An-Najah National University

Faculty of Graduate Studies

TAILORED FUNCTIONALIZATION OF HETEROCYCLIC DERIVATIVES OF CARBOXYLIC ACIDS AND IMINES: SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL IMPLICATIONS

By

Inas Ibrahim Mahmoud Bsharat

Supervisor

Prof. Mohammed Al-Nuri

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This Thesis was Defended Successfully on 5/10/2023 and approved by

Prof. Mohammed Al-Nuri Supervisor	Signature
Dr. Orwa Houshia External Examiner	Signature
Dr. Mohyeddin Assali Internal Examiner	Signature
Dr. Nawaf Al-Maharik Internal Examiner	Signature

Dedication

I dedicate this thesis to my beloved mother and dear father who supported me a lot during the work of this thesis and I hope they will always be proud of me. I also dedicate it to myself, and I hope that it will be the beginning of achieving my dream in the research aspect, and I hope that it will be useful to others who are interested in this field.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

TAILORED FUNCTIONALIZATION OF HETEROCYCLIC DERIVATIVES OF CARBOXYLIC ACIDS AND IMINES: SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL IMPLICATIONS

I declare that 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: Inas Ibrahim Mahmoud Bsharat

Signature: Inas

Date: 5/10/2023

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Abstract

Background: A large number of different natural and synthetic chemicals depend on heterocyclic molecules as key building blocks due to their different structures and properties. In the presence of many drugs based on heterocyclic structures, the study of heterocyclic compounds is a prominent area in organic chemistry and has several uses in numerous industries.

Objectives: In this study, a group of ester, amide and imine compounds were prepared, and they were studied as antibacterial and anticancer, and some of them were studied as antioxidants.

Methodology: All the novel ester, amide and imine compounds in this study were prepared by condensation reactions of heterocyclic compounds, and then the new compounds were identified through FT-IR and NMR analysis, and their biological properties were tested and studied.

Results: All prepared heterocyclic compounds showed effective biological activities in varying proportions. Ee1 and E4 were the best bioactive esters in all assays. Amide A1 showed remarkable biological activity, and imines 4 and 6 were the best imines in all biological tests.

Conclusion: In this study, thymol ester E2 was better at preserving normal muscle cells than thymol. The E4 carvacrol ester compound was the best compound in its group, as it showed anti-cancer activity against HepG2 liver cancer cells in cytotoxic assay and against MCF7 breast cancer cells in cytostatic assay and also gave a distinctive activity against all types of bacteria studied. Ester compound Ea was more effective against MCF7 breast cancer cells in cytotoxic assay and against PC3 prostate cancer cells in

cytostatic assay. The ester compound Eb performed better in cytotoxic and cytostatic tests on HepG2 liver cancer cells and MCF7 breast cancer cells, respectively. Ester compound Ec was more effective against Hela cancer cells and MCF7 breast cancer cells, respectively, in cytotoxic and cytostatic experiments. Also in both cytotoxic and cytostatic experiments, the ester compound Ed was more efficient against HepG2 liver cancer cells. Flavonoid ester Ee1 gave a distinctive activity in all biological tests. Amide compound A1 was the best of the compounds of its group on all the studied biological activities, as were imine 4 and imine 6 as well.

Keywords: Heterocyclic compounds; Antibacterial; Anticancer; Antioxidant.

Chapter One

Introduction

1.1 Definition of the Condensation Reaction

In a broad meaning, the wording "condensation reaction" describes a set of processes in which two or more molecules come together to form a larger molecule while removing a smaller molecule, like water or alcohol. Depending on the scientific discipline or context, the term "condensation reaction" may have different meanings.

For example, a condensation reaction in organic chemistry often includes the combining of two molecules while losing a minor molecule, frequently water. As a result of this process, a bigger molecule known as a condensation product is formed. The preparation of an ester from a carboxylic acid and an alcohol is an illustration of a condensation reaction in organic chemistry [1]. Condensation reactions in biochemistry are frequently referred to as dehydration synthesis reactions. These reactions usually include the elimination of a water molecule while forming a covalent connection between two chemicals. The preparation of biological macromolecules including proteins, nucleic acids, and polysaccharides depends on biochemical condensation processes [2]. Condensation reaction, as used in atmospheric science, is the process by which water vapor condenses to form liquid water or ice in the atmosphere. Clouds, fog, and precipitation are created when water vapor molecules join together under the right temperature and pressure circumstances to produce liquid droplets or ice crystals [3]. Moreover, in the field of polymer chemistry, condensation polymerization is a well-known reaction wherein monomers undergo a chemical reaction, often accompanied by the release of a small compound such as water or methanol. This transformative process results in the preparation of a polymer chain comprising recurring units. Prominent examples of condensation polymers encompass polyesters and polyamides [4]. Pharmaceutical synthesis heavily relies on condensation processes to prepare complex chemical compounds. Through condensation processes, amino acids are combined to form peptide bonds, leading to the synthesis of notable examples such as peptides. Peptides play a crucial role in the production of pharmaceuticals and serve as therapeutic agents with great significance. Condensation reactions find application in the production of diverse dyes and pigments. In the production of azo dyes, which are

widely used in the textile industry, condensation reactions between diazonium salts and aromatic amines result in the required chemicals. Many different adhesives, including polyurethane and epoxy resins, are produced using condensation processes. These reactions help build strong connections between various materials, which aids in the manufacturing of adhesives with outstanding bonding strength and longevity. In the production of resins like urea-formaldehyde resins and phenolic resins, condensation processes also play a big part. In the manufacture of particleboard, plywood, and molded goods, these resins, known for their exceptional adhesive characteristics, are widely used [5].

1.2 Types of Chemical Condensation Reactions

There are many examples of condensation reactions including: Claisen condensation, Aldol condensation, Perkin condensation, Dieckmann condensation, Knoevenagel condensation, esterification, amidation, and the preparation of imines [1, 6].

1.2.1 Esterification Reaction

Fischer esterification, which includes condensing a carboxylic acid and an alcohol, is a typical method for producing esters. Emil Fischer, the reaction's discoverer, is honored with a name for it. A carboxylic acid and an alcohol interact during Fischer esterification, which uses an acid as a catalyst. Alcohol functions as a nucleophile while the acid catalyst protonates the carbonyl oxygen in carboxylic acid to make it more vulnerable to nucleophilic assault. As a result, an intermediate species known as an "acyl" or "carboxylic acid ester" is formed. This intermediate species then goes through a proton transfer and the consequent elimination of water to produce the ester. Reactant concentrations, temperature, and the presence of too much of an alcohol can all affect the equilibrium between the reactants and products in this reversible process. A strong acid catalyst, such as sulfuric acid or p-toluenesulfonic acid, contributes in shifting the balance in the direction of product synthesis by stimulating the protonation of the carbonyl oxygen. It is important to emphasize that an excess of alcohol is frequently used in Fischer esterification to facilitate the formation of the ester. Additionally, in order to produce water as a byproduct of the reaction and encourage the equilibrium's progression, the reaction is often conducted under reflux [6].

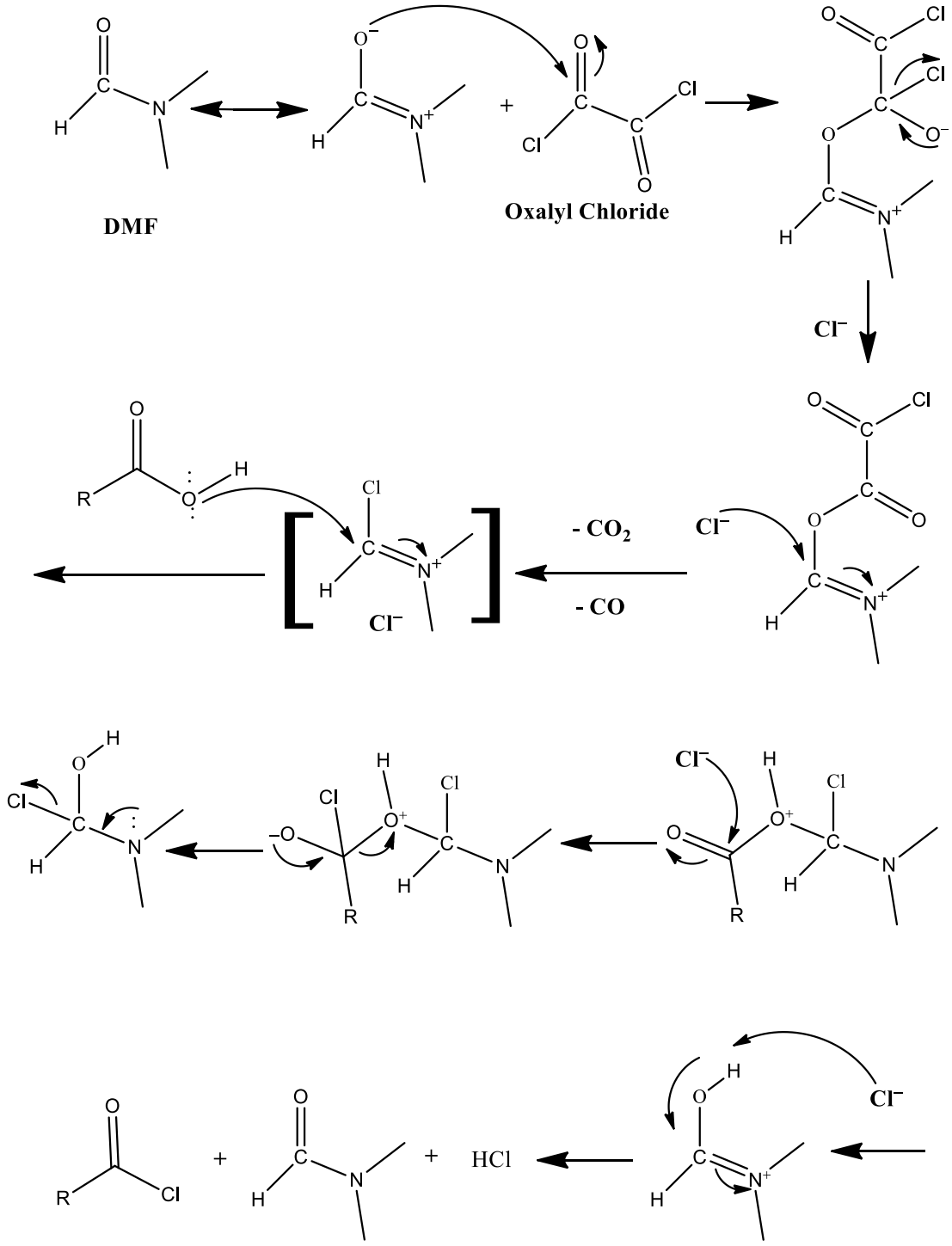
Another way to produce an ester is to combine an alcohol and an acid chloride. When an acid chloride (RCOCl) and an alcohol (ROH) react together, the reaction takes place in the presence of a base as a catalyst, usually a base such as pyridine or triethylamine. The reaction can be described as follows:

$$RCOCl + R'OH \longrightarrow RCOOR' + HCl \quad (1.1)$$

In the above chemical equation, R represents the alkyl or aryl group of the acid chloride, and R' also represents the alkyl or aryl groups that arise from the alcohol. The acid chloride and the alcohol undergo a chemical transformation, resulting in the formation of an ester (RCOOR') and the formation of hydrochloric acid (HCl) as a by-product. To maximize the production of the desired ester, the reaction is usually performed under controlled conditions, such as low temperatures or in the presence of an inert gas [7, 8]. The Scheme 1.1.A below shows the mechanism of acid chloride preparation in the presence of dimethylformamide with oxalyl chloride then Scheme 1.1.B shows the mechanism of ester preparation from acid chloride with alcohol.

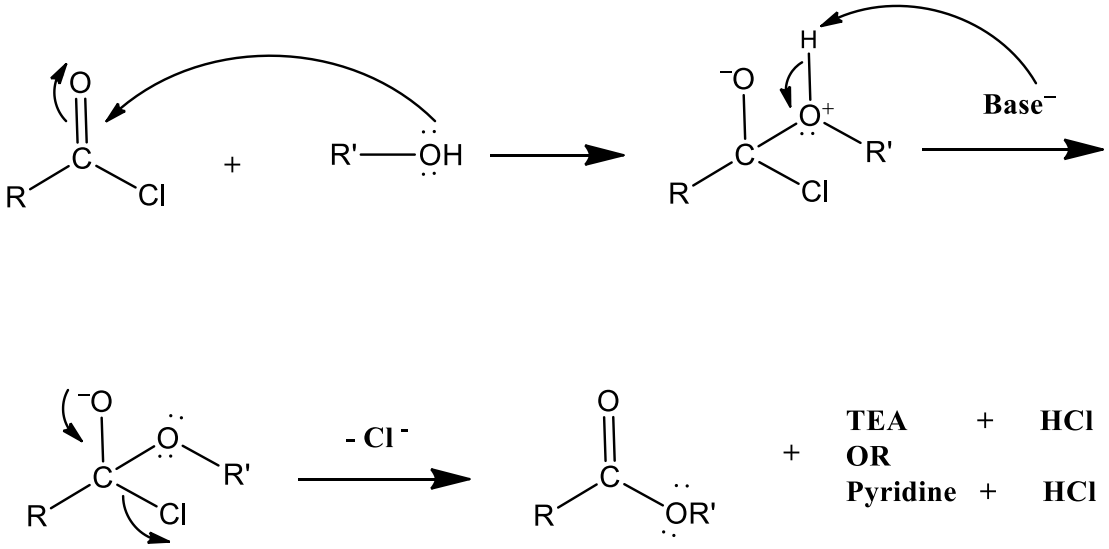
Scheme 1.1.A

Synthesis of Acid Chloride in Presence of DMF and Oxalyl Chloride



Scheme 1.1.B

Synthesis of Ester. Base= $N(CH_2CH_3)_3$ or Pyridine



It should be emphasized that the categorization of esters as aromatic or aliphatic depends on the substituents linked to the carboxylate group and the alcohol functional group that used in the ester preparation. Aromatic and aliphatic esters can have different characteristics and uses. Because of their pleasant and distinctive smells, aromatic esters are frequently utilized in perfumes, fragrances, and flavorings [9]. On the other hand, aliphatic esters are frequently employed as solvents, particularly in paints, varnishes, and adhesives [10]. Esters are utilized in the manufacturing of drugs [11], resins [12], polymers [13], the cosmetics sector [14], and uses in fertilizer [15]. Aliphatic esters frequently have lower boiling points compared to aromatic esters, which may have an impact on both their physical properties and applications. Additionally, the presence of one or more hetero-atoms has a substantial impact on the biological properties and utilization of the ester molecule.

The biological effects of ester compounds have been studied in the past and have included anticancer [16], antibacterial [17], antioxidant [18], anti-inflammatory [18], and antifungal activity [19].

1.2.2 Amidation Reaction

A carboxylic acid or ester interacts with an amine or ammonia to result in the amidation reaction, which is frequently aided by a catalyst or activating substance. The reaction can proceed through a variety of processes, depending on the particular circumstances and reagents used. In the amidation reaction, which produces an amide from carboxylic acids, the hydroxyl group (-OH) of the acid is swapped out for the amino group ($-NH_2$) of the amine. A coupling reagent, such as dicyclohexylcarbodiimide (DCC) or N,N-diisopropylcarbodiimide (DIC), is typically required for the reaction in order to activate the carboxylic acid and allow the nucleophilic attack by the amine. In the process of ester amidation, an ester is first hydrolyzed to produce a carboxylic acid, which then goes through the same process as the previous one. Alternatively, in the presence of a catalyst or activator, the ester can directly react with an amine [6].

Acid chloride and primary or secondary amine can be combined to form an amide. The Schotten-Baumann reaction or amide synthesis is the name of this reaction. To form the amide compound, the acid chloride (RCOCl) reacts with a primary or secondary amine (R'NH2 or R'NHR"), respectively. The reaction frequently takes place in the presence of a base like triethylamine (Et_3N) or pyridine (C_5H_5N) in order to neutralize the hydrogen chloride (HCl) byproduct. The general equation shown below can be used to illustrate the reaction:

$$RCOCl + R'NH_2 \text{ (or } R'NHR'') + Base \rightarrow RCONHR' \text{ (or } RCONR'R'') + HCl \quad (1.2)$$

The synthesis of a new carbon-nitrogen bond and the production of HCl occur as a result of the nucleophilic acyl substitution of the amine with the acid chloride's carbonyl carbon in this reaction [6]. Amides are prepared by the same mechanism as shown in the Scheme 1.2.A and Scheme 1.2.B in the previous section, but by replacing the alcohol with an amine.

Amides are flexible substances with several uses and applications in a variety of industries. Amides are frequently utilized as building blocks in pharmaceutical manufacturing for producing new drugs. They act as crucial functional groups and are included into the chemical structure of many medications. For instance, penicillin and amoxicillin are two antibiotics that have the amide bond [20]. Amides have a significant

role in the formation of synthetic polymers. Through a procedure known as polycondensation, amides are transformed into nylon, a well-known synthetic fiber. Another significant amide-based polymer is polyacrylamide, which is employed in numerous applications such increased oil recovery, soil conditioning, and water treatment [21, 22]. N,N-dimethylacetamide (DMAc) and N,N-dimethylformamide (DMF) are two examples of amides that are frequently utilized as solvents in chemical processes. These solvents can dissolve a variety of organic and inorganic compounds and have high boiling temperatures and good solvency powers. Amides are used to produce agrochemicals such as insecticides, fungicides, and herbicides. In the manufacture of these agricultural chemicals, they can act as either active ingredients or intermediates [23]. Amides are crucial to many biological functions. The structure and operation of living organisms depend on the presence of peptides and proteins, which are made up of amino acids connected by amide bonds. Nucleic acids (DNA and RNA), which are essential for the storage and transmission of genetic information, are also produced by amino acids [24]. Moreover, in industrial applications, amides are used as intermediates in the synthesis of pigments and dyes. In numerous fields, including plastics, textiles, and cosmetics, they are also utilized as additives [25, 26].

1.2.3 Synthesis of Schiff Bases

Schiff bases are compounds that are formed when an aldehyde or ketone (-C=O) and a primary amine ($-NH_2$) condense together and water molecule is removed (dehydrated), that leads to the production of an imine functional group (R-C=N-R'), also known as a Schiff base. Carbonyl compounds that are both aromatic and aliphatic can be converted into Schiff bases. The main amine and the carbonyl molecule combine to produce the imine or azomethine bond. Due to their stability and reactivity, aromatic carbonyl compounds, including benzaldehyde, are frequently utilized in Schiff base reactions. However, the condensation reaction that generates Schiff bases can also involve aliphatic aldehydes and ketones [27, 28]. The chemical equation for the formation of Schiff bases can be represented as follows:

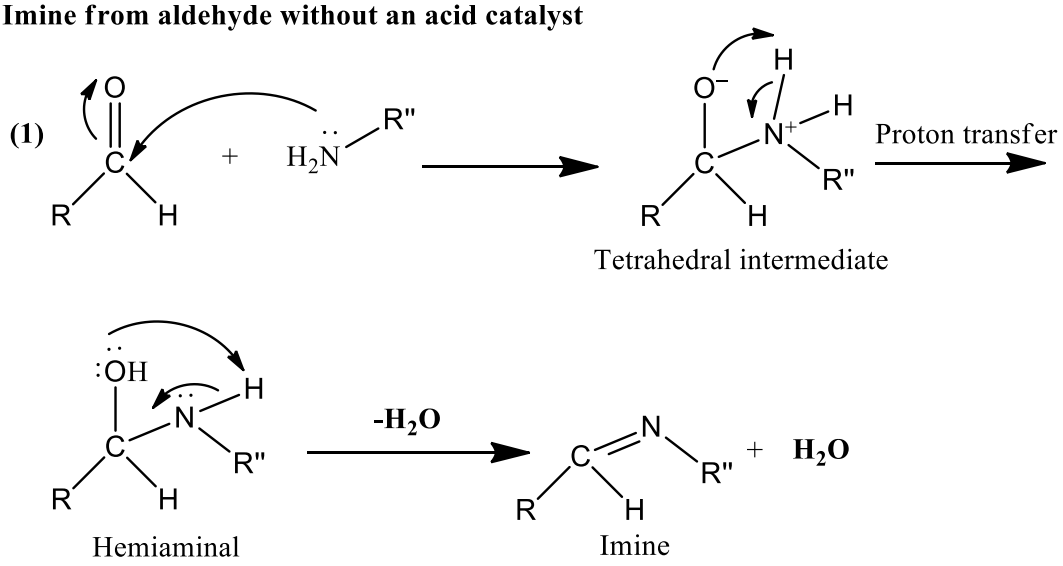
$$RCHO/\ RCR'O + R''NH_2 \rightarrow RCH{=}N\text{-}R''/\ RCR'{=}NR'' + H_2O \qquad (1.3)$$

In this equation, the letters R, R', and R" stand for an organic group (alkyl/aryl) attached to the carbonyl carbon of the aldehyde or ketone, the carbonyl carbon of the ketone, and the nitrogen atom of the primary amine, respectively. The reaction results in the formation of an imine (RCH=N-R') or (RCR'=NR") and a molecule of water (H_2O) as a byproduct. The Scheme 1.1.C below shows the mechanism of imine preparation without the presence of a catalyst at one time (1) then in the presence of an acid catalyst (H_2SO_4) at the other time (2).

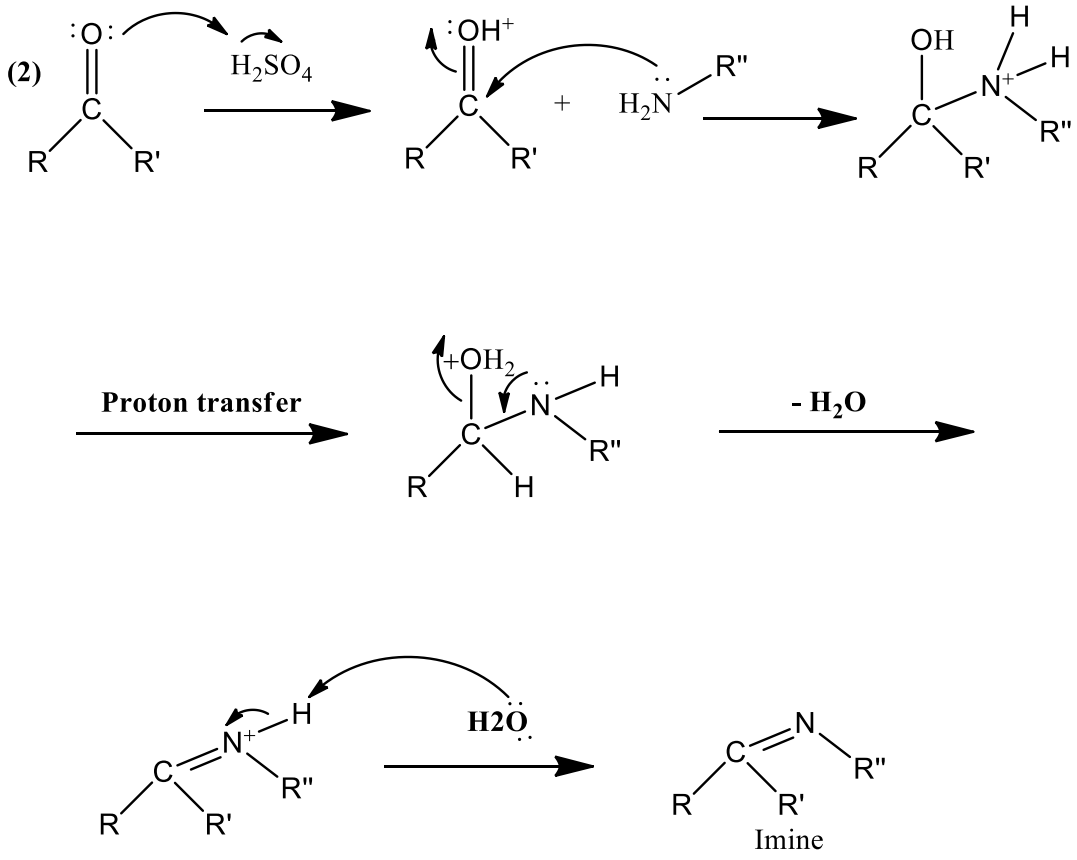
Scheme 1.1.C

Synthesis of Schiff bases (imines)

Imine from aldehyde without an acid catalyst



Imine from ketone in presence of an acid catalyst



Hugo Schiff, a German scientist, is credited with discovering the Schiff bases in 1864. It's also important to note that imines and azomethines are both names for compounds with the C=N functional group [29, 30].

The lone pair of electrons on the nitrogen atom in imine compounds plays a significant role in their reactivity and makes them useful for various applications. Imine compounds have the ability to serve as catalysts in many different chemical reactions. The activation of reactants and the rapidity of the reaction are made possible by the lone pair of electrons' ability to interact with transition metal ions. Imine-based catalysts are advantageous in asymmetric synthesis and other transformations because of these characters [31]. Imine compounds' lone pair of electrons can take part in complexation processes with specific metal ions or other analytes. Imines are advantageous for the development of sensors and detection systems because of this feature. One can adjust the selectivity and sensitivity of the sensor towards particular analytes by changing the imine structure [32]. Moreover, imine groups are present in many medications. The nitrogen atom's lone pair of electrons can interact with biological targets like enzymes or receptors to produce certain pharmacological actions [33-35].

In biological applications such as antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties, Schiff base ligands containing hetero-atoms (N, O, P, and S) have been employed [36-39]. Various studies have looked at the anticancer properties of Schiff base metal complexes in relation to numerous cancer cell types, including leukemia, breast cancer, lung cancer, colon cancer, and prostate cancer. These complexes' modes of action can differ and may involve several pathways. The capacity of Schiff base metal complexes to interact with biomolecules like DNA and proteins is one of the main factors driving interest in them as potential anticancer treatments. In cancer cells, some complexes have been found to attach to DNA and cause DNA damage, which causes cell cycle arrest and apoptosis (programmed cell death). These interactions may disrupt the transcription and replication of DNA, ultimately preventing the growth of cancer cells [40, 41]. Additionally, it has been noted that Schiff base metal complexes have anti-oxidant properties and can scavenge reactive oxygen species (ROS). The development and spread of cancer can be facilitated by excessive ROS production, which can also damage cellular components and produce oxidative stress. Schiff base metal complexes

can help insulate cells from oxidative damage and possibly stop the growth of cancer cells by lowering ROS levels [42].

1.3 Heterocyclic Compounds

Heterocyclic compounds refer to organic compounds that possess one or more ring structures comprising carbon atoms along with atoms of various other elements, such as nitrogen (N), oxygen (O), or sulfur (S) and other atoms like phosphorus (P) and boron (B) can also be present in some cases. These additional elements, called hetero-atoms, give heterocyclic compounds with distinctive chemical characteristics and frequently contribute to their broad range of biological activities. Heterocyclic compounds are categorized according to the ring's atom count and the specific type of heteroatom it contains. Various sizes of rings are commonly encountered, such as those comprising three atoms (referred to as three-membered rings) like epoxide, thiirane, selenirane and aziridine, five-membered rings like pyrrole, furan, thiophene, pyrazole, imidazole and oxazole, six-membered rings like pyridine, pyrimidine, pyrazin, piprazine and pyridazine, and so forth. It is important to note that the number of heteroatoms within the ring can also differ. Based on whether or not the ring structure contains aromaticity, heterocyclic compounds can be further divided into aromatic and non-aromatic groups. According to Hückel's rule, aromatic heterocycles are similar to benzene in that they have a conjugated ring structure with 4n + 2 -electrons (where n is an integer). Moreover, based on how the fused rings are arranged, heterocyclic compounds can be classed. The way the rings are joined to one another in heterocyclic compounds is referred to as the arrangement of fused rings. A bigger cyclic structure with a variety of chemical characteristics is produced by the joining of several rings. In heterocyclic compounds, the following forms of fused ring systems are typical: fused rings in a linear fashion like naphthalene and anthracene, fused rings in a bridged fashion like spiro[4.5]decane, spiro[4.4]nonane and bicyclo[2.2.2]octane, fused rings in a fused fashion like indole (benzene and pyrrole rings fused), benzothiophene (benzene and thiophene rings fused) and quinoline (benzene and pyridine rings fused) or fused rings with a common atom like isoquinoline (benzene ring and a pyridine ring share a common carbon atom) and indoline (benzene ring and a pyrrole ring share a common carbon atom) [43].

The first natural products were isolated and studied by chemists in the early 19th century, which is when heterocyclic chemistry began. The German chemist Friedrich Wöhler, who is credited with creating urea in 1828 by synthesizing it from inorganic materials, was one of the forerunners in this subject. This synthesis disproved vitalism, which claimed that only living things could produce organic molecules, by demonstrating that organic compounds could be formed from inorganic sources [44].

In the middle of the 19th century, August Wilhelm von Hofmann, a pupil of Friedrich Wöhler, made significant contributions to the field of heterocyclic chemistry. Hofmann and his colleagues created a range of heterocyclic chemicals, including pyridine and quinoline derivatives. These compounds have attracted a lot of research due to their prevalence in natural goods and their possible health advantages [45].

Heterocyclic chemistry made progress during the 19th and 20th century. In the late 19th century, chemists like Adolf von Baeyer and Albert Ladenburg made major progress in the synthesis and characterization of many heterocyclic molecules. Baeyer produced a number of important heterocyclic chemicals, including indole, pyrimidine, and pyrazole. His research laid the foundation for the knowledge we have today on aromaticity in heterocyclic systems [46].

The discovery of various naturally occurring heterocyclic compounds and the investigation of novel synthetic methods led to significant developments in the field of heterocyclic chemistry in the early 1900s. By creating colors from heterocyclic molecules, well-known chemist Paul Ehrlich made a significant contribution to medical chemistry. His ground-breaking work paved the way for the creation of essential drugs like the potent antimalarial quinine [47].

Due to improvements in spectroscopic methods like infrared and nuclear magnetic resonance (NMR) spectroscopy, the subject of heterocyclic chemistry expanded quickly in the middle of the 20th century. These methods made it possible to characterize heterocyclic compounds' structures more precisely, which advanced both the synthesis of these compounds and our understanding of their reactivity. Researchers from all over the world are currently working on the synthesis, characterization, and uses of novel heterocyclic molecules, maintaining heterocyclic chemistry as an active field of study. The following are some of aromatic or aliphatic heterocyclic compounds that are

considered the basis for the chemical composition of the starting materials that were used in all the chemical reactions through which the compounds were synthesized in this study:

1.3.1 Pyrrole

Pyrrole, also known as azacyclopenta-2,4-diene or cyclopentadiene with an NH group in place of a CH unit and it is a heterocyclic aromatic organic molecule having the chemical formula C_4H_5N. A five-membered ring made up of four carbon atoms and one nitrogen atom makes up this compound. Due to the presence of an electron pair on the nitrogen atom, pyrrole is extremely reactive and serves as a flexible building block in organic chemistry [48].

At room temperature, pyrrole is a colorless liquid, but it can turn dark when exposed to air and light. It has bad and terrible smell. Various natural resources, including coal tar, crude oil, and some fruits and vegetables, contain pyrrole. F. F. Runge discovered it for the first time in 1834 [49].

Agrochemicals, materials research, and pharmaceuticals all frequently use pyrrole derivatives. They are capable of acting as precursors in the manufacture of medicines, dyes, pigments, and polymers and exhibit biological activities. Although pyrrole has few uses by itself, it is an essential component in the production of chemical compounds. Due to their wide range of biological functions, pyrrole derivatives have been extensively researched and used in pharmaceuticals. A wide range of pharmacological properties, such as antibacterial, antifungal, anti-inflammatory, analgesic, anticancer, and antiviral activities, are displayed by pyrrole derivatives [50]. Pyrrole derivatives are used in agrochemicals as active components in pesticides, herbicides, and fungicides. These substances aid in crop protection by preventing weeds, fungus, and pests from growing. For instance, the natural pyrrole molecule pyrrolnitrin is utilized as a biocontrol agent against many plant infections. It has antibacterial capabilities [51]. Moreover, in materials science, pyrrole-based dyes and pigments are used in textiles, printing inks, paints, and coatings and provide a wide range of colors. On the other hand, pyrrole monomers can be polymerized to produce conducting polymers with great electrical conductivity, such polypyrrole. Electronics, sensors, batteries, and electrochromic materials all use these conducting polymers [52].

Pyrrole exhibits various common reactions, including: electrophilic aromatic substitution reaction, where the electron-rich nature of the pyrrole ring makes it susceptible to attack by electrophile (for example, nitration of pyrrole can be achieved by treating it with a mixture of concentrated nitric acid and sulfuric acid); Vilsmeier-Haack reaction, pyrrole can react with the Vilsmeier-Haack reagent, which is a mixture of dimethylformamide (DMF) and phosphorus oxychloride ($POCl_3$) (this reaction leads to the formation of an N-acylpyrrole, where the nitrogen atom is attached to an acyl group); and pyrrole can participate in the Diels-Alder cycloaddition reaction, where it acts as a diene. The diene character of pyrrole arises from the electron-rich nature of the nitrogen atom and the aromaticity of the ring. This reaction can lead to the formation of fused ring systems and the introduction of new substituents [48].

1.3.2 Furan

Oxacyclopentadiene, known as furan, is a heterocyclic organic molecule with four carbon atoms and one oxygen atom in its aromatic ring. C_4H_4O is its chemical formula. Due to their involvement in the delocalized electron system, the lone pairs of electrons on the oxygen atom contribute to the stability and aromaticity of furan [48]. Furan is a colorless, flammable liquid that has a peculiar smell that borders on the ethereal. Heinrich Limpricht synthesized it for the first time in 1870 [53].

Furan derivatives exhibit a wide range of bioactivities and have been extensively studied in various fields of medicinal and pharmaceutical research such as antimicrobial, anticancer, anti-inflammatory, antioxidant, antidiabetic, antiviral and neuroprotective [54-56].

Furan exhibits various common reactions, including: electrophilic aromatic substitution reaction, where the electron-rich furan ring can react with electrophiles, such as nitronium ion (NO_2^+), to substitute a hydrogen atom with the electrophile, resulting in the formation of substituted furan derivatives; Furan can undergo a Diels-Alder reaction with dienophiles, which are compounds containing double bonds. This reaction leads to the formation of cycloadducts, known as adducts, by forming two new carbon-carbon bonds. Additionally, metalation reaction of furan especially lithiation reaction is a common reaction of furan; it is typically performed using a strong base, such as lithium

diisopropylamide (LDA) or n-butyllithium (n-BuLi) to introduce substituents onto the furan ring as lithiofuran [48].

1.3.3 Thiophene

The five-membered ring of the heterocyclic organic molecule thiophene has one sulfur atom and four carbon atoms. It is a benzene derivative in which a sulfur atom replaces one of the carbon atoms. Thiophene has the chemical formula C_4H_4S. Similar resonance forms to those that exist in pyrrole and furan also contribute to the molecular structure of thiophene, an aromatic molecule. But since sulfur is less electronegative than oxygen, thiophene and furan differ from one another. Thiophene's chemistry therefore resembles that of pyrrole more so than that of furan. For instance, in order to drive a cycloaddition between thiophene and maleic anhydride, very harsh circumstances, high pressure (15 bar), and a temperature of 100°C, are required therefore thiophene does not readily enter into [4 + 2] cycloaddition reactions. Thiophene exhibits a colorless to pale yellow appearance in a liquid state, accompanied by a recognizable yet slightly disagreeable scent. It possesses a high flammability and may toxic when consumed or excessively inhaled. While thiophene does not readily dissolve in water, it exhibits solubility in various organic solvents including ethanol, ether, and benzene [48].

Thiophene is an essential component of numerous significant molecules and is a widely used building block in chemical synthesis. Numerous medicines, agrochemicals, pigments, polymers, and other organic compounds can be made using it as a substrate. Its distinct electrical characteristics make it useful in materials research, particularly in the development of organic semiconductors and conductive polymers [57, 58].

Thiophene can undergo electrophilic aromatic substitution reactions similar to benzene. For example, it can react with nitric acid or sulfuric acid in the presence of a catalyst to form nitro- or sulfonic acid derivatives, respectively. In oxidation reaction, thiophene can be oxidized to form thiophene oxide or other oxidized derivatives. This reaction is often carried out using strong oxidizing agents, such as hydrogen peroxide or peracids. Moreover, thiophene can be directly metalated using strong bases, such as n-butyl lithium (n-BuLi) or lithium diisopropylamide (LDA), these bases deprotonate the thiophene at the 2-position, resulting in the formation of the corresponding metal-

thiophene complex. The choice of base and reaction conditions depends on the desired metalation site and the subsequent reactions [48].

1.3.4 Pyridine

A heterocyclic organic molecule with the chemical formula C_5H_5N is pyridine (azabenzene). It is a colorless liquid with a distinct odor, soluble in water, alcohol, and most organic solvents. The substitution of more electronegative N for the aromatic CH results in a dipole moment of 2.2 D, which indicates a shift in the electron density away from the ring and towards the nitrogen atom. The nitrogen atom of pyridine has a partial negative charge, and the carbons 2 or 6 and 4 hold partial positive charges, according to the valence bond (resonance) description. Pyridine possesses characteristics of a basic compound due to the presence of a lone pair of electrons on its nitrogen atom. This lone pair enables it to act as a Lewis base by donating electrons to electron-deficient species like a proton (H^+), resulting in the formation of a coordinate covalent bond. However, pyridine's basicity is comparatively weaker than that of other common bases like amines. This is mainly because of the delocalization of the lone pair over both the nitrogen atom and the aromatic ring of pyridine. The delocalization phenomenon reduces the availability of the lone pair for protonation, thereby making the nitrogen atom less basic compared to a simple amine [48].

Because of their numerous bioactivities, pyridine derivatives are important compounds in pharmaceutical and medicinal chemistry. Numerous pyridine derivatives have antibacterial properties that make it possible for them to stop the development of bacteria, fungus, and other microbes. Examples include quinolones, a class of antibiotics, and isoniazid, a popular antitubercular medication [59, 60]. Some pyridine derivatives have shown promising anticancer activity. For instance, erlotinib and gefitinib are pyridine-containing drugs used for the treatment of certain types of lung cancer by inhibiting the epidermal growth factor receptor (EGFR) [61]. Moreover, some of pyridine derivatives exhibit anti-inflammatory properties, which can be useful in the treatment of inflammatory conditions. Diclofenac, a nonsteroidal anti-inflammatory drug (NSAID), contains a pyridine ring and is commonly used to relieve pain and inflammation [62].

Pyridine displays a range of reactions, encompassing nucleophilic substitution reactions at the C-2, C-4, and C-6 positions. Additionally, similar to benzene, pyridine can participate in electrophilic aromatic substitution reactions. The nitrogen atom in pyridine serves as a nucleophile, allowing it to attack electrophiles. Common electrophilic substitution reactions comprise nitration, halogenation, sulfonation, as well as Friedel-Crafts acylation or alkylation. Furthermore, pyridine can undergo acylation and alkylation reactions akin to benzene. When treated with acylating agents (such as acyl chlorides) or alkylating agents (like alkyl halides) in the presence of a Lewis acid catalyst, acyl or alkyl groups can be introduced onto the pyridine ring [48].

1.3.5 Indole

Indole is a solid heterocyclic chemical molecule having a bicyclic structure made up of a five-membered nitrogen-containing pyrrole ring joined to a six-membered benzene ring with a chemical formula C_8H_7N. It falls within the category of a bicyclic aromatic compounds, the aromatic nature of indole emerges from the existence of a conjugated π-electron system formed by the overlapping p-orbitals of the carbon and nitrogen atoms within the ring. Two carbon atoms are shared by the benzene ring and the pyrrole ring, leading to the dispersal of π-electrons throughout the entire structure. This generates a resonance structure that stabilizes the molecule and imparts it with distinct properties [48].

The antimicrobial effects of indole derivatives are demonstrated against a variety of microorganisms, such as bacteria, fungus, and protozoa. They can stop germs from growing and surviving by interfering with vital cellular functions [63]. Indole compounds have shown promising anticancer activity by various mechanisms. They have the ability to prevent the growth of cancer cells, trigger apoptosis (programmed cell death), and obstruct tumor angiogenesis (the development of blood vessels to support tumor growth). Indole-3-carbinol (I_3C), a compound found in cruciferous vegetables, is known for its potential cancer-preventive properties and it can modulate estrogen metabolism, potentially reducing the risk of certain hormone-related cancers [64]. Moreover, certain indole compounds exhibit antioxidant properties, enabling them to eliminate free radicals and safeguard cells against oxidative harm. These compounds hold promise for potential utilization in the prevention or treatment of disorders linked to oxidative stress, including cardiovascular diseases and specific types of cancer. [65].

The Mannich and Vilsmeier reactions, as well as a number other electrophilic aromatic substitution processes, are all carried out on indole. In electrophilic aromatic substitution processes, an electrophile is used to replace an atom or a group in a molecule. The C3 position of the pyrrole ring, which is the most electron-rich location, is the most frequent site of electrophilic substitution in the case of indole. An amine, a carbonyl molecule, and an acid catalyst are joined in the Mannich reaction of indole to produce a Mannich base. An attack on the carbonyl carbon of the carbonyl compound is caused by the nucleophilic nitrogen of the indole ring, which is followed by a proton transfer and the subsequent elimination of a water molecule. An N-alkylated indole derivative is the end result. Moreover, the formylating agent and the Lewis acid catalyst interact during the Vilsmeier reaction of indole to produce the formyl cation, an electrophilic species. The indole ring's C3 position is subsequently attacked by the formyl cation, which results in the insertion of a formyl group (CHO) there [48].

1.3.6 Chromone-4-one

Chromones, also known as benzopyran-4-ones or $C_9H_6O_2$ coumarin isomers, are a subset of the larger class of aromatic heterocyclic chemicals. They are distinguished by a fused ring structure made up of a benzene ring fused to an oxygen-containing pyran ring. In accordance with the patterns of substitution and the presence of extra functional groups, chromone can exist in a variety of structural variations [48].

Natural products belonging to the chromone family have a variety of biological effects, such as anticancer, anti-inflammatory, antibacterial, antiviral, atypical antipsychotic, and anti-platelet effects. This is largely because of their well-known antioxidant properties, which result from their capacity to neutralize active forms of oxygen and to stop free radical processes [66-68].

Chromone derivatives can undergo electrophilic aromatic substitution reactions. The carbonyl group in chromone can act as a nucleophile and react with electrophilic reagents. This can lead to the introduction of various functional groups, such as alkyl, acyl, or halogen substituents, at different positions of the chromone ring. Also, Mannich reaction for chromone proceeds in the presence of the amine and the acidic component. The amine acts as a nucleophile and attacks the electrophilic carbonyl carbon, resulting in the formation of an imine intermediate. Subsequent protonation of the imine by the

acidic component and subsequent tautomerization leads to the formation of the β-amino carbonyl product [48].

1.3.7 Pyrrolidine

Tetrahydropyrrole, also known as pyrrolidine, is an aliphatic heterocyclic organic molecule having the chemical formula $(CH_2)_4NH$. It has a five-membered ring made up of four carbon atoms and one nitrogen atom. The ring structure of pyrrolidine is similar to that of pyrrole, but with saturated carbon atoms. It is a colorless liquid that dissolves in water and in the most of the organic solvents and it has a distinctive odor. Because the nitrogen atom has a single pair of electrons, pyrrolidine can function as a Lewis base by giving its electron pair to an acid or proton to generate a coordinate covalent bond. Because of this interaction, pyrrolidine can engage in acid-base processes and accept a proton to transform into the positively charged molecule pyrrolidinium [43].

Pyrrolidine derivatives have shown neuroprotective properties in various studies. They can potentially protect neurons from damage caused by oxidative stress, inflammation, and neurodegenerative disorders [69]. Pyrrolidine compounds have been found to possess antioxidant properties. They can scavenge free radicals and inhibit oxidative stress, which is associated with various diseases, including cardiovascular disorders, cancer, and aging-related conditions [70]. The growth of bacteria, fungus, and other microbes has also been found to be inhibited by a few pyrrolidine derivatives, demonstrating antibacterial characteristics. These substances might interfere with microbial cell membranes or stop vital enzymes from functioning [71].

Many chemical processes, particularly N-alkylation and acylation reactions, contain the molecule pyrrolidine. With alkyl halides or alkylating agents, pyrrolidine can undergo a N-alkylation reaction. In order to deprotonate the nitrogen and boost its nucleophilicity, this reaction requires replacing the hydrogen atom on the nitrogen atom with an alkyl group, and it is commonly conducted in the presence of a base like sodium hydroxide (NaOH). In addition, pyrrolidine is capable of going through N-acylation processes, in which the nitrogen atom is given an acyl group. In order to perform this reaction, pyrrolidine is often treated with an acylating agent, such as an acyl chloride (for example, acetyl chloride) or an acid anhydride (for example, acetic anhydride).

Frequently, a base, such as pyridine or triethylamine, acts as the catalyst for the reaction [43].

1.3.8 Piperazine

The non-aromatic heterocyclic compound class known as the diamines includes piperazine, an organic chemical part. A six-membered ring containing two nitrogen atoms at positions 1 and 4, separated by two carbon atoms, makes up the compound. The chemical formula for piperazine is $C_4H_{10}N_2$ [43].

A versatile substance, piperazine is used in many different industries. It can be found in the production of many different medications and is frequently employed as a pharmaceutical intermediary. Piperazine derivatives have been utilized as anthelmintic medications in the medical field to treat parasitic worm infections. They function by paralyzing the worms so that the body can get rid of them more easily [72]. A few compounds of piperazine have demonstrated anticancer effects [73]. Additionally, piperazine derivatives can alter the release, reuptake, or metabolism of neurotransmitters via interacting with neurotransmitter receptors in the brain. The central nervous system may be affected in a number of ways by this modulation, including by stimulating or inhibiting particular signaling pathways [74].

Alkylating agents or alkyl halides can react with piperazine to produce an alkylated form. Usually, the reaction takes place at one of the nitrogen atoms, replacing a hydrogen atom with an alkyl group. Alkylation of piperazine can yield a range of alkylpiperazines with diverse properties. Also piperazine can undergo acylation reactions, where an acyl group (R-CO-) replaces one of the nitrogen atoms. This reaction is commonly employed to introduce functional groups onto the piperazine ring. For example, treatment of piperazine with acetic anhydride or acyl chlorides can lead to the formation of acylpiperazines. Moreover, piperazine can undergo reductive amination reactions, where an aldehyde or ketone reacts with piperazine in the presence of a reducing agent. This reaction leads to the formation of secondary amines, introducing additional functionality to the piperazine molecule [43].

1.4 The objectives of this study

1. To synthesize a novel heterocyclic esters derived from several alcohols and heterocyclic carboxylic acids.
2. To synthesize a novel heterocyclic amides derived from heterocyclic amines and carboxylic acids.
3. To synthesize a novel heterocyclic imine compounds derived from several heterocyclic carbonyl compounds.
4. To identify all the synthesized compounds by using FT-IR, ^{1}H and ^{13}C NMR analysis.
5. To evaluate the biological activities of all synthesized compounds icluding (anticancer, antibacterial and antioxidant activities).

Chapter Two

Experimental part

2.1 Chemistry

2.1.1 Chemicals and Instruments

All of the chemicals and solvents were bought from Chemical Science Company and Sigma-Aldrich Chemical Company, respectively. Without additional purification, materials and solvents were used. Nuclear magnetic resonance spectra (^{1}H-NMR and^{13}C-NMR) were recorded on Bruker 500MHz-Avance III at the Chemistry Department at University of Jordan. Infrared spectroscopy (FTIR) was recorded on Fourier transform infrared spectrophotometer (Nicolet 1s5-Id3) at the Chemistry Department at An-Najah National University. Also Waters 1525 binary HPLC pump and Waters 2998 photodiode array detector were used in this research. The glassware of reactions was dried by using Bunsen burner flame or oven and cooled under vacuum. All reactions were done under nitrogen gas. TLC analysis was performed on silica gel plates pre-coated with Merck Kiesel gel 60 F254 and was purchased from Sigma-Aldrich Chemical Company also visualization was done by using UV lamp. Rotary evaporator with membrane pump at 30-50mbar was used to remove the solvents. Using the Stuart Melting Point Apparatus (R00102618), melting points were measured. Moreover, flash column chromatography was used with silica gel from Sigma-Aldrich, 60°A pore size and 230-400 mesh, 40-63µm particle under 5 psi compressed air.

2.1.2 Synthetic procedures

The compounds prepared in this thesis were divided into seven categories. Each category of compounds was prepared in the same way and studied using the same biological tests. It can be seen that the compounds are not sequentially numbered (compound symbols) because each category of compounds was prepared separately. Table 2.1 shows the prepared categories and the symbols of the compounds to which they belong.

Table 2.1

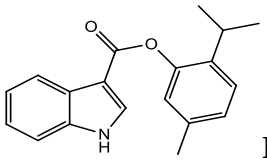
Categories of prepared compounds

Category number	Category name	Symbol of compound/s
1	Thymol ester	E2
2	Carvacrol esters	E4, E5, E6, E7, and E8
3	Esters	Ea, Eb, Ec, and Ed
4	Flavonoid esters	Ee1 and Ee2
5	Amides	A1, A2, Aa, and Ab
6	Schiff bases	3, 4, 7, and 8
7	Schiff bases	1, 2, 5, and 6

2.1.2.1 Synthesis of Thymol Ester (E2)

In a 100 ml round bottom flask under nitrogen atmosphere and in an iced water bath, indole-3-carboxylic acid (1 eq.) and 2 drops of dry dimethylformamide were dissolved in 10 mL of dry dichloromethane. Oxalyl chloride (2 eq.) was added to the mixture which was then stirred for 3 hours. After that, the solvent was evaporated under vacuo. A solution of thymol (1eq.) and triethylamine (1eq.) was added to a stirred solution of residue in 10 ml of dry DCM under nitrogen atmosphere and the mixture was stirred for 24 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate and extracted three times with DCM (1:1). The combined organic layer was dried with $MgSO_4$ drying agent, then the sample was filtered and the solvent was evaporated under vacuum. Through the use of flash chromatography, the product was purified by using a hexane: ethyl acetate (7:3) as an eluant system. Crystals were obtained from the final product through crystalization by the use of dichloromethane and hexane solvents (4.5:0.5) to produce **E2** pure compound.

2-Isopropyl-5-methylphenyl 1H-indole-3-carboxylate (E2)



E2

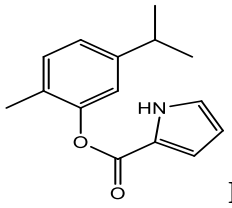
Purified by flash chromatography using a hexane: ethyl acetate (7:3) as an eluant. Light pink colored crystal; 77.8% yield; Melting point 183-184°C; IR main functional groups: 3273.66 cm^{-1} $_{N\text{-}H\ indole}$, 2961.49 cm^{-1} $_{C\text{-}H\ aromatic}$, 2869.82 cm^{-1} $_{C\text{-}H\ aliphatic}$, 1690.16 cm^{-1} $_{C=O\ ester}$, 1619.9 cm^{-1} $_{C=C\ thymol}$, 1581 cm^{-1} $_{C=C\ indole}$. ^1H NMR (500 MHz, DMSO, ppm): 12.14 (1H, s, broad), 8.31 (1H, d, J = 2.3 Hz), 8.01 (1H, d, J = 7.6 Hz), 7.54 (1H, d, J =

7.6Hz), 7.23-7.26 (3H, m), 7.06 (1H, d, J = 7.9), 6.95 (1H, s), 3.03 (1H, sept), 2.29 (3H, s), 1.14 (6H, d, J = 6.9). ^{13}C NMR: (ppm), 136.39 (1C, -C=O), 148.34 (1C, -C-O-), 137.62 (1C, -NH-C=C- indole), 137.04 (1C, -O-C=C-CH(CH_3)$_2$), 136.49 (1C, -C=C-CH_3), 126.26 (1C, -NH-C=C- indole), 105.9 (1C, -C=C-C=O- indole), 133.36 (1C, -NH-C=C-C=O- indole), 128.55 (1C, CH aryl thymol), 127.2 (1C, CH aryl thymol), 123.6 (1C, CH aryl thymol), 122.22 (1C, CH aryl indole), 120.6 (1C, CH aryl indole), 118.1 (1C, CH aryl indole), 112.5 (1C, CH aryl indole), 26.9 (1C, -CH(CH_3)$_2$), 23.02 (2C, 2CH_3), 21.82 (1C, CH_3) (see Fig. 1, 25(a) and 25(b) in Appendix A).

2.1.2.2 Synthesis of Carvacrol Esters (E4, E5, E6, E7 and E8)

A 100 ml round bottom flask was placed in an ice water bath with a solution of carboxylic acid (1 equivalent) and 2 drops of dry dimethylformamide in 10 mL of dry dichloromethane under nitrogen atmosphere. The mixture was then supplemented with oxalyl chloride (2 equivalent), and the mixture was stirred for 3 hours. Following that, the solvent was evaporated in a vacuum before the products were used without being purified in the synthesis of esters. Carvacrol (1 equivalent) and triethylamine (1 equivalent) were added to a stirred solution of an acid chloride in 10 ml of dry DCM under nitrogen atmosphere, and the combination was stirred continuously at room temperature for 24 hours. The reaction was observed by TLC. Then the reaction was extracted by using saturated sodium bicarbonate solution and DCM (1:1). Following separation, the organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent was then evaporated under vacuum. Through the use of flash chromatography, the products were purified.

5-Isopropyl-2-methylphenyl 1H-pyrrole-2-carboxylate (E4)

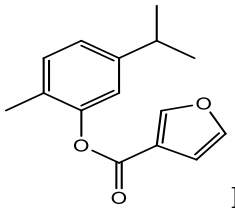


E4

Purified by flash chromatography using a hexane : ethyl acetate (8:2) as an eluant. Pale yellow colored liquid; 50% yield; IR main functional groups: 3250.01 cm $^{-1}$ $_{\text{N-H pyrrole}}$, 2961.74 cm^{-1} $_{\text{C-H aromatic}}$, 2869.66 cm^{-1} $_{\text{C-H aliphatic}}$, 1740.81 cm^{-1} $_{\text{C=O ester}}$, 1619 cm^{-1} $_{\text{C=C}}$

$_{carvacrol}$, 1576.28 cm^{-1} $_{C=C\ pyrrole}$. ^{1}H NMR (500 MHz, DMSO, ppm): 8.52 (1H, s), 7.21 (1H, d, J = 7.8 Hz), 7.04 (2H, m), 6.92 (1H, d, J = 7.6 Hz), 6.62 (1H, s), 6.54 (1H, d, J = 7.6 Hz), 2.86 (1H, sept, J = 7 Hz), 2.08 (3H, s), 1.17 (6H, d, J= 6.9 Hz) (see Fig. 2 and 26 in Appendix A).

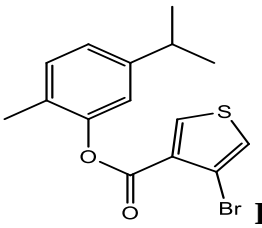
5-Isopropyl-2-methylphenyl furan-3-carboxylate (E5)



E5

Purified by flash chromatography using a dichloromethane : ethyl acetate (9.5:0.5) as an eluant. Colorless liquid; 55% yield; IR main functional groups: 2961.21 cm^{-1} $_{C-H\ aromatic}$, 2869 cm^{-1} $_{C-H\ aliphatic}$, 1740.55 cm^{-1} $_{C=O\ ester}$, 1619 cm^{-1} $_{C=C\ carvacrol}$, 1570.22 cm^{-1} $_{C=C\ furan}$. ^{1}H NMR (500 MHz, DMSO, ppm): 8.64 (1H, s), 7.9 (1H, q, J = 7.8 Hz), 7.21 (1H, d, J = 7.7 Hz), 7.07 (1H, dt, J = 7.8 Hz), 7.01 (1H, d, J = 1.9 Hz), 6.94 (1H, d, J = 7.7 Hz), 2.86 (1H, sept, J = 7 Hz), 2.08 (3H, s), 1.18 (6H, dd, J = 6.9 Hz) (see Fig. 3 and 27 in Appendix A) .

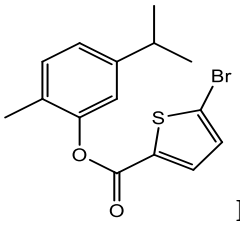
5-Isopropyl-2-methylphenyl 4-bromothiophene-3-carboxylate (E6)



E6

Purified by flash chromatography using a hexane : dichloromethane (6:4) as an eluant. Colorless liquid; 60.5% yield; IR main functional groups: 2959.21 cm^{-1} $_{C-H\ aromatic}$, 2866.5 cm^{-1} $_{C-H\ aliphatic}$, 1741.36 cm^{-1} $_{C=O\ ester}$, 1615.83 cm^{-1} $_{C=C\ carvacrol}$, 1495.41 cm^{-1} $_{C=C\ thiophene}$. ^{1}H NMR (500 MHz, DMSO, ppm): 8.76 (1H, m), 7.9 (1H, dd, J = 3.4 Hz), 7.23 (1H, d, J = 7.8 Hz), 7.09 (1H, d, J = 7.8 Hz, Ar-H), 7.05 (1H, s), 2.88 (1H, sept, J = 7.1 Hz), 2.11 (3H, s), 1.19 (6H, m) (see Fig. 4 and 28 in Appendix A) .

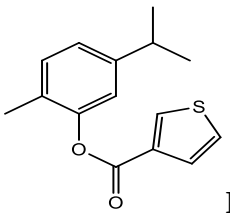
5-Isopropyl-2-methylphenyl 5-bromothiophene-2-carboxylate (E7)



E7

Purified by flash chromatography using a hexane : dichloromethane (6:4) as an eluant. Pale yellow colored liquid; 62.5% yield; IR main functional groups: 2962.61 cm^{-1} $_{C-H\ aromatic}$, 2866.49 cm^{-1} $_{C-H\ aliphatic}$, 1725.61 cm^{-1} $_{C=O\ ester}$, 1619.61 cm^{-1} $_{C=C\ carvacrol}$, 1547.67 cm^{-1} $_{C=C\ thiophene}$. ^{1}H NMR (500 MHz, DMSO, ppm): 7.86 (1H, dd, J = 4.1), 7.47 (1H, dd, J = 4.1Hz), 7.23 (1H, d, J = 7.8 Hz), 7.09 (2H, m), 2.87 (1H, sept, J = 7.1 Hz), 2.09 (3H, s), 1.17 (6H, d, J = 6.9 Hz) (see Fig. 5 and 29 in Appendix A).

5-Isopropyl-2-methylphenyl thiophene-3-carboxylate (E8)



E8

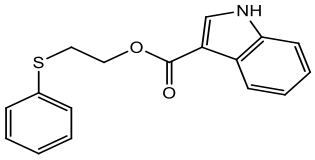
Purified by flash chromatography using a dichloromethane : ethyl acetate (9.5:0.5) as an eluant. Colorless liquid; 65% yield; IR main functional groups: 2960.49 cm^{-1} $_{C-H\ aromatic}$, 2865.8 cm^{-1} $_{C-H\ aliphatic}$, 1732.46 cm^{-1} $_{C=O\ ester}$, 1619 cm^{-1} $_{C=C\ carvacrol}$, 1578.03 cm^{-1} $_{C=C\ thiophene}$. ^{1}H NMR (500 MHz, DMSO, ppm): 8.6 (1H, dt, J = 3.9 Hz), 7.74 (1H, dt, J = 3.9 Hz), 7.61 (1H, dd, J = 5 Hz), 7.22 (1H, d, J = 7.8 Hz), 7.08 (1H, d, J = 7.8 Hz), 7.03 (1H, d, J = 2.2 Hz), 2.87 (1H, sept, J = 7 Hz), 2.09 (3H, s), 1.18 (6H, dd, J = 6.9) (see Fig. 6 and 30 in Appendix A) .

2.1.2.3 Synthesis of Esters (Ea, Eb, Ec and Ed)

Carboxylic acid compound (1 eq.) and 2 drops of dry dimethylformamide were dissolved in 10 mL of dry DCM and placed in a 100 ml round bottom flask under nitrogen atmosphere and at cooled water bath. Two equal parts of oxalyl chloride was added to the mixture, which was then stirred for three hours. The solvent then evaporated under vacuum after that. A solution of alcohol (1 eq.) and triethylamine

(1 eq.) were added to a stirred solution of residue in 10 ml of dry DCM under nitrogen atmosphere and the mixture was stirred for 48 hours at room temperature. The sample was then extracted three times using a 1:1 mixture of DCM and saturated $NaHCO_3$ solution. The organic layer was collected and magnesium sulfate drying agent was added, then the sample was filtered and the solvent was removed under vacuum. Finally, the product was purified by using flash chromatography technique.

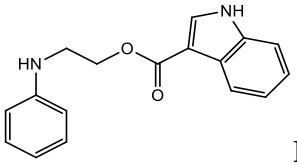
2-(phenylthio)ethyl 1H-indole-3-carboxylate (Ea)



Ea

Purified by flash chromatography using a ethyl acetate : hexane (1:9) as an eluant. Colorless liquid; 50% yield; IR main functional groups: 2977.31cm^{-1} C-H aromatic, 2888.65cm^{-1} C-H aliphatic, 1713.98cm^{-1} C=O ester, 1583.1cm^{-1} C=C Aryl, 1480.09cm^{-1} C=C indole, 3053.9cm^{-1} NH indole. ^{1}H NMR (500 MHz, DMSO, ppm): 8.36 (1H, dd), 8.15 (1H, m), 7.65 (1H, dd), 7.48 (1H, dd), 7.3 (1H, m), 7.1 (2H, m), 6.66 (4H, m), 4.34 (2H, t), 4.13 (2H, dt) (see Fig. 7 and 31 in Appendix A) .

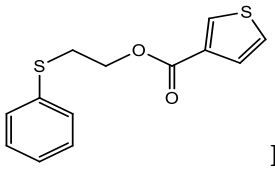
2-(phenylamino)ethyl 1H-indole-3-carboxylate (Eb)



Eb

Purified by flash chromatography using a ethyl acetate : dichloromethane (1:9) as an eluant. Off-white colored solid; 60% yield; Melting point 82-84°C; IR main functional groups: 2971.84cm^{-1} C-H aromatic, 2955.15cm^{-1} C-H aliphatic, 1678.14cm^{-1} C=O ester, 1602.82cm^{-1} C=C Aryl, 1531.5cm^{-1} C=C indole, 3377.92cm^{-1} NH indole, 3050.13cm^{-1} NH amine. ^{1}H NMR (500 MHz, DMSO, ppm): 11.99 (1H, m), 8.13 (1H, d), 8.03 (1H, m), 7.5 (1H, m), 7.18 (4H, m), 6.75 (2H, d), 6.63 (1H, t), 5.76 (1H, t), 4.37 (2H, t), 3.46 (2H, q) (see Fig. 8 and 32 in Appendix A).

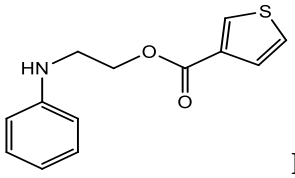
2-(phenylthio)ethyl thiophene-3-carboxylate (Ec)



Ec

Purified by flash chromatography using a ethyl acetate : dichloromethane (1:9) as an eluant. Colorless liquid; 55% yield; IR main functional groups: 3053.13cm^{-1} C-H aromatic, 2957.18cm^{-1} C-H aliphatic, 1718.77cm^{-1} C=O ester, 1582.63cm^{-1} C=C Aryl, 1521.6cm^{-1} C=C thiophene. ^{1}H NMR (500 MHz, DMSO, ppm): 8.23 (1H, m), 7.64 (2H, dd), 7.39 (4H, m), 7.23 (1H, m), 4.33 (2H, t), 3.72 (2H, t) (see Fig. 9 and 33 in Appendix A).

2-(phenylamino)ethyl thiophene-3-carboxylate (Ed)



Ed

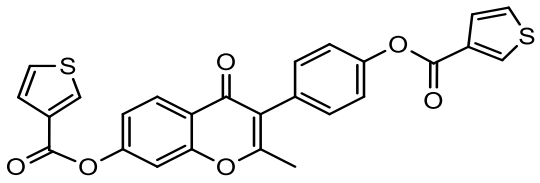
Purified by flash chromatography using a ethyl acetate : dichloromethane (1:9) as an eluant. Olive colored liquid; 53% yield; IR main functional groups: 2928.32cm^{-1} C-H aromatic, 2850.66cm^{-1} C-H aliphatic, 1714.57cm^{-1} C=O ester, 1602.17cm^{-1} C=C Aryl, 1519.4cm^{-1} C=C thiophene, 3109.75cm^{-1} NH amine.^{1}H NMR (500 MHz, DMSO, ppm): 8.35 (1H, dd), 7.65 (1H, dd), 7.48 (1H, dd), 7.08 (2H, m), 6.64 (2H, tt), 6.55 (1H, t), 5.78 (1H, t), 4.33 (2H, t), 3.3 (2H, q) (see Fig. 10 and 34 in Appendix A).

2.1.2.4 Synthesis of Flavonoid Esters (Ee1 and Ee2)

10 mL of dry dichloromethane was used to dissolve two equivalents of the carboxylic acid compound, along with two drops of dry dimethylformamide. This mixture was then put in a 100 ml round-bottom flask under nitrogen atmosphere in an ice bath. Following the addition of 2.5 equivalents of oxalyl chloride, the mixture was stirred for 3 hours. After then, the solvent was vacuum-evaporated. A solution of residue of acid chloride in 5 ml of dimethylformamide was mixed with a solution of one equivalent of 7-hydroxy-3-(4-hydroxyphenyl)-2-methyl-4H-chromen-4-one and 2.2 equivalents of sodium tert-butoxide in 5 ml of dimethylformamide . The mixture was stirred for 24 hours at room

temperature. The product was extracted using ethyl acetate, and the ethyl acetate layers were then washed three times with brine solution. The organic layer was collected, $MgSO_4$ anhydrous salt was added, the sample was filtered, and the solvent was evaporated under vacuum. Utilizing flash chromatography, the products were purified.

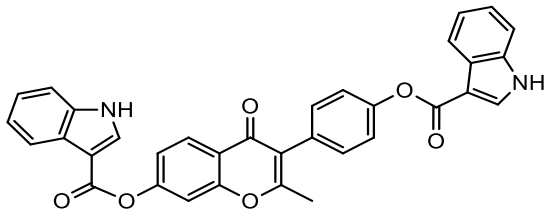
2-methyl-4-oxo-3-(4-((thiophene-3-carbonyl)oxy)phenyl)-4H-chromen-7-yl thiophene-3-carboxylate (Ee1)



Ee1

Purified by flash chromatography using a DCM : ethyl acetate (9:1) as an eluant. Pale yellow colored liquid; 47% yield; IR main functional groups: 2925.27cm^{-1} C-H aromatic, 2854.06cm^{-1} C-H aliphatic, 1715.88cm^{-1} C=O ester, 1622.43cm^{-1} C=C Aryl, 1514.2cm^{-1} C=C thiophene.^{1}H NMR (500 MHz, DMSO, ppm): 8.45 (3H, m), 8.29 (2H, m), 8.00 (2H, m), 7.7 (2H, m), 7.35 (2H, m), 6.86 (2H, m), 2.27 (3H, s) (see Fig. 11 and 35 in Appendix A).

4-(7-((1H-indole-3-carbonyl) oxy)-2-methyl-4-oxo-4H-chromen-3-yl)phenyl 1H-indole-3-carboxylate (Ee2)



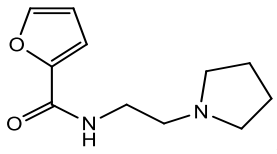
Ee2

Purified by flash chromatography using a DCM : ethyl acetate (6:4) as an eluant. Pale yellow colored liquid; 44% yield; IR main functional groups: 2922.8cm^{-1} C-H aromatic, 2854.36cm^{-1} C-H aliphatic, 1733.6cm^{-1} C=O ester, 1638.15cm^{-1} C=C Aryl, 1515.34cm^{-1} C=C indole, 3289.64cm^{-1} N-H indole.^{1}H NMR (500 MHz, DMSO, ppm): 12.77 (2H, d), 9.6 (2H, dd), 8.65 (5H, m, 7.7 (6H, m), 6.82 (4H, m), 2.51 (3H , s) (see Fig. 12 and 36 in Appendix A).

2.1.2.5 Synthesis of Amides (A1, A2, Aa and Ab)

One equivalent of carboxylic acid compound and 2 drops of dry dimethylformamide were dissolved in 10 mL of dry dichloromethane and placed in a 100 ml round-bottom flask under nitrogen atmosphere in an iced water bath. After that (1.3 equivalent) of oxalyl chloride was added, and the mixture was stirred for three hours. The solvent was then evaporated under vacuum. A stirred solution of residue in 10 ml of dry DCM under nitrogen atmosphere was combined with a solution of aliphatic primary amine (1 equivalent) and triethylamine (1 equivalent), and the mixture was stirred for 24 hours at room temperature. After the solvent was evaporated, DCM and a 10% potassium carbonate solution (1:1) were used to extract the sample. $MgSO_4$ anhydrous salt was added after the organic layer was collected, then the sample was filtered, and the solvent was evaporated under vacuum. The products were purified by using prep TLC plate or flash chromatography technique.

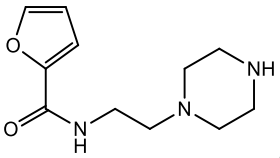
N-(2-(pyrrolidin-1-yl)ethyl)furan-2-carboxamide (A1)



A1

Purified by prep TLC plate using a dichlromethane : methanol (6:4) as an eluant. Yellow gumy solid; 78% yield; IR main functional groups: 3305.79 cm^{-1} $_{N\text{-}H\ amide,}$ 2962.08 cm^{-1} $_{C\text{-}H\ aromatic}$, 2794.17 cm^{-1} $_{C\text{-}H\ aliphatic}$, 1651.73 cm^{-1} $_{C=O\ amide}$, 1573.14 cm^{-1} $_{C=C\ furan}$. ^{1}H NMR (500 MHz, DMSO, ppm): 8.24 (1H, t, J= 5.7 Hz), 7.8 (1H, s), 7.06 (1H, d, J = 3.5 Hz), 6.59 (1H, d, J= 3.5Hz), 3.32 (2H, q, J = 6.5 Hz), 2.49 (4H, m), 2.54 (2H, t, J =6.9 Hz), 1.66 (4H, q, J = 3.1 Hz) (see **Fig. 13** and **37** in Appendix A) .

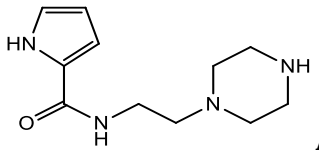
N-(2-(piperazin-1-yl)ethyl)furan-2-carboxamide (A2)



A2

Purified by prep TLC plate using a dichlromethane : methanol (6:4) as an eluant. Brown gumy solid; 79% yield; IR main functional groups: 3307.9 cm^{-1} N-H amide, 3117.88 cm^{-1} N-H piprazine, 2939.31 cm^{-1} C-H aromatic, 2815.71 cm^{-1} C-H aliphatic, 1647.49 cm^{-1} C=O amide, 1572.26cm^{-1} C=C furan. ^{1}H NMR (500 MHz, DMSO, ppm): 8.25 (1H, t, J = 5.8 Hz), 7.81 (1H, d, J = 3.8 Hz), 7.03 (1H, d, J= 3.8 Hz), 6.6 (1H, m), 3.64 (3H, m), 2.48 (8H, m), 2.8 (2H, t, J = 6.9 Hz) (see Fig. 14 and 38 in Appendix A).

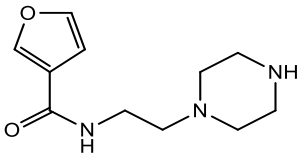
N-(2-(piperazin-1-yl)ethyl)-1H-pyrrole-2-carboxamide (Aa)



Aa

Purified by flash chromatography using an ethyl acetate : methanol (9:1) as an eluant. Yellow gumy solid; 70% yield; IR main functional groups: 3238.15 cm^{-1} N-H amide, 3100.5 cm^{-1} N-H pyrrole, 2945.36 cm^{-1} C-H aromatic, 2809.5 cm^{-1} C-H aliphatic, 1730.46 cm^{-1} C=O amide, 1564.96-1587.34cm^{-1} C=C pyrrole. ^{1}H NMR (500 MHz, DMSO, ppm): 11.4 (1H, s), 7.92 (1H, t, J = 5.6 Hz), 6.84 (1H, dq, J = 17.1Hz), 6.56 (1H, m), 6.08 (1H, m), 3.67 (4H, m), 3.36 (6H, m), 2.47 (3H, m) (see Fig. 15 and 39 in Appendix A).

N-(2-(piperazin-1-yl)ethyl)furan-3-carboxamide (Ab)



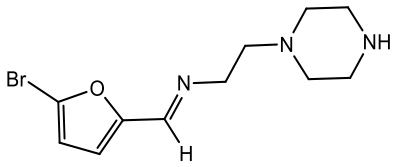
Ab

Purified by flash chromatography using an ethyl acetate : methanol (7:3) as an eluant. Yellow gumy solid; 67% yield; IR main functional groups: 3333.15 cm^{-1} N-H amide, 3050.13 cm^{-1} N-H piprazine, 2921.67 cm^{-1} C-H aromatic, 2852.79 cm^{-1} C-H aliphatic, 1624.89 cm^{-1} C=O amide, 1572.09cm^{-1} C=C furan. ^{1}H NMR (500 MHz, DMSO, ppm): 8.10 (1H, m), 7.71 (1H, m), 6.82 (1H, s), 6.63 (1H, s), 3.55 (3H, t, J = 4.8 Hz), 2.48 (10H, m) (see Fig. 16 and 40 in Appendix A).

2.1.2.6 Synthesis of Schiff bases (3, 4, 7 and 8)

2 mmol (0.35g) of 5-bromo-2-furaldehyde was incorporated into a 100 ml RBF along with 2 mmol (0.26g) of 1, 2-diaminoethyl piperazine or 2 mmol (0.21g) of diethylenetriamine in 20 ml of dichloromethane. The mixture was stirred for 48 hours at room temperature under nitrogen atmosphere. TLC was used to monitor the reaction while using the eluant [ethyl acetate: hexane, 8:2]. Dichloromethane and water were used to extract the organic layer, and the organic layer's water was subsequently removed using a drying agent such $MgSO_4$. By utilizing [DCM: methanol, (96:4)] as an eluant in a prep TLC plate, the crude compound was purified. Oily products were produced after the solvent was evaporated in a vacuum. The procedure was carried out again for 2 mmol (0.22 g) of thiophene-2-carbaldehyde with 2 mmol (0.21 g) of diethylenetriamine or with 2 mmol (0.18 g) of N,N-dimethylethylenediamine.

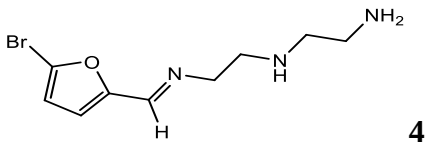
(E)-N-((5-bromofuran-2-yl)methylene)-2-(piperazin-1-yl)ethanamine (3)



3

Orange colored liquid; 87% yield; IR main functional groups: 2940.97 $cm^{-1}_{\text{C-H aromatic}}$, 2811.28 $cm^{-1}_{\text{C-H aliphatic}}$, 1647.24 $cm^{-1}_{\text{C=N}}$, 1478.81 $cm^{-1}_{\text{C=C}}$, 3270.49 $cm^{-1}_{\text{N-H}}$ of primary amine was disappeared.^{1}H NMR (500 MHz, DMSO, ppm): 8.08 (1H, s, azomethine), 6.92 and 6.72 (2H, dd, furan), 4.04 (2H, t, -C=N-C$\underline{H}_2$), 3.6 (1H, s, NH), 3.5 (4H, m, -N-C$\underline{H}_2$-CH_2-NH-, piperazine), 3.01 (4H, m, -N-CH_2-C$\underline{H}_2$-NH-,piperazine), 2.82 (2H, t, CH_2-C$\underline{H}_2$-N-). ^{13}C NMR (500 MHz, DMSO, ppm): 153.82 (-$\underline{C}$-CH=N-, furan), 150.05 (-C-$\underline{C}$H=N-, azomethine), 125.33 (Br-$\underline{C}$=C-C=C-, furan) , 115.73 (2C, Br-C=$\underline{C}$-$\underline{C}$=C-, furan), 79.43 (-CH_2-$\underline{C}H_2$-N-), 58.4 (=N-$\underline{C}H_2$-CH_2-N-), 52.5 (2C,-N-$\underline{C}H_2$-CH_2-NH, piperazine), 48.16 (2C,-N-$\underline{C}H_2$-CH_2-NH, piperazine) (see Fig. 17 in Appendix A and Table B.1 in Appendix B).

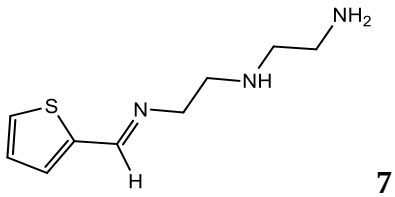
(E)-N^1-(2-aminoethyl)-N^2-((5-bromofuran-2-yl)methylene)ethane-1,2-diamine (4)



4

Orange colored liquid; 90% yield; IR main functional group: peak of two spikes at 3278.18 $cm^{-1}_{N-H}$of primary amine, 2971.29 $cm^{-1}_{C-H\ aromatic}$, 2900.9 $cm^{-1}_{C-H\ aliphatic}$, 1646.83 $cm^{-1}_{C=N}$, 1478.65 $cm^{-1}_{C=C}$.^{1}H NMR (500 MHz, DMSO, ppm): 8.02 (1H, s, azomethine), 6.72 and 6.45 (2H, dd, furan), 3.74 (2H, t, -C=N-C$\underline{H}_2$), 2.89 (2H, t, =N-CH_2-C$\underline{H}_2$-NH-), 2.75 (2H, m, -CH_2-C$\underline{H}_2$-NH_2), 2.72 (2H, m, -NH-C$\underline{H}_2$-CH_2-NH_2), 2.63 (1H, m, -NH-), 1.32 (2H, t, -NH_2). ^{13}C NMR (500 MHz, ppm): 164.89 (-C=$\underline{C}$-C=N-, furan), 161.82(-C=C-$\underline{C}$=N-, azomethine), 134.87(Br-$\underline{C}$=C-C-, furan), 119.37 (Br-C=C-$\underline{C}$-, furan), 114.69 (Br-C=$\underline{C}$-C-, furan) , 55.4(-C=N-$\underline{C}H_2$-), 47.9(-NH-$\underline{C}H_2$-CH_2-NH_2), 45.6 (=N-CH_2-$\underline{C}H_2$-NH-), 42.6 (-NH-CH_2-CH_2-NH_2) (see Fig. 18 in Appendix A and Table B.1 in Appendix B).

(E)-N1-(2-aminoethyl)-N2-(thiophen-2-ylmethylene)ethane-1,2-diamine (7)

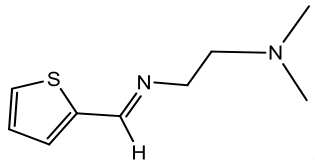


7

Yellow colored liquid; 88% yield; IR main functional group: peak of two spikes at 3270.54 $cm^{-1}_{N-H}$of primary amine, 2933.31 $cm^{-1}_{C-H\ aromatic}$, 2833.46 $cm^{-1}_{C-H\ aliphatic}$, 1632 $cm^{-1}_{C=N}$, 1432.83 $cm^{-1}_{C=C}$.^{1}H NMR (500 MHz, DMSO, ppm): 8.05 (1H, s, azomethine), 7.94-7.99 (1H, dd, -CH=C$\underline{H}$-S-, thiophene), 7.34 (1H, s, -C=C$\underline{H}$-CH=CH-S-, thiophene), 7.26-7.3 (1H, dd, -C$\underline{H}$=CH-S-, thiophene), 3.86 (2H, t, -CH=N-C$\underline{H}_2$-CH_2-NH-), 3.08-3.16 (2H, td, -CH=N-CH_2-C$\underline{H}_2$-NH-), 2.8-2.82 (2H, m, -NH-C$\underline{H}_2$-CH_2-NH_2), 2.74 (2H, m, -NH-CH_2-C$\underline{H}_2$-NH_2), 2.66 (1H, m, -N$\underline{H}$-), 1.03 (2H, t, -N$\underline{H}_2$). ^{13}C NMR (500 MHz, DMSO, ppm): 161.80 (CH, azomethine), 157.29 (-$\underline{C}$=CH-CH=CH-S-, thiophene), 132.6 (-CH=$\underline{C}$H-S-, thiophene), 132.5 (-C=$\underline{C}$H-CH=CH-S-, thiophene), 131.8 (-$\underline{C}$H=CH-S-, thiophene), 67.9 (-CH=N-$\underline{C}H_2$-CH_2-NH-), 56.8 (-NH-$\underline{C}H_2$-CH_2-

NH_2), 51.5 (-CH=N-CH_2-$\underline{C}H_2$-NH-), 44.98 (-NH-CH_2-$\underline{C}H_2$-NH_2) (see Fig. 19 in Appendix A and Table B.1 in Appendix B).

(E)-N1-dimethyl-N2-(thiophen-2-ylmethylene) ethane-1,2-diamine (8)



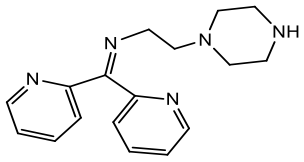
8

Light yellow colored liquid; 85% yield; IR main functional group: 2968.72 $cm^{-1}_{C\text{-}H\ aromatic}$, 2810.08 $cm^{-1}_{C\text{-}H\ aliphatic}$, 1632.94 $cm^{-1}_{C=N}$, 1432.94 $cm^{-1}_{C=C}$, 3356.43 $cm^{-1}_{N\text{-}H}$ of primary amine was disappeared.^{1}H NMR (500 MHz, DMSO, ppm): 8.3 (1H, s, azomethine), 7.24-7.25 (1H, d, -C=CH-CH=C$\underline{H}$-S-, thiophene), 7.18-7.19 (1H, d, -C=C$\underline{H}$-CH=CH-S-, thiophene), 6.1 (1H, t, -C=CH-C$\underline{H}$=CH-S-, thiophene), 3.47-3.49 (2H, t, -CH=N-C$\underline{H}_2$-CH_2-N-), 2.71 (2H, t, -CH=N-CH_2-C$\underline{H}_2$-N-), 0.93 (6H, s, $2CH_3$). ^{13}C NMR (500 MHz, DMSO, ppm): 154.78 (CH, azomethine), 144.78 (-$\underline{C}$=CH-CH=CH-S-, thiophene), 131.8 (-C=CH-CH=$\underline{C}$H-S-, thiophene),131.45 (-C=$\underline{C}$H-CH=CH-S-, thiophene), 115.89 (-C=CH-$\underline{C}$H=CH-S-, thiophene), 58.87 (-CH=N-CH_2-$\underline{C}H_2$-N-), 53.31 (-CH=N-$\underline{C}H_2$-CH_2-N-), 12.17 ($2CH_3$) (see Fig. 20 in Appendix A and Table B.1 in Appendix B) .

2.1.2.7 Synthesis of Schiff bases (1, 2, 5 and 6)

1 equivalent each of primary amine and heterocyclic ketone were dissolved in 15 ml of ethanol and 3 drops of sulfuric acid were then added to 100 ml of RBF. For 24 hours at 80°C, the mixture was refluxed under nitrogen atmosphere. In vacuum, the solvent was evaporated. The sulfuric acid catalyst was then removed from the sample using diethyl ether, and the solvent was then evaporated in *vacuo*. Using a prep TLC plate or a flash chromatography method, the products were purified.

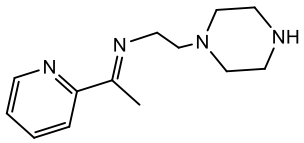
N-(di(pyridin-2-yl)methylene)-2-(piperazin-1-yl)ethanamine (1)



1

Purified by a flash chromatography using an ethyl acetate : hexane (9:1) as an eluant. White colored solid; 63% yield; Melting point 315-318°C; IR main functional groups: 2963.48 $cm^{-1}_{C\text{-}H\ aromatic}$, 2776.74 $cm^{-1}_{C\text{-}H\ aliphatic}$, 1629.96 $cm^{-1}_{C=N}$, 1459.33 $cm^{-1}_{C=C}$, 3270.49 $cm^{-1}_{N\text{-}H}$ of primary amine was disappeared.^{1}H-NMR (500 MHz, DMSO, ppm): 8.66 (2H, dd, pyridine), 7.97 (4H, m, pyridine), 7.4 (2H, m, pyridine), 3.72 (2H, t, -C=N-C$\underline{H}_2$-CH_2-), 2.49 (1H, q, N-H piperazine), 1.09 (10H, m, -C=N-CH_2-C$\underline{H}_2$-N- and 4CH_2 piperazine) (see Fig. 21 and 41 in Appendix A).

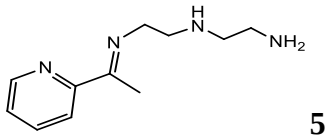
Z)-2-(piperazin-1-yl)-N-(1-(pyridin-2-yl)ethylidene)ethanamine (2)



2

Purified by flash chromatography using an ethyl acetate : hexane (9:1) as an eluant. Off-white colored solid; 67% yield; Melting point 266-267°C; IR main functional groups: 2980.47 $cm^{-1}_{C\text{-}H\ aromatic}$, 2765.67 $cm^{-1}_{C\text{-}H\ aliphatic}$, 1629.21 $cm^{-1}_{C=N}$, 1459.22 $cm^{-1}_{C=C}$, 3270.49 $cm^{-1}_{N\text{-}H}$ of primary amine was disappeared.^{1}H-NMR (500 MHz, DMSO, ppm): 9.97 (1H, d, pyridine), 7.93 (1H, t, pyridine), 7.54 (1H, d, pyridine), 7.13 (1H, t, pyridine),3.72 (2H, t, -C=N-C$\underline{H}_2$-CH_2-), 3.5 (1H, p, N-H piperazine), 2.94 (10H, m, -C=N-CH_2-C$\underline{H}_2$-N- and 8CH_2 piperazine), 2.18 (3H, s, CH_3) (see Fig. 22 and 42 in Appendix A) .

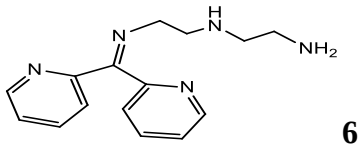
(Z)-N^1-(2-aminoethyl)-N^2-(1-(pyridin-2-yl)ethylidene)ethane-1,2-diamine (5)



5

Purified by prep TLC plate using an ethyl acetate : methanol (9:1) as an eluant. Burgundy colored liquid; 75% yield; IR main functional groups: 2967.34 $cm^{-1}_{C\text{-}H\ aromatic}$, 2888.13 $cm^{-1}_{C\text{-}H\ aliphatic}$, 1639.51 $cm^{-1}_{C=N}$, 1466.38 $cm^{-1}_{C=C}$, 3288.26 $cm^{-1}_{N\text{-}H}$ of primary amine.^{1}H-NMR (500 MHz, DMSO, ppm): 8.55 (1H, m, pyridine), 8.00 (1H, m, pyridine), 7.8 (1H, m, pyridine), 7.37 (1H, m, pyridine), 3.56 (2H, t, =N-C$\underline{H}_2$-CH_2-NH-), 3.39 (2H, q, =N-CH_2-C$\underline{H}_2$-NH-), 3.18 (2H, m, =N-CH_2-CH_2-NH-C$\underline{H}_2$-CH_2-NH_2), 2.85 (2H, tt, =N-CH_2-CH_2-NH-CH_2-C$\underline{H}_2$-NH_2), 2.28 (1H, p, NH), 1.69 (3H, s, CH_3), 1.04 (2H, t, NH_2) (see Fig. 23 and 43 in Appendix A) .

N^1-(2-aminoethyl)-N^2-(di(pyridin-2-yl)methylene)ethane-1,2-diamine (6)



6

Purified by prep TLC plate using an ethyl acetate : methanol (9:1) as an eluant. Burgundy colored liquid; 82% yield; IR main functional groups: 2929.57 $cm^{-1}_{C\text{-}H\ aromatic}$, 2800 $cm^{-1}_{C\text{-}H\ aliphatic}$, 1634 $cm^{-1}_{C=N}$, 1463.52 $cm^{-1}_{C=C}$, 3288.42 $cm^{-1}_{N\text{-}H}$ of primary amine.^{1}H-NMR (500 MHz, DMSO, ppm): 8.36 (2H, dd, pyridine), 7.5 (4H, m, pyridine), 7.03 (2H, m, pyridine), 4.3 (2H, t, C=N-C$\underline{H}_2$-CH_2-), 3.39 (2H, td, =N-CH_2-C$\underline{H}_2$-NH-), 2.81 (2H, m, =N-CH_2-CH_2-NH-C$\underline{H}_2$-CH_2-NH_2), 2.58 (2H, m, =N-CH_2-CH_2-NH-CH_2-C$\underline{H}_2$-NH_2), 2.46 (1H, tt, NH), 0.97 (2H, t, NH_2) (see Fig. 24 and 44 in Appendix A).

2.2 Anticancer Activity

Human cervix cancer cells (ATCC number:CCL-2, from a cervical carcinoma), human breast cancer cells MCF7 (ATCC number:HTB-22), human prostate cancer cells (PC3, ATCC number:CRL-1435, human, from human prostate), human liver cancer cells (HepG2, ATCC number:HB-8065, human, from the epithelial cells of the liver) and

normal muscle cells (L6, ATCC number:CRL-1458, human, from the skeletal muscle) were grown in RPMI medium supplemented with 10% fetal calf serum, 1% l-glutamine, 1% non-essential amino acid, 1% amphotericin B and 1% penicillin streptomycin. At 37°C, a humidified environment composed of 95% air and 5% CO2 was used to grow all of the cell lines. All chemicals used were purchased from Biological Industries but both of the amphotericin B and MTT reagent were purchased from SIGMA Company. All of the five cell lines were purchased from ATTC. Cell viability was assessed using the MTT test, and absorbance at 570 nm was measured using a microplate reader (Labtech, UK).

2.2.1 MTT assay

The ability of functioning mitochondria to convert the yellow, water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to purple, water-insoluble formazan crystals allows for a quantitative assessment of the viability of cells. After the cells were plated in a 96-well dish with 100µl of DMEM medium, they were treated for 24 hours with varying concentrations of the stock solution of the chemical complexes before being incubated for a further 24 hours at 37 °C. MTT (0.5 mg/ml) was added to 100µl of treatment solution and incubated for 4 hours at 37^{o}C. After 4 hours, the MTT solution was discarded, and 100µl of a 9:1 solution of isopropyl alcohol and formic acid was added to dissolve the formazan crystals. This mixture was then incubated for 15 minutes at room temperature and in the dark. Cell viability was then calculated as a proportion of absorbance of treated cells to the control, and the optical density of the MTT formazan was measured at 570 nm in an ELISA reader.

2.2.2 Cytotoxicity assay

After removing the culture media and adding 0.05% trypsin-EDTA, cells at 70–80% confluence were detached from the culture flask. Viable cells were then suspended in 100µl (2.0×104 cells/well) and seeded in a 96-well plate. The cells were then incubated for 24 hours at 37°C. Following the removal of the media, cells were exposed to a 100µl stock solution that had been serially diluted to concentrations of 500, 250, 125, 62.5 and 31.25µg/ml fro synthesized compounds. Cells were then incubated for 24 hours at 37°C to conduct an MTT test.

2.2.3 Cytostatic assay

A reduced number of cells (1.0×104 cells/well) were seeded in each well to ascertain the chemical complexes' cytostatic effect, and the wells were incubated for 24 hours at 37 degrees Celsius. Following the removal of the media, 100µl of the chemical complexes at the same concentrations as those used in the preceding paragraph were applied to the cells, and they were then incubated at 37°C for 24 hours to conduct the MTT experiment.

2.3 Antibacterial Activity

The synthetic compounds' antibacterial efficacy was assessed against several bacterial isolates from the American Type Culture Collection (ATCC). Escherichia coli (ATCC# 25922), Klebsiella pneumoniae (ATCC# 13883), Proteus vulgaris (ATCC# 8427), Pseudomonas aeruginosa (ATCC# 9027), Staphylococcus aureus (ATCC# 6538P), and Staphylococcus epidermis (ATCC# 12228) are a few examples of bacteria. On nutrient agar plates, the tested bacteria were cultivated for the night. To achieve 0.5 McFarland (1.5×10^8 CFU/ml) of broth turbidity. After that, saline was used to dilute it to get 1×10^6 CFU/ml.

2.3.1 Disk diffusion method

The disk diffusion method was used to assess the examined compounds antibacterial activity [75]. Seven isolates of bacteria were used to create the inoculum, which was applied by swabbing the entire sterile agar surface with the swab. To achieve a uniform dispersion of the inoculum, this process was performed twice more by streaking, rotating the plate by about (60°) each time. The rim of the agar was also swabbed as a last step. Then, 0.025% from each produced chemical was placed onto 6 mm disks. By placing the loaded disks onto the surface of inoculated agar plates, the antibacterial activity of all produced compounds under study was evaluated. The plates were incubated at (37°C) for (16–24 h) after standing at room temperature for 15 minutes. The plates were then measured for the inhibition zone diameter (IZD) to the nearest millimeter in order to check for bacterial growth inhibition.

2.3.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

By calculating the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC), the antibacterial efficacy of the produced compounds against the seven selected isolates of bacteria was examined [76]. In the Muller Hinton broth medium, Schiff bases were diluted twice in succession. The 96-well micro-titer plates were prepared with the antibacterial agent dilution vertically in the first row and duplicate, 1 μL of the adjusted bacterial concentration inoculums (1×10^6 CFU/mL), and Muller Hinton broth medium with the tested bacterial concentrations in the growth control wells. While only sterile Muller Hinton broth was present in the sterility control wells. The plates were then kept at 37°C for overnight incubation. So, the MIC was defined as the lowest concentration that prevented bacterial growth. The lack of turbidity is a sign of this. After determining the MIC, any wells that do not exhibit any bacterial growth after being incubated on agar plates are streaked, and then the wells are incubated at 37°C for 16 to 24 hours. The MBC was defined as the lowest concentration that completely eliminated the initial bacterial population while leaving no colonies on the agar.

2.4 Antioxidant Assay

A 96-well microplate was used for the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical test [77]. In a nutshell, 100 μL of 0.01% methanolic DPPH solution was mixed with 100 μL of various methanol compound concentrations (ranging from 500-0.5 μg/ml). Using a microplate reader (Labtech, UK), the absorbance at 540 nm was measured after the plate had been incubated for 30 min at room temperature in the dark. As a standard, ascorbic acid was employed at various doses (500–0.5 μg/mL). Following is how the DPPH radical scavenging activity (%) was determined:

DPPH scavenging activity (%) is calculated as follows: 100 × [(A control - A sample) / A control] whereas the absorbance of the sample was [DPPH + Sample (compound/standard)] and the absorbance of the control was [DPPH + Methanol free from sample]. Using non-linear regression and Microsoft Excel, the concentration (IC50) that inhibits the DPPH radical scavenging activity by 50% was determined.

Chapter Three

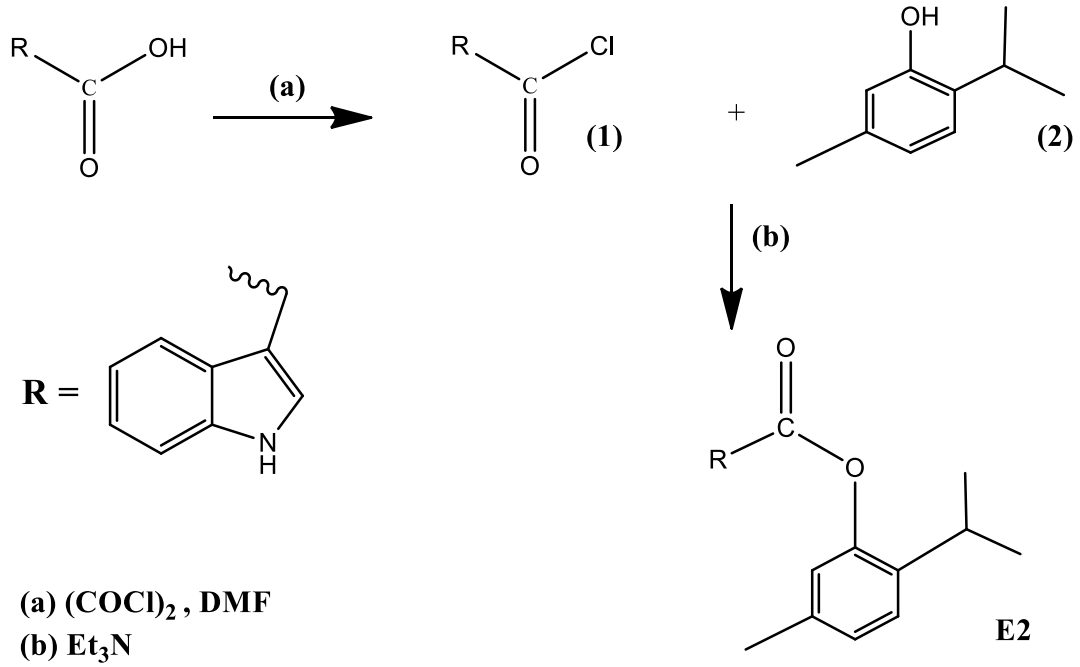
Results and Discussion

3.1 Chemistry

Thymol ester compound E2 was prepared through a condensation reaction of 1 eq. of 1H-indole-3-carbonyl chloride (1) with 1 eq. of thymol (2) and 1 eq. of triethylamine (b) which afforded the good yield formation of 2-isopropyl-5-methylphenyl 1H-indole-3-carboxylate E2 (77.8%) Scheme3.1. By using FT-IR, ^{1}H and ^{13}C NMR analysis, the chemical structure of the produced thymol ester compound was identified. The creation of the thymol ester E2 was satisfactorily demonstrated by all available spectrum data. The purity of E2 was 100% as indecated by HPLC (see Fig. 45 in Appendix A).

Scheme 3.1

Synthesis of thymol ester (E2)

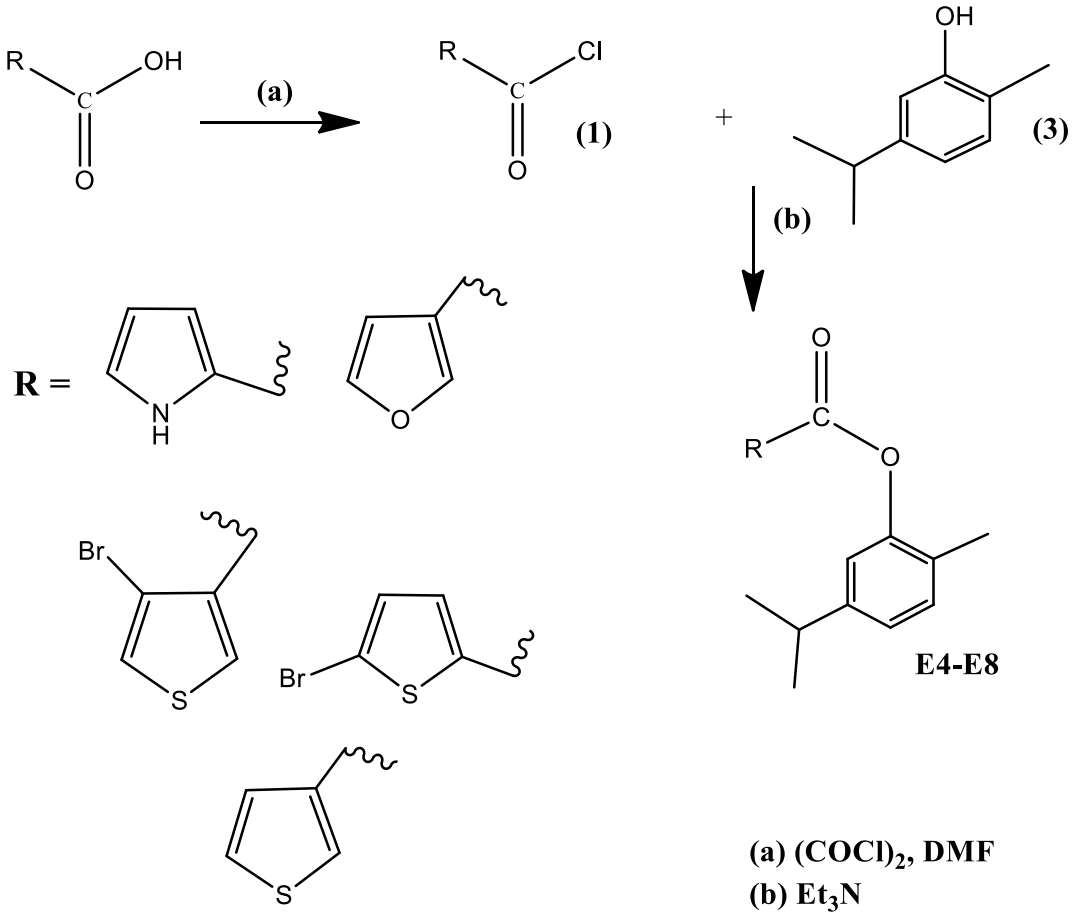


Carvacrol ester compounds were synthesized by condensing 1 eq. of 1H-pyrrole-2-carbonyl chloride or 1 eq. of furan-3-carbonyl chloride or 1 eq. of 4-bromothiophene-3-carbonyl chloride or 5-bromothiophene-2-carbonyl chloride or thiophene-3-carbonyl chloride (1) with 1 eq. of carvacrol (3) and 1 eq. of triethylamine (b) which produced the good yields of 5-isopropyl-2-methylphenyl 1H-pyrrole-2-carboxylate E4 (50%), 5-isopropyl-2-methylphenyl furan-3-carboxylate E5 (55%), 5-isopropyl-2-methylphenyl

4-bromothiophene-3-carboxylate E6 (60.5%), 5-isopropyl-2-methylphenyl 5-bromothiophene-2-carboxylate E7 (62.5%) and 5-isopropyl-2-methylphenyl thiophene-3-carboxylate E8 (65%), respectively Scheme 3.2. By using FT-IR and ^{1}H-NMR analysis, the chemical structures of the produced carvacrol ester compounds were identified. All spectrum data indicated that the successful synthesis of all carvacrol esters (E4, E5, E6, E7, and E8).

Scheme 3.2

Synthesis of carvacrol esters (E4, E5, E6, E7 and E8)

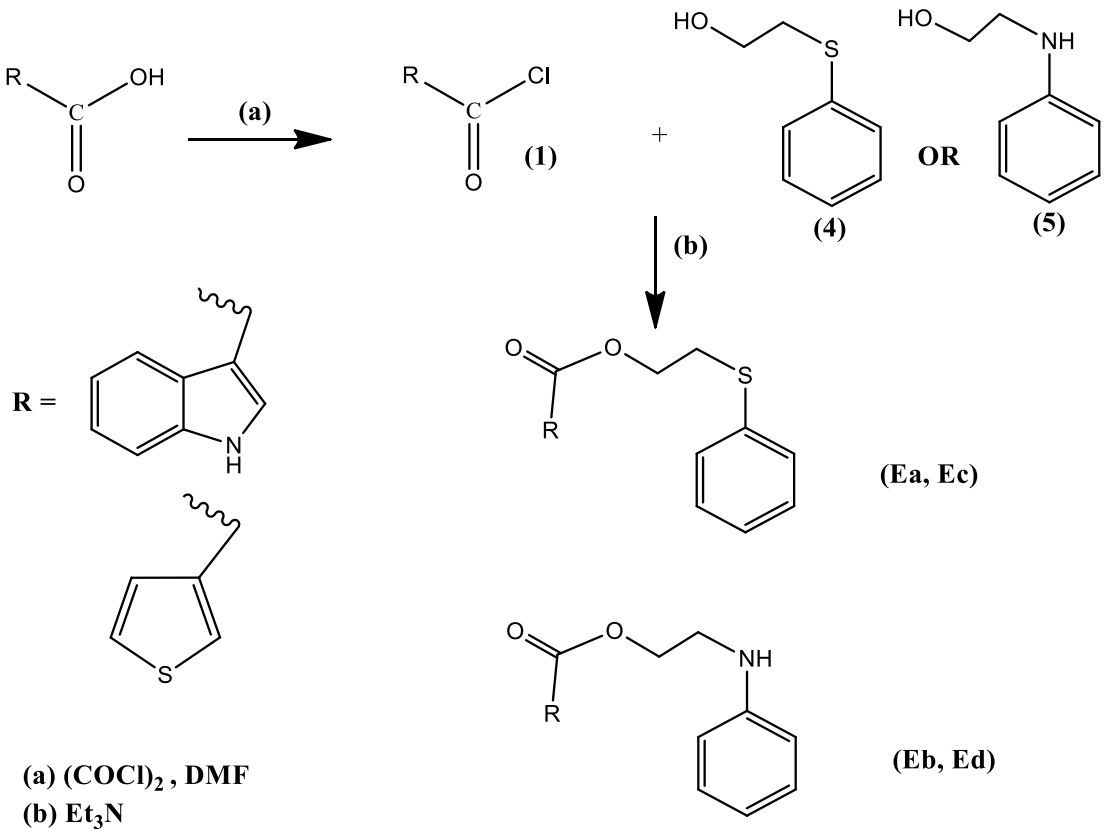


The condensation reaction of 1 equivalent of Indole-3-carbonyl chloride or 1 equivalent of thiophene-3-carbonyl chloride (1) with 1 equivalent of 2-(phenyl thio)ethanol (4) or N-(2-Hydrroxyethyl)aniline (5) and 1 equivalent of triethylamine (b) produced a good yields of 2-(phenylthio)ethyl 1H-indole-3-carboxylate Ea (50%), 2-(phenylamino)ethyl 1H-indole-3-carboxylate Eb (60%), 2-(phenylthio)ethyl thiophene-3-carboxylate Ec (55%) and 2-(phenylamino)ethyl thiophene-3-carboxylate Ed (53%), respectively

Scheme 3.3. The chemical structures of the produced ester compounds were determined using FT-IR and ^{1}H-NMR investigations. The successful synthesis of all ester compounds (Ea, Eb, Ec, and Ed) was demonstrated by all spectrum data.

Scheme 3.3

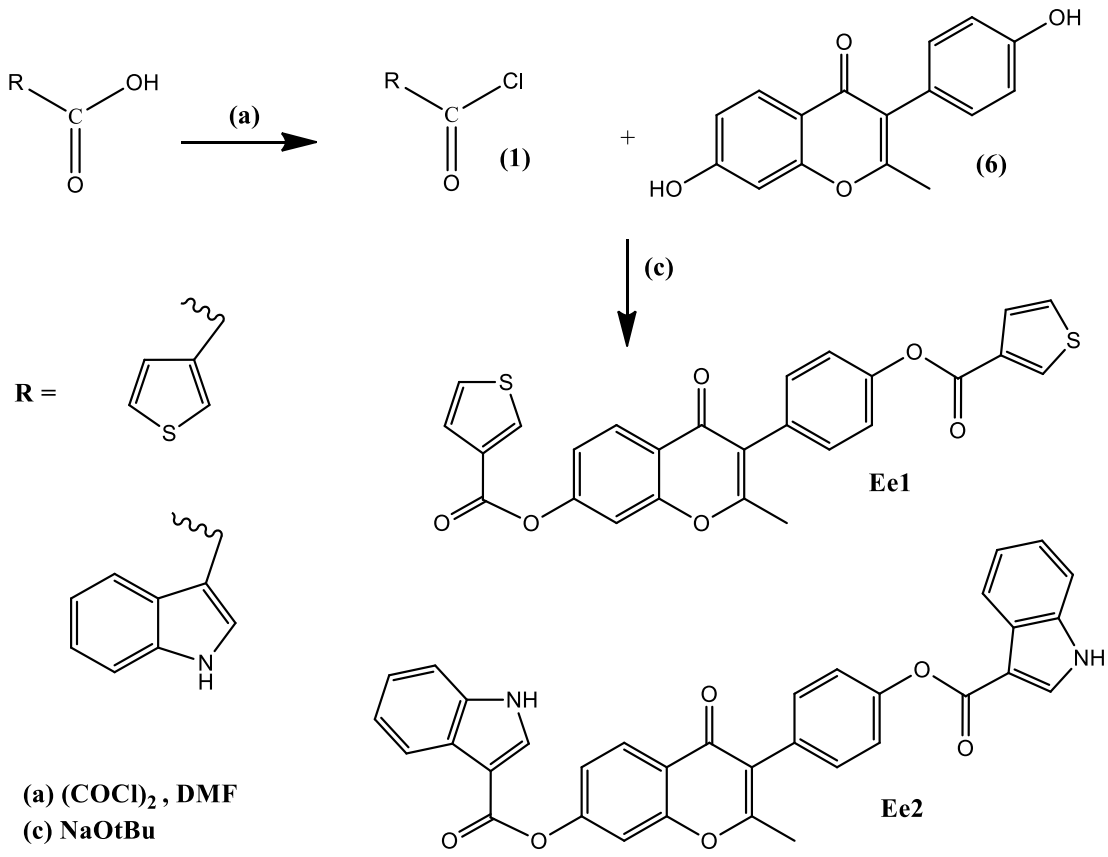
Synthesis of ester compounds (Ea, Eb, Ec and Ed)



The condensation reaction of 2 equivalents of thiophene-3-carbonyl chloride or 2 equivalents of indole-3-carbonyl chloride (1) with 1 equivalent of 7-hydroxy-3-(4-hydroxyphenyl)-2-methyl-4H-chromen-4-one (6) and 2.2 equivalents of sodium tert-butoxide base (c) produced a good yields of 2-methyl-4-oxo-3-(4-((thiophene-3-carbonyl)oxy)phenyl)-4H-chromen-7-yl thiophene-3-carboxylate Ee1 (47%) and 4-(7-((1H-indole-3-carbonyl)oxy)-2-methyl-4-oxo-4H-chromen-3-yl)phenyl 1H-indole-3-carboxylate Ee2 (44%), respectively Scheme 3.4. The chemical structures of the produced ester compounds were determined using FT-IR and ^{1}H-NMR studies. Both ester compounds (Ee1 and Ee2) were effectively synthesized, according to all available spectrum data.

Scheme 3.4

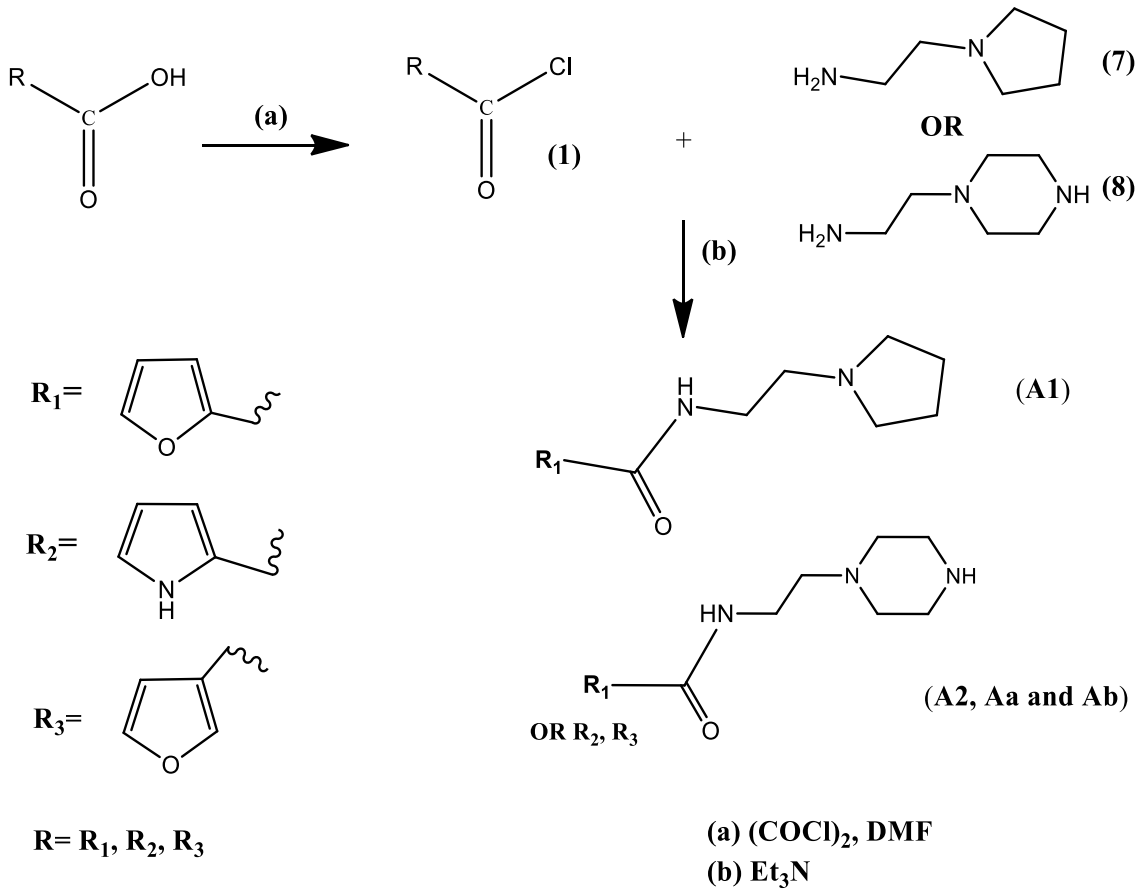
Synthesis of Flavonoid ester compounds (Ee1 and Ee2)



The condensation reaction of 1 equivalent of furan-2-carbonyl chloride, 1 equivalent of 1H-pyrrole-2-carbonyl chloride, or 1 equivalent of furan-3-carbonyl chloride (1) with 1 equivalent with aliphatic primary amines (7) or (8) and 1 equivalent of triethylamine (b) resulted a good yields of N-(2-(pyrrolidin-1-yl)ethyl)furan-2-carboxamide A1 (78%), N-(2-(piperazin-1-yl)ethyl)furan-2-carboxamide A2 (79%), N-(2-(piperazin-1-yl)ethyl)-1H-pyrrole-2-carboxamide Aa (70%) and N-(2-(piperazin-1-yl)ethyl)furan-3-carboxamide Ab (67%), respectively Scheme 3.5. By using FT-IR and ^{1}H-NMR analyses, the chemical structures of the synthesized amide compounds were identified. All spectrum data indicated that the successful synthesis of all amide compounds (A1, A2, Aa, and Ab). The purity of A1 was 100% and the purity of A2 was 100% as indecated by HPLC (see Fig. 46 and 47 in Appendix A). It is important to point that both compounds A1 and A2 have a CAS number on Google, but I could not be able to obtain any scientific paper in which they were prepared.

Scheme 3.5

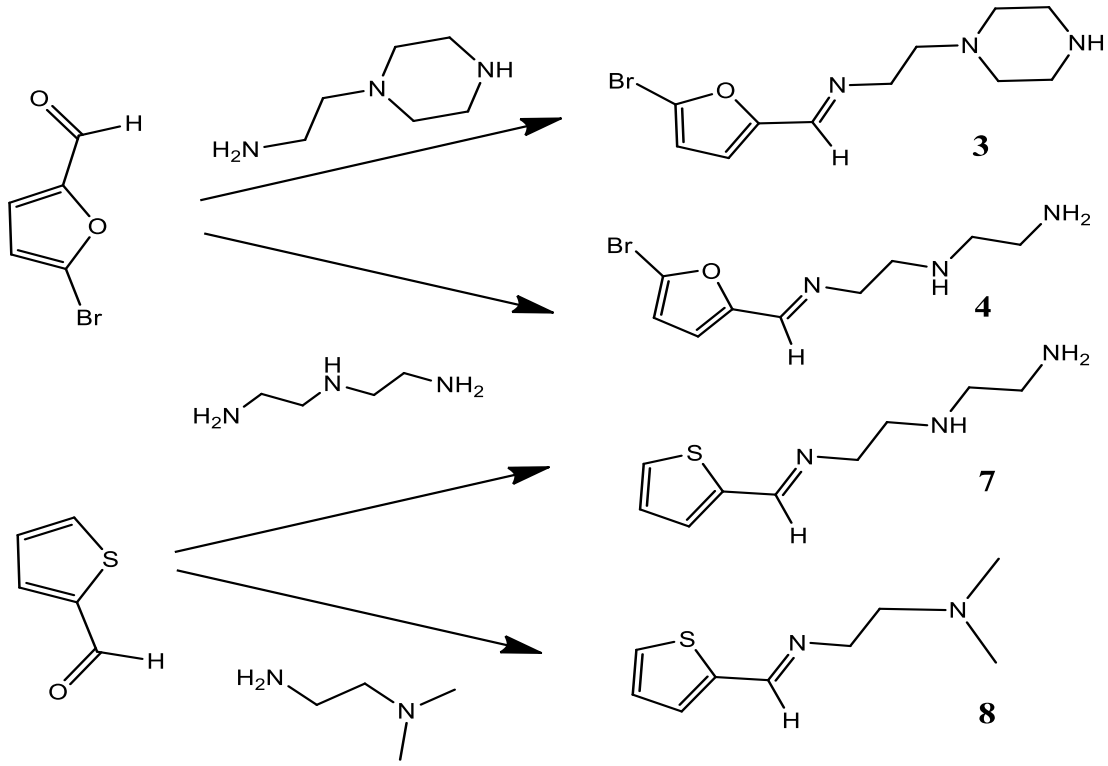
Synthesis of amide compounds (A1, A2, Aa and Ab)



In order to form imine compounds, 2 mmol of 5-bromo-2-furaldehyde were condensed with 2 mmol of 1, 2-aminoethyl piperazine and 2 mmol of diethylenetriamine. Similarly, 2 mmol of thiophene-2-carbaldehyde were combined with 2 mmol of diethylenetriamine and 2 mmol of N, N-dimethylethylenediamine to produce (E)-2-(piperazin-1-yl)ethanamine 3 (87%), (E)-N1-(2-aminoethyl)-N2-((5-bromofuran-2-yl)methylene)ethane-1,2-diamine 4 (90%), (E)-N1-(2-aminoethyl)-N2-(thiophen-2-ylmethylene) ethane-1,2-diamine 7 (88%) and (E)-N1-dimethyl-N2-(thiophen-2-ylmethylene) ethane-1,2-diamine 8 (85%), respectively Scheme 3.6. FT-IR, ^{1}H NMR, and ^{13}C NMR analysis were used to describe the chemical composition of the produced imines. All spectrum data indicated that all imines (3, 4, 7 and 8) had been synthesized effectively.

Scheme 3.6

Synthesis of imines (3, 4, 7 and 8)

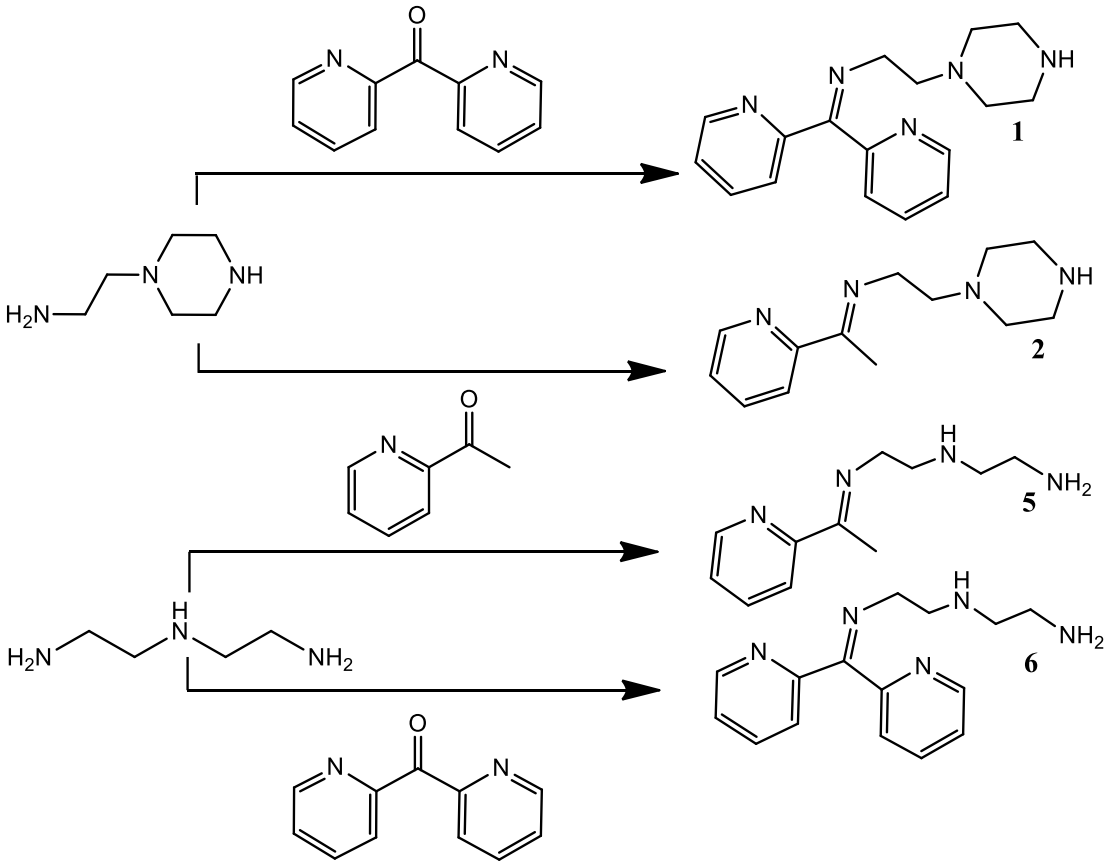


Imine compounds were synthesized through condensing 1 equivalent of di(2-pyridyl)ketone with 1 equivalent of 1, 2-aminoethyl piperazine, 1 equivalent of 2-acetopyridine with 1 equivalent of 1, 2-aminoethyl piperazine, 1 equivalent of 2-acetopyridine with 1 equivalent of diethylenetriamine, and 1 equivalent of di(2-pyridyl)ketone with 1 equivalent of diethylenetriamine, which produced the good yields of of N-(di(pyridin-2-yl)methylene)-2-(piperazin-1-yl)ethanamine 1 (63%), Z)-2-(piperazin-1-yl)-N-(1-(pyridin-2-yl)ethylidene)ethanamine 2 (70%), (Z)-N1-(2-aminoethyl)-N2-(1-(pyridin-2-yl)ethylidene)ethane-1,2-diamine 5 (75%) and N1-(2-aminoethyl)-N2-(di(pyridin-2-yl)methylene)ethane-1,2-diamine 6 (80%), respectively Scheme 3.7. The products were analyzed by using FTIR and ^{1}H-NMR spectroscopy, the chemical structures of the produced imines were identified. All spectral data indicated that all imines (1, 2, 5 and 6) had been produced effectively. Imine compound 2 was prepared in two previous studies, and the results of FT-IR and H-NMR are consistent with the results of IR and H-NMR in the current study. The experimental FT-IR spectra of imine 2 in the previous studies show the C=N, C=C absorption at 1654cm^{-1}, 1459cm^{-1} [78], 1640cm^{-1}, 1464.94cm^{-1} [79], and in this study at 1629.2cm^{-1},

1459.22cm^{-1}, respectively. Moreover, the signal of CH_3 protons in this compound was appeared at 2.01ppm [78], 1.21ppm [79], and at 2.18ppm in this study.

Scheme 3.7

Synthesis of imines (1, 2, 5 and 6)



3.2 Biology

3.2.1 Anticancer activity

We evaluated the in *vitro* anticancer activity of the thymol ester molecule E2 against the Hela cancer cell line, the MCF7 breast cancer cell line, the PC3 prostate cancer cell line, and the HepG2 liver cancer cell line. The results showed that the four cancer cell lines were cytotoxic to various doses of the produced thymol ester E2. The viability of Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (38.6-42.6%), (28-33%), (28-30.3%), and (30.8-33.2%), respectively, within the studied concentration range. Cell proliferation reduced when the concentration of the thymol ester molecule E2 increased.

Ester compound E2 decreased the viability of the tested Hela cancer cells in a cytotoxic assay from 38.6% at 500µg/ml to 42.6% at 31.25µg/ml (Fig. 1a in Appendix A). In a cytostatic assay, ester compound E2 decreased the viability of the tested Hela cancer cells from 56.7% at 500µg/ml to 62% at 31.25µg/ml (Fig. 1b in Appendix A). The prepared thymol ester compound E2 is more effective than thymol in cytotoxic assay and cytostatic assay on Hela cancer cells at all tested concentrations because thymol decreased the viability of the tested Hela cancer cells from 58% at 500 µg/ml to 63% at 31.25 µg/ml and from 69.7% at 500 µg/ml to 73.5% at 31.25 µg/ml, respectively, (Fig. 1a and 1b in Appendix A).

As the new ester compound E2 and thymol compound were studied on Hela cancer cells, they were also studied on one type of living cells which is L6 normal muscle cells. Ester compound E2 decreased the viability of the tested L6 normal cells in a cytotoxic assay from 50% at 500µg/ml to55.3% at 31.25µg/ml. In a cytostatic assay, ester compound E2 decreased the viability of the tested L6 normal cells from 54.4% at 500µg/ml to 60% at 31.25µg/ml. Thymol compound is Slightly more effective than thymol ester compound E2 in cytotoxic assay and cytostatic assay on L6 normal cells at all tested concentrations because thymol decreased the viability of the tested L6 normal cells from 61.05% at 500 µg/ml to 66.5% at 31.25 µg/ml and from 62.5% at 500 µg/ml to 64.2% at 31.25 µg/ml, respectively, (Fig. 1c and 1d in Appendix A). This does not negate the effectiveness of the ester compound E2, especially if it is used in low concentrations (less than 31.25µg/ml).

When investigated cytotoxic assay on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells, ester compound E2 reduced their viability from 28% at 500µg/ml to 33% at 31.25µg/ml, 28% at 500µg/ml to 30.3% at 31.25µg/ml and 30.8% at 500µg/ml to 33.2% at 31.25µg/ml, respectively. Ester compound E2 reduced the viability of investigated MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay from 55.4% at 500µg/ml to 57.5% at 31.25 µg/ml, 57.46% at 500 µg/ml to 60.8% at 31.25 µg/ml and 60.57% at 500µg/ml to 63.44% at 31.25µg/ml, respectively, (Fig. 1e and 1f in Appendix A). This is consistent with previous studies that proved that several indole-derived compounds have a great potential as anti-cancer agents [80-82].

The results showed that Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells are all sensitive to the cytotoxic and cytostatic assays of the produced thymol ester compound E2. Thymol and its produced ester E2 have IC_{50} values for Hela cancer cells of 13.9µg/ml and 14.5µg/ml in cytotoxic assay, respectively. The IC_{50} values for thymol and synthetic thymol ester E2 on Hela cancer cells in cytostatic assay are as follows: thymol 0.18µg/ml and E2 0.0937µg/ml Table 3.1. The IC_{50} values of the synthesized thymol ester E2 on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay were as follows: 17.21µg/ml, 19.98µg/ml and 17.13µg/ml, respectively. Moreover, the IC_{50} values of the synthesized thymol ester E2 on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay were as follows: 7.08µg/ml, 10.26µg/ml and 13.04µg/ml, respectively, Table 3.1. Thus, in a cytotoxic assay, thymol and its produced ester E2 have IC_{50} values for L6 normal cells of 9.2 µg/ml and 8.066µg/ml, respectively. Additionally, in a cytostatic assay, the IC_{50} values for thymol and synthetic thymol ester E2 were 5.37µg/ml and 7.26µg/ml, respectively, for L6 normal cells Table 3.1.

Table 3.1

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of the synthesized thymol ester compound (E2) on Hela cancer cells, L6 normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells

Assay	Thymol	E2
Hela Cytotoxic	13.9	14.5
Hela Cytostatic	0.18	0.094
L6 Cytotoxic	9.27	8.06
L6 Cytostatic	5.37	7.26
MCF7 Breast Cytotoxic	-	17.21
MCF7 Breast Cytostatic	-	7.08
PC3 Prostate Cytotoxic	-	19.98
PC3 Prostate Cytostatic	-	10.26
HepG2 Liver Cytotoxic	-	17.13
HepG2 Liver Cytostatic	-	13.04

The in *vitro* anticancer properties of the five carvacrol ester compounds were tested using the Hela cancer cell line, MCF7 breast cancer cell line, PC3 prostate cancer cell line, and HepG2 liver cancer cell line. The results showed that the four cancer cell lines were lethal to various doses of the produced carvacrol esters. The viability of Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver

cancer cells were measured as (24.5-44.6%), (27.9-36%), (24-31.9%), and (24-33.2%), respectively, within the investigated concentration range. Cell proliferation reduced as the amount of carvacrol ester compounds increased.

The carvacrol ester compounds E4 and E6 perform significantly better in a cytotoxic assay in terms of potency against Hela cancer cells than the other investigated carvacrol ester compounds E5, E7, and E8 at all dosages. When assessed on Hela cancer cells in a cytotoxic assay, ester compound E4 decreased viability from 25.4% at 500µg/ml to 28.8% at 31.25µg/ml, while ester compound E6 decreased viability from 24.5% at 500µg/ml to 29% at 31.25µg/ml. Compared to carvacrol ester E5, the carvacrol esters E7 and E8 are more effective against Hela carcinoma cells. From 28.4% at 500µg/ml to 32% at 31.25µg/ml and from 31.4% at 500µg/ml to 35.4% at 31.25µg/ml, respectively, they both decreased the viability of the tested Hela cancer cells. The ester compound E5 decreased the viability of the tested Hela cancer cells from 39.9% at 500µg/ml to 44.6% at 31.25µg/ml (Fig. 2a in Appendix A). In the cytostatic assay, the ester compound E6 had a greater viability for Hela cancer cells than the carvacrol ester E5, then E7, E4, and E8, as shown in (Fig. 2b in Appendix A). The produced ester compounds (E4, E6, E7, and E8) are more effective on Hela cancer cells at all tested concentrations since carvacrol decreased the viability of the tested Hela cancer cells in a cytotoxic assay from 37% at 500µg/ml to 46.9% at 31.25µg/ml (Fig. 2a Appendix A).

When it comes to potency against MCF7 breast cancer cells in a cytotoxic assay, the carvacrol ester compounds E5 and E8 perform significantly better than the other tested carvacrol ester compounds E4, E6, and E7 at all doses. When evaluated on MC7 breast cancer cells, ester compound E5 decreased viability from 27.9% at 500µg/ml to 30.7% at 31.25µg/ml, while ester compound E8 decreased viability from 28% at 500µg/ml to 32.7% at 31.25µg/ml. Compared to carvacrol ester E4, the carvacrol esters E6 and E7 are more effective against MCF7 breast cancer cells. They both reduced the viability of the tested Hela cancer cells from 30.8% at 500µg/ml to 33.5% at 31.25µg/ml and from 29.6% at 500µg/ml to 32.9% at 31.25µg/ml, respectively. The viability of the tested MCF7 breast cancer cells was reduced by ester compound E4 from 32% at 500µg/ml to 36% at 31.25µg/ml (Fig. 2e in Appendix A). Additionally as shown in (Fig. 2f in Appendix A), the ester compound E4 had a higher vitality for MCF7 breast cancer cells in the cytostatic assay than the carvacrol ester E6, then E8, E5, and E7.

In a cytotoxic assay using PC3 prostate cancer cells, the carvacrol ester compounds E6 and E8 performed better than the other investigated carvacrol ester compounds E4, E5, and E7 at all doses. Ester compound E6 and ester compound E8 both reduced the viability of the PC3 prostate cancer cells in a cytotoxic assay from 25.4% at 500µg/ml to 28.2% at 31.25 µg/ml and 24% at 500µg/ml to 28% at 31.25µg/ml, respectively. Compared to carvacrol ester E5, the carvacrol esters E4 and E7 are more effective against PC3 prostate cancer cells. The viability of the tested PS3 prostate cells was decreased by both of them from 28% at 500µg/ml to 30.8% at 31.25µg/ml and from 24% at 500µg/ml to 28% at 31.25µg/ml, respectively. Ester compound E5 decreased the viability of the PC3 prostate cancer cells under test from 30.9% at 500µg/ml to 34.4% at 31.25µg/ml (Fig. 2g in Appendix A). In the cytostatic assay, the ester compound E6 had a greater vitality for PC3 prostate cancer cells than the ester compound E8, followed by E4, E5, and E7, as shown in (Fig. 2h in Appendix A).

The carvacrol ester compound E4 performance is much better than the other tested carvacrol ester compounds E5, E6, E7, and E8 at all dosages in a cytotoxic assay using HepG2 liver cancer cells. In a cytotoxic assay, ester compound E4 decreased the viability of Hep G liver cancer cells from 24% at 500µg/ml to 27.5% at 31.25µg/ml. The synthesized carvacrol esters E6, E7, and E8 are more efficient against HepG2 liver cancer cells than carvacrol ester E5. The viability of the tested HepG2 liver cells was decreased by E6 from 27.6% at 500µg/ml to 29.6% at 31.25µg/ml, by E7 from 28% at 500µg/ml to 32% at 31.25µg/ml and by E8 from 26% at 500µg/ml to 30% at 31.25µg/ml. The test HepG2 liver cancer cells viability reduced from 30.6% at 500µg/ml to 33.2% at 31.25µg/ml due to ester compound E5 (Fig. 2i in Appendix A). As indicated in (Fig. 2j in Appendix A), the ester compound E4 had a higher vitality for HepG2 liver cancer cells in the cytostatic assay than the ester compound E8, which was followed by E6, E5, and E7.

The five heterocyclic carvacrol ester compounds were tested on L6 normal cells as well as Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells. (Fig. 2c and 2d in Appendix A) illustrate the results of L6 normal cells, which demonstrated that all carvacrol esters investigated have good cytotoxic and cytostatic effects. The % of living cells for E8 in the cytotoxic assay of L6 normal cells was 62.4% for the concentration of 500µg/ml and 67.2% for the concentration of

31.25µg/ml. In a cytotoxic assay for L6 normal cells, produced E5 and E4 perform better than E7 and E6. Carvacrol esters E5 and E4 have % of living cells in cytotoxic assay from 61% for 500µg/ml concentration to 67.4% for 31.25µg/ml and 59.8% for 500µg/ml concentration to 64.2% for 31.25µg/ml concentration, respectively. In addition, the % of living cells for E7 and E6 in the cytotoxic assay of L6 normal cells from 55.33% for the concentration of 500µg/ml and 58.8% for the concentration of 31.25µg/ml and 51.6% for the concentration of 500µg/ml and 59.33% for the concentration of 31.25µg/ml, respectively. According to (Fig. 2d in Appendix A), ester compound E8 performed better than ester compound E5 in the cytostatic assay for L6 normal cells, which was followed by E6, E7 and then E4. It is important to note that all carvacrol esters prepared in this study (E4, E5, E6, E7 and E8) were more effective at preserving L6 normal cells than carvacrol. Carvacrol killed a higher proportion of the tested L6 normal cells in a cytotoxic assay (the % of living cells for carvacrol in the cytotoxic assay of L6 normal cells from 49.3% for the concentration of 500µg/ml and 54.5% for the concentration of 31.25µg/ml) (Fig. 2c in Appendix A).

The findings demonstrated that all synthesized carvacrol ester compounds have cytotoxic and cytostatic effects on Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells at all concentrations. Carvacrol and its synthesized esters have the following IC_{50} values on Hela cancer cells: carvacrol 69.78µg/ml, E4 23.26µg/ml, E5 11.12µg/ml, E6 15.5µg/ml, E7 13.2µg/ml and E8 13.68µg/ml. Carvacrol and synthesized carvacrol esters have IC_{50} values as follows: carvacrol 5.97µg/ml, E4 6.4µg/ml, E5 2.65µg/ml, E6 3.47µg/ml, E7 10.9µg/ml, and E8 10.2µg/ml on Hela cancer cells in the cytostatic assay. The IC_{50} values of the synthesized carvacrol esters on MCF7 breast cancer cells in cytotoxic assay were as follows: E4 10µg/ml, E5 11.76µg/ml, E6 13.57µg/ml, E7 2.03µg/ml and E8 21.33µg/ml. While the IC_{50} values of the synthesized carvacrol esters on MCF7 breast cancer cells in cytostatic assay were as follows: E4 6.88µg/ml, E5 9.58µg/ml, E6 2.29µg/ml, E7 0.57µg/ml and E8 5.79µg/ml. On PC3 prostate cancer cells, carvacrol esters have the following IC_{50} values: E4 8.22µg/ml, E5 12.74µg/ml, E6 6.75µg/ml, E7 7.84µg/ml, and E8 21.89µg/ml. Carvacrol esters exhibit IC_{50} values for PC3 prostate cancer cells as follows: E4 7.45µg/ml, E5 6.03µg/ml, E6 5µg/ml, E7 3.08µg/ml, and E8 2.99 µg/ml. Meanwhile, Carvacrol esters have the following IC_{50} values on HepG2 liver

cancer cells: E4 0.139µg/ml, E5 10.78µg/ml, E6 12.48µg/ml, E7 13.87µg/ml, and E8 22.8µg/ml. Also carvacrol esters have IC_{50} values: E4 .088µg/ml, E5 2.2µg/ml, E6 11.72µg/ml, E7 0.87µg/ml, and E8 2.79µg/ml on HepG2 liver cancer cells in the cytostatic assay as shown in Table 3.2.

Table 3.2

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of the produced carvacrol ester compounds (E4, E5, E6, E7 and E8) on Hela cancer cells, L6 normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells

Assay	Carvacrol	E4	E5	E6	E7	E8
Hela Cytotoxic	69.78	23.26	11.12	15.5	13.2	13.7
Hela Cytostatic	5.97	6.4	2.65	3.47	10.9	10.2
L6 Cytotoxic	7.82	4.13	12.45	13.95	15.9	4.05
L6 Cytostatic	1.05	2.92	6.9	7.87	2.28	2.79
MCF7 Breast Cytotoxic	-	10	11.76	13.57	2.03	21.3
MCF7 Breast Cytostatic	-	6.88	9.58	2.29	0.57	5.8
PC3 Prostate Cytotoxic	-	8.22	12.7	6.75	7.84	21.89
PC3 Prostate Cytostatic	-	7.45	6.03	5	3.08	2.99
HepG2 Liver Cytotoxic	-	0.139	10.78	12.48	13.87	22.8
HepG2 Liver Cytostatic	-	0.088	2.2	11.72	0.57	2.79

All carvacrol ester compounds were investigated on L6 normal cell line in addition to the four cancer cell lines. At all concentrations, carvacrol and its ester compounds show both cytotoxic and cytostatic effects on L6 normal cells. Carvacrol and its esters have IC_{50} values as follows: carvacrol 7.82µg/ml, E4 4.13µg/ml, E5 12.45µg/ml, E6 13.95µg/ml, E7 15.9µg/ml, and E8 4.05µg/ml on L6 normal cells in the cytotoxic assay. As indicated in Table 3.2, the carvacrol and produced carvacrol esters have IC_{50} values in cytostatic assay: carvacrol 1.05µg/ml, E4 2.92µg/ml, E5 6.9µg/ml, E6 7.87µg/ml, E7 2.28µg/ml, and E8 2.79µg/ml on L6 normal cells. Anyway, among the carvacrol esters investigated, E6 exhibited highest cytotoxic and cytostatic effects on Hela cancer cells. The highest cytotoxic and cytostatic effects on MCF7 breast cancer cells are E5 and E4, respectively. The most potent cytotoxic and cytostatic effects on PC3 prostate cancer cells are exhibited by E8 and E6, respectively. Additionally, among the carvacrol esters investigated, E4 exhibited the strongest both cytotoxic and cytostatic effects on HepG2 liver cancer cells. Five membered rings heterocyclic compounds, such as pyrrole, thiophene and furan effective at constructing chemicals that bond with receptors and block the biological processes linked to the growth of cancer [83, 84].

Ester compounds (Ea, Eb, Ec and Ed) were tested in a variety of doses as anticancer agents against the Hela cancer cell line, the MCF7 breast cancer cell line, the PC3 prostate cancer cell line, and the HepG2 liver cancer cell line. In contrast, they were also investigated using the L6 muscle normal cell line. The percentage of living cells of Ea compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells, respectively, was measured as (28-31.5%), (52-53.8%), (27.2-31.2%), (28.2-31%), and (29.3-31.8%) (Fig. 3a in Appendix A); however, in cytostatic assay was measured as (54.7-57.2%), (67.5-70.6%),(57.3-59.9%), (52-55.5%), and (59.5-61.9%), respectively, (Fig. 3b in Appendix A). Results showed that the ester compound Ea was more effective against MCF7 breast cancer cells in cytotoxic assay and against PC3 prostate cancer cells in cytostatic assay.

The percentage of living cells of Eb compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (28-31.5), (51.1-54.7%), (30.8-34.4%), (29.6-32.1%), and (27-30.5%), respectively, (Fig. 3c in Appendix A). However, in cytostatic assay was measured as (54.8-57.1%), (70.1-74.7%), (53.3-56.3%), (54.7-57.4%), and (56.2-59.6%), respectively, (Fig. 3d in Appendix A). The ester compound Eb performed better in cytotoxic and cytostatic tests on HepG2 liver cancer cells and MCF7 breast cancer cells, according to the results. The percentage of living cells for Ec compound in cytotoxic assays against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (27-30.8%), (54.3-56.5%),(28-32%), (29.3-31.1%), and (29-30.5%), respectively, (Fig. 3e in Appendix A). However, in cytostatic assays, it was measured as (55.4-57.9%), (70.1-74.2%),(53.5-56.6%), (60.6-62%), and (62.5-65.1%), respectively, (Fig. 3f in Appendix A). The results demonstrated that the ester compound Ec was more effective against Hela cancer cells and MCF7 breast cancer cells, respectively, in cytotoxic and cytostatic experiments. Moreover, for Ed ester compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells, the% of living cells was measured as (29.3-32.9%), (53.3-55.4%),(25.7-29.6%), (26.88-29.4%), and (25.44-28.7%), respectively, (Fig. 3g in Appendix A); but in cytostatic assay was measured as (52-

54.7%), (64-68%),(52.1-55.1%), (57.2-60.5%), and (52.2-53.5%), respectively, (Fig. 3h in Appendix A). Results showed that in both cytotoxic and cytostatic experiments, the ester compound Ed was more efficient against HepG2 liver cancer cells.

Ester compounds in a cytotoxic assay, the IC_{50} values for Ea, Eb, Ec, and Ed were 5.59µg/ml, 7.3µg/ml, 3.9µg/ml, and 1.957µg/ml, respectively, for Hela cancer cells. In a cytostatic experiment, the IC_{50} values for Ea, Eb, Ec, and Ed on Hela cancer cells were 4.96µg/ml, 5µg/ml, 3.55µg/ml, and 0.454µg/ml, respectively. According to cytotoxic and cytostatic experiments, the ester compounds exhibited IC_{50} values of Ea 2.44µg/ml, Eb 0.723µg/ml, Ec 1.942µg/ml and Ed 0.95µg/ml for MCF7 breast cancer cells in cytotoxic assay, but Ea 1.7µg/ml, Eb 0.238µg/ml, Ec 1.857µg/ml and Ed 0.131µg/ml in cytostatic assay. Additionally, the ester compounds' IC_{50} values in cytotoxic and cytostatic experiments on PC3 prostate cancer cells were found as follows: In the cytotoxic assay, Ea 8.25µg/ml, Eb 5.88µg/ml, Ec 12.42µg/ml and Ed 8.45µg/ml, but in the cytostatic assay, Ea 6.824µg/ml, Eb 0.219µg/ml, Ec 11.393µg/ml and Ed 7.945µg/ml. The IC_{50} values for the ester compounds against HepG2 liver cancer cells in cytotoxic assay were Ea 3µg/ml, Eb 22.55µg/ml, Ec 31.53µg/ml and Ed 10.63µg/ml, while Ea 2.635µg/ml, Eb 17.87µg/ml, Ec 7.06µg/ml and Ed 6.4µg/ml in cytostatic assay Table 3.3.

Table 3.3

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of the ester compounds (Ea, Eb, Ec and Ed) on Hela cancer cells, L6 normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells

Assay	Ea	Eb	Ec	Ed
Hela Cytotoxic	5.59	7.3	3.9	1.96
Hela Cytostatic	4.96	5	3.55	0.454
L6 Cytotoxic	13.375	24.72	1.759	7.78
L6 Cytostatic	6.424	8.722	1.6	0.773
MCF7 Breast Cytotoxic	2.44	0.723	1.942	0.95
MCF7 Breast Cytostatic	1.7	0.238	1.857	0.131
PC3 Prostate Cytotoxic	8.25	5.88	12.42	8.45
PC3 Prostate Cytostatic	6.824	0.219	11.393	7.945
HepG2 Liver Cytotoxic	3	22.55	31.53	10.63
HepG2 Liver Cytostatic	2.6351	7.87	7.06	6.4

The Hela cancer cell line, the MCF7 breast cancer cell line, the PC3 prostate cancer cell line, and the HepG2 liver cancer cell line were used as test subjects for the flavonoid ester molecules Ee1 and Ee2 in vitro anticancer activities. The outcomes demonstrated that the flavanoid esters Ee1 and Ee2 produced at different concentrations were cytotoxic to the four cancer cell lines. Within the investigated concentration range (500-31.25µg/ml), the viability of Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (27-31.3%), (27.2-30.9%), (27-29.6%), and (28-30.8%), respectively, for Ee1. For Ee2, the viability was measured as (32.3-36.6%), (32.3-37.5%), (33.33-36.8%), and (29.6-31.8%), respectively. As flavonoid ester molecules concentration rose, cell growth decreased.

As evaluated on Hela cancer cells in a cytotoxic experiment, the ester compounds Ee1 and Ee2 reduced their viability from 27% at 500µg/ml to 31.3% at 31.25µg/ml and from 32.3% at 500µg/ml to 36.6% at 31.25µg/ml, respectively (Fig. 4a in Appendix A). The viability of the studied Hela cancer cells decreased in a cytostatic experiment from 39.4% at 500µg/ml to 42.8% at 31.25µg/ml for Ee1 and from 43.5% at 500µg/ml to 46.7% at 31.25µg/ml for Ee2 (Fig. 4b in Appendix A). The prepared flavonoid ester compounds Ee1 and Ee2 are more effective than flavonoid in cytotoxic assay and cytostatic assay on Hela cancer cells, particularly at low concentrations because flavonoid reduced the viability of the tested HeLa cancer cells from 31% at 500µg/ml to 45% at 31.25µg/ml and from 46.5% at 500µg/ml to 49.5% at 31.25µg/ml, respectively (Fig. 4a and 4b in Appendix A).

In a cytotoxic assay, the ester compounds Ee1 and Ee2 affected the viability of MCF7 breast cancer cells, reducing it from 27.2% at 500µg/ml to 30.9% at 31.25µg/ml and from 32.3% at 500µg/ml to 37.5% at 31.25µg/ml, respectively (Fig. 4e in Appendix A). (Fig. 4f in Appendix A) shows that in a cytostatic experiment, the viability of the tested MCF7 breast cancer cells decreased from 53.2% at 500µg/ml to 57.5% at 31.25µg/ml for Ee1 and from 55.2% at 500µg/ml to 59.7% at 31.25µg/ml for Ee2. Particularly at low doses, the synthetic flavonoid ester compounds Ee1 and Ee2 performed better than flavonoid in cytotoxic assay and cytostatic assay on MCF7 breast cancer cells due to the evaluated MCF7 breast cancer cells' viability for flavonoid decreased from 31.1% at 500µg/ml to 38.8% at 31.25µg/ml and from 56.2% at 500µg/ml to 61.4% at 31.25µg/ml, respectively.

The PC3 prostate cancer cells' viability was impacted in a cytotoxic experiment by the ester compounds Ee1 and Ee2, which decreased it from 27% at 500µg/ml to 29.6% at 31.25µg/ml and from 33.33% at 500µg/ml to 36.8% at 31.25µg/ml, respectively (Fig. 4g in Appendix A). In a cytostatic experiment, (Fig. 4h in Appendix A) demonstrates that the viability of the evaluated PC3 prostate cancer cells reduced from 52.1% at 500µg/ml to 56.2% at 31.25µg/ml for Ee1 and from 62.3% at 500µg/ml to 65.4% at 31.25µg/ml for Ee2. In both the cytotoxic and cytostatic tests on PC3 prostate cancer cells, the synthetic flavonoid ester compound Ee1 performed better than flavonoid at all doses because the PC3 prostate cancer cells' viability for flavonoid decreased from 30.8% at 500µg/ml to 32.7% at 31.25µg/ml and from 61.5% at 500µg/ml to 65.5% at 31.25µg/ml, respectively.

The viability of HepG2 liver cancer cells was impacted by the ester compounds Ee1 and Ee2 in a cytotoxic assay, decreasing it from 28% at 500µg/ml to 30.8% at 31.25µg/ml and from 29.6% at 500µg/ml to 31.8% at 31.25µg/ml, respectively (Fig. 4i in Appendix A). In a cytostatic experiment, the viability of the tested HepG2 liver cancer cells lowered from 55.1% at 500µg/ml to 57.3% at 31.25µg/ml for Ee1 and from 61.5% at 500µg/ml to 63.6% at 31.25µg/ml for Ee2 as shown in (Fig. 4j in Appendix A). The viability of the investigated HepG2 liver cancer cells for the flavonoid decreased from 27.2% at 500µg/ml to 29.4% at 31.25µg/ml, making the flavonoid better than the flavonoid ester compounds Ee1 and Ee2 by a small percentage at all doses in the cytotoxic assay. However, the investigated HepG2 liver cancer cells' viability for flavonoid decreased from 62.6% at 500µg/ml to 64.7% at 31.25µg/ml, meaning that at all doses, the flavonoid esters Ee1 and Ee2 performed better than flavanoid in the cytotstatic experiment on hepG2 liver cancer cells (Fig. 4i and 4j in Appendix A).

The novel ester compounds Ee1, Ee2, and flavonoid were tested on cancer cells as well as on L6 normal muscle cells, a type of live cell. According to a cytotoxic experiment, the ester compounds Ee1 and Ee2 reduced the viability of the tested L6 normal cells from 56% at 500µg/ml to 58.3% at 31.25µg/ml and from 54.8% at 500µg/ml to 57.1% at 31.25µg/ml, respectively. The viability of the investigated L6 normal cells reduced in a cytostatic experiment from 67.5% at 500µg/ml to 70.3% at 31.25µg/ml and from 68.1% at 500µg/ml to 71.2% at 31.25µg/ml, respectively. The viability of the tested L6 normal cells was reduced by a flavonoid compound in cytotoxic assay and cytostatic

assay, respectively, from 49.6% at 500µg/ml to 53.2% at 31.25µg/ml and from 60.3% at 500µg/ml to 64% at 31.25µg/ml (Fig. 4c and 4d in Appendix A). This indicates that in terms of maintaining normal cells, the novel ester compounds Ee1 and Ee2 greatly perform better than the flavonoid compound.

According to the findings, Hela cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells are all affected by the cytotoxic and cytostatic effects of the synthesized flavonoid ester compounds Ee1 and Ee2. In a cytotoxic experiment, flavonoid and the esters Ee1 and Ee2 that it produces exhibit IC_{50} values for Hela carcinoma cells of 31.84µg/ml, 8.35µg/ml and 5.08µg/ml, respectively. In a cytostatic experiment, flavonoid and its synthetic esters Ee1 and Ee2 had IC_{50} values of 1.16µg/ml, 1.88µg/ml and 4.15µg/ml, respectively, for Hela cancer cells. As for the other cancer cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells; the values of IC_{50} for each of the flavonoid, Ee1 and Ee2 in cytotoxic assay were sequentially: 31.6µg/ml, 4.85µg/ml and 0.25µg/ml; 20.82µg/ml, 1.61µg/ml and 28.63µg/ml; 2.64µg/ml, 19.4µg/ml and 0.8µg/ml. In cytostatic assay, the value of IC_{50} for each flavonoid, Ee1 and Ee2 were, in order: 9.05µg/ml, 2.25µg/ml and 0.13µg/ml; 9.55µg/ml, 1.47µg/ml and 4.86µg/ml; 1.29µg/ml, 0.33µg/ml and 0.028µg/ml; on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells, respectively Table 3.4.

Table 3.4

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of flavonoid and the synthesized flavonoid ester compounds (Ee1 and Ee2) on Hela cancer cells, L6 normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells

Assay	Flavanoid	Ee1	Ee2
Hela Cytotoxic	31.84	8.35	5.08
Hela Cytostatic	1.16	1.88	4.15
L6 Cytotoxic	11.41	4.98	4.4
L6 Cytostatic	1.22	0.19	3.04
MCF7 Breast Cytotoxic	31.6	4.85	0.25
MCF7 Breast Cytostatic	9.05	2.25	0.13
PC3 Prostate Cytotoxic	20.82	1.61	28.63
PC3 Prostate Cytostatic	9.55	1.47	4.86
HepG2 Liver Cytotoxic	2.64	19.4	0.8
HepG2 Liver Cytostatic	1.29	0.33	0.028

Amide compounds were studied as anticancer agents against the Hela cancer cell line, the MCF7 breast cancer cell line, the PC3 prostate cancer cell line, and the HepG2 liver cancer cell line on several concentrations. Compared to that, they were studied on the L6 muscle normal cell line also. The %of living cells of A1 compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (35-41%), (63.7-67.5%),(29.3-32%), (31.34.2%), and (28-30.8%), respectively (Fig. 5a in Appendix A); but in cytostatic assay was measured as (51.8-55.8%), (65.3-69%),(58.6-60.5%), (53.2-57%), and (56.2-59.3%), respectively (Fig. 5b in Appendix A). Accordingly, results were appeared that the amide compound A1 was more effective on HepG2 liver cancer cells in cytotoxic assay and it was more effective on Hela cancer cells in cytostatic assay.

The % of living cells of A2 compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells was measured as (35.4-41%), (54.3-58.7%),(32-34.5%), (33.3-36%), and (28.2-29.9%), respectively (Fig. 5c in Appendix A); but in cytostatic assay was measured as (55.5-58.7%), (61-65%),(53.6-56%), (55.5-58.5%), and (60.63%), respectively (Fig. 5d in Appendix A). According to the findings, the amide compound A2 was more effective in cytotoxic assay on HepG2 liver cancer cells and in cytostatic assay on MCF7 breast cancer cells. For Aa compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells, the % of living cells was measured as (25-29.5%), (51-56.3%),(33.3-36%), (28.2-31.9%), and (31.3-33%), respectively (Fig. 5e in Appendix A); but in cytostatic assay was measured as (55-58%), (71-75.4%),(57.5-60%), (60-63.7%), and (59.6-61.8%), respectively (Fig. 5f in Appendix A). The results showed that in both cytotoxic and cytostatic assays, the amide compound Aa was more efficient on Hela cancer cells. Moreover, for Ab compound in cytotoxic assay against Hela cancer cells, L6 muscle normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells, and HepG2 liver cancer cells, the % of living cells was measured as (23-26%), (71-75%),(28-31.6%), (26-30.7%), and (26.8-30.5%), respectively (Fig. 5g in Appendix A); but in cytostatic assay was measured as (51-54.8%), (61-62.5%),(56.6-59.7%), (58.66-61.4%), and (52-55.7%), respectively (Fig. 5h in Appendix A). The

outcomes demonstrated that the amide compound Ab was more effective on Hela cancer cells in both cytotoxic and cytostatic assays.

Amide compounds A1, A2, Aa and Ab have IC_{50} values for Hela cancer cells of 32.65µg/ml, 16.65µg/ml, 7.19µg/ml and 3.5µg/ml in cytotoxic assay, respectively. The IC_{50} values for A1, A2, Aa and Ab on Hela cancer cells in cytostatic assay are as follows: 4.23µg/ml, 1.65µg/ml, 5.11µg/ml and 3.29µg/ml, respectively. The IC_{50} values of the amide compounds on MCF7 breast cancer cells in cytotoxic and cytostatic assays were as follows: A1 1.5µg/ml, A2 6.03µg/ml, Aa 6.15µg/ml and Ab 19.2µg/ml in cytotoxic assay; but A1 1.02µg/ml, A2 2.64µg/ml, Aa 1.68µg/ml and Ab 9.43µg/ml in cytostatic assay. Moreover, the IC_{50} values of the amide compounds on PC3 prostate cancer cells in cytotoxic and cytostatic assays were as follows: A1 4.92µg/ml, A2 14.93µg/ml, Aa 6.05µg/ml and Ab 25.8µg/ml in cytotoxic assay; but A1 4.77µg/ml, A2 5.73µg/ml, Aa 5.58µg/ml and Ab 2.23µg/ml in cytostatic assay. Finally, A1 14.66µg/ml, A2 14.34µg/ml, Aa 17.06µg/ml and Ab 24.68µg/ml were the IC_{50} values for the amide compounds against HepG2 liver cancer cells in cytotoxic assay and A1 0.016µg/ml, A2 6.79µg/ml, Aa 7.89µg/ml and Ab 10.56µg/ml in cytostatic assay Table 3.5.

Table 3.5

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of the amide compounds (A1, A2, Aa and Ab) on Hela cancer cells, L6 normal cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells

Assay	Aa	Ab	Ac	Ad
Hela Cytotoxic	32.65	16.65	7.19	3.5
Hela Cytostatic	4.23	1.65	5.11	3.29
L6 Cytotoxic	2.42	5.47	3.23	14.38
L6 Cytostatic	2.07	3.3	2.03	1.56
MCF7 Breast Cytotoxic	1.5	6.03	6.15	19.2
MCF7 Breast Cytostatic	1.02	2.64	1.68	9.43
PC3 Prostate Cytotoxic	4.92	14.93	6.05	25.8
PC3 Prostate Cytostatic	4.77	5.73	5.58	2.23
HepG2 Liver Cytotoxic	14.66	14.34	17.06	24.68
HepG2 Liver Cytostatic	0.016	6.79	7.89	10.56

The four imine compounds in *vitro* anticancer effects on the Hela carcinoma cell were assessed. The findings demonstrated that different doses of the synthesized compounds have lethal effects on Hela cancer cells. Within the investigated concentration range, Hela cancer cells' cytotoxic assay viability ranged from 23 to 43.6%. As the concentration of the produced imine compounds rose, cell growth decreased.

In a cytotoxic assay, the imine compound 4 outperformed the other tested imine compounds 3, 7, and 8 against Hela carcinoma cells at all doses. In a cytotoxic assay, imine compound 4 reduced the viability of the tested Hela cancer cells from 23% at a concentration of 500µg/ml to 31.8% at a concentration of 31.25µg/ml. In a cytotoxic experiment, imines 7 and 8 from the produced imines are better than imine 3 against Hela cancer cells. They both reduced the viability of the tested Hela cancer cells, which went from 31% at 500µg/ml to 40% at 31.25µg/ml and from 34% at 500µg/ml to 37% at 31.25µg/ml, respectively. Imine 3 reduced the viability of the tested Hela cancer cells from 40% at a concentration of 500µg/ml to 43.6% at a concentration of 31.25µg/ml. Moreover, the viability of Hela cancer cells in cytostatic assay was higher for imine 4 than for the imine 3, then for imine 7 and imine 8 (Fig. 6a and 6b in Appendix A).

The four imine compounds were investigated on L6 normal cells as well as Hela carcinoma cells. According to the findings of L6 normal cells, all investigated imines exhibit good cytotoxic and cytostatic effects, as shown in (Fig. 6c and 6d in Appendix A). Imine 4 caused 67.8% of the surviving cells in the cytotoxic assay using L6 normal cells to remain alive at 500µg/ml and 73.03% at 31.25µg/ml. Additionally, in the cytostatic test using L6 normal cells, the proportion of living cells for imine 4 was 74.06% at 500µg/ml and 79.32% at 31.25µg/ml. Among the generated imines, imines 7 and 8 are comparable to imine 3 in cytotoxic and cytostatic experiments for L6 normal cells. Imines 7 and 8 exhibit cytotoxic assay results that range from 55.65% at 500µg/ml to 59.2% at 31.25µg/ml and 66.46% at 500µg/ml to 73.15% at 31.25µg/ml, respectively, in terms of the percentage of living cells. In the cytostatic assay of L6 normal cells, the proportion of live cells ranged from 69.04% at 500µg/ml to 75.35% at 31.25µg/ml for imine 7. For imine 8, the percentage of live cells ranged from 74.06% at 500µg/ml to 78.15% at 31.25µg/ml. In the case of imine 3, the percentage of living cells in the cytotoxic assay ranged from 54.19% at 500µg/ml to 58.3% at 31.25µg/ml, while

the percentage of living cells in the cytostatic assay ranged from 62.4% at 500µg/ml to 67.75% at 31.25µg/ml.

The outcomes demonstrated that Hela cancer cells are cytotoxic and cytostatic to all produced imine compounds at all doses. In a cytotoxic study, the following compounds had IC_{50} values for Hela cancer cells: compound 3 is 16.45µg/ml, compound 4 is 24.7µg/ml, compound 7 is 14.518µg/ml and compound 8 is 2.6µg/ml. As indicated in Table 3.6, the produced imines' IC50 values for killing Hela cancer cells were as follows: compound 3 is 3.745µg/ml; compound 4 is 7.829µg/ml; compound 7 is 0.1148µg/ml and compound 8 is 2.144µg/ml. They were tested on L6 normal cells in the same way that all imine compounds were studied on Hela cancer cells. At all doses, the synthesized imine compounds have cytotoxic and cytostatic effects on L6 normal cells.The IC_{50} values of the prepared imines on L6 normal cells in cytotoxic assay were; compound 3 is 6.725µg/ml, compound 4 is 6.213µg/ml, compound 7 is 12.86µg/ml and compound 8 is 19.5µg/ml. While the IC_{50} values of the prepared imines on L6 normal cells in cytostatic assay were as follows: compound 3 is 6.07µg/ml, compound 4 is 3.59µg/ml, compound 7 is 4.07µg/ml and compound 8 is 0.755µg/ml as shown in and Table 3.6. Anyway, imine 4 showed the highest cytotoxic and cytostatic effect among the tested imines against Hela cancer cells. Comparing, Imine 4 compound to control L6 normal cells, which displayed cytotoxic and cytostatic effects with IC_{50} values of 6.213µg/ml and 3.59µg/ml, respectively, Imine 4 compound revealed IC_{50} values of 24.7µg/ml and 7.829µg/ml at nontoxic levels. Imine 4 has the ability to fit well into the binding site, interact with the receptor site of Hela cancer cells, and interact through hydrogen bonding with functional groups present in the receptor site due to the presence of the oxygen heteroatom, bromine atom, and nitrogen atoms [85].

Table 3.6

Cytotoxic and cytostatic effect of prepared imine compounds (3, 4, 7 and 8) on Hela cancer cells and L6 normal cells as IC_{50} values

Assay	3	4	7	8
Hela Cytotoxic	16.45	24.7	14.518	2.6
Hela Cytostatic	3.745	7.829	0.1148	2.144
L6 Cytotoxic	6.72	6.213	12.86	19.5
L6 Cytostatic	6.07	3.59	4.07	0.755

Four imine compounds (1, 2, 5 and 6) were investigated for their in vitro anticancer properties against the Hela cancer cell. The findings demonstrated that different doses of the synthesized compounds have cytotoxic effects on Hela cancer cells. Within the investigated concentration range (31.25-500 µg/ml), the vitality of Hela cancer cells in cytotoxic assay ranged from 20.6 to 52.76%. As the concentration of the synthesized imine compounds rose, cell proliferation decreased (Fig. 7a in Appendix A).

In a cytotoxic study, the imine compound 6 performed better than the other tested imine compounds 1, 2, and 5 against Hela cancer cells at all doses. In a cytotoxic assay, imine compound 6 reduced the viability of the investigated Hela cancer cells from 20.6% to 23.74 at the investigated concentration range from 31.25µg/ml to 500µg/ml. Meanwhile, in a cytotoxic study, the synthesized imines 2 and 5 perform better than imine 1 against Hela cancer cells. They reduced the viability of the investigated Hela cancer cells from 32.7% to 35.4% and from 36.7% f to 40.3%, respectively. With regard to imine 1, the evaluated Hela cancer cells' viability reduced from 44.8% to 52.76% (Fig. 7a in Appendix A). Additionally, as shown in (Fig. 7b in Appendix A), the vitality of Hela cancer cells in the cytostatic experiment was significantly greater for imine 5 than for imine 2, then for imine 6, and at last for imine 1.

The four imine compounds were investigated on L6 normal cells as well as Hela cancer cells. According to the findings in (Fig. 7c and 7d in Appendix A), all imines investigated have significant cytotoxic and cytostatic effects on L6 normal cells. In the cytotoxic experiment on L6 normal cells, the percentage of living cells of imine 1 was 70.23% at 500µg/ml and 75.45% at 31.25µg/ml. Additionally the percentage of alive cells for imine 1 in the cytostatic experiment of L6 normal cells was 73.2% at 500µg/ml and 77.5% at 31.25µg/ml. As for the other imine compounds (2, 5 and 6), their effectiveness on L6 normal cells was close to some extent. In cytotoxic assay on L6

normal cells, the % of living cells for imine 2, imine 5 and imine 6 was from 52% to 53.8%, 55.7% to 57.5% and 50.79% to 54.5%, respectively (Fig. 7c in Appendix A). Moreover, as shown in (Fig. 7d in Appendix A), the vitality of L6 normal cells in the cytostatic experiment was significantly greater for imine 6 than for imine 5 and then for imine 2.

The outcomes demonstrated that Hela cancer cells are cytotoxic and cytostatic to all synthesized imine compounds at all doses. The following are the imines' IC_{50} values for cytotoxic assay on Hela cancer cells: Compounds 1 and 2 are each 25.16µg/ml and 22.79 µg/ml meanwhile Compound 5 and Compound 6 are each 2.36µg/ml and 14.63µg/ml. According to Table 3.7, compound 1 had an IC_{50} value of 24.39µg/ml, compound 2 had a value of 12.26µg/ml, compound 5 had a value of 1.11µg/ml and compound 6 had a value of 9.18µg/ml when tested on Hela cancer cells in cytostatic assay. At all doses, the synthesized imine compounds have cytotoxic and cytostatic effects on L6 normal cells. In a cytotoxic experiment, the synthesized imines had IC_{50} values of compound 1 of 43.16µg/ml, compound 2 of 23.6µg/ml, compound 5 of 16.23µg/ml, and compound 6 of 0.83µg/ml on L6 normal cells. According to Table 3.7, compound 1 had an IC_{50} value of 19.7µg/ml, compound 2 had a value of 2.04µg/ml, compound 5 had a value of 15.875µg/ml, and compound 6 had a value of 0.409µg/ml on L6 normal cells in cytostatic experiment. All of these imine compounds have the ability to fit well into the binding site, engage with the receptor site of Hela cancer cells, and interact through hydrogen bonding with functional groups present in the receptor site due to the presence of nitrogen atoms [85]. Although imine compound 6 was more effective on HeLa cancer cells in the cytotoxic assay, and imine compound 1 was more effective on L6 normal cells in both the cytostatic and cytotoxic assays, it is because of the different results of these compounds, it is difficult for us to distinguish between the effectiveness of these compounds on cancer cells and normal cells, and the difference between them may be clear in subsequent studies.

Table 3.7

IC_{50} values in µg/ml for the cytotoxic and cytostatic effects of the imine compounds (1, 2, 5 and 6) on Hela cancer cells and L6 normal cells

Assay	1	2	5	6
Hela Cytotoxic	25.16	22.79	2.36	14.63
Hela Cytostatic	24.39	12.26	1.11	9.18
L6 Cytotoxic	43.16	23.6	16.23	0.83
L6 Cytostatic	19.7	2.04	15.875	0.409

3.2.2 Antibacterial activity

Agar disk diffusion method of E2 derivative and thymol in this study showed antibacterial activity against the screened bacterial isolates. (Fig. 1g in Appendix A) illustrates that the E2 derivative produced the highest antibacterial activity against tested Gram positive bacteria (S. aureus and S. epidermis) compared to thymol, as it produced 12 mm and 15 mm inhibition zones against both isolates, respectively. Even more, the inhibition zone (15 mm) that resulted from the E2 derivative was larger than Gentamicin inhibition zone (7 mm) against S. Epidermis (Fig. 1g in Appendix A).

The antimicrobial activity of the E2 derivative and thymol under study was quantitatively assessed by determining the MIC concentration for each compound against the five bacterial isolates. (Fig. 1h in Appendix A) clearly showed that the E2 derivative was more effective than thymol against Gram positive bacteria in this experiment. Hence, 15.625µg/mL MIC value was recorded for E2 against S. aureus and 62.5µg/mL against S. epidermis.

The minimum bacteriocidal concentrations (MBC) for all concentrations that were given inhibitory effects in this research were determined. (Fig. 1h in Appendix A) showed that the E2 derivative was the best as it killed the S. epidermis at a low concentration (31.25µg/mL) which is the lowest among the screened compounds.

Thymol exhibits its antibacterial and bactericidal activities by several mechanisms, including, cell membrane disruption, biofilm reduction, inhibition of motility, inhibition of membrane bound ATPases and inhibition of efflux pumps [86]. In addition, thymol possesses antibacterial activity against a wide range of species, including biofilm-embedded microorganisms. In spite of its antibacterial activity, the use of thymol as an antibacterial agent is limited. This is due to the requirement for a high dose of thymol

for its bactericidal action besides its toxicity to animal cells. So, there is a need for additional studies to achieve optimal antimicrobial activity by using nontoxic concentrations of thymol [87]. In this study, a newly synthesized thymol ester derivative with an indole functional group E2 was investigated for its antibacterial behavior against different bacterial species. This compound E2 displayed antibacterial activity against the studied bacterial species with a remarkable effect against Gram positive species. As thymol and its derivatives are terpenes, they tend to affect Gram-positive bacteria more than Gram-negative ones [88]. In general, the lipophilic nature of terpenes is responsible for their antimicrobial mechanism of action. Moreover, the impact of monoterpenes on membrane structure occurs by increasing its fluidity and permeability, changing the topology of its proteins and causing disturbances across the respiration chain [88]. Considering the chemical structure of E2, a previous study dealing with thymol and its ester derivatives highlighted that the most enhanced activity of these esters is against Gram positive bacteria. Indeed, other study focused on the thymol derivatives with blocked phenolic hydroxyl group that linked to different five-membered heterocyclic moieties. These derivatives offer dual antimicrobial and insecticidal activities [89]. Nagle group also synthesized thymol containing pyridine moieties that showed better antibacterial and antifungal activity than thymol [90]. In addition, it is well known that imidazoles and triazoles comprise the largest group of antifungal agents [91]. Similar to E2, all these compounds contain N in their structure, which may be the cause of their antibacterial effectiveness. On top of that, as E2 contains indole, studies showed that both the indole ring and the side chains are important for bioactivity [92]. Based on that, indole derivatives are effective and suitable for further development [93].

The in *vitro* biological screening effects of the investigated compounds were tested by the agar disk diffusion method. The compounds under study possessed potential antibacterial activity against some reference bacteria (Fig. 2k in Appendix A). The obtained results showed that the studied compounds acted as antibacterial agents with different behaviors. It is clearly noticeable that K. pneumoniae was the most susceptible isolates, as most of the tested compounds inhibited its growth. In general, E. coli is weakly affected by E4 (8 mm) and E7 (8 mm) inhibition zones. The same observation was recorded for S. aureus, as compounds E4 and E6 inhibited its growth with (9 mm)

inhibition zones for both. The most active compounds against S. epidermis were E4 (10 mm IZD) and E8 (9 mm IZD). The P. aeruginosa reference isolate showed high susceptibility to E4 (16 mm IZD) and E5 (11 mm IZD) which are approximately equal to or greater than the inhibition zones obtained from the broad spectrum antibiotic Gentanicin (13 mm IZD). It is clearly recognized that compound E4 exhibits a broad spectrum antibacterial activity against all tested bacteria. So, it's the best among the examined compounds in the running experiment.

All compounds under study were further tested for their minimum inhibitory concentration (MIC) against all bacteria in this experiment (Table B.2 in Appendix B). Results showed a strong inhibitory effect of compound E4 against all studied bacteria with MIC range (62.5-250µg/mL). Moreover, the E4 compound (MIC 62.5µg/mL) was more effective than carvacrol (MIC 125µg/mL) against the gram negative bacteria K. pneumoniae. Likewise, the gram positive bacteria S. epidermis was inhibited by the action of compound E4 (MIC 125µg/mL) which is lower than the MIC value of the broad spectrum antibiotic Gentamicin (500µg/mL). In addition to that, the other tested compounds provided a lower inhibitory effect against the examined bacteria. Compound E5 inhibited K. pneumoniae and P. aeruginosa, and compound E7 inhibited E. coli at 250µg/mL. In addition to that, the MIC values of compound E6 against S. aureus and compound E8 against S. epidemis equal to 250µg/mL. Furthermore, the bactericidal effect of all compounds that had an inhibitory effect was evaluated by measuring their minimum bactericidal concentration (MBC) (Table B.2 in Appendix B). MBC results provided that compound E4 was the most powerful bactericidal agent as it killed all tested bacteria in this research at the MBC range (125-250µg/mL). Additionally, S, epidermis was more responsive to the bactericidal effects of compounds E4 (MBC 125µg/mL) and E8 (MBC 125µg/mL) than the Gentamicin antibiotic (MBC 1000µg/mL). With respect to carvacrol (MBC 250µg/mL) the same observation was noticed for the E4 compound (MBC 125µg/mL) against S. aureus.

Recently, scientists have shifted their focus towards alternative therapies for treating infections caused by multidrug resistant bacteria [94]. Therefore, finding potential alternatives to antibiotics was a real challenge. On account of their therapeutic properties, essential oils constituents have gained attention as an alternative approach in traditional medicine. In this field, carvacrol has been a key player, as it has shown

potent antimicrobial activity against a wide range of gram positive and gram negative bacteria. However, the molecular mechanism of action of carvacrol is not completely known, it is proposed to include the alteration of membrane structure, the interference with nucleic acid synthesis, the coagulation of cytoplasm and loss of its constituents, the imbalance of metabolism, and the hindering of bacterial communication [95, 96]. In spite of its potential antibacterial effect, carvacrol displays many unfavorable physico-chemical properties that limit its usage. These properties include high volatility, instability and low water-solubility [97]. Based on that, researchers highlighted their work on carvacrol and its several derivatives to improve its therapeutic profile. In this study, five new esters of carvacrol (E4, E5, E6, E7 and E8) were synthesized and tested against the five reference bacterial isolates (E. coli, K. pneumoniae, P. aeruginosa, S. aureus and S. epidermis). All compounds under study are esters. According to previous studies, there is an association between the presence of the hydroxyl group and the good activity of carvacrol against microbes like E. coli and S. aureus [98]. This bioactivity is noticed in the conducting research when comparing carvacrol with other derivatives. Further studies proposed that the substitution of the hydroxyl group with an ester moiety increases the activity of the originating compounds [99]. Moreover, the presence of carbonyl group in terpenoids improves their bacteriostatic action [95], whereas, the substitution of carvacrol hydroxyl with an amino function leads to a complete loss of its activity [97]. Accordingly, the essential requirements for good activity of carvacrol derivatives are based on the type of substituents in addition to their appropriate positions. In this regard, data highlighted that ester derivatives resulted by the introduction of some moieties like propionate and others in the carvacrol aromatic ring because of a weak antimicrobial effect [100]. In general, results recorded that there is a bioactivity reduction in some ester derivatives, which confirmed the important role of the free hydroxyl group in carvacrol [97]. Besides, the obtained results revealed that the E4 compound was the best antibacterial agent among other synthesized ones due to its broad spectrum of action against all screened bacterial isolates. This compound like others in this study; is a heterocyclic derivative of carvacrol. In the previous literature, this derivative type can be used as antiviral, antiulcer and analgesic agents [101]. Also, some compounds combined with various N and S-nucleophiles exhibit promising antiviral and antiulcer activity. Consequently, those heterocyclic derivatives mainly ester derivatives with N-containing heterocycles, are important and highly motivating

compounds for the further development of antiviral agents and other antimicrobials [102].

This study was performed to evaluate the antibacterial efficacy of two flavonoid esters, Ee1 and Ee2 against two Gram-negative bacteria, *E. coli* and *K. pneumoniae* and two Gram-positive bacteria, *S. aureus* and *S. epidermis*. (Fig. 4k in Appendix A) shows the results of the Kirby-Bauer disk diffusion method of both flavonoid esters in addition to flavonoid and a broad spectrum antibiotic Gentamicin against all studied bacteria. The obtained results indicated that both esters were able to act against the studied bacteria even more than flavonoid. The best activity for Ee1 was against *S. epidermis* (14 mm IZD) and for Ee2 was against *K. pneumoniae* (15 mm IZD).

The MIC results indicated that all examined flavonoid esters were able to act against all studied bacteria, with some variation among them (Fig. 4k in Appendix A and Table B.3 in Appendix B). Moreover, Ee2 manifested the best inhibitory behavior. As it inhibited the growth of all tested bacterial isolates at (15.625 - 31.25µg/mL) concentration range. Apparently, Ee2 inhibition power toward all tested bacteria was greater than flavonoid. Furthermore, in comparison to flavonoid, the inhibitory activity of Ee1 against *S. aureus* (MIC=15.625µg/mL) was better than that of flavonoid (MIC=62.5µg/mL). In addition to that the obtained MBC results confirmed that Ee2 flavonoid ester was a potent bactericidal agent as it killed the tested bacteria at 62.5µg/mL concentration. It is interesting to find that both flavonoid esters showed superior bactericidal activity against *S. epidermidis* that exceeded the bactericidal activity of the Gentamicin antibiotic by two to three folds.

The spread of multidrug-resistant bacteria represents an important medical need for new antimicrobial agents. This prompted researchers to seek novel antibacterial remedies from natural products. Naturally occurring flavonoids are good candidates to fight against pathogens because of their wide range of biological activities. Also, semi-synthetic or synthetic flavonoids have great potential to work as bacteriostatic and bactericidal agents at low concentrations [103]. There are three major classes of flavonoids; flavonoids, isoflavonoids and neoflavonoids [104]. In this study, two new synthetic derivatives of flavonoids Ee1 and Ee2 were explored for their antibacterial potential. Results indicated that both compounds demonstrated a broad spectrum of

antibacterial action. Literature supports these findings, as isoflavonoids use different mechanisms to attack bacteria including, membrane disruption, bio-film formation, inhibition of cell envelope synthesis and inhibition of nucleic acid synthesis [105]. Moreover, the antimicrobial activity of synthetic flavonoids is most often related to the cell membrane interaction [106], inhibition of key enzymes of the bacterial metabolism like gyrase [107], topoisomerase [108] and tyrosine kinase [109]. According to the Ee1 structure, the presence of a thiophene moiety may be associated with a broad spectrum of antibacterial activity. This moiety is found as an integral part of the structure of some antibiotics like Ticarcillin, Cefoxitin, Cephalothin, Temocillin, and Cephalorodine [110]. Likely, the chemical structure of Ee2 indicated the presence of indole. Given that indole is a heterocyclic group, it is possible to infer that all of the numerous properties of the functional groups or derivatives included in this classification are organically dynamic and are crucial in therapeutic science [111]. In this regard, daptomycin antibiotic is an example of an indole-containing alkaloid that has already been utilized in clinical practice to treat complex skin and tissue infections, endocarditis, and bacteremia [112]. Over that, the presence of carboxylic acid is the antibacterial agent that is usually applied as disinfectant [113]. However, due to the vast variety of substituents, it is difficult to draw definitive conclusions concerning the relationship between their structure and activity. On the basis of the obtained findings, both studied compounds can be considered as promising antibacterial candidates.

In order to estimate the antimicrobial activity, amides (A1, A2, Aa and Ab) were tested against several bacterial isolates (*B. subtillus, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus* and *S. epidermis*) using Kirby-Bauer disk diffusion susceptibility method (Fig. 5i in Appendix A). The highest recorded activity is for A1 amide, as it showed broad spectrum antibacterial activity with an inhibition zone range of 10-14 mm. It was observed that A2 and Aa amides produced weak to moderate inhibition activity against the tested bacteria. The results revealed that Ab amide acted as an excellent antibacterial agent against *S. aureus* (16 mm inhibition zone). Remarkably, all tested amides A1, A2, Aa and Ab were able to target *S. epidermis* with inhibition zones of 13, 9, 11 and 11 mm, respectively, which were more than the Gentamicin antibiotic inhibition zone (7 mm).

The obtained MIC results revealed that all amides under study obtained good bacteriostatic activity against the examined bacterial isolates, with some observed variation among them (Table B.4 in Appendix B). Moreover, the A1 amide exhibited the best inhibitory behavior among the others. It inhibited the growth of all tested bacterial isolates in a concentration range between 3.90625µg/mL and 62.5µg/mL. Furthermore, the lowest MIC value (3.90625µg/ml) was recorded for the A1 amide against *E. coli*. However, the other amides displayed noticeable bacteriostatic potential with a concentration range (7.8125µg/mL-62.5µg/mL). Even more, A1 amide work better than Gentamicin against *B. subtillus, E. coli* and *S. epidermis*. The same observation was reported for the A2 amide against *E. coli, P. aeruginosa* and *S. epidermis,* Aa against *B. subtillus* and *S. epidermis,* and Ab against *P. vulgaris*. In addition to that, the bactericidal effect of all amides that exhibited inhibitory effects was determined by measuring their MBC values (Table B.4 in Appendix B). The obtained MBC results confirmed that the A1 amide was the most potent one as it had a bactericidal effect at concentration range (7.8125µg/mL-125µg/mL). It is worth pointing out that all amides in this study kill *S. epidermis* at lower concentrations (62.5µg/mL-250µg/mL) than Gentamicin antibiotic (500µg/mL).

The emergence of multidrug-resistant bacterial isolates and the low efficacy of currently available drugs promote the necessity for the discovery of novel antibacterial chemical compounds that inhibit these multidrug-resistant microorganisms. This has prompted a search for alternative forms of therapy [114]. Among these alternative therapies, a large available number of bioactive compounds are with heterocyclic scaffolds that mostly contain nitrogen or oxygen [115]. An important group of heterocyclic compounds with significant biological characteristics are furan derivatives. In view of their bioactivity, many drugs containing furan rings have been clinically approved (ceftiofur, furazolidone, nifuroxazide, cefuroxime, nifurtoinol, nitrofurantoin and nitrofurazone) [116]. In addition, the other heterocyclic scaffold pyrrolidine emerges as a versatile molecule that builds compounds with a broad spectrum of biological activities, including antimicrobials [117]. Piperazine belongs to the anthelmentics medicines family [118]. This molecule and its derivatives are found in a large number of antibiotics that contain amide linkage. In this aspect, amides possess various and excellent biological activities, like antibacterial ones [119]. As well as pyrroles and their

fused derivatives are a significant class of compounds with a wide-range of biological activities [120]. Besides these heterocyclic compounds, the antibacterial agent carboxylic acids are frequently used as disinfectants.

Based on the previous mentioned information, the four compounds in this study are amides containing carboxylic acid in combination with two of the following: furan, pyrrolidine, piperazine and pyrrole. Noteworthy, the incorporation of these molecules has demonstrated an ability to modulate a wide range of targets to produce excellent *in vitro* antibacterial activity, primarily against Gram-positive and Gram-negative bacteria. A1 and A2 amides were formerly known; chemically characterized and some bioassays showed their ability to act as antibacterial agents. More specifically, A1 amide inhibits *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [121]. Likewise, A2 amide exhibits the same activity against these microorganisms [122].

The outcomes of this study revealed that the investigated amides (A1, A2, Aa and Ab) could contribute to overcoming the problem of antibiotic resistance and the critical requirement to create novel therapies for bacterial infections. Accordingly, further research is needed to explore the precise mechanisms for the antibacterial behavior of these compounds.

The bioactivity of the four newly synthesized Schiff bases was investigated in the current investigation for any potential antibacterial activity against a variety of bacterial isolates, including B. subtilis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus, and S. epidermis. Different bactericidal effects were achieved via disk diffusion (Fig. 6e in Appendix A). It's interesting to note that Schiff bases like 4 and 7 had high antibacterial activity that was even more potent than that of the all-purpose antibiotic Gentamicin.

The micro-broth dilution method was used to measure the MIC and MBC values for all produced Schiff bases in order to further analyze the antibacterial activity. All four Schiff bases exhibit bacteriostatic action against the bacterial isolates under study, according to the results of the micro-broth dilution assay (Table B.5 and B.6 in Appendix B). In this experiment, the obtained MIC results indicated that Schiff base 7 was the highly effective one as it inhibited the growth of all tested bacteria at very low concentrations within 0.078125 to 0.3125µg/ml range. Moreover, Schiff base 8

inhibited the growth of all examined bacteria with MIC values range between 0.078125µg/ml and 0.625µg/ml. In this research, the examined isolate *S. epidermis* was the most responsive to the inhibitory action of synthesized Schiff base 3. In addition, *B. subtilis* was the most sensitive to Schiff base 4. Besides MIC measurements, MBC measurements for all four Schiff bases were also investigated. The MBC results revealed that Schiff base 7 worked as bactericidal agent at a concentration range between 0.15625µg/ml and 1.25µg/ml against all tested bacteria.

The results showed that both Gram-positive and Gram-negative bacteria might be affected by the four Schiff bases. This explains the wide-ranging antibacterial properties of these newly synthesized Schiff bases, which may be mediated by a number of mechanisms that target crucial stages in bacterial growth. These processes include one of the following, according to earlier work [123, 124]. The first involves stopping a key metabolic enzyme in the target microorganism. Interference with the production of the cell wall is the second proposed mechanism. This might alter how permeable cells are or disrupt lipoproteins, which would kill cells. The third process is the breakdown of certain proteins found in microbial cell walls. This in turn leads to harmful effects by the alteration in the normal cellular processes. The fourth is based on the structure of the azomethine group, which forms hydrogen bonds with the active centers of microbial cell components, causing a conflict with regular cellular operations.

Additionally, scientists discovered that Schiff bases with nitrogen, oxygen, and sulfur atoms exhibit significant biological activity, such as their role as antibacterial agents [125].

The current study was conducted to explore the antibacterial potential of newly synthesized Schiff bases, which are 1, 2, 5 and 6. The *in vitro* biological screening effect of the investigated Schiff bases was tested by the agar disk diffusion method. Schiff bases under study possessed potential antibacterial activity against most tested bacterial isolates (Fig. 7e in Appendix A). The obtained results showed that the studied Schiff bases acted as antibacterial agents with different behaviors. It is clearly noticed that *K. pneumoniae* (ATCC# 13883), *P. vulgaris* (ATCC# 8427), *P. aeruginosa* (ATCC# 9027) and *S. epidermis* (ATCC# 12228) were the most sensitive isolates to compound 6 as it was more efficient than the broad specific antibiotic Gentamicin. The

other examined Schiff bases revealed low to moderate antibacterial activity with inhibition zones ranging between 7 mm and 16 mm.

The antibacterial activity was examined by measuring the MIC and MBC values for all previously mentioned Schiff bases (Table B.7 in Appendix B). The obtained MIC results revealed that compound 6 exhibited excellent inhibitory activity against all studied bacterial isolates. The promising bacteriostatic effect of this liquid compound 6 was against Gram negative bacteria, as the MIC values against *E. coli, K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa* were 0.07813, 0.03906, 0.15625 and 0.01953µg/ml respectively. In addition to that, Compounds 2 and 5 displayed good bacteriostatic effects with MIC ranges (31.25 µg/ml-62.5 µg/ml) for compound 2 and (31.25 µg/ml-125 µg/ml) for Compound 5. On the other hand, compound 1 showed a moderate bacteriostatic effect against all bacterial isolates under investigation, with MIC values ranges (31.25µg/ml- 250µg/ml). Moreover, the bactericidal effect of all synthesized Schiff bases was also measured in the current study. Among them, compound 6 was the most potent bactericidal agent, as it killed all the studied bacterial isolates at a concentration range (0.03906-0.3125µg/ml).

The emergence of multidrug-resistant bacteria and their emphasis on health care costs have provoked an interest in the design and development of novel and cost effective antimicrobial agents with increased bioactivity against resistant bacteria [126]. Schiff bases as well as their metal complexes have a wide application in the medical microbiology field. Those compounds may come to be an excellent alternative to antibiotics [127]. Therefore, the *in vitro* antibacterial activity of four new Schiff bases was studied in the present study. The variable antibacterial activity of different Schiff bases may be due to their different structures. It was reported that there was a relationship between the structure of Schiff bases and their activity. For example, the elongation of hydrophobic alkyl chains causes an increase in antibacterial activity [128]. Another important factor controlling antimicrobial activity is hydrophobicity. Thus, the penetration of Schiff bases through the cell membrane into the microbial cell may depend on the degree of their hydrophobicity. The hydrophobic nature of the bacterial cell membrane will enhance the interaction with nonpolar compounds and favor their penetration into the cytoplasm, leading to cell death [129]. Furthermore, it is worth mentioning that the mode of antibacterial action of the Schiff bases may involve the

formation of a hydrogen bond between the azomethine group and some cell constituents. This in turn leads to interference with normal cell processes. Moreover, the variation in the effectiveness of the different Schiff bases against different microorganisms depends on the impermeability of microbial cells or differences in their ribosome's [130]. The presence of numerous scaffolds such as aromatic and substituted aromatic compounds in the structure of Schiff base may enhance their beneficial impact. Besides that, the presence of electron rich species such as nitrogen (N), oxygen (O), sulfur (S) in the Schiff base molecule are reported to increase the applications of Schiff bases [131]. This may explain the valuable antibacterial effect of all prepared Schiff bases in the running experiment as they contain at least one electron rich species a long with aromatic compounds.

3.2.3 Antioxidant activity

The DPPH free radical scavenging assay is the antioxidant assay that is commonly utilized in laboratories to find out the ability of natural products to scavenge free radicals. The findings from this experiment showed that E2 ester derivative illustrated a moderate antioxidant power when compared to the ascorbic acid. Otherwise, the low concentrations of this derivative (16-0.5µg/mL) displayed better scavenging power than thymol (Fig. 1h in Appendix A).

Thymol's phenolic structure and redox properties are crucial in the adsorption and neutralization of free radicals or decomposing peroxides. These features are attributed to its antioxidant activity [132]. Moreover, the capability of thymol derivatives with different structures to scavenge free radicals was investigated. The antioxidant ability of these derivatives is due to the formation of an intramolecular hydrogen bond [133].

The Antioxidant activity of the two flavonoid esters (Ee1 and Ee2) was measured by their ability to scavenge DPPH free radicals. The obtained results revealed that both examined flavonoid esters showed low to moderate antioxidant activity (Fig. 4l in Appendix A). Ee1 had good antioxidant activity (28 %), which was higher than that of isoflavonoid (18.5 %) but lower than that of ascorbic acid (87.2%) at 500µg/mL.

In general, flavonoids considered powerful antioxidants. Their actions include the inhibition of enzymes responsible for superoxide anion production [134], enzymes involved in the generation of reactive oxygen species [135]. Also, some flavonoids chelate trace metals that play an important role in oxygen metabolism [136]. This support to some degree the results obtained in this research.

3.3 Conclusion

Twenty-four heterocyclic compounds distributed into seven groups were prepared by condensation reaction. TLC and FTIR spectroscopy were used to monitor the reaction during the synthesis. FTIR and ^{1}H-NMR spectroscopy were used to characterize all of compounds. The biological properties (such as antibacterial, anticancer, or antioxidants) of all the prepared compounds were studied and compared between them, each according to its group. All the prepared compounds showed good biological activity in varying proportions due to the presence of the heterocyclic part in each compound. Thymol ester E2 which has an indole was better at preserving normal muscle cells than thymol. The E4 carvacrol ester compound which has pyrrole was the best compound in its group, as it showed anti-cancer activity against HepG2 liver cancer cells in cytotoxic assay and against MCF7 breast cancer cells in cytostatic assay and also gave a distinctive activity against all types of bacteria studied. Also E4 showed a strong inhibitory effect against all studied types of bacteria. Ester compound Ea was more effective against MCF7 breast cancer cells in cytotoxic assay and against PC3 prostate cancer cells in cytostatic assay. The ester compound Eb performed better in cytotoxic and cytostatic tests on HepG2 liver cancer cells and MCF7 breast cancer cells, respectively. Ester compound Ec was more effective against Hela cancer cells and MCF7 breast cancer cells, respectively, in cytotoxic and cytostatic experiments. In addition, in both cytotoxic and cytostatic experiments, the ester compound Ed was more efficient against HepG2 liver cancer cells. Flavonoid ester Ee1 gave a distinctive activity in all biological tests. Moreover, amide compound A1 was the best compound in its group on all the studied biological activities, as were imine 4 and imine 6 as well. To understand the mechanism of the effect of these compounds on different biological cells, it would be good to conduct complementary studies in the future.

List of Abbreviations

Abbreviation	Meaning
Fig.	Figure
DMSO	Dimethyl sulfoxide
DCM	Dichloromethane
TLC	Thin layer chromatography
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
DMF	Dimethylformamide
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
μ	Micro
IZD	Diameter of the zone of inhibition
IR	Infrared spectroscopy
NMR	Nuclear magnetic resonance spectra
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
IC_{50}	The concentration of an inhibitor where the response is reduced by half
eq.	equivalent

References

[1] Reinhard, B. (2002). Advanced Organic Chemistry (First ed.). *San Diego, California: Harcourt Academic Press.* p. 414–427.

[2] Voet, D., Voet, J., & Pratt, C. (2008). Fundamentals of Biochemistry. *Hoboken, NJ: John Wiley & Sons, Inc*. p. 88.

[3] Lee, J. K., Han, H. S., Chaikasetsin, S., Marron, D. P., Waymouth, R. M., Prinz, F. B., & Zare, R. N. (2020). Condensing water vapor to droplets generates hydrogen peroxide. *Proceedings of the National Academy of Sciences*, *117*(49), 30934-30941.

[4] Noll, O., & Ritter, H. (1996). Polymerizable oligoesters from cholic acid via lipase catalyzed condensation reactions. *Macromol Rapid Commun*, *17*, 553-557.

[5] Dunky, M. (1998). Urea–formaldehyde (UF) adhesive resins for wood. *International Journal of Adhesion and Adhesives*, *18*(2), 95-107.

[6] Wade, L. G. (2012). Organic Chemistry Book. p. 961-1002.

[7] Hubbard, P., & Brittain, W. J. (1998). Mechanism of amine-catalyzed ester formation from an acid chloride and alcohol. *The Journal of Organic Chemistry*, *63*(3), 677-683.

[8] Hajipour, A. R., & Mazloumi, G. (2002). An efficient and simple procedure for preparation of esters and anhydrides from acid chlorides in the presence of 1, 4-diazabicyclo [2.2.2] octane (DABCO) under solvent-free conditions. *Synthetic communications*, *32*(1), 23-30.

[9] Dhake, K. P., Thakare, D. D., & Bhanage, B. M. (2013). Lipase: A potential biocatalyst for the synthesis of valuable flavour and fragrance ester compounds. *Flavour and Fragrance Journal*, *28*(2), 71-83.

[10] Phenix, A. (2002). The swelling of artists' paints in organic solvents. Part 2, Comparative swelling powers of selected organic solvents and solvent mixtures. *Journal of the American Institute for Conservation*, *41*(1), 61-90.

[11] Chen, X. Y., Wang, R. F., & Liu, B. (2015). An update on oligosaccharides and their esters from traditional Chinese medicines: Chemical structures and biological activities. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1-23.

[12] Hamerton, I., & Hay, J. N. (2016). Recent technological developments in cyanate ester resins. *High Performance Polymers*, *10*(2), 163–174.

[14] Heller, J., Barr, J., Ng, S. Y., Abdellauoi, K. S., & Gurny, R. (2002). Poly (ortho esters): synthesis, characterization, properties and uses. *Advanced drug delivery reviews*, *54*(7), 1015-1039.

[14] Keng, P. S., Basri, M., Zakaria, M. R. S., Rahman, M. A., Ariff, A. B., Rahman, R. A., & Salleh, A. B. (2009). Newly synthesized palm esters for cosmetics industry. *Industrial crops and products*, *29*(1), 37-44.

[15] Islam, M. R., Trivedi, P., Palaniappan, P., Reddy, M. S., & Sa, T. (2009). Evaluating the effect of fertilizer application on soil microbial community structure in rice based cropping system using fatty acid methyl esters (FAME) analysis. *World Journal of Microbiology and Biotechnology*, *25*, 1115-1117.

[16] Mooney, A., Corry, A. J., Ruairc, C. N., Mahgoub, T., O'Sullivan, D., O'Donovan, N., & Kenny, P. T. (2010). Synthesis, characterisation and biological evaluation of N-(ferrocenyl) naphthoyl amino acid esters as anticancer agents. *Dalton Transactions*, *39*(35), 8228-8239.

[17] Chu, M. J., Wang, W., Ren, Z. L., Liu, H., Cheng, X., Mo, K., & Lv, X. H. (2019). Discovery of novel triazole-containing pyrazole ester derivatives as potential antibacterial agents. *Molecules*, *24*(7), 1311.

[18] Noureen, A., Saleem, S., Fatima, T., Siddiqi, H. M., & Mirza, B. (2013). Synthesis, characterization, biological evaluation and QSAR of some Schiff base esters: promising new antitumor, antioxidant and anti-inflammatory agents. *Pak. J. Pharm. Sci*, *26*(1), 113-124.

[19] Jiménez, F., Cruz, M. D. C., Zúniga, C., Martínez, M. A., Chamorro, G., Díaz, F., & Tamariz, J. (2010). Aryloxyacetic esters structurally related to α-Asarone as potential antifungal agents. *Medicinal chemistry research*, *19*, 33-57.

[20] Ferraz, R., Silva, D., Dias, A. R., Dias, V., Santos, M. M., Pinheiro, L., & Branco, L. C. (2020). Synthesis and antibacterial activity of ionic liquids and organic salts based on penicillin g and amoxicillin hydrolysate derivatives against resistant bacteria. *Pharmaceutics*, *12*(3), 221.

[21] Li, C., Li, S., Zhao, J., Zhang, Z., Zhang, J., & Yang, W. (2014). Synthesis and characterization of aliphatic poly (amide urethane) s having different nylon 6 segments through non-isocyanate route. *Journal of Polymer Research*, *21*, 1-10.

[22] Hao, Y., Chen, M., Zhao, J., Zhang, Z., & Yang, W. (2013). Synthesis and properties of polyesteramides having short nylon-610 segments in the main chains through polycondensation and chain extension. *Industrial & Engineering Chemistry Research*, *52*(19), 6410-6421.

[23] Tsikolia, M., Bernier, U. R., Coy, M. R., Chalaire, K. C., Becnel, J. J., Agramonte, N. M., & Bloomquist, J. R. (2013). Insecticidal, repellent and fungicidal properties of novel trifluoromethylphenyl amides. *Pesticide biochemistry and physiology*, *107*(1), 138-147.

[24] Sigel, H., & Martin, R. B. (1982). Coordinating properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands. *Chemical Reviews*, *82*(4), 385-426.

[25] Mallakpour, S., Rafiemanzelat, F., & Faghihi, K. (2007). Synthesis and characterization of new self-colored thermally stable poly (amide-ether-urethane) s based on an azo dye and different diisocyanates. *Dyes and pigments*, *74*(3), 713-722.

[26] Burnett, C. L., Heldreth, B., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., & Andersen, F. A. (2017). Safety assessment of amino acid alkyl amides as used in cosmetics. *International journal of toxicology*, *36*(1_suppl), 17S-56S.

[27] Cimerman, Z., Miljanić, S., & Galić, N. (2000). Schiff bases derived from aminopyridines as spectrofluorimetric analytical reagents. *Croatica Chemica Acta*, *73*(1), 81-95.

[28] Abdel-Magid, A. F., Carson, K. G., Harris, B. D., Maryanoff, C. A., & Shah, R. D. (1996). Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. studies on direct and indirect reductive amination procedures1. *The Journal of organic chemistry*, *61*(11), 3849-3862.

[29] Qin, W., Long, S., Panunzio, M., & Biondi, S. (2013). Schiff bases: A short survey on an evergreen chemistry tool. *Molecules*, *18*(10), 12264-12289.

[30] Zafar, H., Ahmad, A., Khan, A. U., & Khan, T. A. (2015). Synthesis, characterization and antimicrobial studies of Schiff base complexes. *Journal of Molecular Structure*, *1097*, 129-135.

[31] Grivani, G., Ghavami, A., Kučeráková, M., Dušek, M., & Khalaji, A. D. (2014). Synthesis, characterization, crystal structure determination, thermal study and catalytic activity of a new oxidovanadium Schiff base complex. *Journal of Molecular Structure*, *1076*, 326-332.

[32] Berhanu, A. L., Mohiuddin, I., Malik, A. K., Aulakh, J. S., Kumar, V., & Kim, K. H. (2019). A review of the applications of Schiff bases as optical chemical sensors. *TrAC Trends in Analytical Chemistry*, *116*, 74-91.

[33] Iacopetta, D., Rosano, C., Sirignano, M., Mariconda, A., Ceramella, J., Ponassi, M., & Longo, P. (2020). Is the way to fight cancer paved with gold? Metal-based carbene complexes with multiple and fascinating biological features. *Pharmaceuticals*, *13*(5), 91.

[34] Ceramella, J., Mariconda, A., Iacopetta, D., Saturnino, C., Barbarossa, A., Caruso, A., & Longo, P. (2020). From coins to cancer therapy: Gold, silver and copper complexes targeting human topoisomerases. *Bioorganic & Medicinal Chemistry Letters*, *30*(3), 126905.

[35] Iacopetta, D., Mariconda, A., Saturnino, C., Caruso, A., Palma, G., Ceramella, J., & Longo, P. (2017). Novel gold and silver carbene complexes exert antitumor effects triggering the reactive oxygen species dependent intrinsic apoptotic pathway. *ChemMedChem*, *12*(24), 2054-2065.

[36] Dhar, D. N., & Taploo, C. L. (1982). Schiff bases and their applications. *J Sci Ind Res*, *41*(8), 501-506.

[37] Przybylski, P., Huczyński, A. W., Pyta, K. K., Brzezinski, B., & Bartl, F. (2009). Biological properties of Schiff bases and azo derivatives of phenols. *Current Organic Chemistry*, *13*(2), 124–148.

[38] Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., & Li, P. (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydrate research*, *342*(10), 1329-1332.

[39] Chandramouli, C., Shivanand, M. R., Nayanbhai, T. B., Bheemachari, B., & Udupi, R. H. (2012). Synthesis and biological screening of certain new triazole Schiff bases and their derivatives bearing substituted benzothiazole moiety. *J. Chem. Pharm. Res*, *4*(2), 1151-1159.

[40] Abd-Elzaher, M. M., Labib, A. A., Mousa, H. A., Moustafa, S. A., Ali, M. M., & El-Rashedy, A. A. (2016). Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety. *beni-suef university journal of basic and applied sciences*, *5*(1), 85-96.

[41] Zhao, J., Li, W., Gou, S., Li, S., Lin, S., Wei, Q., & Xu, G. (2018). Hypoxia-targeting organometallic Ru (II)–arene complexes with enhanced anticancer activity in hypoxic cancer cells. *Inorganic Chemistry*, *57*(14), 8396-8403.

[42] Warad, I., Ali, O., Al Ali, A., Jaradat, N. A., Hussein, F., Abdallah, L., ... & Alharthi, F. A. (2020). Synthesis and spectral Identification of three Schiff bases with a 2-(piperazin-1-yl)-N-(thiophen-2-yl methylene) ethanamine moiety acting as novel pancreatic lipase inhibitors: Thermal, DFT, antioxidant, antibacterial, and molecular docking investigations. *Molecules*, *25*(9), 2253.

[43] Joule, J. A., & Mills, K. (2008). Heterocyclic Chemistry, 5th edition. *Wiley-Blackwell : New York, 89*(11), 155–1350.

[44] Wohler, F. (1828). Ueber kunstliche Bildung des Harnstoffs. *Ann. Phys. Chem.*, *37*, 330-333.

[45] Kaufman, T. S., & Rúveda, E. A. (2005). The quest for quinine: those who won the battles and those who won the war. *Angewandte Chemie International Edition*, *44*(6), 854-885.

[46] Baeyer, A., & Emmerling, A. (1869). Synthese des indols. *Berichte der deutschen chemischen Gesellschaft*, *2*(1), 679-682.

[47] Wainwright, M., & Kristiansen, J. E. (2011). On the 75th anniversary of Prontosil. *Dyes and Pigments*, *88*(3), 231-234.

[48] Malcolm, S. (2001). Heterocyclic Chemistry Book. p. 18-100.

[49] Runge, F. F. (1834). Ueber einige produkte der steinkohlendestillation. *Annalen der Physik*, *107*(5), 65-78.

[50] Kaur, R., Rani, V., Abbot, V., Kapoor, Y., Konar, D., & Kumar, K. (2017). Recent synthetic and medicinal perspectives of pyrroles: An overview. *J Pharm Chem Chem Sci* , *1*(1), 17-32.

[51] Di Santo, R., Costi, R., Artico, M., Massa, S., Lampis, G., Deidda, D., & Pompei, R. (1998). Pyrrolnitrin and related pyrroles endowed with antibacterial activities against Mycobacterium tuberculosis. *Bioorganic & medicinal chemistry letters*, *8*(20), 2931-2936.

[52] Huang, Z. B., Yin, G. F., Liao, X. M., & Gu, J. W. (2014). Conducting polypyrrole in tissue engineering applications. *Frontiers of Materials Science*, *8*, 39-45.

[53] Limpricht, H. (1870). Ueber das Tetraphenol, C4H4O. *Berichte der deutschen chemischen Gesellschaft*, *3*(1), 90-91.

[54] Loğoğlu, E., Yilmaz, M., Katircioğlu, H., Yakut, M., & Mercan, S. (2010). Synthesis and biological activity studies of furan derivatives. *Medicinal chemistry research*, *19*, 490-497.

[55] Alizadeh, M., Jalal, M., Hamed, K., Saber, A., Kheirouri, S., Pourteymour Fard Tabrizi, F., & Kamari, N. (2020). Recent updates on anti-inflammatory and antimicrobial effects of furan natural derivatives. *Journal of Inflammation Research*, 451-463.

[56] Lin, Y., Ahmed, W., He, M., Xiang, X., Tang, R., & Cui, Z. N. (2020). Synthesis and bioactivity of phenyl substituted furan and oxazole carboxylic acid derivatives as potential PDE4 inhibitors. *European Journal of Medicinal Chemistry*, *207*, 112795.

[57] Kamboj, A., & Randhawa, H. (2012). Pharmacological action and sar of thiophene derivatives: A review. *J. Pharm. Res*, *5*(5), 2676-2682.

[58] Turkoglu, G., Cinar, M. E., & Ozturk, T. (2019). Thiophene-based organic semiconductors. *Sulfur Chemistry*, 79-123.

[59] Hazarika, K. K., Baruah, N. C., & Deka, R. C. (2009). Molecular structure and reactivity of antituberculosis drug molecules isoniazid, pyrazinamide, and 2-methylheptylisonicotinate: a density functional approach. *Structural Chemistry*, *20*, 1079-1085.

[60] Gutierrez, A., Stokes, J. M., & Matic, I. (2018). Our evolving understanding of the mechanism of quinolones. *Antibiotics*, *7*(2), 32.

[61] Bronte, G., Rolfo, C., Giovannetti, E., Cicero, G., Pauwels, P., Passiglia, F., & Russo, A. (2014). Are erlotinib and gefitinib interchangeable, opposite or complementary for non-small cell lung cancer treatment? Biological, pharmacological and clinical aspects. *Critical reviews in oncology/hematology*, *89*(2), 300-313.

[62] Beck, A., Krischak, G., Sorg, T., Augat, P., Farker, K., Merkel, U., & Claes, L. (2003). Influence of diclofenac (group of nonsteroidal anti-inflammatory drugs) on fracture healing. *Archives of orthopaedic and trauma surgery*, *123*, 327-332.

[63] Kumar, S. (2020). A brief review of the biological potential of indole derivatives. *Future Journal of Pharmaceutical Sciences*, *6*(1), 1-19.

[64] Weng, J. R., Tsai, C. H., Kulp, S. K., & Chen, C. S. (2008). Indole-3-carbinol as a chemopreventive and anti-cancer agent. *Cancer letters*, *262*(2), 153-163.

[65] Andreadou, I., Tasouli, A., Bofilis, E., Chrysselis, M., Rekka, E., Tsantili-Kakoulidou, A., & Kremastinos, D. T. (2002). Antioxidant activity of novel indole derivatives and protection of the myocardial damage in rabbits. *Chemical and pharmaceutical bulletin*, *50*(2), 165-168.

[66] Pires, A. D. R. A., Lecerf-Schmidt, F., Guragossian, N., Pazinato, J., Gozzi, G. J., Winter, E., & Pérès, B. (2016). New, highly potent and non-toxic, chromone inhibitors of the human breast cancer resistance protein ABCG2. *European journal of medicinal chemistry*, *122*, 291-301.

[67] Kumar, V., Guru, S. K., Jain, S. K., Joshi, P., Gandhi, S. G., Bharate, S. B., ... & Vishwakarma, R. A. (2016). A chromatography-free isolation of rohitukine from leaves of Dysoxylum binectariferum: Evaluation for in vitro cytotoxicity, Cdk inhibition and physicochemical properties. *Bioorganic & medicinal chemistry letters*, *26*(15), 3457-3463.

[68] Huang, M., Li, J., Liu, L., Yin, S., Wang, J., & Lin, Y. (2016). Phomopsichin A–D; four new chromone derivatives from mangrove endophytic fungus Phomopsis sp. 33#. *Marine drugs*, *14*(11), 215.

[69] Kamiński, K., Wiklik, B., & Obniska, J. (2014). Synthesis, Anticonvulsant Properties, and SAR Analysis of Differently Substituted Pyrrolidine-2, 5-diones and Piperidine-2, 6-diones. *Archiv der Pharmazie*, *347*(11), 840-852.

[70] Tumosienė, I., Jonuškienė, I., Kantminienė, K., Mickevičius, V., & Petrikaitė, V. (2021). Novel N-substituted amino acid hydrazone-isatin derivatives: Synthesis, antioxidant activity, and anticancer activity in 2D and 3D models in vitro. *International journal of molecular sciences*, *22*(15), 7799.

[71] Muneer, S., Memon, S., Pahnwar, Q. K., Bhatti, A. A., & Khokhar, T. S. (2017). Synthesis and investigation of antimicrobial properties of pyrrolidine appended calix [4] arene. *Journal of Analytical Science and Technology*, *8*, 1-6.

[72] Banerjee, J., & Dey, B. K. (2019). ANTIHELMINTHIC ACTIVITY OF DIFFERENT EXTRACTS OF DENDROBIUM OCHREATUM. *Pharmacophore*, *10*(3), 1-4.

[73] Yurttaş, L., Demirayak, Ş., Ilgın, S., & Atlı, Ö. (2014). In vitro antitumor activity evaluation of some 1, 2, 4-triazine derivatives bearing piperazine amide moiety against breast cancer cells. *Bioorganic & Medicinal Chemistry*, *22*(22), 6313-6323.

[74] Fiorino, F., Severino, B., Magli, E., Ciano, A., Caliendo, G., Santagada, V., & Perissutti, E. (2014). 5-HT1A receptor: an old target as a new attractive tool in drug discovery from central nervous system to cancer. *Journal of Medicinal Chemistry*, *57*(11), 4407-4426.

[75] Wayne, P. A. (2002). National committee for clinical laboratory standards. *Performance standards for antimicrobial disc susceptibility testing*, *12*, 01-53.

[76] MA, W. (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. *Clsi (Nccls)*, *26*, M7-A7.

[77] Lubna, A., jafar Amany, A., Asmaa, F., Basel, J., Rahaf, A., Layla, A., & Wejdan, A. (2021). Bioactivity of three medicinal plants leaf aqueous extracts growing wild in Palestine. *Research Journal of Biotechnology*, *16*, 9.

[78] Salga, S. M., Ali, H. M., Abdullah, M. A., Abdelwahab, S. I., Wai, L. K., Buckle, M. J., & Hadi, A. H. A. (2011). Synthesis, characterization, acetylcholinesterase inhibition, molecular modeling and antioxidant activities of some novel schiff bases derived from 1-(2-ketoiminoethyl) piperazines. *Molecules*, *16*(11), 9316-9330.

[79] Gwaram, N. S., Ali, H. M., Khaledi, H., Abdulla, M. A., Hadi, A. H. A., Lin, T. K., & Ooi, C. L. (2012). Antibacterial evaluation of some Schiff bases derived from 2-acetylpyridine and their metal complexes. *Molecules*, *17*(5), 5952-5971.

[80] Dadashpour, S., & Emami, S. (2018). Indole in the target-based design of anticancer agents: A versatile scaffold with diverse mechanisms. *European journal of medicinal chemistry*, *150*, 9-29.

[81] Wan, Y., Li, Y., Yan, C., Yan, M., & Tang, Z. (2019). Indole: A privileged scaffold for the design of anti-cancer agents. *European journal of medicinal chemistry*, *183*, 111691.

[82] Ahmad, A., A Sakr, W., & Wahidur Rahman, K. M. (2010). Anticancer properties of indole compounds: mechanism of apoptosis induction and role in chemotherapy. *Current drug targets*, *11*(6), 652-666.

[83] Zhang, L., Zhang, Z., Chen, F., Chen, Y., Lin, Y., & Wang, J. (2016). Aromatic heterocyclic esters of podophyllotoxin exert anti-MDR activity in human leukemia K562/ADR cells via ROS/MAPK signaling pathways. *European journal of medicinal chemistry*, *123*, 226-235.

[84] Markman, M., & Mekhail, T. M. (2002). Paclitaxel in cancer therapy. *Expert opinion on pharmacotherapy*, *3*(6), 755-766.

[85] Ahmed, M. H., El-Hashash, M. A., Marzouk, M. I., & El-Naggar, A. M. (2020). Synthesis and antitumor activity of some nitrogen heterocycles bearing pyrimidine moiety. *Journal of Heterocyclic Chemistry*, *57*(9), 3412-3427.

[86] Kachur, K., & Suntres, Z. (2020). The antibacterial properties of phenolic isomers, carvacrol and thymol. *Critical reviews in food science and nutrition*, *60*(18), 3042-3053.

[87] Alibi, S., Crespo, D., & Navas, J. (2021). Plant-derivatives small molecules with antibacterial activity. *Antibiotics*, *10*(3), 231.

[88] Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: substances useful in human healthcare. *Archivum immunologiae et therapiae experimentalis*, *55*, 315-327.

[89] El-Miligy, M. M., Hazzaa, A. A., El-Zemity, S. R., & Al-Kubeisi, A. K. (2019). Synthesis of thymol derivatives as potential non-irritant antimicrobial and insecticidal agents. *Current Bioactive Compounds*, *15*(1), 125-137.

[90] Nagle, P. S., Pawar, Y. A., Sonawane, A. E., Bhosale, S. M., & More, D. H. (2012). Synthesis and evaluation of antioxidant and antimicrobial properties of thymol containing pyridone moieties. *Medicinal Chemistry Research*, *21*, 1395-1402.

[91] Odds, F. C., Brown, A. J., & Gow, N. A. (2003). Antifungal agents: mechanisms of action. *Trends in microbiology*, *11*(6), 272-279.

[92] Shirinzadeh, H., Süzen, S., Altanlar, N., & Westwell, A. D. (2018). Antimicrobial activities of new indole derivatives containing 1, 2, 4-triazole, 1, 3, 4-thiadiazole and carbothioamide. *Turkish Journal of Pharmaceutical Sciences*, *15*(3), 291-297.

[93] Varvaresou, A., Tsantili-Kakoulidou, A., Siatra-Papastaikoudi, T., & Tiligada, E. (2000). Synthesis and biological evaluation of indole containing derivatives of thiosemicarbazide and their cyclic 1, 2, 4-triazole and 1, 3, 4-thiadiazole analogs. *Arzneimittelforschung*, *50*(01), 48-54.

[94] Fattahi, S., Zabihi, E., Abedian, Z., Pourbagher, R., Ardekani, A. M., Mostafazadeh, A., & Akhavan-Niaki, H. (2014). Total phenolic and flavonoid contents of aqueous extract of stinging nettle and in vitro antiproliferative effect on hela and BT-474 Cell lines. *International journal of molecular and cellular medicine*, *3*(2), 102-107.

[95] Sivropoulou, A., Kokkini, S., Lanaras, T., & Arsenakis, M. (1995). Antimicrobial activity of mint essential oils. *Journal of Agricultural and Food chemistry*, *43*(9), 2384-2388.

[96] Knobloch, K., Pauli, A., Iberl, B., Weigand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of essential oil research*, *1*(3), 119-128.

[97] Marinelli, L., Di Stefano, A., & Cacciatore, I. (2018). Carvacrol and its derivatives as antibacterial agents. *Phytochemistry reviews*, *17*, 903-921.

[98] Griffin, S. G., Wyllie, S. G., Markham, J. L., & Leach, D. N. (1999). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Fragrance Journal*, *14*(5), 322-332.

[99] Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, *88*(2), 308-316.

[100] Mathela, C. S., Singh, K. K., & Gupta, V. K. (2010). Synthesis and in vitro antibacterial activity of thymol and carvacrol derivatives. *Acta Pol Pharm*, *67*(4), 375-380.

[101] Sokolova, A. S., Yarovaya, O. I., Shtro, A. A., Borisova, M. S., Morozova, E. A., Tolstikova, T. G., & Salakhutdinov, N. F. (2017). Synthesis and biological activity of heterocyclic borneol derivatives. *Chemistry of Heterocyclic Compounds*, *53*, 371-377.

[102] Sokolova, A. S., Yarovaya, O. I., Bormotov, N. I., Shishkina, L. N., & Salakhutdinov, N. F. (2018). Discovery of a New Class of Inhibitors of Vaccinia Virus Based on (−)-Borneol from Abies sibirica and (+)-Camphor. *Chemistry & Biodiversity*, *15*(9), 1-13, e1800153.

[103] Sarbu, L.G., Bahrin, L.G., Babii, C., Stefan, M., Birsa, M.L. (2019). Synthetic flavonoids with antimicrobial activity: a review, *Journal of Applied Microbiology*, *127* (5), 1282-1290.

[104] Narayana, K.R., Reddy, M.S., Chaluvadi, M.R. and Krishna, D.R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol*, *33* (1), 2-16.

[105] Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry reviews*, *18*, 241-272.

[106] Babii, C., Mihalache, G., Bahrin, L. G., Neagu, A. N., Gostin, I., Mihai, C. T., & Stefan, M. (2018). A novel synthetic flavonoid with potent antibacterial properties: In vitro activity and proposed mode of action. *PLoS One*, *13*(4), e0194898.

[107] Lal, K., Yadav, P., Kumar, A., Kumar, A., & Paul, A. K. (2018). Design, synthesis, characterization, antimicrobial evaluation and molecular modeling studies of some dehydroacetic acid-chalcone-1, 2, 3-triazole hybrids. *Bioorganic chemistry*, *77*, 236-244.

[108] Yadav, P., Lal, K., Kumar, L., Kumar, A., Kumar, A., Paul, A. K., & Kumar, R. (2018). Synthesis, crystal structure and antimicrobial potential of some fluorinated chalcone-1, 2, 3-triazole conjugates. *European journal of medicinal chemistry*, *155*, 263-274.

[109] Cushman, M., Nagarathnam, D., Burg, D. L., & Geahlen, R. L. (1991). Synthesis and protein-tyrosine kinase inhibitory activities of flavonoid analogs. *Journal of medicinal chemistry*, *34*(2), 798-806.

[110] Baldo, B. A., & Pham, N. H. (2020). Drug allergy: clinical aspects, diagnosis, mechanisms, structure-activity relationships. *Springer NewYork Heidelberg Dordrecht: London, 2017*, 134-162.

[111] Qin, H. L., Liu, J., Fang, W. Y., Ravindar, L., & Rakesh, K. P. (2020). Indole-based derivatives as potential antibacterial activity against methicillin-resistance Staphylococcus aureus (MRSA). *European journal of medicinal chemistry*, *194*, 112245.

[112] Hair, P. I., & Keam, S. J. (2007). Daptomycin: a review of its use in the management of complicated skin and soft-tissue infections and Staphylococcus aureus bacteraemia. *Drugs*, *67*(10), 1483-1512.

[113] Hirshfield IN, Terzulli S, O'Byrne C. (2003). Weak Organic Acids: A Panoply of Effects on Bacteria. *Science Progress*, *86*(4), 245-270.

[114] Sari, E., Aslan, H., Dadi, S., Öktemer, A., & Loğoğlu, E. (2017). Biological Activity Studies of Some Synthesized Novel Furan and Pyran Derivatives. *Gazi University Journal of Science, 30*, 49-55.

[115] Raimondi MV, Presentato A, Li Petri G, Buttacavoli M, Ribaudo A, De Caro V, Alduina R, Cancemi P. (2020). New Synthetic Nitro-Pyrrolomycins as Promising Antibacterial and Anticancer Agents. *Antibiotics*, *9*(6), 292.

[116] Saeid, H., Al-sayed, H., Bader, M. (2023). A Review on Biological and Medicinal Significance of Furan. *Alq J Med App Sci*, *6(*1), 44-58.

[117] Li Petri, G., Raimondi, M.V., Spanò, V., Ralph, H., Paola, B., Alessandra, M. (2021). Pyrrolidine in Drug Discovery: A Versatile Scaffold for Novel Biologically Active Compounds. *Top Curr Chem (Z)*, *379*(34), 1-46.

[118] Walker, M. A. (2014). Novel tactics for designing water-soluble molecules in drug discovery, *Expert Opin. Drug Discov., 9*(12), 1421-1433.

[119] Gore, RP. (2014). Synthesis, characterization and antimicrobial activity of N'-(substituted phenyl)-2-(1H-azol-1-yl) acetamides. *Der Pharma Chemica*, *6*(6), 35-38.

[120] Mohamed MSS, Fathallah SSS. (2014). Pyrroles and Fused Pyrroles: Synthesis and Therapeutic Activities. *Mini Rev Org Chem, 6*,477-507.

[121] National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 1089162, N-(2-pyrrolidin-1-ylethyl)furan-2-carboxamide. Retrieved June 25, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/N-_2-pyrrolidin-1-ylethyl_furan-2-carboxamide.

[122] National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 28273862, N-[2-(piperazin-1-yl)ethyl]furan-2-carboxamide. Retrieved June 25, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/N-_2-piperazin-1-yl_ethyl_furan-2-carboxamide.

[123] Abou-Hussein, A. A., & Linert, W. (2015). Synthesis, spectroscopic studies and inhibitory activity against bactria and fungi of acyclic and macrocyclic transition metal complexes containing a triamine coumarine Schiff base ligand. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *141*, 223-232.

[124] Taghizadeh, L., Montazerozohori, M., Masoudiasl, A., Joohari, S., & White, J. M. (2017). New tetrahedral zinc halide Schiff base complexes: Synthesis, crystal structure, theoretical, 3D Hirshfeld surface analyses, antimicrobial and thermal studies. *Materials Science and Engineering: C*, *77*, 229-244.

[125] Pramanik, H. A., Das, D., Paul, P. C., Mondal, P., & Bhattacharjee, C. R. (2014). Newer mixed ligand Schiff base complexes from aquo-N-(2′-hydroxy acetophenone) glycinatocopper (II) as synthon: DFT, antimicrobial activity and molecular docking study. *Journal of Molecular Structure*, *1059*, 309-319.

[126] Cloete, T.E. 2003. Resistance mechanisms of bacteria to antimicrobial compounds ,International Biodeterioration & Biodegradation. *51*(4), 277-282.

[127] Siddappa, K., Mane, S. B., & Manikprabhu, D. (2014). (E) 3-2-(1-(2, 4-dihydroxyphenyl) ethyldeneamino) phenyl)-2-methylquinazoline-4 (3H)-one schiff base and its metal complexes: a new drug of choice against methicillin-resistant Staphylococcus aureus. *Bioinorganic Chemistry and Applications*, *2014*, 343540.

[128] Goszczyńska, A., Kwiecień, H., & Fijałkowski, K. (2015). Synthesis and antibacterial activity of Schiff bases and amines derived from alkyl 2-(2-formyl-4-nitrophenoxy) alkanoates. *Medicinal Chemistry Research*, *24*, 3561-3577.

[129] Fonkui, T. Y., Ikhile, M. I., Ndinteh, D. T., & Njobeh, P. B. (2018). Microbial activity of some heterocyclic Schiff bases and metal complexes: A review. *Tropical Journal of Pharmaceutical Research*, *17*(12), 2507-2518.

[130] Mishra, L. and Singh, V. K. (1993). Synthesis, structural and antifungal studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with new Schiff bases bearing benzimidazoles. *Indian J. Chem*, *32*A, 446-449.

[131] Rauf, A., Shah, A., Khan, A. A., Shah, A. H., Abbasi, R., Qureshi, I. Z., & Ali, S. (2017). Synthesis, pH dependent photometric and electrochemical investigation, redox mechanism and biological applications of novel Schiff base and its metallic derivatives. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *176*, 155-167.

[132] Kumar, D., & Rawat, D. S. (2013). Synthesis and antioxidant activity of thymol and carvacrol based Schiff bases. *Bioorganic & medicinal chemistry letters*, *23*(3), 641-645.

[133] Javan, A. J., & Javan, M. J. (2014). Electronic structure of some thymol derivatives correlated with the radical scavenging activity: Theoretical study. *Food chemistry*, *165*, 451-459.

[134] Hanasaki, Y., Ogawa, S., & Fukui, S. (1994). The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free radical biology & medicine*, *16*(6), 845-850.

[135] Brown, J. E., Khodr, H., Hider, R. C., & Rice-Evans, C. A. (1998). Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. *The Biochemical journal*, *330 (*Pt 3), 1173-1178.

[136] Morris, C. J., Earl, J. R., Trenam, C. W., & Blake, D. R. (1995). Reactive oxygen species and iron--a dangerous partnership in inflammation. *The international journal of biochemistry & cell biology*, *27*(2), 10A9-122.

Appendices

Appendix A

Figures of Study

IR spectrum

Figure 1

IR spectrum for thymol ester E2

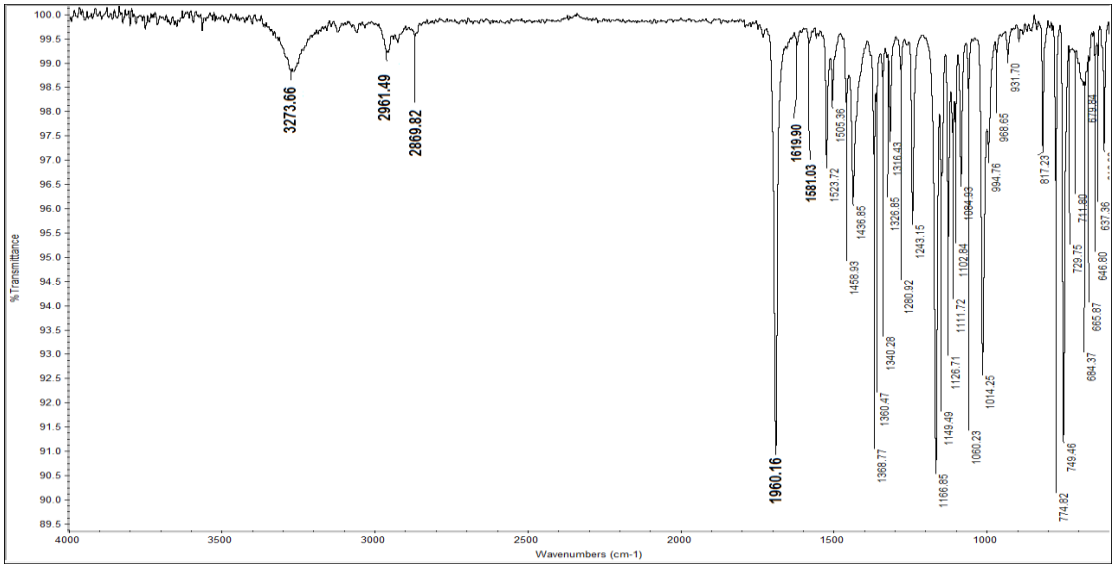


Figure 2

IR spectrum of carvacrol ester E4

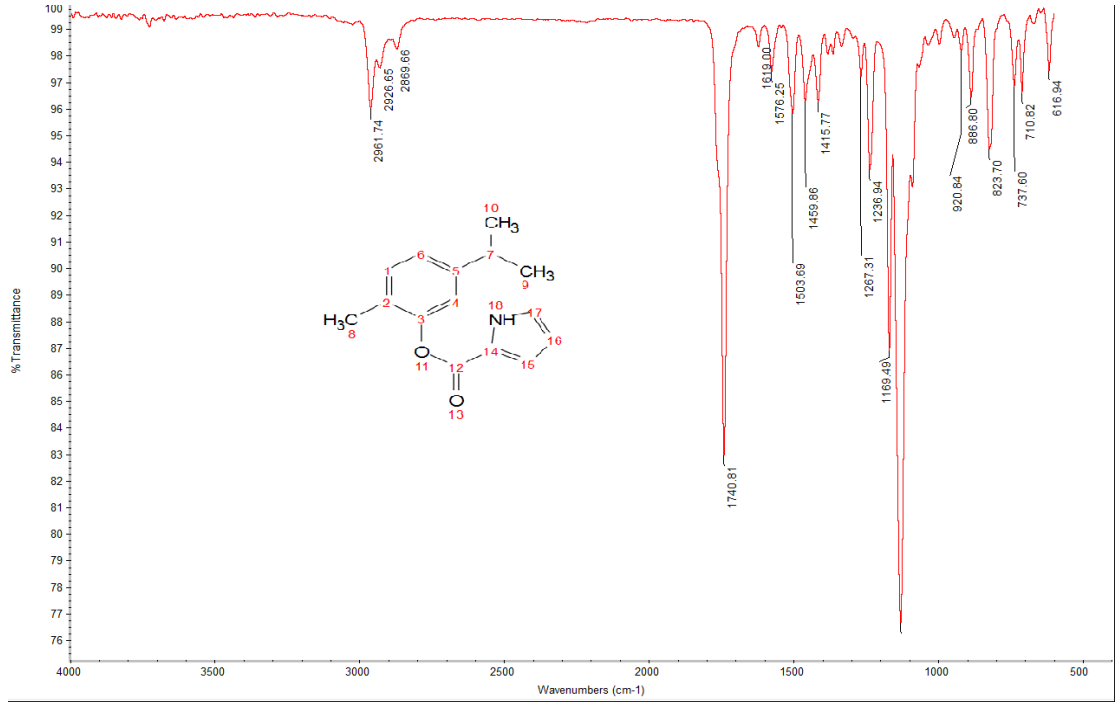


Figure 3

IR spectrum for carvacrol ester E5

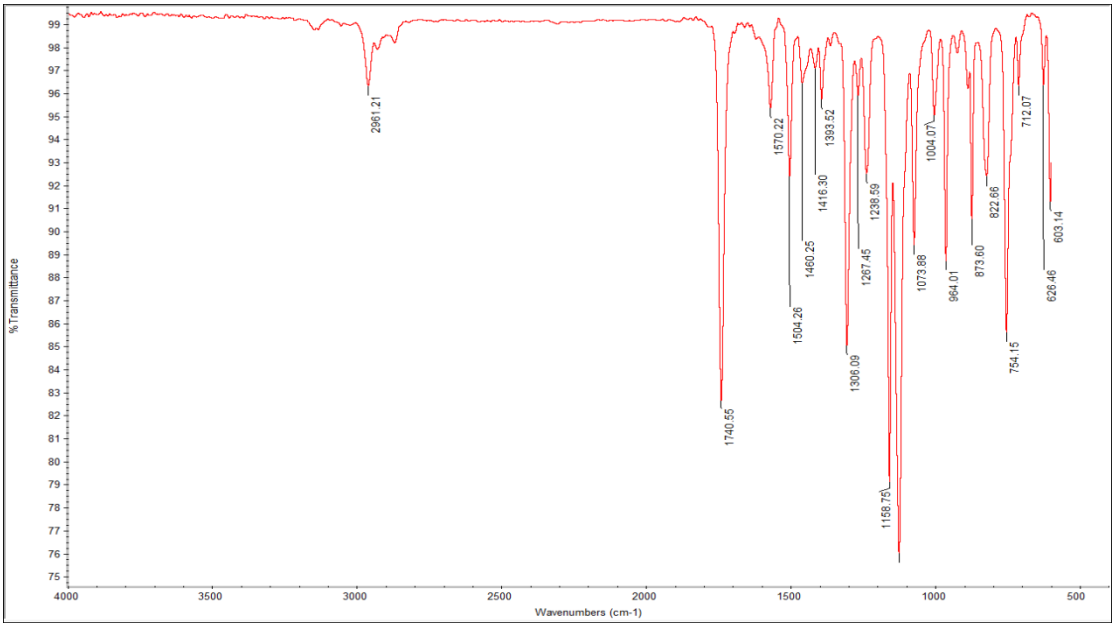


Figure 4

IR spectrum for carvacrol ester E6

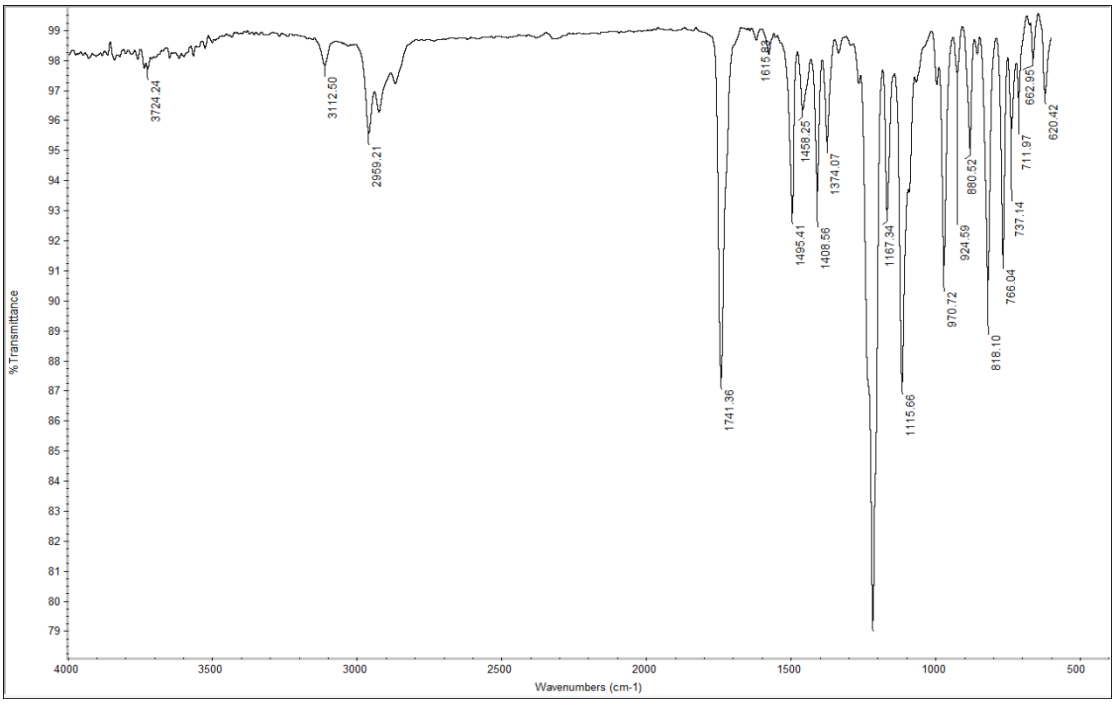


Figure 5

IR spectrum for carvacrol ester E7

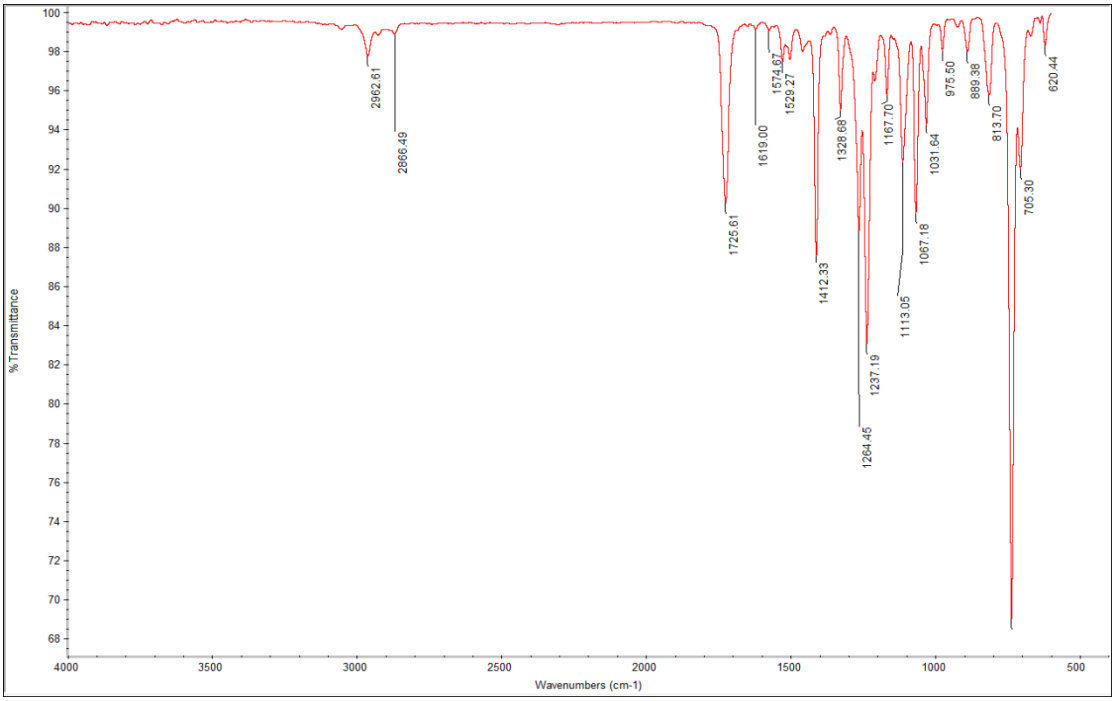


Figure 6

IR spectrum for carvacrol ester E8

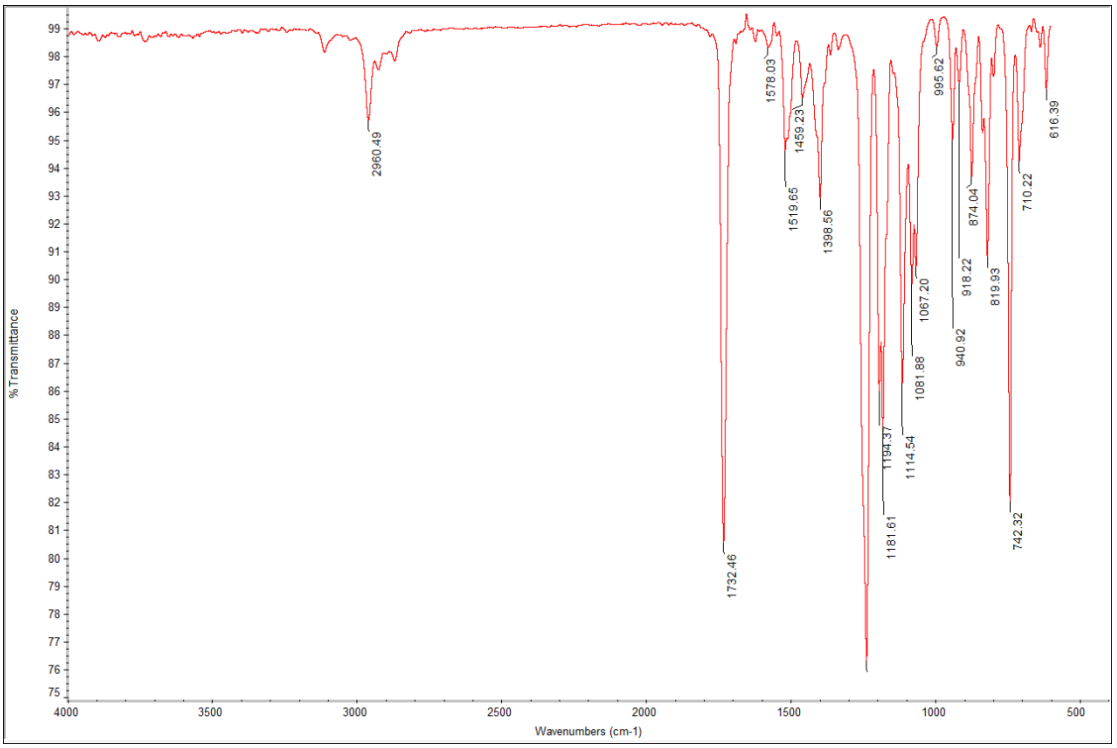


Figure 7

IR spectrum for ester Ea

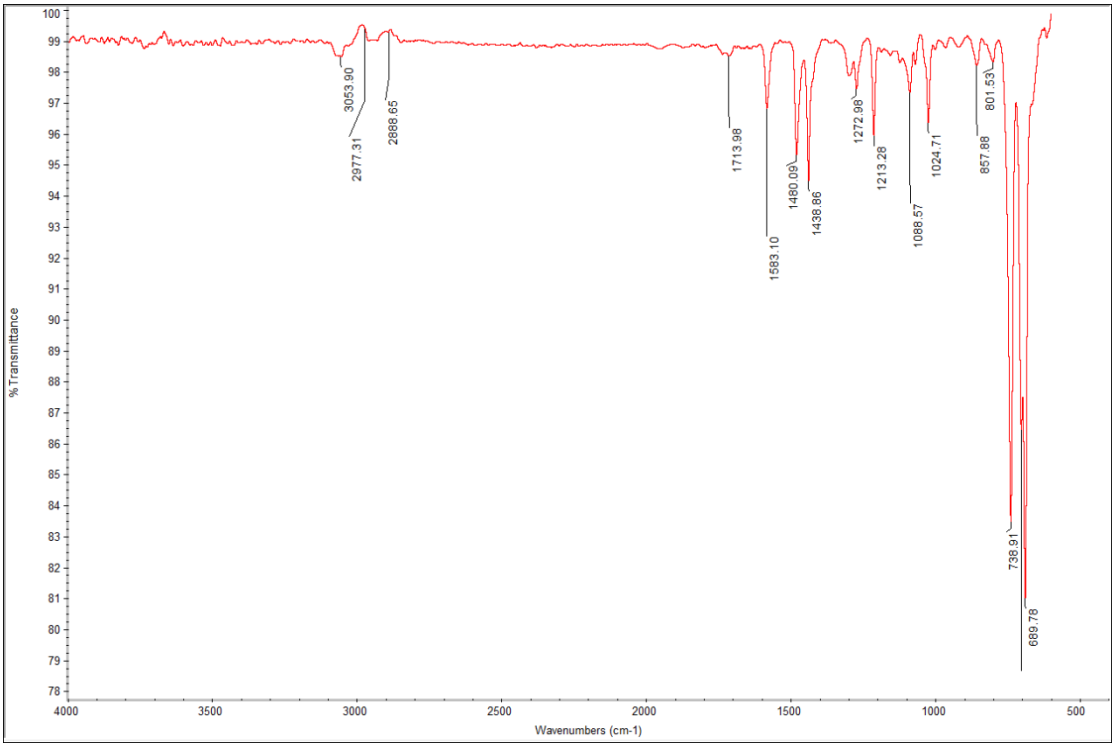


Figure 8

IR spectrum for ester Eb

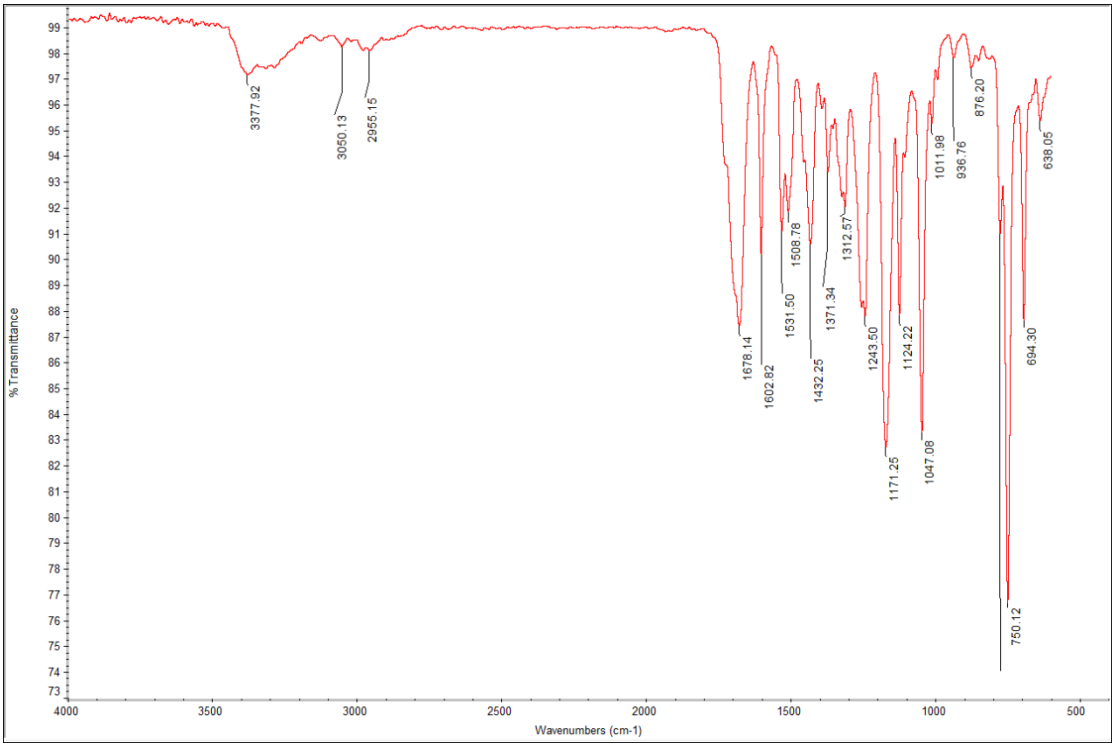


Figure 9

IR spectrum for ester Ec

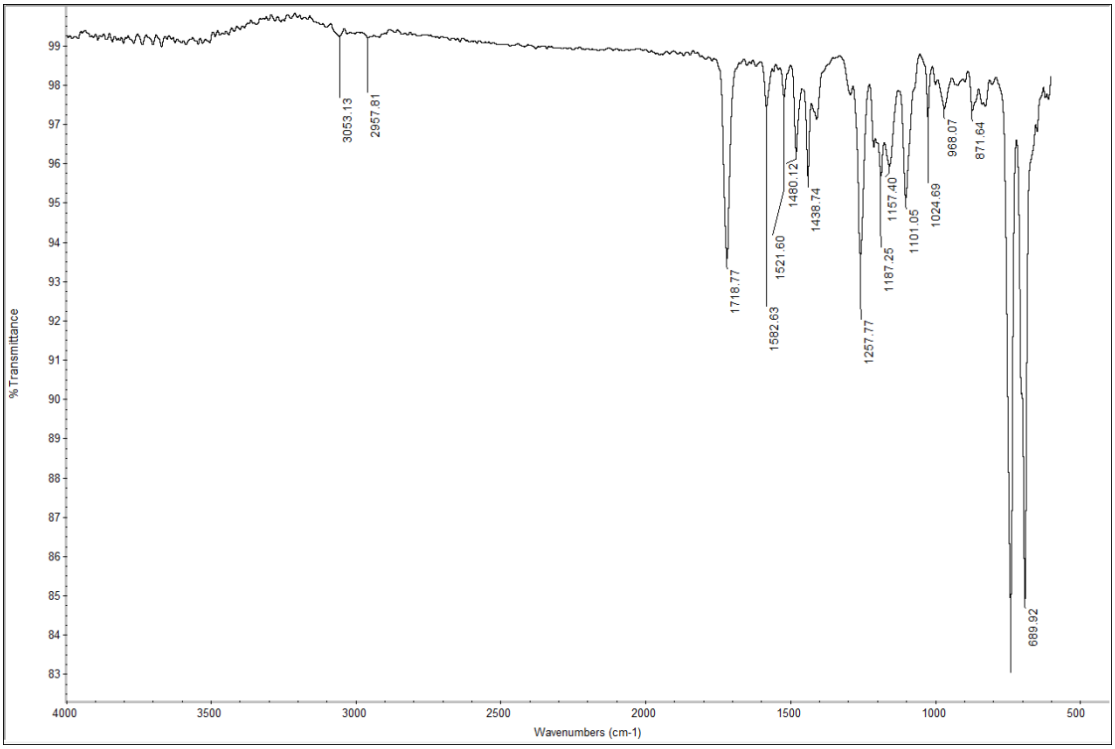


Figure 10

IR spectrum for ester Ed

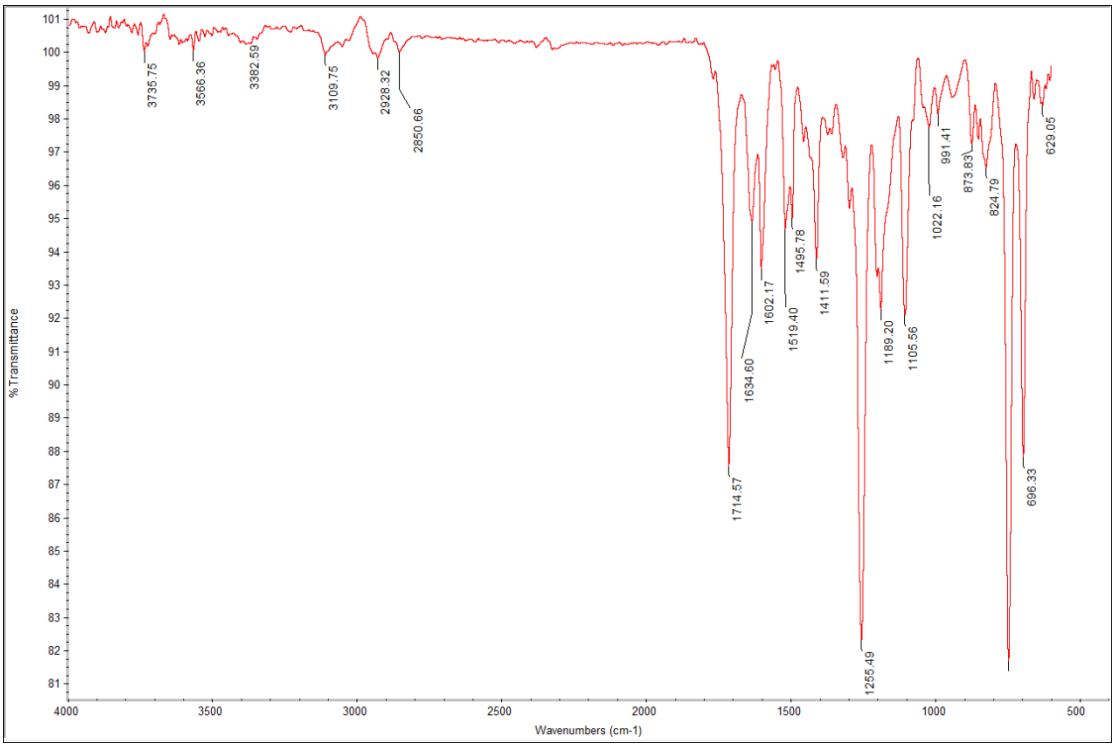


Figure 11

IR spectrum for ester Ee1

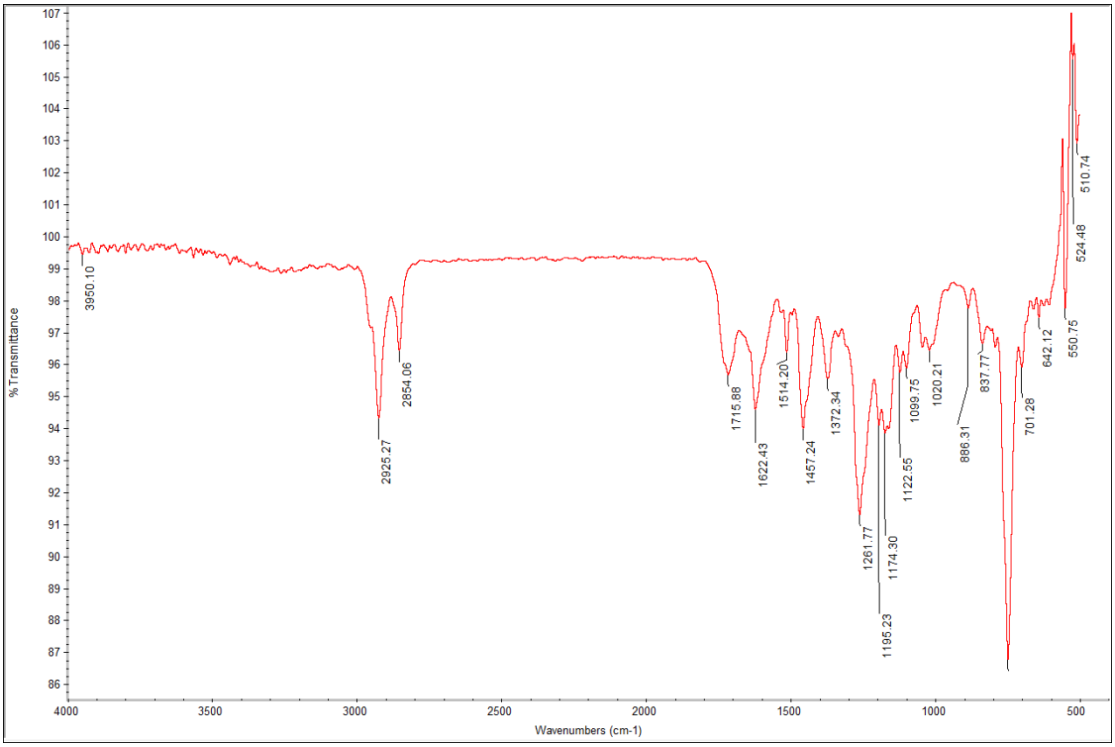


Figure 12

IR spectrum for ester Ee2

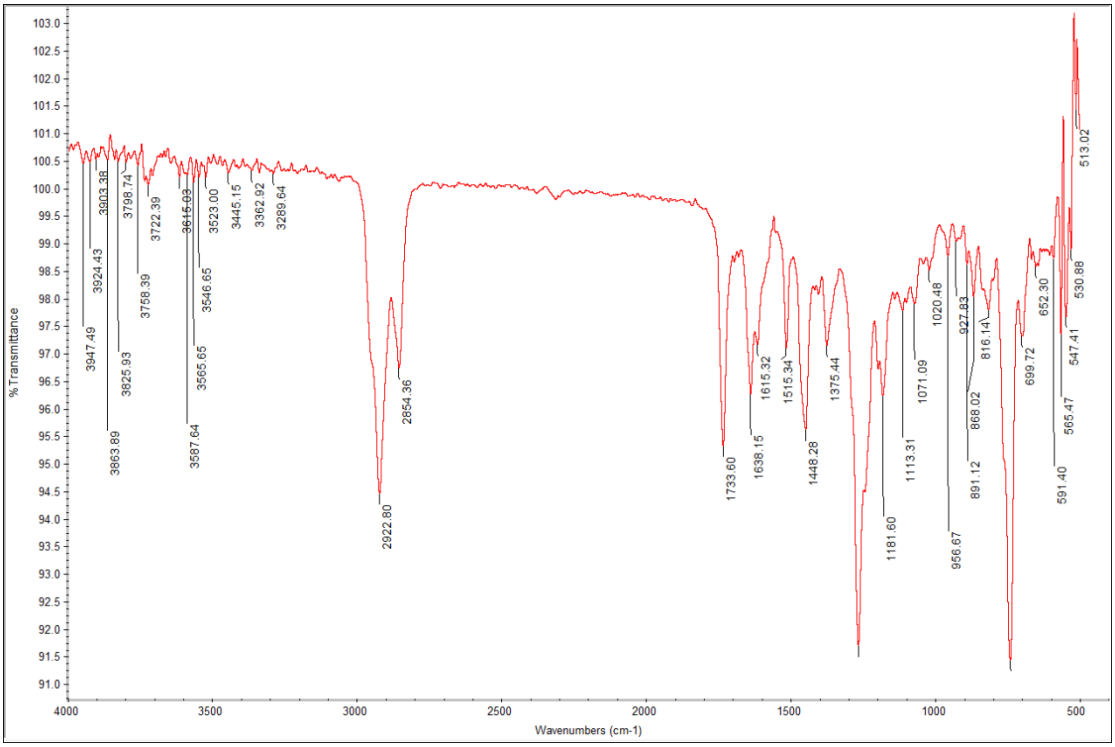


Figure 13

IR spectrum for amide A1

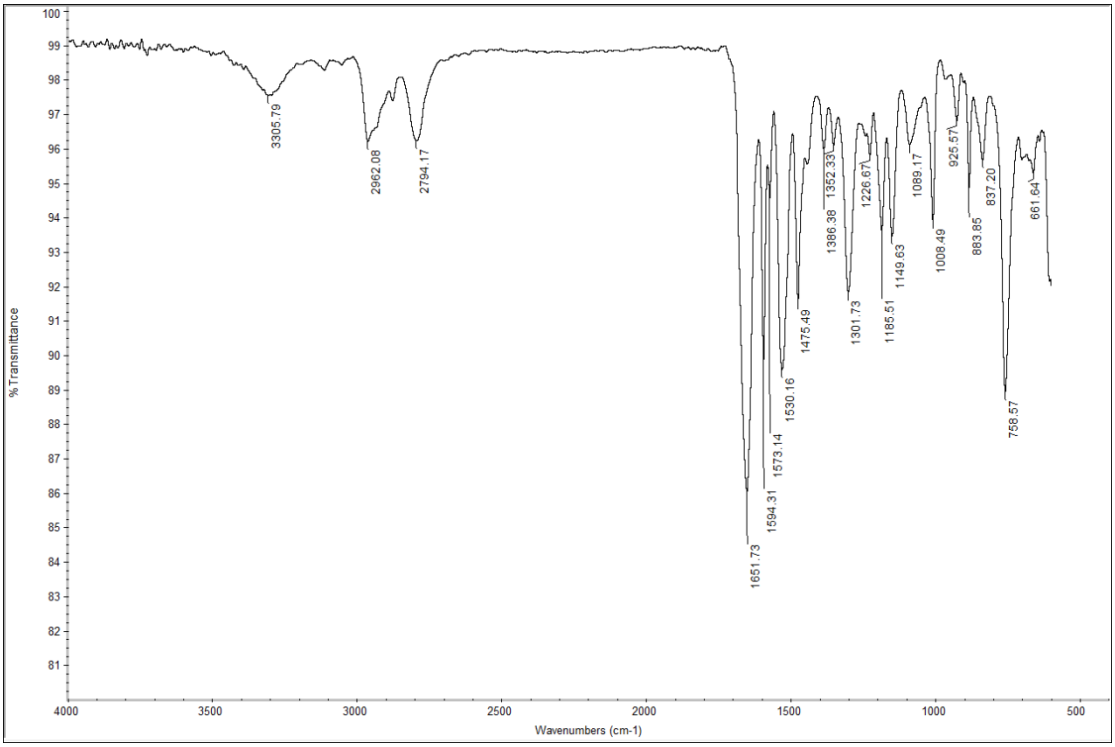


Figure 14

IR spectrum for amide A2

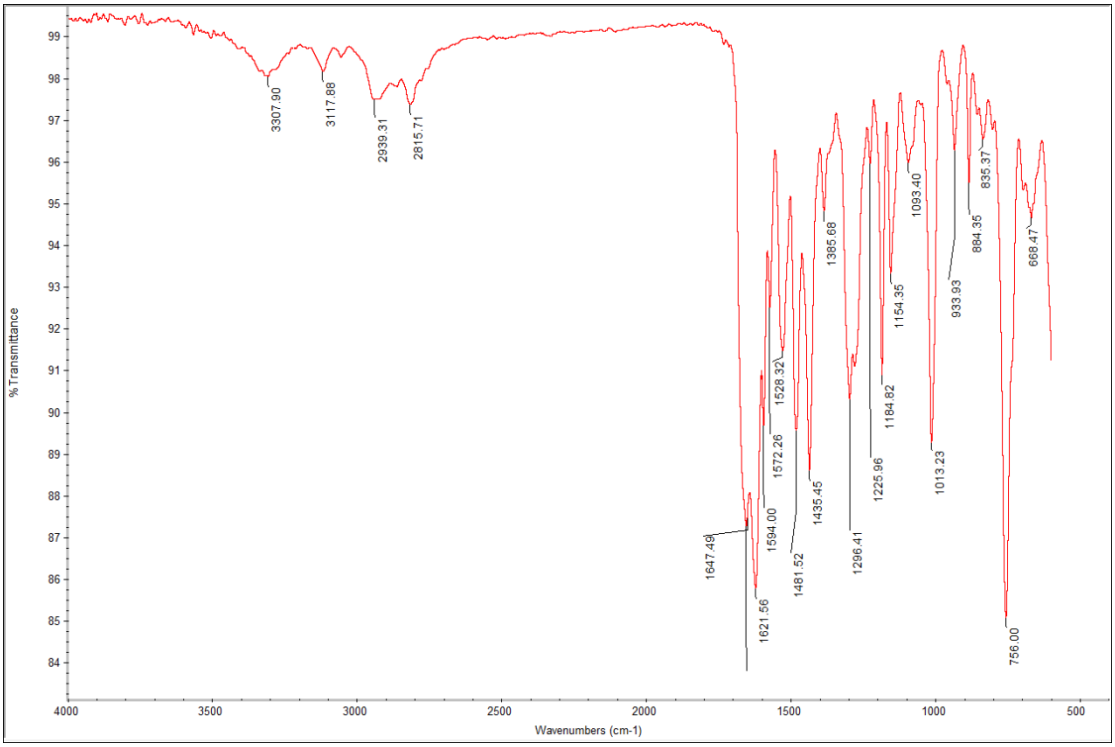


Figure 15

IR spectrum for amide Aa

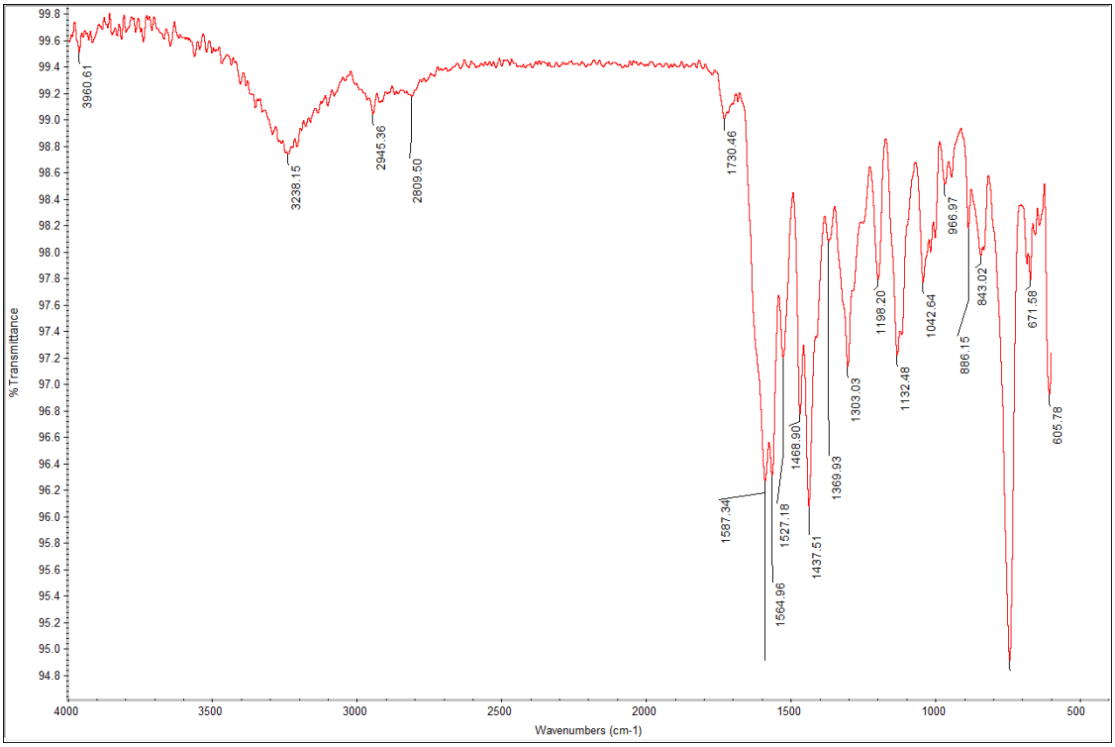


Figure 16

IR spectrum for amide Ab

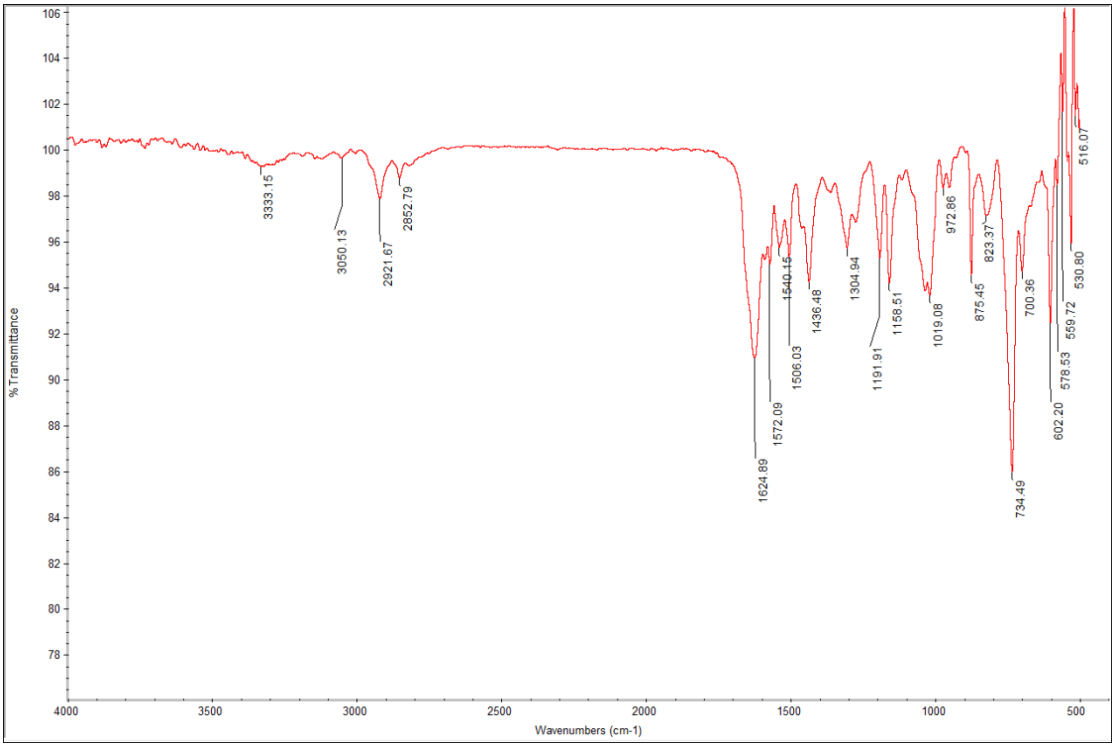


Figure 17

IR spectrum for imine 3

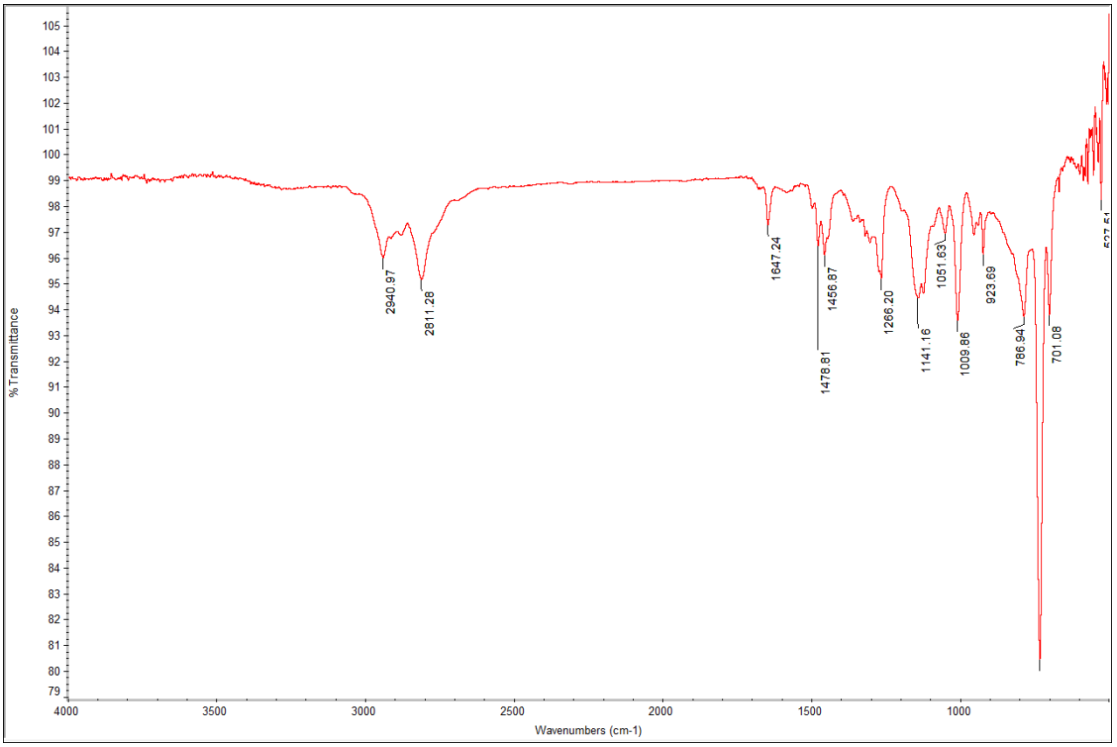


Figure 18

IR spectrum for imine 4

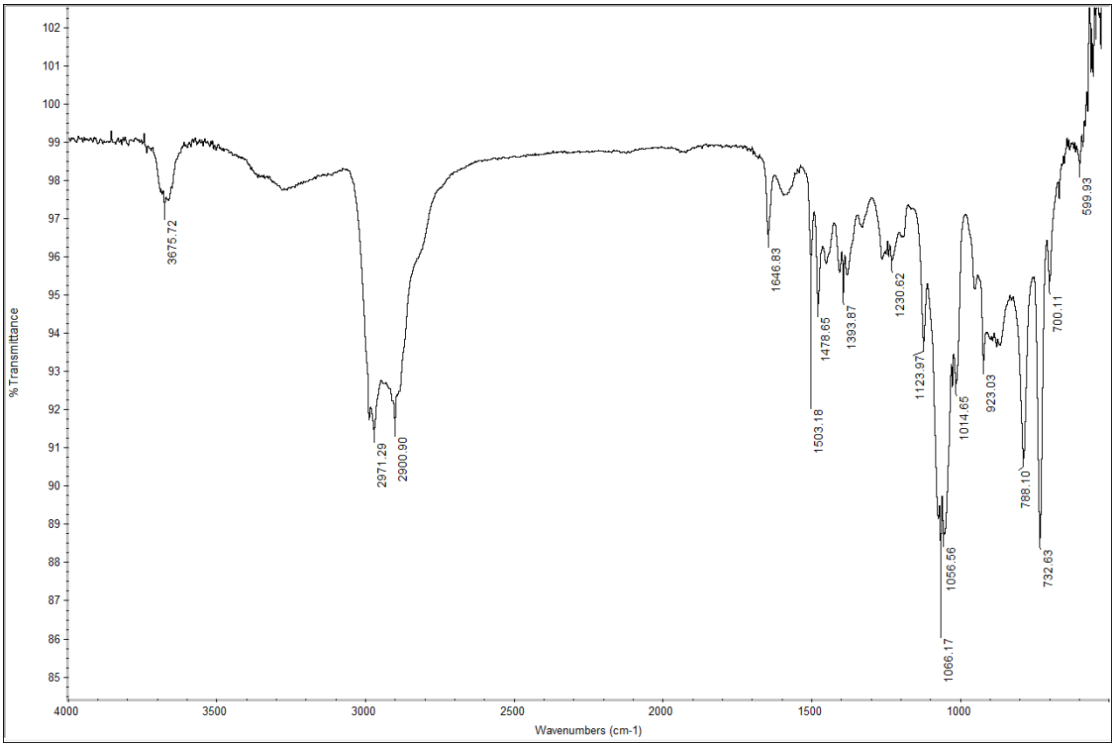


Figure 19

IR spectrum for imine 7

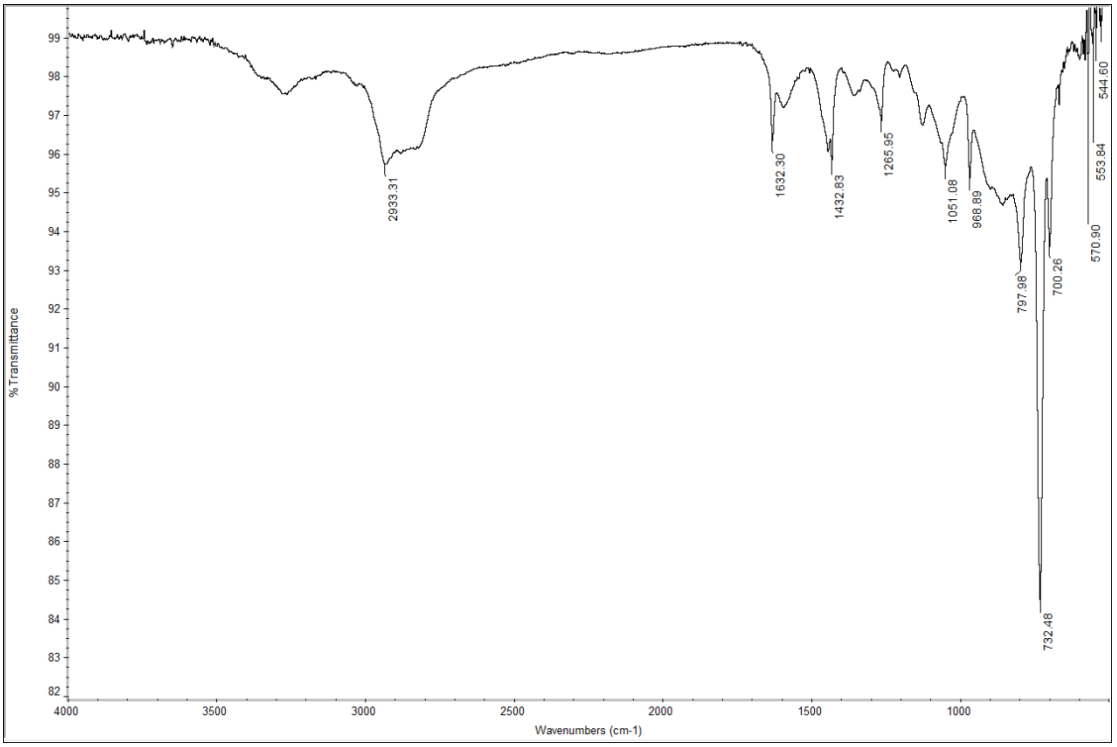


Figure 20

IR spectrum for imine 8

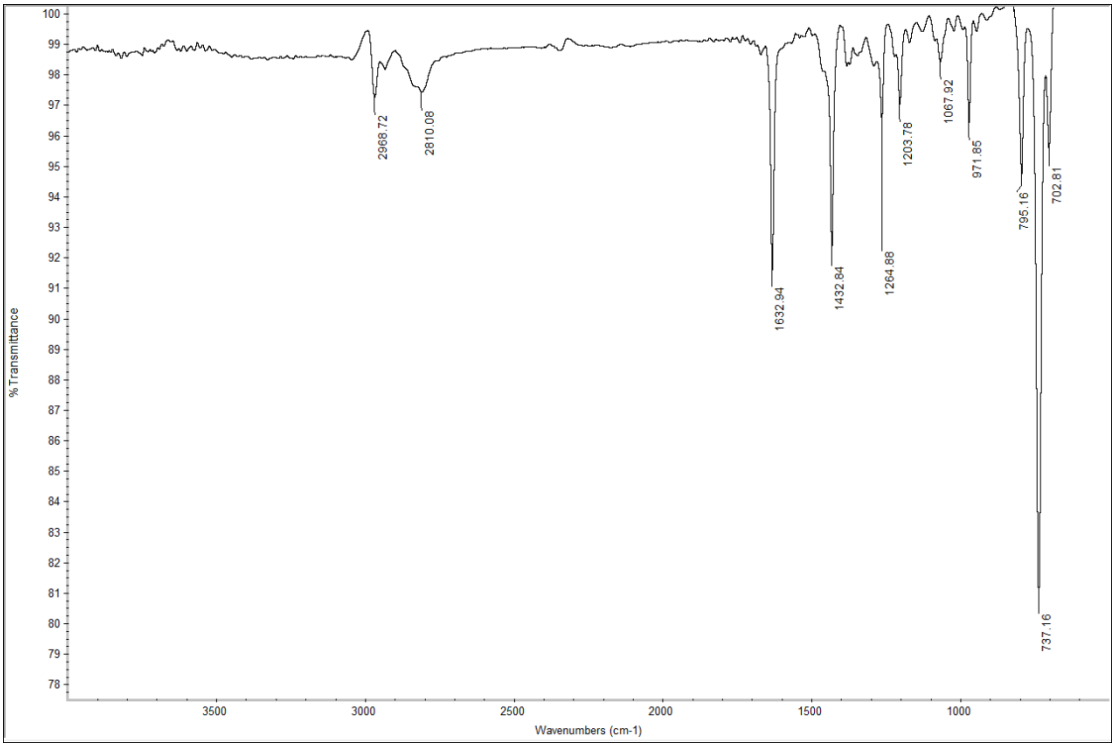


Figure 21

IR spectrum for imine 1

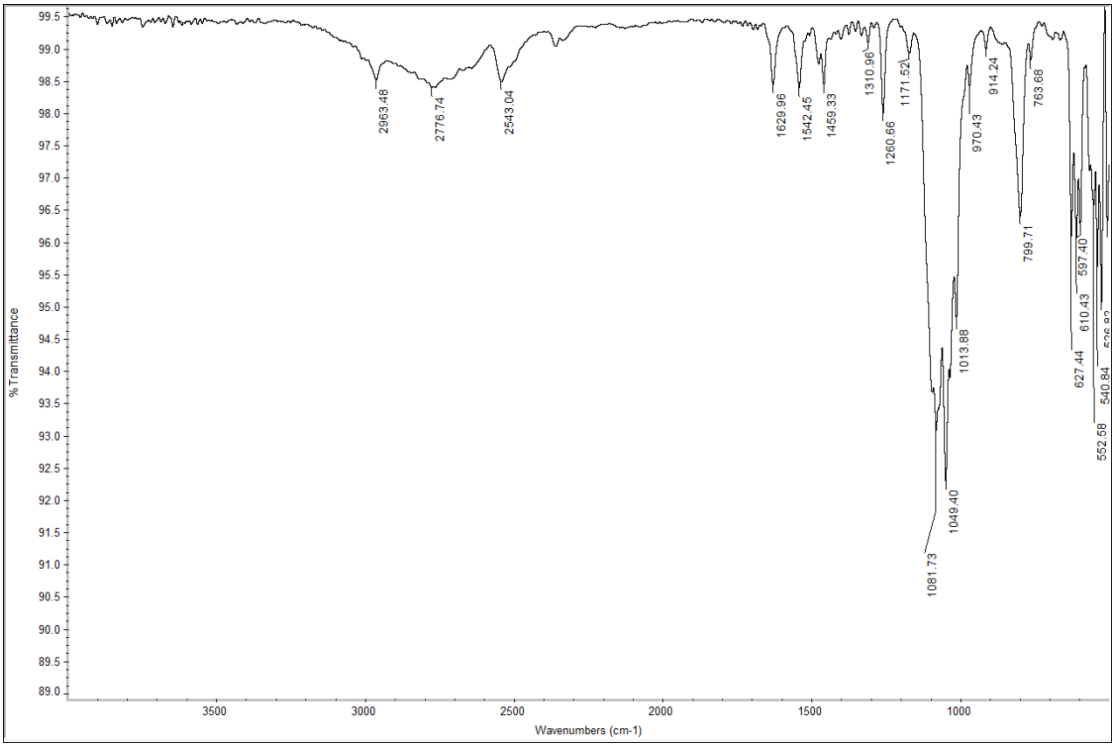


Figure 22

IR spectrum for imine 2

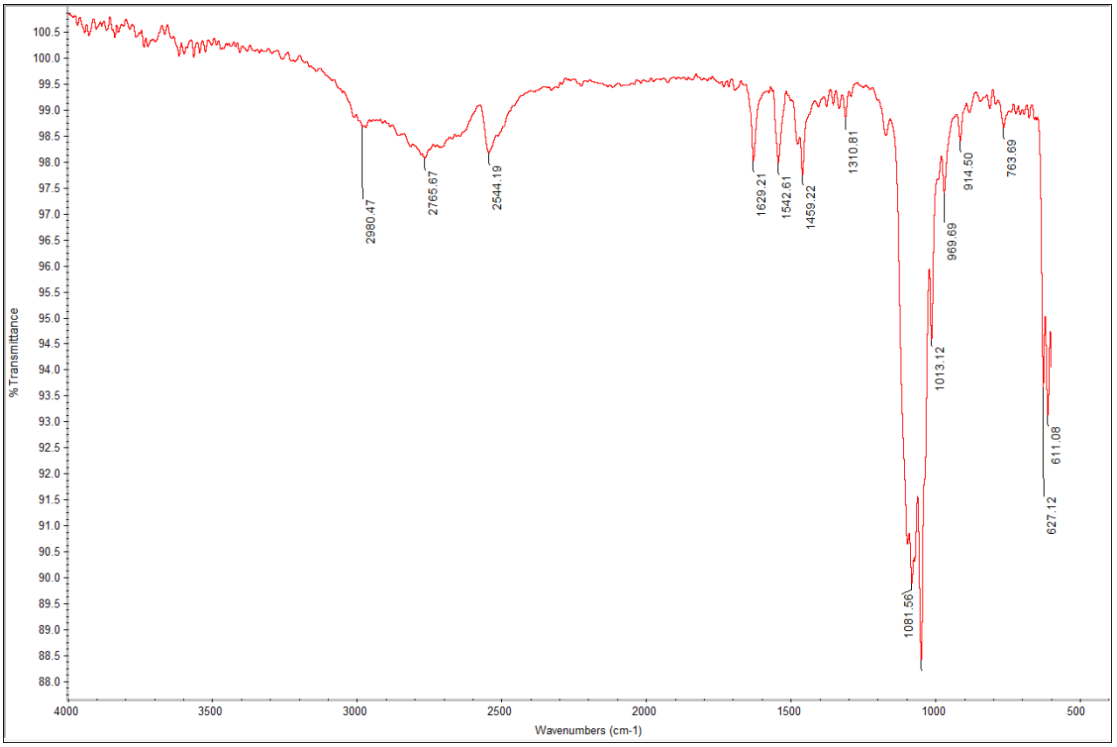


Figure 23

IR spectrum for imine 5

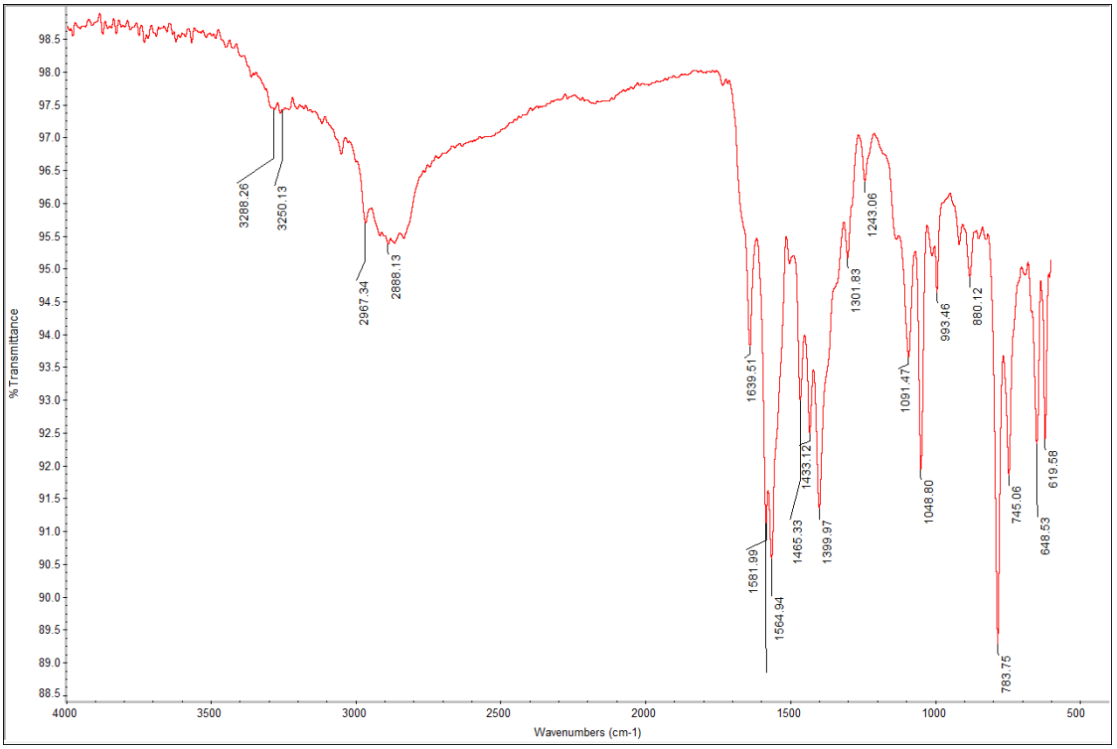
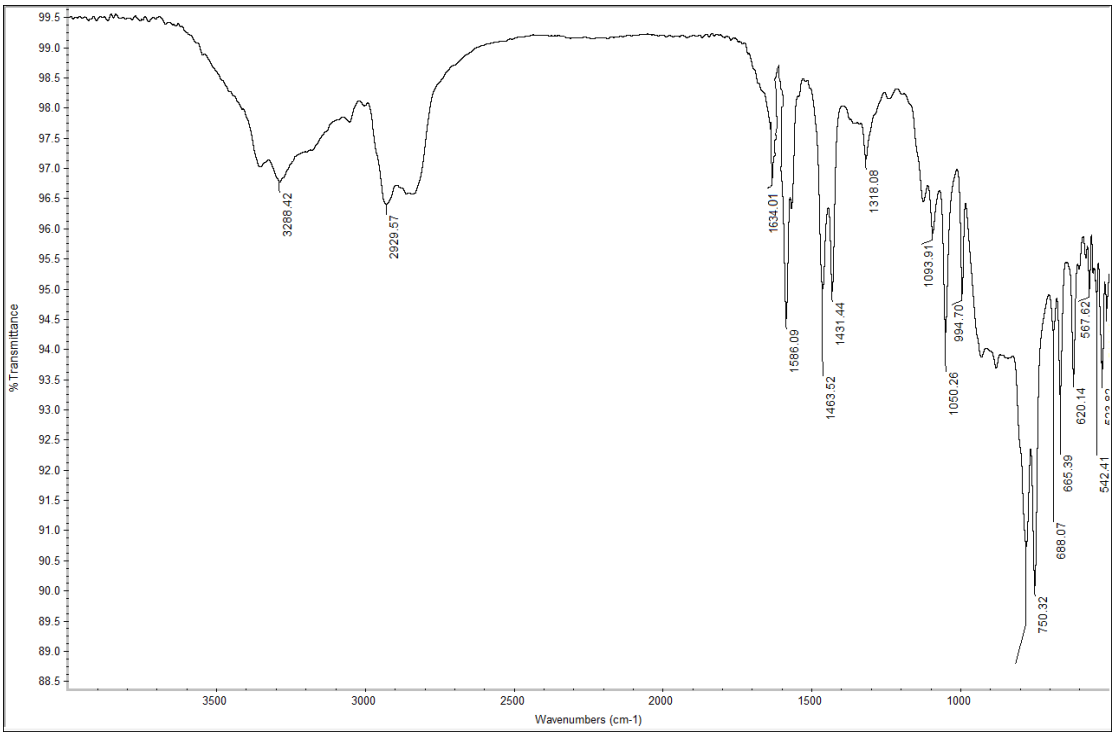


Figure 24

IR spectrum for imine 6



NMR

Figure 25(a)

^{1}H-NMR for ester E2

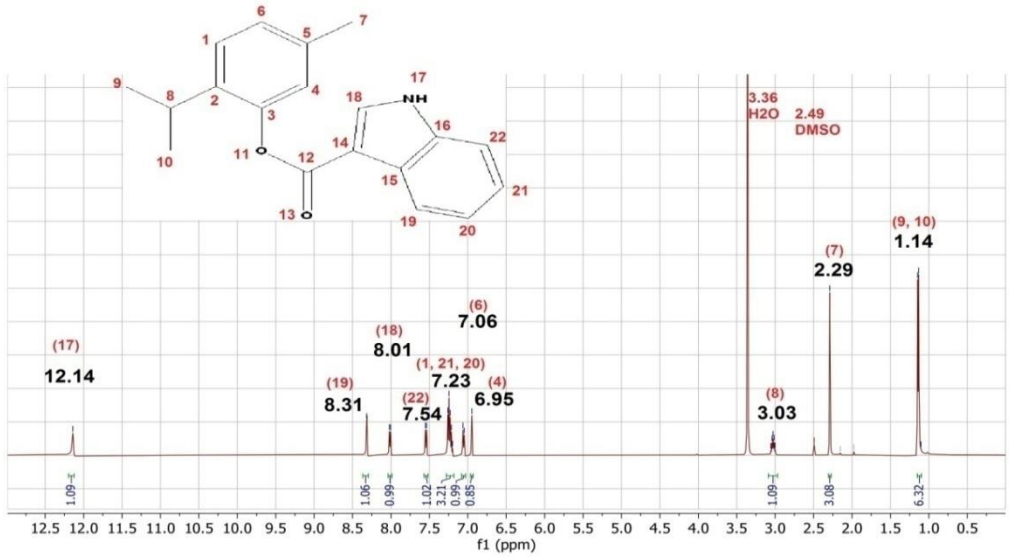


Figure 25(b)

^{13}C-NMR for ester E2

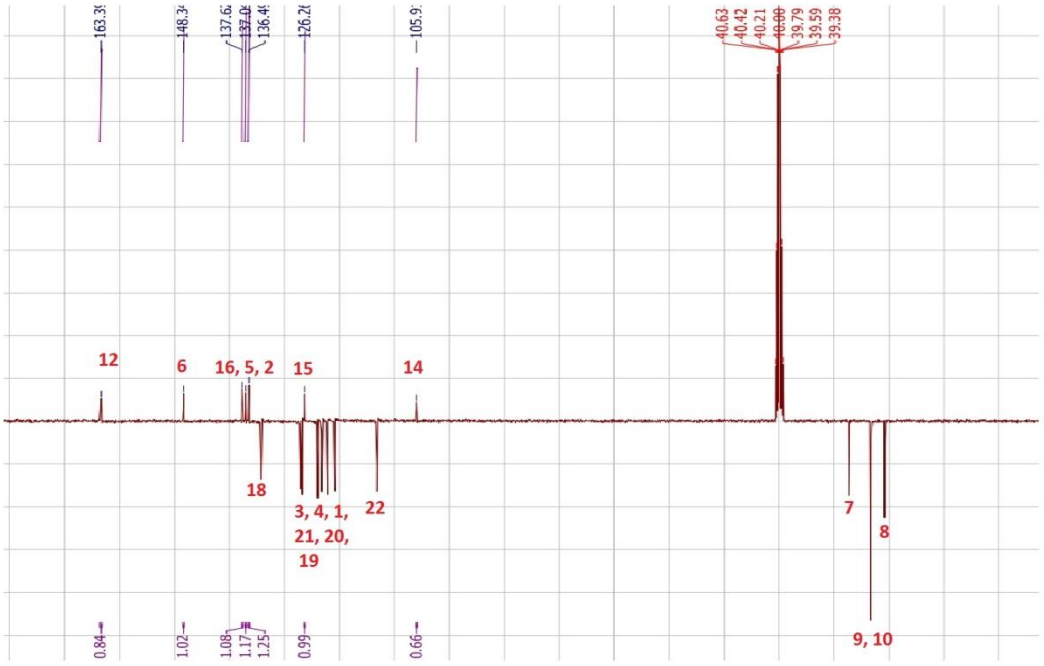


Figure 26

[1]H-NMR for ester E4

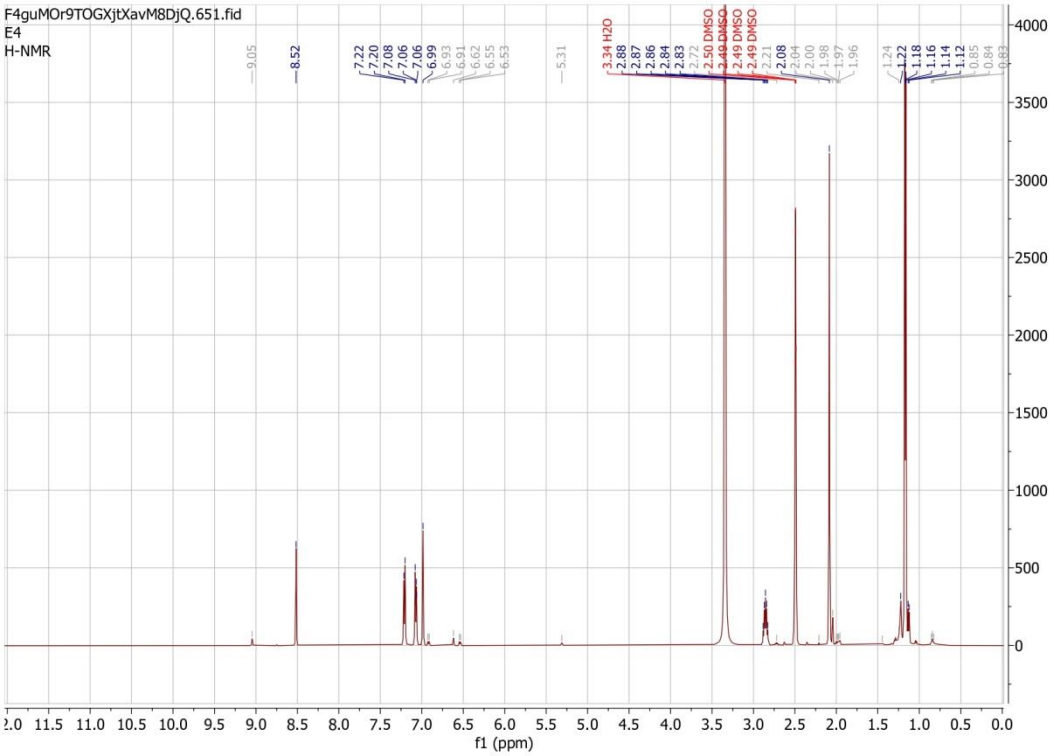


Figure 27

[1]H-NMR for ester E5

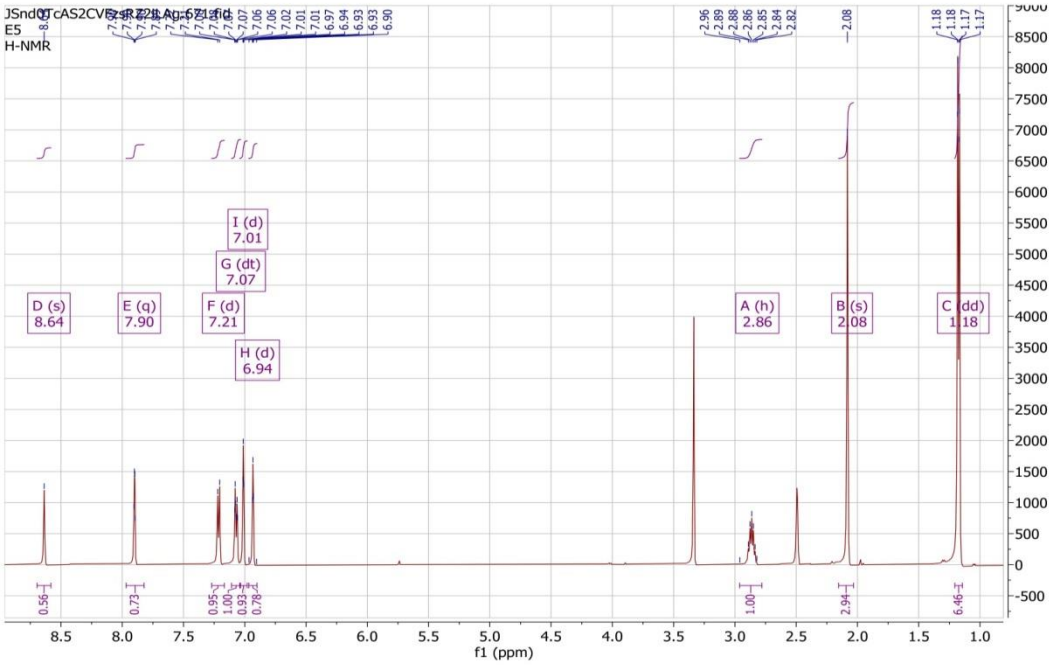


Figure 28

[1]H-NMR for ester E6

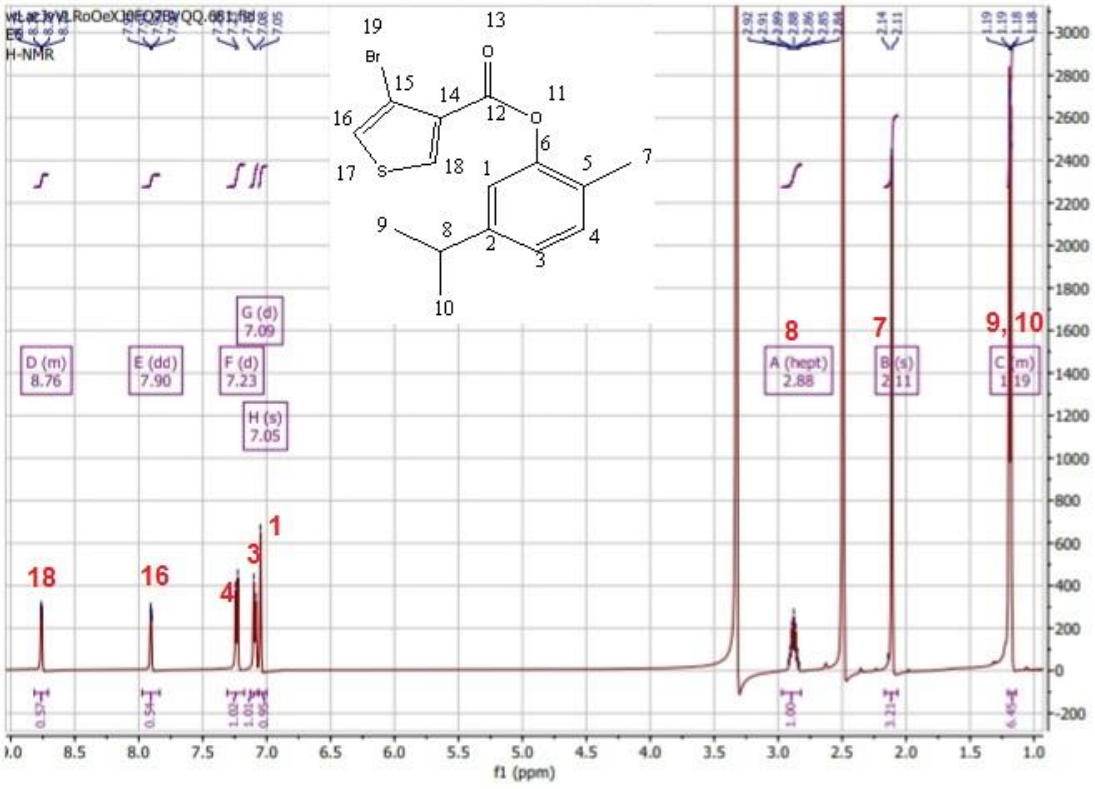


Figure 29

[1]H-NMR for ester E7

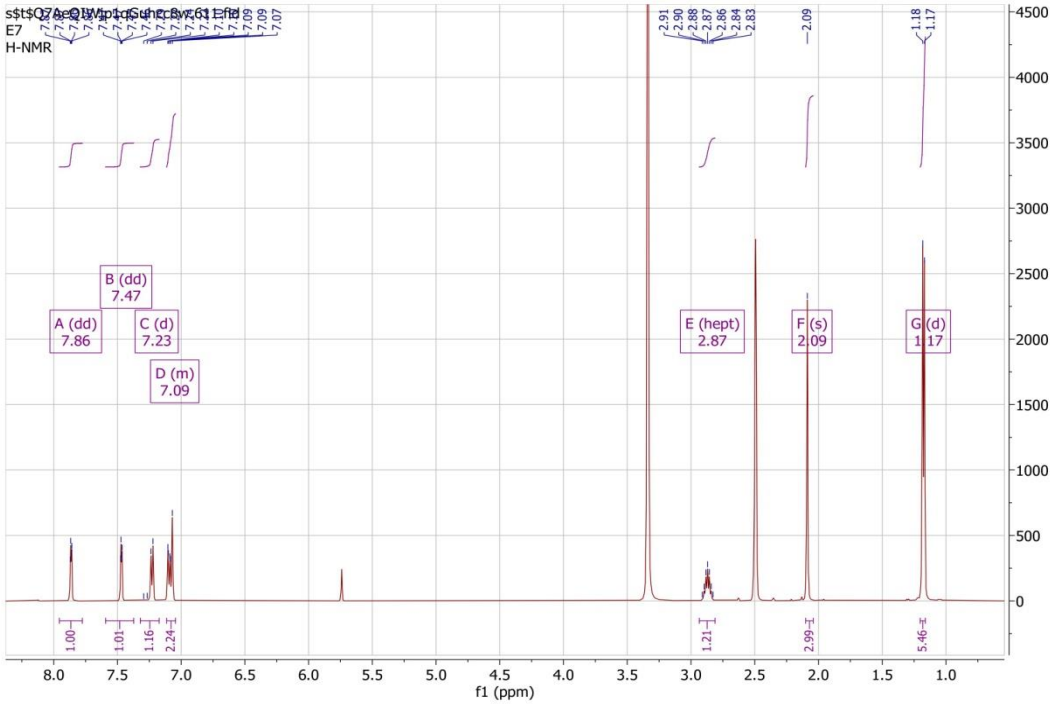


Figure 30

[1]H-NMR for ester E8

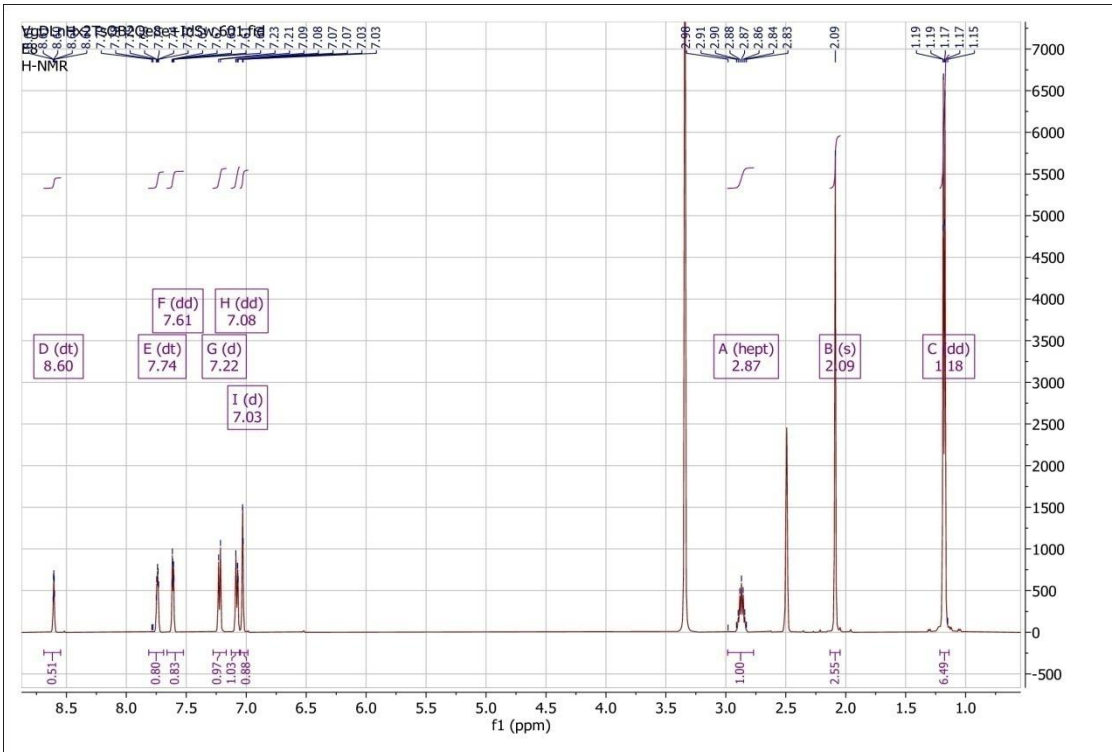


Figure 31

[1]H-NMR for ester Ea

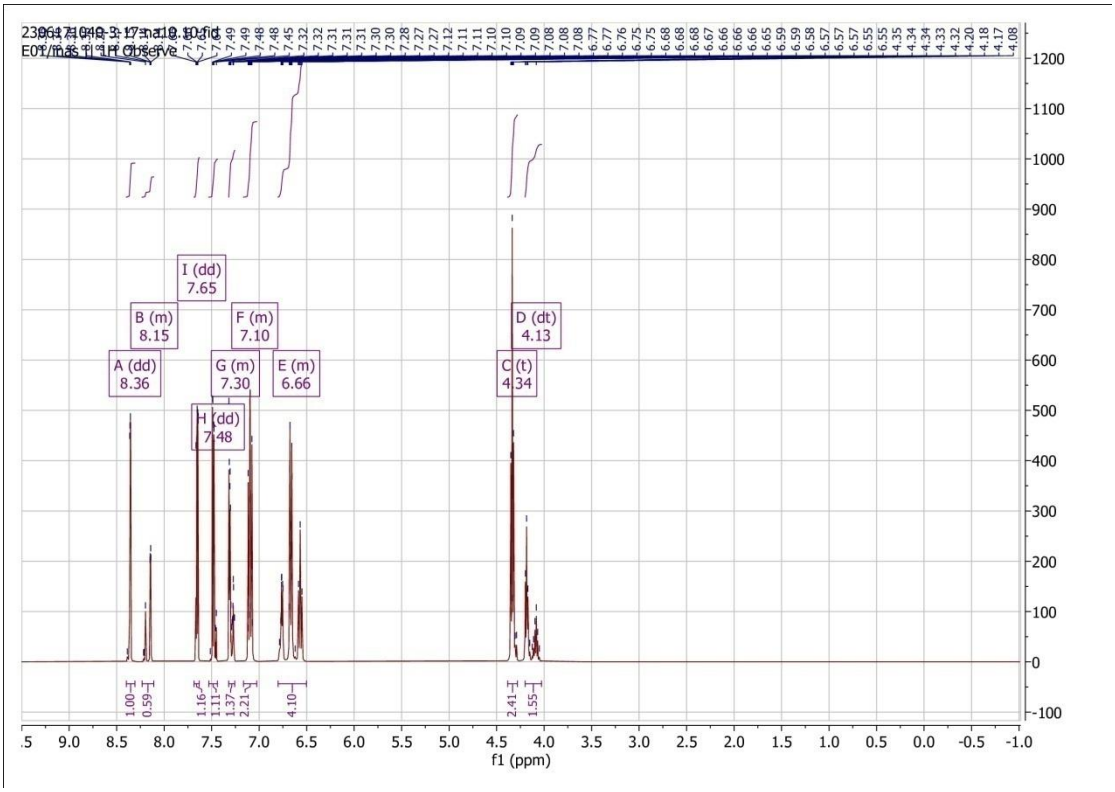


Figure 32

[1]H-NMR for ester Eb

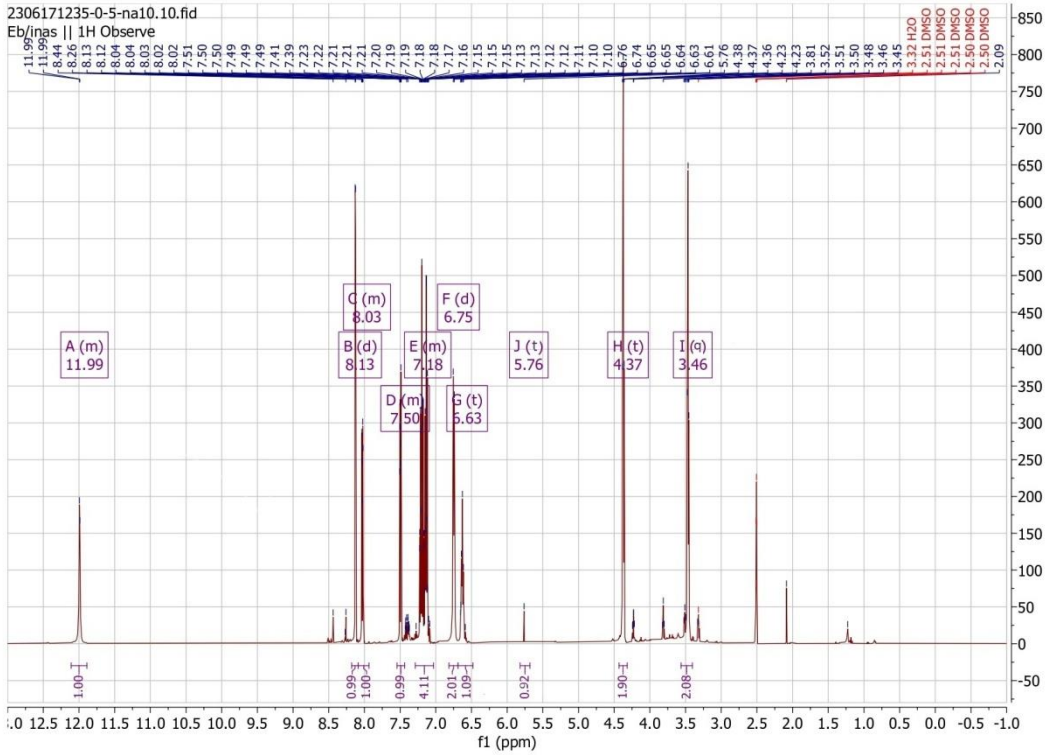


Figure 33

[1]H-NMR for ester Ec

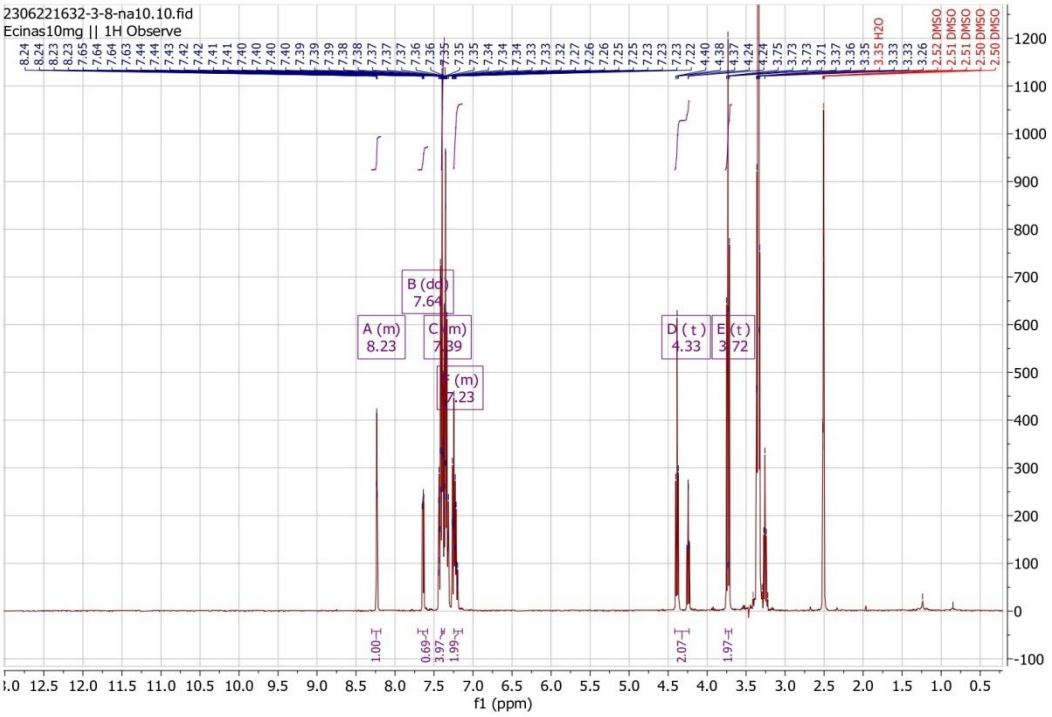


Figure 34

[1]H-NMR for ester Ed

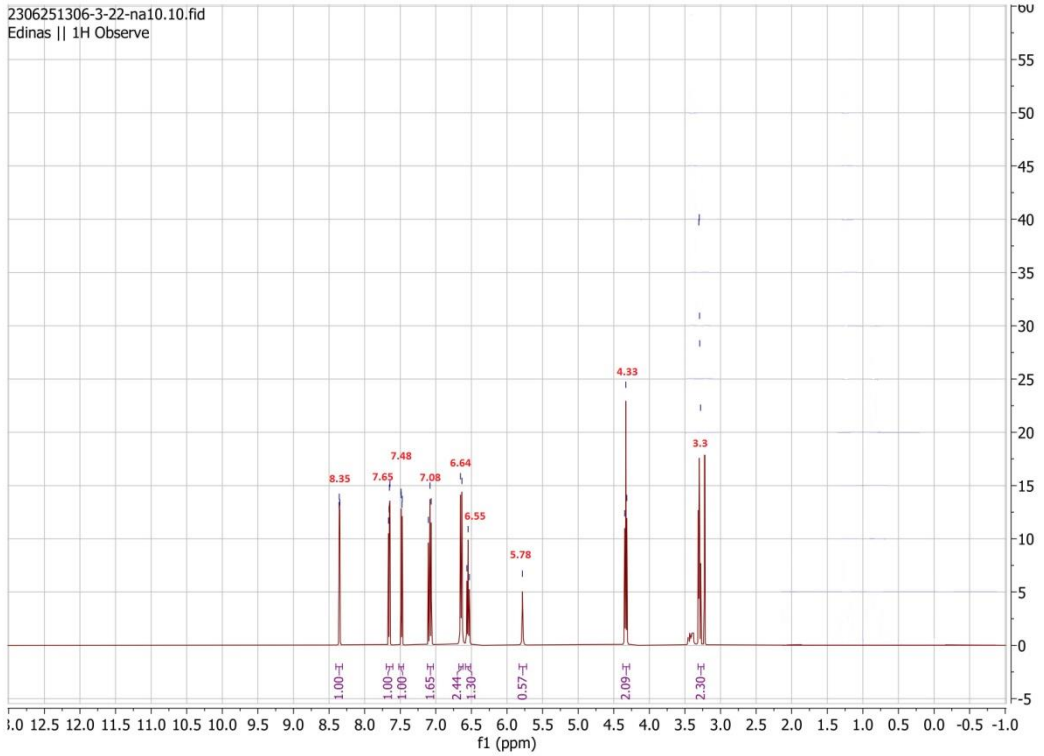


Figure 35

[1]H-NMR for ester Ee1

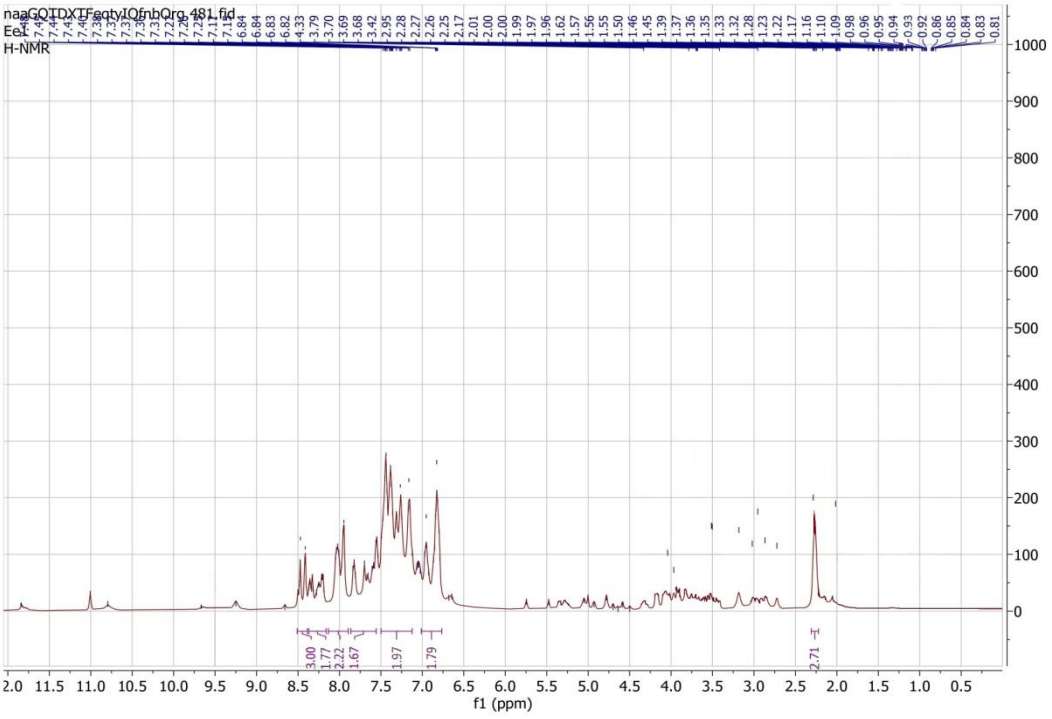


Figure 36

[1]H-NMR for ester Ee2

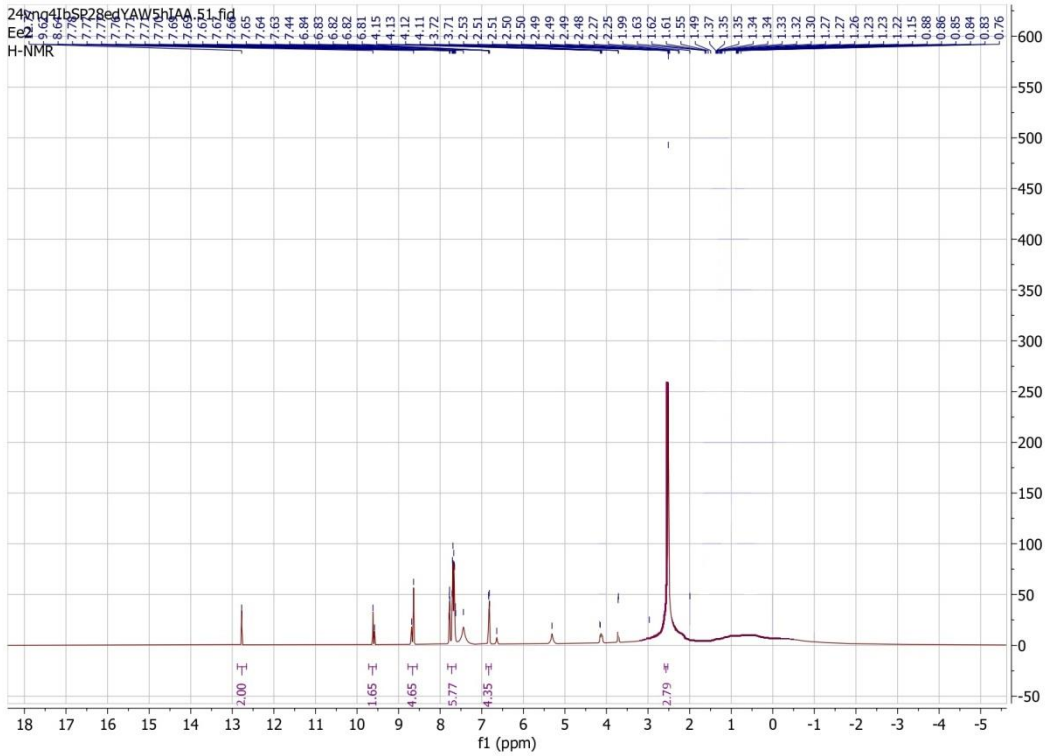


Figure 37

[1]H-NMR for amide A1

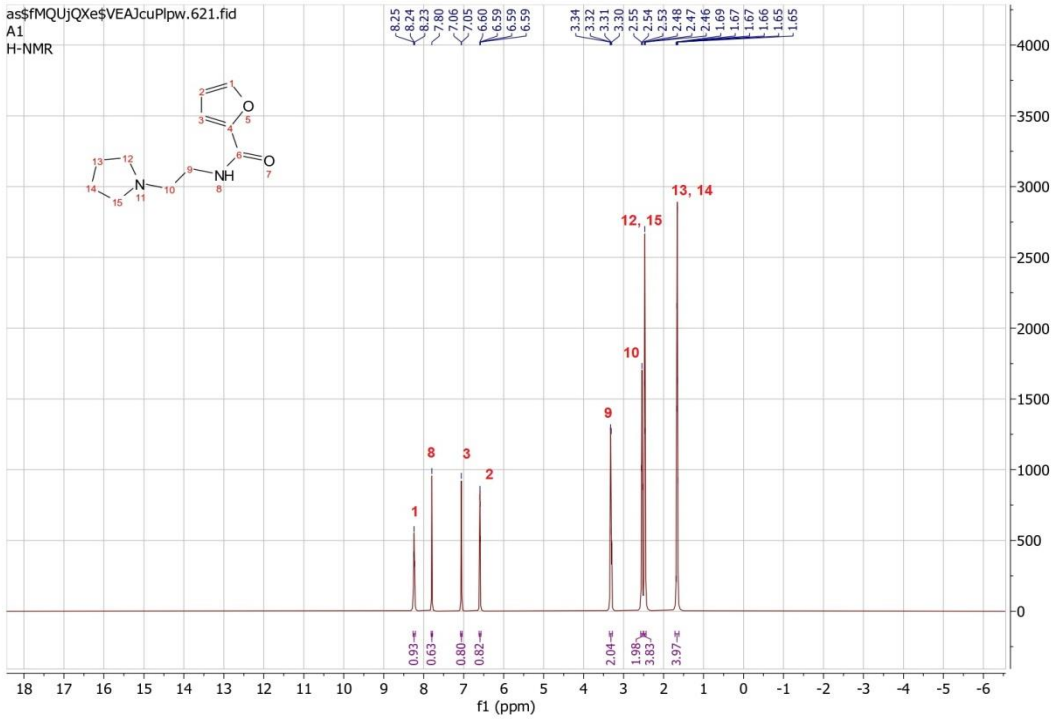


Figure 38

[1]H-NMR for amide A2

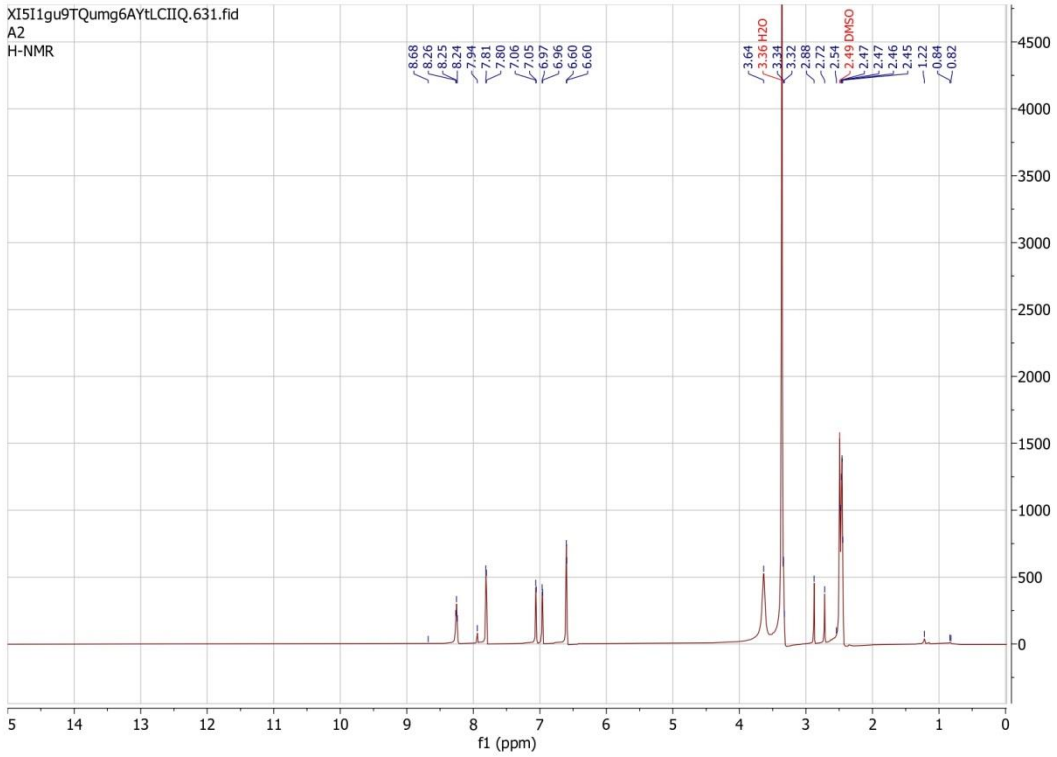


Figure 39

[1]H-NMR for amide Aa

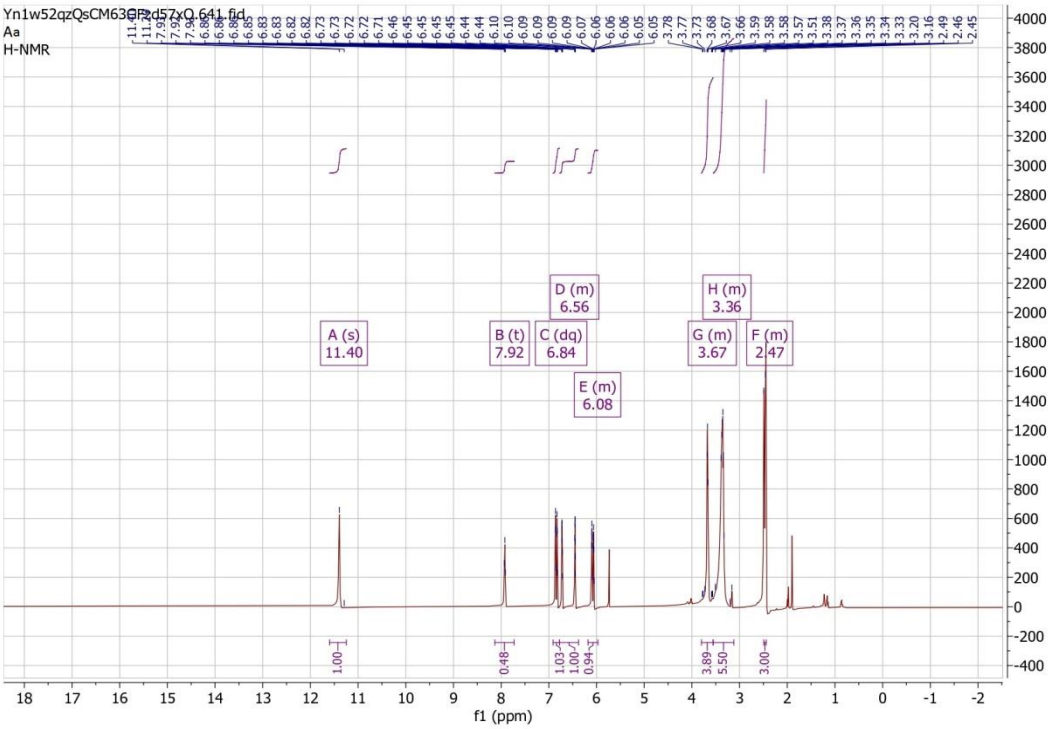


Figure 40

^{1}H-NMR for amide Ab

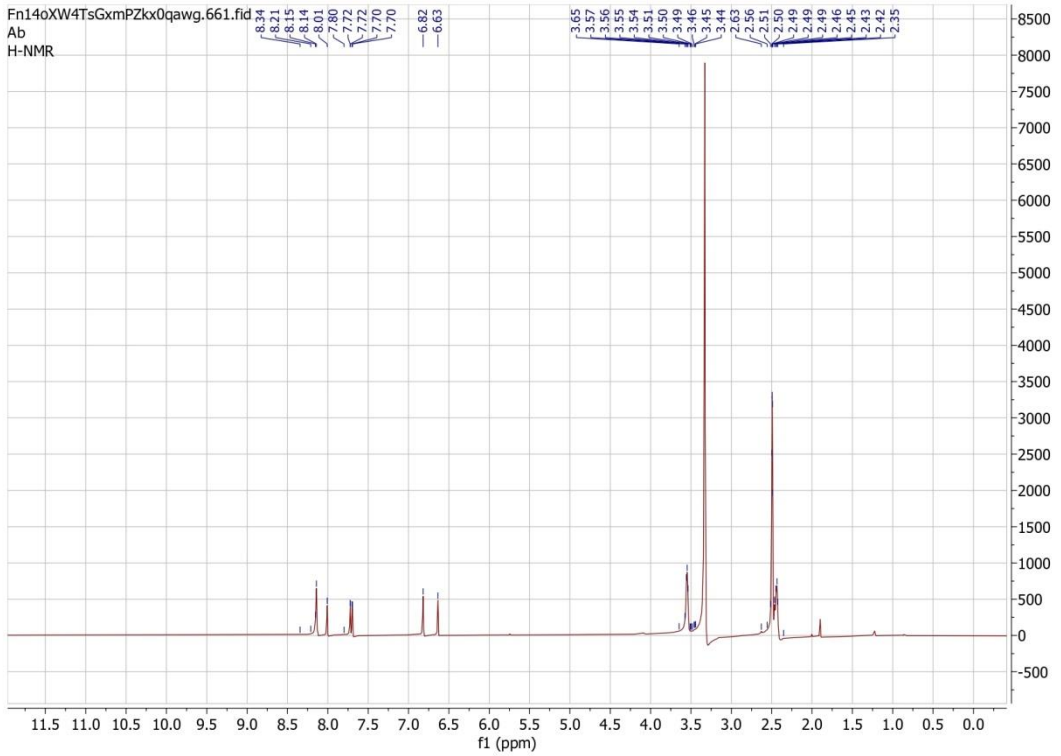


Figure 41

^{1}H-NMR for imine 1

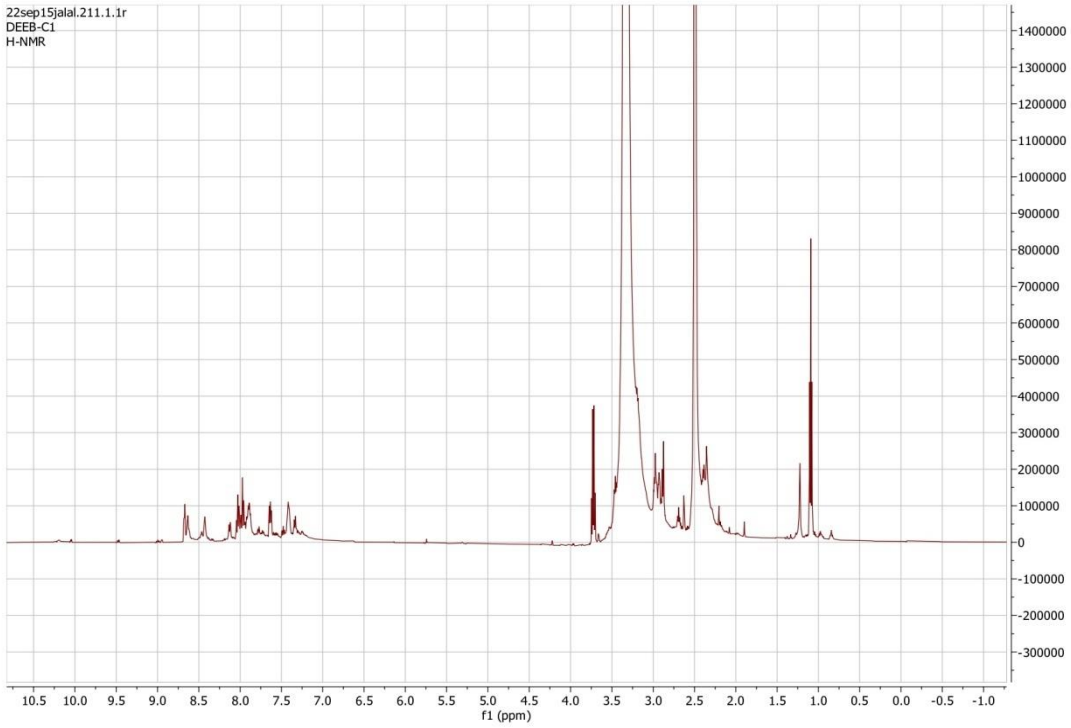


Figure 42

[1]H-NMR for imine 2

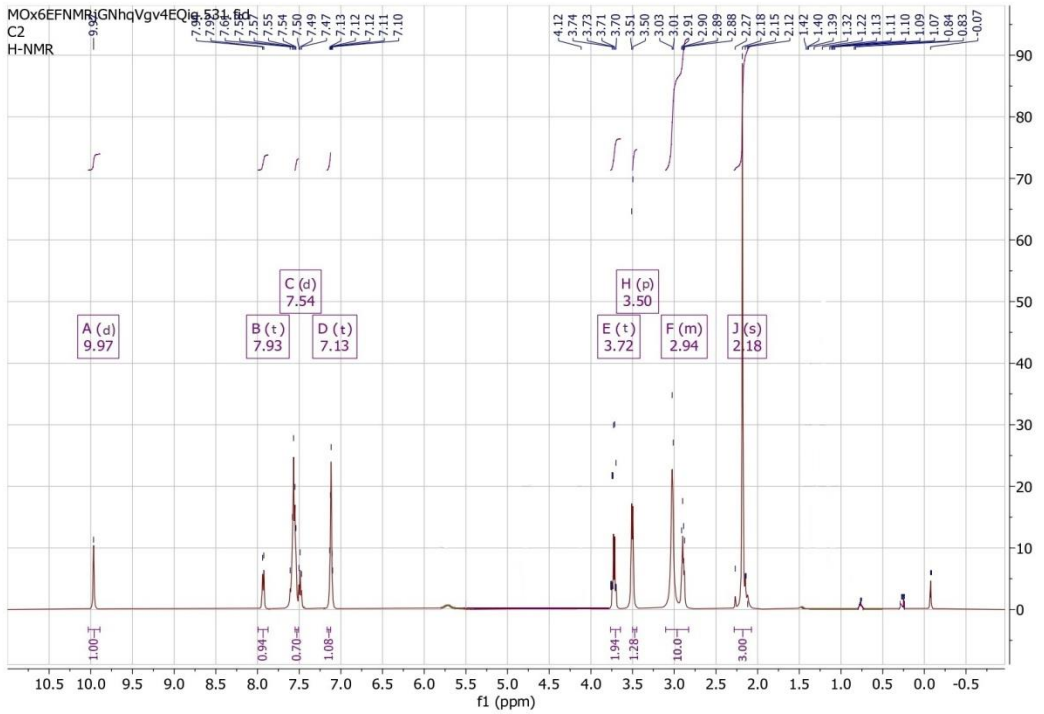


Figure 43

[1]H-NMR for imine 5

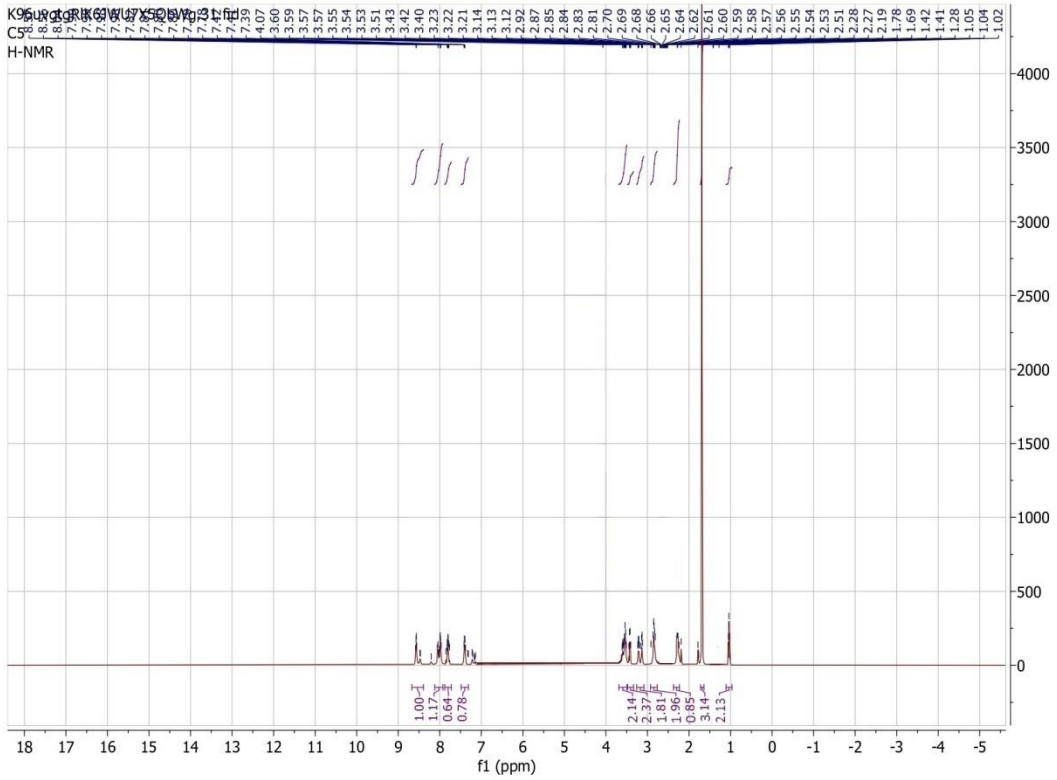
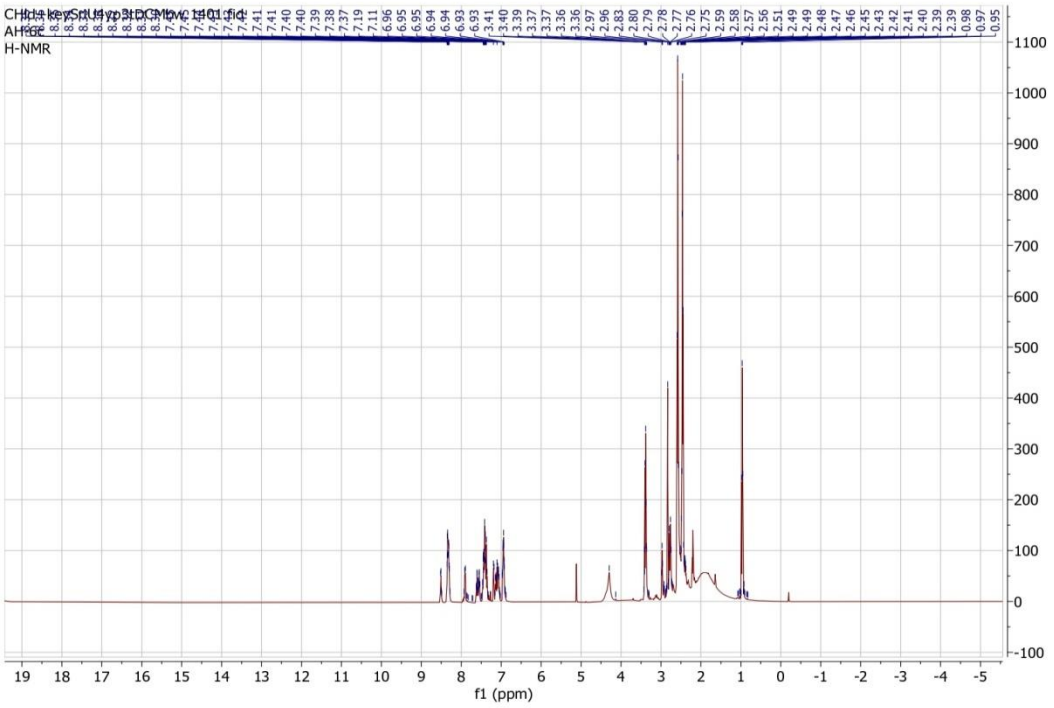


Figure 44

^{1}H-NMR for imine 6



HPLC

Figure 45

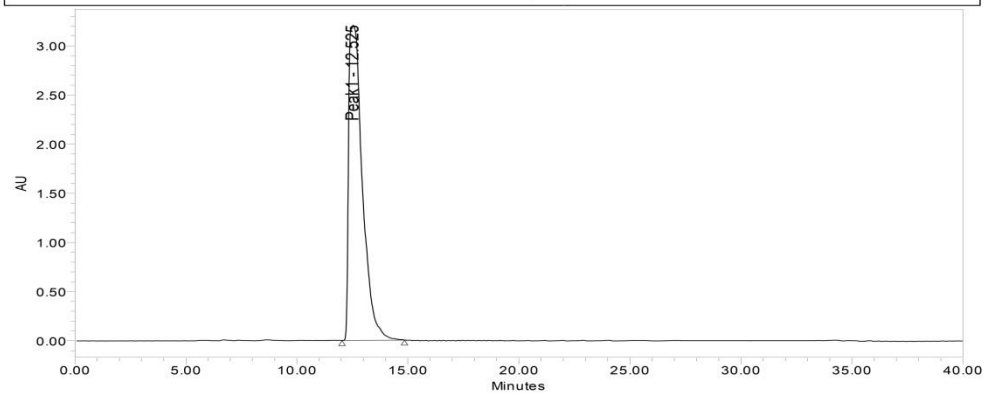
HPLC results for ester E2

An-Najah University
Project Name Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

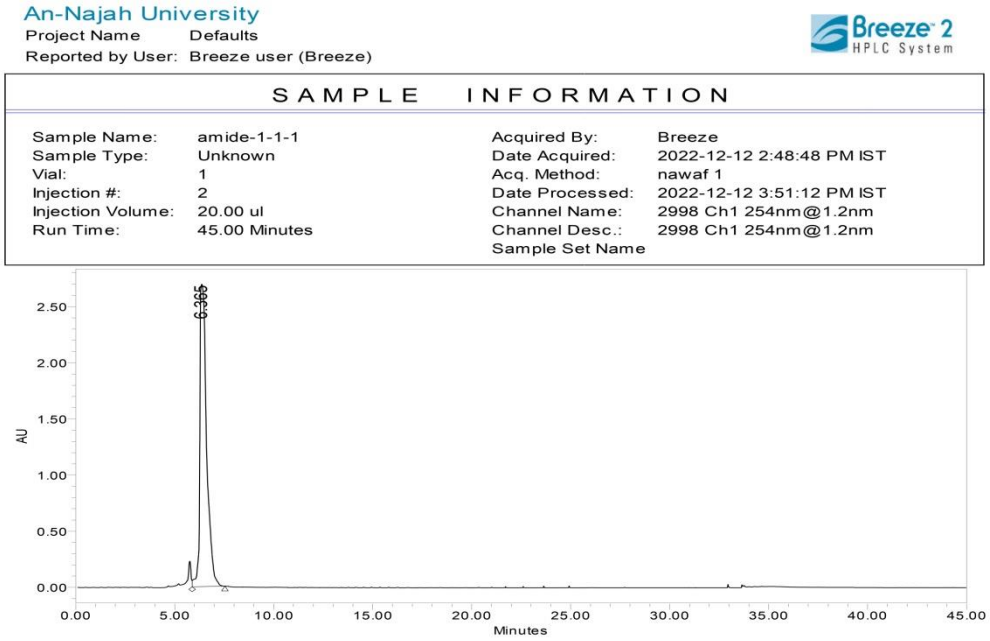
Sample Name:	E2	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	2023-02-01 3:08:49 PM IST
Vial:	1	Acq. Method:	nawaf 1
Injection #:	1	Date Processed:	2023-02-01 3:50:05 PM IST
Injection Volume:	20.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	40.00 Minutes	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Peak1	12.525	137571520	100.00	3204063	100.00

Figure 46

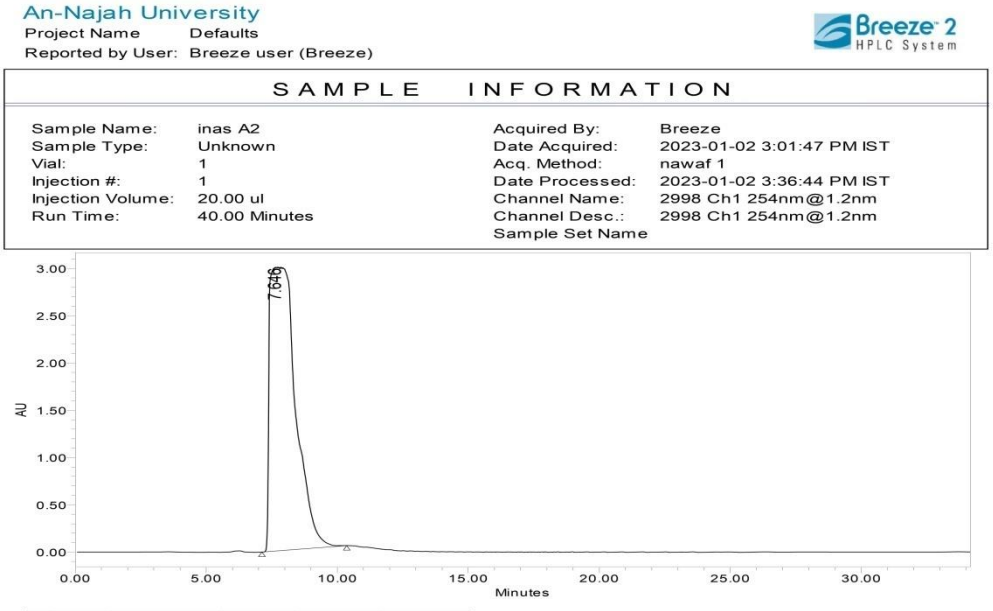
HPLC results for amide A1



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	6.365	64270212	100.00	2692447	100.00

Figure 47

HPLC results for amide A2



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	7.646	207507821	100.00	3002103	100.00

Anticancer activity

Figure 1a

In vitro effect of thymol and thymol ester compound (E2) on Hela cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

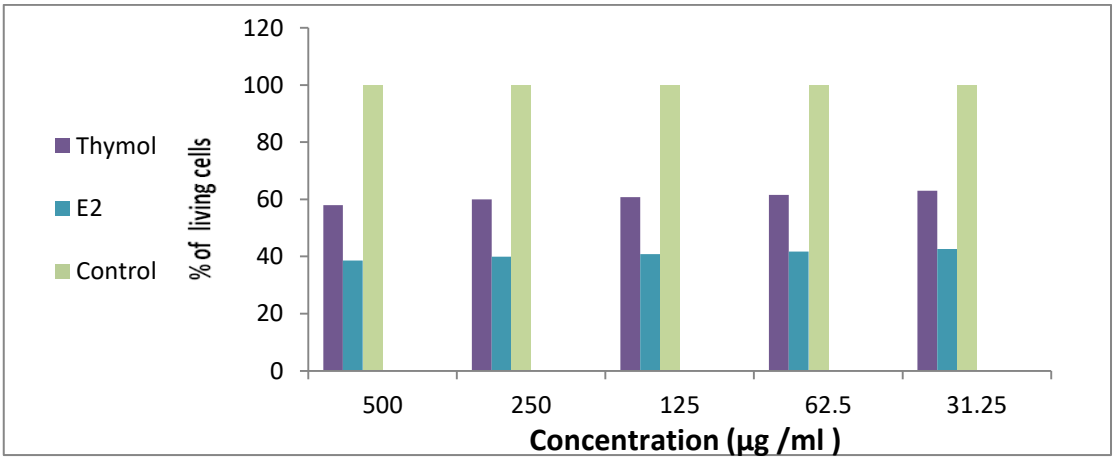


Figure 1b

In vitro effect of thymol and thymol ester compound (E2) on Hela cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

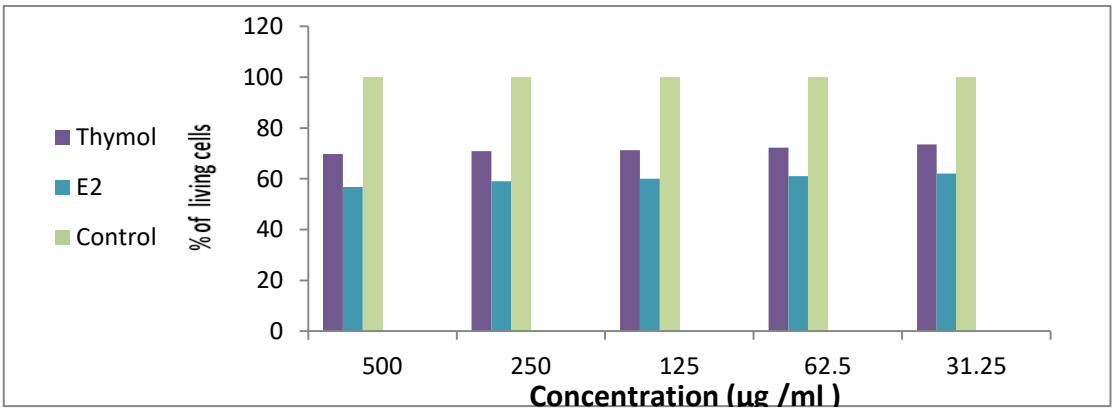


Figure 1c

*In vitro effect of thymol and thymol ester compound (**E2**) on L6 normal cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)*

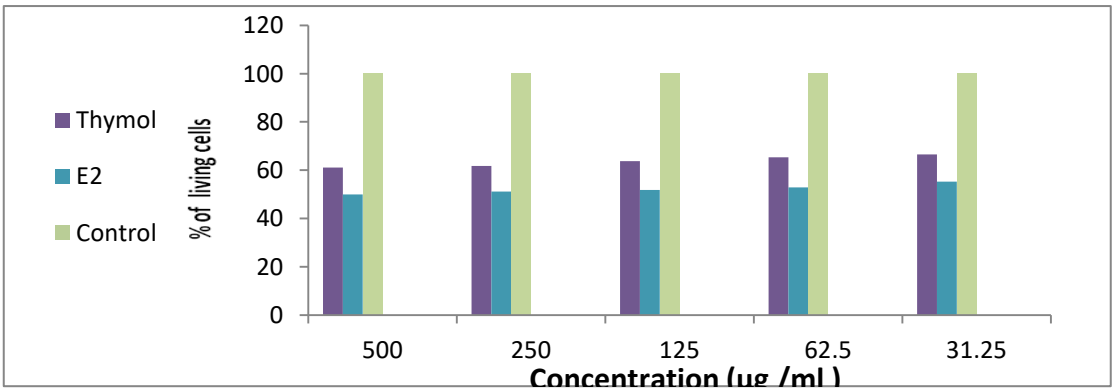


Figure 1d

In vitro effect of thymol and thymol ester compound (E2) on L6 normal cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

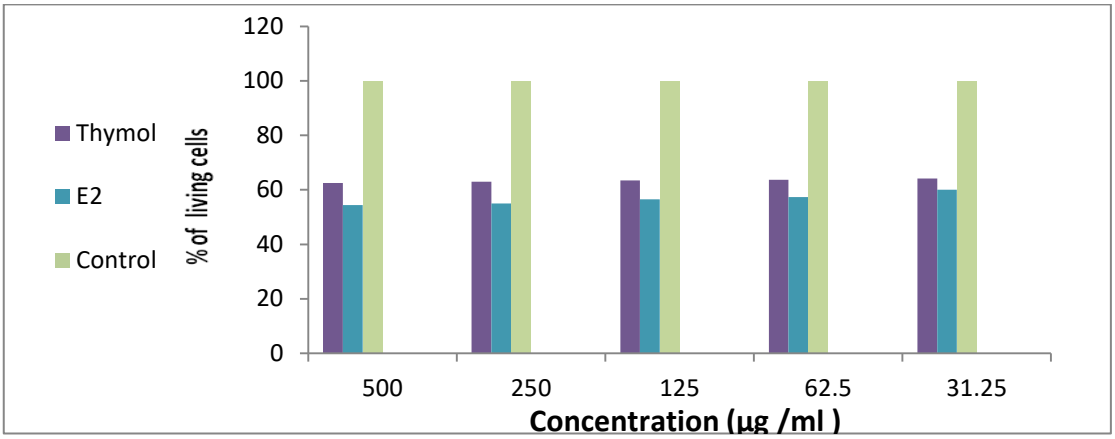


Figure 1e

In vitro effect of thymol ester compound (E2) on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

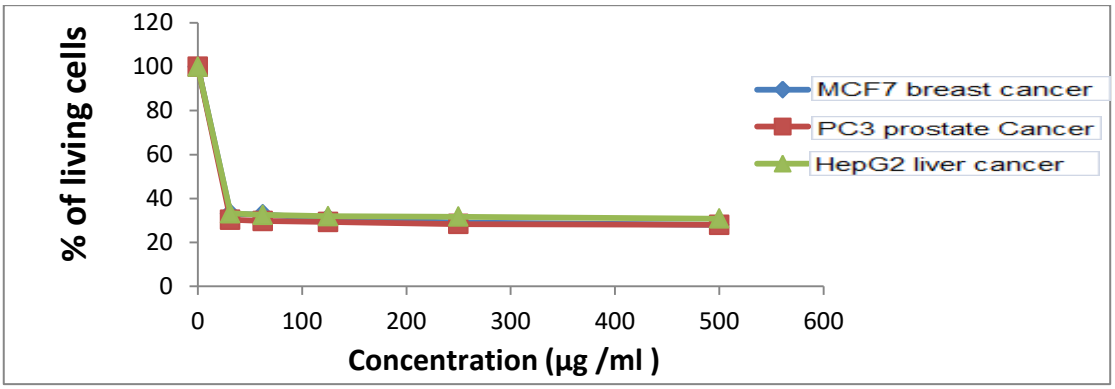


Figure 1f

In vitro effect of thymol ester compound (E2) on MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

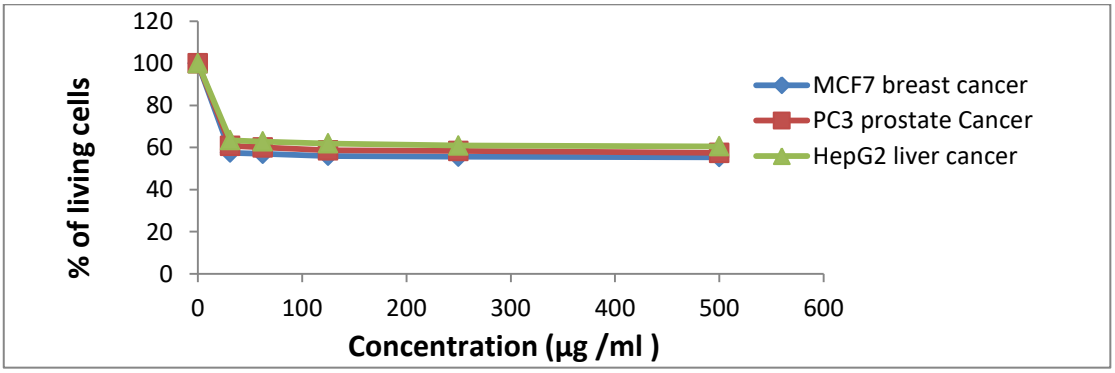


Figure 2a

In vitro effects of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) on Hela cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

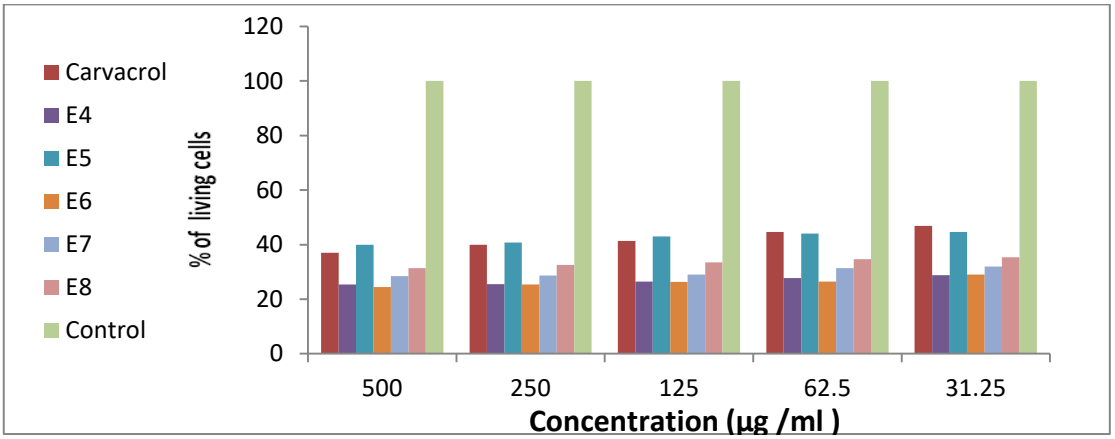


Figure 2b

In vitro effects of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) on Hela cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

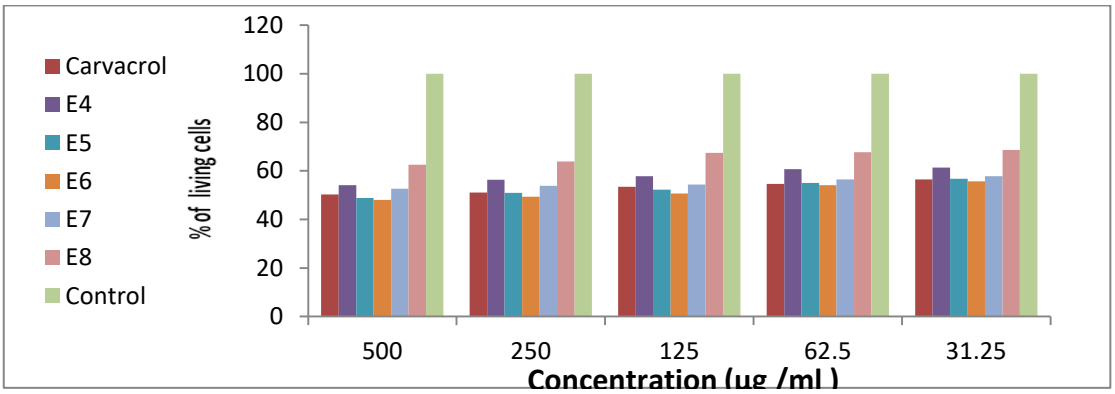


Figure 2c

In vitro effects of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) on L6 normal cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

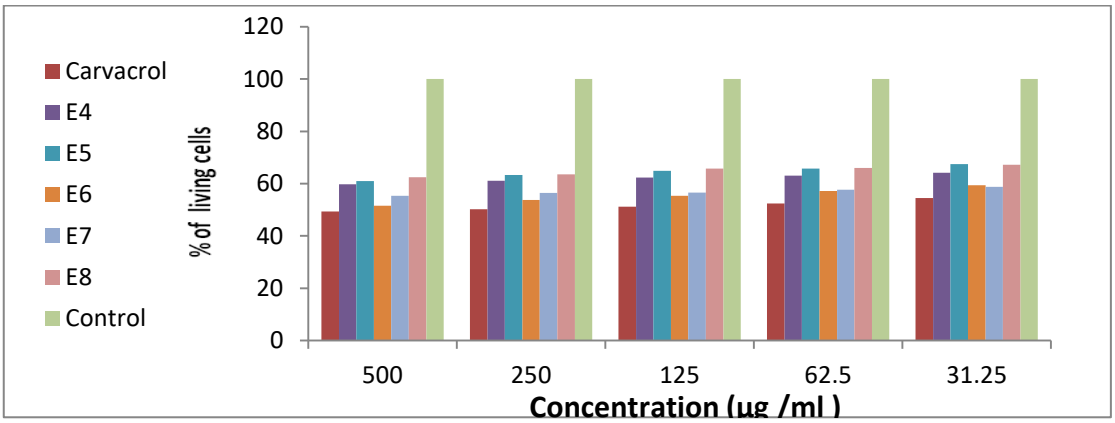


Figure 2d

In vitro effects of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) on L6 normal cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

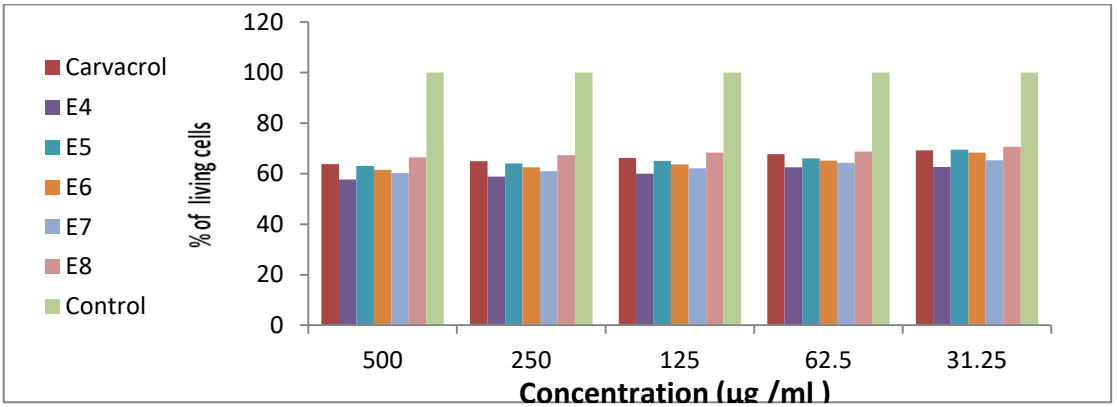


Figure 2e

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on MCF7 breast cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

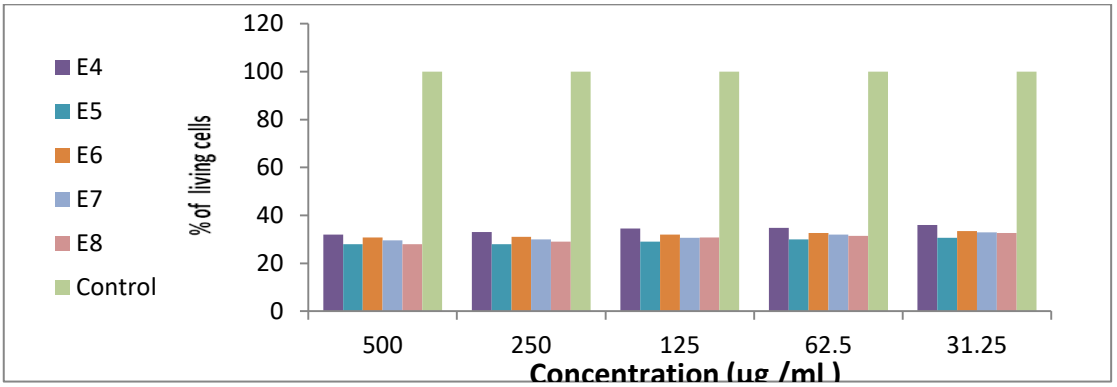


Figure 2f

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on MCF7 breast cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

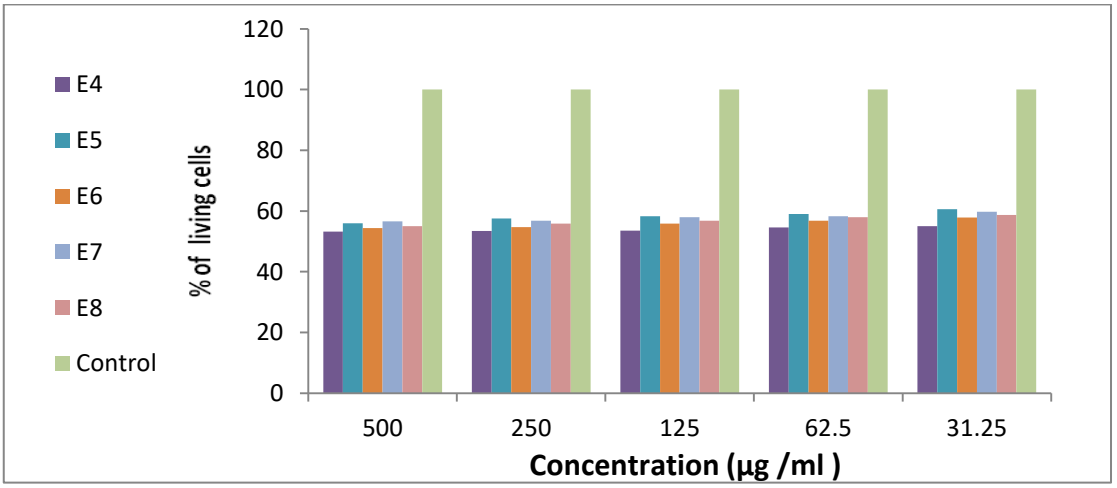


Figure 2g

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on PC3 prostate cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

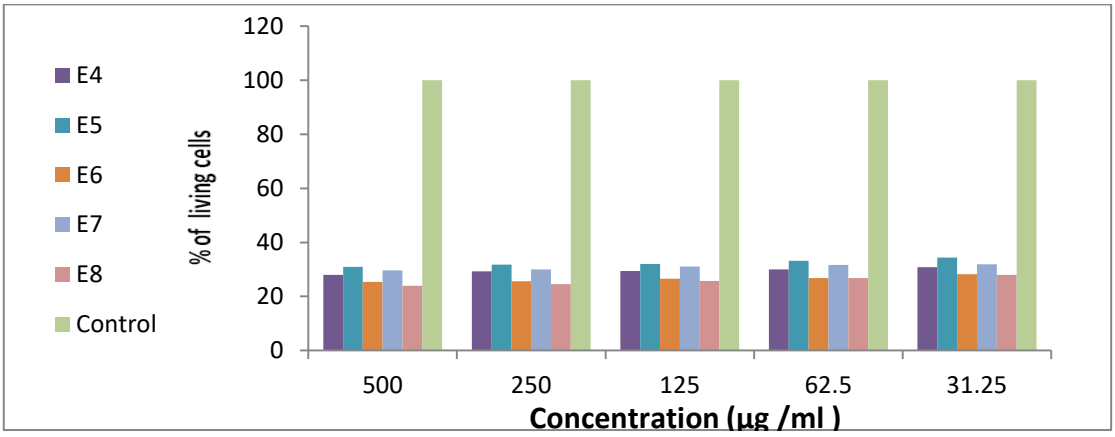


Figure 2h

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on PC3 prostate cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

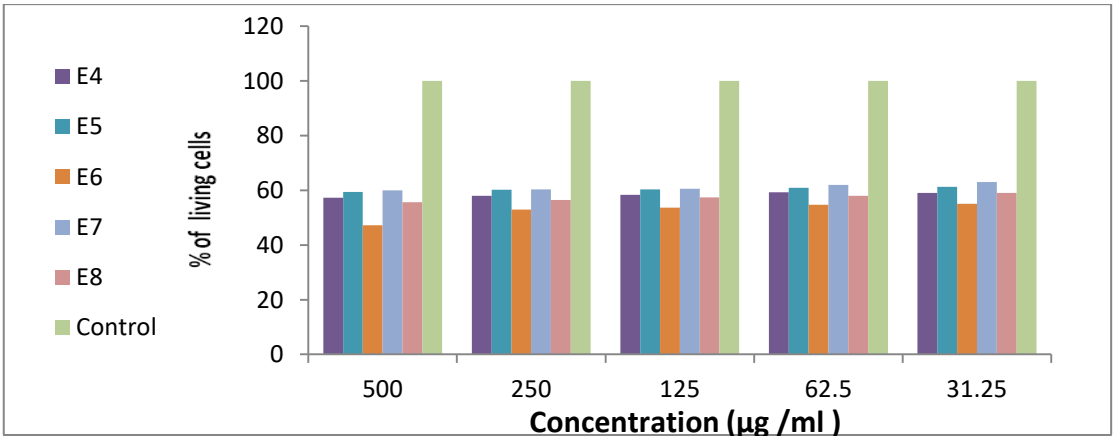


Figure 2i

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

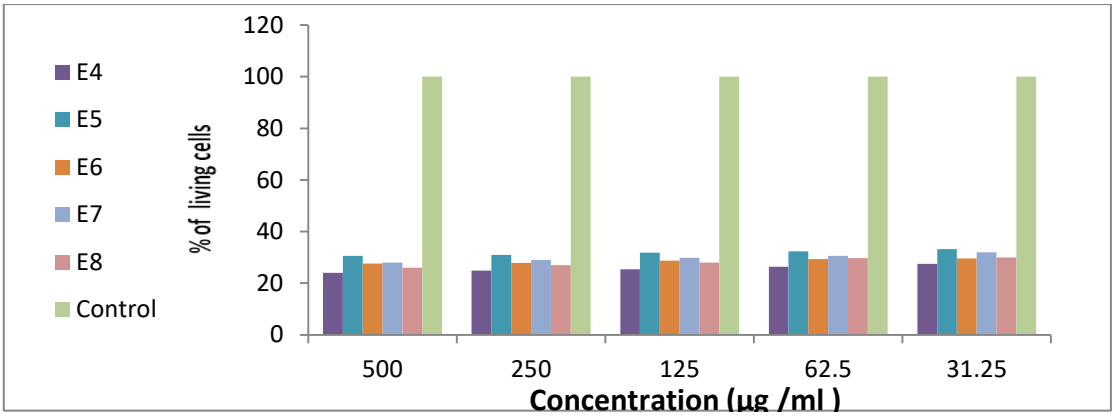


Figure 2j

In vitro effects of carvacrol ester compounds (E4, E5, E6, E7 and E8) on HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

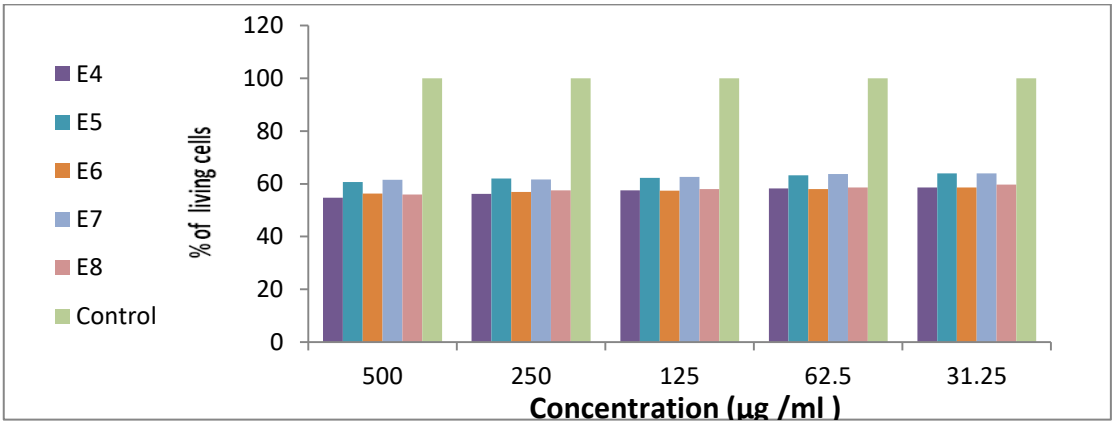


Figure 3a

In vitro effect of ester compound (Ea) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

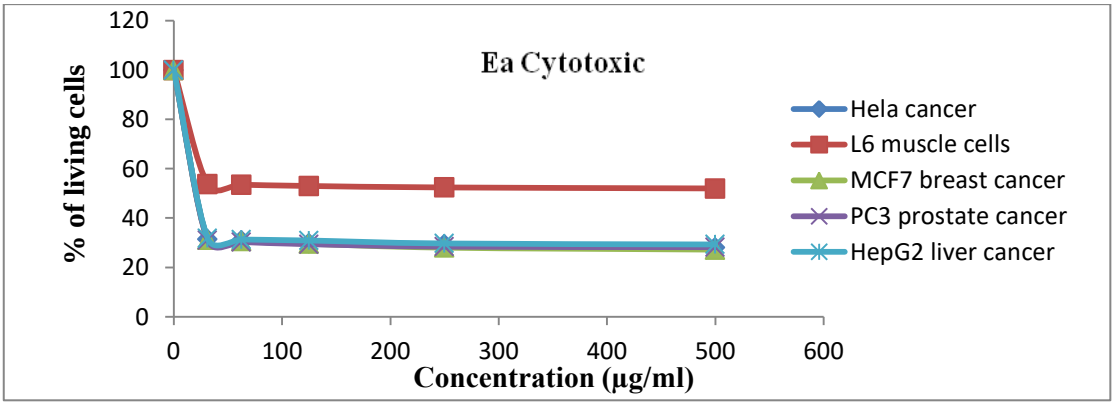


Figure 3b

In vitro effect of ester compound (Ea) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

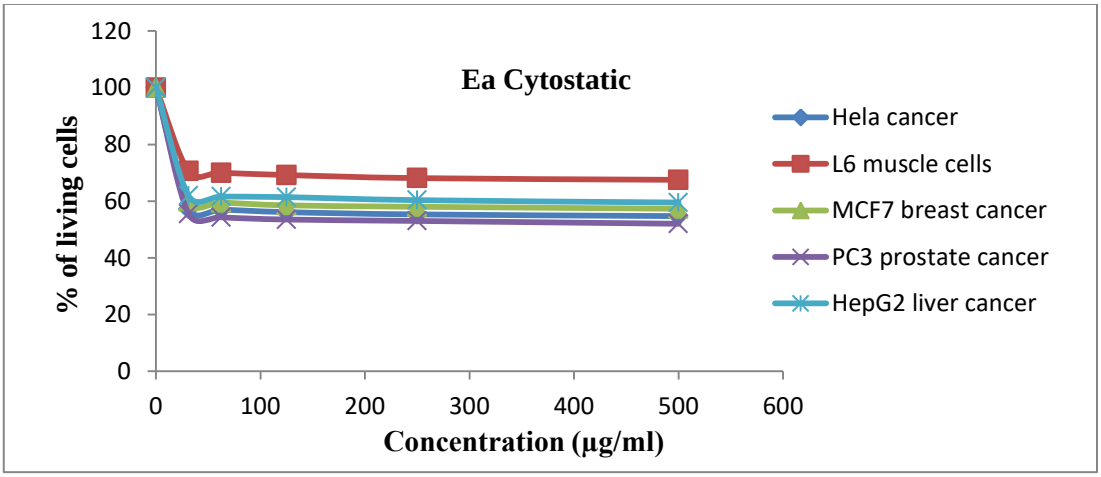


Figure 3c

In vitro effect of ester compound (Eb) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

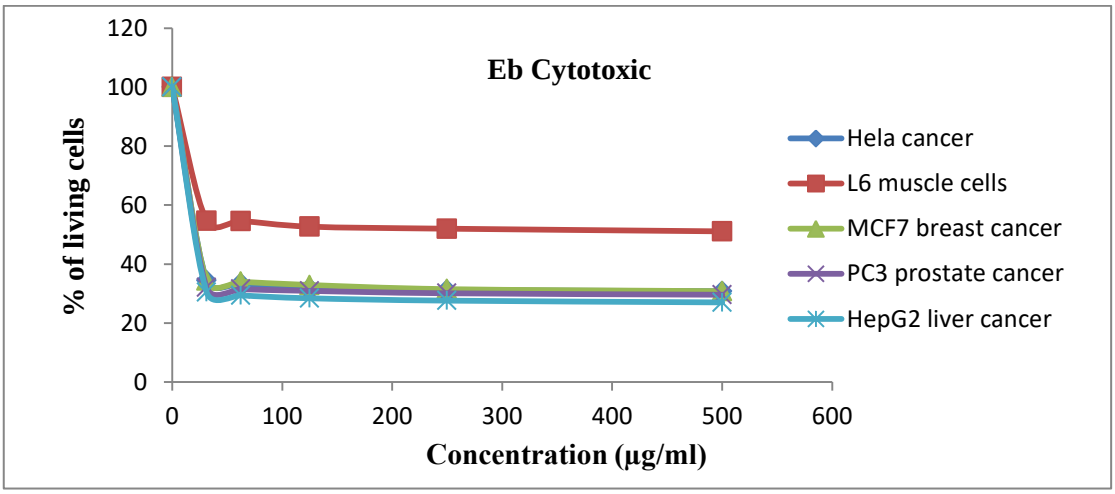


Figure 3d

In vitro effect of ester compound (Eb) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

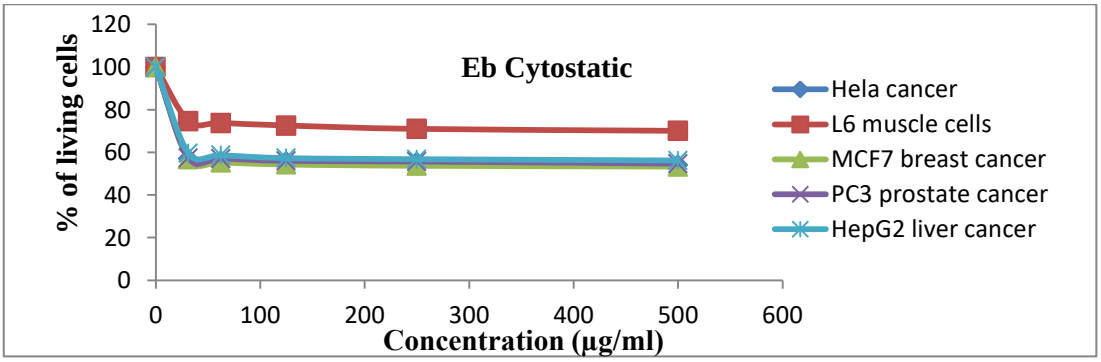


Figure 3e

In vitro effect of ester compound (Ec) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

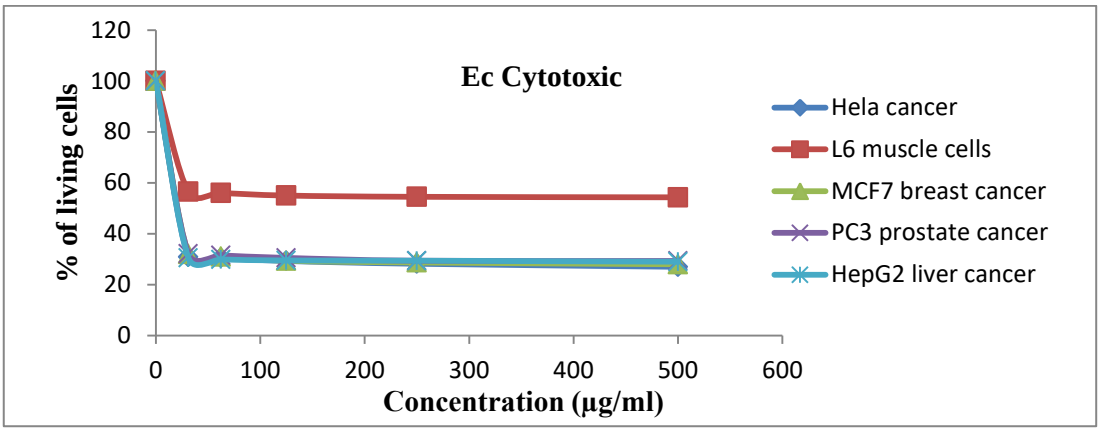


Figure 3f

In vitro effect of ester compound (Ec) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

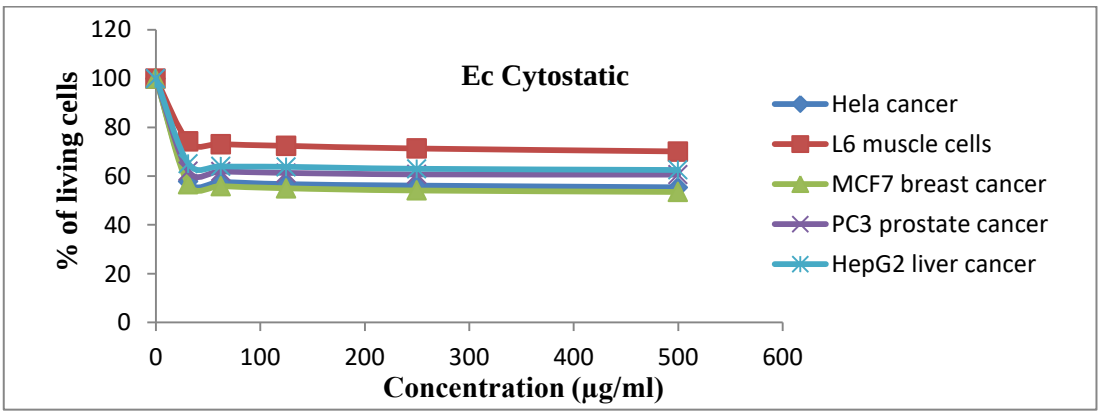


Figure 3g

In vitro effect of ester compound (Ed) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

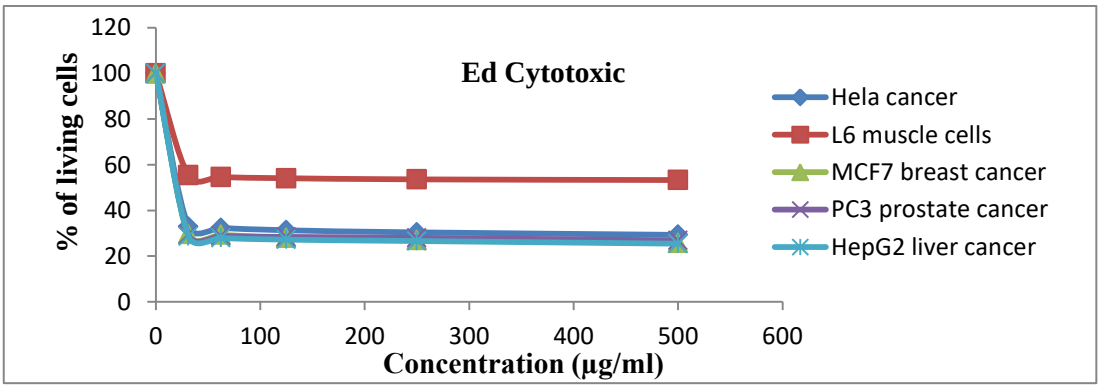


Figure 3h

In vitro effect of ester compound (Ed) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

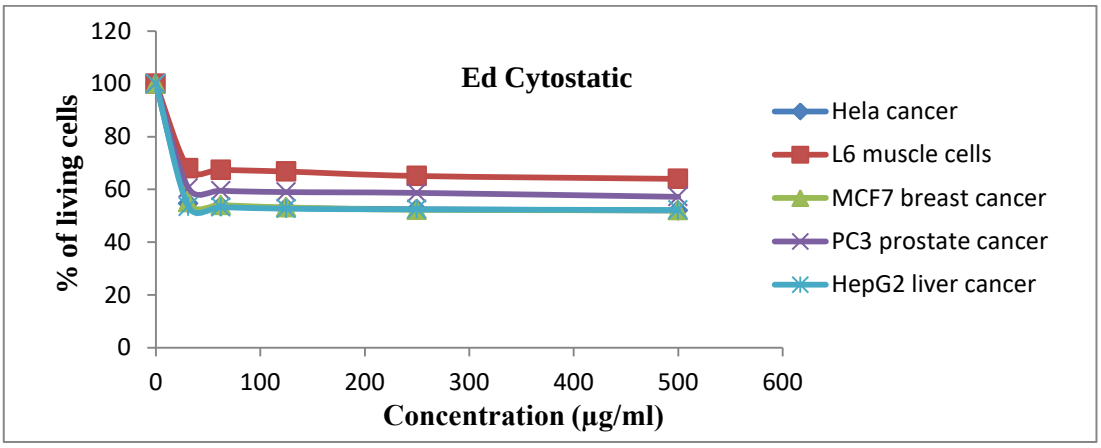


Figure 4a

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on Hela cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

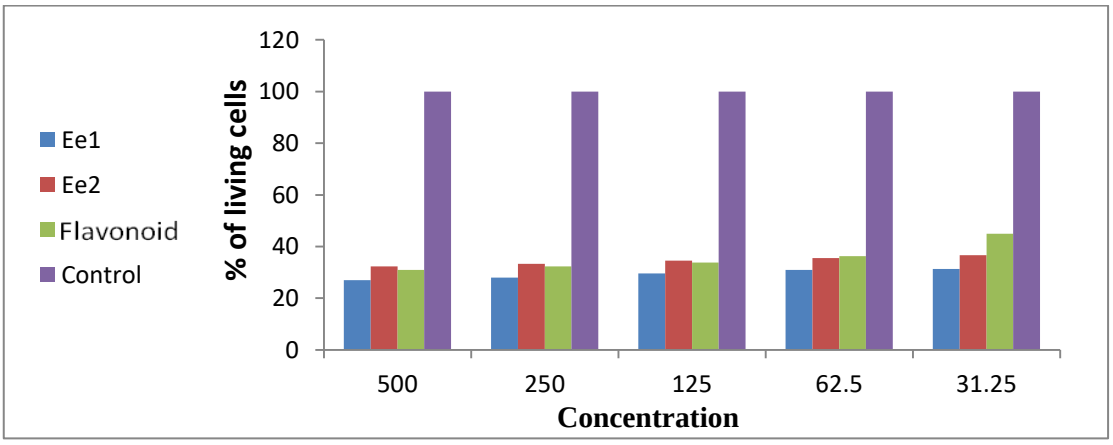


Figure 4b

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on Hela cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

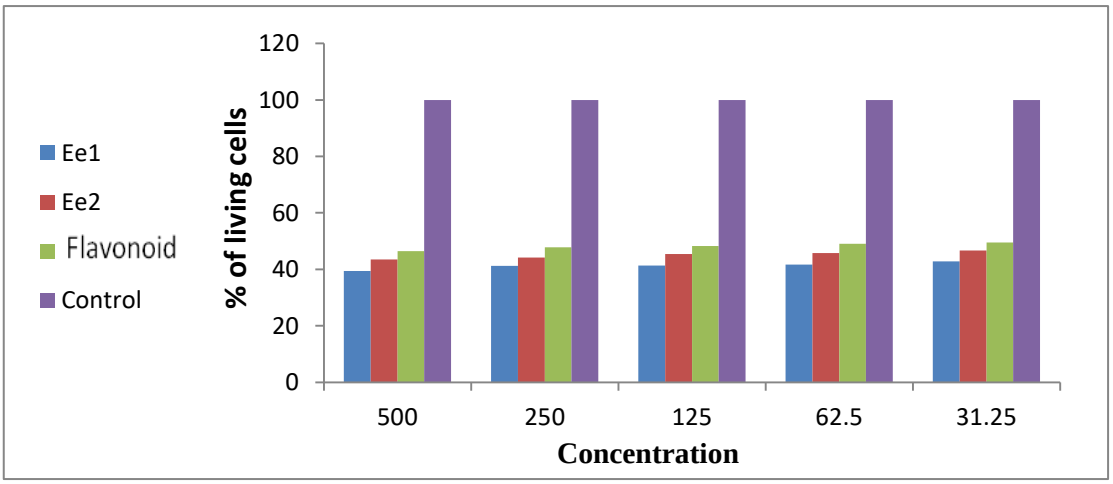


Figure 4c

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on L6 normal cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

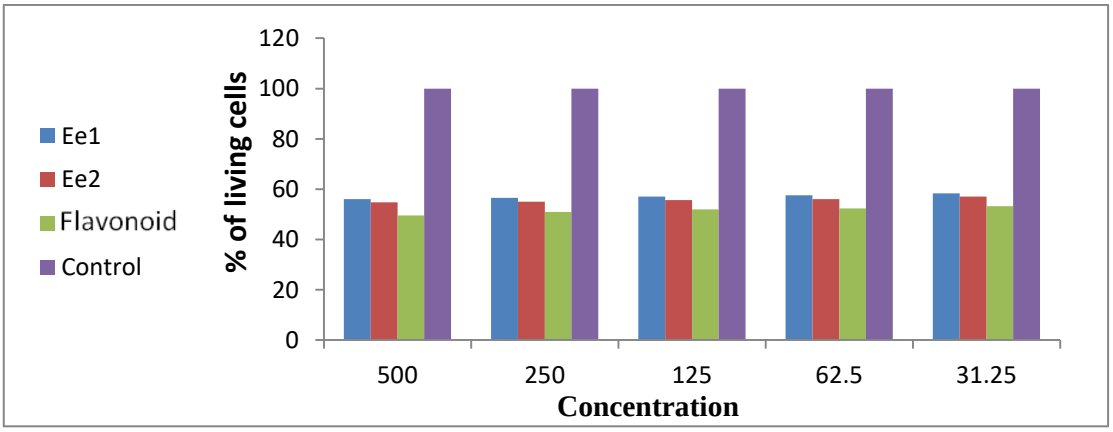


Figure 4d

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on L6 normal cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

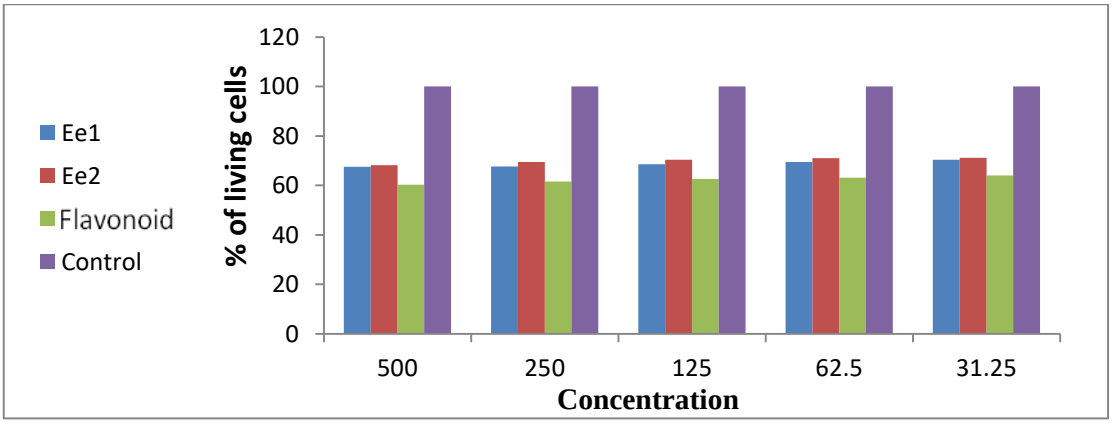


Figure 4e

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on MCF7 breast cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

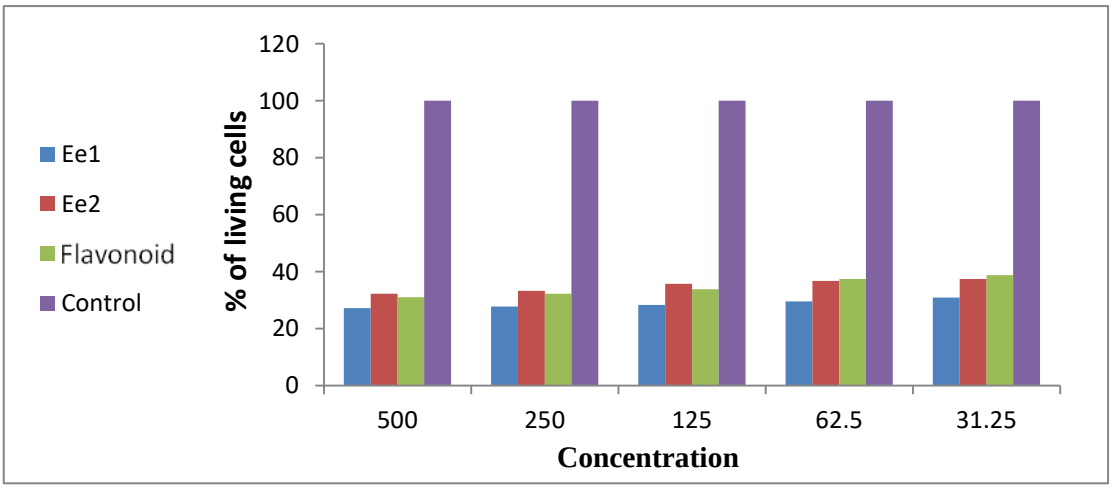


Figure 4f

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on MCF7 breast cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

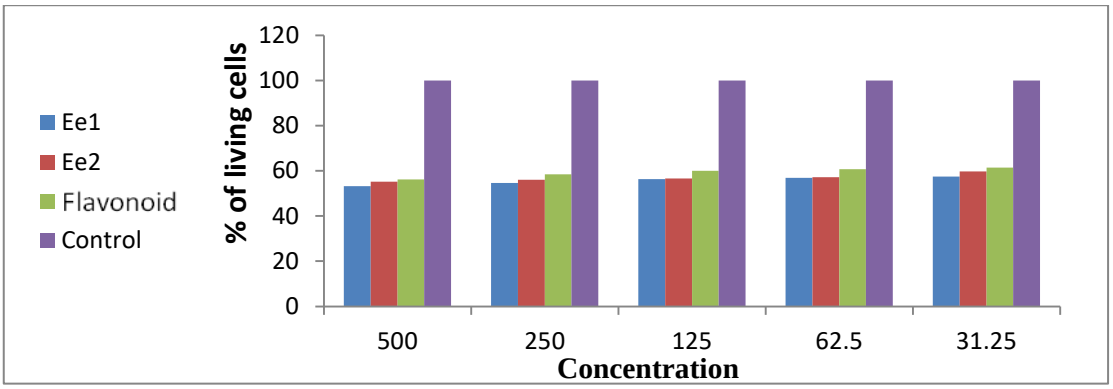


Figure 4g

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on PC3 prostate cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

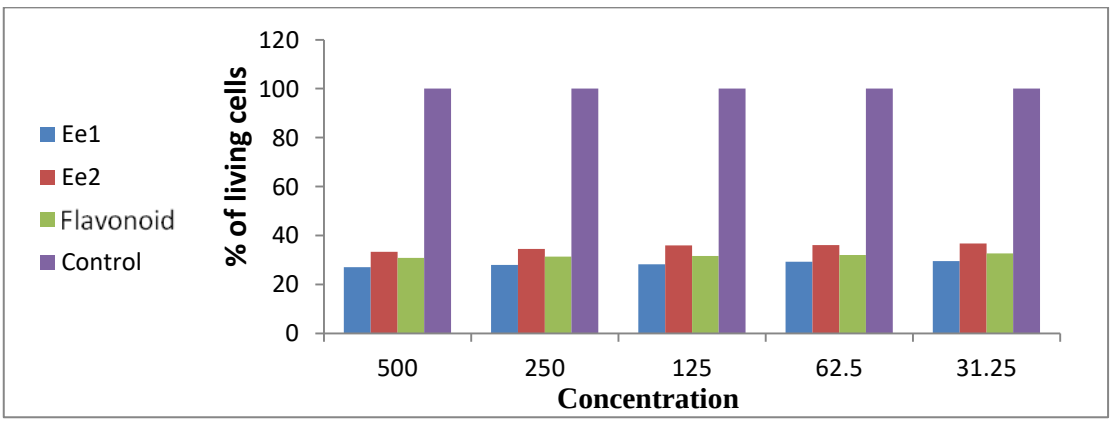


Figure 4h

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on PC3 prostate cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml

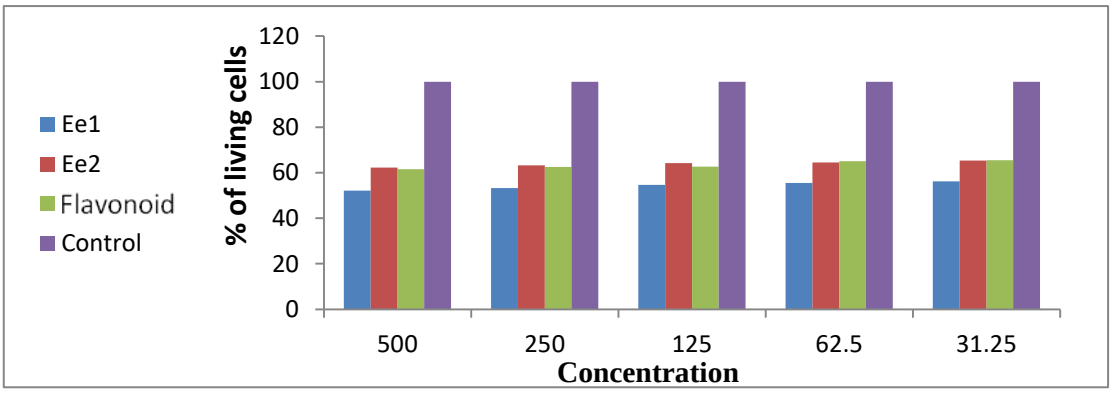


Figure 4i

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

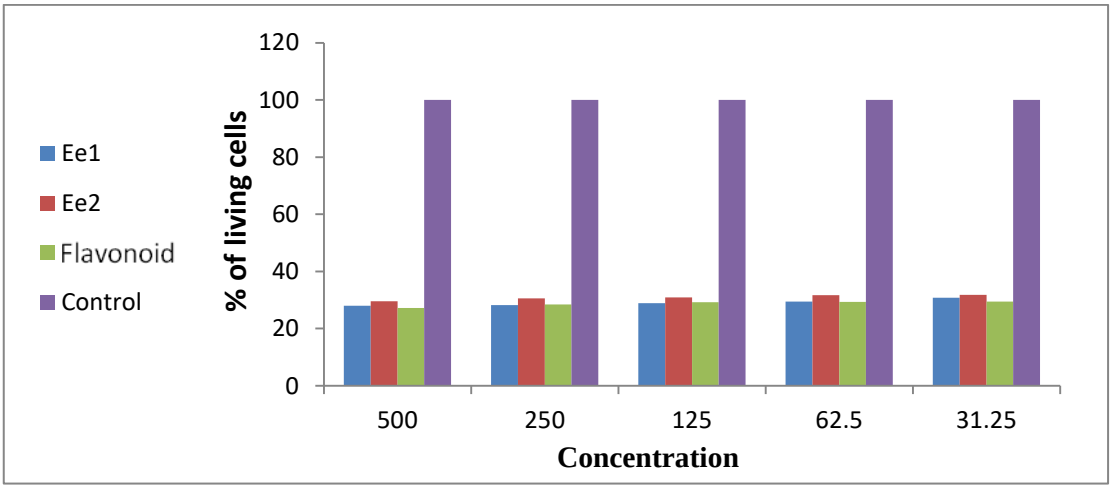


Figure 4j

In vitro effect of flavonoid and flavonoid ester compounds (Ee1 and Ee2) on HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

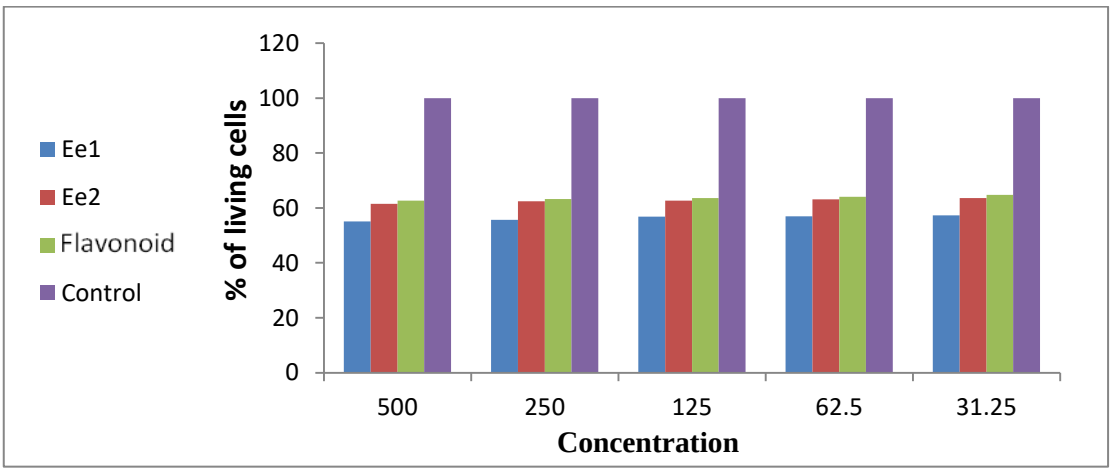


Figure 5a

In vitro effect of amide compound (A1) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

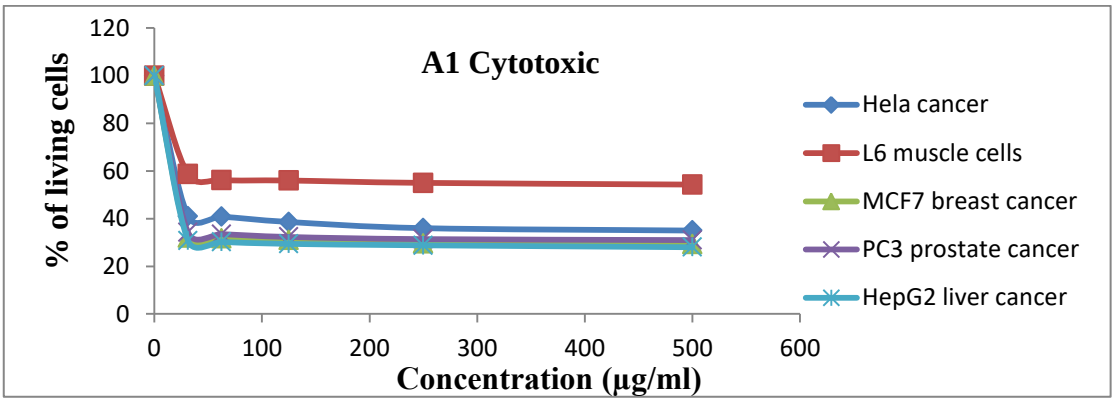


Figure 5b

In vitro effect of amide compound (A1) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

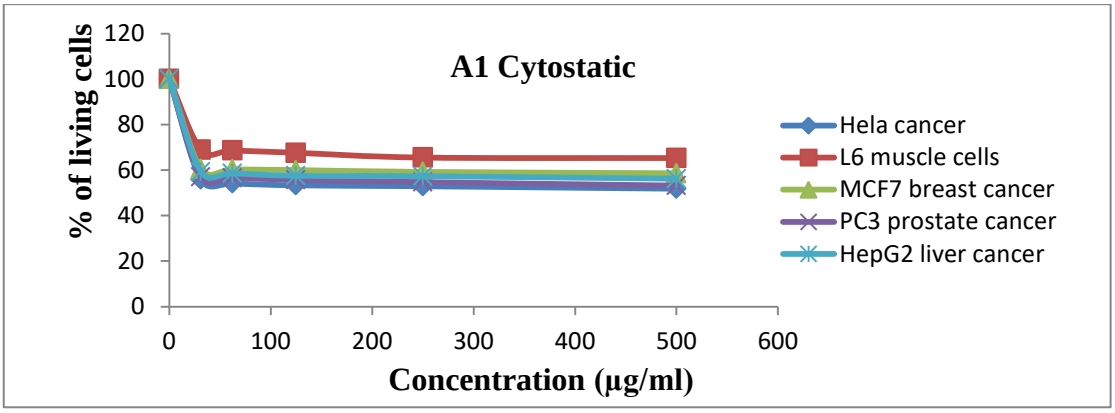


Figure 5c

In vitro effect of amide compound (A2) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

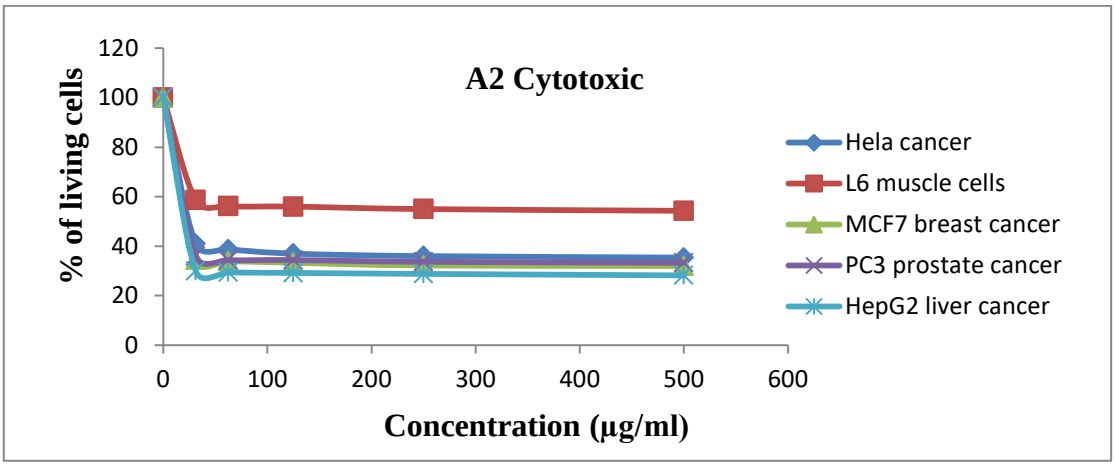


Figure 5d

In vitro effect of amide compound (A2) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

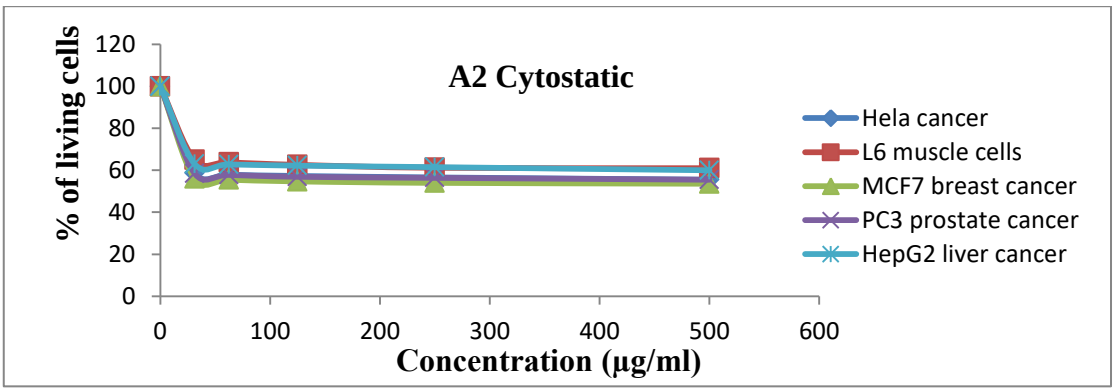


Figure 5e

In vitro effect of amide compound (Aa) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

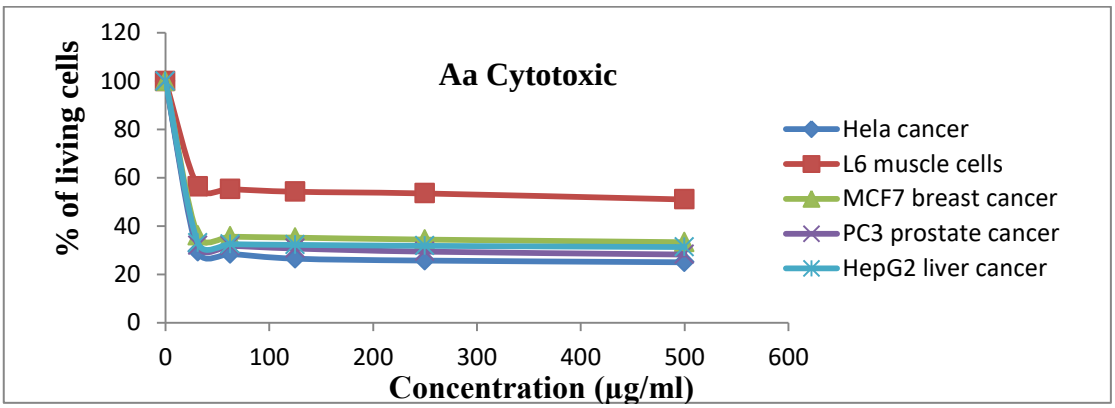


Figure 5f

In vitro effect of amide compound (Aa) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

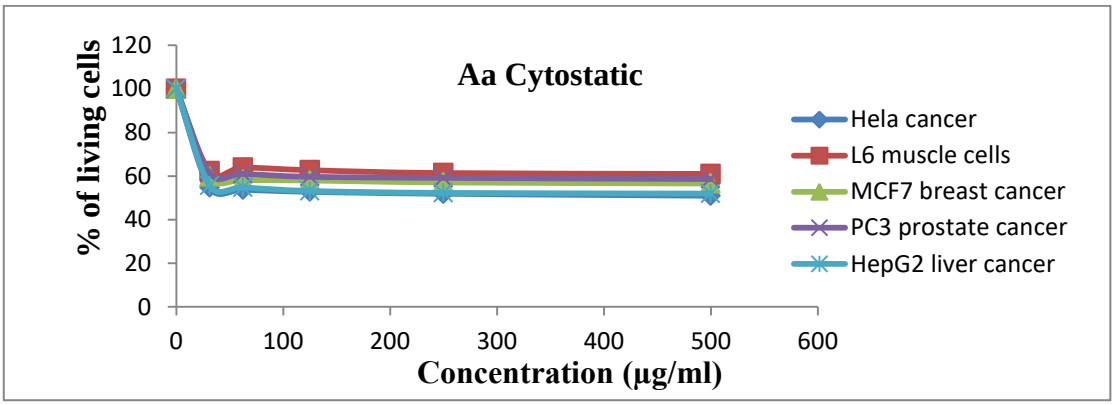


Figure 5g

In vitro effect of amide compound (Ab) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytotoxic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

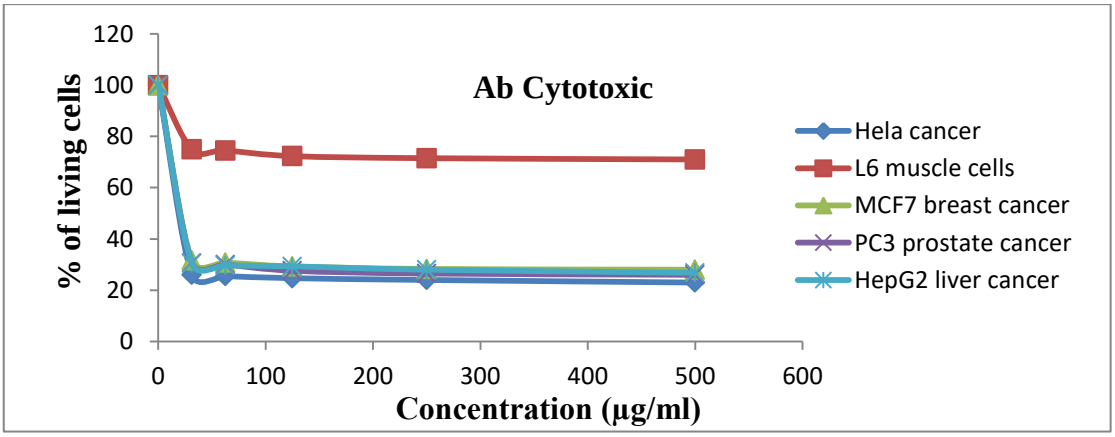


Figure 5h

In vitro effect of amide compound (Ab) on Hela cancer, L6 muscle cells, MCF7 breast cancer cells, PC3 prostate cancer cells and HepG2 liver cancer cells in cytostatic assay at various concentrations (500, 250, 125, 62.5 and 31.25µg/ml)

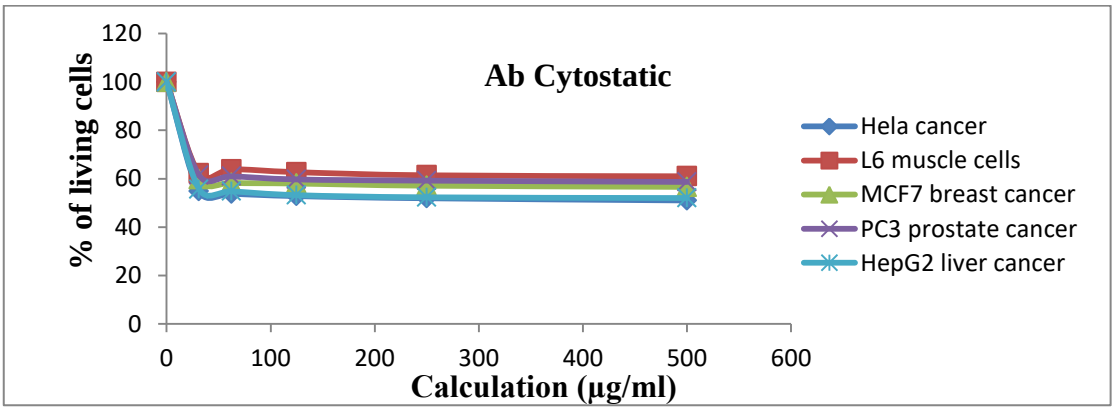


Figure 6a

Cytotoxic effects of the prepared imine compounds (3, 4, 7 and 8) on Hela cancer cells

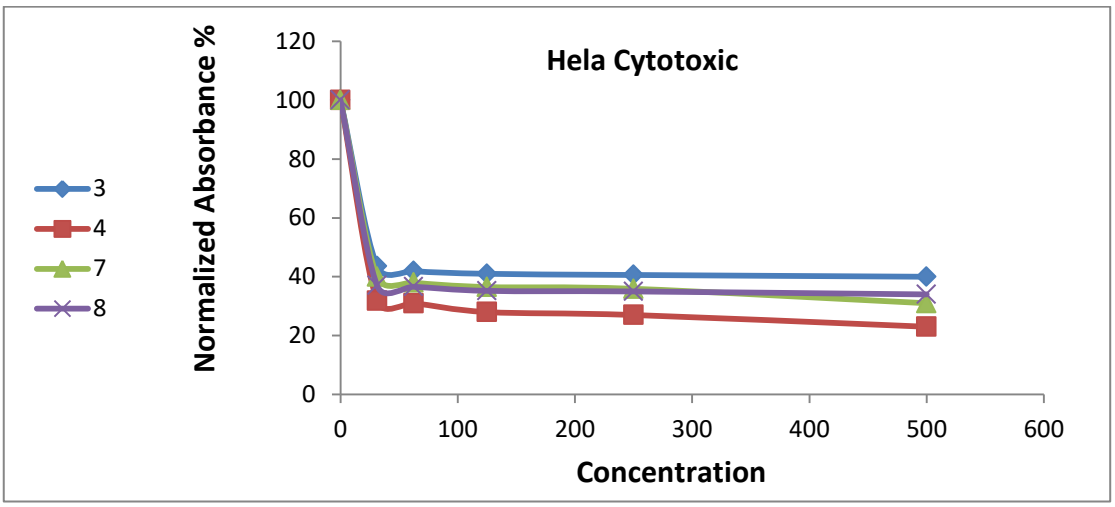


Figure 6b

Cytostatic effects of the prepared imine compounds (3, 4, 7 and 8) on Hela cancer cells

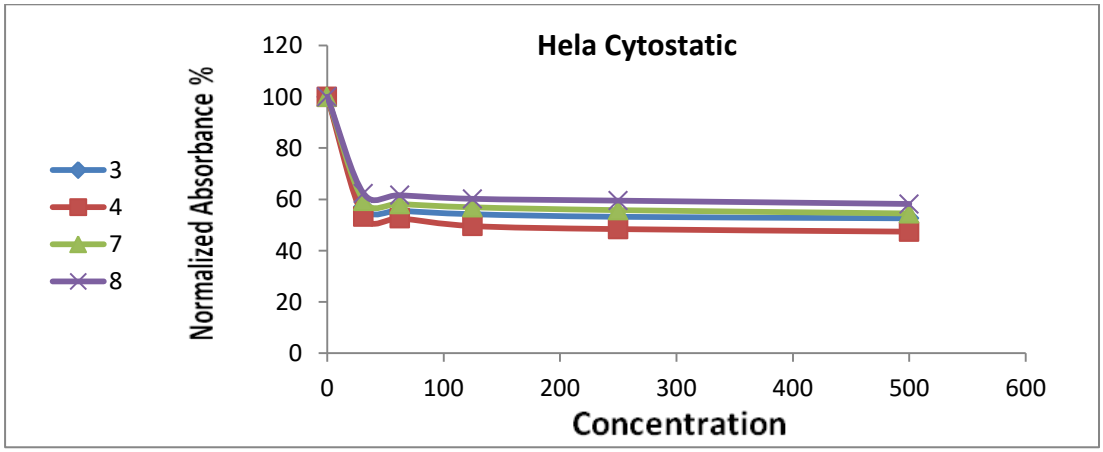


Figure 6c

Cytotoxic effects of the prepared imine compounds (3, 4, 7 and 8) on L6 normal cells

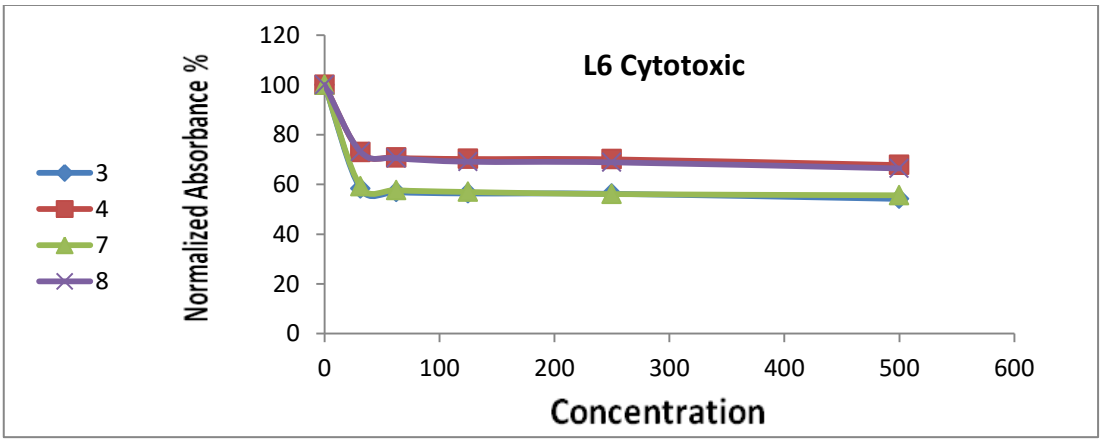


Figure 6d

Cytostatic effects of the prepared imine compounds (3, 4, 7 and 8) on L6 normal cells

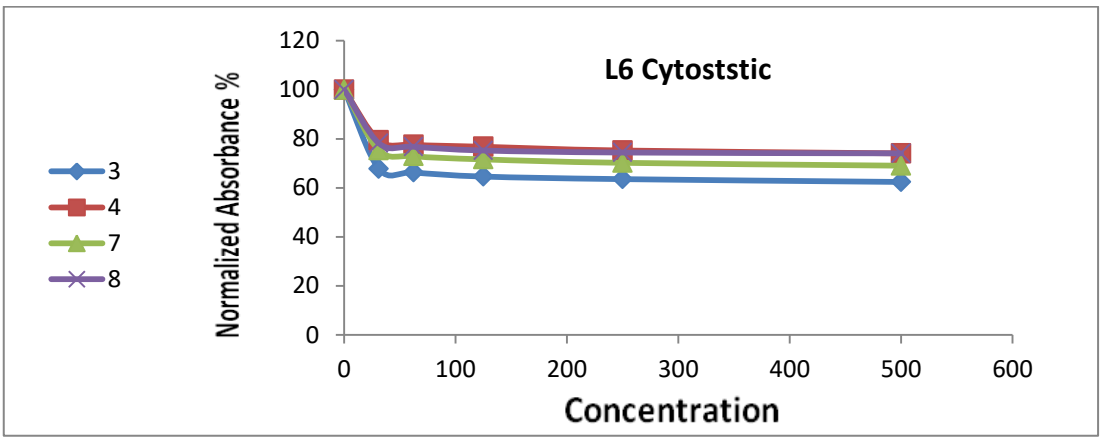


Figure 7a

Cytotoxic effects of the prepared imine compounds (1, 2, 5 and 6) on Hela cancer cells

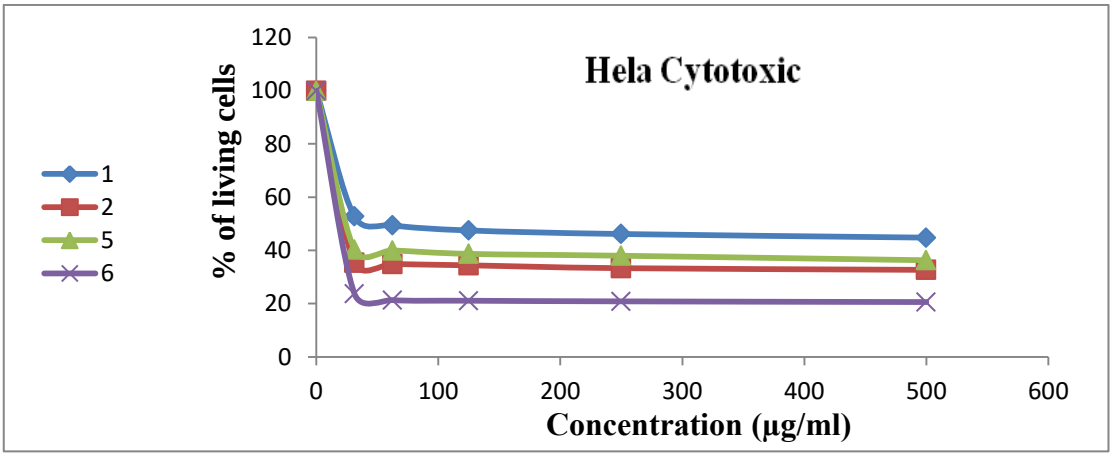


Figure 7b

Cytostatic effects of the prepared imine compounds (1, 2, 5 and 6) on Hela cancer cells

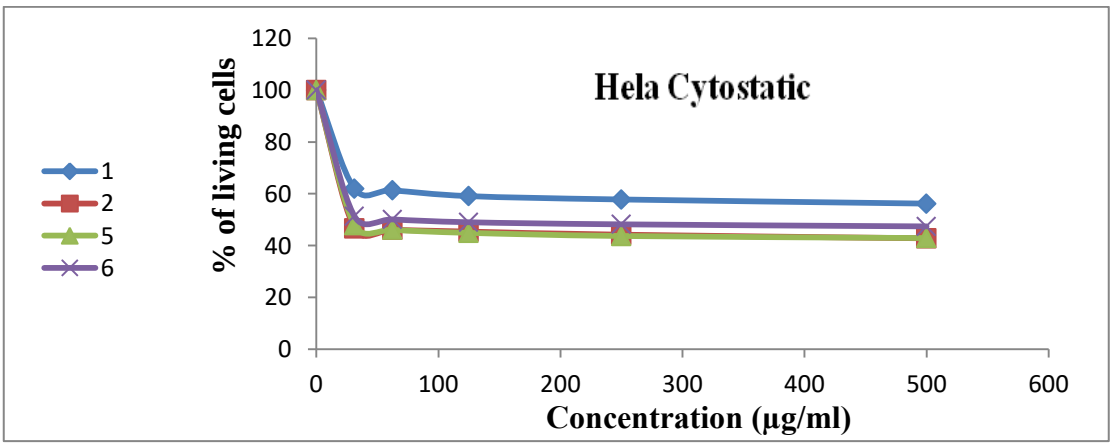


Figure 7c

Cytotoxic effects of the prepared imine compounds (1, 2, 5 and 6) on L6 normal cells

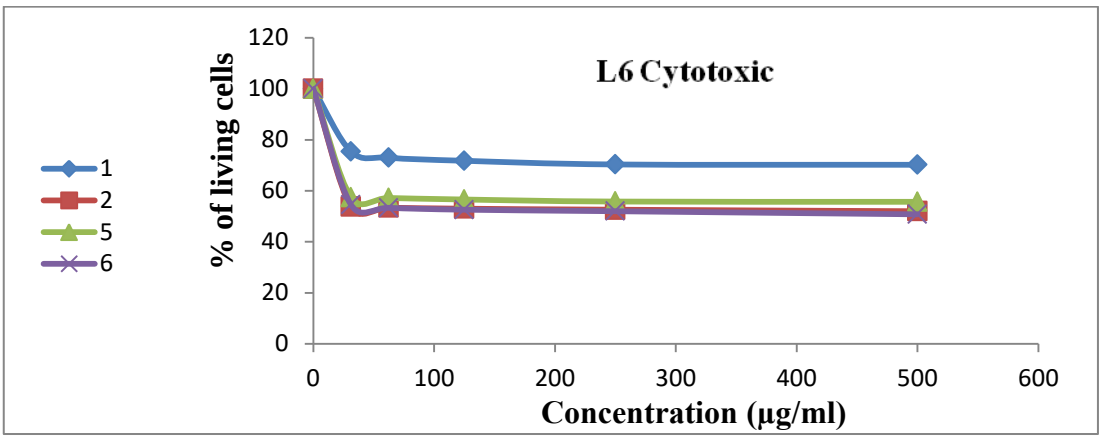
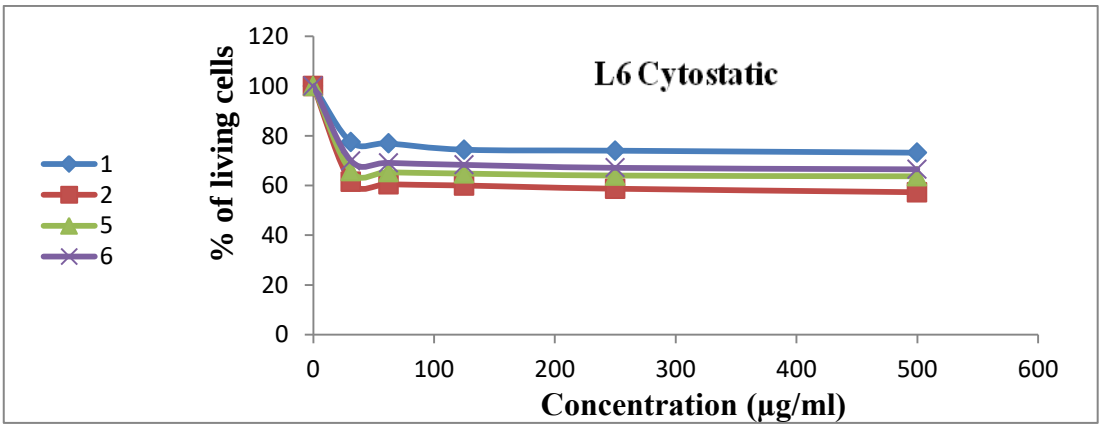


Figure 7d

Cytostatic effects of the prepared imine compounds (1, 2, 5 and 6) on L6 normal cells



Antibacterial activity

Figure 1g

Antibacterial activity of thymol and E2 derivative against E. coli, K. pneumoniae, P. aeruginosa, S. aureus and S. epidermis using disk diffusion method; (IZD) Inhibition zone diameter (mm)

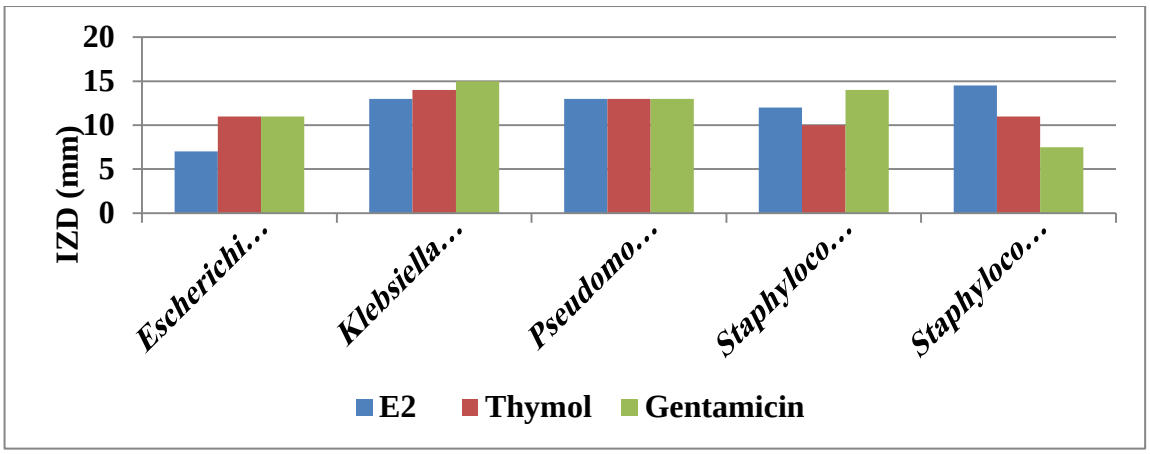


Figure 1h

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in µg/mL of thymol and E2 derivative against E. coli, K. pneumoniae, P. aeruginosa, S. aureus and S. epidermis using micro-broth dilution method

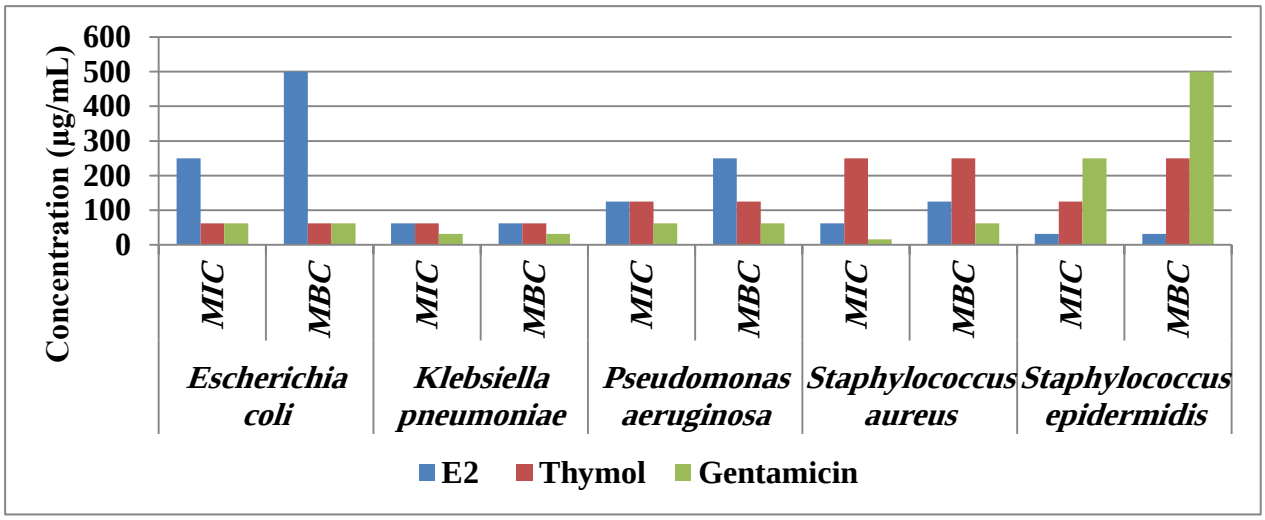


Figure 2k

Antibacterial activity of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) against E. coli, K. pneumoniae, P. aeruginosa, S. aureus and S. epidermis using disk diffusion method; (IZD) Inhibition zone diameter (mm)

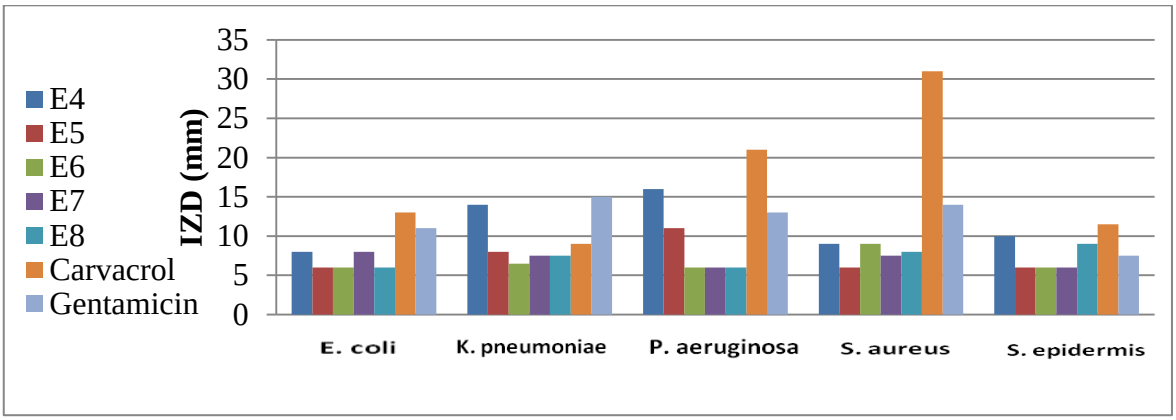


Figure 4k

Antibacterial activity of flavonoid esters Ee1 and Ee2 (0.2 mg/disk) using Kirby-Bauer disk diffusion method; (IZD) inhibition zone diameter

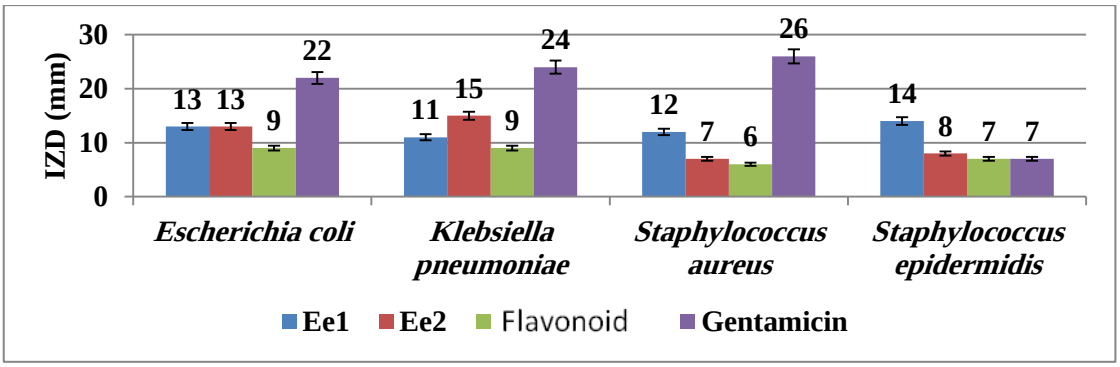


Figure 5i

Susceptibility of B. subtillus, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus and S. epidermis to A1, A2, Aa and Ab amides and Gentamicin; IZD (inhibition zone diameter)

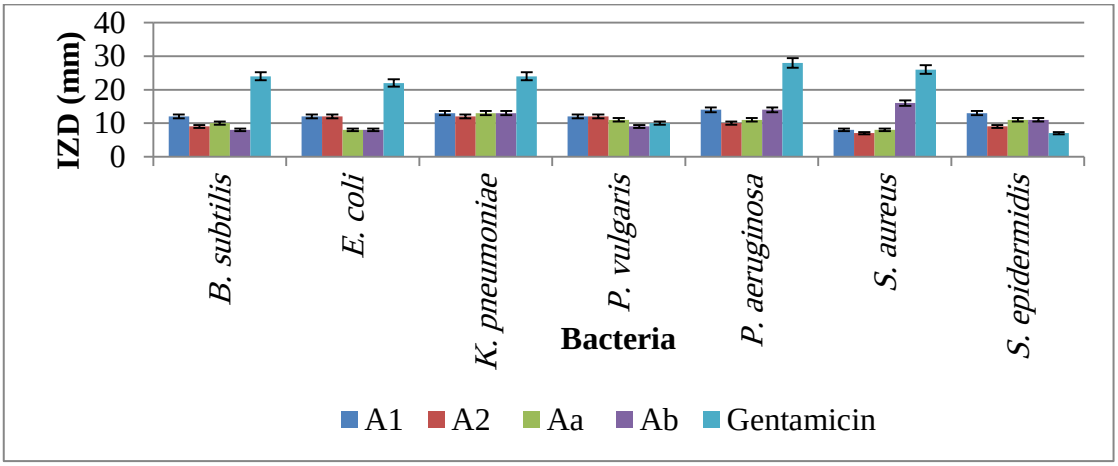


Figure 6e

Antibacterial activity of Schiff bases (3, 4, 7 and 8) against B. subtilis,E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureusand S. epidermis using disk diffusion method; (IZD) Inhibition zone diameter (mm)

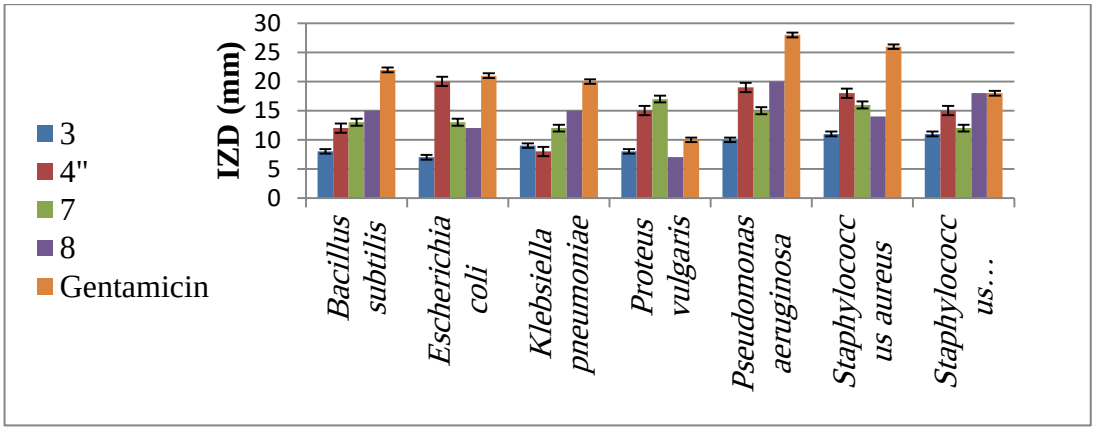


Figure 7e

Antibacterial activity of Schiff bases (1, 2, 5 and 6) against B. subtillis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus and S. epidermis using disk diffusion method; (IZD) Inhibition zone diameter (mm)

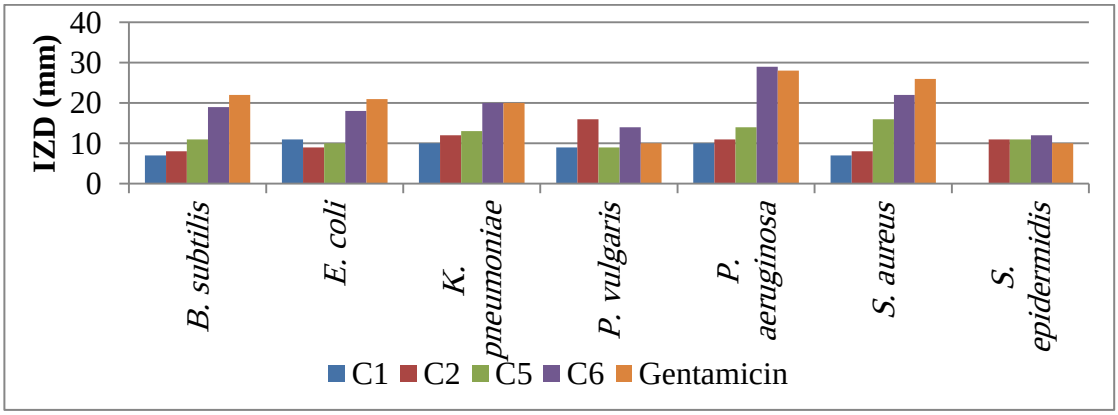
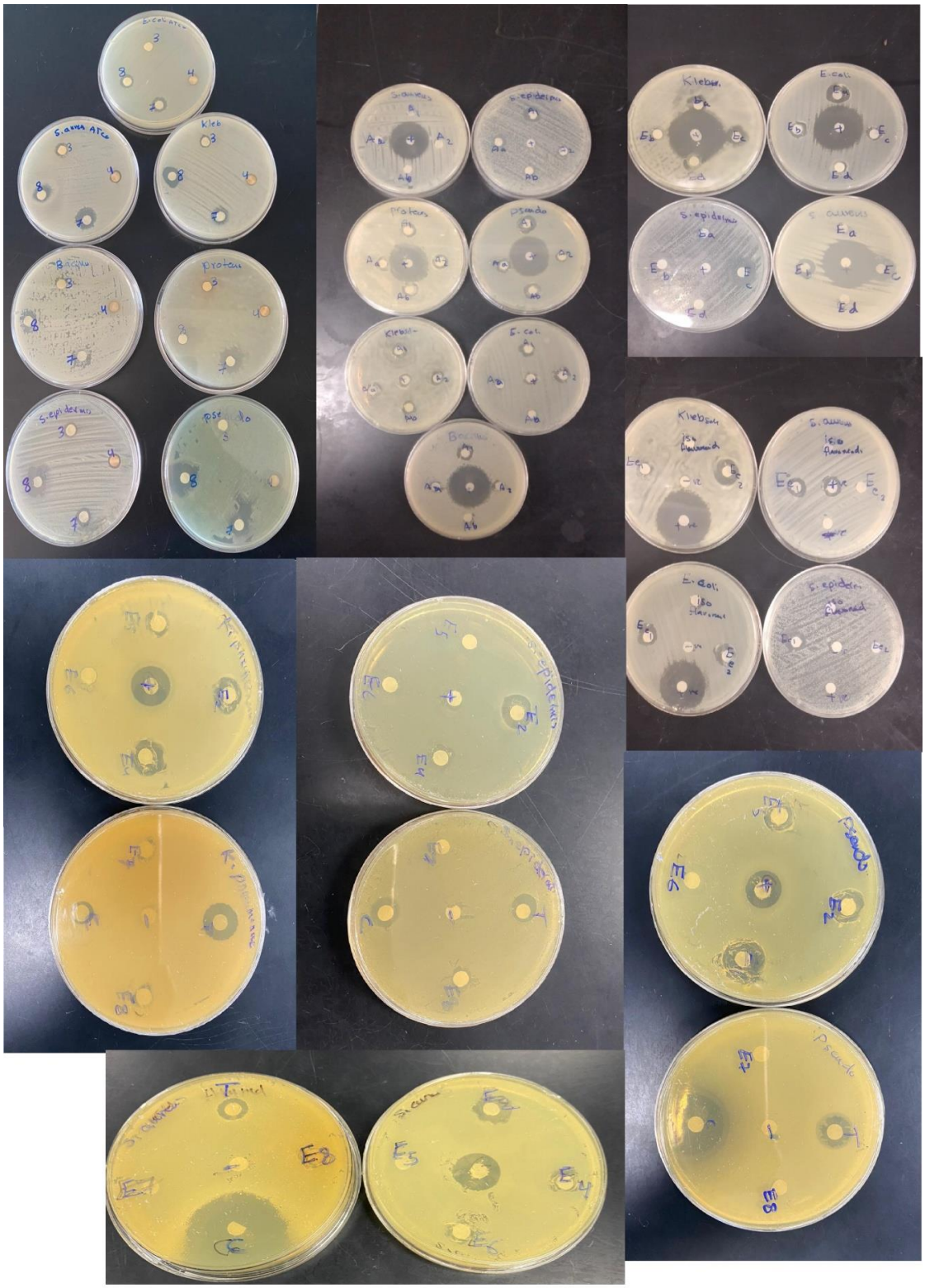


Figure 48

Anti-bacterial tests for all synthesized compounds in disk diffusion method



Antioxidant activity

Figure 1h

Percentage of DPPH inhibition activity of thymol, E2 derivative and ascorbic acid

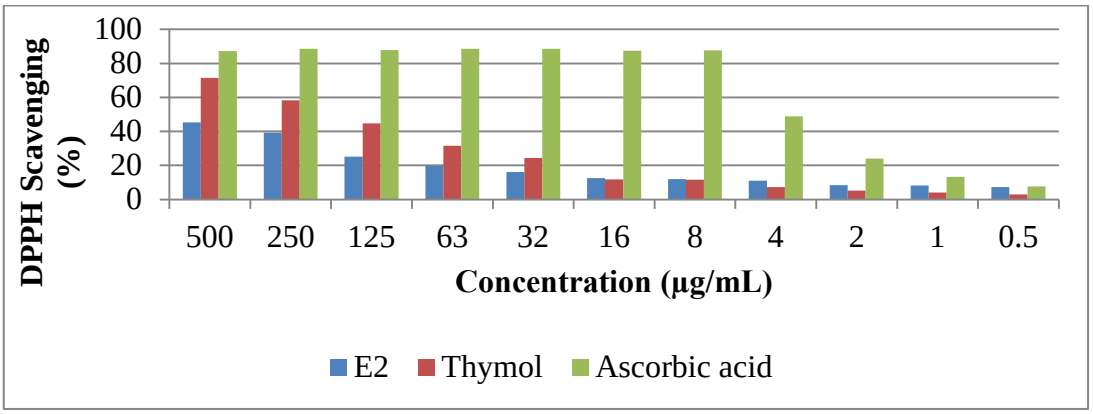
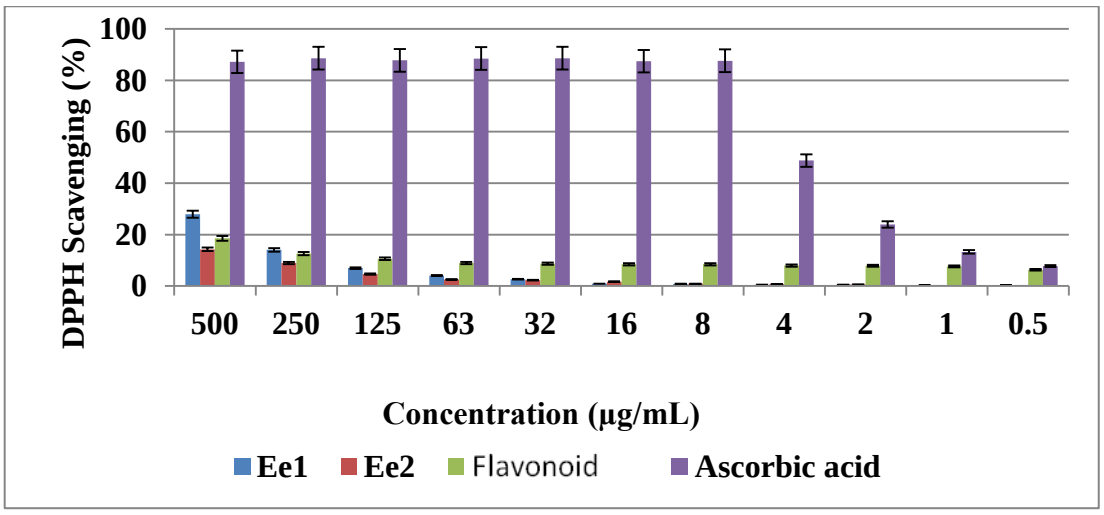


Figure 4l

Percentage of DPPH inhibition activity of flavonoid, Ee1, Ee2 derivatives and ascorbic acid



Appendix B

Tables of Study

Table B.1

*^{1}H and ^{13}C NMR Spectral Data in ppm for imine compounds (**3**, **4**, **7** and **8**)*

Compound	^{1}H NMR $\underline{CH}_{azomethine}$	^{13}C NMR $\underline{C}\text{-H}_{azomethine}$
3	8.08	153.82
4	8.02	164.89
7	8.05	161.80
8	8.30	154.78

Table B.2

Antibacterial activity of carvacrol and carvacrol ester compounds (E4, E5, E6, E7 and E8) in µg/mL against E. coli, K. pneumoniae, P. aeruginosa, S. aureus and S. epidermis using micro-broth dilution method

	E 4		E 5		E 6		E 7		E 8		Carvacrol		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	125	250	500	1000	500	500	125	250	500	1000	62.5	125	62.5	62.5
Klebsiella pneumoniae	62.5	125	250	500	500	1000	500	1000	500	1000	125	250	31.25	31.25
Pseudomonas aeruginosa	62.5	125	250	500	500	1000	500	1000	500	1000	31.25	62.5	62.5	62.5
Staphylococcus aureus	250	250	500	500	250	250	500	500	500	500	7.8125	31.25	15.625	62.5
Staphylococcus epidermidis	125	125	500	1000	500	1000	500	1000	250	500	125	250	500	1000

Table B.3

Antibacterial activity of flavonoid and flavonoid ester compounds (Ee1 and Ee2) in µg/mL against E. coli, K. pneumoniae, S. aureus and S. epidermis using micro-broth dilution method

	Ee1		Ee2		Flavonoid		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	62.5	125	31.25	62.5	62.5	125	62.5	62.5
Klebsiella pneumoniae	62.5	62.5	31.25	62.5	62.5	62.5	31.25	31.25
Staphylococcus aureus	15.625	62.5	15.625	62.5	62.5	62.5	15.625	62.5
Staphylococcus epidermidis	62.5	125	31.25	62.5	62.5	125	250	500

Table B.4

Antibacterial activity of A1, A2, Aa and Ab amides (µg/mL) against B. subtillus, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus and S. epidermis using micro-broth dilution method

	A1		A2		Aa		Ab		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. subtilis	7.8125	7.8125	62.5	500	7.8125	31.25	62.5	250	15.625	62.5
E. coli	3.90625	7.8125	31.25	125	62.5	125	62.5	125	62.5	62.5
K. pneumoniae	31.25	125	31.25	250	31.25	250	62.5	62.5	31.25	31.25
P. vulgaris	62.5	62.5	62.5	62.5	31.25	62.5	15.625	15.625	31.25	125
P. aeruginosa	62.5	125	15.625	125	62.5	250	62.5	500	62.5	62.5
S. aureus	62.5	62.5	31.25	31.25	62.5	250	62.5	250	15.625	62.5
S. epidermidis	62.5	62.5	62.5	250	15.625	62.5	62.5	250	250	500

Table B.5

MIC values of Schiff bases (3, 4, 7 and 8) against B. subtilis,E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. aureusand S. epidermis using micro-broth dilution method

	Bacillus subtilis	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
3	1.25	0.3125	0.15625	0.625	0.3125	0.15625	0.078125
4	0.3125	0.625	0.625	1.25	1.25	1.25	1.25
7	0.3125	0.078125	0.078125	0.078125	0.15625	0.078125	0.15625
8	0.625	0.15625	0.15625	0.3125	0.3125	0.078125	0.078125

Table B.6

MBC values of Schiff bases (3, 4, 7 and 8) against B. subtilis,E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. aureusand S. epidermis using micro-broth dilution method

	Bacillus subtilis	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
3	5	0.625	0.625	2.5	0.625	1.25	0.3125
4	2.5	0.625	1.25	2.5	2.5	2.5	2.5
7	1.25	0.15625	0.15625	0.3125	0.3125	0.15625	0.3125
8	5	0.3125	0.15625	0.625	0.3125	0.15625	0.625

Table B.7

Antibacterial activity of Schiff bases (1, 2, 5 and 6) against B. subtillis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus and S. epidermis using micro-broth dilution method

	1		2		5		6		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. subtilis	62.5	62.5	31.25	250	31.25	250	0.078125	0.15625	15.625	62.5
E.coli	250	250	15.625	62.5	62.5	125	0.078125	0.15625	62.5	62.5
K. pneumoniae	125	250	62.5	62.5	31.25	62.5	0.039063	0.078125	31.25	31.25
P. vulgaris	31.25	125	15.625	62.5	31.25	62.5	0.15625	0.3125	31.25	125
P.aeruginosa	125	125	15.625	125	125	250	0.019531	0.039063	62.5	62.5
S.aureus	125	125	62.5	125	31.25	125	0.039063	0.078125	15.625	62.5
S. epidermidis	125	500	62.5	125	31.25	125	0.15625	0.15625	250	500

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

الوظائف المخصصة للمشتقات الحلقية غير المتجانسة للأحماض الكاربوكسيليه والإمينات: التوليف والتوصيف والآثار البيولوجية

إعداد

إيناس إبراهيم محمود بشارات

إشراف

أ. د. محمد عبد الله النوري

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

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الملخص

خلفية الدراسة: المركبات الحلقيّة غير المتجانسة هي عناصر أساسية في مجموعة واسعة من المواد الكيميائية الطبيعية والمصنعة بسبب تراكيبها وخصائصها المختلفة. مع وجود العديد من الأدوية التي تعتمد على الهياكل الحلقيّة غير المتجانسة، تعد دراسة المركبات الحلقيّة غير المتجانسة مجالًا بارزًا في الكيمياء العضوية ولديها مجموعة واسعة من التطبيقات في مختلف المجالات .إنها توفر كإطار أساسي لمجموعة واسعة من المركبات الصيدلانية، بما في ذلك الأدوية للجهاز العصبي المركزي (CNS)، ومضادات الجراثيم، ومضادات الفيروسات، وعلاجات السرطان.

أهداف الدراسة: في هذه الدراسة تم تحضير مجموعة من مركبات الإستر ومركبات الأميد ومركبات الإيمين من المركبات الحلقيّة غير المتجانسة المختلفة وقد تم دراسة المركبات الجديدة كمضادات للبكتيريا والسرطان وبعضها تمت دراستها كمضادات للأكسدة.

منهجية الدراسة: تم تحضير جميع مركبات الإستر والأميد والإيمين الجديدة في هذه الدراسة من خلال تفاعلات التكثيف للمركبات الحلقيّة غير المتجانسة، ثم تم التعرف على المركبات الجديدة من خلال تحليلات FT-IR و NMR، ثم تمت دراسة خصائصها البيولوجية.

نتائج الدراسة: أظهرت جميع المركبات الحلقيّة غير المتجانسة المحضّرة في هذه الدراسة أنشطة بيولوجية فعّالة بنسب متفاوتة . كان Ee1 و E4 أفضل استرات نشطة بيولوجيًا في جميع الفحوصات البيولوجية. أظهر مركب الأمايد A1 نشاطًا بيولوجيًا ملحوظاً. كان لكل من الإيمين 4 والإيمين 6 نشاطات مميزة في جميع الاختبارات البيولوجية.

الإستنتاجات: قد تساهم هذه النتائج في فهم أعمق لفعاليّة المركبات الحلقيّة غير المتجانسة في الأنشطة البيولوجية المختلفة كمضادات للسرطان والبكتيريا ومضادات الأكسدة.

كلمات مفتاحيّة: المركبات الحلقيّة غير المتجانسة، مضادات الجراثيم، مضادات السرطان، مضادات الأكسدة.