T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND ACTIVITY STUDIES ON NOVEL 6-METHOXYBENZOTHIAZOLE-PIPERAZINE DERIVATIVES WITH PROPANAMIDE CHAIN

MASTER OF SCIENCE THESIS

NESRIN ALHUSADI, MSc.

İSTANBUL-2018

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TEZ ONAYI FORMU

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Program : Farmasötik Kimya Yüksek Lisans Programı

Tez Başlığı : Propanamit Zinciri Taşıyan Yeni 6-Metoksibenzotiyazol-piperazin Türevleri Üzerine Sentez ve Aktivite Çalışmaları

Tez Sahibi : Nesrin ALHUSADI

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Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Yüksek Lisans Tezi olarak kabul edilmiştir.

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 20./07./2018... tarih ve 2018/13-03.... sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Master's Programme in Pharmaceutical Chemistry

Title of the Thesis : Synthesis and Activity Studies On Novel 6-Methoxybenzothiazole-Piperazine Derivatives With Propanamide Chain

Owner of the Thesis : Nesrin ALHUSADI

Examination Date : 19.07.2018

This study have been approved as a Master Thesis in regard to content and quality by the Jury.

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Supervisor:	Dr. Lec. Enise Ece GÜRDAL Yeditepe University Faculty of Pharmacy	
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Member/Examiner:	Assoc. Prof. Dr. Gökçen EREN Gazi University Faculty of Pharmacy	
Member/Examiner:	Assoc. Prof. Dr. Filiz Esra ÖNEN BAYRAM Yeditepe University Faculty of Pharmacy	

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 20/07/2018... and numbered 2018/13-03

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

19.07.2018

Nesrin Alhusadi

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LIST OF ABBREVIATIONS

AChE	Acetylcholine Esterase
ATP	Adenosine Triphosphate
CAS No	Chemical Abstracts Service number
CCRF-CEM	T lymphoblastoid Cell Line
CTAB	Cetyltrimethylammonium Bromide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DU-145	Prostate Cancer Cell Line
FACS	The American College of Surgeons
FT-IR	Fourier Transform Infrared
G_1	Gap 1 Phase
GABA	Gamma-Aminobutyric Acid
GI_{50}	Growth Inhibitory Concentration
$HepG_2$	Hepatocellular Carcinoma Cell Line
MCF-7	Breast Adenocarcinoma Cell Line
HOP-92	Non-small Cell Lung Cancer Cell Line
HUH-7	Hepatocarcinoma Cell Line
IC_{50}	The Half Maximal Inhibitory Concentration
MeOH	Methanol
MS	Mass Spectroscopy
MBC	Minimum Bactericidal Concentration

MET	Maximum Electric Shock
MIC	Minimum Inhibitory Concentration
M.P.	Melting Point
NMR	Nuclear Magnetic Resonance
NCI	National Cancer Institute
TEA	Triethylamine
ppm	Parts Per Million
R_f	Retention Factor
RMSD	Root Mean Square Deviation
S	Solvent Systems
SP	Standard Precision
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet

ABSTRACT

Alhusadi, N. A. (2018). Synthesis and Activity Studies on Novel 6-Methoxybenzothiazole-piperazine Derivatives with Propanamide Chain. Yeditepe University Institute of Health Sciences, Department of Pharmaceutical Chemistry, M.Sc. Thesis, Istanbul.

In this study, ten novel compounds, bearing *N*-(6-methoxybenzothiazol-2-yl)-3-(4-substituedpiperazinyl)propanamide backbone were synthesized. *In vitro* antibacterial and antifungal activity were determined using of Ofloxacin and Nystatin as standards respectively.

To obtain starting compound, 2-amino-6-methoxybenzothiazole was acylated with 3-chloropropionyl chloride. After substitution of chlorine in 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide by different piperazine derivatives, the target compounds were obtained.

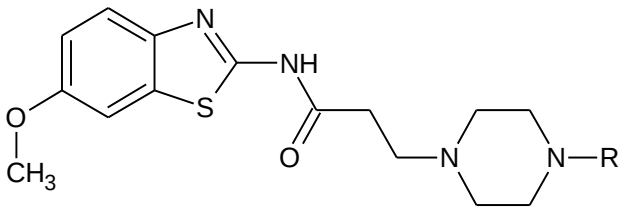
Structure elucidation of the synthesized compounds were confirmed by UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analysis methods. In addition, their antibacterial and antifungal activity were evaluated *in vitro* by agar-based disc diffusion and minimum inhibitory concentration assays against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229) and *Candida albicans* (ATCC 10231) strains.

According to activity results, the most active compounds were compounds **2** and **5** against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* with (inhibition zone: 12 mm).

Docking studies were performed to predict the interactions of compounds in test set with DNA gyrase subunit B of *S. aureus.* Under light of docking studies, a new compound with promising GyrB inhibition was designed.

Keywords: Antimicrobial, benzothiazole, piperazine, antibacterial, antifungal.

Table 1.1. Structure of the synthesized compounds



Compound	R	M.P. (°C)	% Yield
1	2,3-Xylyl	195	19.6
2	3-Hydroxyphenyl	215	37.5
3	4-Nitrophenyl	218	25.7
4	4-Chlorophenyl	200.2	54.8
5	2,3-Dichlorophenyl	185.7	33.8
6	3,4-Dichlorophenyl	220.2	33.8
7	4-Methylphenyl	184.5	29
8	2-Cyanophenyl	163.3	63.6
9	2-Pyridinyl	175	18.17
10	Tetrahydrofuroyl	144.3	30.8

ÖZET

Alhusadi, N. A. (2018). Propanamit Zinciri Taşıyan Yeni 6-Metoksibenzotiyazol-piperazin Türevleri Üzerine Sentez ve Aktivite Çalışmaları Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Anabilim Dalı, Yüksek Lisans Tezi, Istanbul.

Bu çalışmada, *N*-(6-metoksibenzotiyazol-2-il)-3-(4-sübstitüepiperazinil) propanamit yapısına sahip on yeni bileşik sentezlenmiştir. Bileşiklerin i*n vitro* antibakteriyel ve antifungal aktiviteleri sırasıyla Oflokzasin ve Nistatin standartlarıyla belirlenmiştir.

Başlangıç bileşiğinin eldesi için 2-amino-6-metoksibenzotiyazol, 3-kloropropiyonil klorür ile açillenmiştir. 3-Kloro-*N*-(6-metoksi-1,3-benzotiyazol-2-il)propanamite ait klorun çeşitli pieprazinler ile yer değiştirmesi sonucu hedef bileşikler elde edilmiştir.

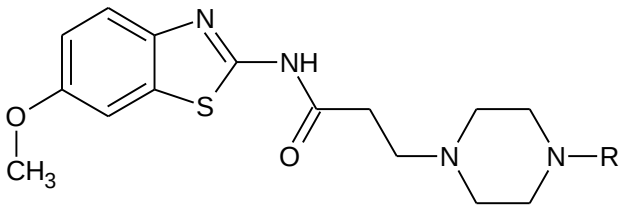
Sentezlenen bileşiklerin yapıları UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR, kütle spektrumları ve elementel analiz yöntemleriyle doğrulanmıştır. Ayrıca, antibakteriyel ve antifungal aktiviteleri *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229) ve *Candida albicans* (ATCC 10231) suşlarına karşı *in vitro* agar disk difüzyon ve minimum inhibitör konsantrasyon testleriyle belirlenmiştir.

Aktivite sonuçlarına göre, bileşik **2** ve **5** en aktif yapılar olup her iki bileşik için de *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans*'a karşı inhibisyon alanı 12 mm olmuştur.

Test dizisindeki bileşiklerin *S. aureus*'un DNA giraz B alt birimi ile etkileşimlerini tahmin etmek için yerleştirme çalışmaları yapılmıştır. Bu çalışmalar ışığında, umut verici GyrB inhibisyonuna sahip yeni bir bileşik tasarlanmıştır.

Anahtar Kelimeler: Antimikrobiyal, benzotiyazol, piperazin, antibakteriyel, antifungal.

Tablo 1.2. Sentezlenen bileşiklerin yapıları



Bileşik	R	E.D. (°C)	% Verim
1	2,3-Ksilil	195	19.6
2	3-Hidroksifenil	215	37.5
3	4-Nitrofenil	218	25.7
4	4-Klorofenil	200.2	54.8
5	2,3-Diklorofenil	185.7	33.8
6	3,4-Diklorofenil	220.2	33.8
7	4-Metilfenil	184.5	29
8	2-Siyanofenil	163.3	63.6
9	2-Piridinil	175	18.17
10	Tetrahidrofuroil	144.3	30.8

1. INTRODUCTION

Public health has always been a critical matter for decades. It has a great impact on the economic status of many countries. The reason behind that is the antibiotic resistance which is considered as a nightmare for the medical team especially for the treatment of the bacterial and fungal infections. Medicinal chemists became successful recently in remodeling the scaffolds of earlier natural and synthetic antibiotics [1, 2].

Benzothiazole is a privileged bicyclic ring system with multiple utilization. Many therapeutic agents are synthesized through the benefit of benzothiazole nucleus [3]. They are known to show an extensive variety of biological attributes including antitumor [4], anticonvulsant [5], antimicrobial [6], anthelmintic [7], antileishmanial [8], schictosomicidal [9], antifungal [10], anti-inflammatory [11], and anti-diabetic activities [12].

In the last years, several 2-substitutedbenzothiazole derivatives have shown antimicrobial activity. Compounds given below were found to inhibit some of Gram-positive, Gram-negative bacteria and *Candida albicans* with minimum inhibitory concentration (MIC) range of 3.12–50 μg/ml [13].

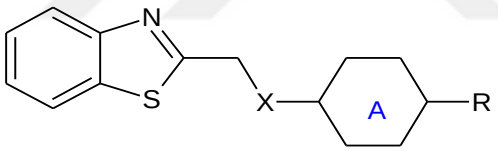


Figure 1.1 2-substitutedbenzothiazole derivatives

X= CH_2, O, S; R= H, Cl; A= cyclohexyl, phenyl

There are numerous researchers that describe features of piperazine in both chemical and biological aspects. Compounds derived from piperazine are recognized to show a large spectrum of biological activity including antimicrobial [14], antidepressant [15], anti-inflammatory [16], anticancer [17], and antihistaminic [18].

A group of new 7,5-dimethoxy-2-(piperazin-1-ylmethyl)-4H-chromen-4-ones were synthesized by Hatnapure *et al*. and tested against bacterial and fungal strains. Below compound was found to have great antibacterial and antifungal activity with around 2 to

2.5-fold more efficacy than both standard drugs ciprofloxacin and miconazole respectively [19].

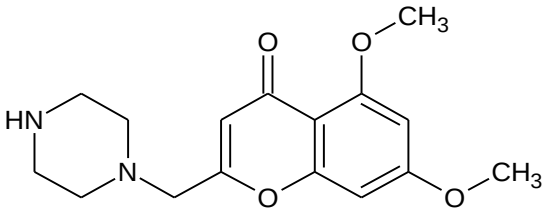


Figure 1.2. 4H-chromen-4-one dervivative

Benzothiazole and piperazine derivatives were prepared by Chaithanya *et al*. and tested against bacterial and fungal strains. Compound **a** presented potent antimicrobial activity in comparison with standard drugs like ofloxacin, ampicillin and fluconazole [20].

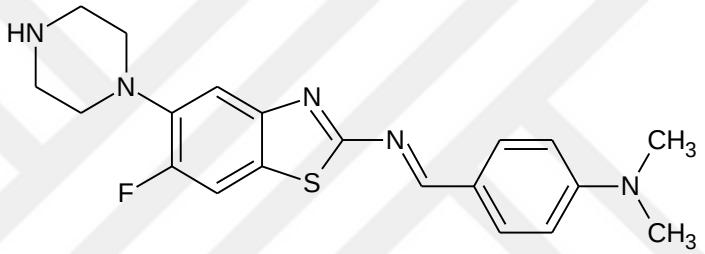
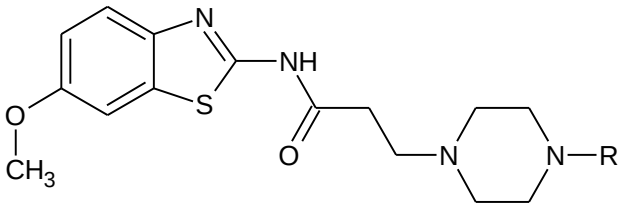


Figure 1.3. Compound **a**

In this study, we aim to report the synthesis and purification of novel compounds bearing *N*-(6-methoxybenzothiazol-2-yl)-3-(4-substitutedpiperazinyl)propanamide backbone with their spectral data. Those compounds were tested for their antimicrobial activities with agar-based disc diffusion assay. We also performed docking studies on DNA gyrase subunit B of *S. aureus* to investigate their possible mechanism of action.

Table 1.1. Structure of the synthesized compounds



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9	2-Pyridinyl	175	18.17
10	Tetrahydrofuroyl	144.3	30.8

2. LITERATURE REVIEW

2.1. Benzothiazole

Benzothiazole is an aromatic heterocyclic compound, and its chemical formula is C_7H_5NS. Benzothiazole ring consists of benzene and thiazole rings [21].

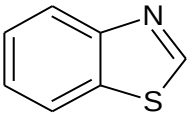
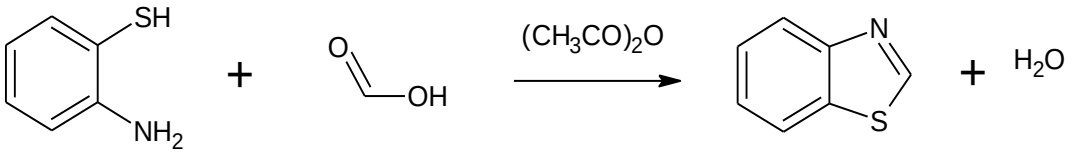


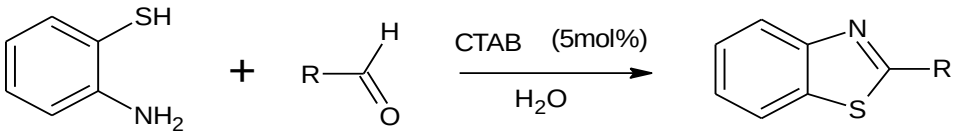
Figure 2.1. Structure of Benzothiazole

2.1.1. Methods of Synthesis

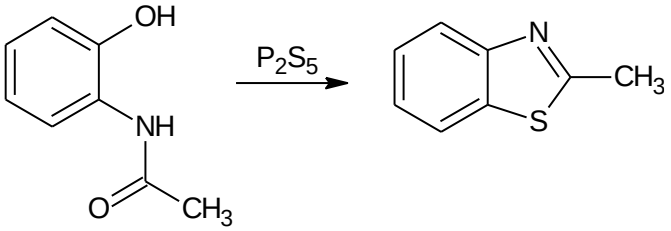
Benzothiazole is formed by reaction of 2-aminothiophenols and formic acid in presence of acetic anhydride [22].



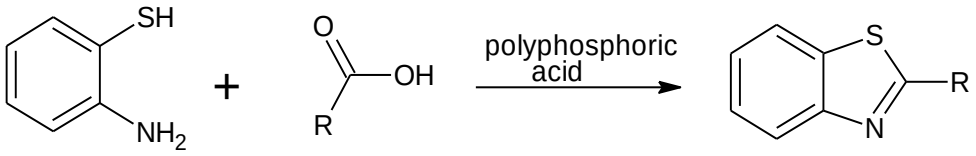
2-substituted benzothiazoles is prepeared through reaction of 2-aminothiophenol with aldehydes (RCHO: R = Alkyl, Aryl, Heteroaryl, 2-Arylformyl) in the existence of cetyltrimethylammonium bromide (CTAB) in water [23].



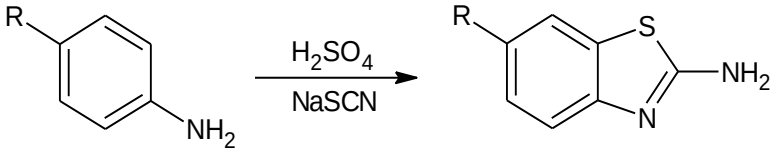
Benzothiazoles are similarly shaped through effect of phosphoruspentasulfide on o-acylaminophenoles [23].



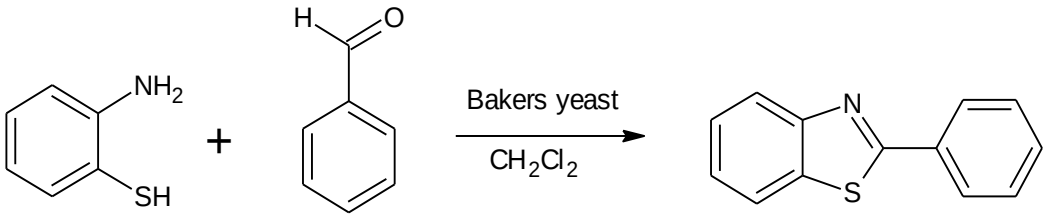
Another method to synthesize 2- substituted benzothiazoles in a good yield is condensation of 2-aminothiophenol with acids in existence of polyphosphoric acid [24].



Sodium thiocyanate and p-substitutedaniline are utilized by Allen *et al*., to produce benzothiazole derivatives in the existence of sulfuric acid as a catalyst [24]



Umesh R. Pratap positively utilized bakers' yeast as a catalyst in reaction of 2-aminothiophenol and aldehydes in presence of dichloromethane (DCM) to give 2-phenylbenzothiazoles [24].



2.1.2. Spectral Properties of Benzothiazole

2.1.2.1. UV Spectroscopy

UV absorption spectra of the benzothiazoles show three absorption bands at wave lengths of 240 nm (A=0.037), 270 nm (A=0.031) and 290 nm (A= 0.030) [25].

2.1.2.2. IR Spectroscopy

IR spectra of benzothiazole shows C-H stretching band approximately at 3050 cm^{-1} . Alicyclic C-H stretching bands are detected at 2880-1160 cm^{-1} [25].

2.1.2.3. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of benzothiazole shows peaks at 8.971, 8.14, 7.94, 7.51 and 7.46 ppm in deuterated chloroform ($CDCl_3$) [26].

2.1.2.4. ^{13}C-NMR Spectroscopy

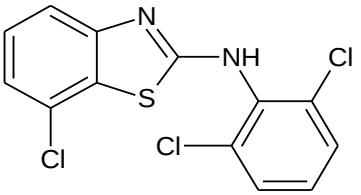
^{13}C-NMR spectra of benzothiazole shows peak at 167.54 ppm as a result of the carbon-nitrogen double bond [27].

2.1.3. Biological Properties of Benzothiazoles

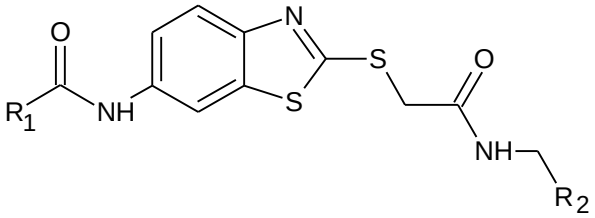
Benzothiazole nucleus is found to have a number of biological activities such as as antitumor [28], anticonvulsant [29], antimicrobial [30], antifungal [31], anti-inflammatory [32] and anti diabetic activities [33].

2.1.3.1. Anticancer activity

7-chloro-N-(2,6-dichlorophenyl)benzothiazol-2-amine has antitumor activity against non-small cell lung cancer HOP-92 cell line with GI_{50} values of 7.18 $x10^{-8}$ M [34].



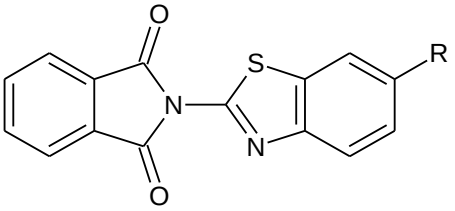
New benzothiazole-2-thiol compounds were prepared by Wang *et al*. and their antiproliferative effects were examined on HepG2 and MCF-7 cells. Most compounds had inhibitor effects on growth of the cell, and some of them were more effective than cisplatin [35].



R_1= H, phenyl, chlorophenyl

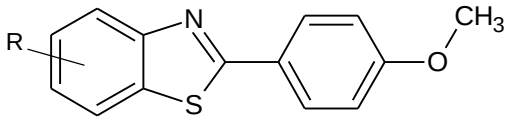
R_2= methyl, phenyl

Benzothiazole containing phthalimide synthesized by Kok *et al*. showed *in vitro* cytotoxicity on human cancer cell lines [35].



R=H, OCF_3

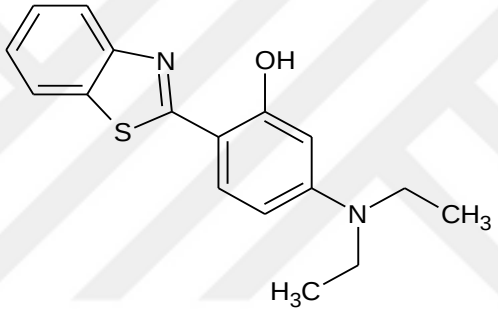
Some benzothiazole derivatives have anticancer activity against MCF-7 cells in addition they have tyrosine kinase inhibitory activity [36].



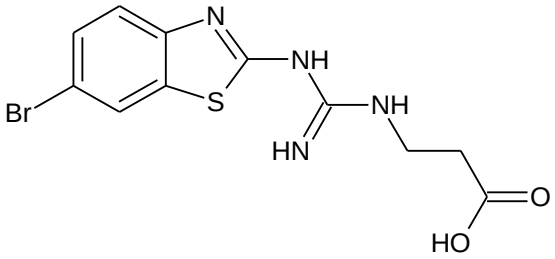
R= 6-Floro, 6-Bromo, 5,6-difloro

2.1.3.2. Antimicrobial activity

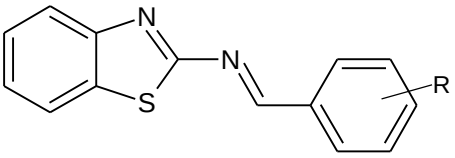
2-Substitutedbenzothiazole compounds have been prepared and reported for their good antifungal activity against *Aspergillus niger* and *Candida albicans* and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [37].



Compounds containing benzothiazole developed by Venkatesh *et al.* were found to have effective antimicrobial activity against both Gram-positive and Gram-negative bacteria [38].



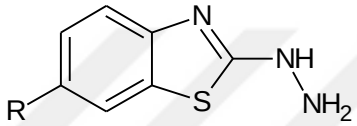
Below benzothiazole derivatives were found to have antibacterial activity against gram positive and gram-negative bacteria with MIC of 0.221 and 0.707 mM respectively by 4-chloro and against both bacteria of 0.46 mM by nitro substitution [39].



R= 2-NO_2, 4-Cl

2.1.3.3. Anthelmintic activity

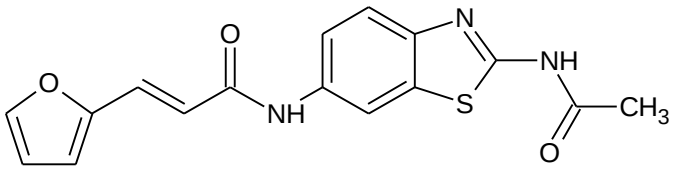
A group of 6-substituted-2-hydrazino-1,3-benzothiazoles were synthesized and evaluated for anthelmintic activity against earthworm species such as *Eudrilus eugeniea* and *Megascoplex konkanensis* [40].



R= H, CH_3, OCH_3, Cl, F

2.1.3.4. Other Activities

Benzothiazole acetamides were reported to have anti-inflammatory and anti-diabetic actions [41].



2.2. Piperazine

Piperazine is a heterocyclic compound containing four carbons and two nitrogen's at 1,4-positions in the ring.

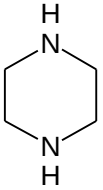
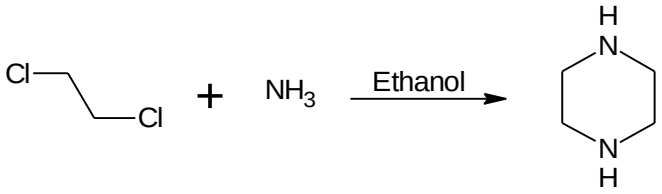


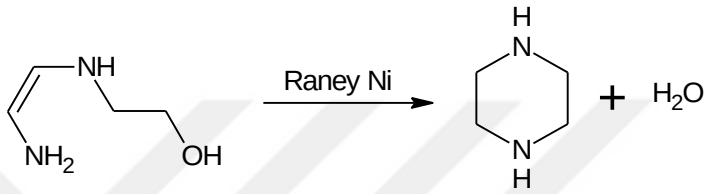
Figure 2.2. Structure of piperazine

2.2.1. Synthesis methods

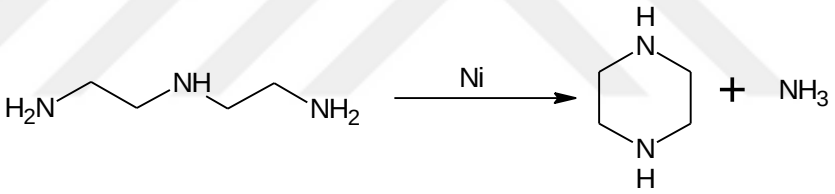
The first method of synthesis of piperazine was performed in 1853 with alcoholic ammonia and ethylene chloride [42].



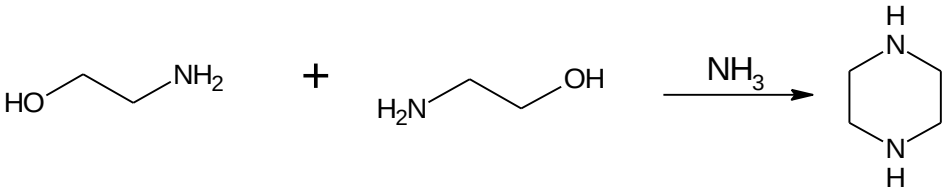
Cyclodehydration of *N*-(2-hydroxyethyl)ethenediamine was accomplished under atmospheric pressure by reflux with several catalysts. The Raney nickel was the first catalyst. Autoclave was utilized to achieve the reaction at 200-300 °C [43].



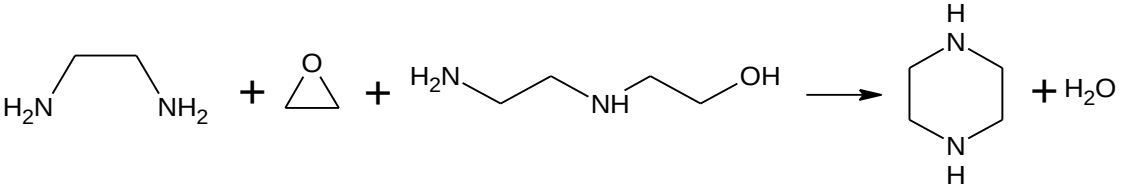
Increased yield of piperazine is achieved by heating diethylenetriamine with Raney nickel in autoclave [43,44].



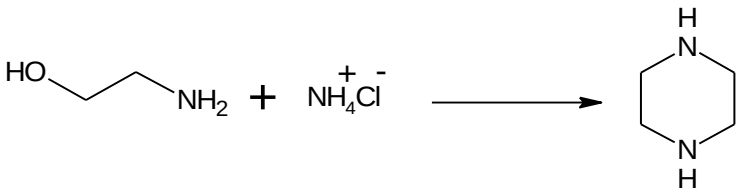
Piperazine is formed by reaction of two moles of 2-aminoethanol in the existence of ammonia at 150-220 °C under 100-200 bar pressure [45].



Piperazine was synthesized in presence of ethylenediamine and oxirane [45].



Piperazine is obtained from heating ethanolamine and ammonium chloride at 250°C [46].



2.2.2. Spectral Properties of Piperazine

2.2.2.1. UV Spectroscopy

UV absorption spectra of the piperazine shows two absorption bands at 260 nm (A=0.0035) and 280 nm (A=0.010) [47].

2.2.2.2. IR Spectroscopy

IR spectra of piperazine shows N-H stretching vibrations as sharp singlet approximately at 3250 cm^{-1}. Alicyclic C-H stretching bands appear at 2950-2700 cm^{-1} [48].

2.2.2.3. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of piperazine shows peaks at 2.84 ppm in deuterated chloroform ($CDCl_3$) [45].

2.2.2.4. ^{13}C-NMR Spectroscopy

^{13}C-NMR spectra of piperazine shows peaks at 47.9 ppm in deuterated chloroform ($CDCl_3$) [45].

2.2.2.5. Mass Spectroscopy

For piperazine.$6H_2O$, four main m/z values are observed in mass spectrum that are 86, 56, 44 and 30. Base peak is m/z=44 which represents fraction of $NHCH_2CH_2]^+$ [49].

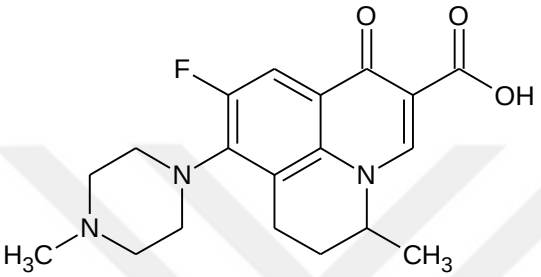
2.2.3. Biological Properties of Piperazines

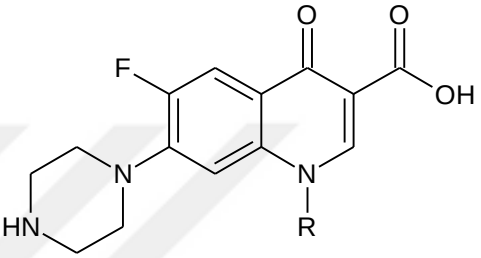
Piperazine was first used as anthelmintic agent as it has inhibitory action on GABA receptor [50].

Piperazine derivatives remain as drug candidates with many activities such as anticancer (*i.e.* imatinib [51]), antibacterial (*i,e*, norfloxacin, ciprofloxacin, levofloxacin [52-56]), antifungal (*i.e*. itraconazole, posaconazole [57]), antihistaminic (*i.e* cinnarizine, cyclizine [51,58,59]).

2.2.3.1 Antibacterial Activity

Examples of piperazine nucleus-bearing drugs used clinically as antibacterial are ciprofloxacin, ofloxacin and norfloxacin [2].

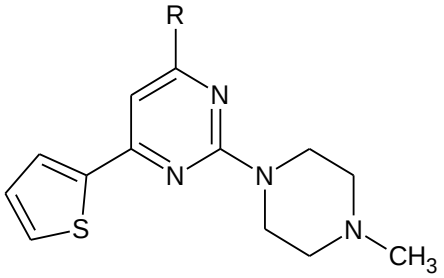




Ciprofloxacin, R= cyclopropyl ofloxacin

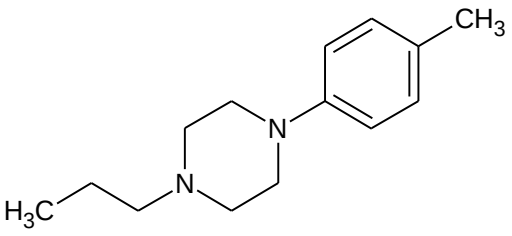
Norfloxacin, R= ethyl

Some piperazinylpyridine derivatives were synthesized and found to have good antibacterial activity [60].

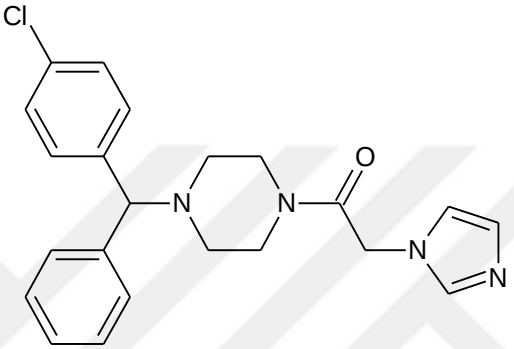


R= phenyl, 2-chloro-3-isoquinolinyl

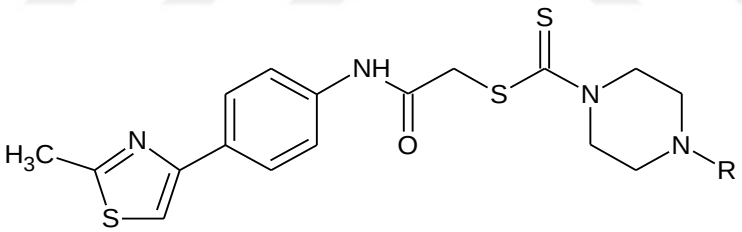
Jain *et al*. synthesized piperazine derivatives with antibacterial activity tested utilizing Ampicillin as standard drug against strains of *S. aureus, S. epidermidis, P. aeruginosa and E. coli.* Compound presented below shows great antibacterial activity when compared with standard drug [60].



Gan *et al.* have designed and synthesized various piperazine containing azole compounds and checked their antibacterial activities. The fundamental results showed that most compounds presented moderate to significant activity [61].



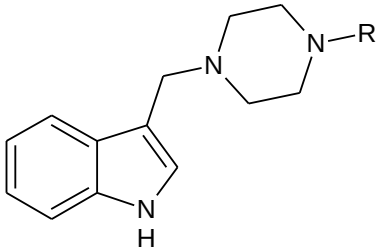
4-Substitutedpiperazine-1-carbodithioate derivatives were synthesized and tested for their growth inhibitory activity against gram positive and gram negative bacteria [62].



R= phenyl, cyclohexyl, 4-florophenyl

2.2.3.2. Anticancer Activity

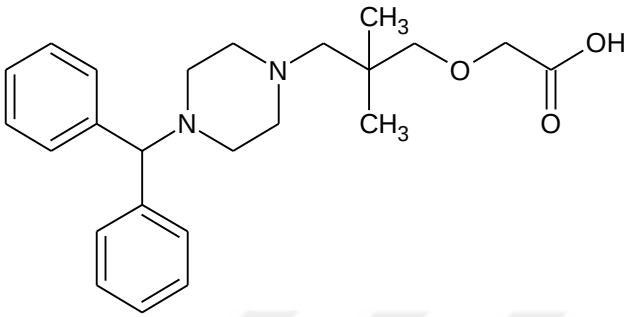
Some piperazinylindole derivatives were synthesized and found to exhibit potent cytotoxicity against human liver (HUH7), breast (MCF-7), and colon (HCT-116) cancer cell lines with IC_{50} concentrations lower than the standard drug 5-fluorouracil [63].



R = 3,4-dichlorobenzyl, 2-flurophenyl, 4-chlorophenyl, 2-methylphenyl

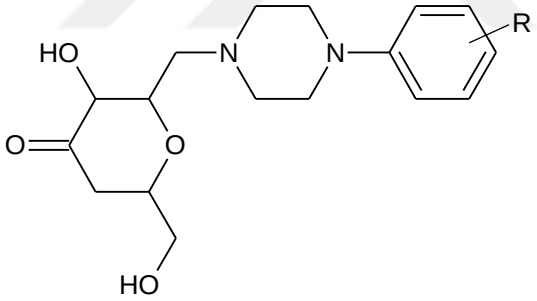
2.2.3.3. Antihistaminic Activity

Benzhydrylpiperazine derivatives are known to have antihistaminic activity [64].



2.2.3.4. Anticonvulsant Activity

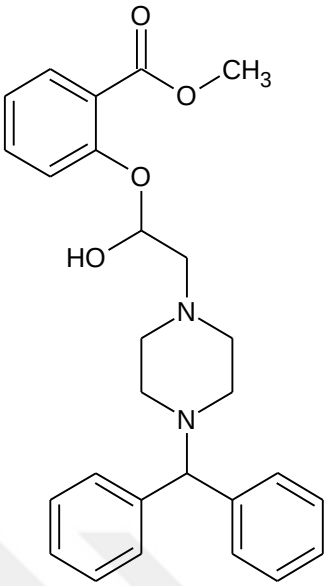
Novel *Mannich* bases of kojic acid that contain piperazine moiety were prepared and evaluated for anticonvulsant activity by maximum electric shock (MES) and subcutaneous Metrazol (scMet) induced seizure tests [65].

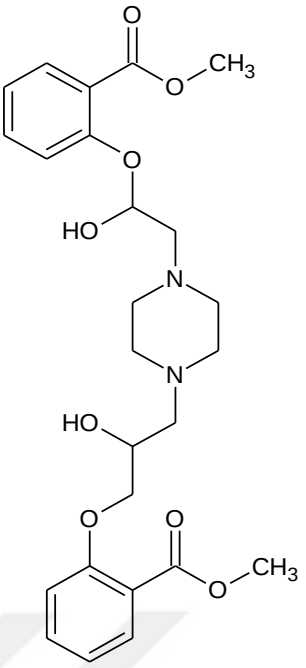


R= 3-trifluromethyl, 2-methoxy, 2-chloro, 4-chloro

2.2.3.4. Anti-inflammatory Activity

Many compounds containing piperazine moiety were synthesized by Jingfen *et al.* and tested for their anti-inflammatory activities in a dose-dependent manner *in vivo*, where, the activities of compounds below were higher than that of aspirin, and even equal to that of indomethacin at the same dose [66].

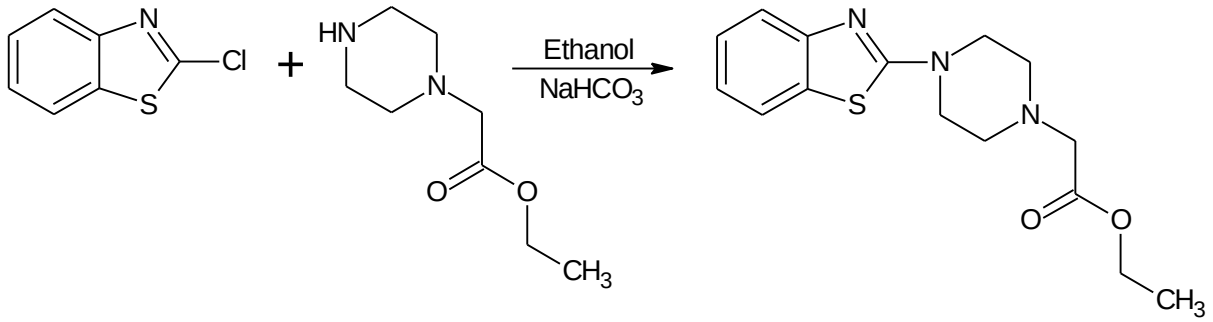




2.3. Benzothiazole-Piperazine Structure

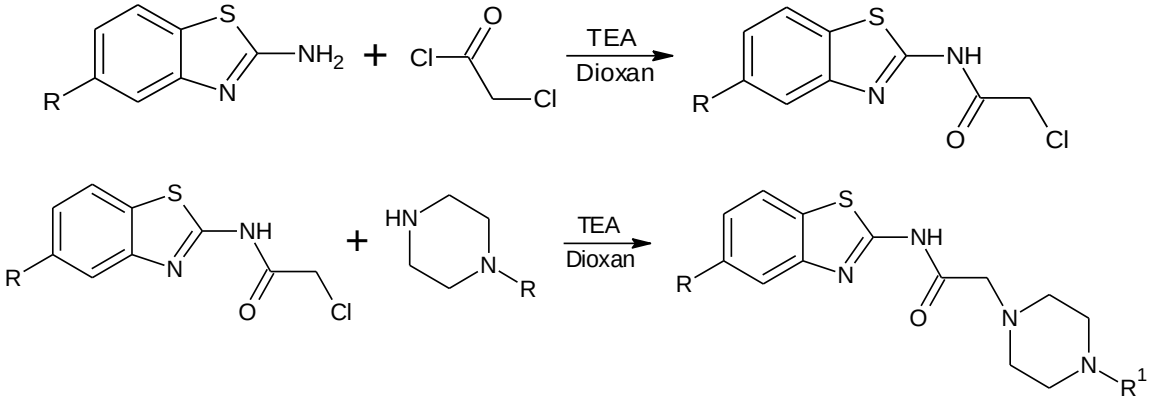
2.3.1. Methods of Synthesis

Ethyl 2-(4-(benzo[d]thiazol-2-yl)piperazin-1-yl)acetate can be synthesized from 2-chlorobenzothiazole and ethyl 2-(piperazin-1-yl)acetate in presence of ethanol and sodium bicarbonate in 24 hours, then solvent is evaporated, residue is extracted with water and $CHCl_3$, and the $CHCl_3$ was evaporated to give ethyl 2-(4-(benzo[d]thiazol-2-yl)piperazin-1-yl)acetate [67].



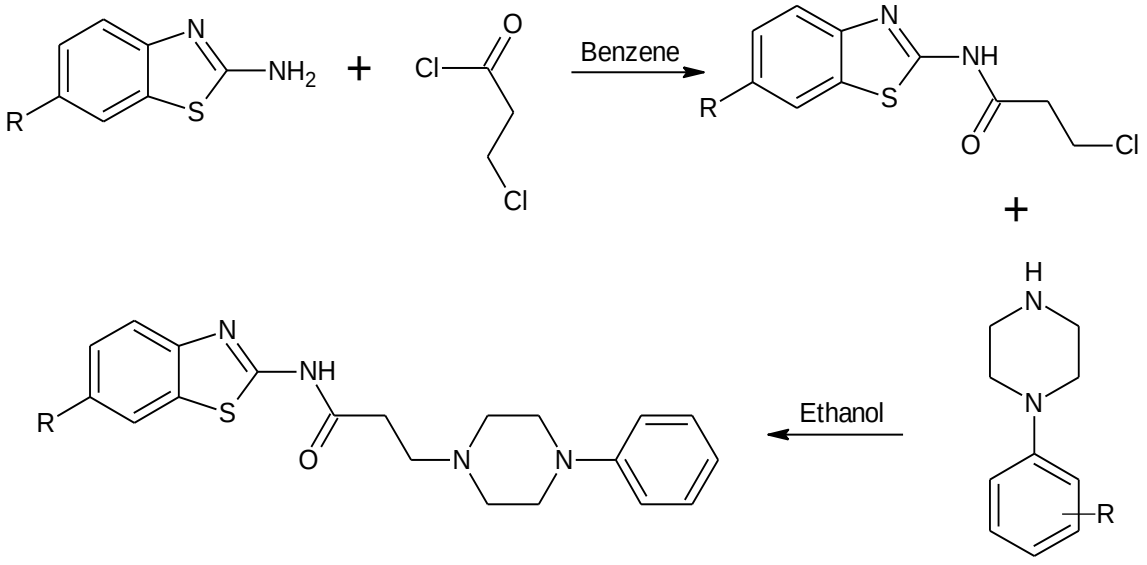
2-Aminobenzothiazoles are reacted with chloroacetyl chloride in presence of triethylamine in dioxane to yield 2-chloro-(*N*-benzo[*d*]thiazol-2-yl)acetamide derivative. In the second step, substituted 2-chloro-(*N*-(benzo[*d*]thiazol-2-yl)acetamides were treated

with various piperazines in presence of triethylamine in dioxane to form corresponding *N*-(benzo[*d*]thiazol-2-yl)-2-(substitutedpiperazin-1-yl)acetamide[68].



R= -H, -OCH_3, -NO_2 R^1= -H, -C_2H_5, -C_6H_5

2-Aminobenzothiazole is acylated with 3-chloropropionyl chloride in dry benzene then product was reacted with 4-phenylpiperazine derivatives in absolute ethanol and alkaline medium. In the first step, the reaction is refluxed in water bath at 80 °C for 3 h. Benzene and 3-chloropropionyl chloride are removed by distillation. The residue is washed with aqueous sodium bicarbonate and cold water. The product is dried and crystallized from ethanol. In the second step, the reaction is heated under reflux in a water bath for 12 h and ethanol is removed by distillation and the residue is washed with sodium bicarbonate and water then the product is dried and crystallized from ethanol [69].



2.3.2. Spectral Properties of Benzothiazole-Piperazine Derivatives

2.3.2.1. UV Spectroscopy

UV spectra of 2-phenylpiperazine-*N*-benzothiazole acetamide shows three absorption bands at 275.5 nm, 226.5 nm and 210 nm respectively [70].

2.3.2.2. IR Spectroscopy

IR spectra shows a sharp band at 1692 cm^{-1}, and N-H stretching vibrations of benzothiazole-piperazine appear as a sharp singlet at 3175 cm^{-1} [70].

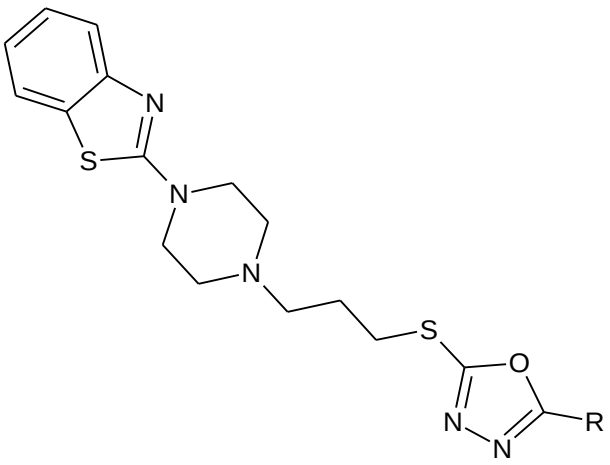
2.3.2.3. ^{1}H-NMR Spectroscopy

In ^{1}H-NMR Spectra of benzothiazole-piperazine derivatives peaks appear at 1.33 (t, 3H, -$OCH_2\underline{CH_3}$) ppm, 3.98 (q, 2H, -$O\underline{CH_2}CH_3$) ppm, 2.59-3.25 (m, 4H, piperazine $H_{2,6}$) ppm, 3.45 (m, 4H, piperazine $H_{3,5}$) ppm, 6.59-7.08 (m, 6H, Ar-H, H_5) ppm and 8.12 (d, 1H, benzothiazole H_4) ppm in deuterated chloroform ($CDCl_3$) [70].

2.3.3. Biological Properties of Benzothiazole-Piperazine

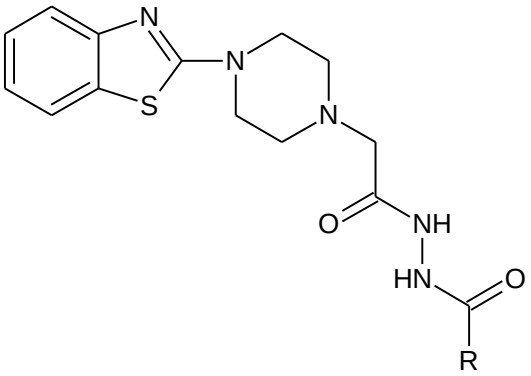
2.3.3.1. Antimicrobial Activity

Raju *et al. s*ynthesized benzothiazole piperazine derivatives, which were tested against *Staphylococcus aureus, Staphylococcus pyogenus* and *Escherichia coli* for their antimicrobial activity [71].



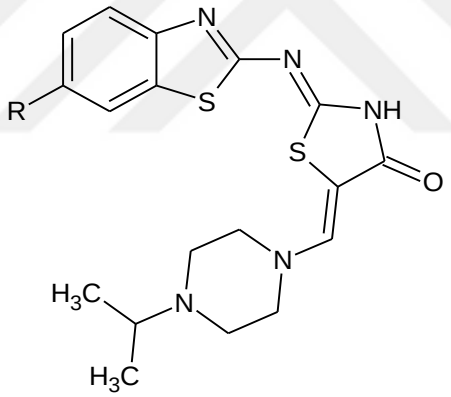
R= 4-nitrobenzene, p-tolyl, 4-phenol

In vitro antibacterial activity of the prepared compounds presented below was examined on *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The minimum inhibitory concentration study showed poor or no activity [67].



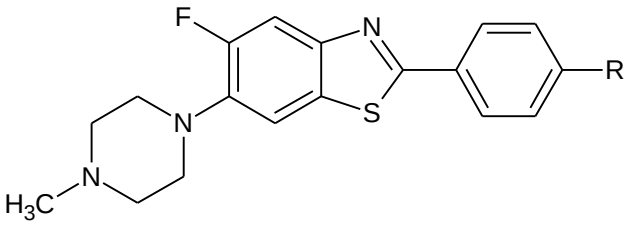
R= phenyl, methyl

A new class of piperazine-based 2-benzothiazolylimino-4-thiazolidinone derivatives have been synthesized and shown as antibacterial against gram positive bacteria (*S. aureus* and *B. subtilis*) gram-negative bacteria (E. coli, P. aeruginosa) with minimum inhibitory concentrations in the range of 4–8 μg /mL and inhibitory zones of 17–22 mm compared with 3.12–6.25 lg/mL and 15–24 mm, respectively, for the control drug ciprofloxacin [72].



R= -H, -Cl, -F, -Br,-NO_2

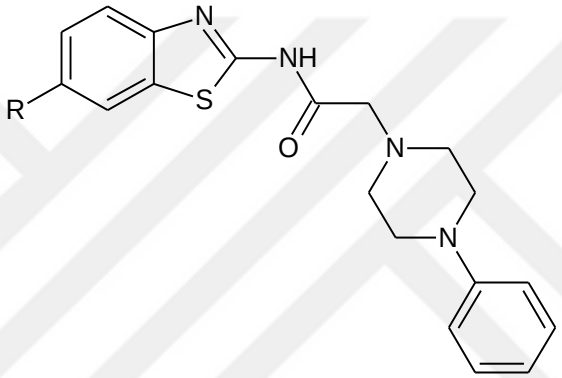
Al-Harthy *et al.* synthesized a novel series of 5-fluoro-6-(4- methylpiperazin-1-yl)-substituted phenylbenzo[d]thiazoles and these compounds were evaluated against different bacterial and fungal strains. Among these, two compounds showed excellent bacterial growth inhibition against *Staphylococcus aureus* with MIC of 32 μg /cm^3 compared to tamoxifen [73]**.**



R= -Cl, -Br

2.3.3.2. Anti-inflammatory Activity

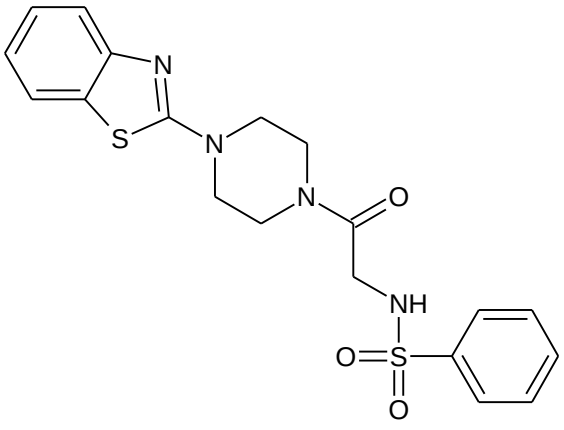
A group of benzothiazole-*N*-phenylpiperazine derivatives have been synthesized and found to be strong anti-inflammatory agents when compared with indomethacin as standard drug [69].



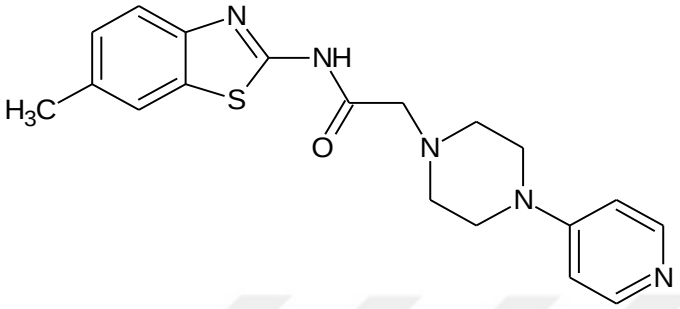
R= -H, -F, -OC_2H_5

2.3.3.3. Anticancer Activity

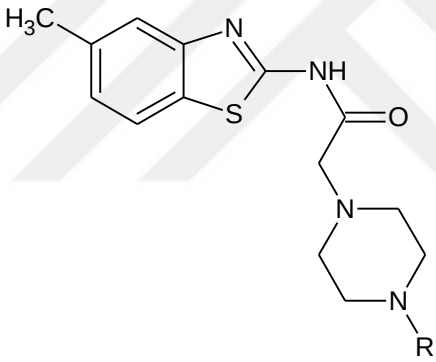
According to anticancer studies of benzothiazole-piperazine structure, arylsulfonamides and arylthiol derivatives have powerful growth inhibitory effect against a large number of cancer cell lines as breast (MCF-7), hepatocellular (HepG-2), prostate (DU-145) cancers and $CD4^+$ human acute T-lymphoblastic leukemia (CCRF-CEM) [74].



Gurdal *et al*., also declared that various benzothiazole-piperazine derivatives have high anticancer activity against liver (HUH-7), breast (MCF-7) and colon (HCT-116) cancer cell lines. Most of the substituted benzothiazole-piperazine derivatives are active against tested cancer cell lines. Further examination of below compound by Hoechst staining and FACS revealed that it causes cell cycle arrest in subG_1 phase [75].



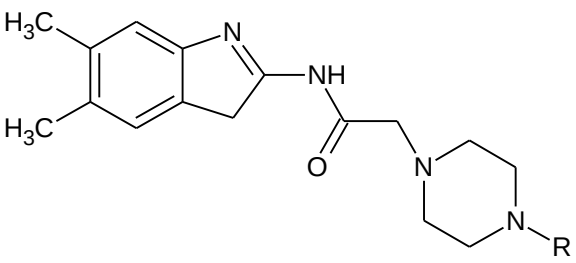
Aroyl substituted benzothiazole-piperazine derivatives were found to cytotoxicity activity against against HUH-7, MCF-7 and HCT-116 cancer cell lines [76].



R= benzoyl, 2-furoyl

2.3.3.4. Antidepressant Activity

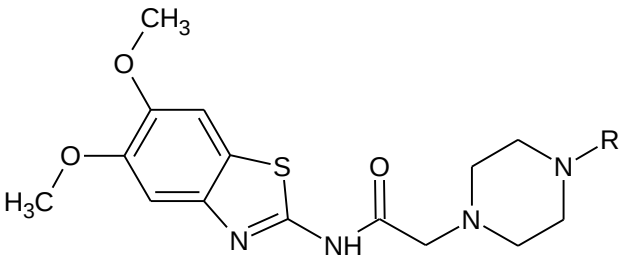
A number of benzothiazole-piperazine derivatives have antidepressant activity when compared with fluoxetine as reference drug [77].



R= methyl, ethyl, 4-florophenyl, 4-florobenzyl

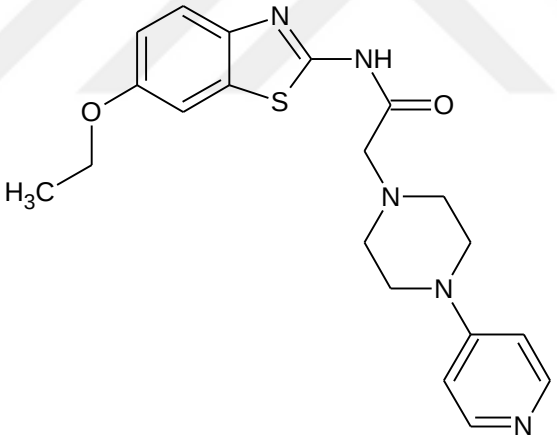
2.3.3.5. Acetylcholinesterase Inhibitory Activity

U. Demir Ozkay *et al*. synthesized and investigated the inhibition potential of a new benzothiazole-piperazine derivatives on acetylcholine esterase (AChE). Below compounds were found to inhibit enzyme comparably with Donepezil [78].



R= N,N-dimethylethylamine, N,N-dimethylpropylamine

Gurdal *et al*., also declared that a new series of benzothiazole-piperazine derivatives have moderate and selective inhibition against AChE in comparision with donepezil [79].



2.4. Biological Activity

2.4.1. Infectious Diseases

Infectious diseases remain one of the leading cause of worldwide death, caused by microorganisms as viruses, bacteria, fungi or parasites and can spread among people. Diseases are caused by the action of microorganisms through activating the immune system leading to many symptoms such as high fever. Infectious illnesses can spread starting with one person then another, by means of contact with bodily fluids, coughing, sneezing, or by a vector such as mosquito. Disease management can be problematic if

resistance is developed against utilized drugs. Such bacteria can undergo mutation in their DNA or acquire a new gene that leads them to survive despite antibiotic therapy [80].

2.4.2. History of Antibiotics

Antibiotics are used in recent years to treat infections. Different molds and plant extracts were utilized for treatment of infections via some of the Egyptians, such as application of moldy bread to infected wounds. However, it was the twentieth century, when infectious illnesses including lung diseases such as pneumonia and others as diarrhea which are caused by means of bacteria, could be treated efficiently.

Paul Ehrlich, a German physician, presented a biochemical named arsphenamine as an active and powerful medicine to treat syphilis. As a result, it turned into a novel antibiotic, or 'chemotherapy' in his own words – using chemicals in the treatment of illness. It is also known that antibiotics were used after some thirty years by a microbiologist, Selman Waksman, who discovered more than twenty antibiotics throughout his life.

In 1928, Alexander Fleming was successful to come up with penicillin. After the Suffolk's trip, the *Penicillium notatum* had affected the plate where the *Staphylococcus* bacterium was being cultivated. The fungus managed to create zones where the bacteria was not present as it was cultivated on the plate. Fleming could segregate and grow the fungus in the culture plate. *P. notatum* showed its high activity by not allowing the *Staphylococcus* growth. In addition, it was not as toxic as the commonly used disinfectants [81].

Following successful production of penicillium, researchers from all around the world contributed to the discovery of new effective antibiotics. Antibiotics of historical breakthrough are listed below in the order of their discovery:

- In 1939 – polypeptides,
- In 1943 – aminoglycosides,
- In 1945 – tetracycline,
- in 1947 – amphenicol.

More than a hundred various antibiotics were defined in 1950. Research for the discovery of new antimicrobials still goes on presently. Today, the number of antibiotics found so far account to several thousands. But, many of them are not applicable in medical practice, because of their high toxicity and side effect potential [82].

2.4.3. Basic Principles of Antimicrobial Therapy

The term chemotherapy describes the use of chemical compounds to attack the organisms invading the body. It is also used to describe the process for both cancer and treating infections.

Antibiotic refers to a substance produced by a microorganism, or to a similar molecule (can be obtained either completely or partially by the chemical synthesis process), which inhibits the growth of other microorganisms in low concentrations.

The term ‘selective toxicity’ means the tendency of the drug to just attack a certain cell or organism without attacking other normal cells.

2.4.3.1. Classification of Antibacterial Agents

Antimicrobials are divided based on several principles, including:

1. The mechanism of action,
2. Spectrum of activity,
3. Bacterial outcome.

2.4.3.1.1. Classification According to Mechanism of Action

Antibiotics have different mechanisms of actions as they have particular structure and the ability to target certain sites on the bacteria including:

1. Synthesis inhibitors of cell wall,
2. Inhibiting the function of cell membranes.
3. Protein’s synthesis inhibitors,
4. Inhibitors of nucleic acid function,
5. Inhibitors of other metabolic processes [83].

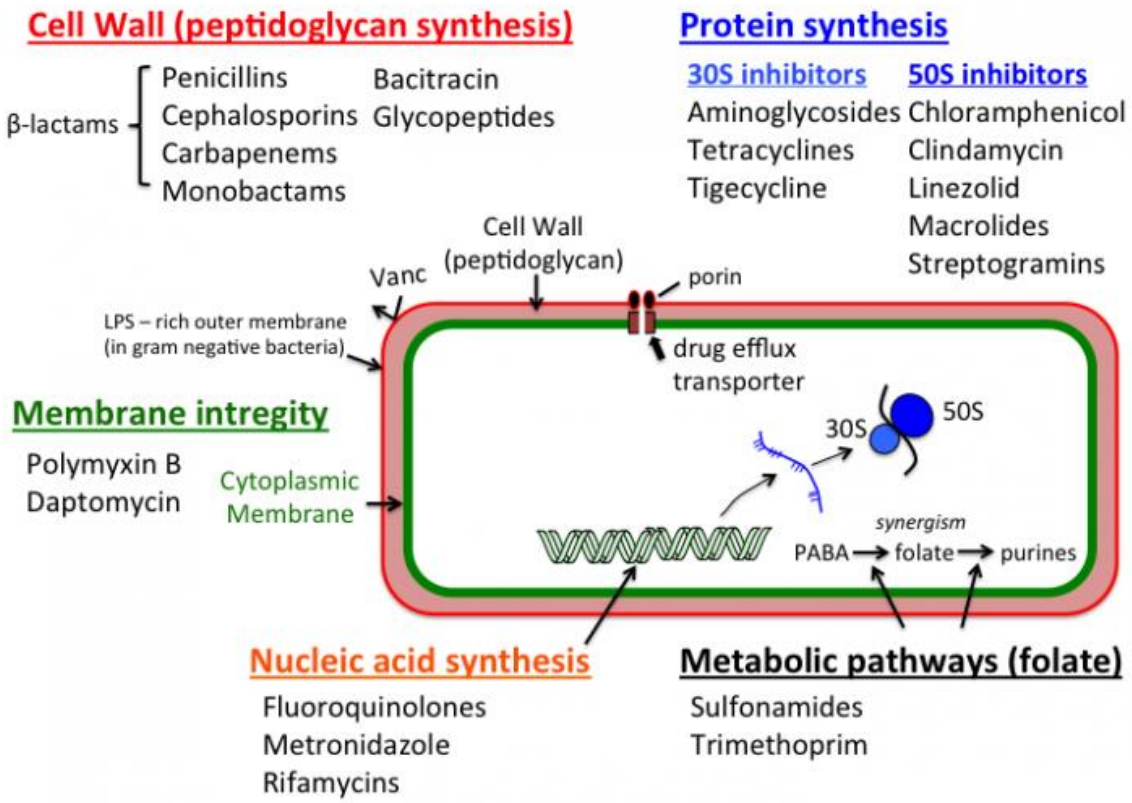


Figure 2.3. Bacterial structure and mechanisms of action of antibacterial drugs [84].

2.4.3.1.2. Classification According to Spectrum of Activity

2.4.3.1.2.1. Narrow Spectrum Antibiotics

The term ‘narrow spectrum’ is used to describe the ability of the compound to attack a single microorganism species. As an example, isoniazid remains active against *Mycobacteria.*

2.4.3.1.2.2. Extended Spectrum Antibiotics

Extended spectrum antibiotics, such as ampicillin, are used against gram positive germs and a number of gram negative germs.

2.4.3.1.2.3. Broad Spectrum Antibiotics

Broad spectrum antibiotics such as *tetracycline* and *chloramphenicol* are successful against a wide range of microorganisms.

2.4.3.1.3. Classification According to Effect on Bacteria

Bacteriostatic drugs such as tetracycline can prevent the growth and replication of bacteria whereas, bactericidal drugs such as aminoglycosides can kill them at achievable drug serum levels.

2.4.3.2. Selection of Antimicrobial Therapy

It is important to know the following before selecting any antimicrobial agent.

1) Identity of the organisms,
2) Susceptibility of the organisms to the agent,
3) The infection sites,
4) Patient's critical factors as age, weight, pregnancy, hepatic and renal status, etc.
5) Agent toxicity,
6) The cost of therapy.

However, some patients may require empiric therapy (umbrella therapy) that is, immediate administration of drug(s) prior to bacterial identification and susceptibility testing.

2.4.3.2.1. Determination of Antimicrobial Susceptibility of Infective Organisms

It is widely known that the susceptibility of both *Streptococcus pyogenes* and *Neisseria meningitidis* can be easily determined but that is not the case for other species. For example, the susceptibility of most gram-negative bacilli and staphylococcal species cannot be easily predicted and needs susceptibility tests to know the best antibiotic to use. In addition, the bactericidal and minimum inhibitory concentration can be determined.

The term minimum inhibitory concentration (MIC) is commonly used to determine the lowest possible concentration of antibiotics which is able to inhibit bacterial growth. while the minimum bactericidal concentration (MBC) refers to the lowest concentration of antibiotics that kills 99.9 of bacteria [83].

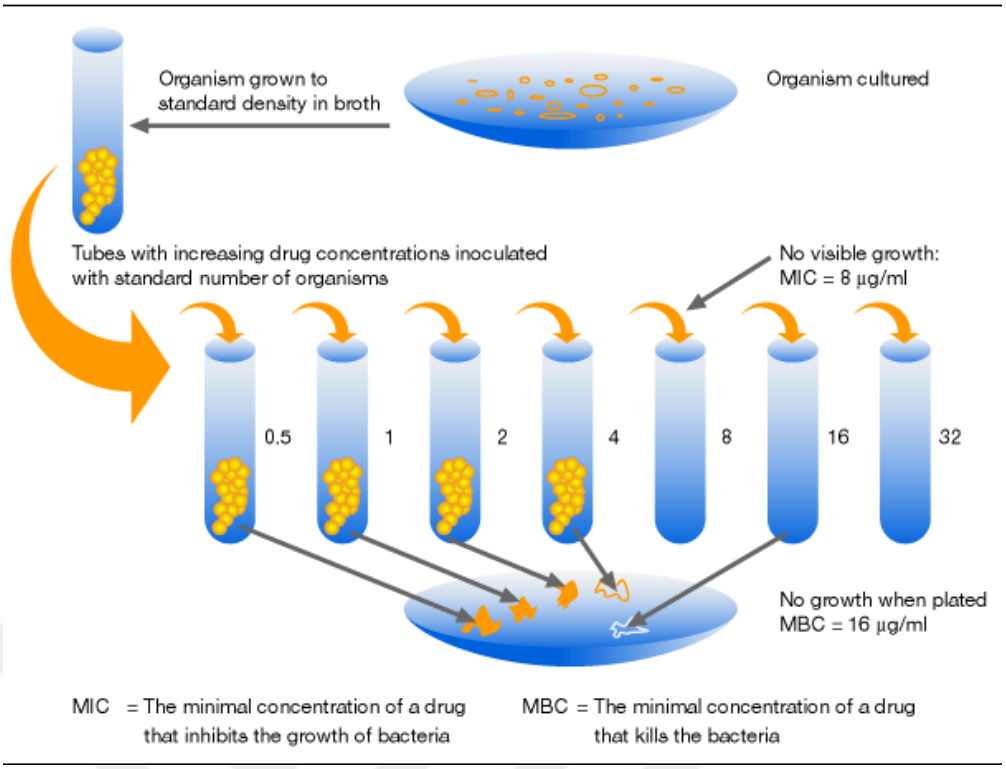


Figure 2.4. Determination of minimum inhibitory concentration and minimum bactericidal concentration [85].

2.4.3.3. Combination of Antimicrobial Drugs

Combinational therapy may result as additive, potentiative or antagonistic.

- Additive response occurs when effect of the total combination is the same to the total effects of the two drugs alone.
- Potentiate interaction occurs when effect of the total combination is much greater than the sum of the effects obtained from the drugs alone.
- In case the combination of two antibiotics is not as effective as the effect of one of the agents alone, antagonist response may occur.

Despite the visible outcomes in therapy, there are certain drawbacks of antibiotic combinations such as,

1) High risk of both toxic and allergic reactions,
2) The possibility of antagonism of antimicrobial effects may occur,
3) High risk of superinfection,
4) High cost of therapy.

2.4.3.4. Drug Resistance

In some cases, microorganisms can be very resistant to the antibiotics even at the maximum concentration tolerated by the host and their growth cannot be stopped. Some microorganisms are inherently resistant to effect of a certain drug. However, sensitive microbial species may develop resistance through spontaneous mutation or acquired resistance.

2.4.3.4.1. Genetic Alteration Leading to Drug Resistance

Spontaneous mutation of DNA occurs by a chromosomal change through insertion, deletion, substitution of one or more nucleotides within the genome.

DNA transfer of drug resistance from one organism to another causes acquired resistance. Resistance gene enters the cells through transduction, transformation, or conjugation.

2.4.3.4.2. Altered Expression of Proteins in Drug Resistant Organisms

Drug resistance may be affected by several mechanisms such as:

a. target site modification,

b. reduced accumulation,

c. enzymatic inactivation.

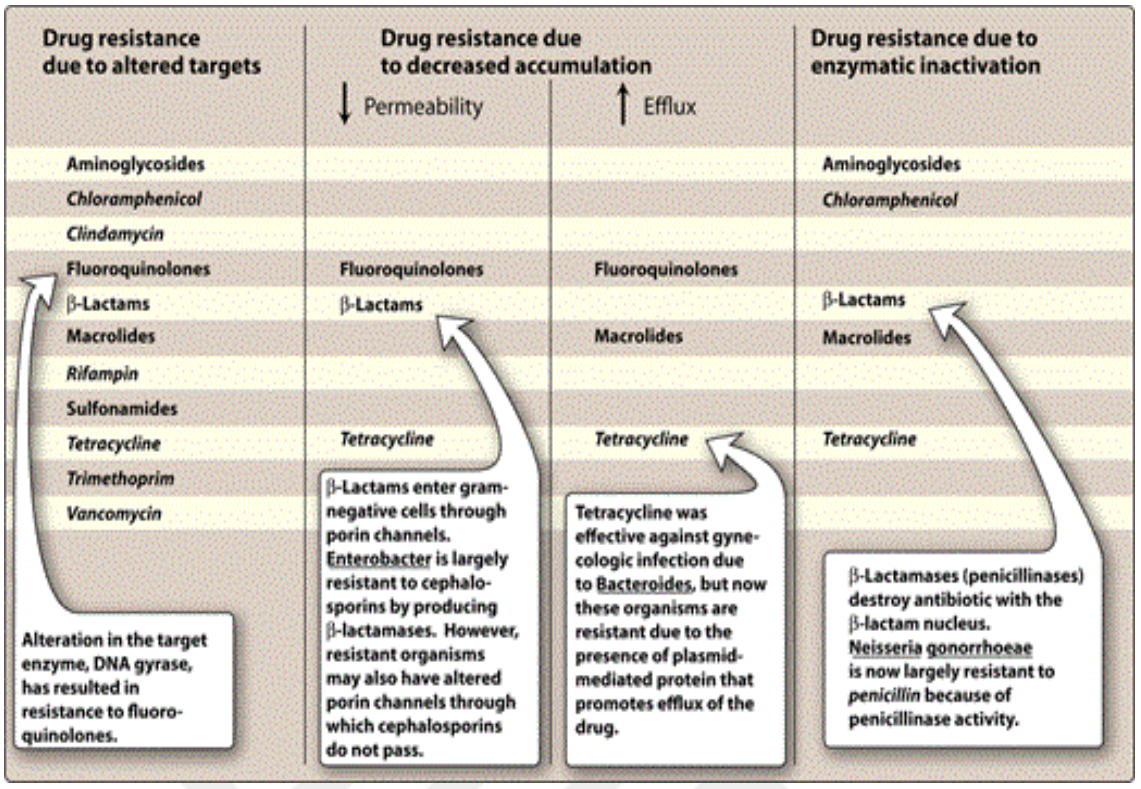


Figure 2.5. Some mechanisms of resistance to antibiotics [83].

2.4.4. Antifungal Agents

Infectious disease caused by fungi are called mycoses, and they are chronic in nature. An antifungal agent, also known as an antimycotic agent, is a pharmaceutical fungicide (kill parasitic fungi or their spores) or fungistatic (inhibits their growth) used to treat and prevent mycosis.

2.4.4.1. Classification of Antifungal Agents According to Mycotic Infection into:

1. Agents for Subcutaneous and Systemic Mycotic Infections**:**

- Amphotericin B (bind to fungal cell membrane ergosterol),
- Caspofungin (inhibit fungal cell wall synthesis),
- Fluconazole (inhibit ergosterol synthesis),
- Flucytocin (inhibit nucleic acid synthesis).

2. Agents for Cutaneous Mycotic Infection:

- Nystatin (bind to fungal cell membrane ergosterol),
- Clotrimazole (inhibit ergosterol synthesis),
- Griseofulvin (disrupt mitotic spindle and fungal mitosis) [83].

2.4.5. DNA Gyrase

DNA gyrase is an attractive target for investigation of new antibacterial agents. The enzyme is composed of GyrA and GyrB subunits as a member of type II topoisomerase family that are responsible with the control of topological state of DNA. It couples ATP hydrolysis through GyrB subunit to super-coiling of DNA in order to maintain DNA topology during replication. Inhibition of enzyme in bacteria leads to interruption of DNA synthesis and ultimately cell death [86].

Naturally occurring coumarins and cyclothialidines produced by certain species of *Streptomyces* compete with ATP for binding to GyrB, and inhibit the ATPase activity of the enzyme. Whereas quinolones and fluoroquinolones are synthetic compounds that preferentially inhibit either prokaryotic or eukaryotic type II topoisomerases. They stabilize the reversible enzyme–DNA covalent intermediate that causes formation of double-stranded breaks in DNA. Resulting protein–DNA adducts also block RNA and DNA polymerases [87].

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

For this study, 2-amino-6-methoxybenzothiazole, 3-chloropropionyl chloride, 1-(2-cyanophenyl)piperazine, 1-(3-hydroxyphenyl)piperazine, 1-(4-nitrophenyl)piperazine, 1-(4-chlorophenyl)piperazine, 1-(3,4-dichlorophenyl)piperazine, 1-(2,3-dichlorophenyl)piperazine, 1-(2,3-xylyl)piperazine, 1-(4-methylphenyl)piperazine, 1-(2-tetrahydrofuroyl)piperazine, 1-(2-pyridyl)piperazine, toluene, benzene, chloroform, absolute ethanol, methanol, ethyl acetate, *n*-hexane, anhydrous potassium carbonate and acetone were purchased from Sigma-Aldrich.

3.1.2. Methods of Synthesis

3.1.2.1. Synthesis Method of Starting Compound: 3-Chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide [69]

2-Amino-6-methoxybenzothiazole 0.0054 mol (1 g) is dissolved in 20 ml toluene. Later, acetylation is performed with 0.00544 mol (1.1 ml) 3-chloropropionyl chloride in room temperature. Because of the highly reactant nature of 3-chloropropionyl chloride, it is added slowly in small amounts, in presence of 0.0068 mol (0.94 g) anhydrous K_2CO_3. Reaction is monitored by TLC with silica gel plate and benzene: methanol (9:1) mobile phase mixture. Reaction is completed in four days at room temperature. Precipitated crude product is filtered, washed with acetone and dried. Ethanol crystallization gives pure product.

3.1.2.2. Synthesis Method of Target Compounds: *N*-(6-methoxybenzothiazol-2-yl)-3-(4-substituedpiperazinyl)propanamide [69]

Target compounds are synthesized in acetone by the reaction of 0.0007 mol (0.2 g) 3-chloro-*N*-(6-methoxybenzothiazol-2-yl)propanamide and 0.0007 mol of suitable piperazine, in presence of 0.0007 mol (0.1 g) anhydrous K_2CO_3. Reactions are monitored by TLC with silica gel plate and benzene:methanol (9:1) mobile phase mixture. Reactions are completed in two days at room temperature. After potassium carbonate residue is taken by filtration, it is washed with distilled water. Water insoluble product is collected

together with solid that remains after filtrate is evaporated to remove acetone. Crude product is crystallized from absolute ethanol or acetone-distilled water mixture.

3.1.3. Analytical Methods

3.1.3.1. Melting Point

Melting points (°C) of the compounds were determined by Melting Point Meter MPM-H2 apparatus and were uncorrected.

3.1.3.2. Controls by Thin Layer Chromatography

Materials:

TLC aluminum sheets 20x20 cm Silica gel 60 F254 (Merck) were used as stationary phase, and preparation of two different solvent systems was done to be applied in chromatographic controls of compounds.

S1: Benzene: Methanol (90:10)

S2: Ethyl acetate: *n*-Hexane (80:20)

For TLC method, solvent systems were poured to chamber and kept 1 hour for adequate and homogeneous saturation. Synthesized compounds and their starting materials were dissolved in suitable solvents and solutions were applied by Pasteur pipettes on the silica gel plates. The compounds on plates were dragged for 10 cm at room temperature, before their R_f values were calculated.

After drying the plates, determination of spots was performed under UV light (254/365 nm).

3.1.3.3. COLUMN CHROMATOGRAPHY

Compounds 1, 9 and 10 were purified by column chromatography using silica gel (60 mesh) as stationary phase and acetone:*n*-hexane (70:30) solution as mobile phase.

Column was filled in accordance with wet method. Elution was controlled with TLC using silica gel plates and ethyl acetate.

3.1.4. Spectral Analyses

Elemental, NMR and LC-MS analyses were conducted in Ankara University, Faculty of Pharmacy.

3.1.4.1. Ultraviolet Spectroscopy

UV Spectra were collected at concentration of 2×10^{-5} M in methanol with quartz cell of 1 cm path length by UV-VIS Agilent 8453 spectrometer.

3.1.4.2. Infrared Spectroscopy

Infrared spectra were collected on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), by applying potassium bromide (KBr) as a background, and the frequencies were shown in cm^{-1}.

3.1.4.3. ^{1}H-NMR Spectroscopy

^{1}H-NMR Spectra were collected with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA USA) by applying of tetramethyl silane (TMS) as the internal reference, with deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as a solvent, and the chemical shifts were reported in parts per million (ppm).

3.1.4.4. ^{13}C-NMR Spectroscopy

^{13}C-NMR spectra were collected with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) by applying tetramethyl silane (TMS) as the internal reference, with deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as a solvent, and the chemical shifts were reported in parts per million (ppm).

3.1.4.5. Mass Spectrometry

Mass spectra were collected with a Waters 2695 Alliance Micromass ZQ LC/MS instrument (Waters Corp., Milford, MA, USA).

3.1.4.6. Elemental Analysis

Elemental analyses were performed on a LECO 932 CHNS (LECO-932, St. Joseph, MI, USA) instrument**.**

3.2. Antimicrobial Activity

3.2.1. Bacterial Cultures

Both the gram positive and gram-negative species were chosen to examine the antibacterial activity. For the antifungal activity, one *Candida* species was selected. All tested strains in the study were *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229) and *Candida albicans* (ATCC 10231).

2.3.3. Agar-Based Disc Diffusion Assay

Disc diffusion method was used to test the antibacterial activity of the samples in 1024 μg/ml concentration against four different microorganisms. Standard discs of antibacterial ofloxacin (5 μg) and antifungal nystatin (100 units) were used as positive controls. The bacterial and fungal suspensions which achieved the turbidity of the 0.5 McFarland standards were inoculated to Mueller Hinton Agar (bacterial) or Sabouraud %2 Dextrose Agar (fungal) with sterile ecuvion sticks. Blanc discs (6 mm in diameter) were impregnated with 20 μL of the samples and placed on the inoculated plates. The antimicrobial activity of the samples was determined by measuring the diameter of zone of inhibition in millimeters after 18-24 hours of incubation [88].

2.3.4. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined by serial tube dilution method. Briefly; ten screw cap test tubes were taken and serially marked as 1, 2, 3, 4, 5, 6 and 7 for samples and the rest three was labeled as TM for medium, TMI for medium & inoculum and TMS for medium & DMSO. Nutrient broth medium of 1 mL was taken in all test tubes and the samples (1024 μg/ml) were only added to the no.1 labelled tube and the tube was shaken gently for proper mixing of the content. From the first tube, 1 ml of the content was added to the no.2 marked one and same was performed up to the no.7 marked tubes. Following proper mixing, 1 mL content from the 7 marked tube was discarded. Later, 10 μl of the bacterial and fungal suspensions, which achieved the turbidity of the 0.5 McFarland standards, was added to no 1, 2, 3, 4, 5, 6, 7 and TMI labelled tubes. Only 1 mL of DMSO was added to TMS labelled tube. Following shaking, 1 mL of the mixture was discarded from the tube. TM labelled tube only contained 1 mL of medium. This process is repeated for all tested substances and microorganisms. All the test tubes were subjected for incubation at 37 °C for 18-24 hours [88].

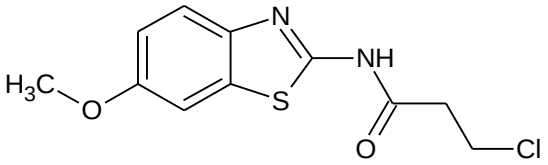
2.4. Docking Studies

For the interaction analysis of compounds with DNA gyrase subunit B of *Staphylococcus aureus*, docking studies were carried out by GLIDE [89] as a standard docking program. To test whether the docking program can correctly reproduce the binding mode and to evaluate docking program, redocking experiments were performed using the co-crystallized inhibitors and the crystal structures. Glide was tested and Glide score (Standard Precision - SP) was chosen as fitness function. The proper pose was evaluated according to the root mean square deviation (RMSD) of predicted conformations versus the corresponding native one; based on the principle of docking poses with RMSD of less than 2.0 Å are in agreement with the X-ray structure. Therefore, this docking program and setup were used in further studies. Then, a dataset of totally 10 benzothiazole-piperazine derivatives was generated. For this dataset, 25 conformers for each molecule were produced using ConfGen [90]. The aim of conformer generation is to generate a set of low energy 3D structures which includes the so-called bioactive conformation of a molecule that is the conformation in which it binds to the target [91]. All conformers were docked with Glide SP [89]. For the selection of the X-ray data to be used in docking studies, co-crystallized ligands were extracted to perform a similarity analysis in comparison with dataset ligands on Canvas [92]. 2D Fingerprints (MACCS) were calculated for all compounds and Similarity/Distance Matrix was created by Tanimoto similarity metric. The X-ray data of 3U2K with co-crystallized ligand that has Tc above 0.5 was selected for further use in docking studies.

4. RESULTS

4.1. Chemical Data

3-Chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide (Starting compound - CAS No: 1016743-82-0)



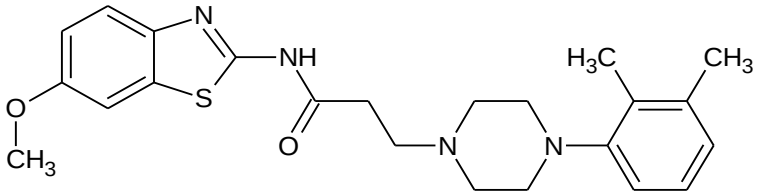
2-Amino-6-methoxybenzothiazole (0.0054 moles, 1 g) and (0.00544 moles, 1.1 ml) 3-chloropropionyl chloride were reacted according to synthesis method at 3.1.2.1. The yield is 0.4 g (13.3 %).

The form of compound is yellowish brown colored irregular crystals and its melting point is 174 °C. It is soluble in hot ethanol, acetone and DMSO. Rf values in TLC at S-1 and S-2 solvent systems are 0.43, 0.8 respectively.

FT-IR (KBr, cm^{-1}); 3251 (N-H), 3079 (C-H, aromatic), 2997 (C-H, aliphatic), 1687 (C=O, amide).

^{1}H-NMR (DMSO, ppm); 2.99 (t, 2H, -CO$\underline{CH_2}CH_2$-, *J=6.4 Hz*), 3.80 (s, 3H, Ar-OCH_3), 3.91 (t, 2H, -$CH_2\underline{CH_2}$Cl, *J=6.4 Hz*), 7.01-7.04 (dd, 1H, benzothiazole H_5, J_1*=2 Hz*, J_2*=8.8 Hz*), 7.57 (d, 1H, benzothiazole H_7, *J=2.4 Hz*), 7.63 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 12.35 (bs, 1H, -CO$\underline{NH}CH_2$-).

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(2,3-xylyl)piperazin-1-yl]propanamide (Compound 1)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.133 g) 1-(2,3-xylyl)piperazine were reacted in acetone with 0.0007 mol (0.18g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.062 g (19.6 %).

The form of compound is white colored regular crystals and its melting point is 195 °C. It is soluble in hot ethanol and chloroform at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.425, 0.3 respectively.

UV (MeOH, λ_{max}, nm); 289 (log ε: 4.13), 269 (log ε: 4.09).

FT-IR (KBr, cm^{-1}); 3244 (N-H), 3063 (C-H, aromatic), 2929 (C-H, aliphatic), 1686 (C=O, amide), 1604 (C=N), 1570 (C=C, aromatic), 1261 (C-N).

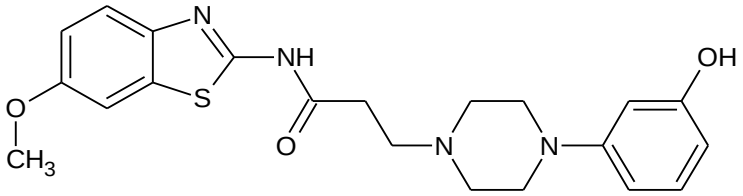
^{1}H-NMR ($CDCl_3$, ppm); 2.25 (s, 3H, Ar-CH_3), 2.29 (s, 3H, Ar-CH_3), 2.69 (t, 2H, -CO$\underline{CH_2}CH_2$N-, *J=5.2 Hz*), 2.83 (bs, 4H, piperazine $H_{2,6}$,), 2.86 (t, 2H, -$COCH_2\underline{CH_2}$N-, *J=5.2 Hz*), 3.11 (t, 4H, piperazine $H_{3,5}$, *J=5.2 Hz*), 3.87 (s, 3H, Ar-OCH_3), 6.94-6.96 (d, 1H, phenyl H_4, *J=7.6 Hz*), 7.01-7.02 (d, 1H, phenyl H_6, *J=2.8 Hz*), 7.03-7.05 (t, 1H, benzothiazole H_5, *J=4 Hz*), 7.13 (t, 1H, phenyl H_5, *J=7.6 Hz*), 7.28-7.29 (d, 1H, benzothiazole H_7, *J=2.4 Hz*), 7.68-7.71 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 12.65 (bs, 1H, -CO$\underline{NH}CH_2$).

MS (m/z); 425.7 (100%, M+H); 235.0 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{23}H_{28}N_4O_2S$ (MW: 424.559 g/mol);

	%C	%H	%N	%S
Calculated	65.07	6.65	13.2	7.55
Found	64.19	6.56	13.23	7.44

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(3-hydroxyphenyl)piperazin-1-yl]-propanamide (Compound 2)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.188 g) 1-(3-hydroxyphenyl)piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.2039 g (37.5 %).

The form of compound is brownish-white colored powder crystals and its melting point is 215 °C. It is soluble in hot ethanol. R_f values in TLC at S-1 and S-2 solvent systems are 0.437, 0.15 respectively.

UV (MeOH, λ_{max}, nm); 289 (log ε: 4.13), 308 (log ε: 4.22).

FT-IR (KBr, cm^{-1}); 3468 (N-H), 3325-2362 (O-H), 3080 (C-H, aromatic), 2941 (C-H, aliphatic), 1692 (C=O, amide), 1608 (C=N), 1542 (C=C, aromatic), 1264 (C-N).

^{1}H-NMR (DMSO, ppm); 2.55 (bs, 4H, -CO$\underline{CH_2CH_2}$N-), 2.69 (bs, 4H, piperazine $H_{2,6}$), 3.06 (bs, 4H, piperazine $H_{3,5}$), 3.79 (s, 3H, Ar-OCH_3), 6.18-6.20 (d, 1H, phenyl H_6, *J=7.6 Hz*), 6.29 (bs, 1H, phenyl H_2), 6.35-6.37 (dd, 1H, phenyl H_4, *J_1=1.6 Hz, J_2=16.4 Hz*), 6.94-6.98 (t, 1H, phenyl H_5, *J=8 Hz*), 6.99-7.02 (dd, 1H, benzothiazole H_5, *J_1=2.4 Hz, J_2=8.6 Hz*), 7.54-7.55 (d,1H, benzothiazole H_7, *J=2.8 Hz*), 7.60-7.62 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 9.09 (s, 1H, Ar-OH), 12.31 (bs, 1H, -CO$\underline{NH}CH_2$-).

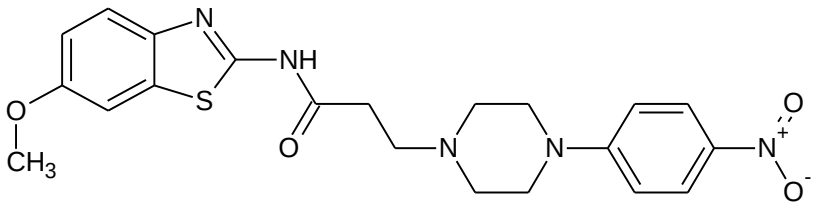
^{13}C-NMR (DMSO, ppm); 32.78 (-CO$\underline{CH_2}CH_2$-); 48.15 (piperazine $C_{2,6}$); 52.33 (piperazine $C_{3,5}$); 53.18 (-$CH_2\underline{CH_2}$N-); 55.58 (Ar-OCH_3); 102.37; 104.69; 106.1; 106.54; 114.81; 121.05; 129.46; 132.70; 142.57; 152.33; 155.74; 156.06 (Ar-C), 158.04 (S-C=N), 170.93 (C=O).

MS (m/z); 413.6 (100%, M^+H); 235.5 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{24}N_4O_3S$ (MW: 412.505 g/mol);

	%C	%H	%N	%S
Calculated	61.14	5.86	13.58	7.77
Found	61.10	6.12	13.60	7.76

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(4-nitrophenyl)piperazin-1-yl]propanamide (Compound 3)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.235g) 1-(4-nitrophenyl) piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.08 g (25.7 %).

The form of compound is brownish yellow colored powder crystals and its melting point is 220 °C. It is soluble in hot ethanol and DMSO. R_f values in TLC at S-1 and S-2 solvent systems are 0.43, 0.112 respectively.

UV (MeOH, λ_{max}, nm); 290 (log ε: 4.01), 308 (log ε: 4.03).

FT-IR (KBr, cm^{-1}); 3613 (N-H), 3079 (C-H, aromatic), 2959 (C-H, aliphatic), 1681 (C=O, amide), 1595 (C=N), 1542 (NO_2), 1328 (NO_2), 1285 (C-N).

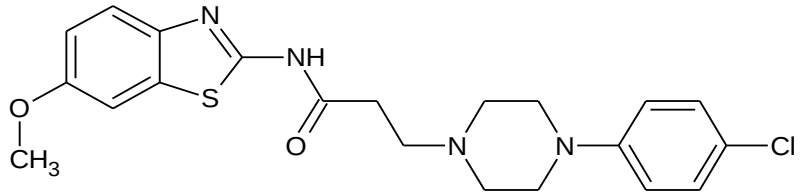
^{1}H-NMR (DMSO, ppm); 2.53 (bs, 4H, piperazine $H_{2,6}$), 2.66-2.71 (m, 4H, -CO$\underline{CH_2}$$\underline{CH_2}$- & -$CH_2$$\underline{CH_2}$N-), 3.43 (bs, 4H, piperazine $H_{3,5}$), 3.78 (s, 3H, -OCH_3), 6.98-7.02 (m, 3H, phenyl $H_{3,5}$, *J=8.4 Hz*), 7.53 (d, 1H, benzothiazole H_5, *J=2.4 Hz*), 7.59 (d, 1H, phenyl H_3, *J=8.4 Hz*), 8.03 (d, 2H, benzothiazole $H_{4,7}$, *J=8.8 Hz*), 12.25 (bs, 1H, -CO$\underline{NH}$$CH_2$-).

MS (m/z); 442.5 (100%, M^+H); 235.2 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{23}ClN_4O_2S$ (MW: 441.50 g/mol);

	%C	%H	%N	%S
Calculated	57.13	5.25	15.86	7.26
Found	56.69	6.10	16.24	7.44

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(4-chlorophenyl)piperazin-1-yl]propanamide (Compound 4)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.22 g) 1-(4-chlorophenyl) piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.17 g (54.8 %).

The form of compound is white colored powder crystals and its melting point is 200.2 °C. It is soluble in hot ethanol. R_f values in TLC at S-1 and S-2 solvent systems are 0.487, 0.212 respectively.

UV (MeOH, λ_{max}, nm); 297 (log ε: 4.13), 308 (log ε: 4.12).

FT-IR (KBr, cm^{-1}); 3247 (N-H), 3069 (C-H, aromatic), 2942 (C-H, aliphatic), 1689 (C=O, amide), 1603 (C=N), 1569 (C=C, aromatic), 1263 (C-N).

^{1}H-NMR (DMSO, ppm); 2.56 (bs, 4H, piperazine $H_{2,6}$), 2.69 (bs, 4H, -CO$\underline{CH_2}CH_2$-&-$CH_2\underline{CH_2}$N-), 3.11 (bs, 4H, piperazine $H_{3,5}$), 3.79 (s, 3H, Ar-OCH_3), 6.92-6.94 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*), 6.99-7.02 (dd, 1H, benzothiazole H_5, *J_1=2.8 Hz*, *J_2=8.8 Hz*), 7.19-7.22 (d, 2H, phenyl $H_{3,5}$, *J=9.2 Hz*), 7.54-7.55 (d,1H, benzothiazole H_7, *J=2.8 Hz*), 7.60-7.62 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 12.30 (bs, 1H, -CO$\underline{NH}CH_2$-).

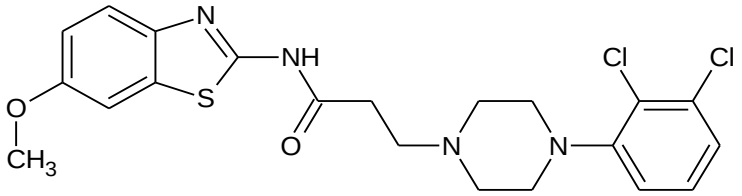
^{13}C-NMR ($CDCl_3$, ppm); 32.79 (-CO$\underline{CH_2}CH_2$-), 47.94 (piperazine $C_{2,6}$), 52.16 (piperazine $C_{3,5}$), 53.14 (-$CH_2\underline{CH_2}$N-), 55.57 (Ar-OCH_3), 104.69, 114.78, 116.74, 121.03, 122.25, 128.52, 132.70, 142.57, 149.71, 155.74, 156.06 (Ar-C), 158.06 (S-C=N), 170.90 (C=O).

MS (m/z); 431.6 (100%, M^+H); 235.5 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{23}ClN_4O_2S$ (MW: 430.950 g/mol);

	%C	%H	%N	%S
Calculated	58.53	5.38	13.00	7.44
Found	58.04	5.68	12.98	7.28

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(2,3-dichlorophenyl)piperazin-1-yl]propanamide (Compound 5)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.22 g) 1-(2,3-dichlorophenyl)piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.16 g (33.8 %).

The form of compound is white colored powder crystals and its melting point is 185.7 °C. It is soluble in hot ethanol. R_f values in TLC at S-1 and S-2 solvent systems are 0.4, 0.15 respectively.

UV (MeOH, λ_{max}, nm); 289 (log ε: 4.12), 308 (log ε: 4.11).

FT-IR (KBr, cm^{-1}); 3242 (N-H), 3066 (C-H, aromatic), 2928 (C-H, aliphatic), 1684 (C=O, amide), 1604 (C=N), 1571 (C=C, aromatic), 1260 (C-N).

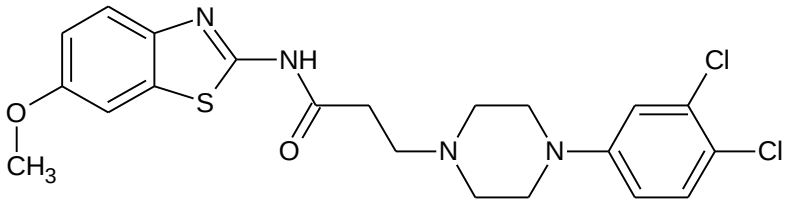
^{1}H-NMR (DMSO, ppm); 2.59 (bs, 4H, piperazine $H_{2,6}$), 2.66 (t, 2H, -CO$\underline{CH_2}$$CH_2$-, *J=5.6 Hz*), 2.73 (t, 2H, -$CH_2\underline{CH_2}$N-, *J=7.2 Hz*), 2.96 (bs, 4H, piperazine $H_{3,5}$), 3.78 (s, 3H, Ar-OCH_3), 6.99-7.01 (dd, 1H, benzothiazole H_5, J_1*=2.4, Hz,* J_2*=9 Hz*), 7.11-7.14 (m, 1H, phenyl H_5), 7.26 (d, 1H, phenyl H_6, *J=3.2 Hz*), 7.27-7.28 (d, 1H, phenyl H_4, *J=1.2 Hz*), 7.53-7.54 (d, 1H, benzothiazole H_7, *J=2.8 Hz*), 7.59-7.61 (d, 1H, benzothiazole H_4, *J=8.4 Hz*), 12.25 (bs, 1H, -CO$\underline{NH}$$CH_2$-).

MS (m/z); 465.5 (100%, M^+); 467.5 (M+2); 235.4 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{22}Cl_2N_4O_2S$ (MW: 465.395 g/mol);

	%C	%H	%N	%S
Calculated	54.20	4.76	12.04	6.89
Found	54.70	4.99	12.05	6.90

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(3,4-dichlorophenyl)piperazin-1-yl]propanamide (Compound 6)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.26 g) 1-(4-chlorophenyl)piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.116 g (33.8 %).

The form of compound is white colored powder crystals and its melting point is 220.2 °C. It is soluble in hot ethanol. R_f values in TLC at S-1 and S-2 solvent systems are 0.375, 0.237 respectively.

UV (MeOH, λ_{max}, nm); 297 (log ε: 4.08), 308 (log ε: 4.06).

FT-IR (KBr, cm^{-1}); 3486 (N-H), 3076 (C-H, aromatic), 2946 (C-H, aliphatic), 1679 (C=O, amide), 1600 (C=N), 1541 (C=C, aromatic), 1229 (C-N).

^{1}H-NMR (DMSO, ppm); 2.54 (t, 4H, piperazine $H_{2,6}$, *J=4.8 Hz*), 2.67 (t, 2H, -CO$\underline{CH_2}CH_2$-, *J=5.6 Hz*), 2.71 (t, 2H, -$CH_2\underline{CH_2}$ N-, *J=9.2 Hz*), 3.16 (t, 4H, piperazine $H_{3,5}$, *J=4.8 Hz*), 3.79 (s, 3H, Ar-OCH_3), 6.89-6.93 (dd, 1H, phenyl H_5, J_1*=3.2 Hz*, J_2*=9.2 Hz*), 6.99 -7.02 (dd, 1H, benzothiazole H_5, J_1*=2 Hz*, J_2*=8.8 Hz*), 7.11-7.13 (d, 1H, phenyl H_6, *J=2.8 Hz*), 7.35-7.37 (d, 1H, phenyl H_2, *J=9.2 Hz*), 7.54-7.55 (d, 1H, benzothiazole H_7, *J=2.8 Hz*), 7.59-7.62 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 12.28 (bs, 1H, -CO$\underline{NH}CH_2$-).

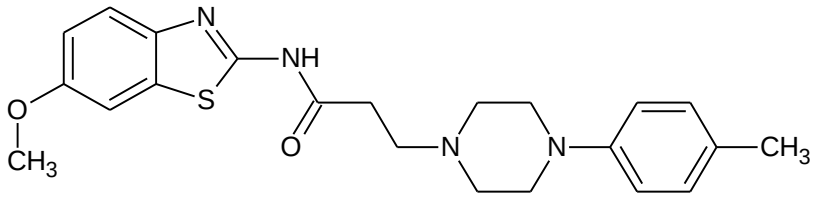
^{13}C-NMR (DMSO, ppm); 32.79 (-CO$\underline{CH_2}CH_2$-), 47.44 (piperazine $C_{2,6}$), 51.99 (piperazine $C_{3,5}$), 53.11 (-$CH_2\underline{CH_2}$N-), 55.55 (Ar-OCH_3), 104.66, 109.49, 114.78, 115.16, 116.12, 119.47, 121.02, 142.57, 128.14, 128.85, 130.34, 131.43, 132.68, 142.55, 150.61, 155.73 (Ar- C), 156.04 (S- C=N), 170.864 (C=O).

MS (m/z); 465.5 (100%, M^+); 467.5 (M+2); 235.4 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{22}Cl_2N_4O_2S$ (MW: 465.395 g/mol);

	%C	%H	%N	%S
Calculated	54.20	4.76	12.04	6.89
Found	54.17	4.907	12.17	6.88

_N_-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(4-methylphenyl)piperazin-1-yl]propanamide (Compound 7)



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.133 g) 1-(4-methylphenyl)piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.087 g (29 %).

The form of compound is white colored regular crystals and its melting point is 184.5°C. It is soluble in hot ethanol. R_f values in TLC at S-1 and S-2 solvent systems are 0.4, 0.225 respectively.

UV (MeOH, λ_{max}, nm); 289 (log ε: 4.14), 308 (log ε: 4.10).

FT-IR (KBr, cm^{-1}); 3432 (N-H), 3070 (C-H, aromatic), 2942 (C-H, aliphatic), 1691 (C=O, amide), 1604 (C=N), 1553 (C=C, aromatic), 1261 (C-N).

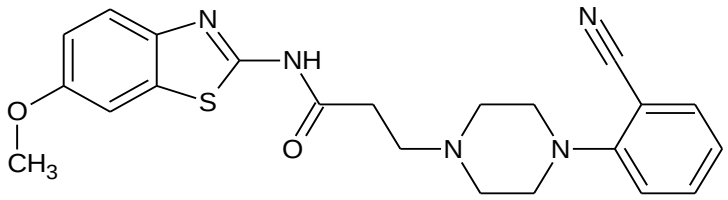
^{1}H-NMR ($CDCl_3$, ppm); 2.29 (s, 3H, Ar-CH_3), 2.72 (t, 4H, piperazine $H_{2,6,}$ *J=6 Hz*) 2.84-2.88 (m, 4H, -CO$\underline{CH_2CH_2}$N-), 3.37 (t, 4H, piperazine $H_{3,5,}$ *J=4.4 Hz*), 3.86 (s, 3H, Ar-OCH_3), 6.89-6.92 (m, 2H, phenyl $H_{3,5}$), 6.99-7.02 (dd, 1H, benzothiazole H_5, J_1*=2.4 Hz,* J_2*=8.8 Hz*), 7.11-7.13 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*), 7.26-7.27 (d, 1H, benzothiazole H_7, *J=2.4 Hz*), 7.64-7.66 (d, 1H, benzothiazole H_4, *J=8.4 Hz*), 12.51 (bs, 1H, -CO$\underline{NH}CH_2$-).

MS (m/z); 411.6 (100%, M+H); 235.5 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{21}H_{22}Cl_2N_4O_2S$ (MW: 410.532 g/mol);

	%C	%H	%N	%S
Calculated	64.36	6.38	13.65	7.81
Found	63.46	6.38	13.77	7.77

_N_-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(2-cyanophenyl)piperazin-1-yl]propanamide (Compound 8)



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.188 g) 1-(2-cyanophenyl)piperazine were reacted in acetone with 0.0007 mol (0.15 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.2050 g (63.6 %).

The form of compound is white colored regular crystals and its melting point is 163.3 °C. It is soluble in hot ethanol and DMSO. R_f values in TLC at S-1 and S-2 solvent systems are 0.425, 0.15 respectively.

UV (MeOH, λ_{max}, nm); 290 (log ε: 4.15), 308 (log ε: 4.18).

FT-IR (KBr, cm^{-1}); 3546 (N-H), 3064 (C-H, aromatic), 2955 (C-H, aliphatic), 2219 (C≡N), 1682 (C=O, amide), 1594 (C=N), 1256 (C-N).

^{1}H-NMR (DMSO, ppm); 2.62 (t, 4H, piperazine $H_{2,6}$, *J=4.4 Hz*), 2.68 (t, 2H, -CO$\underline{CH_2}CH_2$-, *J=6 Hz*), 2.76 (t, 2H, -$CH_2\underline{CH_2}$N-, *J=6 Hz*), 3.14 (t, 4H, piperazine $H_{3,5}$, *J=4.4 Hz*), 3.79 (s, 3H, Ar-OCH_3), 6.99-7.02 (dd, 1H, benzothiazole H_5, J_1*=2.4 Hz*, J_2*=8.6 Hz*), 7.075 (t, 1H, phenyl H_4, *J=7.6 Hz*), 7.13-7.15 (d, 1H, phenyl H_3, *J=8 Hz*), 7.55.7.56 (d, 1H, phenyl H_5, *J=2.4 Hz*), 7.59-7.60 (d,1H, benzothiazole H_7, *J=1.6 Hz*), 7.62-7.60 (d, 1H, benzothiazole H_4, *J=8.8 Hz*), 7.67-7.69 (dd, 1H, phenyl H_6, J_1*=1.6 Hz*, J_2*=7.6 Hz*), 12.28 (bs, 1H, -CO$\underline{NH}CH_2$-).

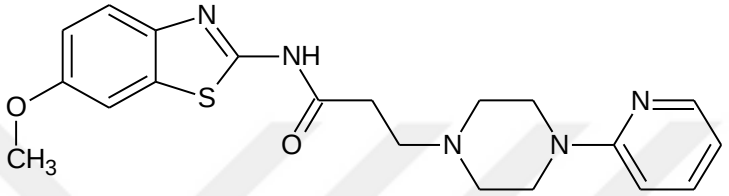
^{13}C-NMR (DMSO, ppm); 32.79 (-CO$\underline{CH_2}CH_2$-); 51.08 (piperazine C2,6); 52.32 (piperazine $C_{3,5}$); 53.09 (-$CH_2\underline{CH_2}$N-); 55.57 (Ar-OCH_3); 104.69; 114.79; 118.21; 119.01; 121.036; 121.91; 132.70; 134.18; 134.26; 142.57; 155.15 (Ar-C); 155.77 (-$\underline{C}$N); 156.06 (S-$\underline{C}$=N); 170.887 (C=O).

MS (m/z); 422.6 (100%, M^+H); 235.2 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{22}H_{23}N_5O_2S$ .H_2O (MW: 439.53 g/mol);

	%C	%H	%N	%S
Calculated	60.12	5.73	15.93	7.30
Found	61.44	5.83	15.83	7.05

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(2-pyridinyl)piperazin-1-yl]propanamide (Compound 9)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.133 g) 1-(2-pyridyl)piperazine were reacted in acetone with 0.0007 mol (0.11g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.0527 g (18.17 %).

The form of compound is white colored regular crystals and its melting point is 175 °C. It is soluble in hot ethanol and chloroform. R_f values in TLC at S-1 and S-2 solvent systems are 0.25, 0.075 respectively.

UV (MeOH, λ_{max}, nm); 291 (log ε: 4.23), 299 (log ε: 4.25).

FT-IR (KBr, cm^{-1}); 3432 (N-H), 3012 (C-H, aromatic), 2943 (C-H, aliphatic), 1673 (C=O, amide), 1603 (C=N), 1591 (C=C, aromatic), 1260 (C-N).

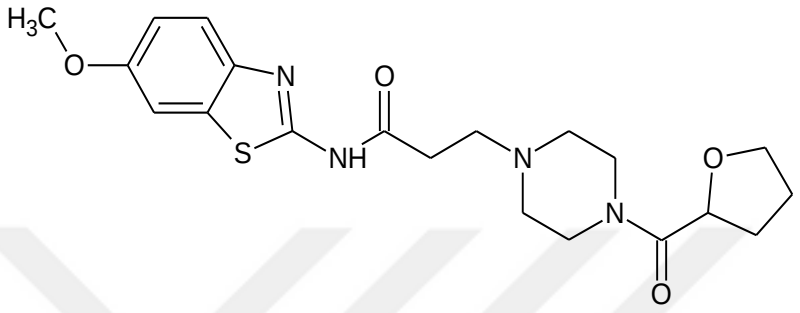
^{1}H-NMR ($CDCl_3$, ppm); 2.72 (t, 2H, -CO$\underline{CH_2}CH_2$-, *J=5.6 Hz*), 2.79 (t, 4H, piperazine $H_{2,6}$, *J=5.2 Hz*), 2.86 (t, 2H, -$CH_2\underline{CH_2}$N-, *J=5.2 Hz*), 3.77 (t, 4H, piperazine $H_{3,5}$, *J=4.4 Hz*), 3.86 (s, 3H, Ar-OCH_3), 6.67-6.72 (m, 2H, pyridine $H_{5,6}$), 6.99-7.02 (dd, 1H, benzothiazole H_5, J_1*=2.8 Hz*, J_2*=9.2 Hz*), 7.26 (d, 1H, benzothiazole H_7, *J=3.2 Hz*), 7.50-7.55 (ddd, 1H, pyridine H_4, J_1*=1.2 Hz*, J_2*=1.6 Hz*, J_3*=15.8 Hz*), 7.64-7.66 (d, 1H, benzothiazole H_4, *J=8.8 Hz*), 8.22-8.24 (dd, 1H, pyridine H_3, J_1*=2 Hz*, J_2*=5 Hz*,), 12.65 (bs, 1H, -CO$\underline{NH}CH_2$).

MS (m/z); 398.6 (100%, M+H); 235.4 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{20}H_{23}N_5O_2S$ .H_2O (MW: 415.51 g/mol);

	%C	%H	%N	%S
Calculated	57.81	6.06	16.85	7.72
Found	58.94	6.20	16.24	7.45

***N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-[4-(2-tetrahydrofuroyl)piperazin-1-yl]propanamide (Compound 10)**



0.0007 Mol (0.2 g) 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide and 0.0007 mol (0.133 g) 1-(2-pyridinylphenyl)piperazine were reacted in acetone with 0.0007 mol (0.136 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.108 g (30.8 %).

The form of compound is white colored regular crystals and its melting point is 175 °C. It is soluble in hot ethanol and chloroform. R_f values in TLC at S-1 and S-2 solvent systems are 0.1375, 0.0375 respectively.

UV (MeOH, λ_{max}, nm); 288 (log ε: 3.87), 308 (log ε: 3.82).

FT-IR (KBr, cm^{-1}); 3417 (N-H), 3073 (C-H, aromatic), 2923 (C-H, aliphatic), 1693 (C=O, amide), 1642 (C=N), 1602 (C=C, aromatic), 1257 (C-N).

^{1}H-NMR (DMSO, ppm); 1.77-1.82 (m, 2H, tetrahydrofuroyl H_4), 1.94-1.99 (m, 2H, tetrahydrofuroyl H_3), 2.34-2.42 (m, 4H, -CO$\underline{CH_2CH_2}$-), 2.62-2.69 (m, 4H, piperazine $H_{2,6}$), 3.39-3.49 (m, 4H, piperazine $H_{3,5}$), 3.69-3.77 (m, 2H, tetrahydrofuroyl H_5), 3.79 (s, 3H, Ar-OCH_3), 4.61-4.65 (dd, 1H, tetrahydrofuroyl H_2, *J_1=6 Hz*, *J_2=7.6 Hz*), 6.99-7.02 (dd, 1H, benzothiazole H_5, *J_1=2.4 Hz*, *J_2=8.6 Hz*), 7.54-7.55 (d, 1H, phenyl H_7, *J=2.8 Hz*), 7.60-7.62 (d, 1H, benzothiazole H_4, *J=9.2 Hz*), 12.26 (bs, 1H, -CO$\underline{NH}CH_2$).

MS (m/z); 419.6 (100%, M+H); 235.2 (6-CH_3O-Ar-$COCH_2CH_2CH_2$+).

Elemental analysis of $C_{20}H_{26}N_4O_4S$ .H_2O (MW: 436.51 g/mol);

	%C	%H	%N	%S
Calculated	55.03	6.47	12.83	7.35
Found	55.34	6.63	12.63	7.31

4.2. Pharmacological Studies

Table 4.1. Antibacterial and Antifungal activities data for compound 1-9 (inhibition zone).

Sample	Sample Concentration	Zone of Inhibition (diameter in mm)			
		S. aureus ATCC 6538	*P. aeruginosa* ATCC 15442	*E. coli* ATCC 11229	*C. albicans* ATCC 10231
1	1024 µg/ml	8	8	8	8
2	1024 µg/ml	12	12	12	10
3	1024 µg/ml	8	8	8	8
4	1024 µg/ml	8	8	8	8
5	1024 µg/ml	12	12	12	10
6	1024 µg/ml	8	8	8	8
7	1024 µg/ml	8	8	8	8
8	1024 µg/ml	10	10	10	8
9	1024 µg/ml	10	10	10	8
Ofloxacin	5 µg	32	32	34	-
Nystatin	100 units	-	-	-	30

Table 4.2. Minimum Inhibitory Concentration data for compounds **1-9** (µg/ml).

Sample	***S. aureus* ATCC 6538**	***P. aeruginosa* ATCC 15442**	***E. coli* ATCC 11229**	***C. albicans* ATCC 10231**
1	1024	1024	1024	1024
2	256	256	256	512
3	1024	1024	1024	1024
4	1024	1024	1024	1024
5	256	256	256	512
6	1024	1024	1024	1024
7	1024	1024	1024	1024
8	512	512	512	1024
9	512	512	512	1024

4.3. Docking Studies on DNA gyrase subunit B (*S. aureus* GyrB)

X-ray data of DNA gyrase subunit B (PDB ID: 3U2K [xx]) was used for docking studies of benzothiazole-piperazine derivatives keeping the conserved water molecule. Redocking study of co-crystallized ligand gave a docking score of -7.838 (RMSD: 0.596) in which the hydrophobic interactions are observed at adenine pocket. 3,4-Dichloro substitution decreases pKa value of NH making it a better H-bond donor to Asp81 in both water mediated and direct interactions. Additional H-bonds are made between a conserved GyrB Arg144 and the carbonyl of amide (Figure 4.1).

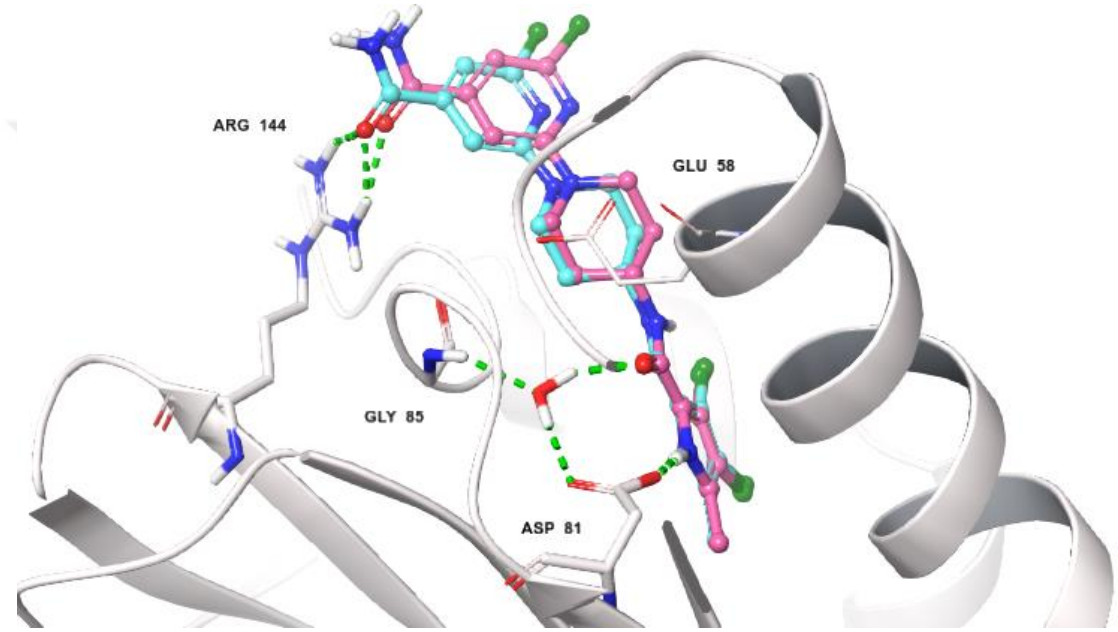


Figure 4.1. X-ray structure of GyrB (PDB ID: 3U2K) with the co-crystallized ligand (cyan) and the generated conformer (pink)

Although the compounds do not make any H-bonds with Asp81, they commonly interact with Arg144 and Glu58 residues. Compound **2**, with the best docking score (-6.286) among all compounds, was given in Figure 4.2 to show the general behavior of the series. Protonated amine of piperazine acts as a H-donor and contributes to a salt bridge with Glu58. In addition, benzothiazole ring makes π-cation and H-bonding interactions with Arg144.

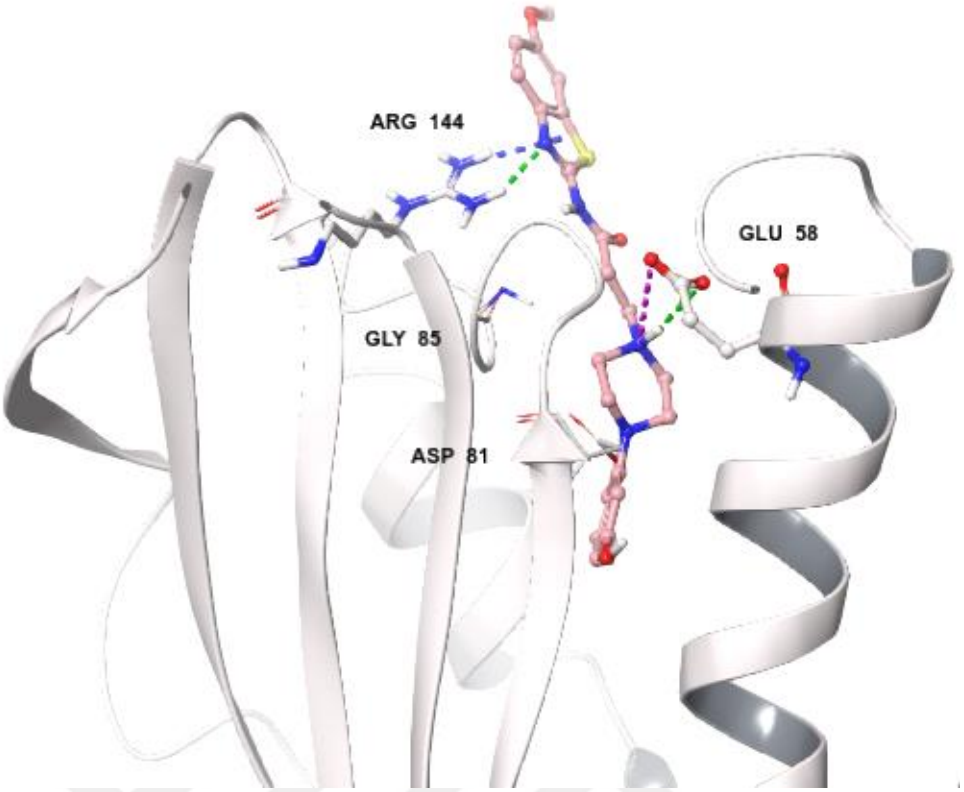
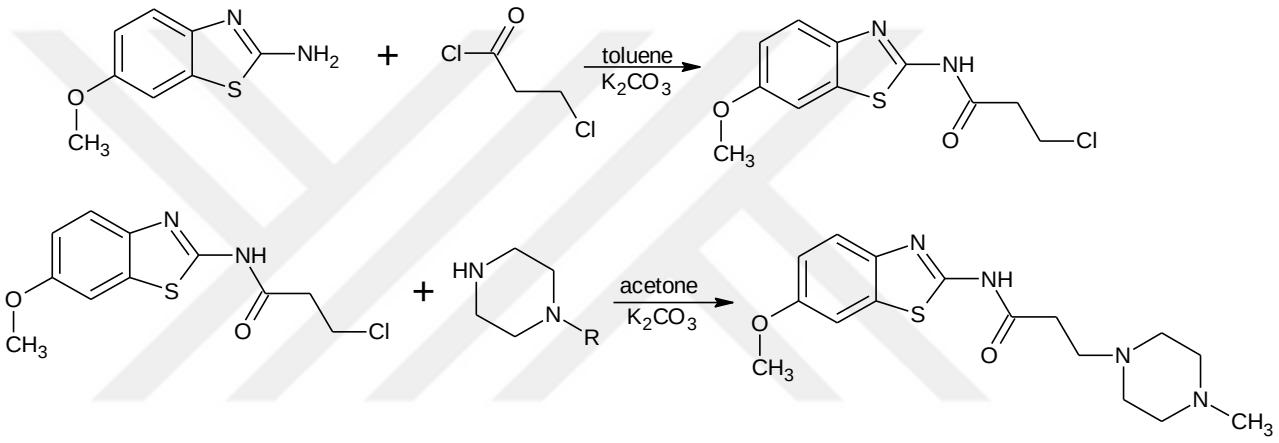


Figure 4.2. Docking pose of compound **2** (PDB ID: 3U2K)

5. DISCUSSION AND CONCLUSION

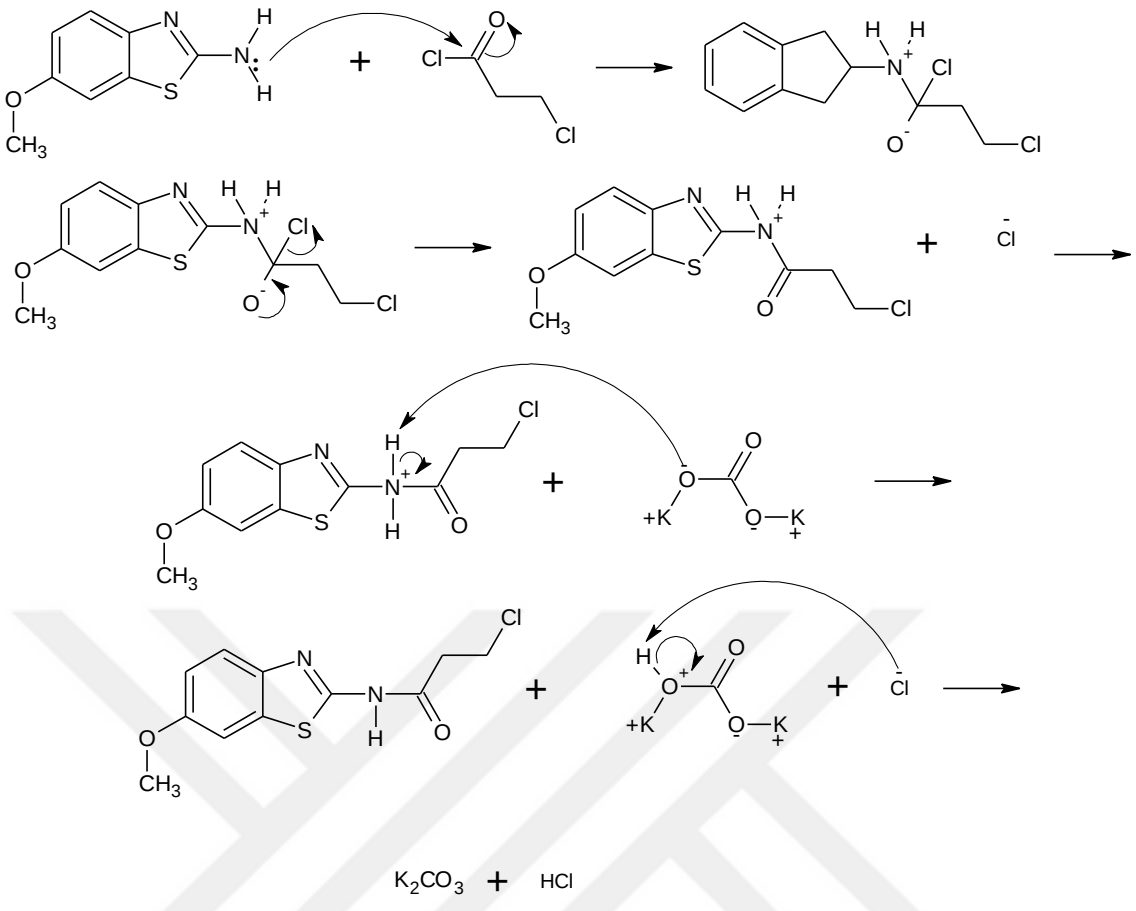
In this study, ten novel compounds having *N*-(6-methoxybenzothiazol-2-yl)-3-(4-substituedpiperazinyl)propanamide were prepared and checked *in vitro* for their antibacterial and antifungal activity against gram positive and gram-negative bacteria and *Candida* strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229) and *Candida albicans* (ATCC 10231). UV, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra were collected for structural analyses. In addition, purity of the compounds was determined by elemental analysis.

Target compounds in this study were synthesized according to the synthetic pathway represented in Scheme 5.1.



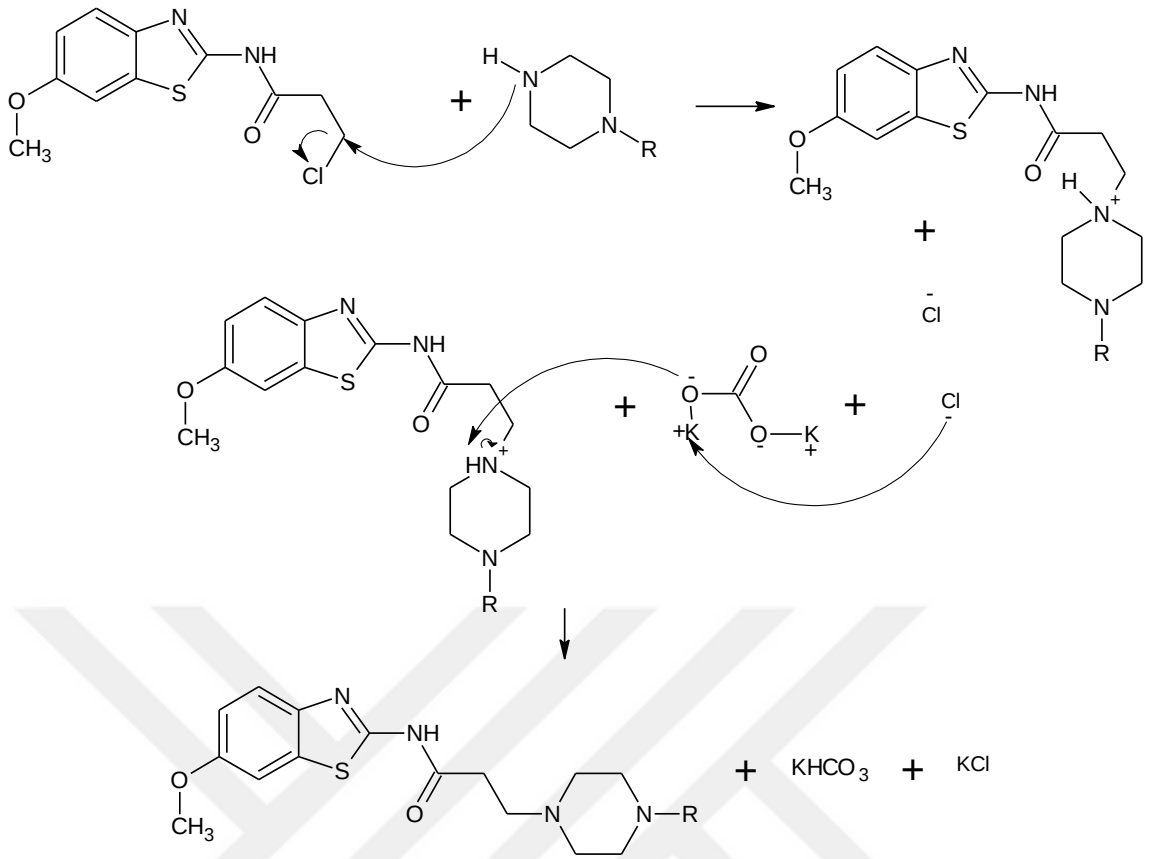
Scheme 5.1. General synthesis pathway of compounds

First stage of synthesis is *N*-acylation of primary amine of 2-amino-6-methoxybenzothiazole by 3-chloropropionyl chloride in alkaline medium to obtain 3-chloro-*N*-(6-methoxy-1,3-benzothiazol-2-yl)propanamide. Acylation reaction starts by a nucleophilic attack on the partially positive carbon of 3-chloropropionyl chloride by the lone pair on nitrogen of primary amine. After the N-C bond formation, nitrogen becomes positively charged and oxygen becomes negatively charged. Subsequently, carbonyl double bond is regained and a chloride ion is extracted.



Scheme 5.2. Mechanism of *N*-acylation reaction

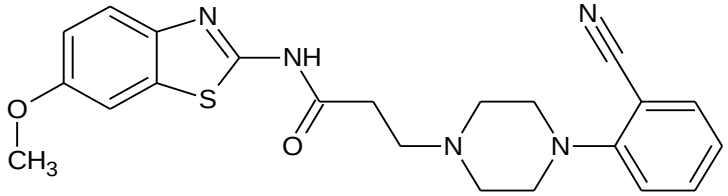
Second stage of synthesis is *N*-alkylation of the piperazine secondary amine to obtain the final compounds. The electrophilic carbon of the alkyl halide is attacked by the amine of piperazine to displace chloride and form a new C-N bond. Later, positively charged ammonium is attacked by potassium carbonate creating the alkylation product, also a tertiary amine is formed.



Scheme 5.3. Mechanism of *N*-alkylation reaction

After the synthesis of target compounds, confirmation of their structures was achieved with spectral analysis. Experimental data was found to be in accordance with expected data for all samples.

UV spectra of compound **8** shows three significant bands at 290 nm (log ε: 4.15), 299 nm (log ε: 4.16) and 308 nm (log ε: 4.18) which represent π→π* transition of aromatic rings and n→π* transition of amide carbonyl group.



Compound **8**

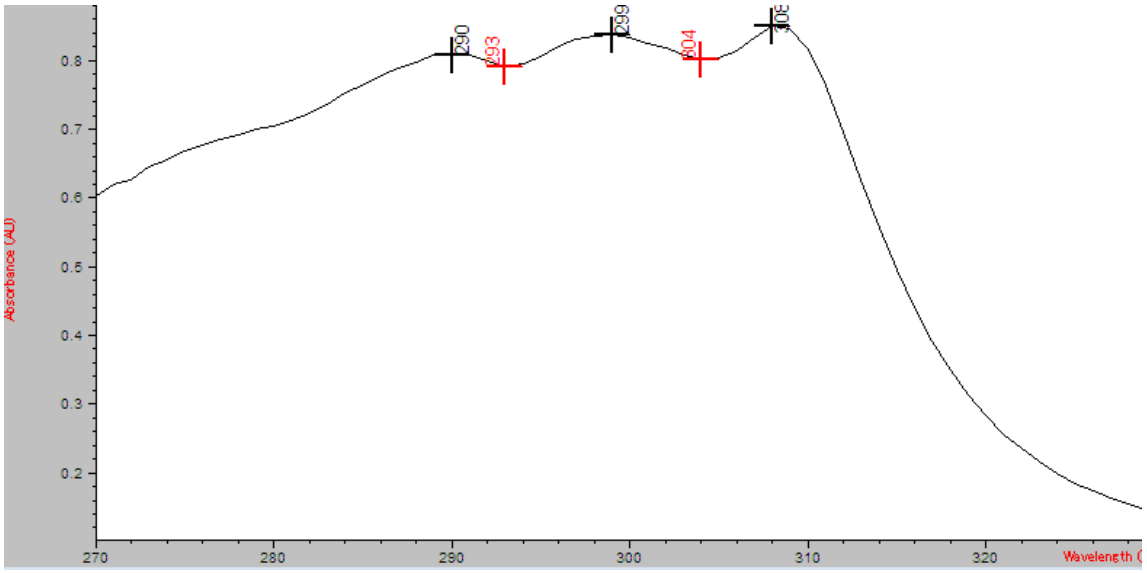


Figure 5.1. Structure and UV spectrum of compound **8**

IR spectrum of compound **8** shows Characteristic N-H stretching band at 3546. Other signals are showed as following: 3064 (C-H, aromatic), 2955 (C-H, aliphatic), 2219 (C≡N), 1682 (C=O, amide), 1594 (C=N), 1256 (C-N).

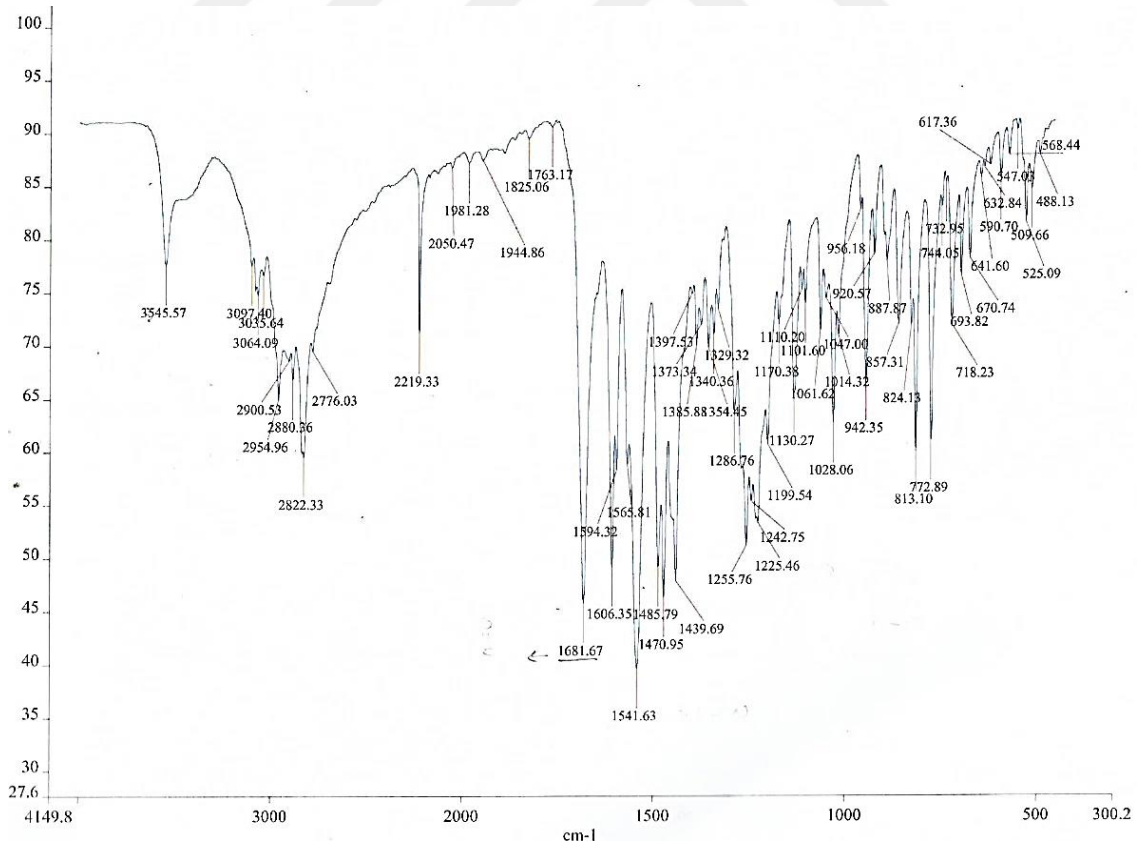
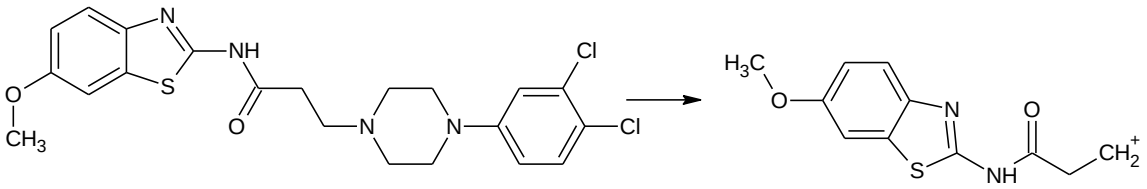


Figure 5.2. IR spectrum of compound **8**

Mass spectra of compound **6** shows molecular ion (M+) peak as base peak at 465.5 (m/z) and fragmentation product gives peak at 235.4 (m/z). The fragmentation pattern is shown in Scheme 5.3.



Scheme 5.3. Mass fragmentation pattern of compound **6**.

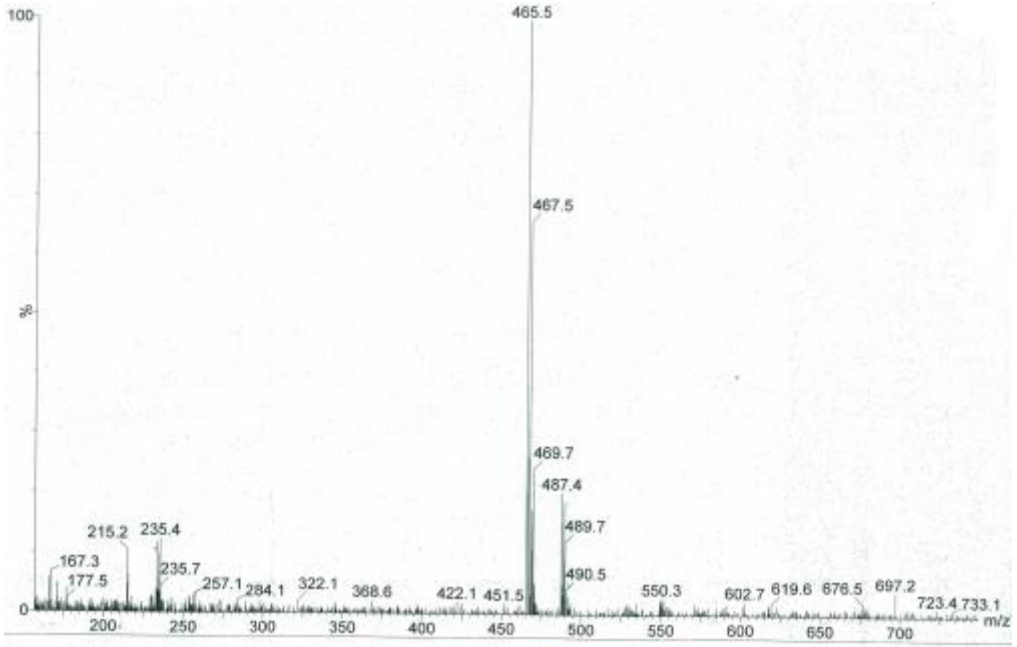
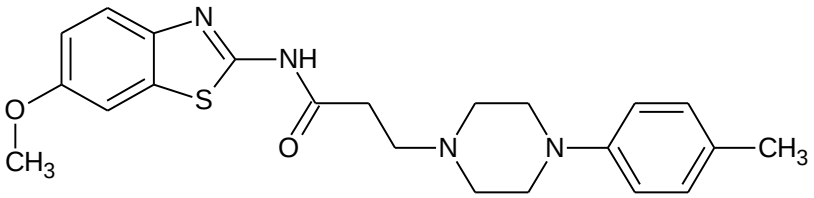


Figure 5.4. Mass spectrum of compound **6**

^{1}H-NMR spectra of compound **7** is described at Figure 5.5 where methyl protons are seen at 2.29 (s, 3H, Ar-CH_3), 2.72 (t, 4H, piperazine $H_{2,6}$, *J=6 Hz*) 2.84-2.88 (m, 4H, -CO$\underline{CH_2CH_2}$N-), 3.37 (t, 4H, piperazine $H_{3,5}$, *J=4.4 Hz*), 3.86 (s, 3H, Ar-OCH_3), 6.89-6.92 (m, 2H, phenyl $H_{3,5}$), 6.99-7.02 (dd, 1H, benzothiazole H_5, J_1*=2.4 Hz*, J_2*=8.8 Hz*), 7.11-7.13 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*), 7.26-7.27 (d, 1H, benzothiazole H_7, *J=2.4 Hz*), 7.64-7.66 (d, 1H, benzothiazole H_4, *J=8.4 Hz*), 12.51 (bs, 1H, -CO$\underline{NH}CH_2$-).



Compound **7**

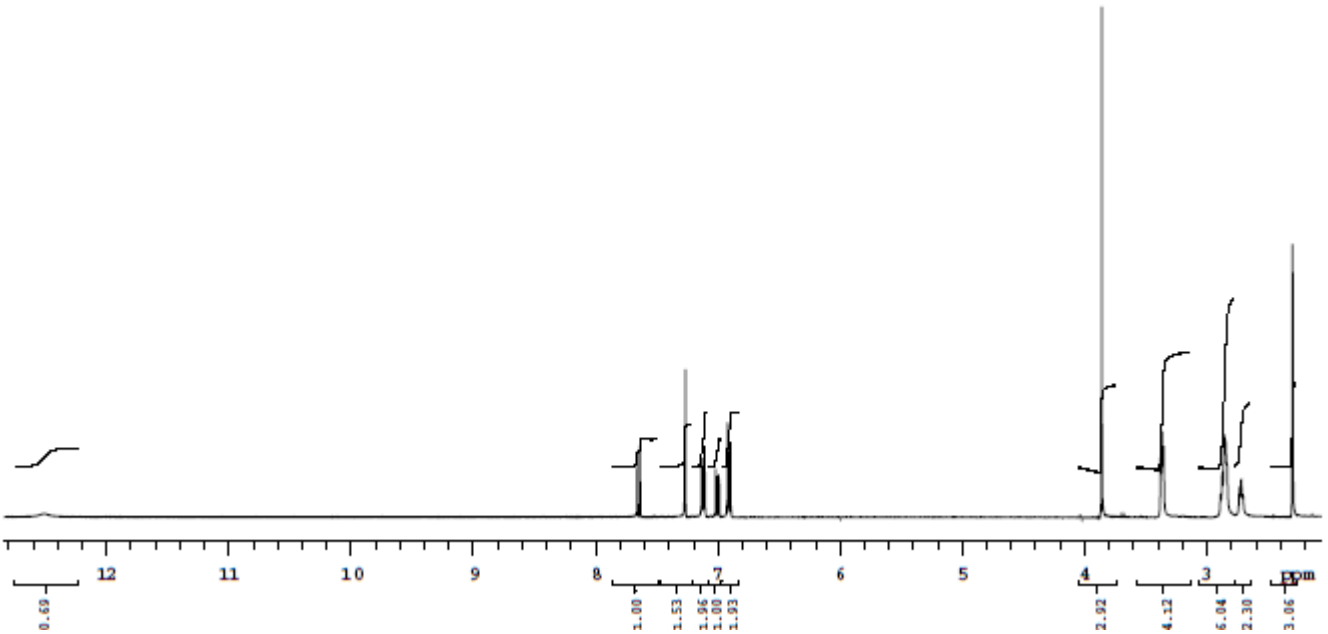
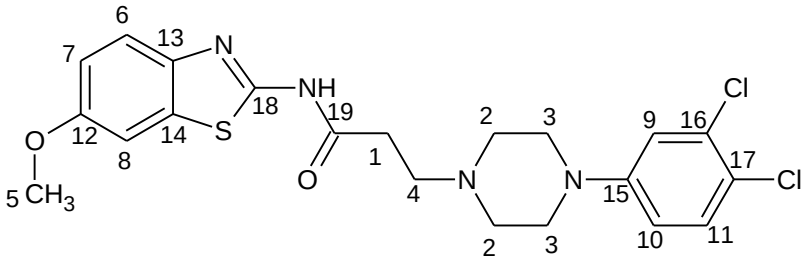


Figure 5.5. Structure and ^{1}H-NMR spectrum of compound **7.**

The ^{13}C-NMR spectrum of the compound **6** was taken in DMSO. Methylenes peak were seen at 32.79 and 53.11 ppm (-CO$\underline{CH_2CH_2}$-N-). 51.99 ppm for Piperazine carbons (piperazine C_2); 52.11 (piperazine C_3). 55.56 ppm for Methoxy peak. Aromatic carbons were seen at 104.67; 114.78; 115.17; 116.12; 119.47; 121.02; 130.34; 131.43; 132.69; 142.55; 150.62; 155.74; 156.04; 170.86. Carbonyl peak was seen at 170.86(C=O).



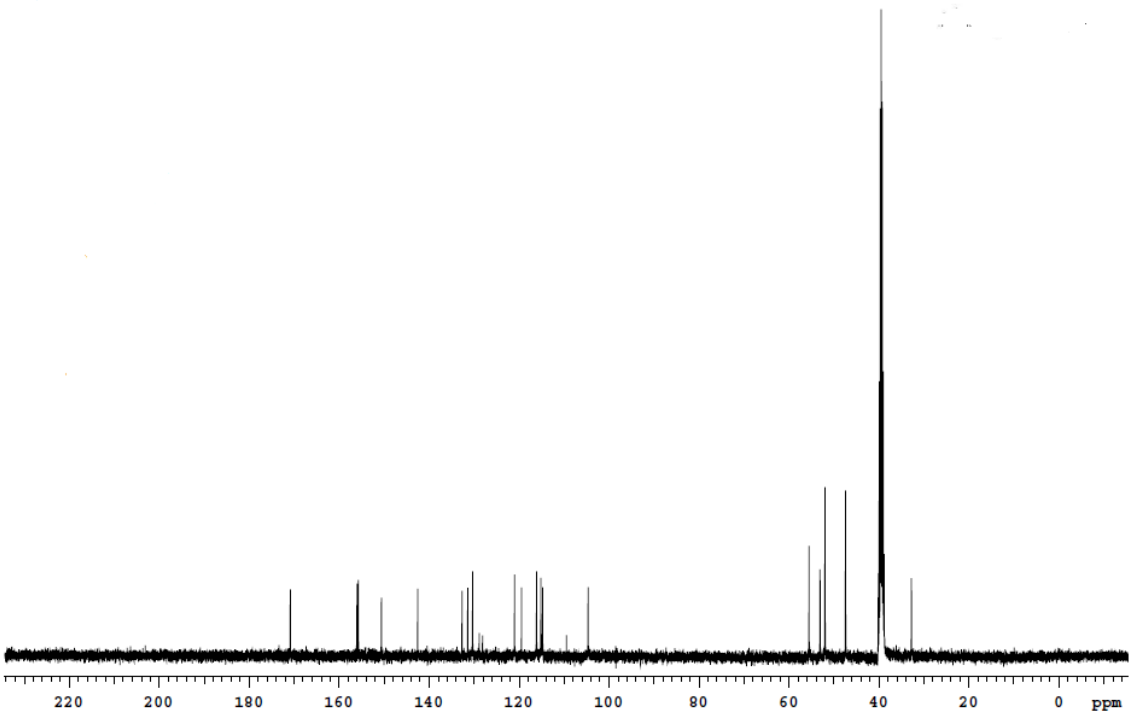


Figure 5.6. ^{13}C-NMR spectrum of compound **6**

C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	32.79	11	121.02
2	47.44	12	130.34
3	51.99	13	131.43
4	53.11	14	132.69
5	55.57	15	142.56
6	104.67	16	150.62
7	114.78	17	155.73
8	115.17	18	156.04
9	116.12	19	170.86
10	119.47		

Table 5.1. ^{13}C-NMR spectrum of compound **6**

Nine of the compounds were evaluated for their *in vitro* antibacterial and antifungal activity by agar-based disc diffusion assay and minimum inhibitory concentration measurement.

According to results given in Tables 4.1 and 4.2., most of the target compounds have antibacterial activity against tested gram-positive and gram-negative species which are *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442),

Escherichia coli (ATCC 11229) and antifungal activity against *Candida albicans* (ATCC 10231).

The antibacterial activity of the samples was evaluated by the disc diffusion assay against gram-positive and gram-negative bacteria using ofloxacin as standards. As shown in Table 4.1, the most active compounds against *S. aureus*, *P. aeruginosa* and *E. coli.* were compounds **2** (3-hydroxyphenyl) and **5** (2,3-dichlorophenyl) (inhibition zone: 12 mm for both). The antifungal activity of the samples was tested against *C. albicans* using nystatin as a standard. Compounds **2** and **5** exerted the highest antifungal activity against *C. Albicans* (inhibition zone: 10 mm).

MIC values were determined by serial dilution methods against the same panel of bacteria and fungus. The minimum inhibitory concentration of the compounds **2** and **5** against *S. aureus*, *P. aeruginosa* and *E. coli* were 256 μg/ml. The minimum inhibitory concentration of the compounds **2** and **5** against *C. albicans* was 512 μg/ml.

Docking studies were performed to analyze the interactions of compounds with DNA gyrase subunit B of *S. aureus*. Compounds generally adopted a similar binding pose with co-crystallized ligand making H-bonds with Arg144 and Glu58 residues (Figure 5.6).

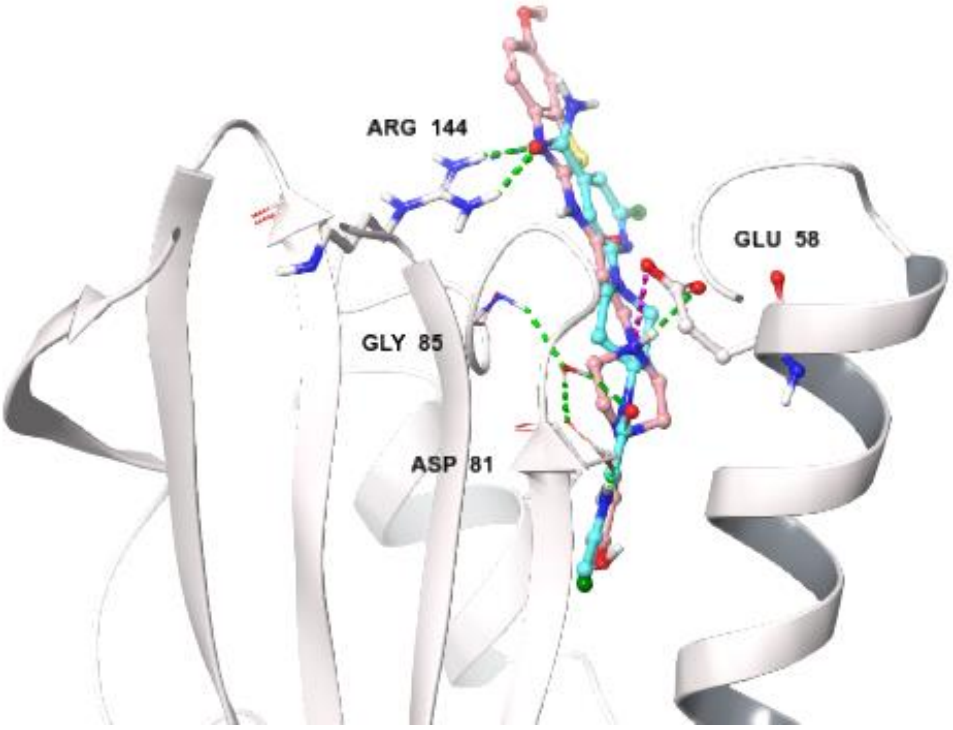


Figure 5.6. X-ray structure of GyrB (PDB ID: 3U2K) with the co-crystallized ligand (cyan) and compound 2 (pink)

In conclusion, we have synthesized ten novel benzothiazole-piperazine derivatives. Their characterization was confirmed by UV, IR, ^{1}H-NMR, ^{13}C-NMR spectroscopies, mass spectrometry and elementary analyses. *In vitro* antibacterial activities were determined for nine compounds in series and compounds **2** and **5** were found to be the most active derivatives. Common interactions were observed for all compounds in docking studies performed on DNA gyrase subunit B of *S. aureus*.

6. REFERENCES

1. Nii-Trebi NI. Emerging and neglected infectious diseases: Insights, advances, and challenges. BioMed Research International, 2017. 1-15.
2. Kharb R, Bansal K, Sharma AK. A valuable insight into recent advances on antimicrobial activity of piperazine derivatives. Der Pharma Chemica. 2012; 4(6): 2470-2488.
3. Keri, R. S., Patil, M. R., Patil, S. A., Budagumpi, S. A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. European journal of medicinal chemistry.2015; 89: 207-251.
4. Shi XH, Wang Z, Xia Y, Ye TH, Deng M, Xu YZ, Wei YQ, Yu LT. Synthesis and Biological Evaluation of Novel Benzothiazole-2-thiol Derivatives as Potential Anticancer Agents. Molecules. 2012; 17: 3933-3944.
5. Siddiqui N, Rana A, Khan SA, Alam O, Ahsan W, Ali R. Design, Synthesis and Anticonvulsant Screening of Newer Benzothiazole Semicarbazones. Asian Journal of Biomedical and Pharmaceutical Sciences. 2012; (10): 8-17.
6. Kaur A, Kumar R, Kalidhar U, Kaur P. Synthesis, Spectral Studies and Biological Evaluation of Schiff Base Derivatives of Benzothiazole for Antimicrobial Activity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(4): 847.
7. Himaja M, Munrjasekhar D, Sunil M. Synthesis and Anthelmintic activity of 2-Amino-6-substituted Benzothiazoles. International Research Journal of Pharmacy. 2011; 2(1): 114-117.
8. Delmas F, Giorgio CD, Azas N, Gasquet M, Detang C, Costa M, Timon-David P, Pierre J. In Vitro Activities of Position 2 Substitution-Bearing 6-Nitro- and 6-AminoBenzothiazoles and Their Corresponding Anthranilic Acid Derivatives against Leishmania infantum and Trichomonas vaginalis. Antimicrobial Agents and Chemotherapy. 2002; 46: 2588–2594.
9. Mahram M, William S, Ramzy F, Sembel F. Synthesis and in vitro Evaluation of New Benzothiazole Derivatives as Schistosomicidal Agents. Molecules. 2007; 12: 622-633.
10. Padalkar VS, Borse BN, GuptaKiran VD, Phatangare RK, Patil VS, Umape PG, Sekar N. Synthesis and antimicrobial activity of novel 2-substituted benzimidazole,

benzoxazole and benzothiazole derivatives. Arabian Journal of Chemistry. 2016; 9: 1125-1130.

11. Verma AK, Martin V, Singh Ak. Synthesis, Characterization and evaluation of Anti-inflammatory and Analgesic activity of Benzothiazole derivatives. Indian Journal of Pharmaceutical and Biological Research. 2014; 2(3): 84-89.
12. Mariappan M, Prabhat P, Sutharson L, Banerjee J, Patangia U, Nath S. Synthesis and Antidiabetic Evaluation of Benzothiazole Derivatives. Journal of the Korean Chemical Society. 2012; 56(2): 251-256.
13. Yilmaz S, Yalcin I, Onurdag FK, Ozgen S, Yildiz I, Akia E. Synthesis and In vitro Antimicrobial Activity of Novel 2-(4-(Substituted-carboxamido)benzyl / phenyl)benzothiazoles. Croatica Chemica Acta. 2013; 86 (2): 223–231.
14. Kulandaivelu U, Shireesha B, Mahesh C, Vidyasagar JV, Rao TR, Jayaveera KN, Jayaprakash V. Synthesis antimicrobial and anticancer activity of N′-arylmethylidene-piperazine-1-carbothiohydrazide. Medicinal Chemistry Research. 2013; 22(6): 2802-2808.
15. Khan I, Tantray MA, Hamid H, Alam MS, Kala A, Shaikh F, Hussain F. Synthesis of Novel Pyrimidin-4-One Bearing Piperazine Ring-Based Amides as Glycogen Synthase Kinase-3β Inhibitors with Antidepressant Activity. Chemical biology & drug design. 2016; 87(5): 764-772.
16. Karthik CS, Manukumar HM, Ananda A. P, Nagashree S, Rakesh KP, Mallesha L, Krishnamurthy NB. Synthesis of novel benzodioxane midst piperazine moiety decorated chitosan silver nanoparticle against biohazard pathogens and as potential anti-inflammatory candidate: A molecular docking studies. International journal of biological macromolecules. 2018; 108: 489-502.
17. Rejmund M, Mrozek-Wilczkiewicz A, Malarz K, Pyrkosz-Bulska M, Gajcy K, Sajewicz M, Polanski J. Piperazinyl fragment improves anticancer activity of Triapine. PloS one. 2018; 13(4): e0188767.
18. Choo HY, Chung BJ, Chung SH. Synthesis of piperazine derivatives and evaluation of their antihistamine and antibradykinin effects. Bioorganic & medicinal chemistry letters. 1999; 9(18): 2727-2730.
19. Hatnapure GD, Keche AP, Rodge AH, Birajdar SS, Tale RH, Kamble VM. Synthesis and biological evaluation of novel piperazine derivatives of flavone as potent anti-inflammatory and antimicrobial agent. Bioorganic & medicinal chemistry letters. 2012; 22(20): 6385-6390.

20. Chaithanya MS, Nagendrappa G, Vaidya VP, Nargund LV. Synthesis, antimicrobial and anti-inflammatory activities of 2- N[aryl-methylidene]-6-fluoro-5-piperazinyl [1, 3]benzothiazole-2- amines. Der Pharma Chemica. 2010; 2(6): 218-223.
21. Hisamoddin SZ, Priyanka S, Yogesh SP, Nilam U. Benzothiazole the molecule of diverse biological activities. An International Journal of Pharmaceutical Sciences. 2014; 5(1): 207-225.
22. Yadav PS, Devprakash D, Senthilkumar GP. Benzothiazole: different methods of synthesis and diverse biological activities. International Journal of Pharmaceutical Sciences and Drug Research. 2011; 3(1):01-07.
23. Yang XL, Xu CM, Lin SM, Chen JX, Ding JC, Wu HY, Su WK. Eco-friendly synthesis of 2-substituted benzothiazoles catalyzed by cetyltrimethyl ammonium bromide (CTAB) in water. Journal of the Brazilian Chemical Society. 2010; 21(1): 37-42.
24. Jaiswal S, Mishra AP, Srivastava A. The Different Kinds of Reaction Involved in Synthesis of 2-Substituted Benzothiazole and Its Derivatives. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(1): 631-641.
25. http://webbook.nist.gov/cgi/cbook.cgi?ID=C95169&Mask=400, 01.02.2018.
26. Issa RS, Khedr AM, Rizk, H. ^{1}H NMR, IR and UV/VIS Spectroscopic studies of some Schiff bases derived from 2-aminobenzothiazole and 2-amino-3-hydroxypyridine. Journal of the Chinese Chemical Society. 2008; 5: 875-884.
27. Alajarín M, Vidal Á, Tovar F, Jones PG, Bautista DA. Novel benzothiazole synthesis by cyclization of ketenimines bearing sulfenylimine fragments. Unexpected sulfur to carbon migration of an imino group. Arkivoc.2005; 9: 39-50.
28. Akhtar J, Khan AA, Ali Z, Haider R, Yar MS. Structure-activity relationship (SAR) study and design strategies of nitrogen-containing heterocyclic moieties for their anticancer activities. European journal of medicinal chemistry. 2017; 125: 143-189.
29. Liu DC, Zhang HJ, Jin CM, Quan ZS. Synthesis and biological evaluation of novel benzothiazole derivatives as potential anticonvulsant agents. Molecules. 2016; 21(3): 164-176.
30. Haroun M, Tratrat C, Kositzi K, Tsolaki E, Petrou A, Aldhubiab B, Elsewedy HS. New Benzothiazole-based Thiazolidinones as Potent Antimicrobial Agents. Design, synthesis and Biological Evaluation. Current topics in medicinal chemistry. 2018; 18(1): 75-87.

31. Zha GF, Leng J, Darshini N, Shubhavathi T, Vivek H.K, Asiri M, Qin HL. Synthesis, SAR and molecular docking studies of benzo [d] thiazole-hydrazones as potential antibacterial and antifungal agents. Bioorganic & Medicinal Chemistry Letters. 2017; 27(14): 3148-3155.
32. Kumar A, Shakya AK, Siddiqui HH. Synthesis and anti-inflammatory activity of some novel 2-aminobenzothiazole derivatives. Indian Journal of heterocyclic chemistry. 2016; 25: 243-249.
33. Bhutani R, Pathak DP, Kapoor G, Husain A, Kant R, Iqbal MA. Synthesis, Molecular modelling studies and ADME prediction of benzothiazole clubbed oxadiazole-Mannich bases, and evaluation of their Anti-diabetic activity through in-vivo model. Bioorganic chemistry. 2018; 77: 6-15.
34. Noolvi MN, Patel HM, Kaur M. Benzothiazoles: search for anticancer agents. European journal of medicinal chemistry. 2012; 54: 447-462.
35. Ali R, Siddiqui N. Biological aspects of emerging benzothiazoles: a short review. Journal of Chemistry. 2013;1-12.
36. Bhuva HA, Kini SG. Synthesis, anticancer activity and docking of some substituted benzothiazoles as tyrosine kinase inhibitors. Journal of Molecular Graphics and Modelling, 2010. 29(1): 32-37.
37. Padalkar VS, Borse BN, Gupta VD, Phatangare KR, Patil VS, Umape PG, Sekar N. Synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives. Arabian Journal of Chemistry. 2016; 9: 1125-1130.
38. Venkatesh P, Tiwari VS. Design and synthesis of Quinazolinone, Benzothiazole derivatives bearing guanidinopropanoic acid moiety and their Schiff bases as cytotoxic and antimicrobial agents. Arabian Journal of Chemistry, 2016. 9: 914-925.
39. Thakkar SS, Thakor P, Ray A, Doshi H, Thakkar VR. Benzothiazole analogues: synthesis, characterization, MO calculations with PM6 and DFT, in silico studies and in vitro antimalarial as DHFR inhibitors and antimicrobial activities. Bioorganic and medicinal chemistry.2017; 25: 5396–5406.
40. Munirajasekhar D, Himaja M, Mali SV, Karigar A, Sikarwar M. Synthesis and anthelmintic activity of 6-substituted 2-hydrazino-1, 3-benzothiazoles. Journal of Pharmaceutical Research.2011; 4(7): 2186-2187.

41. Verma AK, Martin A, Singh Sr AK. Synthesis, Characterization and evaluation of Anti-inflammatory and Analgesic activity of Benzothiazole derivatives. Indian Journal of Pharmaceutical and Biological Research. 2014; 2(3): 84.
42. Kour H, Paul S, Singh PP, Gupta M, Gupta RA. Simple one-pot method for the mercuric oxide mediated synthesis of piperazines via oxidative deamination of olefins. Tetrahedron Letters. 2013; 54: 761-764.
43. Martin WB, Martell AE. Preparation of piperazine. Journal of American Chemical Society. 1948; 70: 1817-1818.
44. Kyrides LP, Groves W. Manufacture of piperazine. US-2267686, 1941.
45. Theophil-Eicher SH. The Chemistry of Heterocycles Structures, Reactions, Synthesis and Applications. Wiley-VCH, 2003.
46. Katrizky AR. Advances in Heterocyclic Chemistry. Academic Press. 1973; 15.
47. http://www.sigmaaldrich.com, 12.12.2016.
48. http://riodb01. ibase.aist.go.jp\sdbs\cgi-bin\direct_frame_top.cgi, 12.12.2016.
49. Kalai T, Khan M, Balog M, Kutala VK, Kuppusamy P, Hideg K. Stracture-activity studies on the protection of Trimetazidine derivatives modifies with nitroxides and their precursors from myocardial ischemia-reperfusion injury. Bioorganic Medicinal Chemistry. 2006; 14: 5510-5516.
50. Rips R, Boschi G, Trinh MC, Cavier R. Phenol-piperazine adducts showing anthelmintic properties. Journal of Medicinal Chemistry. 1973; 16(6): 725-728.
51. Shaquiquzzaman M, Verma G, Marella A, Akhter M, Akhtar W, Khan MF, Tasneem S, Alam MM. Piperazine Scaffold: A remarkable tool in generation of diverse pharmacological agents. European Journal of Medicinal Chemistry. 2015; 102: 487-529.
52. Kerns RJ, Rybak MJ, Kaatz GW, Vaka F, Cha R, Grucz RG, Diwadkar VU, Ward TD. Piperazinyl-linked fluroquinolone dimers possessing potent antibacterial activity against drug-resistant strains of *staphylococcus aureus.* Bioorganic Medicinal Chemistry Letters. 2003; 13: 1745-1749.
53. Foroumdi A, Soltani F, Moshafi MH, Asharf-Askari R. Synthesis and in vitro antibacterial activity of some *N*-(5-aryl-1,3,4-thiadiazole-2-yl) piperazinyl quinolone derivatives. II Farmaco. 2003; 58: 1023-1028.
54. Foromudi A, Emami S, Mansouri S, Javidnia A, Saeid-Adeli N, Shirazi F, Shafiee A. Synthesis and antibacterial activity of levofloxacin derivatives with certain bulky

residues on piperazine ring. European Journal of Medicinal Chemistry. 2007; 42: 985-992.

55. Thomas KD, Adhikari AV, Chowdhury IH, Sumesh E, Pal NK. New quinolone -4-yl-1, 2, 3-triazoles carrying amides, sulphonamides, and amidopiperazines as potential antitubercular agents. European Journal of Medicinal Chemistry. 2001; 46:2503-2512.
56. Appelbaum PC, Hunter PA. The fluroquinolone antibacterial: past, present, and future perspectives. International Journal of Antimicrobial Agents. 2000; 16: 5-15.
57. Upadhayaya RS, Sinha N, Jain S, Kishore N, Chandra R, Arora SK. Optically active antifungal azoles: synthesis and antifungal activity of (2R,3S)-2-(2,4-diflurophenyl)-3-(5-{2-[4-arryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl\1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol. Bioorganic Medicinal Chemistry. 2004; 12: 2225-2238.
58. Saxena M, Gaur S, Prathipat, P, Saxena A. Synthesis of some substituted pyrazinopyridoindoles and 3D QSAR studies with related compounds: piprezines, piperidines, pyrazinoisoquinolines, and diphenylhydramine, and its semi-rigid analogs as antihistamines (H-1). Bioorganic Medicinal Chemistry. 2006; 14: 8249-8258.
59. Park Choo HY, Chung BJ, Chung SH. Synthesis of piperazine derivatives and evaluation of their antihistamine and antibradykinin effects. Bioorganic Medicinal Chemistry letters. 1999; 9: 2727-2730.
60. Thriveni KS, Padmashali B, Siddesh MB, Sandeep C. Synthesis of pyrimidine incorporated piperazine derivatives and their antimicrobial activity. Indian journal of pharmaceutical sciences. 2014; 76(4): 332.
61. Gan LL, Fang B, Zhou CH. Synthesis of Azole-Containing Piperazine Derivatives and Evaluation of Their Antibacterial, Antifungal and Cytotoxic Activities. Bulletin of the Korean Chemical Society. 2010; 31(12): 3684-3692.
62. Mohsen UA. Synthesis and antimicrobial activity of some piperazine dithiocarbamate derivatives. Turkish Journal of Pharmaceutical Science. 2014; 11: 347-354.
63. Akkoç MK, YükseL MY, Durmaz İ, Talay RÇ. Design, synthesis, and biological evaluation of indole-based 1, 4-disubstituted piperazines as cytotoxic agents. Turkish Journal of Chemistry. 2012; 36(4): 515-525.

64. Choo HY, Chung BJ, Chung SH. Synthesis of piperazine derivatives and evaluation of their antihistamine and antibradykinin effects. Bioorganic & medicinal chemistry letters. 1999; 9(18): 2727-2730.
65. Aytemir MD, Septioğlu E, Çaliş Ü. Synthesis and anticonvulsant activity of new kojic acid derivatives. Arzneimittelforschung. 2010; 60(01): 22-29.
66. Li J, Yin Y, Wang L, Liang P, Li M, Liu X, Yang H. Synthesis, Characterization, and Anti-Inflammatory Activities of Methyl Salicylate Derivatives Bearing Piperazine Moiety. Molecules. 2016; 21(11):1544.
67. Al-Talib M, Al-Soud YA, Abussaud M, Khshashneh S. Synthesis and biological evaluation of new benzothiazoles as antimicrobial agents. Arabian Journal of Chemistry. 2016; 9: S926-S930.
68. Raghavendra NM, Jyothsna A, Rao AV, Subrahmanyam CV. Synthesis, pharmacological evaluation and docking studies of N-(benzo [d] thiazol-2-yl)-2-(piperazin-1-yl) acetamide analogs as COX-2 inhibitors. Bioorganic & medicinal chemistry letters. 2012; 22(2): 820-823.
69. Papadopoulou C, Geronikaki A, Litina DH. Synthesis and biological evaluation of new thiazolyl/benzothiazolyl-amides, derivatives of 4-phenyl-piperazine. II Farmaco. 2005; 60: 969–973.
70. Al-Soud YA, Al-Sa'doni HH, Amajaour HA, Salih KM, Mubarak MS, Al-Masoudi, NC, Jaber IH. Synthesis, characterization and anti-HIV and antitumor activities of new coumarin derivatives. Z Naturforsch. 2008; 63b:83-89.
71. Raju GN, Prathyusha TG, Sowmya PL, Mounika SJ, Nadendla RR. Synthesis, characterization and biological activity of some 1, 3, 4-oxadiazole derivatives with benzothiazole moiety. Der Pharmacia Sinica.2015; 6(6): 1-8.
72. Patel RV, Park SW. Catalytic N-formylation for synthesis of 6-substituted-2-benzo-thiazolylimino-5-piperazinyl-4-thiazolidinone antimicrobial agents. Research on Chemical Intermediates. 2015; 41(8): 5599-5609.
73. Al-Harthy T, Zoghaib WM, Stoll R, Abdel-Jalil R. Design, synthesis, and antimicrobial evaluation of novel 2-arylbenzothiazole analogs bearing fluorine and piperazine moieties. Monatshefte fuer Chemie-Chemical Monthly. 2018; 149(3): 645-651.
74. Al-Soud YA, Al-Sa'doni HH, Saeed B, Jaber IH, Beni-Khalid MO, Al-Masoudi, NA, Loddo R. Synthesis and in vitro antiproliferative activity of new benzothiazole derivatives. Arkivoc. 2008; 15: 225-238.

75. Gurdal EE, Durmaz I, Cetin-Atalay R, Yarim M. Cytotoxic activities of some benzothiazole-piperazine derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry. 2015; 30(4): 649-654.
76. Gurdal EE, Buclulgan E, Durmaz I, Cetin-Atala, R, Yarim M. Synthesis and anticancer activity evaluation of some benzothiazole-piperazine derivatives. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2015; 15(3): 382-389.
77. Demir Özkay Ü, Kaya C, Acar Çevik U, Can ÖD. Synthesis and Antidepressant Activity Profile of Some Novel Benzothiazole Derivatives. Molecules. 2017; 22(9): 1490.
78. Özkay ÜD, Can ÖD, Sağlık BN, Cevik UA, Levent S, Özkay Y, Atlı Ö. Design, synthesis, and AChE inhibitory activity of new benzothiazole–piperazines. Bioorganic & medicinal chemistry letters. 2016; 6(22): 5387-5394.
79. Gurdal EE, Turgutalp B, Gulcan HO, Erçetin T, Sahin MF, Durmaz I, Yarim M. Synthesis of Novel Benzothiazole-Piperazine Derivatives and Their Biological Evaluation as Acetylcholinesterase Inhibitors and Cytotoxic Agents. Anti-cancer agents in medicinal chemistry.2017; 17(13): 1837-1845.
80. https://www.yourgenome.org/facts/what-are-infectious-diseases.25.6.2018.
81. https://microbiologysociety.org/education-outreach/antibiotics-unearthed/antibiotics-and-antibiotic-resistance/the-history-of-antibiotics.html. 25.6.2018.
82. Aminov R. History of antimicrobial drug discovery: Major classes and health impact. Biochemical pharmacology. 2017; 133: 4-19.
83. Clark M, Finkel R, Rey J, Whalen K. Chemotherapeutics Drugs Principle of Antimicrobial Therapy. In Lippincott Williams & Wilkins, USA, pp 370-380, 2012.
84. https://www.google.com.tr/search?biw=1600&bih=720&tbm=isch&sa=1&ei=A88FW43rOpGNmgWg85PYDg&q=mechanism+of+action+of+antibiotics&oq=mechof+antibiotics+&gs_l=img.1.0.0i7i30k1l4j0i8i7i30k1l6.15881.27788.0.30294.6.6.0.0.0.0.447.1180.0j5j4-1.6.0....0...1c.1.64.img.0.4.904....0.aD8zwZJN9x0#imgrc=-rk7YEOiDl2NJM:, 10.5.2018.
85. https://www.google.com.tr/search?q=Determination+of+minimum+inhibitory+concentration+and+minimum+bactericidal+concentration.&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjXyKnj2JzbAhXBxaYKHaJVAA8Q_AUICigB&biw=1600&bih=720#imgrc=ZiVcDVOkBEVsyM:., 10.5.2018.

86. Eakin AE, Green O, Hales N, Walkup GK, Bist S, Singh A, Breeze A. Pyrrolamide DNA gyrase inhibitors: fragment-based nuclear magnetic resonance screening to identify antibacterial agents. Antimicrobial agents and chemotherapy. 2012; 56(3): 1240-1246.
87. Chatterji M, Unniraman S, Mahadevan S, Nagaraja V. Effect of different classes of inhibitors on DNA gyrase from Mycobacterium smegmatis. Journal of Antimicrobial Chemotherapy. 2001; 48(4): 479-485.
88. Balkan İA, Taşkın T, Doğan HT, Deniz İ, Akaydın G, Yesilada E. A comparative investigation on the in vitro anti-inflammatory, antioxidant and antimicrobial potentials of subextracts from the aerial parts of Daphne oleoides Schreb. subsp. oleoides. Industrial crops and products. 2017; 95: 695-703.
89. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra precision Glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. Journal of Medicinal Chemistry. 2006; 49: 6177-6196.
90. Watts KS, Dalal P, Murphy RB, Sherman W, Friesner RA, Shelley JC. ConfGen: A conformational search method for efficient generation of bioactive conformers. Journal of Chemical Information and Modeling. 2010; 50: 534-546.
91. Schwab CH. Conformations and 3D pharmacophore searching. Drug Discovery Today: Technologies. 2010; 7: 245-253.
92. Duan J, Dixon SL, Lowrie JF, Sherman W. Analysis and comparison of 2D fingerprints: Insights into database screening performance using eight fingerprint methods. Journal of Molecular Graphics and Modeling. 2010; 29: 157-170.

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