REPUBLIC OF TURKEY

ANKARA UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

SYNTHESIS, STRUCTURAL ANALYSIS, AND BIOLOGICAL ACTIVITIES OF NOVEL BENZIMIDAZOLE DERIVATIVES, AND MOLECULAR DOCKING STUDIES

Yemna ABBADE

PHARMACEUTICAL CHEMISTRY DEPARTMENT

MASTER DEGREE PROGRAM

SUPERVISOR

Prof. Dr. Zeynep ATEŞ ALAGÖZ

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ETHICAL STATEMENT

To Ankara University Health Sciences Institute Directorate,

The thesis titled 'Synthesis, Structural Analysis, and Biological Activities of Novel Benzimidazole Derivatives, and Molecular Docking Studies', which I prepared and presented as a master thesis; Written by me in accordance with scientific morals and values. The idea/hypothesis of my thesis is entirely my own and my thesis advisor. The experimental work/research in the thesis was made by me, and all sentences and comments are my own. I declare that the above-mentioned points are correct.

Student's Name and Surname: Yemna Abbade

Date: 03.02.2022

Signature:

ACCEPTANCE AND APPROVAL

Ankara University Institute of Health Sciences
In the Department of Pharmaceutical Chemistry
Prepared by Yemna ABBADE

The thesis study named 'The Synthesis, Structure Clarification and Investigation of Anticancer Effects of New Indole-Benzimidazole Derived Compounds' was accepted/rejected unanimously/by majority of votes as a MASTER THESIS by the following jury

Thesis Dissertation Date: 03.02.2022

Prof. Dr. Hakan GÖKER
Ankara University
Chairman of the Jury

Prof. Dr. Zeynep ATEŞ ALAGÖZ
Ankara University
Member

Prof. Dr.Sultan BAYTAŞ
Gazi University
Member

The decision of the jury about the thesis was approved by the Ankara University Health Sciences Institute Board of Directors.

Signature
Prof. Dr. Fügen AKTAN
Director of health Sciences Institute

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PREFACE

This master thesis is the result of a study that started with synthesizing Benzimidazole derivative compounds that may have anticancer effects. The work primarily consisted in elucidating their structures, conducting molecular docking studies and investigating their activities. Several persons have contributed to the development and refinement of this work in this preface, I would like to take the opportunity to thank them.

To begin with, I would like to express my gratitude and appreciation for my thesis advisor Prof. Dr. Zeynep ATEŞ ALAGÖZ whose guidance, support and encouragement has been invaluable throughout my master's degree program. This study could not have been possible without her.

A lot of gratitude is also owed to Mr. Mehmet Murat KIŞLA, Ph.D. candidate student, for his precious help. He provided me with all the tools that I needed to choose the right direction and successfully complete my thesis.

Furthermore, the efforts of the head of the Pharmaceutical Chemistry Department Prof. Dr. Oya BOZDAĞ-DÜNDAR and Prof.Dr. Hakan GÖKER for the analysis of ^{1}H-NMR, ^{13}C-NMR, LC-MS, are greatly appreciated.

Thanks for assisting in the anticancer activity determination studies, Middle East University Molecular Biology and Genetics Department Assoc. Dr. Pelin MUTLU and Dr. Tuğba SOMAY-DOĞAN.

Thanks for assisting in the antibacterial activity determination studies, Ankara University Faculty of Pharmacy, Department of Pharmaceutical Microbiology Assoc. Dr. Banu KAŞKATEPE.

To my faculty members of the Department of Pharmaceutical Chemistry, to whom I have always received their interest and support, to my fellow Research Assistants, to my friends who are doctoral and master's students, and to all Department staff,

I send all my gratitude to Dr. Firat YERLIKAYA, manager director and Pelin GENÇER, manager of the analytical chemistry department of Elixir Pharmaceutical Research and Development Corporation Company, for allowing me to complete my master thesis while working.

Thanks also to my husband, Bilal ABBADI, the one who believed in me. He gives me all the possibilities to fulfill my thesis with his patience and comprehension.

SYMBOLS AND ABBREVIATIONS

CC	Column Chromatography
$CDCl_3$	Chloroform-d
DMF	N,N-Dimethylformamide
DMSO-d*6*	Dimethyl sulfoxide d-6
EtOH	Ethanol
HPLC	High Performance Liquid Chromatography
HIV	Human Immunodeficiency Virus
LC-MS	Liquid Chromatography- Mass Spectrometry
MeOH	Methanol
NMR	Nuclear Magnetic Resonance
TB	Tuberculosis
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane

FIGURES

CHARTS

1. INTRODUCTION

Cancer is a group of diseases, which consists of uncontrolled proliferation of cells in different organs, and whose clinical appearance, treatment and approach are different from each other. It is a disease caused by the transformation of cells which become abnormal and proliferate excessively. These disordered cells sometimes end up forming a mass called a malignant tumor. Cancer cells tend to invade nearby tissues and break away from the original tumor. They then migrate through blood vessels and lymphatic vessels to form another tumor (metastasis) (Foye, 1995).

Nowadays, cancer chemotherapy is insufficient with existing drugs and their combinations and treatment cannot be provided in many types of cancer. Cancer and its treatment are among the most important health problems of the people of developed countries. Today, while the success of treatment with surgery alone or radiation is 20%, the success of treatment with chemotherapy has reached up to 75%. However, despite all these developments, cancer is still the second most common cause of death after diseases of the cardiovascular system (Foye, 1995).

Most of the current anticancer drugs show undesirable side effects such as low bioavailability, toxicity and drug resistance. Despite intensive research, the development of effective, selective and low toxicity anticancer drugs has not been possible. Chemotherapy has turned to the use of low molecular weight drugs to selectively destroy tumor cells or limit their proliferation. Side effects of cytotoxic agents include bone marrow suppression, gastrointestinal lesions, hair loss, nausea, and clinical resistance. The main reason for the occurrence of the aforementioned side effects is that these agents do not have a selective effect (Thurston, 2007). The beginning of chemotherapy is based on nitrogen mustards, which began to be used in the 1940s. These derivatives cause a very strong antimetabolite effect. Based on the success of this initial method, many anticancer agents have been developed (Shewach

and Kushta, 2009). These drugs are classified according to their mechanism of action as agents that interact with DNA, antimetabolites, anti-tubulin agents, molecular targeting agents, hormones, monoclonal antibodies, and other biological agents (Thurston, 2007). Therefore, new drugs and treatment methods should be developed urgently.

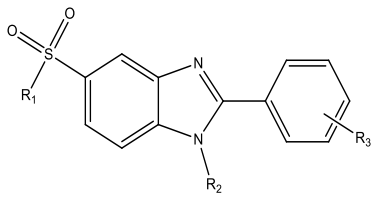
Benzimidazole ring is in the structure of many drug groups currently in use. The main structure of benzimidazole is the structures recognized by the living organism. Indole and benzimidazole rings are isosteres of the basic structures of DNA bases carrying purine and pyrimidine nuclei, and they can be purine antimetabolites. Therefore, it is thought that benzimidazole rings can easily interact with biopolymers in living systems (El Rashedy, 2012).

Benzimidazole and its derivatives are antitumor, antiproliferative, anticancer (Li, 2011; Refaat, 2010; Demirayak, 2011; Moriarty, 2010), anti-inflammatory (Sondhi, 2010), antiviral (Demirayak, 2002; Sharma, 2009), antibacterial (Kumar, 2006; Hosamani, 2009; Guven, 2007), antifungal (Goker, 2002) and antioxidant (Kerimov, 2007; Kilcigil, 2004) are compounds that have attracted a lot of attention and have been studied extensively in recent years (Rashid, 2012).

In this study, it is aimed to design and synthesize new anticancer, antimicrobial agents having 2-phenyl-benzimidazole moieties as main nuclei, which have already reported their potent anticancer and antimicrobial activities. Main target of this project is to obtain novel target compounds that have potent anticancer, antimicrobial activities. To achieve this goal, the specific aims are: Develop a method for synthesizing compounds in gram quantities; *In vitro* perform their anticancer, antimicrobial activities. Perform structure-activity studies to investigate the mechanism of anticancer, antimicrobial activities.

The synthesis of compounds was initiated from 4-chloro-benzenesulfonyl chloride. 4-(alkylsulfonyl)-1-chlorobenzene and 4-(alkylsulfonyl)-1-chloro-2-nitrobenzene were synthesized according to our previous publications (Zengin-Karadayı et.al, 2020, Zengin-Karadayı et.al, 2021). To a solution of 4-(alkylsulfonyl)-1-chloro-2-nitrobenzene (5 mmol) in ethanol (5 mL), amine derivative (15 mmol) was added and heated under reflux until the starting material was consumed (determined by TLC, 8–48 h). Upon cooling the mixture, water was added. The resultant yellow residue was crystallized from ethanol or purified by column chromatography (cc) by using a mixture of hexane and ethyl acetate in varying concentrations as eluent (Goker, 2009). Then nitro compound (3.5 mmol) in EtOH (75 mL) was reduced by hydrogenation using 40 psi of H_2 and 10% Pd/C (40 mg) until cessation of H_2 uptake to obtain the catalyst before filtering off on a bed of celite and washing with EtOH and concentrating the filtrate in vacuo. The crude amine was used without purification. The mixture of the sodium metabisulfite adduct of substitute-benzaldehyde (2 mmol) and the appropriate 1,2-phenylenediamine (2 mmol) in DMF (5 mL) were heated at 130 ^{o}C. The reaction was terminated after observing the end of the onsets with TLC. It was precipitated by pouring on ice. The precipitate was filtered off and purified by column chromatography (Ates-Alagoz, 2005).

Following the synthesis procedure, which was given, the structure elucidation of the synthesized compounds was achieved by their elemental analysis, ^{1}H-NMR, ^{13}C-NMR, and Mass (ESI +) data. Antimicrobial and/or anti-cancer activities of the synthesized compounds were evaluated as *in vitro.* Tube dilution method and MTT test were used for screening the antimicrobial and anticancer activities of the synthesized compounds, respectively.



Compound	R_1	R_2	R_3
23	CH_3	Cyclohexyl	4-chloro
24	CH_3	Cyclohexyl	3,4-difluoro
25	C_2H_5	Cyclohexyl	4-chloro
26	C_2H_5	Cyclohexyl	3,4-difluoro
27	CH_3	3,4-difluorobenzyl	3,5-difluoro
28	C_2H_5	3,4-difluorobenzyl	2,5-difluoro
29	CH_3	C_2H_5	4-acetamido
30	CH_3	C_2H_5	4-fluoro
31	C_2H_5	C_4H_9	4-fluoro
32	C_2H_5	C_4H_9	4-acetamido
33	CH_3	3,4-difluorobenzyl	4-$COOCH_3$
34	C_3H_7	C_3H_7	4-chloro
35	CH_3	p-fluorobenzyl	2,5-difluoro
36	CH_3	p-fluorobenzyl	4-acetamido

Figure 1.1. Synthesis of 1*H*-benzo[d]imidazole derivatives

1.1. History of Benzimidazole Rings

The benzimidazole ring is one of the oldest known nitrogen-bearing heterocyclic ring systems. It was first synthesized by Hobrecker (Hobrecker, 1872) and later synthesized by Ladenberg (Ladenberg, 1875) and Wundt (Wundt, 1878).

The heterocyclic portion of the benzimidazole ring system has been variously named as glyoxaline (Debus, 1858), iminazole, 1,3-diazole, and imidazole (Grimmett, 1997). The term imidazole, which defines a 5-membered heterocyclic moiety with a tertiary nitrogen and imino group, is the most used of these. The benzimidazole core was revealed to have therapeutic potential when Woolley explained in 1944 that it could have a biological purine-like effect (Woolley, 1944). 5 years later, Brink found the 5,6-dimethyl-1-(α-D-ribofuranozyl)benzimidazole structure to be a degradation

product of vitamin B12 and defined that derivative of this structure have vitamin B12-like effects (Brink, 1949). Over the years, it has been revealed that the benzimidazole ring has many biological activities.

Benzimidazole and its derivatives are an important group of bioactive molecules in terms of their pharmacological effects (Valdez et al., 2002). Besides its antimicrobial, anti-inflammatory, potential antitumor, antiparasitic, and antiprotozoal effects, it has antiviral activity against many viruses such as HIV, herpes (HSV-1), influenza and human cytomegolavirus. It has also been reported to have anthelmintic and antiulcer activities (Patil et al., 2008).

Many drug molecules used today have been obtained by substitutions made on the benzimidazole core. Studies on the biological activities of benzimidazole nuclei have increased considerably in the last 10 years.

1.2. Structure of Benzimidazole Rings

The benzimidazole ring system is a planar heterocyclic structure formed by the bonding of the imidazole ring with the benzene ring at the 4th and 5th positions. It is also called 1,3-benzodiazole. The IUPAC name is 1H-benzo[d]imidazole.

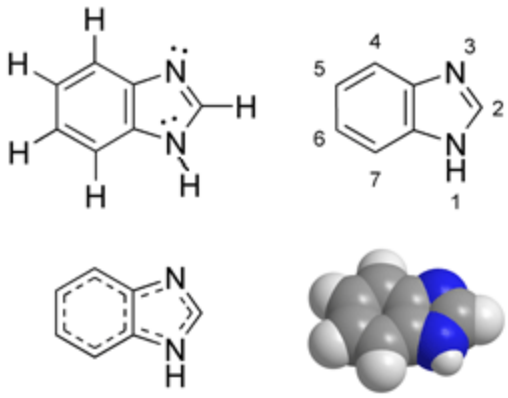


Figure 1.2. Structure of Benzimidazole

The numbering of the benzimidazole ring starts from the nitrogen carrying the hydrogen atom and continues as the number 3 is given to the other nitrogen.

The benzimidazole ring system carries nitrogen atoms in two different structures. One of these is nitrogen, which carries hydrogen on it and is described as 'pyrrole nitrogen' or 'imino nitrogen'. The other is nitrogen that does not carry hydrogen and exists in tertiary structure and is called 'pyridine nitrogen' or 'tertiary nitrogen'. Benzimidazoles with free imino hydrogen show tautomeric character.

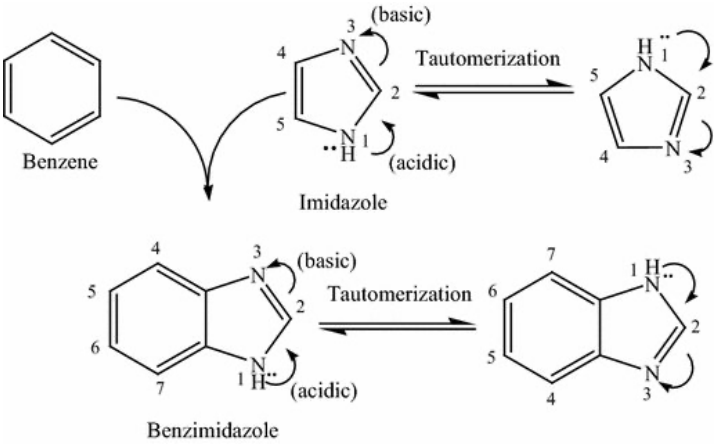


Figure 1.3. Tautomerism of benzimidazole compound

Although the free H in the benzimidazole ring is shown on the N1 nitrogen, there is actually a constant and very rapid exchange between the –NH- and =N- atoms. Therefore, the benzimidazole ring is drawn as 2 tautomers (Figure 1.3). Substitution of this free hydrogen eliminates the possibility of tautomerism and it becomes possible to define the exact structure. In such a case, the numbering is done starting from the substituted nitrogen (Hoffmann, 1953).

The benzimidazole ring shows both acidic and basic character. The N-H group shows strong acidic properties as well as weakly basic properties. Benzimidazoles are

solid compounds with very high melting and boiling points. E.g; benzimidazole itself melts at 170°C. These compounds are highly soluble in polar solvents and less soluble in non-polar solvents, and free imino hydrogen is found in association in polar solvents. Substitution of the imino hydrogen significantly lowers the boiling and melting points (Hoffmann, 1953).

1.3. Anticancer Activity of Benzimidazole Rings

Cancer is a disease that requires urgent treatment because it causes so many deaths worldwide and its incidence is increasing. For this reason, it is very important to find compounds with less anticancer effects than drugs with side effects.

It has been reported that glutamic acid and its derivatives, curcumin-I and Knoevenagel condensates, and Schiff's bases and platinum complexes have anticancer activity (Ali et al., 2013). Leukemia, sarcoma, lymphoma and carcinoma are types of cancer that affect various organs. Thalidomide, which was withdrawn due to its teratogenic effect, was later accepted for use in the treatment of metastatic prostate cancer, multiple myeloma and Kaposi's sarcoma.

1,3-Diarylpyrazino[1,2-a]benzimidazole derivatives were synthesized by Demirayak et al., and their cytotoxicity and cell growth inhibitory effects were tested in various cancer cell lines using melphalan and cisplatin as standard. In the study, 2-aryloylimidazole derivatives were used as the starting compound and 1-(2-aryl-2-oxoethyl)-2-aryloylbenzimidazole (2a-o) compounds were obtained by treating with various 2-bromoacetophenones. By treating these derivatives with ammonium acetate, 1,3-diarylpyrazino[1,2-a]benzimidazole (3a-o) compounds (Figure 1.4), which are diketo derivatives, were obtained. The synthesized compounds were tested for their anticancer activities at 10-5 M concentrations in 6 different human tumor cell lines. It was found that 2c and 2h compounds showed very good activity in the leukemia cell line with a cell growth value of 25.51% (Demirayak et al., 2011).

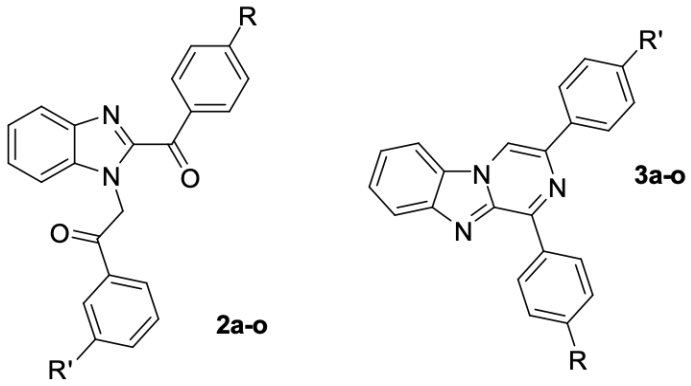


Figure 1.4. 1-(2-Aryl-2-oxoethyl)-2-aryloylbenzimidazole and 1,3-diarylpyrazino[1,2-a]benzimidazole derivative compounds

Hranjeck et al. Schiff base-substituted derivatives of benzimidazoles were synthesized and their antiproliferative activities were screened in various human cancer cell lines. Antiproliferative effect studies for 14 synthesized compounds were performed in both cervical cancer cell line and breast cancer cell line. Cytotoxicity experiments were also performed on normal fibroblast cells. Studies have shown that compounds with Ar group (2-hydroxy-4-diethylamino)phenyl and R group -CN and –CH3 have strong antiproliferative effects, but are very toxic in normal human cells (Figure 1.5, Hranjeck et al., 2011).

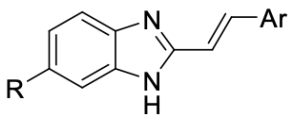


Figure 1.5. Benzimidazole derivatives substituted with Schiff bases

Wang et al. 2-benzylbenzofuran-benzimidazole hybrids (Figure 1.6) were synthesized by and these compounds were evaluated in vitro in human cancer cell lines. In vitro cytotoxicity tests were performed for the synthesized compounds in HL-60 (leukemia), A549 (lung cancer), SW480 (colon cancer), MCF-7 (breast cancer) and SMMC-7721 (liver cancer) cell lines using cisplatin as a standard. Strong cytotoxic effect was observed in all compounds. Most of the compounds showed better efficacy than cisplatin, which is used as a standard. The compound carrying the 4-

methoxyphenylacyl substituent (IC50=1.02 μM) at the 3rd position of benzimidazole and the compound carrying the naphthylacyl substituent (IC50=3.57 μM) also showed cytotoxic effects in 5 cancer cell lines. Compounds with benzyl and 4-bromophenylacyl at position 3 were found to be the most potent compounds, especially in MCF-7 and SMMC-7721 cell lines.

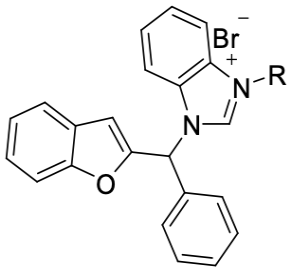


Figure 1.6. 2-Benzylbenzofuran-benzimidazole hybrids

Omar et al. (2021) synthesized benzimidazole-pyrrole conjugates (Figure 1.7) and their antitumor activities were evaluated in A549 and MCF-7 cancer cell lines. Cytotoxicity tests of the synthesized compounds were performed using mitomycin C and natural bis-benzoxazole compound (UK-1) as a standard in the A549 cell line. Compound with 4-pyridine substituent (IC50 = 1.1 μM) was found to be more effective than UK-1 compound (IC50 = 2.0 μM) in A549 cell line. The cytotoxicity test of the synthesized compounds in the MCF-7 cell line was performed using tamoxifen and doxorubicin as standard. Except for the compound carrying the 3-bromophenyl substituent, other compounds showed higher activity than tamoxifen (IC50 = 1.82 μM) (Omar et al., 2012).

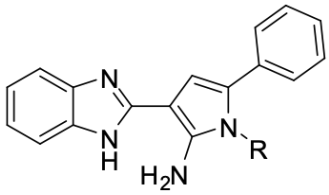


Figure 1.7. Benzimidazole-pyrrole conjugates

1.4. Antibacterial Activity of Benzimidazole Rings

Microbial resistance is one of the extreme troubles facing clinical practice and finding new effective compounds in opposition multi-resistant pathogens is one of the primary goals in actual biomedical research (Breijyeh et al.,2020). The discovery of new antimicrobial and antiviral compounds is a main aim in the context of cutting-edge COVID-19 pandemic (Balasubramaniam et al., 2021).

Coburn et al. (1989) and Badawey et al. (1987) mentioned that many derivatives of benzimidazole are known as antimicrobial and antifungal (Garuti et al., 1987) agents and exhibited a large spectrum of biological activity in addition to anthelmintic activity. The synthesis of the compounds having both fluorine and piperazine substituents at 5- and 6-positions has been suggested (El-Abadelah et al., 1995) due to the impact on antibacterial activity of these groups for the second-generation fluoroquinolones. El-Abadelah et al., synthesised new benzimidazole carbamate, benzimidazole acetate and acetamide derivatives having 5-fluoro and 6-substitute piperazine, piperidine or morpholine and evaluated their antimicrobial and antifungal activities against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Candida albicans*.

Kus et al. (2001) additionally confirmed *in vitro* antibacterial and antifungal activities towards some benzimidazole derivatives. Their activities were evaluated for their in against B. *subtilis, S. aureus, E. coli, and C. albicans* by the agar diffusion method using a 2000 µg/ml solution in propylene glycol. All the compounds exhibited increase inhibition zone against *C. albicans*. Amongst them, methyl 5-fluoro-6-(4-methylpiperidin-1-yl)-1H-benzimidazole-2-carbamate (Figure 1.8) showed the best antifungal activity with 15 mm inhibition zone diameter, which was less than those of fluconazole and ketoconazole (Kus et al. 2001).

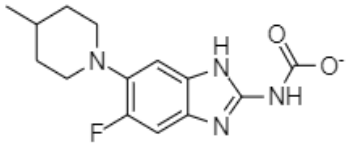


Figure 1.8. Structure of compound 5-fluoro-6-(4-methylpiperidin-1-yl)-1H-benzimidazole-2-carbamate

Consequently, Göker et al. in 2019, have reported the synthesis of a series of 1,2-disubstituted-1*H*-benzimidazole-*N*-alkylated-5-carboxamidine derivatives and their very potent antibacterial activities towards *S. aureus* and methicillin resistant *S. aureus*. The study revealed that compounds I–IV (Figure 1.9) exhibited the best activity, with MIC values of 0.78 - 0.39 μg/mL against these species.

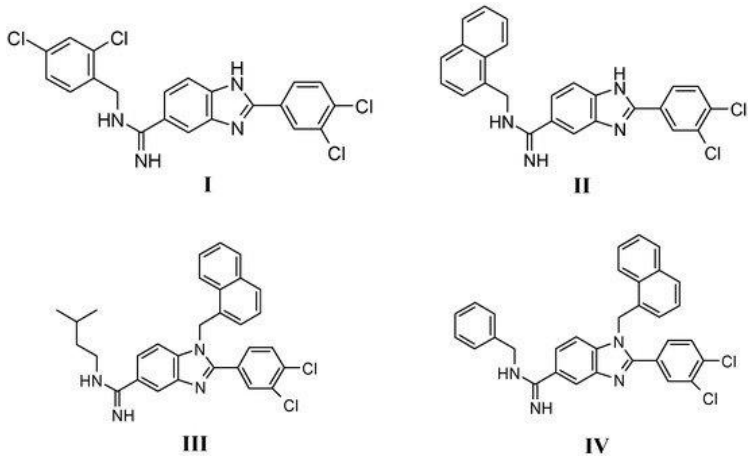


Figure 1.9. Structure of compounds I-IV

As part of a continuing program focused on development of new antimicrobial benzimidazole carboxamidines, Göker et al., synthesised 22 novel 1,2-disubstituted-1*H*-benzimidazole-N-alkylated-5-carboxamidine derivatives. The compounds were tested through the macro-broth dilution assay for *in vitro* antibacterial activity against Gram positive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA, a clinical isolate from a wound), *Enterococcus faecalis* and Gram-negative *Escherichia coli* and for antifungal activity against *Candida albicans*. 1-(2,4-dichlorobenzyl)-*N*-(2-diethylaminoethyl)-1H-benzimidazole-5-carboxamidine (Figure 1.10) compound, with a 3,4-dichlorophenyl group at the C-2 position,

displayed the greatest activity (MIC = 3.12 µg/mL against both some bacteria and the fungus C. albicans) (Göker et al., 2005).

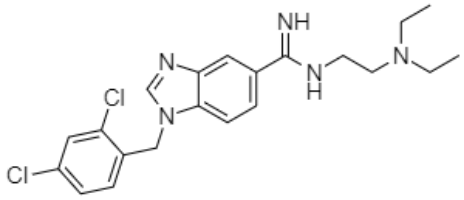


Figure 1.10. Structure of compound 1-(2,4-dichlorobenzyl)-N-(2-diethylaminoethyl)-1H-benzimidazole-5-carboxamidine

The work of Marinescu et al. in 2020, summarizes the syntheses of benzimidazole–pyrazole compounds with antimicrobial properties, in addition to their biological activities mentioned within the literature. From the data presented, it could be concluded that hybrids with pyrazole moiety in position "4" ("7") possess the strongest antimicrobial properties. It has been proven that he presence of some groups grafted on the benzimidazole and pyrazole nuclei, such as -$COOCH_3$, -NHCO, -CHO, -CF_3, -NO_2, -CN, -F, -Cl, -OH, OCH_3, -$N(CH_3)_2$ as well as other heterocycles in the molecule (pyridine, pyrimidine, thiazole, indole), increases the antimicrobial activity of the compounds. Additionally, the antimicrobial activity is improved if the molecule contains linker groups such as carbonyl (CO), amide (NHCO), or other heteroatoms. Among the compounds, mannich base **M-1** shows the best antimicrobial activity. The presence of saturated heterocycles such as 1-methylpiperazine and morpholine has been observed to cause a increase biological activity compared to aromatic amino analogues (Marinescu, 2020).

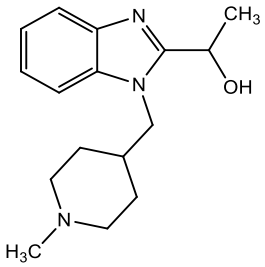


Figure 1.11. Structure of compound **M-1**

In another study (Govindaraj, 2010), a series of novel Schiff bases of isatin were synthesized by the condensation of imesatin with various aromatic aldehydes. Compounds were tested for analgesic activity by the tail-immersion method at a dose of 200 mg/kg body weight. Among the compounds tested, 3-(4-(4-hydroxy-3-methoxylbenzylideneamino) phenylimino) indoline-2-one (Figure 1.12) showed the best analgesic activity compared to standard pentazocine. From the above results it can be concluded that compounds containing electron-donating groups exhibit better analgesic activity than the electron-withdrawing groups.

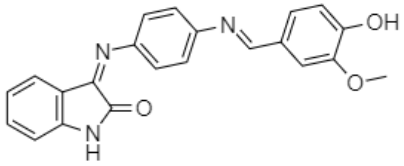


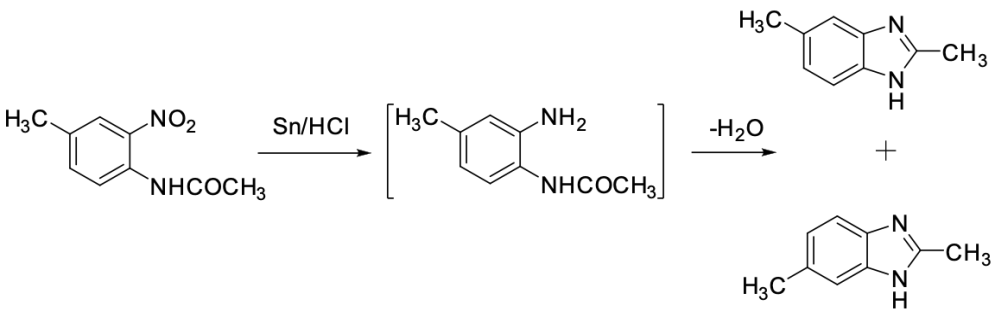
Figure 1.12. Structure of compound 3-(4-(4-hydroxy-3-methoxylbenzylideneamino) phenylimino) indoline-2-one

1.5. Synthesis of Benzimidazole Rings

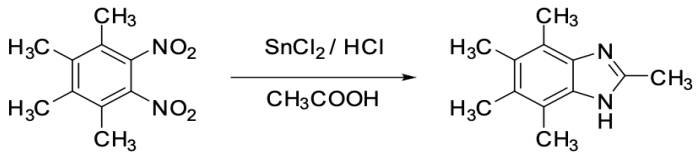
In the method for synthesizing the benzimidazole ring, o-phenylenediamine or its substituted derivatives thereof are mostly used as starting material.

1.5.1. From acylated o-nitroarylamines

The first synthesis of benzimidazole was carried out in 1872 by the reduction of 2-nitro-4-methyl acetanilide, to obtain a derivatives of 2,5 (or 2.6) dimethyl (Wright, 1951).



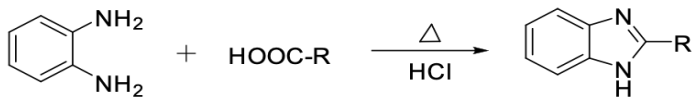
Reduction of *o*-dinitrobenzene derivatives with stannous chloride in the presence of hydrochloric acid and acetic acid without isolation allows the formation of benzimidazole structure. 2,4,5,6,7-pentamethyl benzimidazole is formed as a result of the treatment of 1,2-dinitro-3,4,5,6-tetramethylbenzene with acetic acid under the same reduction conditions (Smith and Harris, 1935; Smith and Moyle, 1936).



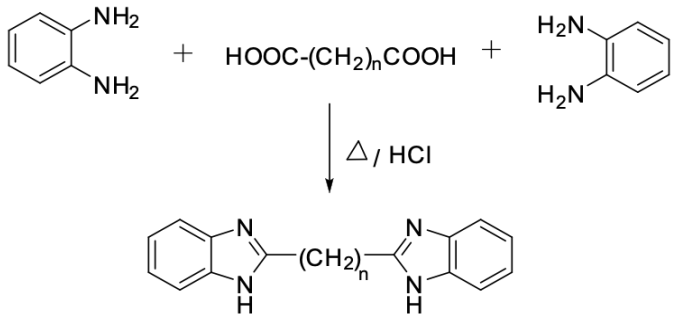
1.5.2. From *o*-phenylenediamines and carboxylic acids, acid anhydrides, esters or amides

Ladenburg realized the first benzimidazole synthesis by the reaction of *o*-phenylenediamine and carboxylic acid. 3,4-diaminotoluene was heated in glacial acetic acid to obtain 2,5-(or 2,6)dimethyl benzimidazole (Ladenburg et al., 1875).

In benzimidazole synthesis, the most widely used method is the reaction of a solution of *o*-phenylenediamines in dilute HCl (usually 4N HCl is used) with a carboxylic acid or acid anhydride. This method is known as Phillips' benzimidazole synthesis (Phillips, 1928[a]; Phillips, 1928[b]).

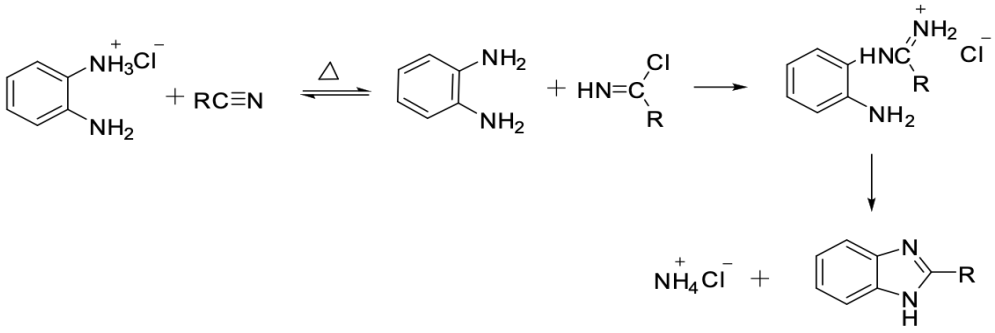


The benzimidazole main ring, which carries no substituents, was synthesized by treating formic acid with o-phenylenediamine (Wright, 1951). 2-alkyl benzimidazoles were also obtained as result of the reaction of *o*-phenylenediamine and carboxylic acids (Pool et al., 1937). Bisbenzimidazoles are obtained as the major product by heating two moles of o-phenylenediamine and one mole of dicarboxylic acid in dilute HCl (Phillips, 1942).



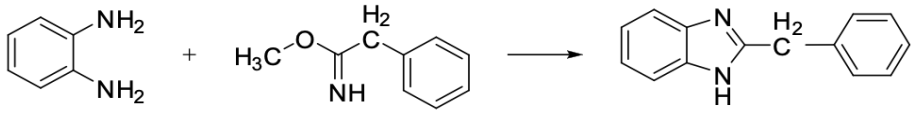
1.5.3. Starting from nitriles with *o*-Phenylenediamine

The 2-alkyl/aryl-substituted benzimidazole structure is obtained by the reaction of the mono HCl salt of *o*-phenylenediamine with an aliphatic or aromatic nitrile at 200°C (Wagner, 1940; Hölljes and Wagner, 1944).



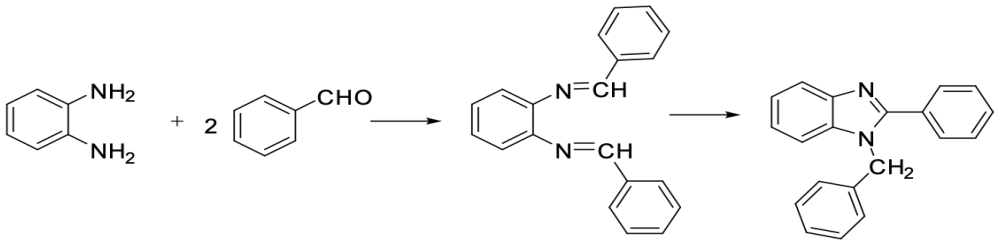
1.5.4. With reference to o-phenylenediamines and iminoethers

2-benzylbenzimidazole is formed as a result of heating the solution of *o*-phenylenediamine and phenacetimino methyl ether in methanol (King and Acheson, 1949).



1.5.5. From aldehydes or ketones with *o*-Phenylenediamine

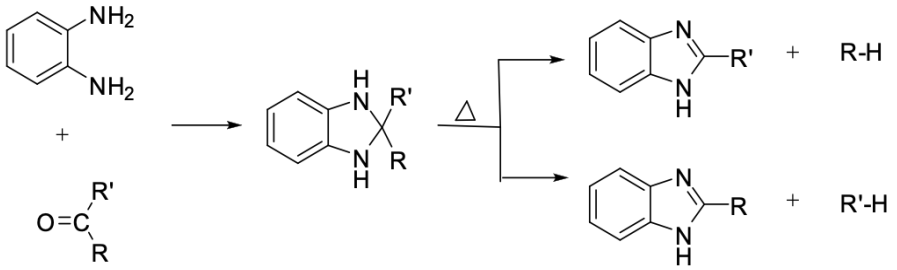
1-benzyl-2-phenylbenzimidazole structure was synthesized by the reaction of formation of benzimidazole structure by following the Schiff base between one mole of *o*-phenylenediamine and two moles of aldehyde (Hinsberg, 1886; Hinsberg, 1887).



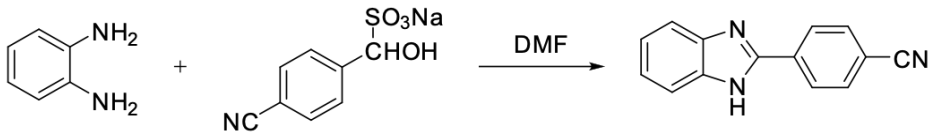
Weidenhagen proposed a method that compensates for the low yield in reactions with aldehydes by using copper acetate as a catalyst (Weidenhagen, 1936).

o-Phenylenediamine gives 2,2-disubstituted-benzimidazolines when treated with ketones, and this product decomposes when heated to give 2-substituted

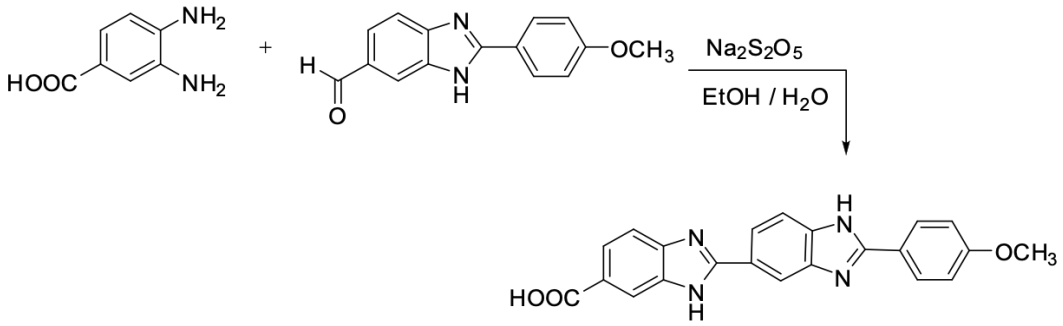
benzimidazole and hydrocarbon structures (Elderfield and Kreysa, 1948; Elderfield and McCarthy, 1951).



4-(1*H*-benzimidazol-2-yl) benzonitrile is obtained by reacting *o*-Phenylenediamine with the sodium bisulfite salt of 4-cyanobenzaldehyde in DMF (Ridley et al., 1965).

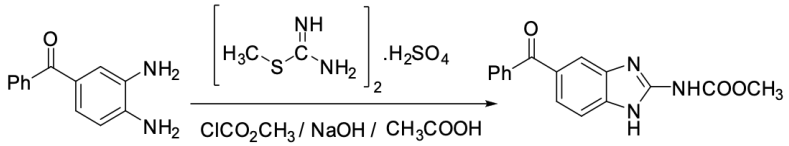


2-(4-Methoxyphenyl) benzimidazole-5-carboxaldehyde reacted with 3,4-diaminobenzoic acid in the presence of sodium pyrosulfite and a bis-benzimidazole derivative compound was synthesized (Ji et al., 2001).

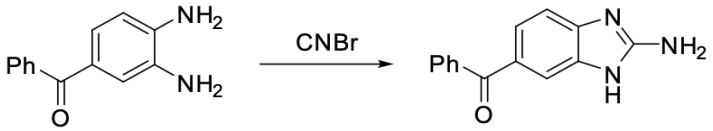


1.5.6. Others

From a mixture of *o*-phenylenediamines and 2-methylthiopseudourea sulfate and methylchloroformate, 1*H*-benzimidazole-2-carbamates are obtained in basic medium. Mebendazole with antihelmentic effect was synthesized in this way (Raeymaekers et al., 1977).



When 3,4-diaminobenzophenone is treated with cyanogen bromide in aqueous medium, 2-amino-5(6)-benzoyl-1*H*-benzimidazole is obtained (Ohemeng and Roth, 1991).



This thesis covers the design and synthesis of new anticancer and antibacterial compounds containing benzimidazole rings, whose anticancer and antibacterial activities have been reported. The main goal is to obtain new target compounds with effective anticancer and antibacterial activities. For this purpose, the anticancer and antibacterial activities of the synthesized compounds were analysed. In order to explain the mechanism of anticancer activity, it was tried to determine the critical targets of the activity by doing structure-activity studies. The purity control and chemical structures of the obtained compounds were elucidated by elemental analysis, ^{1}H, ^{13}C-NMR and mass spectroscopy methods, respectively.

2. MATERIALS & METHODS

2.1. Synthesis Methods of Benzimidazole Derivatives

2.1.1. Synthesis of 1-Chloro-4-(alkylsulfonyl)benzene (2-3) Derivatives

The synthesis of the designed derivatives was started with the starting material 4-chlorobenzenesulfonylchloride (1). Alkyl sulfonyl derivatives were obtained by treating 4-chlorobenzene-sulfonylchloride with alkyl iodides in the presence of tellurium, rongalite and 1M sodium hydroxide solution (Figure 2.1). 1-(methylsulfonyl)-4-chlorobenzene was purchased commercially and used in the experiments. The purity controls of the compounds obtained in this way were made with TLC, melting point and the expected chemical structures were proved by ^{1}H and ^{13}C NMR, Mass spectral analyzes.

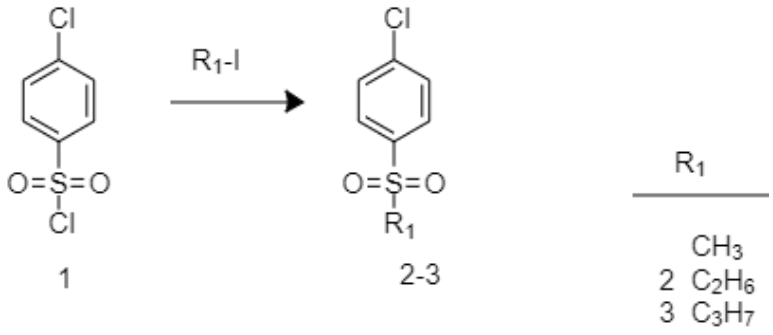


Figure 2.1. Synthesis of 1-chloro-4-(alkyl sulphonic)benzene (**2-3**) derivatives. **Reagents:** Tellurium, rongalite, NaOH, THF, TEBAC,

2.1.2. Synthesis of 1-chloro-4-(alkyl sulfonic)-2-nitrobenzene (4-6) Derivatives

For the synthesis of 1-chloro-4-(alkylsulfonyl)-2-nitrobenzene derivatives, the resulting 1-chloro-4-(alkylsulfonyl) benzene derivatives were nitrated from Position 2 by treatment with potassium nitrate and sulfuric acid.

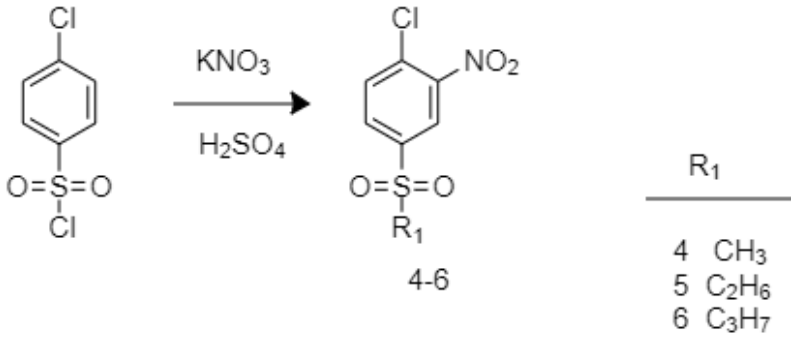
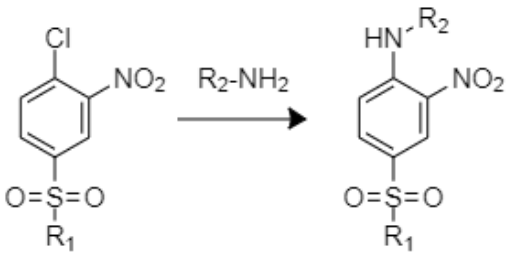


Figure 2.2. Synthesis of 1-Chloro-4-(alkyl sulfonic)-2-nitrobenzene (**4-6**) derivatives. **Reagents:** KNO_3, H_2SO_4

2.1.3. Synthesis of *N*-substituted-4-(alkyl sulfonic)-2-nitroaniline Derivatives (7-14)

The obtained 1-chloro-4-(alkylsulfonyl)-2-nitrobenzene derivatives are dissolved in DMF and treated with appropriate alkyl amines to the resultant products to be synthesized to obtain the desired *N*-substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives as a result of aromatic nucleophilic substitution reaction.



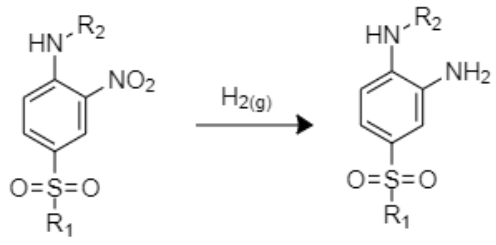
7-14

	R_1	R_2
7	CH_3	Cyclohexyl
8	C_2H_5	Cyclohexyl
9	CH_3	3,4-difluorobenzyl
10	C_2H_5	3,4-difluorobenzyl
11	CH_3	C_2H_5
12	C_2H_5	C_4H_9
13	C_3H_7	C_3H_7
14	CH_3	p-fluorobenzyl

Figure 2.3. Synthesis of *N*-Substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives (**7-14**). **Reagent:** DMF

2.1.4. Synthesis of N^1-substituted-4-(alkylsulfonyl) benzene-1,2-diamine (15-22) Derivatives

N^1-substituted-4-(alkylsulfonyl) benzene-1,2-diamine derivatives were obtained by reducing the nitro group in *N*-substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives by catalytic hydrogenation.



15-22

	R_1	R_2
15	CH_3	Cyclohexyl
16	C_2H_5	Cyclohexyl
17	CH_3	3,4-difluorobenzyl
18	C_2H_5	3,4-difluorobenzyl
19	CH_3	C_2H_5
20	C_2H_5	C_4H_9
21	C_3H_7	C_3H_7
22	CH_3	p-fluorobenzyl

Figure 2.4. Synthesis of *N*-Substituted-4-(alkylsulfonyl) benzene-1,2-diamine (**15-22)** derivatives. **Reagents:** EtOH, Pd/C 10%

2.1.5. Synthesis of alkylsulfonyl 1*H*-benzo[d]imidazole (23-36) Derivatives

The mixture of the sodium metabisulfite adduct of benzaldehyde (2 mmol) and the appropriate 1,2-phenylenediamine (2 mmol) in DMF (5 mL) were reacted to get final products (**23-36).** Purity controls were made by thin layer chromatography, melting point, and their expected chemical structures were proven by ^{1}H and ^{13}C NMR, Mass spectral analyzes.

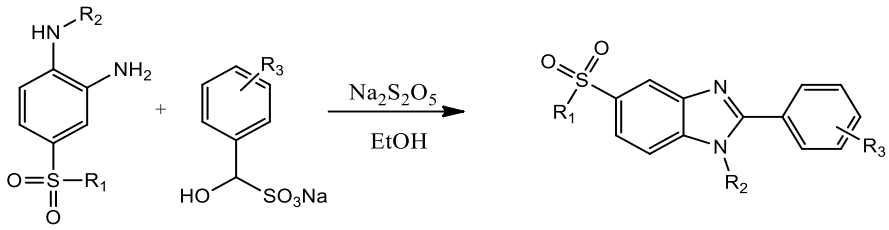


Figure 2.5. Synthesis of 1-(substituted)-1H-benzo[d]imidazole (**23-36**) Derivatives **Reagents:** $Na_2S_2O_5$, EtOH

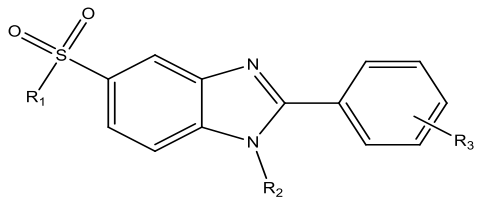
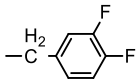
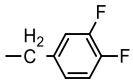
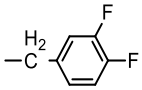
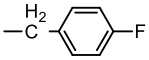
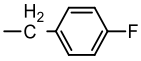


Table 2.1. Synthesis of 1*H*-benzo[d]imidazole (**23**-**36**) derivative compounds

Compound	R_1	R_2	R_3
23	-CH_3	-Cyclohexyl	4-chloro
24	-CH_3	-Cyclohexyl	3,4-difluoro
25	-C_2H_5	-Cyclohexyl	4-chloro
26	-C_2H_5	-Cyclohexyl	3,4-difluoro
27	-CH_3		3,4-difluoro
28	-C_2H_5		3,4-difluoro
29	-CH_3	-C_2H_5	4-acetamido
30	-CH_3	-C_2H_5	4-fluoro
31	-C_2H_5	-C_4H_9	4-fluoro
32	-C_2H_5	-C_4H_9	4-acetamido
33	-CH_3		4-$COOCH_3$
34	-C_3H_7	-C_3H_7	4-chloro
35	-CH_3		2,5-difluoro
36	-CH_3		4-acetamido

2.2. Analytical Methods of Synthesis Benzimidazole Derivatives

2.2.1. Chromatographic Analyses

Thin Layer Chromatography (TLC) was used in order to monitor the progress of the reactions during the synthesis studies and to determine the purity of the products obtained. For this aim, Kieselgel-60 GF254 coated aluminium plates (Merck) was used. The spots were identified under a UV light of 254 nm (Camag UV Lamp). For the column chromatography, silica gel 60 (0,040-0,060 mm, 230-400 mesh) was used.

Table 2.2. Solvent systems used in TLC studies

	Solvents	**Ratio**
1	Hexane : Ethyl acetate	1:1, 2:1, 3:1, 4:1, 1:2, 0.5:1, 0:1, 10:1
2	Ethyl acetate : i-propanol	5:1
3	Ethyl acetate: Cloroform	2:1

2.2.2. Melting Point Measurements

The melting points of the synthesized compounds were measured by using Buchi B540 apparatus. The results were given without correction.

2.2.3. Spectral Analysis

2.2.3.1. Mass Spectrometer (MS)

Mass analyzes were performed in Waters ZQ Micromass LC-MS spectrometer using Electrospray Ionization (ESI) method.

2.2.3.2. Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{1}H and ^{13}C NMR Spectra were performed on the Varian Mercury-400 FT-NMR spectrometer. Tetramethylsilane (TMS) was used as internal standard material, $CDCl_3$ and DMSO-d_6 were used as solvents.

2.2.4. Elementel Analyis

Elemental analyzes were performed with the Leco CHNS 932 Elemental Analyzer.

2.3. Chemical Substances Used during the Synthesis Process

4-Chlorobenzenesulfonylchloride (Sigma-Aldrich), iodoethane (Sigma-Aldrich), 1-iodo-propane (Sigma-Aldrich), tellurium (Sigma-Aldrich), sodium hydroxymethanesulfinate (Sigma Aldrich), sodium hydroxide (Sigma-Aldrich), tetrahydrofuran (Sigma-Aldrich), ammonium chloride (Sigma-Aldrich), benzene (Sigma-Aldrich), toluene (Sigma-Aldrich), sulfuric acid (Sigma-Aldrich), potassium nitrate (Sigma-Aldrich) , ethanol (Sigma-Aldrich), ammonia (Sigma-Aldrich), triethylamine (Sigma-Aldrich), methylamine (Sigma-Aldrich), ethylamine (Sigma-

Aldrich), propylamine (Sigma-Aldrich), butylamine (Sigma-Aldrich), cyclohexylamine (Sigma-Aldrich), benzylamine (Sigma-Aldrich), 4-fluorobenzylamine (Sigma-Aldrich), 3,4-difluorobenzylamine (Sigma-Aldrich), 4-chlorobenzylamine (Sigma-Aldrich), 3,4-dichlorobenzylamine (Sigma) -Aldrich), phosphorus(V)oxychloride (Sigma-Aldrich), dichloromethane (Sigma-Aldrich), hexane (Sigma-Aldrich), ethyl acetate (SigmaAldrich), 2-isopropanol (Sigma-Aldrich), methanol (Sigma-Aldrich), N,N-dimethylformamide (Sigma-Aldrich), chloroform (Sigma-Aldrich).

2.4. Detection of Antimicrobial Activities of Synthesized Compounds

The antibacterial activity tests of the synthesized compounds were performed using the liquid dilution method in accordance with EUCAST recommendations (ISO 20776-1 (2006). For this purpose, double-layer serial dilutions were prepared by adding 100 µL of synthesized compound to the first well of 96-well micro-plates containing 100 µL of Mueller Hinton Broth with cation addition in each well. Then, fresh bacterial suspensions prepared according to McFarland 0.5 turbidity were diluted 1/100 and 100 µL was added to all wells and the plates were incubated at 37C for 16-20 hours. The lowest concentration at which growth was inhibited after incubation was determined as the Minimum Inhibitory Concentration (MIC). Ciprofloxacin was used as a control and ciprofloxacin MIC values were evaluated using EUCAST quality control tables (EUCAST, 2019).

2.5. Detection of Anticancer Activities of Synthesized Compounds

2.5.1. Cell culture

MCF-7 human epithelial breast adenocarcinoma cell line was provided by SAP Institute (Ankara, Turkey). The cells were grown in RPMI-1640 medium (Biological Industries, Israel). Cells were supplemented with 10% fetal bovine serum stabilized

with 1.5% L-glutamine and 0.1 mg / mL gentamicin (Sigma-Aldrich, USA) in a 5% CO_2 incubator at 37°C.

2.5.2. Cytotoxicity Assay

The xCELLigence Real Time Quantitative Cell Analyzer (Agilent, USA) was used for cytotoxicity analysis. Real-time cell viability analyzes were performed on 16-well plates specially designed for the device. The center of the system is the integrated microelectronic cell sensor array at the bottom of specially manufactured well plates. Cell index expressed as unitless (CI); used to measure the relative change in electrical impedance indicating cell state. Briefly, cells were seeded in 16-well plates at a density of $10x10^3$ cells/well in 200 µL of complete medium and allowed to grow overnight for attachment onto the wells. Then the cells were treated with three different concentrations (5µM, 50µM and 100µM) of the compounds for a period of 72 h. vincristine (1µM) was used as a positive control.

2.6. Molecular Docking

Crystallographic data files for *S.aureus* DNA gyrase B subunit (PDB ID:3ttz, resolution: 1.63 Å), *E. faecalis* DNA gyrase B subunit (PDB ID:4k4o, resolution: 1.30 Å), and human DNA topoisomerase IIalpha-DNA complex (PDB ID:5gwk, resolution: 3.15 Å) were obtained from the RCSB Protein database website (Berman et al., 2000). AutoDockTools 1.5.6. (Morris et al., 2009) was used for deleting water molecules and defining the gridbox parameters according to the bound co-ligands. After this process, polar hydrogens and Gasteiger charges were added. The 2D structures of the compounds were drawn on ChemDraw Ultra 12.0, minimized with MMFF94 and UFF forcefields (number of steps: 5000 with steepest descent algorithm and convergence value of 10e-7) and then these files were converted to pdb files using Avogadro software (Hanwell et al., 2012). Subsequently, Gasteiger charges and torsion were added to ligand files with AutoDockTools. The prepared ligands were docked with Autodock Vina (Trott and Olson, 2010),

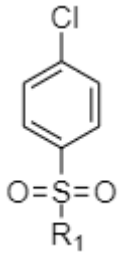
interaction diagrams were created and investigated using Discovery Studio Visualizer Ligand interaction module (Dassault Systèmes BIOVIA, 2020).

2.7. Determination of ADME Properties

For the calculation of the ADME properties of the synthesized benzimidazole derivatives, SwissADME online software was used (Daina et al., 2017). To generate an input for this program, SMILES codes were generated with the ACD/ChemSketch and the codes for the commercialized compounds were obtained from PubChem. All of them were submitted as input to the SwissADME online program, and molecular parameters such as log *P*, BBB permeation, P-glycoprotein substrate characteristics, and suitability to the Lipinski and Veber filters were evaluated (Lipinski et al., 2001, Veber et al., 2002). CYP-450 enzyme inhibition was also calculated with this program to identify candidates that have tendency towards drug-drug interactions.

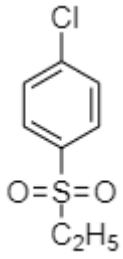
3. RESULTS

3.1. General Synthesis Method of 1-Chloro-4-(alkylsulfonyl)benzene (2-3) Derivatives



1.28 grams (10 mmol) of tellurium and 7.71 grams (50 mmol) of rongalite were heated in 25 ml of 1M sodium hydroxide (NaOH) solution at 70 °C until red colored sodium telluride was obtained. This resulting mixture was added dropwise to a mixture of 3.02 grams (10 mmol) of p-chlorobenzenesulfonylchloride and 0.23 grams (0.1 mmol) of triethylbenzyl ammonium chloride (TEBAC) in 30 ml of THF. After stirring at room temperature for 5 minutes, 50 mmol of iodoethane/iodopropane in 3 ml of THF was added. It was controlled with TLC and refluxed for 5 hours at 90 °C. After the time, the solvent was evaporated and the residue was treated with aqueous NH_4Cl. It was purified by column chromatography by extraction with benzene (hexane:ethyl acetate 3:1).

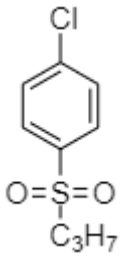
3.1.1.1-Chloro-4-(ethylsulfonyl)benzene (2)



According to the general synthesis method, 0.37 g of product was obtained with 18% yield.

^{1}H NMR Spectrum (CdCl3) δ ppm: 1.28 (t, 3H, CH_3), 3.13 (q, 2H, CH_2), 7.56 (dd, *J*=6.0 Hz, *J*=2.0 Hz, 2H, CH-3, CH-5), 7.85 (dd, *J*=6.4 Hz, *J*=2.0 Hz, 2H, CH-2, CH-6).

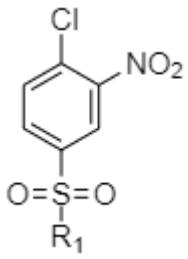
3.1.2. 1-Chloro-4-(propylsulfonyl)benzene (3)



According to the general synthesis method, 0.63 g of product was obtained with a yield of 29%.

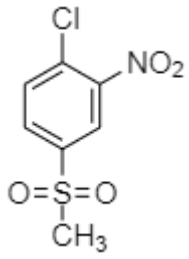
^{1}H NMR Spectrum (DMSO-d6) δ ppm: 0.94 (t, 3H, CH_3), 1.50-1.59 (m, 2H, CH_2), 2.91 (t, 2H, CH_2), 7.28-7.34 (m, 4H,).

3.2. Synthesis Method of 1-Chloro-4-(alkylsulfonyl)-2-nitrobenzene (4-6) Derivatives



A solution of 6.2 mmol 1-(alkylsulfonyl)-4-chlorobenzene in 6.4 ml (12 mmol) H_2SO_4 was heated to 80 °C. KNO_3 was added little by little (1,212 grams) at 80 °C. The reaction was continued for 2 hours at 90 °C, controlled by TLC. The yellow precipitate formed by pouring into iced water was filtered off. Purified by column chromatography (hexane:ethyl acetate 2:1).

3.2.1. 1-Chloro-4-(methylsulfonyl)-2-nitrobenzene (4)

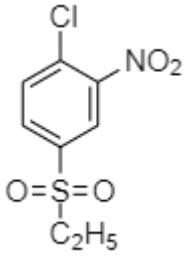


According to the general synthesis method, 1.28 g of product was obtained with 88% yield.

Melting Point: 204 ºC.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 4.14 (s, 3H, CH_3), 8.09 (d, *J*=8.8 Hz, 1H, CH-6), 8.21 (dd, *J*=8.6 Hz, *J*=2.0 Hz, 1H, CH-5), 8.60 (d, *J*=2.0 Hz, 1H, CH-3).

3.2.2. 1-Chloro-4-(ethylsulfonyl)-2-nitrobenzene (5)

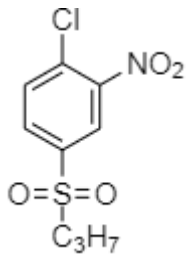


According to the general synthesis method, 0.98 g of product was obtained with a yield of 63%.

Melting Point: 97 ºC.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 1.14 (t, 3H, CH_3), 3.46 (q, 2H, CH_2), 8.09 (d, *J*=8.4 Hz, 1H, CH-6), 8.18 (dd, *J*=8.4 Hz, *J*=2.0 Hz, 1H, CH-5), 8.56 (d, *J*=2.0 Hz, 1H, CH-3).

3.2.3. 1-Chloro-4-(propylsulfonyl)-2-nitrobenzene (6)



According to the general synthesis method, 1.44 g of product was obtained with 88% yield.

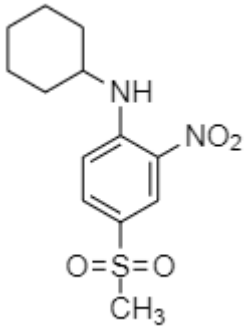
Melting Point: 79 ^{o}C.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 0.91 (t, 3H, CH_3), 1.54-1.59 (m, 2H, $\underline{CH_2}$-CH_3), 3.39-3.43 (m, 2H, $\underline{CH_2}$-CH_2), 8.05 (d, *J*=8.0 Hz, 1H, CH-6), 8.15 (dd, *J*=8.6 Hz, *J*=2.0 Hz, 1H, CH-5), 8.53 (d, *J*=2.0 Hz, 1H, CH-3).

3.3. Synthesis Method of *N*-Substituted-4-(alkylsulfonyl)-2-nitroaniline Derivatives (7-14)

4-(Alkylsulfonyl)-1-chloro-2-nitrobenzene (1 mmol) and the appropriate alkyl amines (3-4 mmol) were dissolved in dimethylformamide (2 ml). It was stirred at 80 °C for 3 hours. Checked with TLC. At the end of the reaction, it was poured on ice and isolated. It was crystallized from ethanol.

3.3.1. *N*-Cyclohexyl-4-(methylsulfonyl)-2-nitroaniline (7)

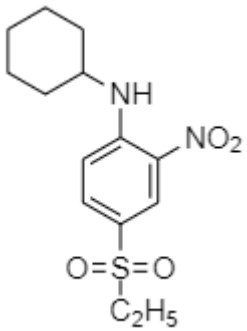


According to the general synthesis method, 0.200 g of product was obtained with 85% yield.

Melting Point: 124 ^{o}C.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 1.24 (m, 1H, cyclohexyl CH), 1.41 (m, 4H, cyclohexyl), 1.56-70 (m, 3H, cyclohexyl), 1.93 (m, 2H, cyclohexyl CH_2), 3.18 (s, 3H, CH_3), 3.73 (m, 1H, cyclohexyl CH), 7.31 (d, *J*=9.2 Hz, 1H, CH-6), 7.88 (dd, *J*=8.6 Hz, *J*=2.4 Hz, 1H, CH-5), 8.30 (brd d, 1H, NH), 8.45 (d, *J*=2.4 Hz, 1H, CH-3)

3.3.2. *N*-Cyclohexyl-4-(ethylsulfonyl)-2-nitroaniline (8)



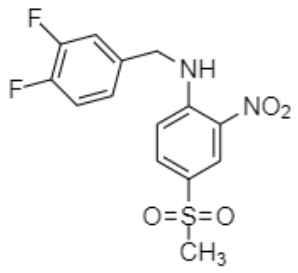
According to the general synthesis method, 0.189 g of product was obtained with 80% yield.

Melting Point: 154 ^{o}C.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 1.11 (t, 3H, CH_3), 1.26-1.27 (m, 1H, cyclohexyl CH), 1.40-1.47 (m, 4H, cyclohexyl), 1.61 (d, 1H, cyclohexyl CH), 1.69-

1.72 (m, 2H, cyclohexyl CH_2), 1.93-1.96 (m, 2H, cyclohexyl CH_2), 3.28 (q, 2H, CH_2), 3.74-3.76 (m, 1H, cyclohexyl CH), 7.34 (d, *J*=9.6 Hz, 1H, CH-6), 7.85 (dd, *J*=9.0 Hz, *J*=2.4 Hz, 1H, CH-5), 8.32 (d, *J*=8.0 Hz, 1H, CH-3), 8.55 (d, *J*=2.4 Hz, 1H. NH).

3.3.3. *N*-(3,4-Difluorobenzyl)-4-(methylsulfonyl)-2-nitroaniline (9)

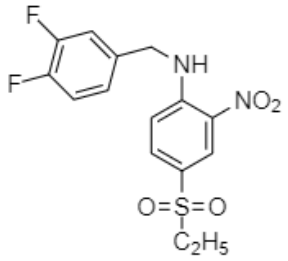


According to the general synthesis method, 0.189 g of product was obtained with 80% yield.

Melting Point: 128 ºC.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 3.16 (s, 3H, CH_3), 4.68 (d, 2H, CH_2), 7.05 (d, *J*=8.8 Hz, 1H, CH-6), 7.21-7.24 (m, 1H), 7.35-7.49 (m, 2H), 7.83 (dd, *J*=9.2 Hz, *J*=2.4 Hz, 1H, CH-5), 8.49 (d, *J*=2.4 Hz, 1H, CH-3), 9.16 (t,1H, NH)

3.3.4. *N*-(3,4-Diflorobenzyl)-4-(ethylsulfonyl)-2-nitroaniline (10)

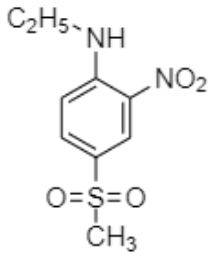


According to the general synthesis method, 0.177 g of product was obtained with 71% yield.

Melting Point: 121 °C.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 1.08 (t, 3H, CH_3), 3.26 (q, 2H, CH_2), 4.70 (d, 2H, NH-$\underline{CH_2}$), 7.07 (d, *J*=9.2 Hz, 1H, CH-6), 7.24-7.26 (m, 1H), 7.37-7.52 (m, 2H), 7.79 (dd, *J*=9.0 Hz, *J*=2.4 Hz, 1H, CH-5), 8.46 (d, *J*=2.4 Hz, 1H, CH-3), 9.20 (t,1H, NH).

3.3.5. *N*-Ethyl-4-(methylsulfonyl)-2-nitroaniline (11)

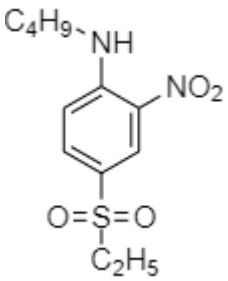


According to the general synthesis method, 0.195 g of product was obtained with 83% yield.

Melting Point: 164 °C.

^{1}H NMR Spektrumu (DMSO-d6) δ ppm: 1.23 (t, 3H, CH_2-$\underline{CH_3}$), 3.21 (s, 3H, CH_3), 3.45-3.52 (m, 2H, CH_2), 7.25 (d, *J*=9.2 Hz, 1H, CH-6), 7.92 (dd, *J*=9.2 Hz, *J*=2.0 Hz, 1H, CH-5), 8.50 (d, *J*=2.4 Hz, 1H, CH-3), 8.60 (brd t, 1H, NH).

3.3.6. *N*-Butyl-4-(ethylsulfonyl)-2-nitroaniline (12)

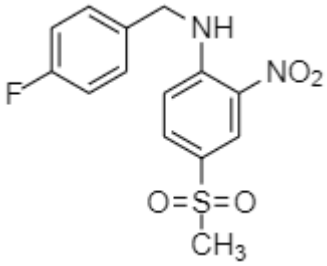


According to the general synthesis method, 0.193 g of product was obtained with 82% yield.

Melting Point: 76 °C.

^{1}H NMR Spectrum (DMSO-d6) δ ppm: 0.92 (t, 3H, CH_2-CH_2-CH_2-$\underline{CH_3}$), 1.11 (t, 3H, CH_2-$\underline{CH_3}$), 1.35-1.40 (m, 2H, CH_2-CH_2-$\underline{CH_2}$-CH_3), 1.57-1.64 (m, 2H, CH_2-$\underline{CH_2}$-CH_2-CH_3), 3.27 (q, 2H, NH-$\underline{CH_2}$), 3.44 (q, 2H, $\underline{CH_2}$-CH_3), 7.25 (d, *J*=8.8 Hz, 1H, CH-6), 7.85 (dd, *J*=9.4 Hz, *J*=2.0 Hz, 1H, CH-5), 8.44 (d, *J*=2.0 Hz, 1H, CH-3), 8.60 (brd t,1H, NH).

3.3.7. *N*-(4-Fluorobenzyl)-4-(methylsulfonyl)-2-nitroaniline (14)

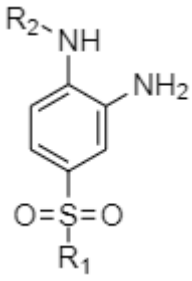


According to the general synthesis method, 0.193 g of product was obtained with 82% yield.

Melting Point: 144 °C.

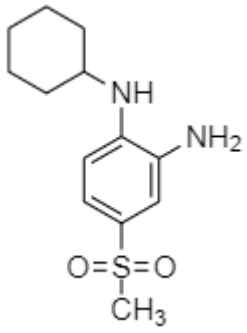
^{1}H NMR Spectrum (DMSO-d6) δ ppm: 3.18 (s, 3H, CH_3), 4.71 (d, 2H, CH_2), 7.09 (d, *J*=9.6 Hz, 1H, CH-6), 7.15-7.20 (m, 2H), 7.44 (m, 2H), 7.85 (dd, *J*=9.6 Hz, *J*=2.4 Hz, 1H, CH-5), 8.52 (d, *J*=2.0 Hz, 1H, CH-3), 9.18 (t,1H, NH)

3.4. Synthesis of *N*^{1}-substituted-4-(alkylsulfonyl)benzene-1,2-diamine (15-22) Derivatives



N-substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives (1.5 mmol) were dissolved in 15 ml of ethanol. It was hydrogenated at 40 psi with 10% Pd/C (80-100 mg) catalyst at room temperature until the hydrogen consumption was over. The catalyst was separated by filtration over celite. The product was obtained by evaporation of ethanol. The structure of the obtained products was clarified by mass analysis.

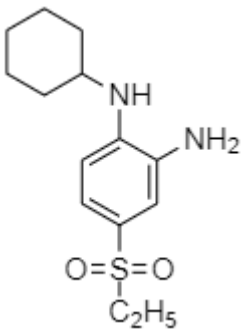
3.4.1. N^1-Cyclohexyl-4-(methylsulfonyl)benzene-1,2-diamine (15)



According to the general synthesis method, 0.402 g of product was obtained with 90% yield.

Mass Spectrum MS (ESI+) m/e: 269.10 (% 100) (M^++H)

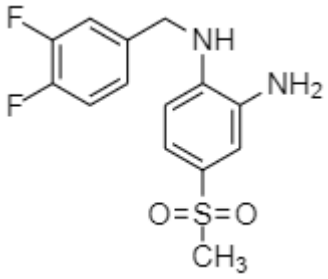
3.4.2. N^1-Cyclohexyl-4-(ethylsulfonyl)benzene-1,2-diamine (16)



According to the general synthesis method, 0.360 g of product was obtained with 80% yield.

Mass Spectrum: MS (ESI+) m/e: 283.48 (% 100) (M^++H)

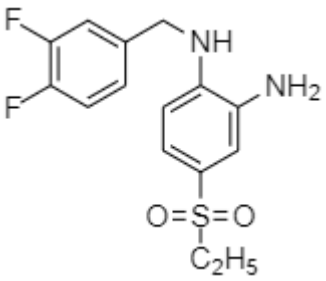
3.4.3. *N*^{1}-(3,4-Difluorobenzyl)-4-(methylsulfonyl)benzene-1,2-diamine (17)



According to the general synthesis method, 0.368 g of product was obtained with 81% yield.

Mass Spectrum: MS (ESI+) m/e: 313.17 (% 20) (M^++H)

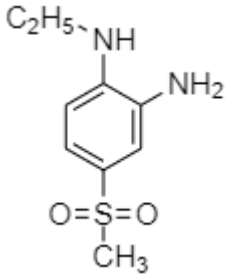
3.4.4. *N*^{1}-(3,4-Difluorobenzyl)-4-(ethylsulfonyl) benzene-1,2-diamine (18)



According to the general synthesis method, 0.346 g of product was obtained with 76% yield.

Mass Spectrum: MS (ESI+) m/e: 327.41 (% 35) (M^++H)

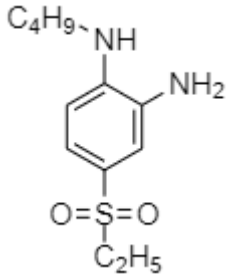
3.4.5. N^1-Ethyl-4-(methylsulfonyl) benzene-1,2-diamine (19)



According to the general synthesis method, 0.379 g of product was obtained with 87% yield.

Mass Spectrum: MS (ESI+) m/e: 215.22 (% 100) (M^++H)

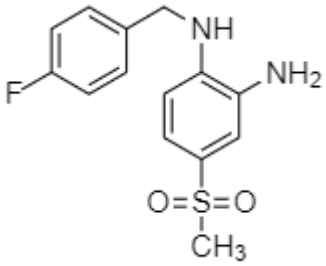
3.4.6. N^1-Butyl-4-(ethylsulfonyl)benzene-1,2-diamine (20)



According to the general synthesis method, 0.374 g of product was obtained with a yield of 84%.

Mass Spectrum: MS (ESI+) m/e: 257.51 (% 100) (M^++H)

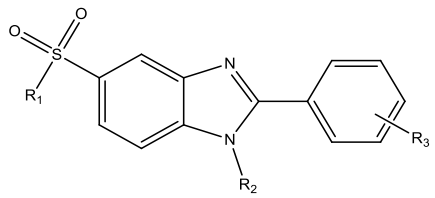
3.4.7. N^1-(4-Fluorobenzyl)-4-(methylsulfonyl) benzene-1,2-diamine (22)



According to the general synthesis method, 0.375 g of product was obtained with 83% yield.

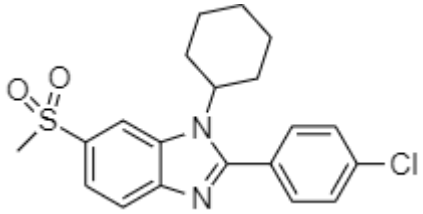
Mass Spectrum: MS (ESI+) m/e: 295.17 (% 20) (M^++H)

3.6. General Synthesis Method of 1*H*-benzo[d]imidazole (23-36) Derivatives



A solution of substituted benzaldehyde (30 mmol) in 20 mL EtOH was added to a solution of $Na_2S_2O_5$ (30 mmol) in 20 mL water and the mixture stirred in an ice-bath to give a white precipitate which was filtered and dried (m.p. decom. > 300 ^oC). The mixture of the sodium metabisulfite adduct of benzaldehyde (2 mmol) and the appropriate 1,2-phenylenediamine (2 mmol) in DMF (5 mL) were heated at 130 ^oC for 4 h. The reaction was terminated after observing the end of the onsets with TLC. It was precipitated by pouring on ice. The precipitate was filtered off and purified by column chromatography. It was crystallized from methanol.

3.6.1. 2-(4-Chlorophenyl)-1-cyclohexyl-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazole (23)



243.1 mg of N^1-cyclohexyl-4-(methylsulfonyl) benzene-1,2-diamine (0.97 mmol) and 238 mg of sodium (4-chlorophenyl)(hydroxy)methanesulfonate (0.97 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:1) solvent system. 71.5 mg of product was obtained in 14.9% yield.

Melting Point: 239.2 ºC.

Mass Spectrum: MS (ESI+) m/e: 389.56 (% 100) (M^++H).

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.34 (m, 3H, cyclohexyl), 1.63 (d, 1H, cyclohexyl), 1.90 (m, 4H, cyclohexyl), 2.29 (m, 2H, cyclohexyl), 3.24 (s, 3H, CH_3), 4.26 (m, 1H, cyclohexyl), 7.71 (m, 4H), 7.80 (dd, *J*=8.4 Hz, *J*=2 Hz, 1H), 8.18 (d, *J*=8.4 Hz, 1H), 8.23 (d, *J*=2 Hz, 1H).

^{13}C NMR (DMSO-d_6) δ ppm 24.27, 25.38, 30. 44, 44.19, 57.10, 113.99, 119.13, 120. 55, 128.80, 128.98, 131.30, 134. 47, 135.14, 136.73, 142.52, 154.95.
Elementel Analysis: $C_{20}H_{21}ClN_2O_2S$

	%C	%H	%N	%S
Calculated:	61.77	5.44	7.20	8.24
Found:	61.51	5.72	7.41	8.12

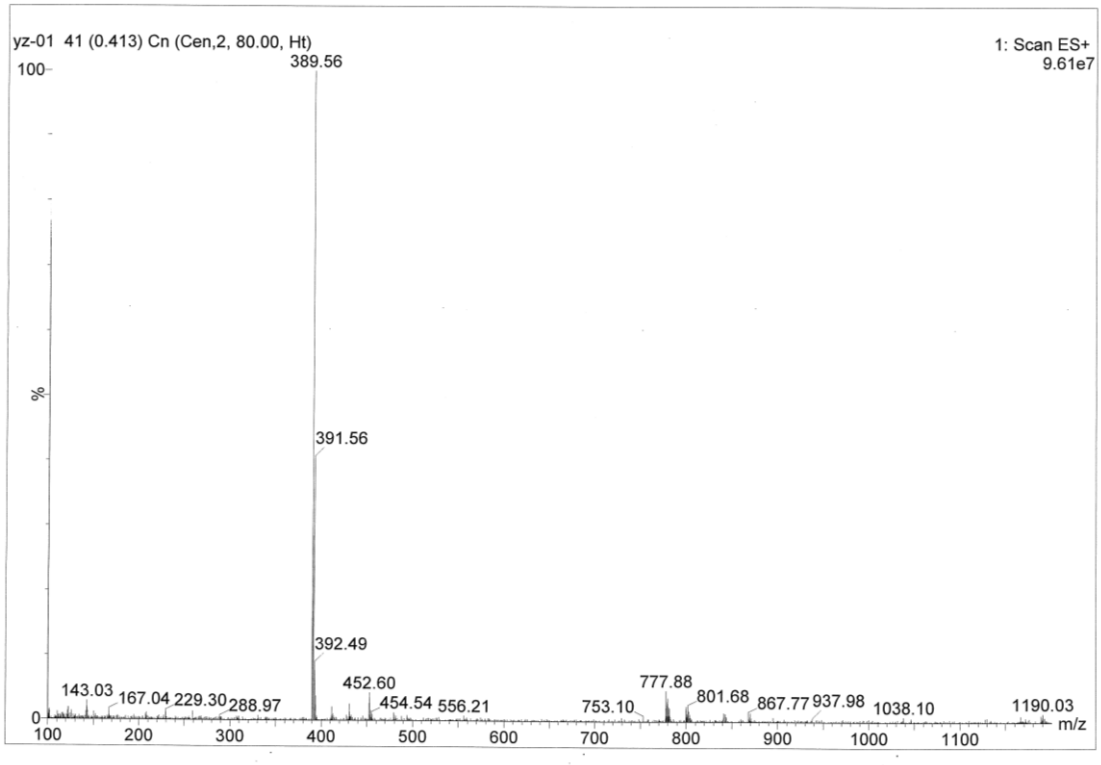


Figure 3.1. Mass spectrum of compound **23**

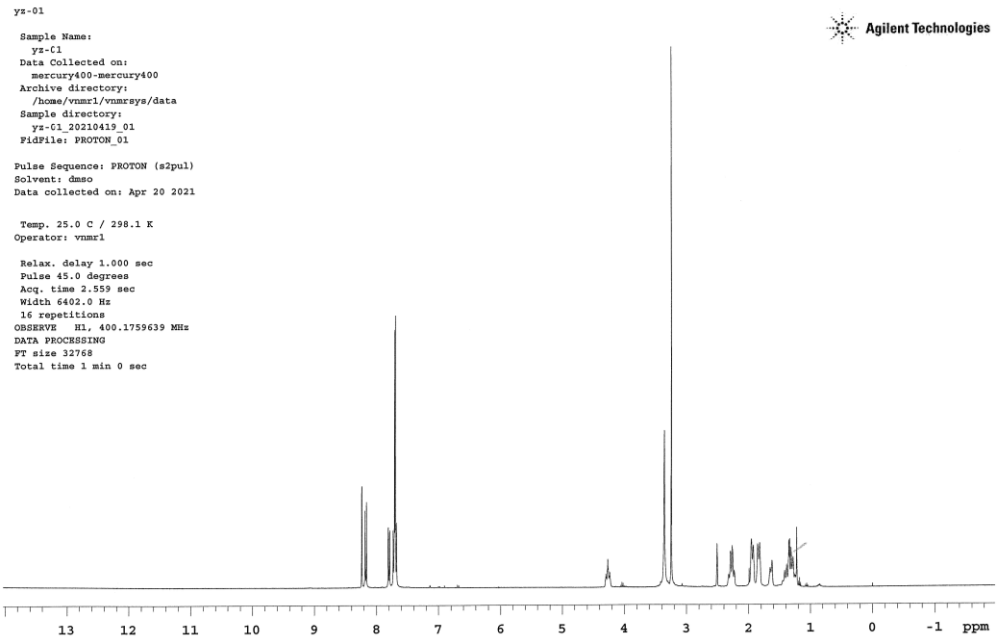


Figure 3.2. ^{1}H NMR spectrum of compound **23**

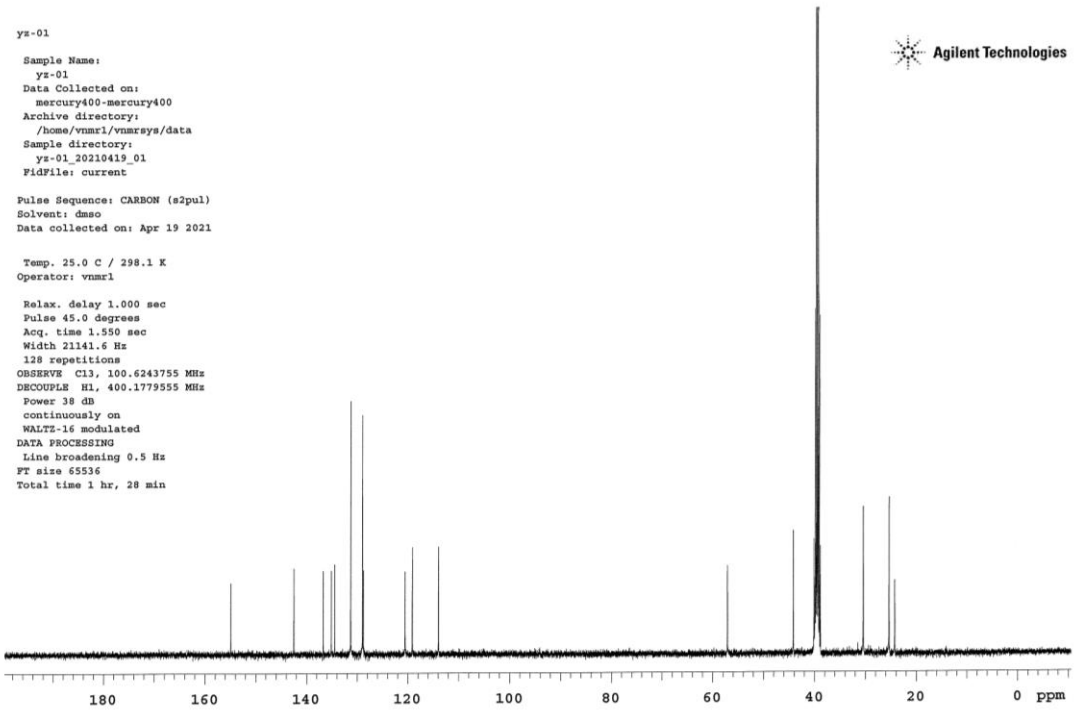
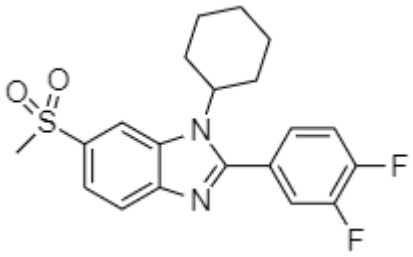


Figure 3.3. ^{13}C NMR spectrum of compound **23**

3.6.2. 1-Cyclohexyl-2-(3,4-difluorophenyl)-5-(methylsulfonyl)–1*H*-benzo[*d*]imidazole (24)



230.7 mg of N^1-cyclohexyl-4-(methylsulfonyl) benzene-1,2-diamine (0.93 mmol) and 229 mg of sodium (3,4-difluorophenyl) (hydroxy) methanesulfonate (0.93 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:1) solvent system. 224 mg of product was obtained in 48.7% yield.

Melting Point: 196.8 ^{o}C.

Mass Spectrum: MS (ESI+) m/e: 391.56 (% 100) (M^++H).

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.33 (m, 3H, cyclohexyl), 1.64 (d, *J*=1.1 Hz 1H, cyclohexyl), 1.83 (d, *J*=1.2 Hz, 2H, cyclohexyl), 1.97 (d, *J*=1.2Hz, 2H, cyclohexyl), 2.26 (m, 2H, cyclohexyl), 3.24 (s, 3H, CH_3), 4.24 (m, 1H, cyclohexyl), 7.54 (m, 1H), 7.71 (m, 1H), 7.80 (m, *2*H), 8.17 (d, *J*=8.8 Hz, 1H), 8.23 (d, *J*=2 Hz, 1H).

Elementel Analysis: $C_{20}H_{20}F_2N_2O_2S$

	%**C**	%**H**	%**N**	%**S**
Calculated:	61.52	5.16	7.17	8.24
Found:	61.81	5.54	7.30	8.18

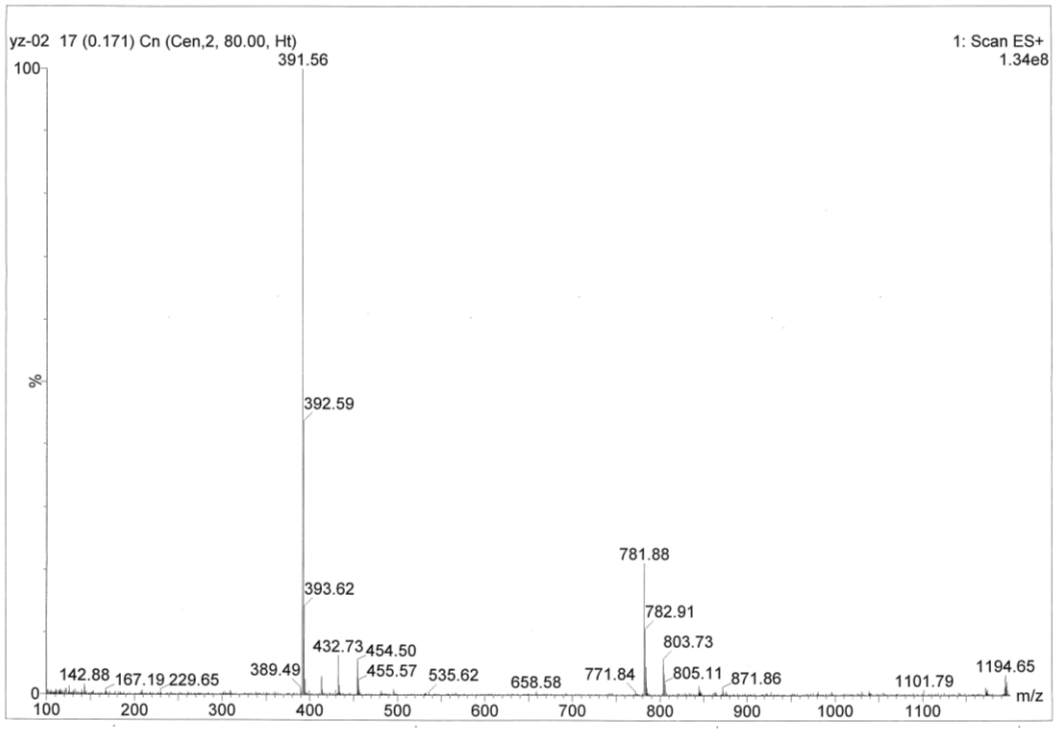


Figure 3.4. Mass spectrum of compound **24**

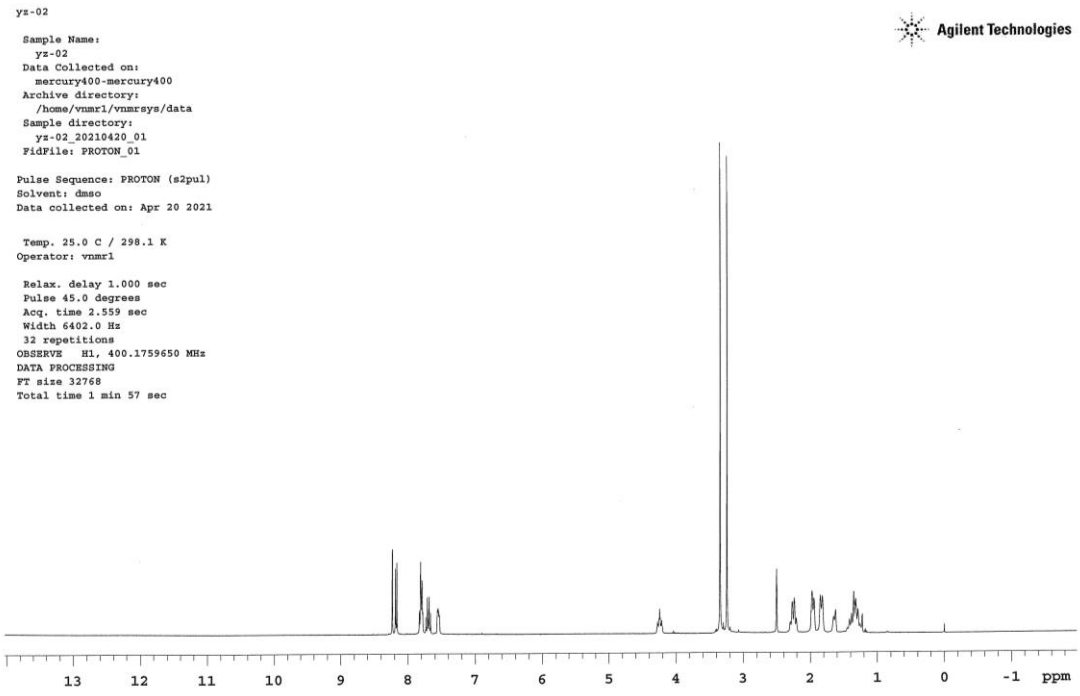
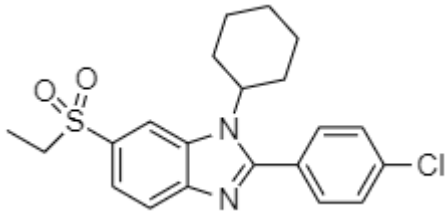


Figure 3.5. ^{1}H NMR spectrum of compound **24**

3.6.3. 2-(4-Chlorophenyl)-1-cyclohexyl-5-(ethylsulfonyl)-1*H*-benzo[*d*]imidazole (25)



253.5 mg of N^1-cyclohexyl-4-(ethylsulfonyl) benzene-1,2-diamine (0.96 mmol) and 236 mg of sodium (4-chlorophenyl) (hydroxy) methanesulfonate (0.96 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (2:1) solvent system. 167 mg of product was obtained in 34.1% yield.

Melting Point: 249.8 ^{o}C.

Mass Spectrum: MS (ESI+) m/e: 403.5 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.10 (t, 3H), 1.29 (m, 3H, cyclohexyl), 1.63 (d, *J*=1.2 Hz, 1H, cyclohexyl), 1.82 (d, *J=1.2* Hz, 2H, cyclohexyl), 1.93 (d, *J*=1.2 Hz, 2H, cyclohexyl), 2.24 (m, 2H, cyclohexyl), 3.29 (q, 2H), 4.25 (m, 1H, cyclohexyl), 7.69 (m, 5H), 8.16 (m, 2H).

^{13}C NMR (DMSO-d_6) δ ppm 7.36, 24.29, 25.39, 30.45, 49.68, 57.12, 114.00, 119.94, 121.31, 128.81, 128.99, 131.33, 131.97, 135.15, 136.87, 142.59, 154.98.

Elementel Analysis: $C_{21}H_{23}ClN_2O_2$

	%C	%H	%N	%S
Calculated:	62.60	5.75	6.95	7.96
Found:	62.77	6.29	7.15	8.03

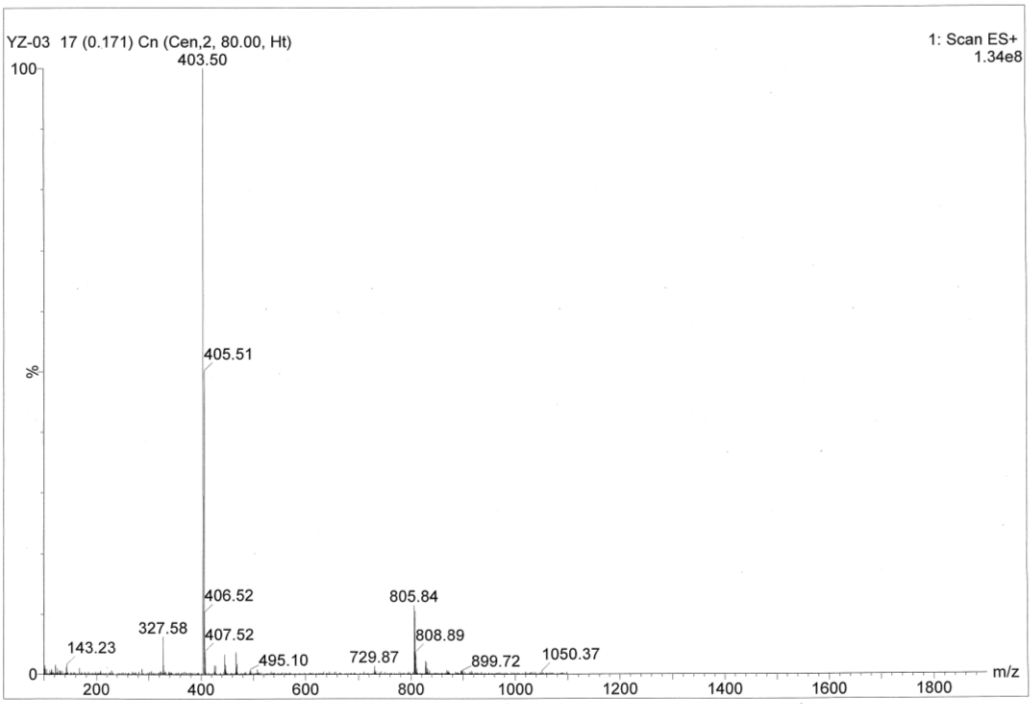


Figure 3.6. Mass spectrum of compound **25**

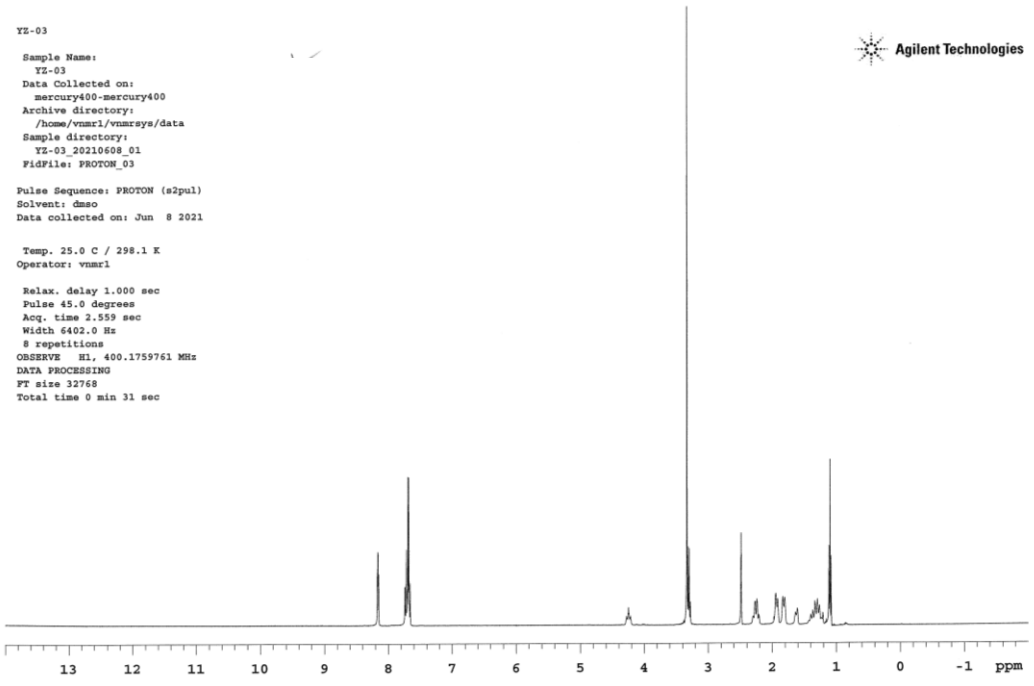


Figure 3.7. ^{1}H NMR spectrum of compound **25**

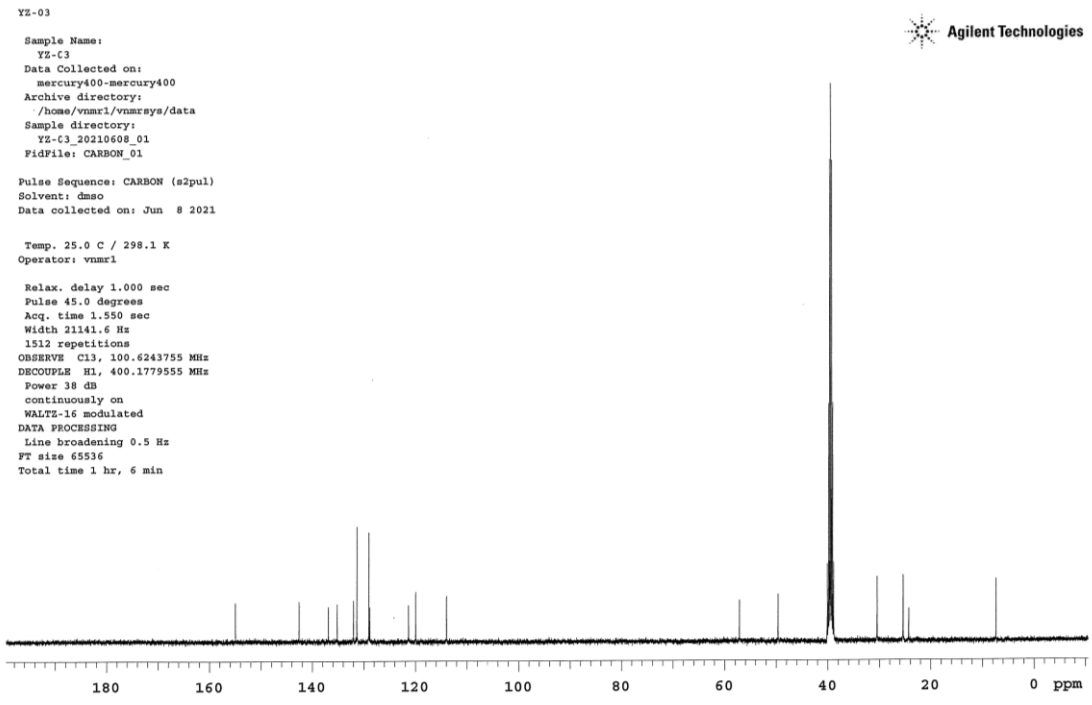
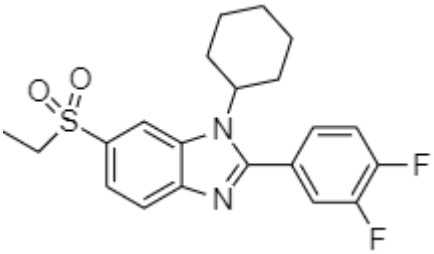


Figure 3.8. ^{13}C NMR spectrum of compound **25**

3.6.4. 1-Cyclohexyl-2-(3,4-difluorophenyl)-5-(ethylsulfonyl)-1*H*-benzo[*d*]imidazol (26)



226.5 mg of N^1-cyclohexyl-4-(ethylsulfonyl) benzene-1,2-diamine (0.86 mmol) and 212 mg of sodium (3,4-difluorophenyl) (hydroxy) methanesulfonate (0.86 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:1) solvent system. 119 mg of product was obtained in 27.1% yield.

Melting Point: 193.0 ^{o}C.

Mass Spectrum: MS (ESI+) m/e: 405.52 (% 100) (M^+)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.09 (t, 3H), 1.33 (m, 3H, cyclohexyl), 1.62 (d, *J*=1.0 Hz, 1H, cyclohexyl), 1.82 (d, *J=1.2* Hz, 2H, cyclohexyl), 1.95 (d, *J*=1.2 Hz, 2H, cyclohexyl), 2.24 (m, 2H, cyclohexyl), 3.33 (q, 2H), 4.22 (m, 1H, cyclohexyl), 7.54 (m, 1H), 7.74 (m, 3H), 8.16 (m, 2H).

Elementel Analysis: $C_{21}H_{17}ClN_2O_2S$

	%C	**%H**	**%N**	**%S**
Calculated:	62.36	5.48	6.93	7.93
Found:	62.61	5.71	7.08	7.91

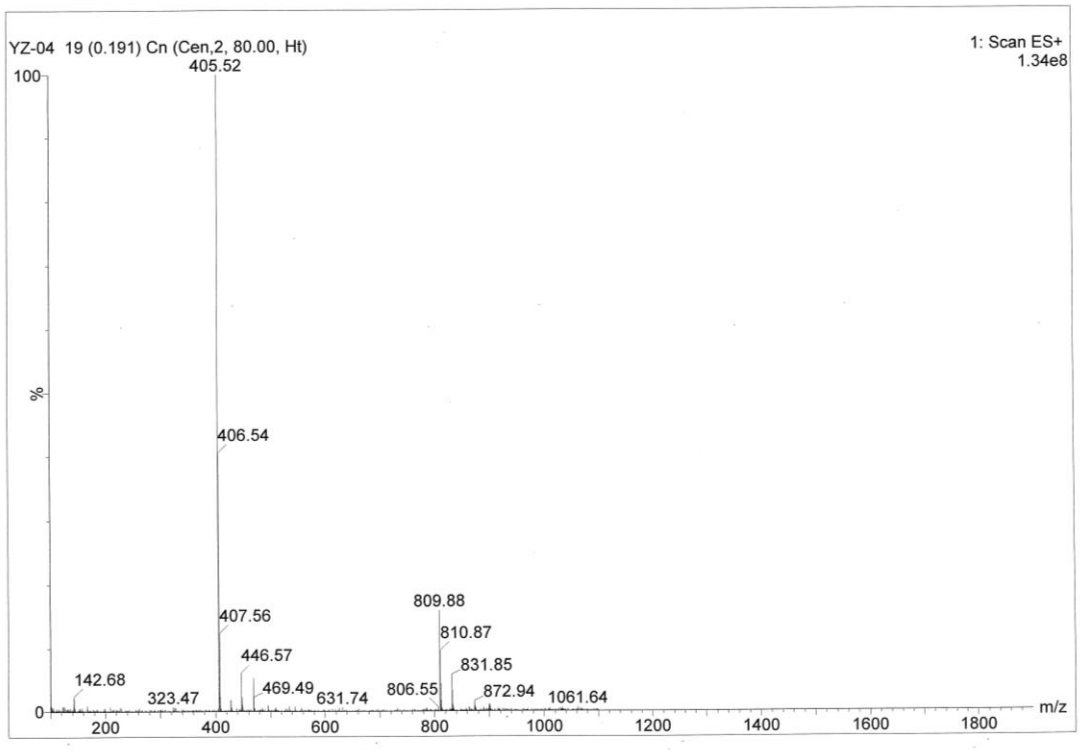


Figure 3.9. Mass spectrum of compound **26**

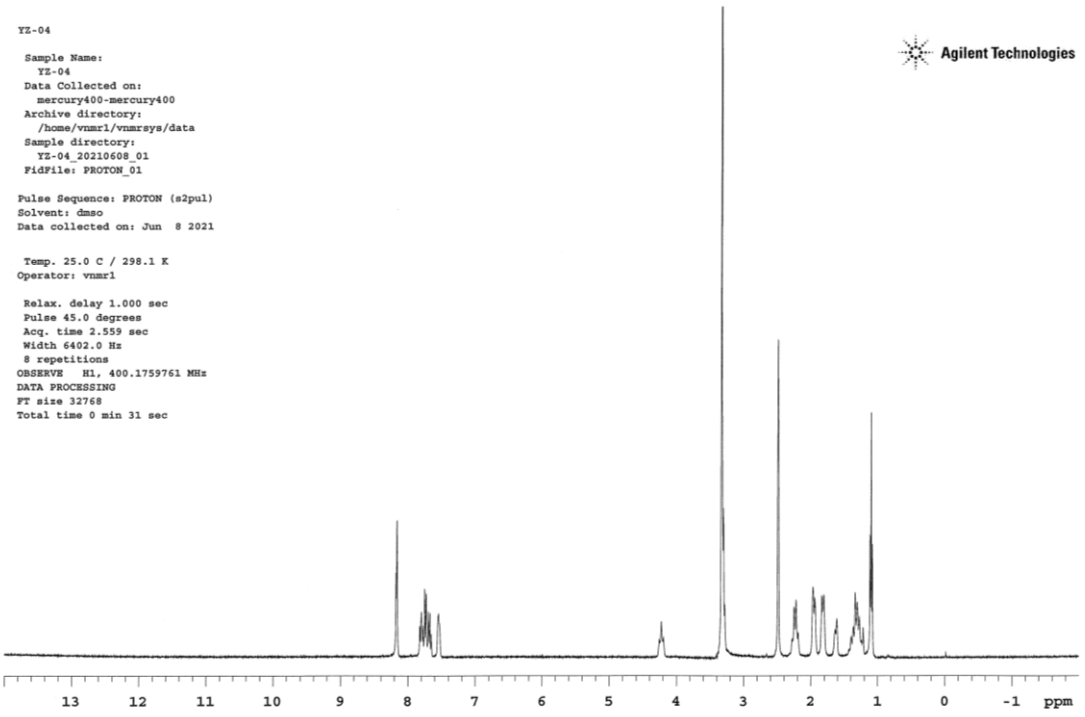
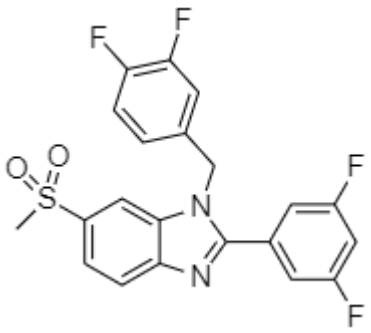


Figure 3.10. ^{1}H NMR spectrum of compound **26**

3.6.5. 1-(3,4-Difluorobenzyl)-2-(3,5-difluorophenyl)-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazole (27)



260.0 mg of N^1-(3,4-difluorobenzyl-4-(methylsulfonyl) benzene-1,2-diamine (0.83 mmol) and 205 mg of sodium (3,5-difluorophenyl) (hydroxy) methanesulfonate (0.83 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:2) solvent system. 115 mg of product was obtained in 24.7% yield.

Melting Point: 170.3 ºC.

Mass Spectrum: MS (ESI+) m/e: 435.65 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.24 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 6.73 (m, 1H), 7.08 (m, 1H), 7.31 (m, 2H), 7.52 (m, 1H), 7.72-7.86 (m, 3H), 8.28 (m, 1H).

Elementel Analysis: $C_{21}H_{14}F_4N_2O_2S$

	%**C**	%**H**	%**N**	%**S**
Calculated:	58.06	3.25	6.45	7.38
Found:	58.29	3.45	6.62	7.58

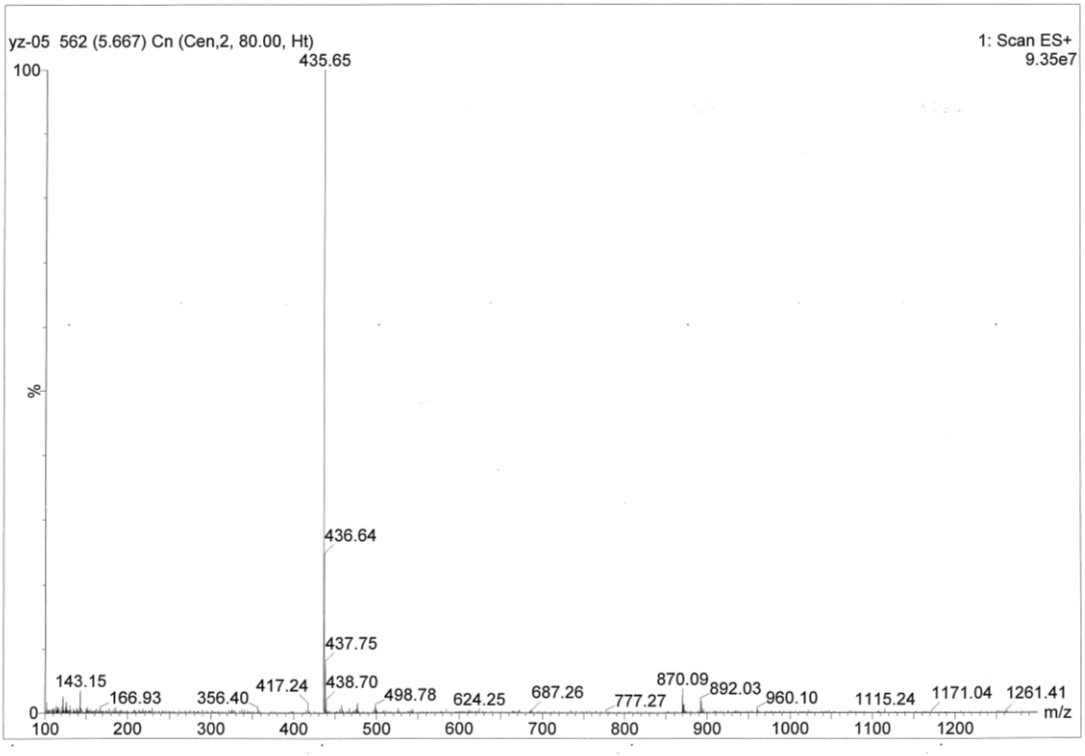


Figure 3.11. Mass spectrum of compound **27**

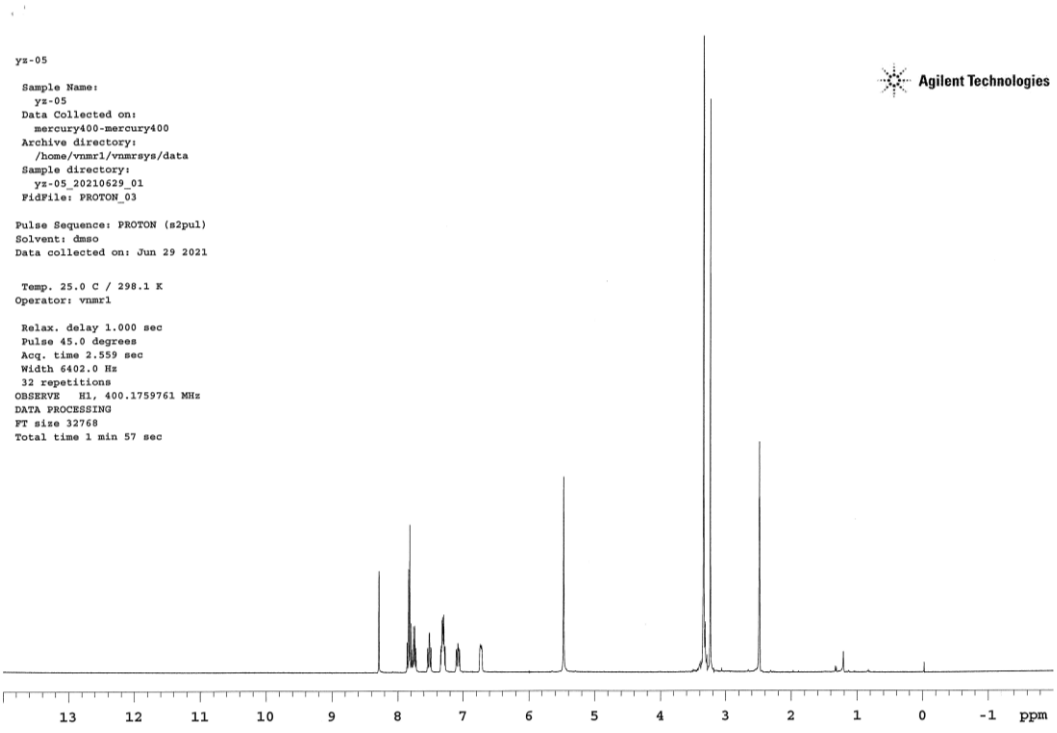
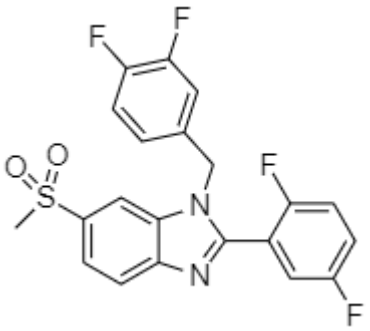


Figure 3.12. ^{1}H NMR spectrum of compound **27**

3.6.6. 1-(3,4-Difluorobenzyl)-2-(2,5-difluorophenyl)-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazol (28)



280.0 mg of N^1-(3,4-difluorobenzyl-4-(methylsulfonyl) benzene-1,2-diamine (0.90 mmol) and 205 mg of sodium (2,5-difluorophenyl) (hydroxy) methanesulfonate (0.90 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:2) solvent system. 50 mg of product was obtained in 10.4% yield.

Melting Point: 168.3 ^{o}C

Mass Spectrum: MS (ESI+) m/e: 435.63 (% 100) (M^{+}+H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.25 (s, 3H, CH_3), 5.51 (s, 2H, CH_2), 6.76 (m, 1H), 7.11 (m, 1H), 7.32 (m, 1H), 7.53 (m, 2H), 7.61 (m, 1H), 7.85 (m, 2H), 8.31 (m, 1H).

Elementel Analysis: $C_{21}H_{14}F_4N_2O_2S$

	%**C**	%**H**	%**N**	%**S**
Calculated:	58.06	3.25	6.45	7.38
Found:	57.79	3.29	6.58	7.36

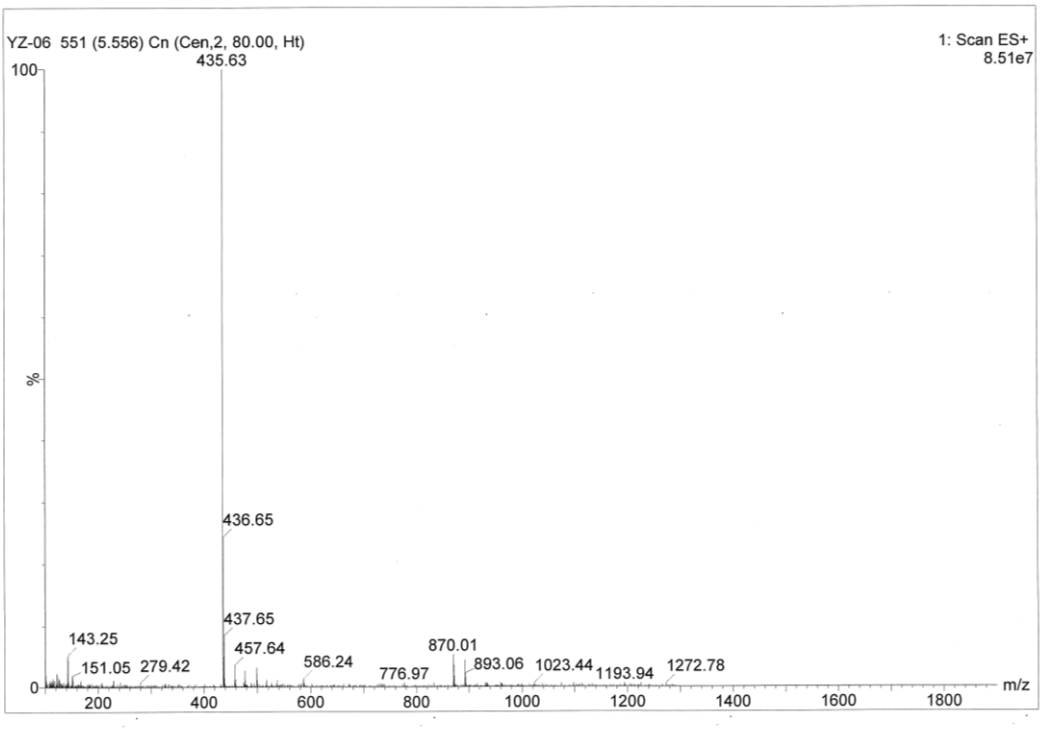


Figure 3.13. Mass Spectrum of compound **28**

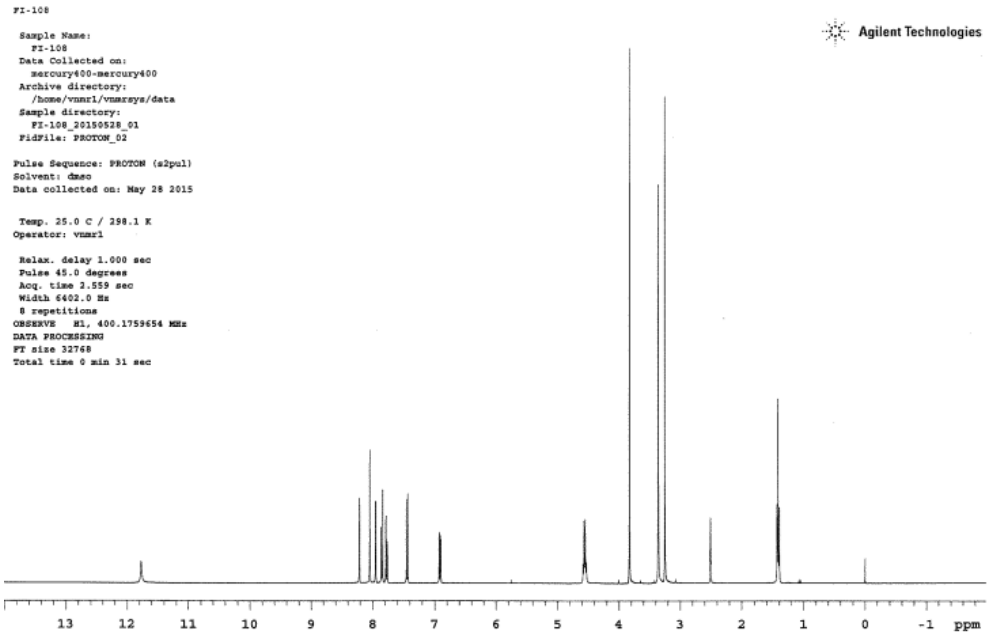
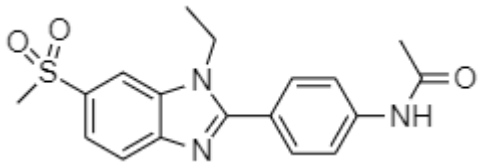


Figure 3.14. ^{1}H NMR spectrum of compound **28**

3.6.7. 4-(1-Ethyl-5-(methylsulfonyl)–1*H*-benzo[*d*]imidazol-2yl)phenyl)acetamide (29)



304.0 mg of N^1-ethyl-(methylsulfonyl) benzene-1,2-diamine (1.42 mmol) and 350.8 mg of sodium (4-acetamidophenyl) (hydroxy) methanesulfonate (1.42 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/isopropyl (5:1) solvent system. 121 mg of product was obtained in 18.5% yield.

Melting Point: 215.1 ºC.

Mass Spectrum: MS (ESI+) m/e: 358.58 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.35 (t, 3H), 2.25 (s, 3H), 3.35 (s, 3H), 4.42 (q, 2H), 7.80 (dd, *J*=8.4 Hz, *J*=2 Hz, 4H), 7.95 (d, *J*=8.4 Hz, 2H), 8.23 (s, 1H), 10.25 (s, 1H).

^{13}C NMR (DMSO-d_6) δ ppm 14.95, 24.18, 44.25, 111.62, 118.57, 118.84, 120.76, 123.77, 129.86, 134.56, 138.55, 141.12, 142.00, 155.47, 168.83.

Elementel Analysis: $C_{18}H_{19}N_3O_3S.\ 0{,}7H_2O$

	%**C**	%**H**	%**N**	%**S**
Calculated:	58.42	5.55	11.35	8.66
Found:	58.26	5.86	11.20	8.46

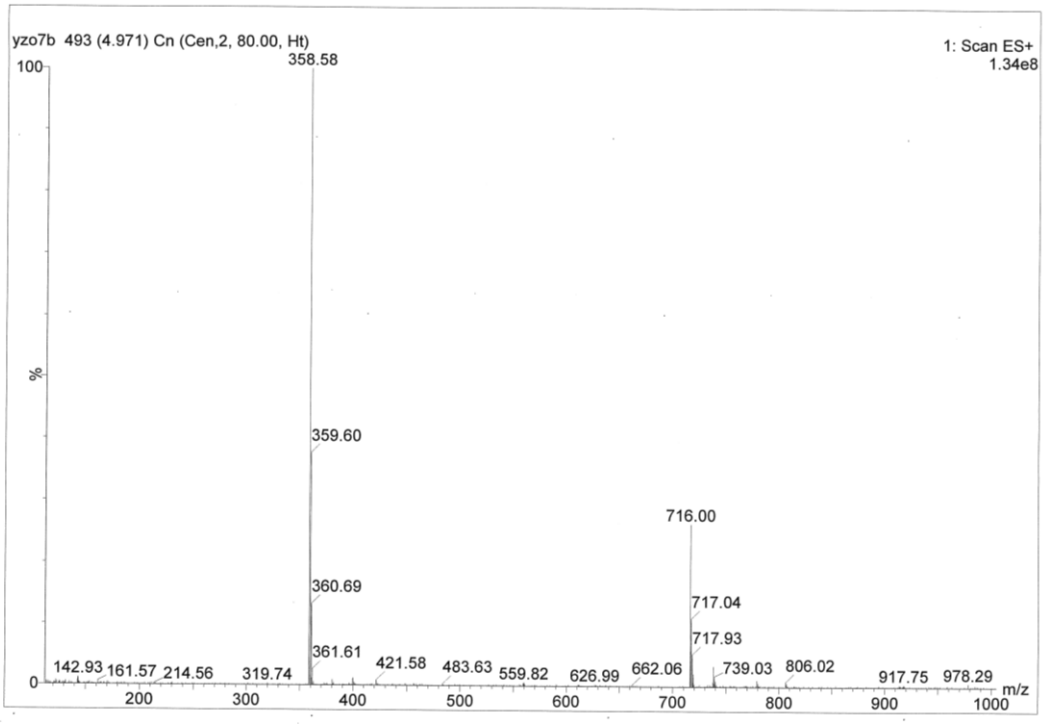


Figure 3.15. Mass spectrum of compound **29**

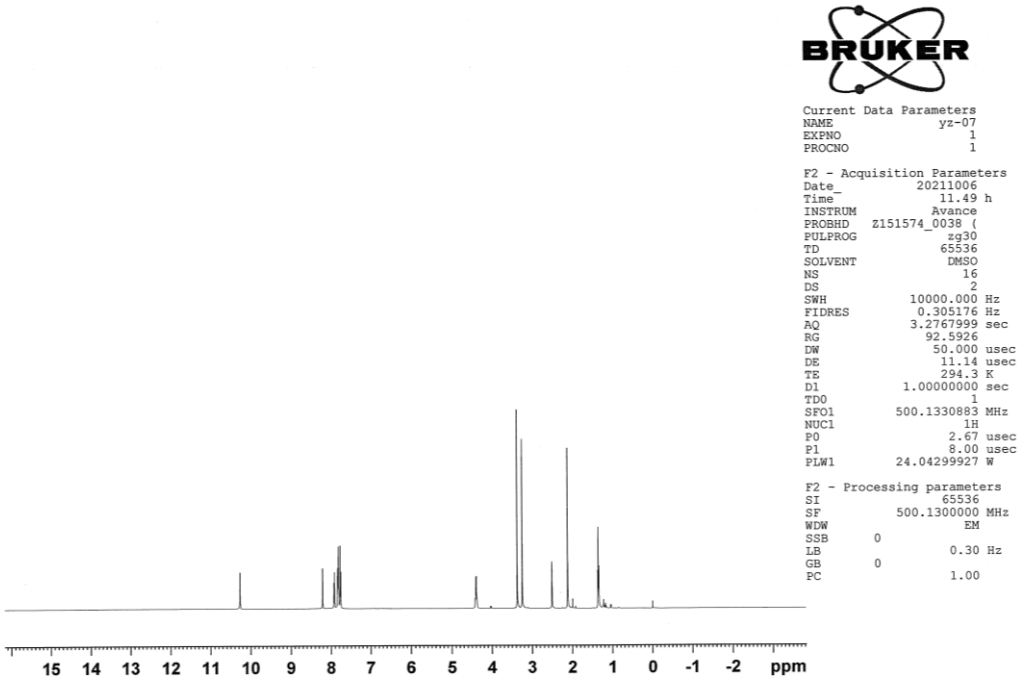


Figure 3.16. ^{1}H NMR spectrum of compound **29**

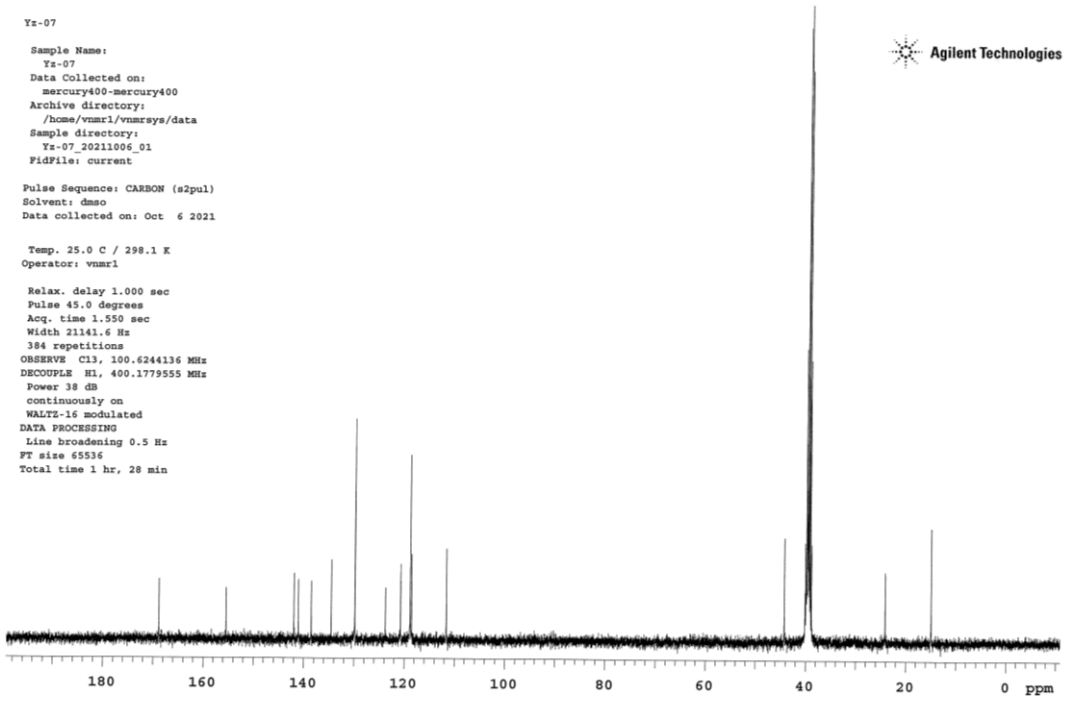
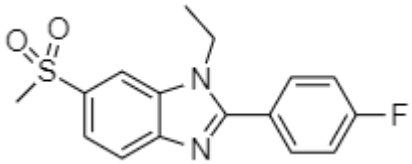


Figure 3.17. ^{13}C NMR spectrum of compound **29**

3.6.8. 1-Ethyl-2-(4-fluorophenyl)-5-(methylsulfonyl)–1*H*-benzo[*d*]imidazol (30)



296 mg of N^1-ethyl-4-(methylsulfonyl) benzene-1,2-diamine (1.38 mmol) and 289 mg of sodium (2,5-difluorophenyl) (hydroxy) methanesulfonate (1.38 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (4:1) solvent system. 48 mg of product was obtained in 8.5% yield.

Melting Point: 198.9 ^{o}C

Mass Spectrum: MS (ESI+) m/e: 319.59 (%100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.31 (t, 3H), 3.23 (s, 3H), 4.36 (q, 2H), 7.43 (t, 2H), 7.85 (m, 3H), 7.94 (d, *J*=8.8 Hz, 1H), 8.23 (s, 1H).

^{13}C NMR (DMSO-d_6) δ ppm 14.89, 44.22, 111.81, 115.93, 116.14, 118.78, 120.96, 126.08, 126.11, 131.65, 131.73, 134.72, 138.41, 141.87, 154.72, 161.06, 164.42.

Elementel Analysis: $C_{16}H_{15}FN_2O_2S$-0,08C_6H_{14}

	%**C**	%**H**	%**N**	%**S**
Calculated:	60.85	4.99	8.61	9.85
Found:	60.88	5.28	8.66	9.68

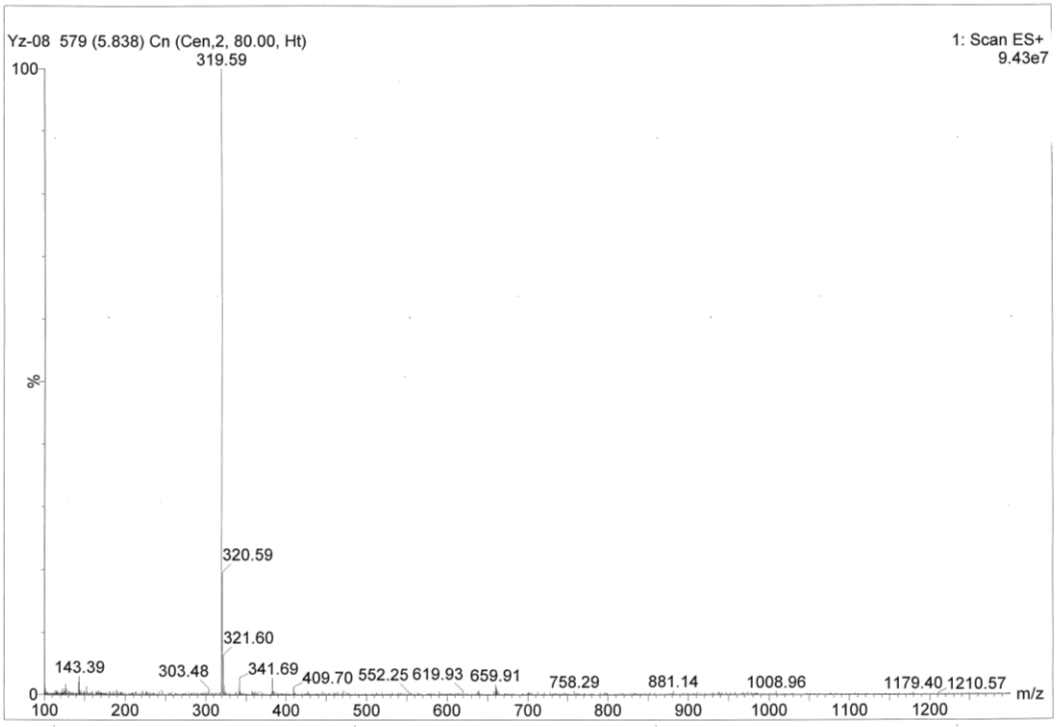


Figure 3.18. Mass spectrum of compound **30**

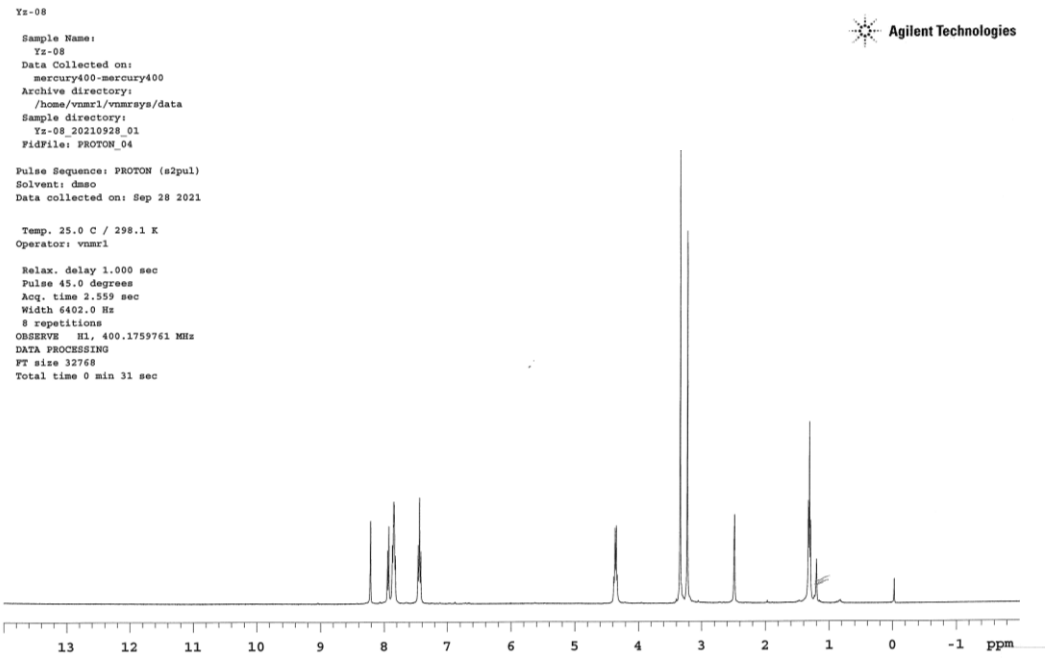


Figure 3.19. ^{1}H NMR spectrum of compound **30**

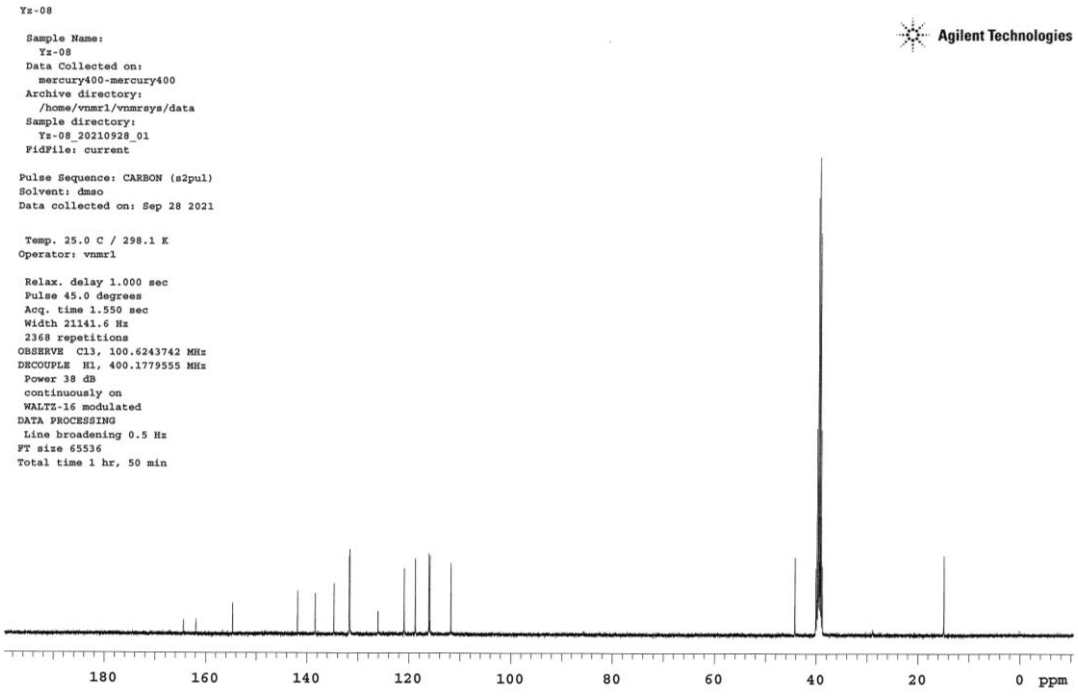
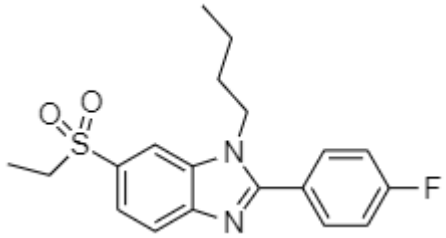


Figure 3.20. ^{13}C NMR spectrum of compound **30**

3.6.9. 1-Butyl-5-(ethylsulfonyl)-2-(4-fluorophenyl)–1*H*-benzo[*d*]imidazol (31)



446.1 mg of 4-(ethylsulfonyl)-N^1-propylbenzene-1,2-diamine (0.14 mmol) and 298.0 mg of sodium (4-fluorophenyl) (hydroxy) methanesulfonate (0.14 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:2) solvent system. 60.8 mg of product was obtained in 8.2% yield.

Melting Point: 146.1 ^{o}C.

Mass Spectrum: MS (ESI+) m/e: 361.65 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.87 (t, 3H), 1.15 (m, 4H), 1.65 (m, 2H), 3.26 (t, 3H), 4.54 (t, 2H), 7.47 (m, 2H), 7.80 (dd, *J*=8.4 Hz, *J*=2 Hz, 1H), 7.83 (m, 2H), 7.95 (d, *J*=8.8 Hz, 1H), 8.18 (s, 1H).

^{13}C NMR (DMSO-d_6) δ ppm 7.85, 13.72, 19.62, 31.56, 44.64, 50.15, 112.42, 116.39, 116.56, 120.08, 122.14, 126.73, 126.75, 132.17, 132.24, 132.61, 139.29, 142.29, 155.45, 162.62, 164.59.

Elementel Analysis: $C_{19}H_{21}FN_2O_2S$

	%C	**%H**	**%N**	**%S**
Calculated:	63.31	5.87	7.77	8.90
Found:	63.45	6.15	7.86	8.85

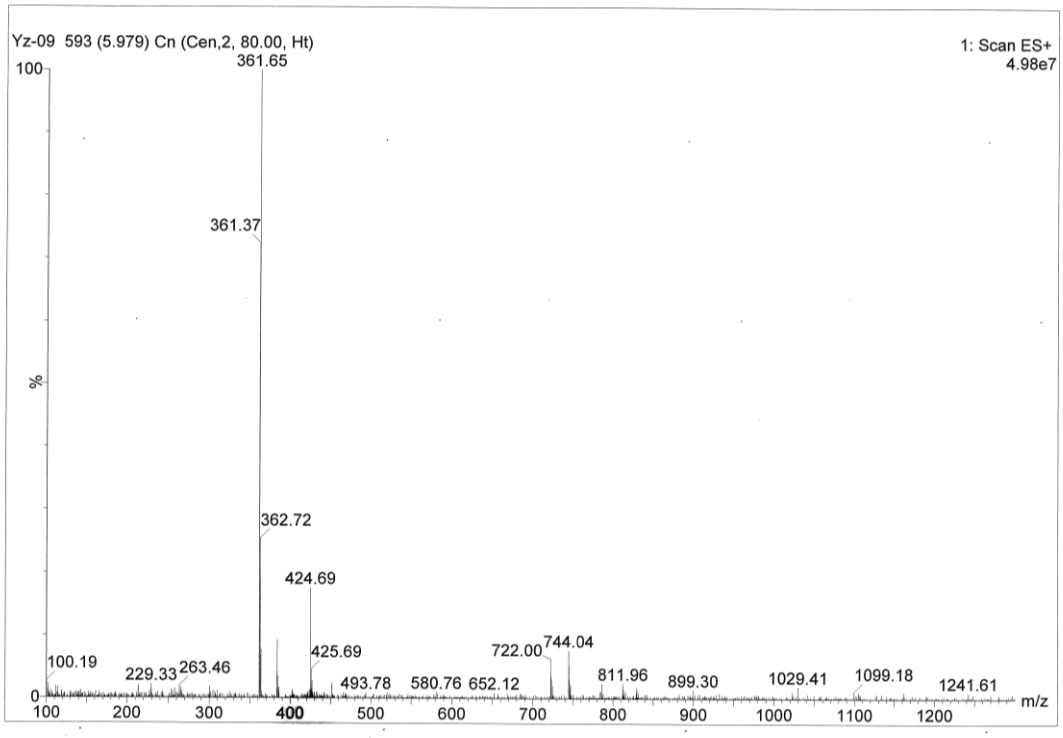


Figure 3.21. Mass spectrum of compound **31**

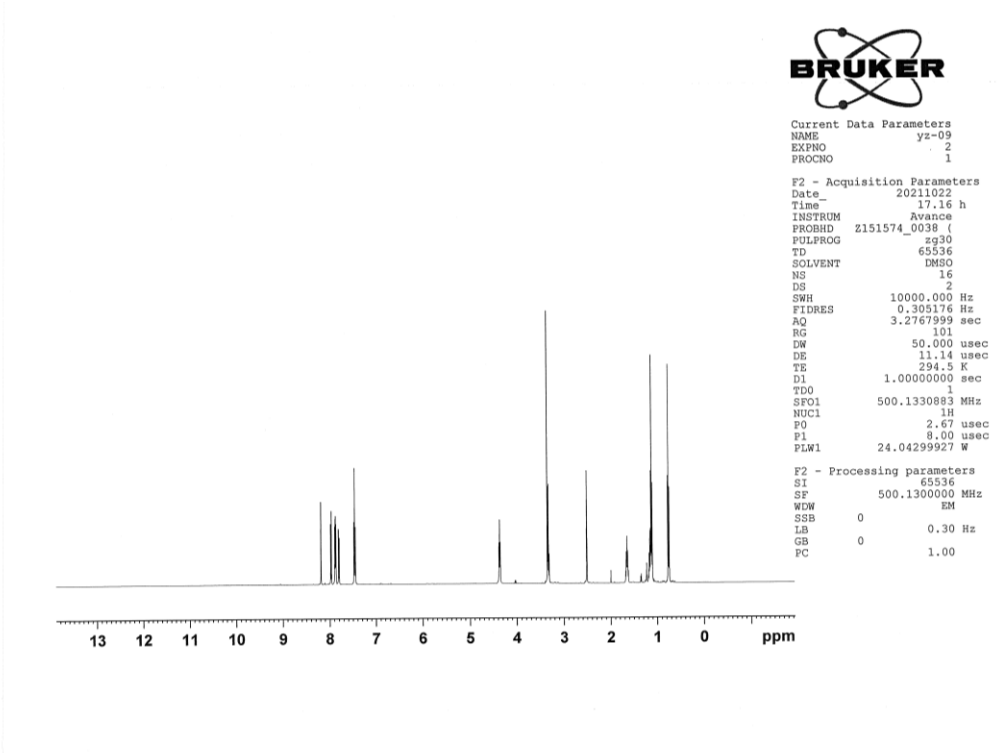


Figure 3.22. ^{1}H NMR spectrum of compound **31**

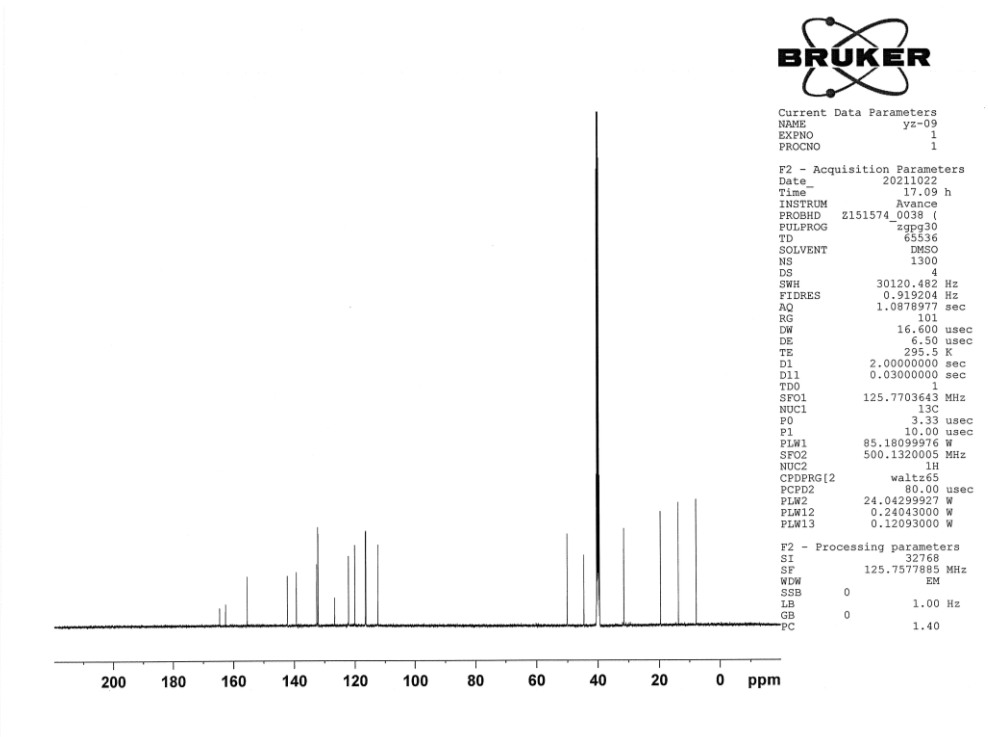
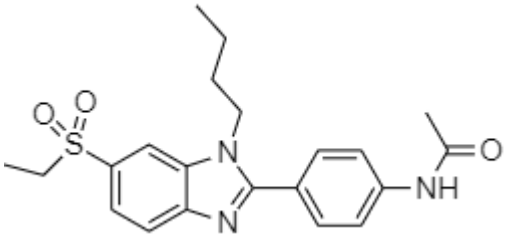


Figure 3.23. ^{13}C NMR spectrum of compound **31**

3.6.10. *N*-(4-(1-Butyl-5-(ethylsulfonyl)-1*H*-benzo[*d*]imidazol-2-yl) phenyl) acetamide (32)



473.1 mg of 4-(ethylsulfonyl)-N^1-butylbenzene-1,2-diamine (0.15 mmol) and 267.2 mg of sodium (4-acetamidophenyl) (hydroxy) methanesulfonate (0.15 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethylacetate/hexane (2:1), then 100% ethylacetate solvent system. 35.10 mg of product was obtained in 4.7 % yield.

Melting Point: 179.9 ^oC

Mass Spectrum: MS (ESI+) m/e: 400.66 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.89 (t, 3H), 1.15 (m, 4H), 1.64 (m, 2H), 2.11 (s, 3H), 3.31 (t, 3H), 4.39 (t, 2H), 7.78 (m, 5H), 7.95 (d, *J*=8.8 Hz, 1H), 8.17 (s, 1H), 10.25 (s, 1H).

^{13}C NMR (DMSO-d_6) δ ppm 7.85, 13.75, 19.65, 24.61, 31.57, 44.67, 50.17, 112.21, 119.24, 119.85, 121.91, 124.42, 130.33, 132.45, 139.42, 141.50, 142.42, 156.20, 169.25.

Elementel Analysis: $C_{21}H_{25}N_3O_3S$

	%**C**	%**H**	%**N**	%**S**
Calculated:	63.13	6.31	10.52	8.03
Found:	63.01	6.43	10.65	8.00

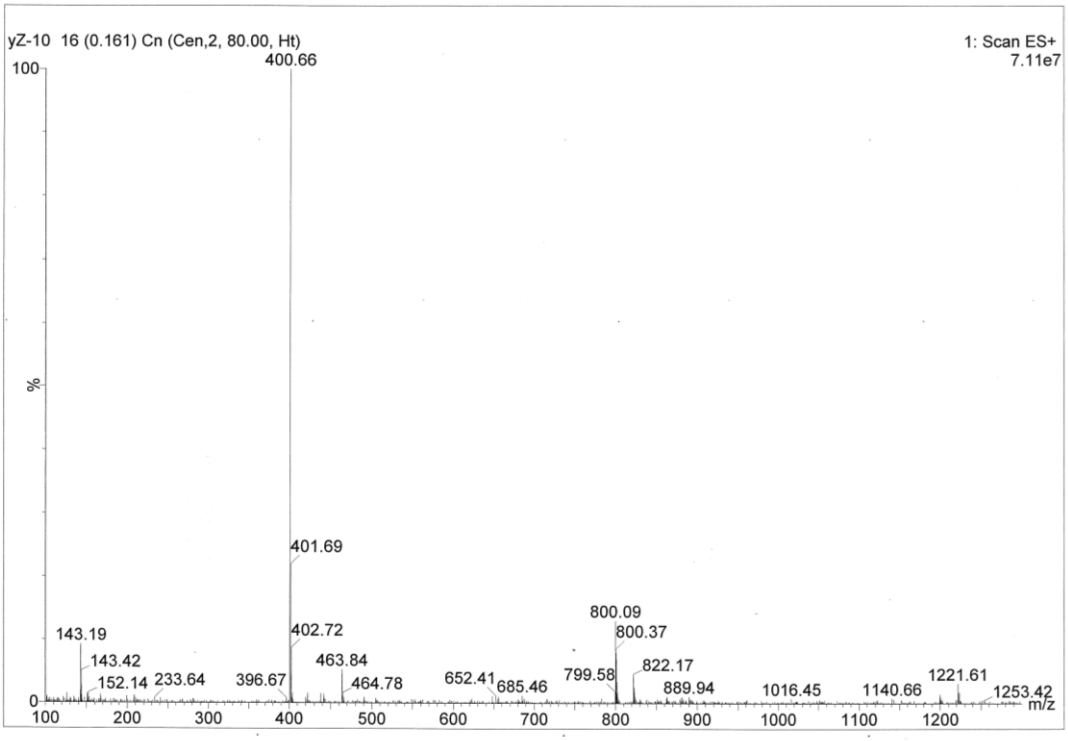


Figure 3.24. Mass spectrum of compound **32**

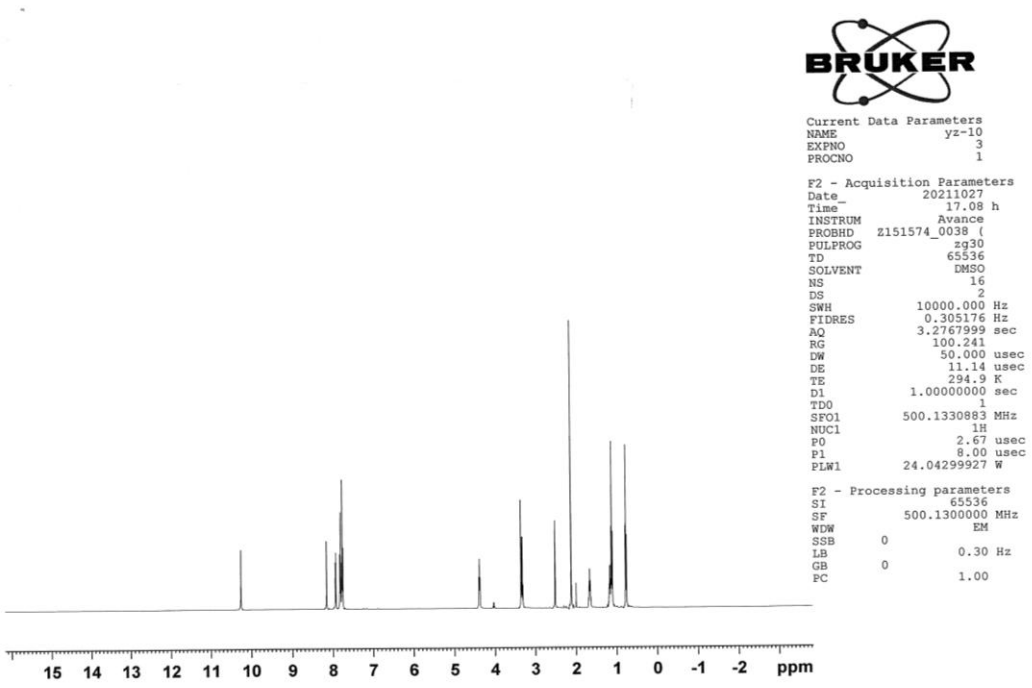


Figure 3.25. ^{1}H NMR spectrum of compound **32**

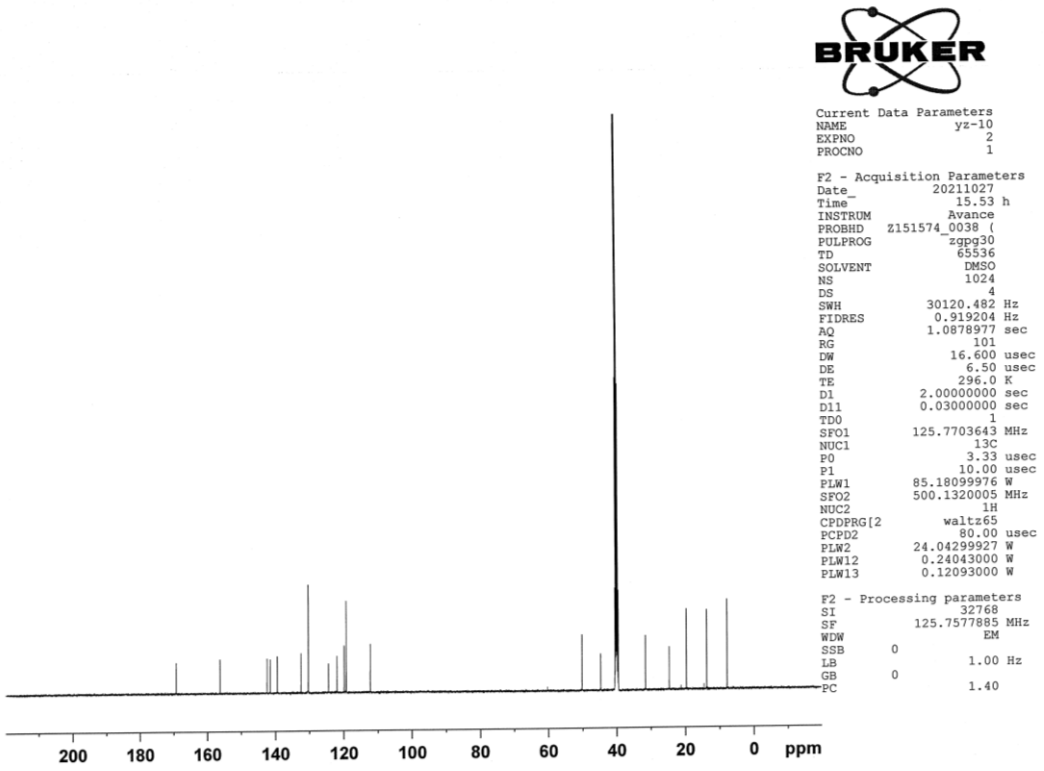
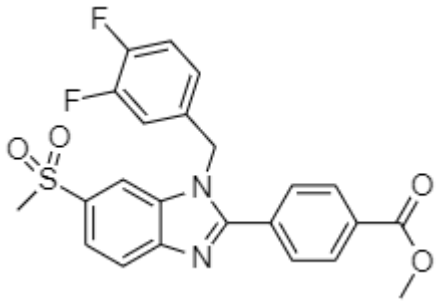


Figure 3.26 ^{13}C NMR spectrum of compound **32**

3.6.11. Methyl 4-(1-(3,4-difluorobenzyl)-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazol-2-yl) benzoate (33)



348.3 mg of N^1-(3,4-difluorobenzyl-4-(ethylsulfonyl) benzene-1,2-diamine (0.13 mmol) and 299 mg of sodium hydroxy (4-(methoxycarbonyl) phenyl) methanesulfonate (0.13 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:3) solvent system. 67.7 mg of product was obtained in 10.5% yield.

Melting Point: 408.7 ^oC

Mass Spectrum: MS (ESI+) m/e: 457.67 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.25 (s, 3H), 3.84 (s, 3H), 5.65 (s, 2H), 6.77 (m, 1H), 7.23 (m, 1H), 7.35 (m, 1H), 7.72 (m, 1H), 7.83 (m, 2H), 8.03 (m, 1H), 8.13 (m, 1H), 8.22 (m, 1H), 8.30 (m, 1H).

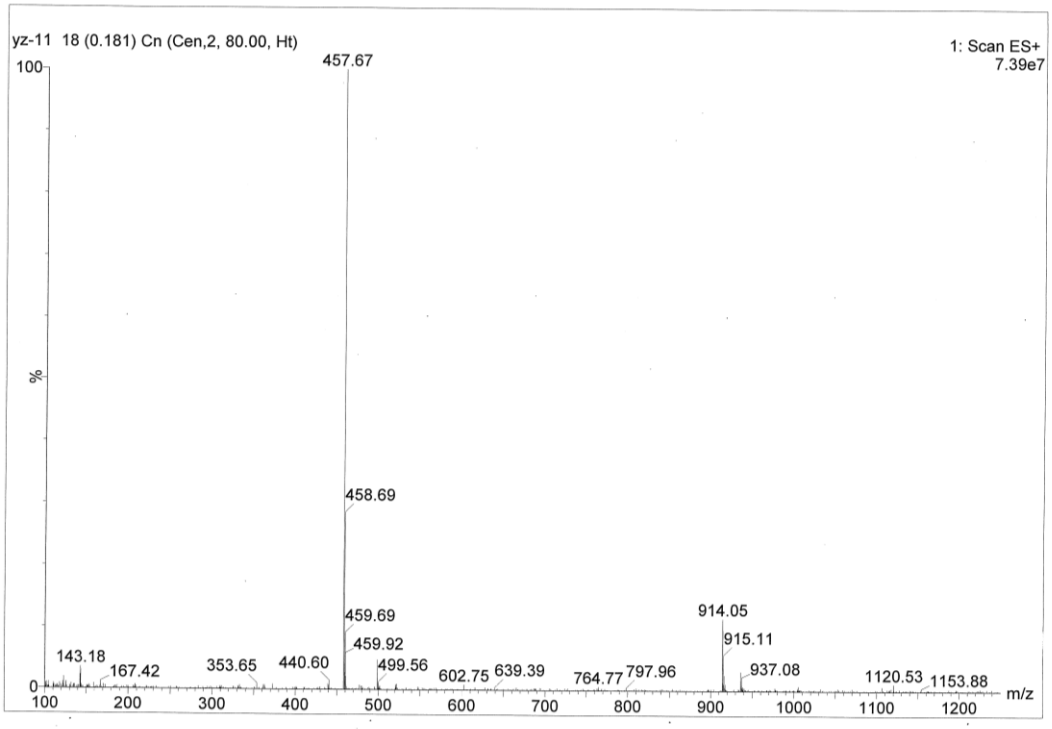


Figure 3.27. Mass spectrum of compound **33**

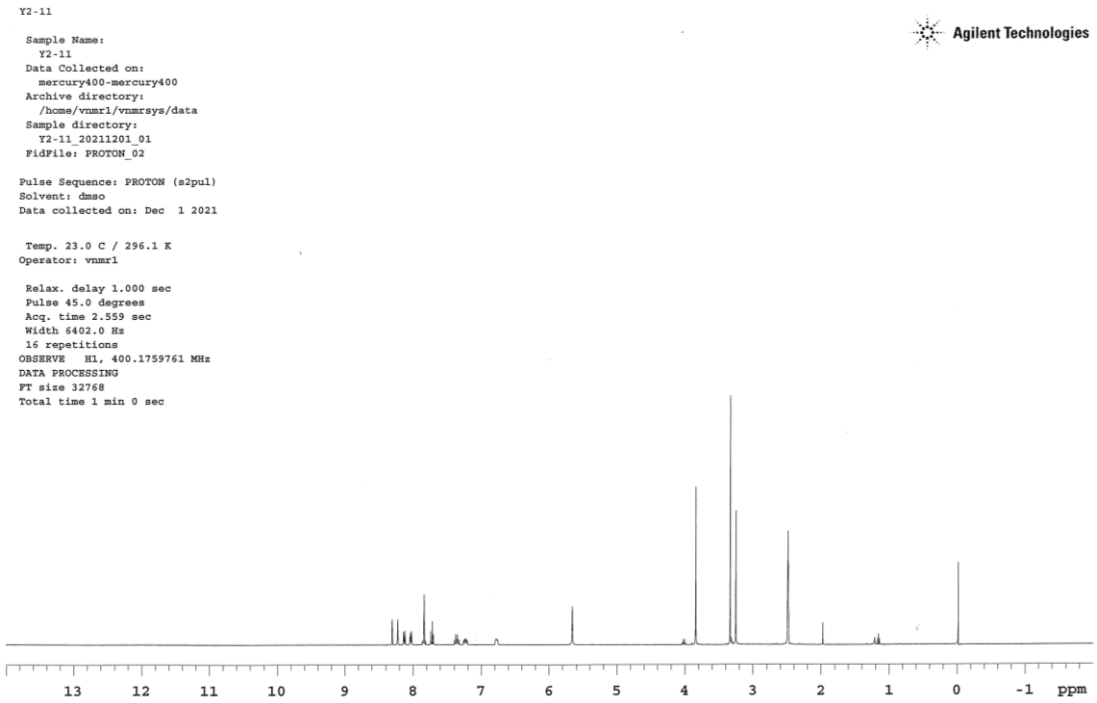
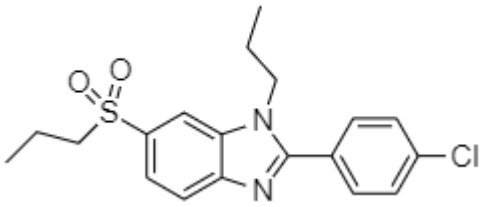


Figure 3.28. ^{1}H NMR spectrum of compound **33**

3.6.12. 2-(4-Chlorophenyl)-1-propyl-5-(propylsulfonyl)–1*H*-benzo[*d*]imidazole (34)



436.6 mg of N^1-propyl-4-(propylsulfonyl) benzene-1,2-diamine (0.15 mmol) and 373.0 mg of sodium (4-chlorophenyl) (hydroxy) methanesulfonate (0.15 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:2) solvent system. 21.67 mg of product was obtained in 2.7% yield.

Melting Point: 172.8 ºC

Mass Spectrum: MS (ESI+) m/e: 377.63 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.74 (t, 3H), 0.91 (t, 3H), 1.57 (m, 2H), 1.68 (m, 2H), 3.31 (m, 2H), 4.34 (t, 2H), 7.68 (m, 2H), 7.79-7.85 (m, 3H), 7.98 (d, *J*=6.8 Hz, 1H), 8.18 (d, *J*=1.1Hz, 1H).

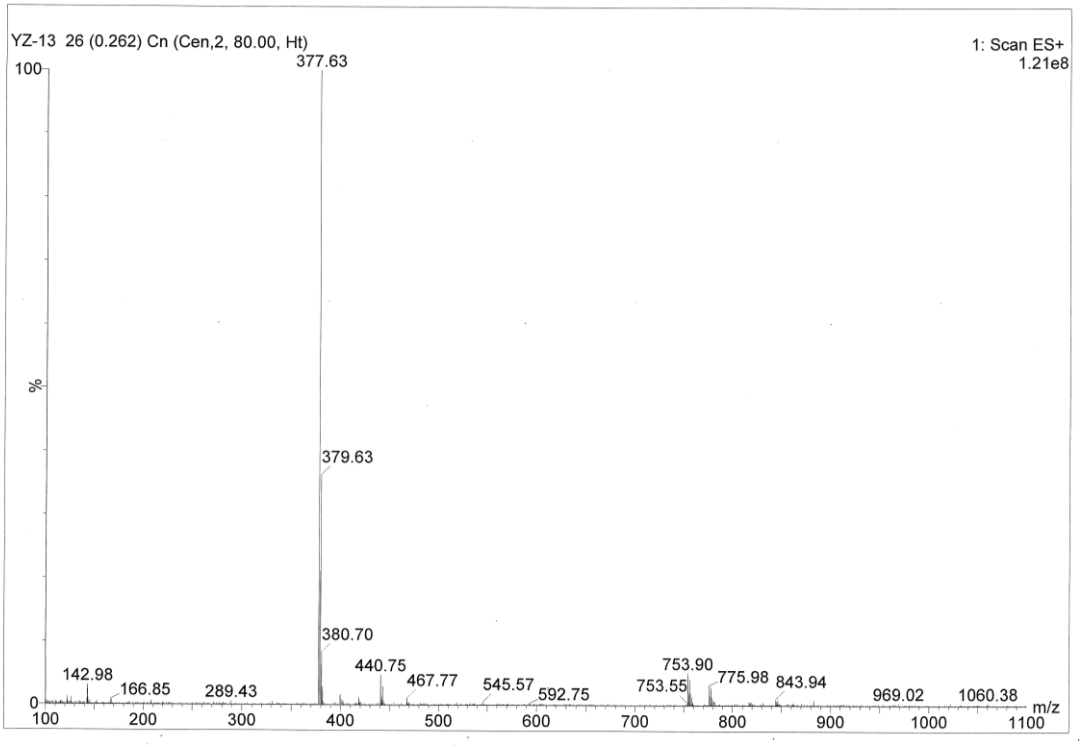


Figure 3.29. Mass spectrum of compound **34**

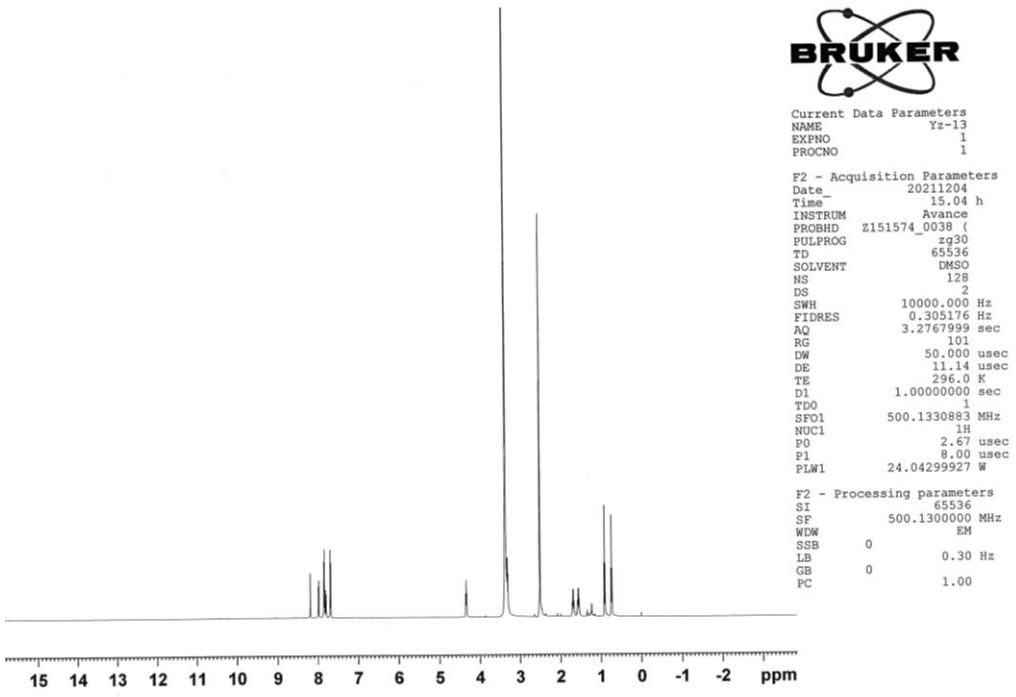
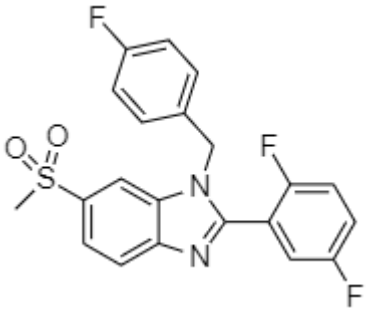


Figure 3.30. ^{1}H NMR spectrum of compound **34**

3.6.13. 2-(2,5-Difluorophenyl)-1-(4-fluorobenzyl)-5-(methylsulfonyl)–1*H*-benzo[*d*]imidazole (35)



199.4 mg of N^1-(4-fluorobenzyl)-4-(methylsulfonyl) benzene-1,2-diamine (0.64 mmol) and 170.6 mg of sodium (2,5-difluorophenyl) (hydroxy) methanesulfonate (0.64 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethylacetate/hexane (1:10) solvent system. 82.3 mg of product was obtained in 22.2% yield.

Melting Point: 236.1 ^{o}C.

Mass Spectrum: MS (ESI+) m/e: 417.43 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.24 (s, 3H), 5.47 (s, 2H), 6.98 (m, 2H), 7.08 (m, 2H), 7.31 (m, 1H), 7.52 (m, 1H), 7.74 (m, 1H), 7.82 (m, 2H), 8.27 (m, 1H).

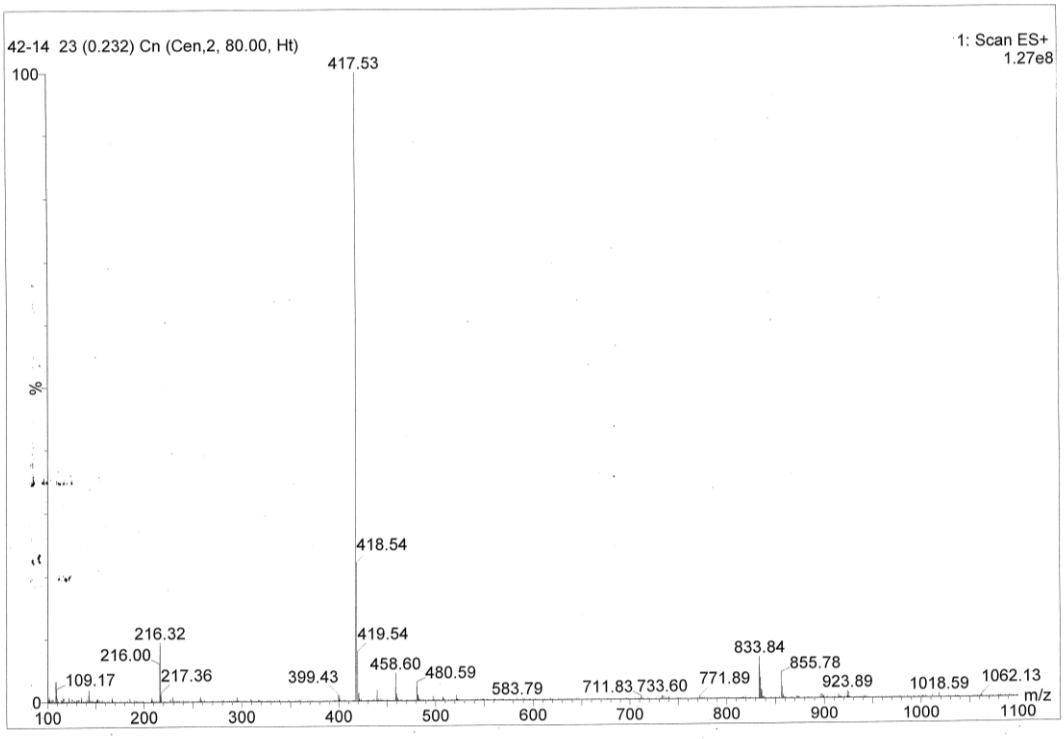


Figure 3.31. Mass Spectrum of compound **35**

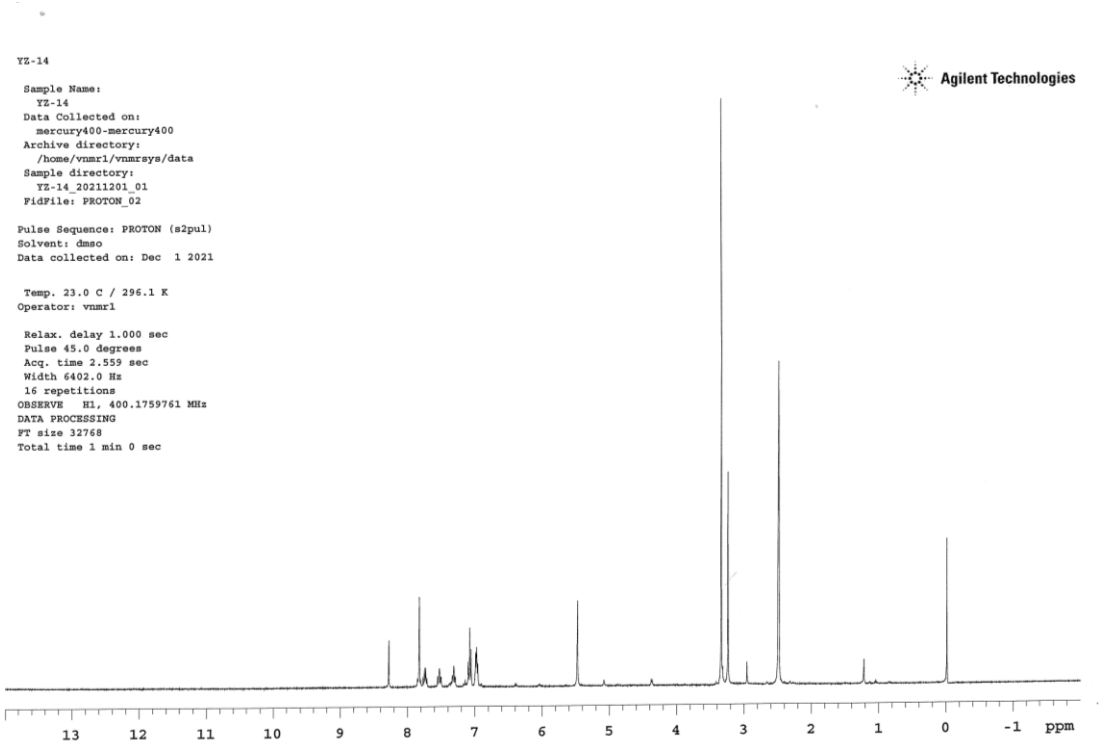
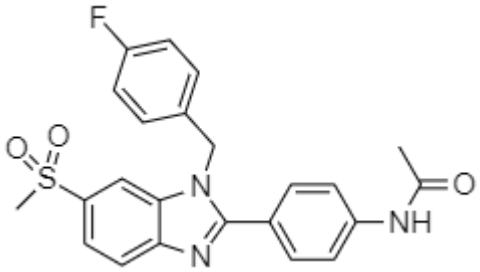


Figure 3.32. ^{1}H NMR spectrum of compound **35**

3.6.14. *N*-(4-(1-(4-fluorobenzyl)-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazol-2-yl) phenyl) acetamide (36)



271.5 mg of N^1-(4-fluorobenzyl)-4-(methylsulfonyl) benzene-1,2-diamine (0.86 mmol) and 179.9 mg of sodium (4-acetamidophenyl)hydroxymethane sulfonate (0.86 mmol) were synthesized from the starting materials. The resulting product was purified by column chromatography using an ethyl acetate/hexane (1:5) solvent system. 80.1 mg of product was obtained in 17.7% yield.

Melting Point: 165.7 ºC.

Mass Spectrum: MS (ESI+) m/e: 438.58 (% 100) (M^++H)

^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.09 (s, 3H), 3.24 (s, 3H), 5.67 (s, 2H), 7.03-7.14 (m, 4H), 7.70-7.81 (m, 6H), 8.25 (m, 1H), 10.15 (s, 1H). **^{13}C NMR** (DMSO-d_6): 24.31, 44.62, 47.59, 112.35, 116.09, 116.27, 119.15, 119.24, 121.56, 123.94, 128.77, 128.83, 130.30, 133.07, 133.093 135.42, 139.46, 141.68, 142.48, 156.36, 160.95, 162.89, 169.25.

Elementel Analysis: $C_{24}H_{20}ClN_3O_3S$-0.75 CH_3OH

	%C	%H	%N	%S
Calculated:	60.66	4.73	8.57	6.54
Found:	60.84	5.30	8.89	6.56

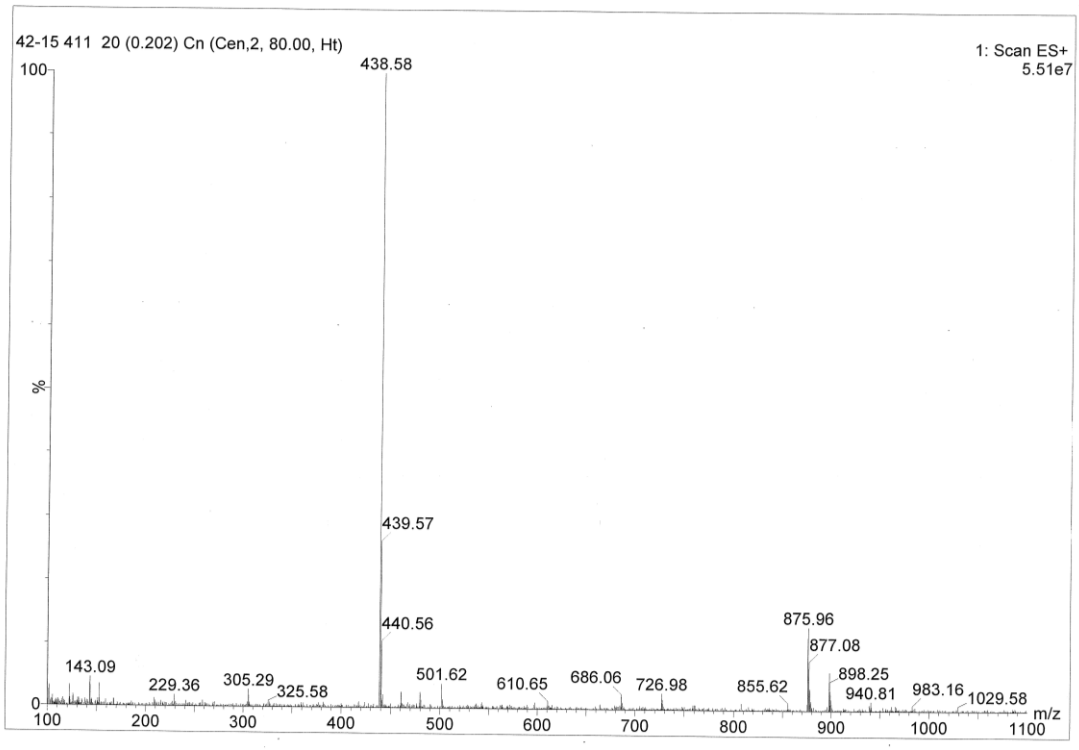


Figure 3.33. Mass spectrum of compound **36**

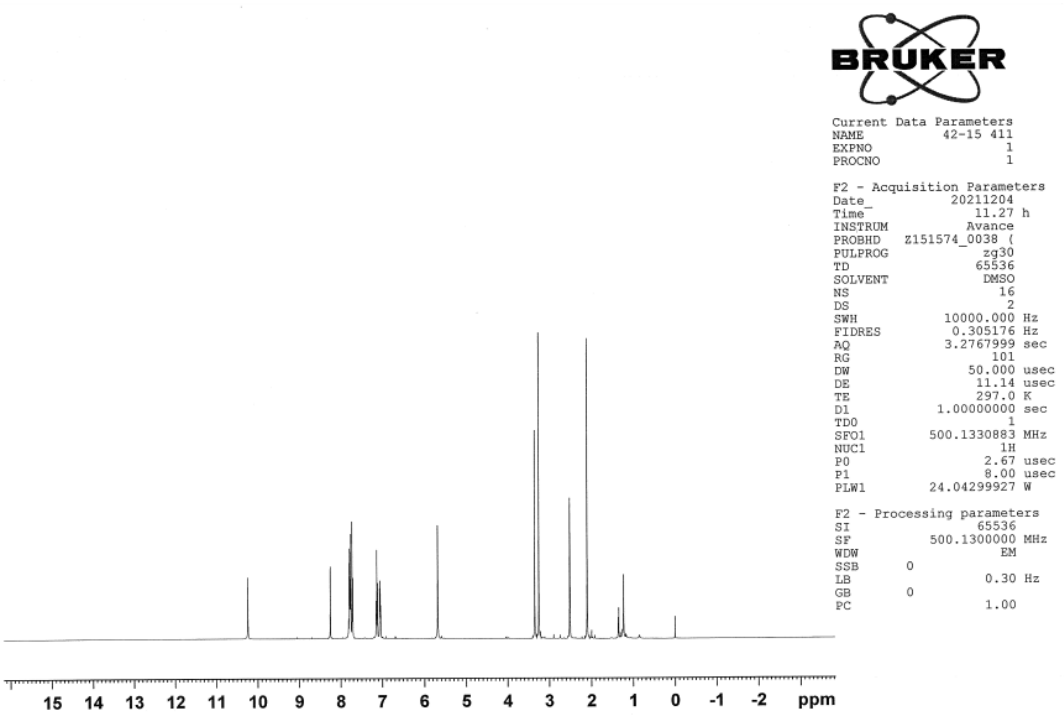


Figure 3.34. ^{1}H NMR spectrum of compound **36**

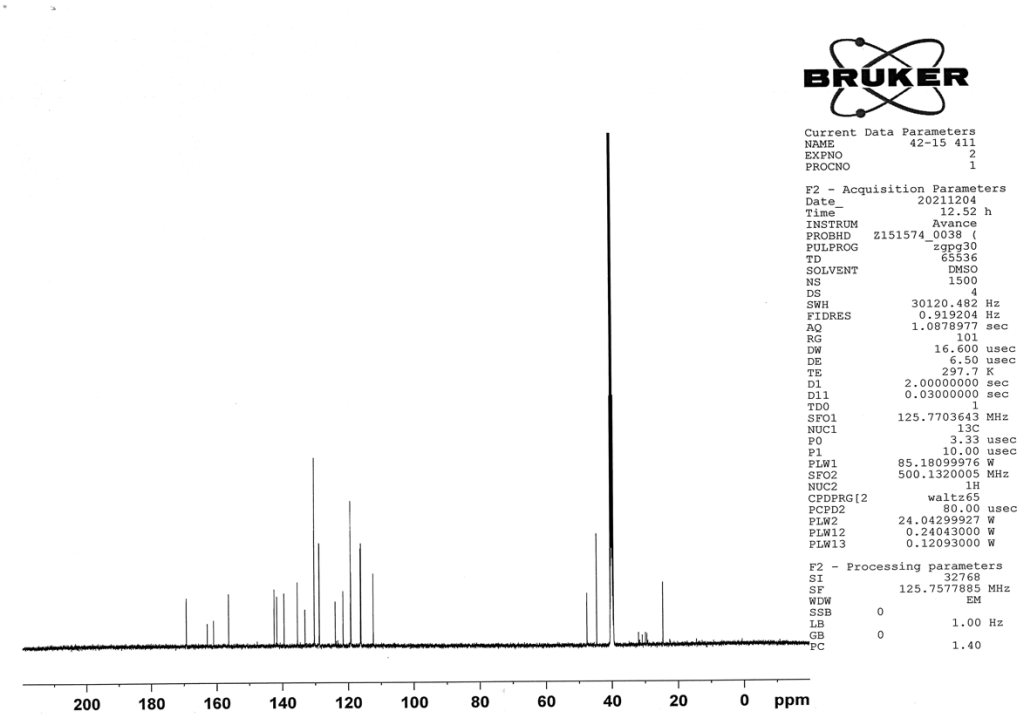


Figure 3.35. ^{13}C NMR spectrum of compound **36**

3.7. Detection of Antimicrobial Activities of Synthesized Compounds

In this thesis study, the antibacterial activity properties of 2-phenylbenzimidazole derivative compounds (23-37) containing different substituents were determined by microdilution method against to *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Methicillin resistant S. aureus (MRSA)* and *Enterococcus faecalis* ATCC 29212 tested on strains. The lowest concentration at which growth was inhibited after incubation was determined as the Minimum Inhibitory Concentration (MIC) (µg/mL). MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. A lower MIC value indicates that less drug is required for inhibiting growth of the organism; therefore, drugs with lower MIC scores are more effective antimicrobial agents. The MIC value of benzimidazole derivatives was determined by the broth microdilution method, was determined by inoculation on the sensitest agar surface. The results obtained are given in Table 3.1.

Table 3.1. Antibacterial activity results of synthesized compounds **23-36** (µg/mL)

Compounds	*Esherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213	Methicillin resistant *S.aureus* ATCC 43300	*Enterococcus faecalis ATCC* 29212
23	50	50	50	50	**25**
24	50	50	50	50	50
25	50	50	**25**	**25**	**25**
26	50	50	50	50	**12.5**
27	50	50	50	**25**	**25**
28	50	50	50	50	50
29	50	50	50	50	50
30	50	50	50	50	50
31	50	50	50	50	50
32	50	50	50	50	50
33	50	50	50	50	25
34	50	50	50	50	**25**
35	50	50	50	**25**	**12.5**
36	50	50	50	50	50
DMSO	50	50	50	50	50
Ciprofloxacin	0.005	0.19	0.39	0.78	0.78

3.8. Anti-cancer activity on MCF-7 cell line

3.8.1. Cytotoxicity Analysis

The propagation of breast cancer model MCF-7 cells was performed in RPMI 1640 medium containing 10% FBS, 1.5% L-glutamine and 0.1 mg/mL gentamicin, at 37°C and in a 5% CO_2 incubator.

A series of experiments were carried out to determine the cytotoxic effect of the compounds to be applied to MCF-7 cells. Vincristine (3 μM), known to be a tubulin inhibitor, was used as a positive control. Based on vincristine IC50 values, low-dose and high-dose values were selected for each of the synthesized compounds, and their toxic effects were evaluated in MCF-7 cells. Drugs were tested in 2 doses as 100 μM and 10 μM as pre-screening (Table 3.2).

The xCELLigence device was used for cytotoxicity analysis. Real-time cell viability analyzes were performed on 16-well plates specially designed for the device. The xCELLigence® (Real Time Cell Analyzer, RTCA) device is a cell-based microelectronic biosensor system that measures the ionic concentration in each well with electrical impedance technology and whether the cells are connected to the electrodes. It is a system used for detecting cell proliferation and morphology change without using markers, making automatic measurements with real-time monitoring without requiring physiological contact, and imaging with high sensitivity and accuracy. The center of the system is the microelectronic cell sensor array integrated at the bottom of the custom-made well plates. This sensor; Measuring the electronic impedance of the electrodes allows detecting and monitoring changes on the electrodes. Cell index expressed without units (cell index; CI); It is used to measure the relative change in electrical impedance indicating the cell state.

Table 3.2. Effective dose range of the 14 synthesized compounds at low and high doses on the MCF-7 cell line with respect to vincristine.

Compounds	10 µM	100 µM	Toxicity range (µM)	Selected Compounds
Vincristine	toxic	toxic	3	Positive control
23	toxic	toxic	1-10	Most toxic*
24	toxic	toxic	10-60	
25	toxic	toxic	10-60	
26	Nontoxic	toxic	20-80	
27	toxic	toxic	5-40	
28	toxic	toxic	5-40	
29	Nontoxic	toxic	20-80	
30	toxic	toxic	5-40	
31	toxic	toxic	5-40	
32	Nontoxic	toxic	20-80	
33	Nontoxic	toxic	20-80	
34	Nontoxic	toxic	20-80	
35	toxic	toxic	5-40	
36	toxic	toxic	1-50	Second most toxic**

3.9. Docking results

In this study, interacting residues of *S.aureus* DNA GyrB subunit were designated as H-bonds with various polar residues such as Asp81, Arg81, Arg144 , Pro87, Ile86, Asn54, Ile175, Ile51, Thr173, Glu58 (Sherer *et al.* 2011). Standard for antibacterial activity (Ciprofloxacin) was re-docked with 3ttz for obtaining corresponding interaction pattern given in **Figure 3.36.C.** Then, benzimidazole derivatives were subjected to above-mentioned docking process to eventually witness perfect alignment in the binding domain of 3ttz (**Figure 3.36.A, Figure 3.36.B**). Binding pattern of Ciprofloxacin included a plethora of interactions such as H-bonds with Ser55, Thr173, Arg84 and halogen interactions with Gly85, Glu58. In addition to these last two aminoacids, Ile86 and Ile102 were bonded to quinoline and cyclopropyl

moieties through hydrophobic interactions. In the interaction pattern for **25**, the important reference residues were existent (**Figure 3.36.D**). Benzimidazole and phenyl rings create interactions with literature amino acids such as Pro87, Glu58, Ile86, and Asn54. H-bond interaction with Arg144 occurs by the acceptor capacity of sulfonyl group in the structure.

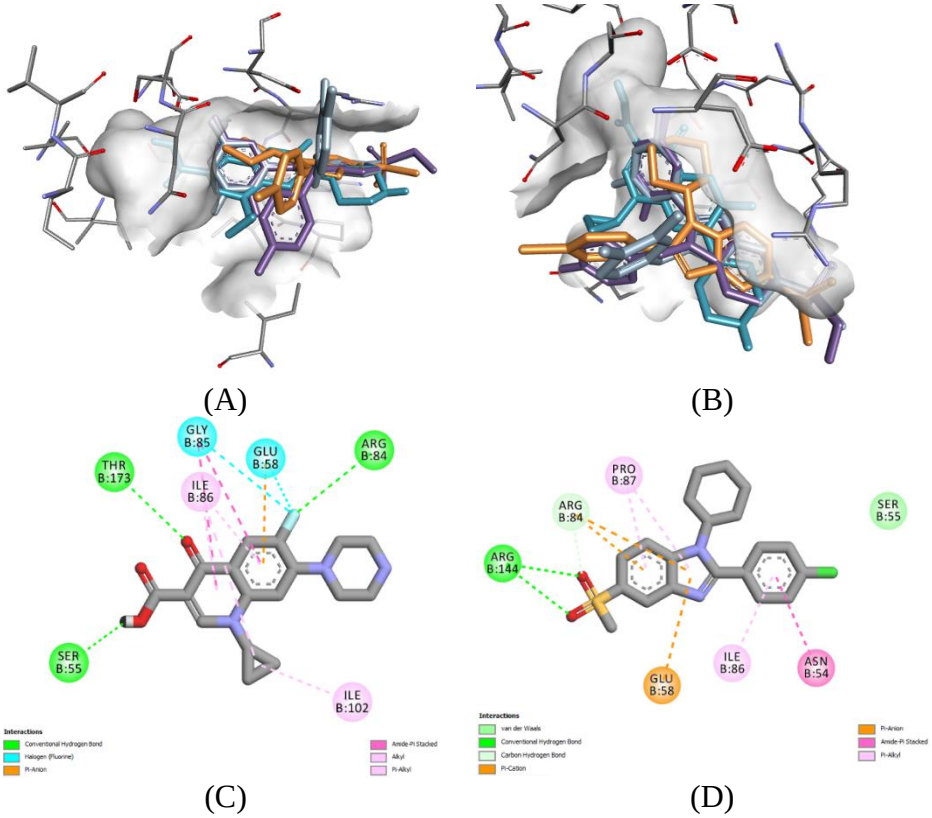


Figure 3.36. Docked conformers (A, B) of **Ciprofloxacin (turquoise)**, **25 (orange)**, **27 (light blue)**, **35 (magenta)** and interaction patterns of **Ciprofloxacin** (C) and **25** (D) with *S.aureus* DNA GyrB subunit (3ttz).

Activity results for various strains of bacteria gave us leads also for *E.faecalis*. Therefore, this outcome prompted us to investigate the binding conformers and interaction patterns with another vital bacterial enzyme *E.faecalis* DNA GyrB subunit (PDB ID: 4k4o). The docked conformers were given in **Figure 3.37.A** and **3.37.B**, with two different angles. H-bond with Adenine-binding Asp residue is important according to the reference study (Tari *et al.* 2013). Ciprofloxacin was able to afford the interaction with Asp75 through mobile hydrogen of -COOH. Moreover, there are several other interactions such as H-bonds with Ile80 and Thr167. Fluorine atom attached to quinoline gives halogen interactions with Arg78 and Gly79. Quinoline itself interacts with Glu52, Ile80, and Pro81 through hydrophobic interactions (**Figure 3.37.C**). Compound **26** stabilized with lesser number of residues such as Val96, Ala55, Ile80, and Glu52. This time, common residue Glu52 was an actor in halogen bond interaction (**Figure 3.37.D**). In addition to common hydrophobic interactions, **35** gave halogen bond interactions with Arg78, and Gly79 through *p*-fluoro substituent (**Figure**

3.37.E). All things considered, halogen incorporation is an important feat for the increased interactions with DNA-gyrase. Benzimidazole ring proved to be useful in mimicking the quinoline ring of Ciprofloxacin against DNA-gyrase for both bacteria strains.

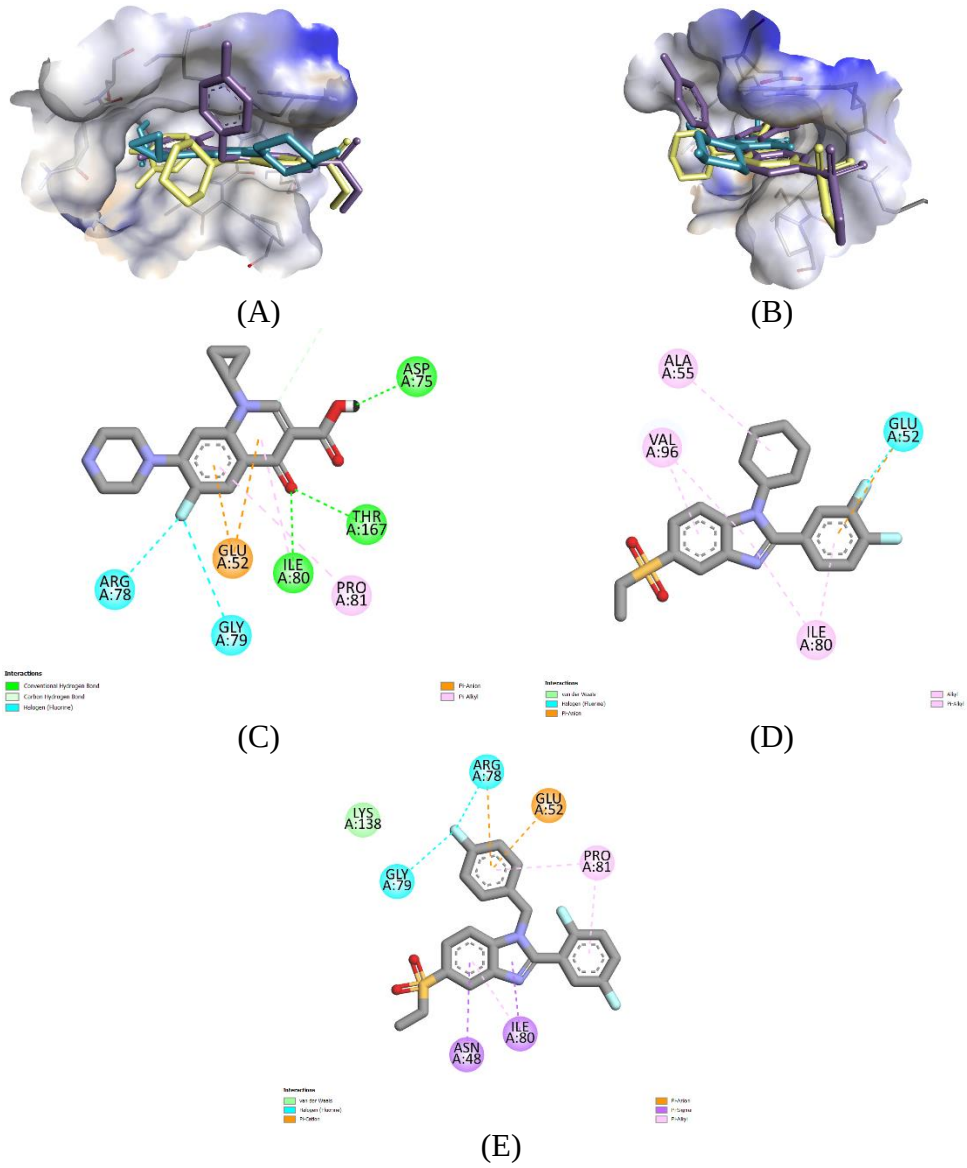


Figure 3.37. Docked conformers (A, B) of **Ciprofloxacin (turquoise)**, **26 (yellow)**, **35 (magenta)** and interaction patterns of **Ciprofloxacin** (C), **26** (D), and **35** (E) with *E. faecalis* DNA GyrB subunit (4k4o).

Molecular docking pipeline was also implemented for benzimidazoles and **Vincristine** and **Etoposide**, against Human DNA Topoisomerase II enzyme. According to reference study, **Etoposide** binds to Topoisomerase-DNA site and interacts with DG13, DT9, DG10, DC8, and amino acids Met766, Arg487, Asp463, and Gly462 (Wang *et al.* 2017). Unfortunately, **Vincristine** was not able to bind to this enzyme. Hence, **Etoposide** was taken as reference in this study since it possesses better docking scores and interactions. Compounds **36** (second most toxic) and **23** (most toxic) were found to be the most potent compounds on MCF-7 cell line. In **Figure 3.38.A.** and **B.**, the residence of ligands at the binding site can be seen. Etoposide offered several interactions with DNA bases DC8, DT9, DG10, DG13, and amino acids Met766, Arg487, Asp463, Gly462 (**Figure 3.38.C.**). For **36** and **23**, amide and sulfonyl groups played crucial roles in H-bond interactions with DNA bases. On the other hand, benzimidazole and phenyl rings were important for both of their Pi interactions. This knowledge on their interacting parts gave us an insight on their preliminary SAR interpretations and in the future, compounds having methylsulfonyl and amide moieties to achieve higher activities and better docking profiles.

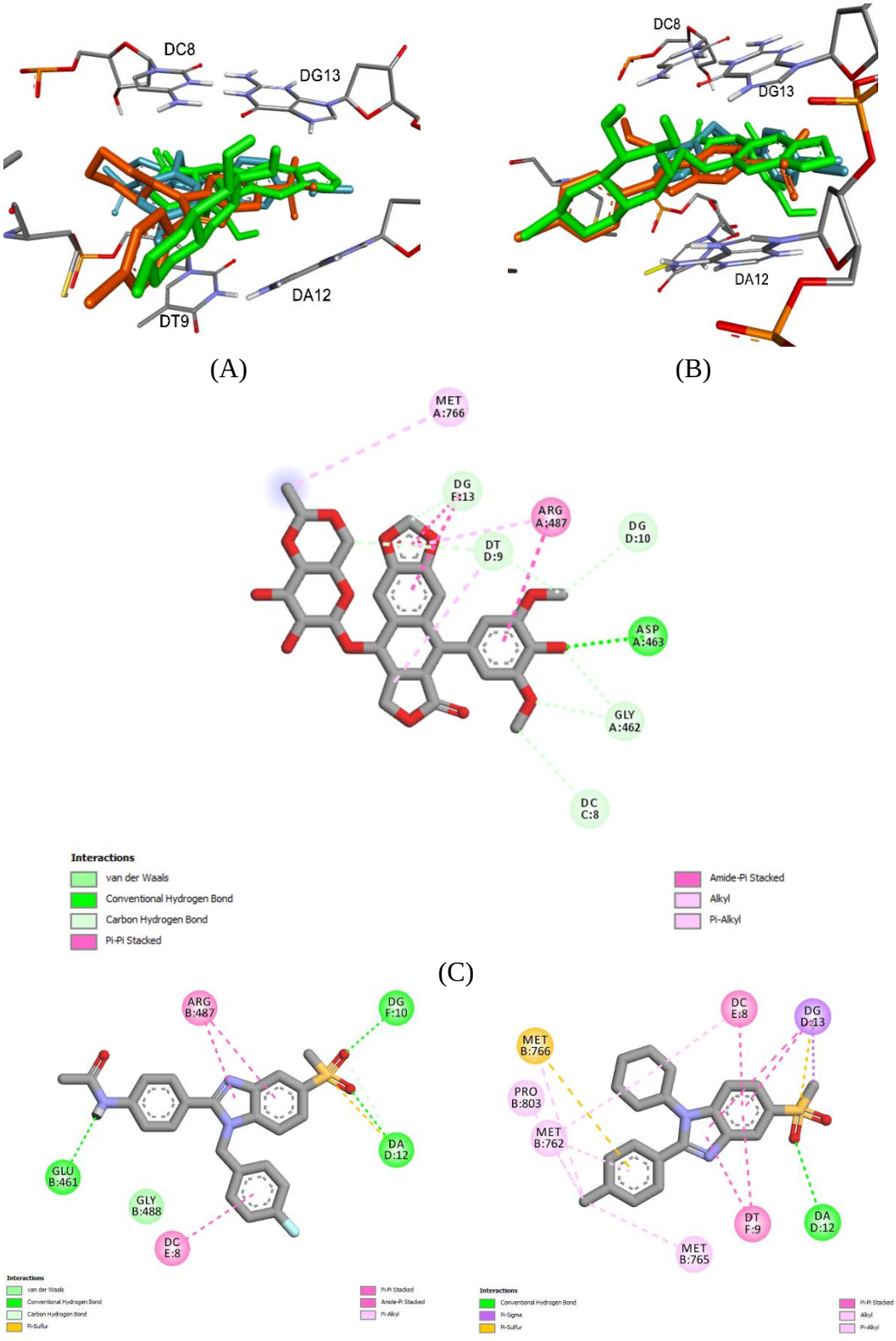


Figure 3.38. Docked conformers (A, B) of **Etoposide (green)**, **36 (blue)**, **23 (orange)** and interaction patterns of **Etoposide** (C), **36** (D), and **23** (E) with *Human DNA-Topoisomerase II complex* (5gwk).

Table 3.3. Docking scores of the synthesized derivatives against DNA Gyrase B subunit (*S.aureus* and *E.faecalis*) and Human DNA Topoisomerase II targets.

Compounds	**Vina scores** (kcal/mol)		
	S. aureus DNA Gyrase B Subunit	***E. faecalis DNA Gyrase B Subunit***	***Human DNA Topoisomerase II***
23	-7.7	-8	-8
24	-8.3	-7.9	-8.3
25	-7.7	-8	-7.9
26	-8.2	-7.7	-10.8
27	-9.2	-8.1	-9
28	-9.1	-7.9	-8.9
29	-8.5	-8.6	-8
30	-7.5	-7.6	-7.6
31	-7.4	-7.4	-7.1
32	-8.1	-8.2	-7.4
33	-9.3	-8.3	-9.4
34	-7.4	-7.2	-7.4
35	-8.6	-8.6	-10.4
36	-9.1	-9.2	-8.5
Vincristine	-	-	-9.1
Etoposide	-	-	-10.9
Ciprofloxacin	-8.1	-7.5	-

3.10. ADME Results

Calculated SwissADME properties may give knowledge on the drug-likeness of benzimidazole derivatives and standard compounds. Log $P_{o/w}$ data for the chemical compounds indicate their lipophilicities, thus bioavailability and toxicities. For starters, compound **28** had the highest log *P* values, suggesting the accumulation in tissues for them would be higher, leading to increased toxicity. Permeation through Blood-Brain Barrier is undesired for the synthesized anti-cancer derivatives since this parameter would influence the cytotoxicity for central nervous system and compounds except **30** were predicted to be impermeable. P-glycoprotein is an important protein that is present on the cell membranes and is also responsible for the transport of the small molecules. Compounds **23, 24, 25, 26** and commercial drugs **Vincristine** and **Ciprofloxacin** were predicted as the substrates to this protein. Moreover, we have calculated the CYP-450 enzyme inhibitions. Compounds **31** and **34** were critical since they are predicted to interact with all these enzymes. This finding may project their

unfavored drug-drug interaction profiles. Except **Vincristine,** all the compounds suited Lipinski and Veber filters, making them suitable drug candidates. All things considered, with a reasonable log *P* and suitability to the parameters, compounds **24, 26, 29** are designated as the best drug-like compounds (Table 3.4).

Table 3.4. Calculated ADME properties for Vincristine, Ciprofloxacin, and benzimidazole derivatives

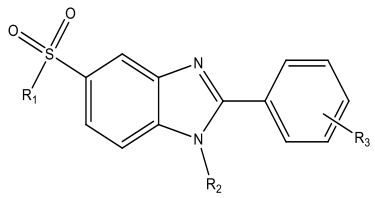
[a] Yes or satisfying the criteria is represented by 1 while 0 is used for defining ‘‘no’’ or not satisfying that rule.

	Consensus log $P_{o/w}$	**Permeation through BBB [a]**	**Substrates to p-gp[a]**	**Inhibition of CYP-450 enzymes (out of 5)**	**Lipinski[a]**	**Veber[a]**
Vincristine	3.41	0	1	1	0	0
Ciprofloxacin	1.10	0	1	0	1	1
23	4.46	0	1	4	1	1
24	4.53	0	1	2	1	1
25	4.8	0	1	3	1	1
26	4.87	0	1	3	1	1
27	4.96	0	0	3	1	1
28	5.67	0	0	3	1	1
29	2.48	0	0	1	1	1
30	3.23	1	0	3	1	1
31	4.24	0	0	5	1	1
32	3.52	0	0	4	1	1
33	4.4	0	0	2	1	1
34	4.46	0	0	5	1	1
35	5.02	0	0	4	1	1
36	3.75	0	0	3	1	1

4. DISCUSSION

In this study, 14 original compounds containing benzimidazole ring system were synthesized. The synthesis of the designed derivatives was started with the starting material 4-chlorobenzenesulfonylchloride (**1**). Alkyl sulfonyl derivatives (**2-3**) were obtained by treating 4-chlorobenzene-sulfonylchloride with alkyl iodides in the presence of tellurium, rongalite and 1M sodium hydroxide solution (Figure 2.1). 1-(Methylsulfonyl)-4-chlorobenzene was purchased commercially and used in the experiments. For the synthesis of 1-chloro-4-(alkylsulfonyl)-2-nitrobenzene derivatives (**4-6**), the obtained 1-chloro-4-(alkylsulfonyl) benzene derivatives were nitrated by treatment with potassium nitrate and sulfuric acid (Figure 2.2). The obtained 1-chloro-4-(alkylsulfonyl)-2-nitrobenzene derivatives are dissolved in DMF and treated with appropriate alkyl amines to the resulting products to be synthesized, and the desired N-substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives as a result of aromatic nucleophilic substitution reaction (**7-14**) was obtained (Figure 2.3). N-Substituted-4-(alkylsulfonyl)benzene-1,2-diamine derivatives (**15-22**) were obtained by reducing the nitro group in N-Substituted-4-(alkylsulfonyl)-2-nitroaniline derivatives by catalytic hydrogenation (Figure 2.4). The mixture of the sodium metabisulfite adduct of benzaldehyde and the appropriate 1,2-phenylenediamine in DMF were reacted to get final products (**23-36,** Figure 2.6). Purity control and chemical structures were elucidated by mass spectroscopy, ^{1}H, ^{13}C-NMR and elemental analysis methods, respectively. Thin Layer Chromatography was used to monitor the progress of the reactions and to determine the purity of the products obtained during the synthesis studies.

Melting point determinations were made and the results were given without correction. A total of 14 new benzimidazole derivative compounds were synthesized, 9 of which are carrying methyl sulfonyl groups, 4 carrying ethyl sulfonyl groups, and 1 carrying propyl sulfonyl groups.



In vitro, the antibacterial activities of 2-phenyl benzimidazole derivative compounds (**23-36**) were determined by microdilution method against 5 different bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Methicillin resistant S.aureus* ATCC 43300, *Enterococcus faecalis ATCC* 29212 strains. The MBC value of benzimidazole derivatives, determined by the broth microdilution method, was determined by inoculation on the sensitest agar surface. The results obtained are given in Table 3.1. With the MIC, the lowest concentration of the antimicrobial agent that inhibits visible growth is determined. Compound **25,** which carries a bulky alkyl group such as cyclohexyl at 1st position, p-chloro phenyl in the 2nd position, ethylsulfonyl in the 5th position of the benzimidazole ring has been found to be effective against *Staphylococcus aureus (S. aureus), Methicillin resistant S.aureus (MRSA), and Enterococcus faecalis (E. Faecalis)*

Compounds **26** and **35**, which had been found the handiest compounds against *E. faecalis* with a MIC value of 12.5 µg/ml, containning 2.5 di fluoro phenyl and 3,4-di-fluoro phenyl in the 2nde position of the benzimidazole ring, respectively. Compounds **25** and **26** have the same R1 and R2 groups. The only difference between them is the substituent on the phenyl ring. Relatively less antimicrobial activity was observed in compound **25** containing p-chloro phenyl group instead of 2,5 di fluoro phenyl in the 2nd position of the benzimidazole ring, indicating that the antimicrobial activity increased as electron affinity increased in the structure. Compounds **23, 27** and **34** were also found to be effective against *E. faecalis*.

Compounds **27** and **35** bearing fluorine substituents in both position 1 and position 2 of the benzimidazole ring have been found to be effective against MRSA.

These again showed that the antimicrobial activity increased as the electron affinity of the compounds increased. Moreover, molecular docking studies show that halogen incorporation is an important feat for the increased interactions with DNA-gyrase inhibitors. Benzimidazole ring proved to be useful in mimicking the quinoline ring of Ciprofloxacin against DNA-gyrase for *S.aureus* and *E.facealis* strains.

The cytotoxic effects of 14 synthesized compounds were analyzed in the MCF-7 cell line using xCELLigence® Real Time Cell Analyzer for 72 hours. The cells were treated with two different concentrations (10µM and 100µM) of the compounds. Vincristine is widely used in breast cancer threapy and known as an effective tubulin polimerase inhibitor was used as a positive control for cytotoxicity. In this way we have determined the most toxic ones with respect to vincristine. Among the synthesized compounds (**23-36**); the candidate compounds **23** (R1=methyl, R2 = cyclohexyl, R3= p-chloro), and **36** (R1=methyl, R2 = p-flourobenzyl, R3= acetamido), which were found to have a significant cytotoxic effect when compared to vincristine, were selected and further studies were continued with these two compounds (Table 3.8.1). They have a serious cytotoxic effect on MCF-7 cells, which is a breast cancer cell line, through apoptosis and cell cycle.

Molecular docking study was also implemented for benzimidazoles and **Vincristine** and **Etoposide**, against Human DNA Topoisomerase II enzyme. For compounds **36** and **23**, amide and sulfonyl groups played crucial roles in H-bond interactions with DNA bases. On the other hand, benzimidazole and phenyl rings were important for both of their Pi interactions. This knowledge on their interacting parts gave us an insight on their preliminary SAR interpretations and in the future, compounds having methylsulfonyl and amide moieties to achieve higher activities and better docking profiles. ADME studies show us that all the compounds suited Lipinski and Veber filters, making them suitable drug candidates. All things considered, with a reasonable log *P* and suitability to the parameters, compounds **24, 26, 29** are designated as the best drug-like compounds.

In the future, by studying the anticancer activities of these compounds in different cancer and healthy cell lines, at the protein level, and *in vivo* animal experiments, broader and detailed data on this subject will be obtained. In this respect, the outputs from this study lead to new studies in the future.

5. RESULTS

In this thesis study, 9 sulfonylmethyl derivatives, 4 sulfonylethyl derivatives and 1 sulfonylpropyl derivative were synthesized. Compounds were synthesized in 5 steps starting from 4-chlorobenzenesulfonylchloride. Purity control and chemical structures were elucidated by elemental analysis, ^{1}H, ^{13}C-NMR and mass spectroscopy methods, respectively. Thin Layer Chromatography was used to monitor the progress of the reactions and to determine the purity of the products obtained during the synthesis studies. Melting point determinations were made and the results were given without correction.

In vitro antibacterial activity properties of 2-phenyl benzimidazole derivative compounds (**23-36**) containing different substituents were determined by microdilution method against to *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Methicillin resistant S.aureus* ATCC 43300, *Enterococcus faecalis ATCC* 29212 strains. The results obtained are given in Table 3.1. Compound **25** has been found to be effective against *S. aureus, MRSA, and E. Faecalis.*

Compounds **26** and **35**, which were found the most effective compounds against *E. faecalis* with a MIC value of 12.5 µg/ml. Compounds **25** and **26** have the same R1 and R2 groups. The only difference between them is the substituent on the phenyl ring. Relatively less antimicrobial activity was observed in compound **25** containing p-chlorophenyl group instead of 2,5-difluorophenyl in the 2nd position of the benzimidazole ring, indicating that the antimicrobial activity increased as electron affinity increased in the structure. Compounds **23, 27** and **34** were also found to be effective against *E. faecalis.* Compounds **27** and **35** were found effective against to MRSA. These showed again, as electron affinity increases in compounds, antimicrobial activity increases.

The cytotoxic effects of synthesized compounds were analyzed in the MCF-7 cell line using xCELLigence® Real Time Cell Analyzer for 72 hours. The cells were treated with two different concentrations (10µM and 100µM) of the compounds. Vincristine was used as a positive control for cytotoxicity. The candidate compounds **23** and **36**, which were found to have a significant cytotoxic effect when compared to vincristine, were selected and further studies were continued with these three compounds (Table 3.8.1). In the future, by studying the anticancer activities of these compounds in different cancer and healthy cell lines, at the protein level, and *in vivo* animal experiments, broader and detailed data on this subject will be obtained. In this respect, the outputs from this study lead to new studies in the future.

SUMMARY

Synthesis, Structural Analysis, and Biological Activities of Novel Benzimidazole Derivatives, and Molecular Docking Studies

In this study, 14 original benzimidazole derivative compounds carrying different groups were synthesized. *In vitro* antibacterial activity properties of 2-phenyl benzimidazole derivative compounds (**23-36**) containing different substituents were determined by microdilution method against to *E. Coli, P. Aeruginosa, S. aureus, MRSA and E. faecalis.* Anticancer activities were determined *in vitro* by extensive screening on MCF-7 cell lines, comparing with clinically commonly used vincristine. The synthesis of the derivatives was started with 4-chlorobenzene-sulfonylchloride **(1),** and N-substituted-4-(alkylsulfonyl) benzene-1,2-diamine derivatives **(15-22)** were obtained in 4 steps. The mixture of the sodium metabisulfite adduct of benzaldehyde and the appropriate 1,2-phenylenediamine were reacted to get final products (**23-36).** After the purity of the synthesized compounds were checked by TLC, their melting points were determined and their structures were elucidated with their ^{1}H-NMR, ^{13}C-NMR spectrum, Mass (ESI+) spectral data and elementel analysis.

Among the synthesized 14 compounds (**23-36**); Compounds **26** and **35**, which were found the most effective compounds against *E. faecalis* with a MIC value of 12.5 µg/ml, possessing 2.5 di-fluoro phenyl and 3,4-di-fluoro phenyl in the second position of the benzimidazole ring, respectively. For anticancer activity; compound **23** (R_1=methyl, R_2 = cyclohexyl, R_3= p-chloro) and **36** (R_1=methyl, R_2 = p-flourobenzyl, R_3= acetamido) have a serious cytotoxic effect on MCF-7 cells, which is a breast cancer cell line, through apoptosis and cell cycle.

Some important structure activity relationships (SARs) resulting from this study: The antimicrobial activity increased as the electron-withdrawing groups increased on the phenyl ring located at the second position of the benzimidazole ring. Compounds with small alkyl group at the R_1 position have been found to have relatively more effective anticancer activity. For example, both compounds **23** and **36** containing methyl group at the R_1 position has been found to be the more effective against MCF-7 cells among these compounds. The groups R_2 and R_3 may exhibit different cytotoxic activity, either individually or in combination. In the future, by studying the anticancer activities of these compounds in different cancer and healthy cell lines, at the protein level, and *in vivo* animal experiments, broader and detailed data on this subject will be obtained. In this respect, the outputs from this study lead to new studies in the future.

Keywords: Anticancer Activity, Antimicrobial Activity, Benzimidazole, Synthesis.

ÖZET

Yeni Benzimidazol Türevlerinin Sentezi, Yapı Aydınlatılması ve Biyolojik Aktiviteleri ve Moleküler Doking Çalışmaları

Bu çalışmada, farklı sübstitüentler taşıyan 14 adet yeni benzimidazol türevi bileşik sentezlenmiştir. Farklı sübstitüentler içeren 2-fenil benzimidazol türevi bileşiklerin (**23-36**) *in vitro* antibakteriyel aktiviteleri mikrodilüsyon yöntemi ile *E. Coli, P. Aeruginosa, S. aureus, MRSA ve E. faecalis*'e karşı belirlenmiştir. Antikanser aktiviteleri, klinikte yaygın olarak kullanılan vinkristin ile karşılaştırılarak, MCF-7 hücre hatları üzerinde *in vitro* olarak belirlenmiştir. Bileşiklerin sentezine 4-klorobenzen-sülfonilklorür (**1**) ile başlanılmış ve 4 aşamada N-sübstitüe-4-(alkilsülfonil) benzen-1,2-diamin türevleri (**15-22**) elde edilmiştir. Benzaldehit türevlerinin sodyum metabisülfit tuzu uygun 1,2-fenilendiaminler ile reaksiyona sokularak sonuç ürünler (**23-36**) elde edilmiştir. Sentezlenen bileşiklerin saflığı TLC ile kontrol edildikten sonra erime noktaları belirlenmiş ve yapıları elementel analiz, ^{1}H-NMR ve ^{13}C-NMR spektrumları ve Mass (ESI+) spektral verileriyle kanıtlanmıştır.

Sentezlenen 14 adet bileşikten (**23-36**); benzimidazol halkasının ikinci konumunda 2,5-diflorofenil taşıyan **26** no lu bileşik ile benzimidazole halkasının 2. Konumda 3,4-diflorofenil içeren **35** no lu bileşik *E. faecalis'e* karşı 12,5 µg/ml MIC değeri en etkili bulunan bileşiklerdir. Yapılan antikanser aktivite çalışmaları ile de bileşik **23** (R_1=metil, R_2 = sikloheksil, R_3= p-kloro) ve bileşik **36** 'nın (R_1=metil, R_2 = p-florobenzil, R_3= asetamido) meme kanseri olan MCF-7 hücreleri üzerinde hücre hattı, apoptoz ve hücre döngüsü yoluyla ciddi sitotoksik etkiye sahip olduklarını bulunmuştur.

Bu çalışmadan elde edilen önemli yapı aktivite ilişkileri (SAR'lar): Benzimidazol halkasının ikinci konumundaki fenil halkasında elektron çekici gruplar arttıkça antimikrobiyal aktivitenin arttığı görülmüştür. R_1 konumunda küçük alkil grubu taşıyan bileşiklerin nispeten daha etkili antikanser aktivitesine sahip olduğu bulunmuştur. Örneğin, R_1 konumunda metil grubu içeren **23** ve **36** no lu bileşiklerin MCF-7 hücrelerine karşı çok etkili olduğu bulunmuştur. R_2 ve R_3 grupları, ayrı ayrı veya kombinasyon halinde farklı sitotoksik aktivite sergileyebilir. Gelecekte bu bileşiklerin farklı kanser ve sağlıklı hücre dizilerinde antikanser aktivitelerinin protein düzeyinde incelenmesi ve *in vivo* hayvan deneyleri ile bu konuda daha geniş ve detaylı veriler elde edilebilecektir. Bu bağlamda, bu çalışmanın gelecekte yeni çalışmalara yol açma potansiyelinin yüksek olduğu düşünülmektedir.

Anahtar Sözcukler: Antikanser Aktivite, Antimikrobiyal Aktivite, Benzimidazol, Sentez,

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RESUME

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