Modulating Dopamine Receptors Subtype Selectivity by Thiophene and Benzothiophene based Derivatives

Dissertation

zur Erlangen des akademischen Grades

Doctor rerum naturalium

(Dr . rer . nat .)

Vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät

Der Friedrich-Schiller- Universität Jena

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geboren am 6. April 1983 in Kairo

1. Gutachter:
2. Gutachter:
3. Gutachter:
Datum der Disputation:

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Abstract

In the course of this work we tried to design and create new ligands acting on the different dopamine receptors but with novel affinity and selectivity profiles so that we could come up with new medical agents characterized by higher curing potential towards different CNS disorders and lower side effects relative to the currently available medications.

In the first part, some thieno and benzothieno azecine derivatives have been synthesized and biologically screened towards the 5 receptor subtypes of dopamine. Among these derivatives, compound **3** has shown to be the first reported azecine to show a unique selectivity profile towards D2 and D5 receptor subtypes with the same order of magnitude (Ki D2: 1.5 nM; D5: 1.9 nM).

In a second part, some arylmethylphenylpiperazine derivatives have been synthesized to serve as D4 acting ligands and had their Ki values towards the 5 dopamine receptor subtypes determined. Among this set of compounds, compounds **32a** and **36a** have shown superior affinity to D4 receptors with Ki values of 0.7 and 0.03 nM respectively. Docking experiments to D4 homology model have revealed a first to report arene cation interaction in which the unique D4 residue Argenine 186 is involved in.

In the last part of this work, some arylamidoalkylphenylpiperazine derivatives were synthesized and tested against the different dopamine receptors for the sake of getting new probes with modulated selectivity towards D3 and D4 receptors rather than D2 subtypes. Among this series, compound **44a** has shown to be 200 times more selective to D4 rather than D2 subtypes and compound **45a** was about 900 times more selective to D4 rather than D2 and 100 times more selective to D3 rather than D2 receptor subtypes.

1. Introduction

1.1 Biosynthesis and metabolic fates of dopamine

Dopamine is a neurotransmitter that belongs to the family of catecholamines and can be released from its neurons either in the central or the peripheral compartments [1]. The biosynthesis of Dopamine starts by the action of tyrosine hydroxylase, also known as tyrosine-3-monoxygenase, on L-tyrosine to yield L- dihydroxyphenyl alanine that is commonly known as L-DOPA which is further subjected to decarboxylation process mediated by aromatic L-amino acid decarboxylase enzyme that is always referred to as dopa decarboxylase to yield dopamine. After biosynthesis, dopamine is stored inside special vesicles in the neurons which are then released into synapse following stimulation through presynaptic action potential [2, 3].

Among the most important biochemical fates of dopamine is its conversion into norepinephrine and epinephrine by the action of dopamine-β-hydroxylase and phenylethanolamine-*N*-methyl transferase enzymes successively [4].

As for the degradation of dopamine, it occurs via the reuptake mechanism either through specific Dopamine transporter known as DAT-1 or through the Norepinephrine transporter NET in the areas where there are very few amounts of dopamine transporter proteins such as prefrontal cortex. After the reuptake, comes the enzymatic degradation of dopamine that is mediated either by monoamine oxidase with its both subtypes MAO-A and MAO-B or catechol-O-methyl transferase (COMT) enzymes, Figure **1**.

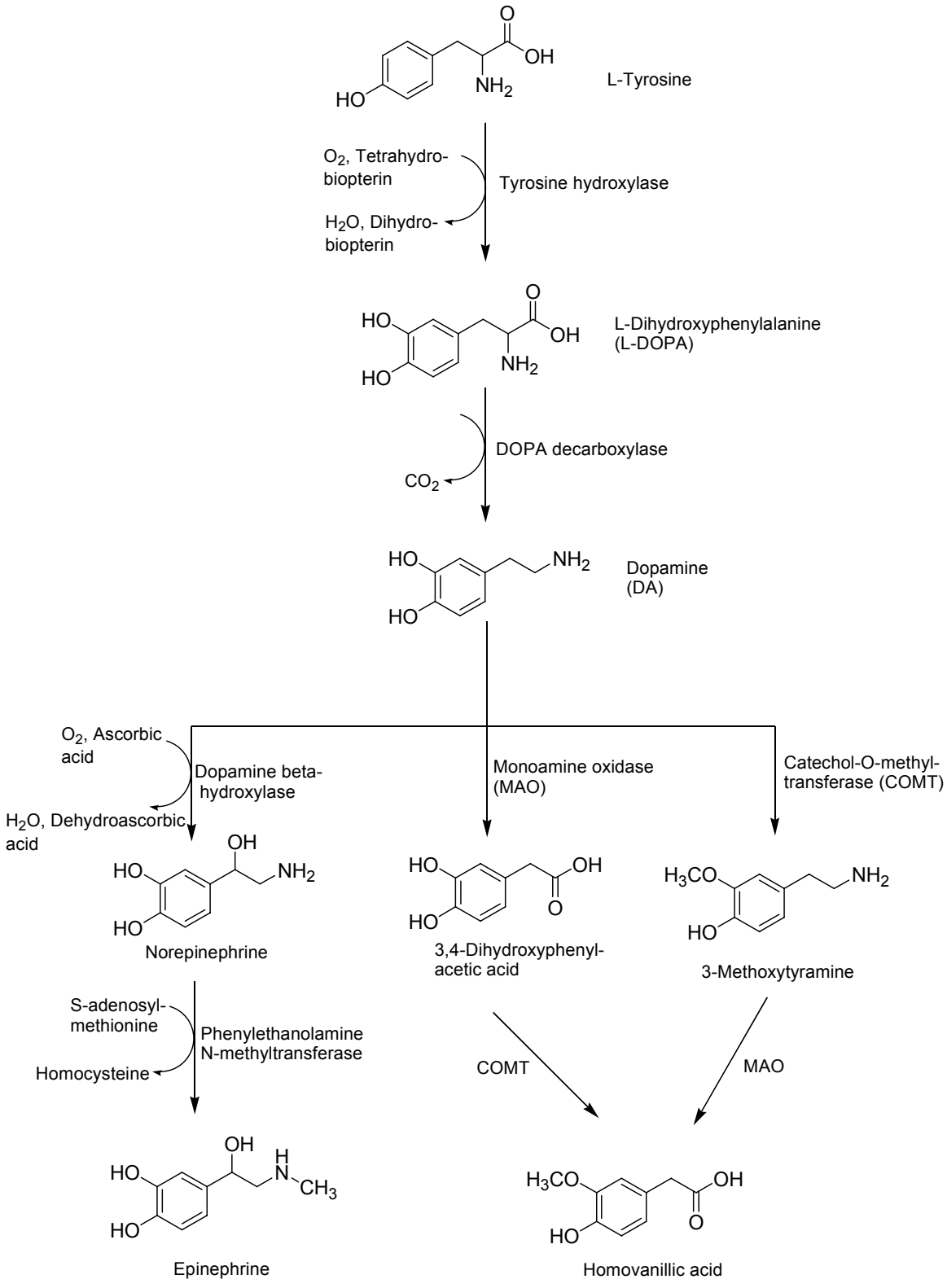


Figure 1: Biosynthesis and metabolism of dopamine

The action of MAO enzyme involves oxidative deamination of dopamine to produce 3,4-dihydroxyphenyl acetic acid, while the COMT enzyme converts

dopamine to 3-methoxy tyramine. Both metabolites are considered inactive when compared to dopamine [5, 6].

1.2 Central functions of dopamine

Central dopaminergic neurons originate mainly at four areas in the brain, namely substantia nigra, pars compacta, ventral tegmental area, and hypothalamus. From these areas, axons extend to many other areas in the brain through four major pathways which are Mesocortical pathway, Mesolimbic pathway, Nigrostriatal pathway, and tuberoinfundibular pathway, Figure 2 [7].

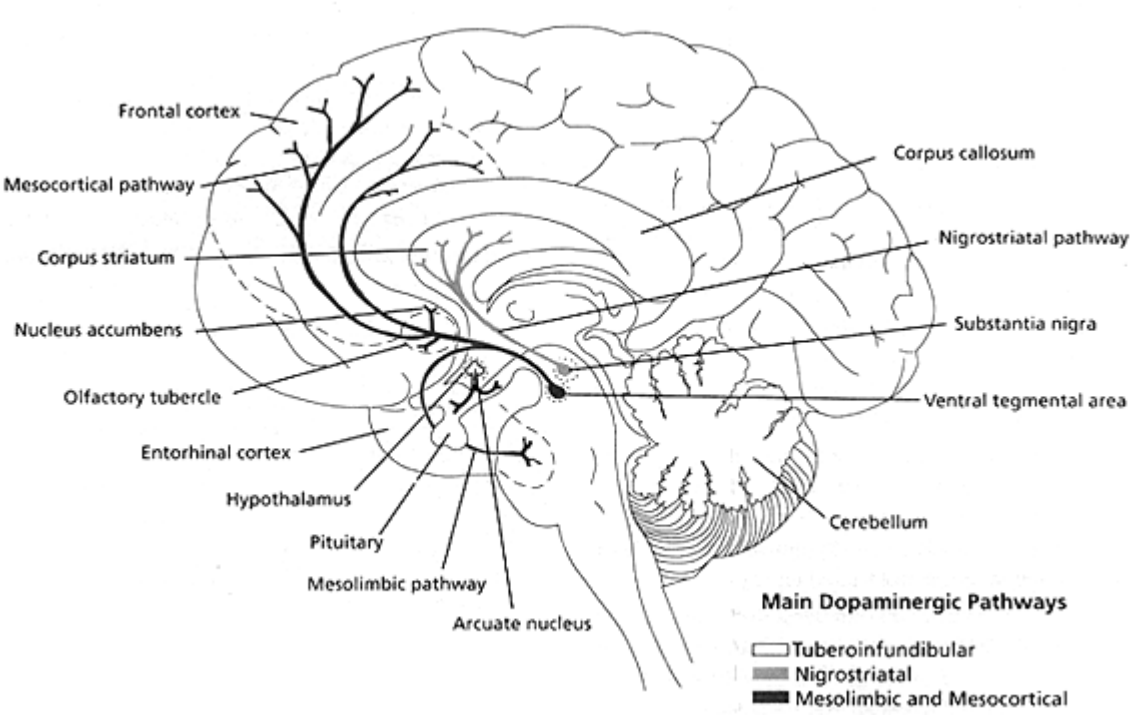


Figure 2: Major dopaminergic pathways in the brain [7]

Among these pathways, the Nigrostriatal and Mesolimbic ones have attracted a plenty of interest due to their assured involvement in lots of pathological conditions related to disturbed Dopamine neurotransmission, where the former is responsible for controlling the motor functions while the latter is

involved in cognition and emotionality. Degeneration of dopaminergic neurons in the nigrostriatal area is the main cause of developing Parkinson's Disease (PA) with its associated symptoms of tremors and rigidity, meanwhile the over activity of Dopamine neurotransmission in the mesolimbic pathway is the major responsible for delusions and hallucinations that are the utmost noticeable signs of Schizophrenia [8, 9].

1.2.1 Control of locomotion and motor functions

Dopamine is considered to be a key regulator for the motor functions in CNS, where it has a stimulatory effect on locomotion via activating D2 receptors and lately it was shown that stimulating D1 and D5 receptors may also show synergetic action regarding controlling the motor functions. Accordingly, Dopamine plays an essential role in the pathogenesis of many motor disorders such as PD, Restless leg syndrome, Tourette's syndrome, and Huntington's disease [8, 10- 12].

1.2.2 Control of cognition

As many neurocognitive functions as memory, attention, and problem solving are under the control of Dopamine, where in the frontal lobes, it controls the flow of the information from other areas of the brain. A Low Dopamine level in the prefrontal cortex is the major contributor to Attention Deficit Hyperactivity Disorder (ADHD) [13].

1.2.3 Prolactin regulation

Dopamine also plays an important role in adjusting the levels of prolactin hormone where it is considered to serve as Prolactin Inhibiting Hormone (PIH) or Prolactostatin as it inhibits the secretion of prolactin from the anterior pituitary gland [(14)].

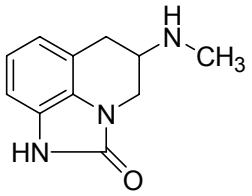
1.2.4 Dopamine and reward system

Dopamine is known to be involved in the brain reward's system as it is released upon rewarding experiences such as sex, food, some drugs such as cocaine, amphetamines, and nicotine that increase the level of Dopamine in brain through blocking its reuptake. Dopamine is then ensuring the feelings of enjoyment and reinforcement to motivate the person to perform certain tasks and activities. Animal studies have been conducted and confirmed the role of Dopamine in motivation, desire and pleasure, where in one of these experiments, rats depleted from Dopamine have shown no longer initiation to eat on their own will.

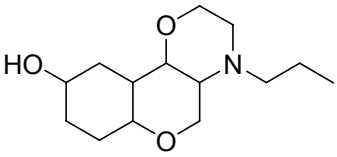
This crucial role of Dopamine has put great value for some dopaminergic receptors agonists and antagonists in the scope of treating cocaine addiction.

Several studies have proved the role of D2, D3, and D5 receptors in this regards as it was found that the signaling mechanisms of D2 and D3 particularly play an integral function in the transduction of cocaine's discriminative stimulus effects. On the other hand D5 receptors were proved to mediate the increase of NMDAR in the ventral tegmental area (VTA), an action by which cocaine promotes synaptic plasticity of VTA neurons and thus leads to development of addictive behaviors at the end [(15)].

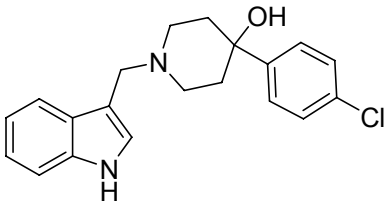
It is worth to mention that both D2 agonist PNU-95666 and D3 agonist PD128907 were able to reproduce and prime cocaine's effects, while both D2 antagonist L-741626 and D3 antagonist PG01037 were managed to attenuate the effects of cocaine [16].



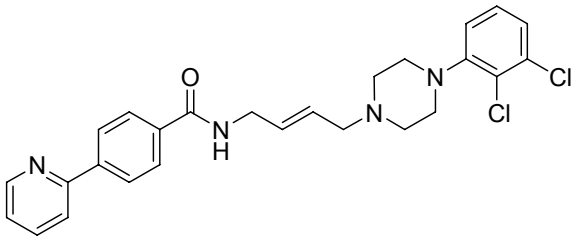
PNU-95666



PD128907



L-741626



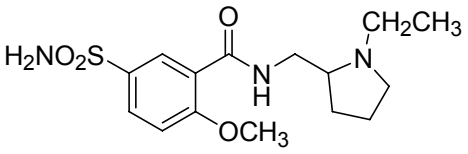
PG01037

1.2.5 Pain processing

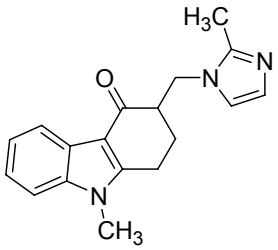
Dopamine has also proved to play a role in pain processing in CNS, where its decreased level in brain was shown to be associated with painful symptoms that accompany PD frequently. It was also shown that disturbed Dopamine neurotransmission is observed in some painful clinical conditions such as burning mouth syndrome and restless leg syndrome. It has been reported that the analgesic effect of Dopamine is mediated most properly through D1 and D2 receptors in CNS [17].

1.2.6 Stimulation of Chemoreceptor Trigger Zone

Dopamine as well as other neurotransmitters such as histamine and serotonin is able to stimulate a certain area in the brain medulla known as chemoreceptor trigger zone (CTZ), an area of the brain located outside the blood brain barrier and communicates with the vomiting center. Stimulation of this area leads mainly to initiation of nausea and vomiting. As many drugs and bacterial toxins as opiates, cardiac glycosides, chemotherapeutics, and staphylococcal enterotoxin are able to stimulate CTZ and initiate emesis, an action that can be offset by the use of Dopamine and/or Serotonin antagonists such as Sulpiride and ondansetron respectively [18].



Sulpiride



Ondansetron

1.3 Peripheral functions of dopamine

The most common peripheral actions of Dopamine are mainly observed on cardiovascular system, kidney, and immune system. Low doses of Dopamine have been proved to be able to dilate renal blood vessels increasing the renal blood flow and hence increase the overall renal perfusion resulting in about five units increase in the urine output, thus Dopamine is said to be of a diuretic effect.

Intermediate doses of Dopamine is shown to activate β1 receptors in the heart leading to positive inotropic and chronotropic effects, meanwhile large doses of Dopamine exert a vasopressor action on the blood vessels through

stimulation of α1 receptors leading to increasing peripheral resistance and blood pressure [19].

Regarding the immuno-regulatory function of Dopamine, it has been shown that some dopaminergic receptors subtypes are expressed on B-cells and natural killers, moderately expressed on neutrophils and esinophils, while with low degree of expression on T-cells and monocytes. Moreover, Dopamine was demonstrated to be synthesized and released from the immune cells themselves.

It was shown that Dopamine activates resting T-cells and in a reverse action inhibits them when they are activated. It is worth to mention that disorders associated with disturbed levels of Dopamine are accompanied with altered immune functions [20].

1.4 Dopaminergic receptors

Dopamine mediates its different pharmacological actions via stimulating five different but closely related receptor subtypes that are classified under two families, the D1-like family which includes D1 and D5 subtypes and the D2-like family that includes D2, D3, and D4. This classification is mainly based on the biochemical way by which Dopamine is able to modulate Adenylyl Cyclase (AC) activity, and accordingly the cAMP production. All of these dopaminergic receptors belong to the super family of G-protein Coupled Receptors (GPCRs) [21].

GPCRs are considered to be a super family of transmembrane receptors that are encoded by about 791 genes. They sense its ligands outside the cell and then activate signal transduction pathways inside, so that cellular responses are ultimately observed. The stimulatory ligands that bind to GPCRs vary in

size from small molecules to peptides to large proteins and in nature from light sensitive compounds to hormones to neurotransmitters. These receptors are involved in many diseases and considered to be the target of for approximately 30% of the newly developed drug candidates [22, 23].

1.4.1 Molecular structure of dopaminergic receptors

Like other GPCRs, dopaminergic receptors are considered to be integral membrane proteins that possess seven transmembrane helices. The extracellular loops of the receptors contain two highly conserved cysteine residues that contrive to afford disulphide bonds to stabilize the structure of the receptor [24].

Deeper view to the structure of GPCRs to which belong the dopaminergic receptors, show that the receptor structure is characterized by the presence of an extracellular N-terminal, followed by seven transmembrane α-helices that are denoted as 7-TM α-helices (TM-1 to TM-7). The seven transmembrane helices are connected together by three intracellular loops and 3 extracellular loops denoted as IL-1 to IL-3 and EL-1 to EL-3 respectively, and finally comes the intracellular C-terminal, Figure **3** [7].

These receptors arrange themselves into a tertiary structure in which the seven transmembrane helices form a cavity within the plasma membrane that serves as the ligand binding domain. This cavity is often covered by EL-2 that resembles the lid that covers the top of the ligand binding site [25, 26].

It's worth to mention that the sequence identity between the members of D1-like and D2-like families is only 44%, where D1-like family receptors have a shorter intracellular third loop than D2- like family receptors. Also the C-

terminal of D1- like family receptors is about seven times longer than that of the D2- like family receptors [24].

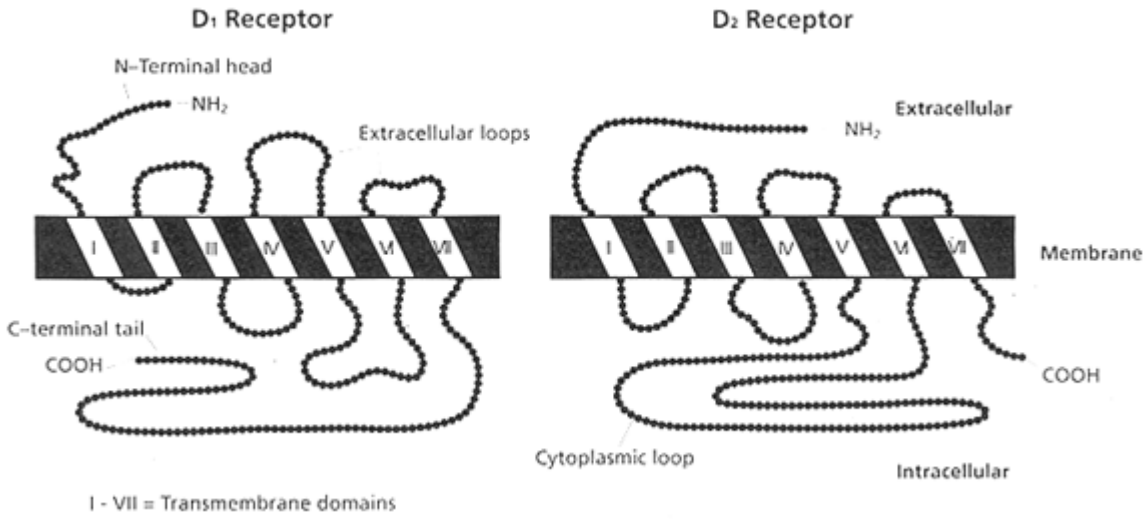


Figure 3: Structural features of D1- like and D2- like receptors [7]

Both D1 and D5 receptors share about 80% degree of homology in their primary sequence. D1 receptors contain 446 amino acids, while D5 have 477 ones. The primary structure of the two subtypes is observed mainly in the third intracellular loop and in the external loop between the transmembrane domain TM-4 and TM-5 [27].

As for the D2- like family, D2 and D3 share about 75% degree of homology, while D2 and D4 share only 53% homology degree. D2 receptor subtype is characterized by having two different variants. These variants have been termed D2S (D2- short) and D2L (D2- long). The D2L differs than the other isoform in regards of the presence of an additional 29 amino acids in the third intracellular loop [28].

As for D4 receptor subtype, several polymorphic variants with a 48-base-pair repeat sequence in the third cytoplasmic loop were described. Some of these

polymorphic variants might have a slightly altered affinity for the antipsychotic clozapine; however no evidence has been reported that indicates an increased incidence of schizophrenia in the subjects with these variants [29].

1.4.2 Mechanisms of dopaminergic receptors signaling

As already mentioned, all dopaminergic receptors belong to the super family of GPCRs that mediates its action via the activation of heterotrimeric G-proteins to induce intracellular signaling mechanisms. Moreover, there is a strong accumulating evidence suggests that these receptors do not signal exclusively through G-proteins but may also be involved in G-protein independent signaling cascades. In general, G-proteins consist of three associated protein subunits termed α, β, and γ and they are classified into four broad classes according to the nature of the α- subunit sequence. These four classes are $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$. Before an agonist binds to its GPCR, α- subunit of the G-protein is bound to GDP and tightly associated with βγ- complex to form the inactive trimeric protein complex. Upon agonist binding, a sequence of events results in GDP release and instead GTP binds to the α- subunit leading to its disconnection from the βγ- complex. Both the α-subunit and the βγ- complex can then transduce signals to activate some effector systems [30, 31].

The D1- like family receptors (D1 and D5) are generally coupled to $G\alpha_{s/olf}$ that their stimulation leads to activation of AC that provokes the production of cAMP secondary messenger that in turn activates Protein Kinase A (PKA). On the contrary, activation of D2- like family (D2, D3, D4) that are coupled to $G\alpha_{i/0}$

leads to negative regulation of the production of cAMP and accordingly a decrease in PKA activity [32, 33].

1.4.2.1 D1- like receptors signaling

As already mentioned before, D1 and D5 are coupled to $G\alpha_{s/olf}$ protein. Upon stimulation of these receptors, $G\alpha_s$ and presumably $G\alpha_{olf}$ bind primarily to C2 cytosolic domain of AC, bringing the C1 and the C2 domains together in a way that enhances the catalytic activity of the enzyme which in turn leads to the conversion of ATP into cAMP that binds to the regulatory subunit of PKA leading to its activation [32, 34-36].

Once activated, PKA phosphorylates a number of proteins involved in signal transduction and regulation of gene expression, Figure **4** [34].

Activation of PKA as a result of D1- like family receptors stimulation not only stimulates PKA catalyzed phosphorylation of numerous protein substrates but also prevents the phosphatase 1 (PP1) catalyzed dephosphorylation of these phosphoproteins through phosphorylating and hence activating Dopamine and Cyclic AMP- regulated Phosphoprotein (DARPP-32) [37, 38].

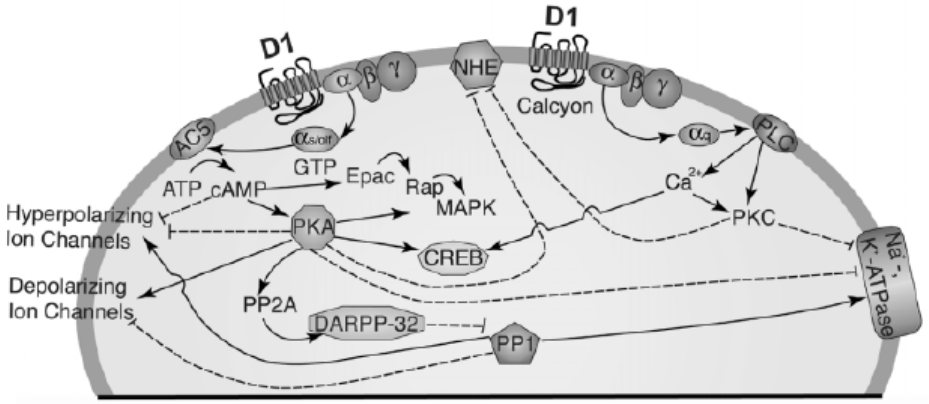


Figure 4: D1- like receptors signaling pathways [34]

Among the substrates that are phosphorylated as a result of PKA activation in response to D1- like receptors stimulation are GABA receptors and the two subtypes of glutamate receptors namely AMPA and NMDA. Moreover, regulation of several ion channels including Na^+, K^+, and Ca^{2+} channels also occur by modulating the phosphorylation states of these ion channels [39].

1.4.2.2 D2- like receptors signaling

The signaling of the D2- like receptors family is mediated mainly through the activation of the heterotrimeric inhibitory G proteins $G\alpha_{i/0}$ [40].

Regarding the D2 receptor subtype, it seems likely that both receptor variants D2L and D2S are able to activate multiple $G\alpha_{i/0}$ including $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_0$ but the interactions with particular G proteins are restricted in a cell type dependent manner according to the availability of appropriate effectors and scaffolding proteins [41].

As for the D4 receptor subtype, it is similar to D2, where it activates multiple G protein subtypes including $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_0$ [42].

Several working groups have identified $G\alpha_0$ to be activated by the D3 receptor and mediating D3 signaling [43].

Contrary to D1- like family, stimulation of D2- like receptors leads to the inhibition of AC activity and thus decreases the phosphorylation of PKA substrate such as DARPP-32 for instance, Figure **5** [34]. It is worth to mention that it was noted that D3 stimulation inhibits AC by a weaker degree than D2 and D4 [44].

Among the other signaling pathways modulated via the activation of D2- like receptors are phospholipases, Na^+, K^+, Ca^{2+} ion channels, as well as NMDA, AMPA, and GABA receptors [45].

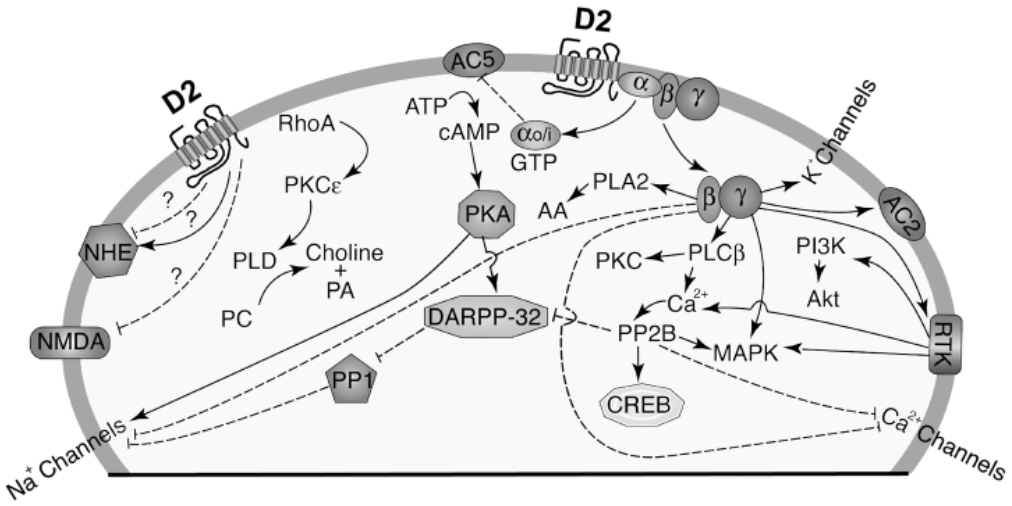


Figure 5: D2- like receptors signaling pathways [34]

1.4.3 Dopaminergic receptors expression and tissue distribution

Dopamine receptors are characterized by having broad expression patterns both in central and peripheral compartments.

In the brain, D1 receptors subtype are expressed at high level in the nigrostraiatal, mesolimbic, and mesocortical areas such as striatum , substantia nigra, amygdale, and the frontal cortex. D1- receptors also show low level of expression in some central areas like hippocampus, cerebellum, and hypothalamic areas. D5 receptors are with relatively lower expression levels but yet in multiple regions of the brain such as prefrontal coretex, substantia nigra, hypothalamus, and hippocampus.

The highest level of D2 receptors are found in striatum, nucleus accumbens. They are also expressed at significant levels in substantia nigra, ventral tegmental area, hypothalamus, and hippocampus.

The D3 receptors show more limited distribution pattern, where the highest level of expression was being observed in the limbic areas such as the

nucleus accumbens, the olfactory tubercle, and the islands of calleja. These receptors are of very low expression in the striatum, ventral tegmental area, and cortical areas.

As for the D4 receptors, they have the lowest level of expression in the brain with confirmed expression in the frontal cortex, hippocampus, hypothalamus, and substantia nigra.

Regarding the periphery, D1, D2, and D4 receptors have been detected in retina. All subtypes of Dopamine receptors have been detected also in kidney, adrenal glands, sympathetic ganglia, gastrointestinal tract, blood vessels, and the heart [25, 46, 47].

1.5 Dopaminergic Ligands

Dopaminergic receptors ligands are structurally diverse, however the majority shares some common structural features which are necessary for binding to the receptor. From a medicinal chemistry perspective, generally when the chemical properties of the agonist for a specific receptor system are compared with those of the corresponding antagonists, the agonists are relatively small molecules and hydrophilic in their chemical nature, while the antagonists are usually larger, more lipophillic, and lacking the essential pharmacophore elements for displaying agonistic properties [48].

Except for D3 receptors, the binding pockets of dopaminergic receptors is still not completely identified, however all of them comprise an Asp residue at position 3.32 which affords ionic interaction with a protonated basic nitrogen of the ligand that is usually surrounded on either sides by two hydrophobic cavities [49, 50].

The majority of the agonists on these receptors share in their structures the common catechol ethylamine skeleton, while the antagonists always lack certain essential pharmacophore element such as the catechol group and the correct conformation and/or distance separating the basic nitrogen from the aromatic moiety. It is worth to mention that this rule is not always equivocal, where some dopamine agonists such as benzergoline derivatives lack the essential catechol group [50- 52].

1.5.1 D1-like family receptors ligands

This part will mainly focus on classes of dopamine receptors ligands bearing the previously mentioned structural features.

1.5.1.1 Phenylbenzazepine derivatives

This class represents one of the most important classes of D1 selective agonists and antagonists that have served a major role in the pharmacology of dopamine receptors. The prototype SCH23390 is the first discovered D1 antagonist showing higher selectivity towards D1 rather than D2 receptor subtypes [53, 54]. All the antagonists of this class bear a halogen substitution at position 7 whereas compounds having the catechol system are serving as potent agonists (SKF series) [55]. Neumeyer *et al* introduced minor changes into the structures of SKF series by changing the substituent at the terminal nitrogen and C6 of the benzazepine moiety as well as the phenyl ring. Most of the resulted compounds have shown more or less high affinities to D1-like family receptor subtypes [56].

Several structural modifications have also been carried out on the prototype D1 selective antagonist SCH23390. The phenyl ring was substituted with a benzofuran and dihydrobenzofuran moiety, resulting in highly selective D1-like

antagonists namely NNC112, NNC687, and NNC756. Further structural variations on SCH23390 included rigidifying the structure and reducing the flexibility of the phenyl ring leading to the development of a new series in which SCH39166 was the prototype [57]. It showed nanomolarar affinities on D1-like receptors that is though weaker than SCH23390, but with higher selectivity over D2-like receptors [58, 59]. Table **1** illustrates the binding affinity constants of some of the compounds belonging to these series [53- 59].

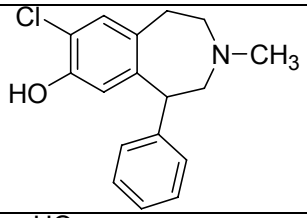
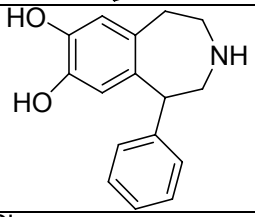
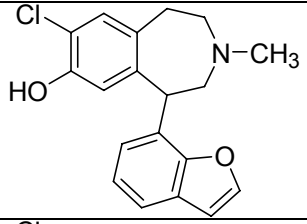
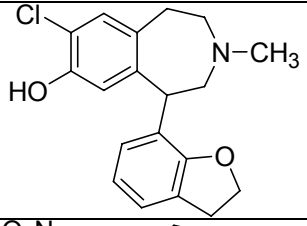
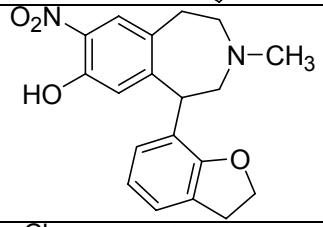
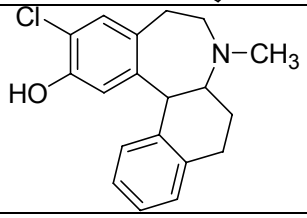
Compound	Structure	Ki (nM)	
		D1	D2
SCH23390		0.35	2145
SKF38393		150	4670
NNC112		0.18	898
NNC756		0.17	942
NNC687		5.8	>10000
SCH39166		1.2	980

Table 1: Binding affinity constants (Ki) values for some Phenylbenzazepine derivatives **(53- 59)**

1.5.1.2 Tetrahydroisoquinoline derivatives

Tetrahydroisoquinoline ring is one of the most frequently encountered components in the structure of a class of dopaminergic ligands that have made a breakthrough in the pharmacology of dopamine receptors. Numerous dopamine agents contain a tetrahydroisoquinoline ring either substituted with phenyl/benzyl group or incorporated in a tetracyclic skeleton with different annulation patterns.

Apomorphine, Dihydrexidine, Dinapsoline, and their derivatives are considered the major representatives of this class [60, 61].

R(-)-apomorphine, the well known agonist, is used in the treatment of Parkinson's disease and erectile dysfunction. It displays high affinities for all dopamine receptors and has a rather interesting binding profile, showing the highest affinity for D4 followed by D5 receptor, with markedly lower affinities for the D1 receptor [62]. Numerous structural variations have been introduced into apomorphine structure for the sake of modulating its special selectivity and affinity profiles. Derivatives bearing only one hydroxyl substituent at position 11 were found to possess antagonistic rather than agonistic properties and this completely matched with the previously mentioned structural features necessary for the functional activity of dopaminergic ligands [63]. The nature of N-alkyl substituent was shown to have also great effect on both the affinity and selectivity of these ligands, where N-propyl substituent was found to be more selective to D2 receptors. Moreover, while the (R) enantiomers generally showed greater affinities than their (S) congers, the latter have shown antagonistic activity rather than agonistic effects. For

instance, S(+)-apomorphine has been reported to possess dopaminergic antagonistic properties [64].

Dihydrexidine is considered to be the first potent D1 full agonist, showing an intrinsic efficacy comparable to dopamine itself. Similar to appomorphine, dihydrexidine has a conformationally rigid structure. Compared to the previously mentioned dopamine ligands, N-methyl substitution of dihydrexidine resulted in the loss of D1 selectivity, while in the N-propyl derivative the affinity for D2 was higher than for D1. Dinapsoline is another conformationally rigid analogue which is similar to dihydrexidine showed potent D1 full agonistic properties [55, 60, 61].

Table **2** sums up the binding affinity constants for some representatives of this class [60- 64].

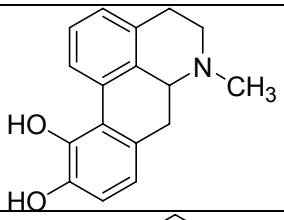
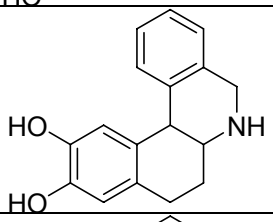
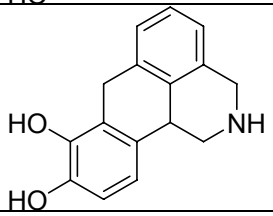
Compound	Structure	Ki(nM)	
		D1	D2
Apomorphine		214	13.2
Dihydrexidine		6.2	58.1
Dinapsoline		5.9	31.3

Table 2: Binding affinity constants (Ki) values for some Tetrahydroisoquinoline derivatives [60- 64]

1.5.1.3 Indolobenzazecines and Dibenzazecines

Indolo[3,2-f]benzazecines and dibenz[d,g]azecines present a structurally novel class of dopamine receptors antagonists with interesting pharmacological profiles. The scaffold of the prototype LE300 has been designed to incorporate the structures of both dopamine and serotonin in a relatively flexible backbone as the more rigid pentacyclic precursor of LE300 was found to be inactive, indicating that a moderate amount of flexibility is crucial for the dopaminergic binding properties of this class of compounds [(65)], Figure **6**.

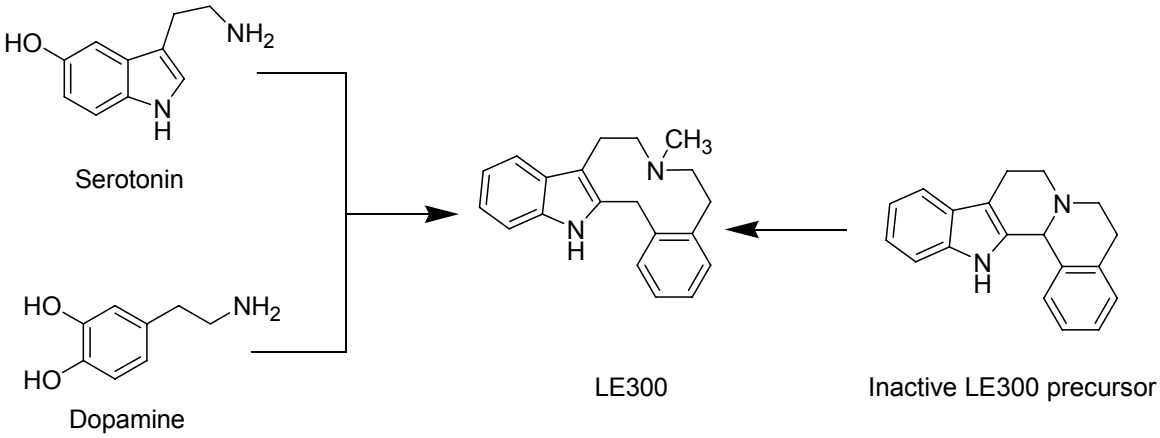


Figure 6: Design of LE300 based on serotonin and dopamine structures

Various modifications have been introduced to the structure of LE300 for the sake of imaging a comprehensive SAR for this class of compounds. The results can be summed up as follows:

Removal of the indole ring as well as substitution of the annulated benzene ring with a phenyl group has abolished the activity.

Replacement of the indole ring with benzene and other aromatic systems keeps the activity.

Methyl substitution of the central alicyclic nitrogen atom was most favored; derivatives with longer alkyl groups or aralkyl ones have shown to be much less active. Methoxylation and/or hydroxylation of the indole ring increased the activity. Ring expansion to an 11-membered central ring showed different effects; members had their tryptamine structure maintained and the ring is elongated from the benzene side possessed similar activity to LE300. On the other hand, expanding the ring from the indole side has much decreased the activity while contraction of the central ring to a 9-membered one lead to almost complete loss of activity [65- 69].

As for the dibenzazecine derivatives, LE410 is considered the prototype, on which numerous modifications have been made to establish the SAR for such a class. 3-Hydroxylated or methoxylated dibenzazecines mostly showed higher affinities. 2,3-dihydroylated or methoxylated derivatives were shown to be with much lower activities. The most active compound within this series was the 4-chloro-3-hydoroxydibenzazecine, which displayed very high affinity towards D1-like family. Like the indolobenzazecine, expansion of the central ring was more or less keeping the activity on the target receptor subtypes [69, 70].

Table **3** summaries the binding affinity constants for some members of this class [65- 70].

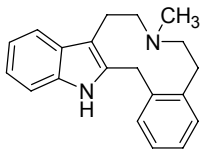
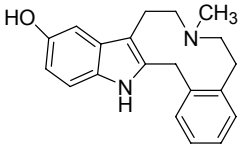
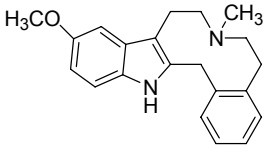
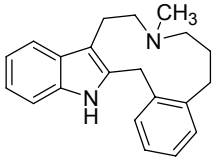
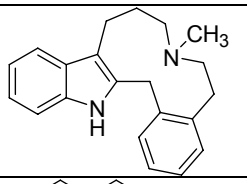
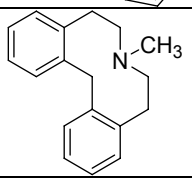
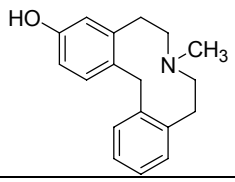
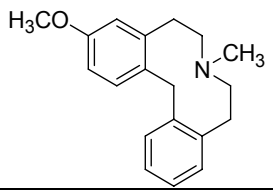
Compound	Structure	Ki(nM)				
		D1	D2	D3	D4	D5
LE300		1.9	44.5	40.3	109	7.5
LE-CE551		0.56	38.4	944	398	0.39
LE-CE550		0.82	11.9	475	266	3.6
LE-CE560		2.2	14.5	277	98.4	0.61
LE-CE580		163.5	143	521	184	92
LE410		4.5	56.5	52	148	11.2
LE405		0.4	44.5	47.5	11.3	1.5
LE425		28.5	13	75.7	43.3	54

Table 3: Binding affinity constants (Ki) values for some Azecine derivatives [65- 70]

1.5.2 D2-like family receptors ligands

Drugs known to activate or block D2-like receptor subtypes are widely used for treatment of several diseases. Majority of the agonists at these receptor subtypes for instance are used for treatment of Parkinson's disease, Restless leg syndrome, other agonists can serve as antiemetic and prolactin inhibitors. Recent studies have also proved the ability of D2-like receptors agonists for counteracting male erectile dysfunction [71, 72]. On the other hand D2-like receptors antagonists are commonly used for reducing symptoms of schizophrenia and counteracting anxiety. Drugs used for treating schizophrenia are classified into typical antipsychotics and atypical antipsychotics based on their receptor subtype selectivity profile and their dissociation rate off the receptor. Typical antipsychotics are known with either limited selectivity and /or slow dissociation rate. They are also characterized by having extrapyramidal adverse effects manifested as some acute dystonic reactions and movement disorders like akinesia, akathisia, dyskinesia, muscular spasms of the neck, and rigidity of tongue and jaws. On the contrary, atypical members show better selectivity towards D3, D4, and/or 5-HT receptor subtypes and/ or rapid dissociation rate off D2 receptor subtype, and much lower extrapyramidal symptoms [73, 74].

Starting with the agonists, lots of D2-like receptors agonists are widely used and already available in the market, Figure **7**.

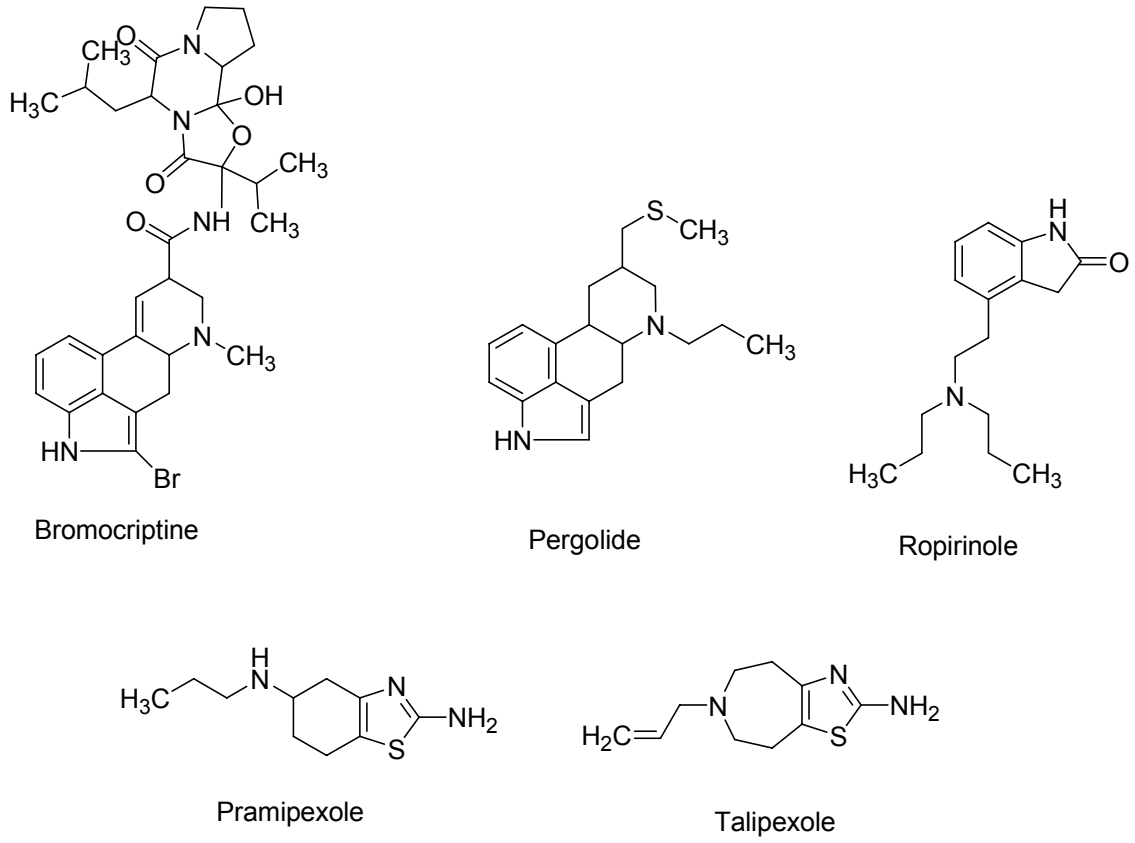


Figure 7: Some D2-like receptors agonists used in medicinal market

Among these agonists come the tetrahydrobenzthiazole derivative Pramipexole, the tetrahydrothiazoloazepine derivative Talipexole, and the indole-2-one derivative Ropirinole that are mainly used for treating PD. Also the ergot alkaloids derivatives Bromocriptine and Pergolide are available for treating male and female sterility associated with hyperprolactinemia [71].

Table **4** lists the binding affinity constants of the previously mentioned agonists towards the D2-like receptor subtypes [71, 75].

Drug	Ki(nM)		
	D2	D3	D4
Pramipexole	6.9	0.9	15
Talipexole	5.8	7	5.2
Ropirinole	7.2	19	>1000
Bromocriptine	10	87	370
Pergolide	4	4	6.2

Table 4: Binding affinity constants (Ki) values for some D2-like receptors agonists [71, 75]

Moving to the antagonists, Figure **8** illustrates variety of D2-like receptors antagonists available in the medicinal market for treating psychosis and anxiety.

Drugs belonging to the structural class known as phenothiazines such as Chlorpromazine are considered the most classical (typical) antipsychotics that have been used widely for counteracting this disorder. Among the other classical antipsychotics come the classes of thioxanthenes such as Chlorprothixene, butyrophenones such as Haloperidole, and the diphenylbutyl piperidines such as Pimozide. Enlarging the middle ring of the phenothiazine and keeping appropriate electron cloud around this ring lead to the development of the non classical (atypical) antipsychotics characterized by their enhanced subtype selectivity to D3/D4 receptors and/or their high dissociation rate off D2 receptors subtype and most importantly minimal exrapyramidal symptoms. Major representatives of this family are Clozapine and Olanzapine [76].

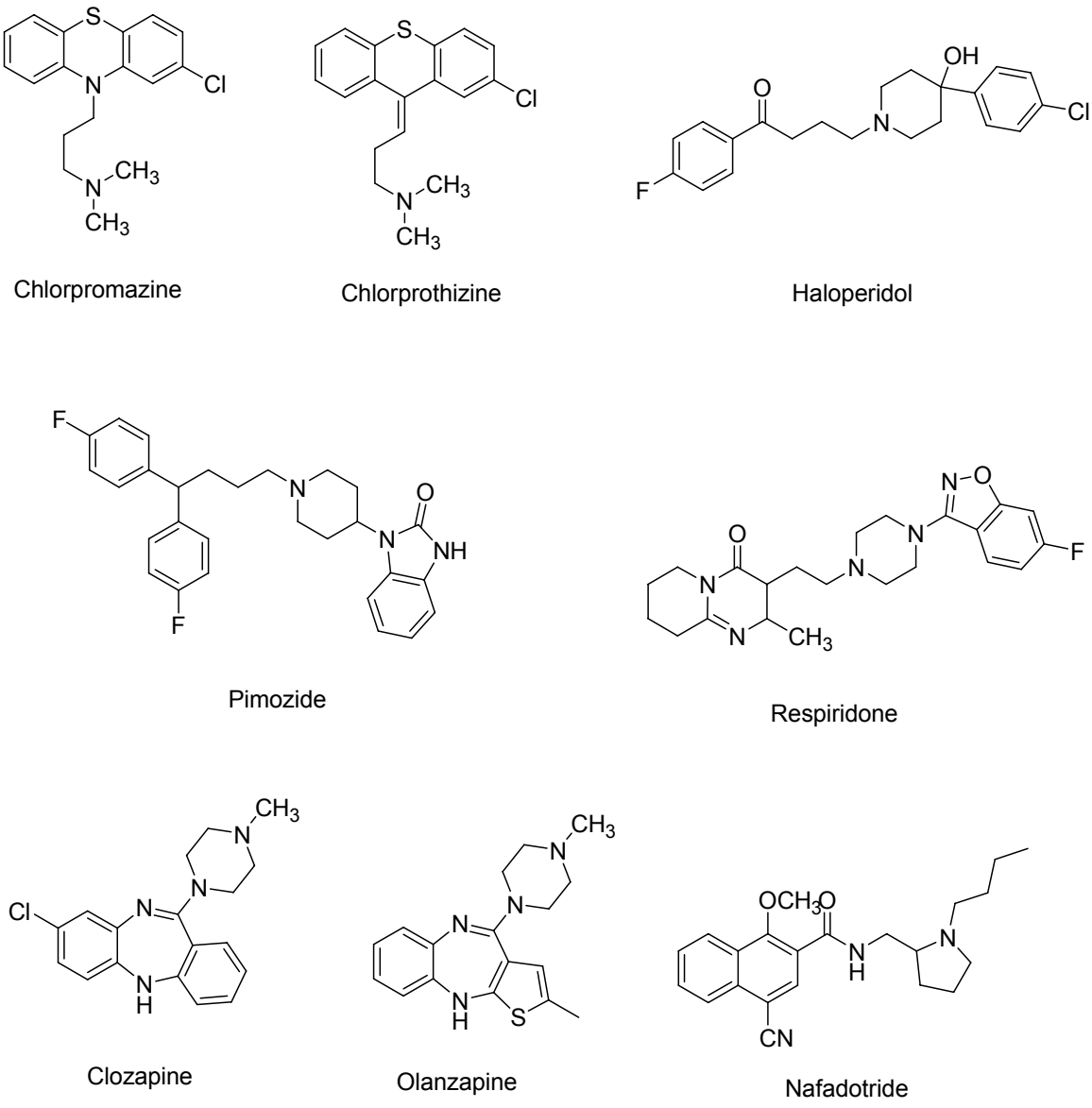


Figure 8: Some D2-like receptors antagonists used in medicinal market

Other structural classes of D2-like antagonists include the Methoxybenzamide derivatives such as Sulpiride and Nafadotride, and the Benzisoxazole derivative Resperidone that has shown to have 5-HT2 antagonistic properties.

Table **5** lists the binding affinity constants of some of the previously mentioned antagonists towards the D2-like receptor subtypes [(76)].

Drug	Ki(nM)		
	D2	D3	D4
Chlorpromazine	5.4	5	15.9
Chlorprothixene	3.3	----	0.64
Pimozide	2.51	2.84	1.8
Sulpiride	51	120	2100
Nafadotride	3	0.31	------
Risperidone	4.9	12.2	7.5

Table 5: Binding affinity constants (Ki) values for some D2-like receptors antagonists [76]

In addition to the drugs already available in the market, lots of ligands belonging to other structural classes are still under investigation. The following context will summarize the most important classes showing appreciable affinity to D2-like family receptor subtypes.

1.5.2.1 4-Phenylpiperidine derivatives

In the search for novel D2-like receptors ligands, Pettersson *et al* have introduced a series of 4-Phenylpiperidine/piperazine derivatives to serve as antagonists at the target receptors subtypes [77].

The key to this approach was to maintain the chemical pharmacophore of the natural substrate, Dopamine, with performing some modifications in such a way that the hydrophilicity is retained or even higher to lead to compounds that antagonize dopamine, but unlike the lipophillic antagonists, lack the ability to stabilize the inactive state of D2 receptors, so that they could exert modulatory effects on dopamine transmission and possibly state dependent activity *invivo*.

The first ligand introduced among this series was 3-(3-hydroxyphenyl)-N-n-propylpiperidine, known as 3-PPP. This candidate has shown partial agonistic activity at the D2 receptor subtype and it was found that its phenolic hydroxyl function is essential for its activity as it affords hydrogen bonding interaction with the target protein. Replacing the 3-OH group of the 3-PPP with the electron withdrawing group, methylsulfonyl, lead to an analogue that showed a unique neurochemical effects, where it displayed an *in vivo* effects similar to classic D2 antagonists such as the increase in the synthesis and turnover of dopamine, but in sharp contrast to these classic antagonists, it could stimulate, suppress, or show no effect on the motor and behavioral symptoms depending on the prevailing dopaminergic tone. Moreover, this compound has also shown to stabilize high state of D2 ($D2^{High}$) rather than the low state ($D2^{Low}$). Therefore, the effects on the motor and behavioral symptoms have been regarded as state dependent and this analogue has been classified as dopaminergic stabilizer. These results have triggered the group to investigate the ability of various ligands bearing the main skeleton to inhibit D2 receptors at different dopamine concentrations [77]. Table **6** shows the Ki^{Low}/Ki^{High} ratio of this series of compounds [77].

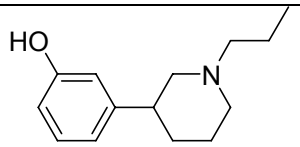
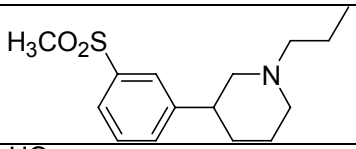
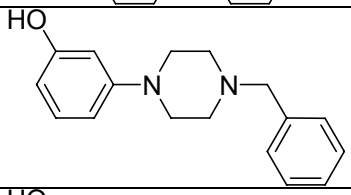
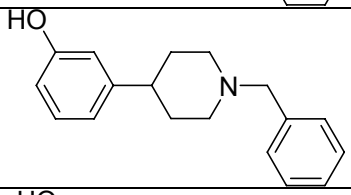
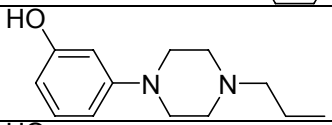
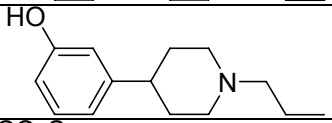
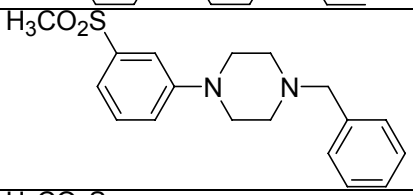
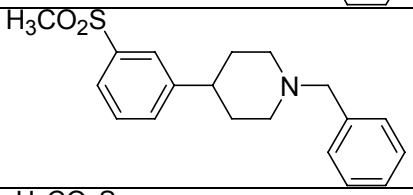
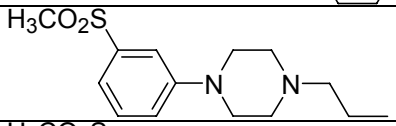
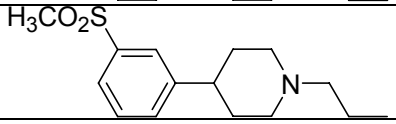
Compound	Structure	D2 Ki ^{Low}/Ki ^{High}
I, 3-PPP		130
II		137
III		25
IV		8.1
V		10
VI		7.3
VII		4.4
VIII		2.1
IX		1.8
X		2.3

Table 6: Ki Low/Ki High ratio of some 4-Phenylpiperidine derivatives [77]

1.5.2.2 Aminotetralin derivatives

A series of aminotetralin derivatives has been designed and synthesized by Cannon *et al* utilizing ligand based drug design approach based on dopamine. It was known from previous studies that dopamine can adopt both the alpha and beta conformations and SAR studies have shown that only the meta hydroxyl group of the catechol system is necessary for dopaminergic activity. The conformational regidification of the ethyl chain of dopamine and inserting N-alkyl substituents on the terminal nitrogen lead to generating this series of compounds, Figure **9** [78].

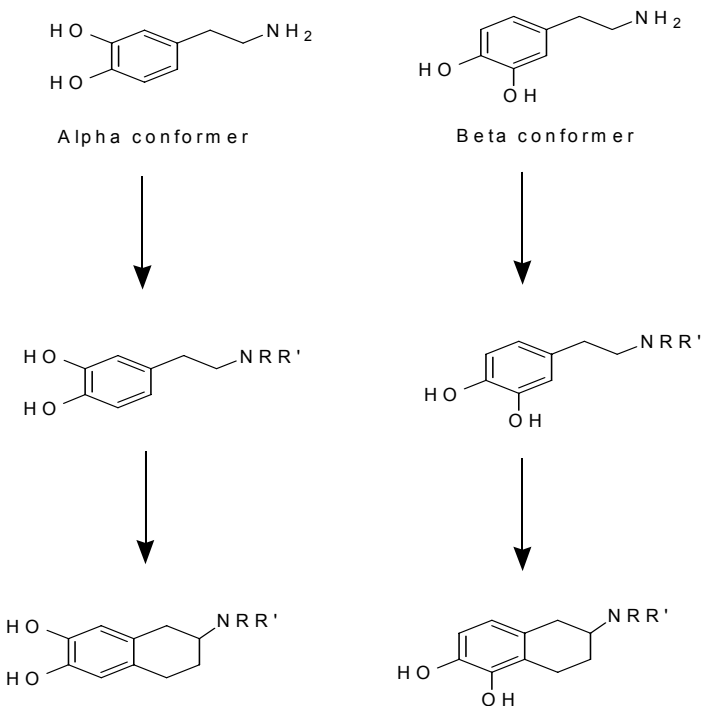


Figure 9: Design of Aminotetralin derivatives [78]

Two alternatives of bridging the dopamine structure are possible resulting in the formation of the 5,6 and the 6,7 dihydroxy derivatives which represent the beta and alpha conformers of dopamine respectively. Interestingly, the 5,6-dihydroxy derivatives bearing n-propyl side chains on the terminal nitrogen were found to be among the most potent derivatives. Among the monohydroxylated dipropylamino tetralins is the 7-hydroxy substituted derivative 7-OH-DPAT that was shown to display high selectivity towards D3 receptors. On the other hand, the 5-OH-DPAT revealed higher potency with mixed D2/D3 affinity profile. In an attempt to improve the pharmacokinetics of DPAT derivatives, several bioisosteric replacements of the hydroxyphenyl structure with substituted and unsubstituted heterocycles have been investigated [78- 80]. Table **7** illustrates the binding affinity constants of some candidates belonging to this series of compounds [78- 80].

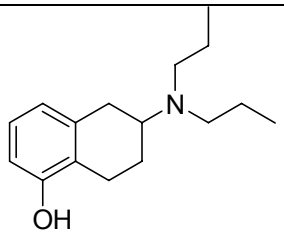
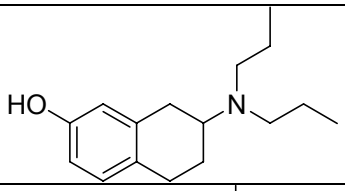
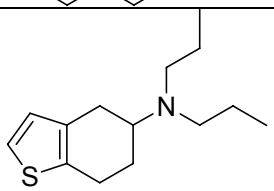
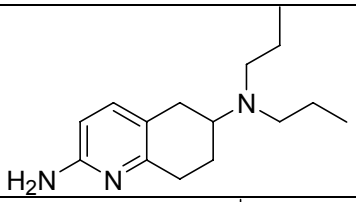
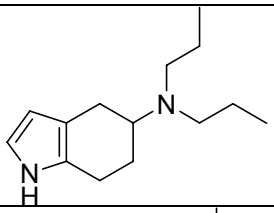
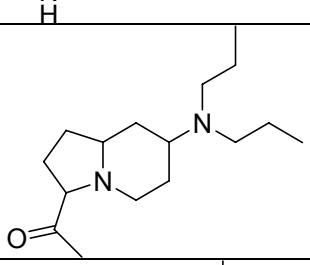
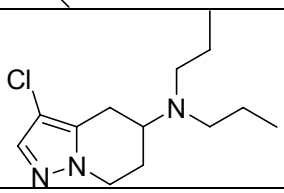
Compound	Structure	Ki (nM)	
		D2	D3
XI		6	0.54
XII		56	0.57
XIII		20	40
XIV		17.1	0.87
XV		9700	38
XVI		90	6
XVII		210	6.1

Table 7: Binding affinity constants (Ki) values of some Aminotetralin derivatives [78- 80]

1.5.2.3 Phenylpiperazine derivatives

Dopaminergic receptors ligands belonging to this structural class are considered among the most widely spread dopamine candidates. The general pharmacophore of this class includes an aryl or heteroaryl ring that is connected via a linker to a 4-aryl substituted piperazine unit [81], Figure **10**.

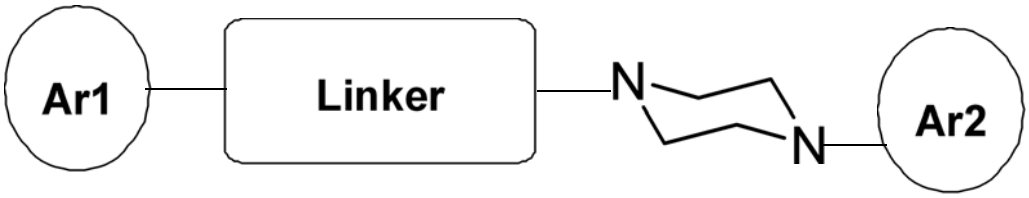


Figure 10: General Pharmacophore of Phenylpiperazines

The structural class of phenylpiperazines is known to be a privileged structural moiety simulating the native biogenic amine, dopamine, where by the virtue of containing an aromatic ring system and basic nitrogen, the phenylpiperazine skeleton can be regarded as the primary recognition element targeting the neurotransmitter binding site on the target receptor [81].

It is worth to mention that the nature of the aryl rings, the positioning of the substituentes on these rings, the nature, length, and the degree of rigidity of the linker are the major contributes to the subtype receptor selectivity and functional activity. Different natured aryl rings have been used to serve as the Ar1 part in this pharmacophore, among of which phenyl, benzothiophene, indole, azaindole, benzimidazole, and pyrazolopyridine systems were identified as excellent hits for maintaining selectivity towards D2-like family receptors [81- 85].

For the linker part, as many suitable units as saturated or unsaturated aliphatic chains, cyclic and bicyclic carbon based systems have been used. It

was found that a short linker such as one methylene unit linking the primary recognition element results in D4 subtype receptor selectivity, while increasing the length to a one propyl unit, shifts selectivity to D2 receptors, however a further increase by using one butyl unit yields highly selective D3 ligands. Degree of unsaturation and rigidity of the linker units also play an important role in directing the subtype receptor selectivity. It was reported that D2 and D3 affinity is strongly reduced upon using propenyl and butenyl linker units respectively. Replacing the butyl chain with a cyclohexyl unit also resulted in reducing D3 binding affinity. However, inserting amide group into the linker units improved the binding affinity on the target receptor over the same analogues deprived of this functional group [81, 82, 86, 87].

The second aromatic moeity that is attached to the piperazine ring is usually represented by a substituted phenyl ring. It was noticed that using 2-methoxy and 2,3-dichloro, and 2,3-dimethylphenyl piperazines resulted in greater D3 subtype receptor selectivity over D2 and D4 [88- 90].

Table **8** shows the binding affinity constants for some phenylpiperazine derivatives at D2-like family receptors [81- 90].

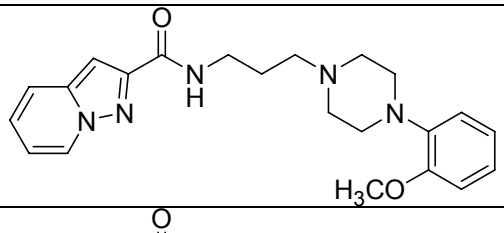
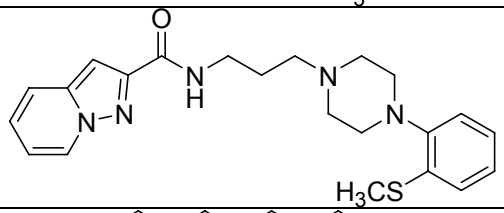
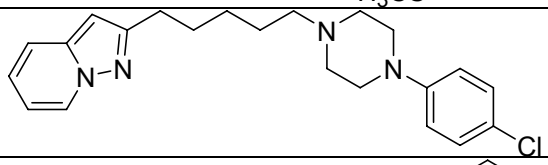
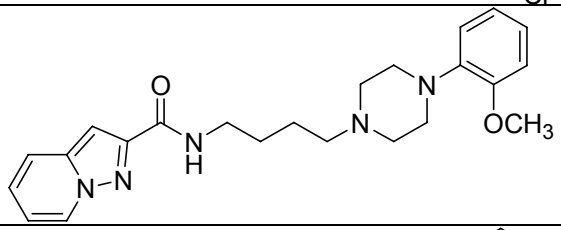
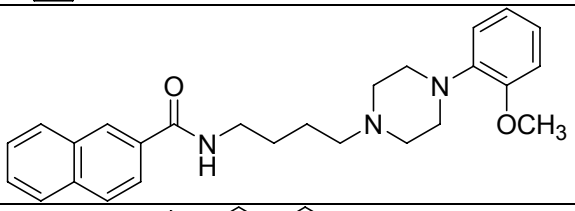
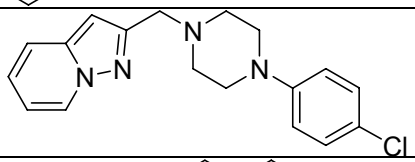
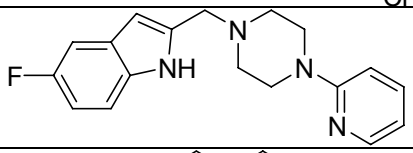
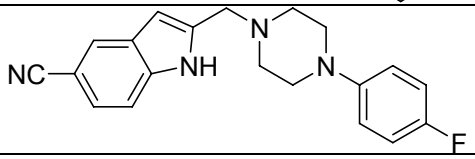
Compound	Structure	Ki (nM)		
		D2	D3	D4
XVIII		11	150	14
XIX		1.4	18	8.8
XX		160	370	46
XXI		310	4.3	130
XXII		220	1.3	44
XXIII		3200	5000	3.1
XXIV		>600	>600	6
XXV		28000	15000	1

Table 8: Binding affinity constants (Ki) values of some Phenylpiperazine derivatives [81-90]

1.6 Binding pockets of some dopaminergic receptors

With the implication of GPCRs – to which dopaminergic receptors belong – in many diseases, the need to solve the 3D structure of this class is crucial for enabling structural based drug design. This lack of GPCRs structures is due to the fact that these receptors are bound to the membrane making it difficult to express in sufficient quantities for crystallization. Moreover, the poor aqueous solubility of membrane proteins makes it difficult to obtain crystal structures of this type of receptors [51].

Dopaminergic receptors are known to share structural homology with rhodopsin and β-adrenergic receptors, on the basis of which comparative molecular modeling of the dopamine receptors and ligands docking have been investigated, where the emergence of the high resolution crystal structures of β-adrenergic receptors has inspired many researchers to use them as a main template for dopaminergic receptors modeling [91, 92].

The results revealed differences in the size and the shape of a common ligand binding site, where it was suggested that particular microdomains in transmembrane helix 2 (TM2), TM3, and TM7 might be relevant for ligand selectivity, while some other amino acids residues in TM3, TM5, and TM6 are necessary for interaction with ligands in all the receptor subtypes [51, 81, 93- 95].

Starting with D2 and D4 receptor subtypes, Ortore *et al*, in agreement with data reported in literature, have proved similar binding fashion between dopamine and the receptor pocket in case of D2 and D4 subtypes, where the *para*-OH and *meta*-OH of dopamine have been found to be hydrogen bonded to Serine residues, Ser 5.43 and 5.46 in the TM5 of the target receptors. The

protonated nitrogen of dopamine afforded ionic interaction with Asp 3.32 and finally the aromatic moiety of dopamine afforded hydrophobic interaction with the amino acid residues constituting the TM6 in the binding pocket of D2 and D4 receptor subtypes. The major residues in this area are Trp 6.48, Phe 6.51, Phe 6.52, and His 6.55 that are conserved in both receptor subtypes. Other amino acid residues in TM7 were found to share to constitute the hydrophobic pocket of the binding site of the two target receptors. These amino acids include Thr 7.39, Trp 7.40, Tyr 7.43, and Ser 7.46 [96- 98], Figure **11** [96].

Regarding the antagonist binding fashion, Kalani *et al* have suggested two binding modes between the antagonist and the D2 receptor subtype [51]. The first binding mode is for clozapine like antagonists, where docking clozapine to the predicted 3D structure of D2 receptors in this work has shown that the ligand occupied the region of the agonist binding site between TM3, TM4, TM5, and TM6. Clozapine has shown to make salt bridge to Asp 114 (3.32), hydrogen bond to Ser 193 in TM5, and a mostly hydrophobic pocket shown in Figure **12** [51] formed by Val 87, Trp 90 (TM2), Phe 110, Leu 113, Val 115, Met 117, and Cys 118 (TM3), Phe 164 (TM4), Phe 189, Val 190, Ser 194, Ser 197 (TM5), Phe 382, Trp 386, Phe 389, and Phe 390 (TM6), and finally Thr 412, Trp 413, Tyr 416, Ser 419 in TM7 [51, 99, 100].

The second binding mode suggested by this group is for haloperidol like antagonists. Docking haloperidol to the predicted 3D structure of D2 receptors

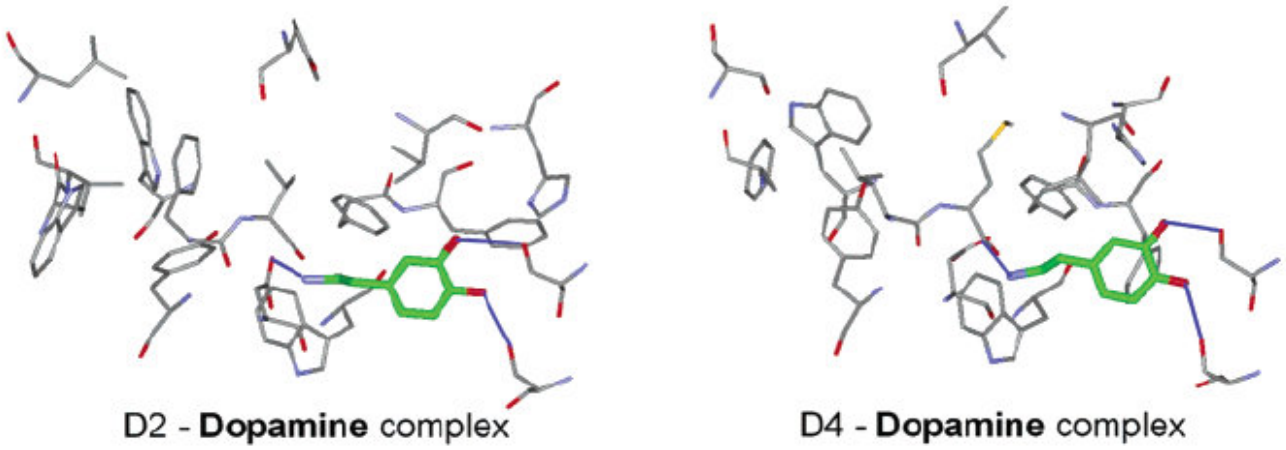


Figure 11: D2 and D4 binding complexes with dopamine [96]

has shown that the ligand occupied the region between TM2, TM3, TM6, and TM7. Haloperidol afforded salt bridge to Asp 114 in TM3, hydrogen bond to Ser 197 in TM5, and a mostly hydrophobic pocket shown in Figure **12** [51] provided by Val 87, Val 91, Leu 94 (TM2), Phe 110, Leu 113, Val 115, Met 117, and Cys 118 (TM3), Trp 160, Phe 164 (TM4), Phe 189, Val 190, Val 196 (TM5), Trp 386, Phe 389, Phe 390, His 393 (TM6), and finally Ser 409, Thr 412, Trp 413, Tyr 416, Val 417 in TM7 [51, 99, 100].

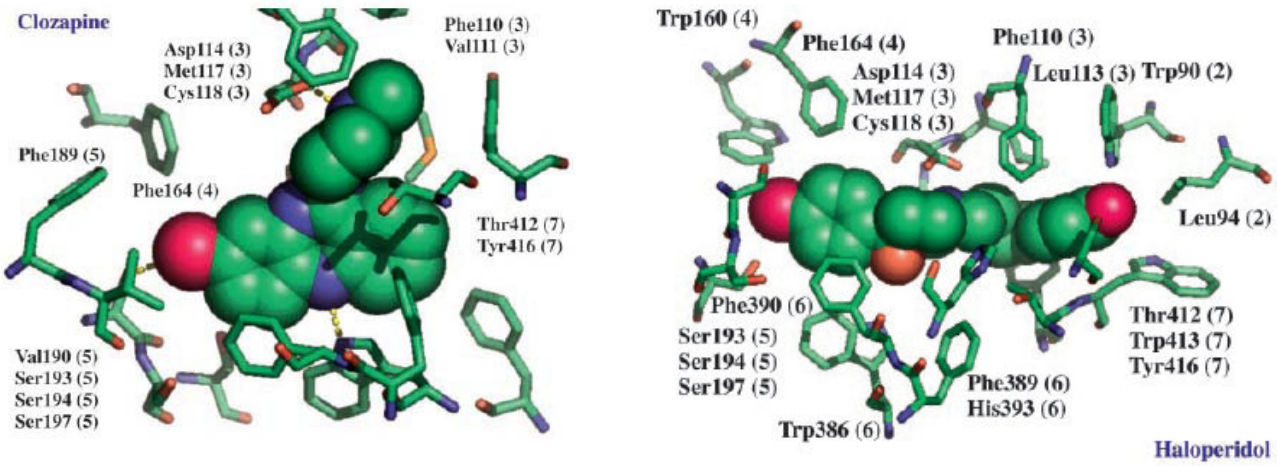


Figure 12: Residues within 5.5 A^0 of Clozapine (left) and Haloperidol (right) bound to human D2 receptor model [51]

As for the D4 antagonist FAUC 213, Lober *et al* have studied the binding fashion of this phenyl piperazine derivative to the D4 receptor binding cavity, Figure **13** [101]. The protonated aliphatic amine of the ligand interacts with Asp 3.32 in TM3. The chlorophenyl moiety is supposed to contribute to D4 affinity and selectivity by recognizing Phe 2.61 in TM2. Finally the azaindole ring of the ligands was bound to Ser 5.46 in TM5 via hydrogen bonding [101].

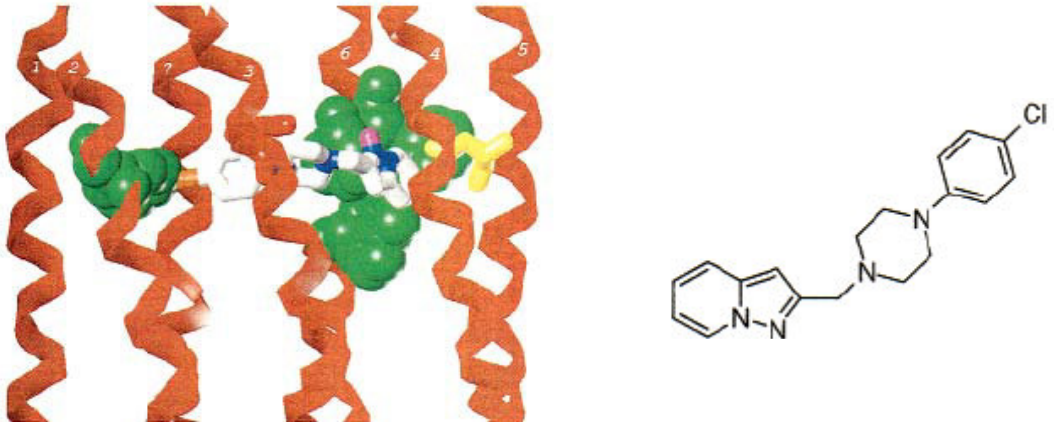


Figure 13: Docking the D4 antagonist FAUC 213 to the binding cavity of human D4 receptor model [101]

In contrast to the binding mode of the agonist, the partial agonist and the antagonist have afforded weaker hydrogen bonding with Ser 5.46 due to higher distance between the ring Nitrogen atom and the OH of the serine residue. This distance can be controlled through varying the positioning of the substituent on the aromatic ring of the ligand [101].

The main difference between D2 and D4 receptors as configured by previous studies seems to be due to the lipophillic region in the binding site which is situated in a different position in the two receptors. In D2 receptors it is due to Phe 3.28 and Tyr 7.35, while in D4 the lipophillic region is due to Phe 2.61. Therefore, the selectivity towards the D4 receptors with respect to the D2 one

could be due to the ability of a D4 selective ligand to interact with the region near Phe 2.61 of the lipophillic region of D4 receptor [96- 98].

A further mutagenesis study done by Ehrlich *et al* has confirmed the role of the amino acids at positions 2.60, 2.61, 3.28, and 3.29 in providing key structural determinants for drug selectivity between D2 and D4 receptor subtypes [81]. The amino acid residues that correspond to these positions are as follow:

- At position 2.60, it is Tryptophan in D2 and Leucine in D4.
- At position 2.61, it is Valine in D2 and Phenylalanine in D4.
- At position 3.28, it is Phenylalanine in D2 and Leucine in D4.
- At position 3.29, it is Valine in D2 and Methionine in D4.

Figure **14** [96] illustrates the subset of residues involved in the ligand binding at D2 and D4 receptor subtypes. This study has proved also the importance of the histidine residue 6.55 as a key residue for the interaction with the primary recognition element of the ligand which was shown to be the phenyl piperazine unit of the used ligands in this study [81].

As for D3 binding pocket, Chien *et al* have managed to crystallize the target receptor bound to the D2/D3 selective antagonist Eticlopride, Figure **15** [102].

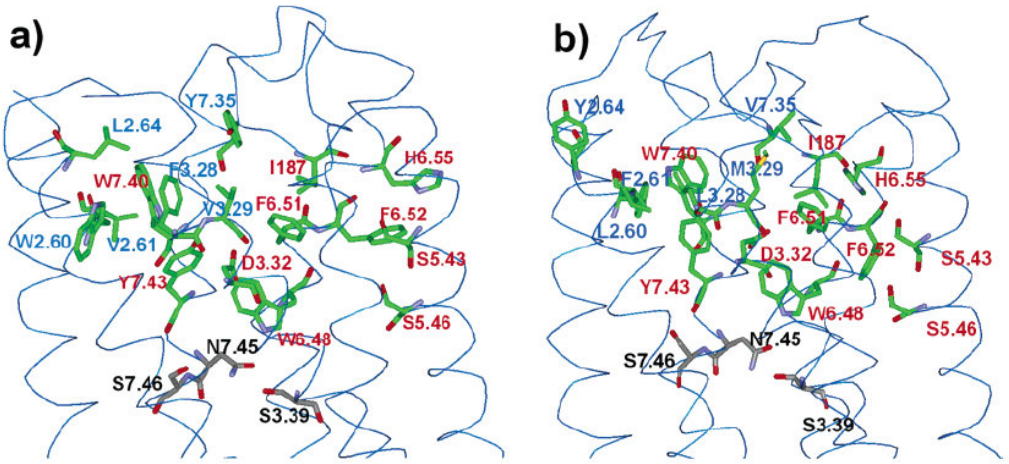


Figure 14: Subset of residues involved in the ligand binding at D2 (a) and D4 (b) receptors [96]

Eticlopride occupies the part of the binding pocket defined by side chains from helices II, III, V, VI, and VII. The tertiary amine in the ethyl-pyrrolidine ring of eticlopride is likely charged at physiological pH and forms a salt bridge to the carboxylate of Asp 3.32, which is highly conserved in all aminergic receptors. This salt bridge is structurally and pharmacologically critical for high-affinity ligand binding to the aminergic subfamily of GPCRs. Another key component of the eticlopride pharmacophore is a substituted aromatic ring connected to the pyrrolidine by an amide bond that fits tightly within a hydrophobic cavity

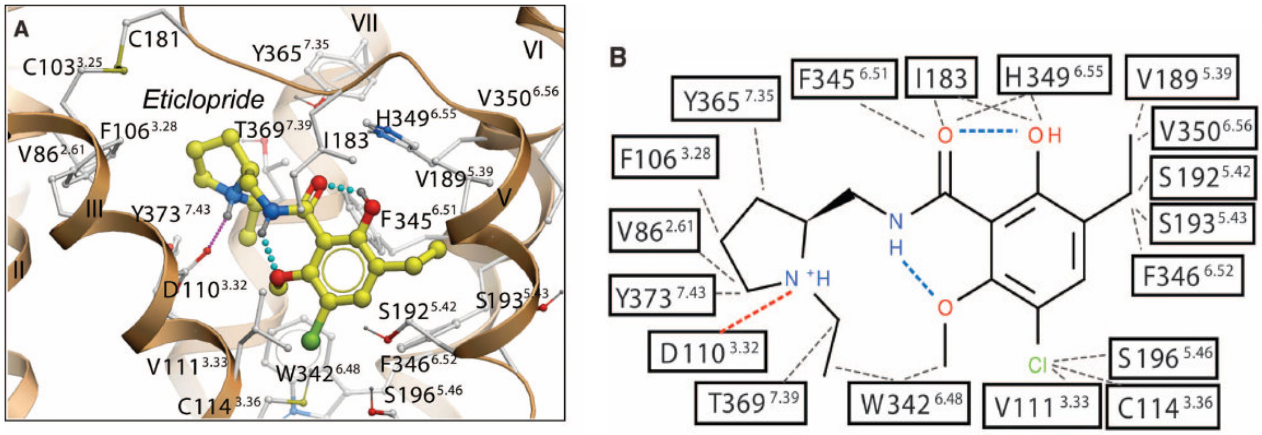


Figure 15: A. Binding cavity of Eticlopride in D3 receptor, B. Interactions of Eticlopride with the amino acid residues in D3 binding cavity [102]

formed by Phe 6.51 and Phe 6.52 in helix VI, Val 5.39, Ser 5.42, and Ser 5.43 in helix V, and Val 3.33 in helix III, as well as Ile 183 in EL2. Polar substituents such as OH, OCH3 in the phenyl ring form intramolecular hydrogen bonds with both the N and O of the amide, thereby maintaining the compound in an almost planar conformation [102, 103].

Of the 18 eticlopride contact residues in the D3 receptor structure, 17 are identical in the D2 receptor (Val 6.56 is an isoleucine in D2 receptors). Qualitatively, this agrees with the finding that eticlopride, and some of its analogs, share similar affinities for the D2 and D3 receptors. The structural determinants of pharmacological specificity in the D2 and D3 receptors are more subtle considering that the residues lining the binding pocket are essentially identical. In accordance with high conservation of the eticlopride binding site between D3 and D2 receptors, the available structure-activity relationship data suggest that, to achieve targeted selectivity, the ligand must extend toward the extracellular opening of the binding pocket. The D3 selective pharmacophore consists of an extended aryl amide connected to an amine-containing scaffold by a relatively flexible four-carbon linker [81-84, 86, 87, 102, 104].

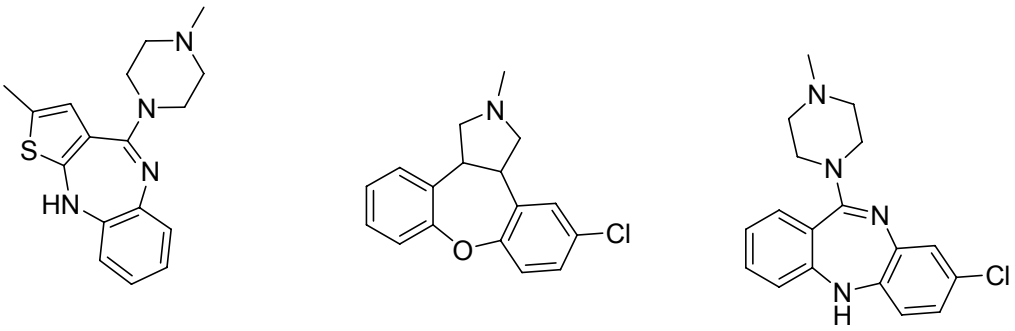
2. Research Objectives

Dopaminergic system plays an important role in regulating neuronal motor control, cognition, emotion, and vascular function. Neuropsychiatric diseases such as schizophrenia, Parkinson's disease, or addiction are strongly related to disturbed dopamine transmission in CNS, thus dopamine receptors are attractive therapeutic targets for ligands design and synthesis. This work aims at developing novel dopaminergic ligands showing better pharmacokinetics relative to the previously prepared ones and with modulated affinity and/or selectivity towards the five subtypes of these receptors, where single subtype selectivity or more favorable combinations of affinities to several subtypes of dopaminergic receptors may reduce the unfavorable side effects and/or potentiate the activity of the classic ligands. Further more the introduction of novel ligands for dopaminergic receptors is still needed to help in studying the molecular structure and/or crystallizing some of the members of this receptors family whose exact molecular structure is still not well figured out.

(1) Modulating affinity and selectivity of LE300 and some of its analogues towards dopamine receptor subtypes by variation of the chemical structure

Annelated azecines represent a new family of dopaminergic antagonists characterized by their high affinity more or less unselectively towards the D1- and the D5-receptor and by moderate to weak affinity towards the D2-like receptors[67- 69]. Designing a highly selective ligand for either one of the two receptor subtypes of the D1-like family still stands as a challenge due to the fact that both receptor subtypes share high level of molecular structure identity within their transmembrane helices [27]. Such D1/D5-subtype-selective ligands may not only serve as novel atypical antipsychotics but also would

contribute to investigate the functions of each receptor subtype separately. Moreover, D1/D5-selective ligands might be of further therapeutic interest after recent studies have shown evidence that these receptors elicit certain effects in different organs, among them the kidney [105]. Furthermore, compounds equipotent at members of both the D1 and the D2 family seem to be favorable with respect to lower undesired side effects as demonstrated by olanzapine, asenapine, and clozapine, Figure **16**.



Olanzapine
D1: 70 nM
D2: 53 nM
D5: 82 nM

Asenapine
D1: 2.9 nM
D2: 1.4 nM
D5: 22.7 nM

Clozapine
D1: 266 nM
D2: 343 nM
D5: 255 nM

Figure 16: Ki values* of olanzapine, asenapine, and clozapine towards some dopaminergic receptors
*values taken from PDSP database; Source: human, cloned.

In this work we tried to modulate the selectivity/affinity profiles of lead azecine derivatives, namely the indolobenzazecine derivative LE 300 and its dibenzo analogues, Figure **17**, for the sake of learning more about SAR of azecine-type dopaminergic ligands and getting derivatives with novel selectivity profiles. These lead compounds exhibit moderate affinity to D2-like receptor subtypes and higher to the D1-like members. Regarding subtype receptor selectivity, it is noticeable that symmetrical dibenzazecine LE 410 is slightly selective for D1, while further increasing the electron cloud on the aromatic

rings by hydroxylation reverses the selectivity pattern and shifts affinity it towards the D5 receptor subtype [67].

In order to achieve a high electron density at one of the aromatic systems without substitution we designed and prepared two regioisomers carrying a thiophene in different orientations, namely the benzo[d]thieno[2,3-g]azecine **1**, and the benzo[d]thieno[3,2-g]azecine **2** and furthermore the benzothiophene derivative **3**.

Furthermore, selection of sulfur containing arene systems was based on the observation that Olanzapine shows for D1, D2, and D5 a much higher affinity than Clozapine.

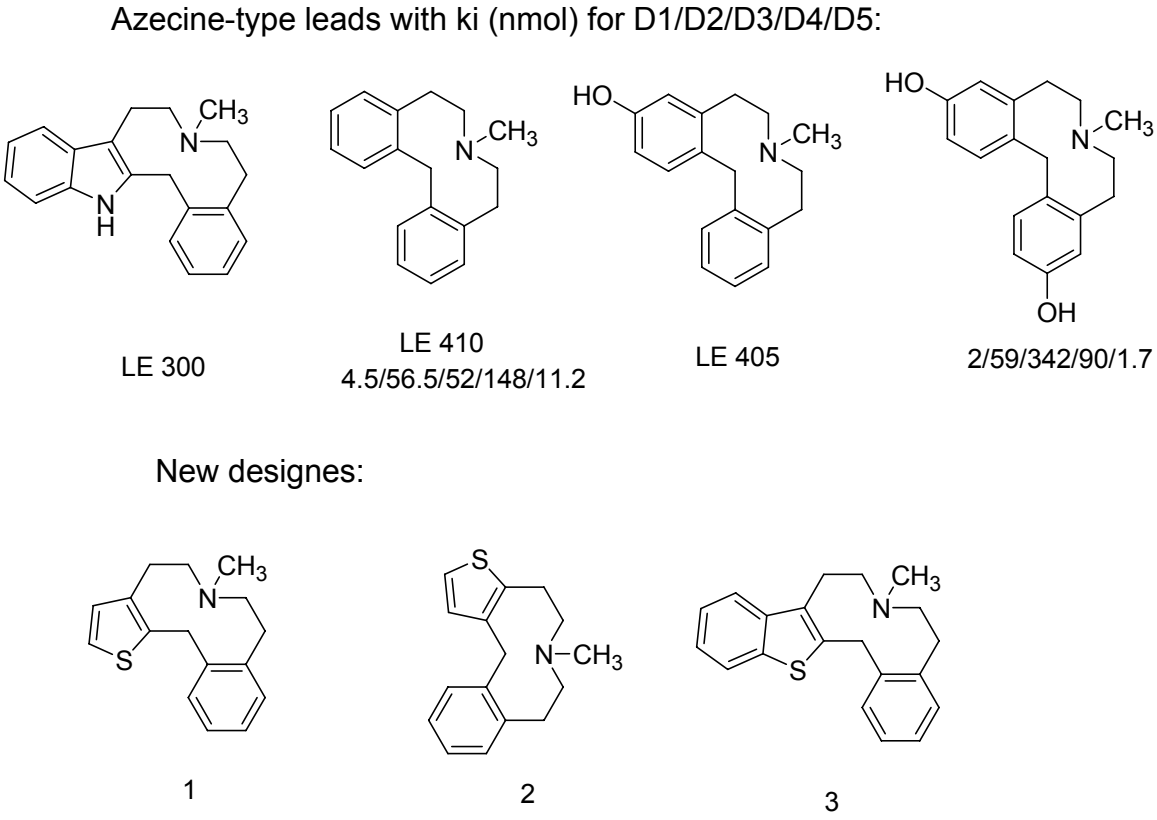


Figure 17: Novel target compounds 1, 2, 3 based on the lead compounds

(2) Synthesis of novel potent and selective D4 arylmethylphenyl-piperazine derivatives

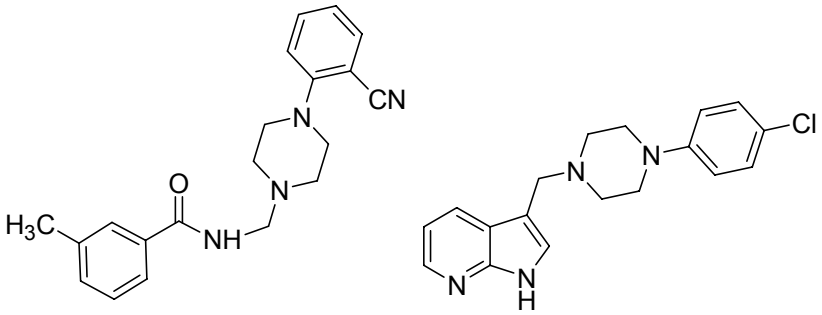
Among the D2-like family, D4 receptor subtypes have recently shown great interest as a result of its involvement in treatment regimes of variety of neuropsychiatric disorders [106]. Beside counteracting Parkinson's disease, selective D4 agonists and partial agonists have proved to be of benefit in relieving Attention Deficit Hyperactivity Disorder, and other mood disturbances [107, 108]. This is related to the contribution of this receptor subtype in some personality traits such as novelty seeking or impulsive, compulsive, and addictive behavior [109]. Moreover, other D4 selective agonists have been reported to induce penile erection in rats when administered in vivo, an action that is inhibited by concurrent ingestion of selective D4 antagonist confirming the mechanistic pathway [72, 110].

Antagonists with remarkable affinity to D4 receptors are considered among the powerful marketed antipsychotic drugs that show minimal Parkinson's like side effects that characterize the classical non selective members [76, 111].

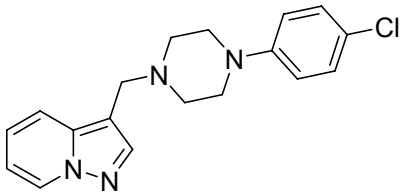
Unlike D3 receptor subtype, the exact molecular structure of the D4 binding pocket is still not characterized by X-ray crystallography leaving the field in a still need for selective chemical probes that might help in identifying the binding fashion to this valuable target protein. Ligands bearing the arylmethylphenylpiperazine scaffold are known with their remarked selectivity and affinity towards D4 receptor subtype [81, 85].

As an example of these ligands, comes PD-168077 that was demonstrated to be unstable in acidic solution, precluding its chances for oral administration [112]. L-745870 is another member that is induced for clinical trials but failed to

exert clinical efficacy [113]. Structure of these compounds and another D4 selective ligand, FAUC 113, are shown in Figure **18**.

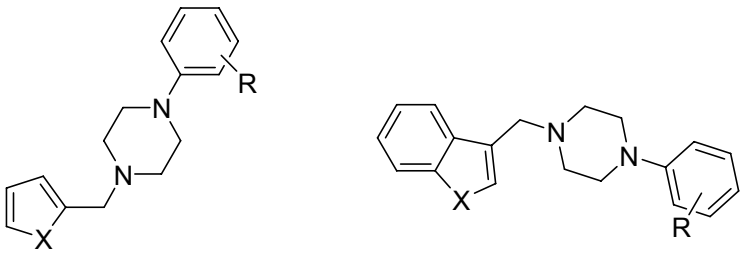


PD168077 **L-745870**



FAUC 113

New Candidates:



X= S, HC═CH ,HC═N X= S, HC═CH ,NH

Figure 18: Some D4 selective Phenylpiperazine derivatives and the new developed candidates

In this work, we would start with synthesizing a series of thiophenylmethylphenylpiperazine derivatives (**31a- j**) bearing the thiophene as the heterocyclic arene moiety and different phenylpiperazine units to serve as the recognition element for the target D4 receptor. Thinking about centrally acting dopaminergic ligands, thiophene system would serve as an optimum aromatic heterocycle moiety as a start for this set of compounds for being more or less electronically similar to the catechole ring of the endogenous

Dopamine (molar refractivity of thiophene is 2.6 versus 3.0 for the catechol system, calculated with molar refractivity descriptor of MOE [114], so that it would provide this area of the compound's scaffold with the optimum electrostatic potential required to ensure the affinity of the designed candidates to the target receptor [101].

Concerning the factor of molar volume and its effect on CNS penetrability, thiophene bearing candidates would have a relative smaller molar volume when compared to those bearing other arenes and thus may show better ability to diffuse through the densely packed cells of brain and blood brain barrier. The molar volume of thiophene ring is shown to be 79.62 versus 129.62 for benzothiophene, 127.50 for indole, 94.37 for benzene, and 86.87 for pyridine, calculated using the volume descriptor of MOE [114].

Relative to other five member arenes such as pyrole (molar volume of 78.12 and molar refractivity of 2.2) and furan (molar volume of 73.6 and molar refractivity of 2.0), thiophene system would stand as an optimum arene to start with.

As for the phenylpiperazine part of the scaffold, we decided to use different substituents on the phenyl ring to investigate the electronic, steric, and polarity impact of this part of the compounds on the affinity and selectivity to D4 receptors.

Based on affinity and selectivity data obtained after testing the synthesized candidates on the five dopaminergic receptor subtypes, some of the synthesized derivatives would have their thiophene ring replaced with other aryl and heteroaryl moieties to demonstrate the effect of this particular part of the structure on the affinity and selectivity towards the target protein, and to

configure out an interactive Structure Affinity Relationship study. In such way, two rounds of structural optimization could be adopted, where compounds bearing the phenylpiperazine units that showed best affinity would have their heteroarene moiety replaced with other ones for the sake of getting the optimum affinity.

(3) Modulating the affinity and selectivity profiles of some previously marketed D2-like family antagonists via developing Thiophenylamido propyl/butylphenylpiperazines

Among the well known D2-like family typical antagonists, comes the butyrophenone derivative Haloperidol as one of the most commonly marketed typical antipsychotic agents [76, 115]. These typical medications are characterized by their ability to block the D2-like receptor subtypes unselectively and though being useful in curing the positive symptoms of psychosis, they possess marked Extra Pyramidal Parkinson's like adverse effects [116]. This undesired propensity is thought to be a result of blocking D2 receptor subtype that is mainly concentrated in striatal areas of the brain [117]. On the contrary, atypical antipsychotic drugs relieve both positive and negative signs of the disease experiencing much lower incidence of the Extra Pyramidal symptoms [118]. This special behavior of atypical antipsychotic drugs is believed to be as mentioned before either due to their ability to block D3/D4 receptor subtypes selectively over D2 ones, or due to their loose binding and their rapid dissociation off D2 receptor subtype, an action that allows normal dopamine transmission and thus this transient binding fashion would be the factor that obviates the Parkinson's like effects [74, 118]. Recent studies have linked this special pharmacological behavior of atypical drugs to their cross interaction with 5-HT2A receptors [73].

From these atypical medications, the thienobenzodiazepine derivative Olanzapine and the benzamide derivative Sulpiride are commonly used. Ketanserine is another ligand characterized by its moderate interaction with D2 receptor subtype while exhibiting much higher binding affinity to D4 and 5HT2A receptors.

Unfortunately, the use of atypical antipsychotic medicines is still limited because of diverse of hazards including increased risk of brain stroke, cardiovascular diseases, metabolic and diabetic complications, weight gain, and impaired sexual function [119] leaving the field in a need of newer probes.

Attempting to develop novel selective D3 and/or D4 ligands, we have designed our probes to serve as hybrids bearing combined structural features from both the typical and atypical lead compounds Haloperidole, Sulpiride, Olanzapiene, and Ketanserine, Figure **19**. These designed probes are also bearing an optimum scaffold in which the aromatic ring appendage and the basic nitrogen of the phenylpiperazine separated with the amidoalkyl linker would keep the primary recognition elements that have been proved to be necessary for fitting into the binding pockets of the target dopaminergic receptors [81].

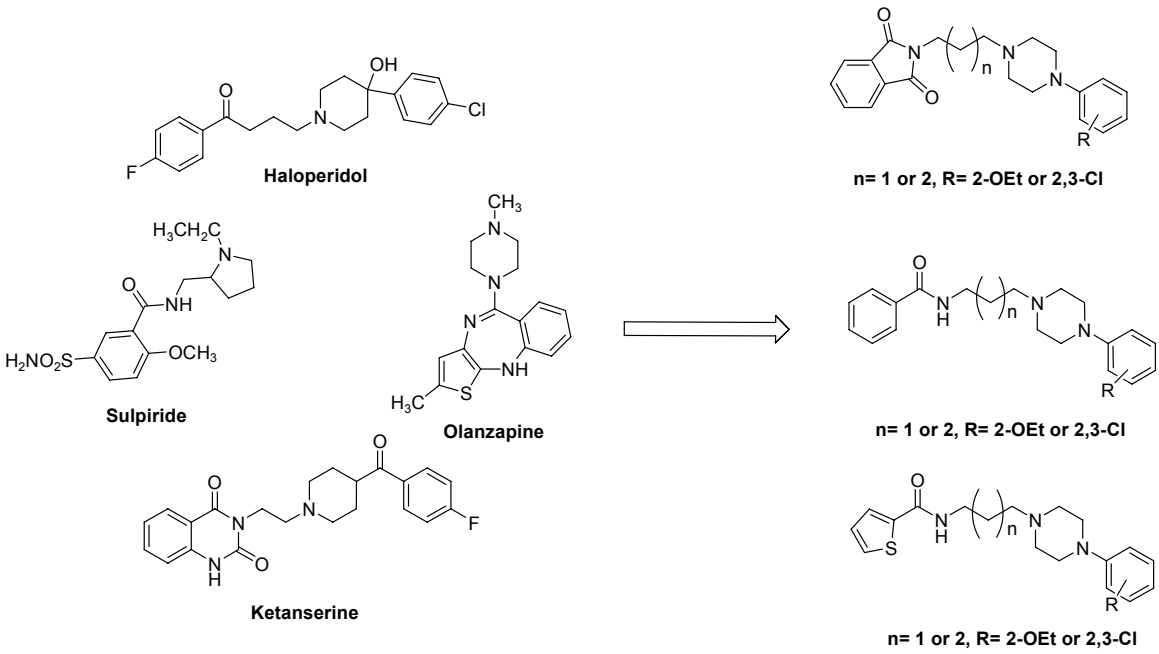


Figure 19: Design of hybrid dopaminergic probes based on marketed typical and atypical antipsychotic agents

We would start with the first group of compounds (**40a, 40b, 41a, 41b**) having the phenylpiperazinylalkylisoindoledione skeleton in which the phenylpiperazin scaffold is substituted once with 2-OEt group and another with 2,3-Cl which have been shown by previous studies to be optimum for targeting dopaminergic receptors [81, 101]. The spacer linking the aromatic appendage to the phenylpiperazine unit would be either a propyl or butyl chain. We would also replace the isoindoledione system with a benzamide one so we would synthesize the second group, compounds (**42a, 42b, 43a, 43b**). Finally, we would go for a further structural modification utilizing a thienoamide appendage in place of the benzamide and accordingly the thienoamide bearing probes (**44a, 44b, 45a, 45b**) would be synthesized.

The scaffold to show to have the best affinity towards the target receptors, would have further modifications at the phenylpiperazine unit through utilizing other different oxygenated and halogenated substituents on that part of the

scaffold in order to investigate the electronic, and steric impact of this part of the scaffold on the affinity and selectivity towards the target receptor subtypes and hence figuring out a comprehensive interactive SAR study.

3. Results and Discussion

3.1 Chemistry

3.1.1 Synthesis of Thieno and Benzothieno based azecine derivatives

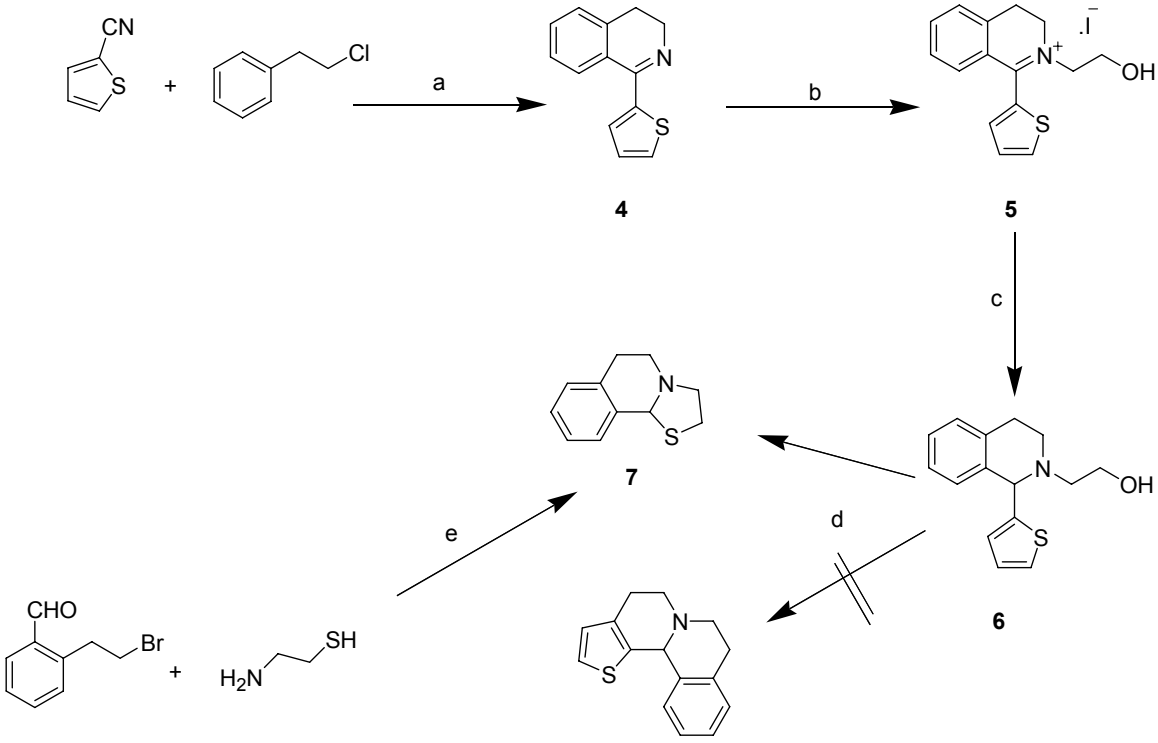
The general strategy adopted for the synthesis of the thieno and benzothieno the azecine derivatives was depending on the separate preparation of the respective β-aryl ethyl amine and the 2-(2-bromoethyl) benzaldehyde which were then reacted together under Pictet-Spengler or modified Pictet-Spengler conditions mainly to afford the formation of the respective quinolizines that were then subjected to N-methylation followed by C-N hydrogenolysis to obtain the desired candidates. The structure elucidation of the synthesized target compounds have been confirmed through IR spectroscopy, GC/Mass spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis.

3.1.1.1 Synthesis of 6-Methyl-4,5,6,7,8,13-hexahydrobenzo[d] thieno [2,3-g] azecine (1)

It was first necessary to prepare the corresponding thieno quinolizine namely, 4,7,8,12b-Tetrahydro-5H-benzo[h]thieno[2,3-a]quinolizine **15**, so that it would be followed with ring opening procedure to get the target azecine derivative.

The synthesis of this quinolizine, as illustrated in scheme **1**, started through reacting 2-thieophene carbonitrile and 2-phenylethylchloride in presence of stannous (IV) chloride to get the 3,4-dihydro-1-(2-thienyl)isoquinoline **4** that in turn reacted with 2-iodoethanol to get the quaternary N-hydroxyethyl isoquinolinium iodide salt **5** [120].

The quaternary salt was then subjected to a reduction procedure using sodium borohydride to get the corresponding 2-(2-hydroxyethyl)-1(2-thienyl)-1,2,3,4-tetrahydroisoquinoline **6** that was in turn entered into a cyclization reaction with polyphosphoric acid to afford the desired quinolizine derivative. Unfortunately, the spectral analysis of the resulted compound showed that it was not bearing the structure of the desired quinolizine. The mass spectrum showed a molecular ion peak of 191 g/mol and the integration of the ^{1}HNMR spectrum could elucidate the structure of 2,3,5,6-Tetrahydro-10b*H*-thiazolo[2,3-a]isoquinoline **7**. To confirm the structure of the resulted compound, we had to synthesize it adopting another route in which 2-(2-bromoethyl) benzaldehyde was reacted with 2-aminoethane- thiol in presence of KOH using ethanol as a solvent. The ^{1}HNMR spectra of the compound prepared from the two routes have been shown to be identical, Figure **20** [120].



Scheme 1: a: $SnCl_4$ b: 2-Iodoethanol, acetone C: $NaBH_4$, MeOH d: PPA, reflux e: KOH, EtOH, RT

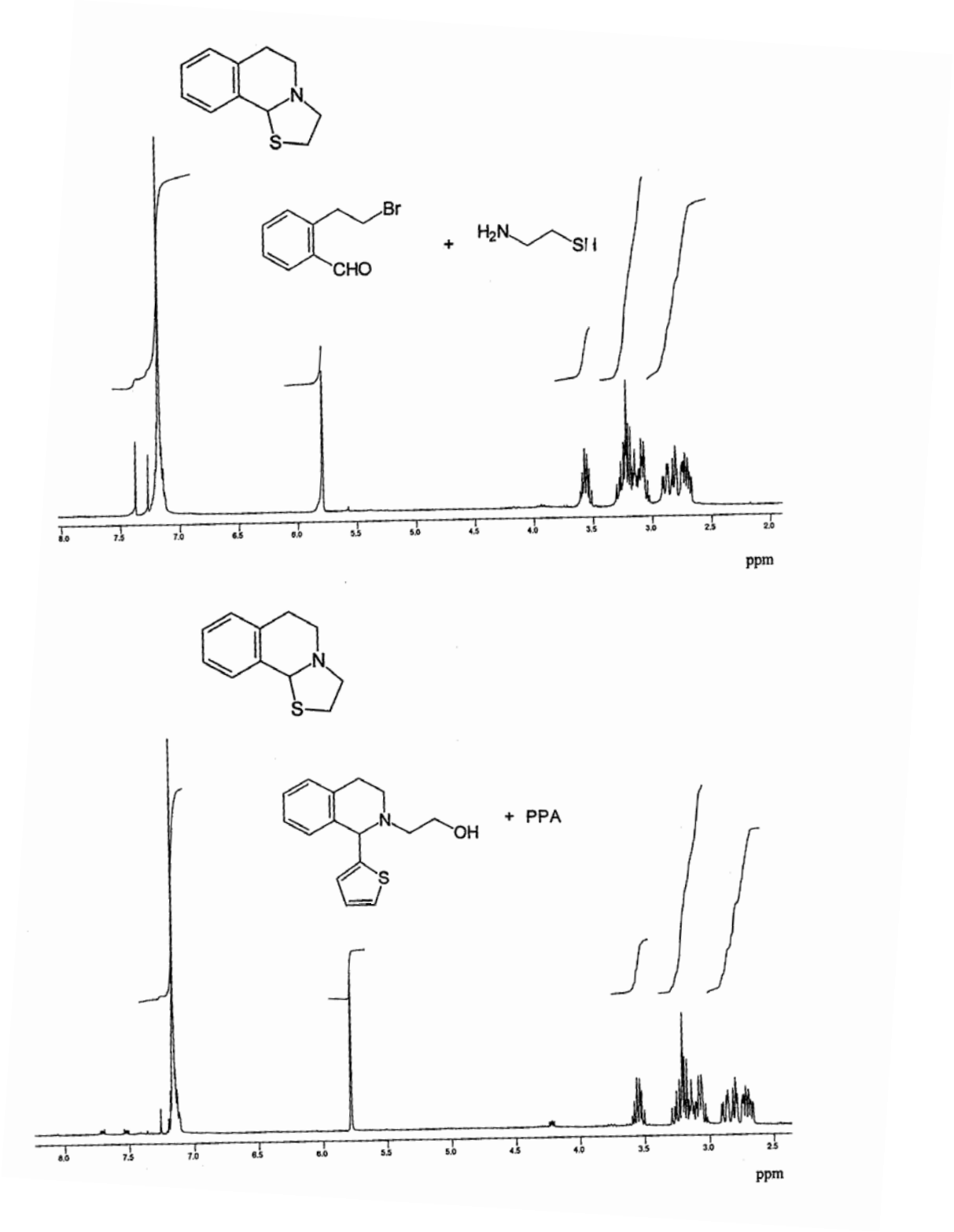


Figure 20: ^{1}HNMR charts of compound 7 synthesized via two different routes

In a trial to understand the mechanism of the formation of the thiazolo[2,3-a]isoquinoline **7**, we searched the organic literature for similar behavior. Interestingly it was reported that in rare cases, the thiophene sulfur atom

could be alkylated under acid catalysis followed by ring degradation [121, 122]. Based on these findings we suggested a proposed mechanism that might explain this strange behavior, Figure **21**.

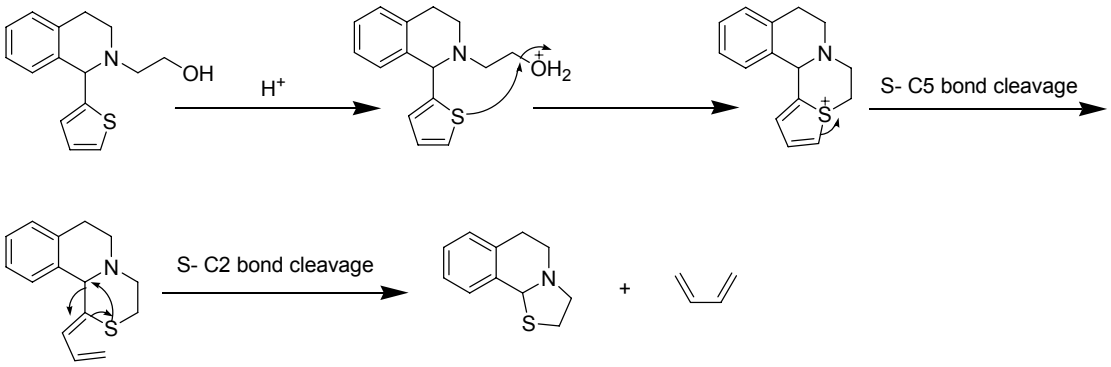


Figure 21: Proposed mechanism for the formation of the thiazolo[2,3-a]isoquinoline derivative

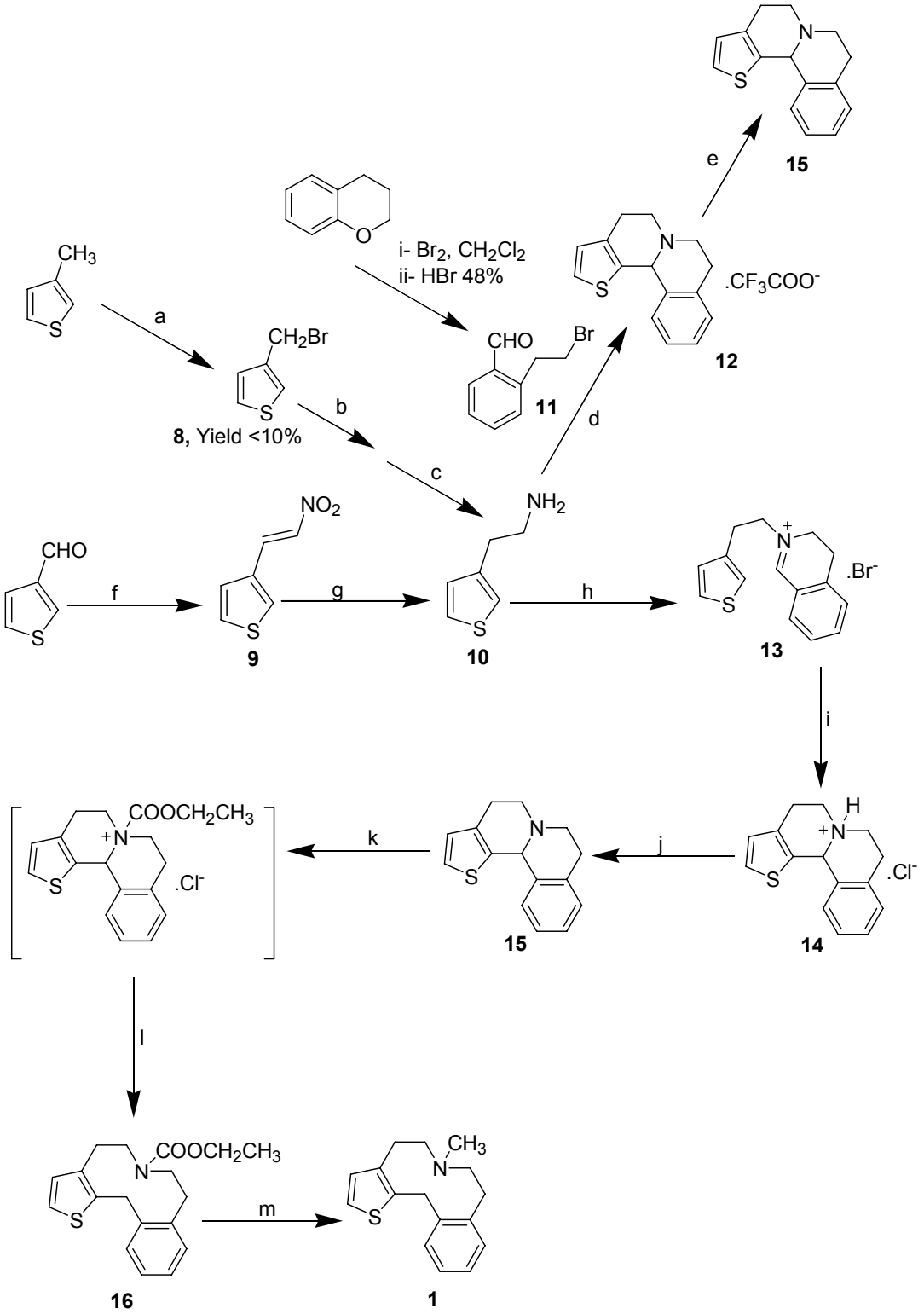
In a second trial to synthesize the target azecine the regular Pictet-Spengler reaction procedure has been adopted.

To prepare the 2-thiophene-3-yl-ethylamine **10**, we started with the bromination of 3-methyl thiophene with NBS in presence of benzoyl peroxide so that the resulted 3-(2-bromomethyl) thiophene **8** is to be subjected to nucleophilic substitution reaction with KCN followed by reduction with $LiAlH_4$ to the respective 2-thiophene-3-yl-ethylamine. Actually the bromination step ended up in a mixture of the desired product and different poly and mono brominated analogues. The desired 3-(2-bromomethyl) thiophene **8** was separated by silica gel column chromatography using etheyl acetate: toluene 1:1 but in an unsatisfactory yield (< 10%). Another route for the preparation of the desired amine was accomplished in which, the thiophene-3-carboxaldhyde was subjected to aldol condensation reaction with nitromethane and ammonium acetate to obtain the 3-(2-nitrovinyl) thiophene **9** that was treated with $LiAlH_4$ in dry THF to get the target amine.

The respective thienoquinolizine derivative was then tried to be prepared using Pictet-Spengler reaction conditions where it has been refluxed with 2-(2-bromoethyl) benzaldehyde **11** in presence of TFA and dry dioxane as a solvent. Unfortunately, the yield of the obtained product was lower than expected. To have our product in a higher yield, a modified Pictet-Spengler reaction was utilized, where the 2-thiophene-3-yl-ethylamine **10** was reacted with 2-(2-bromoethyl) benzaldehyde **11** in dry dioxane for 2-3 hours and the resulted imminum bromide salt was subjected to cyclization using 6M HCl and reflux for 8 hours. This resulted in the formation of the respective quinolizine in the form of its HCl salt, from which the free base was liberated using 33% NH_3 solution with more satisfactory yield.

The ring opening was done through reacting the respective thienoquinolizine with chloroethylformate in dry THF at -80 ^{o}c, using dry ice in methanol for 4 hours followed by the addition of $NaBH_3CN$ to afford the ring opening and obtaining the corresponding carbamate derivative **16** that in turn was reduced with $LiAlH_4$ in dry THF to get the desired benzo[d]thieno[2,3-g] azecine target candidate.

Scheme **2** illustrates the adopted pathway for the synthesis of the target candidate [67- 69, 123, 124]



Scheme 2: a: NBS, benzoyl peroxide, benzene reflux b: KCN, TEBA, H_2O c: $LiAlH_4$, dry THF d: TFA, dry Dioxane e: 33% NH_3 f: $NH4COOCH_3$, CH_3NO_2 g: $LiAlH_4$, dry THF h: 2-(2-bromoethyl) benzaldehyde, dry Dioxane i: 6M HCl j: 33% NH_3 k: $ClCOOCH_2CH_3$, dry THF l: $NaBH_3CN$, dry THF m: $LiAlH_4$, dry THF

3.1.1.2 Synthesis of 11-Methyl-4,9,10,11,12,13-hexahydrobenzo [d] thieno[3,2-g] azecine (2)

This started by Reacting 2-thiophene methanol and $SOCl_2$ together resulting in the formation of 2-chloromethylthiophene **17** that was converted into the desired 2-thiophen-2-yl-ethylamine **19** adopting similar procedure as before.

Reacting the previously synthesized 2-thiophen-2-yl-ethylamine with 2-(2-bromoethyl) benzaldehyde and TFA in dry dioxane adopting Pictet-Spengler or modified Pictet-Spengler reaction to prepare the respective quinolizine, 4,7,8,12b-Tetrahydro-5H-benzo[h]thieno[3,2-a]quinolizine **23** was unsuccessful trial. This is could be related to the low possibility of the thienyl C3 attack on the imminium quaternary nitrogen as the negative charge that affords this attack is predominantly localized on thienyl C2 so that the electrophillic substitution reaction could not have been happened.

Alternatively, 3-Thiophenecarbonitril was reacted with 2-Phenylethylchloride in presence of stannous (IV) chloride to afford the formation of the oily 3,4-dihydro-1-(3-thienyl)isoquinoline **20**, that in turn reacted with 2-iodoethanol to give the corresponding N-hydroxyrthyl isoquinolinium iodide salt **21**.

The resulted quaternary salt was then subjected to a reduction reaction using sodium borohydride to get the corresponding 2-(2-hydroxyethyl)-1-(3-thienyl)-1,2,3,4-tetrahydroisoquinoline **22** that was in turn entered into a cyclization reaction with polyphosphoric acid to afford the desired quinolizine derivative as major product that was isolated from other minor side products via recrystallization from ethanol .

In similar procedure as before, the obtained quinolizine derivative was subjected to ring opening procedure via its reaction with chloro ethylformate and $NaBH_3CN$ to get the corresponding carbamate that was then reduced

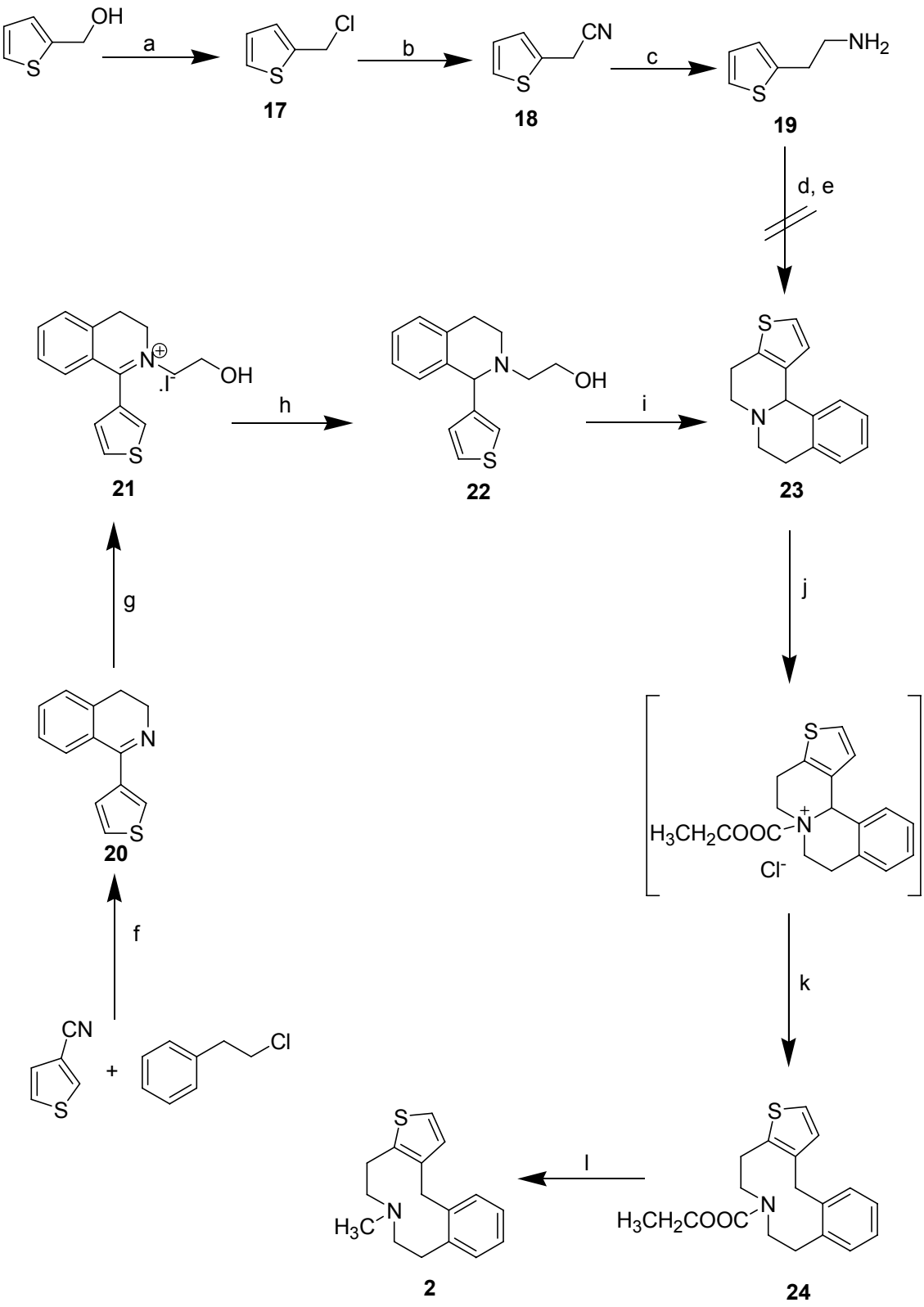
with $LiAlH_4$ in dry THF to have the desired benzo[d]thieno[3,2-g] azecine derivative [120].

Scheme **3** illustrates the synthesis of the desired benzo[d]thieno[3,2-g] azecine.

3.1.1.3 Synthesis of 8-Methyl-6,7,8,9,10,15-hexahydrobenzo [d][1] benzothieno [2,3-g]azecine (3)

Scheme **4** illustrates the pathway utilized to synthesize the desired benzo[d][1] benzothieno[2,3-g] azecine derivative.

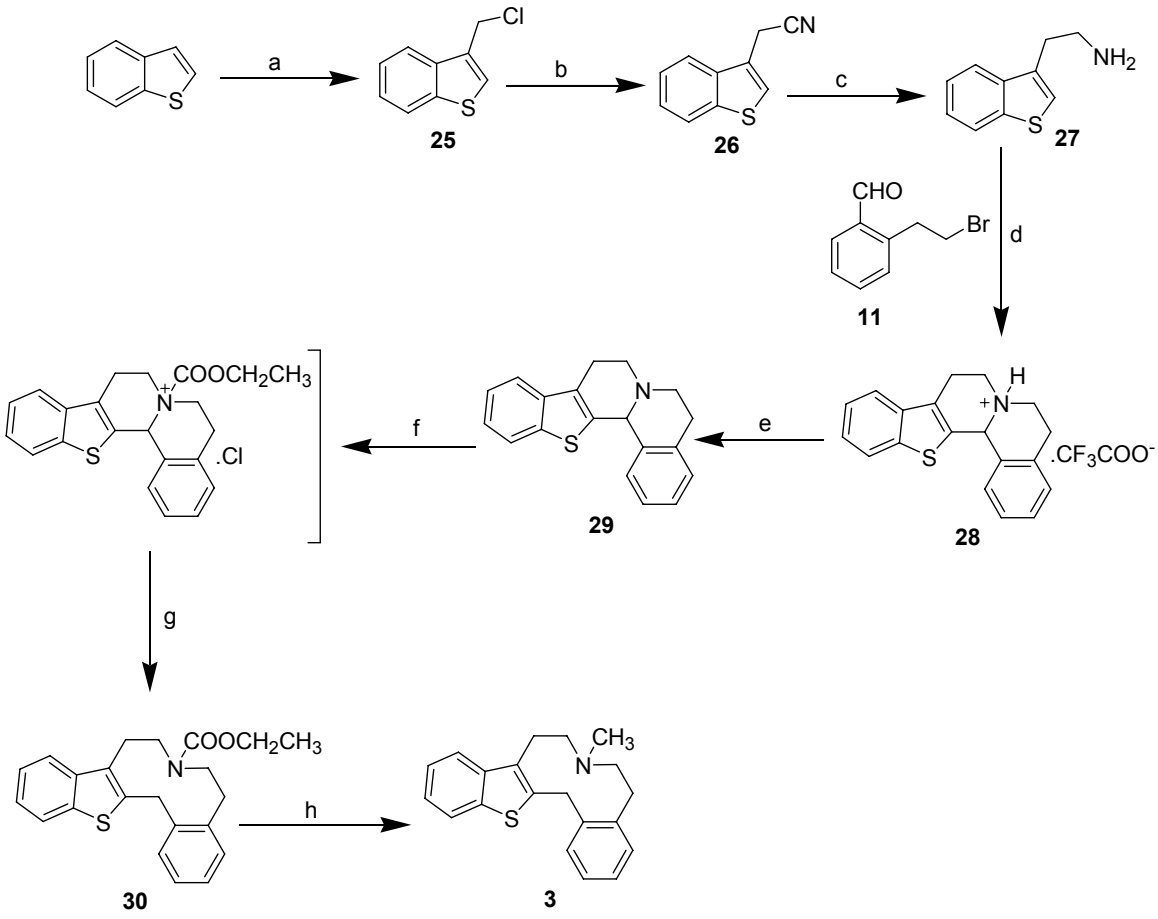
First, the 2-benzo[b]thiophen-3-yl-ethylamine **27** was prepared starting from bezothiophene that was subjected to chloromethylation reaction using HCOH 37% under continuous HCl gas passage to get the 3-choromethylbenzothiophene **25**, which in turn reacted with KCN and TEBA to afford the formation benzothiophene-3-yl-acetonitrile **26** that was converted to the desired amine under reduction conditions using $LiAlH_4$ in dry THF.



Scheme 3: a: $SOCl_2$ b: KCN, TEBA, H_2O c: $LiAlH_4$, dry THF d: 2-(2-bromoethyl) benzaldehyde, dry Dioxane, TFA e: 33% NH_3 f: $SnCl_4$ g: 2-Iodoethanol, acetone h: $NaBH_4$, MeOH i: PPA j: $ClCOOCH_2CH_3$, dry THF k: $NaBH_3CN$, dry THF l: $LiAlH_4$, dry THF

As for the preparation of the benzothienoquinolizine derivative **29**, similar previously prepared analogues were obtained via reacting the respective β-aryl ethyl amine with isochromanone to afford the formation of the corresponding benzamide derivative which in turn was subjected to cyclization under Bieschler-Napieralski conditions to get the desired quinolizine. Here in, we adopted one step preparation for the desired quinolizine utilizing Pictet-Spengler reaction conditions, where the previously prepared 2-benzo[b]thiophen-3-yl-ethylamine **27** was refluxed with 2-(2-bromoethyl) benzaldehyde **11** in presence of TFA in dry dioxane to get the desired quinolizine in its triflouroacetate salt **28**, from which the free base is liberated using 33% NH_3 solution. The resulted quinolizine prepared using this method was formed with a satisfactory yield of 81%.

Again, the ring opening was done through reacting the benzothieno quinolizine with chloroethylformate in dry THF at -80 ^{o}c, using dry ice in methanol for 4 hours to yield the very unstable carbamate chloride salt that was not isolated but to which directly added the milder reducing agent $NaBH_3CN$ to afford the ring opening and obtaining the corresponding carbamate derivative **30** that in turn was reduced with $LiAlH_4$ in dry THF to get the desired benzo[d][1]benzothieno- [2,3-g] azecine target candidate in a good yield [125- 127].



Scheme 4: a: $SOCl_2$, CH_2Cl_2 b: KCN, TEBA, H_2O c: $LiAlH_4$, dry THF d: TFA, dry Dioxane e: 33% NH_3 f:$ClCOOCH_2CH_3$, dry THF g: $NaBH_3CN$, dry THF h: $LiAlH_4$, dry THF.

3.1.2 Synthesis of Phenylpiperazine derivatives

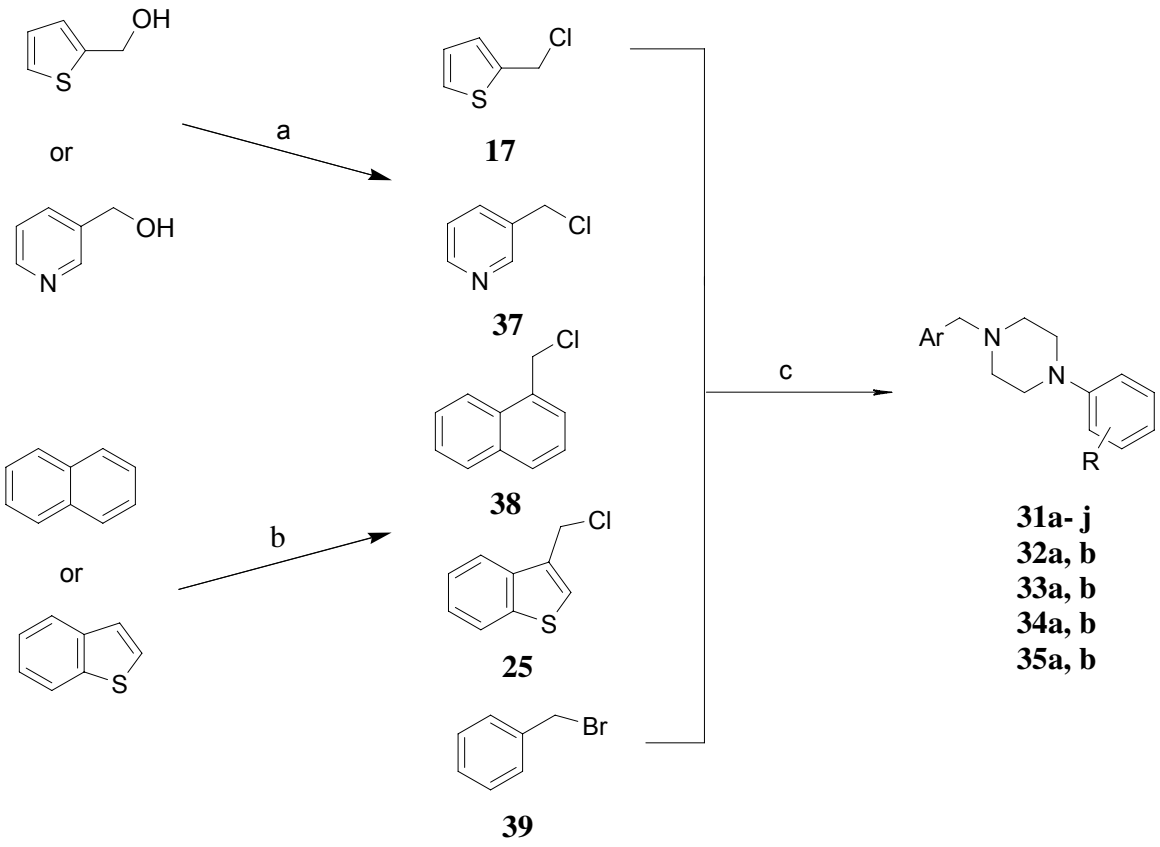
Ligands bearing an aryl moiety separated with an alkyl spacer from a phenyl piperazine nucleus serve as good candidates for binding to the D2- receptors family. In this work, three sets of compounds bearing this skeleton have been synthesized with varying spacers between the aryl group and the phenyl piperazine moiety to manipulate selectivity towards the different subtypes of D2- receptors family.

3.1.2.1 Synthesis of Arylmethylphenylpiperazine derivatives

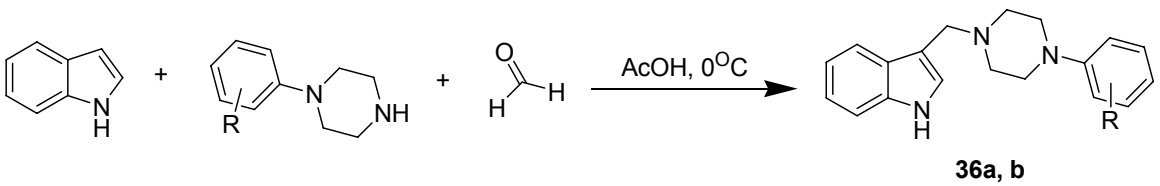
The synthesis of the arylmethylphenylpiperazine derivatives as shown in scheme **5** depended on the reaction between the halomethylarene with the corresponding phenylpierazine derivative in TEA and acetonitrile under inert atmosphere [128].

Except for the commercially available benzylbromide **39** all other halomethylarenes have been prepared by our group. The 1-Chloromethylnaphthalene **38** and 3-Chloromethylbenzothiophene **25** were prepared utilizing chloromethylation reaction between the corresponding arene and HCHO 37% with continuous passage of HCl gas during the whole reaction time. The 2-Chloromethylthiophene **17** and the 3-Chloromethylpyridine **37** building units have been prepared starting from the corresponding arenemethanol through reaction with $SOCl_2$ in CH_2Cl_2.

Similar reaction between Indole-3-methanol and $SOCl_2$ did not afford the corresponding chloromethyl derivative. This may be related to the high reactivity of indole ring, where it could happen that the formed 3-chloromethylindole product affords electrophillic substitution reaction at position 2 of another mole leading to polymerization. Inspired by Mannich synthesis of Gramine [129], the desired indolomethylphenylpiperazine derivatives were prepared as shown in scheme **6** in one step reaction between the indole, the corresponding phenylpiperazine free base, and HCOH 37% in acetic acid at 0^oC for one hour.



Scheme 5: a: $SOCl_2$, CH_2Cl_2 b: 37%HCHO, HCl gas, CH_2Cl_2 c: Corresponding phenylpiperazine, TEA, MeCN

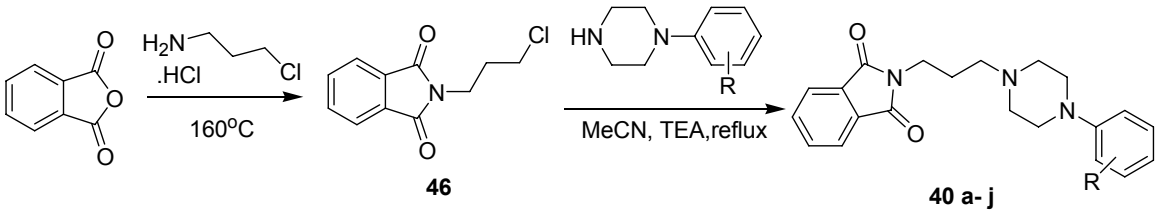


Scheme 6: Synthesis of 3-[4-Phenylpiperazin-1-ylmethyl]-1H-indole derivatives

3.1.2.2 Synthesis of Phenylpiperazinylpropyl/butylisoindole-1,3-dione and Arylpropyl/butylamidophenylpiperazine derivatives

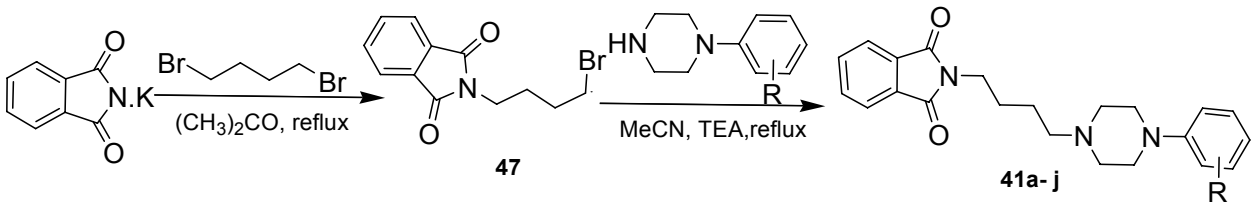
The synthesis of the phenylpiperazinylpropylisoindoledione derivatives as illustrated in scheme **7** has started from a fusion reaction between phethalic anhydride and 3-Chloropropylamine hydrochloride salt to afford the formation of 2-(3-Chloropropyl)-isoindole-1,3-dione **46** which in turn was subjected to a

nucleophilic substitution reaction with the corresponding phenylpiperazine to obtain the desired derivatives in a suitable yield [130, 131].



Scheme 7: Synthesis of Phenylpiperazinylpropylisoindole1,3dione derivatives

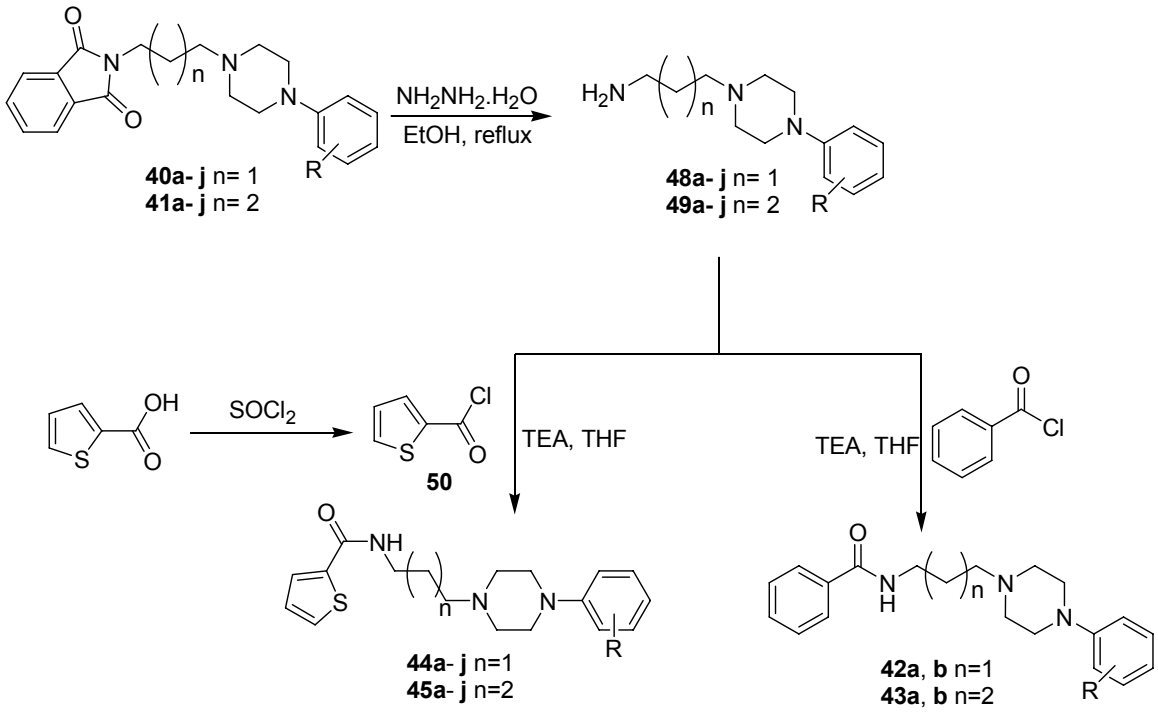
Due to the non availability of the 4-Chlorobutylamine hydrochloride salt, the synthesis of the phenylpiperazinylbutylisoindoledione derivatives was done adopting Gabriel synthesis [132] in which the phethalimide potassium salt was N-alkylated with 1,4-dibromobutane to give 2-(4-Bromobutyl)isoindole-1,3-dione **47** which was again subjected to a nucleophilic substitution reaction with the corresponding phenylpiperazine to yield the desired candidates, scheme **8**.



Scheme 8: Synthesis of Phenylpiperazinylbutylisoindole1,3dione derivatives

The synthesis of the benzamides and thienoamides as depicted in scheme **9** was carried out starting from the corresponding previously synthesized phenylpiperazinylalkylisoindoledione derivatives, where they were subjected to Ing-Mansk reaction that involves refluxing with aqueous hydrazine in 95% ethanol to afford the corresponding primary amine which in turn was reacted with the corresponding acylchloride in presence of TEA to afford the formation

of the desired amide derivatives [133]. Figure **22** illustrates the mechanisms of the Gabriel and Ing-Mansk reactions.



Scheme 9: Synthesis of benzamide and thienoamide derivatives

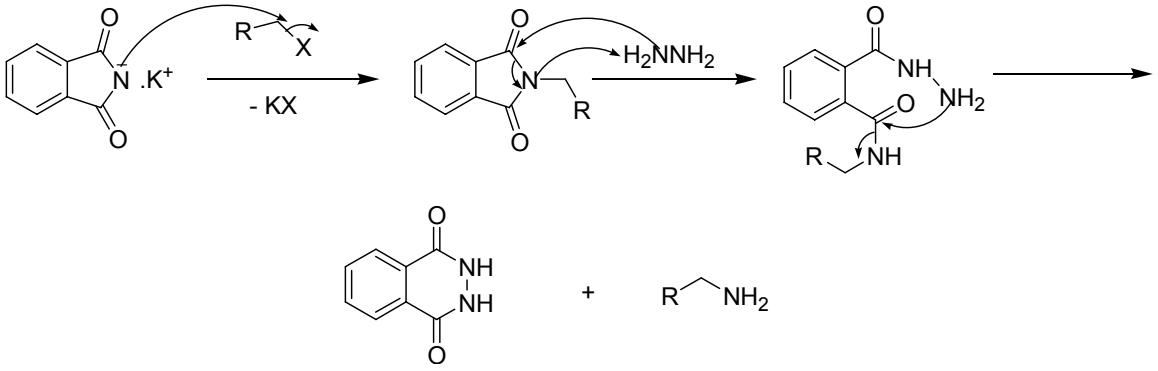


Figure 22: Mechanism of Gabriel and Ing-Mansk reactions for the synthesis of primary amines

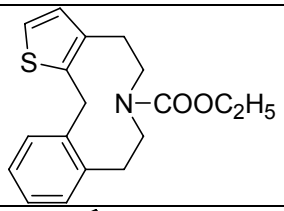
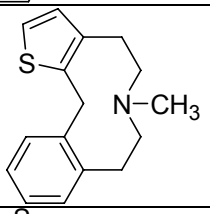
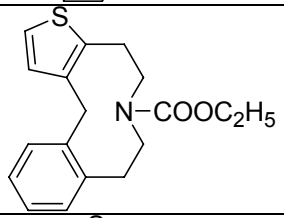
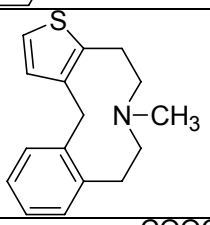
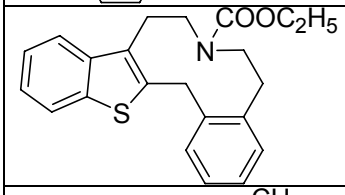
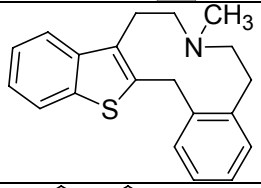
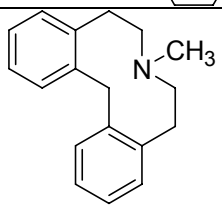
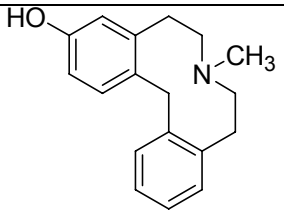
3.2 Pharmacology

The target prepared compounds were screened for binding affinity towards human stably cloned dopamine receptor subtypes D1, D2, D3, D4, and D5 utilizing radioligand binding studies according to our developed protocol [66]. [^{3}H]SCH23390 was used as radioligand for the D1-like family, and [^{3}H]Spiperone for the D2-like family. Incubations at 27^{o}C were terminated after 90 minutes by rapid filtration with a Perkin-Elmer Mach III harvester. At least two independent experiments, each in triplicate, were carried out.

3.2.1 Binding affinity data of Thieno and Benzothieno azecine derivatives

The target azecines including their carbamate precursors were screened for binding affinity towards the 5 dopamine receptors subtypes. Ki values for our azecine derivatives **1**, **2**, **3** and their carbamate precursors are listed in table **9** and compared with clozapine, olanzapine and asenapine. The respective Ki values are taken from the PDSP database and are also from human cloned receptors.

The rational on which the thiophene scaffold was selected is based on the comparison of clozapine and its thiophene congener olanzapine, which has higher affinities for D1, D2 and D5 receptors than clozapine. For this couple not only the affinities are increased by the bioisosteric replacement of benzene to thiophene, but also the selectivity profile changed: Clozapine has a slightly higher affinity for D1 (266 nM) and D5 (255 nM) than for the D2 receptor (343 nM), whereas olanzapine has a higher affinity for D2 (53 nM) than for D1 and D5 (70 and 82 nM).

Compound	D1	D2	D3	D4	D5
	>1000	>1000	>1000	>1000	>1000
	60 ±4.2	45.9 ±2.7	24 ±1.5	188 ± 17	3.1 ± 1.7
	>1000	>1000	>1000	>1000	>1000
	4.1± 0.4	190 ±2.7	87 ± 6	99± 21	15± 3.2
	>1000	>1000	>1000	>1000	>1000
	40 ± 1.5	1.5 ±0.02	18 ± 2	72 ± 7	1.9 ± 0.5
 LE410	4.5	56.5	52	148	11.2
 LE405	0.4	44.5	47.5	11.3	1.5

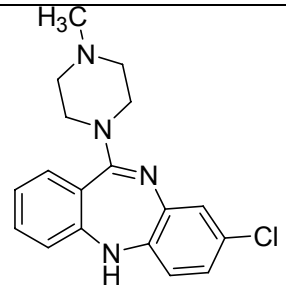
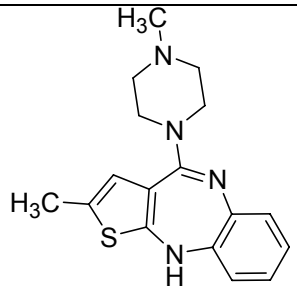
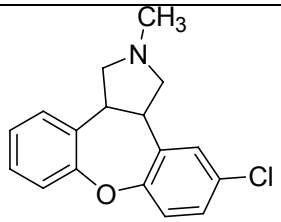
 Clozapine	260	343	---	---	255
 Olanzapine	70	53	---	---	82
 Asenapine	2.9	1.4	---	---	22.7

Table 9: Binding affinity data of compounds 1, 2, 3, and their carbamate precursors to human cloned dopamine receptors subtypes compared to Clozapine, Olanzapine, and Asenapine
At least two independent experiments were carried out in triplicate each.

Selent *et al*. thoroughly analyzed the binding mechanisms of both compounds for 14 different homology models of GPCRs including various serotonin receptors, some dopamine receptors, but not the D1 and the D5 receptor [(134)]. In their models, the binding cavity is lined by the following amino acids positions: 3.26, 3.28, 3.29, 3.32, 3.33, 3.36, 3.37, 3.40, 5.38, 5.39, 5.42, 5.43, 5.46, 5.47, 6.44, 6.48, 6.51, 6.52, 6.55, 7.35, 7.39 and 7.43. We now extended their observation to the dopamine D1 family. The amino acids that surround the docked ligand in < 4.5 Å proximity are compared for the receptors that are the focus of our interest, Figure **23**.

	3.26	3.28	3.29	3.32	3.33	3.36	3.37	3.40	5.38	5.39	5.42	5.43	5.46	5.47	6.44	6.48	6.51	6.52	6.55	7.35	7.39	7.43
D1:	N	W	V	D	V	S	T	I	Y	A	S	S	S	F	F	W	F	F	N	F	V	W
D5:	D	W	V	D	V	S	T	I	Y	A	S	S	S	F	F	W	F	F	N	F	V	W
D2:	D	F	V	D	V	C	T	I	F	V	S	S	S	F	F	W	F	F	H	Y	T	Y

Figure 23: AlignMent of amino acid positions that are found in < 4.5 Å proximity to clozapine or olanzapine docked into 14 different GPCRs [134].Amino acids that are different in either receptor are highlighted in red and could be responsible for a certain selectivity profile

This alignMent again shows the very high sequence identity between the D1 and the D5 receptor. The only difference is position 3.26 with an asparagine at the D1 and an aspartate at the D5. Asparagine can act as an H-bond donor and acceptor whereas aspartate can only accept H bonds.

There are 5 of 20 amino acids that make up a difference between the D1 family and the D2 receptor and it was hypothesized that the SH/N motif (S 5.43 and H or N at 6.55) forms additional polar interactions with the sulfur of the thiophene moiety, which in turn explains the higher affinity of olanzapine. Comparing our two thienoazecine regioisomers to olanzapine, in the case of compound **1**, the sulfur is in a similar position. Surprisingly compound **2**, in which the sulfur occupies a different position than in olanzapine, shows for the D1 distinctively higher affinities than the positional isomer where the thiophene has the same orientation like in olanzapine. This indicates a different binding mode.

Regarding the molecular structures of the D1-like receptors, D1 and D5, neither homology models nor molecular docking in-silico data are so far available for the target proteins but Surgand *et al* have reported the most important amino acid residues involved in the binding pockets of the target receptors using alignMent studies relying on human beta-2 adrenergic

receptor as a template. According to this study, the most important amino acid residues thought to constitute the binding cavities of the respective receptors were residues 1.35, 1.39, 1.42, 2.58, 3.32, 4.56, 4.60, 5.38, 5.42, 5.43, 5.46, 6.44, 6.48, 6.51, 6.52, 7.35, 7.39, 7.43, 7.45 [135]. For us to study the rare different amino acid residues in and near by the suggested binding sites of D1 and D5, we aligned the reported D1 and D5 sequences according to Ballesteros and Weinreb numbering and via the aid of the sequence of the recently co-crystallized human D3 receptors we were able to define the beginning and the ending of D1 and D5 helices [134, 136].

As expected, very few amino acids were found to be different between D1 and D5 in and surrounding the binding cavities, namely Phenylalanine 2.69 in D1 faces Tyrosine in D5, Proline 4.39 in D1 faces Glutamine in D5, Lysine 5.67 in D1 faces Valine in D5, Aspartic 7.31 faces Serine in D5, and finally Aspergine 7.33 faces Therionine in D5. In terms of the chemical nature of these different amino acids, it is noticeable that Tyrosine, Glutamine, Serine, and Therionine residues in D5 could serve as hydrogen bond acceptors from ligands owning electron donating moieties contrary to their counterparts in the D1 binding site. Also Valine residue 5.67 in D5 would afford better hydrophobic interaction with the binding ligand rather than its Lysine counterpart. Based on these finding we suggested that ligands bearing thiophene scaffold might serve as a D1 D5 distinguishing aid with enhanced preferentiality to D5 subtype receptors, where the electron pair of the thiophene sulfur would role as the donor moiety. The use of the thiophene ring was also suggested in order to generally make the best use of the hydrophobic region of the studied binding pockets, where the large atomic polarizability of the sulfur atom and

the electron rich thiophene system would provide higher dispersion forces compared to benzene which may lead to better π - π stacking and/or van der waals interaction with the hydrophobic residues lining the hydrophobic pocket of the target receptors.

Examining the binding affinity data of the test compounds versus the leads we observed that compound **1** showed to have the best selectivity towards the D5 receptor subtype with D5/D1 selectivity index of 0.3 versus 2.5 for LE 410 and 3.75 for LE 405 and D1/D5 of 3.1 versus 0.4 for LE 410 and 0.26 for LE 405. Shifting the position of the sulfur atom of the thiophene ring in the regioisomer **2** has completely reversed the selectivity pattern, where it showed the best selectivity to D1 receptors subtype with D1/D5 selectivity index of 0.3 versus 0.4 for LE 410 and 0.26 for LE 405 and D5/D1 selectivity index of 3.3 versus 2.5 for LE 410 and 3.75 for LE 405 revealing the importance of the role and the position of the thiophene sulfur atom for binding D5 receptors subtype.

It is also noticeable that generally, the developed compounds **1**, and **2** exhibited lower affinity to the target receptors pointing out the importance of the molar volume of the ring adjacent to the azecine scaffold, so we suggested that using other aromatic systems with similar electronic effect but larger molar volume would serve best in this case. Accordingly, we have synthesized the benzothienobenzoazecine derivative **3**.

Interestingly, examining the binding affinity data of the benzothienobenzoazecine derivative **3** versus compound **1** could show a comparable selectivity profile towards the D5 receptor subtype over D1 receptors with D5/D1 Selectivity Index of 0.05 showing considerable higher selectivity towards D5 over D1 and confirming the importance of sulfur atom

position and the value of enlarging the size of the aromatic ring at this area of the compound scaffold.

As for the affinity of the test compounds to the D2-like receptors, only compounds **1**, **3** with the regular thiophene positioning were those that showed better affinity to D2 and D3 receptor subtypes when compared to the lead compounds LE 410 and LE 405. Compound **1** showed Ki values of 45.6 and 24 nM for D2 and D3 respectively versus 60 nM on D1 receptor subtype. The most interesting compound **3**, was found to be much more selective to D2 and D3 receptors with Ki values of 1.5 and 18 nM respectively versus 40 nM on D1 receptor subtype showing to be the first azecine derivative with this reversed selectivity pattern and thus considered to be the first azecine derivative that is able to serve as prominent antipsychotic agent. Again this could be rationalized based on the molecular structure of the recently co-crystallized human D3 receptors where it was approved that Serine residues, Ser 192, Ser 193, and Ser 196 serve as hydrogen bond acceptors in the D3 binding pockets [(102)]. Also the developed homology model by Kalani *et al* for the D2 receptors subtype with the antagonist clozapine showed that Serine residues, Ser 193 and Ser 197 contribute similarly to the interaction between ligand and the target protein suggesting a similar binding fashion of our thieno and benzothienobenzoazecine derivatives **1, 3** [(51)]. Reversing the position of the sulfur atom in compound **2** again reversed the affinity profile on the D2-like members with more or less similar binding affinity data to the lead compounds.

As for the carbamate precursors, all showed very poor affinity towards all receptor subtypes revealing the importance of specific steric and hydrophobic features at this part of the structure.

3.2.2 Binding affinity data of Arylmethylphenyl-piperazine derivatives

Examining the binding affinity data shown in table **10** the ten thienylmethyl phenylpiperazine ligands belonging to this series **31a**- **31j** showed high to moderate affinity towards D4 receptor subtype with Ki values ranging from 3.9 to 214 nM and selectivity towards D4 subtypes over D2 and D3 with selectivity indices as high for certain candidate **31f** as >1351 making it one of the most selective D4 ligands reported so far.

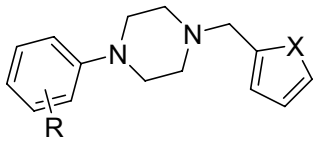
Regarding the length of the spacer linking the arene moiety to the phenylpiperazine one, it was meant to be designed as methylene linker as ligands bearing such a feature are highly selective to D4 receptor subtypes over D2 and D3 ones due to the fact that these short ligands bearing only one carbon as a spacer between the aryl and the phenylpiperazine moieties can not stretch that far into the hydrophobic pocket at the transmembrane helix 2 TM2/TM3 interface [81].

Herein, the effects of two major factors on the affinity and selectivity towards D4 receptor subtypes can be possibly studied, namely the nature of the arene moiety in terms of size and electrostatic potential, the nature and the position of the substituent on the phenylpiperazine scaffold.

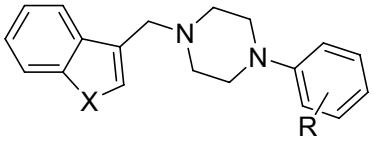
Starting with the effect of the nature of the substituent of the phenylpiperazine scaffold on the affinity, it was found that ligands bearing ortho oxygenated substituents have shown better affinity towards D4 receptors rather than those

bearing halogenated substituents at this area of the structure. Compound **31e** having 2-OEt showed the best D4 binding affinity among this series followed by **5f** (2-OH), **5d** (2,3-Cl), **5c** (2-Cl), **5b** (2-F) with Ki values of 3.9, 7.4, 49.9, 52, 57 nM respectively. This affinity pattern of the newly synthesized ligands may be rationalized in view of the results of recent mutagenesis studies that have confirmed that this part of the compound structure is supposed to interact with His 6.55 residue in the binding pocket of D4 receptors and accordingly the nature of the substituent at this area could affect such an interaction as shown by the binding data of our synthesized ligands, where the electron donating oxygenated substituent would ensure an electron rich aromatic system that is able to provide higher dispersion forces compared to that provided with a system bearing the electron withdrawing halogenated substituents thus leading to better Van der Waals interaction with the imidazole ring of His 6.55 [137, 138].

Moreover, the substituent on the phenylpiperazine scaffold can influence the pKa of the basic piperazine nitrogen and hence affecting the key salt bridge interaction with Asp 3.32 residue, an interaction reported to be afforded by all aminergic GPCRs and their corresponding ligands [81, 94, 97, 102]. Comparing the pKa values of the candidates bearing oxygenated phenylpiperazine scaffold versus those having the halogenated substituted scaffold would show that these pKa values followed the order 31f~31e>31b>31c>31d (7.74, 7.73, 7.47, 7.24, and 7.13 respectively) explaining why compounds with ortho oxygenated substituents on the phenylpiperazine exhibited higher affinity to D4 receptors than those with halogenated phenylpiperazine scaffold.



Formula 1



Formula 2

Compound	Structure			Ki-values [nM] Average ± SEM				
	Formula	X	R	D1	D2	D3	D4	D5
31a	1	S	H	>10000	>10000	1648 ± 126	65 ± 13	>10000
31b	1	S	2-F	>10000	>10000	1899± 97	57 ± 15	>10000
31c	1	S	2-Cl	>10000	2179 ± 569	427 ± 14	52 ± 13.5	>10000
31d	1	S	2,3-Cl	>10000	1545 ± 75	44.5 ± 0.5	49.9 ± 5.9	1743
31e	1	S	2-OEt	>10000	162 ± 42	112 ± 13	3.9± 0.3	>10000
31f	1	S	2-OH	>10000	>10000	>10000	7.4 ± 1.8	>10000
31g	1	S	4-F	>10000	>10000	>10000	105 ± 10	2059 ± 484
31h	1	S	4-Cl	>10000	>10000	>10000	214 ± 4.5	3523
31i	1	S	3,4-Cl	2541	1593 ± 123	62 ± 4.1	78 ± 4.6	1534
31j	1	S	4-OH	>10000	2167 ± 58	221± 31	152 ± 12.2	>10000
32a	1	HC=N	2-OEt	>10000	255 ± 2	42 ± 1	0.7 ± 0.1	>10000
32b	1	HC=N	2,3-Cl	>10000	327± 6.5	41 ± 2.5	4.4 ± 0.8	>10000
33a	2	HC=CH	2-OEt	>10000	264 ± 6	59 ± 1	2 ± 0.7	>10000
33b	2	HC=CH	2,3-Cl	>10000	351 ± 4	92 ± 3	31 ± 5	>10000
34a	2	S	2-OEt	>10000	219 ± 6	45.1 ± 2	4.5 ± 0.2	>10000
34b	2	S	2,3-Cl	>10000	383 ± 25	52.5 ± 5	22.5 ± 1	>10000
35a	1	HC=CH	2-OEt	>10000	217 ± 5	42.7 ± 2	3 ± 1	>10000
35b	1	HC=CH	2,3-Cl	>10000	242 ± 5.5	41± 0.5	11 ± 0.5	>10000
36a	2	NH	2-OEt	>10000	473 ± 18	103 ± 12	0.03 ± 0	>10000
36b	2	NH	2,3-Cl	>10000	502 ± 13	123 ± 7	6.2 ± 0.1	>10000
	FAUC 113			--------	3200 ± 58	5000 ± 121	3.1± 0.3	-------

Table 10: Binding affinity data of Arylmethylphenylpiperazine derivatives to cloned human dopamine receptor subtypes.
At least two independent experiments were carried out in triplicate each.

Regarding the position of the substituent of the phenylpiperazine scaffold on the affinity, compounds bearing an ortho positioned substituent on the phenyl-piperazine scaffold have exhibited much better affinity to D4 than their para substituted congeners e.g. **31c** versus **31h** and **31f** versus **31g**. This ortho substituent would affect the degree of non co-planarity between the phenyl and the piperazine rings, a feature that seems crucial for the affinity aspects to the target receptor. This is obvious when comparing the dihedral angles of compounds **31c** and **31f** to their para substituted analogues **31h** and **31j** respectively; a dihedral angle of about 60^{o} seems optimal for activity. This is confirmed by that the smaller in size fluorine atom in the ortho position of compound **31b** did not greatly affect the dihedral angle and hence activity has not been reduced so much in its para analogue **31g**, Figure **24**.

From receptor subtype selectivity perspective, compound **31f** has shown to be highly selective towards D4 receptors over D2 with selectivity index D2/D4 of > 1351. Compounds **31a** and **31b** showed to be more than 100 folds more selective to D4 receptors over D2 with D2/D4 of 152 and 175 respectively, while other derivatives showed also appreciable selectivity with D2/D4 values ranging from 14 to 95.

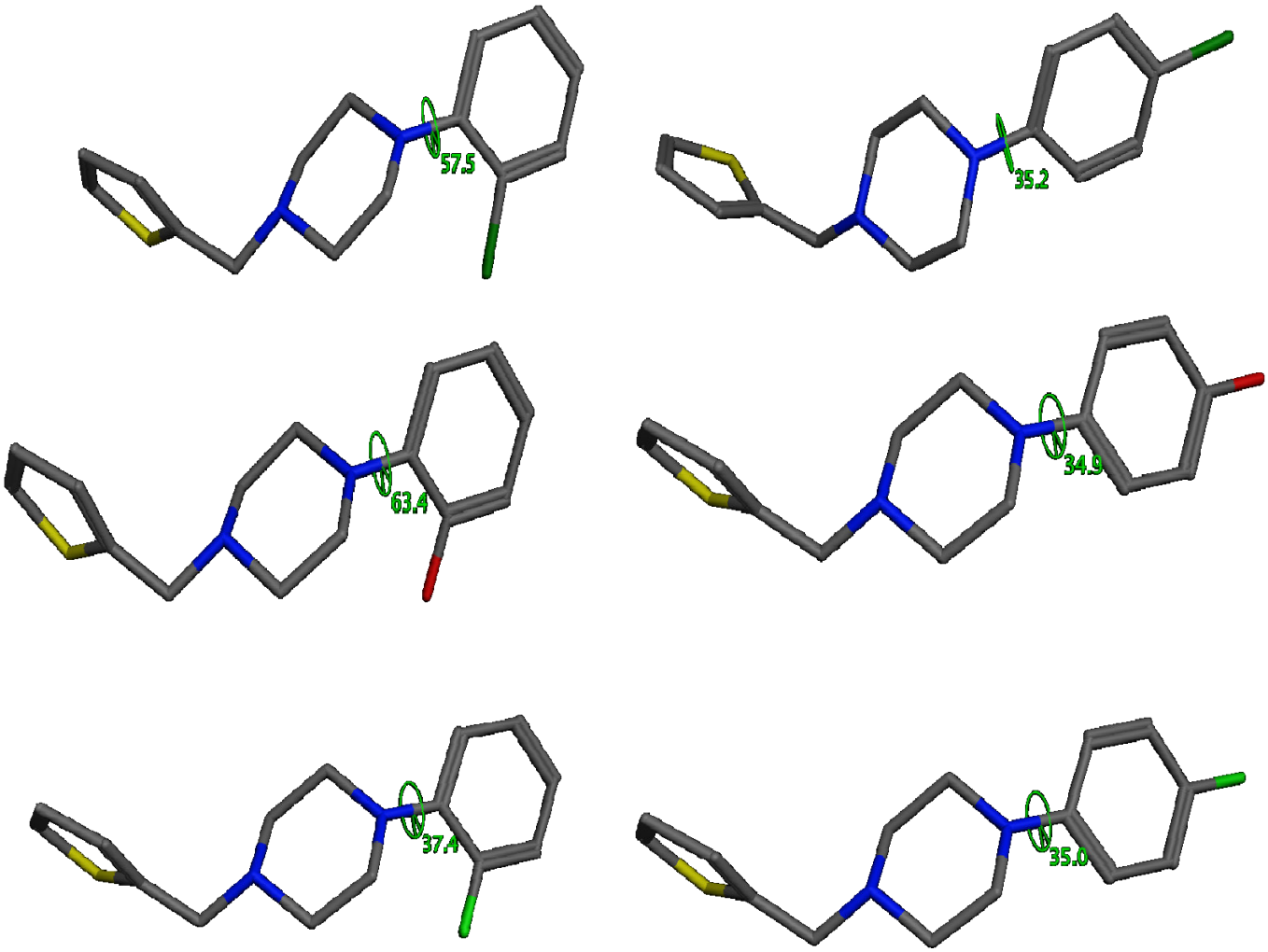


Figure 24: Dihedral angles of compounds 31c (top left), 31h (top right), 31f (middle left), 31j (middle right), 31b (bottom left), and 31g (bottom right)

As for D4 over D3 selectivity, Compound **31f** again showed to be >1351 folds more selective to D4 over D3. Compounds **31a**, **31b**, **31e**, **31g**, **31h** also showed appreciable selectivity towards D4 with D3/D4 values ranging from 25 to 95, while compounds **31c**, **31j** showed lower degree of selectivity to D4 receptor subtype with D3/D4 values of 8 and 1.5 respectively. The most interesting compounds **31d** and **31i** bearing a dichlorinatedphenylpiperazine skeleton have shown to be with better affinity on D3 rather than D4 with Ki values of 44.5, 62.3 nM, respectively on D3 versus 49.9, and 78 nM

respectively on D4 receptors showing to be the first ligands of this class to violate the common selectivity pattern and pointing out that the nature of the substituents on the phenylpiperazine scaffold may control also ligand subtype selectivity.

In an attempt to rationalize the selectivity pattern of the synthesized compounds among this series towards D4 receptor, we have docked all the synthesized compounds to the D4 homology model developed before [139] and also to the recently co-crystallized D3 receptor model with the antagonist eticlopride (PDB ID: 3PBL). The docking poses showed ionic salt bridge interaction with Asp 3.32 in D3 (Asp 110) and D4 (Asp 115), indicating the importance of the basicity of this piperazine nitrogen upon affinity to D4 receptors.

Figure **25** shows the docking poses of the highest affinitive D4 ligand from each series to the D4 receptor model. Figure **26** shows the 3D structures of the highest affinitive D4 ligands from each series relayed over each other while docked into the binding pocket of D4 receptor model. Figure **27** illustrates the docking of compounds **31d** and **31i** that showed better D3 affinity rather than D4 to D3 receptor model.

Viewing the binding pockets of the D3 and D4 models could show that amino acid residues 2.61 and 3.28 are resembling Valine 86 and Phenylalanine 106 in D3 while Phenylalanine 91 and Leucine 111 in D4 respectively and thus might manipulate the ligands' selectivity towards either subtype and come in accordance with the results of the previous mutagenesis studies [81].

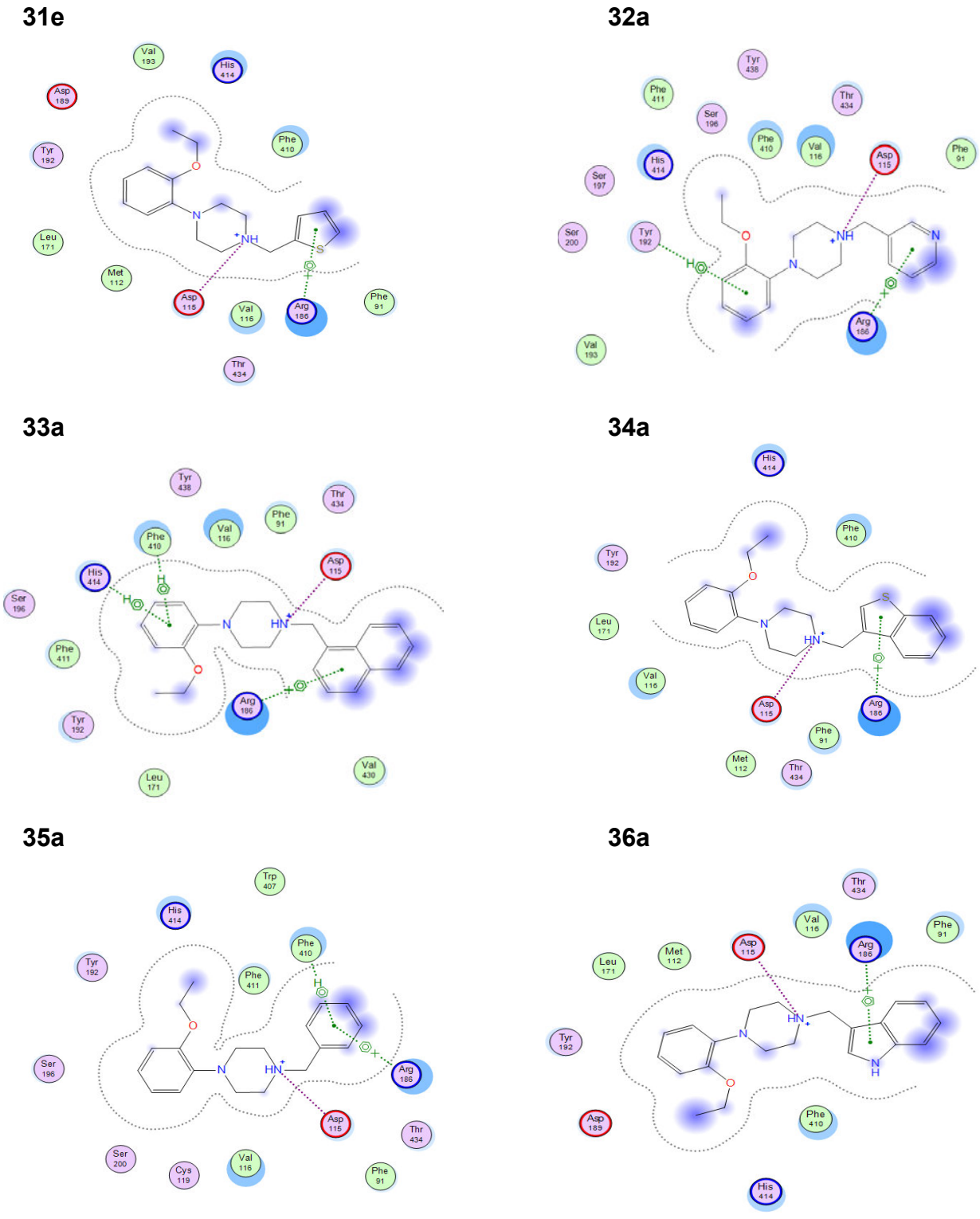


Figure 25: 2D interactions of the highest affinitive D4 compound from each series docked to human D4 model showing arene cation interaction between the ligands' arene moiety and the unique D4 residue Arg 186. Tyr 192 is in contact to the phenylpiperazine unit of the ligands

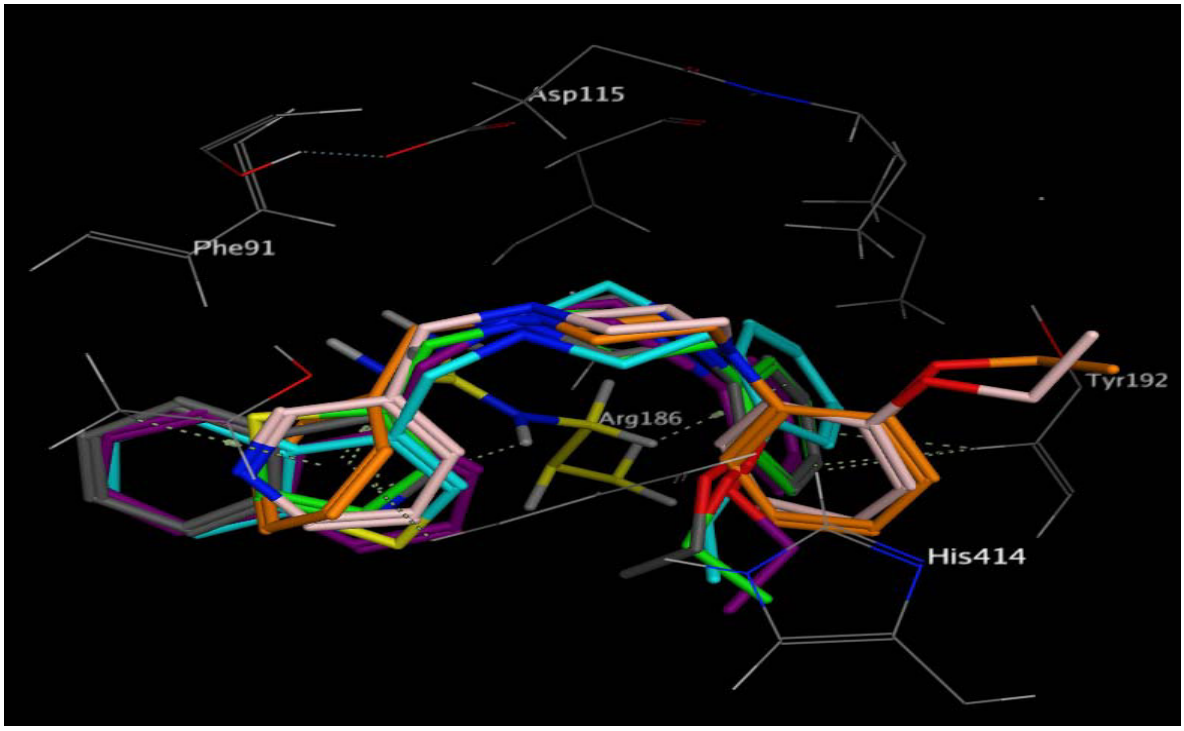


Figure 26: 3D structures of the highest affinitive D4 compound from each series over relayed each other in the binding pocket of D4 receptor model

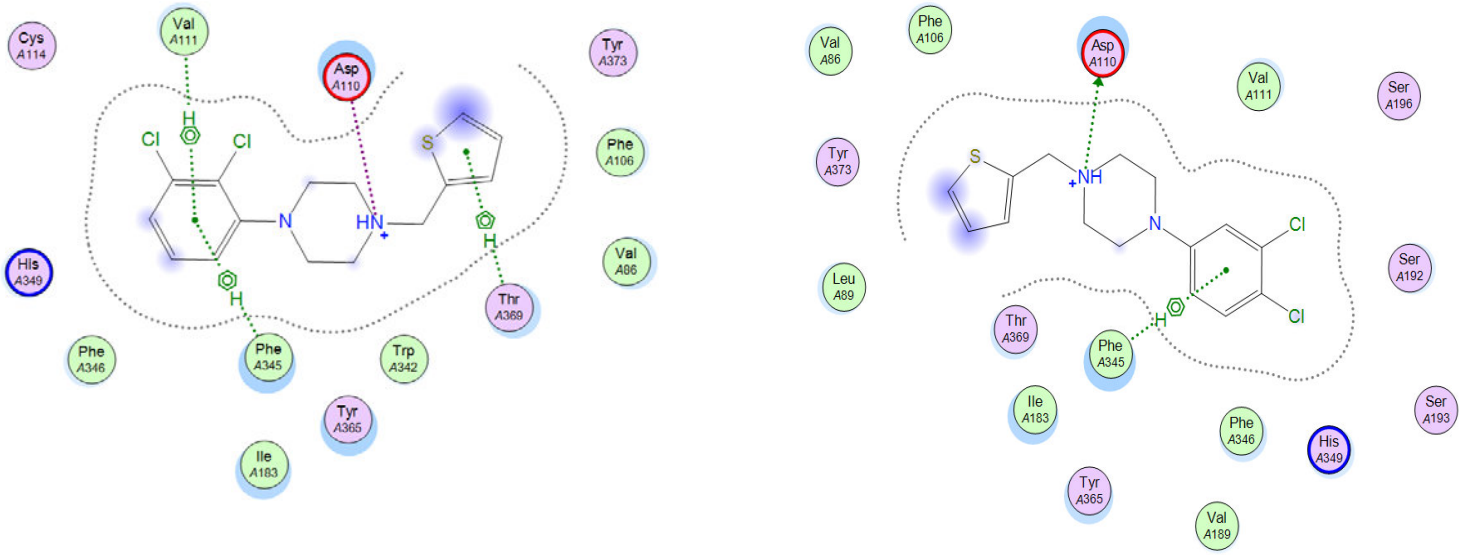


Figure 27: 2D interactions of compounds 5d (left) and 5i (right) docked to D3 binding pocket. Amino acid residue Val 86 is conserved in the binding pocket

Furthermore, we have observed that two other amino acid residues contributing to the binding pocket of the receptor subtypes were found to be different among them. The first one is the EL2 residue Arginine 186 in D4 that faces Serine 182 in D3 receptor subtypes. The second one is the residue 5.38 that resembles Phenylalanine 188 in D3 subtype, while faces Tyrosine 192 in D4 receptors. Examining the docking poses of D4 receptor model, Figure **25** could show that the unique D4 Tyrosine 192 is facing the phenylpiperazine unit of the docked ligands. The chemical nature of this amino acid enables it to afford hydrogen bond interaction with the 2-OH group of compound **31f** providing a possible explanation for its superior selectivity towards D4 receptor subtypes. It is also important to note that this selectivity was dramatically reduced when shifting the hydroxyl function to the para position as in compound **31j**.

Comparing the selectivity data of compound **31f** to that of **31b**, **31c** and **31e** would reveal decrease in selectivity towards the D4 receptors over the other two subtypes. This might emphasis the contribution of the Tyrosine 192 residue in controlling the subtype receptor selectivity of the synthesized candidates, where this specific residue faces Phenylalanine 188 in D3 and Phenylalanine 189 in D2 subtypes. These phenylalanine residues in D2 and D3 receptors would resemble a sort of incompatibility with the 2-OH group of compound **31f** while afford better hydrophobic interaction with the more lipophillic 2-F, 2-Cl and the 2-OEt groups of compounds **31b**, **31c**, and **31e** respectively explaining the better affinity of these compounds to the other two receptor subtypes relative to compound **31f** itself.

Viewing the docking poses could also show that the Phenylalanine 91 residue is conserved in the binding pocket of D4 receptor and faces the also conserved and the less bulky Valine 86 in D3 binding pocket. This may provide a possible explanation why the relatively more bulky dihalogenated bearing compounds **31d** and **31i** have shown the best affinity towards D3 (with a relative larger pocket than D4 subtypes) among the thienylmethyl-phenylpiperazine series.

Moving to the effect of the heteroarene moiety on the binding affinity and selectivity to D4 receptors, we have replaced the thiophene ring of compound **31e** that showed the best affinity to the target protein among this series and compound **31d** that showed better affinity to D3 rather than D4 receptor subtypes with benzene, benzothiophene, naphthalene, pyridine, and indole, so that factors like ring size and ring electron density could be tested and thus picturing the second round of our optimization plan.

Relative to **31e**, compounds **34a** and **35a** having benzothiophene and benzene had Ki values of 4.5 and 3 nM respectively that is almost similar to that of **31e** (3.9 nM). Compound **33a** with naphthalene ring showed little better affinity with Ki value of 2 nM. The binding affinity of compound **31e** have been very much developed using pyridine ring in compound **32a** that showed Ki value of 0.7 nM. Compound **36a** having the indole ring as the heteroarene moiety was much more superior with Ki as low as 0.03 nM showing to be about 100 folds more affinitive to D4 than compounds **31e** and the reference compound, FAUC 113. These findings could support the assumption that a specific negative electrostatic potential of the ring at this area of the compound structure does really matter rather than ring size, where the best

D4 affinity was obtained by the compounds bearing arenes with the highest negative electrostatic potential, namely compounds **32a** and **36a** having pyridine and indole arenes respectively.

Interestingly all the docked compounds to D4 receptors model have afforded a first to report arene-cation interaction through their arene moiety with the unique Arginine 186 in EL2 of the binding pocket of the D4 receptors, Figure 25. Such an interaction could be strengthen via increasing the negative electrostatic potential at this area of the compound skeleton explaining the high affinity and selectivity of compounds **32a** and **36a** towards D4 subtypes.

Neither one of the **31d** five analogues showed to bind preferentially to D3 over D4 like compound **31d** itself; however they showed considerably good affinity to D3 with Ki values ranging from 41 to 123 nM.

3.2.3 Binding affinity data of Phenylpiperazinylalkyl-isoindoledione and Arylmethylphenylpiperazine derivatives

Viewing the Ki affinity binding data illustrated in table **11** could show that our designed probes lack the affinity towards D1-Like family receptor subtypes while exhibiting appreciable binding affinity towards D2-like family receptors showing a diverse of affinity and selectivity patterns.

Starting with the isoindole-1,3-dione derivatives, the propyl derivatives **40a** and **40b** have shown lower affinity to the D2-like receptors and also fair selectivity to either D3 or D4 over D2 receptors relative to their longer butyl analogues **41a** and **41b**.

In the first round of optimizing affinity and selectivity of our probes, we decided to decrease the ring size and go for an open chain amide rather than the lactam system while keeping the linker as either propyl or butyl.

Accordingly, we have prepared the benzamide derivatives **42a**, **42b**, **43a**, **43b**.

Compounds **42a** and **42b** have shown increase in affinity to D2 and D4 receptor subtypes, while a very slight improvement in affinity to D3 receptors when compared to compounds **40a** and **40b**. While the benzamidobutyl-phenylpiperazine counterparts, **43a** and **43b**, have shown grater improvement in affinity to all the D2-like members with preferentiality towards D3 and D4 subtypes.

In a second round of optimization we went for the thienoamide skeleton instead of the benzamide one, compounds **44a**, **44b**, **45a**, **45b**. As already mentioned before, theiophene has smaller molar volume than benzene (molar volume of thiophene is 79.6 versus 94.3 for benzene, calculated using MOE [114] volume descriptor) giving the candidates the privilege of better CNS penetrability through the densely packed cells of brain and blood brain barrier.

Relative to the benzamide derivatives **42a** and **42b**, the binding affinity data of the thienoamidopropylphenylpiperazine analogues **44a** and **44b** have shown slight improvement in affinity to all the D2-like receptor family members.

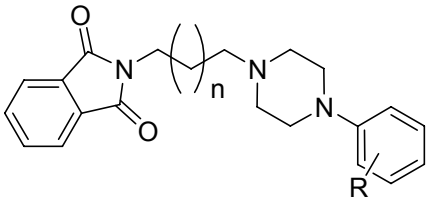
The butyl linker bearing analogues **45a** and **45b** have also shown slight improvement in affinity to the target receptors and greater selectivity to D3 and D4 over D2 receptor subtypes when compared to their benzamide counterparts **43a** and **43b**.

We then decided to investigate the effect of changing the nature and the position of the substituent placed at the phenylpiperazine unit on the affinity and selectivity of the thienoamide derivatives, so we have synthesized

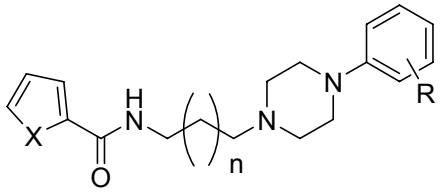
candidates **44c** - **44j** and **45c** - **45j**. The binding affinity data of these candidates is listed in table **11**.

From the obtained binding data we could discuss the effect of three major factors on the affinity and selectivity pattern of our synthesized probes.

Starting with the length of the spacer separating between the aromatic appendage and the phenylpiperazine unit, it is obvious that compounds bearing the butyl linker among the whole series were having better binding data to the D2-like receptors than their propyl analogues. In terms of affinity, the length of the spacer could have a prominent effect on the pKa and hence the ionization of the basic nitrogen of the phenylpiperazine unit that is reported to be involved in a key salt bridge interaction with Asp 3.32 of the target receptors [81, 135, 102]. From the calculated pKa values of this nitrogen atom in the synthesized ligands we could notice that this value ranges from 6.8 to 7.8 in the compounds with the propyl linker while ranges from 7.3 to 8.3 in their analogues with the butyl one. This could point out a better ionization and better ionic interaction with Asp 3.32 among the butyl derivatives and hence explain their better binding to the target receptors.



Formula I



Formula II

Cpd No.	Structure				Ki± SEM [nM]				
	Formula	n	X	R	D1	D2	D3	D4	D5
40a	I	1	-	2-OEt	> 10000	1095± 28	810± 4	118± 1	> 10000
40b	I	1	-	2,3-Cl	> 10000	1393± 40	806±12	124± 10	> 10000
41a	I	2	-	2-OEt	> 10000	770± 4	39± 0.5	10.7± 0.2	> 10000
41b	I	2	-	2,3-Cl	> 10000	793± 2	40± 1.5	11± 2	> 10000
42a	II	1	HC=CH	2-OEt	> 10000	185±9	797± 16	0.75± 0.02	> 10000
42b	II	1	HC=CH	2,3-Cl	> 10000	273± 24	780± 20	7.3± 0.1	> 10000
43a	II	2	HC=CH	2-OEt	> 10000	29.5± 0.7	0.93± 0.01	0.64± 0.02	> 10000
43b	II	2	HC=CH	2,3-Cl	> 10000	34± 1	1.5± 0.2	0.66± 0.1	> 10000
44a	II	1	S	2-OEt	> 10000	138± 6	765± 13	0.62± 0.04	> 10000
44b	II	1	S	2,3-Cl	> 10000	147± 4	754± 13	6.5± 0.3	> 10000
44c	II	1	S	2-F	> 10000	253± 2	851± 8	14.7± 1.5	> 10000
44d	II	1	S	2-Cl	> 10000	309± 27	805± 3	14.1± 0.3	> 10000
44e	II	1	S	2-OH	> 10000	591± 26	1027± 9	13.8± 3.5	> 10000
44f	II	1	S	4-F	> 10000	850± 45	1028± 10	63.2± 2.8	> 10000
44g	II	1	S	4-Cl	> 10000	637± 3	872± 23	62.3± 0.7	> 10000
44h	II	1	S	3,4-Cl	> 10000	290± 9	770± 14	60.1± 1.3	> 10000
44i	II	1	S	4-OH	> 10000	618± 25	900± 12	60.9± 0.8	> 10000
44j	II	1	S	H	> 10000	253± 9	907± 4	15.1± 0.4	> 10000
45a	II	2	S	2-OEt	> 10000	28.5± 0.4	0.26± 0.02	0.03± 0.01	> 10000
45b	II	2	S	2,3-Cl	> 10000	27.2± 0.2	0.5± 0.01	0.54± 0.01	> 10000
45c	II	2	S	2-F	> 10000	34± 0.2	1.04± 0.02	6.6± 1.2	> 10000
45d	II	2	S	2-Cl	> 10000	29± 1	0.7± 0.01	5.9± 0.6	> 10000
45e	II	2	S	2-OH	> 10000	54±1	0.53± 0.03	6± 0.1	> 10000
45f	II	2	S	4-F	> 10000	53± 2	33± 3	31.6± 0.5	> 10000
45g	II	2	S	4-Cl	> 10000	38± 0.4	20± 1	30.9± 0.1	> 10000
45h	II	2	S	3,4-Cl	> 10000	40± 0.5	9.6± 0.4	29.3± 1.2	> 10000
45i	II	2	S	4-OH	> 10000	65± 5	1.07± 0.03	30.4± 0.6	> 10000
45j	II	2	S	H	> 10000	33± 0.7	1± 0.01	6.8± 0.3	> 10000
	Haloperidol				3.93± 1.7	0.28± 0.2	0.18± 0.03	3.53± 0.9	4.17± 0.3

Table 11: Binding affinity data of Phenylpiperazinylalkylisoindoledione and Arylamidoalkylphenylpiperazine derivatives to cloned human dopamine receptors
At least two independent experiments were carried out in triplicate each.

In terms of selectivity, the length of the spacer might play a crucial rule in enabling the ligand's aromatic appendage to afford the hydrophobic interaction with the corresponding amino acids that are reported to figure the D2/D3 subtype receptor selectivity in the EL2 of these receptors. Namely, these amino acids are Glu 181 and Ile 183 in D2 that are occupied with Val 180 and Ser 182 in D3 [81].

It is clear from the obtained data that the butyl linker bearing candidates among all series have shown to be 20 to 110 times more selective to D3 over D2 receptor subtypes. In accordance with the previously published mutagenesis and docking results [81], this selectivity may be explained by the idea that the longer butyl linker bearing ligands have been able to stretch farther toward the extracellular side EL2 of the binding pocket of the target receptors to afford hydrophobic interaction between the aromatic appendage and Val 180 residue in D3 that faces the more polar Glu 181 residue in D2.

On the other hand the propyl linker bearing ligands in the benzamide and the thienoamide series have shown to have preferentiality towards D2 rather than D3 receptor subtypes. This again might be explained in view of the results of the recent modeling studies where this selectivity pattern could be contributed to the Ile 183 residue in D2 that affords much better hydrophobic interaction with the ligand's aromatic appendage than its Ser 182 counterpart in D3 receptor subtype.

As for the D4 selectivity over D2 receptor subtypes, both propyl and butyl linker bearing ligands among all series have shown to keep moderate to appreciable selectivity to D4 over D2 receptor subtypes. It was important to figure out the amino acids occupying the residues proved to manipulate the

D2/D3 selectivity at the D4 binding site. It was found that Ile 183 of D2 is occupied with Arg 186 in D4 and the Glu 181 in D2 is occupied with Val 184 in D4 receptor subtypes. Both Val and Arg residues are able to interact properly with the aromatic appendage of our probes and this might explain the noticeable preferentiality of both propyl and butyl linker bearing ligands towards D4 receptor subtypes rather than D2 ones.

It is noteworthy to mention that the general enhanced binding affinity of all the butyl linker bearing candidates towards all the D2-like members emphasizes the role of the butyl spacer in enabling the compound to afford a specific folded conformation stabilized by an intramolecular hydrogen bond between the amide carbonyl and the protonated piperazine. This conformation is supposed to possess optimum distance between the pharmacophores leading to optimum binding affinity to the target receptor subtypes [140].

Moving to the second factor affecting the affinity and selectivity of the synthesized ligands towards the target receptor, it is worth to consider the effect of changing the aromatic appendage responsible for attaining the required hydrophobic interaction with the target receptor. Among the whole series, the thienoamide system has shown the highest binding affinity at all the D2-Like receptor subtypes followed by the benzamide and finally came the largest in size isoindole-1,3-dione system with the least binding affinity data. As already mentioned before, the superiority of the thiophene system relative to the benzene ones may be a function of better hydrophobic interaction at the binding pocket. This is mainly due to the fact that the thiophene system has higher electron rich properties as the lone pair of electrons in the P orbital of the sulfur atom contributes to the Huckel aromatic

sixtet and pushes high electron density toward the ring carbons that accordingly acquire partial negative charge. Thus, it was suggested that the large atomic polarizability of the sulfur atom and the electron rich thiophene system would provide higher dispersion forces compared to benzene which may lead to better π - π stacking and/or van der Waals interaction [141] with the hydrophobic residues lining the hydrophobic pocket of the target receptors.

Pushed by the binding affinity pattern of the synthesized compounds, we were determined to carry out some in-silico experiments to configure the binding fashion of these compounds to the target receptor subtypes. The synthesized probes have been docked to the human D3 model (PDB ID: 3PBL), and the validated D2 and D4 homology models developed before [139].

Compound **44a** that showed the highest preferentiality to D2 over D3 among all derivatives bearing the propyl linker was docked to the D2 receptor homology model and showed the contact between the ligand's arene and Ile 183 residue. Docking the same compound to D3 receptor model have illustrated the contact between the ligand's arene and the Ser 182 in the EL2 of the binding site of the D3 receptors that is occupied with the more hydrophobic Ile 183 in the D2 receptor subtype confirming the rule of this amino acid in manipulating the ligands' preferentiality towards D2/D3. The other propyl linker bearing compounds **42a**, **42b**, **44b** have been also docked to D2 and D3 receptor models and showed to over relay compound **44a** in the binding site of the target receptors, Figure **28.**

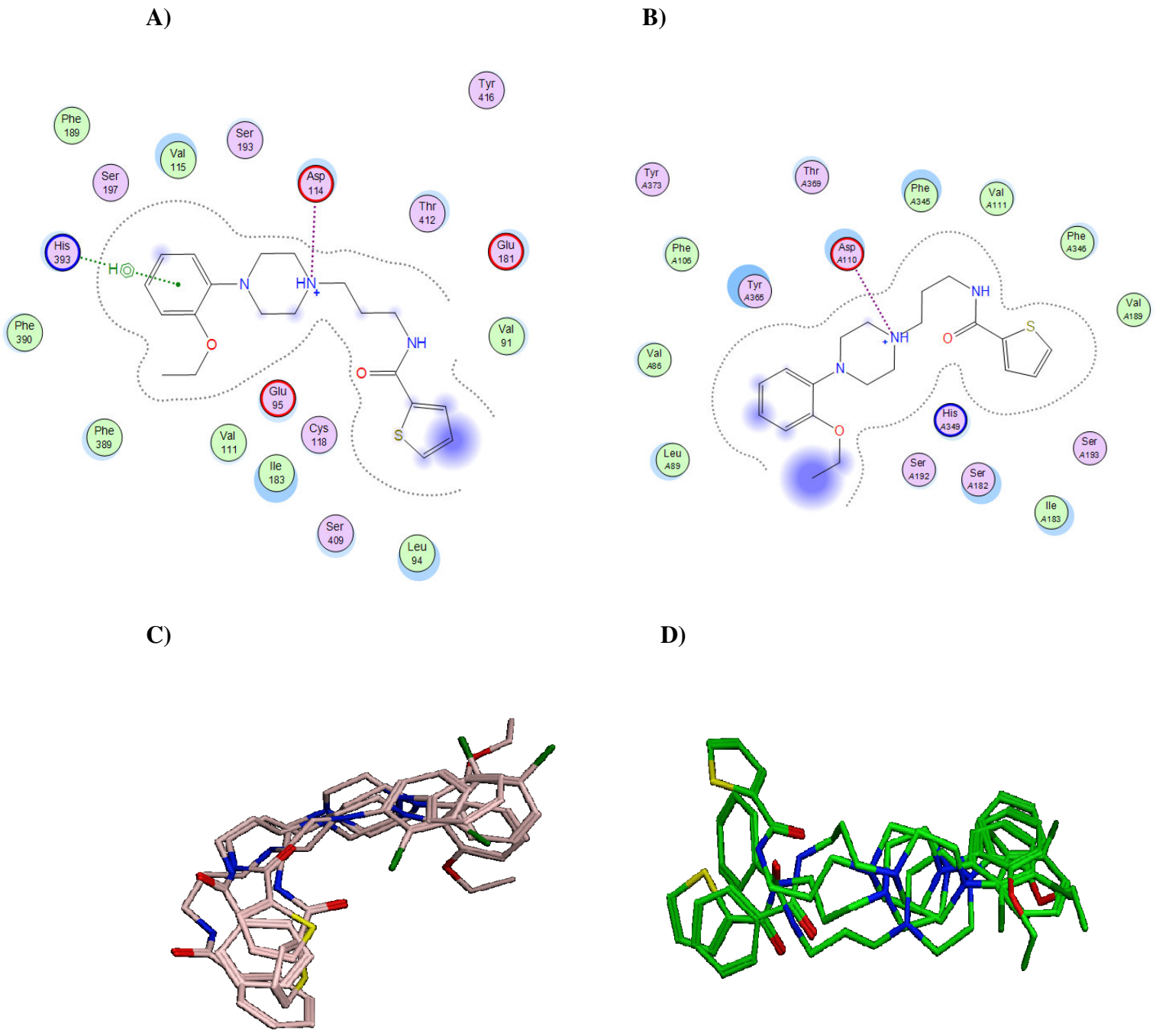


Figure 28: A) 2D interactions of compound 44a docked to human D2 model showing the key salt bridge interaction with Asp 3.32 (Asp 114) and the ligands' aromatic appendage in contact to Ile 183 in EL2. B) 2D interactions of compound 44a docked to human D3 model showing the key salt bridge interaction with Asp 3.32 (Asp 110) and the ligands' aromatic appendage in contact to Ser 182 in EL2. C) Compounds 42a, 42b, 44b over relayed compound 44a in the binding site of D2 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity. D) Compounds 42a, 42b, 44b over relayed compound 9a in the binding site of D3 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity

Compound **45a** that showed the highest preferentiality to D3 over D2 among all the derivatives bearing the butyl linker has docked to both D2 and D3 models. The docking revealed the rule of the butyl spacer to get the ligands' arene in contact to the Glu 181 that is conserved in the binding site of the D2 subtype and faces the more hydrophobic Val 180 in D3. Although Val 180 is conserved in the binding site of D3 receptor subtypes, our 2D interactions of compound **45a** with D3 receptor model did not show this residue in the binding site. The other butyl linker bearing compounds **43a**, **43b**, **45b** have been also docked to D2 and D3 models and showed to over relay compound **45a** in the binding site of the target receptors, Figure **29.**

As for D4 receptor subtypes, docking both compounds **44a** and **45a** that were among the probes with the highest D4 affinity through over the whole series have configured the rule of the unique D4 amino acid residue Arg 186 that turned out to be involved in affording hydrogen bond interaction with the carbonyl moiety of the synthesized ligands. The other propyl linker bearing compounds **42a**, **42b**, **44b** and the butyl linker bearing compounds **43a**, **43b**, and **45b** have been also docked to D4 model and showed to over relay compounds **44a** and **45a** respectively in the binding site of the target receptor, Figure **30**.

Finally comes the effect of the substituent on the phenylpiperazine scaffold upon the affinity to the target receptors, it is noticeable that the propyl linker bearing ligands, **44c**- **44j**, have shown appreciable affinity to D4 receptor subtypes. The best affinity was obtained by ligands bearing ortho oxygenated and di-halogenated derivatives followed by those having an ortho monohalogenated substituent.

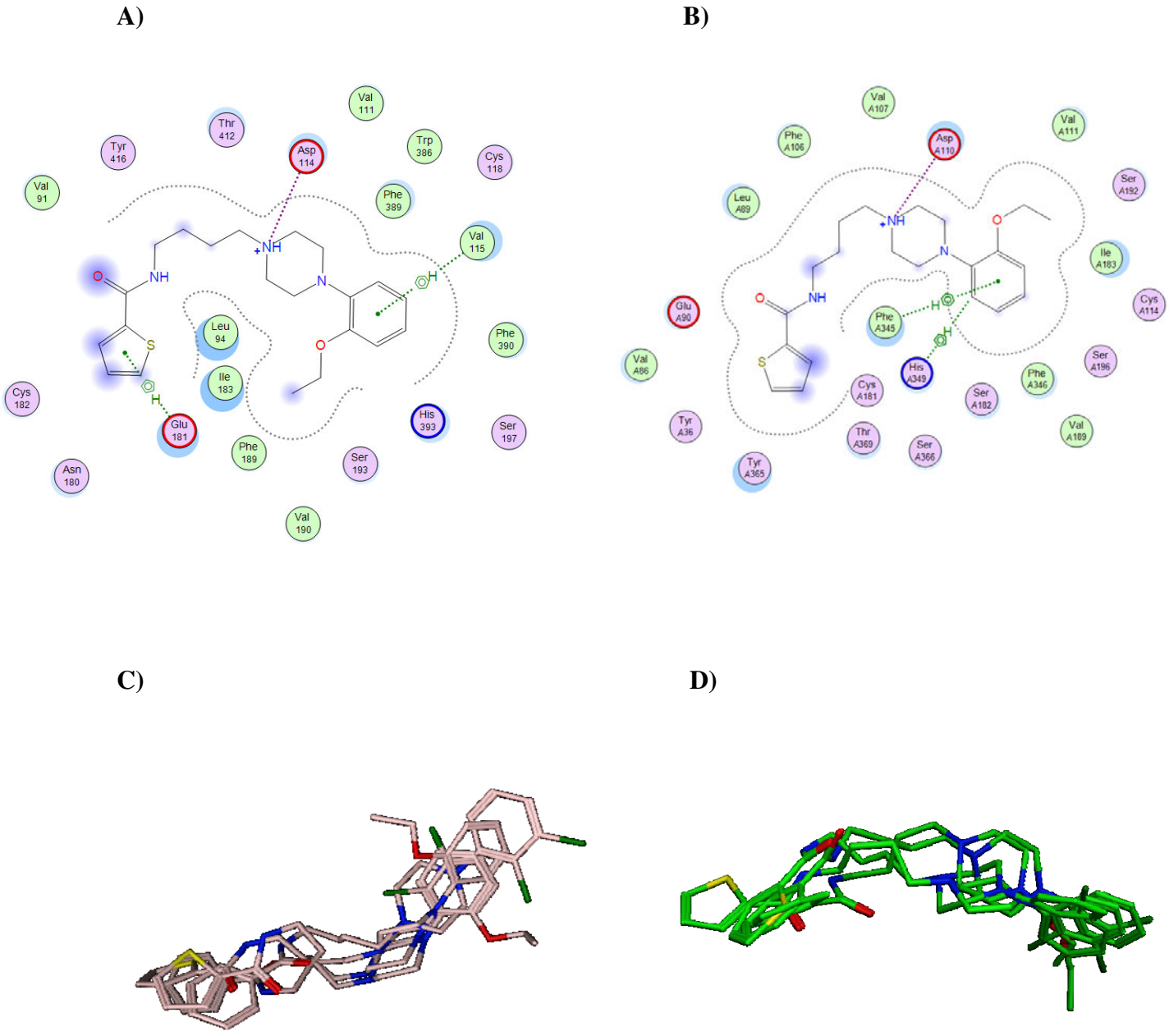


Figure 29: A) 2D interactions of compound 45a docked to human D2 model showing the key salt bridge interaction with Asp 3.32 (Asp 114) and the ligands' aromatic appendage in contact to Glu 181 in EL2. B) 2D interactions of compound 45a docked to human D3 model showing the key salt bridge interaction with Asp 3.32 (Asp 110). C) Compounds 43a, 43b, 45b over relayed compound 45a in the binding site of D2 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity. D) Compounds 43a, 43b, 45b over relayed compound 45a in the binding site of D3 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity

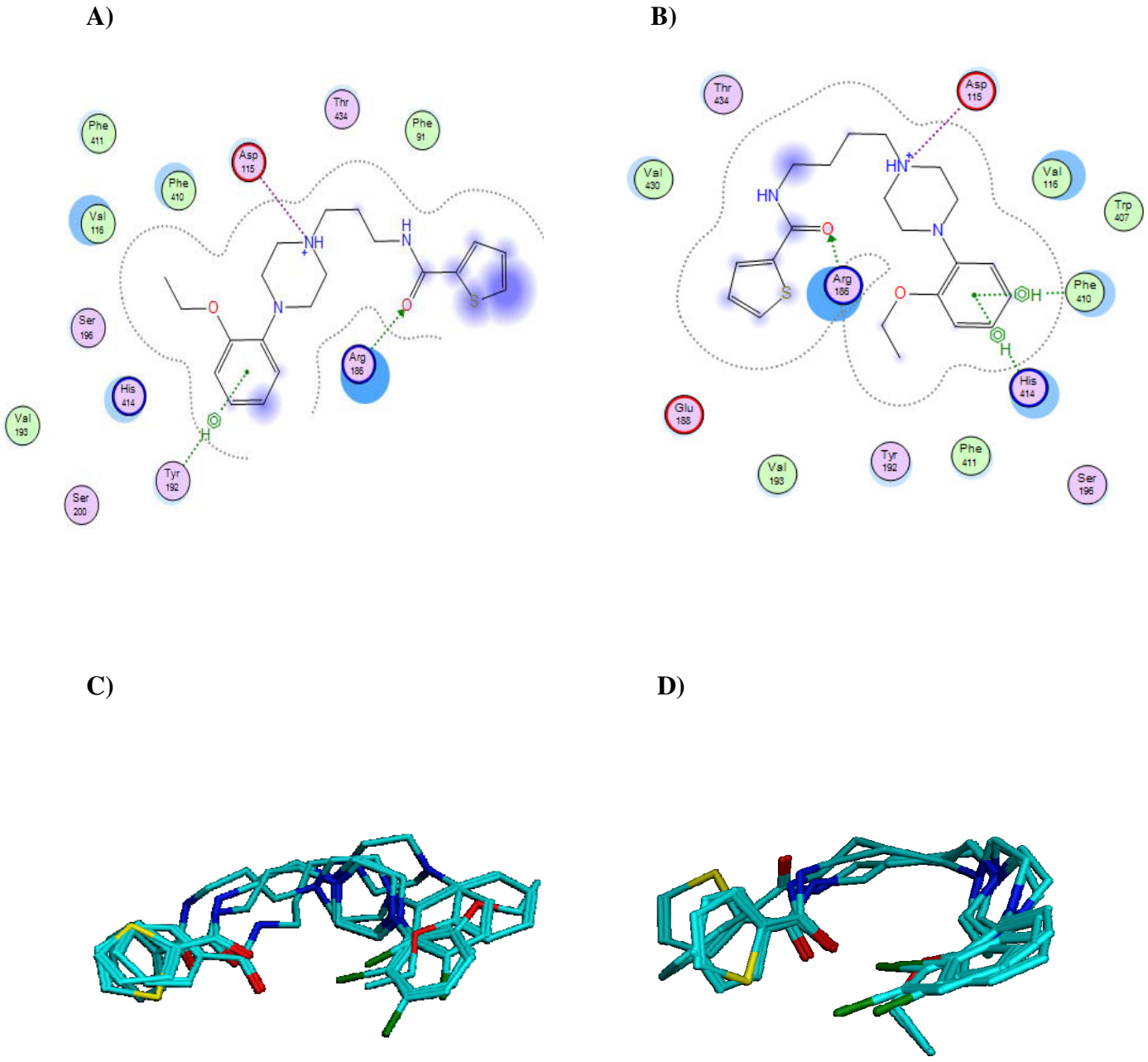


Figure 30: 2D interactions of compounds 44a (A) and 45a (B) docked into human D4 model showing the key salt bridge interaction with Asp 3.32 (Asp 115) and hydrogen bond interaction between the ligand's carbonyl and the unique D4 residue Arg 186.
C) Compounds 42a, 42b, 44b over relayed compound 44a in the binding site of D4 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity. D) Compounds 43a, 43b, 45b over relayed compound 45a in the binding site of D4 receptor model. Hydrogen atoms of the ligands and the amino acid residues have been removed for clarity

The affinity of the synthesized ligands exhibited four to ten folds decrease when the substituent was shifted to the para position. This is clear when comparing the Ki value data of compounds **44b**, **44c**, **44d**, and **44e** to their para substituted analogues **44h**, **44f**, **44g**, and **44i**. This would point out the importance of having an ortho substituted phenylpiperazine unit with a specific electrostatic potential to manipulate affinity towards D4 receptor subtypes.

Regarding the butyl linker bearing ligands, **45c**- **45j**, all of them have shown superior affinity to both D3 and D4 receptor subtypes relative to D2 ones. Again the nature and position of the substituent at the phenylpiperazine scaffold manipulated the affinity of these derivatives in a similar fashion exhibited by their propyl counterparts.

4. Experimental

4.1 Chemistry

4.1.1 General experimental details

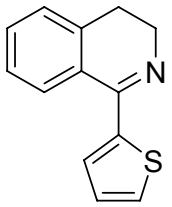
1. Melting points were measured in open capillary tubes using a Gallenkamp melting-point apparatus and were not corrected.
2. IR data were obtained from a Magna-IR FT-IR spectrometer, system 550 by Nicolet (WI). KBr disc used for sample preparation.
3. ^{1}H-NMR and ^{13}C-NMR spectra were obtained from Bruker Avance 250 and Avance 400 spectrometers (250 MHz, 400 MHz, respectively) using $CDCl_3$ or DMSO-d_6 as a solvent; chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS; multiplicities are abbreviated as: s: singlet; d: doublet; q: quartret; m: multiplet; dd: doublet of doublet; brs: broad singlet.
4. MS data were determined by GC/MS, using a Hewlett-Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). For all compounds M^+ was corresponding to the respective Mwt. of the compound. GC/MS was used to obtain MS data and so retention times are not given here.
5. Elemental analyses were performed by Institute of Organic Chemistry, Jena University, and were performed on a Hereaus Vario EL apparatus.
6. Column chromatography was performed using silica-gel 60 63-200µm.
7. Reaction progress was monitored by TLC using fluorescent precoated plates and detection of the components was made by short UV light.

8. All starting materials were obtained from Sigma –Aldrich or Alpha Acer and were used without further purification.

4.1.2 Methods

Procedure for the preparation of 3,4-dihydro-1(2-thienyl)isoquinoline (4) [120]

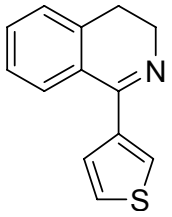
2-Thiophene carbonitrile (11g, 100 mmol) was added gradually with stirring at room temperature to 21.6g (100 mmol) of stannous (IV) chloride. The temperature was raised gradually to 90^{o}C, and 14g (100 mmol) of 2-Phenyl ethylchloride was added gradually to the reaction mixture. After 3 hours of reaction at a temperature of 100-120 ^{o}C, the mixture was allowed to cool and 20% Sodium hydroxide solution was added gradually till brown oil is separated. The crude oily product is then left to dry under vacuum overnight. Yield and spectral data of the prepared intermediate matched data described in literature [120].



Procedure for the preparation of 3,4-dihydro-1(3-thienyl)isoquinoline (20) [120]

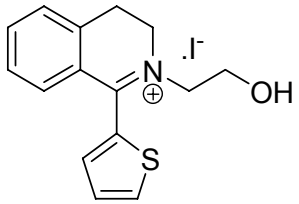
3-thiophene carbonitrile (11g, 100 mmol) was added gradually with stirring at room temperature to 40g (185 mmol) of stannous (IV) chloride. The temperature was raised gradually to 90^{o}C, and 14g (100) mmol of 2-phenyl ethylchloride was added gradually to the reaction mixture. After 3 hours of reaction at a temperature of 100-120 ^{o}C, the mixture was allowed to cool and 20% Sodium hydroxide solution was added gradually till brown oil is separated. The crude oily product is then left to dry under vacuum overnight.

Yield and spectral data of the prepared intermediate matched data described in literature [120].



Procedure for the preparation of 2-(2-hydroxyethyl)-3,4-dihydro-1(2-thienyl) isoquinolinium iodide (5) [120]

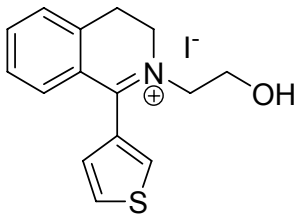
3,4-dihydro-1(2-thienyl)isoquinoline (1.5g, 7 mmol) was dissolved in 50 ml dry acetone and to the solution was gradually added 2.5g (14 mmol) of 2-iodoethanol. The reaction mixture was stirred under nitrogen atmosphere at 90 ^{o}C for 48 hours. The solvent was then evaporated under reduced pressure and the resulted compound was then washed with 50 ml acetone and dried under vacuum. Yield and spectral data of the prepared intermediate matched data described in literature [120].



Procedure for the preparation of 2-(2-hydroxyethyl)-3,4-dihydro-1(3-thienyl) isoquinolinium iodide (21) [120]

3,4-dihydro-1(3-thienyl)isoquinoline (10g, 47 mmol) was dissolved in 100 ml dry acetone and to the solution was gradually added 10g (58 mmol) of 2-iodoethanol. The reaction mixture was stirred under nitrogen atmosphere at 90 ^{o}C for 48 hours. The solvent was then evaporated under reduced pressure and the resulted compound was then washed with 50 ml acetone and dried

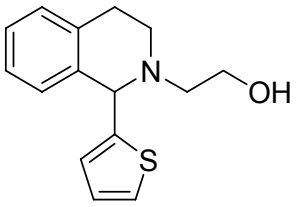
under vacuum. Yield and spectral data of the prepared intermediate matched data described in literature [120].



Procedure for the preparation of 2-(2-hydroxyethyl)-1(2-thienyl)-1,2,3,4-tetrahydroisoquinoline (6) [120]

N-hydroxyethyl-3,4-dihydro-1(2-thienyl) isoquinolinium iodide (5g, 130 mmol) were dissolved in methanol and to the solution was added gradually 8g (212 mmol) of sodium borohydride over a period of 2 hours. The reaction mixture was allowed to reflux for further half an hour. After cooling, the solvent was removed under reduced pressure. The residue was then suspended in water and the mixture was extracted with ethyl acetate (2 X 100 ml).

The collected organic layers were evaporated under vacuum to give a crude residue from which the target compound was recrystallized from ethanol. Yield and spectral data of the prepared intermediate matched data described in literature [120].

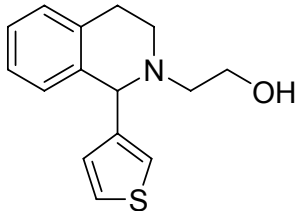


Procedure for the preparation of 2-(2-hydroxyethyl)-1(3-thienyl)-1,2,3,4-tetrahydroisoquinoline (22) [120]

N-hydroxyethyl-3,4-dihydro-1(3-thienyl) isoquinolinium iodide (5g, 130 mmol) were dissolved in methanol and to the solution was added gradually 8g (212 mmol) of sodium borohydride over a period of 2 hours. The reaction mixture was allowed to reflux for further half an hour. After cooling, the solvent was

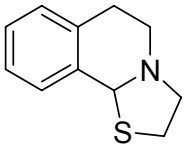
removed under reduced pressure. The residue was then suspended in water and the mixture was extracted with ethyl acetate (2 X 100 ml).

The collected organic layers were evaporated under vacuum to give a crude residue from which the target compound was recrystallized from ethanol. Yield and spectral data of the prepared intermediate matched data described in literature [120].



Procedure for the preparation of 2,3,5,6-tetrahydro-10b*H*-thiazolo[2,3-a] isoquinoline (7) [120]

To a solution of 30 mg of potassium hydroxide in 50 ml ethanol, was added 1g (88 mmol) of 2-aminoethanthiol and 1.87g (88 mmol) of 2-(2-bromoethyl)benzaldehyde. The reaction mixture was allowed to stir at room temperature for 24 hours. The precipitated compound was filtered and dried under vacuum.



Yield: 77%, white crystals

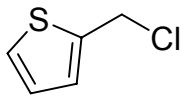
m.p.: 90- 91 ^{o}C

^{1}HNMR ($CDCl_3$): 2.60- 2.95 (m, 3H, aliphatic), 3.01- 3.33 (m, 4H, aliphatic), 3.57- 3.60 (m, 1H, aliphatic) 5.79 (s, 1H, C10b), 7.00- 7.25 (m, 4H, aromatic)

Elemental analysis: for $C_{11}H_{13}NS$: calcd. C 69.11, H 6.49, N 7.30 ; found C 69.07; H 6.80; N 6.88

Procedure for the preparation of 2-Chloromethylthiophene (17)

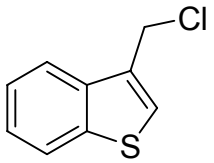
To a 100-ml round bottom flask in an ice bath containing 0.45g (4 mmol) of thiophene-2-methanol was added slowly and with continuous stirring 10g (85 mmol) of thionyl chloride. After the complete addition, the ice bath was removed and the reaction mixture was allowed to reflux for 3 hours. The flask was then cooled to room temperature, the solution was neutralized with saturated solution of sodium bicarbonate, and the desired organic product was extracted with methylene chloride (2X50 ml). The organic layers were collected and dried over anhydrous sodium sulphate and evaporated under reduced pressure. The final product was retrieved in a form of brownish black oil and was used for the further reaction without extra purification. Yield and spectral data of the prepared intermediate matched data described in literature [126].



Procedure for the preparation of 3-Chloromethyl benzo[b]thiophene (25)

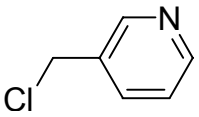
To benzo[b]thiophene (13.5g, 100 mmol) was added 15g (500 mmol) 37% HCHO solution and 15g (411 mmol) 36% HCl solution. To the mixture, HCl gas that is generated from the reaction between H_2SO_4 and NaCl in a separate connected flask was then passed to the reaction mixture that was stirred under the temperature of 60 ^oC for 6 hours. The reaction mixture was then allowed to cool to room temperature and then poured into 50 ml of water and extracted with diethyl ether (2X5O ml). The combined organic layers were then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield straw yellow oil that was directly used without further purification. Yield

and spectral data of the prepared intermediate matched data described in literature [125].



Procedure for the preparation of 3-Chloromethylpyridine (37)

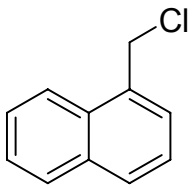
To an ice cooled solution of 5.5g (50 mmol) of pyridine-3-methanol in 40 ml methylene chloride was added in a dropwise manner a solution of 1g thionyl chloride in 10 ml methylene chloride. The reaction mixture was allowed to stir for 15 minutes in the ice path and then 20 minutes under reflux. The solution was then neutralized with saturated solution of sodium bicarbonate and extracted twice with 40 ml methylene chloride. The combined organic layers were then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield yellow oil that was directly used without further purification. Yield and spectral data of the prepared intermediate matched data described in literature [126].



Procedure for the preparation of 1-Chloromethylnaphthalene (38)

To a solution of 13g (100 mmol) naphthalene in 60 ml methylene chloride was added while cooling in ice bath 15g (500 mmol) 37% HCHO solution and 15g (411 mmol) 36% HCl solution. To the mixture, HCl gas that is generated from the reaction between H_2SO_4 and NaCl in a separate connected flask was then passed to the reaction mixture that was stirred at room temperature for 6 hours. The reaction mixture was then poured into 50 ml of water and extracted with diethyl ether (2X5O ml). The combined organic layers were

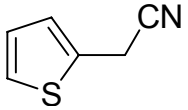
then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield yellow oil that was directly used without further purification. Yield and spectral data of the prepared intermediate matched data described in literature [125].



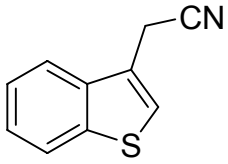
General Procedure for the preparation of Thiophene-2-yl-acetonitrile and Benzo[b]thiophene-3-yl-acetonitrile

To an ice-cooled solution of 6.5g (100 mmol) potassium cyanide and 22g (20 mmol) triethylbenzylammonium chloride in water, was added 100 mmol of **(17)** or **(25)** respectively over a period of 5 minutes. The ice path was then replaced with water path that was heated to 90-95 ^oC. The reaction mixture was allowed to stir under this temperature for 2 hours and it was then allowed to cool to room temperature, diluted with 50 ml of water, and extracted with methylene chloride (4X30 ml). The combined organic layers were then dried over Na_2SO_4 and evaporated under reduced pressure. The obtained black oil was then subjected to purification on silica gel column chromatography using a mixture of Hexane: Acetone 9:1 as an eluent. Yield and spectral data of the prepared intermediates matched data described in literature [126].

Thiophene-2-yl-acetonitrile (18)

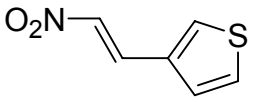


Benzo[b]thiophene-3-yl-acetonitrile (26)



Procedure for the preparation of 3-(2-nitrovinyl) thiophene (9)

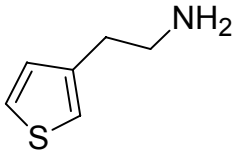
To a solution of 1g (10 mmol) of thiophene-3-carboxaldhyde in 30 ml of nitromethane, was added 0.4g (5 mmol) of ammonium acetate. The mixture was heated to 110 ^{o}C in an open flask for 4 hours. The excess nitromethane was then evaporated under reduced pressure. The residue was then poured into crushed ice and the separated product was filtered off, washed with water (2X50 ml), and dried under vacuum.



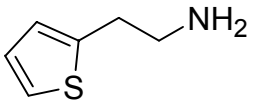
General procedure for the preparation of the β-aryl ethylamine (10, 19, 27)

To an ice-cooled suspension of $LiAlH_4$ (2.3g, 60 mmol) in 250 ml dry THF, was added slowly and with stirring under inert atmosphere over a period of 15 minute a solution of 20 mmol of the respective aryl acetonitrile precursor **(2 or 15)** or the 3-(2-nitrovinyl) thiophene **(9)** in 20 ml dry THF. The ice bath was then removed and the reaction mixture was heated to reflux for 10 hours. It was then cooled to room temperature and the excess $LiAlH_4$ was quenched by the careful addition of saturated Rochelle solution under inert atmosphere and with cooling in an ice bath till no H_2 evolves. The reaction mixture was then filtered, washed with dry THF, and the filtrate was evaporated under reduced pressure to yield an oil of the respective β-aryl ethylamine. The desired amine product was introduced to the following step without further purification. Yield and spectral data of the prepared intermediates matched data described in literature [126].

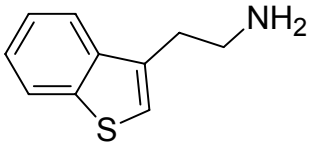
2-(thiophene-3-yl) ethylamine (10)



2-(thiophene-2-yl) ethylamine (19)

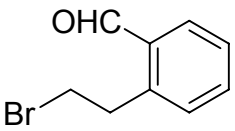


2-(benzo[b]thiophene-3-yl) ethylamine (27)



Procedure for the preparation of 2-(2-bromoethyl) benzaldehyde (11)

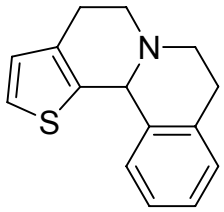
To an ice-cooled solution of isochroman (12.5g, 93 mmol) in methylene chloride (50 ml), was added bromine solution (5g, 31.2 mmol) slowly over a period of 5 minutes. The ice bath was removed and the reaction was refluxed until it becomes pale yellow (about 4 hours). The reaction was then allowed to reach room temperature and the solvent was removed under reduced pressure. To the obtained yellow oil, 48% HBr solution (30 ml) was added and the mixture was refluxed for additional 20 minutes. The mixture was then allowed to reach room temperature, extracted with tert. butyl methyl ether (2X30 ml). The organic layer was then washed with water (2X30 ml), dil. $NaHCO_3$ (2X50 ml), and then dried over anhydrous Na_2SO_4. Evaporation of the organic solvent under reduced pressure produced an irritant, lacrimatory orange oil of the desired aldehyde in 82% yield which was used without further purification.



Procedure for the preparation of 4,5,7,8 tetrahydro-12bH-benzo[h] thieno [2,3-*a*]quinolizine (15)

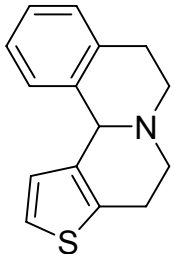
To a solution of 2.8g (13 mmol) of 2-(2-bromoethyl) benzaldehyde **(11)** in 40 ml of dry dioxane, was added slowly a solution of 1.4g (11 mmol) of the 2-(thiophene-3-yl) ethylamine **(10)** in 15 ml of dry dioxane under inert atmosphere. The reaction mixture was allowed to stir at room temperature till the separation of the corresponding imminium bromide salt in the form of thick oily syrup. After further 3 hours of stirring at the same conditions, the syrup was separated, and washed with dry dioxane (2X10 ml) and then with diethyl ether (2X15 ml). The obtained syrup was then immediately dissolved in 20 ml of 6M HCl and the reaction mixture was heated to reflux for 24 hours to afford the formation of the halo salt of the corresponding quinolizine.

The excess HCl was then evaporated under reduced pressure and the residue obtained was suspended in 15 ml of water, treated with drops of 33% NH_3 solution, and extracted with diethyl ether (2X15 ml). The organic solvent was evaporated under reduced pressure to obtain a gummy residue. Recrystallization from petroleum ether produced the respective desired quinolizines. Yield and spectral data of the prepared intermediate matched data described in literature [127].



Procedure for the preparation of Benzo[a]-5,6,8,9-tetrahydro-12bH-thieno[2,3-*h*]quinolizine (23)

A solution of 1.8g (7 mmol) of 2-(2-hydroxyethyl)-1-(3-thienyl)-1,2,3,4-tetrahydroiso- quinoline in 40 ml polyphosphoric acid was heated to reflux for 6 hours under inert atmosphere. The reaction mixture was then poured into ice water and extracted with 50 ml diethyl ether. The organic layer was then neutralized with 1M sodium hydroxide solution and again extracted with 50 ml diethyl ether twice. The collected organic layers were collected and dried over anhydrous sodium sulphate, evaporated under reduced pressure, and the product was recrystallized from ethanol. Yield and spectral data of the prepared intermediate matched data described in literature [120].



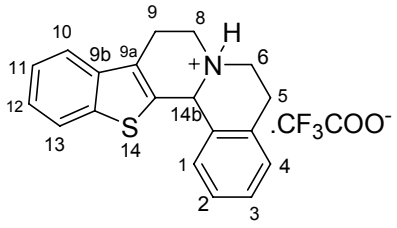
Procedure for the preparation of 5,6,8,9-tetrahydro-14bH-benzo[h]benzothieno[2,3-*a*]quinolizine (29)

To a solution of 1.9g (11 mmol) of 2-(benzo[b]thiophene-3-yl) ethyl amine **(27)** in 40 ml of dry dioxane, was added 2.8g (13 mmol) of 2-(2-bromoethyl) benzaldehyde **(11)** and 1.3g (11 mmol) of TFA. The reaction mixture was then heated to reflux under inert atmosphere for 6 hours. The produced pale yellow precipitate was then filtered off, washed with dry dioxane, and dried under vacuum to yield the respective quinolizine in its trifluoro acetate salt.

This salt was then suspended in water, treated with drops of 33% NH_3 solution, and then extracted with diethyl ether (2X15 ml). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4 and the organic solvent was evaporated under reduced pressure to produce a gummy

flakes residue. Re-crystallization from petroleum ether yielded pale yellow crystals of the respective quinolizine. Yield and spectral data of the prepared intermediate matched data described in literature [127].

5,6,8,9-tetrahydro-14bH-benzo[h]benzothieno[2,3-*a*]quinolizinium trifluoroacetate (28)

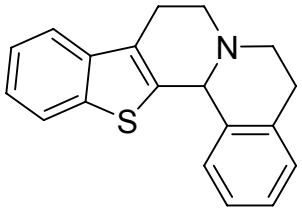


Yield: 3.5g, 81%, buff crystals

m.p. 218- 220 ^{0}C

^{1}HNMR (DMSO-d_6) 2.96- 3.61 (m, 8H, 4 X CH_2), 6.20 (s, 1H, C14b), 7.30-7.94 (m, 8H, aromatic), 12.54 (s, 1H, N^+H)

5,6,8,9-tetrahydro-14bH-benzo[h]benzothieno[2,3-*a*]quinolizine (29)

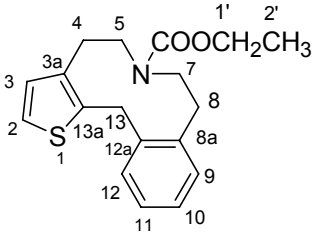


General Procedure for the preparation of the carbamate derivatives (16, 24, 30)

A solution of 2 mmol of the respective quinolizine derivative in 30 ml dry THF was cooled in methanol/dry ice at -80^{o}C. To the solution was added 1g (10 mmol) of ethylchloroformate under inert atmosphere. The reaction mixture was stirred for 4 hours and then a solution of 0.37g (6 mmol) of sodium cyanoborohydride in 20 ml dry THF was added after cooling again to -80 ^{o}C. The reaction mixture was allowed to reach to room temperature and stirred for 48 hours. It was then treated with 100 ml 2N NaOH, and the organic layer was

separated, washed with brine solution (2X 30 ml), and the organic solvent was then evaporated under reduced pressure. The residue obtained was purified on silica gel column chromatography using Hexane: Ethyl acetate 3:1.

4,5,6,7,8,13-hexahydrobenzo[d]thieno[2,3-*g*]azecine-6-carboxylic acid ethylester (16)



Yield: 0.5g, 78%, yellow resin

^{1}HNMR ($CDCl_3$): 0.92 (t, 3H, J= 7 Hz -CH_2**CH_3**), 2.66- 3.57 (m, 8H, 4X CH_2), 3.73 (q, 2H, J= 7 Hz, -**CH_2**CH_3), 4.09- 4.17 (d, 2H, C13), 6.69 (d, 1H, J= 5 Hz, aromatic), 6.79 (d, 1H, J= 5 Hz, aromatic), 7.00- 7.20 (m, 4H, aromatic)

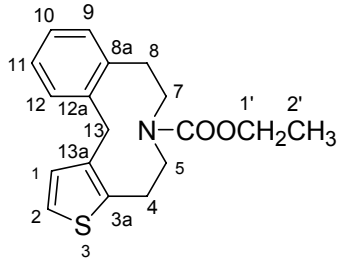
^{13}CNMR ($CDCl_3$): 14.20 (C**2**'), 28.47 (C4), 32.50 (C13), 32.83 (C8), 34.05 (C**1**'), 53.92 (C5), 61.00 (C7), 122.54 (C3a), 126.73 (C10), 126.98 (C11), 130.19 (C9), 130.36 (C12), 131.14 (C 3), 135.87 (C2), 138.45 (C13a), 138.59 (C8a), 139.27 (C12a), 156.48 (C=O)

IR (cm^{-1}): 1680 (C=O)

GC/MS: m/z 104 (100%), m/z 115 (80 %), m/z 184 (60%), m/z 211 (30%), 315 (M^+, 20%)

Elemental analysis: for $C_{18}H_{21}NO_2S$: calcd. C 68.54, H 6.71, N 4.44 ; found C 68.31; H 6.41; N 4.42

4,5,6,7,8,13-hexahydrobenzo[d]thieno[3,2-*g*]azecine-6-carboxylic acid ethylester (24)



Yield: 0.47g, 75%, yellow resin

^{1}HNMR ($CDCl_3$): 0.95 (t, 3H, J= 7 Hz -CH_2**CH_3**), 2.66- 3.56 (m, 8H, 4X CH_2), 3.75 (q, 2H, J= 7 Hz, -**CH_2**CH_3), 3.96 (d, 2H, C13), 6.82- 7.28 (m, 6H, aromatic)

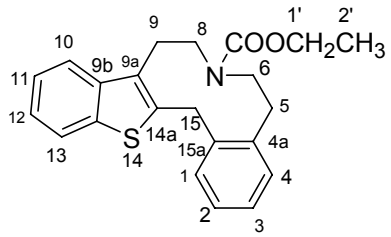
^{13}CNMR ($CDCl_3$): 14.20 (C**2**'), 27.83 (C8), 31.57 (C13), 32.72 (C4), 33.99 (C**1**'), 54.23 (C7), 60.98 (C5), 121.58 (C13a), 126.60 (C10), 126.94 (C11), 130.23 (C9), 130.58 (C12), 131.28 (C1), 136.65 (C2), 137.14 (C3a), 139.04 (C8a), 139.19 (C12a), 156.50 (C=O)

IR (cm^{-1}): 1684 (C=O)

GC/MS: m/z 104 (100%), m/z 115 (85 %), m/z 184 (70%), m/z 211 (25%), 315 (M^+, 15%)

Elemental analysis: for $C_{18}H_{21}NO_2S$: calcd. C 68.54, H 6.71, N 4.44 ; found C 68.21; H 6.53; N 4.35

5,6,7,8,9,15-hexahydrobenzo[d]benzothieno[2,3-*g*]azecine-7-carboxylic acid ethylester (30)



Yield: 0.64g, 88%, pale yellow resin

^{1}HNMR ($CDCl_3$): 0.82- 0.93 (t, 3H, -CH_2**CH_3**), 2.06 (brs, 2H, -**CH_2**CH_3), 2.75- 3.73 (m, 8H, 4X CH_2), 4.26 (d, 2H, C15), 7.14- 7.78 (m, 8H, aromatic)

^{13}CNMR ($CDCl_3$): 13.16 (C**2'**), 26.13 (C9), 32.81 (C15), 33.07 (C5), 34.34 (C**1'**), 54.17 (C8), 60.91 (C6), 120.53 (C13), 121.96 (C9a), 123.68 (C10), 123.79 (C11), 126.71 (C12), 127.03 (C3), 127.32 (C2), 129.02 (C9b), 130.29 (C4), 130.66 (C1), 138.28 (C13a), 138.96 (C14a), 140.00 (C4a), 141.32 (C15a), 156.40 (C=O)

IR (cm^{-1}): 1692 (C=O)

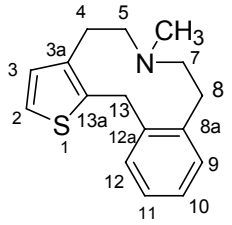
GC/MS: m/z 104 (100%), m/z 115 (75 %), m/z 234 (80%), m/z 249 (70%), 365 (M^+, 35%)

Elemental analysis: for $C_{22}H_{23}NO_2S$: calcd. C 72.30, H 6.34, N 3.83 ; found C 71.93; H 6.36; N 3.72

General procedure for the preparation of the azecine derivatives (1, 2, 3)

To an ice-cooled suspension of 1g (2.6 mmol) $LiAlH_4$ in 15 ml dry THF was added a solution of 6 mmol of the respective carbamate derivative in 10 ml dry THF, while stirring under inert atmosphere. The ice bath was then removed and the reaction mixture was heated to reflux for 3 hours. It was then allowed to cool to room temperature and the excess un-reacted $LiAlH_4$ was quenched with the careful addition of saturated Rochelle solution under inert atmosphere and with cooling in an ice bath till no H_2 evolves. The reaction mixture was then filtered, washed with dry THF, and the filtrate was evaporated under reduced pressure. The obtained residue was subjected to purification process on silica gel chromatography using Hexane: Ethyl acetate 3:2.

6-Methyl-4,5,6,7,8,13-hexahydrobenzo[d]thieno[2,3-*g*]azecine (1)



Yield: 1.07g, 70%, yellow resin

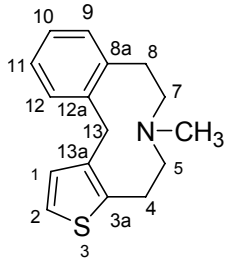
^{1}HNMR ($CDCl_3$): 2.22 (s, 3H, N-CH_3), 2.59- 2.87 (m, 8H, 4X CH_2), 4.35 (s, 2H, C13), 6.72 (d, 1H, J= 5Hz, aromatic), 7.03 (d, 1H, J= 5Hz, aromatic), 7.06- 7.18 (m, 4H, aromatic)

^{13}CNMR ($CDCl_3$): 29.42 (C4), 32.84 (C13), 35.00 (C8), 46.20 (N-CH_3), 59.51 (C5), 59.59 (C7), 121.63 (C3a), 126.35 (C10), 126.50 (C11), 129.93 (C9), 130.21 (C12), 130.30 (C 3), 137.37 (C2), 139.66 (C13a), 139.78 (C8a), 140.28 (C12a)

GC/MS: m/z 115 (95%), m/z 152 (90%), m/z 165 (70%), m/z 184 (100%), m/z 199 (60%), 257 (M^+, 25%)

Elemental analysis: for $C_{16}H_{19}NS$: calcd. C 74.66, H 7.44, N 5.44 ; found C 74.59; H 6.98; N 5.38

6-Methyl-4,5,6,7,8,13-hexahydrobenzo[d]thieno[3,2-*g*]azecine (2) [67]



Yield: 1.12g, 73%, yellow resin

^{1}HNMR ($CDCl_3$): 2.26 (s, 3H, N-CH_3), 2.65- 3.09 (m, 8H, 4X CH_2), 4.34 (s, 2H, C13), 6.92 (d, 1H, J= 5.2 Hz, aromatic), 7.01- 7.26 (m, 4H, aromatic) 7.31 (d, 1H, J= 5.8 Hz, aromatic),

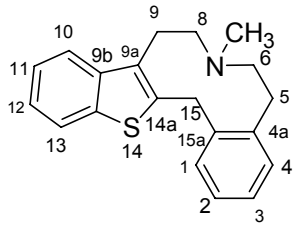
^{13}CNMR ($CDCl_3$): 29.91 (C8), 33.71 (C13), 34.88 (C4), 46.29 (N-CH_3), 59.41 (C7), 60.67 (C5), 121.45 (C13a), 126.28 (C10), 129.93 (C11), 130.37 (C9), 130.49 (C12), 131.36 (C1), 137.77 (C2), 138.32 (C3a), 139.94 (C8a), 140.35

(C12a)

GC/MS: m/z 115 (90%), m/z 152 (80%), m/z 165 (60%), m/z 184 (100%), m/z 199 (40%), 257 (M^+, 25%)

Elemental analysis: for $C_{16}H_{19}NS$: calcd. C 74.66, H 7.44, N 5.44 ; found C 74.38; H 7.05; N 5.48

7-Methyl-5,6,7,8,9,15-hexahydrobenzo[d]benzothieno[2,3-*g*]azecine (3)



Yield: 1.25g, 68%, yellowish white crystals

m.p. 96-98 ^{0}C

^{1}HNMR ($CDCl_3$): 2.18 (s, 3H, N-CH_3), 2.71- 2.94 (m, 8H, 4X CH_2), 4.47 (s, 2H, C15), 7.09- 7.45 (m, 6H, aromatic), 7.57 (dd, 1H, J= 1.2, 8 Hz, aromatic), 7.77 (dd, 1H, J= 1.5, 7 Hz, aromatic)

^{13}CNMR ($CDCl_3$): 25.63 (C9), 33.80 (C15), 34.93 (C5), 46.38 (N-CH_3), 59.22 (C8), 59.50 (C6), 121.01 (C13), 122.26 (C9a), 123.55 (C10), 123.75 (C11), 126.47 (C12), 126.84 (C3), 127.30 (C2), 130.39 (C9b), 130.55 (C4), 130.83 (C1), 138.80 (C13a), 138.92 (C14a), 140.25 (C4a), 140.36 (C15a)

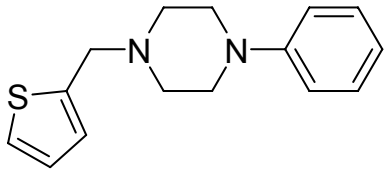
GC/MS: m/z 115 (90 %), m/z 234 (100%), m/z 249 (40%), 307 (M^+, 40%)

Elemental analysis: for $C_{20}H_{21}NS$: calcd. C 78.13, H 6.88, N 4.56 ; found C 78.48; H 7.10; N 4.13

General procedure for the preparation of Arylmethylphenylpiperazine derivatives

To 5 mmol of the halomethylaryl derivative was added 5 mmol of the corresponding phenylpiperazine, 1.5g (15 mmol) TEA, and 40 ml Acetonitrile. The reaction mixture was allowed to reflux for 48 hours under inert atmosphere then left to cool to room temperature. The organic solvent was then evaporated under reduced pressure and the oily residue obtained was subjected to purification on Silica gel column chromatography eluting with Methylene chloride: Methanol 100: 1.

1-Phenyl-4-thiophen-2-yl-methylpiperazine (31a)



Yield: 0.9g, 71%, brown crystals

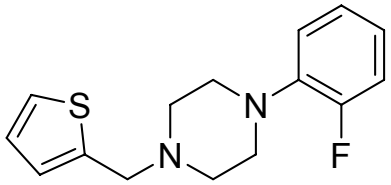
m.p.: 75-77 ^{o}C

^{1}H-NMR ($CDCl_3$): 2.68 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.24 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 4.08 (s, 2H, -CH_2), 6.85- 7.01 (m, 5H, aromatic), 7.25- 7.32 (m, 3H, aromatic)

GC/MS: m/z 97 (100%), m/z 125 (15 %), m/z 258 (M^+, 10%).

Elemental analysis: for $C_{15}H_{18}N_2S.0.25H_2O$: calcd. C 68.57, H 6.85, N 10.66 ; found C 68.49; H 7.16; N 10.45.

1-(2-Fluoro-phenyl)-4-thiophen-2-ylmethylpiperazine (31b)



Yield: 1g, 78%, brown crystals.

m.p.: 60- 62 ^{o}C.

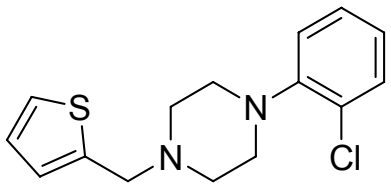
^{1}H-NMR ($CDCl_3$): 2.71 (t, 4H, J= 4.7Hz, 2 X –CH_2, piperazine), 3.16 (t, 4H, J= 4.7Hz, 2 X –CH_2, piperazine), 3.82 (s, 2H, -CH_2), 6.66- 7.05 (m, 7H, aromatic)

IR (cm^{-1}): 1203 (C-F)

GC/MS: m/z 97 (100%), m/z 125 (70%), m/z 179 (20%), m/z 276 (M^+, 20%).

Elemental analysis: for $C_{15}H_{17}FN_2S.0.1H_2O$ calcd. C 64.79, H 6.11, N 10.07 ; found C 64.75; H 6.09; N 9.83.

1-(2-Chlorophenyl)-4-thiophen-2-ylmethylpiperazine (31c)



Yield: 1g, 71%, brown crystals.

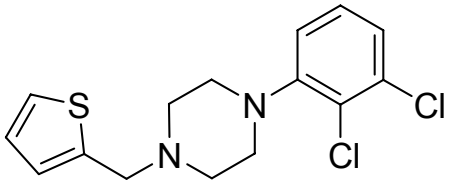
m.p.: 68-70 ^{o}C.

^{1}H-NMR ($CDCl_3$): 2.72 (t, 4H, J= 4.5Hz, 2 X –CH_2, piperazine), 3.10 (t, 4H, J= 4.5Hz, 2 X –CH_2, piperazine), 3.81 (s, 2H, -CH_2), 6.93-7.07 (m, 4H, aromatic), 7.19- 7.26 (m, 3H, aromatic)

GC/MS : m/z 97 (100%), m/z 125 (90%), m/z 195 (25%), m/z 292 (M^+, 10%).

Elemental analysis: for $C_{15}H_{17}ClN_2S.0.2H_2O$ calcd. C 60.89, H 5.75, N 9.47 ; found C 60.58; H 5.39; N 9.22.

1-(2,3-Dichlorophenyl)-4-thiophen-2-ylmethylpiperazine (31d)



Yield: 1.2g, 70%, brown crystals

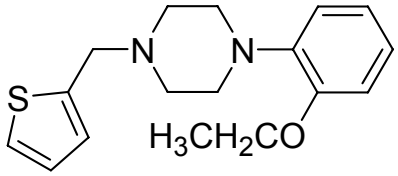
m.p.: 64- 66 ^{o}C.

^{1}H-NMR ($CDCl_3$): 2.69 (brs, 4H, 2 X –CH_2, piperazine), 3.08 (brs, 4H, 2 X –CH_2, piperazine), 3.81 (s, 2H, -CH_2), 6.94-6.97 (m, 3H, aromatic), 7.13- 7.26 (m, 3H, aromatic)

GC/MS : m/z 97 (100%), m/z 125 (30%), m/z 174 (10%), m/z 326 (M^+, 5%).

Elemental analysis: for $C_{15}H_{16}Cl_2N_2S.0.7H_2O$ calcd. C 53.16, H 4.72, N 8.26 ; found C 52.83; H 4.64; N 8.15.

1-(2-Ethoxyphenyl)-4-thiophen-2-ylmethylpiperazine (31e)



Yield: 1.1g, 72%, brown crystals.

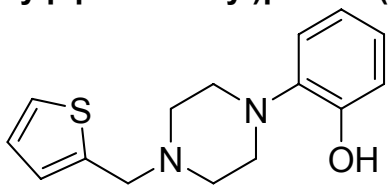
m.p.: 105-107 ^{o}C.

^{1}H-NMR ($CDCl_3$): 1.40 (t, 3H, J= 7Hz, -OCH_2**CH_3**), 2.73 (brs, 4H, 2 X –CH_2, piperazine), 3.15 (brs, 4H, 2 X –CH_2, piperazine), 3.81 (s, 2H, -CH_2), 4.05 (q, 2H, J= 7Hz, 2H, -O**CH_2**CH_3), 6.82- 7.26 (m, 7H, aromatic)

GC/MS : m/z 97 (100%), m/z 120 (65%), m/z 150 (60%), m/z 302 (M^+, 10%).

Elemental analysis: for $C_{17}H_{22}N_2OS.0.95H_2O$ calcd. C 63.92, H 6.89, N 8.77 ; found C 63.53; H 7.18; N 8.97.

2-(4-Thiophen-2-ylmethylpiperazin-1-yl)phenol (31f)



Yield: 0.8g, 63%, brown crystals

m.p.: 99-101 ^{o}C.

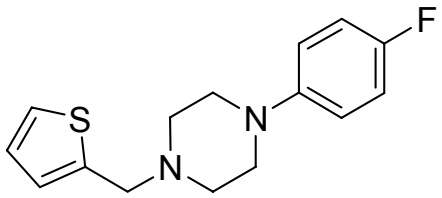
^{1}H-NMR ($CDCl_3$): 2.63 (brs, 4H, 2 X –CH_2, piperazine), 3.08 (brs, 4H, 2 X –CH_2, piperazine), 4.28 (s, 2H, -CH_2), 6.72- 7.02 (m, 7H, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3235 (OH)

GC/MS : m/z 97 (100%), m/z 120 (70%), m/z 274 (M^+, 15%).

Elemental analysis: for $C_{15}H_{18}N_2OS$ calcd. C 65.66, H 6.03, N 10.21 ; found C 65.93; H 6.15; N 9.98.

1-(4-Fluorophenyl)-4-thiophen-2-ylmethylpiperazine (31g)



Yield: 1g, 74%, brown crystals.

m.p.: 73-75 ^{o}C.

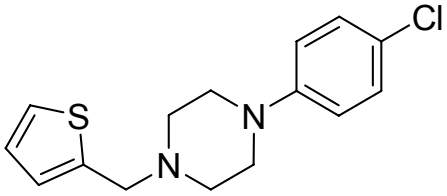
^{1}H-NMR ($CDCl_3$): 2.65 (t, 4H, J= 4.85Hz, 2 X –CH_2, piperazine), 3.12 (t, 4H, J= 4.85Hz, 2 X –CH_2, piperazine), 3.78 (s, 2H, -CH_2), 6.83- 6.25 (m, 7H, aromatic)

IR (cm^{-1}): 1210 (C-F)

GC/MS : m/z 97 (100%), m/z 125 (40%), m/z 179 (15%), m/z 276 (M^+, 15%).

Elemental analysis: for $C_{15}H_{17}FN_2S.0.7H_2O$ calcd. C 62.29, H 5.89, N 9.70 ; found C 62.18; H 5.85; N 9.29.

1-(4-Chlorophenyl)-4-thiophen-2-ylmethylpiperazine (31h)



Yield: 0.9g, 67%, brown crystals.

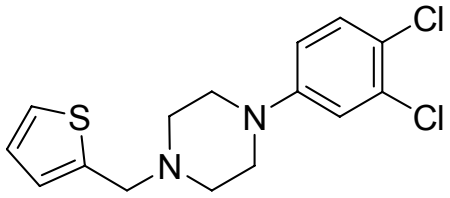
m.p.: 76-78 ^oC.

^{1}H-NMR ($CDCl_3$): 2.64 (t, 4H, J= 5Hz, 2 X $-CH_2$, piperazine), 3.17 (t, 4H, J= 5Hz, 2 X $-CH_2$, piperazine), 3.78 (s, 2H, $-CH_2$), 6.89 (d, 2H, J= 7Hz, aromatic), 6.96 (d, 2H, J= 7Hz, aromatic), 7.16- 7.25 (m, 3H, aromatic)

GC/MS : m/z 97 (100%), m/z 125 (90%), m/z 195 (55%), m/z 292 (M^+, 40%).

Elemental analysis: for $C_{15}H_{17}ClN_2S.0.2H_2O$ calcd. C 60.89, H 5.75, N 9.47 ; found C 60.82; H 6.26; N 9.46.

1-(3,4-Dichlorophenyl)-4-thiophen-2-ylmethylpiperazine (31i)



Yield: 0.9g, 61%, brown crystals.

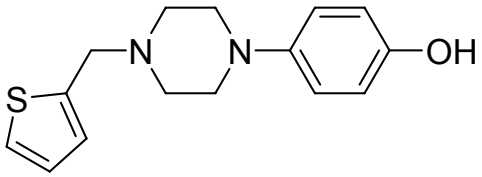
m.p.: 67-69 ^oC.

^{1}H-NMR ($CDCl_3$): 2.64 (t, 4H, J= 5Hz, 2 X $-CH_2$, piperazine), 3.19 (t, 4H, J= 5Hz, 2 X $-CH_2$, piperazine), 3.67 (s, 2H, $-CH_2$), 6.72 (dd, 1H, aromatic), 6.93- 6.98 (m, 3H, aromatic), 7.24- 7.27 (m, 2H, aromatic)

GC/MS : m/z 97 (100%), m/z 125 (70%), m/z 172 (30%), m/z 326 (M^+, 10%).

Elemental analysis: for $C_{15}H_{16}Cl_2N_2S.0.2H_2O$ calcd. C 54.61, H 4.85, N 8.49 ; found C 54.42; H 5.04; N 8.05.

4-(4-Thiophen-2-ylmethylpiperazin-1-yl)phenol (31j)



Yield: 0.9g, 68%, brown crystals

m.p.: 101- 103 ^oC.

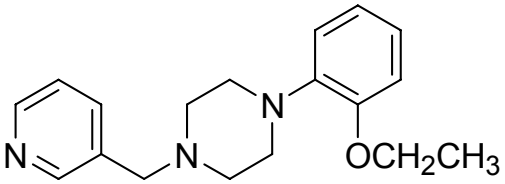
^{1}H-NMR ($CDCl_3$): 2.64 (brs, 4H, 2 X $-CH_2$, piperazine), 3.09 (brs, 4H, 2 X $-CH_2$, piperazine), 4.30 (s, 2H, $-CH_2$), 6.71-6.96 (m, 7H, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3200 (OH)

GC/MS : m/z 97 (100%), m/z 120 (40%), m/z 274 (M^+, 15%).

Elemental analysis: for $C_{15}H_{18}N_2OS$ calcd. C 65.66, H 6.03, N 10.21 ; found C 65.86; H 6.23; N 9.94.

1-(2-Ethoxyphenyl)-4-pyridin-3-ylmethylpiperazine (32a)



Yield: 1.3g, 85%, yellow crystals

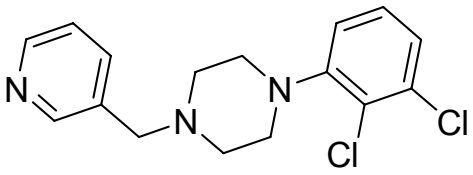
m.p. 82- 84 ^{o}C

^{1}H-NMR ($CDCl_3$): 1.44 (t, 3H, J= 6.75Hz, -OCH_2**CH_3**), 2.68 (t, 4H, 2 X –CH_2, piperazine), 3.13 (brs, 4H, 2 X –CH_2, piperazine), 3.62 (s, 2H, -CH_2), 4.05 (q, 2H, J= 6.75Hz, 2H, -O**CH_2**CH_3), 6.82- 6.99 (m, 4H, aromatic), 7.25- 7.30 (m, 1H, aromatic), 7.75 (d, 1H, J= 7.75, aromatic), 8.52 (d, 1H, J= 3.5, aromatic).

GC/MS : m/z 92 (85%), m/z 120 (100%), m/z 150 (60%), m/z 297 (M^+, 10%).

Elemental analysis: for $C_{18}H_{23}N_3O$ calcd. C 72.70, H 7.80, N 14.13 ; found C 72.28; H 8.26; N 13.76.

1-(2,3-Dichlorophenyl)-4-pyridin-3-ylmethylpiperazine (32b)



Yield: 1.3g, 83%, yellowish white crystals

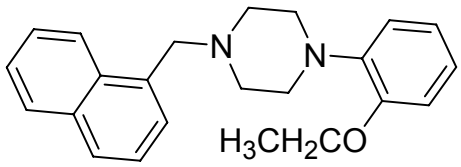
m.p. 78- 80 ^{o}C

^{1}H-NMR ($CDCl_3$): 2.75 (t, 4H, J= 4.5, 2 X –CH_2, piperazine), 3.16 (t, 4H, J= 4.5, 2 X –CH_2, piperazine), 3.69 (s, 2H, -CH_2), 7.01-7.56 (m, 4H, aromatic), 7.81 (d, 1H, J= 7.75, aromatic), 8.62 (d, 2H, J= 4, aromatic).

GC/MS : m/z 92 (90%), m/z 120 (100%), m/z 174 (30%), m/z 321 (M^+, 10%).

Elemental analysis: for $C_{16}H_{17}Cl_2N_3$ calcd. C 59.64, H 5.32, N 13.04 ; found C 59.33; H 5.70; N 13.04.

1-(2-Ethoxyphenyl)-4-naphthalen-1-ylmethylpiperazine (33a)



Yield: 1.4g, 84%, yellow crystals

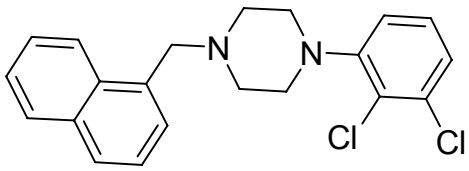
m.p. 90-92^{o}C

^{1}H-NMR ($CDCl_3$): 1.47 (t, 3H, J= 7Hz, -OCH_2**CH_3**), 2.74 (t, 4H, 2 X –CH_2, piperazine), 3.12 (brs, 4H, 2 X –CH_2, piperazine), 4.01 (s, 2H, -CH_2), 4.08 (q, 2H, J= 7Hz, 2H, -O**CH_2**CH_3), 6.83- 7.01 (m, 4H, aromatic), 7.40- 7.57 (m, 4H, aromatic), 7.82- 8.35 (m, 3H, aromatic).

GC/MS : m/z 65 (20%), m/z 115 (45%), m/z 141 (100%), m/z 346 (M^+, 5%).

Elemental analysis: for $C_{23}H_{26}N_2O$ calcd. C 79.73, H 7.56, N 8.09 ; found C 79.20; H 7.98; N 8.23.

1-(2,3-Dichlorophenyl)-4-naphthalen-1-ylmethylpiperazine (33b)



Yield: 1.5g, 81%, yellow crystals

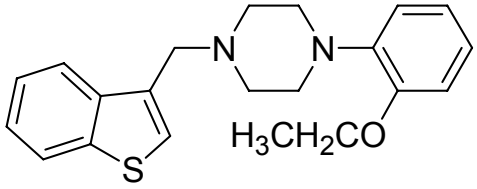
m.p. 83-85 ^{o}C

^{1}H-NMR ($CDCl_3$): 2.72 (brs, 4H, 2 X –CH_2, piperazine), 3.05 (brs, 4H, 2 X –CH_2, piperazine), 4.03 (s, 2H, -CH_2), 6.91- 7.14 (m, 3H, aromatic), 7.39- 7.56 (m, 4H, aromatic), 7.78- 8.34 (m, 3H, aromatic).

GC/MS : m/z 115 (95%), m/z 141 (100%), m/z 370 (M^+, 5%).

Elemental analysis: for $C_{21}H_{20}Cl_2N_2$ calcd. C 67.93, H 5.43, N 7.54 ; found C 67.46; H 5.87; N 7.68.

1-Benzo[b]thiophen-3-ylmethyl-4-(2-ethoxyphenyl)piperazine (34a)[128]



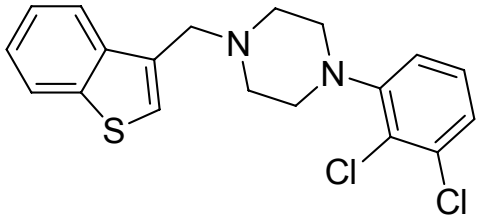
Yield: 1.3g, 77%, yellow resin

^{1}H-NMR ($CDCl_3$): 1.46 (t, 3H, J= 7Hz, -OCH_2**CH_3**), 2.73 (brs, 4H, 2 X –CH_2, piperazine), 2.74 (brs, 4H, 2 X –CH_2, piperazine), 3.83 (s, 2H, -CH_2), 4.07 (q, 2H, J= 7Hz, 2H, -O**CH_2**CH_3), 6.83- 8.03 (m, 9H, aromatic).

GC/MS : m/z 122 (100%), m/z 210 (30%), m/z 352 (M^+, 15%).

Elemental analysis: for $C_{21}H_{24}N_2OS$ calcd. C 71.55, H 6.86, N 7.95 ; found C 71.08; H 6.68; N 7.72.

1-Benzo[b]thiophen-3-ylmethyl-4-(2,3-dichlorophenyl)-piperazine (34b)[128]



Yield: 1.5g, 79%, pale yellow crystals

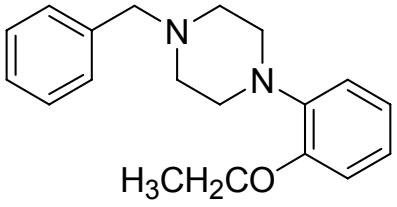
m.p. 98- 100 ^{O}C

^{1}H-NMR ($CDCl_3$): 2.71 (brs, 4H, 2 X –CH_2, piperazine), 3.07 (brs, 4H, 2 X –CH_2, piperazine), 3.84 (s, 2H, -CH_2), 6.92- 7.86 (m, 9H, aromatic).

GC/MS : m/z 122 (100%), m/z 237 (20%), m/z 377 (M^+, 10%).

Elemental analysis: for $C_{19}H_{18}Cl_2N_2S$ calcd. C 60.48, H 4.81, N 7.42 ; found C 60.22; H 4.88; N 7.43.

1-Benzyl-4-(2-ethoxyphenyl)piperazine (35a)



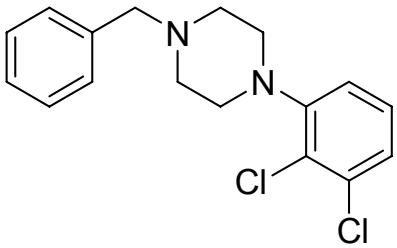
Yield: 1.1g, 77%, yellow resin.

^{1}H-NMR ($CDCl_3$): 1.40 (t, 3H, J= 7Hz, -OCH_2**CH_3**), 2.67 (t, 4H, 2 X –CH_2, piperazine), 3.13 (brs, 4H, 2 X –CH_2, piperazine), 3.59 (s, 2H, -CH_2), 4.02 (q, 2H, J= 7Hz, 2H, -O**CH_2**CH_3), 6.83- 7.00 (m, 4H, aromatic), 7.26- 7.36 (m, 5H, aromatic)

GC/MS : m/z 91 (100%), m/z 150 (90%), m/z 296 (M^+, 20%)

Elemental analysis: for $C_{19}H_{24}N_2O$ calcd. C 76.99, H 8.16, N 9.45 ; found C 76.58; H 8.15; N 9.38.

1-Benzyl-4-(2,3-dichlorophenyl)piperazine (35b)



Yield: 1.2g, 74%, pale yellow crystals

m.p. 107-109 ^{O}C

^{1}H-NMR ($CDCl_3$): 2.67 (t, 4H, J= 4.75Hz, 2 X –CH_2, piperazine), 3.07 (t, 4H, J= 4.75Hz, 2 X –CH_2, piperazine), 3.59 (s, 2H, -CH_2), 6.97 (dd, 1H, J= 3.5Hz, 7Hz, aromatic), 7.15 (d, 2H, J= 7Hz, aromatic), 7.26- 7.36 (m, 5H, aromatic)

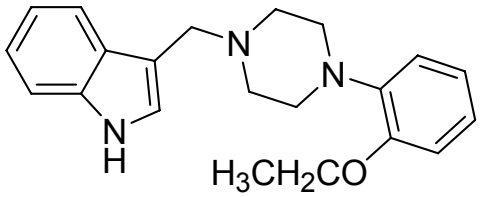
GC/MS : m/z 91 (100%), m/z 174 (10%), m/z 320 (M^+, 5%)

Elemental analysis: for $C_{17}H_{18}Cl_2N_2$ calcd. C 63.56, H 5.65, N 8.72 ; found C 63.62; H 5.81; N 9.06.

Procedure for the preparation of 3-[4-Phenylpiperazin-1-ylmethyl]-1H-indole derivatives

Indole (1.2g, 10 mmol) together with 10 mmol of the corresponding phenyl piperazine free base and 10g (300 mmol) of HCHO 37% were mixed with 5 ml glacial acetic acid at 0 ^{O}C for 1 hour. The mixture was then alkalinized with 5M NaOH and extracted twice with 30 ml diethyl ether. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude residue was then subjected to Silica gel column chromatography eluting with Methylene chloride: Methanol 9.5: 0.5.

3-[4-(2-Ethoxyphenyl)-piperazin-1-ylmethyl]-1H-indole (36a)



Yield: 2.2g, 66%, yellowish brown crystals

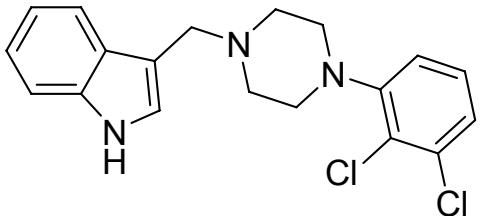
m.p. 52- 54^{o}C

^{1}H-NMR ($CDCl_3$): 1.36 (t, 3H, J= 7Hz, -OCH_2**CH_3**), 3.15 (brs, 4H, 2 X –CH_2, piperazine), 3.23 (brs, 4H, 2 X –CH_2, piperazine), 3.98 (q, 2H, J= 7Hz, 2H, -O**CH_2**CH_3), 4.41 (s, 2H, -CH_2), 6.77- 6.96 (m, 4H, aromatic), 7.09- 7.16 (m, 2H, aromatic), 7.26- 7.56 (m, 3H, aromatic), 8.78 (s, 1H, NH).

GC/MS : m/z 77 (60%), m/z 120 (100%), m/z 164 (70%), m/z 206 (40%), m/z 335 (M^+, 2%).

Elemental analysis: for $C_{21}H_{25}N_3O$ calcd. C 75.19, H 7.51, N 12.53 ; found C 74.53; H 7.90; N 12.94.

3-[4-(2,3-Dichlorophenyl)-piperazin-1-ylmethyl]-1H-indole (36b)



Yield: 2.1g, 58%, yellowish white crystals

m.p. 49-51 ^{o}C

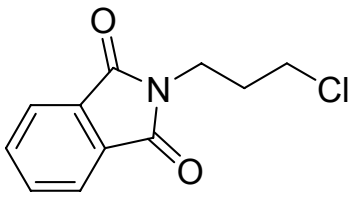
^{1}H-NMR ($CDCl_3$): 2.06 (brs, 4H, 2 X $-CH_2$, piperazine), 2.28 (brs, 4H, 2 X $-CH_2$, piperazine), 3.84 (s, 2H, $-CH_2$), 6.93- 7.79 (m, 8H, aromatic), 8.24 (brs, 1H, NH).

GC/MS : m/z 77 (40%), m/z 120 (100%), m/z 231 (80%), m/z 206 (40%), m/z 360 (M^+, 1%).

Elemental analysis: for $C_{19}H_{19}Cl_2N_3$ calcd. C 63.34, H 5.32, N 11.66 ; found C 63.93; H 5.65; N 12.01.

Procedure for the preparation of 2-(3-Chloropropyl)-isoindole-1,3-dione (46)

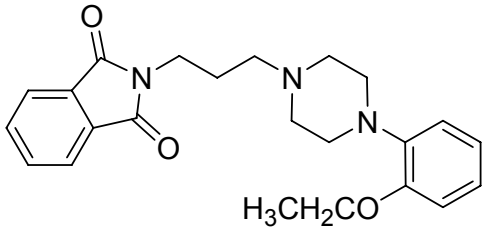
A mixture of 1.48g (10 mmol) of Phthalic anhydride and 1.43g (11 mmol) of 3-Chloropropylamine hdrochloride salt was heated in an oil bath at 160 ^{o}C till fusion occurred. The fused mixture was maintained at the same temperature for 15 minutes. The reaction mixture was cooled to room temperature and 30 ml water was added just before solidification to form slurry. The product was filtered, washed twice with water and purified on Silica gel column chromatography eluting with Methylene chloride. The yield and Spectroscopy data of the intermediate matched data found in literature [142].



General procedure for the preparation of 2-[3-(4-Arylpiperazin-1-yl)-propyl]isoindole-1,3-dione derivatives

To a solution of 1.0g (4.5 mmol) of **(46)** in 40 ml dry acetonitrile, were added 4.5 mmol of the corresponding phenyl piperazine and 1.5g (15 mmol) of TEA. The reaction mixture was allowed to reflux under innert atmosphere for 48 hours and then left to cool to room temperature. The organic solvent was removed under reduced pressure and the remained residue was subjected to purification on Silica gel column chromatography eluting with Methylene chloride: Methanol 100: 2.

2-{3-[4-(2-Ethoxyphenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40a)



Yield: 1.3g, 71%, yellow crystals

m.p. 109- 111 ^{o}C

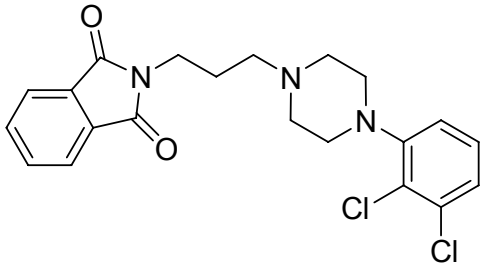
^{1}H-NMR ($CDCl_3$): 1.43 (t, 3H, J= 7 Hz, -OCH_2**CH_3**), 1.89- 1.95 (m, 2H, CH_2, C2, propyl), 2.50 (t, 2H, J= 6.9 Hz, CH_2, C3, propyl), 2.59 (brs, 4H, 2 X –CH_2, piperazine), 2.96 (brs, 4H, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 6.9 Hz, CH_2, C1, propyl), 4.05 (q, 2H, J= 7Hz, -O**CH_2**CH_3), 6.78-6.96 (m, 4H, aromatic), 7.68- 7.82 (m, 4H, aromatic)

IR (cm^{-1}): 1708, 1737 (2 X C=O)

GC/MS : m/z 77 (10%), m/z 104 (5%), m/z 130 (10%), m/z 191 (60%), m/z 219 (90%), m/z 378 (40%), m/z 393 (M^+, 100%)

Elemental analysis: for $C_{23}H_{27}N_3O_2$ calcd. C 70.21, H 6.92, N 10.68 ; found C 69.74; H 7.08; N 10.89.

2-{3-[4-(2,3-Dichlorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40b)[142]



Yield: 1.3g, 70%, yellowish white crystals

m.p. 103- 105 ^{o}C

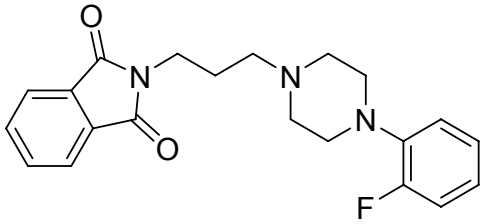
^{1}H-NMR ($CDCl_3$): 1.88- 1.96 (m, 2H, CH_2, C2, propyl), 2.51 (t, 2H, J= 6.9 Hz, CH_2, C3, propyl), 2.57 (brs, 4H, 2 X –CH_2, piperazine), 2.90 (brs, 4H, 2 X –CH_2, piperazine), 3.80 (t, 2H, J= 6.9 Hz, CH_2, C1, propyl), 6.80- 7.15 (m, 3H, aromatic), 7.70 (dd, 2H, J= 3, 5.5 Hz, aromatic), 7.85 (dd, 2H, J= 3, 5.5 Hz, aromatic)

IR (cm^{-1}): 1711, 1766 (2 X C=O)

GC/MS : m/z 77 (20%), m/z 104 (100%), m/z 130 (40%), m/z 174 (10%), m/z 243 (80%), m/z 269 (20%), m/z 417 (M^+, 90%), m/z 419 (M^++2, 55%), m/z 421 (M^++4, 20%)

Elemental analysis: for $C_{21}H_{21}Cl_2N_3O_2$ calcd. C 60.30, H 5.06, N 10.05 ; found C 60.44; H 4.90; N 9.98.

2-{3-[4-(2-Fluorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40c)



Yield: 1.2g, 71%, yellow crystals

m.p. 96-98 ^{o}C

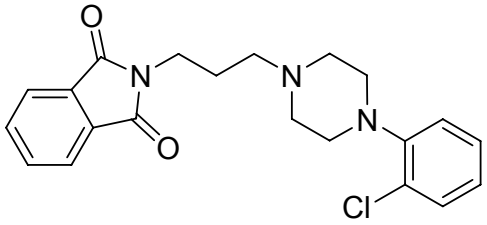
^{1}H-NMR ($CDCl_3$): 1.84- 1.95 (m, 2H, CH_2, C2, propyl), 2.48 (t, 2H, J= 6.8 Hz, CH_2, C3, propyl), 2.56 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 2.94 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 6.8 Hz, CH_2, C1, propyl), 6.79- 7.05 (m, 4H, aromatic), 7.67- 7.72 (m, 4H, aromatic), 7.67- 7.72 (m, 4H, aromatic)

IR (cm^{-1}): 1709, 1765 (2 X C=O), 1200 (C-F)

GC/MS : m/z 77 (80%), m/z 104 (100%), m/z 130 (20%), m/z 193 (60%), m/z 352 (10%), m/z 368 (M^+, 30%)

Elemental analysis: for $C_{21}H_{22}FN_3O_2$ calcd. C 68.65, H 6.04, N 11.44 ; found C 69.02; H 6.11; N 11.41.

2-{3-[4-(2-Chlorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40d)



Yield: 1.1g, 66%, yellow crystals

m.p. 127-129 ^oC

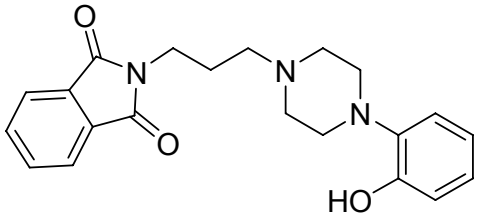
^{1}H-NMR ($CDCl_3$): 1.84- 1.95 (m, 2H, CH_2, C2, propyl), 2.49 (t, 2H, J= 6.85 Hz, CH_2, C3, propyl), 2.56 (brs, 4H, 2 X –CH_2, piperazine), 2.89 (brs, 4H, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 6.85 Hz, CH_2, C1, propyl), 6.87- 7.20 (m, 4H, aromatic), 7.26- 7.72 (m, 4H, aromatic)

IR (cm^{-1}): 1714, 1779 (2 X C=O)

GC/MS : m/z 77 (35%), m/z 104 (100%), m/z 130 (20%), m/z 209 (85%), m/z 348 (20%), m/z 383 (M^+, 35%), m/z 385 (M^++2, 10%)

Elemental analysis: for $C_{21}H_{22}ClN_3O_2$ calcd. C 65.71, H 5.78, N 10.95 ; found C 65.32; H 5.41; N 10.76.

2-{3-[4-(2-Hydroxyphenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40e)



Yield: 1.0g, 60%, yellow crystals

m.p. 135- 137 ^oC

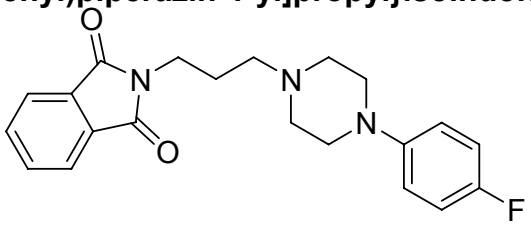
^{1}H-NMR ($CDCl_3$): 1.88- 1.93 (m, 2H, CH_2, C2, propyl), 2.49 (t, 2H, J= 6.7 Hz, CH_2, C3, propyl), 2.53 (brs, 4H, 2 X –CH_2, piperazine), 2.70 (brs, 4H, 2 X –CH_2, piperazine), 3.81 (t, 2H, J= 6.7 Hz, CH_2, C1, propyl), 6.81- 7.04 (m, 4H, aromatic), 7.74 (dd, 2H, J= 3, 5.4 Hz, aromatic), 7.87 (dd, 2H, J= 3, 5.4 Hz, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3220 (-OH), 1712, 1769 (2 X C=O)

GC/MS : m/z 77 (20%), m/z 104 (10%), m/z 163 (100%), m/z 217 (95%), m/z 230 (40%), m/z 350 (30%), m/z 365 (M^+, 20%)

Elemental analysis: for $C_{21}H_{23}N_3O_3$ calcd. C 69.02, H 6.34, N 11.50 ; found C 68.78; H 6.32; N 11.24.

2-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40f)



Yield: 1.2g, 72%, brown crystals

m.p. 103-105 ^oC

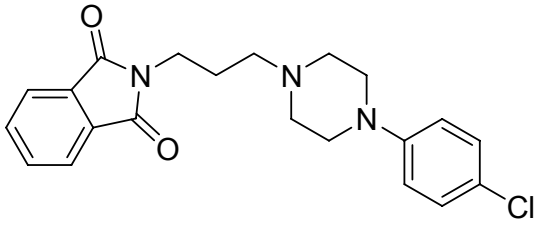
^{1}H-NMR ($CDCl_3$): 1.76- 1.95 (m, 2H, CH_2, C2, propyl), 2.47 (t, 2H, J= 6.9 Hz, CH_2, C3, propyl), 2.53 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 2.95 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 6.9 Hz, CH_2, C1, propyl), 6.76- 6.96 (m, 4H, aromatic), 7.68 (dd, 2H, J= 3, 5.5 Hz Aromatic), 7.83 (dd, 2H, J= 3, 5.5 Hz Aromatic)

IR (cm^{-1}): 1707, 1758 (2 X C=O), 1214 (C-F)

GC/MS : m/z 77 (90%), m/z 104 (100%), m/z 130 (50%), m/z 193 (30%), m/z 352 (15%), m/z 368 (M^+, 35%)

Elemental analysis: for $C_{21}H_{22}FN_3O_2$ calcd. C 68.65, H 6.04, N 11.44 ; found C 68.70; H 5.80; N 11.07.

2-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40g)



Yield: 1.3g, 72%, pale yellow crystals

m.p. 119-121 ^{o}C

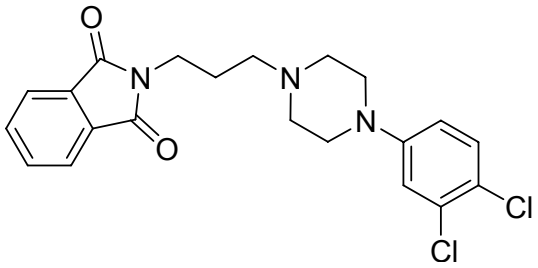
^{1}H-NMR ($CDCl_3$): 1.84- 1.95 (m, 2H, CH_2, C2, propyl), 2.46 (t, 2H, J= 7 Hz, CH_2, C3, propyl), 2.52 (t, 4H, J= 5.1 Hz, 2 X –CH_2, piperazine), 2.98 (t, 4H, J= 5.1 Hz, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 7 Hz, CH_2, C1, propyl), 6.75 (d, 2H, J= 4.7 Hz, aromatic), 7.16 (d, 2H, J= 4.7 Hz Aromatic), 7.66- 7.84 (m, 4H, aromatic)

IR (cm^{-1}): 1701, 1774 (2 X C=O)

GC/MS : m/z 77 (40%), m/z 104 (100%), m/z 130 (10%), m/z 209 (90%), m/z 348 (15%), m/z 383 (M^+, 50%), m/z 385 (M^++2, 15%)

Elemental analysis: for $C_{21}H_{22}ClN_3O_2$ calcd. C 65.71, H 5.78, N 10.95 ; found C 65.83; H 5.68; N 11.37.

2-{3-[4-(3,4-Dichlorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40h)



Yield: 1.3g, 67%, yellow crystals

m.p. 88- 90 ^{o}C

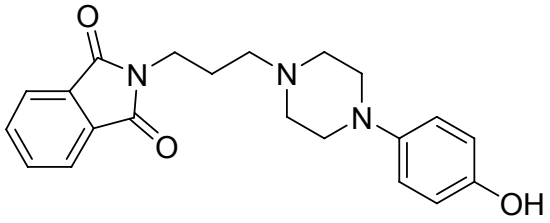
^{1}H-NMR ($CDCl_3$): 1.83- 1.94 (m, 2H, CH_2, C2, propyl), 2.46 (t, 2H, J= 6.8 Hz, CH_2, C3, propyl), 2.51 (t, 4H, J= 5.2 Hz, 2 X –CH_2, piperazine), 2.99 (t, 4H, J= 5.2 Hz, 2 X –CH_2, piperazine), 3.79 (t, 2H, J= 6.8 Hz, CH_2, C1, propyl), 6.67 (dd, 1H, J= 2.85, 9 Hz, aromatic), 6.85 (d, 1H, J= 2.85 Hz, aromatic), 7.23 (d, 1H, J= 9 Hz, aromatic), 7.67- 7.83 (m, 4H, aromatic)

IR (cm^{-1}): 1713, 1760 (2 X C=O)

GC/MS : m/z 77 (50%), m/z 104 (100%), m/z 130 (10%), m/z 174 (20%), m/z 243 (90%), m/z 269 (15%), m/z 417 (M^+, 90%), m/z 419 (M^++2, 55%), m/z 421 (M^++4, 20%)

Elemental analysis: for $C_{21}H_{21}Cl_2N_3O_2$ calcd. C 60.30, H 5.06, N 10.05 ; found C 60.20; H 4.95; N 9.99.

2-{3-[4-(4-Hydroxyphenyl)piperazin-1-yl]propyl}isoindole-1,3-dione (40i)



Yield: 1.0g, 62%, brown crystals

m.p. 145- 147 ^oC

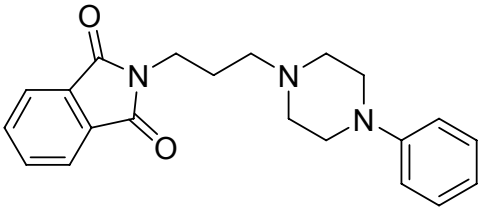
^{1}H-NMR ($CDCl_3$): 1.85- 1.96 (m, 2H, CH_2, C2, propyl), 2.51 (t, 2H, J= 6.9 Hz, CH_2, C3, propyl), 2.55 (t, 4H, J= 4.7 Hz, 2 X –CH_2, piperazine), 2.90 (t, 4H, J= 6.7 Hz, 2 X –CH_2, piperazine), 3.78 (t, 2H, J= 6.9 Hz, CH_2, C1, propyl), 6.69- 7.77 (m, 4H, aromatic), 7.69 (dd, 2H, J= 3, 5.5 Hz, aromatic), 7.83 (dd, 2H, J= 3, 5.5 Hz, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3209 (-OH), 1704, 1759 (2 X C=O)

GC/MS : m/z 77 (20%), m/z 104 (10%), m/z 163 (100%), m/z 217 (80%), m/z 230 (50%), m/z 350 (40%), m/z 365 (M^+, 25%)

Elemental analysis: for $C_{21}H_{23}N_3O_3$ calcd. C 69.02, H 6.34, N 11.50 ; found C 68.71; H 6.24; N 11.62.

2-[3-(4-Phenyl piperazin-1-yl)propyl]isoindole-1,3-dione (40j)



Yield: 1.2g, 76%, yellow crystals

m.p. 105-107 ^oC

^{1}H-NMR ($CDCl_3$): 1.86- 1.97 (m, 2H, CH_2, C2, propyl), 2.50 (t, 2H, J= 7 Hz, CH_2, C3, propyl), 2.56 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.06 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.80 (t, 2H, J= 7 Hz, CH_2, C1, propyl), 6.80- 7.26 (m, 5H, aromatic), 7.68 (dd, 2H, J= 3, 5.4 Hz, aromatic), 7.84 (dd, 2H, J= 3, 5.4 Hz, aromatic)

IR (cm^{-1}): 1703, 1760 (2 X C=O)

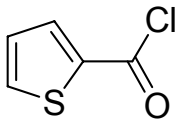
GC/MS : m/z 77 (95%), m/z 104 (100%), m/z 130 (50%), m/z 160 (60%), m/z 175 (30%), m/z 349 (M^+, 10%)

Elemental analysis: for $C_{21}H_{23}N_3O_2$ calcd. C 72.18, H 6.63, N 12.03 ; found C 72.09; H 6.67; N 11.98.

General procedure for the preparation of 3-(4-Arylpiperazin-1-yl) propylamine derivatives (48a-j)

A solution of 2 mmol of the corresponding isoindole-1,3-dione derivative and 0.25g (6 mmol) hydrazine hydrate 80% in 20 ml ethanol was heated to reflux for 5 hours. After cooling to room temperature, any insoluble material was filtered off, washed with ethanol (2 X 20 ml) and the filterate was evaporated under reduced pressure. The product was extracted with chloroform (2 X 30 ml) and the desired amine obtained was introduced to the following reaction without further purification.

Procedure for the preparation of Thiophene-2-carbonyl chloride (50)



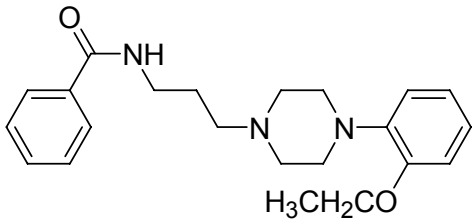
To a 100-ml round bottom flask containing 0.5g (4 mmol) of thiophene-2-carboxylic acid was added slowly and with continuous stirring 10g (85 mmol) of thionyl chloride. After complete addition, the reaction mixture was allowed to reflux for 6 hours. The flask was then cooled to room temperature, 50 ml of water was added, and the desired organic product was extracted with

Methylene chloride (2X50 ml). The organic layers were collected and dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The final product was retrieved in a form of brownish black oil and was used for the further reaction without extra purification.

General procedure for the preparation of Arylamidopropylphenyl-piperazin derivatives

A solution of 2 mmol of thiophene-2-carbonyl chloride (0.30g) or benzoyl chloride (0.28g) in 10 ml dry THF was added slowly to a solution of the corresponding amine derivative (2.3 mmol) and TEA (0.5g, 5 mmol) in dry THF (30 ml) at 0 ^oC. The mixture was then allowed to stir at room temperature for 5 hours. The reaction mixture was then poured into 30 ml water and extracted with Methylene chloride (2 X 40ml). The organic layers were collected and dried over anhydrous Na_2SO_4 and evaporated to yield a residue of the desired product that was purified on Silica gel column chromatography using Methylene chloride : Methanol 200 : 3 as mobile phase.

N-{3-[4-(2-Ethoxyphenyl)piperazin-1-yl]propyl}benzamide (42a)



Yield: 0.53g, 72%, yellow crystals

m.p. 121- 123 ^oC

^{1}H-NMR ($CDCl_3$): 1.44 (t, 3H, J= 7 Hz, -OCH_2**CH_3**), 1.80- 1.87 (m, 2H, CH_2, C2, propyl), 2.64 (t, 2H, J= 5.75 Hz, CH_2, C3, propyl), 2.71 (brs, 4H, 2 X –CH_2, piperazine), 3.11 (brs, 4H, 2 X –CH_2, piperazine), 3.59 (q, 2H, J= 5.5 Hz, CH_2, C1, propyl), 4.06 (q, 2H, J= 7 Hz, -O**CH_2**CH_3), 6.84- 6.99 (m, 4H, aromatic), 7.35- 7.50 (m, 3H, aromatic), 7.74- 7.83 (m, 2H, aromatic), 8.31 (brs, 1H,

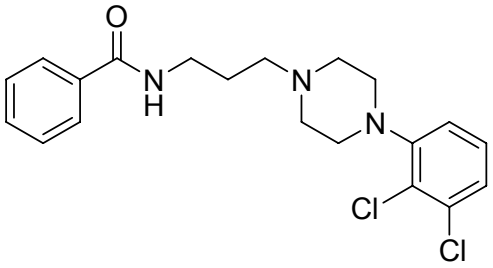
-NH)

IR (cm^{-1}): 1637 (C=O)

GC/MS : m/z 77 (100%), m/z 105 (95%), m/z 120 (95%), m/z 219 (20%), m/z 352 (15%), m/z 367 (M^+, 10%)

Elemental analysis: for $C_{22}H_{29}N_3O_2$ calcd. C 71.90, H 7.95, N 11.43 ; found C 71.38; H 7.74; N 10.81.

N-{3-[4-(2,3-Dichlorophenyl)piperazin-1-yl]propyl}benzamide (42b)



Yield: 0.6g, 78%, yellowish white crystals

m.p. 112- 114 ^oC

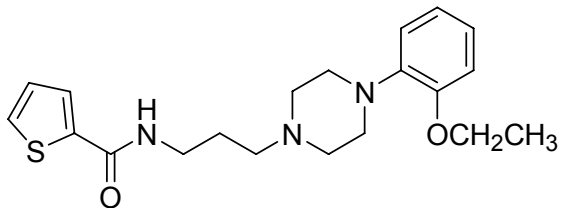
^{1}H-NMR ($CDCl_3$): 1.81- 1.86 (m, 2H, CH_2, C2, propyl), 2.65 (t, 2H, J= 6.25 Hz, CH_2, C3, propyl), 2.70 (brs, 4H, 2 X –CH_2, piperazine), 3.04 (brs, 4H, 2 X –CH_2, piperazine), 3.60 (q, 2H, J= 5.75 Hz, CH_2, C1, propyl), 6.86- 6.90 (m, 1H, aromatic), 7.12- 7.20 (m, 2H, aromatic), 7.37- 7.51 (m, 3H, aromatic), 7.81- 7.85 (m, 2H, aromatic), 8.11 (brs, 1H, -NH)

IR (cm^{-1}): 1640 (C=O)

GC/MS : m/z 77 (80%), m/z 105 (100%), m/z 219 (30%), m/z 375 (15%), m/z 392 (M^+, 10%)

Elemental analysis: for $C_{20}H_{23}Cl_2N_3O$ calcd. C 61.23, H 5.91, N 10.71 ; found C 60.76; H 5.84; N 10.47.

Thiophene-2-carboxylic acid {3-[4-(2-ethoxyphenyl)piperazin-1-yl]propyl} amide (44a)



Yield: 0.5g, 68%, yellow resin

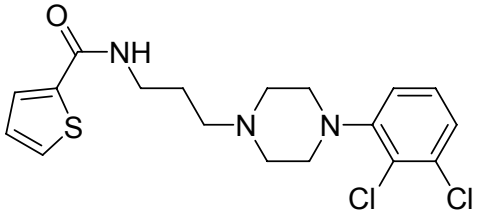
^{1}H-NMR ($CDCl_3$): 1.45 (t, 3H, 7 Hz, -OCH_2**CH_3**), 1.76- 1.86 (m, 2H, CH_2, C2, propyl), 2.62 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.71 (brs, 4H, 2 X –CH_2, piperazine), 3.17 (brs, 4H, 2 X –CH_2, piperazine), 3.57 (q, 2H, J= 5.6 Hz, CH_2, C1, propyl), 4.07 (q, 2H, J= 7 Hz, -O**CH_2**CH_3), 6.84- 7.04 (m, 5H, aromatic), 7.41 (dd, 1H, J= 1.05, 5 Hz, aromatic), 7.56- 7.58 (m, 1H, aromatic), 7.79 (brs, 1H, -NH)

IR (cm^{-1}): 1636 (C=O)

GC/MS : m/z 77 (20%), m/z 111 (35%), m/z 168 (30%), m/z 219 (70%), m/z 358 (100%), m/z 372 (M^+, 50%)

Elemental analysis: for $C_{20}H_{27}N_3O_2S$ calcd. C 64.31, H 7.29, N 11.25 ; found C 64.72; H 7.25; N 11.25.

Thiophene-2-carboxylic acid {3-[4-(2,3-dichlorophenyl)piperazin-1-yl]-propyl}amide (44b)



Yield: 0.52g, 65%, yellow resin

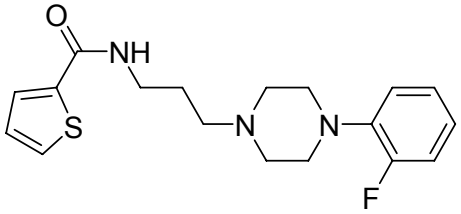
^{1}H-NMR ($CDCl_3$): 1.80- 1.87 (m, 2H, CH_2, C2, propyl), 2.64 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.71 (brs, 4H, 2 X –CH_2, piperazine), 3.11 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 3.58 (q, 2H, J= 5.6 Hz, CH_2, C1, propyl), 6.91- 6.95 (m, 1H, aromatic), 7.06 (dd, 1H, J= 3.6, 5 Hz, aromatic), 7.12- 7.20 (m, 2H, aromatic), 7.43 (dd, 1H, J= 1.15, 5 Hz, aromatic), 7.57 (dd, 1H, J= 1.15, 3.6 Hz, aromatic), 7.85 (brs, 1H, -NH)

IR (cm^{-1}): 1639 (C=O)

GC/MS : m/z 77 (20%), m/z 111 (80%), m/z 168 (80%), m/z 197 (100%), m/z 381 (20%), m/z 398 (M^+, 10%)

Elemental analysis: for $C_{18}H_{21}Cl_2N_3OS$ calcd. C 54.27, H 5.31, N 10.55 ; found C 54.11; H 5.36; N 10.52.

Thiophene-2-carboxylic acid {3-[4-(2-fluorophenyl)piperazin-1-yl]propyl} amide (44c)



Yield: 0.48g, 69%, brown resin

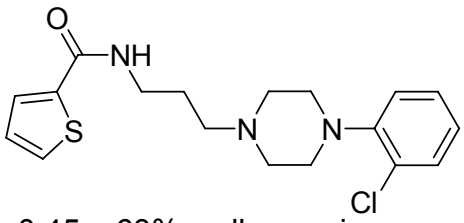
^{1}H-NMR ($CDCl_3$): 1.79- 1.84 (m, 2H, CH_2, C2, propyl), 2.63 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.70 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 3.16 (t, 4H, J= 4.8 Hz, 2 X –CH_2, piperazine), 3.57 (q, 2H, J= 5.5 Hz, CH_2, C1, propyl), 6.90- 7.06 (m, 5H, aromatic), 7.40- 7.56 (m, 2H, aromatic), 7.98 (brs, 1H, -NH)

IR (cm^{-1}): 1643 (C=O), 1201 (C-F)

GC/MS : m/z 77 (15%), m/z 111 (100%), m/z 122 (80%), m/z 193 (40%), m/z 331 (40%), m/z 347 (M^+, 10%)

Elemental analysis: for $C_{18}H_{22}FN_3OS$ calcd. C 62.22, H 6.38, N 12.09 ; found C 62.47; H 6.29; N 12.07.

Thiophene-2-carboxylic acid {3-[4-(2-chlorophenyl)piperazin-1-yl]propyl} amide (44d)



Yield: 0.45g, 63%, yellow resin

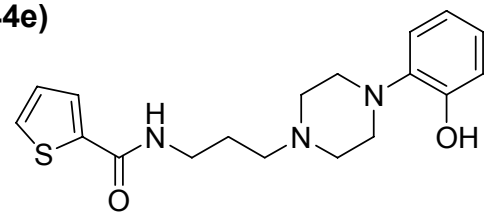
^{1}H-NMR ($CDCl_3$): 1.78- 1.96 (m, 2H, CH_2, C2, propyl), 2.60 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.91 (brs, 4H, 2 X –CH_2, piperazine), 3.25 (brs, 4H, 2 X –CH_2, piperazine), 3.62 (q, 2H, J= 5.5 Hz, CH_2, C1, propyl), 6.99- 7.28 (m, 4H, aromatic), 7.35- 7.68 (m, 3H, aromatic), 8.09 (brs, 1H, -NH)

IR (cm^{-1}): 1634 (C=O)

GC/MS : m/z 77 (25%), m/z 111 (85%), m/z 168 (100%), m/z 197 (95%), m/z 347 (40%), m/z 364 (M^++2, 10%)

Elemental analysis: for $C_{18}H_{22}ClN_3OS$ calcd. C 59.41, H 6.09, N 11.55 ; found C 59.56; H 6.04; N 11.52.

Thiophene-2-carboxylic acid {3-[4-(2-hydroxyphenyl)piperazin-1-yl]propyl}amide (44e)



Yield: 0.47g, 68%, yellow resin

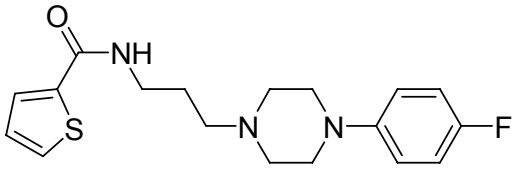
^{1}H-NMR ($CDCl_3$): 1.78- 1.85 (m, 2H, CH_2, C2, propyl), 2.63 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.66 (brs, 4H, 2 X –CH_2, piperazine), 2.97 (brs, 4H, 2 X –CH_2, piperazine), 3.58 (q, 2H, J= 5.5 Hz, CH_2, C1, propyl), 6.88- 7.61 (m, 7H, aromatic), 7.95 (brs, 1H, -NH). Phenolic proton signal was not shown.

IR (cm^{-1}): 3208 (-OH), 1647 (C=O)

GC/MS : m/z 77 (15%), m/z 111 (75%), m/z 168 (80%), m/z 197 (100%), m/z 330 (10%), m/z 345 (M^+, 15%)

Elemental analysis: for $C_{18}H_{23}N_3O_2S$ calcd. C 62.58, H 6.71, N 12.16 ; found C 62.51; H 6.78; N 12.24.

Thiophene-2-carboxylic acid {3-[4-(4-fluorophenyl)piperazin-1-yl]propyl} amide (44f)



Yield: 0.48g, 70%, yellowish white crystals

m.p. 103-105 ^oC

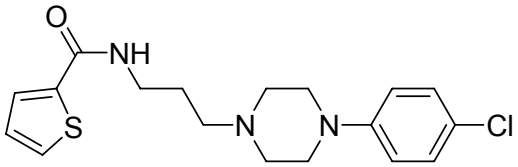
^{1}H-NMR ($CDCl_3$): 1.79- 1.86 (m, 2H, CH_2, C2, propyl), 2.60 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.66 (t, 4H, J= 5.1 Hz, 2 X –CH_2, piperazine), 3.16 (t, 4H, J= 5.1 Hz, 2 X –CH_2, piperazine), 3.57 (q, 2H, J= 5.6 Hz, CH_2, C1, propyl), 6.84- 7.03 (m, 5H, aromatic), 7.38- 7.53 (m, 2H, aromatic), 7.82 (brs, 1H, -NH)

IR (cm^{-1}): 1648 (C=O), 1221 (C-F)

GC/MS : m/z 77 (20%), m/z 111 (100%), m/z 122 (50%), m/z 197 (90%), m/z 331 (60%), m/z 347 (M^+, 20%)

Elemental analysis: for $C_{18}H_{22}FN_3OS$ calcd. C 62.22, H 6.38, N 12.09 ; found C 62.65; H 6.52; N 12.30.

Thiophene-2-carboxylic acid {3-[4-(4-chlorophenyl)piperazin-1-yl]propyl} amide (44g)



Yield: 0.54g, 74%, pale yellow crystals

m.p. 144-146 ^{o}C

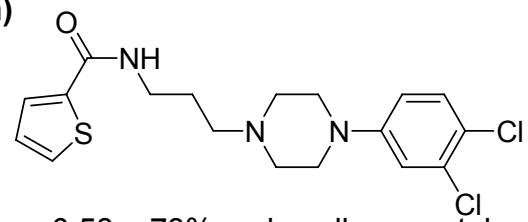
^{1}H-NMR ($CDCl_3$): 1.79- 1.86 (m, 2H, CH_2, C2, propyl), 2.60 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.66 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.20 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.57 (q, 2H, J= 5.7 Hz, CH_2, C1, propyl), 6.83 (d, 2H, J= 9 Hz, aromatic), 7.01 (dd, 1H, J= 3.7, 5 Hz, aromatic), 7.21 (d, 2H, J= 9 Hz, aromatic), 7.38-7.52 (m, 2H, aromatic), 7.78 (brs, 1H, -NH)

IR (cm^{-1}): 1641 (C=O)

GC/MS : m/z 77 (20%), m/z 111 (55%), m/z 168 (70%), m/z 197 (100%), m/z 347 (35%), m/z 362 (M^+, 20%)

Elemental analysis: for $C_{18}H_{22}ClN_3OS$ calcd. C 59.41, H 6.09, N 11.55 ; found C 59.45; H 6.21; N 11.62.

Thiophene-2-carboxylic acid {3-[4-(3,4-dichlorophenyl)piperazin-1-yl]-propyl}amide (44h)



Yield: 0.58g, 73%, pale yellow crystals

m.p. 107-109 ^{o}C

^{1}H-NMR ($CDCl_3$): 1.76- 1.86 (m, 2H, CH_2, C2, propyl), 2.58 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.64 (t, 4H, J= 5.2 Hz, 2 X –CH_2, piperazine), 3.20 (t, 4H, J= 5.2 Hz, 2 X –CH_2, piperazine), 3.56 (q, 2H, J= 5.7 Hz, CH_2, C1, propyl), 6.73 (dd, 1H, J= 2.8, 8.8 Hz, aromatic), 6.94 (d, 1H, J= 2.8 Hz, aromatic), 7.02 (dd, 1H, J= 3.6, 5 Hz, aromatic), 7.28 (d, 1H, J= 8.8 Hz, aromatic), 7.40 (dd,

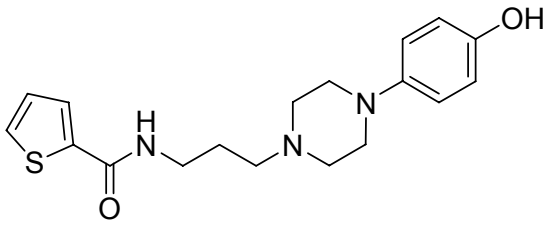
1H, J= 1, 5 Hz, aromatic), 7.52 (d,, J= 3.6 Hz, aromatic), 7.78 (brs, 1H, -NH)

IR (cm^{-1}): 1649 (C=O)

GC/MS : m/z 77 (20%), m/z 111 (55%), m/z 168 (70%), m/z 197 (100%), m/z 381 (30%), m/z 398 (M^+, 15%)

Elemental analysis: for $C_{18}H_{21}Cl_2N_3OS$ calcd. C 54.27, H 5.31, N 10.55 ; found C 54.20; H 5.29; N 10.57.

Thiophene-2-carboxylic acid {3-[4-(4-hydroxyphenyl)piperazin-1-yl] propyl}amide (44i)



Yield: 0.51g, 74%, creamy white crystals

m.p. 156- 158 ^oC

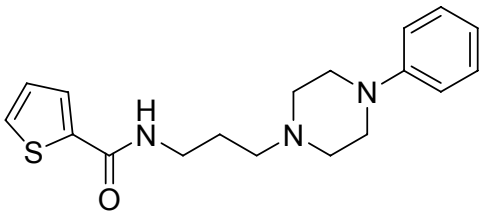
^{1}H-NMR ($CDCl_3$): 1.77- 1.87 (m, 2H, CH_2, C2, propyl), 2.70 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.75 (t, 4H, J= 4.9 Hz, 2 X –CH_2, piperazine), 3.23 (t, 4H, J= 4.9 Hz, 2 X –CH_2, piperazine), 3.57 (q, 2H, J= 5.5 Hz, CH_2, C1, propyl), 6.97- 7.38 (m, 7H, aromatic), 8.01 (brs, 1H, -NH). Phenolic proton signal was not shown.

IR (cm^{-1}): 3217, (-OH), 1639 (C=O)

GC/MS : m/z 77 (20%), m/z 111 (35%), m/z 168 (40%), m/z 197 (100%), m/z 330 (70%), m/z 345 (M^+, 80%)

Elemental analysis: for $C_{18}H_{23}N_3O_2S$ calcd. C 62.58, H 6.71, N 12.16 ; found C 62.46; H 6.80; N 12.17.

Thiophene-2-carboxylic acid [3-(4-phenyl piperazin-1-yl)propyl]amide (44j)



Yield: 0.5g, 76%, creamy white crystals

m.p. 162-164 ^{0}C

^{1}H-NMR ($CDCl_3$): 1.77- 1.87 (m, 2H, CH_2, C2, propyl), 2.61 (t, 2H, J= 6 Hz, CH_2, C3, propyl), 2.67 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.25 (t, 4H, J= 5 Hz, 2 X –CH_2, piperazine), 3.56 (q, 2H, J= 5.6 Hz, CH_2, C1, propyl), 6.85- 7.02 (m, 4H, aromatic), 7.25- 7.40 (m, 3H, aromatic), 7.55 (d, 1H, aromatic), 7.97 (brs, 1H, -NH)

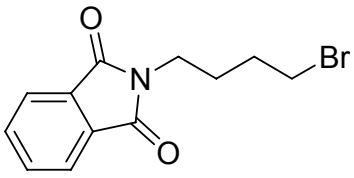
IR (cm^{-1}): 1635 (C=O)

GC/MS : m/z 77 (95%), m/z 111 (100%), m/z 132 (40%), m/z 175 (30%), m/z 197 (25%), m/z 314 (15%), m/z 329 (M^+, 10%)

Elemental analysis: for $C_{18}H_{23}N_3OS$ calcd. C 65.62, H 7.04, N 12.75 ; found C 65.61; H 6.89; N 12.57.

Procedure for the preparation of 2-(4-Bromobutyl) isoindole-1,3-dione (47)

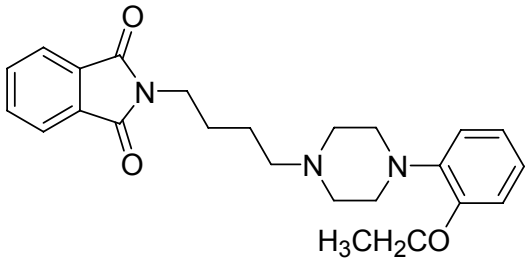
To 1.85g (10 mmol) of phthalimide potassium salt was added slowly over a period of 10 minutes to a solution of 2.41g (11 mmol) of 1,4 dibromobutane in 60 ml of acetone. The reaction mixture was refluxed for 24 hours and was then hot filtered. The filtrate was evaporated under reduced pressure and the pale yellow oil produced was subjected to column chromatography on Silica gel eluting with Methylene chloride to get creamy white crystals of the desired product. The yield and spectroscopy data of the intermediate matched what found in literature [142].



General procedure for the preparation of 2-[4-(4-Aryl piperazin-1-yl)butyl] isoindole-1,3-dione derivatives

To a solution of 1.2g (4.5 mmol) of **(47)** in 40 ml dry acetonitrile, were added 4.5 mmol of the corresponding phenylpiperazin and 1.5g (15 mmol) of TEA. The reaction mixture was allowed to reflux under innert atmosphere for 48 hours and then left to cool to room temperature. The organic solvent was removed under reduced pressure and the remained residue was subjected to purification on Silica gel column chromatography eluting with Methylene chloride: Methanol 100: 2.

2-{4-[4-(2-Ethoxyphenyl)piperazin-1-yl]butyl}isoindole-1,3-dione (41a)



Yield: 1.2g, 68%, orange oil

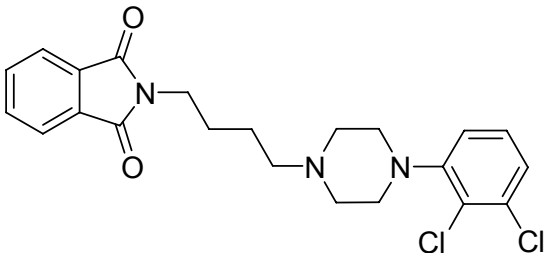
^{1}H-NMR ($CDCl_3$): 1.45 (t, 3H, J= 7 Hz, -OCH_2**CH_3**), 1.64- 1.74 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.45 (t, 2H, J= 7.2 Hz, CH_2, C4, butyl), 2.64 (brs, 4H, 2 X CH_2, piperazine), 3.11 (brs, 4H, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.2 Hz, CH_2, C1, butyl), 4.06 (q, 2H, J= 7 Hz, -O**CH_2**CH_3), 6.82-6.92 (m, 4H, aromatic), 7.71 (dd, 2H, J= 3, 5.5 Hz, aromatic), 7.84 (dd, 2H, J= 3, 5.5 Hz, aromatic)

IR (cm^{-1}): 1705, 1741 (2 X C=O)

GC/MS : m/z 70 (40%), m/z 104 (20%), m/z 130 (70%), m/z 160 (100%), m/z 172 (30%), m/z 407 (M^+, 15%)

Elemental analysis: for $C_{24}H_{29}N_3O_3$ calcd. C 70.74, H 7.17, N 10.31 ; found C 71.03; H 7.42; N 9.96.

2-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-isoindole-1,3-dione (41b) [142]



Yield: 1.3g, 69%, yellow crystals

m.p. 119- 121 ^{o}C

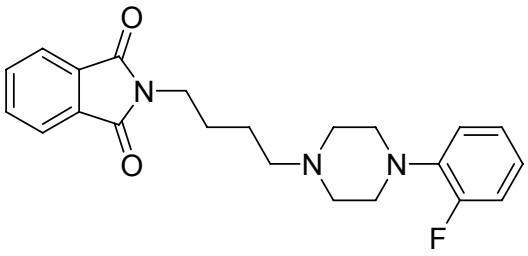
^{1}H-NMR ($CDCl_3$): 1.57- 1.77 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.44 (t, 2H, J= 7 Hz, CH_2, C4, butyl), 2.61 (brs, 4H, 2 X CH_2, piperazine), 3.04 (brs, 4H, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7 Hz, CH_2, C1, butyl), 6.92- 6.14 (m, 3H, aromatic), 7.69- 7.83 (m, 4H, aromatic)

IR (cm^{-1}): 1710, 1744 (2 X C=O)

GC/MS : m/z 70 (60%), m/z 104 (30%), m/z 130 (50%), m/z 160 (100%), m/z 172 (30%), m/z 243 (50%), m/z 432 (M^+, 5%)

Elemental analysis: for $C_{22}H_{23}Cl_2N_3O_2$ calcd. C 61.12, H 5.36, N 9.72 ; found C 60.73; H 5.42; N 9.93.

2-{4-[4-(2-Fluorophenyl)piperazin-1-yl]butyl} isoindole-1,3-dione (41c)



Yield: 1.2g, 72%, yellowish white crystals

m.p. 120- 122 ^{o}C

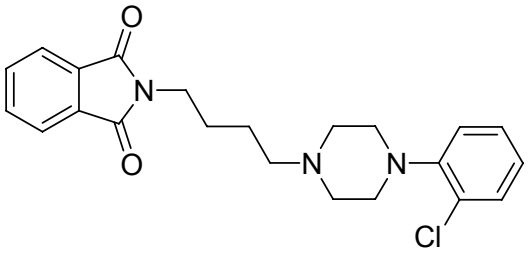
^{1}H-NMR ($CDCl_3$): 1.51- 1.79 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.43 (t, 2H, J= 7 Hz, CH_2, C4, butyl), 2.61 (t, 4H, J= 4.7 Hz, 2 X CH_2, piperazine), 3.09 (t, 4H, J= 4.7 Hz, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7 Hz, CH_2, C1, butyl), 6.87- 6.07 (m, 4H, aromatic), 7.70 (dd, 2H, J= 3, 5.5 Hz Aromatic), 7.81 (d, 2H, J= 4 Hz, aromatic)

IR (cm^{-1}): 1703, 1746 (2 X C=O), 1206 (C-F)

GC/MS : m/z 70 (90%), m/z 104 (30%), m/z 122 (90%), m/z 160 (100%), m/z 193 (80%), m/z 366 (5%), m/z 381 (M^+, 10%)

Elemental analysis: for $C_{22}H_{24}FN_3O_2$ calcd. C 69.27, H 6.34, N 11.02 ; found C 69.43; H 6.03; N 10.95.

2-{4-[4-(2-Chlorophenyl)piperazin-1-yl]butyl} isoindole-1,3-dione (41d)



Yield: 1.3g, 74%, yellowish white crystals

m.p. 141- 143 ^oC

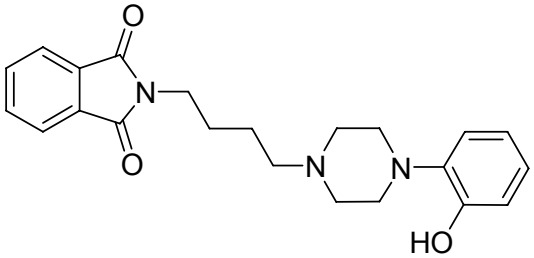
^{1}H-NMR ($CDCl_3$): 1.57- 1.77 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.45 (t, 2H, J= 7.1 Hz, CH_2, C4, butyl), 2.62 (brs, 4H, 2 X CH_2, piperazine), 3.06 (brs, 4H, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.1 Hz, CH_2, C1, butyl), 6.92- 6.35 (m, 4H, aromatic), 7.71 (dd, 2H, J= 3, 5.5 Hz Aromatic), 7.83 (d, 2H, J= 3 Hz, aromatic)

IR (cm^{-1}): 1704, 1745 (2 X C=O)

GC/MS : m/z 70 (50%), m/z 104 (30%), m/z 138 (50%), m/z 160 (100%), m/z 209 (70%), m/z 382 (2%), m/z 397 (M^+, 5%)

Elemental analysis: for $C_{22}H_{24}ClN_3O_2$ calcd. C 66.41, H 6.08, N 10.56 ; found C 66.20; H 6.02; N 10.43.

2-{4-[4-(2-Hydroxyphenyl)piperazin-1-yl]butyl}isoindole-1,3-dione (41e)



Yield: 1.0g, 63%, yellowish white crystals

m.p. 118- 120 ^{o}C

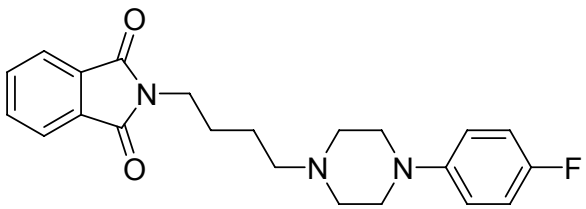
^{1}H-NMR ($CDCl_3$): 1.57- 1.75 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.45 (t, 2H, J= 7.3 Hz, CH_2, C4, butyl), 2.61 (brs, 4H, 2 X CH_2, piperazine), 2.91 (brs, 4H, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.3 Hz, CH_2, C1, butyl), 6.81- 6.17 (m, 4H, aromatic), 7.70- 7.86 (m, 4H, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3215 (-OH), 1716, 1741 (2 X C=O)

GC/MS : m/z 70 (50%), m/z 104 (35%), m/z 120 (100%), m/z 160 (90%), m/z 191 (20%), m/z 364 (5%), m/z 379 (M^+, 5%)

Elemental analysis: for $C_{22}H_{25}N_3O_3$ calcd. C 69.64, H 6.64, N 11.07; found C 69.31; H 6.83; N 10.89.

2-{4-[4-(4-Fluorophenyl)piperazin-1-yl]butyl} isoindole-1,3-dione (41f)



Yield: 1.3g, 76%, pale yellow crystals

m.p. 116- 118 ^{o}C

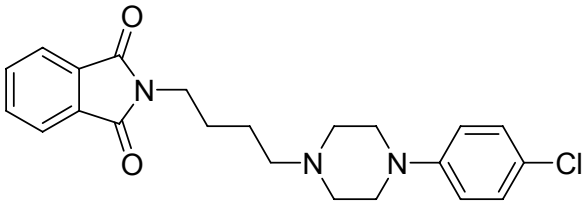
^{1}H-NMR ($CDCl_3$): 1.53- 1.80 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.43 (t, 2H, J= 7.1 Hz, CH_2, C4, butyl), 2.58 (t, 4H, J= 5.1 Hz, 2 X CH_2, piperazine), 3.10 (t, 4H, J= 5.1 Hz, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.1 Hz, CH_2, C1, butyl), 6.82- 6.98 (m, 4H, aromatic), 7.71 (dd, 2H, J= 3, 5.5 Hz Aromatic), 7.81 (d, 2H, J= 4 Hz, aromatic)

IR (cm^{-1}): 1717, 1776 (2 X C=O)

GC/MS : m/z 70 (80%), m/z 104 (30%), m/z 122 (80%), m/z 160 (100%), m/z 193 (70%), m/z 366 (5%), m/z 381 (M^+, 10%)

Elemental analysis: for $C_{22}H_{24}FN_3O_2$ calcd. C 69.27, H 6.34, N 11.02 ; found C 69.48; H 6.01; N 10.92.

2-{4-[4-(4-Chlorophenyl)piperazin-1-yl]butyl} isoindole-1,3-dione (41g)



Yield: 1.4g, 77%, pale yellow crystals

m.p. 149- 151 ^{o}C

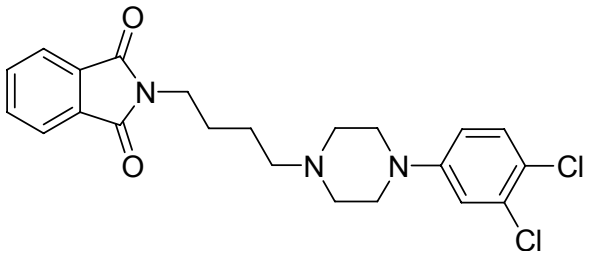
^{1}H-NMR ($CDCl_3$): 1.56- 1.77 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.42 (t, 2H, J= 7.3 Hz, CH_2, C4, butyl), 2.57 (t, 4H, J= 4.8 Hz, 2 X CH_2, piperazine), 3.14 (t, 4H, J= 4.8 Hz, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.3 Hz, CH_2, C1, butyl), 6.82 (d, 2H, J= 9 Hz, aromatic), 7.19 (d, 2H, J= 9 Hz Aromatic), 7.71 (dd, 2H, J= 3, 5.5 Hz, aromatic), 7.84 (dd, 2H, J= 3, 5.5 Hz, aromatic)

IR (cm^{-1}): 1707, 1755 (2 X C=O)

GC/MS : m/z 70 (60%), m/z 104 (30%), m/z 138 (40%), m/z 160 (100%), m/z 209 (50%), m/z 382 (5%), m/z 397 (M^{+}, 15%)

Elemental analysis: for $C_{22}H_{24}ClN_3O_2$ calcd. C 66.41, H 6.08, N 10.56 ; found C 66.14; H 6.04; N 10.43.

2-{4-[4-(3,4-Dichlorophenyl)piperazin-1-yl]butyl}-isoindole-1,3-dione (41h)



Yield: 1.4g, 72%, yellow crystals

m.p. 125- 127 ^{o}C

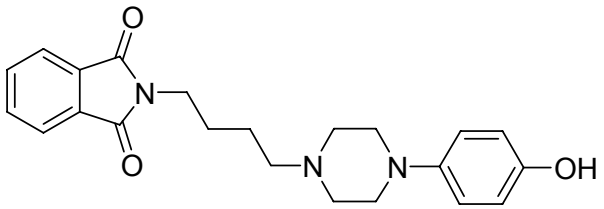
^{1}H-NMR ($CDCl_3$): 1.56- 1.77 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.41 (t, 2H, J= 7.3 Hz, CH_2, C4, butyl), 2.55 (t, 4H, J= 5.25 Hz, 2 X CH_2, piperazine), 3.14 (t, 4H, J= 5.25 Hz, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7.3 Hz, CH_2, C1, butyl), 6.72 (dd, 1H, J= 2.75, 9 Hz, aromatic), 6.93 (d, 1H, J= 2.75 Hz Aromatic), 7.25 (d, 1H, J= 9 Hz, aromatic), 7.71 (dd, 2H, J= 3, 5 Hz, aromatic), 7.84 (dd, 2H, J= 3, 5 Hz, aromatic)

IR (cm^{-1}): 1711, 1742 (2 X C=O)

GC/MS : m/z 70 (50%), m/z 104 (35%), m/z 130 (60%), m/z 160 (100%), m/z 172 (60%), m/z 243 (50%), m/z 432 (M^+, 15%)

Elemental analysis: for $C_{22}H_{23}Cl_2N_3O_2$ calcd. C 61.12, H 5.36, N 9.72 ; found C 61.16; H 5.49; N 10.17.

2-{4-[4-(4-Hydroxyphenyl)piperazin-1-yl]butyl}isoindole-1,3-dione (41i)



Yield: 1.0g, 62%, yellow crystals

m.p. 151- 153 ^oC

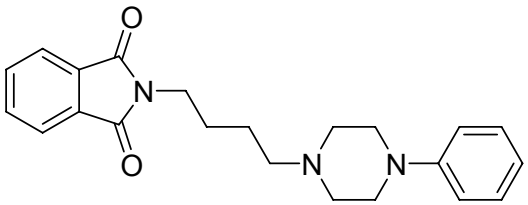
^{1}H-NMR ($CDCl_3$): 1.65- 1.78 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.40 (t, 2H, J= 7 Hz, CH_2, C4, butyl), 2.57 (brs, 4H, 2 X CH_2, piperazine), 3.01 (brs, 4H, 2 X CH_2, piperazine), 3.64 (t, 2H, J= 7 Hz, CH_2, C1, butyl), 6.68 (d, 2H, J= 9 Hz, aromatic), 6.78 (d, 2H, J= 9 Hz, aromatic), 7.68- 7.76 (m, 4H, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3217 (-OH), 1712, 1748 (2 X C=O)

GC/MS : m/z 70 (15%), m/z 104 (5%), m/z 120 (20%), m/z 160 (25%), m/z 191 (100%), m/z 364 (20%), m/z 379 (M^+, 90%)

Elemental analysis: for $C_{22}H_{25}N_3O_3$ calcd. C 69.64, H 6.64, N 11.07; found C 69.84; H 7.03; N 10.79.

2-[4-(4-Phenyl piperazin-1-yl)butyl] isoindole-1,3-dione (41j)



Yield: 1.2g, 73%, yellow crystals

m.p. 137- 139 ^{o}C

^{1}H-NMR ($CDCl_3$): 1.54- 1.80 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.43 (t, 2H, J= 7 Hz, CH_2, C4, butyl), 2.59 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.18 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.73 (t, 2H, J= 7 Hz, CH_2, C1, butyl), 6.81- 6.93 (m, 3H, aromatic), 7.22- 7.28 (m, 2H, aromatic), 7.69- 7.84 (m, 4H, aromatic)

IR (cm^{-1}): 1710, 1756 (2 X C=O)

GC/MS : m/z 77 (95%), m/z 104 (100%), m/z 130 (40%), m/z 160 (80%), m/z 175 (70%), m/z 348 (5%), m/z 363 (M^+, 15%)

Elemental analysis: for $C_{22}H_{25}N_3O_2$ calcd. C 72.70, H 6.93, N 11.56 ; found C 72.41; H 6.96; N 11.53.

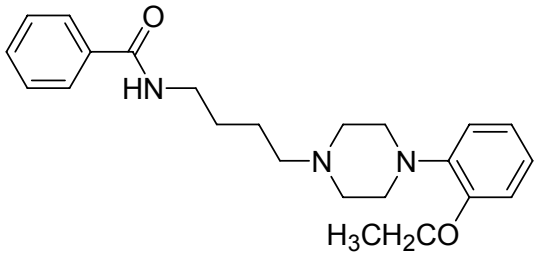
General procedure for the preparation of 4-(4-Aryl piperazin-1-yl)-butylamine derivatives (49a-j)

A solution of 2 mmol of the corresponding 1,3 isoindole dione derivative and 0.25g (6 mmol) hydrazine hydrate 80% in 20 ml ethanol was heated to reflux for 5 hours. After cooling to room temperature, any insoluble material was filtered off, washed with ethanol (2 X 20 ml) and the filterate was evaporated under reduced pressure. The product was extracted with chloroform (2 X 30 ml) and the desired amine obtained was introduced to the following reaction without further purification.

General procedure for the preparation of Arylamidobutylphenyl-piperazine derivatives

A solution of 2 mmol of thiophene-2-carbonyl chloride (0.30g) or benzoyl chloride (0.28g) in 10 ml dry THF was added slowly to a solution of the corresponding amine derivative (2.3 mmol) and 0.5g TEA (5 mmol) in dry THF (30 ml) at 0 ^{o}C. The mixture was then allowed to stir at room temperature for 5 hours. The reaction mixture was then poured into 30 ml water and extracted with Methylene chloride (2 X 40ml). The organic layers were collected and dried over anhydrous Na_2SO_4 and evaporated to yield a residue of the desired product that was purified on Silica gel column chromatography using Methylene chloride : Methanol 200 : 3 as mobile phase.

N-{4-[4-(2-Ethoxyphenyl)piperazin-1-yl]butyl}benzamide (43a)



Yield: 0.5g, 66%, pale yellow crystals

m.p. 106- 108 ^{o}C

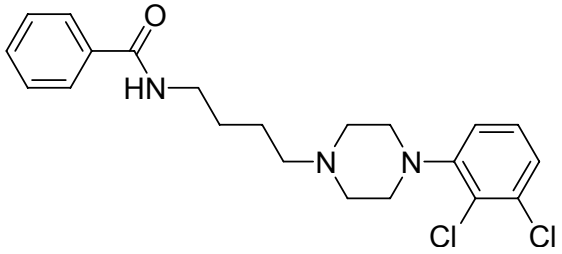
^{1}H-NMR ($CDCl_3$): 1.44 (t, 3H, J= 7 Hz, $OCH_2\mathbf{CH_3}$), 1.60- 1.71 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.53 (t, 2H, J= 6.75 Hz, CH_2, C4, butyl), 2.70 (brs, 4H, 2 X CH_2, piperazine), 3.12 (brs, 4H, 2 X CH_2, piperazine), 3.48 (q, 2H, J= 6.2 Hz, CH_2, C1, butyl), 4.06 (q, 2H, J= 7 Hz, $O\mathbf{CH_2}CH_3$), 6.82- 6.97 (m, 5H, aromatic and NH), 7.38- 7.48 (m, 3H, aromatic), 7.76- 7.87 (m, 2H, aromatic)

IR (cm^{-1}): 1641 (C=O)

GC/MS : m/z 77 (60%), m/z 105 (100%), m/z 120 (30%), m/z 219 (20%),m/z 297 (10%), m/z 366 (10%), m/z 382 (M^+, 5%)

Elemental analysis: for $C_{23}H_{31}N_3O_2$ calcd. C 72.41, H 8.19, N 11.01; found C 71.87; H 7.98; N 10.87.

N-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}benzamide (43b) [143]



Yield: 0.64g, 80%, yellowish white crystals

m.p. 128- 130 ^{o}C

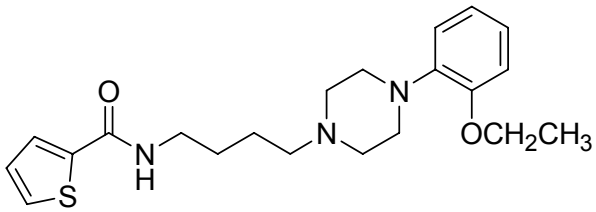
^{1}H-NMR ($CDCl_3$): 1.62- 1.72 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.47 (t, 2H, J= 6.9 Hz, CH_2, C4, butyl), 2.62 (brs, 4H, 2 X CH_2, piperazine), 3.02 (brs, 4H, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 6.25 Hz, CH_2, C1, butyl), 6.69 (brs, 1H, NH), 6.87- 6.91 (m, 1H, aromatic), 7.09- 7.17 (m, 2H, aromatic), 7.38- 7.51 (m, 3H, aromatic), 7.74- 7.82 (m, 2H, aromatic)

IR (cm^{-1}): 1643 (C=O)

GC/MS : m/z 77 (100%), m/z 105 (95%), m/z 172 (80%), m/z 205 (70%),m/z 243 (60%), m/z 410 (M^{+}+4, 5%)

Elemental analysis: for $C_{21}H_{25}Cl_2N_3O$ calcd. C 62.07, H 6.20, N 10.34; found C 61.84; H 6.66; N 10.34.

Thiophene-2-carboxylic acid {4-[4-(2-ethoxyphenyl)piperazin-1-yl]butyl} amide (45a)



Yield: 0.57g, 74%, orange oil

^{1}H-NMR ($CDCl_3$): 1.45 (t, 3H, J= 7 Hz, $OCH_2\mathbf{CH_3}$), 1.67- 1.85 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.47 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.65 (brs, 4H, 2 X CH_2, piperazine), 3.12 (brs, 4H, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 5.8 Hz, CH_2, C1, butyl), 4.06 (q, 2H, J= 7 Hz, $O\mathbf{CH_2}CH_3$), 6.46 (brs, 1H, NH), 6.82- 7.07 (m, 5H, aromatic), 7.43- 7.49 (m, 2H, aromatic)

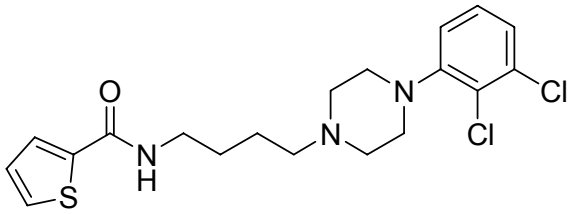
IR (cm^{-1}): 1641 (C=O)

GC/MS : m/z 70 (30%), m/z 111 (100%), m/z 121 (40%), m/z 134 (20%),m/z 219 (20%), m/z 372 (5%), m/z 387 (M^{+},

5%)

Elemental analysis: for $C_{21}H_{29}N_3O_2S$ calcd. C 65.08, H 7.54, N 10.84; found C 64.73; H 7.28; N 10.47.

Thiophene-2-carboxylic acid {4-[4-(2,3-dichlorophenyl)piperazin-1-yl]-butyl}amide (45b)



Yield: 0.58g, 71%, yellowish brown resin

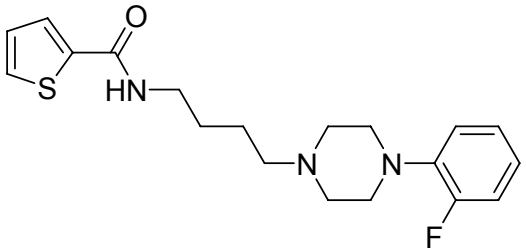
^{1}H-NMR ($CDCl_3$): 1.77- 1.94 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.95 (t, 2H, J= 6.8 Hz, CH_2, C4, butyl), 3.12 (brs, 4H, 2 X CH_2, piperazine), 3.36 (brs, 4H, 2 X CH_2, piperazine), 3.53 (q, 2H, J= 6.2 Hz, CH_2, C1, butyl), 6.96 (brs, 1H, NH), 6.99- 7.16 (m, 4H, aromatic), 7.46 (d, 1H, J= 3 Hz, aromatic), 7.51 (d, 1H, J= 2.3 Hz, aromatic)

IR (cm^{-1}): 1637 (C=O)

GC/MS : m/z 70 (50%), m/z 130 (60%), m/z 160 (100%), m/z 174 (40%),m/z 243 (40%), m/z 397 (5%), m/z 412 (M^+, 5%)

Elemental analysis: for $C_{19}H_{23}Cl_2N_3OS$ calcd. C 55.34, H 5.62, N 10.19; found C 55.44; H 5.62; N 9.94.

Thiophene-2-carboxylic acid {4-[4-(2-fluorophenyl)piperazin-1-yl]butyl} amide (45c)



Yield: 0.51g, 71%, creamy white crystals

m.p. 135- 137 ^oC

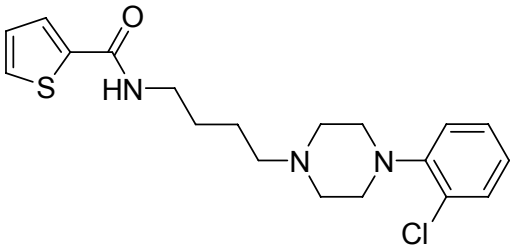
^{1}H-NMR ($CDCl_3$): 1.60- 1.65 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.45 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.62 (t, 4H, J= 4.9 Hz, 2 X CH_2, piperazine), 3.10 (t, 4H, J= 4.9 Hz, 2 X CH_2, piperazine), 3.46 (q, 2H, J= 6.3 Hz, CH_2, C1, butyl), 6.44 (brs, 1H, NH), 6.89- 7.07 (m, 5H, aromatic), 7.44 (d, 1H, J= 4.3 Hz, aromatic), 7.50 (d, 1H, J= 3.6 Hz, aromatic)

IR (cm^{-1}): 1634 (C=O), 1200 (C-F)

GC/MS : m/z 70 (50%), m/z 111 (100%), m/z 122 (60%), m/z 193 (40%),m/z 211 (20%), m/z 346 (10%), m/z 361 (M^+, 5%)

Elemental analysis: for $C_{19}H_{24}FN_3OS$ calcd. C 63.13, H 6.69, N 11.62; found C 62.84; H 6.72; N 12.03.

Thiophene-2-carboxylic acid {4-[4-(2-chlorophenyl)piperazin-1-yl]butyl} amide (45d)



Yield: 0.53g, 71%, yellowish brown oil

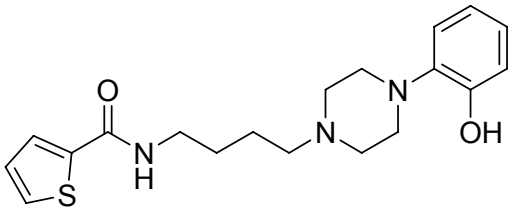
^{1}H-NMR ($CDCl_3$): 1.67- 1.80 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.47 (t, 2H, J= 6.8 Hz, CH_2, C4, butyl), 2.65 (brs, 4H, 2 X CH_2, piperazine), 3.08 (brs, 4H, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 6 Hz, CH_2, C1, butyl), 6.39 (brs, 1H, NH), 6.93- 7.46 (m, 7H, aromatic)

IR (cm^{-1}): 1649 (C=O)

GC/MS : m/z 70 (70%), m/z 111 (100%), m/z 138 (50%), m/z 194 (10%),m/z 211 (40%), m/z 362 (5%), m/z 377 (M^+, 5%)

Elemental analysis: for $C_{19}H_{24}ClN_3OS$ calcd. C 60.38, H 6.40, N 11.12; found C 60.01; H 6.39; N 10.79.

Thiophene-2-carboxylic acid {4-[4-(2-hydroxyphenyl)piperazin-1-yl]butyl} amide (45e)



Yield: 0.46g, 64%, reddish brown oil

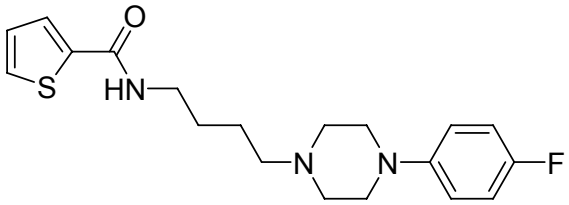
^{1}H-NMR ($CDCl_3$): 1.66- 1.69 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.52 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.68 (brs, 4H, 2 X CH_2, piperazine), 2.94 (t, 4H, J= 4.75 Hz, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 5.8 Hz, CH_2, C1, butyl), 6.56 (brs, 1H, NH), 6.82- 6.95 (m, 2H, aromatic), 7.04- 7.14 (m, 3H, aromatic), 7.45 (d, 1H, J= 4.8 Hz, aromatic), 7.54 (d, 1H, J= 3.8 Hz, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3225 (OH), 1635 (C=O)

GC/MS : m/z 70 (40%), m/z 111 (50%), m/z 148 (25%), m/z 199 (40%),m/z 211 (100%), m/z 355 (10%), m/z 359 (M^+, 5%)

Elemental analysis: for $C_{19}H_{25}N_3O_2S$ calcd. C 63.48, H 7.01, N 11.69; found C 63.11; H 6.95; N 11.72.

Thiophene-2-carboxylic acid {4-[4-(4-fluorophenyl)piperazin-1-yl]butyl} amide (45f)



Yield: 0.53g, 74%, greish white crystals

m.p. 143- 145 ^oC

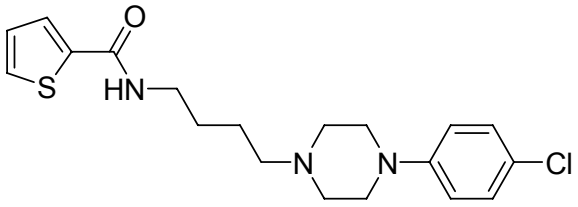
^{1}H-NMR ($CDCl_3$): 1.61- 1.67 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.46 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.62 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.12 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.45 (q, 2H, J= 6.3 Hz, CH_2, C1, butyl), 6.40 (brs, 1H, NH), 6.83- 7.06 (m, 5H, aromatic), 7.44 (d, 1H, J= 4.9 Hz, aromatic), 7.50 (d, 1H, J= 3.8 Hz, aromatic)

IR (cm^{-1}): 1644 (C=O), 1218 (C-F)

GC/MS : m/z 70 (35%), m/z 111 (100%), m/z 122 (35%), m/z 193 (20%),m/z 211 (15%), m/z 346 (5%), m/z 361 (M^+, 5%)

Elemental analysis: for $C_{19}H_{24}FN_3OS$ calcd. C 63.13, H 6.69, N 11.62; found C 62.94; H 6.83; N 11.35.

Thiophene-2-carboxylic acid {4-[4-(4-chlorophenyl)piperazin-1-yl]butyl} amide (45g)



Yield: 0.57g, 76%, creamy white crystals

m.p. 158- 160 ^oC

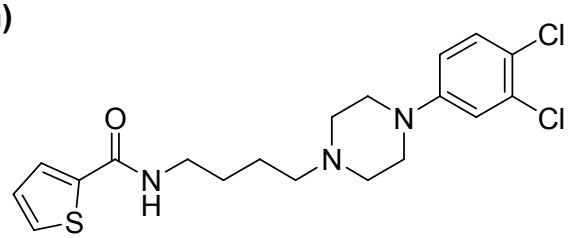
^{1}H-NMR ($CDCl_3$): 1.66- 1.74 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.46 (t, 2H, J= 6.8 Hz, CH_2, C4, butyl), 2.60 (t, 4H, J= 4.8 Hz, 2 X CH_2, piperazine), 3.16 (t, 4H, J= 4.8 Hz, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 6 Hz, CH_2, C1, butyl), 6.29 (brs, 1H, NH), 6.83 (d, 2H, J= 9 Hz, aromatic), 7.04- 7.07 (m, 1H, aromatic), 7.20 (d, 2H, J= 9 Hz, aromatic), 7.44- 7.48 (m, 2H, aromatic)

IR (cm^{-1}): 2887 (-CH Aliphatic), 1649 (C=O)

GC/MS : m/z 70 (70%), m/z 111 (100%), m/z 140 (35%), m/z 196 (10%),m/z 211 (45%), m/z 362 (5%), m/z 377 (M^+, 5%)

Elemental analysis: for $C_{19}H_{24}ClN_3OS$ calcd. C 60.38, H 6.40, N 11.12; found C 60.80; H 6.55; N 10.82.

Thiophene-2-carboxylic acid {4-[4-(3,4-dichlorophenyl)piperazin-1-yl] butyl}amide (45h)



Yield: 0.62g, 76%, pale yellow crystals

m.p. 132- 134 ^oC

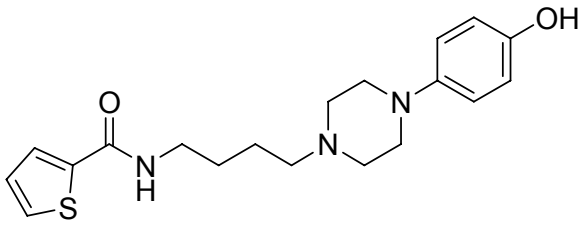
^{1}H-NMR ($CDCl_3$): 1.65- 1.74 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.43 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.57 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.16 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 6.2 Hz, CH_2, C1, butyl), 6.28 (brs, 1H, NH), 6.72 (dd, 1H, J= 3, 8.8 Hz, aromatic), 6.94 (d, 1H, J= 3 Hz, aromatic), 7.06 (d, 1H, J= 8.8 Hz, aromatic), 7.24- 7.47 (m, 3H, aromatic)

IR (cm^{-1}): 1641 (C=O)

GC/MS : m/z 70 (80%), m/z 130 (40%), m/z 160 (100%), m/z 174 (30%),m/z 243 (50%), m/z 397 (5%), m/z 416 (M^++4, 5%)

Elemental analysis: for $C_{19}H_{23}Cl_2N_3OS$ calcd. C 55.34, H 5.62, N 10.19; found C 55.54; H 5.67; N 10.15.

Thiophene-2-carboxylic acid {4-[4-(4-hydroxyphenyl)piperazin-1-yl]butyl} amide (45i)



Yield: 0.46g, 64%, buff crystals

m.p. 139- 141 ^oC

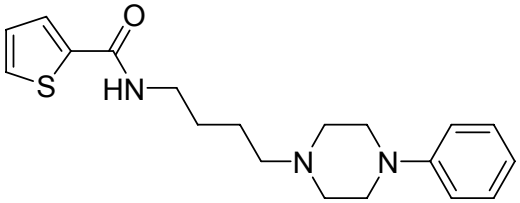
^{1}H-NMR ($CDCl_3$): 1.66- 1.69 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.52 (t, 2H, J= 6.7 Hz, CH_2, C4, butyl), 2.68 (brs, 4H, 2 X CH_2, piperazine), 2.95 (brs, 4H, 2 X CH_2, piperazine), 3.47 (q, 2H, J= 5.8 Hz, CH_2, C1, butyl), 6.56 (brs, 1H, NH), 6.82- 7.55 (m, 7H, aromatic). Phenolic proton signal was not shown.

IR (cm^{-1}): 3236 (OH), 1633 (C=O)

GC/MS : m/z 70 (60%), m/z 111 (90%), m/z 148 (30%), m/z 199 (45%),m/z 211 (100%), m/z 355 (20%), m/z 359 (M^+, 10%)

Elemental analysis: for $C_{19}H_{25}N_3O_2S$ calcd. C 63.48, H 7.01, N 11.69; found C 63.87; H 6.65; N 11.75.

Thiophene-2-carboxylic acid [4-(4-phenylpiperazin-1-yl)butyl]amide (45j)



Yield: 0.5g, 73%, creamy white crystals

m.p. 115- 117 ^{o}C

^{1}H-NMR ($CDCl_3$): 1.65- 1.68 (m, 4H, 2 X CH_2, C2, C3, butyl), 2.46 (t, 2H, J= 6.8 Hz, CH_2, C4, butyl), 2.63 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.19 (t, 4H, J= 5 Hz, 2 X CH_2, piperazine), 3.46 (q, 2H, J= 6.2 Hz, CH_2, C1, butyl), 6.42 (brs, 1H, NH), 6.83- 7.45 (m, 8H, aromatic)

IR (cm^{-1}): 1641 (C=O)

GC/MS : m/z 70 (30%), m/z 104 (40%), m/z 111 (50%), m/z 132 (60%), m/z 211 (100%), m/z 328 (50%), m/z 343 (M^+, 30%)

Elemental analysis: for $C_{19}H_{25}N_3OS$ calcd. C 66.44, H 7.34, N 12.23; found C 66.56; H 7.36; N 12.07.

4.2 Radioligand binding assay

4.2.1 Radioligand binding of dopamine receptors in intact HEK293 cells

One T75 flask of HEK293 cells (~90% confluent) recombinantly expressing the respective receptor was harvested by trypsinisation (0.05% trypsin/0.02% EDTA), and cells were resuspended in phosphate-buffered saline (PBS), pelleted and rinsed once with PBS. Pelleted cells were then resuspended in Krebs-HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 11.7 mM D-glucose, 1.3 mM $CaCl_2$, 10 mM HEPES, pH 7.4) and evenly distributed into pretreated microcentrifuge tubes (sigmacote; protein content: ~90 µg/tube). Binding assays were carried out in triplicate in a total volume of 1 ml. Incubation was initiated by addition of 100 µl of [^{3}H]ligand and was carried out at 27°C for 1 h. It was stopped by rapid filtration through pretreated glass fibre filters (0.25% polyethyleneimin), followed by 2×5-ml washes with ice-cold water. Radioactivity retained on the filters was counted by liquid scintillation spectroscopy using a Beckman scintillation counter. For binding studies at the hD1 and hD5 receptors, [N-methyl-^{3}H]SCH23390 (83.0 Ci/mmol; Nycomed Amersham, Buckinghamshire, UK) was used. [^{3}H]Spiperone (97.0 Ci/mmol; Nycomed Amersham) was used for binding studies at the hD2L and hD3 receptors [66].

4.2.2 Radioligand binding of dopamine receptors in CHO cells

One T75 flask of CHO cells (~90% confluent) recombinantly expressing the respective receptor was harvested by trypsinisation (0.05% trypsin/0.02% EDTA), and cells were resuspended in ice-cold 50 mM Tris-HCl/5 mM $MgCl_2$, pH 7.4. Cells were then disrupted using a Polytron on ice. After centrifugation at 40,000 g, supernatant was discarded, and pellet was 3 times washed with ice-cold Tris-HCl/$MgCl_2$ buffer. Eventually, the pellet was resuspended in Tris-HCl/$MgCl_2$ buffer and evenly distributed into pretreated microcentrifuge tubes (sigmacote; protein content: ~120 µg/tube). Binding assays were carried out in triplicate in a total volume of 1 ml as described for HEK293 cells [66].

4.3 Molecular Modeling

4.3.1 Energy minimization procedure

The compounds were drawn on ChemSketch 11 and saved as mol file, the latter were subjected to energy minimization using Force Field MMFF94x by Molecular Operating EnvironMent (MOE) [114] software, MOE, Chemical Computing Group Inc. http://www.chemcomp/com and the basic piperazine nitrogen is protonated.

4.3.2 Source of target proteins

The Crystal structure of Human D3 receptors complexed with its antagonist eticlopride (PDB ID code: 3PBL), was downloaded from the Protein data bank and opened with MOE software. The Homology model of human D4 receptor was downloaded from the supporting information of the article published by McRobb *et al* [139].

4.3.3 Docking procedure of D3 receptors

The co-crystallized compound was selected, and the binding site was identified according to residues in 4A^{o} proximity to those interacted with the co-crystallized antagonist eticlopride. Ligand interactions were computed for the X-ray co-crystallized compound, eticlopride, to reveal the different types of interaction as a validation for the coming docking procedure. Default settings of MOE-Dock [114] were used, including “Rotate Bonds” option in order to allow flexible ligand-rigid receptor docking. The scoring function was London dG with a replacement of Triangle Matcher. Thirty poses of each ligand docked to the identified binding site were retained and ranked in order of increasing

scoring function. The 2D ligand-receptor interactions of these poses were viewed using the "compute ligand interaction" option of MOE.

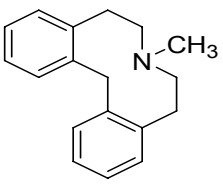
4.3.4 Docking procedure of D2 and D4 receptors

The binding site was isolated off the validated homology model using the default settings of the site finder panel option of MOE [114]. Again the default settings of MOE-Dock were used, including “Rotate Bonds” option in order to allow flexible ligand-rigid receptor docking. The scoring function was London dG with a replacement of Triangle Matcher. Thirty poses of each ligand docked to the identified binding site were retained and ranked in order of increasing scoring function. The 2D ligand-receptor interactions of these poses were viewed using the "compute ligand interaction" option of MOE [114].

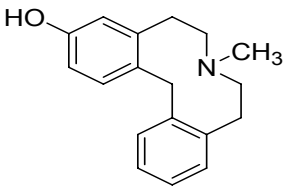
5. Conclusion

This work aimed at modulating the selectivity and affinity of some dopaminergic ligands towards the different subtypes of the two families of dopamine receptors; the D1-like and the D2-like receptors *via* developing new thieno and benzothieno ligands so that we could achieve novel receptor subtype selectivity and/or better pharmacokinetic properties.

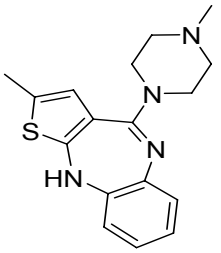
In the first part of the work we have targeted the D1-like receptor subtypes; D1 and D5 that are characterized by sharing high level of molecular structure identity within their transmembrane helices. We aimed at developing new azecine- bearing ligands characterized by being sub selective to either one of the two D1 family subtypes and/or with cross affinity to D2 family members. The work has started by observing that increasing the electron cloud of the D1 selective dibenzazecine LE410 by hydroxylation (LE405) shifts the affinity towards the D5 receptor subtype. We have also noticed that the thiophene containing Olanzapine shows for D1, D2, and D5 a much higher affinity than Clozapine. Thus we have prepared two regioisomers carrying a thiophene in different orientations, namely the benzo[d]thieno[2,3-g]azecine **1**, and the benzo[d]thieno[3,2-g]azecine **2** and furthermore the benzothiophene derivative **3**, Schemes **2**, **3**, **4**.



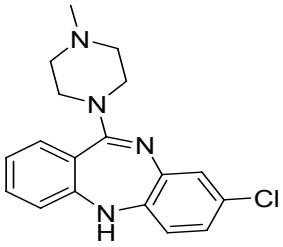
LE 410



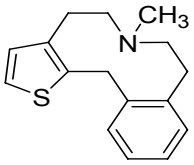
LE 405



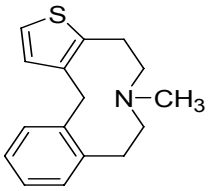
Olanzapine



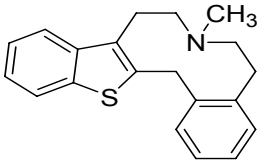
Clozapine



1



2



3

Based on the results obtained in this work, the following conclusions can be made:

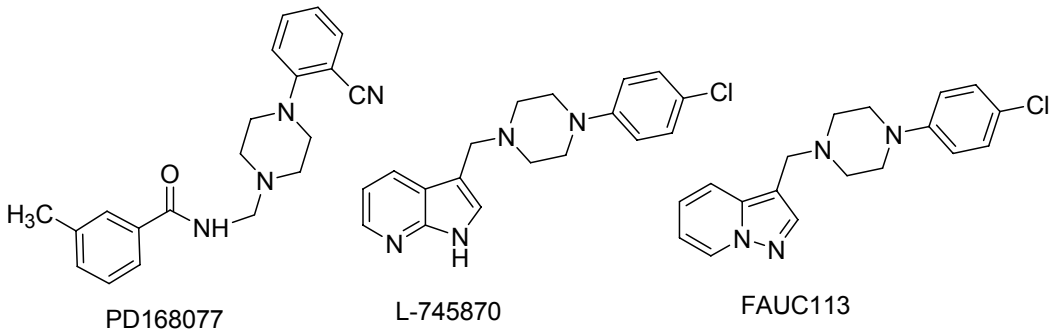
- The bioisosteric replacement of the benzene (LE410) with thiophene or benzothiophene in right orientation (compounds **1**, **3**) have shifted the selectivity from D1 to D5 receptor subtypes, the same effect achieved by replacing benzene with hydroxybenzene (LE405), suggesting that increasing the electron cloud on the ligand's scaffold could be one of the prominent factors to enhance the ligand's affinity at D5 receptor subtypes. In this case thiophene system would ensure better pharmacokinetics over its hydroxybenzene analogue.
- Reversing the orientation of the thiophene ring (compound **2**) has restored back the original selectivity pattern of LE410 towards D1 rather than D5 subtypes revealing the importance of the position of the thiophene sulfur atom in binding to D5 subtypes.
- Aligning the reported D1 and D5 sequences according to Ballesteros and Weinreb numbering [134- 136] reveled that Tyrosine, Glutamine, Serine, and Therionine residues in D5 could serve as hydrogen bond acceptors from ligands owning electron donating moieties contrary to

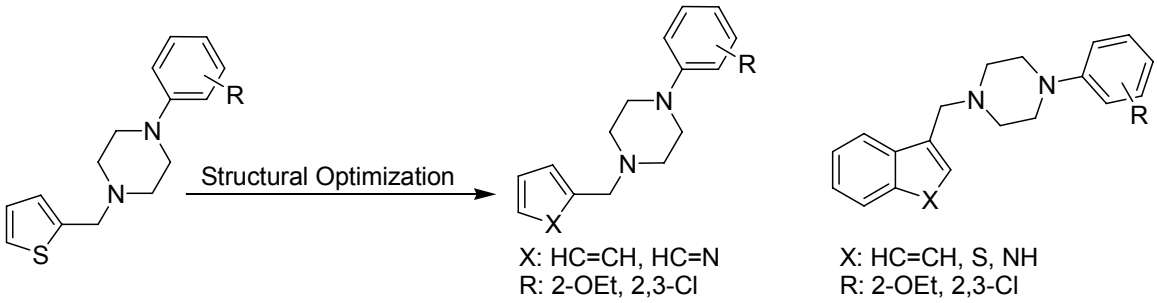
their counterparts in the D1 binding site, thus providing a possible explanation for the importance of having the thiopene ring in the appropriate orientation [134- 136].

- Compounds **1**, **3** with the appropriate thiophene positioning were those that showed better affinity to D2 and D3 receptor subtypes when compared to the lead compounds LE410 and LE405. Compound 3 was found to be much more selective to D2, and D3 receptors with Ki values of 1.5, and 18 nM respectively versus 40 nM on D1 receptor subtype showing to be the first azecine derivative with this reversed selectivity pattern. It also has a Ki of 1.9 nM at D5 receptors showing to be the first azecine with comparable cross affinity between the two families of dopamine receptors.
- Based on the suggested molecular structure of the D2 and D3, it was proved that Serine residues contribute to protein-ligand interaction serving as hydrogen bond acceptors, concluding the importance of having the regularly positioned thiophene or benzothiophene as electron donating moieties.

In the second part of this work, our interest was shifted towards D2-like family members, where we aimed at developing new phenylpiperazine bearing ligands with subtype receptor selectivity towards D3/D4 rather than D2 receptor subtypes. We started with a series of 10 thienomethylphenylpiperazines serving as D4 selective analogues for PD168077, L-745870, and FAUC113. Based on the binding affinity data obtained, some of the synthesized derivatives had their thiophene ring replaced with other aryl and heteroaryl moieties to demonstrate the effect of

this particular part of the structure on the affinity towards the target protein and to configure out an interactive Structure Affinity Relationship study, thus another 10 analogues have been prepared, Schemes **5**, **6**.

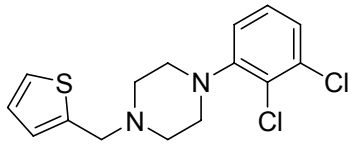




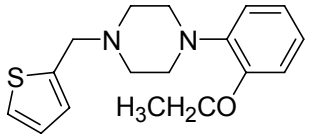
Based on the results obtained in this work, the following conclusions can be made:

- Presence of oxygenated rather than halogenated substituent on the phenylpiperazine unit is crucial for getting optimum D4 affinity. Compounds with unsubstituted phenylpiperazine unit shown even lower affinity to the target receptor. This is may be due to the influence of this substituent either on the pKa of the basic piperazine that affords salt bridge interaction with Aspartate 115 residue in the D4 binding pocket or on the dispersion force of the phenyl ring that is necessary to afford Van der Waals interaction with the imidazole ring of Histidine 414 residue.

- Compound **31e** having 2-OEt substituent on the phenypiperazine unit showed the best affinity to D4 receptors among the thienylmethylphenyl- piperazine series, while compound **31d** having the 2,3-Cl was shown to have better affinity towards D3 rather than D4, indicating that the nature of the substituent at this part of the scaffold could also manipulate receptor subtype selectivity.



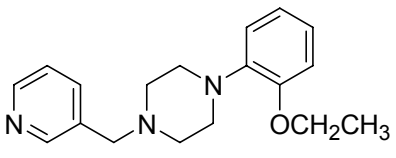
31d



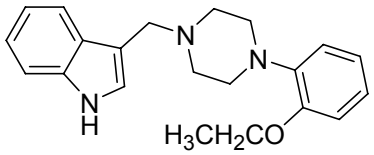
31e

- Viewing the binding pockets of D4 versus D3 model could show that the Phenylalanine 91 residue is conserved in the D4 pocket and faces the also conserved and the less bulky Valine 86 in D3. Thus, we could conclude that the relatively more bulky dihalogenated bearing compound **31d** is more likely to show better affinity towards D3 (with a relative larger pocket than D4 subtypes) among this series.
- The position of the substituent on the phenylpiperazine unit seems to be also essential for modulating ligands' affinity towards D4 receptors, where ortho substituted ligands were found to be 2- 10 folds more affinitive to D4 receptors than their para substituted congeners. A certain degree of non coplanarity achieved through a dihedral angle of about 60^{o} between the phenyl and the piperazine rings lead to optimum D4 affinity.
- Replacing the thiophene system with more or less electronically similar arene systems such as benzene, naphthaline, and benzothiophene lead to ligands with similar binding affinity data towards D4, while

replacing the thiophene system with relatively more electronegative arene moieties such as pyridine (compound **32a**) and indole (compound 36a) lead to dramatic increase in D4 affinity and selectivity over D2 and D3 receptor subtypes. Compound **36a** had Ki value of 0.03 nM on D4 receptors showing to be 100 times more potent than the D4 selective FAUC113.



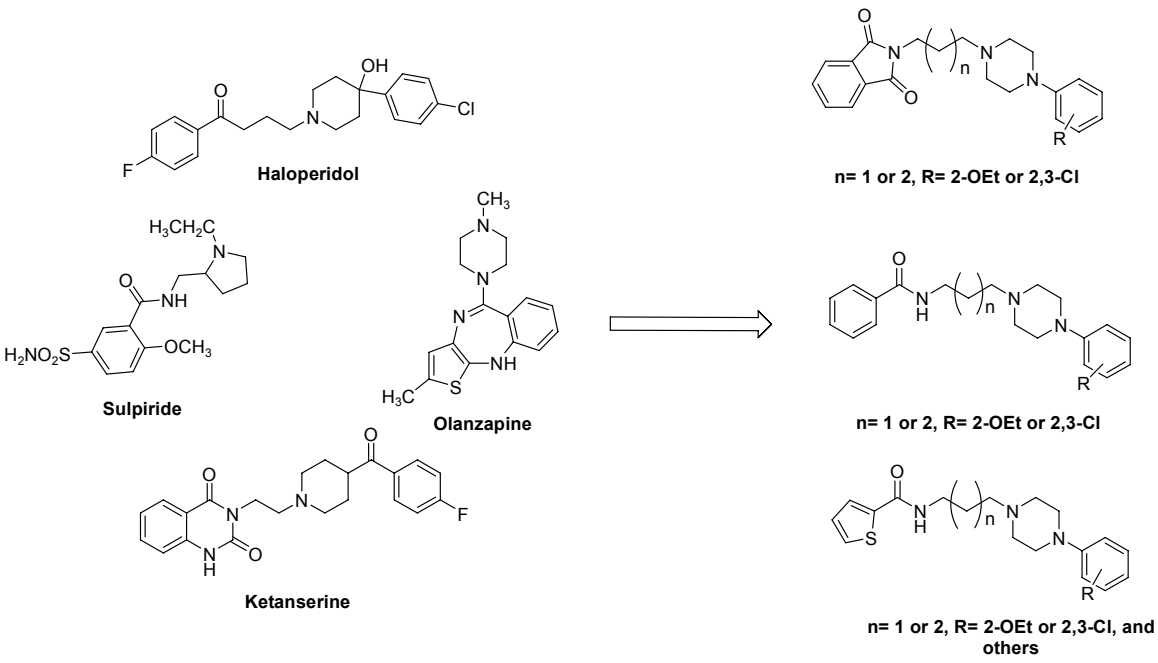
32a



36a

- Molecular docking studies at D4 receptor model revealed the involvement of the D4 unique residue Arginine 186 in affording a first to report cation arene interaction with the docked ligands, providing a possible explanation why compounds bearing pyridine and indole systems were among the highest D4 affinitive ligands.

In a further attempt to develop D3/D4 selective ligands we have prepared three series of compounds bearing related chemical scaffolds, namely phenylpiperazinylalkylisoindoledione; compounds **40a- b**, **41a- b**, benzamido alkylphenylpiperazine, compounds **42a**- **b**, **43a**- **b**, and thienoamidophenyl-piperazine, compounds **44a**- **b**, **45a**- **b**, Schemes **7**, **8**, **9**. The design of these probes was inspired from the structures of some typical and atypical antipsychotic agents having variable affinity and selectivity patterns towards different dopamine and/or serotonin receptor subtypes, so that our prepared probes could be able to serve as hybrids of these marketed agents.



Based on the results obtained in this work, the following conclusions can be made:

- Compounds bearing propyl linker between the phenylpiperazine unit and the terminal arene unit were shown to have better affinity at D2 subtypes, while those having butyl linker were much selective to D3 subtypes. Both types of compounds have shown appreciable affinity at D4 receptor subtypes.
- Molecular docking studies at the D2, D3, and D4 models have revealed that probes with propyl linker would have their terminal aromatic appendage in contact to Isoleucine 183 in D2 receptors that faces Serine 182 in D3 receptors. The chemical nature of Isoleucine enables it to be more hydrophobic than Serine and thus would afford better hydrophobic interaction with the ligands' aromatic appendage explaining the better affinity of the compounds bearing the propyl linker towards D2 receptor subtypes.

- Docking also has shown that butyl linker bearing probes are able to deliver their aromatic appendage to interact with the Valine 180 residue in the EL2 of D3 receptors that faces the more polar Glutamate 181 in D2 subtypes. Again the more hydrophobic nature of Valine relative to Glutamate residues would ensure better hydrophobic interaction with ligands' aromatic appendage. This might explain the better affinity of the compounds bearing the butyl linker towards D3 receptor subtypes.
- It was found that Isoleucine 183 of D2 is occupied with Arginine 186 in D4 and the Glutamate 181 in D2 is occupied with Valine 184 in D4 receptor subtypes. Both Arginine 186 and Valine 184 residues in the D4 binding site are able to interact properly with the aromatic appendage of our probes and this might explain the noticeable preferentiality of both propyl and butyl linker bearing ligands towards D4 receptor subtypes rather than D2 ones. Furthermore, the carbonyl group of the synthesized propes was found to afford hydrogen bonding interaction with the unique Arginine 186 in D4 binding site.
- Thiophene bearing probes showed the best affinity towards the target receptor subtypes among the whole series.
- As for the phenylpiperazine part of the scaffold, substitution with 2-OEt or 2,3-Cl was optimum for the affinity of the synthesized probes towards the target receptor subtypes.

6. Zusammenfassung

Ziel dieser Arbeit war es, potente Liganden mit neuen Subtypselektivitäts-Mustern an Dopamin Rezeptoren zu finden. Eine Chance dazu, wurde in der Etablierung des Thiophenringes in bioaktiven, aber Thiophen-freien Strukturen gesehen. Methodisch enthält die Arbeit in erster Linie, Strukturdesign, Synthese, truktursicherung, Radioligandbindungsexperimente zur Bestimmung der Rezeptoraffinitäten und Molecular Modelling zur Analyse der Struktur-Wirkungsbeziehungen.

Im ersten Teil der Arbeit wurden neue Substanzen mit Selektivität zu den D1-like Rezeptoren (D1, D5) angestrebt, wobei hier eine interne Subtyp-Selektivität problematisch ist und bisher kaum verwirklicht wurde, da D1 and D5 ein hohes Maß an struktureller Identität aufweisen. Die Idee der Fokussierung auf Thiophenderivate ergab sich z.B. aus der Beobachtung, dass bei den in unserer Arbeitsgruppe enwickelten Dopamin Rezeptor Antagonisten des Azecin-Typs die Erhöhung der Elektronendichte eines der beiden Benzenringe im Dibenzo-azecin-Derivat LE 410 durch Hydroxylierung zu LE 405, eine Verschiebung der Affinität hin zu D5 bewirkte. Die Frage stellt sich, ob die Erhöhung der Elektronendichte hierfür der auslösende Faktor ist und ob dann auch der Ersatz des Benzenringes durch elektronenreiches Thiophen zum Ziel führt. Motivierend war auch, dass das Thiophenderivat Olanzapin an den Subtypen D1, D2, und D5 sehr viel affiner ist als das Benzen-analoge Clozapin. Aus diesen Gründen wurden in dieser Arbeit zwei regioisomere Azecine mit einem Thiophenring in unterschiedlicher Orientierung, nämlich das Benzo[d]thieno[2,3-g]azecine **1** und das Benzo[d]thieno[3,2-g]azecine **2**, darüber hinaus auch noch des

Benzothiophen-Derivat **3** jeweils in Mehourstufensynthesen hergestellt, wie sie in Schemata **2**, **3**, und **4** wiedergegeben sind. Deren Affinitäten wurden durch Radioligand-Bindungsexperimente an klonierten humanen Dopamin Rezeptoren bestimmt. Im Rahmen der Synthesearbeit wurden auch ein ungewöhnlicher Abbau des Thiophenringes beobachtet und damit neue Erkenntnisse zur Thiophenchemie erhalten.

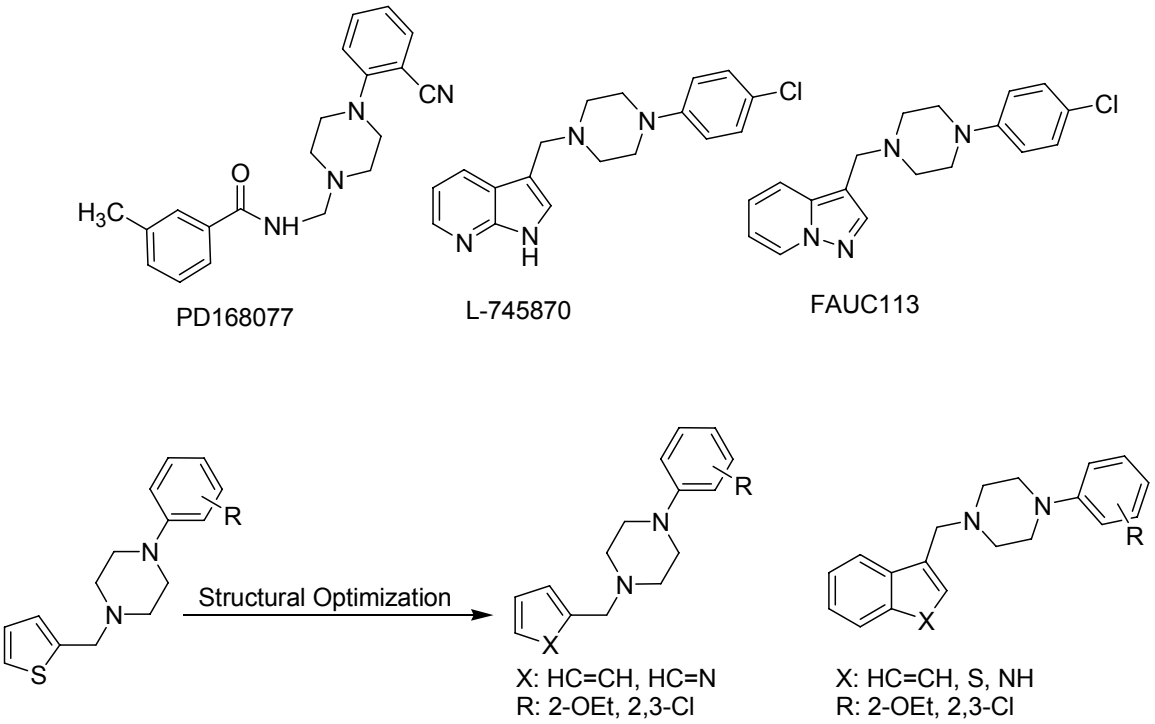
Basierend auf den Ergebnissen dieses Arbeitsabschnittes können hier die folgenden Schlüsse gezogen werden.

- Der bioisostere Austausch des Benzens in LE410 gegen Thiophen oder Benzothiophen in der Orientierung der Verbindungen 1 und 3 führt zu Hochaffinen Substanzen und verschiebt die Selektivität vom D1 zum D5 Subtyp. Ähnliches war schon bei der Hydroxylierung von LE 410 zu LE 405 zu beobachten, was den Schluß naheleg, dass die Steigerung der Elektronendichte an einem der Aromaten generell eine D5 Affinität begünstigt. Aus pharmakokinetischer Sicht sollte bei einem ZNS-Target der Einbau von Thiophen vorteilhafter sein als die Hydroxylierung, die praktisch schon einen ersten Schritt eines metabolischen Abbaus darstellt.
- Die Umkehrung der Orientierung des Thiophenringes (Verbindung 2 im Vergleich zu 1 und 3) bringt das bekannte Selektivitätsmuster von LE410 mit Bevorzugung von D1 gegenüber D5 wieder zurück, d.h., die Position des Schwefels ist relevant.
- Aus der von Ballesteros and Weinreb (134- 136) publizierten Sequenz in der Bindungstasche des D5 Rezeptors mit den Aminosäuren

Tyrosin, Glutamin, Serin, und Threonin läßt sich ebenfalls eine Präferenz von Thiophenderivaten ableiten.

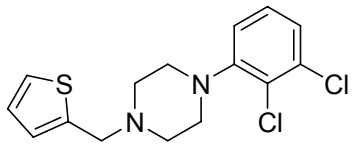
- Die Verbindungen **1**, **3** mit vorteilhafter Thiophen Positionierung zeigen höhere Affinität zu D2 und D3 im Vergleich zu den Azecin-leads LE410 und LE405. 3 war dabei hochaffin an D2 und D3 mit Ki Werten von 1.5 und 18 nM, gegenüber nur 40 nM an D1. Bei zugleich sehr hoher Affinität zu D5 mit Ki = 1.9 nM steht fest, dass Verbindung **3** das erste cross-affinity Azecin ist, die Substanz ist gleichermaßen hochaffin an D2 und D5, belegt also beide Dopamin Rezeptor Familien.
- Die vorgeschlagenen molekularen Strukturen von D2 und D3 Rezeptor legen nahe, dass die Aminosäure Serin als Wasserstoffbrückenakzeptor wesentlich zur Protein-Ligand Interaktion beiträgt und dass regular positioniertes Thiophen einen bedeutenden Beitrag zur Interaktion leisten kann.

Im zweiten Teil der Arbeit focussierten wir uns mehr auf Liganden an den Rezeptoren der D2-Familie und synthetisierten zunächst 10 verschiedene Thienomethylphenylpiperazine, die als Thiophenanaloga der Thiophen-freien D4 selektiven Liganden PD168077, L-745870, und FAUC113 betrachtet werden können. Weiterhin wurde bei einigen der Syntheseprodukte der Thiophenring wieder gegen andere Aryl,bzw. Heteroaryl-Reste ausgetauscht um den Einfluss des Strukturelementes Thiophen noch besser erkennen zu können, Schemata **5**, **6**.

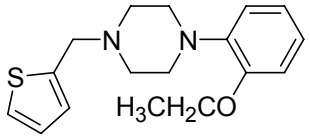


Folgende Erkenntnisse konnten in dieser Piperazinreihe gewonnen werden.

- Ethoxy am Phenylpiperazine-Teil sind offensichtlich günstiger als Halogen, d.h. optimale Affinitäten am D4 Rezeptor werden mit den Ethoxyderivaten erreicht. Dies mag am positiven Einfluss der Alkoxygruppe auf die Basizität der Piperazin-Stickstoffe liegen, wodurch die Interaktion mit dem Aspartat 115 der Bindungstasche begünstigt wird oder an der Verstärkung der Möglichkeit des Phenylrestes zur van der Waals Interaktion mit dem Histidin 414 .
- Das 2-Ethoxy-thienomethylphenylpiperazin Derivat **31e** zeigt die höchste Affinität zum humanen D4 Rezeptor, während die Affinität des strukturell analogen 2,3-Dichlor-thienomethylphenylpiperazin **31d** am D4 sogar geringer als die am D3 Rezeptor ist.

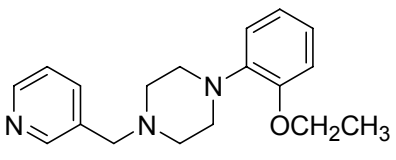


31d

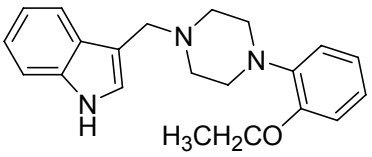


31e

- Ein Vergleich der Bindungstaschen zeigt dass ein Phenylalanin 91 in D4 einem weniger raumerfüllenden Valin 86 in D3 entspricht. So leuchtet ein, dass Verbindungen mit dem sterisch anspruchsvolleren Dihalogenphenyl-rest wie in **31d** mit D3 besser zur Interaktion kommen als mit D4.
- Die Position der Substituenten an der Phenylpiperazin-Teilstruktur moduliert ebenfalls die Affinität zu D4. Orthosubstituierte Derivate sind 2-10 fach günstiger als parasubstituierte. Ein gewisses Verdrehen aus der Koplanarität durch die Orthosubstituenten - z.B. ca. 60° Diederwinkel zwischen Phenyl- und Piperazinring - führt zur optimalen D4 Affinität in der Verbindungsgruppe.
- Der Austausch von Thiophen mit elektronisch relativ ähnlichen Arenen, wie Benzen, Naphthalin und Benzothiophen führt auch zu Liganden mit ähnlicher D4 Affinität. Ersatz des Thiophens durch Pyridin (**32a**) oder Indol (**36a**) steigert die D4 Affinität und die Selektivität gegenüber D2 und D3 dramatisch. Tatsächlich hat Verbindung (**36a**) einen Ki Wert von 0.03 nM am D4 Rezeptor und ist damit 100x potenter als der D4 selektive Ligand **FAUC113**.



32a



36a

- Molecular docking Studien am D4 Rezeptor Modell belegen die Beteiligung eines D4 spezifischen Arginin 186 in Form einer bisher nicht berichteten Kation-Aren-Wechselwirkung mit dem angedockten Liganden. Dies könnte erklären, dass die Verbindungen mit Pyridin und Benzothiophen Komponenten zu den höchstaffinen D4 Liganden gehören.

In Fortsetzung der Suche nach D3/D4 selektiven Liganden wurden drei weitere Serien von Phenylpiperazinen synthetisiert, nämlich Phenylpiperazinylalkyl-isoindoledione (**40a- b**, **41a- b**), Benzamidoalkylphenylpiperazine (**42a- b**, **43a- b**), and Thienoamidophenylpiperazine (**44a- b**, **45a- b**), Schemata **7**, **8**, **9**. The Design dieser Kandidaten wurde durch Strukturelemente von typischen und atypischen Antipsychotika inspiriert, deren aromatische/heteroaromatische Teilstrukturen über Linker als aromatische Endgruppen an den Phenylpiperazinteil eingebracht wurde, so dass von Hybridverbindungen gesprochen werden kann.

Die Ergebnisse dieses Arbeitsabschnittes lassen wie folgt zusammenfassen:

- Verbindungen mit einem Propyllinker zwischen Phenylpiperazin und der terminalen Areneinheit entwickeln höhere D2, die mit einem Butyllinker höhere D3 Affinität. Beide Typen zeigen dazu akzeptable D4 Affinität.
- Molecular docking Studien an D2, D3, and D4 Modellen ergaben, dass die Strukturen im Falle eines Propyllinkers ihren terminalen aromatischen Teil in Kontakt mit dem Isoleucine 183 im D2 receptors bringen können. Strukturen mit Butyllinker wiederum ermöglichen eine

gute Interaktion der aromatischen Endgruppe mit Valin 180 im EL2 des D3 Rezeptors.

- Durch Docking wurde auch gefunden, dass das Isoleucin 183 in D2 durch Arginin 186 in D4 und das Glutamate 181 in D2 durch Valine 184 in D4 ersetzt ist. Beide Varianten ermöglichen eine Wechselwirkung mit den aromatischen Endgruppen unserer Versuchsubstanzen und dies mag die Affinität sowohl der Propyllinker als auch der Butyllinker Derivate vorzugsweise zu D4 und weniger zu D2 erklären. Hinzu kommt, dass die Carbonylgruppen der Testverbindungen eine Wasserstoffbrückenbindung mit dem typischen Arginine 186 in der D4 Bindungstasche ausbilden können.
- Generell zeigen die Thiophen-haltigen Strukturen die höchsten Affinitäten gegenüber den verschiedenen Targets, was das Ausgangskonzept dieser Arbeit unterstützt.
- Im Phenylpiperazine-Teil ist tatsächlich die Substitution mit 2-OEt oder 2,3-Cl das Optimum.

7. References

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List of Abbreviations

AC:	Adenylyl cyclase.
ADHD:	Attention deficit hyperactivity disorder.
AMPA:	Alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP:	Adenosine triphosphate.
cAMP:	Cyclic adenosine monophosphate.
CNS:	Central nervous system.
COMT:	Catechol - o - methyl transferase.
CTZ:	Chemoreceptor trigger zone.
DARPP-32:	Dopamine and cyclic AMP- regulated phosphoprotein.
DAT-1:	Dopamine transporter
DMSO:	Dimethyl sulfoxide.
GABA:	Gama amino butyric acid.
GPCRs:	G protein coupled receptors.
L-DOPA:	L-3,4-dihydroxyphenylalanine.
m.p.:	Melting point.
MAO:	Monoamine oxidase.
MOE:	Molecular Operating EnvironMent.
MS:	Mass spectrometry.
NET:	Norepinephrine transporter.
NMDARs:	N-methyl D-aspartate receptors.
NMR:	Nuclear magnetic resonance.
PD:	Parkinson's disease.
PDB:	Protein data bank.
PET:	Positron emission tomography.
PIH:	Prolactin inhibiting hormone.
PKA:	Protein kinase A.
PP 1:	Phosphatase 1.
R_f:	Retention factor.
VTA:	Ventral tegmental area.

List of Publications

Manuscripts

Ashraf H. Abadi,Dalal A. Abouel-Ella, Jochen Lehmann, Heather N. Tinsley, Bernard D. Gary, Gary A. Piazza, and Mohammed A. O. Abdel-Fattah, "Discovery of colon tumor cell growth inhibitory agents through a combinatorial approach", Eur. J. Med. Chem., 45, 2010, 90- 97.

Ismail Salama, Mohamed A. O. Abdel-Fattah, Marwa S. Hany, Shaimaa A. El-Sharif, Mahmoud A. M. El-Naggar, Rasha M. H. Rashied, Gary A. Piazza, and Ashraf H. Abadi, "CoMFA and CoMSIA Studies of 1,2-dihydropyridine Derivatives as Anticancer Agents", Med Chem, 8, 2012, 372- 83.

Mohamed A. O. Abdelf-Fattah, Mahmoud A. M. El-Naggar, Rasha M. H. Rashied, Bernard D. Gary, Gary A. Piazza, and Ashraf H. Abadi " Four-Component Synthesis of 1,2-Dihydropyridine Derivatives and their Evaluation as Anticancer Agents", Med. Chem., 8, 2012, 392- 400.

Mohamed A.O. Abdel-Fattah, Jochen Lehmann, and Ashraf H. Abadi “Discovery of Highly Potent and Selective D4 ligands by Interactive SAR Study”, Bioorg. Med. Chem. Lett., 23, 2013, 5077- 81

Mohamed A. O. Abdel-Fattah,Jochen Lehmann, and Ashraf H. Abadi “Adopting an Interactive SAR Approach to Discover Novel Hybrid Thieno Probes as Ligands for D2-Like Receptors with Affinities in the Subnanomolarar Range”, submitted to Chemistry and Biodiversity.

Mohamed A. O. Abdel-Fattah, Christoph Enzensperger, Peter Schweikert, Ashraf H. Abadi, Jochen Lehmann “Synthesis and pharmacology of thieno-azecine derivatives as dopamine receptor ligands with novel subtype-selectivity profile”, submitted to J. Med. Chem.

Posters

Mohammed A. O. Abdel-Fattah, ,Dalal A. Abouel-Ella, Jochen Lehmann, Heather N. Tinsley, Bernard D. Gary and Gary A. Piazza, and Ashraf H. Abadi, "Discovery Of Novel Phosphodiestrase 3 and Colon Tumor Cell Growth Inhibitory Agents through a Combinatorial Approach", Deutsche Pharmazeutische Gesellschaft, 2009, Fredrich – Schiller University, Jena, Germany.

Selbstständigkeitserklärung

Hiermit erkläre ich, dass mir die geltende Promotionsordnung der Fakultät bekannt ist.

Die vorliegende Arbeit habe ich selbstständig und ausschließlich unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt.

Ich habe weder die Hilfe eines Promotionsberaters in Anspruch genommen, noch unMittelbar oder mittelbar geldwerte Leistungen im Zusammenhang mit dem Inhalt meiner Dissertation an Dritte erbracht.

Die vorliegende Dissertation habe ich ausschließlich an der Friedrich-Schiller-Universität als Prüfungsarbeit eingereicht.

Jena, im Juni 2013

Curriculum Vitae

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Acknowledgement

All my gratitude to **Allah** (Arabic name of the God), to Whom goes all my thanks and appreciation, and to Whom I owe the courage and strength to complete my thesis.

This Work is the come out of four years of work that took place at the Pharmacy Institute, Jena University and the Faculty of Pharmacy and Biotechnology, German University in Cairo.

My endless appreciation to my thesis advisors **Prof. Dr. Jochen Lehmann**, Professor of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller Universität, Jena, Germany; and **Prof. Dr. Ashraf H. Abadi**, Professor of Pharmaceutical Chemistry, Dean of Faculty of Pharmacy and Biotechnology, The German University in Cairo, for suggesting the point of the research, constructive supervision, enthusiasm, inspiration, providing me with immense knowledge in the subject matter, and for their sincere efforts in revising this text. I was really blessed to have such professional and talented advisors who gave me the chance to join their research group to finish this work, impressed me with their kind hospitality and provided all necessary facilities to come up with this work. I will always owe to them.

I would also like to heartily thank the helpful and cheerful group of people in Philosophenweg 14 whom I have been blessed with and on whose assistance I could always rely on. My deepest thanks and appreciation are due to **Dr. Christoph Enzensperger** whose ideas and suggestions have contributed a lot to this research work. His friendly and easy going way of dealing with people has encouraged me to ask about and discuss a lot of issues with him. He has really taught me a lot.

In particular I would like to thank my dearest friends **Dina Robaa** and **Robert Otto** for their continuous support and being always there in tough times. I am very grateful to **Mrs. Katrin Fischer** and **Mrs. Monika Listing** who were always there to answer my technical questions and **Mrs. Heidi Traber** and **Mrs. Petra Wiecha** for their help in biological assays.

My sincere thanks would fly to all my colleagues at the Pharmaceutical Chemistry Department, the German University in Cairo for the emotional support, comradely, and encouragement during the completion of this work.

Lastly, and most importantly, I wish to thank my parents and family. They bore me, raised me, supported me, taught me, loved me and gave me a lot. To them I dedicate this thesis.