An-Najah National University

Faculty of Graduate Studies

Design, Synthesis, Antimicrobial and Cytotoxicity of Curcumin Based Benzodiazepines, Diazepines, Diazoles and Amines

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This thesis was Defended Successfully on 17/2/2021, and approved by:

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4.Prof. Ghaleb Adwan/ Internal Examiner	Ghaleb Adwan .....

Dedication

To those who gave me the endless love, and gave me a chance to prove and improve myself through all my walks of life, and support me to chase my dreams.

To the best gifts that ever happened to my life …to my parents

To my sisters (Rana, Roaa, Rasha, and Subhiah), my brothers (Mahmoud, Amjad and Emad), friends and all my family

To my little nieces Shams and Meera

To uncle Saadeh Mustafa Irshaid and all his family

To uncle Azzam Alshawah and all his family

To uncle Salah Al-Mallah

To my doctors

To my students

To those who are looking forward for more knowledge

Rola Sultan Al-Kerm

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Rola Sultan Al-Kerm

الاقرار

أنا الموقعة أدناه، مقدّمة الرسالة التي تحمل العنوان:

Design, Synthesis, Antimicrobial and Cytotoxicity of Curcumin Based Benzodiazepines, Diazepines, Diazoles and Amines

أقر بأن ما اشتملت عليه هذه الأطروحة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة إليه حيثما ورد. وأن هذه الرسالة كاملة، أو اي جزء منها لم يقدم من قبل لنيل اي درجة او لقب علمي او بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

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

Student's Name: اسم الطالبة: رود سلطان الكرم

Signature: التوقيع: Rola

Date: التاريخ: 17/2/2021

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List of Schemes

List of Abbreviations

Abbreviation	Full name
FT-IR	**Fourier -Transform Infrared Spectroscopy**
NMR	**Nuclear Magnetic Resonance**
DMSO	**Dimethyl Sulfoxide**
EtOAc	**Ethyl Acetate**
mmol	**Millimole**
MHA	**Mueller-Hinton agar**
NB	**Nutrient Broth**
EMB	**Eosin methylene blue agar**
S. aureus	***Staphylococcus aureus***
MRSA	**Methicillin-resistant *S. aureus***
K. pneumoniae	**Klebsiella pneumoniae**
E. coli	***Escherichia coli***
CFU	**Colony-forming units**
CLSI	**Clinical and Laboratory Standard Institute**
MIC	**Minimum Inhibitory concentration**
MBC	**Minimum Bactericidal Concentration**
ERIC	**Enterobacterial repetitive intergenic consensus**
H-curcumin	**Hydrogenated curcumin**
TEA	**Tri ethanol amine**
DIPA	**diisopropylamine**
DNA	**Deoxyribonucleic acid**
TLC	**Thin Layer Chromatography**

Design, Synthesis, Antimicrobial and Cytotoxicity of Curcumin Based Benzodiazepines, Diazepines, Diazoles and Amines

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Abstract

In this study novel sets of curcumin-based benzodiazepine, diazepine, diazoles and amines were prepared using simple and convenient condensation reaction; by reacting curcumin with various types of 1,2-diamino and hydrazine compounds. The prepared compounds were characterized using FT-IR, ^{1}H NMR, and ^{13}C NMR.

Several simple and convenient synthetic methods were employed in order to prepare the desired products. In the first method, curcumin-based benzodiazepine, diazepine, diazoles, and amines were prepared by condensation reaction of curcumin with various 1,2 diamino and hydrazine compounds in either ethanol which was used as a solvent and H_2SO_4 as a catalyst.

Another new set of curcumin-based benzodiazepine, diazepine, diazoles and amines were prepared using Knoevenagel Condensation that involves two-fold mechanism; The first step includes the formation of α,β-unsaturated intermediate **20 A** using Knoevenagel condensation; by mixing curcumin with benzaldehyde in ethanol solvent, in the presence of catalytic amount of diisopropylamine (DIPA) base. Meanwhile, acid-catalyzed condensation cyclization reactions of the curcumin carbonyl groups of the

synthesized intermediate with various 1,2-diamino or hydrazine compounds were involved in the second step to produce the desired products. The Knoevenagel condensation was employed in this work to prevent keto-enol tautomerization, which expected to enhance the yield.

H-Curcumin-based amine was prepared by condensation reaction of H-curcumin with ammonium acetate in two steps; the first step involved heating the resulting mixture at 60 °C until complete solvent evaporation. Followed by heating the resulted compound again at 60 °C for an hour.

Then a selected set of the prepared curcumin-based compounds was functionalized with methoxy group. Where; the phenolic groups of the selected compounds were alkylated by reacting with iodomethane in the presence of sodium hydroxide. Functionalization of the prepared curcumin-based compounds with alkyl groups was employed in this work in order to determine the effect of the hydroxyl group on the antimicrobial activity.

Antibacterial activities of selected set of the prepared curcumin-based heterocycles were evaluated against four types of bacteria strains: *S. aureus,* MRSA, *E. coli* and *K. pneumoniae*.

The antimicrobial activity occurred by inhibition of growth of the tested strains with MIC values ranging between 1.56-200 μg/mL. In general, the potency against S. aureus and Klebsiella is greater than that against MRSA and E. coli.

However, the highest activity was shown by compound **9** as it showed MIC of (1.56, 1.56, 50 μg/ mL) against S. aureus, MRSA, and Klebsiella respectively. And it is the only compound that showed activity against MRSA with MIC of (1.56 μg/ mL).

Moreover, the prepared curcumin-based heterocycle diazepines (compounds **2, 4, 10, 35**) showed synergistic effects with ampicillin antibiotics. Furthermore, the Genotoxicity test was performed on compound **8.** Results showed that compound **8** did not interact with the DNA molecule in *E. coli* strains. Thus, it is considered as a non-mutagenic or non-genotoxic agent. This study revealed that, the prepared curcumin-based diazepine could be a promising design for a potentially active antibacterial synergized agent with conventional antibiotics.

CHAPTER ONE

INTRODUCTION

1. Background

Bacterial resistance toward available antibacterial agents is rapidly becoming a worldwide and an urgent problem. Accordingly, there is a growing interest in the development of new antibacterial agents with various functionalities and novel mechanisms of action. So, in this work a group of curcumin-based heterocycles was prepared and their antibacterial activities against various Gram-positive and Gram-negative bacteria are reported [1-4].

1.1 Gram positive and gram-negative Bacteria

Gram positive and gram-negative bacteria are two types of bacteria, differentiated based on their reaction to the Gram stain test and cell wall composition. The Gram staining test, developed by Hans Christian Gram in the 1800s, identifies bacteria based on the reaction of their cell walls to certain chemicals and dyes (crystal violet dye). Gram-positive bacteria retain a purple-colored stain as shown in figure 1.1. This occurred because their cell walls are composed mostly of a thick layer of a unique substance known as peptidoglycan. In contrast, the cell walls of Gram-negative bacteria have a thin layer of peptidoglycan and an outer membrane with a lipopolysaccharide (LPS) which is not exist in Gram positive bacteria, so they appear pinkish or red after Gram staining figure 1.1 [5-7].

Fig. 1.1 The Gram-positive bacteria retain a purple-colored stain, while the Gram-negative bacteria lose the crystal violet and stain pinkish or red [8].

The gram-positive bacteria differ from the gram-negative ones in the components present in their cell wall. The cell wall of Gram-positive Bacteria consists of several thick layers (15-80 nanometers) of peptidoglycan figure 1.2. Peptidoglycan is a macromolecule composed of disaccharides (glycan) and short chains of amino acids (peptide) which are cross-linked together forming several types of peptidoglycan. Peptidoglycan protects bacteria and defines their shape. Gram positive cell walls also contain a group of molecules called teichoic acids that extend from the plasma membrane and running perpendicular to the peptidoglycan sheets. These acids are unique to the cell walls of Gram-positive bacteria and help them to infect cell and cause disease [8-9].

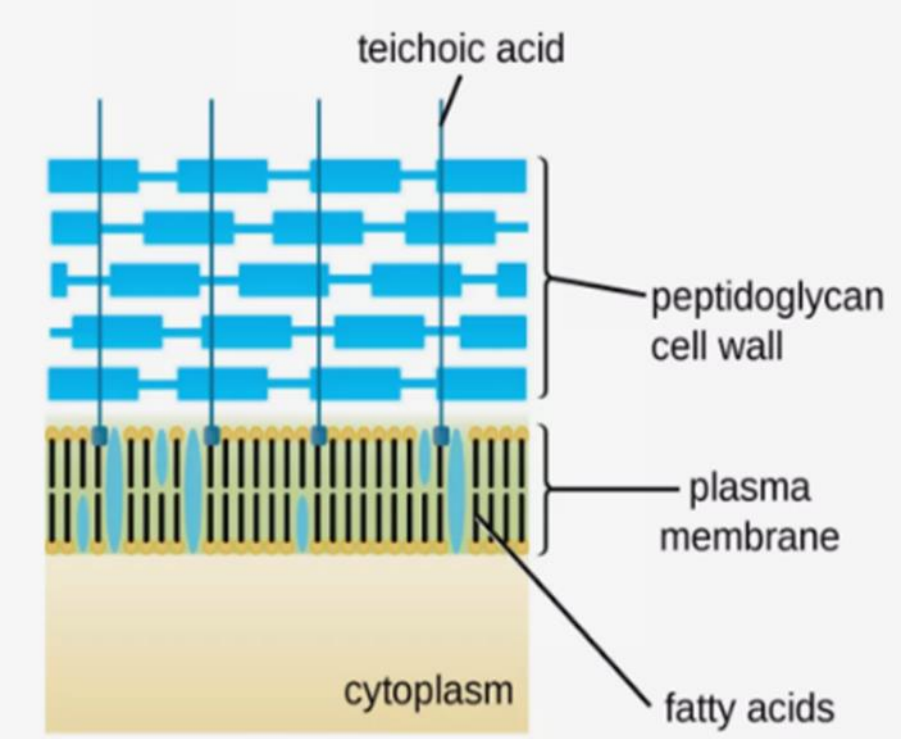


Fig. 1.2 the composition of the Gram-positive bacteria cell wall [5].

The cell wall of Gram-negative Bacteria is more complex than that of Gram positive one, the Bactria structure is shown in figure 1.3. It consists of a single, thin layer (10 nm) of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria has a unique component, lipopolysaccharide (LPS). LPS refers to endotoxin is a large glycolipid complex composed of three different parts; a hydrophobic component known as lipid A, a hydrophilic core oligosaccharide, and O-specific oligosaccharide chain. LPS works like an impenetrable shield to protect bacteria from harmful substances [8-12].

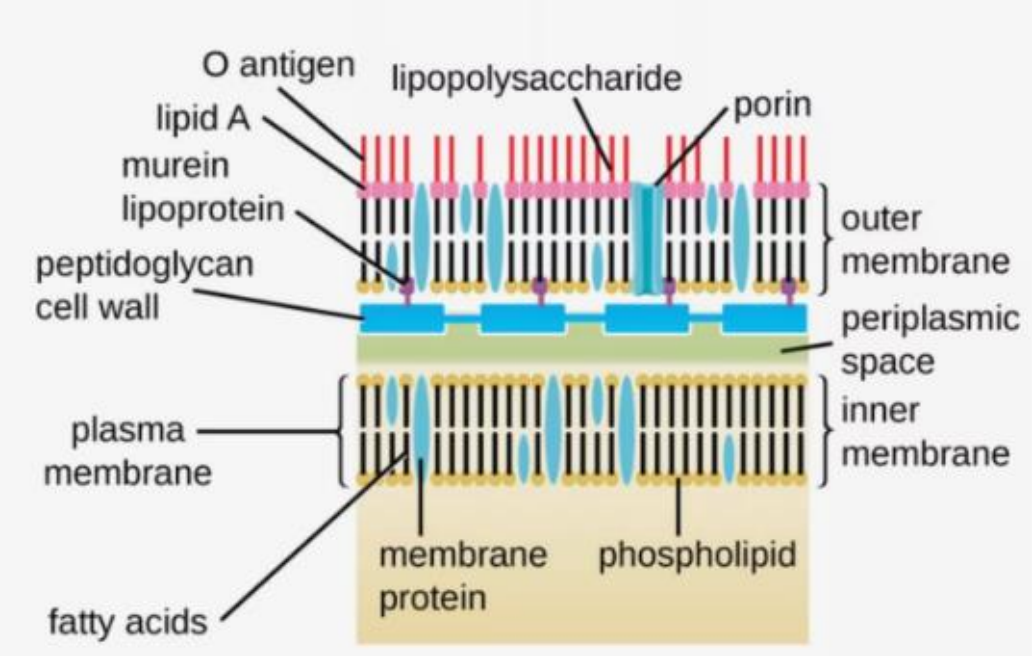


Fig. 1.3 the composition of the Gram-negative bacteria cell wall [5].

Gram-negative bacteria are more dangerous than Gram positive ones, 90-95% of Gram-negative bacteria are pathogenic due to the presence of LPS in their outer membrane, an endotoxin which increases the severity of inflammation. On the other hand, many Gram-positive bacteria are non-pathogenic as they contain an outer peptidoglycan layer that can be easily dissolved by lysozyme [13,14]. Examples on gram negative and gram-positive bacteria are listed below.

1.1.1 Staphylococcus aureus

Staphylococcus aureus is a pathogenic Gram-positive bacterium with diameters of 0.5–1.5 μm and characterized by individual cocci, which divide in more than one plane forming microscopic grape-like clusters as shown in figure 1.4. The staphylococcus is facultative anaerobic, non-motile, non-spore forming that grow by fermentation or by aerobic

respiration. It can grow over a wide range of temperature (7–48 °C), with an optimum temperature around 37 °C. And over the pH range 4–10, with an optimum at 6–7 [15-17]. The Staphylococcus aureus cells can also tolerate high salinities up to 20%. Furthermore, it is found on human nose, armpit, groin, and carriage via skin, sneezing and coughing. It can also cause more serious infections like; endocarditis, bloodstream infections, bone and joint infections, and pneumonia [17,18].

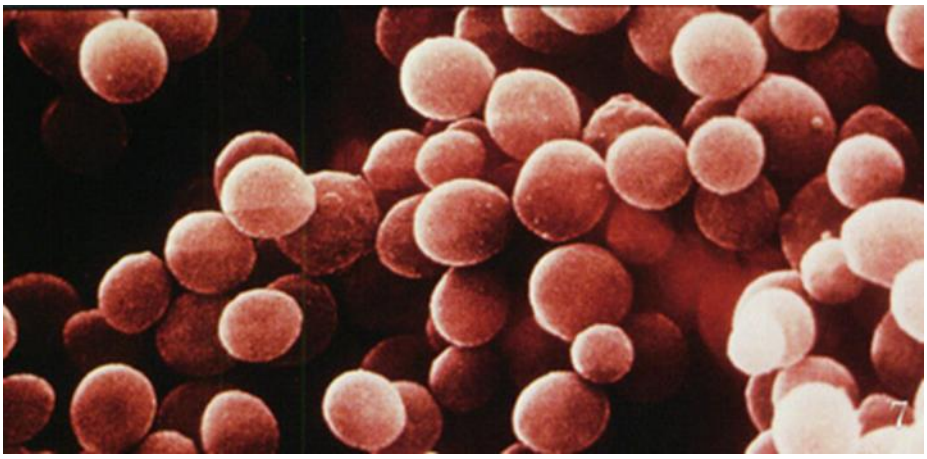


Fig. 1.4 Staphylococcus aureus Gram-positive bacteria [9].

1.1.2 Methicillin-resistant Staphylococcus aureus

Methicillin-resistant Staphylococcus aureus (MRSA) is a virulent and an opportunistic pathogen often carried asymptomatically on the human body. These strains figure 1.5 have acquired a gene that makes them resistant to almost all beta-lactam antibiotics. MRSA is currently one of the most common causes of nosocomial and significant infections, including; pneumonia, endocarditis, skin and soft tissue infections, necrotizing fasciitis, severe sepsis, osteomyelitis, and toxinoses such as toxic shock syndrome. MRSA infections occur mostly among in-patients who have

been in hospitals or who have weakened immune systems it's known as health care-associated MRSA (HA-MRSA). While these strains are generally easier to treat, some may move into hospitals and become increasingly resistant to drugs other than beta-lactams [19-21].

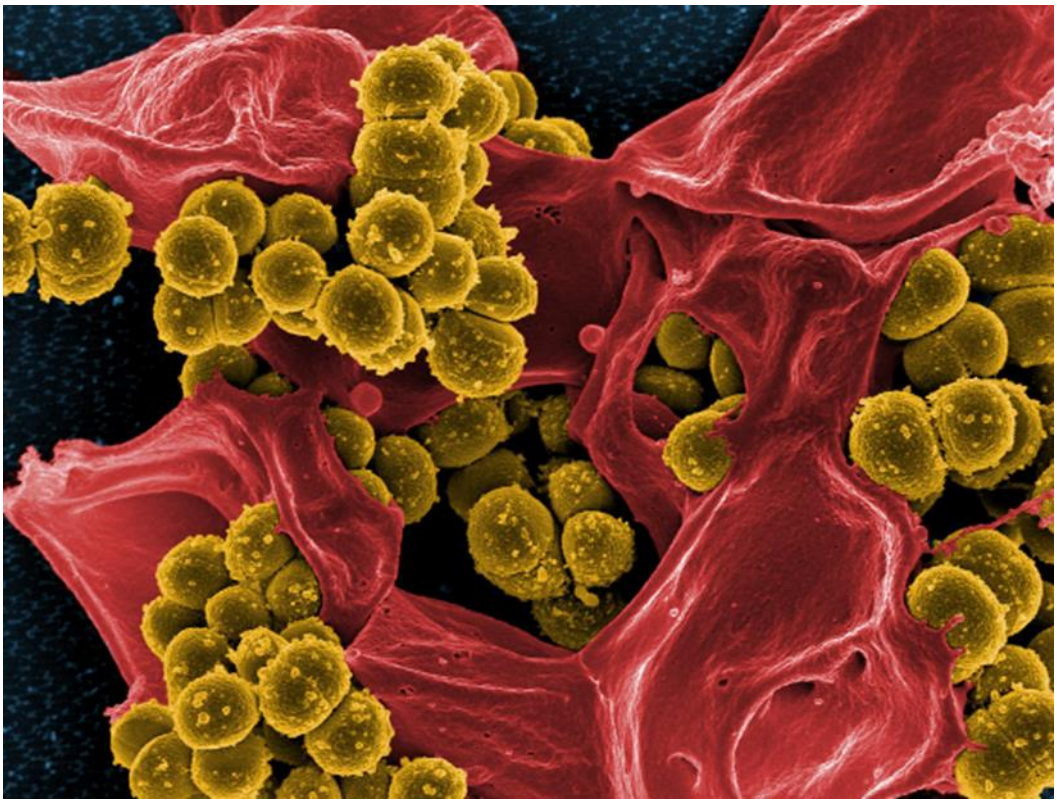


Fig.1.5 Methicillin-resistant Staphylococcus aureus (MRSA) [22].

1.1.3 Klebsiella

Klebsiella is a rod-shaped gram-negative anaerobic, non-motile, and encapsulate bacterium, that is a normal commensal living in the skin, gut, and mouth figure 1.6. However, Klebsiella becomes pathogenic when it becomes transported elsewhere. Klebsiella usually associated with liver abscess, pneumonia, urinary tract infections (UTIs), meningitis, bloodstream infections, respiratory infections, and wounds Commonly, K.

pneumoniae is an opportunistic pathogen mostly affects patients who has an immunodeficiency, and people who are taking long courses of particular antibiotics, and tends to cause hospital-acquired (HA) infections. K. pneumoniae is provided by two factors make them chronic and antibiotic resistance; the biofilms that formed in vivo protect the pathogen from being attacked by the antibiotics and host immune responses. And the extended-spectrum β-lactamases that is commonly make them effective against beta-lactam antibiotics and show multidrug-resistance [23-27].

Fig.1.6 Klebsiella is Gram-negative bacteria [28].

1.1.4 Escherichia coli

Escherichia coli, commonly known as E. coli, is the most common rod-shaped gram-negative bacteria. Commensal of warm-blooded animals and humans. They are nonsporulating, non-encapsulated, facultative anaerobes, and they could be either motile or non-motile figure 1.7. Toxic strains of E.

coli can cause chronic illness, including severe diarrhea and in some cases kidney damage and it is the leading cause of meningitis during the neonatal period [29-32].

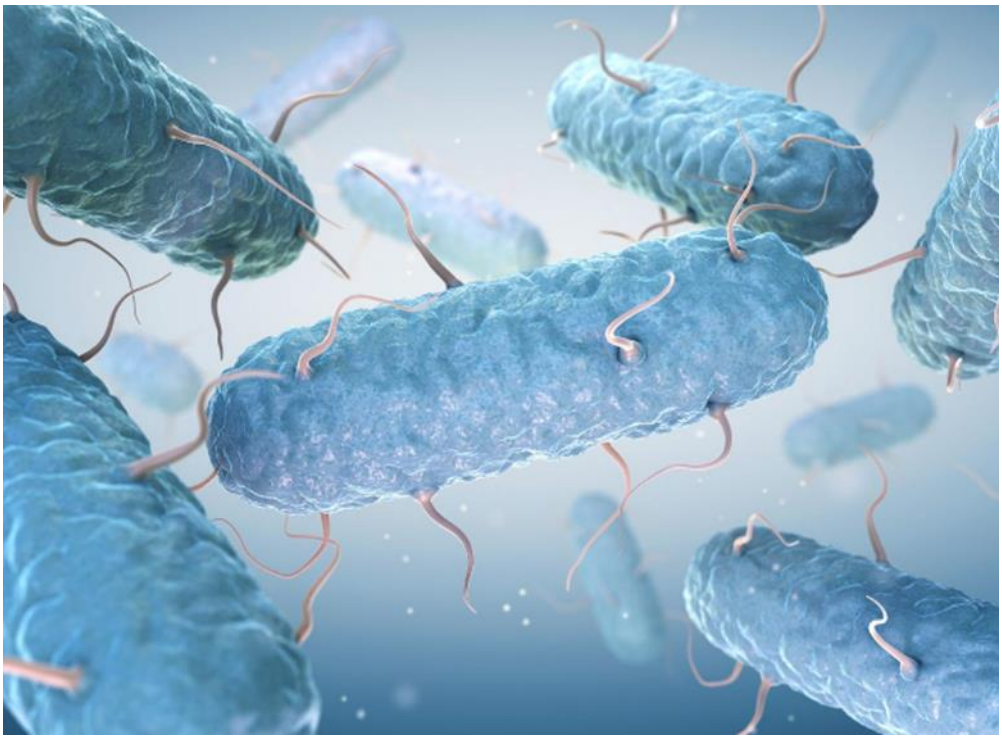


Fig. 1.7 Escherichia coli Gram-negative bacteria [33].

1.2 Antibiotics

Antibiotics are molecules that kill, or inhibit microorganism's growth, including both fungi and bacteria. They are the most potent chemotherapeutics among drugs. While some antibiotics can completely kill bacteria by inhibiting cell wall synthesis and are called "bactericidal". Some are only stop their growth by interfering with DNA replication, bacterial protein production, or other aspects of bacterial cellular metabolism and these are called "bacteriostatic". Initially, antibiotics were considered as organic compounds produced by different microorganisms's

species (fungi, bacteria, and actinomycetes) that selectively inhibits another microorganisms' growth and may eventually destroy them. Chemically related antibiotics to the natural ones were then prepared wholly or partly through artificial means [34-36].

1.2.1 Classification of Antibiotics

The main standard classes of antibiotics are based on molecular and chemical structures including Beta-lactams, Macrolides, Quinolones, Tetracyclines, Aminoglycosides, Glycopeptides, Sulphonamides, and Oxazolidinones [36].

1.2.1.1 Beta-lactam antibiotics and their Mechanism of action

The structures figure 1.8 of this antibiotics class compose of a 1-nitrogen and a 3-carbon ring that is highly reactive. The mechanism of action of antibiotics agents can be classified according to the agent's function as follows; (1) inhibition of the cell wall synthesis, this class of antibiotics involve vancomycin and β-lactam antibiotics like penicillins, carbapenems, cephalosporins. (2) Inhibition of DNA synthesis like sulfonamides and fluoroquinolones and (3) Protein synthesis inhibition such as aminoglycosides, tetracyclines, chloramphenicol, and macrolides [34,37,38].

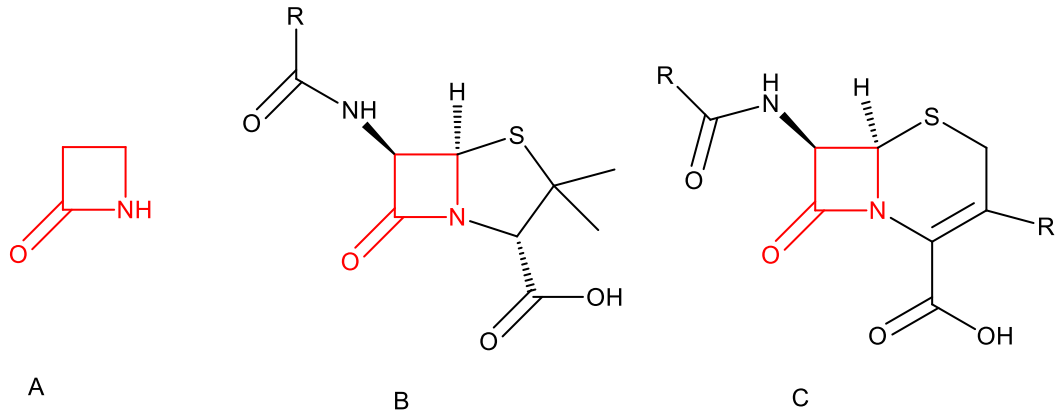


Fig. 1.8 Chemical structure of beta-lactam ring (A) Core structure of penicillin (B) and cephalosporins (C).

The beta (β)-lactam antibiotics constitute the vast majority of antibiotics that have low toxicity. They contain all antibiotics that involve a β-lactam ring in their molecular structure such as; penicillin derivatives (penams), monobactams, cephalosporins (cephems), and carbapenems figure 1.9. They are well known to interfere with the cell wall of the bacteria assembly as part of their mode-of-action. Most bacterial cells are surrounded by a polysaccharide cell wall that prevents the osmotic rupture of their cytoplasmic membrane. The cell wall is synthesized by cross linking of peptidoglycans of one carboxyl of D-alanine in one peptidoglycan chain with an amino group in the next chain using transpeptidase catalyst. This process is essential as it causes the rigidity of the cell wall. Antibiotic binding to the transpeptidase enzyme forming an acyl enzyme complex via the antibiotic β-lactam ring cleavage, causes inactivation of transpeptidase enzyme that leads to loss of osmotic support and finally cell lysis [34,37,38].

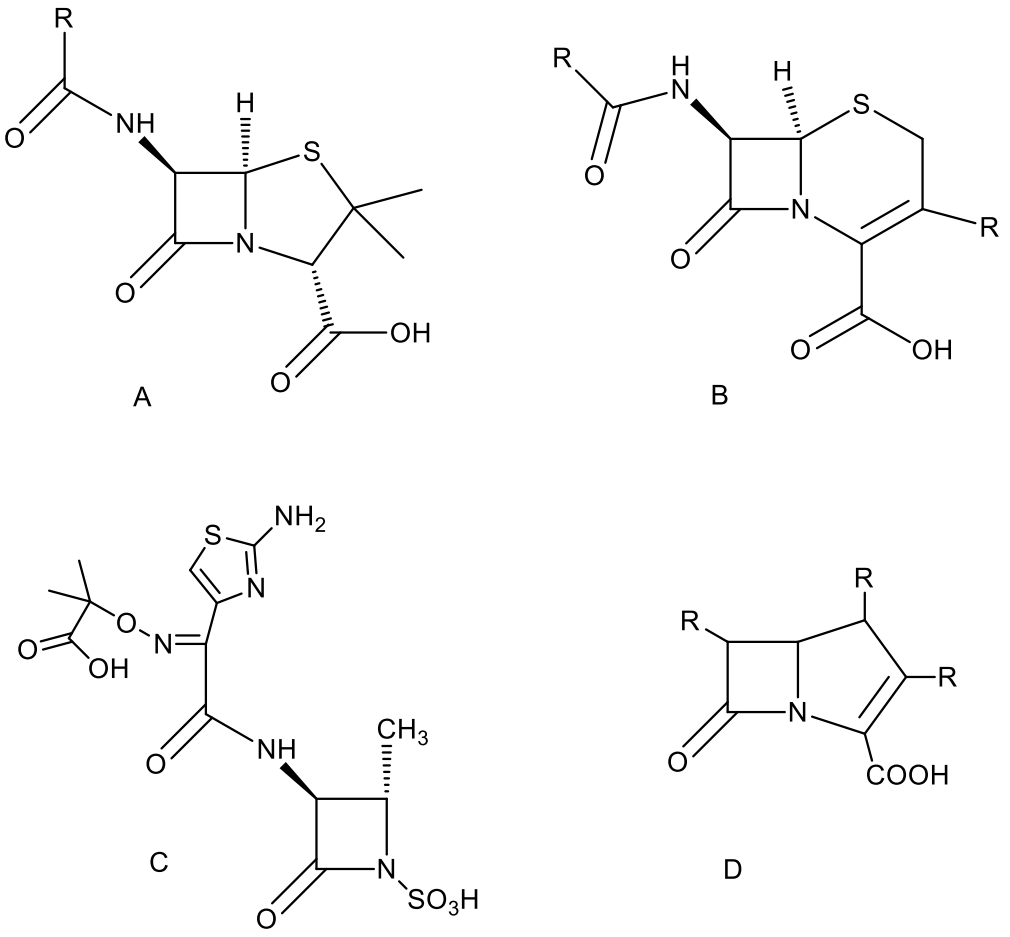


Fig. 1.9 Structure of penicillin (A), cephalosporins (B), monobactams (C), and carbapenems (D).

1.2.1.2 Penicillin

Penicillin (beta-lactam) is the oldest available antibiotic which was discovered in 1929 by Alexander Fleming, and it is still one of the most widely used antibiotics. Chemically it is a heterocyclic compound composed of a 5-membered thiazolidine ring fused to a 4-membered b-lactam ring with an acyl side chain figure 1.10. The R group's nature determines the antibiotic's stability to acidic and enzymatic hydrolysis and its activity against different bacteria. Changing the R group by adding functional groups or bulky side chains gives different penicillins' derivatives with enhanced activity, more resistance to acids and

penicillinase. The low toxicity (unless high dosage is used) of penicillins is one of their most remarkable properties. Penicillins are effective in the treatment of throat, nose, genitourinary tract soft tissue infections and lower respiratory tract [34, 39, 40].

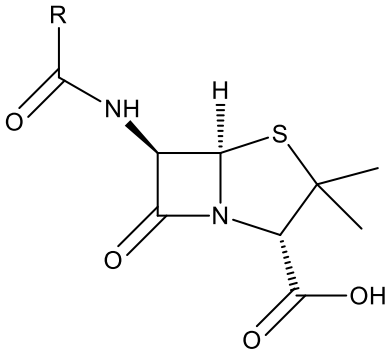


Fig. 1.10 Penicillin (beta-lactam) antibiotic [39].

1.2.1.3 Ampicillin

Ampicillin figure 1.11 is one of the penicillin derivatives that is prepared by adding amino group to benzylpenicillin (amino-penicillin). It is a well-known antibiotic that is active against a wide range of Gram-positive and Gram-negative bacteria. Ampicillin is available as parenteral and oral dosage forms and is administered every 6 hours. It is used to treat various bacterial infections, including; gonorrhea and infections of the intestinal, urinary, and respiratory tracts [34, 41].

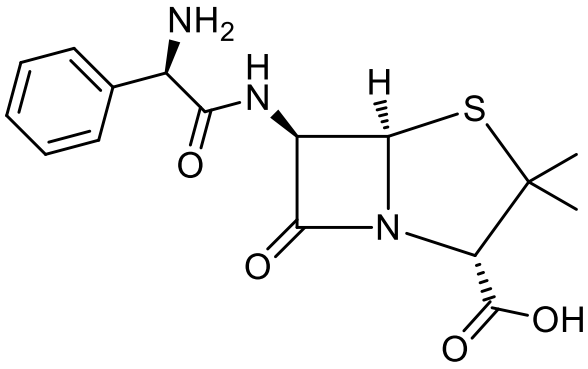


Fig. 1.11 Ampicillin antibiotic [34].

1.2.1.4 Macrolides

The macrolides' structure composes of 14-, 15-, or 16- membered macrocyclic lactose rings with D-desosamine and unusual deoxy sugars L-cladinose attached figure 1.12. They have a mor comprehensive range of antibiotic activity than pinicillins, and they mainly administered to patients with penicillin allergy [36].

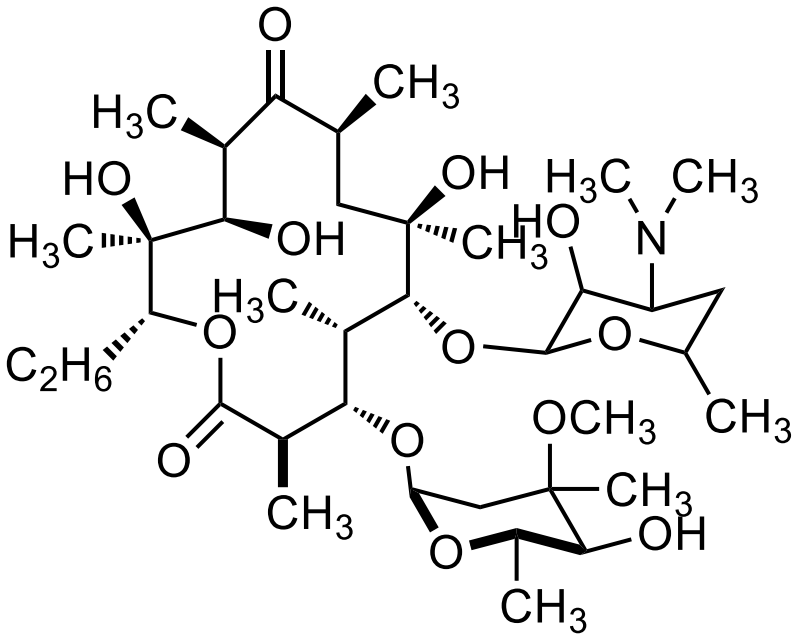


Fig. 1.12 Structure of Macrolide

1.2.1.5 Tetracyclines

The structure of tetracyclines contains four hydrocarbon rings as shown in figure 1.13, and their names end with the suffix "-cyline" [36].

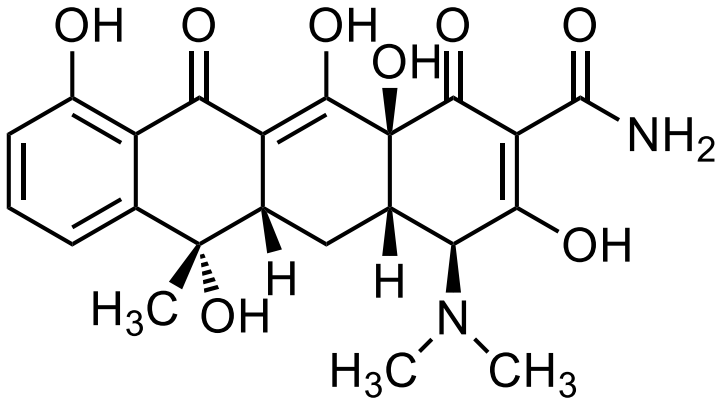


Fig. 1.13 Structure of Tetracycline

Other classes of antibiotics including Quinolones, Sulphonamides, Aminoglycosides, Glycopeptides and Oxazolidinones (Linezolid), are shown in figure 1.14 [36].

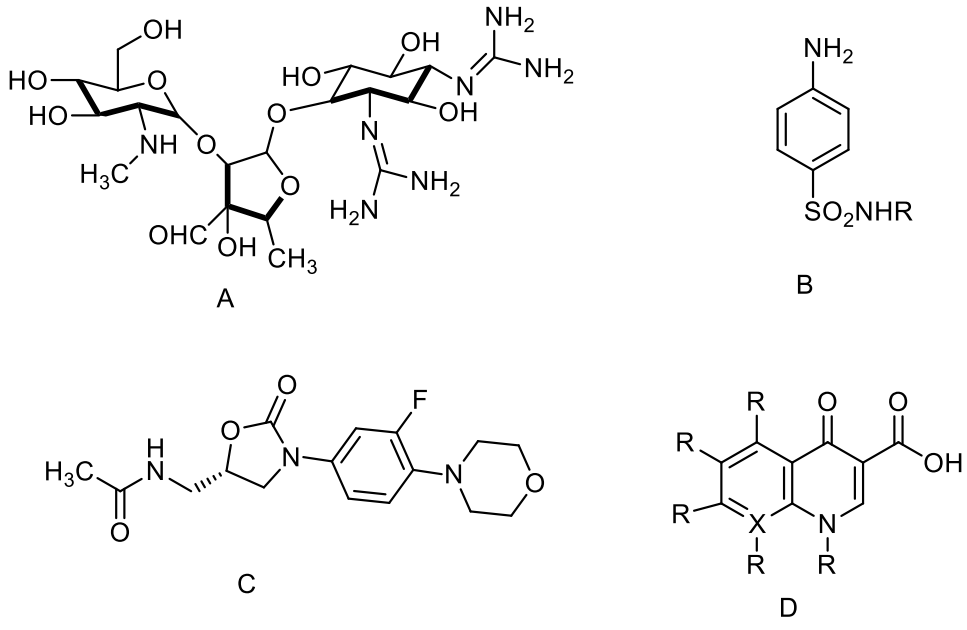


Fig. 1.14 The structures of Aminoglycosides (A), Sulphonamides(B), Linezolid (C), and Quinolones (D).

1.3 Synergistic and Antagonistic Drug Combinations

Multi-drugs combination is a promising and widely used approaches to treat variety of serious diseases including; infectious diseases, cancer, 2 diabetes, and inflammation. Combinations of multi-drugs can also be used in antibiotics to combat highly resistance bacteria to the individual use of normal antibiotics. The efficacy of the combination may be enhanced and more significant than the sum of each drug's effect individually, which is called synergism, conversely, when the efficacy of the combination reduces the effect of each drug individually this is called antagonism. Using various drugs with several mechanisms and modes of actions may interacts against the target in many unexpected ways and more effectively. There are several possibilities for synergism such as; (1) Enhancing the efficacy and the specificity of the drug, (2) reducing the dosage while maintaining or increasing the efficacy to avoid toxicity, (3) slowing down the drug resistance development, (4) providing selective synergism against target by doing both toxicity antagonism, and efficacy synergism [42-44].

1.4 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a powerful, and rapid method for *in vitro* amplifying specific DNA sequences, to generate more than 100 billion similar copies. Repetitive cycles including denaturation of the template, annealing of the primer (oligonucleotide) and annealed primers extension using DNA polymerase, to produce unlimited copies of DNA segment from a single copy of initial DNA figure 1.15. in this method the

synthesized products of primer extension in one cycle can serve as a template in the next one. Thus, the number of target DNA copies duplicates at every cycle. Since its discovery PCR has had such versatile applications in both diagnostic and basic aspects of molecular biology [45].

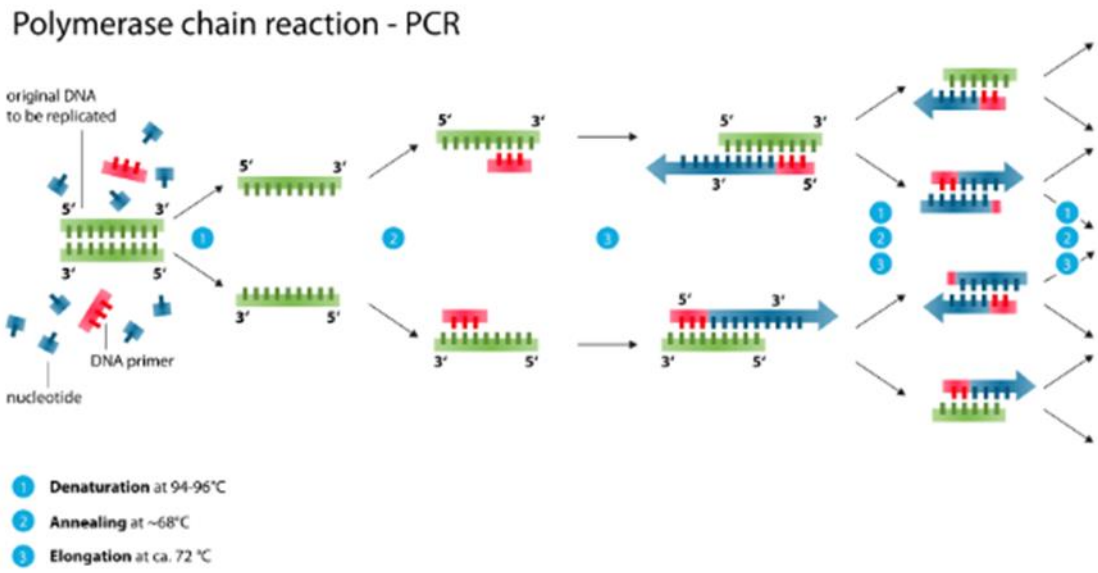


Fig. 1.15 Steps of PCR [46].

1.5 Electrophoresis

Electrophoresis is the migration of charged molecules or particles in aqueous solution as a result of applying an electric field. The speed of migration depends on the charges of the molecules and the applied electric field strength. Hence, different charged particles and molecules form individual zones during their migration. Electrophoresis is accomplished in an anti-convective medium like a gel matrix or a viscous fluid. Thus, the speed of migration also depends on the size of the charged particles and molecules. In this way high resolution fractionation of a mixture of substances is achieved [47].

1.5.1 Agarose gel electrophoresis

Agarose gel electrophoresis is a simple, and one of the most known electrophoresis techniques that possesses great resolving power. The agarose gel contains microscopic pores that work like a molecular sieve used to separate molecules based upon the size, charge, and shape. Agarose gel electrophoresis is an efficient separation technique that is mostly used in analyzing DNA fragments of diverge sizes ranging from 100 bp to 25 kb that is generated by restriction enzymes. DNA fragments larger than 25 kb is separated using pulse-field gel electrophoresis, meanwhile, polyacrylamide gel electrophoresis is used to effectively separate DNA fragments smaller than 100 bp or more. Agarose gel electrophoresis is also used to separate other charged biomolecules like proteins and RNA. To separate DNA using this technique, the DNA is loaded into pre-cast wells in the gel then a current is applied. The phosphate backbone of the DNA and RNA molecules is negatively charged; thus, the DNA fragments migrate to the positively charged anode when placed in an electric field. DNA molecules are separated according to their size as they have constant mass/charge ratio. The migration rate of DNA molecules through a gel depend on the following; (1) The size of DNA molecules, (2) DNA conformation, (3) type and concentration of agarose, (4) electrophoresis buffer, (5) voltage applied. The DNA molecules can be then visualized using UV light after separation and staining with a specific dye [48, 49].

1.6 Genotoxicity

Genotoxicity refers to processes that change the structure, segregation, information content of cell genetic materials (DNA, RNA) that affects the cell integrity. Genotoxins involve both radiation as well as chemical substances. Genotoxins are mutagens that can cause mutation by damaging the DNA or the chromosomal material. All mutagens are genotoxic; but not all genotoxic substances are mutagenic. According to their effects Genotoxins can be classified in to: (1) Cancer causing agents "Carcinogens", (2) mutation causing agents "Mutagens" (3) congenital disabilities causing agents "Teratogens". Heritable mutations result from the genetic damage of the germ cells causing congenital disabilities, while malignancy (cancer) results from the damage of genetic material of somatic cells in eukaryotic organisms. Mutations have many forms including; duplication, deletion or insertion of genetic information [50, 51].

1.6.1 Importance of Genotoxicity testing

Genotoxicity studies are the diverse in-vitro and in-vivo tests used to identify any compound or substance that may damage the genetic material by several mechanisms directly or indirectly. These tests help to identify the hazard results from DNA damage and fixation. Genotoxicity tests play a crucial role in predicting if the new chemical entities can cause carcinogenicity or Genotoxicity by testing them positive. Regulatory authorities worldwide require information about the genotoxic potential of

the new chemical entities and drugs as a part of safety evaluation process [50, 51].

1.7 Natural-Based Antimicrobial Agents

Most of the currently available antibacterial and antifungal agents have undesirable side effects, moreover, excessive use of these drugs has caused rapid development of drug-resistant strains including; Methicillin-resistant Staphylococcus aureus (MRSA). As a result, most of commonly used antibiotics become ineffective in both agricultural and clinical applications. Development of new natural-based drugs with minimal or no side effect of the currently used drugs, and that overcome the bacterial resistance to current antimicrobial drugs is emerging [52].

1.7.1 Alkaloid

Alkaloids are naturally occurring organic bases containing one or more nitrogen atom in their structures. Alkaloids have several important physiological effects on humans and other animals as well. The most commonly known alkaloids including morphine, quinine, strychnine, nicotine, ephedrine and caffeine figure 1.16.

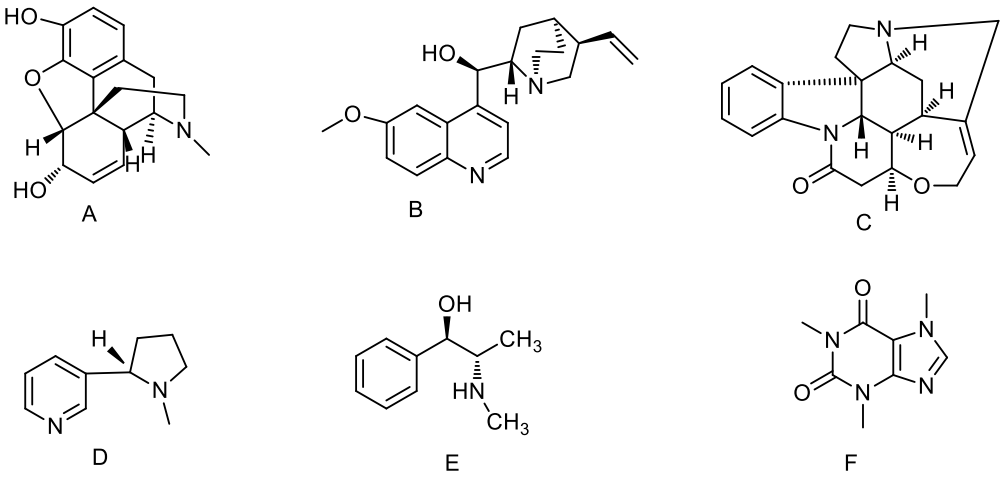


Fig. 1.16 structures of morphine (A), quinine (B), strychnine (C), nicotine (D), ephedrine (E) and caffeine (F).

Alkaloids readily form hydrogen bonds with enzymes, proteins, and receptors and this is mainly due to the presence of a proton-accepting nitrogen atom, and one or more proton-donating functional groups in their structures. Possessing a proton-donating and - accepting functional groups like polycyclic moieties and phenolic hydroxyl, explains the alkaloids superior bioactivity. Pharmacological properties including central nervous depressant (morphine), central nervous stimulant (brucine), anticholinergic (atropine), analgesic (codeine), antihypotensive (ephedrine), antipyretic (quinine), antiemetic (scopolamine), antihypertensive (reserpine), antitumor (vinblastine), vasoconstrictor oxytocic (eg. ergometrine), antimalarial (quinine) activities. figure 1.17 shows the structure of the mentioned alkaloids [53].

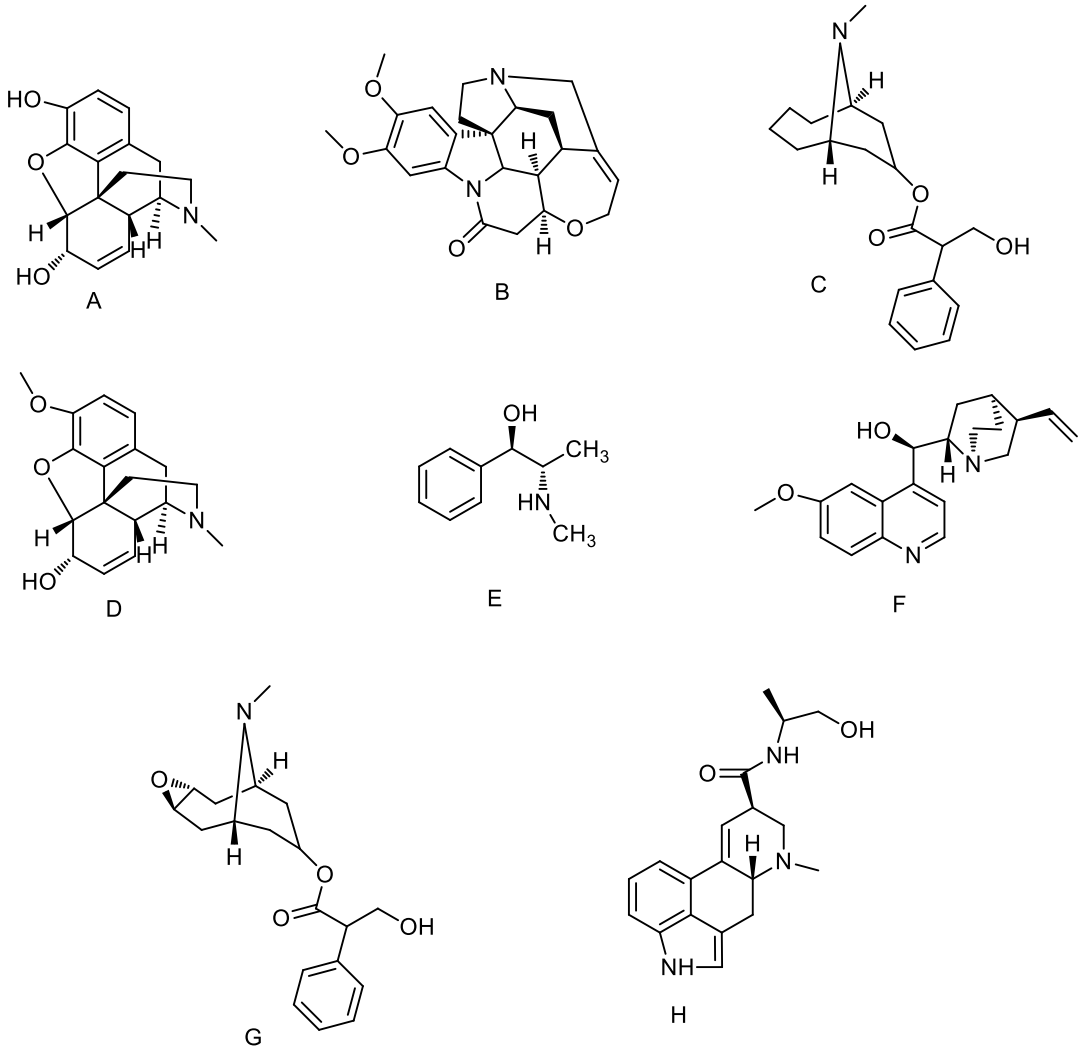


Fig. 1.17 Chemical Structures of morphine (A), brucine (B), atropine (C), codeine (D), ephedrine (E), quinine (F), scopolamine (G), ergometrine (H).

1.7.2 Phenolic compounds

Phenolic compounds are common phytochemicals found in all plants. That contain an aromatic ring bearing one or more hydroxyl groups figure 1.18. Based on the number of phenol units in the molecule, phenolic compounds are categorized as simple phenols or polyphenols. Plant phenolics include simple phenols, lignins, lignans, coumarins, phenolic acids and flavonoids, condensed and hydrolysable tannins. Phenolics act as antioxidants that

reduce inflammation, prevent heart disease, lower the incidence of diabetes and cancers, and reduce rates of mutagenesis in human cells [54,55].

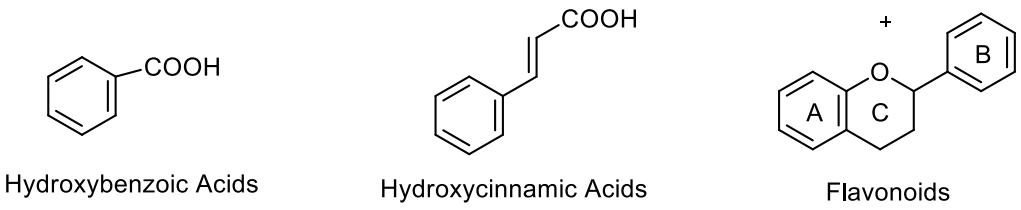


Fig. 1.18 Basic structures of phenolic acids, and flavonoids**.**

1.7.2.1 Curcumin

Natural plants products have traditionally been a magnificent source of bioactive agents for centuries. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione), is a natural yellow pigments and food-coloring agents isolated from the spice turmeric of the Asian herb Curcuma longa [56-60]. Turmeric has been used for thousands of years as a dye, dietary spice, food preservative, additives in cosmetics, as a remedy for variety minor and major ailments, and therapeutic in the treatment of cuts, wounds, jaundice, and rheumatoid arthritis [57, 61-65]. Curcumin, the principal and the major bioactive component of turmeric, possesses several engaging biological activities, such as anti-inflammatory, antioxidant, anticancer, antimalarial, antibacterial, antiviral, antifungal, anti-HIV-1 integrase, anti- Parkinson's, anti-Alzheimer's, anti-arthritic, anti-angiogenic, anti-diabetic, anti-depressant, anti-venom, free-radical scavenging activity, and anti-protozoan [56, 65-71]. The non-toxic natural origin and wide range of pharmaceutical drug properties of curcumin make it a promising lead molecule for medicinal chemistry [58, 60-62].

Chemically, curcumin has three critical functional groups that can contribute to its biological activities: An orthomethoxy phenolic hydroxyl group, α,β-unsaturated β-diketo group, and a seven carbon linker figure 1.19.

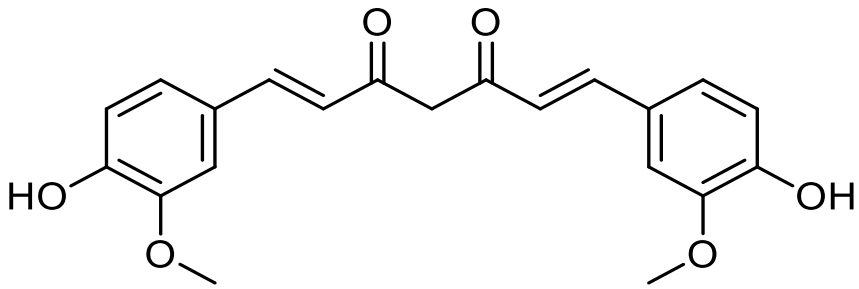


Fig. 1.19 The structure of Curcumin.

Structurally, curcumin exists in the keto and the enol tautomeric form in equilibrium as shown in figure 1.20 due to intra-molecular hydrogen bonding between keto carbonyl oxygen and an enolic hydrogen atom [60, 61, 65, 67]. Keto and enol forms remain in an equilibrium driven by solvent polarity and pH value. The enol form predominates in an aqueous solution under alkaline conditions, and in some organic polar solvents (e.g., alcohols, and dimethyl sulfoxide (DMSO), as a result of intramolecular hydrogen bonding and conjugation that increase the stability of this form. Meanwhile, the keto form predominates in solid phase, nonpolar solvents, and in acidic or neutral pH values [61, 72-78].

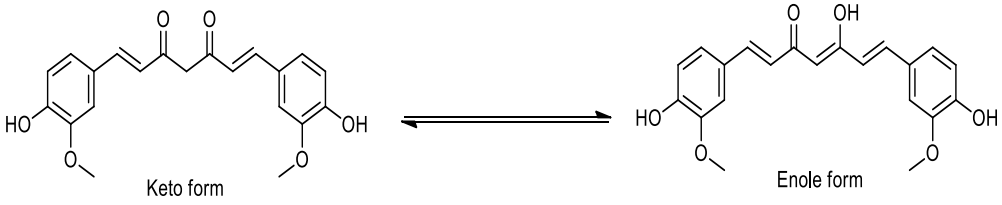


Fig. 1.20 keto-enol tautomerism**.**

The crystalline curcumin powder is a relatively hydrophobic, indicating selective solubility; it is readily soluble in organic solvents such as methanol, ethanol, acetone and DMSO. Whereas, it has a low aqueous solubility of 0.6 μg/mL. Due to, its relatively low solubility at physiological conditions, poor chemical stability low absorption from the gut, fast metabolism and rapid plasma elimination, and high intestinal degradation, limit its therapeutic usage and clinical applicability [64, 71, 73-77]. Therefore, numerous strategies have been developed to overcome these limitations such as nanoparticle or ceramic particles and formulations in micellar, liposomal, and phospholipid complex. Moreover, several strategies have also been done to chemically modify curcumin structures and their biological activity by designing and synthesis new curcumin analogues and derivative. Such structural modifications to the parent skeleton will not only improve therapeutic and physicochemical properties but also enhance the efficacy as well [79-82].

1.7.2.1.1 Antibacterial activity of curcumin

The increase of Gram-positive and Gram-negative bacteria resistance to current antimicrobial drugs have caused an urgent need to assess and identify alternative drugs including plant- based materials that has low human cytotoxicity. Curcumin is a safe molecule with no toxicity or side effect on human health even when taken at high dosage of 8.0 g per day [83].

So far, curcumin has been used for diverse clinical applications. Several studies have shown that curcumin has a therapeutic effect in different diseases such as inflammatory disorders, diabetes, and different types of cancers. Moreover, curcumin showed an in vitro anti-microbial activity against a wide variety of microorganisms such as Gram-positive and Gram-negative bacteria and fungi as well [83].

Recent study investigated that curcumin suppresses the Streptococcus mutant's adherence to extra-cellular matrix protein and surfaces of human tooth. Many studies have indicated that curcumin show a synergistic effect with different important antibiotics including vancomycin, tetracycline, and cefixime against S. aureus [83].

In a published work [84] a new series of curcumin-based heterocyclic systems including isoxazole, pyrazole, and diazepine were synthesized using two different new methods shown in figure 1.21. The prepared compounds were tested for their antimicrobial activity against two types of gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis)* and two types of gram-negative bacteria (*pseudomonas aeruginosa, and Escherichia coli*). The prepared compounds showed low to high potency against the tested strains with MIC values ranging between 1.5 and 6.25µg/mL for *S. aureus.* Meanwhile, Gram- negative bacteria were found to be higher resistant than gram-positive ones. Among the prepared compounds, diazepine showed the highest antibacterial activity. Based on these results, a novel set of curcumin-based compounds were

prepared using a simple and convenient synthetic method (condensation reaction of curcumin with diverse 1,2 diamino compounds) to study their antimicrobial activity against four different types of bacteria.

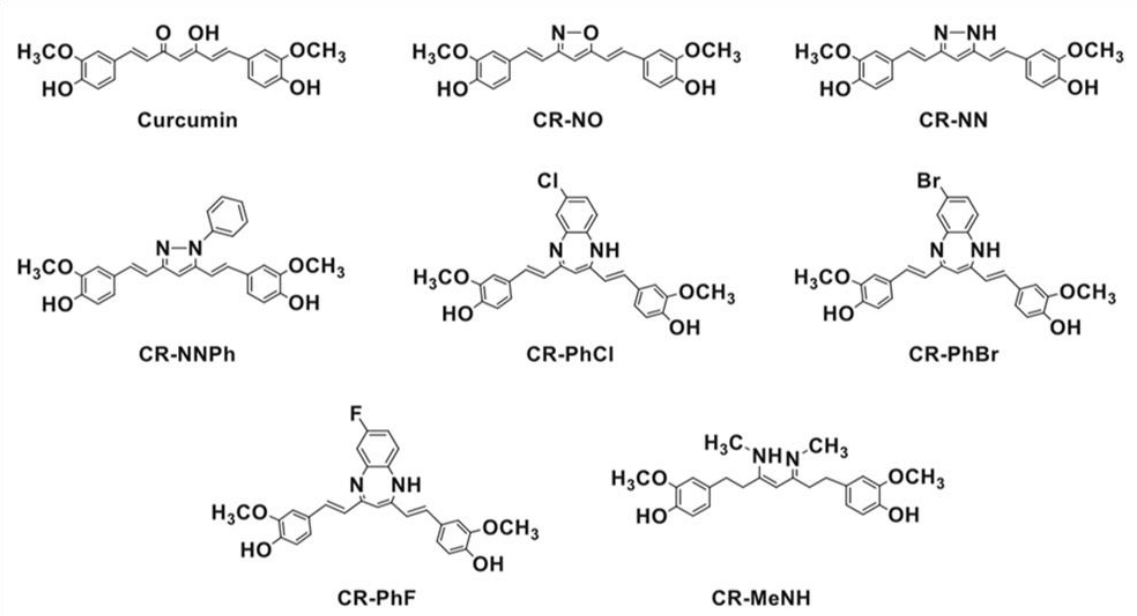


Fig. 1.21 The chemical structures of the prepared curcumin derivatives [84].

1.8 Specific aims

Curcumin is a component of the Indian spice turmeric, isolated from the rhizome of the perennial herb Curcuma longa L. Since the second half of the last century, this naturally occurring polyphenol has attracted enormous attention because of its multiple biological activities, and pharmacological applications, including anti-oxidation, anti-bacterial activities, anti-fungal, anti-tumor, anti-inflammatory antiviral and anti-cancer activities [71, 79, 85, 86]. So, the specific aims of this study are:

1. To prepare novel sets of curcumin-based diazepines, benzodiazepines, diazoles, and amines.

2. To prepare curcumin-based compounds with capped phenolic hydroxyl group.
3. To characterize the prepared curcumin-based compounds using IR, H-NMR, and C13.
4. To identify the antibacterial activities and genotoxicity of the prepared compounds.
5. To investigate the synergistic effect of curcumin-based heterocyclics with ampicillin antibiotic.

CHAPTER TWO

EXPERIMENTAL PART

1. Materials and procedures

All chemical including solvents and reagents were purchased from Aldrich Chemical Company and used without any further purification unless otherwise specified. Some of these reagents include ethanol, methanol, acetone, dimethyl sulfoxide (DMSO), H_2SO_4, glacial acetic acid, hexane, diethyl ether, ethyl acetate, NaOH, $NaHCO_3$, $MgSO_4$, curcumin, diaminomaleonitrile, diaminopyridien, 5, 6-diamino-2, 3-pyrazindicarbonitrile, 2-Hydrazinopyrimidine hydrate, ethylene diamine, phenylenediamine, 2-chlorophenylhydrazin hydrogen chloride, 2, 3-diamino-5-bromopyridine, 2, 3-diamino-5-bromopyrizine, Methyl iodide, TLC plates pre-coated with Merck Kieselgel 60 F254, opened capillary tubes, molecular sieve beads.

Infrared (IR) spectra were recorded by using FTIR Spectrum 820 PC FT-IR, Shimadzu.

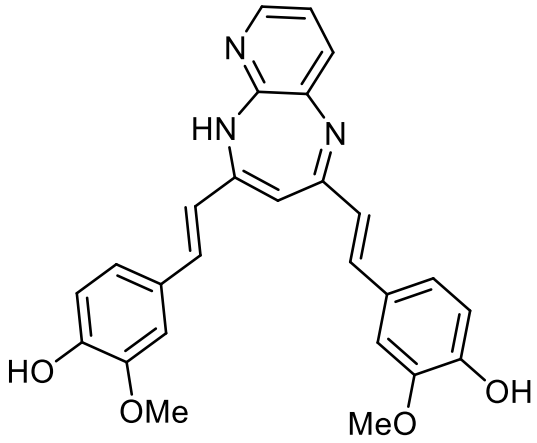
All ^1H NMR experiments are reported in δ units, parts per million (ppm) downfield from tetramethylsilane (internal standard) and were measured relative to the signal for DMSO-d6 (2.5ppm). Nuclear Magnetic Resonance spectra were recorded on a Varian VXR S400 NMR spectrometer with a proton resonance frequency of 400 MHz.

1.1 Preparation of curcumin-based benzodiazepines

General Procedure A

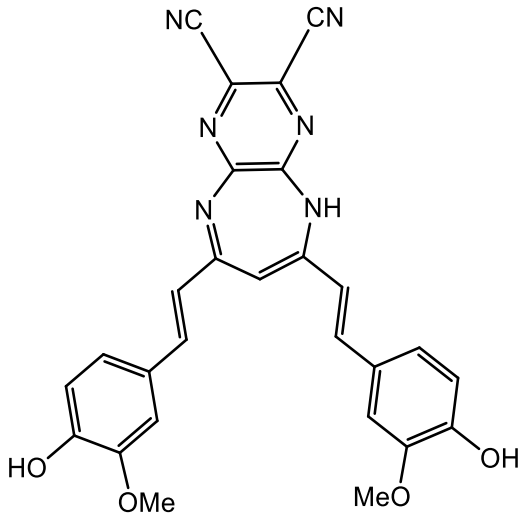
To a solution of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmole) in ethanol (30.0 mL) in a single-neck round bottom flask diamino compound (1.357 mmole) and a catalytic amount of H_2SO_4 (3-5 drops) were added. The reaction mixture was stirred under reflux for (12-60 h) at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as an eluting solvent. After cooling the reaction down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with sodium bicarbonate ($NaHCO_3$, 5%) to remove the excess acid, filtered, washed again several times with water, dried. The purification was completed by washing the solid product with diethyl ether and ethyl acetate several times and dried.

1.1.1 4-[(E)-2-{2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-5H-pyrido[2,3-b] [1,4] diazepin-4-yl}ethenyl]-2-methoxyphenol (2)



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 2,3-diaminopyridine (0.148 g, 1.357 mmol) was added followed by H_2SO_4 (3 drops) **According to procedures A**. Produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.35 g (Yield 58.5%) of yellow solid, mp. 118-120 °C, IR: ν_{max} cm^{-1} 3344 (O-H, and N-H stretching), 3022 (=C-H), 2974 (C-H, aliphatic), 1605 (C=N), 1584 (C=C, conjugated), 1389 (C-N), 1085 (C-O ether and alcohol). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3), 4.0 (bs, 1H, NH), 5.06 (1H, d), 5.35 (bs, 2H, OH), 5.67 (d, 1H, *J = 15.1 Hz*), 6.80-6.98 (m, 7H); 7.13 (m, 1H), 7.26 (m, 2H); 7.36 (d, 1H, *J = 7.5 Hz*); 8.12 (d, 1H).^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.1, 88.69, 111.9, 113.0, 116.8, 122.9, 124.0, 127.6, 132.6, 135.0, 138.1, 146.6, 147.9, 149.1, 149.5, 160.0, 164.6.

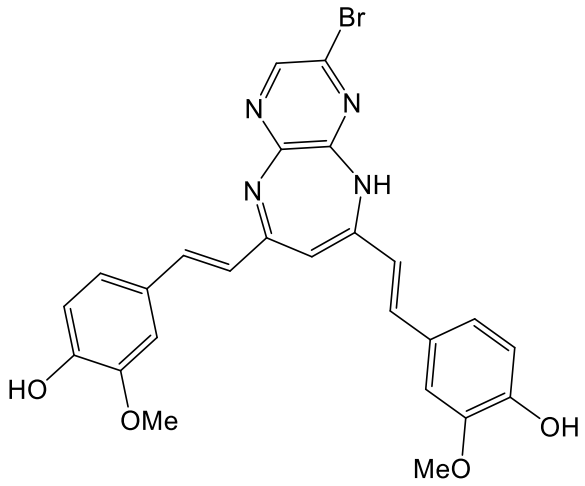
1.1.2 6,8-bis[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-5H-pyrazino [2,3-b][1,4]diazepine-2,3-dicarbonitrile (3).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 5,6-diamino-2,3-pyrazindicarbonitrile (0.217g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.67g (Yield 99.55%) of brown solid, mp 206-208°C, IR ν_{max} cm^{-1}: 3338.69 (N-H), 3157.25 (=C-H), 2961.48 (C-H), 2231.89 (C≡N stretch), 1671 (C=N), 1628.62 (C=C). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.81 (s, 6H, 2 OCH_3), 4.1 (bs, 1H, NH), 5.09 (1H, s), 5.41 (bs, 2H, OH), 5.67 (d, 1H, *J = 15.1 Hz*), 6.81 (m, 3H), 6.83 (d, 1H); 6.88 (d, 1H); 6.99 (d, 2H); 7.21 (d, 2H, *J = 7.5 Hz*).^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.2, 112.1, 117.3, 122.9,

127.9, 131.3, 135.1, 124.4, 137.9, 148.1, 149.3, 149.7, 154.5, 147.5, 155.2, 160.2, 164.5.

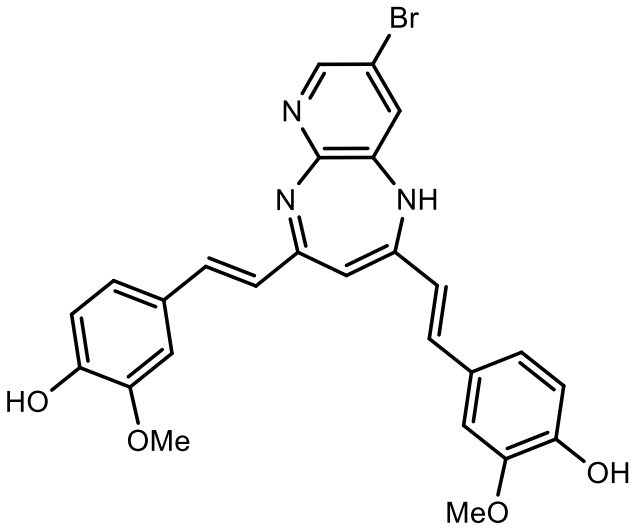
1.1.3 4-[(E)-2-{3-bromo-8-[(E)-2-(4-hydroxy-3-ethoxyphenyl)ethenyl]-5H-pyrazino[2,3-b][1,4]diazepin-6-yl}ethenyl]-2-methoxyphenol (4).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.25 g, 0.6785 mmol), was dissolved in ethanol (15.0 mL) and then, 2, 3-diamino-5-bromopyrizine (0.1276 g, 0.678 mmole) was added followed by H_2SO_4 (1 drop) According to procedures A. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.31g (Yield 87.85%), of yellow solid, mp 88-90°C, IR (KBr): ν_{max} cm^{-1} 3384.14 (-C-N-H), 1621.33 (-C=N), 542.15 (C-Br). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.82 (s, 6H, 2 OCH3), 3.98 (bs, 1H, NH), 5.06 (1H, s), 5.35 (bs, 2H, OH), 5.67 (d, 1H, J = 15.1 Hz), 6.79 (m, 2H), 6.85 (d, 1H, J = 15.1 Hz), 6.86 (d,

2H); 6.99 (d, 2H); 7.16 (d, 2H, J = 7.5 Hz), 7.96 (s, 1H).^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.2, 103.1, 111.5, 116.4, 121.2, 122.5, 124.2, 135.6, 139.3, 147.2, 149.3, 150.6, 159.3, 164.8.

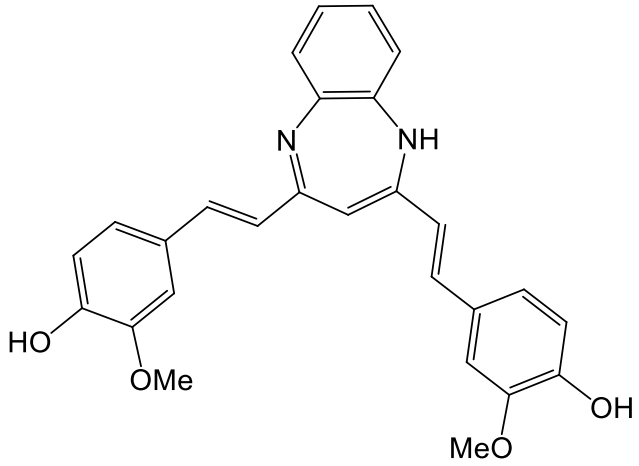
1.1.4 4-[(E)-2-{8-bromo-4-[(E)-2-(4-hydroxy-3- ethoxyphenyl) ethenyl]-1H-pyrido[2,3-b][1,4]diazepin-2-yl}ethenyl]-2-methoxyphenol (5).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 2, 3-diamino-5-bromopyridine (0.2538 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.34 g (yield 48.14%) of brown solid. mp 108-110°C, IR (KBr): ν_{max} cm^{-1} 1623.19 (-C=N), 3374.27 (–C–NH), 568.88 (C-Br), and 1030.66 (C-O ether) of (–O-CH_3). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3), 4.02 (bs,

1H, NH), 5.05 (1H, s), 5.33 (bs, 2H, OH), 5.68 (d, 1H, J = 15.1 Hz), 6.81 (m, 4H), 6.87 (d, 1H, J = 15.1 Hz); 6.97 (d, 2H, J = 7.5 Hz); 7.16 (s, 2H), 7.67 (s, 1H), 8.14 (s, 1H).^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.2, 103.1, 111.5, 116.4, 121.2, 122.5, 123.2, 124.2, 135.6, 139.3, 147.2, 149.3, 150.6, 159.3, 164.8.

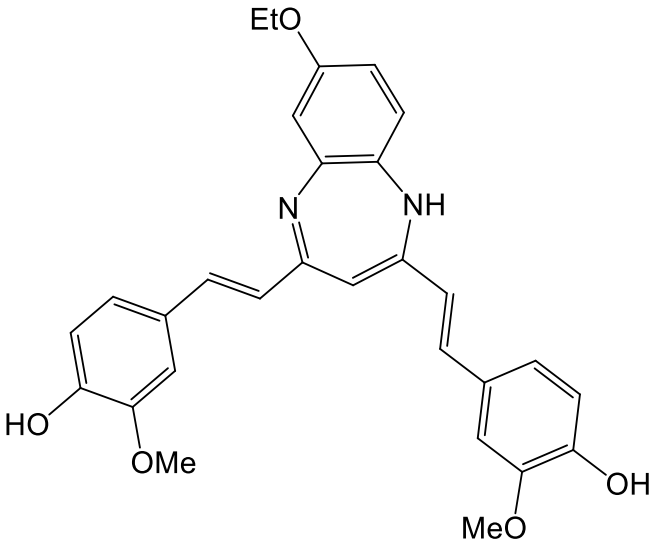
1.1.5 4-[(E)-2-{4-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-1,5-benzodiazepin-2-yl}ethenyl]-2-methoxyphenol (6).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, phenylenediamine (0.239 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.5 g (yield 83.6%) of yellow solid. Melting point is 144 -145°C. IR: ν_{max} cm^{-1} 3350 (-C-OH), 3020 (=C-H), 1640 (-C=N), 1600 (C=C), 1180 (C-O ether), 1220

(C-N). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.89 (s, 6H, 2 OCH_3), 5.54 (bs, 2H, OH), 5.90 (s, 1H), 6.78 (d, 2H, J = 17.6), 6.80 (d, 2H, J = 6.95 (d, 2H, J = 17.6), 7.05- 7.14 (4H, m), 7.33-7.41 (m, 4H, J = 12.3 Hz), 7.68 (d, 1H, J = 12.3 Hz). ^{13}C-NMR (400 MHz, DMSO-d6) δ: 28.8, 733., 45.3, 56.42, 113.7, 121.5, 125.3, 130.0, 133.1,140.5, 144.9, 147.9, 165.5.

1.1.6 4-[(E)-2-{7-ethoxy-2-[(E)-2-(4-hydroxy-3- ethoxyphenyl)ethenyl]-1H-1,5-benzodiazepin-4-yl}ethenyl]-2-methoxyphenol (7).

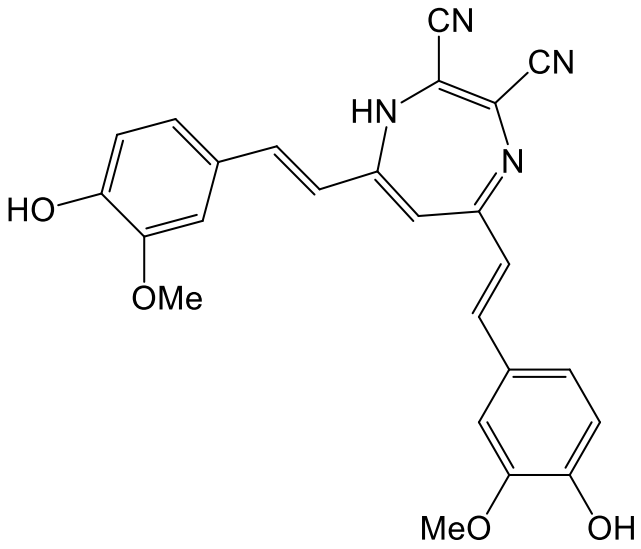


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 4-ethoxybenzene-1,2-diamine (0.205g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.48 g (yield 73.1%) of yellow solid. Melting point is 208 - 210°C. IR: ν_{max} cm^{-1}

3394.26 (O-H, N-H stretching), 1624.17 (C=N), 1513.50 (C=C conjugated), 1085 (C-O ether and alcohol). ^{1}H-NMR (400 MHz, DMSO-d6) δ: ^{1}HNMR δ:1.32 (t, 3H, CH_3 methyl), 3.83 (s, 6H, 2 OCH3, methyl), 4.0 (s, 1H, NH), 4.09 (q, 2H, CH_2, methylene), 5.06 (s, 1H, CH), 5.35 (s, 2H, OH), 5.67 (d, 1H, CH), 6.02 (d, 1H, CH), 6.62 (s, 1H, CH), 6.74 (d, 1H, CH), 6.79 (d, 2H, CH benzene), 6.81 (d, 1H, CH), 6.85 (d, 1H, CH), 6.99 (d, 2H, CH benzene), 7.16 (s, 2H, CH benzene). ^{13}C-NMR (400 MHz, DMSO-d6) δ: 14.8, 56.1, 64.6, 86.6, 109.2, 111.9, 113.7, 116.6, 116.8, 118.2, 122.9, 124.3, 127.6, 129.7, 135.3, 138.0, 138.1, 147.9, 148.7, 149.1, 155.4, 164.6.

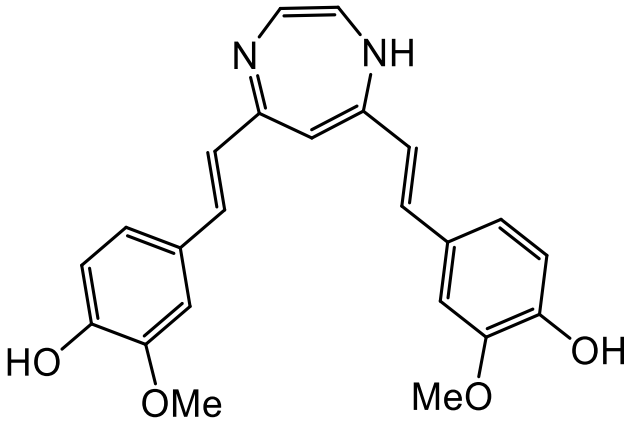
1.2 Preparation of curcumin based diazepines

1.2.1.1 5,7-bis[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-1,4-diazepine-2,3-dicarbonitrile (8).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, diaminomaleonitrile (0.146 g, 1.357 mmole) was added, followed by H_2SO_4 (3 drops). **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.137 g, (Yield 22.9 %) of yellow solid, mp 250-254 °C, IR: ν_{max} cm^{-1} 3417.74 (O-H), 3366.66 (N-H), 2362.39 (C≡N), 1650.55 (C=N), 1558.32 (C=C). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.82 (s, 6H, OCH3), 4.05 (bs, 1H, NH), 5.10 (1H, s), 5.41 (bs, 2H, OH), 5.71 (d, 1H, J = 15.1 Hz), 6.82 (m, 4H), 7.03 m, 3H; 7.25 (d, 2H, J = 7.5 Hz).^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.1, 103.6, 105.0, 11.3, 138, 1147.9, 115.2, 116.8, 120.2, 122.9, 124.3, 127.6, 135, 149.1, 149.4, 164.6.

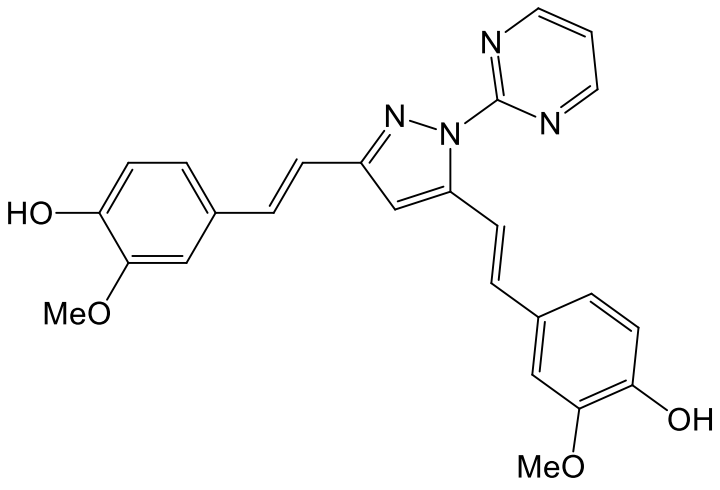
1.2.1.2 4-[(E)-2-{5-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-2,3-dihydro-1H-1,4-diazepin-7-yl}ethenyl]-2-methoxyphenol (9).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, ethylenediamine (4 drops, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.27 g, (Yield 50.7%) of brown solid, mp 198-200°C, IR: ν_{max} cm^{-1}: 3299.21 (N-H), 1512.99 (C=N), 1584.71 (C=C), 1277.27 (C-N), 817.81 (C-H). ^{1}HNMR (400 MHz, DMSO-d6) δ: 3.16 (s, 2H, CH_2), 3.764 (s, 4H, CH_2CH_2); 3.78 (s, 6H, 2 OCH_3), 5.72 (s, 2H, OH), 6.85 (m, 2H), 6.91 (d, 2H, J = 15.2 Hz), 7.05 (d, 2H, J = 12.1 Hz), 7.20 (s, 2H), 7.40- 7.60 (m, 2H). ^{13}CNMR (400 MHz, DMSO-d6) δ: 24.5, 48.3, 56.4, 112.9, 116.3, 120.6, 122.4, 127.8, 129.3, 148.2, 149.3, 165.8.

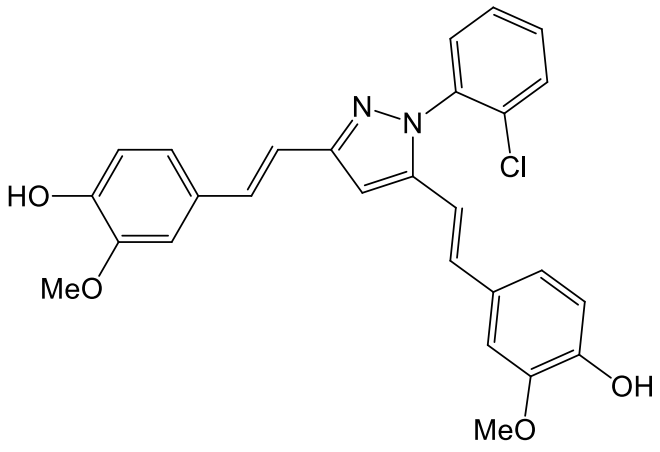
1.2.2 Five membered heterocyclic compounds

1.2.2.1 4-[(E)-2-{5-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1-(pyrimidin-2-yl) -1H-pyrazol-3-yl}ethenyl]-2-methoxyphenol (10).



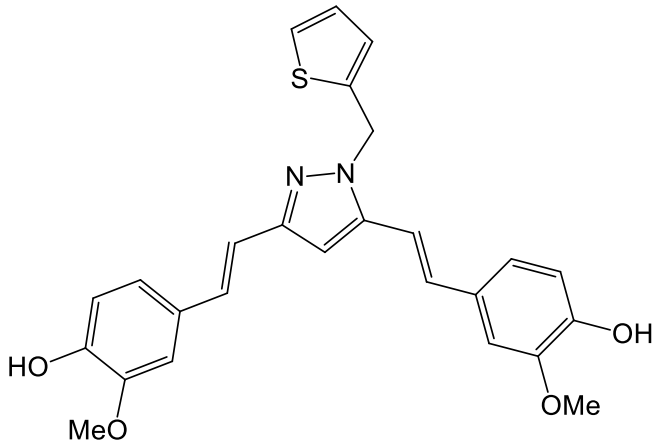
1,7- Bis (4- hydroxy -3- methoxyphenyl) hepta -1, 6- diene -3,5 - dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 2-Hydrazinopyrimidine hydrate (0.15 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.4 g, (Yield 66.66%) of yellow solid, mp 88-90°C, IR (KBr): ν_{max} cm^{-1} 1639.9 (-C=N), 1061 (C-O ether) of (–O-CH_3), and 1214.80 (N-N). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.81 (s, 6H, OCH3), 5.38 (sb, 2H, OH), 6.76 (s, 1H), 6.97 (m, 6H); 7.12 (d, 2H, J = 7.7 Hz), 7.18 (m, 2H, J = 7.7 Hz); 7.68 (m, 1H), 8.83 (d, 2H, J = 7.9 Hz). ^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.2, 107.5, 109.3, 116.4, 116.6, 118.5, 122.7, 123.8, 130.3, 131.4, 147.3, 147.5. 148.1, 149.3, 155.7, 156.6.

1.2.2.2 4- [(E)-2- [1- (2-chlorophenyl) -5- [(E) -2- (4- hydroxy-3-methoxyphenyl) ethenyl] -1H-pyrazol-3-yl] ethenyl] -2-methoxyphenol (11).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 2-chlorophenylhydrazin hydrogen chloride (0.252 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops). **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.61 g (Yield 80.3 %) of yellow solid, mp 124-126 °C, IR: ν_{max} cm^{-1} 1637.15 (-C=N), 1098.91 (C-O ether) of (–O-CH_3). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, OCH_3), 5.37 (sb, 2H, OH), 6.79 (s, 1H), 6.93 (m, 4H); 7.14 (d, 2H, *J = 7.8 Hz*), 7.17 (d, 2H); 7.36-7.61 (m, 4H); ^{13}C-NMR (400 MHz, DMSO-d6) δ: 56.4, 109.1, 110.5, 116.6, 116.7, 119.3. 123.1, 123.6, 127.7, 130.3, 133.2, 139.8, 143.5, 147.7, 149.3, 154.2.

1.2.2.3 4- [(E)-2- {5- [(E)-2- (4-hydroxy-3-methoxyphenyl) ethenyl] -1-[(thiophen-2-yl) methyl] -1H-pyrazol-3-yl} ethenyl] -2- methoxyphenol (12).

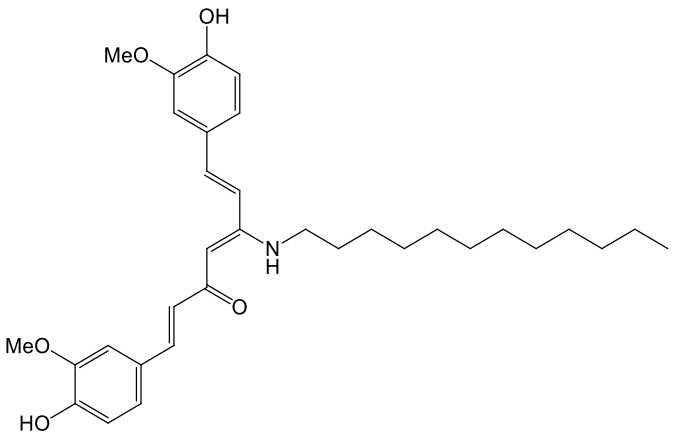


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, (2-thienylmethyl)hydrazine hydrochloride (0.224 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops). **According to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.51 g (yield 74 %) of brown solid, m.p 130-133 °C, IR: ν_{max} cm^{-1} 3406.93 (O-H), 1578 (C=N), 1513.90 (C=C), 1029.11 (C-O). ^{1}HNMR δ: 3.83 (s, 6H, 2 OCH_3), 4.99 (s, 2H, CH_2, methylene), 5.35 (s, 2H, OH), 6.53 (s, IH, CH 1-pyrazole), 6.83 (d, IH, CH 2-thiophene), 6.88 (d, 1H, CH 1-benzene), 6.93 (t, IH, CH 2-thiophene), 6.95 (d, 2H), 6.99 (d, 2H), 7.12 (d, 2H, CH benzene), 7.16 (s, 2H, CH 1-benzene), 7.40 (d, IH, CH 2-thiophene).

^{13}CNMR δ: 52.9, 56.1, 108.1, 109.2, 116.1, 116.8, 122.9, 123.5, 125.5, 126.7, 127.0, 130.5, 131.2, 138.2, 139.4, 147.9, 149.1, 152.2.

1.3 Preparation of curcumin-based amines

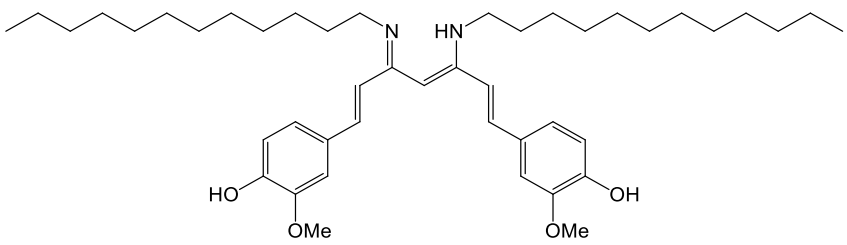
1.3.1 1E,5E,6E)-5-(dodecylimino)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (13).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, dodecyl amine (0.28 g, 1.357 mmole) was added followed by H_2SO_4 (3 drops), **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.82 g (yield 99.2 %) of yellow solid. Melting point is 115 -117 °C, IR: ν_{max} cm^{-1} 3300 (O-H, and N-H stretching), 3022 (=C-H), 2730- 2914 (C-H, aliphatic), 1562 (C=C, conjugated), 1389 (C-N), 1085 (C-O ether and alcohol). ^{1}HNMR (400 MHz, DMSO-d6) δ: 0.88 (t, 3H, CH_3 methylene), 1.26-2.87 (m, 22H, CH_2 methylene), 2.0 (s, 1H, NH), 3.83 (s, 6H, 2 OCH_3), 5.35 (s, 2H, OH), 5.48 (s, 1H, CH), 6.79 (d, 2H, CH), 6.81 (d, 1H, CH), 6.85 (d, 1H, CH), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H, CH), 7.16 (d, 2H, CH benzene), 7.82 (d, 1H, CH ethylene).

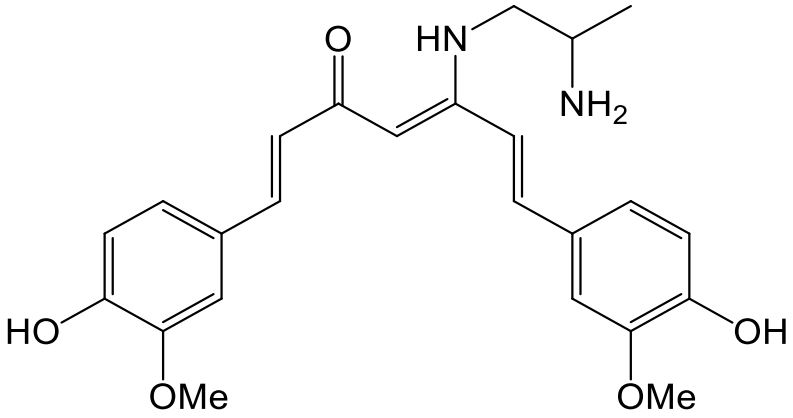
^{13}CNMR (400 MHz, DMSO-d6) δ: 14.1, 22.7, 27.1, 29.3, 29.6, 31.0, 31.9, 44.3, 56.1, 105.6, 111.9, 116.8, 122.9, 123.3, 124.3, 127.6, 135.3, 142.2, 147.9, 149.1, 172.4, 188.6.

1.3.2 4- [(1E,3Z,5E,6E) -3,5- bis (dodecylimino)-7- (4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-1-yl]-2- methoxyphenol (14).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, dodecyl amine (2.714 mmol, 0.56 g) was added followed by H_2SO_4 (3 drops), **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.69 g (yield 95.1 %) of brown solid. Melting point is 212 - 213 °C, IR: ν_{max} cm^{-1} 3343 (O-H, and N-H stretching), 3012 (=C-H), 2713- 2880 (C-H, aliphatic), 1620 (C=C, conjugated), 1389 (C-N), 1060 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 0.88 (t, 6H, CH_3 methylene), 1.26 (t of t, 12H, CH_2 methylene), 1.29-1.52 (m, 40H, CH_2 methylene, 2.0 (bs, 1H, NH), 2.87 (t, 2H, CH_2), 3.83 (s, 6H, 2 OCH_3), 4.12 (s, 1H, CH), 5.35 (bs, 2H, OH), 5.67 (d, 1H, CH), 6.79 (d, 1H, CH), 6.81 (d, 1H, CH), 6.85 (d, 1H, CH), 6.88 (d, 2H, CH benzene), 6.99 (d, 2H, CH benzene), 7.16 (s, 2H, CH benzene). ^{13}CNMR (400 MHz, DMSO-d6) δ:14.1, 22.7, 27.1, 27.2, 29.3, 29.6, 31.0, 31.9, 32.1, 44.3, 44.7, 56.1, 94.5, 111.9, 116.6, 116.8, 124.3, 122.9, 127.6, 135.3, 138.1, 147.9, 149.1, 153.1, 164.6.

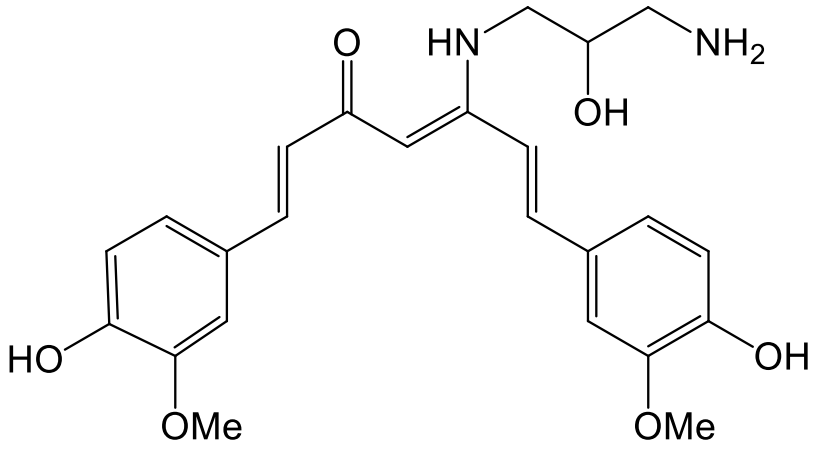
1.3.3 (1E,5E,6E) -5- [(2-aminopropyl) imino] -1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (15).



1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 1,2-diaminopropane (0.115 mL, 0.1005g, 1.357 mmole) was added followed by H_2SO_4 (3 drops) **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.4 g (yield 69.5 %) of yellow solid. Melting point is 170 -172 °C, IR: ν_{max} cm^{-1} 3407 (N-H stretching, aliphatic primary amine), 3310 (O-H, and N-H stretching), 3020 (=C-H), 2780 (C-H, aliphatic), 1611 (C=C, conjugated), 1370 (C-N), 1034 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 1.12 (d, 3H, CH_3), 2.0 (bt, 1H, NH), 2.75, 3.03 (m, 1H, CH), 3.83 (s, 6H, 2 OCH_3), 5.11(bd, 2H, NH_2), 5.48 (s, 1H), 6.79 (d, 1H, CH), 6.81 (d, 1H, CH), 6.85 (d, 1H), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H) 7.18 (s, 1H, CH benzene), 7. 3 (d, 1H, CH benzene), 7.82 (d, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 21.2, 48.0, 53.7, 56.1, 105.6, 111.9, 116.8, 122.9, 123.3, 124.3, 127.6, 135.3, 142.2, 147.9, 149.1, 172.4, 188.6.

1.3.4 1E, 5E, 6E) -5- [(3-amino-2-hydroxypropyl) imino] -1,7- bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (16).



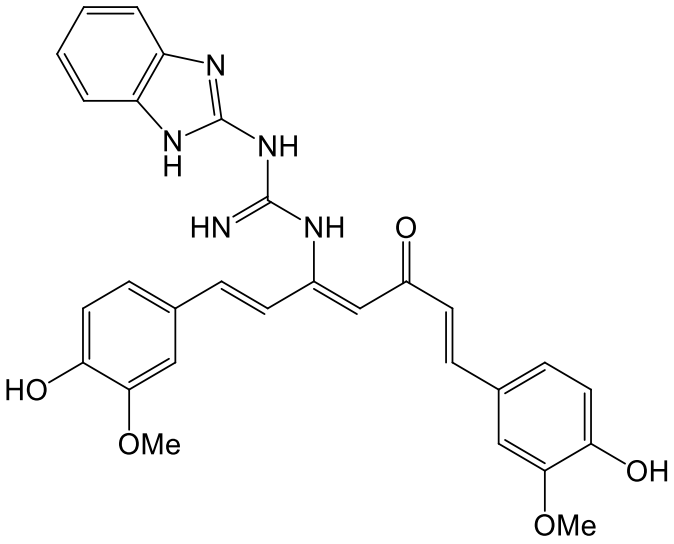
1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, 1,3-diamino-2-propanol (0.122 g, 1.357 mmole) was added followed by H_2SO_4 (2 drops) **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.32 g (yield 53.5 %) of yellow solid. Melting point is 240 -242 °C, IR: ν_{max} cm^{-1} 3370 (N-H stretching, aliphatic primary amine), 3293 (O-H, and N-H stretching), 3035 (=C-H), 2780 (C-H, aliphatic), 1617 (C=C, conjugated), 1345 (C-N), 1025 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 2.92 & 2.67 (m, 2H, CH_2), 2.98 & 2.73 (m, 2H, CH_2), 3.58 (bd, 1H, OH alcohol), 2.0 (bt, 1H, NH), 3.69 (m, 1H,

CH), 3.83 (s, 6H, 2 OCH_3), 5.11(bt, 2H, NH_2), 5.35 (bs, 2H, OH), 5.48 (s, 1H), 6.79 (d, 2H, CH benzene) 6.81 (d, 1H, CH), 6.85 (d, 1H), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H), 7.16 (s, 2H, CH benzene), 7.81 (d, 1H), 7.82 (d, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 47.5, 49.6, 56.1, 74.1, 105.6, 111.9, 116.8, 122.9, 123.3, 124.3, 127.6, 135.3, 142.2, 147.9, 149.1, 172.4, 188.6.

1.3.5 (E)-N-(1H-1,3-benzodiazol-2-yl) -N'-[(1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl) -5-oxohepta-1,6-dien-3-ylidene]guanidine (17).

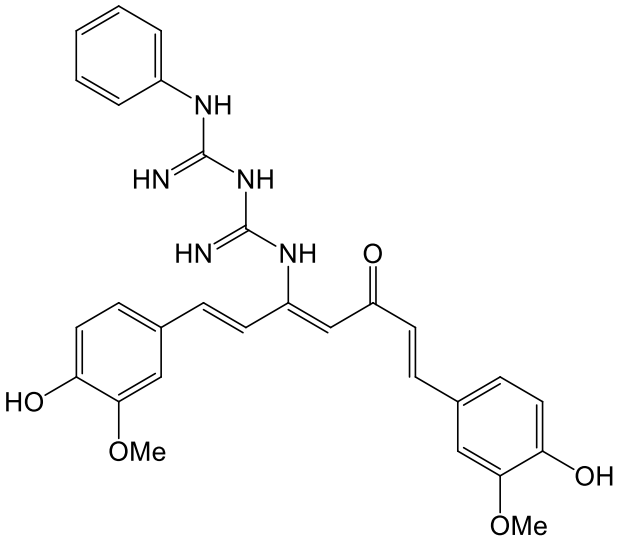


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, Guanidinobenzimidazole (0.473g, 1.357 mmole) was added followed by H_2SO_4 (2 drops) **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.56 g (yield 78.6 %) of brown solid. Melting point is 330-333°C, IR: ν_{max} cm^{-1} 3333

(N-H stretching), 3288 (O-H, and N-H stretching), 3033 (=C-H), 1654 (C=N, imine), 1614 (C=C, conjugated), 1345 (C-N stretching), 1025 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (bs, 1H, NH Amine), 3.83 (s, 6H, 2 OCH_3), 4 (bs, 1H, NH Amine), 5 (bs, 1H, NH benzimidazole), 5.35 (bs, 2H, OH), 6.75 (s, 1H), 6.79 (d, 1H, CH 1-benzene), 6.81 (d, IH), 6.82 (d, IH), 6.85 (d, 1H), 6.99 (d, 1H, CH 1-benzene), 7.03 (d, 1H), 7.12 (d, 2H, CH benzene), 7.16 (d, 2H, CH 1-benzene), 7.22 (d, 2H, CH 1-benzene), 7.82 (d, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 105.6, 111.9, 116.8, 115.2, 122.9, 123, 123.3, 124.3, 127.6, 135.3, 142.2, 147.9, 149.1,158.5, 172.4, 188.6.

1.3.6 N-[(1E,3E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,6-dien-3-ylidene]-1-(N'-phenylcarbamimidamido)methanimidamide (18).

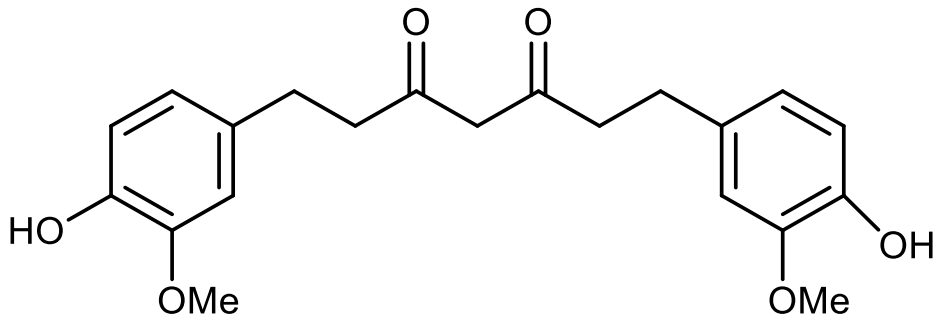


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, Phenylbiguanide (0.48 g, 1.357 mmole) was added followed by H_2SO_4 (2 drops) **according to procedures A**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.62 g (yield 86.7 %) of brown solid. Melting point is 323-326 °C, IR: ν_{max} cm^{-1} 3390 (N-H stretching), 3260 (O-H, and N-H stretching), 3047 (=C-H), 1662 (C=N, imine), 1614 (C=C, conjugated), 1288 (C-N stretching), 1027 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (bs, 2H, NH Amine), 3.83 (bs, 6H, OCH3, methyl), 4 (bs, 1H, NH Amine), 5.35 (bs, 2H, OH), 6.75 (s, 1H), 6.81 (d, IH), 6.81(t, 1H, CH benzene), 6.85 (d, 1H), 6.79 (d, 1H, CH 1-benzene), 6.99 (d, 1H, CH 1-benzene), 7.03 (d, 1H), 7.16 (d, 2H, CH 1-benzene), 7.2 (t, 2H, CH 1-benzene), 7.77 (d, 2H, CH benzene), 7.82 (d, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 105.6, 111.9, 116.8, 121.5, 122.4, 122.9, 123.3, 124.3, 127.6, 129.5, 135.3, 138.5, 142.2, 147.9, 149.1, 158.5, 172.4, 188.6

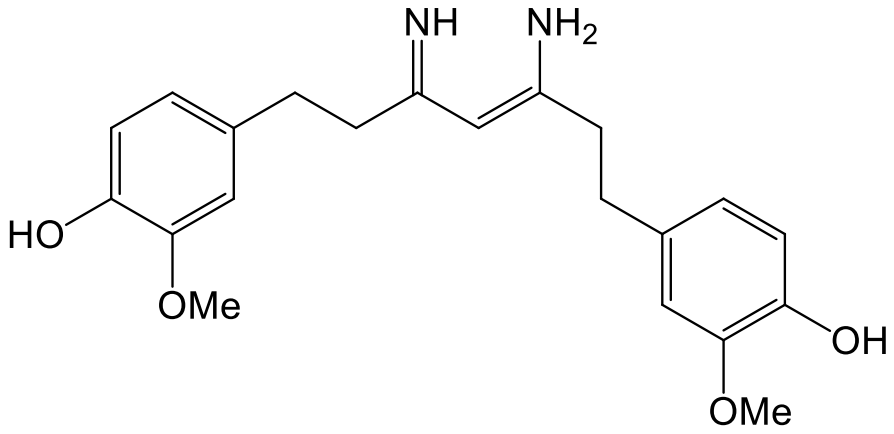
1.4 Preparation of H-Curcumin Based Amines

1.4.1 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-dione (19a).



A low-pressure reaction bottle was charged with a solution of curcuminoids 5.0 g in absolute ethanol (100 ml) and in the presence of Pd/C (0.3 g) which was used as a heterogeneous catalyst. The bottle was attached to the low-pressure hydrogenation apparatus and evacuated, and then hydrogen was admitted to a pressure slightly above 3 atm. The contents of the flask were shaken until absorption of hydrogen stopped (about 4 hrs). The catalyst was removed by filtration and ethanol was removed under vacuum to afford 4.6 g (91.8%) of pale-yellow gummy material. The gummy material was purified by flash chromatography using ethyl acetate as eluent. The produced THC 19a, was analyzed by 1H NMR and 13C NMR. ^{1}H NMR (300 MHz) ($CDCl_3$): 6.8 (d, *J* = 8.24, 2H), 6.62 (d, *J* = 8.42 =2H), 6.6 (s, 2H), 5.6 (bs, 2H, OH), 5.4 (s, 0.75 H, vinylic), 3.90 (s, 0.5H, diketone), 3.85 (s, 6H, 2 OCH_3), 2.9 (t, *J* = 7.97, 3H), 2.6 (t, *J* = 7.14, 3H). ^{13}C NMR ($CDCl_3$ d6) δ: 193.2, 144.5, 143.9, 132.5, 120.7, 114.3, 110.9, 99.8, 55.8, 40.4, 31.1.

1.4.2 4-[(4Z)-5-amino-7-(4-hydroxy-3-methoxyphenyl)-3-iminohept-4-en-1-yl]-2-methoxyphenol (19).



To a 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (H-curcumin) compound **19a** (0.5g, 12.5 mL, 1.357 mmol) in a single-neck round bottom flask, Ammonium acetate (0.313g, 1.357 mmol) was added. The mixture was stirred under reflux at 60 °C until complete solvent evaporation. Then it was heated again at 60°C for an hour. The mixture was regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system to a H-curcumin reference. The residue was washed with water and dried. The dry solid was washed with diethyl ether and ethyl acetate several times and dried. Then it was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.3 g (yield 60 %) of yellow solid. Melting point is 160-163°C, IR: ν_{max} cm^{-1} 3216.78 (C-NH), (C=N), 2939.29 (C-N), 1605.51 (C=C), 1515.17 (C=N).

^{1}HNMR (400 MHz, DMSO-d6) δ: 1.86 (t, 2H, CH_2), 2.29 (t, 2H, CH_2), 2.56 (t, 4H, CH_2), 3.83 (s, 6H, 2 OCH_3), 3.88 (s, 1H), 5.35 (bs, 2H, 2OH),

6.68 (d, 2H, CH benzene), 6.71 (s, 2H, CH benzene), 6.79 (d, 2H, CH benzene) 8.56 (bs, 2H, NH_2).

^{13}CNMR (400 MHz, DMSO-d6) δ: 29.7, 34.8, 35.7, 36.7, 51.7, 56.1, 113.2, 115.5, 122.5, 133.0, 145.9, 147.1, 147.4, 164.6.

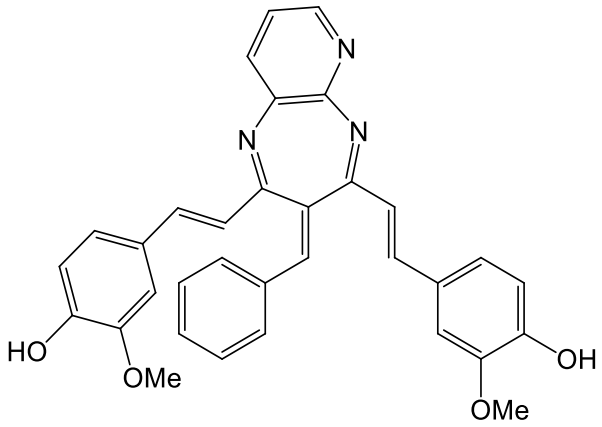
1.5 Preparation of curcumin -based compounds using Knoevenagele Doebner Condensation

General Procedure B

To a solution 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmole) in ethanol (30.0 mL) in a single-neck round bottom flask, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5mI) or tri ethanol amine (TEA) (4 drops), were added. The mixture was stirred under reflux for 30 h at 120°C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as an eluting solvent. then diamino compound (1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours). The reaction progress was monitored using TLC to a curcumin reference. After cooling the reaction down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with sodium bicarbonate ($NaHCO_3$, 5%) to remove the excess acid, filtered, washed again several times with water, dried. The purification was completed by washing the solid product with diethyl ether and ethyl acetate several times and dried.

1.5.1 Seven Membered Curcumin-Based Benzodiazepines

1.5.1.1 4-[(E)-2-[(3E)-4-[(E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl]-3 (phenylmethylidene) -3H -pyrido [2,3-b] [1,4] diazepin-2-yl] ethenyl]-2 methoxyphenol (20).

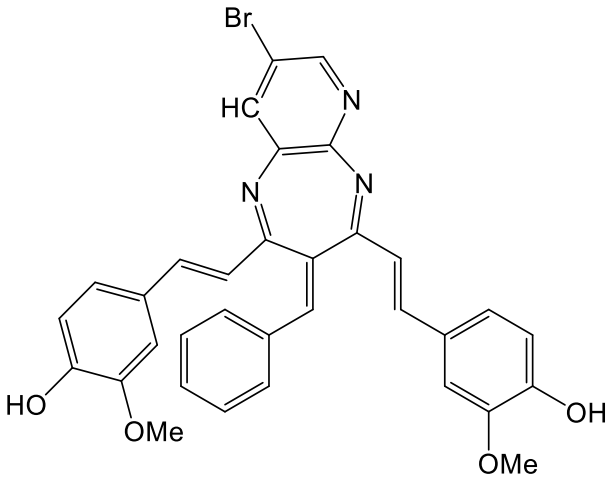


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5mI) were added. The Solution was refluxed for 30 h at 120 °C. Then 2,3-diaminopyridien (1.357 mmole, 0.15 g), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.6 g (yield 83.6%) of brown solid. Melting point is 302 -303 °C. IR: ν_{max} cm^{-1}: 3200 (O-H), 3039 (=C-H), 1616 (C=N), 1598 (C=C, conjugated), 1303 (C-N stretching), 1031 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, OCH3, methyl), 4.0 (bs, 1H, NH), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H, CH benzene), 7.10 (d, 1H, CH 2-

pyridine), 7.18 (s, 1H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.42 (t, 1H, CH 2-pyridine), 7.6 (d, 2H, CH), 8.16 (d, 1H, CH 2-pyridine).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 97.7, 111.9, 116.6, 116.8, 120.2, 121.7, 122.9, 124.3, 125.9, 127.6, 127.9, 128.5, 128.6, 131.1, 135.2, 135.3, 137.3, 138.1, 142.1, 147.9, 149.1,153.0, 158.4, 164.6.

1.5.1.2 4-[(E)-2-[(3E)-8-bromo-4-[(E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl]-3-(phenylmethylidene) -3H-pyrido [2,3-b] [1,4] diazepin-2-yl]ethenyl]-2-methoxyphenol (21).

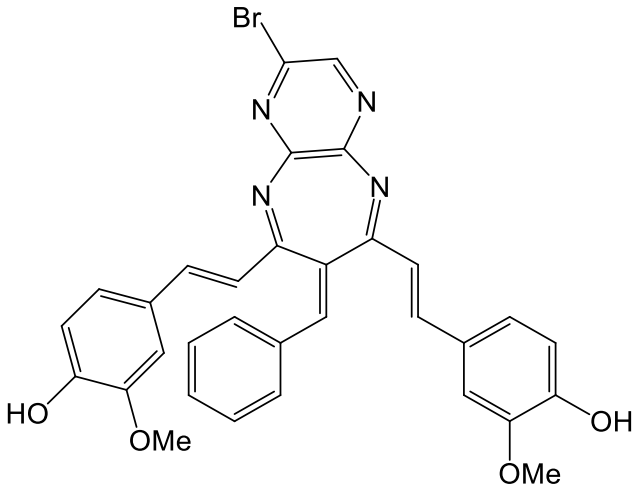


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5 mI) were added. The Solution was refluxed for 30 h at 120 °C. Then 2, 3-diamino-5-bromopyridine (0.26 g, 1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B**. The produced solid was recrystallized from

hexane/EtOAc (2:1 by volume) to give 0.76 g (yield 92%) of yellow solid. Melting point is > 350 °C. IR: ν_{max} cm^{-1}: 3200 (O-H), 3039 (=C-H), 1616 (C=N), 1598 (C=C, conjugated), 1303 (C-N stretching), 1031 (C-O ether of (–O-CH_3)), 573 (C-Br stretching).^{1}HNMR (400 MHz, DMSO-d6) δ: 4.0 (bs, 1H, NH), 3.83 (s, 6H, OCH3, methyl), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.81 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H, CH benzene), 7.18 (d, 2H, CH benzene), 7.23 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (s, 2H, CH 2-pyridine), 7.6 (d, 2H, CH benzene), 8.16 (s, 1H, CH 2-pyridine).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 97.7, 111.9, 114.5, 116.6, 116.8, 122.9, 123, 124.3, 125.9, 127.6, 127.9, 128.5, 128.6, 130, 131.1, 135.2, 135.3, 138.1,143.3, 147.9, 149.1,153.0, 157.5, 164.6.

1.5.1.3 4-[(E)-2-[(7Z)-2-bromo-8-[(E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl]-7-(phenylmethylidene)-7H-pyrazino[2,3-b] [1,4] diazepin-6-yl] ethenyl] -2-methoxyphenol (22).



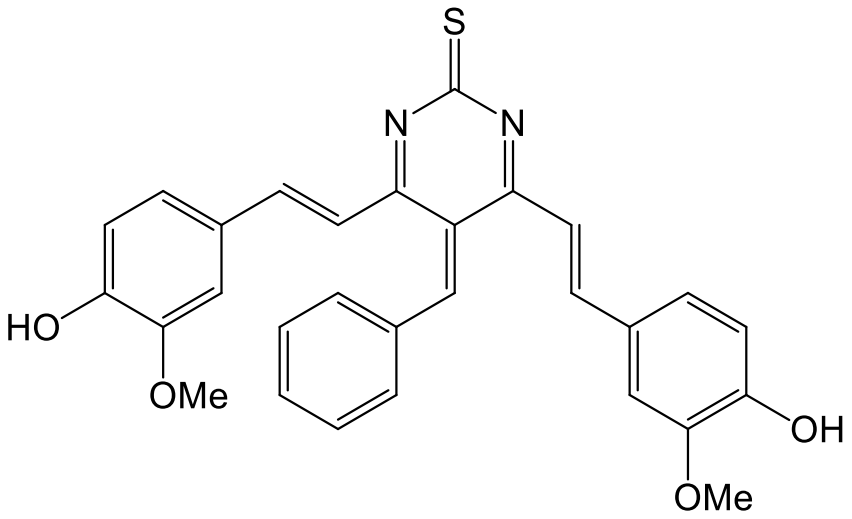
1,7-Bis(4- hydroxy-

3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5mI) were added. The Solution was refluxed for 30 h at 120 °C. Then 2, 3-diamino-5-bromopyrizine (0.26 g, 1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.73 g (yield 88.5 %) of yellow solid. Melting point is > 350 °C. IR: ν_{max} cm^{-1}: 3281 (O-H), 3043 (=C-H), 1607 (C=N), 1582 (C=C, conjugated), 1293 (C-N stretching), 1211 (C-O ether of (–O-CH_3)), 584 (C-Br stretching).^{1}HNMR (400 MHz, DMSO-d6) δ: 4.0 (bs, 1H, NH), 3.83 (s, 6H, OCH3, methyl), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH benzene), 6.81 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H, CH benzene), 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (s, 2H, CH 2-pyrazine), 7.6 (d, 2H, CH benzene), 7.96 (s, 1H, CH 2-pyrazine).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 97.7, 111.9, 116.6, 116.8, 121.0, 122.9, 124.3, 124.5, 125.9, 127.6, 127.9, 128.5, 128.6, 135.2, 135.3, 138.1,147.9, 149.1, 150.2, 151.1, 153.0, 164.6.

1.5.2 Five Membered Curcumin-Based Diazepines

1.5.2.1 4,6 - bis [(E) -2- (4- hydroxy -3- methoxyphenyl) ethenyl] -5- (phenylmethylidene) -2, 5 -dihydropyrimidine-2-thione (23).



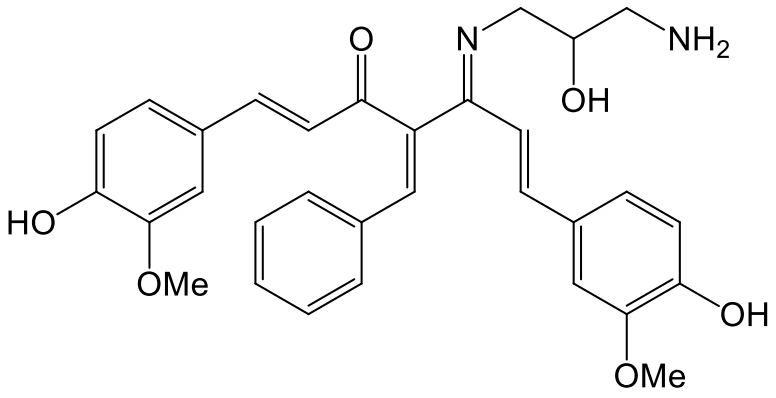
To a 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmole) in ethanol (30.0 mL) in a single-neck round bottom flask, thiourea (0.103 g, 1.357 mmole), benzaldehyde (3 drops), and a catalytic amount of tri ethanol amine (TEA) (4 drops), were added. The mixture was stirred under reflux for 30 h at 120°C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system to a curcumin reference. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with sodium bicarbonate ($NaHCO_3$, 5%) to remove the excess acid, filtered, washed again several times with water, dried. The purification was completed by washing the solid product with diethyl ether and ethyl acetate several times

and dried. Then it was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.61 g (yield 90.2%) of yellow solid. Melting point is 280-284 °C. IR: ν_{max} cm^{-1}: 3250 (O-H), 3034 (=C-H), 1611 (C=N), 1667 (C=N),1211 (C-O ether of (–O-CH_3)), 1587 (C=C, conjugated). ^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, OCH3, methyl), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH benzene), 6.81 (d, 2H, CH), 6.85 (d, 2H, CH), 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (d, 2H, CH benzene), 13.76 (bs, 1H, NH).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 111.9, 116.6, 116.8, 122.9, 124.3, 125.9, 127.6, 127.9, 128.5, 128.6, 131.1, 135.2, 135.3, 138.1,147.9, 148.7,149.1, 164.6. 180.4, 98.4

1.5.3 Curcumin Based Amines

1.5.3.1 (1E,4E,5Z,6E)-5-[(3-amino-2-hydroxypropyl)imino]-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(phenylmethylidene)hepta-1,6-dien-3-one (24).



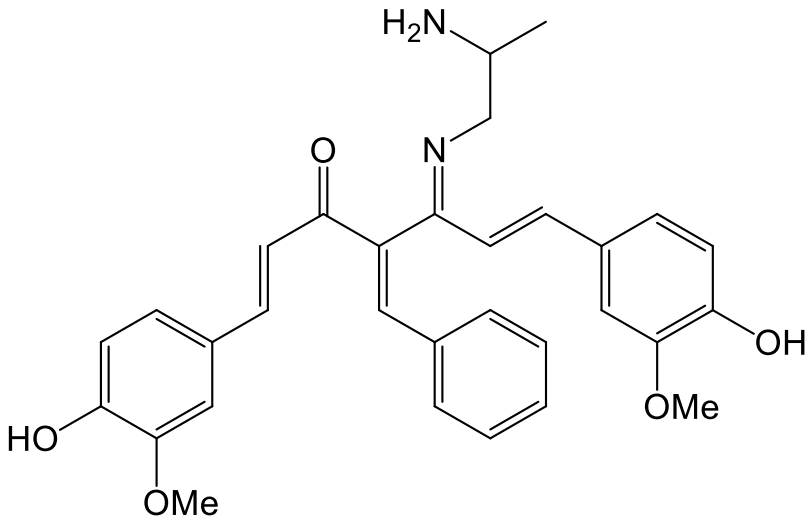
1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (20.0 mL) and then,

benzaldehyde (0.5 mL), and a catalytic amount of tri ethanol amine (TEA) (4 drops) were added. The Solution was refluxed for 30 h at 120 °C. Then 1,3-diamino-2-propanol (0.122 g, 1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (12 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.35 g (yield 52.7 %) of yellow solid. Melting point is 120 -123 °C. IR: ν_{max} cm^{-1} 3028.39 (N-H), 1574.58 (C=N), 1513.53 (C=C), 1031.78 (C-O).

^{1}HNMR (400 MHz, DMSO-d6) δ:1.4 & 1.6 (m, 2H, CH_2), 2.92 & 2.765 (m, 2H, CH_2), 3.5 (m, 1H, CH), 3.58 (bd, 1H, OH), 3.83 (s, 6H, OCH3, methyl), 5.11(bt, 2H, NH_2), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH benzene), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H) 7.18 (d, 2H, CH benzene), , 7.33 (t, 1H, CH benzene), 7.4 (t, 2H CH benzene), 7.45 (s, 1H), 7.6 (d, 2H, CH benzene), 7.82 (d, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 47.6, 54.1, 56.1, 77.5, 111.9, 116.6, 116.8, 122.9, 125.4, 127.6, 127.7, 127.9, 128.5, 128.6, 132.9, 138.1, 142.2, 147.9, 149.1, 151.3, 164.6, 183.7.

1.5.3.2 (1E,4E,5Z,6E)-5-[(2-aminopropyl)imino]-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(phenylmethylidene)hepta-1,6-dien-3-one (25).



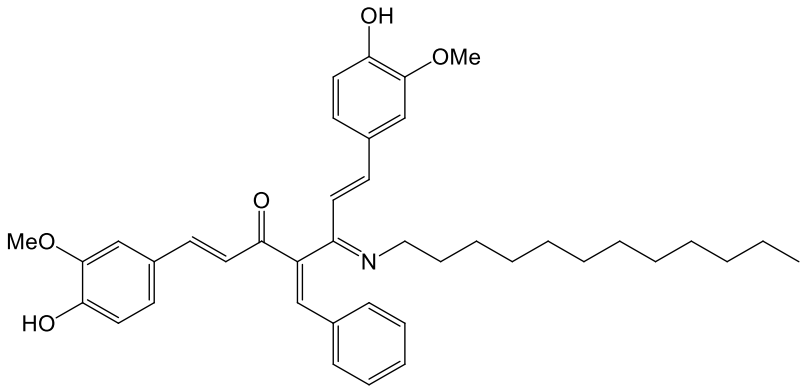
1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (20.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of tri ethanol amine (TEA) (4 drops) were added. The Solution was refluxed for 30 h at 120 °C. Then 1,2-diaminopropane (1.357 mmole, 2 drops), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (12 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.53 g (yield 73 %) of yellow solid. Melting point is 113 - 115 °C. IR: ν_{max} cm^{-1} 3276.79 (O-H), 1736.24 (C=O), 1575.56 (C=N), 1513.20 (C=C), 1032.11 (C-O).

^{1}HNMR (400 MHz, DMSO-d6) δ: 1.12 (d, 3H, CH_3), 1.4 & 1.7 (d, 2H, CH_2), 2.8 (m 1H, CH), 3.83 (s, 6H, OCH3, methyl), 5.11(bd, 2H, NH_2), 5.35 (bs, 2H, OH benzene), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH

benzene), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H) 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (m, 2H, CH), 7.6 (d, 2H, CH), 7.82 (d, 1H) 7.91 (s, 1H).

^{13}CNMR (400 MHz, DMSO-d6) δ: 21.3, 49.2, 54.4, 56.1, 111.9, 116.6, 116.8, 122.9, 125.4, 127.6, 127.7, 127.9, 128.5, 12.8.6, 132.9, 138.1, 142.2, 147.9, 149.1, 151.3, 164.6, 183.7.

1.5.3.3 1E, 4Z, 5Z, 6E) -5- (dodecylimino) - 1,7- bis (4-hydroxy -3-methoxyphenyl) -4- (phenylmethylidene) hepta-1,6-dien-3-on (26).

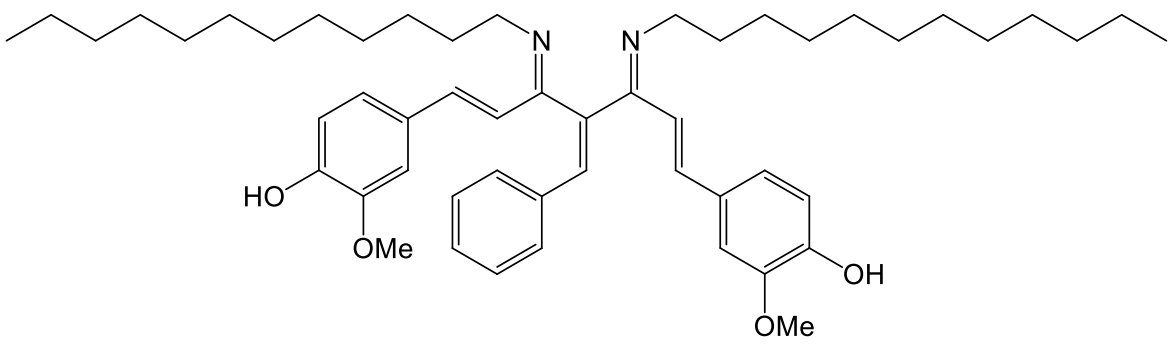


1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5mI) were added. The Solution was refluxed for 30 h at 120 °C. Then dodecyl amine (0.26 g, 1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc

(2:1 by volume) to give 0.72 g (yield 85.1%) of yellow solid. Melting point is 290-293 °C. IR: ν_{max} cm^{-1}: 3202 (O-H), 3034 (=C-H), 2798 (C-H, aliphatic), 1650 (C=O), 1610 (C=N), 1592 (C=C, conjugated), 1203 (C-O ether of (–O-CH_3)).^{1}HNMR (400 MHz, DMSO-d6) δ: 0.88 (t, 3H, CH_3 methyl), 1.26-1.52 (m, 20H, CH_2 methylene), 2.0 (s, 1H, NH), 2.78 (t of d 2H, CH_2 methylene), 3.83 (s, 6H, OCH3, methyl), 5.35 (bs, 2H, OH), 6.79 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H, CH benzene), 7.03 (d, 1H, CH), 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (d, 2H, CH), 7.82 (d, 1H, CH ethylene),

^{13}CNMR (400 MHz, DMSO-d6) δ: 14.1, 22.7, 27.1, 29.3, 29.6, 31.0, 31.9, 44.7, 56.1, 111.9, 116.8, 119.2, 122.9, 124.3, 125.4, 125.9, 127.6, 127.9, 128.5, 128.6, 131.1, 135.2, 135.3, 142.2, 147.9, 149.1, 165.4, 183.7.

1.5.3.4 4- [(1E,3E,4Z,5Z,6E)-3- (dodecylimino)-7- (4-hydroxy-3-methoxyphenyl) -4- (phenylmethylidene) -5-(undecylimino) hepta-1,6-dien-1-yl] -2-methoxyphenol (27).



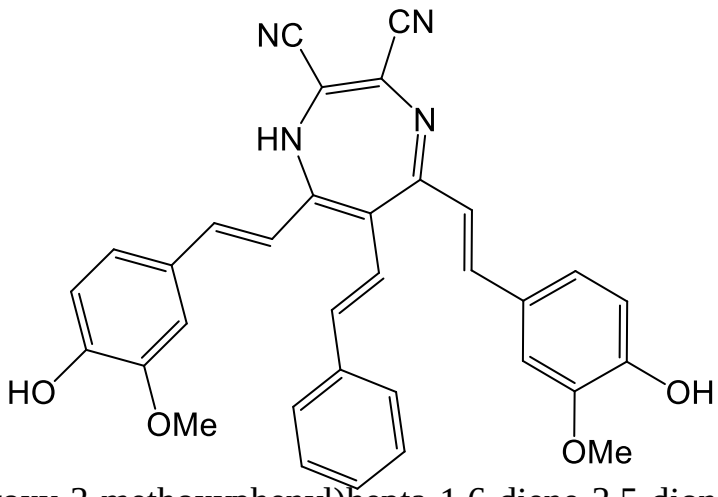
1,7- Bis (4- hydroxy-3- methoxyphenyl) hepta-1,6- diene-3,5- dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5 mI) was added. The Solution was refluxed for 30 h at 120 °C. Then dodecyl amine (0.51 g, 2.714 mmol), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.72 g (yield 68.3%) of yellow solid. Melting point is 311-314 °C. IR: ν_{max} cm^{-1}: 3198 (O-H), 3002 (=C-H), 2820 (C-H, aliphatic), 1630 (C=N), 1582 (C=C, conjugated), 1211 (C-O ether of (–O-CH_3).

^{1}HNMR (400 MHz, DMSO-d6) δ: 0.88 (t, 6H, CH_3 methylene), 1.26-1.52 (m, 42H, CH_2 methylene), 2.0 (bs, 1H, NH), 2.87 (t, 2H, CH_2), 3.83 (s, 6H, OCH3, methyl), 5.35 (bs, 2H, OH), 5.67 (d, 1H, CH), 6.79 (d, 1H, CH), 6.79 (d, 2H, CH benzene), 6.81 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H,

CH benzene), 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (d, 2H, CH).

^{13}CNMR (400 MHz, DMSO-d6) δ:14.1, 22.7, 24.3, 27.1, 27.2, 29.3, 29.6, 31.0, 31.9, 32.1, 44.7, 45.1, 56.1, 100, 111.9, 116.6, 116.8, 124.3, 122.9, 125.9, 127.6, 127.9, 128.5, 128.6, 131.1, 135.2, 135.3, 138.0, 145.1, 146.1, 147.9, 149.1, 164.6.

1.5.3.5 5,7- bis [(E)-2- (4-hydroxy-3-methoxyphenyl) ethenyl] -6-(phenylmethylidene) -6H-1,4- diazepine-2,3-dicarbonitrile (28).



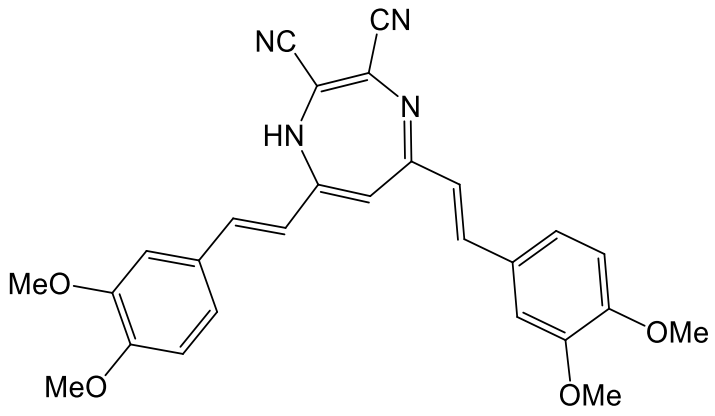
1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), was dissolved in ethanol (30.0 mL) and then, benzaldehyde (0.5 mL), and a catalytic amount of diisopropylamine (0.5 mI) were added. The Solution was refluxed for 30 h at 120 °C. Then diaminomaleonitrile (0.15 g, 1.357 mmole), and glacial acetic acid (1 mL) were added to the mixture and refluxed again for (20 hours) **according to procedures B**. The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.64 g (yield 89.3%) of brown solid. Melting point

is 294-297 °C. IR: ν_{max} cm-1: 3203 (O-H), 3010 (=C-H), 2220 (C≡N, stretching), 1633 (C=N), 1582 (C=C, conjugated), 1211 (C-O ether of (–O-CH3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (bs, 1H, NH), 3.83 (s, 6H, 2 OCH_3), 5.35 (bs, 2H, OH), 5.67 (d, 1H), 6.79 (d, 1H), 6.79 (d, 2H, CH benzene), 6.81 (d, 2H, CH), 6.85 (d, 2H, CH), 6.99 (d, 2H, CH benzene), 7.16 (s, 2H, CH benzene), 7.33 (t, 1H, CH), 7.4 (t, 2H, CH), 7.6 (d, 2H, CH).

^{13}CNMR δ: 56.1, 95.7, 105.1, 111.9, 115.2, 116.6, 120.2, 121.5, 122.9, 124.3, 125.9, 127.6, 127.9, 128.5, 128.6, 131.1, 135.2, 135.3, 138.1, 142.4, 147.9, 149.1, 164.6.

1.6 Functionalization of the prepared curcumin -based compounds with methoxy group

1.6.1 5,7-bis [(E)-2-(3,4-dimethoxyphenyl) ethenyl] -6H-1,4-diazepine-2,3- dicarbonitrile (29).

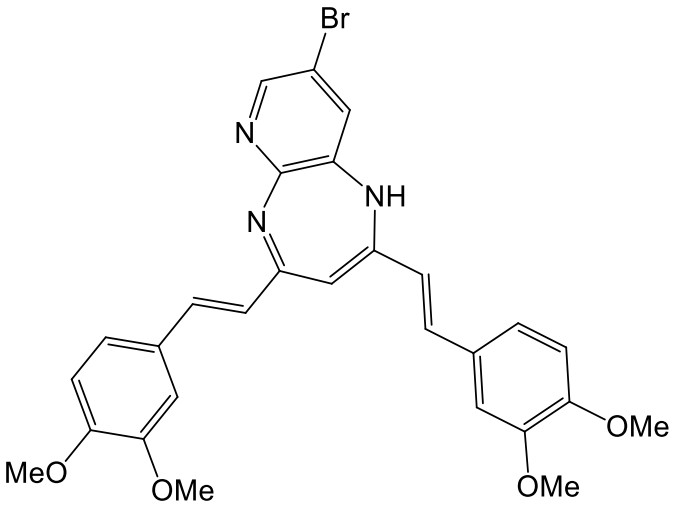


To a 45,7- bis [(E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl] -1H-1,4-diazepine-2,3-dicarbonitrile (C8) (0.2 g, 0.45 mmol) in ethanol (20.0 mL)

in a single-neck round bottom flask, NaOH (0.04 g, 1 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (1.6 mmol, 2 drops) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 79.9% (0.17g) of orange solid, m.p 133-135 °C, IR: ν_{max} cm-1: 3000 (=C-H), 2238 (C≡N, stretching), 1627 (C=N), 1601 (C=C, conjugated), 1209 (C-O ether of (–O-CH3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (s, 1H, NH), 3.83 (s, 12H, OCH3, methyl), 5.06 (s, 1H), 5.67 (d, 1H), 6.79 (d, 1H), 6.81 (d, 1H, CH), 6.85 (d, 1H, CH), 6.94 (d, 2H, CH benzene), 7.18 (d, 2H, CH benzene), 7.22 (s, 2H, CH benzene).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 86.6, 105.1, 111.5, 111.7, 111.9, 115.2, 116.6, 116.8, 120.2, 121.5, 122.5, 122.9, 124.3, 127.3, 127.6, 135.3, 138.1, 147.1, 147.9, 149.0, 149.3, 149.4, 149.7, 164.6.

1.6.2 8-bromo -2,4- bis [(E) -2- (3,4-dimethoxyphenyl) ethenyl]-3H-pyrido [2,3-b] [1,4] diazepine (30).

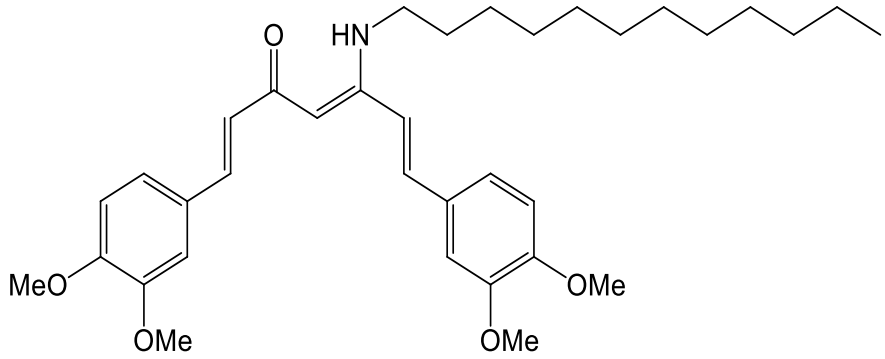


To a 4-[(E)-2-{8-bromo-4-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-pyrido[2,3-b][1,4]diazepin-2-yl}ethenyl]-2-methoxyphenol (C5) (0.09 g, 0.17 mmol) in ethanol (20.0 mL) in a single-neck round bottom flask, NaOH (0.02 g, 0.5 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (1.6 mmol, 2 drops) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 21.1% (0.02 g) of a yellow solid, m.p 260-264 °C, IR: ν_{max} cm^{-1}: 3009 (=C-H), 1617 (C=N), 1604 (C=C, conjugated), 1213 (C-O ether of (–O-CH_3)) 650 (C-Br stretching).

^{1}HNMR (400 MHz, DMSO-d6) δ: 4.0 (s, 1H, NH), 3.83 (s, 12H, OCH3, methyl), 5.06(s, 1H, CH), 5.67 (d, 1H), 6.79 (d, 1H), 6.81 (d, 1H), 6.85 (d, 1H), 6.94 (d, 2H, CH benzene), 7.6 (s, 1H, CH, 2-pyridine), 7.18 (d, 2H, CH benzene), 7.22 (s, 2H, CH benzene), 8.16 (s, 1H, CH, 2-pyridine).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 88.6, 111.5, 111.7, 114.5, 116.6, 122.5, 123.0, 124.3, 127.3, 130, 135.3, 138.1, 143.3, 149.0, 149.7, 157.5, 160, 164.6.

1.6.3 (1E,5E,6E)-1,7-bis(3,4-dimethoxyphenyl)-5-(dodecylimino)hepta-1,6-dien-3-one (31).



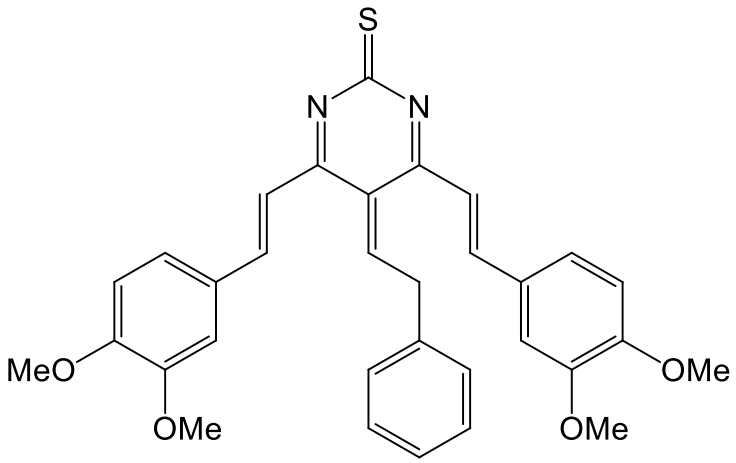
To a 1E,5E,6E) -5- (dodecylimino) -1,7- bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (C13) (0.2 g, 0.373 mmol) in ethanol (20.0 mL) in a single-neck round bottom flask, NaOH (0.033 g, 0.825 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (3 drops, 2.4 mmol) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by

washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 80.7 % (0.17 g) of yellow solid, m.p 164-166 °C, IR: ν_{max} cm^{-1}: 3028 (=C-H), 2813 (C-H, aliphatic), 1631 (C=O), 1611 (C=N), 1601 (C=C, conjugated), 1207 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 0.88 (t, 3H, CH_3 methylene), 1.26-1.52 (m, 20H, CH_2 methylene), 2.0 (s, 1H, NH), 2.87 (m, 2H, CH_2 methylene), 3.83 (s, 12H, OCH3, methyl), 5.48 (s, 1H, CH), 6.81 (s, 1H, CH), 6.85 (s, 1H, CH), 6.94 (d, 2H, CH benzene), 7.03 (d, 1H, CH), 7.18 (d, 2H, CH benzene), 7.22 (s, 2H, CH benzene). 7.82 (s, 1H, CH ethylene),

^{13}CNMR (400 MHz, DMSO-d6) δ: 14.1, 27.1, 29.3, 29.6, 31.0, 31.9, 42.2, 44.3, 56.1, 105.6, 111.5, 111.7, 122.5, 123.3, 124.3, 127.3, 135.3, 149, 149.7, 172.4, 188.6.

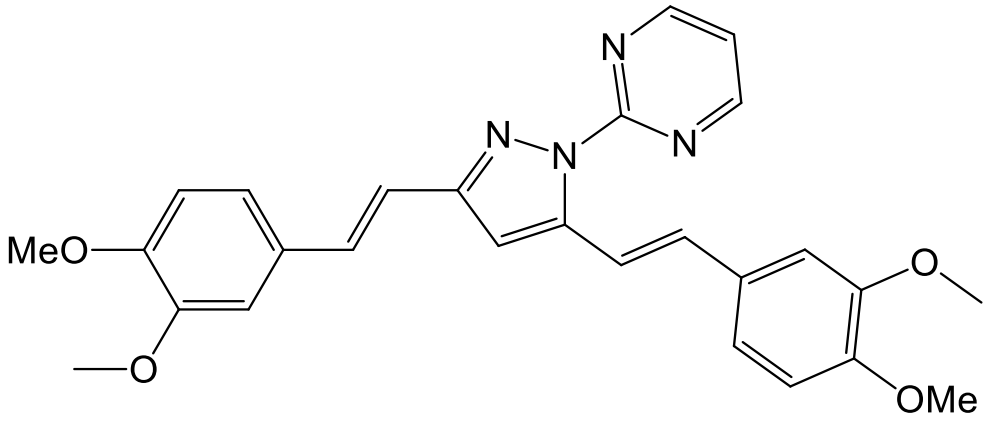
1.6.4 4,6-bis [(E) -2 - (3, 4- dimethoxyphenyl) ethenyl] -5- (phenylmethylidene) -2,5- dihydropyrimidine-2-thione (32).



To a 4,6 -bis [(E) -2- (4- hydroxy- 3- methoxyphenyl) ethenyl] -5- (phenylmethylidene) -2,5- dihydropyrimidine-2-thione (C23) (0.5 g, 1 mmol) in ethanol (20.0 mL) in a single-neck round bottom flask, NaOH (0.09 g, 2.25 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (4 drops, 3.2 mmol) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120°C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 95.0% (0.5 g) of a brown solid, m.p 336-339 °C, IR: ν_{max} cm^{-1}: 3020 (=C-H), 1609 (C=N), 1597 (C=C, conjugated), 1204 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 3.21 (d, 2H, CH_2), 3.83 (s, 12H, OCH3, methyl), 5.67 (d, 2H), 6.36 (t, 1H, CH), 6.79 (d, 2H), 6.94 (d, 2H, CH benzene), 7.18 (d, 2H, CH benzene), 7.22 (s, 2H, CH benzene), 7.23 (d, 2H, CH), 7.26 (t 1H, CH), 7.33 (t, 1H, CH). ^{13}CNMR (400 MHz, DMSO-d6) δ: 35, 56.1, 111.5, 111.7, 112.4, 116.6, 122.5, 125.7, 127.3, 128.6, 129, 134.5, 138.1, 141.4, 149, 149.7, 164.6, 232.

2-(3,5-bis((E)-3,4-dimethoxystyryl)-1H-pyrazol-1-yl)pyrimidine (33).



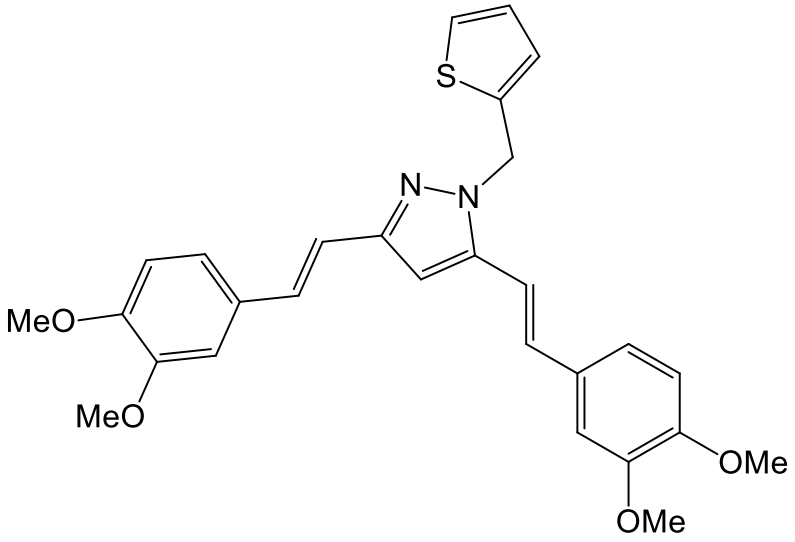
To a 4-[(E)-2- {5- [(E)-2- (4-hydroxy-3- methoxyphenyl) ethenyl] -1- (pyrimidin-2-yl) - 1H-pyrazol-3-yl} ethenyl]-2-methoxyphenol (C10) (0.5 g, 1.1 mmmol) in ethanol (20.0 mL) in a single-neck round bottom flask, NaOH (0.1 g, 2.5 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (4 drops, 3.2 mmol) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to

room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 50.3 % (0.29 g) of a brown solid, m.p 177-178 °C, IR: ν_{max} cm^{-1} 1634.7 (-C=N), 1619 (C=C, conjugated), 1063 (C-O ether) of (–O-CH_3), and 1217.33 (N-N).

^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 12H, 2 OCH_3), 4.06 (t, 4H, CH_2), 6.56 (s, IH, CH 1-pyrazole), 6.94 (d, 1H, CH 1-benzene), 6.95 (d, 2H), 6.99 (d, 2H), 7.18 (d, 2H, CH benzene), 7.22 (s, 2H, CH 1-benzene), 7.71 (t, IH, CH 2-pyrimidine), 8.85 (d, 2H, CH, 2-pyrimidine).

^{13}CNMR (400 MHz, DMSO-d6) δ: 56.1, 68.7, 107.7, 111.3, 111.6, 116.1, 118.3, 121.8, 123.5, 129.8, 131.2, 146.7, 147.1, 149.1, 155.9, 156.1.

1.6.6 3,5-bis [(E)-2-(3,4-dimethoxyphenyl) ethenyl]-1-[(thiophen-2-yl) methyl]-1H-pyrazole (34).

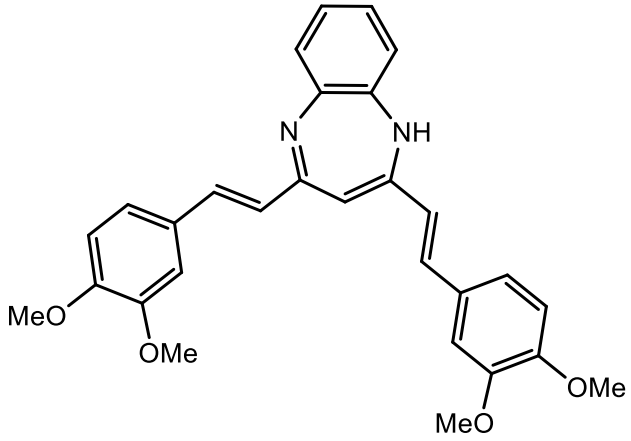


To a 4-[(E)-2-{5- [(E) -2- (4-hydroxy-3- methoxyphenyl) ethenyl]-1-[(thiophen-2-yl) methyl]-1H-pyrazol-3-yl}ethenyl]-2-methoxyphenol (C12) (0.2 g, 0.43 mmol) in ethanol (20.0 mL) in a single-neck round bottom flask, NaOH (0.04 g, 1 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (4 drops, 3.2 mmol) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120 °C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. Yield 56.6 % (0.12 g) of a yellow solid, m.p 371-373 °C, IR: ν_{max} cm^{-1} 1630.2 (-C=N), 1611.4 (C=C, conjugated), 1053.7 (C-O ether) of (–O-CH_3), and 1220.1 (N-N).

^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 12H, OCH3, methyl), 4.99 (s, 2H, CH_2, methylene), 6.53 (s, IH, CH 1-pyrazole), 6.83 (d, 2H, CH 2-thiophene), 6.94 (d, 1H, CH 1-benzene), 6.93 (t, IH, CH 2-thiophene), 6.95 (d, 2H), 6.99 (d, 2H), 7.22 (s, 2H, CH 1-benzene), 7.40 (d, IH, CH 2-thiophene).

^{13}CNMR (400 MHz, DMSO-d6) δ: 52.9, 56.1, 108.1, 108.7, 111.7, 116.1, 122.5, 123.5, 125.5, 126.7, 127.0, 130.2, 131.2, 138.2, 139.4, 149, 149.7, 152.2.

1.6.7 2,4-bis [(E) -2- (3, 4 -dimethoxyphenyl) ethenyl] - 1H- 1,5-benzodiazepine (35).



To a 4-[(E)-2-{4-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-1,5-benzodiazepin-2-yl}ethenyl]-2-methoxyphenol (C6) (0.445 g, 1.011 mmole) in ethanol (10.0 mL) in a single-neck round bottom flask, NaOH (0.09 g, 2.25 mmol) was added. The solution was stirred for (30 mins) at room temperature. Methyl iodide (2 drops, 1.6 mmol) were added dropwise over (10 mins), then the mixture was stirred under reflux for 15 h at 120°C and regularly monitored for reaction progress by TLC using hexane: ethyl acetate (6: 4) as the solvent system. After cooling down to room temperature, the resulting solution was evaporated to dryness under reduced pressure and residue was purified by washing with distilled water, filtered, washed again several times with diethyl ether, dried. The purification was completed by washing the solid product with ethyl acetate several times with stirring for several hours and dried over $MgSO_4$. The produced solid was collected by filtration. Yield 36.73 % (0.174 g) of the

yellow solid, mp 174-175 °C, IR (KBr): ν_{max} cm^{-1} 3346.97 (N-H), 2963.36(C-H), 1640. (C=N), 1598.44 (C=C), ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.78 (s, 12H, OCH3), 4.02 (bs, 1H, NH), 5.09 (s, 1H), 5.67 (d, 1H, J =15.1), 6.79 (m, 4H), 6.95 (d, 2H, J =7.5), 7.12 (d, 2H, J = 7.5), 7.16 (d, 2H, J =7.5), 7.22 (m, 2H), 7.32 (d, 1H, J = 7.5 Hz). ^{13}C-NMR (400 MHz, DMSO-d6) δ: 28.9, 33.8, 45.1, 56.40, 113.9, 121.3, 125.2, 130.1, 132.9,140.3, 144.7, 147.8, 165.3.

2. Antibacterial activities of the prepared curcumin-based heterocyclics.

2.1 Materials and procedures

2.1.1 Media and solutions preparation:

2.1.1.1 Nutrient broth (NB)

Nutrient broth (ACUMEDIA, USA) was prepared according to manufacturer's instructions labeled on the bottle. In a 0.5 L bottle, 2 g of NB and 248 ml deionized water were mixed and dissolved well. The broth was then distributed into tubes to have 7 ml each and plugged with cotton. The tubes were autoclaved at 121°C for 15min, allowed to cool and then kept at 4-6°C in refrigerator.

2.1.1.2 Nutrient Agar (NA)

Nutrient agar (ACUMEDIA, USA) was prepared according to manufacturer's instructions. In a 1 L bottle, 500 ml deionized water were heated and mixed with 11.5 g NA until the agar dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 45°C. The agar was poured into sterile Petri dishes to have 20 ml each and left overnight at room temperature. The following morning the Petri dishes were turned upside down and stored at 4°C.

2.1.1.3 Normal Saline (0.9% NaCl)

Normal saline solution (0.9% NaCl, MWt 58.44) was prepared in a 0.5 L bottle, a 2.25 g NaCl was dissolved in a 250 ml deionized water. The saline solution was then distributed into tubes to have 5-10 ml each and plugged with cotton. The tubes were autoclaved at 121°C for 15 min, allowed to cool and then refrigerated.

2.1.1.4 Dimethyl sulfoxide (10% DMSO)

Dimethyl sulfoxide (10%, Mwt 78.14, Sigma) solution was prepared in 0.25 L bottle, 10 ml Dimethyl sulfoxide and 90 ml distilled water were mixed. The solution was autoclaved at 121°C for 15 min, allowed to cool and then kept at room temperature.

2.1.1.5 Preparation of McFarland turbidity standard No. 0.5

McFarland 0.5 turbidity standard was prepared by mixing 50 µl of a 1.175% (w/v) barium chloride dihydrate ($BaCl_2.2H_2O$) solution and 9.95 ml of 1% (v/v) sulfuric acid. The tube which had the 0.5 McFarland standard was then sealed with parafilm to prevent evaporation and stored in the dark at room temperature. The 0.5 McFarland standard was vigorously mixed on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland Standard is comparable to a bacterial suspension of 1.5 X 10^8 colony-forming units (CFU)/ml [87].

Three to four colonies of each bacteria were transferred into tubes had 10 mL of sterile normal saline (0.9%), the turbidity of the bacterial

suspensions was adjusted to have similar turbidity of 0.5 McFarland standard with bacterial suspension of about 1.5 x10^8 cfu/mL.

2.1.2 Microorganisms used

Four types of bacteria strains have been used in this study. Two of these strains are Gram-positive bacteria which are *Staphylococcus aureus (S. aureus)* ATCC 6538P and clinical isolate of Methicillin-resistant *S. aureus* (MRSA*),* while the other 2 strains are Gram-negative bacteria included *Klebsiella pneumoniae (K. pneumoniae* ATCC 13883) and *Escherichia coli (E. coli)* ATCC 25922.

2.1.3 Determination of minimum inhibitory concentration (MIC)

The MIC of curcumin-based heterocyclics was determined by the two-fold serial dilution method in sterile 96- microwell plates according to the Clinical and Laboratory Standard Institute (CLSI) [88]. The curcumin-based heterocyclics (400 μg/ml of 10% DMSO) and negative control of 10% DMSO were two-fold-serially diluted in nutrient broth in the wells of the plates in a final volume of 100 μL. After that, a bacterial inoculum size of 1.0 x 10^5CFU/ml was added to each well. A100 μL NB only, or 100 μL DMSO with bacterial inoculum, or curcumin-based heterocyclics and nutrient broth without bacteria were included as a negative control wells in these experiments. Each curcumin-based heterocyclic compound was run in duplicate. The microwell plates were then covered and incubated at 37°C for 24 h. The highest dilution (lowest concentration) of the curcumin-based

heterocyclic compound that resulted in inhibition of bacterial growth was considered as the MIC.

2.1.4 Determination of minimum bactericidal concentration (MBC)

The wells that showed inhibition the bacterial growth, 10 μL from these wells were transferred by disposable inoculating loop and subcultured on NA plates. Then these plates were incubated at 37ºC for 24 hours. The highest dilution (the lowest concentration) of curcumin-based heterocyclic compound that required to kill a specific bacterial strain was considered as MBC.

2.1.5 Determination of minimum inhibitory concentration (MIC) of Ampicillin antibiotic

The MIC of Ampicillin antibiotic was determined by the two-fold serial dilution method in sterile 96- microwell plates according to the CLSI [88]. The Ampicillin antibiotic (100 μg/ml) were two-fold-serially diluted in NB in the wells of the plates in a final volume of 100 μL. After that, *E. coli* ATCC 25922 inoculum size of 1.0 x 10^5 CFU/ml was added to each well. Each Ampicillin antibiotic concentration was run in duplicate. The microwell plates were then covered and incubated at 37°C for 24 h. The highest dilution (lowest concentration) of the antibiotic that resulted in inhibition of bacterial growth was considered as the MIC.

2.1.6 Synergistic effect of curcumin-based heterocyclics with ampicillin antibiotic

The synergistic effect of four selected curcumin-based heterocyclics (compound **2, 4, 10, 35**) with ampicillin antibiotic was determined by the two-fold serial dilution method in sterile 96- microwell plates according to the CLSI [88]. The curcumin-based heterocyclics (400 μg/ml of 10% DMSO) were two-fold serially diluted in nutrient broth in the wells of the plates in a final volume of 100 μL. After that, a sub-MIC of ampicillin antibiotic (0.39125 μg/ml) was added to each dilution of curcumin-based heterocyclic compound. Then, *E. coli* ATCC 25922 inoculum size of 1.0 x 10^5 CFU/ml was added to each well. Each curcumin-based heterocyclic compound with the antibiotic was run in duplicate. The microwell plates were then covered and incubated at 37°C for 24 h. Detection the effect of the drugs together, fractional inhibitory concentration (FIC) for both ampicillin antibiotic and curcumin-based heterocyclic compound was calculated with the equation $\Sigma FIC = FIC_A + FIC_B = (C_A/MIC_A) + (C_B/MIC_B)$, where MIC_A and MIC_B are the MICs of drugs A and B alone, respectively, and C_A and C_B are the concentrations of the drugs in combination, respectively. Synergy is interpreted when the FIC index is $\leq$ 0.5, indifference or no interaction corresponds to the FIC index > 0.5–4.0 and antagonism when the FIC index is > 4.0 [89].

2.2 Evaluation of the Genotoxic Potential of curcumin-based heterocyclic on *E. coli* ATCC 25922 strain

2.2.1 Inoculation of E. coli

Few colonies from 24-hour old *E. coli* strain growth culture plated on NA medium were sub-cultured in a bottle containing 25 mL of nutrient broth under sterile conditions and incubated for an hour at 37°C with continuous shaking. Then 1 mL of the one-hour old *E. coli* culture was added to each of four sterile bottles each containing 24 mL of nutrient broth medium under aseptic conditions. The four bottles were then incubated for an hour at 37°C with continuous shaking. Different concentrations of compound **8**: zero µg/ml (10% DMSO), 25µg/ml, 50µg/ml and 100µg/ml of 10% DMSO) were each added into one of the four bottles of the *E. coli* broth culture. The zero µg/ml concentration of compound **8** with a specific volume of 10% DMSO was considered as a negative control (untreated).

2.2.2 DNA extraction

For enterobacterial repetitive intergenic consensus (ERIC) PCR test a genome of *E. coli* was prepared according to the method described previously [90] A four mL of each of the four samples of the *E. coli* growth culture was taken after 3h, 5h, and 24h, centrifuged at 14.000 rpm for five minutes where the supernatant of each sample was discarded. Each bacterial sample pellet was re-suspended in 1 mL of Tris-EDTA (10 mMTris-HCl, 1 mM EDTA [pH 8]), centrifuged at 14.000 rpm for five minutes, then the supernatant was discarded. The pellet of each bacterial sample was re-suspended in a 350 µl of sterile distilled water, boiled for 15 min, then the mixture was incubated in ice for five minutes. The samples were then pelleted by centrifugation for five minutes at 14.000 rpm, the

supernatant of each sample was then transferred to a new eppendorf tube. The DNA concentration for each sample was then determined using nanodrop spectrophotometer (GenovaNano, Jenway), the DNA samples were then stored for ERIC-PCR analysis at - 20 ºC.

2.2.3 Enterobacterial repetitive intergenic consensus (ERIC) PCR analysis

The ERIC-PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3` and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3`. Each PCR reaction mix (25 µl) was composed of 10 mM PCR buffer pH 8.3; 3 mM $MgCl_2$; 0.4 mM of each dNTP; 0.8 µM of each primer; 1.5U of Taq DNA polymerase, 5% DMSO and fixed amount of DNA template (30µg-35µg). The thermal cycler (Mastercycler personal, Eppendorf, Germany) was then used for DNA amplification as the following thermal conditions: initial denaturation for 3 min at 94ºC; followed by 40 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 1 min and extension at 72ºC for 1 min, followed by a final extension step at 72ºC for 5 min. Then, the PCR products were analyzed by electrophoresis through 1.5% agarose gel.

2.3.1 Agarose gel electrophoresis

A 1.5% agarose gel was used to analyze ERIC-PCR products. A DNA marker of 100 bp was also used on the same gel including ERIC-PCR products. Electrophoresis was run using 1X TAE electrophoresis working

buffer (50x: 242g Tris base, 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA [pH 8.0]) for 75 min at 80 V. Then the gel was stained with ethidium bromide (0.5 μg/ml of water) for 10 min. The ERIC-PCR profile was visualized using UV trans-illuminator and photographed. Changes in ERIC-PCR banding pattern profiles following curcumin-based heterocyclic compound number **8** treatments, including variations in band intensity as well as gain or loss of bands, were taken into consideration [91, 92].

CHAPTER THREE

RESULTS AND DISCUSSION

In a previous work [84] curcumin was employed as a naturally occurring skeleton for preparing many different heterocyclic compounds including diazepine, pyrazole and isoxazole using two new different methods; in the first method, curcumin was mixed with several diamines in ethanol using sulfuric acid as a catalyst. While, in the second method, a mixture of curcumin and diamine compounds was heated gradually to 160 ˚C and maintained at 160 ˚C for 30 min. The antibacterial activity of the prepared curcumin-based compounds was tested against four types of bacteria; gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis)* and gram-negative bacteria (*Pseudomonas aeruginosa, and Escherichia coli*). The tested compounds showed good antibacterial activities against gram positive bacteria and moderate activity against gram negative ones. Among the prepared compounds, diazepines derivatives showed the highest antibacterial activity. In continuation of this work and for developing more efficient curcumin-based compounds against several types of microorganisms, novel sets of curcumin-based benzodiazepine, diazepine, diazoles and amines were prepared using simple and convenient condensation reaction that includes reacting curcumin with various types of 1,2-diamino and hydrazine compounds.

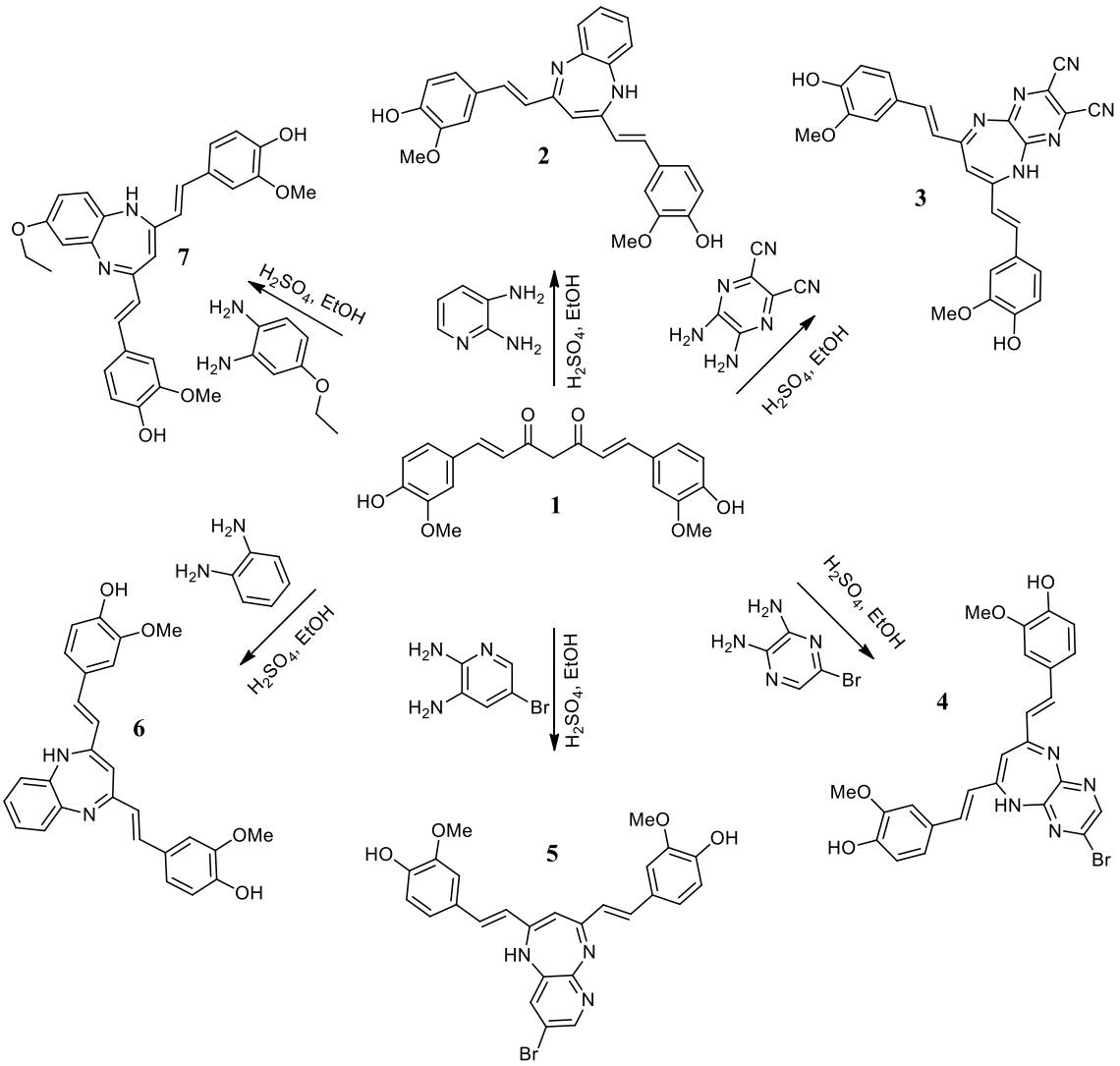
Schemes 1-5 summarize the prepared compounds structures and reaction conditions. As well as, H-curcumin (Hydrogenated curcumin **19 a**) based

amino compound was prepared from reaction of H-curcumin with ammonium acetate as shown in scheme 6. Moreover, different types of curcumin-based benzodiazepine, diazepine, and amin were prepared using Knoevenagel Condensation schemes 7-14. The second step involves etherification of the prepared curcumin-based compounds with methyl group as shown in schemes 15-21.

3.1 Curcumin-based benzodiazepines

3.1.1 Preparation curcumin-based benzodiazepines

Curcumin-based benzodiazepines were prepared by condensation reaction of curcumin with various diamines in a 1:1 ratio using ethanol as a solvent and H_2SO_4 as a catalyst as shown in scheme 1. The reaction progress was monitored using TLC. The products were purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR. The results are summarized in the experimental part. The yields of some curcumin-based benzodiazepines were quantitative; it ranged from 48.14 % to 99.55%.



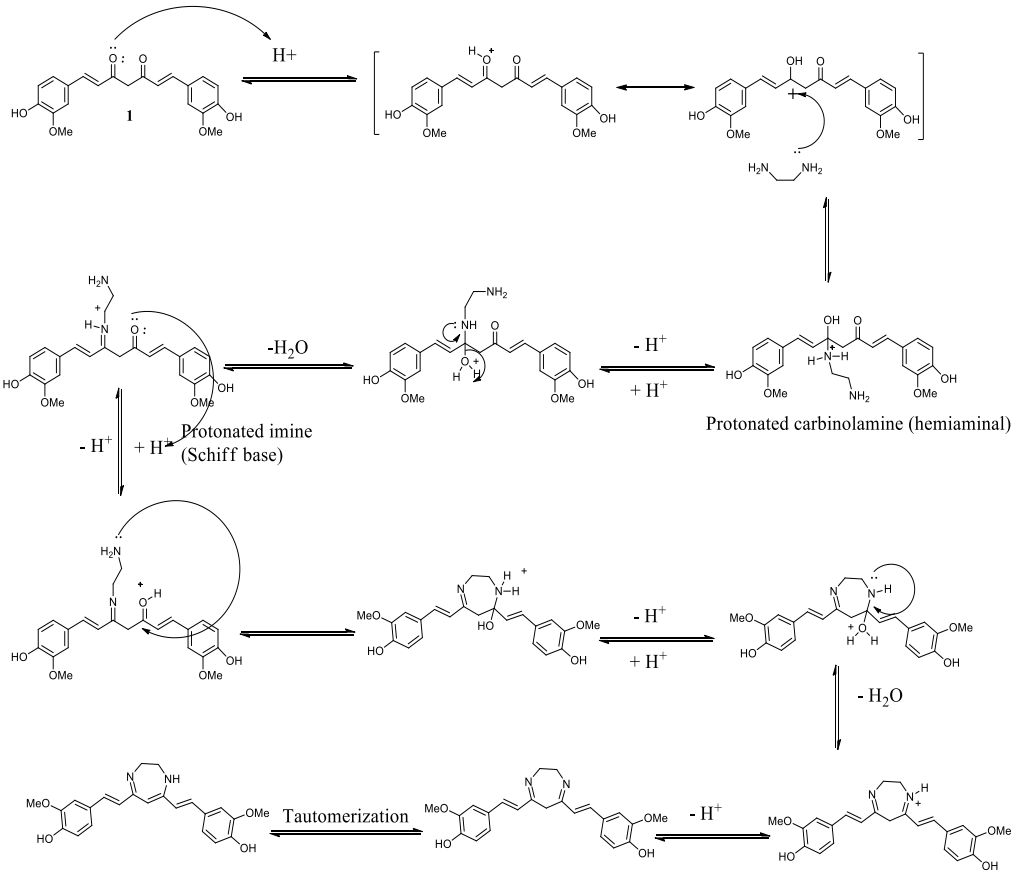
Scheme 1: Chemical structures and reaction conditions for making curcumin-based benzodiazepines.

3.1.2 Reaction mechanism of making curcumin-based benzodiazepines

A detailed mechanism of the condensation cyclization of curcumin with various 1,2 diamino compounds to form curcumin-based benzodiazepines is shown in scheme 2. The first step is a typical acid-catalyzed nucleophilic addition of the diamine to one of the curcumin carbonyl groups. The acid catalyst protonates the carbonyl group, and the diamine or hydrazine (weak nucleophiles) attacks the protonated, activated carbonyl. Loss of a proton

from the positively charged intermediate gives a hemiaminal (carbinolamine).

The second step is acid catalyzed dehydration where the carbinolamine converts to an imine by losing water and forming a double bond. This dehydration follows the same mechanism as the acid-catalyzed dehydration of an alcohol. Protonation of the hydroxy group converts it to a good leaving group, and it leaves as water. The resulting cation is stabilized by resonance forms, including one with all octets filled and the positive charge on nitrogen. Loss of a proton gives an imine intermediate. Acid-catalyzed nucleophilic attack by the second amino group on the intermediate **imine** followed by a loss of a second water molecule results in the diazepine formation. For clarification purpose ethylene diamine was used in the reaction mechanism depicted in scheme 2.



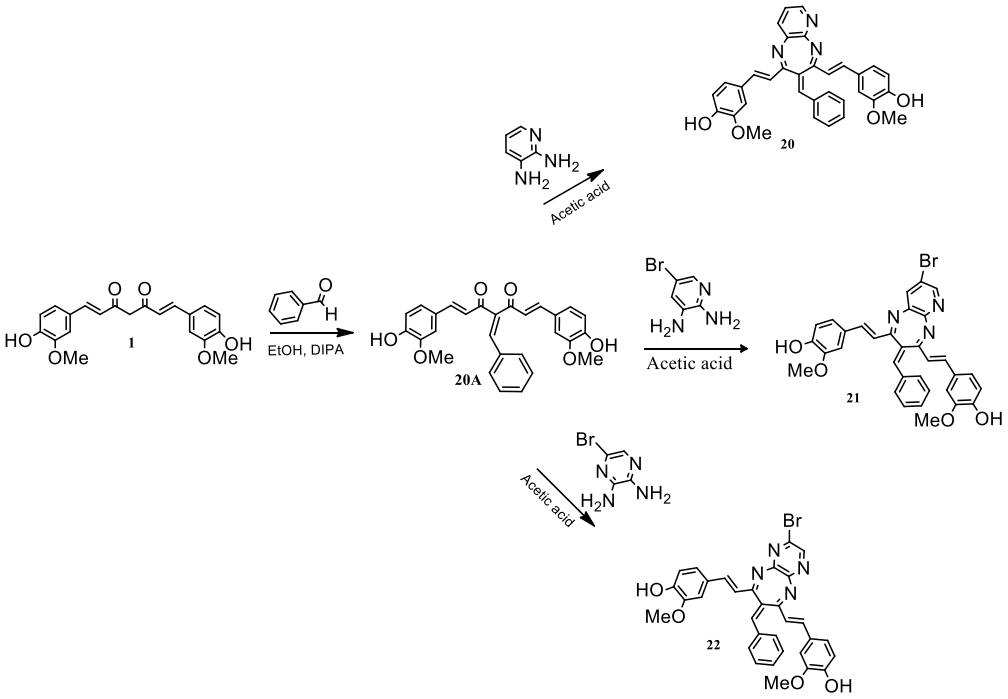
Scheme 2: A detailed mechanism of the condensation cyclization of curcumin with various 1,2 diamino and hydrazine compounds to form curcumin-based benzodiazepines.

3.1.3 Knoevenagel Condensation of Curcumin-Based Benzodiazepines

Curcumin-based benzodiazepines **20, 21**, and **22** were prepared using a two-steps method; in the first step, the curcumin was reacted with benzaldehyde in ethanol solvent, in the presence of diisopropylamine (DIPA) base, which was used as a catalyst to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation, scheme 3.

The second step involved acid-catalyzed condensation cyclization reactions of the curcumin carbonyl groups of the prepared intermediate **20A** with several 1,2-diamino or hydrazine compounds to produce the desired compounds. Excess amount of acetic acid was added to the prepared

intermediate which played a dual function; to neutralize the excess base from the first step, and as a catalyst to protonate the carbonyl oxygen and activate the carbon atom. followed by adding 2,3-diaminopyridien, 2, 3-diamino-5-bromopyridine, or 2, 3-diamino-5-bromopyrizine in (1:1 ratios) to produce benzodiazepines **20, 21**, and **22** respectively scheme 3. The reaction progress was monitored using TLC. The products were purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yields of 83.6%, 92% and 88.5 % for compounds **20, 21** and **22,** respectively.

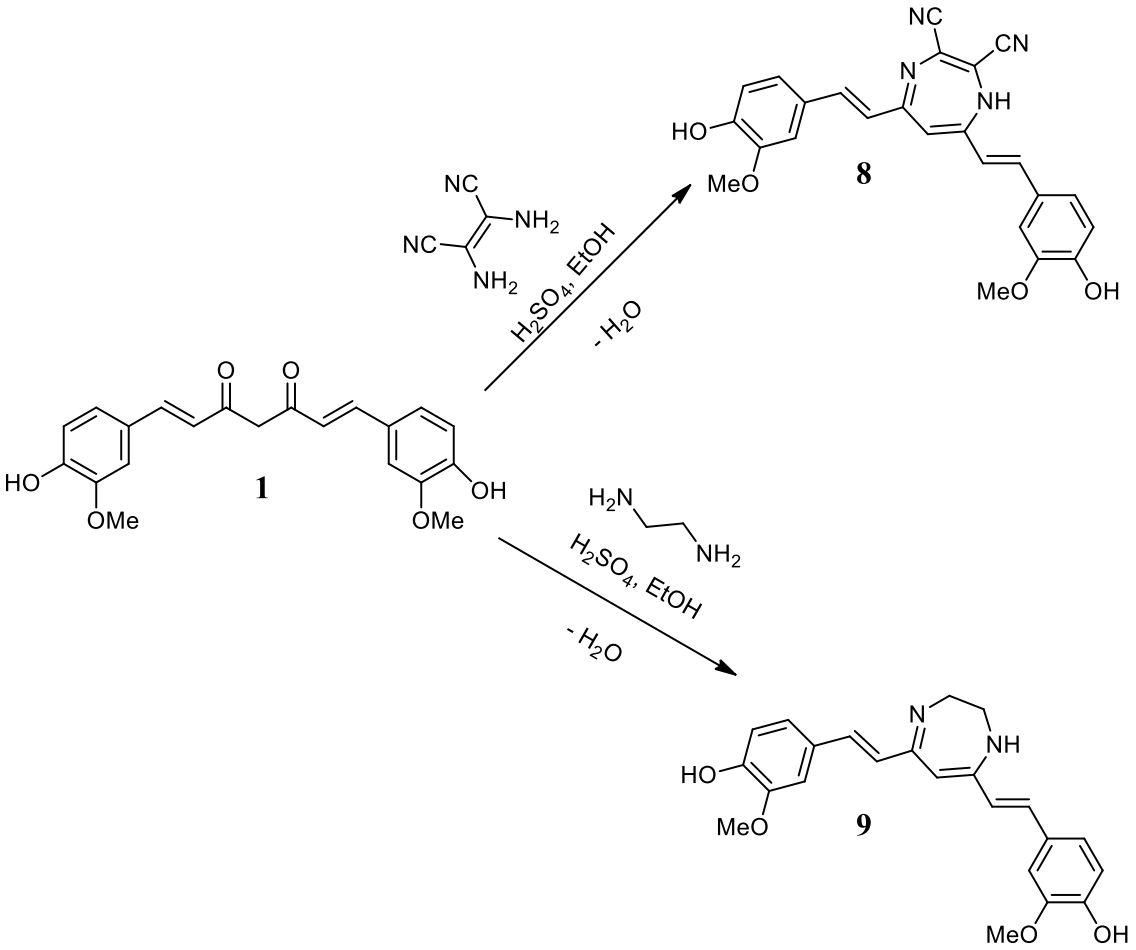


Scheme 3: Chemical structures and reaction conditions for making curcumin-based benzodiazepines using Knoevenagel Condensation.

3.2. Curcumin based diazepines

Curcumin-based diazepines were prepared using condensation cyclization reaction by mixing curcumin and diaminomaleonitrile in a 1:1 ratio using a ethanol as a solvent and H_2SO_4 as a catalyst as shown in scheme 3. The reaction progress was monitored using TLC. The product **8** was purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; yield 22.9%.

While product **9** was prepared using the same procedures used to prepare curcumin-based benzodiazepines; by mixing the curcumin with ethylene diamine in a 1:1 ratio in ethanol as a solvent and H_2SO_4 was used as a catalyst scheme 3. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield 50.7%.



Scheme 4: Chemical structures and reaction conditions for making curcumin-based diazepines.

3.3 Curcumin-based diazoles

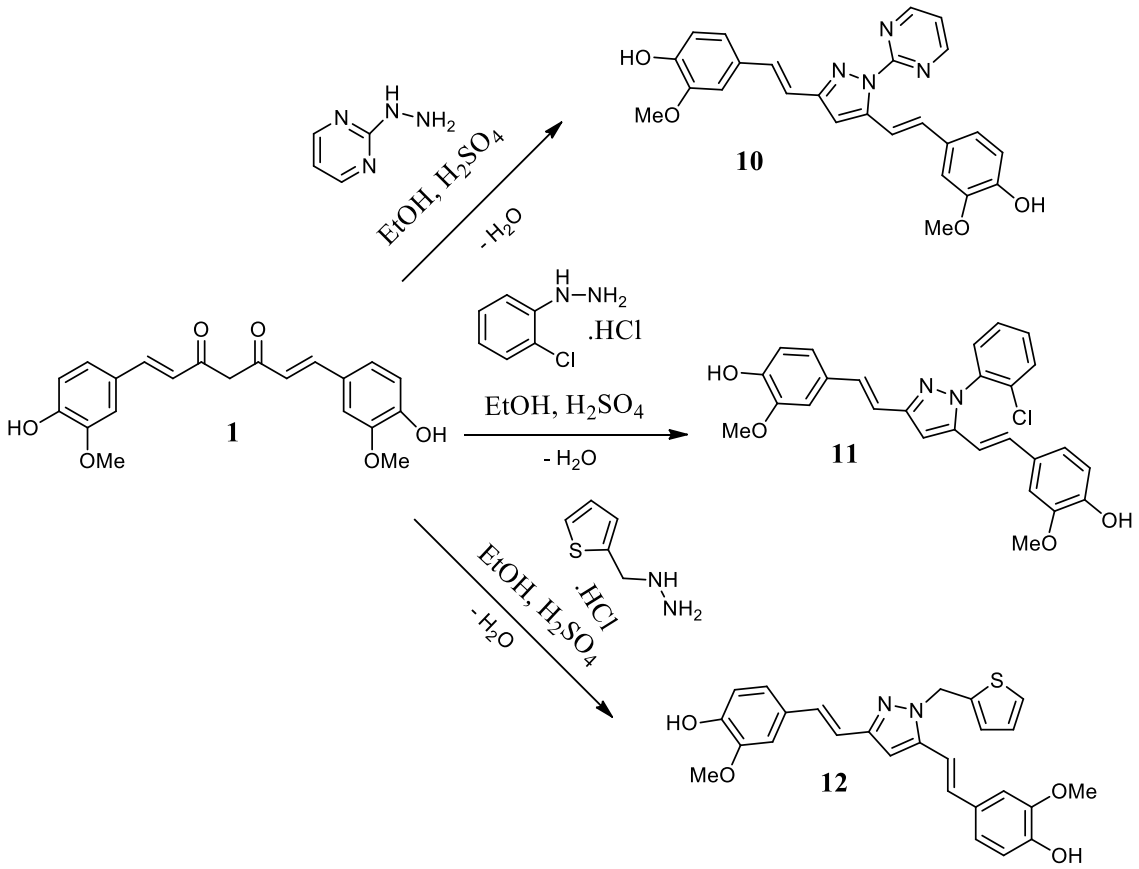
3.3.1 Preparation of curcumin-based diazoles

Curcumin-based diazoles were prepared using condensation cyclization reaction of curcumin with 2-hydrazinopyrimidinehydrate to produce compound **10** using the same procedures used to prepare curcumin-based benzodiazepines and diazepines; by mixing the curcumin with 2-hydrazinopyrimidinehydrate (1:1 ratio) using ethanol as a solvent and H_2SO_4 as a catalyst as shown in scheme 4. The reaction progress was monitored using TLC. The product was purified as described in the

experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 66.66%.

The condensation cyclization reactions of curcumin with 2-chlorophenylhydrazin hydrogen chloride and (2-thienylmethyl)hydrazine hydrochloride to produce compounds **11** and **12,** respectively were carried out in ethanol solvent using H_2SO_4 as a catalyst scheme 4. The reaction progress was monitored using TLC. The products **11** and **12** were purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; yields of 80.3 % and 74 % respectively.

Preparation of curcumin-based benzodiazepines and diazepines required more reflux time (60-90 hrs) than the preparation of curcumin-based diazoles which required (12-30 hrs). for all prepared curcumin-based benzodiazepines, diazepines and diazoles the yields were good and quantitative.



Scheme 5: Chemical structures and reaction conditions for making curcumin-based diazoles.

3.4 Amino Curcumin

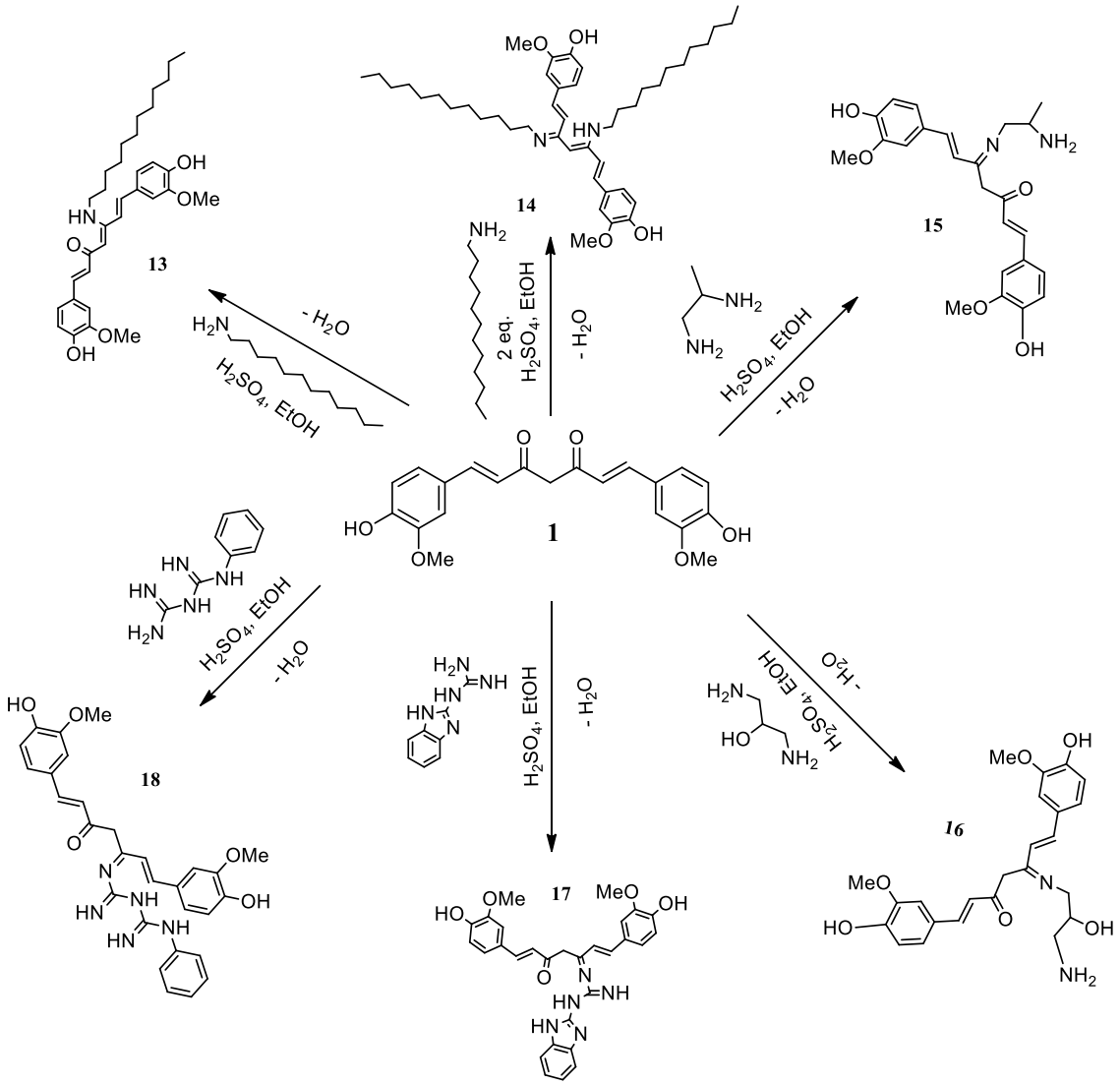
3.4.1 Preparation of amino curcumin

Curcumin amines were prepared by a one-step condensation reaction of curcumin with dodecyl amine to produce amino compounds **13** and **14**. The preparation was done using the same procedures described previously for the preparation of curcumin-based benzodiazepines. Curcumin and 2-dodecyl amine were mixed in a 1:1 ratio and in the second reaction, they were mixed in a 1:2 ratio, in ethanol that was used as a solvent in the presence of H_2SO_4 catalyst, as summarized in scheme 5. The reaction progress was monitored using TLC. The products were purified as described in the experimental part, analyzed using melting point, TLC, IR,

NMR; results are also summarized in the experimental part, Yields of 99.2 % and 95.1 % for compounds **13** and **14** respectively.

The condensation reactions of curcumin with 1,2-diaminopropane and 1,3-diamino-2-propanol to produce compounds **15** and **16** respectively were carried out in ethanol that was used as a solvent in the presence of H_2SO_4 that was used as a catalyst as shown in scheme 5. The reaction progress was monitored using TLC. The products **15** and **16** were purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; yields of 69.5% and 53.5 % respectively.

In the same way, the condensation reactions of curcumin with 2-Guanidinobenzimidazole and Phenyl biguanide to produce compounds **17** and **18** respectively were carried out in ethanol that was used as a solvent in the presence of H_2SO_4 that was used as a catalyst as shown in scheme 5. The reaction progress was monitored using TLC. The products **17** and **18** were purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; yields of 78.6% and 86.7 % respectively.



Scheme 6: Chemical structures and reaction conditions for making curcumin-based amines.

3.5 H-Curcumin Based Amines

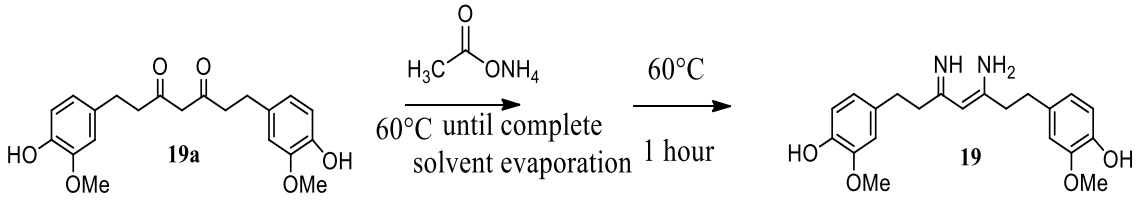
3.5.1 Hydrogenetaed curcumin (H-Curcumin)

Tetrahydrocurcuminoids (THC) compound **19a** was prepared by hydrogenation in the presence of Pd/C as a catalyst, the reaction was carried out in ethanol as a solvent. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic

techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 91.8%

3.5.2 Amination of H-Curcumin

H-Curcumin-based amine compound **19** was prepared by condensation reaction of H-curcumin **19 a** with Ammonium acetate (1:1 ratio) as the following; the mixture was heated at 60 °C until complete solvent evaporation. Then it was heated again at 60 °C for an hour as shown in scheme 6. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 60%.



Scheme 7: Chemical structure and reaction condition for making H-curcumin-based amine.

3.6 Curcumin-based compounds using Knoevenagel Condensation

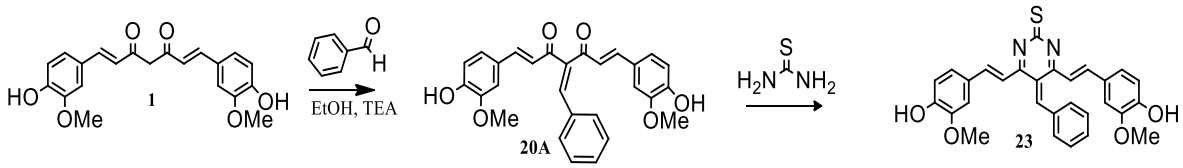
3.6.1 Preparation of curcumin-based compounds using Knoevenagel Condensation

Curcumin-based benzodiazepines, diazepines, diazoles, and amines were prepared using two-steps method; in the first step, the curcumin was reacted with benzaldehyde in ethanol solvent, and in the presence of

several organic bases such as diisopropylamine (DIPA), or tri-ethanol amine (TEA) which were used as catalysts to produce α,β-unsaturated intermediates using Knoevenagel condensation schemes 7-14. The second step involves acid-catalyzed condensation reactions of the prepared intermediate's curcumin carbonyl groups with several 1,2-diamino or hydrazine compounds to produce the desired compounds.

3.6.1.2 Derivatization of curcumin-based diazepines via Knoevenagel reaction

Curcumin-based diazepine compound **23** was prepared using a one-step process condensation cyclization reaction. Where the curcumin was mixed with thiourea in (1:1 ratio), benzaldehyde, tri ethanol amine (TEA) in ethanol 30 mL scheme 8. Benzaldehyde and (TEA) were employed to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 90.2%.

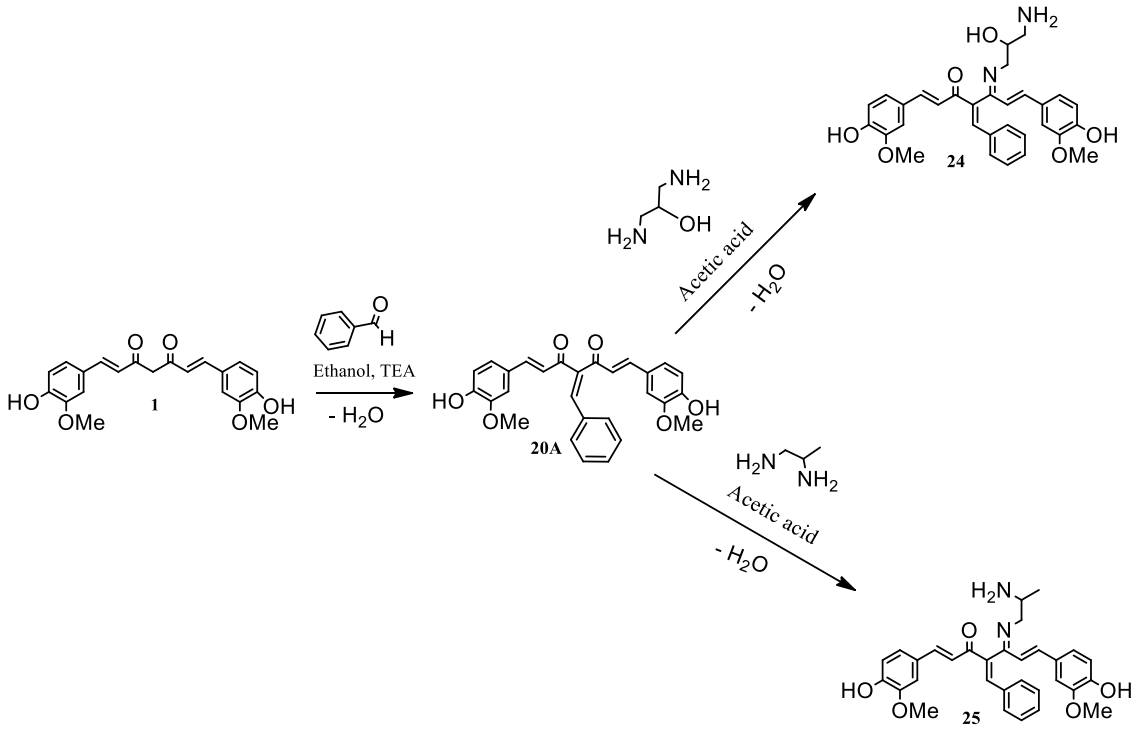


Scheme 8: Chemical structures and reaction conditions for making curcumin-based diazepines using Knoevenagel Condensation.

3.6.1.3 Derivatization of Curcumin-Based Amines using Knoevenagel reaction

Curcumin-based amines **24** and **25**, were prepared using two-steps method; in the first step, the curcumin was reacted with benzaldehyde in ethanol solvent, in the presence of tri ethanol amine (TEA) base, which was used as a catalyst to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation, scheme 9. The second step involves acid-catalyzed condensation reactions of one of the curcumin carbonyl groups of the prepared intermediate **20 A** with several amino compounds to produce the desired compounds.

An excess amount of acetic acid was added to the prepared intermediate which played a dual function; to neutralize the excess base from the first step, and as a catalyst to protonate the carbonyl oxygen and activate the carbon atom. followed by adding 1,3-diamino-2-propanol or 1,2-diaminopropane in (1:1 ratios) to produce amines **24**, and **25** respectively scheme 9. The reaction progress was monitored using TLC. The products were purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yields of 52.7 % and 73 % for compounds **24** and **25** respectively.

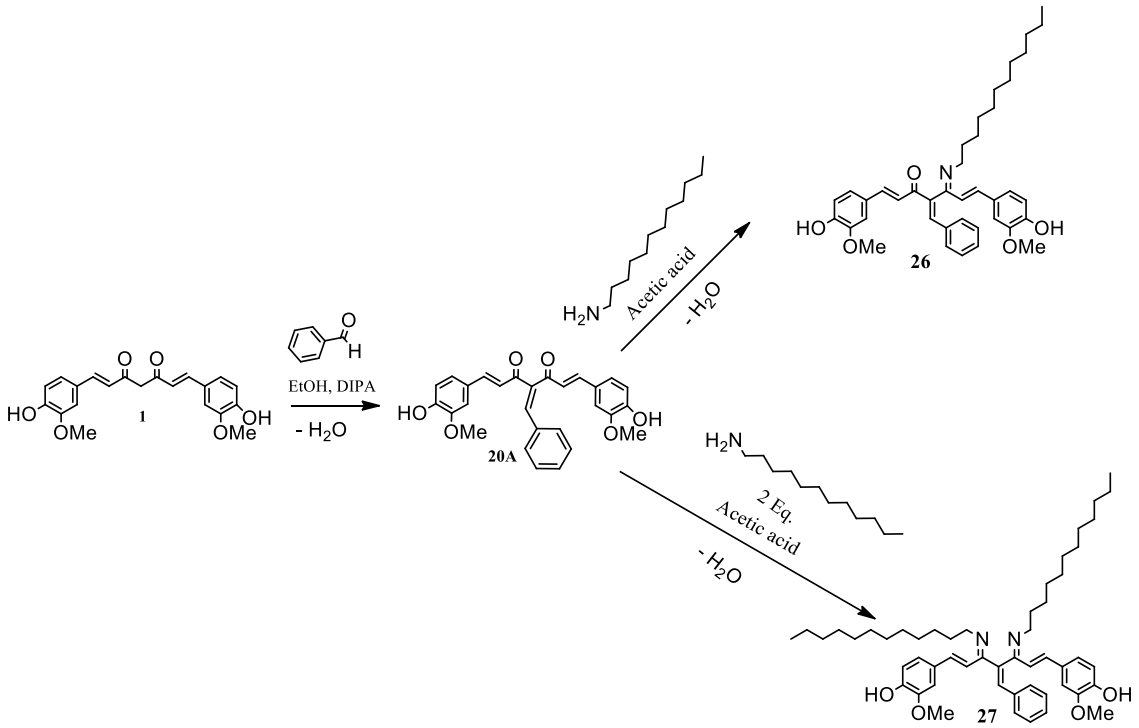


Scheme 9: Chemical structures and reaction conditions for making curcumin-based amines using Knoevenagel Condensation.

Curcumin-based amines **26** and **27** were prepared using two-steps method; in the first step, the curcumin was reacted with benzaldehyde in ethanol solvent, in the presence of diisopropylamine base, which was used as a catalyst to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation, scheme 10. The second step involves acid-catalyzed condensation reactions of one of the curcumin carbonyl groups of the prepared intermediate **20 A** with amino compound with two ratios to produce the desired compounds.

An excess amount of acetic acid was added to the prepared intermediate which played a dual function; to neutralize the excess base from the first step, and as a catalyst to protonate the carbonyl oxygen and activate the carbon atom. followed by adding dodecyl amine in (1:1 and 1:2 ratios) to

produce amines **26**, and **27** respectively scheme 10. The reaction progress was monitored using TLC. The products were purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yields of 85.1 and 86.3 % for compounds **26** and **27** respectively.

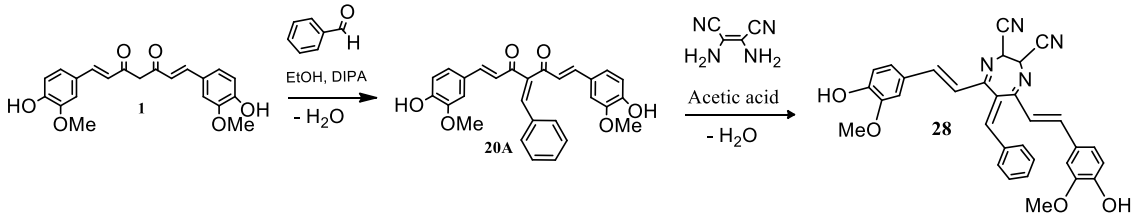


Scheme 10: Chemical structures and reaction conditions for making curcumin-based amines using Knoevenagel Condensation.

Curcumin-based diazepine **28**, was prepared using two-steps method; in the first step, the curcumin was reacted with benzaldehyde in ethanol solvent, in the presence of diisopropylamine base, which was used as a catalyst to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation scheme 11. The second step involves acid-catalyzed condensation cyclization reaction of the curcumin carbonyl groups of the

prepared intermediate **20 A** with hydrazine to produce the desired compound.

An excess amount of acetic acid was added to the prepared intermediate which played a dual function; to neutralize the excess base from the first step, and as a catalyst to protonate the carbonyl oxygen and activate the carbon atom. The diaminomaleonitrile in (1:1 ratio) was added to produce diazepine **28** scheme 11. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 89.3%.



Scheme 11: Chemical structures and reaction conditions for making curcumin-based diazepine using Knoevenagel Condensation.

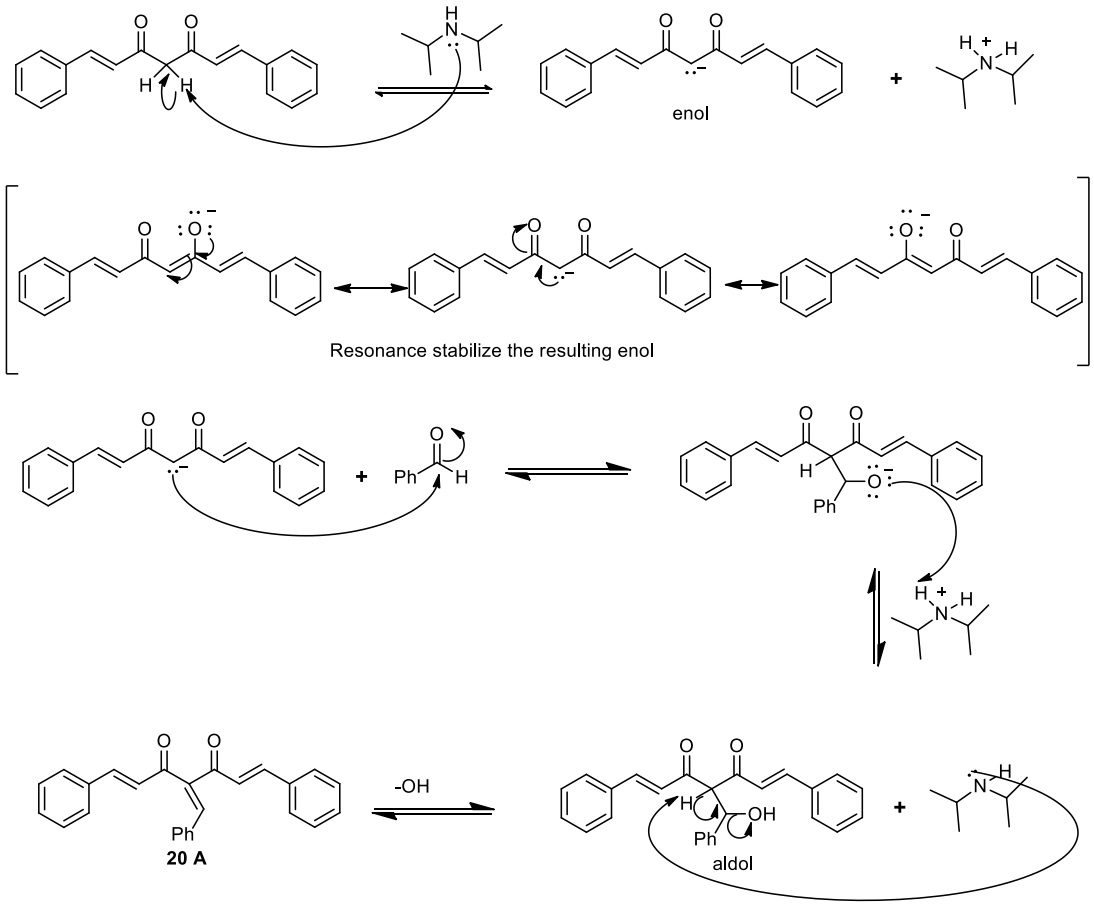
3.6.2 Stepwise mechanism of Knoevenagel condensation reaction of Curcumin

A detailed mechanism of the Knoevenagel condensation of curcumin with benzaldehyde in the presence of diisopropylamine base to form α,β-unsaturated curcumin-based compound **20A** is shown in scheme 12. The general mechanism of Knoevenagel condensation is an aldol like reaction between 1,3-dicarbonyl (nucleophile) and an aldehyde (which acts as electrophile) requiring an amine as a base. Moreover, this reaction proceeds

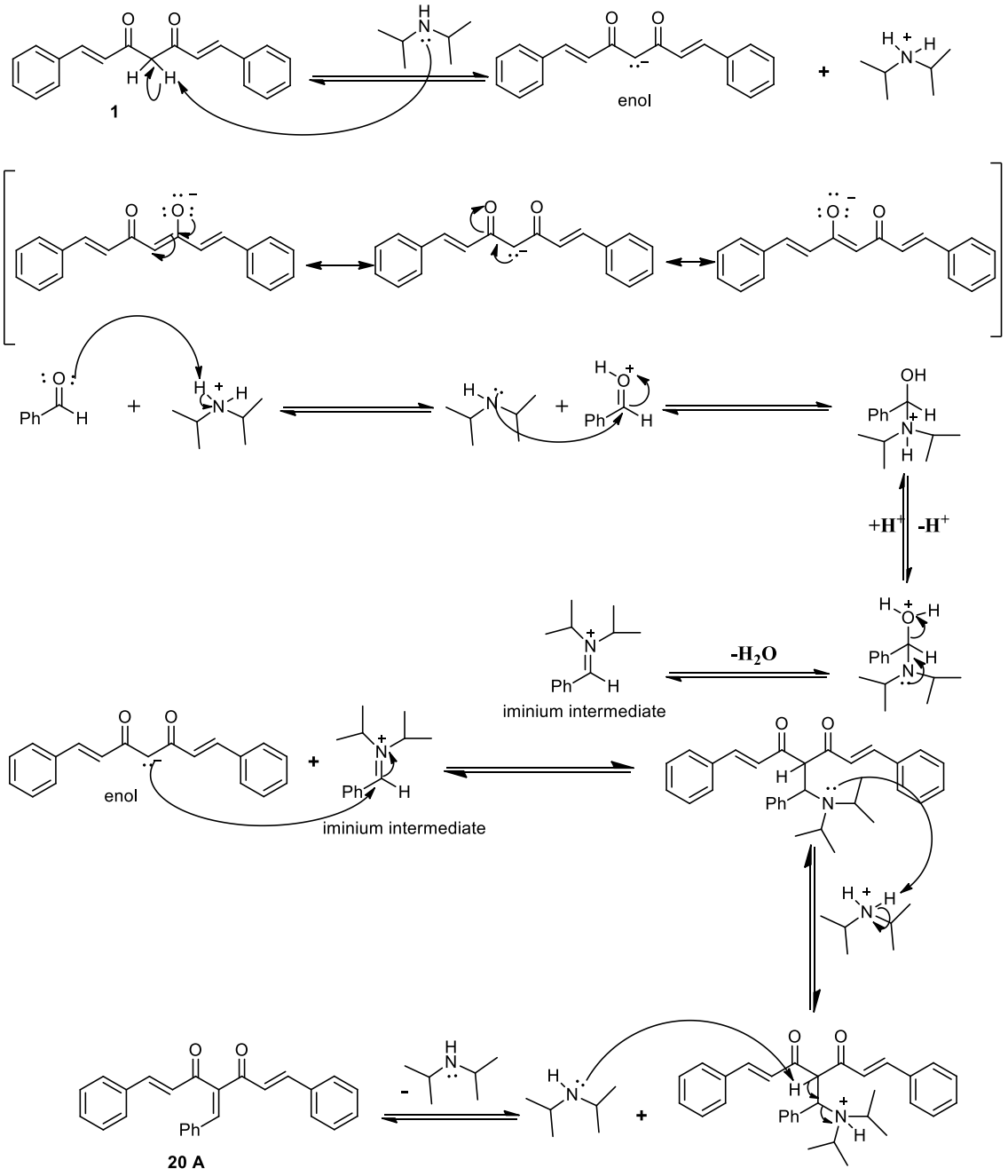
with loss of water. The Knoevenagel condensation was employed to prevent keto-enol tautomerization, which expected to enhance the yield.

An enol intermediate is formed initially, where the diisopropylamine acts as a base abstracting the α-hydrogen of curcumin molecule. The resulting enol is stabilized by the resonance structures that are shown in scheme 12. The resulting enol reacts with the benzaldehyde, and the resulting aldol undergoes subsequent base-induced elimination.

A reasonable variation of the mechanism, in which the diisopropylamine acts as organocatalyst, involves the corresponding iminium intermediate as the electrophile scheme 13.



Scheme 12: A stepwise mechanism of the Knoevenagel condensation of curcumin with benzaldehyde in the presence of diisopropylamine base to form α,β-unsaturated curcumin-based compound 20A.

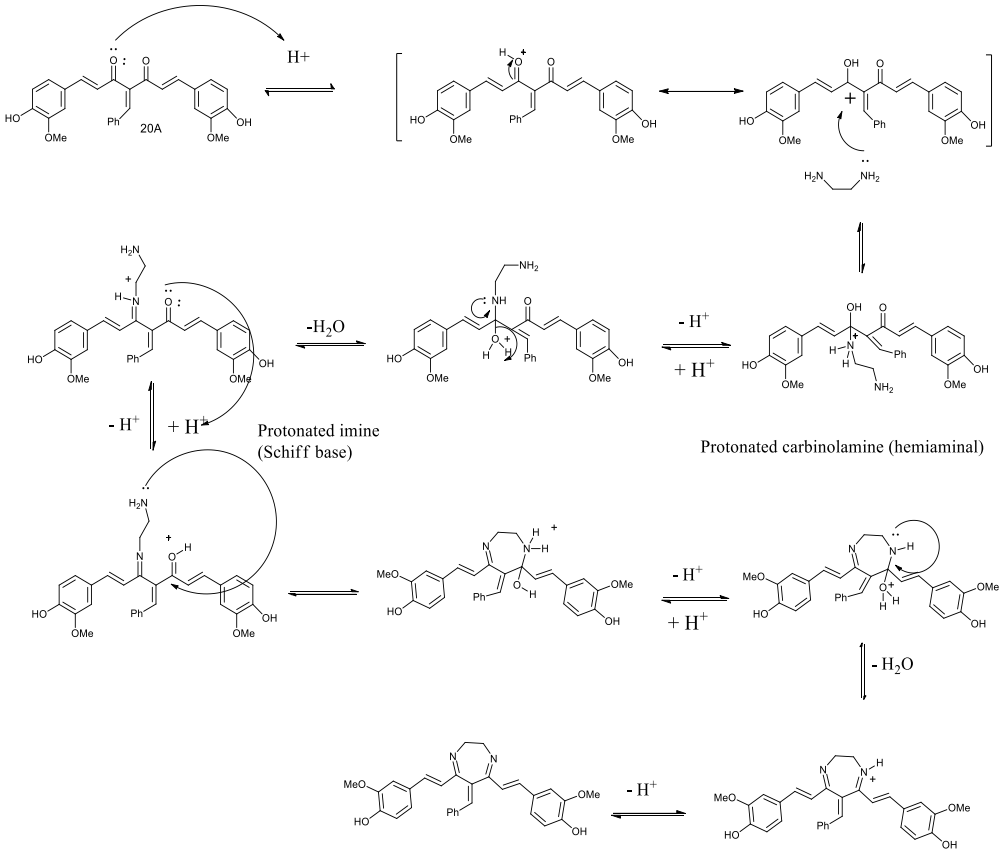


Scheme 13: A stepwise mechanism of the Knoevenagel condensation of curcumin with benzaldehyde where the diisopropylamine acts as organocatalyst to form α,β-unsaturated curcumin-based compound 20A.

The second step of the mechanism is an acid-catalyzed condensation reaction of α,β-unsaturated curcumin-based intermediate compound **20A** with various 1,2 diamino and hydrazine compounds to form α,β-unsaturated curcumin-based benzodiazepines, diazepines and amines as

shown in scheme 14. The first step is a typical acid-catalyzed nucleophilic addition of the diamine to one of the curcumin carbonyl groups. The acid catalyst protonates the carbonyl group, and the diamine or hydrazine (weak nucleophiles) attacks the protonated, activated carbonyl. Loss of a proton from the positively charged intermediate gives a hemiaminal **(carbinolamine).**

The second step is an acid catalyzed dehydration where the carbinolamine is converted to an imine by losing of water and forming a double bond. This dehydration follows the same mechanism as the acid-catalyzed dehydration of an alcohol. Protonation of the hydroxy group converts it to a good leaving group, and it leaves as water. The resulting cation is stabilized by resonance forms, including one with all octets filled and the positive charge on nitrogen. Loss of a proton gives the imine intermediate. Acid-catalyzed nucleophilic attack by the second amino group on the intermediate **imine** followed by a loss of a second water molecule results on the formation of the product.

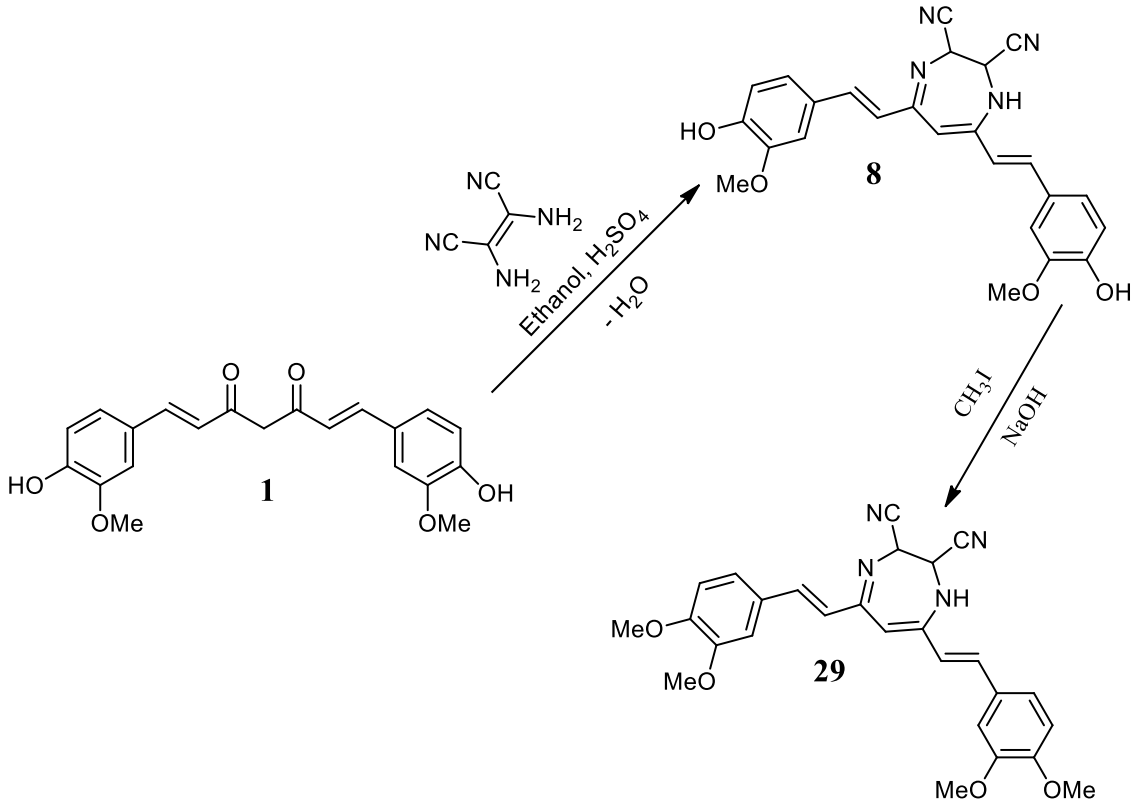


Scheme 14: A detailed mechanism of the condensation reaction of α,β-unsaturated curcumin-based intermediate compound 20A with diverse 1,2 diamino and hydrazine compounds to form α,β-unsaturated curcumin-based benzodiazepines, diazepines, and amines.

3.7 Methylation of the curcumin phenolic hydroxy

Diazepine **29** was prepared in a two-step process, as shown in scheme 15. The first step involved the formation of compound **8** by condensation cyclization reaction between curcumin and diaminomaleonitrile as described before. In the second step, the phenolic groups of compound **8** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of

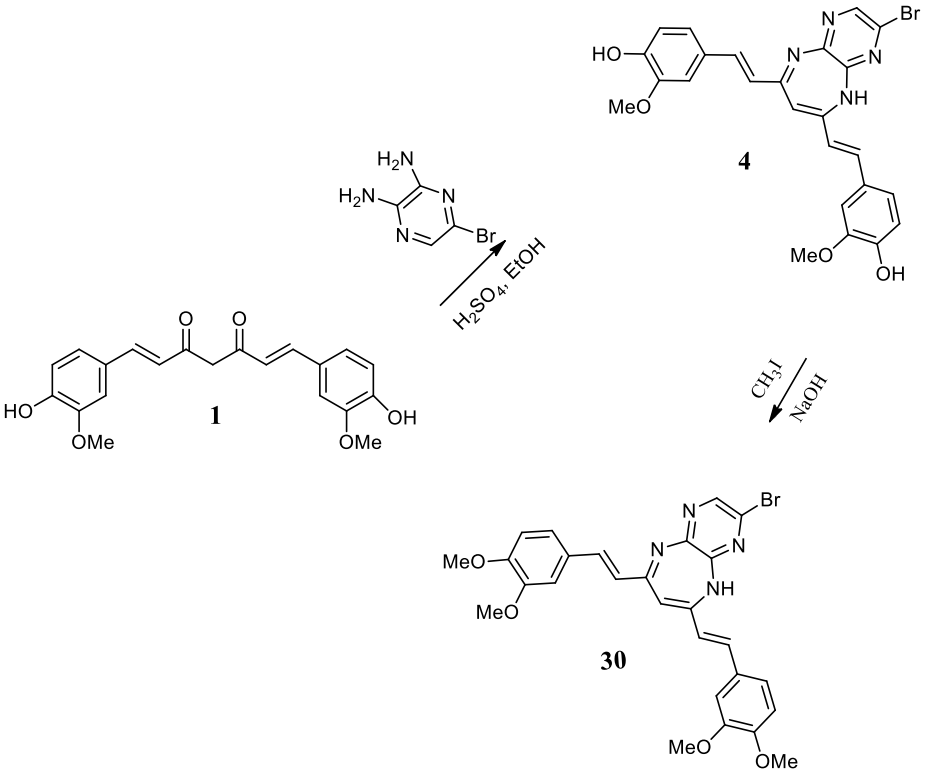
79.9%. Compound **29** was prepared in order to determine the effect of the hydroxyl group on the antimicrobial activity.



Scheme 15: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize Diazepine 29.

Benzodiazepine **30** was prepared in a two-step process, as shown in scheme 16. The first step involved the formation of compound **4** by condensation cyclization reaction between curcumin and 2,3-diamino-5-bromopyrazine as described before. In the second step, the phenolic groups of compound **4** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR, obtained results are also summarized in the experimental part. The

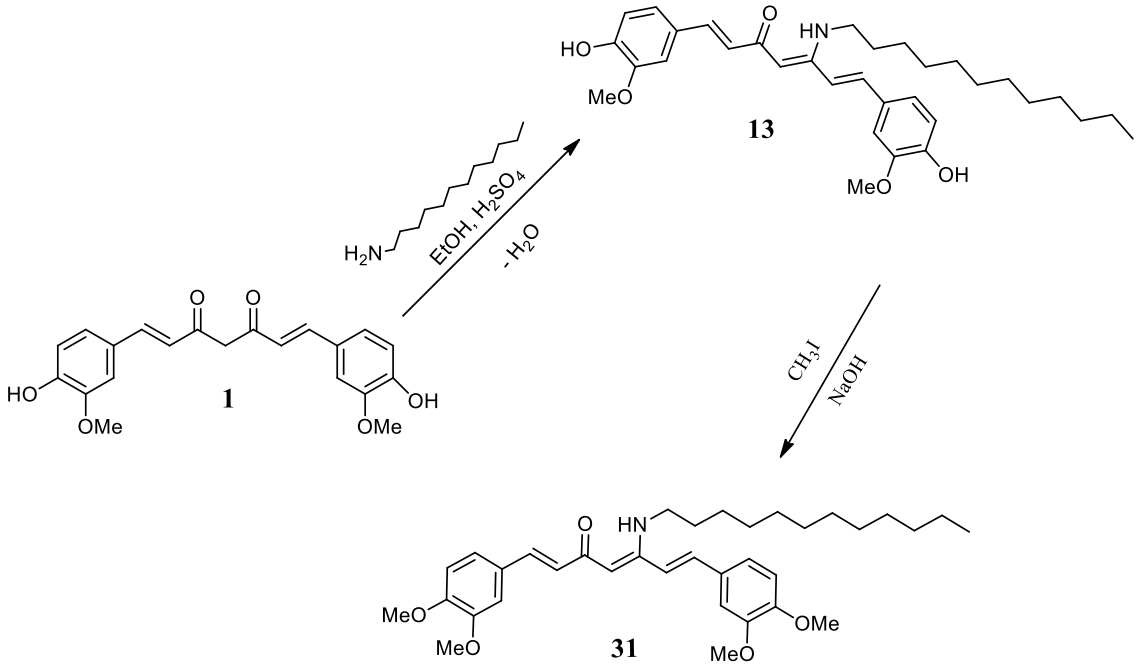
yield was about 21.1%. Compound **30** was prepared in order to determine the effect of the hydroxyl group on the antimicrobial activity.



Scheme 16: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize Benzodiazepine 30.

Amine **31** was synthesized in a two-step process, as shown in scheme 17. The first step involved the formation of compound **13** by condensation reaction between curcumin and dodecylamine as described before. In the second step, the phenolic groups of compound **13** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 80.7%. Compound

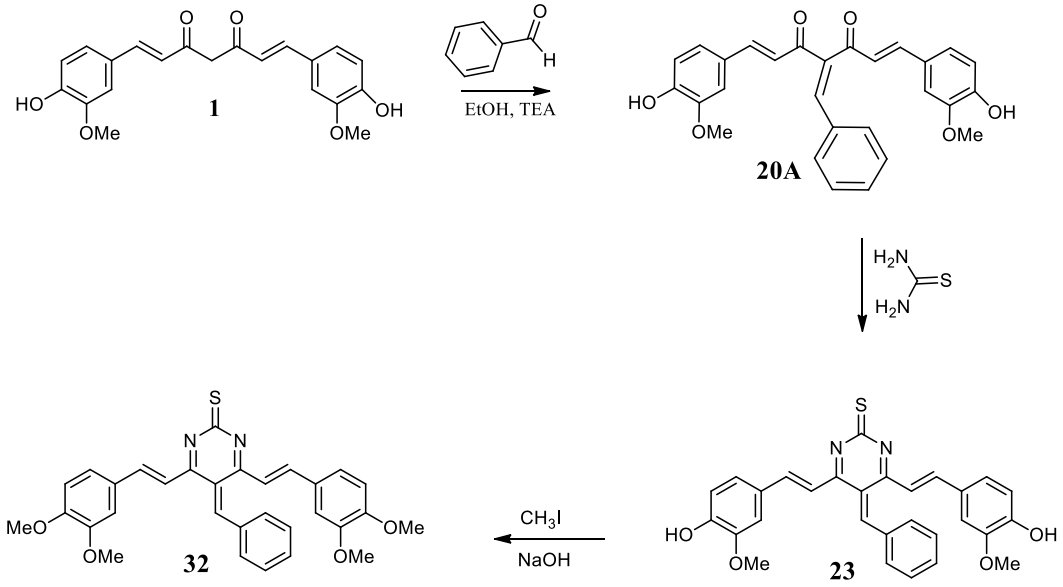
31 was prepared to determine the effect of the hydroxyl group on the antimicrobial activity.



Scheme 17: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize amine 31.

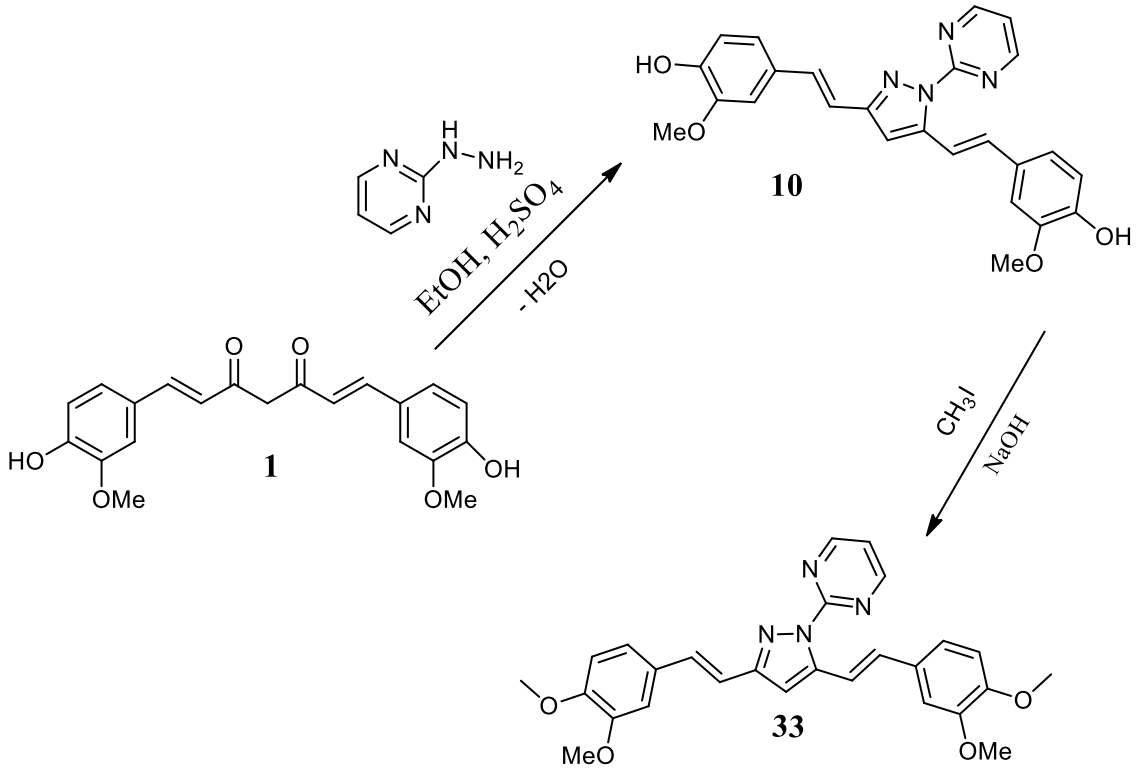
Compound **32** was prepared in a three-step process, the reaction is shown in scheme 18, initially compound **23** was prepared using two-step process between curcumin and benzaldehyde using Knoevenagel condensation to produce intermediate compound **20A** followed by acid-catalyzed condensation cyclization reaction of the prepared intermediate **20A** with thiourea as described before. In the third step, the phenolic groups of compound **23** was alkylated by reacting with it iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including

melting point, TLC, IR, NMR; results are also summarized in the experimental part, yield of 95% compound **32** was prepared to determine the effect of the hydroxyl group on the antimicrobial activity.



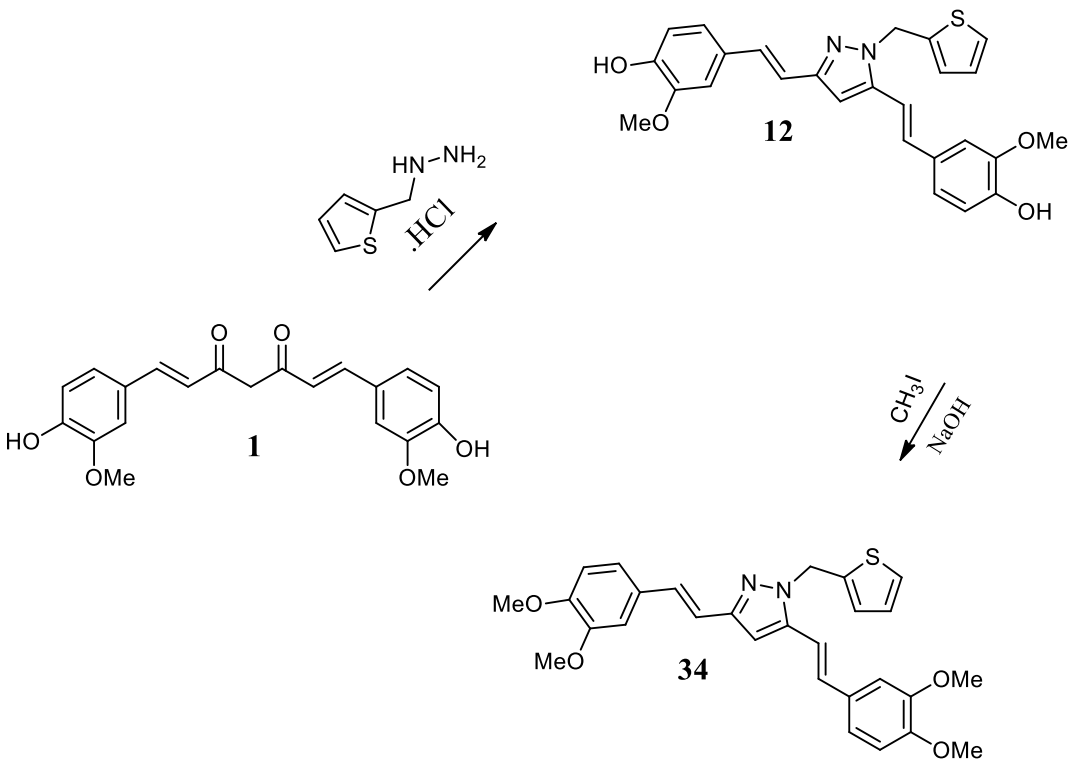
Scheme 18: Chemical structures and reaction conditions for the three-fold mechanism used to synthesize compound 32.

Diazole **33** was prepared in a two-step process, as shown in scheme 19. The first step involved the formation of compound **10** by condensation cyclization reaction between curcumin and 2-hydrazinopyrimidine hydrate as described before. In the second step, the phenolic groups of compound **10** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 50.3 %. Compound **33** was prepared to determine the effect of the hydroxyl group on the antimicrobial activity.



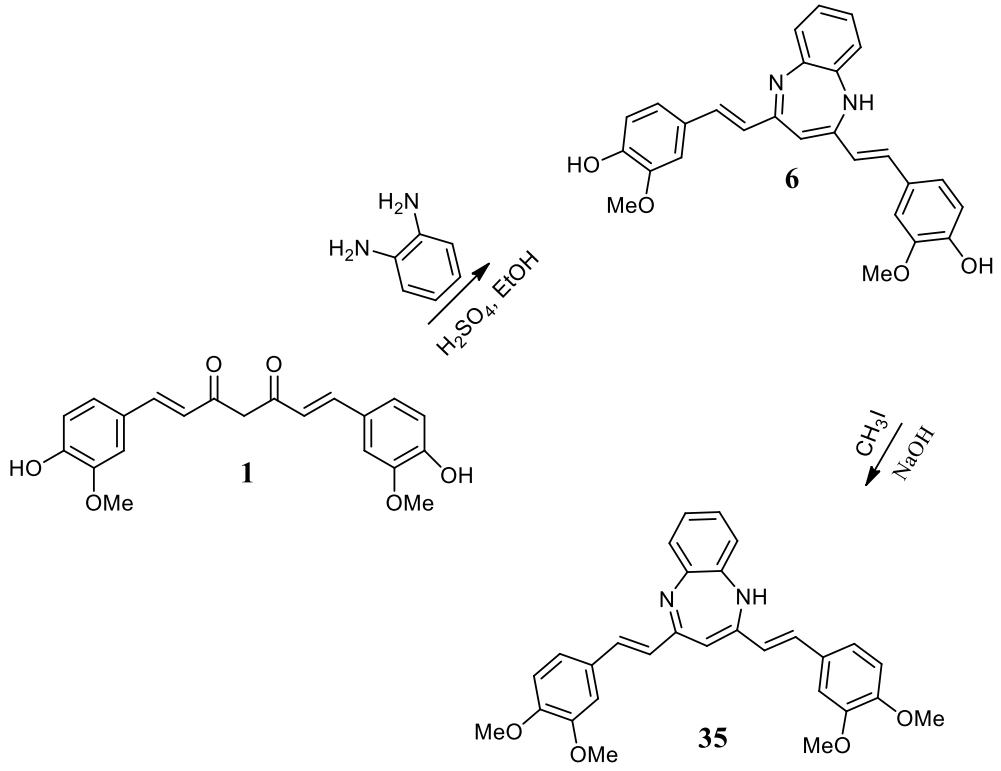
Scheme 19: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize diazole 33.

Diazole **34** was prepared in a two-step process, as shown in scheme 20. The first step involved the formation of compound **12** by condensation cyclization reaction between curcumin and (2-thinylmethyl) hydrazine hydrochloride as described before. In the second step, the phenolic groups of compound **12** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using melting point, TLC, IR, NMR; results are also summarized in the experimental part, Yield of 56.6%. Compound **34** was prepared to determine the effect of the hydroxyl group on the antimicrobial activity.



Scheme 20: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize diazole 34.

Benzodiazepine **35** was prepared in a two-step process, as shown in scheme 21. The first step involved the formation of compound **6** by condensation cyclization reaction between curcumin and 1,2- phenylenediamine as described before. In the second step, the phenolic groups of compound **6** were alkylated by reacting with iodomethane in the presence of sodium hydroxide. The reaction progress was monitored using TLC. The product was purified as described in the experimental part, analyzed using several analytical and spectroscopic techniques, including melting point, TLC, IR, NMR; results are also summarized in the experimental part, yield of 36.73%. Compound **35** was prepared in order to determine the effect of the hydroxyl group on the antimicrobial activity.



Scheme 21: Chemical structures and reaction conditions for the two-fold mechanism used to synthesize benzodiazepine 35.

3.8 Antibacterial Activities of the Prepared Curcumin-Heterocyclics

The in-vitro minimum inhibition concentration (MIC) test was subjected to the synthesized curcumin-based heterocycles against four types of bacteria strains: S. aureus, E. coli, MRSA, and Klebsiella using the two-fold serial dilutions method in sterile 96- microwell plates according to the CLSI [88]. Results are summarized in Tables 3.1-3.6. As shown in the tables, all of the tested curcumin-based heterocycles showed varying degree of inhibition against the tested bacteria strains. In general, the potency against S. aureus and Klebsiella is greater than that against MRSA and E. coli. Compounds **2, 4, 5, 10, 11, 13, 16, 19, 35** showed MIC values in a range of (12.5- 100 μg/mL), meanwhile, compounds **3, 8, 9, and 25** showed lower MIC value

(1.56 µg/mL) against S. aureus, However, the highest activity was shown by compound **9** as it showed MIC of (1.56, 1.56, 50 µg/ mL) against S. aureus, MRSA, and Klebsiella respectively. And it is the only compound that showed activity against MRSA with MIC of (1.56 µg/ mL).

Table 3.1: MIC values (µg/ml) for studied curcumin-based benzodiazepine against different species of bacteria

Curcumin derivative	**MIC value (µg/ml)**			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
2	12.5	-	50	200
3	1.56	-	12.5	-
4	12.5	-	200	200
5	100	-	200	-
7	-	-	25	-

Table 3.2: MIC values (µg/ml) for studied curcumin-based diazepine against different species of bacteria

Curcumin derivative	**MIC value (µg/ml)**			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
8	1.56	-	100	100
9	1.56	1.56	50	-
23	12.5	-	200	-

Table 3.3: MIC values (µg/ml) for studied curcumin-based diazoles against different species of bacteria

Curcumin derivative	MIC value (µg/ml)			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
10	25	-	200	200
11	50	-	-	-
12	-	-	100	-

Table 3.4: MIC values (µg/ml) for studied curcumin-based amines against different species of bacteria

Curcumin derivative	MIC value (µg/ml)			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
13	-	-	50	50
14	-	-	50	50
15	200	-	100	200
16	100	100	-	-
25	1.56	-	25	-
26	-	-	-	200
27	-	100	100	25

Table 3.5: MIC values (µg/ml) for studied H-curcumin-based amines against different species of bacteria

Curcumin derivative	MIC value (µg/ml)			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
19	100	-	200	-

Table 3.6: MIC values (µg/ml) for studied functionalized curcumin-based compounds with alkyl group against different species of bacteria

Curcumin derivative	MIC value (µg/ml)			
	***S. aureus* ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae* ATCC 13883**	***E. coli* ATCC 25922**
29	-	-	-	-
30	-	-	-	-
31	-	-	-	-
32	-	-	-	-
33	-	-	-	-
34	-	-	-	200
35	25	-	25	200

The MBC of curcumin-based heterocycles that showed inhibition to bacterial growth was also determined. Majority of compounds showed high potency against S. Aureus and Klebsiella and showed no activity against MRSA, **C24** showed inhibition to E. coli Table 3.7.

Compound **9** has the highest potency against S. aureus (6.25 µg/mL) while compound **3** has the highest potency against Klebsiella (12.5 µg/mL).

In general, among the tested curcumin-based heterocyclic compounds, diazepines and benzodiazepines showed higher activity than diazoles and amines.

Among the tested diazoles compound **10** showed higher activity than compound **11 and 12**; and this could be attributed to the heterocyclic nature (presence of nitrogen atoms) on the ring attached to the diazole ring. Blocking the phenolic hydroxyl group with a methyl or other alkyl groups compounds **29-35** reduced both MIC and MBC values. Which investigate the effect of the hydroxyl group on the antimicrobial activity.

Table 3.7: MBC values (µg/ml) for studied curcumin-based heterocyclics against different species of bacteria

Curcumin derivative	MBC values (µg/ml)			
	S. aureus **ATCC** 6538P	**MRSA (clinical isolate)**	***K. pneumoniae*** **ATCC 13883**	***E. coli*** **ATCC 25922**
2	100-200	-	-	-
3	-	-	12.5	-
4	-	-	-	-
5	100	-	200	-
8	-	-	200	-
9	6.25	-	100	-
10	-	-	-	-
11	-	-	-	-
24	-	-	-	200
35	-	-	-	-

The MIC values of Ampicillin and Gentamicin antibiotics were determined by the two-fold serial dilutions method against MRSA and E. coli strains

Table 3.8. Both antibiotics showed high activity of (1.56 µg/ mL) against both strains.

Table 3.8: MIC values (µg/ml) for Ampicillin and Gentamicin Antibiotics against MRSA and E. Coli

Antibiotic	MRSA	E. Coli
Ampicillin	1.56	1.56
Gentamicin	1.56	1.56

The combination between each of four selected curcumin-based heterocyclics compound **2, 4, 10**, and **35** which had MIC 200 µg/mL and a sub-MIC of ampicillin antibiotic (0.39 µg/ml) decreased the MIC of curcumin-based heterocyclic compounds from 200 µg/mL into less than 1.56 µg/mL Table 3.9. The FIC index for both ampicillin antibiotic and curcumin-based heterocyclic compounds showed that these compounds with ampicillin antibiotic have a synergistic effect (FIC index ≤0.5).

Table 3.9: Synergistic effect of curcumin-based heterocyclics with ampicillin antibiotic.

Conc. (µg/ml	C2	C4	C10	C35
200	-	-	-	-
100	-	-	-	-
50	-	-	-	-
25	-	-	-	-
12.5	-	-	-	-
6.25	-	-	-	-
3.125	-	-	-	-
1.56	-	-	-	-

Blocking the genotoxicity test was performed on compound **8** using the ERIC-PCR profile for DNA extracted from *E. coli*. The genotoxicity test results on treated, and untreated *E. Coli* with compound **8** at different time intervals are shown in Fig. 3.1 The obtained ERIC-PCR profile showed no change in band intensity and the number of bands and their lengths compared to the treated and untreated samples at a specific time interval. According to this test, compound **8** does not interact with the DNA molecule of the *E. coli*. It is considered a non-mutagenic or non-genotoxic agent.

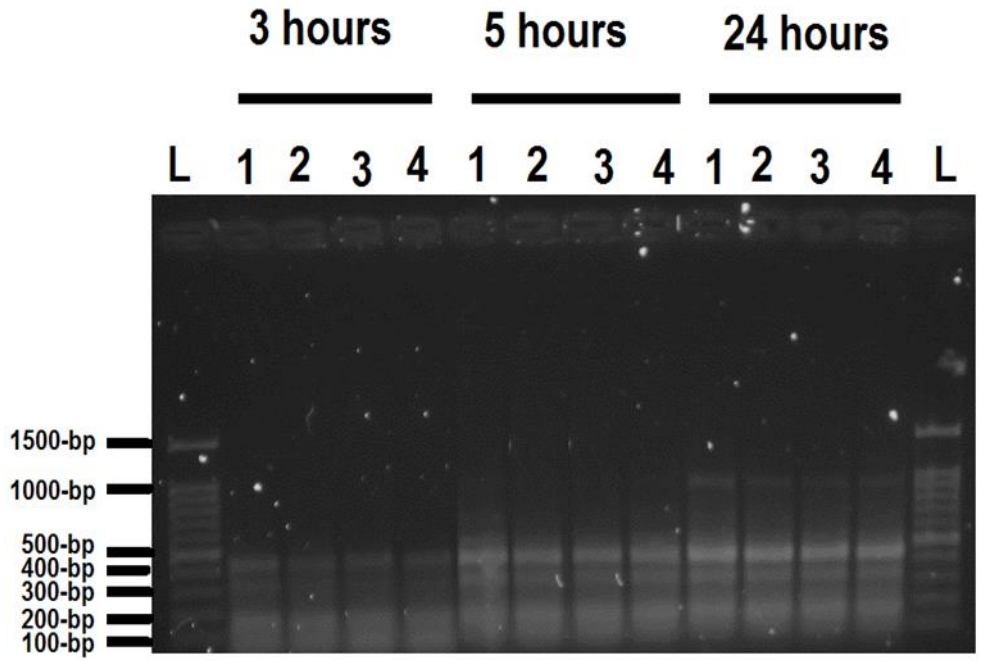


Fig. 3.1 ERIC-PCR profile of *E. coli* ATCC 25922 strain treated and untreated with different concentrations of compound **8** at different time intervals. Lanes L are 100-bp DNA ladder. Lanes 1 treated with DMSO as negative control, lanes 2, 3 and 4 are ERIC-PCR products from *E. coli* ATCC 25922 treated with compound 4 using 100 μg/ml, 50 μg/ml and 25 μg/ml, respectively.

CONCLUSION

- A novel set of curcumin-based heterocycles were prepared using simple and convenient methods.
- The antibacterial activities of a selected set of the prepared heterocycles against gram-positive and gram-negative bacteria were evaluated.
- The vast majority of the tested heterocycles effectively inhibit *S. aureus and K. pneumonia with MIC* values ranging between (1.56-200 μg/mL).
- Among the tested heterocycles, (compounds **2, 4, 10, 35**) showed inhibition against *E. coli* with MIC of (200 μg/ mL).
- The prepared curcumin-based heterocycles (compounds **2, 4, 10, 35**) showed a synergistic effect with ampicillin antibiotic.
- The MIC of curcumin-based heterocyclic compounds was decreased from 200 μg/mL into less than (1.56 μg/mL). Besides, such combinations can be used against several bacterial infections.
- Genotoxicity test revealed that compound **8** did not interact with DNA molecule in *E. coli* and considered as a non-mutagenic or non-genotoxic agent.
- Typically speaking, the prepared curcumin-based heterocycles are promising for designing a potentially active antibacterial synergized agent with the conventional antibiotics.

SUGGESTION FOR FURTHER WORK

The following recommendations are suggested for the further works:

1. Test the antibacterial activity of the rest of the prepared curcumin-based heterocycles against gram-positive and gram-negative bacteria.
2. Test the antibacterial activity of the prepared curcumin-based heterocycles against other different strains of gram-positive and gram-negative bacteria.
3. Test the synergistic effects of the prepared curcumin-based heterocycle with other conventional antibiotics
4. Test the anticancer and antiviral activity prepared curcumin-based heterocycles.
5. Employ the synthesized curcumin-based heterocycles (compounds **3, 4, 5, 10, 11, 12, 14, 20, 21, 22, 27, 30, 33,** and **34**) as a ligand for complex formation with different metals.
6. Functionalize the rest of the synthesized curcumin-based heterocycles with methoxy or butoxy groups.
7. Functionalize the synthesized curcumin-based heterocycles with different alkyl groups.

References

[1] Seelam N., Shrivastava S.P., Prasanthi S., **Synthesis and antimicrobial activity of some novel fused heterocyclic moieties,** *Org. Commun.*, **6(2)**, P 78-85, (2013).

[2] Mustafa Y. F., Synthesis, ***characterization and antibacterial activity of novel heterocycle, coumacine, and two of its derivatives,*** **Saudi Pharmaceutical Journal, 26** , P 870–875, (2018).

[3] Hamed O.A., Mehdawia N., Abu Taha A., Hamed E.M., Al-Nuria M.M. Hussein A.S., ***Synthesis and Antibacterial Activity of Novel Curcumin Derivatives Containing Heterocyclic Moiety***, **Iranian Journal of Pharmaceutical Research, 12 (1)**, P 47-56, (2013).

[4] Adwan G., Abu-Shanab B., Adwan K., ***Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant Pseudomonas aeruginosa strains,*** **Asian Pacific Journal of Tropical Medicine,** P 266-269, (2010).

[5] Bailey, R., "**Gram Positive vs. Gram Negative Bacteria**.", *Thought Co*, (2020) thoughtco.com/gram-positive-gram-negative-bacteria-4174239.

[6] Doi Y., Bonomo R.A., Hooper D.C., Kaye K.S., Johnson J.R., Clancy C.J, Thaden J.T., Stryjewski,7 and David M.S., Duin D.V, **Gram-**

Negative Bacterial Infections: Research Priorities, Accomplishments, and Future Directions of the Antibacterial Resistance Leadership Group, *CID*, **64**, P 1-6, (2017).

[7] Panawala L., **Difference Between Gram Positive and Gram-negative Bacteria,** (2017).

[8] Steward K., **Gram Positive vs Gram Negative**, (2019).

[9] Todar K., **Structure and Function of Bacterial Cells, Todar's Online Textbook of Bacteriology,** p 1-10, (2019).

[10] Rhee S.H., **Lipopolysaccharide: Basic Biochemistry, Intracellular Signaling, and Physiological Impacts in the Gut**, *Intest Res*, **12(2)**, P 90-95, (2014).

[11] Esteban J., Tanoira R.P., Jorge-Peremarch C.P, Barrena E.G., **Bacterial Adherence to Biomaterials Used in Surgical Procedures**, *Microbiology for Surgical Infections Diagnosis, Prognosis and Treatment* , P 41-57, (2014).

[12] Wang X., P.J. Quinn, **Endotoxins: Lipopolysaccharides of Gram-Negative Bacteria**, *Sub-cellular biochemistry*, **53(3)**, P 1-24, (2010).

[13] Al-Saadi A.G., Fazaa S., Abbas A.F, ***The Prevalence of the Gram Positive and Gram Negative Bacteria in Open Fractures and their Resistance Profiles to Antimicrobial Agents,*** **Journal of Global Pharma Technology, 12(9)**, P 83-90, (2018).

[14] Tilton J., **What Types of Bacteria Are Pathogenic?, Infection Control & First Aid Blog**, (2017).

[15] Harris L.G., Foster S.J, Richards R.G., **An introduction to staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: Review**,*European Cells and Materials*, **4**, P 39-60, (2002).

[16] G.Wirtanen ,S.Salo, **Chapter 5 - Biofilm Risks**, *Woodhead Publishing Series in Food Science, Technology and Nutrition*, (2016), Pages 55-79.

[17] Soniat T., 10 - **Managing molluscan shellfish-borne microbial diseases,** *Woodhead Publishing Series in Food Science, Technology and Nutrition*, P 248-269, (2009).

[18] Bitrus A.A., Peter O.M., Abbas M.A., Goni M.D., **Staphylococcus aureus: A Review of Antimicrobial Resistance Mechanisms**, *Veterinary Sciences: Research and Reviews*, **4(2)**, P 43-54, (2018).

[19] Jones J., **Chapter 11 - INFECTIOUS DISEASES**, *Essential Human Disease for Dentists*, P 177-194, (2006).

[20] Pruthi S., **MRSA infection, Mayo Foundation for Medical Education and Research (MFMER)**, (2011).

[21] Garoy E.Y, Gebreab Y.B., Achila O.O., Tekeste D.G., Kesete R., Ghirmay R., Kiflay R., Tesfu T., ***Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients—A Multicenter Study in Asmara, Eritrea***, **Canadian Journal of Infectious Diseases and Medical Microbiology,** P1-9 , (2019).

[22] Fact Sheet: Methicillin-Resistant Staphylococcus Sureus (MRSA), **Hamilton county public health,** https://www. flickr.com/ photos/ niaid/ 8436193898 Accessed on 2019, December, 24.

[23] Buckle J., Chapter 7 – Infection, **Clinical Aromatherapy (Third Edition)Essential Oils in Healthcare,** P 130-167, (2015).

[24] Klebsiella pneumoniae in Healthcare Settings, **Centers for Disease Control and Prevention,** (2010).

[25] Podschun R., Ullmann U., Klebsiella spp. **as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors,** *CLINICAL MICROBIOLOGY REVIEWS*, **Vol. 11, No. 4**, p. 589–603, (1998).

[26] Caneiras C., Lito L., Cristino J.M., Duarte A., **Community- and Hospital-Acquired Klebsiella pneumoniae Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance**, *microorganisms*, **7(38)**, P1-14, (2019).

[27] Ashurst J.V, Dawson A., Klebsiella Pneumonia, **StatPearls Publishing,** (2020).

[28] Cohen J, **Scientists engineer a powerful new weapon against antibiotic-resistant bacteria**, (2018).

[29] Makvana S., Krilov L.R., **Escherichia coli Infections**, *pediatric in review*, **36 (4)**, P 167-171. (2015).

[30] Kabiru L.M., Bello M., Kabir J., Grande L., Morabito S., **Detection of Pathogenic Escherichia coli in Samples Collected at an Abattoir in Zaria, Nigeria and at Different Points in the Surrounding Environment**, *Int. J. Environ. Res. Public Health*, **12**, P 679-691, (2015).

[31] Woodward S., E. ***coli: A brief overview***, **British journal of nursing**, **24(3)**, P.158-160, (2015).

[32] Crawford S.E., Daum R.S., **Chapter 35 - Bacterial Pneumonia, Lung Abscess, and Empyema,** *Pediatric Respiratory Medicine*, P. 501-553, (2008).

[33] Cullough M., E. coli: **What you need to know**, (2019).

[34] Jumaa S., Karaman R., Antibiotics, *In:* **Commonly Used Drugs**, P. 1-35, (2015).

[35] Etebu E., Arikekpar I., Antibiotics: **Classification and mechanisms of action with emphasis on molecular perspectives**, *IJAMBR*, **4**, P. 90-10, (2016).

[36] Etebu E., Arikekpar I., **Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives**, *IJAMBR*, **4**, P 90-101, (2016).

[37] Dowling A., Dwyer J. O', Adley C.C., **Antibiotics: Mode of action and mechanisms of resistance,** P 1-11, (2017).

[38] Cho H., Uehara T., Bernhardt T.G., **Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery**, *Cell*, **159(6)**, P 1300–1311, (2014).

[39] Dumancas G., Penicillins, **Encyclopedia of Toxicology**, **3**, P 768–772, (2015).

[40] Canzani D., Aldeek F., ***Penicillin G's function, metabolites, allergy, and resistance,*** **Journal of Nutrition and Human Health**, **1(1)**, (2017).

[41] Khan E., Shukla A., Srivast A., Tandon P., ***Molecular structure, spectral analysis and hydrogen bonding analysis of ampicillin trihydrate: A combined DFT and AIM approach***, **New Journal of Chemistry**, P1-13, (2013).

[42] Chou T.C., **Synergism and antagonism in chemotherapy**, *Pharmacol*, **58(3)**, P 621–681, (2006).

[43] Yin N., Ma W., Pei J., Ouyang Q., Tang C., Lai L., **Synergistic and Antagonistic Drug Combinations Depend on Network Topology**, *PLOS ONE*, **9(4)**, P1-7, (2014).

[44] Tekin E., Beppler C., White C., Mao Z., Savage V.M., Yeh P.J., Enhanced identification of synergistic and antagonistic emergent interactions among three or more drugs, *J. R. Soc. Interface*, **13**, P 1-11, (2016).

[45] Atawodi S., Atawodi J.C. Dzikwi A.A., ***POLYMERASE CHAIN REACTION: THEORY, PRACTICE AND APPLICATION: A REVIEW,*** **Sahel Medical Journal**, 13(2), P 1-13, (2011).

[46] Aryal S., **Polymerase Chain Reaction (PCR)- Principle, Procedure, Types, Applications and Animation**, (2018).

[47] Westermeier R., Gel Electrophoresis, **Encyclopedia of life science**, John Wiley and Sons, P1-7, (2005).

[48] Tankeshwar A., Agarose gel electrophoresis: **Principle, Procedure and Results**, *Molecular Biology*, (2019)

[49] Lee P.Y., Costumbrado J., Hsu C.Y., Kim Y.H., ***Agarose Gel Electrophoresis for the Separation of DNA Fragments,*** **Journal of Visualized Experiments**, **62**, P1-5, (2012).

[50] SAKS M., Upreti S., Rajendra S.V., Dang R., Genotoxicity: ***Mechanisms, Testing Guidelines and Methods***, **Global journal of Pharmacy & pharmaceutical Science, 5**, P1-6, (2017).

[51] Savale S.K., Genotoxicity of Drugs: ***Introduction, Prediction and Evaluation,*** **Asian Journal of Biomaterial Research**, **4(6)**, P 1-29, (2018).

[52] Saleem M.,Nazir M., Ali M.S., Hussain H., Lee Y.S., Riaza N., Jabbar A., **Antimicrobial natural products: an update on future antibiotic drug candidates,** *Natural Product Reports*, **27**, P 238–254, (2010).

[53] Cushnie T., Cushnie B., Lamb A.J., Alkaloids: ***an overview of their antibacterial, antibiotic-enhancing, and antivirulence activities,*** **International Journal of Antimicrobial Agents**, **44(5)**, P 1-45, (2014).

[54] Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A., **Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview**, *Medicines*, **5(93)**, P 1-16, (2018).

[55] Khoddami A., Wilkes M.A., Roberts T.H., **Techniques for Analysis of Plant Phenolic Compounds,** *Molecules*, **18**, P 2328-2375, (2013).

[56] K. Indira Priyadarsini, **Chemical and Structural Features Influencing the Biological Activity of Curcumin**, *Current Pharmaceutical Design*, 2013, **19**, 2093-2100.

[57] Jianguo Fang, Jun Lu, and Arne Holmgren, ***Thioredoxin Reductase Is Irreversibly Modified by Curcumin,*** **THE JOURNAL OF BIOLOGICAL CHEMISTRY**, **280**, 2005, pp. 25284–25290.

[58] Mohamed Jawed Ahsan, Habibullah Khalilullah, Sabina Yasmin, Surender Singh Jadav, and Jeyabalan Govindasamy, Synthesis, Characterisation, and In Vitro Anticancer Activity of Curcumin Analogues Bearing Pyrazole/Pyrimidine Ring Targeting EGFR Tyrosine Kinase, *BioMed Research International*, Volume **2013**, Article ID 239354, 14 pages. http://dx.doi.org/10.1155/2013/239354

[59] Rita M. Borik, Nagwa M. Fawzy, Sherifa M. Abu-Bakr and Magdy S. Aly, Design, Synthesis, **Anticancer Evaluation and Docking Studies of Novel Heterocyclic Derivatives Obtained via Reactions Involving Curcumin,** *Molecules*, 2018, **23**, 1398. doi:10.3390/molecules23061398

[60] Roxana Ciochina, Chasity Savella, Brianna Cote. **Davis Chang, and Deepa Rao, Synthesis and Characterization of New Curcumin Derivatives as Potential Chemotherapeutic and Antioxidant Agents,** *DRUG DEVELOPMENT RESEARCH*, **75**, (2014) ,88–96.

[61] Mahmood Ahmed, Muhammad Abdul Qadir, Muhammad Imtiaz Shafiq, Muhammad Muddassar, Abdul Hameed, Muhammad Nadeem Arshad, Abdullah M. Asiri, Curcumin: **Synthesis optimization and in silico interaction with cyclin dependent kinase,** *Acta Pharm.*, **67** (2017) 385–395.

[62] Shiyou Li, Wei Yuan, Guangrui Deng, Ping Wang, Peiying Yang, **Chemical composition and product quality control of turmeric (Curcuma longa L.)**, (2011). Faculty Publications. Paper 1. http://scholarworks. sfasu. edu/ agriculture_facultypubs/1

[63] Erika Ferrari, Francesca Pignedoli, Carol Imbriano, Gaetano Marverti, Valentina Basile, Ettore Venturi, and Monica Saladini, Newly **Synthesized Curcumin Derivatives: Crosstalk between Chemico-physical Properties and Biological Activity**, *Med. Chem.*, 2011, **54**, 8066−8077.

[64] Nikola Hadzi-Petrushev, Jane Bogdanov, Jovanka Krajoska, Jovana Ilievska, Biljana Bogdanova-Popov, Elizabeta Gjorgievsk, Vadim Mitrokhinc, Ramadan Sopie, Hristo Gagov, Andre Kamkin, Mitko Mladenov, **Comparative study of the antioxidant properties of monocarbonyl curcumin analogues C66 and B2BrBC in isoproteranol induced cardiac damage**, *Life Sciences*, **197** (2018) 10–18.

[65] Jaggi Lal , Sushil K. Gupta , D. Thavaselvam, Dau D. Agarwal, **Synthesis and pharmacological activity evaluation of curcumin derivatives**, *Chinese Chemical Letters*, **27** (2016) 1067–1072.

[66] Jun Li, Yun Wang, Chaozhe Yang, Pengfei Wang, Denise K. Oelschlager, Yong Zheng,

De-An Tian, William E. Grizzle, Donald J. Buchsbaum, and Mei Wan, **Polyethylene Glycosylated Curcumin Conjugate Inhibits Pancreatic Cancer Cell Growth through Inactivation of Jab1**, *Mol Pharmacol*, **76**, 81–90, 2009.

[67] Qingyong Li, Jian Chen, Shuyue Luo, Jialin Xu, Qiaoxian Huang, Tianyu Liu, ***Synthesis and assessment of the antioxidant and antitumour properties of asymmetric curcumin analogues***, **European Journal of Medicinal Chemistry** (2015.), doi: 10.1016/j.ejmech.2015.02.005.

[68] Grzegorz Litwinienko, and K. U. Ingold, **Abnormal Solvent Effects on Hydrogen Atom Abstraction. 2.Resolution of the Curcumin Antioxidant Controversy.** The Role of Sequential Proton Loss Electron Transfer, *J. Org. Chem.*, **69**, 5888-5896, 2004.

[69] Leila Ma'mani, Safoora Nikzad, Hamidreza Kheiri-manjili, Sharafaldin al-Mussawi, Mina Saeedi, Sonia Askarlou, Alireza Foroumadi, Abbas Shafiee, ***Curcumin-Loaded guanidine functionalized PEGylated I3ad mesoporous silica nanoparticles***

KIT-6: practical strategy for the breast cancer therapy, **European Journal of Medicinal Chemistry,** (2014), doi: 10.1016/j .ejmech. 2014.06.069.

[70] Satyendra Mishra, Upma Narain, Roli Mishra and Krishna Misra, **Design, development and synthesis of mixed bioconjugates of piperic acid–glycine, curcumin–glycine/alanine and curcumin glycinepiperic acid and their antibacterial and antifungal properties,** *Bioorganic & Medicinal Chemistry*, **13**, (2005) 1477–1486.

[71] Luyang Ding, Shuli Ma, Hongxiang Lou, Longru Sun and Mei Ji, **Synthesis and Biological Evaluation of Curcumin Derivatives withWater-Soluble Groups as PotentialAntitumor Agents: An in Vitro Investigation Using Tumor Cell Lines**, *Molecules*, **20**, 21501–21514, 2015; doi:10.3390/molecules201219772.

[72] Satyendra Mishra, Sejal Patel, and Chandni G Halpani, **Recent Updates In Curcumin Pyrazole and Isoxazole Derivatives: Synthesis and Biological Application, Chem**. Biodiversity 10.1002/ cbdv.201800366.

[73] P.V.Sri Ramya, Lalita Guntuku, Srinivas Angapelly, Chander Singh Digwal, Uppu Jaya Lakshmi, Dilep Kumar Sigalapalli, Bathini Nagendra Babu, V.G.M. Naidu, Ahmed Kamal, ***Synthesis and biological evaluation of curcumin inspired imidazo[1,2-a]pyridine***

analogues as tubulin polymerization inhibitors, **European Journal of Medicinal Chemistry** (2017), doi: 10.1016/j.ejmech.2017.11.010.

[74] Mustafa Usta, Heleen M. Wortelboer, Jacques Vervoort, Marelle G. Boersma, Ivonne M. C. M. Rietjens, Peter J. van Bladeren, and Nicole H. P. Cnubben, **Human Glutathione S Transferase-Mediated Glutathione Conjugation of Curcumin and Efflux of These Conjugates in Caco-2 Cells,** *Chem. Res. Toxicol*. 2007, **20**, 1895–1902.

[75] Sujuan Wang, Xixi Peng, Liangliang Cui, Tongtong Li, Bei Yu, Gang Ma, Xinwu Ba, **Synthesis of water-soluble curcumin derivatives and their inhibition on lysozyme amyloid fibrillation**, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2017), doi: 10.1016/j. saa. 2017.09.010.

[76] Siti Noor Hajar Zamrus, Muhammad Nadeem Akhtar, Swee Keong Yeap, Ching Kheng Quah, Wan‑Sin Loh, Noorjahan Banu Alitheen, Seema Zareen, Saiful Nizam Tajuddin, Yazmin Hussin and Syed Adnan Ali Shah, ***Design, synthesis and cytotoxic effects of curcuminoids on HeLa, K562, MCF‑7 and MDA‑MB‑231 cancer cell lines***, **Chemistry Central Journal**, (2018) 12-31.

[77] Wenjie Zhang, Heyuan Bai, Liqiang Han, Han Zhang, Bo Xu, Jingrong Cui, Xin Wang, Zemei Ge, Runtao Li, **Synthesis and biological evaluation of curcumin derivatives modified with α-**

amino boronic acid as proteasome inhibitors, Bioorganic & Medicinal Chemistry Letters, **28**, (2018) 2459–2464.

[78] Rama I. Mahran,Magda M. Hagras, Duxin Sun, and Dean E. Brenner, ***Bringing Curcumin to the Clinic in Cancer Prevention: a Review of Strategies to Enhance Bioavailability and Efficacy***, **The AAPS Journal,** (# 2016) DOI: 10.1208/s12248-016-0003-2.

[79] Volker Lorenz, Phil Liebing, Markus Suta, Felix Engelhardt, Liane Hilfert, Sabine Busse,Sida Wang, Claudia Wickleder, Frank T. Edelmann, ***Synthesis, structure, complexation, and luminescence properties of the first metal-organic curcumin compound Bis(4-triphenylsiloxy) curcumin,*** **Journal of Luminescence**, **211** (2019) 243–250.

[80] Fiona C. Rodrigues, N.V. Anil Kumar, Goutam Thakur, ***Developments in the anticancer activity of structurally modified curcumin: An up-to-date review,*** **European Journal of Medicinal Chemistry**, **177** (2019) 76-104.

[81] A. Golonko, H. Lewandowska, R. _Swisłocka, U.T. Jasi_nska, W. Priebe, W. Lewandowski, ***Curcumin as tyrosine kinase inhibitor in cancer treatment,*** **European Journal of Medicinal Chemistry**, **181** (2019) 111512.

[82] Sawsan A. Noureddin, Reda M. El-Shishtawy, Khalid O. Al-Footy, ***Curcumin analogues and their hybrid molecules as multifunctional***

drugs, **European Journal of Medicinal Chemistry**, **182** (2019) 111631.

[83] Tyagi P., Singh M., Kumari H., Kumari A., Mukhopadhyay K., **Bactericidal Activity of Curcumin I Is Associated with Damaging of Bacterial Membrane,** *PLOS ONE*, **10(3)**, P 1-16, (2015).

[84] Fares O., "**Design, Synthesis and Biological Activities of Curcumin Based Alkaloids"**, Master's thesis, An-Najah National University, Nablus, Palestine, (2018).

[85] Taki Sato, Mayumi Hotsumi, Koki Makabe, Hiroyuki Konno, Design, **synthesis and evaluation of curcumin-based fluorescent probes to detect Aβ fibrils,** Bioorganic & Medicinal Chemistry Letters, **28** (2018) 3520–3525.

[86] Wing-Hin Lee, Ching-Yee Loo, Mary Bebawy, Frederick Luk, **Rebecca S Mason, and Ramin Rohanizadeh, Curcumin and its Derivatives: Their Application in Neuropharmacology and Neuroscience in the 21st Century,** Current Neuropharmacology, **2013**, 11, 338-378.

[87] Andrews, J. M., ***BSAC standardized disc susceptibility testing method (version 5),*** **Journal of Antimicrobial Chemotherapy**,**58(3)**, P 511-529, (2006).

[88] Clinical and Laboratory Standards Institute (CLSI). **Performance standards for antimicrobial susceptibility testing.** 28th ed. CLSI supplement. M100. Wayne, PA, USA; 2018.

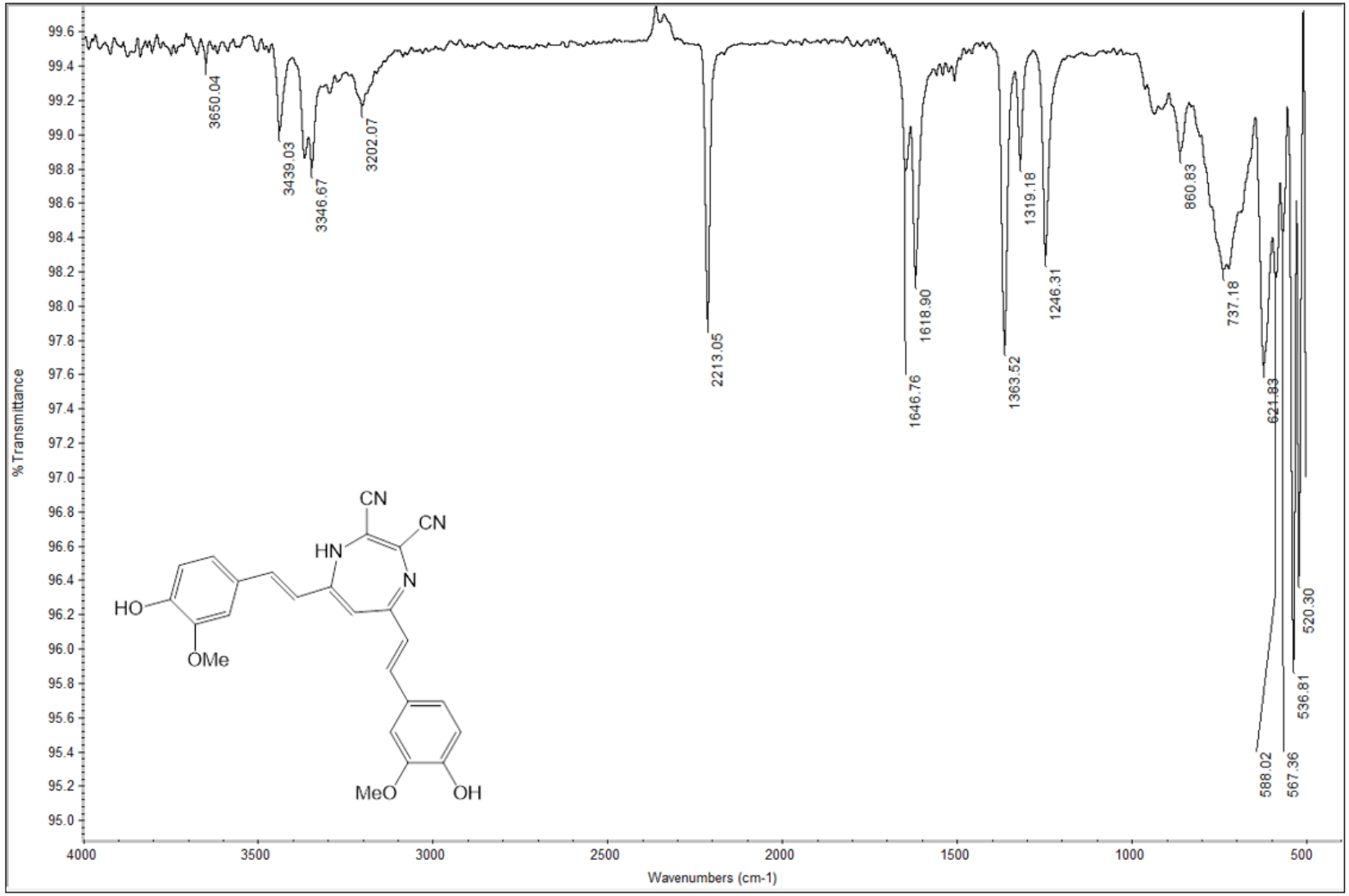
[89] Laishram S., Pragasam A.K., Bakthavatchalam Y.D., **Veeraraghavan B., An update on technical, interpretative and clinical relevance of antimicrobial synergy testing methodologies,** *Indian J Med Microbiol*, **35(4)**, P 445-468, (2017).

[90] Adwan K., Jarrar N., Abu-Hijleh A., ***Molecular analysis and susceptibility patterns of methicillin-resistant Staphylococcus aureus strains causing community- and health care-associated infections in the northern region of Palestine.*** **American Journal of Infection Control**. **41(3)**, P 195-198, (2013) . https://doi.org/10.1016/j.ajic.2012.03.040

[91] Abu-Hijleh A., Adwan G., Abdat W., **Biochemical and molecular evaluation of the plant *Ecballium elaterium* extract effects on *Escherichia coli*.** J Adv Biol Biotechnol, **19(2)**, P 1-11, (2018).

[92] Atienzar F.A., Venier P., Jha A.N., Depledge,MH. **Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations,** *Mutat Res*., **521(1-2)**, P 151-163, (2002).

APPENDIX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1319.18
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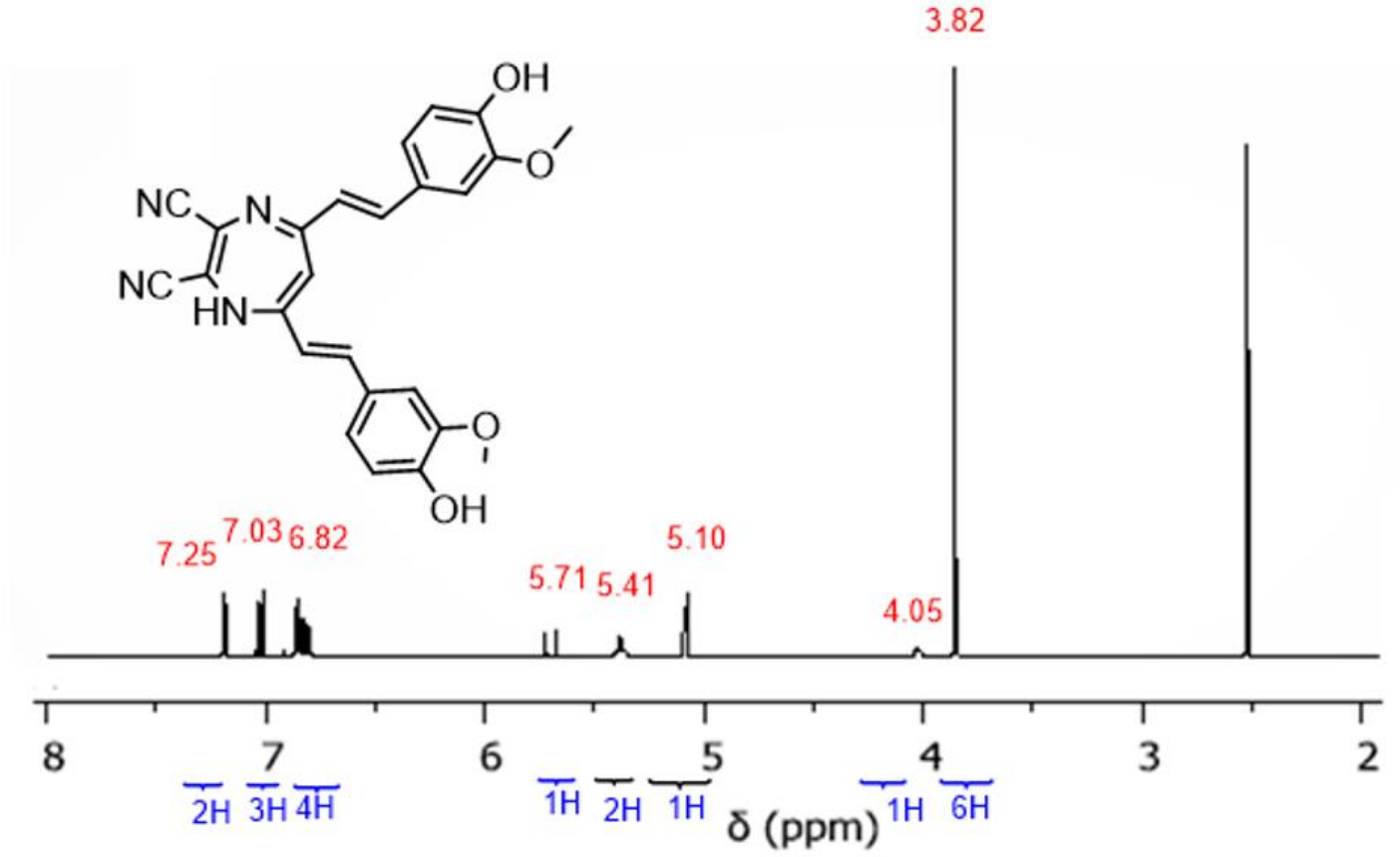
HO
OMe
MeO
OH



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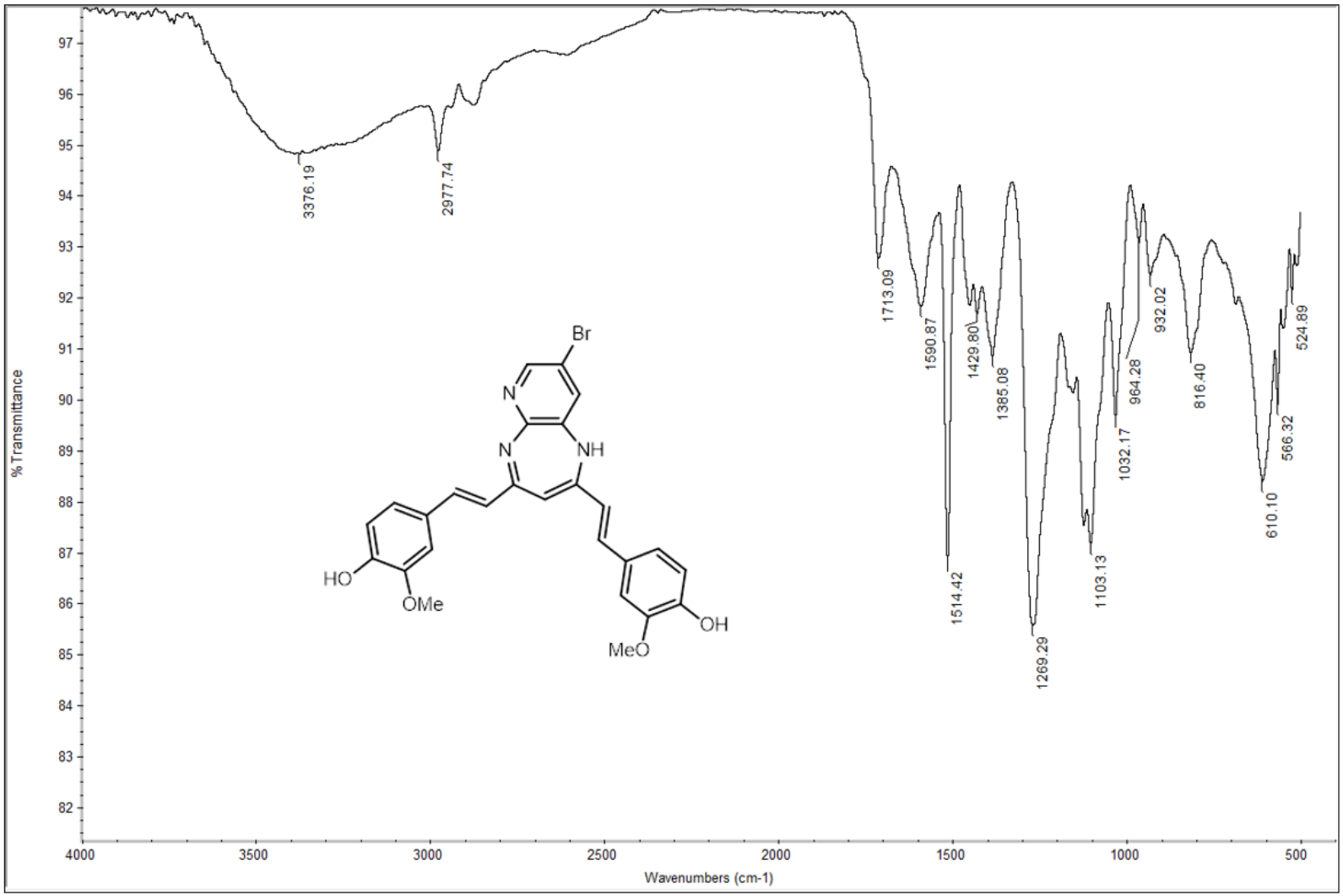
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1103.13
1032.17
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N
N
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OMe
MeO
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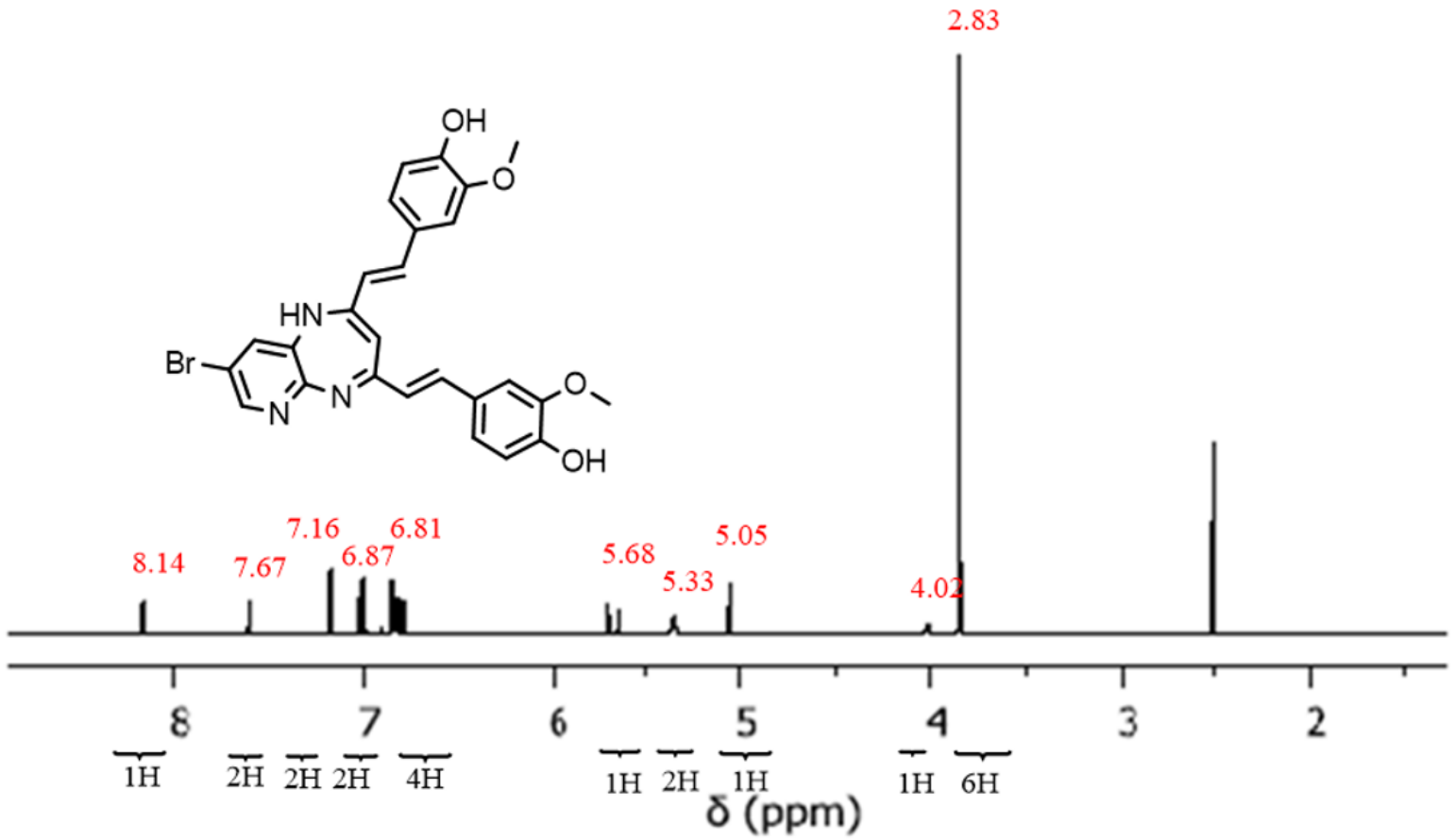

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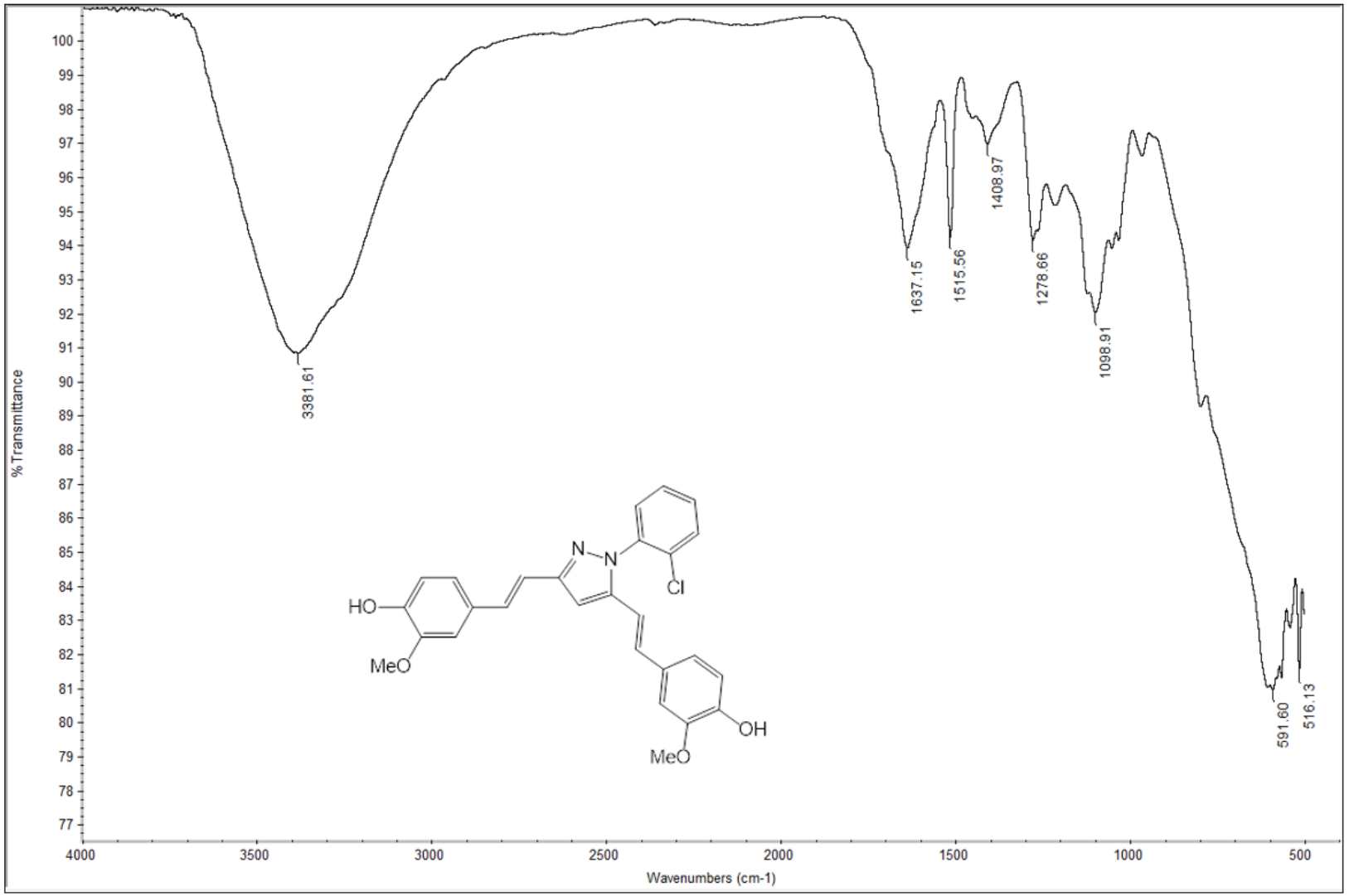
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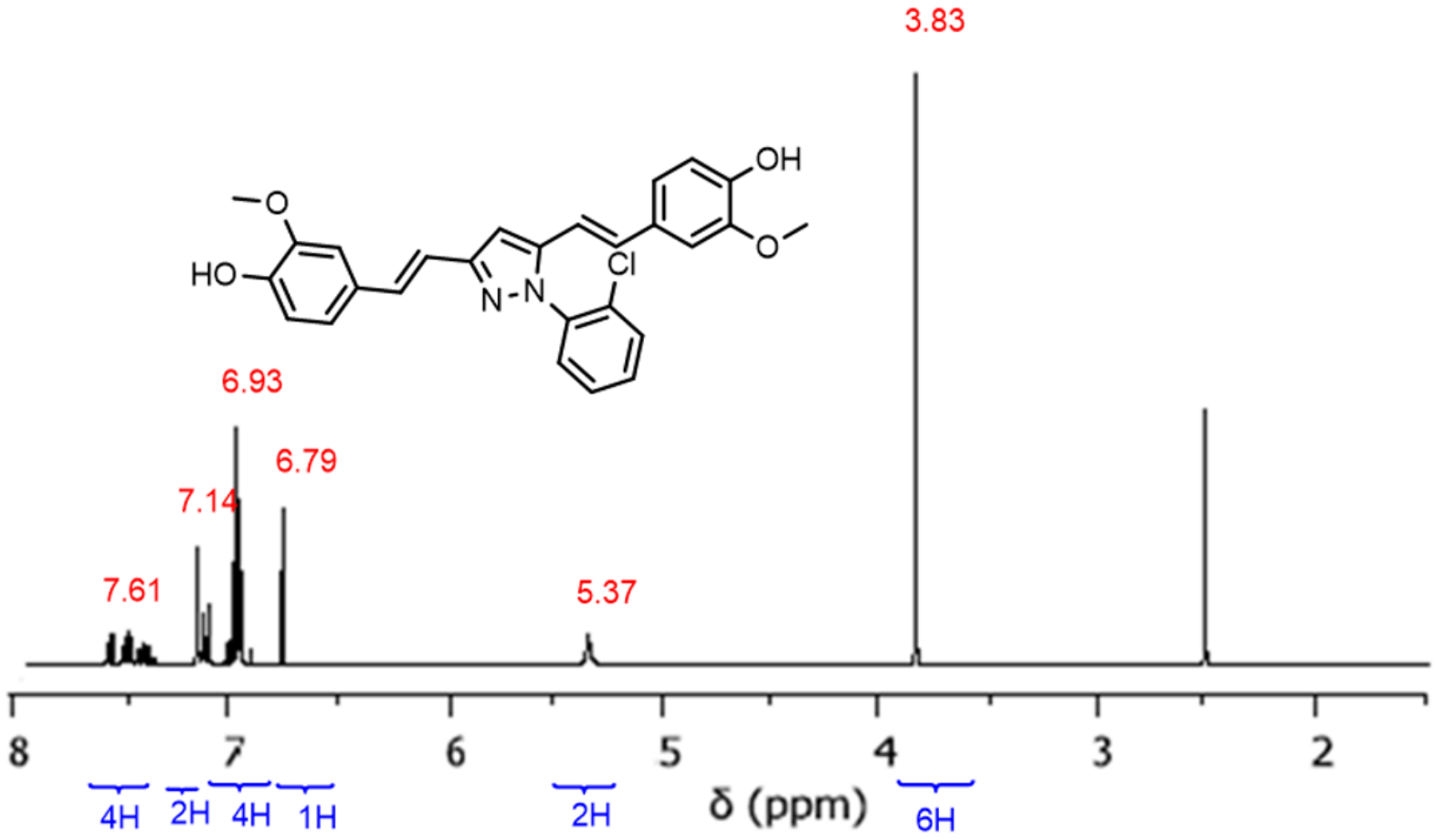
N
Cl
OH
MeO



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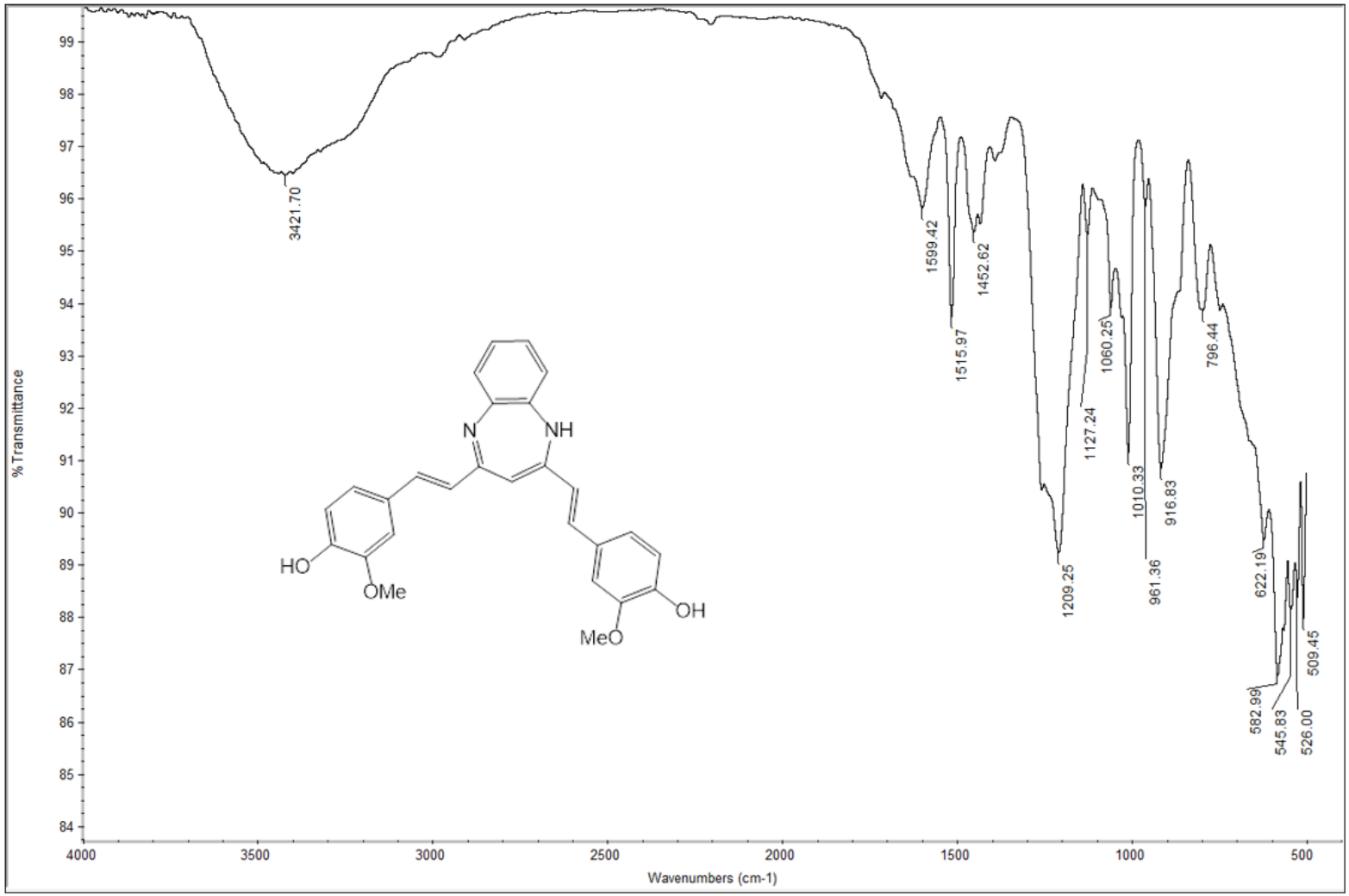
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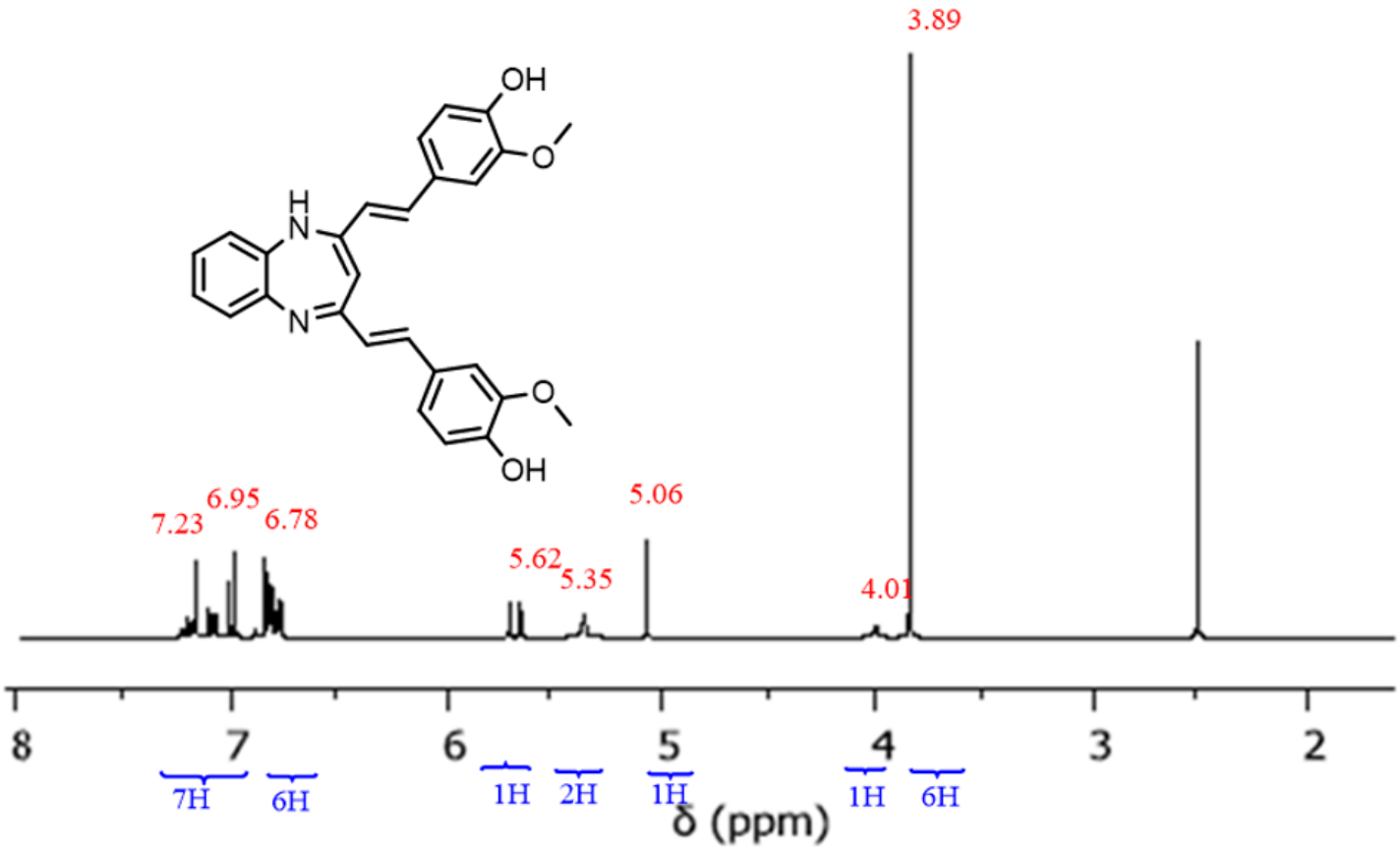
OMe
MeO
OH



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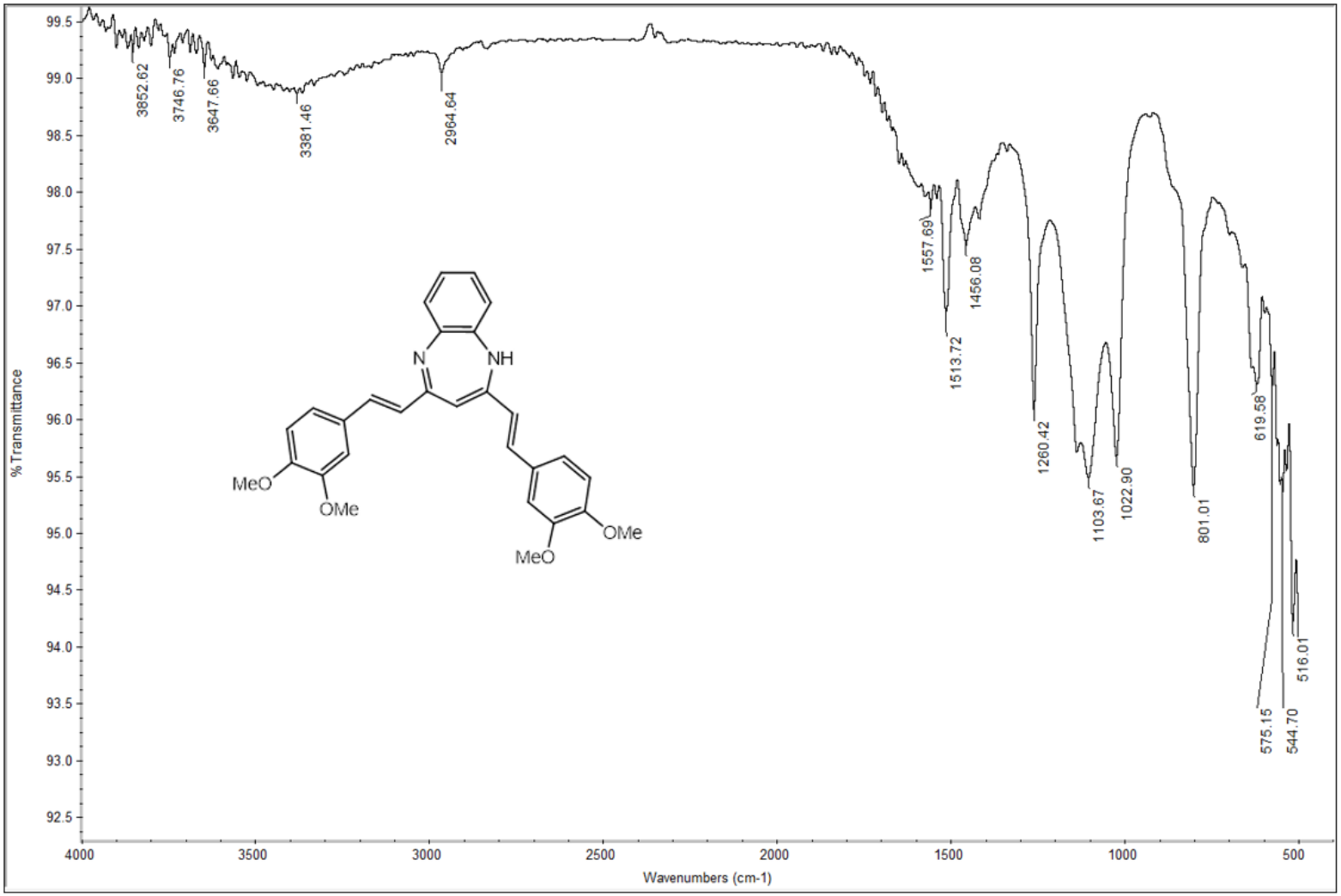
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2964.64
1557.69
1513.72
1456.08
1260.42
1103.67
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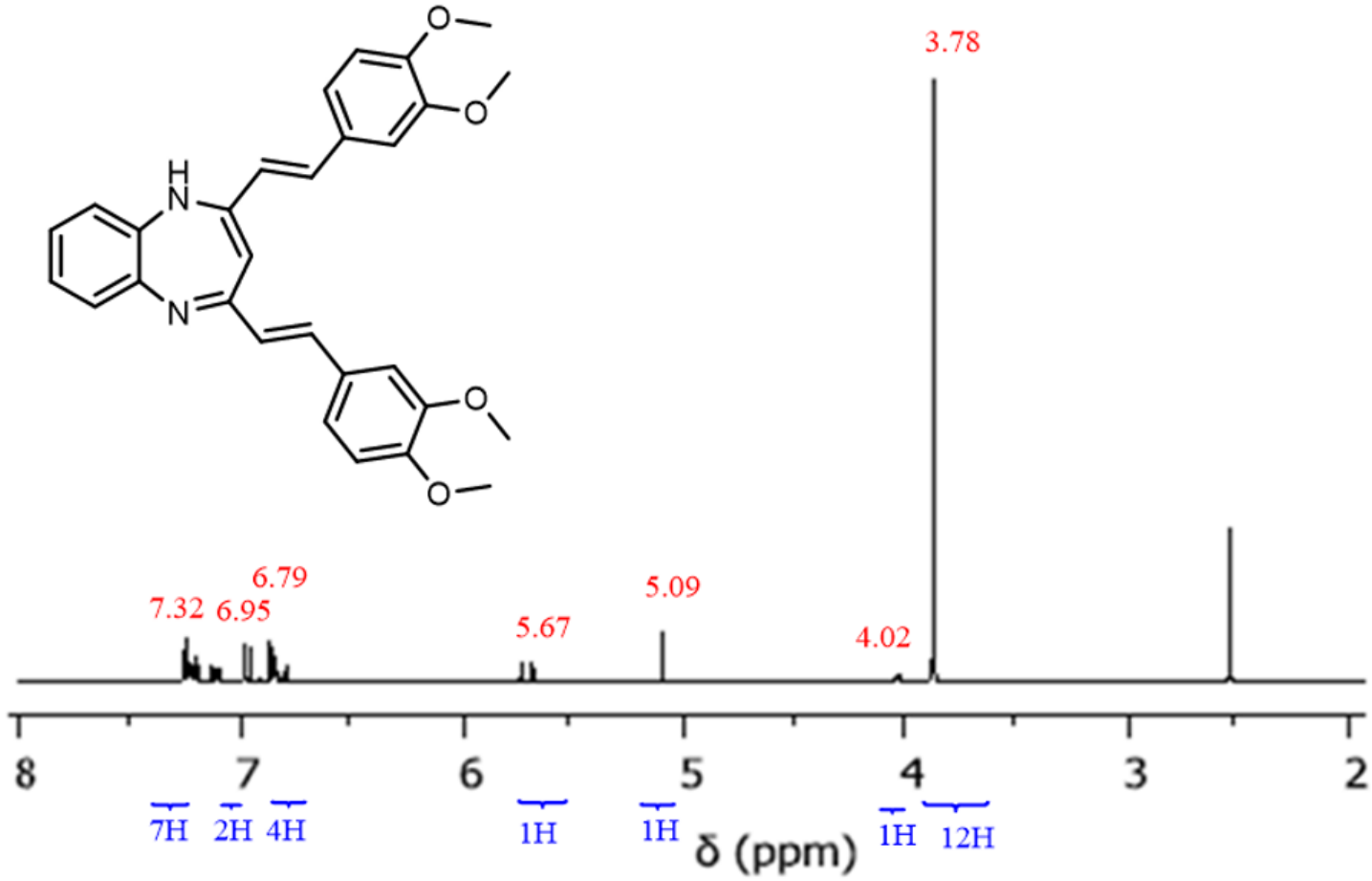
801.01
619.58
575.15
544.70
516.01
% Transmittance
Wavenumbers (cm-1)
N
NH
MeO
OMe
OMe
MeO



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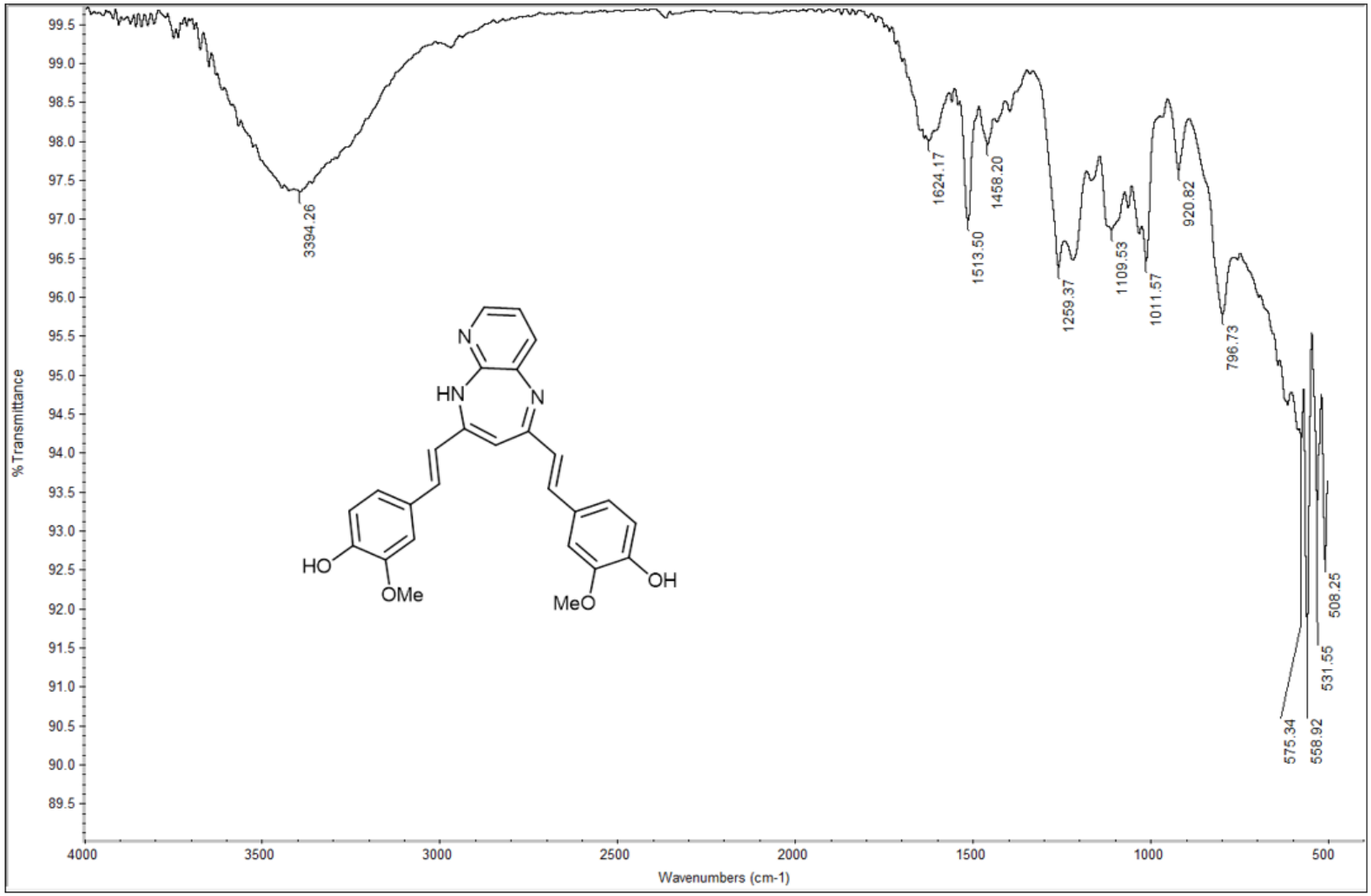
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Wavenumbers (cm-1)
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OMe
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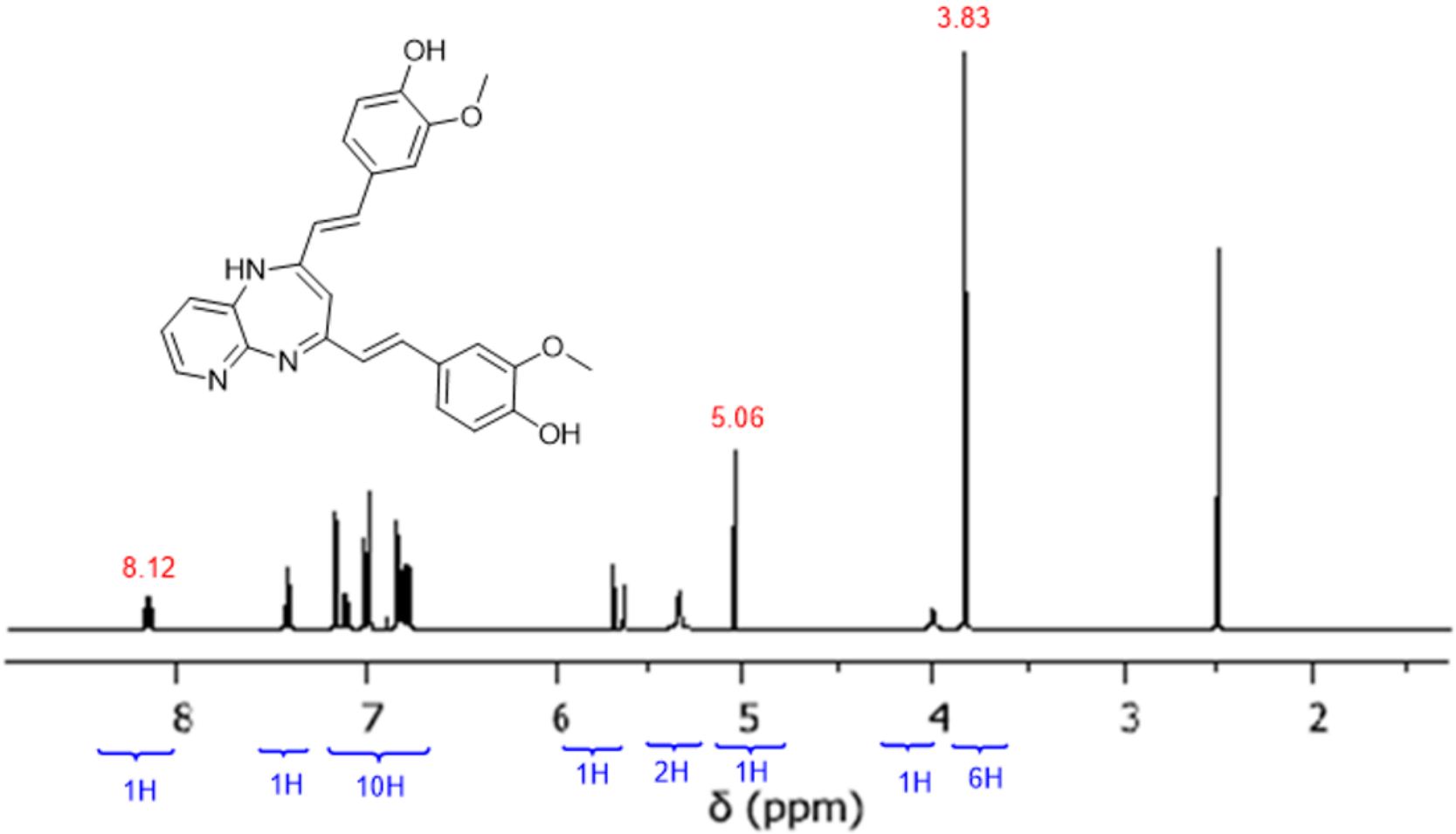
N



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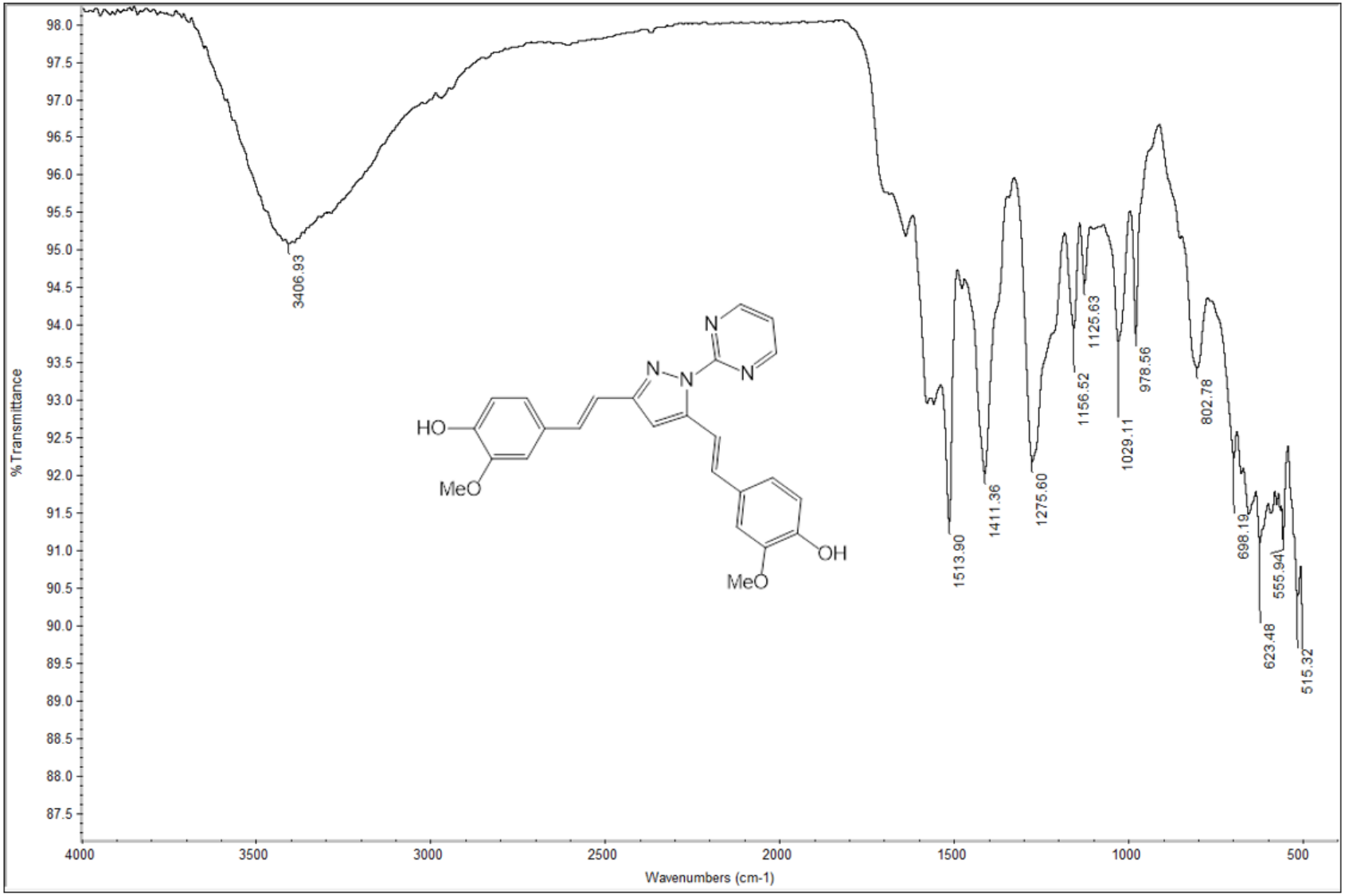
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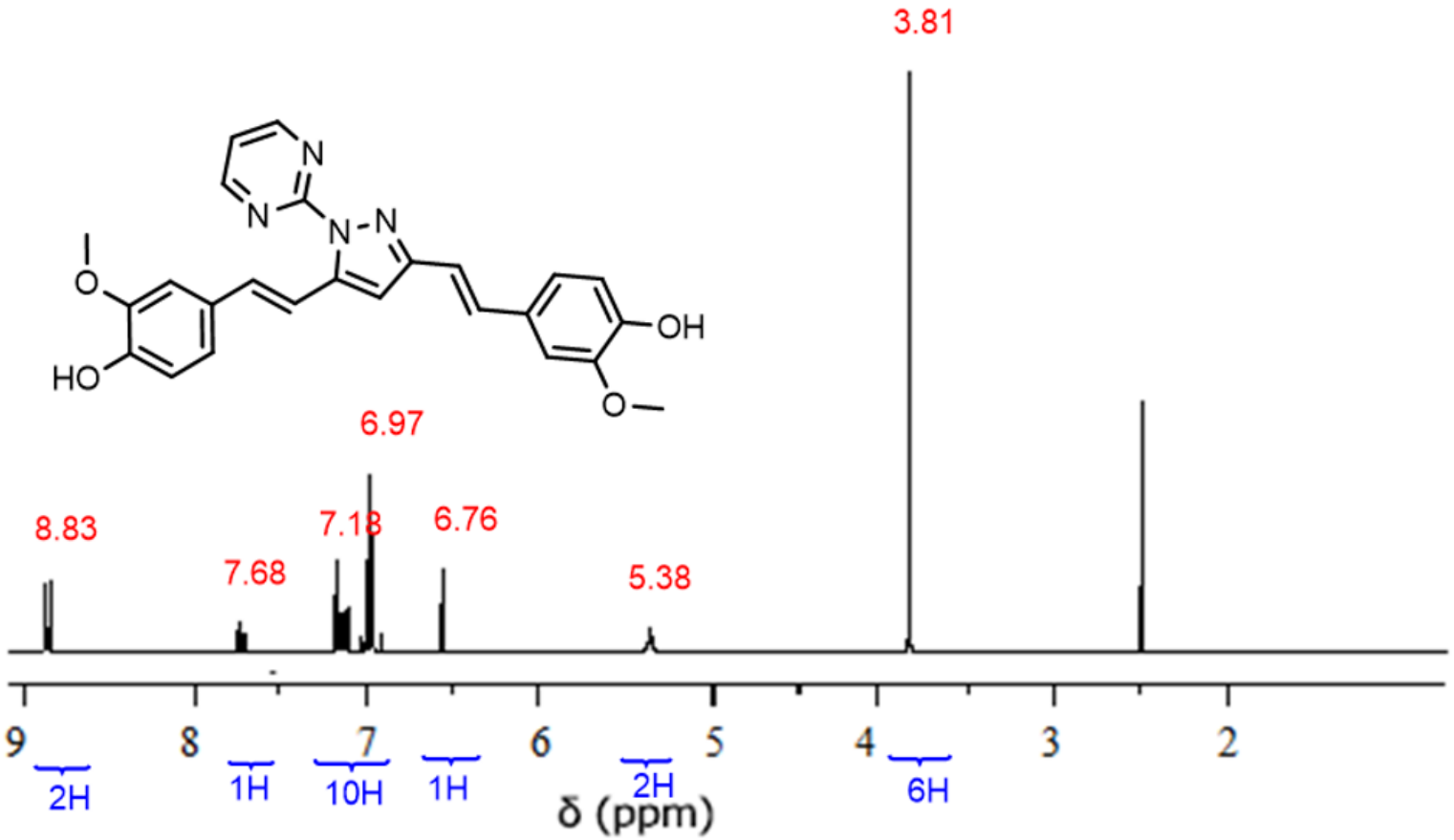
MeO
MeO
OH
N
N
N



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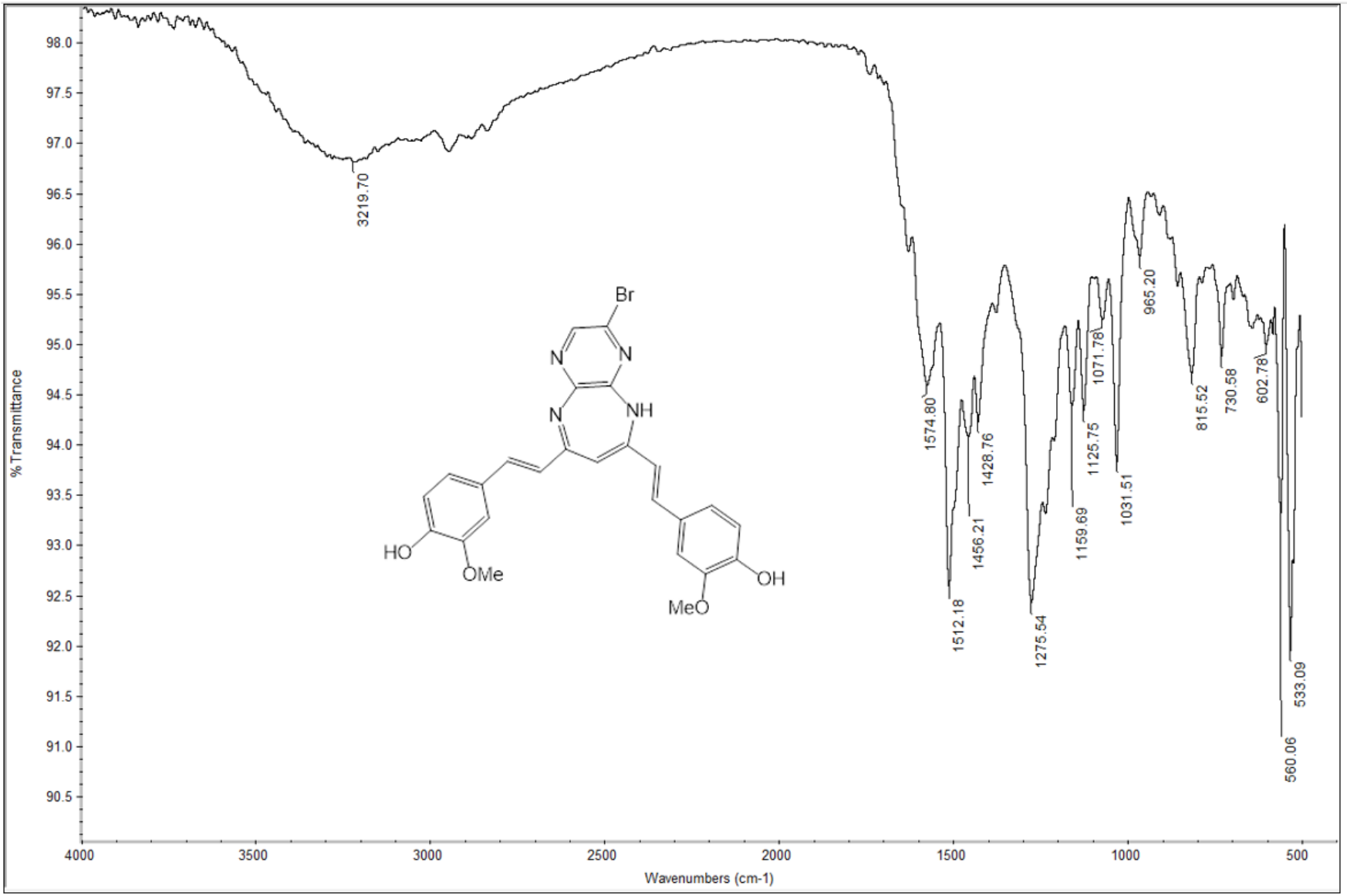
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Wavenumbers (cm-1)
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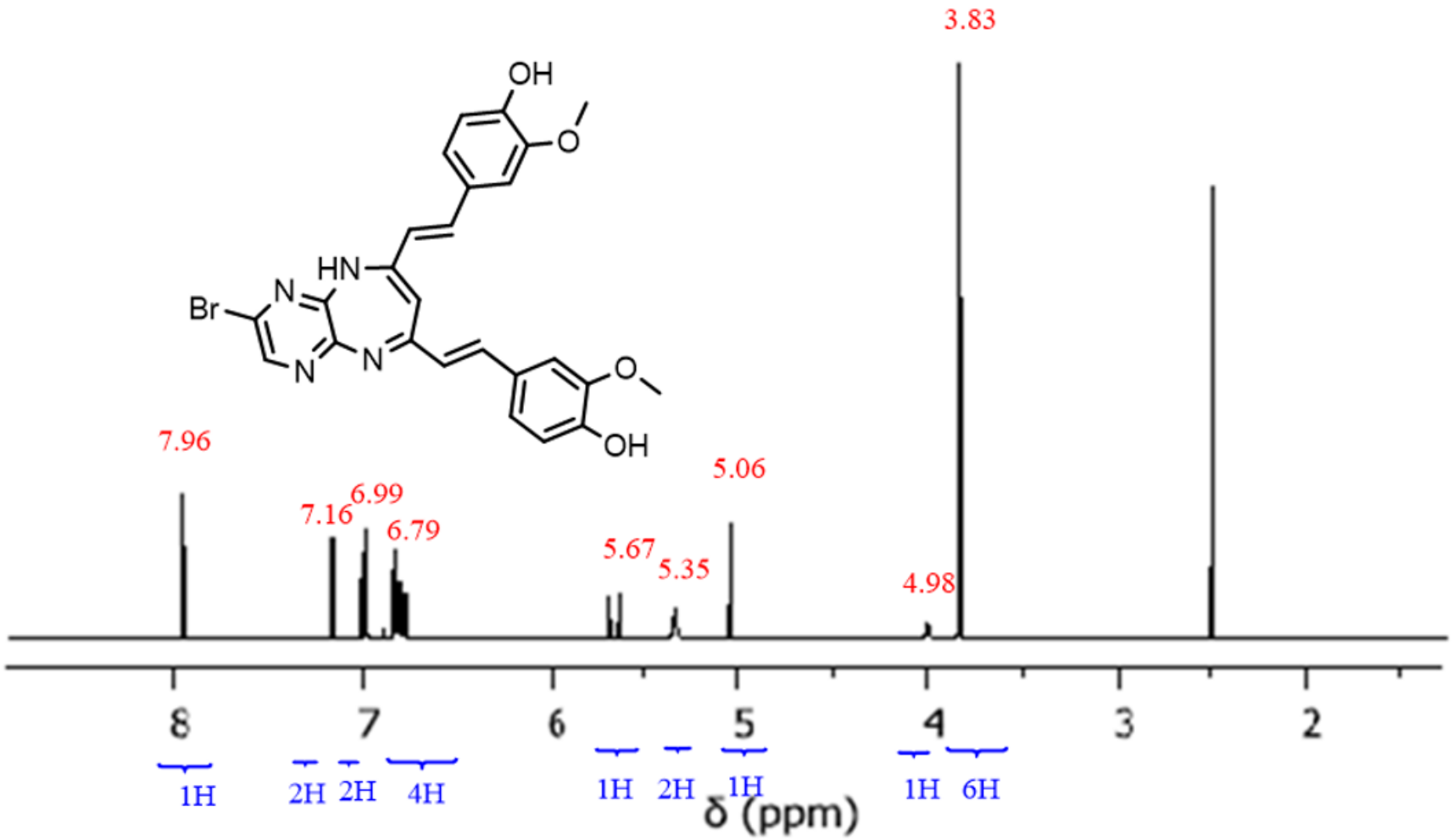

^{1}HNMR spectrum

جامعة النجاح الوطنية

كلية الدراسات العليا

تصميم وتحضير مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز من الكركمين ودراسة النشاط المضاد للبكتيريا والسمية الوراثية لها

اعداد

رولا سلطان محمود الكرم

اشراف

أ.د.عثمان حامد

د. محمد قنيبي

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الدكتوراه في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس – فلسطين.

2021

تصميم وتحضير مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز من الكركمين ودراسة النشاط المضاد للبكتيريا والسمية الوراثية لها

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د. محمد قنيبي

الملخص

تم تحضير مجموعة جديدة من مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز من الكركمين، باستخدام طرق بسيطة ومباشرة. حيث تم مفاعلة الكركمين مع انواع مختلفة من مركبات الهيدرازين ومركبات ثنائي الأمين. وتم تحليل المركبات الناتجة باستخدام تقنيات التحليل الطيفي المختلفة، مثل:(T-IR) و(^{13}C NMR) و (^{1}H NMR).

تم توظيف العديد من الطرق السهلة والبسيطة من أجل الحصول على المركبات المطلوبة:

في الطريقة الاولى تم تحضير مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز ، باستخدام تفاعلات التكثيف حيث تم دمج الكركمين مع انواع مختلفة من مركبات ثنائي الامين والهيدرازين بوجود الايثانول وكمية محفزة من حمض الكبريتيك.

هذا وقد تم استخدام طريقة ثانية من اجل الحصول على مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز وهي طريقة نوفينيغل للتكثيف وقد تم استخدام هذه الطريقة من اجل الحصول على كمية اكبر من النواتج.

بالاضافة الى تحضير احد مركبات الامين من خلال استخدام الكركمن المهدرج حيث تم مفاعلة اسيتات الامونيوم مع الكركمن المهدرج لانتاج المركب المطلوب.

ومن ناحية اخرى، تم اختيار مجموعة من المركبات المحضرة عشوائيا ومن ثم تم حجب مجموعة الهيدروكسيد الفينولي بمجموعات الكيل مختلفة لدراسة اهمية مجموعة الهيدروكسيد بالنشاط الحيوي.

تم اختبار النشاط المضاد للبكتيريا لمجموعة من المركبات التي تم تحضيرها ضد أربعة أنواع مختلفة من البكتيريا وهي (*S. aureus, MRSA, E. coli , K. pneumoniae*)

وأظهرت النتائج ان المركبات المحضرة لها فعالية أكبر ضد (*S. aureus, and K. pneumoniae*) من فعاليتها ضد (*MRSA,and E. coli*) . وقد تبين ان المركب **9** له اكبر فعالية من بين المركبات التي تم اختبارها حيث كانت قيم (MIC) لمركب **9** هي (1.56, 1.56, 50 µg/ mL) ضد (S. aureus, MRSA, and Klebsiella) على التوالي.

وقد أظهرت نتائج دراسة النشاط الحيوي لاربعة من المركبات المحضرة وهي (**2, 4, 10, 35**) مع احد المضادات الحيوية الموجودة بالفعل وهو (ampicillin) ضد بكتيريا (*E.coli*) ان هذه المركبات لها تآزر حيوي كبير مع المضاد الحيوي المستخد حيث اظهرهذا التآزر انخفاض كبير وملحوظ على قيم ال (MIC) حيث انخفضت من (200 µg/mL) الى اقل من (1.56 µg/mL).

علاوة على ذلك، اظهرت نتائج دراسة السمية الوراثية ان المركب **8** المحتوي على مجموعة (Diazepine) لم يتفاعل مع (DNA) لبكتيريا (*E.coli*) وبناءا على هذه النتائج تم تصنيف هذا المركب **8** كمركب غير سام وراثيا.

وقد اظهرت هذه الدراسة ان المركبات التي تم تحضيرها هي مركبات واعدة للاستخدام كمضادات حيوية بالتآزر مع المضادات الحيوية المستخدمة حاليا.