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

SYNTHESIS AND ACTIVITY STUDIES ON SOME NOVEL BENZOTHIAZOLE-PIPERAZINE DERIVATIVES

MASTER OF SCIENCE THESIS

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Yüksek Lisans (Master) öğrencisi. Ebru Buclulğan'ın çalışması jürimiz tarafından Farmasötik Kimya Anabilim Dalı Master tezi olarak uygun görülmüştür.

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ONAY

Yukarıdaki jüri kararı Enstitü Yönetim Kurulu'nun 17/06/2014 tarih ve ....14-1..... sayılı kararı ile onaylanmıştır.

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Müdür V.

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Dedicated to my parents;

Aysel & Nihat Buclulgan

ÖZET

Buclulgan E., Bazı Yeni Benzotiyazol-Piperazin Türevleri Üzerine Sentez ve Aktivite Çalışmaları. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Yüksek Lisans Tezi, İstanbul, 2014.

Bu çalışmada, *N*-(4-metil-1,3-benzotiyazol-2-il)-2-(*N*-sübstitüepiperazin-1-il)asetamit yapılarına sahip, dokuzu orjinal, on bileşik sentezlenmiştir. Bu bileşiklerin *in vitro* sitotoksik aktivitelerine sülforodamin B testi ile bakılmıştır.

Başlangıç maddesi 2-amino-4-metilbenzotiyazol, benzen:trietilamin karışımında çözülmüş ve 2-kloro-*N*-(4-metil-1,3-benzotiyazol-2-il)asetamit sentezi için kloroasetil klorür ile asetillenmiştir. *N*-(4-metil-1,3-benzotiyazol-2-il)-2-(*N*-sübstitüepiperazin-1-il)asetamit, *N*-sübstitüe piperazin ve 2-kloro-(4-metil-1,3-benzotiyazol-2-il)asetamitin susuz K_2CO_3 ile aseton içindeki reaksiyonu ile sentezlenmiştir.

Bileşiklerin yapıları UV, IR, ^{1}H-NMR, ^{13}C-NMR, kütle spektrumları ve elementel analiz ile aydınlatılmış, fiziksel özellikleri ve ince tabaka kromotografisindeki R_f değerleri belirlenmiştir. *In vitro* sitotoksik aktivite çalışmaları, sülforodamin B yöntemi ile meme (MCF7), karaciğer (HUH7) ve kolorektal (HCT116) kanser hücre hatlarında yapılmıştır.

HCT116 kanser hücre hattına karşı genel olarak, aroil sübstitüe türevler daha etkin bulunmuştur. Bu hücre hattına en yüksek aktivite gösteren bileşikler; bileşik **1** (GI_{50}= 3.5 μM), bileşik **8** (GI_{50}= 0.9 μM) ve bileşik **10** (GI_{50}= 1.3 μM)'dur.

MCF7 hücre hattı üzerinde en yüksek aktiviteli bileşik *N*-(4-metil-1,3-benzotiyazol-2-il)-2-[4-(2-furoil)piperazin-1-il]asetamit (bileşik **10**; GI_{50}= 4.3 μM) olmuştur. Diğer yüksek aktiviteli türevler (bileşik 3; GI_{50}= 9.7 μM) ve (bileşik **8**; GI_{50}= 9.2 μM)'tür. Bileşikler **5** ve **9** bu hücre hattına karşı sitotoksisite göstermemektedir.

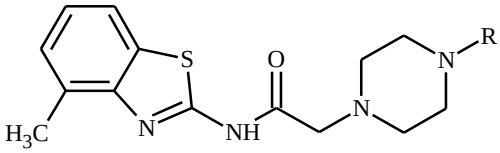
HUH7 hücre hattına karşı aroil sübstitüe türevler diğerlerinden daha etkin bulunmuştur. Bu hücre hattına karşı en yüksek aktiviteye sahip bileşik *N*-(4-metil-1,3-benzotiyazol-2-il)-2-(4-benzoilpiperazin-1-il)asetamit (bileşik **8**; GI_{50}= 0.7 μM)

olmuştur. Ayrıca alkil türevleri, aril türevlerine göre daha az sitotoksisite göstermektedirler.

Aroil sübstitüe bileşikler **8** ve **10** en aktif türevler olarak bulunmuştur. Ayrıca bileşik **8** ve **10** için Hoechst boyama ve FACS testleriyle yapılan ileri analizler ile bu bileşiklerin hücre siklusunu $subG_1$ fazında durdurarak apoptoza neden oldukları açıklanmıştır.

Anahtar kelimeler: Piperazin, benzotiyazol, sitotoksik aktivite, kanser hücre hattı.

Tablo. Sentezi gerçekleştirilen bileşiklerin **(1-10)** kimyasal formülleri.



Bileşik	R	E. D. (°C)	Verim (%)
1*	4-Florofenil	218.9	53
2	2-Hidroksifenil	162.0	30
3	3,4-Diklorofenil	178.6	84
4	4-Nitrofenil	177.6	12
5	4-Metilfenil	202.5	31
6	4-Klorobenzil	148.3	56
7	Metil	>300	35
8	Benzoil	175.0	80
9	Asetil	186.1	39
10	2-Furoil	159.6	75

(*) Bileşik **1**; CAS No: 946808-47-5

ABSTRACT

Buclulgan E., Synthesis and Activity Studies On Some Novel Benzothiazole-Piperazine Derivatives. Yeditepe University Institute of Health Sciences, MSc Thesis of Pharmaceutical Chemistry Programme, Istanbul, 2014.

In this study, ten compounds with structures of *N*-(4-methyl-1,3-benzothiazole-2-yl)-2-(*N*-substitutedpiperazine-1-yl)acetamide were prepared, of those, nine compounds are original. *In vitro* cytotoxic activities of these compounds were determined by sulphorodamine B assay.

Starting material 2-amino-4-methylbenzothiazole was dissolved in benzene:triethylamine mixture and acetylated with chloroacetyl chloride to give 2-chloro-*N*-(4-methyl-1,3-benzothiazole-2-yl)acetamide. *N*-(4-methyl-1,3-benzothiazole-2-yl)-2-(*N*-substitutedpiperazine-1-yl)acetamide was synthesized with reaction of *N*-substituted piperazine and 2-chloro-*N*-(4-methyl-1,3-benzothiazole-2-yl)acetamide in acetone and with anhydrous K_2CO_3.

Structure of compounds were elucidated with UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and elemental analysis; also their physical characteristics and R_f values on thin layer chromatography were determined. *In vitro* cytotoxic activity screening of the compounds were performed with sulphorodamine B assay against breast (MCF7), hepatocellular (HUH7) and colorectal (HCT-116) cancer cell lines.

Against HCT-116 cell line, in general, aroyl substituted derivatives were found to be more potent than others. The most active compounds against this cell line are compound **1** (GI_{50}= 3.5 μM), compound **8** (GI_{50}= 0.9 μM) ve compound **10** (GI_{50}= 1.3 μM).

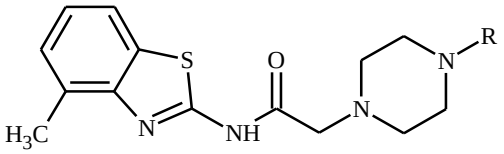
Against MCF7 cell line, *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(2-furoyl)piperazin-1-yl]acetamide (compound **10**; GI_{50}= 4.3 μM) is the most potent derivative. Other highly active derivatives are compound **6 (**GI_{50}= 9.7 μM) and compound **8 (**GI_{50}= 9.2 μM). Compounds **5** and **9** do not show cytotoxicity against this cell line.

Against HUH7 cell line, aroyl substituted derivatives were more potent than others. The most potent compound against this cell line is *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-benzoylpiperazin-1-yl)acetamide (compound **8**; GI_{50}= 0.7 μM). In addition, alkyl derivatives have less cytotoxicity than aryl derivatives.

Aroyl substituted compounds **8** and **10** were found to be the most active derivatives. In addition, further investigation of compounds **8** and **10** by Hoechst staining and FACS revealed that these compounds cause apoptosis by cell cycle arrest at $subG_1$ phase.

Keywords: Piperazine, benzothiazole, cytotoxic activity, cancer cell line.

Table. Structures of the synthesized compounds (**1-10**).



Compound	R	M.P. (°C)	Yield (%)
1*	4-Fluorophenyl	218.9	53
2	2-Hydroxyphenyl	162.0	30
3	3,4-Dichlorophenyl	178.6	84
4	4-Nitrophenyl	177.6	12
5	4-Methylphenyl	202.5	31
6	4-Chlorobenzyl	148.3	56
7	Methyl	>300	35
8	Benzoyl	175.0	80
9	Acetyl	186.1	39
10	2-Furoyl	159.6	75

(*) Compound **1**; CAS No: 946808-47-5

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ABBREVIATIONS

ADP	Adenosine diphosphate
AK	Adenylate Kinase
ATP	Adenosine triphosphate
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
FACS	Fluorescence Activated Cell Sorting
G_1	Gap 1 Phase
G_2	Gap 2 Phase
GABA	Gamma amino butyric acid
GADPH	Glyceraldehyde-3-phosphate Dehydrogenase
GI_{50}	Growth Inhibitory Concentration 50 percent
IR	Infrared
LDH	Lactate Dehydrogenase
M	Mitosis Phase
MeOH	Methanol
MS	Mass Spectroscopy
MTS	Cell Proliferation Assay
NCI	National Cancer Institute
NMR	Nuclear Magnetic Resonance

PMS	Phenazine Methosulfate
ppm	parts per million
R_f	Retention factor
S	Synthesis Phase
SRB	Sulphorhadamine
TCA	Trichloroacetic acid
TEA	Triethylamine
TLC	Thin Layer Chromatography
UV	Ultraviolet
WHO	World Health Organization
XTT	Cell Viability Kit

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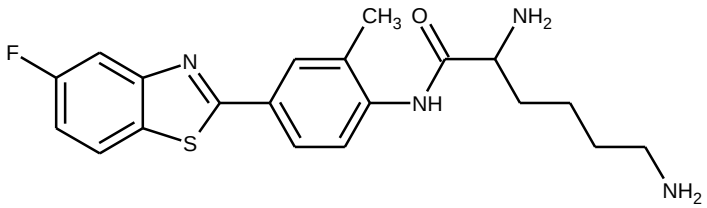
1. INTRODUCTION AND AIM

Cancer remains to be the leading cause of deaths throughout the world. The disease occurs due to uncontrolled cell growth. Cancerous malignant cells may spread all parts of body through the lymphatic system and bloodstream. Benign tumors are not cancerous, and they do not spread throughout the body [1].

Cancer chemotherapeutic agents cure specific cancer type, but the agents are cytotoxic also to normal cells. These agents effect especially resting cells (G_0) such as hair, bone marrow cells and cells lining the gastrointestinal tract [2]. Moreover, common side effect of cytotoxic agents is immune system depression together with nausea and diarrhea [3]. Another problem about chemotherapy is the development of drug resistance. For instance, most drugs are very effective, subsequent therapy may be ineffective, because tumor cells could be non-sensitive to drug [4]. Therefore, drug discovery studies in cancer area remain prevalent in order to decrease side effects, and prevent drug resistance.

Benzothiazole is a heterocyclic ring, that is investigated frequently in the different fields of therapy. Benzothiazole derivatives have many activities such as anticancer [5-9], antifungal [10], antioxidant [11], antimicrobial [12-15], antiinflammatory [16], anthelmintic [17], anticonvulsant [18], and central nervous system activities [19].

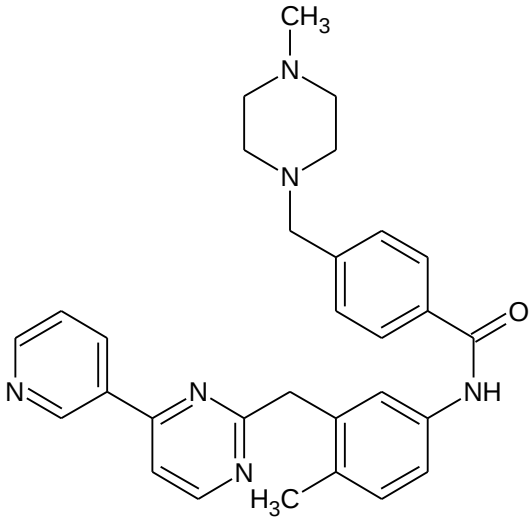
Phortress is a chemotherapeutic agent that contains benzothiazole ring. It has a considerably selective anticancer activity. It effects as a selective antitumour response via a mechanism of action distinct from any clinically used chemotherapeutic agent [20].



Phortress

Another important ring structure for the drug studies is piperazine. The compounds carrying piperazine ring structure have many activities such as antiviral [21], antipsychotic [22], antimicrobial [23], anthelmintic [24], antimalarial [25] and anticancer activities [26-28]. A very well known piperazine derivative anticancer drug is imatinib (Gleevec®) [29].

Imatinib (Gleevec®) is a molecularly targeted therapy for the treatment of a specific cancer such as chronic myeloid leukaemia. Today, the drug is used clinical trials of molecularly targeted. It is evaluated for different tumors. Imatinib inhibits a tyrosine kinase enzyme which is significant factor for cancer development, thus preventing the growth of cancer cells and leading to their death by apoptosis [29].



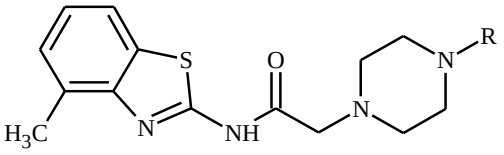
Imatinib

Benzothiazole and piperazine rings are combined in many drug discovery studies. For instance, Ozkay et al, reported AchE inhibitory benzothiazole-piperazine derivatives to be effective for Alzheimer [30]. Some benzothiazole-piperazine compounds also have anticancer activity. The derivatives have potent cytotoxicity against cancer cell lines such as breast (MCF-7), hepatocellular (HepG-2), prostate (DU-145) cancers and CD4+ human acute T-lymphoblastic leukaemia (CCRF-CEM) [31].

In this study, novel derivatives benzothiazole-piperazine structures are synthesized and characterized. The cytotoxic activities of these novel compounds are evaluated on breast (MCF-7), colorectal (HCT116) and hepatocellular (HUH7) cell lines by Sulphorodamine B assay.

To examine the anticancer mechanism of action, further evaluation of apoptosis and cell-cycle arrest were conducted by Hoechst staining and flow cell cytometry.

Table 1.1. Structures of the synthesized compounds **(1-10)**



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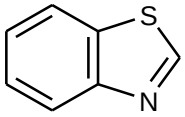
9	Acetyl	186.1	39
10	2-Furoyl	159.6	75

(*) compound **1**; CAS No: 946808-47-5

2. GENERAL DESCRIPTION

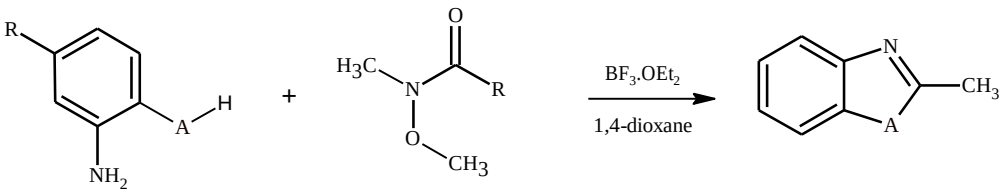
2.1. Benzothiazole

Benzothiazole is an aromatic heterocyclic compound, and its chemical formula is C_7H_5NS. Benzothiazole ring is formed by thiazole ring fusion with benzene ring [32].



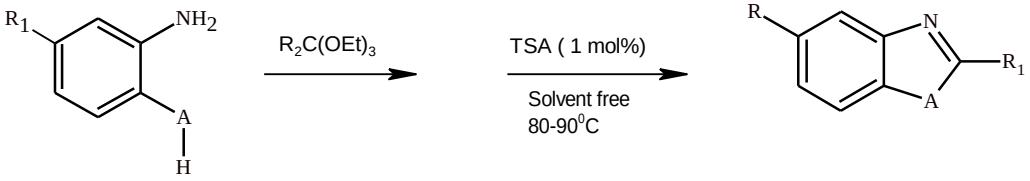
2.1.1. Methods of Synthesis

2-Substitued benzothiazole is synthesized with a condensation reaction by cyclization with *o*-diaminoarene or *o*-aminothiophenol. Presence of boron trifluoride etherate in 1,4- dioxane for 60 min provides high yields of product [33].



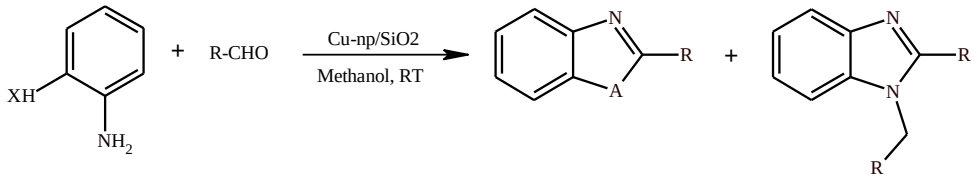
A= O, S

Moreover, benzothiazole ring is synthesized by Tungstate sulfuric acid (TSA), starting with *o*-aminophenols or *o*-aminothiophenols [34].



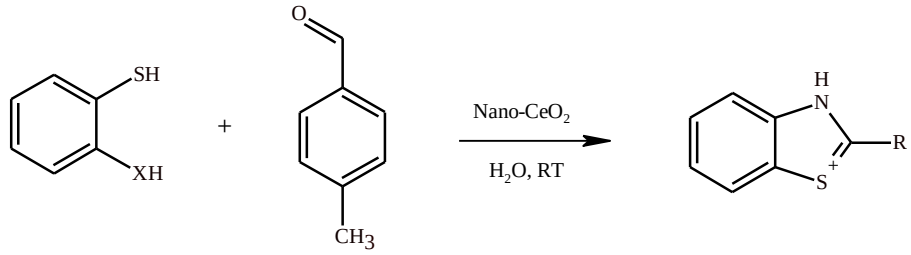
A= O,S

Benzothiazole is also synthesized with silica supported silica nano-cupper(II) oxide as a catalyst [35].



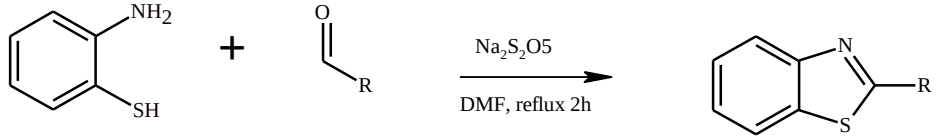
R= Aromatic, Heteroaromatic, Aliphatic
X= NH,S,O

Nano Cerium Oxide was used as efficient heterogeneous agent for benzothiazole synthesis [36].



R= aryl,heteroaryl,aliphatics

Benzothiazole is also synthesized with sodium metabisulfide [37].



R= substitued aryl

2.1.2. Spectral Properties of Benzothiazole

2.1.2.1. UV Spectroscopy

Benzothiazole has three absorption bands in the UV region at 240 nm (A=0.037), 270 nm (A=0.031) and 290 nm (A= 0.030) [38].

2.1.2.2. IR Spectroscopy

Aromatic ring of benzothiazole has C-H stretching band nearly at 3050 cm^{-1}. Alicyclic C-H stretching bands are observed at 2880-1160 cm^{-1} [38].

2.1.2.3. ^{1}H-NMR Spectroscopy

Benzothiazole, C-H protons give peaks at 8.971, 8.14, 7.94, 7.51 and 7.46 ppm in deuterated chloroform ($CDCl_3$) [39].

2.1.2.4. ^{13}C-NMR Spectroscopy

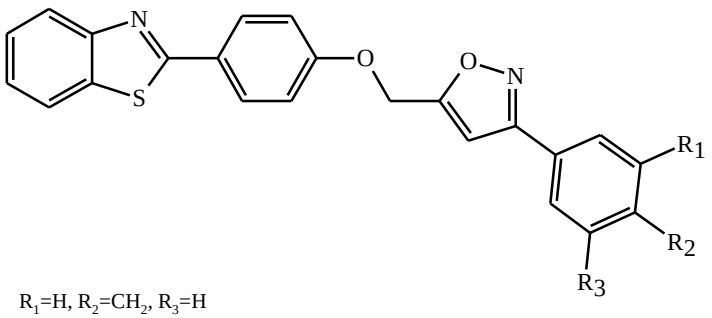
In the ^{13}C-NMR spectrum, benzothiazole carbon atoms appear at 167.54 ppm due to the presence of carbon-nitrogen double bond [40].

2.1.3. Biological Properties of Benzothiazoles

Benzothiazole has a considerable role in many drug candidates to show substantial therapeutic activities. Many research about benzothiazole is made to investigate anticancer [41-50], antimicrobial [48] and other activities such as antibacterial activity, antimicrobial activity and antiinflammatory activity[51-53].

2.1.3.1. Anticancer Activity

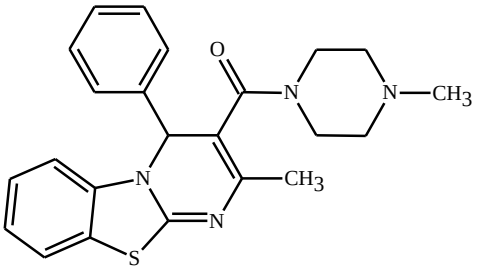
2-Phenylbenzothiazole derivatives (1) have shown good cytotoxicity against Colo-205 and A549 cells [4].



R_1=H, R_2=CH_2, R_3=H

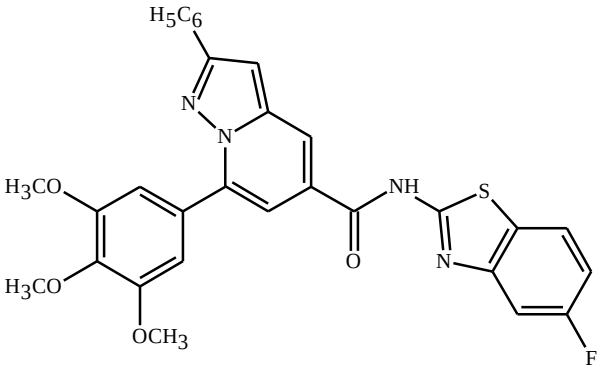
(1)

Benzo[4,5]thiazolo[1,2-a]pyrimidine derivatives have significant cytotoxic effect against human breast adenocarcinoma (MCF-7) [4].



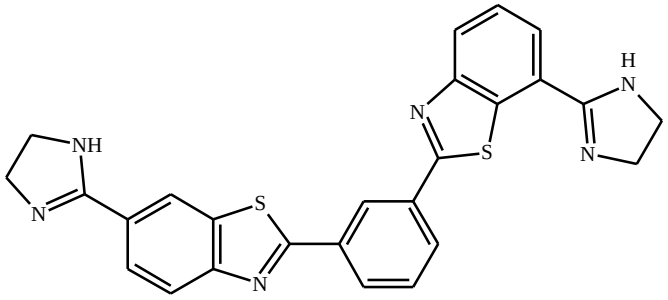
(2)

Pyrozolo[1,5]pyrimidine with 2-aminobenzothiazole derivatives have significant anticancer activity against different human cancer cell lines [5].



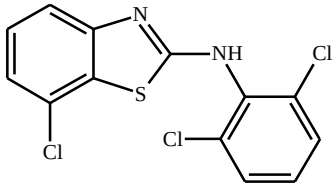
(3)

Bis(benzothiazolyl)phenylenes have strong antiproliferative effect on ovarian, breast, renal and colon carcinoma human tumor cell lines [45].



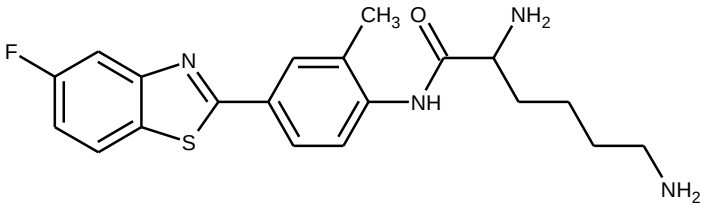
(4)

4-Aminobenzothiazoles (2) have strong cytotoxic activity for lung cancer lines [45].



(5)

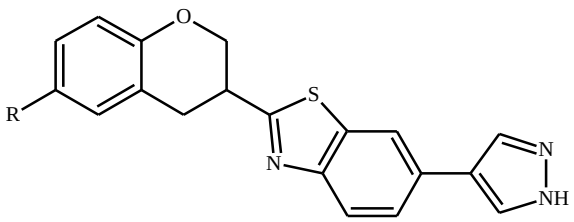
2-(4-Aminophenyl)benzothiazole derivative Phortress causes accumulation of DNA adducts in sensitive tumor cells and apoptosis [54].



(6)

Furthermore, derivatives of benzothiazole (7) have ROCK (Rho associated protein kinase) kinase inhibitory activity. Those type of kinases have roles in diverse

biological processes like growth, differentiation, metabolism and apoptosis for cancer cells [55].

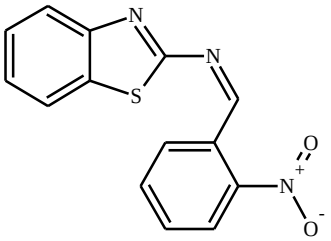


ROCK kinase inhibitory (7)

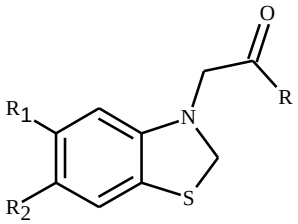
2.1.3.2. Other Activities

Benzothiazoles have antibacterial activity against *Bacillus subtilis*, *Salmonella typhi* and *Shigella Dysenter* [12].

2-Nitrophenyl substituted 2-aminobenzothiazole derivatives have good antimicrobial activity [12]. Benzothiazole derivatives were synthesized and examined for their anthelmintic activity against earthworm, *Perituma posthuma*. In recent years a number of benzothiazole derivatives have been synthesized and investigated for anti inflammatory activity [56].



Antimicrobial (8)



Anthelmintic (9)

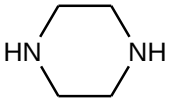
R= -OCH_3, -OC_2H_5

R_1= H,Cl / R_2=H, CH_3, C_6H_5, CO

Antioxidants are therapeutic agents in many diseases. Free radicals have a significant role, and they were discovered in cancer, diabetes, autoimmune diseases, new medical insight. Minimizing a oxidative damage prevents or treatment of these diseases [50].

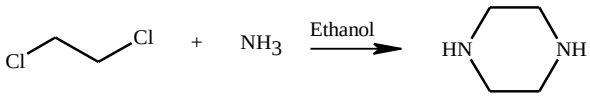
2.2. Piperazine

Piperazine has a six membered ring structure, and contains two nitrogen atoms at 1,4- position in the ring. Piperazine can be synthesized by different methods [57].

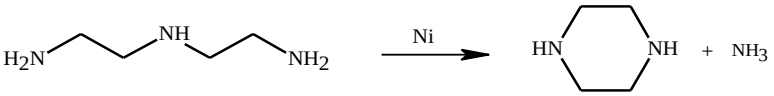


2.2.1. Methods of Synthesis

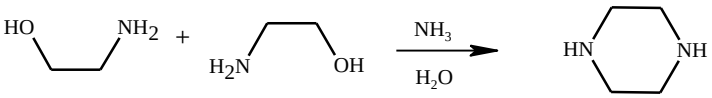
Piperazine was first synthesized by Cloez *et al.,* in 1853 from alcoholic ammonia and ethylene chloride [58].



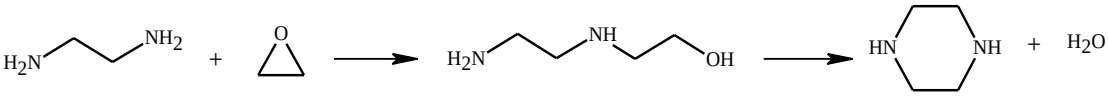
Martin and his colleagues synthesized piperazine from diethylenetriamine by using Raney nickel as a catalyst. Autoclave was used for increasing the yields of reaction. Kyrides *et al.,* in 1948 synthesized piperazine with similar way; nickel catalysts were used at 73 atm with 236 °C [59].



Piperazine is also synthesized from 2-aminoethanol in ammonia at 150-200 °C and 100-200 bar [60].



The other way of synthesizing piperazine is in the presence of ethylendiamine and oxirane [57].



2.2.2. Spectral Properties of Piperazine

2.2.2.1. UV Spectroscopy

Two absorption bands appear for piperazine structure in the UV region at 260 nm (A= 0.0035) and 280 nm (A=0.010) [57].

2.2.2.2. IR Spectroscopy

N-H strecthing vibrations of piperazine appear as sharp singlet nearly at 3250 cm^{-1}. Alicyclic C-H stretching bands appear at 2950-2700 cm^{-1} [61].

2.2.2.3. ^{1}H-NMR Spectroscopy

C-H protons of piperazine are shown at 2.84 ppm in deuterated chloroform ($CDCl_3$) [57].

2.2.2.4. ^{13}C-NMR Spectroscopy

Piperazine carbon atoms appear at 47.9 ppm in deuterated chloroform ($CDCl_3$) [57].

2.2.2.5. Mass Spectroscopy

For piperazine, four main m/z values are observed. $6H_2O$ mass spectrum that are 86, 56, 44 and 30. Base peak is m/z=44 which represents fraction of $NHCH_2CH_2]^+$ [62].

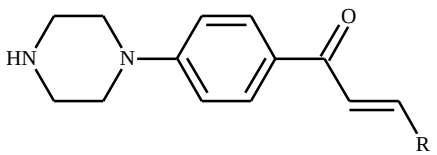
2.2.3. Biological Properties of Piperazines

Piperazine was first used as anthelmintic agent as it has inhibitory action on GABA receptor [63].

Piperazine derivatives are used in various therapeutic fields for their activities such as anti-HIV [64], antidepressant [65], antibacterial [66], anticancer [27, 67-69] and antihypertensive [70].

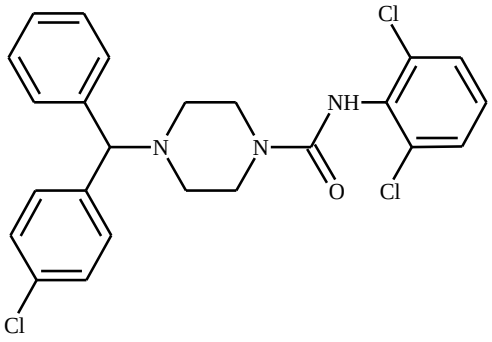
2.2.3.1. Anticancer Activity

Various piperazine derivatives (10) have anticancer activity. For instance, MTT assay performed on various human cancer cell lines such as MCF-7 (breast cancer), HepG2 (hepatocellular cancer), HeLa (cervix cancer), brain cancer, and colorectal cancer show significant growth inhibitory (GI_{50}) action of chalcone derivative piperazine species [71].



(10)

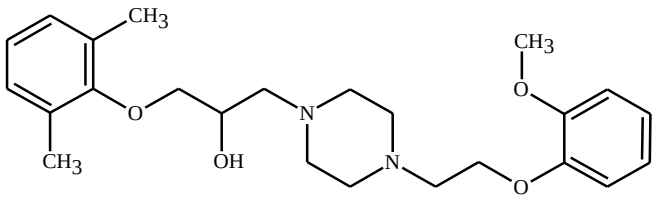
Yarim *et al.*, previously reported many piperazine derivatives (11) with high cytotoxic activity against liver (HUH-7, FOCUS, MAHLAVU, HepG-2, Hep-3B), breast (MCF-7, BT20, T47D), colon (HCT-116), gastric (KATO-3), cervix (HeLa) and endometrial (MFE-296) cancer cell lines [27, 68-69].



Anticancer (11)

2.3.2.2. Other Activities

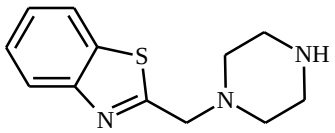
1,4-Substitued piperazine derivatives (12) also have antiarrhytmic and antihypertensive activities. Synthesized compound provides fall in blood pressure [70]. Piperazine and its derivatives with heterocycles have anti HIV-1 activity [64].



Antiarrhytmic (12)

2.3. Benzothiazole-Piperazine Structure

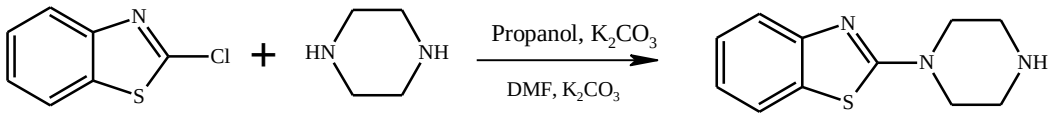
Benzothiazole can be combined with piperazine structure by different methods [72].



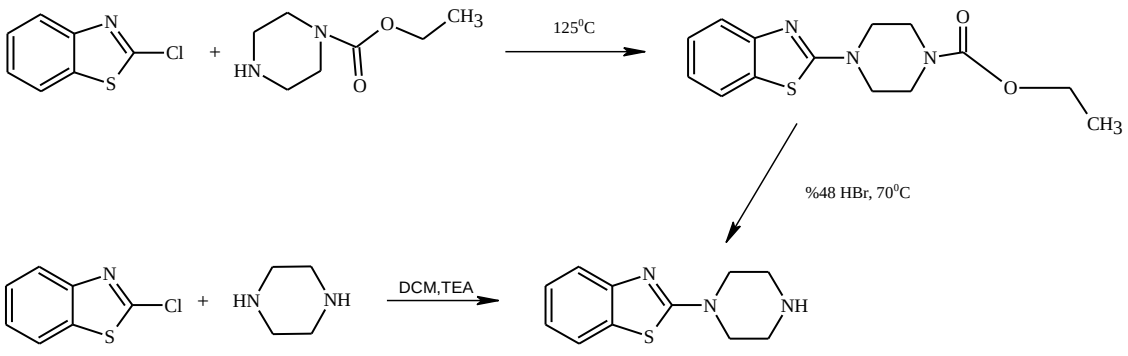
(13)

2.3.1. Methods of Synthesis

2-Piperazinylbenzothiazole can be synthesized from 2-chlorobenzothiazole and piperazine in presence of 2-propanol and potassium carbonate. For this method, dimethylformamide is used as a solvent to obtain the target compounds with 90 % yield [72].

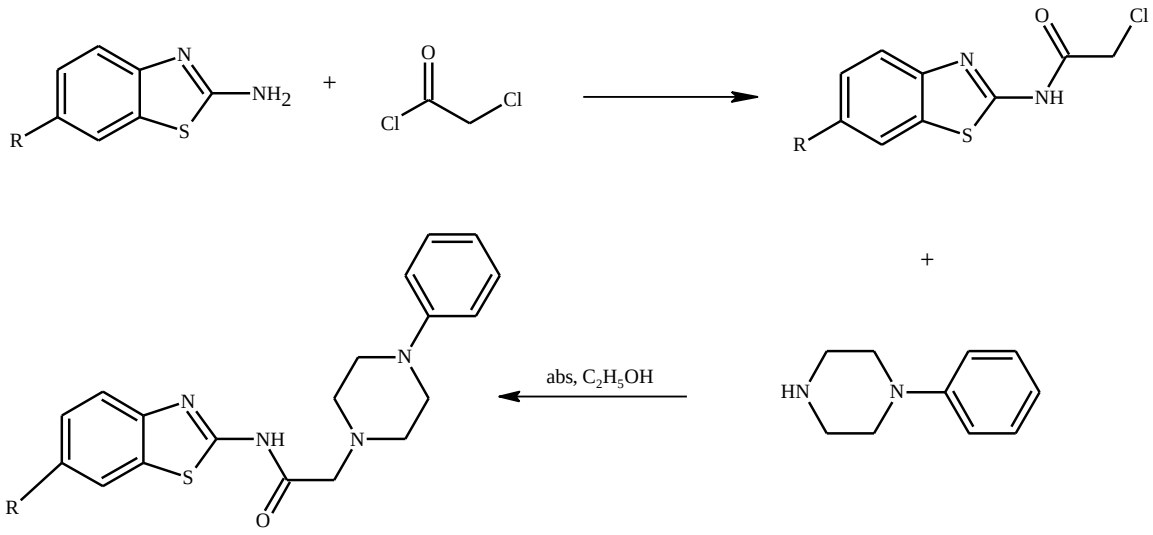


Synthesis of 1-(benzothiazol-2-yl)piperazine can be conducted in two different ways. 2-Chlorobenzothiazole and *N*-ethoxycarbonylpiperazine are heated at 125 °C for 1-5 h. Then, piperazine is liberated by acid hydrolysis. Also, piperazine and 2-chlorobenzothiazole may be reacted in dichloromethane with triethylamine to produce 1-(benzothiazole-2-yl)piperazine [73].



2-Aminobenzothiazole is acetylated with chloroacetyl chloride and reacted with 1-substituted piperazine in absolute ethanol. The reaction was refluxed in water bath at

80 °C for 3 h. Benzene and 3-chloropropionyl chloride were removed by distillation. It was washed with aqueous sodium bicarbonate and cold water. The product was dried and crystallized from ethanol [74].



2.3.2. Spectral Properties of Benzothiazole-Piperazine

2.3.2.1. UV Spectroscopy

Three absorption bands appear for 2-phenylpiperazine-*N*-benzothiazole acetamide at 275.5 nm, 226.5 nm and 210 nm respectively [75].

2.3.2.2. IR Spectroscopy

IR spectra gives a sharp band at 1692 cm^{-1}, and N-H stretching vibrations of benzothiazole-piperazine give a sharp singlet at 3175 cm^{-1} [75].

2.3.2.3. ^{1}H-NMR Spectroscopy

C-H protons of benzothiazole-piperazine appear at 1.33 (t, 3H, $-OCH_2CH_3$) ppm, 3.98 (q, 2H, $-OCH_2CH_3$) 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, H, benzothiazole H_4) ppm in deuterated chloroform ($CDCl_3$) [75].

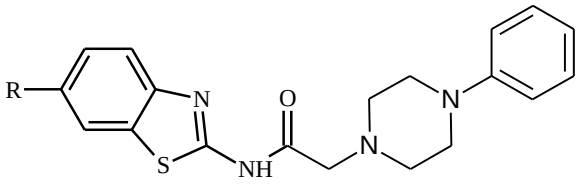
2.3.3. Biological Properties of Benzothiazole-Piperazine

2.3.3.1. Anticancer Activity

Anticancer activity of benzothiazole-piperazine backbone has been researched. Arylsulphonamides and arylthiol derivatives have potent cytotoxicity against a large scale of cancer cell lines such as breast (MCF-7), hepatocellular (HepG-2), prostate (DU-145) cancers and $CD4^+$ human acute T-lymphoblastic leukaemia (CCRF-CEM) [71-73, 75].

2.3.3.2. Other Activities

In recent studies, compounds bearing both benzothiazole and piperazine rings in their structure have been found as new and potent anti-Alzheimer agents with their AchE inhibitory activities. Benzothiazole-piperazine derivatives also have inhibitory effect on 11β- hydroxysteroid dehydrogenase 1 (11β-HSD1) which is responsible with conversion of cortisone to cortisol [74, 75].



(14)

2.4. Cancer

Cancer arises from uncontrolled cell growth. Many factors are known to increase the risk of cancer, such as tobacco use, diet factors, certain infections, exposure to

radiation, lack of physical activity, obesity, and environmental pollutants [4]. Genes are damaged directly from these factors, and genetic faults cause cancerous mutation. Today, number of ways exist to detect cancer signs and symptoms, screening tests, or medical imaging. Chemotherapy, radiation therapy and surgery are methods of cancer treatment.

Cancers are mostly environmental disease with 90–95% and 5–10% due to genetics. Cancer is a disease of tissue growth regulation failure. Genes are effected divided into two categories. Oncogenes are genes which promote cell growth and reproduction. Tumor suppressor genes that inhibit cell division and survival. Changes in many genes are required to transform a normal cell into a cancer cell [76].

Nowadays, 20-25% of deaths arises from malignant tumors in developed countries. Cancer is known as fatal disease and rising potential life threatening with each passing days [76].

According to WHO report, cancer caused 8.2 million deaths in 2012 throughout the world [77].

Table 2.1. Cancer case frequencies in men (Turkey, 2009).

Location	**Case frequency (reported data, %)**
Trachea, Bronchi, Lung	66.0
Prostate	36.1
Bladder	21.4
Colorectal	21.0
Stomach	16.2
Non-Hodgkin Lymphoma	7.2
Kidney	6.3
Pancreas	5.4

Table 2.2. Cancer case frequencies in women (Turkey, 2009).

Location	**Case frequency (reported data, %)**

Breast	40.6
Tiroid	18.6
Colorectal	13.4
Trachea, Bronchi, Lung	8.1
Stomach	8.1
Ovarian	6.9
Non-Hodgkin Lymphoma	5.3

2.4.1. Principles on the Cell and Cancer

There are two important matter of cellular life which are DNA synthesis and mitosis to produce new cells and cell differantion to produce specialized cells. The cells cycle mechanisms are regulated by chemical signals such as growth factors. Normally, negative feedback loop is applied by effects of growth factor. Production of growth factors decreases and proliferation increases when organ is damaged until lost cells. In cancer cells process of binding to cell surface are abnormal. Thus, cancer cells have grown uncontrollably [4].

Genetic changes can occur in the nucleotide sequence of genomic DNA that is called mutations. Mutation may be deletion or gain partial of chromosome.

Tyrosine kinase is an enzyme that provides to transfer a phosphate group from ATP to a protein in a cell. Kinases have significant role in signal transmission that provides regulating cellular activity, cell division or appoptosis. This system has an effect cancer treatment, such as imatinib that is kinase inhibitors [55].

For the cell cycle mechanism, cytotoxic agents act at specific phases, and they have activity against the cells that are in divison process. Normal tissue may be effected by the damages of chemotherapy. Cell cycle during division process may be characterized in various different cell types.

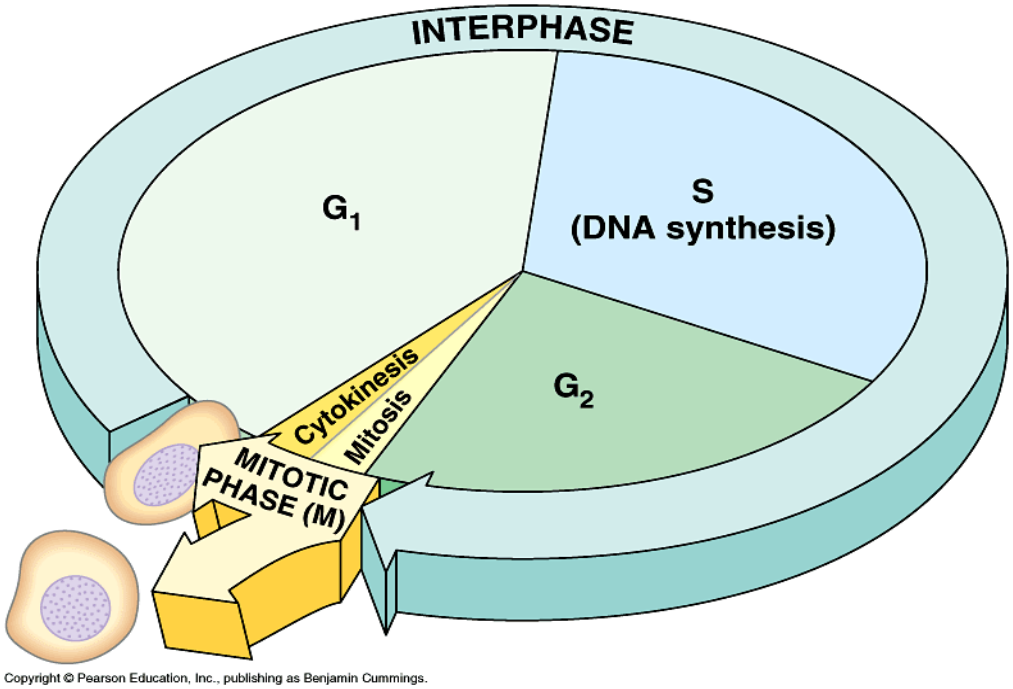
The phases of cell division are as following [78];

1. Presynthetic phase - Gap 1 phase (G_1); a newly created cell is born. In this phase, time period depends on the tissue type and whether it is a normal or tumor cells.

2. Synthesis phase (S); DNA is replicated, and two copies of DNA are present in the cell at the end of the phase.

3. Postsynhetic phase - Gap 2 phase (G_2); the termination of DNA synthesis, which preparations are made for the mitosis.

4. Mitosis (M) phase; containing a double complement of DNA divides into two daughter G_1 cells.

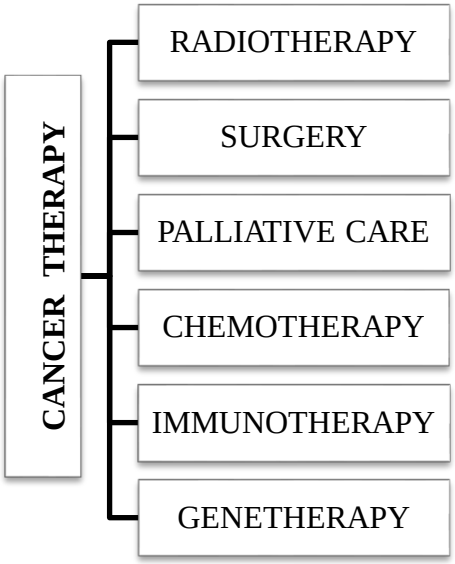


Scheme 2.1. The cell cycle

Metastasis is the spread of cancer to other locations in the body via blood or lymphatic system. The new tumors arise in a side of body different from primary site, that are called metastatic tumors. Different types of cancers tend to metastasize to

particular organs, but the most common metastases occur in lung, liver, brain and the bones [79].

2.4.2. Cancer Treatment



Scheme 2.2. Types of cancer therapy

There are fundamental techniques to treat cancer such as surgery, radiation, therapy, immunologic treatment, gene therapy and chemotherapy. Generally, surgery and chemotherapy are preferred to be used in combination for treatment [80].

2.4.2.1. Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents). Traditional chemotherapeutic agents act by killing cells that divide rapidly.

The efficacy of chemotherapy depends on the type of cancer and the stage. In combination with surgery, chemotherapy has proven useful in a number of different cancer types including: breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer, and certain lung cancer. Chemotherapy may be useful to reduce symptoms like pain or to reduce the size of an inoperable tumor in the hope that surgery will be possible in the future [81].

2.4.2.2. Radiation

Radiation therapy involves the use of ionizing radiation to improve the symptoms of cancer or to cure it. Radiation therapy damages the DNA of cancerous tissue leading to cellular death [80].

2.4.2.3. Surgery

Surgery is the primary method of treatment solid tumor. Biopsies are applied for making the definitive diagnosis and staging [80].

2.4.2.4. Palliative care

Palliative care is treatment which makes the person feel better and may or may not be combined with an attempt to treat the cancer. Aim of this treatment is to improve the person's quality of life [80].

2.4.2.5. Immunotherapy

Immunotherapy is a treatment way to enforce immune system of patient who received chemotherapy. It helps to attack cancer cells, and it prevents getting infection after surgery [81].

2.4.2.6. Gene Therapy

Defective genes cause to cancer. Gene therapy is a treatment that corrects defective and missing genes in cancer cells. Genes can attack existing cancer at the molecular level [81].

2.4.3. Cytotoxicity Analysis

The compounds which effect cellular integrity and decrease cell growth rate or cause cell death [82]. There are several techniques for cytotoxicity analysis.

2.4.3.1. Sulphorhodamine B Assay

Sulphorhodamine B ($C_{27}H_{30}N_2O_7S_2$) is a kind of fluorescent dye used for to the quantification of cellular proteins of cultured cells. The dye has maximal absorbance at 565 nm light and maximal fluorescence emission at 585 nm light. The assay is used for cell density determination, based on the measurement of cellular protein content [83].

2.4.3.2. Hoechst Assay

Hoechst staining is a kind of fluorescent dye used to label DNA and dye mitochondria. Dye is excited by ultraviolet light at around 350 nm, and both emit blue/cyan fluorescent light around an emission maximum at 461 nm. The fluorescence intensity of Hoechst dyes also increases with the pH of the solvent. The dyes generally bind to double strand of DNA, and the dye mostly binds to A-T rich double stranded DNA strands [83].

2.4.3.3. Flow Cytometry

Flow Cytometry is a method to analyze physical characteristics of a particle such as size, relative granularity or internal complexity. It is used for cell counting, cell sorting, and biomarkers detection. Cells flow in a fluid through a beam of light. Three main systems are used for flow cytometry that are fluidics, optics, and electronics [83].

2.4.3.4. Dye Exclusion Test

Dye exclusion test analyzes characteristics of viable cells. For the test, trypan blue, naphtalene black and erythrosine are used as a dye. When the membrane integrity of cells is degraded, dye is uptaken into the cells. Thus, viable cells are observed clear with refractile ring around them, whereas nonviable cells appear dark blue coloured without any refractle ring around them [83].

2.4.3.5. XTT/ PMS Assay

XTT assay is another cytotoxity assay quantitating and viability testing of cells. It is used for growth factors, cytokines or media components. It is used to determine the number of viable cells. 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) is used to measure reduced state. XTT is metabolically reduced by the mitochondria in viable cells to form orange colored water soluble dye [83].

2.4.3.6. MTS/PMS Assay

The assay involves the biological reduction by viable cells of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)

-2H-tetrazolium (MTS). The MTS assay reagent is composed of MTS and the electron coupling agent phenazine methosulfate (PMS). MTS over XTT has a big advantage as it is more soluble and nontoxic [82].

2.4.3.7. Neutral Red Assay

Neutral red (3-amino-7-dimethylamino-2-methylphenazine HCl) provides measurement for cell viability in many cell lines. It stains lysosomes red. Live cells incorporate the dye in their lysosomes, when the cells begin to die neutral red starts to diminish in the lysosomes [84].

2.4.3.8. ATP Cell Viability Assay

Using the luciferase-luciferin reagent provides to quantifiy ATP by a luminometer. ATP levels decreases for apoptotic cells. This assay is formed from two main steps. In the first step, ADP is added as a substrate for adenylate kinase and ATP is produced. In the second step, the enzyme luciferase catalizes the formation of light from ATP and luciferin [85].

2.4.3.9. Enzyme Release-Based Cytotoxicity Assays

Cellular component from degraded cells into the culture medium is measured for cell death assesment. Lactate deheydrogenase (LDH), adenylate kinase (AK) and glyceraldehyde-3-phosphate dehydrogenase (GADPH) are used as a marker of cell death for *in vitro* models [82].

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

For this study, 2-amino-4-methylbenzothiazole, chloroacetyl chloride, 1-(4-fluorophenyl)piperazine, 4-chlorobenzyl piperazine, 1-(2-chlorophenyl)piperazine monohydrochloride, 2-(1-piperazinyl)phenol, 3-(1-piperazinyl)phenol, 1-benzoylpiperazine hydrochloride, 1-acetylpiperazine, cyclohexylpiperazine, 4-hydroxyphenylpiperazine, 1-(3,4-dichlorophenyl)piperazine, 4-nitrophenylpiperazine, 1-methylpiperazine, 1-butylpiperazine, 1-(4-methylphenyl)piperazine, 1-(2-furoyl)piperazine, 1-ethylpiperazine, 1-(2-pyridyl)piperazine, 1-isopropylpiperazine, 1-piperonylpiperazine, 1-(2-ethoxyethyl)piperazine, benzene, triethylamine, absolute ethanol, methanol, ethyl acetate, n-hexane, anhydrous potassium carconate acetone were purchased from Sigma-Aldrich.

3.1.2. Methods of Synthesis

3.1.2.1. Synthesis of *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide [74]

0.006 mol (1.0159 g) 2-amino-4-methylbenzothiazole was dissolved in benzene: triethylamine mixture (20:1). Then, 0.0066 mol (0.53 ml) chloroacetyl chloride was used for acetylation at room temperature. Chloroacetyl chloride was added slowly by small portions. This reaction was completed in four days. The product is beige-colored powder. M.P.: 195.7 °C. FT-IR (KBr, cm^{-1}); 3138 (NH), 3026 (C-H, aromatic), 1665 (C=O), 1594 (C=N).

3.1.2.2. Synthesis of the Target Compounds (1-10)

N-(4-Methyl-1,3-benzothiazol-2-yl)-2-substitued piperazinyl acetamides were synthesized in acetone by the reaction of 0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide, 0.0025 mol appropriate piperazine, and in presence of

0.0025 mol (0.345g) anhydrous K_2CO_3. The products were recrystallized with absolute ethanol.

3.1.3. Analytical Methods

3.1.3.1. Melting Point Determination

Melting points (°C) of the synthesised compounds were detected by using a Mettler Toledo FP62 capillary melting point apparatus.

3.1.3.2. Controls by Thin Layer Chromatography

Materials:

TLC aluminum sheets 20x20 cm Slica gel 60 F_{254} (Merck) as a plates, and two different solvent systems were used for chromatographic controls of compounds as a solvent system;

S1: Benzene: Methanol (90:10)

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

Method:

Solvent systems were poured to chamber and waited 1 hour for adequate and homogeneous saturation.

For TLC method, after dissolving the snythesized compounds and starting materials with suitable solvents and the solvents were applied with Pasteur pipettes on the silica gel plates. The compounds on plates were dragged 10 cm at room temperature, then R_f values of compounds were calculated.

After drying of plates, stains of synthesized compounds and their starting materials were determined by UV light (254/365 nm).

3.1.4. Spectrometric Analyses

Elemental, NMR and LC-MS analyses were conducted in Ankara University, Faculty of Pharmacy by Prof. Dr. Hakan Göker.

3.1.4.1. Ultraviolet Spectra

UV Spectra were recorded on Evolution^{TM} 201/220 UV-visible spectrophotometer and ethanol was used as a solvent.

3.1.4.2. Infrared Spectra

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

3.1.4.3. ^{1}H-NMR Spectra

The ^{1}H-NMR Spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA USA) using tetramethylsilane (TMS) as the internal reference, with dimethylsulfoxide (DMSO-d_6) and chloroform as a solvent, and the chemical shifts were reported in parts per million (ppm).

3.1.4.4. ^{13}C-NMR Spectra

The ^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane (TMS) as the internal reference, with dimethylsulfoxide (DMSO-d_6) as solvent, the chemical shifts were reported in part per million (ppm).

3.1.4.5. Mass Spectra

The mass spectra were recorded 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 LECO 932 CHNS (LECO-932, St. Joseph, MI, USA) instrument.

3.2. Cytotoxic Activity

The cytotoxic activity of the synthesized compounds was investigated on liver (HUH-7), breast (MCF-7) and colon (HCT-116) cancer cell lines, by means of sulphorhodamine B (SRB) assays in triplicate. Serial dilutions from 100 µM to 2.5 µM were used, 5-fluorouracil (5-FU) was the reference compound and camptothecin (CPT) was the positive control for the cytotoxic effect. Biological activity screening studies were conducted in Bilkent University, Department of Molecular Biology and Genetics, by Assoc. Prof. Dr. Rengül Çetin Atalay and Irem Durmaz, MSc.

3.2.1. Cell Culture

The human cancer cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. Each cell line was maintained in an incubator at 37 °C supplied with 5% CO_2 and 95% air.

3.2.2. NCI-60 Sulphorhodamine B (SRB) Assay

Cancer cells (range of 2000 cell/well to 5000 cell/well) were inoculated into 96-well plates in 200 µl of media and incubated in 37 °C incubators containing 5% CO_2 and 95% air. After a 24h incubation period, one plate for each cell line was fixed with 100 µl 10% ice-cold trichloroacetic acid (TCA). This plate represents the behaviour of the cells just prior to drug treatment and is accepted as the time-zero plate. The compounds to be tested were solubilized in DMSO to a final concentration of 40 mM and stored at +4 °C. While treating the cells with the compounds, the corresponding volume of the compound was applied to the cell to achieve the desired drug concentration and diluted through serial dilution. After drug treatment, the cells were incubated in 37 °C incubators containing 5% CO_2 and 95% air for 72 hours. Following the termination of the incubation period after drug treatment, the cells were fixed with 100 µl 10% ice-cold TCA and incubated in the dark at +4 °C for 1 hour. Then the TCA was washed away with ddH_2O five times and the plates were left to air dry. For the final step, the plates were stained with 100 µl of 0.4% sulphorhodamine B (SRB) solution in

1% acetic acid solution. Following staining, the plates were incubated in dark for 10 min at room temperature. The unbound dye was washed away using 1% acetic acid and the plates were left to air dry. To measure the absorbance results, the bound stain was then solubilized using 200 µl of 10 mM Tris-Base. The OD values were obtained at 515 nm [73].

3.2.3. Hoechst Staining Analysis

Cells were seeded on coverslips in 6-well plates. After overnight culture, cells were exposed to compounds at a concentration of their GI_{50} values for 72 hour. To determine nuclear condensation by Hoechst 33258 (Sigma-Aldrich) staining, coverslips were washed twice with icecold PBS, fixed in 1 ml of cold methanol for 10 min, and then incubated with 3 Ig/ml of Hoechst 33258 for 5 min in darkness. The coverslips were then rinsed with distilled water, mounted on glass microscopic slides using 50% glycerol, and examined under fluorescent microscopy (40x).

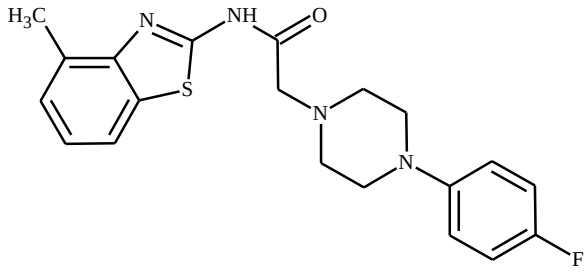
3.2.4. Fluorescence-Activated Cell Sorting Analysis (FACS)

Human cancer cell line (HUH-7) of interest were inoculated into 100-mm culture dishes (300.000 cells/dish). Twenty-four hours later, cells were then treated with the desired compounds according to their GI_{50} values and incubated for 72 hour before. Cells were then collected by trypsinization and the pellets were fixed in ice-cold 70% ethanol and stored at -20 °C. Before the analysis, the samples were stained with MUSE cell cycle reagent (contains propidium iodide solution) according to the manufacturer's protocol. Cell cycle analysis was conducted with MUSE cell cycle analyzer.

4. EXPERIMENTAL

4.1. Chemical Data

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(4-fluorophenyl)piperazin-1-yl] acetamide (Compound 1; CAS No: 946808-47-5)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.4598 g) 1-(4-fluorophenyl)piperazine were reacted in acetone with 0.0025 mol (0.3455 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.2050 g (53 %).

The form of compound is white colored irregular crystals and melting point of the compound is 218.9 °C. It is soluble with hot ethanol and in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.80 and 0.84.

FT-IR (KBr, cm^{-1}); 3296 (N-H), 3057 (C-H, aromatic), 2957 (C-H, aliphatic), 1698 (C=O, amide), 1589 (C=N), 1515 (C=C, aromatic), 1310 (C-N).

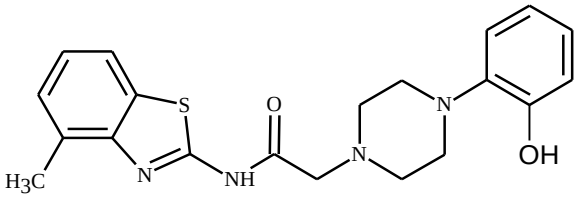
^{1}H-NMR ($CDCl_3$, ppm); 2.66 (s, 3H, Ar-CH_3), 2.82 (t, 4H, piperazine $H_{2,6}$, *J=10 Hz*), 3.24 (t, 4H, piperazine $H_{3,5}$, *J=8 Hz*), 3.36 (s, 2H, -CO$\underline{CH_2}$N-), 6.89-7.02 (m, 4H, phenyl), 7.20-7.26 (m, 2H, benzothiazole $H_{5,6}$), 7.66 (dd, 1H, benzothiazole H_7, J_1*=1.2, Hz,* J_2*=7.6 Hz*), 10.36 (bs, 1H, -$\underline{NH}$COCH$_2$-).

^{13}C-NMR ($CDCl_3$, ppm); 18.17 (-CH_3); 50.18 (piperazine $C_{2,6}$); 53.72 (piperazine $C_{3,5}$); 61.28 (-NHCO$\underline{C}$H$_2$-); 115.55; 115.77; 118.14; 118.22; 118.86; 124.01; 126.93; 130.92; 132.05; 147.56; 147.58; 147.64; 156.03; 156.24; 158.62 (Ar-C), 168.85 (C=O).

Elemental analysis of $C_{20}H_{21}FN_4OS$ (MW: 384.47 g/mol);

	% C	%H	%N	%S
Calculated	62.48	5.51	14.57	8.24
Found	61.27	5.50	14.85	8.29

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(2-hydroxyphenyl)piperazin-1-yl)]acetamide (Compound 2)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.4546 g) 2-(1-piperazinyl)phenol were reacted in acetone and with presence of 0.0025 mol (0.3455 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.1150 g (30 %).

The form of compound is white colored soft crystals and melting point of the compound is 162 °C. It is soluble in hot ethanol and methanol in medium, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.80 and 0.81.

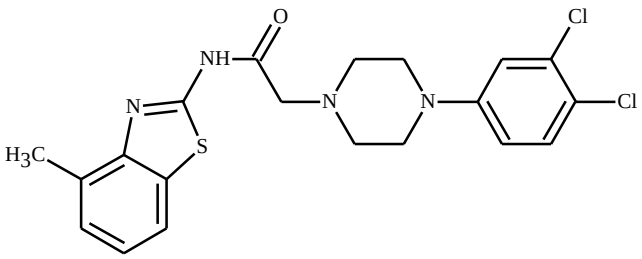
FT-IR (KBr, cm^{-1}); 3318-2750 (O-H), 3317 (N-H), 3060 (C-H; aromatic), 2937 (C-H; aliphatic), 1706 (C=O; amide), 1592 (C=N), 1542 (C=C; aromatic), 1262 (C-N), 1235 (C-O).

^{1}H-NMR (DMSO, ppm); 2.69 (s, 2H, -$\underline{CH_3}$), 2.85 (bs, 4H, piperazine $H_{2\text{-}6}$), 3.04 (t, 4H, piperazine $H_{3\text{-}5}$, *J=4.4 Hz*), 3.38 (s, 2H, -CO$\underline{CH_2}$N-), 6.89-6.99 (m, 3H benzothiazole $H_{5,6}$), 7.09-7.28 (m, 4H, phenyl), 7.67 (dd, 1H, benzothiazole H_7, J_1=2 *Hz* J_2= 7.6 *Hz*), 10.35 (bs, 1H, -$\underline{NH}$COCH$_2$-).

Elemental analysis of $C_{20}H_{22}N_4O_2S$ (MW: 382.48g/mol);

	% C	%H	%N	%S
Calculated	62.80	5.80	14.65	8.38
Found	62.65	5.81	14.57	8.32

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(3,4-dichlorophenyl)piperazin-1-yl]-acetamide (Compound 3)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.5836 g) 1-(3,4-dichlorophenyl)piperazine were reacted in acetone with 0.0025 mol (0.345 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.465 g (84 %).

The form of compound is white colored regular crystals and melting point of the compound is 178.6 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.81 and 0.14.

FT-IR (KBr, cm^{-1}); 3318 (N-H), 3015 (C-H; aromatic), 2947 (C-H; aliphatic), 1702 (C=O; amide), 1688 (C=N), 1591 (C=C; aromatic), 1260 (C-N).

^{1}H-NMR ($CDCl_3$, ppm); 2.65 (s, 2H, -CH_3), 2.79 (t, 4H, piperazine $H_{2\text{-}6}$, *J= 12 Hz*), 3.29 (t, 4H, piperazine $H_{3\text{-}5}$, *J=12 Hz*), 3.35 (s, 2H -CO$\underline{CH_2}$N-), 6.76 (dd, benzothiazole H_5, 1H, *J_1=2.8 Hz, J_2=7.2 Hz*), 6.98 (d, 1H, benzothiazole H_6, *J=2.4 Hz*),

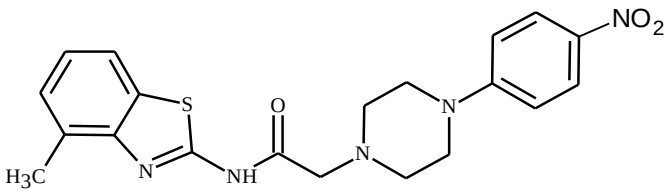
7.20-7.31 (m, 3H, phenyl), 7.66 (dd, 1H, benzothiazole H_7, J_1=1.6 Hz, J_2=6.8 Hz), 10.30 (bs, 1H, -NHCOCH$_2$-).

^{13}C-NMR ($CDCl_3$, ppm); 18.15 (-CH_3); 48.71 (piperazine $C_{2,6}$); 53.37 (piperazine $C_{3,5}$); 61.25 (-NHCOCH$_2$-); 76.69; 77.00: 77.32; 115.60; 117.60; 118.85; 122.82; 124.04; 124.04; 126.93; 130.55; 130.95; 132.05; 132.91; 147.63; 150.27; 155.95; 168.58 (C=O).

Elemental analysis of $C_{20}H_{20}Cl_2N_4OS$ (MW: 435.37 g/mol);

	% C	%H	%N	%S
Calculated	55.17	4.63	12.87	7.36
Found	54.97	4.80	13.24	7.37

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(4-nitrophenyl)piperazin-1-yl]acetamide (Compound 4)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.5341 g) 1-(4-nitrophenyl)piperazine were reacted in acetone with 0.0025 mol (0.345 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.050 g (12 %).

The form of compound is orange colored tiny crystals and melting point of the compound is 177.6 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.77 and 0.77.

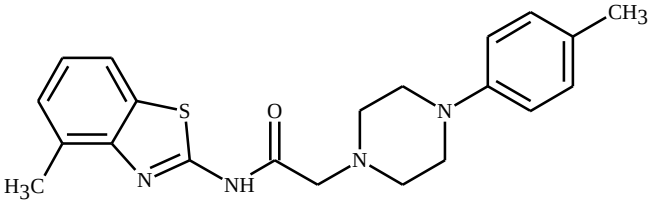
FT-IR (KBr, cm^{-1}); 3262 (N-H), (C-H; aromatic), 2845 (C-H; aliphatic), 1691 (C=O; amide), 1597 (C=N), 1532 (C=C; aromatic), 1508 (N=O, asym.), 1318 (N=O, sym.), 1247 (C-N).

^{1}H-NMR ($CDCl_3$, ppm); 2.65 (s, 2H, -CH_3), 2.82 (t, 4H, piperazine $H_{2,6,}$ *J_1=5.2 Hz*), 3.38 (s, 2H, -CO$\underline{CH_2}$N-), 3.56 (t, 4H, piperazine $H_{3,5,}$ *J=4.8 Hz*), 6.86-6.89 (m, 2H phenyl $H_{2,6}$), 7.21-7.26 (m, 3H, benzothiazole $H_{5,6}$), 7.67 (dd, benzothiazole H_7, 1H, *J_1=1.6 Hz, J_2=7.4 Hz*), 8.15 (dd, 2H, phenyl H_3, *J_1*=3.6, *J_2*=12.2 Hz), 10.29 (bs, 1H, -$\underline{NH}COCH_2$-).

Elemental analysis of $C_{20}H_{21}N_5O_3S$ (MW: 411.48g/mol);

	% C	%H	%N	%S
Calculated	58.38	5.14	17.02	7.79
Found	58.12	4.95	17.24	7.37

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-[1-(4-methylphenyl)piperazine-1-yl)acetamide (Compound 5)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.4496 g) 1-(4-methylphenyl)piperazine were reacted in acetone with 0.0025 mol (0.345 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.118 g (31 %).

The form of compound is white colored soft crystals and melting point of the compound is 202.5 °C. It is soluble in hot ethanol and methanol, in chloroform and

acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.72 and 0.45.

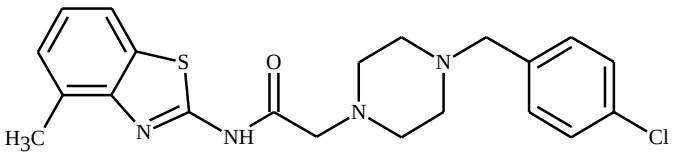
FT-IR (KBr, cm^{-1}); 3279 (N-H), 3035 (C-H; aromatic), 2975 (C-H; aliphatic), 1707 (C=O; amide), 1688 (C=N), 1590 (C=C; aromatic), 1267 (C-N).

^{1}H-NMR (DMSO, ppm); 2.29 (s, 2H, -CH_3), 2.66 (s, 2H, -CH_3), 2.81 (t, 4H, piperazine $H_{2,6}$, *J=8 Hz*), 3.27 (t, 4H, piperazine $H_{3,5}$, *J=10 Hz*), 3.35 (s, 2H -CO$\underline{CH_2}$N-), 6.86-6.89 (m, 2H phenyl $H_{3,5}$), 7.10 (d, 2H, phenyl $H_{2,6}$, *J=8 Hz*), 7.19-7.26 (m, 2H, benzothiazole $H_{5,6}$), 7.66 (dd, benzothiazole H_7, J_1*=1.6 Hz*, J_2*=17.2 Hz*), 10.37 (bs, 1H, -$\underline{NH}$COCH$_2$-).

Elemental analysis of $C_{21}H_{24}Cl_2N_4OS$ (MW: 380.51/mol);

	% C	%H	%N	%S
Calculated	66.29	6.36	14.72	8.43
Found	66.58	6.50	14.24	8.37

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-chlorobenzylpiperazin-1-yl)acetamide (Compound 6)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.5430g) 1-(4-chlorobenzyl)piperazine were reacted in acetone and with 0.0025 mol (0.3455 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.2340 g (56 %).

The form of compound is yellow colored irregular crystals and melting point of the compound is 148.3 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.80 and 0.56.

FT-IR (KBr, cm^{-1}); 3312 (N-H), 3043 (C-H; aromatic), 2940 (C-H; aliphatic), 1707 (C=O; amide), 1589 (C=N), 1525 (C=C; aromatic), 1263 (C-N).

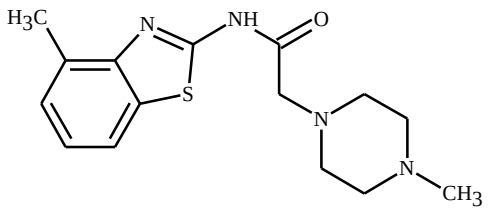
^{1}H-NMR (DMSO, ppm); 2.39 (bs, 4H, piperazine $H_{3,5}$), 2.55 (s, 3H, Ar-CH_3), 3.27 (bs, 4H, piperazine $H_{2,6}$), 3.31 (s, 2H, -CO$\underline{CH_2}$N-), 3.44 (s, 2H, -CH_2)- 7.16-7.24 (m, 2H, benzothiazole $H_{5,6}$), 7.29-7.36 (m, 4H, phenyl), 7.75 (d, 1H, benzothiazole H_7, *J=7.6 Hz*).

MS (m/z); 415.0 (M^+, 100%); 417.0 (M^{+2}, 1/3); 249.1 (4-CH_3-Ar-$NHCOCH_2NH(CH_3)_2$]$^+$); 208.2 (Ar-$NHCOCH_2NH_2$]$^+$).

Elemental analysis of $C_{20}H_{21}ClN_4OS$ (MW: 400.93 g/mol);

	% C	%H	%N	%S
Calculated	59.91	5.28	13.97	8.00
Found	60.88	5.38	13.58	7.69

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (Compound 7)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.2530 g) 1-methylpiperazine were reacted in acetone with 0.0025 mol (0.345 g)

anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.107 g (35 %).

The form of compound is white colored regular crystals and melting point of the compound is decomposed above 300 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.31 and 0.02.

UV (MeOH, λ_{max}, nm); 280 ($n \rightarrow \pi^*$, log ε: 4.90).

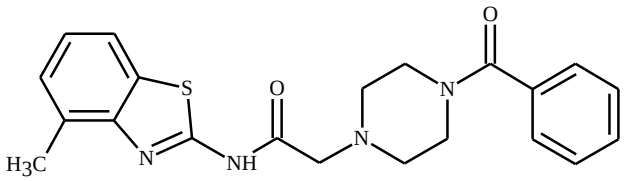
FT-IR (KBr, cm^{-1}); 3378 (N-H), 3038 (C-H; aromatic), 2917 (C-H; aliphatic), 1698 (C=O; amide), 1596 (C=N), 1560 (C=C; aromatic), 1262 (C-N).

^{1}H-NMR (DMSO, ppm); 2.57 (s, 3H, $-CH_3$), 3.04 (bs, 4H, piperazine $H_{2,6}$), 3.39 (s, 3H, $-CH_3$), 3.56 (s, 2H, $-CO\underline{CH_2}N-$), 3.75-3.78 (m, 4H, piperazine $H_{3,5}$), 7.18-7.30 (m, 2H, benzothiazole $H_{5,6}$), 7.77 (d, 1H, benzothiazole H_7, *J=8 Hz*), 13.17 (bs, 1H, $-\underline{NH}COCH_2-$).

Elemental analysis of $C_{15}H_{20}N_4OS$ (MW: 304.41 g/mol);

	% C	%H	%N	%S
Calculated	59.18	6.62	18.41	10.53
Found	58.94	6.52	17.55	10.11

_N_-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-benzoylpiperazin-1-yl)-acetamide (Compound 8)



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.5843 g) 1-benzoylpiperazine hydrochloride were reacted in acetone with 0.0025 mol (0.3455 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.3180 g (80 %).

The form of compound is white colored regular crystals and melting point of the compound is 175 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.70 and 0.59.

UV(MeOH, λ_{max}, nm); 280 ($n \rightarrow \pi^*$, log ε: 4.91).

FT-IR (KBr, cm^{-1}); 3157 (N-H), 3058 (C-H; aromatic), 2989 (C-H; aliphatic), 1699 (C=O; amide), 1608 (C=N), 1542 (C=C; aromatic), 1258 (C-N).

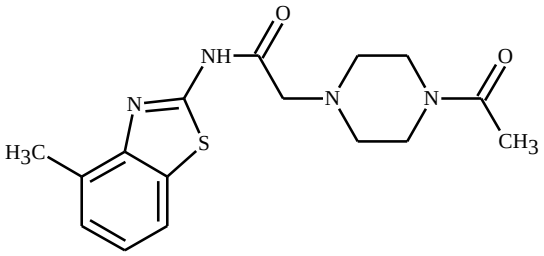
^{1}H-NMR (DMSO, ppm); 2.69 (s, 3H, -$\underline{CH_3}$) 2.84 (bs, 4H, piperazine $H_{2,6}$), 3.03 (t, 4H, piperazine $H_{3,5}$, *J=4.4 Hz*), 3.38 (s, 2H, -CO$\underline{CH_2}$N-), 6.89-6.98 (m, 2H, benzothiazole $H_{5,6}$), 7.09-7.27 (m, 5H, phenyl), 7.66-7.67 (m, 1H benzothiazole H_7), 10.35 (bs, 1H, -$\underline{NH}$COCH$_2$-).

MS (m/z); 395.2 (M^+, 100%); 249.3 (4-CH_3-Ar-NHCOCH$_2$NH$(CH_3)_2$$^+$); 208.0 (Ar-NHCOCH$_2NH_2$$^+$).

Elemental analysis of $C_{21}H_{22}N_4O_2S$ (MW: 394.49 g/mol);

	% C	%H	%N	%S
Calculated	63.94	5.62	14.20	8.13
Found	63.36	5.75	14.04	8.13

***N*-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-acetylpiperazin-1-yl)-acetamide (Compound 9)**



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.3237 g) 1-acetylpiperazine were reacted in acetone with 0.0025 mol (0.345 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.132 g (39 %).

The form of compound is yellow colored regular crystals and melting point of the compound is 186.1 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0.5 and 0.86

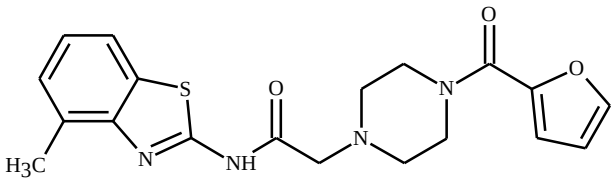
FT-IR (KBr, cm^{-1}); 3451 (N-H), 3292 (C-H; aromatic), 2920 (C-H; aliphatic), 1698 (C=O; amide), 1638 (C=N), 1593 (C=C; aromatic), 1264 (C-N).

^{1}H-NMR (DMSO, ppm); 2.11 (s, 3H, -CO$\underline{CH_3}$), 2.60-2.64 (m, 4H, piperazine $H_{2,6}$), 2.65 (s, 3H, Ar-CH_3), 3.31 (s, 2H, -CO$\underline{CH_2}$N-), 3.58-3.76 (m, 4H, piperazine $H_{3,5}$), 7.19-7.26 (m, 2H, benzothiazole $H_{5,6}$), 7.65 (dd, 1H, benzothiazole H_7, *J_1= 1.2 Hz, J_2=7 Hz*), 10.27 (bs, 1H, -$\underline{NH}$COCH$_2$-).

Elemental analysis of $C_{16}H_{20}N_4O_2S$ (MW: 332.42 g/mol);

	% C	%H	%N	%S
Calculated	57.81	6.06	16.85	9.65
Found	57.35	5.70	16.52	9.48

***N*-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(2-furoyl)piperazin-1-yl]-acetamide (Compound 10)**



0.0025 mol (0.6018 g) *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide and 0.0025 mol (0.4644 g) 1-(2-furoyl)piperazine were reacted in acetone with 0.0025 mol (0.345 g) anhydrous K_2CO_3 according to general synthesis method at 3.1.2.2. The yield is 0.320 g (75 %).

The form of compound is white colored regular crystals and melting point of the compound is 159.6 °C. It is soluble in hot ethanol and methanol, in chloroform and acetone at room temperature. R_f values in TLC at S-1 and S-2 solvent systems are 0 and 8.5.

FT-IR (KBr, cm^{-1}); 3328 (N-H), 3074 (C-H; aromatic), 2995 (C-H; aliphatic), 1708 (C=O; amide), 1620 (C=O; amide), 1592 (C=N), 1532 (C=C; aromatic), 1266 (C-N).

^{1}H-NMR ($CDCl_3$ ppm); 2.72 (t, 4H, piperazine $H_{2,6}$, *J=4.8 Hz*), 3.35 (s, 3H, -CH_3), 3.96 (bs, 4H, piperazine $H_{3,5}$), 4.33 (s, 2H, -CO$\underline{CH_2}$N-), 6.50 (dd, 1H, furyl H_4, *J_1=1.6 Hz, J_2=3.6 Hz*), 7.06 (d, 1H, furyl H_5, *J=3.2 Hz)*, 7.21-7.26 (m, 2H, benzothiazole $H_{5,6}$), 7.49-7.5 (m, 1H, furyl H_3), 7.66-7.68 (m, 1H, benzothiazole H_7), 10.29 (bs, 1H, -$\underline{NH}$COCH$_2$-).

Elemental analysis of $C_{19}H_{20}N_4O_3S$ (MW: 384.45g/mol);

	% C	%H	%N	%S
Calculated	59.36	5.24	14.57	8.34
Found	60.37	5.60	14.14	8.27

4.2. Biological Data

Table 4.1. Cytotoxic activity data for compounds **1-10**.

Compounds	Cancer Cell Line (GI_{50}, μM)		
	HCT-116	**MCF-7**	**HUH-7**
1	3.5	14.8	4.8
2	10.2	9.7	7.8
3	36.5	46.3	16.4
4	18.2	23.2	11.3
5	10.2	*NI*	10.3
6	10.9	15.3	8.4
7	39.2	15.7	20.9
8	0.9	9.2	0.7
9	46.0	*NI*	13.9
10	1.3	4.3	4.5
5-FU	30.66	3.51	18.67

NI: Not inhibitory.

Table 4.2. Comparison of cytotoxic activity of compound **10** against non-tumorigenic epithelial mammary cell line and breast cancer cell line (GI_{50}, μM).

Compound	MCF7	MCF12A
10	4.3	7.9

DMSO **Compound 8** **Compound 10**

Figure 4.1. Fluorescence images of liver cancer (HUH7) cells treated with compounds **8** and **10** or DMSO control.

DMSO **Compound 8** **Compound 10**

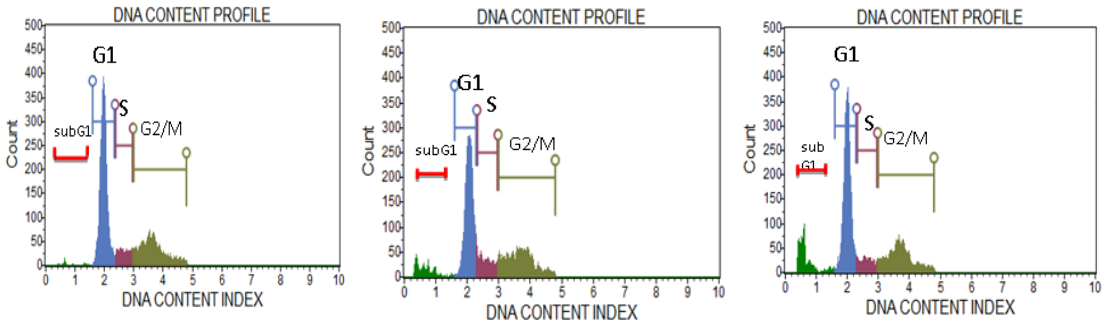


Figure 4.2. Cell cycle distribution analysis of compounds **8 and 10** with DMSO control on HUH7 cancer cells

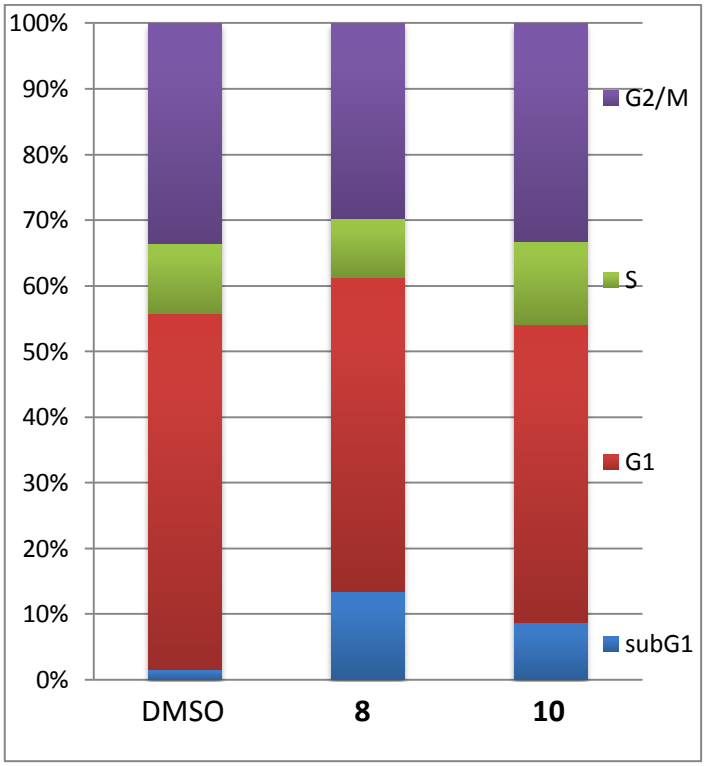
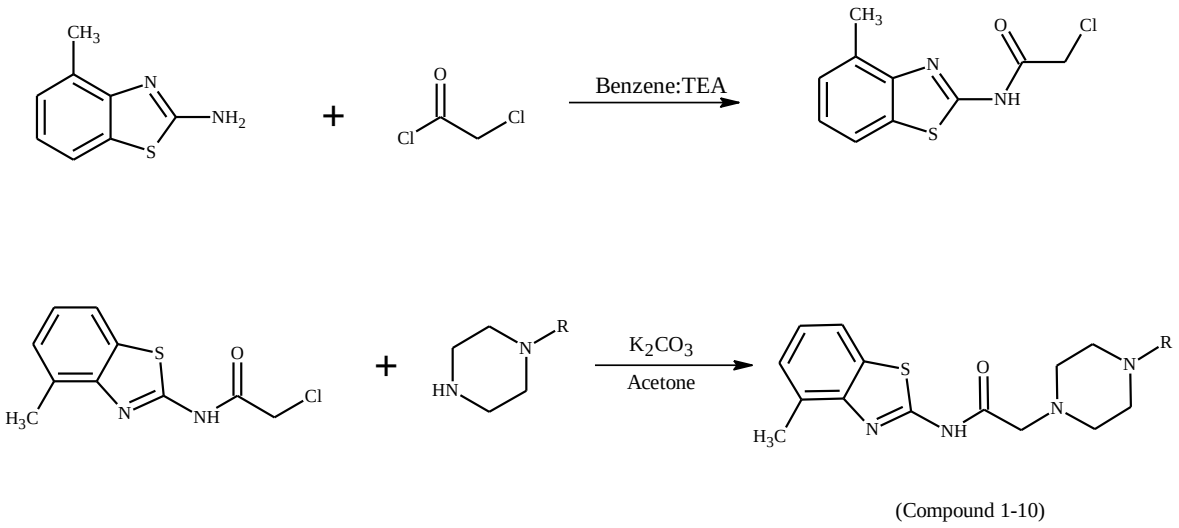


Figure 4.3. Cell cycle distribution analysis on HUH7 cancer cells

5. RESULTS AND DISCUSSION

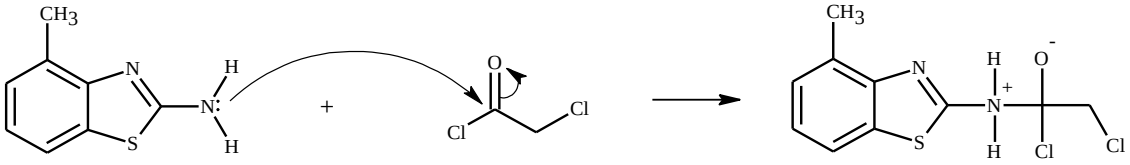
In this study, original compounds that contain *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-(substitutedpiperazin-1-yl)acetamide structure were synthesized according to synthetic pathway in Scheme 5.1. For the confirmation of each structure, UV, IR, ^{1}H-NMR,^{13}C-NMR and elementary analyses were conducted.



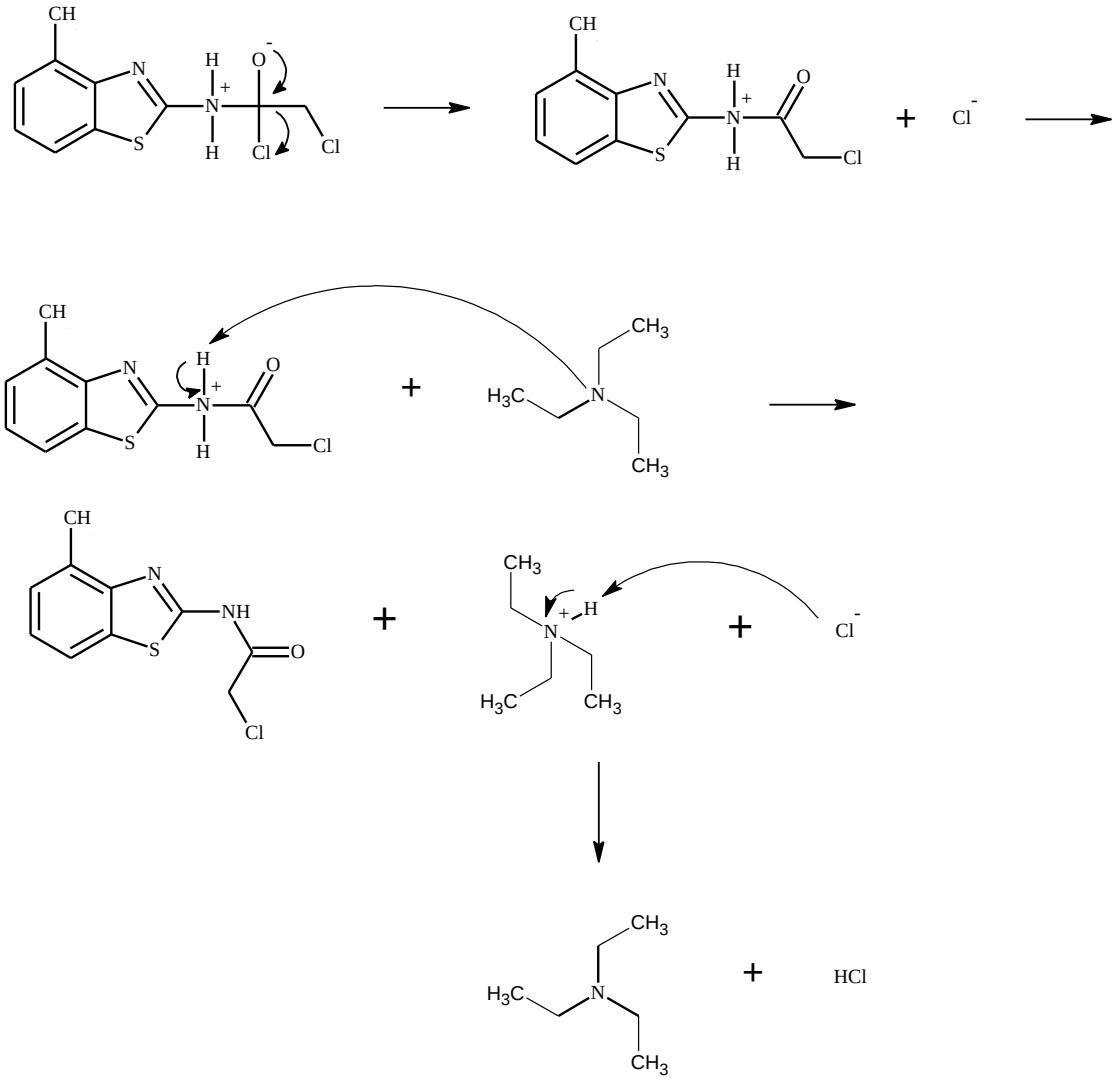
(Compound 1-10)

Sheme 5.1. General synthesis pathway of the compounds

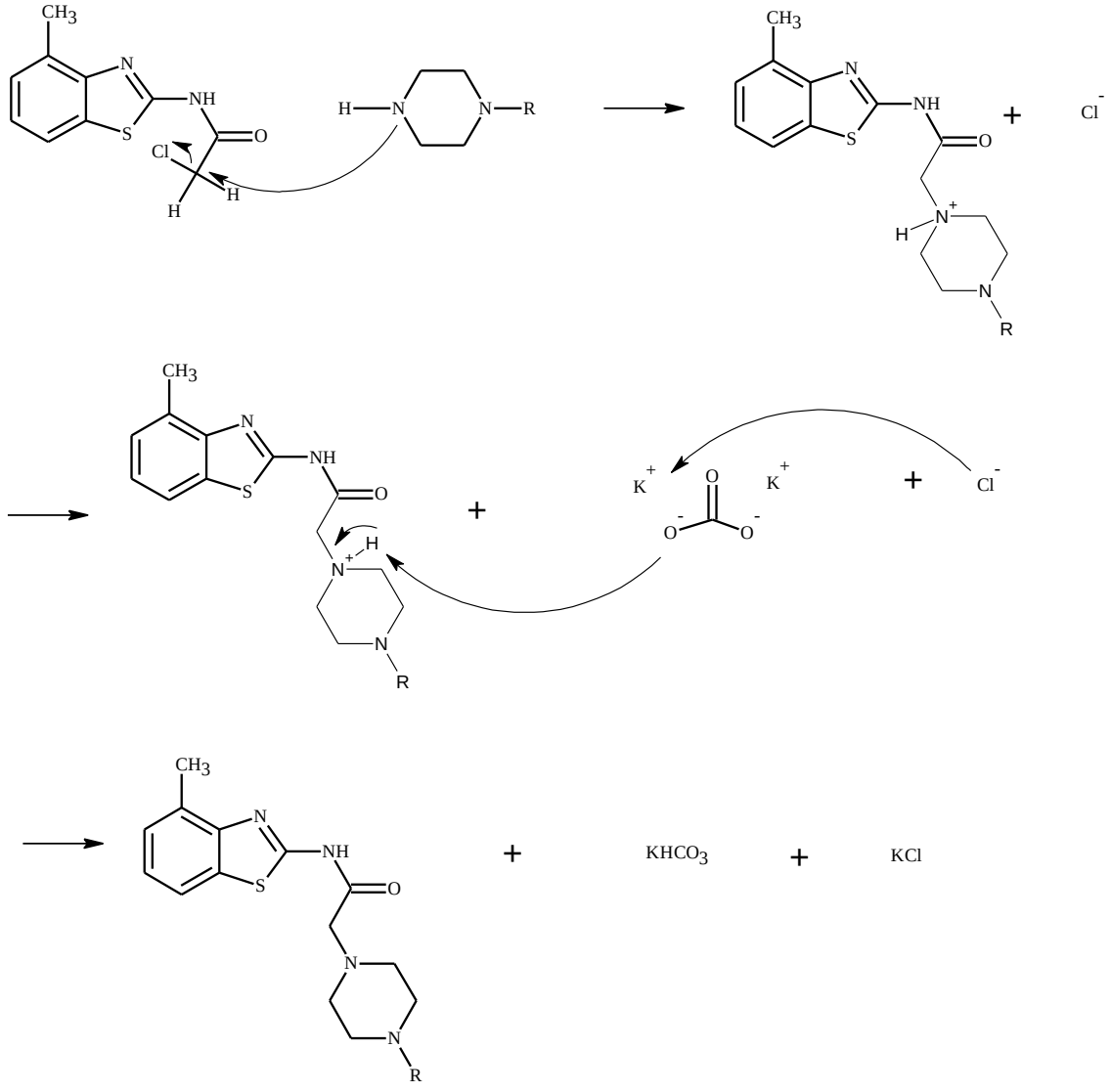
The two stages of synthesis are *N*-acetylation of the primary amine and *N*-alkylation of the secondary amine to obtain the final compounds. First of all, it is necessary to synthesize *N*-(4-methyl-1,3-benzothiazol-2-yl)acetamide from 2-amino-4-methylbenzothiazole. The acetylation reaction starts with nucleophilic attack on the positive carbon atom of chloroacetylchloride by the lone pair on nitrogen in the benzothiazole ring. After the band formation, nitrogen is positively charged and oxygen is negatively charged.



Carbonyl double bond is regained and a chloride ion is extracted. Nucleophilic attack occurs to the acidic hydrogen by the lone pair of nitrogen in TEA to form 2-chloro-*N*-(4-methylbenzothiazole-1-yl)acetamide. Then, chloride ions attack hydrogen atom on TEA. Finally, TEA and HCl are formed again.



For the piperazine ring addition or substitution, the amine nitrogen attacks the electrophilic carbon of the alkyl halide to displace chloride and new C-N bond is formed. Potassium carbonate attacks the positively charged ammonium creating the alkylation product, also a tertiary amine is formed.



The final products were confirmed by UV, IR, ^{1}H-NMR and ^{13}C-NMR, mass spectrometries and elemental analyses.

In UV spectrum of compound **8** there is one significant band at 280 nm which represents n→π* transition of the series.

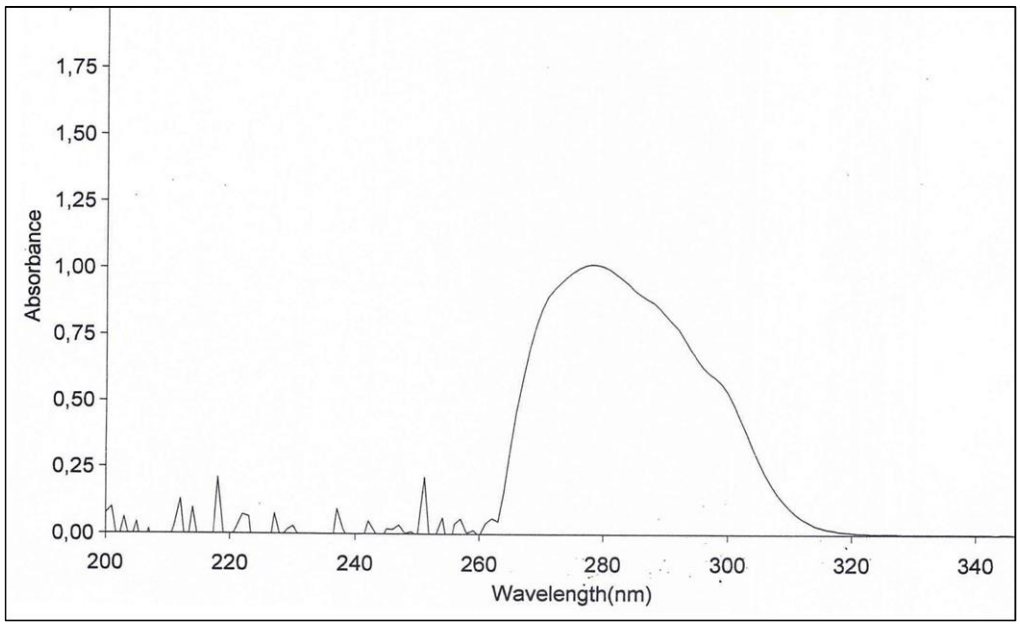


Figure 5.1. UV spectrum of compound **8**

In UV spectrum of compound **7** there is one significant band at 280 nm which represents n→π* transition of the series.

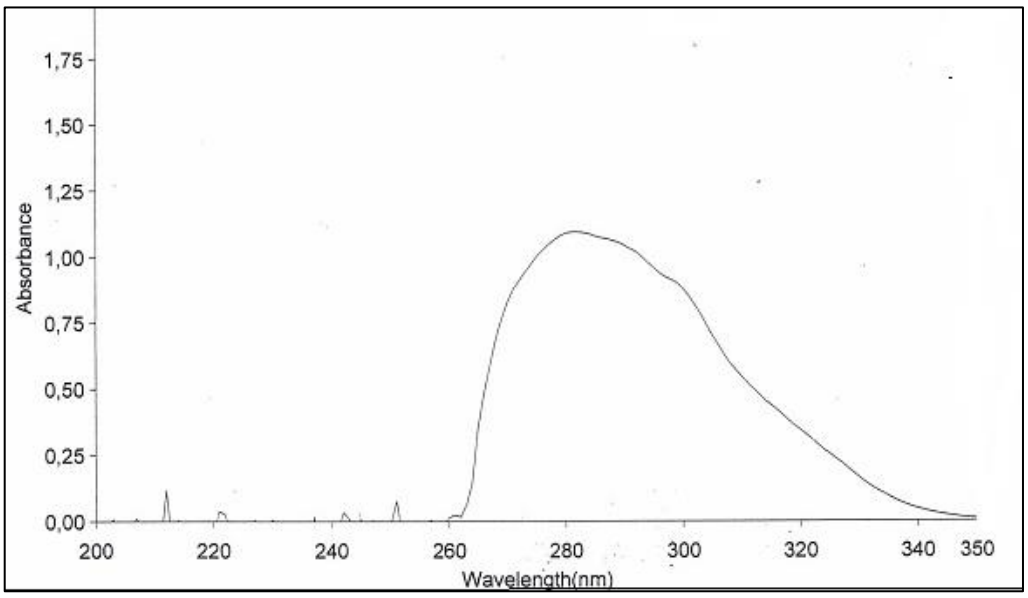


Figure 5.2. UV spectrum of compound **7**

IR spectrum of compound **9** shows the IR absorption bands of benzothiazole-piperazine derivatives. Characteristic N-H stretching band is observed at 3450 cm^{-1}. Other stretching bands are observed at 3292 cm^{-1} (C-H; aromatic), 2920 cm^{-1} (C-H; aliphatic), 1698 cm^{-1} (C=O; amide), 1536 cm^{-1} (C=C; aromatic), 1264 cm^{-1} (C-N).

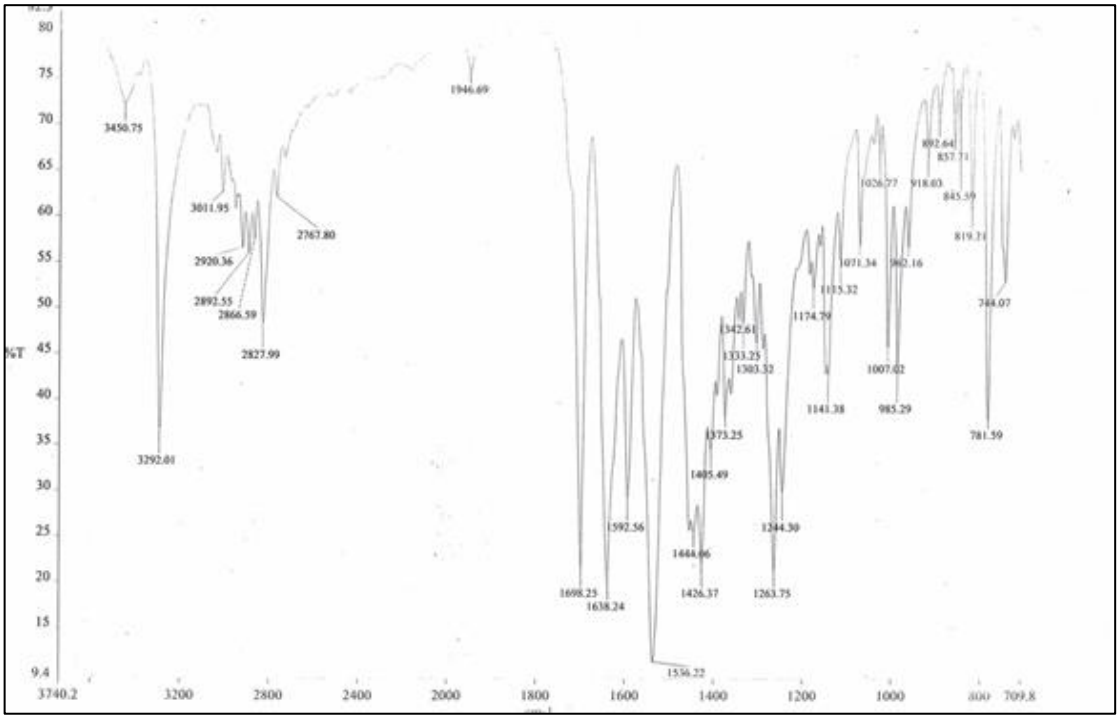


Figure 5.3. IR spectrum of compound **9**

IR spectrum of compound **3** shows the IR absorption bands of series. Characteristic N-H stretching band is observed at 3317 cm^{-1}. Other stretching bands are observed approximately at 3015 cm^{-1} (C-H; aromatic), 2882 cm^{-1} (C-H; aliphatic), 1702 cm^{-1} (C=O; amide), 1531 cm^{-1} (C=C; aromatic), 1260 cm^{-1} (C-N).

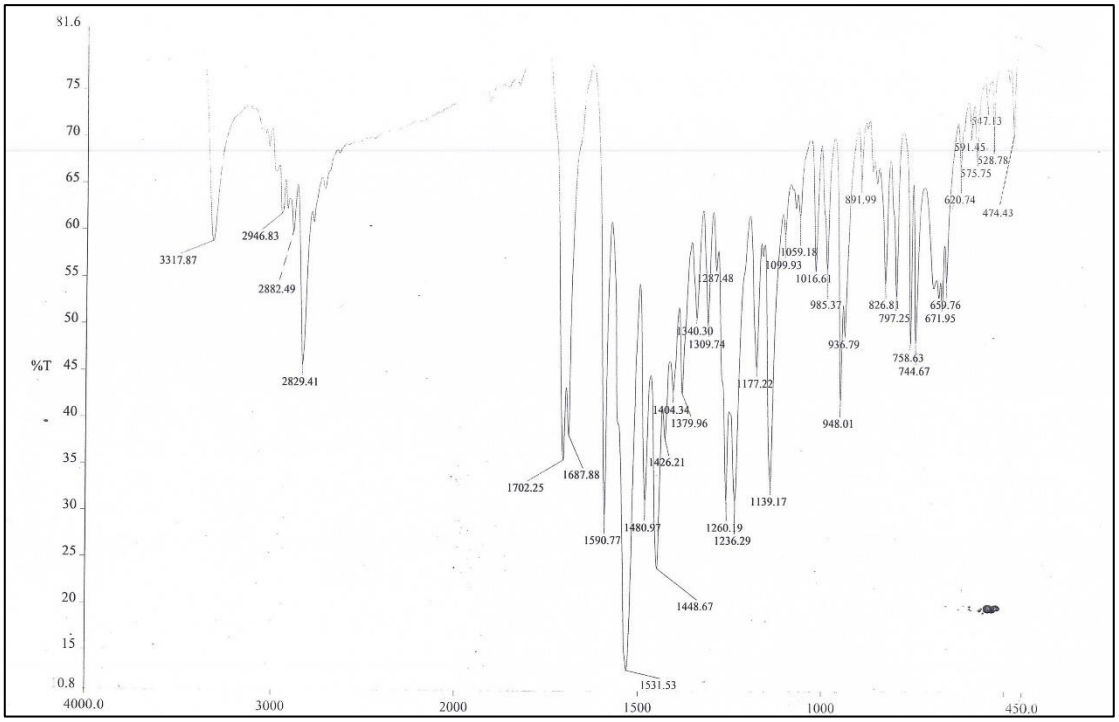


Figure 5.4. IR spectrum of compound **3**

^{1}H-NMR spectrum of benzothiazole-piperazine derivatives are represented by compound **1** in figure 5.5. Methyl protons are observed at 2.66 ppm as singlet. The protons of piperazine are seen at 2.82 (t, 4H, $H_{2\text{-}6}$, *J= 10 Hz)* ppm, 3.24 (t, 4H, piperazine $H_{3\text{-}5}$, *J=8 Hz*) ppm. Methylene protons of -CO$\underline{CH_2}$N- group are observed at 3.36 (s, 2H) ppm. Phenyl group gives 6.89-7.02 (m, 4H), and protons of benzothiazole ring gives multiplet at 7.20-7.26 (m, 2H) and 7.66 (dd, 1H, J_1*=1.2, Hz*) NH group gives peak at 10.36 (bs, 1H, -$\underline{NH}$COCH$_2$-).

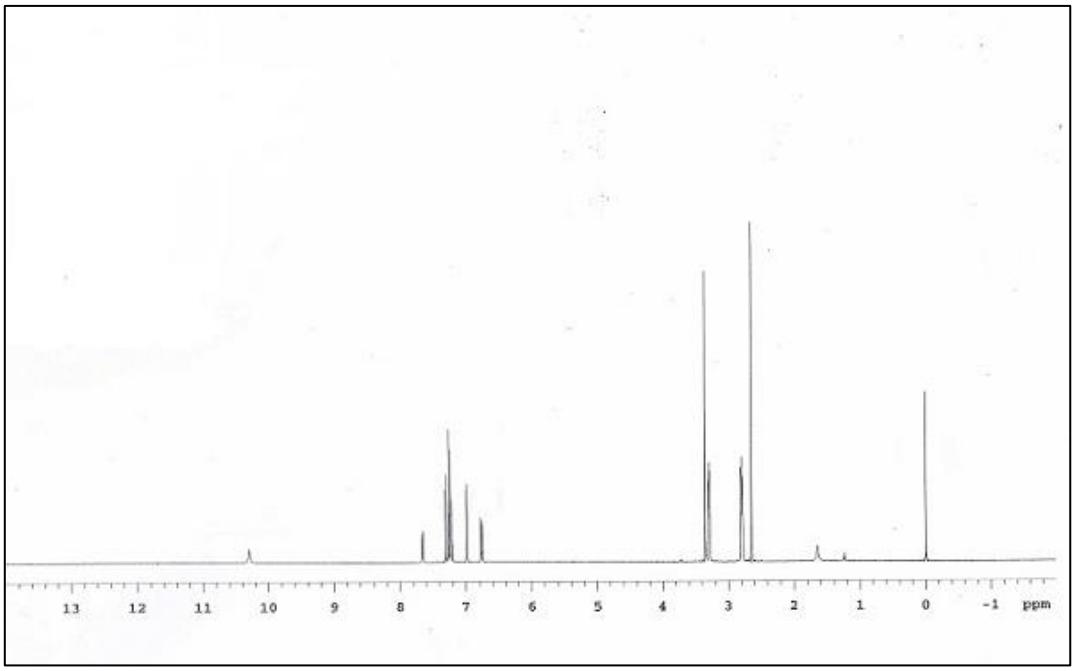


Figure 5.5. ^{1}H-NMR spectrum of compound **1**

^{1}H-NMR spectrum of compound **9** is represented at Figure 5.6. Acetyl protons are observed at 2.11 ppm as singlet. The methyl protons of benzothiazole are seen at 2.65 (s, 2H, -CH_3) ppm, protons of piperazine are seen at 2.60-2.64 (m, 4H, piperazine $H_{2,6}$) ppm and 3.58-3.76 (m, 4H, piperazine $H_{3,5}$) ppm. Methylene protons of -CO$\underline{CH_2}$N- group are observed at 3.31 (s, 2H) ppm. Benzothiazole ring gives signals at 7.19-7.26 (m, 2H, $H_{5,6}$) ppm and 7.65 (dd, 1H, H_7, J_1*= 1.2 Hz*, J_2*= 7 Hz*) ppm. NH group gives peak at 10.27 (bs, 1H, -$\underline{NH}COCH_2$-) ppm.

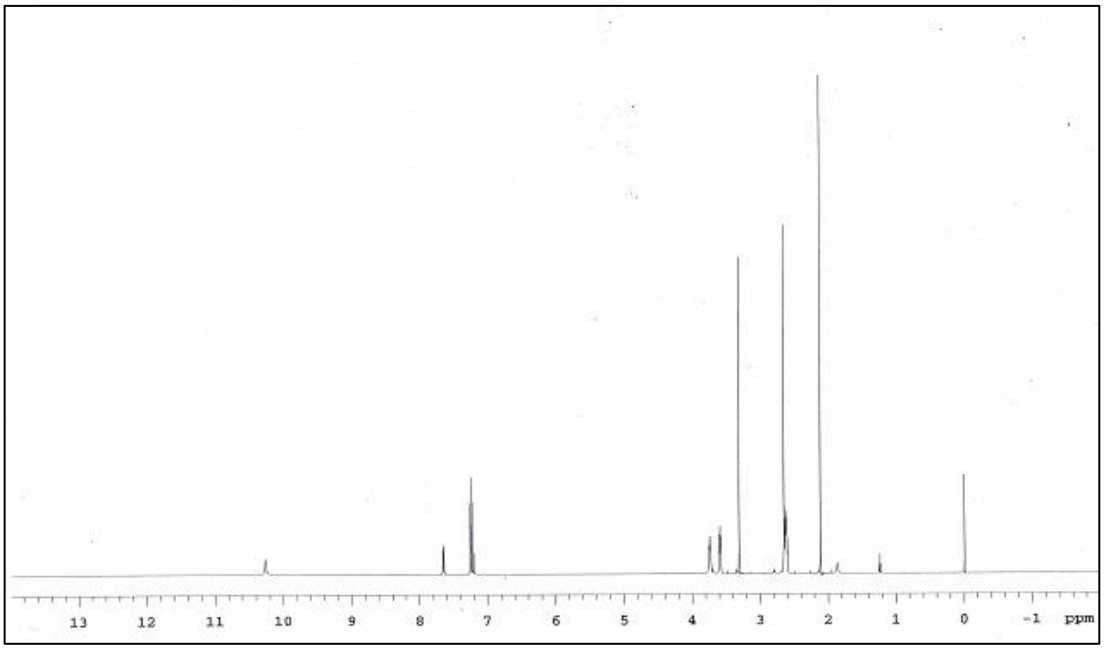


Figure 5.6 ^{1}H-NMR spectra of compound **9**

The ^{13}C-NMR spectrum of the compound **1** was taken in $CDCl_3$. Methyl peak was observed at 18.17 (C_1) ppm, piperazine ring peak was observed at 50.18 (C_2) and 53.71 (C_3) ppm, methylene group at 61.28 (C_4) ppm and carbonyl group at 168.85 (C_9) as seen at figure 5.7.

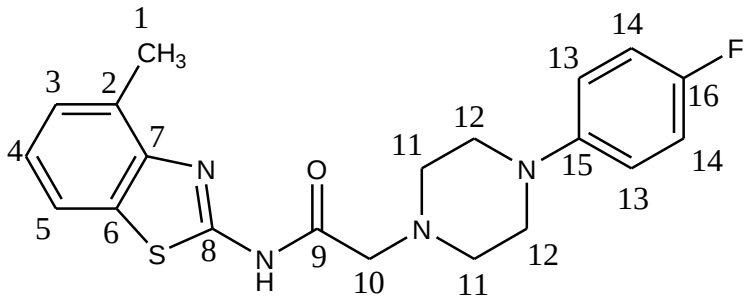


Table 5.1. ^{13}C-NMR spectrum of compound **1.**

C	Chemical Shift δ	C	Chemical Shift δ
1	18.17	9	168.85
2	132.05	10	61.28
3	130.91	11	53.71
4	126.93	12	50.18
5	118.86	13	115.55
6	124.01	14	118.14
7	147.58	15	147.56
8	158.62	16	147.64

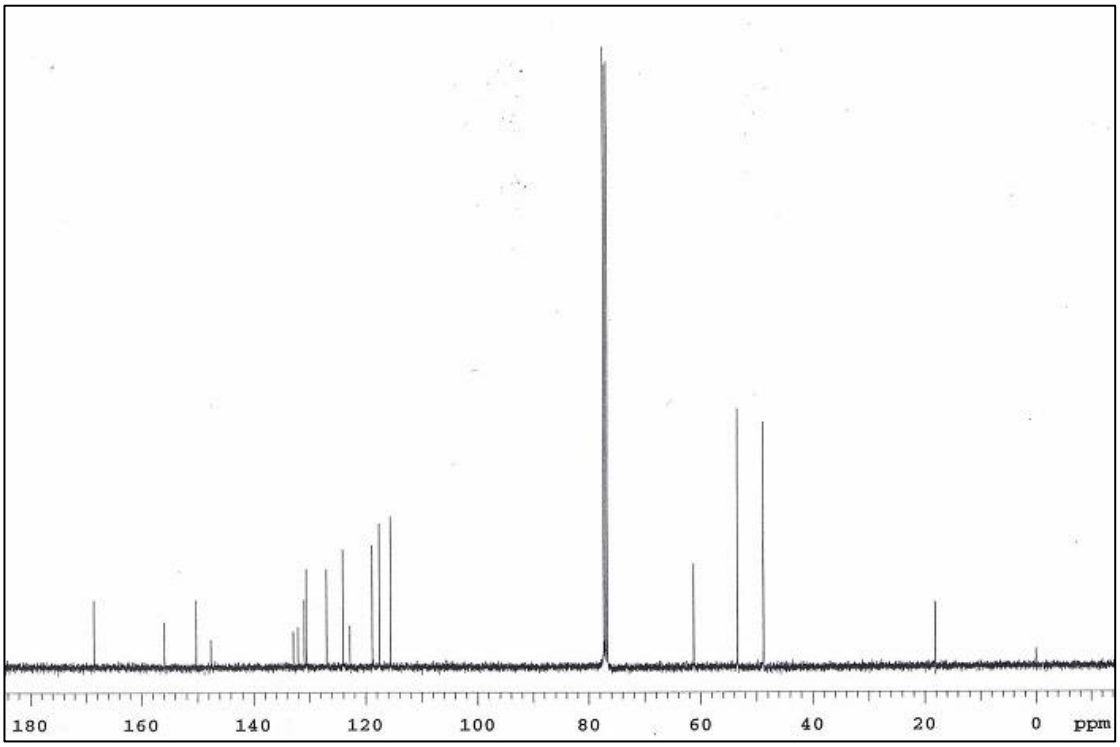
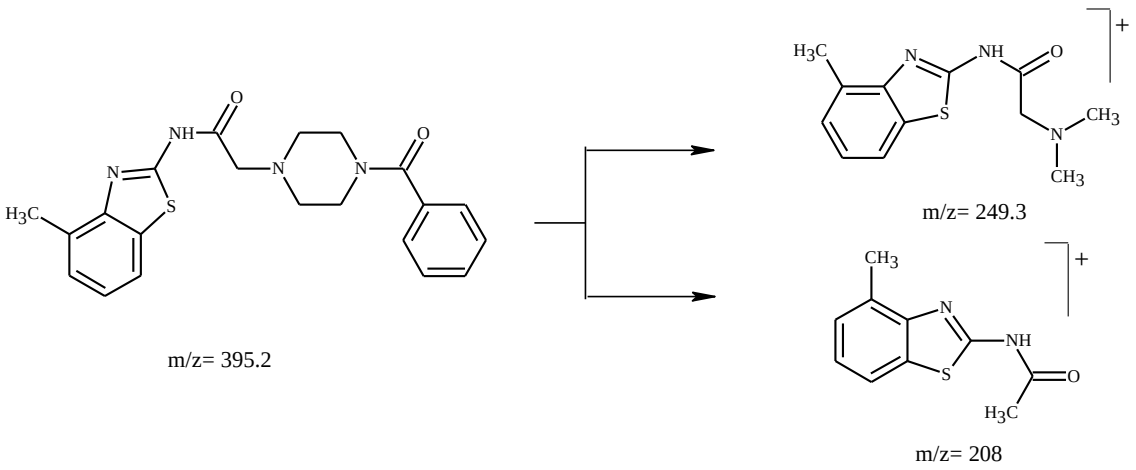


Figure 5.7. ^{13}C-NMR spectrum of the compound **1**

Mass spectrum of benzothiazole-piperazine derivatives are examplified by compound **8.** Molecular ion (M^+) peak is observed as base peak at 395.2 (m/z) and fragmentation products give peaks at 249.3 and 208.0 (m/z). The fragmentation pattern is illustrated in scheme 5.2.



Scheme 5.2. Mass fragmentation pattern of compound **8**.

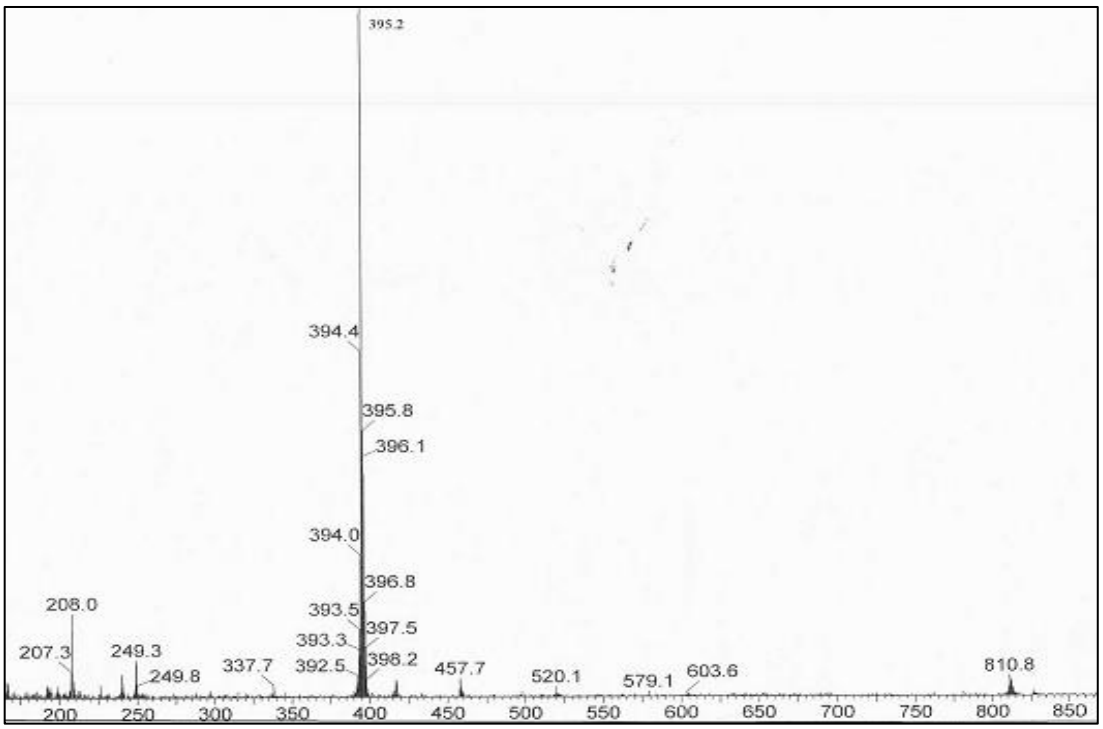


Figure 5.8. Mass spectrum of compound **8**

All of the synthesized compounds were evaluated for their *in vitro* cytotoxic activity by Sulphorhodamine B assay against hepatocellular (HUH7), breast (MCF7) and colon (HCT116) cancer cell lines.

According to results shown in Table 4.1., synthesized compounds are generally cytotoxic against tested cancer cell lines. Also, generally aryl substituted derivatives have higher cytotoxicity against these cancer cell lines.

The most active compounds against HCT116 cancer cell line are *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-benzoylpiperazin-1-yl) acetamide compound **8** (GI_{50}= 0.9 μM) and compound **10** (GI_{50}= 1.3 μM) which have an aroyl structure in common. Another highly cytotoxic derivative is compound **1** (GI_{50}= 3.5 μM). Fluorine may be useful for its highly electron withdrawing nature and its small volume.

The most active compounds against MCF7 cancer cell line is again *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-[4-(2-furoyl)piperazin-1-yl] acetamide compound **10** (GI_{50}= 4.3 μM). Other moderately active derivatives are compound **2** (GI_{50}= 9.7 μM) and compound **4** (GI_{50}= 9.2 μM). Compounds **5** and **9** show no cytotoxicity against MCF7. When 4-methylphenylpiperazine bearing compound compound **5** is compared with 4-fluorophenylpiperazine bearing compound compound **1** (GI_{50}= 14.8 μM) it is concluded that electron donating methyl group results in loss of cytotoxic activity. Also when aroyl substituted compound **8** (GI_{50}= 9.2 μM) and compound **10** (GI_{50}=4.3 μM) are compared with acetyl substituted compound **9**, it is again concluded that acetyl group results in loss of cytotoxic activity. In addition, the most cytotoxic derivative against MCF7 cell line, compound **10**, has been also investigated for selective cytotoxicity against normal breast cell line (MCF12A). It is seen that, inhibition of cell growth is more pronounced in the MCF7 cells compared to the MCF12A cells by compound **10** (Table.4.2.).

The most active compounds against HUH7 cancer cell line is *N*-(4-methyl-1,3-benzothiazol-2-yl)-2-(4-benzoylpiperazin-1-yl) acetamide compound **8** (GI_{50}= 0.7 μM) which indicates that aroyl structure may promote activity also in HUH7 cancer cell line. Also alkyl substituted derivatives have lower cytotoxicity than aryl substituted counterparts.

Compounds **8** and **10** are found to be the most active derivatives among the series. Therefore, further investigation with Hoechst staining (Figure 4.1) and Fluorescence-Activated Cell Sorting Analysis (FACS, Figures 4.2 and 4.3) were done for compounds **8** and **10**.

To examine the type of cell death, presence of apoptopic induction was investigated by Hoechst staining. Human liver cancer cell line HUH-7 was treated with compounds **8** and **10**. Hoechst staining showed condensed nuclei that indicated apoptopic cells in treated samples. Whereas in HUH-7 cell group treated with DMSO, no apoptopic cells were present. This result indicated that apoptosis is most likely to be thcell death type induced by compounds **8** and **10**.

DMSO **Compound 8** **Compound 10**

Figure 4.1. Fluorescence images of liver cancer (HUH7) cells treated with compounds **8** and **10** or DMSO control.

The effect of the compounds **8** and **10** on the cell cycle was further characterized by FACS analysis, using a propidium iodide stain. This analysis revealed elevated $subG_1$ phased cells indicating the $subG_1$ cell cycle arrest compared to control cells treated with DMSO. This result indicates that the presence of cells arrested at $subG_1$ phase supports the induction of apoptopic cell death in those cells treated with compounds **8** and **10**.

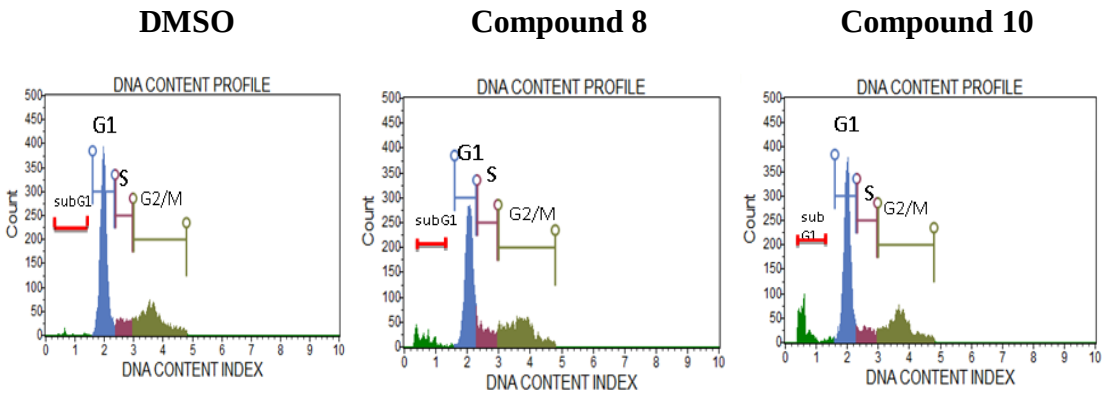


Figure 4.2. Cell cycle distribution analysis of compounds **8** and **10** with DMSO control on HUH7 cancer cells

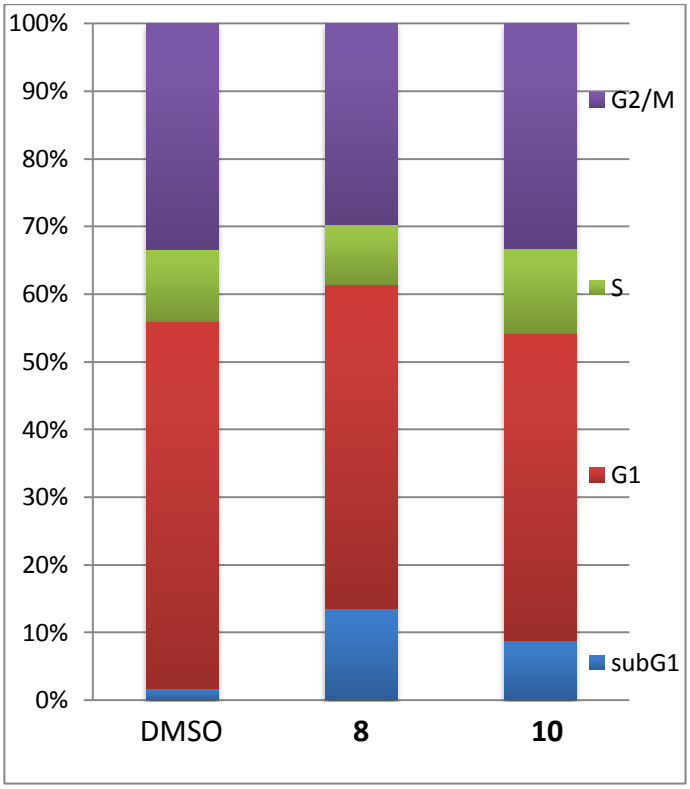


Figure 4.3. Cell cycle distribution analysis on HUH7 cancer cells

6. CONCLUSION

In summary, we have synthesized ten benzothiazole-piperazine derivatives. Their characterization were performed by UV, IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy, mass spectrometry and elementary analyses. *In vitro* cytotoxic activities were screened against colorectal (HCT116), breast (MCF7), and hepatocellular (HUH7) cancer cell lines by sulphorhodamine B assay. The advanced cytotoxicity analysis, Hoechst staining and Fluorescence-activated cell sorting analysis, were also performed. Most of the compounds showed high cytotoxic activity against tested cancer cell lines. Aroyl substituted derivatives compounds **8** and **10** were found to be the most active derivatives. In addition, further investigation of compounds **8** and **10** by Hoechst staining and FACS revealed that these compounds cause apoptosis by cell cycle arrest at subG_1 phase.

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