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SYNTHESIS AND ACTIVITY STUDIES ON SOME NOVEL 1,4-DISUBSTITUTED PIPERAZINES

DOCTOR OF PHILOSOPHY THESIS

ENİSE ECE GÜRDAL, B.Pharm.

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ADVISOR

Assoc. Prof. Dr. MİNE YARIM YÜKSEL

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Doktora öğrencisi Enise Ece GÜRDAL'ın çalışması jürimiz tarafından Farmasötik Kimya Anabilim Dalı Doktora tezi olarak uygun görülmüştür.

İMZA

Başkan : Prof. Dr. Hülya AKGÜN
Üniversite : Yeditepe Üniversitesi .....

Üye : Prof. Dr. Fügen ÖZKANLI
Üniversite : Hacettepe Üniversitesi .....

Üye : Doç Dr. Mine YARIM (Danışman)
Üniversite : Yeditepe Üniversitesi .....

Üye : Doç. Dr. Meriç KÖKSAL
Üniversite : Yeditepe Üniversitesi ----------

Üye :Yrd. Doç. Dr. Filiz Esra Önen BAYRAM
Üniversite : Yeditepe Üniversitesi ----------

ONAY

Yukarıdaki jüri kararı Enstitü Yönetim Kurulu'nun ..24/04/2012 tarih ve .....4-3..... sayılı kararı ile onaylanmıştır.

Prof. Dr. Selçuk YILMAZ
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Dedicated to my grandmother,

Enise Altınok, B.Pharm.

ÖZET

Gürdal, E. E., Bazı Yeni 1,4-Disübstitüepiperazin Türevleri Üzerine Sentez ve Aktivite Çalışmaları. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Doktora Tezi, İstanbul, 2012.

Bu çalışmada, *N*-sübstitüe-4-[(4-sübstitüedifenil)metil]piperazin-1-karboksamit/karbotiyoamit ve *N*-[sübstitüebenzoil/fenilsülfonil]-4-[4-sübstitüe-difenil)metil]piperazin yapılarına sahip, kırk yedisi orijinal, elli bir bileşik sentezlenmiştir. *İn vitro* sitotoksik aktiviteleri kamptotesin (pozitif kontrol) ve 5-florourasil (referans) ile karşılaştırılarak belirlenmiştir.

Başlangıç maddesi olarak kullanılan 4-sübstitüebenzofenon, sodyum borohidrür ile redüklenerek sübstitüe benzhidrol türevleri elde edilmiştir. Sübstitüe benzhidrol türevleri kalsiyum klorür ve hidroklorik asitle kaynatılarak veya tiyonil klorür ile soğuk ortamda muamele edilerek benzhidril klorür türevleri sentezlenmiştir. Benzhidrilpiperazin, 4-klorobenzhidrilpiperazin ve 4,4'-diflorobenzhidrilpiperazin türevleri, piperazin ve ilgili benzhidril klorürlerin bazik ortamda kaynatılmasıyla elde edilmiştir. Benzhidrilpiperazin türevlerinin, oda sıcaklığında, trietilamin varlığında, uygun izosiyanat, izotiyosiyanat, benzoil klorür ve sülfonil klorürlerle reaksiyonları ile bileşikler sentezlenmiştir.

Bileşiklerin yapıları IR, ^{1}H-NMR, ^{13}C-NMR, kütle spektroskopileri, X-ışını kristallografisi ve elementel analiz metotları ile aydınlatılmış, fiziksel özellikleri ve ince tabaka kromatografisinde R_f değerleri belirlenmiştir. *İn vitro* sitotoksik aktivite çalışmaları, sülforodamin B yöntemi ile meme kanseri (MCF-7), hepatoselüler karsinom (HUH-7) ve kolorektal karsinom (HCT-116) hücre hatlarına karşı yapılmıştır.

HUH-7 hücre hattına karşı genel olarak 4-klorobenzhidrilpiperazin türevlerinin diğer türevlere göre daha yüksek etki gösterdiği gözlenmiştir. Bu hücre hattı için en etkin bileşik *N*-(4-siyanofenil)-4-[(4-klorofenil)(fenil)metil]piperazin-1-karboksamit (bileşik **25**; IC_{50} = 1.29 μM) olarak bulunmuştur. Fenil taşıyan tüm karboksamit türevleri arasında en iyi etkiyi, halka üzerinde elektron çekici sübstitüent (4-bromo, 4-siyano, 2,6-dikloro) içeren bileşikler (**15**, **21**, **23**, **25**) göstermiştir. Diğer bileşiklerin aynı hücre hattına karşı aktiviteleri incelendiğinde ise, tiyoamit türevlerinin iyi,

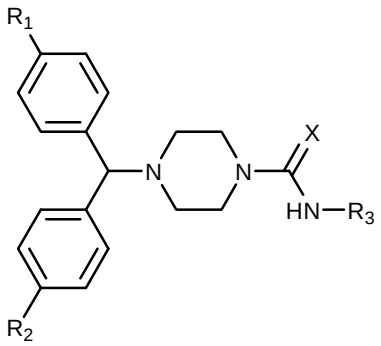
benzoilpiperazin türevlerinin orta, sülfonilpiperazin türevlerinin ise düşük etki gösterdiği görülmüştür.

MCF-7 hücre hattına karşı en yüksek etkiyi 1-(4-bromobenzoil)-4-[bis(4-florofenil)metil]piperazin HCl (bileşik **36**; IC_{50} = 2.21 μM) göstermiştir. Fenil halkası üzerinde elektron çekici sübsitüent taşıyan karboksamit ve benzoilpiperazin türevi bileşikler, genel olarak yüksek aktivite gösterirken, alkil sübstitüe karboksamit türevleri düşük, tiyoamit türevleri ise değişken değerlerde etki göstermiştir. Sülfonilpiperazin türevlerinde, bu hücre hattına karşı etki düşük bulunmuş veya hiç gözlenmemiştir.

HCT-116 hücre hattına karşı en etkili bileşikler *N*-*ter*-butil-4-(difenilmetil)piperazin-1-karboksamit (bileşik **2**; IC_{50} = 1.01 μM) ve *N*-(4-siyanofenil)-4-[(4-klorofenil)(fenil)metil]piperazin-1-karboksamit (bileşik **25**; IC_{50} = 1.81 μM) olarak bulunmuştur. 4-Klorobenzhidril yapısı taşıyan karboksamit türevleri diğer karboksamit türevlerine göre daha etkili görülmüştür. Tiyoamit ve benzoilpiperazin türevleri ise, genel olarak bu hücre hattına karşı yüksek etki gösterirken, sülfonilpiperazin türevleri düşük etkili veya etkisiz bulunmuştur.

Anahtar kelimeler: Piperazin, 1-benzhidrilpiperazin, izosiyanat, izotiyosiyanat, benzoil klorür, sülfonil klorür, sitotoksik aktivite

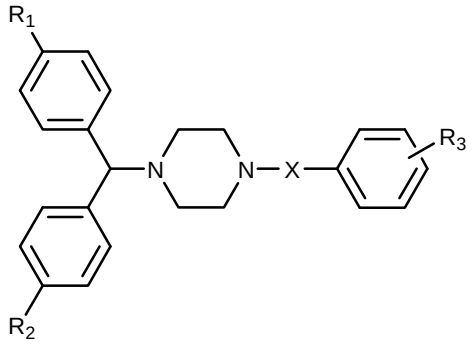
Tablo. Sentezi gerçekleştirilen bileşiklerin (**1-51**) kimyasal formülleri.



Bileşik	X	R_1	R_2	R_3	E. D. (°C)	Verim (%)
1*	O	- H	- H	*sek*-Butil	198.4	68
2	O	- H	- H	*ter*-Butil	192.4	62

3	O	- H	- H	İzopropil	220.4	94
4	O	- H	- H	Etil	208.9	84
5	O	- H	- H	2,6-Diklorofenil	234.6	88
6	O	- H	- H	2-Benzilfenil	192.1	89
7*	O	- H	- H	Etilasetato	150.0	69
8*	O	- H	- H	Allil	213.6	96
9	O	- F	- F	*sek*-Butil	157.7	54
10	O	- F	- F	*ter*-Butil	162.4	82
11	O	- F	- F	Butil	132.9	45
12	O	- F	- F	Etil	175	83
13	O	- F	- F	İzopropil	169.9	92
14	O	- F	- F	Etilasetato	152.3	20
15	O	- F	- F	4-Bromofenil	210.9	67
16	O	- Cl	- H	*sek*-Butil	> 300 (dek.)	62
17	O	- Cl	- H	*ter*-Butil	190.3	36
18	O	- Cl	- H	Etil	288.6 (dek.)	17
19	O	- Cl	- H	İzopropil	198.6	34
20	O	- Cl	- H	Allil	172.7	27
21	O	- Cl	- H	2,6-Diklorofenil	224.6	38
22	O	- Cl	- H	2-Feniletil	147.8	49
23	O	- Cl	- H	4-Bromofenil	195.5	37
24	O	- Cl	- H	2-Benzilfenil	174.6	44
25	O	- Cl	- H	4-Siyanofenil	196.8	26
26	S	- F	- F	*ter*-Butil	176.8	14

27	S	- F	- F	Siklohekzil	198.2	50
28	S	- Cl	- H	Etil	150.6	15
29	S	- Cl	- H	İzopropil	252.4 (dek.)	39
30	S	- Cl	- H	Allil	139.4	10
31	S	- Cl	- H	Benzil	157.2	23
32	S	- Cl	- H	Butil	125.5	20



Bileşik	**X**	**R_1**	**R_2**	**R_3**	**E. D. (°C)**	**Verim (%)**
33	C=O	- H	- H	5-Floro-2-metil	> 300 (dek.)	95
34	C=O	- F	- F	2-Bromo	189.7	35
35	C=O	- F	- F	3-Bromo	151.4	27
36	C=O	- F	- F	4-Bromo	> 300 (dek.)	19
37	C=O	- F	- F	3-Kloro	177.5	47
38	C=O	- Cl	- H	2-Metoksi	120	33
39	C=O	- Cl	- H	3-Nitro	196.1	24
40	C=O	- Cl	- H	3,4-Dimetoksi	148.6	11
41	C=O	- Cl	- H	4-Etil	206.4	22
42	SO_2	- H	- H	2-Triflorometoksi	205.8	10

43	SO_2	- F	- F	2-Triflorometil	135.6	22
44	SO_2	- F	- F	2,4,5-Trikloro	> 300 (dek.)	11
45	SO_2	- F	- F	3,4-Dikloro	145.1	42
46	SO_2	- F	- F	2-Metil	117.7	10
47*	SO_2	- F	- F	4-Nitro	224.5	13
48	SO_2	- F	- F	2,5-Dikloro	116.1	23
49	SO_2	- Cl	- H	2,4,5-Trikloro	151.1	19
50	SO_2	- Cl	- H	3,4-Dikloro	107.1	25
51	SO_2	- Cl	- H	4-Nitro	209.3	37

(*) Bileşik 1, CAS No: 1071382-92-7; Bileşik 7, CAS No: 1350123-57-7; Bileşik 8, CAS No: 1349487-56-4; Bileşik 47, CAS No: 1286459-36-6

ABSTRACT

Gürdal, E. E., Synthesis and Activity Studies On Some Novel 1,4-Disubstitutedpiperazines. Yeditepe University Institute of Health Sciences, Ph. D. Thesis of Pharmaceutical Chemistry Programme, Istanbul, 2012.

In this study, fifty one compounds with structures of *N*-substituted-4-[(4-substituteddiphenyl)methyl]piperazine-1-thioamide/carbothioamide and *N*-[substituted benzoyl/phenylsulfonyl]-4-[(4-substituteddiphenyl)methyl]piperazine were prepared. Forty seven compounds are original. *In vitro* cytotoxic activities were screened in comparison with the reference drugs camptothecin (positive control) and 5-fluorouracil (reference).

Starting material 4-substitutedbenzophenone was reduced with sodium borohydride to yield substituted benzhydrole derivatives. These compounds were refluxed with calcium chloride in hydrochloric acid or reacted with thionyl chloride to obtain benzhydryl chloride derivatives. Benzhydrylpiperazine, 4-chlorobenzhydrylpiperazine and 4,4'-difluorobenzhydrylpiperazine were synthesized by reaction of piperazine and suitable benzhydryl chlorides in alkali medium. The target compounds were synthesized with reactions of benzhydrylpiperazine derivatives with suitable isocyanates, isothiocyanates, benzoyl chlorides and sulfonyl chlorides in room temperature in the presence of triethylamine.

Structures of the compounds were elucidated with IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopies, X-Ray crystallography and elemental analyses, 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 method against breast cancer (MCF-7), hepatocellular carcinoma (HUH-7) and colorectal carcinoma (HCT-116) cell lines.

Against HUH-7 cell line, in general, 4-chlorobenzhydrylpiperazine derivatives were more potent than other derivatives. The most potent compound against this cell line was *N*-(4-cyanophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (compound **25**; IC_{50} = 1.29 μM). The most active compounds among phenyl bearing carboxamides carry electron withdrawing substituents (compounds **15**,

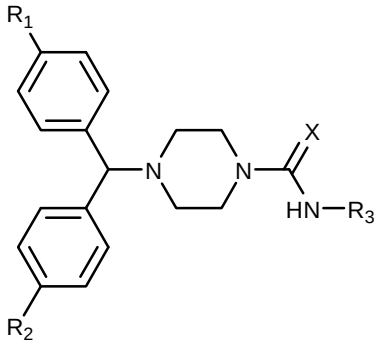
21, **23**, **25**). Considering the activities of other compounds against the same cell line, it was observed that thioamides showed high, benzoylpiperazines showed moderate and sulfonylpiperazine derivatives showed low potency.

The most potent compound against MCF-7 cell line was 1-(4-bromobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine HCl (compound **36**; IC_{50} = 2.21 μM). Carboxamide and benzoylpiperazine derivatives that contain electron withdrawing substituents on phenyl ring were generally highly active, however alkyl substituted carboxamide derivatives were low in activity and thioamide derivatives showed variable values of potency. Sulfonylpiperazine derivatives showed low or no inhibition against this cell line.

The most potent compounds against HCT-116 cell line were *N*-*tert*-butyl-4-(diphenylmethyl)piperazine-1-carboxamide (compound **2**; IC_{50} = 1.01 μM) and *N*-(4-cyanophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (compound **25**; IC_{50} = 1.81 μM). Carboxamide derivatives bearing 4-chlorobenzhydryl moiety were found to be more potent than other carboxamide derivatives. Thioamide and benzoylpiperazine derivatives were generally highly potent whereas sulfonylpiperazine derivatives had low or no inhibition against this cell line.

Keywords: Piperazine, 1-benzhydrylpiperazine, isocyanate, isothiocyanate, benzoyl chloride, sulfonyl chloride, cytotoxic activity

Table. Structures of the synthesized compounds **1-51**.



Compound	X	R_1	R_2	R_3	M. P. (°C)	Yield (%)
1*	O	- H	- H	*sec*-Butyl	198.4	68

2	O	- H	- H	*tert*-Butyl	192.4	62
3	O	- H	- H	Isopropyl	220.4	94
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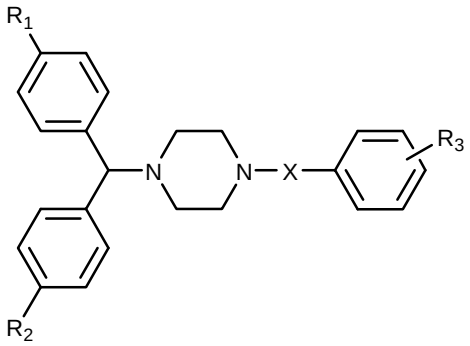

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43	SO_2	- F	- F	2-Trifluoromethyl	135.6	22
44	SO_2	- F	- F	2,4,5-Trichloro	> 300 (dec.)	11
45	SO_2	- F	- F	3,4-Dichloro	145.1	42
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48	SO_2	- F	- F	2,5-Dichloro	116.1	23
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50	SO_2	- Cl	- H	3,4-Dichloro	107.1	25
51	SO_2	- Cl	- H	4-Nitro	209.3	37

(*) Compound 1, CAS No: 1071382-92-7; Compound 7, CAS No: 1350123-57-7; Compound 8, CAS No: 1349487-56-4; Compound 47, CAS No: 1286459-36-6

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ABBREVIATIONS

5-FU	5-Fluorouracil
5-HT	5-Hydroxytryptamine
AK	Adenylate kinase
ATP	Adenosine-5'-triphosphate
CB_1	Cannabinoid 1 Receptor
CPT	Camptothecin
DCM	Dichloromethane
DHFR	Dihydrofolate reductase
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
EGF	Epidermal Growth Factor
FAP	Familial Adenomatous Polyposis
FSH	Follicle Stimulating Hormone
FT	Fourier Transform
G_1	Gap 1 Phase
G_2	Gap 2 Phase
GABA	Gamma-aminobutiric acid
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
H_1	Histamine 1 Receptor
HCC	Hepatocellular Cancer
HCMV	Human Cytomegalovirus

hERG	Human *Ether-à-go-go*-Related Gene
HIV	Human Immunodeficiency Virus
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
IC_{50}	The half maximal inhibitory concentration
IR	Infrared
LDH	Lactate dehydrogenase
LC	Liquid Chromatography
LEV	Levamisole
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
LV	Leucovorin
M	Mitosis Phase
MEM	Methoxyethoxymethyl
MeOH	Methanol
MS	Mass Spectroscopy
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulphophenyl)-*2H*-tetrazolium
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAD	Nicotinamide adenine dinucleotide
NCI	National Cancer Institute
NMDA	*N*-methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
PAF	Platelet Activating Factor

PMS	Phenazine methosulphate
ppm	parts per million
PTC	Phase transfer catalyst
QSAR	Quantitative structure activity relationship
R_f	Retention factor
RNA	Ribonucleic Acid
S	Synthesis Phase
SERM	Selective Estrogen Receptor Modulator
S_Ni	Internal Nucleophilic Substitution
SRB	Sulphorhodamine
TCA	Trichloroacetic acid
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMEDA	Tetramethylenediamine
TMS	Tetramethylsilane
UV	Ultraviolet
WHO	World Health Organisation
X-RAY	X-Radiation
XTT	2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-*2H*-tetrazolium-5-carboxanilide sodium salt

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1. INTRODUCTION AND AIM

Cancer is the disease resulting from abnormal cells with abilities of uncontrolled dividing and invasion to other tissues through blood and lymph systems [1]. Cancer still continues to be the leading cause of deaths worldwide and is expected to increase from 2007 to 2030 at a rate of 45% partly due to the growing and aging population [2].

Sulfur mustards, originally used as chemical warfare agents during World War I, are known to be the first remedy against cancer. Autopsy of a mustard gas exposure case revealed that drug inhibited bone marrow formation (aplasia) leading these agents to be used in treatment of leukemia. In 1942, the first clinical trials with nitrogen mustards took place, hence the era of cancer chemotherapy began.

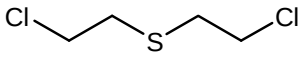


Figure 1.1. Structure of sulfur mustard, bis(2-chloroethyl)sulfide.

Cancer chemotherapeutic agents are intended to cure a specific cancer, reduce the size of a tumor prior to surgery, make tumors sensitive to radiation therapy or to eradicate microscopic metastases after tumors are surgically removed.

As a major problem, cancer chemotherapeutics are basically cytotoxic thus they kill normal and malignant cell types without total selectivity. Rapidly proliferating cancer cells and normal cells take up cytotoxic agents in higher rates than resting cells (G_0). These rapidly proliferating normal cells include hair, bone marrow cells and cells lining the gastrointestinal tract. Therefore common side effects of cytotoxic agents are hair loss, immune system depression together with nausea or diarrhea [3]. Another serious problem in cancer chemotherapy is the development of drug resistance. Initially, most drugs are effective however the tumor cells become non-sensitive to the drug after subsequent administration. Furthermore multidrug resistance to a group of mechanistically distinct antitumor agents has recently evolved [4]. Hence the discovery of novel cytotoxic drugs has always been a hot topic of medicinal chemistry.

Piperazine is a significant heterocycle for lead compounds. There are many studies that describe its features in both chemical and biological aspects. Anticancer activities of piperazine bearing compounds are often reported [5-9], also a well-known anticancer piperazine derivative exists in the market under the name of imatinib, Gleevec® (Fig. 1.1.). Furthermore, thioamide [10-12], carboxamide [13-17], sulfonamide [18-26] and acyl [27-30] moieties are commonly addressed in the literature surveying anticancer compounds.

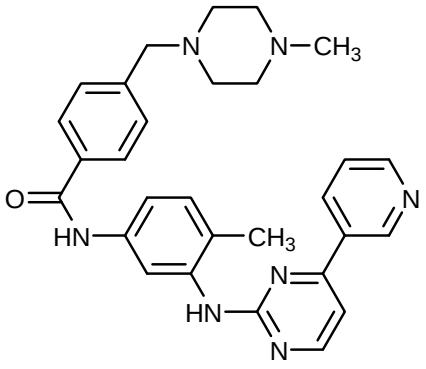


Figure 1.2. Structure of imatinib.

Benzhydrylpiperazines are popular with their antihistaminic activities [31-33], in addition, they are extensively used in treatment of allergies *i.e.* cetirizine, Zyrtec® (Fig. 1.2.). Literature search reveals many other activities of benzhydrylpiperazine derivatives including calcium channel blocking [34-36], dopaminergic [37, 38], antimicrobial (39-41) and antiviral [42, 43] activities.

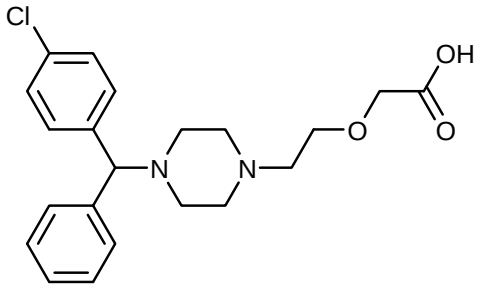
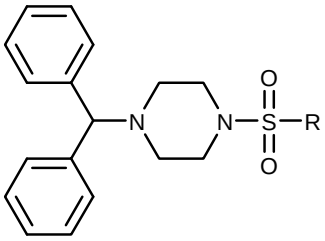
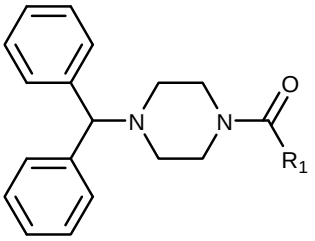


Figure 1.3. Structure of cetirizine.

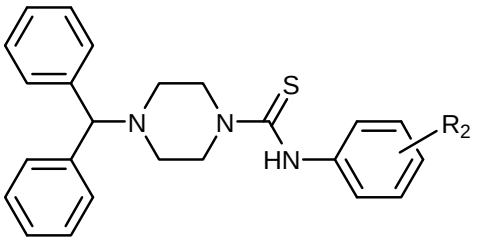
Anticancer activity of benzhydrylpiperazines has recently become important [44-47]. Kumar *et al*. has performed cytotoxicity assays to several 1-benzhydrylpiperazine derivatives substituted with variable sulfonyl chlorides, acid chlorides and isothiocyanates. These derivatives have potent cytotoxicity over breast cancer (MCF-7), hepatocellular carcinoma (HepG-2), cervix carcinoma (HeLa) and colon carcinoma (HT-29) cell lines [44]. This study has urged us to prepare variable benzhydrylpiperazines substituted with isocyanates, isothiocyanates, benzoyl chlorides and sulfonyl chlorides.



R = 4-chloro-2-fluorophenyl; camphoryl; phenyl; 2,2,2-trifluoroethyl



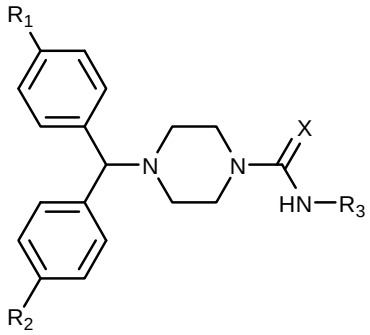
R_1 = isoxazol-5-yl; morpholin-4-yl; pyrrolidinyl; cyclopropyl



R_2 = 2-methoxy; 3-methoxy; 4-methoxy; 2-chloro; 3-chloro; 4-chloro; 4-fluoro; 2,4-dichloro

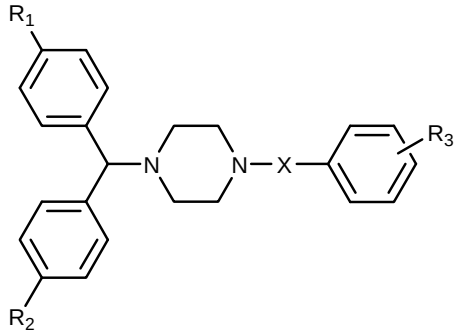
In this study, we reported the synthesis and purification of novel compounds bearing benzhydrylpiperazine backbone coupled with their spectral data and crystal structures. Those compounds are tested for their cytotoxic activities with sulphorhodamine B assay. We aimed to develop a structure activity relationship for benzhydrylpiperazine derivatives in accordance with their cytotoxic activity results.

Table 1.1. Structures of the synthesized compounds



Compound	X	R_1	R_2	R_3	M. P. (°C)	Yield (%)
1*	O	- H	- H	*sec*-Butyl	198.4	68
2	O	- H	- H	*tert*-Butyl	192.4	62
3	O	- H	- H	Isopropyl	220.4	94
4	O	- H	- H	Ethyl	208.9	84
5	O	- H	- H	2,6-Dichlorophenyl	234.6	88
6	O	- H	- H	2-Benzylphenyl	192.1	89
7*	O	- H	- H	Ethylacetato	150.0	69
8*	O	- H	- H	Allyl	213.6	96
9	O	- F	- F	*sec*-Butyl	157.7	54
10	O	- F	- F	*tert*-Butyl	162.4	82
11	O	- F	- F	Butyl	132.9	45
12	O	- F	- F	Ethyl	175	83
13	O	- F	- F	Isopropyl	169.9	92
14	O	- F	- F	Ethylacetato	152.3	20

15	O	- F	- F	4-Bromophenyl	210.9	67
16	O	- Cl	- H	*sec*-Butyl	> 300 (dec.)	62
17	O	- Cl	- H	*tert*-Butyl	190.3	36
18	O	- Cl	- H	Ethyl	288.6 (dec.)	17
19	O	- Cl	- H	Isopropyl	198.6	34
20	O	- Cl	- H	Allyl	172.7	27
21	O	- Cl	- H	2,6-Dichlorophenyl	224.6	38
22	O	- Cl	- H	2-Phenylethyl	147.8	49
23	O	- Cl	- H	4-Bromophenyl	195.5	37
24	O	- Cl	- H	2-Benzylphenyl	174.6	44
25	O	- Cl	- H	4-Cyanophenyl	196.8	26
26	S	- F	- F	*tert*-Butyl	176.8	14
27	S	- F	- F	Cyclohexyl	198.2	50
28	S	- Cl	- H	Ethyl	150.6	15
29	S	- Cl	- H	Isopropyl	252.4 (dec.)	39
30	S	- Cl	- H	Allyl	139.4	10
31	S	- Cl	- H	Benzyl	157.2	23
32	S	- Cl	- H	Butyl	125.5	20



Compound	X	R_1	R_2	R_3	M. P. (°C)	Yield (%)
33	C=O	- H	- H	5-Fluoro-2-methyl	> 300 (dec.)	95
34	C=O	- F	- F	2-Bromo	189.7	35
35	C=O	- F	- F	3-Bromo	151.4	27
36	C=O	- F	- F	4-Bromo	> 300 (dec.)	19
37	C=O	- F	- F	3-Chloro	177.5	47
38	C=O	- Cl	- H	2-Methoxy	120	33
39	C=O	- Cl	- H	3-Nitro	196.1	24
40	C=O	- Cl	- H	3,4-Dimethoxy	148.6	11
41	C=O	- Cl	- H	4-Ethyl	206.4	22
42	SO_2	- H	- H	2-Trifluoromethoxy	205.8	10
43	SO_2	- F	- F	2-Trifluoromethyl	135.6	22
44	SO_2	- F	- F	2,4,5-Trichloro	> 300 (dec.)	11
45	SO_2	- F	- F	3,4-Dichloro	145.1	42
46	SO_2	- F	- F	2-Methyl	117.7	10
47*	SO_2	- F	- F	4-Nitro	224.5	13
48	SO_2	- F	- F	2,5-Dichloro	116.1	23

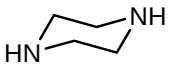
49	SO_2	- Cl	- H	2,4,5-Trichloro	151.1	19
50	SO_2	- Cl	- H	3,4-Dichloro	107.1	25
51	SO_2	- Cl	- H	4-Nitro	209.3	37

(*) Compound 1, CAS No: 1071382-92-7; Compound 7, CAS No: 1350123-57-7; Compound 8, CAS No: 1349487-56-4; Compound 47, CAS No: 1286459-36-6

2. GENERAL DESCRIPTION

2.1. Piperazine

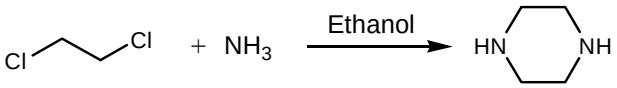
Piperazine prefers a chair conformation having bond lengths 154.0 pm (C–C) and 146.7 pm (C–N) together with bond angles 110° (C–C–N) and 109° (C–N–C). The N–H bonds prefer the equatorial position which also applies to *N*-substituents in *N*-alkyl and *N*,*N*-dialkylpiperazines [48].



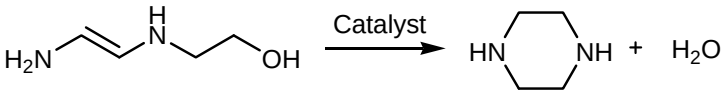
Piperazine shows the properties of a secondary amine and is a weaker base (pKa = 9.8) than piperidine (pKa = 11.2) due to the inductive effect of the second heteroatom [48].

2.1.1. Methods of synthesis

Piperazine was first synthesized by Cloëz *et al*. in 1853 from alcoholic ammonia and ethylene chloride [49].

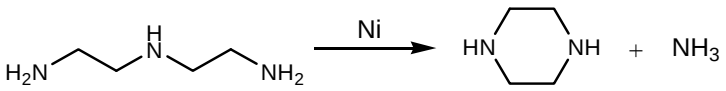


Catalytic cyclodehydration of *N*-(2-hydroxyethyl)ethenediamine was carried out under atmospheric pressure by reflux with various catalysts. Raney nickel was the first catalyst of choice. Autoclave was optionally utilized to perform the reaction at 200-300°C [50].

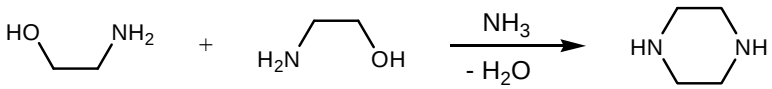


Martin *et al*. synthesized piperazine from diethylenetriamine with Raney nickel catalyst under various experimental conditions. Reaction was carried out in autoclave to increase the yields [51]. In a similar study by Kyrides *et al*., in 1938, piperazine was

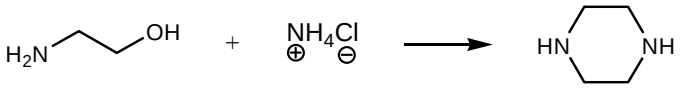
obtained in good yield when nickel catalysts were used at 73 atm pressure in autoclave with temperature as high as 236°C [52].



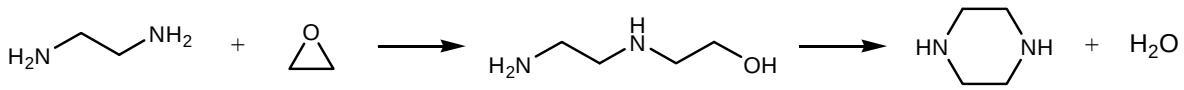
Piperazine is commercially synthesized from 2-aminoethanol in the presence of ammonia at 150-220°C and 100-200 bar [48].



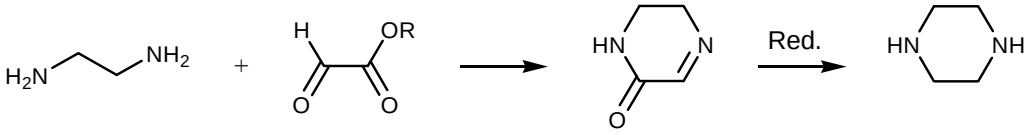
Heating of ethanolamine and ammonium chloride at 250°C also gives piperazine [53].



An alternative commercial synthesis of piperazine is carried out in the presence of ethylenediamine and oxirane [48].



In another method ethylenediamine is reacted with an ester to produce 3,4-dehydropiperazine-2-one, subsequently a reducing agent such as lithium aluminium hydride ($LiAlH_4$), sodium borohydride ($NaBH_4$), aluminium hydride (AlH_3), potassium borohydride (KBH_4) or borane (B_2H_6) is used to obtain piperazine [54].



2.1.2. Spectral Properties of Piperazine

2.1.2.1. UV Spectroscopy

Piperazine has two absorption bands in the UV region at 260 nm (A = 0.035) and 280 nm (A = 0.010) [55].

2.1.2.2. ^{1}H-NMR Spectroscopy

C–H protons of piperazine appear at 2.84 ppm in deuterated chloroform ($CDCl_3$). The difference in the chemical shifts concerning the equatorial and axial protons is 0.16 ppm [48].

2.1.2.3. ^{13}C-NMR Spectroscopy

Piperazine carbon atoms appear at 47.9 ppm in deuterated chloroform ($CDCl_3$) [48].

2.1.2.4. IR Spectroscopy

N-H stretching vibrations of piperazine give sharp singlet approximately at 3250 cm^{-1}. Alicyclic C-H stretching bands appear at 2950-2700 cm^{-1} [55].

2.1.2.5. Mass Spectroscopy

Piperazine.$6H_2O$ mass spectrum shows four main m/z values; 86 (M^+), 56, 44 and 30. Base peak is m/z = 44 which corresponds to the fraction $NHCH_2CH_2]^+$ [56].

2.1.3. Biological Properties of Piperazine and Piperazine Derivatives

Piperazine was first introduced as an anthelmintic drug. Various piperazine compounds have anthelmintic action. The activity is mediated by the agonist effect on the inhibitory GABA (γ-aminobutyric acid) receptor. Piperazine anthelmintic compounds are selective over helminths because GABA receptor of helminth is a different isoform of the vertebrate's [57-59].

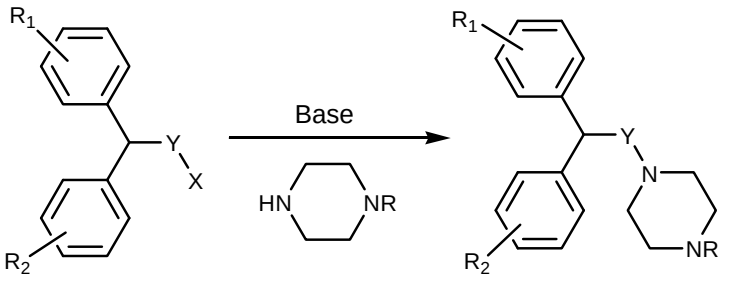
Piperazine derivatives are common drugs and drug candidates with many activities such as antianginal (*i.e.* trimetazidine [60], ranolazine [61]), anxiolytic and antidepressant [62-67] (*i.e.* amoxapine, buspirone), antipsychotic [68-72] (*i.e.* blonanserin, loxapine, perphenazine, clozapine, olanzapine), antihistaminic [73-76] (*i.e.* cyclizine, cinnarizine), antibacterial [77-83] (*i.e.* norfloxacin, ciprofloxacin, levofloxacin) [84, 85], anticancer [5-9, 86-93] (*i.e.* imatinib, dasatinib), anti-inflammatory [94-98] (*i.e.* antrafenine [99]), psychostimulant [100, 101] (*i.e.* benzylpiperazine) [102], anti-erectile dysfunction [103] (*i.e.* sildenafil, vardenafil), antifungal [104-108] (*i.e.* itraconazole, posaconazole, terconazole) and antiparkinson (*i.e.* piribedil [109]), pardoprunox [110]).

2.2. Diphenylalkylpiperazines

2.2.1. Diphenylalkylpiperazine Derivatives

2.2.1.1. Methods of Synthesis

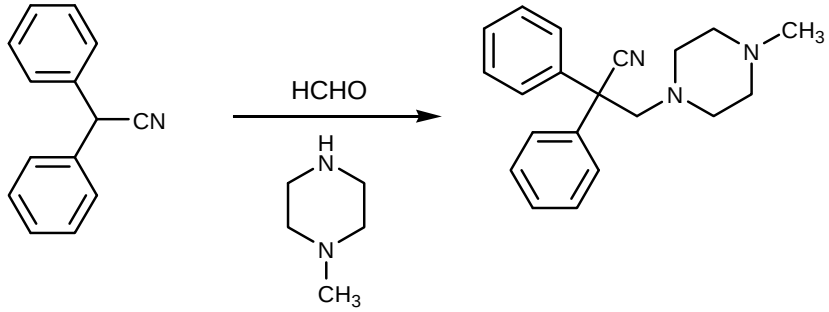
Most of the diphenylalkylpiperazines in the literature are prepared from diphenylalkyl or diphenylacyl halides and appropriate piperazines in basic media and hot environment [111-114].



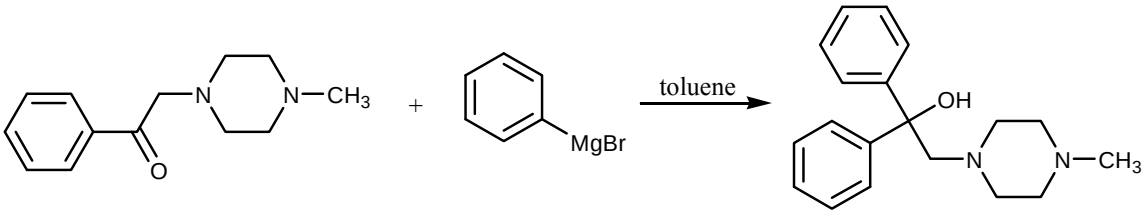
X = Halogen
R_1, R_2 = H, halogen, alkyl, alkoxy, aryl, acyl

Y = $(CH_2)_n$, $(CH_2)_nCO$
R = H, alkyl, acyl

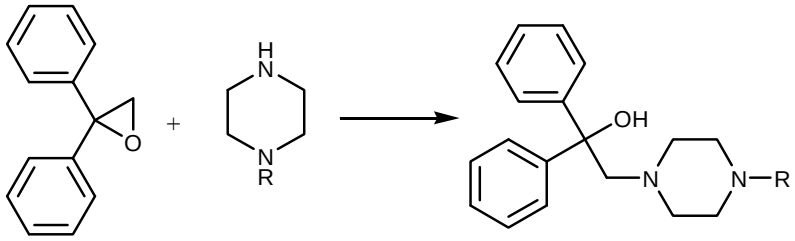
α,α-Diphenyl-β-(4-methyl-1-piperazino)propionitrile was prepared by *Mannich* reaction of diphenylacetonitrile and formaldehyde with *N*-methylpiperazine [115].



N-(β,β-Diphenyl-β-hydroxy)ethyl-*N*'-methylpiperazine synthesis was reportedas *Grignard* reaction of phenylmagnesium bromide and *N*-methyl-*N*'-phenacylpiperazine in toluene [116-118].

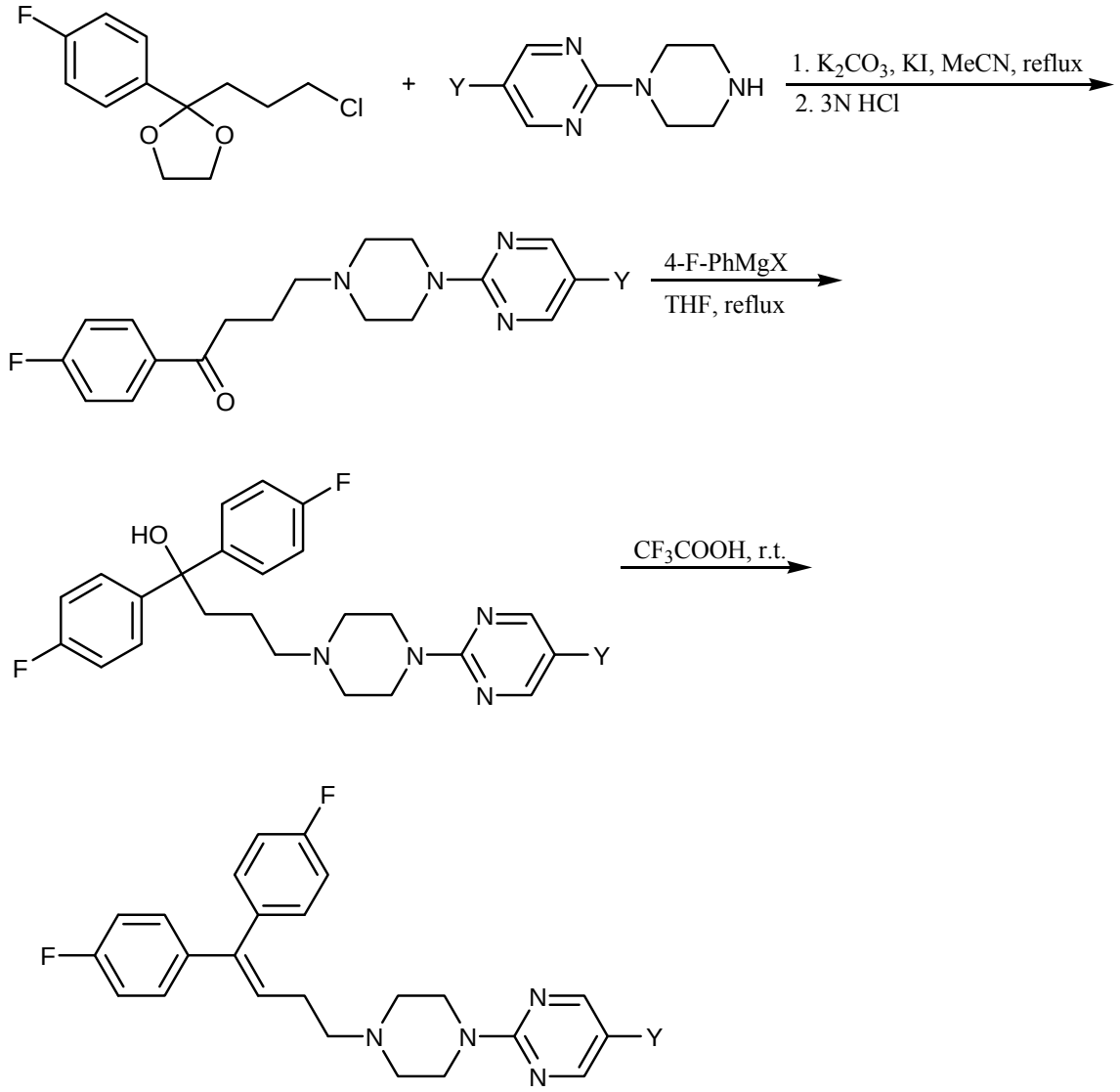


Zaugg and co-workers prepared diphenylalkylpiperazines with the reaction of 1,1-diphenylethyleneoxide and piperazines. They observed formation of the symmetrical by-product which was diminished by using eqimolar quantities of piperazine with 1,1-diphenylethyleneoxide [119].



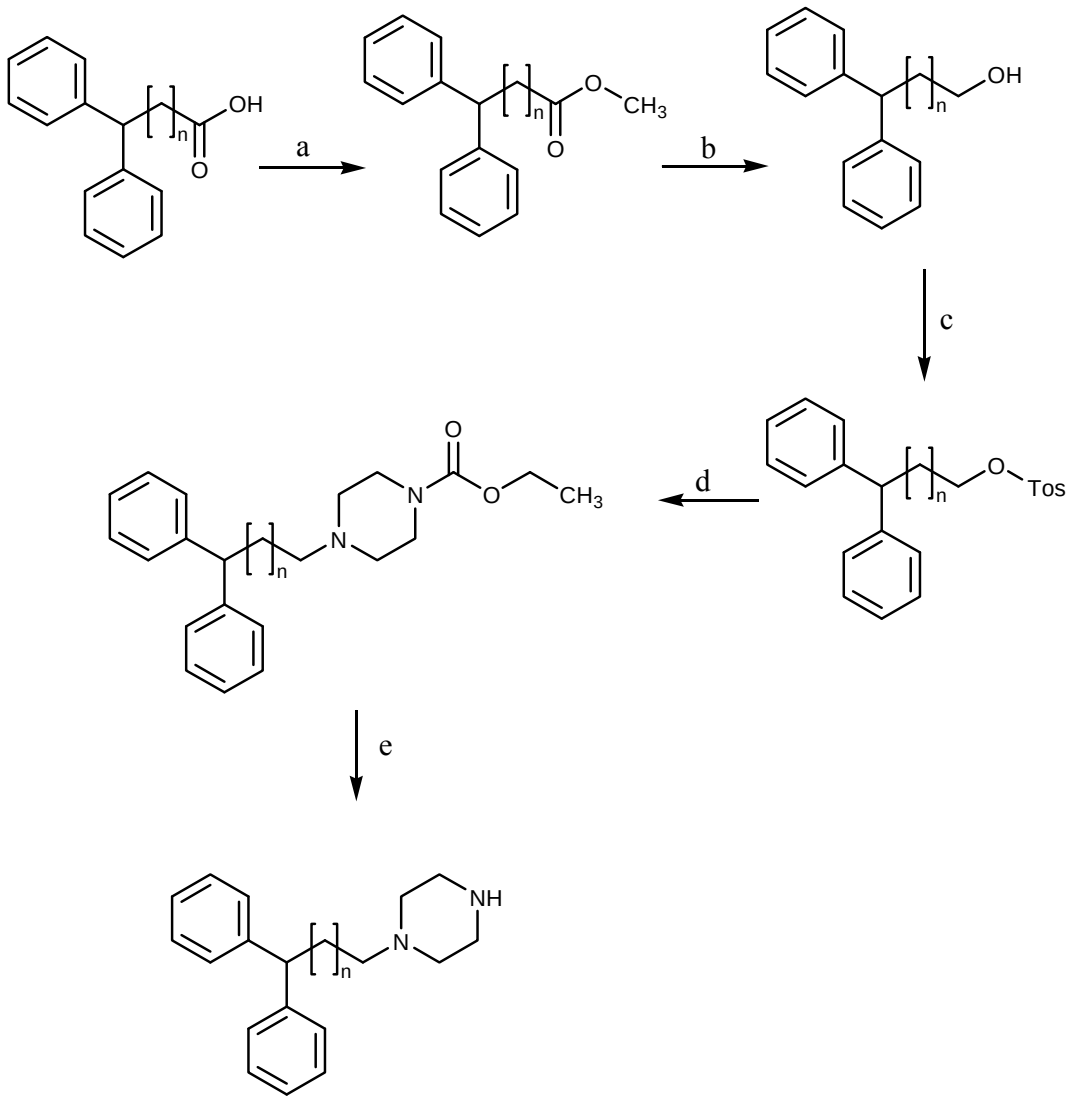
Yevich *et al*. synthesized some diphenylalkylpiperazine derivatives and their olefin analogues, starting from alkylation of 1-(pyrimidine-2-yl)piperazine with the ethylene ketal derivative of γ-chloro-*p*-fluorobutyrophenone to obtain ketone compounds. Reactions of

these ketones with *Grignard* reagents led to the formation of diphenylalkylpiperazine derivatives. Olefin analogues were afforded by the acid-catalyzed dehydration of these compounds [120].



Some diphenylalkylpiperazines were synthesized by reaction of diphenylalkyl-*p*-toluenesulfonates with ethyl *N*-piperazinecarboxylate. At first, Schmidt and co-workers obtained diphenylacetic acid or propionic acid methyl esters. Esters were reduced simultaneously with lithium aluminium hydride to the corresponding alcohols and transferred into tosylates with carbon chain length of 2 and 3. The methyl esters were

reduced to free alcohols and converted to tosylates with carbon chain length of 4 and 5. Related tosylates were converted without solvent and two-fold molar quantity of ethyl *N*-piperazinecarboxylate followed by removal of protection groups with basic hydrolysis yielding the diphenylalkylpiperazine moieties [121].



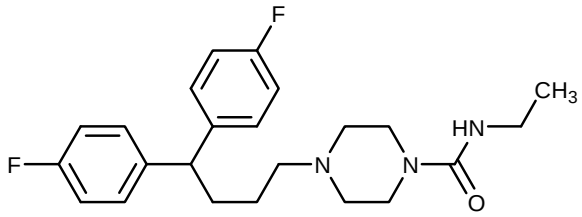
n=0,1

a. MeOH, $H_2SO_4/CHCl_3$, reflux; b. $LiAlH_4/Et_2O$, rt; c. Tos-Cl, TEA/$CHCl_3$, rt; d. Ethyl *N*-piperazinecarboxylate, 100°C; e. KOH/EtOH, H_2O, reflux

2.2.1.2. Biological Properties

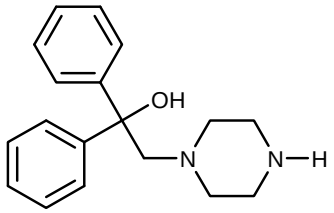
2.2.1.2.1. Central Nervous System Activity

Amperozide is an atypical antipsychotic in the diphenylbutylpiperazine class acting as an antagonist at the 5-HT_{2A} receptor [122].

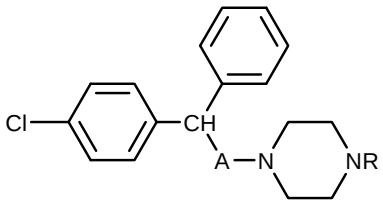


Amperozide

N-(β,β-Diphenyl-β-hydroxyethyl)piperazine was reported to be useful for controlling tremors observed with Parkinson's disease [123].

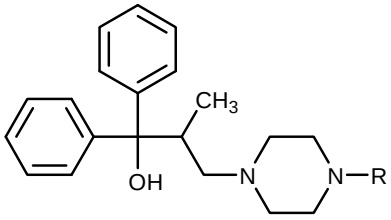


Some *N*-[1-(4-chlorophenyl)(phenyl)]alkyl]piperazine derivatives were synthesized and found to show good to moderate activity as central nervous system depressants [124].



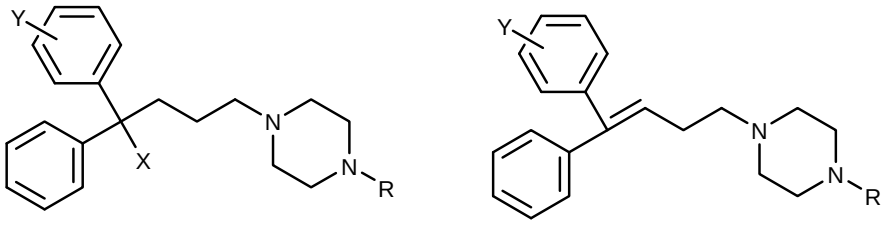
A = CH_2CO, CH_2CH_2, $O(CH_2)_2$, $O(CH_2)_4$, $S(CH_2)_2$, $NH(CH_2)_2$ or $N(CH_3)CH_2CH_2$
R = Alkyl, cycloalkyl, aralkyl, aryl and heterocyclic groups

Various substituted 1,1-diphenyl-3-piperazinylpropanols were prepared and their central nervous system activities were assessed. These compounds showed potent anticholinergic and anticonvulsant activities [125-127].



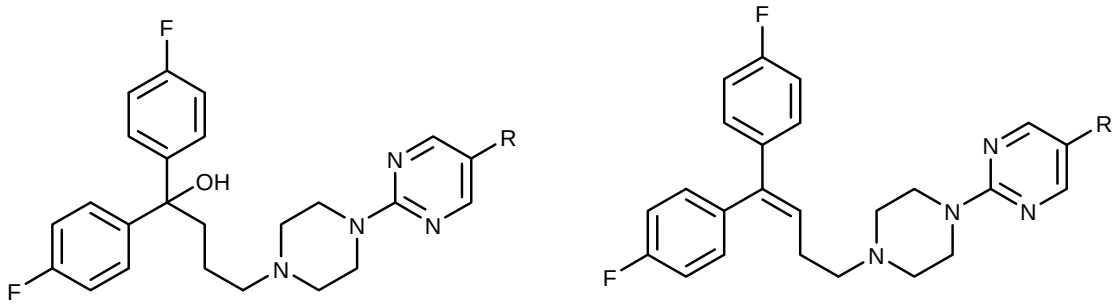
R = Methyl, phenyl, 4-methoxyphenyl, 4-chlorophenyl

Kaiser *et al*. synthesized a group of chlorpromazine analogs bearing diphenylalkyl structure and reported with their good to moderate psychotropic activities [128].



Y = H, 2-Cl, 3-Cl, 2-CF_3, 3-CF_3; X = H, OH; R = CH_3, $(CH_2)_2OCH_2Ph$, $(CH_2)_2OH$

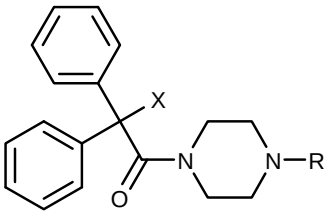
1-(Pyrimidin-2-yl)piperazinebutanol derivatives prepared by Yevich *et al*. showed low psychotropic activity [120].



R = H, F

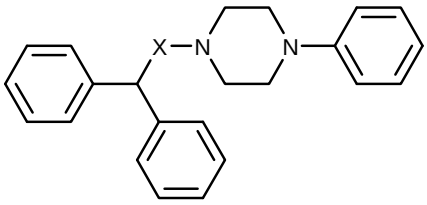
2.2.1.2.2. Cardiovascular Activity

Some diphenylethylpiperazine derivatives have been published for their variable degrees of hypocholesteremic activity [129].



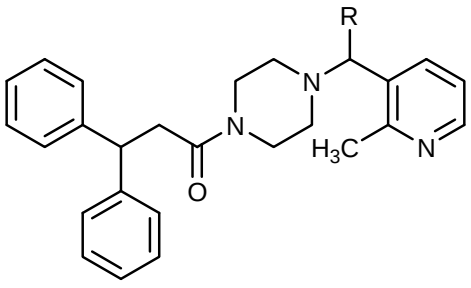
X = H, -OH, -Cl, -OCH_3; R = -H, Methyl, Benzyl

A series of 1,4-disubstituted piperazines were synthesized to investigate their antihypertensive activity, considering diphenylethylpiperazine derivatives, amide bearing compound produced a large unsustained fall in blood pressure [130].



X = CH_2, C=O

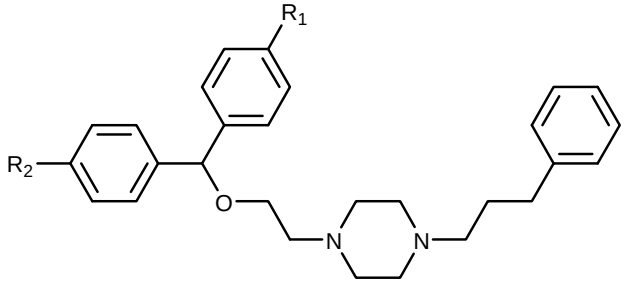
Carceller *et al*. synthesized potent and orally active platelet activating factor (PAF) antagonists bearing diphenylalkylpiperazine moiety [131, 132].



R = -COOEt, $(CH_3)C=N(OCH_3)$, -C≡CH

2.2.1.2.3. Dopaminergic Activity

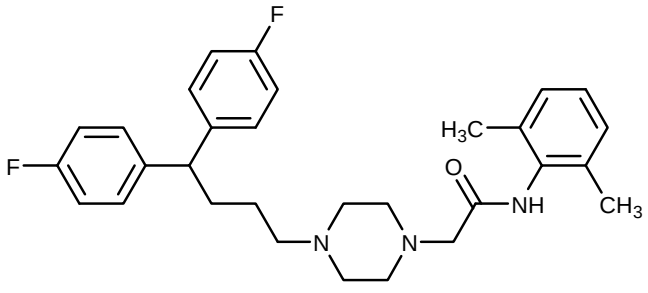
Van Der Zee *et al*., in 1980, prepared some diphenylalkylpiperazine derivatives that are well inhibitory to dopamine uptake. Among these series, GBR12909 (*i.e.* vanoxerine) and GBR12935 were reported as the most potent compounds. Many further studies exist including the derivatives of these inhibitors [133-142].



R_1, R_2 = H; GBR12909
R_1, R_2 = F; GBR12935

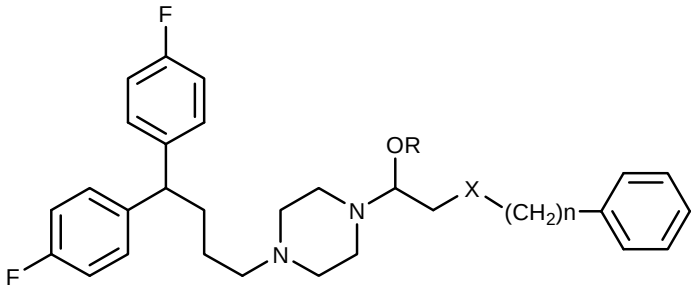
2.2.1.2.4. Calcium Channel Blocking Activity

Lidoflazine is an antianginal calcium channel blocker drug also known as a significant blocker of the hERG K+ channels, which is related to drug induced QT interval prolongation and ventricular arrythmia [143].



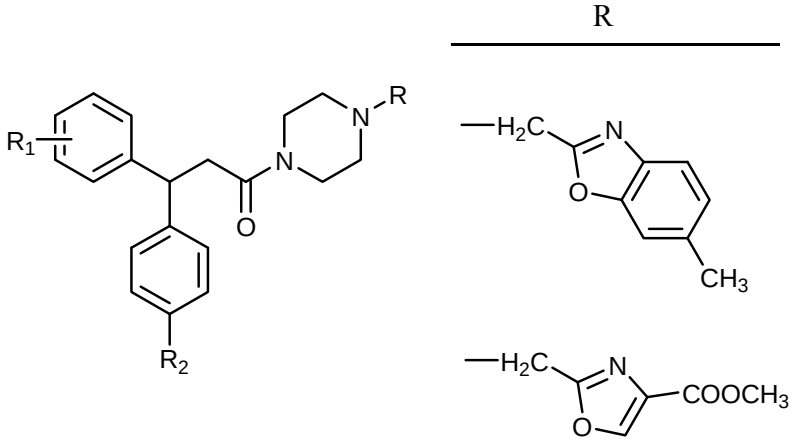
Lidoflazine

Some 4-(4,4-difluorophenyl)butylpiperazine derivatives have been synthesized and found to show high calcium antagonistic activity [144].



n = 0, 1; X = S, $NCOCH_3$, NH, NCH_3; R = H, Methyl, Acetyl

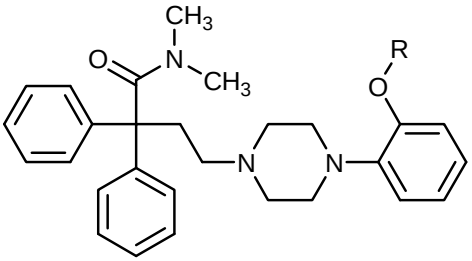
3,3-Diphenylpropanoylpiperazine derivatives have been recently prepared and reported as potent blockers of T-type calcium channel, also known as low voltage activated calcium channel [114].



R_1 = H, 3-F, 4-F; R_2 = F, Cl, Br, CF_3, Methoxy

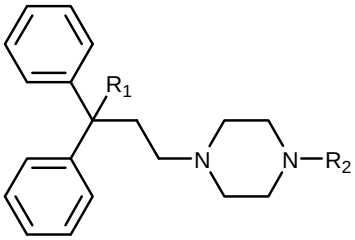
2.2.1.2.5. Other Activities

Highly potent μ-opioid receptor agonist diphenylalkylpiperazines with phenoxyethanol and phenoxyacetic acid moiety have been published [145].



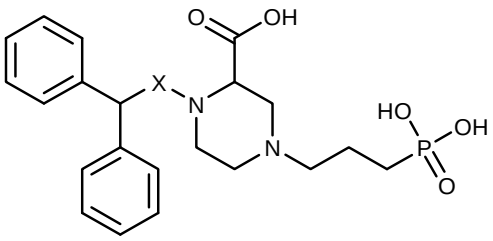
R = -CH_2CH_2OH, -CH_2COOH

Some diphenylpropylpiperazine derivatives have been reported as analgesics [146].



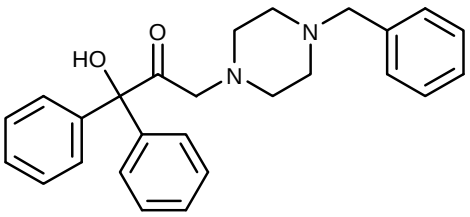
R_1 = H, CN; R_2 = 2-Pyrimidyl, 2-pyrazinyl, 3-pyridazinyl

Some diphenylalkylpiperazines were reported to have low antagonism to *N*-methyl-D-aspartic acid (NMDA) receptor [147].

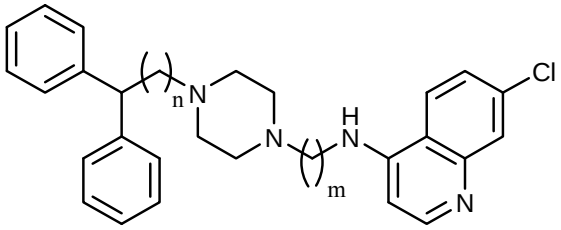


X = $(CH_2)_4$, $(CH_2)_2$, $=CH(CH_2)_2$

3-(4-Benzylpiperazinyl)-1,1-diphenyl-1-hydroxy-2-propanone was synthesized and found to have good antimuscarinic activity [148].

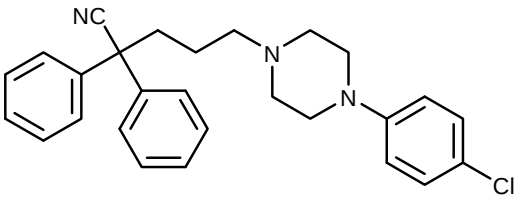


Recently, some chloroquine analogues of benzhydryl and diphenylethylpiperazines with antimalarial activity have been reported by Burgess *et al* [149].

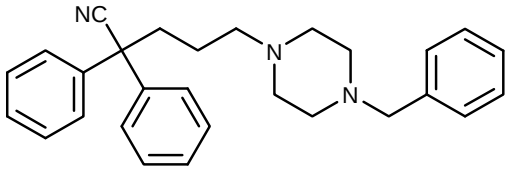


n = 0, 1; m = 3, 4

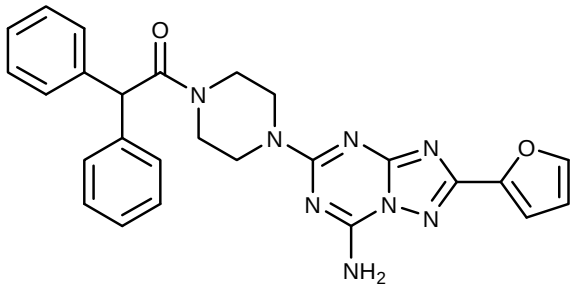
Low inverse agonistic activity of human cytomegalovirus (HCMV) encoded chemokine receptor has been reported for a diphenylalkylpiperazine derivative [150].



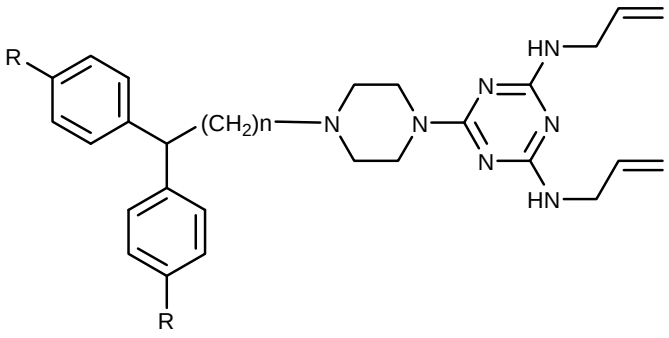
De Lucca *et al*. studied some diphenylalkylpiperazines and reported their moderate CC chemokine receptor-3 (CCR3) antagonism [151].



A potent human A_{2A} adenosine receptor antagonist of diphenylalkylpiperazine has been reported [152].



Some diphenylalkylpiperazines were found to be active as multidrug-resistance modulator [153].

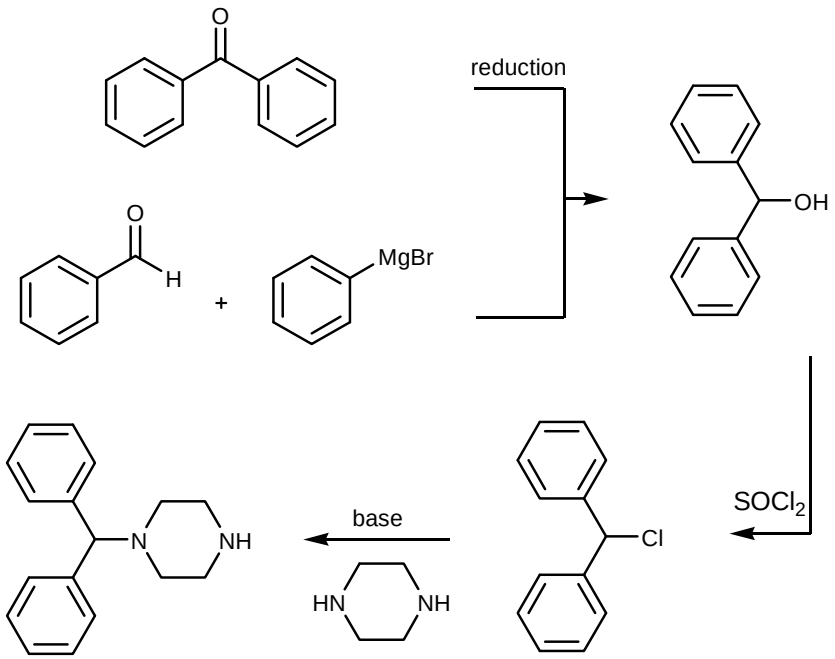


R_1 = -H, -F, -Cl ; n = 0, 2, 3

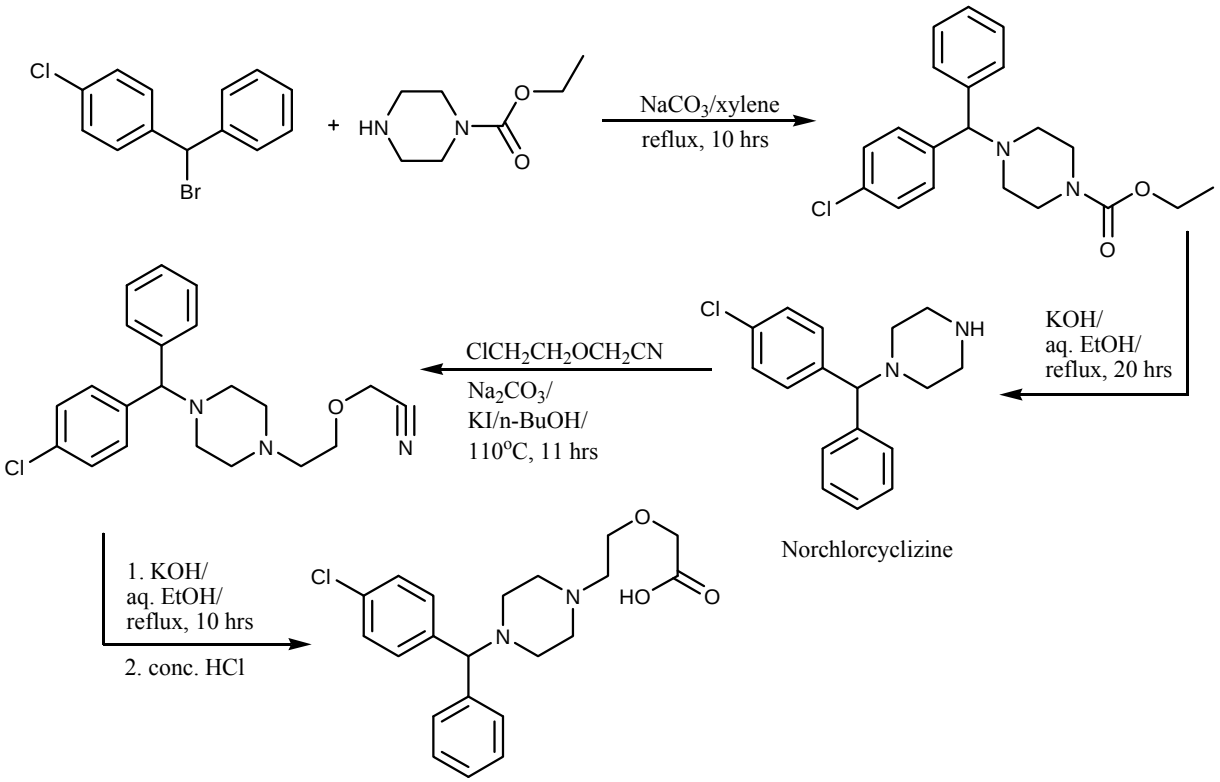
2.2.2. Benzhydrylpiperazines

2.2.2.1. Methods of Synthesis

Benzhydrole derivatives are generally prepared with *Grignard* reagents and benzaldehydes or by reduction of benzophenones. Further halogenation of the alcohol is provided with thionyl chloride or hydrochloric acid. Piperazine is later treated with benzhydryl chlorides in hot and alkali medium [42, 154-159].

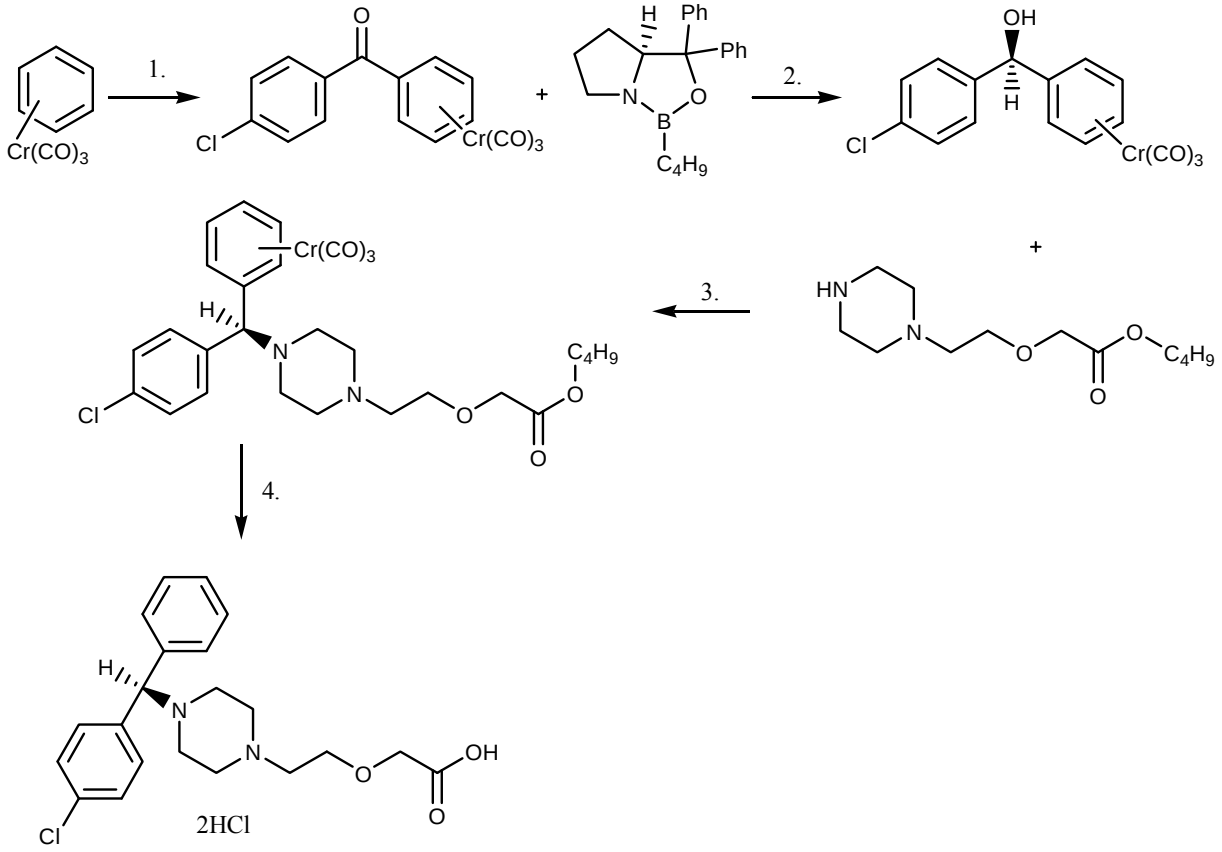


Preparation of antihistaminic drug cetirizine was first described in a patent application. 1-[(4-Chlorophenyl)(phenyl)methyl]piperazine was synthesized by alkylation of the *N*-ethoxycarbonylpiperazine with 4-chlorobenzhydryl bromide and subsequent hydrolysis of piperazine intermediate. Treatment of norchlorcyclizine with 2-(2-chloroethoxy)acetonitrile in the presence of sodium carbonate, potassium iodide and *n*-butanol at 110°C for 11 hours afforded nitrile bearing derivative which was hydrolysed to yield cetirizine [160].



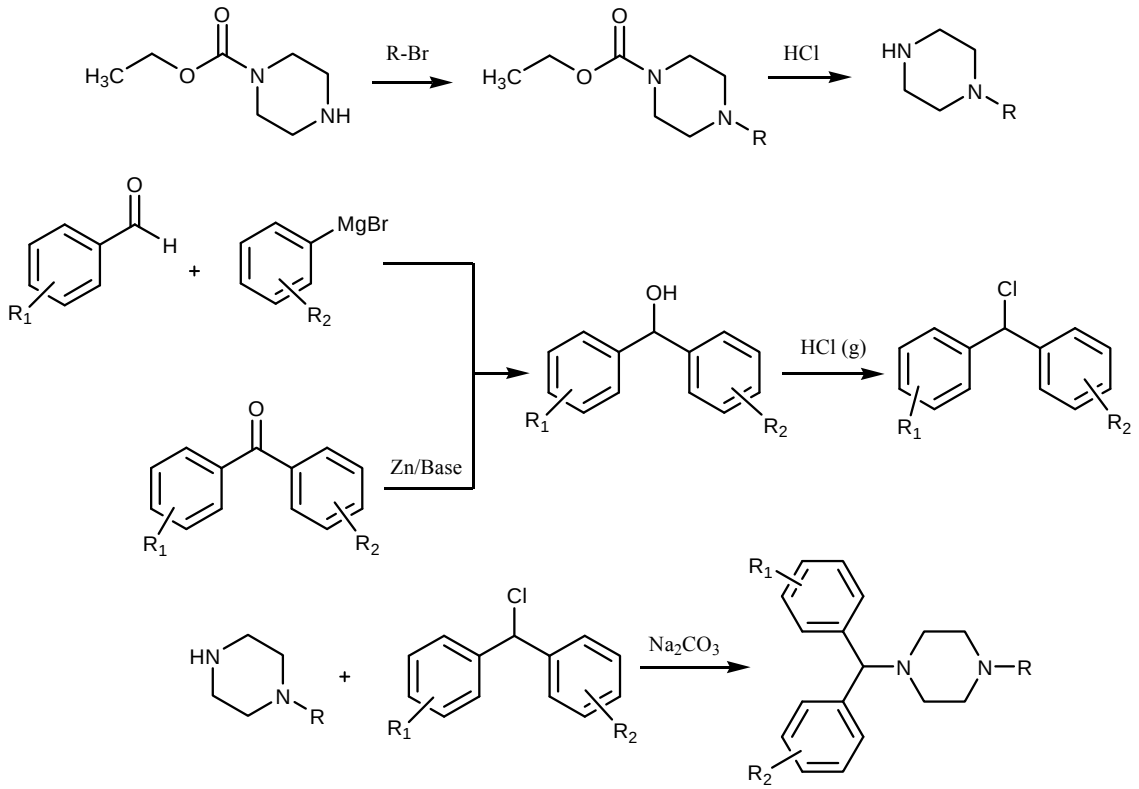
Cetirizine

Synthesis of chiral derivative, (*S*)-levocetirizine was also reported (160-163). According to the literature, lithiation reaction of benzene chromium tricarbonyl with *n*-butyllithium, followed by copper (I) bromide - dimethylsulphide complex and *p*-chlorobenzoyl chloride yielded a ketone intermediate. Addition of ketone to catecholborane at -78°C afforded benzhydrole structure. Optically active benzhydrylpiperazine analog was formed by an instantaneous reaction with piperazine susbtituents in tetrafluoroboric acid-diethyl ether mixture at -60°C. The resulting compound was refluxed and hydrolysed in hydrochloric acid to obtain (-)-cetirizine hydrochloride [160].



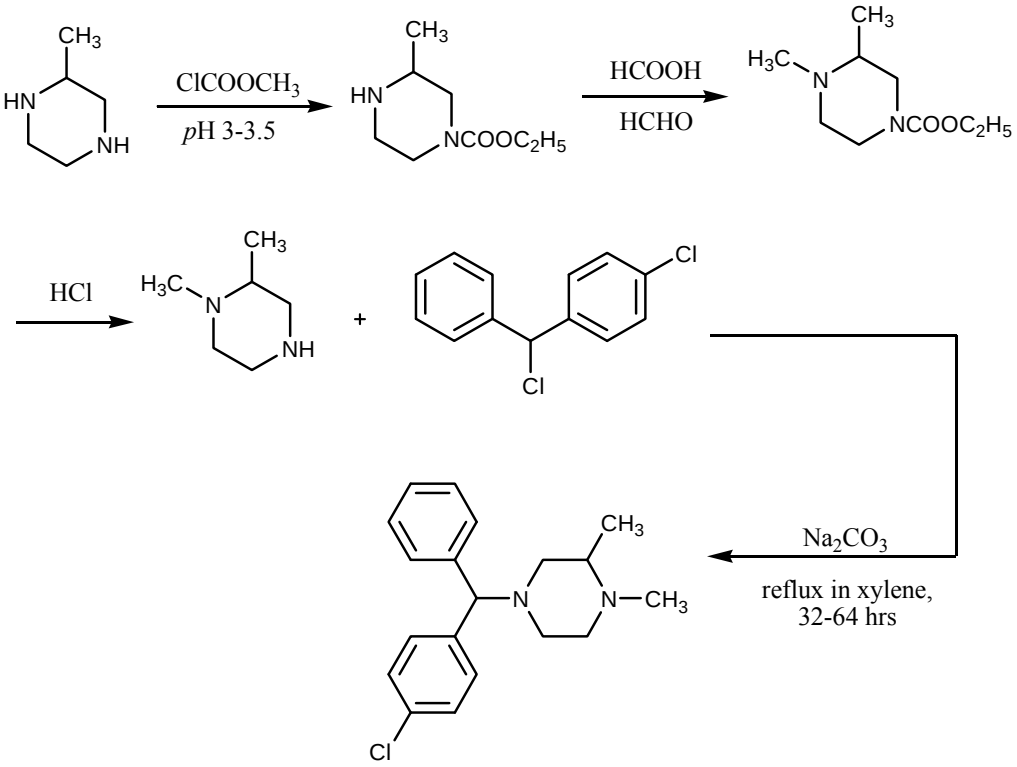
1.a. *n*-BuLi/TMEDA/THF; 1.b. CuBr-Me_2S/THF; 1.c. *p*-ClC_6H_4COCl/THF 78%
2. Catecholborane/toluene 99%; 3. HBF_4-Et_2O/CH_2Cl_2; 4.a. Pyridine 92%; 4.b. 2M HCl 86%

Hamlin *et al*. prepared chlorocyclizine and some 4-substitutedbenzhydryl-piperazines from 1-carbethoxypiperazine. Reaction was started from the addition of alkyl halides to 1-carbethoxypiperazine. Then, deprotection of the compound gave *N*-alkylpiperazine. As a second step, Hamlin and co-workers synthesized benzhydryl chlorides from benzhydroles which was prepared in two distinct pathways. The researchers either preferred *Grignard* addition of phenylmagnesium bromide derivatives with appropriate benzaldehydes, or benzophenone reduction by zinc in alkali medium. Benzhydroles were treated with hydrochloric acid gase in the presence of anhydrous calcium carbonate for the synthesis of benzhydryl chlorides. Subsequently, prepared benzhydryl chlorides were added to the substituted piperazines using sodium carbonate as acid binding agent [154].

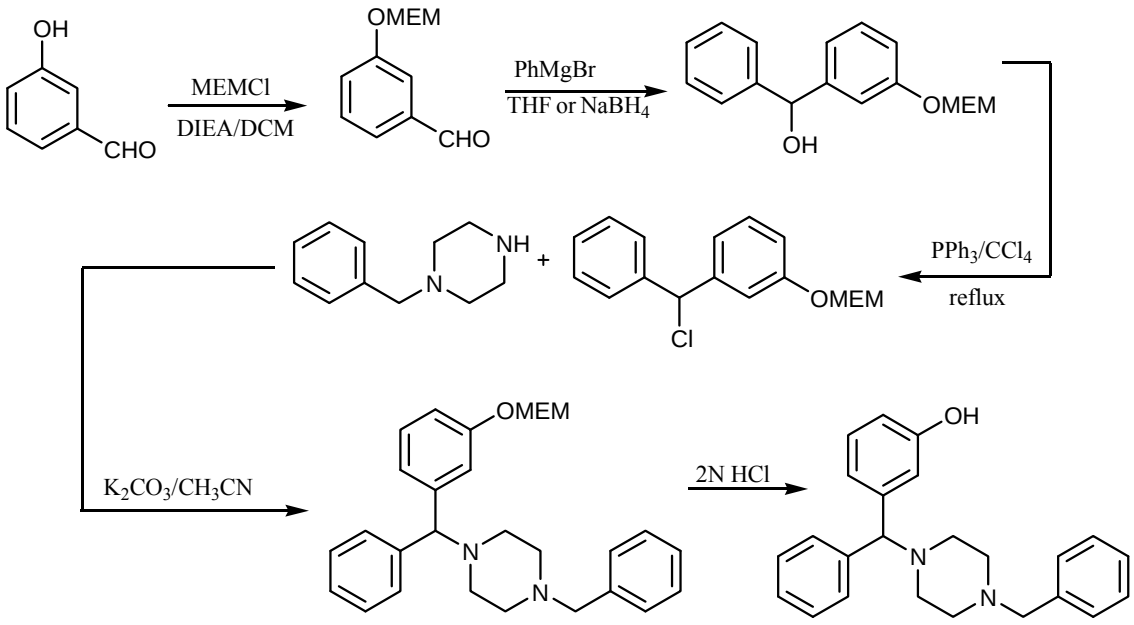


R = alkyl ; R_1, R_2 = I, Cl, Br, F, methyl, methoxy

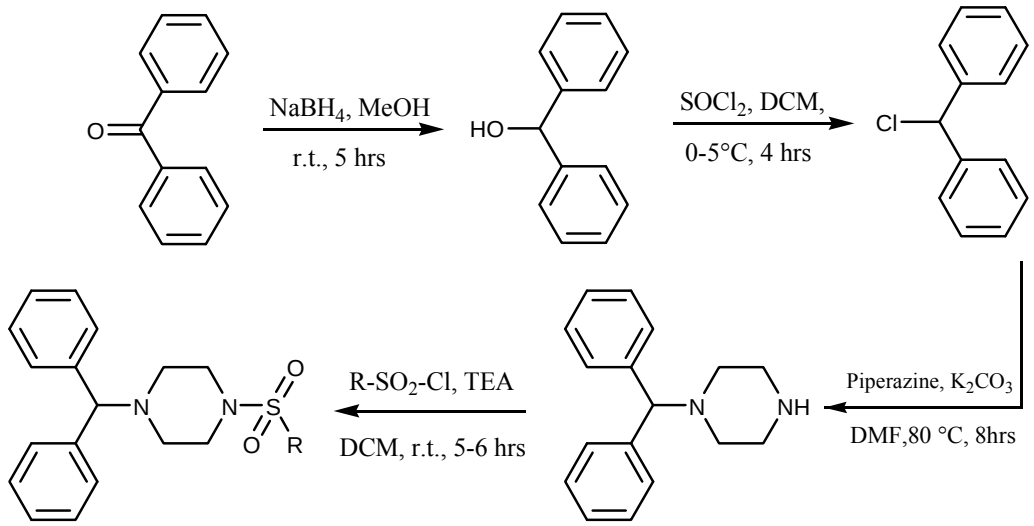
Chlorocyclizine analogs with various methyl substitutions on piperazine ring were synthesized with *Eschweiler-Clarke* methylation after carbethoxy protection on piperazine [164].



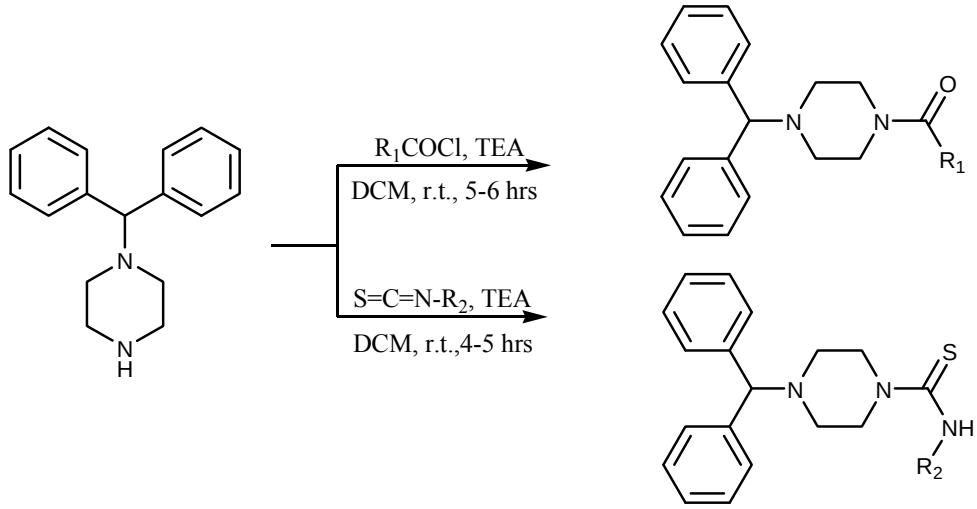
1-(3-Hydroxybenzhydryl)-4-benzylpiperazine was synthesized according to following description. The hydroxyl group of 3-hydroxybenzaldehyde was protected as the methoxyethoxymethyl ether (MEM). The aldehyde was later treated with corresponding *Grignard* reagents. The formed alcohol group was converted to chloride under neutral conditions in the presence of triphenylphosphine. Following treatment with 1-benzylpiperazine in the presence of potassium carbonate by reflux produced piperazine derivative. In order to remove the protecting group (MEM), compound was subjected to hydrochloric acid in methanol/dioxane mixture to yield the corresponding compound as hydrochloride salt [165].



Kumar *et al*. synthesized the benzhydrol intermediate by reduction of benzophenone with sodium borohydride and reported to obtain the product in good yields. Benzhydrol was then treated with thionyl chloride to collect benzhydryl chloride which was reacted with piperazine and anhydrous potassium carbonate in dimethylformamide at 80°C to afford 1-benzhydrylpiperazine. The researchers prepared sulfonyl derivatives by nucleophilic substitution reactions with different sulfonyl chlorides in the presence of triethylamine and dichloromethane as solvent [166].

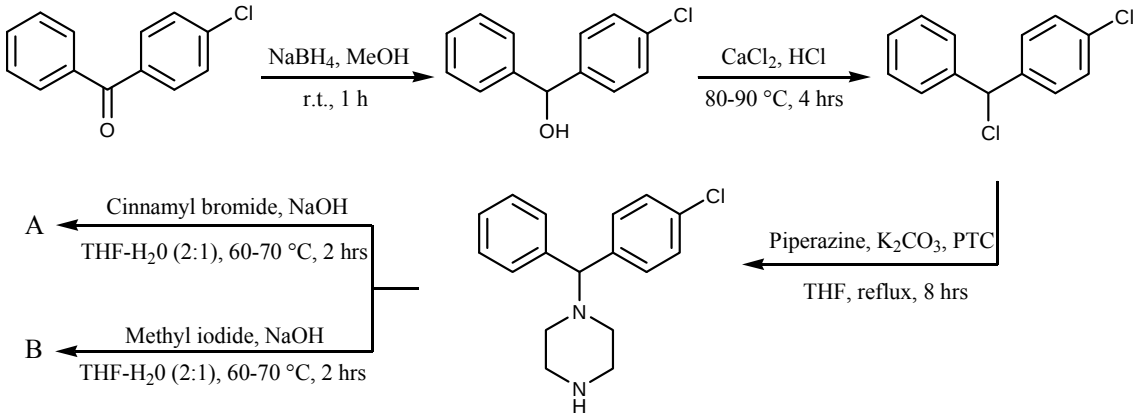


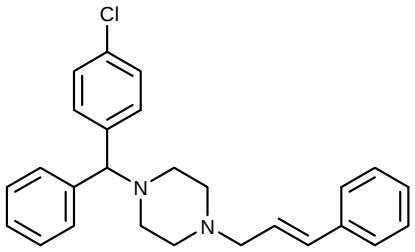
Kumar *et al*. later synthesized the acyl and thioamide derivatives from 1-benzhydrylpiperazine which is treated subsequently with acid chlorides and isothiocyanates in the presence of triethylamine and dichloromethane [44].



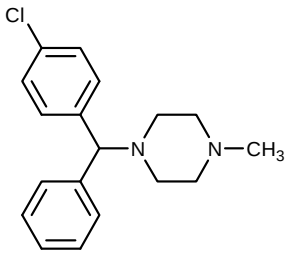
An efficient synthesis of derivatives clocinizine and chlorcyclizine, known as first generation antihistaminic agents, has recently been reported by Venkat Narsaiah *et al*. According to the literature, starting material 1-[(4-chlorophenyl)(phenyl)]methanone was

treated with sodium borohydride in methanol at room temperature to obtain the alcohol derivative, 1-[(4-chlorophenyl)(phenyl)]methanol which was reacted with hydrochloric acid in the presence of calcium chloride to afford the corresponding compound, 1-[(4-chlorophenyl)(phenyl)]methyl chloride. This compound was reacted with piperazine in the presence of potassium carbonate and phase-transfer catalyst, tetrabutylammonium iodide to yield 1-[(4-chlorophenyl)(phenyl)methyl]piperazine. In order to obtain clocinizine, piperazine compound was treated with cinnamyl bromide and sodium hydroxide in THF-water mixture at 60-70°C. On the other hand, to afford the chlorcyclizine, piperazine derivative is treated with methyl iodide at the same conditions with clocinizine [167].





A: Clocinizine



B: Chlorcyclizine

2.2.2.2. Spectral Properties

2.2.2.2.1. ^{1}H-NMR Spectra

N-H proton of 1-benzhydrylpiperazine gives peak at 2.2 ppm [168]. Piperazine ring shows two broad singlets at 2.8-2.9 ppm and 2.41-2.60 ppm respectively, with integration of 4 hydrogens each. Diphenylmethyl C-H gives a singlet at 4.32-4.62 ppm. 1-Benzhydryl-piperazine derivatives present phenyl rings as a triplet at 7.16 ppm of 2 hydrogens, a triplet at 7.25 ppm of 4 hydrogens with a doublet at 7.4 ppm of 4 hydrogens [159, 169].

Piperazine protons of 1-[bis(4-fluorophenyl)methyl]piperazine derivatives give two broad singlets at 2.00 and 3.50 ppm of 4 hydrogens each. Diphenylmethyl C-H gives a singlet at 4.15-5.15 ppm. Phenyl protons have multiplet peaks at 7.15-7.40 ppm of 8 hydrogens [170, 171].

Piperazine protons of 1-[(4-chlorophenyl)(phenyl)methyl]piperazine derivatives has two broad singlets at 2.55 and 3.55 ppm of 4 hydrogens each. Diphenylmethyl C-H gives a singlet peak at 4.25-5.2 ppm and aromatic rings have multiplet peaks at 7.15-7.35 ppm of 9 hydrogens [41, 167, 172].

N-H hydrogen of benzhydrylpiperazine carboxamide derivatives gives peak at 4.21 ppm [173].

2.2.2.2.2. ^{13}C-NMR Spectra

Piperazine carbon atoms of 1-benzhydrylpiperazine derivatives give peaks at 50-54.0 ppm, diphenylmethyl carbon atom gives peak at 75-85 ppm [170, 174].

In a similar manner, 1-[bis(4-fluorophenyl)methyl]piperazine derivatives give peaks at 52.5 ppm and 54.0 ppm that belong to piperazine and a peak at 84.5 ppm regarding diphenylmethyl carbon atom [170].

2.2.2.2.3. IR Spectra

1-[(4-Chlorophenyl)(phenyl)methyl]piperazine N-H stretching vibrations appear at 3422 cm^{-1} [167].

C=O stretching vibrations of benzhydrylpiperazine carboxamide appear at 1630-1670 cm^{-1}. Benzhydrylpiperazine sulfonamide O=S=O presents asymmetric stretching frequency at 1350-1370 cm^{-1} combined with symmetric stretching frequency at 1270-1290 cm^{-1} [169].

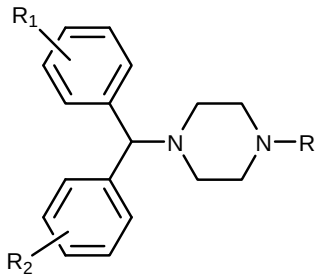
2.2.2.2.4. Mass Spectra

1-[(4-Chlorophenyl)(phenyl)methyl]piperazine molecular ion has two peaks at 287 (60%) and 289 (30%) m/z, in detail relative abundancy values represent the existence of chloride. 4-Chlorobenzhydryl moiety gives the base peak at 201 m/z [167].

2.2.2.3. Biological Properties

Most of the benzhydrylpiperazines in therapy belong to the antihistaminics family. Cyclizine, chlorcyclizine, meclizine, buclizine and cinnarazine are counted in first generation antihistaminics whereas hydroxyzine, cetirizine and oxatomide are known as second generation antihistaminics. Especially, the first generation compounds have been used for motion sickness, since they can act as central anti-emetics. Additionally, antihistaminic benzhydrylpiperazines are familiar for their anticholinergic side effects and drowsiness.

Table 2.1. Therapeutically used drugs bearing benzhydrylpiperazine structure



Compound	R_1	R_2	R
Cyclizine	-H	-H	methyl
Chlorcyclizine	-H	4-Cl	methyl
Meclizine	-H	4-Cl	3-methylbenzyl
Buclizine	-H	4-Cl	4-(*tert*-butyl)benzyl
Cinnarazine	-H	-H	1-(phenyl)propen-3-yl
Hydroxyzine	-H	4-Cl	2-(2-hydroxyethoxy)ethyl
Cetirizine	-H	4-Cl	2-(carboxymethoxy)ethyl
Oxatomide	-H	-H	3-(1,3-dihydro-*2H*-benzimidazol-2-on-1-yl)propyl
Flunarizine	4-F	4-F	1-(phenyl)propen-3-yl
Lomerizine	4-F	4-F	2,3,4-trimethoxybenzyl
Tamolarizine	-H	-H	[2-(3,4-dimethoxyphenyl)-2-methyl]ethyl
Lifarizine	-H	-H	[(4-methyl-2-phenyl)imidazole-5-yl]methyl

Second generation antihistaminic drugs are devoid of side effects connected to central nervous system. Hydroxyzine is metabolically oxidized to its carboxyl derivative cetirizine, that is polar and amphoteric, *i.e.* able to form a zwitterion having both the tertiary amine and carboxylic acid functional group. Advantageously, the drug can not pass the blood-brain barrier in turn sedative effects are decreased [175, 176].

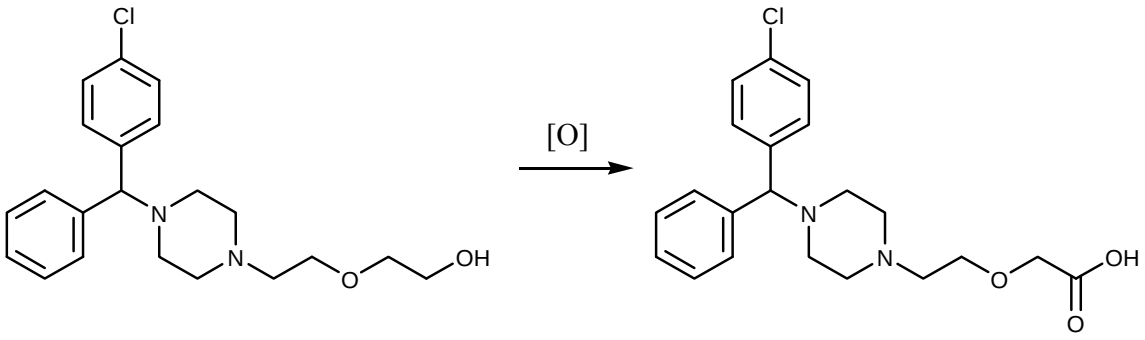


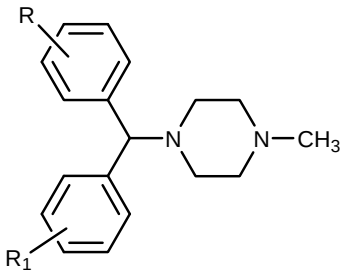
Figure 2.1. Oxidative biotransformation of hydroxyzine to cetirizine.

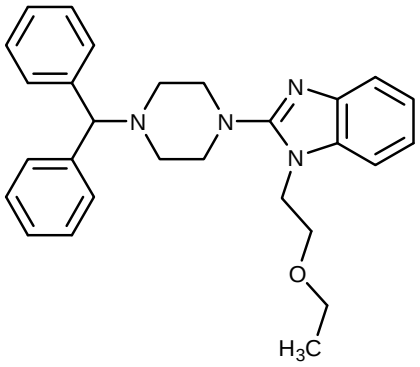
Cetirizine is highly selective for H_1 receptors. No cardiotoxicity has been reported however, some drowsiness is common. Cetirizine is marketed as a racemic mixture of levocetirizine and dextrocetirizine. Its binding affinity, pKi, over human H_1 receptor is 8.2, whereas hydroxyzine has binding affinity of 8.7 and for levocetirizine this value appears to be 8.5. Optically pure levorotatory (-)-cetirizine is also reported to provide treatment lacking adverse effects such as sedation, somnolence, headache, gastrointestinal disturbance, anticholinergic effects, dizziness, cardiac arrythmias and other cardiovascular effects observed with administration of racemic mixture of cetirizine [160, 176, 177].

Other benzhydrylpiperazine agents used in therapy are calcium channel blockers, *i.e.* flunarizine, lomerizine, tamolarizine and lifarizine [34, 144, 178]. Cinnarazine and oxatomide also have calcium channel blocker activity in addition to their antihistaminic actions [175, 176].

2.2.2.3.1. Antihistaminic Activity

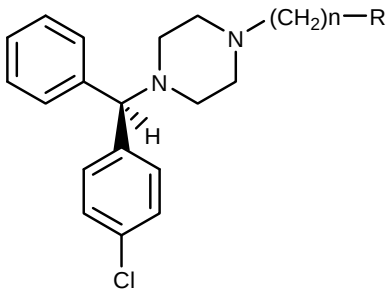
Many benzhydrylpiperazines are documented for their good antihistaminic activities [31-33, 164, 179].





R = H; 2-Cl; 3-Cl; 4-Cl; 2-Br; 4-Br; 4-CH_3; 2-OCH_3; 4-OCH_3; 3-OC_2H_5; R_1 = H; 4-Cl; 4-Br;

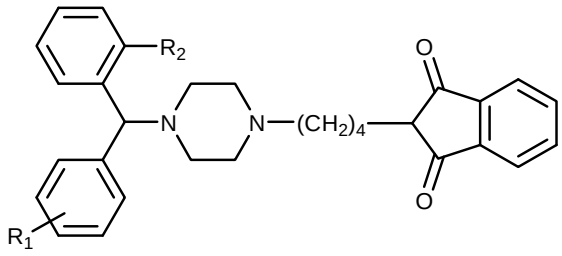
Currently, piperazine H_1 receptor antagonists with higher affinity to H_1 receptors than histamine are often clinically used in treatment of allergies. Among these drugs levocetirizine is mostly known for its low side effects and high potency. There is an extensive research on series of levocetirizine derivatives to be screened for potential antihistaminic activites [180].

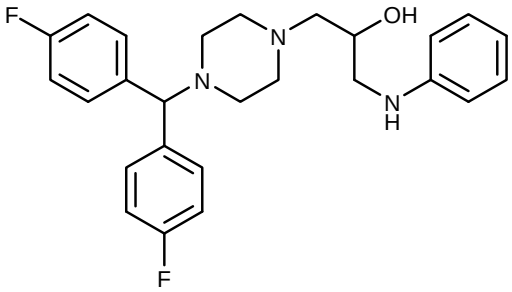


n= 2,3; R = sulfonamides

2.2.2.3.2. Dopaminergic Activity

Many benzhydrylpiperazines in the literature have good dopaminergic activity [37, 38, 137, 181].

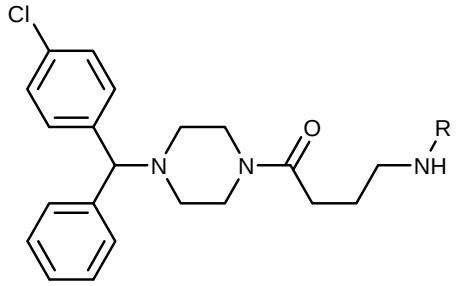




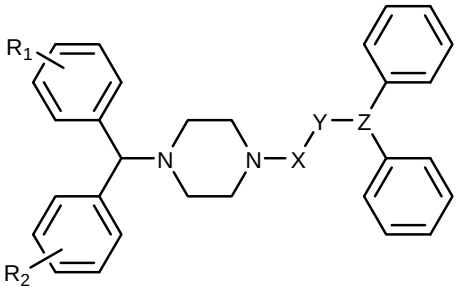
R_1 = H, 4-Cl, 2-OCH_3
R_2 = H, -OCH_3

2.2.2.3.3. Calcium Channel Blocking Activity

Flunarizine, lomerizine, tamolarizine and lifarizine were mentioned previously as calcium channel blockers in therapy. Benzhydrylpiperazine derivatives exhibit a large area in the search of potent calcium channel blockers [34-36, 144, 182-185].



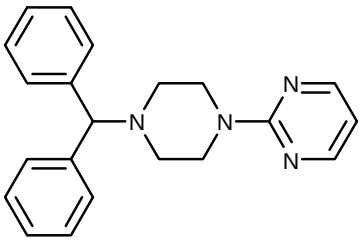
R = -H, alkyl, substitutedbenzyl



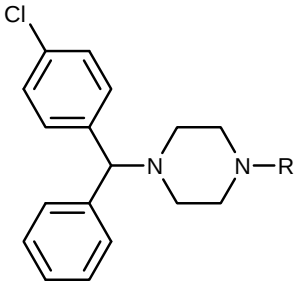
R_1 = H; R_2 = H, halogen, methyl;
X = CO, CH; Y = NH, CO, CH;
Z = CH, N

2.2.2.3.4. Cardiovascular Activity

Regnier *et al*. synthesized a benzhydrylpiperazine derivative carrying a pyrimidyl ring and reported that the structure owned vasodilator action [186].

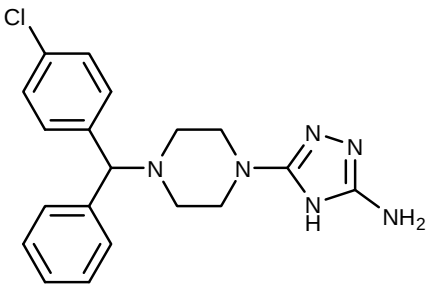


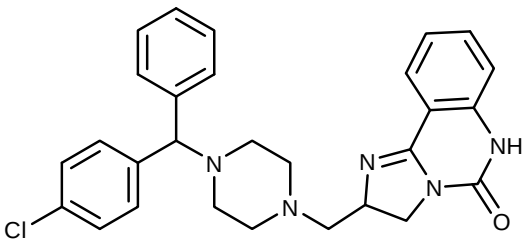
Several *N*-[(4-chlorophenyl)(phenyl)methyl]piperazine derivatives were published as moderately acting hypocholesteremic agents [129].



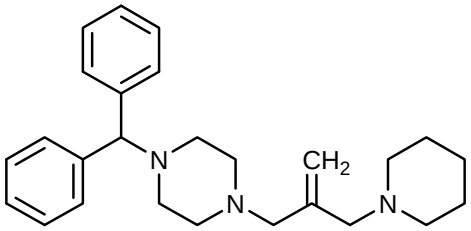
R = -CH_2CN; -$CH_2CH_2NH_2$; -CH_2CONH_2; -$COCH_3$; -$COCH_3Cl$;
-COC_6H_5; -$CH_2COOC_2H_5$; -CH_3; -$CH_2CH{=}CHC_6H_5$

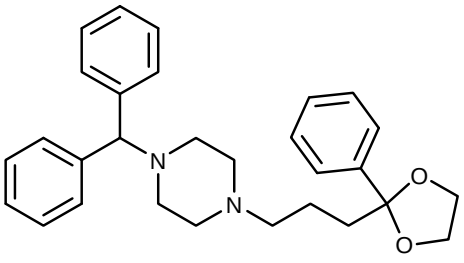
N-[(4-chlorophenyl)(phenyl)methyl]piperazine derivatives were reported to have moderate antihypertensive activities [187, 188].

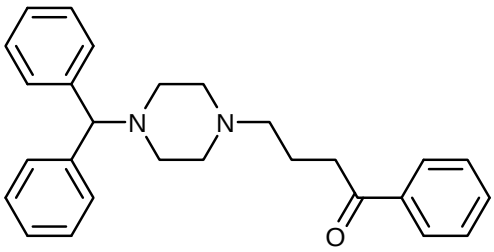




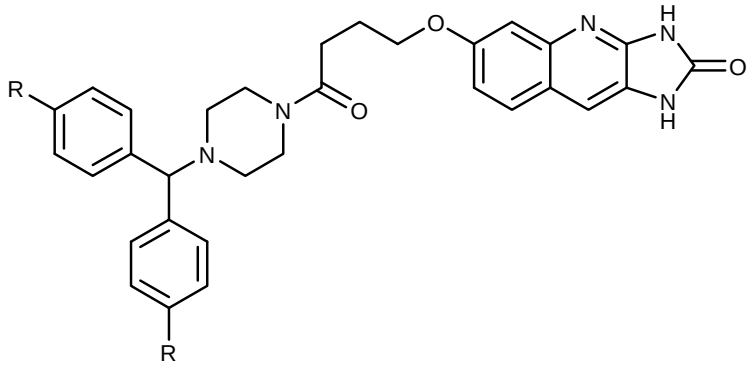
Some benzhydrylpiperazine derivatives were found to increase cerebral blood flow [189].





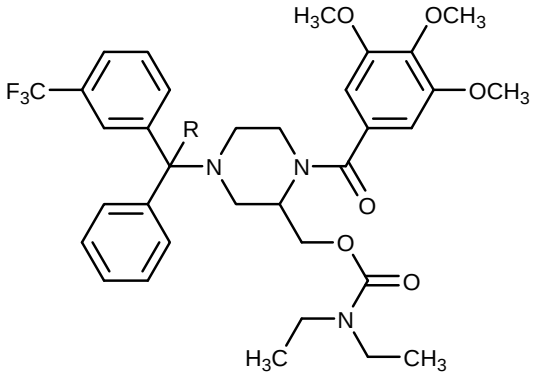


Some benzhydrylpiperazines were reported to inhibit blood platelet cAMP phosphodiesterase in nanomolar range [190].



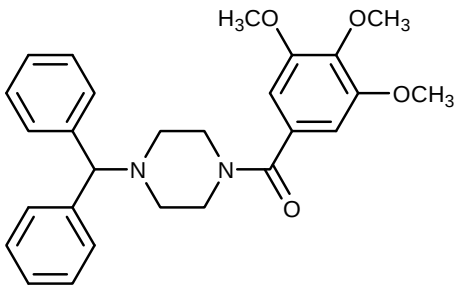
R = -H, -F

Some benzhydrylpiperazine carboxamides have been published for their high platelet activating factor inhibition (anti-PAF) property [191].

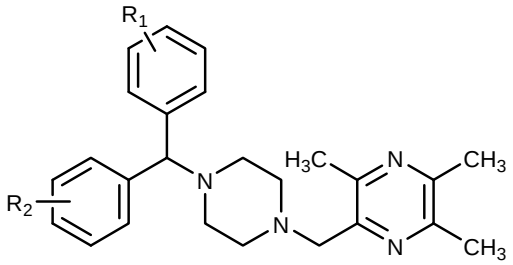


R = -H, -CN

Heymans *et al*. also reported a benzhydrylpiperazine derivative to have platelet activating factor antagonistic (anti-PAF) activity [192].

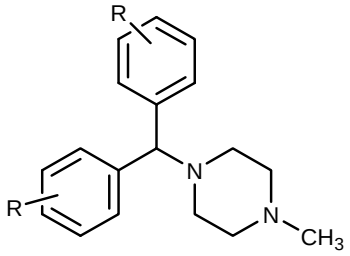


Several benzhydrylpiperazine derivatives were found to be highly active for protecting damaged human umbilical vascular endothelial cells (ECV-304 cells) [193].

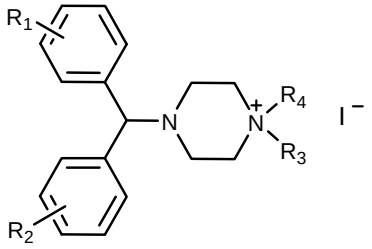


R_1 = -H, 4-F, 4-Cl; R_2 = -H, 4-F

Some benzhydrylpiperazines were reported for their high anti-fibrillatory and spasmolytic actions [194, 195].



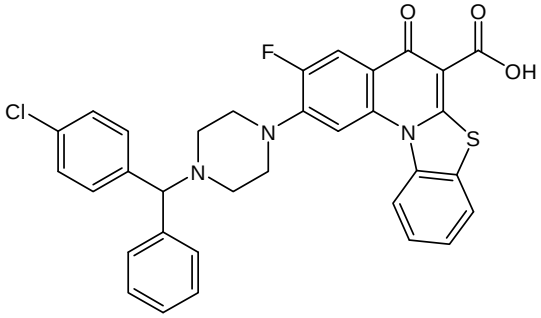
R = 2-CH_3; 4-CH_3; 2-C_2H_5

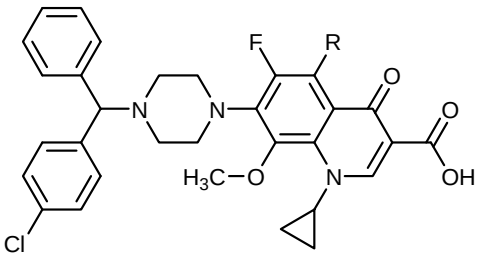


R_1 = -H; 2-Cl, 3-Cl; 4-Cl; 2-2-Br; 4-NO_2; 2-CH_3; 3-CH_3; 2-OCH_3; 3-OC_2H_5
R_2 = -H; 4-Cl; R_3 = -CH_3; -C_2H_5
R_4 = -CH_3; -C_2H_5; *i*-C_3H_7; *n*-C_4H_9

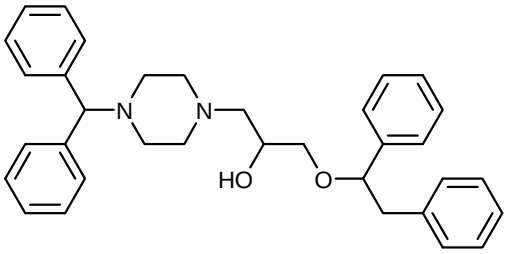
2.2.2.3.5. Antimicrobial Activity

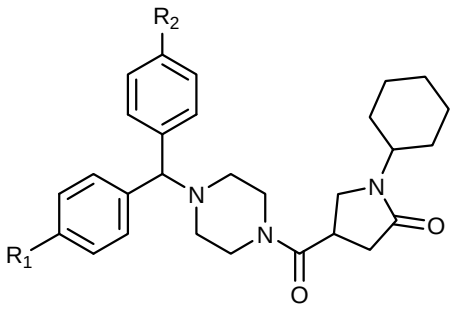
Many benzhydrylpiperazine derivatives were reported for their good antimicrobial activities against *Mycobacterium tuberculosis* [39-41, 172, 174, 196-202]. Some of these highly active derivatives are depicted below.





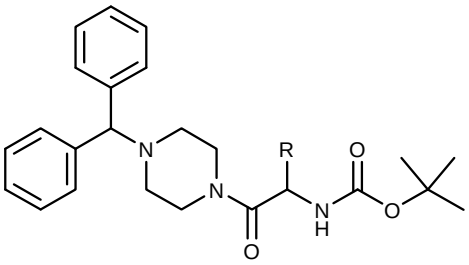
R = -H, -NO_2

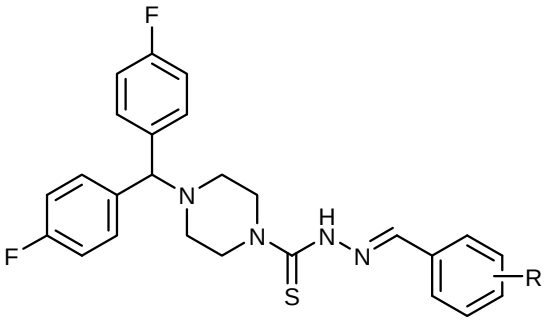




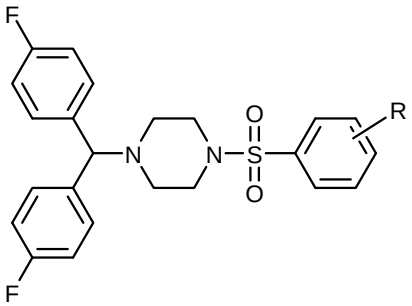
R_1 = -Cl, -F, -CH3
R_2 = -H, -F

Other bacterial strains that benzhydrylpiperazine derivatives had good activity were reported as *Staphylococcus aureus, Klebesiella pnemoniae, Pseudomonas auregenosa, Escherichia coli*. Benzhydrylpiperazines were also found to have good antifungal activity against *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Fusarium monoliforme* [159, 203-207].

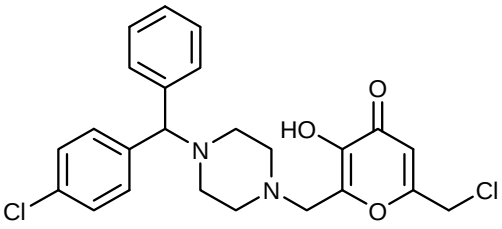




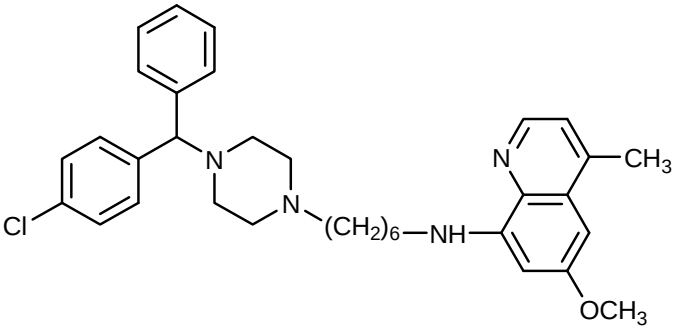
R = 2-Cl, 3- NO_2, 4-NO_2, 4-Br, 4-Cl, 4-CH_3



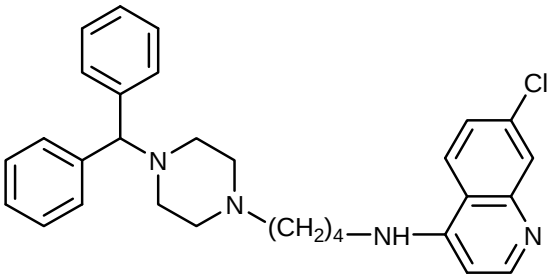
R = 2,5-Cl, 4-Cl, 4-CH_3, 4-$C(CH_3)_3$



Following benzhydrylpiperazine derivative was reported to have high antileishmanial activity [208].

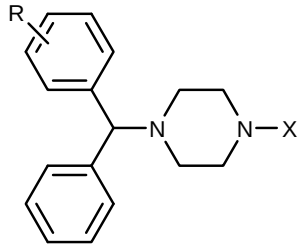


Burgess *et al*. prepared a 4-chloroquine derivative benzhydrylpiperazine compound with highly potent antimalarial activity [149].



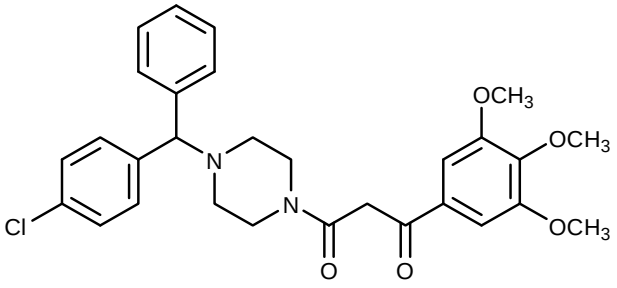
2.2.2.3.6. Central Nervous System Activity

Several benzhydrylpiperazine derivatives were synthesized and their mild psychomotor stimulant and anticonvulsant activities were reported [209].

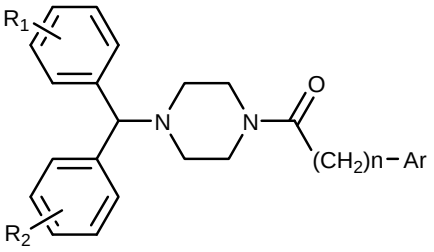


R = H, 2-Cl, 4-Cl
X = $CH_2CHOHCH_2OH$; $CON(C_2H_5)_2$; $CH_2C(Br)=CH_2$; $CSNHC_6H_5$; $CH_2CONHCONHCH_3$; $(CH_3)CHCONHNONHCH_3$

Following *N*-[(4-chlorophenyl)(phenyl)methyl]piperazine derivative was found to have low central nervous system depressant activity [210].

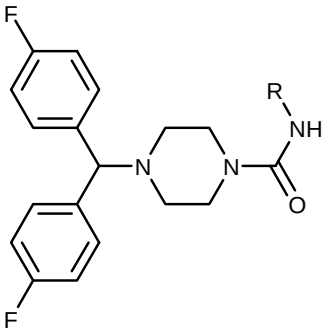


Empfield *et al*. stated that cannabinoid receptor CB_1 agonist benzhydrylpiperazines are potentially useful in treatment of obesity, psychiatric and neurologic disorders [211].



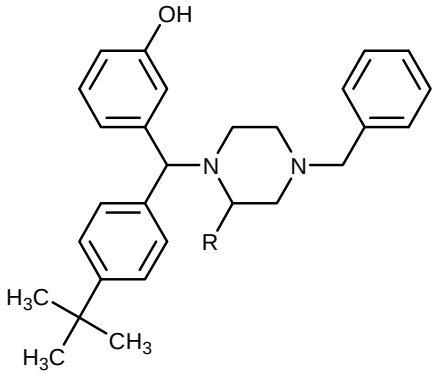
n = 0,1
R_1, R_2 = -H, -F, -Cl, -Br, methyl, trifluoromethyl, methoxy
Ar = phenyl, $C_{3\text{-}5}$ heteroaryl

Some benzhydrylpiperazine derivatives were reported as carnitine palmitoyl transferase inhibitors with good antidiabetic or anti-obesity activities [212].



R = 2-phenylethyl, 2-ethylphenyl, 4-heptyloxyphenyl, 4-chlorophenyl, 4-methoxybenzyl

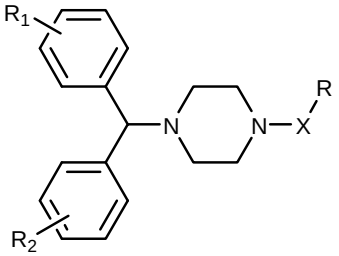
Some benzhydrylpiperazine derivatives were found to be highly selective and potent δ-opioid receptor agonists [213].



R = benzyloxymethyl, methyl, benzyl, hydroxymethyl

2.2.2.3.7. Bradykinin Inhibitory Activity

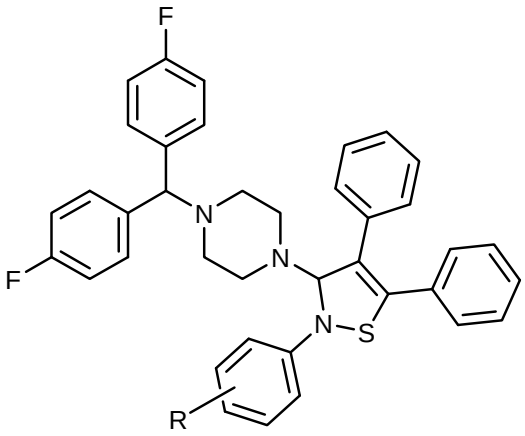
Benzhydrylpiperazines have been reported to have remarkable antagonistic effects on bradykinin receptors [214, 215].



R_1 = 4-Cl; 4-F
R_2 = -H; 4-F
X = $COCH_2CH_2CO$; $COCH_2CH(CH_3)CH_2CO$; $COCH_2C(CH_3)_2CH_2CO$; COCH=CHCO, CO
R = alkylamino, arylamino, acylamino

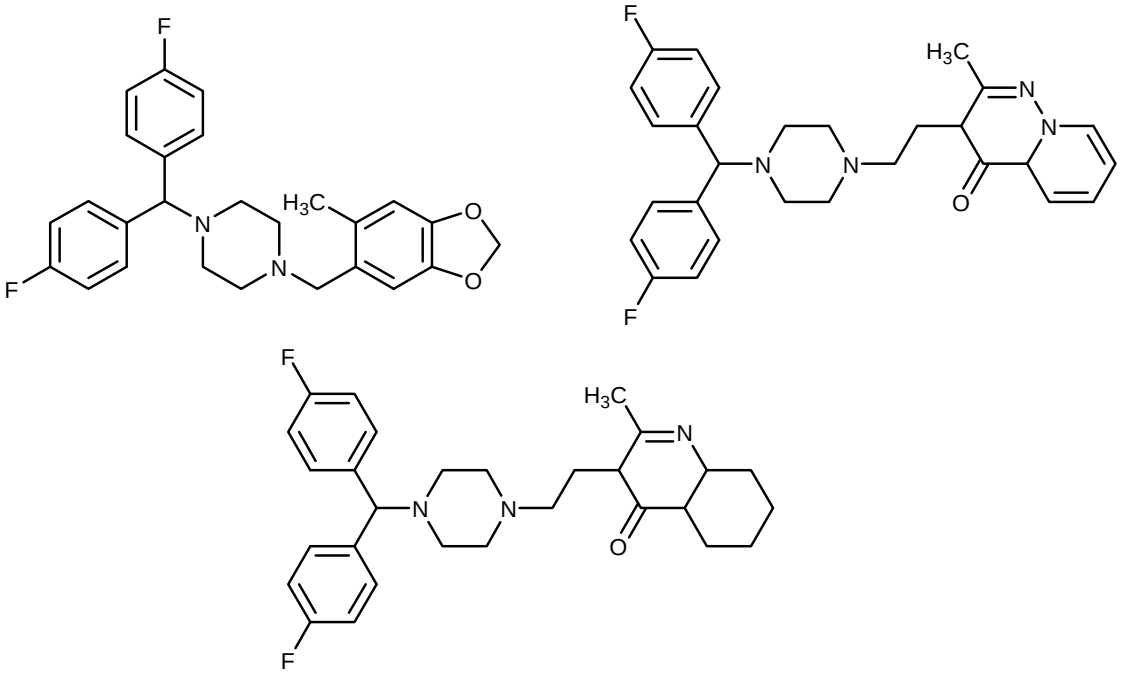
2.2.2.3.8. Anticholinesterase Activity

Benzhydrylpiperazine derivatives bearing isothiazole moiety were reported as moderately active acetylcholinesterase inhibitors [216].



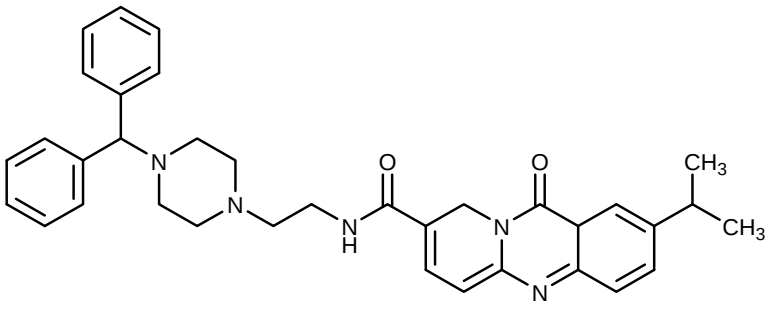
R = 2-NO_2, 2-F

Several 1-[bis(4-fluorophenyl)methyl]piperazines have been recently published for their good anticholinesterase activities [217].

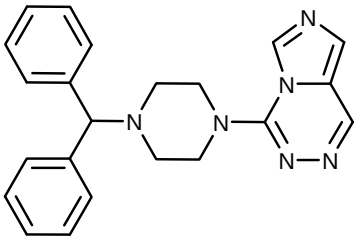


2.2.2.3.9. Antiallergic Activity

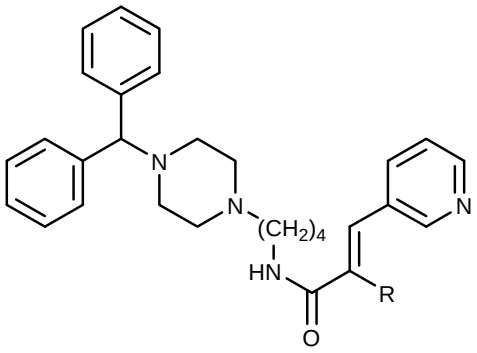
Tilley *et al*. published a benzhydrylpiperazine derivative capable of relieving anaphylaxis [218].



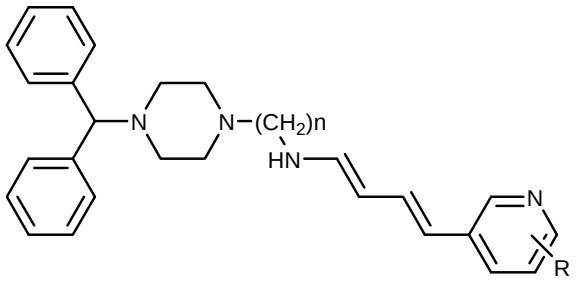
Good antiasthma activity of a benzhydrylpiperazine derivative was also reported [219].



Benzhydrylpiperazines bearing pyridyl moiety were synthesized and documented as highly active antiallergic compounds [220].



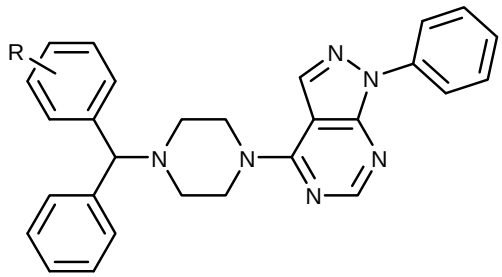
R = -H, Methyl, ethyl, n-propyl, phenyl



n = 3,4
R = -H; 6-CH_3; 4-CH_2OH, 5-OH, 6-CH_3

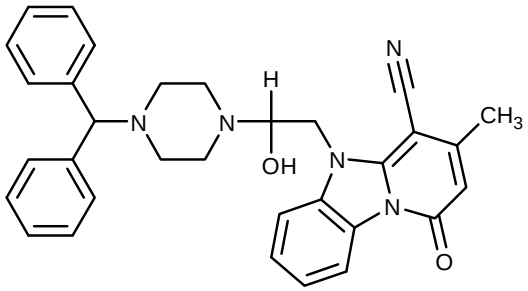
2.2.2.3.10. Antiviral Activity

Some benzhydrylpiperazines are published as potent enterovirus inhibitors [42].



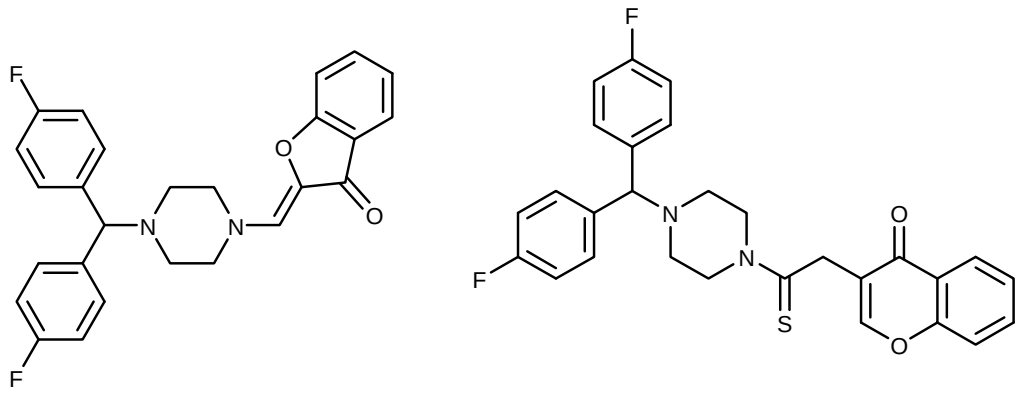
R = -H, 4-Cl, 4-Br, 4-CH_3, 4-C_6H_5, 4-CN, 3-CN, 2-CH_3

A benzhydrylpiperazine derivative has recently been reported to have good antiviral potency against HIV-1 human immunodeficiency virus [43].

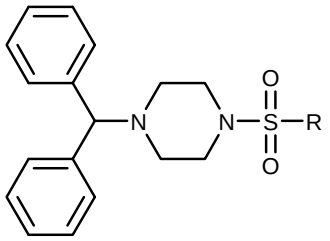


2.2.2.3.11. Anticancer / Cytotoxic Activity

To evaluate cytotoxic activity, MTT assay was applied to a benzhydrylpiperazine derivative and the compound was discovered to have high antitumor activities against HCCLM-7 (hepatoma carcinoma cell), Hep-2 (laryngocarcinoma cell), MDA-MB-435S (mammary adenocarcinoma cell) and SW-480 (colon carcinoma cell). Huang *et al.* applied flow-activated cell sorting analysis which revealed that compound arrests the cell cycle in G_0/G_1 phase and displayed apoptosis-inducing effect on Hep-2 cells [45]. Huang *et al.* subsequently reported a chromone bearing benzhydrylpiperazine derivative which also has high antiproliferative activity [46].

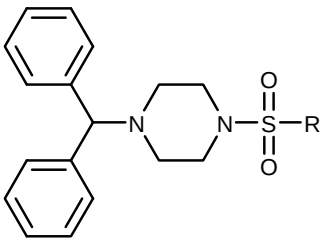


Kumar *et al*. synthesized 1-benzhydrylpiperazine sulfonamide derivatives and assessed their antiproliferative activities with MTS assay against MDA-MB-231 human breast cancer cell. The synthesized compounds had good inhibition values [166].

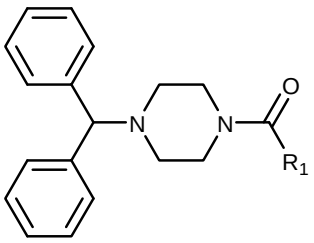


R = methyl, toluyl, 4-chlorophenyl, 4-*tert*-butylphenyl, (3,5-dimethyl)isoxazol-4-yl

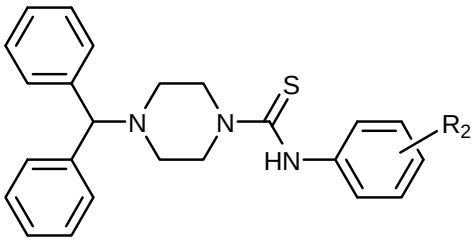
In another study, MTT cytotoxicity assay was performed to different sulfonyl chlorides, acid chlorides and isothiocyanates containing substituted 1-benzhydryl-piperazines by Kumar *et al*. These derivatives were potently cytotoxic against MCF-7 (breast carcinoma cell line), HepG-2 (hepatocellular carcinoma cell line), HeLa (cervix carcinoma cell line) and HT-29 (colon carcinoma cell line). It was stated that the nature of the *N*-terminal on the 1-benzhydrylpiperazine exerted a remarkable effect on antiproliferative activity [44].



R = 4-chloro-2-fluorophenyl;
camphoryl; phenyl; 2,2,2-trifluoroethyl

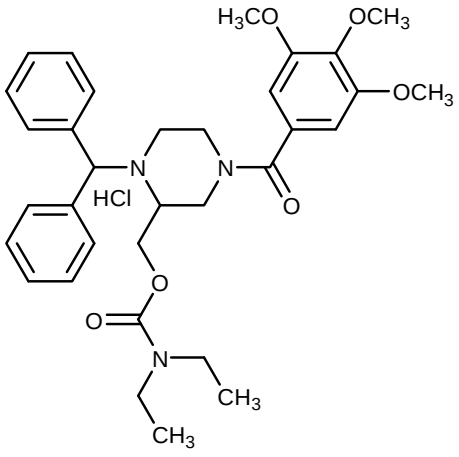


R_1 = isoxazol-5-yl; morpholin-4-yl;
pyrrolidinyl; cyclopropyl



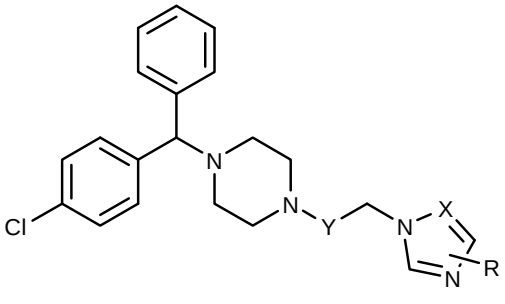
R_2 = 2-methoxy; 3-methoxy; 4-methoxy; 2-chloro; 3-chloro; 4-chloro; 4-fluoro; 2,4-dichloro

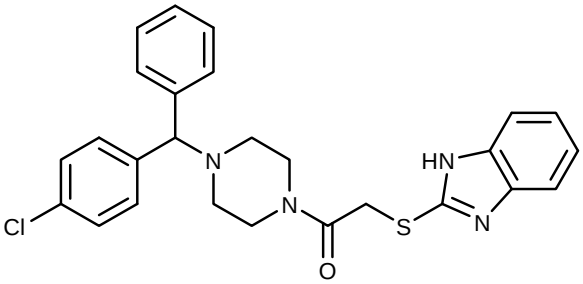
2-(*N*,*N*-Diethylaminocarbonyloxymethyl)-1-benzyhydryl-4-(3,4,5-trimethoxybenzoyl)piperazine hydrochloride (PMS-1077) has been studied with several assays against human Burkitt's lymphoma cells. The compound is responsible for cell cycle arrest at G_0/G_1 phase which forces cancer cells to undergo apoptosis [221].



PMS-1077

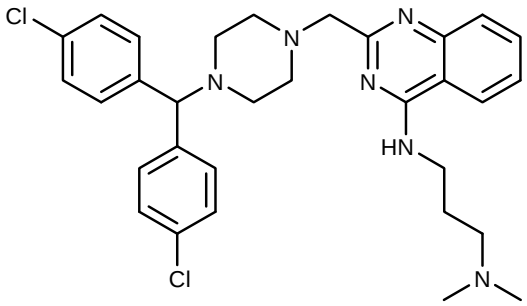
In a recent study, Gan *et al*. have synthesized some benzhydrylpiperazines and evaluated their cytotoxic activities by MTT assay and reported them as mild inhibitors of cell growth [47].





X = N, C ; Y = CH2, C(O)
R = H; 4-NO_2; 2-CH_3-4-NO_2; 2-C_6H_5; 2-CH_3

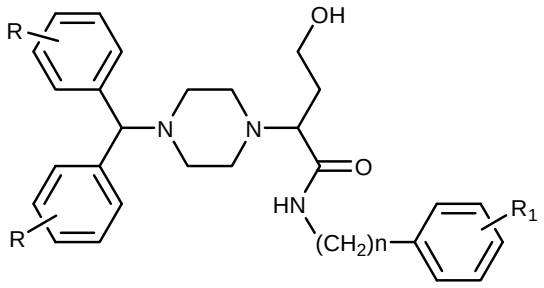
1-[Bis(4-chlorophenyl)methyl]piperazine derivative, SCH529074, is reported as an activator of mutant p53. Activated p53 leads to apoptosis in p53 mutant tumor cells [222].



SCH529074

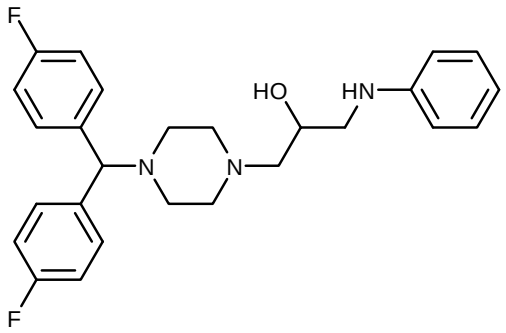
2.2.2.3.12. Other Activities

Several benzhydrylpiperazine derivatives have been published as good inhibitors of GABA uptake [170].

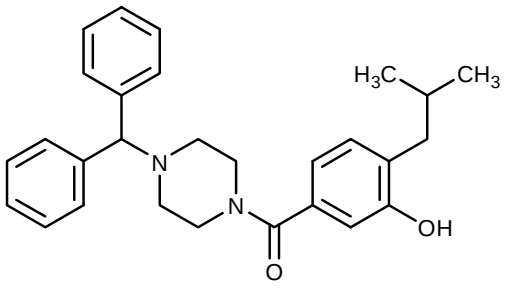


R = -H, 4-F
R_1 = -H, 2-Cl, 4-Cl, 2-F, 4-F, 4-CH3, 4-OCH3
n = 1,2

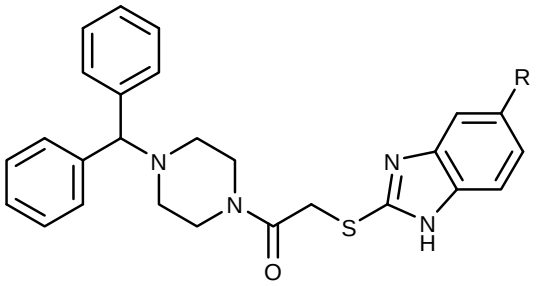
Kimura *et al*. synthesized a benzhydrylpiperazine derivative and reported its high antioxidant activity [138].



A benzhydrylpiperazine derivative was reported for its good anti-ulcer, acid secretion inhibition and antibacterial activity against *Helicobacter pylori*. The compound was also tested for its cell damaging effect with MTT assay and found to be safe [223].

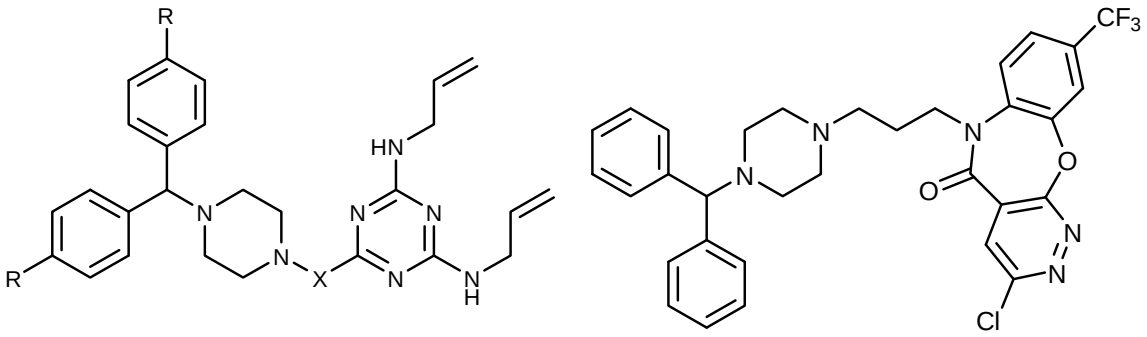


Some benzhydrylpiperazines with anthelmintic activity were published [224].



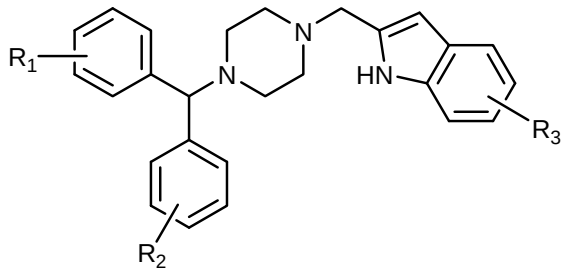
R = -H, -CH_3

Some benzhydrylpiperazines were found effective for multidrug-resistance modulation [153, 225].



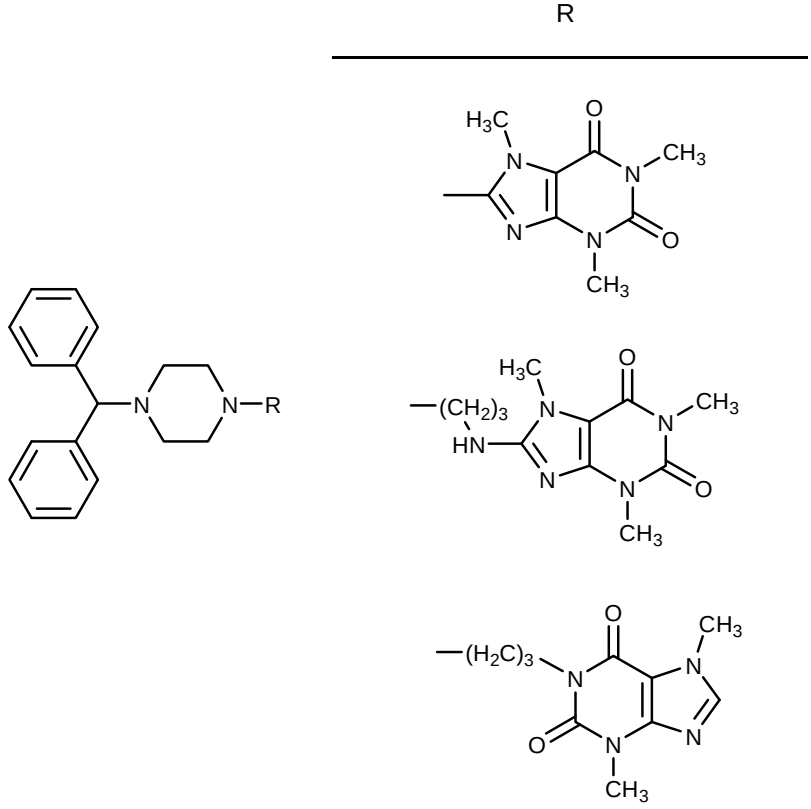
R = -F, -Cl
X = -NH-, no spacer

Silverman *et al*. synthesized some benzhydrylpiperazine derivatives with good insecticidal activity [226].

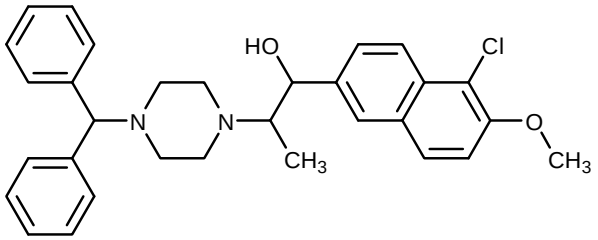


R_1 = -H; 4-F; 4-Cl; 4-OCH_3; 4-CH_3; 4-OCF_3; 2,4-Cl_2; 3,4-Cl_2; 3,5-Cl_2
R_2 = 4-F; 4-Cl; 4-CH_3; 4-OCF_3
R_3 = 2-CH_3; 4-Cl; 4-F; 4-OCH_3; 4-$O(CH2)_3CH_3$; 4-OSO_2CF_3; 4-$OCOCH_3$; 4-$NHCOC_6H_5$

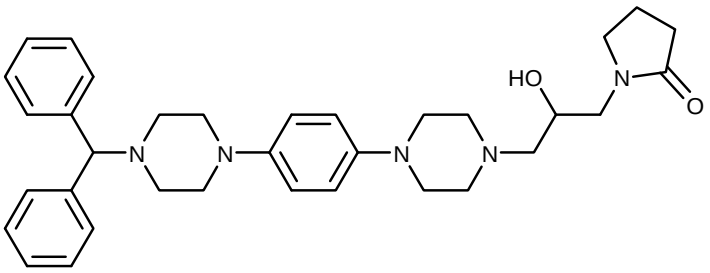
Several benzhydrylpiperazines with xanthine moiety have been reported as high affinity compounds to 5-HT_{1A} and 5-HT_{1B} receptors [227].



Chen and co-workers published a benzhydrylpiperazine as an inhibitor for 5-HT and noradrenaline reuptakes [228].



Another benzhydrylpiperazine derivative was reported as a good α_1-adrenoceptor antagonist [229].



2.3. Biological Activity

2.3.1. Cancer

Cancer is explained as a genetic disease of abnormally excessive cell proliferation. Aberrant genes that control cellular proliferation lead to the unrestricted growth which characterizes the malignant cell. The objective of most cancer therapy is to reduce the number of tumor cells and prevent their further accumulation [230].

20-25% of deaths in developed countries result from malignant tumors. Today cancer is accepted as a common fatal disease and cancer cases keep rising day after day. Despite the rapidly increasing medicinal research and treatment facilites (such as early diagnosis or the controlled treatment system), recovery of a cancer patient can be achieved merely as 20-25% [231].

WHO reports that cancer caused 7.6 million deaths (around 13% of all deaths) in 2008 throughout the world. The main types of cancer are mentioned as lung (1.4 million deaths, 14% of cancer caused deaths), stomach (740,000 deaths, 10% of cancer caused deaths), liver (700,000 deaths, 9% of cancer caused deaths), colorectal (610,000 deaths, 8% of cancer caused deaths) and breast cancer (460,000 deaths, 6% of cancer caused deaths) [232].

Table 2.2. Some prevalent cancer case frequencies in men (Turkey, 2004-2006) [233].

Location	Case frequency (reported data, %)
Trachea, Bronchi, Lung	27.0
Prostate	10.9
Skin	9.4
Colon	**4.1**
Rectum	**2.9**
Hepatocellular	**1.5**
Breast	**0.3**

Table 2.3. Some prevalent cancer case frequencies in women (Turkey, 2004-2006) [233].

Location	Case frequency (reported data, %)
Breast	**23.8**
Skin	11.6
Thyroid	6.3
Uterine corpus	5.0
Colon	**4.9**
Rectum	**3.0**
Hepatocellular	**1.1**

2.3.1.1. Important Principles on the Cell and Cancer

Two key matters of cellular life are

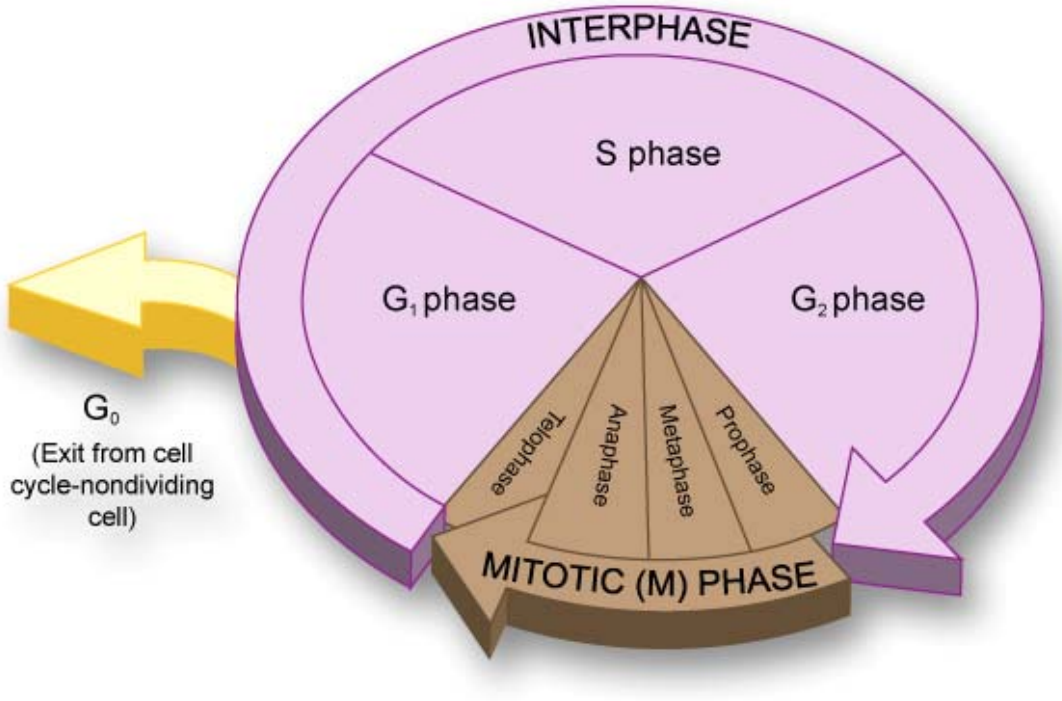
- DNA synthesis and mitosis in order to produce new cells,
- Cell differentiation in order to produce specialized cells.

The mechanisms for cell cycle and proliferation are regulated through chemical signals such as growth factors or growth inhibitors. Growth factors are produced for growth stimulation in normal cells. Simultaneously, many cells apply a negative feedback loop to counterbalance the effects of growth factors. If an organ is damaged, production of growth inhibitor decreases and the rate of proliferation increases until the lost cells are replaced [3].

Growth factors and growth inhibitors exert their effects by binding to cell surface receptors. In cancer cells, these regulatory processes are abnormal. For instance, cancer cells may over-produce growth factors (*i.e.* epidermal growth factor, EGF), under-express growth inhibitors (*i.e.* p53) or over-express growth factor receptors. The aberrant activation of growth factors or decreased expression of inhibitors will lead to abnormal and increased cell proliferation. The origin of these abnormalities, at the cellular level, has not been completely determined. However, it is believed that proto-oncogenes, which control normal proliferation and differentiation, are transformed into oncogenes. As a result, oncogenes alter normal cellular control mechanisms by stimulating processes that support cellular proliferation [3].

Many of the most potent cytotoxic agents act at specific phases of the cell cycle, therefore, they have activity only against the cells that are in process of division. Consequently, normal tissues that proliferate rapidly (*i.e.* bone marrow, hair follicles and intestinal epithelium) are effected by the damages of chemotherapy. On the contrary, slow-growing tumors (*e.g.* carcinomas of the colon and lung) are often unresponsive to cytotoxic drugs. Despite these variations between cells of different types, all cells follow a similar cell cycle pattern during the division process which may be characterized as follows:

1. Presynthetic phase – Gap 1 phase (G_1); a newly created cell is born. The time period a cell remains in this phase depends on the tissue type and whether it is a normal cell or a tumor cell.
2. Synthesis phase (S); DNA is replicated, and at the end of this phase two copies of DNA are present in the cell.
3. Postsynthetic phase – Gap 2 phase (G_2); an interval follows the termination of DNA synthesis, which preparations are made for the mitosis.
4. Mitosis (M) phase – the G_2 cell, containing a double complement of DNA divides into two daughter G_1 cells [234].



Scheme 2.1. The cell cycle [3]

2.3.1.2. Investigated Cancer Types

2.3.1.2.1. Breast Cancer

Female breast cancer is a major medical problem concerning public health and society. It appears that breast cancer cases have increased and become more complex during the last 25 years.

Among many risk factors, gender and age are the most striking ones. Male breast cancer case number is too small to compare with its female counterpart. Considering the age factor, it is absolute that breast cancer cases increase dramatically at ages more than 60 especially compared with ages between 20 and 30.

Women of higher economic class and educational status are under risk due to lifestyle related reasons such as diet, exogenous hormonal use and alcohol consumption.

Different ethnic groups express varying case numbers examplary the incidence of Asian - Pacific groups is much lower than that of Western European origin.

Approximately one-third of women suffering breast cancer have a family history of one or more first-degree relatives.

Endocrine and reproductive risk factors are strongly reliable for many women. Estrogen is sufficiently determinant for breast cancer as it is required for optimal development of mammary carcinomas in experimental systems, also men do not suffer as much as women do because of this type of cancer. Ovarian ablation supplies protectivity for women against breast cancer. Menopause occuring after age 54 is another risk factor. Women who have their first pregnancy after age 35 have three times higher incidence of breast cancer than women who have their first pregnancy before age 18.

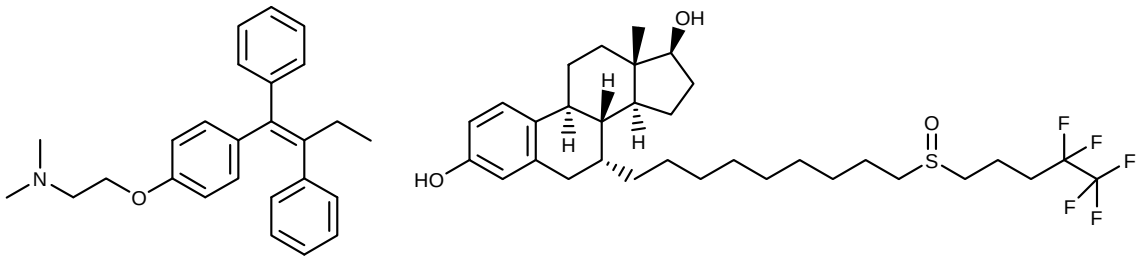
Other risk factors include use of exogenous hormones such as oral contraceptives, environmental factors, obesity, radiation exposure, benign breast disease and history of ovarian, uterine or bowel cancers.

Chemotherapy of breast cancer involves inhibition of hormone synthesis, blockage of hormone receptors and killing cancer cells.

Hormone synthesis is chemically inhibited with selective aromatase inhibitors and inhibitors of pituitary function. Aromatase is an enzyme responsible for the conversion of

androstenedione to estrone and subsequently to estradiol at peripheral tissues. Aromatase inhibitors decrease serum and tumor estrogen levels in postmenopausal patients making it applicable only to postmenopausal or oophorectomized women in whom estrogen is supplied from external sources. Luteinizing hormone-releasing hormone (LHRH) analogs produce long-lasting inhibition of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release after a transient initial increase. These drugs are primarily effective in premenopausal patients.

Tamoxifen, a familiar example of selective estrogen receptor modulators (SERMs), is capable of binding to estrogen receptor competitively. Another important drug of this type is fulvestrant which blocks estrogen receptor activaton. Fulvestrant is reported to be effective in tamoxifen-resistant cell lines.



Tamoxifen Fulvestrant

Figure 2.2. Structures of tamoxifen and fulvestrant.

Breast cancer is moderately sensitive to several chemotherapeutic agents including anthracyclines (*e.g.* doxorubicin), alkylating agents (*e.g.* cyclophosphamide), anthraquinones (*e.g.* mitoxantrone), antimetabolites (*e.g.* methotrexate), Vinca alkaloids (*e.g.* vincristine) and taxanes (*e.g.* paclitaxel).

Synthetic progestational agents, estrogens, corticosteroids, antiandrogens and antiprogestins are also under evaluation for treatment of breast cancer [235].

2.3.1.2.2. Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the third main type of cancer caused deaths in men globally and one of the most widespread cancers counted fifth for men and eighth for women worldwide.

More than 80% of liver cancer cases appear in Asia and Africa, mostly triggered of hepatitis B infection along with aflatoxines exposed from nutrition. In western countries, liver cirrhosis due to chronic alcohol consumption accounts for the main ethiological factor. Propagation of hepatitis C virus is responsible for increased rates of hepatocellular carcinoma in USA and Europe [236].

Regarding drug therapy, since HCC takes up its blood supply primarily from hepatic artery, wheras normal hepatocytes are sustained by portal vein, arterial infusion therapy is advantageous for increasing local drug delivery while potentially lowering systemic and hepatic toxicity. Intraarterial infusions of chemotherapeutics such as doxorubicin, cisplatin, floxuridine, epirubicin and mitomycin are mostly effective for localized HCC.

Systematic therapy for metastatic disease of HCC is gained with chemotherapeutics including anthracyclines, doxorubicin, 4-epidoxorubicin and mitoxantrone.

In many cases hepatic tumor size is decreased with drugs to be able to proceed with hepatic resection. 5-Fluorouracil and oxaliplatin are known to be effective for this methodology. Recurrence rates after resection of liver are extremely high leading patients to undergo adjuvant 5-fluorouracil based chemotherapy. However this type of medication is not offered to patients who received chemotherapy before surgery [237].

2.3.1.2.3. Colorectal Cancer

Colorectal cancer exhibits 9.4% of all cancer cases which states approximately 1,000,000 new cases. Colon cancer has nearly same incidence rate for women and men, however rectum cancer is more prevalent among men.

Worldwide variations are dramatic, reaching up to 25 fold more incidence rates in developed countries. These variations are mostly based on diet and life style differences, especially alcohol consumption and physical passivity.

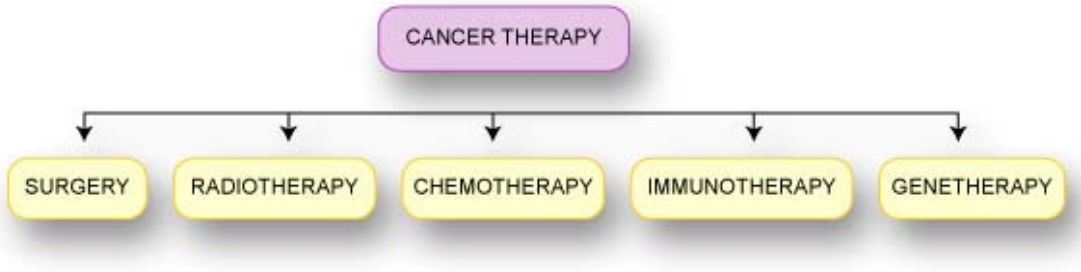
Family history is important due to high risk of two hereditary diseases namely, familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC).

Randomized trials have shown that faecal occult blood test reduces mortality related to colorectal cancer. Colonoscopy is the most reliable method for early diagnosis of colorectal cancer. Sigmoidoscopy is a recent method for screening of patient against cancerous polyps [238].

Early stage colorectal cancer is curable with resection. Stage III colorectal cancer presents high possibility of recurrence. For this reason, patients should be treated with adjuvant chemotherapy of 5-fluorouracil and leucovorin (5-FU/LV) or 5-fluorouracil and levamisole (5-FU/LEV) combinations. Treatment of stage IV colorectal cancer is mostly palliative as metastases reduce survival rates. Patients receive 5-FU/LV plus irinotecan or oxaliplatin as supportive care [239].

2.3.1.3. Cancer Treatment

Specific approach of cancer treatment varies upon the type, location and stage of the cancer. Regardless of these variations, several fundamental techniques are available to treat cancer including surgery, radiation therapy, immunologic treatment, gene therapy and chemical based approaches. Usually, a combination of these methods is preferred, for example surgery in combination with chemotherapy. A chemical component will exist in most therapeutic methods of cancer treatment [3].



Scheme 2.2. Types of cancer therapy [3]

2.3.1.4. Cancer Chemotherapy

2.3.1.4.1. Drugs Interacting Directly with DNA

Alkylating agents (*i.e.* nitrogen mustards, aziridines, nitrosoureas, triazenes, hydrazines, methanesulphonate esters) and some anticancer antibiotics (*e.g.* streptozotocin), damage DNA by covalent bond formation. *cis*-Platinium co-ordination complexes modify the DNA structure by binding of the metal ions directly to DNA. Some antibiotic agents (*e.g.* bleomycin) cause the breakdown of the DNA molecule. Intercalating agents (*e.g.* doxorubicin) impair DNA function by intercalating between the base pairs. Antisense anticancer agents (*e.g.* peptide nucleic acids) exert their action on nucleic acids [4].

2.3.1.4.2. Drugs Interfering with DNA Synthesis

Anticancer agents responsible for the inhibition of the enzymes involved in DNA synthesis are specific for the S-phase of the cell-cycle. Target enzymes of these inhibitors are as follows; dihydrofolate reductase (DHFR) in tetrahydrofolate synthesis (*e.g.* methotrexate); phosphoribosyl-pyrophosphate amidotransferase in purine synthesis (*e.g.* 6-mercaptopurine); thymidylate synthase in generation of thymidine monophosphate (dTMP); DNA and RNA polymerases (*e.g.* cytarabine); the enzyme ribonucleotide reductase catalyzing the conversion of all ribonucleotides into the corresponding deoxyribonucleotides (*e.g.* hydroxyurea) [4].

2.3.1.4.3. Mitotic Inhibitors

These agents are M phase specific because mitosis takes place during the M phase of the cell cycle. By the action of mitotic inhibitors, chromatides, separated in the metaphase, are prevented from migration toward the opposite poles in the following anaphase (*e.g.* vincristine, vinblastine) [4].

2.3.1.5. *In vitro* Cytotoxicity Assays

If a compound or treatment effects cellular integrity, highly alters morphology, decreases cell growth rate or causes cell death then, it is considered to be cytotoxic [240].

2.3.1.5.1. Dye Exclusion Test

Dye exclusion test is based on the characteristic of viable cells to be impermeable to trypan blue, naphtalene black, erythrosine and other dyes. If the membrane integrity of cells is compromised, uptake of the dye into the cells occur, so that unstained viable cells appear clear with a refractile ring around them; whereas nonviable cells appear dark blue coloured without any refractle ring around them. Trypan blue is the most commonly used dye during this assay [241].

2.3.1.5.2. XTT/PMS Assay

Internal environment of proliferating cells is more reduced than the nonviable cells. Tetrazolium salts such as 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-*2H*-tetrazolium-5-carboxanilide sodium salt (XTT) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) are used to measure this reduced state. XTT and MTT are metabolically reduced by the mitochondria in viable cells to a coloured formazan product for which the intensity can be measured spectrophotometrically. XTT is preferred to MTT because it is more soluble. Electron coupling reagent phenazine methosulphate (PMS) is required for efficient reduction with XTT. However, both of these reagents are dependent on mitochondrial function, so variations in cellular levels of NADH, glucose and other factors cause variable results and a false result may be gained as if the cells are nonviable or not proliferating [241, 242].

2.3.1.5.3. MTS/PMS Assay

3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-*2H*-tetrazolium, inner salt MTS, is bioreduced by viable cells into a formazan product soluble in culture media in the presence of PMS. The advantage of MTS over XTT is that it is more soluble and nontoxic, so that cells can be returned to culture for further evaluation [241].

2.3.1.5.4. Neutral Red Assay

Neutral red, (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) is also measured for cell viability as an indicator of cytotoxicity in cultures of many cell lines. It is

taken up by living cells and subsequent accumulation of dye in the lysosomes takes place [243, 244].

2.3.1.5.5. AlamarBlue Assay

AlamarBlue is used to observe the reducing environment of proliferating cells. Since it is not toxic, cells exposed to it, can be returned to culture or used for other purposes. AlamarBlue utilizes the mitochondrial reductase to convert nonfluorescent resazurin to fluorescent resorufin [241].

2.3.1.5.6. ATP Cell Viability Assay

ATP can be quantified in a luminometer by measuring the light generated using the luciferase-luciferin reagent. Apoptotic cells are known to exhibit a significant decrease in ATP levels due to loss of cell integrity. This assay is based on two 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. The intensity of light emitted is measured with a luminometer or a β counter [241].

2.3.1.5.7. [^{3}H]-Thymidine Incorporation Assay

Proliferating cells can incorporate [^{3}H]-thymidine into replicating DNA. Despite its precision to give accurate data on DNA synthesis, this assay is disadvantageous as it uses radioactivity and requires extensive sample preparation [241].

2.3.1.5.8. Sulphorhodamine B (SRB) Assay

SRB assay is a widely used method for *in vitro* cytotoxicity screening developed in 1990 by Skehan *et al* [245].

Sulphorhodamine B is a bright-pink aminoxanthene dye bearing two sulphonic groups that bind stoichiometrically to basic amino acid residues under mild acidic conditions, and dissociate under basic conditions. Colorimetric measurement provides an estimate of total protein mass, which is related to cell number.

The greater the number of cells, the greater the amount of dye is taken up, then after fixing with trichloroacetic acid (TCA), as the cells are lysed, the released dye will give a more intense colour and greater absorbance.

This assay has many advantages over other tests including better linearity, higher sensitivity, a stable end point that does not require time-sensitive measurement and lower cost. However the limitation of this assay is the need for the addition of TCA for cell fixation. It is important to add TCA gently, or otherwise, it may dislodge cells before they become fixed, which in turn, can affect the results [241, 242, 246].

SRB assay can be utilized for cytotoxicity testing as well as testing of drug efficacy against pathogens and viruses, it is also effective for *in vitro* evaluation of cancer cell sensitivity to radiation, and for the study of interactions between radiotherapy and chemotherapy [246].

2.3.1.5.9. Enzyme Release-Based Cytotoxicity Assays

The leakage of cellular components from compromised cells into the culture medium is also measured for assessment of cell death. Common enzymes favored as a marker of cell death for *in vitro* models are lactate dehydrogenase (LDH), adenylate kinase (AK) and glyceraldehyde-3-phosphate dehydrogenase (GADPH) [240].

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

In this work, benzophenone, 4-chlorobenzophenone, 4,4-difluorobenzophenone, sodium borohydride, thionyl chloride, piperazine, *sec*-butyl isocyanate, *tert*-butyl isocyanate, isopropyl isocyanate, ethyl isocyanate, 2,6-dichlorophenyl isocyanate, 2-benzylphenyl isocyanate, ethylacetato isocyanate, allyl isocyanate, butyl isocyanate, 4-bromophenyl isocyanate, phenethyl isocyanate, 4-cyanophenyl isocyanate, *tert*-butyl isothiocyanate, cyclohexyl isothiocyanate, ethyl isothiocyanate, allyl isothiocyanate, benzyl isothiocyanate, butyl isothiocyanate, 5-fluoro-2-methylbenzoyl chloride, 2-bromobenzoyl chloride, 3-bromobenzoyl chloride, 4-bromobenzoyl chloride, 3-chlorobenzoyl chloride, *o*-anisoyl chloride, 3-nitrobenzoyl chloride, 3,4-dimethoxybenzoyl chloride, 4-ethylbenzoyl chloride, 2-trifluoromethylbenzenesulfonyl chloride, 2,4,5-trichlorobenzenesulfonyl chloride, 3,4-dichlorobenzenesulfonyl chloride, 2-methylbenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, 2,5-dichlorobenzenesulfonyl chloride, 1-benzhydryl-piperazine, sulphorhodamine B, absolute ethanol, methanol, ethyl acetate, *n*-hexane and dichloromethane were purchased from Sigma-Aldrich. 1-[Bis(4-fluorophenyl)-methyl]piperazine was purchased from Chemicals International Türkiye. 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was purchased from Chemicaline Products Co. Dimethylformamide was purchased from Carlo Erba Reagenti. Potassium carbonate, anhydrous sodium sulphate, ammonium chloride, aqueous hydrochloric acid (37%), benzene, toluene and diethylamine were purchased from Riedel de Häen. Anhydrous calcium chloride and triethylamine were purchased from J. T. Baker.

3.1.2. Methods of Synthesis

3.1.2.1. Synthesis of the starting materials

3.1.2.1.1. Benzhydrole derivatives [247]

Benzhydrole was synthesized according to following procedure. 10 mmol (2.2 g) Benzophenone was dissolved in 10 ml ethanol. In a separate flask, 11 mmol (0.4 g) sodium borohydride ($NaBH_4$) was dissolved in 2 ml ethanol. Sodium borohydride solution was slowly added to benzophenone solution with a Pasteur pipette. Reaction mixture was allowed to continue stirring for a further 30 minutes. For the work up of reaction, 2 ml of concentrated HCl was added to a 20 ml ice-water solution. Reaction mixture was poured into this ice cold solution slowly with stirring. White solid product was collected with vacuum filtration and washed twice with distilled water. 4-Chlorobenzophenone and 4,4'-difluorobenzophenone were also reacted with sodium borohydride to give 4-chlorobenzhydrole and 4,4'-difluorobenzhydrole respectively according to above procedure.

3.1.2.1.2. Benzhydryl chloride derivatives

3.1.2.1.2.1. With thionyl chloride [44]

10 mmol (1.84 g) Benzhydrole was dissolved in 20 ml dry dichloromethane. Temperature was dropped to 0-5°C with NaCl-ice mixture. Afterwards 15 mmol (1.1 ml) thionyl chloride was added slowly and reaction was continued for 6 hours. After reaction ended the product was collected under vacuo as brown liquid. 4-Chlorobenzhydryl chloride and 4,4'-difluorobenzhydryl chloride were also synthesized from 4-chlorobenzhydrole and 4,4'-difluorobenzhydrole according to above procedure.

3.1.2.1.2.2. With hydrochloric acid and anhydrous calcium chloride [167]

10 mmol (1.84 g) Benzhydrole was added to 15 ml of aqueous HCl (37%). 10 mmol (1.1 g) anhydrous calcium chloride was added to the mixture to be refluxed at 85°C for 4 hours with stirring. After reaction completed, the flask was cooled to room temperature and extracted twice with 20 ml ethyl acetate. Organic layers were combined together, washed

with brine and water, then dried over anhydrous sodium sulfate. Followed by the concentration under vacuo, the product was collected as brown liquid. 4-Chlorobenzhydryl chloride and 4,4'-difluorobenzhydryl chloride were also synthesized from 4-chlorobenzhydrole and 4,4'-difluorobenzhydrole according to above procedure.

3.1.2.1.3. Benzhydrylpiperazine derivatives [44]

9 mmol (0.78 g) Piperazine was dissolved in dimethylformamide. Anhydrous potassium carbonate was added to the solution and stirred for 10 minutes. Followed by the addition of 9 mmol (1.82 g) benzhydryl chloride, reaction mixture was heated at 80°C for 8 hours. After completion, dimethylformamide was removed under vacuo, then residue was taken in water and extracted with ethyl acetate. Organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated and white solid product was obtained. 1-[(4-Chlorophenyl)(phenyl)methyl]piperazine and 1-[bis(4-fluoro-phenyl)methyl]piperazine were also synthesized from 4-chlorobenzhydryl chloride and 4,4'-difluorobenzhydryl chloride consecutively according to above procedure.

3.1.2.2. Synthesis of the Target Compounds

3.1.2.2.1. *N*-Alkyl-4-[(diphenyl)/((4-chlorophenyl)(phenyl))methyl]piperazine-1-carboxamides

2 mmol (1 mole, 0.515 g) 1-Benzhydrylpiperazine or 0.872 mmol (1 mole, 0.2632 g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 20 mL dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (3 moles) was added to the solution. Ice bath was removed after 10 minutes and appropriate isocyanate derivative (n moles) was added. Reaction was mixed overnight at room temperature. After reaction completed, solution was extracted with water and ammonium chloride solution (10%), respectively. Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. Solvent was evaporated under vacuo and solid product was crystallized with ethanol/water.

3.1.2.2.2. *N*-Alkyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamides

1.7 mmol (0.515 g) 1-[Bis(4-fluorophenyl)methyl]piperazine was dissolved in 30 ml dimethylsulfoxide. Reaction flask was taken into ice bath and 5.1 mmol (0.7 mL) triethylamine was added to the solution and ice bath was removed immediately. 10 Minutes later, 1.7 mmol appropriate isocyanate derivative was added. Reaction was stirred overnight at room temperature. After reaction was completed, 60 ml of water was added and the solution was taken to a separatory funnel. Ethyl acetate was added to the flask and extraction was applied furtherly with ammonium chloride solution (10%). Ethyl acetate layer was washed with water again and dried with anhydrous sodium sulphate. Solvent was evaporated under vacuo and solid product was crystallized with ethanol/water.

3.1.2.2.3. *N*-Alkyl-4-[bis(4-fluorophenyl)/((4-chlorophenyl)(phenyl))methyl]-piperazine-1-carbothioamides

1.7 mmol (1 mole, 0.515 g) 1-[Bis(4-fluorophenyl)methyl]piperazine or 0.872 mmol (1 mole, 0.2632 g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 75 ml dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (3 moles) was added to the solution. 10 minutes later, ice bath was removed and suitable isothiocyanate derivative (1 mole) was added. Reaction was stirred overnight at room temperature. After reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. Solvent was evaporated under vacuo and solid product was crystallized with ethanol/water.

3.1.2.2.4. 1-[(5-Fluoro-2-methyl)benzoyl]-4-(diphenylmethyl)piperazine hydrochloride

2 mmol (0.515 g) 1-Benzhydrylpiperazine was dissolved in 20 ml dry dichloromethane. Reaction flask was taken into ice bath and 6 mmol (0.86 ml) triethylamine was added to the solution. Ice bath was removed after 10 minutes and 2 mmol (0.28 ml) (5-fluoro-2-methyl)benzoyl chloride was added. Reaction was stirred overnight at room temperature. After reaction was completed, solution was extracted in order with water

and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. The mixture was concentrated in vacuo and oily product was dissolved in diethylether. HCl gas was passed through the solution and solid hydrochloride salt of product was obtained. Compound needed no further purification.

3.1.2.2.5. 1-(Substitutedbenzoyl)-4-[bis(4-fluorophenyl)/((4-chlorophenyl)-(phenyl))methyl]piperazine hydrochlorides

1.7 mmol (1 mole, 0.515 g) 1-[Bis(4-fluorophenyl)methyl]piperazine or 0.872 mmol (1 mole, 0.2632 g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 50 mL dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (3 moles) was added to the solution. 10 minutes later ice bath was removed and appropriate benzoyl chloride derivatives (1 mole) were added. Reaction was stirred overnight at room temperature. After the reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. The mixture was concentrated in vacuo and product was dissolved in ethyl acetate. Column chromatography was applied with *n*-hexane-ethyl acetate (80:20) mixture in silica gel column. Oily products were dissolved in diethylether and HCl gas was passed through the solutions to obtain solid hydrochloride salt of compounds.

3.1.2.2.6. 1-[2-Trifluoromethoxyphenyl]sulfonyl-4-(diphenylmethyl)piperazine hydrochloride

1 mmol (0.2575 g) 1-Benzhydrylpiperazine was dissolved in 20 mL dry dichloromethane. Reaction flask was taken into ice bath and 3 mmol (0.43 ml) triethylamine was added to the solution, 10 minutes later, 1.1 mmol (0.18 ml) 2-trifluoromethoxybenzenesulfonyl chloride was added. Ice bath was removed 2 hours later and reaction was stirred overnight at room temperature. After reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. The mixture was concentrated in vacuo and oily product was dissolved in

diethylether. HCl gas was passed through the solution and solid hydrochloride salt of compound was obtained.

3.1.2.2.7. 1-(Substitutedphenyl)sulfonyl-4-[bis(4-fluorophenyl)/((4-chloro-phenyl)(phenyl))methyl]piperazines

1.7 mmol (1 mole, 0.515 g) 1-[(Bis-4-fluorophenyl)methyl]piperazine or 0.872 mmol (1 mole, 0.2632 g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 50 ml dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (3 moles) was added to the solution. 10 minutes later, appropriate sulfonyl chloride derivatives (1 mole) were added. Ice bath was removed after 2 hours and reaction was stirred overnight at room temperature. After reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. Solvent was evaporated under vacuo and solid product was crystallized with ethanol/water.

3.1.3. Analytical Methods

3.1.3.1. Melting Point Determination

Melting points (°C) of the compounds were determined by using a Mettler Toledo FP62 capillary melting point apparatus and are uncorrected.

3.1.3.2. Controls by Thin Layer Chromatography

Material:

Plates: TLC aluminum sheets 20x20 cm Silica gel 60 F_{254} (Merck).

Solvent systems: Three different solvent systems were prepared to be used in chromatographic controls of compounds.

S-1: Benzene:Methanol (90:10)

S-2: *n*-Hexane:Ethyl acetate (80:20)

S-3: Toluene:Ethyl acetate:Diethylamine (75:25:10)

Method:

Dragging conditions: Solvent systems were poured to chambers and kept for 1 hour for adequate saturation.

Reactions were monitored with TLC after dissolving the synthesized compounds and starting materials with suitable solvents and application of them with Pasteur pipettes onto silica gel plates. The plates were dragged for 10 cm at room temperature. R_f values of compounds were calculated.

Stain determination: Stains of the synthesized compounds and their starting materials were determined by UV light (254/365 nm) and *Dragendorff* reagent was sprayed over the plate in order to visualize piperazine residues.

Dragendorff Reagent [248]:

Solution I: 2 g bismuth subnitrate is dissolved in 25 ml acetic acid and 100 ml distilled water.

Solution II: 40 g potassium iodide is dissolved in 100 ml distilled water.

Spray solution: 10 ml of solution I, 10 ml of solution II and 20 ml acetic acid are mixed and diluted with 100 ml distilled water.

3.1.3.3. Purification by Column Chromatography

Materials:

Stationary phase: Silica gel 60 mesh.

Mobile phase: *n*-Hexane:Ethyl acetate (80:20).

Method:

Column was filled in accordance with wet method. Elution was controlled with TLC using silica gel plates and benzene:methanol (90:10) mobile phase.

3.1.4. Spectrometric Analyses

3.1.4.1. Infrared Spectra

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

3.1.4.2. ^{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) as solvent, the chemical shifts were reported in parts per million (ppm).

3.1.4.3. ^{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 parts per million (ppm).

3.1.4.4. Mass Spectra

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

3.1.4.5. Elemental Analyses

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

3.1.4.6. X-Ray Crystallography

Crystals of compounds **43** and **48** suitable for X-ray crystallography were selected and data collection were performed on a STOE IPDS II diffractometer [249] with graphite monochromated Mo-K_α (λ = 0.71073 Å) radiation at 296 K. The structures were solved by

direct-methods using SHELXS-97 and refined by full-matrix least-squares methods on F^2 using SHELXL-97 [250] from within the WINGX [251] suite of software. All non-hydrogen atoms were refined with anisotropic parameters. Hydrogen atoms bonded to carbon were refined using a riding model, with C–H=0.93–0.97 Å. The constraint $U_{iso}(H)=1.2U_{eq}$ (C and CH_2) was applied. Molecular diagrams were created using ORTEP-III [252]. Geometric calculations were performed with PLATON [253].

3.2. Cytotoxic Activity

3.2.1. Cytotoxicity Analyses of the Compounds

The cytotoxic activity of the synthesized compounds was investigated initially 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.

3.2.2. Sulphorhodamine B Assay

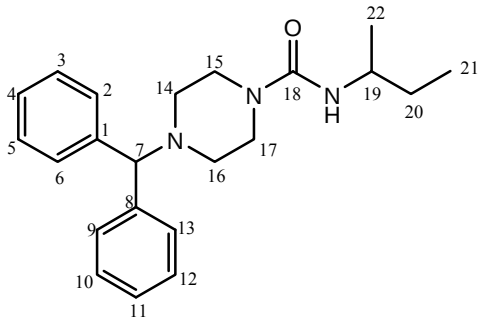
Cancer cells (range of 2000 cell/well to 5000 cell/well) were inoculated into 96-well plates in 200 μl of media and incubated in 37°C incubators containing 5% CO_2 and 95% air. After a 24 h incubation period, one plate for each cell line was fixed with 100 μl 10% ice-cold trichloroacetic acid (TCA). This plate represents the behavior 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.

4. EXPERIMENTAL

4.1. Chemical Data

***N-sec*-Butyl-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 1, CAS No: 1071382-92-7)**



Diphenylmethylpiperazine (2 mmol, 0.515 g), *sec*-butyl isocyanate (2 mmol, 0.23 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.240 g (68%).

The form of compound is white, opaque, needle-shaped crystals and the compound has a melting point of 198.4°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.57, 0.78 and 0.07 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 5.17), 224 (log ε : 4.69).

FT-IR (KBr, cm^{-1}); 3342 (N-H), 3022 (C-H, aromatic), 2959 (C-H, aliphatic), 1619 (C=O, amide), 1540 (C=C, aromatic), 1246 (C-N).

^{1}H-NMR (DMSO, ppm); 0.78 (t, 3H, -CH_2-$\underline{CH_3}$, *J=7.6 Hz*); 0.98 (d, 3H, -CH-$\underline{CH_3}$, *J=6.8 Hz*); 1.35 (m, 2H, -$\underline{CH_2}$-CH_3); 2.23 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.28 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.53 (m, 1H, -NH-$\underline{CH}$-); 4.29 (s, 1H, $(Ar)_2CH$-); 6.02 (d, 1H, -CONH-, *J=7.6 Hz*); 7.20 (m, 2H, diphenyl H_4, $H_{4'}$); 7.30 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=7.6 Hz*); 7.43 (t, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=7.2 Hz*).

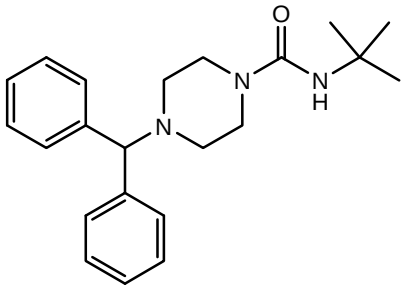
^{13}C-NMR (DMSO, ppm); 11.43 (C_{21}); 21.45 (C_{22}); 29.90 (C_{20}); 44.25 ($C_{14,16}$); 47.82 (C_{19}); 52.06 ($C_{15,17}$); 75.59 (C_7); 127.56 ($C_{4,11}$); 128.29 ($C_{2,6,9,13}$); 129.20 ($C_{3,5,10,12}$); 143.30 ($C_{1,8}$); 157.76 (C_{18}).

MS (m/z); 352.8 (M^+); 253.7 ($((C_6H_5)_2CHN(C_2H_4)_2NH^+$); 167.5 ($((C_6H_5)_2CH^+$).

Elemental analysis of $C_{22}H_{29}N_3O$ (MW: 351.49 g/mol);

	% C	% H	% N
Calculated	75.18	8.32	11.96
Found	75.12	8.27	11.85

N-tert-Butyl-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 2)



Diphenylmethylpiperazine (2 mmol, 0.515 g), *tert*-butyl isocyanate (2 mmol, 0.24 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.436 g (62%).

This compound forms white, opaque, needle-shaped crystals and has a melting point of 192.4°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.7, 0.82 and 0.18.

UV (MeOH, λ_{max}, nm); 206 (log ε : 5.13), 227 (log ε : 4.62).

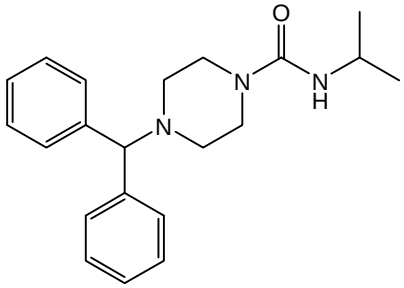
FT-IR (KBr, cm^{-1}); 3322 (N-H), 3023 (C-H, aromatic), 2970 (C-H, aliphatic), 1621 (C=O, amide), 1536 (C=C, aromatic), 1260 (C-N).

^{1}H-NMR (DMSO, ppm); 1.22 (s, 9H, -$C(CH_3)_3$); 2.23 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.25 **(**t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 4.29 (s, 1H, $(Ar)_2CH$-); 5.68 (s, 1H, CONH); 7.19 (m, 2H, diphenyl H_4, $H_{4'}$); 7.30 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=7.6 Hz*); 7.43 (t, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=7.2 Hz*).

Elemental analysis of $C_{22}H_{29}N_3O$ (MW: 351.49 g/mol);

	% C	% H	% N
Calculated	75.18	8.32	11.96
Found	74.60	8.21	11.84

_N_-Isopropyl-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 3)



Diphenylmethylpiperazine (2 mmol, 0.515 g), isopropyl isocyanate (2 mmol, 0.2 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.318 g (94%).

The form of compound is white, opaque, clustered crystals and the compound has a melting point of 220.4°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.61, 0.76 and 0.07 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.21), 227 (log ε : 4.81).

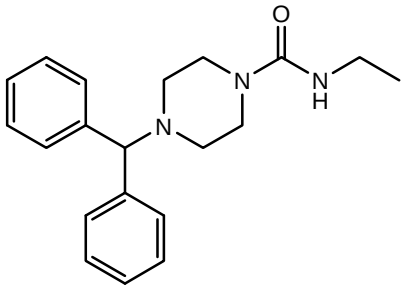
FT-IR (KBr, cm^{-1}); 3367 (N-H), 3060 (C-H, aromatic), 2964 (C-H, aliphatic), 1611 (C=O, amide), 1538 (C=C, aromatic), 1254 (C-N).

^{1}H-NMR (DMSO, ppm); 0.98 (d, 6H, -CH($\underline{CH_3}$)$_2$, *J=6.8 Hz*); 2.19 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.25 (t, 4H, piperazine H_2, H_6, *J=5.2 Hz*); 3.68 (m, 1H, -$\underline{CH}$(CH_3)$_2$); 4.25 (s, 1H, (Ar)$_2$CH-); 6.05 (d, 1H, CONH, *J=7.6 Hz*); 7.15 (m, 2H, diphenyl H_4, $H_{4'}$); 7.26 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=7.2 Hz*); 7.39 (t, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=6.8 Hz*).

Elemental analysis of $C_{21}H_{27}N_3O$ (MW: 337.46 g/mol);

	% C	% H	% N
Calculated	74.74	8.06	12.45
Found	74.89	7.73	12.30

_N_-Ethyl-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 4)



Diphenylmethylpiperazine (2 mmol, 0.515 g), ethyl isocyanate (2 mmol, 0.16 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.294 g (84%).

The form of compound is white, shiny, flat crystals and the compound has a melting point of 208.9°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.41, 0.63 and 0.01 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 5.11), 221 (log ε : 4.58).

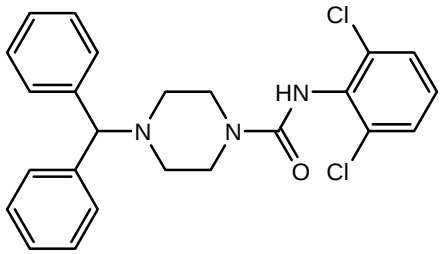
FT-IR (KBr, cm^{-1}); 3365 (N-H), 3024 (C-H, aromatic), 2978 (C-H, aliphatic), 1622 (C=O, amide), 1545 (C=C, aromatic), 1259 (C-N).

^{1}H-NMR (DMSO, ppm); 0.98 (t, 3H, -CH_3, *J=7.6 Hz*); 2.23 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.01 (m, 2H, -CH_2-); 3.28 (t, 4H, piperazine H_2, H_6, *J=5.2 Hz*); 4.28 (s, 1H, $(Ar)_2$CH-); 6.41 (t, 1H, CONH, *J=5.2 Hz*); 7.20 (m, 2H, diphenyl H_4, $H_{4'}$); 7.29 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=8 Hz*); 7.43 (t, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=7.2 Hz*).

Elemental analysis of $C_{22}H_{29}N_3O$ (MW: 351.49 g/mol);

	% C	% H	% N
Calculated	74.27	7.79	12.99
Found	73.77	7.46	12.93

_N_-(2,6-Dichlorophenyl)-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 5)



Diphenylmethylpiperazine (2 mmol, 0.515 g), 2,6-dichlorophenyl isocyanate (2 mmol, 0.3837 g) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.386 g (88%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 234.6°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.79, 0.91 and 0.08 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.32), 226 (log ε : 4.72).

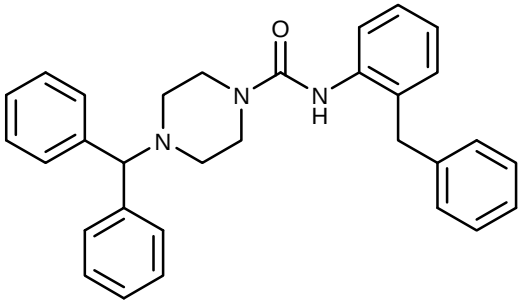
FT-IR (KBr, cm^{-1}); 3237 (N-H,), 3025 (C-H, aromatic), 2967 (C-H, aliphatic), 1638 (C=O, amide), 1528 (C=C, aromatic), 1255 (C-N).

^{1}H-NMR (DMSO, ppm); 2.29 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.44 (t, 4H, piperazine H_2, H_6, *J=4 Hz*); 4.33 (s, 1H, $(Ar)_2CH$-); 7.15-7.3 (m, 10H, diphenyl); 7.40-7.47 (m, 3H, 2,6-dichlorophenyl); 8.34 (s, 1H, CONH).

Elemental analysis of $C_{24}H_{23}Cl_2N_3O$ (MW: 440.36 g/mol);

	% C	% H	% N
Calculated	65.46	5.26	9.54
Found	65.37	5.36	9.62

_N_-(2-Benzylphenyl)-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 6)



Diphenylmethylpiperazine (2 mmol, 0.515 g), 2-benzylphenyl isocyanate (2 mmol, 0.38 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.412 g (89%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 192.1°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.78, 0.82 and 0.02 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 5.12), 224 (log ε : 4.26).

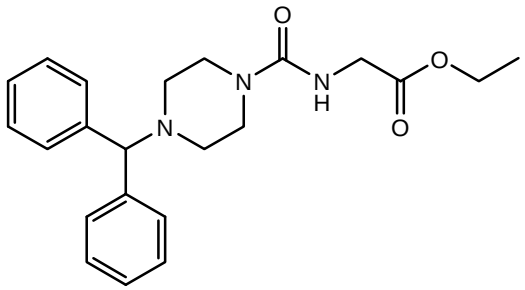
FT-IR (KBr, cm^{-1}); 3251 (N-H), 3060 (C-H, aromatic), 2954 (C-H, aliphatic), 1637 (C=O, amide), 1524 (C=C, aromatic), 1253 (C-N).

^{1}H-NMR (DMSO, ppm); 2.24 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.37 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.91 (s, 2H, -CH_2-); 4.29 (s, 1H, $(Ar)_2CH$-); 7.05-7.25 (m, 10H, diphenyl); 7.30 (m, 5H, phenyl); 7.44 (m, 4H, N-phenyl); 7.97 (s, 1H, CONH).

Elemental analysis of $C_{31}H_{31}N_3O$ (MW: 461.60 g/mol);

	% C	% H	% N
Calculated	80.66	6.77	9.10
Found	80.90	6.48	9.13

Ethyl 2-[4-(diphenylmethyl)piperazino]carbamoyl]acetate (Compound 7, CAS No: 1350123-57-7)



Diphenylmethylpiperazine (2 mmol, 0.515 g), ethyl isocyanatoacetate (2 mmol, 0.24 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.263 g (69%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 150°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.55, 0.63 and 0.06 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.87), 223 (log ε : 4.35).

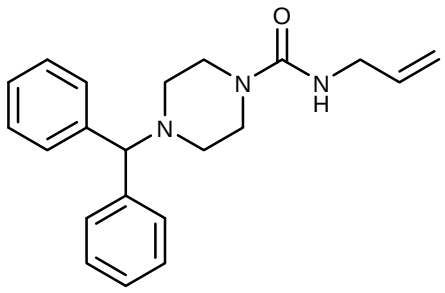
FT-IR (KBr, cm^{-1}); 3360 (N-H), 3026 (C-H, aromatic), 2986 (C-H, aliphatic), 1755 (C=O, ester), 1636 (C=O, amide), 1531 (C=C, aromatic), 1192 (C-O), 1147 (C-N).

^{1}H-NMR (DMSO, ppm); 1.17 (t, 3H, -CH_2-$\underline{CH_3}$, *J=6.8 Hz*); 2.25 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 3.31 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.68 (d, 2H, -NH-$\underline{CH_2}$-, *J=5.6 Hz*); 4.05 (q, 2H, -O-$\underline{CH_2}$-); 4.30 (s, 1H, $(Ar)_2CH$-); 6.93 (t, 1H, CONH, *J=6 Hz*); 7.19 (t, 2H, diphenyl H_4, $H_{4'}$, *J=7.2 Hz*); 7.29 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=7.2 Hz*); 7.44 (d, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=7.6 Hz*).

Elemental analysis of $C_{22}H_{27}N_3O_3$ (MW: 381.47 g/mol);

	% C	% H	% N
Calculated	69.27	7.13	11.02
Found	69.24	6.96	10.96

***N*-Allyl-4-(diphenylmethyl)piperazine-1-carboxamide (Compound 8, CAS No: 1349487-56-4)**



Diphenylmethylpiperazine (2 mmol, 0.515 g), allyl isocyanate (2 mmol, 0.18 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (25 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.323 g (96%).

The form of compound white, shiny, flat crystals and the compound has a melting point of 213.6°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.56, 0.66 and 0.07 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.32), 226 (log ε : 4.75).

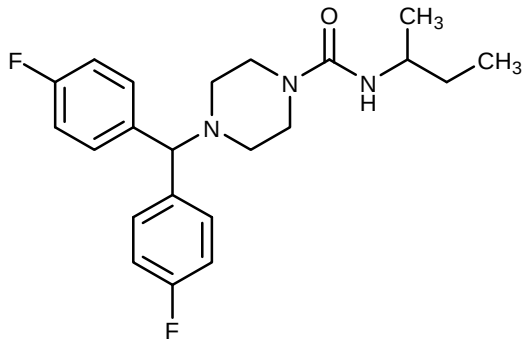
FT-IR (KBr, cm^{-1}); 3343 (N-H), 3027 (C-H, aromatic), 2954 (C-H, aliphatic), 1625 (C=O, amide), 1546 (C=C, aromatic), 1255 (C-N).

^{1}H-NMR (DMSO, ppm); 2.23 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.30 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.63 (t, 2H, -$\underline{CH_2}$-, *J=5.2 Hz*); 4.29 (s, 1H, $(Ar)_2CH$-); 5.0 (dd, 2H, -CH=$\underline{CH_2}$, J_1*=17.2 Hz,* J_2*=8 Hz,* J_3*=1.6 Hz*); 5.78 (m, 1H, -$\underline{CH}$=CH_2); 6.61 (t, 1H, CONH, *J=5.2 Hz*); 7.17 (t, 2H, diphenyl H_4, $H_{4'}$, *J=7.6 Hz*); 7.29 (t, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$, *J=7.6 Hz*); 7.43 (d, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$, *J=8.8 Hz*).

Elemental analysis of $C_{21}H_{25}N_3O$ (MW: 335.44 g/mol);

	% C	% H	% N
Calculated	75.19	7.51	12.53
Found	75.09	7.25	12.46

N-sec-Butyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 9)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), *sec*-butyl isocyanate (1.7 mmol, 0.2 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.208 g (54%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 157.7°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.35, 0.68 and 0.10 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.24), 225 (log ε : 4.71).

FT-IR (KBr, cm^{-1}); 3310 (N-H), 3076 (C-H, aromatic), 2965 (C-H, aliphatic), 1615 (C=O, amide), 1548 (C=C, aromatic), 1247 (C-N), 1223 (C-F).

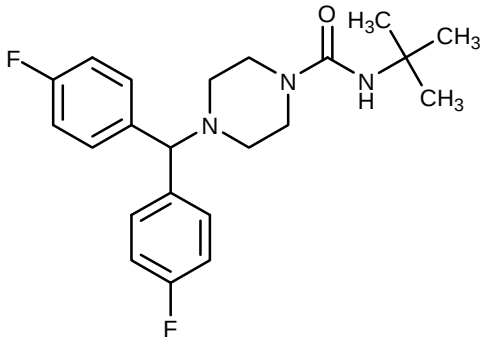
^{1}H-NMR (DMSO, ppm); 0.8 (t, 3H, -$CH_2\underline{CH_3}$, *J=7.2 Hz*); 0.98 (d, 3H, -CH-$\underline{CH_3}$, *J=6.8 Hz*); 2.24 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 2.5 (m, 2H, -CH-$\underline{CH_2}$-CH_3); 3.28 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.54 (m, 1H, -NH-$\underline{CH}$-); 4.38 (s, 1H, $(Ar)_2CH$-); 6.04 (d, 1H, CONH, *J=7.6 Hz*); 7.10-7.16 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.41-7.45 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

MS (m/z); 388.95 (M^+); 290.00 ((4-F-C_6H_5)$_2$CH[N(C_2H_4)$_2$N]H$^+$); 203.5 (100%, (4-F-C_6H_5)$_2$CH$^+$).

Elemental analysis of $C_{22}H_{27}F_2N_3O$ (MW: 387.46 g/mol);

	% C	% H	% N
Calculated	68.20	7.02	10.84
Found	67.44	7.01	10.89

N-tert-Butyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 10)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), *tert*-butyl isocyanate (1.7 mmol, 0.21 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.317 g (82%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 162.4°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.47, 0.60 and 0.13 respectively.

UV (MeOH, λ_{max}, nm); 208 (log ε : 5.32), 227 (log ε : 4.78).

FT-IR (KBr, cm^{-1}); 3332 (N-H), 3046 (C-H, aromatic), 2968 (C-H, aliphatic), 1623 (C=O, amide), 1537 (C=C, aromatic), 1259 (C-N), 1219 (C-F).

^{1}H-NMR (DMSO, ppm); 1.22 (s, 9H, C(CH_3)$_3$); 2.20 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.24 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.38 (s, 1H, (Ar)$_2$CH-); 5.68 (s,

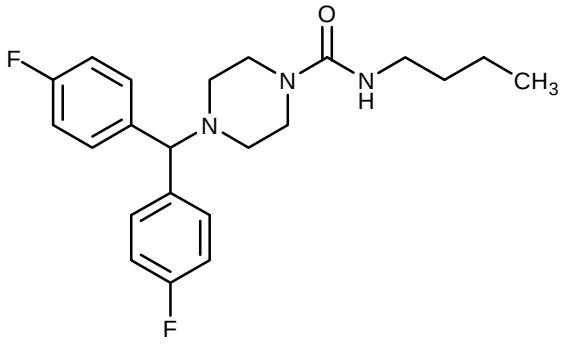
1H, CONH); 7.10-7.16 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.41-7.45 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

MS (m/z); 388.88 (100%, M^+); 203.51 ($(4\text{-F-}C_6H_5)_2CH^+$).

Elemental analysis of $C_{22}H_{27}F_2N_3O$ (MW: 387.46 g/mol);

	% C	% H	% N
Calculated	68.20	7.02	10.84
Found	67.96	7.32	10.87

_N_-Butyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 11)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), butyl isocyanate (1.7 mmol, 0.20 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.174 g (45%).

The form of compound is white, opaque, flat crystals and the compound has a melting point of 132.9°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.35, 0.52 and 0.06 respectively.

UV (MeOH, λ_{max}, nm); 209 (log ε : 5.43), 226 (log ε : 4.83).

FT-IR (KBr, cm^{-1}); 3402 (N-H), 3073 (C-H, aromatic), 2962 (C-H, aliphatic), 1629 (C=O, amide), 1531 (C=C, aromatic), 1251 (C-N), 1217 (C-F).

^{1}H-NMR (DMSO, ppm); 0.85 (t, 3H, -CH_3, *J=7.2 Hz*) 1.20-1.27 (m, 2H, -$\underline{CH_2}$-CH_3); 1.31-1.37 (m, 4H, -$\underline{CH_2}\underline{CH_2}CH_3$-); 2.21 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*);

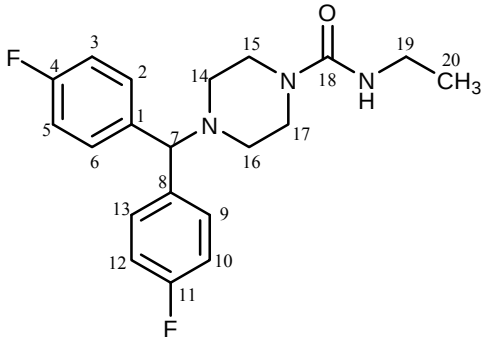
2.95-3.06 (q, 2H, -NH-$\underline{CH_2}$-); 3.27 (t, 4H, piperazine H_2, H_6, *J=5.2 Hz*); 4.38 (s, 1H, $(Ar)_2CH$-); 6.38 (t, 1H, CONH); 7.1-7.15 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.41-7.45 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

MS (m/z); 388.93 (100%, M^+); 203.55 ($(4\text{-F-}C_6H_5)_2CH^+$).

Elemental analysis of $C_{22}H_{27}F_2N_3O$ (MW: 387.46 g/mol);

	% C	% H	% N
Calculated	68.20	7.02	10.84
Found	67.92	6.82	10.85

_N_-Ethyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 12)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), ethyl isocyanate (1.7 mmol, 0.14 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.297 g (83%).

The form of compound is white, opaque, cottonlike crystals and the compound has a melting point of 175°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.21, 0.50 and 0.01 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.39), 225 (log ε : 4.81).

FT-IR (KBr, cm^{-1}); 3349 (N-H), 3060 (C-H, aromatic), 2972 (C-H, aliphatic), 1617 (C=O, amide), 1544 (C=C, aromatic), 1253 (C-N), 1216 (C-F).

^{1}H-NMR (DMSO, ppm); 0.98 (t, 3H, -CH_3, *J=6.8 Hz*); 2.21 (t, 4H, piperazine H_3, H_5, *J=4 Hz*); 3.05-2.98 (m, 2H, -CH_2-); 3.28 (t, 4H, piperazine H_2, H_6, *J=4 Hz*); 4.38 (s, 1H, $(Ar)_2CH$-); 6.42 (t, 1H, CONH, *J=5.2 Hz*); 7.11-7.15 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.42-7.45 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

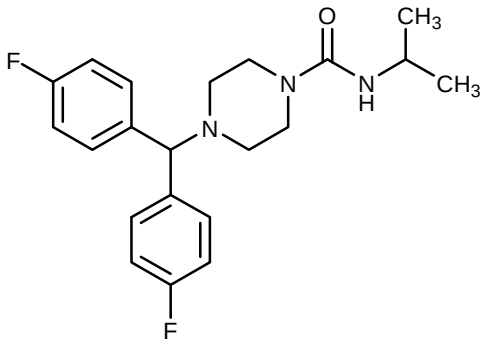
^{13}C-NMR (DMSO, ppm); 16.26 (C_{20}); 35.44 (C_{19}); 44.03 ($C_{14,16}$); 51.84 ($C_{15,17}$); 73.48 (C_7); 115.91-116.12 ($C_{3,5,10,12}$); 130.03-130.12 ($C_{2,6,9,13}$); 139.20, 139.17 ($C_{1,8}$); 157.96-160.52 ($C_{4,11}$); 162.95 (C_{18}).

MS (m/z); 360.85 (M^+); 203.53 (100%, $(4\text{-F-}C_6H_5)_2CH^+$)

Elemental analysis of $C_{20}H_{23}F_2N_3O$ (MW: 359.41 g/mol);

	% C	% H	% N
Calculated	66.84	6.45	11.69
Found	66.44	6.28	11.68

_N-Iso_propyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 13)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), isopropyl isocyanate (1.7 mmol, 0.17 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.345 g (92%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 169.9°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.35, 0.65 and 0.04.

UV (MeOH, λ_{max}, nm); 205 (log ε : 5.25), 223 (log ε : 4.47).

FT-IR (KBr, cm^{-1}); 3331 (N-H), 3074 (C-H, aromatic), 2976 (C-H, aliphatic), 1615 (C=O, amide), 1547 (C=C, aromatic), 1252 (C-N), 1215 (C-F).

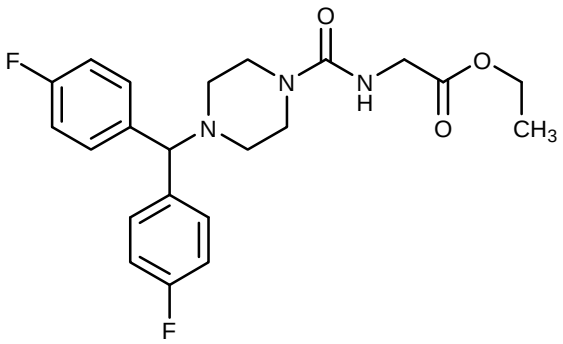
^{1}H-NMR (DMSO, ppm); 1.01 (d, 6H, -CH($\underline{CH_3}$)$_2$, *J=6.8 Hz*); 2.21 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 3.28 (t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 3.68-3.76 (m, 1H, -$\underline{CH}$(CH_3)$_2$); 4.38 (s, 1H, (Ar)$_2$CH-); 6.10 (d, 1H, CONH, *J=7.6 Hz*); 7.11-7.15 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.42-7.45 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

MS (m/z); 374.87 (M^+); 289.72 ((4-F-C_6H_5)$_2$CHN(C_2H_4)$_2$NH$^+$); 203.54 (100%, (4-F-C_6H_5)$_2$CH$^+$).

Elemental analysis of $C_{21}H_{25}F_2N_3O$ (MW: 373.44 g/mol);

	% C	% H	% N
Calculated	67.54	6.75	11.25
Found	67.87	6.64	11.20

Ethyl 2-[bis(4-fluorophenyl)methyl]piperazino]carbamoylacetate (Compound 14)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), ethyl isocyanatoacetate (1.7 mmol, 0.21 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.08 g (20%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 152.3°C. It is soluble in ethanol in hot medium; DMSO and

acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.52, 0.64 and 0.03 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 4.89), 221 (log ε : 4.29).

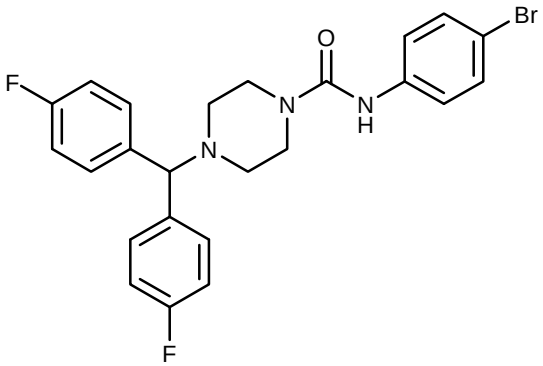
FT-IR (KBr, cm^{-1}); 3359 (N-H), 3070 (C-H, aromatic), 2978 (C-H, aliphatic), 1748 (C=O, ester), 1640 (C=O, amide), 1602 (C=C, aromatic), 1224 (C-O), 1198 (C-N), 1153 (C-F).

^{1}H-NMR (DMSO, ppm); 1.17 (t, 3H, -CH_3, *J=7.2 Hz*); 2.23 (t, 4H, piperazine H_3, H_5, *J=5.2 Hz*); 3.11 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 3.68 (d, 2H, -NH-$\underline{CH_2}$-, *J=6 Hz*); 4.03-4.08 (q, 2H, -O-CH_2-); 4.39 (s, 1H, $(Ar)_2CH$-); 6.93 (t, 1H, CONH, *J=6 Hz*); 7.11-7.16 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.42-7.46 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

Elemental analysis of $C_{22}H_{25}F_2N_3O_3$ (MW: 417.45 g/mol);

	% C	% H	% N
Calculated	63.30	6.04	10.07
Found	63.46	6.05	10.02

_N_-(4-Bromophenyl)-4-[bis(4-fluorophenyl)methyl]piperazine-1-carboxamide (Compound 15)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 4-bromophenyl isocyanate (1.7 mmol, 0.34 g) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.2. The yield is 0.325 g (67%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 210.9 °C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.72, 0.65 and 0.16 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.31), 237 (log ε : 4.15) , 246 (log ε : 4.12).

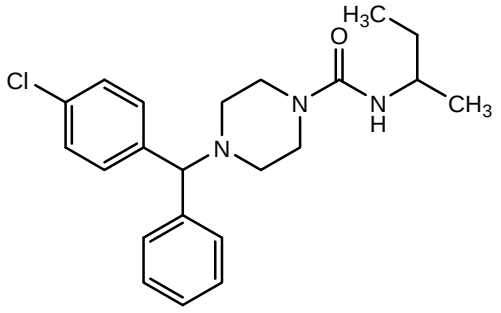
FT-IR (KBr, cm^{-1}); 3290 (N-H), 3044 (C-H, aromatic), 2999 (C-H, aliphatic), 1646 (C=O, amide), 1506 (C=C, aromatic), 1246 (C-N), 1224 (C-F).

^{1}H-NMR (DMSO, ppm); 2.29 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 3.45 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.44 (s, 1H, $(Ar)_2CH$-); 7.12-7.47 (m, 12H, aromatic H's); 8.61 (s, 1H, CONH).

Elemental analysis of $C_{24}H_{23}BrClN_3O$ (MW: 484.82 g/mol);

	% C	% H	% N
Calculated	59.27	4.56	8.64
Found	59.02	4.38	8.73

N-sec-Butyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 16)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), *sec*-butyl isocyanate (0.8717 mmol, 0.1 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.240 g (62%).

The form of compound is white, shiny, clustered crystals and the compound has a melting point above 300°C. It is soluble in ethanol in hot medium; DMSO and acetone

at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.59, 0.76 and 0.09 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.32), 226 (log ε : 4.51).

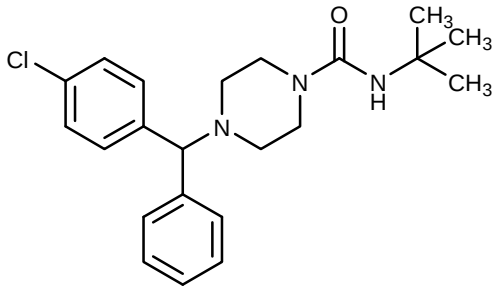
FT-IR (KBr, cm^{-1}); 3393 (N-H), 3027 (C-H, aromatic), 2970 (C-H, aliphatic), 1618 (C=O, amide), 1533 (C=C, aromatic), 1246 (C-N), 1091 (C-Cl).

^{1}H-NMR (DMSO, ppm); 0.78 (t, 3H, -CH_2-$\underline{CH_3}$, *J=7.6 Hz*); 1.00 (d, 3H, -CH-$\underline{CH_3}$, *J=6.8 Hz*); 1.32-1.40 (m, 2H, -CH-$\underline{CH_2}$-CH_3); 2.22 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 3.28 (t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 3.51-3.55 (m, 1H, -NH$\underline{CH}$); 4.35 (s, 1H, $(Ar)_2CH$-); 6.03 (d, 1H, CONH, *J=8 Hz*); 7.18-7.46 (m, 9H, diphenyl).

Elemental analysis of $C_{22}H_{28}ClN_3O$ (MW: 385.93 g/mol);

	% C	% H	% N
Calculated	68.47	7.31	10.89
Found	68.65	7.20	10.93

N-tert-Butyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 17)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), *tert*-butyl isocyanate (0.8717 mmol, 0.1 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.137 g (36%).

The form of compound is white, shiny, flat crystals and the compound has a melting point of 190.3°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.70, 0.84 and 0.21 respectively.

UV (MeOH, λ_{max}, nm); 207 (log ε : 5.29), 225 (log ε : 4.52).

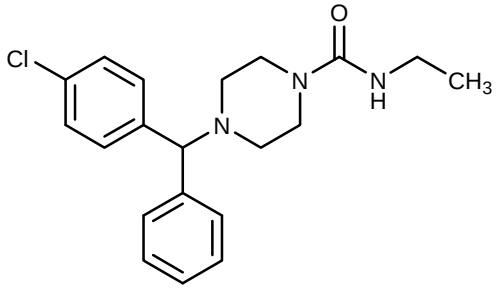
FT-IR (KBr, cm^{-1}); 3371 (N-H), 3027 (C-H, aromatic), 2968 (C-H, aliphatic), 1629 (C=O, amide), 1538 (C=C, aromatic), 1257 (C-N), 1092 (C-Cl).

^{1}H-NMR (DMSO, ppm); 1.19 (s, 9H, -C(CH_3)$_3$); 2.19 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.21 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.31 (s, 1H, $(Ar)_2CH$-); 5.65 (s, 1H, CONH); 7.17-7.42 (m, 9H, diphenyl).

Elemental analysis of $C_{22}H_{28}ClN_3O$ (MW: 385.93 g/mol);

	% C	% H	% N
Calculated	68.47	7.31	10.89
Found	68.67	7.23	10.93

_N_-Ethyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 18)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), ethyl isocyanate (0.8717 mmol, 0.07 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.06 g (17%).

The form of compound is white, shiny, clustered crystals and the compound has a melting point of 288.6°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.41, 0.56 and 0.05 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 5.24), 224 (log ε : 4.46).

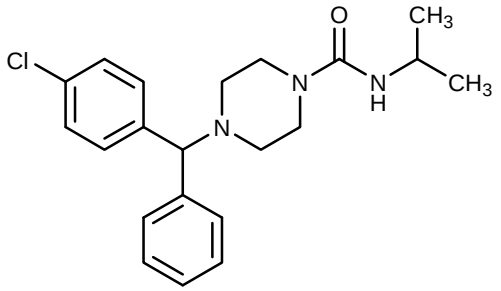
FT-IR (KBr, cm^{-1}); 3363 (N-H), 3020 (C-H, aromatic), 2970 (C-H, aliphatic), 1620 (C=O, amide), 1539 (C=C, aromatic), 1254 (C-N), 1090 (C-Cl).

^{1}H-NMR (DMSO, ppm); 0.98 (t, 3H, -CH_3, *J=7.2 Hz*); 2.22 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 3.00-3.03 (m, 2H, -$\underline{CH_2}$-); 3.27 (t, 4H, piperazine H_2, H_6, *J=5.2 Hz*); 4.34 (s, 1H, $(Ar)_2CH$-); 6.41 (t, 1H, CONH, *J=5.6 Hz*); 7.18-7.46 (m, 9H, diphenyl).

Elemental analysis of $C_{20}H_{24}ClN_3O$ (MW: 357.88 g/mol);

	% C	% H	% N
Calculated	67.12	6.76	11.74
Found	67.22	6.69	11.79

_N_-Isopropyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 19)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), isopropyl isocyanate (0.8717 mmol, 0.09 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.128 g (34%).

The form of compound is white, shiny, flat crystals and the compound has a melting point of 198.6°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.5, 0.65 and 0.07 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 5.15), 223 (log ε : 4.45).

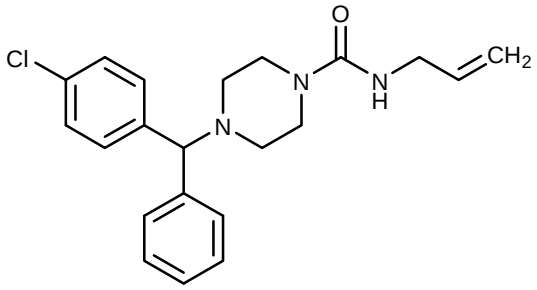
FT-IR (KBr, cm^{-1}); 3390 (N-H), 3020 (C-H, aromatic), 2969 (C-H, aliphatic), 1617 (C=O, amide), 1532 (C=C, aromatic), 1252 (C-N), 1092 (C-Cl).

^{1}H-NMR (DMSO, ppm); 1.01 (d, 6H, -CH(CH$_3$)$_2$, *J=6.8 Hz*); 2.22 (t, 4H, piperazine H$_3$, H$_5$, *J=4.4 Hz*); 3.27 (t, 4H, piperazine H$_2$, H$_6$, *J=5.2 Hz*); 3.68-3.75 (m, 1H, -CH(CH$_3$)$_2$); 4.34 (s, 1H, (Ar)$_2$CH-); 6.08 (d, 1H, CONH, *J=7.6 Hz*); 7.18-7.46 (m, 9H, diphenyl).

Elemental analysis of $C_{21}H_{26}ClN_3O$ (MW: 371,9 g/mol);

	% C	% H	% N
Calculated	67.82	7.05	11.30
Found	67.88	7.11	11.35

_N_-Allyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 20)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), allyl isocyanate (0.8717 mmol, 0.08 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.1 g (27%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 172.7°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.50, 0.65 and 0.04.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.11), 225 (log ε : 4.38).

FT-IR (KBr, cm^{-1}); 3356 (N-H), 3027 (C-H, aromatic), 2981 (C-H, aliphatic), 1622 (C=O, amide), 1543 (C=C, aromatic), 1252 (C-N), 1094 (C-Cl).

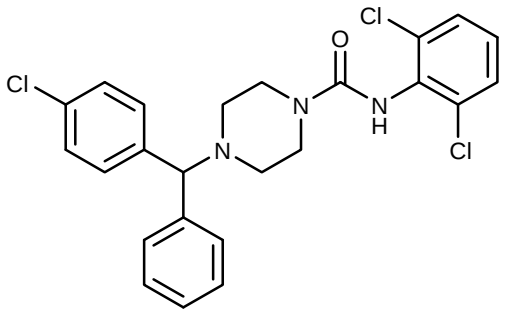
^{1}H-NMR (DMSO, ppm); 2.23 (t, 4H, piperazine H$_3$, H$_5$, *J=4.8 Hz*); 3.30 (t, 4H, piperazine H$_2$, H$_6$, *J=4.8 Hz*); 3.63 (t, 2H, NH-CH$_2$-CH=, *J=4.8 Hz*); 4.34 (s, 1H,

$(Ar)_2CH$-); 4.97-5.08 (dd, 2H, -CH=$\underline{CH_2}$ *J_1=16 Hz, J_2=10 Hz, J_3=1.6 Hz*); 5.75-5.82 (m, 1H, -$\underline{CH}$=CH_2); 6.62 (t, 1H, CONH); 7.18-7.46 (m, 9H, diphenyl).

Elemental analysis of $C_{21}H_{24}ClN_3O$ (MW: 371.9 g/mol);

	% C	% H	% N
Calculated	68.19	6.54	11.36
Found	68.52	6.43	11.43

_N_-(2,6-Dichlorophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 21)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), 2,6-dichlorophenyl isocyanate (0.8717 mmol, 0.1673 g) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.178 g (38%).

The form of compound is white, shiny, powdered crystals and the compound has a melting point of 224.6°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.65, 0.80 and 0.13 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 4.47), 245 (log ε : 4.12).

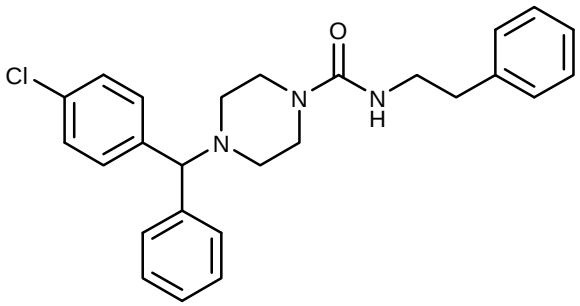
FT-IR (KBr, cm^{-1}); 3316 (N-H), 3020 (C-H, aromatic), 2963 (C-H, aliphatic), 1645 (C=O, amide), 1519 (C=C, aromatic), 1254 (C-N), 1089 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.31 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.46 (t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 4.41 (s, 1H, $(Ar)_2CH$-); 7.21-7.49 (m, 12H, aromatic H's); 8.37 (s, 1H, CONH).

Elemental analysis of $C_{24}H_{22}Cl_3N_3O$ (MW: 474.81 g/mol);

	% C	% H	% N
Calculated	60.71	4.67	8.85
Found	60.70	4.77	9.18

_N_-(2-Phenylethyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 22)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), phenethyl isocyanate (0.8717 mmol, 0.12 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.212 g (49%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 147.8°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.68, 0.85 and 0.11 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 4.52), 245 (log ε : 4.07).

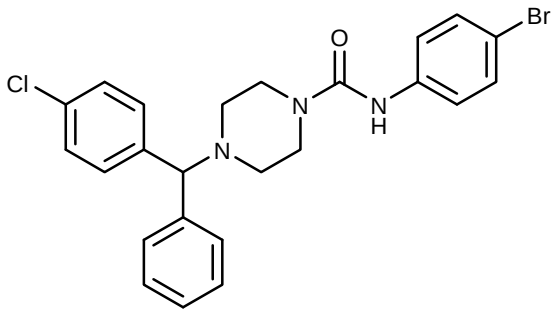
FT-IR (KBr, cm^{-1}); 3307 (N-H), 3022 (C-H, aromatic), 2955 (C-H, aliphatic), 1617 (C=O, amide), 1543 (C=C, aromatic), 1256 (C-N), 1091 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.22 (t, 4H, piperazine H_3, H_5, *J=4.4 Hz*); 2.69 (t, 2H, -$\underline{CH_2}$-C_6H_5, *J=6.8 Hz*); 3.19 (q, 2H, -NH$\underline{CH_2}$); 3.28 (t, 4H, piperazine H_2, H_6, *J=5.2 Hz*); 4.34 (s, 1H, $(Ar)_2CH$-); 6.55 (t, 1H, CONH, *J=5.6 Hz*); 7.15-7.46 (m, 14H, aromatic H's).

Elemental analysis of $C_{26}H_{28}ClN_3O$ (MW: 433.97 g/mol);

	% C	% H	% N
Calculated	71.96	6.50	9.68
Found	72.04	6.72	9.70

_N_-(4-Bromophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 23)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), 4-bromophenyl isocyanate (0.8717 mmol, 0.1744 g) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.180 g (37%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 195.5°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.71, 0.73 and 0.20 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 4.33), 236 (log ε : 4.11).

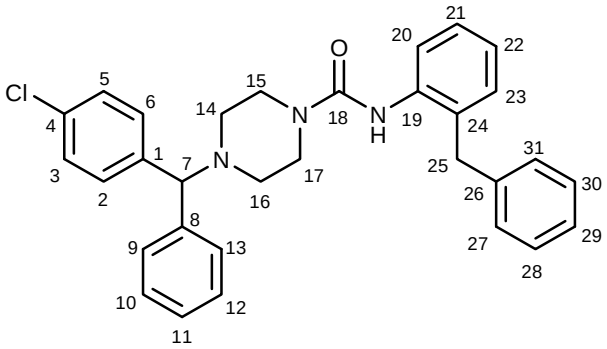
FT-IR (KBr, cm^{-1}); 3316 (N-H), 3028 (C-H, aromatic), 2966 (C-H, aliphatic), 1634 (C=O, amide), 1537 (C=C, aromatic), 1243 (C-N), 1089 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.30 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.45 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.39 (s, 1H, $(Ar)_2CH$-); 7.21-7.47 (m, 13H, aromatic H's); 8.6 (s, 1H, CONH).

Elemental analysis of $C_{24}H_{23}BrClN_3O$ (MW: 484.82 g/mol);

	% C	% H	% N
Calculated	59.46	4.78	8.67
Found	59.43	4.97	8.84

_N_-(2-Benzylphenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 24)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), 2-benzylphenyl isocyanate (0.8717 mmol, 0.17 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.219 g (44%).

The form of compound is white, shiny, needle-shaped crystals and the compound has a melting point of 174.6°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.81, 0.85 and 0.17 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 4.57), 224 (log ε : 4.21).

FT-IR (KBr, cm^{-1}); 3332 (N-H), 3026 (C-H, aromatic), 2967 (C-H, aliphatic), 1626 (C=O, amide), 1523 (C=C, aromatic), 1251 (C-N), 1089 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.23 (bs, 4H, piperazine H_3, H_5); 3.36 (bs, 4H, piperazine H_2, H_6); 3.91 (s, 2H, -CH_2-); 4.34 (s, 1H, $(Ar)_2CH$-); 7.05-7.47 (m, 18H, aromatic H's); 7.96 (s, 1H, CONH).

^{13}C-NMR (DMSO, ppm); 37.74 (C_{25}); 44.46 ($C_{14,16}$); 51.91 ($C_{15,17}$); 74.48 (C_7); 125.46 (C_{20}); 126.52 (C_{22}); 126.97 (C_{29}); 127.23 (C_{21}); 127.79 ($C_{27,31}$); 128.31 (C_{11}); 128.91 ($C_{9,13}$); 129.23 (C_{23}); 129.33 ($C_{28,30}$); 129.42 ($C_{10,12}$); 130.11 ($C_{3,5}$); 130.64 ($C_{2,6}$);

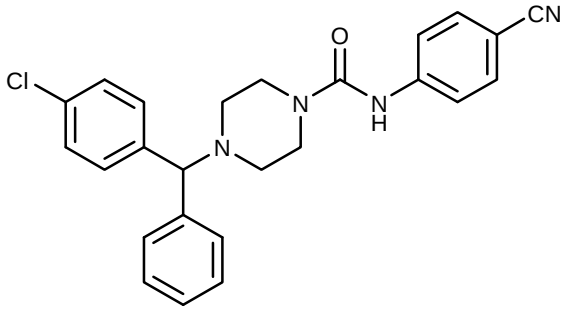
132.09 (C_4); 136.87 (C_{19}); 138.23 (C_1); 141.05 (C_{26}); 142.26 (C_8); 142.64 (C_{24}); 156.06 (C_{18}).

MS (m/z); 496.9 (M^+, 100%); 498.9 (M+2, 33%); 287.8 ((4-Cl-C_6H_5)(C_6H_5)CH-$N(C_2H_4)_2N^+$); 201.6 ((4-Cl-C_6H_5)(C_6H_5)CH^+).

Elemental analysis of $C_{31}H_{30}ClN_3O$ (MW: 496.04 g/mol);

	% C	% H	% N
Calculated	75.06	6.10	8.47
Found	75.13	6.28	8.54

_N_-(4-Cyanophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (Compound 25)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), 4-cyanophenyl isocyanate (0.8717 mmol, 0.1295 g) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.1. The yield is 0.114 g (26%).

The form of compound is white, shiny, powdered crystals and the compound has a melting point of 196.8°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.53, 0.54 and 0.11 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.82), 270 (log ε : 4.59).

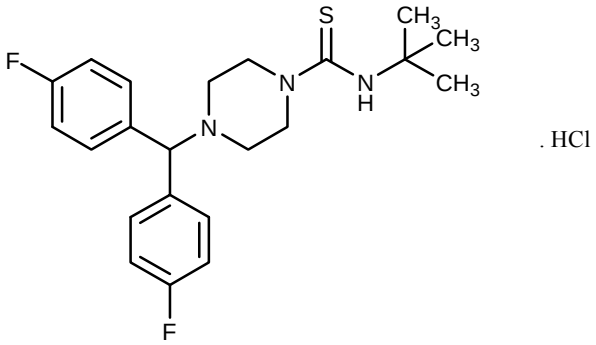
FT-IR (KBr, cm^{-1}); 3278 (N-H), 3027 (C-H, aromatic), 2951 (C-H, aliphatic), 2221 (C≡N), 1653 (C=O, amide), 1513 (C=C, aromatic), 1245 (C-N), 1089 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.32 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.48 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.41 (s, 1H, $(Ar)_2CH$-); 7.21-7.47 (m, 9H, diphenyl); 7.61-7.67 (m, 4H, 4-cyanophenyl); 8.97 (s, 1H, CONH).

Elemental analysis of $C_{25}H_{23}ClN_4O$ (MW: 430.93 g/mol);

	% C	% H	% N
Calculated	69.68	5.38	13.00
Found	69.74	5.50	13.06

N-tert-Butyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carbothioamide hydrochloride (Compound 26)



. HCl

1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), *tert*-butyl isothiocyanate (1.7 mmol, 0.22 mL) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (60 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.06 g (14%).

The form of compound is yellowish white, opaque, powdered crystals and the compound has a melting point of 176.8°C. It is soluble in ethanol and acetone in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.78, 0.79 and 0.25 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 4.23), 223 (log ε : 4.11).

FT-IR (KBr, cm^{-1}); 3258 (N-H), 3057 (C-H, aromatic), 2972 (C-H, aliphatic), 1606 (C=C, aromatic), 1288 (C-N), 1236 (C=S, thioamide), 1189 (C-F).

^{1}H-NMR (DMSO, ppm); 1.45 (s, 9H, $-C(CH_3)_3$); 2.96-3.15 (m, 4H, piperazine H_3, H_5); 3.65 (t, 4H, piperazine H_2, H_6); 4.59 (d, 1H, $(Ar)_2CH$-, *J=14.4 Hz*); 5.75 (d, 1H,

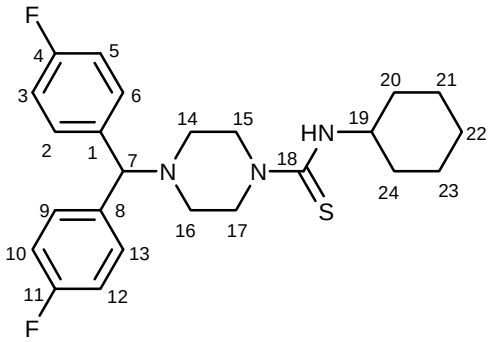
CSNH, *J=8.8 Hz*); 7.17-7.33 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.95 (bs, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$); 12.55 (bs, 1H, N-H salt).

MS (m/z); 404.90 (100%, M^+ - Cl); 205.3 ($(4\text{-F-}C_6H_5)_2CH^+$).

Elemental analysis of $C_{22}H_{28}ClF_2N_3S$ (MW: 439.99 g/mol);

	% C	% H	% N	% S
Calculated	60.05	6.41	9.55	7.29
Found	59.55	6.45	9.47	6.64

_N_-Cyclohexyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carbothioamide (Compound 27)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), cyclohexyl isothio-cyanate (1.7 mmol, 0.25 mL) and triethylamine (5.1 mmol, 0.70 mL) in DMSO (40 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.214 g (50%).

The form of compound is white, shiny, needle-shaped crystals and the compound has a melting point of 198.2°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.81, 0.81 and 0.23 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.10), 224 (log ε : 4.02), 248 (log ε : 3.88).

FT-IR (KBr, cm^{-1}); 3328 (N-H), 3060 (C-H, aromatic), 2996 (C-H, aliphatic), 1603 (C=C, aromatic), 1299 (C-N), 1221 (C=S, thioamide), 1104 (C-F).

^{1}H-NMR (DMSO, ppm); 1.13-1.21 (m, 5H, cyclohexyl); 1.53-1.79 (m, 6H, cyclohexyl); 2.22 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.71 (t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 4.12 (s, 1H, CSNH); 4.40 (s, 1H, $(Ar)_2CH$-); 7.09-7.14 (m, 4H, diphenyl H_2, H_6, $H_{2'}$, $H_{6'}$); 7.39-7.43 (m, 4H, diphenyl H_3, H_5, $H_{3'}$, $H_{5'}$).

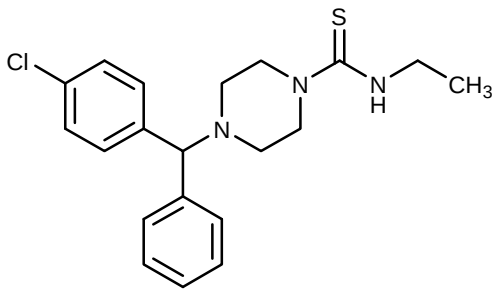
^{13}C-NMR (DMSO, ppm); 24.99 ($C_{21,23}$); 25.18 (C_{22}); 31.97 ($C_{20,24}$); 47.06 ($C_{14,16}$); 50.85 ($C_{15,17}$); 54.28 (C_{19}); 72.32 (C_7); 115.14 ($C_{10,12}$); 115.35 ($C_{3,5}$); 129.35 ($C_{9,13}$); 129.43 ($C_{2,6}$); 138.12 (C_8); 138.15 (C_1); 159.79 (C_{11}); 162.21 (C_4); 180.14 (C_{18}).

MS (m/z); 430.95 (100%, M^+); 203.65 ($(4\text{-F-}C_6H_5)_2CH^+$)

Elemental analysis of $C_{24}H_{29}F_2N_3S$ (MW: 429.57 g/mol);

	% C	% H	% N	% S
Calculated	67.10	6.80	9.78	7.46
Found	66.94	6.94	9.89	7.42

_N_-Ethyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carbothioamide (Compound 28)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), ethyl isothiocyanate (0.8717 mmol, 0.08 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.056 g (15%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 150.6°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.65, 0.66 and 0.14 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 4.25), 225 (log ε : 4.13).

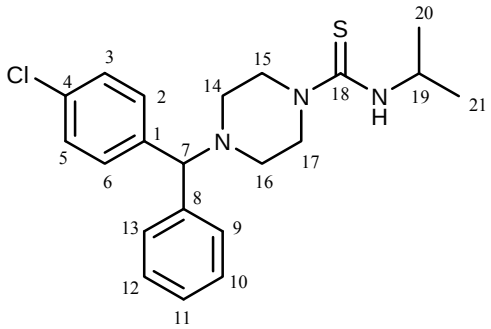
FT-IR (KBr, cm^{-1}); 3294 (N-H), 3020 (C-H, aromatic), 2966 (C-H, aliphatic), 1531 (C=C, aromatic), 1255 (C-N), 1229 (C=S, thioamide), 1289 (C-N), 1091 (C-Cl).

^{1}H-NMR (DMSO, ppm); 1.06 (t, 3H, -CH_3, *J=6.8 Hz*); 2.27 (t, 4H, piperazine H_3, H_5, *J=5.2 Hz*); 3.52-3.45 (m, 2H, -CH_2-); 3.75 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.39 (s, 1H, $(Ar)_2CH$-); 7.19-7.46 (m, 9H, diphenyl); 7.61 (t, 1H, CSNH).

Elemental analysis of $C_{20}H_{24}ClN_3S$ (MW: 373.94 g/mol);

	% C	% H	% N	% S
Calculated	64.24	6.47	11.24	8.57
Found	64.44	6.19	11.35	8.67

_N_-Isopropyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carbothioamide (Compound 29)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), isopropyl isothiocyanate (0.8717 mmol, 0.1 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.15 g (39%).

The form of compound is white, shiny, needle-shaped crystals and the compound has a melting point of 252.4°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.72, 0.81 and 0.22 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 4.31), 223 (log ε : 4.15).

FT-IR (KBr, cm^{-1}); 3371 (N-H), 3059 (C-H, aromatic), 2967 (C-H, aliphatic), 1539 (C=C, aromatic), 1270 (C-N), 1232 (C=S, thioamide), 1232 (C-N), 1001 (C-Cl).

^{1}H-NMR (DMSO, ppm); 1.09 (d, 6H, -$(CH_3)_2$, *J=6.8 Hz*); 2.27 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.76 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.39 (s, 1H, $(Ar)_2$CH-); 4.44-4.53 (m, 1H, -CH$(CH_3)_2$); 7.19-7.46 (m, 10H, diphenyl H's + NH).

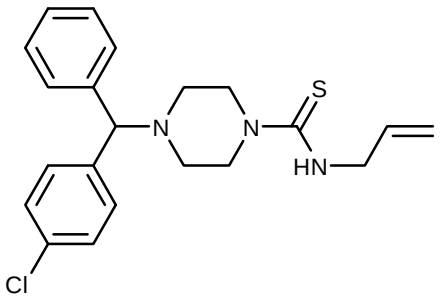
^{13}C-NMR (DMSO, ppm); 38.79-40.05 ($C_{20,21}$); 46.93-47.01 ($C_{14,15,16,17}$); 50.93 (C_{19}); 73.36 (C_7); 127.05 (C_{11}); 127.57 ($C_{9,13}$); 128.42 ($C_{10,12}$); 128.52 ($C_{3,5}$); 129.35 ($C_{2,6}$); 131.32 (C_4); 141.28 (C_8); 141.64 (C_1); 180.17 (C_{18}).

MS (m/z); 388.8 (M^+, 100%); 390.8 (M+2, 33%); 201.5 (4-Cl-C_6H_5)(C_6H_5)CH$^+$)

Elemental analysis of $C_{21}H_{26}ClN_3S$ (MW: 387.97 g/mol);

	% C	% H	% N	% S
Calculated	65.01	6.75	10.83	8.26
Found	64.88	6.88	10.87	8.29

_N_-Allyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carbothioamide (Compound 30)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), allyl isothiocyanate (0.8717 mmol, 0.09 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.040 g (10%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 139.4°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.71, 0.74 and 0.18 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 4.27), 225 (log ε : 4.13).

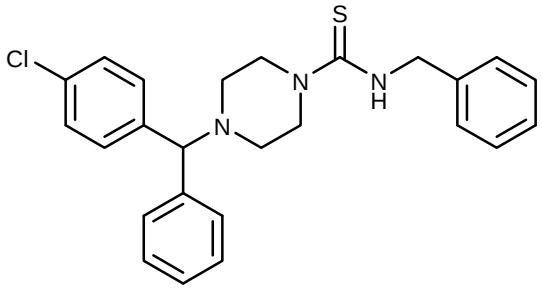
FT-IR (KBr, cm^{-1}); 3296 (N-H), 3023 (C-H, aromatic), 2960 (C-H, aliphatic), 1528 (C=C, aromatic), 1252 (C-N), 1224 (C=S, thioamide), 1223 (C-N), 1090 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.28 (t, 4H, piperazine H_3, H_5, *J=5.2 Hz*); 3.79 (t, 4H, piperazine H_2, H_6, *J=4 Hz*); 4.15 (t, 2H, -$\underline{CH_2}$-CH=CH_2, *J=5.6 Hz*); 4.39 (s, 1H, $(Ar)_2$CH-); 5.01-5.11 (dd, 2H, -CH=C$\underline{H_2}$, J_1*=17.2 Hz*, J_2*=8.6 Hz*, J_3*=1.6 Hz*); 5.80-5.90 (m, 1H, -$\underline{CH}$=CH_2); 7.19-7.46 (m, 9H, diphenyl); 7.80 (t, 1H, CSNH, *J=5.6 Hz*).

Elemental analysis of $C_{21}H_{24}ClN_3S$ (MW: 385.95 g/mol);

	% C	% H	% N	% S
Calculated	65.35	6.27	10.89	8.31
Found	65.71	6.44	11.01	8.28

_N_-Benzyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carbothioamide (Compound 31)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), benzyl isothiocyanate (0.8717 mmol, 0.12 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.1 g (23%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 157.2°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.8, 0.78 and 0.23 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 4.51), 226 (log ε : 4.33).

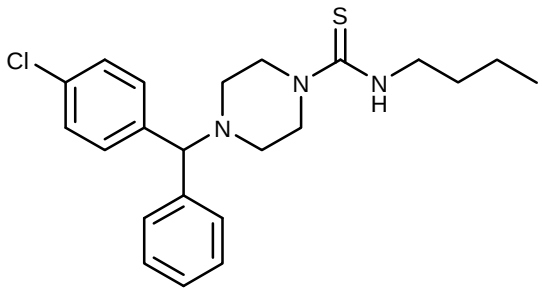
FT-IR (KBr, cm^{-1}); 3236 (N-H), 3020 (C-H, aromatic), 2813 (C-H, aliphatic), 1539 (C=C, aromatic), 1246 (C-N), 1211 (C=S, thioamide), 1246 (C-N), 1001 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.31 (t, 4H, piperazine H_3, H_5, *J=4.8 Hz*); 3.83 (t, 4H, piperazine H_2, H_6, *J=4.4 Hz*); 4.41 (s, 1H, $(Ar)_2CH$-); 4.79 (d, 2H, -CH_2-, *J=5.2 Hz*); 7.19-7.47 (m, 14H, aromatic H's); 8.19 (t, 1H, CSNH, *J=5.6 Hz*).

Elemental analysis of $C_{25}H_{26}ClN_3S$ (MW: 436.01 g/mol);

	% C	% H	% N	% S
Calculated	68.87	6.01	9.64	7.35
Found	69.02	5.98	9.80	7.46

_N_-Butyl-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carbothioamide hydrochloride (Compound 32)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.8717 mmol, 0.2632 g), butyl isothiocyanate (0.8717 mmol, 0.1 mL) and triethylamine (2.6151 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.3. The yield is 0.080 g (20%).

The form of compound is white, opaque, featherlike crystals and the compound has a melting point of 125.5°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.75, 0.78 and 0.25 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 4.43), 227 (log ε : 4.35).

FT-IR (KBr, cm^{-1}); 3261 (N-H), 3028 (C-H, aromatic), 2958 (C-H, aliphatic), 1541 (C=C, aromatic), 1298 (C-N), 1201 (C=S, thioamide), 1201 (C-N), 1001 (C-Cl).

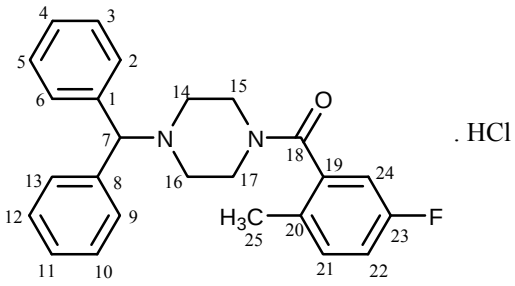
^{1}H-NMR (DMSO, ppm); 0.87 (t, 3H, -$CH_2CH_2\underline{CH_3}$, *J=7.2 Hz*); 1.20-1.29 (m, 2H, -$CH_2\underline{CH_2}CH_3$); 1.44-1.52 (m, 2H, -$\underline{CH_2}CH_2CH_3$); 2.27 (t, 4H, piperazine H_3, H_5,

J=4.8 Hz); 3.42-3.47 (q, 2H, -NH$\underline{CH_2}$-); 3.75 (t, 4H, piperazine H_2, H_6, *J=4.8 Hz*); 4.39 (s, 1H, $(Ar)_2CH$-); 7.19-7.46 (m, 9H, diphenyl); 7.58 (t, 1H, CSNH, *J=5.6 Hz*).

Elemental analysis of $C_{22}H_{28}ClN_3S$ (MW: 401.17 g/mol);

	% C	% H	% N	% S
Calculated	65.73	7.02	10.45	7.98
Found	66.06	7.07	10.56	8.05

1-((5-Fluoro-2-methyl)benzoyl)-4-(diphenylmethyl)piperazine hydrochloride (Compound 33)



1-Diphenylmethylpiperazine (2 mmol, 0.515 g), (5-fluoro-2-methyl)benzoyl chloride (2 mmol, 0.28 mL) and triethylamine (6 mmol, 0.86 mL) in dry DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.4. The yield is 0.402 g (95%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point above 300°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.79, 0.86 and 0.24 respectively.

UV (MeOH, λ_{max}, nm); 205 (log ε : 5.22), 224 (log ε : 4.74).

FT-IR (KBr, cm^{-1}); 3423 (N-H), 3043 (C-H, aromatic), 2955 (C-H, aliphatic), 1654 (C=O, amide), 1612 (C=C, aromatic), 1293 (C-N), 1261 (C-F).

^{1}H-NMR (DMSO, ppm); 2.13 (s, 3H, $-CH_3$); 3.2 (bs, 4H, piperazine H_3, H_5); 3.76 (bs, 4H, piperazine H_2, H_6); 5.64 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.1-7.91 (m, 13H, aromatic H's); 12.61 (bs, 1H, N-H salt).

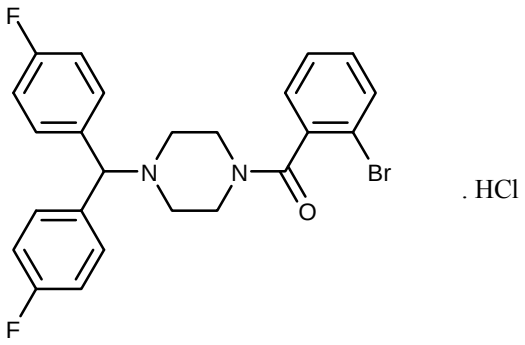
^{13}C-NMR (DMSO, ppm); 37.98 (C_{25}); 43.09 ($C_{14,16}$); 51.49-51.74 ($C_{15,17}$); 75.28 (C_7); 113.31-113.54 ($C_{2,6,9,13}$); 116.36-116.56 ($C_{3,5,10,12}$); 129.17(C_4); 129.55 (C_{11}); 129.97 (C_1); 130.73 (C_8); 132.9 (C_{21}); 132.98 (C_{22}); 136.15 (C_{24}); 137.43 (C_{20}); 159.60 (C_{19}); 162.02(C_{23}); 167.86 (C_{18}).

MS (m/z); 389.8 (100%, M^+ - Cl); 167.5 (($C_6H_5)_2CH^+$)

Elemental analysis of $C_{25}H_{26}ClFN_2O$ (MW: 424.94 g/mol);

	% C	% H	% N
Calculated	70.66	6.17	6.59
Found	69.77	5.94	6.63

1-(2-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine hydrochloride (Compound 34)



. HCl

1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 2-bromobenzoyl chloride (1.7 mmol, 0.38 g) and triethylamine (5.1 mmol, 0.70 mL) in DCM (40 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.3 g (35%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 189.7°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.57, 0.53 and 0.19.

UV (MeOH, λ_{max}, nm);205 (log ε : 5.27), 223 (log ε : 4.49).

FT-IR (KBr, cm^{-1}); 3423 (N-H), 3006 (C-H, aromatic), 2924 (C-H, aliphatic), 1643 (C=O, amide), 1606 (C=C, aromatic), 1292 (C-N), 1232 (C-F).

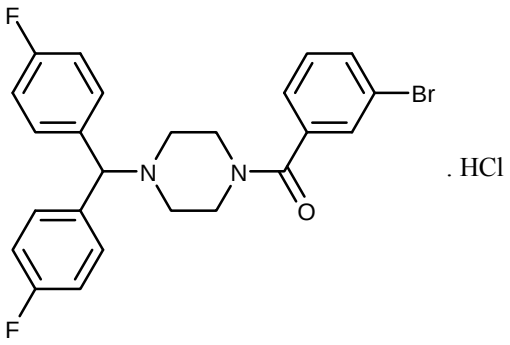
^{1}H-NMR (DMSO, ppm); 3.03 (bs, 4H, piperazine H_3, H_5); 3.67 (bs, 4H, piperazine H_2, H_6); 5.59 (s, 1H, $(Ar)_2CH$-); 7.27-7.91 (m, 17H, aromatic H's); 12.8 (bs, 1H, N-H salt).

MS (m/z); 471.92 (90%, M^+ - Cl), 473.92 (89%, M+2), 203.60 ((100%, $(4\text{-}F\text{-}C_6H_5)_2CH^+$)

Elemental analysis of $C_{24}H_{22}BrClF_2N_2O$ .H_2O (MW: 524.07 g/mol);

	% C	% H	% N
Calculated	54.82	4.60	5.33
Found	54.45	4.83	5.50

1-(3-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine hydrochloride (Compound 35)



. HCl

1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 3-bromobenzoyl chloride (1.7 mmol, 0.23 mL) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (40 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.23 g (27%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 151.4°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.66, 0.66 and 0.28 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.09), 223 (log ε : 4.51).

FT-IR (KBr, cm^{-1}); 3425 (N-H), 3068 (C-H, aromatic), 2924 (C-H, aliphatic), 1638 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F).

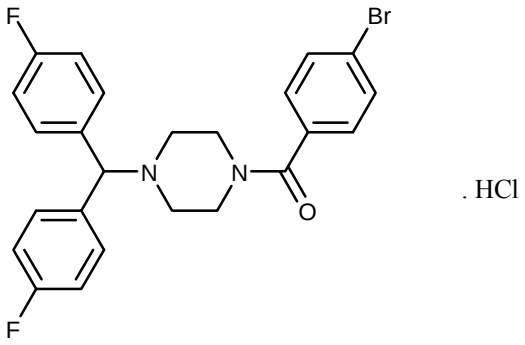
^{1}H-NMR (DMSO, ppm); 3.11 (bs, 4H, piperazine H_3, H_5); 3.67 (bs, 4H, piperazine H_2, H_6); 5.59 (bs, 1H, $(Ar)_2CH$-); 7.27-7.93 (m, 13H, aromatic H's); 12.63 (bs, 1H, N-H salt).

MS (m/z); 471.91 (M^+ - Cl, 95%), 473.91 (M+2, 94%), 203.61 (100%, $(4\text{-}F\text{-}C_6H_5)_2CH^+$)

Elemental analysis of $C_{24}H_{22}BrClF_2N_2O$ .H_2O (MW: 524.07 g/mol);

	% C	% H	% N
Calculated	54.82	4.60	5.33
Found	54.81	4.59	5.60

1-(4-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine hydrochloride (Compound 36)



. HCl

1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 4-bromobenzoyl chloride (1.7 mmol, 0.38 g) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (40 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.1654 g (19%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point higher than 300°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.65, 0.65 and 0.29 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.23), 224 (log ε : 4.46).

FT-IR (KBr, cm^{-1}); 3437 (N-H), 3008 (C-H, aromatic), 2950 (C-H, aliphatic), 1640 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F).

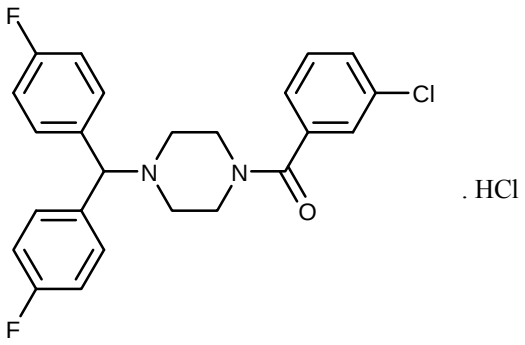
^{1}H-NMR (DMSO, ppm); 3.09 (bs, 4H, piperazine H_3, H_5); 3.68 (bs, 4H, piperazine H_2, H_6); 5.58 (bs, 1H, $(Ar)_2CH$-); 7.15-7.91 (m, 12H, aromatic H's); 12.69 (bs, 1H, N-H salt).

MS (m/z); 471.92 (68%, M^+ - Cl), 473.92 (67%, M+2), 203.59 (100%, $(4\text{-}F\text{-}C_6H_5)_2CH^+$).

Elemental analysis of $C_{24}H_{22}BrClF_2N_2O$ .H_2O (MW: 524.07 g/mol);

	% C	% H	% N
Calculated	54.82	4.60	5.33
Found	55.02	4.84	5.62

1-(3-Chlorobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine hydrochloride (Compound 37)



. HCl

1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 3-chlorobenzoyl chloride (1.7 mmol, 0.23 mL) and triethylamine (6 mmol, 0.86 mL) in DCM (40 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.3663 g (47%).

The form of compound is yellowish, opaque, powdered crystals and the compound has a melting point of 177.5°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.67, 0.70 and 0.32 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.14), 226 (log ε : 4.29).

FT-IR (KBr, cm^{-1}); 3423 (N-H), 3007 (C-H, aromatic), 2925 (C-H, aliphatic), 1639 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F).

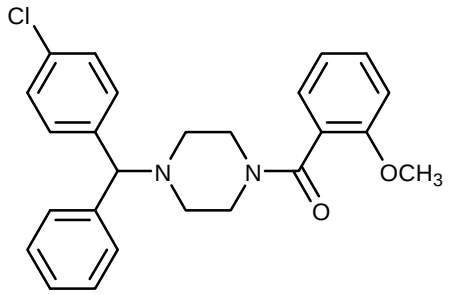
^{1}H-NMR (DMSO, ppm); 3.13 (bs, 4H, piperazine H_3, H_5); 3.65-4.44 (m, 4H, piperazine H_2, H_6); 5.62 (bs, 1H, $(Ar)_2CH$-); 7.25-7.97 (m, 12H, aromatic H's); 12.72 (bs, 1H, N-H salt).

MS (m/z); 427.97 (98%, M^+ - Cl); 429.96 (32%, M+2), 203.61 (100%, $(4\text{-}F\text{-}C_6H_5)_2CH^+$).

Elemental analysis of $C_{24}H_{22}Cl_2F_2N_2O$ .H_2O (MW: 480.12 g/mol);

	% C	% H	% N
Calculated	59.88	5.03	5.82
Found	59.93	5.06	5.98

1-(2-Methoxybenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine (Compound 38)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.872 mmol, 0.2632 g), 2-anisoyl chloride (0.872 mmol, 0.12 mL) and triethylamine (2.62 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.140 g (33%).

The form of compound is white, shiny, needle-shaped crystals and the compound has a melting point of 120°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.68, 0.71 and 0.07 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.78), 275 (log ε : 3.36).

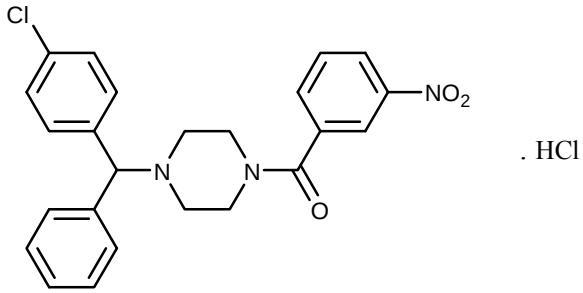
FT-IR (KBr, cm^{-1}); 3449 (N-H), 3029 (C-H, aromatic), 2996 (C-H, aliphatic), 1626 (C=O, amide), 1602 (C=C, aromatic), 1296 (C-O), 1247 (C-N), 1000 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.18-2.33 (m, 4H, piperazine H_3, H_5); 3.63 (m, 2H, piperazine H_2); 3.14 (m, 2H, piperazine H_6); 3.75 (s, 3H, -OCH_3); 4.39 (s, 1H, $(Ar)_2CH$-); 6.94-7.45 (m, 13H, aromatic H's).

Elemental analysis of $C_{25}H_{25}ClN_2O_2$ (MW: 420.93 g/mol);

	% C	% H	% N
Calculated	71.33	5.99	6.66
Found	70.78	6.41	6.73

1-(3-Nitrobenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine hydrochloride (Compound 39)



. HCl

1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.872 mmol, 0.2632 g), 3-nitro-benzoyl chloride (0.872 mmol, 0.12 mL) and triethylamine (2.62 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.112 g (24%).

The form of compound is yellowish orange, opaque, powdered crystals and the compound has a melting point of 196.1°C. It is soluble in ethanol and acetone in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.8, 0.79 and 0.08 respectively.

UV (MeOH, λ_{max}, nm); 203 (log ε : 4.33), 227 (log ε : 4.02).

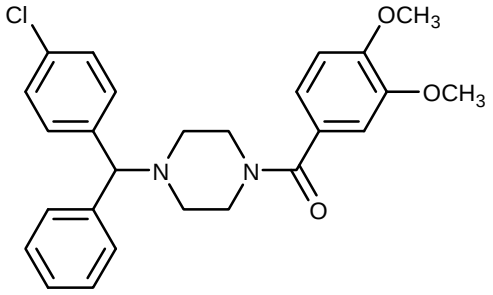
FT-IR (KBr, cm^{-1}); 3423 (N-H), 3063 (C-H, aromatic), 2924 (C-H, aliphatic), 1644 (C=O, amide), 1533 (N=O), 1291 (C-N), 1092 (C-Cl).

^{1}H-NMR (DMSO, ppm); 3.17 (bs, 4H, piperazine H_3, H_5); 3.83 (bs, 4H, piperazine H_2, H_6); 5.59 (s, 1H, $(Ar)_2CH$-); 7.3-8.5 (m, 13H, aromatic H's); 12.71 (s, 1H, N-H salt).

Elemental analysis of $C_{24}H_{23}Cl_2N_3O_3 .H_2O$ (MW: 490.38 g/mol);

	% C	% H	% N
Calculated	58.78	5.14	8.57
Found	58.57	5.52	8.64

1-(3,4-Dimethoxybenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine (Compound 40)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.872 mmol, 0.2632 g), 3,4-dimethoxybenzoyl chloride (0.872 mmol, 0.1804 g) and triethylamine (2.62 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.050 g (11%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 148.6°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.63, 0.69 and 0.04 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 4.59), 276 (log ε : 3.45).

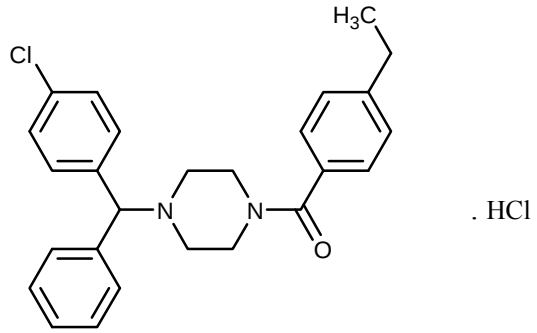
FT-IR (KBr, cm^{-1}); 3082 (C-H, aromatic), 2966 (C-H, aliphatic), 1621 (C=O, amide), 1583 (C=C, aromatic), 1268 (C-O), 1230 (C-N), 1027 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.31 (bs, 4H, piperazine H_3, H_5); 3.52 (bs, 4H, piperazine H_2, H_6); 3.75 (s, 3H, -OCH_3); 3.76 (s, 3H, -OCH_3); 4.39 (s, 1H, $(Ar)_2CH$-); 6.91-7.46 (m, 12H, aromatic H's).

Elemental analysis of $C_{26}H_{27}ClN_2O_3$ (MW: 450.96 g/mol);

	% C	% H	% N
Calculated	69.25	6.03	6.21
Found	69.03	6.32	6.30

1-(4-Ethylbenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine hydrochloride (Compound 41)



. HCl

1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.872 mmol, 0.2632 g), 4-ethylbenzoyl chloride (0.872 mmol, 0.13 mL) and triethylamine (2.62 mmol, 0.36 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.5. The yield is 0.1 g (22%).

The form of compound is yellow, opaque, powdered crystals and the compound has a melting point of 206.4°C. It is soluble in ethanol and acetone in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.78, 0.85 and 0.23 respectively.

UV (MeOH, λ_{max}, nm): 204 (log ε : 4.56), 225 (log ε : 4.11).

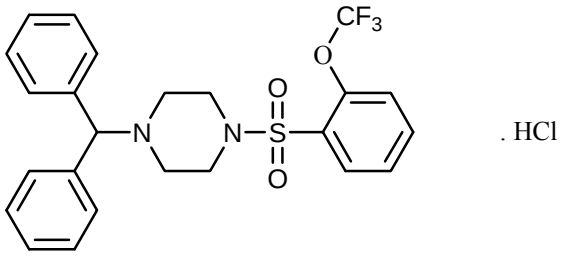
FT-IR (KBr, cm^{-1}); 3422 (N-H), 3010 (C-H, aromatic), 2966 (C-H, aliphatic), 1634 (C=O, amide), 1287 (C-N), 1092 (C-Cl).

^{1}H-NMR (DMSO, ppm); 1.15 (t, 3H, -CH_3, *J=8 Hz*); 2.63 (q, 2H, -CH_2-); 3.13 (s, 4H, piperazine H_3, H_5); 3.79 (s, 4H, piperazine H_2, H_6); 5.63 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.27-7.90 (m, 13H, aromatic H's); 12.63 (s, 1H, N-H salt).

Elemental analysis of $C_{26}H_{28}Cl_2N_2O$ .H_2O (MW: 473.43 g/mol);

	% C	% H	% N
Calculated	65.96	6.39	5.92
Found	66.33	6.73	5.98

1-[2-(Trifluoromethoxy)phenylsulfonyl]-4-(diphenylmethyl)piperazine hydrochloride (Compound 42)



. HCl

1-Diphenylmethylpiperazine (1 mmol, 0.2575 g), 2-(trifluoromethoxy)benzene-sulfonyl chloride (1.1 mmol, 0.2956 g) and triethylamine (3 mmol, 0.43 mL) in DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.6. The yield is 0.05 g (10%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 205.8°C. It is soluble in ethanol in hot medium; DMSO and methanol at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.93, 0.88 and 0.44 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.24), 226 (log ε : 4.87).

FT-IR (KBr, cm^{-1}); 3445 (N-H), 3007 (C-H, aromatic), 2909 (C-H, aliphatic), 1590 (C=C, aromatic), 1353 (S=O, asym.), 1282 (C-O), 1248 (C-F), 1209 (C-N), 1167 (S=O, sym.).

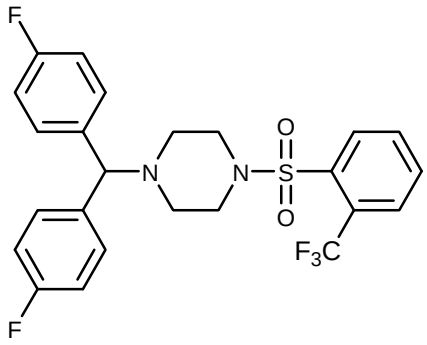
^{1}H-NMR (DMSO, ppm); 3.21-3.38 (m, 4H, piperazine H_3, H_5); 3.74 (m, 4H, piperazine H_2, H_6); 5.57 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.31-7.89 (m, 14H, aromatic H's); 12.5 (bs, 1H, N-H salt).

MS (m/z); 477.8 (100%, M^+ - Cl); 167.6 ($(C_6H_5)_2CH^+$).

Elemental analysis of $C_{24}H_{24}ClF_3N_2O_3S$ (MW: 512.97 g/mol);

	% C	% H	% N	% S
Calculated	56.19	4.72	5.46	6.25
Found	55.85	4.82	5.80	6.21

1-[2-(Trifluoromethyl)phenylsulfonyl]-4-[(bis(4-fluorophenyl)methyl]piperazine (Compound 43)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 2-(trifluoromethyl)-benzenesulfonyl chloride (1.87 mmol, 0.3 mL) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.112 g (22%).

The form of compound is colourless, shiny, prism-shaped crystals and the compound has a melting point of 135.6°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.88, 0.91 and 0.35 respectively.

UV (MeOH, λ_{max}, nm): 203 (log ε : 5.16), 225 (log ε : 4.67).

FT-IR (KBr, cm^{-1}); 3074 (C-H, aromatic), 2917 (C-H, aliphatic), 1604 (C=C, aromatic), 1370 (S=O, asym.), 1284 (C-N), 1218 (C-F), 1144 (S=O, sym.).

^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 3.18 (bs, 4H, piperazine H_2, H_6); 4.45 (s, 1H, $(Ar)_2CH$-); 7.09-7.43 (m, 8H, diphenyl); 7.91-8.06 (m, 4H, 2-trifluoromethylphenyl).

Elemental analysis of $C_{24}H_{21}F_5N_2O_2S$ (MW: 496.49 g/mol);

	% C	% H	% N	% S
Calculated	58.06	4.26	5.64	6.46
Found	58.19	4.22	5.79	6.58

X-RAY;

Table 4.1. Parameters for data collection and structure refinement of compound **43**.

Chemical formula	$C_{24}H_{21}F_5N_2O_2S$
Crystal shape/colour	Prism, colourless
Formula weight	496.49
Crystal system	Triclinic
Space group	P-1
Unit cell parameters	a= 10.8935 (5)Å b= 11.1103 (5)Å c= 11.5213 (5)Å
Temperature (K)	296
Volume	1135.57 (9)Å^3
Z	2
D_x (Mg m^{-3})	1.452
μ (mm^{-1})	0.21
F_{000}	512
Crystal size (mm)	0.44 × 0.39 × 0.20
Data collection	
Diffractometer/meas.meth	STOE IPDS II/w-scan
Absorption correction	Integration
T_{min}	0.8269
T_{max}	0.9300
No. of measured, independent and observed reflections	16429, 4700, 4036
Criterion for observed reflection	$I > 2\sigma(I)$
R_{int}	0.084
θ_{max} (º)	26.5
Refinement	
Refinement on	F^2
R[$F^2 > 2\sigma(F^2)$], wR(F^2), S	0.044, 0.11, 1.06
No. of reflections	4700
No. of parameters	307
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0481P)^2+0.202P]$ $P=(F_0^2+2F_C^2)/3$
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (eÅ^{-3})	0.18, -0.48

Table 4.2. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($Å^2$)

	x	y	z	U
C1	-0.53375 (18)	0.1231 (2)	-0.08845 (18)	0.0560 (4)
C2	-0.4061 (2)	0.2547 (2)	-0.03055 (19)	0.0606 (4)
H22	-0.4047	0.3363	0.0190	0.073
C3	-0.27866 (18)	0.26391 (19)	-0.04732 (17)	0.0541 (4)
H24	-0.1907	0.3532	-0.0092	0.065
C4	-0.27999 (16)	0.14172 (17)	-0.12027 (14)	0.0414 (3)
C5	-0.41171 (17)	0.01042 (18)	-0.17511 (16)	0.0500 (4)
H25	-0.4143	-0.0728	-0.2226	0.060
C6	-0.54002 (19)	0.0005 (2)	-0.16062 (19)	0.0596 (4)
H26	-0.6288	-0.0879	-0.1992	0.072
C7	-0.14222 (15)	0.14886 (16)	-0.14361 (13)	0.0408 (3)
H7	-0.1605	0.0492	-0.1666	0.049
C8	-0.10842 (15)	0.19927 (17)	-0.25300 (13)	0.0420 (3)
C9	-0.0594 (2)	0.34295 (19)	-0.24357 (16)	0.0529 (4)
H17	-0.0479	0.4102	-0.1690	0.063
C10	-0.0276 (2)	0.3874 (2)	-0.34293 (18)	0.0594 (4)
H15	0.0063	0.4840	-0.3360	0.071
C11	-0.04701 (19)	0.2862 (2)	-0.45181 (17)	0.0584 (4)
C12	-0.0944 (2)	0.1450 (2)	-0.46558 (17)	0.0654 (5)
H30	-0.1063	0.0787	-0.5410	0.078
C13	-0.1247 (2)	0.1013 (2)	-0.36475 (16)	0.0557 (4)
H20	-0.1563	0.0049	-0.3724	0.067
C14	-0.04611 (17)	0.17916 (19)	0.06746 (14)	0.0471 (3)
H14A	-0.0625	0.0813	0.0369	0.057
H14B	-0.1338	0.1708	0.0861	0.057
C15	0.07934 (17)	0.2719 (2)	0.18579 (14)	0.0486 (4)
H15A	0.0928	0.3683	0.2195	0.058
H15B	0.0583	0.2263	0.2481	0.058
C16	0.11483 (16)	0.25234 (19)	-0.05669 (14)	0.0447 (3)
H16A	0.1354	0.2928	-0.1222	0.054
H16B	0.0989	0.1546	-0.0867	0.054
C17	0.24358 (16)	0.34759 (18)	0.05867 (14)	0.0451 (3)
H17A	0.3295	0.3520	0.0390	0.054
H17B	0.2624	0.4465	0.0873	0.054
C18	0.40847 (16)	0.52526 (17)	0.36449 (13)	0.0419 (3)
C19	0.32788 (18)	0.5373 (2)	0.44684 (14)	0.0510 (4)
H28	0.2596	0.4538	0.4588	0.061
C20	0.3478 (2)	0.6716 (2)	0.51115 (17)	0.0641 (5)
H33	0.2952	0.6787	0.5678	0.077
C21	0.4445 (3)	0.7935 (2)	0.49172 (19)	0.0712 (6)
H34	0.4538	0.8829	0.5314	0.085
C22	0.5284 (2)	0.7848 (2)	0.41362 (18)	0.0649 (5)
H32	0.5960	0.8696	0.4029	0.078

C23	0.51471 (17)	0.65260 (18)	0.35043 (14)	0.0484 (4)
C24	0.62501 (19)	0.6600 (2)	0.28074 (18)	0.0613 (5)
N1	0.21262 (13)	0.28613 (14)	0.15737 (11)	0.0421 (3)
N2	-0.01724 (13)	0.24297 (14)	-0.03011 (11)	0.0395 (3)
O1	0.46103 (13)	0.33646 (14)	0.22380 (11)	0.0578 (3)
O2	0.29148 (15)	0.25526 (14)	0.34992 (12)	0.0610 (3)
F1	0.56806 (12)	0.58424 (14)	0.15747 (10)	0.0713 (3)
F2	0.70498 (12)	0.61218 (17)	0.32411 (12)	0.0838 (4)
F3	0.71967 (15)	0.79829 (15)	0.29140 (14)	0.0959 (5)
F4	-0.01832 (16)	0.32940 (17)	-0.55116 (12)	0.0882 (4)
F5	-0.65948 (13)	0.11260 (15)	-0.07299 (14)	0.0818 (4)
S1	0.34985 (4)	0.34041 (4)	0.27468 (3)	0.04514 (12)

Table 4.3. Bond Lengths (Å) for Compound **43**.

C1—F5	1.3551 (19)	C14—H14B	0.9700
C1—C2	1.363 (3)	C15—N1	1.4692 (19)
C1—C6	1.365 (3)	C15—H15A	0.9700
C2—C3	1.383 (2)	C15—H15B	0.9700
C2—H22	0.9300	C16—N2	1.4665 (18)
C3—C4	1.392 (2)	C16—C17	1.508 (2)
C3—H24	0.9300	C16—H16A	0.9700
C4—C5	1.379 (2)	C16—H16B	0.9700
C4—C7	1.524 (2)	C17—N1	1.4647 (19)
C5—C6	1.382 (2)	C17—H17A	0.9700
C5—H25	0.9300	C17—H17B	0.9700
C6—H26	0.9300	C18—C19	1.389 (2)
C7—N2	1.4752 (18)	C18—C23	1.403 (2)
C7—C8	1.521 (2)	C18—S1	1.7862 (16)
C7—H7	0.9800	C19—C20	1.379 (3)
C8—C13	1.382 (2)	C19—H28	0.9300
C8—C9	1.389 (2)	C20—C21	1.358 (3)
C9—C10	1.377 (2)	C20—H33	0.9300
C9—H17	0.9300	C21—C22	1.373 (3)
C10—C11	1.365 (3)	C21—H34	0.9300
C10—H15	0.9300	C22—C23	1.386 (3)
C11—C12	1.354 (3)	C22—H32	0.9300
C11—F4	1.365 (2)	C23—C24	1.503 (2)
C12—C13	1.387 (3)	C24—F2	1.330 (2)
C12—H30	0.9300	C24—F1	1.337 (2)
C13—H20	0.9300	C24—F3	1.342 (2)
C14—N2	1.4656 (18)	N1—S1	1.6348 (12)
C14—C15	1.511 (2)	O1—S1	1.4196 (12)
C14—H14A	0.9700	O2—S1	1.4249 (13)

Table 4.4. Bond Angles (°) for Compound **43**

F5—C1—C2	118.97 (17)	N1—C15—H15B	109.8
F5—C1—C6	118.49 (17)	C14—C15—H15B	109.8
C2—C1—C6	122.53 (16)	H15A—C15—H15B	108.2
C1—C2—C3	118.34 (16)	N2—C16—C17	111.23 (12)
C1—C2—H22	120.8	N2—C16—H16A	109.4
C3—C2—H22	120.8	C17—C16—H16A	109.4
C2—C3—C4	121.06 (16)	N2—C16—H16B	109.4
C2—C3—H24	119.5	C17—C16—H16B	109.4
C4—C3—H24	119.5	H16A—C16—H16B	108.0
C5—C4—C3	118.37 (14)	N1—C17—C16	109.36 (13)
C5—C4—C7	119.17 (13)	N1—C17—H17A	109.8
C3—C4—C7	122.44 (14)	C16—C17—H17A	109.8
C4—C5—C6	121.07 (16)	N1—C17—H17B	109.8
C4—C5—H25	119.5	C16—C17—H17B	109.8
C6—C5—H25	119.5	H17A—C17—H17B	108.3
C1—C6—C5	118.60 (16)	C19—C18—C23	119.13 (15)
C1—C6—H26	120.7	C19—C18—S1	114.01 (13)
C5—C6—H26	120.7	C23—C18—S1	126.55 (12)
N2—C7—C8	111.23 (12)	C20—C19—C18	120.83 (17)
N2—C7—C4	111.80 (11)	C20—C19—H28	119.6
C8—C7—C4	110.66 (11)	C18—C19—H28	119.6
N2—C7—H7	107.6	C21—C20—C19	119.96 (18)
C8—C7—H7	107.6	C21—C20—H33	120.0
C4—C7—H7	107.6	C19—C20—H33	120.0
C13—C8—C9	118.47 (15)	C20—C21—C22	120.13 (18)
C13—C8—C7	119.84 (14)	C20—C21—H34	119.9
C9—C8—C7	121.69 (14)	C22—C21—H34	119.9
C10—C9—C8	121.03 (16)	C21—C22—C23	121.48 (19)
C10—C9—H17	119.5	C21—C22—H32	119.3
C8—C9—H17	119.5	C23—C22—H32	119.3
C11—C10—C9	118.33 (18)	C22—C23—C18	118.29 (16)
C11—C10—H15	120.8	C22—C23—C24	116.48 (17)
C9—C10—H15	120.8	C18—C23—C24	125.00 (16)
C12—C11—F4	118.77 (18)	F2—C24—F1	106.89 (17)
C12—C11—C10	122.84 (17)	F2—C24—F3	105.87 (16)
F4—C11—C10	118.38 (18)	F1—C24—F3	105.10 (15)
C11—C12—C13	118.55 (17)	F2—C24—C23	112.42 (15)
C11—C12—H30	120.7	F1—C24—C23	114.33 (14)
C13—C12—H30	120.7	F3—C24—C23	111.59 (17)
C8—C13—C12	120.77 (17)	C17—N1—C15	111.30 (11)
C8—C13—H20	119.6	C17—N1—S1	117.92 (10)
C12—C13—H20	119.6	C15—N1—S1	117.72 (10)
N2—C14—C15	111.47 (13)	C14—N2—C16	107.73 (11)
N2—C14—H14A	109.3	C14—N2—C7	109.53 (11)
C15—C14—H14A	109.3	C16—N2—C7	110.55 (11)
N2—C14—H14B	109.3	O1—S1—O2	119.74 (8)

C15—C14—H14B	109.3	O1—S1—N1	107.38 (7)
H14A—C14—H14B	108.0	O2—S1—N1	106.27 (7)
N1—C15—C14	109.40 (12)	O1—S1—C18	110.17 (7)
N1—C15—H15A	109.8	O2—S1—C18	106.83 (7)
C14—C15—H15A	109.8	N1—S1—C18	105.54 (7)

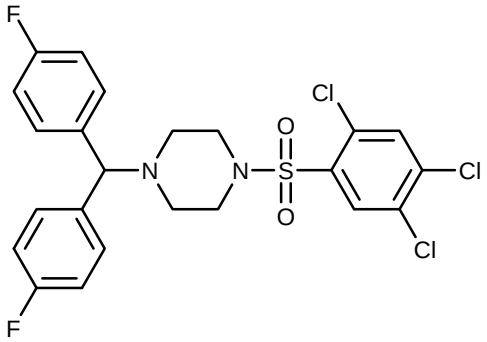
Table 4.5. Torsion Angles (°) for Compound **43**

F5—C1—C2—C3	-179.89 (17)	C21—C22—C23—C24	-172.54 (18)
C6—C1—C2—C3	0.8 (3)	C19—C18—C23—C22	-4.1 (2)
C1—C2—C3—C4	-0.7 (3)	S1—C18—C23—C22	169.12 (13)
C2—C3—C4—C5	-0.4 (3)	C19—C18—C23—C24	170.19 (15)
C2—C3—C4—C7	178.32 (16)	S1—C18—C23—C24	-16.6 (2)
C3—C4—C5—C6	1.4 (3)	C22—C23—C24—F2	118.08 (19)
C7—C4—C5—C6	-177.33 (16)	C18—C23—C24—F2	-56.3 (2)
F5—C1—C6—C5	-179.12 (17)	C22—C23—C24—F1	-119.81 (18)
C2—C1—C6—C5	0.2 (3)	C18—C23—C24—F1	65.8 (2)
C4—C5—C6—C1	-1.3 (3)	C22—C23—C24—F3	-0.7 (2)
C5—C4—C7—N2	-141.49 (14)	C18—C23—C24—F3	-175.10 (15)
C3—C4—C7—N2	39.8 (2)	C16—C17—N1—C15	-56.99 (16)
C5—C4—C7—C8	93.92 (17)	C16—C17—N1—S1	162.50 (10)
C3—C4—C7—C8	-84.78 (18)	C14—C15—N1—C17	56.47 (17)
N2—C7—C8—C13	125.26 (15)	C14—C15—N1—S1	-162.93 (11)
C4—C7—C8—C13	-109.83 (16)	C15—C14—N2—C16	59.61 (16)
N2—C7—C8—C9	-53.95 (18)	C15—C14—N2—C7	179.91 (12)
C4—C7—C8—C9	70.96 (17)	C17—C16—N2—C14	-60.04 (16)
C13—C8—C9—C10	0.0 (2)	C17—C16—N2—C7	-179.69 (12)
C7—C8—C9—C10	179.21 (15)	C8—C7—N2—C14	-172.08 (12)
C8—C9—C10—C11	0.8 (3)	C4—C7—N2—C14	63.66 (15)
C9—C10—C11—C12	-0.8 (3)	C8—C7—N2—C16	-53.52 (15)
C9—C10—C11—F4	178.69 (16)	C4—C7—N2—C16	-177.78 (12)
F4—C11—C12—C13	-179.40 (17)	C17—N1—S1—O1	-45.77 (13)
C10—C11—C12—C13	0.1 (3)	C15—N1—S1—O1	176.25 (11)
C9—C8—C13—C12	-0.7 (3)	C17—N1—S1—O2	-175.03 (11)
C7—C8—C13—C12	-179.95 (16)	C15—N1—S1—O2	46.99 (14)
C11—C12—C13—C8	0.7 (3)	C17—N1—S1—C18	71.75 (12)
N2—C14—C15—N1	-58.32 (17)	C15—N1—S1—C18	-66.23 (13)
N2—C16—C17—N1	59.30 (16)	C19—C18—S1—O1	-161.91 (11)
C23—C18—C19—C20	2.2 (2)	C23—C18—S1—O1	24.56 (15)
S1—C18—C19—C20	-171.87 (13)	C19—C18—S1—O2	-30.36 (13)
C18—C19—C20—C21	1.8 (3)	C23—C18—S1—O2	156.11 (13)
C19—C20—C21—C22	-3.7 (3)	C19—C18—S1—N1	82.46 (12)
C20—C21—C22—C23	1.7 (3)	C23—C18—S1—N1	-91.07 (14)
C21—C22—C23—C18	2.2 (3)		

Table 4.6. Hydrogen Bonds for Compound **43** (Å and °)

D-H· · ·A	D-H	H· · ·A	D· · ·A	D-H· · ·A
C17-H15B· · ·O2	0.97	2.5	3.058(2)	117

1-[(2,4,5-Trichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (Compound 44)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 2,4,5-trichlorobenzenesulfonyl chloride (1.87 mmol, 0.5511 g) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.060 g (11%).

The form of compound is white, shiny, needle-shaped crystals and the compound has a melting point over 300°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.91, 0.94 and 0.66 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 5.09), 225 (log ε : 4.79).

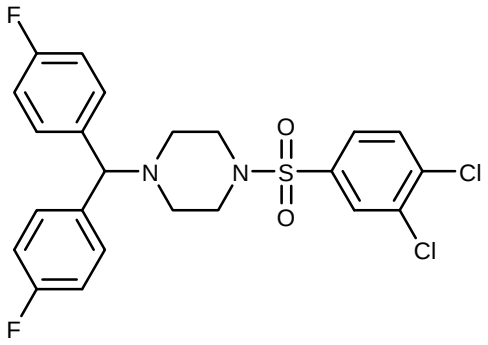
FT-IR (KBr, cm^{-1}); 3089 (C-H, aromatic), 2969 (C-H, aliphatic), 1602 (C=C, aromatic), 1373 (S=O, asym.), 1282 (C-N), 1219 (C-F), 1153 (S=O, sym.), 1097 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.32 (bs, 4H, piperazine H_3, H_5); 3.24 (bs, 4H, piperazine H_2, H_6); 4.45 (s, 1H, $(Ar)_2CH$-); 7.09-7.43 (m, 8H, diphenyl); 8.05 (s, 1H, 2,4,5-trichlorophenyl, H_6); 8.17 (s, 1H, 2,4,5- trichlorophenyl, H_2).

Elemental analysis of $C_{23}H_{19}Cl_3F_2N_2O_2S$ (MW: 531.83 g/mol);

	% C	% H	% N	% S
Calculated	51.94	3.60	5.27	6.03
Found	51.73	3.82	5.56	6.10

1-[(3,4-Dichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (Compound 45)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 3,4-dichlorobenzenesulfonyl chloride (1.87 mmol, 0.31 mL) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.210 g (42%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 145.1°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.88, 0.92 and 0.53.

UV (MeOH, λ_{max}, nm); 203 (log ε : 5.35), 227 (log ε : 4.46).

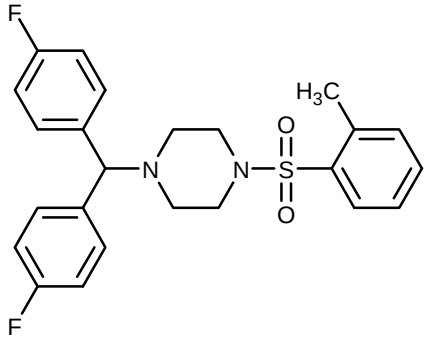
FT-IR (KBr, cm^{-1}); 3088 (C-H, aromatic), 2979 (C-H, aliphatic), 1602 (C=C, aromatic), 1355 (S=O, asym.), 1286 (C-N), 1220 (C-F), 1175 (S=O, sym.), 1031 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 2.98 (bs, 4H, piperazine H_2, H_6); 4.42 (s, 1H, $(Ar)_2CH$-); 7.07-7.40 (m, 8H, diphenyl); 7.69-7.97 (m, 3H, 3,4-dichlorophenyl).

Elemental analysis of $C_{23}H_{20}Cl_2F_2N_2O_2S$ (MW: 497.38 g/mol);

	% C	% H	% N	% S
Calculated	55.54	4.05	5.63	6.45
Found	55.70	4.11	5.92	6.57

1-[(*o*-Toluyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (Compound 46)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), (*o*-toluene)sulfonyl chloride (1.87 mmol, 0.28 mL) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.05 g (10%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 117.7°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.87, 0.91 and 0.44 respectively.

UV (MeOH, λ_{max}, nm); 206 (log ε : 5.32), 225 (log ε : 4.67).

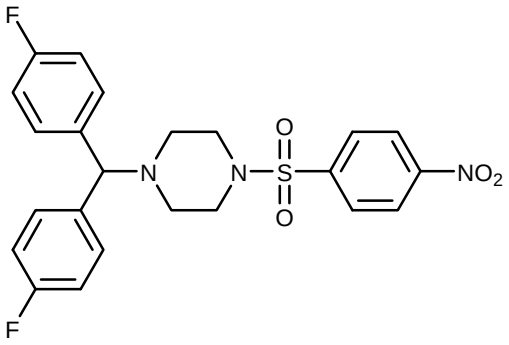
FT-IR (KBr, cm^{-1}); 3068 (C-H, aromatic), 2968 (C-H, aliphatic), 1601 (C=C, aromatic), 1342 (S=O, asym.), 1229 (C-N), 1221 (C-F), 1156 (S=O, sym.).

^{1}H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H_3, H_5); 2.55 (s, 3H, -CH_3); 3.04 (bs, 4H, piperazine H_2, H_6); 4.41 (s, 1H, $(Ar)_2CH$-); 7.00-7.77 (m, 12H, aromatic H's).

Elemental analysis of $C_{23}H_{20}Cl_2F_2N_2O_2S$ (MW: 497.38 g/mol);

	% C	% H	% N	% S
Calculated	65.14	5.47	6.33	7.25
Found	65.51	5.30	6.56	7.37

1-[(4-Nitrophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (Compound 47, CAS No: 1286459-36-6)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 4-nitrobenzenesulfonyl chloride (1.87 mmol, 0.4272 g) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.06 g (13%).

The form of compound is yellowish orange, shiny, powdered crystals and the compound has a melting point of 224.5°C. It is soluble in ethanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.91, 0.91 and 0.38 respectively.

UV (MeOH, λ_{max}, nm): 203 (log ε : 5.23), 225 (log ε : 4.39).

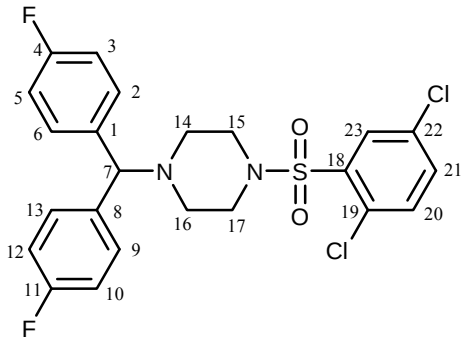
FT-IR (KBr, cm^{-1}); 3070 (C-H, aromatic), 2996 (C-H, aliphatic), 1604 (C=C, aromatic), 1527 (N=O), 1359 (S=O, asym.), 1224 (C-N), 1213 (C-F), 1172 (S=O, sym.).

^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 2.99 (bs, 4H, piperazine H_2, H_6); 4.42 (s, 1H, $(Ar)_2CH$-); 7.06-7.38 (m, 8H, diphenyl); 7.99 (d, 2H, 4-nitrophenyl H_2, H_6, *J=9.2 Hz*); 8.47 (d, 2H, 4-nitrophenyl H_3, H_5, *J=8.8 Hz*).

Elemental analysis of $C_{23}H_{21}F_2N_3O_4S$ (MW: 473.49 g/mol);

	% C	% H	% N	% S
Calculated	58.34	4.47	8.87	6.77
Found	57.75	4.56	8.91	6.83

1-[(2,5-Dichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (Compound 48)



1-[Bis(4-fluorophenyl)methyl]piperazine (1.7 mmol, 0.515 g), 2,5-dichlorobenzene-sulfonyl chloride (1.87 mmol, 0.4685 g) and triethylamine (5.1 mmol, 0.70 mL) in dry DCM (50 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.115 g (23%).

The form of compound is colourless, shiny, prism-shaped crystals and the compound has a melting point of 116.1°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.87, 0.91 and 0.52 respectively.

UV (MeOH, λ_{max}, nm): 205 (log ε : 5.19), 224 (log ε : 4.91).

FT-IR (KBr, cm^{-1}); 3003 (C-H, aromatic), 2966 (C-H, aliphatic), 1603 (C=C, aromatic), 1375 (S=O, asym.), 1284 (C-N), 1218 (C-F), 1178 (S=O, sym.), 1009 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H_3, H_5); 3.23 (bs, 4H, piperazine H_2, H_6); 4.44 (s, 1H, $(Ar)_2CH$-); 7.09-7.43 (m, 8H, diphenyl); 7.75-7.81 (m, 2H, 2,5-dichlorophenyl H_3, H_4); 7.9 (d, 1H, 2,5-dichlorophenyl H_6, *J=2.4 Hz*).

^{13}C-NMR (DMSO, ppm); 46.39 ($C_{14,16}$); 51.28 ($C_{15,17}$); 72.82 (C_7); 115.95-116.16 ($C_{3,10}$); 130.02-130.10 ($C_{2,9}$); 130.48 (C_{20}); 131.61 (C_{21}); 132.99 (C_{23}); 134.79 (C_{22}); 135.02 (C_{19}); 137.34 (C_{18}); 138.83-138.85 ($C_{1,8}$); 160.57-162.99 ($C_{4,11}$).

MS (m/z); 497.98 (25%, M^+); 499.8 (12%, M+2); 203.5 (100%, (4-F-$C_6H_5)_2CH^+$).

Elemental analysis of $C_{23}H_{20}Cl_2F_2N_2O_2S$ (MW: 497.38 g/mol);

	% C	% H	% N	% S
Calculated	55.54	4.05	5.63	6.45
Found	55.55	3.89	5.89	6.58

X-RAY;

Table 4.7. Parameters for data collection and structure refinement of Compound **48**

Chemical formula	$C_{23}H_{20}Cl_2F_2N_2O_2S$
Crystal shape/colour	Prism, colourless
Formula weight	497.37
Crystal system	Triclinic
Space group	P-1
Unit cell parameters	a= 9.1771 (6) Å b= 11.0806 (7) Å c= 13.0463 (8) Å
Temperature (K)	296
Volume	1162.51 (13) $Å^3$
Z	2
D_x (Mg m^{-3})	1.421
μ (mm^{-1})	0.41
F_{000}	512
Crystal size (mm)	0.49 × 0.34 × 0.16
Data collection	
Diffractometer/meas.meth	STOE IPDS II/w-scan
Absorption correction	Integration
T_{min}	0.8135
T_{max}	0.9434
No. of measured, independent and observed reflections	14073, 4577, 3250
Criterion for observed reflection	$I > 2\sigma(I)$
R_{int}	0.039
θ_{max} (º)	26.0
Refinement	
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.047, 0.124, 1.05
No. of reflections	4577
No. of parameters	289
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0617P)^2+0.15482P]$ $P=(F_0^2+2F_C^2)/3$

$\Delta\rho_{max}$, $\Delta\rho_{min}$ (eÅ^{-3})	0.40, -0.53

Table 4.8. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	y	Z	U
Cl1	0.40264 (12)	-0.24307 (9)	1.00534 (6)	0.0949 (3)
Cl2	0.01369 (11)	0.04354 (8)	0.75315 (9)	0.1029 (3)
O1	0.2165 (2)	-0.24963 (16)	0.59565 (12)	0.0680 (5)
O2	0.0225 (2)	-0.14130 (19)	0.55737 (16)	0.0806 (6)
C1	0.8955 (3)	0.3721 (2)	0.73209 (17)	0.0520 (5)
C2	1.0193 (3)	0.3906 (3)	0.6870 (2)	0.0643 (6)
H2	0.9984	0.3927	0.6138	0.077
C3	1.1745 (3)	0.4061 (3)	0.7485 (3)	0.0787 (8)
H3	1.2582	0.4199	0.7179	0.094
C4	1.2016 (3)	0.4006 (3)	0.8545 (2)	0.0721 (7)
C5	1.0841 (3)	0.3839 (3)	0.9030 (2)	0.0707 (7)
H5	1.1068	0.3818	0.9762	0.085
C6	0.9304 (3)	0.3699 (3)	0.84156 (18)	0.0615 (6)
H6	0.8489	0.3589	0.8739	0.074
C7	0.7258 (3)	0.3560 (2)	0.66356 (17)	0.0523 (5)
H7	0.7184	0.3400	0.5861	0.063
C8	0.7027 (3)	0.4837 (2)	0.69706 (17)	0.0502 (5)
C9	0.7391 (3)	0.5814 (3)	0.6406 (2)	0.0657 (7)
H9	0.7721	0.5658	0.5798	0.079
C10	0.7268 (4)	0.7011 (3)	0.6737 (2)	0.0745 (8)
H10	0.7520	0.7665	0.6363	0.089
C11	0.6769 (3)	0.7212 (3)	0.7622 (2)	0.0671 (7)
C12	0.6388 (3)	0.6283 (3)	0.8197 (2)	0.0651 (6)
H12	0.6049	0.6447	0.8799	0.078
C13	0.6520 (3)	0.5094 (2)	0.78599 (19)	0.0571 (6)
H13	0.6262	0.4450	0.8241	0.069
C14	0.6167 (3)	0.1175 (2)	0.65495 (19)	0.0567 (6)
H14A	0.7258	0.1303	0.6990	0.068
H14B	0.6058	0.0900	0.5782	0.068
C15	0.4902 (3)	0.0114 (2)	0.68198 (19)	0.0578 (6)
H15A	0.5032	-0.0718	0.6652	0.069
H15B	0.5067	0.0361	0.7599	0.069
C16	0.4330 (3)	0.2192 (2)	0.6030 (2)	0.0596 (6)
H16A	0.4248	0.1861	0.5276	0.072
H16B	0.4182	0.3023	0.6094	0.072
C17	0.3014 (3)	0.1195 (2)	0.6316 (2)	0.0611 (6)
H17A	0.3085	0.1529	0.7067	0.073
H17B	0.1940	0.1054	0.5835	0.073
C18	0.1840 (3)	-0.1189 (2)	0.7629 (2)	0.0586 (6)
C19	0.2682 (3)	-0.1818 (2)	0.82167 (18)	0.0590 (6)

H19	0.3086	-0.2357	0.7862	0.071
C20	0.2931 (4)	-0.1652 (3)	0.9329 (2)	0.0696 (7)
C21	0.2360 (4)	-0.0861 (3)	0.9868 (3)	0.0896 (9)
H21	0.2539	-0.0751	1.0617	0.108
C22	0.1525 (4)	-0.0234 (3)	0.9304 (3)	0.0902 (10)
H22	0.1138	0.0307	0.9675	0.108
C23	0.1237 (3)	-0.0385 (3)	0.8178 (3)	0.0719 (7)
F1	1.3528 (2)	0.4123 (2)	0.91457 (17)	0.1107 (7)
F2	0.6654 (3)	0.83933 (17)	0.79559 (16)	0.0989 (6)
N1	0.5953 (2)	0.24286 (18)	0.67671 (14)	0.0492 (4)
N2	0.3226 (2)	-0.00744 (18)	0.61928 (14)	0.0548 (5)
S1	0.17393 (8)	-0.13926 (6)	0.62196 (5)	0.05911 (18)

Table 4.9. Bond Lengths (Å) for Compound **48**

Cl1—C20	1.737 (3)	C12—H12	0.9300
Cl2—C23	1.731 (3)	C13—H13	0.9300
O1—S1	1.4264 (17)	C14—N1	1.464 (3)
O2—S1	1.4223 (19)	C14—C15	1.499 (3)
C1—C2	1.376 (3)	C14—H14A	0.9700
C1—C6	1.389 (3)	C14—H14B	0.9700
C1—C7	1.521 (3)	C15—N2	1.473 (3)
C2—C3	1.384 (4)	C15—H15A	0.9700
C2—H2	0.9300	C15—H15B	0.9700
C3—C4	1.357 (4)	C16—N1	1.465 (3)
C3—H3	0.9300	C16—C17	1.500 (3)
C4—C5	1.356 (4)	C16—H16A	0.9700
C4—F1	1.361 (3)	C16—H16B	0.9700
C5—C6	1.377 (3)	C17—N2	1.476 (3)
C5—H5	0.9300	C17—H17A	0.9700
C6—H6	0.9300	C17—H17B	0.9700
C7—N1	1.473 (3)	C18—C19	1.380 (3)
C7—C8	1.517 (3)	C18—C23	1.399 (4)
C7—H7	0.9800	C18—S1	1.793 (2)
C8—C13	1.381 (3)	C19—C20	1.383 (3)
C8—C9	1.394 (3)	C19—H19	0.9300
C9—C10	1.384 (4)	C20—C21	1.361 (4)
C9—H9	0.9300	C21—C22	1.360 (5)
C10—C11	1.361 (4)	C21—H21	0.9300
C10—H10	0.9300	C22—C23	1.398 (4)
C11—F2	1.363 (3)	C22—H22	0.9300
C11—C12	1.365 (4)	N2—S1	1.631 (2)
C12—C13	1.381 (3)		

Table 4.10. Bond Angles (°) for Compound **48**

C2—C1—C6	118.2 (2)	N2—C15—C14	111.18 (18)
C2—C1—C7	120.5 (2)	N2—C15—H15A	109.4
C6—C1—C7	121.3 (2)	C14—C15—H15A	109.4
C1—C2—C3	121.1 (2)	N2—C15—H15B	109.4
C1—C2—H2	119.4	C14—C15—H15B	109.4
C3—C2—H2	119.4	H15A—C15—H15B	108.0
C4—C3—C2	118.4 (3)	N1—C16—C17	110.05 (18)
C4—C3—H3	120.8	N1—C16—H16A	109.7
C2—C3—H3	120.8	C17—C16—H16A	109.7
C5—C4—C3	122.6 (2)	N1—C16—H16B	109.7
C5—C4—F1	118.7 (3)	C17—C16—H16B	109.7
C3—C4—F1	118.7 (3)	H16A—C16—H16B	108.2
C4—C5—C6	118.6 (2)	N2—C17—C16	109.3 (2)
C4—C5—H5	120.7	N2—C17—H17A	109.8
C6—C5—H5	120.7	C16—C17—H17A	109.8
C5—C6—C1	121.0 (2)	N2—C17—H17B	109.8
C5—C6—H6	119.5	C16—C17—H17B	109.8
C1—C6—H6	119.5	H17A—C17—H17B	108.3
N1—C7—C8	110.01 (18)	C19—C18—C23	118.8 (2)
N1—C7—C1	111.46 (18)	C19—C18—S1	115.83 (18)
C8—C7—C1	109.27 (17)	C23—C18—S1	125.3 (2)
N1—C7—H7	108.7	C18—C19—C20	120.6 (2)
C8—C7—H7	108.7	C18—C19—H19	119.7
C1—C7—H7	108.7	C20—C19—H19	119.7
C13—C8—C9	118.0 (2)	C21—C20—C19	120.8 (3)
C13—C8—C7	121.72 (19)	C21—C20—Cl1	119.5 (2)
C9—C8—C7	120.2 (2)	C19—C20—Cl1	119.7 (2)
C10—C9—C8	120.9 (2)	C22—C21—C20	119.5 (3)
C10—C9—H9	119.6	C22—C21—H21	120.2
C8—C9—H9	119.6	C20—C21—H21	120.2
C11—C10—C9	118.5 (2)	C21—C22—C23	121.4 (3)
C11—C10—H10	120.7	C21—C22—H22	119.3
C9—C10—H10	120.7	C23—C22—H22	119.3
C10—C11—F2	118.8 (2)	C22—C23—C18	118.9 (3)
C10—C11—C12	122.8 (2)	C22—C23—Cl2	117.9 (2)
F2—C11—C12	118.4 (3)	C18—C23—Cl2	123.2 (2)
C11—C12—C13	118.1 (2)	C14—N1—C16	106.93 (17)
C11—C12—H12	121.0	C14—N1—C7	113.43 (18)
C13—C12—H12	121.0	C16—N1—C7	111.56 (17)
C12—C13—C8	121.7 (2)	C15—N2—C17	111.83 (18)
C12—C13—H13	119.1	C15—N2—S1	116.00 (15)
C8—C13—H13	119.1	C17—N2—S1	118.09 (16)
N1—C14—C15	110.03 (19)	O2—S1—O1	119.64 (12)

N1—C14—H14A	109.7	O2—S1—N2	108.18 (11)
C15—C14—H14A	109.7	O1—S1—N2	106.77 (11)
N1—C14—H14B	109.7	O2—S1—C18	110.06 (13)
C15—C14—H14B	109.7	O1—S1—C18	105.46 (10)
H14A—C14—H14B	108.2	N2—S1—C18	105.89 (10)

Table 4.11. Torsion Angles (°) for Compound **48**

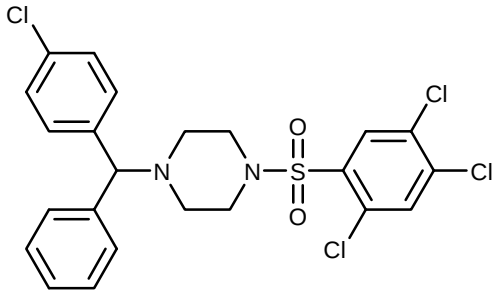
C6—C1—C2—C3	0.2 (4)	C19—C20—C21—C22	0.5 (5)
C7—C1—C2—C3	179.7 (2)	Cl1—C20—C21—C22	178.9 (3)
C1—C2—C3—C4	1.0 (4)	C20—C21—C22—C23	0.1 (5)
C2—C3—C4—C5	-1.6 (5)	C21—C22—C23—C18	-0.7 (5)
C2—C3—C4—F1	178.3 (3)	C21—C22—C23—Cl2	179.1 (3)
C3—C4—C5—C6	1.0 (4)	C19—C18—C23—C22	0.8 (4)
F1—C4—C5—C6	-179.0 (3)	S1—C18—C23—C22	-174.6 (2)
C4—C5—C6—C1	0.4 (4)	C19—C18—C23—Cl2	-179.0 (2)
C2—C1—C6—C5	-0.9 (4)	S1—C18—C23—Cl2	5.6 (3)
C7—C1—C6—C5	179.7 (2)	C15—C14—N1—C16	-62.8 (2)
C2—C1—C7—N1	133.1 (2)	C15—C14—N1—C7	173.80 (17)
C6—C1—C7—N1	-47.5 (3)	C17—C16—N1—C14	65.0 (2)
C2—C1—C7—C8	-105.1 (2)	C17—C16—N1—C7	-170.42 (19)
C6—C1—C7—C8	74.3 (3)	C8—C7—N1—C14	-175.10 (17)
N1—C7—C8—C13	38.9 (3)	C1—C7—N1—C14	-53.7 (2)
C1—C7—C8—C13	-83.8 (3)	C8—C7—N1—C16	64.1 (2)
N1—C7—C8—C9	-143.7 (2)	C1—C7—N1—C16	-174.56 (18)
C1—C7—C8—C9	93.6 (2)	C14—C15—N2—C17	-52.5 (3)
C13—C8—C9—C10	0.8 (4)	C14—C15—N2—S1	168.07 (15)
C7—C8—C9—C10	-176.6 (2)	C16—C17—N2—C15	53.5 (2)
C8—C9—C10—C11	-0.5 (4)	C16—C17—N2—S1	-167.97 (15)
C9—C10—C11—F2	179.7 (2)	C15—N2—S1—O2	-179.85 (17)
C9—C10—C11—C12	0.1 (4)	C17—N2—S1—O2	43.3 (2)
C10—C11—C12—C13	0.1 (4)	C15—N2—S1—O1	-49.86 (18)
F2—C11—C12—C13	-179.5 (2)	C17—N2—S1—O1	173.31 (16)
C11—C12—C13—C8	0.2 (4)	C15—N2—S1—C18	62.18 (19)
C9—C8—C13—C12	-0.7 (4)	C17—N2—S1—C18	-74.65 (18)
C7—C8—C13—C12	176.8 (2)	C19—C18—S1—O2	147.01 (19)
N1—C14—C15—N2	57.3 (2)	C23—C18—S1—O2	-37.5 (3)
N1—C16—C17—N2	-60.5 (3)	C19—C18—S1—O1	16.7 (2)
C23—C18—C19—C20	-0.2 (4)	C23—C18—S1—O1	-167.8 (2)
S1—C18—C19—C20	175.6 (2)	C19—C18—S1—N2	-96.3 (2)
C18—C19—C20—C21	-0.4 (4)	C23—C18—S1—N2	79.2 (2)
C18—C19—C20—Cl1	-178.8 (2)		

Table 4.12. Hydrogen Bonds for Compound **48** (Å and °)

D-H· · ·A	D-H	H· · ·A	D· · ·A	D-H· · ·A
C15-H15A· · ·F2^{i}	0.97	2.51	3.192(3)	127
C17-H17A· · ·Cl2	0.97	2.82	3.344(3)	115
C17-H17B· · ·O2^{ii}	0.97	2.53	3.457(3)	159

i: x,+y-1,+z **ii:** -x,-y,-z+1

1-[(2,4,5-Trichlorophenyl)sulfonyl]-4-[(4-chlorophenyl)(phenyl)methyl]piperazine (Compound 49)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.87 mmol, 0.2632 g), 2,4,5-trichlorobenzenesulfonyl chloride (0.96 mmol, 0.2827 g) and triethylamine (2.6 mmol, 0.36 mL) in dry DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.100 g (19%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 151.1°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.88, 0.92 and 0.70 respectively.

UV (MeOH, λ_{max}, nm): 205 (log ε : 4.66), 234 (log ε : 4.09).

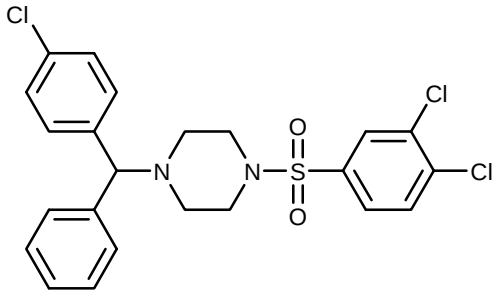
FT-IR (KBr, cm^{-1}); 3091 (C-H, aromatic), 2967 (C-H, aliphatic), 1568 (C=C, aromatic), 1352 (S=O, asym.), 1283 (C-N), 1164 (S=O, sym.), 1066 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.29 (bs, 4H, piperazine H_3, H_5); 3.21 (bs, 4H, piperazine H_2, H_6); 4.37 (s, 1H, $(Ar)_2CH$-); 7.14-7.39 (m, 9H, diphenyl); 8.016 (s, 1H, 2,4,5-trichlorophenyl H_5); 8.14 (s, 1H, 2,4,5- trichlorophenyl H_3).

Elemental analysis of $C_{23}H_{20}Cl_4N_2O_2S$ (MW: 530.29 g/mol);

	% C	% H	% N	% S
Calculated	52.09	3.80	5.28	6.05
Found	52.22	4.00	5.52	6.19

1-[(3,4-Dichlorophenyl)sulfonyl]-4-[(4-chlorophenyl)(phenyl)methyl]piperazine (Compound 50)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.87 mmol, 0.2632 g), 3,4-dichlorobenzenesulfonyl chloride (0.96 mmol, 0.16 mL) and triethylamine (2.6 mmol, 0.36 mL) in dry DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0.123 g (25%).

The form of compound is white, opaque, powdered crystals and the compound has a melting point of 107.1°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.88, 0.91 and 0.61 respectively.

UV (MeOH, λ_{max}, nm); 204 (log ε : 4.35), 236 (log ε : 4.11).

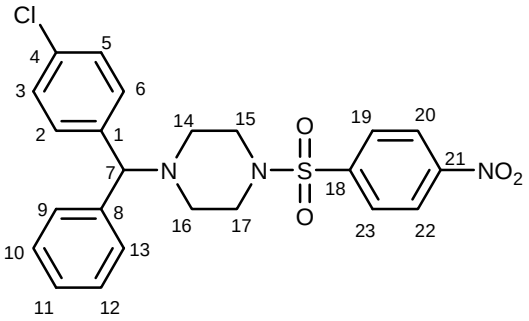
FT-IR (KBr, cm^{-1}); 3063 (C-H, aromatic), 2965 (C-H, aliphatic), 1560 (C=C, aromatic), 1356 (S=O, asym.), 1281 (C-N), 1172 (S=O, sym.), 1033 (C-Cl).

^{1}H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H_3, H_5); 2.98 (bs, 4H, piperazine H_2, H_6); 4.37 (s, 1H, $(Ar)_2CH$-); 7.16-7.40 (m, 9H, diphenyl); 7.69-7.97 (m, 3H, 3,4-dichlorophenyl).

Elemental analysis of $C_{23}H_{21}Cl_3N_2O_2S$ (MW: 495.85 g/mol);

	% C	% H	% N	% S
Calculated	55.71	4.27	5.65	6.47
Found	55.82	4.35	5.91	6.51

1-[(4-Nitrophenyl)sulfonyl]-4-[(4-chlorophenyl)(phenyl)methyl]piperazine (Compound 51)



1-[(4-Chlorophenyl)(phenyl)methyl]piperazine (0.87 mmol, 0.2632 g), 4-nitro-benzenesulfonyl chloride (0.96 mmol, 0.2192 g) and triethylamine (2.6 mmol, 0.36 mL) in dry DCM (20 mL) were reacted according to the general synthesis method at 3.1.2.2.7. The yield is 0,175 g (37%).

The form of compound is yellowish orange, opaque, cottonlike crystals and the compound has a melting point of 209.3°C. It is soluble in ethanol and methanol in hot medium; DMSO and acetone at room temperature. R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.88, 0.91 and 0.51 respectively.

UV (MeOH, λ_{max}, nm); 202 (log ε : 4.53), 231 (log ε : 4.18), 264 (log ε : 3.82)

FT-IR (KBr, cm^{-1}); 3055 (C-H, aromatic), 2947 (C-H, aliphatic), 1606 (C=C, aromatic), 1534 (N=O), 1356 (S=O, asym.), 1281 (C-N), 1170 (S=O, sym.), 1088 (C-Cl)

^{1}H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H_3, H_5); 2.99 (bs, 4H, piperazine H_2, H_6); 4.38 (s, 1H, $(Ar)_2CH$-); 7.15-7.38 (m, 9H, diphenyl); 8.01 (d, 2H, 4-nitrophenyl H_2, H_6, *J=8.8 Hz*); 8.46 (d, 2H, 4-nitrophenyl H_3, H_5, *J=8.8 Hz*).

^{13}C-NMR (DMSO, ppm); 46.67 ($C_{14,16}$); 50.81 ($C_{15,17}$); 73.69 (C_7); 125.38 ($C_{20,22}$); 127.79 (C_{11}); 128.08 ($C_{10,12}$); 129.18 ($C_{19,23}$); 129.29 ($C_{9,13}$); 129.81 ($C_{2,6}$); 129.89 ($C_{3,5}$); 132.11 (C_4); 140.99 (C_1); 141.92 (C_8); 142.29 (C_{18}); 150.76 (C_{21}).

MS (m/z); 472.8 (25%, M^+); 474.8 (12%, M+2); 201.5 (100% (4-Cl-C_6H_5)-(C_6H_5)$\underline{CH}^+$); 203.6 (38%).

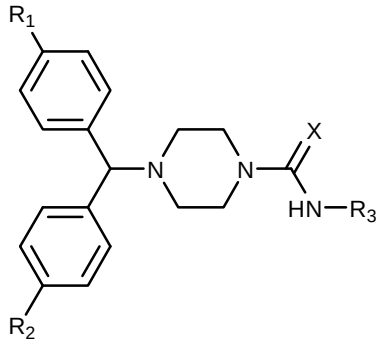
Elemental analysis of $C_{23}H_{22}ClN_3O_4S$ (MW: 471.96 g/mol);

	% C	% H	% N	% S
Calculated	58.53	4.70	8.90	6.79
Found	58.56	4.83	8.99	6.84

4.2. Pharmacological Studies

Cytotoxic activity results of the synthesized molecules are given at Table 4.13.

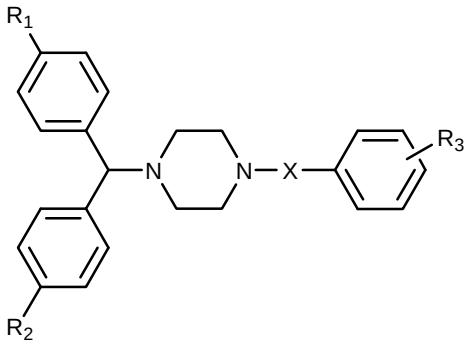
Table 4.13. IC_{50} values of compounds screened in liver (HUH-7), breast (MCF-7) and colon (HCT-116) cancer cell lines by means of sulphorhodamine B assay.



Compound	X	R_1	R_2	R_3	Cancer Cell Line IC_{50} (µM)		
					HUH-7	MCF-7	HCT-116
1	O	- H	- H	*sec*-Butyl	-	-	-
2	O	- H	- H	*tert*-Butyl	-	-	1,01
3	O	- H	- H	Isopropyl	-	-	-
4	O	- H	- H	Ethyl	-	25,7	-
5	O	- H	- H	2,6-Dichlorophenyl	-	-	-

6	O	- H	- H	2-Benzylphenyl	-	-	-
7	O	- H	- H	Ethylacetato	-	-	-
8	O	- H	- H	Allyl	-	10,91	-
9	O	- F	- F	*sec*-Butyl	13,85	-	24,48
10	O	- F	- F	*tert*-Butyl	29,96	-	28,4
11	O	- F	- F	Butyl	13,39	19,03	16,24
12	O	- F	- F	Ethyl	34,84	-	17,98
13	O	- F	- F	Isopropyl	36,57	45,23	20,94
14	O	- F	- F	Ethylacetato	-	36,14	-
15	O	- F	- F	4-Bromophenyl	9,46	8,68	8,87
16	O	- Cl	- H	*sec*-Butyl	13,03	11,39	9,33
17	O	- Cl	- H	*tert*-Butyl	10,88	8,77	9,33
18	O	- Cl	- H	Ethyl	20,92	60,24	10,78
19	O	- Cl	- H	Isopropyl	15,36	13,16	17,12
20	O	- Cl	- H	Allyl	16,29	9,12	10,14
21	O	- Cl	- H	2,6-Dichlorophenyl	6,44	6,14	8,93
22	O	- Cl	- H	2-Phenylethyl	13,18	8,51	5,72
23	O	- Cl	- H	4-Bromophenyl	8,54	9,28	7,34
24	O	- Cl	- H	2-Benzylphenyl	17,22	16,91	4,76
25	O	- Cl	- H	4-Cyanophenyl	1,29	6,34	1,81
26	S	- F	- F	*tert*-Butyl	5,97	10,62	13,09
27	S	- F	- F	Cyclohexyl	25,8	-	-
28	S	- Cl	- H	Ethyl	10,81	-	13,75
29	S	- Cl	- H	Isopropyl	6,20	11,47	14,98

30	S	- Cl	- H	Allyl	9,95	4,94	8,85
31	S	- Cl	- H	Benzyl	22,59	23,00	12,68
32	S	- Cl	- H	Butyl	8,10	14,80	13,91



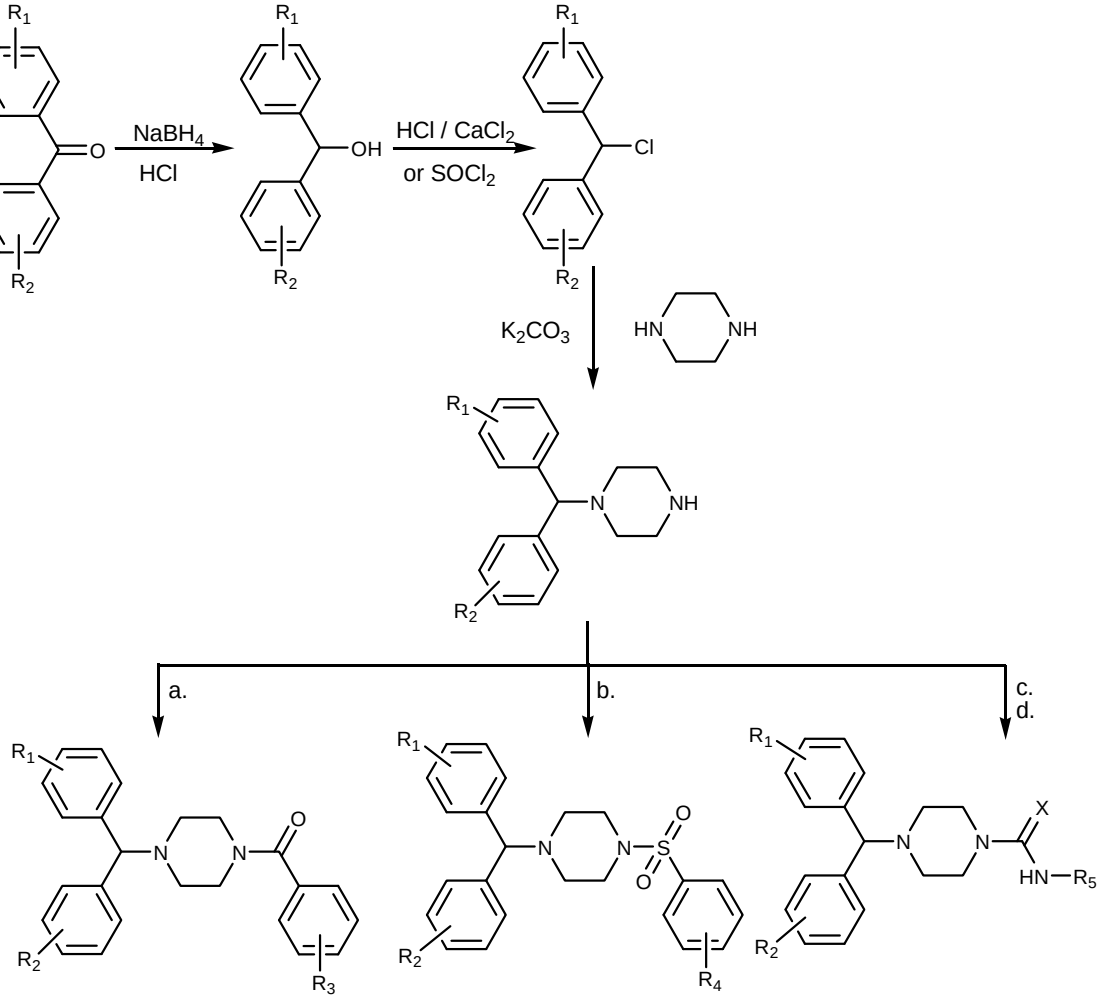
Compound	**X**	**R_1**	**R_2**	**R_3**	**Cancer Cell Line IC_{50} (µM)**		
					HUH-7	**MCF-7**	**HCT-116**
33	C=O	- H	- H	5-Fluoro-2-methyl	10,80	10,44	11,34
34	C=O	- F	- F	2-Bromo	20,89	6,05	12,78
35	C=O	- F	- F	3-Bromo	11,72	5,95	9,10
36	C=O	- F	- F	4-Bromo	12,12	2,21	12,16
37	C=O	- F	- F	3-Chloro	11,16	5,87	8,95
38	C=O	- Cl	- H	2-Methoxy	8,49	17,88	11,00
39	C=O	- Cl	- H	3-Nitro	13,23	22,72	13,85
40	C=O	- Cl	- H	3,4-Dimethoxy	10,81	16,09	10,54
41	C=O	- Cl	- H	4-Ethyl	10,91	-	9,45
42	SO_2	- H	- H	2-Trifluoromethoxy	-	4,50	21,09
43	SO_2	- F	- F	2-Trifluoromethyl	-	-	-
44	SO_2	- F	- F	2,4,5-Trichloro	-	-	-
45	SO_2	- F	- F	3,4-Dichloro	15,23	56,02	-

46	SO_2	- F	- F	2-Methyl	17,65	17,10	27,41
47	SO_2	- F	- F	4-Nitro	-	-	-
48	SO_2	- F	- F	2,5-Dichloro	-	-	-
49	SO_2	- Cl	- H	2,4,5-Trichloro	54,41	11,16	31,41
50	SO_2	- Cl	- H	3,4-Dichloro	10,88	-	53,06
51	SO_2	- Cl	- H	4-Nitro	39,95	17,22	97,74
5-FU	-	-	-	-	30,66	3,51	18,67
CPT	-	-	-	-	*n.d.*	*n.d.*	*n.d.*

n.d.: Not determined (Camptothecin was cytotoxic at concentrations below 2.5 µM.)

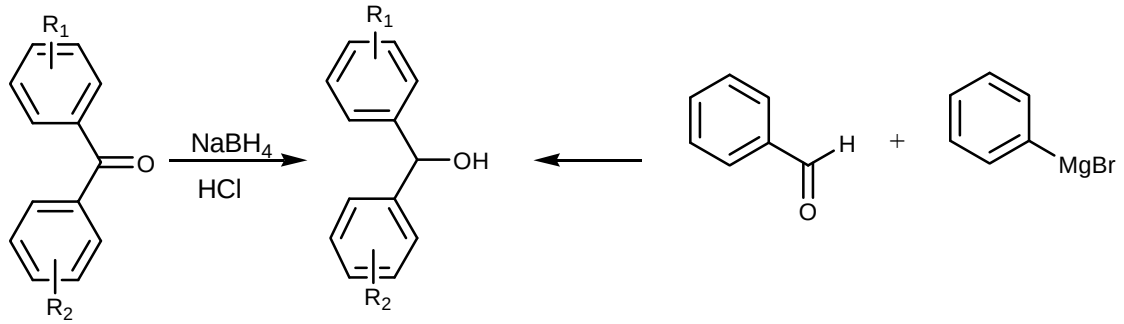
5. RESULTS AND DISCUSSION

In this study, novel compounds having benzhydrylpiperazine, 4,4-difluoro and 4-chloro benzhydrylpiperazine derivatives containing carboxamide, thioamide, benzoyl and sulfonyl structures were prepared and evaluated for their *in vitro* cytotoxic activity on breast (MCF-7), hepatocellular (HUH-7) and colorectal (HCT-116) carcinoma cell lines. UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and X-Ray crystallography assisted the confirmation of the structures. In addition purity of the compounds was determined by elemental analysis. The target compounds mentioned in this study were prepared according to the synthetic pathway depicted in Scheme 5.1.

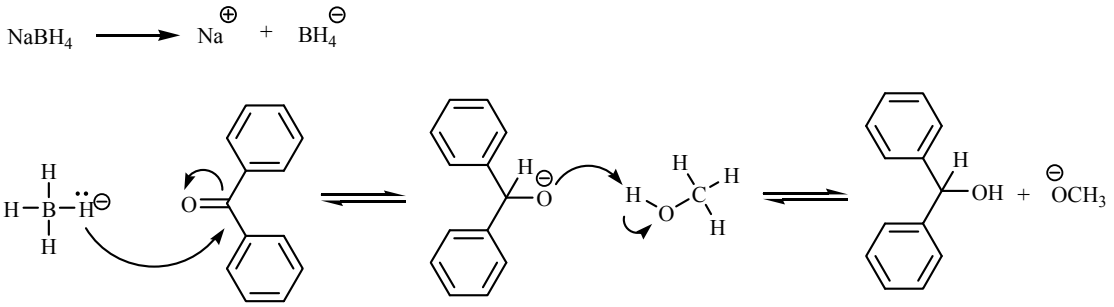


Scheme 5.1. General synthesis pathway of the compounds **1-51**. [**a**. Benzoyl chlorides, TEA, DCM (compounds **33-41**); **b.** Sulfonyl chlorides, TEA, DCM (compounds **42-51**); **c.** Isocyanates, TEA, DCM or DMSO, X=O (compounds **1-25**); **d.** Isothiocyanates, TEA, DCM; R_1 = H, F, Cl; R_2 = H, F, X=S (compounds **26-32**).]

The final compounds are obtained by nucleophilic substitution of piperazine N-H with appropriate sulfonyl chlorides and benzoyl chlorides or nucleophilic addition to several isocyanates and isothiocyanates. According to literature, in order to obtain final compounds, it is necessary to synthesize 1-benzhydrylpiperazines starting from benzhydroles prepared by optionally two distinct pathways. One of the strategies includes *Grignard* reaction of benzaldehyde and phenylmagnesium bromide, however, it is reported that yield is lower than that of the second strategy. We preferred that second strategy which is reduction of benzophenones with sodium borohydride producing pure and high yield (90-95%) benzhydroles [166]. The reaction was not time consuming and its work up was easy to carry out.

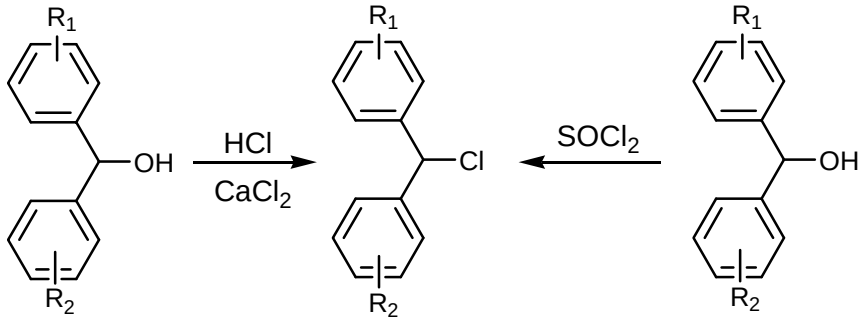


Mechanism of benzophenone reduction is proposed as:

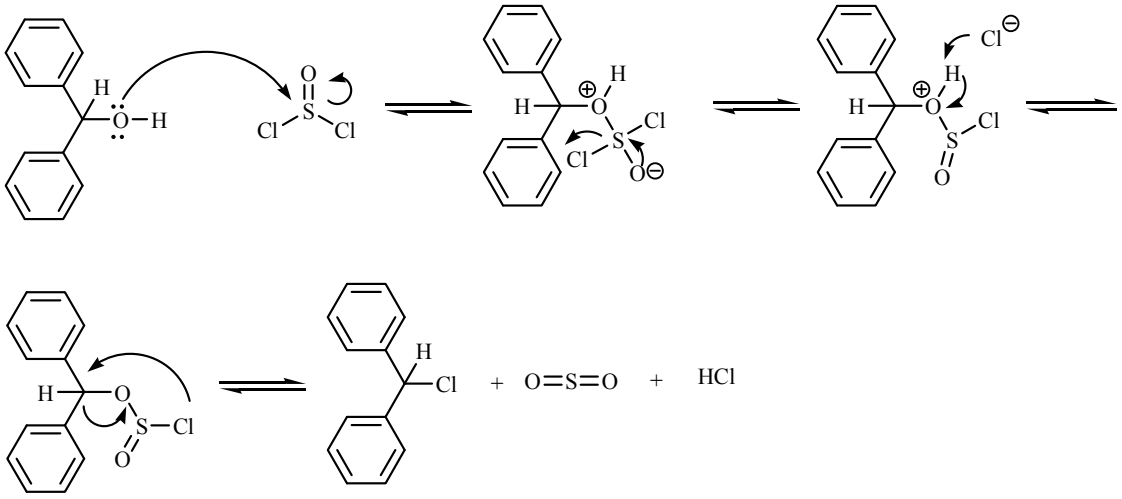


Methodology to synthesize 1-benzhydrylpiperazines includes the preparation of benzhydryl chlorides from benzhydroles. We applied two previously reported methods to achieve this. At first, Kumar *et al*. reported that, benzhydrole is treated with thionyl chloride in ice bath, however, the products were not pure due to possible reasons of thionyl chloride [44]. Then we synthesized benzhydryl chloride derivatives in a manner reported by Narsaiah *et al*., including the treatment of benzhydroles with anhydrous

calcium chloride and aqueous hydrochloric acid with reflux at 80°C [167]. Reaction work up was easy to handle also resulting products are pure and in good yields (90-95%).

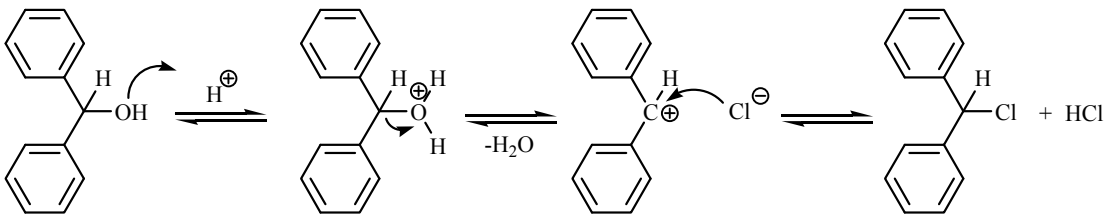


Mechanism of benzhydrole chlorination with $SOCl_2$ is proposed to be internal nucleophilic substitution (S_Ni) depicted as:

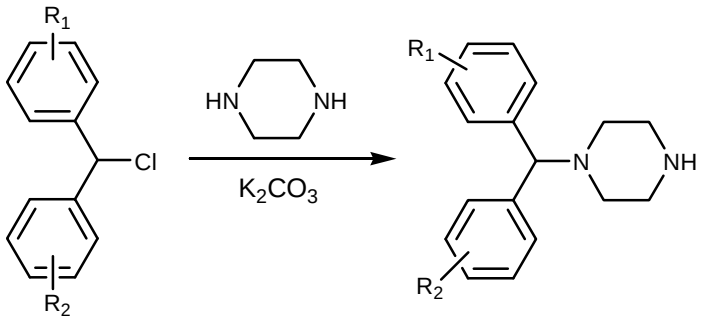


Mechanism of benzhydrole chlorination with HCl and $CaCl_2$ is suggested as:

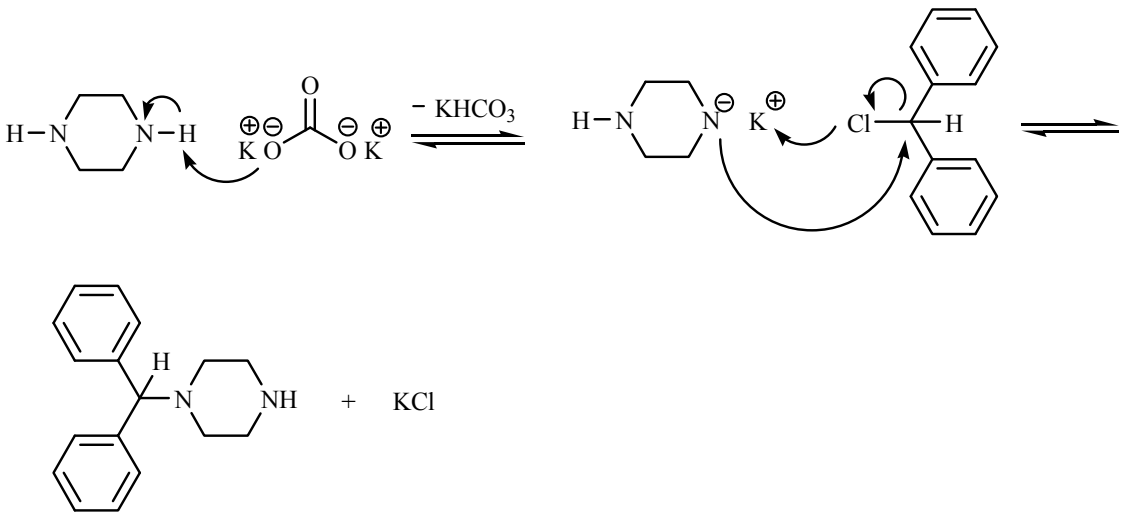
$$CaCl_2 + HCl \,.\, H_2O \rightleftharpoons CaCl_2 \,.\, H_2O + HCl\,(g)$$



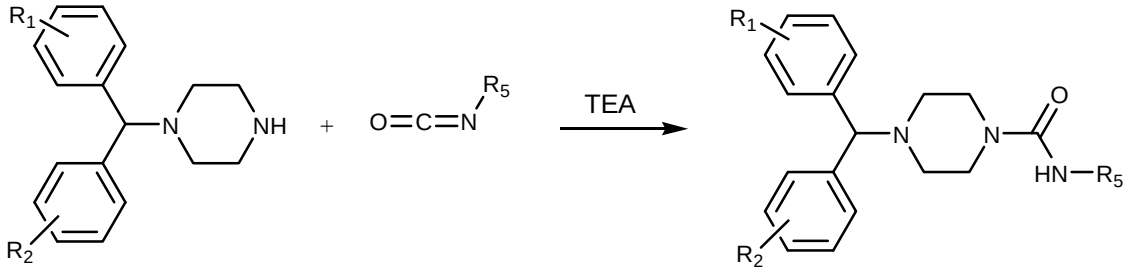
Piperazine was refluxed with benzhydryl chlorides in alkali medium in order to prepare 1-benzhydrylpiperazines. The reaction conditions were easy to perform and compounds were produced in yields of 40-50%.



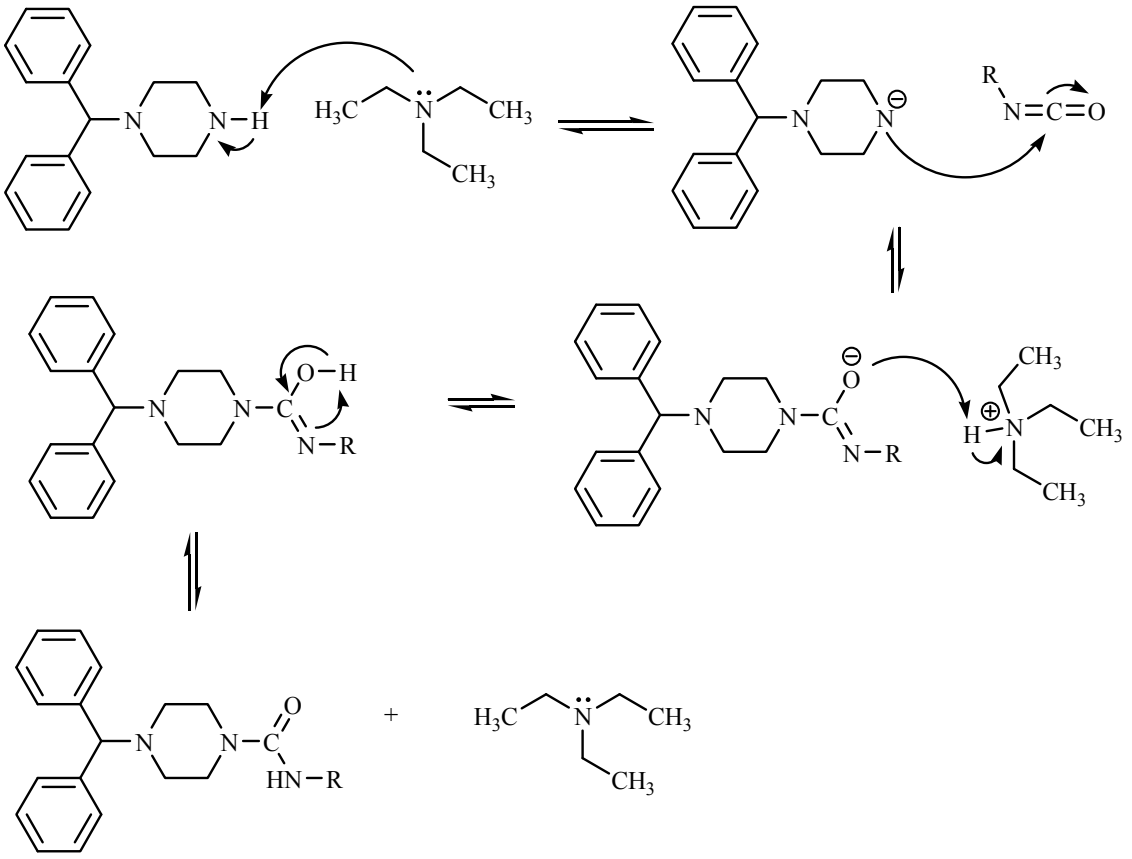
Mechanism of *N*-alkylation of piperazine is proposed to be nucleophilic substitution depicted as:



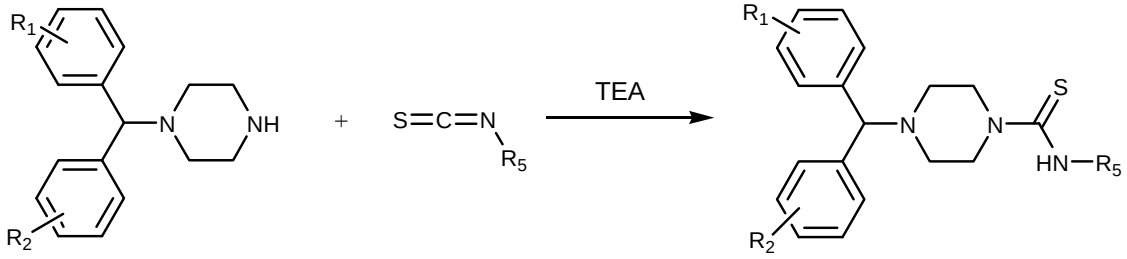
To obtain carboxamide derivatives, 1-benzhydrylpiperazines were reacted with appropriate isocyanates in alkali medium. Nucleophilic addition reaction afforded solid products easily purifiable with recrystallization from ethanol and water in variable yields (20-96%).



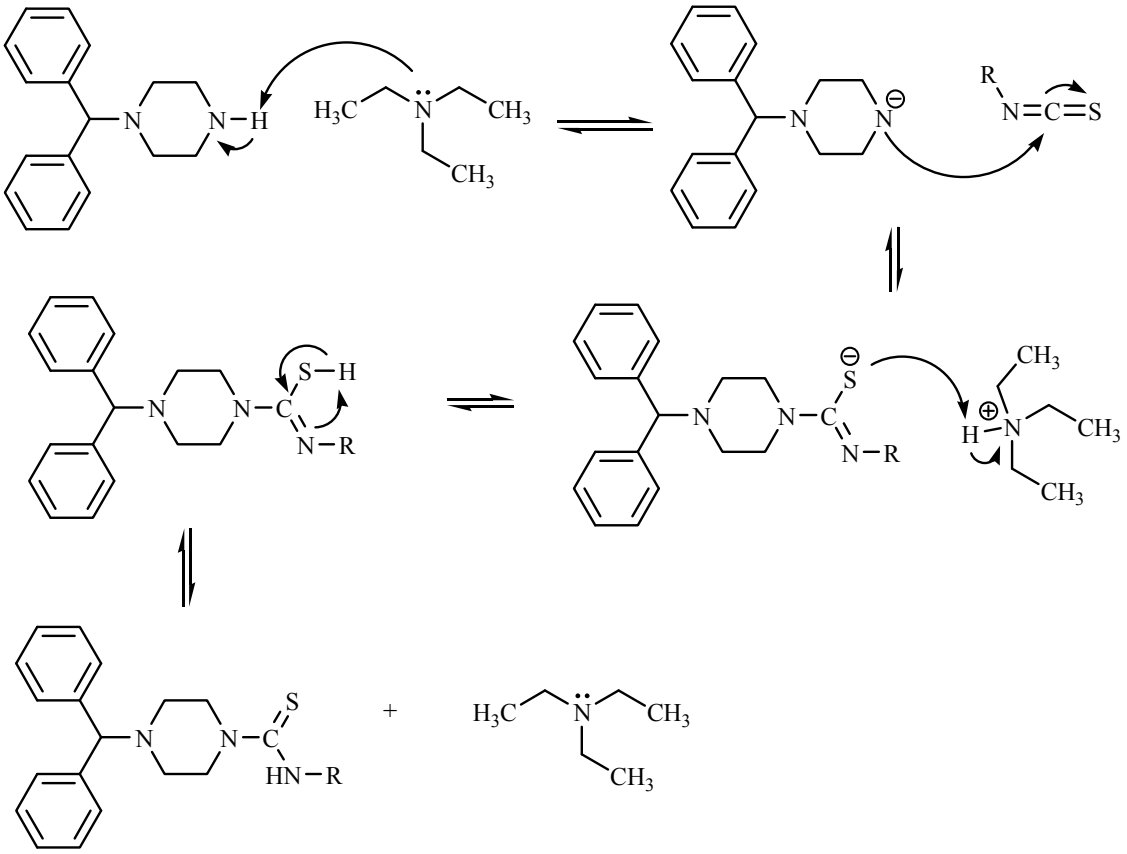
Mechanism of the carboxamide formation with isocyanate derivatives is proposed to be nucleophilic addition depicted as:



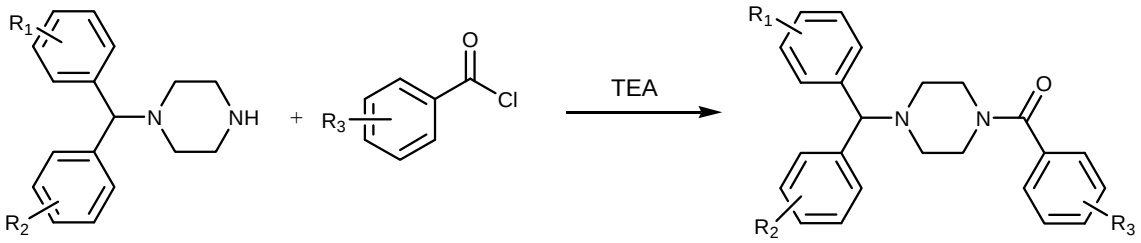
In order to gain thioamide derivatives, 1-benzhydrylpiperazines were reacted with appropriate isothiocyanates in alkali medium. Nucleophilic addition reaction afforded solid products easily purifiable with recrystallization from ethanol and water in variable yields (10-50%).



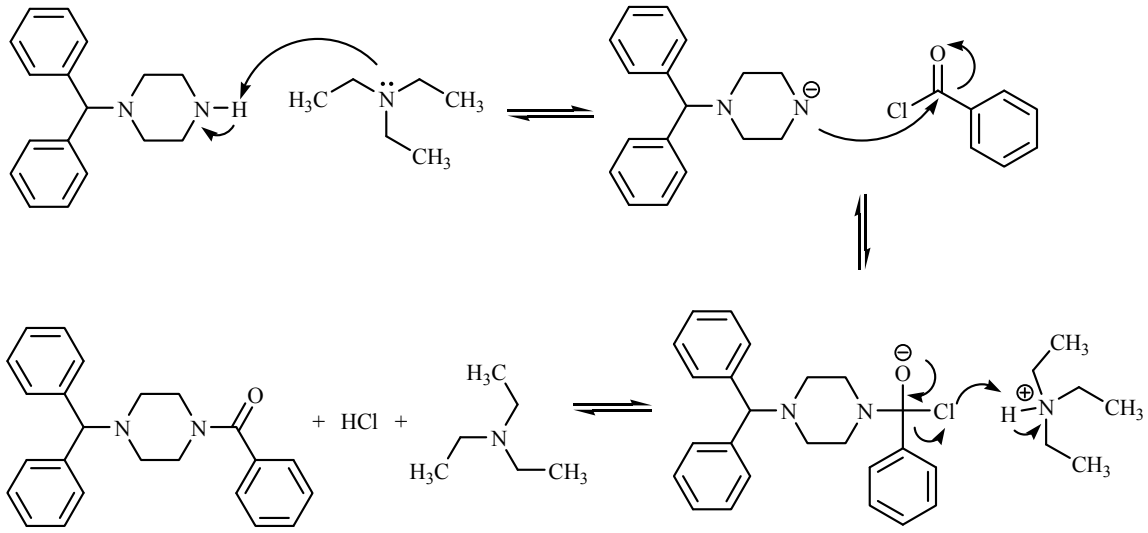
Mechanism of the thioamide formation of benzhydrylpiperazine is proposed to be nucleophilic addition depicted as:



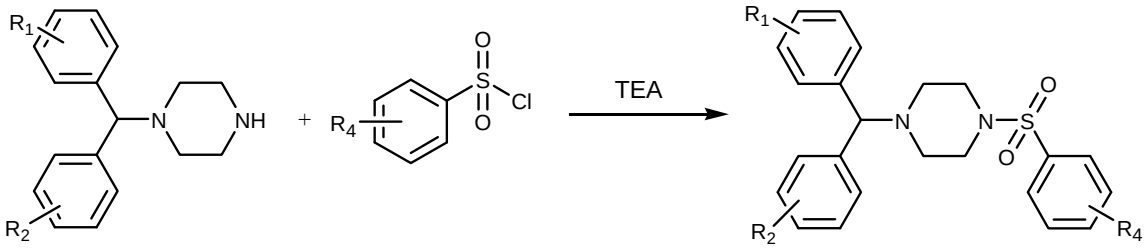
Nucleophilic substitution reactions of 1-benzhydrylpiperazines with different benzoyl chlorides were carried out in alkali medium to produce liquid or solid benzoylpiperazine derivatives which were easily purified by recrystallization or column chromatography. Liquid benzoyl derivatives were solidified by hydrochloride salt formation. Yields of benzoyl derivatives were variable (11-95%).



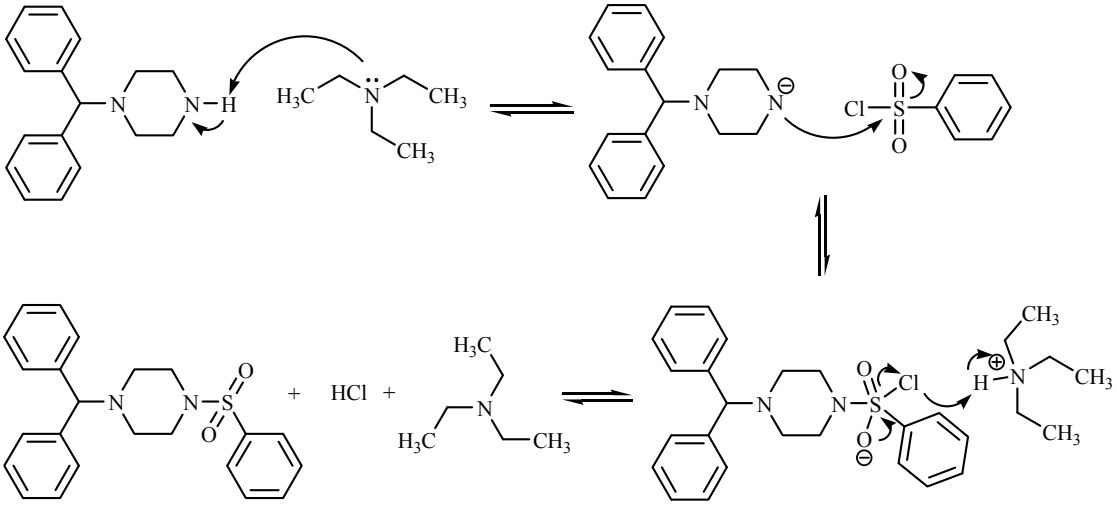
Mechanism of benzhydrylpiperazine *N*-acylation is proposed to be nucleophilic substitution depicted as:



With the intention to synthesize sulfonylpiperazine derivatives, 1-benzhydrylpiperazines were treated with suitable sulfonyl chlorides in alkali medium. Nucleophilic substitution reaction afforded appropriate solid products easily purifiable with recrystallization from ethanol and water in variable yields (10-42%).



Mechanism of the sulfonamide formation of benzhydrylpiperazine is proposed to be nucleophilic substitution depicted as:



Following the synthesis of the compounds, elucidation of their structures with spectral analysis was carried out. All spectral data are in accordance with the assumed structures.

In UV spectra of compound **1** there are two significant bands at 205 and 224 nm which represent π→π* and n→π* transitions of the carboxamide series.

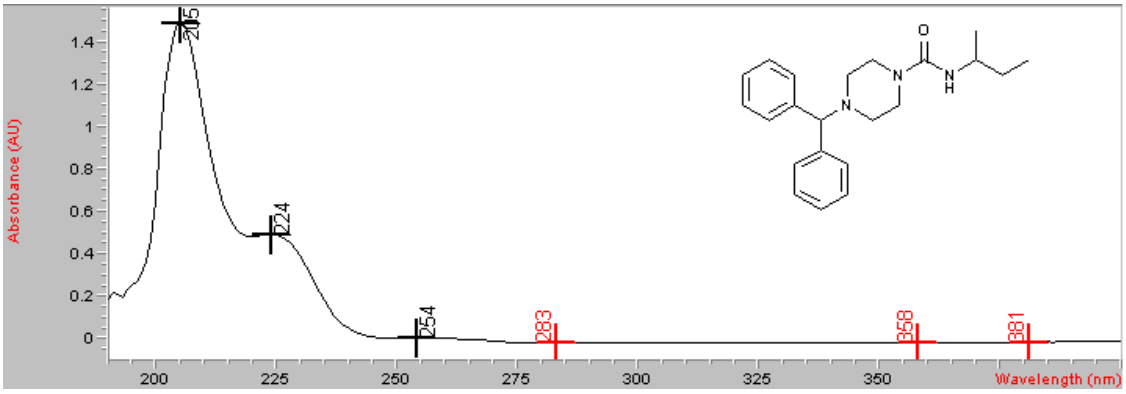


Figure 5.1. UV spectrum of compound **1**.

In UV spectra of compound **27** there are three significant bands at 202, 224 and 248 nm which represent π→π* and n→π* transitions of the thioamide series.

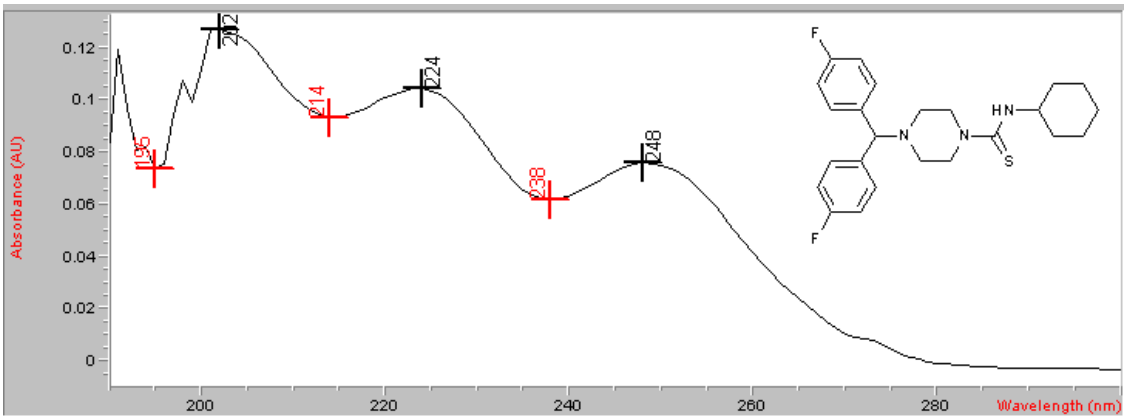


Figure 5.2. UV spectrum of compound **27**.

In UV spectra of compound **33** there are two significant bands at 205 and 224 nm which represent π→π* and n→π* transitions of the benzoylpiperazine series.

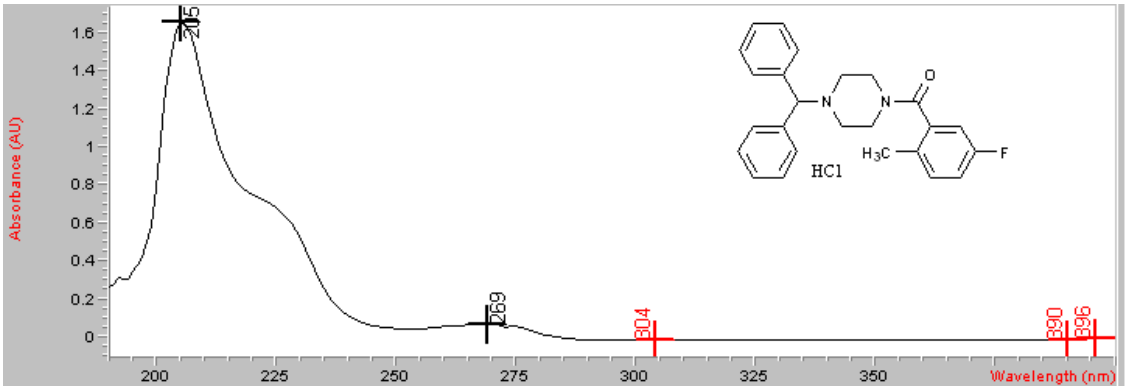


Figure 5.3. UV spectrum of compound **33**.

In UV spectra of compound **48** there are two significant bands at 205 and 224 nm which represent π→π* and n→π* transitions of the sulfonylpiperazine series.

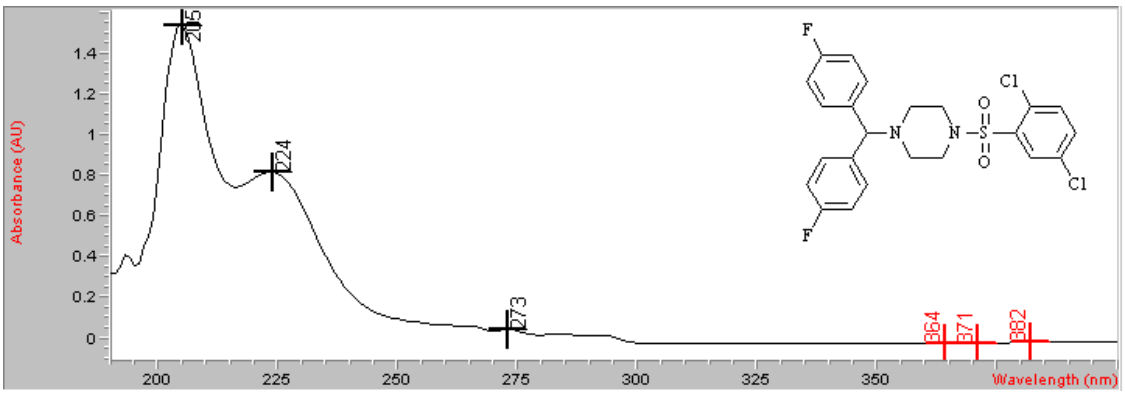


Figure 5.4. UV spectrum of compound **48**.

IR spectrum of compound **24** represents the IR absorption bands of carboxamide series. Characteristic N-H stretching band is observed at 3332 cm^{-1}. Other stretching bands are observed at 3026 cm^{-1} (C-H; aromatic), 2967 cm^{-1} (C-H; aliphatic), 1626 cm^{-1} (C=O; amide), 1523 cm^{-1} (C=C; aromatic), 1251 cm^{-1} (C-N) and 1089 cm^{-1} (C-Cl).

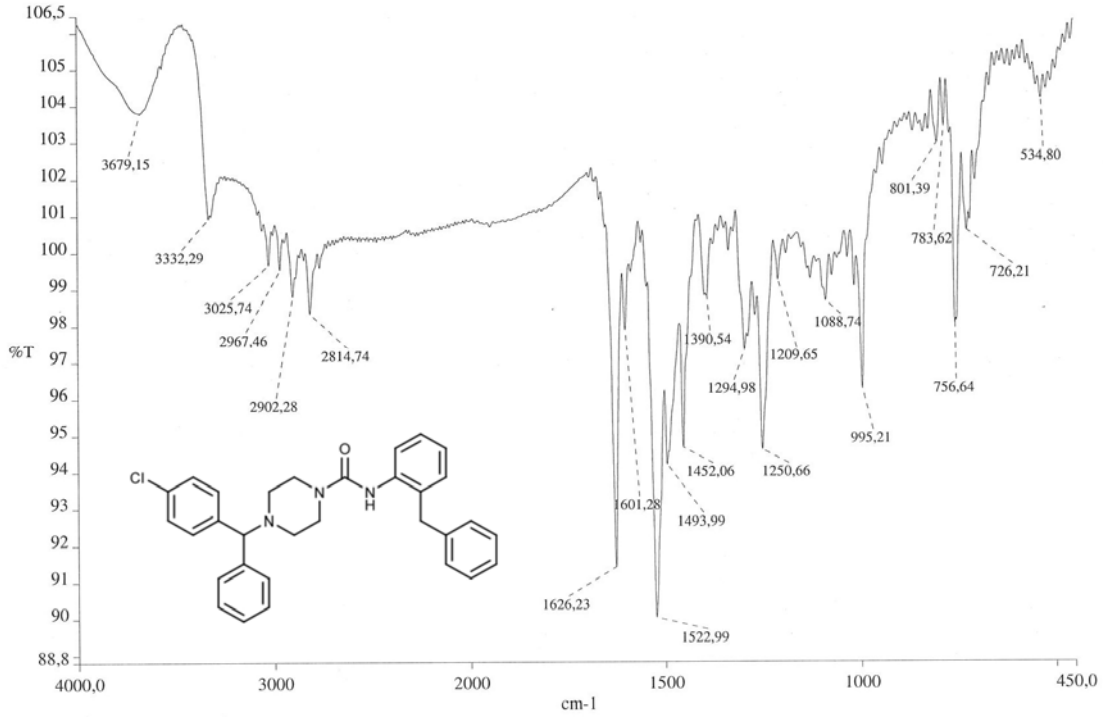


Figure 5.5. IR spectrum of compound **24**.

IR spectrum of compound **27** represents the IR absorption bands of thioamide series. Characteristic N-H stretching band is observed at 3328 cm^{-1}. Other stretching bands are observed at 3060 cm^{-1} (C-H; aromatic), 2996 cm^{-1} (C-H; aliphatic), 1603 cm^{-1} (C=C; aromatic), 1299 cm^{-1} (C-N) and 1221 cm^{-1} (C=S) and 1104 cm^{-1} (C-F).

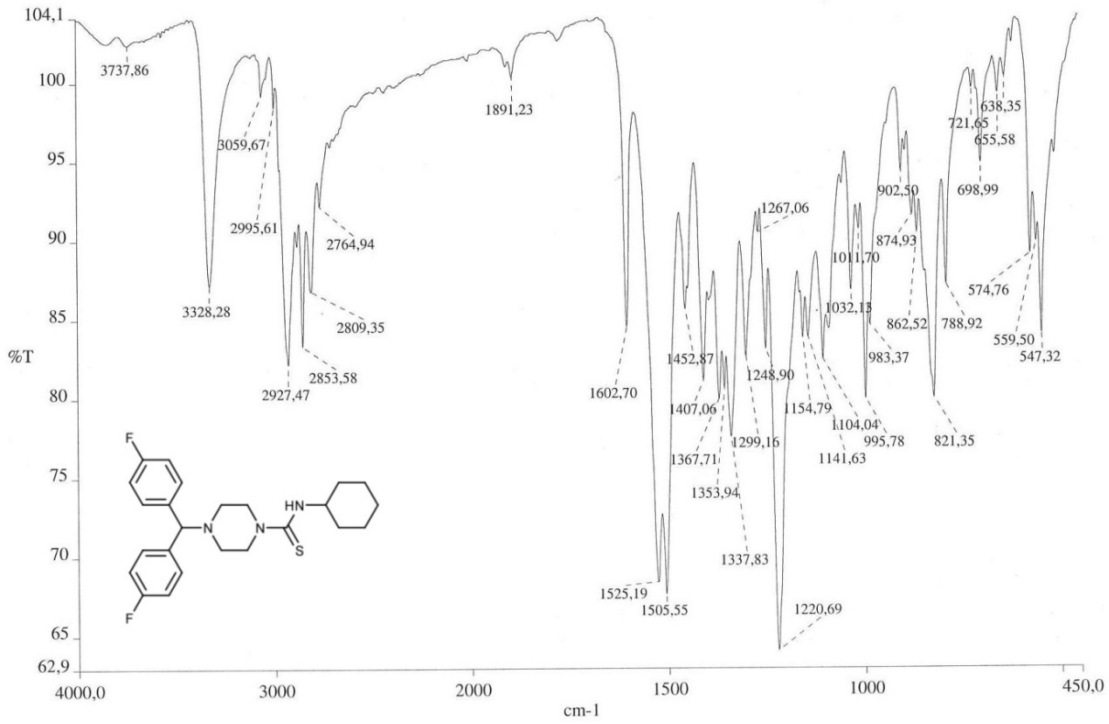


Figure 5.6. IR spectrum of compound **27**.

IR spectrum of compound **40** illustrates the loss of an absorption band in the 3100-3400 cm^{-1} area indicating the successful substitution of piperazine N-H to give benzoylpiperazine derivatives. The stretching bands are observed at 3082 cm^{-1} (C-H; aromatic), 2966 cm^{-1} (C-H; aliphatic), 1621 cm^{-1} (C=O), 1583 cm^{-1} (C=C; aromatic), 1268 cm^{-1} (C-O), 1230 cm^{-1} (C-N) and 1027 cm^{-1} (C-Cl).

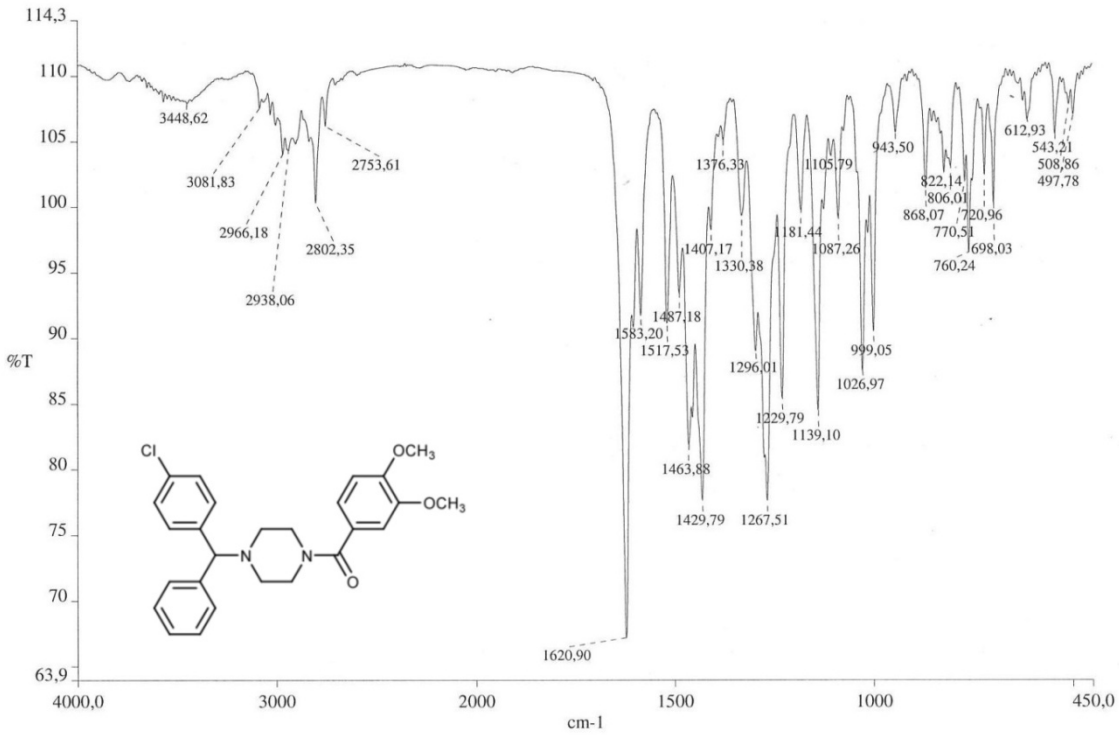


Figure 5.7. IR spectrum of compound **40**.

IR spectrum of compound **51** represents the loss of an absorption band in the 3100-3400 cm^{-1} area indicating the successful substitution of piperazine N-H to give sulfonylpiperazine derivatives. The stretching bands are observed at 3055 cm^{-1} (C-H; aromatic), 2947 cm^{-1} (C-H; aliphatic), 1606 cm^{-1} (C=C; aromatic), 1534 cm^{-1} (N=O), 1356 (S=O; asym.), 1281 cm^{-1} (C-N), 1170 cm^{-1} (S=O; sym.) and 1088 cm^{-1} (C-Cl).

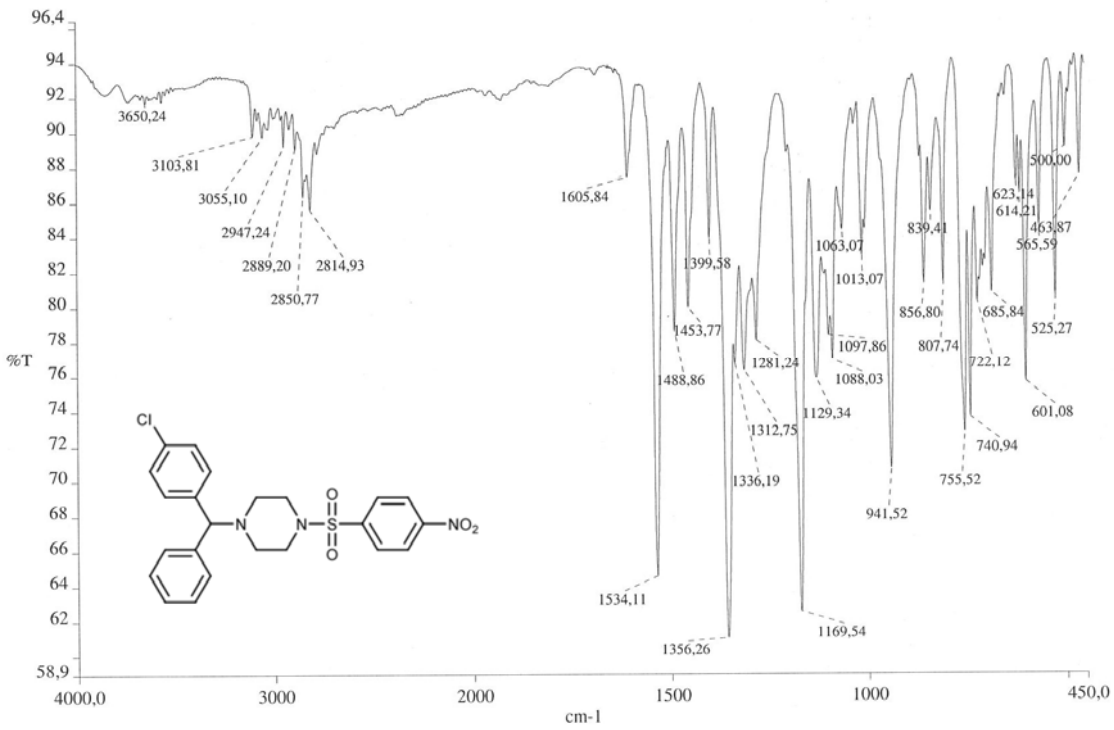
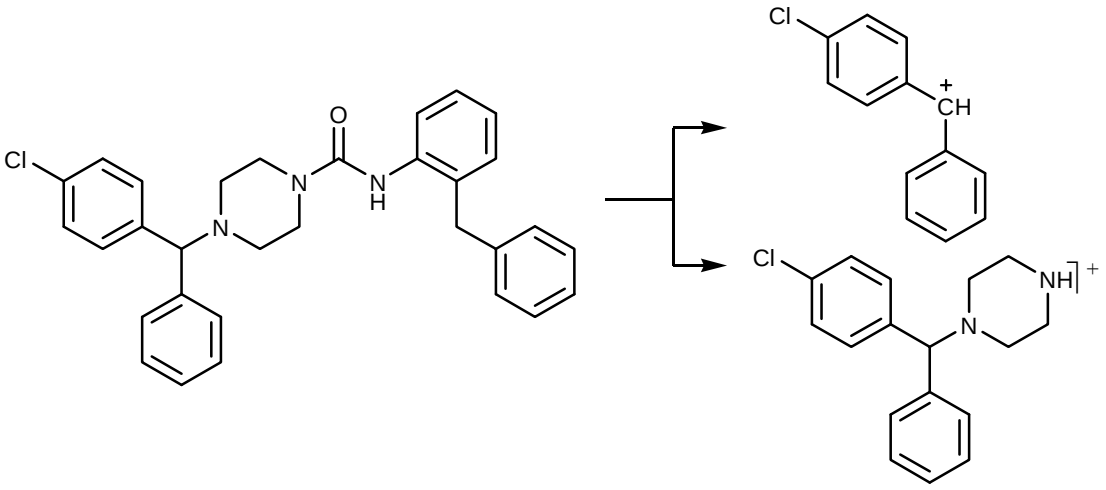


Figure 5.8. IR spectrum of compound **51**.

Mass spectra of carboxamide derivatives are illustrated with compound **24**. M^+ peak is observed as base peak at 496.9 (m/z) and fragmentation products give peaks at 287.8 and 201.6 (m/z). M+2 peak is well recognizable which corresponds to chloride isotope of ≈ 33% abundance. The fragmentation pattern is illustrated in scheme 5.2.



Scheme 5.2. Mass fragmentation pattern of compound **24**.

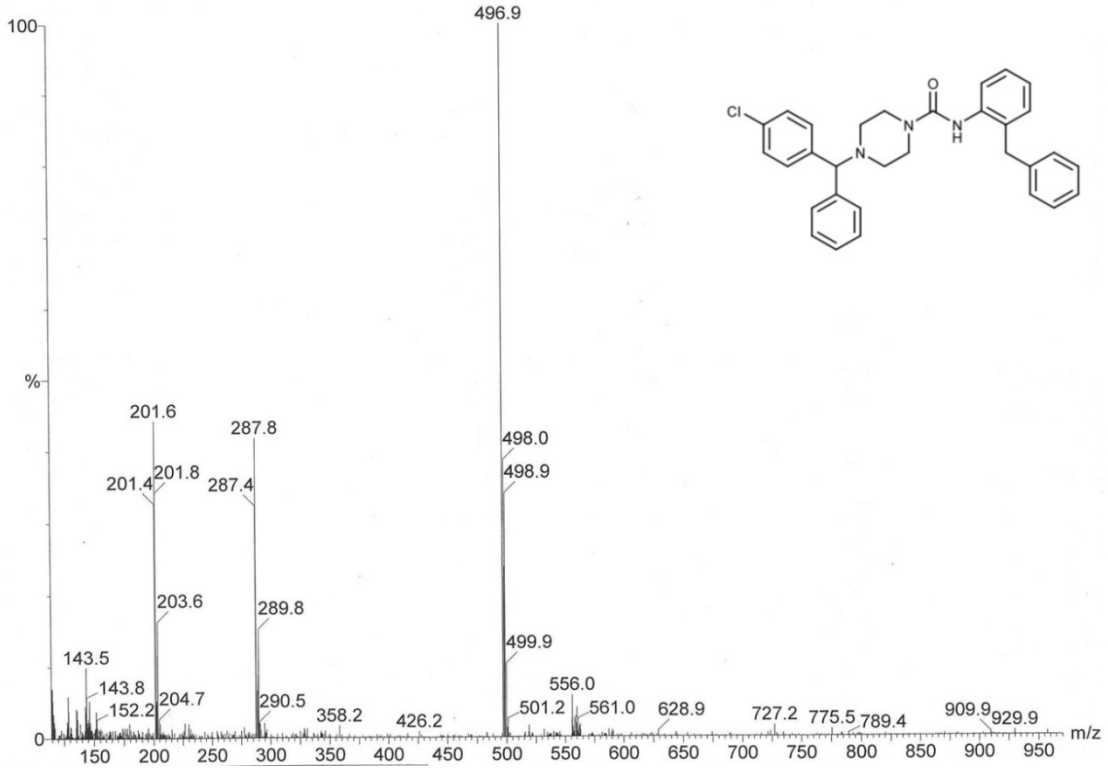
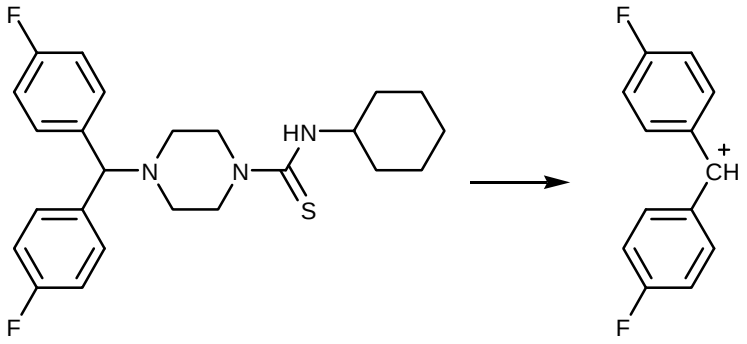


Figure 5.9. Mass spectrum of compound **24**.

Mass spectra of thioamide derivatives are illustrated with compound **27**. M^+ peak is observed as base peak at 430.95 (m/z) and fragmentation product gives peak at 203.65 (m/z). The fragmentation pattern is illustrated in scheme 5.3.



Scheme 5.3. Mass fragmentation pattern of compound **27**.

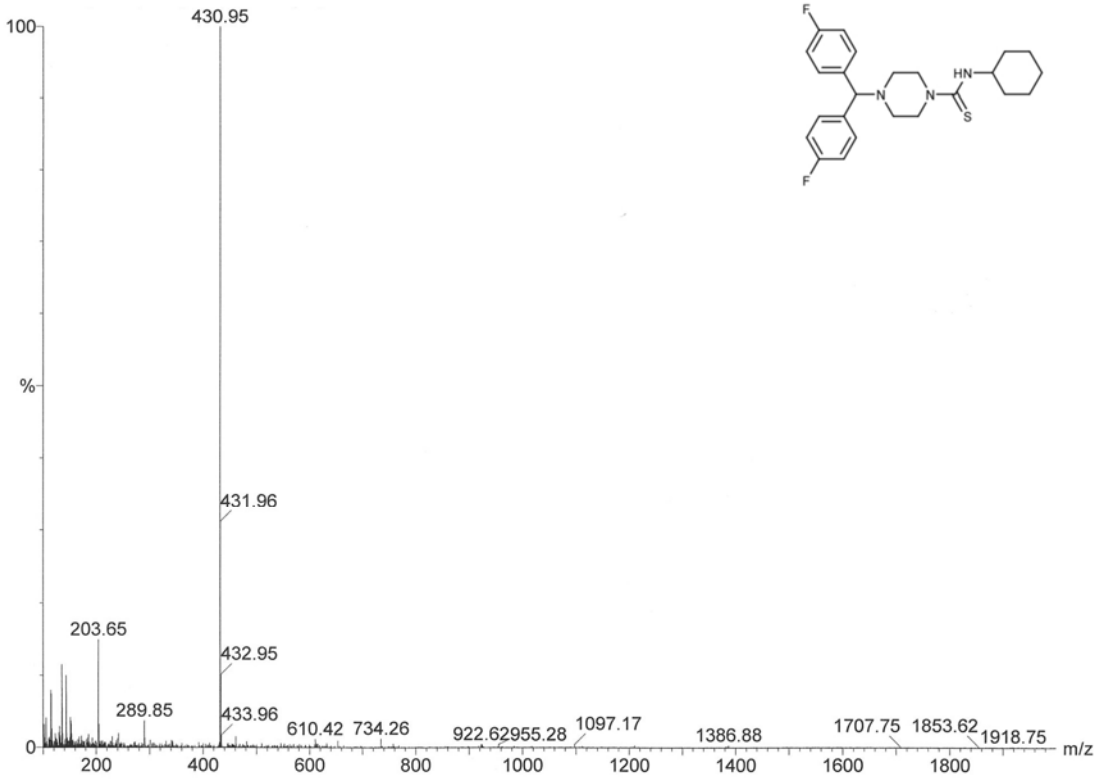
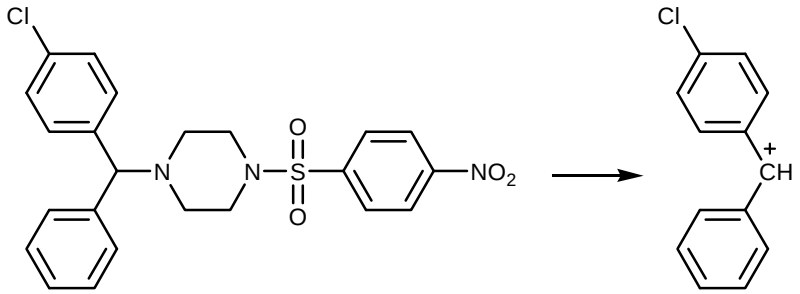


Figure 5.10. Mass spectrum of compound **27**.

Mass spectra of sulfonylpiperazine derivatives are illustrated with compound **51**. M^+ peak is observed at 472.8 (m/z, ≈ 25%) and fragmentation product gives base peak at 201.5 (m/z). M+2 peak is well recognizable which corresponds to chloride isotope of ≈ 12% abundance. The fragmentation pattern is illustrated in scheme 5.4.



Scheme 5.4. Mass fragmentation pattern of compound **51**.

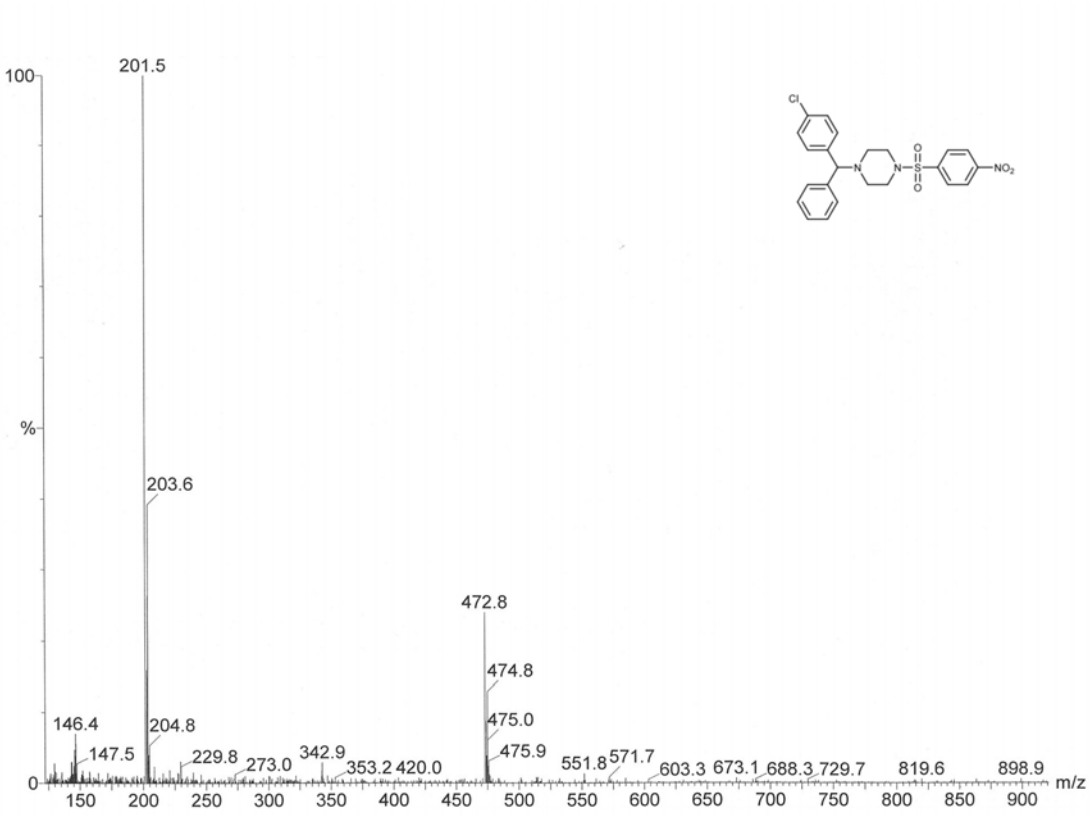


Figure 5.11. Mass spectrum of compound **51**.

H^1-NMR spectra of carboxamide derivatives are represented with compound **24**. The protons of piperazine are seen at 2.23 (bs, 4H, H^1) ppm and 3.36 (bs, 4H, H^2) ppm respectively. Benzyl methylene gives singlet at 3.91 ppm. Diphenylmethyl C-H gives singlet at 4.34 ppm. Aromatic rings give multiplet at 7.05-7.47 ppm. Amide N-H gives singlet at 7.96 ppm.

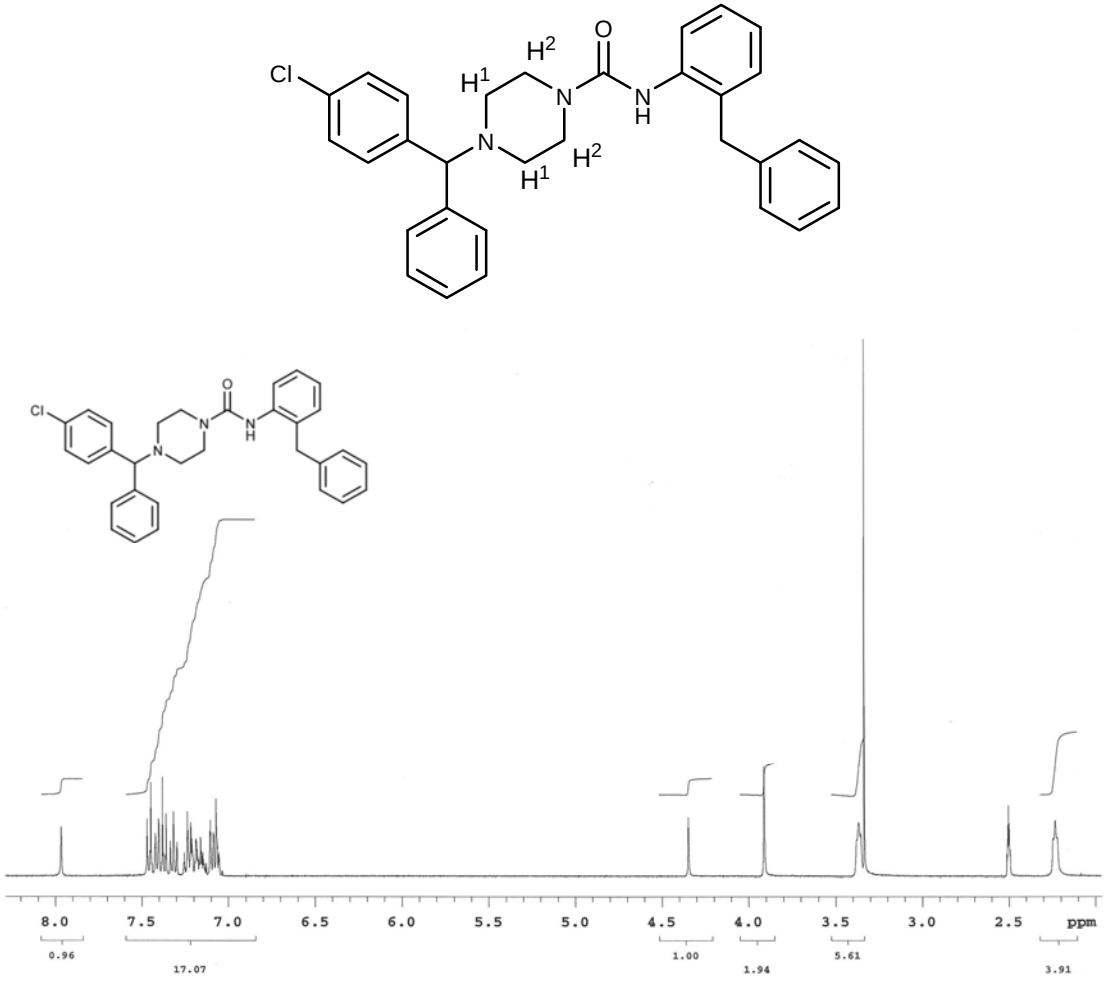


Figure 5.12. H^1-NMR spectrum of compound **24**.

H^1-NMR spectra of thioamide derivatives are represented with compound **30**. The protons of piperazine are seen at 2.28 (t, 4H, H^2, J = 5.2 Hz) ppm and 3.79 (t, 4H, H^1, *J=4 Hz*) ppm respectively. Methylene protons of –NH$\underline{CH_2}$CH= group are observed at 4.15 ppm (t, 2H, *J=5.6 Hz*). Diphenylmethyl C-H gives singlet at 4.39 ppm. Allyl group has characteristic peaks observed at 5.80-5.90 (m, 1H, H_X) ppm and at 5.01-5.11 (dd, 2H, J^{M-X}*=17.2 Hz*, J^{M-A}*=1.6 Hz*, J^{A-X}*=8.6 Hz*) ppm. Protons of aromatic rings give multiplet at 7.19-7.46 ppm. Thioamide N-H is observed at 7.80 ppm (t, 1H, *J=5.2 Hz*).

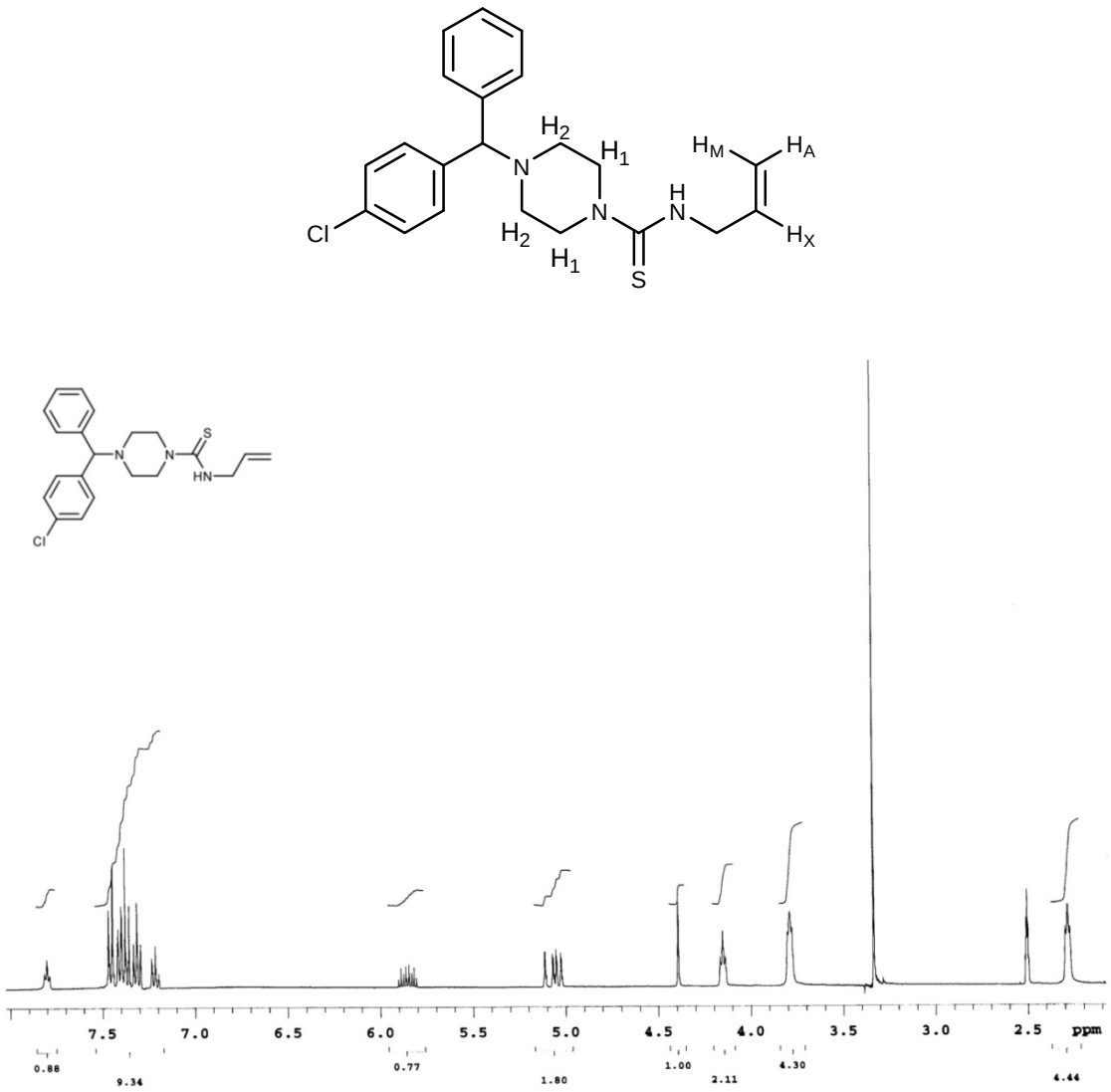


Figure 5.13. H^1-NMR spectrum of compound **30**.

H^1-NMR spectra of benzoylpiperazine derivatives are represented with compound **40**. Protons of piperazine ring are seen at 2.31 ($H^{2,6}$) ppm and 3.52 ($H^{3,5}$) ppm as broad singlets. Methoxy protons give two singlets at 3.75 ppm and 3.76 ppm. Diphenylmethyl C-H is observed as a singlet at 4.39 ppm. Protons of aromatic rings give multiplet peaks at 6.91-7.46 ppm.

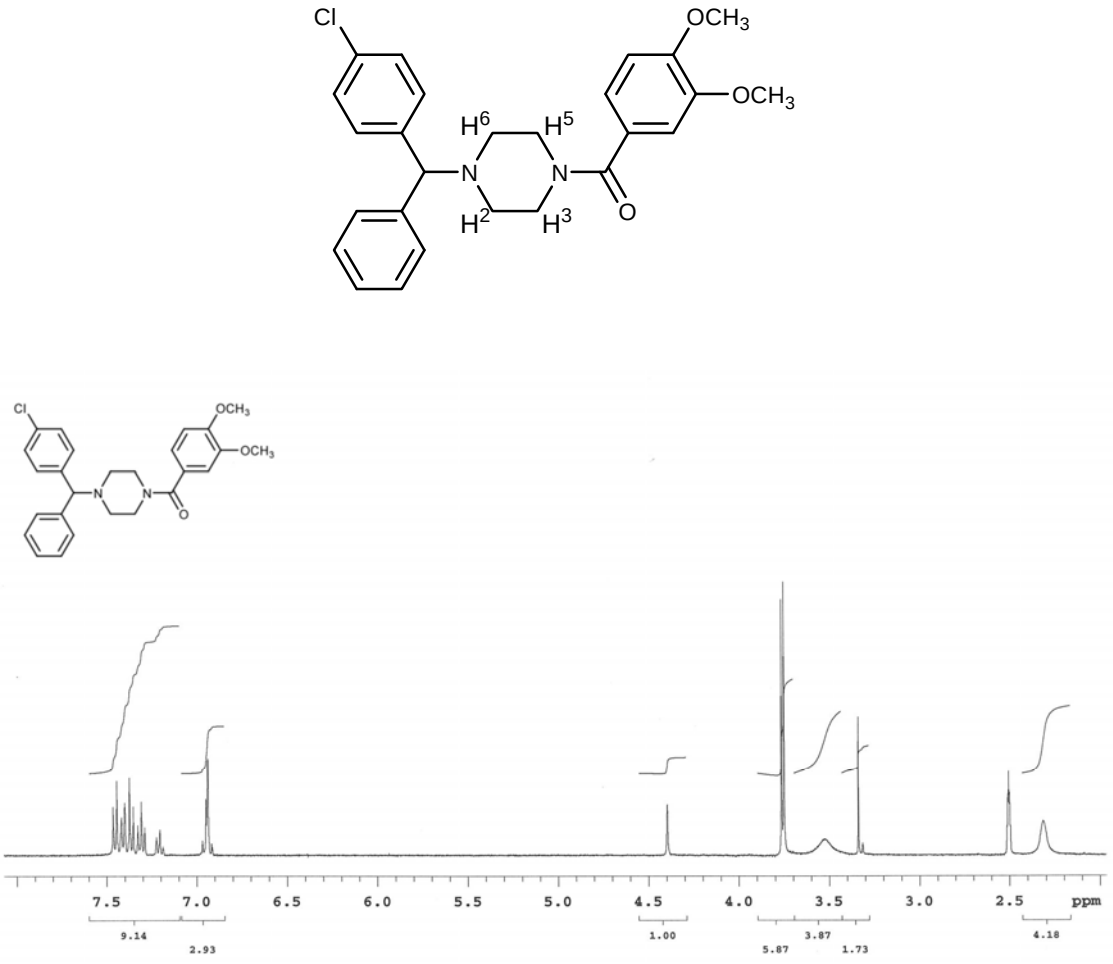
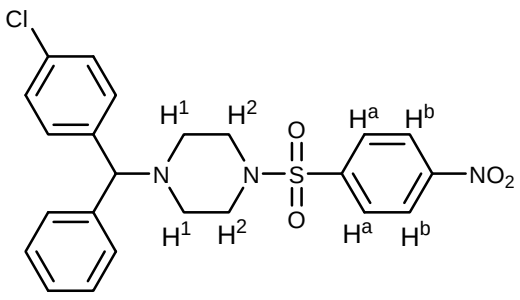


Figure 5.14. H^1-NMR spectrum of compound **40**.

H^1-NMR spectra of sulfonylpiperazine derivatives are represented with compound **51**. Protons of piperazine moiety are observed at 2.36 (H^1) ppm and 2.99 (H^2) ppm as broad singlets. Diphenylmethyl C-H is recognized as a singlet at 4.38 ppm. Protons of 4-chlorobenzhydryl group are seen as multiplets at 7.15-7.38 ppm. Protons of 4-nitrophenyl ring are observed at 8.46 (d, 2H, H^b, *J=8.8 Hz*, *J=2.4 Hz*) ppm and 8.01 (d, 2H, H^a, *J=8.8 Hz*, *J=2.8 Hz*) ppm.



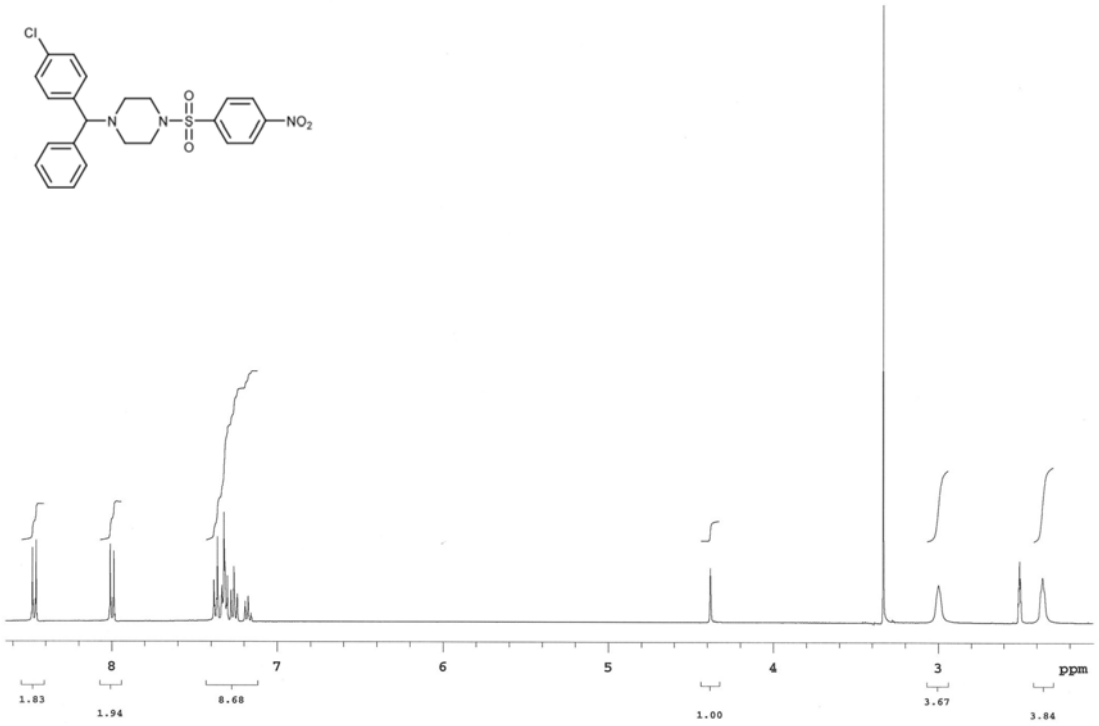
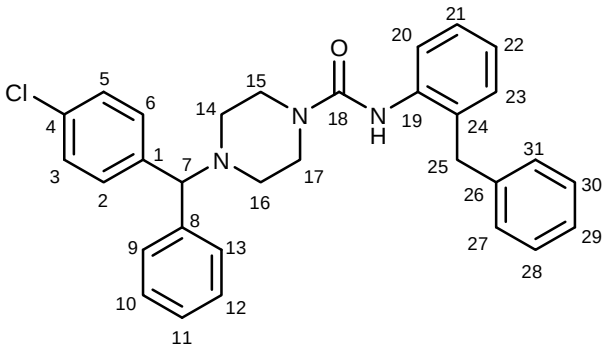


Figure 5.15. H^1-NMR spectrum of compound **51**.

The ^{13}C-NMR spectrum of the compound **24** was taken in dimethylsulfoxide-d_6 (DMSO-d_6). Characteristic peaks of the carboxamide derivatives were observed at 44.46-51.91 ppm for piperazine ring, 74.48 ppm for diphenylmethyl carbon and 156.06 ppm for carbonyl group.



C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	138.23	17	51.91
2	130.64	18	156.06
3	130.11	19	136.87

4	132.09	20	125.46
5	130.11	21	127.23
6	130.64	22	126.52
7	74.48	23	129.23
8	142.26	24	142.64
9	128.91	25	37.74
10	129.42	26	141.05
11	128.31	27	127.79
12	129.42	28	129.33
13	128.91	29	126.97
14	44.46	30	129.33
15	51.91	31	127.79
16	44.46		

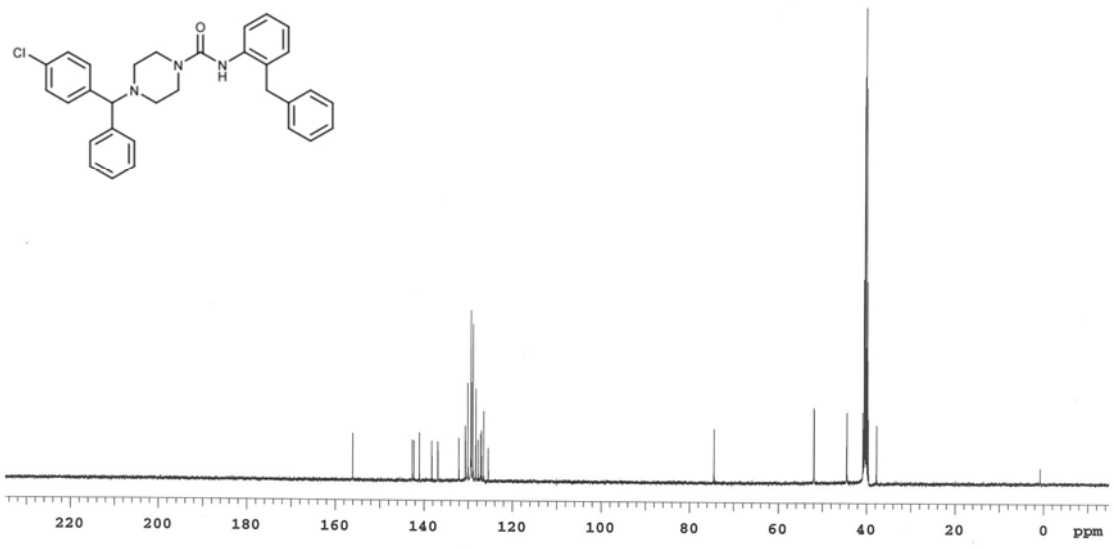
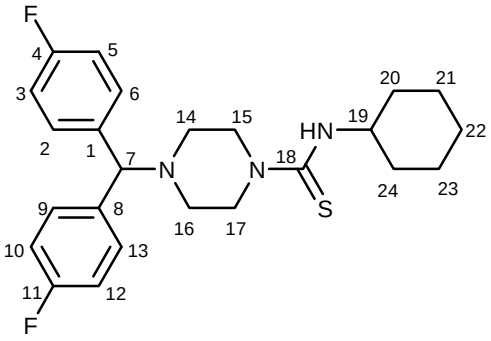


Figure 5.16. ^{13}C-NMR spectrum of compound **24**.

The ^{13}C-NMR spectrum of the compound **27** was taken in dimethylsulfoxide-d_6 (DMSO-d_6). Characteristic peaks of the thioamide derivatives were observed at 47.06 and 50.85 ppm for piperazine ring, 72.32 ppm for diphenylmethyl carbon and 180.14 ppm for carbonyl group.



C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	138.15	13	129.35
2	129.43	14	47.06
3	115.35	15	50.85
4	162.21	16	47.06
5	115.35	17	50.85
6	129.43	18	180.14
7	72.32	19	54.28
8	138.12	20	31.97
9	129.35	21	24.99
10	115.14	22	25.18
11	159.79	23	24.99
12	115.14	24	31.97

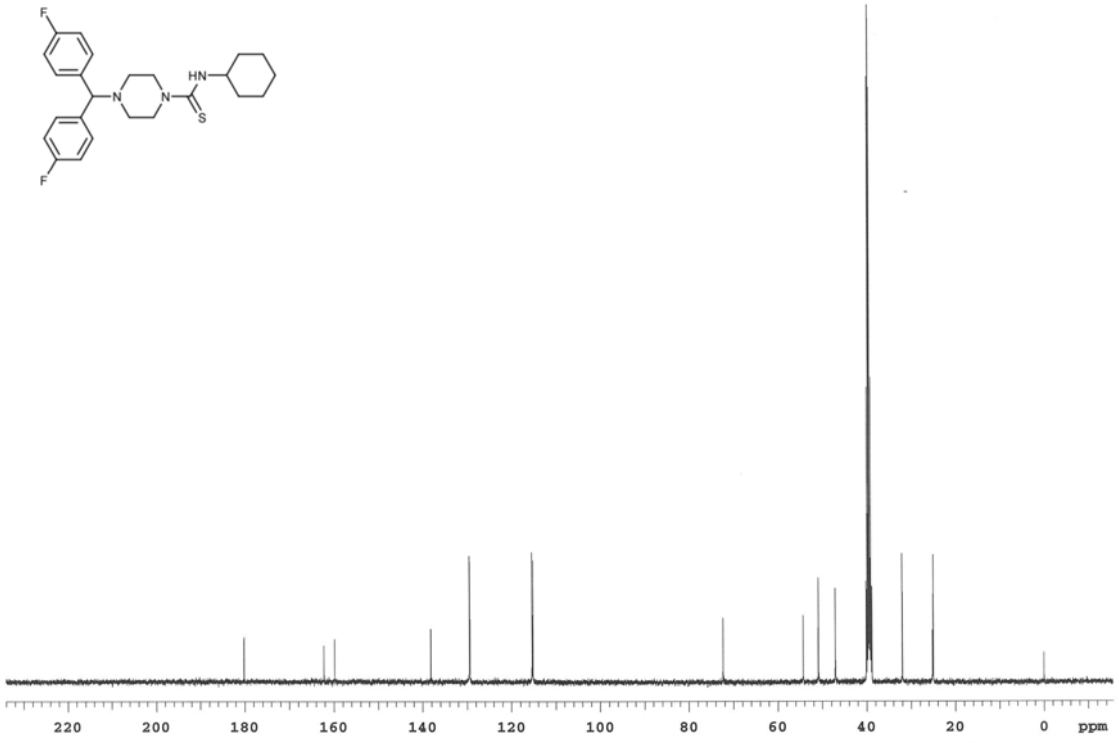
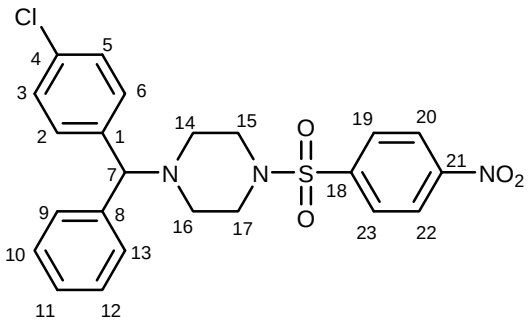


Figure 5.17. ^{13}C-NMR spectrum of compound **27**.

The ^{13}C-NMR spectrum of the compound **51** was taken in dimethylsulfoxide-d_6 (DMSO-d_6). Characteristic peaks of the sulfonamide derivatives were observed at 46.67 and 50.81 ppm for piperazine ring and 73.69 ppm for diphenylmethyl carbon.



C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	140.99	13	129.29
2	129.81	14	50.81
3	129.89	15	46.67
4	132.11	16	50.81
5	129.89	17	46.67

6	129.81	18	142.29
7	73.69	19	129.18
8	141.92	20	125.38
9	129.29	21	150.76
10	128.08	22	125.38
11	127.79	23	129.18
12	128.08		

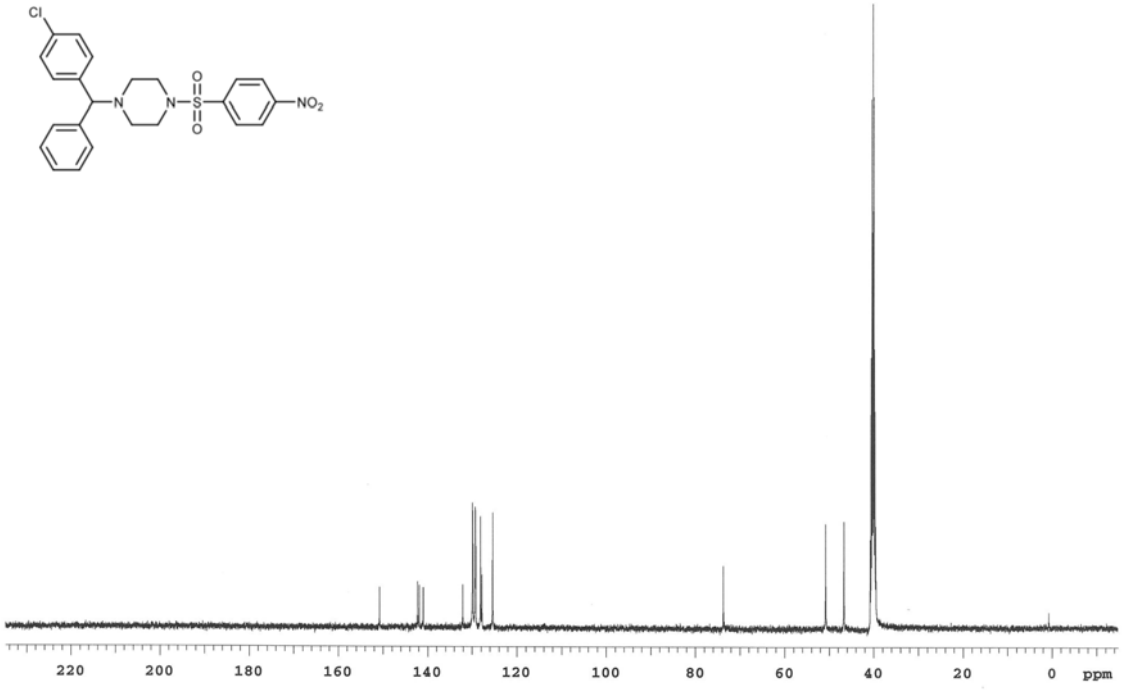


Figure 5.18. ^{13}C-NMR spectrum of compound **51**.

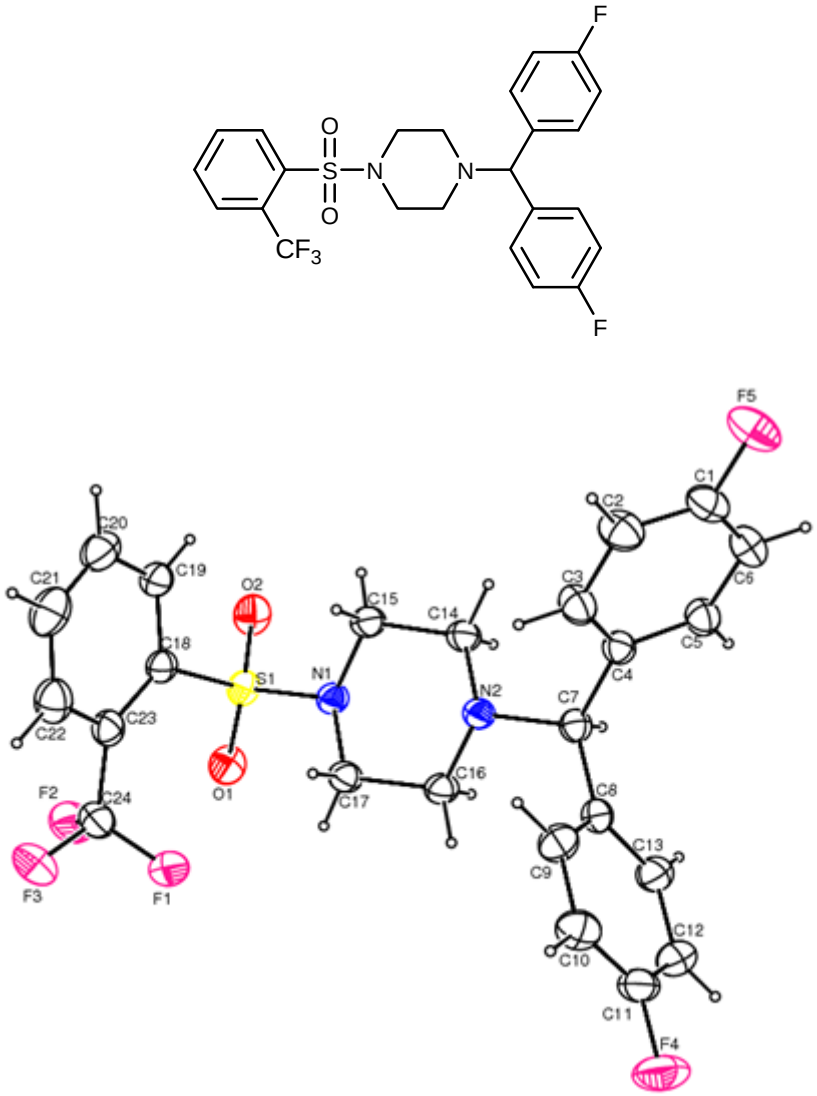


Figure 5.19. Atom-labelling scheme of compound **43**. Displacement ellipsoids are drawn at the 30% probability level.

The molecular structure of compound **43** and the atom-labelling scheme are shown in Fig. 5.19. The molecule is not planar. The C1-C6, C8-C13 and C18-C23 rings are essentially planar (r.m.s. deviations 0.0054, 0.0037 and 0.0171Å respectively). The dihedral angles between the mean planes of C1-C6 (A), C8-C13 (B) and C18-C23 (C) rings are 70.44(06)˚ (A/B), 34.48(07)˚ (B/C) and 37.81(07)˚ (A/C). The N1-C15-C14-N2-C16-C17 ring exhibits a puckered conformation, with puckering parameters Q=0.5861(19)Å, θ=177.45(17)˚ and Ø=13(5)˚, which indicates that the N1-C15-C14-N2-C16-C17 ring has a chair conformation.

The compound contains an intramolecular hydrogen bond (C17-H15B· · ·O2). This hydrogen bond produces S(5) ring. The crystal packing is stabilized by van der Waals interactions.

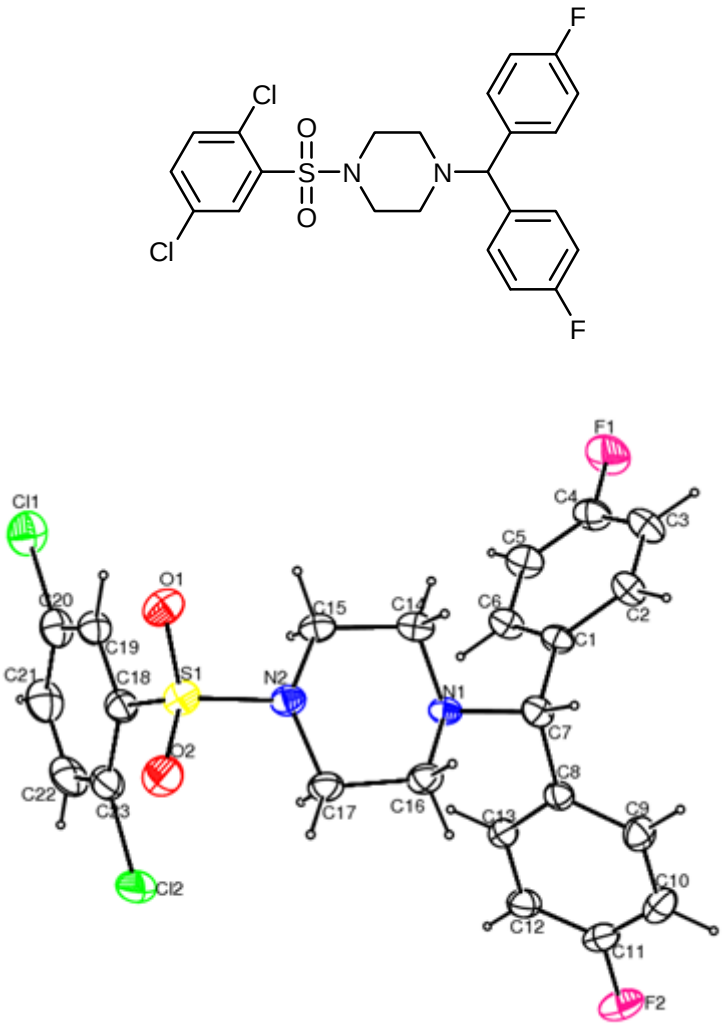


Figure 5.20. Atom-labelling scheme of compound **48**. Displacement ellipsoids are drawn at the 20% probability level.

The molecular structure of compound **48** and the atom-labelling scheme are shown in Fig. 5.20. The molecule is not planar. The C1-C6, C8-C13 and C18-C23 rings are essentially planar (r.m.s. deviations 0.0055, 0.0024 and 0.0030Å respectively). The dihedral angles between the mean planes of C1-C6 (A), C8-C13 (B) and C18-C23 (C) rings are 72.70(10)˚ (A/B), 39.28(12)˚ (B/C) and 45.84(10)˚ (A/C). The N1-C15-C14-N2-C16-C17 ring exhibits a puckered conformation, with puckering parameters

Q=0.5928(25)Å, θ= 172.34(22)˚ and Ø= 164.6(19)˚, which indicates that the N1-C15-C14-N2-C16-C17 ring has a chair conformation.

The compound contains an intramolecular hydrogen bond (C17-H17A· · ·Cl2). This hydrogen bond produces S(7) ring. The compound also contains two intermolecular hydrogen bonds. Firstly, atom C10 in the asymmetric unit acts as hydrogen-bond donor, *via* H11A, connecting this molecule to O1 in a symmetry related molecule at (x,+y-1,+z), forming a C(10) chain running paralel to the [010] direction. Secondly, atom C17 acts as a hydrogen-bond donor, *via* atom H17B, to atom O2 in a symmetry related molecule at (-x,-y,-z+1), so forming a centrosymmetric $R_2^2(10)$ ring. The combination of these two hydrogen bonds produces $R_4^4(30)$ rings running paralel to the [010] direction (Fig. 5.21.).

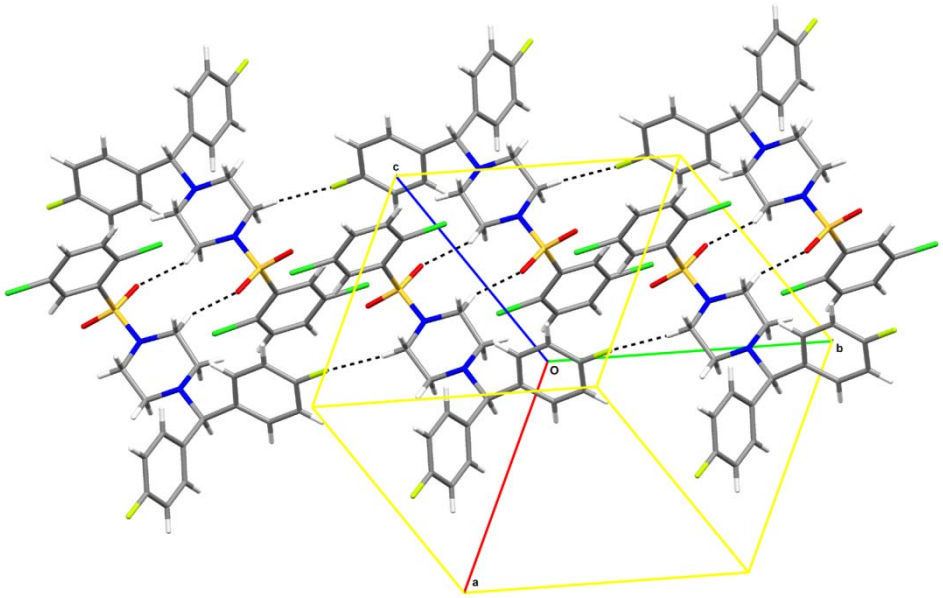
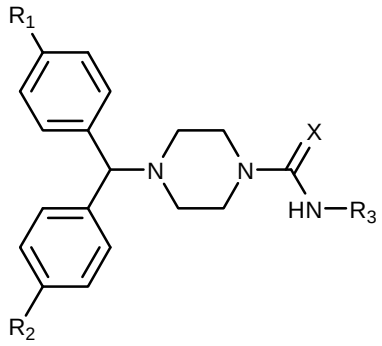


Figure 5.21. Part of the crystal structure of compound **48**, showing the formation of a chain of edge-fused $R_4^2(20)$ rings[b].

All of the synthesized compounds have been evaluated for *in vitro* cytotoxic activity by sulphorhodamine B (NCI-SRB) assay.

According to results shown in table 4.13., it is markedly evident that most of the nonsubstituted benzhydrylpiperazine derivatives are inactive or they have low activities against all cancer cell lines. It should also be noted that, in general, 4-chloro-benzhydrylpiperazine derivatives have higher activities becoming superior over their

4,4-difluoro and nonsubstituted counterparts. Moreover, thioamide derivatives are more potent than carboxamide derivatives against all cancer cell lines. Corresponding compound groups representing these findings are shown below.



Comp.	X	R_1	R_2	R_3	HUH-7	MCF-7	HCT-116
3	O	- H	- H	Isopropyl	-	-	-
13	O	- F	- F	Isopropyl	36,57	45,23	20,94
19	O	- Cl	- H	Isopropyl	15,36	13,16	17,12
29	S	- Cl	- H	Isopropyl	6,20	11,47	14,98
5	O	- H	- H	2,6-Dichlorophenyl	-	-	-
21	O	- Cl	- H	2,6-Dichlorophenyl	6,44	6,14	8,93
6	O	- H	- H	2-Benzylphenyl	-	-	-
24	O	- Cl	- H	2-Benzylphenyl	17,22	16,91	4,76
8	O	- H	- H	Allyl	-	10,91	-
20	O	- Cl	- H	Allyl	16,29	9,12	10,14
30	S	- Cl	- H	Allyl	9,95	4,94	8,85

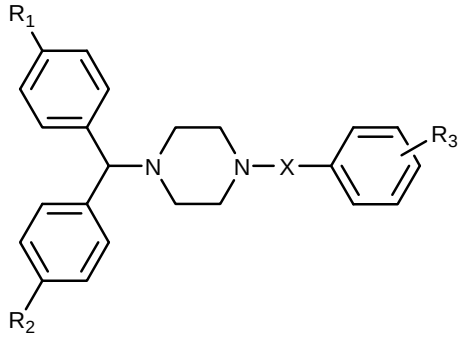
Table 5.1. IC_{50} (μM) values of some carboxamide and thioamide derivatives.

Compounds **3**, **13**, **19** and **29** have the same substituents on NH group. Compound **3** (R_1, R_2=H, R_3=isopropyl, X=O) has no cytotoxicity against any of these cancer cell lines. However compound **13** (R_1, R_2=F, R_3=isopropyl, X=O) has slight cytotoxicity, compound **19** (R_1=Cl, R_2=H, R_3=isopropyl, X=O) has good cytotoxicity and compound **29** (R_1=Cl, R_2=H, R_3=isopropyl, X=S) has the highest cytotoxicity against all three cancer cell lines.

Compounds **5** and **21** have the same substituents on NH group. Compound **5** (R_1, R_2=H, R_3=2,6-dichlorophenyl, X=O) has no cytotoxicity against any of the cancer cell lines. Interestingly, compound **21** (R_1=Cl, R_2=H, R_3=2,6-dichlorophenyl, X=O) has increased cytotoxicity against all the cancer cell lines.

Compounds **6** and **24** have the same substituents on NH group. Compound **6** (R_1, R_2=H, R_3=2-benzylphenyl, X=O) has no cytotoxicity against none of these cancer cell lines. However compound **24** (R_1=Cl, R_2=H, R_3=2-benzylphenyl, X=O) has good cytotoxicity against all the cancer cell lines.

Compounds **8**, **20** and **30** have the same substituents on NH group. Compound **8** (R_1, R_2=H, R_3=allyl, X=O) has no cytotoxicity against HUH-7 and HCT-116 cell lines wheras the compound has good cytotoxicity against MCF-7 cell line. However compound **20** (R_1=Cl, R_2=H, R_3=allyl, X=O) has good cytotoxicity and compound **30** (R_1=Cl, R_2=H, R_3=allyl, X=S) has the highest cytotoxicity against all three cancer cell lines.



Comp.	X	R_1	R_2	R_3	HUH-7	MCF-7	HCT-116
44	SO_2	- F	- F	2,4,5-Trichloro	-	-	-
49	SO_2	- Cl	H	2,4,5-Trichloro	54,41	11,16	31,41
47	SO_2	- F	- F	4-Nitro	-	-	-
51	SO_2	- Cl	H	4-Nitro	39,95	17,22	97,74

Table 5.2. IC_{50} (μM) values of some sulfonylpiperazine derivatives.

Compounds **44** and **49** have the same substituents on phenyl group. Compound **44** (R_1, R_2=F, R_3=2,4,5-trichloro, X=SO_2) has no cytotoxicity against any of the cancer cell lines. Wheras, compound **49** (R_1=Cl, R_2=H, R_3=2,4,5-trichloro, X=SO_2) has good to moderate cytotoxicity against all the cancer cell lines.

Compounds **47** and **51** have the same substituents on phenyl group. Compound **47** (R_1, R_2=F, R_3=4-nitro, X=SO_2) has no cytotoxicity against any of the cancer cell lines. Whereas, compound **51** (R_1=Cl, R_2=H, R_3=4-nitro, X=SO_2) has variable cytotoxicity against all the cancer cell lines.

It is useful to categorize the compounds as carboxamides, thioamides, benzoylpiperazines and sulfonylpiperazines to discuss the structure activity relationships for each cell line in detail.

In general, nonsubstituted benzhydryl derivatives are inactive or have low inhibition whereas 4-chlorobenzhydryl derivatives are more active than other compounds against HUH-7 cell line.

The most active compounds against HUH-7 cell line are *N*-(4-cyanophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (compound **25**; IC_{50}=1.29 μM) and *N*-*tert*-butyl-4-[bis(4-fluorophenyl)methyl]piperazine-1-carbothioamide HCl (compound **26**; IC_{50}=5.97 μM).

Among the carboxamide derivatives, compounds bearing electron withdrawing substituents on phenyl ring such as **15** (IC_{50}=9.46 μM), **21** (IC_{50}=6.44 μM), **23** (IC_{50}=8.54 μM) and **25** (IC_{50}=1.29 μM) have high activities against HUH-7 cell line. In addition, alkyl substituted derivatives, except thioamide derivatives, have no activities (compounds **1-4**, **8**, **14**) or low activities (compounds **9-13**, **16-20**) .

Thioamide derivatives generally have good activity values on HUH-7 cell line. It can be noted that thioamides show higher activity than their carboxamide derivatives which can be exemplified by compounds **10** (IC_{50}=29.96 μM) compared with **26** (IC_{50}=5.97 μM), **18** (IC_{50}=20.92 μM) compared with **28** (IC_{50}=10.81 μM), **19** (IC_{50}=15.36 μM) compared with **29** (IC_{50}=6.20 μM) and **20** (IC_{50}=16.29 μM) compared with **30** (IC_{50}=9.95 μM).

Benzoylpiperazines are moderately active on HUH-7 cell line and compound **38** (IC_{50}=8.49 μM) is the most active compound of the series.

Sulfonylpiperazines show low activity or no inhibition on HUH-7 cell line in general. Compound **50** (IC_{50}=10.88 μM) has the highest activity value among the sulfonylpiperazines.

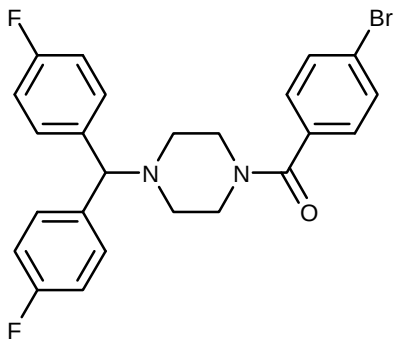
The most active compounds against MCF-7 cell line are 1-(4-bromobenzoyl)-4-[bis(4-fluorophenyl)methyl]piperazine HCl **36** (IC_{50}=2.21 μM) and 1-[2-(trifluoromethoxy)phenylsulfonyl]-4-(diphenylmethyl)piperazine HCl **42** (IC_{50}=4.50 μM).

Against MCF-7 cell line, nonsubstituted benzhydryl carboxamide derivatives (except compounds **4** and **8**) and compounds **9**, **10**, **12**, **27**, **28** together with some of the sulfonylpiperazines (compounds **43**, **44**, **47**, **48**, **50**) show no inhibition.

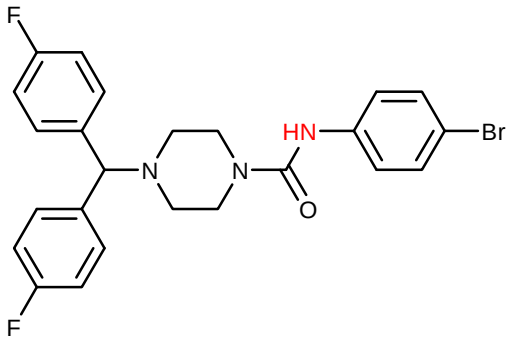
Regarding MCF-7 cell line, the most active compound among the carboxamide derivatives is compound **25** (IC_{50}=6.34 μM) bearing electron withdrawing cyano group on phenyl ring. Alkyl substituted carboxamide derivatives have low activity values such as compounds **4** (IC_{50}=25.7 μM), **11** (IC_{50}=19.03 μM), **13** (IC_{50}=45.23 μM), **14** (IC_{50}=36.14 μM), **18** (IC_{50}=60.24 μM). Thioamide derivatives have variable activities. Compound **30** (IC_{50}=4.94 μM) is the most active compound in the carboxamide series.

On MCF-7 cell line, electron withdrawing halogen substitution on phenyl ring of benzoylpiperazine derivatives has elevated activity values as can be seen for compounds **34** (IC_{50}=6.05 μM), **35** (IC_{50}=5.95 μM), **36** (IC_{50}=2.21 μM) and **37** (IC_{50}=5.87 μM).

4-Bromo substituted carboxamide derivative compound **15** (IC_{50}=8.68 μM) has lower activity than N-H deficient benzoylpiperazine compound **36** (IC_{50}=2.21 μM) against MCF-7 cell line.



Compound **36**
IC_{50} = 2.21 μM



Compound **15**
IC_{50} = 8.68 μM

Against HCT-116 cell line, *N*-*tert*-butyl-4-(diphenylmethyl)piperazine-1-carboxamide **2** (IC_{50}=1.01 μM) and *N*-(4-cyanophenyl)-4-[(4-chlorophenyl)(phenyl)-methyl]piperazine-1-carboxamide **25** (IC_{50}=1.81 μM) are the most active derivatives.

With the exception of compound **2**, nonsubstituted benzhydryl carboxamide derivatives present no inhibition against HCT-116 cell line. 4-Chlorobenzhydryl carboxamide derivatives are higher in activity than 4,4-difluorobenzhydryl carboxamide derivatives demonstrated with compounds **9** (IC_{50}=24.48 μM) and **16** (IC_{50}=9.33 μM) or compounds **10** (IC_{50}=28.4 μM) and **17** (IC_{50}=9.33 μM) or compounds **12** (IC_{50}=17.98 μM) and **18** (IC_{50}=10.78 μM).

Thioamides and benzoylpiperazines generally show good activity values considering HCT-116 cell line. Compounds **35** (IC_{50}=9.10 μM), **37** (IC_{50}=8.95 μM) and **41** (IC_{50}=9.45 μM) are the highly active molecules in benzoylpiperazines.

Generally sulfonylpiperazines present low or no inhibition on HCT-116 cell line.

In summary, we have synthesized fifty one derivatives of benzhydrylpiperazines which have been tested for their cytotoxic activites on several cell lines of breast (MCF-7), hepatocellular (HUH-7) and colorectal (HCT-116) cancer families. Future synthesis of similar derivatives will take place to create a larger set of compounds in order to produce a rational quantitative structure-activity relationship (QSAR) mapping. Since 4-chlorobenzhydryl-piperazine derivatives are chiral compounds, further exploration of chiral separation methods will be performed. The primary ambition regarding future research is to evaluate the mechanism of cytotoxicity.

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