An-Najah National University

Faculty of Graduate Studies

BIOBASED IMIDAZOLE DERIVATIVES WITH VERSATILE BIOACTIVITIES

By

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This Dissertation is Submitted in Partial Fulfillment of the Requirements for the Degree of PhD of Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2024

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In accordance with An-Najah National Univesity Deans Council Regulations for the award of Doctor of Philosophy, the following papers have been published after their extraction from the dissertation:

1. **New Zwitterionic Imidazolones with Enhanced Water Solubility and Bioavailability: Synthesis, Anticancer Activity, and Molecular Docking.**

Dedication

I sincerely dedicate this thesis to my parents for their unending love, support, and inspiration. I wish them both long lives and good health. I also dedicate it to my wife and my son Amro. I also want to dedicate it to my brother Islam and his wife and kids, I also dedicate it to my friends. Finally, I dedicate it to everyone who has helped, supported, and encouraged me throughout my studies.

Acknowledgment

Praise and appreciation to the Almight Allah for assisting me in completing my thesis, without his assistance, my effort would have been futile. This thesis would be difficult to complete without the assistance and support of various people. First, I want to thank my great advisor, Prof. Othman Hamed, for his wonderful supervision during this project. I appreciate his upbeat approach and words of encouragement and support, which kept me going during this study.

I'd like to thank my co-supervisor, Dr. Mohyeddin Assali, for his ongoing assistance and direction during my study. I want to express my gratitude to him for his unending assistance, as well as his useful knowledge and advice. I'd like to express my gratitude to Mr. Ameed Amireh, the chemical lab supervisor at An-Najah National University, for his collaboration and assistance during this project. A special thanks and appreciation to Mr. Ahmed Ghreeb for his time, advice, and knowledge and his great work on cancer related material in this thesis. Special thanks to Prof. Avni for the molecular docking work. Many thanks to Al-Najah National University's Chemistry Department, which was represented by the department's chairman, lecturers, professors, and laboratory technicians. Finally, thanks to all my friends and special thanks to every person who helped me.

Abu Jabal Saber

Declaration

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

BIOBASED IMIDAZOLE DERIVATIVES WITH VERSATILE BIOACTIVITIES

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: Saber Mahmoud Abu-Jabal

Signature: 

Date: 07-03-2024

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Abstract

Imidazole is known to be a highly momentous biomolecule since it is demonstrated to possess exceptional biological properties, like antimicrobial, antifungal, inhibition of nitric oxide synthase, and cytotoxic activities. Vanillin was used as the starting material in this study, it is a natural phenolic substance, and it makes up the majority of the vanilla orchid's bean and pod. It is used as a flavoring agent and as a preservative in the pharmaceutical industry and cosmetics.

This work aims to synthesize a novel natural vanillin-based imidazolone derivatives and zwitterionic vanillin-based imidazolone derivatives with alkyl sulfonate moiety starting from the natural product vanillin. Thirty new derivatives were synthesized.

Various spectroscopic methods analyzed all prepared derivatives. The anticancer properties of all derivatives were evaluated against two liver cancer cell lines (Hep-3B, LX2) and cervical cancer (HeLa). Moreover, the molecular docking between some of the prepared compounds and DNA was performed.

The study discovered that a few of these substances have high impact on preventing the growth of two types of cancer cells liver cancer (Hep-3B) and cervical cancer (HeLa), and compounds with the numbers (3, 4, 5, 7, 8, 9, 11**,** 13, 14, 17, 24, 26) shown extraordinary efficiency against these cancer cells with low viability against the normal cells. A molecular docking study showed excellent interaction of some of these derivatives with the DNA through forming strong H-bonding. Prepared compounds with the alkyl sulfonate moieties showed improvement in the anticancer efficiency.

In conclusion, a new novel set of imidazoles and synthesis of imidazolone with alkyl sulfonate moiety were successfully prepared. The anticancer activities were very promising, which makes the synthesized compounds possible future drugs for the treatment of various cancers.

Keywords: Hippuric acid; oxazolone; imidazolones; anticancer; Benzimidazole; Schiff.

Chapter One

Introduction

1.1 Heterocyclic compounds

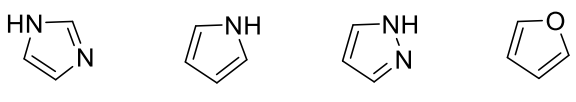
Organic molecules having a ring structure with at least one of the following elements in the ring: O, N, or S, are known as heterocyclic compounds. Rings with five or six atoms are the most common cycles, and they are more stable than rings with three, four, seven, or more atoms. There are numerous heterocyclic compounds, nevertheless, that contain fewer than five or six atoms. It is possible for heterocyclic rings to be aromatic or non-aromatic. The majority of non-aromatic heterocyclic compounds share chemical characteristics with acyclic compounds (1).

A single cycle or many cycles, isolated or condensed, may be present in heterocyclic compounds. It is also expected to find compounds with carbon aromatic cycles condensed with heterocycles. Heteroarenes are occasionally used to describe polycyclic aromatic compounds with heterocycles (PHAs).

The heterocyclic compound class is the largest and most varied family of organic compounds. In addition to being naturally occurring, heterocyclic compounds have been synthesized in a large variety. A heterocyclic ring system is found in many compounds, including various synthetic medications and dyes, alkaloids, antibiotics, amino acids, nucleic acids, hemoglobin, hormones, and perfumes. Simple O & N N-containing compounds are very important among the heterocyclic compounds. Imidazole 1, Pyrrole 2, Pyrazole 3, and Furan 4 instances of basic heterocyclic systems are displayed in Figure 1.1

Figure 1.1

Simple heterocyclic system examples



1 **2** **3** **4**

1.2 Biological importance of heterocyclic compounds

Most medicines fall within the category of heterogeneous substances. Heterocyclic compounds, many of which are five- and six-membered molecules with one to three heteroatoms in the nucleus, are essential to metabolizing all living cells. The molecules might be the pyrimidine and purine bases of DNA, and these heterocyclic molecules could be single molecules or combined heterocyclic systems. Some common heterocyclic compounds used in pharmaceuticals are the amino acids proline, histidine, and tryptophan; they also include the vitamin and coenzyme precursors thiamine, riboflavin, pyridoxine, folic acid, biotin, B12, and E families of the vitamins. Several heterocyclic compounds are pharmacologically active, and many of them are often used in therapeutic settings (2).

An analysis of the available literature reveals that certain condensed ring heterocyclic compounds perform a variety of physiological roles. N-Benzylidene derivatives and condensed triazolo- pyrimidines demonstrate antifungal (3), anti-inflammatory (4), antibacterial (5) , anticonvulsant (6), antiallergic (7), and anticancer activities (8).

Numerous active medicines and natural items containing standard cores are heteroatoms and heterocyclic scaffolds. Statistics show that more than 85% of all compounds having physiological activity are heterocycles or contain one, with nitrogen heterocycles being the mainly prevalent backbone in their intricate structures. These data highlight heterocycles' crucial role in contemporary drug discovery and design (9).

Heterocycles can be categorized as nitrogen, oxygen, or sulfur-based on the heteroatoms in their ring structures. Compounds are categorized within each class based on how the number of atoms affects the ring structure's size (10).

1.3 Nitrogen-based heterocycles

For synthetic organic chemists, nitrogen heterocyclic molecules have long been desired targets because they demonstrate a variety of biological functions. Many of them are found in natural products, especially alkaloids, which is why the synthetic community is very interested in them, especially individuals engaged in fully synthesizing of natural products (11). The first thorough examination of the heterocycles based on nitrogen was published by Njardarson *et al*. in 2014 (12). This research demonstrated that, an N-based heterocycle serves as the common architectural core in around 60% of small-

molecule medicines. Regardless of the types of heterocycles used, Baumann *et al*. (2011) gave a summary of the primary synthesis pathways for the most widely used five-membered ring heterocyclic medicines (13). The same authors then summarized the synthetic routes to the top-selling medications, including six-membered heterocyclic systems in 2013.

Among the heterocyclic nitrogen containing compounds imidazole receives a lot on interest form scientists due to versatile bioactivities.

Among the most significant heterocyclic substances that have unlimited numbers of applications is imidazole.

1.4 Imidazole

An organic substance having the chemical formula $C_3N_2H_4$. It is a colorless or white solute that makes water slightly alkaline. With nitrogen atoms in meta-substitution that are not adjacent, it is an aromatic heterocycle. and is classified as a diazole in chemistry.

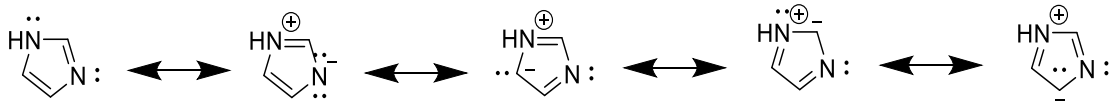
The imidazole **1** ring is found in many natural compounds, particularly alkaloids. These imidazoles have different substituents but the same 1,3-C_3N_2 ring. This ring structure is present in significant biological building blocks such as histidine and the related hormone histamine. Numerous medications, including some antifungal medications, the nitro imidazole family of antibiotics, and the sedative midazolam, include an imidazole ring (14).

1.4.1 Structures and properties

Due to the fact that hydrogen can be linked to either one or both of the nitrogen atoms, imidazole 1 is a five-member planar ring with two equivalent tautomeric forms. With an electric dipole moment of 3.67 D and a high solubility in water, imidazole is a strongly polar molecule. The chemical has a planar ring with six-electrons, which is what distinguishes it as an aromatic compound (two electrons from the nitrogen atom that has been protonated and one from each of the other four atoms in the ring) (15). Below are several imidazole resonance and structures of imidazole as shown in Figure 1.2.

Figure 1.2

Imidazole resonance structures



1.4.2 Physical properties

It has a greater boiling point than any other 5-membered heterocyclic complex, 256°C, because of intermolecular H-bonding, where molecules are connected linearly.

In dioxane, imidazoles have a significant dipole moment of 4.8 D. It is more amphoteric than pyrazole and pyridine and has a pKa of 7.2. The resonance value of imidazoles, an aromatic molecule, is 14.2 K cal/mol, or approximately half that of pyrazole. In imidazoles, nucleophilic substitution occurs when an electron withdrawing group is present in the nucleus, while electrophilic substitution occurs often in imidazoles. Imidazoles have a melting point of 90°C, are weak bases, and are tautomeric compounds since positions 4 and 5 are comparable (16).

1.4.3 Synthesis of imidazoles

There are many ways to synthesis imidazole. By changing the functional groups on the parent molecule. Numerous of these synthesises can also be applied to other derivatives and substituted imidazoles. There are several methods for creating imidazoles, including the Radiszewski and Debus synthesis methods, dehydrogenation of imidazolines, Wallach method, from alpha halo ketones, and Marckwald method (17). Below are descriptions of a few of the imidazole synthetic processes**.**

1. Debus method

Glyoxal 5 and formaldehyde 6 were used to synthesize imidazole in ammonia, as shown in Scheme 1.1. Despite having very low yields, this procedure is nevertheless utilized to make C-substituted imidazoles 7 (18).

2. Radiszewski method (19)

In the presence of ammonia, benzyl 8 and Benzaldehyde 9 condensate to produce 2,4,5-triphenylimidazole 10, according to Radiszewski as shown in Scheme 1.1.

3. Dehydration of imidazoline

Barium manganate has been described as a gentler reagent to convert imidazolines to imidazoles when sulphur is present by Knapp and colleagues. When 1,2-ethanediamine 11 and alkyl nitriles like 12 are combined, the resulting imidazolines produce 2-substituted imidazoles 13 as shown in Scheme 1.1(20).

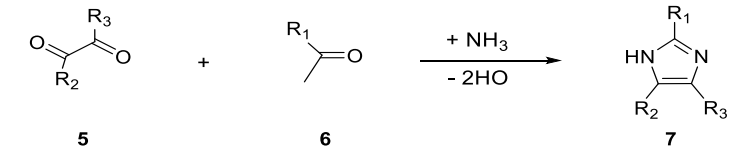
4. Wallach method

Wallach used the phosphorus pentachloride to treat N,N- dimethyloxamide 14 to produce a chemical 15 that contained chlorine, which when reduced with hydroiodic acid, produced N- methyl imidazole 16 as shown in Scheme 1.1, N,N-diethyloxamide was transformed into a chlorine compound under the same conditions, and when that molecule was reduced, it produced 1-ethyl-2-methyl imidazole (21).

Scheme 1.1

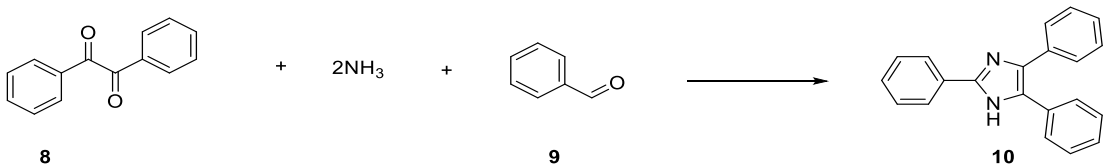
Synthesis of imidazole: A-Debus method, B- Radiszewski method, C- Dehydration of imidazoline, D-Wallach method

A- Debus method



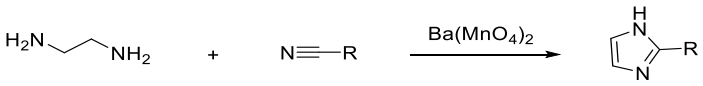
5 6 7

B- Radiszewski method



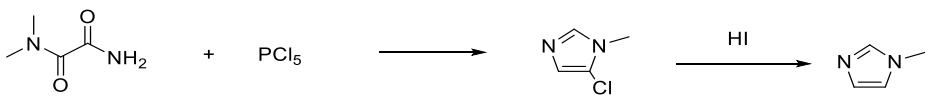
8 9 10

C- Dehydration of imidazoline



11 12 13

D- Wallach method



14 15 16

5. Starting with α-halo ketone (20)

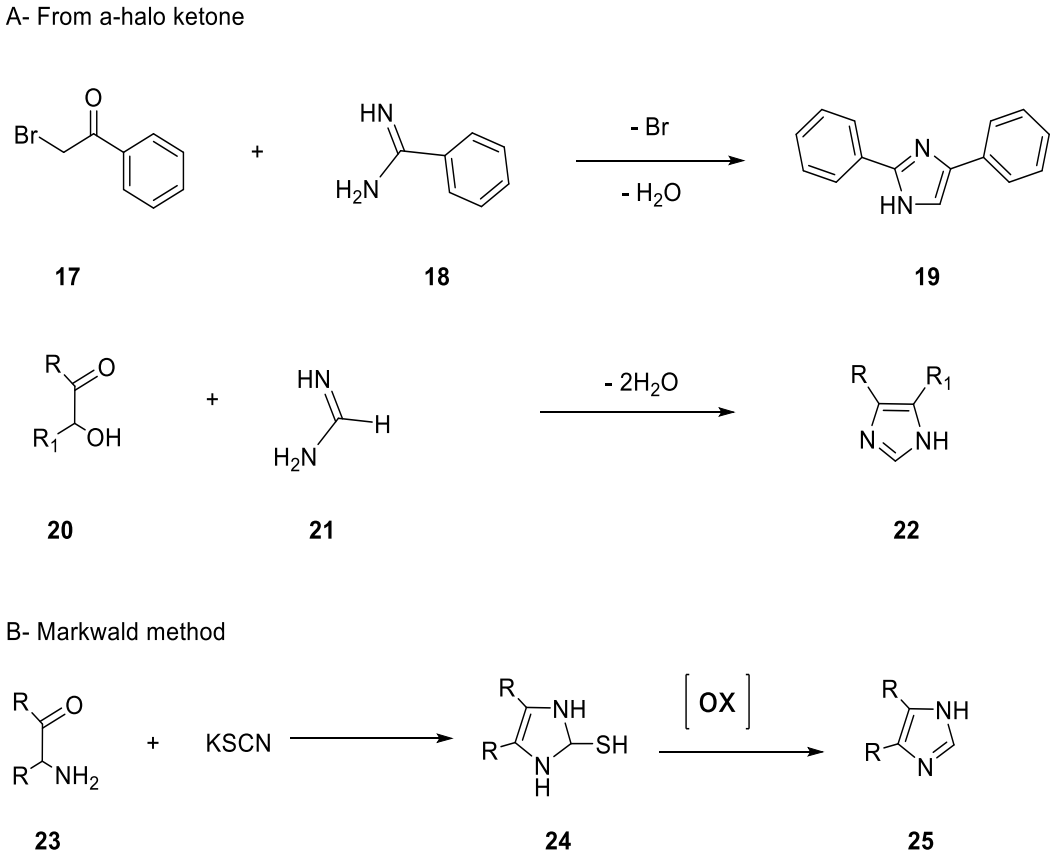
This approach is based on the interaction of imidine 18 with alpha halo ketones 17. The 2,4- or 2,5-biphenyl imidazole 19 has been effectively synthesized using this approach. A similar reaction occurs when acyloin 20 is combined with amidine 21 to produce imidazoles 22 as shown in Scheme 1.2.

6. Markwald method (20)

To synthesize 2-thiol substituted imidazoles 24, 2-mercaptoimidazoles are made from amino ketones 23 or aldehydes and KSCN. To produce the required imidazoles 25, the sulfur may easily be removed using a number of reactive methods as demonstrated in Scheme 1.2.

Scheme 1.2

Formation of imidazole using α-halo ketone or Markwald methods



7. α-Acylaminoketones cyclization

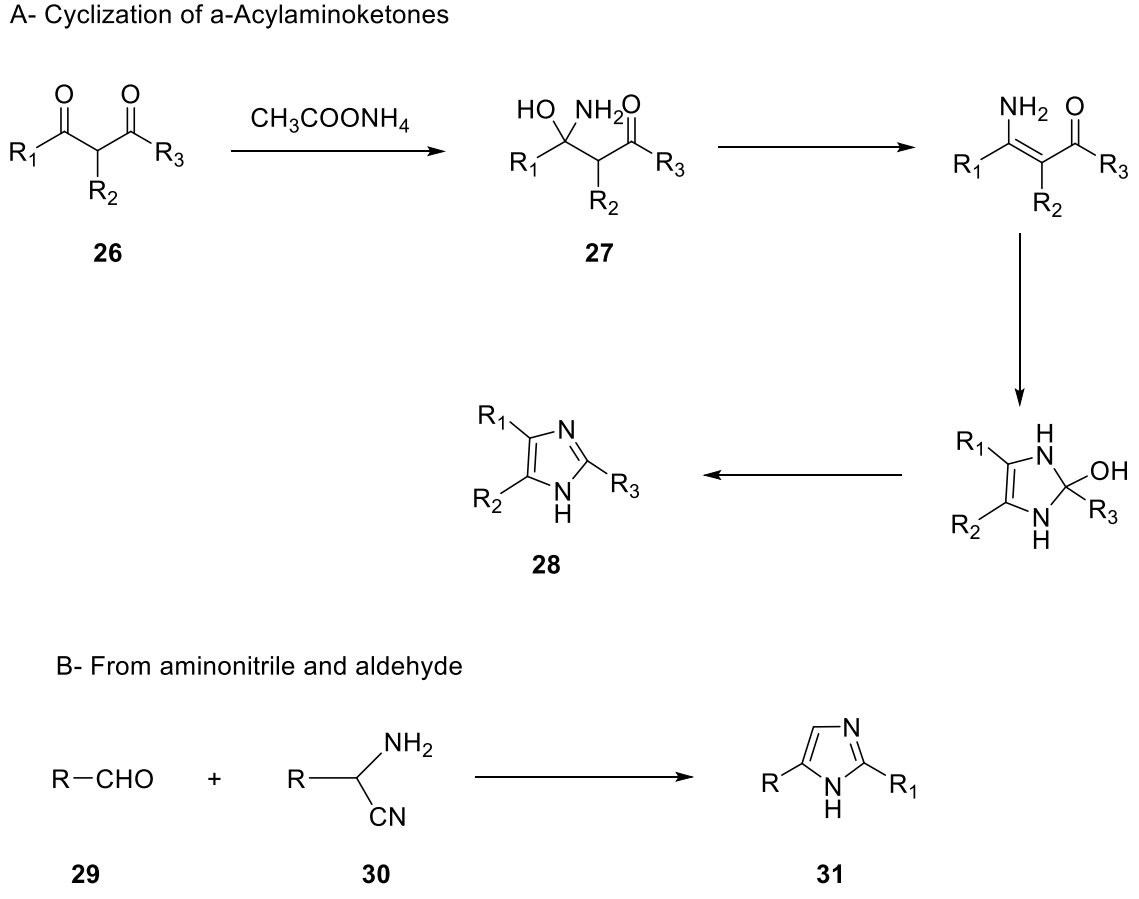
The 1,4-diketo molecule 27, acylaminoketones 26, likewise exhibit this behavior. This substance caused a quick cyclization 28, followed by the existence of ammonium acetate in the presence of anhydride as shown in Scheme 1.3 (22).

8. From aminonitrile and aldehyde (23)

Aldehyde 29 and aminonitrile 30 mixture condensed into substituted imidazole 31 under the ideal reaction circumstances, as demonstrated below in Scheme 1.3.

Scheme 1.3

Formation of imidazole from A- Cyclization of α-Acylaminoketones, B- Aminonitrile and Aldehyde

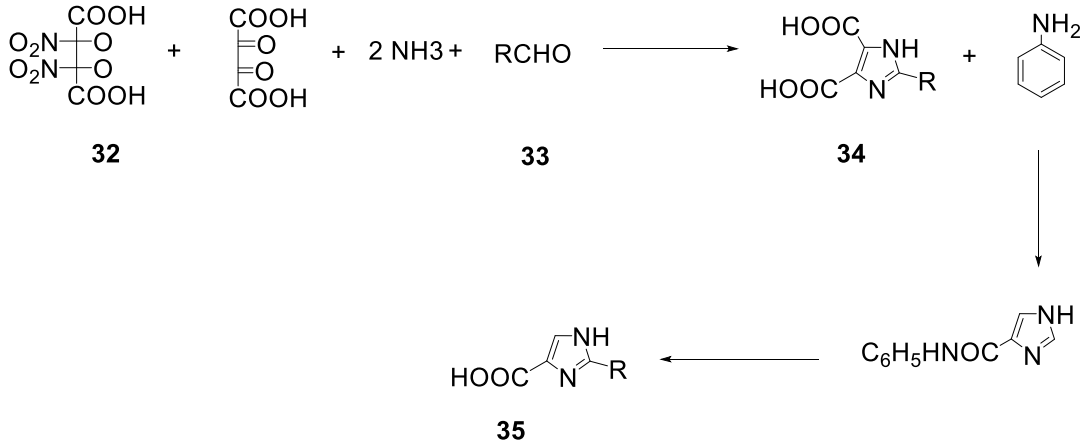


9. Starting with formaldehyde and tartaric acid dinitrate (22).

The most practical method for producing imidazole is to heat the resulting dicarboxylic acid with quinoline in the presence of copper to create compound 34 after reacting ammonia with a mixture of formaldehyde 33 and tartaric acid dinitrate 32., finally compound 34 combined with aniline to produce substituted benzamide 35 as demonstrated in Scheme 1.4

Scheme 1.4

Formation of imidazole from formaldehyde and tartaric acid



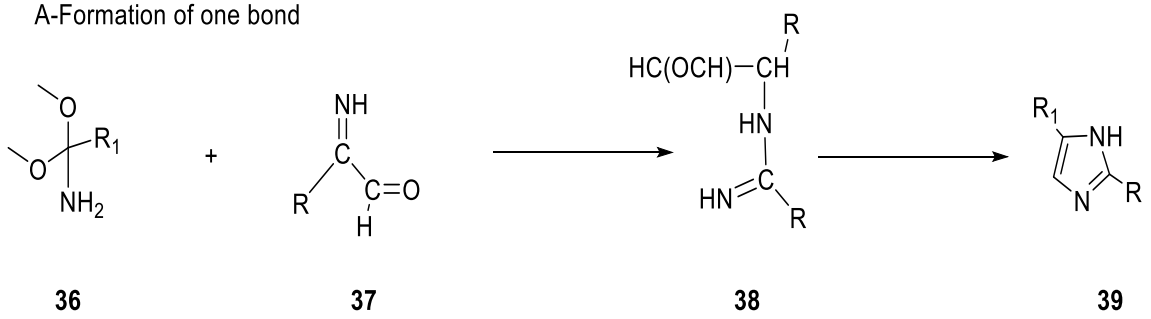
10. By the formation of one bond

Aminoacetal 36 and compound 37 which is called imidate were combined to give an imidine 38, which converted to an imidazole-like 39 through cyclization reaction. Imidazole synthesis by this methods is represented by the example shown below in Scheme 1.5 (22).

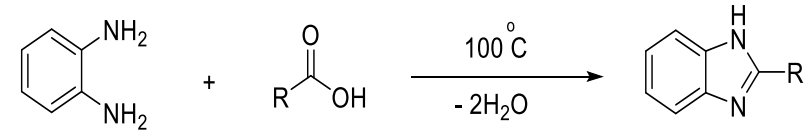
11. When heated in an acidic solution, o-phenylenediamine 40 condenses with a carboxylic acid like 41 to produce benzimidazole 42 which is more significant than imidazole because the former occurs in vitamin B12 as demonstrated in Scheme 1.5 (24).

Scheme 1.5

Formation of imidazole by A- the formation of one bond, B-condensation reaction



36 **37** **38** **39**



40 **41** **42**

1.4.4 Imidazole derivative synthesis using microwave reactions

Because microwave assisted reactions have demonstrated noteworthy advantages over conventional techniques such as ease of setup, higher yield, and environmental friendliness. Many efforts have been directed in this direction. Some examples are provided below:

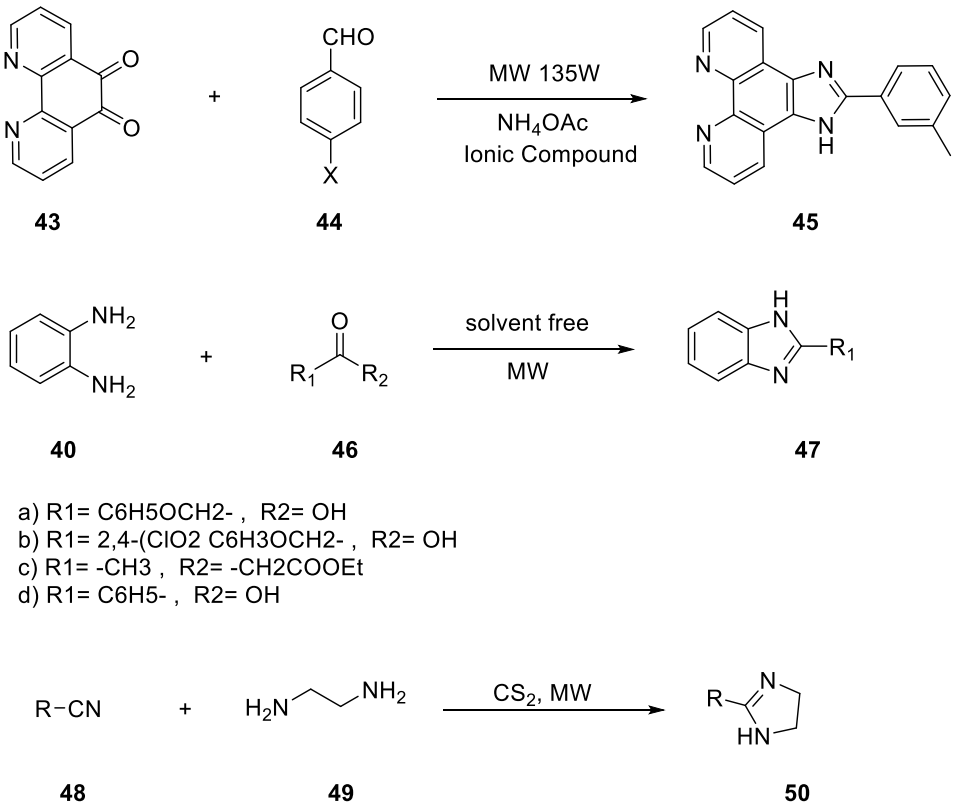
Dicarbonyl compound 43 and *p*-substituted benzaldehyde 44 were combined to create 2-phenylimidazol phenanthroline derivatives as shown in 45 Scheme 1.6. Without solvent and microwave-assisted conditions, to produce a good yield when there is a neutral ionic liquid called 1-methyl-3-heptylimidazolium tetrafluoroborate [(HeMIM) BF4]. This specific reaction has all the advantages of microwave reactions, including simple setup, increased yield, and environmentally benign reaction (19).

It was found that benzimidazoles and trisubstituted imidazoles 47 may be synthesized quickly and efficiently with microwave assistance as shown in Scheme 1.6. Without the use of a catalyst, 1,2-phenylenediamine 40, carboxylic acids, and acetoacetic ester 46 were condensed to produce three benzimidazoles (25).

Alkyl cyanide 48 and ethylenediamine 49 were reported to react with each other when carbon is present disulfide to produce 2-substituted 2-imidazolines 50 when exposed to microwave radiation as shown in Scheme 1.6. This procedure results in much higher product yields and shorter reaction times (26).

Scheme 1.6

Synthesis of 2-substituted 2-imidazolines



N-Cbz amino acid, which are widely accessible and reasonably priced, were used as the starting point for a simple 4-step reaction sequence to create imidazole derivatives. N-Cbz amino acid 51 using as a starting point, imidazole derivatives were easily created in four steps. For the production of these imidazole derivatives 52, the condensation of the appropriate α-bromo ketones with formamidine acetate in liquid ammonia was shown to be effective in Scheme 1.7. The compounds created in are structurally linked to histamine (27).

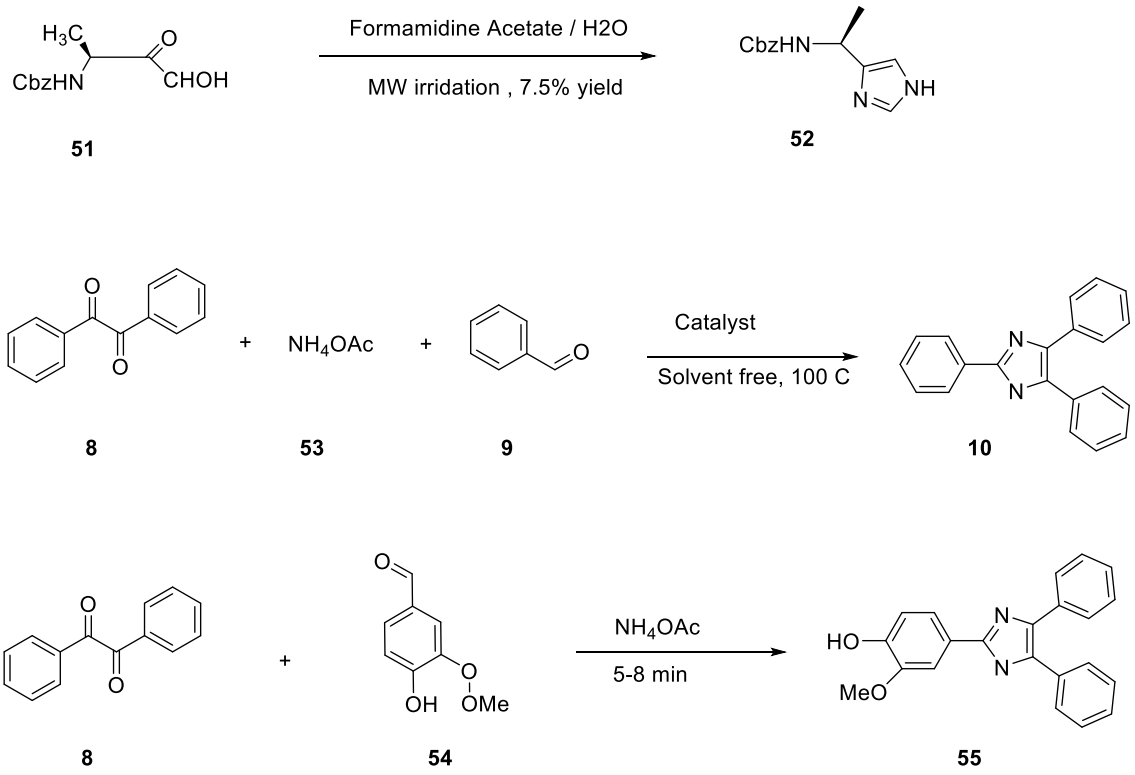
By using microwave irradiation and a three-component, one-pot condensation of benzyl 8 benzaldehyde 9 and ammonium acetate 53 in solvent-free conditions, using $(NH_4)_6Mo_7O_{24}.4H_2O$ acted as a powerful catalyst for an enhanced and speedy synthesis

of 2,4,5-triphenyl imidazole 10 as shown in Scheme 1.7. The reactions under standard heating settings and the reactions aided by microwaves were contrasted (28).

3-methoxy-4-hydroxy benzaldehyde 54 and benzyl 8 were condensed under microwave irradiation, an effective and environmentally friendly method for producing 2, 4, 6-triaryl1H-imidazole 55 as shown in Scheme 1.7 in polyethylene glycol with brilliant yield has been established. The chemical polyethylene glycol is non-toxic, recyclable, affordable, and widely accessible (29).

Scheme 1.7

Synthesis of Imidazole derivatives



1.4.5 Reactivity of imidazole

Pyrrole 2 and pyridine might be thought of as having qualities that are comparable to imidazole 1. Since the 'pyrrole' molecule is a member of the aromatic sextet, the electrophilic reagent would attack the unshared electron pair on N-3 but not the one on the nitrogen. While it is possible to attack the imidazole ring electrophilically on an annular carbon, it is considerably less likely to be engaged in a nucleophilic substitution process unless the ring contains other highly electron-withdrawing substituents. The

location most vulnerable to a nucleophilic assault in the absence of such activation is C-2. In Benzimidazole, the fused benzene ring offers enough electrons with drawl to for a variety of nucleophilic substitution reactions at C-2.

The collections of resonance structures where the dipolar contributors have a limited significance are where imidazoles and benzimidazoles' overall reactivity is derived. These predict nucleophilic assault at C-2 or C-1, electrophilic attack at N-3 or any other ring carbon atom in imidazole, as well as the molecule's amphoteric character. The nucleophilic assault on C-2 in benzimidazole is anticipated. Compared to the neutral molecule, the benzimidazole ion's C-2 location is more reactive with nucleophiles (30).

1.4.6 Biological significance of imidazole

Imidazole compounds have a variety of pharmacological outcomes, containing antifungal and antibacterial properties, analgesic and anti-inflammatory activities, anti-tubercular, anti-depressant, anti-cancer, and anti-viral activities.

A molecule with an imidazole moiety and a 2,4-dienone motif with substantial efficacy against many fungi was found while searching for novel antifungal agents. Then, a total of 26 derivatives of this molecule were created, produced, and tested due to their capacity to impede fungal growth in both in *vitro* and in *vivo* experiments (31).

Since millions of individuals throughout the world get bacteria and fungi every year, bacterial and fungal infections both cause illnesses and place a heavy socioeconomic cost on society. As a result, several antimicrobial medications that are crucial for treating infections have been mentioned (32).

Future access to medications for treating common illnesses is threatened by rising antifungal resistance (33), Therefore, the search for novel chemicals having antibacterial and antifungal properties is crucial (34), especially those whose modes of action differ from those of the well-known groups of antifungal medications (35).

Recent years have seen a lot of interest in imidazole compounds that contain two nitrogen atoms within a five-membered aromatic azole ring. As was previously mentioned, the fascinating application of creating medicines with neuroprotective action frequently employs imidazole rings as spin-trapping species (36). These imidazole-based derivatives also possess a number of beneficial qualities, including high tissue

permeability and penetrability, exceptional bioavailability, and a comparatively low frequency of negative and toxic side effects, indicating that they have a great deal of development potential in the fields of medicinal chemistry, materials science, and chemistry (37).

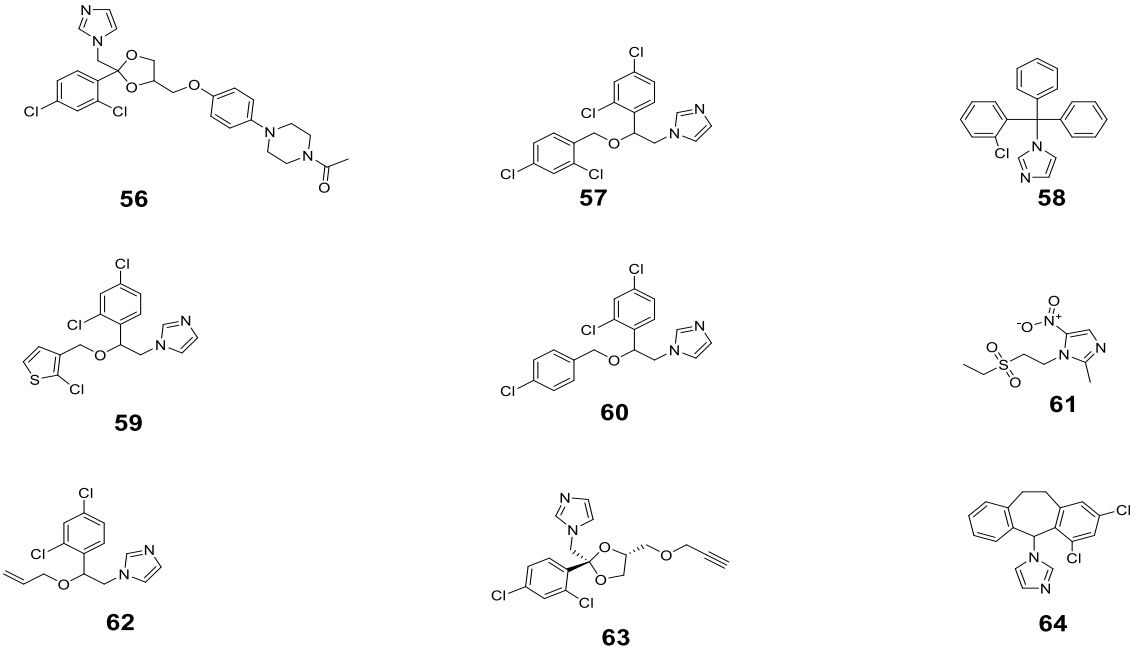
1.4.6.1 Antifungal drugs

Numerous compounds of imidazole that are used as antifungal medications include ketoconazole 56, miconazole 57, clotrimazole 58, tioconazole 59, econazole 60, tinidazole 61, enilconazole/imazalil 62, parconazole 63, eberconazole 64, fenticonazole, lanoconazole, sulconazole, lombazole, bifonazole, and sertaconazole are some examples of the antibiotics. Some of these compounds' chemical structures are presented in Figure 1.3 (38).

It has been shown that the imidazole ring serves as a flexible core for a variety of physiologically active compounds, particularly those having antifungal characteristics.

Figure 1.3

Example on marketed imidazole drugs



The antifungal drug ketoconazole 56, The molecular arrangement displayed in Figure 1.3 which was invented in 1977 and approved in 1981, is commonly applied to the treatment of several fungal diseases. The primary application of ketoconazole is the treatment of fungal skin diseases including tinea, cutaneous candidiasis, pityriasis,

dandruff, and versicolor. 454 Nizoral is another brand name used for ketoconazole. In reality, ketoconazole has the chemical name *cis*-1-acetyl-4-[4-[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolan-4- ylmethyl]phenyl] piperazine.

One of the biggest risks to human health is cancer, which has gotten unusually much attention globally. Effective anticancer therapies have been the subject of extensive research, involving the combined use of surgical and radiation procedures, chemotherapy and therapy. According to extensive study, imidazole compounds have been shown to have significant promise as anticancer medications. Typically, imidazoles have the potential to halt cell division and growth by interfering with DNA synthesis *via* weak interactions like - π–π stacking, coordination bonds, and hydrogen bonds (39).

1.4.6.2 Antiviral drugs

The five-membered cyclic structure of imidazoles and their fused derivatives provides them a distinct identity in the antiviral field medications (40). They are superior to other recognized moieties due to the distinct structural characteristics of the imidazole and benzimidazole rings and their favorable electron-rich features, which allow them to bind to a range of targets (41). To help the medical department, Numerous studies have been conducted to find more imidazole derivatives or moieties that include imidazole (42). Several antiviral medications based on imidazole are shown in Figure B.1(appendix B).

Acyclovir 65, the chemical structure shown in Figure B.2 (appendix B), a medication included in this class of imidazole derivatives, received FDA clearance for medicinal use in 1981 and was given a patent in 1974(43). It is a drug that fights viruses (44). The virus's DNA synthesis is decreased as a result. It treats infections caused by the herpes simplex virus and chickenpox. Acyclovir, also known as 9-[(2-hydroxyethoxy) methyl]guanine (ACV), is a medication that is marketed under the trade name Zovirax (45). It selectively affects herpes cells by inhibiting the capacity of the herpes virus to proliferate. Acyclovir, the first antiviral drug to be effective, is in fact an analog of a nucleoside.

1.4.6.3 Anticancer drugs

The imidazole moiety has only recently been researched as a crucial component of anticancer or antitumor agents. The varied substitutions at various moiety locations are mostly important. Numerous indole-imidazole compounds were also discovered, and they demonstrated strong in *vitro* antiproliferative effects on cancer cell lines, encompassing multidrug resistance (MDR) phenotypes. Prolonged administration of specific drugs to cancer cells can lead cells to develop acquired resistance to multiple drugs (46). The chemical indole-pyridoimidazole has demonstrated efficacy in opposition to every cell line, including the multidrug-resistant MES-SA/DX5 and HL60/TX1000 cell lines that did not respond well to treatment with Taxol. Additional research was done to ascertain the different substitution effects that influence these compounds' mechanisms of action, which may help develop future medications that are effective against MDR-carcinoma cells.

Numerous imidazole derivatives, such as dacarbazine, azathioprine, zoledronic acid, tipifarnib and nilotinib, have been successfully employed in the clinic as anticancer medications which shown in Figure B.3 (appendix B). They have been essential in the management of several malignancies.

To look into the anticancer activities, Numerous novel imidazole-(Benz) azole and imidazole epiperazine derivatives were synthesized by Yusuf Ozkay *et al*. The results of the anticancer activity screening indicate that compounds 66A, 66B, 66C were the series' most active chemicals. Cisplatin served as the benchmark medication (47), as shown in Figure B.4 (appendix B).

A series of 1, 4-diarylimidazole-2(3H)-one derivative and their 2-thione analogs were synthesized by Cenzo Congiu *et al*. and their antitumor activity was assessed. Compound 67 (1-(4-chlorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-imidazol-2(3H)-one), the chemical structure shown in Figure B.4 (appendix B) showed potent antitumor activity.

Also a number of 2-substituted benzimidazole series were synthesized by Hanan M. Refaat. When anticancer screening was conducted on a number of the synthesized products, it was discovered that every tested compound showed antitumor activity against human hepatocellular carcinoma, breast, human colon carcinoma and

adenocarcinoma (48). Some of these compounds 68, 69 are shown in Figure B.4 (appendix B).

1.5 Target Forecasts for a Range of Imidazole Derivatives

Figure B.5 (appendix B) presents suggested mechanisms for a few imidazole derivatives. Compound 70 was found to inhibit Topoisomerase IIR, while compound 71's mechanism was proposed to maintain DNA c-MYC G-quadruplex by modulating ion-channel. Ultimately, it was reported that compound **72** functions by binding to one of the kinase enzymes, GSK-3β (49).

1.6 Vanillin

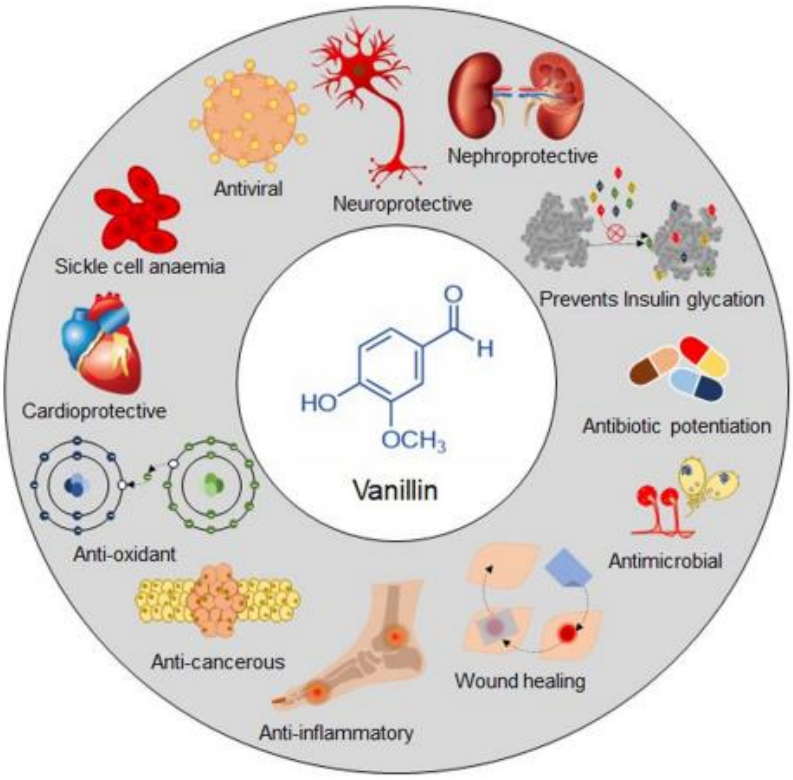
Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the primary constituent of natural vanilla, a flavoring agent that is extensively utilized and significant globally (50). It is a white to slightly yellow crystalline powder that dissolves quickly in boiling water but at room temperature, it has a somewhat low solubility in aqueous solution. It also has an intriguing sweet and remarkable fragrance (51). It is composed of three functional groups that are arranged around an arene: ether, hydroxyl, and aldehyde (52).

Vanillin usually comes from three sources: natural, chemical/synthetic, and biotechnological. It is classified as either a natural or artificial flavor, based on the synthesis technique and source. Of these, the majority of food control authorities worldwide classify Vanillin as a food-grade additive produced both naturally and biotechnologically (using ferulic acid as a substrate)(53).

It possesses a variety of biological properties, including anticancer (54), antioxidant (55), antibacterial (56), antiviral (57), and antideperessant properties (58). In addition, it has been discovered that vanillin and its synthetic analogs control gene expression and have biological properties (53), as shown in Figure 1.4.

Figure 1.4

Vanillin bioactivities



1.6.1 Pharmacological Activity of Vanillin Derivatives

Vanillin and the aromatic amine reacted to produce 4-(((4-butylphenyl) amino)methyl)-2-methoxyphenol 73 Figure B.6 (Appendix B). The produced substance was examined against Micrococcus and *E. coli*. *Bacillus subtilis and luteus bacteria*, as ascertained using the broth micro dilution method. The longer aliphatic chain and butyl group, which improved the antibacterial activity of vanillin derivatives, were the cause of the higher activity (59).

4-(((4-Fluorobenzyl)- imino)-methyl)-2-methoxy-6-nitrophenyl acetate 74 and 2-Methoxy-6-nitro-4-(((2-(pyridin-2-yl)-ethyl)-imino)-methyl)- phenyl acetate 75 are the new acetyl vanillin derivatives were produced by reacting acetyl nitro vanillin with various amines, the well diffusion method was used to test them, and the results showed that they had significant antibacterial activity against *E. coli*. The compounds 74 and 75 exhibited a strong antibacterial action because the substituted amine's para position contains electron-withdrawing substituents (60) as shown in Figure B.6 (Appendix B).

A derivative of vanillin and substituted sulfanilamide reacted to produce in vitro cytotoxic potential compounds such as (E)- 4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino)-N-(pyridin-2-yl)benzenesulfonamide 76, (*E*)-2-methoxy-4-(((4-(N-(pyridin-2-yl)sulfamoyl)phenyl)imino)methyl) phenyl acetate 77 and (*E*)-2-methoxy-4-(((4-(N-(pyridin-2-yl) sulfamoyl)phenyl)imino)methyl)phenyl isobutyrate 78 Figure B.6 (Appendix B). Subsequently, it was shown that these substances had notable activity against the human breast cancer cell line MCF-7 (61).

4-(1H-imidazo[4,5-f][1,10]-phenanthrolin-2-yl)-2-methoxyphenol 79 Figure B.6 (Appendix B) was created through the reaction of vanillin with 1,10-phenanthroline-5,6-dione, and it was tested on human colon cancer-causing cell lines, including HT29 and HCT116 cells. The test compound demonstrated a strong anti-colorectal cancer effect by blocking the Wnt/β-catenin signaling pathway.

Research utilizing enhanced molecular docking interactions has exhibited the compound's strong anti-tumor properties (62).

1.7 Biological activity of Heterocyclic drugs

Many of heterocyclic drugs have an antihistamine activity , and used to treat allergic rhinitis such as, Azelastine, Loratadin, Cetirizine, The strucrural formula of these drugs are shown in Figure B.7 (Appendix B). Azelastine is a medication which is primarily used as a nasal spray to treat allergic rhinitis .Azelastine was first patented in 1971 and came into medical use under the trade name of Optivar in 1986 (63). Loratadine (has been shown to be effective in treating hives and allergic rhinitis, among other types of allergies. It was granted a patent in 1980 and went on sale in 1988 under the brand name Claritin(43). As loratadine/pseudoephedrine, it is also offered in conjunction with the decongestant pseudoephedrine. In actuality, second-generation antihistamines such as loratadine, cetirizine, and astemizole have replaced first-generation antihistamines like diphenhydramine and ketotifen (64).

A second-generation antihistamine called cetirizine is used to treat allergic rhinitis, dermatitis, and urticarial. Although it was granted a patent in 1981, Zyrtec (Zyrtec, Zirtec) was only introduced to the market in 1987(43). Cetirizine's pharmacological, medical, and therapeutic efficacy have already been discussed. Cetirizine is made as a racemic mixture that can be split into its isomers.

Also there are many of heterocyclic drugs used as ACE inhibitor medication, or used to prevent cardiovascular disease, to prevent nausea and vomiting caused by cancer chemotherapy, to treat high blood pressure, to treat attention deficit hyperactivitydisorder and high blood pressure, All of these heterocyclic drugs are shown in Figure B.8 (Appendix B).

The ACE inhibitor medication family includes lisinopril, which is primarily used to treat hypertension, heart failure, and is routinely taken following a heart attack(65). Chemically, lisinopril is known as N2-[(1S)-1-carboxy-3-phenylpropyl]-l-lysyl-l-proline, but Merck sells it under the trade name PRINVIL. In the United States, lisinopril was authorized for medicinal use in 1987 after receiving a patent in 1978.

Under the trade name Lipitor, atorvastatin is one of several oral statin medications that doctors may give. By lowering blood levels of low density lipoprotein (LDL) cholesterol, it is known to prevent cardiovascular disease. Atorvastatin was patented in 1986, received US government certification for prescription use in 1996, and is now available as a generic medication(66). Butler and colleagues first achieved and reported an effective synthetic method for atorvastatin in 1989.

Ondansetron, 1,2,3,9-tetrahydro-9-methyl-3-(2-methyl-1*H*-imidazol-1-ylmethyl)-4*H*-carbazol-4-one hydrochloride dihydrate , commercialized under the brand name Zofran. Ondansetron was patented in 1984 and approved for medical use in 1990. It is a medication used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, or surgery(67). The pharmacologic and therapeutic applications of ondansetron have extensively been reviewed(68).

Losartan, was patented in 1986, commercialized under the trade name Cozaar, among other names, and FDA-approved for prescription use in 1995(63). It is a medication that is typically used to treat high blood pressure.

Clonidine, (2-(2,6-dichlorophenylamino)imidazoline is marketed in addition under the name Catapres. It is frequently used to treat attention deficit hyperactivity disorder and high blood pressure. In 1961, clonidine received patent approval, and it hit the market in 1966(69). This anti-hypertensive drug has a distinct central site of action. Kobinger (70) and Walland have written extensively about the pharmacology of clonidine.

A well-known class of chemicals known as benzodiazepinesexhibits a wide range of central nervous system (CNS)-related functions(71). They exhibited various kinds of biological potencies, for example antitumoral and anticonvulsive activities.

Also, among nitrogen-based heterocycles more complex scaffolds have been gaining terrain in medicinal chemistry studies over the last decade. For example, triazolothiadiazoles and triazolothiadiazines, which are cleverly designed polycyclic scaffolds arranged by combining triazoles to thiadiazoles or thiadiazines, have become important biologically relevant scaffolds in cancer(72). basic scaffold to other heterocyclic moieties including fused rings has led Asif Husain and colleagues to the synthesis of benzimidazole hybrid heterocycles clubbed with triazolo-thiadiazoles and triazolo-thiadiazines, in an attempt to produce improved pharmacological compounds(73).

Novel triazolothiadiazines, triazolothiadiazoles, and 1,2,4-triazoles have recently been synthesized, and their anticancer potential has been assessed (74). Seven compounds with significant cytotoxic activity against a variety of cancer cell lines (NUGC; DLD1, HA22T, HEPG2, HONE1, MCF7) were discovered by Kamel and colleagues. Among these, compound 6-(4-chlorophenyl)-3-(pyridin4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole Figure B.9 (Appendix B) demonstrated an IC50 of 25 n (NUGC).Noteworthy, toxicological testing employing normal fibroblast cells (WI38), in order to assess potential side effects, demonstrated significant differences, being approximately 400-fold less toxic to normal cells compared to the NUGC cell line(74).

Paclitaxel, an oxygen-based heterocycle drug with an incorporated oxetane ring, has emerged as a key drug in cancer therapy.In a process similar to that of microtubule associated proteins (MAPs), Paclitaxel prevents the de-polymerization of microtubule polymers to exert its therapeutic effects. However, this process is irreversible, which prevents the progression of mitosis in cancer cells. Despite the basic advancements in cancer therapy that have been made since the discovery of PTX, there are still a number of problems that need to be solved. In particular, PTX and the development of multidrug resistance profiles have been closely connected with cellular modifications of tubulin structure and the amplification of efflux pumps, burdening oncology-related research and clinical treatments(75).

Oxygen-based heterocycles make for about 8% of the heterocycles with anticancer characteristics that the FDA has approved since 2010. The most recent medications approved were Cabazitaxel and Eribulin Figure B.10 (Appendix B).

Cabaditaxel, also known as Jevtana, is recommended for the treatment of patients with castrate-resistant metastatic prostate cancer. Cabazitaxel, a taxane derivative, prevents cell division by inhibiting microtubules (76).Eribulin (Halaven), on the other hand, is a non-taxane medication that works as a microtubule inhibitor and is prescribed to patients with metastatic breast cancer(77) .

1.8 Recent application of imidazole

The study of medicinal chemistry focuses on determining how chemical structure affects biological activity. The field evolved from an empirical one that involved the organic synthesis of novel compounds, mostly by the modification of structure before determining their biological function. The study of medicinal chemistry is concerned with the molecular level identification, development, interpretation, and discovery of physiologically active molecules. A nitrogen-containing heterocyclic ring with five members is present in the structures of some synthetic substances that are physiologically active. It has been stated that structural frameworks are preferred structures, and that N-containing polycyclic structures in particular are linked to a variety of biological activities. Among the five membered heterocyclic structures, the imidazole nucleus exhibits a variety of characteristics. The great therapeutic potential of medications related to imidazoles has inspired medicinal chemists to create a wide range of innovative chemotherapy medicines (78). Some imidazole drugs, at high concentrations, could exert direct inhibitory action on membranes, without interference with sterols and sterol esters (79). Microbes are the source of infectious microbial diseases globally because they have a longer resistance to treatment or prophylaxis than any other form of life. Multidrug-resistant microbe issues have gotten alarmingly worse in many nations worldwide in recent decades. An increasing number of significant worldwide issues are brought about by anti-microbial agent resistance to various bacterial species and β-lactam antibiotics, macrolides, quinolones, and vancomycin, among others (80) . Imidazole and its derivatives are effective in treating a variety of disorders due to their physiological and pharmacological properties. The imidazole core scaffold's biological and physiochemical features have led to various applications in

chemical, optical, pharmaceutical, and other fields. The imidazole moiety consists of three carbon atoms and two nitrogen atoms, with electronic-rich This heterocyclic ring has unique properties that enable it to bind with a wide range of enzymes, proteins, and receptors, distinguishing it from others. The imidazole ring, found in natural products, plays a crucial role in medicinal chemistry. It has been used to treat a variety of diseases, and new derivatives are being synthesized globally for medicinal applications (81). These derivatives constitutes a very basic structural and fundamental building block of the various type of medical scaffolds demonstrated promising anti-cancer, antimicrobial, and anti-inflammatory activities (82). The imidazole ring's structural features allow for various drug-ligand interactions through vander Waals forces, hydrophobic forces, and hydrogen bonds (83). The imidazole moiety is found in various natural substances, including histamine, histidine, biotin, alkaloids, and nucleic acid, as well as FDA-approved pharmaceuticals. Fused imidazole derivatives have been widely employed in medicine due to their medicinal characteristics (84). Over the past few decades, scientists and researchers from all over the world have been drawn to the imidazole molecule because due to its highly prized chemical and biological qualities. The heterocyclic chemical class known as imidazoles is intriguing. It possesses a wide range of biological properties, including antimicrobial, antibacterial, antifungal, anticancer, anti-inflammatory, antiparasitic, antiviral, anti-HIV, anticonvulsant, and antiulcer properties. These days, trichomonacide applications have been the focus of imidazole-based antibacterial medications such as 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and 2-nitroimidazole. Moreover, certain imidazoles, including misonidazole, metronidazole, clotrimazole, and metrazole, are essential anticancer medications (85). Polycyclic aromatic compounds are extensively used in medicinal and dye industry. In specific, nitrogen containing compound like aminonaphthalimide, [3] 2-(2’-hydroxy-5’- chlorophenyl)-6-chloro-4(3H)-quinazolinone are used as fluorophores (86). Luminescent chemicals have found widespread use due to their varied uses in molecular logic gates, light emitting diodes (LEDs), and biosensors. Certain polycyclic aromatic compounds with an imidazole base have their luminosity features identified. Generally speaking, the luminous characteristics of tiny heterocyclic aromatic compounds combined with polycyclic aromatic compounds with structures like phenanthrene and anthracene were superior(87). The antibacterial activity of several imidazoquinolines functionalized with thio-, chloro-, and hydroxyl groups was investigated by Velmurugan et al. against a

range of bacterial pathogens. They proposed that imidazoquinoline 80 Figure B.11 (Appendix B).-containing electron-withdrawing (-Cl) substitutuent had more antibacterial and antioxidant activity than other imidazoquinolines and attained the standard level of efficacy (88). A novel series of 1-methyl-2,6-diphenylbenzoimidazole and 1-methyl-phenyl(o-tolyl) benzo [d]imidazole derivatives were synthesized from 4-bromobenzene-1,2-diamine and benzoic acid using palladium (II) acetate have been developed by Havale et al (88). Using the broth-dilution method, the synthetic compounds' antibacterial activity against Gram-positive S. aureus and Gram-negative E. coli bacteria was assessed. Out of all the compounds studied, compounds 81 and 82 Figure B.11 (Appendix B). demonstrated the highest efficacy against S. aureus and E. coli germs. The biological activity of certain imidazole derivatives have been described by Zina et al. When compared to the antibiotic tetracycline, antibacterial research showed strong efficacy against S. aureus and E. coli of 83, 84, and 85 Figure B.11 (Appendix B).compounds (89). Magar et al. developed a new series of 2, 4, and 5-trisubstituted imidazole derivatives employing ultrasonication and green synthesis using various aldehydes as substitutes. The compounds were tested for anti-microbial activity against Staphylococcus aureus, Bacillus subtilis, and Gram-negative Escherichia coli using cup-plate agar diffusion. 5-(4-methoxyphenyl)-2,4-diphenyl-1H-imidazole 86 Figure B.11 (Appendix B). showed the highest activity (90). Quasar et al. were employed a multistep synthetic protocol to accomplish the synthesis of (2Z)-2-((E)-4-(benzylideneamino) phenyl)-3-(1-methyl-1H-imidazol-2-yl) acrylonitrile derivatives. The synthesized compounds were screened for in vitro antitubercular activity in which the compounds 87, 88 and 89 Figure B.11 (Appendix B). appeared promising activity (90). Three imidazoles: bis-imidazole, phenylsubstituted 1H-imidazole, and thiopene-imidazole for cellular toxicity were evaluated by Oluyomi et al(91) . The compounds were assessed for in vitro cytotoxic action. Their findings showed dose-dependent cellular toxicity involves likely impairment to redox balance and mitochondrial membrane potential in living cells, and altogether may boost their prospects as new and alternative anti-protozoan (90, 91 , 92) Figure B.12 (Appendix B). Trypanosoma spp. cause both animal and human trypanosomiasis, which has a significant health and economic impact in developing countries. Oluyomi et al. found that novel imidazoles can effectively reduce parasite burden in infected rats. Their in vivo study revealed that the imidazole compound 93 Figure B.12 (Appendix B) not only cleared the systemic parasite burden but cured infected rats after no death was recorded

(92). Toxoplasmosis is a common parasitic disease caused by Toxoplasma gondii. Adeyemi et al. were synthesized a series of new imidazole derivatives: bis-imidazoles, phenyl-substituted 1H-imidazole, and thiopene-imidazoles. 94 Figure B.12 (Appendix B) exhibited significantly high selectivity towards the parasite versus the host cells. Deblina et al. developed a library of quinoline − imidazole hybrid compounds which have significant antimalarial activity was evaluated in both drug-sensitive and –multi drugresistant (MDR) P. falciparum strain. The enantiomer 95 Figure B.12 (Appendix B) demonstrated more antimalarial activity than the other isomer, with an IC_{50} of 0.10 µM (32). Systemic mycoses are a significant cause of morbidity and mortality in the elderly. Fatima et al. studied the mechanism of action of three (Z)-5-amino-N'-aryl1-methyl-1H-imidazole-4-carbohydrazonamides (96,97,98) Figure B.13 (Appendix B) that have high antifungal activity against Candida krusei and C. albicans ATCC strains(93). A series of new imidazole-1,2,3-triazole derivatives were designed and synthesized by Blewi et al. in 2021. The resulted adducts were investigated for their anticancer activity against four cancer cell lines (Caco-2, HCT-116, HeLa, and MCF-7) by the MTT assay. Their investigation showed 99 Figure B.14 (Appendix B) displayed potent cytotoxic activity against the cancer cell lines, especially MCF-7. The hedgehog (Hh) signaling pathway drives oncogenic transformation for a wide range of cancers, and it is therefore a promising target in cancer therapy. Chiyu et al. designed and synthesized a series of Hh signaling pathway inhibitors with phenyl imidazole scaffold, which were biologically evaluated. Ali et al. developed a new family of N-1 arylidene amino imidazole-2 thiones. The cytotoxic effect of the produced compounds was tested on three cancer cell lines: MCF-7 breast cancer, HepG2 liver cancer, and HCT-116 colon cancer. Imidazole derivative 100 Figure B.14 (Appendix B) demonstrated the highest potency against three cancer cell lines (94). Cius et al. produced benzenesulfonamide-containing imidazole derivatives with 4-chloro and 3,4-dichloro substituents in the benzene ring and 2-ethylthio and 3-ethyl groups in the imidazole ring. They tested their cytotoxicity against human triple-negative breast cancer. The study examined whether 4-chloro substituents in the benzene ring 100 and 2-ethylthio in the imidazole ring 101 Figure B.14 (Appendix B) could contribute to the strong anticancer activity (95). Ali et al. presented a one-pot three-component reaction of 1,2-diketone with aldehydes and ammonium acetate, catalyzed by ZnO nanocatalyst, to synthesize bis- and poly(imidazoles). The compounds were tested for anticancer activity against MCF-7, HepG-2, and CaCO-2 cell lines. The chemical 102 Figure B.14 (Appendix B) with a

bis(imidazole) analog and 4,5-difuran rings showed the best activity against HepG-2 cancer cells with high selectivity index (96). PI3K is a key therapeutic target for cancer treatment. Yang et al. produced 2-arylthio and 2-arylamino-1H-benzo[d]imidazole derivatives of dehydroabietic acid. The study found that certain imidazole moiety had considerable inhibitory activity against four cancer cell lines (HCT-116, MCF-7, HeLa, and HepG2). Compound 103 Figure B.14 (Appendix B) showed the highest potency against all four cancer cell lines, making it a promising PI3Kα inhibitor candidate (97). Zhang and colleagues developed a simple and effective technique for synthesizing pyrrole-imidazole via a post-Ugi cascade reaction. Compound 104 Figure B.14 (Appendix B) showed significant anticancer activity in PANC and ASPC-1 cell lines (98). Using privileged scaffolds in medicinal chemistry helps scientists discover new and improved therapeutic compounds. A library of 2,4,5- triphenyl imidazole derivatives were synthesized and evaluated in vitro as Xanthine oxidase (XO) inhibitors as well as antiproliferative agents. Compound 105 Figure B.14 (Appendix B) was the most effective XO inhibitor, having an IC50 of 85.8 µg/ml. Overall, of the six tested malignant cell lines. molecule 106 Figure B.14 (Appendix B) was the most antiproliferative compounds (99).

1.9 Aims of the project

The aim of this work is to synthesize new imidazole derivatives with a natural basis that exhibit anti-cancer properties. The following are the study's main goals:

1. Construct a novel class of biobased imidazoles and synthesize them.

2. Use spectroscopic methods such as NMR (1H and 13C) and IR to analyze the prepared derivatives' structure and physical characteristics.

3. Assess the prepared compounds' in *vitro* cytotoxicity and anticancer efficacy against various tumor cell lines.

1.10 Long-term objectives

Provide interested pharmaceutical companies with multi-gram quantities of the prepared derivatives for full scale testing in large animal models, clinical evaluation, and subsequent commercial development.

1.11 Literature review and novelty

The current research will present a method of synthesizing novel vanillin-based imidazole. The new method comprises a multistep process that involves reacting vanillin with various commercially available reagents and compounds. The new vanillin derivatives are anticipated to be valuable drugs with versatile bioactivities. The derivatives will be evaluated as anticancers. From an economic point of view there is a great financial benefit from using low-cost, safe, natural, and biodegradable starting material for making drugs.

In this project, a new set of the natural product vanillin-based imidazole derivatives were synthesized as versatile bioactive compounds. To the best of our knowledge this type of imidazole derivatives have never been presented in the literature.

Chapter Two

Materials and Methods

2.1 Reagents and Instrumentations

Every item that was used in the experiments was of analytical grade. The Glycine, 4-Chloroaniline, 2-Aminopyridine, 2-Guanidinobenzimidazole, 2-Picolylamine, 2-Mercaptobenzimidazole, 4-Amino benzoic acid, O-phenylenediamine, Thiogycolic acid, Ethylchloro acetate, Hydrazine hydrate, 2-(2-Pyridyl)ethylamine, acquired from (Sigma-Aldrich, Germany). 4-Bromoaniline, *p*-phenylenediamine, 4-Aminophenol, 4-Nitroaniline 3-Aminobenzoic acid, 4-Bromoaniline, acquired from (Alfa Aesar company, England). Benzoyl chloride purchased from (RiedeldeHaen company, Germany). Vanillin, Isatin, purchased from (Thermo company).

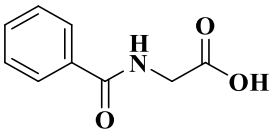
A Stuart Scientific Melting Point SMP3 apparatus was employed for the melting point determinations. All melting points are reported in Celsius (ºC). (FT-IR) was performed on Nicolet iS5 (Thermo Fisher Scientific Company, USA). Nuclear Magnetic Resonance (NMR) spectra were obtained using Bruker Avance (500MHz spectrometers, Switzerland, University of Jordan, Amman).

Dulbecco's free Ca^{+2} phosphate-saline buffer (REF # 02-023-1A), Pen-Strep Solution (catalogue #030311B), and L-glutamine solution (REF # 03-020-1B) were obtained from the Biological Industries (Jerusalem). Sigma Life Science provided us with a Trizma base (Lot SLBF2864V).

Esco CO_2 cell culture incubator was used to incubate the cell line. Also, an Accumax Variable micropipette with normal and long narrow gel loading tips was used. Eppendorf Thermo mixer dry, Shaker, PH/ORP meter (by HANNA), Benchtop UV Transilluminator, Photodoc-it™imaging system, XB-30 Flak Ice maker M.R.C LTD.

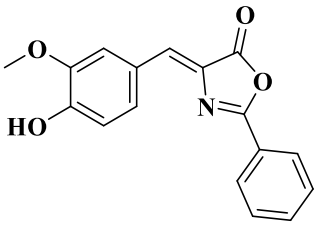
2.2 Chemical Synthesis

2.2.1 Synthesis of benzoyl glycine (1).



Glycine (0.5 g, 6.6 mmol) was dissolved in a solution of $NaHCO_3$ (10.0 mL,10%) and benzoyl chloride (1.0 g, 7.11 mmol) was added to the solution. The solution was mixed vigorously until the smell of the benzoyl chloride disappeared completely. The $NaHCO_3$ was neutralized by the addition of concentrated HCl dropwise. The produced was filtered, collected, and then boiled in water (about 20.0 mL) before being recrystallized. The produced benzoylglycine was collected by suction filtration and dried in an oven at 60°C under vacuum. The mass of the product was 0.85 g (yield 71%), m.p =188-190 °C. IR (ν in cm^{-1}): 3339 (N-H stretching), 3071 (OH stretch of carboxylic acid), 2930-2670 (CH_2 group), 1743 (C=O), 1600-1490 (aromatic ring), 600-580 (C-C=O) Figure A.1 (Appendix A).

2.2.2 Preparation of (Z)-4-(4-hydroxy-3-methoxybenzylidene)-2-phenyloxazol-5(4H)-one (2).



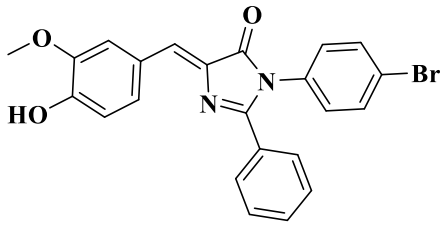
Benzoyl glycine **1** (0.4 g, 2.23 mmol) and vanillin (0.34 g, 2.23 mmol) were placed in a round-bottomed flask (50.0 mL), followed by the addition of acetic anhydride (3.0 mL, 6.69 mmol**)** and sodium acetate (0.18 g, 2.23 mmol). The mixture was stirred for 2 h at 110 °C. After cooling to room temperature, 10.0 mL ethanol was added at once. The round-bottomed flask was then placed in a refrigerator overnight, the formed precipitate (yellow solid) was collected by suction filtration and dried in an oven at 60 °C. The Product mass was 0.35 g (yield 54%), melting point =189-190 °C. R_f 0.65 (Hexane/Ethylacetate: 4:2). IR (ν in cm^{-1}): 3569 (OH), 1796-1755 (C=O, lactone), 1650 (C=N), 1601-1557 (C=C**)**, 1326-1286 (C-N), 1270 (C-O, OCH_3), 1119 (C-O-C). Figure A.2 (Appendix A). ^1H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.81 (s, 1H, OH), 8.09 (s,

1H, CH=C), 7.62-7.51 (m, 5H, Ar), 7.26-6.96 (m, 3H, Ar), 3.91 (s, 3H, OCH_3) Figure A.33 (Appendix A). ^{13}CNMR (500 MHz, DMSO-d6) δ in *ppm*: 167.3(O-C=O), 163.6 (O-C=N), 151.4 (Ar-O), 142.0 (Ar-OH), 133.4-132.7 (=C-N), 130.5-126.0 (C_6H_5-), 56.3 (CH_3) Figure A.58 (Appendix A).

General procedure for synthesis of imidazolones

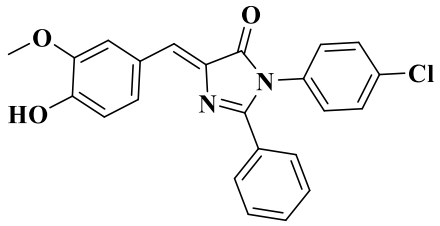
A solution of amine (5.0 mmol), ethanol (20.0 mL), and acetic acid (3 drops) was prepared in a round-bottomed flask (50.0 mL), oxazolone 2 (5.0 mmol) was added to the solution. After cooling to room temperature and being refluxed for two hours, the reaction mixture was refrigerated for two hours. The resultant solid was collected by filtration, dried, and recrystallized from methanol.

2.2.3 Preparation of (*Z*)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3).



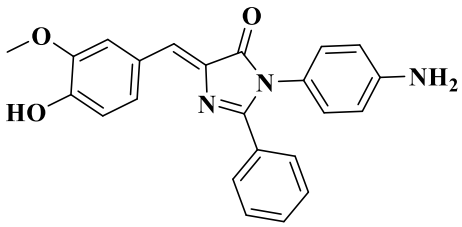
Adhering to the standard protocol for imidazolone synthesis, pure product 3 was obtained. Product mass was 1.5 g (yield 68 %), m.p 238-240 °C. R_f 0.41 (Hexane/Ethyl acetate 4:2). IR (ν cm^{-1}): 3400-3260 (OH), 2929 (C-H, OCH_3), 1758 (C=O, lactam), 1640 (C=N), 1617-1510 (C=C), 1315 (C-N), 1280-1160 (CO, Ar-O-CH_3), 1200 (C-O,OH), 800-600 (C-Br) Figure A.3 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.19 (s, 1H, OH), 8.06 (d, 2H, J = 7.6Hz, Ar-Br), 7.73 (d, 2H, J = 8.8Hz, Ar-Br), 7.61-7.51 (m, 5H, Ar), 7.42 (s, 1H, CH=C), 7.25 (d, 1H, J = 8.4Hz, Ar-H-C-OH), 7.19 (s, 1H, Ar-H-C-OCH_3), 7.12 (d, 1H, J = 8.0Hz, Ar-H-C-C=C), 3.62 (s, 3H, OCH_3) Figure A.34 (Appendix A). ^{13}CNMR (500 MHz, DMSO-d6) δ in *ppm*: 166.5 (N-C=O), 164 (N-C=N), 139.99 (Ar-OH), 151.06 (Ar-O), 132.39-131.83 (=C-N), 131.28-128.37 (C_6H_5-), 122.54 (Ar-Br), 55.96 (CH_3) Figure A.59 (Appendix A).

2.2.4 Preparation of (*Z*)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (4).



Adhering to the standard protocol for imidazolone synthesis, pure product 4 was obtained. Product mass was 1.5 g (yield 74 %), m.p =248-250 °C. R_f 0.41 (Hexane/Ethyl acetate: 4:2). IR (ν in cm^{-1}): 3400-3220 (OH), 2968 (=C-H), 2880 (C-H,OCH_3), 1759 (C=O, lactam), 1639 (C=N), 1617-1510 (C=C), 1315 (C-N), 1280-1160 (CO,Ar-O-CH_3), 1215 (C-O,OH), 800-600 (C-Cl) Figure A.4 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.20 (s, 1H, OH), 8.06 (d, 2H, J = 7.6Hz, Ar-Cl), 7.78 (d, 2H, J = 8.4Hz, Ar-Cl), 7.61-7.37 (m, 5H, Ar), 7.29 (s, 1H, CH=C), 7.25 (d, 1H, J = 8.4Hz, Ar-H-C-OH), 7.20 (s, 1H, Ar-H-C-OCH_3), 7.12 (d, 1H, J = 8.3Hz, Ar-H-C=C), 3.62 (s, 3H, OCH_3) Figure A.35 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 166.54 (N-C=O), 164.86 (N-C=N), 139.99 (Ar-OH), 151.07 (Ar-O), 133.43 (Ar-Cl), 133.43- 132.40 (=C-N), 131.27-128.37 (C_6H_5-), 55.95 (CH_3) Figure A.60 (Appendix A).

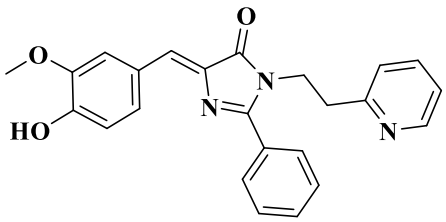
2.2.5 Preparation of(*Z*)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5).



Adhering to the standard protocol for imidazolone synthesis, pure product 5 was obtained. Product mass was 1.00 g (yield 52%), m.p =208-212 °C. R_f 0.33 (Ether/Ethyl acetate: 6:4). IR (ν in cm^{-1}): 3620 (OH), 3367-3261 (primary amine), 3060-3020 (=C-H), 2881-2837(C-H, OCH_3), 1764 (C=O, lactam), 1640 (C=N), 1614-1511 (C=C), 1322 (C-N), 1276-1162 (C-O,Ar-O-CH_3), 1204 (C-O,OH) Figure A.5 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.73 (s, 1H, OH), 7.60-7.50 (m, 5H, Ar), 7.39

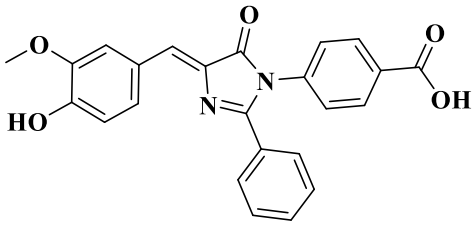
(s, 1H, CH=C) 7.33 (d, 1H, J = 8.2Hz, Ar-H-C=C), 7.25 (s, 1H, Ar-H-C-OCH_3) 7.20 (d, 1H, J = 10.1Hz, Ar-H-C-OH), 7.09 (d, 2H, J = 8.3Hz, Ar-NH_2), 6.53 (d, 2H, J = 8.2Hz, Ar-NH_2), 4.89(s, 2H, NH_2), 3.58 (s, 3H, OCH_3) Figure A.36 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 168.92 (N-C=O), 163.76 (N-C=N), 145.51 (Ar-OH), 151.00 (Ar-O), 140 (C_6H_4-NH_2), 133.70-132.26 (=C-N), 131.54-128.27 (C_6H_5-), 123.36-122.52 (OH-C6H4-), 55.89 (CH_3) Figure A.61 (Appendix A).

2.2.6 Preparation of (*Z*)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (6).



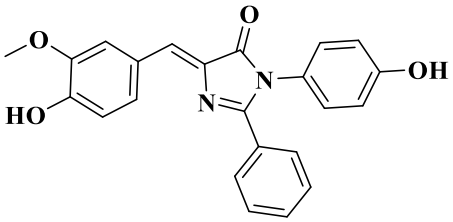
Adhering to the standard protocol for imidazolone synthesis, pure product 6 was obtained. Product mass was 1.00 g (yield 50%), m.p =147-151 °C. R_f 0.33 (Ethyl acetate/Methanol 9.5:0.5). IR (ν in cm^{-1}): 3732 (OH), 3209 (tertiary amine of pyridine), 2900 (=C-H), 2800 (C-H, OCH3), 1762 (C=O, lactam), 1641 (C=N), 1619-1513 (C=C), 1311 (C-N), 1209 (C-O, OH), 1200-1163 (C-O, Ar-O-CH_3) Figure A.6 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.98 (s, 1H, OH), 8.04 (d, 1H, J = 8.0Hz, Pyridine-H), 7.67-7.54 (m, 5H, Ar), 7.52 (t, 1H, J = 7.5Hz, Pyridine-H), 7.34 (s, 1H, CH=C), 7.28-719 (m, 2H, Pyridine-H), 7.14-7.06 (m, 3H, Ar- OCH_3), 3.53 (m, 2H, Pyridine-CH_2), 3.36 (s, 3H, OCH_3), 2.93 (q, 2H , Pyridine-CH_2) Figure A.37 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 166.36 (N-C=O), 159.78 (=C-N in pyridine), 149.39 (N-C=N), 150.99 (Ar-OH), 131.33-130.98 (=C-N), 133.96-128.3 (C_6H_5-), 55.85 (CH_3) Figure A.62 (Appendix A).

2.2.7 Preparation of (*Z*)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoic acid (7).



Adhering to the standard protocol for imidazolone synthesis, pure product 7 was obtained. Product mass was 1.1 g (yield 53%), m.p =220-225 °C. R_f 0.58 (Ethyl acetate/Methanol 9.5:0.5). IR (ν in cm^{-1}): 3600 (OH), 3300 (OH carboxylic acid), 3050 (=C-H), 1731 (C=O, lactam), 1671 (C=N), 1600-1519 (C=C), 1320 (C-N), 1287-1157 (C-O,Ar-O-CH_3), 1264 (C-O,OH) Figure A.7 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.31 (s, 1H, COOH), 9.98(s, 1H, OH), 8.20 (s, 1H, CH=C), 8.01-7.85 (m, 4H, Ar-COOH), 7.66-7.39 (m, 5H, Ar), 7.25-6.85 (m, 3H, Ar-OCH_3), 3.85 (s, 3H, OCH_3) Figure A.38 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 169.91 (N-C=O), 169.36 (COOH) ,185.67 (N-C=N), 147.86 (Ar-OH), 149.29 (Ar-O), 131.62-130.5 (=C-N), 135.94-130.67 (C_6H_5-), 130- 123.85 (-C_6H_4-COOH), 55.69 (CH_3) Figure A.63 (Appendix A).

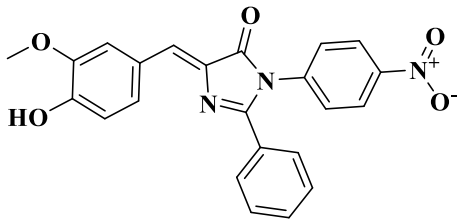
2.2.8 Preparation of (*Z*)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl) -2-phenyl-3,5-dihydro-4H-imidazol-4-one (8).



Adhering to the standard protocol for imidazolone synthesis, pure product 8 was obtained. Product mass was 1.3 g (yield 67%), m.p=208-212 °C. R_f 0.82 (Ethyl acetate/Methanol 9.5:0.5). IR (ν in cm^{-1}): 3430 (OH), 3270 (tertiary amine), 3100-3020 (=C-H), 2842 (C-H, OCH_3), 1770- 1735 (C=O, lactam), 1638 (C=N), 1612-1509 (C=C), 1320 (C-N), 1273-1161 (C-O, Ar-O-CH_3), 1211 (C-O,OH) Figure A.8 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.09 (s, 1H, OH), 9.89 (s, 1H, N-Ar-OH), 9.23 (s, 1H, CH=C), 8.05-7.57(m, 4H, Ar-OH), 7.54-7.40 (m, 5H, Ar), 7.20 (s, 1H, Ar-

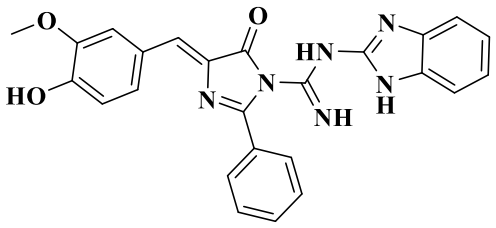
H-C-OCH_3), 7.10 (d, 1H, J = 8.2Hz, Ar-H-C=C), 6.73 (d, 1H, J = 8.3Hz, Ar-H-C-OH), 3.59 (s, 3H, OCH_3) Figure A.39 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 168.9 (N-C=O), 164.08 (N-C=N), 154.04 (-C_6H_5-OH) , 151.01 (Ar-O), 132.29-131.23 (=C-N), 133.63-128.31 (C_6H_5-), 56.52 (CH_3) Figure A.64 (Appendix A).

2.2.9 Preparation of (*Z*)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (9).



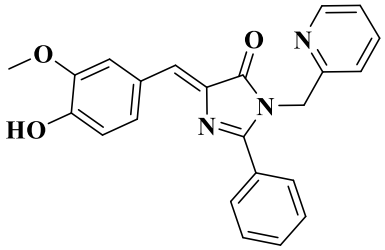
Adhering to the standard protocol for imidazolone synthesis, pure product 9 was obtained after purified by silica column chromatography. Product mass was 1.5 g (yield 72%), m.p =105-109 °C. R_f 0.46 (Hexane/Ethyl acetate: 4:2). IR (ν in cm^{-1}): 3433 (OH), 3354 (tertiary amine), 3059-2990 (=C-H), 2895-2841 (C-H, OCH_3), 1766- 1714 (C=O, lactam), 1665 (C=N), 1600-1508 (C=C), 1579-1377 (NO_2), 1377 (C-N), 1271-1129 (C-O, Ar-O-CH_3), 1242 (C-O,OH) Figure A.9 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.16 (s, 1H, OH), 9.96 (s, 1H, CH=C), 7.99(d, 2H, J = 7.7Hz, Ar-NO_2), 7.94 (d, 2H, J = 8.8Hz, Ar-NO_2), 7.60-7.29 (m, 5H, Ar), 7.12 (d, 1H, J = 8.3Hz, Ar-H-C-OH), 6.73 (s, 1H, Ar-H-C-OCH_3), 6.60 (d, 1H, J = 8.8Hz, Ar-H-C=C), 3.62 (s, 3H, OCH_3) Figure A.40 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 168.86 (N-C=O), 156.2 (N-C=N), 151.1 (Ar-O), 140.53 (Ar-NO_2), 132.41 (=C-N), 136.09-128.08 (C_6H_5-), 56.02 (CH_3) Figure A.65 (Appendix A).

2.2.10 Preparation of (*Z*)-N-(1H-benzo[d]imidazol-2-yl)-4-(4-hydroxy-3-methoxybenzylidene) -5- oxo -2-pheny -4,5- dihydro -1H- imidazole -1-carboximidamide (10).



Adhering to the standard protocol for imidazolone synthesis, pure product 10 was obtained. Product mass was 1.00 g (yield 44%), m.p =292-295 °C. IR (ν in cm^{-1}): 3442 (OH), 3329 (tertiary amine), 1657 (C=N), 1597-1511 (C=C), 3164 (=C-H), 2667 (C-H, OCH_3), 1739 (C=O, lactam), 1219 (C-O, OH), 1284-1016 (C-O, Ar-O-CH_3), 1358 (C-N) Figure A.10 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 11.68 (s, 1H, NH-imidazole), 11.01 (s, 1H, C-NH-C=), 9.07 (s, 1H, OH), 8.52 (s, 1H, C=NH), 7.99 (s, 1H, CH=C), 7.85-7.52 (m, 5H, Ar), 7.43-7.33 (m, 4H, Ar-imidazole), 7.19-6.79 (m, 3H, Ar- OCH_3), 3.56 (s, 3H, OCH_3) Figure A.41 (Appendix A).

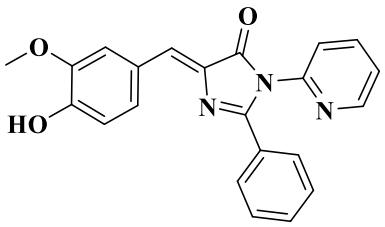
2.2.11 Preparation of (*Z*)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (11).



Adhering to the standard protocol for imidazolone synthesis, pure product 11 was obtained. Product mass was 1.2 g (yield 62.5%). R_f 0.37 (Ethyl acetate/Methanol 9.5:0.5). IR (ν in cm^{-1}): 3430 (OH), 3288 (tertiary amine), 3064 (=C-H), 2936 (C-H, OCH_3), 1763 (C=O, lactam), 1644 (C=N), 1598-1512 (C=C), 1371 (C-N), 1201 (C-O, OH) Figure A.11 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.98 (s, 1H, OH), 8.83-8.09 (m, 4H, Pyridine), 7.76-7.39 (m, 5H, Ar), 8.23 (s, 1H, CH=C), 7.24-7.07 (m, 3H, Ar- OCH_3), 6.77 (d, 2H, Pyridine-CH_2), 3.56 (s, 3H, OCH_3) Figure A.42 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 168.91 (N-C=O), 159.16 (N-C=N), 151.04 (Ar-O), 132.30 (=C-N), 137.06-128.06 (C_6H_5-), 56.55 (CH_3) Figure A.66

(Appendix A).

2.2.12 Preparation of (*Z*)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (12).

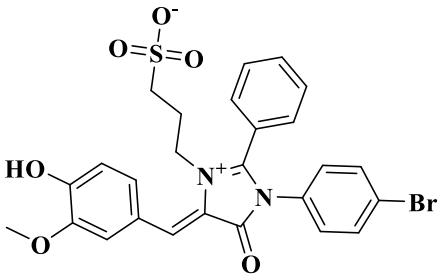


Adhering to the standard protocol for imidazolone synthesis, pure product 12 was obtained. Product mass was 1.00 g (yield 55%). R_f 0.40 (Ethyl acetate/Methanol 9.5:0.5). IR (ν in cm^{-1}): 3370(OH), 3167 (tertiary amine of pyridine), 2980 (=C-H), 2880 (C-H,OCH_3), 1700 (C=O, lactam), 1644 (C=N), 1567-1500 (C=C), 1410 (C-N), 1286-1165 (C-O, Ar-O-CH_3), 1244 (C-O, OH) Figure A.12 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.00 (s, 1H, OH), 8.04-7.87 (m, 4H, Pyridine), 7.58-7.47 (m, 5H, Ar), 7.45 (s, 1H, CH=C), , 7.15-6.48 (m, 3H, Ar- OCH_3), 3.58 (s, 3H, OCH_3) Figure A.43 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 166.80 (N-C=O), 160.01 (N-C=N), 149.72 (Ar-O), 132.34 (=C-N), 137.74-128.03 (C_6H_5-), 55.64 (CH_3) Figure A.67 (Appendix A).

General procedure for synthesis of imidazolone with alkyl sulfonate moiety.

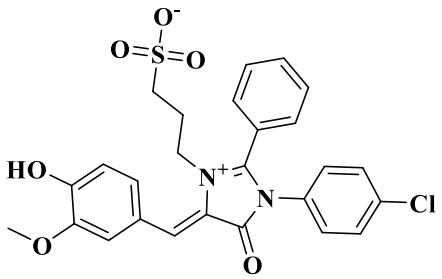
A solution of imidazolone (0.245 mmol) in THF (15.0 mL) was prepared in a round-bottom flask (50.0 mL). 1,3-propaneSultone or 1,4-butaneSultone (0.245 mmol) was added to the solution in the flask. The reaction mixture was agitated at room temperature while being watched over by TLC for one hour, or until the starting material was completely gone. A rotary evaporator was then used to remove the solvent at a lower pressure. The collected solid was rinsed with ethanol to remove residual starting materials.

2.2.13 Preparation of (*Z*)-3-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5- oxo-2- phenyl-4,5- dihydro-1H- imidazole -3-ium-3-yl) propane-1-sulfonate (13).



Following the general procedure for the synthesis of imidazolone with propyl sulfonate, pure product 13 was obtained, and the product mass was 0.1 g (yield 71.5%). IR (ν in cm^{-1}): 3500-3230 (OH), 2930 (=C-H), 1758 (C=O, lactam), 1639 (C=N), 1618-1531 (C=C), 1315 (C-N), 1248 (C-O,OH), 1278-1160 (CO, Ar-O-CH_3), 1194 (SO_3^- group), 800-600 (C-Br) Figure A.13 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.28 (s, 1H, OH), 8.04 (d, 2H, J = 7.7Hz, Ar-Br), 7.71 (d, 2H, J = 8.6Hz, Ar-Br), 7.60-7.40 (m, 5H, Ar), 7.35 (s, 1H, CH=C), 7.27-7.21 (m, 1H, Ar-H-C-OH), 7.17 (s, 1H, Ar-H-C-OCH_3), 7.11 (d, 1H, J = 8.2Hz, Ar-H-C-C=C), 4.45 (t, 2H, J=6.8Hz, propyl group $C\underline{H_2}CH_2CH_2$), 3.61 (s, 3H, OCH_3), 3.51-3.40 (m, 2H, propyl group $CH_2CH_2C\underline{H_2}$), 2.00-1.67(m, 2H, propyl group $CH_2C\underline{H_2}CH_2$) Figure A.44 (Appendix A).

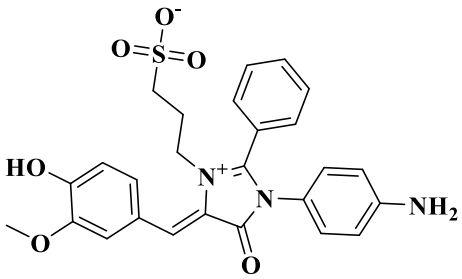
2.2.14 Preparation of (*Z*)-3-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl 4,5-dihydro -1H- imidazole -3- ium-3-yl) propane-1-sulfonate (14).



Following the general procedure for the synthesis of imidazolone with propyl sulfonate, pure product 14 was obtained, and the product mass was 0.1 g (yield 77%). IR (ν in cm^{-1}): 3363-3287 (OH), 2976 (=C-H), 1759 (C=O, lactam), 1641 (C=N), 1617-1508 (C=C), 1302 (C-N), 1280 (C-O, OH), 1280-1088 (CO, Ar-O-CH3), 1194 (SO_3^- group),

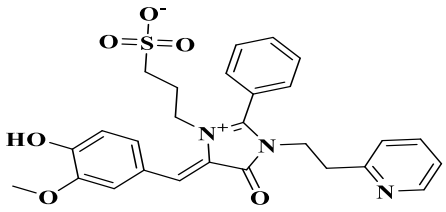
800-600 (C-Cl) Figure A.14 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.18 (s, 1H, OH), 8.04 (d, 2H, J = 7.7Hz, Ar-Cl), 7.75 (d, 2H, J = 8.4Hz, Ar-Cl), 7.59-7.35 (m, 5H, Ar), 7.00 (s, 1H, CH=C), 7.23 (d, 1H, J = 8.2Hz, Ar-H-C-OH), 7.15 (s, 1H, Ar-H-C-OCH_3), 7.11 (d, 1H, J = 8.2Hz, Ar-H-C=C), 4.45 (t, 2H, J=6.7Hz, propyl group $C\underline{H_2}CH_2CH_2$), 3.69 (s, 3H, OCH_3), 3.38 (t, 2H,J=7.7Hz, propyl group $CH_2CH_2C\underline{H_2}$), 2.23-1.96(m, 2H, propyl group $CH_2C\underline{H_2}CH_2$) Figure A.45 (Appendix A).

2.2.15 Preparation of (*Z*)-3-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5- dihydro -1H- imidazole -3- ium -3- yl) propane-1-sulfonate (15).



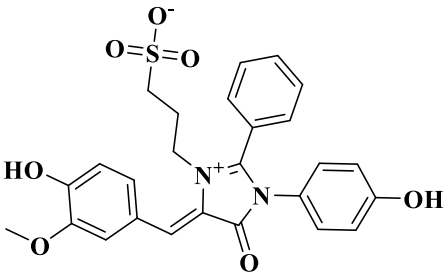
Following the general procedure for the synthesis of imidazolone with propyl sulfonate, pure product 15 was obtained, and the product mass was 0.09 g (yield 75%). IR (ν in cm^{-1}): 3597 (OH), 3300-3212 (primary amine), 2973 (=C-H), 1734 (C=O, lactam), 1635 (C=N), 1640-1511 (C=C), 1388 (C-N), 1209 (C-O,OH), 1290-1200 (C-O,Ar-O-CH_3), 1140 (SO_3^- group) Figure A.15 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm* : 10.04 (s, 1H, OH), 9.80 (s, 1H, CH=C),8.07-7.81 (m, 5H, Ar), 7.61-740 (m, 3H, Ar-OCH_3), 7.33-7.07 (m, 2H, Ar-NH_2), 6.78 (d, 2H, J = 8.1Hz, Ar-NH_2), 3.91 (s, 2H, NH_2), 4.05 (t, 2H, J=6.7Hz, propyl group $CH_2CH_2CH_2$) 3.91 (s, 3H, OCH_3), 2.69 (t, 2H, J=6.7Hz, propyl group $CH_2CH_2CH_2$), 2.03-1.94 (m, 2H, propyl group $CH_2CH_2CH_2$) Figure A.46 (Appendix A).

2.2.16 Preparation of (*Z*)-3-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (16).



Following the general procedure for the synthesis of imidazolone with propyl sulfonate, pure product 16 was obtained, and the product mass was 0.08 g (yield 63%). IR (ν in cm^{-1}): 3502 (OH), 3255 (tertiary amine of pyridine), 2973 (=C-H), 1752 (C=O, lactam), 1642 (C=N), 1594-1516 (C=C), 1353 (C-N), 1210 (C-O,OH), 1210-1185 (C-O, Ar-O-CH_3), 1185 (SO_3^- group) Figure A.16 (Appendix A).^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.86 (s, 1H, OH), 9.04 (dd, 1H, J = 29.3,6.2 Hz, Pyridine-H), 8.14-7.88 (m, 5H, Ar), 7.59 (t, 1H, J = 7.5Hz, Pyridine-H), 7.55 (s, 1H, CH=C), 7.53-7.44 (m, 2H, Pyridine-H), 7.19-7.12 (m, 3H, Ar- OCH_3), 4.45 (t, 2H, J=6.7Hz, propyl group $C\underline{H_2}CH_2CH_2$) 3.50 (s, 3H, OCH_3), 3.44-3.25 (m, 4H, Pyridine-CH_2CH_2), 2.03-1.88(m, 4H, propyl $CH_2C\underline{H_2}C\underline{H_2}$) Figure A.47 (Appendix A).

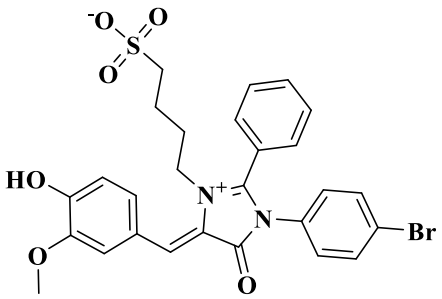
2.2.17 Preparation of (*Z*)-3-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (17).



Following the general procedure for the synthesis of imidazolones with propyl sulfonate, pure product 17 was obtained, and the product mass was 0.08 g (yield 65%). IR (ν in cm^{-1}): 3660 (OH), 2971 (=C-H), 1712 (C=O, lactam), 1641 (C=N), 1600-1515 (C=C), 1381 (C-N), 1210 (C-O, OH), 1286-1164 (C-O, Ar-O-CH_3), 1164 (SO_3^- group) Figure A.17 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.92 (s, 1H, OH), 9.81 (s, 1H, N-Ar-OH), 8.66 (s, 1H, CH=C), 8.06-7.98 (m, 4H, Ar-OH), 7.60-7.49 (m,

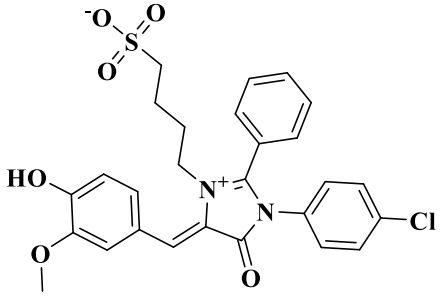
5H, Ar), 7.32 (s, 1H, Ar-H-C-OCH_3), 7.17 (d, 1H, J = 8.3Hz, Ar-H-C=C), 7.11 (d, 1H, J = 8.3Hz, Ar-H-C-OH), 4.44 (t, 2H, J=6.6Hz, propyl group $C\underline{H_2}CH_2CH_2$), 3.72 (s, 3H, OCH_3), 3.3 (t, 2H, J=7.8Hz, propyl group $CH_2CH_2C\underline{H_2}$), 2.03-1.92(m, 2H, propyl $CH_2C\underline{H_2}CH_2$) Figure A.48 (Appendix A).

2.2.18 Preparation of (*Z*)-4-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (18).



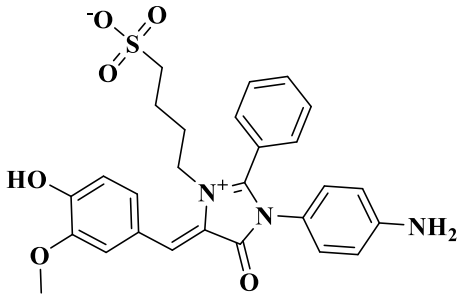
Following the general procedure for the synthesis of imidazolone with butyl sulfonate, pure product 18 was obtained, and the product mass was 0.15 g (yield 71.5%). IR (ν in cm^{-1}): 3520-3240 (OH), 2971 (=C-H), 1758 (C=O, lactam), 1640 (C=N), 1618-1532 (C=C), 1350 (C-N), 1248 (C-O, OH), 1275-1169 (CO, Ar-O-CH_3), 1191 (SO_3^- group), 800-600 (C-Br) Figure A.18 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.18 (s, 1H, OH), 8.04 (d, 2H, J = 7.6Hz, Ar-Br), 7.71 (d, 2H, J = 8.5Hz, Ar-Br), 7.60-7.41 (m, 5H, Ar), 7.28 (s, 1H, CH=C), 7.23-7.21 (m, 1H, Ar-H-C-OH), 7.17 (s, 1H, Ar-H-C-OCH_3), 7.11 (d, 1H, J = 8.3Hz, Ar-H-C-C=C), 4.46 (t, 2H, J=5.3Hz, butyl group $C\underline{H_2}CH_2CH_2CH_2$), 3.61 (s, 3H, OCH_3), 3.29 (t, 2H, J=6.1Hz, butyl group $CH_2CH_2CH_2C\underline{H_2}$), 2.06 (m, 2H, butyl group $CH_2CH_2C\underline{H_2}CH_2$), 1.75(m, 2H, butyl group $CH_2C\underline{H_2}CH_2CH_2$) Figure A.49 (Appendix A).

2.2.19 Preparation of (*Z*)-4-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (19).



Following the general procedure for the synthesis of imidazolone with butyl sulfonate, pure product 19 was obtained, and the product mass was 0.14 g (yield 70%). IR (ν in cm^{-1}): 3331-3202 (OH), 2971 (=C-H), 1764 (C=O, lactam), 1644 (C=N), 1596-1535 (C=C), 1302 (C-N), 1247 (C-O, OH), 1281-1169 (CO, Ar-O-CH3), 1190 (SO_3^- group), 800-600 (C-Cl) Figure A.19 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.29 (s, 1H, OH), 8.04 (d, 2H, J = 7.6Hz, Ar-Cl), 7.76 (d, 2H, J = 8.4Hz, Ar-Cl), 7.60-7.35 (m, 5H, Ar), 7.22 (s, 1H, CH=C), 7.23 (d, 1H, J = 8.2Hz, Ar-H-C-OH), 7.17 (s, 1H, Ar-H-C-OCH_3), 7.14-7.08 (m, 1H, Ar-H-C=C), 4.46 (t, 2H, J=5.4Hz, butyl group $C\underline{H_2}CH_2CH_2CH_2$), 3.61 (s, 3H, OCH_3), 3.32-3.26 (m, 2H, butyl group $CH_2CH_2CH_2C\underline{H_2}$), 2.06 (dq, 2H, J=12.2,5.6Hz, butyl group $CH_2CH_2C\underline{H_2}CH_2$), 1.75 (p, 2H, J=5.5Hz,butyl group $CH_2C\underline{H_2}CH_2CH_2$) Figure A.50 (Appendix A).

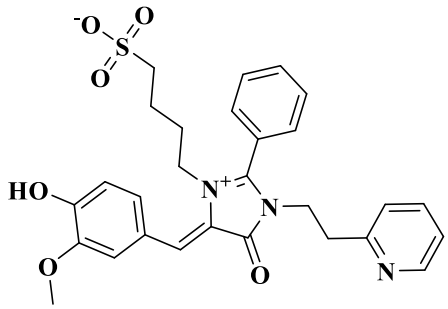
2.2.20 Preparation of (*Z*)-4-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (20).



Following the general procedure for the synthesis of imidazolone with butyl sulfonate, pure product 20 was obtained, and the product mass was 0.12 g (yield 63%). IR (ν in cm^{-1}): 3726 (OH), 3367-3205 (primary amine), 2970 (=C-H), 2887 (C-H,OCH_3), 1766

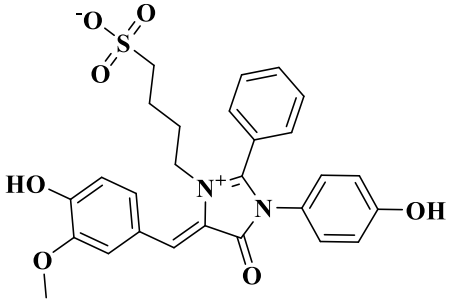
(C=O, lactam), 1638-1513 (C=C), 1404 (C-N),1267 (C-O, OH), 1267-1151 (C-O, Ar-O-CH_3), 1194 (SO_3^- group) Figure A.20 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.29 (s, 1H, OH), 9.85 (s, 1H, CH=C),8.07-7.81 (m, 5H, Ar), 7.40 (d, 1H, J = 9.6, Ar-H-C=C), 7.30 (d, 1H, J = 15.2Hz, Ar-H-C-OH), 7.19 (s, 1H, Ar-H-C-OCH_3) 7.14–7.06 (m, 4H, Ar-NH_2), 4.46 (s, 2H, NH_2), 3.60 (s, 3H, OCH_3), 3.28 (t, *J* = 5.9 Hz, 2H, butyl group $CH_2CH_2CH_2CH_2$), 2.49-2.23 (m, 2H, butyl group $CH_2CH_2CH_2CH_2$), 2.09–2.03 (m, 2H, butyl group $CH_2CH_2CH_2CH_2$), 1.78 – 1.72 (m, 2H, butyl group $CH_2CH_2CH_2$ CH_2). Figure A.51 (Appendix A).

2.2.21 Preparation of (*Z*)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (21).



Following the general procedure for the synthesis of imidazolone with butyl sulfonate, pure product 21 was obtained, and the product mass was 0.11 g (yield 61%). IR (ν in cm^{-1}): 3615 (OH), 3262 (tertiary amine of pyridine), 2888 (=C-H), 1763 (C=O, lactam), 1649 (C=N), 1622-1511 (C=C), 1302 (C-N), 1265 (C-O,OH), 1265-1121 (C-O, Ar-O-CH_3), 1190 (SO_3^- group) Figure A.21 (Appendix A).^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 9.98 (s, 1H, OH), 8.03 (d, 1H, J = 7.9Hz, Pyridine-H), 87.72-7.65 (m, 5H, Ar), 7.59 (t, 1H, J = 7.4Hz, Pyridine-H), 7.34 (s, 1H, CH=C), 7.29-7.25 (m, 2H, Pyridine-H), 7.20-7.13 (m, 3H, Ar- OCH_3), 4.46 (t, 2H, J=5.4Hz, butyl C$\underline{H_2}$$CH_2CH_2CH_2$) 3.35 (s, 3H, OCH_3), 3.29 (t, 4H , J=6.1 Pyridine-CH_2CH_2), 2.94(t, 2H, J=7.3Hz,butyl group $CH_2CH_2CH_2C\underline{H_2}$), 2.84-2.20(m, 2H, butyl group $CH_2CH_2C\underline{H_2}CH_2$), 2.00-1.93(m, 2H, butyl group $CH_2C\underline{H_2}CH_2$ CH_2) Figure A.52 (Appendix A).

2.2.22 Preparation of (*Z*)-4-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (22).



Following the general procedure for the synthesis of imidazolone with butyl sulfonate, pure product 22 was obtained, and the product mass was 0.13 g (yield 68.5%). IR (ν in cm^{-1}): 3593-3383 (OH), 3317 (tertiary amine), 3043 (=C-H), 2963 (C-H, OCH_3), 1757 (C=O, lactam), 1632 (C=N), 1632-1509 (C=C), 1350 (C-N),1217 (C-O, OH), 1267-1169 (C-O, Ar-O-CH_3), 1194 (SO_3^- group) Figure A.22 (Appendix A). ^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.08 (s, 1H, OH), 9.88 (s, 1H, N-Ar-OH), 9.22 (s, 1H, CH=C), 8.07-7.50 (m, 4H, Ar-OH), 7.59-7.50 (m, 5H, Ar), 7.39 (s, 1H, Ar-H-C-OCH_3), 7.20 (d, 1H, J = 9.9Hz, Ar-H-C=C), 6.71 (d, 1H, J = 8.3Hz, Ar-H-C-OH), 4.49-4.43 (m, 2H, butyl group $C\underline{H_2}CH_2CH_2CH_2$) 3.59 (s, 3H, OCH_3), 3.29 (t, 2H, J=6.1Hz, butyl $CH_2CH_2CH_2C\underline{H_2}$), 2.23-1.75(m, 4H, butyl group $CH_2C\underline{H_2}C\underline{H_2}CH_2$) Figure A.53 (Appendix A).

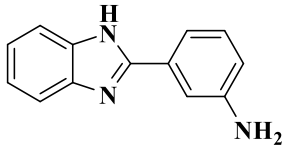
General procedure for synthesis of compounds (23, 25)

O-phenylenediamine (4.62 mmol), m-aminobenzoic acid (7.29 mmol) to yield compound 23, *p*-aminobenzoic acid (7.29 mmol) to yield compound 25, were placed in a round-bottom flask (100.0 mL) followed by addition of O-phosphoric acid (10.0 mL). The mixture was refluxed for 2 h at (180-200) °C. After cooling the mixture to a temperature of roughly 50 °C, it was poured over crushed ice and neutralized with a solution of NaOH (20.0 mL, 10%). The precipitate that had formed was gathered using suction filtration, cleaned with water and an excess of NaOH (10%) solution, dried, and then recrystallized from ethanol.

General procedure for synthesis of compounds (24, 26)

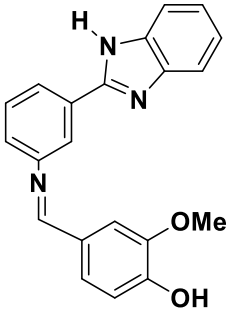
Vanillin (2.39 mmol), 3-(1H-benzo[d]imidazol-2-yl) aniline 23 (2.39 mmol) to yield compound 24, 4-(1H-benzo[d]imidazol-2-yl)aniline 25 (2.39 mmol) to yield compound 26 , were placed in a round-bottom flask (100.0 mL), followed by addition of acetic acid (10.0 mL) and a pinch of silica jel as catalyst. For 2 h, the mixture was refluxed. The mixture was then given partial cooling. The product was then obtained by distilling the solvent in a rotary evaporator at a low temperature.

2.2.23 Preparation of 3-(1H-benzo[d]imidazol-2-yl)aniline (23).



Following the general procedure , pure product 23 was obtained, product mass was 0.5 g (yield 52%), m.p =245-248 °C. IR (ν in cm^{-1}): 3374 (NH benzimidazole); 3243, 3113 (NH_2 aminophenyl), 2924 (CH arom), 1641 (C=N), 1611 (C=C arom) Figure A.23 (Appendix A).

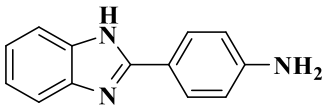
2.2.24 Preparation of 4-(((3-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (24).



Following the general procedure, pure product of 24 was obtained, product mass was 0.1 g (yield 62.5%), m.p =187-191°C. IR (ν in cm^{-1}): 3735 (NH benzimidazole), 3601 (OH), 3070 (CH arom), 1670 (C=N), 1591 (C=C arom), 1288 (C-O, OH), 1288-1125 (C-O, Ar-O-CH_3) Figure A.24 (appendix A).^{1}H NMR (500 MHz, DMSO-D6) δ in *ppm*: 12.91 (s, 1H, NH), 9.75 (s, 1H, OH), 8.49 (s, 1H, CH=N), 8.03-7.11(m, 10H, Ar-H), 6.94 (s, 1H, Ar-H-C-OCH_3), 3.16 (s, 3H, OCH_3) Figure A.54 (Appendix A). ^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 169.05 (CH=N), 151.67 (C-N=C), 148.7(Ar-C-OH),

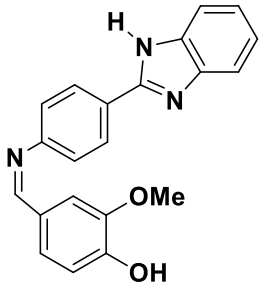
148.45(Ar-C-OCH_3), 140.30 (C=C of imidazole ring), 129.75-111.09 (C_6H_5-) , 56.18 (CH_3) Figure A.68 (Appendix A).

2.2.25 Synthesis of 4-(1H-benzo[d]imidazol-2-yl)aniline (25).



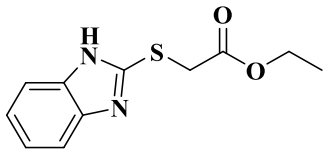
Following the general procedure , pure product 25 was obtained, product mass was 2.5 g (yield 62%), melting point =239-243 °C. IR (ν in cm^{-1}): 3375 (NH benzimidazole), 3230-3167 (NH_2 aminophenyl), 2923 (CH arom), 1639 (C=N), 1611 (C=C arom) Figure A.25 (Appendix A).

2.2.26 Preparation of (*Z*)-4-(((4-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (26).



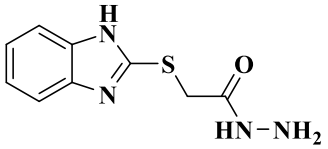
Following the general procedure, pure product of 26 was obtained, product mass was 0.45 g (yield 55%), and melting point =228-234 °C. IR (ν in cm^{-1}): 3506 (NH benzimidazole); 3201 (OH), 2837 (C-H, OCH_3), 1665 (C=N), 1590-1509 (C=C), 1298 (C-N), 1265 (C-O, OH), 1298-1123 (C-O,Ar-O-CH_3) Figure A.26 (Appendix A).^{1}H NMR (500 MHz, DMSO-d6) δ in *ppm*: 10.21 (s, 1H, NH), 9.73 (s, 1H, OH), 7.95 (s, 1H, CH=N), 7.76-7.2 (m, 10H, Ar-H), 6.96 (s, 1H, Ar-H-C-OCH_3), 3.82 (s, 3H, OCH_3) Figure A.55 (Appendix A).^{13}CNMR (125 MHz, DMSO-d6) δ in *ppm*: 171.25 (CH=N), 153.53 (C-N=C), 148.60 (Ar-C-OH), 144.22(Ar-C-OCH_3), 139.29 (C=C of imidazole ring), 129.14-111.05 (C_6H_5-) , 56.02 (CH_3) Figure A.69 (Appendix A).

2.2.27 Preparation of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate (27).



2-Mercaptobenzimidazole (10 g, 66.66 mmol) and potassium hydroxide (3.7 g, 6.07 mmol) were placed in a round–bottom flask (500 mL), followed by the addition of ethanol (140.0 mL). The mixture was refluxed for 15 min at 80–90 °C along with stirring. After that a 7.0 mL Ethyl chloroacetate was added at once, which caused an exothermic reaction and a temperature increase of 30 to 40 °C. The mixture was added to 100 g of ice after being stirred for 24 h at 18 to 20 °C. The mixture was continuously stirred for 30 minutes while keeping the temperature 0–10 °C. The formed precipitate (white solid) was collected by suction filtration, washed to render it free of chloride, dried and recrystallized from ethanol. The product mass was 9 g (yield 57%) , melting point = 96-99 °C. IR (ν in cm^{-1}): 3044 (NH benzimidazole), 2929 (CH arom), 1742 (C=O), 1617 (C=N), 1494-1222 (C=C), 1299 (C-N), 617 (C-S). Figure A.27 (Appendix A).

2.2.28 Preparation of 2-((1H-benzo[d]imidazol-2-yl)thio) acetohydrazide (28).



Ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate 27 (3.5g, 14.82 mmol), and hydrazine hydrate (4.3 mL, 88.94 mmol) were placed in a round-bottom flask (250 ml), followed by addition of ethanol 30.0 mL. The mixture was refluxed for 2.5h. The reaction progress was followed by TLC. The mixture was concentrated and kept overnight in a refrigerator. The formed precipitate (white) was separated from the mother liquor, dried and recrystallized from boiling water in order to obtain the pure compound 28. Product weight was 1.6 g (yield 50%), melting point = 164-167 °C. IR (ν in cm^{-1}): 3314 (NH benzimidazole), 3265 (primary amine), 2989 (CH arom), 1664 (C=O) ,1656 (C=N), 1457-1226 (C=C), 1267 (C-N), 620 (C-S). Figure A.28 (Appendix A).

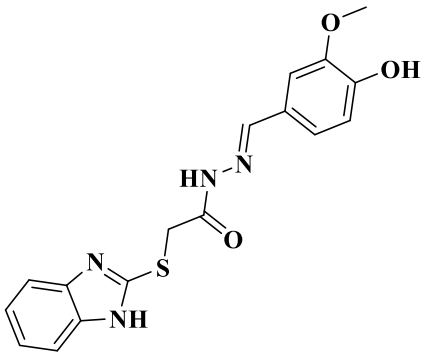
General procedure for synthesis of compounds (29, 31)

Ethyl-2-(1H-benzo[d] imidazol-2-ylthio) acetohydrazide 28 (0.4g, 1.8 mmol), vanillin (0.27g, 1.8 mmol) to yield compound 29, 6-chloroindol-3-carboxaldehyde (0.32g, 1.8 mmol) to yield compound 31 and 25 mL ethanol were placed in a round-bottom flask (100.0 mL), followed by addition few drops of glacial acetic acid as a catalyst. The mixture was refluxed overnight. TLC verified that the reaction had finished. The excess solvent was distilled off in a rotating evaporator at a low temperature. The final product was washed with dilute ethyl alcohol and recrystallized from rectified spirit.

General procedure for synthesis of compounds (30, 32)

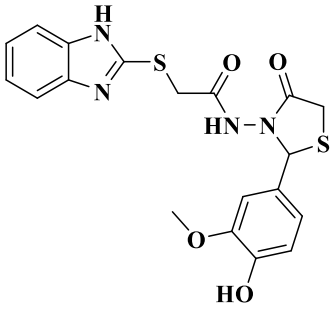
Products (30, 32)were synthesized by refluxing the appropriate Schiff base (29, 0.015 M) to yield compound 30, (31, 0.015 M) to yield compound 32 with thioglycolic acid (0.015 M) for 8–10 h in 20.0 mL acetic acid using a pinch of silica gel as a catalyst. Using TLC, the reaction's endpoint was determined. The mixture was then allowed to cool to room temperature. The product was then obtained by distilling the solvent in a rotary evaporator at a low temperature.

2.2.29 Preparation of Schiff's bases (*E*)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-(4-hydroxy-3- methoxybenzylidene) acetohydrazide (29).



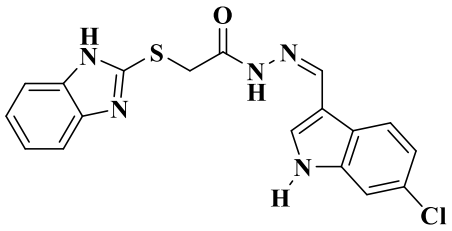
Following the general procedure, pure product 29 was obtained, product mass was 0.4 g (yield 63 %). IR (ν in cm^{-1}): 3360 (NH benzimidazole), 3050 (OH), 2970 (C-H, OCH_3), 1669 (C=O for secondary amide), 1677 (C=N), 1582-1515 (C=C), 1281 (C-N), 1212 (C-O, OH), 1281-1121 (C-O, Ar-O-CH_3), 616 (C-S). Figure A.29 (Appendix A).

2.2.30 Preparation of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide (30).



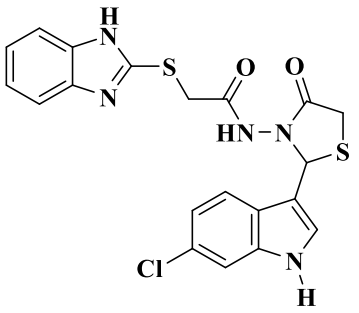
Following the general procedure , pure product 30 was obtained after refined through the use of silica gel column chromatography. Product mass was 0.25 g (yield 52 %), melting point =120-125 °C. IR (ν in cm^{-1}): 3379 (NH benzimidazole), 3252 (OH), 2917 (C-H, OCH_3), 1661 (C=N), 1584 (C=O of thiazolidinone), 1584-1511 (C=C), 1476 (ring of thiazolidinone), 1280 (C-N), 1280-1031 (C-O, Ar-O-CH_3),612 (OCN). Figure A.30 (Appendix A). ^{1}HNMR (500 MHz, DMSO-d6) δ in *ppm*: 9.93 (s, 1H, NH benzimidazole), 9.68 (s, 1H, NH of amide), 7.92 (s, 1H, OH), 7.42 – 6.81 (m, 8H, aromatic), 6.78 (s, 1H, CH of thiazolidinone), 3.86 (s, 2H, -CH_2), 3.47 (s, 3H, OCH_3). Figure A.56 (Appendix A).

2.2.31 Preparation of (*Z*)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-((6-chloro-1H-indol-3-yl)methylene)acetohydrazide (31).



Following the general procedure, pure product 31 was obtained. Product mass was 0.4 g (yield 65.5%). IR (ν in cm^{-1}): 3362 (NH benzimidazole), 2974 (C-H, OCH_3), 1679 (C=O for secondary amide), 1634 (C=N), 1615-1549 (C=C), 1318 (C-N),1248 (C-O, OH), 1248-1077 (C-O, Ar-O-CH_3), 631 (C-S), 600-800 (C-Cl). Figure A.31 (Appendix A).

2.2.32 Preparation of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(6-chloro-1H-indol-3-yl)-4-oxothiazolidin-3-yl)acetamide (32).



Following the general procedure, pure product 32 was obtained after purified by silica gel column chromatography. Product mass was 0.25 g (yield 53 %). IR (ν in cm^{-1}): 3420 (NH benzimidazole), 2986 (C-H, OCH_3), 1641 (C=N), 1575 (C=O of thiazolidinone), 1575-1527 (C=C), 1453 (ring of thiazolidinone), 1283 (C-N),1267 (C-O,OH), 1267-1061 (C-O, Ar-O-CH_3), 612 (OCN), 600-800 (C-Cl). Figure A.32 (Appendix A). ^{1}HNMR (500 MHz, DMSO-d6) δ in *ppm*: 12.42 (s, 1H, NH benzimidazole), 9.92 (s, 1H, NH of amide), 9.67 (s, 1H, NH of pyrrole), 7.58-6.91 (m, 8H, aromatic), 6.05 (s, 1H, CH of thiazolidinone), 3.87 (s, 2H, -CH_2), Figure A.57 (Appendix A).

2.3 Anticancer activity

2.3.1 Cell line

We analyzed the cytotoxicity of our samples on multiple cancer types cell lines, human liver cancer cell (Hep3B) cells, cervical adenocarcinoma (HeLa) cells, and normal liver cells (LX2).

2.3.2 Cell culture and cytotoxicity test

The cell line was cultured in T-175 cell culture flasks with RPMI basal medium supplemented with 1% L-glutamine as the initial step in the cell culture process, FBS (10%), and penicillin/streptomycin (1%). At 37°C and 99% humidity, the cells were maintained at 5% CO_2 in a typical cell culture incubator.

After removing the medium, it was cleaned using Ca^{2+}-free PBS before subculture. After that, cells were kept in an incubator containing 0.025% trypsin for a maximum of five minutes, or until a sufficient number of cells detached. Trypsin was rendered

inactive *via* CGM. Trypan blue stain was used to count the viable cells in the cell suspension after they were collected. This was done before adjusting the cell concentration to 74, 59, and 103 for HeLa, LX2, and Hep3B cells respectively. In the 96-well plate, cells were seeded at 5000 cells per well. Before conducting the tests, the cells were given an overnight period to adhere and accommodate. After 24 hours, we prepared different concentrations of our extract. These concentrations are 500 ug/ml, 250 ug/ml, 125 ug/ml, 62.5 ug/ml, and 31.25 ug/ml under 7.4 pH conditions. In addition to that we added 500 ug/ml of 5-FU as a positive control to our experiment. After that, we added 100 µl of each concentration per well. After 48 hours, we added 20 µl of MTS reagent to each well and incubated it for 2 hours in the incubator. Then, we measured the absorbance at 490 nm using the pleat reader.

Chapter Three

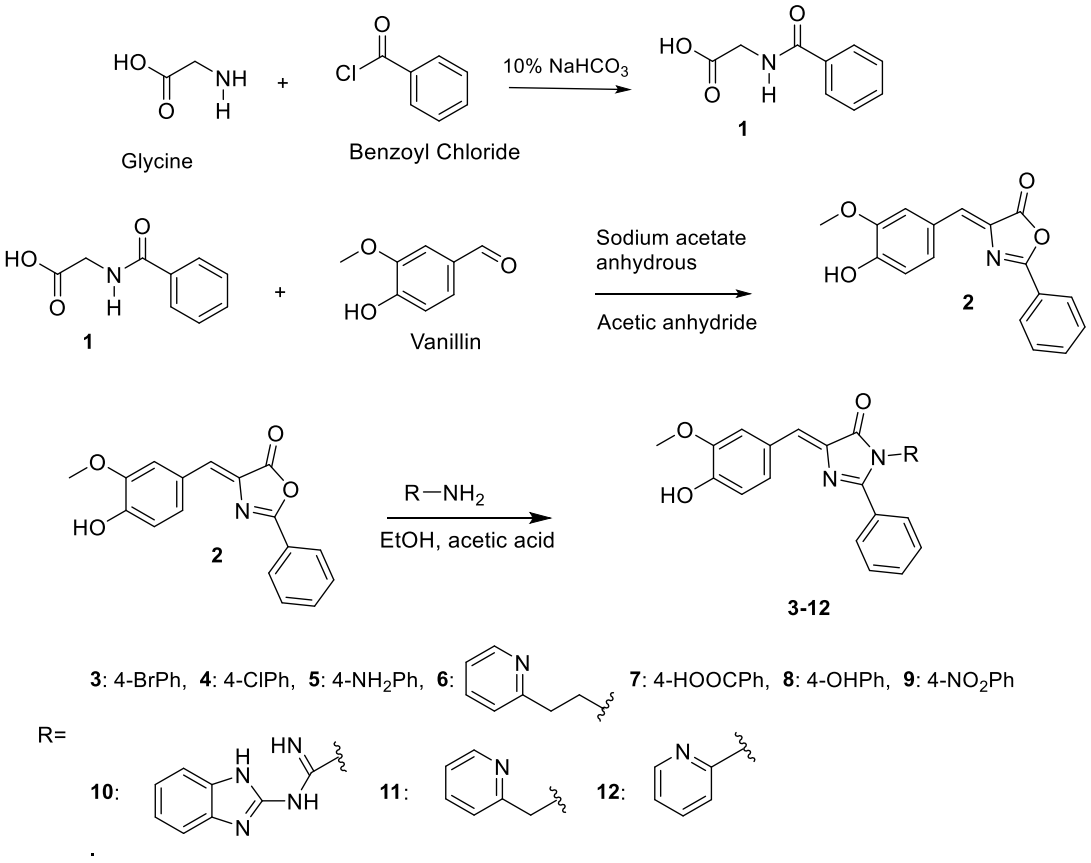
Results and Discussion

3.1 Synthesis of imidazolones

The imidazolone derivatives presented in this work were synthesized according to the strategy summarized in Scheme 3.1. The multistep process involved three steps with a total yield ranging from 50 to 70%. The first step involved a direct condensation reaction between glycine and benzoyl chloride to form hippuric acid 1. The second step involved preparation of 5-oxazolone 2 from reacting hippuric acid 1 with vanillin in the presence of sodium acetate and acetic anhydride used as dehydrating agent. This step involved a condensation cyclization reaction with a loss of water molecules from 5-oxazolone 2. Compound 2 was then converted to the final product imidazolones (3-12) by reacting them with various primary aromatic amines. During the reaction, amine makes a nucleophilic addition at the carbonyl group in compound 2 causing it to undergo ring opening, followed by condensation cyclization that results in a loss of water molecule and formation of the target product. Several spectroscopic techniques were used to identify the structures of each derivative. Various aromatic primary amines were utilized to investigate how different substituents affect the bioactivity.

Scheme 3.1

Synthesis of imidazolones derivatives



3.1.1 Mechanisms of reactions

3.1.1.1 Synthesis of benzoylglycine (1)

In the first stage, the carbonyl group of benzoyl chloride is attacked by the nitrogen of glycine. Then, there is a loss of Cl^- group as a good leaving group, and the reaction is completed by the abstraction of a proton to yield the product as demonstrated in Figure C.1 (Appendix C).

3.1.1.2 Synthesis of Oxazolone (2)

First, sodium acetate removes a proton from benzoylglycine to form oxide. Next, an oxide attacks the carbonyl group of acetic anhydride, followed by protonation to form a double bond. The aldehyde's carbonyl is then attacked, and oxazolone 2 is produced as a result of the loss of one water molecule as shown in Figure C.2 (Appendix C).

3.1.1.3 Synthesis of imidazolones

The first step in the mechanism is protonation, then the amine makes a nucleophilic addition at the carbonyl group in oxazolone 2 cause it to undergo ring opening, followed by condensation cyclization that results on a loss of water molecule and formation of the target product as shown in Figure C.3 (Appendix C).

3.1.2 Characterization

The IR spectra of benzoyl glycine 1 showed an absorption band at 3339cm^{-1} due to N-H stretching of amide, 3071 cm^{-1} corresponding to O-H stretch of carboxylic acid, 2930-2670 cm^{-1} due to CH_2 group, 1743 cm^{-1} due to carbonyl stretching, 1600-1490 cm^{-1} due to aromatic ring, and finally 600-580 cm^{-1} due to C-C=O. The IR spectra of oxazolone 2 revealed an absorption band at 1790-1750 cm^{-1} due to carbonyl stretching of lactone , 1650 cm^{-1} due to -C=N stretching, 1606-1553 cm^{-1} due to C=C aromatic stretching, 1119 cm^{-1} due to C-O-C stretching, 1326-1286 cm^{-1} due to C-N stretching, and finally 1271 cm^{-1} due to C-O, OCH_3 stretching .

The ^{13}CNMR (δ) spectra for compound 2 showed peak at 167.32 *ppm* due to carbonyl group of oxazolone, 163.61 *ppm* due to -O-C=N, 151.45 *ppm* due to Ar-C-OCH_3, 142.06 *ppm* due to Ar-C-OH, 133.4-132.71 *ppm* due to =C-N, 130.5-126.02 *ppm* due to phenyl group, 56.33 *ppm* due to substituted methyl group. The ^{1}HNMR (δ) spectra showed peak at 9.81 due to *ortho*- substituted OH, 8.09 due to –CH=, 7.62-6.96 due to phenyl group, 3.91 due to methoxy group.

Followed by oxazolone 2 was condensed with a series of primary amines that yielded corresponding substituted imidazolones derivatives 3-12. The IR spectra of derivatives revealed an absorption band at 1770-1731 cm^{-1} due to carbonyl stretching of lactam,1671-1638 cm^{-1} due to -C=N stretching, 1617-1500 cm^{-1} due to C=C aromatic stretching, 1410-1311 cm^{-1} due to -C-N stretching. and finally 1271-1016 cm^{-1} due to

substituted methyl group .

The ^{13}CNMR (δ) spectra showed peak at 169.91-166.5 *ppm* due to carbonyl group of imidazolone, 185.67-156.2 ppm due to N-C=N, 151.07-149.29 ppm due to Ar-C-OCH_3, 154.04-139.99 *ppm* due to Ar-C-OH, 133.7-130.5 *ppm* due to =C-N, 137.74-128.03 *ppm* due to phenyl group, 56.55-55.64 ppm due to substituted methyl group . The ^{1}HNMR (δ) spectra of all the derivatives were showed peak at 10-9.22 due to *ortho*-substituted OH, 9.6-8.44 due to –CH=, 7.65-6.5 due to the phenyl group, 3.85-3.57 due to methoxy group. 3,4,5,7,8 ,9 showed peak at 8-6.5 due to substituted phenyl group, while compounds 6, 10, 11, 12 showed a peak at 8.83-7.19 due to the substituted pyridine ring .

3.1.3 Biological activity

All prepared compounds were submitted to the pharmaceutical Department at An-Najah University (Nablus, Palestine) for anticancer activity against the liver cancer cell (Hep3B) cells, cervical adenocarcinoma (HeLa) cells and the normal liver cells LX2. The preliminary *in vitro* anticancer screening results are summarized in Table 3.1. The test was carried out in five doses against the three selected cell lines using a five series of two-fold dilutions from a stock solution with 500 μg/mL (500.0, 250.0, 125.0, 65.5, and 32.25 μg/mL). A 48 h of incubation with the tumor cell lines was conducted. The results were expressed for each cell line from triplicate response parameters, IC_{50} (concentration required to kill 50% of cancer cells) was determined. Results are summarized in Table 3.1.

Table 3.1

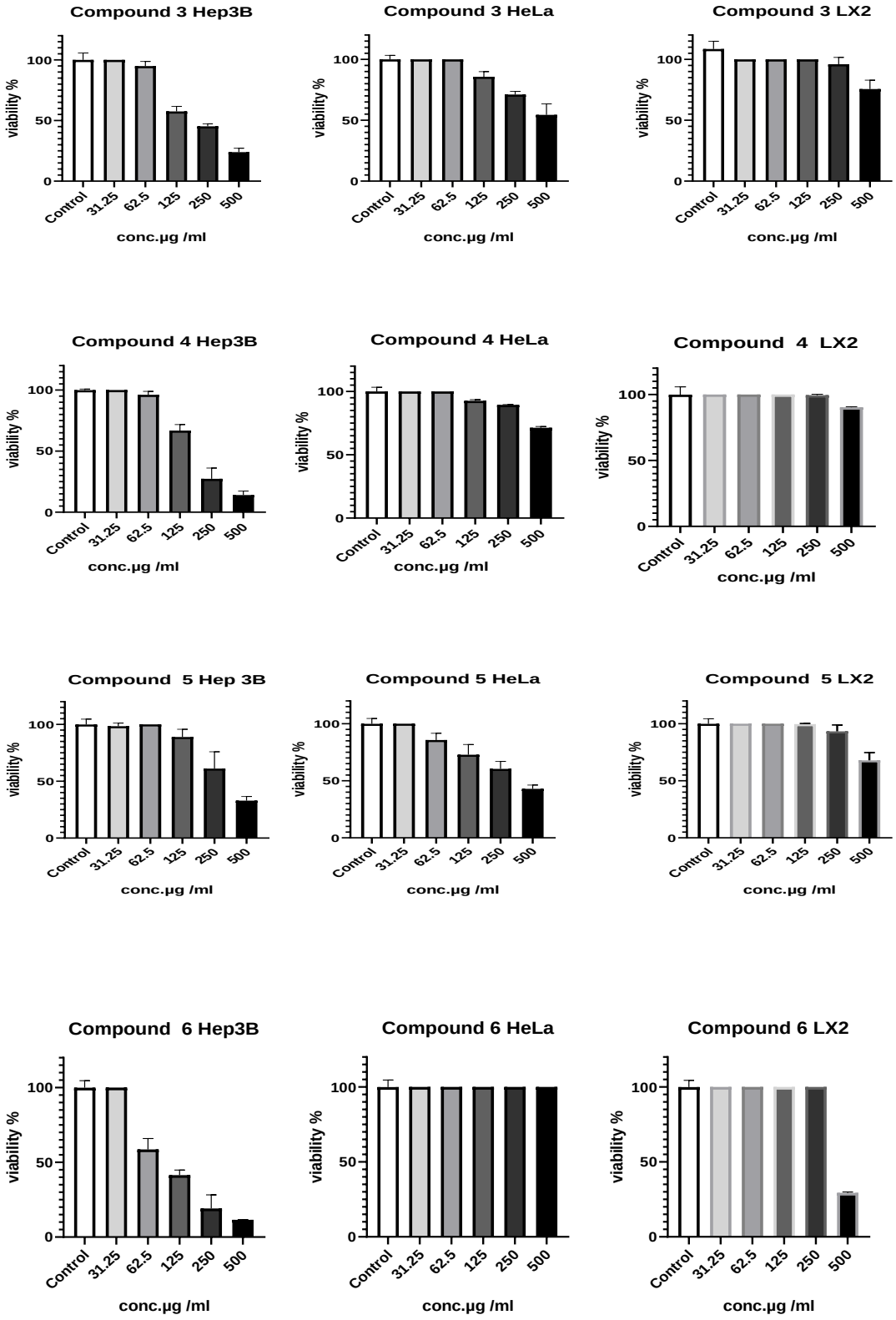
IC_{50} imidazolones derivatives compounds on different cancer cells (Hep3B,HeLa and LX2 normal cell)

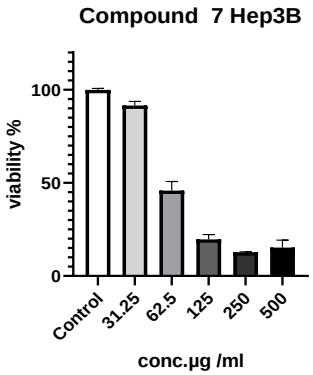
Imidazolone	IC_{50} µg/mL		
	Hep3B	HeLa	LX2
3	120.11 ± 0.15	227.56 ± 0.02	613.62 ± 0.23
4	140.55 ± 0.23	931.25 ± 0.17	1234.21 ± 0.03
5	292.15 ± 0.35	293.35 ± 0.35	256.71 ± 0.08
6	102.15 ± 0.25	-	479.60 ± 0.21
7	64.45 ± 0.28	82.89 ± 0.04	99.76 ± 0.08
8	492.32 ± 0.21	131.44 ± 0.39	780.22 ± 0.16
9	55.74 ± 0.14	98.28 ± 0.08	65.66 ± 0.45
10	141.81 ± 0.07	139.33 ± 0.47	110.10 ± 0.51
11	131.38 ± 0.28	32.80 ± 0.11	65.81 ± 0.22
12	677.25 ± 0.03	586.90 ± 0.05	384.35 ± 0.31

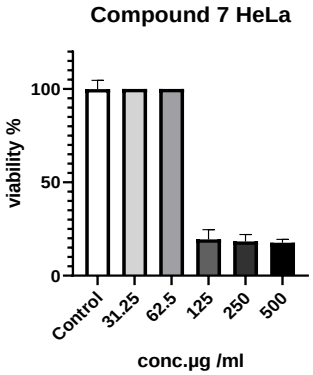
The majority of the prepared imidazolones 3, 4, 5, 7, 8, 9, 11 with the substituents -Br, -Cl, $-NH_2$, $-NO_2$, -COOH, -OH on the 4-position of imidazolone ring and the2-methyl pyridine group demonstrated strong anti-cancer properties against the examined cancer cells. (Hep3B and Hela cell) with low cytotoxicity against the normal cells. Imidazolone 6 with the 2-ethyl pyridine group showed no activity against HeLa cell, compound 12 with a pyridine ring showed poor activity against Hep3B and HeLa cell. Compound 11 with a similar structure to 12 with an extra methylene group showed remarkable activity against Hela cells. It exhibited remarkable growth inhibition with an IC_{50}=32.80 ± 0.11 µg/mL and cytotoxicity over 95% at a concentration of 250 µg/ml**.** These results could be attributed to the steric hindrance that prevented the pyridine for associating with the target. However adding methylene groups extended the chain length, reduced the steric hindrance and enhanced the interaction between the pyridine ring and the target. The results also demonstrated that, compound 9 with a NO_2 group on position 4 showed the highest anticancer activity against Hep3B cells with **(**IC_{50} = 55.75 ± 0.14 µg/mL), it showed a growth inhibition of tested cancer cells of over 85% at 250 µg/mL**.** The presence of the nitro group, the results could be related to the high interaction with target of the tested cancer cell. The results also indicate very low cytotoxicity of the tested compounds 3,4,5,6, the cytotoxicity against the tested normal cells LX2 was very low for most compounds as shown in Figure 3.1.

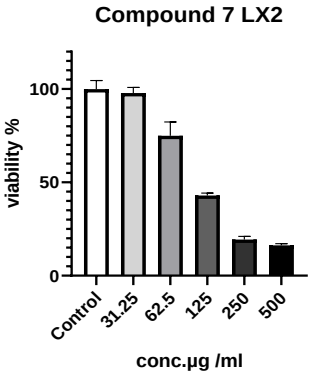
Figure 3.1

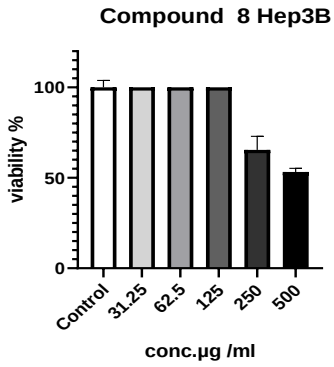
Imidazolones compounds viability against the tested cells

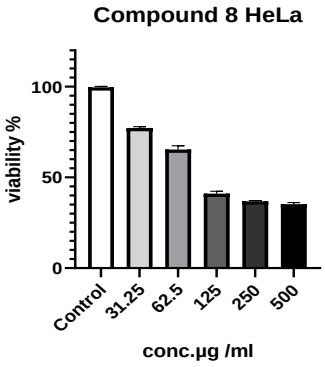


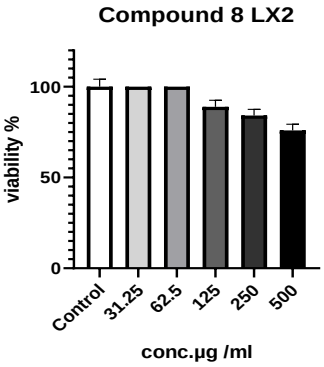

Compound 7 Hep3B
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

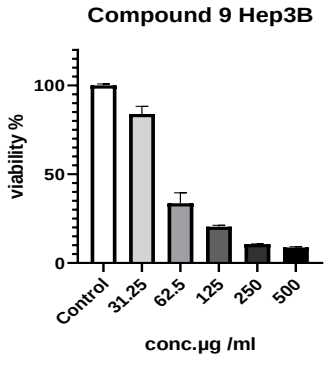

Compound 7 HeLa
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

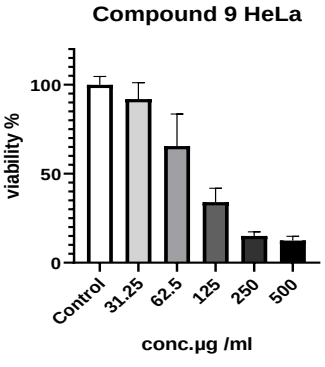

Compound 7 LX2
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

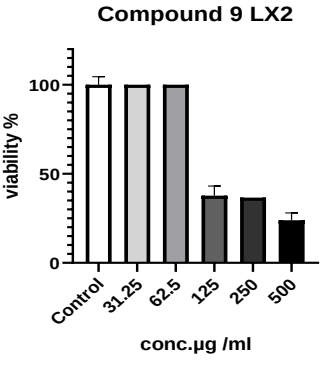

Compound 8 Hep3B
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

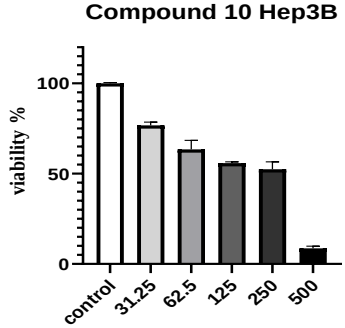

Compound 8 HeLa
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

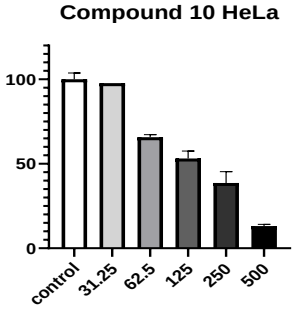

Compound 8 LX2
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

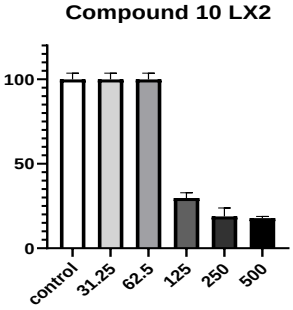

Compound 9 Hep3B
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml

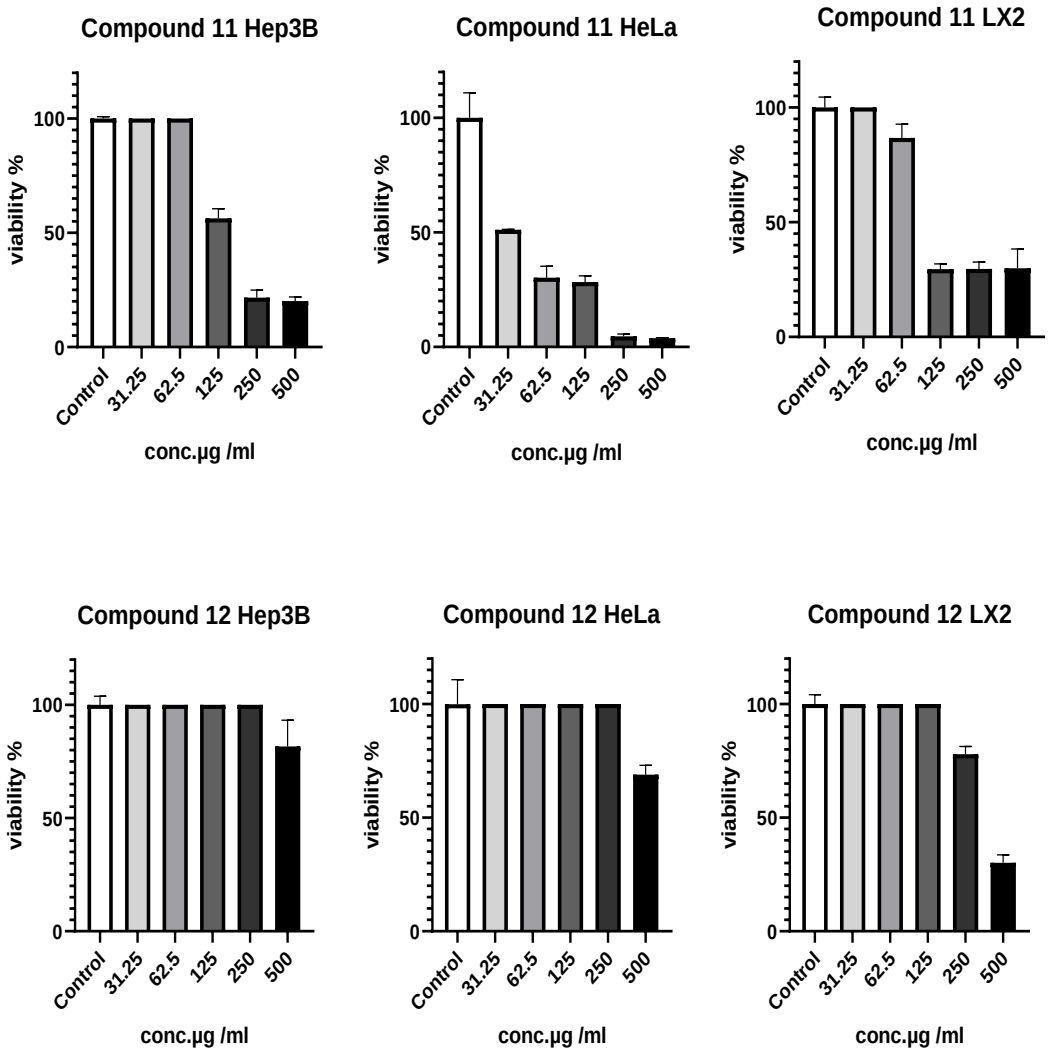

Compound 9 HeLa
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml


Compound 9 LX2
viability %
100
50
0
Control
31.25
62.5
125
250
500
conc.µg /ml


Compound 10 Hep3B
viability %
100
50
0
control
31.25
62.5
125
250
500


Compound 10 HeLa
100
50
0
control
31.25
62.5
125
250
500


Compound 10 LX2
100
50
0
control
31.25
62.5
125
250
500

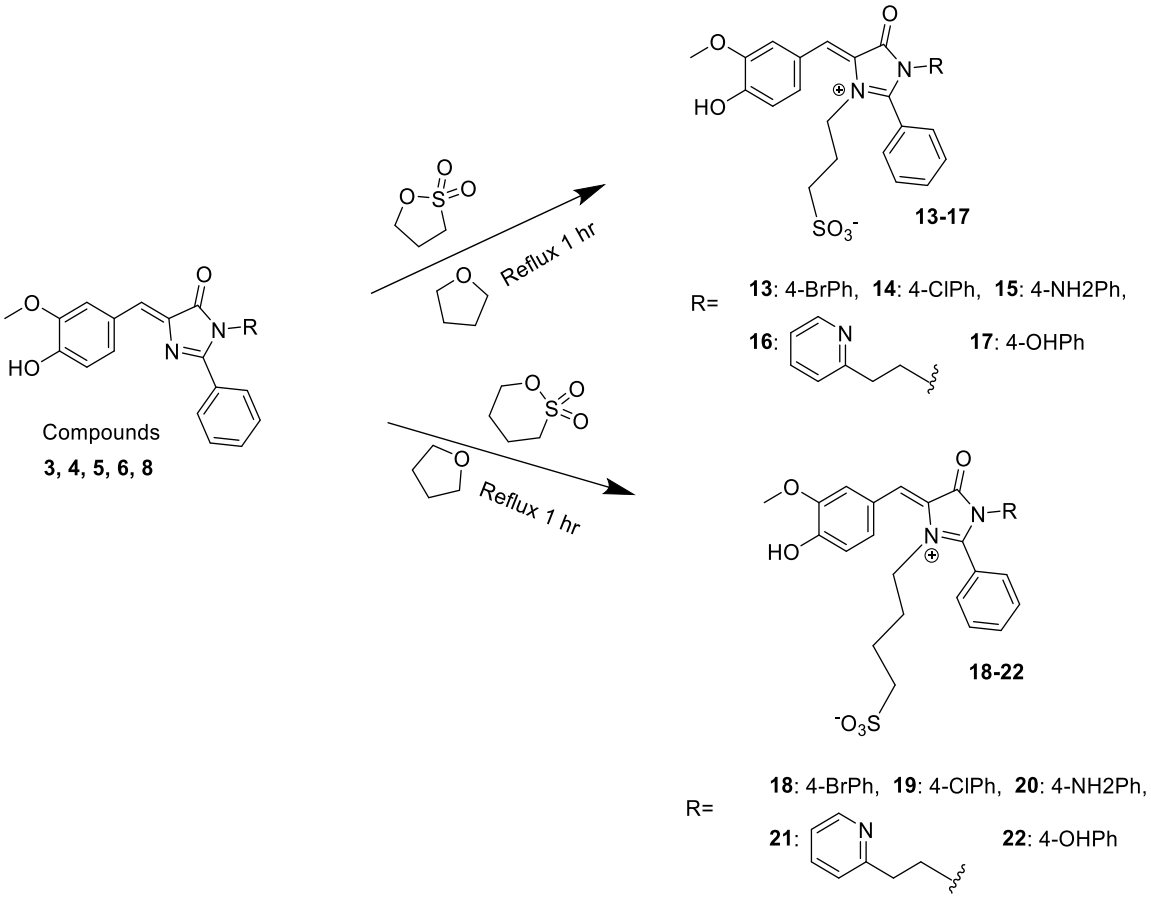


3.2 Synthesis of imidazolone with alkyl sulfonate moiety

After testing all the prepared imidzolone, we decided to test the possibility of enhancing the bioactivity of some imidazolone like compound 3 by adding another functionality that makes it more polar and water soluble with high bioavailability. An alkyl Sulfonate moiety was added to some imidazolones by reacting them with various Sultones. The reaction was performed by mixing equimolar of imidazolone with the Sultone in THF and refluxing the solution for one hour. The yield of synthesized benzimidazole ranged from 61% to 77%. A summary of the reaction and generated substances are enumerated in Scheme 3.2.

Scheme 3.2

Formation of imidazolone with alkyl sulfonate moiety



3.2.1. Mechanism of reaction

Imidazolone undergoes S_N2 reaction with Sultone to produce the target product in excellent yield as shown in Figure C.4 (Appendix C).

3.2.2. Characterization

3.2.2.1 Imidazolones with 1,3-Propane Sultone derivatives (13-17).

The derivatives' infrared spectra revealed an absorption band at 3660-3220 cm^{-1} due to OH substituted, 2973-2900 cm^{-1} due to –CH=, 1760-1712 cm^{-1} due to carbonyl stretching of lactam, 1645-1631 cm^{-1} because of -C=N stretching, 1640-1500 cm^{-1} because of C=C aromatic stretching, 1388-1300 cm^{-1} because of -C-N stretching, and finally 1194-1140 cm^{-1} due to SO_3^- group. The ^{1}HNMR (δ) spectra of all the derivatives showed peak at 10.28-9.81 due to *ortho* substituted OH, 8.51-8.04 due to –CH=, 7.94-6.69 due to phenyl group , 4.47-1.98 due to CH_2-CH_2-CH_2-SO_3^-, 3.91-3.50 due to methoxy group.

3.2.2.2 imidazolones with 1,4-Butane sultone derivatives (18-22)

The derivatives' infrared spectra revealed an absorption band at 3720-3200 cm^{-1} due to OH substituted, 3043-2800 cm^{-1} due to –CH=, 1761-1750 cm^{-1} due to carbonyl stretching of lactam, 1645-1632 cm^{-1} because of -C=N stretching, 1638-1509 cm^{-1} because of C=C aromatic stretching, 1404-1310 cm^{-1} because of -C-N stretching, and finally 1195-1185 cm^{-1} due to SO_3^- group. The ^{1}HNMR (δ) spectra of all the derivatives showed peak at 10.29-9.98 due to ortho substituted OH, 9.88-8.06 due to –CH=, 7.83-6.71 due to phenyl group, 4.49-1.18 due to CH_2-CH_2-CH_2-CH_2-SO_3^-, 3.64-3.35 due to methoxy group.

3.2.3 Anticancer evaluation for zwitterionic imidazolones

Selective imidazolnes with 1,3-propane Sultone moiety were synthesized 13, 14, 17. All showed improved anti-cancer activities against the tested cancer cells (Hep3B and HeLa cell). Compound 13 with 1,3-prpoane Sultone showed remarkable activity against HeLa cells compared to compound 3, it exhibited remarkable growth inhibition (IC_{50}= 105.4 ± 0.10 µg/mL) Table 3.2 with a cytotoxicity over 70% at a concentration of 250 µg/mL. The results also showed that compound 14 with the sulfate group showed the highest anticancer activity against Hep3B cells with IC_{50} of 134.70 ± 0.31 µg/mL, it showed a growth inhibition of tested cancer cells of over 67% at 250 µg/mL**.** Its activity toward the HeLa cells improved a lot by adding the alkyl sulfonate group. The improved in the activity could be related to the increase in polarity and water solubility of the compounds that enhanced the interaction with target of the tested cancer cell. Table 3.2 summarizes the value of IC_{50} of compounds with alkyl sulfonate group. Figure 3.2 shows the viability against tested cells for compounds 13,14,17.

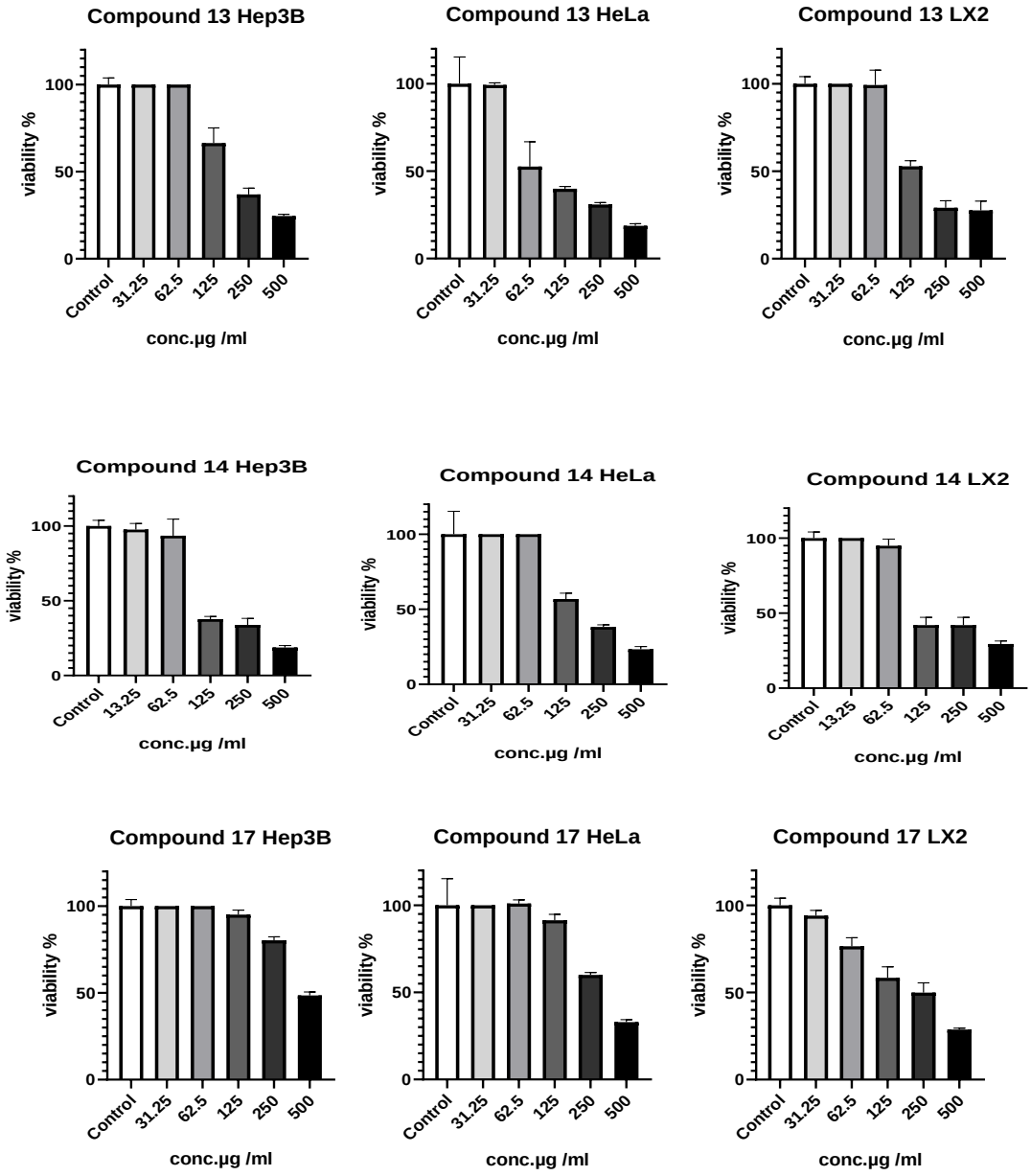
Table 3.2

IC_{50} for compounds with alkyl sulfonate group on different cancer cells (Hep3B,HeLa and LX2 normal cell)

Compound no.	IC_{50} µg/mL		
	Hep3B	HeLa	LX2
13	224.91 ± 0.24	105.40 ± 0.10	167.30 ± 0.14
14	134.70 ± 0.31	202.20 ± 0.15	183.30 ± 0.07
15	524.50 ± 0.08	590.40 ± 0.10	1665.25 ± 0.49
16	664.11 ± 0.04	369.21 ± 0.14	622.20 ± 0.05
17	484.90 ± 0.11	333.40 ± 0.21	214.52 ± 0.33
18	509.70 ± 0.22	570.60 ± 0.45	120.60 ± 0.13
19	519.50 ± 0.26	739.50 ± 0.35	130.70 ± 0.24
20	-	530.80 ± 0.12	1349.22 ± 0.17
21	523.80 ± 0.14	515.10 ± 0.49	486.30 ± 0.20
22	495.10 ± 0.32	306.30 ± 0.17	525.33 ± 0.31

Figure 3.2

The viability of compounds (13, 14, 17) against tested cells



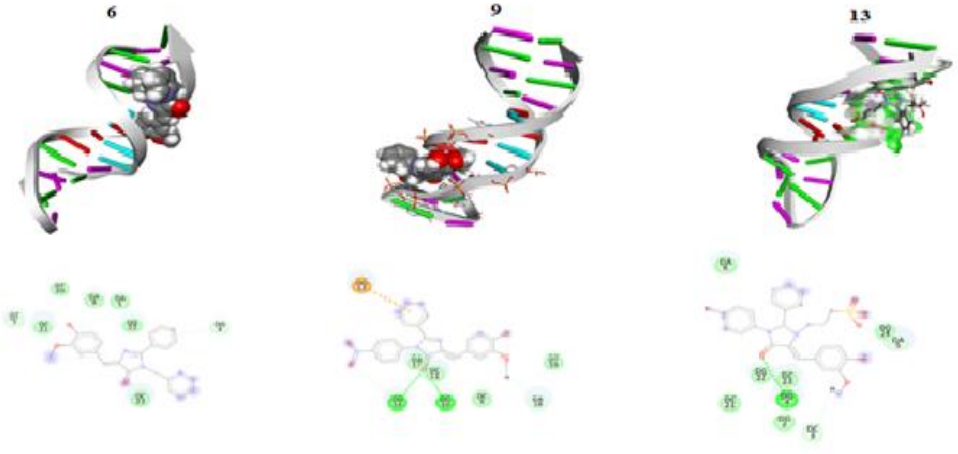
3.3 Molecular Docking

The ability to calculate and interpret the interactions between proteins and small molecules is extremely important in the fields of biology and the development of pharmaceuticals. This prophetic ability gives scientists the ability to deeply investigate the complexities of biological mechanisms, providing a profound understanding that forms the foundation to further the field of pharmaceutical research and fostering innovation. By precisely anticipating these interactions, researchers can access a

multitude of insights in the complex field of biology. They are able to recognize the minute relationships between tiny molecules and proteins, shedding light on the intricate molecular choreography governing various cellular functions. Furthermore, this foresight enables scientists to identify possible targets for therapeutic interventions, ushering in opportunities for creating novel drugs and treatments. The importance of this predictive capability resonates widely across the board in the scientific community, serving as a pivotal element in unraveling the multifaceted fabric of cellular mechanisms. Predictive modeling of protein-small molecule interactions plays a key role in improving our understanding of these complex biological processes, whether it is through illuminating the complexities of cellular signaling pathways, understanding the finely tuned mechanisms governing protein regulation, or discovering the intricate pathways underlying different diseases. Essentially, it acts as a guiding beacon, illuminating the path forward in the pursuit of innovations and novel treatments in the medical field (100, 101). Furthermore, accuracy in predicting the interactions between small molecules and proteins is a crucial prerequisite in the field of drug development. This precision equips researchers with the necessary tools to identify potential drug targets and refine the formulation of innovative medications. Understanding these relationships enables researchers to influence particular biological processes, which makes it easier to develop highly specialized medications that can precisely regulate protein activities to meet therapeutic goals.

Figure 3.3

Corresponding docking poses in 2D and 3D for interaction of the three synthesized drugs with the PDB id strands 1BNA DNA strands



The precise three-dimensional DNA coordinates were successfully retrieved from the Protein Data Bank (PDB) database (102). Subsequently, the three synthesized drug molecules underwent a thorough assessment to determine possible connections to particular protein structures, as elaborated in Figure 3.3. The resulting docking scores provided insights into their respective affinities for these proteins. Predicting how molecules and proteins will interact with one another holds significant importance in unraveling a broad spectrum of biological processes, comprehending protein functions, and facilitating drug development. Blind docking of proteins and ligands stands as a potent technique for exploring the locations where receptors bind and the orientations of ligand binding (103, 104). This approach has gained widespread acceptance in the fields of pharmacology and biology, and CB-Dock2 was employed for this purpose.

The findings gain considerable strength from the drug's capacity to establish hydrogen bonds with DNA chains throughout the docking procedure, which is in complete agreement with the extraordinarily high docking score values noted in other instances. (104, 105). Figure 3.3 visually illustrates the molecule's robust binding affinity to the DNA loop, emphasizing a remarkable docking score ranging from approximately -6.8 to -8.7 kcal/mol for both protein structures. The electron pair at the oxygen atoms and the amino group of the drug facilitate the molecule's natural ability to form hydrogen bonds, which is the primary mechanism underlying this remarkable interaction between the molecule and the target proteins. Additionally, the molecule actively engages in π-alkyl interactions through its aromatic rings. In summary, these results not only underscore the drug's potential as a promising candidate for DNA binding but also provide insight into the intricate molecular mechanisms behind its potent adherence to particular protein targets. This paves the path for additional investigation and advancement in the area of medicinal research.

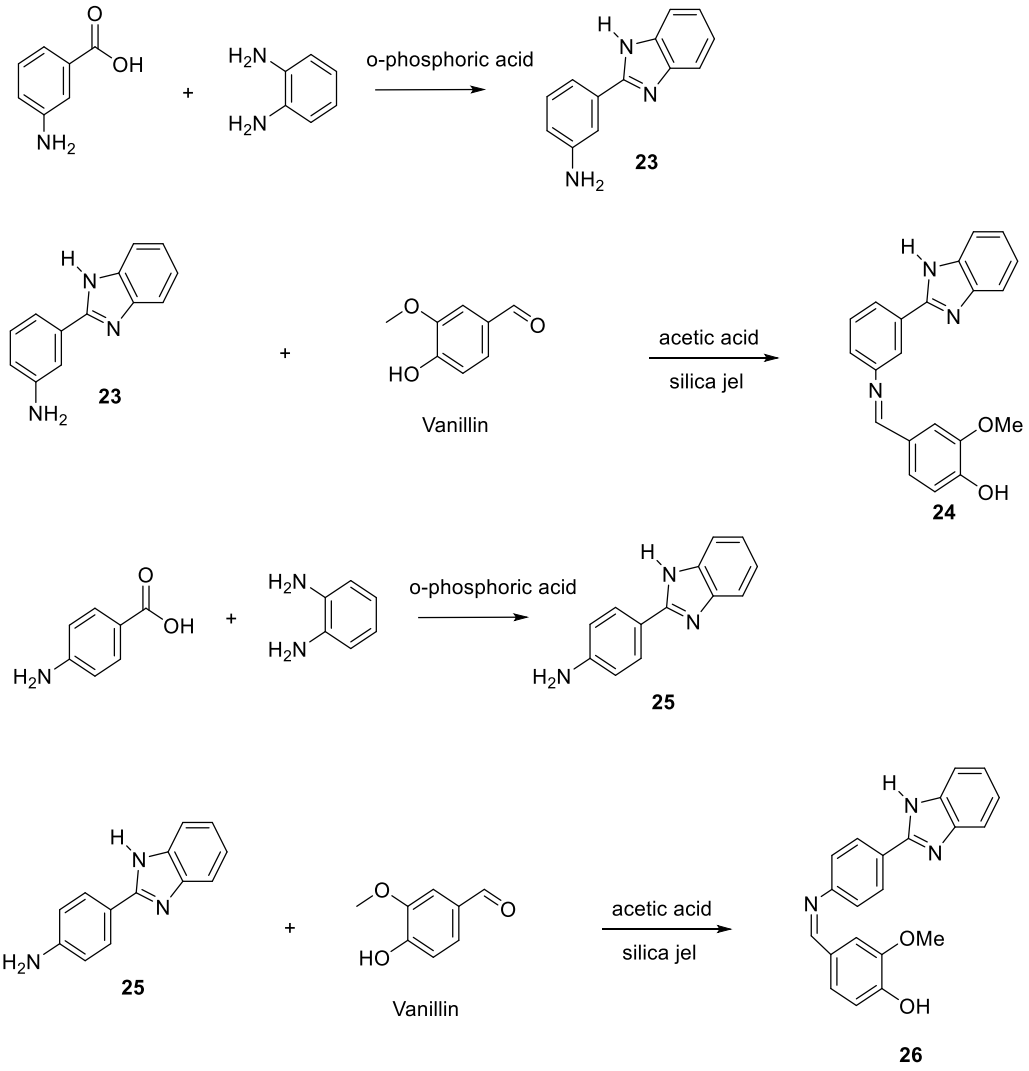
3.4 Synthesis of Benzimidazole derivatives

In the present study, a series of 2-substituted Benzimidazole having imine linkage were synthesized by two step reactions. According to Scheme 3.3. Firstly, Aminophenyl Benzimidazole was created by condensing O-phenylenediamine with *para* and *meta* amino benzoic acid in o-phosphoric acid. In the second step, aminophenyl Benzimidazole is treated with vanillin to form substituted Benzimidazole having imine linkage**.** The yield of synthesized benzimidazole

ranged from 55% to 62%.

Scheme 3.3

Synthesis of benzimidazole derivatives



3.4.1 Mechanism

Synthesis of aminophenyl Benzimidazole (23, 25).

The amino group's nitrogen on phenylenediamine attacks the carbonyl group, followed by an oxygen atom abstracting a proton, followed by the loss of one molecule of H_2O. The nitrogen of the other amino group then attacks the carbonyl group to produce an imidazole ring, after that one molecule of H_2O is lost to obtain the product as shown in Figure C.5 (appendix C).

3.4.2 Characterization

The IR spectra of compounds 23, 25 revealed an absorption band at 3375-3374 cm^{-1} because of (NH benzimidazole), 3243-3113 cm^{-1} due to (NH_2 aminophenyl), 2924-2923cm^{-1} due to (CH arom), 1641-1639 cm^{-1} because of (C=N), 1611 cm^{-1} because of (C=C Ar). While for compounds 24, 26 the IR spectra showed absorption band at3735-3506cm^{-1} due to (NH benzimidazole), 3601-3201cm^{1} due to (OH), 3100-3070 cm^{-1} due to (CH Ar), 1670-1665 cm^{-1} because of (C=N), 1591-1509 cm^{-1} because of (C=C Ar), 1288-1265 cm^{-1} because of (C-O, OH), 1298-1123 cm^{-1} because of (C-O, Ar-O-CH_3).

The ^{13}CNMR (δ) spectra showed peak at 171.25-169.05 *ppm* due to CH=N, 153.53-151.67 *ppm* due to C-N=C,148.70-148.60 *ppm* due to Ar-C-OH, 148.45-148.29 *ppm* due to Ar-C-OCH_3, 143.70-140.30 *ppm* due to C=C of imidazole ring, 129.03-111.05 *ppm* due to phenyl group , 56.18-56.02 ppm due to substituted methyl group .

The ^{1}HNMR (δ) spectra of 24, 26 showed peak at 12.91-10.14 due to NH of imidazole ring, 9.75-9.73 *ortho* substituted OH, 8.49-7.95 due to –CH=, 8.04-7.11 due to phenyl group , 3.82-3.81 due to methoxy group .

3.4.3 Biological activity

Compounds 23, 24, 25, and 26 demonstrated strong anti-tumor activity against the examined cancer cells. (Hep3B and HeLa cells). According to results in Table 3.3, compound 23 has shown the maximum anticancer activity against Hep3B and HeLa cells with **(**IC_{50}=38.05 ± 0.23 µg/mL**),** and **(**IC_{50}=95.50 ± 0.15 µg/mL**)** respectively. However, these compounds didn't show high selectivity in comparison to the normal liver cell LX2 except compound 26 which has shown high viability of LX2 cells on 125 µg/mL while killing more than 50% of the cancer cells as shown in Figure 3.4.

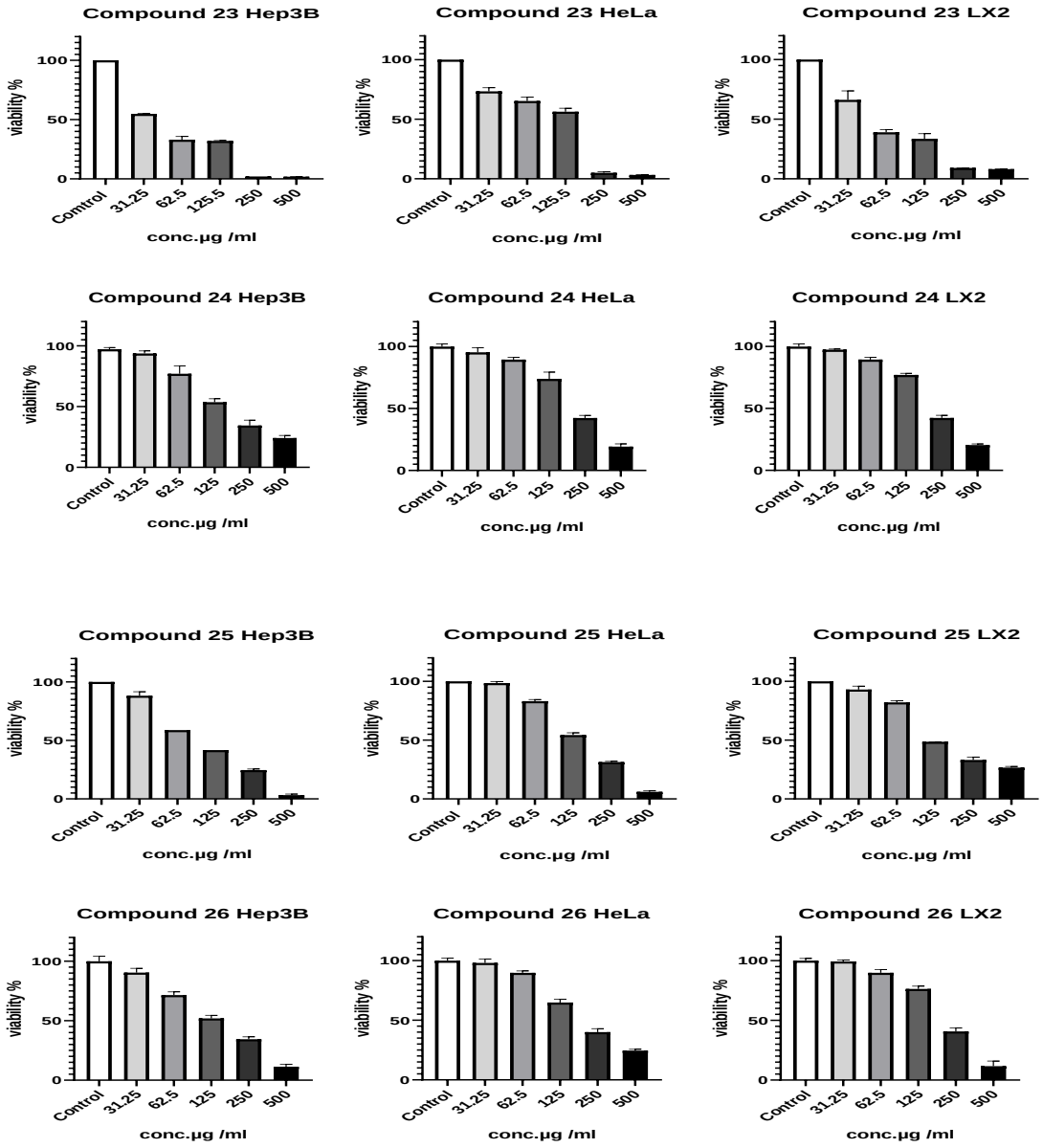
Table 3.3

IC_{50} benzimidazole derivatives compounds on different cancer cells (Hep3B,HeLa and normal cell)

Compound no.	IC_{50} µg/mL		
	Hep3B	HeLa	LX2
23	38.05 ± 0.23	95.50 ± 0.15	50.07 ± 0.18
24	167.10 ± 0.10	204.40 ± 0.03	220.70 ± 0.28
25	98.17 ± 0.25	155.60 ± 0.35	115.50 ± 0.11
26	134.40 ± 0.42	210.60 ± 0.28	210.60 ± 0.21

Figure 3.4

Benzimidazole derivatives viability against the tested cells

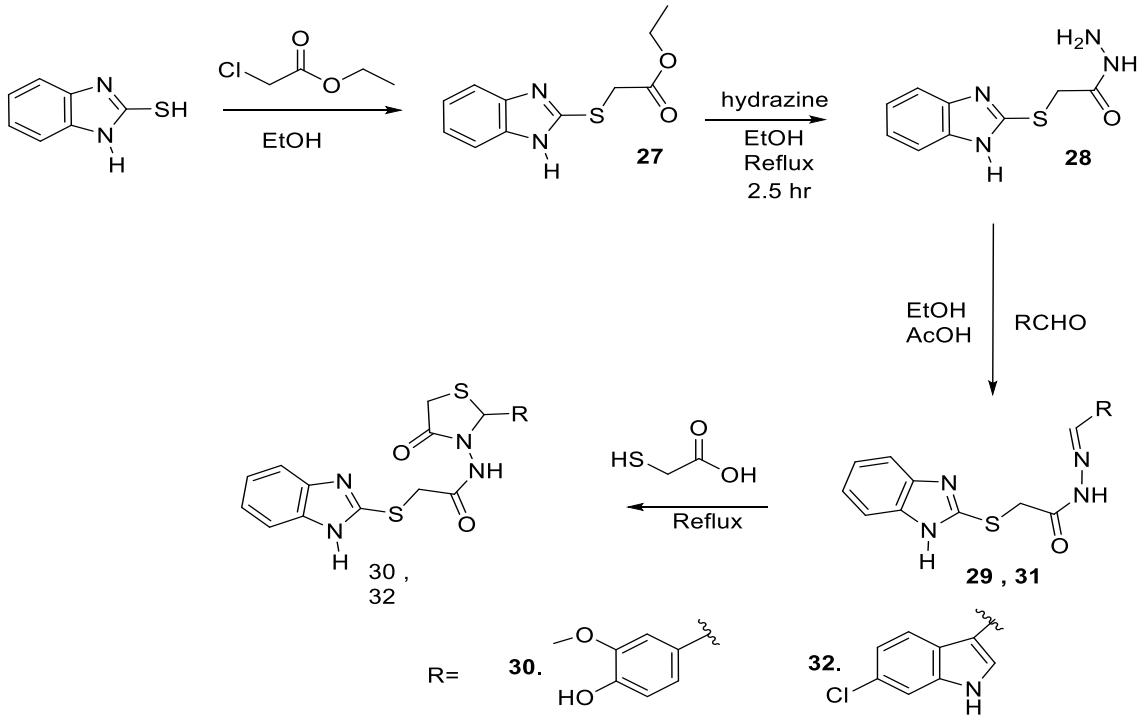


3.5 Synthesis of benzimidazole-substituted-1,3-thiazolidin-4-ones.

In this study 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide 30, 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(6-chloro-1H-indol-3-yl)-4-oxothiazolidin-3-yl)acetamide 32, were synthesized according to strategy summarized in Figure 3.5. The process involved four steps with a yield (52-53%). The first step was alkylation of the 2- mercaptobenzimidazole with ethyl chloroacetate to obtain thioether compound 27. In the second step compound 27 and hydrazine were refluxed to obtain thioacetohydrazide 28. The third step involving preparation of the Schiff bases 29, 31 by refluxing a solution containing equimolar quantities of vanillin and 6-chloroindol-3-carboxaldehyde respectively, with compound 28 in presence of acetic acid. Finally compound 30, 32 were synthesized by refluxing appropriate Schiffbase 29, 31 respectively with thioglycolic acid in acetic acid using a pinch of silica gel as catalyst.

Figure 3.5

Synthesis of 1,3 thiazolidin-4 ones substituted with benzimidazole



3.5.1 Mechanisms of reactions

3.5.1.1 Synthesis of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate (27).

To generate the product, the sulfur atom first attacks the carbon atom next to the carbonyl group, and then the chloride ion abstracts a proton as shown in Figure C.6 (Appendix C).

3.5.1.2 Synthesis of (E)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-(4-hydroxy-3-methoxybenzylidene) acetohydrazide (29).

To obtain the product, first a nitrogen atom of hydrazine binds to the carbonyl of compound 27, then ethoxy group leaves as a good leaving group to obtain compound 28. After that the nitrogen of compound 28 attacks the carbonyl of vanillin, then an OH group absorbs a proton to make an H_2O molecule, which serves as a good leaving group.. Finally, proton is lost to form a C=N as shown in Figure C.7 (Appendix C).

3.5.1.3 Synthesis of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide (30).

To obtain the product, first a nitrogen atom binds to the carbonyl of thioglycolic acid, then an OH group absorbs a proton to make an H_2O molecule, which serves as a good leaving group, and then a proton is lost to form a carbocation. Finally, the sulfide ion hits a positively charged carbon to produce the thiazolidin ring as shown in Figure C.8 (Appendix C).

3.5.2 Characterization

Compound **27's** infrared spectra revealed an absorption band at 3044 cm^{-1} because of (NH benzimidazole), 2929 cm^{-1} because of (CH arom), 1742 cm^{-1} because of (C=O), 1617 cm^{-1} because of (C=N), 1494-1222 cm^{-1} because of (C=C), 1299 cm^{-1} because of (C-N), 617 cm^{-1} because of (C-S), while Compound 28's infrared spectrum revealed an absorption band at 3314 cm^{-1} because of (NH benzimidazole), 3265 cm^{-1} because of (primary amine), 2989 cm^{-1} because of (CH Ar), 1664 cm^{-1} because of (C=O),1656 cm^{-1} because of (C=N), 1457-1226 cm^{-1} because of (C=C), 1267 cm^{-1} because of (C-N), 620 cm^{-1} because of (C-S). Compounds 29, 31**,** IR spectra showed absorption band at 3360 cm^{-1} because of (NH benzimidazole), 3050 cm^{-1} due to (OH), 2970 cm^{-1} because of (C-

H, OCH_3), 1669 cm^{-1} because of (C=O for secondary amide), 1677 cm^{-1} because of (C=N), 1582-1515 cm^{-1} because of (C=C), 1281 cm^{-1} because of (C-N), 1212 cm^{-1} because of (C-O, OH), 1281-1121 cm^{-1} because of (C-O, Ar-O-CH_3), 616 cm^{-1} because of (C-S).

Finally compounds 30, 32**,** IR spectra showed absorption band at 3379 cm^{-1} because of (NH benzimidazole), 3250 cm^{-1} because of (OH), 2917 cm^{-1} because of (C-H, OCH_3), 1661 cm^{-1} because of (C=N), 1584 cm^{-1} because of (C=O of thiazolidinone), 1584-1511 cm^{-1} because of (C=C), 1453 cm^{-1} because of (ring of thiazolidinone), 1274 cm^{-1} because of (C-N), 1280 cm^{-1} because of (C-O, OH), 1280-1031 cm^{-1} because of (C-O, Ar-O-CH_3), 612 cm^{-1} because of (OCN).

The ^{1}HNMR (δ) spectra of 30, 32, showed peak at: 9.93 due to NH of imidazole ring, 9.68 due to NH of amide, 7.92 due to OH, 7.45– 6.92 due to Ar-H, 6.70 due to S-CH-N of thiazolidinone ring, 3.86 due to S-CH-C=O, 3.84 due to S-CH-C=O of thiazolidinone ring, 3.54 due to methoxy group.

3.5.3 Biological activity

Compound 32 showed poor activity against anticancer Hep3B and HeLa cells, while compound 30 has good anticancer activities against the same cells. According to the results in Table 3.4, compound 30 has shown maximum anticancer activity against Hep3B cells with (IC_{50}=246.50 µg/mL) with little selectivity on the normal liver cells as shown in Figure 3.6.

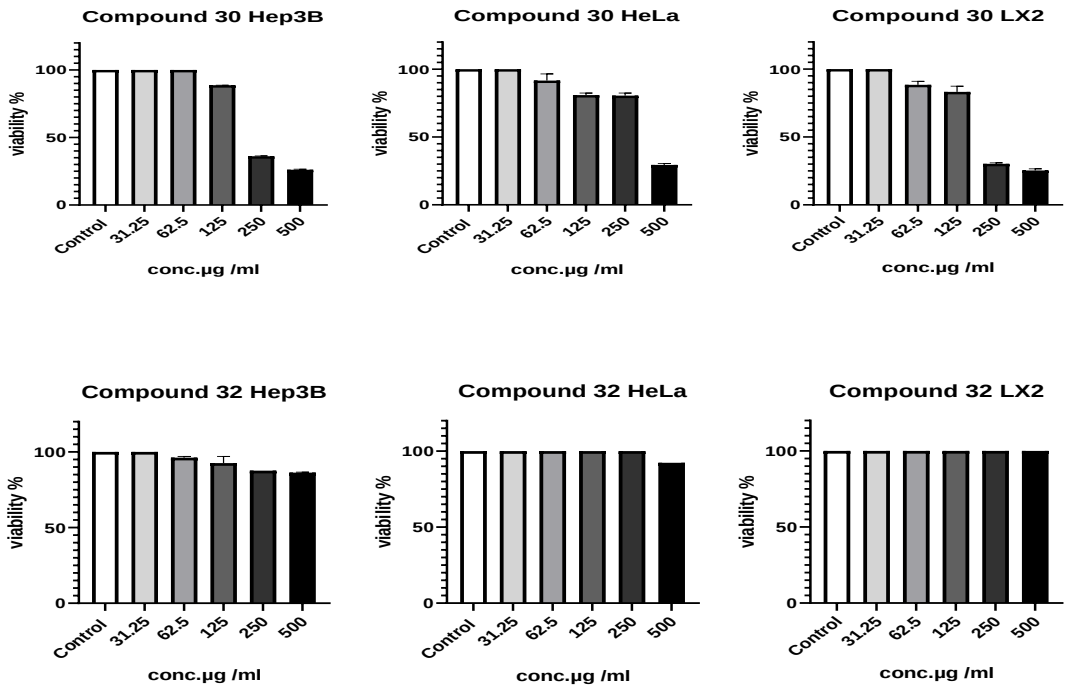
Table 3.4

IC_{50} benzimidazole -substituted-1,3-thiazolidin-4-ones *compounds on different cancer cells (Hep3B,HeLa and normal cell)*

Compound no.	IC_{50} µg/mL		
	Hep3B	HeLa	LX2
30	246.50 ± 0.35	367.10 ± 0.19	207.80 ± 0.14
32	-	-	-

Figure 3.6

The viability of compounds (30, 32) against tested cells.



3.6 Conclusion

Imidazolone derivatives of a new series were created. The prepared imidazolone's anticancer properties against the two cancer cells, HeLa cells and human liver cancer cell (Hep3B), as well as the normal cell LX2, were investigated. Compound 9 demonstrated remarkable activity against Hep3B cells with an IC_{50} of 55.75 µg/mL and a viability of over 85% against both tested cells with low cytotoxicity. Most of the prepared compounds had some activity against the tested cell. Compound 11 exhibited noteworthy efficacy against Hela cells, exhibiting an IC_{50} of 32.8µg/mL. Alkyl sulfonate moiety, when added to imidazolone, improves the drug's water solubility and bioactivities against cancer cells under test. In the molecular docking assessment, some of the prepared imidazolne and sulfonate demonstrated an impressive docking score ranging from roughly -6.8 to -8.7 kcal/mol .

Additionally, two-step reactions created a series of 2-substituted Benzimidazoles with imine linkage, these compounds exhibited some activity against the tested cell.

List of Abbreviations

Abbreviation	Meaning
FT-IR	Fourier -Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
DMSO	Dimethyl Sulfoxide
mL	Milliliter
NB	Nutrient Broth
IC_{50}	Molar concentration required to kill 50% of cancer cells was determined
DNA	Deoxyribonucleic acid
m.p	Melting point
CO_2	Carbon dioxide
$NaHCO_3$	Sodium bicarbonate
TLC	Thin Layer Chromatography
NH_4OAc	Ammonium acetate
ppm	Part per million
R_f	Retention Factor
5-FU	Five-Fluorouracil
s	Singlet
d	Doublet
m	Multiplet

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Appendices

Appendix A

Figures of Chemistry Study

Figure A.1

FT-IR of benzoyl Glycine (1)

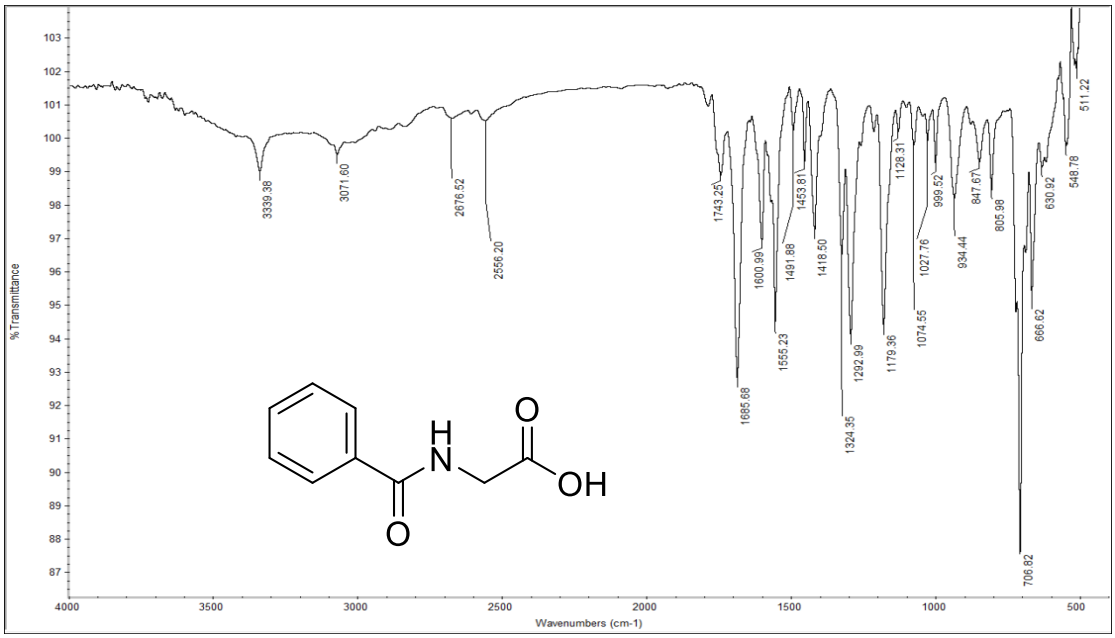


Figure A.2

FT-IR Spectra of (Z)-4-(4-hydroxy-3-methoxybenzylidene)-2-phenyloxazol-5(4H)-one (2)

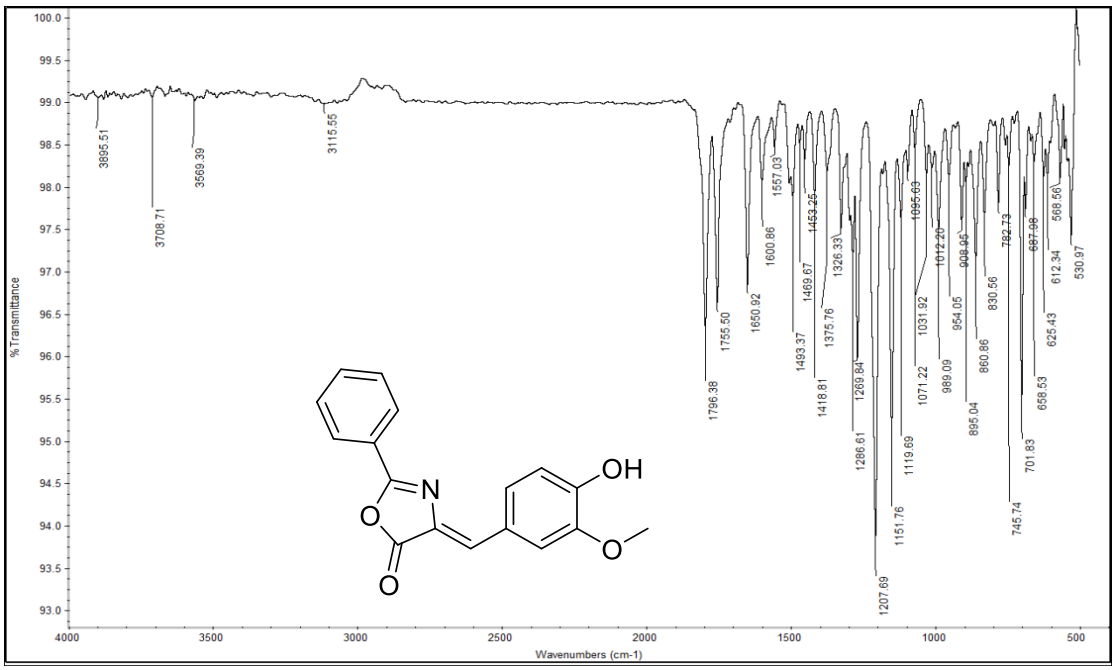


Figure A.3

FT-IR of (Z)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3)

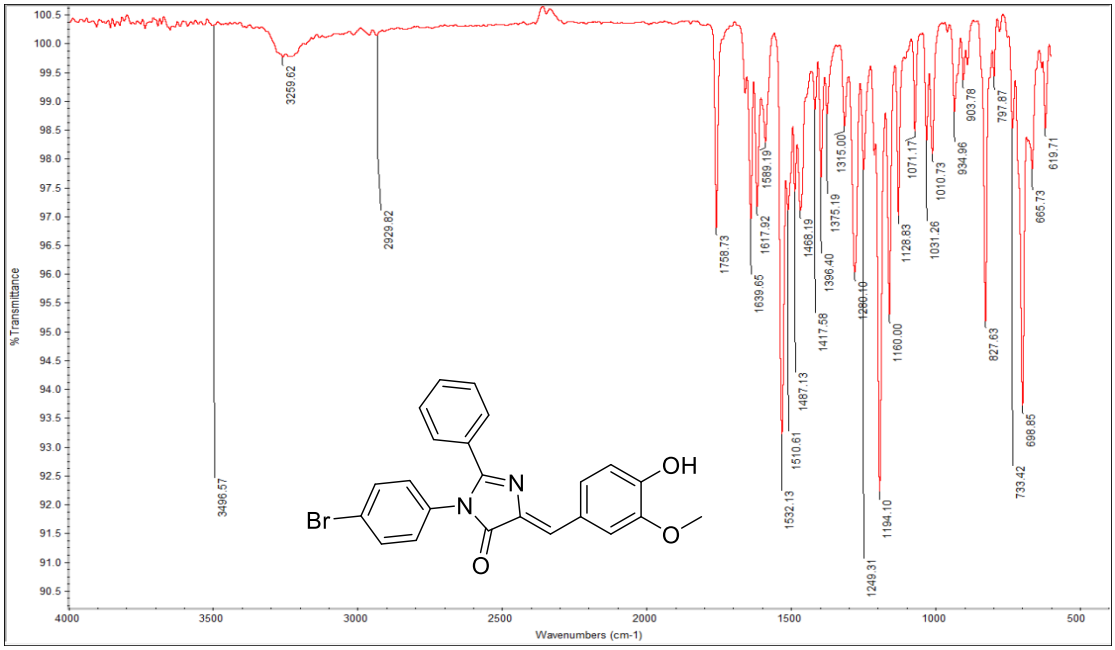


Figure A.4

FT-IR Spectra of (Z)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (4)

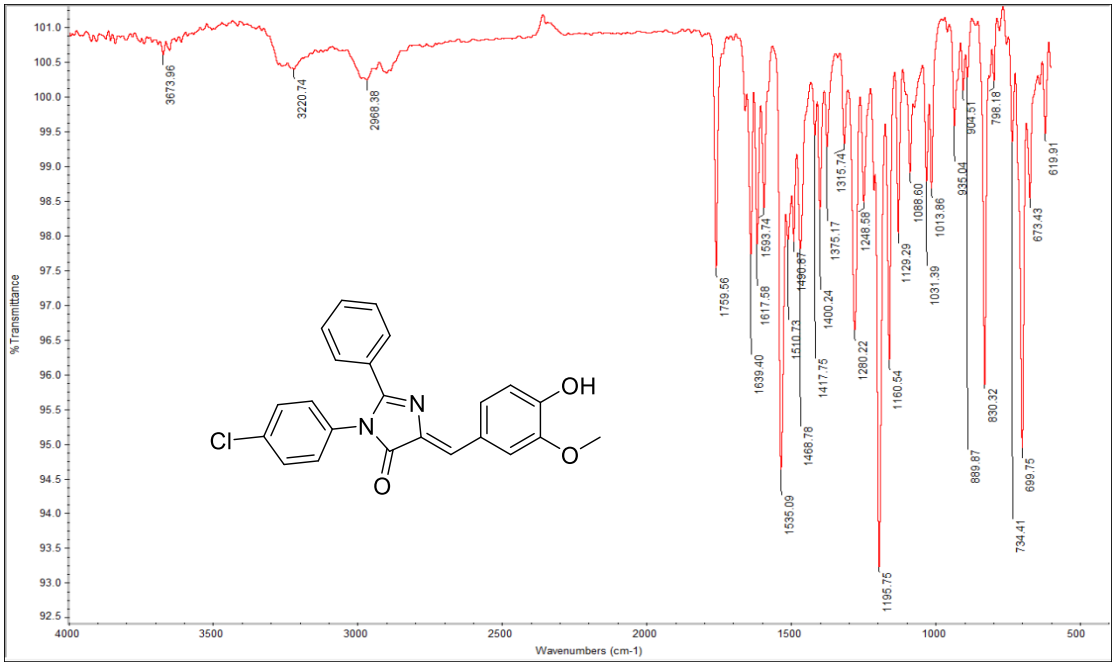


Figure A.5

FT-IR Spectra (Z)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5)

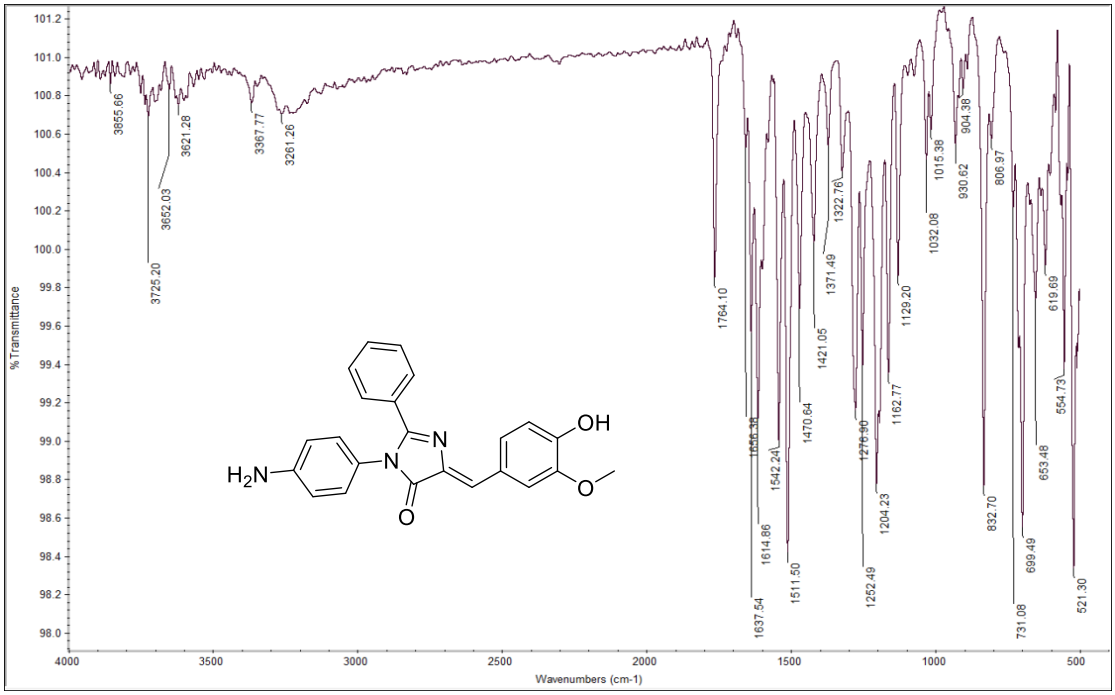


Figure A.6

FT-IR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (6)

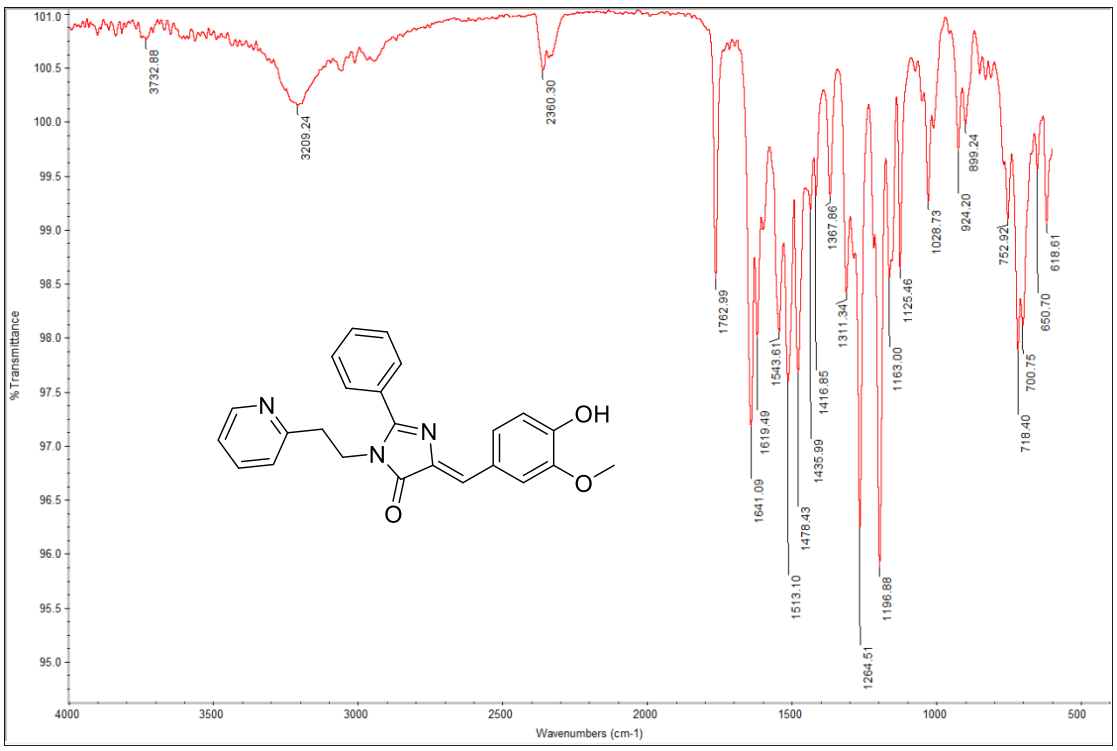


Figure A.7

FT-IR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoic acid (7)

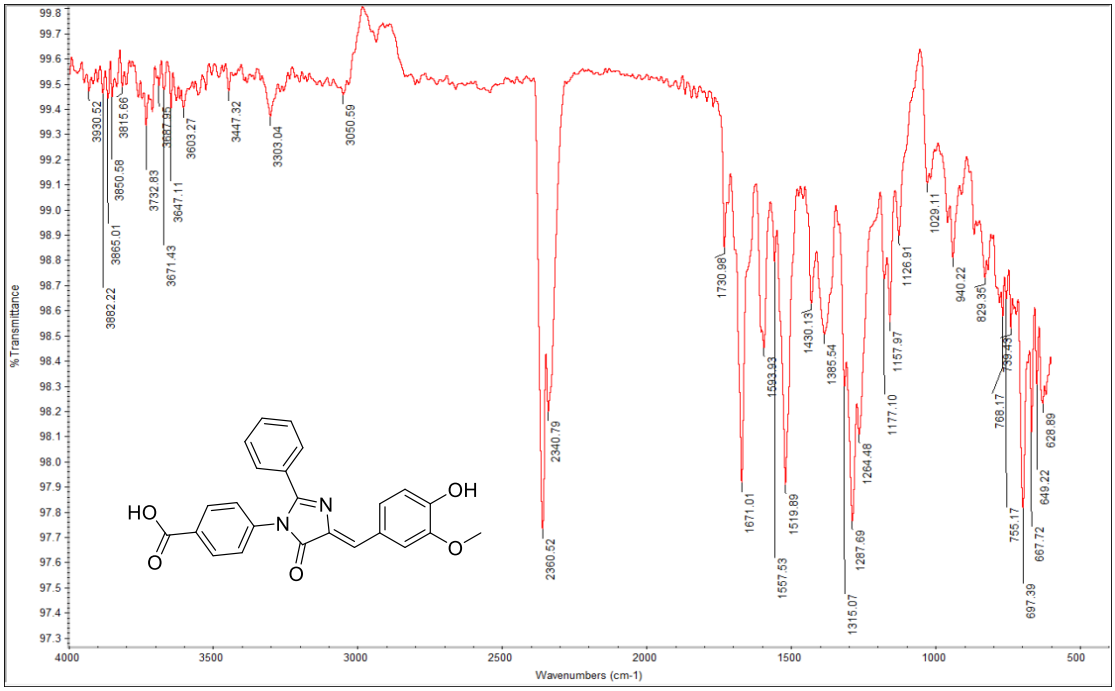


Figure A.8

FT-IR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (8)

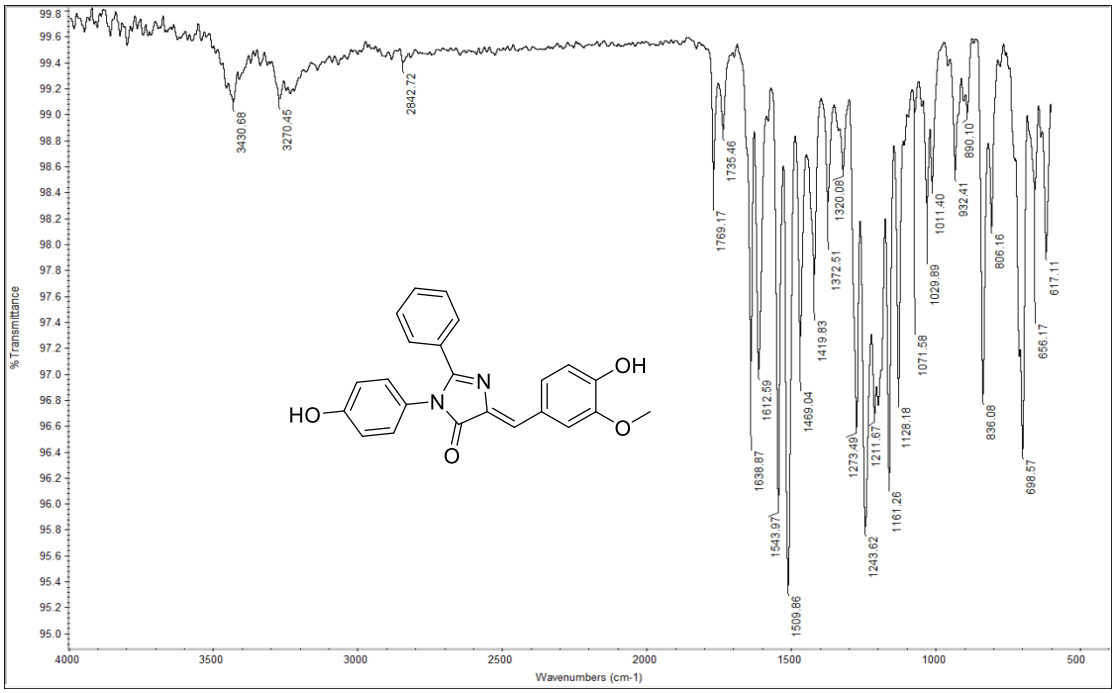


Figure A.9

FT-IR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (9)

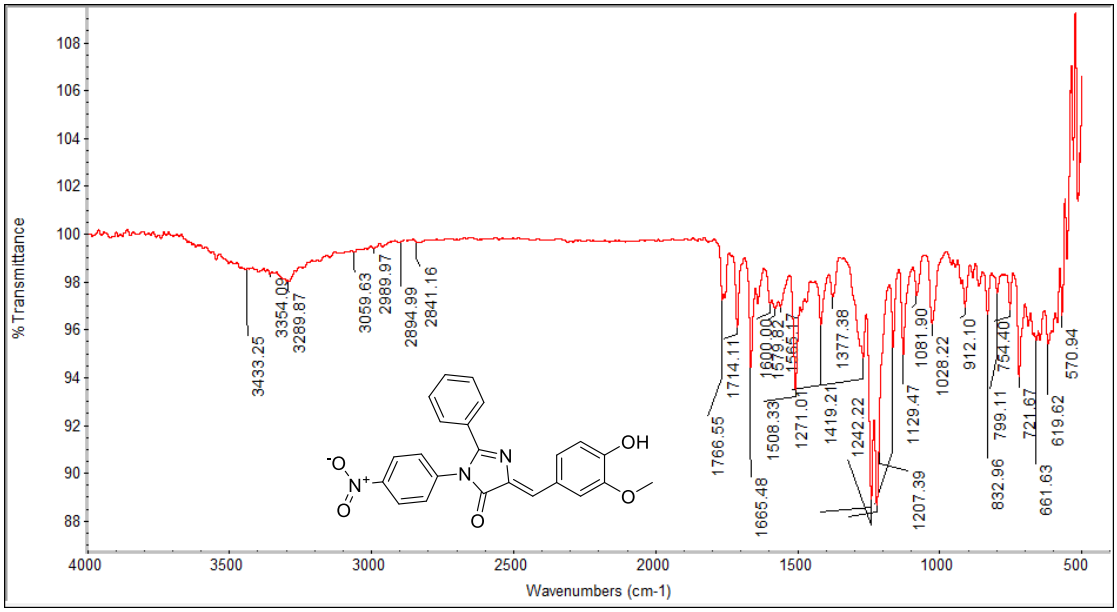


Figure A.10

FT-IR Spectra of (Z)-N-(1H-benzo[d]imidazol-2-yl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazole-1-carboximidamide (10)

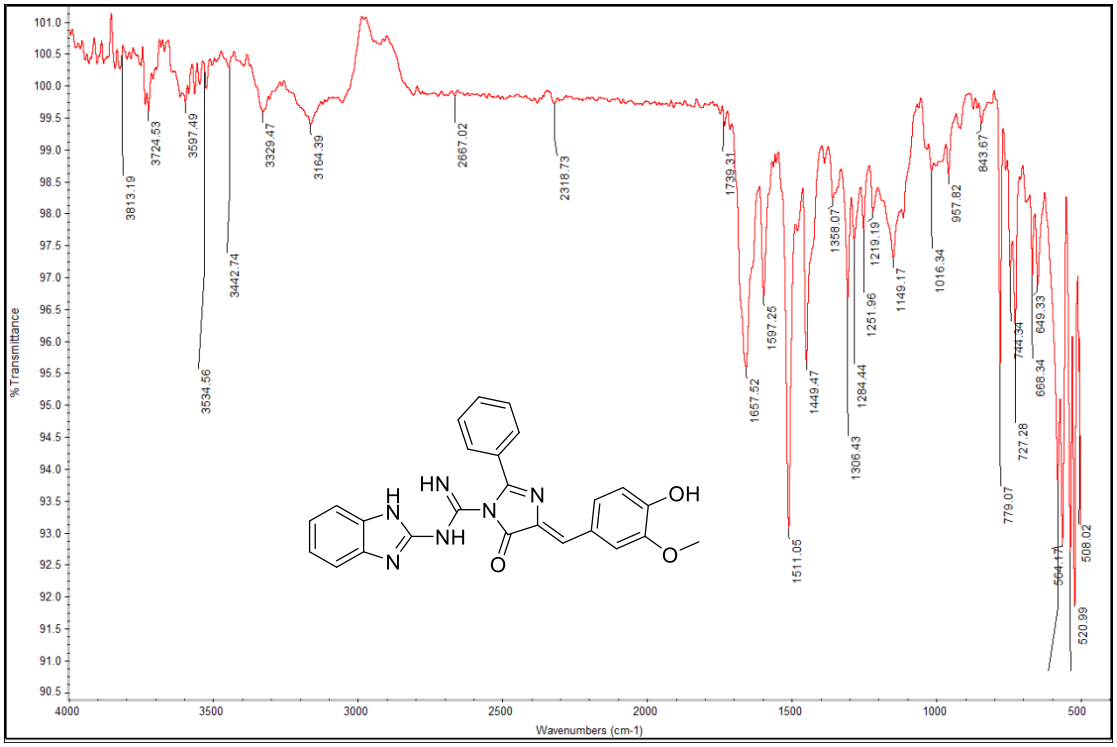


Figure A.11

FT-IR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (11)

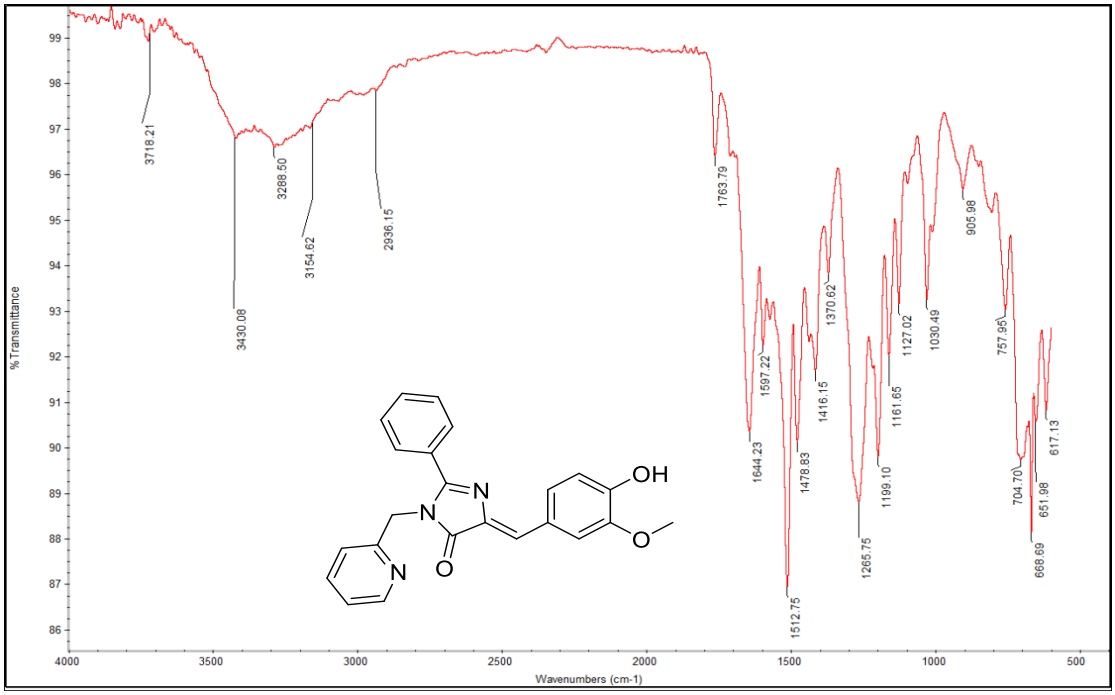


Figure A.12

FT-IR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (12)

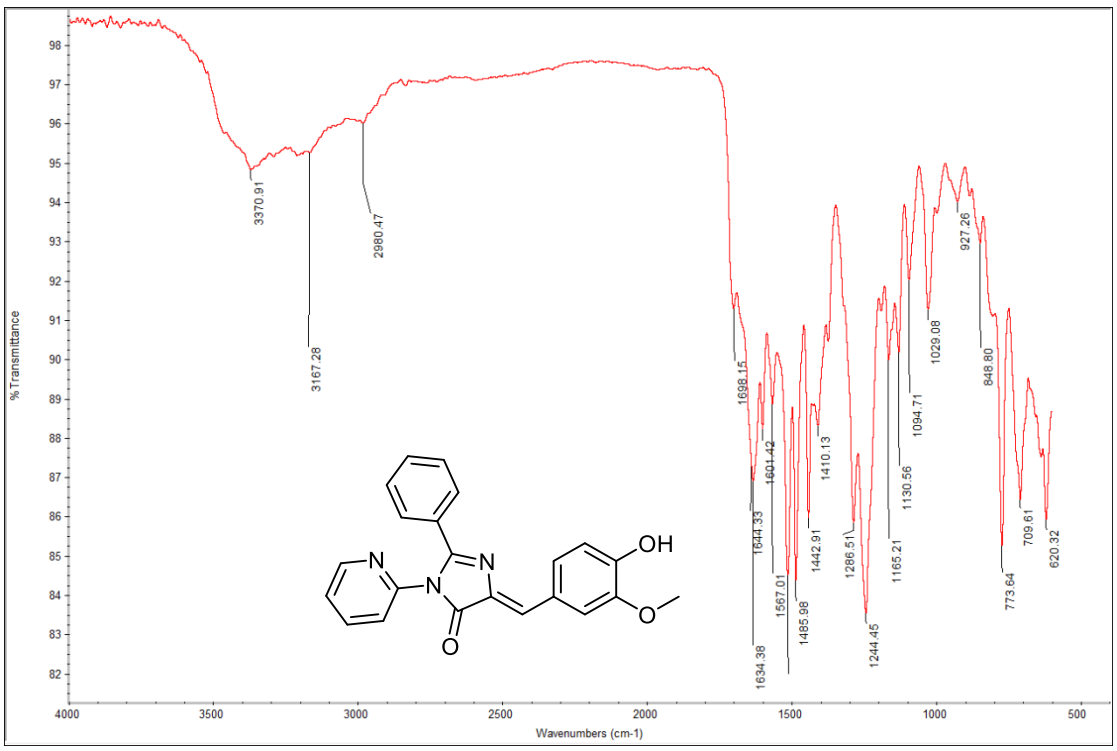


Figure A.13

FT-IR Spectra of (Z)-3-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (13)

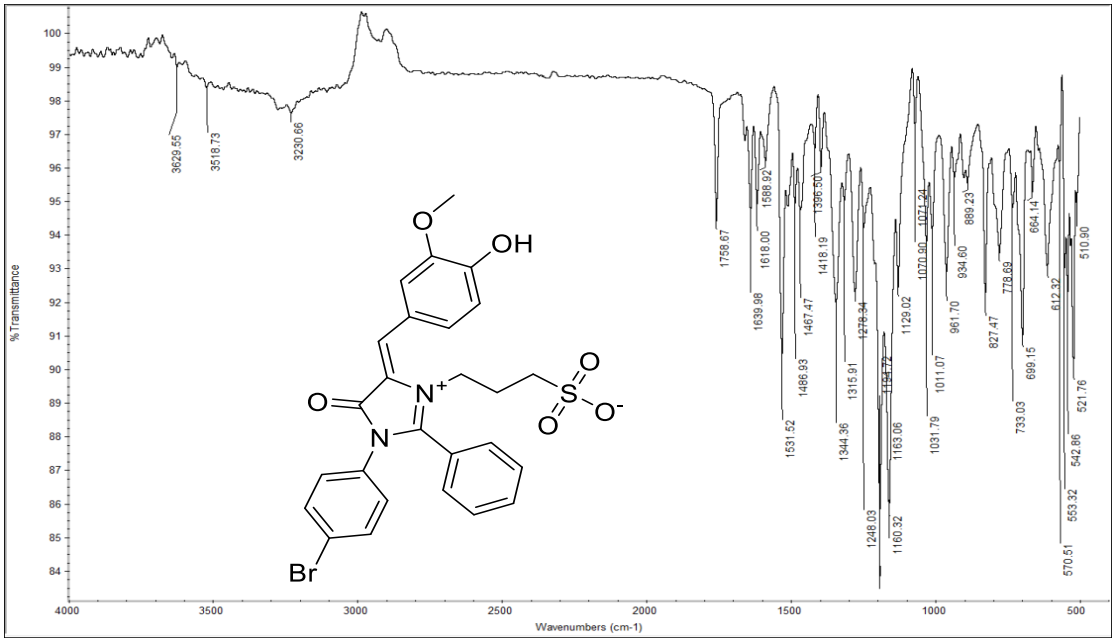


Figure A.14

FT-IR Spectra of (Z)-3-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (14)

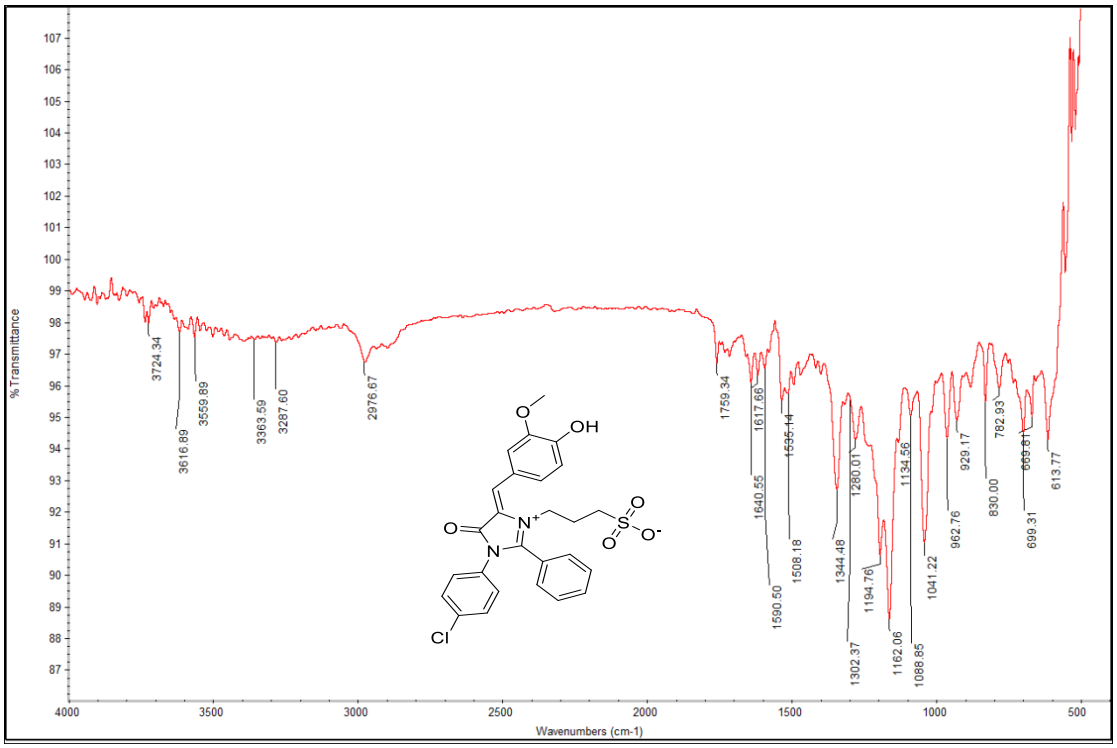


Figure A.15

FT-IR Spectra of (Z)-3-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (15)

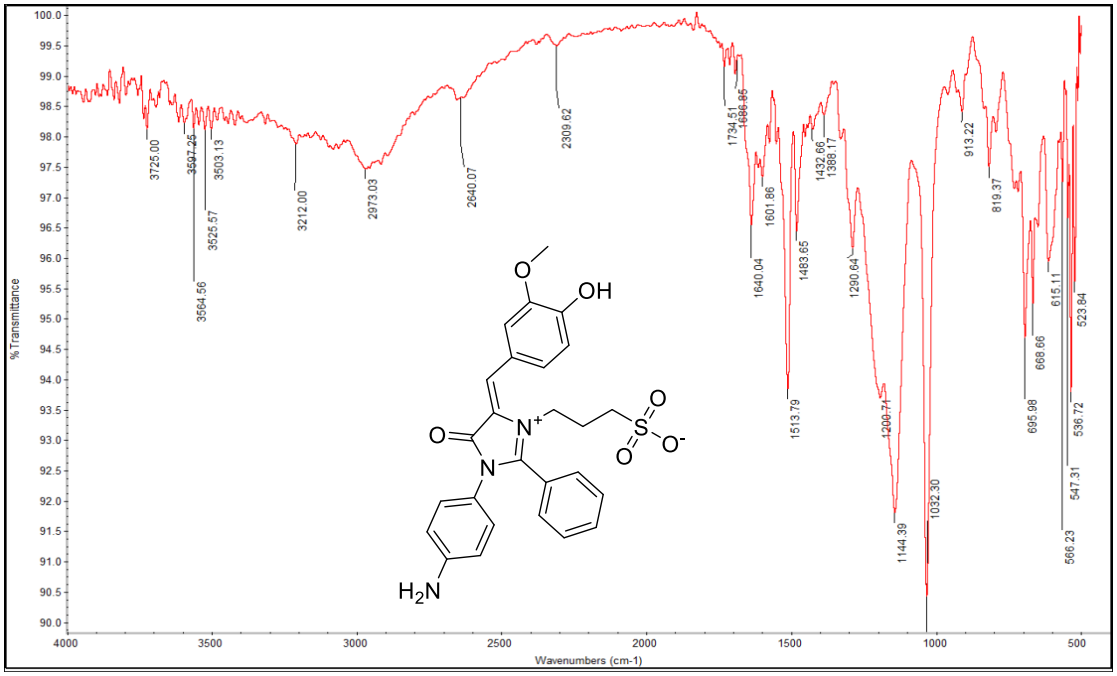


Figure A.16

FT-IR Spectra of (Z)-3-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (16)

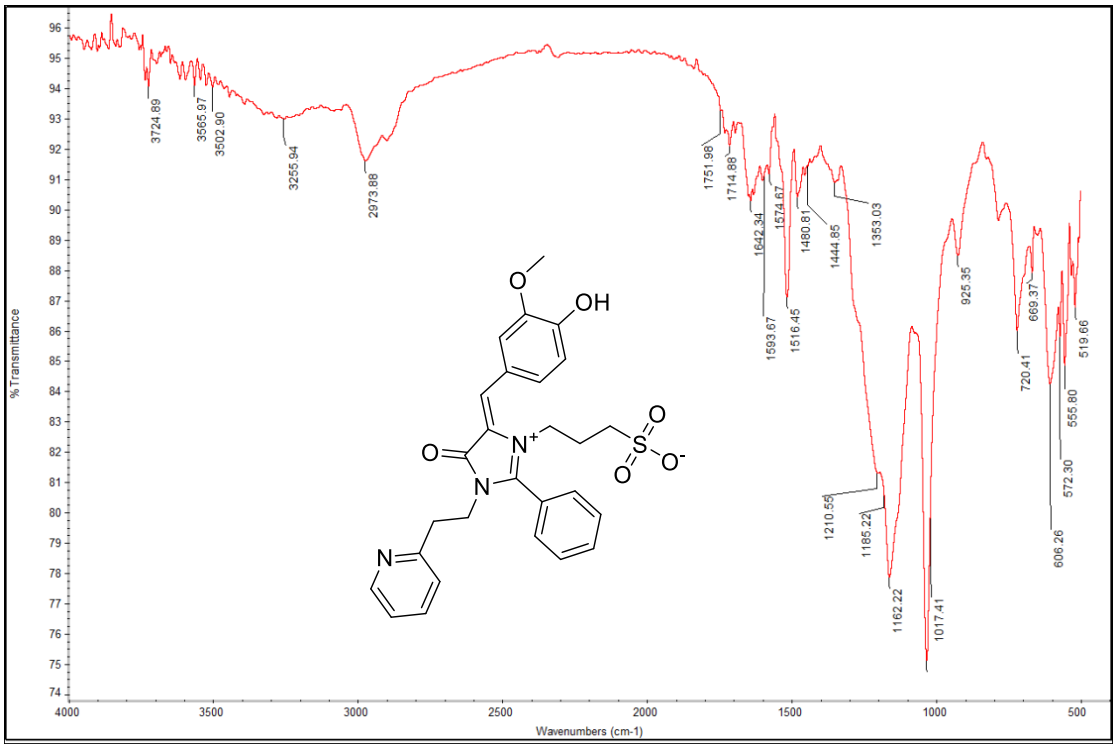


Figure A.17

FT-IR Spectra of (Z)-3-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (17)

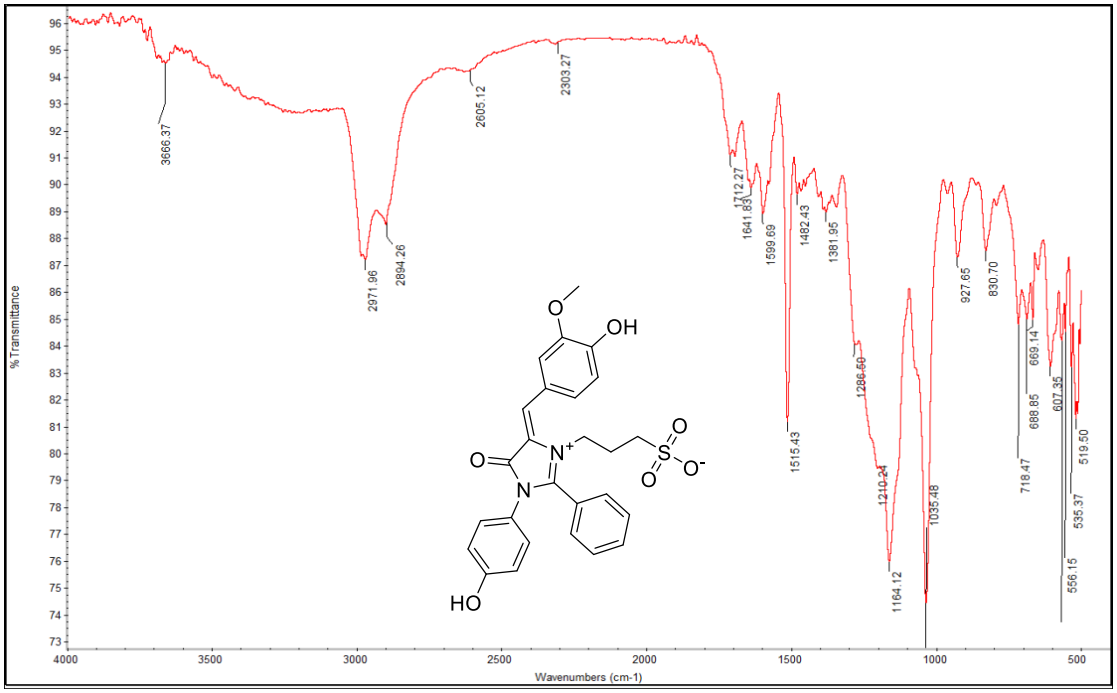


Figure A.18

FT-IR Spectra of (Z)-4-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (18)

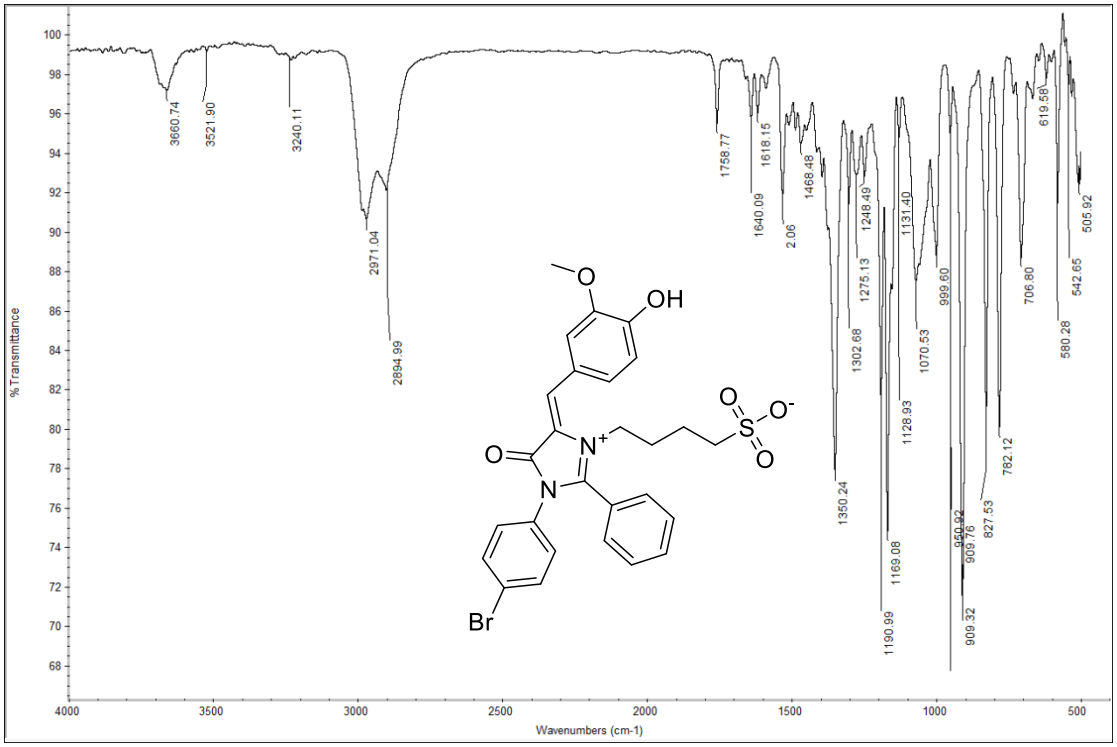


Figure A.19

FT-IR Spectra of (Z)-4-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (19)

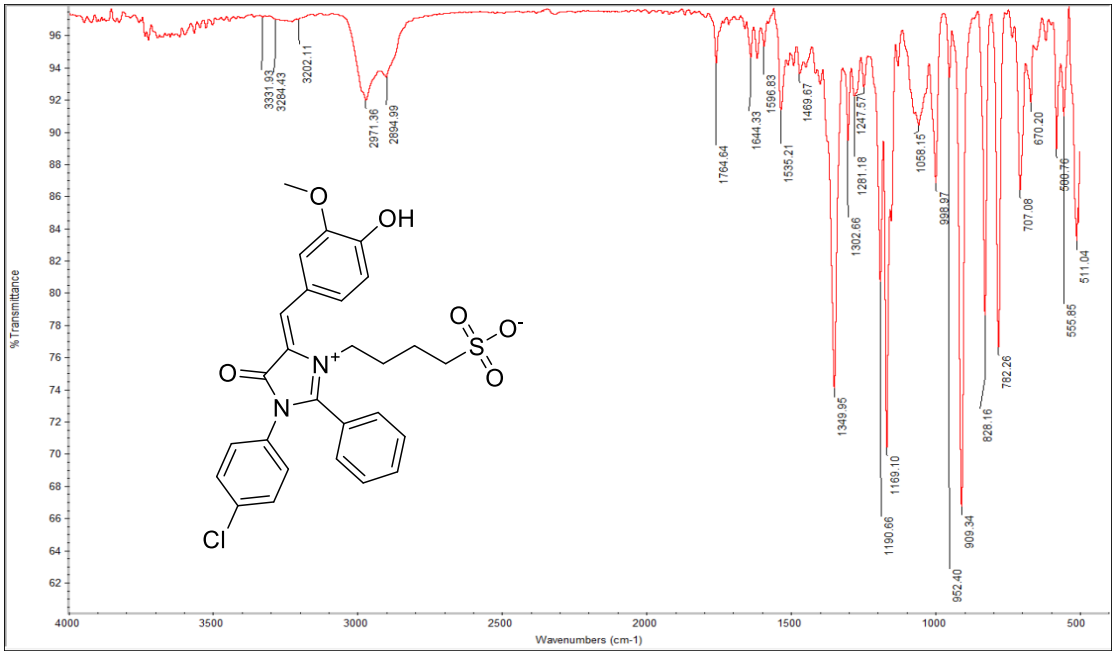


Figure A.20

FT-IR Spectra of (Z)-4-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (20)

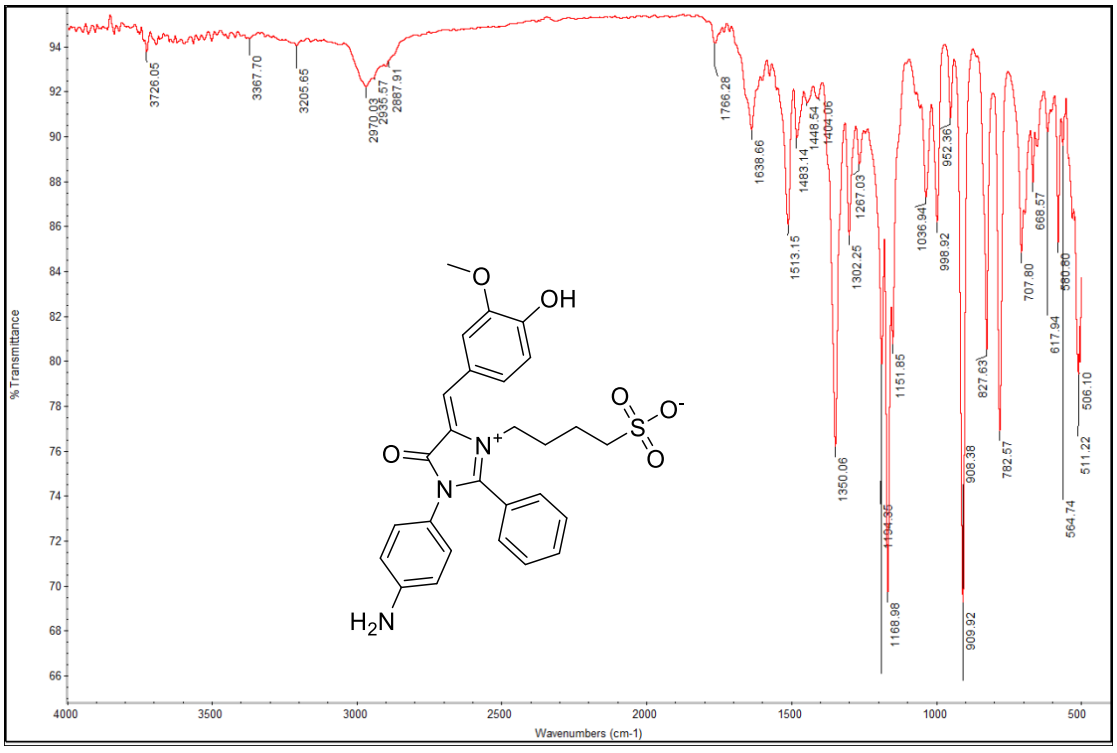


Figure A.21

FT-IR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (21)

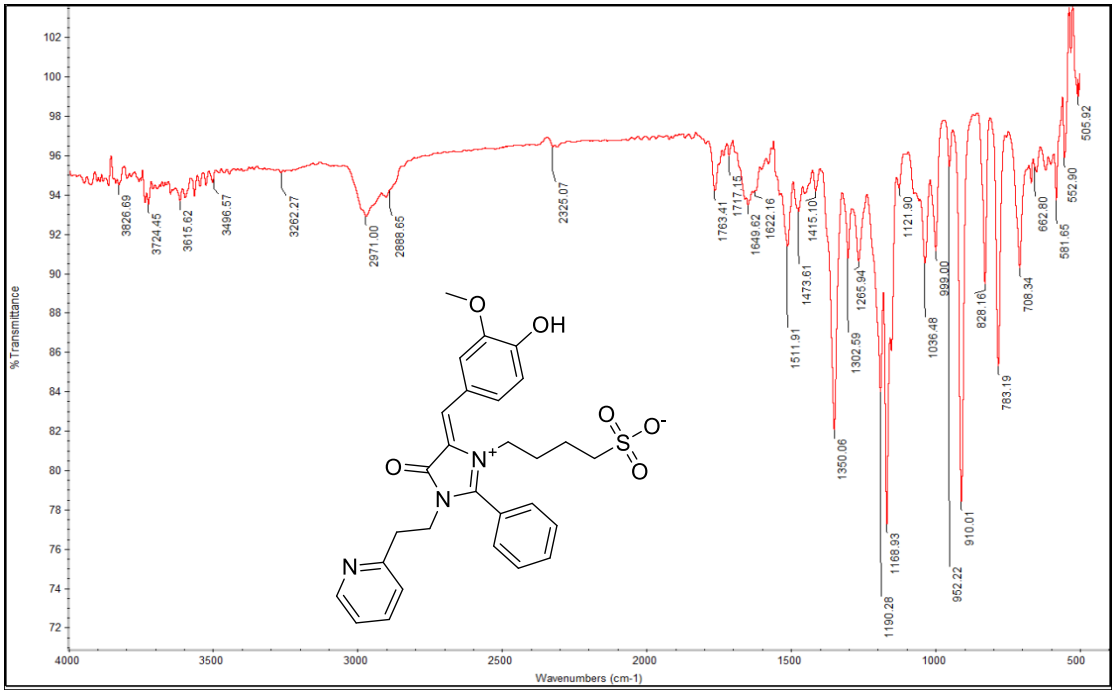


Figure A.22

FT-IR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (22)

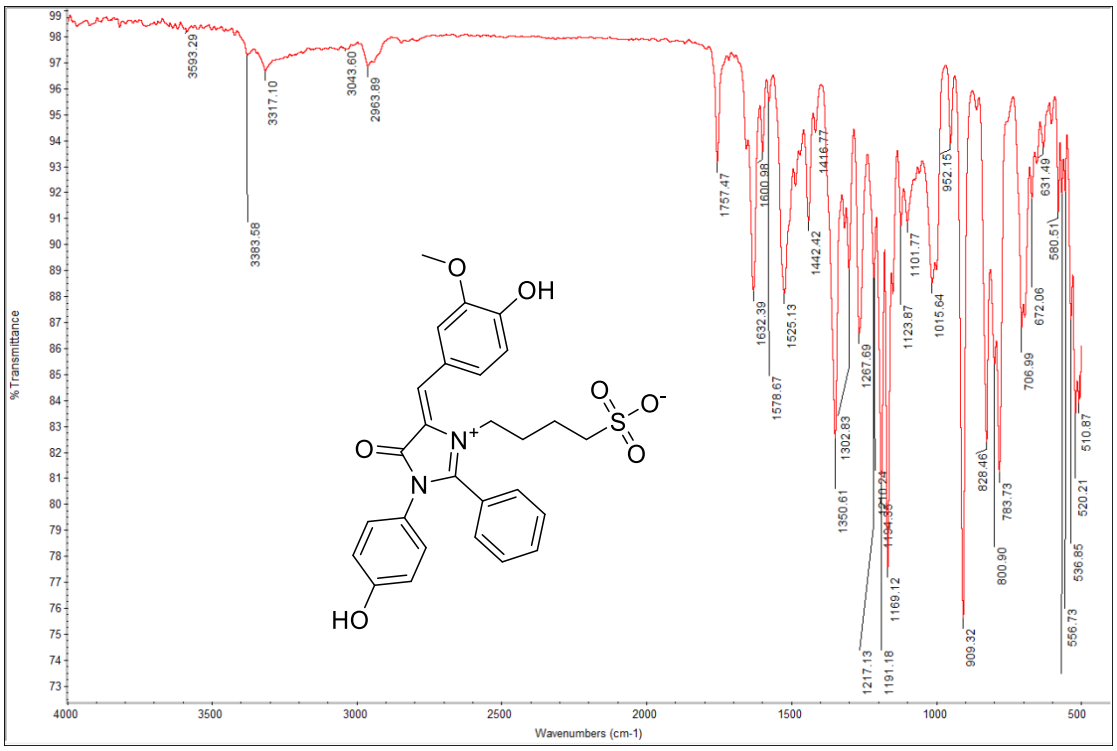


Figure A.23

FT-IR Spectra of 3-(1H-benzo[d]imidazol-2-yl)aniline (23)

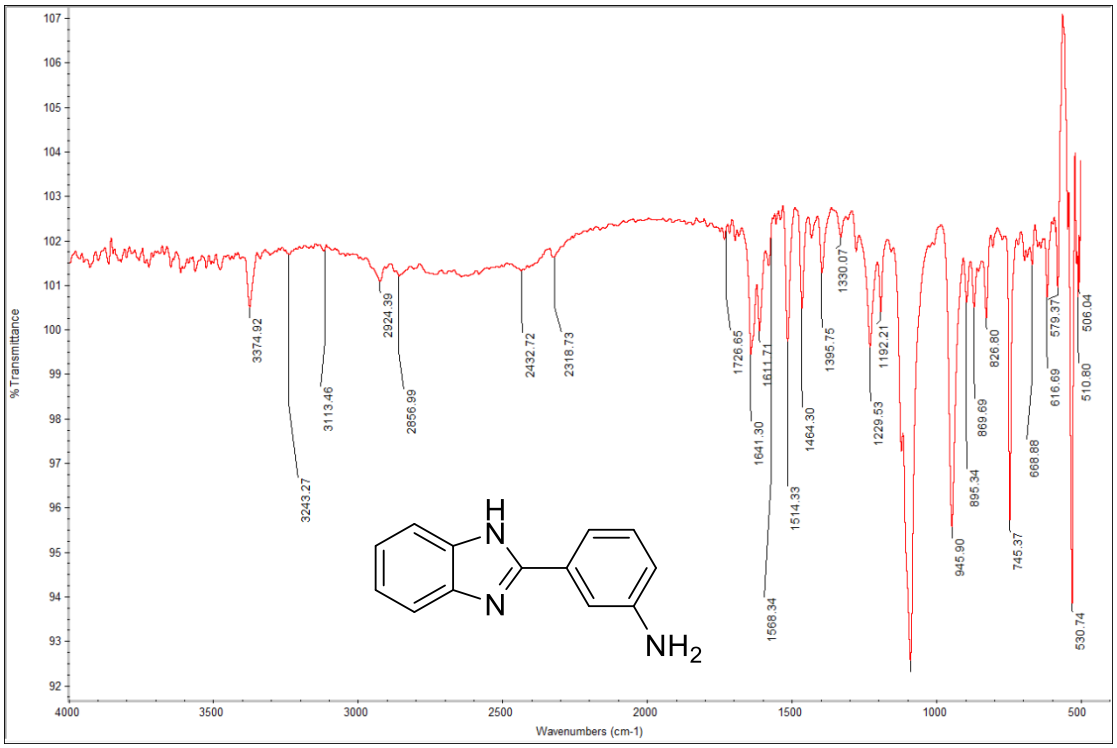


Figure A.24

FT-IR Spectra of 4-(((3-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (24)

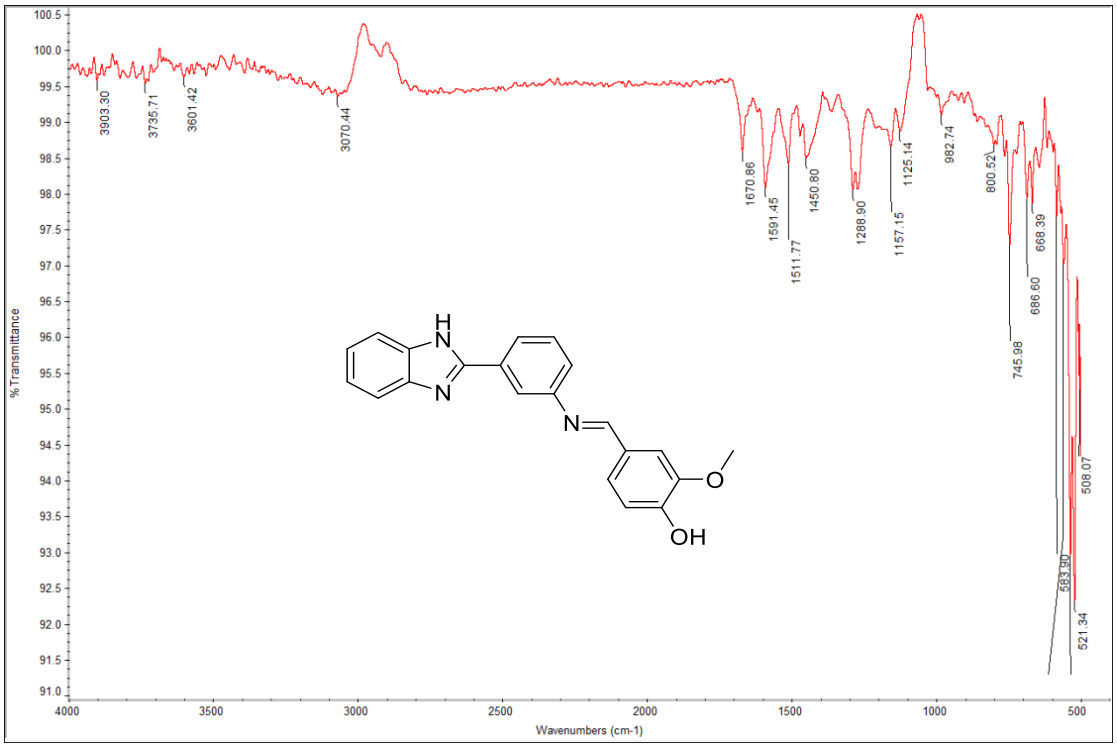


Figure A.25

FT-IR Spectra of 4-(1H-benzo[d]imidazol-2-yl)aniline (25)

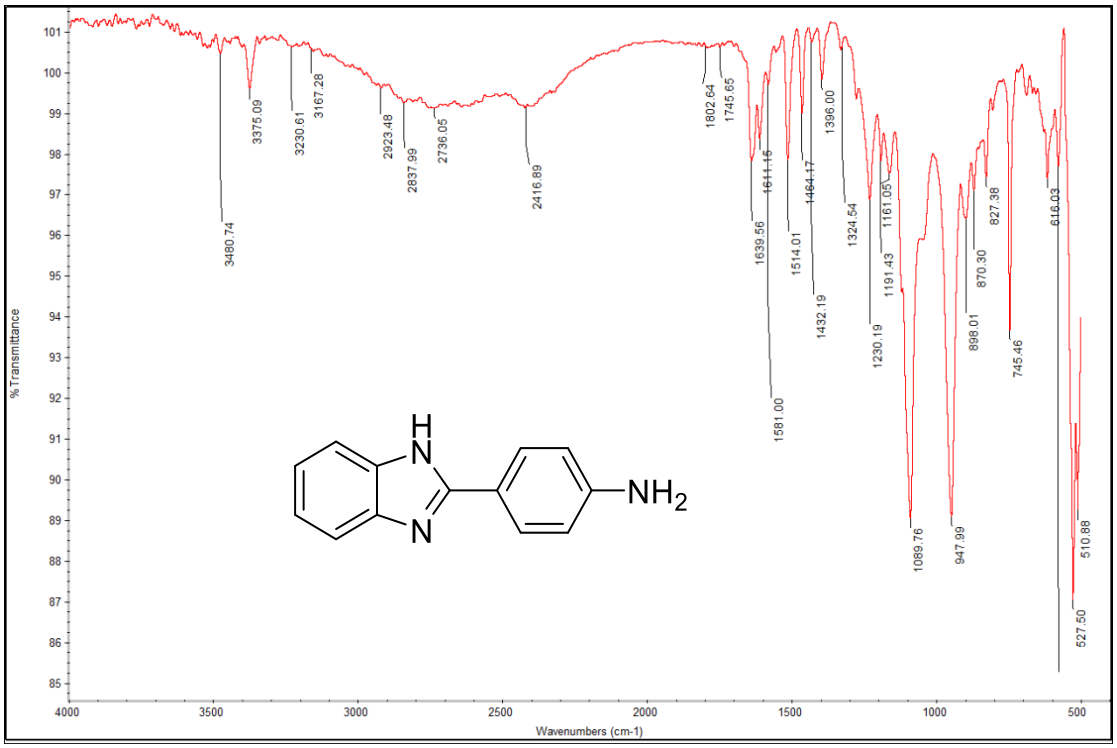


Figure A.26

FT-IR Spectra of (Z)-4-(((4-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (26)

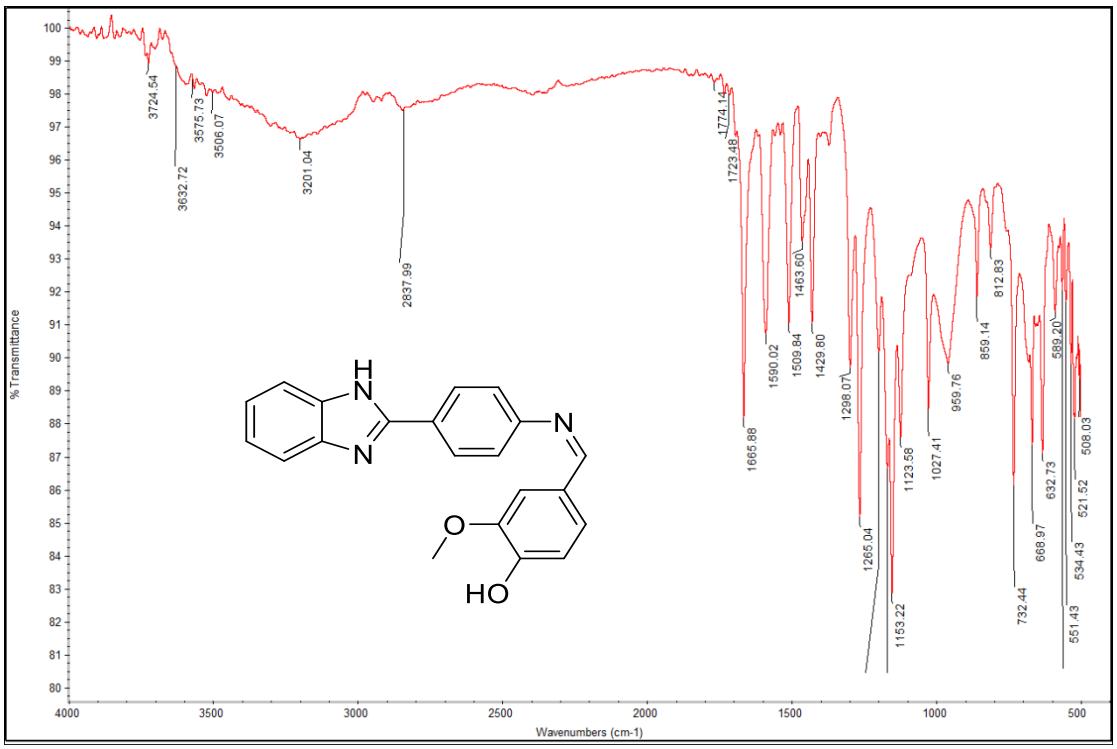


Figure A.27

FT-IR Spectra of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate (27)

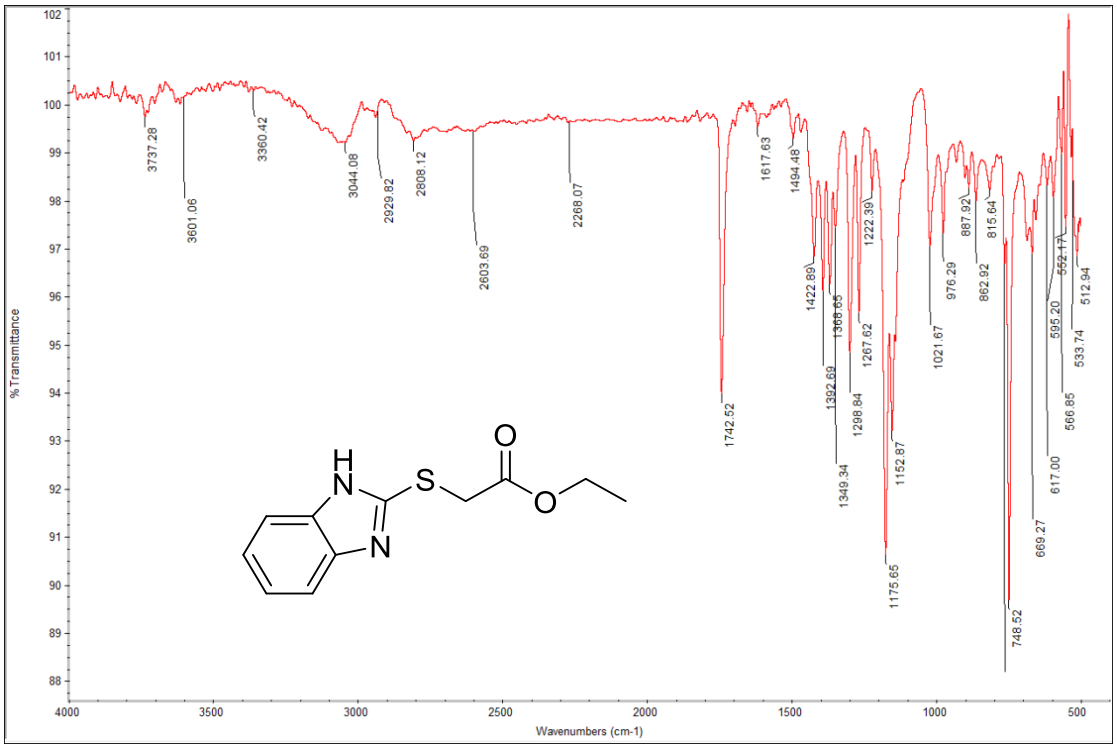


Figure A.28

FT-IR Spectra of 2-((1H-benzo[d]imidazol-2-yl)thio)acetohydrazide (28)

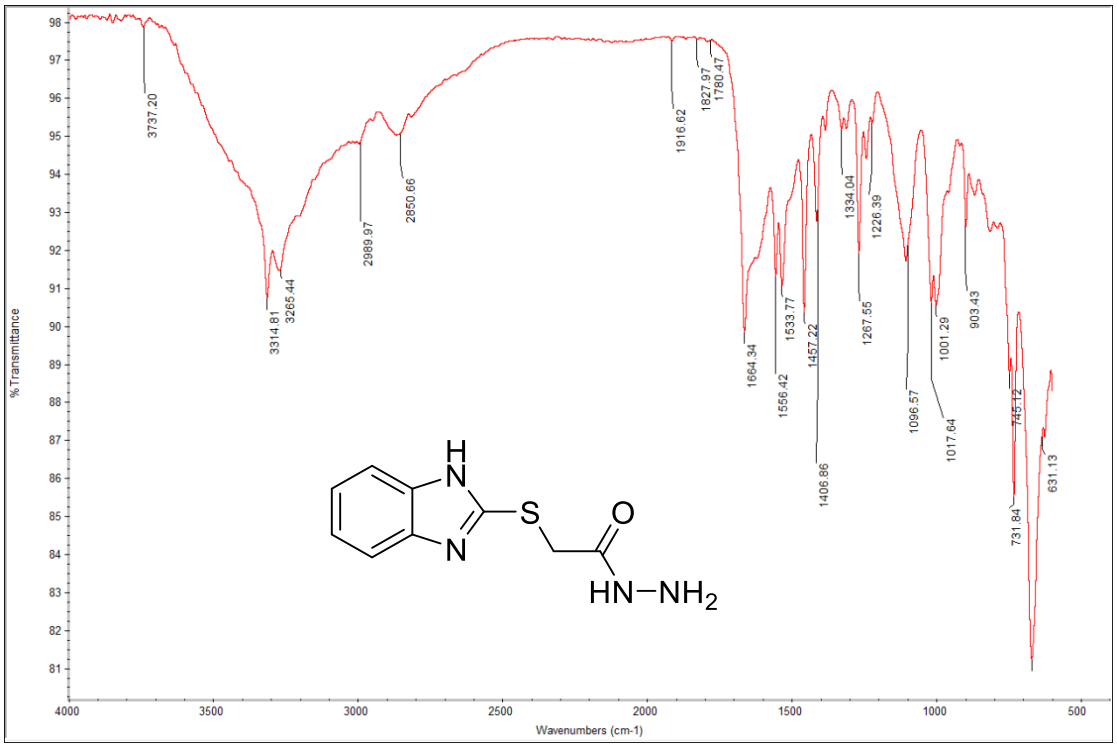


Figure A.29

FT-IR Spectra of (E)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-(4-hydroxy-3-methoxybenzylidene)acetohydrazide (29)

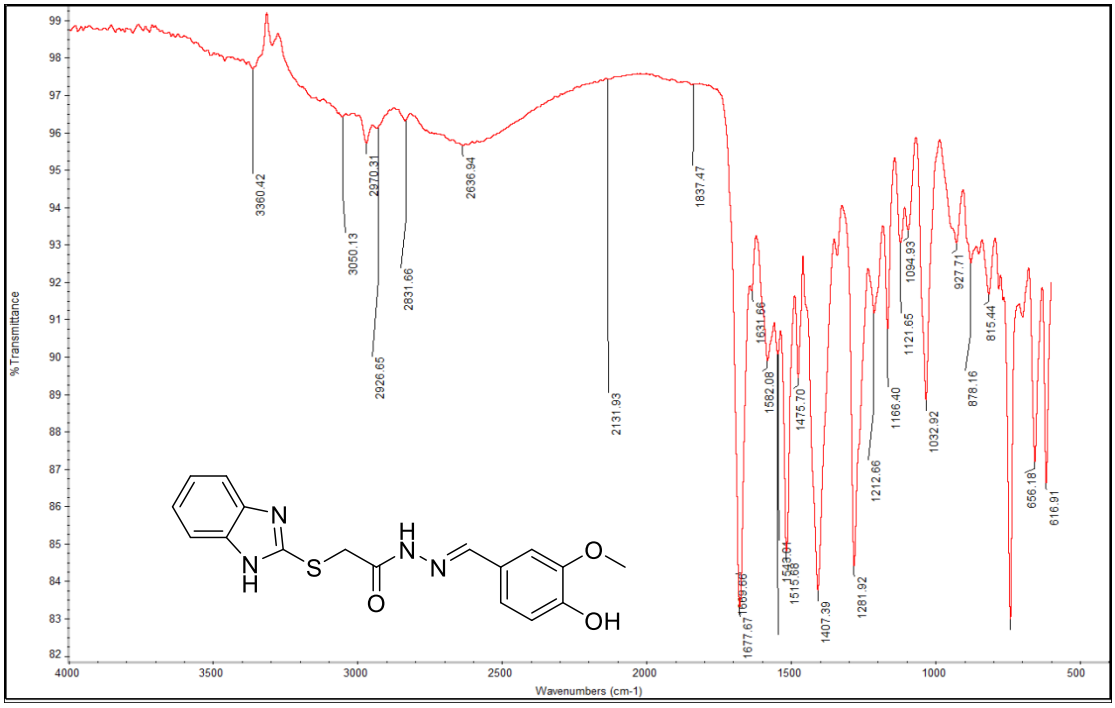


Figure A.30

FT-IR Spectra of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide (30).

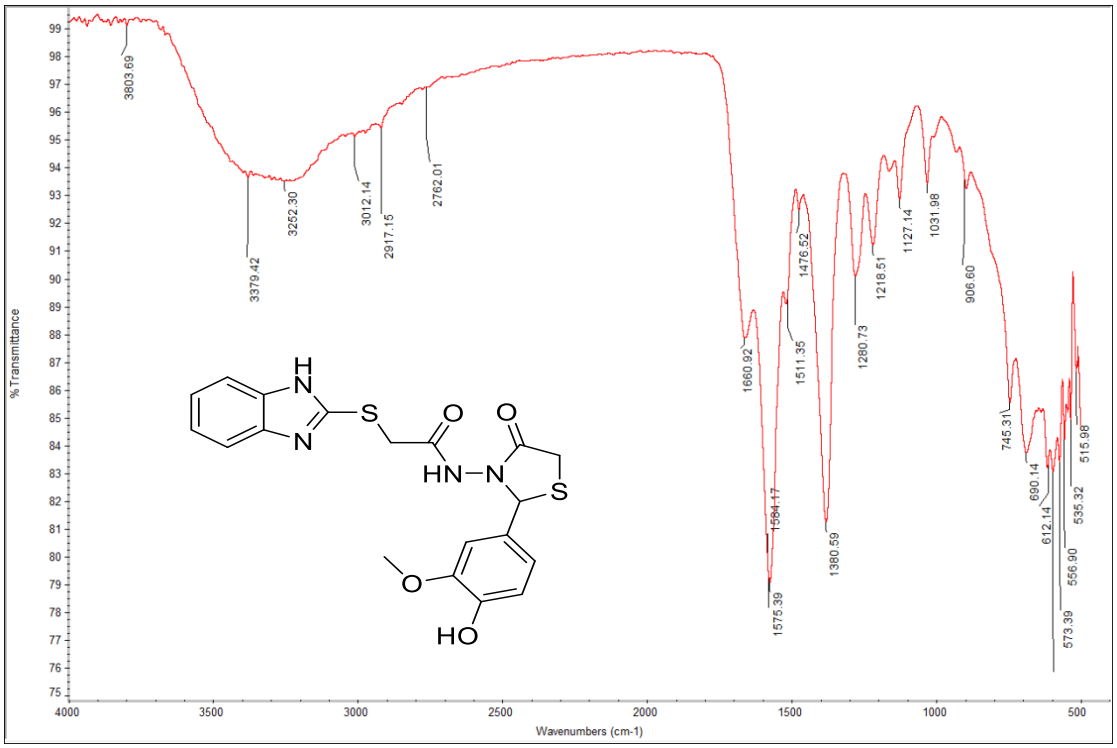


Figure A.31

FT-IR Spectra of (Z)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-((6-chloro-1H-indol-3-yl)methylene)acetohydrazide (31)

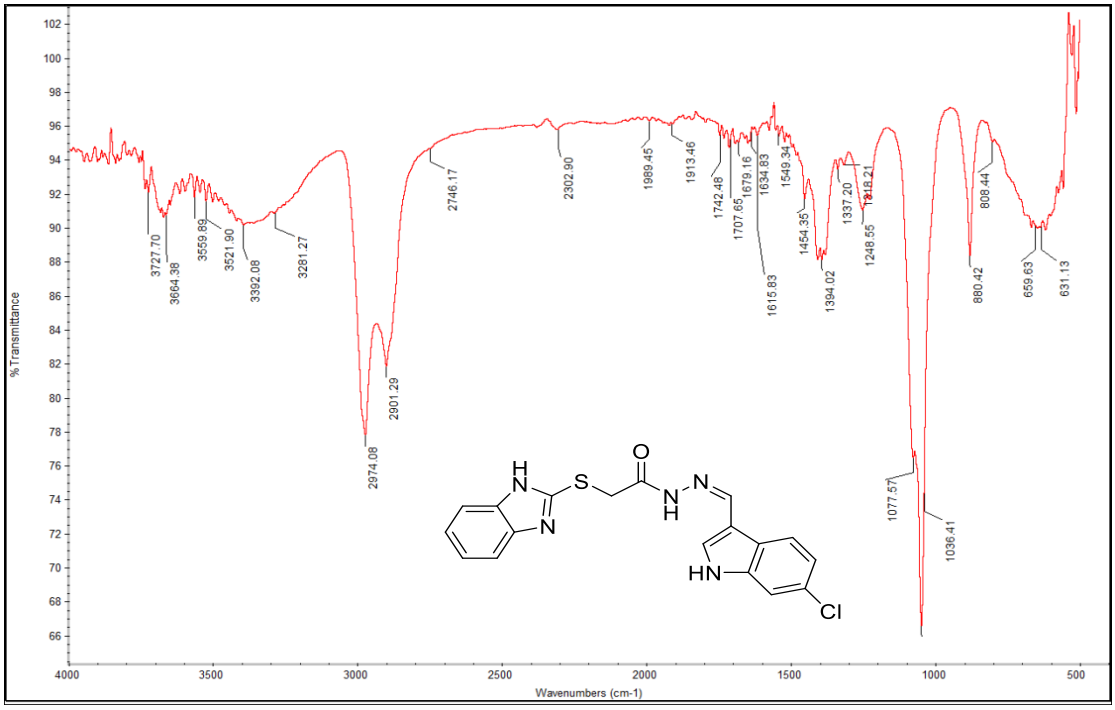


Figure A.32

FT-IR Spectra of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(6-chloro-1H-indol-3-yl)-4-oxothiazolidin-3-yl)acetamide (32)

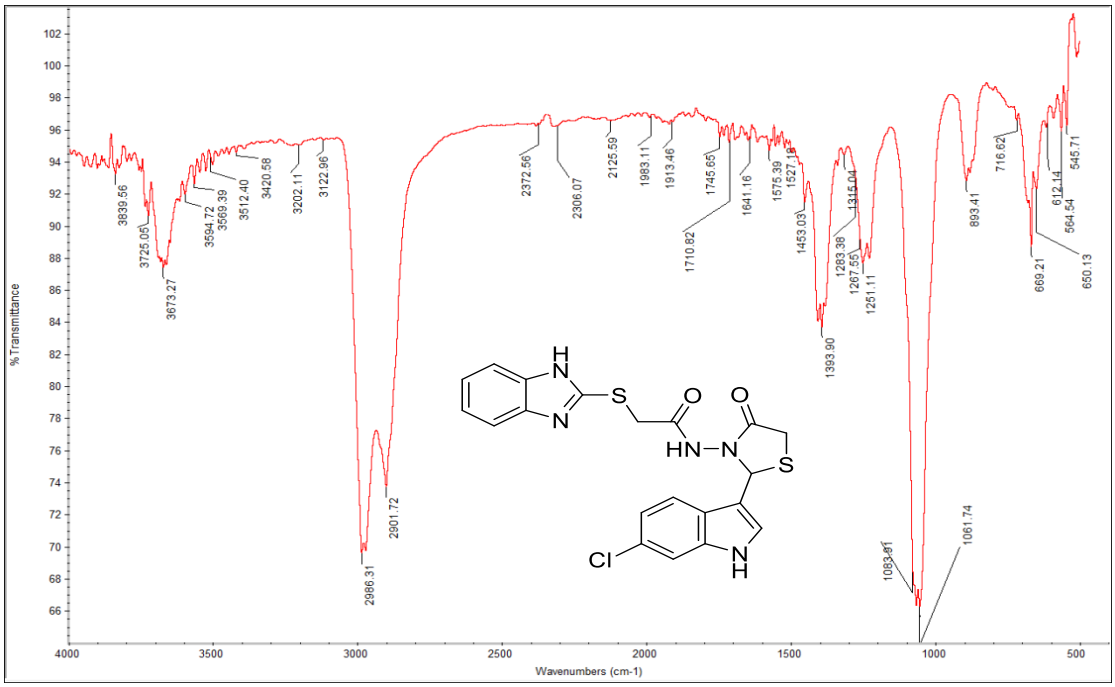


Figure A.33

[1]H-NMR Spectra of (Z)-4-(4-hydroxy-3-methoxybenzylidene)-2-phenyloxazol-5(4H)-one (2)

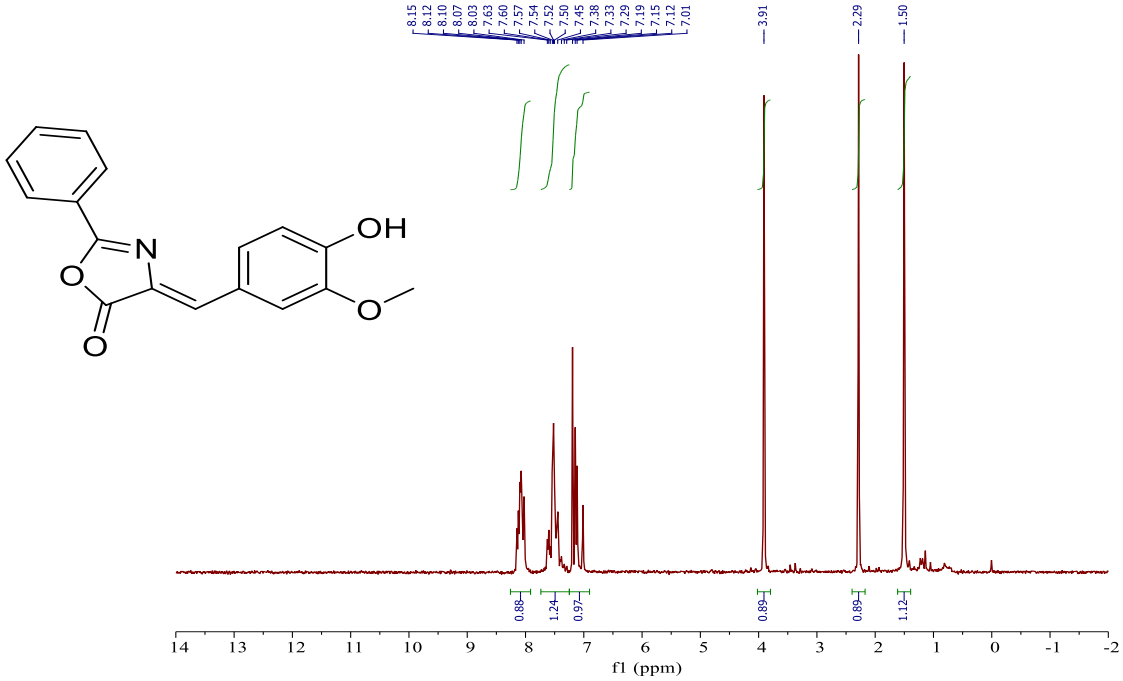


Figure A.34

[1]H-NMR Spectra of (Z)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3)

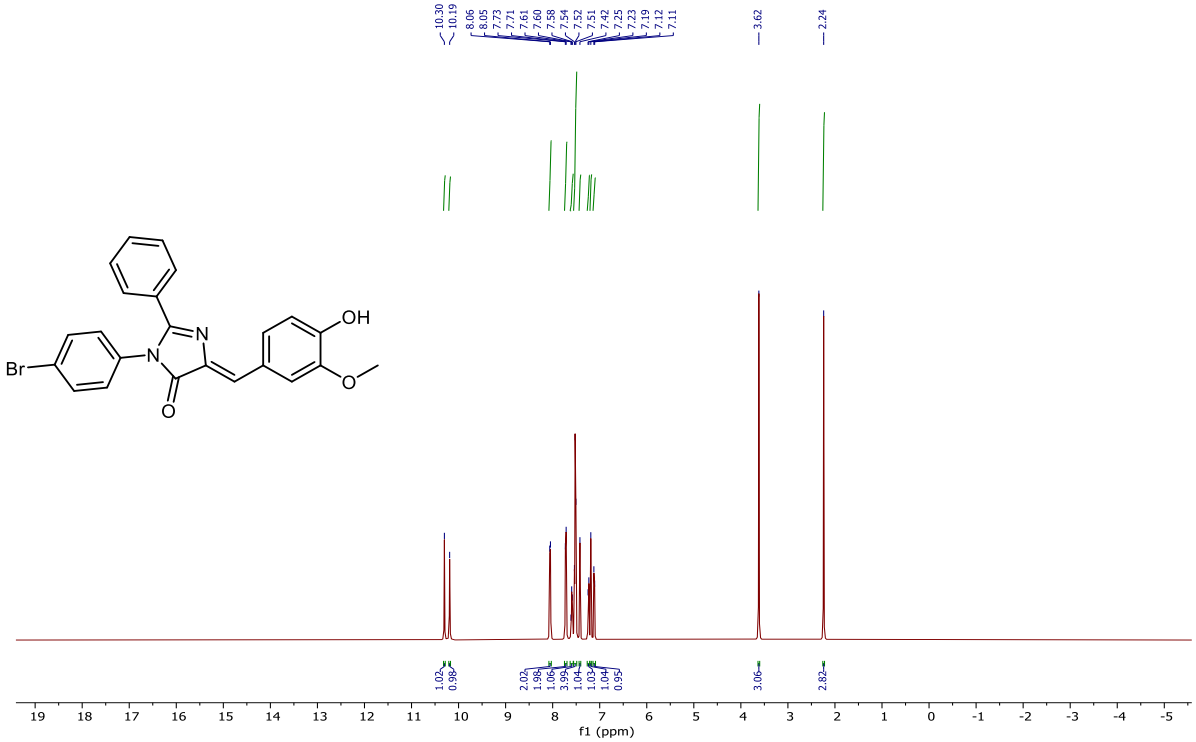


Figure A.35

^{1}H-NMR Spectra of (Z)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (4)

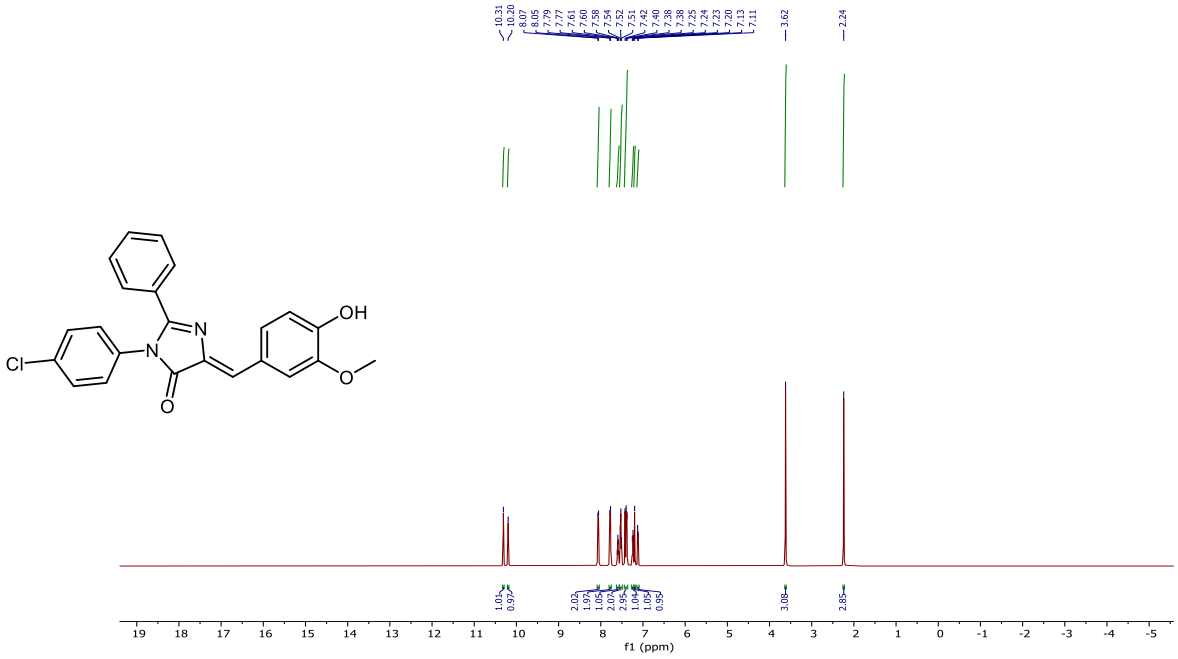


Figure A.36

^{1}H-NMR Spectra of (Z)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5)

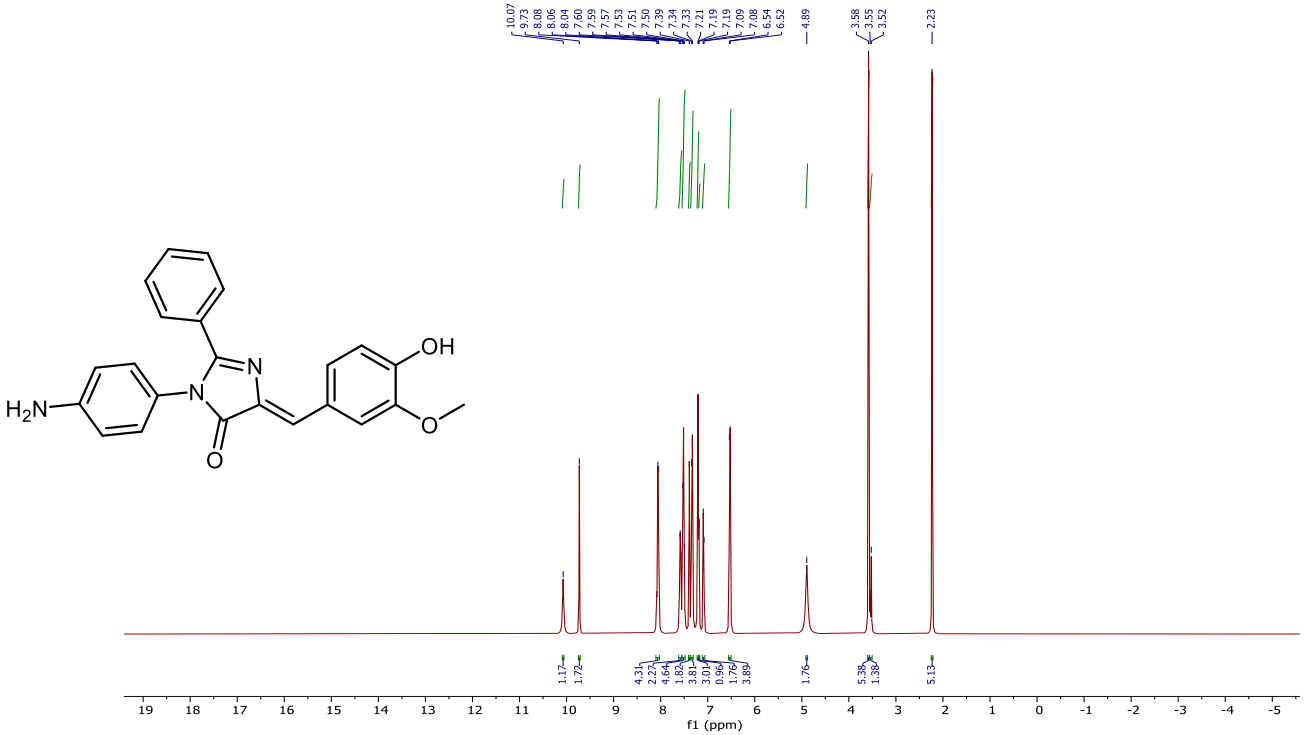


Figure A.37

^{1}H-NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (6)

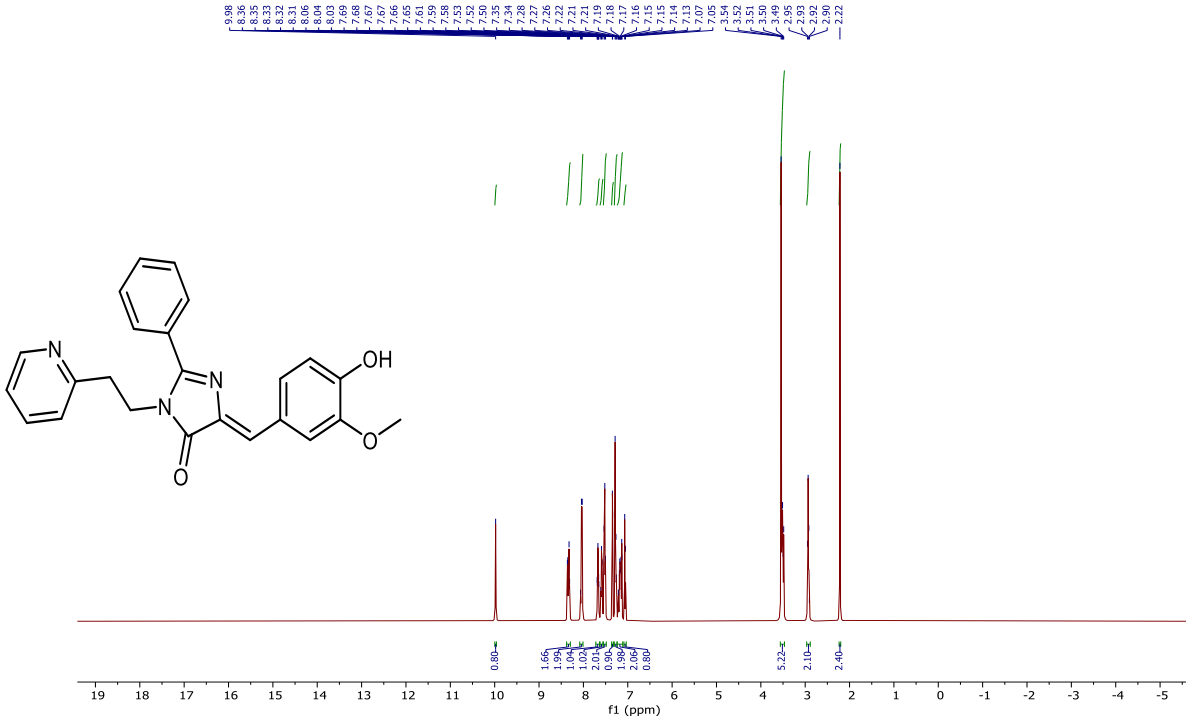


Figure A.38

^{1}H-NMR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoic acid (7)

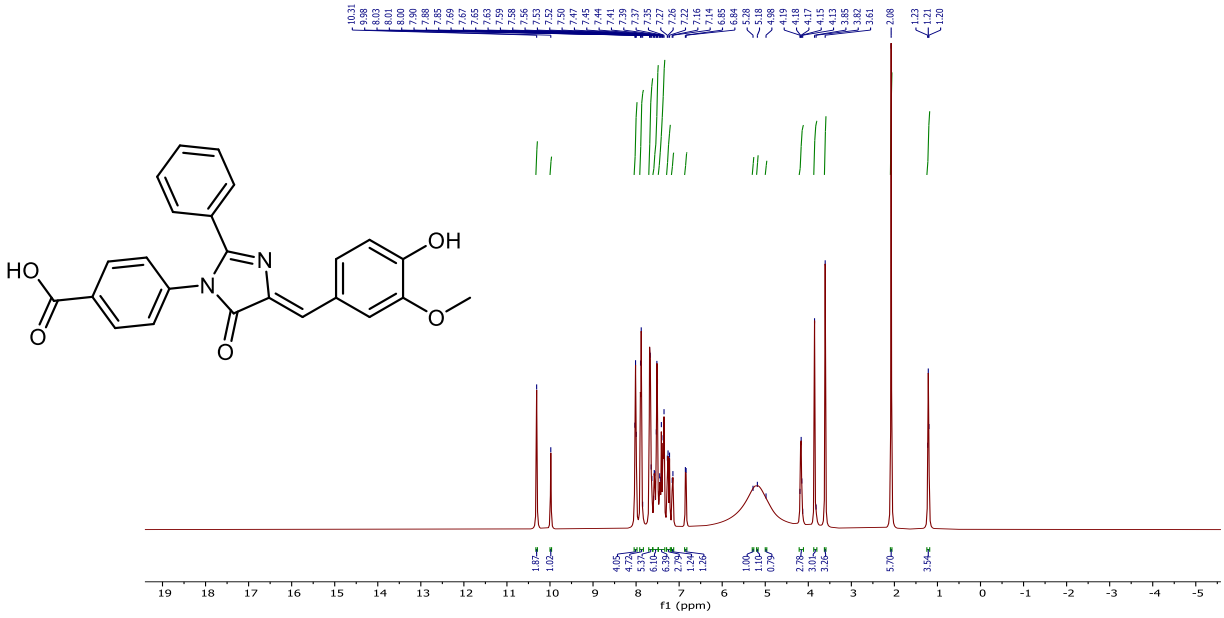


Figure A.39

^{1}H-NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (8)

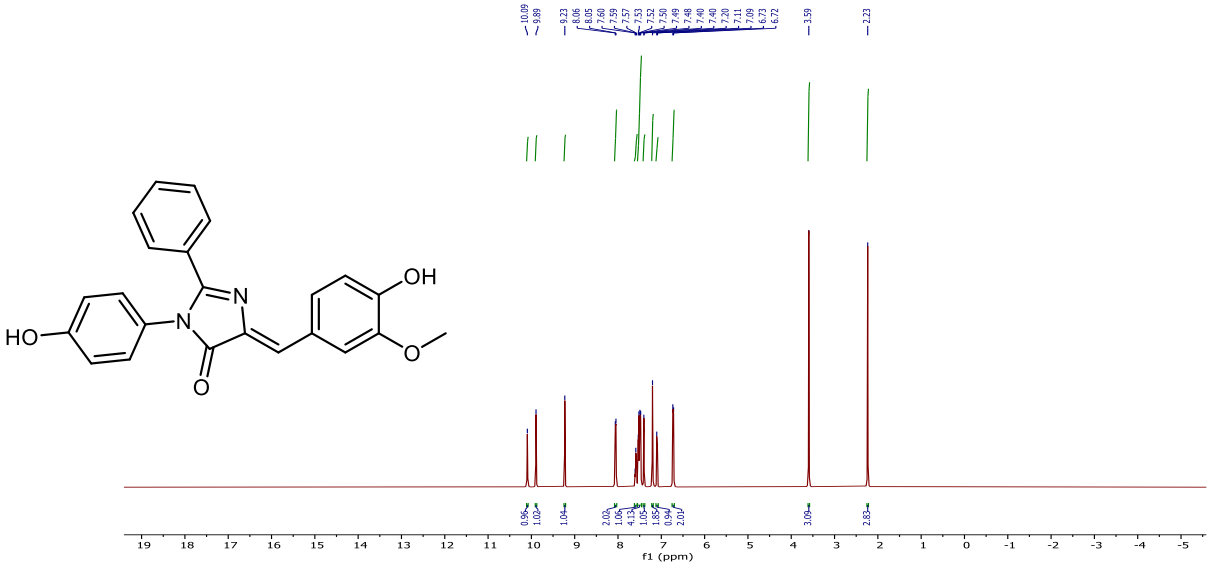


Figure A.40

^{1}H-NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (9)

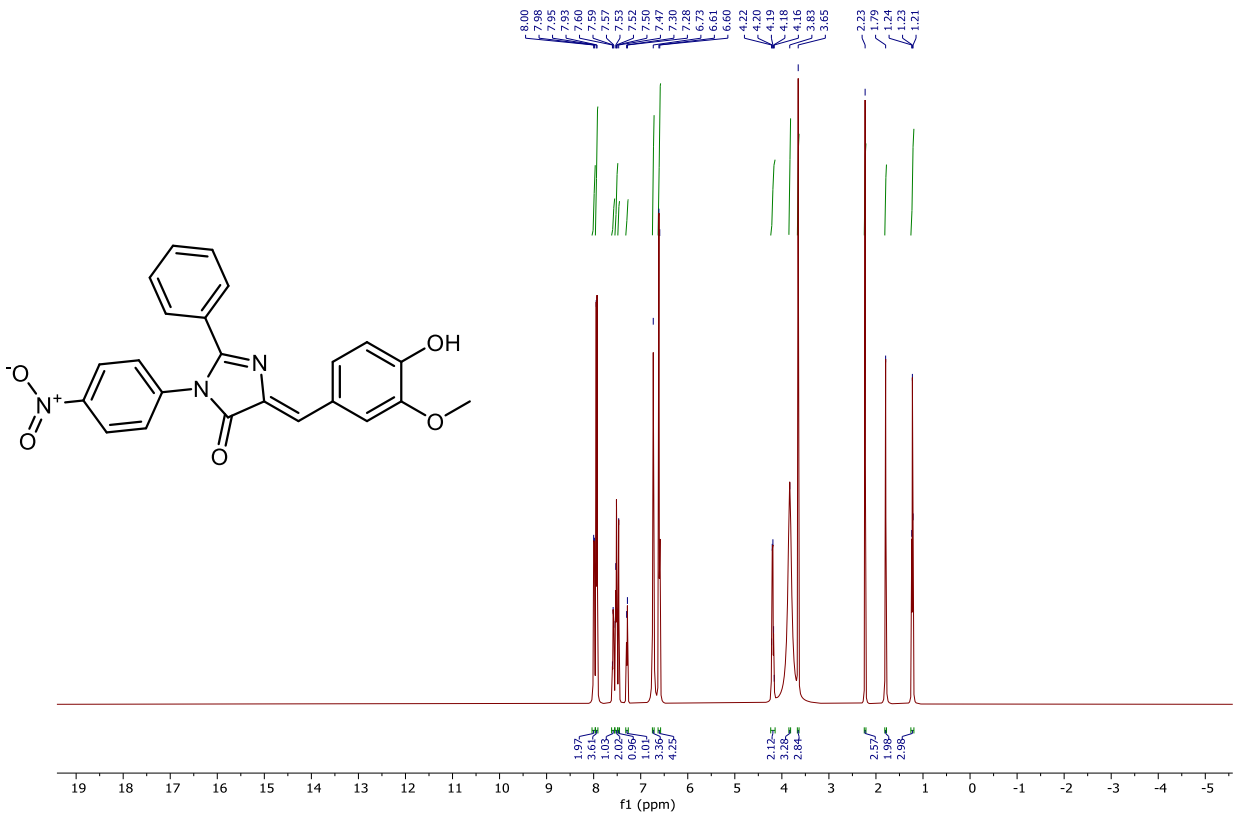


Figure A.41

^{1}H-NMR Spectra of (Z)-N-(1H-benzo[d]imidazol-2-yl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazole-1-carboximidamide (10)

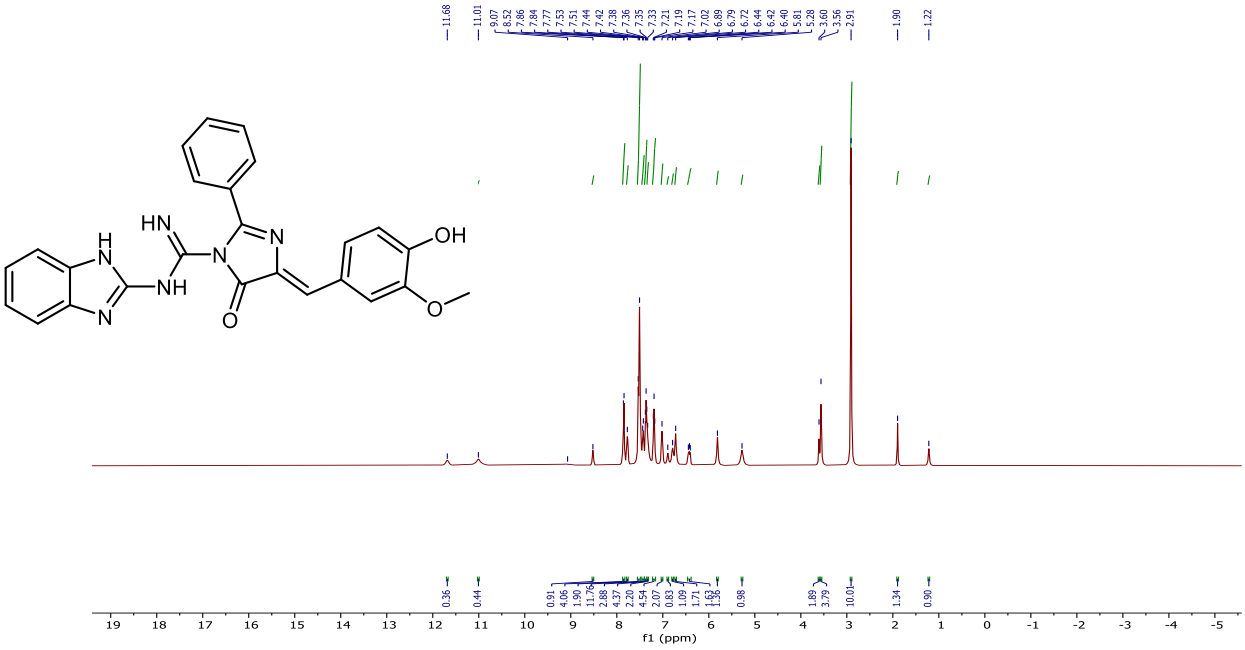


Figure A.42

^{1}H-NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (11)

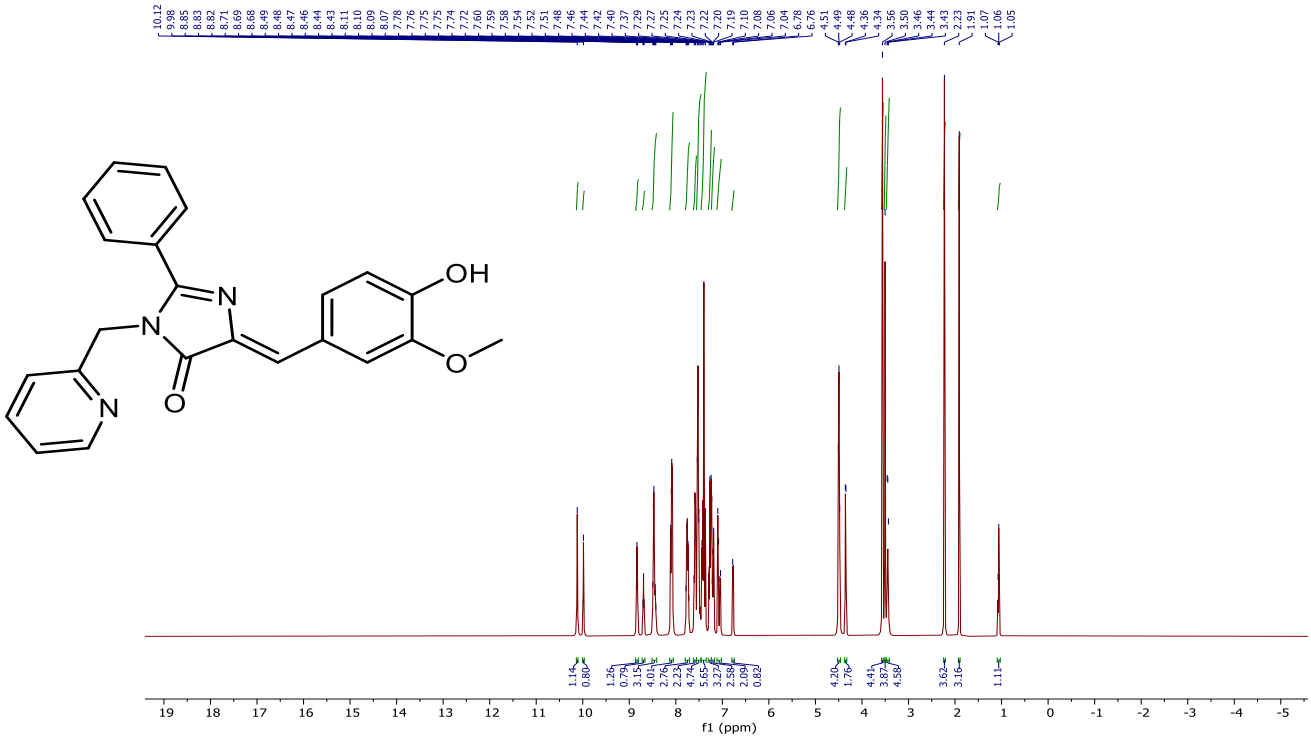


Figure A.43

[1]H-NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (12)

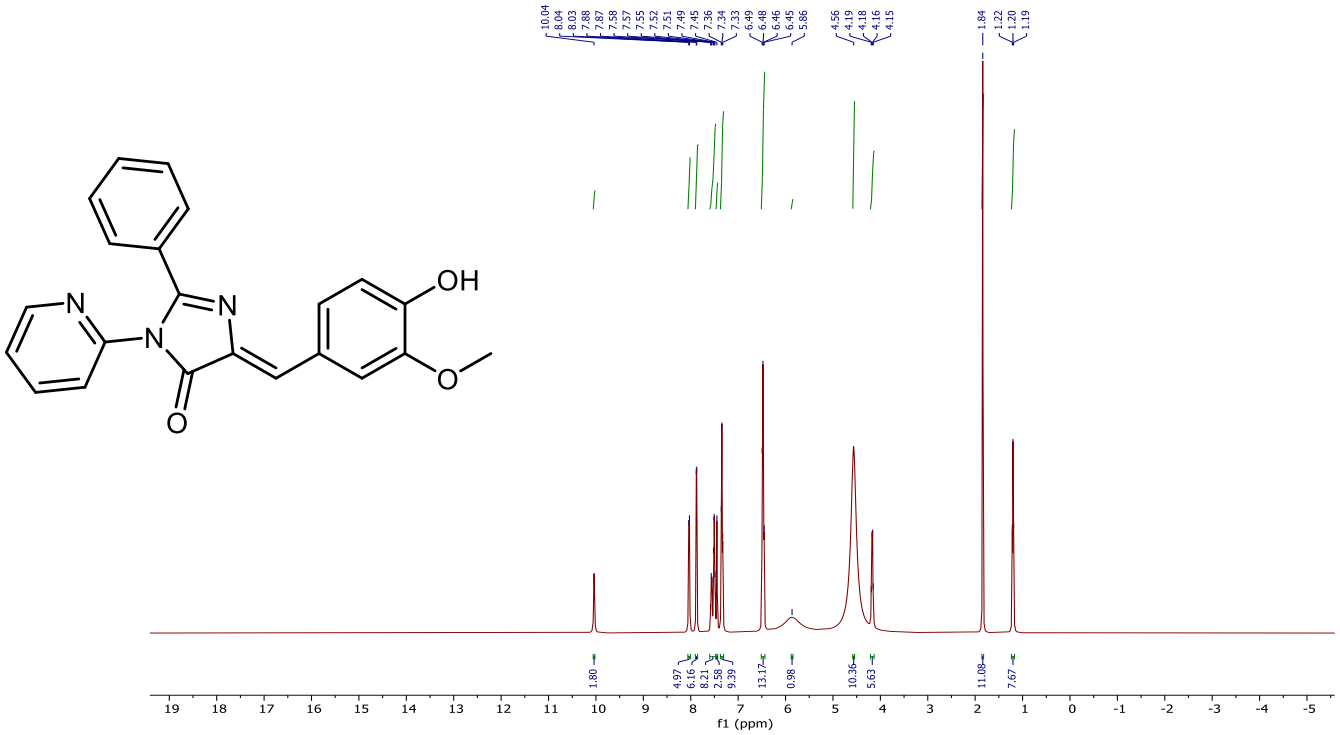


Figure A.44

[1]H-NMR Spectra of (Z)-3-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (13)

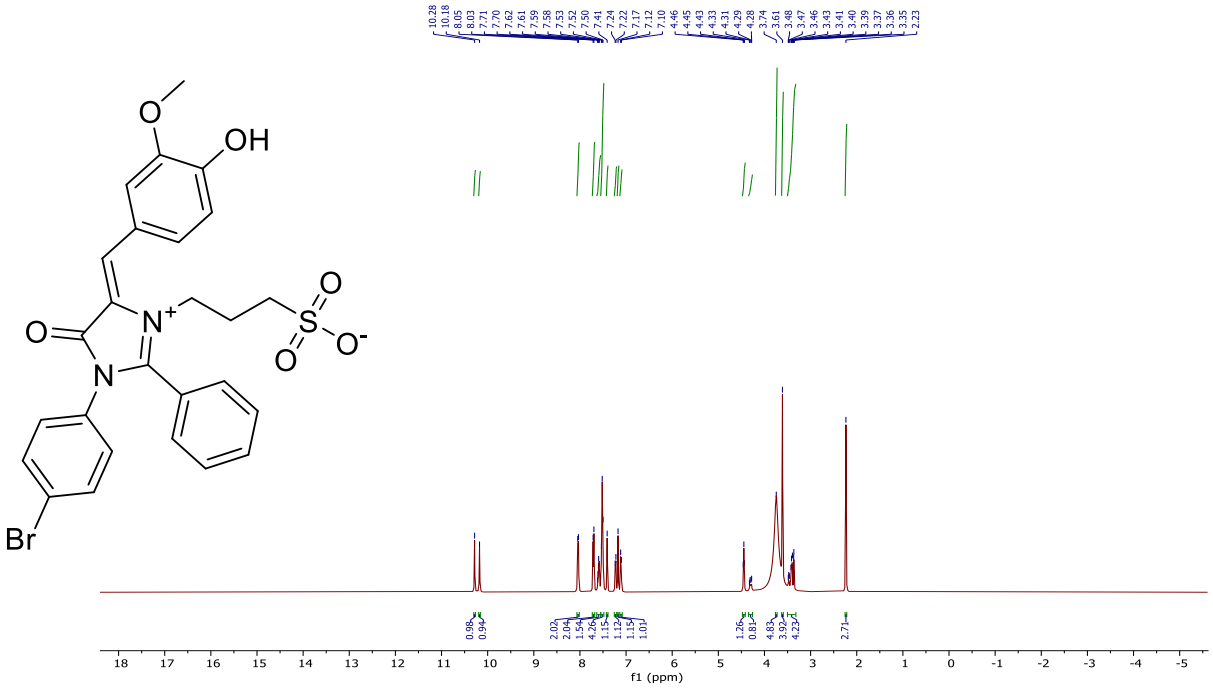


Figure A.45

^{1}H-NMR Spectra of (Z)-3-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (14)

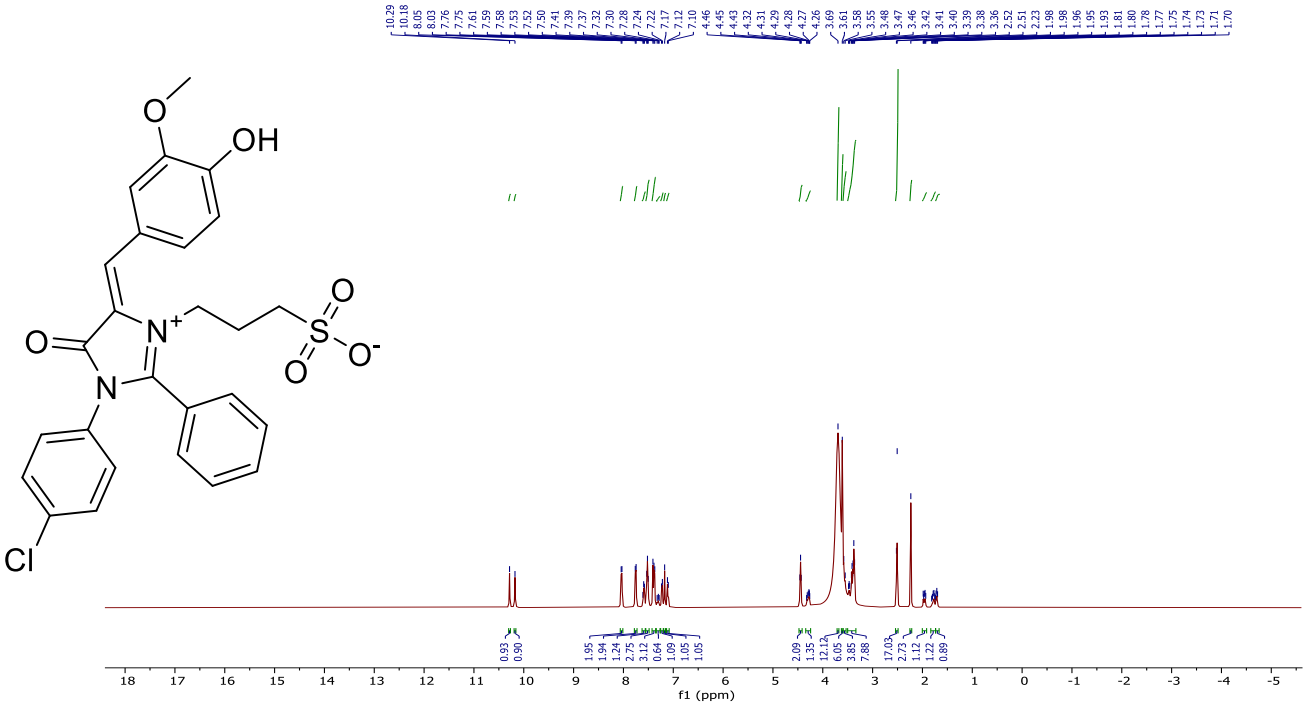


Figure A.46

^{1}H-NMR Spectra of (Z)-3-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (15)

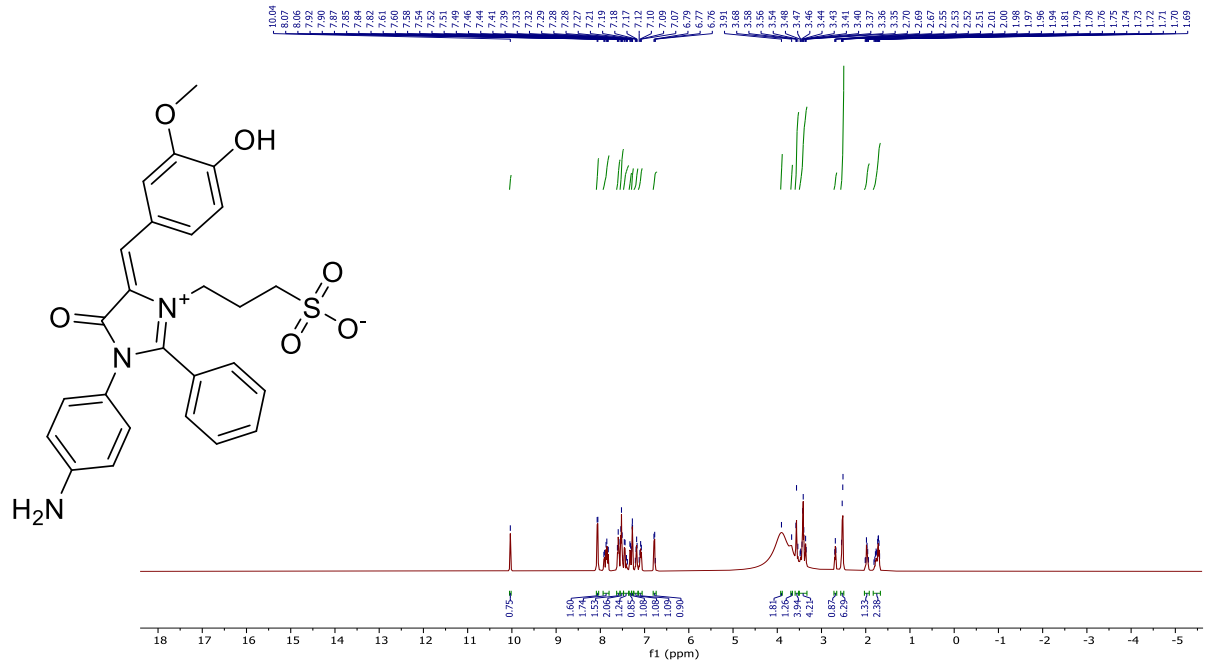


Figure A.47

^{1}H-NMR Spectra of (Z)-3-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (16)

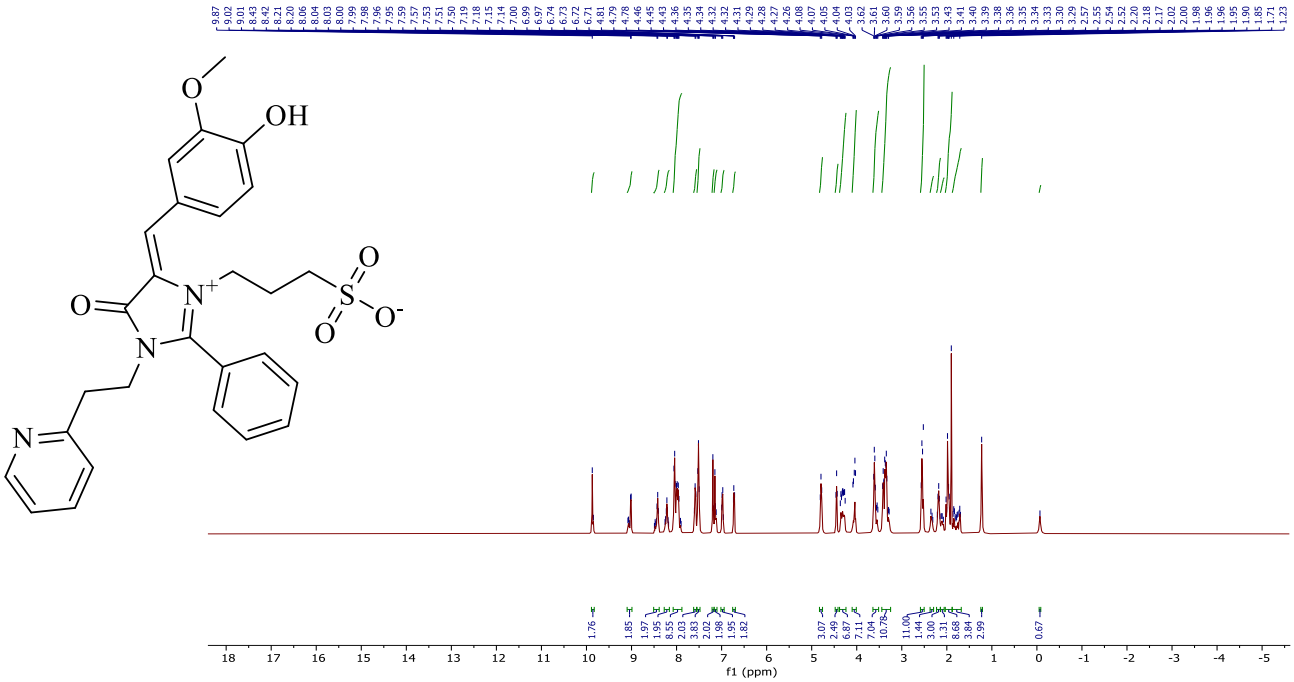


Figure A.48

^{1}H-NMR Spectra of (Z)-3-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (17).

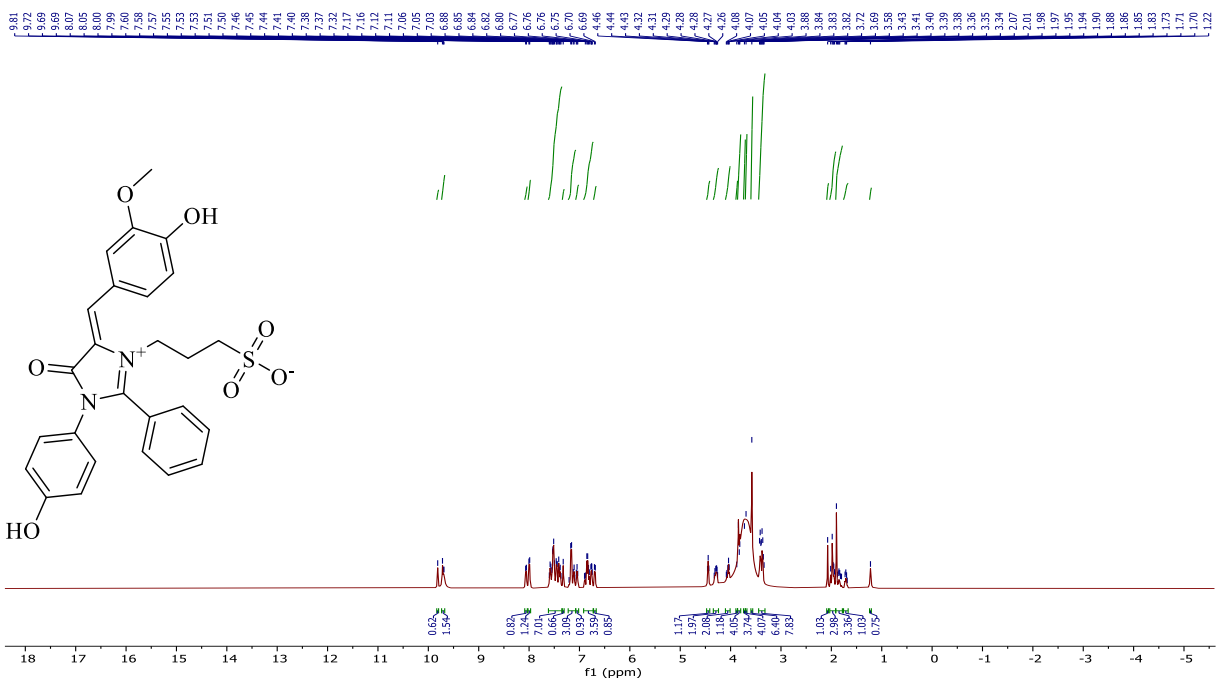


Figure A.49

^{1}H-NMR Spectra of (Z)-4-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (18)

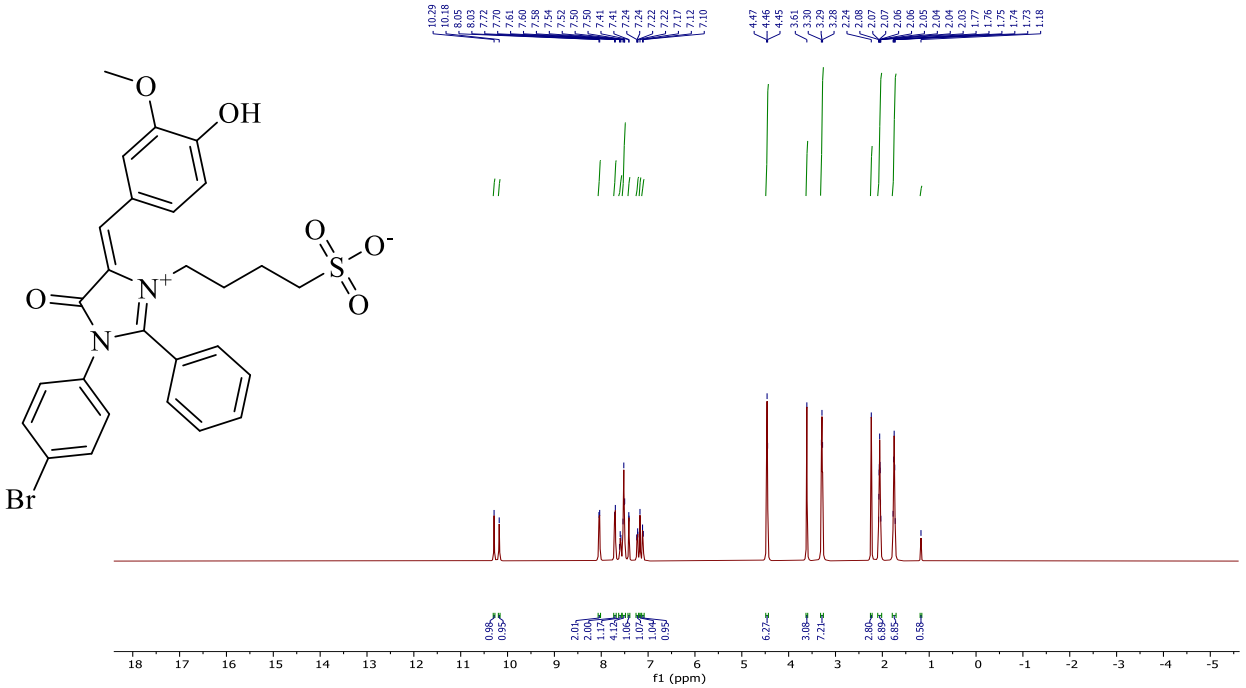


Figure A.50

^{1}H-NMR Spectra of (Z)-4-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (19)

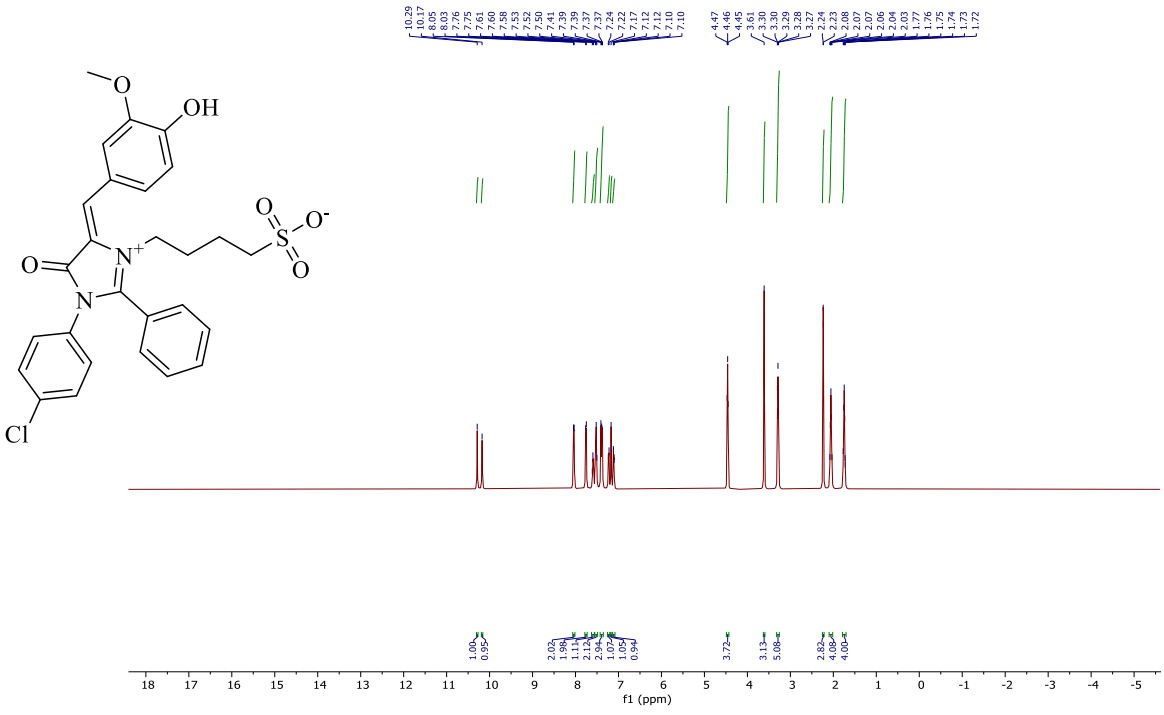


Figure A.51

^{1}H-NMR Spectra of (Z)-4-(1-(4-aminophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (20)

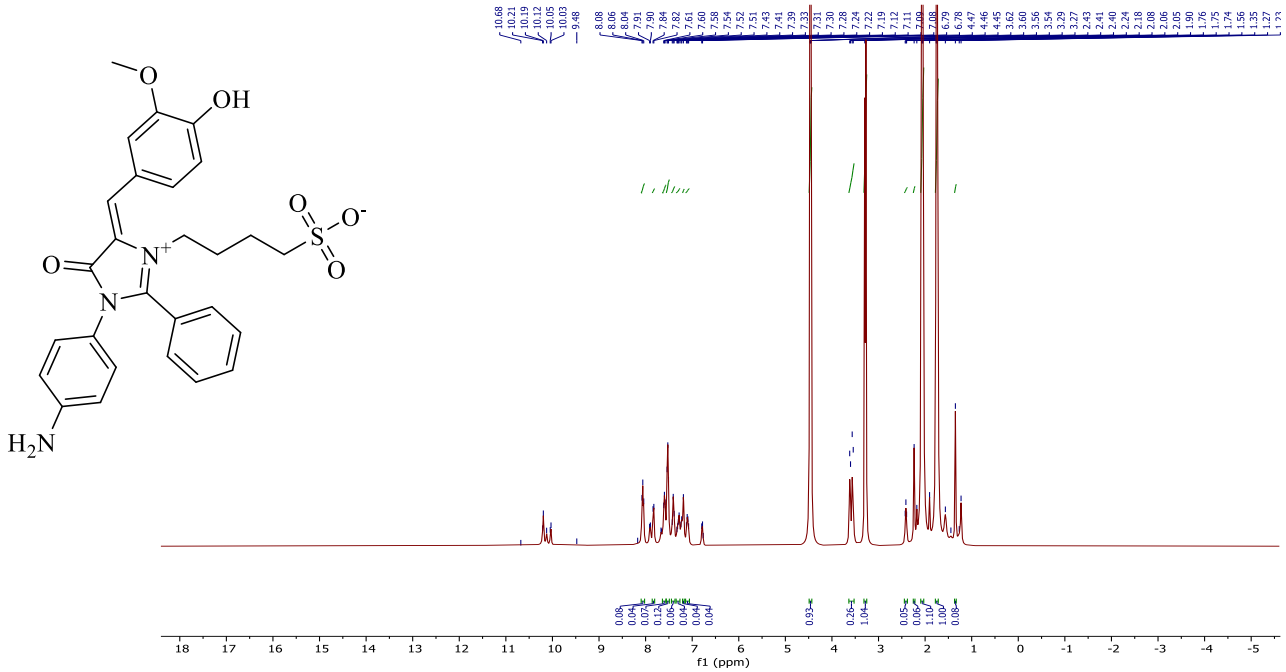


Figure A.52

^{1}H-NMR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-1-(2-(pyridin-2-yl)ethyl)-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (21)

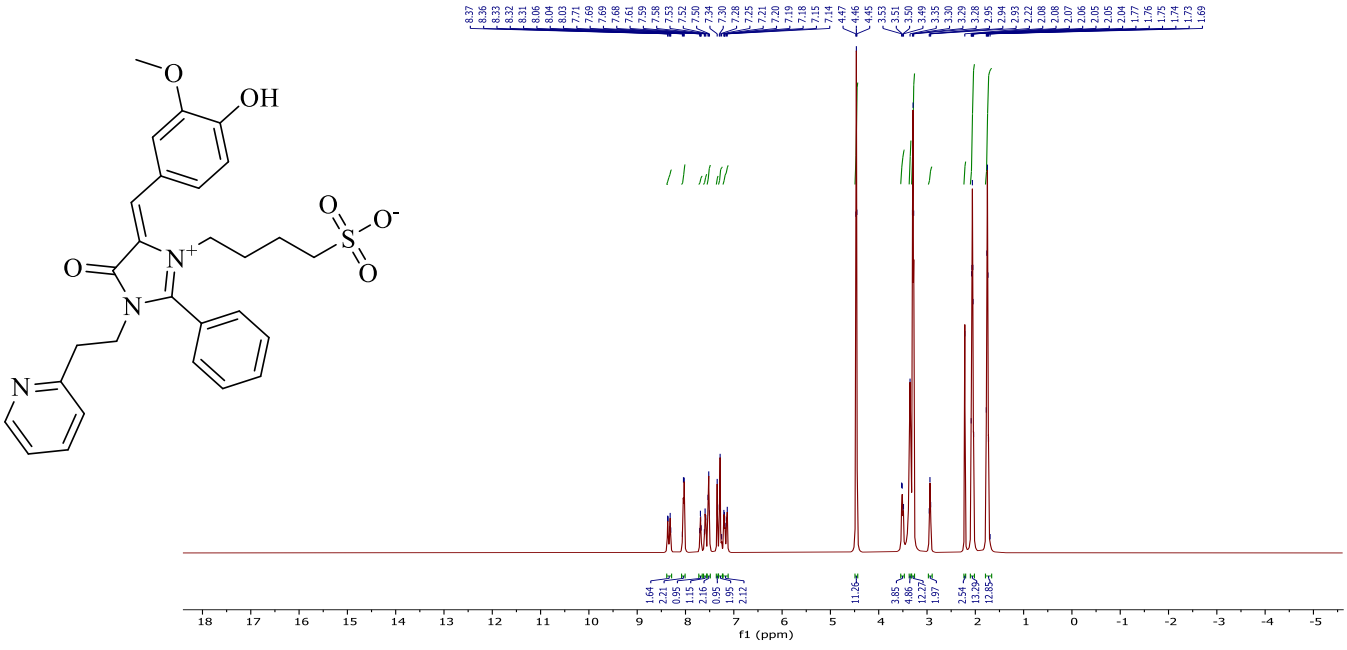


Figure A.53

^{1}H-NMR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)butane-1-sulfonate (22)

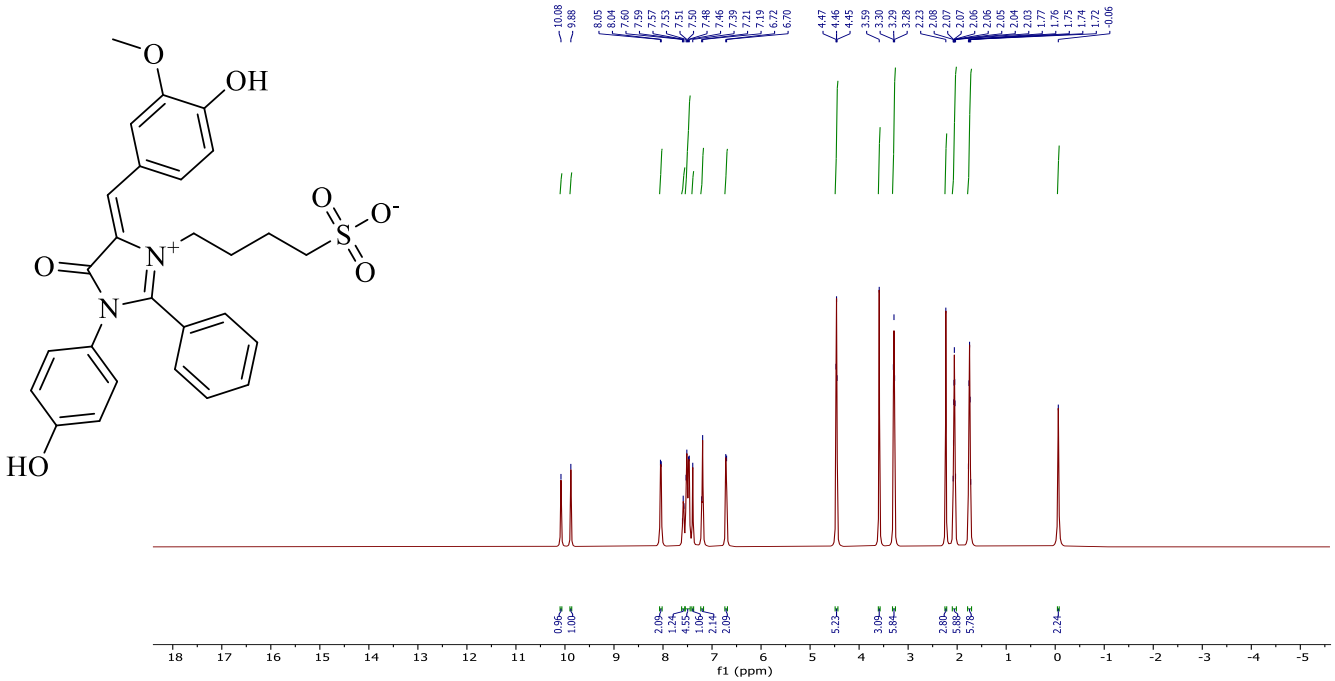


Figure A.54

^{1}H-NMR Spectra of 4-(((3-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (24).

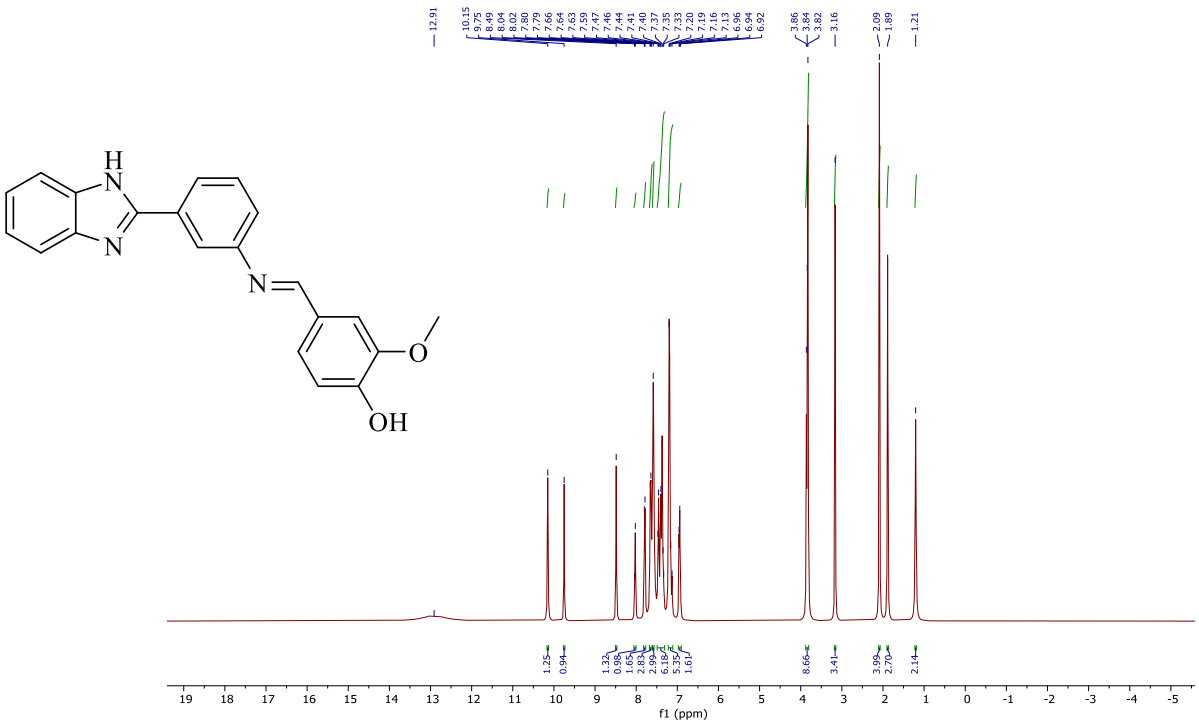


Figure A.55

^{1}H-NMR Spectra of (Z)-4-(((4-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (26)

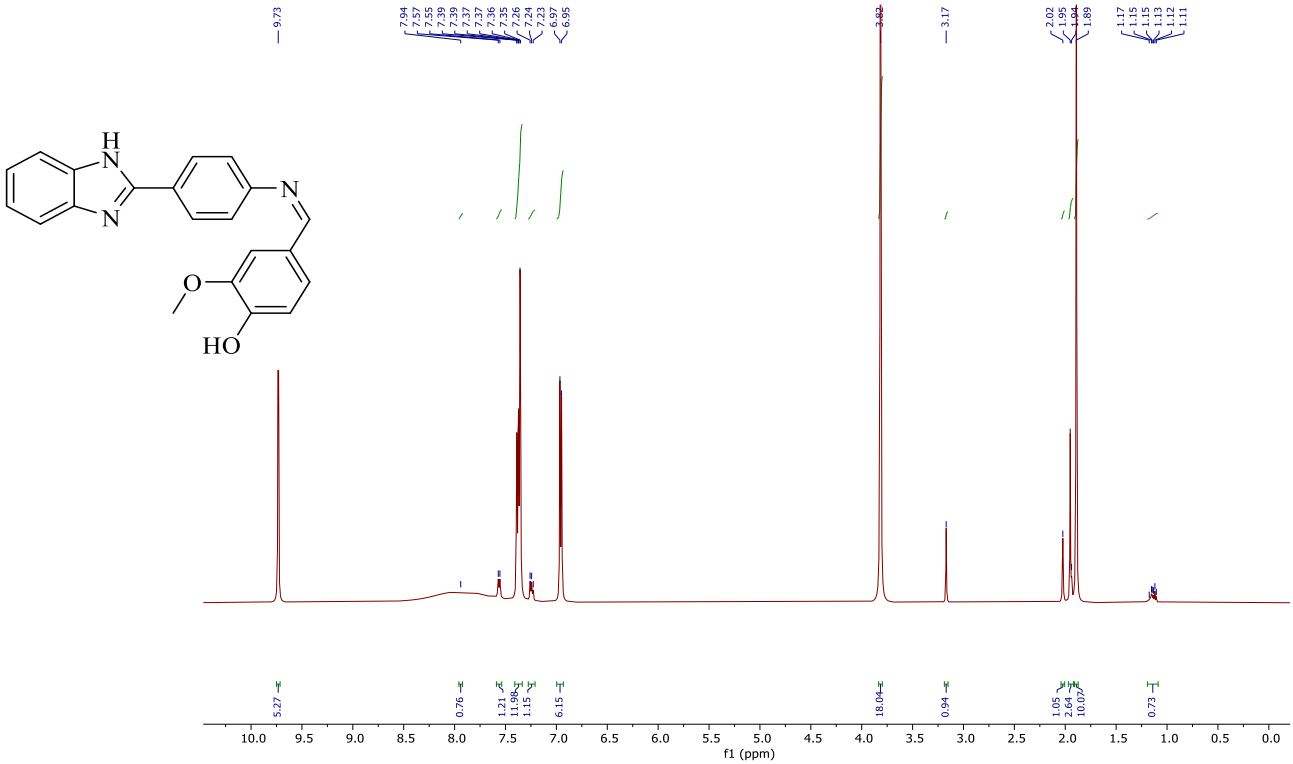


Figure A.56

^{1}H-NMR Spectra of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide (30).

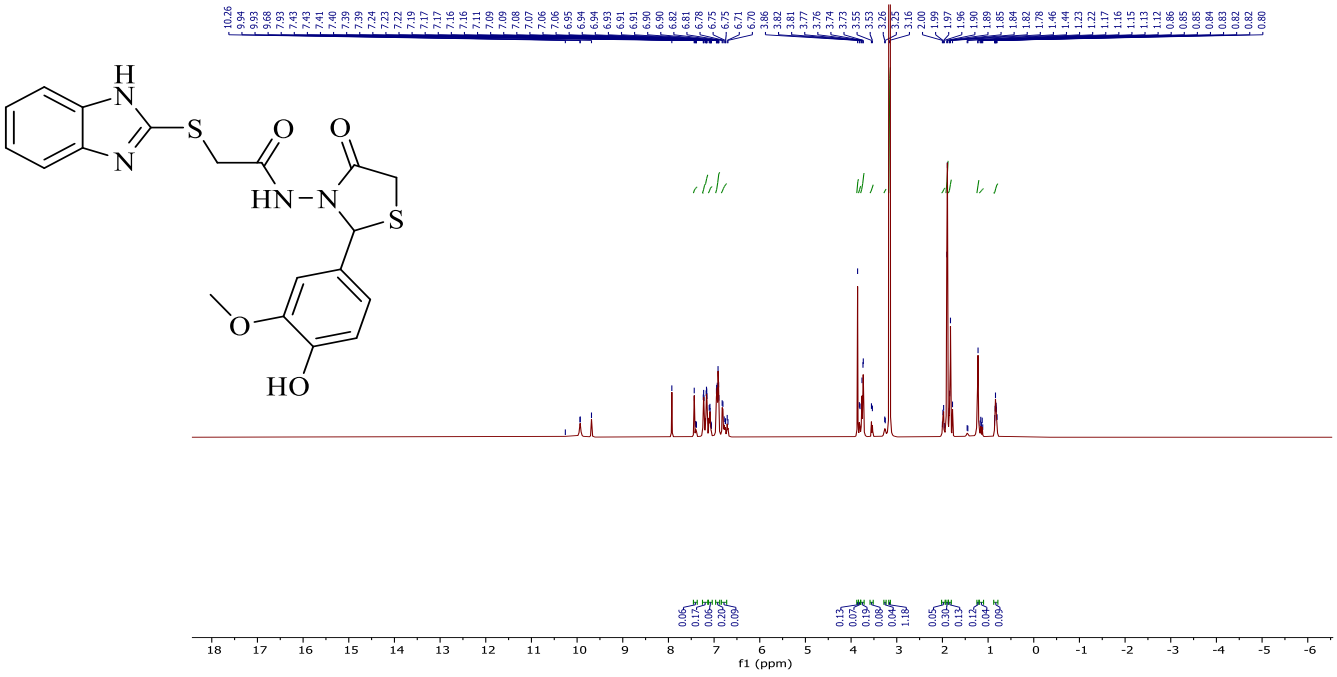


Figure A.57

[1]H-NMR Spectra of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(6-chloro-1H-indol-3-yl)-4-oxothiazolidin-3-yl)acetamide(32)

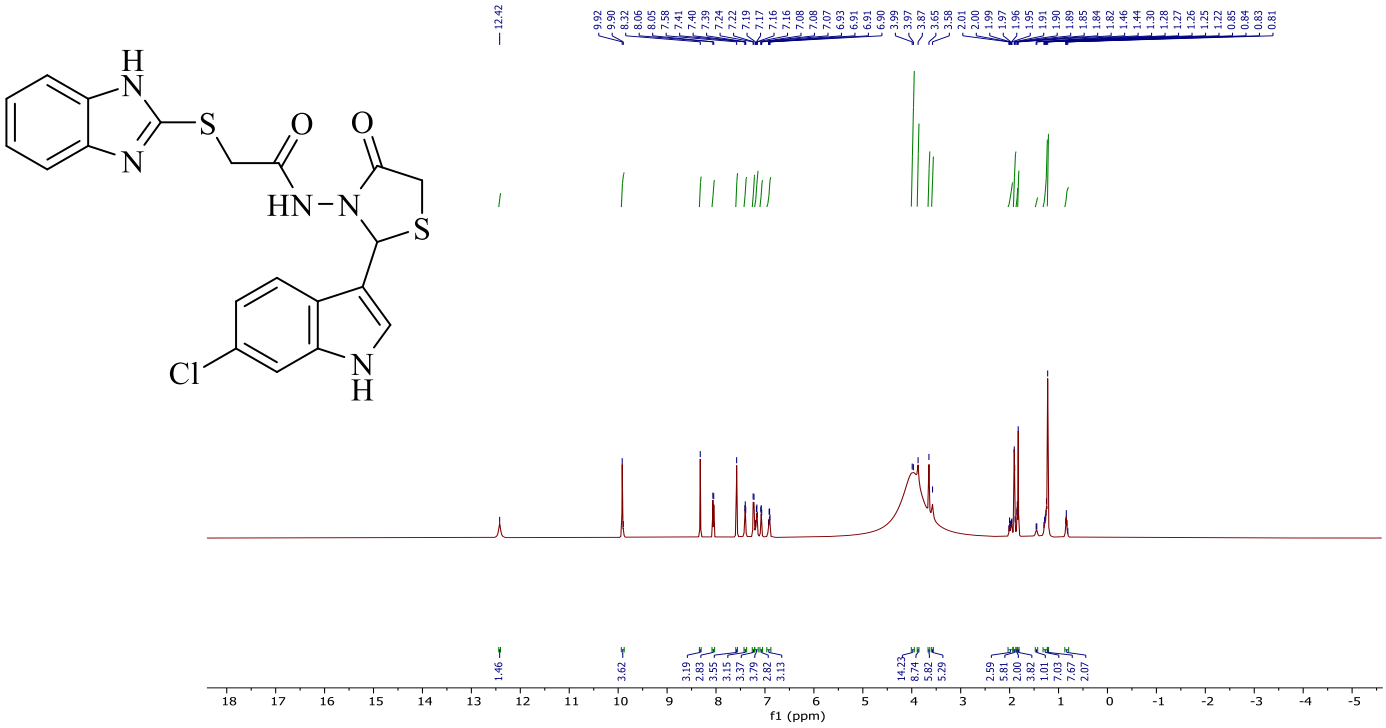


Figure A.58

[13]CNMR NMR Spectra of (Z)-4-(4-hydroxy-3methoxybenzylidene)-2-phenyloxazol-5(4H)-one (2)

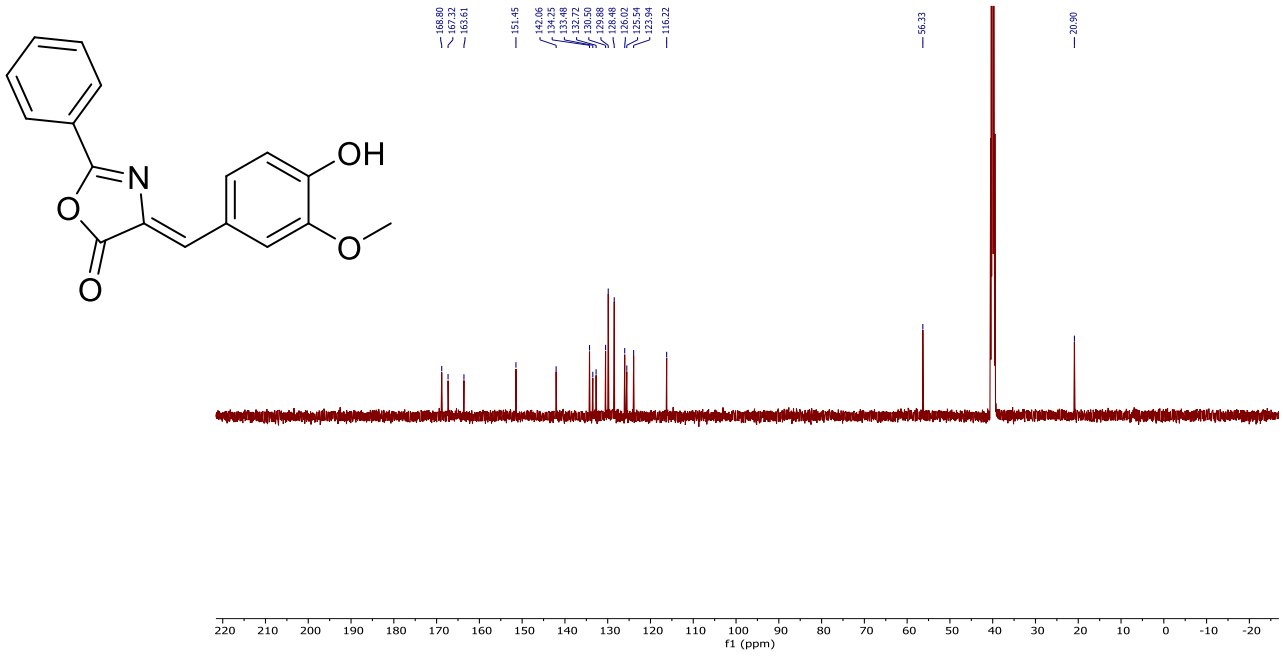


Figure A.59

^{13}CNMR NMR Spectra of (Z)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3)

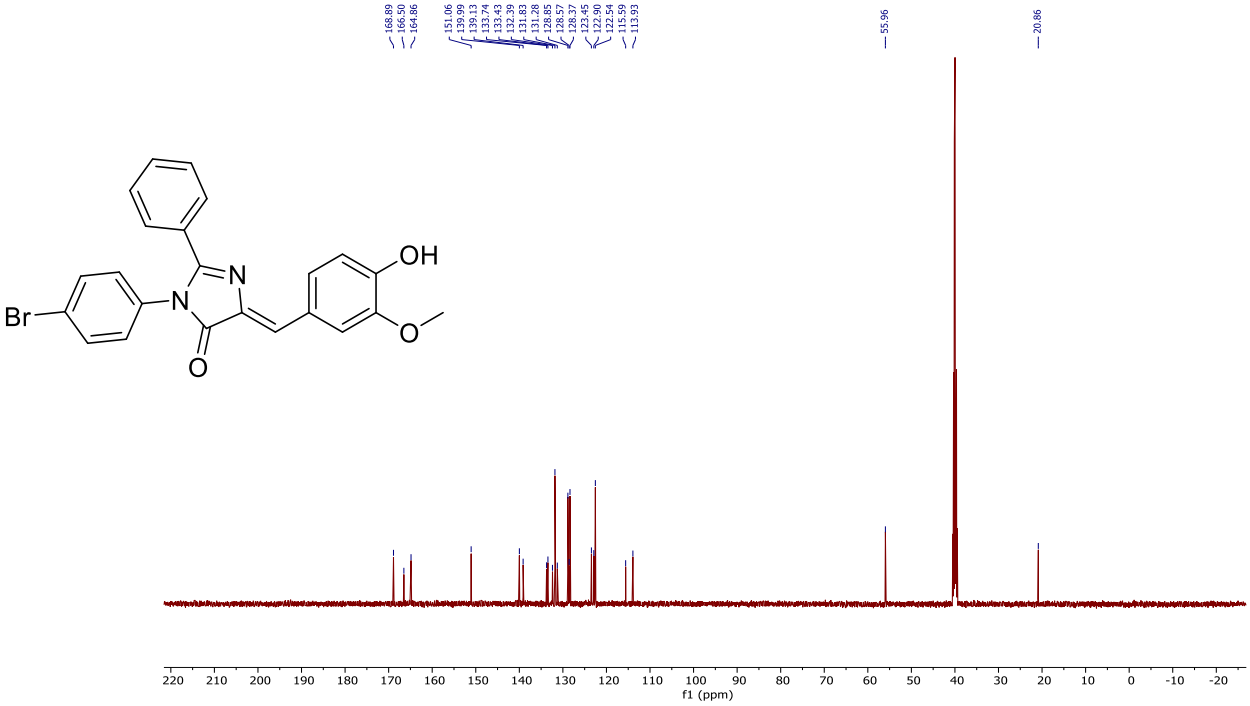


Figure A.60

^{13}CNMR NMR Spectra of (Z)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (4)

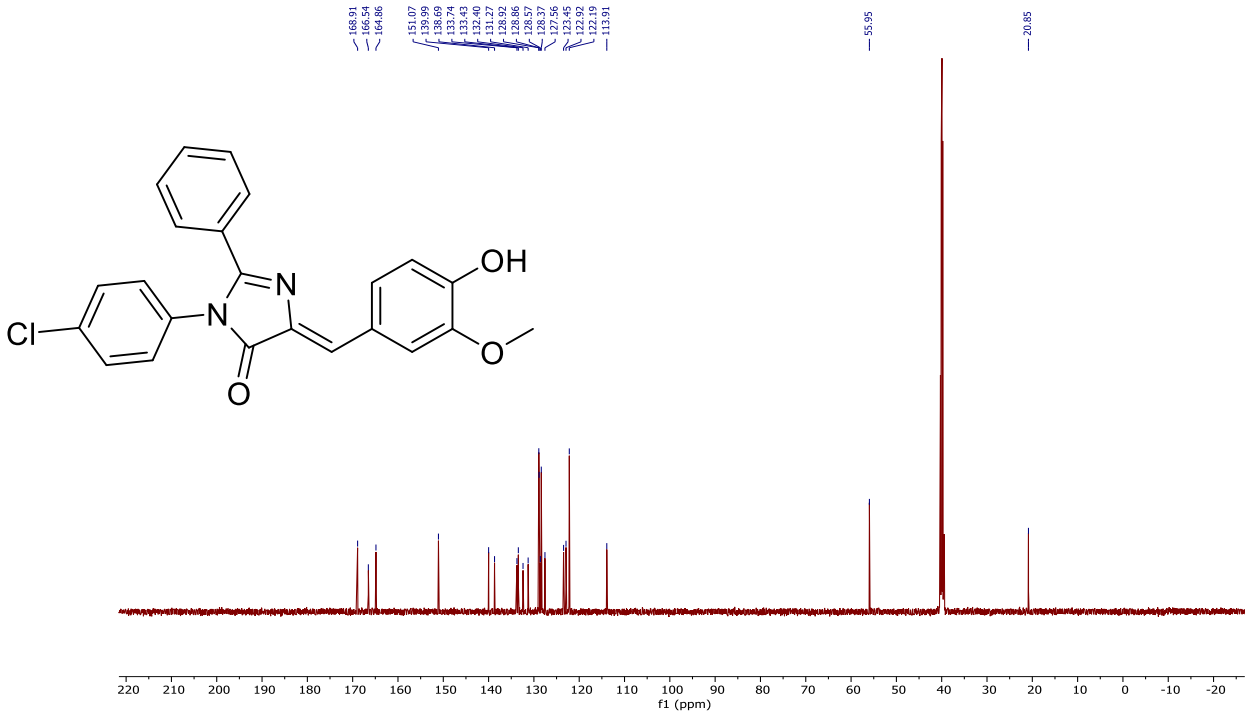


Figure A.61

^{13}CNMR NMR Spectra of (Z)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5)

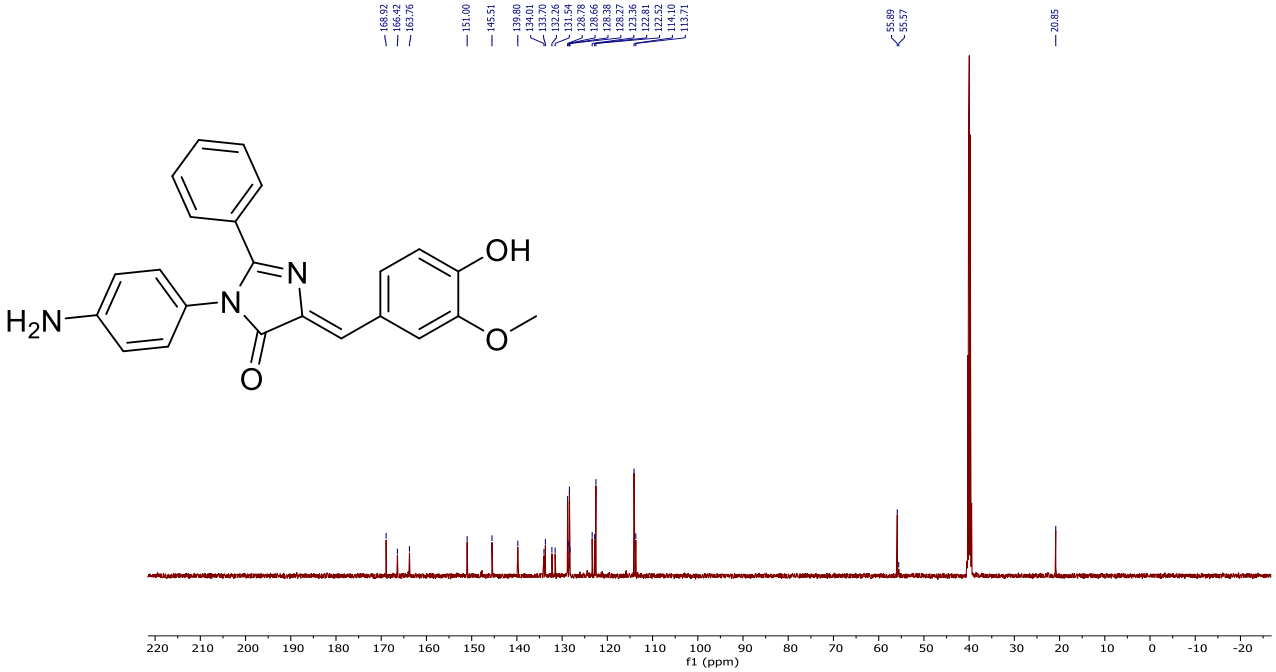


Figure A.62

^{13}CNMR NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (6)

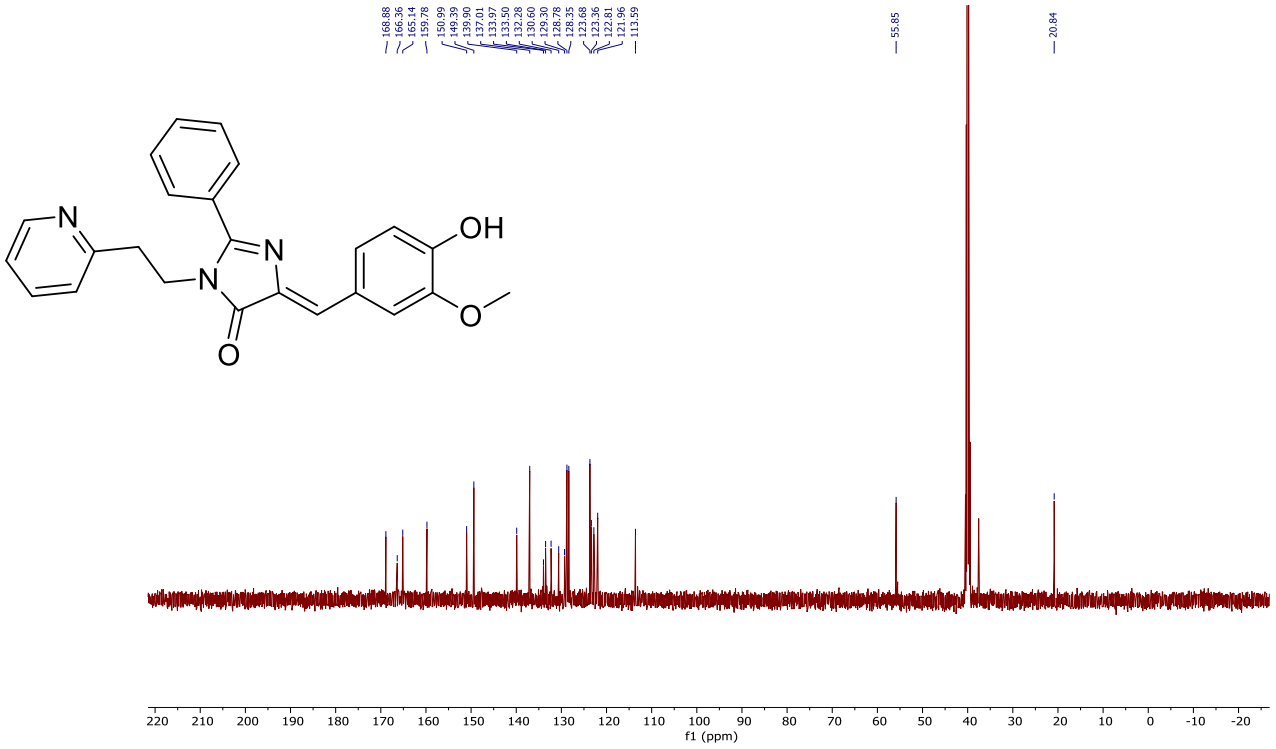


Figure A.63

^{13}CNMR NMR Spectra of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoic acid (7)

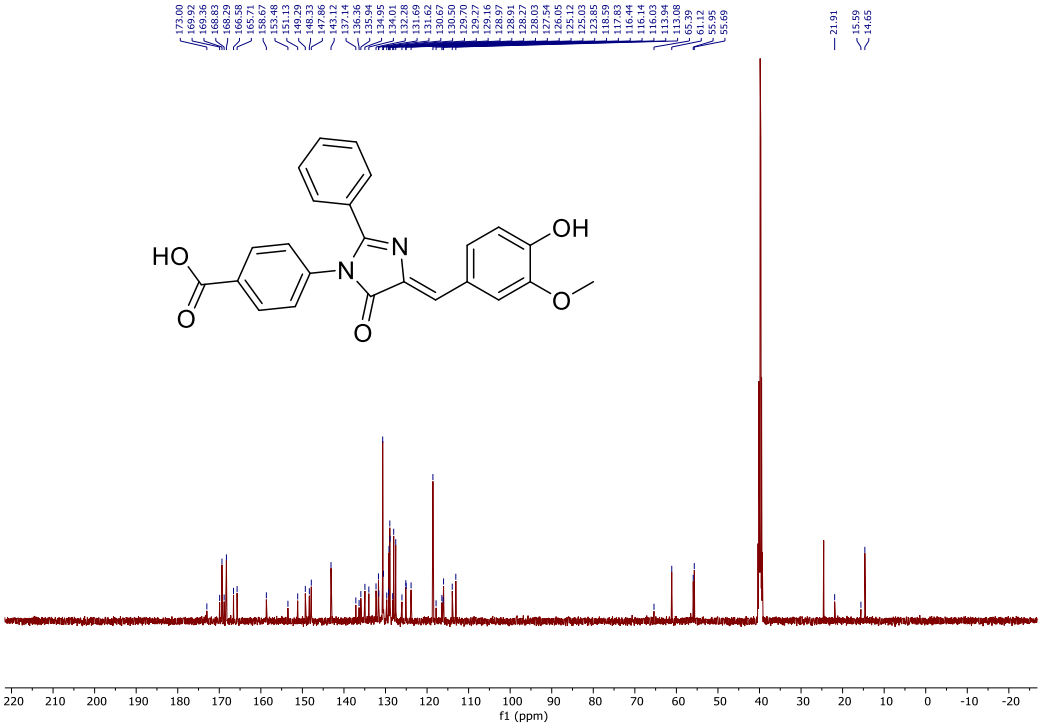


Figure A.64

^{13}CNMR NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (8)

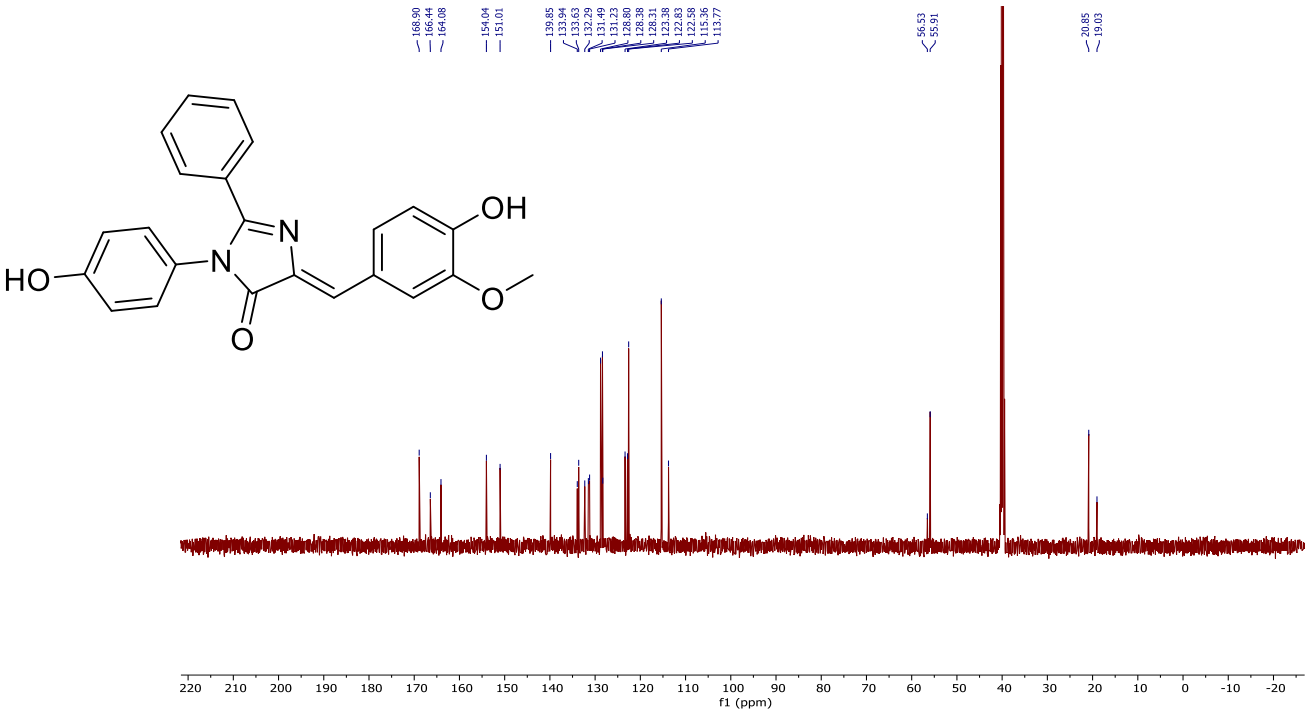


Figure A.65

^{13}CNMR NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (9)

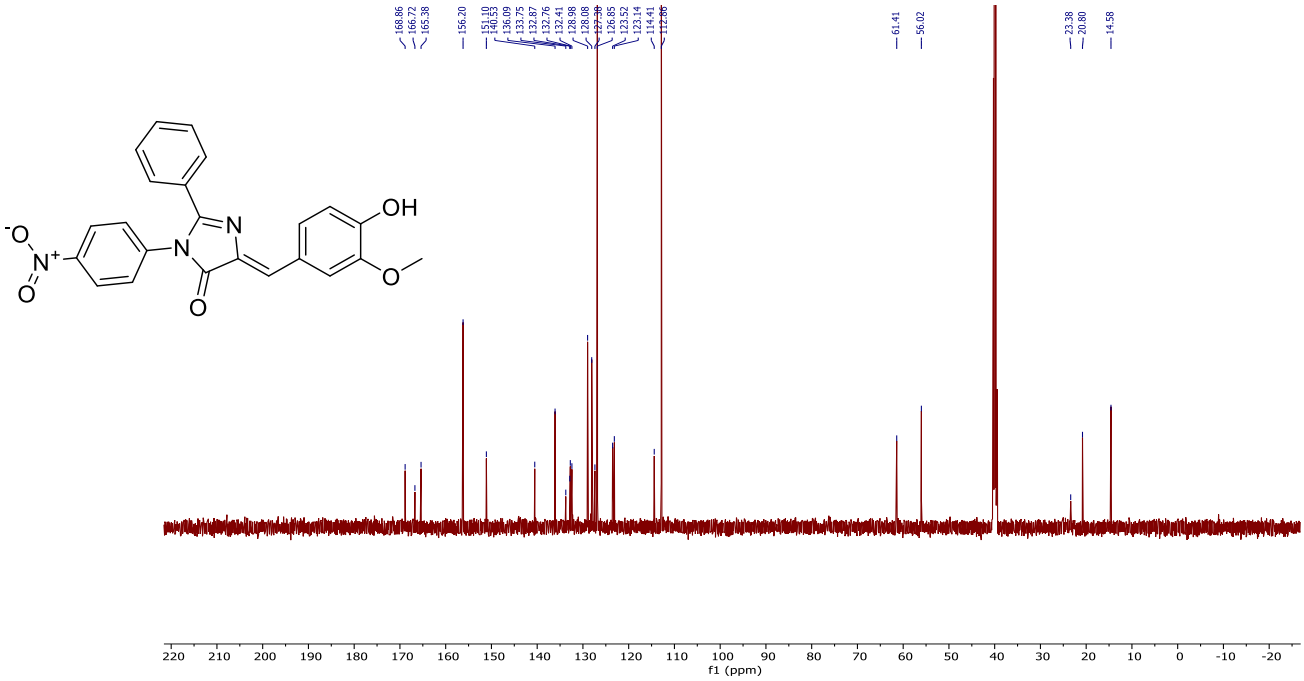


Figure A.66

^{13}CNMR NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (11)

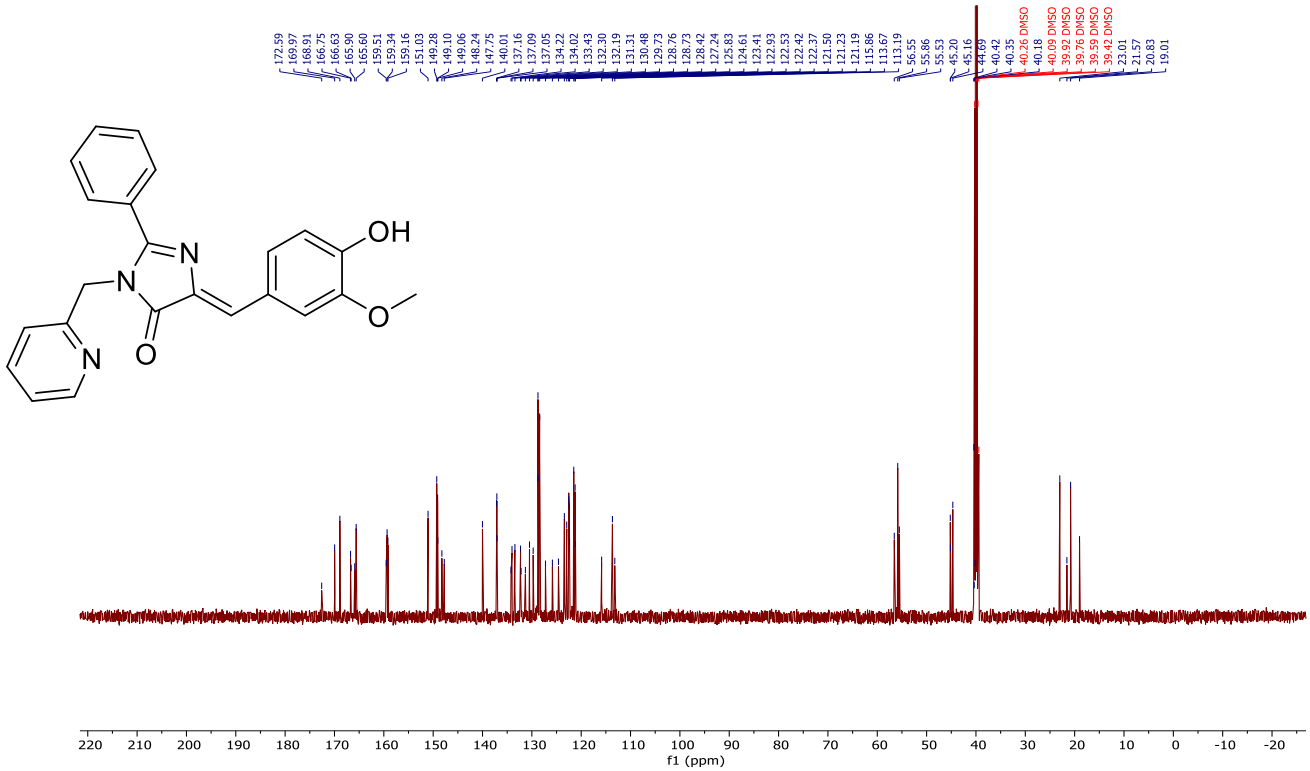


Figure A.67

^{13}CNMR NMR Spectra of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (12)

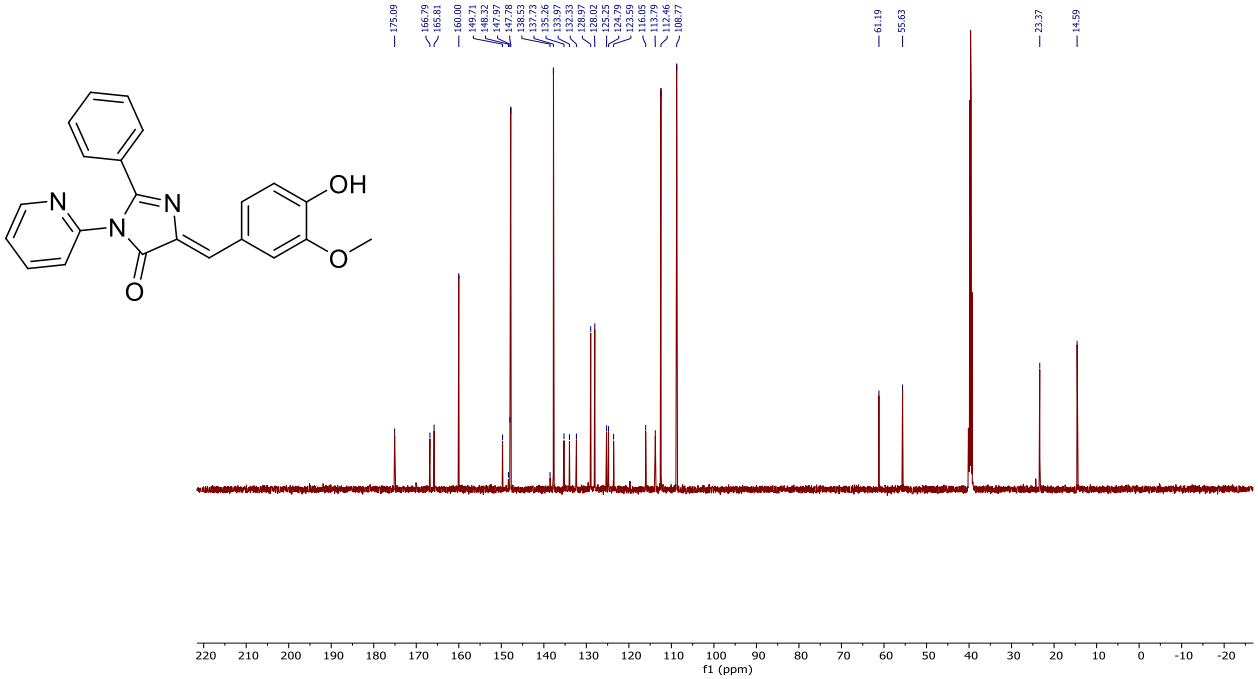


Figure A.68

^{13}CNMR NMR Spectra of 4-(((3-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (24)

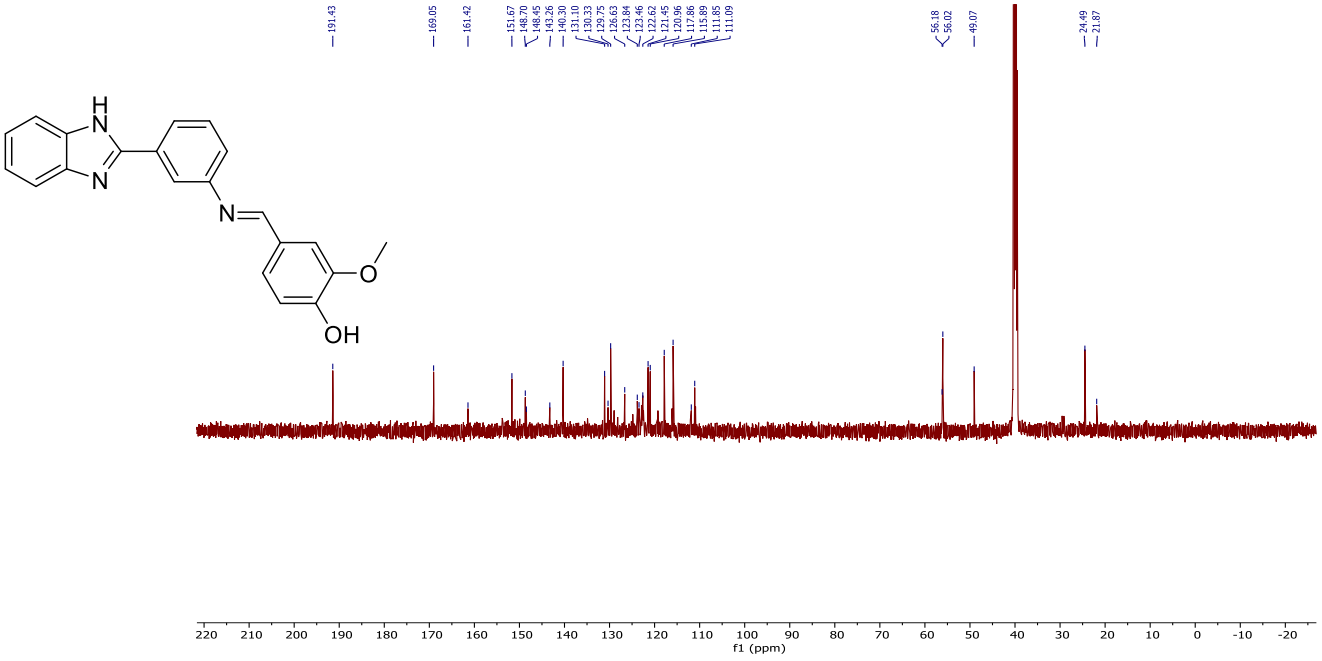
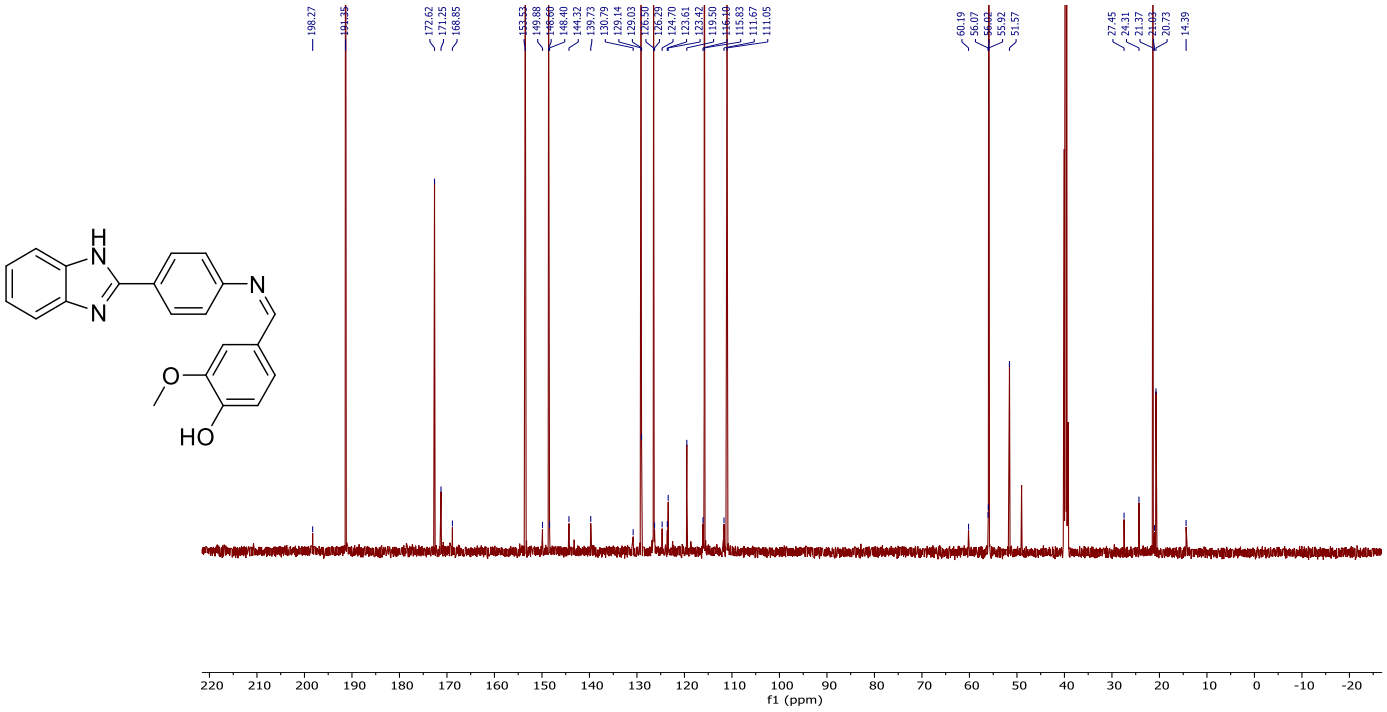


Figure A.69

^{13}CNMR NMR Spectra of (Z)-4-(((4-(1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (26)



Appendix B

List of previously synthesized compounds

Figure B.1

Several imidazole based antiviral drugs

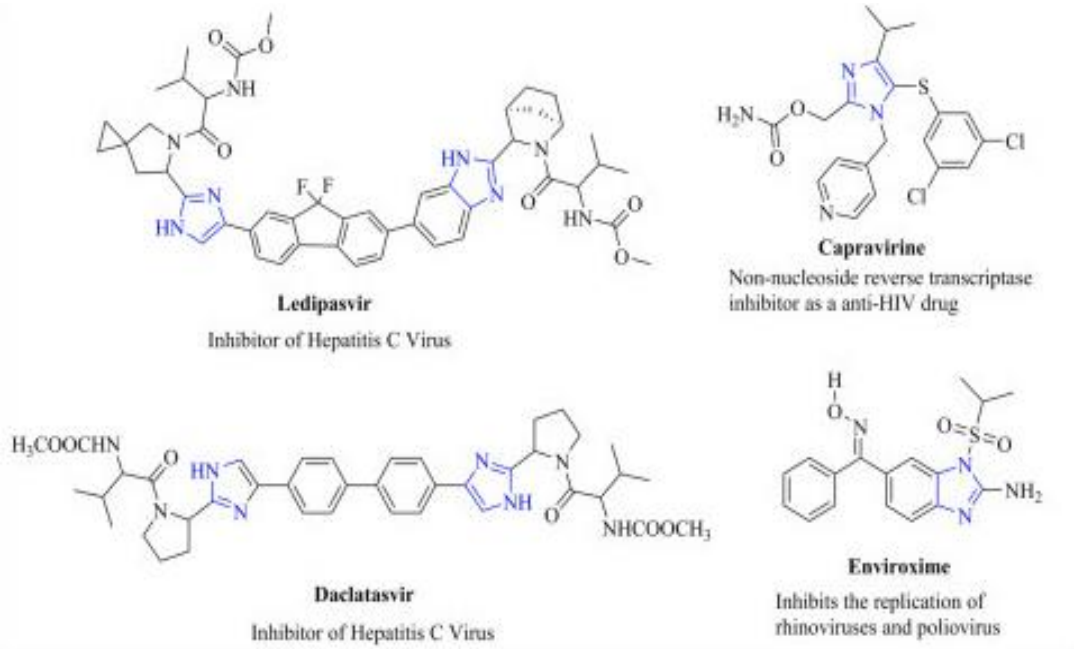


Figure B.2

Chemical structure of Acyclovir

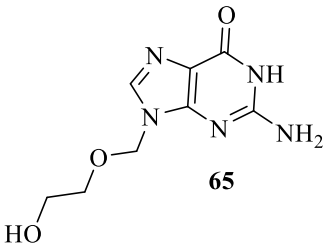


Figure B.3

Some imidazole-based anticancer drugs

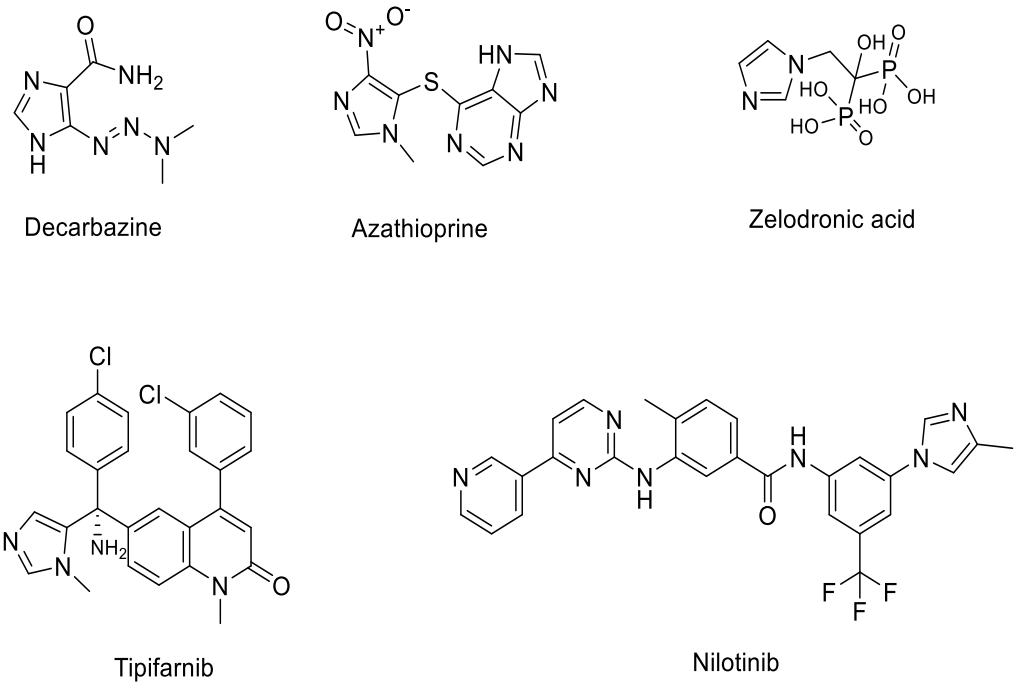


Figure B.4

Compounds have anticancer activity

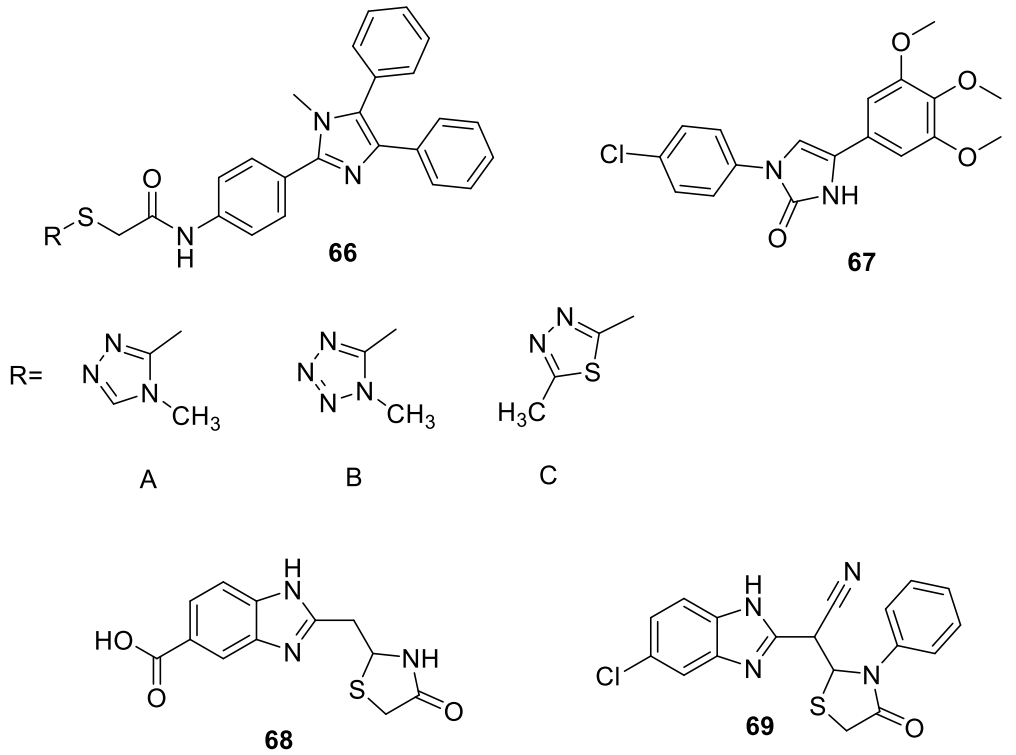


Figure B.5

Proposed mechanisms for imidazole derivatives

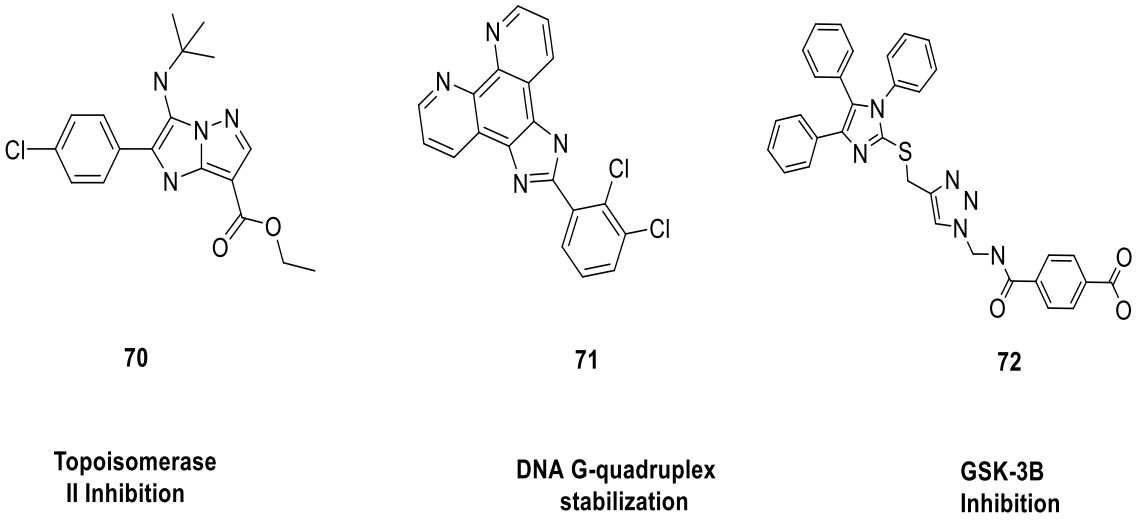


Figure B.6

Compounds with antimicrobial (73-75), Compounds with anticancer (76-79)

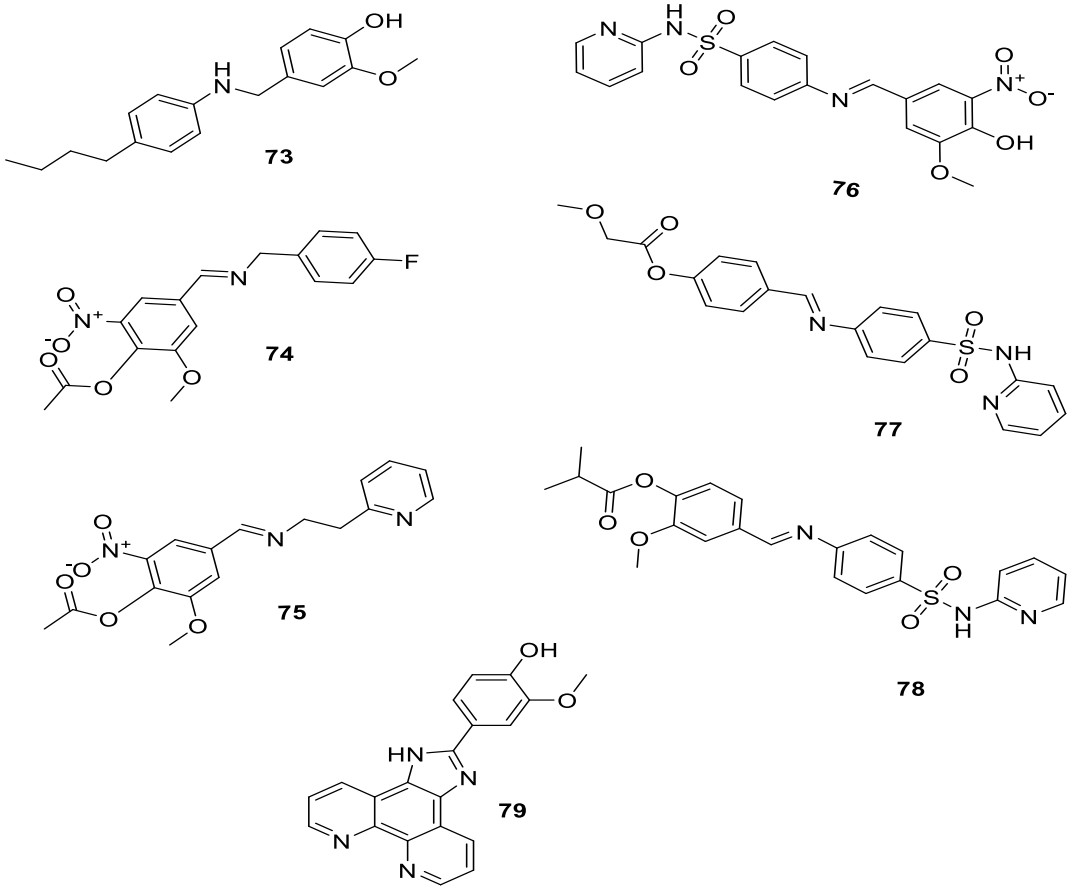
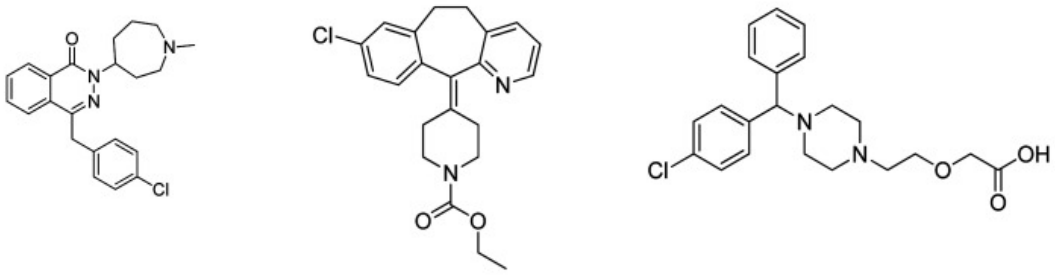


Figure B.7

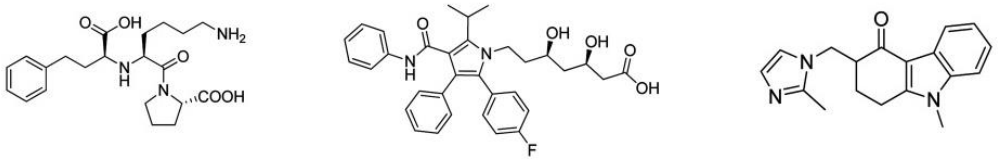
Drugs used to treat allergic rhinitis



Azelastine Loratadine Cetirizine

Figure B.8

Heterocylic dugs have different activity



Lisinopril Atorvastatin Ondansetron

Losartan Clonidine

Figure B.9

Chemical structure of 6-(4-chlorophenyl)-3-(pyridin4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole with proven antiproliferative activity, with superior selectivity for gastric cancer cell lines

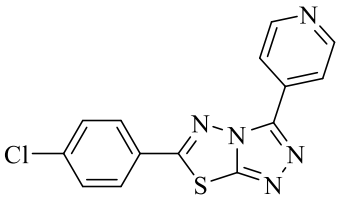
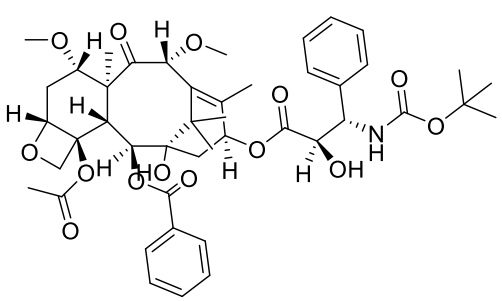
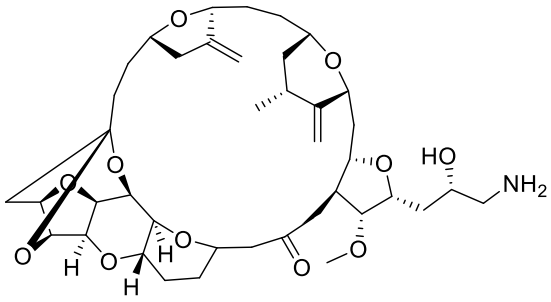


Figure B.10

Chemical structure representation of FDA approved oxygen-based heterocycles, Cabazitaxel , with an oxetane ring, and Eribulin with tetrahydrofuran and tetrahydropyran rings(76).



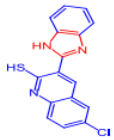
Cabazitaxel



Eribulin

Figure B.11

Compounds have antibacterial activity



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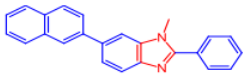

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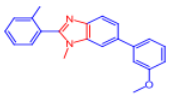

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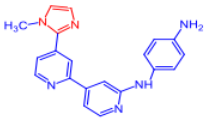

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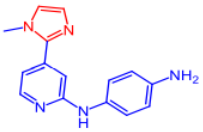

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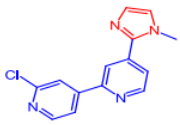

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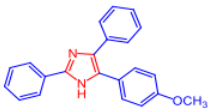

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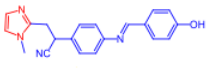

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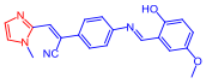

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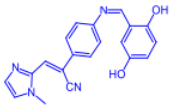
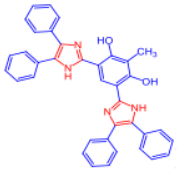


89

Figure B.12

Compounds with Anti-protozoan (90-92), Compounds with anti-parasitic (93-94),Compound with *anti-malaria (95*)



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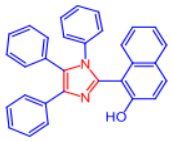

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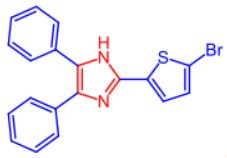

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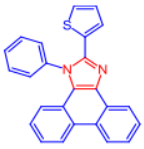

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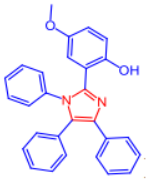

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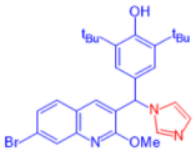
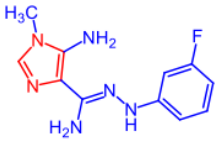


95

Figure B.13

Compounds with Antifungal activity



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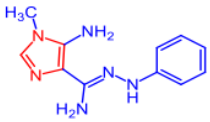

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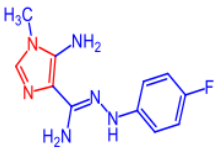

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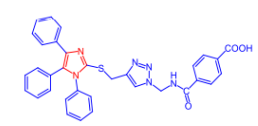
Figure B.14

Compounds with Anticancer activity



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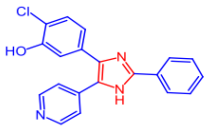

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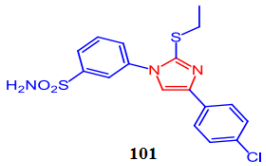

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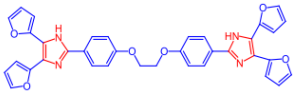

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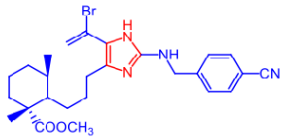

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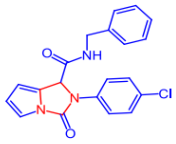

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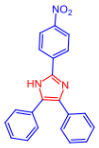

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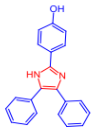


106

Appendix C

List of reactions mechanism

Figure C.1

Reaction mechanism for the synthesis of benzoylglycine

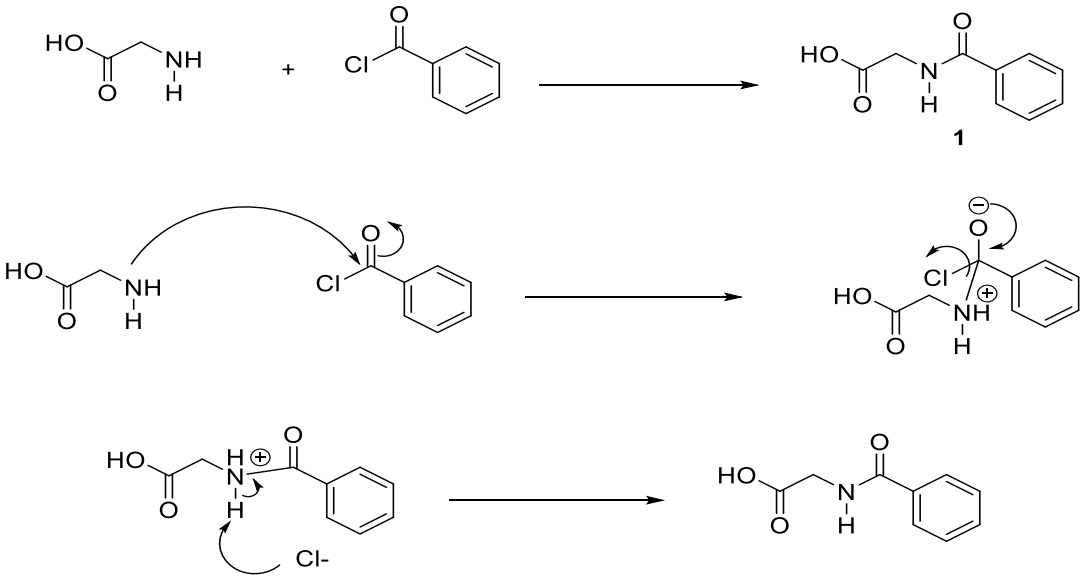


Figure C.2

Reaction mechanism for the synthesis of oxazolone

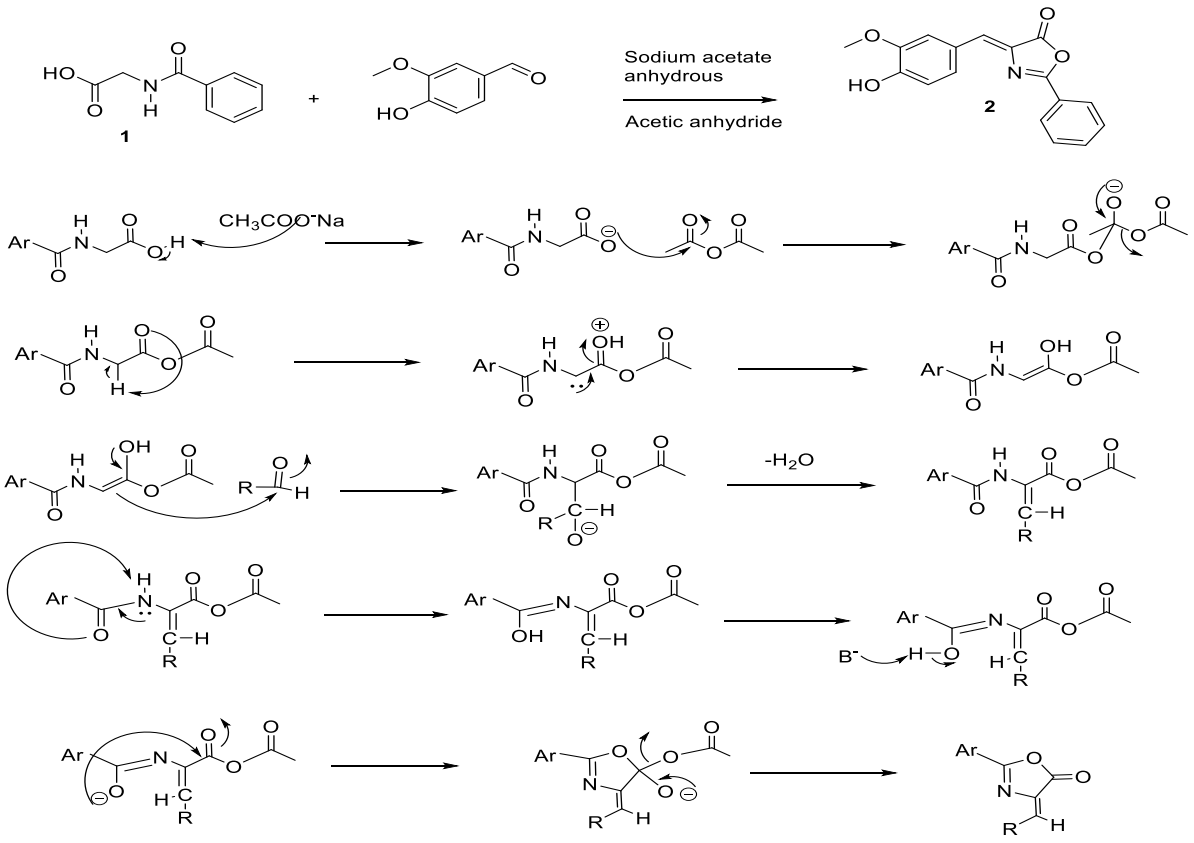


Figure C.3

Reaction mechanism for the synthesis of imidazolones

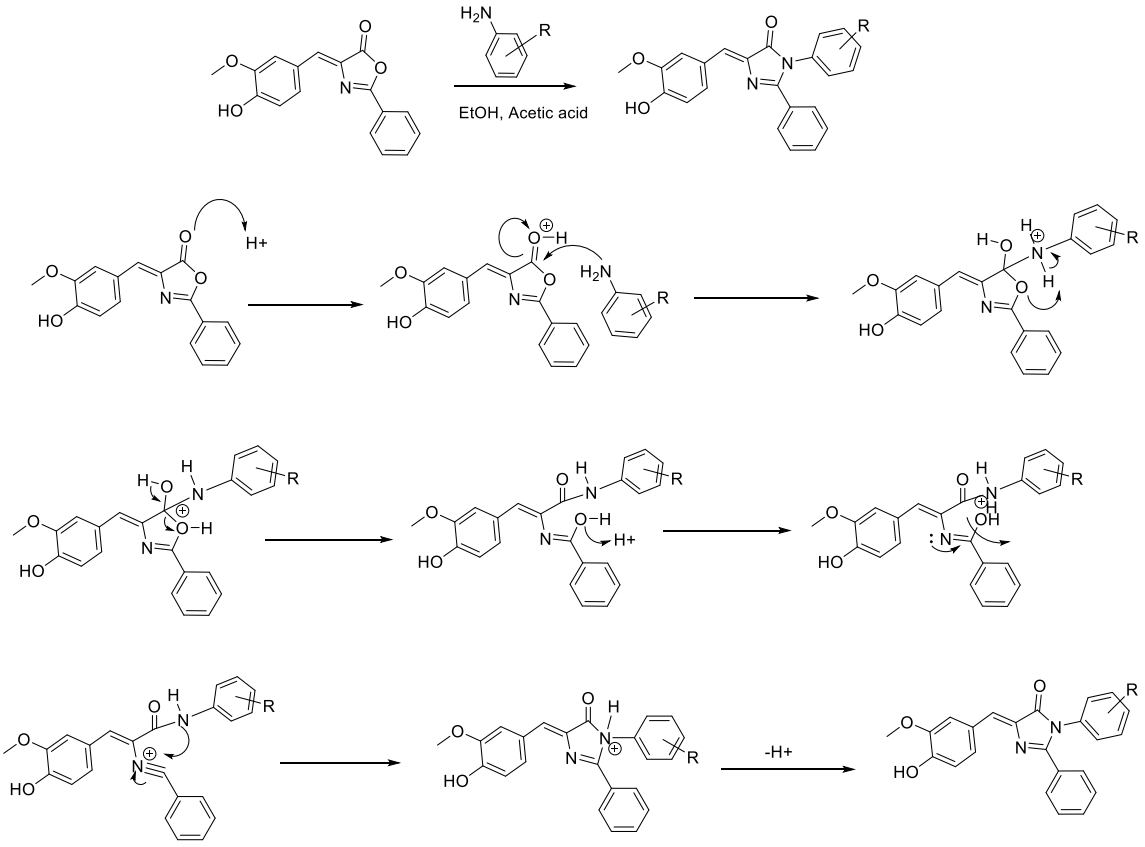


Figure C.4

Reaction mechanism for the synthesis of imidazolone with alkyl sulfonate moiety

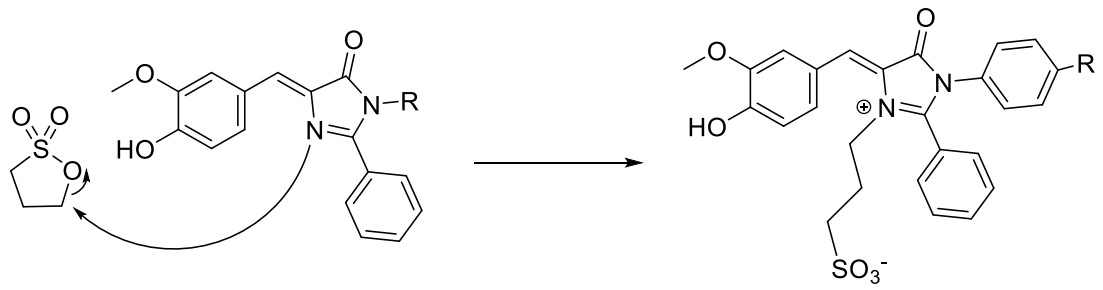


Figure C.5

Mechanism for the synthesis of benzimidazole

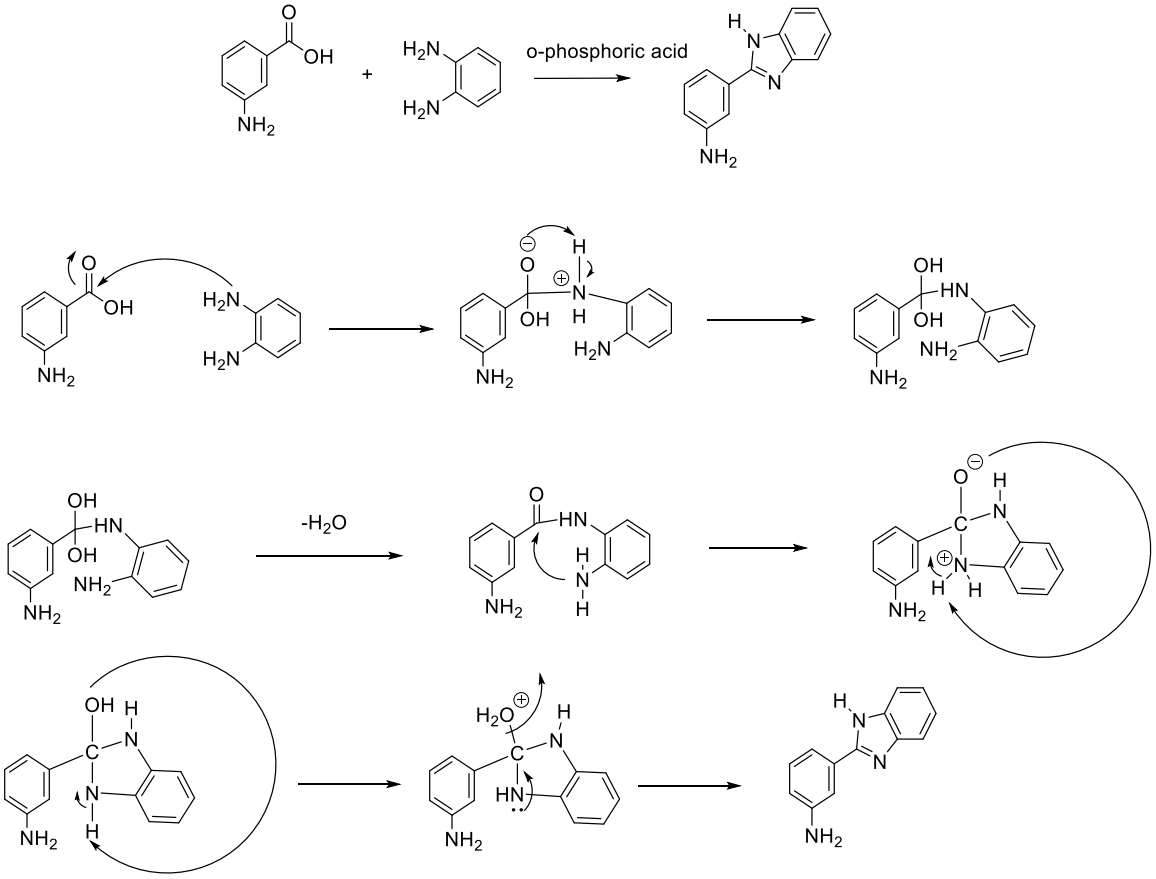


Figure C.6

Reaction mechanism for the synthesis of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate

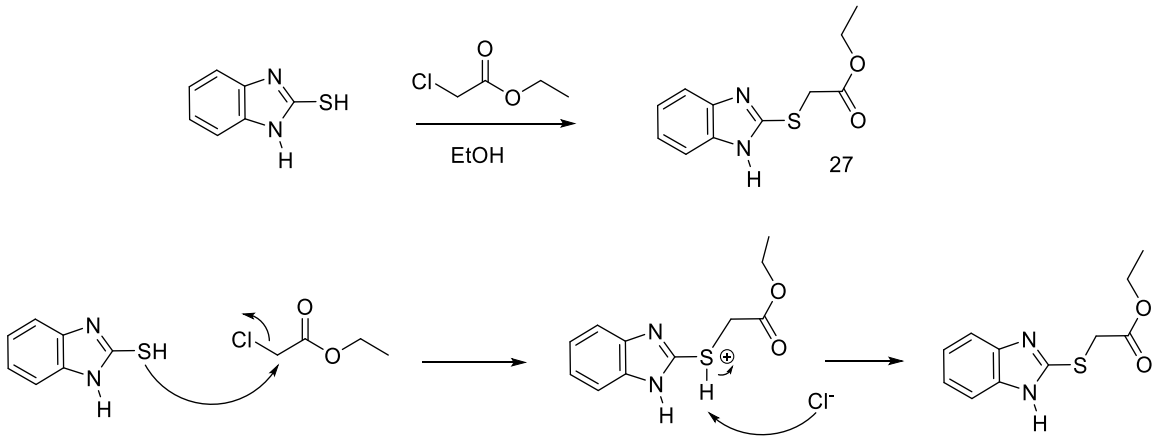


Figure C.7

Reaction mechanism for the synthesis of (E)-2-((1H-benzo[d]imidazol-2-yl)thio)-N'-(4-hydroxy-3- methoxybenzylidene)acetohydrazide

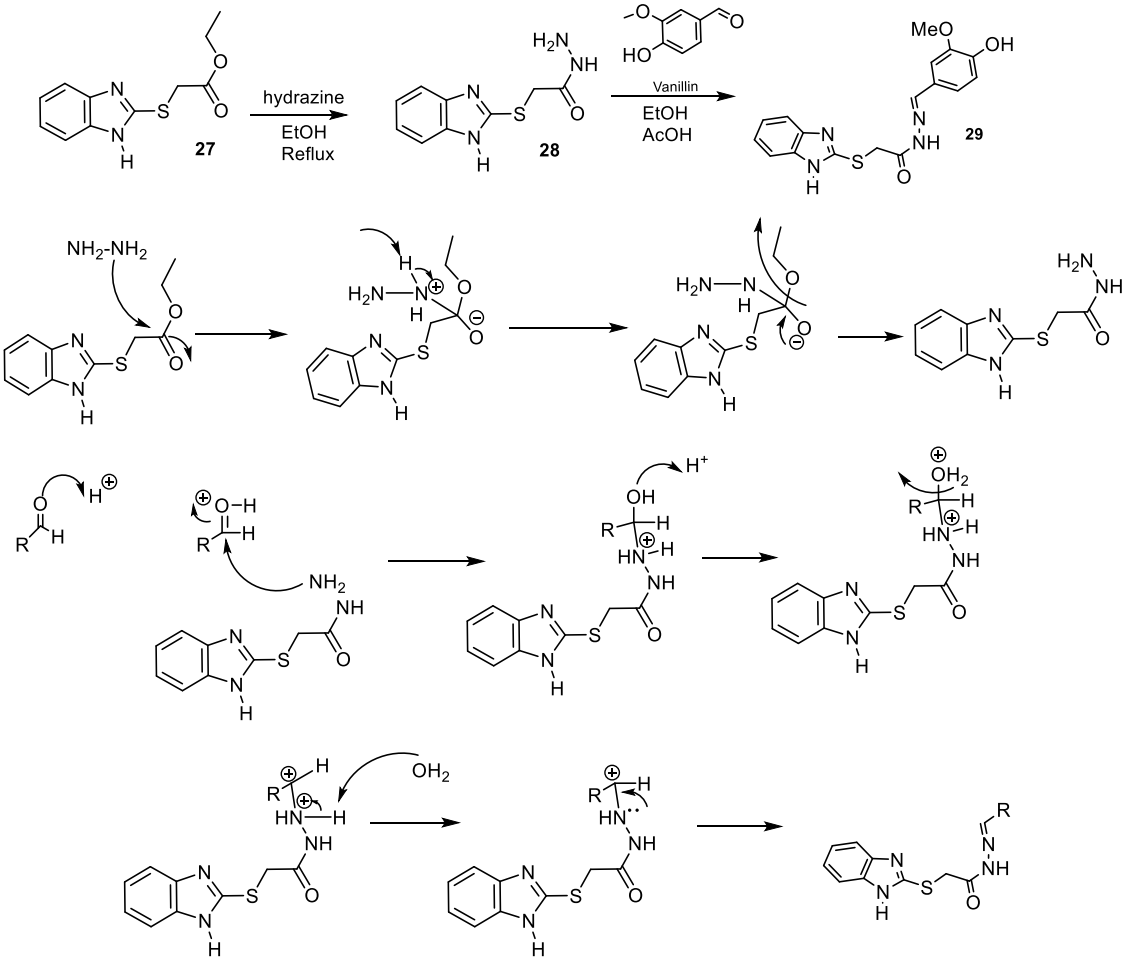
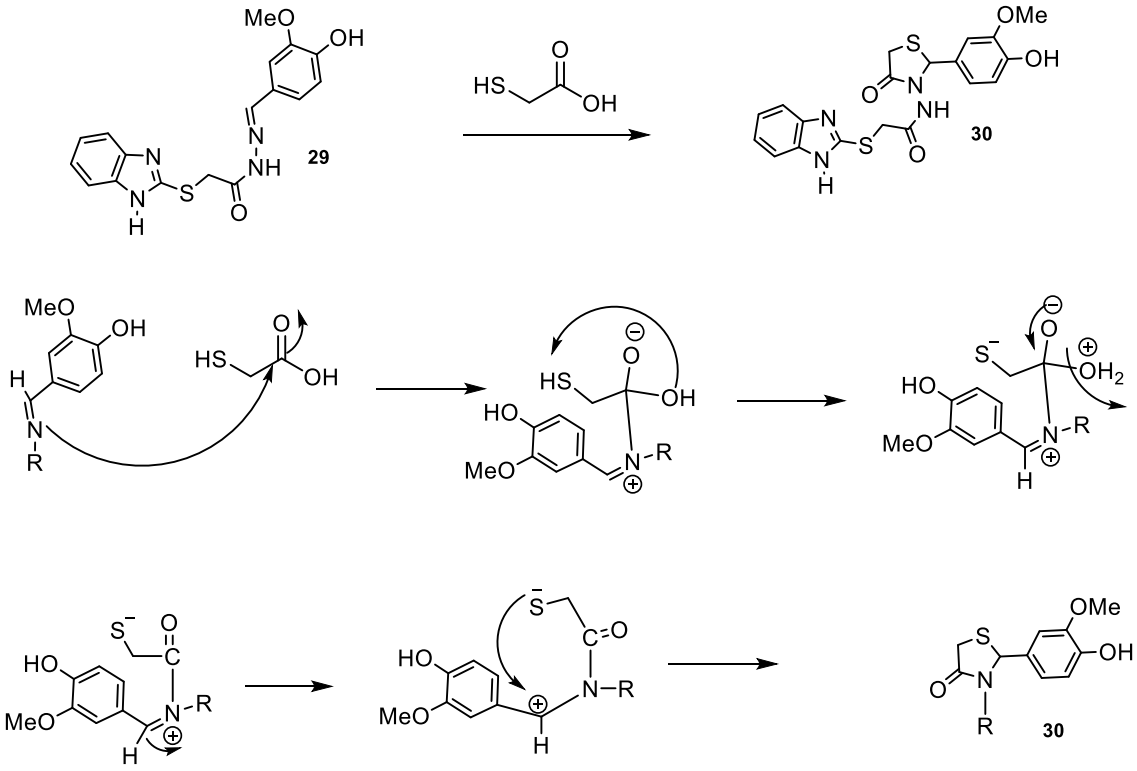


Figure C.8

Reaction mechanism for the synthesis of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide



Appendix D

Certificate of acceptance of the research extracted from the dissertation
Research title: New Zwitterionic Imidazolones with Enhanced Water Solubility and Bioavailability: Synthesis, Anticancer Activity, and Molecular Docking

جـامعـــة النجاح الوطنية
كليـــة الدراســـات العليـــا

مشتقات الايميدازول المستمدة حيويا ذات الأنشطة الحيوية المتنوعة

إعداد
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إشراف
أ. د. عثمان حامد
د. محيي الدين العسالي

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

2024

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الملخص

من المعروف أن الايميدازول جزيء حيوي بالغ الأهمية، لأنه يظهر أنشطة بيولوجية ملحوظة مثل مضادات الميكروبات، ومضادات الفطريات وتثبيط سينيز أكسيد النيتريك، والأنشطة السامة للخلايا. تم استخدام الفانيلين كمادة أولية في هذه الدراسة، وهو مركب فينولي طبيعي يتم استخدامه كمادة حافظة في صناعة الأدوية ومستحضرات التجميل.

الغرض من هذه الدراسة، تصنيع مشتقات ايميدازولون طبيعية جديدة أساسها الفانيلين، ومشتقات ايميدازولون أخرى أساسها الفانيلين مع سلفونات الالكيل، ولقد قمنا بتصنيع ثلاثين مركباً جديداً.

تم تحليل جميع المشتقات بالطرق الطيفية المختلفة. بعد تشخيص هذه المركبات، تم تقييم فعاليتها ضد عدة خلايا سرطانية. حيث أظهرت الدراسة أن لهذه المركبات تأثيراً على تثبيط نمو أنواع مختلفة من الخلايا السرطانية مثل سرطان الكبد (Hep-3B) وسرطان عنق الرحم (HeLa). علاوة على ذلك قمنا بعمل الالتحام الجزيئي بين بعض المركبات المحضرة والحمض النووي.

وأظهرت الدراسة أن بعض هذه المركبات لها تأثير كبير في منع نمو نوعين من الخلايا السرطانية، سرطان الكبد (Hep-3B) وسرطان عنق الرحم (HeLa)، والمركبات ذات الارقام (3, 4, 5, 7, 8, 9, 11, 13, 14, 17, 24, 26) أظهرت كفاءة غير عادية ضد هذه الخلايا السرطانية، مع انخفاض

قابليتها للحياة ضد الخلايا الطبيعية. أظهرت دراسة الالتحام الجزيئي تفاعلا ممتازا لبعض هذه المشتقات مع الحمض النووي، كما أظهرت المركبات المحضرة بأجزاء سلفونات الألكيل تحسنا في الكفاءة المضادة للسرطان.

تم تحضيرها مجموعة جديدة من الايميدازولات وتصنيع الايميدازولون مع سلفونات الالكيل. وكانت النتائج واعدة للغاية، مما يجعل من هذه المركبات أدوية مستقبلية محتملة لعلاج أنواع مختلفة من السرطان.

الكلمات المفتاحية: أوكسازولون، ايميدازولون، بنزيميدازول، مضاد للسرطان.