8 Preparation of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone

Curcumin (1.5 mmole, 0.5 g) was dissolved in glacial acetic acid (8.0 mL), and 2- Furoic hydrazide (1.5mmole,0.2 g) was added to the solution.. The solution was refluxed for 5 hrs, Then neutralized with ammonium hydroxide to produce dark yellow precipitate which was collected after evaporation in rotary evaporator under vacuum and purified by flash chromatography (hexane/EtOAc 6:4).

The product weight was 0.45g (65.2%), m.p. 138-141 ^{o}C. IR : (at 1750 cm^{-1} (C=O), 1580 cm^{-1} (C=C) aromatic rings, 1080 cm^{-1} (C-O) of the OCH_3 and

1300 cm^{-1} (C-O) of the five-membered ring, 3600 (O-H) phenol; ^{1}H NMR (300 MHz, DMSO-d_6): 3.78 (s, 6H, OCH_3), 6.0 (s, 2H, OH), 6.5-7.3 (m, 12 H), 7.5 (d, 2H, *J*= 16.15Hz), 7.9 (s, 1H, N-H). ^{13}C NMR (300 MHz, DMSO-d_6): 56.03, 99.79, 101.4, 11.74, 112.43, 115.18, 116.01, 116.14, 116.37, 120.56, 121.52, 123.67, 126.74, 128.79, 130.06, 141.18, 146.32, 146.67, 148.32, 148.46, 149.89, 157.7, 183.3.

Anal. Calcd for $C_{26}H_{24}N_2O_6$: C 68.11, H 4.84, N 6.11, O 21; found: C 67.04, H 5.24, N 5.61, O 22 .



(3,5-bis((*E*)-4-hydroxy-3-methoxystyryl)-1*H*-pyrazol-2(3*H*)-yl) (furan-2-yl)methanone

2.4 Testing for Antibacterial Activity :

2.4.1Materials:

Culture media: Mueller-Hinton**,** Tryptic Soy Broth **(Hylabs, Israel)**

2.4.2 Microorganisms used:

Bacterial strains used in the study were clinical isolates of *Staphylococcus aureus*, *Escherechia coli*, *Proteus mirabilis*, and *Pseudomonas aeroginosa*, all of them were isolated from patients suffering from bacterial infections with the relevant bacteria.

2.4.3Testing Procedure

These isolates were tested for their susceptibility to the prepared curcumin derivatives as follows:

Solutions of these derivatives have been prepared in concentration of 4 mg of derivative per 1 ml of dimethyl sulfoxide (DMSO) solvent, then incubated for 24 hours at 37 ^{o}C.

2.4.4 Screening for Antimicrobial activity :

Drugs were screened for antimicrobial activity by using the well diffusion method reported in the literature by Perez et al. [73]

1. Three colonies of bacteria were transferred to sterile tubes each containing 5 ml of Tryptic Soy Broth.
2. Turbidity of the bacterial suspensions was adjusted to reach an optical density equivalent to a 0.5 McFarland standard to give a bacterial suspension of 10^{8} cfu/ml. (cfu : colony forming unit)

3. Mueller-Hinton agar plates were inoculated by streaking bacterial swabs over the entire surface of the plates.
4. Plates were allowed to dry at room temperature.
5. Six millimeter wells were punched in the plates.
6. Fifty microliters of 4 mg/ml solutions of each of the curcumin derivatives were added into duplicate wells.
7. Plates were allowed to stand at room temperature to let the tested derivative diffuse into the agar, and afterwards, they were incubated at 37 ^{o}C for 18 to 24 hours.
8. Plates were examined for bacterial growth inhibition and zones of inhibition were measured in millimeters.

2.4.5 Determination of Minimum Inhibitory Concentration (MIC)[74] .

MIC was determined by broth dilution method as shown below:

1. Drugs with inhibitory zones against the above mentioned bacterial strains were used in this part.
2. Two-fold serial dilutions were prepared from the drugs in Tryptic Soy Broth.
3. Duplicate tubes of each dilution were inoculated with $5x10^5$ of the bacterial strains.
4. All tubes were incubated at 37 ^{o}C for 18 to 24 hours.
5. The highest dilution of the drug that resulted in inhibition of bacterial growth was considered as the MIC.

2.4.6 Determination of Minimal Bactericidal Concentration (MBC):

MBC was determined by the following method:

1. Subcultures from the above dilutions were done on Muller-Hinton plates and incubated at 37 ^{o}C for 18 to 24 hours.
2. The highest dilution that resulted in total inhibition of bacterial growth was considered the MBC.

CHAPTER THREE

RESULTS AND DISCUSSION

The objectives of this work are to design and prepare novel natural product based heterocyclic compounds with bioactivity against bacteria that exceeds current antimicrobial reagents and are less toxic and eco friendly reagents.

Heterocyclic compounds, in general, are very important class of organic compounds, a lot of them are naturally found. Natural heterocyclics have shown a broad spectrum of bioactive ranging from antibacterial to anticancer. Hence, they were chosen for this study.

Curcumin (**9**) was chosen as the base of the heterocyclic derivatives due to several reasons among which mentioned earlier in the introduction. Curcumin was modified with hydrazine derivatives to form various heterocyclic compounds such as pyrazoles and isoxazoles. Both pyrazoles and isoxazoles are heterocyclic aromatic compounds with large number of medical applications as mentioned earlier in the introduction .

3.1 Preparation of Curcumin Based Heterocyclic Compounds:

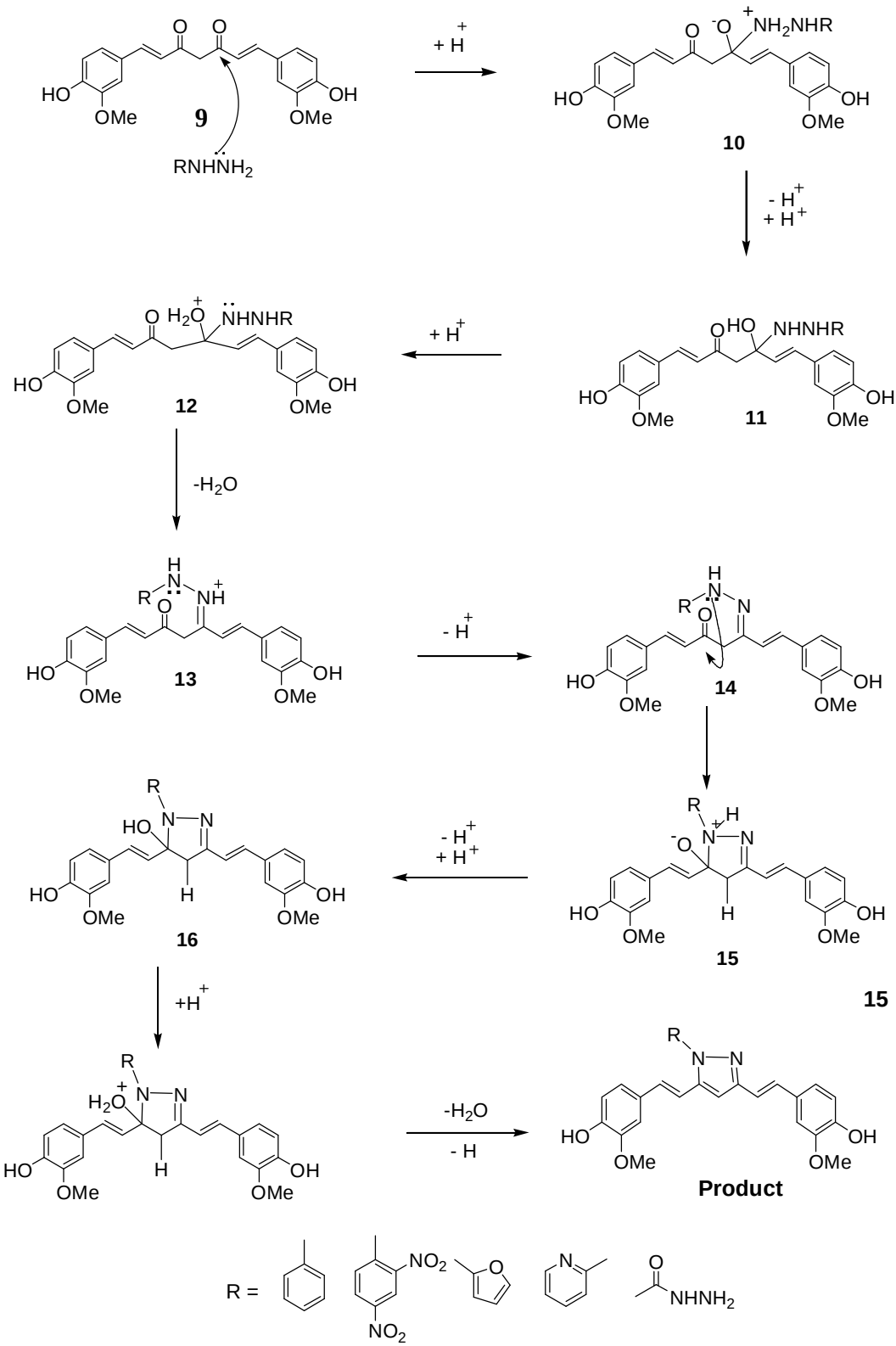
Compounds used in this study were prepared following literature procedure with minor modification. In this procedure, curcumin was refluxed with various hydrazines in the presence of acid, which performs dual function as catalyst and solvent. Acid used for this purpose was either glacial acetic acid or polysulfuric acid. The progress of the reaction was monitored by

TLC. Some reactions required more reflux time than others. In all cases 7 hr period reflux was optimum time for the completion of the reaction.

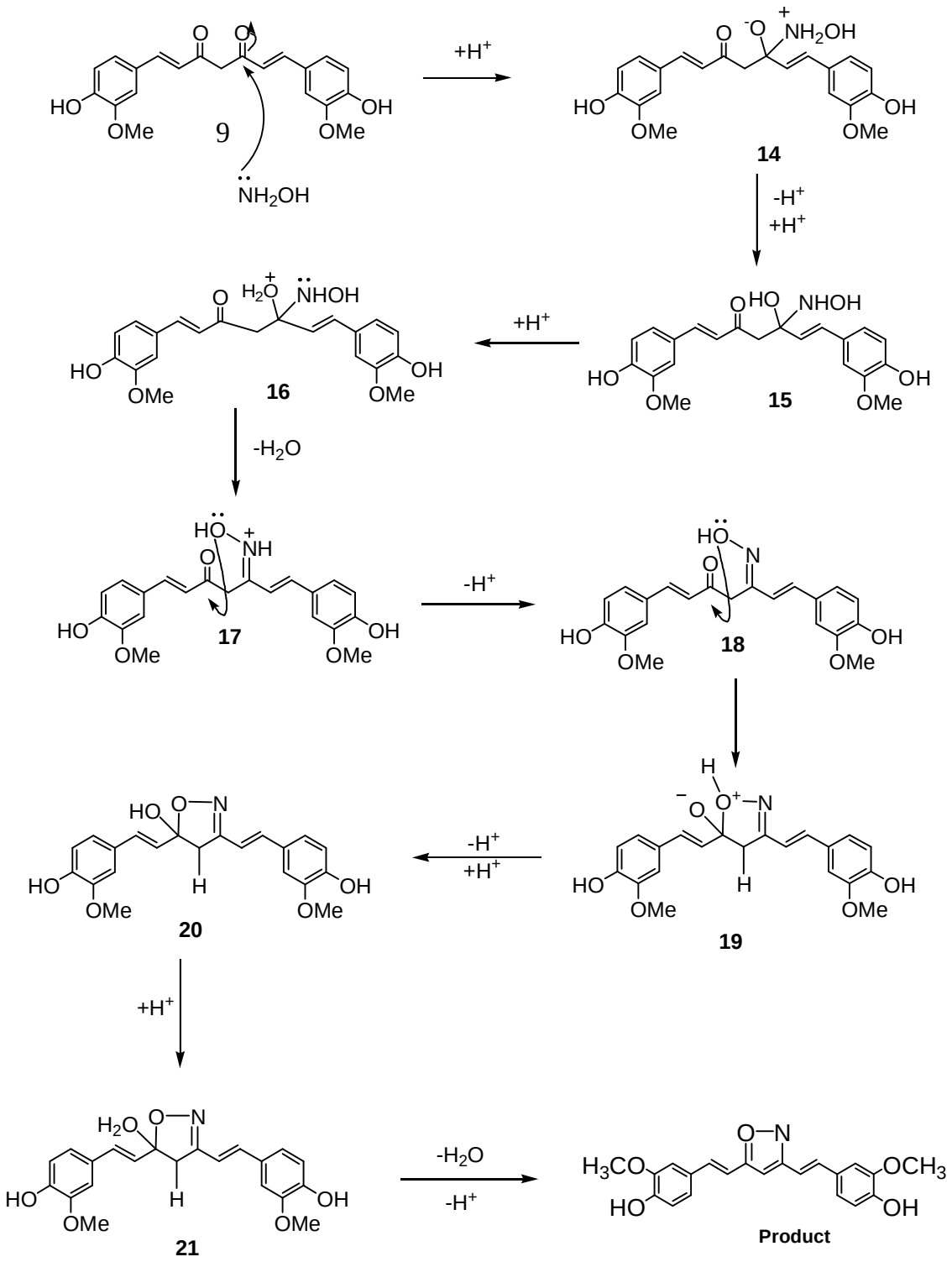
The followed procedure produced only the expected product, some starting materials were also observed. Purification of the products were performed by either flash chromatography or recrystallization from suitable solvents. Purification of the crude products of all reaction by column chromatography or recrystallization gave only one major fraction which characterized through TLC then analyzed by various spectroscopic techniques, such as; melting point, LC/MS and elemental analysis. In all cases results are consistent with the expected structures. All compounds were obtained in acceptable yield (65.2 % to 89.5%).

Mechanistically, the coupling between curcumin and hydrazines is expected to proceed as shown in scheme (4). Both amino groups of hydrazine make nucleophilic attack on the carbonyl groups of the curcumin followed by loss of two water molecules.

Coupling between hydroxylamine and curcumin is shown in scheme 5. It is expected to proceed in a similar fashion to that shown in scheme 4.



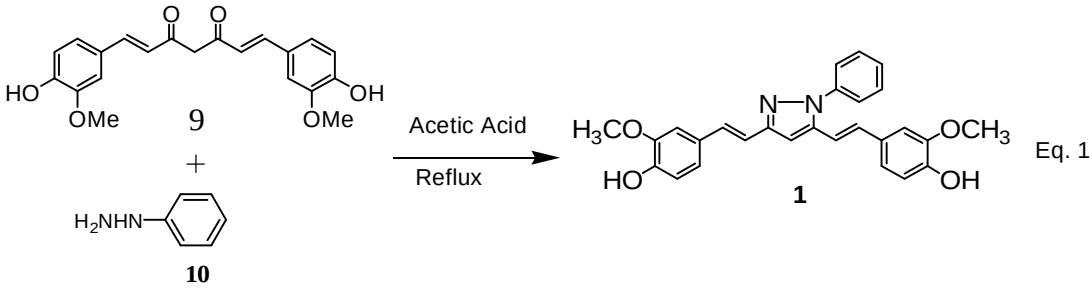
Scheme 4 : Mechanism of coupling between curcumin and hydrazines:
(phenyl hydrazine, 2,4-dinitrophenyl hydrazine, carbohydrazide, 2-hydrazino pyridine,2-Furoic hydrazide)



Scheme 5 :Coupling between hydroxylamine and curcumin.

3.1.1 Preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (1)

Pyrazole (1) was prepared by reacting curcumin (9) with phenyl hydrazine (10) in glacial acetic acid. The reactants were refluxed for 4 hr. The reaction is summarized in eq. 1.



Eq. 1

Pyrazole (1) is green crystals with a melting point range of 127-130 ^{o}C. The elemental analysis and spectral data are shown in the experimental part.

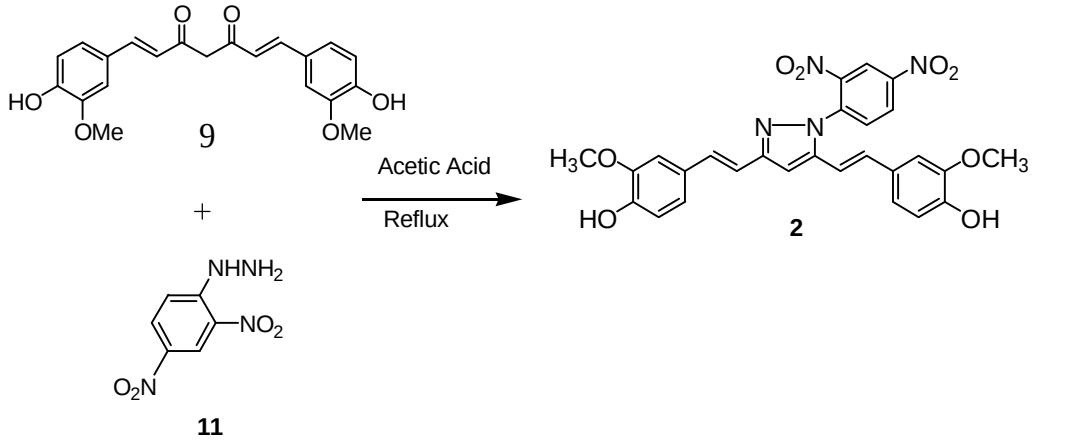
The structure of pyrazole (1) was confirmed by IR and NMR spectroscopy. In the IR spectrum, Fig (3.1) shows strong absorption band at 1700 cm^{-1} , due toC=N vibrational stretching, the band 1080 cm^{-1} is characteristic for (C-O ether) of methoxy group. Bands at 3100 and 1600 cm^{-1} are for =C-H and C=C in aromatic, respectively. Another broad band showed at 3600 cm^{-1} that is a characteristic O-H phenolic group.

In the ^{1}H NMR spectrum Fig(3.2), the aromatic rings protons gave signals in the aromatic region (δ 7.06-7.6), which appeared as multiplet. A singlet signal attributed to OCH_3 attached to the ring appeared at δ 3.72, the vinylic protons of C1, C2, C-6, and C-7 appeared at δ 6. 7 and C-4 proton appeared at δ 5.8.

The IR spectral data along with ^{1}H and ^{13}C Fig (3.3) , are summarized in the experimental section confirm the proposed structure for pyrazole (**1**).

3.1.2 Preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H- pyrazol-3-yl)vinyl)-2-methoxyphenol (2)

Pyrazole (2) was prepared by reacting curcumin (9) with 2,4-dinitrophenyl hydrazine (11) in glacial acetic acid. The reactants were refluxed for 7 hr. The reaction is summarized in eq. 2.



Eq. 2

Compound (2) produced as orange-red crystals with a melting point range of 92-95 ^{o}C. The elemental analysis data and spectra are shown in the experimental section.

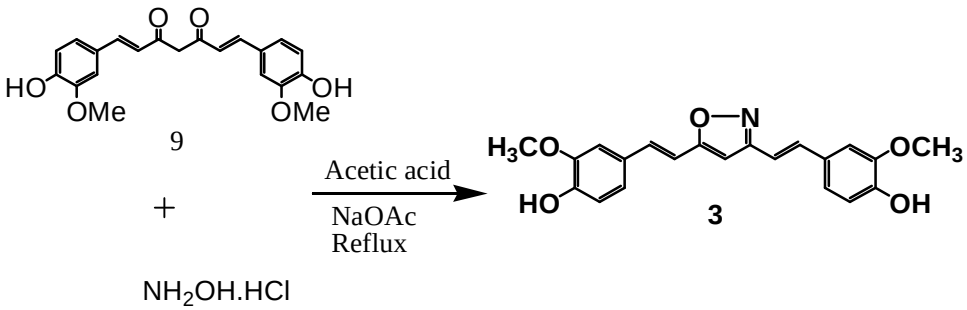
The structure of pyrazole (2) was confirmed by NMR and IR spectroscopic analysis. IR spectrum, Fig (3.4) shows an absorption band at 1690 cm^{-1}, due to (C=N). Band 1590 cm^{-1} for (C=C aromatic), band at 1080 cm^{-1} is characteristic for (C-O ether) of methoxy group, band at 1350 cm^{-1} and 1520 cm^{-1} for nitro groups, strong absorption at 3600 cm^{-1} is due to stretching of (O-H) of phenol group.

^{1}H NMR spectrum of compound **2**, Fig(3.5), shows the following signals δ : 3.780 (s, 3H, OCH3), 3.80 (s, 3H, OCH_3), 6.0 (s, 2H, OH), 8.80 (s, 1H, C-4H) 6.83(m, 4H, C1-H, C2-H, C-6-H, and, C7-H), 6.9-7.4(m, 6H, Ar-H), 8.0 (d, 1 H, Ar-H containing NO_2), 8.43 (d, 1H, Ar-H containing NO_2), 8.9

(d, 1H, Ar-H containing NO_2). The IR spectral data along with ^1H and ^{13}C Fig (3.6), are summarized in the experimental section confirm the proposed structure for pyrazole (2).

3.1.3 Preparation of 4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol (3)

Pyrazole (3) was prepared in the same manner as compound **2** by reacting curcumin (9) with hydroxylamine hydrochloride in glacial acetic acid. The reactants were refluxed for 7 hr. The reaction is summarized in eq. 3.



Eq. 3

Compound (3) was produced as dark grey crystals with a melting point range from 116-119 ^oC. The elemental analysis data are shown in the experimental section.

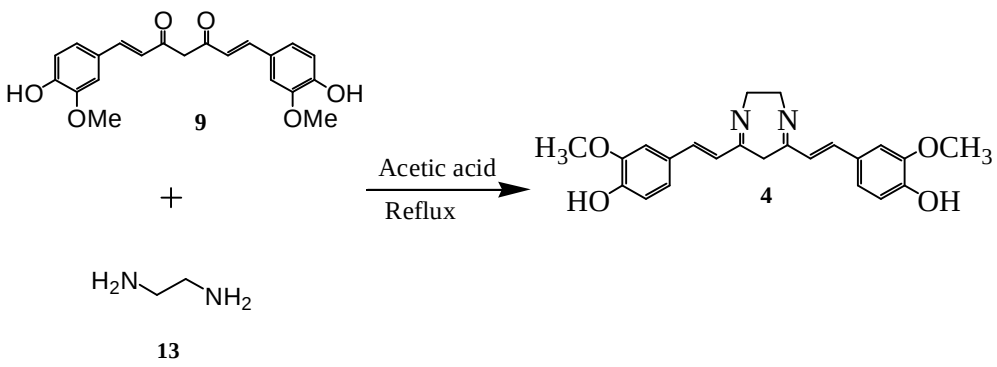
The IR spectrum Fig (3.7), shows a strong absorption at 3700 cm^{-1}which is due to (O-H) of phenol group, a band at 1615 cm^{-1} for (C=N) group, 1580 cm^{-1} for (C=C) of aromatic rings, and a band at 1350 cm^{-1} that is characteristic for (C-O ether) of methoxy group. In the ^1H NMR spectrum for compound **3** is shown in Fig(3.8), the aromatic rings protons gave multiplet signals in the aromatic region δ 7.04-7.15 and another multiplet at δ 7.16 for protons of the aromatic rings. A singlet signal attributed to OCH_3

attached to the ring appeared at δ 3.85, the vinylic protons of C-1, C-2, C-6, and C-7 appeared at δ 6.84-7.01- and C-4 proton appeared at δ 6.65.

The IR spectral data along with ^{1}H and ^{13}C Fig (3.9) are summarized in the experimental along with the section confirm the proposed structure for isoxazole (3).

3.1.4 Preparation of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol)(4)

Pyrazole (4) was prepared by reacting curcumin (9) with ethylene diamine (13) in glacial acetic acid. The reactants were refluxed for about 4 hr. The reaction is summarized in eq. 4.



Eq.4

Compound (4) produced as red crystals with a melting point range of 65- 68 ^{o}C.

The IR spectrum of compound 4 is shown in Fig (3.10). The IR spectrum show strong absorption at 1700 cm^{-1} due to two imine C=N groups, weak band at 1580 cm^{-1} for C=C of aromatic, band at 1080 cm^{-1} C-O of methoxy group, and band at 3700 cm^{-1} for O-H of phenol. ^{1}H NMR spectrum for compound **4,** Fig **(**3.11**)** shows the following NMR data which are consistent

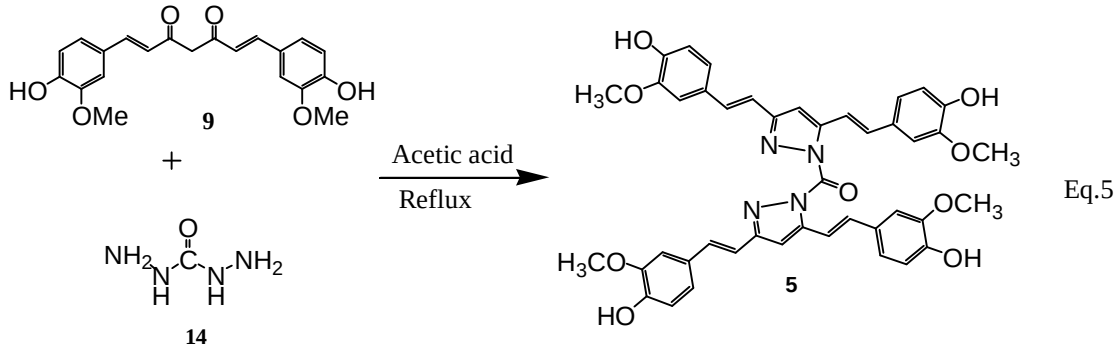
with the structure of compound **4**. δ 1.764 (s, 4H, CH_2CH_2); 3.78 (s, 6H, OCH_3), 5.72 (s,2H, OH), 6.81-6.85 (m, 3H), 6.91 (d, 2H, J = 15.2 Hz), 7.05 (d, 2H, J = 12.1 Hz), 7.2 (s, 2H), 7.4- 7.6 (m, 2H)

Diazepine **4** was also analyzed by LC/MS, LC of diazepine **4** is shown in Fig (3.12). MS spectrum shows a [M + 1] at 394.0 which is consistent with compound **4**, Fig (3.13).

The IR spectral data along with ^{1}H NMR and LC/MS results are summarized in the experimental section and they confirm the proposed structure for isoxazole (4).

3.1.5 Preparation of 3,5-bis(4-hydroxy-3-methoxystyryl)pyrazolidine-1-carbohydrazide(5)

Pyrazole (5) was prepared by reacting curcumin (9) with carbohydrazide (14) in glacial acetic acid. The reactants were refluxed for 5 hr. The reaction is summarized in eq. 5.



Eq.5

Compound (5) was as brown crystals with a melting point range of 151- 154 ^{o}C. The elemental analysis data and spectra are shown in the experimental section.

IR spectrum Fig (3.14) show strong absorption band at 1550 cm^{-1} which is due

to (C=C) stretching of the aromatic rings, band at 1600 $cm^{-1}$for (C=O) stretching vibration, and 3300 cm^{-1} for (O-H phenol).

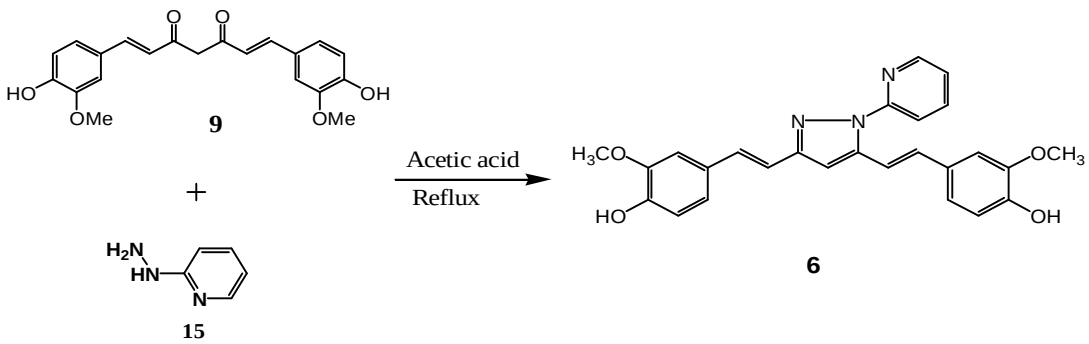
^{1}H NMR spectrum for compound **5,** Fig. (3.15) shows the following NMR data which are consistent with the structure of compound **5**. δ 3.78 (s, 12H, OCH_3), 6.5-7.4 (m, 22H), 8.8(s, 4H, OH).

The IR spectral data along with ^{1}H and ^{13}C Fig (3.16), are summarized in the experimental section along with the section confirm the proposed structure for pyrazolidine (5). Compound **5** has a unique structure, in this case curcumin reacted with two equivalents of carbazide to produce compound with double heterocyclic rings.

3.1.6 Preparation of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (6)

Pyrazole (6) was prepared from reacting curcumin (9) with 2-hydrazino pyridin (15) in glacial acetic acid. The reactants were refluxed for 4 hr.

The reaction is summarized in eq. 6.



Eq.6

Compound (6) was produced as dark orange crystals with a melting point

range of 122- 125 ^{o}C. The elemental analysis and the spectral data are shown in the experimental section.

IR spectrum Fig (3.17) shows absorption at 1580 cm^{-1}, due to (C=C) stretching of the aromatic rings, band at 1080 cm^{-1} for (C-O ether) of methoxy group, band at1700 cm^{-1}indicates (C=N), and band at 3600 cm^{-1} for (O-H) phenol.

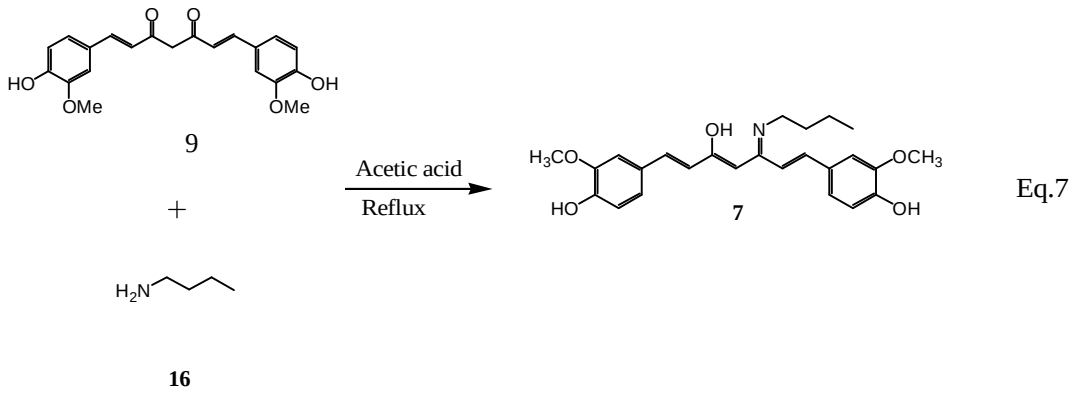
^{1}H NMR spectrum for compound **6**, Fig (3.18) shows the following NMR data which are consistent with the structure of compound **6**. ^{1}H NMR signals are: δ 3.78 (s, 6H, 2OCH_3), 6.0 (2H, OH), 6.65 (s, 1H, C_4-H), 6.75 (t, 2H), 6.95 (t, 2H,), 7.05 (d, 1H), 7.1 (t, 2H), 7.25 (d, 1H), 7.38 (t, 1H), 7.5 (d, 1H), 7.72 (d, 1H), 7.74 (d, 1H), 8.0 (t, 1H), 8.5 (d, 1H).

The IR spectral data along with ^{1}H and ^{13}C Fig (3.19) are summarized in the experimental part along with the section, confirm the proposed structure for pyrazole (6).

3.1.7 Preparatin of 4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol (7)

Pyrazole (7) was prepared by reacting curcumin (9) with n-butyl amine (16) in glacial acetic acid. The reactants were refluxed for 4 hr.

The reaction is summarized in eq. 7.



Eq.7

Compound (7) was produced as dark brown crystals with a melting point range of 106- 109 ^{o}C. The elemental analysis data and spectral results are shown in the experimental section.

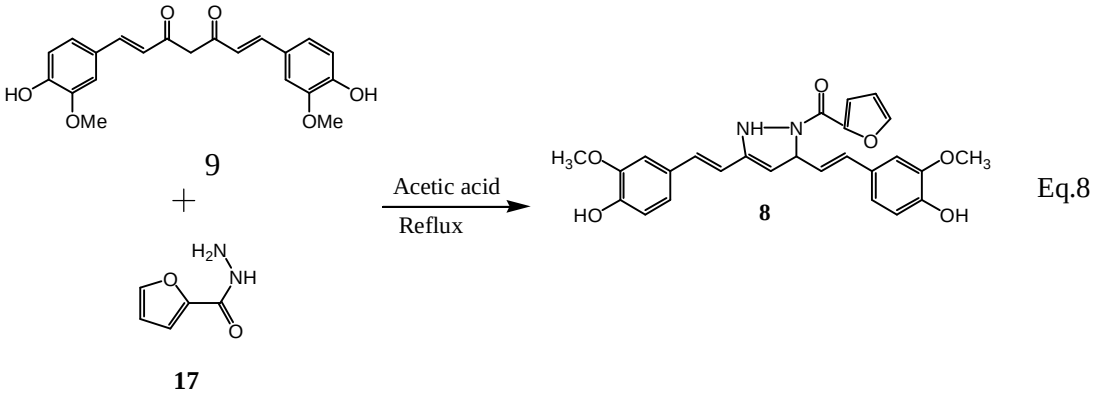
IR spectrum Fig (3.20) shows strong absorption band at 1550 cm^{-1}, due to (C=C) stretching of the aromatic rings, band at 1080 cm^{-1} due to (C-O ether) of methoxy group, band at 1360 cm^{-1} for (C-H alkane) of methyl group, band at 1220 for (C-N aliphatic amine) and a band at 3600 cm^{-1} for (O-H) phenol group. ^{1}H NMR spectrum for compound **7,** Fig (3.21) shows the following NMR data which are consistent with the structure of compound **7**. The NMR signals are δ 0.79 (m, 5H), 1.1 (m, 2H), 1.3 (m, 3H), 3.78 (s, 6H, OCH_3), 6.62 (s, 1H, C_4-H), 6.84 (m, 4H, C_2-H, C_6-H), 7.1(m, 2H), 7.2 (m, 2H), 7.6 (d, 2H) .

The IR spectral data along with ^{1}H and ^{13}C Fig (3.22), are summarized in the experimental along with the section, confirm the proposed structure for pyrazole (**7**).

3.1.8 Preparation of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone(8)

Pyrazole (8) was prepared by reacting curcumin (9) with 2-Furoic hydrazide (17) in glacial acetic acid. The reactants were refluxed for 5 hr.

The reaction is summarized in eq. 8.



Eq.8

Compound (8) produced as yellow crystals with a melting point range of 138- 141 ^{o}C. The elemental analysis and spectral data are shown in the experimental section.

IR spectrum Fig (3.23) shows strong absorption at 1750 cm^{-1}, due to (C=O), band at 1580 cm^{-1} for (C=C) stretching of the aromatic rings, a band at 1080 cm^{-1} for (C-O) of the methoxy group and a band at1300 cm^{-1} for (C-O) of the five-membered ring, the band at 3600 for (O-H) of phenol. ^{1}H NMR spectrum for compound **8**, Fig (3.24) shows the following NMR data which are consistent with the structure of compound **8**. The NMR signals are δ 3.78 (s, 6H, OCH_3), 6.0 (s, 2H, OH), 6.5-7.3 (m, 12 H), 7.5 (d, 2H, *J*= 16.15), 7.9 (s, 1H, N-H). The IR spectral data along with ^{1}H and ^{13}C Fig (3.25), are

summarized in the experimental part along with the section, confirm the proposed structure for pyrazole (8).

3.2 Antibacterial Activity

After the preparation and characterization of the curcumin derivatives using NMR, IR and elemental analysis, they were tested for their antimicrobial activity against four types of bacteria : *S. aureus*, *E. coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

These bacterial strains were clinical isolates; all of the strains were isolated from patients suffering from bacterial infections with the relevant bacteria.

3.2.1 Screening Results :

Curcumin and Curcumin derivatives were screened for antimicrobial activity by using the well diffusion method reported in the literature by Perez et al[73], the efficiency of the drug was measured by the zone of inhibition in millimeters of the bacteria cultured on the Mueller-Hinton agar plate. The results are summarized in table 1. As can be seen in table 1, curcumin and all of its derivatives were inactive (zones of inhibition were zero), for *Proteus mirabilis* and *Pseudomonas aeruginosa*. However they showed some activities against *E. coli*, except curcumin and derivatives number 4 and 7 which showed no activity as shown in table 1, zone of inhibition for compound 4 and 7 were 0 mm. The highest activity agianst *E. coli* was obtained from compound 2, with zone of inhibition 13 mm.

The best activity showed by curcumin derivatives was against *S. aureus.* All compounds showed intermediate to susceptible activities, according to interpretive criteria defined by the National Committee for Clinical Laboratory Standards [75], that divide the activity of compounds to bacteria as susceptible, intermediate and resistant.

the zones of inhibition varied from 11mm to 18mm. Compound 4 showed the highest zone of inhibition, it was about 27 mm, and its effect is at least three fold more than that of the other derivatives.

Table (1) : the antimicrobial activity of curcumin and its derivatives against various types of bacteria.

Screening results (Zone of inhibition in mm)

compound (4mg/ml)	*S. aureus*	*E. coli*	*P. mirabilis*	*P. aeroginosa*
curcumin	11	0	0	0
1	11	11	0	0
2	14	13	0	0
3	16	12	0	0
4	27	0	0	0
5	18	12	0	0
6	16	10	0	0
7	11	0	0	0
8	15	12	0	0

1=**4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol**

2 = **4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H- pyrazol-3-yl)vinyl)-2-methoxyphenol**

3=**4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol**

4=**4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol)**

5 = **3,5-bis(4-hydroxy-3-methoxystyryl)pyrazolidine-1-carbohydrazide**

6 = **4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol** (6)

7 = **4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol (7)**

8 = **1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone**

3.2.2 Determination of Minimum Inhibitory Concentration (MIC). The aim of this test was to determine the minimum concentration of each drug that inhibits the growth of bacteria. Curcumin was also evaluated, MIC was determined by broth dilution method. Two solutions were used to obtain correct results; one is positive control solution (contains bacteria alone) where bacterial growth always positive, another is sterility control solution (contains curcumin alone) where bacterial growth always negative. The results obtained from curcumin are summarized in table 2. As shown in table 2 , MIC of curcumin was 1mg/mL, which is at least four fold higher than the MIC of the other curcumin derivatives shown in table2. The results may indicate that curcumin has slight activity against the bacteria used in this study.

Table (2): MIC Determination of Curcumin and its derivatives(1 -8)

Conc. (µg /mL)	**Curcumin**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
4 x 10^3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2 x 10^3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
1 x 10^3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
500	**+ve**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
250	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
125	+ve	**+ve**	**+ve**	**+ve**	-ve	-ve	-ve	-ve	-ve
62.5	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
31.25	+ve	+ve	+ve	+ve	-ve	**+ve**	**+ve**	**+ve**	**+ve**
15.625	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
7.8125	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
3.9062	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
1.953125	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
0.976563	+ve	+ve	+ve	+ve	**+ve**	+ve	+ve	+ve	+ve
Positive control	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Sterility control	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Bacterial growth (+ve or -ve)

3.2.3 Determination of Minimal Bactericidal Concentration (MBC):

The concentration of each curcumin derivatives that results in a total inhibition of bacterial growth was considered the MBC.

Table 3 summarizes MIC and MBC of each drug.

Table (3) :MBC results for *S. aureus* bacteria

Compound no.	**MIC (mg\mL)**	**MBC (mg\mL)**
curcumin	1	1
1	0.25	1
2	0.25	1
3	0.25	1
4	0.0019	0.0075
5	0.0625	1
6	0.0625	1
7	0.0625	1
8	0.0625	1

The highest MIC result was for derivative 4 which was about 1.9 µg \mL**.** For compounds 1, 2 and 3 the MIC was equal 0.25 mg\mL, derivatives 5,6, 7 and 8 showed MIC of 0.0625 mg\mL. These results indicate that compounds 5,6, 7 and 8 have higher activities than compounds 1,2, and 3.

However, the MBC results for all compounds and curcumin was about 1 mg\ml, except for compound 4, MBC was 0.0075 mg\mL.

In conclusion, all curcumin derivatives showed antimicrobial activity against the isolated strains of *S. aureus*, and *E. coli* except for derivatives no.4 and 7. But for clinical isolates of *Proteus mirabilis* and *Pseudomonas aeruginosa*, they showed no activity.

Screening results against *S. aureus* were better than screening of *E. coli*, that means the prepared curcumin derivatives are more efficient against gram-positive bacteria than gram-negative.

Derivative number 4, 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol), showed the highest efficiency against *S. aureus* bacteria.

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Appendix of Spectral Data

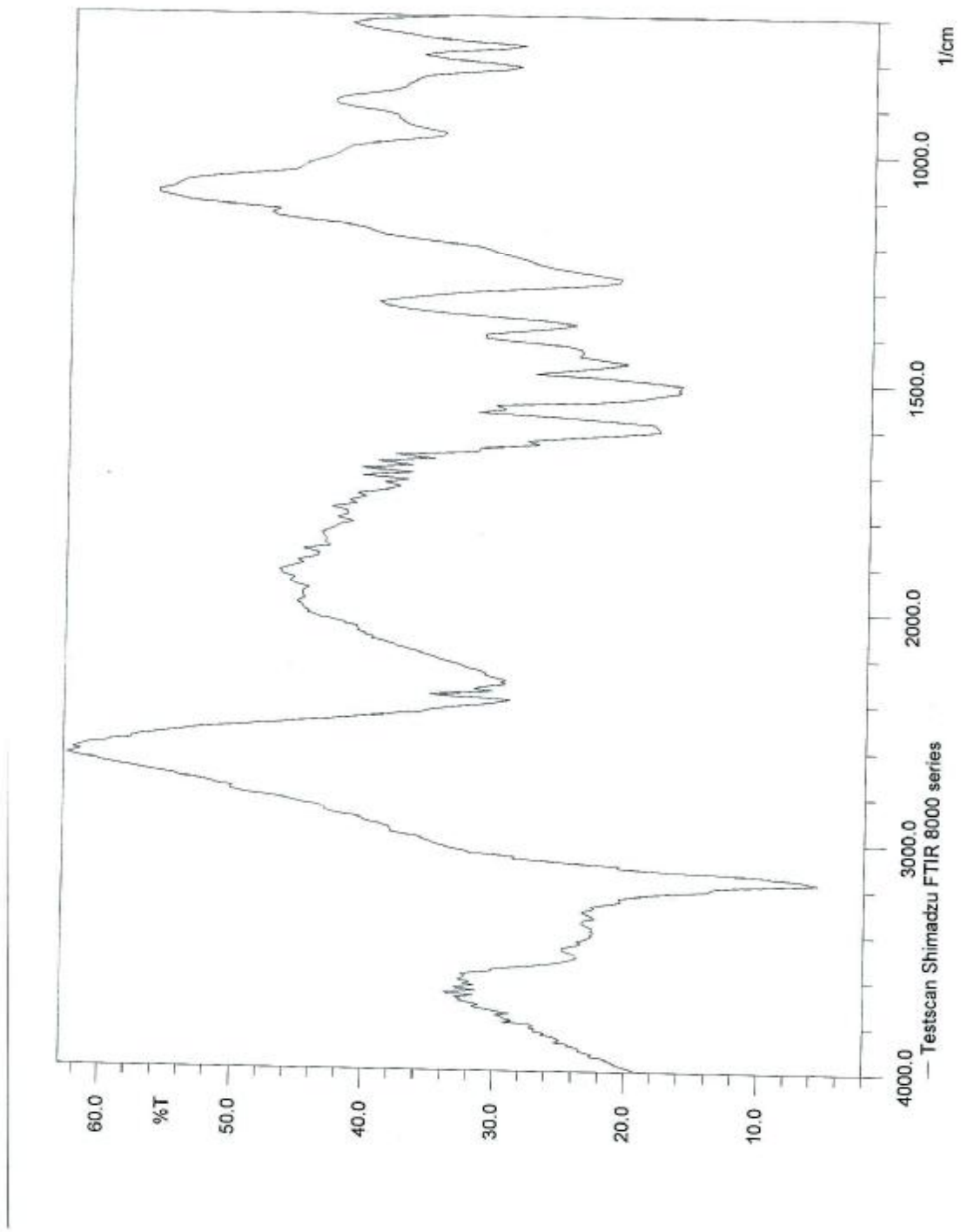

Fig 3.1: FT-IR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**1**)

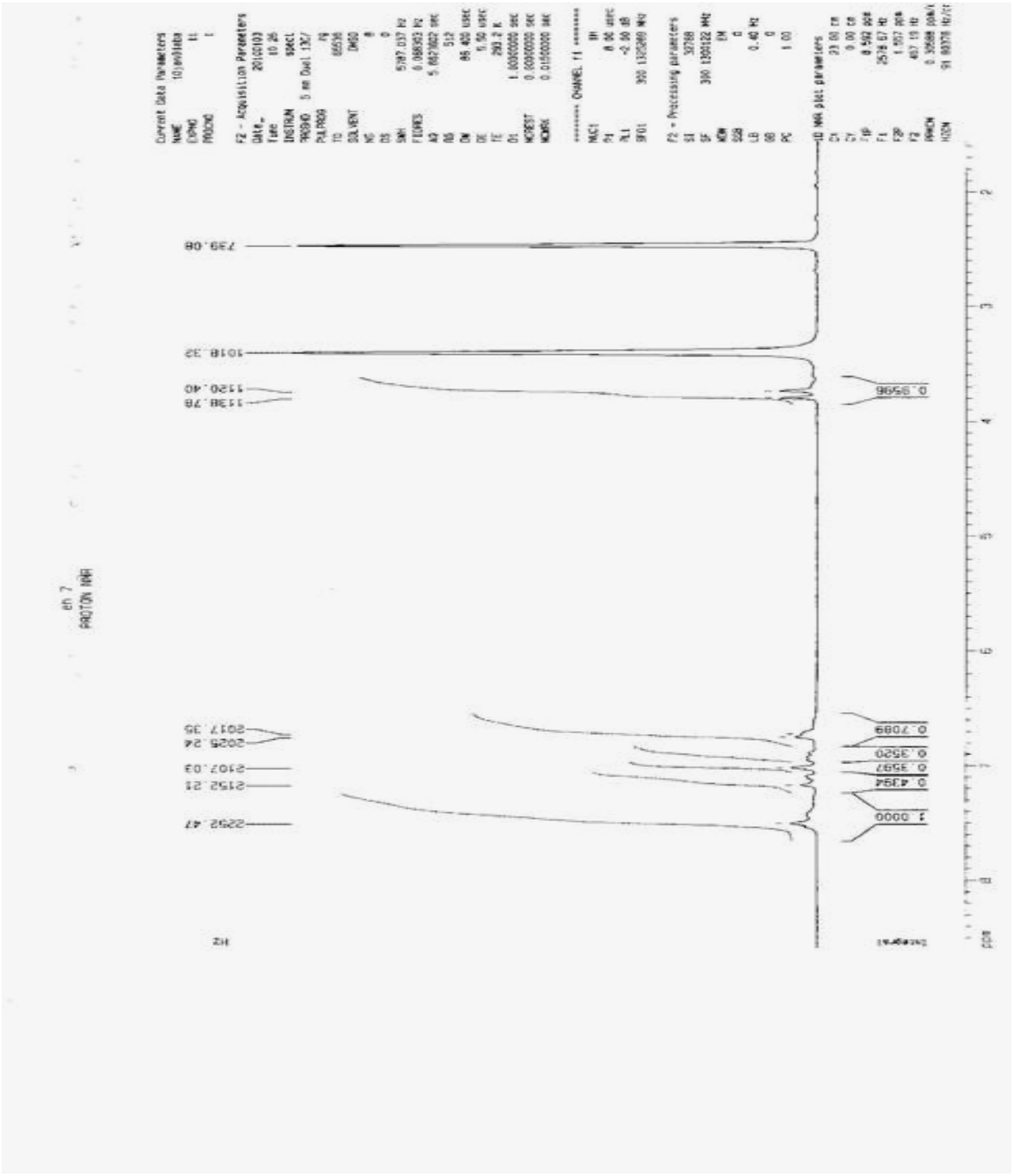

Fig 3.2 : ^{1}H NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)- 2-methoxyphenol (**1**)

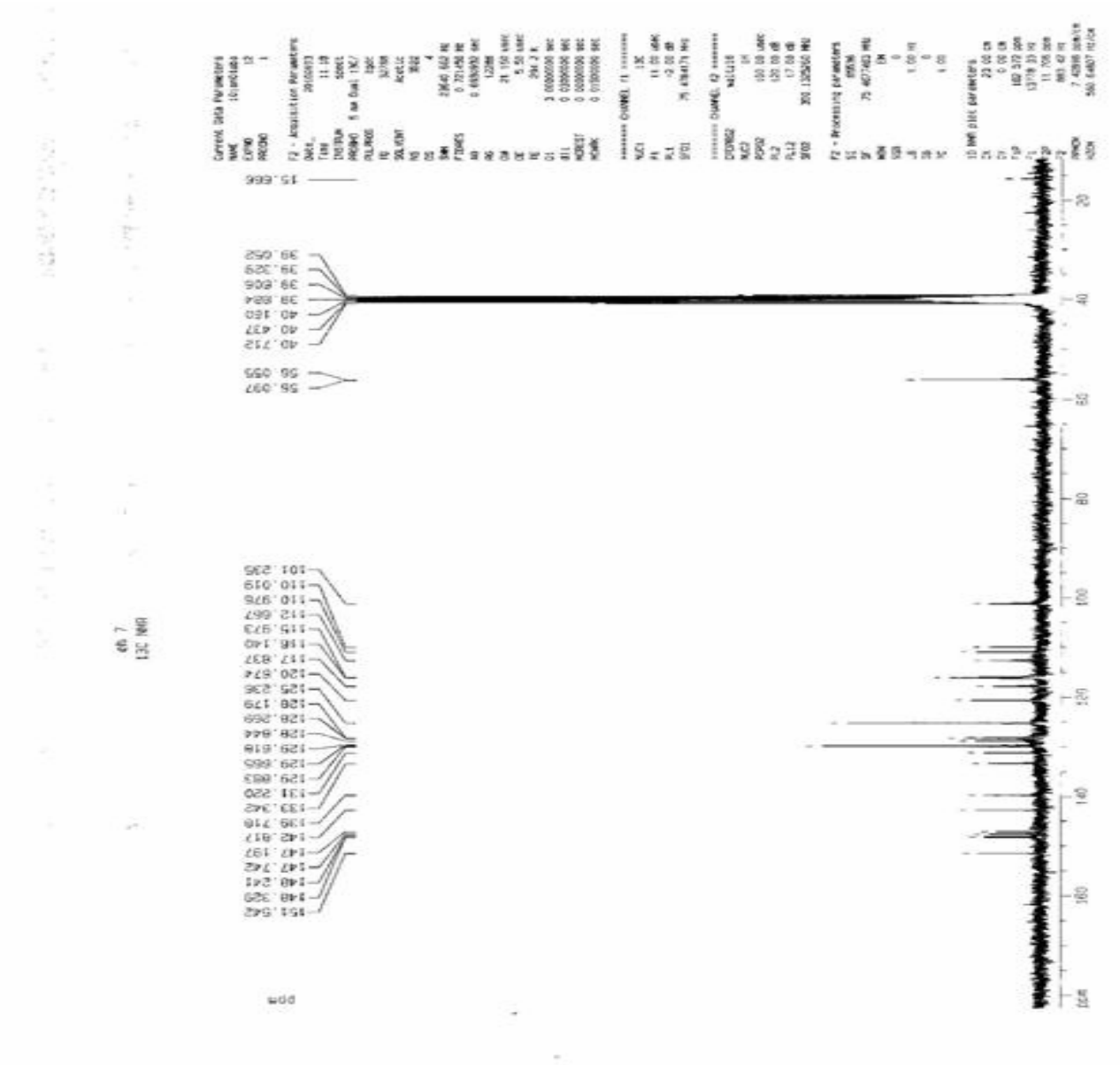

Fig 3.3(a) : ^{13}C NMR of 4-((1E)-2-(5-(4-hydroxy-3-ethoxystyryl)- 1-phenyl-1H-pyrazol-3-yl)vinyl)- 2-methoxyphenol (**1**)

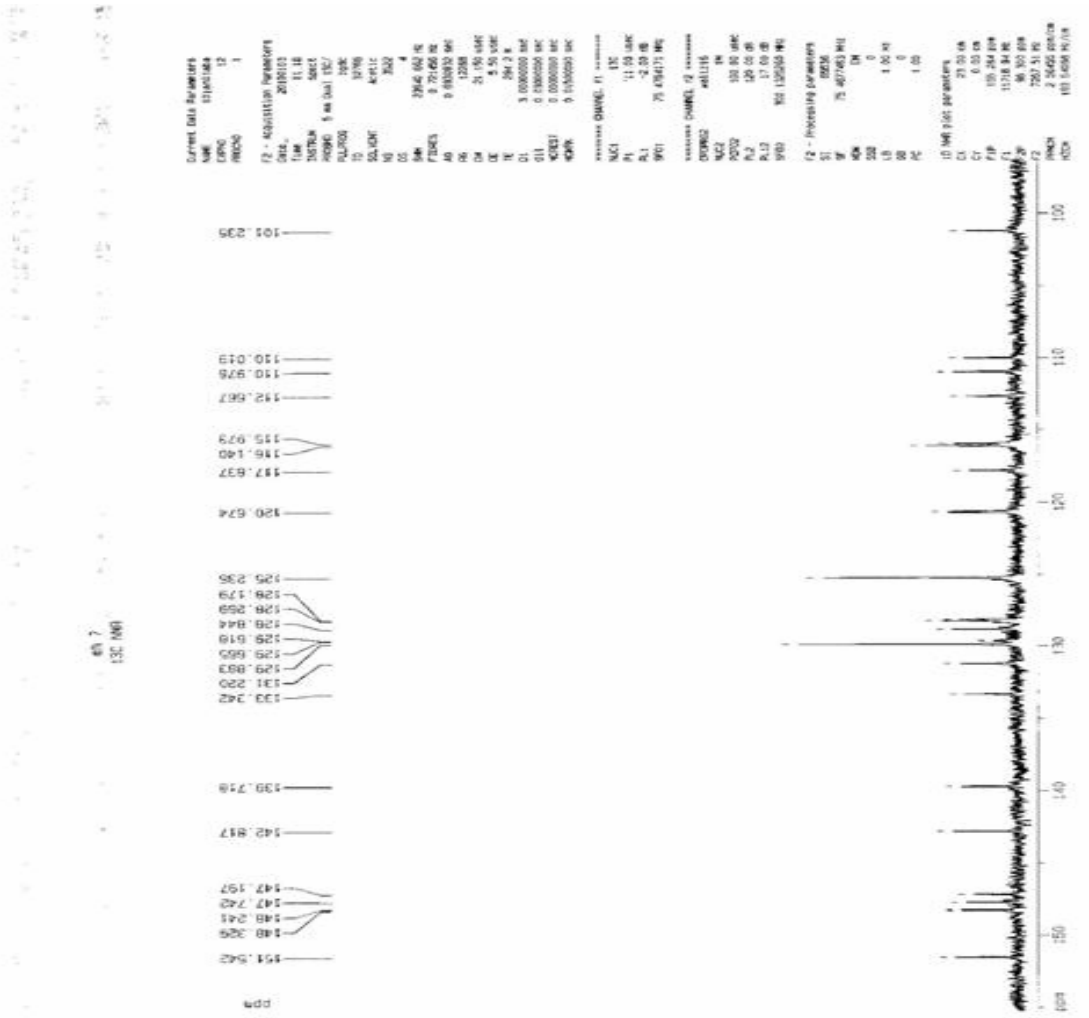

Fig 3.3(b) : ^{13}C NMR of 4-((1E)-2-(5-(4-hydroxy-3- ethoxystyryl)-1-phenyl-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**1**)

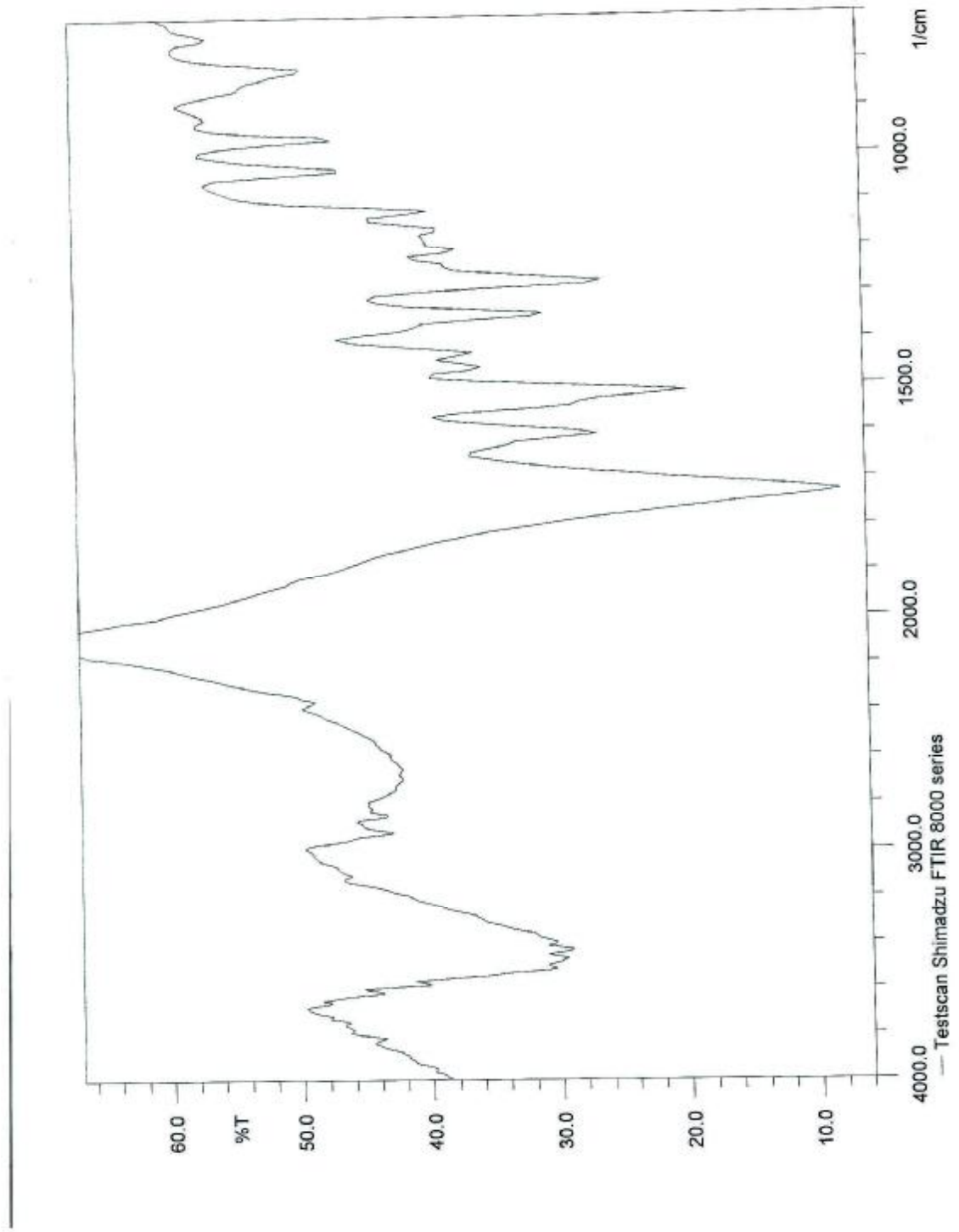

Fig 3.4 : FT-IR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H- pyrazol-3-yl)vinyl)-2-methoxyphenol (**2**)

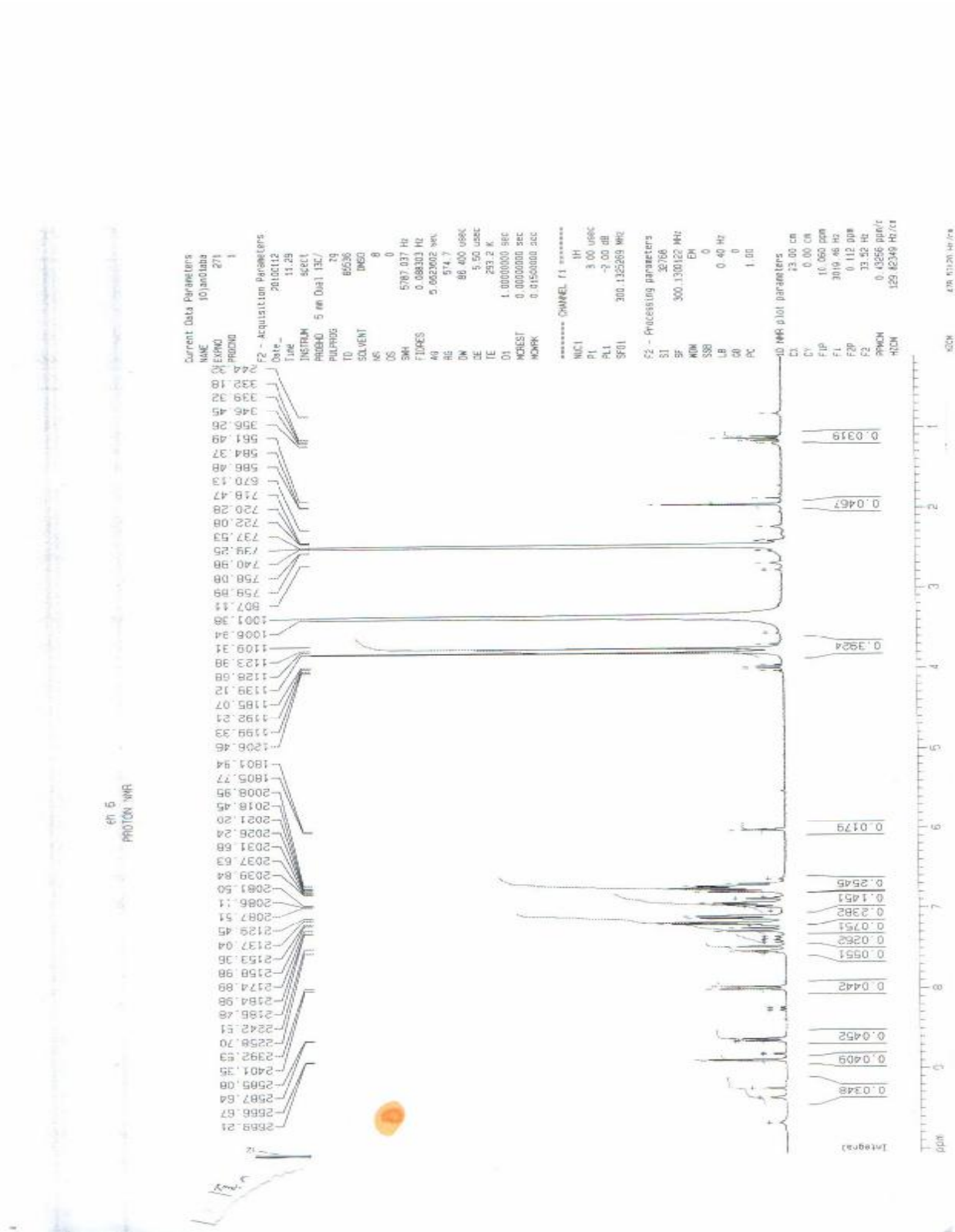

Fig 3.5 :1H NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**2**)

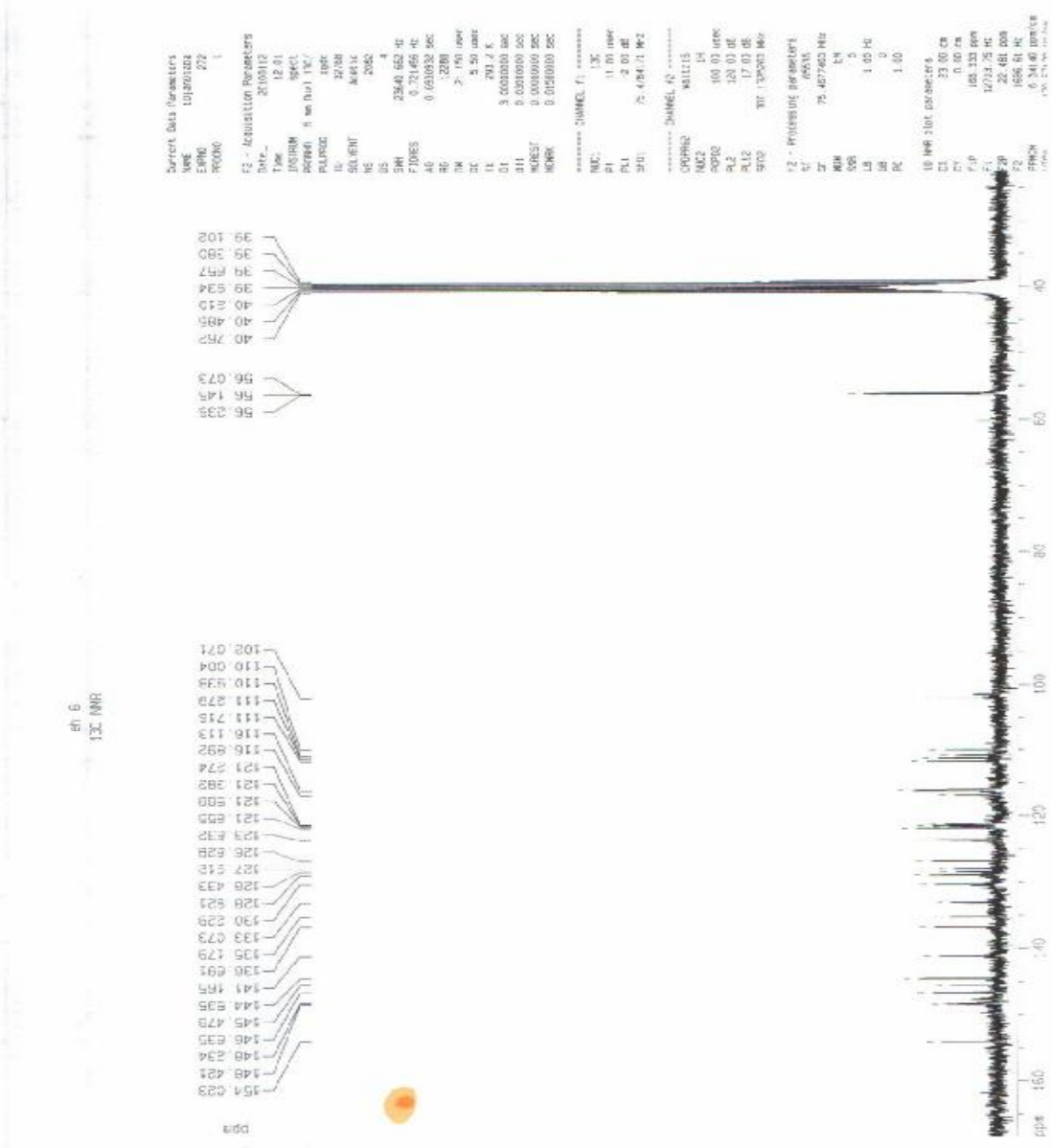

Fig 3.6 : ^{13}C NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(2,4-dinitrophenyl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**2**)

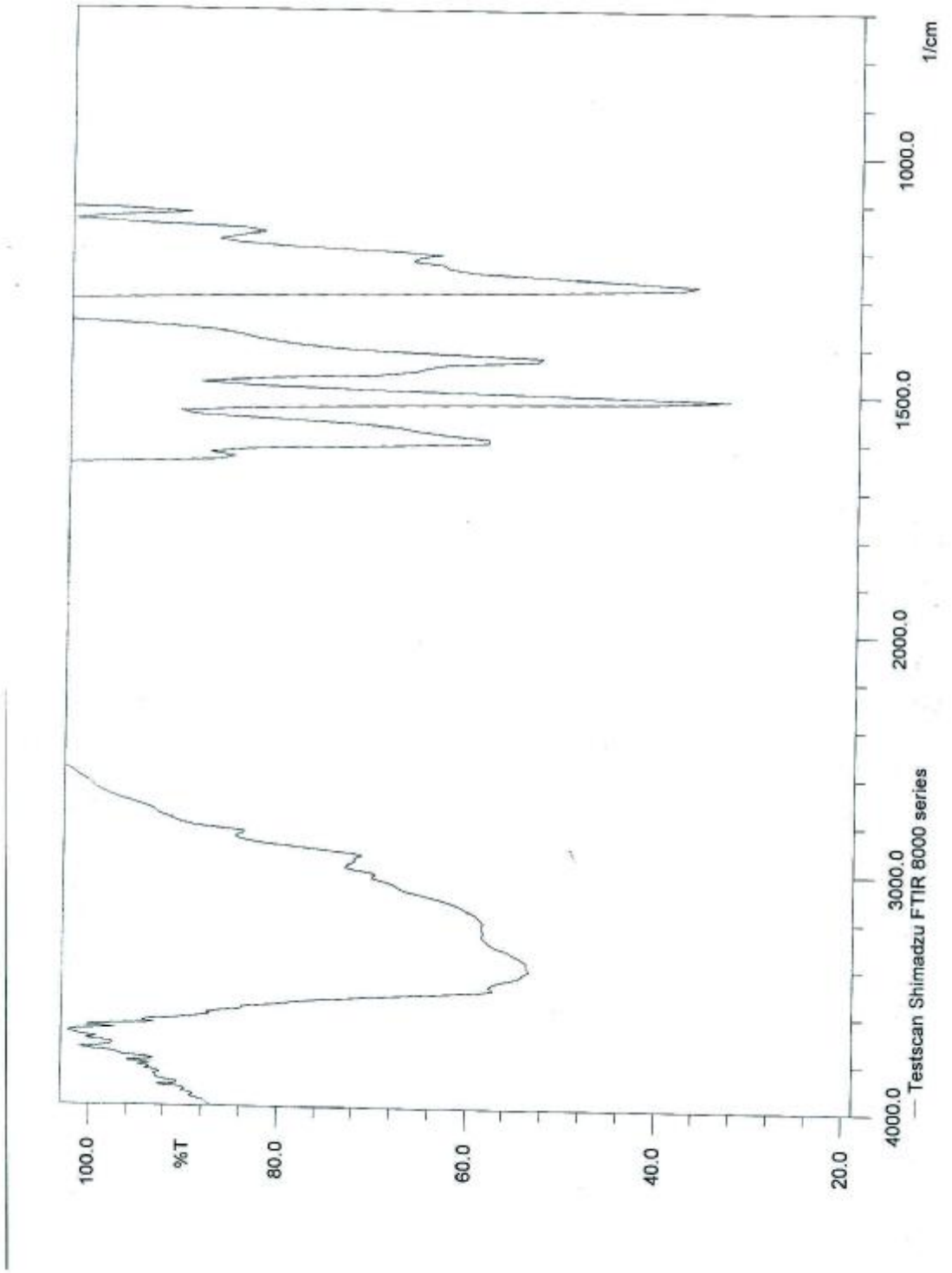

Fig 3.7 : FT-IR of 4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol (**3**)

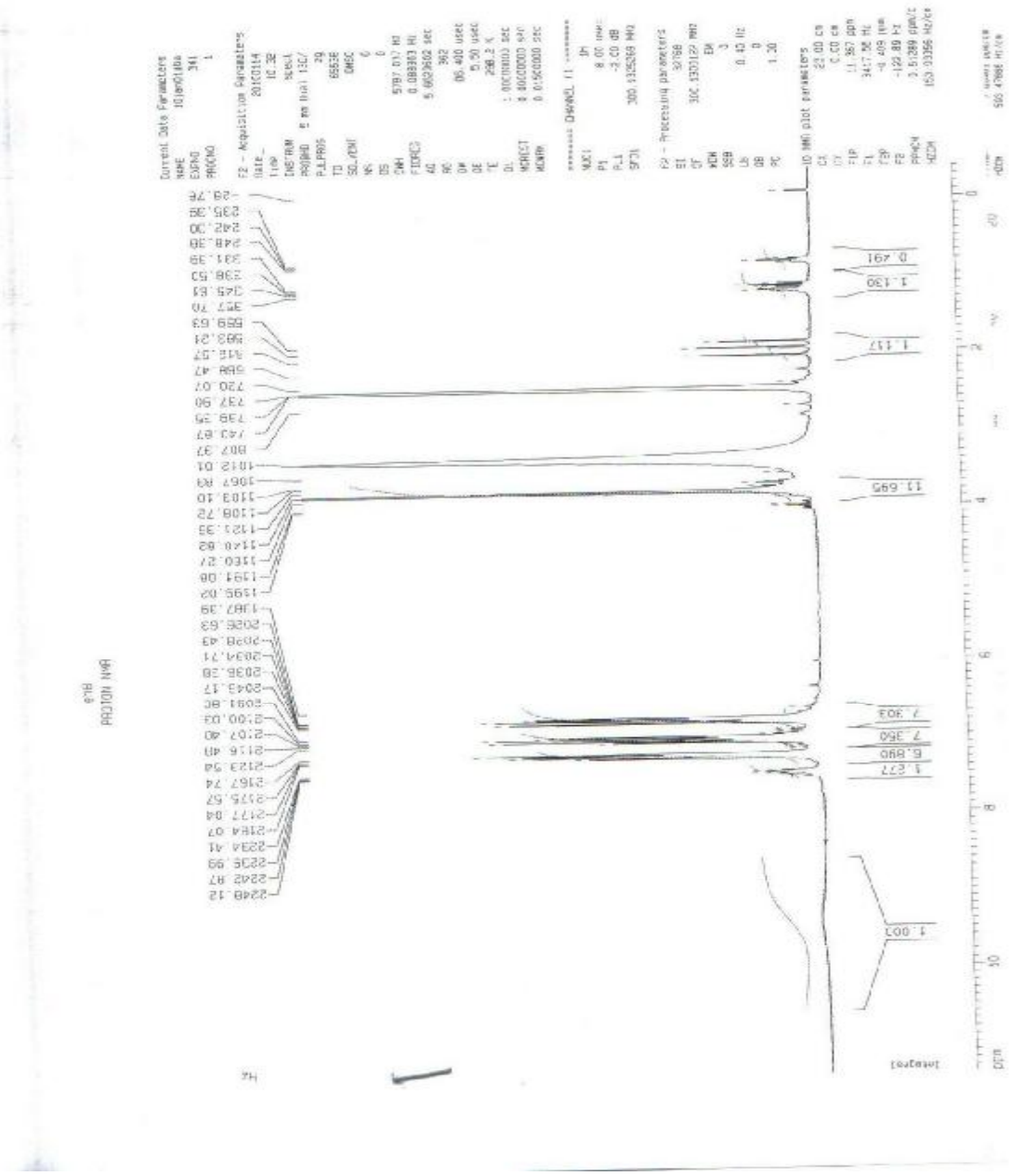

Fig 3.8 : ^{1}H NMR of 4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol (3)

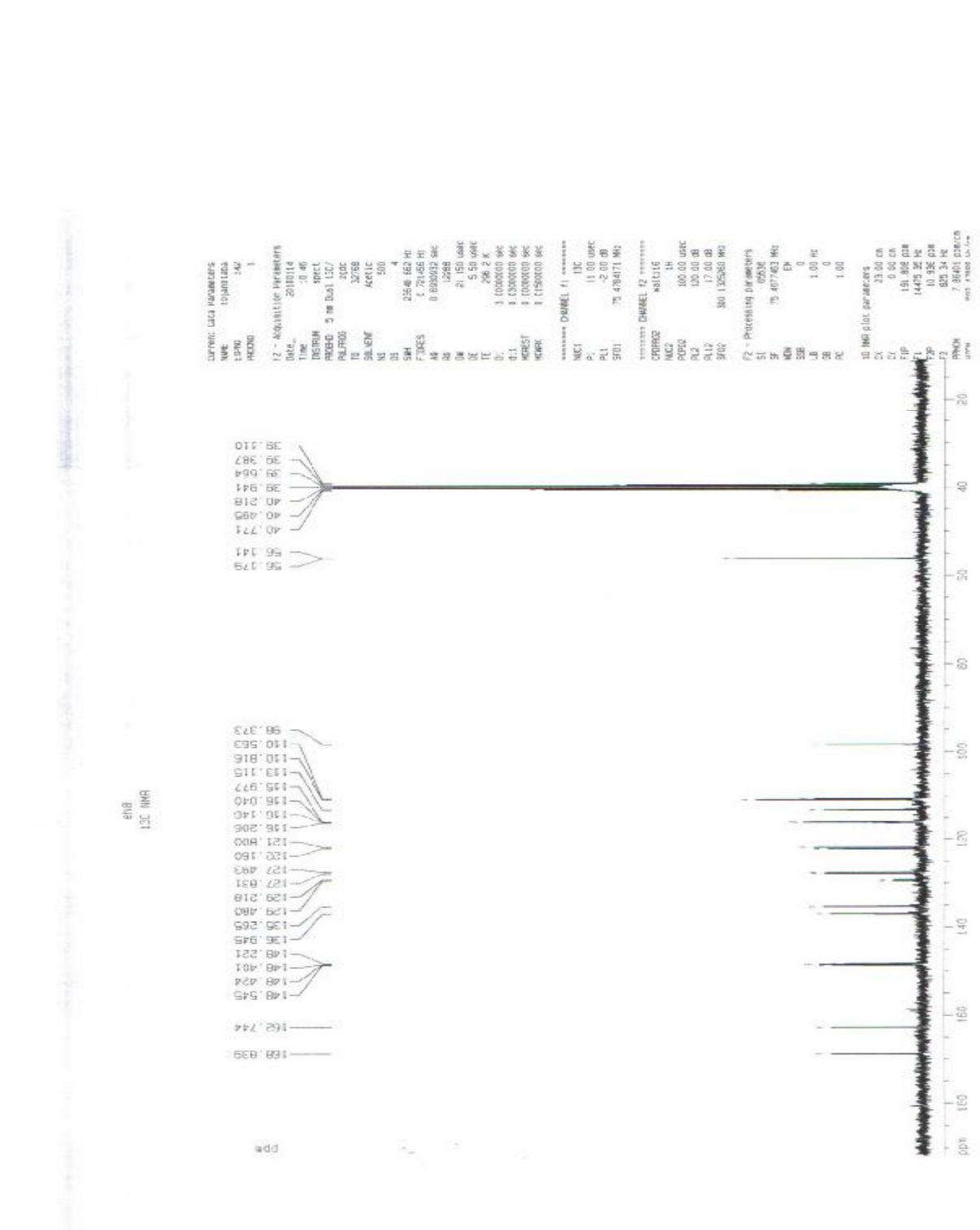

Fig 3.9 : ^{13}C NMR of 4-((1*E*)-2-(3-(4-hydroxy-3-methoxstyryl)-4,5-dihydroxyisoxazole-5-yl)vinyl)-2-methoxyphenol (**3**)

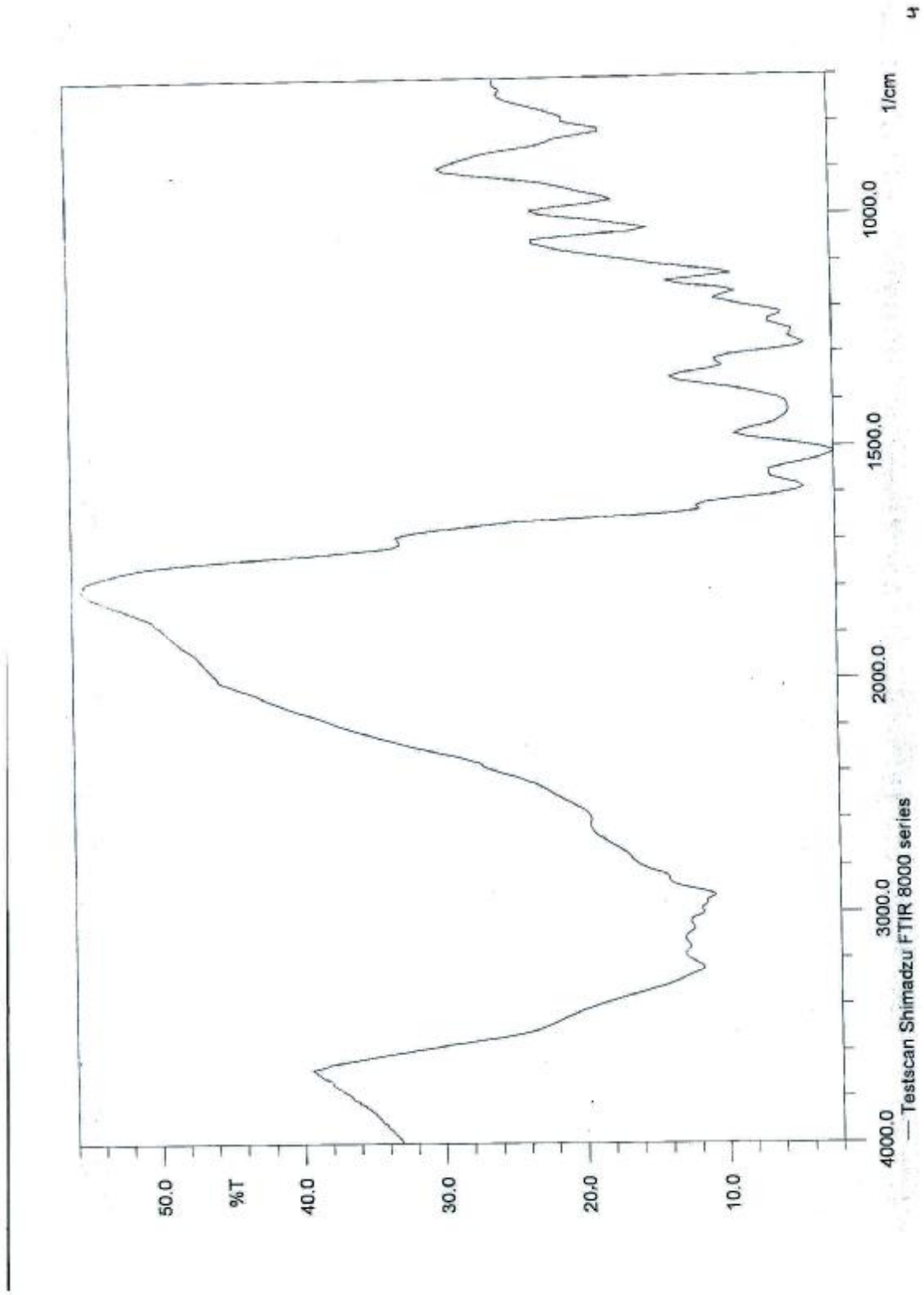

Fig 3.10 : FT-IR of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)

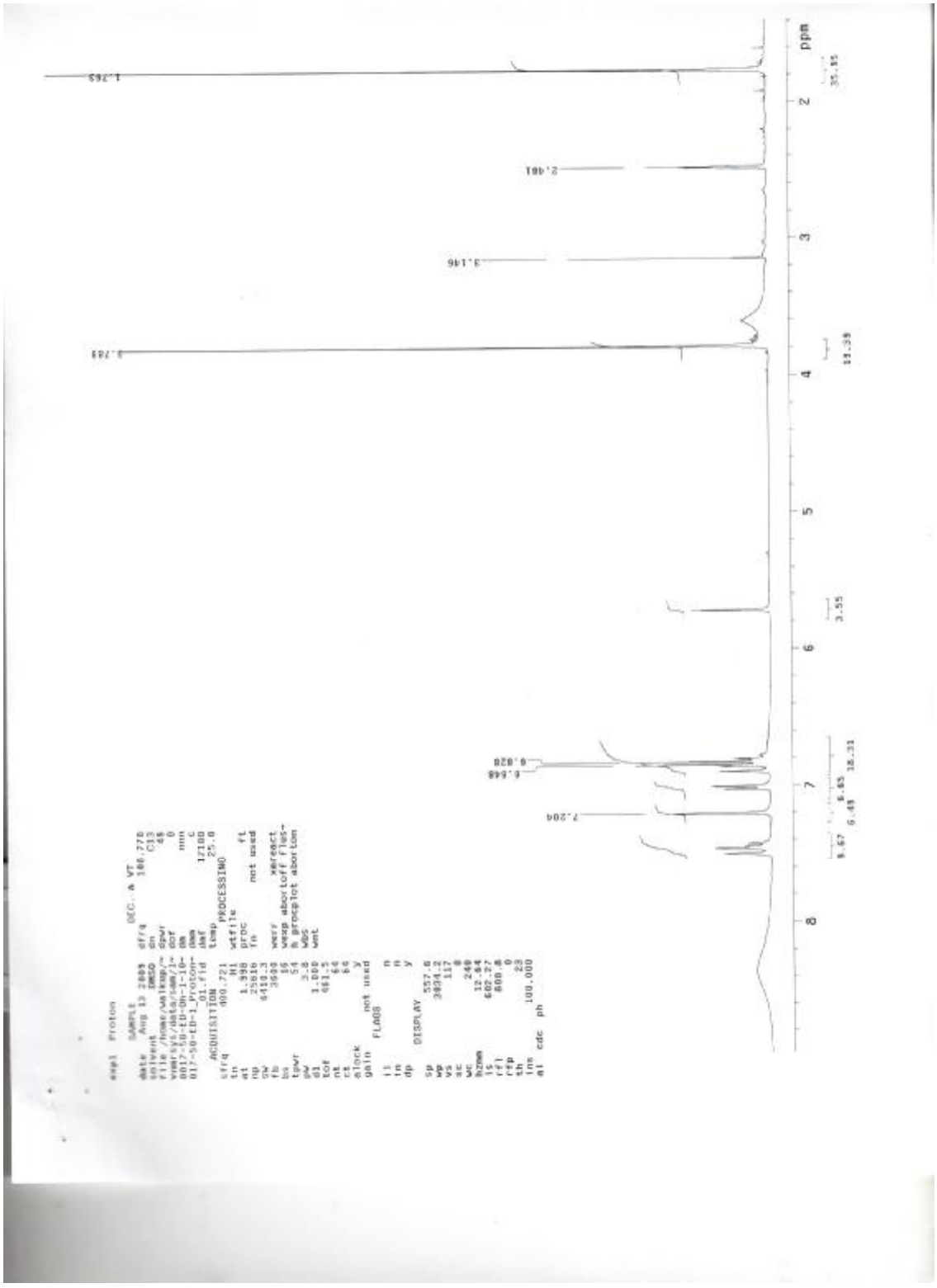

Fig 3.11 (a) : ^{1}H NMR of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)

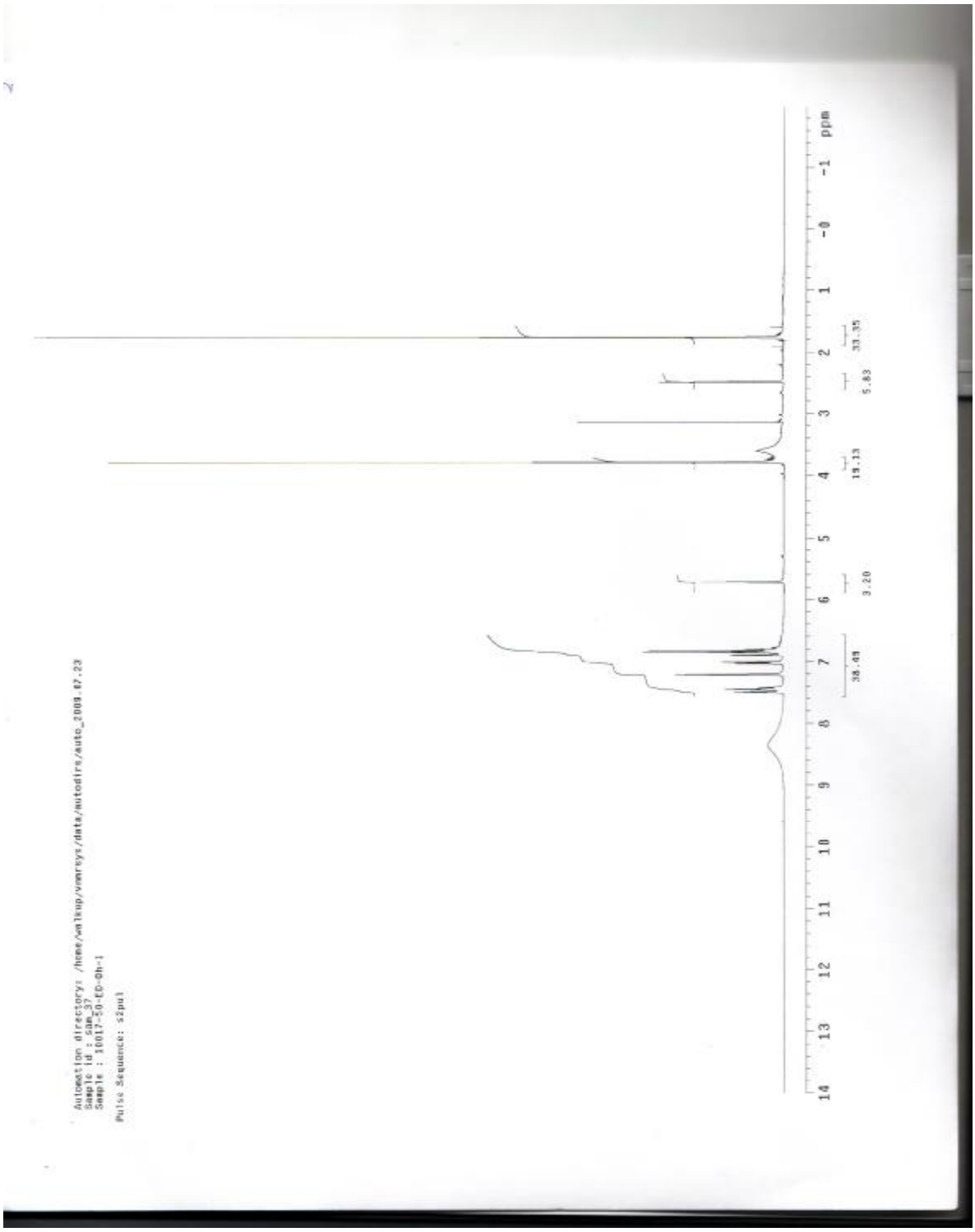

Fig 3.11 (b) : ^{1}H NMR of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)

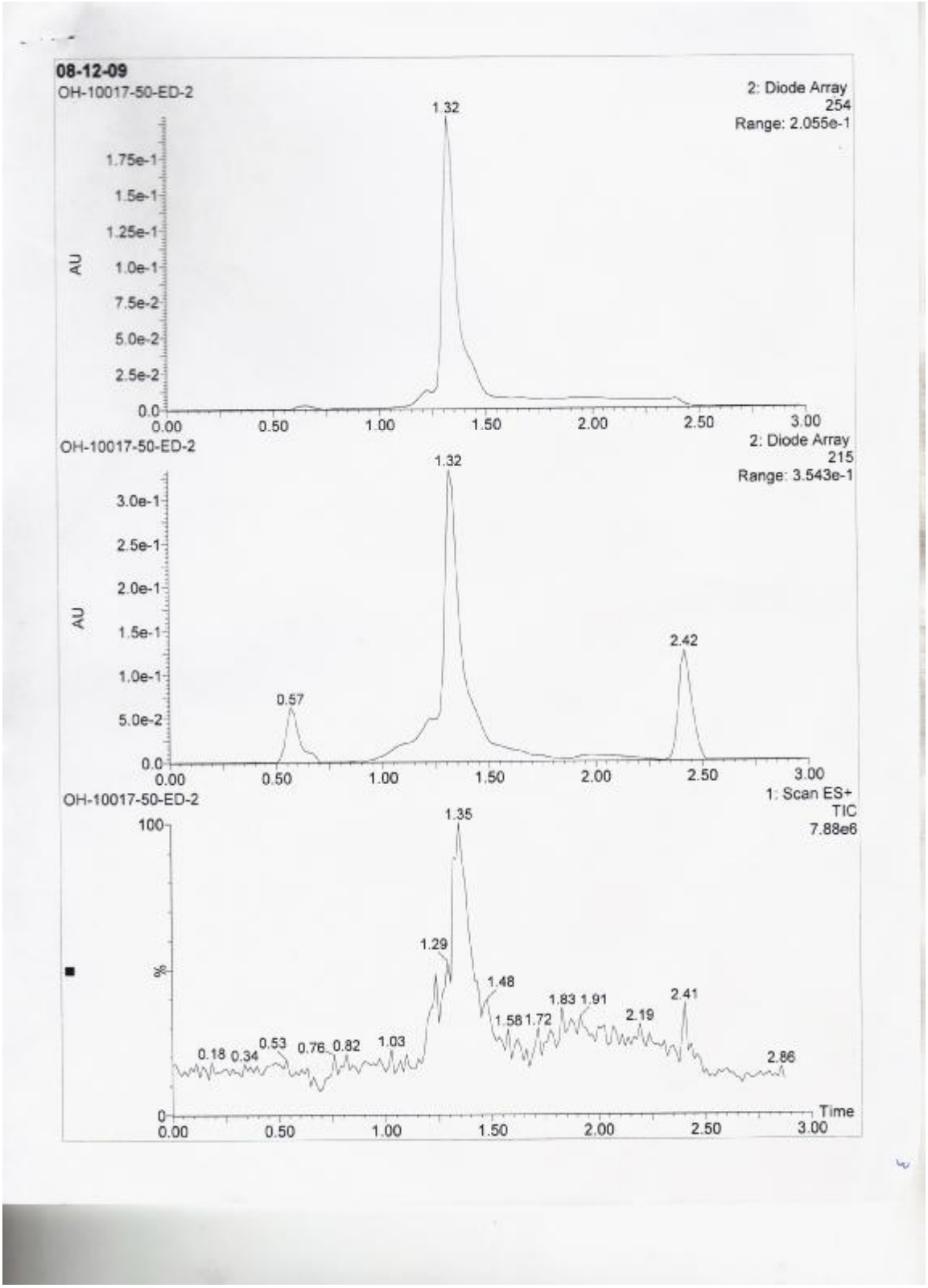

Fig 3.12 : LC of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)

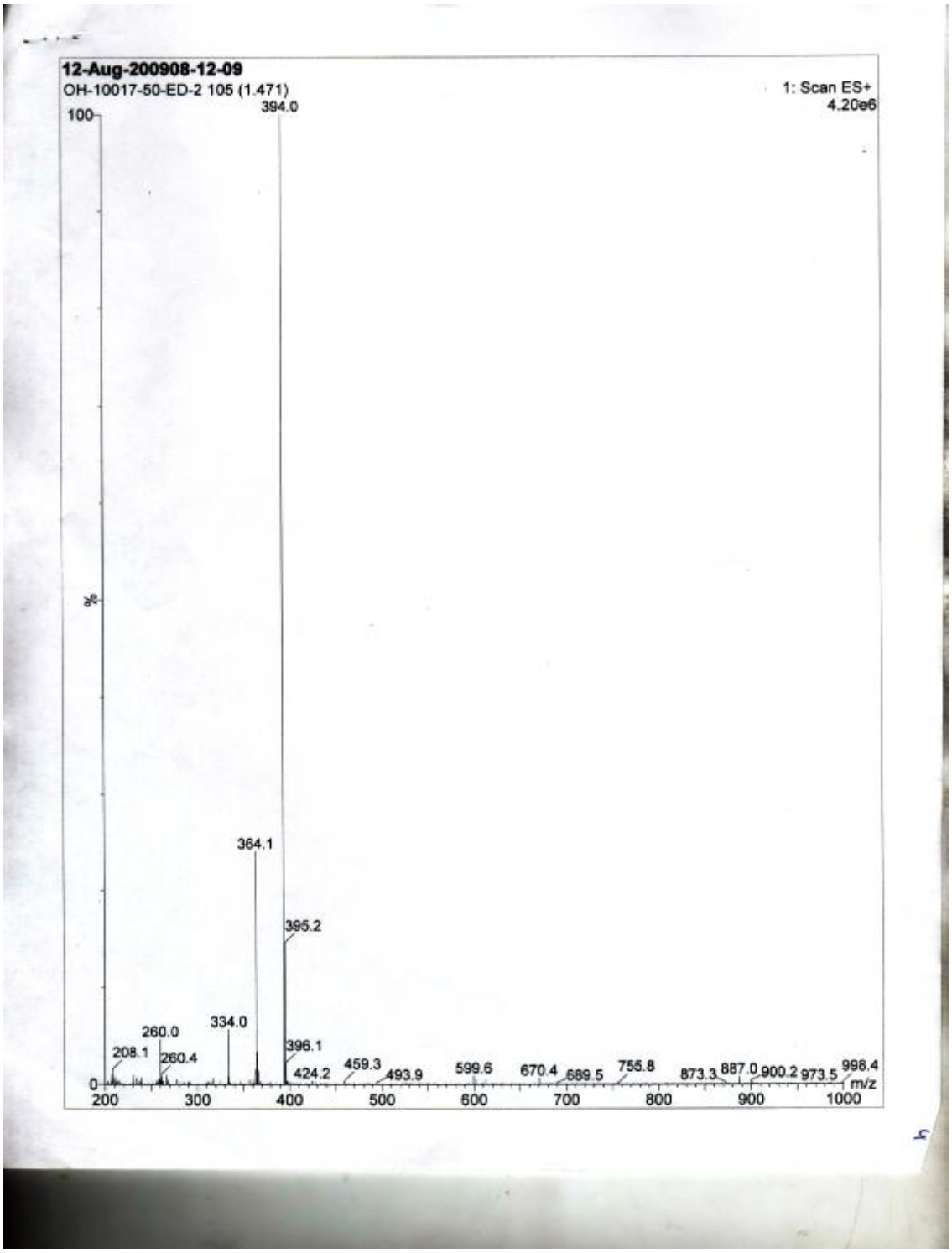

Fig 3.13 : Mass Spectra of 4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)

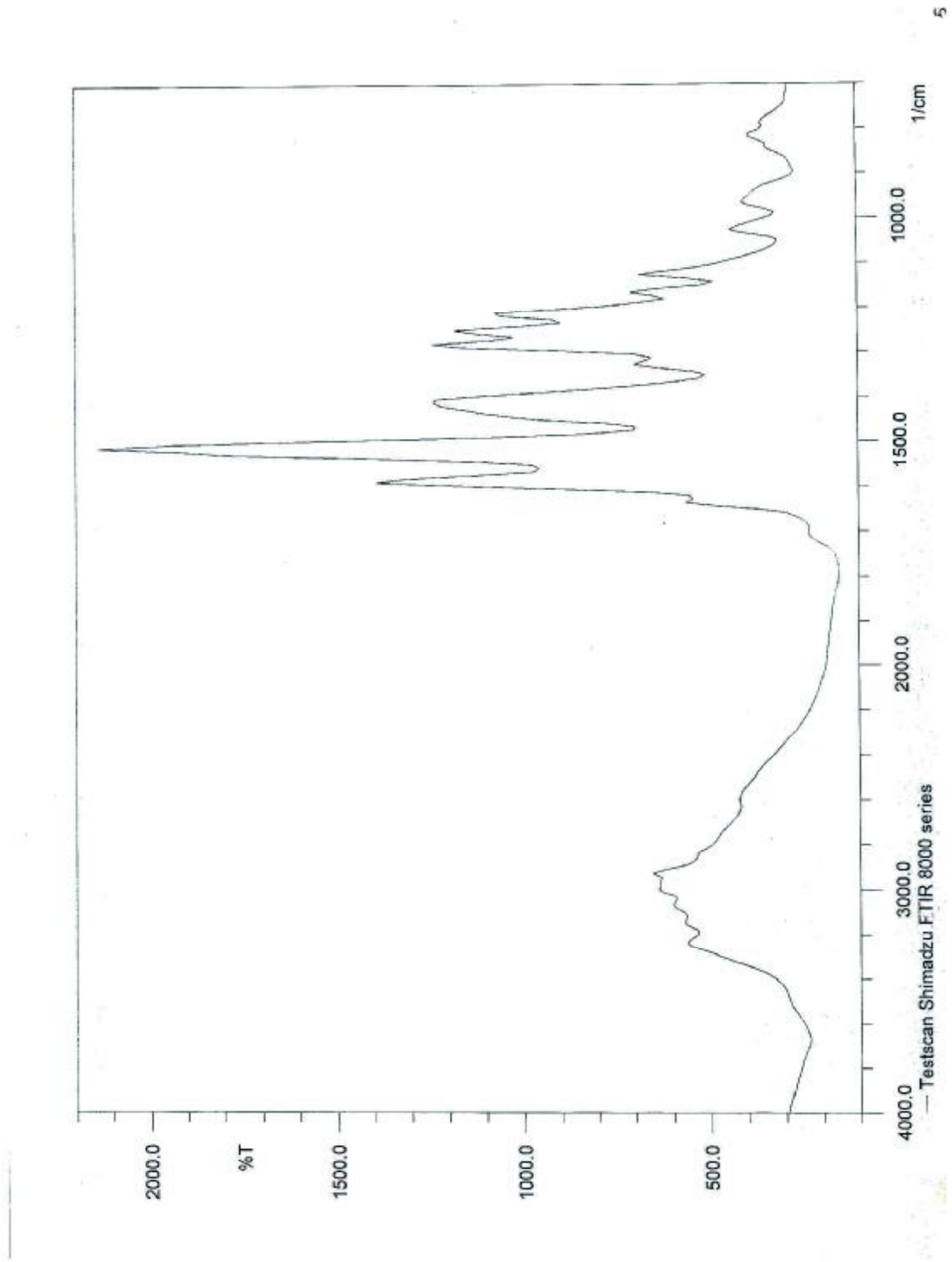

Fig 3.14: FT-IR of 3,5-bis(4-hydroxy-3-methoxystyryl)pyrazolidine-1-carbohydrazide (**5**)

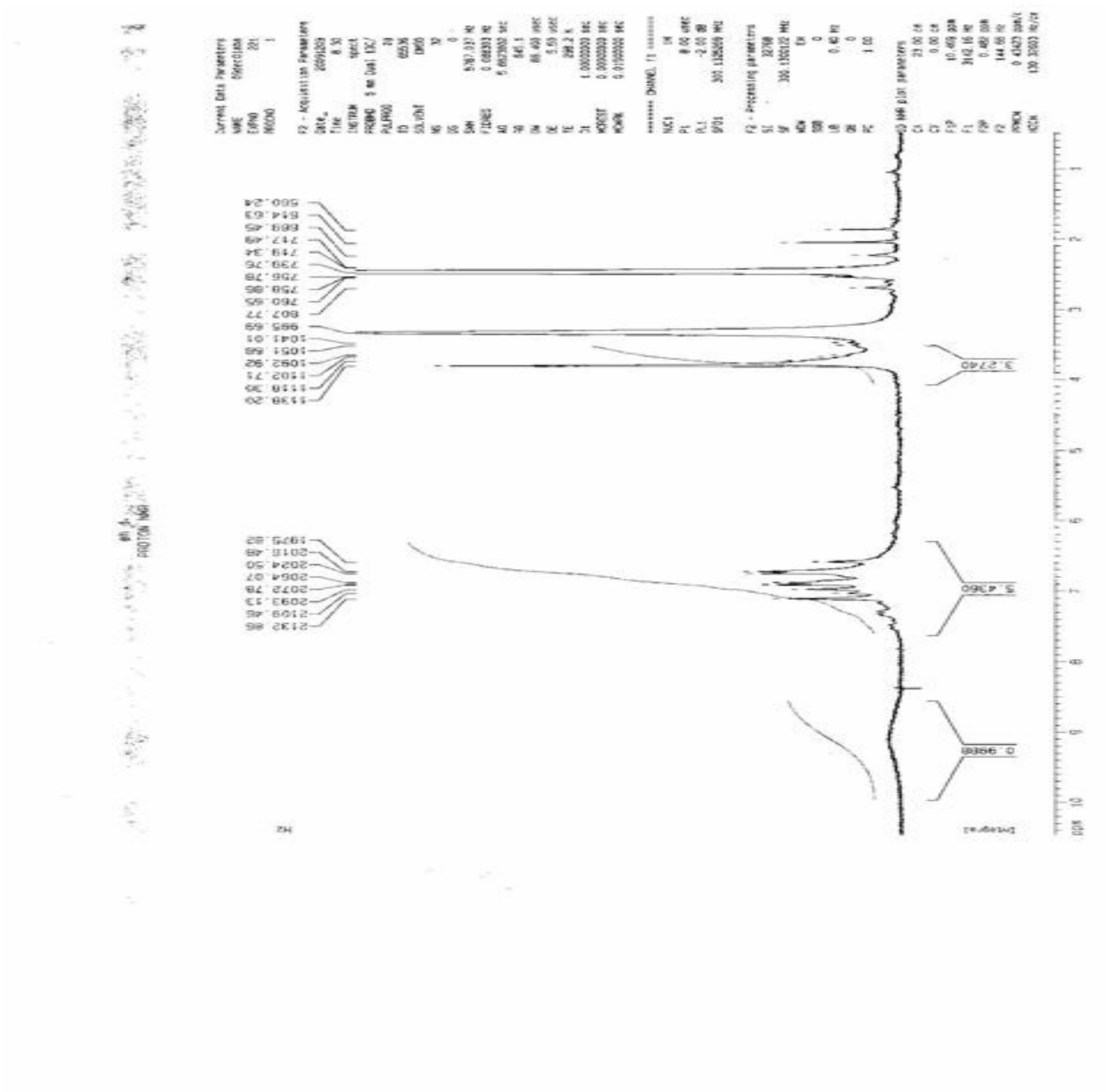

Fig 3.15: ^{1}H NMR of 3,5-bis(4-hydroxy-3- methoxystyryl) pyrazolidine- 1-carbohydrazide (**5**)

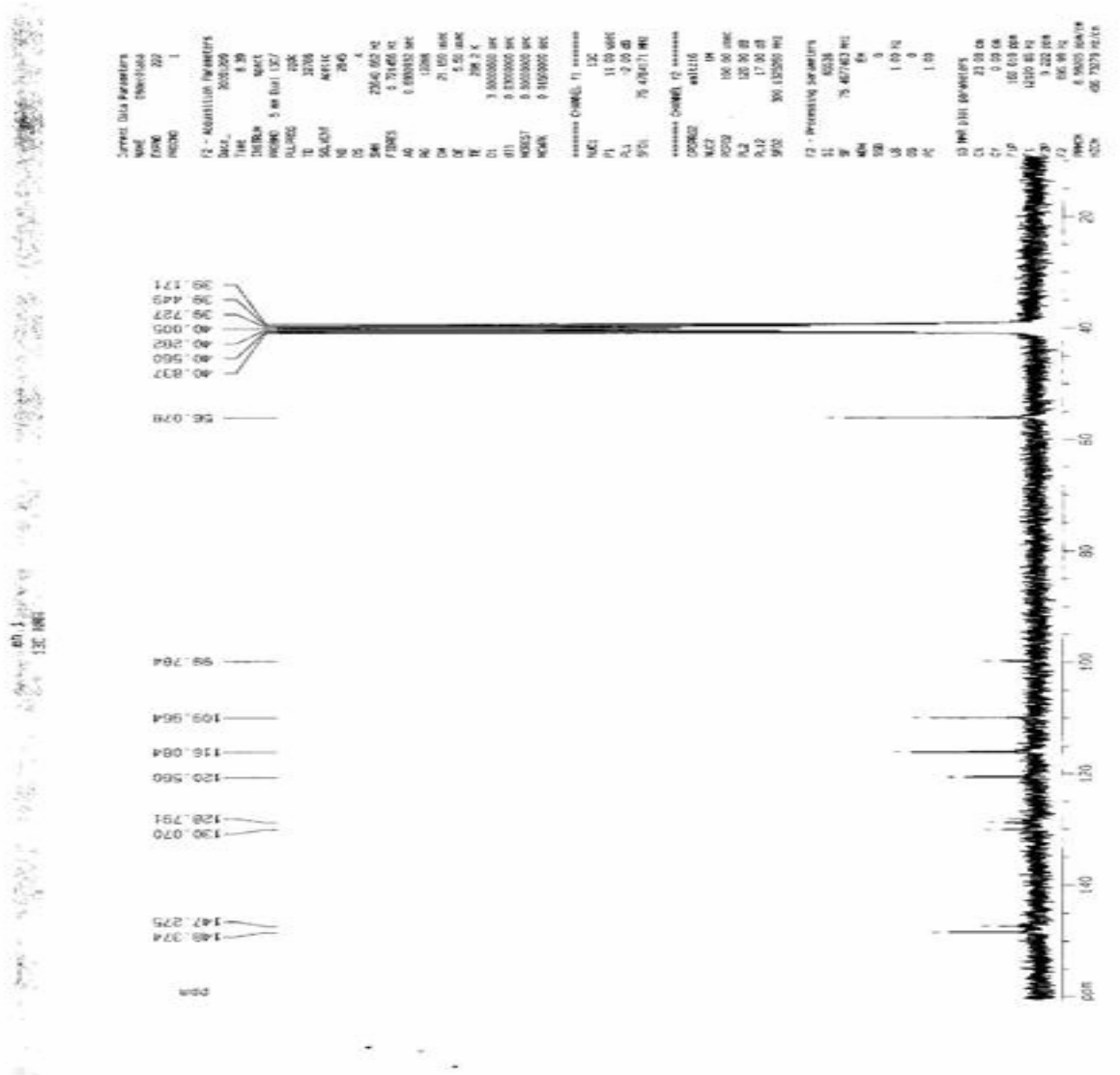

Fig 3.16 : ^{13}C NMR of 3,5-bis(4-hydroxy-3-methoxystyryl) pyrazolidine-1-carbohydrazide (**5**)

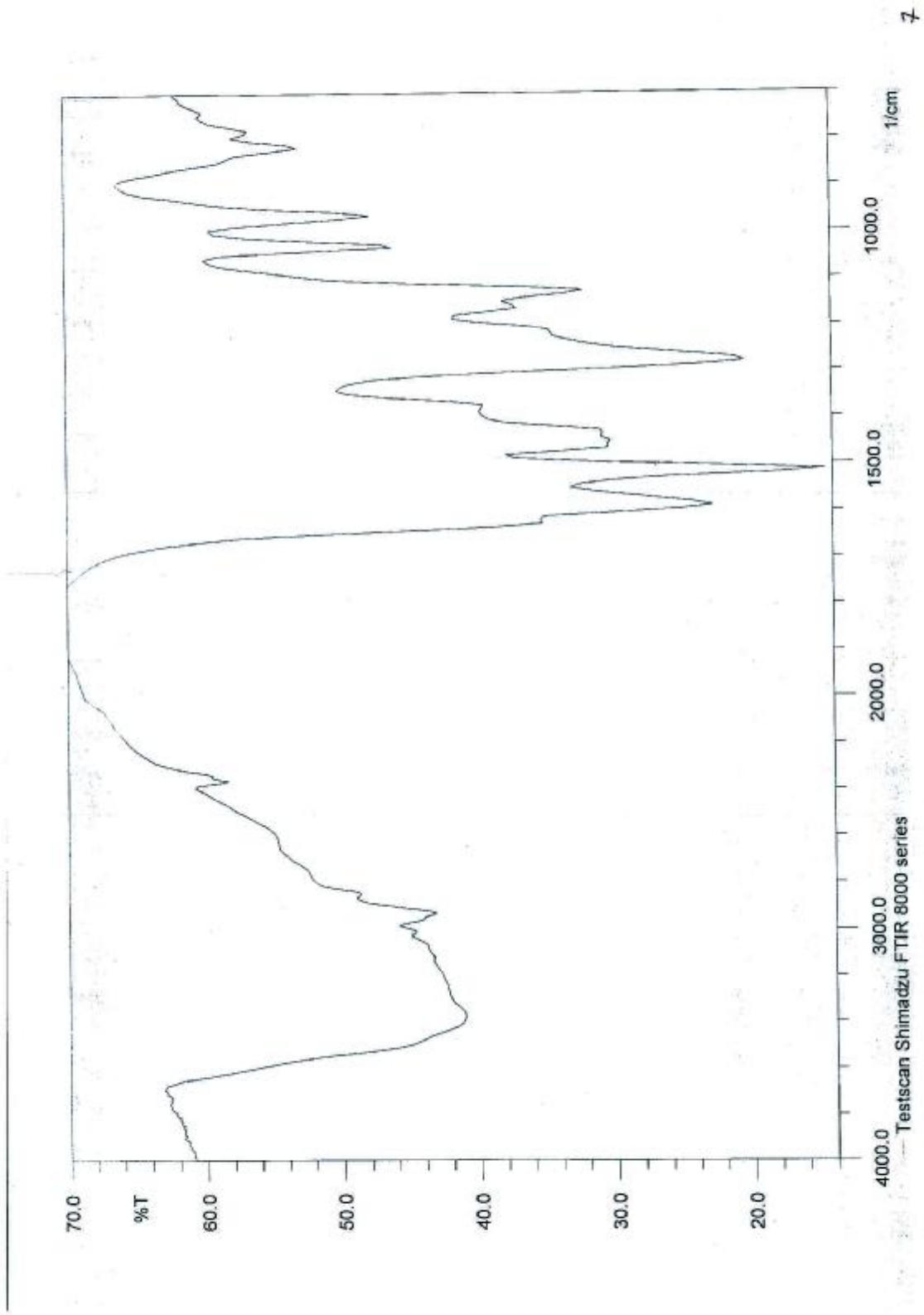

Fig 3.17 : FT-IR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (6)

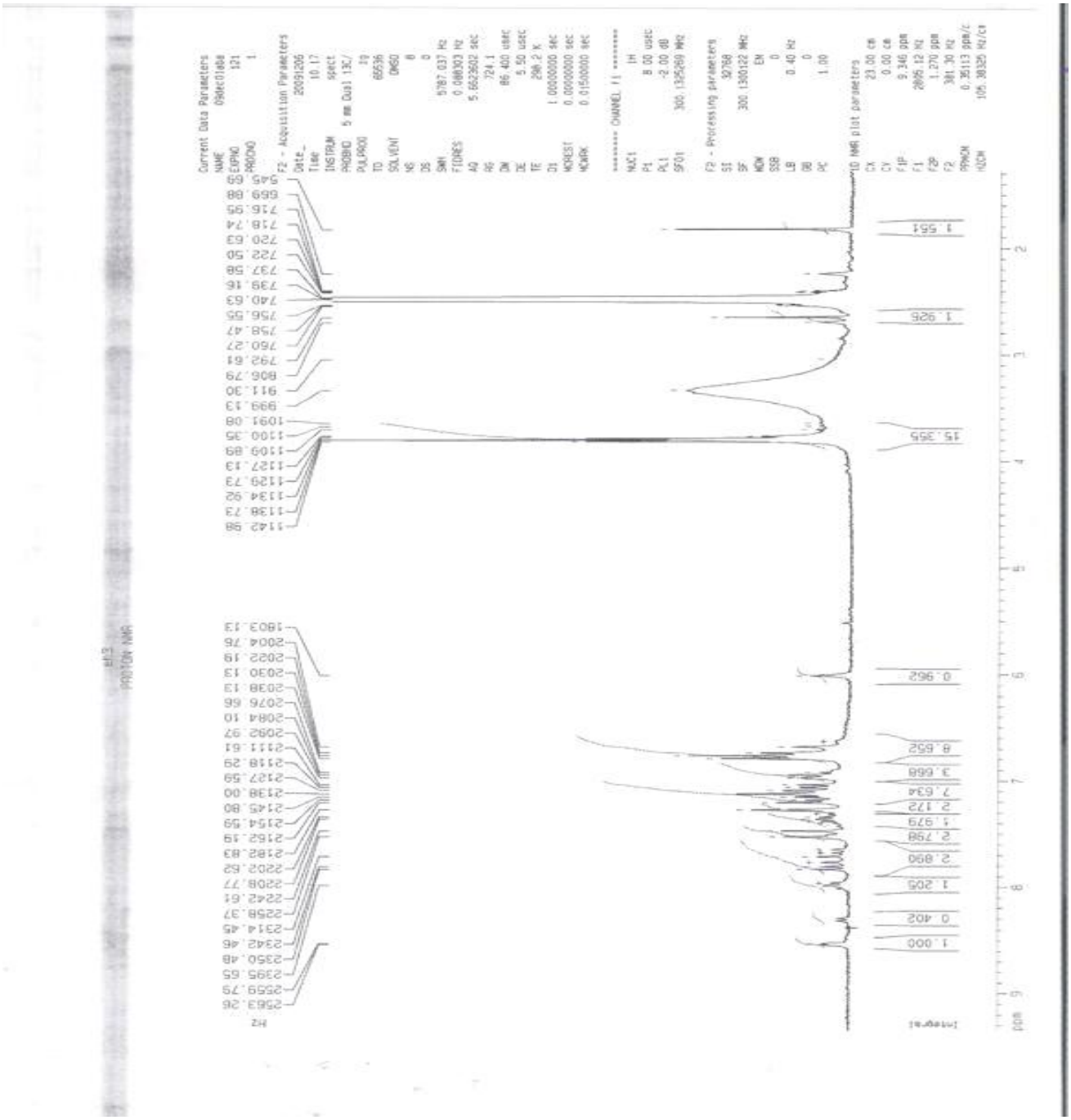

Fig 3.18(a) : ^{1}H NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol- 3-yl)vinyl)-2-methoxyphenol (**6**)

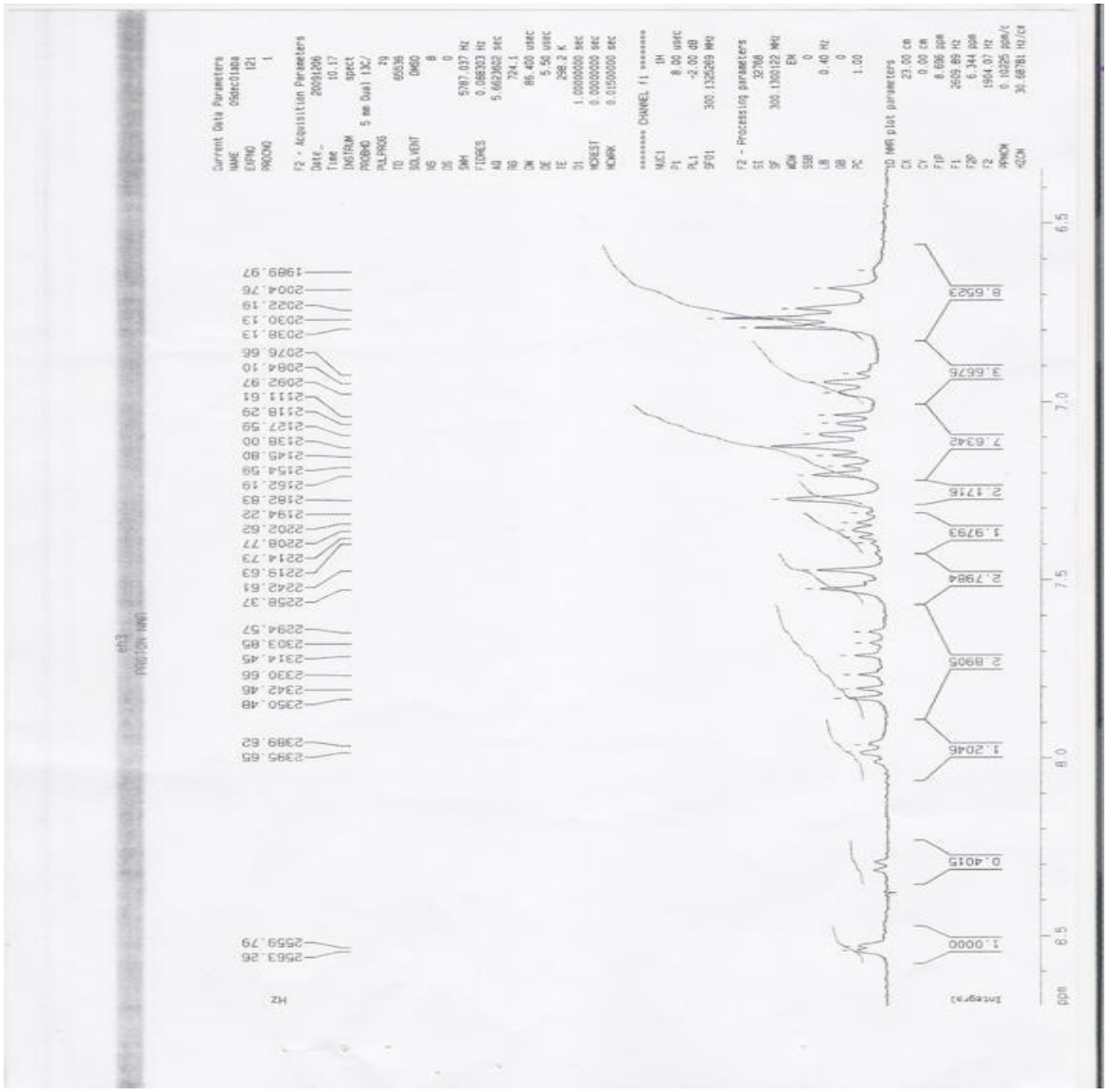

Fig 3.18(b): ^{1}H NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**6**)

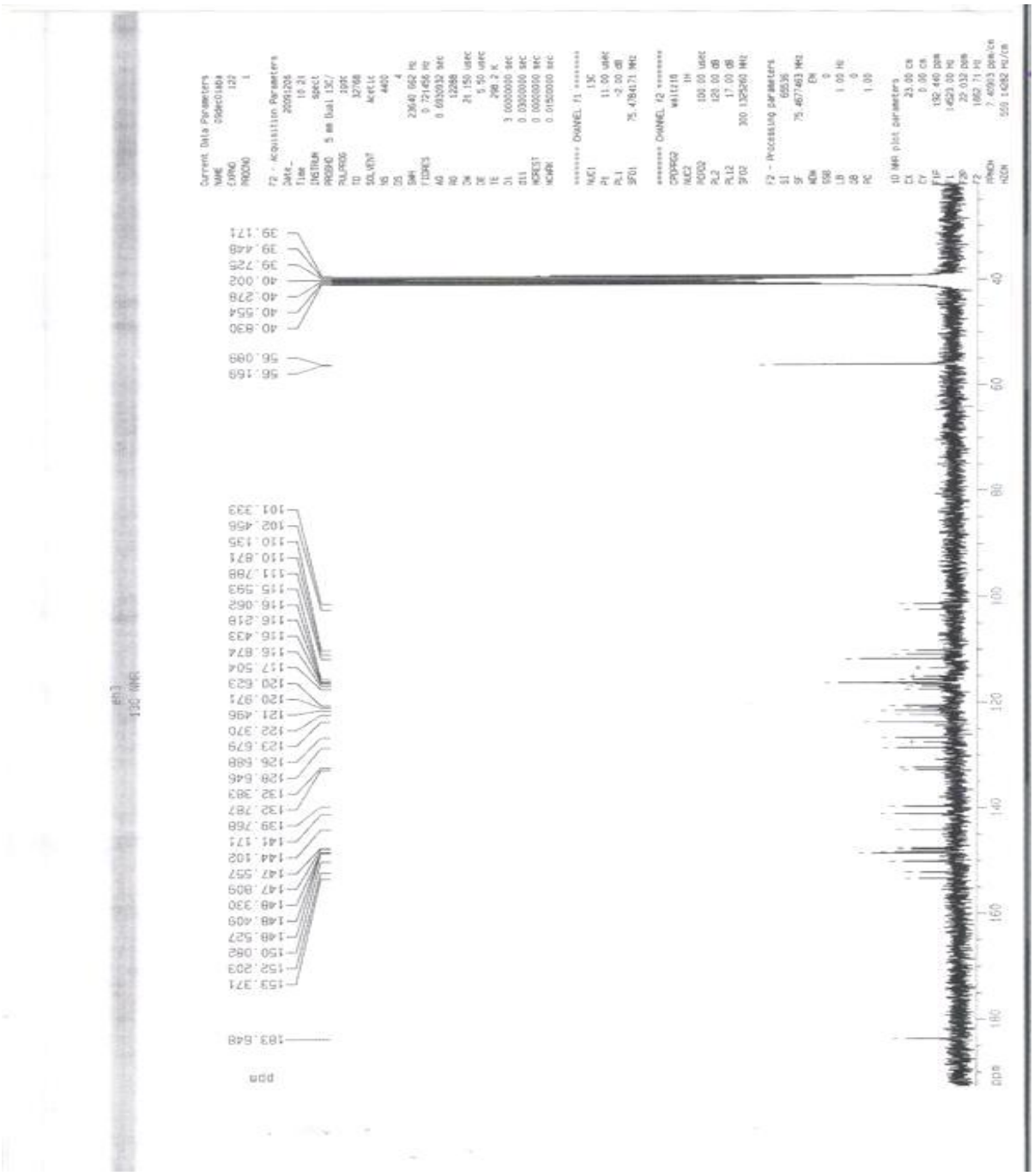

Fig 3.19(a) : ^{13}C NMR of **4**-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**6**)

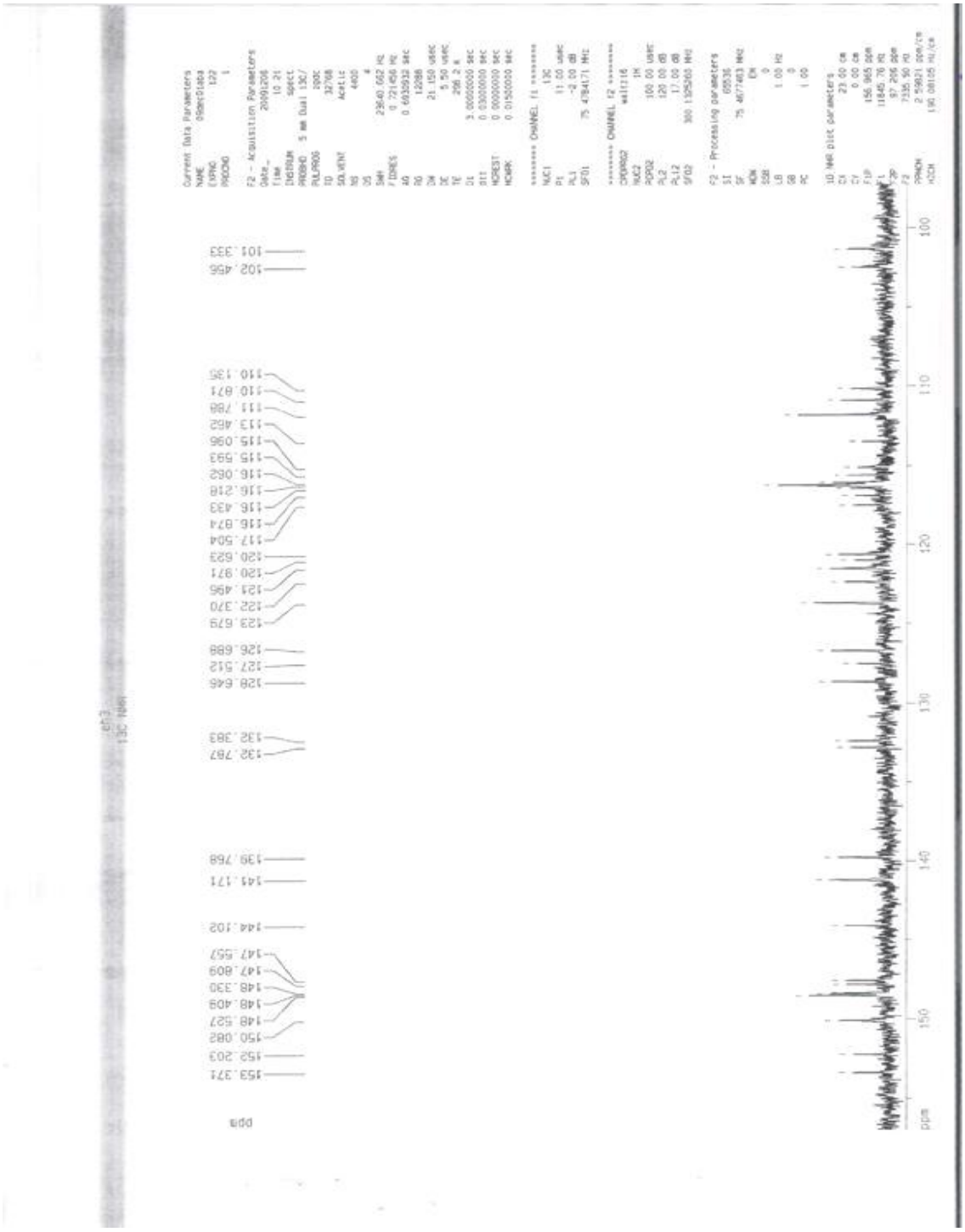

Fig 3.19(b): ^{13}C NMR of 4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl)-1-(pyridin-2-yl)-1H-pyrazol-3-yl)vinyl)-2-methoxyphenol (**6**)

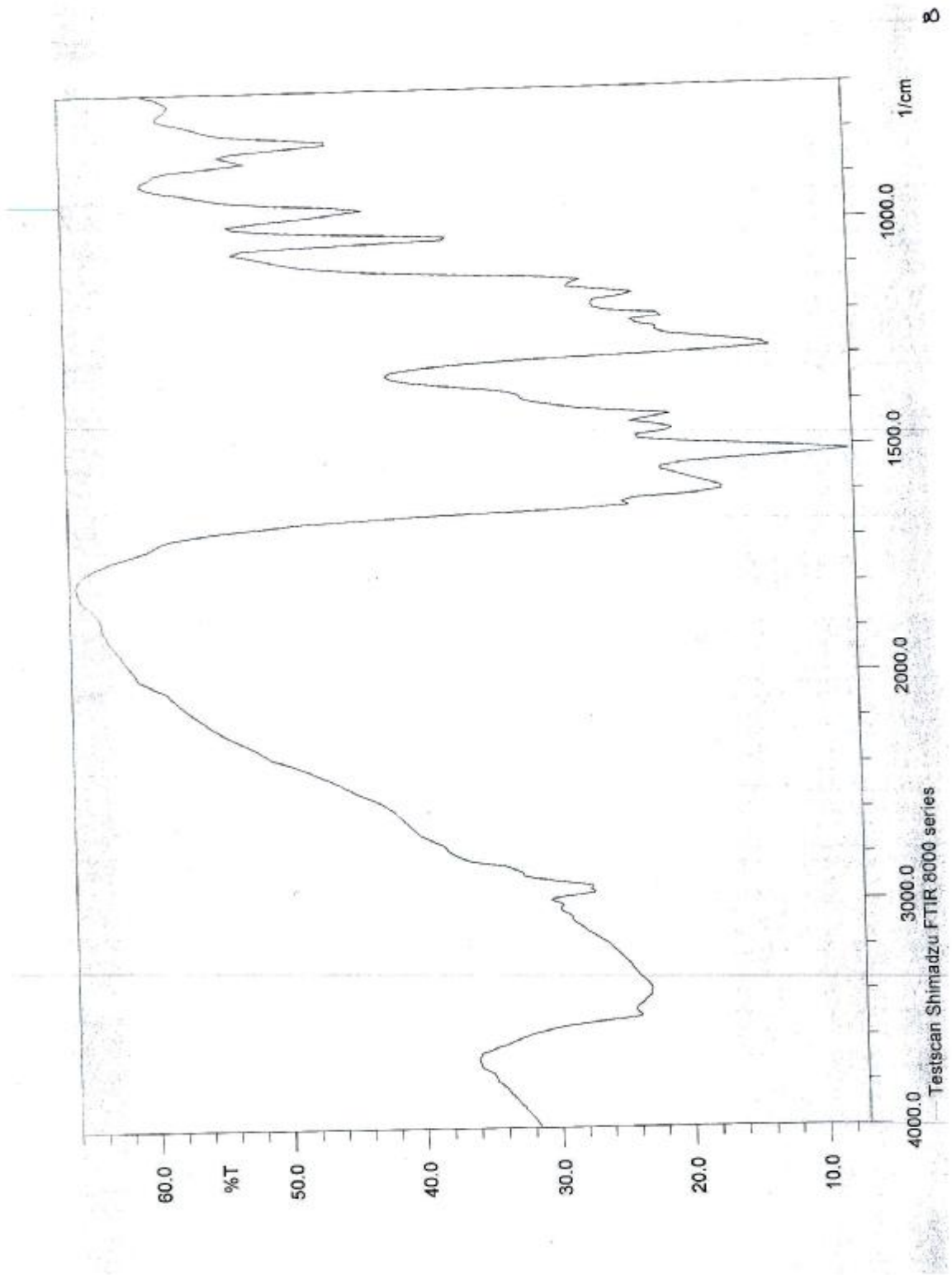

Fig 3.20 : FT-IR of 4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol-5-yl)vinyl)-2-methoxyphenol (**7**)

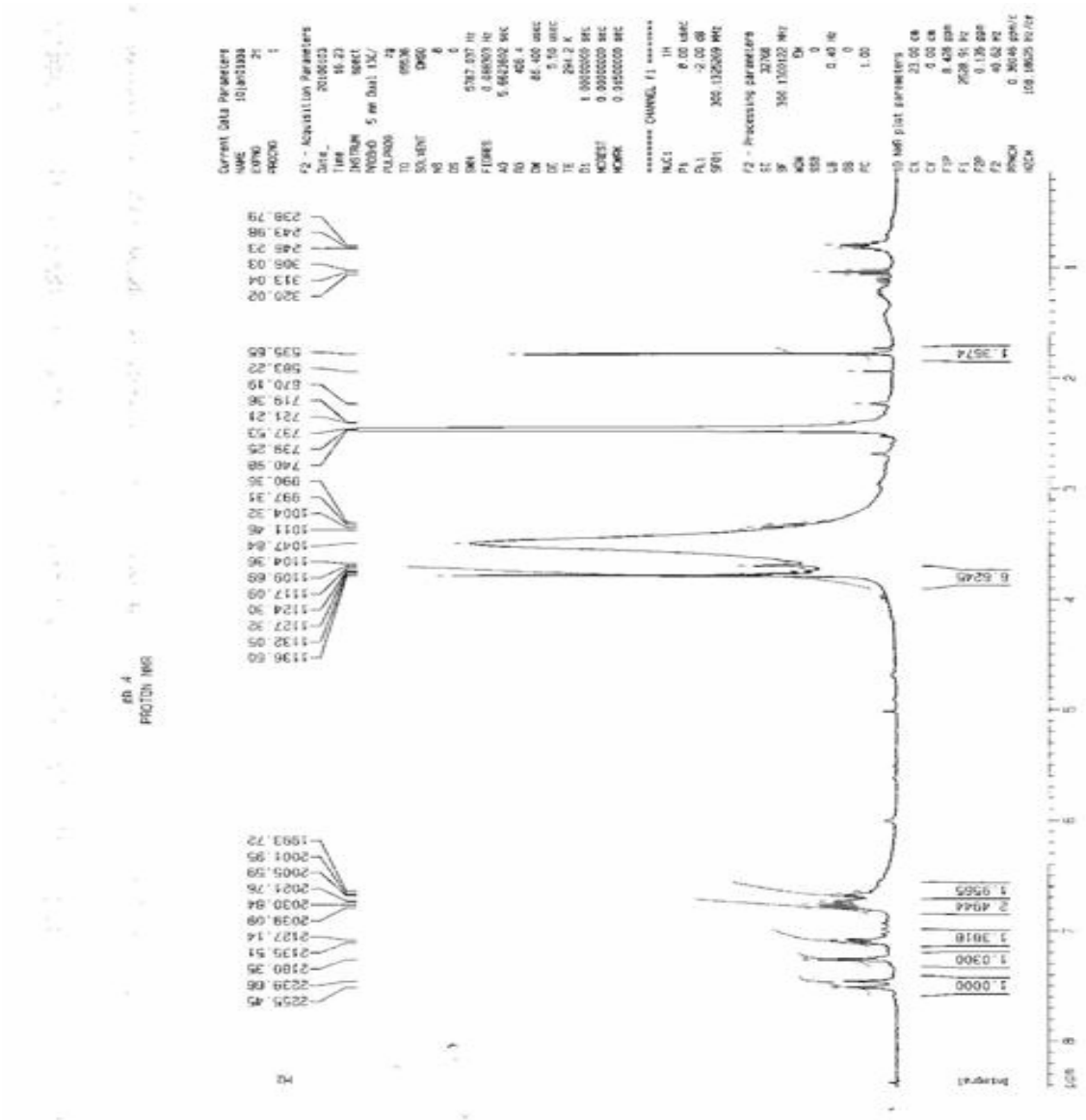

Fig 3.21 :^{1}H NMR of 4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol- 5-yl)vinyl)-2-methoxyphenol (**7**)

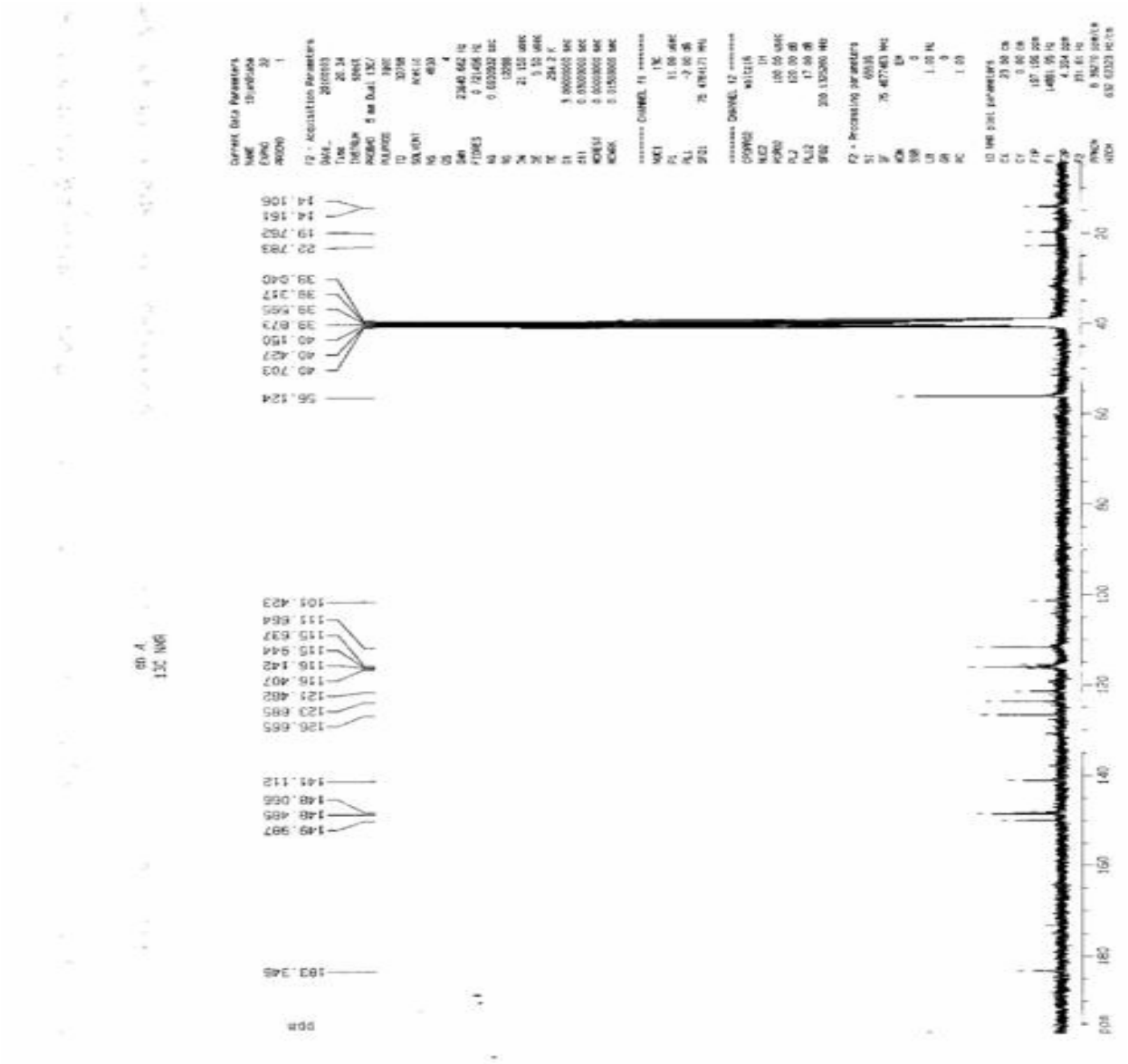

Fig 3.22 : ^{13}C NMR of 4-((1E)-2-(3-(4-hydroxy-3-methoxystyryl)-2-butyl-2,3-dihydroisoxazol- 5-yl)vinyl)-2-methoxyphenol (**7**)

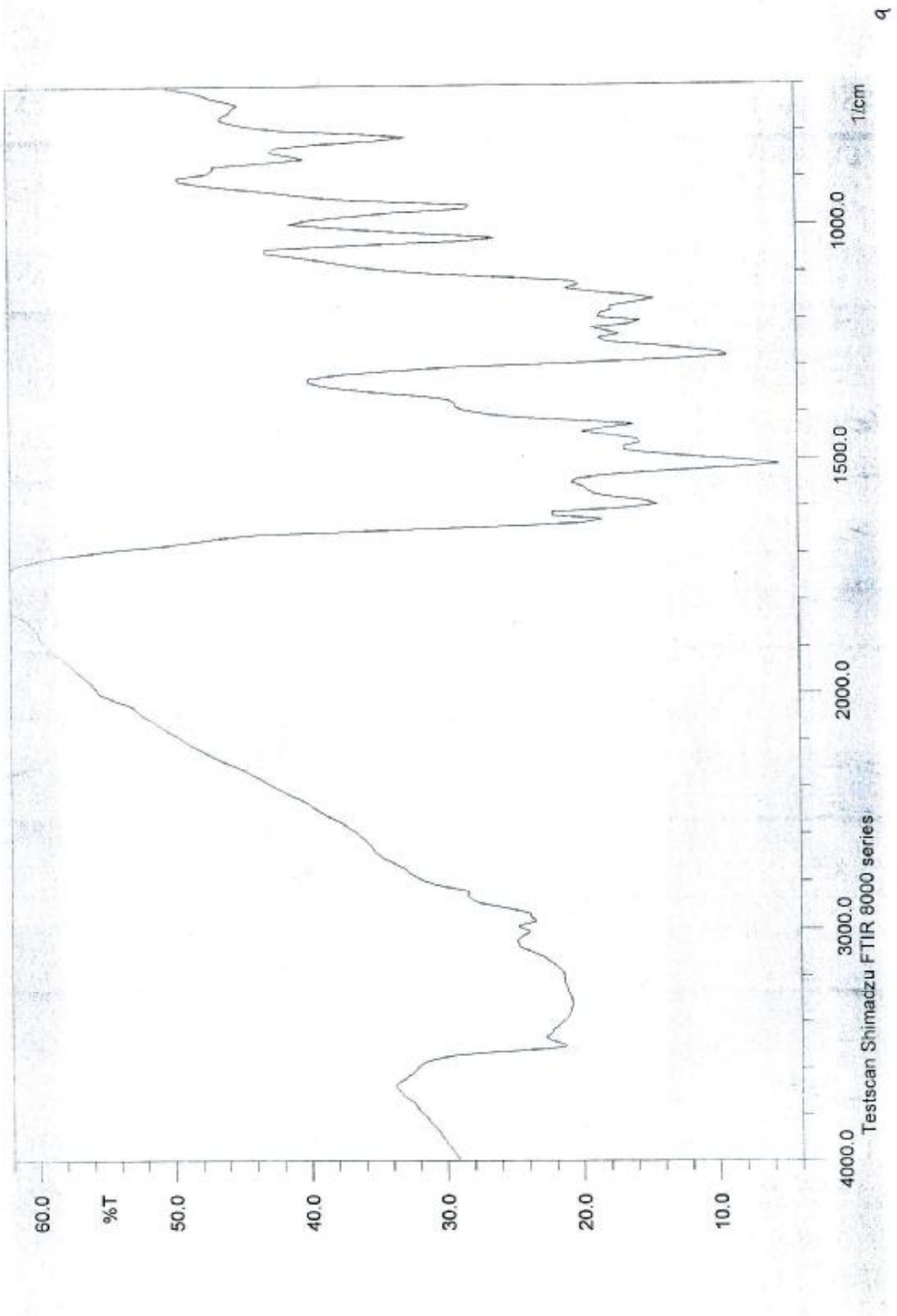

Fig 3.23: FT-IR of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone (**8**)

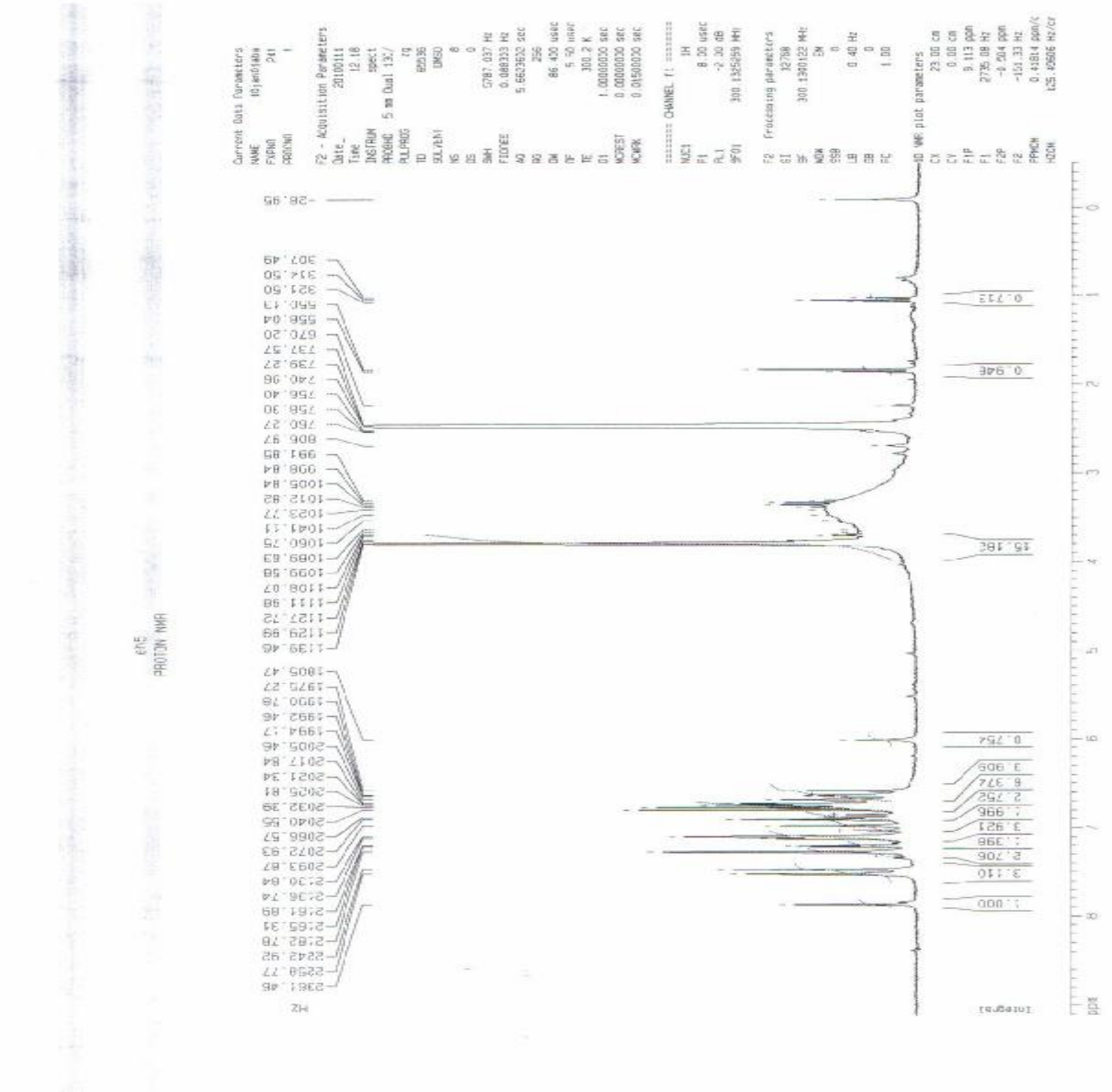

Fig 3.24 : ^{1}H NMR of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone (**8**)

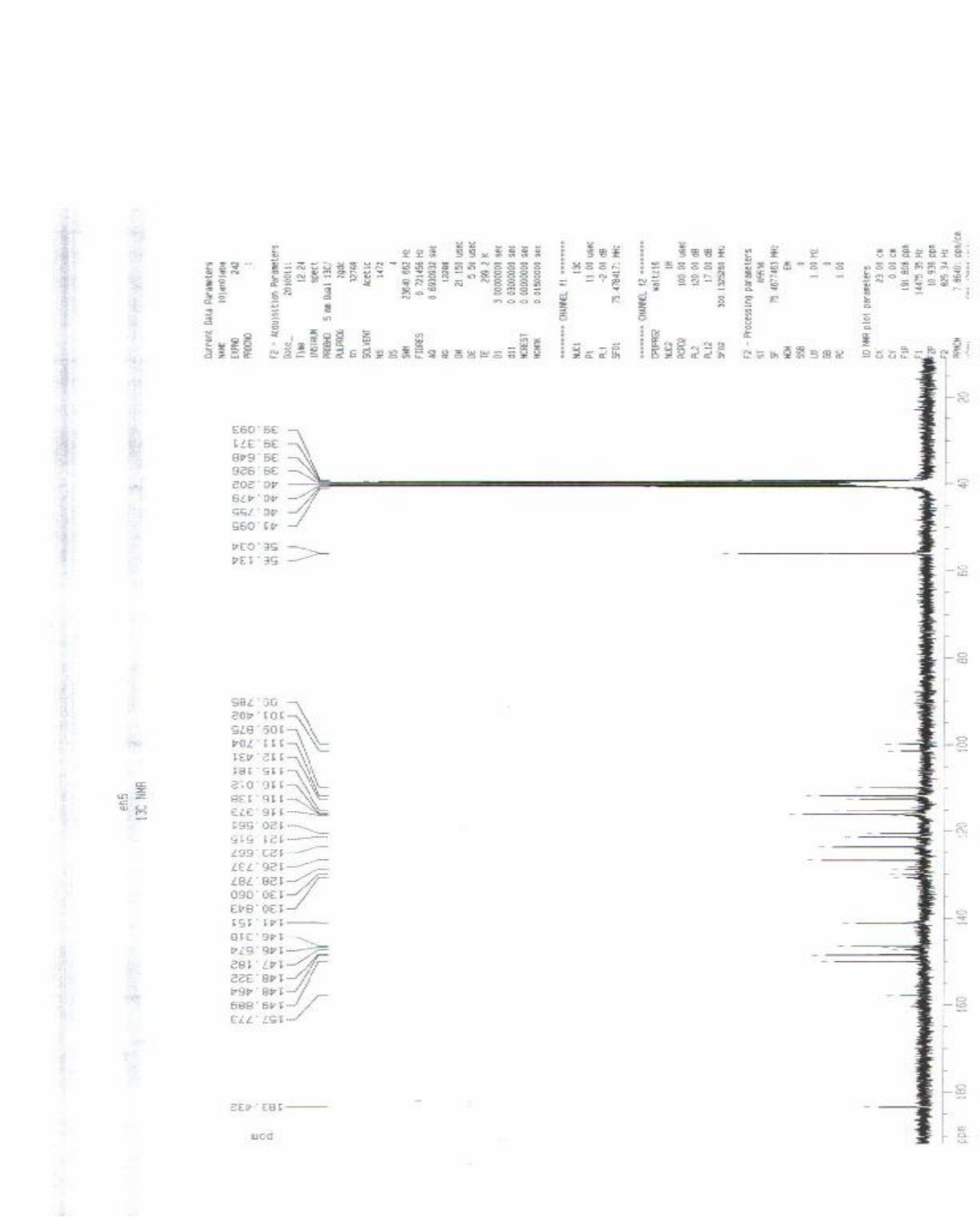

Fig 3.25(a): ^{13}C NMR of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone (**8**)

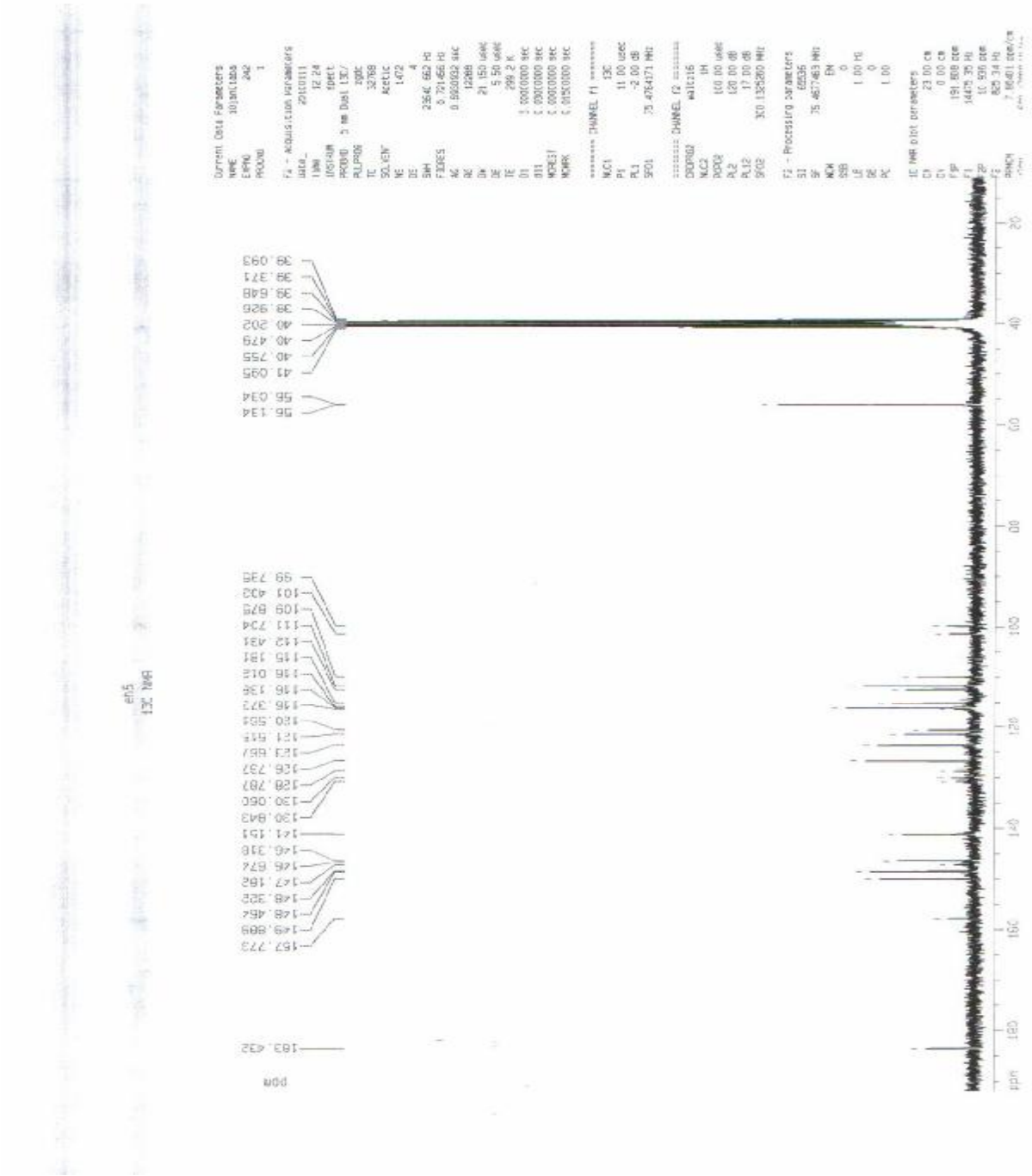

Fig 3.25(b): ^{13}C NMR of 1-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-2(3H)-yl)ethanone (**8**)

جامعة النجاح الوطنية
كلية الدراسات العليا

مشتقات الكركم من ديازول و أوكسازول ومدى تأثيرها ضد البكتيريا

إعداد
نهى عبد الرحمن حسن مهداوي

إشراف
د. عثمان حامد
د. أيمن حسين

قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2010م

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الملخص

سلسلة جديدة من المركبات الحلقية من مشتقات الكركم من البايرزول و الايزوكسازول و غيرها تم تحضيرها عن طريق تفاعلات الكركم مع الهادرزين .

هذه التفاعلات تمت بوجود حامض الأستيك أسيد كمنشط و مذيب للتفاعل. بالإضافة لهذه المركبات الحلقية، فقد تم تحضير مركب مشتق الكركم من بيتاــ ايمينو كحول،وهو مركب رقم [7].

تفاصيل عمليات التحضير موضحة بالتفصيل في الجزء العملي.

ومن خلال هذا البحث تم التأكد من أشكال المركبات الجديدة وذلك باستخدام عدة طرق

التحليل بواسطة الأشعة تحت الحمراء، أطياف الرنين المغناطيسي، التحليل الدقيق للعناصر وأخيراً أطياف الكتلة.

وبعد ذلك تم فحص هذه المركبات لفاعليتها ضد أنواع البكتيريا التالية : ستافيلوكوكس اوريس، ايشيرشيا كولاي، بروتيس ميرابيلس وبسودومونس اريجنوزا.

وقد لوحظ أنه لم تكن هناك أية فاعليه لهذه المركبات ضد بروتيس ميرابيلس وبسودومونس اريجنوزا، ولكن بعض منها فعال ضد ايشيرشيا كولاي، أما فاعليتها ضد ستافيلوكوكس اوريس، كانت كلها فعاله، أما مركب رقم [4] كان أكثرها فاعلية.

4-((1*E,1'E*)-(3,6-dihydro-2H-1,4-diazepine-5,7-diyl)bis(ethene-2,1-diyl))bis(2-methoxyph وهو :