T.C.

REPUBLIC OF TURKEY

HACETTEPE UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

SYNTHESIS AND INVESTIGATION OF TYROSINASE INHIBITORY ACTIVITY OF SOME NEW BENZIMIDAZOLE DERIVATIVES

Pharm. Leen Mohammad Amin Farhan KHRAISAT, MS

Pharmaceutical Chemistry Program

MASTER OF SCIENCE THESIS

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This thesis study has been approved and accepted as a Master dissertation in "Pharmaceutical Chemistry Program" by the assessment committee, whose members are listed below, on 21.07.2023.

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ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor (Prof. Dr. Oya ÜNSAL TAN) and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

(Signature)

Leen Mohammad Amin Farhan KHRAISAT

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ABSTRACT

Khraisat, L., Synthesis and Investigation of Tyrosinase Inhibitory Activity of Some New Benzimidazole Derivatives, Hacettepe University Graduate School of Health Sciences, Faculty of Pharmacy Department of Pharmaceutical Chemistry, Master of Science Thesis, Ankara, 2023. In this study, 4 new 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-one (**2a-d**) and 7 new 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one (**3a-g**) were synthesized and their tyrosinase inhibitory activity were investigated. The structures of the target compounds were elucidated using $^{1}H/^{13}C$-NMR, IR and MS. The structure of **2b** was also characterized using HSQC NMR technique. The inhibitory activity of the compounds against tyrosinase was evaluated and kojic acid was used as positive control in the activity studies. Among the target compounds, **3b-g** demonstrated stronger tyrosinase inhibitory activity (IC_{50} values for **3b-g** ranged from 80.93 to 119.20 μM), compared to the positive control kojic acid (IC_{50}: 125.08 μM). With IC_{50} value of 80.93 μM, 5-(2-(4-(1H-benzimidazol-1-yl)phenyl)-4-oxothiazolidin-3-yl)-2-methylbenzenesulfonamide **3g** was found to be the most active derivative of the series. The MTT assay studies used to determine the cytotoxicity of **3b-g** showed that **3c**, **3d**, **3f** and **3g** were not cytotoxic in the range of 0-200 μM. Considering its tyrosinase inhibitory activity and cytotoxic effect, **3g** exhibits promising potential for further research and development as a novel tyrosinase inhibitor.

Key Words: Benzimidazole, Thiazolidinone, Tyrosinase inhibitory activity, Cytotoxicity, HSQC.

ÖZET

Khraisat, L., Bazı Yeni Benzimidazol Türevlerinin Sentezi ve Tirozinaz İnhibitör Aktivitesinin İncelenmesi, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, Eczacılık Fakültesi Farmasötik Kimya Anabilim Dalı, Yüksek Lisans Tezi, Ankara, 2023. Bu çalışmada 4 yeni 2-(4-(1*H*-benzimidazol-1-il)fenil)-3-(sübstitüefenilamino)tiyazolidin-4-on (**2a-d**) ve 7 yeni 2-(4-(1*H*-benzimidazol-1-il)fenil)-3-(sübstitüefenil)tiyazolidin-4-on (**3a-g**) bileşiği sentezlenmiş ve tirozinaz inhibitör aktiviteleri araştırılmıştır. Hedef bileşiklerin yapıları, $^{1}H/^{13}C$-NMR, IR ve MS kullanılarak aydınlatılmıştır. Bileşik **2b**'nin yapısı ayrıca HSQC NMR tekniği kullanılarak karakterize edilmiştir. Bileşiklerin tirozinaza karşı inhibitör aktivitesi değerlendirilmiş ve aktivite çalışmalarında pozitif kontrol olarak kojik asit kullanılmıştır. Hedef bileşiklerden **3b-g**'nin (IC_{50} değerleri 80,93-119,20 μM aralığında) pozitif kontrol kojik asitle (IC_{50}: 125,08 μM) karşılaştırıldığında daha güçlü tirozinaz inhibitör aktiviteye sahip oldukları gözlenmiştir. 80,93 μM IC_{50} değeri ile 5-(2-(4-(1*H*-benzimidazol-1-yl)fenil)-4-oxotiyazolidin-3-il)-2-metil benzensülfonamit **3g** serinin en aktif türevi olarak bulunmuştur. **3b-g**'nin sitotoksisitesini belirlemek için yapılan MTT çalışmaları, **3c**, **3d**, **3f** ve **3g**'nin 0-200 μM aralığında sitotoksik olmadıklarını göstermiştir. Tirozinaz inhibitör aktivitesi ve sitotoksik etkisi göz önüne alındığında, **3g**'nin, yeni tirozinaz inhibitörü geliştirme çalışmaları için umut verici bir bileşik olduğu düşünülmektedir.

Anahtar Kelimeler: Benzimidazol, Tiyazolidinon, Tirozinaz inhibitör aktivite, Sitotoksisite, HSQC.

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ABBREVIATIONS

AcOH	Acetic acid
CNBr	Cyanogen bromide
COSY	Homonuclear correlation spectroscopy
D_2O	Deuterium oxide
DCC	Dicyclohexylcarbodimide
DIEA	*N*,*N*-Diisopropylethylamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOPA	Dihydroxyphenylalanine
DPBS	Dulbecco's phosphate-buffered saline
EtOH	Ethanol
H_2O_2	Hydrogen peroxide
HCl	Hydrogen chloride
HCN	Hydrogen cyanide
HSDD	Hypoactive sexual desire disorder
HSQC	Heteronuclear single quantum coherence spectroscopy
IC_{50}	Half-maximal inhibitory concentration
IR	Infrared
$KHSO_5$	Potassium peroxymonosulfate
$KMnO_4$	Potassium permanganate
$LiAlH_4$	Lithium aluminum hydride

M.A.	Molecular weight
MHz	Megahertz
mL	Milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWI	Microwave irradiation
NaH	Sodium hydride
$NaIO_4$	Sodium periodate
NF1	Neurofibromatosis type 1
NMR	Nuclear Magnetic Resonance
pH	Potential hydrogen
PPA	Polyphosphoric acid
PPM	Parts per million
PPO	Polyphenol oxidase
Raney Ni	Raney nickel
Sn	Tin element
SD	Standard deviation
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
UVB	Ultraviolet B radiation

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1. INTRODUCTION

Enzymes are biomolecules that are essential in biological systems and play a critical role in maintaining life. Their utility extends to numerous fields such as the chemical industry, cleaning agents, food production, medicine, leather processing, paint manufacturing, biology, biotechnology, agriculture, and veterinary sciences. Enzymes are utilized in various aspects of life across these domains [1, 2].

In the realm of pharmaceuticals, enzymes play a significant role in the mechanisms of action for many drugs. Therefore, the study of enzyme inhibition and activation holds a key position in identifying and discovering new drug molecules [3].

The presence of enzymes, particularly tyrosinase, in food items can lead to darkening, resulting in a decrease in product quality. When vegetables and fruits come into contact with oxygen, the activation of the tyrosinase enzyme causes the product to turn black. This enzymatic darkening not only compromises the product's quality but also leads to financial losses. Tyrosinase also lead to dermatological conditions because of excessive melanin production including hyperpigmentation, melasma, and lentigo. To prevent browning and spoilage in foods, and to treat these dermatological conditions tyrosinase has been considered an important target for developing therapeutic agents [4]. Additionally, in the cosmetic industry, these inhibitors are effective in removing facial freckles, treating pregnancy-related and age-related skin discoloration, mitigating sun-induced dark spots on sensitive skin, and addressing acne. In scientific research, tyrosinase inhibitors can be obtained naturally or synthesized, although only a few of these products can be used due to their toxicity [5-8].

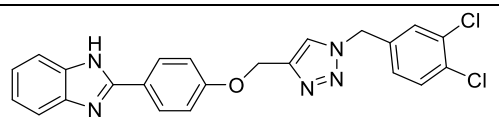
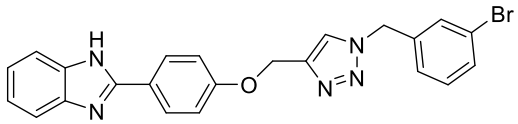
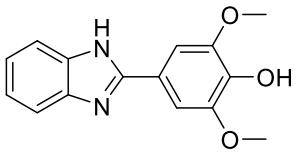
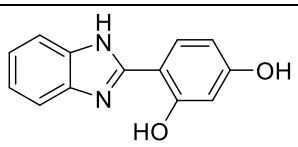
Tyrosinase inhibitors generally bind to the enzyme's active site or the enzyme-substrate complex, effectively preventing the formation of melanosomes and reducing or halting tyrosinase enzyme activity. Consequently, melanin production is inhibited. Compounds that activate tyrosinase can be utilized in the treatment of hypopigmentation, whereas those that inhibit it are employed in addressing hyperpigmentation. Tyrosinase inhibitors are employed as skin-whitening agents. Well-known tyrosinase inhibitors include hydroquinone, arbutin, vitamin C, and kojic acid. However, it is important to note that these inhibitors may also have side effects

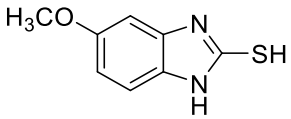
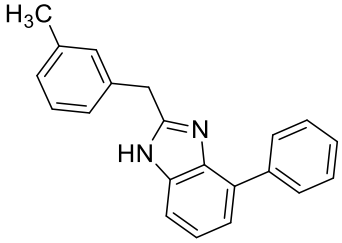
such as skin irritation, mutagenic activity in mammalian cells, and cytotoxicity in melanocytes. Hydroquinone, in particular, has limitations in its use in cosmetic products due to stability and cytotoxicity concerns. Prolonged use of kojic acid has been associated with a potential carcinogenic effect on cells. Consequently, there is a need to explore new tyrosinase inhibitors that are reliable and stable, addressing these issues.[5, 9-11].

Extensive research has been conducted on the pharmacophore of benzimidazole, which has demonstrated its potential for biomedical applications. Notably, this class of compounds exhibit a broad range of biological activities, including anti-inflammatory [12] , antidiabetic [13], antimicrobial [14] , anticonvulsant [15], antioxidant [16], antitubercular [17, 18], antiprotozoal [19], anticancer [20], antiulcer [21], antiviral [22], antihypertensive [23], antimalarial [24], and acetyl-cholinesterase inhibitory activities for Alzheimer's disease [25].

Significant activity has been observed in numerous compounds with benzimidazole structures during recent studies on tyrosinase inhibitors in drug development. (**Table 1.1**).

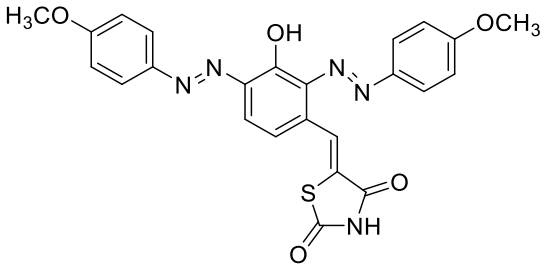
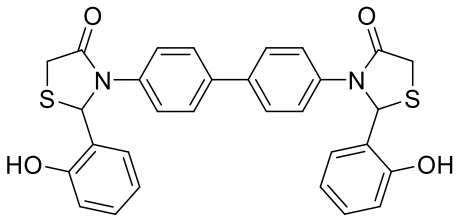
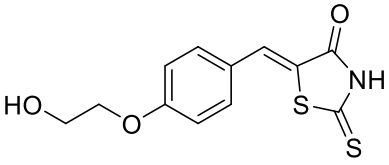
Table 1.1. Compounds with benzimidazole structure having tyrosinase inhibition activity.

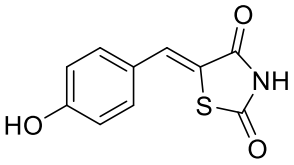
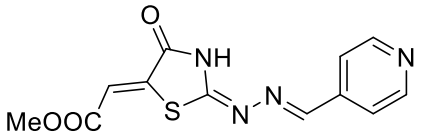
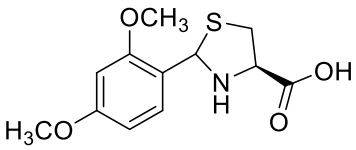
Compounds	**Tyrosinase Inhibitory Activity**
	IC_{50}= 9.42 μM [26]
	IC_{50}= 10.34 μM [26]
	98% percentage inhibition at 50 μM [27]
	78% percentage inhibition at 50 μM [27]

	IC_{50}= 60 nM [28]
	IC_{50}= 37.86 µM [29]

On the other hand, sulfur containing heteroaromatic compounds might have the capability to engage with copper ions or amino acid residues within the active site of tyrosinase, resulting in potent inhibition of tyrosinase activity [30]. Studies in which compounds with thiazolidinones structure have tyrosinase inhibition are shown in **Table 1.2**.

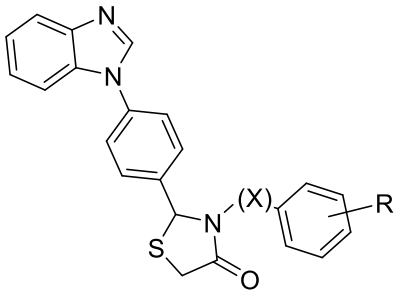
Table 1.2. Compounds with thiazolidinone structure having tyrosinase inhibition activity.

Compounds	**Tyrosinase Inhibitory Activity**
	53.21% percentage inhibition at 40 µM [31]
	99.08% percentage inhibition at 40 µM [32]
	IC_{50}= 0.56 µM [33]

	IC_{50}= 13.36 μM [33]
	IC_{50}= 3.17 μM [34]
	IC_{50}= 5.05 μM [34]

Based on these studies, a set of compounds was designed by combining benzimidazole and thiazolidinone structures, which is expected to have a tyrosinase inhibitory effect. The compounds were designed and synthesized in the form of 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-one (**2a-d)** and 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one (**3a-g)**. The structures of the target compounds were characterized. Their tyrosinase inhibitory activity and cytotoxicity were evaluated. The target compounds can be found in **Table 1.3.**

Table 1.3. The structures of the target compounds 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-one (**2a-d**) and 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one (**3a-g**).



Compounds	**(X)**	**R**
2a	NH	H
2b	NH	4-CH_3
2c	NH	4-OCH_3
2d	NH	4-CN
3a	-	H
3b	-	4-F
3c	-	4-CH_3
3d	-	3-OCH_3
3e	-	4-OCH_3
3f	-	3,4-diOCH_3
3g	-	3-CH_3, 4-SO_2NH_2

2. LITERATURE REVIEW

2.1. Benzimidazoles

Benzimidazoles are a class of organic compounds that contain a benzene ring fused with the 4th and 5th positions of imidazole to form the bicyclic heteroaromatic molecule (**Figure 2.1**) [35].

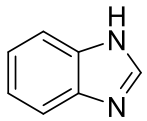


Figure 2.1. Benzo[*d*]imidazole

In organic chemistry, benzimidazoles have a long history that dates to the late 1800s. It was first synthesized by Hoebrecker who reduced 2-nitro-4-methylacetanilide to produce 2,5 (or 2,6)-dimethylbenzimidazole and later by Ladenberg and Wundt between 1872-1878 (**Figure 2.2**) [36, 37]

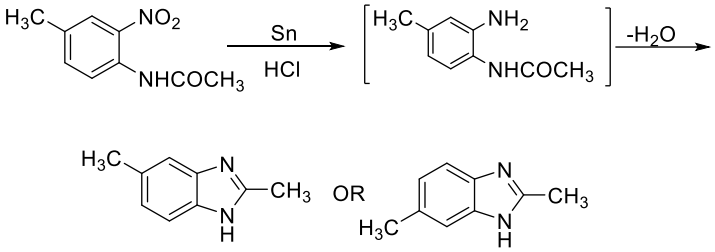


Figure 2.2. Synthesis of 2,5 (or 2,6)-dimethylbenzimidazole

The IUPAC's numbering system for the benzimidazoles is depicted in (**Figure 2.3**).

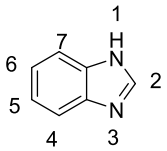


Figure 2.3. Numbering of benzo[*d*]imidazole

Despite being commonly represented with a proton attached to the N_1 nitrogen atom, benzimidazole is in fact subject to rapid proton exchange between its nitrogen atoms (-NH and =N) **Figure 2.4**. This leads to the existence of two possible tautomers for the molecule. In *N*-substituted benzimidazoles, tautomerism is eliminated, and it becomes possible to isolate and characterize two separate, non-equivalent isomers. This is because the *N*-substituent blocks the proton exchange between the nitrogen atoms in the imidazole ring, resulting in the presence of two distinct and stable forms of the molecule [36, 37].

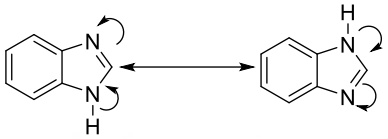


Figure 2.4. Tautomeric forms of benzimidazole

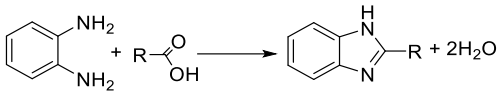
2.1.1. Synthesis Methods of Benzimidazoles

Typically, the production of benzimidazoles can be categorized into different groups based on the starting materials employed. Among them, the most commonly utilized method involves the synthesis from *o*-phenylenediamine. To generate benzimidazole derivatives, there are two primary traditional methods of synthesis. In the first approach, *o*-phenylenediamine is combined with carboxylic acids or their derivatives such as nitriles, amides, esters, or acid halides. The second method involves oxidative cyclodehydrogenation, followed by condensation reactions that include dehydrogenated coupling of *o*-phenylenediamine and aldehydes or alcohols [38].

By reaction *o*-phenylenediamines with acids and its derivatives

O-phenylenediamines have a high propensity to react with most carboxylic acids, leading to the formation of 2-substituted benzimidazoles with exceptional yields. The common approach for carrying out this reaction involves the application of different heating methods, such as a steam bath, reflux heating, elevated temperature heating, or heating in a sealed tube. These techniques have demonstrated a high level of efficacy in generating the desired 2-substituted benzimidazoles, making

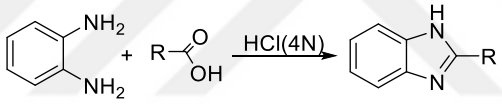
them an invaluable tool in numerous applications across diverse fields (**Figure 2.5**) [39, 40].



R= Alkyl, aryl

Figure 2.5. Benzimidazole synthesis from *o*-phenylenediamine and carboxylic acids

The synthetic method referred to as *Phillip's* method [41] is the most frequently utilized approach for creating a wide variety of benzimidazoles. The process involves the reaction of *o*-phenylenediamine derivatives with non-aromatic carboxylic acid derivatives in the presence of an acid catalyst; typically, dilute HCl (commonly 4N HCl is employed). This condensation reaction leads to the synthesis of the benzimidazole ring (**Figure 2.6**).



R= Alkyl

Figure 2.6. *Phillips* benzimidazole synthesis method

Rithe et al. [42] reported that different 2-substituted benzimidazole derivatives were produced in a moderate to fair yield by condensing *o*-phenylenediamine and different aromatic acids, which was facilitated by ammonium chloride as a catalyst. Saberi [43] has recently reported the synthesis of 2-benzimidazoles using *o*-phenylenediamine and a variety of aromatic, aliphatic, and heterocyclic carboxylic acids under solvent-free conditions and microwave irradiation. The reaction was catalyzed by alumina, and silica gel.

Benzimidazoles can be synthesized by reacting acid anhydrides with *o*-phenylenediamines. A complete conversion of *o*-phenylenediamines into 2-methylbenzimidazole can be achieved by heating them under reflux for several hours with acetic anhydride (**Figure 2.7**) [39, 44].

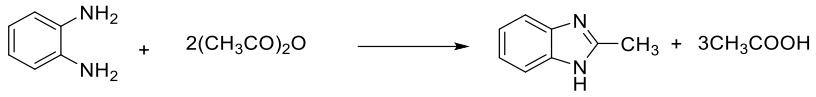


Figure 2.7. Benzimidazole synthesis from *o*-phenylenediamine and anhydrides

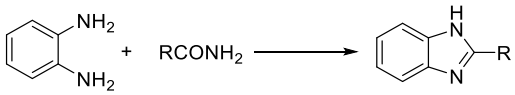
Von Niementowski was the pioneering researcher who initially explored the chemical reaction between esters and *o*-phenylenediamines, leading to the formation of benzimidazoles. The benzimidazole core without any substituents was synthesized using formic acid ester in combination with *o*-phenylenediamine. Alternatively, when other esters were employed, the reaction resulted in the formation of benzimidazole compounds substituted at the 2nd position (**Figure 2.8**) [45, 46].

R= H, aryl, alkyl

R'= Alkyl

Figure 2.8. Benzimidazole synthesis from *o*-phenylenediamine and esters

The use of amides for synthesizing benzimidazoles has been relatively uncommon, although favorable yields have been generally obtained. Notable instances of amides employed in this context include $HCONH_2$, CH_3CONH_2, and $C_6H_5CONH_2$ (**Figure 2.9**) [39].



R= H, alkyl, aryl

Figure 2.9. Benzimidazole synthesis from *o*-phenylenediamine and amides

Benzimidazoles can be synthesized by subjecting *o*-phenylenediamines to the reaction with acid chlorides. When acetyl chloride and 3,4-diaminotoluene (4-methyl-*o*-phenylenediamine) are combined in a benzene solution, the resulting product is 2,5-dimethylbenzimidazole (or 2,6-dimethylbenzimidazole) (**Figure 2.10**) [39, 47].

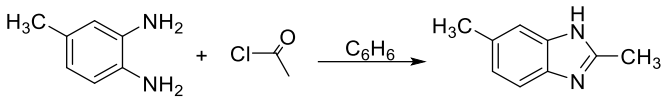


Figure 2.10. Benzimidazole synthesis from *o*-phenylenediamine and acid chlorides

By reacting *o*-phenylenediamines with cyanogen bromide, 2-aminobenzimidazoles can be obtained with high yields (**Figure 2.11**) [48, 49].

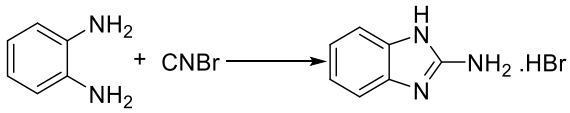
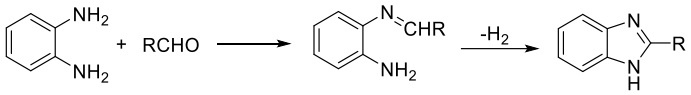


Figure 2.11. Benzimidazole synthesis from *o*-phenylenediamine and nitriles

By reaction *o*-phenylenediamines with carbonyl compounds

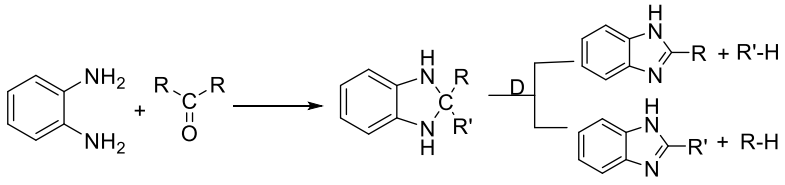
Aldehydes can react with *o*-phenylenediamines to produce 2-substituted benzimidazoles under the right circumstances. The reaction works better under oxidative circumstances because an oxidation is involved. Although air can induce the oxidation, it is more advantageous to utilize an oxidizing agent such as cupric acetate [40]. The latter reagent was first introduced by Weidenhagen. By employing Weidenhagen's technique, it is possible to obtain remarkable yields of 2-substituted benzimidazoles (**Figure 2.12**) [50].



R= alkyl, aryl

Figure 2.12. Benzimidazole synthesis from *o*-phenylenediamine and aldehydes

Elderfield and Kreysa conducted the reaction between *o*-phenylenediamines and ketones, which results in the formation of benzimidazoles. Specifically, they observed that *o*-phenylenediamine reacts with ketones to form 2,2-disubstituted-benzimidazolines. These benzimidazolines can then decompose when exposed to heat, ultimately leading to the production of a 2-substituted benzimidazole and a hydrocarbon (**Figure 2.13**) [51]



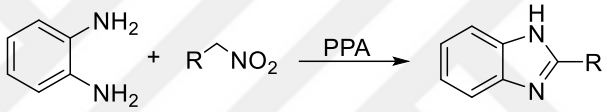
R= H, Alkyl

R'= Alkyl

Figure 2.13. Benzimidazole synthesis from *o*-phenylenediamine and ketones

Synthesis from Nitro Compounds

In the presence of polyphosphoric acid, the reaction between *o*-phenylenediamines and nitro compounds leads to the formation of benzimidazoles (**Figure 2.14**) [52].



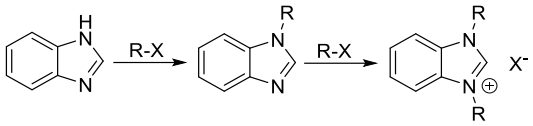
R= Alkyl, Aryl

Figure 2.14. Benzimidazole synthesis from *o*-phenylenediamine and nitro compounds

2.1.2. Chemical Properties of Benzimidazoles

N-Alkylation Reactions

The alkylation of benzimidazoles has been extensively investigated, particularly by O. Fischer. When benzimidazoles react with alkyl halides, they form 1-alkylbenzimidazoles. In a basic environment, benzimidazoles are alkylated specifically at position 1 using alkyl halides. Furthermore, when subjected to even more intense conditions, 1,3-dialkylbenzimidazolium halides are generated (**Figure 2.15**) [37, 53, 54].



R= Alkyl

Figure 2.15. *N*-alkylation reaction of benzimidazole

***N*-Acylation Reactions**

Through the utilization of acyl chlorides or anhydrides, benzimidazoles can undergo a conversion into *N*-acylbenzimidazoles. This chemical transformation typically occurs in an anhydrous environment, devoid of any water. However, it is important to note that in the presence of water, particularly in an alkaline solution, the imidazole ring can undergo cleavage. Additionally, to obtain 1-acetylbenzimidazole involves the application of heat to 2-benzimidazolecarboxylic acid in the presence of acetic anhydride. This process induces decarboxylation and ultimately yields 1-acetylbenzimidazole (**Figure 2.16**) [37, 55].

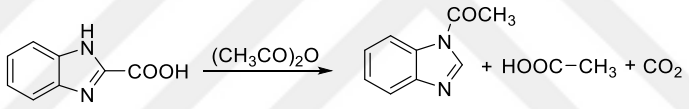
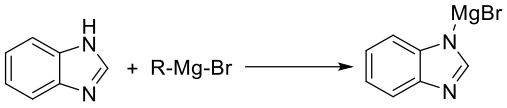


Figure 2.16. *N*-acylation reaction of benzimidazole

Reaction with Grignard Reagent

Grignard reagents react with the active hydrogen in the 1st position of benzimidazole (**Figure 2.17**) [37].



R= Alkyl, Aryl

Figure 2.17. Reaction of benzimidazoles with Grignard reagents

Mannich Reactions

The Mannich reactions involving benzimidazoles were extensively investigated and elucidated by Bachman and Heisey in the past. When equimolar quantities of benzimidazole, formaldehyde, and pyrrolidine are combined, a

remarkable yield of 1-(pyrrolidin-1-yl-methyl)-1*H*-benzimidazole is obtained (**Figure 2.18**) [57].

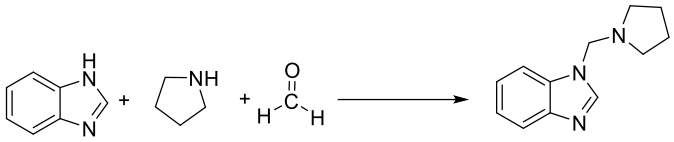


Figure 2.18. Mannich reaction of benzimidazoles

Halogenation Reactions

A well-documented reaction involving benzimidazoles is halogenation. Treatment of benzimidazoles with a saturated solution of calcium hypochlorite at 35°C results in the formation of 1-chloro-2,5 (or 2,6)-dimethylbenzimidazole (**Figure 2.19**) [54].

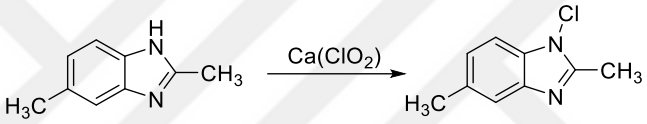


Figure 2.19. Halogenation of 2,5 dimethyl benzimidazole

2.1.3. Spectral Properties of Benzimidazoles

IR Spectrum

The examination of the IR spectra of benzimidazoles revealed the presence of specific peaks indicating various molecular vibrations. The N-H stretching vibrations appeared within the range of 3500-3300 cm^{-1} [24, 41, 48, 49, 58-60] while the aromatic C-H stretching vibrations were observed between 3100-3000 cm^{-1} [41, 52, 60, 61]. Within the 1650-1400 cm^{-1} range, distinct peaks corresponding to C=N and C=C stretching vibrations were detected [41, 48, 49, 60, 62]. Furthermore, it was reported that peaks associated with C-N stretching vibrations were observed in the range of 1250-1000 cm^{-1} [53, 61, 62], while C-H bending vibrations were observed between 900-600 cm^{-1} [53, 62].

^{1}H-NMR Spectrum

In the ^{1}H-NMR spectra of benzimidazole derivatives lacking a substituent at position 1, a broad peak around 13 ppm corresponding to the NH proton can be detected [58, 62-64]. Interestingly, this peak vanishes when deuterated solvents are utilized in these compounds [65].

Ozden et al. conducted a study to examine the relationship between tautomeric and non-tautomeric forms of benzimidazoles using NMR spectra. They focused on a specific compound, 5,6-dichloro-2-{[4-(2-methoxyphenyl)piperazin-1-yl]carbonyl}-1*H*-benzimidazole (referred to as Compound X), which exhibited a mixture of tautomers. However, the NMR spectra of Compound X were not sufficiently clear under standard conditions due to the presence of tautomeric species. This indicated that Compound X displayed a slow-motion tautomeric effect. In **Figure 2.20**, the ^{1}H NMR spectra of Compound X were depicted. At low concentrations in DMSO-d_6, the 1,3-tautomerism was suppressed, resulting in Compound X existing as a single isomer. Consequently, the protons H-4 and H-7 appeared as two distinct singlets (**Figure 2.20A**). However, when the solution concentration was increased, the exchange rate of the imidazole proton accelerated, leading to the broadening of signals from H-4 and H-7 (**Figure 2.20B**). In contrast, by eliminating the tautomeric effects through the addition of a small amount of dry NaH and a few drops of D_2O, all chemically equivalent protons were observed as a single sharp singlet in the NMR spectra (**Figure 2.20C**).

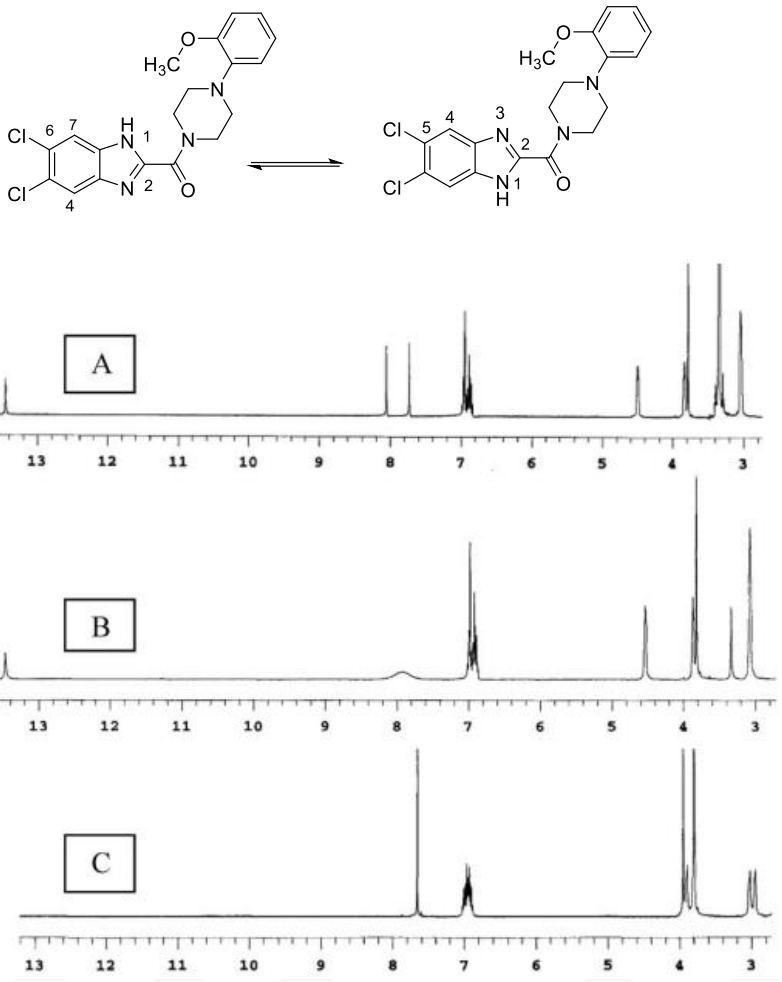


Figure 2.20. (A) ^{1}H NMR spectra of compound X in low concentration. (B) ^{1}H NMR spectra of compound X in high concentration. (C) ^{1}H NMR spectra of compound X + NaH + D_2O.

Doganc et al. [66] conducted an examination of the ^{1}H-NMR spectrum of methyl-1-(2,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate. Their findings revealed that in the benzimidazole section of the compound, the H_4 proton exhibited a peak at 7.85 ppm, the H_6 proton at 7.82 ppm, and the H_7 proton at 6.91 ppm. In order to further clarify the aromatic protons in this compound, a COSY (Correlated Spectroscopy-2D-NMR technique) spectrum was recorded. The analysis of the COSY spectrum, presented in **Figure 2.21**, confirms the assignment of the resonances H-6/H7 as neighboring hydrogens.

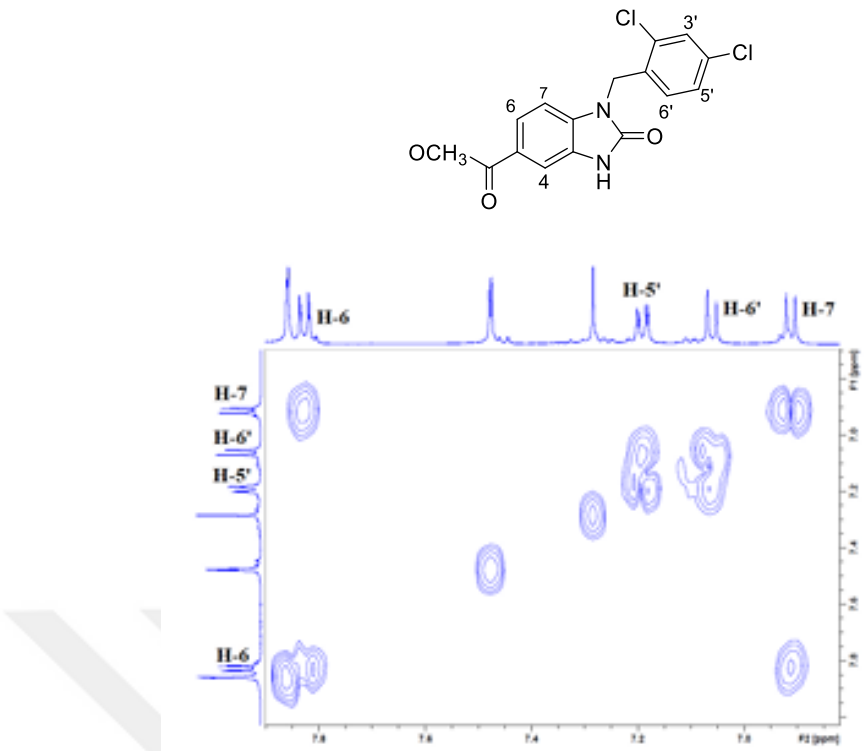


Figure 2.21. COSY spectrum of methyl 1-(2,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate

^{13}C-NMR Spectrum

Distinct carbon peaks can be observed in the spectrum within the 0 to 200 range, exhibiting dissimilarities compared to the reference compound tetramethylsilane (TMS).

Pilyugin et al. reported that when they examined the ^{13}C-NMR spectrum of benzimidazole derivatives, they observed peaks within specific ranges for carbon atoms C_2, C_4, C_5, C_6, C_7, C_{7a}, and C_{3a} of the benzimidazole ring. They stated that these carbon atoms exhibited peaks in the following ranges: 144-156 ppm for C_2, 112-115 ppm for C_4, 120-126 ppm for C_5, 121-131 ppm for C_6, 112-115 ppm for C_7, 129-136 ppm for C_{7a}, and 127-135 ppm for C_{3a}. [67]

In their study, Ceniceros-Gomez et al. observed distinctive peaks in the ^{13}C-NMR spectrum of 2,6-bis(benzimidazol-2-yl) pyridine. The C_2 carbon exhibited a peak at 151 ppm, the C4 carbon at 120 ppm, the C_5 carbon at 123 ppm, the C_6 carbon at 124 ppm, the C_7 carbon at 112 ppm, the C_{7a} carbon at 135 ppm, and the C_{3a} carbon at 145 ppm [68]. (**Figure 2.22**)

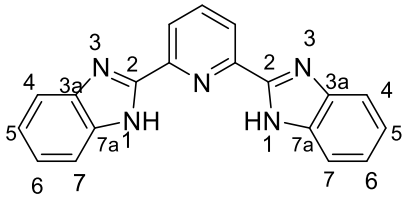


Figure 2.22. 2,6-Bis(benzimidazol-2-yl) pyridine

Mass Spectrum

Mass spectrometry involves the generation of ions from a given sample for analysis purposes. These ions are subsequently separated and quantitatively analyzed. When mobile ions are exposed to electric and/or magnetic fields, they display different paths depending on their mass-to-charge (*m/z*) ratios. Mass spectrometry allows for the accurate characterization and quantification of ions inside a sample by taking use of their divergent paths [69]. The degradation pathways of benzimidazoles bear resemblance to those of imidazoles. When examining the spectrum of benzimidazole, one can observe the stepwise elimination of two hydrogen cyanide (HCN) molecules from the molecular ion. The initial elimination event is nonspecific in nature. Consequently, the spectrum of benzimidazole exhibits peaks corresponding to $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$, representing the molecular ion combined with hydrogen, sodium, and potassium ions, respectively [70-72].

2.1.4. Biological Properties of Benzimidazoles

Benzimidazoles can act as purine antimetabolites due to their structural similarity to the fundamental building blocks of DNA bases, specifically adenine and guanine. These properties enable them to potentially interact more effectively with biopolymers in living systems. As a result, benzimidazole is widely acknowledged as a vital component that plays a significant role in various medicinal applications (**Figure 2.23**) [73, 74].

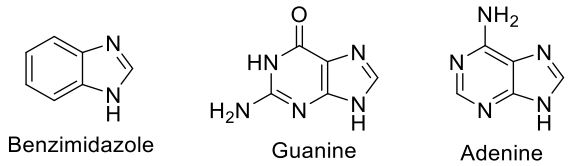
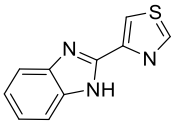
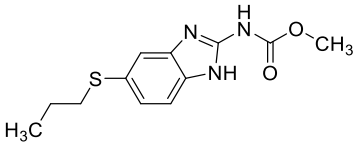
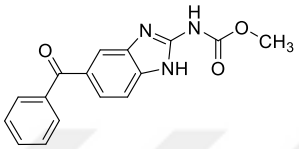
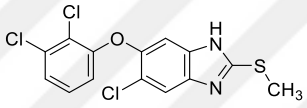
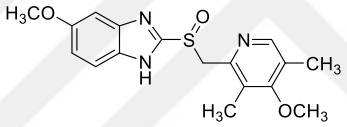
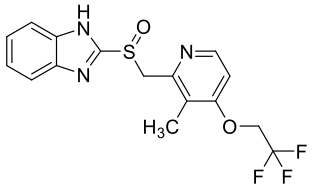
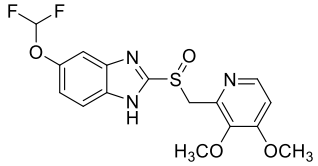
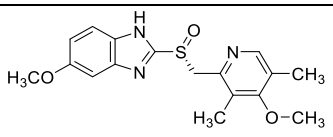
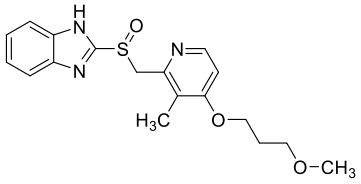
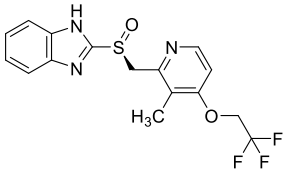
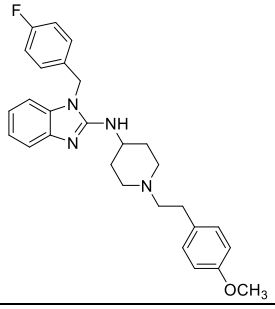
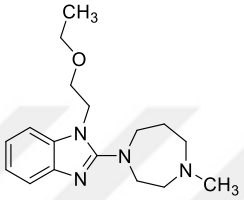
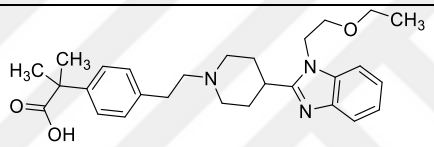
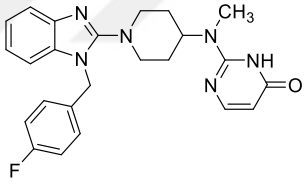
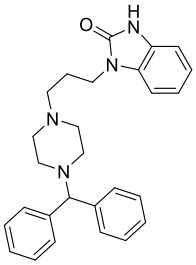
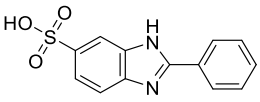
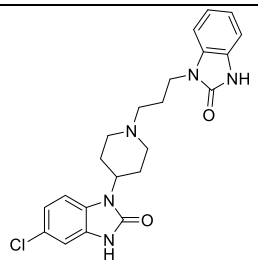


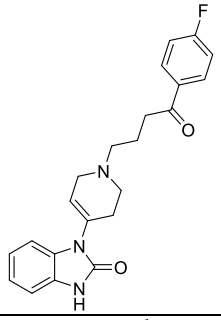
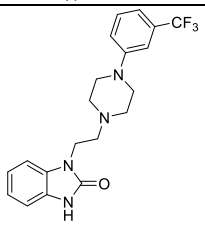
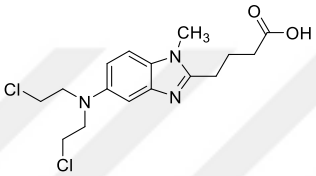
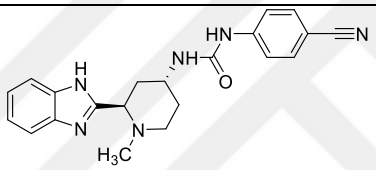
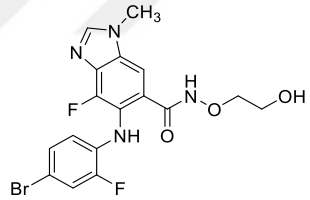
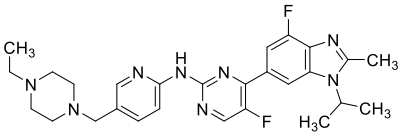
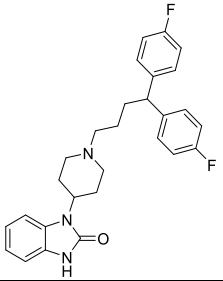
Figure 2.23. Benzimidazole and DNA bases

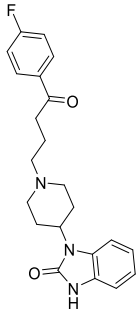
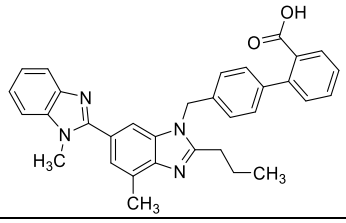
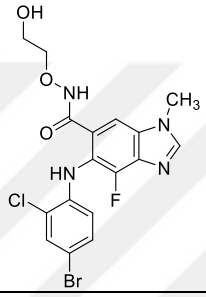
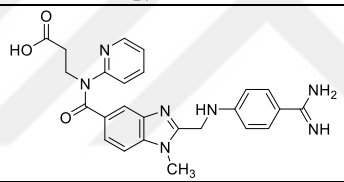
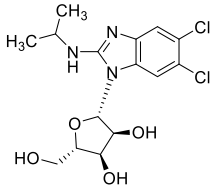
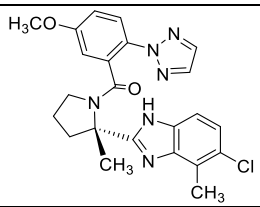
Extensive research on benzimidazoles has uncovered their diverse range of properties, including their effects as antitubercular, anti-inflammatory, analgesic, antifungal, antiviral, antihypertensive, anthelmintic, antidiabetic, and contraceptive agents. [73, 75-77]. The benzimidazoles employed as approved medications were acquired through the utilization of a chemical structure search tool in DRUGBANK [78] and are shown in **Table 2.1.** In 1962, Thiabendazole achieved a significant milestone as the first benzimidazole compound developed and authorized for human use. Since then, numerous benzimidazole derivatives have emerged and obtained clinical approval for various therapeutic applications. Remarkable examples include albendazole, mebendazole, and triclabendazole, which effectively combat parasitic worms. Furthermore, benzimidazole derivatives such as omeprazole, lansoprazole, pantoprazole, esomeprazole, rabeprazole, and dexlansoprazole are recognized as proton pump inhibitors. The antihistaminic properties of benzimidazole compounds like astemizole, emedastine, bilastine, mizolastine, and oxatomide are well-documented. Moreover, ensulizole acts as a sunscreen by absorbing UVB radiation, while domperidone and droperidol serve as antiemetic agents. Flibanserin is specifically used to treat acquired, generalized hypoactive sexual desire disorder (HSDD) in select premenopausal women. Benzimidazole compounds such as bendamustine, glasdegib, binimetinib, and abemaciclib are utilized as anticancer agents, and pimozide and benperidol exhibit antipsychotic effects. Telmisartan has received approval as an antihypertensive agent, while selumetinib is employed in the treatment of neurofibromatosis type 1 (NF1). Dabigatran functions as an anticoagulant, and maribavir demonstrates antiviral activity. Additionally, daridorexant has been approved to address insomnia in adults.

Table 2.1. Approved Benzimidazole drugs [78].

COMPOUND NAME	CHEMICAL STRUCTURE	AREA OF USE
Thiabendazole		Worms infection
Albendazole		Anthelmintic
Mebendazole		Anthelmintic
Triclabendazole		Anthelmintic
Omeprazole		Proton pump inhibitors
Lansoprazole		Proton pump inhibitors
Pantoprazole		Proton pump inhibitors
Esomeprazole		Proton pump inhibitors
Rabeprazole		Proton pump inhibitors

Dexlansoprazole		Proton pump inhibitors
Astemizole		Antihistamine
Emedastine		Antihistamine
Bilastine		Antihistamine
Mizolastine		Antihistamine
Oxatomide		Antihistamine
Ensulizole		Sunscreens to absorb UVB radiation.
Domperidone		Anti-emetic

Droperidol		Anti-emetic
Flibanserin		Women with HSDD
Bendamustine		Antineoplastic agent
Glasdegib		Acute myeloid leukemia
Binimetinib		Anti-cancer
Abemaciclib		Anti-cancer
Pimozide		Antipsychotic

Benperidol		Antipsychotic
Telmisartan		Antihypertensive
Selumetinib		Neurofibromatosis type 1
Dabigatran		Anticoagulant
Maribavir		Antiviral
Daridorexant		Insomnia

2.2. 4-Thiazolidinones

4-Thiazolidinones are characterized by their sulfur and nitrogen atoms, along with a carbonyl group located at the 4th positions within the ring. The ring's numbering begins from the sulfur atom, progressing towards the nitrogen atom and concluding with the carbonyl group **(Figure 2.24)** [79].

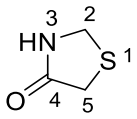
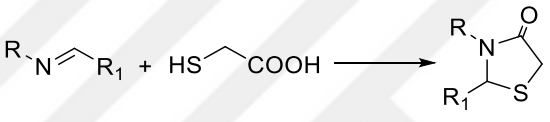


Figure 2.24. Numbering of the 4-Thiazolidinone ring.

2.2.1. Synthesis Methods of 4-Thiazolidinones

The formation of 4-thiazolidinone derivatives occurs through the reaction of *Schiff* bases or hydrazones, with mercaptoacetic acid dissolved in solvents like dry benzene or toluene. Aforementioned *Schiff* bases are generated by combining aliphatic amines [60], arylamines [80-82], or aryl hydrazines [83] with aromatic aldehyde derivatives. (**Figure 2.25**).



R= Alkyl, aryl, aryl amine
R_1= Alkyl, aryl

Figure 2.25. Synthesis of 4-Thiazolidinone by reaction of mercaptoacetic and *Schiff* base/hydrazones.

By heating equimolar quantities of imines and thiolactic acid in an anhydrous benzene solution, 3-aryl-2-(2-pyrrolyl)-4-thiazolidinones were successfully synthesized with favorable yields. (**Figure 2.26**) [84]

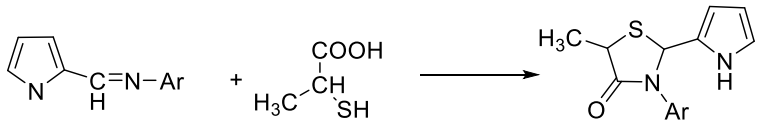


Figure 2.26. Synthesis of 4-Thiazolidinone by refluxing equimolar amounts of the imine and thiolactic acid in dry benzene

The optimized procedure utilized microwave irradiation to heat a mixture of amine hydrochloride, aldehyde, and mercaptoacetic acid in the presence of 1.25 equivalents of *N*,*N*-diisopropylethylamine (DIEA) base in ethanol at 120°C for 30 minutes under atmospheric pressure (**Figure 2.27**) [84, 85].

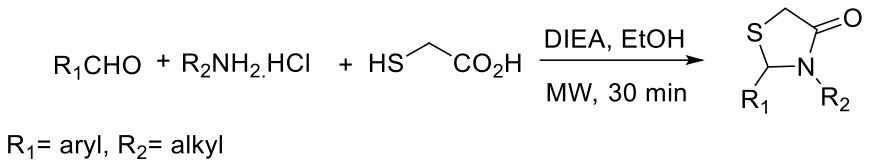


Figure 2.27. Synthesis of 4-Thiazolidinone by subjecting a mixture of amine hydrochloride, aldehyde, and mercaptoacetic acid to microwave irradiation.

Sriram et al. [86] conducted a study where they synthesized a series of 1,3-thiazolidin-4-ones containing diaryl rings at the C-2 and N-3 positions. The method employed for synthesizing the 2,3-diaryl-1,3-thiazolidin-4-ones involved a reaction between a substituted benzaldehyde and an equimolar quantity of a suitable substituted aromatic amine. This reaction took place in the presence of an excess of mercaptoacetic acid and toluene, utilizing microwave irradiation (**Figure 2.28**).

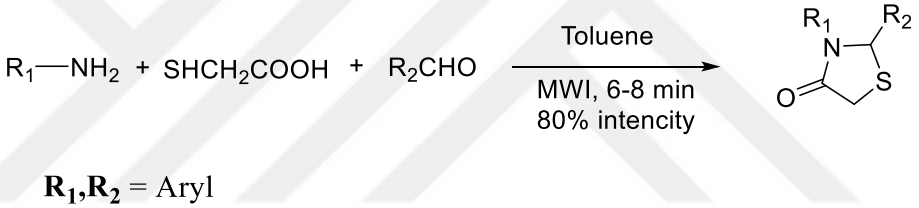
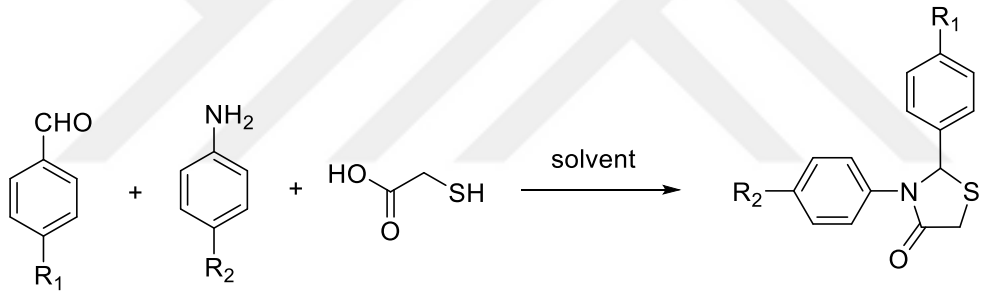


Figure 2.28. Synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones utilizing microwave irradiation

Problems in synthesis methods including lengthy reaction times, low yields, and the use of hazardous solvents, have made a demand for a more convenient approach to the synthesis of 4-thiazolidinones. As a result, a novel three-component, one-pot synthetic method has been developed. Numerous techniques have been documented for synthesizing 4-thiazolidinones. The novel synthetic pathways to 1,3-thiazolidin-4-ones involve a three-component reaction between an amine, a compound containing a carbonyl group, and a mercaptocarboxylic acid. This conventional approach can be carried out either as a one-pot three-component condensation or as a two-step process. Mechanistically, these reactions commence with the creation of an imine, followed by the nucleophilic attack of the nitrogen atom from the amine component on the carbonyl carbon of the aldehyde or ketone. Subsequently, the resulting imine is subjected to a nucleophilic attack by a sulfur nucleophile, and an

intramolecular cyclocondensation reaction occurs, ultimately yielding the desired product. [87]

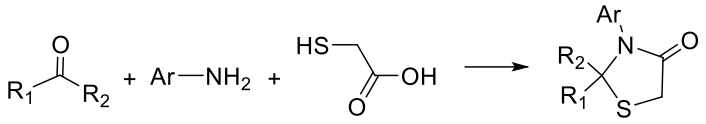
In recent times, there has been a growing interest in utilizing ionic liquids for the production of thiazolidinone derivatives. Ionic liquids refer to salts that possess a liquid state at room temperature. They have emerged as viable alternatives to traditional solvents in organic synthesis due to several advantageous characteristics. These include their non-volatile nature, non-explosiveness, ease of handling, heat resistance, and recyclability, all of which surpass those of conventional organic solvents [88]. Zhang et al. [89] conducted a study wherein they employed the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF6]) as a solvent in the synthesis of thiazolidinone derivatives. By utilizing a one-pot reaction scheme involving [bmim][PF6], aromatic aldehyde, amine, and mercaptoacetic acid, the researchers successfully synthesized 2,3-disubstituted-4-thiazolidinone derivatives with a high yield. (**Figure 2.29)**



R_1= NO_2; F; Cl; Br; CH_3
R_2= CH_3; Cl; Br

Figure 2.29. Synthesis of 4-thiazolidinone from aromatic aldehyde, aromatic amine, and mercaptoacetic acid using ionic liquid as a solvent

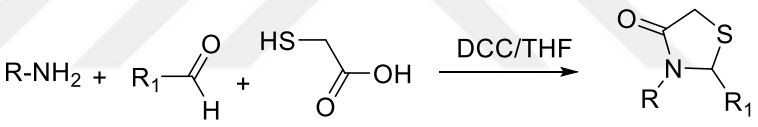
Lingampalle et al. utilized an innovative approach to synthesize thiazolidinone derivatives by employing a compound featuring a carbonyl group, along with mercaptoacetic acid and primary aryl amine compounds. They achieved remarkable results by utilizing an ionic liquid derived from pyridine and methyl-4-toluene sulfonate. This unique methodology allowed them to carry out the synthesis process within a single container (**Figure 2.30**) [90].



R_1,R_2= H, aryl

Figure 2.30. Synthesis of 4-thiazolidinones from carbonyl compounds, aromatic amines and mercaptoacetic acid in ionic liquid.

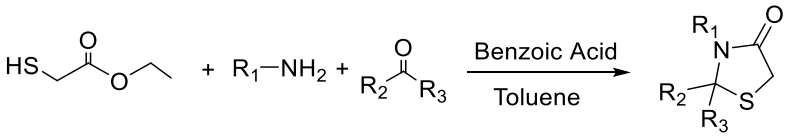
In their study, Srivastava et al. [91] successfully synthesized 4-thiazolidinone compounds using a three-component one-pot approach. They employed aromatic aldehyde, aromatic amine, and mercaptoacetic acid as reactants, along with dicyclohexylcarbodiimide as a catalyst, while maintaining a reaction temperature of 0°C. The researchers discussed the impact of using aromatically and sterically hindered amines, which led to reduced yields due to the rate-determining step of the reaction involving the attack of the amine nitrogen on the carbonyl carbon. However, the incorporation of dicyclohexylcarbodiimide effectively resolved this issue (**Figure 2.31).**



R= Cyclohexyl; n-butyl; n-octyl; isopropyl; C_6H_5; $CH_2C_6H_5$; $CH(CH_2C_6H_5)COOCH_3$
R_1 = C_6H_5; 4-ClC_6H_4; 2-$OCH_3C_6H_4$; 4-CNC_6H_4; 1-naphthyl

Figure 2.31. Synthesis of 4-thiazolidinone from aromatic aldehyde, aromatic amine, and mercaptoacetic acid

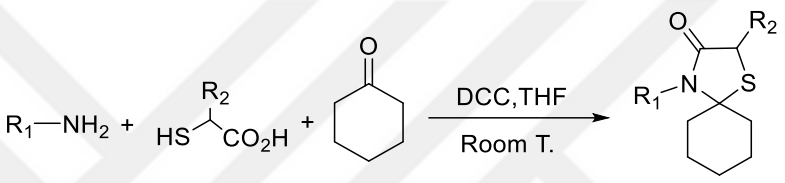
Pelletier et al. [92] demonstrated that an extensive range of carbonyl compounds and amines can be effectively transformed into 4-thiazolidinones utilizing benzoic acid, a reagent previously unexplored in this type of reaction. Remarkably, they achieved the synthesis of 4-thiazolidinone-derived compounds with an average yield of 74% by substituting mercaptoacetic acid with its ethyl ester **(Figure 2.32)**.



R_1,R_2,R_3= Aryl

Figure 2.32. Synthesis of 4-thiazolidinone Derived Compounds in the Presence of Benzoic Acid

Srivastava et al. [93] successfully synthesized 4-thiazolidinone derivatives by reacting a mixture of amine, cyclohexanone, and mercaptoacetic acid at room temperature. The reaction was facilitated by the presence of dicyclohexylcarbodimide (DCC) and tetrahydrofuran (THF) as reagents **(Figure 2.33)**.



R_1,R_2 = Aryl

Figure 2.33. Synthesis of 4-thiazolidinone derived compound in the presence of DCC and THF

2.2.2. Chemical Properties of 4-Thiazolidinone

A. Oxidation Reactions

The impact of an oxidizing agent on a derivative of 4-thiazolidinone is influenced by various factors, including the substituent groups connected to the thiazolidine ring, the specific chemical properties of the oxidizing agent, and the conditions in which it interacts with the heterocyclic compound.

Under the reaction conditions described by Raza et al. [94] 2,3-disubstituted-4-thiazolidinone sulfoxide derivatives were synthesized through the reaction of 2,3-disubstituted-4-thiazolidinones with potassium peroxymonosulfate (oxone) in a mixture of methanol and water (1:1) at temperatures ranging from -5 to -10°C. Subsequently, by maintaining the same reaction conditions, the sulfoxides obtained

were further converted into 2,3-disubstituted-4-thiazolidinone sulfone derivatives (as shown in **Figure 2.34**).

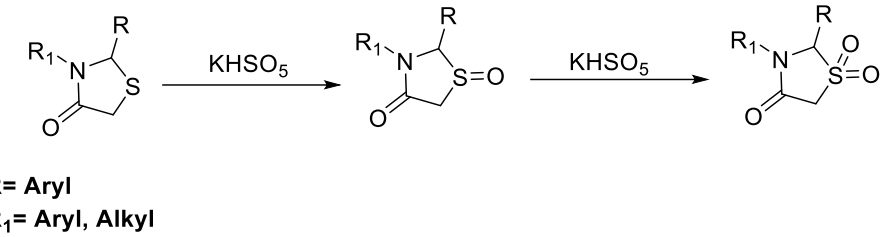


Figure 2.34. Reaction of 2,3-disubstituted-4-thiazolidinone derivatives with potassium peroxymonosulfate.

Sulfones were obtained as a result of the oxidation of 2,3-disubstituted-4-thiazolidinones in acetic anhydride and acetic acid with hydrogen peroxide at 55ºC or with potassium permanganate in acetic acid (**Figure 2.35**) [95].

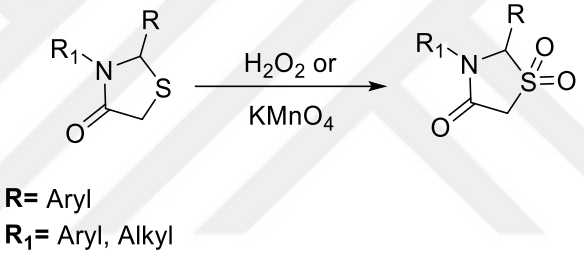


Figure 2.35. Reaction of 2,3-disubstituted-4-thiazolidinone derivatives with hydrogen peroxide or potassium permanganate.

By subjecting 2,3-disubstituted-4-thiazolidinones to different reaction conditions, including hydrogen peroxide in acetic acid, peroxyacetic acid at room temperature, potassium peroxymonosulfate in a mixture of methanol and water (1:1) at temperatures ranging from -5 to -10°C, or sodium metaperiodate in an aqueous methanol solution, the formation of sulfoxide derivatives of 4-thiazolidinone was observed. [96, 97] (**Figure 2.36**).

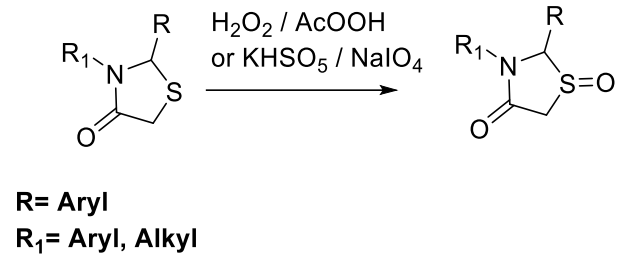


Figure 2.36. Reaction of 2,3-disubstituted-4-thiazolidinone derivatives with hydrogen peroxide, peroxyacetic acid or potassium peroxymonosulfate.

B. Reduction Reactions

Previous studies have indicated that upon treating 4-thiazolidinone derivatives with Raney nickel in ethanol, the removal of the carbon atom and sulfur atom located in the second position of the 4-thiazolidinone ring leads to the formation of amide derivatives (**Figure 2.37**) [98].

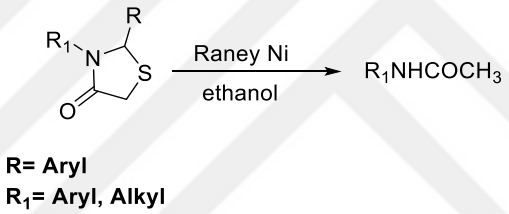


Figure 2.37. Reaction of 4-Thiazolidinone derivatives with Raney nickel.

According to reported findings, the utilization of lithium aluminum hydride in the reduction of 4-thiazolidinones results in the conversion of the carbonyl group into a methylene group, while simultaneously causing the opening of the heterocyclic ring between the sulfur atom and the second carbon atom (**Figure 2.38**) [98].

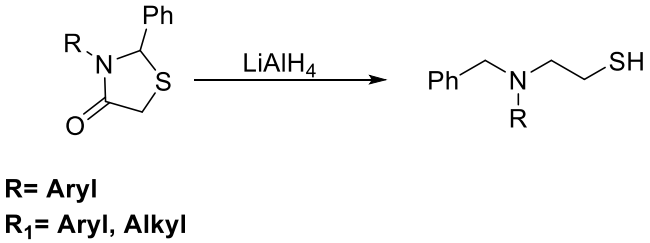


Figure 2.38. Reaction of 4-Thiazolidinone derivatives with lithium aluminum hydride.

2.2.3. Spectral Properties of 4-Thiazolidinones

IR Spectrum

Upon analyzing the IR spectra of compounds containing the 4-thiazolidinone structure, distinct spectral features emerge. The C=O stretching of the lactam group is observed within the range of (1750-1660) cm^{-1} [99]. Aquino et al. conducted a study exploring the IR spectra of derivative compounds of 2- [(substituted phenylmethylene) hydrazine]-4-oxo-3-phenyl-5-thiazolidine acetic acid. Their findings revealed distinct absorption bands attributed to the C=O stretching vibrations, which were observed within the range of 1731-1707 cm^{-1} [100]. Kouznetsov et al. conducted a thorough investigation of the IR spectra of derivatives of 1,2/1,4-Bis-[2-aryl4-oxo-1,3-thiazolidin-3-yl] ethane/butane. Their findings revealed the presence of distinctive absorption bands associated with the C=O stretching vibrations. These bands were observed in the range of 1680-1643 cm^{-1}, highlighting the characteristic spectral signature of this functional group [101]. In their study, Vicini et al. [102] examined the infrared spectra of a derivative compound called 5-aryl-2-(benzo[*d*]thiazol-2-illimino)thiazolidin-4-one. Their findings revealed that the N-H group located at the 3rd position of the thiazolidinone ring exhibited stretching vibrations within the range of 3230-3070 cm^{-1}.

^{1}H-NMR Spectrum

The ^{1}H-NMR spectra of derivatives of 5-fluoro-*N*-(5-methyl-4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-3-phenyl-1*H* indole-2-carboxamide were obtained using DMSO-d_6 as the solvent. The H_2 proton of the thiazolidinone ring appeared as a double singlet or a singlet and doublet at 5.78 and 5.99 ppm, respectively. Similarly, the H_5 proton exhibited a double quartet or a quartet and a doublet of a quartet at 4.03 and 4.21 ppm, respectively. These splitting patterns were attributed to distant interactions occurring at 4 bond distances between the H_2 and H_5 protons, with an interaction constant of *J = 1.0-1.5 Hz*. Furthermore, the methyl protons located at the fifth position of the thiazolidinone ring were observed at 1.50-1.54 ppm, appearing as a doublet or multiplet. This splitting pattern was attributed to a distant interaction with the H_2 proton, resulting in an interaction constant of *J* = 6.7-7.3 *Hz* [103].

In a study examining the ^{1}H-NMR spectra of a series of 2-substituted-3-[[4-(4-methoxybenzoylamino) benzoyl] amino]-4-thiazolidinone derivatives using (DMSO-d_6) as the solvent, it was found that the peaks corresponding to the methylene protons at the 5th position of the thiazolidinone ring appeared around 3.80 and 3.90 ppm. These protons exhibited a double doublet splitting pattern and the interaction constant between these protons was determined to be J = 15.9 *Hz* [104].

When examining the ^{1}H-NMR spectrum peaks taken in $CDCl_3$ of 2,3-Diaryl-5-methyl-thiazolidin-4-one derivative compounds, important observations have been made. The methyl protons located at the fifth position of the thiazolidinone ring are observed as doublets at 1.62-1.79 ppm, with a interaction constant (*J*) of *6.8-7.2 Hz*. The H_2 protons appear as a singlet in the range of 6.50-7.19 ppm. Additionally, the H_5 protons are observed as quartets at 4.10-4.20 ppm, with a interaction constant (*J*) of (*6.8-7.2 Hz*) [105].

Patel et al. conducted an analysis of the ^{1}H-NMR spectra for a derivative series of 4-[4-dimethylamino-6-[4-(4-oxo-2-arylthiazolidin-3-yl)-phenylamino]- [1,3,5] triazin-2-yloxy]-1-methyl-1*H*-quinolin-2-one using DMSO-d_6 as the solvent. Their investigation revealed that the protons situated at the 5th position of the thiazolidinone ring exhibited a doublet pattern with peaks observed around 3.60 and 3.70 ppm in the spectra and the interaction constant between these protons was determined to be J = *12.1 Hz* [106].

^{13}C-NMR Spectrum

Upon analyzing the ^{13}C-NMR spectrum peaks obtained in DMSO-d_6 of 2,3-diaryl-5-methyl-thiazolidin-4-one derivative compounds, notable observations have been made. The carbon signals for C_2, C_4, and C_5 appear in the range of 60.00-61.21 ppm, 171.96-172.56 ppm, and 38.31-39.48 ppm, respectively. Additionally, the carbons associated with the methyl group at position 5 are detected between 19.97-20.71 ppm. It has been reported that two peaks are observed for these carbons, indicating the presence of a mixture of cis-trans isomers [107].

Mass Spectrum

When analyzing the mass spectra of 3-substituted-2-adamantyl-4-thiazolidinone derivatives, several significant observations were made. Apart from the

fragmentation products resulting from the separation of substituents at the 2 and/or 3 positions from the molecule, additional groups were observed. These included CH_2S fragments originating from the thiazolidinone ring (breakage from the 1-2 and 4-5 bonds), as well as CH_2CO groups (breakage from the 1-5 and 3-4 bonds). Furthermore, the spectra exhibited breaks in the 1-2 and 3-4 bonds, along with peaks indicating proton migration and SCHCO cleavage [108].

Upon analyzing the mass spectrum of the 3-benzyl-2-(4-methylthiophenyl)-1,3-thiazolidin-4-one derivative, notable observations were made regarding its decomposition products. The spectrum revealed fragmentation products resulting from the separation of substituents at the 2 or 3 positions of the molecule. Additionally, peaks corresponding to the separation of the $COCH_2$ group from the thiazolidinone ring (breaking the 1-5 and 3-4 bonds) were also observed [99].

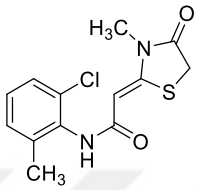
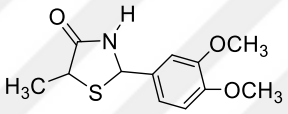
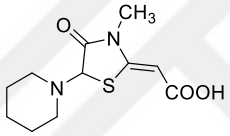
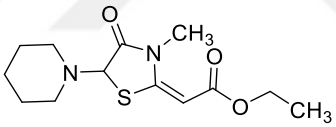
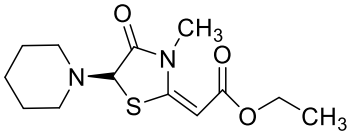
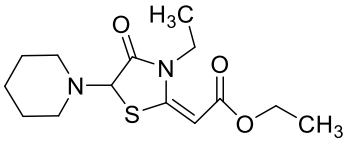
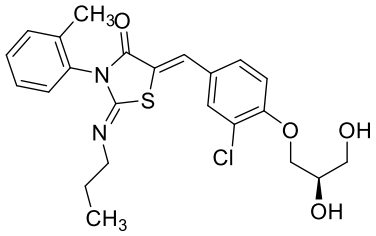
2.2.4. Biological Properties of 4-Thiazolidinones

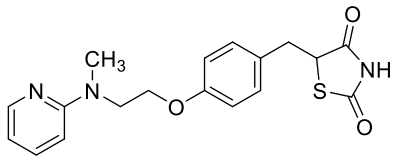
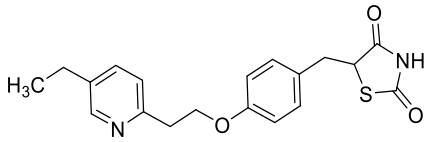
Thiazolidinones, classified as heterocycles, have captured considerable attention due to their extensive range of biological activities. These activities encompass antifungal, antibacterial, antihistaminic, antimicrobial, anticonvulsant, anticancer, and anti-inflammatory properties. [109] The remarkable diversity exhibited in their biological response profile has sparked the interest of organic chemists, leading them to explore the full potential of this molecular framework across various biological activities [110].

The thiazolidinone employed as approved medications were acquired through the utilization of a chemical structure search tool in DRUGBANK. Among the diverse range of activities exhibited by thiazolidinone derivatives, Ralitoline stands out as an effective anticonvulsant. Mezolidone demonstrates antiulcer properties, while Ozolinone and Etozoline act as diuretics. Dexetazoline is recognized for its antihypertensive effects, and Piprozoline functions as a choleretic agent. Ponesimod is specifically indicated for the treatment of multiple sclerosis. Additionally, the oral antidiabetic drugs Rosiglitazone and Pioglitazone, both belonging to the 4-thiazolidindione derivative class, are widely utilized in medical practice. These

compounds exemplify the therapeutic versatility of thiazolidine derivatives and their significant contributions to various fields of medicine [111-113]. **Table 2.2.** provides a comprehensive listing of approved drug compounds belonging to the class of thiazolidinone derivatives [78]

Table 2.2. Approved Thiazolidinone drugs [78].

COMPOUND NAME	CHEMICAL STRUCTURE	ARE OF USE
Ralitoline		Anticonvulsant
Mezolidone		Antiulcer
Ozolinone		Diuretic
Etozoline		Diuretic
Dexetozoline		Antihypertension
Piprozoline		Choleretic
Ponesimod		Multiple Sclerosis

Rosiglitazone		Antidiabetic
Pioglitazone		Antidiabetic

2.3. Tyrosinase Inhibitors

Tyrosinase (EC 1.14.18.1) is an enzyme that contains two copper atoms and functions as a monooxygenase. It is widely distributed across various organisms, ranging from simple to complex ones, and is also known by alternative names such as polyphenol oxidase (PPO) and catechol oxidase.[114] Its primary role is to regulate the rate of melanin production in a process called melanogenesis, which occurs in the skin. Melanogenesis begins with the hydroxylation of tyrosine, catalyzed by tyrosinase, leading to the formation of L-3,4-dihydroxyphenylalanine (DOPA). Tyrosinase also facilitates the subsequent conversion of DOPA into dopaquinone through enzymatic reactions. [114-117]

Tyrosinase is the rate-limiting enzyme for melanin production. Melanin, a pigmented heteropolymer, is synthesized by specialized cellular organelles called melanosomes within differentiated melanocytes. The entire process of melanin formation is known as melanogenesis, which commences with the initial step of tyrosine oxidation to dopaquinone, catalyzed by the enzyme tyrosinase. While the primary function of melanin in human skin is photoprotection, an excessive accumulation of melanin in certain areas can lead to the development of more pigmented patches, posing aesthetic concerns. Conditions such as melasma, freckles, large-scale brown spots on the face and body, as well as age-related pigmentation spots, arise due to abnormal melanin pigmentation in humans and can significantly impact aesthetics.[118, 119] Furthermore, tyrosinase is responsible for undesirable browning reactions observed in vegetables and fruits. These reactions typically occur when fruits and vegetables are left on the shelf for extended periods or undergo injuries during post-harvest processing. Such browning reactions contribute to the

deterioration of tissue integrity, resulting in visible color changes on the surface of these products. Not only does this affect their visual appeal, but it also negatively impacts their taste and overall quality. As a consequence, the usability of these products is limited, leading to significant economic losses due to decreased shelf life [120].

The use of tyrosinase inhibitors as medications can effectively hinder the synthesis of melanin by targeting the enzyme's mono and diphenolase activities through various mechanisms. These inhibitors can either directly inhibit the enzymatic activities of tyrosinase or convert the generated *o*-quinone intermediates into colorless conjugates. By doing so, these inhibitors play a crucial role in regulating and controlling melanin production, providing a potential avenue for treating conditions associated with abnormal pigmentation or hyperpigmentation [121, 122]. Tyrosinase has been also implicated in the development and progression of neurodegenerative diseases, by catalyzes the formation of melanin from dopamine in the brain. The excessive amount of melanin formed can cause Parkinson's disease and neurodegenerative movement disorders related to this disease [123].

In nerve cells, the conversion of tyrosine to L-DOPA occurs, followed by its transformation into dopamine through a decarboxylation reaction [124]. In the event of damage to dopaminergic neurons in the brain, the synthesis of catecholamines is maintained by an enzyme called tyrosinase, as illustrated in **Figure 2.39.** Oxidation of dopamine leads to the formation of dopamine quinones, which are known as oxidized derivatives and have been associated with neurotoxicity in Parkinson's disease. Consequently, the inhibition of tyrosinase represents a potential novel approach for the treatment of Parkinson's disease [125, 126] .

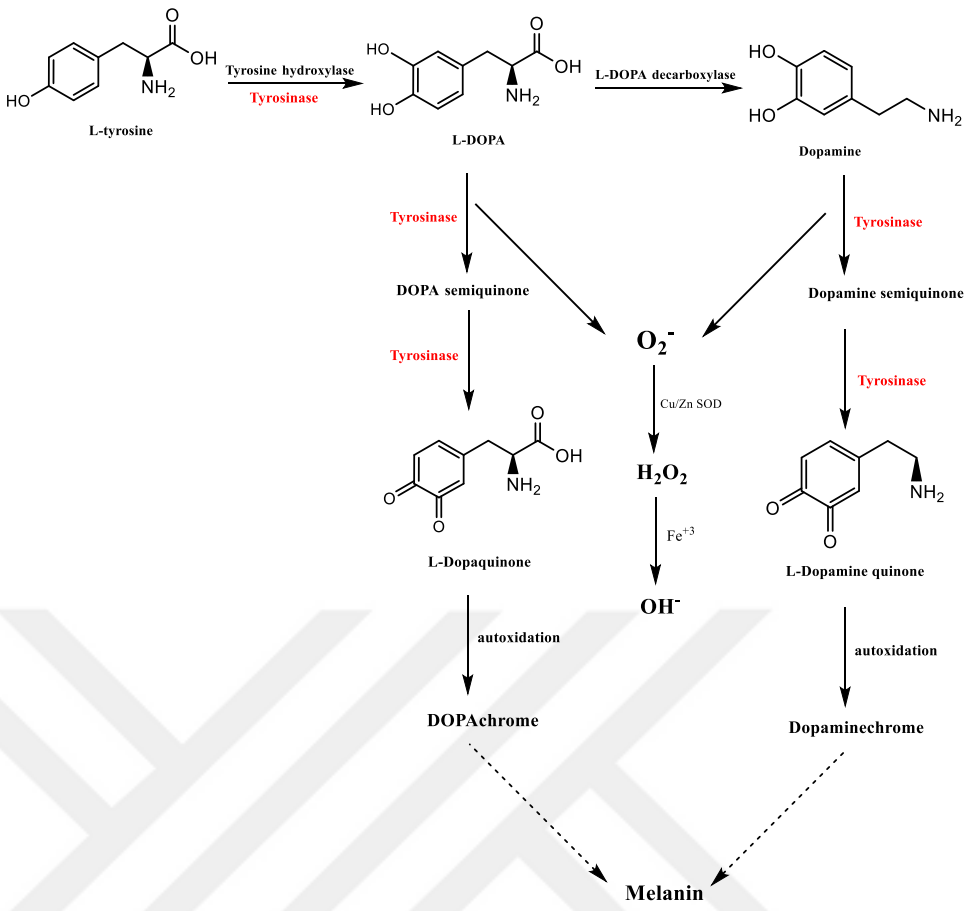


Figure 2.39. Production of catechol o-quinones and other reactive oxygen species during the enzymatic action of tyrosinase (from Hasegawa, [126])

Moreover, dysregulated tyrosinase activity in the skin can contribute to the development of cancer. Consequently, it is crucial to regulate melanin synthesis and, consequently, tyrosinase activity in order to safeguard human health, particularly in individuals who have a predisposition or are at risk of developing such diseases [127, 128] The significance of tyrosinase inhibitors has grown significantly due to their ability to impede melanin synthesis. They have found widespread applications in various fields, including the cosmetic industry, pharmaceuticals, and the food industry, owing to their potential for reducing pigmentation. As a result, there is a strong interest in developing efficient and safe tyrosinase inhibitors in the cosmetic, agricultural, and medical sectors.[129, 130]

Various tyrosinase inhibitors, including hydroquinone, arbutin, kojic acid, ellagic acid, and tranexamic acid, have been employed as skin-whitening agents in commercial products (**Figure 2.40.**). However, these inhibitors are accompanied by

specific drawbacks and potential side effects. These include concerns of carcinogenicity, chemical instability, and limited bioavailability, which can impact their safety and effectiveness as long-term solutions [10, 117].

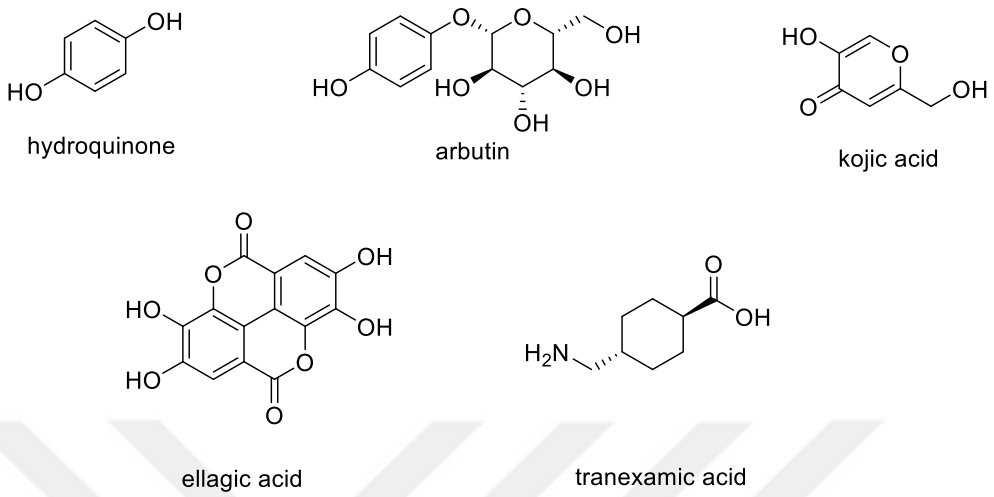


Figure 2.40. Structures of various tyrosinase inhibitors

Kojic acid has emerged as the most extensively studied tyrosinase inhibitor. Presently, it finds application as a skin whitening agent in cosmetic products and as a food additive to prevent enzymatic browning in the food industry. It was demonstrated that kojic acid, a recognized inhibitor of fungal tyrosinase, exhibits competitive inhibition on monophenolase activity. Additionally, it exerts a mixed inhibition effect on diphenolase activity. Polyphenols are a widely studied class of tyrosinase inhibitors, characterized by their multiple phenolic functional groups. Among them, flavonoids have received considerable attention, and their structures have been extensively elucidated. These compounds are commonly found in various plant parts such as leaves, seeds, flowers, and bark. To date, more than 4000 flavonoids with clarified structures have been identified through research. Flavonoids serve as natural defense mechanisms in plants, protecting them against UV rays, pathogens, and herbivores. Plants of the Morus genus, in particular, exhibit high polyphenol content. Extracts derived from different parts of these plants have been purified to obtain numerous inhibitors, which are widely used in skin whitening due to their non-toxic effects [124, 131]

In a study conducted by Lee et al. in 2002, it was found that moracin (M-6,3'-*o*-*p*-glucopyranoside) extracted from Morus leaves exhibited a 4-5 times stronger

inhibitory effect on the diphenolase activity of tyrosinase compared to kojic acid. Another study by Ryu et al. in 2008 revealed that norartocarpetin (5,2,7',4'-tetrahydroxyflavone) purified from the bark of Morus plants demonstrated a remarkable inhibitory effect that was 10.4 times more potent than kojic acid against fungal tyrosinase activity.

Considering the information provided, the potential applications of tyrosinase inhibitors can be summarized as follows: [132]

I. In the food industry, both synthetic and natural tyrosinase inhibitors are extensively utilized either individually or in combination. Their purpose is to prevent undesired color changes that may occur due to enzymatic reactions catalyzed by polyphenol oxidase (PPO).

II. Tyrosinase inhibitors have significant cosmetic and therapeutic applications. They are employed in the treatment of Parkinson's disease, as well as for addressing hyperpigmentation and leprosy in individuals. These inhibitors offer potential benefits in managing and improving the conditions associated with these disorders, both for cosmetic purposes and therapeutic interventions.

3. MATERIALS AND METHODS

3.1. Chemical Studies

3.1.1. Materials

1*H*-Benzimidazole, 4-fluorobenzaldehyde, phenylhydrazine, 4-methylphenylhydrazine, 4-methoxyphenylhydrazine, 4-cyanophenylhydrazine, aniline, 4-fluoroaniline, *p*-toluidine, 3-methoxyaniline, 4-methoxyaniline, 3,4-dimethoxyaniline, 5-amino-2-methylbenzenesulfonamide, and mercaptoacetic acid used in our studies are products of Sigma Aldrich, Merck companies. Solvents used for the thin layer chromatography (TLC) system are n-hexane, ethyl acetate and methanol are products of Isolab. Tyrosinase from mushroom is product of Merck, Dulbecco's phosphate-buffered saline (DPBS) is product of Biological Industries.

3.1.2 General Synthesis Methods

4-(1*H*-Benzimidazol-1-yl) benzaldehyde (1)

To a solution of 20 mmol of 1*H*-benzimidazole and 20 mmol of 4-fluorobenzaldehyde in 20 ml of DMSO is added 22 mmol of potassium carbonate. It is mixed in the ultrasonic bath until it becomes a gel, then the temperature of the mixture is increased to 100-140^{o} C, then it is reduced to 60^{o} C. The mixture is poured into 400 ml of distilled water. The precipitate formed is filtered to obtain a solid. Melting point for 4-(1*H*-benzimidazol-1-yl)benzaldehyde is 162-164°C [133].

General Synthesis of 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino) thiazolidin-4-one (2a-d) and 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one (3a-g)

2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde (**1**) and 2 mmol of appropriate phenylhydrazine or aniline are heated under reflux for 4 hours in 10 ml of methanol in the presence of 1 drop of acetic acid. The solvent is evaporated and the obtained solid is heated with mercaptoacetic acid (1 ml) at 70°C for 8 hours. After the

mixture is cooled to room temperature, 10 ml of ethyl acetate are added to the reaction medium and extracted 3 times with 20 ml of sodium bicarbonate. After drying the organic phase with sodium sulfate, it is filtered and concentrated in vacuo. The resulting oily part is solidified by cold treatment with n-hexane, filtered and purified by column chromatography over silica gel using ethyl acetate / n-hexane /methanol 60:30:10 (v/v/v) as eluent.

3.1.3. Analytical Methods

Melting Point

Determination the melting points of the synthesized compounds were measured with the “Thomas Hoover Capillary Melting Point Apparatus” melting point detector. The melting points given are uncorrected values.

3.1.4. Spectroscopic Methods

IR Spectrum

The IR spectra of the compounds were obtained from the “Perkin Elmer FT-IR System Spectrum BX” spectrophotometer, “Reduced Total Reflection” (ATR) apparatus (MIRacleTM PIKE Technologies, zinc selenide (ZnSe) crystal) at Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Chemistry Research Laboratory. It was measured with the help of the wave number and evaluated in terms of the number of waves (cm^{-1}).

^{1}H-NMR, ^{13}C-NMR and HSQC spectrum

The ^{1}H-NMR, ^{13}C-NMR and HSQC spectra of the compounds were measured with Bruker Avance Neo 500 MHz spectrometer devices in deuterated dimethylsulfoxide (DMSO-d_6, Merck) or deuterated chloroform ($CDCl_3$, Merck) solution at Ankara University Faculty of Pharmacy Central Laboratory, values are given in Hz and the spectra are evaluated on the δ (ppm) scale.

Mass Spectrum

The mass spectra of the compounds were measured in the "Micromass ZQ LC-MS Spectrometer" device with the ESI method in Hacettepe University Faculty of

Pharmacy, Department of Pharmaceutical Chemistry Research Laboratory and were obtained using the "Mass Lynx" software.

3.2. Biological Impact Studies

Tyrosinase inhibitory activity and cytotoxicity assays of synthesized compounds were carried out in the Hacettepe University Toxicology Research Laboratory (Sihhiye, Ankara, Turkey).

3.2.1. Tyrosinase inhibition assay

L-DOPA was used to assess the tyrosinase inhibitory effects of the compounds [11] . For a screening experiment involving numerous samples, the 96-well microplate technique is particularly practical. In summary, the following reaction mixtures were placed in the twelve wells designated for A (three wells), B (three wells), C (three wells), and D (three wells): A, 120 ml of a 1/15 M phosphate buffer (pH 6.8) and 40 ml of tyrosinase (46 units/ml) in the same buffer; B, 160ml of the same buffer; C, 80ml of the same buffer, 40 ml of mushroom tyrosinase (46 units/ml) in the same buffer, 40 ml of an appropriate amount of the sample-buffer solution containing 5% DMSO to dissolve the sample; D, 120 ml of the same buffer and 40 ml of the same amount of the sample solution containing 5% DMSO. Each well's contents were combined, and after 10 minutes of incubation at 23°C, 3 mM of L-DOPA was added to each well. The absorbances were measured at 475 nm following a 10 minutes' incubation period at 23°C. The percentage inhibition of the tyrosinase activity was calculated by the following equation:

$$\{[(A-B) -(C-D)]/(A-B)\}x100$$

3.2.2. Cytotoxicity Assay

Several methodologies are employed in toxicological screening to assess cell viability or proliferation when exposed directly or indirectly to substances. Among these methods, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test stands out as one of the most commonly utilized and is widely regarded as the gold standard for cytotoxicity measurements [134]. The MTT method, developed by Mossman in 1983 [135], involves a reaction depicted in **Figure 3.1.**

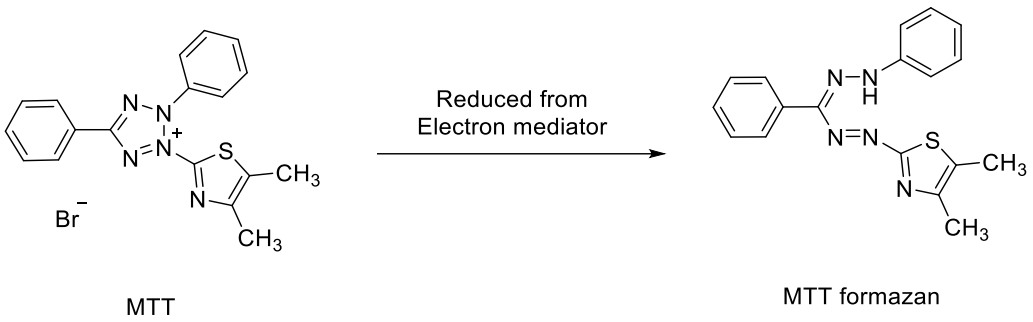


Figure 3.1. Reduction of MTT to MTT formazan [135]

This test is performed in 96-well plates, and its primary objective is to quantitatively assess the absorbance of purple formazan crystals, composed of tetrazolium and mitochondrial reductase enzymes, using a spectrophotometer. The increased dehydrogenase activity in proliferating living cells leads to the production of these colored crystals, allowing for colorimetric measurements. To evaluate the impact of the compounds on the viability of murine fibroblast (3T3) cells, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay described by Ohguro et al. with slight modifications was employed [136]. After a two-week growth period, the cells were seeded in 96-well plates at a concentration of 10,000 cells per well and incubated for an additional 24 hours. Subsequently, the cells were exposed to the compounds at concentrations ranging from 1.56 µM to 200 µM for 24 hours. Following the treatment, the culture medium in each well was substituted with 100 µl of a 1 mg/ml MTT solution in DPBS. The cells were then incubated for an additional 3 hours at 37°C. Subsequently, 100 µl of DMSO was added to dissolve the formazan crystals, and the optical density was measured at 570 nm using a microplate reader (SpectraMax M2 Molecular Devices Limited, Berkshire, UK). The cytotoxicity of the compounds was determined by calculating the percentage of cell viability in relation to the untreated (control) cells (% cell viability). Each experiment was conducted twice, and the results were reported as the mean ± standard deviation (SD).

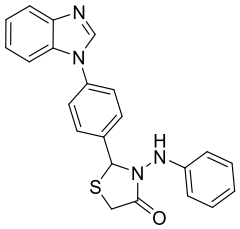
3.2.3. Statistical analysis

Statistical analysis was performed using student's T-test in the Microsoft Excel Windows 10 in order to compare the cytotoxic potentials of the compounds.

4. RESULTS

4.1. Chemical Studies

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(phenylamino)thiazolidin-4-one (2a)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of phenylhydrazine with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.160g (41.40%)

It is a white compound, and its melting point is 233-235 °C.

FT-IR spectrums (cm^{-1}); υ 3160 (N-H stretch), 2950 (aliphatic C-H stretch), 1698 (C=O stretch), 1513, 1493 (C=N and C=C stretch), 1266 (C-N stretch).

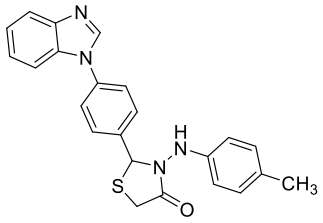
^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.84 (1H; d; thiazolidine $H_{5a,}$ *J= 15.80 Hz*), 3.98-4.01 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.80,* J_2 *= 1.70 Hz*), 6.03 (1H; d; thiazolidine H_2, *J= 1.30 Hz*), 6.70 (2H; d; Ar- $H_{2'',6'',}$ *J= 7.70 Hz*), 6.78 (1H; t; Ar- $H_{4''}$*)*, 7.19 (2H; t; Ar- $H_{3'',5''}$), 7.31-7.37 (2H; m; benzimidazole $H_{5,6}$), 7.62-7.64 (1H; dd; benzimidazole $H_{7,}$ J_1 *=7.00,* J_2 *= 1.65 Hz*), 7.67 (2H; d; Ar- $H_{3',5'}$ *J= 8.55 Hz*), 7.72 (2H; d; Ar- $H_{2',6'}$, *J= 8.55 Hz*), 7.78-7.80 (1H; dd; benzimidazole $H_{4,}$ J_1 *=6.98,* J_2 *= 1.80 Hz*), 8.29 (1H; s; NH), 8.58 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 29.28 (thiazolidine C_5), 61.24 (thiazolidine C_2), 111.18, 112.69, 119.83, 120.47, 123.02, 124.02, 124.20, 129.31, 129.46, 133.40, 136.47, 140.25, 143.74, 144.30, 146.92, 169.57 (CO) ppm.

ESI- MS (*m/z*): 387.33 $[M+H]^+$ (100 %), 409.30 $[M+Na]^+$.

Analysis: $C_{22}H_{18}N_4OS$ (M.A. 386.47 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-((4-methylphenyl)amino) thiazolidin-4-one (2b)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 4-methylphenylhydrazine with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.083g (20.64%)

It is a white compound, and its melting point is 155-157 °C.

FT-IR spectrums (cm^{-1}); υ 3241 (N-H stretch), 2922 (aliphatic C-H stretch), 1687 (C=O stretch), 1513, 1455 (C=N and C=C stretch), 1229 (C-N stretch).

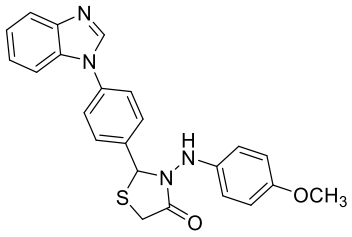
^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.19 (3H; s; CH_3), 3.80 (1H; d; thiazolidine $H_{5a,}$ *J= 15.80 Hz*), 3.96-4.00 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.80,* J_2 *= 1.55 Hz*), 6.00 (1H; d; thiazolidine H_2, *J= 0.95 Hz*), 6.61 (2H; d; Ar- $H_{2'',6'',}$ *J= 8.20 Hz*), 6.99 (2H; d; Ar- $H_{3'',5'',}$ *J= 8.35 Hz*), 7.32-7.35 (2H; m; benzimidazole $H_{5,6}$), 7.61-7.66 (3H; m; benzimidazole $H_{7,}$ Ar- $H_{3',5'}$), 7.71 (2H; d; Ar- $H_{2',6'}$, *J= 8.45 Hz*), 7.79 (1H; d; benzimidazole $H_{4,}$ *J =7.68 Hz*), 8.11 (1H; s; NH), 8.58 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR & HSQC (DMSO-d_6, 125 MHz) δ 20.60 (CH_3), 29.32 (thiazolidine C_5), 61.30 (thiazolidine C_2), 111.17 (benzimidazole C_7), 112.92 ($C_{2'', 6''}$), 120.46 (benzimidazole C_4), 123.01 (benzimidazole C_5), 124.02 (benzimidazole C_6), 124.20 ($C_{2', 6'}$), 128.52 ($C_{4''}$), 129.30 ($C_{3', 5'}$), 129.83 ($C_{3'', 5''}$), 133.41 ($C_{1'}$), 136.45 (benzimidazole C_{7a}), 140.30 ($C_{4'}$), 143.74 (benzimidazole C_2), 144.30 (benzimidazole C_{3a}) 144.56 ($C_{1''}$), 169.54 (CO) ppm.

ESI- MS (*m/z*): 401.34 $[M+H]^+$ (100 %), 423.31 $[M+Na]^+$.

Analysis: $C_{23}H_{20}N_4OS$ (M.A. 400.50 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-((4-methoxyphenyl)amino) thiazolidin-4-one (2c)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl) benzaldehyde and 2 mmol of 4-methoxyphenylhydrazine with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.09g (21.61%)

It is a white compound, and its melting point is 158-160 °C.

FT-IR spectrums (cm^{-1}); υ 3348 (N-H stretch), 2851 (aliphatic C-H stretch), 1660 (C=O stretch), 1590, 1494, 1445 (C=N and C=C stretch), 1220 (C-N stretch), 1114 (C-O stretch).

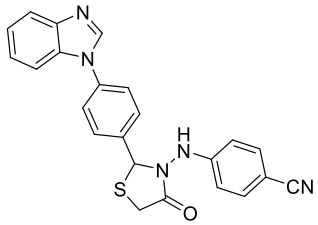
^{1}H-NMR (DMSO-d_6, 500 MHz) δ 3.67 (3H; s; -OCH_3), 3.80 (1H; d; thiazolidine H_{5a}, *J*= 15.75 *Hz*), 3.95-3.99 (1H; dd; thiazolidine H_{5b}, J_1 =15.75, J_2 = 1.75 *Hz*), 6.01 (1H; d; thiazolidine H_2, *J* =1.55 *Hz*), 6.68 (2H; d; Ar- $H_{3'',5''}$, *J*= 6.7 *Hz*), 6.80 (2H; d; Ar- $H_{2'',6''}$, *J*= 7.0 *Hz*), 7.32-7.35 (2H; m; benzimidazole $H_{5,6}$), 7.62-7.66 (3H; m; benzimidazole H_7, Ar- $H_{3',5'}$), 7.71 (2H; d; Ar- $H_{2',6'}$, *J*= 7.60 *Hz*), 7.79 (1H; d; benzimidazole H_4, *J*= 7.4 *Hz*), 7.94 (1H; s; NH), 8.58 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 29.37 (thiazolidine C_5), 55.76 (-OCH_3), 61.39 (thiazolidine C_2), 111.17, 114.37, 114.91, 120.47, 123.01, 124.01, 124.19, 129.30, 133.42, 136.43, 140.35, 140.56, 143.73, 144.30, 153.64,169.58 (CO) ppm.

ESI- MS (*m/z*): 417.32 $[M+H]^+$ (100 %), 439.29 $[M+Na]^+$.

Analysis: $C_{23}H_{20}N_4O_2S$ (M.A. 416.50 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-((4-cyanophenyl)amino) thiazolidin-4-one (2d)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 4-cyanophenylhydrazine with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.091g (22.15%)

It is a white compound, and its melting point is 145-146 °C.

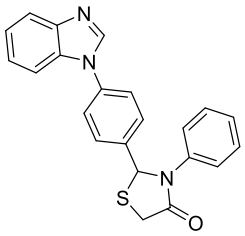
FT-IR spectrums (cm^{-1}); υ 3294 (N-H stretch), 2850 (aliphatic C-H stretch), 2380 (C≡N stretch), 1677 (C=O stretch), 1607, 1508, 1487, 1454 (C=N and C=C stretch), 1227 (C-N stretch).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.86 (1H; d; thiazolidine $H_{5a,}$ *J= 16.15 Hz*), 3.92-3.96 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=16.22*, J_2 *= 1.75 Hz*), 5.86 (1H; d; thiazolidine H_2, *J= 1.50 Hz*), 6.81 (2H; d; Ar- $H_{2'',6'',}$ *J= 8.80 Hz*), 6.93 (1H; s; NH), 7.41-7.43 (2H; m; benzimidazole $H_{5,6}$), 7.52-7.62 (7H; m; benzimidazole H_7, Ar-$H_{2',3',5',6',3'',5''}$), 7.90-7.92 (1H; m; benzimidazole H_4), 8.30 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR ($CDCl_3$, 125 MHz) δ 29.46 (thiazolidine C_5), 61.90 (thiazolidine C_2), 104.22, 110.62, 113.11, 119.14, 120.17, 123.89, 124.51, 124.63, 129.37, 132.93, 133.87, 136.85, 138.46, 141.56, 148.93, 169.52 (CO) ppm.

Analysis: $C_{23}H_{17}N_5OS$ (M.A. 411.48 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-phenylthiazolidin-4-one (3a)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of aniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.188g (50.61%)

It is a white compound, and its melting point is 153-155 °C.

FT-IR spectrums (cm^{-1}); υ 3050 (aromatic C-H stretch), 2985 (aliphatic C-H stretch), 1692 (C=O stretch), 1514, 1478, 1455 (C=N and C=C stretch), 1229 (C-N stretch).

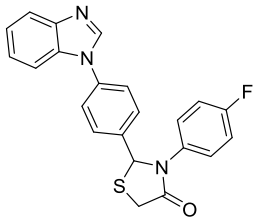
^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.95 (1H; d; thiazolidine $H_{5a,}$ *J= 15.85 Hz*), 4.04-4.07 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.87,* J_2 *= 1.57 Hz*), 6.25 (1H; s; thiazolidine H_2), 7.21-7.26 (3H; m; Ar- $H_{3'',4'',5''}$), 7.33-7.40 (4H, m, Ar- $H_{2',6',2'',6''}$), 7.48-7.56 (5H, m; benzimidazole $H_{5,6,7,}$ Ar- $H_{3',5'}$), 7.90-7.92 (1H, dd, benzimidazole $H_{4;}$ J_1 *=7.70*, J_2 *= 1.70 Hz*), 8.23 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR ($CDCl_3$, 125 MHz) δ 33.47 (thiazolidine C_5), 64.81 (thiazolidine C_2), 110.58, 120.33, 123.52, 124.27, 124.31, 125.55, 127.37, 128.80, 129.35, 133.09, 136.37, 137.27, 139.77, 141.68, 142.69, 170.88 (CO) ppm.

ESI- MS (*m/z*): 372.32 $[M+H]^+$ (100 %).

Analysis: $C_{22}H_{17}N_3OS$ (M.A. 371.46 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(4-fluorophenyl)thiazolidin-4-one (3b)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 4-fluoroaniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.178g (45.70%)

It is a white compound, and its melting point is 169-170 °C.

FT-IR spectrums (cm^{-1}); υ 3079 (aromatic C-H stretch), 1694 (C=O stretch), 1503, 1482, 1457 (C=N and C=C stretch), 1227 (C-N stretch).

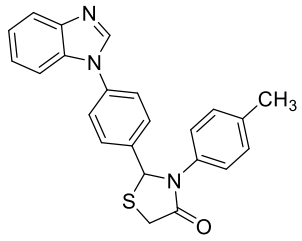
^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.95 (1H; d; thiazolidine $H_{5a,}$ *J= 15.90 Hz*), 4.03-4.06 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.92,* J_2 *= 1.55 Hz*), 6.17 (1H; s; thiazolidine H_2), 7.02-7.06 (2H; t; Ar- $H_{3'',5''}$), 7.19-7.22 (2H; m; Ar- $H_{2'',6''}$), 7.37-7.40 (2H; m; benzimidazole $H_{5,6}$), 7.49-7.55 (5H; m; benzimidazole $H_{7,}$ Ar- $H_{2',3',5',6'}$), 7.89-7.91 (1H; dd; benzimidazole $H_{4,}$ J_1 *=6.05,* J_2 *= 2.30 Hz*), 8.27 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR ($CDCl_3$, 125 MHz) δ 33.34, 42.08 (thiazolidine C_5), 64.94 (thiazolidine C_2), 110.58, 116.31, 116.49, 120,27, 123.59, 124.33, 124.39, 127.58, 127.65, 128.94, 133.14, 136.52, 139.44, 141.89, 142.56, 160.28, 162.25, 170.98, 172.56 (CO) ppm.

ESI- MS (*m/z*): 390.30 $[M+H]^+$ (100 %), 412.27 $[M+Na]^+$.

Analysis: $C_{22}H_{16}FN_3OS$ (M.A. 389.45 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(4-methylphenyl)thiazolidin-4-one (3c)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 4-methylaniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.100g (25.94%)

It is a white compound, and its melting point is 95-97 °C.

FT-IR spectrums (cm^{-1}); υ 3077 (aromatic C-H stretch), 1681 (C=O stretch), 1514, 1480 (C=N and C=C stretch), 1230 (C-N stretch).

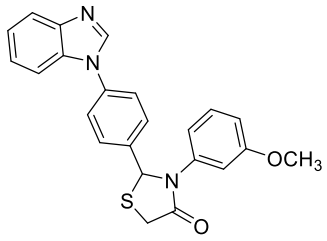
^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.30 (3H; s; CH_3), 3.95 (1H; d; thiazolidine $H_{5a,}$ *J= 15.65 Hz*), 4.03-4.07 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.80,* J_2 *= 1.70 Hz*), 6.25 (1H; d; thiazolidine H_2, *J= 1.05 Hz*), 7.11 (2H; d; Ar- $H_{3'',5'',}$ *J= 8.55 Hz*), 7.15 (2H; d; Ar- $H_{2'',6'',}$ *J= 8.35 Hz*), 7.37-7.40 (2H; m; benzimidazole $H_{5,6}$), 7.48-7.56 (5H; m; benzimidazole $H_{7,}$ Ar- $H_{2',3',5',6'}$), 7.91 (1H; d; benzimidazole $H_{4,}$ *J= 7.75 Hz*), 8.27 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR ($CDCl_3$, 125 MHz) δ 21.06 (thiazolidine C_5), 33.44 (CH_3), 64.90 (thiazolidine C_2), 110.66, 120.18, 123,64, 124.30, 124.36, 125.60, 128.90, 130.03, 133.04, 134.54, 136.24, 137.46, 140.00, 141.75, 142.29,170.92 (CO) ppm.

ESI- MS (*m/z*): 386.33 $[M+H]^+$ (100 %), 408.30 $[M+Na]^+$.

Analysis: $C_{23}H_{19}N_3OS$ (M.A. 385.49 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(3-methoxyphenyl)thiazolidin-4-one (3d)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 3-methoxyaniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.063g (15.69%)

It is a white compound, and its melting point is 120-122 °C.

FT-IR spectrums (cm^{-1}); υ 2931 (aliphatic C-H stretch), 1681 (C=O stretch), 1602, 1514, 1493, 1454 (C=N and C=C stretch), 1229 (C-N stretch).

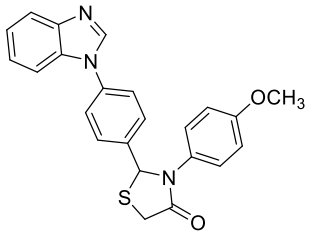
^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.70 (3H; s; -OCH_3), 3.92 (1H; d; thiazolidine $H_{5a,}$ *J= 15.75 Hz*), 4.09 (1H; d; thiazolidine $H_{5b,}$ *J =15.80*), 6.69 (1H; s; thiazolidine H_2), 6.75-6.77 (1H; dd; Ar- $H_{4''}$, *J_1 =8.30, J_2 =2.15 Hz*), 6.97-6.99 (1H; dd; Ar- $H_{6''}$, *J_1 =8.05, J_2 =0.80 Hz*), 7.03 (1H; s; Ar- $H_{2''}$), 7.22-7.26 (1H; m; Ar- $H_{5''}$), 7.30-7.31 (2H; m; benzimidazole $H_{5,6}$), 7.58 (2H; d; Ar- $H_{3',5'}$ *J= 7.20 Hz*), 7.62-7.67 (3H; m; benzimidazole $H_{7,}$ Ar- $H_{2',6'}$), 7.75-7.77 (1H; dd; benzimidazole $H_{4,}$ *J_1 =8.20, J_2 = 2.10 Hz*), 8.55 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 33.17 (thiazolidine C_5), 55.67 (OCH_3), 63.14 (thiazolidine C_2), 111.17, 111.97, 112.27, 117.99, 120.44, 122.99, 123.98, 124.12, 129.03, 130.04, 133.23, 136.32, 139.12, 140.14, 143.69, 144.28, 159.86, 170.97 (CO) ppm.

ESI- MS (*m/z*): 402.36 $[M+H]^+$ (100 %), 424.33 $[M+Na]^+$.

Analysis: $C_{23}H_{19}N_3O_2S$ (M.A. 401.48 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(4-methoxyphenyl)thiazolidin-4-one (3e)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 4-methoxyaniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.200g (49.81%)

FT-IR spectrums (cm^{-1}); υ 3063 (aromatic C-H stretch), 2835 (aliphatic C-H stretch), 1681 (C=O stretch), 1510, 1455 (C=N and C=C stretch), 1231 (C-N stretch).

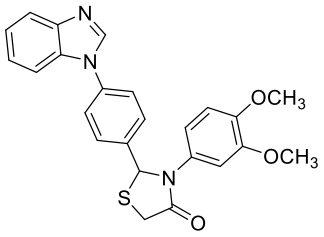
^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.69 (3H; s; OCH_3), 3.91 (1H; d; thiazolidine $H_{5a,}$ *J= 15.65 Hz*), 4.06 (1H; dd; thiazolidine $H_{5b,}$ J_1*= 15.65 Hz*, J_2 *=1.55 Hz*), 6.57 (1H; d; thiazolidine $H_{2,}$ *J =1.2 Hz*), 6.89 (2H; d; Ar- $H_{3'',5''}$), 7.28-7.32 (4H; m; benzimidazole $H_{5,6,}$ Ar- $H_{2',6'}$), 7.57-7.64 (5H; m; benzimidazole $H_{7,}$ Ar- $H_{3',5',2'',6''}$), 7.75-7.77 (1H; dd; benzimidazole $H_{4,}$ J_1 *=7.68*, J_2 *= 2.35 Hz*), 8.54 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 33.02 (thiazolidine C_5), 55.64 (OCH_3), 63.47 (thiazolidine C_2), 111.18, 114.35, 114.53, 120.44, 121.27, 122.99, 123.98, 124.09, 127.64, 129.19, 130.68, 133.24. 136.33, 140.22, 143.68, 144.29, 158.01, 170.90 (CO) ppm.

ESI- MS (*m/z*): 402.32 $[M+H]^+$ (100 %), 424.29 $[M+Na]^+$.

Analysis: $C_{23}H_{19}N_3O_2S$ (M.A. 401.48 g/mol)

2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-3-(3,4-dimethoxyphenyl) thiazolidin-4-one (3f)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 3,4-dimethoxyaniline with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.105g (24.33%)

It is a white compound and its melting point is 125-126 °C.

FT-IR spectrums (cm^{-1}); υ 2929 (aliphatic C-H stretch), 1677 (C=O stretch), 1512, 1453 (C=N and C=C stretch), 1231 (C-N stretch).

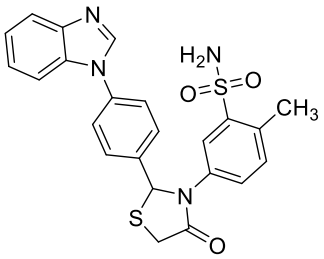
^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.68 (3H; s; OCH_3), 3.69 (3H; s; OCH_3), 3.91 (1H; d; thiazolidine $H_{5a,}$ *J= 15.65 Hz*), 4.06-4.09 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.67,* J_2 *= 1.55 Hz*), 6.58 (1H; s; thiazolidine H_2), 6.86-6.89 (2H; m; Ar- $H_{5'',6''}$), 7.00 (1H; d; Ar- $H_{2'',}$ *J= 2.10 Hz*), 7.29-7.32 (2H; m; benzimidazole $H_{5,6}$), 7.57-7.59 (1H; dd; benzimidazole $H_{7,}$ J_1 *=7.38,* J_2 *= 1.90 Hz*), 7.63 (2H; d; Ar- $H_{3',5'}$ *J= 7.65 Hz*), 7.66 (2H; d; Ar- $H_{2',6'}$, *J= 7.65 Hz*), 7.75-7.77 (1H; dd; benzimidazole H_4 ,J_1 *=7.20,* J_2 *= 1.50 Hz*), 8.55 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 33.50 (thiazolidine C_5), 55.91 (OCH_3), 56.10 (OCH_3), 63.57 (thiazolidine C_2), 110.64, 111.16, 111.84, 118.89, 120.44, 123.00, 123.99, 124.06, 129.28, 130.77, 133.24, 136.33, 140.28, 143.69, 144.28, 147.76, 148.97, 170.85 (CO) ppm.

ESI- MS (*m/z*): 432.31 $[M+H]^+$ (100 %), 454.28 $[M+Na]^+$.

Analysis: $C_{24}H_{21}N_3O_3S$ (M.A. 431.51 g/mol)

5-(2-(4-(1*H*-Benzimidazol-1-yl)phenyl)-4-oxothiazolidin-3-yl)-2-methylbenzenesulfonamide (3g)



It was synthesized from 2 mmol of 4-(1*H*-benzimidazol-1-yl)benzaldehyde and 2 mmol of 5-amino-2-methylbenzenesulfonamide with mercaptoacetic acid according to the general method given in 3.1.2 and purified by column chromatography over silica gel using ethyl acetate / *n*-hexane /methanol 60:30:10 (v/v/v) as eluent. Yield: 0.143g (30.76%)

It is a white compound, and its melting point is 165-167 °C.

FT-IR spectrums (cm^{-1}); υ 3237 (N-H stretch), 3069 (aromatic C-H stretch), 2925 (aliphatic C-H stretch), 1677 (C=O stretch), 1515, 1491, 1455 (C=N and C=C stretch), 1323, 1159 (SO_2 symmetric and antisymmetric stretch), 1231 (C-N stretch).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.67 (3H; s; CH_3), 3.94 (1H; d; thiazolidine $H_{5a,}$ *J= 15.70 Hz*), 4.14-4.15 (1H; dd; thiazolidine $H_{5b,}$ J_1 *=15.80,* J_2 *= 1.40 Hz*), 6.67 (1H; d; thiazolidine $H_{2,}$ *J= 0.70*), 7.29-7.35 (3H; m; benzimidazole $H_{5,6,}$ Ar- $H_{5''}$), 7.46-7.48 (3H, m; $NH_{2,}$ Ar- $H_{6''}$), 7.59-7.68 (5H; m; benzimidazole $H_{7,}$ Ar- $H_{2',3',5',6'}$), 7.75-7.77 (1H; m; benzimidazole H_4), 7.96 (1H; d; Ar- $H_{2''}$, *J= 2.30*), 8.54 (1H; s; benzimidazole H_2) ppm.

^{13}C-NMR (DMSO-d_6, 125 MHz) δ 19.75 (CH_3), 33.04 (thiazolidine C_5), 63.06 (thiazolidine C_2), 111.24, 120.43, 123.01, 123.99, 124.23, 124.63, 128.71, 129.12, 133.18, 133.23, 134.57, 135.84, 136.47, 139.75, 142.92, 143.69, 144.28, 171.25 (CO) ppm.

ESI- MS (*m/z*): 465.36 $[M+H]^+$ (100 %), 487.34 $[M+Na]^+$.

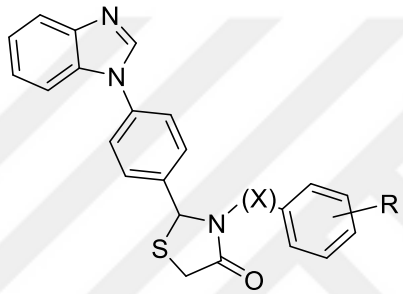
Analysis: $C_{23}H_{20}N_4O_3S_2$ (M.A. 464.56 g/mol)

4.2 Biological Activity Studies

4.2.1 *In vitro* Tyrosinase Inhibitory Activity Studies

The *in vitro* tyrosinase inhibitory activities of the target compounds (**2a-d** and **3a-g**) were tested and kojic acid used as positive control in the activity studies. The % inhibition values of the target compounds and the IC_{50} values of the compounds with a high inhibition (more than 80%) are given in **Table 4.1.**

Table 4.1. Tyrosinase percentage inhibition at 100 µM of **2a-d** and **3a-g** and IC_{50} values (µM) of **3b-g**



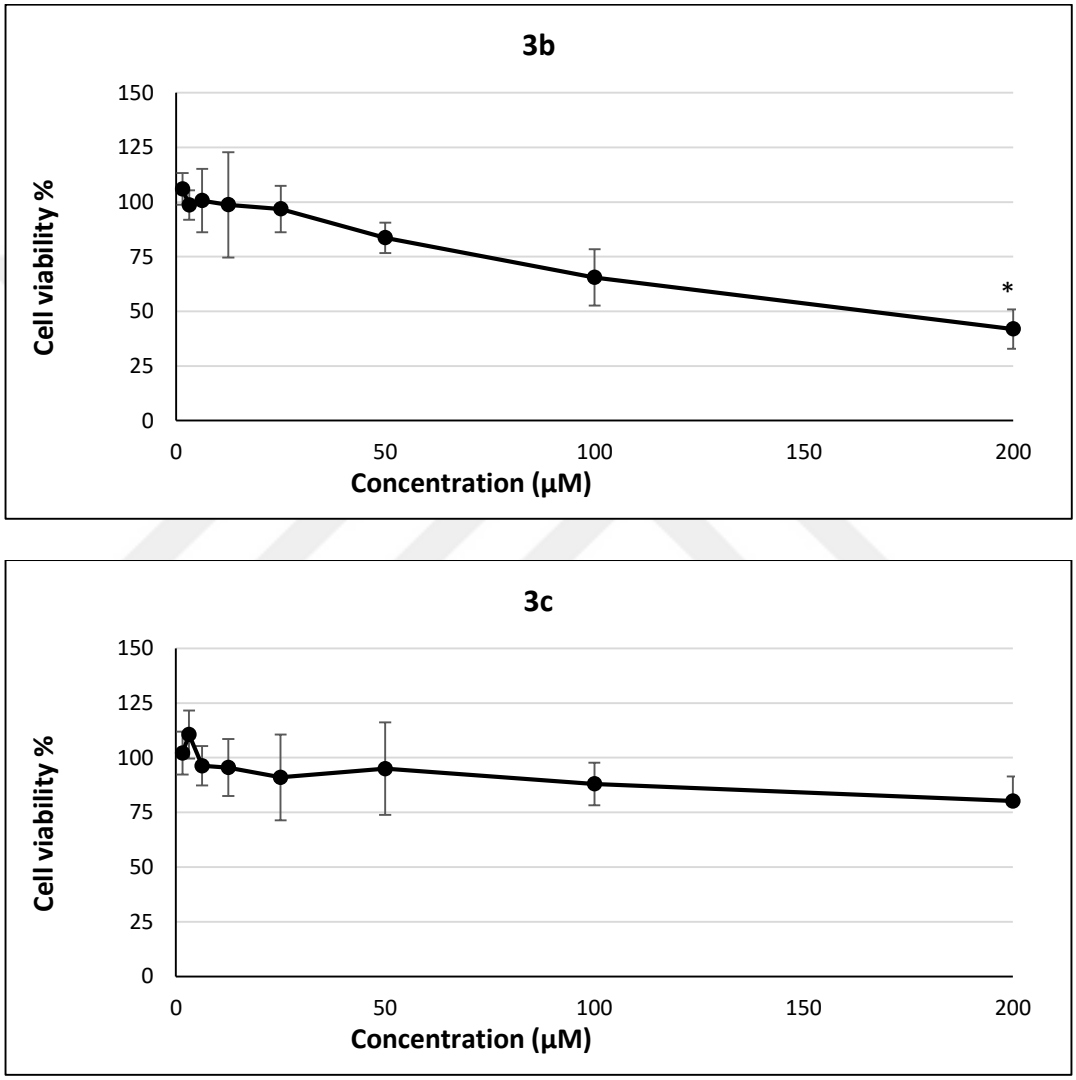
Compounds	**(X)**	**R**	**% inhibition[a]**	**IC_{50} (µM)**
2a	NH	H	44.63	-
2b	NH	4-CH_3	61.08	-
2c	NH	4-OCH_3	28.01	-
2d	NH	4-CN	71.57	-
3a	-	H	65.21	-
3b	-	4-F	92.95	113.69
3c	-	4-CH_3	90.66	103.95
3d	-	3-OCH_3	89.45	86.78
3e	-	4-OCH_3	88.88	119.20
3f	-	3,4-diOCH_3	88.48	106.25
3g	-	3-CH_3,4-SO_2NH_2	82.69	80.93
Kojic acid[b]			83.03	125.09

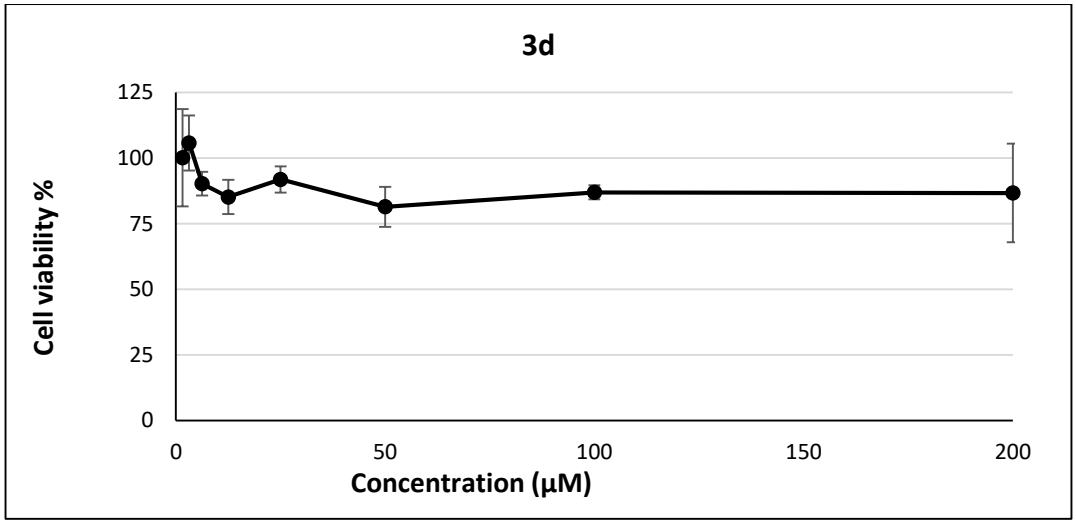
[a] at 100 µM

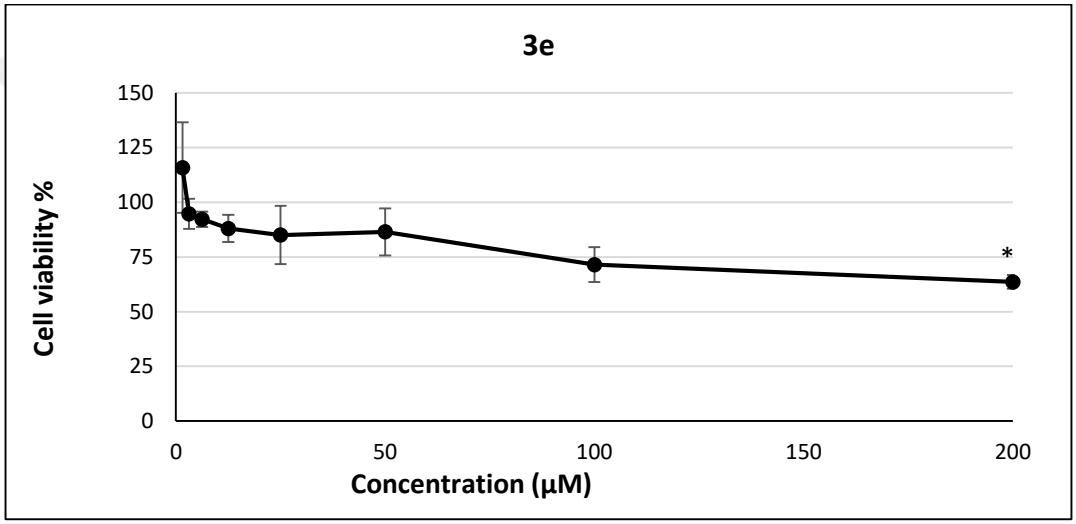
[b] at 50 µM

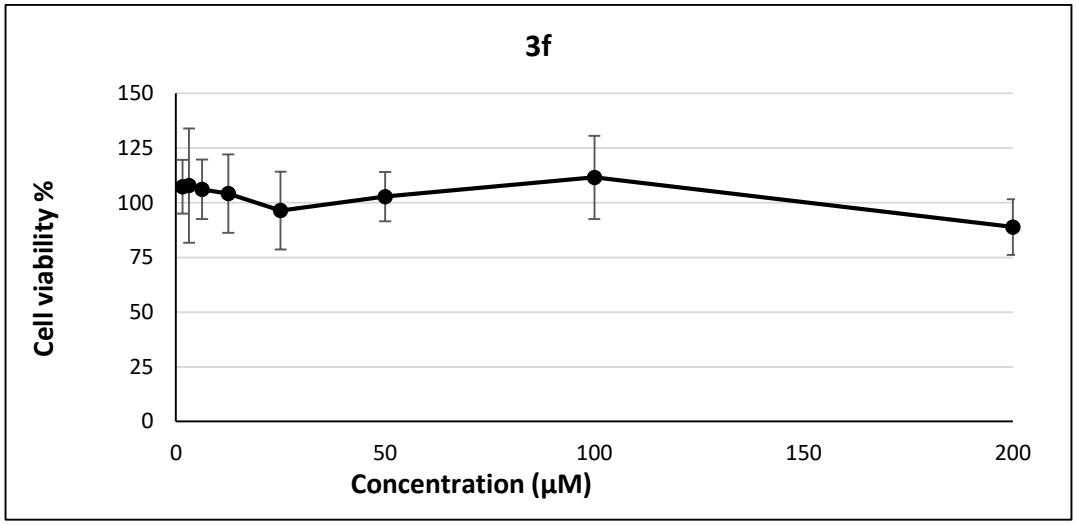
4.2.2. Cytotoxicity Assay

Compounds **3b-g** were evaluated for their cytotoxic effects against murine fibroblast 3T3 cell lines at concentrations between 1.56–200 μM at the end of a 24 h treatment by measuring % cell viabilities according to the MTT assay and the results are given in **Figure 4.1.**




3d
Cell viability %
125
100
75
50
25
0
0
50
100
150
200
Concentration (µM)


3e
Cell viability %
150
125
100
75
50
25
0
*
0
50
100
150
200
Concentration (µM)


3f
Cell viability %
150
125
100
75
50
25
0
0
50
100
150
200
Concentration (µM)

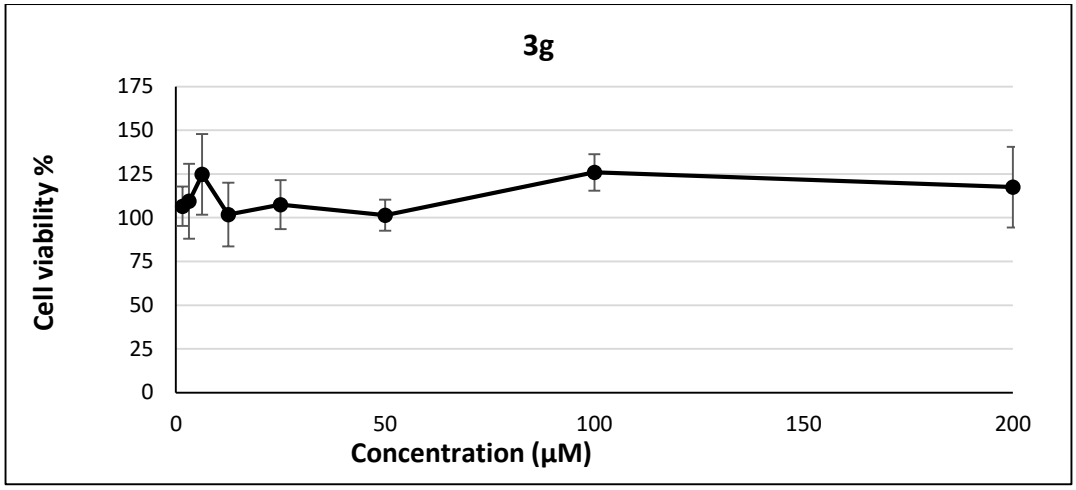


Figure 4.1. Cytotoxicity of target compounds **(*p<0.05)**

5. DISCUSSION

In this study, 4 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-one (**2a-d**) and 7 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one (**3a-g**) derivatives were synthesized. The molecular structures of these compounds were confirmed through analyses of IR, ^{1}H-NMR, ^{13}C-NMR and MS. Structure of one of the target compounds, **2b** was also characterized using HSQC NMR technique. Subsequently, their tyrosinase inhibitory activity and cytotoxic effects were investigated.

5.1 Chemical Studies

5.1.1 General Synthesis Procedures

The synthesis methods used in the preparation of the target compounds are shown below. **Figure 5.1.**

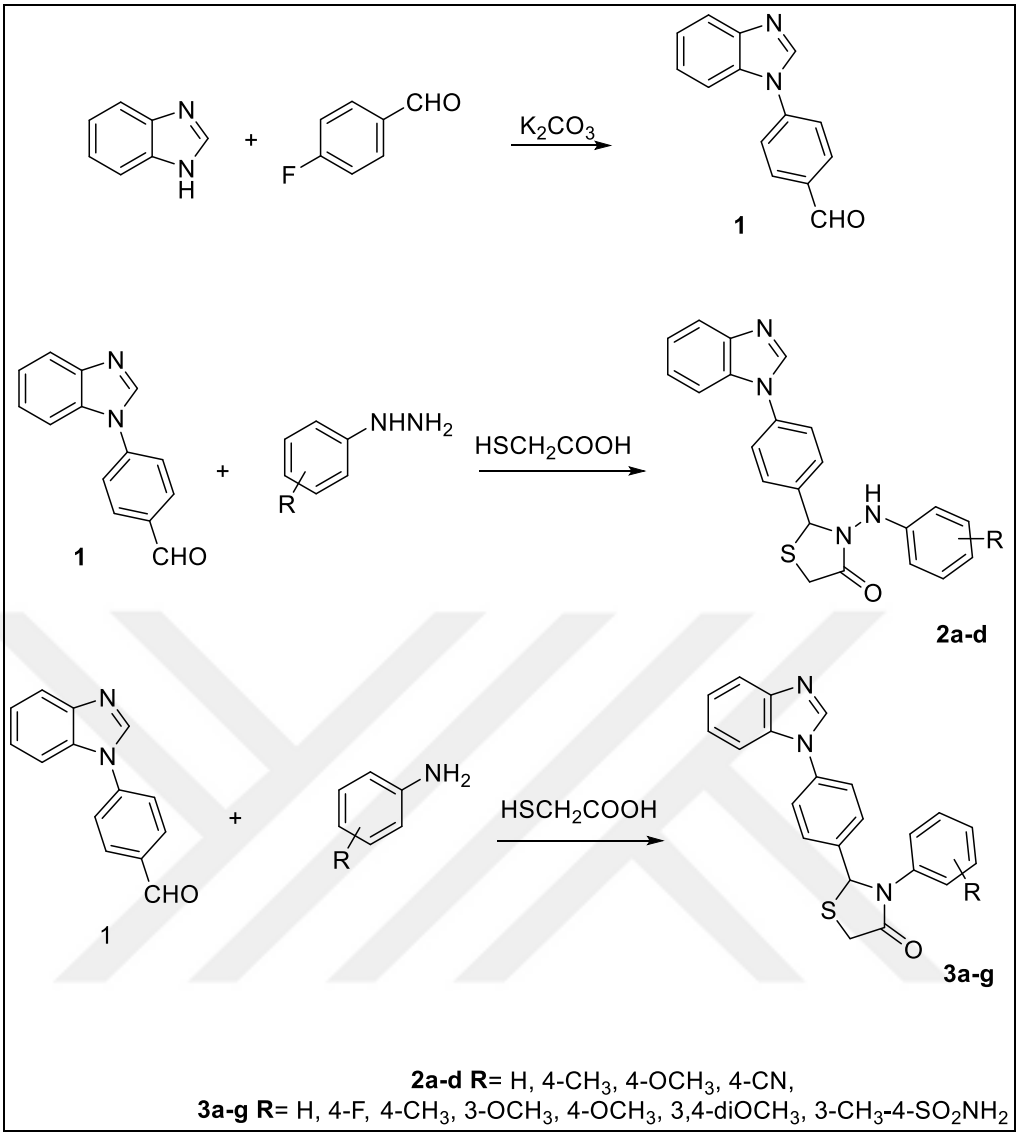


Figure 5.1. Synthesis of target compounds **2a-d** and **3a-g**

4-(1*H*-Benzimidazol-1-yl)benzaldehyde **1**, an intermediated compound, was prepared by reacting 4-fluorobenzaldehyde with 1*H*-benzimidazole in the presence of potassium carbonate as a catalyst. The melting point of **1** matched the data reported in the literature [133]. The 4-(1*H*-Benzimidazol-1-yl)benzaldehyde obtained during the initial stage of the synthesis studies are believed to be formed through a two-step aromatic nucleophilic substitution reaction initiated by 4-fluorobenzaldehyde. In the first step of the reaction, carbanion formation takes place when the nitrogen atom in the benzimidazole attacks the aryl carbon linked to the fluorine atom, utilizing its unpaired electrons. In the second step, the fluorine ion is separated from the carbanion,

resulting in the formation of the 4-(1*H*-Benzimidazol-1-yl)benzaldehyde molecule **Figure 5.2.**

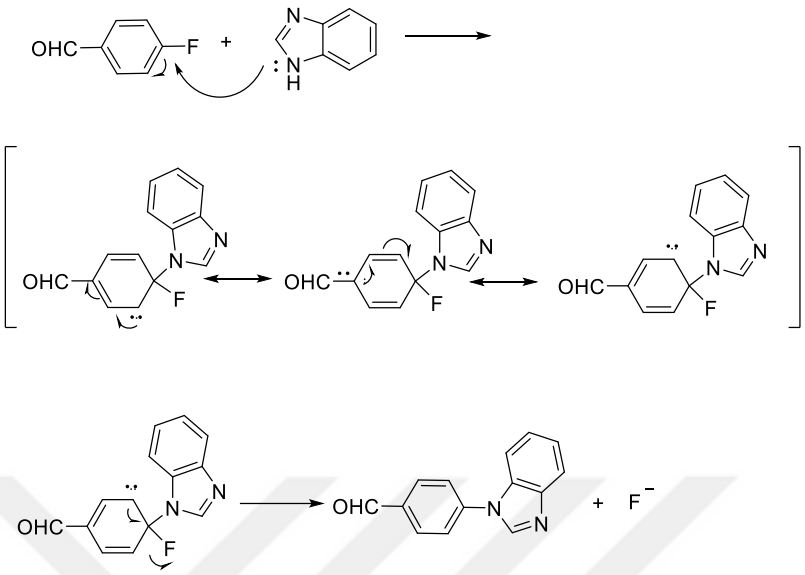


Figure 5.2. Proposed mechanism for the synthesis of 4-(1*H*-benzimidazol-1-yl)benzaldehyde

To obtain 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino) thiazolidin-4-one **(2a-d)** and 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one **(3a-g),** the aforementioned benzaldehyde was subjected to heat and acetic acid catalysis in the presence of appropriate phenylhydrazine or aniline derivatives. Collected after filtration, the precipitate was reacted with an excess of mercaptoacetic acid under heat conditions resulting in a yield ranging from 15 to 50%.

The initial stage of the 4-thiazolidinone cyclization involves the creation of *Schiff* bases. These *Schiff* bases are synthesized through a two-step process of nucleophilic addition to the carbonyl group, where the nucleophile is the amine. In the first step, the condensation of the carbonyl group with the primary amine results in the formation of a carbinolamine intermediate. Subsequently, in the second step, the intermediate undergoes dehydration to produce the *Schiff* base (**Figure 5.3.**) [137].

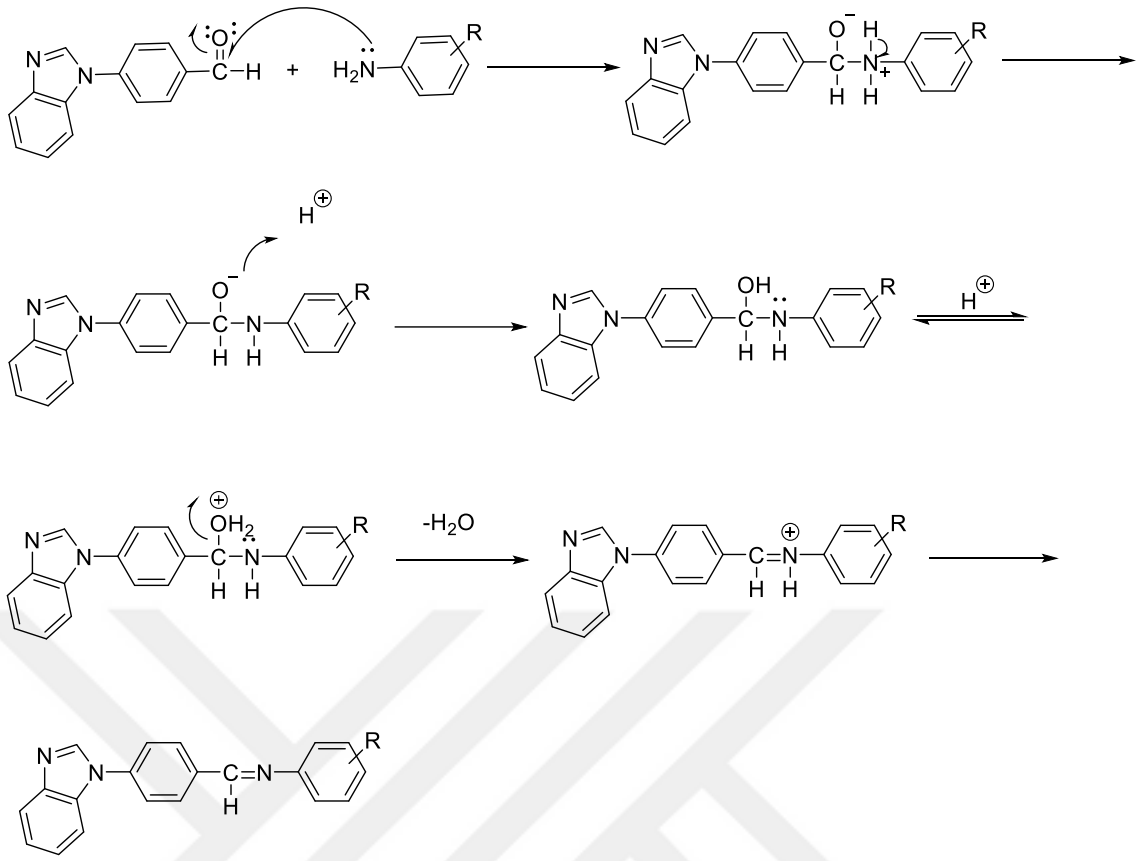


Figure 5.3. Proposed mechanism for the synthesis of *Schiff* bases

The formation of the 4-thiazolidinone ring occurred through a cycloaddition reaction of mercaptoacetic acid and a *Schiff* base, as illustrated in **Figure 5.4**[138].

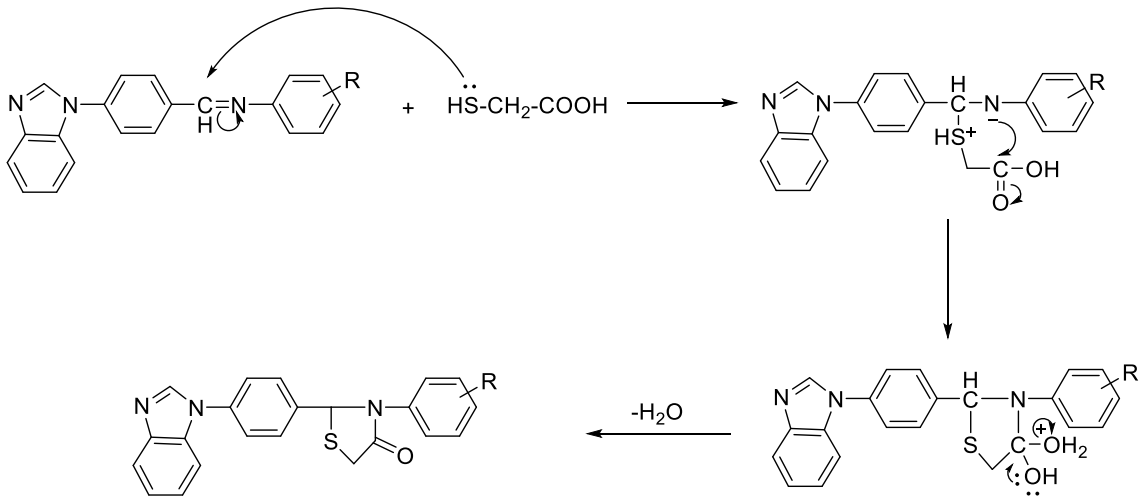
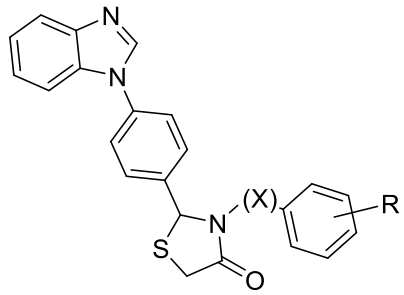


Figure 5.4. Proposed mechanism for the synthesis of 2-(4-(1*H*-benzoimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one

The melting points and % yields of the synthesized compounds are given in **Table 5.1.**

Table 5.1. The reaction yields and melting points of the synthesized compounds.



Compound	(X)	R	Yield (%)	Melting Point (°C)
2a	NH	H	41.40	233-235
2b	NH	4-CH_3	20.64	155-157
2c	NH	4-OCH_3	21.61	158-160
2d	NH	4-CN	22.15	145-146
3a	-	H	50.61	153-155
3b	-	4-F	45.70	169-170
3c	-	4-CH_3	25.94	95-97
3d	-	3-OCH_3	15.69	120-122
3e	-	4-OCH_3	49.81	97-98
3f	-	3,4-diOCH_3	24.33	125-126
3g	-	3-CH_3,4-SO_2NH_2	30.76	165

5.1.2 Spectral characterization of the Structures of 2a-d and 3a-g

In the IR spectra of the target compounds, displayed peaks around 1680 (C=O stretch) and 3200 (N-H stretch) cm^{-1} for 3-(phenylamino)thiazolidin-4-ones **2a-d** and 1680 (C=O stretch) for 3-(phenyl)thiazolidin-4-one **3a-g** indicated the formation of a thiazolidinone ring.

The ^{1}H-NMR spectra of the target compounds demonstrate the appearance of peaks belonging to the protons of the benzimidazole and phenyl rings, as well as the

peaks of the protons at the 2nd and 5th positions of the thiazolidinone ring. The H_b proton at position 5 of the thiazolidinone ring exhibited interactions with H_a (*J: 15.65 - 16.15 Hz*) and the proton at position 2 (*J: 1.40 - 1.70 Hz*), resulting in the formation of a double doublet at approximately 3.96-4.00 ppm. The H_a proton appeared as a doublet (*J: 15.80 Hz*) around 3.80 ppm. Furthermore, the peak attributed to the proton at position 2 of the ring appeared as a doublet (*J: 1.50 Hz*) at approximately 6.00 ppm. Protons at the 5th and 6th positions of the benzimidazole ring were generally seen as multiplet at around 7.30 ppm, while the protons at the 7th, 4th, and 2nd positions were seen at around 7.60, 7.80, 8.50 ppm respectively. The presence of these protons provides confirmation of the compounds' formation.

The ^{13}C-NMR spectra data reveals that the chemical shifts of the C_5 atom in the thiazolidinone moiety were observed within the range of 29.37-33.47 ppm. Furthermore, the C_2 atom of the thiazolidinone ring exhibited signals in the range of 60.40-61.90 ppm. As expected, the peak corresponding to the C=O functional group appears the farthest downfield, approximately around 170 ppm. In order to identify proton-bearing carbons and establish their correlation with the attached protons, HSQC technique was used in compound **2b**. The HSQC NMR spectrum shows a correlation between the single peak of methyl protons at 2.19 ppm and the peak of methyl carbon at 20.60 ppm. Also, the signal observed at δ 29.32 ppm in the HSQC spectrum of the compound coincided with the ^{1}H-NMR signals observed at δ 3.80-4.00 ppm, corresponding to the protons on the same carbon. The carbon nucleus, which signals at 61.30 ppm, is understood to belong to thiazolidinone carbon number 2, as it interacts with the proton number 2 at 6.00 ppm.

In the mass spectra of the target compounds obtained through the Electrospray Ionization (ESI) technique, the presence of $[M+Na]^+$ and $[M+H]^+$ peaks confirmed the structural composition of these compounds.

5.2 Biological Activity Studies

The aim of this study was to evaluate the *in vitro* tyrosinase inhibitory activity of two sets of synthesized compounds: 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-ones **2a-d** and 2-(4-(1*H*-benzimidazol-1-

yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-ones **3a-g**. The experimental procedure outlined in the Materials and Methods section was followed.

Based on the percentage of inhibition, it was observed that the 3-(phenyl)thiazolidin-4-one derivatives **3a-g** displayed a higher percentage of inhibition compared to the 3-(phenylamino)thiazolidin-4-one derivatives **2a-d**. However, **3a** was an exception to this trend. These findings indicated that the absence of the NH group in the structure led to an increase in the inhibition percentage. Indeed, when considering the effect of substituents on the phenyl ring in the 3-(phenyl)thiazolidin-4-one derivatives **3a-g**, it can be concluded that compounds lacking substituents (**3a**) displayed lower inhibition. Conversely, the inhibition activity increased as substituents were introduced into the structure of the compounds.

Furthermore, the inhibitory activity of **3b-g** was quantitatively assessed by determinig their IC_{50} values in μM and comparing them to the positive control, kojic acid. The IC_{50} values for **3b-g** ranged from 80.93 to 119.20 μM, while kojic acid exhibited an IC_{50} value of 125.08 μM. This indicates that **3b-g** demonstrated stronger tyrosinase inhibitory activity compared to the positive control. A slight contribution to the activity was observed when a substituent was present at the third position of the phenyl ring as seen in **3d**, **3f** and **3g** (IC_{50} =86.78, 106.25 and 80.93 respectively). Notably, among the tested compounds, 5-(2-(4-(1*H*-benzimidazol-1-yl)phenyl)-4-oxothiazolidin-3-yl)-2-methylbenzenesulfonamide **3g** demonstrated the most promising inhibitory activity.

The MTT assay was used to determine the cytotoxicity of **3b-g**. At the end of cytotoxicity studies, it was observed that all studied compounds were not cytotoxic in the range of 0-200 μM, except for **3b** and **3e**. These two compounds were found to be significantly (p<0.05) different from the control at 200μM concentration and were found to be cytotoxic.

6. CONCLUSION

To summarize, in this study, 4 new 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenylamino)thiazolidin-4-one **2a-d** and 7 new 2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(substitutedphenyl)thiazolidin-4-one **3a-g,** which are expected to show tyrosinase inhibitory activity, were synthesized and their structures were proved with the help of data from IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy.

The inhibitory activity of the compounds against tyrosinase was assessed and kojic acid was used as positive control in the activity studies. Evaluating the percentage of inhibition revealed that the 3-(phenyl)thiazolidin-4-one derivatives **3a-g** exhibited a higher level of inhibition compared to the 3-(phenylamino)thiazolidin-4-one derivatives **2a-d**, with the exception of compound **3a**. These results suggested that the absence of the NH group in the structure contributed to an increase in the inhibition percentage. Among the compounds tested, 5-(2-(4-(1*H*-benzoimidazol-1-yl)phenyl)-4-oxothiazolidin-3-yl)-2-methylbenzenesulfonamide **3g** exhibited the most significant inhibition, as indicated by its IC_{50} value. According to the MTT assay, **3g** did not show significant toxicity up to 200 μM. Considering its tyrosinase inhibitory activity and cytotoxic effect, **3g** exhibits promising potential for further research and development as a novel tyrosinase inhibitor.

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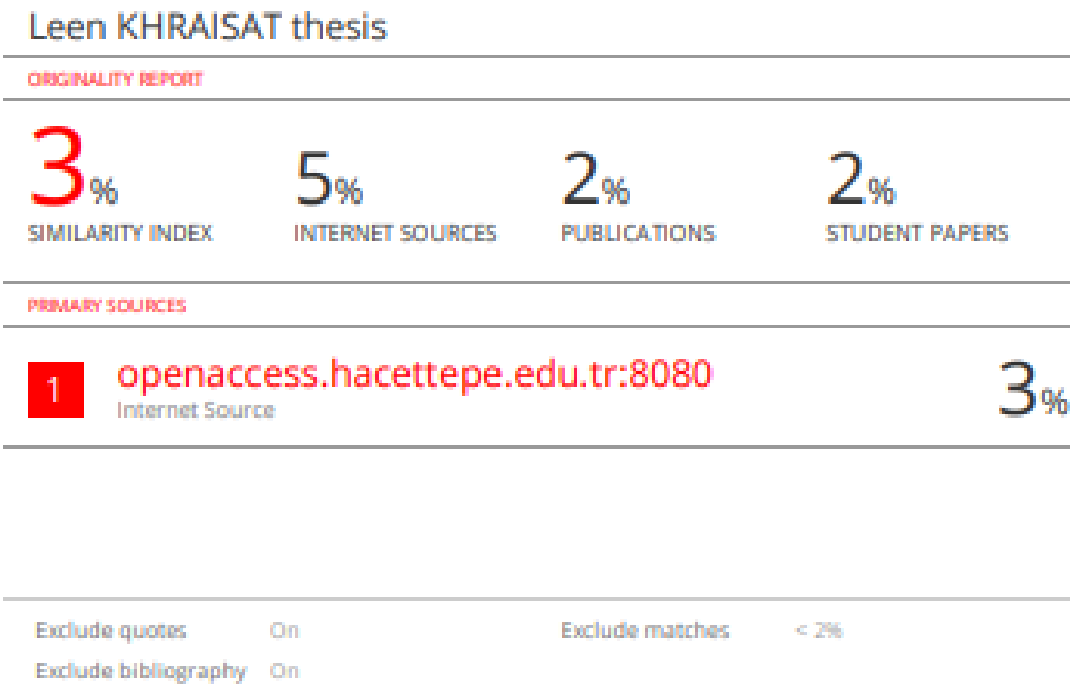
8. APPENDIX

APPENDIX-1: Turnitin originality report



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APPENDIX-2: Digital Turnitin originality report

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Submission title:	Leen KHRAISAT thesis
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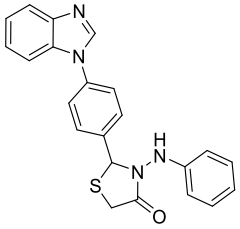
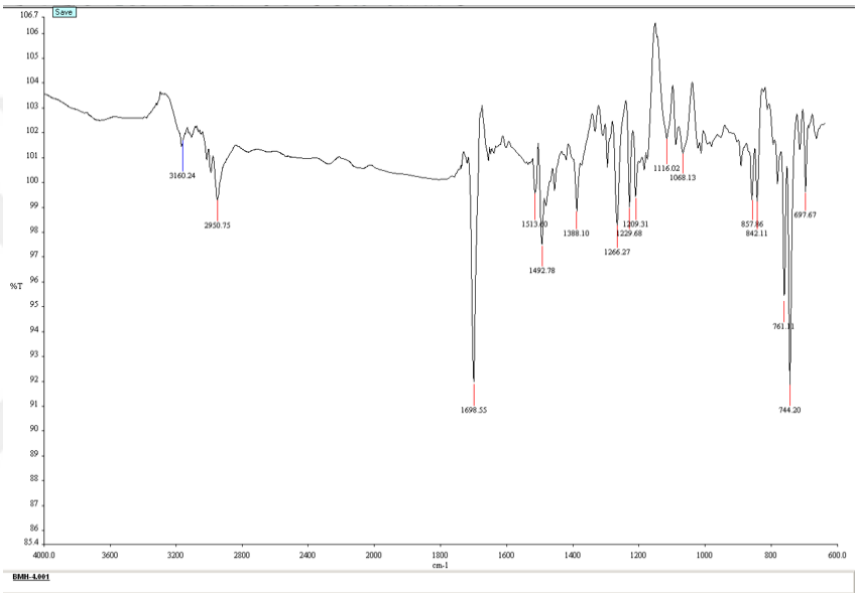
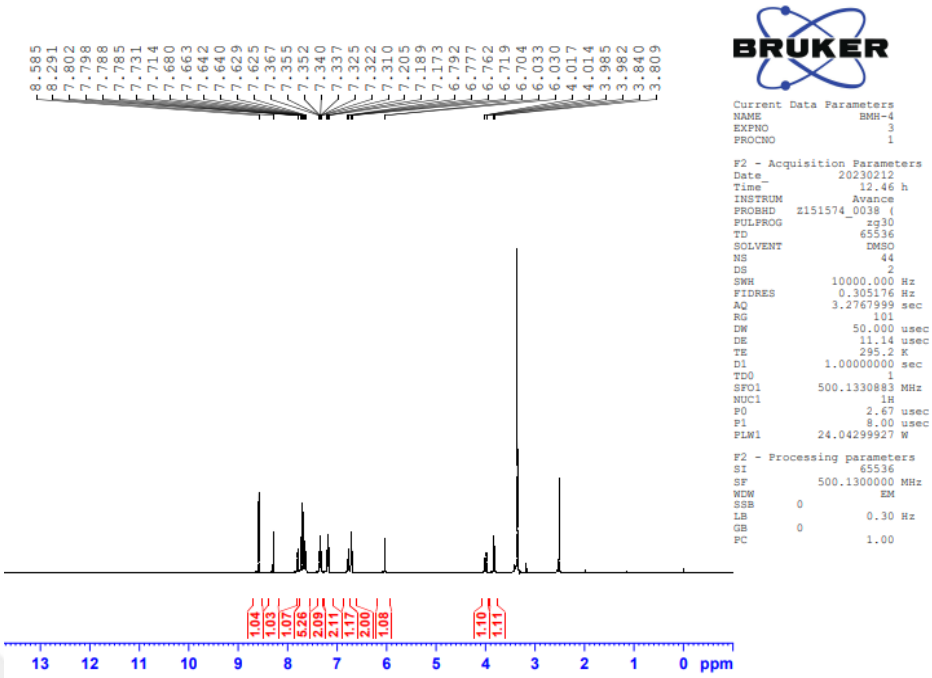
File size:	4.09M
Page count:	105
Word count:	15,517
Character count:	87,544
Submission date:	24-Jul-2023 02:41PM (UTC+0300)
Submission ID:	2136048816

APPENDIX-3: Spectral Data of Compounds Synthesized within the Scope of the Thesis

2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(phenylamino)thiazolidin-4-one (2a)







BRUKER

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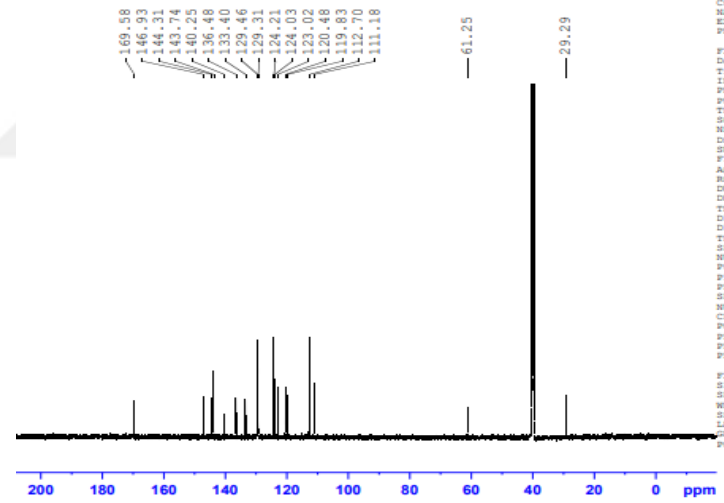
Current Data Parameters
NAME                  BMH-4
EXPNO                     3
PROCNO                    1

F2 - Acquisition Parameters
Date_              20230212
Time                 12.46 h
INSTRUM              Avance
PROBHD    Z151574_0038 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       44
DS                        2
SWH              10000.000 Hz
FIDRES               0.305176 Hz
AQ                3.2767999 sec
RG                      101
DW                   50.000 usec
DE                   11.14 usec
TE                   295.2 K
D1                1.00000000 sec
TD0                       1
SFO1           500.1330883 MHz
NUC1                     1H
P0                    2.67 usec
P1                    8.00 usec
PLW1           24.04299927 W

F2 - Processing parameters
SI                    65536
SF               500.1300000 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```



BRUKER

```
Current Data Parameters
NAME                  BMH-4
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20230212
Time                 12.40 h
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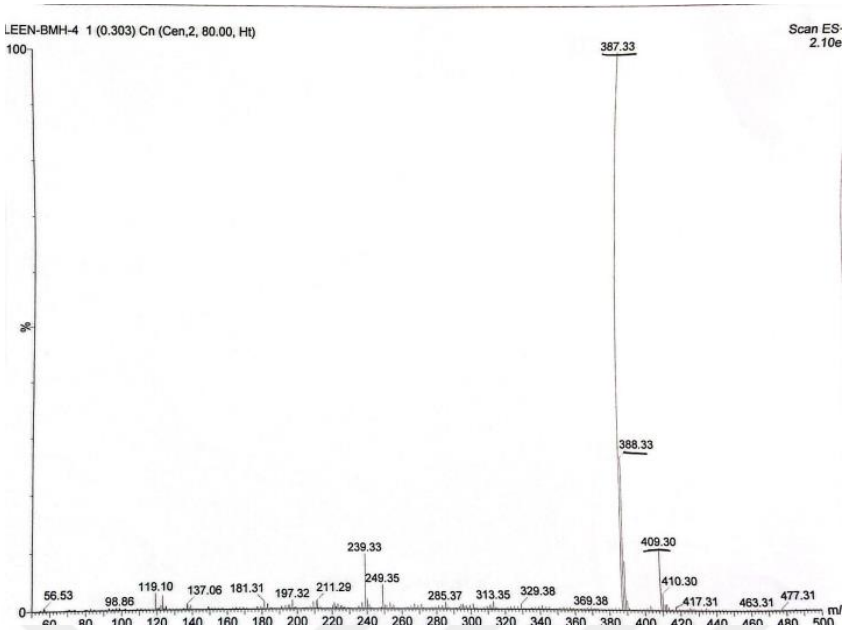
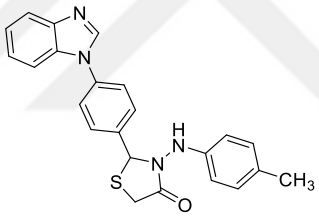
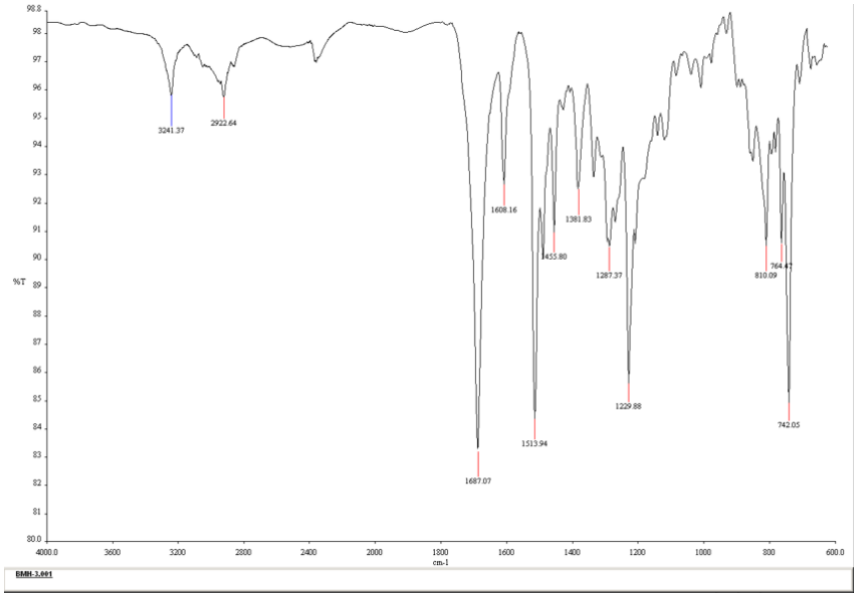
PROBHD    Z151574_0038 (
PULPROG             zgpg30
TD                    65536
SOLVENT                DMSO
NS                     1024
DS                        4
SWH              30120.482 Hz
FIDRES               0.919204 Hz
AQ                1.0878977 sec
RG                      101
DW                   16.600 usec
DE                    6.50 usec
TE                   295.2 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1
SFO1           125.7703643 MHz
NUC1                     13C
P0                    3.33 usec
P1                   10.00 usec
PLW1           85.18099976 W
SFO2           500.1320005 MHz
NUC2                     1H
CPDPRG[2            waltz65
PCPD2                80.00 usec
PLW2           24.04299927 W
PLW12           0.24043000 W
PLW13           0.12093000 W

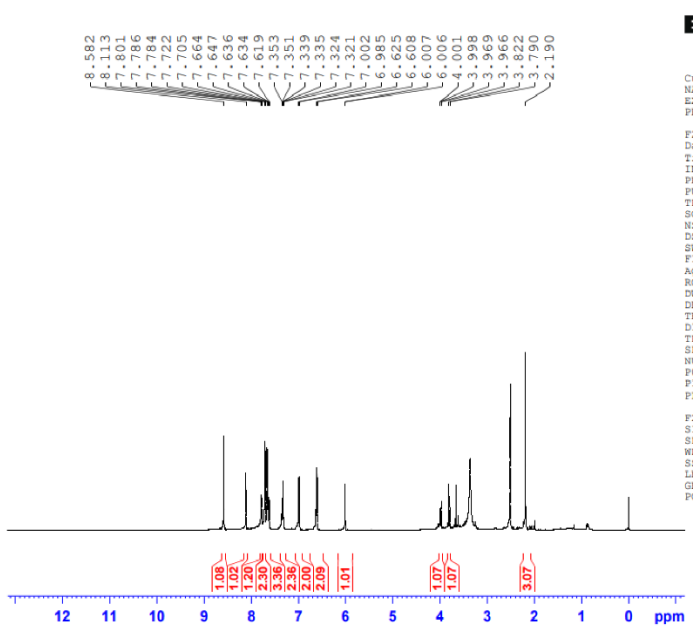
F2 - Processing parameters
SI                    32768
SF               125.7577885 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.40
```



2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(4-methylphenylamino) thiazolidin-4-one (2b)







BRUKER

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Current Data Parameters
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EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
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Time               14.18 h
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PROBHD    Z151574_0038 (
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       44
DS                        2
SWH             10000.000 Hz
FIDRES              0.305176 Hz
AQ                3.2767999 sec
RG                      101
DW                 50.000 usec
DE                 11.14 usec
TE                 295.2 K
D1              1.00000000 sec
TD0                       1
SFO1           500.1330883 MHz
NUC1                    1H
P0                 2.67 usec
P1                 8.00 usec
PLW1           24.04299927 W

F2 - Processing parameters
SI                    65536
SF             500.1300000 MHz
WDW                      EM
SSB          0
LB                 0.30 Hz
GB           0
PC                 1.00
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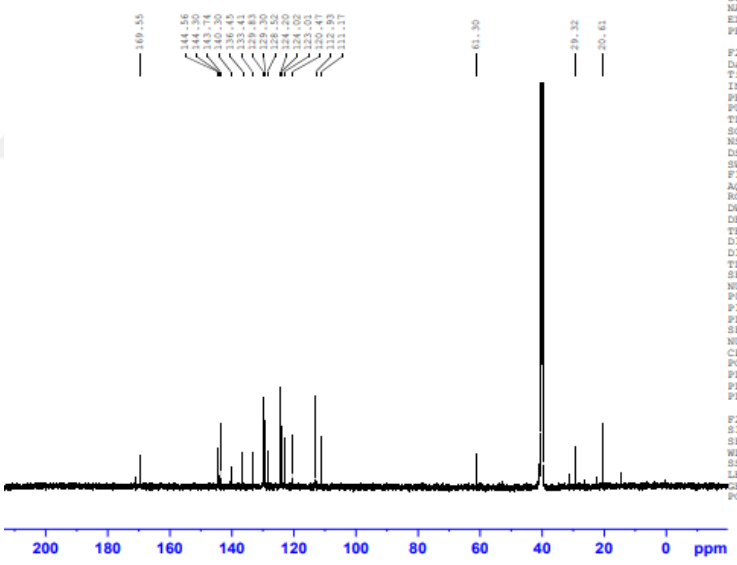

BRUKER

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Current Data Parameters
NAME                  BMH-3
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20230211
Time               13.35 h
INSTRUM              Avance
PROBHD    Z151574_0038 (
PULPROG             zgpg30
TD                    65536
SOLVENT                DMSO
NS                      1024
DS                        4
SWH             30120.482 Hz
FIDRES              0.919204 Hz
AQ                1.0878977 sec
RG                      101
DW                 16.600 usec
DE                  6.50 usec
TE                 295.2 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                       1
SFO1           125.7703643 MHz
NUC1                    13C
P0                 3.33 usec
P1                10.00 usec
PLW1           85.18099976 W
SFO2           500.1320005 MHz
NUC2                    1H
CPDPRG[2           waltz65
PCPD2                80.00 usec
PLW2           24.04299927 W
PLW12           0.24043000 W
PLW13           0.12093000 W

F2 - Processing parameters
SI                    32768
SF             125.7577885 MHz
WDW                      EM
SSB          0
LB                 1.00 Hz
GB           0
PC                 1.40
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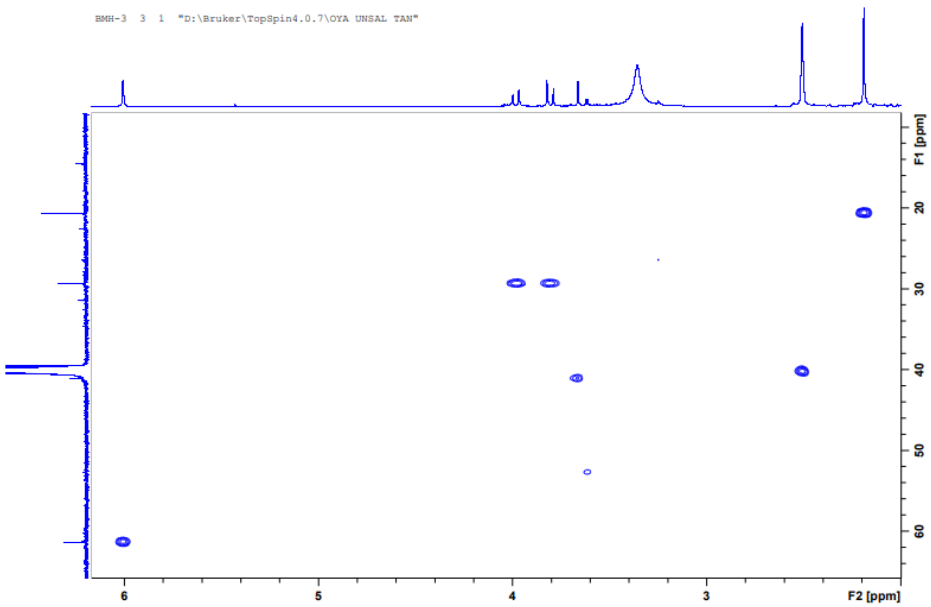
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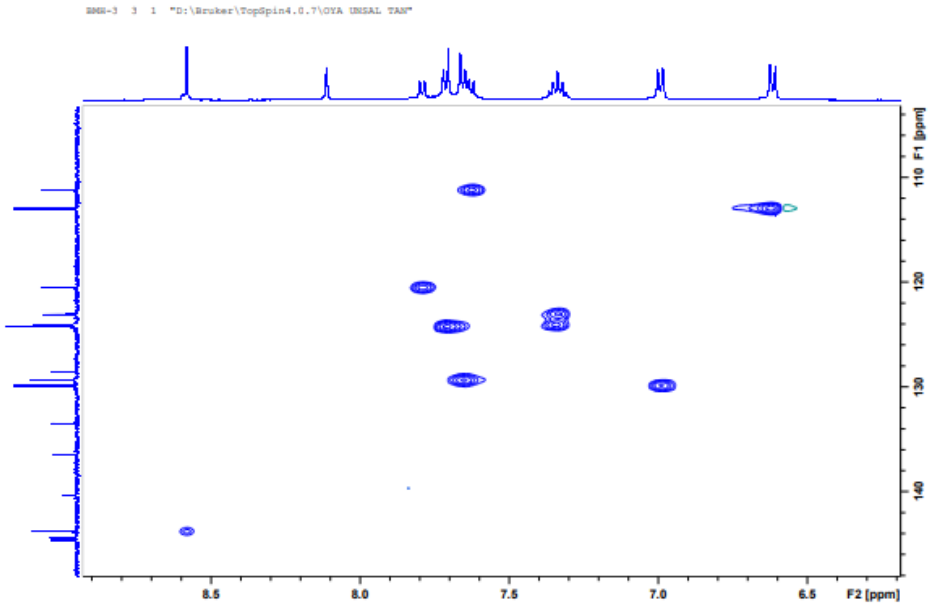
20
30
40
50
60
6
5
4
3
F2 [ppm]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120
130
140
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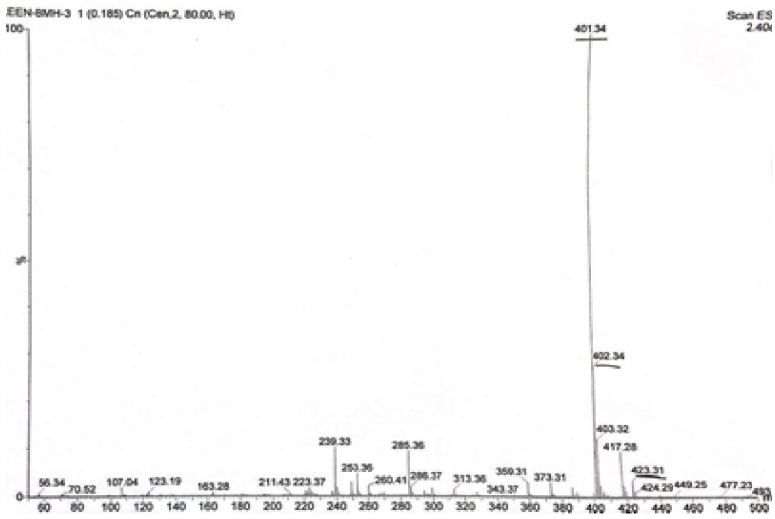
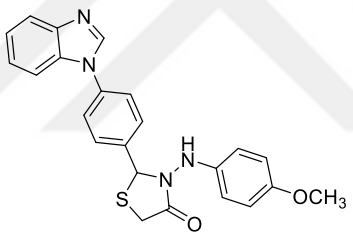
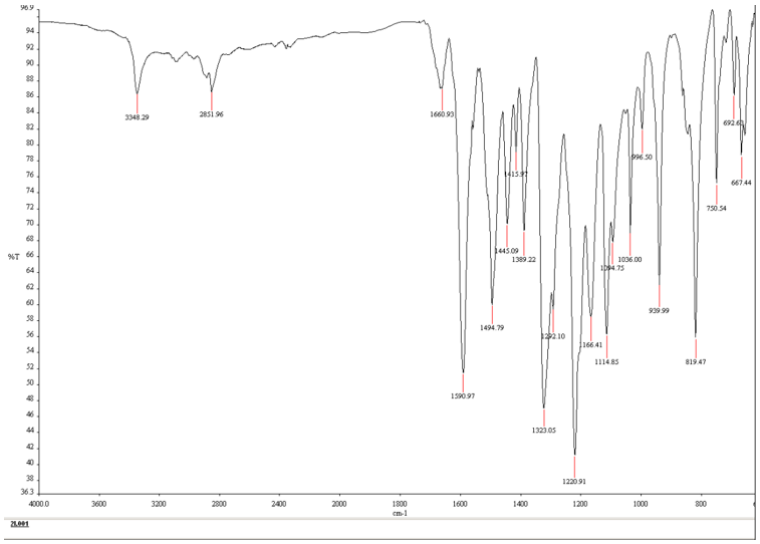
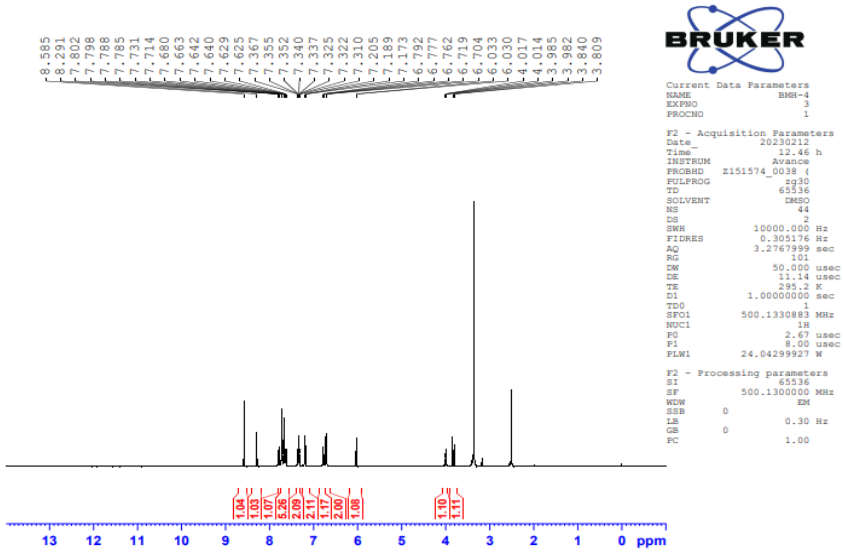
8.0
7.5
7.0
6.5
F2 [ppm]



2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-((4-methoxyphenyl)amino) thiazolidin-4-one (2c)



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7.798
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7.785
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7.680
7.663
7.642
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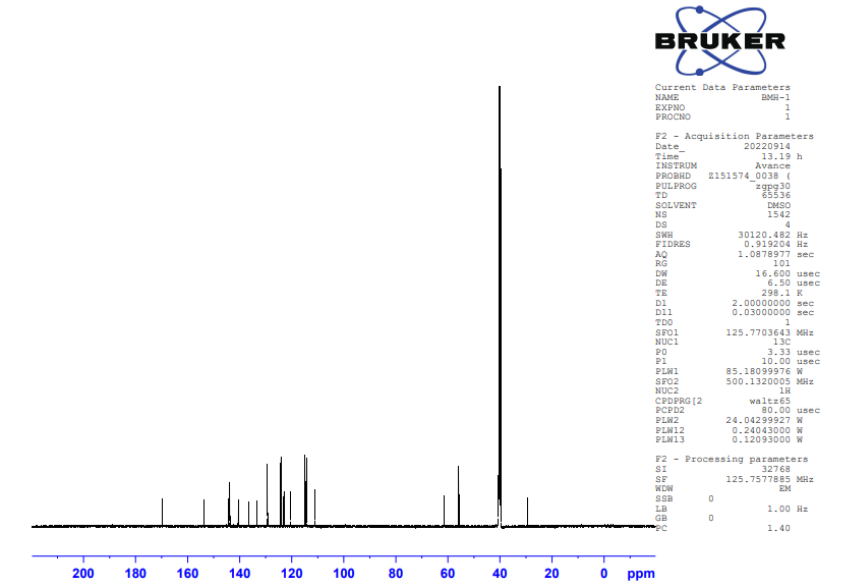
7.625
7.367
7.355
7.352
7.340
7.337
7.325
7.322
7.310
7.205
7.189
7.173
6.792
6.777
6.762
6.719
6.704
6.033
6.030
4.017
4.014
3.985
3.982
3.840
3.809
1.04
1.03
1.07
5.26
2.09
2.11
1.17
2.00
1.08
4.10
4.11
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
BRUKER
Current Data Parameters
NAME BMH-4
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230212
Time 12.46 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 44
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 101
DW 50.000 usec
DE 11.14 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 24.04299927 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00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BRUKER
Current Data Parameters
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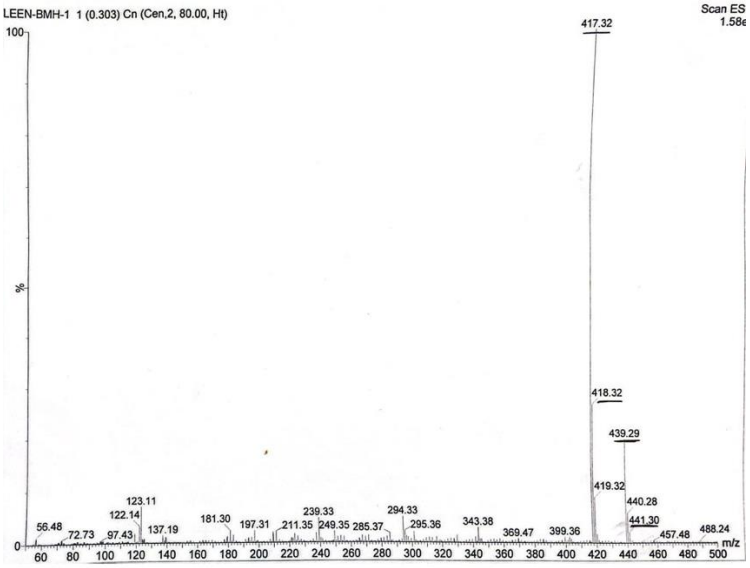
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220914
Time 13.19 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1542
DS 4
SWH 30120.482 Hz
FIDRES 0.919204 Hz
AQ 1.0878977 sec
RG 101
DW 16.600 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 85.18099976 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 24.04299927 W
PLW12 0.24043000 W
PLW13 0.12093000 W
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40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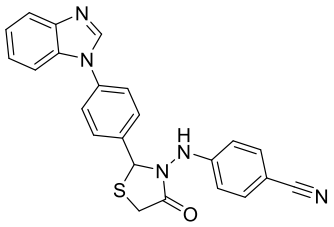
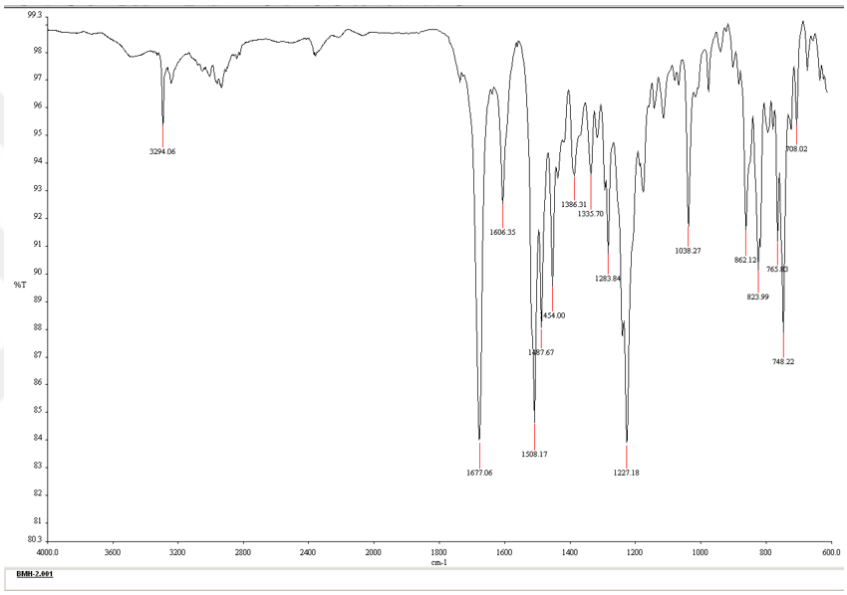
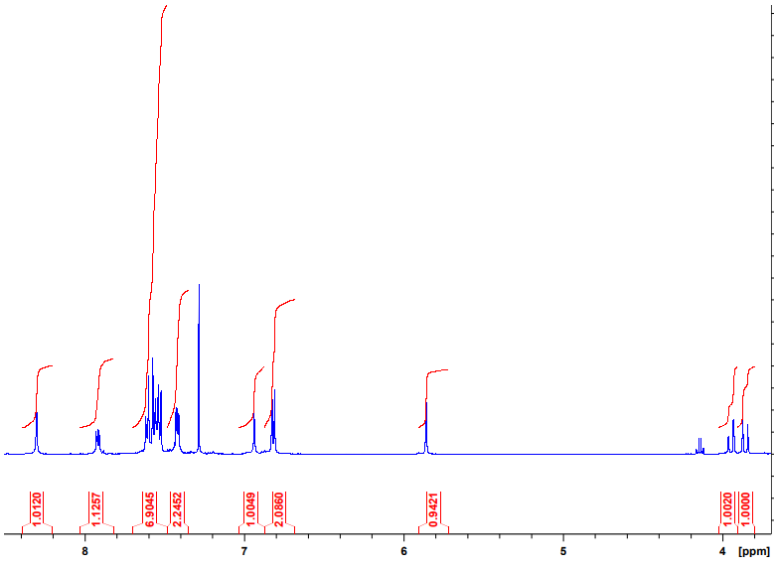
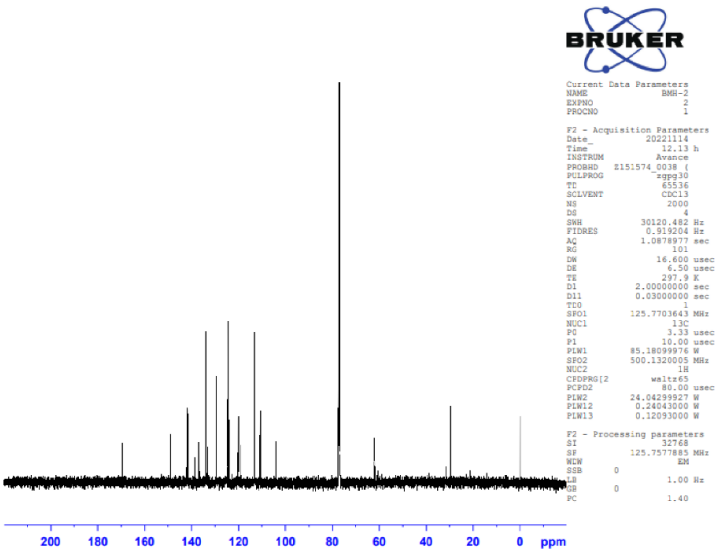
100
%
0
417.32
418.32
439.29
419.32
440.28
441.30
457.48
488.24
123.11
122.14
56.48
72.73
97.43
137.19
181.30
197.31
211.35
239.33
249.35
285.37
294.33
295.36
343.38
369.47
399.36
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z

2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-((4-cyanophenyl)amino) thiazolidin-4-one (2d)

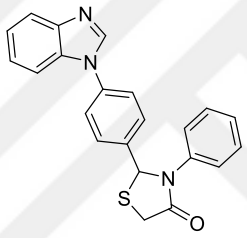


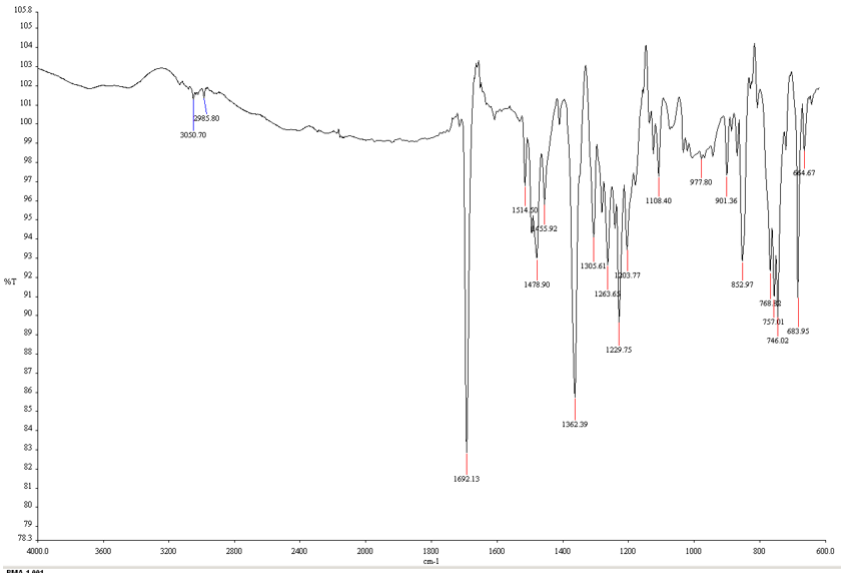


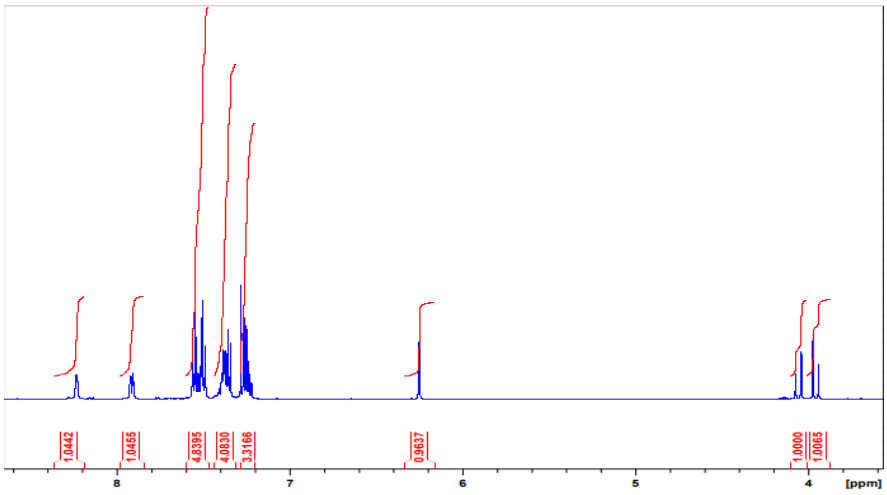


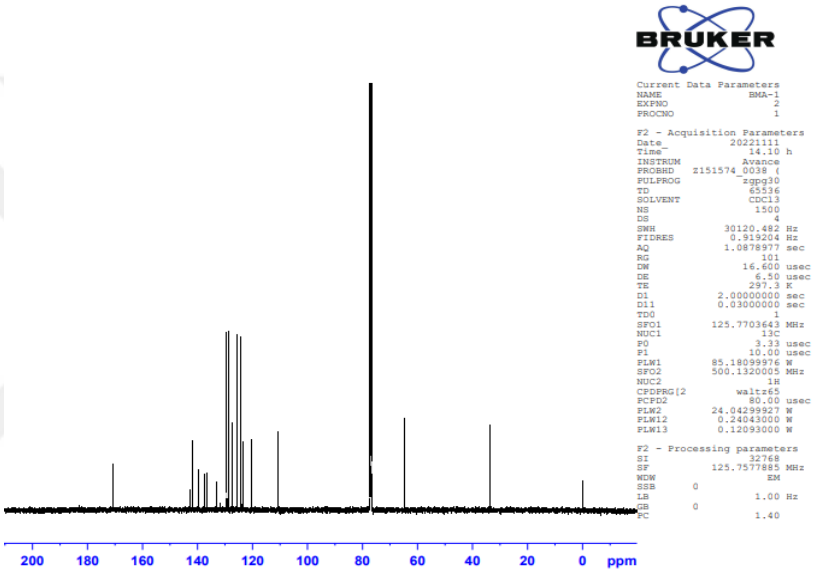


2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-phenylthiazolidin-4-one (3a)









BRUKER

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EXPNO                     2
PROCNO                    1

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Time                 14.10 h
INSTRUM                Avance
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PULPROG              zgpg30
TD                    65536
SOLVENT                CDCl3
NS                     1500
DS                        4
SWH              30120.482 Hz
FIDRES           0.919204 Hz
AQ               1.0878977 sec
RG                      101
DW                 16.600 usec
DE                  6.50 usec
TE                  297.3 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                       1
SFO1           125.7703643 MHz
NUC1                    13C
P0                   3.33 usec
P1                  10.00 usec
PLW1           85.18099976 W
SFO2           500.1320005 MHz
NUC2                    1H
CPDPRG[2             waltz65
PCPD2                80.00 usec
PLW2           24.04299927 W
PLW12           0.24043000 W
PLW13           0.12093000 W

F2 - Processing parameters
SI                    32768
SF             125.7577885 MHz
WDW                     EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```
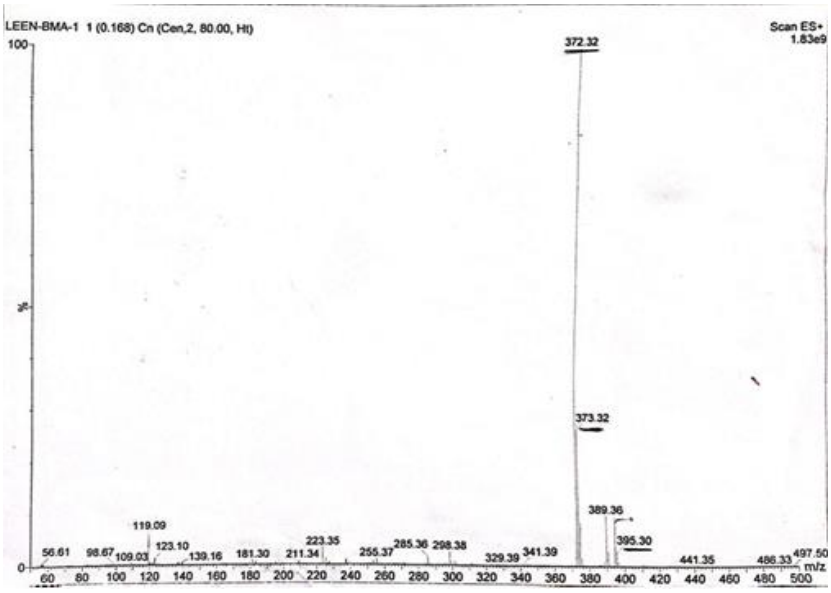
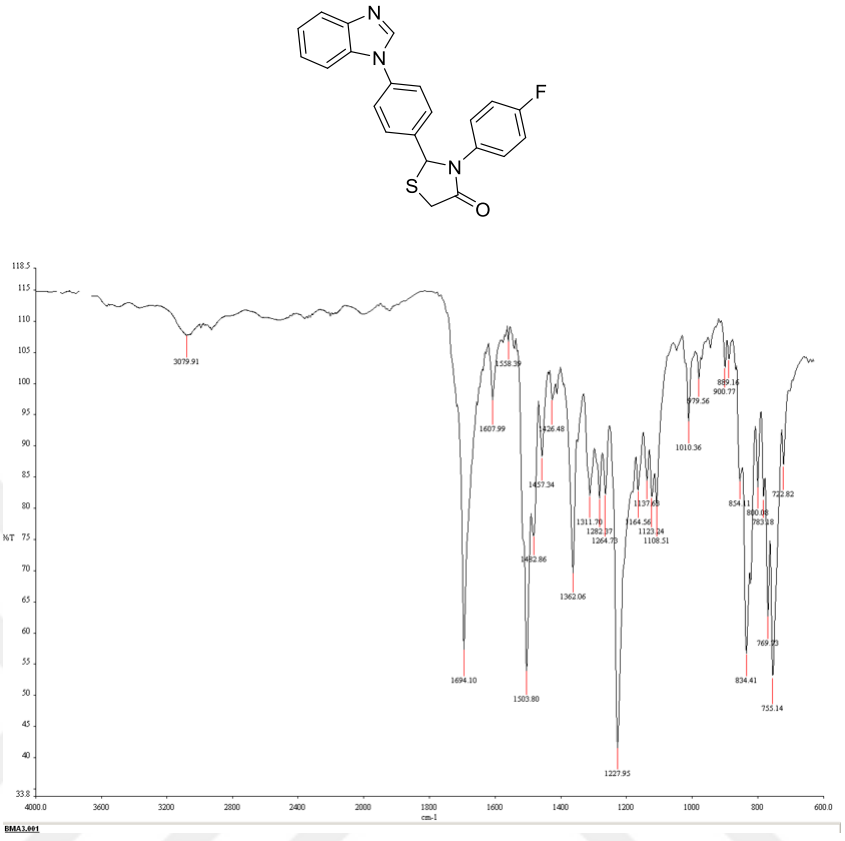
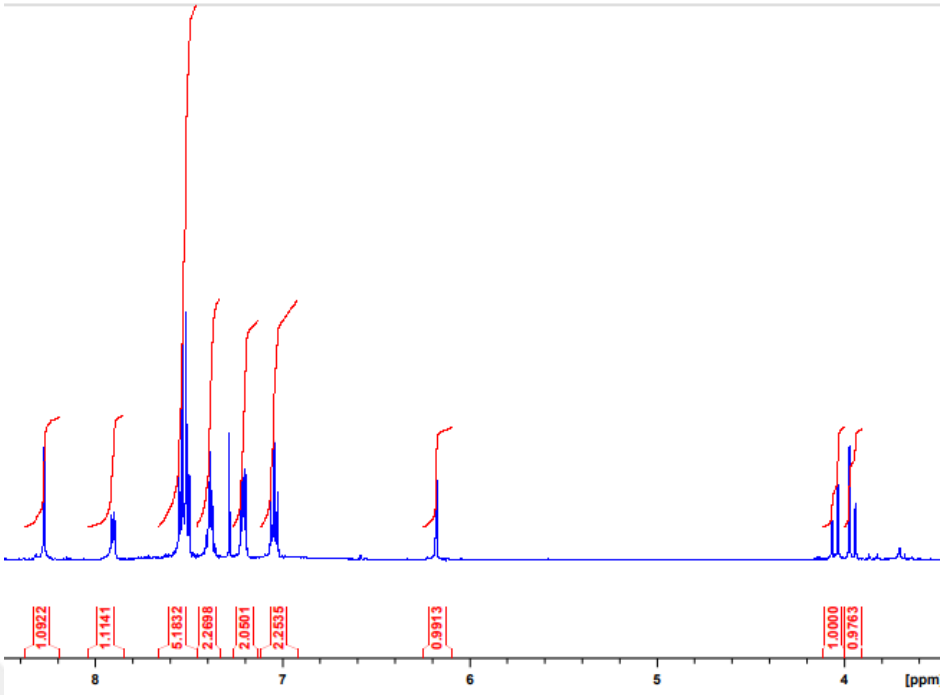
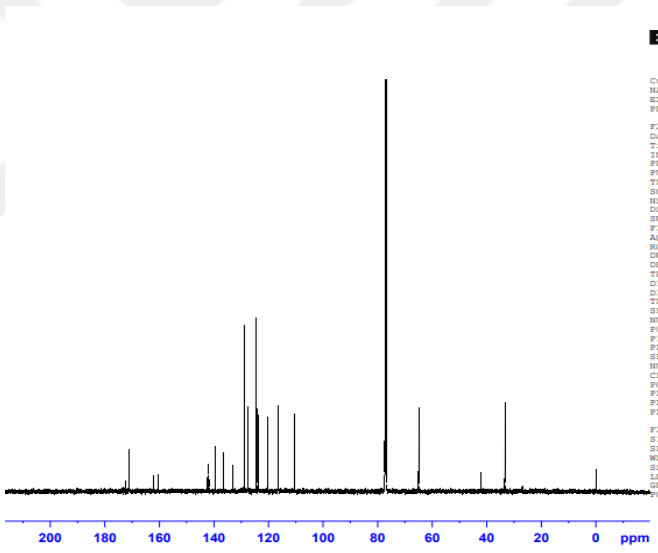



2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(4-fluorophenyl)thiazolidin-4-one (3b)







BRUKER

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Current Data Parameters
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EXPNO                     2
PROCNO                    1

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Time                 12.42 h
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PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                     1500
DS                        4
SWH               30120.482 Hz
FIDRES            0.919204 Hz
AQ                1.0878977 sec
RG                      101
DW                 16.600 usec
DE                  6.50 usec
TE                  298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                       1
SFO1          125.7703643 MHz
NUC1                    13C
P0                   3.33 usec
P1                  10.00 usec
PLW1          85.18099976 W
SFO2          500.1320005 MHz
NUC2                    1H
CPDPRG[2             waltz65
PCPD2               80.00 usec
PLW2          24.04299927 W
PLW12          0.24043000 W
PLW13          0.12093000 W

F2 - Processing parameters
SI                    32768
SF            125.7577885 MHz
WDW                      EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
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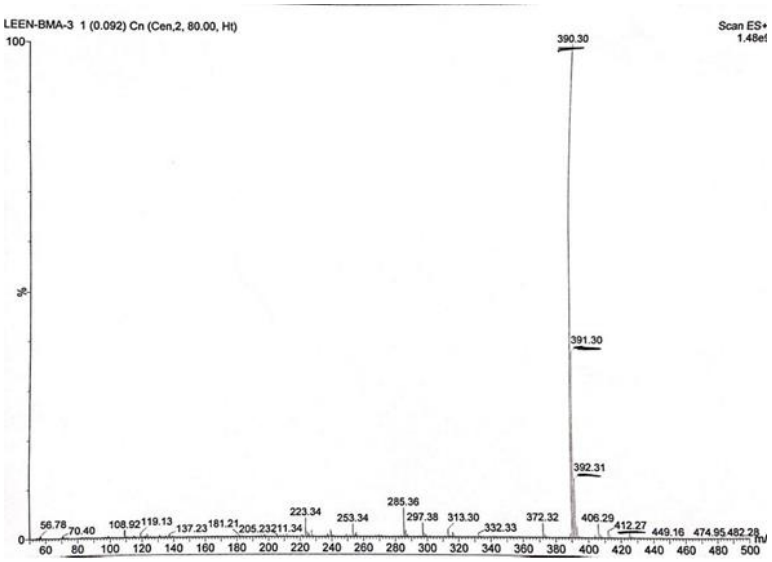
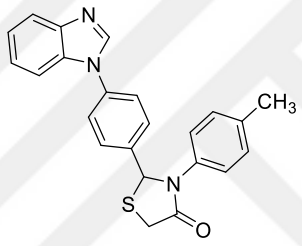
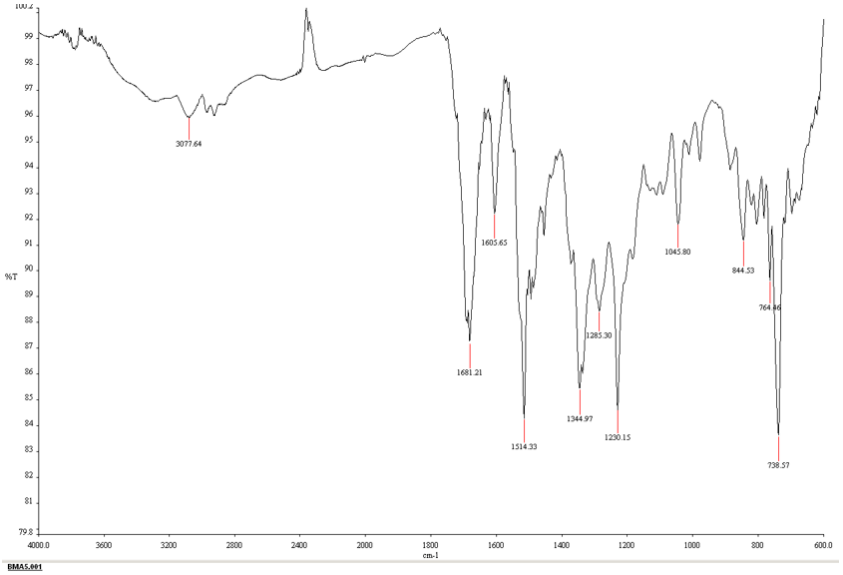
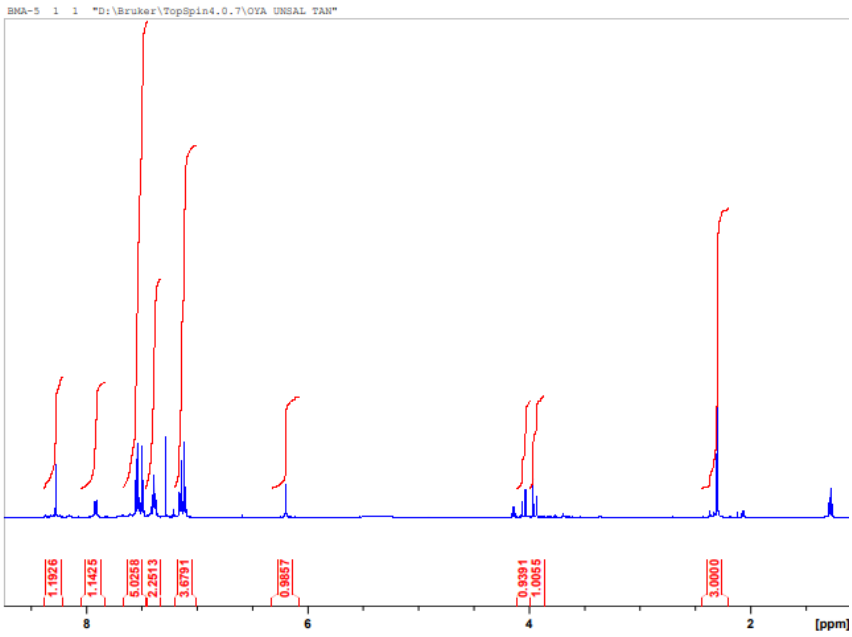
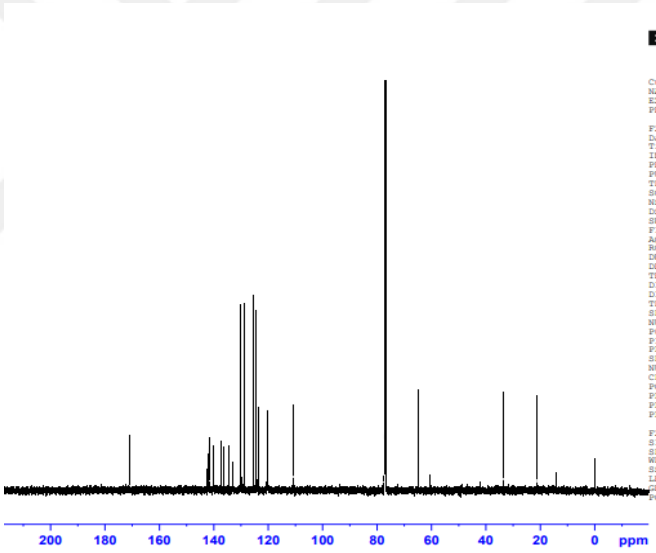


2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(4-methylphenyl)thiazolidin-4-one (3c)







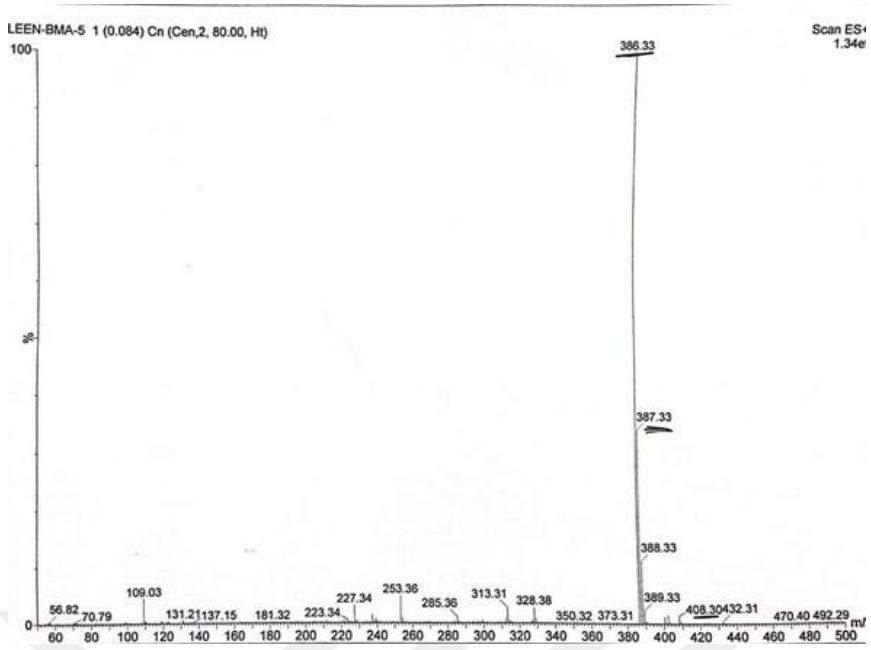


BRUKER

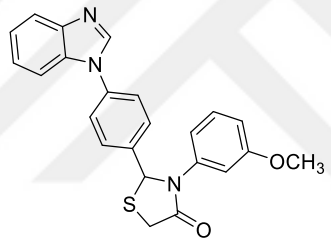
Current Data Parameters
NAME BMA-5
EXPNO 2
PROCNO 1

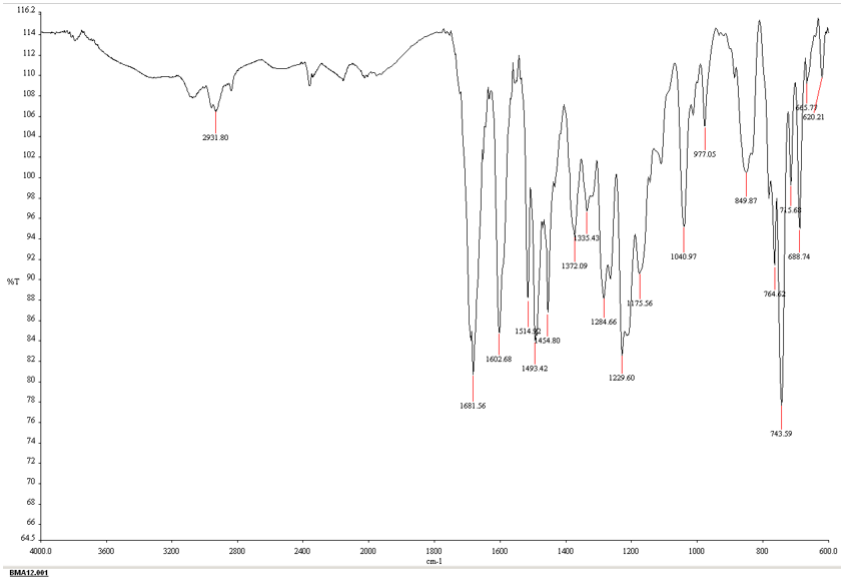
F2 - Acquisition Parameters
Date 20221112
Time 14.17 h
INSTRUM Avance
PROBHD 2151574_0038 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 919
DS 4
SWH 30120.482 Hz
FIDRES 0.919204 Hz
AQ 1.0878977 sec
RG 101
DW 16.600 usec
DE 6.50 usec
TE 297.4 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 85.18099976 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 24.04299927 W
PLW12 0.24043000 W
PLW13 0.12093000 W

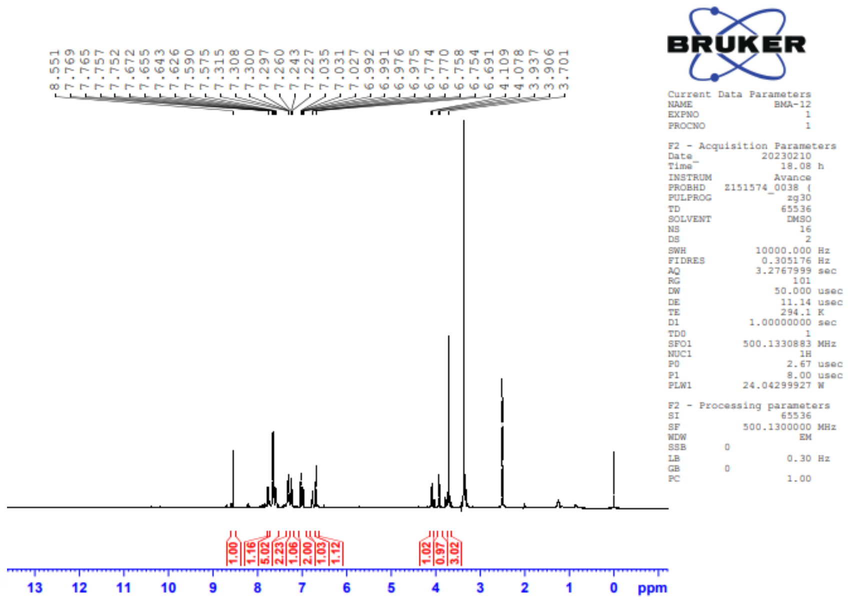
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

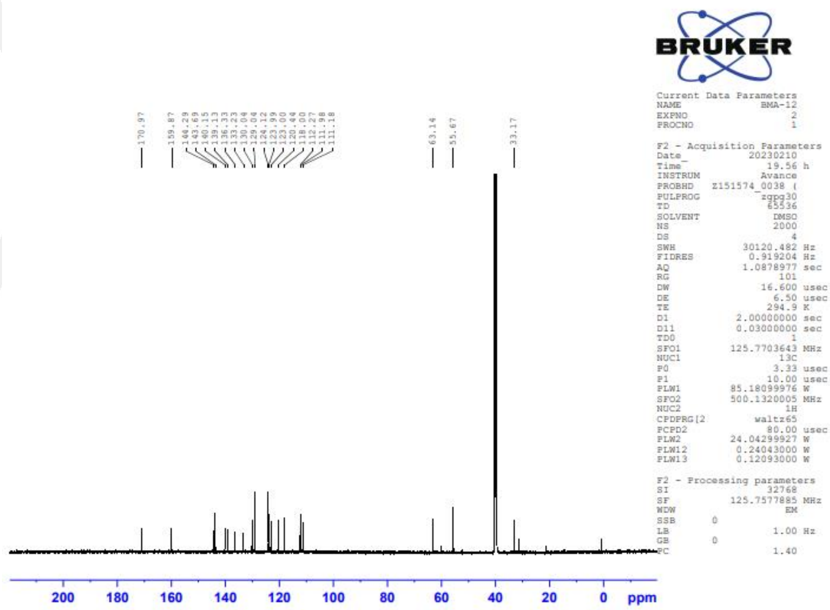


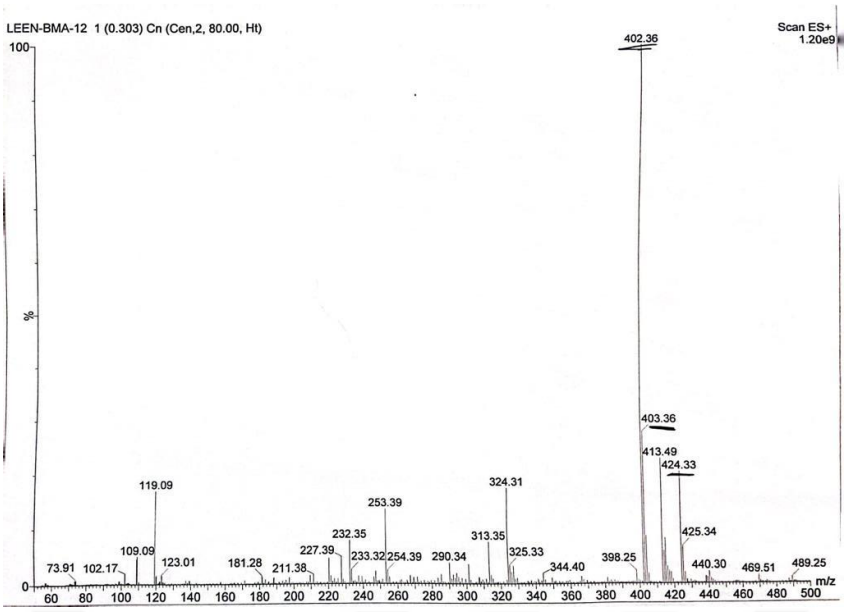
2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(3-methoxyphenyl)thiazolidin-4-one (3d)



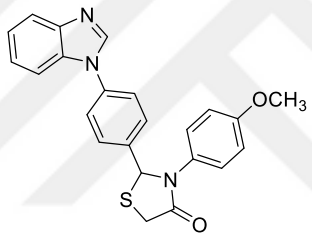


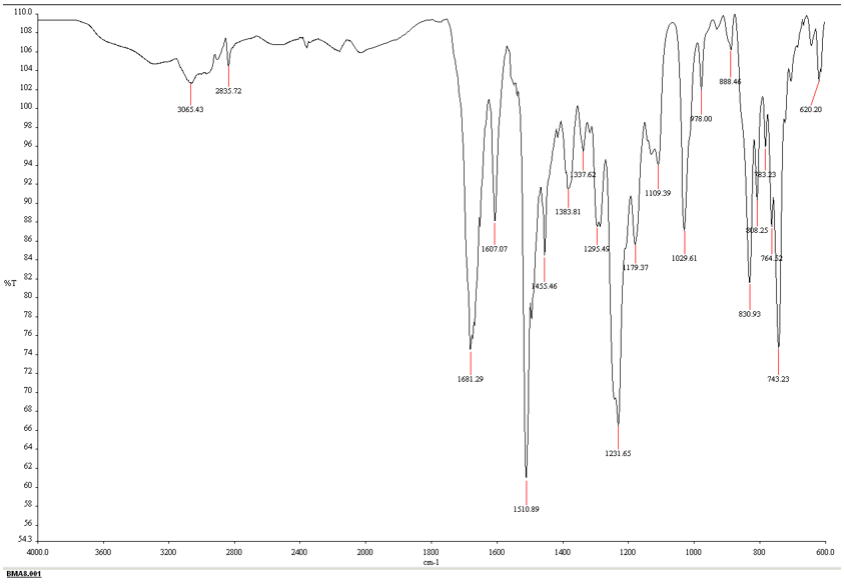

8.551
7.769
7.765
7.757
7.752
7.672
7.655
7.643
7.626
7.590
7.575
7.315
7.308
7.300
7.297
7.260
7.243
7.227
7.035
7.031
7.027
6.992
6.991
6.976
6.975
6.774
6.770
6.758
6.754
6.691
4.109
4.078
3.937
3.906
3.701
BRUKER
Current Data Parameters
NAME BMA-12
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230210
Time 18.08 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 101
DW 50.000 usec
DE 11.14 usec
TE 294.1 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 24.04299927 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.00
1.16
5.02
2.23
1.06
2.00
1.03
1.12
1.02
0.97
3.02
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

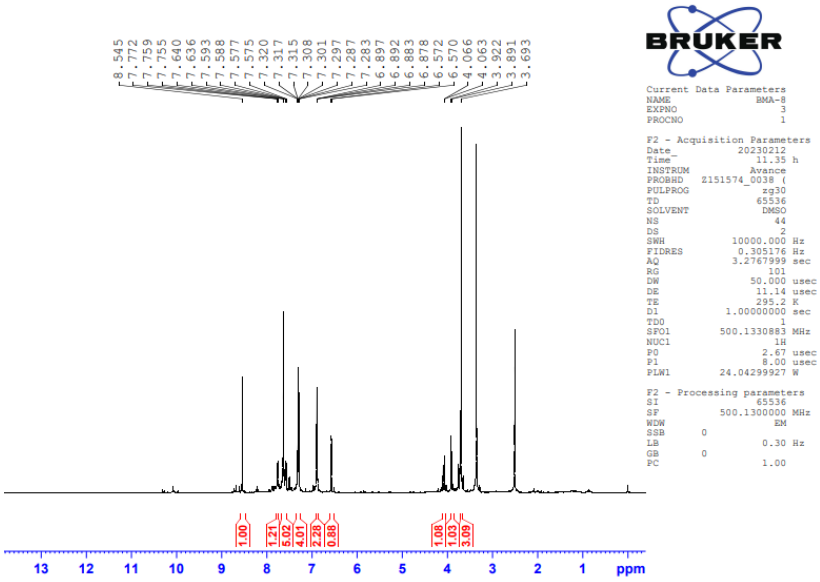

170.97
159.87
144.29
143.69
140.15
139.13
136.33
133.23
130.04
128.12
123.99
123.00
120.44
118.00
112.27
111.38
63.14
55.67
33.17
BRUKER
Current Data Parameters
NAME BMA-12
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230210
Time 19.56 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2000
DS 4
SWH 30120.482 Hz
FIDRES 0.919204 Hz
AQ 1.0878977 sec
RG 101
DW 16.600 usec
DE 6.50 usec
TE 294.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 85.18099976 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 24.04299927 W
PLW12 0.24043000 W
PLW13 0.12093000 W
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm



2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(4-methoxyphenyl)thiazolidin-4-one (3e)



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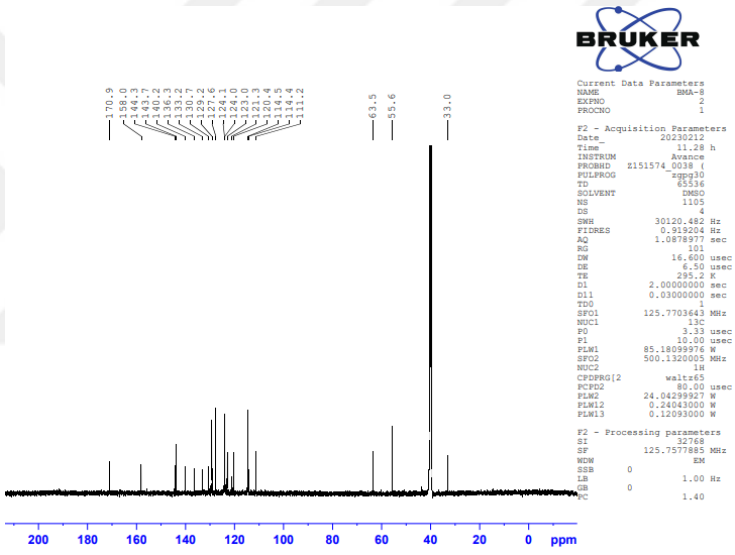
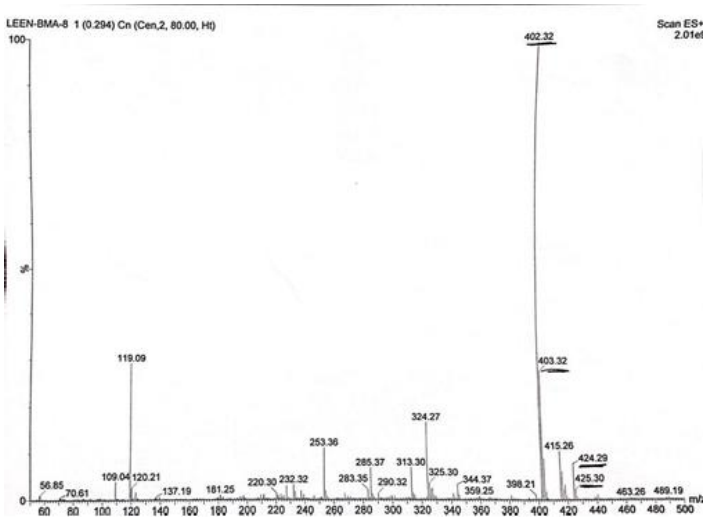
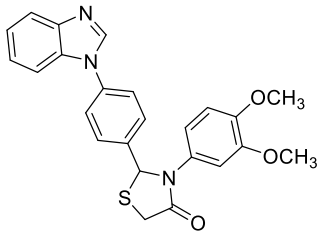
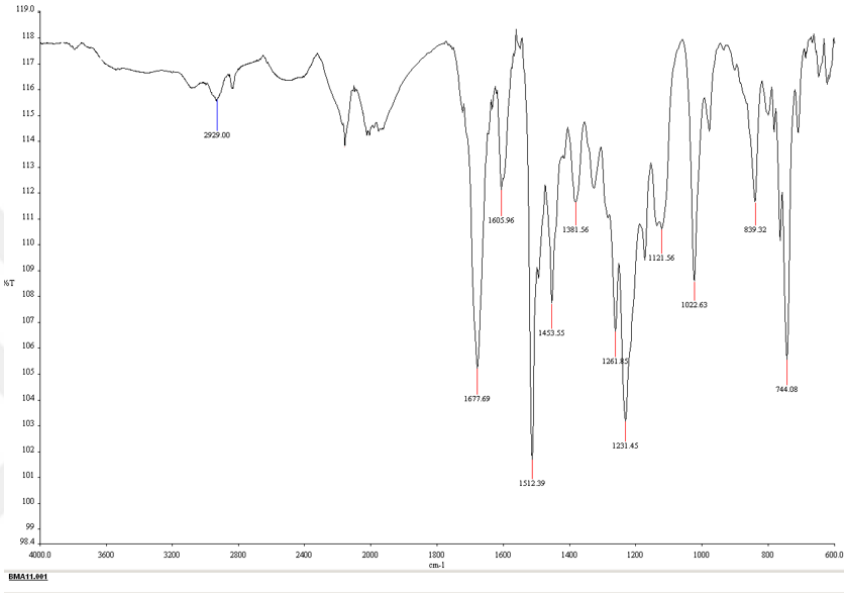
7.772
7.759
7.755
7.640
7.636
7.593
7.588
7.577
7.575
7.320
7.317
7.315
7.308
7.301
7.297
7.287
7.283
6.897
6.892
6.883
6.878
6.572
6.570
4.066
4.063
3.922
3.891
3.693
1.00
1.21
5.02
4.01
2.28
0.88
1.08
1.03
3.09
13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
BRUKER
Current Data Parameters
NAME BMA-8
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230212
Time_ 11.35 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 44
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 101
DW 50.000 usec
DE 11.14 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 24.04299927 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

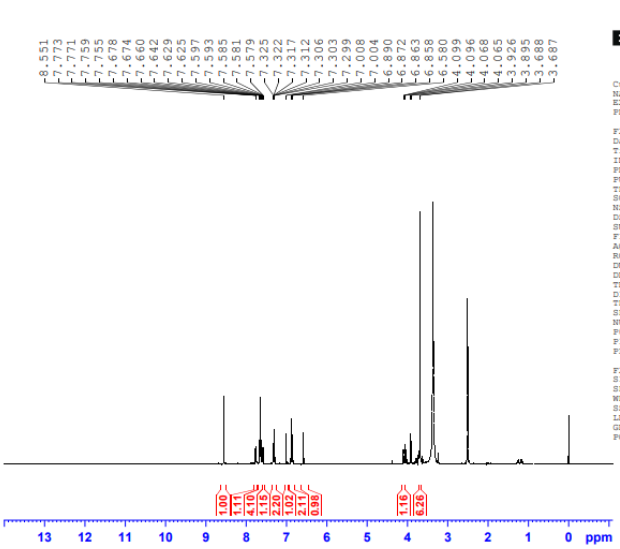

170.9
158.0
144.3
143.7
140.2
140.1
133.2
130.7
129.2
127.6
124.1
124.0
123.0
121.3
120.4
114.5
114.4
111.2
63.5
55.6
33.0
200 180 160 140 120 100 80 60 40 20 0 ppm
BRUKER
Current Data Parameters
NAME BMA-8
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230212
Time_ 11.28 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1105
DS 4
SWH 30120.482 Hz
FIDRES 0.919204 Hz
AQ 1.0878977 sec
RG 101
DW 16.600 usec
DE 6.50 usec
TE 295.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 85.18099976 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 24.04299927 W
PLW12 0.24043000 W
PLW13 0.12093000 W
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

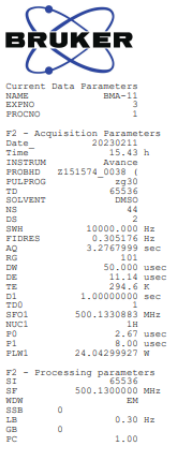

LEEN-BMA-8 1 (0.294) Cn (Cen,2, 80.00, Ht)
Scan ES+
2.01e8
100
%
0
402.32
403.32
119.09
324.27
253.36
415.26
424.29
425.30
285.37
313.30
325.30
344.37
359.25
283.35
290.32
398.21
463.26
489.19
56.85
70.61
109.04
120.21
137.19
181.25
220.30
232.32
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 m/z

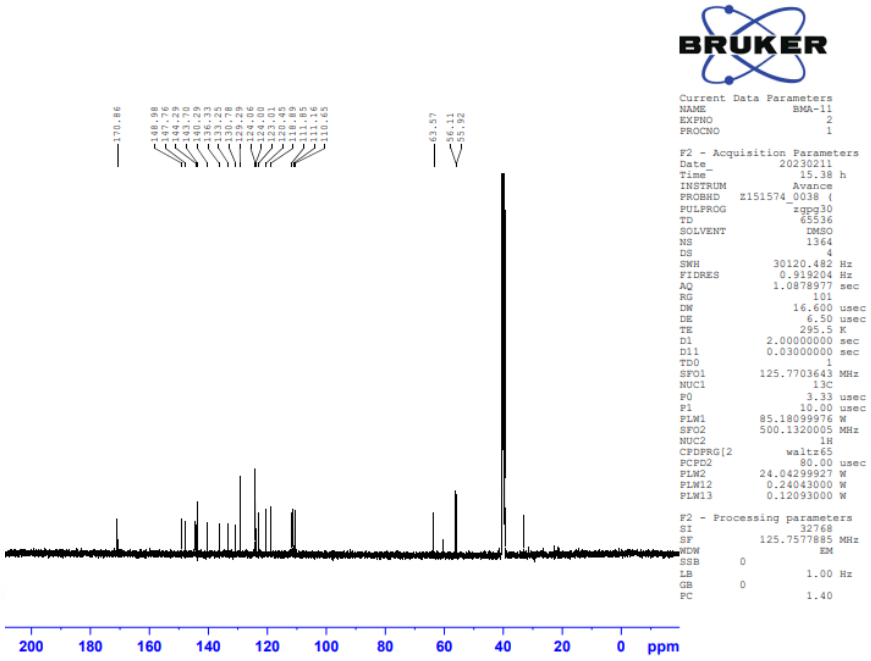
2-(4-(1*H*-benzimidazol-1-yl)phenyl)-3-(3,4-dimethoxyphenyl) thiazolidin-4-one (3f)

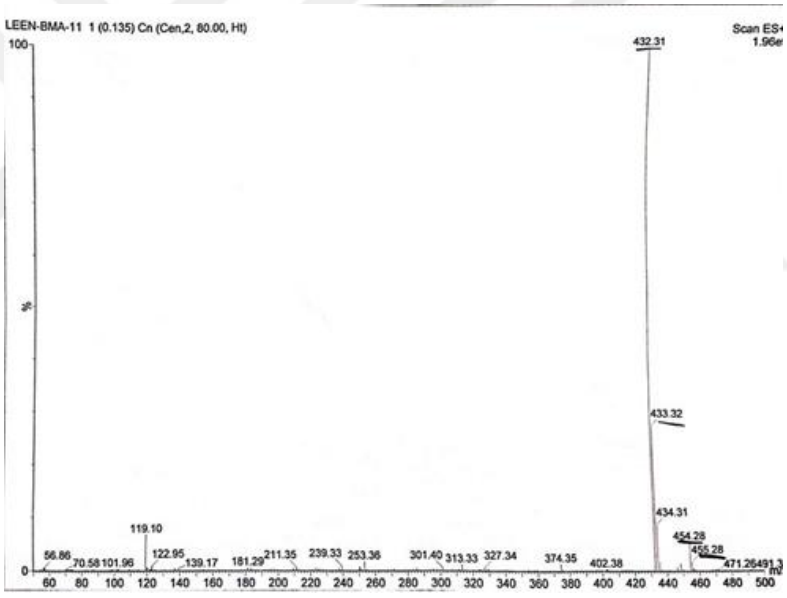




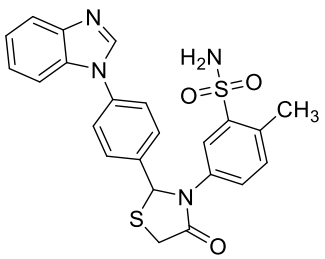


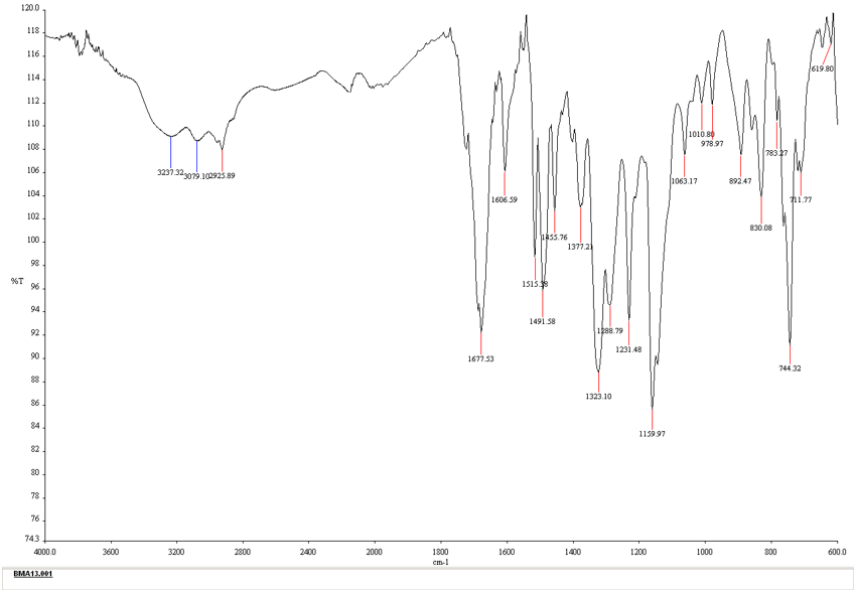


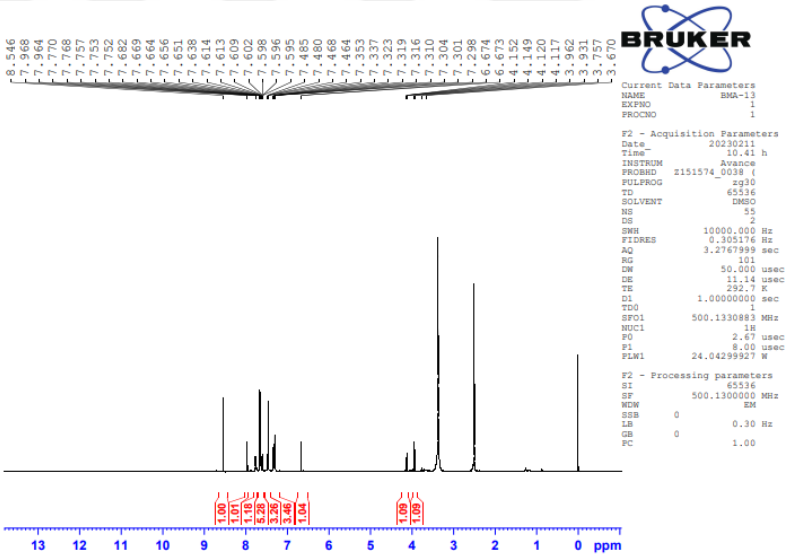


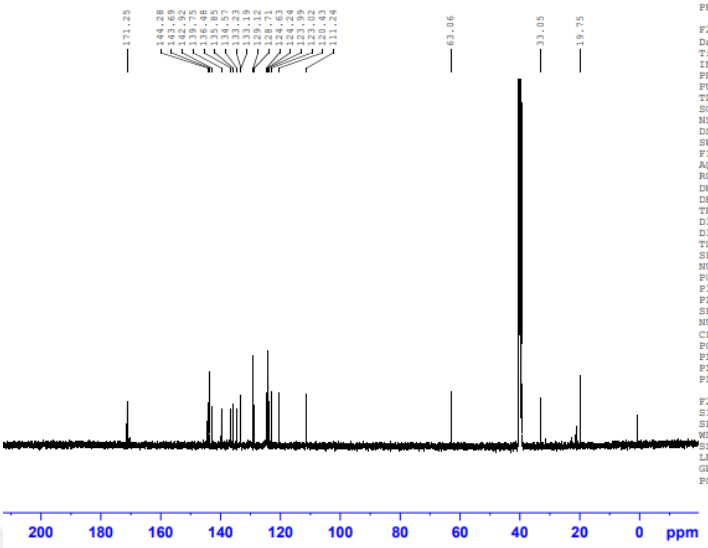


5-(2-(4-(1*H*-benzimidazol-1-yl)phenyl)-4-oxothiazolidin-3-yl)-2-methylbenzenesulfonamide (3g)




%T
cm-1
3237.32
3079.10
2925.89
1677.53
1606.59
1515.28
1491.58
1455.76
1377.21
1323.10
1288.79
1231.48
1159.97
1063.17
1010.88
978.97
892.47
830.08
783.27
744.32
711.77
619.80
BMA13.001

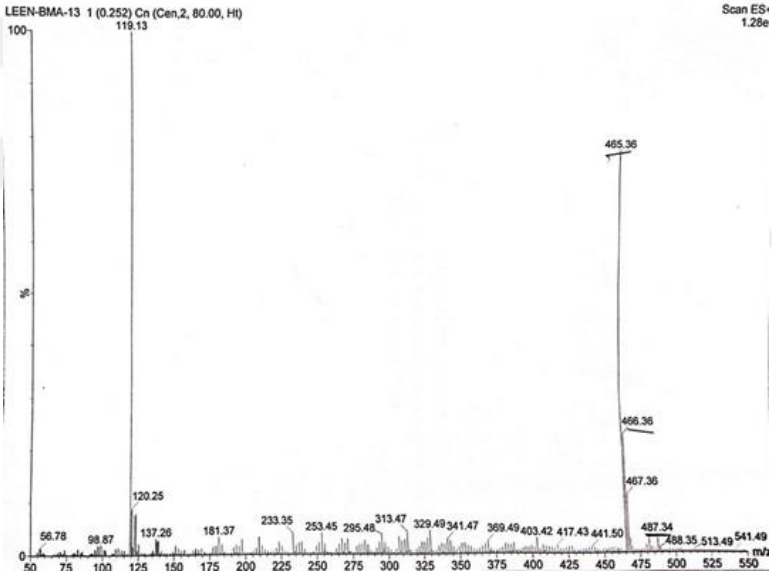

BRUKER
Current Data Parameters
NAME BMA-13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20230211
Time 10.41 h
INSTRUM Avance
PROBHD Z151574_0038 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 55
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 101
DW 50.000 usec
DE 11.14 usec
TE 292.7 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 24.04299927 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.00
1.01
1.01
5.28
3.36
3.46
1.04
1.00
1.00
13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm




```
Current Data Parameters
NAME                  BMA-13
EXPNO                      2
PROCNO                     1

F2 - Acquisition Parameters
Date_              20230211
Time_                 12.04 h
INSTRUM              Avance
PROBHD    Z151574_0038 (
PULPROG                zgpg30
TD                     65536
SOLVENT                DMSO
NS                      1500
DS                         4
SWH               30120.482 Hz
FIDRES             0.919204 Hz
AQ                 1.0878977 sec
RG                       101
DW                    16.600 usec
DE                     6.50 usec
TE                   295.2 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                        1
SFO1              125.7703643 MHz
NUC1                     13C
P0                     3.33 usec
P1                    10.00 usec
PLW1              85.18099976 W
SFO2              500.1320005 MHz
NUC2                     1H
CPDPRG[2              waltz65
PCPD2                  80.00 usec
PLW2              24.04299927 W
PLW12              0.24043000 W
PLW13              0.12093000 W

F2 - Processing parameters
SI                       32768
SF                125.7577885 MHz
WDW                         EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```



9. CURRICULUM VITAE