An-Najah National University

Faculty of Graduate Studies

Design, Synthesis and *In vitro* Evaluation of Anticancer Activity of Curcumin Based Benzodiazepines, Diazepines, Diazoles and Amines

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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 (Rola, 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

Rana Sultan Al-Kerm

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Rana Sultan Al-Kerm

الاقرار

أنا الموقعة أدناه، مقدّمة الرسالة التي تحمل العنوان:

Design, Synthesis and *In vitro* Evaluation of Anticancer Activity 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:** رنا سلطان بكوم

Signature: **التوقيع:**

Date: **التاريخ:** 18/2/2021

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List of Abbreviations

Abbreviation	Full name
FT-IR	Fourier -Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
DMSO	Dimethyl Sulfoxide
EtOAc	Ethyl Acetate
mmol	millimole
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
DIPN	Dipropylenetriamine
DMEM	Dulbecco's Modified Eagle's medium
TSCs	Thiosemicarbazones
UV	Ultra-violet
µL	Microliter
DNA	Deoxyribonucleic acid
CRC cells	Colorectal cancer cell
RNA	Ribonucleic acid
THF	Tetrahydrofuran
IC50	The half maximal inhibitory concentration
TLC	Thin Layer Chromatography
DIPA	Diisopropylamine
TEA	Tri ethanol amine

Design, Synthesis and *In vitro* Evaluation of Anticancer Activity of Curcumin Based Benzodiazepines, Diazepines, Diazoles and Amines

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Abstract

In this work new sets of curcumin-based diazoles, benzodiazepines, diazepines, and amines were synthesized using unsophisticated and convenient condensation reaction; by coupling curcumin with different hydrazine and 1,2 diamino compounds. The synthesized compounds were characterized using melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR.

The desired products were synthesized using convenient and simple synthetic methods. The first method involves refluxing the curcumin with various 1,2 diamino compounds or hydrazines in a glacial acetic acid, which played a dual function as a catalyst and as a solvent.

On the other hand, another new set of curcumin-based benzodiazepine, diazepine, diazoles and amines were synthesized using Knoevenagel Condensation that involves two-steps mechanism; α,β-unsaturated intermediate **20 A** was formed in the first step; by mixing curcumin with benzaldehyde in toluene 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 have been involved in the second step to produce the desired products. The

Knoevenagel condensation is supposed here to enhance the yield by preventing keto-enol tautomerization.

A two-steps method has been used to prepare H-Curcumin-based amine by condensation reaction of H-curcumin with ammonium acetate; in the first step the solvent was evaporated by heating the reaction mixture at 60 °C, then the heating was continued at the same temperature for an hour in the second step.

To investigate the effect of the hydroxyl group on the bioactivity of the synthesized curcumin-based compounds, a selected set of the prepared curcumin-based compounds was functionalized with butoxy groups. Where, the phenolic groups of the selected compounds were alkylated by reacting with chlorobutane in the presence of sodium hydroxide.

A novel set of seven curcumin-based benzodiazepine, diazepines, diazoles, and H-curcumin based amine were randomly chosen and screened for in vitro anticancer activity against HeLa cancer cells.

The results indicate that all the prepared curcumin-based heterocyclics have varying cytotoxic effect on the HeLa cells at different concentrations. The viability of HeLa cells has reduced in the range of 4.48- 14.57%.

In general, cell growth has been decreased as the concentration of the prepared curcumin-based heterocyclics increased.

Among the prepared curcumin-based compounds (**3** and **5**) are more effective against HeLa cancer cells than other tested compounds. As they

reduced the viability of the tested HeLa cells in range of 4.78 % and 4.63% for the 400 μg /ml concentration to 5.44% and 5.12% for the 12.5 μg /ml concentration respectively.

H-curcumin-based amine **19** showed the lowest cytotoxic effect on the HeLa cells at all concentrations among the prepared heterocyclics. It reduced the viability of the tested HeLa cells in range of 5.43 % for the 400 μg /ml concentration to 14.57% for the 12.5 μg /ml concentration.

Strictly speaking all the prepared curcumin-based compounds exhibited promising anticancer activity against HeLa cancer cells, which indicates that these compounds have anticancer effect at nontoxic concentrations.

CHAPTER ONE

INTRODUCTION

1. Background

Cancer is a significant universal problem that annually causes more than eight million deaths. Developing countries are at higher risk of cancer than other countries. Previous studies indicated that 63% of cancer-related deaths were from developing countries. Cancer is an abnormal condition in which the cells in a part of the body begin to grow with no stopping and spread out of control figure 1.1 disregard the physiological cell growth and division rules in an uncontrolled manner [1-3].

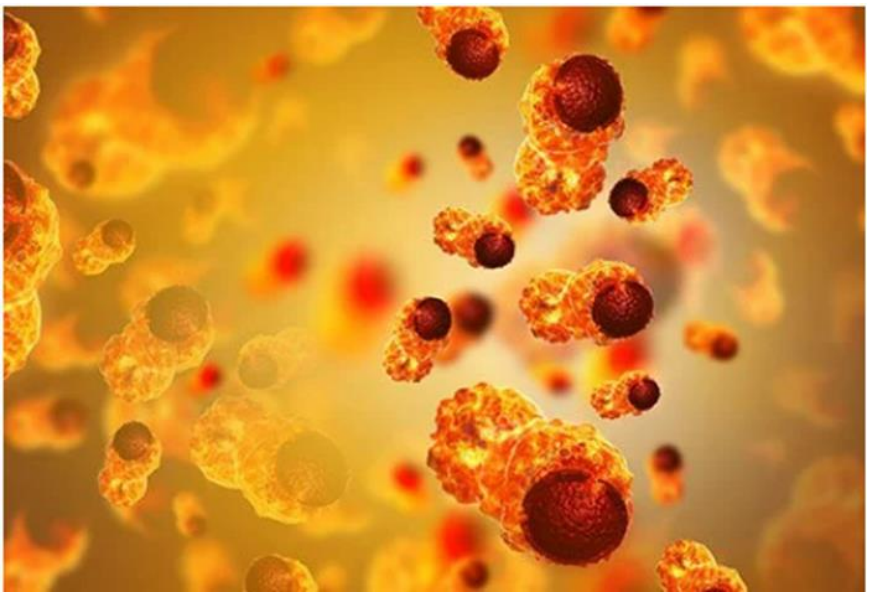


Fig. 1.1 Cancer cells [4].

1.1 Understanding the cancer

Human body is made up of trillions of living cells. Typically, these living cells grow, divide to form new cells and to replace damaged, worn-out, or dying cells, and die in an organized way. if this organized process is broken

down, a cancer is developed. Cancer begins when the damaged and old cells in a part of the body survive and grow out of control when they should die. There are many kinds of cancer, and they can start almost anywhere in the human body, but they all start due to this uncontrolled growth of abnormal cells. These abnormal cells can divide with no stopping and in most cases, they form growths called a tumor (solid masses of tissue). Some cancers, like leukemia, do not form solid tumors generally. Instead, such cancer cells form in the bone marrow and blood [1-3].

Cancer cells that can invade, and spread into nearby tissues in the way that normal cells cannot do are called malignant. Moreover, these cancer cells can break off and spread to other parts of the body through the lymph vessels or the bloodstream and form new tumors far from the original. If cancer cells′ proliferation continues to other tissues and starts to grow and form new tumors that replace normal ones in fetal way this process is known as metastasis (a secondary cancer) figure 1.2 [1-3].

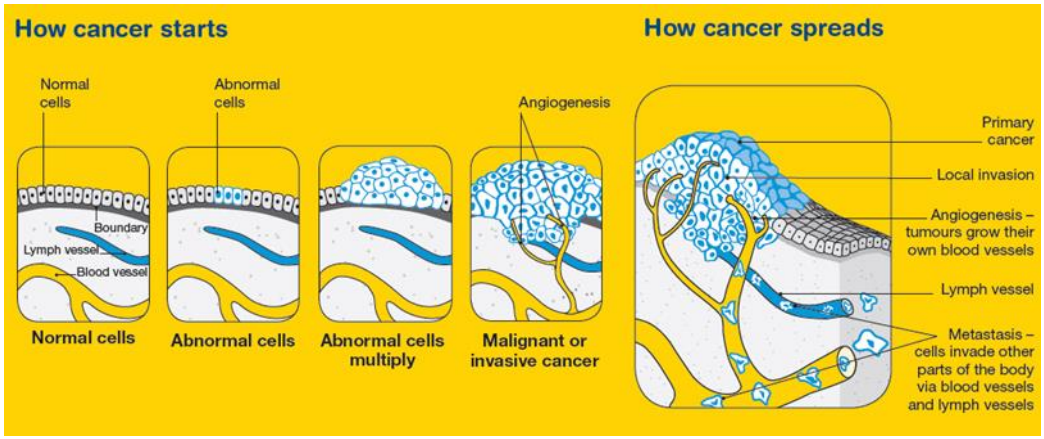


Fig. 1.2 A metastatic cancer [5].

No matter where cancer proliferates, metastatic cancer retains the original cancer (the primary one). For instance, lung cancer that has spread to the bones is known as metastatic lung cancer, not a bones cancer, though the person may be suffering symptoms caused by bones problems. Likewise, breast cancer spread to the liver is not called liver cancer, but breast cancer instead. Each type of cancer behaves differently than other cancers; for example, breast cancer and lung cancer are entirely different diseases. They grow at different rates and they can be treated differently [1-3,5]. Not all tumors are cancerous. Tumors that are confined to one area are called benign. benign tumors do not invade other parts of the body and cannot spread to the nearby tissues, unlike malignant tumors figure 1.3. In some case benign tumors grow to form a large solid mass, that usually doesn't grow back when removed. At the same time, malignant tumors in some cases do. Benign brain tumors may be life threatening sometimes, unlike most benign tumors in the other parts of the body [1-3,5].

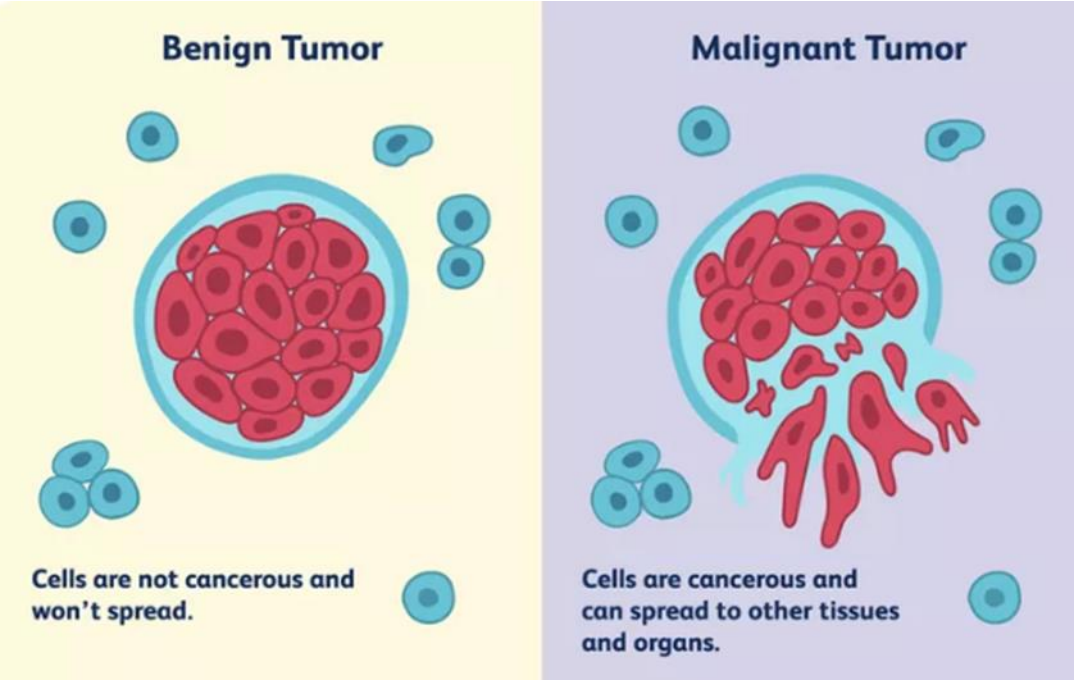


Fig. 1.3 Differences between a benign tumor and a malignant tumor [6].

1.2 Differences between Cancer Cells and Normal Cells

Cancer cells differ from normal cells in several ways

1.2.1 Growth

Cancer cells continue to grow out of control and with no stopping even when there enough cells present and become invasive. This overgrowth causes a tumor formation. On contrast, normal cells stop growing when there are enough cells figure 1.4 [2,3,7,8].

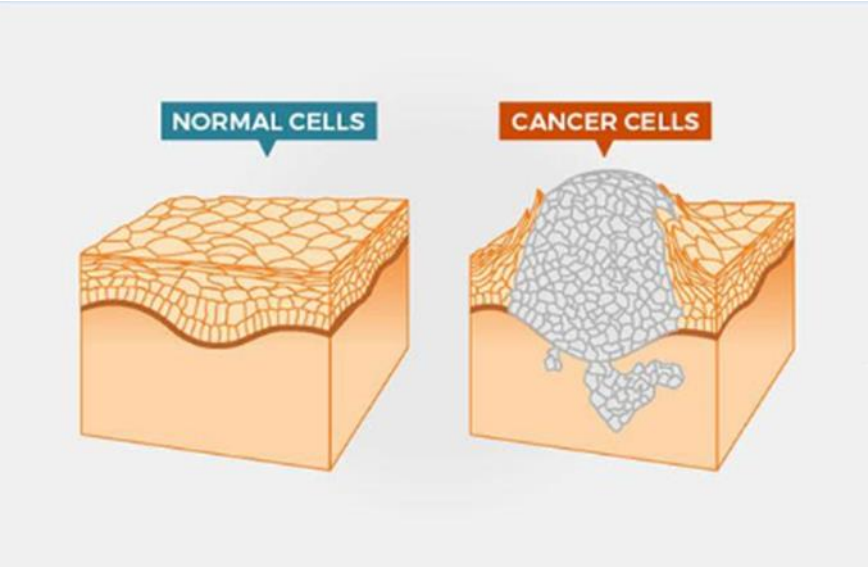


Fig. 1.4 uncontrollable growth of cancer cells various the normal cells [3].

1.2.2 Maturation

Normal cells are specialized and mature into very distinct cell types, whereas cancer cells are less specialized than normal ones. As they proliferate and continue to divide without stopping before cells are fully mature, they remain immature [2,3,7,8].

1.2.3 Communication

Normal cells interact with other cells while cancer cells do not. Moreover, normal cells respond to nearby cells signals that tell cells to stop growing and dividing or begin the programmed cell death or apoptosis that the body uses to dispose the worn-out, damaged, and unneeded cells. Whereas cancer cells disregard the physiological rules of the cell division and ignore signals from nearby cells warning overgrowth, and uncontrollably grow [2,3,7,8].

1.3 How Cancer Arises

Cancer is a genetic disease that results due to changes in a gene control the cell functions, particularly the cell growth and division. These changes may be inherited from parents or may appear throughout a person's lifetime due to errors happen during cell division or as a result of DNA damage due to many factors that may arise inside the cell such as hormones, immune conditions, and mutations figure 1.5. Moreover, external factors may also cause damage in DNA including environmental exposures to chemicals, smoking, ultraviolet radiations from the sun, and infectious organism [2,3].

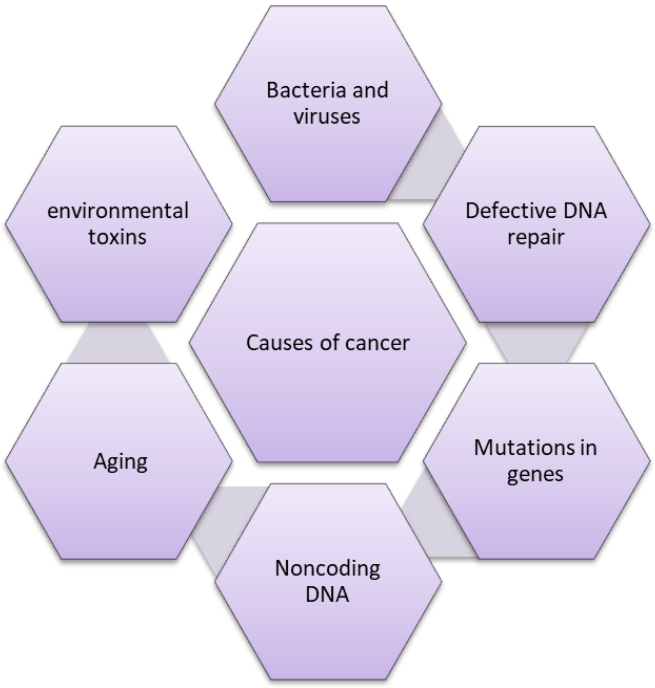


Fig. 1.5 Causes of cancer [2].

Mutations in tumor oncogenes and suppressor is the main causation of cancer development. As well as the mutation in a master gene controlling cells division may shepherd normal cells to abnormal chromosomal replication, leading to deletion or duplication of the entire chromosomes'

sections figure 1.6. The abnormal amount of a particular protein is produced regardless of the actual need, due to this genetic change. When the protein that plays a critical role in the cell cycle is affected by any chromosomal aberration qualitatively or quantitatively a cancer may arise [2,3].

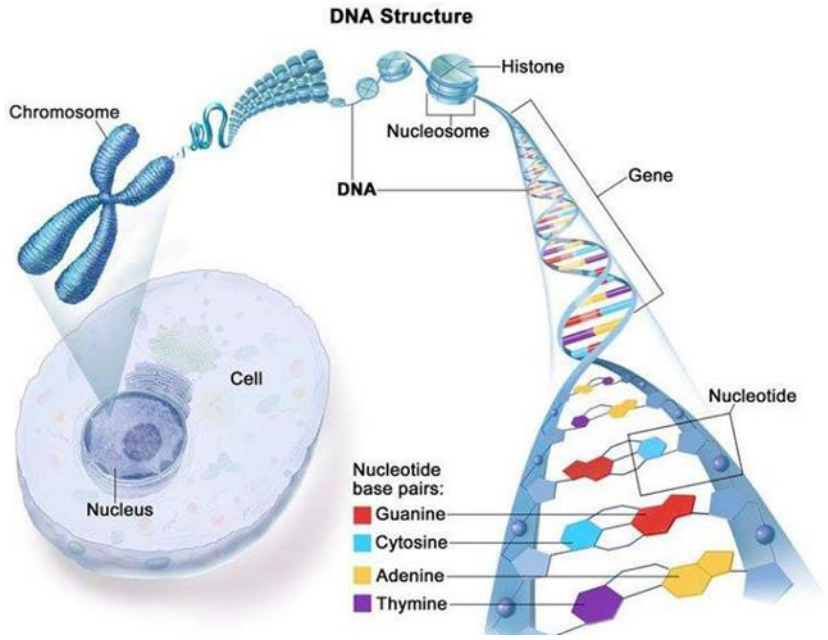


Fig. 1.6 Cancer is caused by certain changes to genes, the basic physical units of inheritance. Genes are arranged in long strands of tightly packed DNA called chromosomes [3].

1.4 The types of cancers

Cancers are mainly named for the tissue and organ where they originate. Moreover, cancers may also be described according to the type of cell initially altered, like a squamous cell or an epithelial cell.

- **Carcinomas** are the most common type of cancer. which are formed by altered epithelial cells.

- **Sarcomas** are abnormalities in the bone, fats, muscle, blood and lymph vessels, and connective tissue.
- **Leukemia,** is cancerous white blood cells originated in the bone marrow, Leukemia is characterized by forming large amount of abnormal white blood cells in the blood and bone marrow instead of solid mass tumors formed in other types of cancer.
- **Lymphoma,** is cancer that arises in lymphatic system. In lymphoma, abnormal lymphocytes are formed in lymph vessels, lymph nodes, and in other body organs.
- **Myelomas,** are cancers in the white blood cells that produce antibodies [2,3].

1.5 Cancer treatment modalities

So far, several types of research have been done to discover novel cancer treatments. Cancer treatments depend on cancer type and progress, as well as its locality. Chemotherapy, surgery, radiotherapy and radiation-based surgical knives are the most common and more widely used cancer treatments.

1.5.1 Surgery

Surgery is a promising and a fundamental method for palliative and curative cancer treatment of most solid organ benign and malignant cancers, like, colon and breast cancers, since they are mostly diagnosed at their early stages. Compared to other treatments like radiotherapy and chemotherapy, surgery assures the least damage to the nearby tissues and

organs, moreover, the tumor can be removed with no risk of tissue damage. Several types of surgeries, either minimally invasive or open, can be performed depending on different factors;

- The surgery reasons
- The region where the surgery will be performed
- The type of solid mass or tumor that will be removed
- The preference of the patients

Depending on the stage of cancer there are various types of surgery

- Remove the entire tumor from a specific part of body.
- Debulk a tumor when its removal may damage a specific organ.
- Relieve the symptoms of cancer when the large tumor causes intense pressure or pain to a specific part of the body [2,9].

1.5.2 Radiation therapy

Radiation therapy is a physical method for killing cancer cells using ionizing particles like protons, electrons, and various ions. The energy of radiation can either kill cancer cells directly or alter them genetically so that they follow the apoptosis and cell death. The mechanism behind genetic alterations using radiation treatment is that the high energy damage DNA, thus stop cancer cells division, and block their ability to proliferate, leading them to death. Radiation therapy shrink the tumor before the surgery, or it can be given after surgery as cancer relapse reducing or destroys the left behind tumor cells [2].

1.5.3 Chemotherapy

Chemotherapy is the most effective and broadly used treatment against most types of cancer. Chemotherapy prevents the progression of the tumor by blocking their ability to divide and enforcing apoptosis and cell death.

The body refreshes its cells by removing damaged cells, excess cells, and old ones and then signaling new cell formation as one of its normal biological functioning. Cancerous cells have an increased ability to grow and divide in an uncontrollable manner disregarding apoptosis. Cancer cells lack the balance between cell proliferation and cell death. Chemotherapy causes changes in the tumor cells, thus halts their growth, division and proliferation. Two types of chemotherapeutic treatment are known, which are cytotoxic and cytostatic (biological drugs). Chemotherapeutic drugs harm the normal cells, leading to several dose dependent side effects including; nausea, hair loss, vomiting, fatigue, and even death in extreme cases as patients became immunocompromised, thus susceptible to infections. Up to now, hundreds of chemotherapeutic drugs are known, which can be used alone or in combination with other therapies. Each of them is different in its composition and chemical structure from the others. In contrast to radiotherapy and surgery which are invasive and have targeted procedures, chemotherapy is mostly systemic, traveling to the tumors and cancer cells through the body [2,9,10]. Examples of some drugs used in the treatment of cancer are shown below.

1.5.3.1 Alkylating agents and Methylating agents and cancer therapy

Different types of traditional chemotherapy drugs are being used for cancer treatments including; topoisomerase inhibitors, alkylating agents, intercalating dugs, antimitotic drugs, and antimetabolites, in addition to the recently identified targeted therapies like various kinase inhibitors. Some types of cancers can be completely cured using these therapies. Yet, the success of cancer treatments depends on the specific type of cancer diagnosed and stage of diagnosis [11-15].

Alkylating agents are earliest and one of the most commonly used anticancer drugs. Basically, alkylating share similar mechanisms of action, but differ in their clinical efficacy. These agents act directly on DNA, resulting in its crosslinking and causing DNA strand breaks, leading to abnormal base pairing and inhibiting cell division, eventually resulting in cell death. Alkylating anticancer agents are effective during all phases of cell cycle and are used to treat several types of cancers. Mainly, these compounds are more effective in treating slow-growing cancers, like solid leukaemia and tumours [11-15].

There are several types of alkylating agents used in chemotherapy treatments:

- Mustard gas derivatives: Mechlorethamine, Chlorambucil, Cyclophosphamide, Melphalan, and Ifosfamide.
- Alkylsulfonates: Busulfan.

- Ethylenimines: Hexamethylmelamine and Thiotepa.
- Nitrosureas: Carmustine, Streptozocin and Lomustine. Nitrosureas are unique because, unlike most types of chemo treatments, they can cross the blood-brain barrier. They can be useful in treating brain tumors.
- Hydrazines and Triazines: Altretamine, Dacarbazine, Procarbazine, and Temozolomide.
- Metal salts: Cisplatin, Carboplatin, and Oxaliplatin [11-15].

1.5.3.2 Cisplatin

Cisplatin, a platinum-based anticancer agent, is one of the most powerful and commonly used chemotherapeutic drugs in treating various human cancers, including lung, bladder, ovarian, head and neck, cervical, esophageal, testicular, and breast cancer. It is also efficient against several types of cancers, such as germ cell tumors, carcinomas, sarcomas, and lymphomas. Cisplatin has considerable efficacy in cancer treatment with both palliative and curative intent. Cisplatin, also known as cis-diamminedichloroplatinum(II), is a metallic coordination compound with a square planar geometry. The platinum (II) complex consists of two chloride ligands in a cis configuration as shown in figure 1.7. At room temperature it is a white or yellow to yellow-orange crystalline powder. It is stable under normal pressures and temperatures, but may convert slowly over

time to the trans-isomer. Cisplatin, is soluble in N,Ndimethylformamide, while, It is slightly soluble in water [14-18].

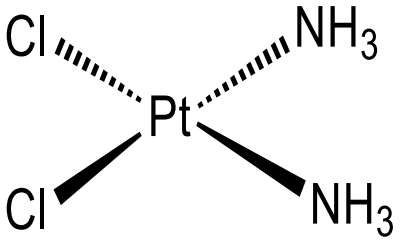


Fig. 1.7 Chemical structure of cisplatin.

Cisplatin, can be used in combination with other types of therapies such as immunotherapy and radiotherapy and monotherapy. Its mechanism of action is associated to its ability to crosslink with the DNA purine bases; interfering with repair mechanisms of DNA, causing DNA damage, and therefore inducing cancer cells apoptosis. It is currently estimated that approximately 50% of cancer patients will be treated with cisplatin in their chemotherapy [14-18].

1.5.3.2.1 Toxicity of Cisplatin

Cisplatin treatment has been associated with several undesirable side effects such as nephrotoxicity, nausea, cardiotoxicity, neurotoxicity and hepatotoxicity. Moreover, decrease immunity to infections, allergic reactions, gastrointestinal disorders, congestive heart failure, hearing loss especially in younger patients, arrhythmias, myocarditis, and electro-cardiographic changes [14,15].

1.5.3.3 Cisplatin and other platinum-containing drugs

Nowadays, thousands of platinum-containing anti-cancer analogs have been prepared and tested for their therapeutic properties. Carboplatin is the only analog that shows advantages over cisplatin and achieved worldwide approval among thirteen analogs that have been evaluated in clinical trials. Currently, nine platinum analogs are in clinical trials all over the world which are; oxaliplatin, ormaplatin (tetraplatin), CI-973 (NK-121), JM-216, lobaplatin, enloplatin, DWA2114R, 254-S, and liposome-entrapped cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexane platinum (II) (LNDDP)]. Four of these analogs are shown in figure 1.8 including ormaplatin, enloplatin, oxaliplatin, and carboplatin [14].

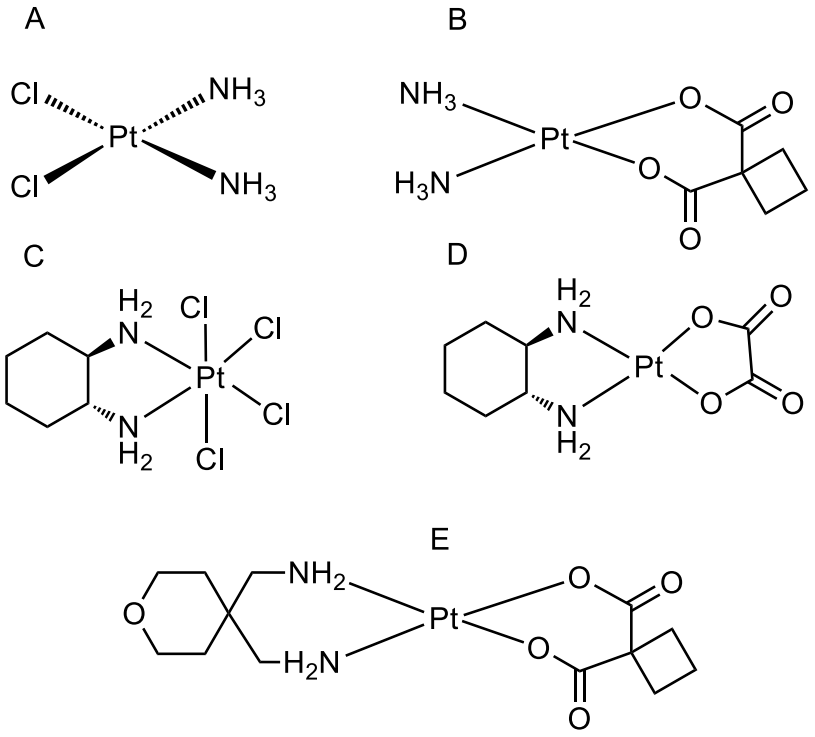


Fig. 1.8 Chemical structures of selected cis-platin analogues: A-cisplatin; B-carboplatin; C-ormaplatin; D- oxaliplatin; E-enloplatin [14].

1.5.3.3.1 Carboplatin

Carboplatin, cis-diammine(1,1-cyclobutanedicarboxylato) platinum (II), is a cisplatin analog that shares cisplatin nearly similar structure, toxicity and mechanism of action figure 1.9. Carboplatin has a lower incidence of nephro-toxicity, neuro-toxicity, and nausea-vomiting. moreover, there is no need for prolonged hydration. However, Carboplatin is active against same tumor range as cisplatin, it is intravenously administered, and it is cross-resistant with cisplatin [19,20,21].

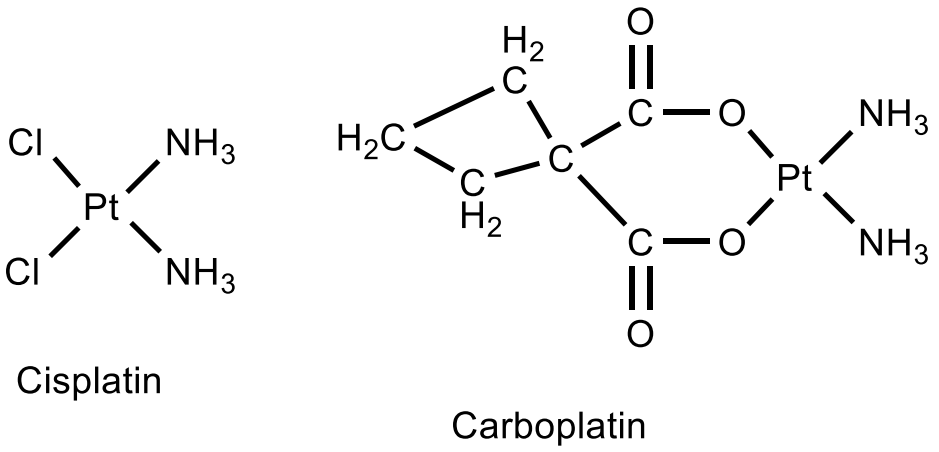


Fig. 1.9 Chemical structure of cisplatin and carboplatin.

1.5.3.3.2 Oxaliplatin

Oxaliplatin (trans-/-diaminocyclohexane oxalatoplatinum) is a third-generation platinum compound consisting of a dicarboxylate leaving group instead of chloride, and diamminocyclohexane carrier ligand instead of ammonia figure 1.10. Oxaliplatin can overcome cisplatin resistance cancer and it is used for treatment of colon cancer. In 2002 oxaliplatin was approved by United States Food and Drug Administration (FDA) for

advanced colorectal cancer treatment. But, oxaliplatin is neurotoxic and it is efficient against limited types of cancer. So, more effective cisplatin analogue anticancer drugs are still needed [19,22].

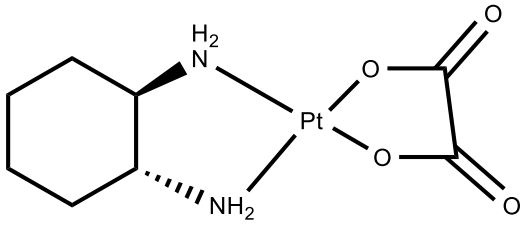


Fig. 1.10 The chemical structure of oxaliplatin

1.5.3.3 Recent Oxaliplatin and Cisplatin Analogues

1.5.3.3.1 Iodido analogue of oxaliplatin

So far, oxaliplatin has been used worldwide for cancer treatment alone or in combination with other drugs. Mainly CapeOx (capecitabine and oxaliplatin) and FOLFOX (5-FU, leucovorin, and oxaliplatin). Despite great success of the clinically established Pt drugs, e.g. cisplatin and oxaliplatin. Their side effects including chronic and acute neurotoxicity limit their clinical use. Therefore, new Pt based drugs are still urgently needed. In this regard a new oxaliplatin analogue {(1R,2R)-cyclohexane-1,2-diamine} diiodidoplatinum(II), (PtI_2(DACH) was developed, its structure is shown figure 1.11. The other one, the two iodides were replaced the oxalate bidentate ligand of oxaliplatin [23].

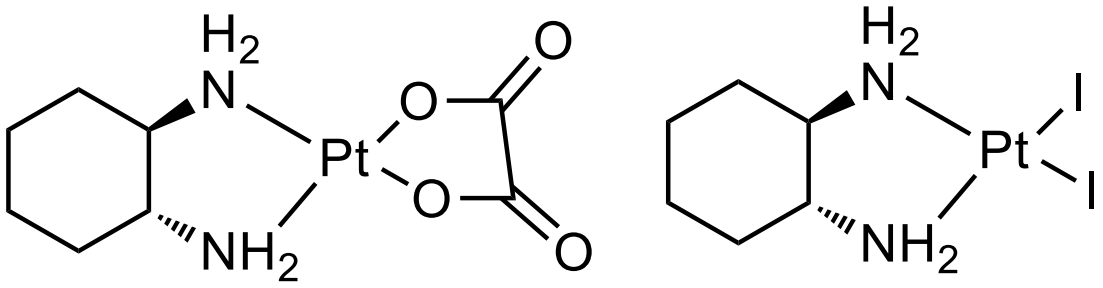


Fig. 1.11 The chemical structure of oxaliplatin and its iodide analogue.

The replacement of the oxaliplatin bidentate oxalate ligand with two iodide ligands make PtI_2(DACH) medicinally crucial since it affects the Pt center activation process, which thermodynamically and kinetically influence its overall biological profile. Moreover, the lipophilic character of the resulting drug of the insertion of two iodides in place of the bidentate oxalate considerably enhances the drug's lipophilic character which increases its bioavailability and cellular uptake.

The effects of PtI_2(DACH) on CRC cells were studied [23]. The results investigated that PtI_2(DACH) induce cell apoptosis similar to cis$PtI_2(NH_3)_2$. Comparable to oxaliplatin, PtI_2(DACH) were roughly induced the cytotoxic properties in CRC cell lines [23].

1.5.3.3.2 Dibromido Analogue of Cisplatin

Notably, The Iodido analogue of oxaliplatin and cisplatin manifests exciting biological and chemical properties, add to that the strong in vitro cytotoxic effects on various cancer cell lines, especially cisplatin resistant cancer cells. This encouraged the preparation and evaluation of new Pt analogues by replacing the two chloride ligands with non-conventional halido ligands particularly bromide. In this framework, the dibromido

analogue of cisplatin, cis$PtBr_2(NH_3)_2$ was prepared figure 1.12 and evaluated against several cancer cell lines [24].

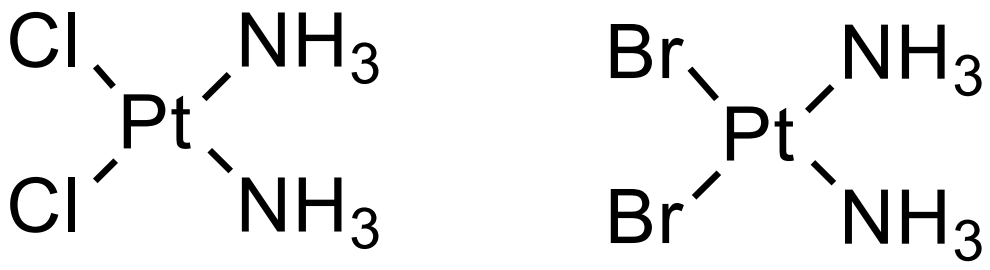


Fig. 1.12 The chemical structure of cisplatin and its dibromido analogue

The results indicated that the tested compound had comparable cytotoxic effects to cisplatin ones or even higher in some cases. Moreover, cis$PtBr_2$ was capable of triggering apoptosis, modulating cell cycle distribution, and reducing cell proliferation to an extent same or even better than oxaliplatin and cisplatin. This can be explained by the lipophilicity of the dibromido-analogue which is higher than cisplatin one. Which is significantly enhanced its bioavailability and cellular uptake as well [24].

1.6 Medicinal plants and cancer

As mentioned before, the chemotherapeutic drugs that have been used previously exhibited relatively high toxicity to the tumor cells and the normal cells of the body where cancer had developed. Moreover, the increase in the incidence of different cancer types creates a need for novel chemotherapeutic drugs from natural sources. So far, several studies have been conducted to develop new plant-derived drugs, not only among terrestrial plants, but in marine environments as well. Several plants have been used in ancient times to treat different diseases. Many different plants

are consumed all over the worldwide for their medical benefits as traditional medicine [25,26].

There are four main classes of natural product-derived anticancer drugs including;

1. Taxanes (paclitaxel, cabazitaxel, and docetaxel) figure 1.13

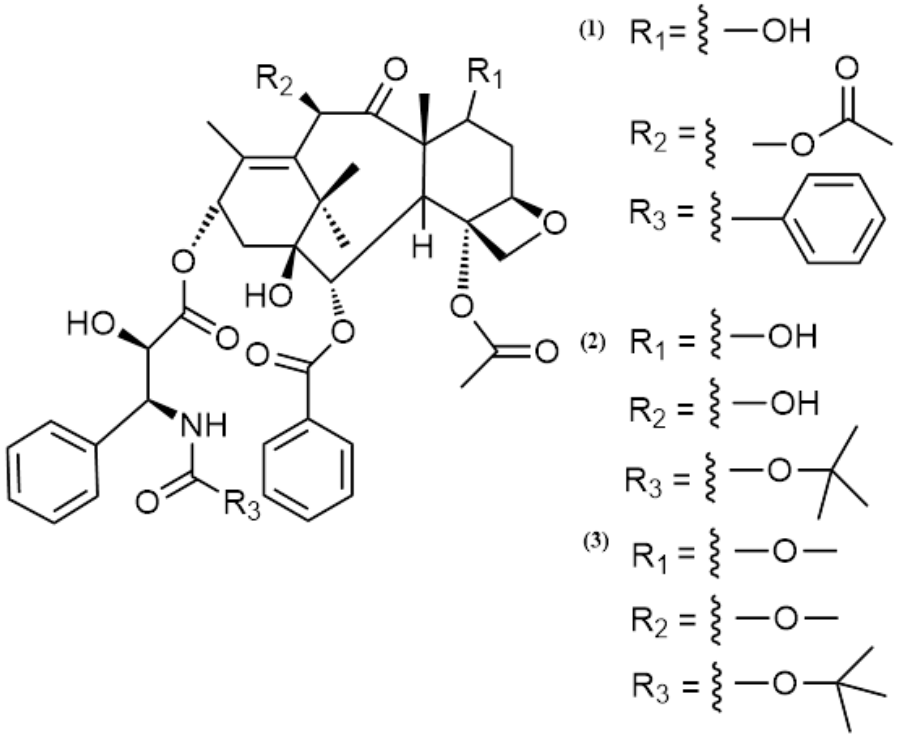


Fig. 1.13 Taxanes: (1) paclitaxel, (2) docetaxel, (3) cabazitaxel [25, 26].

2. The vinca alkaloids (vinblastine, vincristine, vindesine, 3. vinorelbine, and (5) vinflunine) figure 1.14

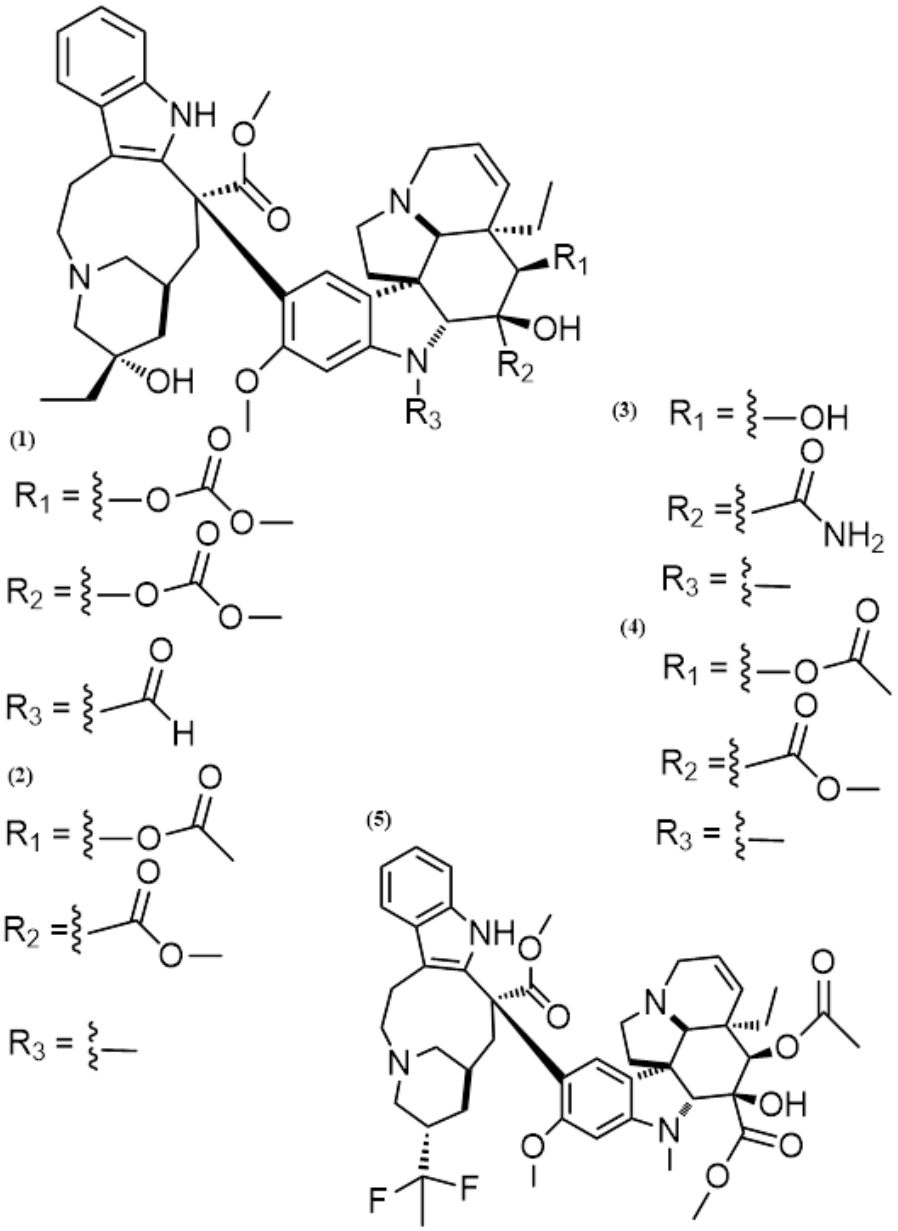


Fig. 1.14 Catharanthus alkaloids: (1) vincristine, (2) vinblastine, (3) vindesine, (4) vinorelbine, and (5) vinflunine [25,26].

3. The camptothecin derivatives (camptotecin, topotecan and irinotecan) figure 1.15.

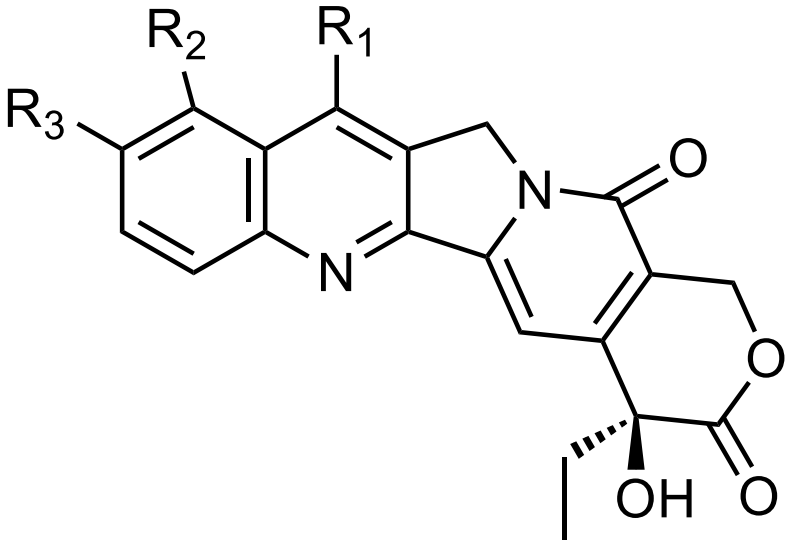


Fig. 1.15 Camptothecin and its derivatives: (1) camptothecin, (R_1; H, R_2:H, R_3:H) (2) irinotecan, (R_1; C_2H_5, R_2:H, R_3:$C_{11}H_{20}N_2O_2$) (3) topotecan (R_1; H, R_2:$CH_2N(CH_3)_2$, R_3: OH) [25,26].

4. The epipodophyllotoxins (etoposide, podophyllotoxin and teniposide) figure 1.16.

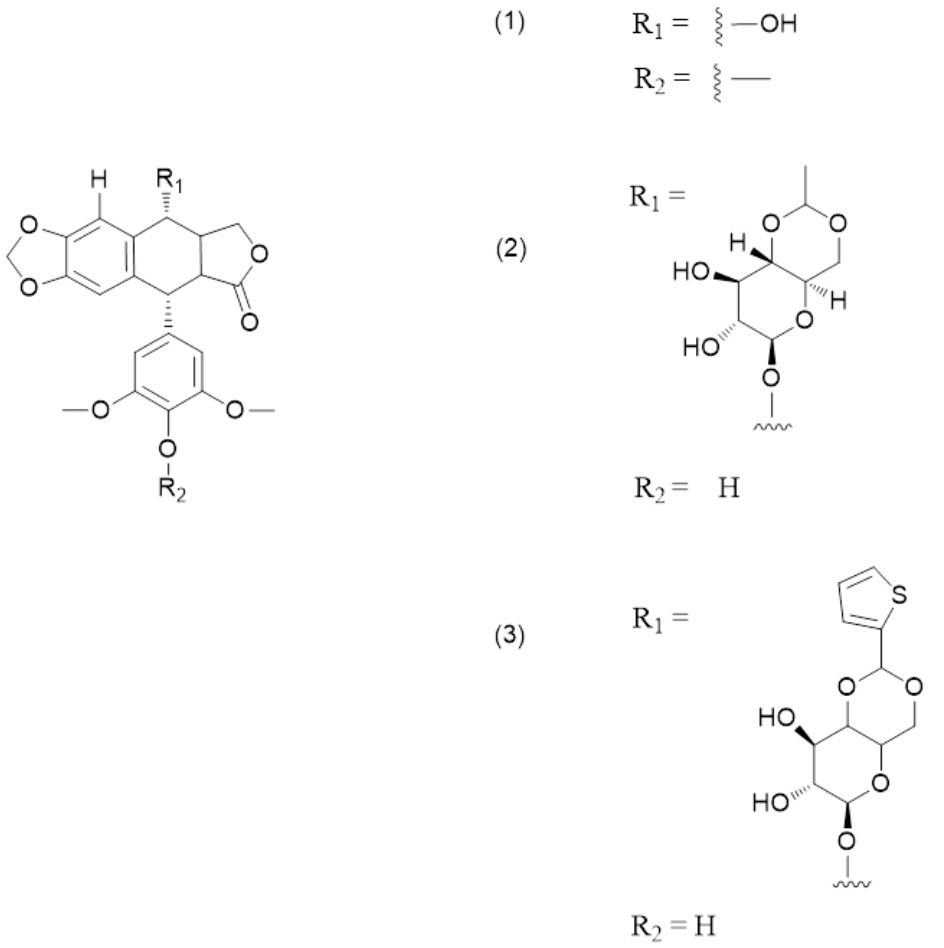


Fig. 1.16 Podophyllotoxin derivatives: (1) podophyllotoxin, (2) etoposide, (3) teniposide [25,26].

1.7 Heterocyclic compounds

Heterocyclic compounds are very widely distributed in nature, they comprise the largest and most diverse organic compounds. Heterocyclic compounds are cyclic organic compounds which contain at least one hetero atom in place of a carbon atom, the most common heteroatoms are Nitrogen, Oxygen and Sulphur but heterocyclic rings containing other hetero atoms are also widely known [27-30].

Examples of natural heterocyclic compounds are chlorophyll, vitamins, hemoglobin, hormones, biological molecules such as DNA and RNA, and

many more contains the heterocyclic ring in their central skeleton. Also, they played an essential role in most field of sciences including medicinal chemistry, due to their activity in multiple illnesses they are essential components of more than 90% of new drugs. Furan, thiazole, pyridine, pyrrolidine, etc. are essential heterocyclic compounds used in synthesis. Heterocycles can be classified according to the heteroatoms present in the ring structures as; nitrogen, oxygen, or sulfur, within each class, compounds are organized based on the ring structure's size (size determined by the total number of atoms) [27-30]. Among all the heterocyclic compounds mentioned above, nitrogen-based heterocycles are most essential in pharmaceutical and agrochemical industries. The biological importance and the structural variety of Nitrogen based Heterocycles have made them attractive targets for synthesis over recent years. They are found in many natural products and have been known as products of biological and chemical importance. Nitrogen containing heterocyclic compounds have been widely studied and used to synthesize diverse alkaloids like: Azine, diazine, pyridine, pyrazine and pyrimidine. Their importance as precursors to numerous biologically active compounds has drawn a significant attention on developing new methods to functionalize these compounds. The synthesis of nitrogen heterocycles and their derivatives takes an essential place in the field of natural and synthetic organic chemistry due to their pharmacological and therapeutic properties. They have arisen as integral backbones of over seven thousand existing drugs so far. In addition to these significant biological applications nitrogen

heterocycles have played a crucial role in making great drugs and inhibitors. Most of the heterocyclic organic compounds based on nitrogen show better biological activity than non-nitrogen heterocyclic compounds [27,31-33].

Several heterocyclic compounds are soluble in hydrophilic solvents forming hydrogen bonds enhancing their interaction with the bioactive sites. Based on this fact, different studies are focusing on developing new natural based drugs. The preparation of curcumin derivatives with many different heterocyclic moieties is an example [34,35].

1.8 Curcumin

Curcumin (diferuloylmethane), is a natural, non-toxic polyphenol yellow powder, extracted from Curcumin longa L [36-41]. It has a long history in use in Southeast Asia, India and China as a food preservative, sauce, spice, curry powder, coloring agent for food and textiles, cosmetics, and traditional medicine for a variety of diseases [41-47]. Curcumin has a simple molecular structure, low toxicity and numerous beneficial biological and pharmacological activities such as antioxidant, antibacterial, anticancer, anti-inflammatory, antiviral, antidiabetic, antimicrobial, antibacterial, and antifungal [36,43-47]. Medicinally, it is useful for numerous diseases, including neurodegenerative diseases, allergy, nephrotoxicity, arthritis, psoriasis, multiple sclerosis, inflammatory bowel disease, cardiovascular disease, diabetes, AIDS and lung fibrosis [39-41,47-49]. It also inhibits lipid peroxidation and scavenges superoxide

anions, nitric oxide, singlet oxygen, and hydroxyl radicals with excellent widespread availability, low cost, and it is safe and tolerable even at high doses [37,38,49-51]. Despite all these benefits, curcumin's potential utility in clinical studies has been slowed down due to its low aqueous solubility at physiological conditions, and poor bioavailability because of its hydrophobic property, rapid metabolism to degradation, low cellular uptake and quick elimination from the body [36-40,45-47,51-52]. Therefore, numerous strategies have been developed to overcome these drawbacks, such as synthesis of curcumin nanoparticle, enhance its solubility, formulations in liposomal, micellar or phospholipid complex, the formation of complexes with metals, combination with adjuvants, synthesis of curcumin derivatives with functional substituents and design hybrid curcumin molecules. These strategies will enhance the physicochemical properties and improve the efficacy simultaneously [37-40, 46]. The therapeutic effect of curcumin results from its essential and straightforward chemical structure and unique physicochemical properties.

Curcumin ($C_{21}H_{20}O_6$) has a melting point of approximately 183 °C and a low molecular mass of 368.37 g/mol. Curcumin (IUPAC: 1E, 6E) _1,7-Bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) is a symmetrical molecule in which two ortho-methoxy phenolic groups are linked by a chain of seven carbon atoms with an α,β-unsaturated β-diketone group figure 1.17 [36,38,39-41].

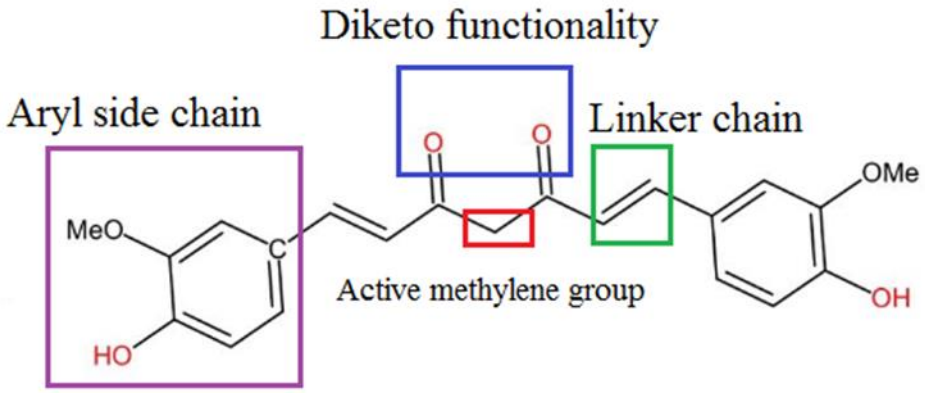


Fig. 1.17 Chemical Structure of curcumin indicating the major reactive sites.

It exists in equilibrium with keto and enol tautomeric conformations figure 1.18 due to the presence of intramolecular hydrogen atoms transfer at the β-diketone chain of curcumin. The keto-enol tautomers also exist in various *cis* and *trans* forms depending on the polarity of solvent, temperature, substituent on the aromatic rings and the pH. In the alkaline conditions and in polar solvents (water, alcohols) the enolic form predominates while in the solid phase and neutral and acidic conditions the keto form predominates. Due to its hydrophobic nature, the crystalline powder of curcumin has a selective solubility where it is poorly soluble in water, yet, it is readily soluble in organic solvent such as methanol, ethanol, acetone, isopropanol, and dimethylsulfoxide (DMSO). Moreover, it is moderately soluble in cyclohexane, dioxane, hexane, and tetrahydrofuran (THF) [45,49,50,53, and 54].

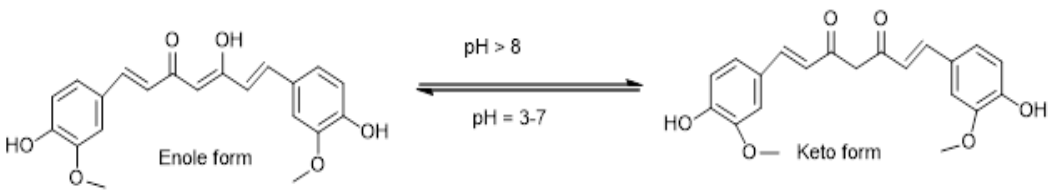


Fig. 1.18 Chemical structure of keto and enol forms of curcumin.

1.8.1 Curcumin as Anti-Cancer agent

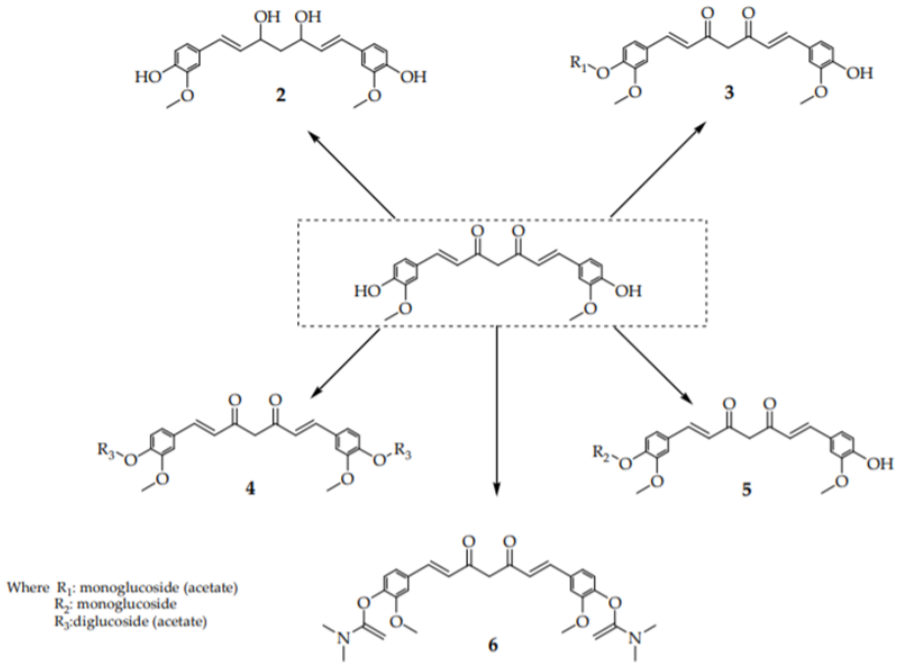
Curcumin is the most natural active ingredient derived from turmeric rhizome or Curcuma longa. Over the past few years, curcumin and its derivatives have drawn significant attention due to their nontoxicity, natural origin and biofunctional properties including antioxidant, anti-tumor, and anti-inflammatory activities. Moreover, curcumin has protective effects against several types of cancer. Since cancer mostly produces under chronic inflammatory conditions. Curcumin and its derivatives have numerous advantages over traditional chemotherapeutic drugs such as less toxic side effect and broad anticancer spectrum. Furthermore, curcumin affects several stages of cancer development including cancer cell proliferation, oncogene activation, apoptosis evasion, anoikis resistance, and metastasis, unlike most chemotherapeutic drugs that act on a specific stage of cancer development, like apoptosis or cell growth. Thus, Curcumin and its derivatives are promising in overcoming chemoresistance which is the major drawback of cancer chemotherapy [55-58].

However, curcumin has many drawbacks that limited its applications including its low chemical stability and low water solubility, moreover the low cellular uptake. Curcumin penetrates the cell membrane and bind with the chains of fatty acyl of membrane lipids through hydrophobic interactions, and hydrogen bonding, leading to curcumin’s low availability inside the cytoplasm [55-58]. Various structural modifications have been

developed to overcome these drawbacks and to enhance stability, increase bioavailability, and enhance selective toxicity towards specific cancer cells.

1.8.1.1 Curcumin Derivatives with Anticancer efficacy

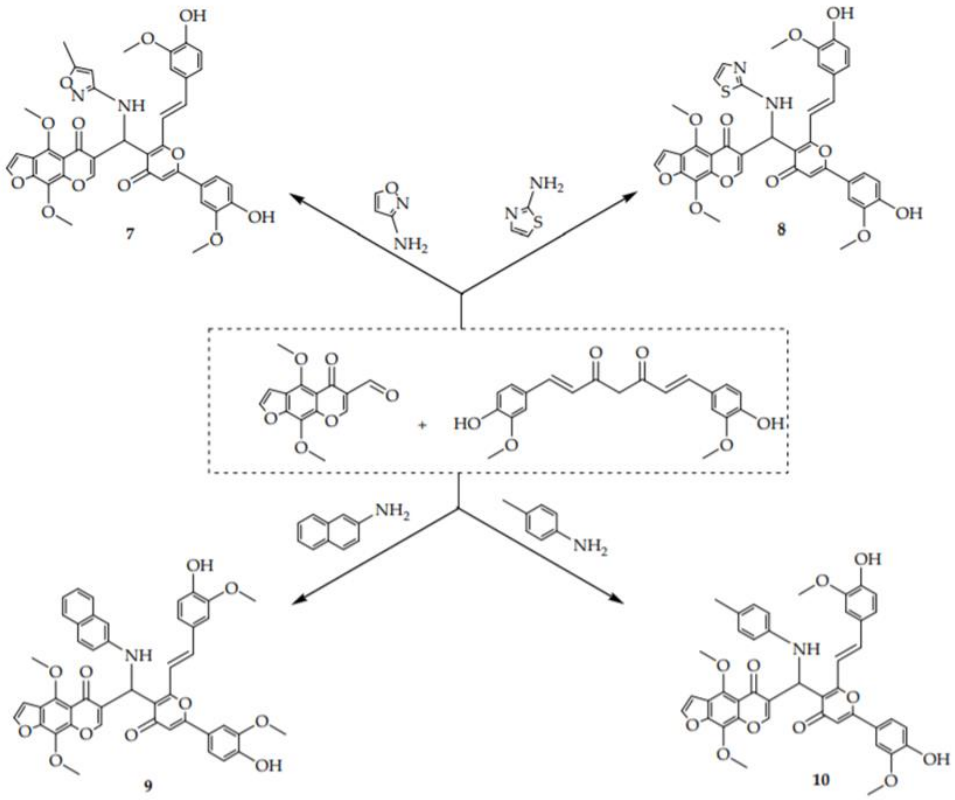
To date, a series of novel curcumin derivatives that exhibit potential synergistic anticancer activity were synthesized. Scheme 1.1 summarizes some of the prepared derivatives that have the ability to inhibit breast cancer stem cells growth by hindering the mediated efflux mechanism of P-glycoprotein (P-gp). Glucoside of curcumin derivatives showed higher affinity to bind with P-glycoprotein than other derivatives of curcumin. Which have reduced the breast cancer stem cells growth [59].



Scheme 1.1 Some of the prepared curcumin derivatives compounds 1–6. [59].

To overcome the toxicity of the current anticancer drugs to normal cells; reducing the number of doses reduces the drug's side effects. So new

heterocyclic curcumin derivatives were prepared scheme 1.2. the prepared derivatives are expected to have low or no side effects to the normal cells and the immune system. The cytotoxicity of the prepared curcumin derivatives was evaluated on breast carcinoma (MCF-7) cell lines. The results investigated that curcumin-based derivative **8** exhibited higher anticancer activity against MCF-7 cell line with an IC50 value of 20 µg/mL compared to derivatives **7, 9**, and **10** that showed relatively no effect on the MCF-7 cell line. Moreover, derivative **8** exhibited a high growth-inhibitory effect on the tested cell line compared to 5-fluorouracil (5-FU) which is used as a reference drug since it has the most effective anticancer effect with 13.35 µg/mL concentration [59].



Scheme 1.2 Some of the prepared curcumin derivatives compounds 7-10. [59].

In this work a new set of curcumin- based benzodiazepines, diazepines, diazoles and amines will be prepared, the compounds are designed to be more selective and potent for the treatment of cancer cells without or with a common side effect of the current drugs.

1.9 The aims of this study

Curcumin, a polyphenolic promising natural product, exhibits a wide range of pharmacological properties against several diseases, its therapeutic activity is attributed mainly to its chemical structure and unique chemical, biological, and physical properties. So far, curcumin has been extensively studied from both chemical and biological point of view as antioxidant, antiviral, anti-inflammatory, antimicrobial, antibacterial, and anti-cancer [60-62].

The purpose of this study is to develop novel curcumin-based heterocycles that are effective in cancer treatment. The overall objectives of the study are:

1. Design, and synthesize a new class of curcumin-based benzodiazepines, diazepines, and amines.
2. Analyze the structure and the physical properties of all curcumin-based heterocycles.
3. Evaluate the prepared compounds for anticancer activities by assessing their in vitro cytostatic and cytotoxic activities using cancer cell lines.

4. Determine the effect of the phenolic hydroxyl on the bioactivity of curcumin by blocking it with certain groups.

CHAPTER TWO

Experimental Part

1. Materials and procedures

All chemical including reagents and solvents were purchased from Aldrich Chemical Company and used with no further purification unless otherwise specified. Some of these reagents include acetone, ethanol, methanol, glacial acetic acid, H_2SO_4, dimethyl sulfoxide (DMSO), hexane, ethyl acetate, diethyl ether, NaOH, $NaHCO_3$, $MgSO_4$, curcumin, diaminomaleonitrile, diaminopyridien, 5,6-diamino -2, 3-pyrazindicarbonitrile, ethylene diamine, 2-chlorophenylhydrazin hydrogen chloride, 2-Hydrazinopyrimidine hydrate, 2, 3-diamino-5-bromopyrizine, 2, 3-diamino-5-bromopyridine, phenylenediamine, Methyl iodide, TLC plates pre-coated with Merck Kieselgel 60 F254, opened capillary tubes, molecular sieve beads.

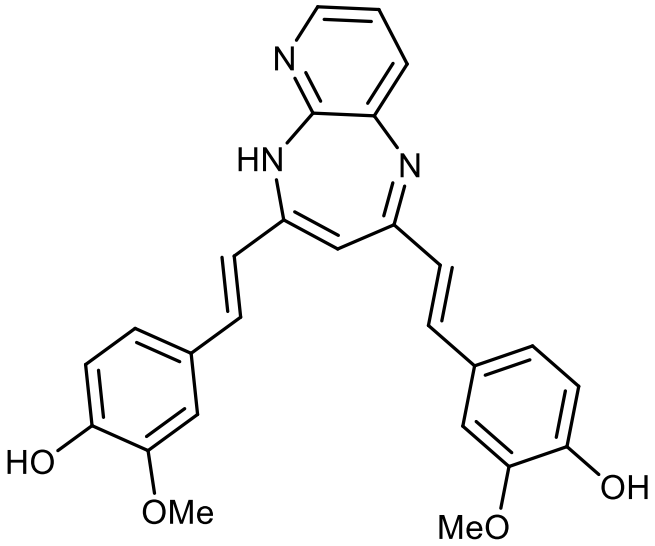
All ^1H NMR experiments are reported in δ units, parts per million (ppm) downfield from tetramethylsilane (internal standard) and were measured relative to the signal for DMSO-d6 (2.5ppm). Nuclear Magnetic Resonance spectra were recorded on a Varian VXR S400 NMR spectrometer with a proton resonance frequency of 400 MHz Infrared (IR) spectra were recorded by using FTIR Spectrum 820 PC FT-IR, Shimadzu.

1.1 Preparation of curcumin-based benzodiazepines

General Procedure A

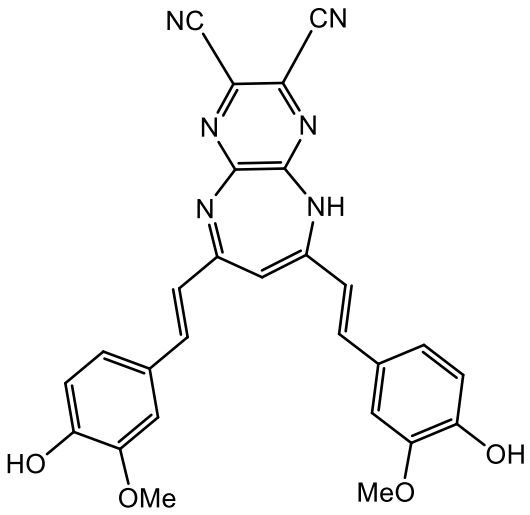
To a round bottom flask (50 mL), 1,7-Bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), diamino compounds (1.357 mmol), glacial acetic acid 30 mL were added. The contents of the flask were refluxed for (12 - 60 hours). The reaction progress was monitored using TLC to a curcumin reference. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with sodium bicarbonate ($NaHCO_3$, 5%) to remove the acid, filtered, washed with water and dried. The dry solid was washed 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).



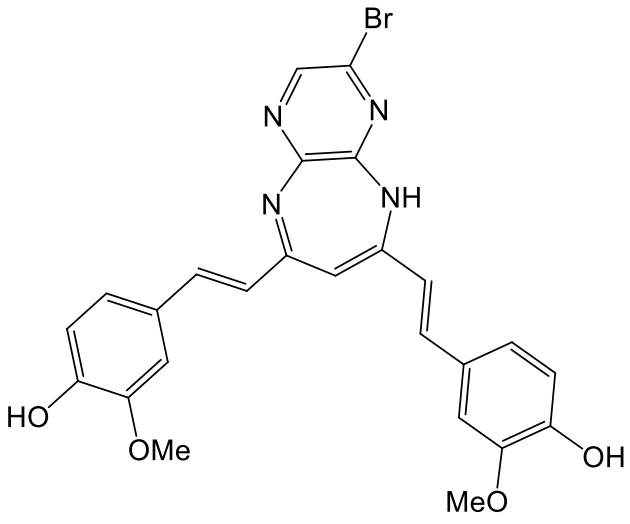
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 2,3-diaminopyridine (0.148 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.4 g (yield 65.5 %) of yellow solid. Melting point is 125-127 °C. IR: ν_{max} cm^{-1} 3350 (O-H, and N-H stretching), 3018 (=C-H), 2978 (C-H, aliphatic), 1601 (C=N), 1580 (C=C, conjugated), 1393 (C-N), 1079 (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 (s, 1H), 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)



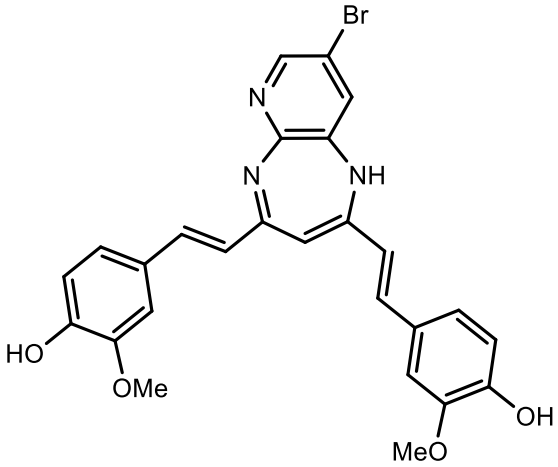
Amixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 5,6-diamino-2,3-pyrazindicarbonitrile (0.217g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.63 g (Yield 98.8 %) of orange solid, m.p. 216-217 °C, IR ν_{max} cm^{-1}: 3340.19 (N-H), 3160.13 (=C-H), 2959.50 (C-H), 2237.18 (C≡N stretch), 1675 (C=N), 1630.43 (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-methoxyphenyl) ethenyl]-5H- pyrazino [2,3-b] [1,4] diazepin-6-yl} ethenyl] -2-methoxyphenol (4).



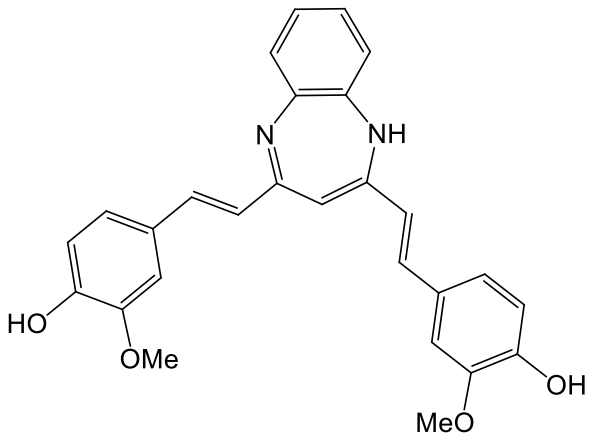
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.25 g, 0.6785 mmol), 2, 3-diamino-5-bromopyrizine (0.1276 g, 0.6785 mmol), and acetic acid (15 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.36 g (Yield 90.1 %) of yellow solid, mp 130 -131 °C, IR (KBr): ν_{max} cm^{-1} 3388.23 (-C-N-H), 1627.11 (-C=N), 549.11 (C-Br). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.82 (s, 6H, 2 OCH_3), 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-methoxyphenyl) ethenyl] -1H-pyrido[2,3-b][1,4]diazepin-2-yl}ethenyl]-2-methoxyphenol (5)



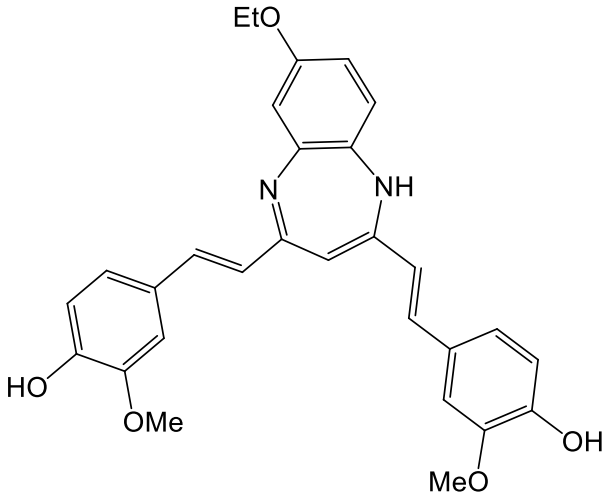
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 2, 3-diamino-5-bromopyridine (0.2538 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.41 g (yield 53.1 %) of brown solid. Melting point is 119 - 122 °C. IR (KBr): ν_{max} cm^{-1} 1612.90 (-C=N), 3368.11 (–C–NH), 553.75 (C-Br), and 1042.45 (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)



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), phenylenediamine (0.239 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.48 g (yield 80.3 %) of yellow solid. Melting point is 148 -149 °C. IR: ν_{max} cm^{-1} 3353 (-C-OH), 3027 (=C-H), 1648 (-C=N), 1607 (C=C), 1185 (C-O ether), 1229 (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-methoxyphenyl) ethenyl]-1H-1,5-benzodiazepin-4-yl}ethenyl]-2-methoxyphenol (7)

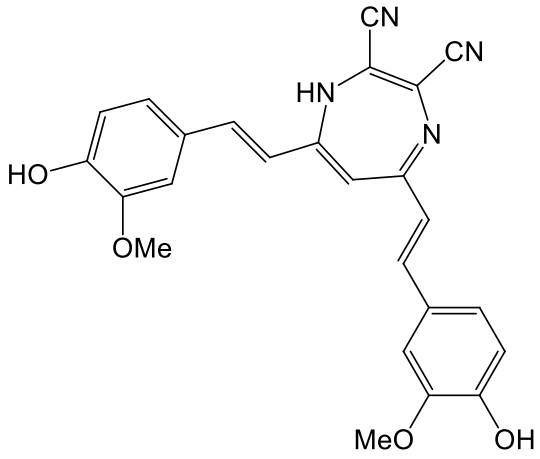


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 4-ethoxybenzene-1,2-diamine (0.205 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.52 g (yield 75.4 %) of yellow solid. Melting point is 211 - 212 °C. IR: ν_{max} cm^{-1} 3395.11 (O-H, N-H stretching), 1629.22 (C=N), 1523.58 (C=C conjugated), 1091 (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

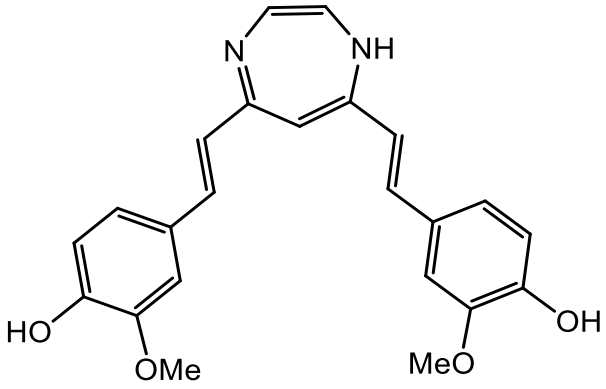
1.2.1 Seven membered heterocyclic compounds

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



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), diaminomaleonitrile (0.146 g, 1.357 mmol), and glacial acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.126 g (yield 21.1 %) of yellow solid. Melting point is 250-254 °C, IR: ν_{max} cm^{-1} 3421.33 (O-H), 3370.17 (N-H), 2368.42 (C≡N), 1658.77 (C=N), 1563.59 (C=C). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.20 (bs, 1H, NH), 3.82 (s, 6H, 2 OCH_3), 5.10 (1H, s), 5.52 (bs, 2H, OH), 5.71 (d, 1H, *J = 15.1 Hz*), 6.78 (m, 3H), 6.81 (d, 1H, *J = 15.1 Hz*), 6.86 (d, 1H); 6.98 (d, 2H); 7.18 (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

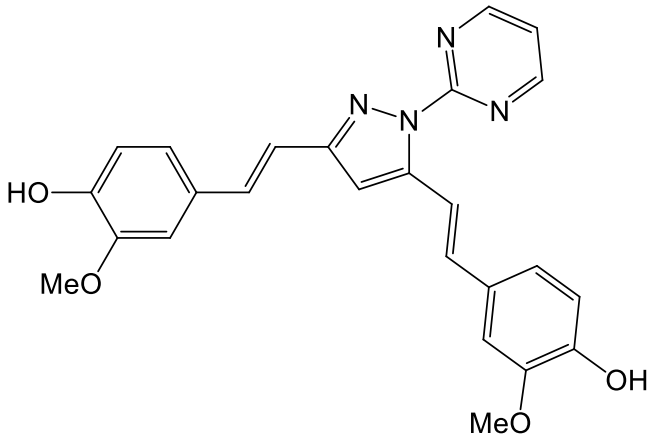
1.2.1.2 4-[(E)-2-{5-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-2,3-dihydro-1H-1,4-diazepin-7-yl}ethenyl]-2-methoxyphenol (9)



Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), ethylene diamine (4 drops, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.24 g, (Yield 48.7 %) of yellow solid. Melting point is 195 - 196 °C. IR: ν_{max} cm^{-1} 3291.99 (O-H, N-H stretching), 1587.83 (C=C conjugated), 1511.88 (C=N), 1273.56 (C-N), 1089 (C-O ether and alcohol), 813.53 (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.

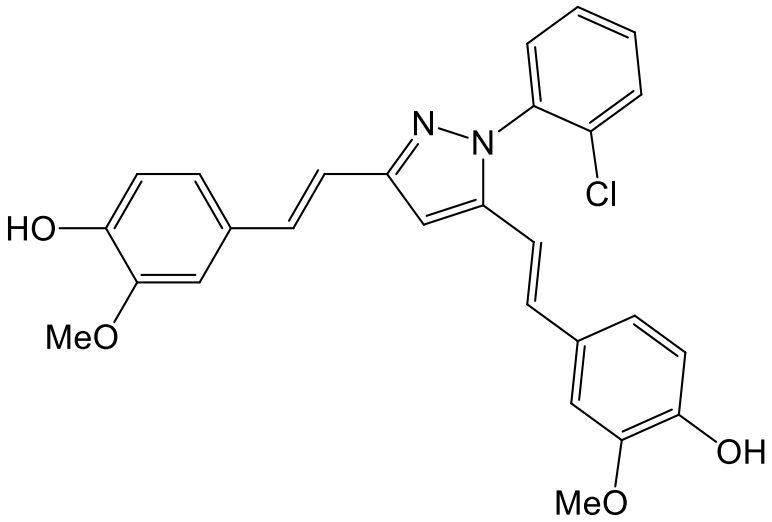
1.2.2 Five membered heterocyclic compounds

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)



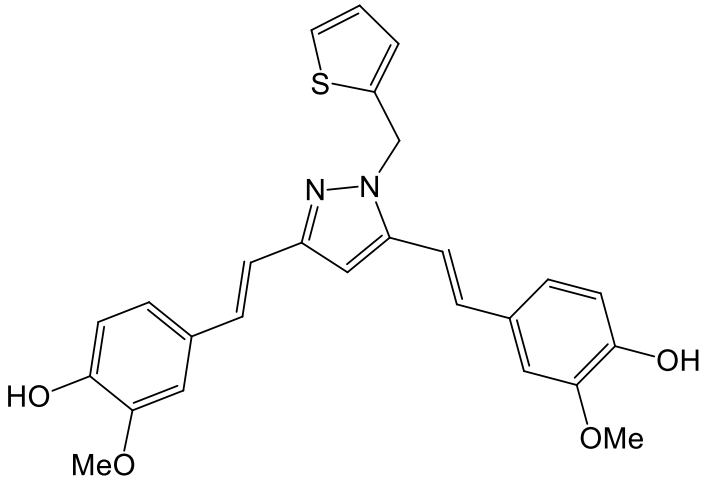
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 2-hydrazinopyrimidinehydrate (0.15 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.43 g (yield 68.03 %) of yellow solid. Melting point is 115 -116 °C. IR (KBr): ν_{max} cm^{-1} 1640.33 (-C=N), 1066.3 (C-O ether) of (–O-CH_3), and 1218.35 (N-N). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.81 (s, 6H, 2 OCH_3), 5.38 (bs, 2H, OH), 6.76 (s, 1H), 6.97 (m, 6H); 7.12 (d, 2H, J = 7.7 Hz), 7.18 (m, 2H); 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).



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 2-chlorophenylhydrazin hydrogen chloride (0.252 g, 1.357 mmol), and glacial acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.589 g (yield 79.12%) of yellow solid. Melting point is 123 -126 °C. IR: ν_{max} cm^{-1} 1633.22 (-C=N), 1093.17 (C-O ether) of (–O-CH_3). ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3), 5.37 (bs, 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).



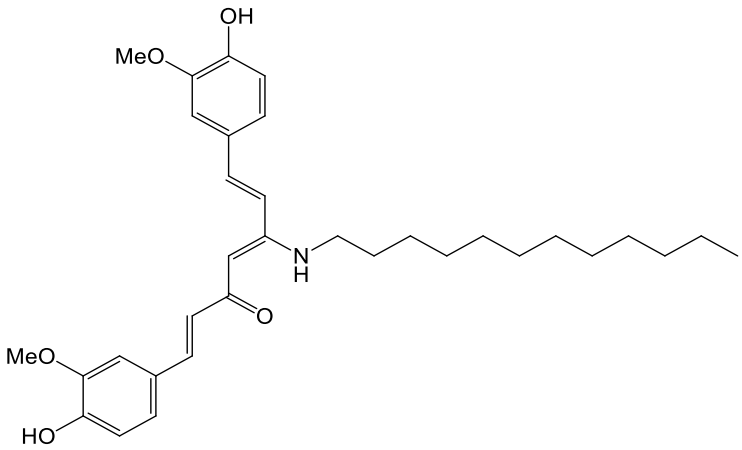
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), (2-thienylmethyl)hydrazine hydrochloride (0.224 g, 1.357 mmol), and glacial acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.45 g (yield 72.1%) of brown solid. Melting point is 126 -128 °C, IR: ν_{max} cm^{-1} 3413.25 (O-H), 1569.11 (C=N), 1515.98 (C=C), 1033.23 (C-O).

^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3), 4.99 (s, 2H, CH_2, methylene), 5.35 (s, 2H, OH), 6.53 (s, 1H, CH 1-pyrazole), 6.83 (d, 1H, CH 2-thiophene), 6.88 (d, 1H, CH 1-benzene), 6.93 (t, 1H, 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, 1H, CH 2-thiophene).^{13}C-NMR (400 MHz,

DMSO-d6) 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

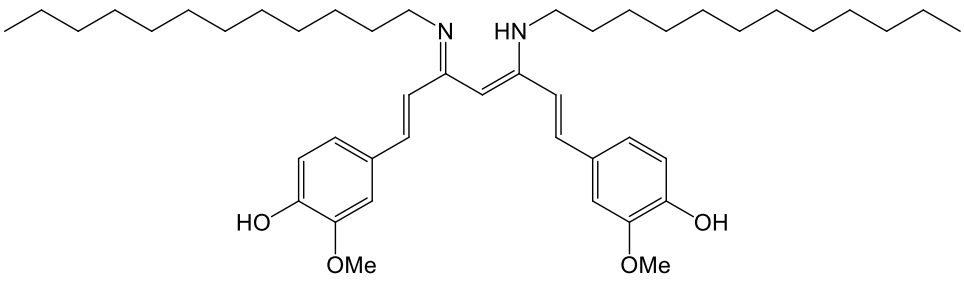
1.3.1 1E,5E,6E)-5-(dodecylimino)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (13).



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), dodecyl amine (0.28 g, 1.357 mmol), and glacial acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.719 g (yield 99 %) of brown solid. Melting point is 99 -100 °C, IR: ν_{max} cm^{-1} 3311.03 (O-H, and N-H stretching), 3027.12 (=C-H), 2732- 2911 (C-H, aliphatic), 1559 (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, methyl), 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.

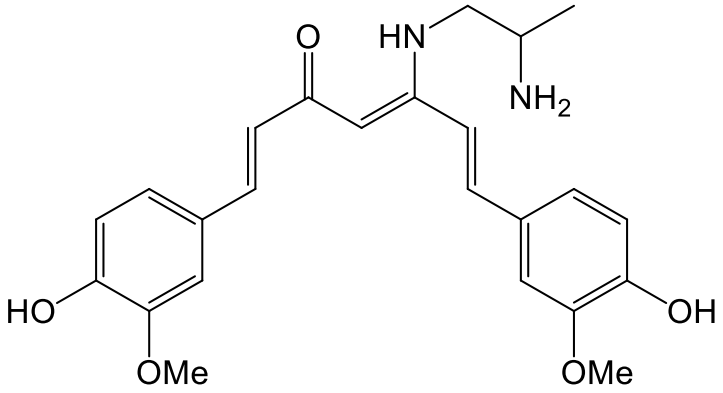
1.3.2 4-[(1E,3Z,5E,6E)-3,5-bis(dodecylimino)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-dien-1-yl]-2-methoxyphenol (14)



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), dodecyl amine (0.56 g, 2.714 mmol), and glacial acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.75 g (yield 98.95 %) of brown solid. Melting point is 220 - 223 °C, IR: ν_{max} cm^{-1} 3351 (O-H, and N-H stretching), 3015 (=C-H), 2726- 2878 (C-H, aliphatic), 1623.1 (C=C, conjugated), 1383.1 (C-N), 1062.3 (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, methyl), 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.

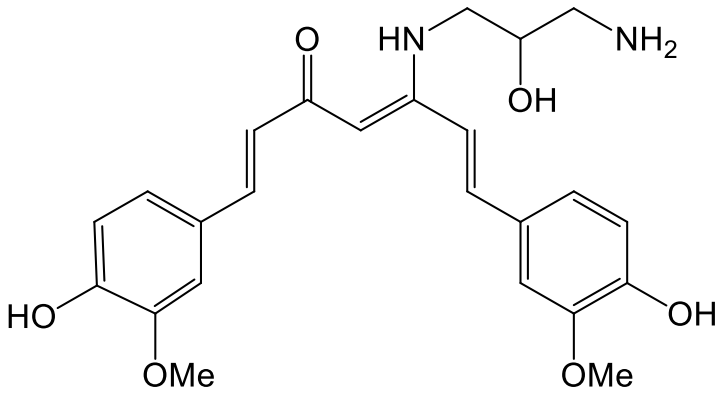
1.3.3 (1E,5E,6E) -5- [(2-aminopropyl) imino]-1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3-one (15).



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 1,2-diaminopropane (0.115 mL, 0.1005 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.37 g (yield 65.3 %) of yellow solid. Melting point is 173 -174 °C, IR: ν_{max} cm^{-1} 3411.07 (N-H stretching, aliphatic primary amine), 3315.3 (O-H, and N-H stretching), 3021.3 (=C-H), 2784.7 (C-H, aliphatic), 1615.7 (C=C, conjugated), 1372.1 (C-N), 1037.1 (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, methyl), 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).

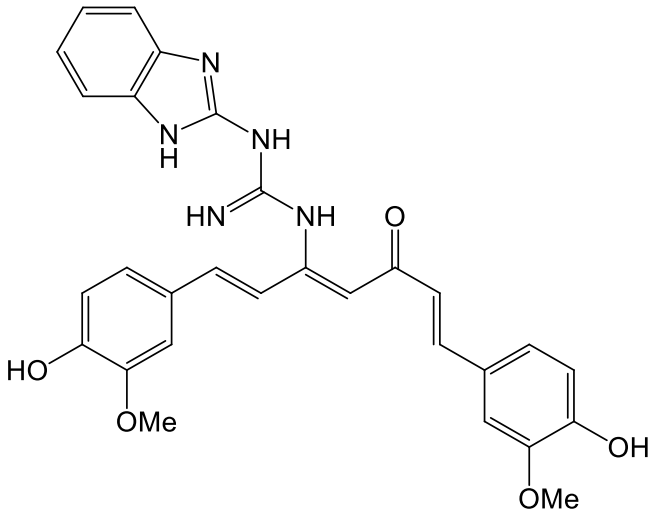


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 1,3-diamino-2-propanol (0.122 g, 1.357 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.34 g (yield 55.11 %) of yellow solid. Melting point is 241 - 242 °C, IR: ν_{max} cm^{-1} 3376.13 (N-H stretching, aliphatic primary amine), 3297.1 (O-H, and N-H stretching), 3036.17 (=C-H), 2789.13 (C-H, aliphatic), 1619.13 (C=C, conjugated), 1342.13 (C-N),

1027.33 (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, methyl), 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).

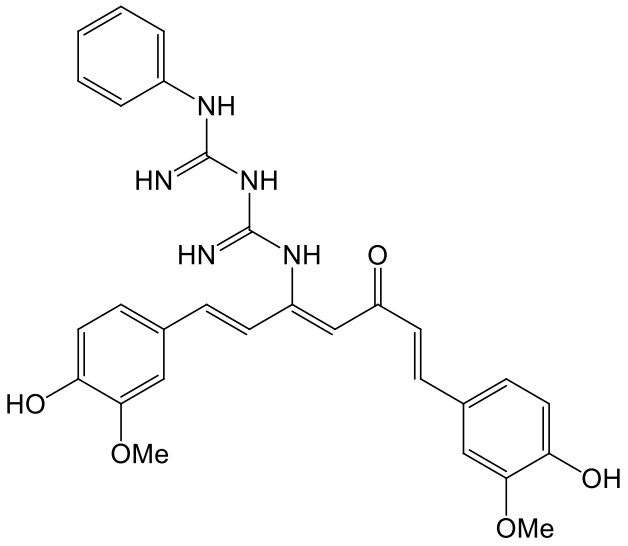


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), 2-Guanidinobenzimidazole (0.473g, 2.714 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from

hexane/EtOAc (2:1 by volume) to give 0.51 g (yield 73.2 %) of brown solid. Melting point is 332-333 °C, IR: ν_{max} cm^{-1} 3339 (N-H stretching), 3282 (O-H, and N-H stretching), 3035 (=C-H), 1661 (C=N, imine), 1611.7 (C=C, conjugated), 1347 (C-N stretching), 1031 (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, methyl), 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.

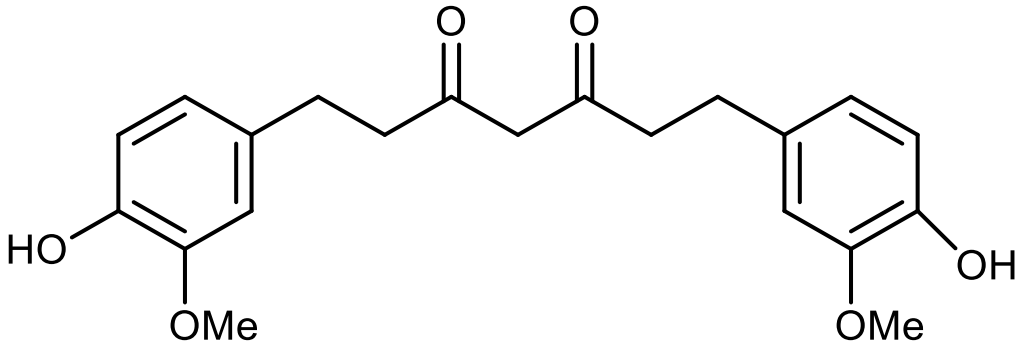
1.3.6 N-[(1E,3E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,6-dien-3-ylidene]-1-(N'-phenylcarbamimidamido) methanimidamide (18).



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), Phenylbiguanide (0.48 g, 2.714 mmol), and acetic acid (30 mL) were refluxed at 120 °C **as described in procedure A.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.60 g (yield 84.9 %) of brown solid. Melting point is 320-321 °C, IR: ν_{max} cm^{-1} 3388.11 (N-H stretching), 3263.17 (O-H, and N-H stretching), 3042.33 (=C-H), 1665.18 (C=N, Imine), 1612.45 (C=C, conjugated), 1287.11 (C-N stretching), 1028.1 (C-O ether of (–O-CH_3)).^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (bs, 2H, NH Amine), 3.83 (bs, 6H, 2 OCH_3, 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.

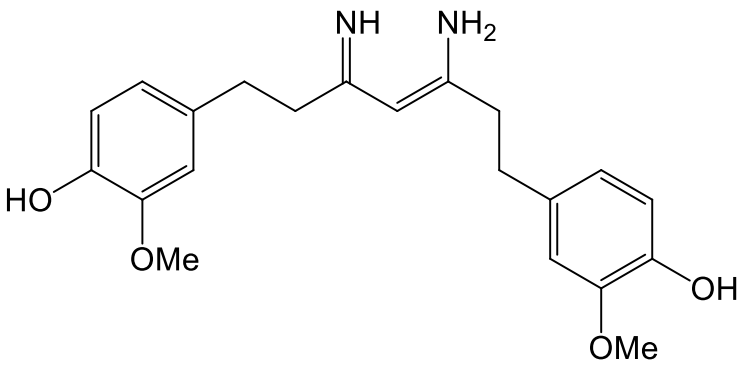
1.4 Preparation of H-Curcumin Based Amines

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) ($CDCl_3$): δ= 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 round bottom flask (50 mL), 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (H-curcumin) compound **19a** (0.5g, 1.357 mmol, 12.5 mL) and Ammonium acetate (0.313g) were added. The contents of the flask were heated at 60°C until complete solvent evaporation. Then it was heated again at 60°C for an hour. The reaction progress was monitored using TLC to a H-curcumin reference. The residue was washed with 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 0.3 g (yield 60 %) of yellow solid. Melting point is 160-163 °C. IR: *v*max cm^{-1} 3216.78 (C-NH), 2939.29 (C-N), 1605.51 (C=C), 1515.17 (C=N), and (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, OCH3, methyl), 3.88 (s, 1H), 5.35 (bs, 2H,

OH), 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.

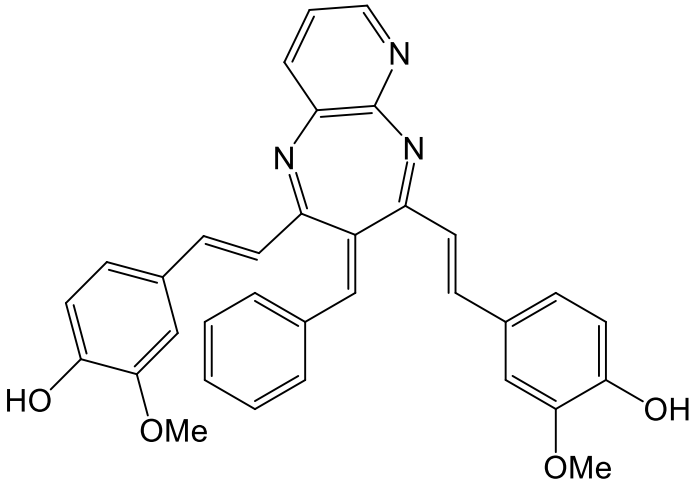
1.5 Preparation of curcumin-based compounds using Knoevenagel Condensation

General Procedure B

To a round bottom flask (50 mL), 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL) or tri ethanol amine (TEA) (4 drops), and toluene 30 mL were added. The contents of the flask were refluxed for 30 hours. Then glacial acetic acid (1 mL) and diamino compound (1.357 mmol) were added to the mixture and refluxed again for (20 hours). The reaction progress was monitored using TLC to a curcumin reference. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with sodium bicarbonate ($NaHCO_3$, 5%) to remove the acid, filtered, washed with water and dried. The dry solid was washed 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).

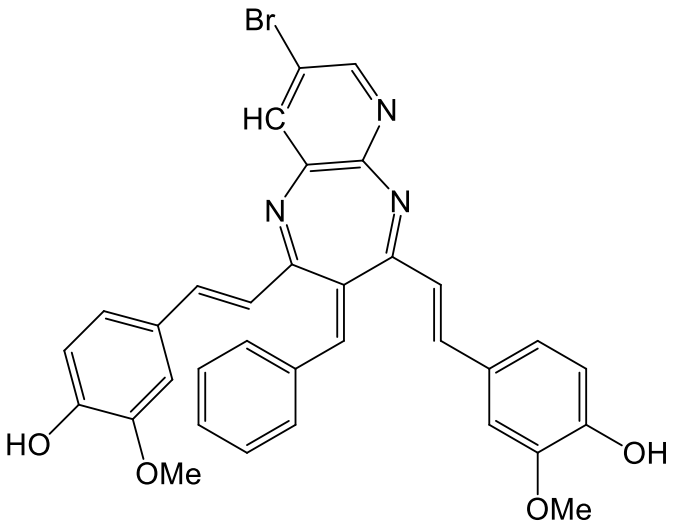


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL), and toluene 30 mL were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and 2,3-diaminopyridine (0.15g 1.357 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.63 g (yield 84.9 %) of orange solid. Melting point is 311 -313 °C. IR: ν_{max} cm^{-1}: 3210 (O-H), 3043.11 (=C-H), 1619 (C=N), 1601 (C=C, conjugated), 1309 (C-N stretching), 1042 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3, 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.

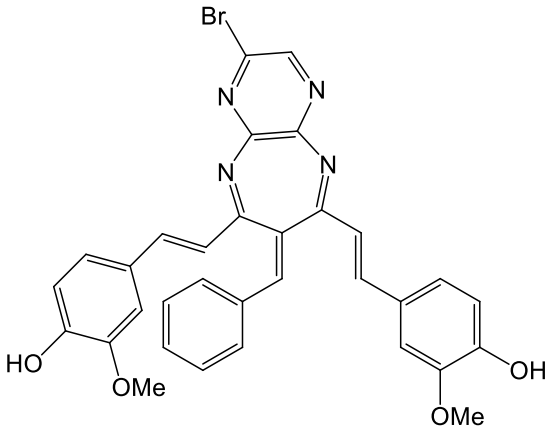
1.5.1.2 4-[(E)-2-[(3E)-8-bromo-4-[(E)-2-(4-hydroxy-3-methoxyphenyl) ethenyl]-3- (phenylmethylidene) -3H-pyrido [2,3-b] [1,4] diazepin -2-yl]ethenyl] -2-methoxyphenol (21).



A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and 2, 3-diamino-5-

bromopyridine (0.26g, 1.357 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.71 g (yield 90.1 %) of yellow solid. Melting point is > 360 °C. IR: ν_{max} cm^{-1}: 3207 (O-H), 3042 (=C-H), 1619 (C=N), 1609 (C=C, conjugated), 1313 (C-N stretching), 1037 (C-O ether of ($-O-CH_3$)), 577 (C-Br stretching). ^{1}HNMR (400 MHz, DMSO-d6) δ: 4.0 (bs, 1H, NH), 3.83 (s, 6H, 2 OCH_3, 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).

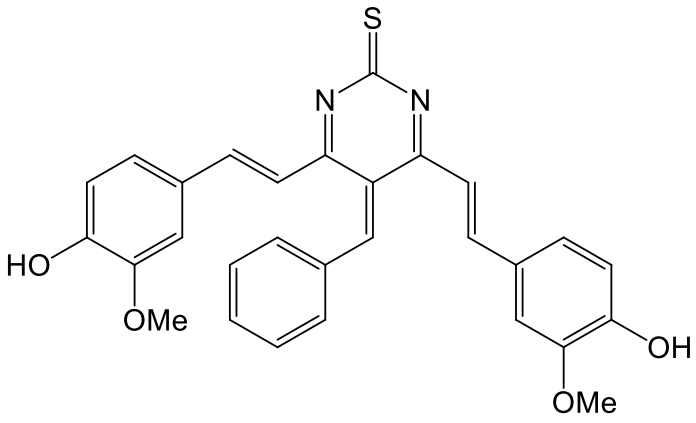


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and 2, 3-diamino-5-bromopyrizine (0.26 g, 1.357 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.70 g (yield 85.6 %) of yellow solid. Melting point is > 350 °C. IR: ν_{max} cm^{-1}: 3279.2 (O-H), 3051.12 (=C-H), 1601.13 (C=N), 1582 (C=C, conjugated), 1298.44 (C-N stretching), 1209.6 (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, 2 OCH_3, 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 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 round bottom flask (50 mL), 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), thiourea (0.103 g, 1.357 mmol), benzaldehyde (3 drops), tri ethanol amine (TEA) (4 drops) and toluene (30 mL) were added. The contents of the flask were refluxed for 30 hours. The reaction progress was monitored using TLC to a curcumin reference. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with sodium bicarbonate ($NaHCO_3$, 5%) to remove the acid, filtered, 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 0.68 g (yield 95.3 %) of yellow solid. Melting point is 279-282 °C. IR: ν_{max} cm^{-1}: 3253.2 (O-H), 3037.12

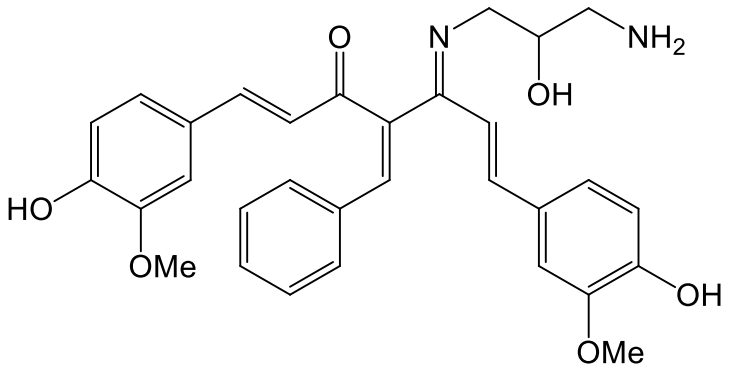
(=C-H), 1615.9 (C=N), 1589.1 (C=C, conjugated), 1662.5 (C=N),1217.9 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 3.83 (s, 6H, 2 OCH_3, 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).

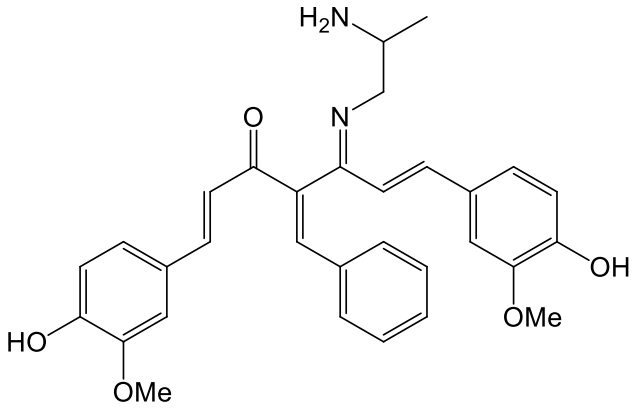


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), tri ethanol amine (TEA) (4 drops), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and 1,3-diamino-2-propanol (0.122 g, 1.357 mmol) were added to the mixture and refluxed again for (12 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.41 g (yield 57.7 %) of yellow solid. Melting point is 113 -116 °C. IR: *v* max cm^{-1} 3030.52 (N-H), 1578.22 (C=N), 1518.62 (C=C), 1039.32 (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, 2 OCH_3, 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).



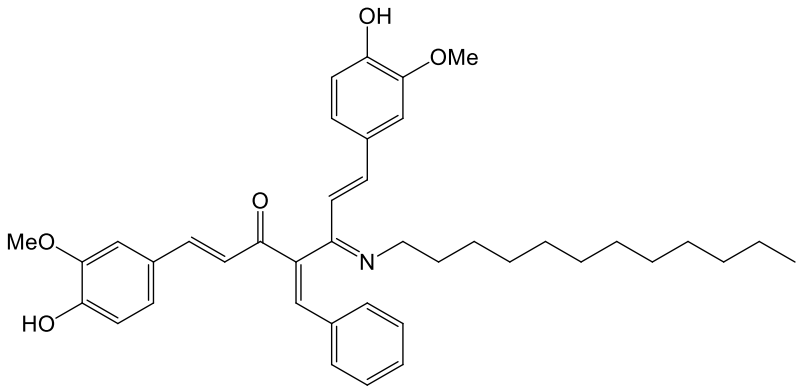
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), tri ethanol amine (TEA) (4 drops), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and 1,2-diaminopropane (2 drops, 1.357 mmol) were added to the mixture and refluxed again for (12 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.5 g (yield 71.9 %) of yellow solid. Melting point is 98 -99 °C. IR: ν_{max} cm^{-1} 3281.35 (O-H), 1740.33 (C=O), 1569.13 (C=N), 1509.55 (C=C), 1028.79 (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, 2 OCH_3, 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).

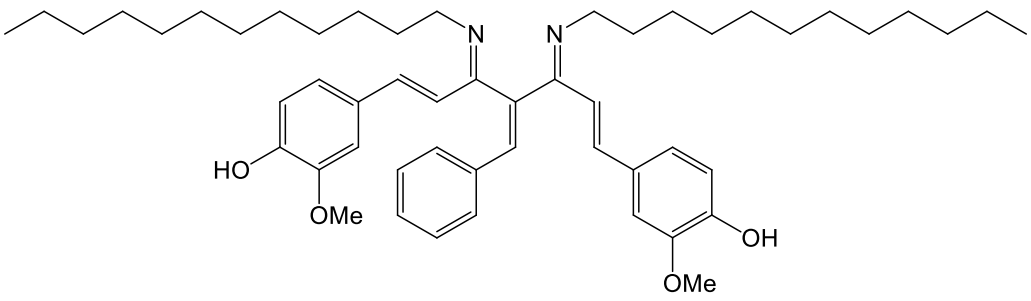


A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and dodecyl amine (0.26 g, 1.357 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/EtOAc (2:1 by volume) to give 0.69 g (yield 83.3 %) of yellow solid. Melting point is 288-291 °C. IR: ν_{max} cm^{-1}: 3213 (O-H), 3039 (=C-H), 2790 (C-H, aliphatic), 1653 (C=O), 1612 (C=N), 1597 (C=C,

conjugated), 1216 (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, 2 OCH_3, 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).



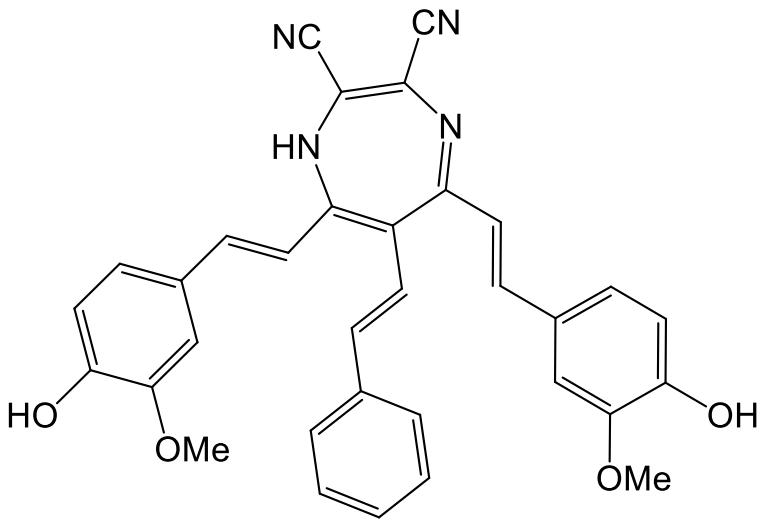
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5 mL), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and dodecyl amine (0.51 g, 2.714 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from

hexane/EtOAc (2:1 by volume) to give 0.75 g (yield 70.9 %) of yellow solid. Melting point is 316-317 °C. IR: ν_{max} cm^{-1}: 3187.2 (O-H), 3011 (=C-H), 2829.29 (C-H, aliphatic), 1635.3 (C=N), 1586.5 (C=C, conjugated), 1216.12 (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, 2 OCH_3, 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.

1.5.4 Seven Membered Curcumin-Based Diazepines

1.5.4.1 5,7-bis [(E) -2- (4- hydroxy-3- methoxyphenyl) ethenyl]-6-(phenylmethylidene) -6H-1,4-diazepine-2,3-dicarbonitrile (28).



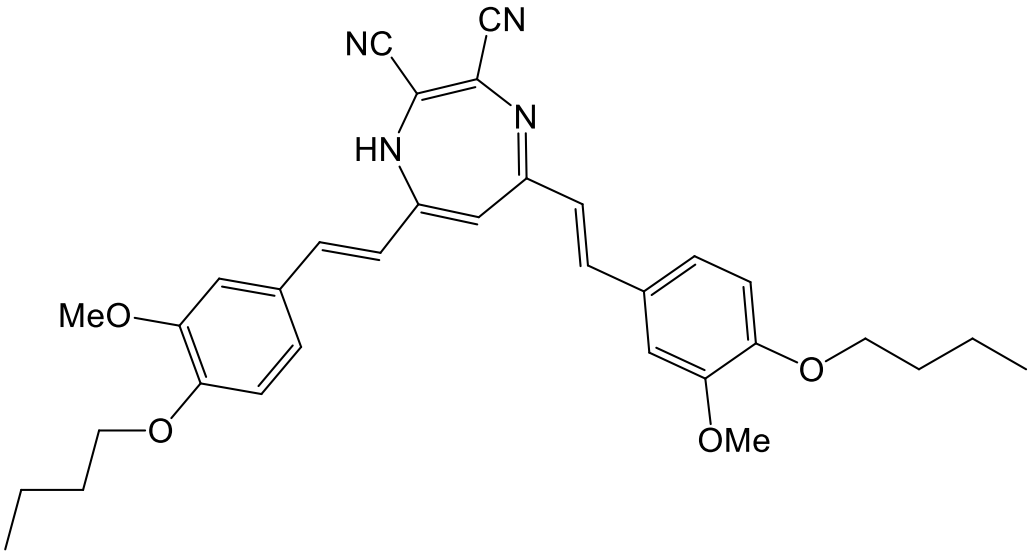
A mixture of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (0.5 g, 1.357 mmol), benzaldehyde (0.5 mL), diisopropylamine (0.5mI), and toluene (30 mL) were refluxed for 30 hours at 120 °C, then glacial acetic acid (1 mL) and diaminomaleonitrile (0.15 g, 1.357 mmol) were added to the mixture and refluxed again for (20 hours) **as described in procedure B.** The produced solid was recrystallized from hexane/ EtOAc (2:1 by volume) to give 0.67 g (yield 91.3%) of orange solid. Melting point is 299 - 301 °C. IR: ν_{max} cm^{-1}: 3226.44 (O-H), 3013.55 (=C-H), 2229.56 (C≡H, stretching), 1639.75 (C=N), 1589.47 (C=C, conjugated), 1259.7 (C-O ether of (–O-CH_3)). ^{1}HNMR (400 MHz, DMSO-d6) δ: 2.0 (bs, 1H, NH), 3.83 (s, 6H, 2 OCH_3, 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 (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 groups

1.6.1 5,7- bis ((E) -4- butoxy-3- methoxystyryl)- 1H-1, 4-diazepine -2 ,3-dicarbonitrile (29).



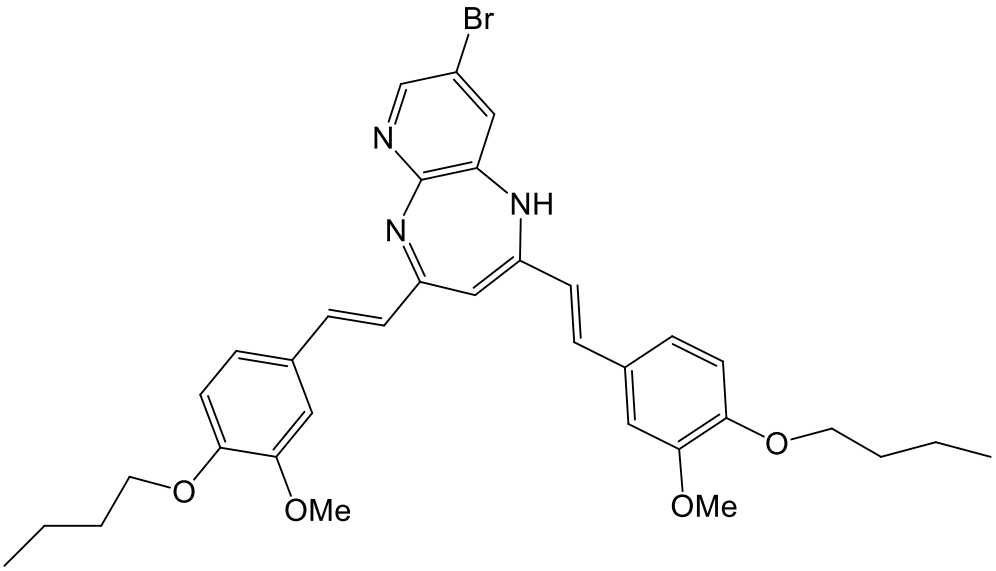
In a round bottom flask (50 mL), 5,7-bis[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-1,4-diazepine-2,3-dicarbonitrile (C8) (0.2 g, 0.45 mmol) was dissolved in ethanol (20.0 mL), NaOH (0.04g, 1 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over

(10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried. Yield 76.2 % (0.13 g) of brown solid, m.p 141-143 °C, IR: ν_{max} cm^{-1}: 3000 (=C-H), 2816 (C-H, aliphatic) 2238 (C≡H, stretching), 1627 (C=N), 1601 (C=C, conjugated), 1209 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 2.0 (s, 1H, NH), 3.83 (s, 6H, OCH_3, 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: 14.7, 19.3, 32.1, 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)-4-butoxy-3-methoxystyryl)-1H-pyrido[2,3-b][1,4]diazepine (30)

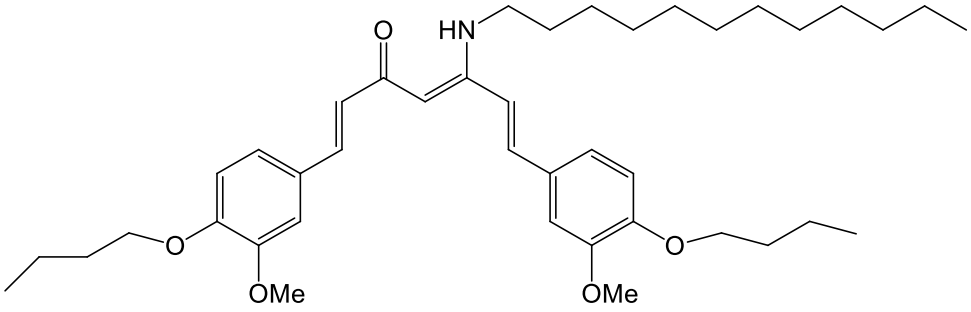


In a round bottom flask (50 mL), 4-[(E)-2-{8-bromo-4-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-pyrido[2,3-b][1,4]diazepin-2-yl}ethenyl]-2-methoxyphenol (C5) (0.09 g, 0.17 mmol) was dissolved in ethanol (20.0mL), NaOH (0.02 g, 0.5 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried. Yield; 60.1 % (0.1 g) of yellow solid, m.p 273 - 275 °C, IR: ν_{max} cm^{-1}: 3009 (=C-H), 2818 (C-H, aliphatic), 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: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 4.0 (s, 1H, NH), 3.83 (s, 6H, OCH_3, 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: 14.5, 20, 32, 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,4Z,6E)-1,7-bis (4-butoxy-3-methoxyphenyl)-5-(dodecylamino) hepta-1,4,6-trien-3-one (31).



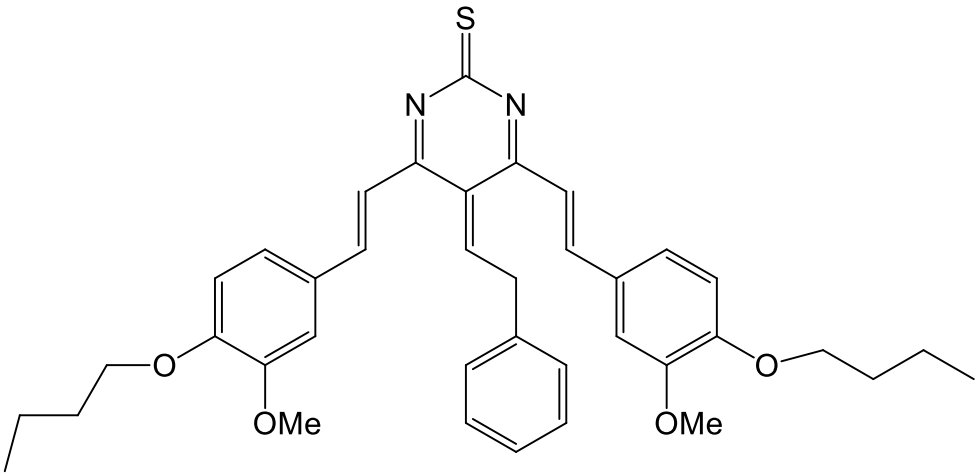
In a round bottom flask (50 mL), 1E,5E,6E)-5-(dodecylimino)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-dien-3-one (C13) (0.2 g, 0.373 mmol) was dissolved in ethanol (20.0 mL), NaOH (0.033 g, 0.825 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was

washed with distilled water and dried. Yield 85.3 % (0.2 g) of yellow solid, m.p 184 - 186 °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, 6H, OCH_3, 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) -4-butoxy -3-methoxystyryl) -5- (2-phenylethylidene) pyrimidine-2(5H)-thione (32).

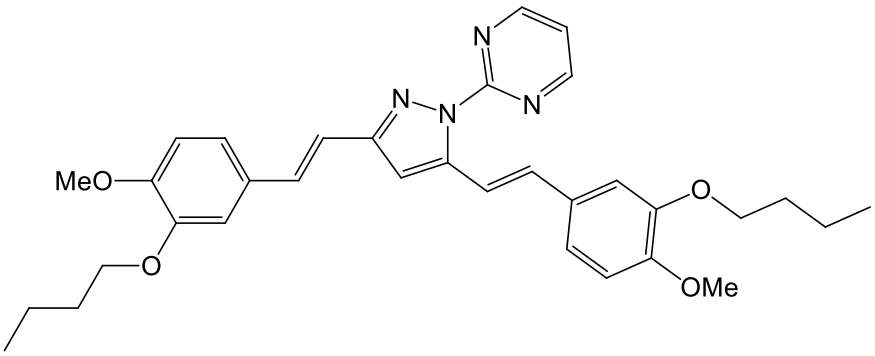


In a round bottom flask (50 mL), 4,6-bis[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-5-(phenylmethylidene)-2,5-dihydropyrimidine-2-thione (C23) (0.5 g, 1 mmol) was dissolved in ethanol (20.0 mL), NaOH (0.09 g, 2.25 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried. Yield 91.0% (0.43 g) of brown solid, m.p 366-368 °C, IR: ν_{max} cm^{-1}: 3020 (=C-H), 2811 (C-H, aliphatic), 1609 (C=N), 1597 (C=C, conjugated), 1204 (C-O ether of (–O-CH_3)).

^{1}HNMR (400 MHz, DMSO-d6) δ: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 3.21 (d, 2H, CH_2), 3.83 (s, 6H, OCH_3, 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: 13.7, 18.6, 31, 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.

1.6.5 2- {3,5-bis [(E)-2- (4-butoxy-3-methoxyphenyl) ethenyl]-1H-pyrazol-1-yl} pyrimidine (33).



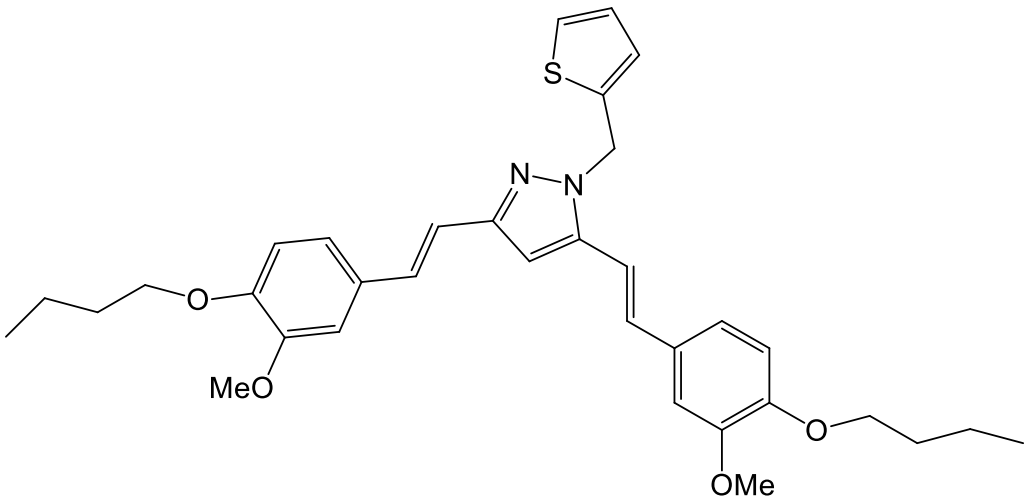
In a round bottom flask (50 mL), 4-[(E)-2-{5-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1-(pyrimidin-2-yl)-1H-pyrazol-3-yl}ethenyl]-2-methoxyphenol (C10) (0.5 g, 1.1 mmol) was dissolved in ethanol (20.0 mL), NaOH (0.1 g, 2.5 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried. Yield; 52.6% (0.33 g) of brown solid, m.p 173-174 °C, IR: ν_{max} cm^{-1} 2813 (C-H, aliphatic), 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: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 3.83 (s, 6H, OCH3, methyl), 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: 14.1,19, 31.8, 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)-4-butoxy-3-methoxystyryl)-1-(thiophen-2-ylmethyl)-1H-pyrazole (34).



In a round bottom flask (50 mL), 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) was dissolved in ethanol (20.0 mL), NaOH (0.04 g, 1 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored

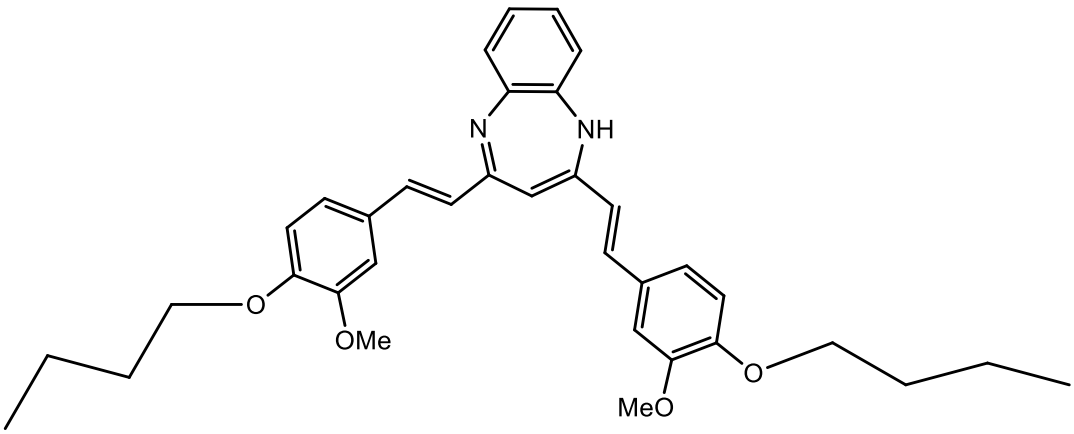
using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried.

Yield; 69.5 % (0.16 g) of yellow solid, m.p > 350 °C, IR: ν_{max} cm^{-1} 2825 (C-H, aliphatic), 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: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 3.83 (s, 6 H, OCH_3, 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: 13, 19, 31.9, 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)-4-butoxy-3-methoxystyryl)-1H-benzo[b][1,4]diazepine (35).



In a round bottom flask (50 mL), 4-[(E)-2-{4-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-1,5-benzodiazepin-2-yl}ethenyl]-2-methoxyphenol (C6) (0.445 g, 0.01mol) was dissolved in ethanol (10.0 mL), NaOH (0.09 g, 2.25 mmol) was added to it. The solution was stirred for (30 mins) at room temperature. 1-Chlorobutane (0.177 mL, 4 drops, 1.92 mmol) were added dropwise over (10 mins), The contents of the flask were refluxed for 15 hours. The reaction progress was monitored using TLC. The excess solvent was removed under reduced pressure (using rotary evaporator), the residue was washed with distilled water and dried. Yield 45.3 % (0.23 g) of yellow solid, mp 188 - 191 °C, IR (KBr): ν_{max} cm^{-1} 3346.97 (N-H), 2963.36 (C-H), 2820 (C-H, aliphatic) 1640. (C=N), 1598.44 (C=C), ^{1}H-NMR (400 MHz, DMSO-d6) δ: 0.9 (t, 6H, CH_3), 1.45-1.76 (m, 2H, CH_2), 3.78 (s, 6H, OCH_3), 4.02 (bs, 1H, NH), 1H 5.09 (s, 1H), 5.67 (d, 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: 14,3, 19.2, 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. Anticancer activities of the prepared curcumin-based heterocyclics

2.1 Materials and procedures

2.1.1 Preparation of stock solutions

Solutions of curcumin-based heterocyclics were prepared at a concentration of 400 μg per 1 mL of dimethyl sulfoxide (DMSO) solvent and then incubated at 4 °C.

Various concentrations of (200, 100, 50, 25, and 12.5 μg /ml) were then prepared using serial dilution method.

2.1.2 Cell lines

The human cervical cancer cell lines (HeLa cells) were obtained from the American Type Culture Collection [ATCC], Manassas, VA, USA. Were grown in RPMI medium supplemented with 10% fetal calf serum, 1% non-essential amino acid, 1% l-glutamine, 1% penicillin streptomycin and 1% amphotericin B. All cells were grown in a humidified atmosphere of 95% air, 5% CO_2 at 37ºC, the culture medium was changed at least twice a week as needed. All chemicals used were purchased from Biological Industries except for the amphotericin B and MTT reagent from SIGMA Aldrich.

For screening experiment, the cells were grown into 12-well plates in 950 μl of RPMI medium (Biological Industries, USA) containing 5% FBS, 2×10^4 cells/well plating density. After that 50μl of diverse concentrations

(400, 200, 100, 50, 25, and 12.5 µg /ml) of curcumin-based heterocyclics was added in duplicates to the prepared 12-well plates and incubated for 24 h at 37ºC, 5% CO_2, 95% air and 100% relative humidity. An inverted microscope (Labomed, USA) was used to observe the morphological changes of the cells.

2.2 Cytotoxicity Assay

Cells at 70-80% confluence were detached from culture flask by removing the culture medium then adding 0.05% trypsin- EDTA and a suspension of 100 µl (2.0×10^4 cells/well) of viable cells were seeded in a 96-well plate and incubated for 24 h at 37ºC. After the removal of media cells were treated with 50 µl stock solution serially diluted to reach concentrations of (400, 200, 100, 50, 25, and 12.5 µg /ml) of curcumin-based heterocyclics, then incubated for 24h at 37ºC to perform the MTT assay.

2.3 MTT assay

The anticancer effect of curcumin-based heterocyclics against HeLa cells was estimated by the 3- [4, 5- dimethylthiazole-2-yl]-2, 5- diphenyltetrazolium bromide (MTT) assay using (cell growth determination kit MTT based, Sigma). Cells (2×10^4 cells/well) for cytotoxic assay and (1.0×10^4 cells/well) for cytostatic Assay

were incubated with various concentrations of the compounds (400, 200, 100, 50, 25, and 12.5 µg /ml) in 5% CO_2, 95% air and 100% relative humidity at 37 ºC for 24 h in an FBS-free medium. Aseptically MTT

solution was added in an amount equal to 10% of the culture volume. Then cultures were returned to incubator and incubated for 4 hours. After the incubation period, the resulting MTT formazan crystals were dissolved by the addition of MTT solvent in an amount equal to the original culture volume. The addition of MTT solvent was performed after the removal and disposal of the culture fluid as HeLa cells were still attached to the culture surface. The absorbance at 570 nm was measured using micro plate reader (Labtech, UK). The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The data are expressed as the mean percentage of viable cells as compared to the respective control.

CHAPTER THREE

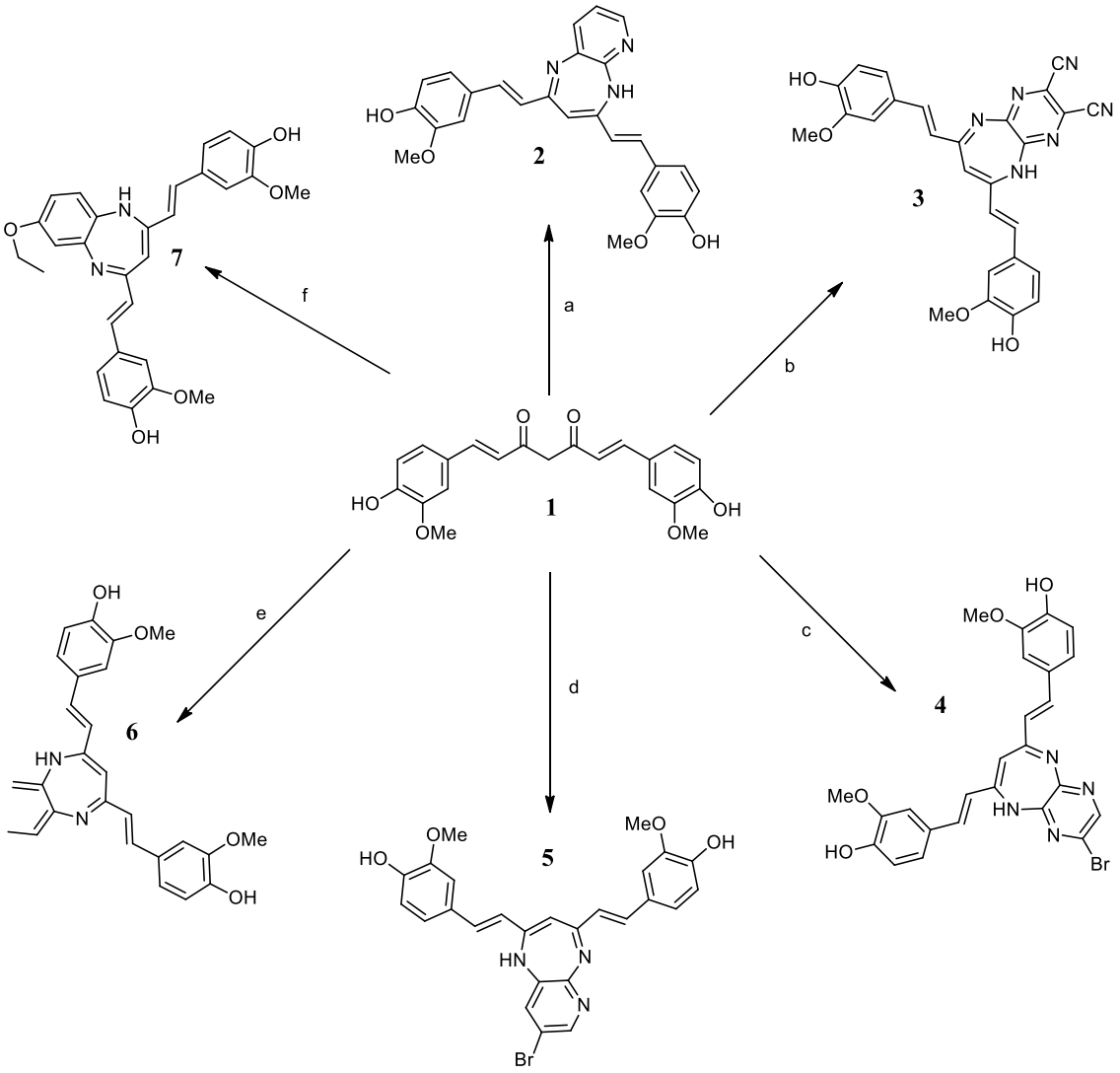
RESULTS AND DISCUSSION

In a preceding study [63] a non-toxic and a naturally occurring curcumin has been used as a skeleton for synthesizing diverse heterocyclic compounds including pyrazole, diazepine, and isoxazole. The compounds were synthesized either by mixing the curcumin with the diamine compounds in the presence of catalytic amount of sulfuric acid and by using ethanol as a solvent. Alternatively, by heating the mixtures of curcumin and diamines to melt, the synthesis was completed by heating the mixture for 30 min at 160°C. The results indicated that diazepine exhibited the excellent, highest potential bioactivity. Based on these results and to extend the work for development of curcumin-based reagents with better bioactivity. Novel sets of curcumin-based diazoles, benzodiazepine, diazepine, and amines were synthesized using an unsophisticated and convenient condensation reaction (by coupling curcumin with diverse types of hydrazine and 1,2-diamino compounds. Schemes 1-5 show a summary of the synthesized compounds reaction conditions and structures. Besides, H-curcumin based amino compound was prepared from reaction of H-curcumin with Ammonium acetate scheme 6. Moreover, some of the prepared curcumin-based heterocycles were further subjected to condensation via Knoevenagel Condensation as shown in schemes 7-14. A blocking of the phenolic hydroxyl groups with butyl groups was also performed. The second step involves etherification of the prepared curcumin-based compounds with butyl groups as shown in schemes 15-21.

3.1 Curcumin-based benzodiazepines

3.1.1 Preparation curcumin-based benzodiazepines

In the preparation of Curcumin-based benzodiazepines, the condensation reactions of curcumin with various diamines with 1:1 ratio were carried out in acetic acid which was used as a solvent and as a catalyst as shown in scheme 1. The progress of reactions was monitored using TLC. The synthesized products were purified as previously described in the experimental part. The structures of the prepared compounds were analyzed and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for all prepared curcumin-based benzodiazepines, they ranged between 48.14 % and 99.55%.



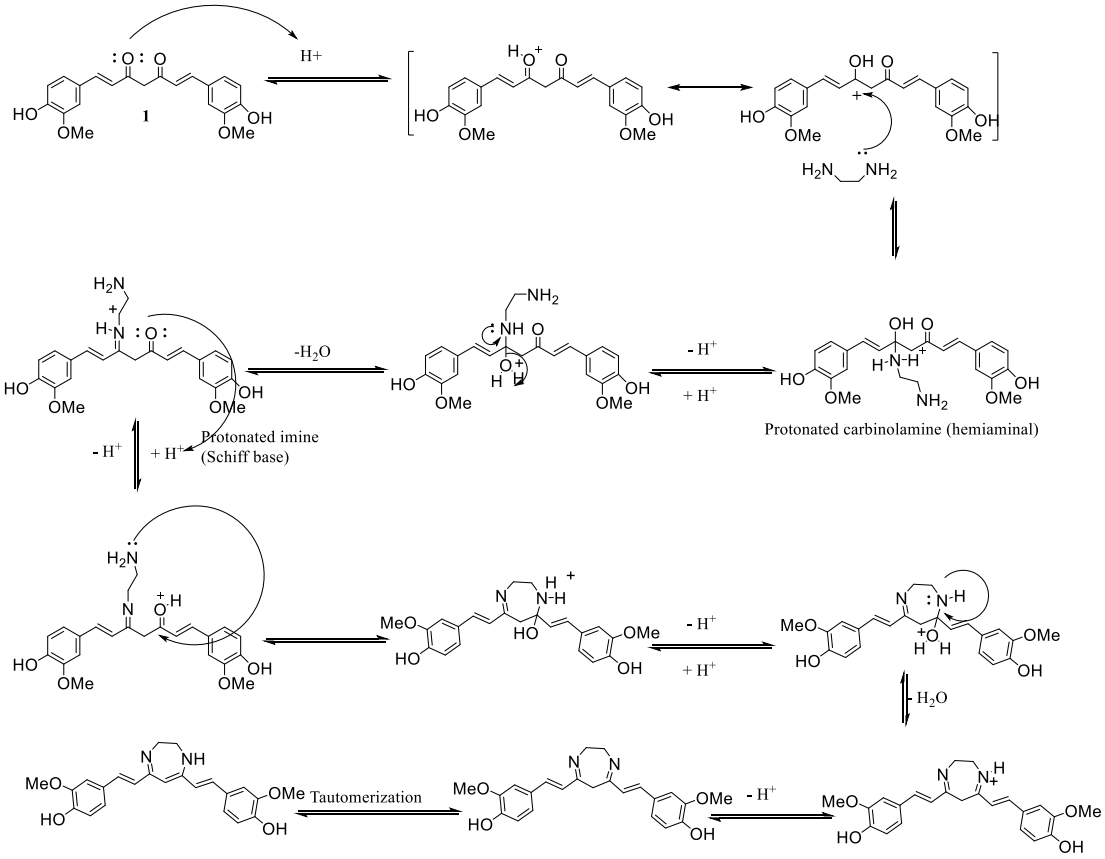
Scheme 1: Chemical structures and for making curcumin-based benzodiazepines.

3.1.2 Curcumin-Based Benzodiazepines reaction mechanism

A detailed mechanism of the condensation cyclization of curcumin with diverse 1,2 diamino and hydrazine compounds to form curcumin-based benzodiazepines is described in scheme 2. The mechanism of benzodiazepine formation begins with an acid-catalyzed nucleophilic addition of the diamino and hydrazine compounds to one of the carbonyl groups. Since 1,2-diamino and hydrazine compounds are weak

nucleophiles activation of carbonyl group is needed to make it susceptible for the attack, followed by deprotonation of the nitrogen atom, gives an unstable intermediate called a carbinolamine (hemiaminal)**.**

The second half of the mechanism converts the carbinolamine (hemiaminal) to the more stable imine. Protonation of the hydroxy group converts it to a good leaving group, followed by water loss, gives a resonance-stabilized carbocation where the positive charge on nitrogen atom with all octets filled, followed by loss of a proton, gives the imine intermediate. After that, **imine** intermediate is attacked by the second amino group followed by a loss of a second water molecule forming the desired product. For clarification purpose ethylene diamine was used in the reaction mechanism depicted in scheme 2



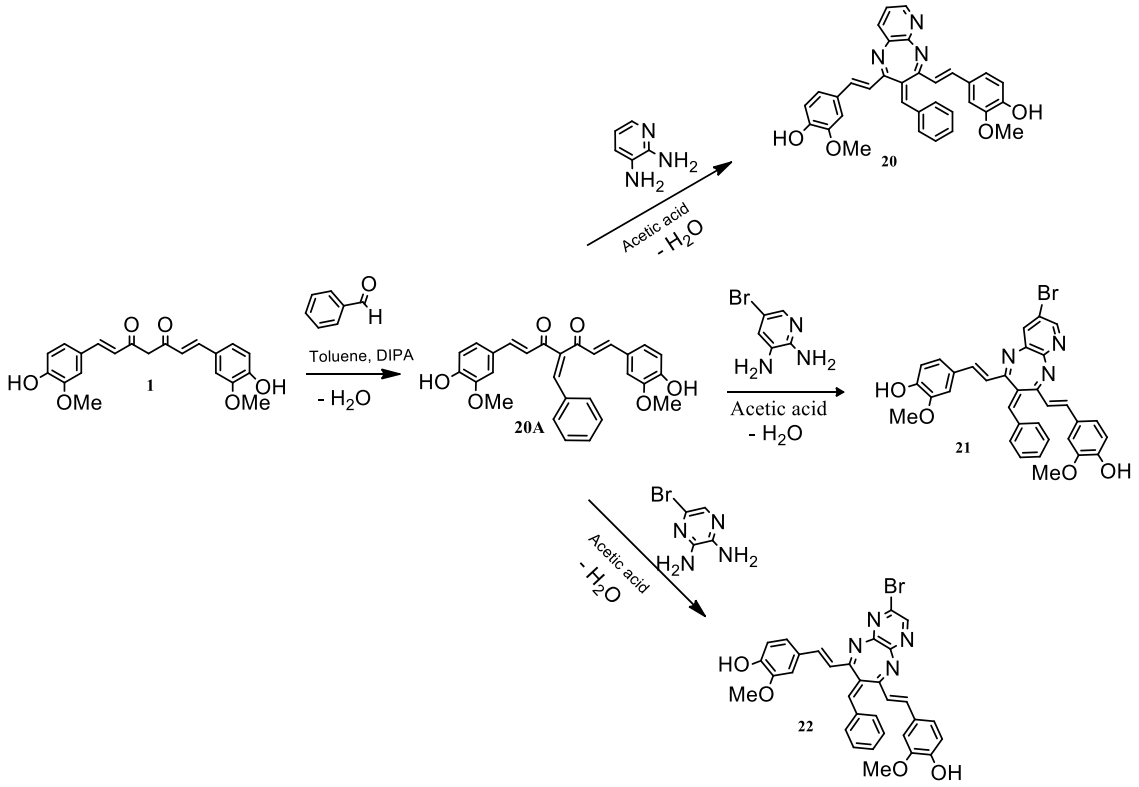
Scheme 2: A detailed mechanism of the condensation cyclization of curcumin with various 1,2 diamino and hydrazine compounds to form curcumin-based benzodiazepines.

3.1.3 Knoevenagel Condensation of Curcumin-Based Benzodiazepines

A two-fold mechanism was used to synthesize Curcumin-based benzodiazepines **20, 21**, and **22**. The first step includes the formation of α,β-unsaturated intermediate **20 A** using Knoevenagel condensation; by mixing curcumin with benzaldehyde in toluene 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 scheme 7.

An excess amount of acetic acid was added to the synthesized intermediate which was used for a couple of purposes; 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 7.

The progress of the reactions was monitored using TLC. The synthesized products **20, 21**, and **22** were purified as previously described in the experimental part. The structures of the prepared compounds were characterized and determined using TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based benzodiazepines **20, 21**, and **22**, and they are found to be of 84.9 %, 90.1 % and 85.6 % respectively.

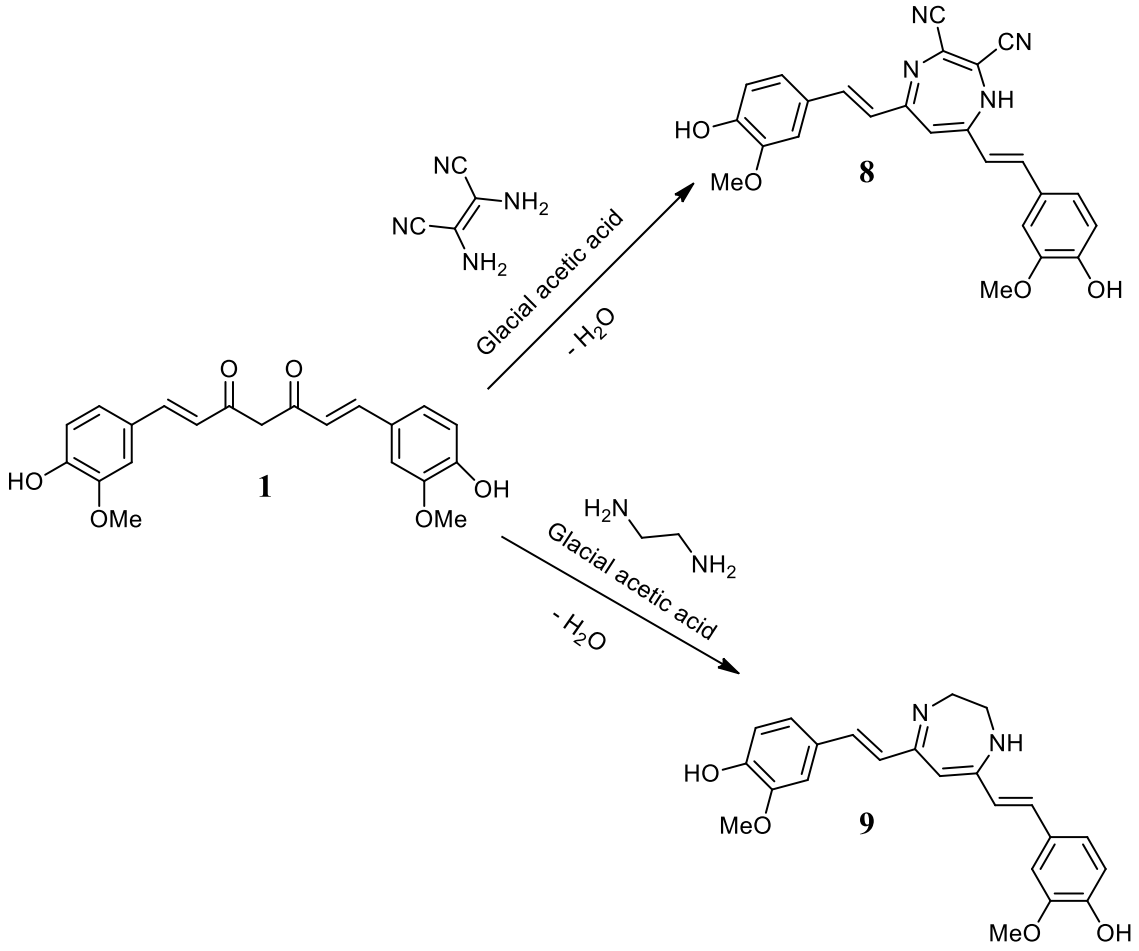


Scheme 7: Chemical structures and reaction conditions for making curcumin-based benzodiazepines using Knoevenagel Condensation.

3.2. Curcumin based diazepines

In the preparation of curcumin-based diazepines, the condensation cyclization reaction of curcumin with diaminomaleonitrile in 1:1 ratio was carried out in glacial acetic acid which was used for a couple of purposes as a solvent and as a catalyst. as shown in scheme 3. The progress of the reaction was monitored using TLC. The synthesized product **8** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared curcumin-based diazepine **8**, and it is found to be 21.1 %.

Meanwhile, compound **9** was synthesized using the same procedures described above “in the preparation of curcumin-based benzodiazepines part”; by mixing the curcumin with ethylene diamine in (1:1 ratio) in acetic acid that was used as a solvent and as a catalyst as shown in scheme 3. The progress of the reaction was monitored using TLC. The synthesized product was purified as previously described in the experimental part. The structure of the prepared compound **9** was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared curcumin-based diazepine compound **9**, and it is found to be 48.7 %.



Scheme 3: Chemical structures and reaction conditions for making curcumin-based diazepines.

3.3 Curcumin-based diazoles

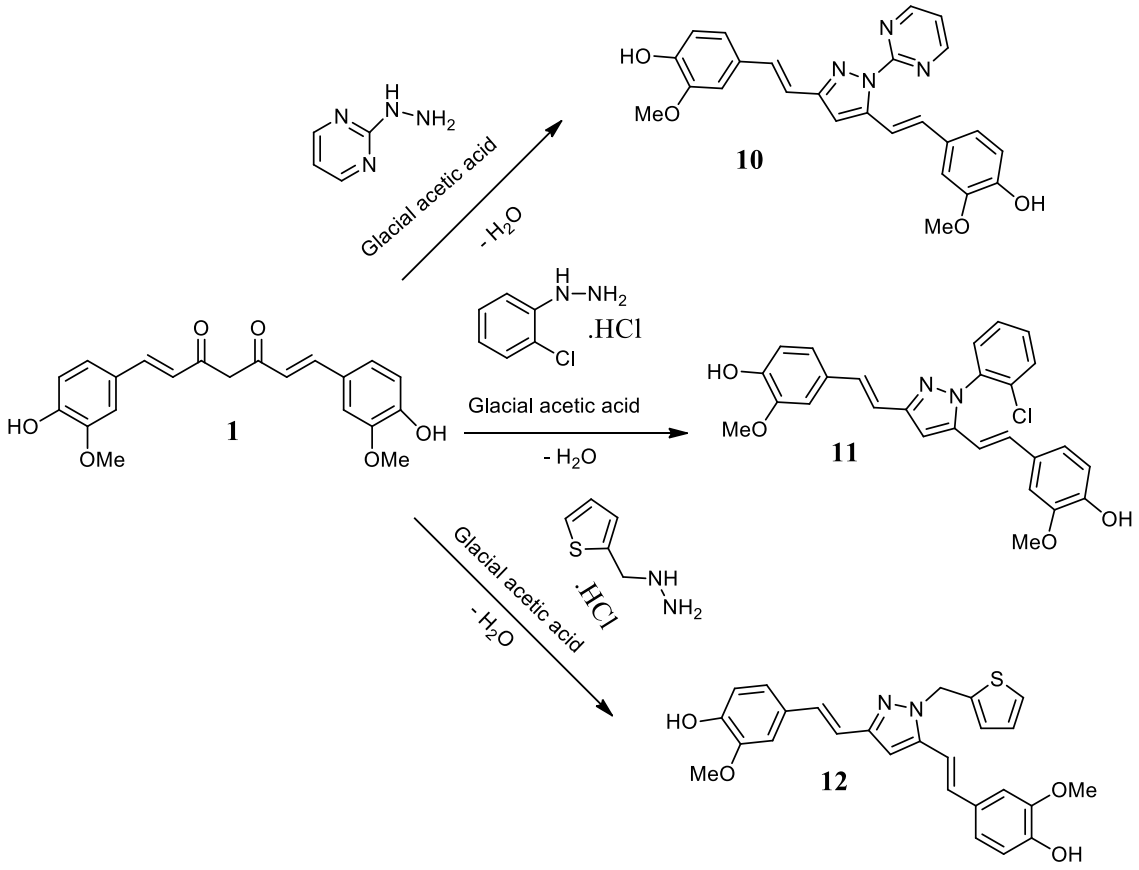
3.3.1 Preparation of curcumin-based diazoles

In the preparation of Curcumin-based diazoles, the condensation cyclization reaction of curcumin with 2-hydrazinopyrimidinehydrate to produce compound **10** was carried out using the same procedures described above "in the preparation of curcumin-based benzodiazepines and diazepines parts"; by mixing the curcumin with 2-hydrazinopyrimidinehydrate (1:1 ratio) in acetic acid which was used as a solvent and as a catalys as shown in scheme 4. The progress of reaction was

monitored using TLC. The synthesized product was purified as previously described in the experimental part. The structure of the prepared compound **10** was characterized and determined using TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared curcumin-based diazole compound **10**, and it is found to be 68.03%.

Compounds **11** and **12** were synthesized by mixing the curcumin with 2-chlorophenylhydrazin hydrogen chloride and (2-thienylmethyl)hydrazine hydrochloride respectively in (1:1 ratios) in glacial acetic acid which was used for a couple of purposes as a solvent and as a catalyst. as shown in scheme 4. The progress of reactions was monitored using TLC. The synthesized products **11** and **12** were purified as previously described in the experimental part. The prepared compounds' structures were characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based diazoles **11** and **12**, and they are found to be 79.2 % and 72.1% 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 4: Chemical structures and reaction conditions for making curcumin-based diazoles.

3.4 Amino Curcumin

3.4.1 Preparation

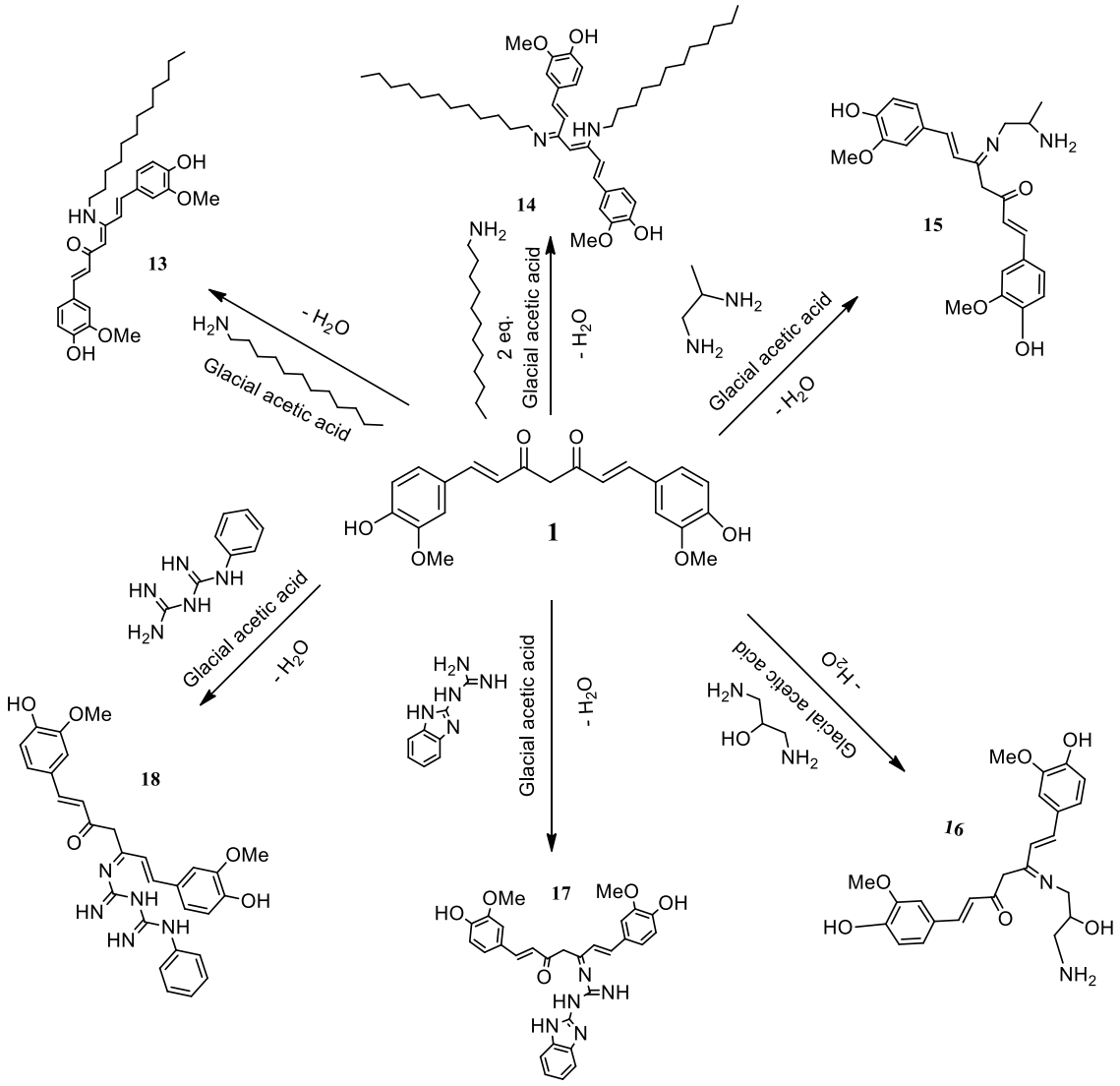
In the preparation of curcumin-based amines, the one-step condensation reactions of curcumin with dodecyl amine to produce compounds **13** and **14** were carried out using the same procedures described above "in the preparation of curcumin-based benzodiazepines, diazepines and diazoles parts"; by mixing the curcumin with 2- dodecyl amine in a 1:1 and in a 1:2 ratio in glacial acetic acid which was used for a couple of purposes as a solvent and as a catalyst, as shown in scheme 5. The progress of reactions was monitored using TLC. The synthesized products **13** and **14** were

purified as previously described in the experimental part. The prepared compounds' structures were characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based amines **13** and **14**, and they are found to be 99 % and 98.95% respectively.

Meanwhile, compounds **15** and **16** were synthesized by mixing the curcumin with 1,2-diaminopropane and 1,3-diamino-2-propanol respectively in (1:1 ratios) in glacial acetic acid which was used as a solvent and as a catalyst as shown in scheme 5. The progress of the reactions was monitored using TLC. The synthesized products **15** and **16** were purified as previously described in the experimental part. The prepared compounds' structures were characterized and determined using TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based amines **15** and **16**, and they are found to be 65.3 % and 55.11 % respectively.

Similarly, compounds **17** and **18** were synthesized by mixing the curcumin with 2-Guanidinobenzimidazole and Phenylbiguanide in a 1:1 ratio in acetic acid which was used as a solvent and as a catalyst as shown in scheme 5. The progress of reactions was monitored using TLC. The synthesized products **17** and **18** were purified as previously described in the experimental part. The structures of the prepared compounds were characterized and determined using several analytical and spectroscopic

techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based amines **17** and **18**, and they are found to be 73.2 % and 84.9 % respectively.



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

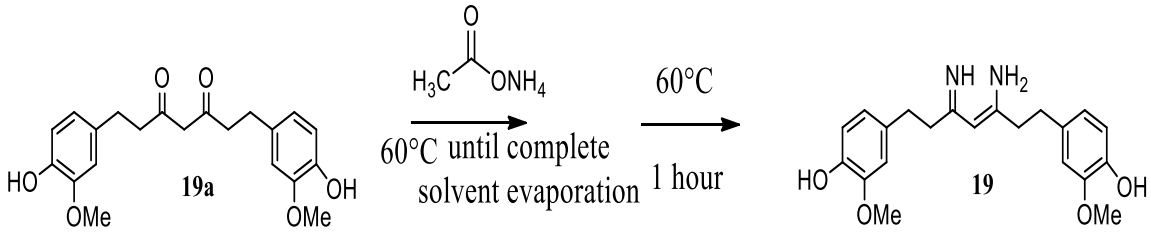
3.5 H-Curcumin Based Amines

3.5.1 Hydrogenetaed curcumin (H-Curcumin)

Tetrahydrocurcuminoids (**H-Curcumin**) 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

In the preparation of H-Curcumin-based amines compound **19**, the condensation reaction was carried out by mixing the H-curcumin 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 progress of reaction was monitored using TLC. The synthesized product was purified as previously described in the experimental part. The structure of the prepared compound **19** was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured and it is found to be 60 %.



Scheme 6: Chemical structure and reaction condition for making H-curcumin-based amine.

3.6 Curcumin-based compounds using Knoevenagel Condensation

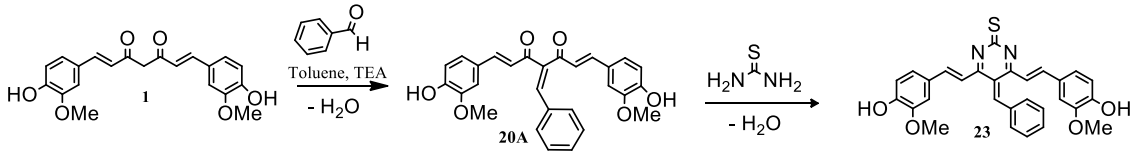
3.6.1 Preparation of curcumin-based compounds using Knoevenagel Condensation

A two-fold mechanism was used to synthesize curcumin-based benzodiazepines, diazepines, diazoles, and amines. The first step includes the formation of α,β-unsaturated intermediates using Knoevenagel condensation; by mixing curcumin with benzaldehyde in toluene solvent, in the presence of catalytic amount of organic bases including tri ethanol amine (TEA) and diisopropylamine (DIPA). Meanwhile, acid-catalyzed condensation reactions of the curcumin carbonyl groups of the synthesized intermediates with various 1,2-diamino or hydrazine compounds were involved in the second step to produce the desired products schemes 7-14.

3.6.1.2 Preparation of Curcumin-Based diazepines via Knoevenagel Condensation

In the preparation of Curcumin-based diazepine compound **23**, the one-step condensation cyclization reaction of curcumin with thiourea in (1:1 ratio) was carried out in the presence of benzaldehyde and tri ethanol amine

(TEA) which were employed to produce α,β-unsaturated intermediate compound **20A** using Knoevenagel condensation in toluene which was used as a solvent scheme 8. The progress of reaction was monitored using TLC. The synthesized product **23** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared curcumin-based diazepine **23**, and it is found to be 95.3 %.

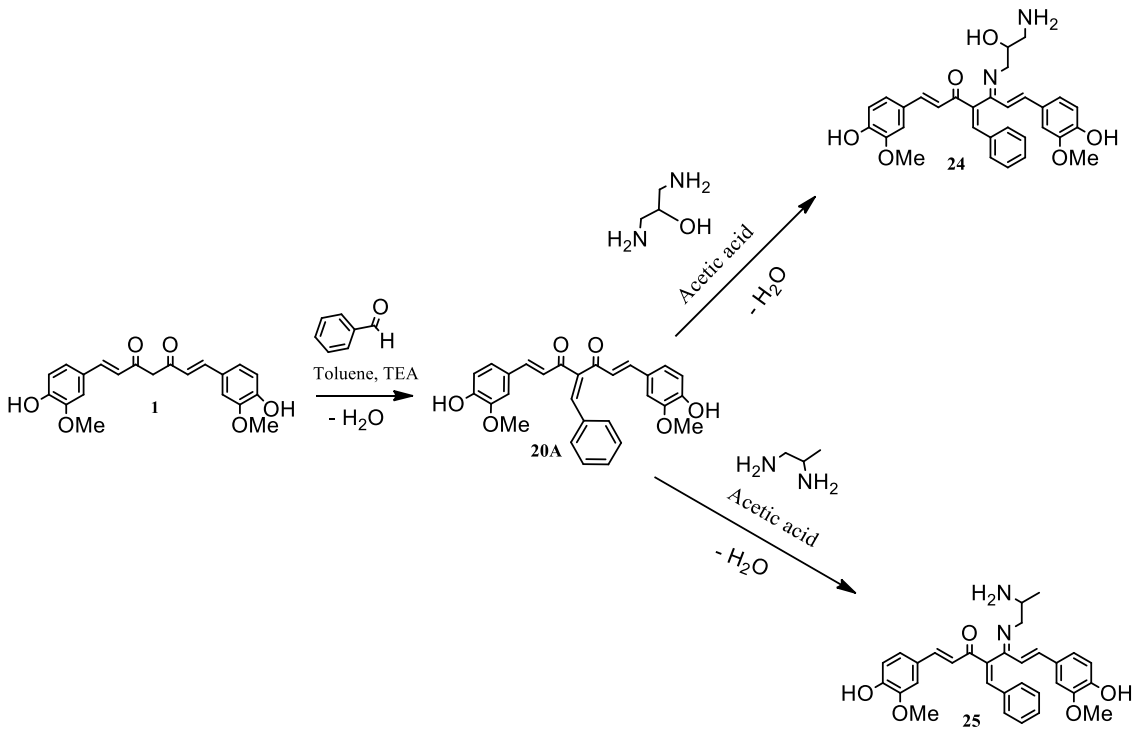


Scheme 8: Chemical structures and reaction conditions for making curcumin-based diazepines using Knoevenagel Condensation.

3.6.1.3 Preparation of Curcumin-Based Amines using Knoevenagel Condensation

Curcumin-based amines **24** and **25** were prepared using two-steps method; in the first step, the curcumin was reacted with benzaldehyde in toluene 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, Yield of 57.7 and 71.9 % for compounds **24** and **25** respectively.



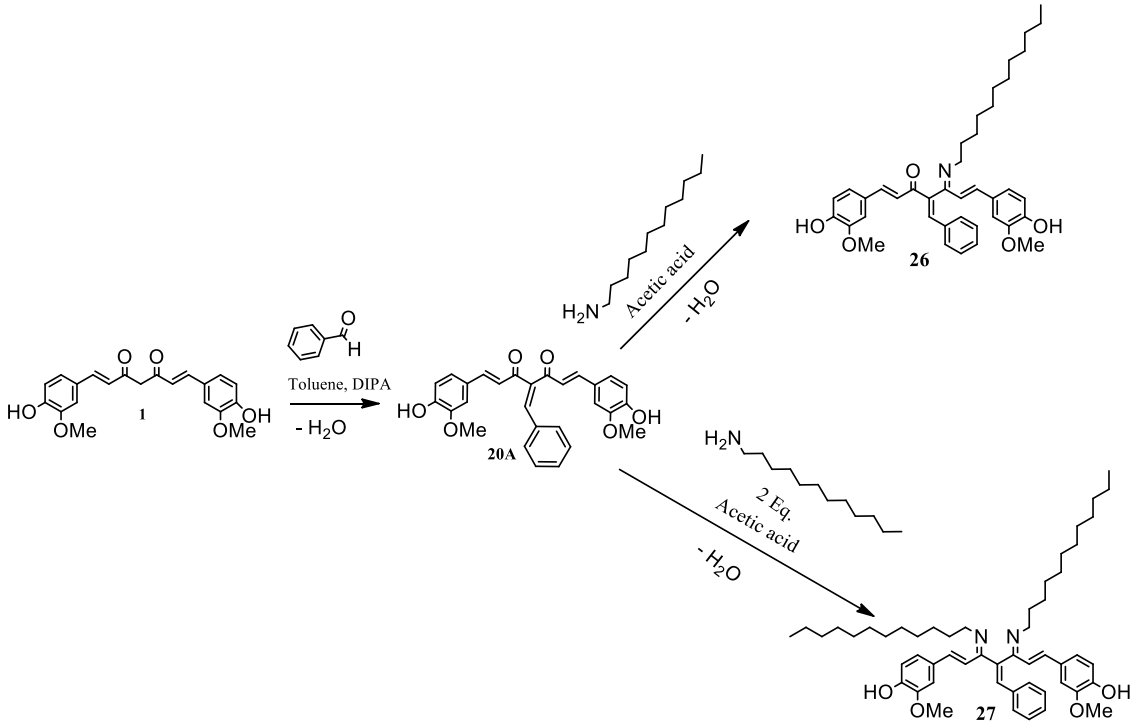
Scheme 9: Chemical structures and reaction conditions for making curcumin-based amines using Knoevenagel Condensation.

A two-fold mechanism was used to synthesize curcumin-based amines **26**, and **27**. The first step includes the formation of α,β-unsaturated

intermediate **20 A** using Knoevenagel condensation; by mixing curcumin with benzaldehyde in toluene solvent, in the presence of catalytic amount of diisopropylamine base. Meanwhile, acid-catalyzed condensation reactions of one of the curcumin carbonyl groups of the synthesized intermediate with amino compound in two ratios were involved in the second step to produce the desired products scheme 10.

An excess amount of acetic acid was added to the synthesized intermediate which was used for a couple of purposes; 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 progress of reactions was monitored using TLC. The synthesized products **26** and **27** were purified as previously described in the experimental part. The structures of the prepared compounds were characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yields were quantitatively measured for the prepared curcumin-based amines **26**, and **27**, and they are found to be of 83.3 % and 70.9 % respectively.



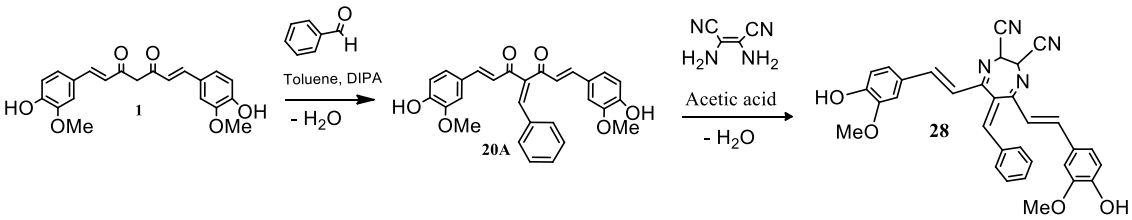
Scheme 10: Chemical structures and reaction conditions for making curcumin-based amines using Knoevenagel Condensation.

A two-fold mechanism was used to synthesize Curcumin-based diazepine **28**. The first step includes the formation of α,β-unsaturated intermediate **20 A** using Knoevenagel condensation; by mixing curcumin with benzaldehyde in toluene solvent, in the presence of catalytic amount of diisopropylamine base. Meanwhile, acid-catalyzed condensation cyclization reaction of the curcumin carbonyl groups of the synthesized intermediate with hydrazine compound was involved in the second step to produce the desired product scheme 11.

An excess amount of acetic acid was added to the synthesized intermediate which was used for a couple of purposes; 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 diaminomaleonitrile in (1:1 ratio) to produce diazepine **28** scheme 11.

The progress of reaction was monitored using TLC. The synthesized product **28** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared curcumin-based diazepine **28**, and it is found to be 91.3 %.



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

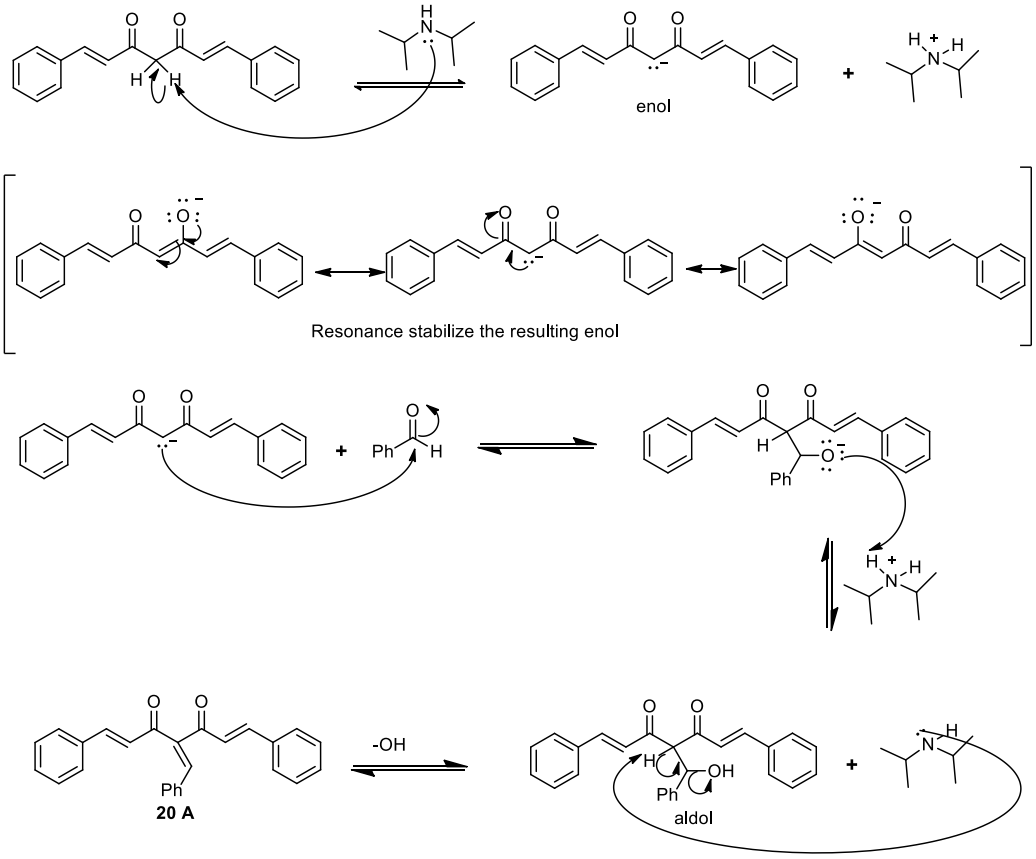
3.6.2 Stepwise mechanism for the preparation of Curcumin-Based compounds using Knoevenagel Condensation

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** is shown in scheme 12. Mainly Knoevenagel condensation mechanism is an aldol like reaction between 1,3-dicarbonyl (nucleophile) and an aldehyde (which acts as electrophile) in the presence of an amine which acts as a base. And this reaction proceeds with loss of water. The Knoevenagel condensation was

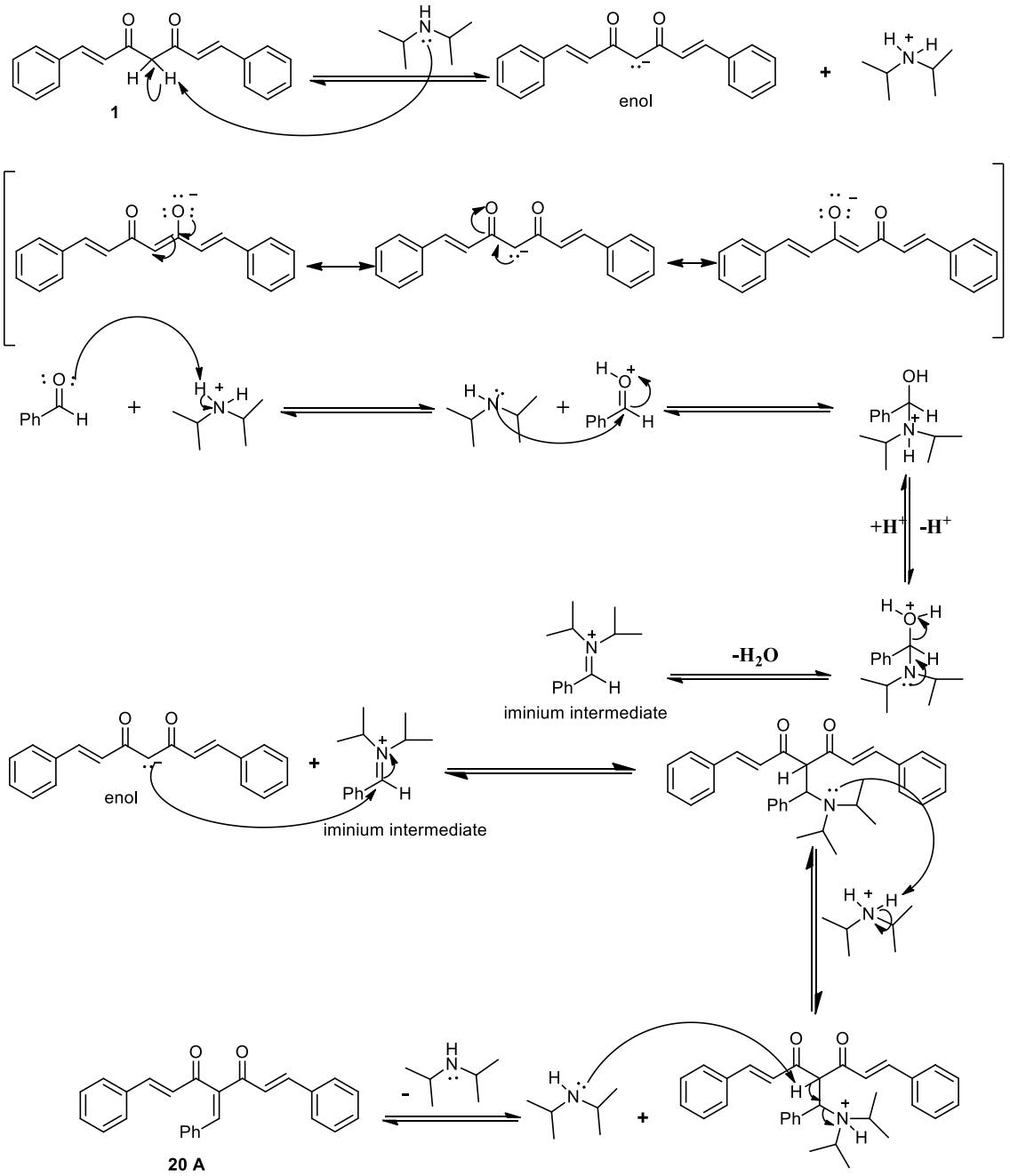
employed in this study to prevent keto-enol tautomerization, which expected to enhance the yield.

In the first step, an enol intermediate is formed, where the diisopropylamine acts as a base abstracting the curcumin α-hydrogen. 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.

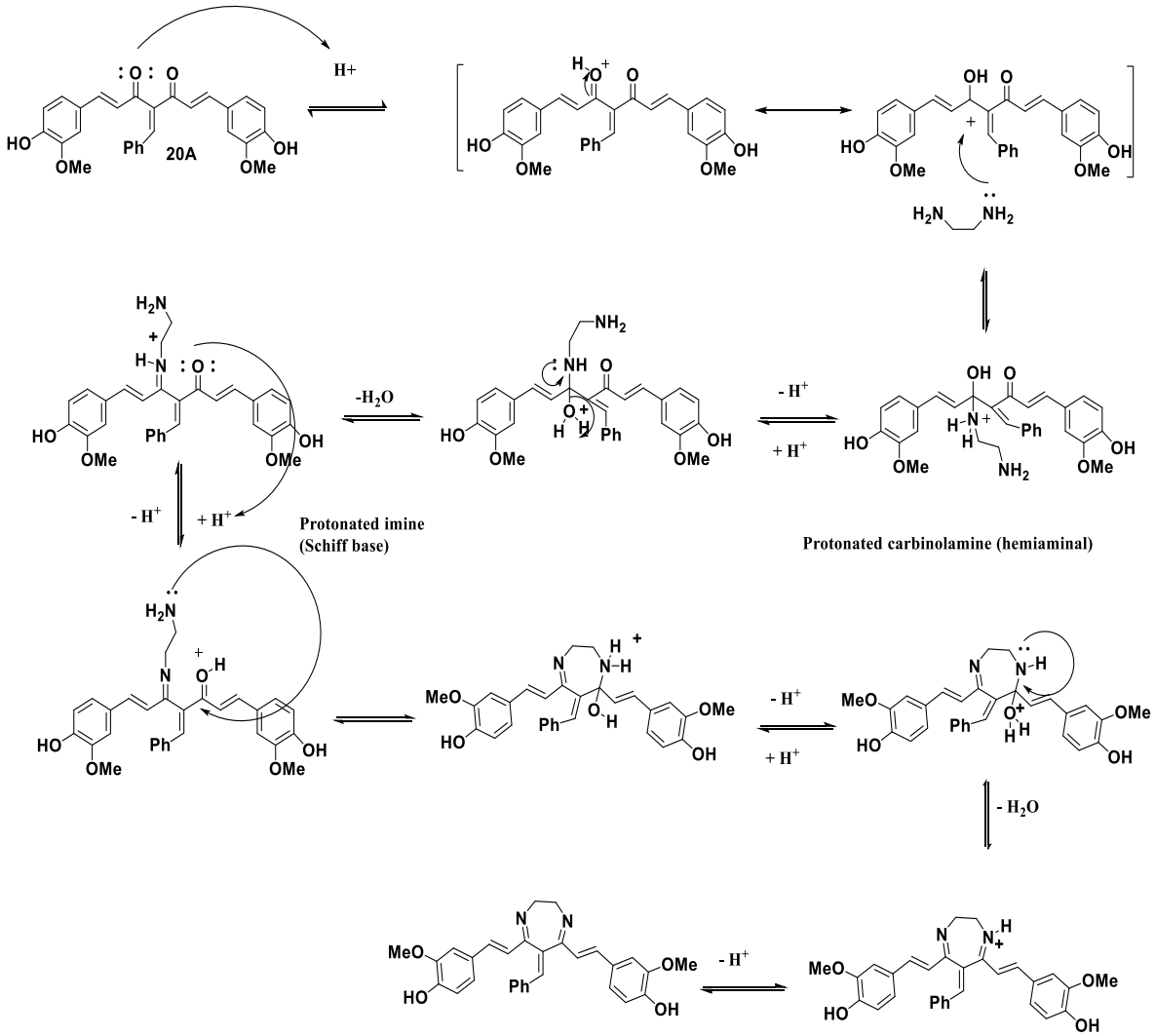


Scheme 13: A stepwise mechanism of the Knoevenagel condensation of curcumin with benzaldehyde where the diisopropylamine acts as organocatalyst to form α,β-unsaturated curcumin-based compound 20A.

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

described in scheme 14. The mechanism of condensation reaction begins with an acid-catalyzed nucleophilic addition of the diamino and hydrazine compounds to one of the curcumin carbonyl groups. Since 1,2-diamino and hydrazine compounds are weak nucleophiles activation of carbonyl group is needed to make it susceptible for the attack, followed by a deprotonation of the nitrogen atom, gives an unstable intermediate called a carbinolamine (hemiaminal).

The second half of the mechanism converts the carbinolamine (hemiaminal) to the more stable imine. Protonation of the hydroxy group converts it to a good leaving group, followed by loss of water, gives a resonance-stabilized carbocation where the positive charge on nitrogen atom with all octets filled, followed by loss of a proton, gives the imine intermediate. After that, imine intermediate is attacked by the second amino group followed by a loss of a second water molecule forming the desired product.

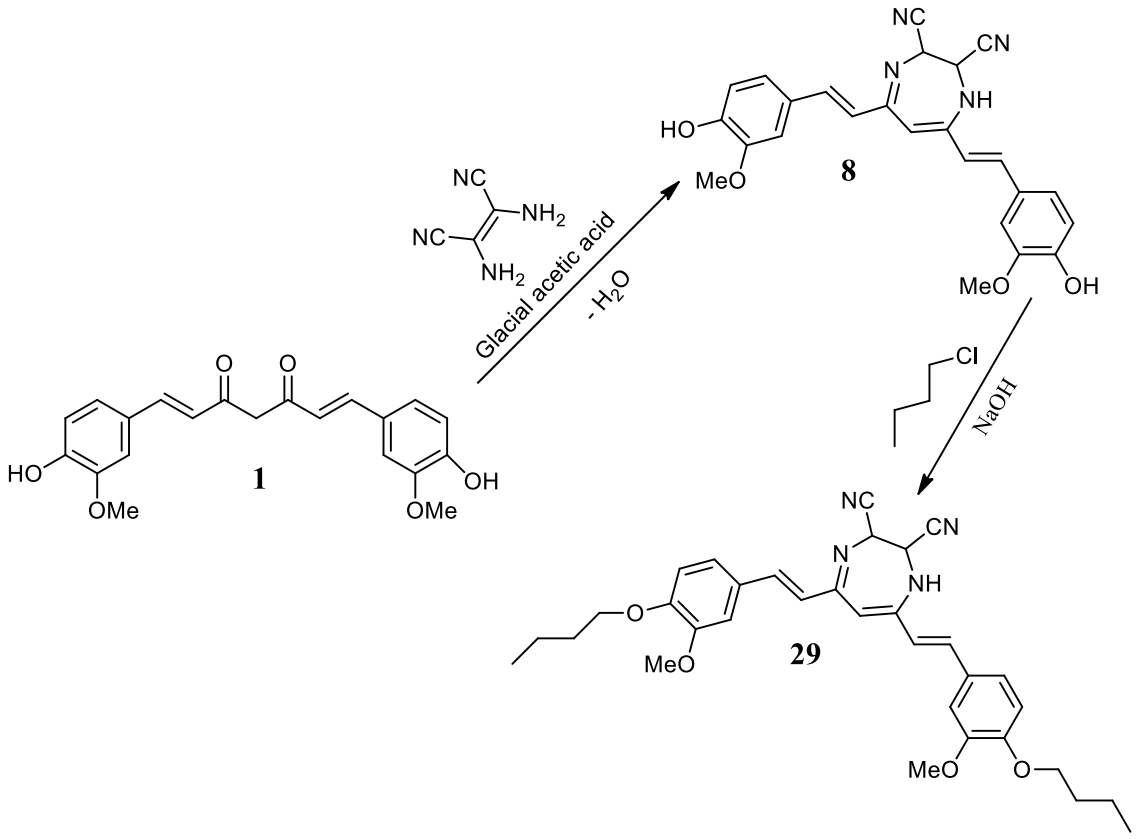


Scheme 14: A detailed mechanism of the condensation reaction of α,β-unsaturated curcumin-based intermediate compound 20A with diverse 1,2 diamino and hydrazine compounds to form α,β-unsaturated curcumin-based benzodiazepines, diazepines, and amines.

3.7 Alkylation of curcumin-based compounds

A two-fold mechanism was used to synthesize diazepine **29**, as shown in scheme 15. The first step involved the formation of compound **8** by condensation cyclization reaction between curcumin and diaminomaleonitrile as described earlier. In the second step, the phenolic groups of compound **8** were alkylated by reacting with 1-chlorobutane in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **29** was purified as previously

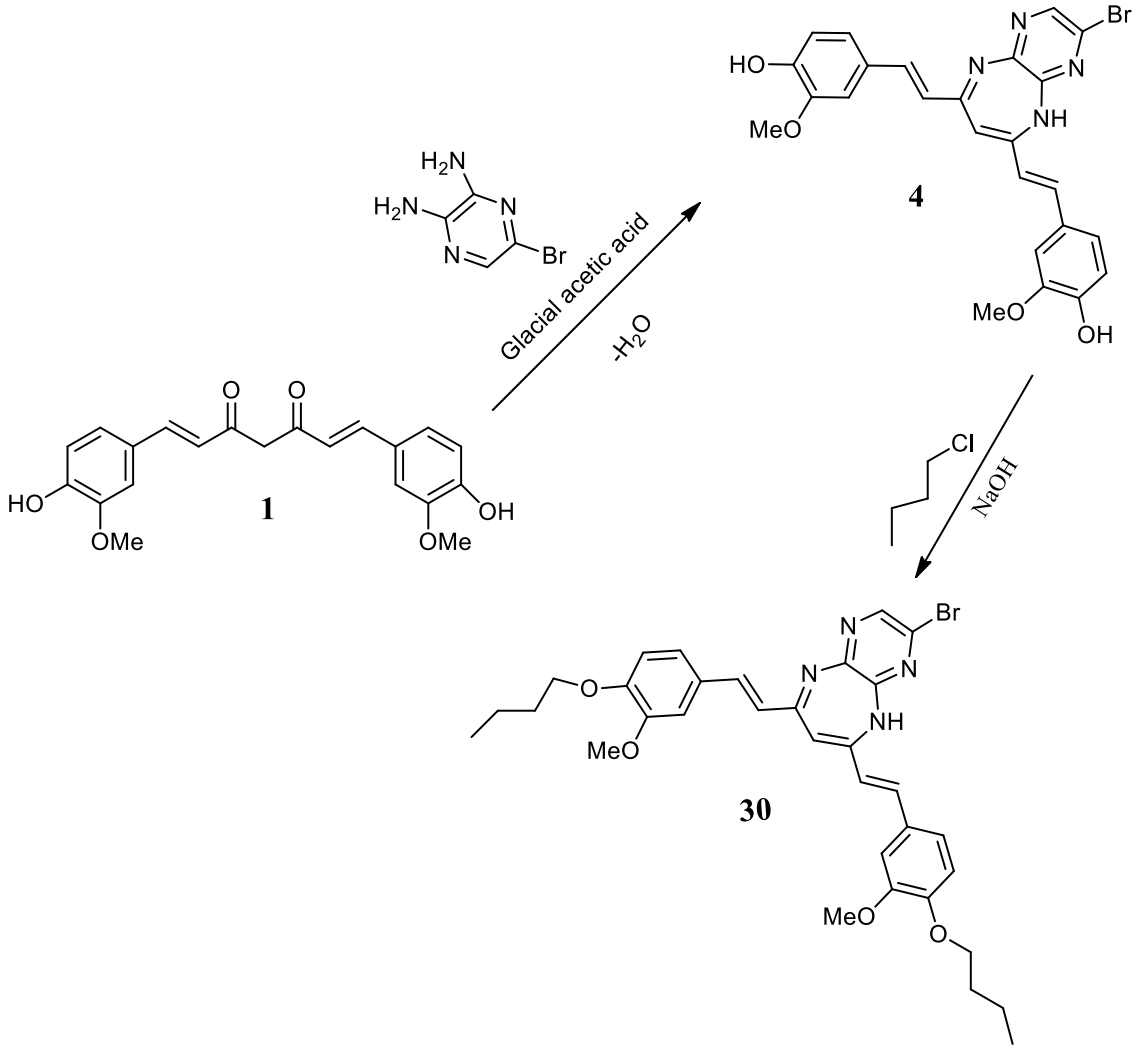
described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared diazepine **29**, and it is found to be 76.2 %. Compound **29** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

A two-fold mechanism was also used to synthesize benzodiazepine **30**, 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 earlier. In the second step, the phenolic groups of compound **4** were alkylated by reacting with 1-chlorobutane in the

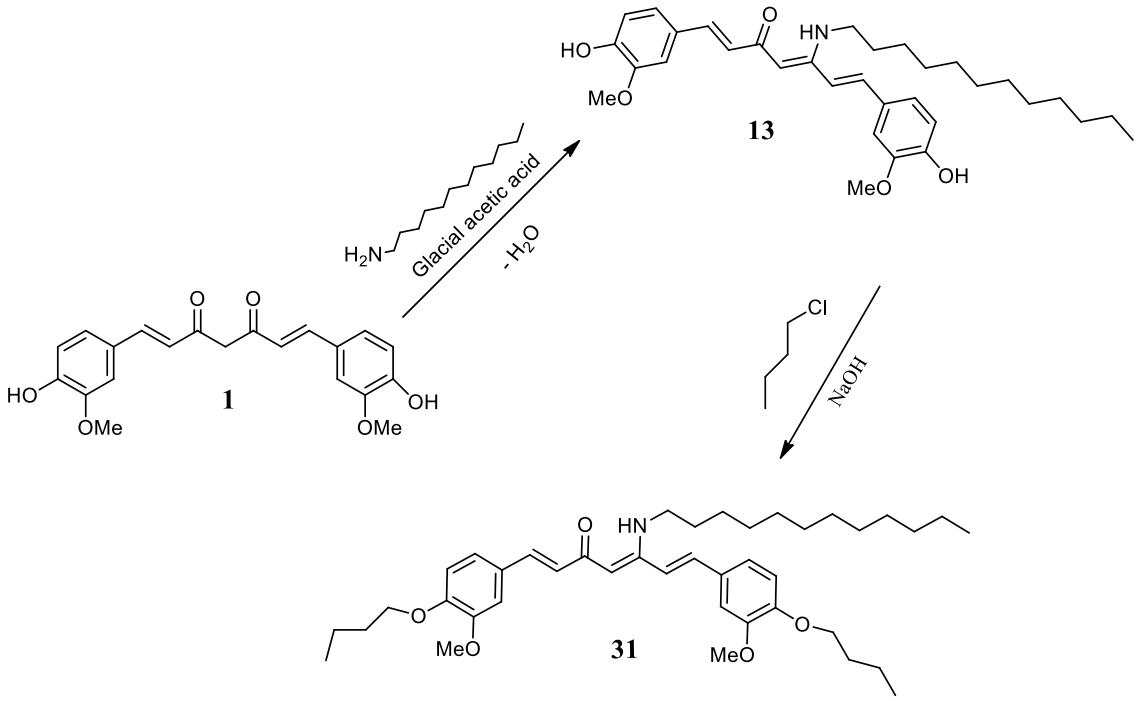
presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **30** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared benzodiazepine **30**, and it is found to be 60.1 %. Compound **30** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

A two-fold mechanism was used to synthesize Amine **31**, as shown in scheme 17. The first step involved the formation of compound **13** by condensation cyclization reaction between curcumin and dodecylamine as described earlier. In the second step, the phenolic groups of compound **13** were alkylated by reacting with 1-chlorobutane in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **31** was purified as previously described in the experimental part. The structure of the prepared compound was

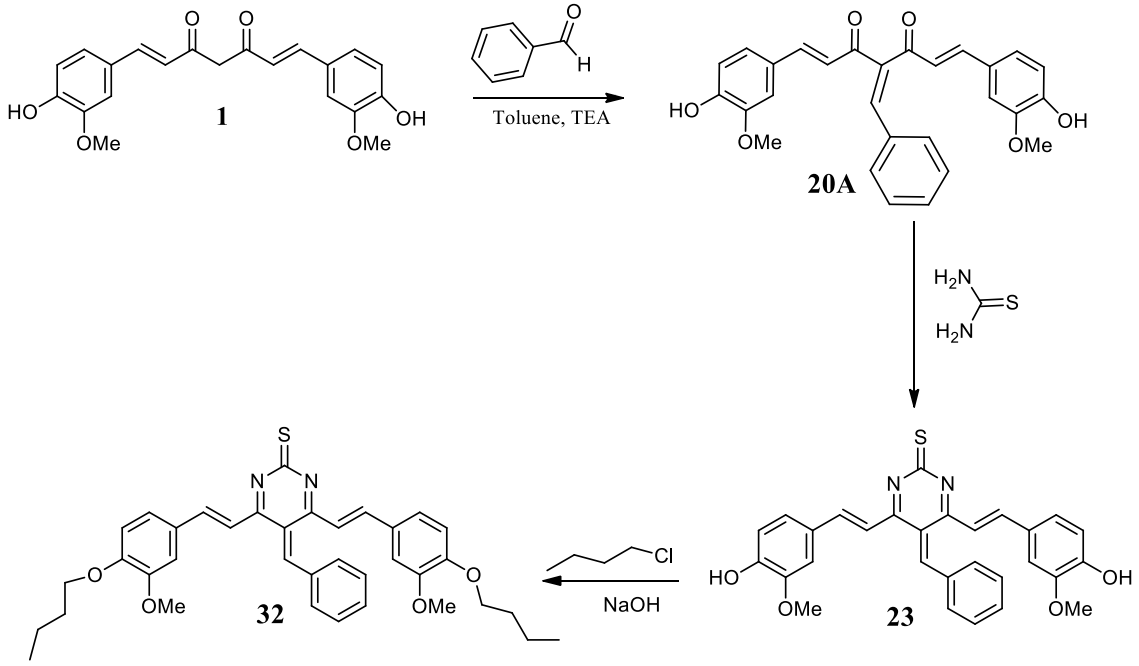
characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared amine **31**, and it is found to be 85.3 %. Compound **31** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

A three-fold mechanism was used to synthesize compound **32** as shown in scheme **18.** Initially compound **23** was prepared using the two-fold mechanism 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 earlier. In the third step, the phenolic groups of compound **23** were alkylated by reacting it with 1-chlorobutane

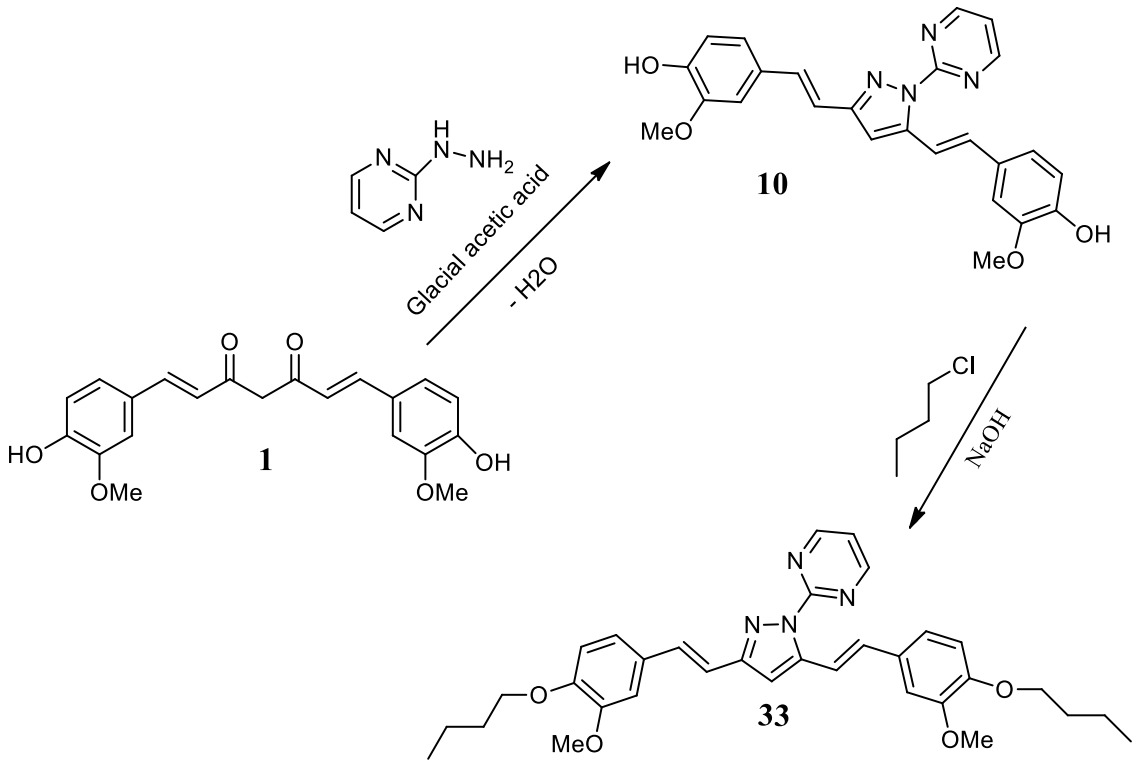
in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **32** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared compound **32**, and it is found to be 91 %. Compound **32** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

A two-fold mechanism was used to synthesize diazole **33** 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 earlier. In the second step, the phenolic groups of compound **10** were alkylated by reacting with

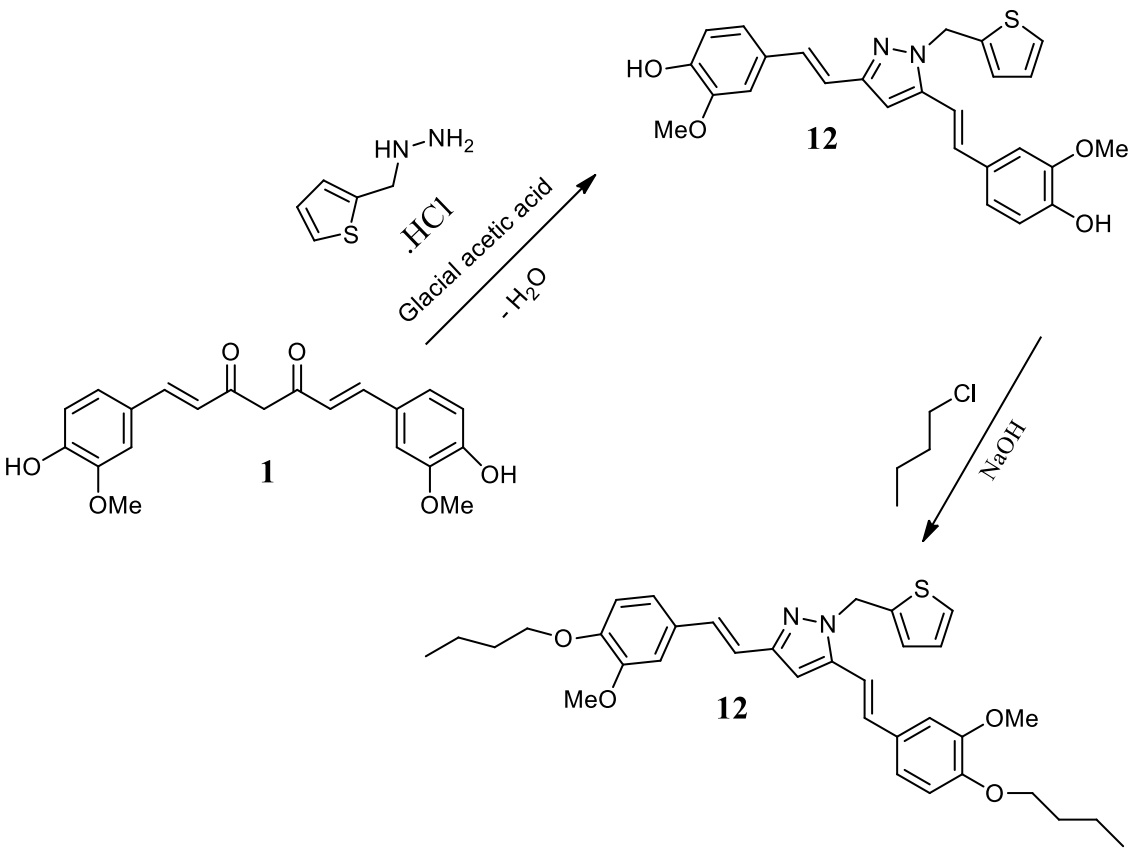
chloromethane in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **33** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared diazole **33**, and it is found to be 52.6%. Compound **33** was prepared to investigate the effect of the phenolic hydroxyl group on the curcumin bioactivity.



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

A two-fold mechanism was used to synthesize compound **34** as shown in scheme 20. The first step involved the formation of compound **12** by

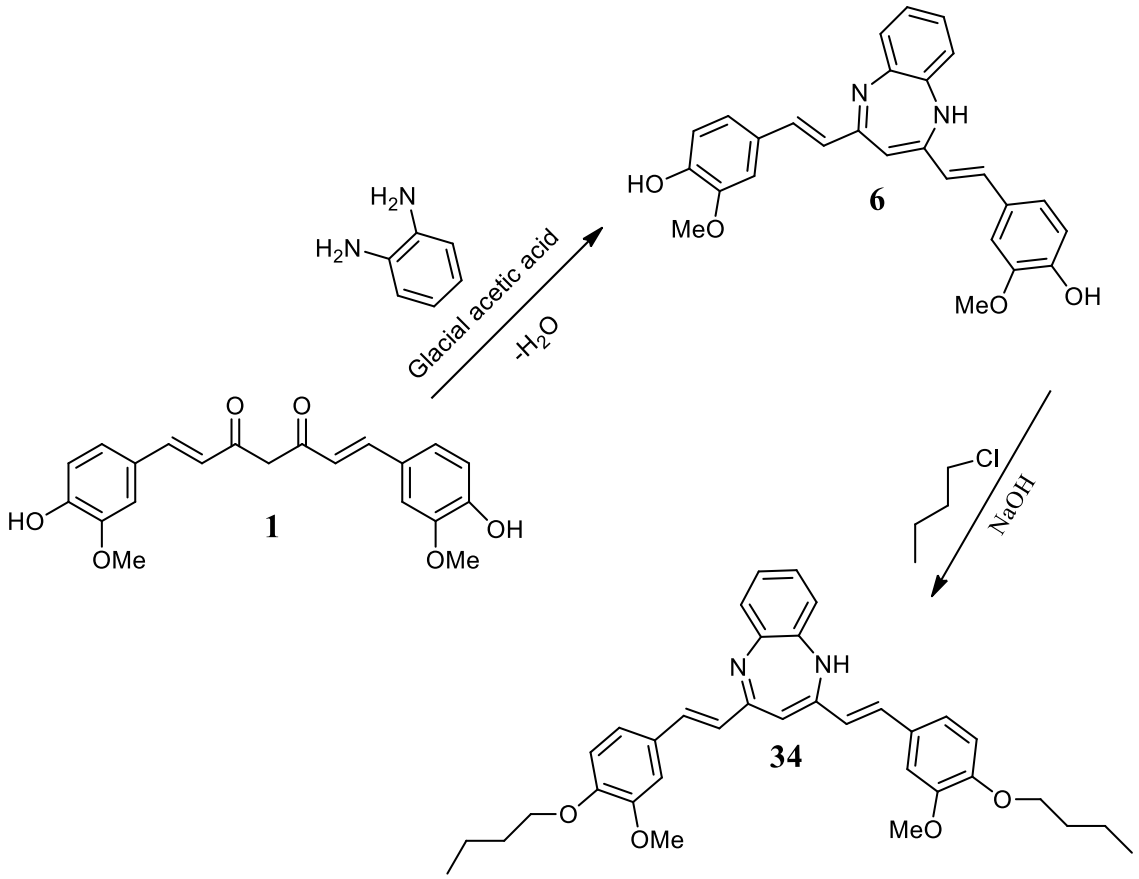
condensation cyclization reaction between curcumin and (2-thinylmethyl) hydrazine hydrochloride as described earlier. In the second step, the phenolic groups of compound **12** were alkylated by reacting with 1-chlorobutane in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **34** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared diazole **34**, and it is found to be 69.5 %. Compound **34** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

A two-fold mechanism was used to synthesize benzodiazepine **35** 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 earlier. in the second step the phenolic groups of compound **6** were alkylated by reacting with 1-chlorobutane in the presence of sodium hydroxide. The progress of reaction was monitored using TLC. The synthesized product **35** was purified as previously described in the experimental part. The structure of the prepared compound was characterized and determined using several analytical and spectroscopic techniques, including TLC, melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR. The yield was quantitatively measured for the prepared

benzodiazepine **35**, and it is found to be 45.3 %. Compound **35** was prepared to investigate the effect of the hydroxyl group on the curcumin bioactivity.



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

3.8 Anticancer activities of the prepared curcumin-based heterocyclic compounds

Curcumin and its derivatives have been known to have a wide variety of therapeutic effects, ranging from anti-inflammatory, chemo-preventive, anti-proliferative, and anti-metastatic. In this work a novel set curcumin-based heterocyclics were screened for their anti-tumor effect against HeLa

cells. The *in vitro* cytotoxic and cytostatic effect of the prepared heterocyclics were evaluated using MTT test.

3.8.1 Cytotoxic effect of the curcumin-based heterocyclics

Cells (2×10^4 cells/well) with 70-80% confluence were seeded on 96-well plate, the cells were then treated with different concentrations of the prepared curcumin-based heterocyclics (400, 200, 100, 50, 25, and 12.5 μg /ml) and incubated for 24 hours.

MTT assay was used to determine the cell viability by adding MTT solution to the plate and incubated for four hours, after that the isopropyl alcohol was added and incubated in dark for 15 minutes. The microplate reader (Labtech, UK) was used to measure the absorbance at 570 nm.

3.8.2 Cytotoxic effect of the curcumin-based heterocyclics on HeLa cells

The in vitro anticancer activity of a novel set of curcumin-based benzodiazepine, diazepines, diazoles, and H-curcumin based amine against HeLa cancer cell were evaluated. The results indicate that all the tested curcumin-based heterocyclics have varying cytotoxic effect on the HeLa cells at different concentrations. The viability of HeLa cells was reduced in the range of 4.48- 14.57% within the studied concentration range Table 3.1. In general, cell growth was decreased as the concentration of the tested curcumin-based heterocyclics increased figure 3.1.

Curcumin-based benzodiazepine and diazepines **2, 3, 4, 5** and **8** showed higher potency on HeLa cancer cells than the other tested heterocyclics **10** and **19**. The viability of HeLa cells that were treated with benzodiazepine and diazepines **2, 3, 4, 5** and **8** was reduced in the range of 4.48- 6.73%. Curcumin-based diazepine **8** showed the highest cytotoxic effect on the HeLa cells at all concentrations. it reduced the viability of the tested HeLa cells in range of 4.84 % for the 400 μg /ml concentration to 4.95% for the 12.5 μg /ml concentration. This is due to the presence of the hetero atom Nitrogen in addition to the halogen atom bromine gives this molecule the ability to accommodate well into the binding site and interact with the receptor site of the cancer cells and interact through H-bonding with functional groups present in the receptor site.

Among the tested curcumin-based benzodiazepine **3** and **5** are more effective against HeLa cancer cells than **2** and **4**. As they reduced the viability of the tested HeLa cells in range of 4.78 % and 4.63% for the 400 μg /ml concentration to 5.44% and 5.12% for the 12.5 μg /ml concentration for **3** and **5** respectively. In case of compounds **2** and **4** more substituents are introduced in the benzodiazepine ring, the steric factor effect becomes the predominant factor that tend to reduce the interaction with the receptor sites and thus the potency drops.

Speaking of the cytotoxic effect of curcumin-based diazole **10** on the HeLa cells. It showed lower effect than curcumin-based benzodiazepine and diazepines **2, 3, 4, 5** and **8**. Diazole **10** reduced the viability of the tested

HeLa cells in range of 4.71 % for the 400 µg /ml concentration to 6.02% for the 12.5 µg /ml concentration.

On the other hand, H-curcumin-based amine **19** showed the lowest cytotoxic effect on the HeLa cells at all concentrations among the prepared heterocyclics. It reduced the viability of the tested HeLa cells in range of 5.43 % for the 400 µg /ml concentration to 14.57 % for the 12.5 µg /ml concentration. This could be due to the loss of the olefinic part of curcumin due to hydrogenation, this functionality is important functionality in anticancer activity.

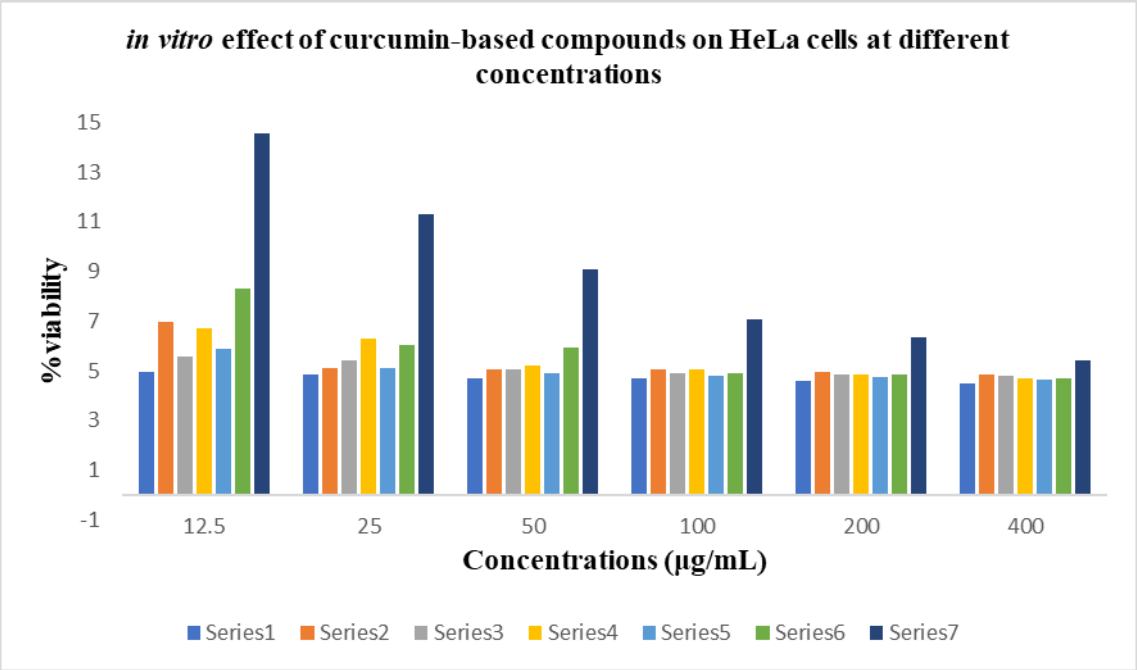


Fig. 3.1: *In vitro* effects of curcumin-based heterocyclics on HeLa cells at different concentrations (µg/ml).

Series 1: compound **8**, series 2: compound **2**, series 3: compound **3**, series 4: compound **4**, series 5: compound **5**, series 6: compound **10**, series 7: compound **19**.

Table 3.1: *In vitro* effects of curcumin-based heterocyclics on the viability of HeLa cells at different concentrations (µg/ml).

Curcumin Derivatives	Concentration (µg/ml)					
	400	200	100	50	25	12.5
2	4.84%	4.95%	5.07%	5.076%	5.096%	6.96%
3	4.78%	4.84%	4.88%	5.07%	5.44%	5.59%
4	4.72%	4.84%	5.07%	5.19%	6.32%	6.73%
5	4.63%	4.75%	4.82%	4.88%	5.12%	5.9%
8	4.48%	4.6%	4.7%	4.72%	4.84%	4.95%
10	4.71%	4.84%	4.9%	5.95%	6.02%	8.31%
19	5.43%	6.37%	7.08%	9.07%	11.28%	14.57%

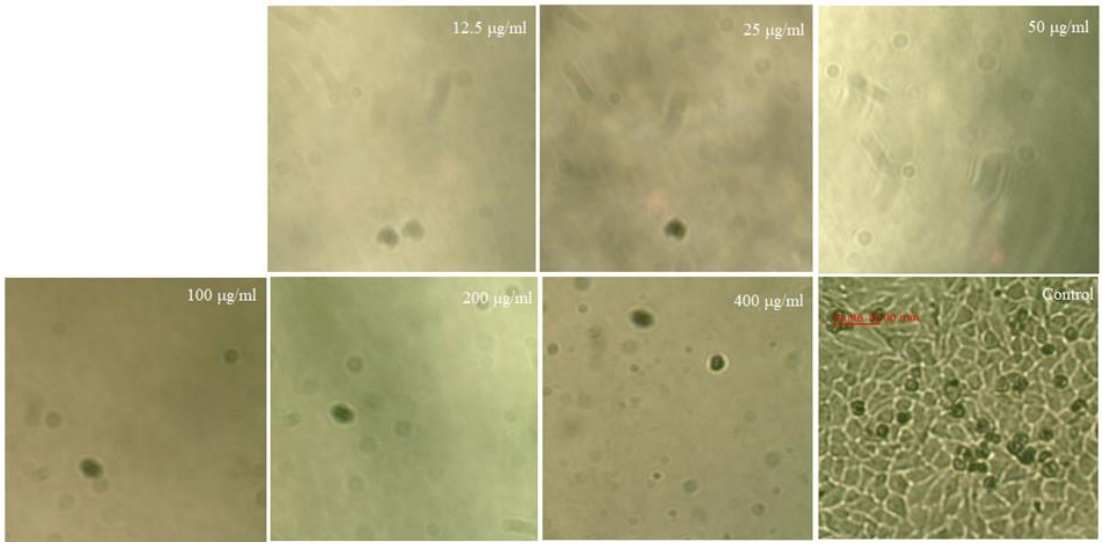


Fig. 3.2: *In vitro* effect of Compound 2 on HeLa cells at different concentrations (µg/ml).

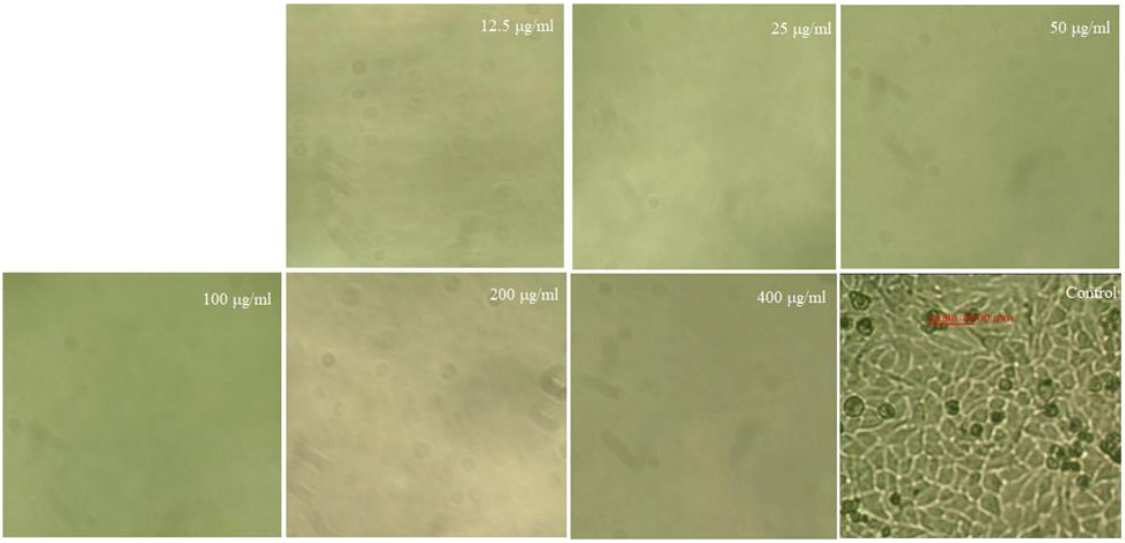


Fig. 3.3: *In vitro* effect of compound 3 on HeLa cells at different concentrations (µg/ml).

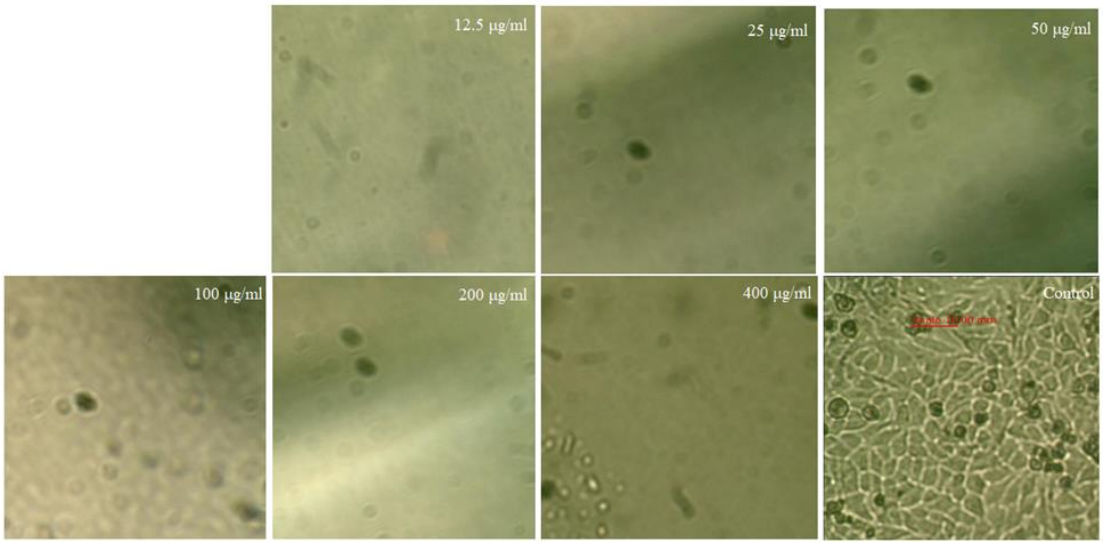


Fig. 3.4: *In vitro* effect of compound 4 on HeLa cells at different concentrations (µg/ml).

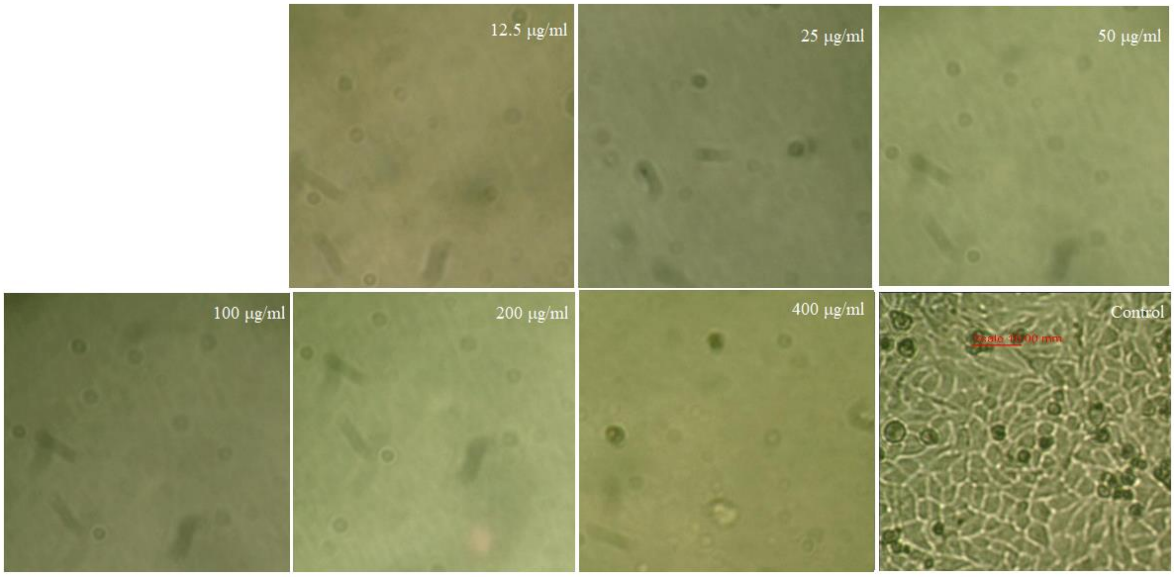


Fig. 3.5: *In vitro* effect of compound 5 on HeLa cells at different concentrations (µg/ml).

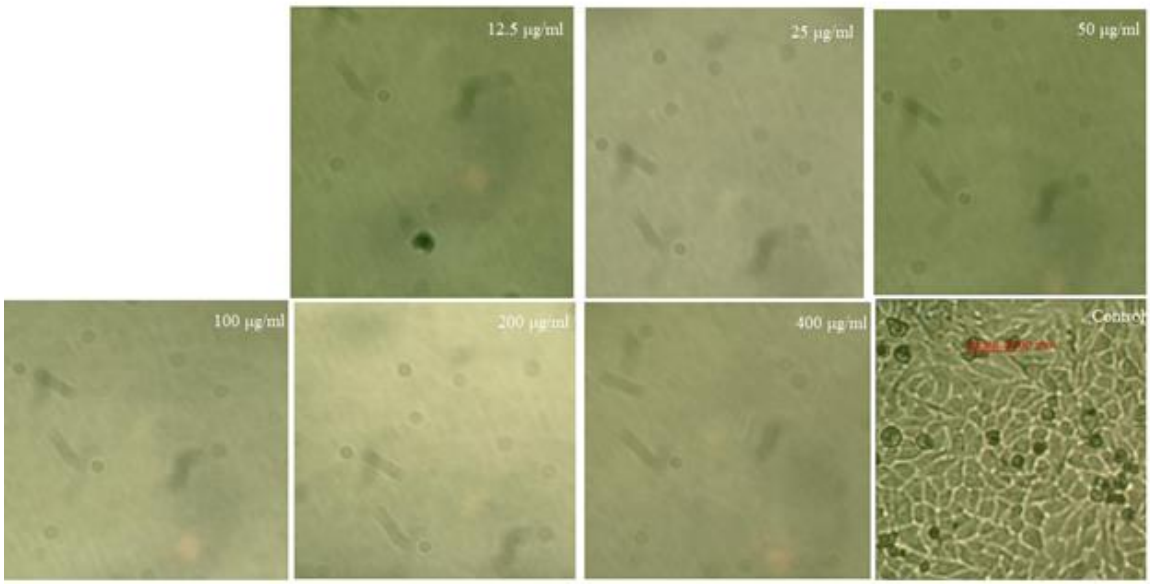


Figure 3.6: *In vitro* effect of compound 8 on HeLa cells at different concentrations (µg/ml).

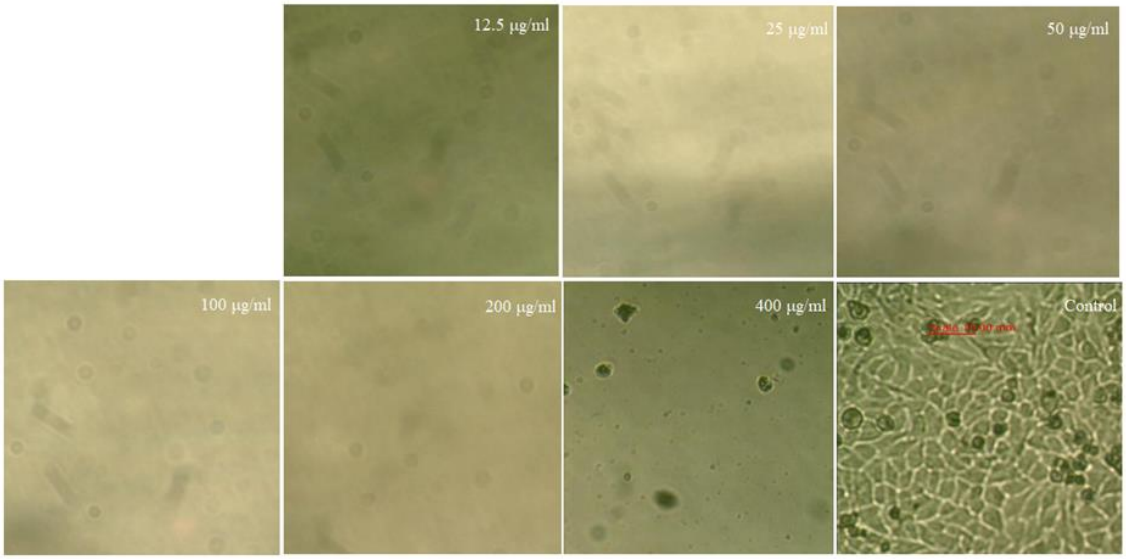


Fig. 3.7: *In vitro* effect of compound 10 on HeLa cells at different concentrations (µg/ml).

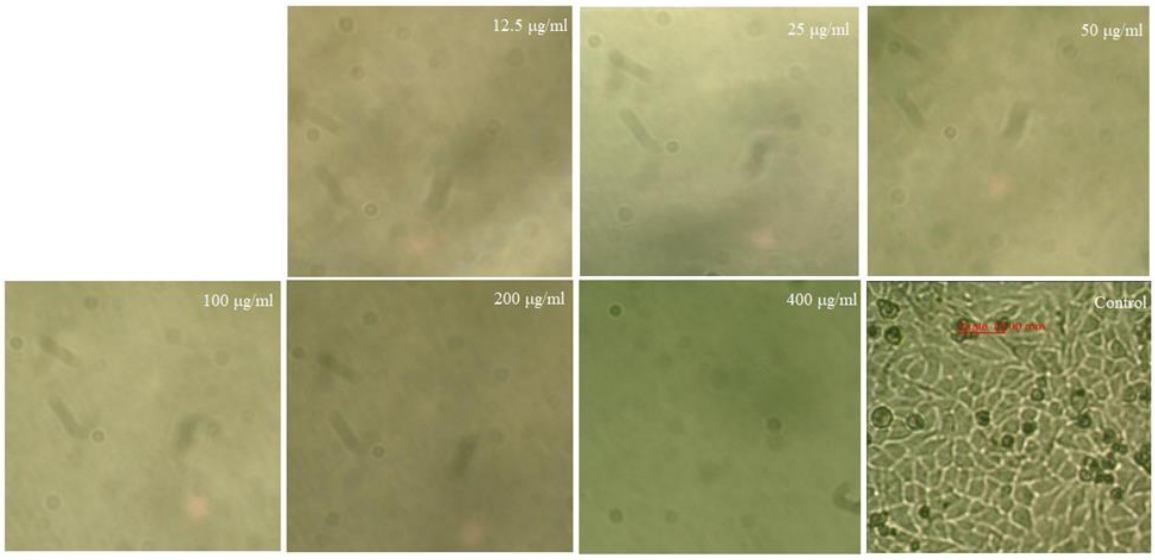


Fig. 3.8: *In vitro* effect of compound 19 on HeLa cells at different concentrations (µg/ml).

CONCLUSIONS

- Convenient and unsophisticated methods were used to synthesis a novel set of curcumin-based heterocycles.
- The anticancer activities of a randomly selected set of the synthesized heterocycles against HeLa cancer cells were evaluated.
- All the tested curcumin-based heterocyclics have varying cytotoxic effect on the HeLa cells at different concentrations.
- The viability of HeLa cells was reduced in the range of 4.48- 14.57% within the studied concentration.
- Cell growth was decreased as the concentration of the prepared curcumin-based heterocyclics increased.
- Curcumin-based benzodiazepine and diazepines showed higher potency on HeLa cancer cells than the other tested heterocyclics.
- The viability of HeLa cells that were treated with benzodiazepine and diazepines was reduced in the range of 4.48- 6.73%.
- Curcumin-based diazepines compound **8** showed the highest cytotoxic effect on the HeLa cells at all concentrations. it reduced the viability of the tested HeLa cells in range of 4.84 % for the 400 μg /ml concentration to 4.95% for the 12.5 μg /ml concentration.
- H-curcumin-based amine compound **19** showed the lowest cytotoxic effect on the HeLa cells at all concentrations among the tested heterocyclics. It reduced the viability of the tested HeLa cells in range of 5.43 % for the 400 μg /ml concentration to 14.57% for the 12.5 μg /ml concentration.

- Strictly speaking all the prepared curcumin-based compounds exhibited promising anticancer activity against HeLa cancer cells, which indicates that these compounds have anticancer effect at nontoxic concentrations.

SUGGESTION FOR FURTHER WORK

The following recommendations are suggested for the further works:

1. Test the anticancer activity of the rest of the synthesized curcumin-based heterocycles against HeLa cancer cells.
2. Test the anticancer activity of lower concentration than 12.5 μg /ml of the tested curcumin-based heterocycles against HeLa cancer cells.
3. Screen the anticancer activity of the synthesized curcumin-based heterocycles against other different cancer cell lines.
4. Test the antibacterial, and antiviral activity of the synthesized 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.

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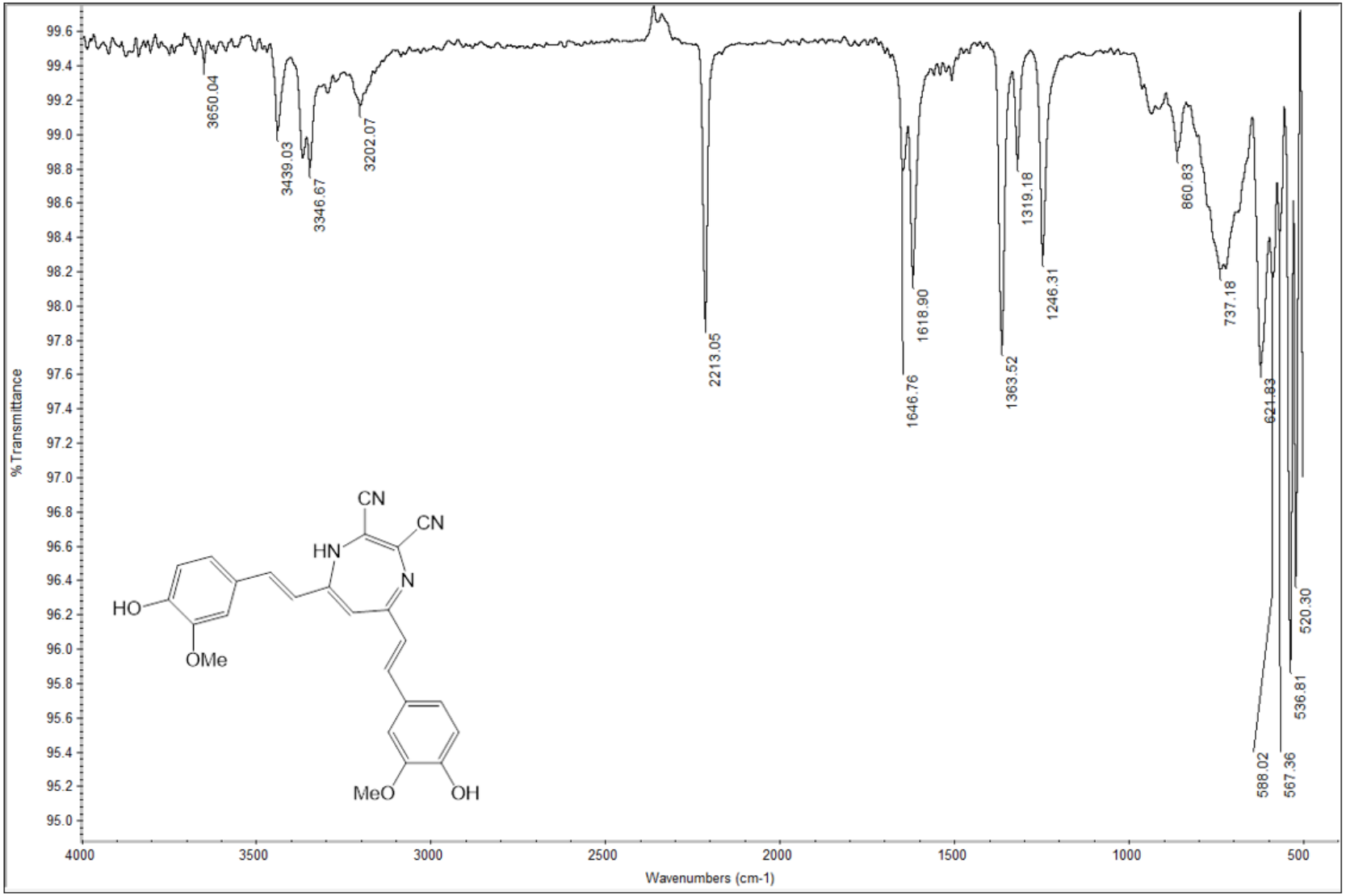
APPANDIX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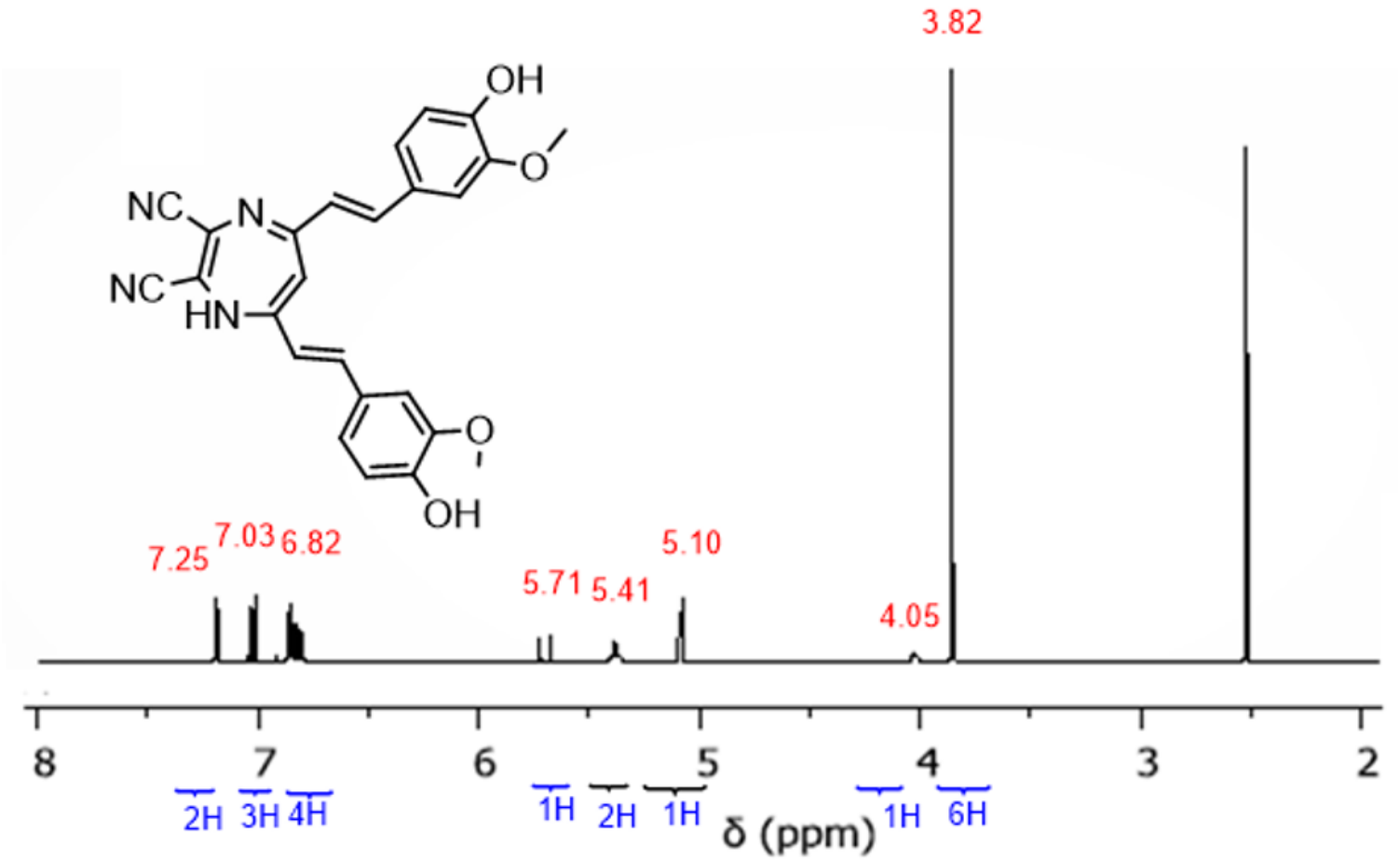
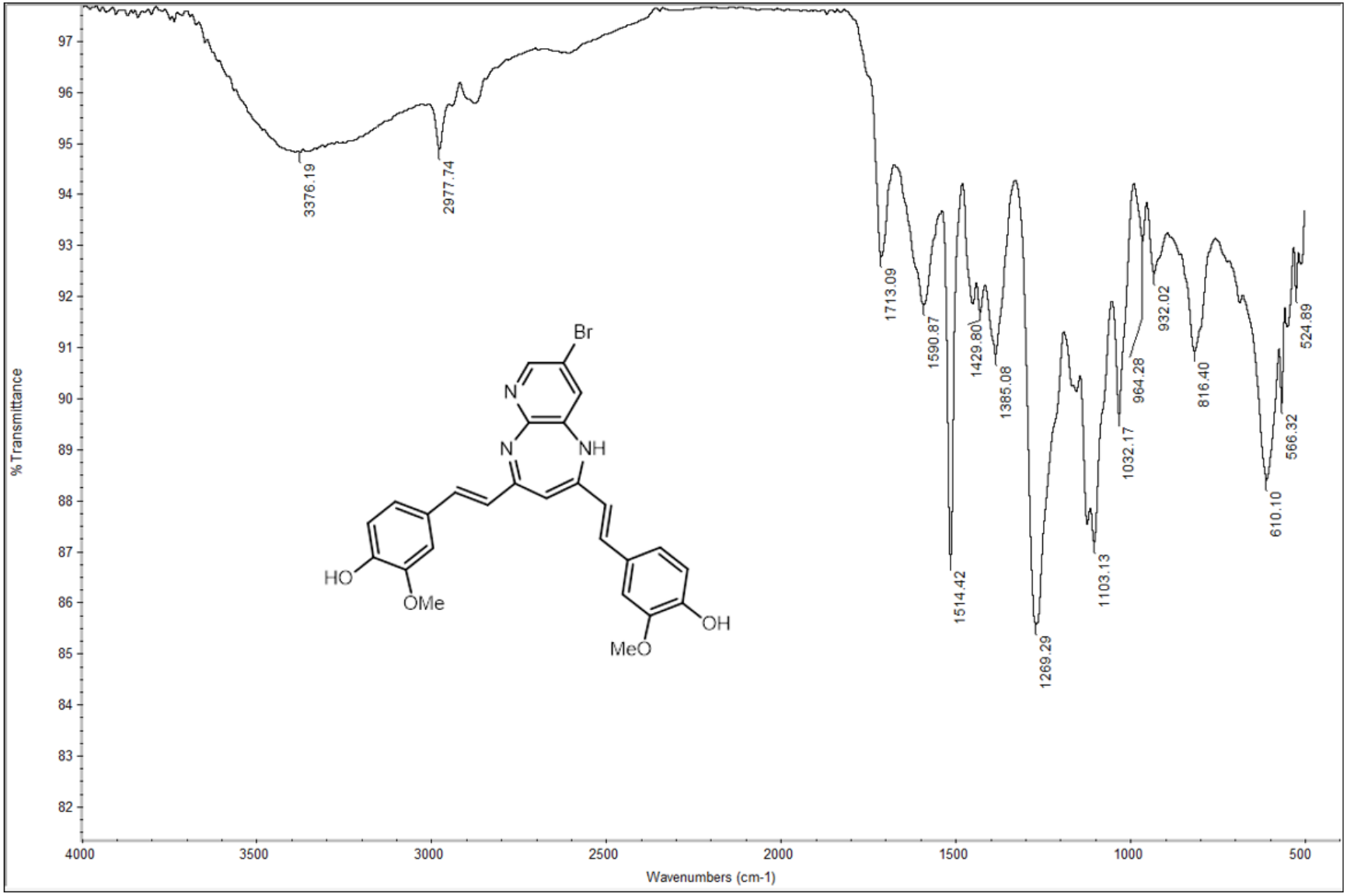
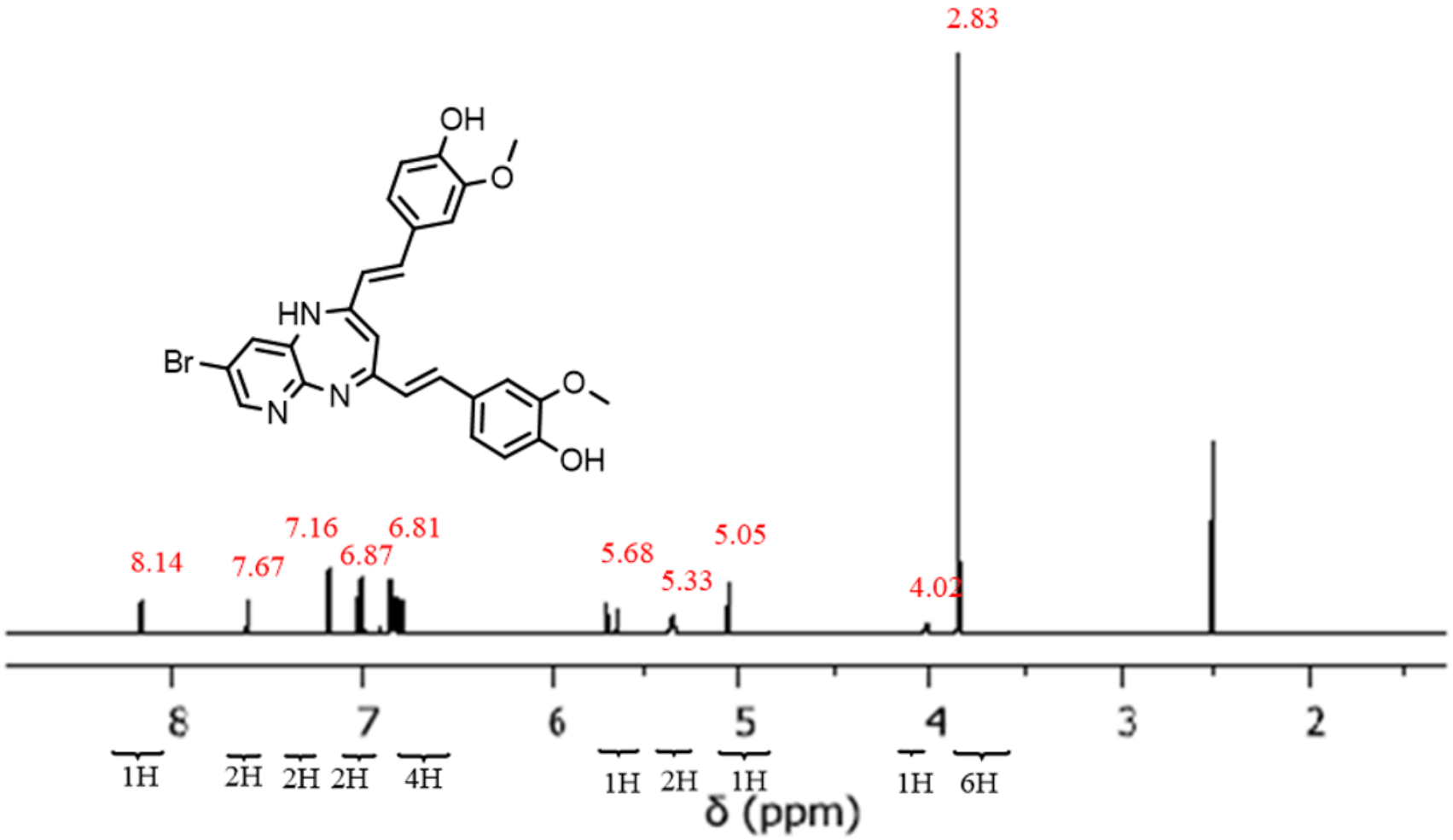
MeO
OH

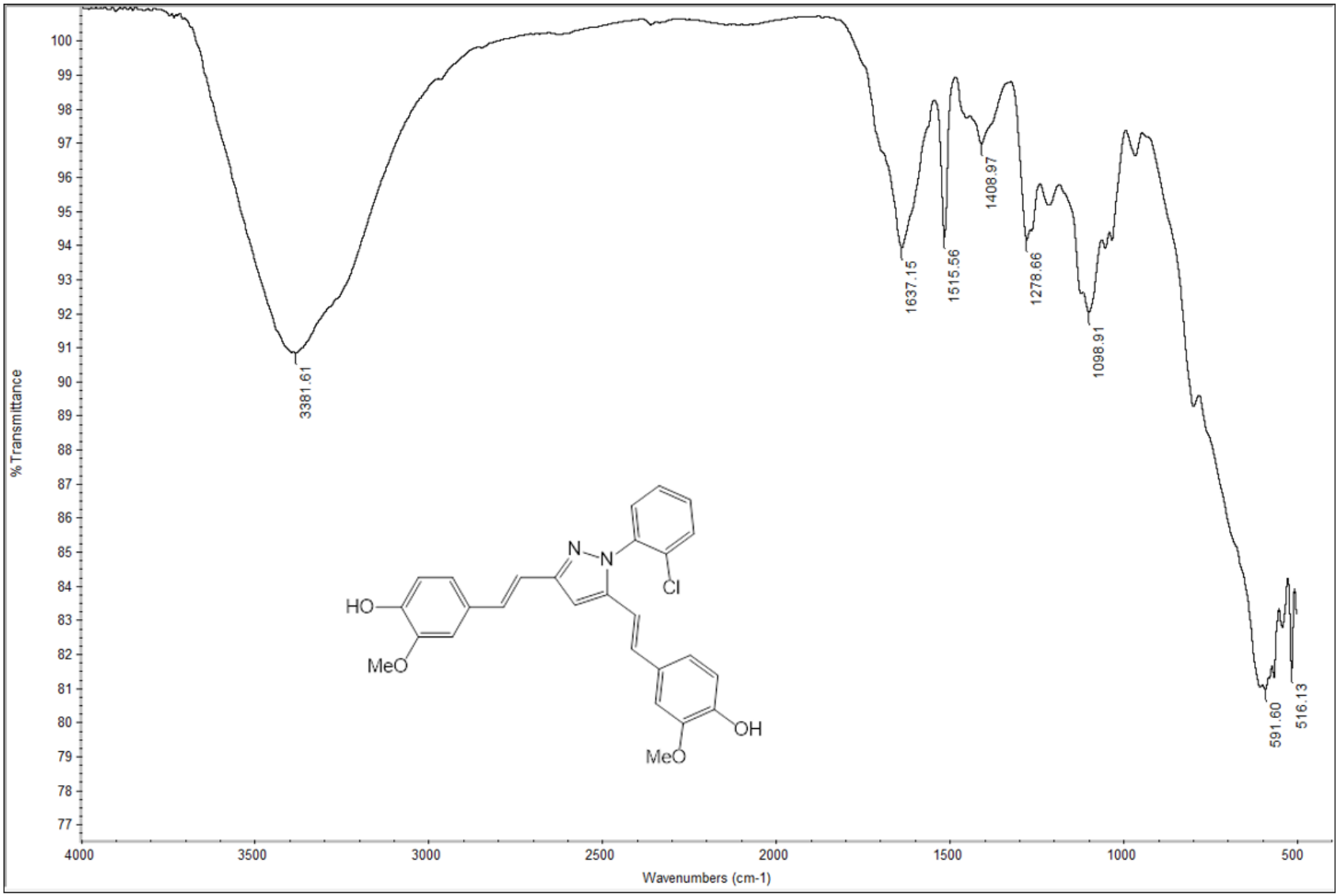


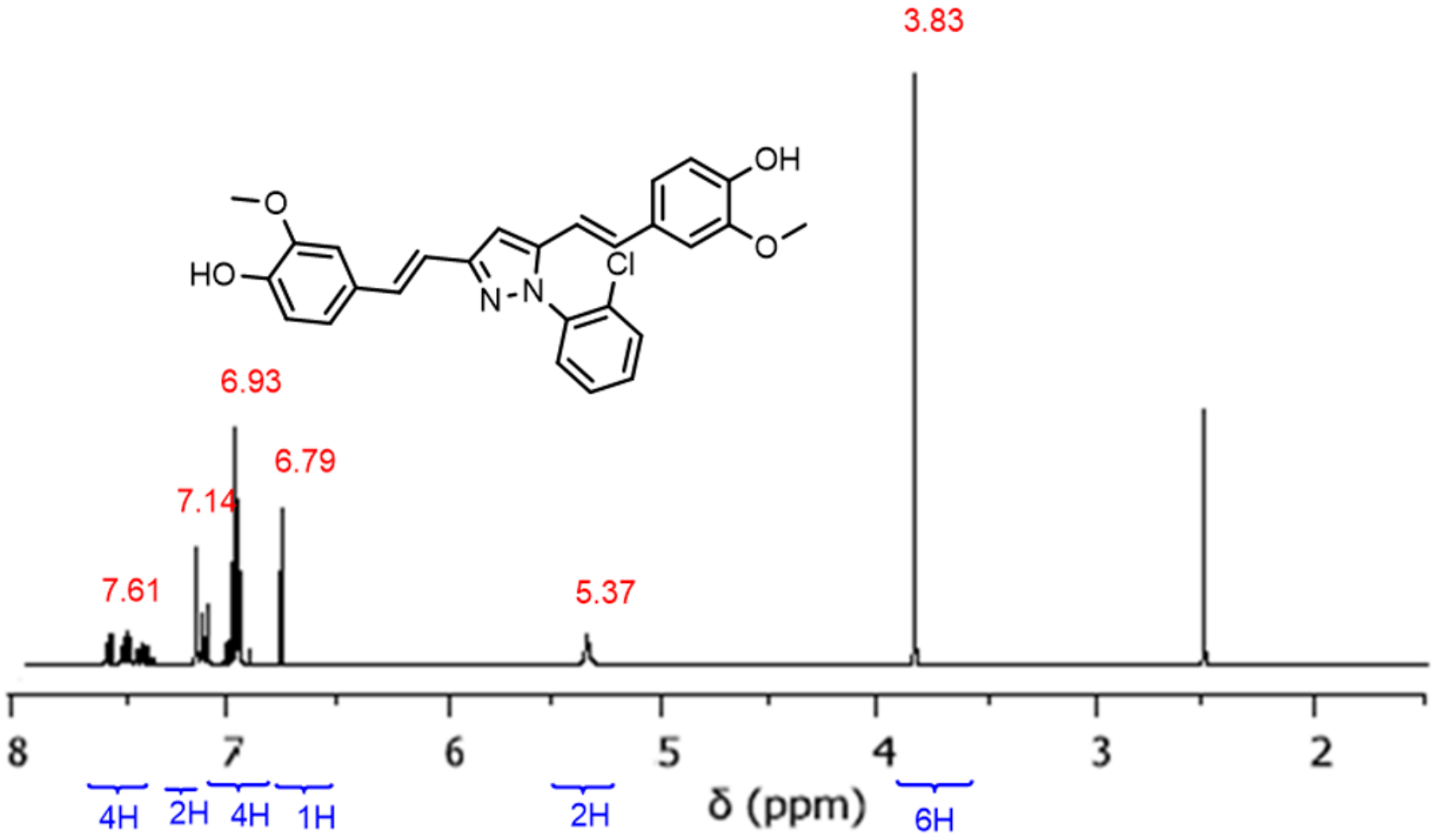
^{1}HNMR spectrum


% Transmittance
Wavenumbers (cm-1)
3376.19
2977.74
1713.09
1590.87
1514.42
1429.80
1385.08
1269.29
1103.13
1032.17
964.28
932.02
816.40
610.10
566.32
524.89
Br
N
N
NH
HO
OMe
MeO
OH

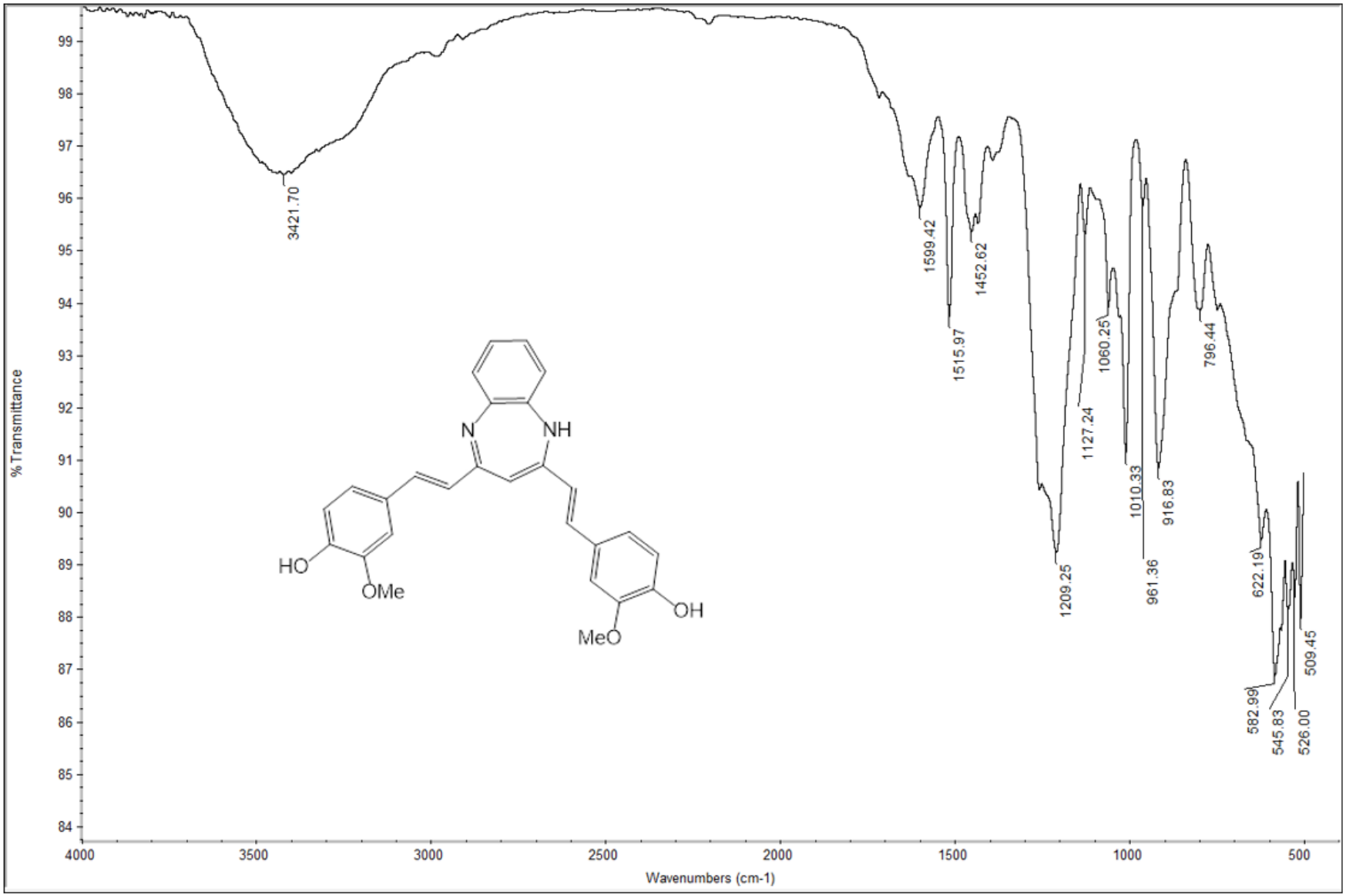


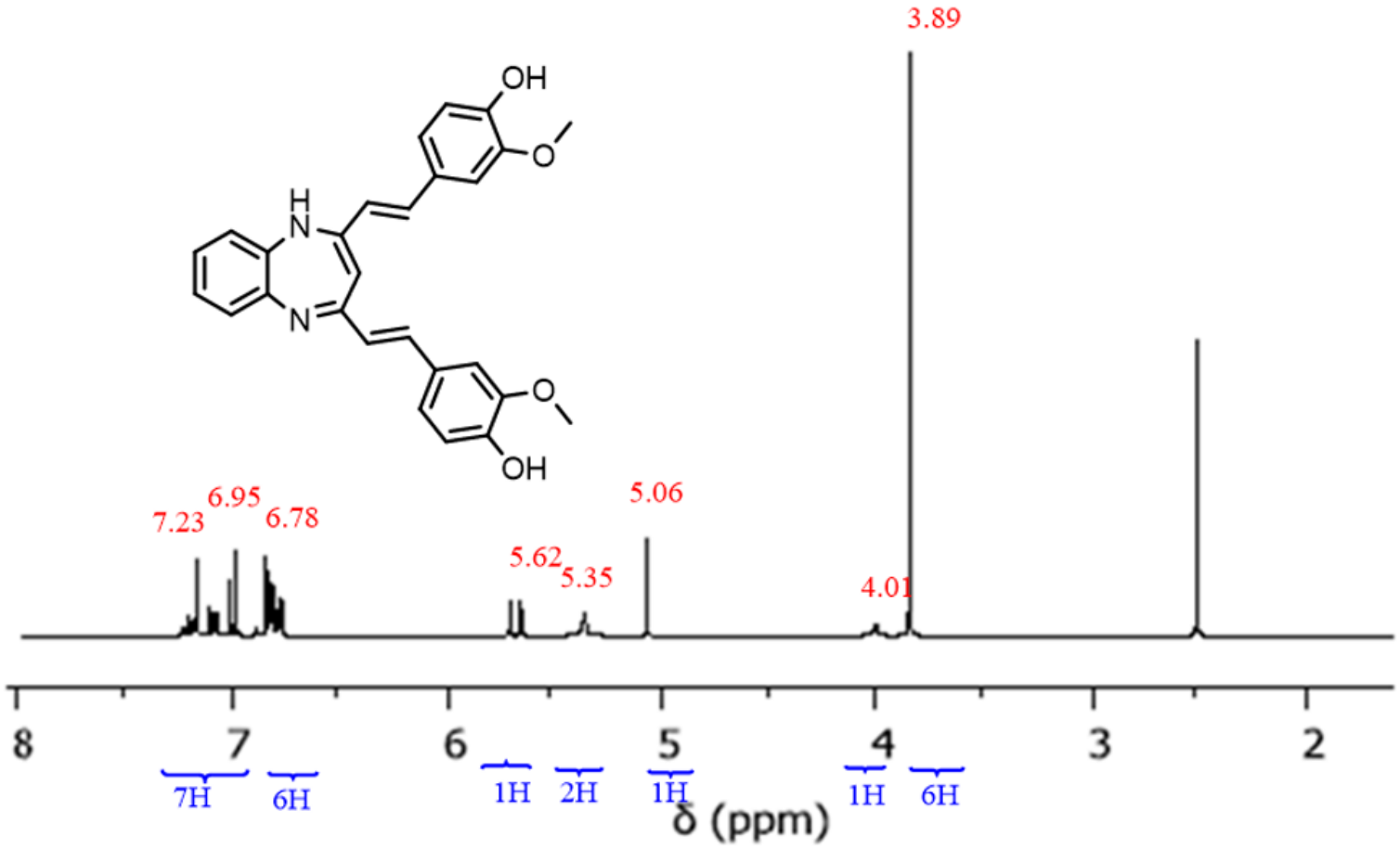
^{1}HNMR spectrum


% Transmittance
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
3381.61
1637.15
1515.56
1408.97
1278.66
1098.91
591.60
516.13
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
HO
MeO
N
N
Cl
OH
MeO

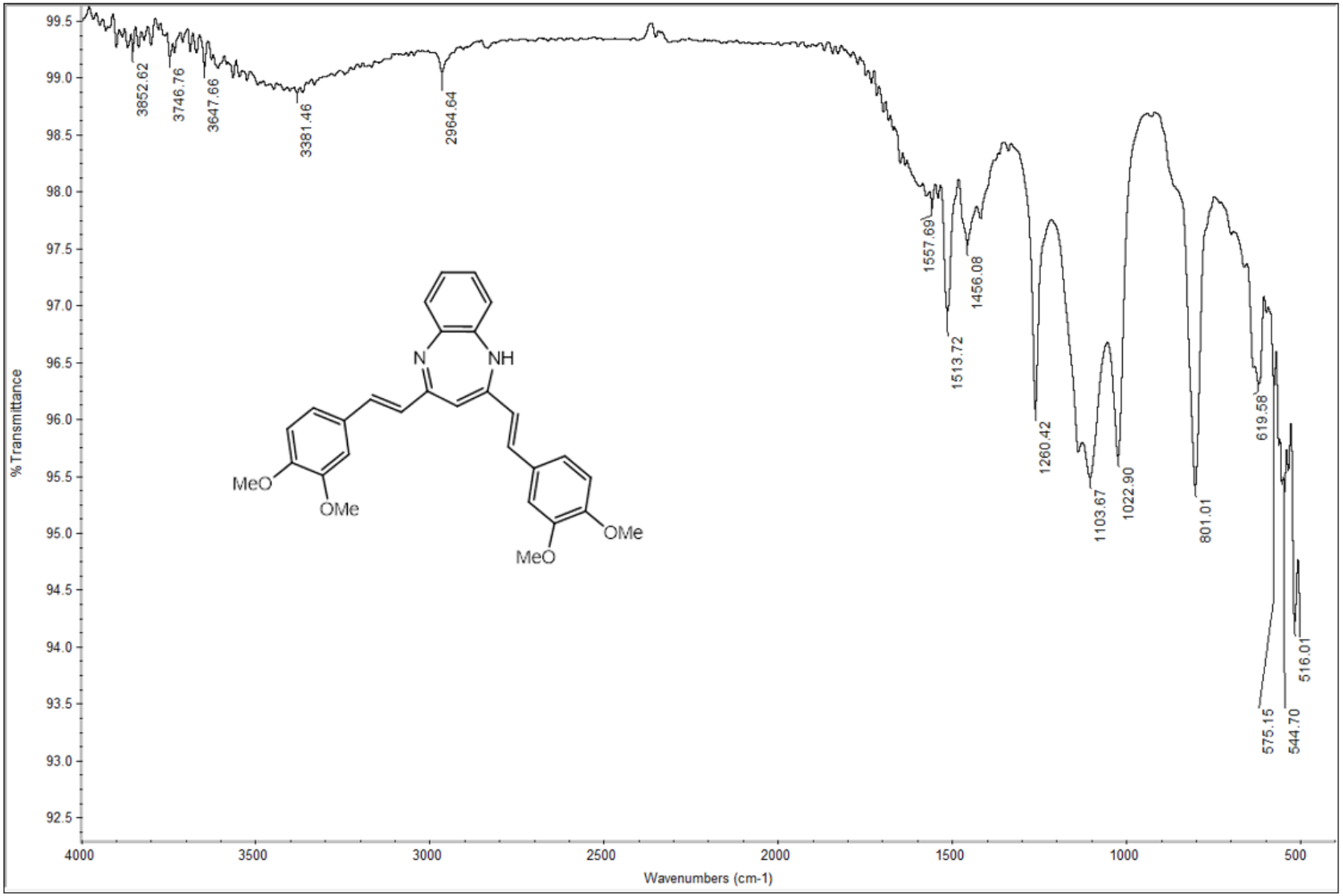


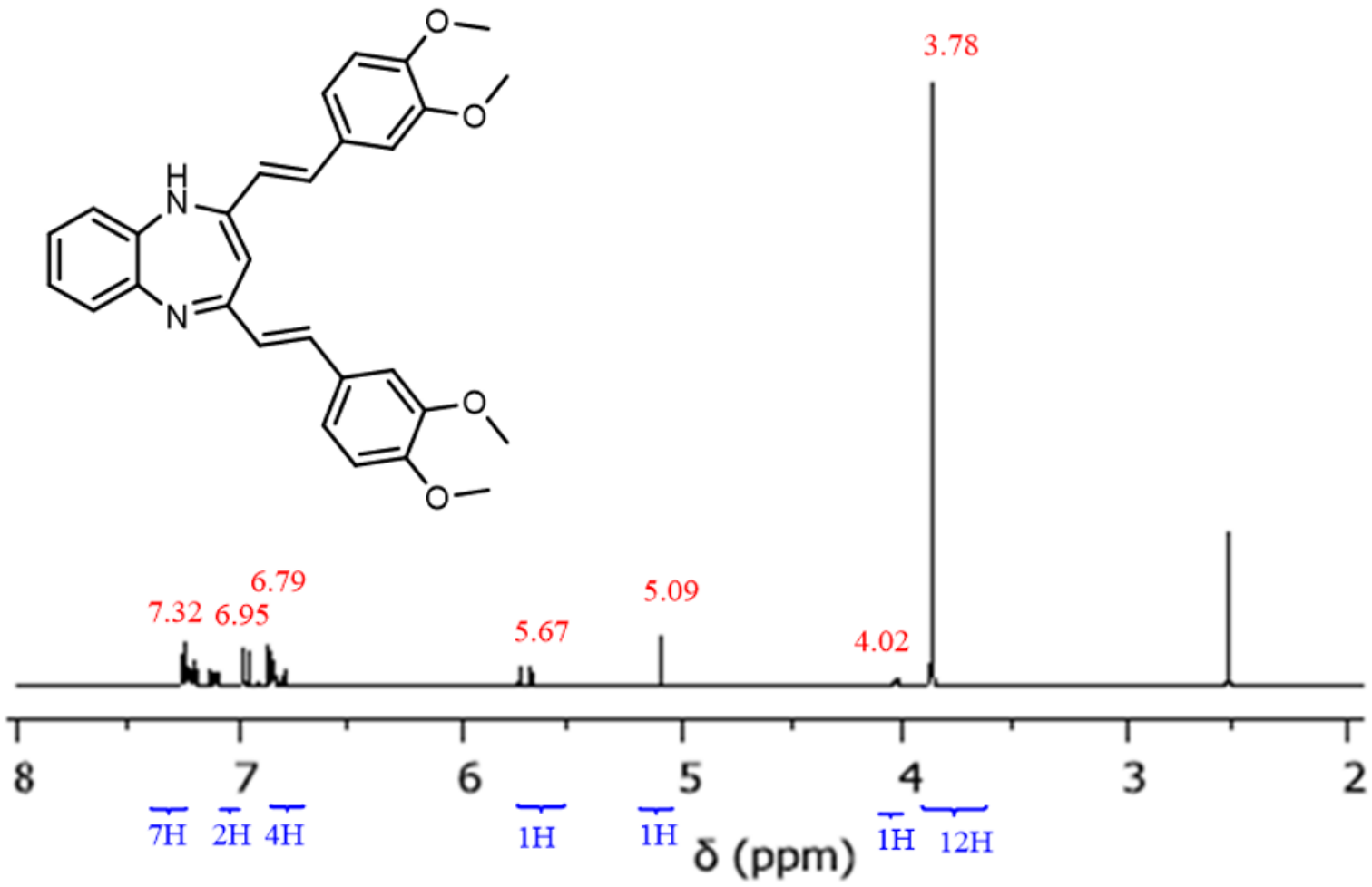
^{1}HNMR spectrum


% Transmittance
Wavenumbers (cm-1)
3421.70
1599.42
1515.97
1452.62
1209.25
1127.24
1060.25
1010.33
961.36
916.83
796.44
622.19
582.99
545.83
526.00
509.45
N
NH
HO
OMe
MeO
OH

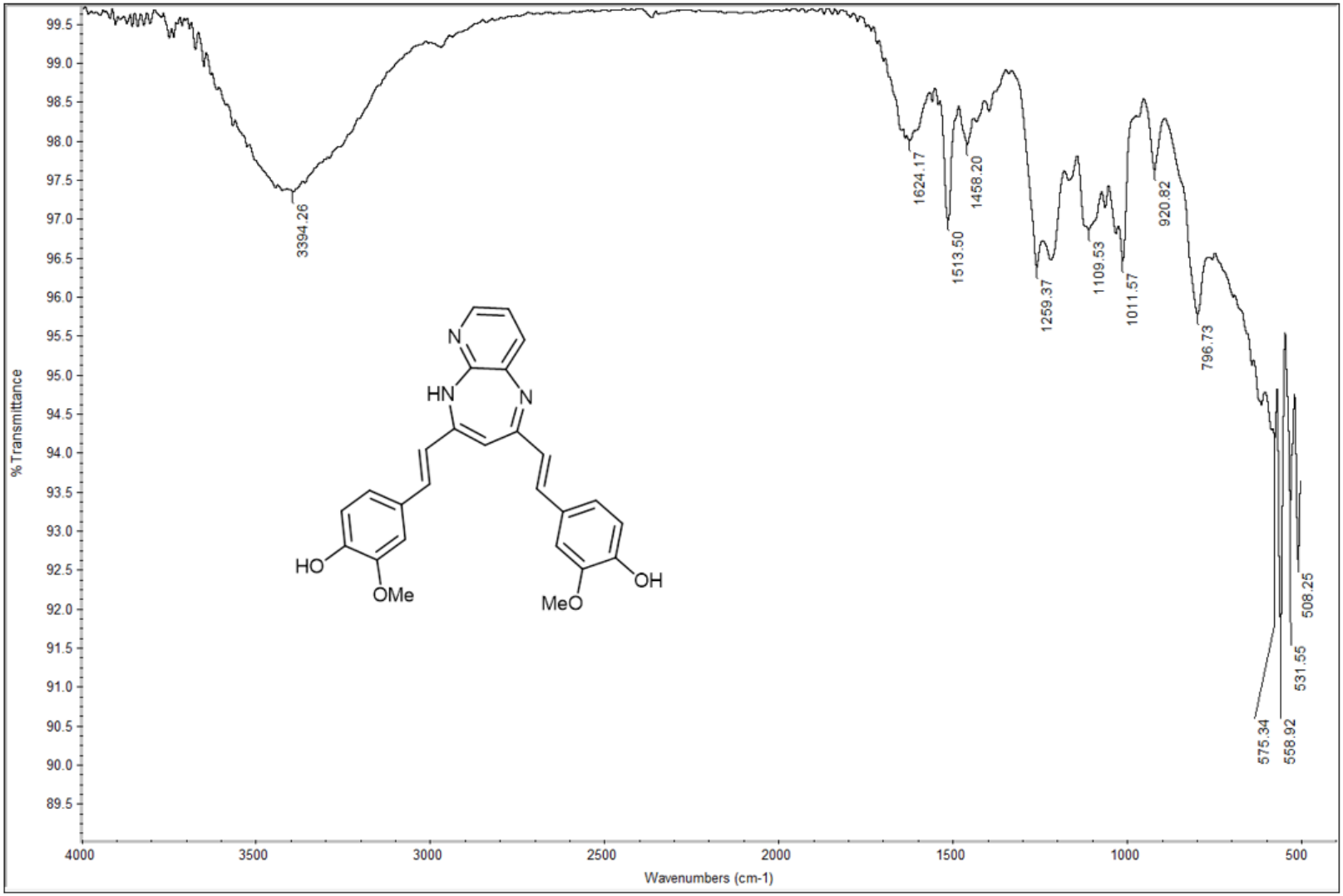


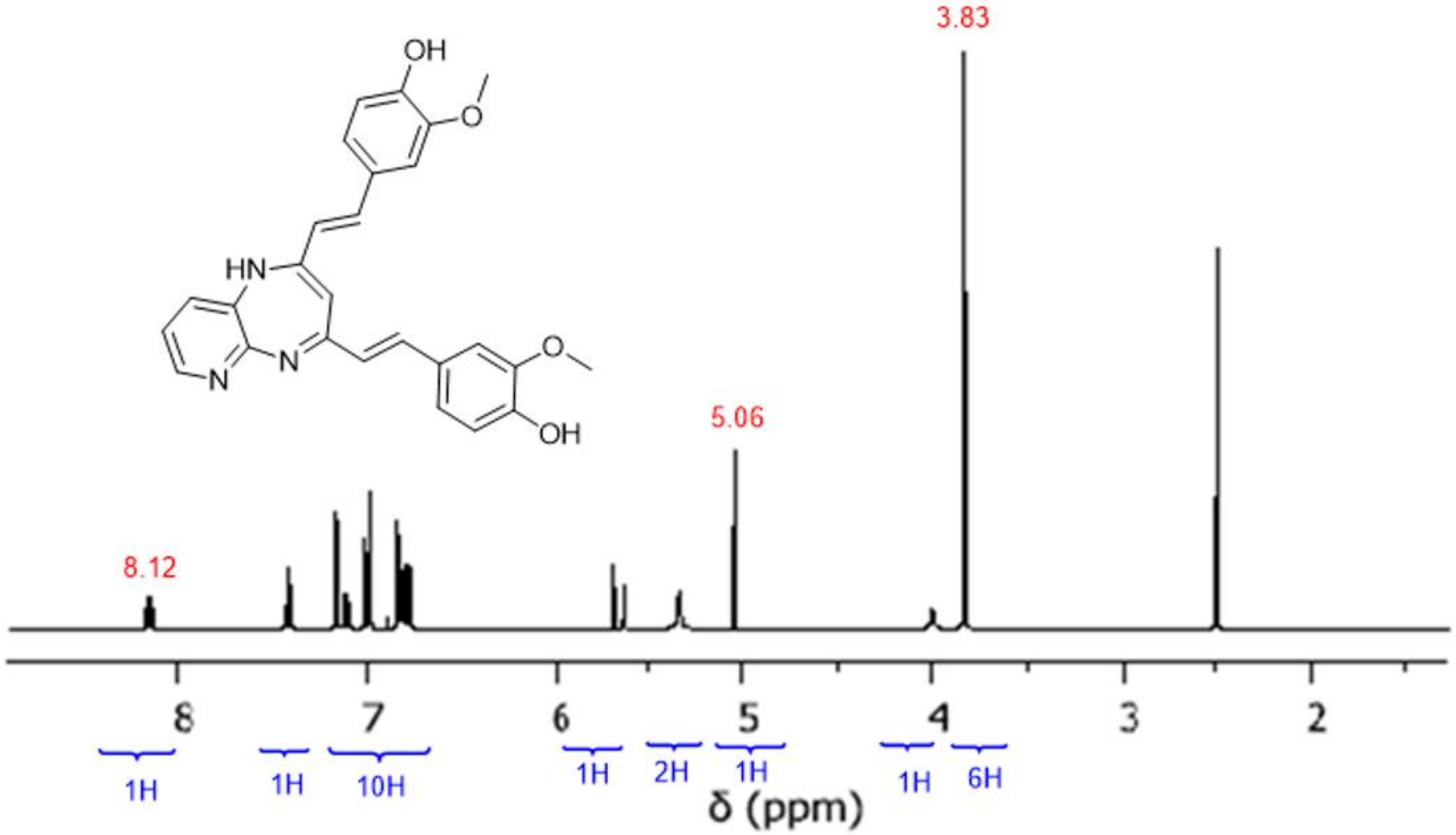
^{1}HNMR spectrum


3852.62
3746.76
3647.66
3381.46
2964.64
1557.69
1513.72
1456.08
1260.42
1103.67
1022.90
801.01
619.58
575.15
544.70
516.01
N
NH
MeO
OMe
OMe
MeO
% Transmittance
Wavenumbers (cm-1)

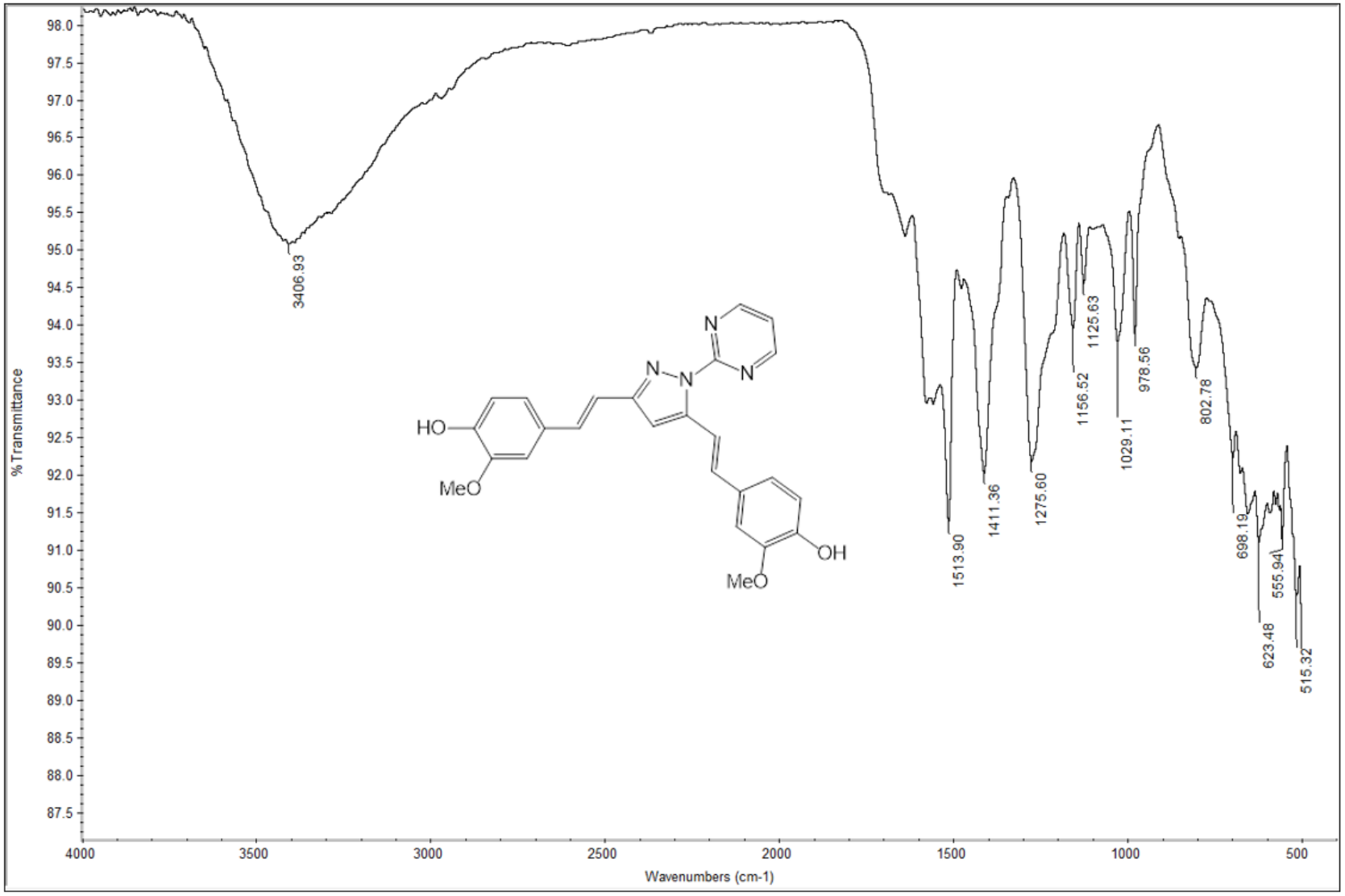


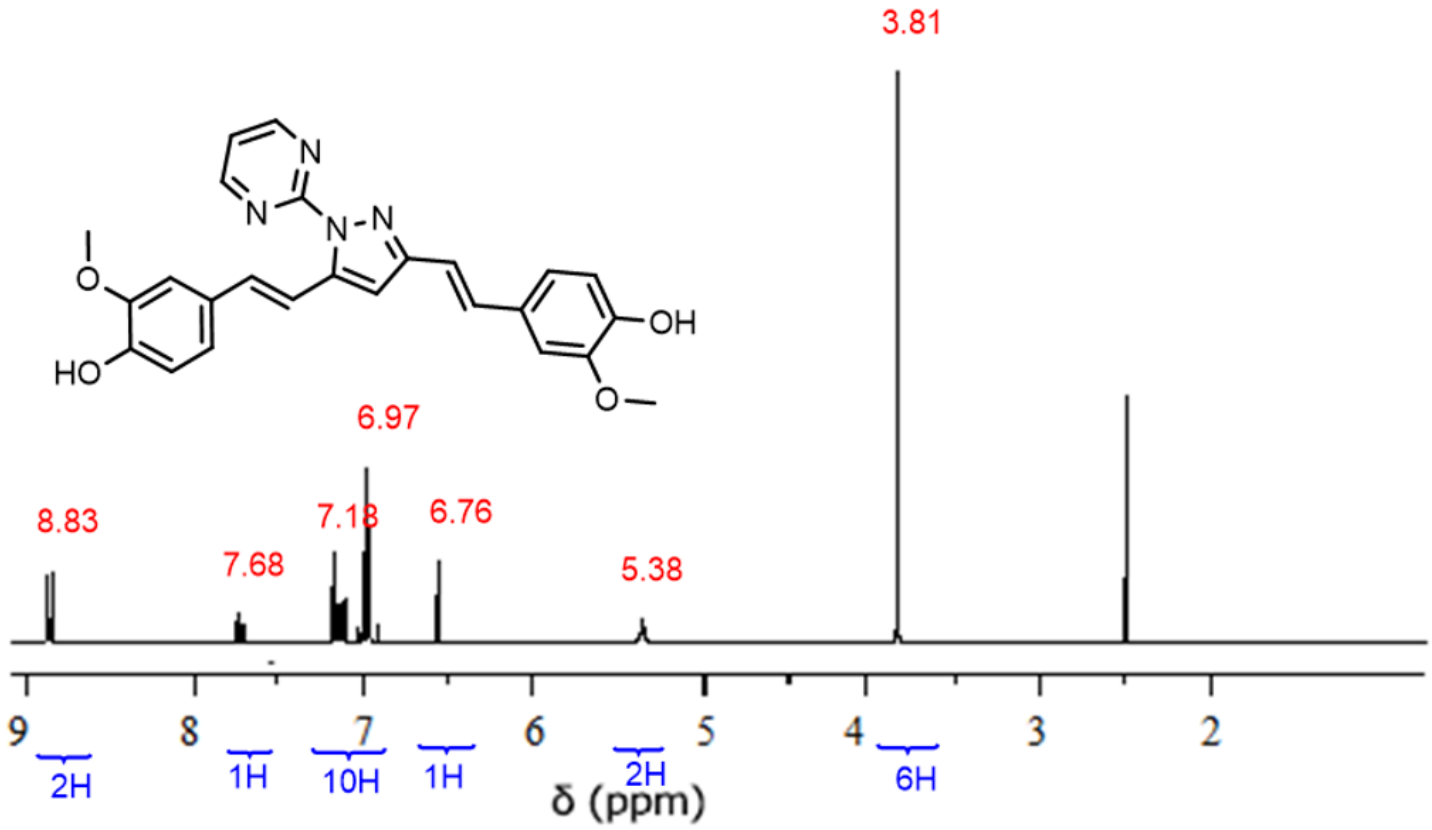
^{1}HNMR spectrum


% Transmittance
Wavenumbers (cm-1)
3394.26
1624.17
1513.50
1458.20
1259.37
1109.53
1011.57
920.82
796.73
575.34
558.92
531.55
508.25
HO
OMe
MeO
OH
HN
N
N

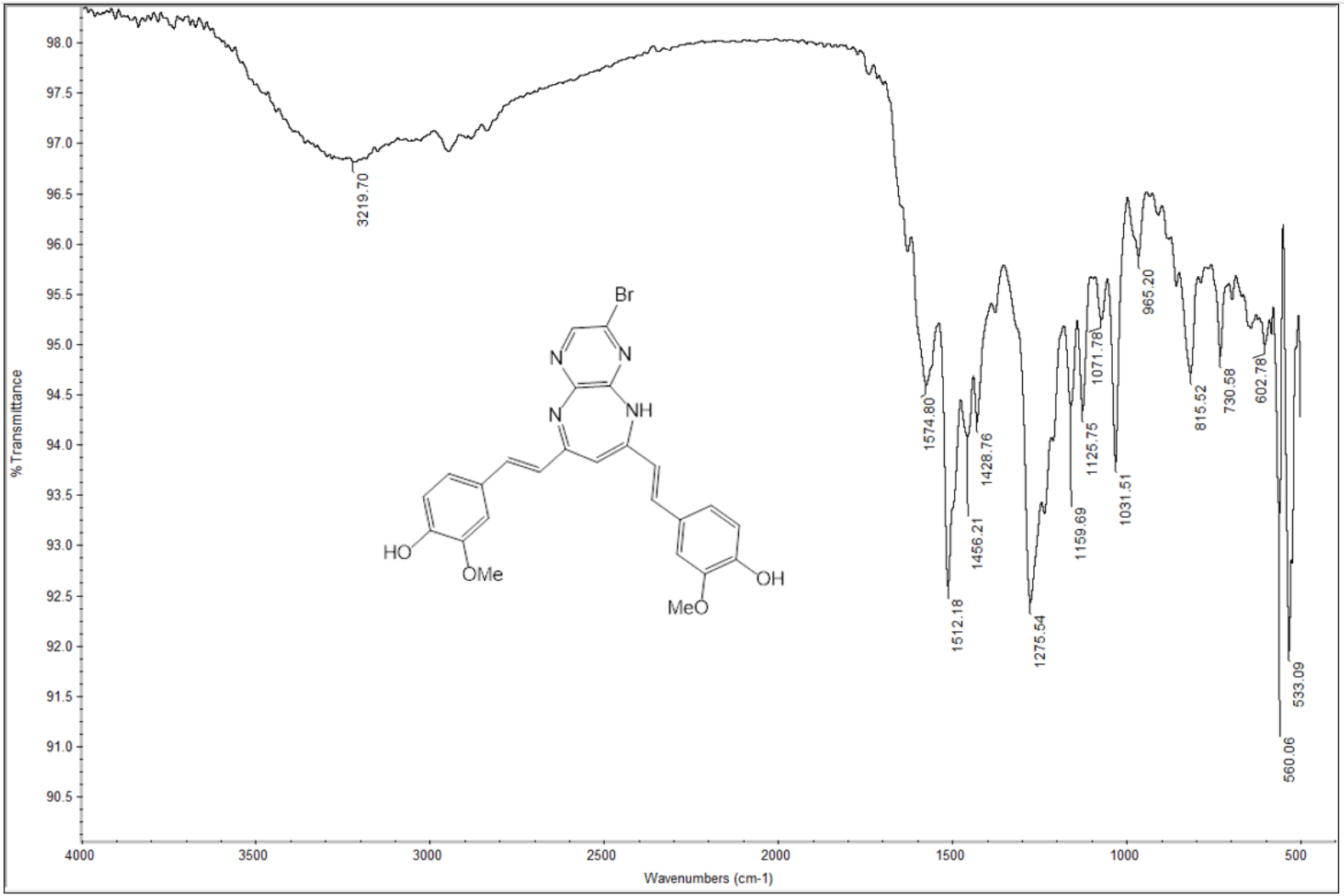


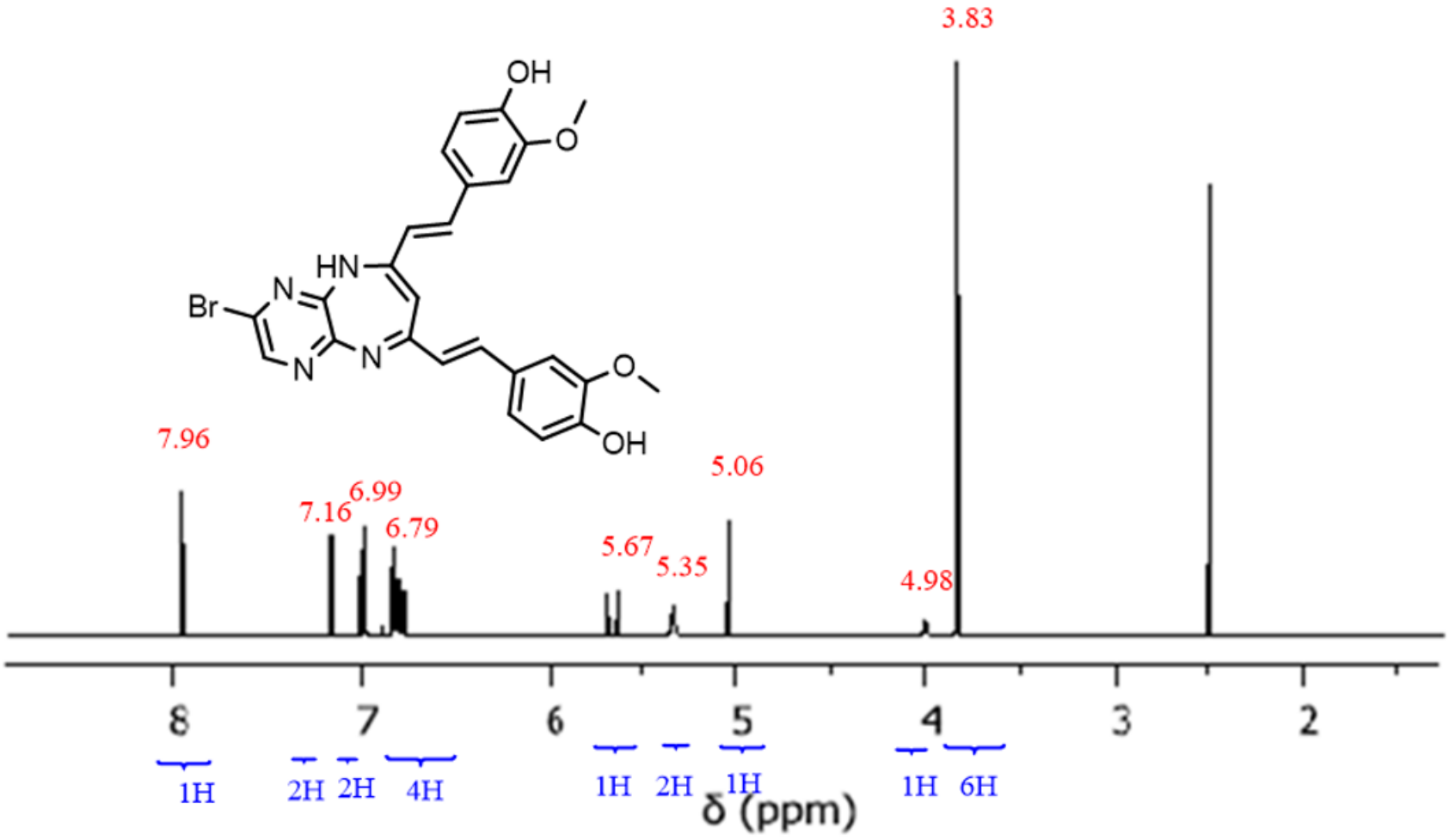
^{1}HNMR spectrum


% Transmittance
Wavenumbers (cm-1)
3406.93
1513.90
1411.36
1275.60
1156.52
1125.63
1029.11
978.56
802.78
698.19
623.48
555.94
515.32
HO
MeO
MeO
OH
N
N
N
N



^{1}HNMR spectrum


%Transmittance
Wavenumbers (cm-1)
3219.70
1574.80
1512.18
1456.21
1428.76
1275.54
1159.69
1125.75
1071.78
1031.51
965.20
815.52
730.58
602.78
560.06
533.09
Br
N
N
N
NH
HO
OMe
MeO
OH



^{1}HNMR spectrum

جامعة النجاح الوطنية

كلية الدراسات العليا

تصميم وتحضير مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز من الكركمين وتقييم نشاطها مخبريا في علاج خلايا السرطان

اعداد

رنا 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 وتقييم نشاطها مخبريا في علاج خلايا السرطان

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اشراف

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د. محمد قنيبي

الملخص

تم تحضير مجموعة مبتكرة من مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز من الكركمين، باستخدام طرق موثوقة وغير معقدة. حيث تم دمج الكركمين مع انواع متنوعة من مركبات ثنائي الأمين ومركبات الهيدرازين. وتم تحليل و دراسة المركبات الناتجة باستخدام تقنيات التحليل الطيفي المختلفة، مثل:(FT-IR) و(^{1}H NMR) و(^{13}C NMR) وقد تم تحضير المركبات المطلوبة باستخدام عدة طرق بسيطة ومباشرة حيث تضمنت الطريقة الاولى تحضير مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز، عن طريق تكثيف الكركمن مع مجموعات متنوعة من مركبات الهيدرازين وثنائي الامين حيث تم استخدام الايثانول كمذيب بوجود كمية قليلة من حمض الكبريتيك الذي تم استخدامه هنا كمحفز للتفاعل، في حين تضمنت الطريقة الثانية تحضير المركبات المطلوبة بوجود حمض الخليك الذي لعب دور المذيب والمحفزللتفاعل في الوقت ذاته.

ومن ناحية اخرى، لقد تم تحضير مجموعة مبتكرة اخرى من مركبات البنزودايزابينز، دايزابينز، دايزولز، وأمينز باستخدام طريقة تكثيف نوفينيغل وقد تم استخدام هذه الطريقة من اجل تحسين كمية النواتج.

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

ولدراسة اهمية مجموعة الهيدروكسيد الفينولي على النشاط الحيوي للمركبات المحضرة، فقد تم حجب مجموعة الهيدروكسيد في عدد من المركبات المحضرة بمجموعات الكيل مختلفة.

هذا وقد تم اختيار سبع مركبات بتراكيز مختلفة (400, 200, 100, 50, 25, and 12.5 µg /ml) من اجل دراسة تأثيرها على الخلايا السرطانية (HeLa cancer cells). أكدت النتائج ان المركبات المحضرة لها فعالية متباينة حسب تراكيزها على الخلايا السرطانية.

اثبتت الدراسة انه بشكل عام كلما زادت تراكيز المركبات المحضرة، تحسنت فعاليتها في تثبيط الخلايا السرطانية. ومن بين المركبات التي تم اختبارها أظهر كل من المركبين (**3** و**5**) فعالية اكبر في تثبيط الخلايا السرطانية.

ومن ثم تم استخدام صبغة MTT لفحص مدى سمية المركبات المحضرة مقارنة ب خلايا سليمة التي استخدمت ك ضابط للاختبار.

وبصورة عامة، اثبتت هذه الدراسة ان المركبات المحضرة لها فعالية واعدة ومبشرة في تثبيط الخلايا السرطانية من نوع (HeLa cancer cells) في مستويات غير سامة للخلايا السليمة.