Published in final edited form as:
Bioorg Med Chem. 2014 June 15; 22(12): 3105–3114. doi:10.1016/j.bmc.2014.04.026.

Identification of a New Selective Dopamine D_4 Receptor Ligand

Dinithia Sampson[a,#], **Xue Y. Zhu**[a,#], **Suresh V. K. Eyunni**[a], **Jagan R. Etukala**[a], **Edward Ofori**[a], **Barbara Bricker**[a], **Nazarius S. Lamango**[a], **Vincent Setola**[b], **Bryan L. Roth**[b], and **Seth Y. Ablordeppey**[a,*]

[a]Division of Basic Pharmaceutical Sciences, Florida A&M University, College of Pharmacy and Pharmaceutical Sciences, Tallahassee, FL 32307, USA

[b]Department of Pharmacology, Medicinal Chemistry and Psychiatry, University of North Carolina at Chapel Hill, School of Medicine, NC 27599, USA

Abstract

The dopamine D_4 receptor has been shown to play key roles in certain CNS pathologies including addiction to cigarette smoking. Thus, selective D_4 ligands may be useful in treating some of these conditions. Previous studies in our laboratory have indicated that the piperazine analog of haloperidol exhibits selective and increased affinity to the DAD_4 receptor subtype, in comparison to its piperidine analog. This led to further exploration of the piperazine moiety to identify new agents that are selective at the D_4 receptor. Compound **27** (K_iD_4 = 0.84 nM) was the most potent of the compounds tested. However, it only had moderate selectivity for the D_4 receptor. Compound **28** (K_iD_4 = 3.9 nM) while not as potent, was more discriminatory for the D_4 receptor subtype. In fact, compound **28** has little or no binding affinity to any of the other four DA receptor subtypes. In addition, of the 23 CNS receptors evaluated, only two, $5HT_{1A}R$ and $5HT_{2B}R$, have binding affinity constants better than 100 nM (Ki < 100 nM). Compound 28 is a potentially useful D_4-selective ligand for probing disease treatments involving the D_4 receptor, such as assisting smoking cessation, reversing cognitive deficits in schizophrenia and treating erectile dysfunction. Thus, further optimization, functional characterization and evaluation in animal models may be warranted.

1. Introduction

Targeting dopamine receptors for drug development has been of interest for decades because of their potential utility in many well-known pathological conditions.[1] Initially, dopamine receptors were classified as D_1-like and D_2-like for many years until it became evident that the D_1-like receptors consisted of D_1 and D_5 subtypes and the D_2-like receptors consisted of D_2, D_3 and D_4 subtypes. By far, the D_2-like receptors have been studied more because of their association with clinically relevant neuropsychiatric conditions, such as schizophrenia,

*Corresponding author.
#Equal Contribution to this manuscript

mood disorders, Parkinson's disease and others. Each of these subtypes is now separately associated with certain pathophysiological conditions. For example, several D_2 agonists and partial agonists have shown beneficial effects in counteracting Parkinson's disease, attention-deficit hyperactivity disorder and certain mood disorders[2] while D_2 antagonism is associated with antipsychotic properties.

Since its cloning, the D_4 receptor has attracted considerable interest. For example, early reports indicated that D_4 antagonists attenuate not only the discriminative-stimulus effects of cocaine and methamphetamine[3–4] but also morphine-induced withdrawal signs induced by naloxone.[5] More recently, there was a report that the D_4 receptor antagonist, L-745,870 (Chart 1) but not PD 168,077, a selective D_4 receptor agonist, attenuated nicotine-induced reinstatement of nicotine seeking behavior.[6] Thus, it would appear that the pharmacological blockade of DAD_4 may serve as a new and potentially effective treatment against relapse to tobacco smoking. On the other hand, PD 168,077 has been shown to induce penile erection in rats when administered in vivo, and L-745,870 was able to block this action, and thus, confirming the D_4 receptor mediated mechanism by which penile erection occured.[7] This potential could place D4 agonists in a strong position to replace the current PDE5 antagonists with a plethora of side-effects.[8] In addition, D4-receptor agonists may be useful in reversing cognitive deficits in schizophrenia.[9] These demonstrated therapeutic potentials have encouraged the search for new D_4 selective ligands in our laboratories.

We have previously carried out several SAR studies that sought to identify structural entities that demonstrate DA receptor subtype selectivity.[10–12] A frequent observation in such studies was the fact that unlike the piperidine analogs of haloperidol, the piperazine analogs demonstrated selective and significant affinities to the D_4 receptor subtype. Chart 1 displays common D_4 selective ligands with the piperazine pharmacophore. The purpose of the current study was to further explore the piperazine ring as a pharmacophore in a search for new entities that are selective to the D_4 receptor subtype using compound **1** (Chart 1), the piperazine analog of haloperidol as the lead.

2. Chemistry

The syntheses of compounds **1–7** (Chart 2) have previously been reported using routine N-alkylation reactions.[9] Compound **8**, the cyclic analog of **1**, was synthesized as depicted in Scheme 1 below. The commercially available 4-chloro-1-(4-fluorophenyl)-butan-1-one (**29**) was converted to 4-chloro-1-(4-fluorophenyl)-2-methylenebutan-1-one (**30**) by refluxing in acetic anhydride in the presence of hexamethylenetriamine.[10,13] Compound **30** was cyclized by heating in concentrated sulfuric acid to produce 2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one (**31**) which was protected by reaction with ethylene glycol to form the 1,3-dioxolane, **32**. Alkylating 4-(4-chlorophenyl)piperazine with **32** and deprotecting the dioxolane resulted in the formation of the desired target compound **8** (Scheme 1).

Compound **9** was similarly obtained using the desfluoro starting material 4-chloro-1-(phenyl)butan-1-one. To obtain compound **10**, the indanone **31** was deoxygenated (**32**) under Clemmensen reaction conditions[14] and then used to alkylate 4-(4-chlorophenyl)piperazine (Scheme 1).

Compounds **11–14** and **16a–c** (Chart 3) were previously synthesized as a part of a drug design effort to obtain novel antipsychotic drugs.[9] In general, an appropriately substituted phenol or benzenethiol (**34**) was alkylated using 3-chloropropan-1-ol (**35**) and the resulting alcohol (**36**) was either tosylated or mesylated and then utilized in alkylating the heteroaryl piperazine (**A–C**) in Scheme 2. The synthesis of compounds **15–16** followed the same procedure reported in Scheme 2.

The syntheses of compounds **17–18** (Chart 4) were also previously reported.[15] To obtain the benzo[d]oxazole analogs, **19–22** (Chart 4, Scheme 3), 2-aminophenol was reacted with chloroalkanoyl alkyl halide (**39**) to form the amide intermediate (**40**) which was then cyclized using polyphosphoric acid (PPA) to obtain 2-(2-chloroalkyl)benzo[d]oxazole, **41**. Compound **41** was used to alkylate 4-chlorophenyl-1-piperazine to yield the desired target compounds **19–22**.

The synthesis of benzofuran moiety in analogs **23–26** (Chart 4), followed a literature procedure.[16] 2-Iodophenol was reacted with an appropriate alkyl-1-yn-1-ol to form the corresponding benzofuran alkanol, which was subjected to a tosylation reaction and the resulting product was used to alkylate an appropriate aryl piperazine or a related analog to form the target compounds as shown in Scheme 4.

Finally, the synthesis of compound **28** (Chart 4) followed the synthetic procedure previously utilized in obtaining compound **27** (Chart 4) as shown in Scheme 5.[15] Briefly, 2-aminobenzenethiol (**46**) was reacted with 4-chlorobutanoyl chloride (**47**) to form 2-(3-chloropropyl)-benzo[d]thiazole, **48**, which was then used to alkylate 2-(piperazin-1-yl)pyrimidine to form the desired target compound, **28**.

3. Results and Discussion

Cigarette smoking is associated with major diseases including cardiovascular problems, stroke and cancers of several organs. In fact, according to the CDC website, cigarette smoking harms nearly every organ of the body and affects a person's overall health.[17] Quitting smoking cuts down on all risks including, cardiovascular risks, stroke, and cancers of the lung, mouth, throat, esophagus and bladder.[18] Unfortunately, smoking cessation is difficult and often requires therapeutic interventions. And yet there have been articles suggesting significant risks associated with some of the current pharmacological interventions.[19] Thus, a search to find new agents that could help those addicted to cigarette smoking to quit the habit is an urgent and important public health need. Similarly, the discovery of the selective D4 agonist, PD168,077 as having the capability of reversing cognitive deficits in schizophrenia[9] and inducing penile erection[7] and hence the potential to treat erectile dysfunction is very interesting and could provide an alternative treatment option in place of the PDE5 antagonists with several known side-effects.[8]

Our laboratory's attention has been drawn to these DAD_4 receptor-mediated pathologies and that has spurred us to mine our databases for new pharmacophores for the D_4 receptor. Previous publications from our lab have identified compounds **1–7** as having low affinities for the D_2 receptor while exhibiting selectivity for the D_4 receptor among D_2-like receptors.

We have now screened these compounds at the D_1-like receptors (Table 1) and the results further demonstrate that their selectivity extends beyond the D_2-like receptors and hence we selected compound **1** as the lead agent to conduct a structure-activity relationship (SAR) study that has led to the identification of potent and selective D_4 ligands. Replacing the carbonyl moiety in compound **1** with a sulfoxide (**2**), methylene (**3**), sulfur (**4**) or oxygen (**5**) group/atom resulted in increasing potency at the D_4 receptor with Ki values from 17.5 nM to 6.9 nM respectively while binding at other DA receptors showed no observable trends. The sulfoxide analog resulted in the lowest affinity binding at the rest of the DA receptors while its reduced counterpart, the sulfide (**4**) had moderate binding except at the D_1 receptor where its affinity binding Ki was 52 nM.

Overall, these compounds have demonstrated better selectivity toward the D_4 receptor when compared to compound **1**. In particular, compound **5**, the oxygen analog, not only had the best binding at the D_4 receptor, but demonstrated the highest selectivity among compounds **1–5**. Further probing of the oxygen analog revealed that removing the *para* fluoro atom (**6**) has little or no contribution to binding to the D_2-like receptors while replacement of N-4 in the piperazine ring with CH (**7**) resulted in a very significant increase in potency for the D_2 receptor with only minimal changes elsewhere. Compound **8** was designed and synthesized to explore the effect of restricting the carbonyl group in an indanone ring, on binding affinity and selectivity. Indeed, this transformation resulted in one of the highest selectivities toward the D_4 receptor; the ratios for the D_2/D_4 and D_3/D_4 being 253 and 406 respectively. Synthesis of **9**, an analog of **8** but without the fluorine atom resulted in a significant decrease in binding at all the DA subtypes including the D_4 suggesting the fluorine may have some important interaction at the binding sites for these indanones. Deoxygenation of the carbonyl to form compound **10** however, produced only minimal changes.

The next evaluations focused on the replacement of the 4-chlorophenyl moiety. Our previous work indicated that pyridine and pyrimidine rings impacted binding to CNS receptors.[9–12] Hence, we first explored replacing the 4-chlorophenyl moiety with the pyridine ring in compounds **3–5** to obtain compounds **11–13** (Chart 2) and the binding affinities are reported in Table 2. Compounds **11–13** bind with very high affinities at D_4 versus D_1–D_3 receptors, and therefore demonstrate decreased selectivity for the D_4 receptor. Compound **14**, an analog of **13** with the fluorine atom replaced with a trifluoromethyl group, displayed significantly lower binding affinities for all DA receptors suggesting either a steric limitation or deleterious electron withdrawing effect or both. Replacement of the pyridine ring with 5-methyl substituted pyridine for the trio (**11–13**) to form compounds **15a–c** were also evaluated. Interestingly, compound **15a** with the oxygen linker resulted in the most potent analog for the D_4 receptor (Ki = 1.1 nM) among the 17 compounds evaluated thus far. In addition, it also displayed the highest selectivity for the D_4 receptor when compared with the D_2 receptor. Not surprisingly, compounds **15b** and **15c**, the sulfur and carbon analogs respectively, have reduced binding for the D_4 receptor but retain similar affinities for the D_1–D_3 receptors.

Finally in this series, compounds **16a–c**, the pyrimidine analogs of **11–13** were synthesized and evaluated. Once again the oxygen analog **16a** displayed the highest potency and selectivity for the D_4R among the three analogs. The carbon analog (**16c**) had a similar

binding profile at the DA subtypes as **16a** although its selectivity for the D_4R is much lower. Meanwhile, the sulfur analog **16b** again demonstrated a 5-fold lower affinity for the D_4R compared to **16a**. These evaluations have clearly demonstrated that the oxygen analogs have the highest potencies for the D_4R within each cohort evaluated. Comparing the pyridine and pyrimidine analogs in this series, the pyridine analogs overall, demonstrated moderate enhancement in binding affinity at the D_4R than the pyrimidine analogs. In addition, among the twenty compounds evaluated, none has a better binding affinity for the D_5 receptor than compound **7** with a Ki of 867 nM. In other words, the compounds have little or no affinity for the D_5R.

The last group of compounds evaluated is **17 – 28** (Chart 4), which may be considered as the heterobicyclic analogs of the compounds in charts 2 and 3, with binding affinities reported in Table 3. Compound **17** and **18** are benzothiazole analogs which were previously synthesized and evaluated for binding to the D_2-like receptors.[15] In this paper, their binding affinities to the D_1-like receptors were evaluated and the results are reported. The results suggest that a chain length of 4 (**17**) produced a more potent agent at the D_4R than a chain length of 3 (**18**). The selectivities of both compounds for the D_4R were unremarkable.

Next, we synthesized and evaluated the benzoxazole analogs, **19 – 22**, by systematically modifying the chain length from 5 to 2 respectively. A chain length of five atoms (**19**) produced a weak binding affinity at the D_4R (Ki = 240 nM) while **20**, with a chain length of 4 atoms produced an 8-fold increase in binding to the D_4 receptor (Ki = 30.6 nM), thus suggesting a chain length of 4 is preferred. Compound **20** however, has similar affinity for the D_3R (Ki = 33 nM) resulting in loss of selectivity. Further comparison of this benzothiazole, **20** with the benzoxazole, **17** shows over 7-fold differential, suggesting the benzothiazole ring with a 4-methylene chain is preferred at the D_4R. There is however no preference at the D_3 receptor since they (**17** & **20**) have similar binding affinities. Compounds **21** and **22**, benzoxazoles with a chain length of 3 and 2 respectively, resulted in compounds with only moderate to very weak binding affinities at all the DA receptors.

We also synthesized and evaluated compounds obtained by replacement of the benzoxazole with a benzofuran, (Chart 4), **23 – 26**, of which **24** may be considered as a restricted analog of the straight chain ether analog in Chart 2. The results indicated that while somewhat selective for the D_4 receptor, the 2 carbon chain analog (**23**) and the 3-chain analog (**24**) have weaker binding affinities for the D_4 receptor. Replacement of the piperazine ring with its bridged counterpart (**25**) and with a homopiperazine (**26**) did not improve potency or selectivity.

Returning to the benzothiazoles, we further explored replacement of the 4-chlorophenyl moiety with 2-(piperazin-1-yl)pyrimidine to obtain the 4-chain (**27**) and 3-chain (**28**) analogs. Compound **27** was the most potent D_4 ligand in this study (Ki = 0.84 nM) as previously reported[15] albeit with diminished selectivity, while **28** has high potency (Ki = 3.9 nM) and is by far the most selective analog at the D_4 receptor when compared with other dopamine subtypes. Taking their binding constants at face value, compound **28** is as potent but more selective than FAUC 113, a previously reported D_4 ligand (Chart 1).[20] In addition, while not as potent as L-745,860,[21] compound **28** is also more selective for the D_4 receptor

among the D_2-like receptors. To determine the extent of compound **28**'s selectivity, we screened its binding affinities at several other CNS receptors and the results are reported in Table 4.

Screening experiments involving a total of 18 other receptors indicated that apart from the $5HT_{1A}$ and $5HT_{2B}$ receptors, where binding affinities were below 100 nM (Ki < 100 nM), compound **28** exhibited significantly poorer affinities for the remainder of the assayed receptors. The above results strongly suggest that compound **28** can be a potentially useful D4-selective ligand for probing D_4R related pathophysiological conditions including smoking cessation, erectile dysfunction and reversal of cognitive deficits in schizophrenia depending on the intrinsic activity.

3.1. Conclusions

This study was initiated to identify selective DAD_4 receptor ligands. The results confirm the piperazine ring as a reliable pharmacophore impacting potency and selectivity for the D_4 receptor. Of the 25 piperazine derivatives evaluated, all displayed higher potencies at the D_4 than at the D_2 receptors. Compound **27** (Ki = 0.84 nM) has the highest potency at the D_4 receptor but displays only moderate selectivity compared to the other DA subtypes. The most significant finding however is the identification of a novel benzothiazole alkyl piperazine, compound **28**, with a binding affinity constant (Ki) of 3.9 nM and no significant binding affinity to any of the other DA receptor subtypes (less than 50% inhibition of the appropriate radioligand at each of the other DA subtypes). In addition, compound **28** has only weak to moderate affinities for eighteen other CNS receptors. These results warrant a more elaborate pharmacological profiling, including functional characterization, which is the focus of our current ongoing studies.

4. Experimental

Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. All NMR spectra were obtained on a Varian 300 MHz Mercury Spectrometer. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA, and are within 0.4% of theory unless otherwise noted. Flash chromatography was performed with Davisil grade 634 silica gel. Starting materials were obtained from Sigma-Aldrich and were used without further purification.

4.1. Synthetic Procedure

4.1.1. Synthesis of 2-(2-chloroethyl)-5-fluoro-indan-1-one, 31—A mixture of 4-chloro-1-(4-fluorophenyl)-butan-1-one, **29** (10 g, 50 mmol), hexamethylenetriamine (10.5 g, 75 mmol) in Ac_2O (25 mL) was refluxed under N_2 for 16 h. After cooling to rt, the mixture was diluted with $CHCl_3$ (500 mL) and then washed with HCl solution (10%, 2×300 mL), H_2O (300 mL), and sat $NaHCO_3$ (300 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, followed by column chromatography on silica gel to afford 4-chloro-1-(4-fluoro-phenyl)-2-methylene-butan-1-one, **30** (2.8 g, 26.4%). ^1H NMR ($CDCl_3$): δ 7.82 (dd, *J* = 9.0, 5.7 Hz, 2H), 7.13 (t, *J* = 9.0 Hz, 2H), 5.99 (s, 1H), 5.73 (s, 1H), 3.72 (t, *J* = 6.6 Hz, 2H), 2.94 (t, *J* = 6.0 Hz, 2H).

Compound **30** (1.2 g, mmol) was dissolved in Conc H_2SO_4 (4 mL) and heated at 60°C for 1 h. After cooling to rt, the mixture was diluted with EtOAc (200 mL) and washed with sat'd $NaHCO_3$ (2×200 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was concentrated under vacuum followed by column chromatography on silica gel to afford 2-(2-chloro-ethyl)-5-fluoro-indan-1-one, **31** in quantitative yield. ^{1}H NMR ($CDCl_3$): δ 7.76 (dd, *J* = 8.4, 5.4 Hz, 1H), 7.05–7.14 (m, 2H), 3.80–3.86 (m, 1H), 3.68–3.78 (m, 1H), 3.42 (dd, *J* = 17.1, 7.8 Hz, 1H), 2.90–2.98 (m, 1H), 2.83 (dd, *J* = 17.1, 4.2 Hz, 1H), 2.38–2.49 (m, 1H), 1.86–1.98 (m, 1H).

4.1.2. 2′-(2-Chloroethyl)-5′-fluoro-2′,3′-dihydrospiro[[1,3]dioxolane-2,1′-indene], 33—A solution of 2-(2-chloro-ethyl)-5-fluoro-indan-1-one (5 g, 23.5 mmol), ethylene glycol (5 mL), TsOH (100 mg) in toluene (50 mL) was refluxed under N_2 for 48 h. Water was removed by azeotropic distillation and the reaction was monitored by ^{1}H NMR. The reaction was quenched by addition of Et_3N (1 mL), diluted with EtOAc (250 mL), washed with sat $NaHCO_3$, (25 mL), H_2O (25 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was concentrated in under vacuum to dryness yielding a mixture of 2-(2-chloro-ethyl)-5-fluoro-indan-1-one and its ethylene acetal in a ratio of 1/4. 2-(2-chloroethyl)-5-fluoro-indan-1-one was removed by reducing to its 2-(2-chloro-ethyl)-5-fluoro-indan-1-ol with $NaBH_4$ in MeOH, followed by column chromatography on silica gel which afforded 2′-(2-chloroethyl)-5′-fluoro-2′,3′-dihydrospiro[[1,3]dioxolane-2,1′-indene] **33** (4.5 g, 75%). ^{1}H NMR ($CDCl_3$): δ 7.24–7.28 (m, 1H), 6.89–6.96 (m, 2H), 4.22–4.28 (m, 1H), 4.07–4.16 (m, 3H), 3.70–3.74 (m, 1H), 3.62–3.68 (m, 1H), 3.55–3.61 (m, 1H), 3.07 (dd, *J* = 14.7, 6.6 Hz, 1H), 2.71–2.75 (m, 1H), 2.60–2.68 (m, 1H), 2.11–2.20 (m, 1H), 1.91–2.00 (m, 1H).

4.1.3. 2-(2-(4-(4-Chlorophenyl)piperazin-1-yl)ethyl)-5-fluoro-indan-1-one hydrochloride, 8—A mixture of **33** (1.2 g, 4.67 mmol), 1-(4-chlorophenyl)piperazine dihydrochloride (1.3 g, 5.6 mmol), KI (100 mg), K_2CO_3 (1.2 g, 8.75 mmol) in DME (10 mL) was heated to reflux under N_2 for 12 h. The mixture was directly purified through column chromatography on silica gel to afford 2-{2-[4-(4-chloro-phenyl)-piperazin-1-yl]-ethyl}-5-fluoro-indan-1-one ethylene acetal. The product was dissolved in wet MeOH and TsOH was added with stirring at rt. After stirring at rt for 12 h, the solution was diluted with EtOAc (450 mL) and washed with saturated $NaHCO_3$ (40 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo* to dryness and column chromatographed on silica gel to give 2-{2-[4-(4-chloro-phenyl)-piperazin-1-yl]-ethyl}-5-fluoro-indan-1-one, **8**. The product was converted to the hydrochloride salt and further crystallization from MeOH-Et_2O afforded the HCl salt (450 mg, yield 28%), mp 202–203 °C. ^{1}H NMR (DMSO-d^6): δ 11.15 (brs, 1H), 7.72 (dd, *J* = 8.7, 4.5 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.24–7.28 (m, 3H), 7.00 (d, *J* = 9.0 Hz, 2H), 3.77–3.80 (m, 2H), 3.53–3.56 (m, 2H), 3.28–3.36 (m, 2H), 3.12–3.22 (m, 4H), 2.81–2.90 (m, 2H), 2.22–2.78 (m, 1H), 1.84–1.96 (m, 2H). *Calcd for* $C_{21}H_{23}Cl_2FN_2O$*:* C 61.62, H 5.66, N 6.84; *Found:* C 61.38, H 5.58, N 6.77.

4.1.4. 2-(2-Chloroethyl)-indan-1-one—A mixture of 4-chloro-1-phenyl-butan-1-one (10 g, 54 mmol), hexanmethylene triamine (10.5 g, 75 mmol) in Ac_2O (25 mL) was refluxed

under N_2 for 18 h. After cooling to rt, the mixture was diluted with $CHCl_3$ (500 mL) and then washed with HCl solution (10%, 2×300 mL), H_2O (300 mL), and saturated aq $NaHCO_3$ (300 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel to afford 4-chloro-2-methylene-1-phenyl-butan-1-one (2.8 g, 26%). ^{1}H NMR ($CDCl_3$): δ 7.75–7.78 (m, 2H), 7.52–7.56 (m, 1H), 7.42–7.47 (m, 2H), 6.01 (s, 1H), 5.78 (s, 1H), 3.73 (t, *J* = 6.6 Hz, 2H), 2.95 (t, *J* = 6.6 Hz, 2H). The 4-chloro-2-methylene-1-phenyl-butan-1-one (1.2 g, 6.15 mmol) was dissolved in conc H_2SO_4 (4 mL) and heated at 60 °C for 1 h. The mixture was allowed to cool to rt, diluted with EtOAc (200 mL), washed with saturated aq $NaHCO_3$ (2×200 mL) and dried over Na_2SO_4. The solution was filtered, solvent was removed in vacuo and the residue was purified by column chromatography on silica gel to afford 2-(2-chloro-ethyl)-indan-1-one in quantitative yield. ^{1}H NMR ($CDCl_3$): δ 7.75 (d, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.2 Hz, 1H), 7.45–7.48 (m, 1H,), 7.38 (t, *J* = 7.2 Hz, 1H), 3.75–3.86 (m, 1H), 3.68–3.74 (m, 1H), 3.39–3.47 (m, 1H), 2.89–2.94 (m, 1H), 2.80–2.88 (m, 1H), 2.38–2.47 (m, 1H), 1.88–1.96 (m, 1H).

4.1.5. 2-{2-[4-(4-Chloro-phenyl)-piperazin-1-yl]-ethyl}-indan-1-one tosylate, 9—

A solution of 2-(2-chloroethyl)-indan-1-one (5 g, 25.6 mmol), ethylene glycol (5 mL), p-toluene sulfonic acid (TsOH, 100 mg) in toluene (50 mL) was refluxed under N_2 for 48 h and water was removed by azeotropic distillation. The reaction was monitored by ^{1}H NMR until a conversion of 80% was achieved. The reaction was quenched by the addition of Et_3N (1 mL), diluted with EtOAc (250 mL), washed with saturated aq $NaHCO_3$, (25 mL) and H_2O (25 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated in vacuo to dryness to afford a mixture of 2-(2-chloro-ethyl)-indan-1-one and its ethylene acetal in a ratio of 1/4. 2-(2-Chloroethyl)-indan-1-one was removed by reducing to its 2-(2-chloro-ethyl)-indan-1-ol with $NaBH_4$ in MeOH, followed by column chromatography on silica gel to afford 2-(2-chloro-ethyl)-indan-1-one ethylene acetal (4.4 g, 72%). ^{1}H NMR ($CDCl_3$):): 7.20–7,32 (m, 4H), 4.28–4.31 (m, 1H), 4.08–4.19 (m, 3H), 3.52–3.63 (m, 1H), 3.65–3.74 (m, 1H), 3.04–3.15 (m, 1H), 2.62–2.74 (m, 2H), 2.12–2.24 (m, 1H), 1.92–2.05 (m, 1H). A mixture of 2-(2-chloroethyl)indan-1-one ethylene acetal (1.0 g, 4.18 mmol), 1-(4-chlorophenyl)-piperazine dihydrochloride (1.4 g, 5.19 mmol), KI (100 mg), K_2CO_3 (1.2 g, 8.75 mmol) in DME (10 mL) was heated to reflux under N_2 for 12 h. The mixture was allowed to cool to rt and then directly purified through column chromatography on silica gel to afford an oily residue. Without characterization, the product was dissolved in MeOH and *p*-toluene sulfonic acid (800 mg) was added with stirring at rt. After stirring for 12 h, the solution was diluted with EtOAc (450 mL) and washed with sat $NaHCO_3$ (40 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated in vacuo to dryness. The resulting residue was purified by column chromatography on silica gel to afford 2-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}indan-1-one. The product was converted to *p*-toluenesulfonate and crystallized in MeOH-Et_2O to afford the *p*-toluenesulfonate salt, **9** (610 mg, 28%). Mp 215–216 °C; ^{1}H NMR (DMSO-d^6): 9.57 (brs, 1H), 7.71 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 9.0 Hz, 2H), 7.08 (d, *J* = 8.1 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.80–3.85 (m, 2H), 3.58–3.62 (m, 2H), 3.25–3.39 (m, 2H), 3.11–3.21 (m, 2H), 2.92–3.00 (m, 2H), 2.80–2.87 (m, 1H), 2.72–2.77 (m, 1H), 2.24 (s, 3H), 2.16–2.22 (m, 1H), 1.84–1.93

(m, 1H). *Calcd for* $C_{28}H_{31}ClN_2O_4S.0.8H_2O$*:* C 62.11, H 6.07, N 5.17; *Found:* C 62.09, H 6.06, N 5.08.

4.1.6. 2-(2-Chloroethyl)-5-fluoroindane, 32—Amalgamated zinc is prepared by stirring a mixture of zinc (1.2 g), $HgCl_2$ (120 mg) in H_2O (5 mL) with conc HCl (0.1 mL) at rt. After stirring for 5 min, the mixture was decanted, followed by the addition of H_2O (1 mL), conc HCl (1.75 mL), toluene (10 mL), and 2-(2-chloroethyl)-5-fluoro-indan-1-one, **31** (2.0 g, 9.43 mmol). The mixture was refluxed with stirring for 12 h, allowed to cool to rt and the solid was filtered off. The collected filtrate was diluted with EtOAc (200 mL), separated and the organic layer was washed with H_2O (50 mL) and saturated aq $NaHCO_3$ (50 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel to afford **32** (1.68 g, 90%). ^{1}H NMR ($CDCl_3$): δ 7.09 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.81–6.88 (m, 2H), 3.60 (t, *J* = 7.2 Hz, 2H), 3.00–3.08 (m, 2H), 2.68–2.73 (m, 1H), 2.54–2.63 (m, 2H), 1.94–2.02 (m, 2H).

4.1.7. 1-(4-Chlorophenyl)-4-(2-(5-fluoro-2,3-dihydro-1H-inden-2-yl)ethyl)piperazine dihydrochloride, 10—A mixture of **32** (0.8 g, 4.0 mmol), 1-(4-chlorophenyl)piperazine dihydrochloride (1 g, 4.3 mmol), KI (150 mg), K_2CO_3 (1.2 g, 8.7 mmol) in DME (10 mL) was heated to reflux under N_2 for 12 h. The mixture was directly purified through column chromatography on silica gel to afford 1-(4-chloro-phenyl)-4-[2-(5-fluoro-indan-2-yl)-ethyl]-piperazine, **9**. The product was converted to the hydrochloride salt immediately and then recrystallized from MeOH-Et_2O (0.78 g, 45%), mp 194–196 °C. ^{1}H NMR (DMSO-d^6): 11.00 (brs, 1H), 7.26 (d, *J* = 9.0 Hz, 2H), 7.17 (dd, *J* = 5.7, 8.1 Hz, 1H), 6.99 (m, 3H), 6.90 (dt, *J* = 2.4, 8.4 Hz, 1H), 3.77 (d, *J* = 11.1 Hz, 2H), 3.53 (d, *J* = 11.1 Hz, 2H), 3.07–3.19 (m, 6H), 2.96–3.05 (m, 4H), 2.45–2.60 (m,1H), 1.88–1.96 (m, 2H). *Calcd for* $C_{21}H_{26}Cl_3FN_2$: C 58.41, H 6.07, N 6.49; *Found:* C 58.56, H 6.01, N 6.49.

4.1.8. 3-(4-Fluorophenoxy)propan-1-ol, 36a—A mixture of 4-fluorophenol (1.12g, 10 mmol), 3-chloropropanol (1.4 g, 15 mmol), KI (50 mg), K_2CO_3 (2.76 g, 20 mmol) in ^{i}PrOH was refluxed under N_2 for 1h. The mixture was diluted with EtOAc (200 mL), washed with H_2O (50 mL) and then brine (50 mL). The organic layer was dried with Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, followed by distillation *in vacuo* to give the intermediate, 3-(4-fluorophenoxy)propan-1-ol, **36a** (1.53 g, 90%). ^{1}H NMR ($CDCl_3$): δ 6.96 (t, *J* = 8.4 Hz, 2H), 6,84 (dd, *J* = 9.0, 4.5 Hz, 2H), 4.09 (t, *J* = 6.0 Hz, 2H), 3.84–3.87 (m, 2H), 1.99–2.07 (m, 2H).

4.1.9. 3-(4-Fluorophenoxy)propyl methanesulfonate, 37a—To a solution of **36a** (1.3 g, 7.6 mmol), Et_3N (3 mL) in CH_2Cl_2 (10 mL) was added at rt MsCl (0.8 mL, 10.3 mmol). The mixture was stirred at rt for 12 h, solvent was removed and the residue was purified through column chromatography on silica gel, to yield 3-(4-fluorophenoxy)propyl methanesulfonate, **37a** (1.79 g, 95%). ^{1}H NMR ($CDCl_3$): δ 6.97 (t, *J* = 8.1 Hz, 2H), 6.83 (dd, *J* = 9.0, 4.5 Hz, 2H), 4.44 (t, *J* = 6.0 Hz, 2H), 4.05 (t, *J* = 6.0 Hz, 2H), 2.18–2.23 (m, 2H).

4.1.10. 3-((4-Fluorophenyl)thio)propan-1-ol, 36b—A mixture of 4-fluorobenzenthiol (1.55g, 12.1 mmol), 3-chloropropanol (2.26 g, 27.65 mmol), KI (100 mg), K_2CO_3 (3.3 g, 23.9 mmol) in ^{i}PrOH (10 mL) was refluxed under N_2 for 1h. The mixture was diluted with EtOAc (200 mL), and washed with water (50 mL), brine (50 mL). The organic layer was dried with Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, and followed by distillation *in vacuo* to give product 3-(4-fluorophenylthio)propan-1-ol, **36b** (1.62 g, 72%). ^{1}H NMR ($CDCl_3$): δ 7.35 (dd, *J* = 8.4, 5.4 Hz, 2H), 6.99 (t, *J* = 8.4 Hz, 2H), 3.76 (t, *J* = 6.0 Hz, 2H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.80–1.89 (m, 2H).

4.1.11. 3-(4-Fluorophenylthio)propyl-4-methylbenzenesulfonate, 37b—To a solution of 3-(4-fluorophenylthio)-propan-1-ol (1 g, 5.4 mmol), Et_3N (2 mL)in CH_2Cl_2 (10 mL) was added at rt TsCl (1.54 g, 8.1 mmol). The mixture was stirred at room temperature for 12 h, and then followed by directly purification through column chromatography on silica gel, and provided 3-(4-fluorophenylthio)propyl 4-methylbenzenesulfonate (1.72 g, 94%). ^{1}H NMR ($CDCl_3$): δ 7.77 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.30 (dd, *J* = 5.4, 8.4 Hz, 2H), 6.97 (d, *J*= 8.7 Hz, 2H), 4.13 (t, *J* = 8.0 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 1.85–1 92 (m, 2H).

4.1.12. 3-(4-Fluorophenylthio)propyl methanesulfonate, 37c—To a solution of 3-(4-fluorophenylthio)propan-1-ol (1.2 g, 6.45 mmol), Et_3N (3 mL) in CH_2Cl_2 (10 mL) was added at rt MsCl (1 mL, 12.7 mmol). The mixture was stirred at room temperature for 12 h, and then followed by directly purification through column chromatography on silica gel, and provided 3-(4-fluorophenylthio)propyl methanesulfonate (1.60 g, 94%). ^{1}H NMR ($CDCl_3$): δ 7.37 (dd, *J* = 9.0, 4.8 Hz, 2H), 7.00 (t, *J* = 9.0 Hz, 2H), 4.30 (t, *J* = 8.0 Hz, 2H), 3.00 (s, 3H), 2.78 (t, *J* = 7.2 Hz, 2H), 1.99–2.04 (m, 2H).

4.1.13. General procedure of alkylation of arylpiperazines, 15a–c—A mixture of aryl piperazines (0.4 mmol, 1 eq.) and K_2CO_3 (4 mmol, 4 eq.) was stirred and refluxed for 20 min in CH_3CN (10 mL). To the mixture, a solution of substituted tosylsulfonates/ mesylates/chlorides (0.53 mmol, 1.3 eq.) in CH_3CN (5 mL) was added drop wise. The reaction mixture was refluxed overnight, diluted with EtOAc (100 mL), filtered and washed with brine. The organic layer was collected, dried (Na_2SO_4), filtered, and the solvent was evaporated. The residue obtained was chromatographed on a silica gel column using hexane and EtOAc combinations as eluent. The final compounds were obtained in moderate yields, converted to HCl salts where necessary and re-crystallized using appropriate solvents.

4.1.14. 1-(3-(4-Fluorophenoxy)propyl)-4-(5-methylpyridin-2-yl)piperazine, 15a—Yield 30 %, mp 79.6 °C. ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.32 (dd, *J* = 8.7, 2.4 Hz, 1H,), 6.99–6.93 (m, 2H), 6.85–6.81 (m, 2H), 6.59 (d, *J* = 8.7 Hz, 1H,), 4.00 (t, *J* = 6.6 Hz, 2H,), 3.52–3.48 (m, 4H), 2.61–2.55 (m, 6H), 2.19 (s, 3H), 2.05–1.96 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 158.74, 158.03, 155.59, 155.07, 155.04, 147.67, 138.41, 122.45, 115.90, 115.60, 115.46, 115.35, 107.03, 66.75, 55.23, 53.04, 45.60, 26.65, 17.34. *Calcd for* $C_{19}H_{24}FN_3O$*:* C 69.28, H 7.34, N 12.76; *Foun:* C 69.47, H 7.30, N 12.50.

4.1.15. 1-(3-((4-Fluorophenyl)thio)propyl)-4-(5-methylpyridin-2-yl)piperazine, 15b—Yield 62 %, mp 85.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, *J* = 2.4 Hz, 1H,), 7.37–7.29 (m, 3H), 7.01–6.96 (m, 2H), 6.58 (d, *J* = 8.7 Hz, 1H), 3.48–3.45 (m, 4H), 2.92 (t, *J* = 7.5 Hz, 2H), 2.54–2.46 (m, 6H), 2.18 (s, 3H), 1.86–1.77 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 163.32, 160.06, 158.04, 147.65, 138.40, 132.20, 132.09, 122.41, 116.14, 115.85, 107.01, 105.00, 57.12, 53.01, 45.62, 32.86, 26.39, 17.34. *Calcd for* $C_{19}H_{24}FN_3S$*:* C 66.05, H 7.00, N 12.16; *Found:* C 66.06, H 7.03, N 12.13.

4.1.16. 1-(4-(4-Fluorophenyl)butyl)-4-(5-methylpyridin-2-yl)piperazine, 15c—Yield 22 %, mp 74 °C ^{1}H NMR ($CDCl_3$) δ 8.01 (d, *J* = 2.7 Hz, 1H,), 7.29 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.14–7.09 (m, 2H), 6.98–6.92 (m, 2H), 6.58 (d, *J* = 8.7 Hz, 1H), 3.50–3.46 (m, 4H), 2.63–2.52 (m, 6H), 2.42–2.37 (m, 2H), 2.18 (s, 3H), 1.68–1.50 (m, 4H). ^{13}C NMR($CDCl_3$): δ 162.78, 159.56, 158.11, 147.67, 138.39, 137.97, 137.93, 129.72, 129.62, 122.37, 115.12, 114.84, 106.99, 58.59, 53.09, 45.65, 34.98, 29.51, 26.34, 17.35. *Calcd for* $C_{20}H_{26}FN_3$: C 73.36, H 8.00, N 12.83; *Found*: C 73.79, H 8.28, N 12.47.

4.1.17. General Procedure for the synthesis of n-chloro-*N*-(2-hydroxy-phenyl)alkylamide, 40, (n=2–5)—For n=2, a solution of 2-aminophenol (1.0 g, 9.16 mmol), 3-chloropropionylchloride, (1.39 g, 11 mmol, 1.2 eq) and Et_3N (1.12 g, 11 mmol, 1.2 eq) in EtOAc (25 mL) was heated to reflux for 6–10 h. After allowing to cool to rt, EtOAc (100 mL) was added and the solution was washed once with 10% HCl (100 mL). The aqueous layer was extracted with EtOAc (3×100 mL) and the combined organic layers was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation under reduced pressure. The pure product, 3-chloro-N-(2-hydroxyphenyl)propanamide, **40** was obtained as a colorless solid by column chromatography using EtOAc: hexane (3:7) as eluent.

4.1.18. General procedure for the synthesis of (n-chloroalkyl)benzoxazole, 41, (n = 2–5)—A mixture of 3-chloro-*N*-(2-hydroxyphenyl)-propanamide (1g, 5.01 mmole) and polyphosphoric acid (PPA, 3g) was heated with magnetic stirring at 130 °C for 3–4 h. The reaction mixture was poured into ice-water (50 mL), neutralized with saturated aq NH_3, and extracted with EtOAc (2 × 50 mL). The combined extract was washed with H_2O, brine, dried (Na_2SO_4), and concentrated to give the crude product which was purified by column chromatography using EtOAc: hexane (1:9) to give 2-(2-chloroethyl)benzoxazole as a pale yellow oily liquid.

4.1.19. General procedure for the synthesis of 2-{2-[4-(4-chloro-phenyl)-piperazin-1-yl]-ethyl}-benzoxazole—A mixture of 2-(2-chloroethyl)benzoxazole, (100 mg, 0.55 mmol), 4-chlorophenyl-piperazine, (108 mg, 0.55 mmol), and K_2CO_3 (433 mg, 3.30 mmol) in CH_3CN (3 mL) was heated at reflux for 12–24 h. After cooling to rt, the solvent was removed under vacuum, H_2O (10 mL) was added and the solution was extracted with EtOAc (3×50 mL). The pooled organic layer was washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation at reduced pressure. The residue was purified by column chromatography using EtOAc:hexane (9:1) as eluent to yield the pure product (2-{2-[4-(4-chlorophenyl)piperazin-1-yl]-ethyl}benzoxazole) as a colorless solid. The other benzoxazoles were similarly prepared.

4.1.20. 2-(5-(4-(4-Chlorophenyl)piperazin-1-yl)pentyl)benzo[d]oxazole, 19— Yield 45%, mp 110–112 °C ^{1}H NMR ($CDCl_3$): δ 7.68–7.65 (m, 1H), 7.50–7.46 (m, 1H), 7.31–7.28 (m, 2H), 7.19 (d, *J* = 9.3 Hz, 2H), 6.82 (d, *J* = 9.3 Hz, 2H), 3.17–3.13 (m, 4H), 2.95 (t, *J* = 7.5 Hz, 2H), 2.59–2.56 (m, 4H), 2.40 (t, *J* = 7.5 Hz, 2H), 1.98–1.91 (m, 2H), 1.66–1.58 (m, 4H). *Calcd for* $C_{22}H_{26}ClN_3O$*:* C 68.83, H 6.83, N 10.95; *Found:* C 69.12, H 6.86, N 10.72.

4.1.21. 2-(4-(4-(4-Chlorophenyl)piperazin-1-yl)butyl)benzo[d]oxazole, 20—Yield 70%, mp 127–129 °C ^{1}H NMR ($CDCl_3$): δ 7.68–7.65 (m, 1H), 7.49–7.46 (m, 1H), 7.31–7.28 (m, 2H), 7.19 (d, *J* = 9.3 Hz, 2H), 6.82 (d, *J* = 9.0 Hz, 2H), 3.14 (t, *J* = 4.8 Hz, 4H), 2.98 (t, *J* = 7.5 Hz, 2H), 2.58 (t, *J* = 4.8 Hz, 4H), 2.45 (t, *J* = 7.5 Hz, 2H), 1.98–1.93 (m, 2H), 1.72–1.62 (m, 2H). *Calcd for* $C_{21}H_{24}ClN_3O$ *0.18* H_2O*:* C 67.60, H 6.48, N 11.26; *Found:* C 67.69, H 6.61, N 10.98.

4.1.22. 2-(3-(4-(4-Chlorophenyl)piperazin-1-yl)propyl)benzo[d]oxazole, 21— Yield 37%, mp 98–99 °C ^{1}H NMR ($CDCl_3$): δ 7.68–7.65 (m, 1H), 7.49–7.46 (m, 1H), 7.30–7.28 (m, 2H), 7.18 (d, *J* = 9.3 Hz, 2H), 6.80 (d, *J* = 9.0 Hz, 2H), 3.09 (t, *J* = 5.1 Hz, 4H), 3.01 (t, *J* = 7.8 Hz, 2H), 2.59 (t, *J* = 5.1 Hz, 4H), 2.53 (t, *J* = 6.9 Hz, 2H), 2.14–2.09 (m, 2H). *Calcd for* $C_{20}H_{22}ClN_3O$*:* C 67.50, H 6.23, N 11.81; *Found:* C 67.28, H 6.35, N 11.56.

4.1.23. 2-(2-(4-(4-Chlorophenyl)piperazin-1-yl)ethyl)benzo[d]oxazole, 22—Yield 58%, mp 110–112 °C ^{1}H NMR ($CDCl_3$): δ 7.69–7.66 (m, 1H), 7.52–7.47 (m, 1H), 7.32–7.30 (m, 2H), 7.19 (d, *J* = 9.3 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 3.18–3.14 (m, 6H), 3.01 (t, *J* = 6.9 Hz, 2H), 2.70 (t, *J* = 5.1 Hz, 4H). *Cacld for* $C_{19}H_{20}ClN_3O$*:* C 66.76, H 5.90, N 12.29; *Found* C 67.06, N 5.97, H 11.90.

4.1.24. General Method for Tosylated alkyl benzofurans—The method reported in Bakunova et al,[16] was followed to construct the benzofuran moiety. A mixture of 2-Iodophenol (3g, 1eq), alkyl-1-yn-1-ol (1.1eq), and copper (I) oxide (1.36 g, 0.7eq) in dry pyridine (15 mL) was stirred at 100–120 °C overnight. The mixture was allowed to cool to rt, diluted with EtOAc (50 mL), filtered through celite and concentrated. The residue was dissolved in EtOAc (100 mL), washed with 2 M HCl (50 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was, then, dissolved in CH_2Cl_2 (10 mL) and TsCl (1.1eq) and Et_3N (1.4eq) were added at rt while stirring overnight. The solution was diluted with EtOAc (50 mL), washed with H_2O (3 × 50 mL) and brine (50 mL). The pooled organic solvent was dried (Na_2SO_4), concentrated under reduced pressure and purified using column chromatography with hexane:EtOAc (7:3) as the eluent.

4.1.25. General Procedure for Benzofuran Coupling—To a stirred solution of CH_3CN (10 mL) and Et_3N (2 eq), the appropriate haloalkylbenzofuran (1eq) and an arylcycloalkylamine (1.1eq) were added and refluxed overnight. The solution was allowed to cool to rt, diluted with EtOAc (50 mL) and washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified using column chromatography with hexane:EtOAc (6:4) as the eluent to afford an orange-yellow solid.

4.1.26. 1-(2-(Benzofuran-2-yl)ethyl)-4-(4-chlorophenyl)piperazine, 23—Yield 93%, mp 166.6 – 168.2 °C ^{1}H NMR ($CDCl_3$): δ 7.49 (dd, *J* = 4.5, 4.5 Hz, 1H), 7.42 (d, *J* = 12.0 Hz, 1H), 7.24 (m, 4H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.46 (s, 1H), 3.19 (t, *J* = 15 Hz, 4H), 3.03 (t, *J* = 9.0 Hz, 2H), 3.85 (t, *J* = 9.0 Hz, 2H), 2.70 (t, *J* = 15 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 157.4, 154.3, 149.98, 128.98, 124.3, 123.2, 122.2, 120.2, 117.3, 110.07, 102.3, 56.1, 52.8, 49.3, 26.2. *Calcd for* $C_{20}H_{21}ClN_2O$*:* C 70.48, H 6.21, N 8.22; *Found:* C 70.63, H 6.30, N 8.25.

4.1.27. 1-(3-(Benzofuran-2-yl)propyl)-4-(4-chlorophenyl)piperazine, 24—Yield 90%, mp 84.8 – 85.9 °C ^{1}H NMR ($CDCl_3$): δ 7.49 (dd, *J* = 6.0, 6.0 Hz, 1H), 7.41 (d, *J* = 12.0 Hz, 1H), 7.19 (m, 4H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.41 (s, 1H), 3.15 (t, *J* = 15 Hz, 4H), 2.83 (t, *J* = 9.0 Hz, 2H), 2.62 (t, *J* = 12.0 Hz, 2H), 2.49 (t, *J* = 9 Hz, 4H), 1.97 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 158.5, 154.5, 149.98, 128.94, 124.25, 123.21, 122.47, 120.24, 117.22, 110.75, 102.1, 57.66, 53.05, 49.11, 26.32, 24.88. *Calcd for* $C_{21}H_{23}ClN_2O{\cdot}0.3H_2O$*:* C 70.01, H 6.43, N 7.78; *Found:* C 69.9, H 6.41, N 7.42.

4.1.28. 2-(3-(Benzofuran-2-yl)propyl)-5-(4-chlorophenyl)-2,5-diazabicyclo[2.2.1]heptane, 25—Yield 70%, mp 88.1 – 89.5 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (dd, *J* = 5.4, 4.5 Hz,1H), 7.19 (m, 1H), 7.17 (m, 1H), 7.13 (d, *J* = 9.6 Hz, 2H), 6.45 (d, *J* = 12.0 Hz, 2H), 6.35 (s, 1H), 4.18 (br s, 1H), 3.57 (br s, 1H), 3.35 (dd, *J* = 4.5, 3.3 Hz, 1H,), 3.22 (d, *J* = 9 Hz, 1H), 2.99 (dd, *J* = 4.5, 4.5 Hz, 1H), 2.78 (t, *J* = 9.0 Hz, 2H), 2.55 (m, 4H), 1.94 (t, *J* = 9 Hz, 2H), 1.84 (t, *J* = 9 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 158.9, 154.2, 145.7, 128.9, 123.3, 122.2, 120.9, 120.05, 113.8, 110.6, 102.1, 61.7, 57.7, 56.9, 52.05, 36.3, 27.1, 26.01. *Calcd for* $C_{22}H_{23}ClN_2O$*:* C 72.02, H 6.32, N 7.64; *Found: C* 71.75, H 6.38, N 7.51.

4.1.29. 1-(3-(Benzofuran-2-yl)propyl)-4-(4-chlorophenyl)-1,4-diazepane dihydrochloride, 26—Yield 89%, mp 138.1 – 139.9 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (dd, *J* = 6.0, 4.8 Hz, 1H), 7.39 (dd, *J* = 6.0Hz, 3.3 Hz, 1H), 7.14–7.22 (m, 2H), 7.12 (d, *J* = 7.2 Hz, 2H), 6.57 (d, *J* = 6.0 Hz, 2H), 6.35 (s, 1H), 3.53 (t, *J* = 6.0 Hz, 2H), 3.44 (t, *J* = 9.0 Hz, 2H), 2.73–2.81 (m, 4H), 2.66 (t, *J* = 6.0 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H), 1.91–2.00 (m, 4H). *Calcd for* $C_{22}H_{27}Cl_3N_2O{\cdot}0.5H_2O$*:* C 63.77, H 6.32, N 6.76; *Found:* C 63.78, H 6.35, N 6.61.

4.1.30. 2-(3-Chloropropyl)benzo[d]thiazole, 48—A mixture of 2-aminobenzenethiol (10 g, 80 mmol) and 4-chlorobutyl chloride (14 g, 99 mmol) in toluene (100 mL) was stirred for 48 h at rt. The mixture was diluted with EtOAc (300 mL) and washed with saturated aq $NaHCO_3$ (2 × 100 mL). The organic layer was dried with Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, followed by chromatography on silica gel to afford 2-(3-chloropropyl)benzo[d]thiazole (13.5 g, 80%.) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 3.69 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 7.5 Hz, 2H), 2.34–2.42 (m, 2H).

4.1.31. 2-(3-(4-(Pyrimidin-2-yl)piperazin-1-yl)propyl)benzo[d]thiazole trihydrobromide, 28—A mixture of 2-(3-chloropropyl)benzothiazole (1.5 g, 7.08 mmol), 2-(piperazin-1-yl)pyrimidine dihydrochloride (1.6 g, 6.7 mmol), KI (200 mg), K_2CO_3 (1.2 g,

8.7 mmol) in DME (10 mL) was heated to reflux under N_2 for 12 h. The mixture was diluted with EtOAc (400 ml) and washed with brine (50 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo* to dryness and the residue was column chromatographed on silica gel to afford 2-[3-(4-pyrimidin-2-yl-piperazin-1-yl)propyl]benzothiazole, **28**. The product was converted into the HBr salt, and recrystallized from MeOH-Et_2O (865 mg, 21%), mp > 260 °C. ^{1}H NMR (DMSO-d^6): δ 12.08 (brs, 2H), 10.13 (brs, 1H), 8.45 (d, *J* = 4.5 Hz, 2H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.48 (dt, *J* = 1.5, 8.1 Hz, 1H), 7.40 (dt, *J* = 1.5, 8.1 Hz, 1H), 6.78 (t, *J* = 4.5 Hz, 1H), 4.68 (d, *J* = 14.1 Hz, 2H), 3.63 (d, *J* = 14.1 Hz, 2H), 3.35–3.44 (m, 2H), 3.20–3.31 (m, 4H), 3.04–3.14 (m, 2H), 2.25–2.35 (m, 2H). *Calcd for* $C_{18}H_{24}Br_3N_5S$*:* C 37.13, H 4.16, N 12.03; *Found:* C 37.22, H 4.11, N 12.01.

4.2. Receptor binding studies

Binding affinities (Ki, nM) reported in Tables 1–4 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) unless otherwise stated. Details of the methods and the radioligands used for the binding assays at each receptor were previously reported.[22]

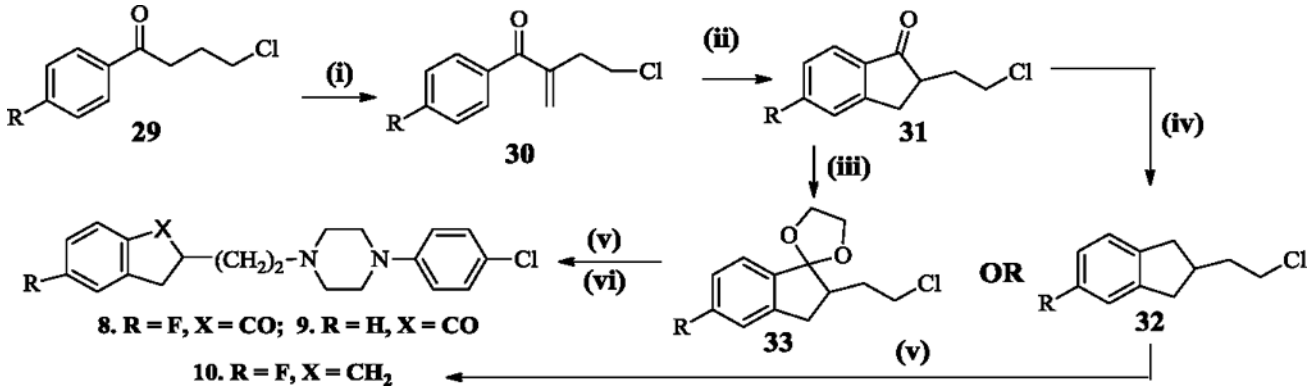
Acknowledgments

This work was supported by NIH grant # 5SC1GM088451-01-04 and a Title III Grant to Florida A&M University. The work was also supported in part by RCMI grant # 5 G12 MD007582-30 and the Pharmaceutical Research Center NIH/NCRR grant # 1C06-RR12512-01.

References

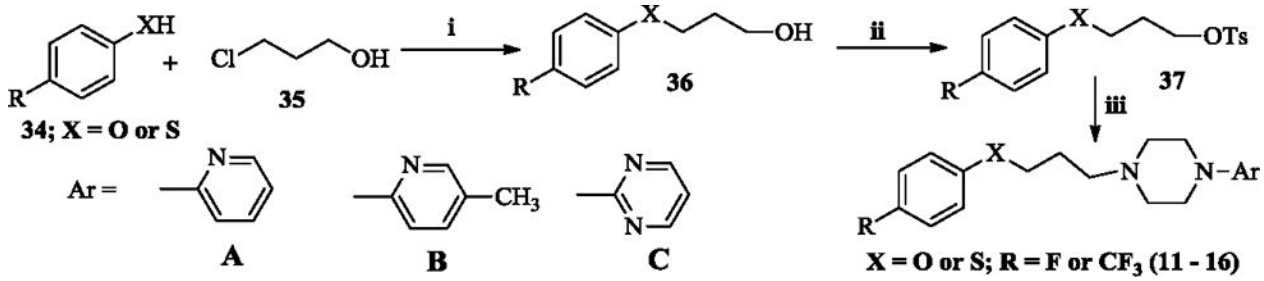
1. a) Seeman P, Van Tol HHM. Trends Pharmacol Sci. 1994; 15:264. [PubMed: 7940991] b) Van Tol HHM, Burnzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. Nature. 1991; 350:610. [PubMed: 1840645] c) Beaulieu J-M, Gainetdinov RR. Pharmacol Rev. 2011; 63:182. [PubMed: 21303898]
2. a) Bergauer M, Hübner H, Gmeiner P. Bioorg Med Chem Lett. 2002; 12:1937. [PubMed: 12113813] b) McGough JJ. Pharmacogenomics. 2012; 13:365. [PubMed: 22379991] c) Lynn DE, Lubke G, Yang M, McCracken JT, McGough JJ, Ishii J, Loo SK, Nelson SF, Smalley SL. Am J Psychiatry. 2005; 162:906. [PubMed: 15863792]
3. Ukai M, Mitsunaga H. Methods Find Exp Clin Pharmacol. 2005; 27:645. [PubMed: 16357950]
4. a) Yan Y, Mizuno T, Nitta A, Yamada K, Nabeshima T. Ann NY Acad Sci. 2004; 1025:274. [PubMed: 15542727] b) Yan Y, Nitta A, Mizuno T, Nakajima A, Yamada K, Nabeshima T. Behav Brain Res. 2006; 173:39. [PubMed: 16857277]
5. Mamiya T, Matsumura T, Ukai M. Ann NY Acad Sci. 2004; 1025:424. [PubMed: 15542745]
6. Yan Y, Pushparaj A, Le Strat Y, Gamaleddin I, Barnes C, Justinova Z, Goldberg SR, Le Foll B. Neuropsychopharmacology. 2012; 37:685. [PubMed: 22030716]
7. a) Sanna F, Corda MG, Melis MR, Piludu MA, Löber S, Hübner H, Gmeiner P, Argiolas A, Giorgi O. Pharmacol Biochem Behav. 2013; 109:59. [PubMed: 23664901] b) Patel MV, Kolasa T, Mortell K, Matulenko MA, Hakeem AA, Rohde JJ, Nelson SL, Cowart MD, Nakane M, Miller LN, Uchic ME, Terranova MA, El-Kouhen OF, Donnelly-Roberts DL, Namovic MT, Hollingsworth PR, Chang R, Martino BR, Wetter JM, Marsh KC, Martin R, Darbyshire JF, Gintant G, Hsieh GC, Moreland RB, Sullivan JP, Brioni JD, Stewart AO. J Med Chem. 2006; 49:7450. [PubMed: 17149874] c) Kolasa T, Matulenko MA, Hakeem AA, Patel MV, Mortell K, Bhatia P, Henry R, Nakane M, Hsieh GC, Terranova MA, Uchic ME, Miller LN, Chang R, Donnelly-Roberts DL, Namovic MT, Hollingsworth PR, Martino B, El Kouhen O, Marsh KC, Wetter JM, Moreland RB, Brioni JD, Stewart AO. J Med Chem. 2006; 49:5093. [PubMed: 16913699]

8. a) Galvez-Ruiz A, Arishi N. Saudi J Ophthalmol. 2013; 27:241–6. [PubMed: 24409087] b) Byoun H-S, Lee Y-J, Yi H-J. J Korean Neurosurg Soc. 2010; 47:210–212. [PubMed: 20379474] c) Buxton N, Flannery T, Wild D, Bassi S. Br J Neurosurg. 2001; 15:347–349. [PubMed: 11599452] Egan RA, Pomeranz H. Neurology. 2002; 59:293–294. [PubMed: 12136078] d) Habek M, Petravic D. Clin Neuropharmacol. 2006; 29:165–167. [PubMed: 16772819] e) Martí I, Martí Massó JF. Neurology. 2004; 63:534. [PubMed: 15304588] f) Monastero R, Pipia C, Camarda LK, Camarda R. J Neurol. 2001; 248:141–142. [PubMed: 11284133]
9. Sood P, Idris NF, Cole S, Grayson B, Neill JC, Young AM. J Psychopharmacol. 2011; 25:792–800. [PubMed: 21088042]
10. a) Peprah K, Zhu XY, Eyunni SVK, Setola V, Roth BL, Ablordeppey SY. Bioorg Med Chem. 2012; 20:1291. [PubMed: 22245230] b) Peprah K, Zhu XY, Eyunni SVK, Etukala JR, Setola V, Roth BL, Ablordeppey SY. Bioorg Med Chem. 2012; 20:1671. [PubMed: 22336245]
11. Sikazwe DMN, Nkansah NT, Altundas R, Zhu XY, Roth BL, Ablordeppey SY. Bioorg Med Chem. 2009; 17:1716. [PubMed: 19155177]
12. Ablordeppey SY, Altundas R, Bricker B, Zhu XY, Eyunni VKSK, Jackson T, Khan A, Roth BL. Bioorg Med Chem. 2008; 16:7291. [PubMed: 18595716]
13. Vaidya T, Eisenberg R, Frontier AJ. ChemCatChem. 2011; 3:1531.
14. Yamamura S, Nishiyama S. Comp Org Syn. 1991; 8:309.
15. Zhu XY, Etukala JR, Eyunni SVK, Setola V, Roth BL, Ablordeppey SY. Eur J Med Chem. 2012; 53:124. [PubMed: 22520153]
16. Bakunova SM, Bakunov SA, Wenzler T, Barszcz T, Werbovetz KA, Brun R, Hall JE, Tidwell RR. J Med Chem. 2007; 50:5807. [PubMed: 17948982]
17. http://www.cdc.gov/tobacco/data_statistics/fact_sheets/health_effects/effects_cig_smoking/ [accessed 2-10-2014]
18. U.S. Department of Health and Human Services. A Report of the Surgeon General: How Tobacco Smoke Causes Disease: What It Means to You. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2010. http://www.cdc.gov/tobacco/datastatistics/sgr/2010/consumerbooklet/pdfs/consumer.pdf[accessed 2-10-2014]
19. a) Eisenberg MJ, Filion KB, Yavin D, Bélisle P, Mottillo S, Joseph L, Gervais A, O'Loughlin J, Paradis G, Rinfret S, Pilote L. CMAJ. 2008; 179:135. [PubMed: 18625984] b) Singh S, Loke YK, Spangler JG, Furberg CD. CMAJ. 2011; 183:1359. [PubMed: 21727225] c) Goldstein MG. J Clin Psychiatry. 1998; 59:66. [PubMed: 9554323] d) Hays JT, Ebbert JO, Sood A. Mayo Clin Proc. 2009; 84:730. [PubMed: 19648390]
20. Abdelfattah MAO, Lehman J, Abadi AH. Bioorg Med Chem Lett. 2013; 23:5077. [PubMed: 23920439]
21. Kulagowski JJ, Broughton HB, Curtis NR, Mawer IM, Ridgill MP, Baker R, Emms F, Freedman SB, Marwood R, Patel S, Patel S, Ragan CI, Leeson PD. J Med Chem. 1996; 39:1941. [PubMed: 8642550]
22. Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, Mailman R. Neuropsychopharmacology. 2003; 28:1400. [PubMed: 12784105]



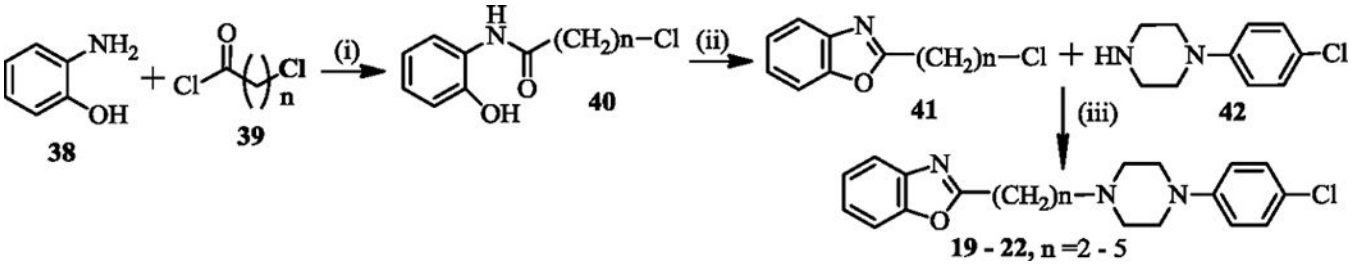
Scheme 1. Synthesis of 4-chlorophenylpiperazine analogs, 8–10

Reagents and conditions: i) HMTA, Ac_2O, Reflux; ii) Conc. H_2SO_4, 60°C; iii) a) Ethylene glycol, TsOH, Reflux, 48 h; b) $NaBH_4$, MeOH; iv) $Zn/HgCl_2$, Conc. HCl, Toluene; v) KI, K_2CO_3, DME, 90°C, 4-(4-chlorophenyl)piperazine, 12 h; vi) TsOH, MeOH, rt



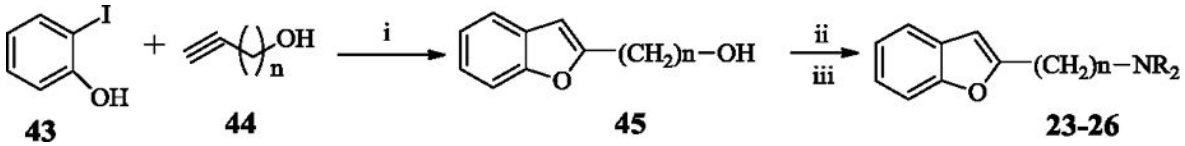
Scheme 2. Synthesis of heteroaryl piperazine analogs. 11–16

Reagents and conditions: i) KI, K_2CO_3, iPrOH or DME, Reflux; ii) MsCl/TsCl, Et_3N, DCM, rt; iii) KI, K_2CO_3, DME, 90°C, heteroaryl piperazine, 12 h.



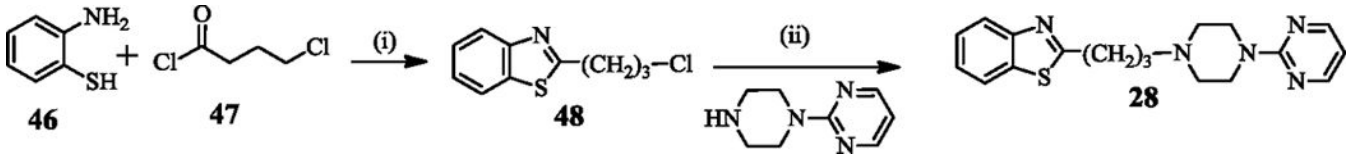
Scheme 3. Synthesis of benzoxazole derivatives, 19–22

Reagents and Conditions: (i) EtOAc, TEA, Reflux; (ii) PPA, 110°C; (iii) CH_3CN, K_2CO_3, KI, Reflux.



Scheme 4. Synthesis of benzofuran analogs, 23–26

Reagents and conditions: (i) An appropriate alkyl-1-yn-1-ol, Cu_2O, pyridine, 100°C, 15h (ii) TosylCl, NEt_3, CH_2Cl_2, rt, overnight; (iii) NHR_2 [Aryl piperazine or related analog], ACN, NEt_3, 60°C, reflux.



Scheme 5. Synthesis of benzothiazole analog, 28

Reagents and conditions: (i) Toluene, rt; (ii) KI, K_2CO_3, CH_3CN, reflux.

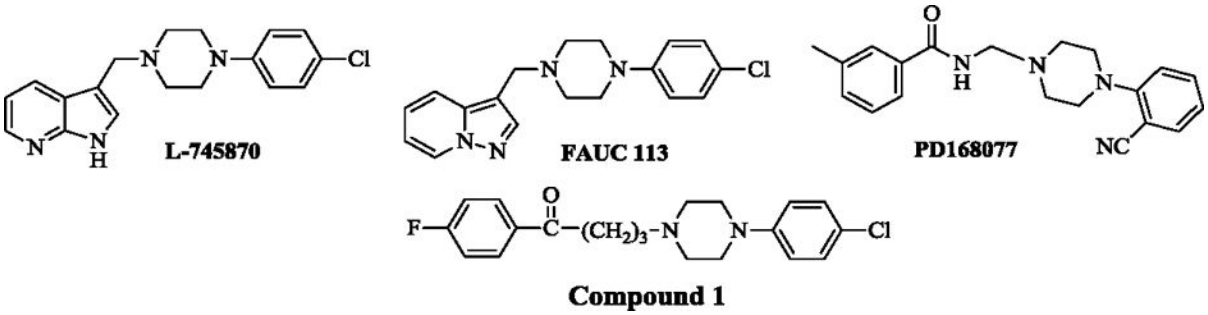


Chart 1.
Known D_4 selective Ligands with a Piperazine Pharmacophore

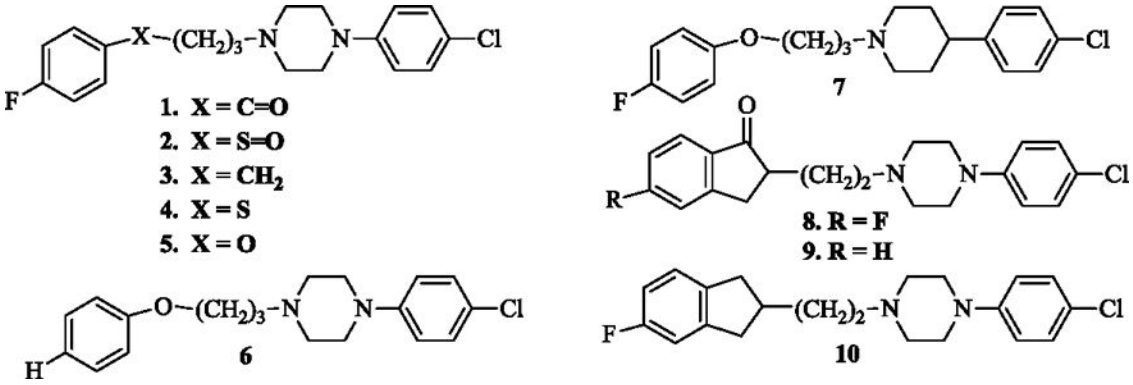
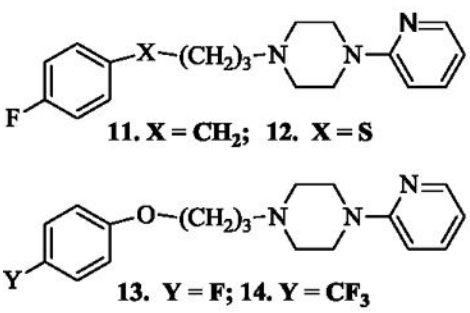


Chart 2.
4-Chlorophenyl piperazine analogs



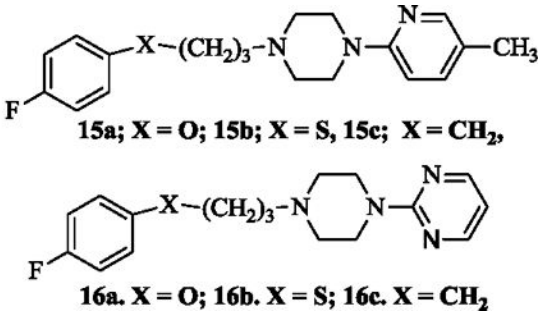


Chart 3.
Heteroaryl piperazine analogs.

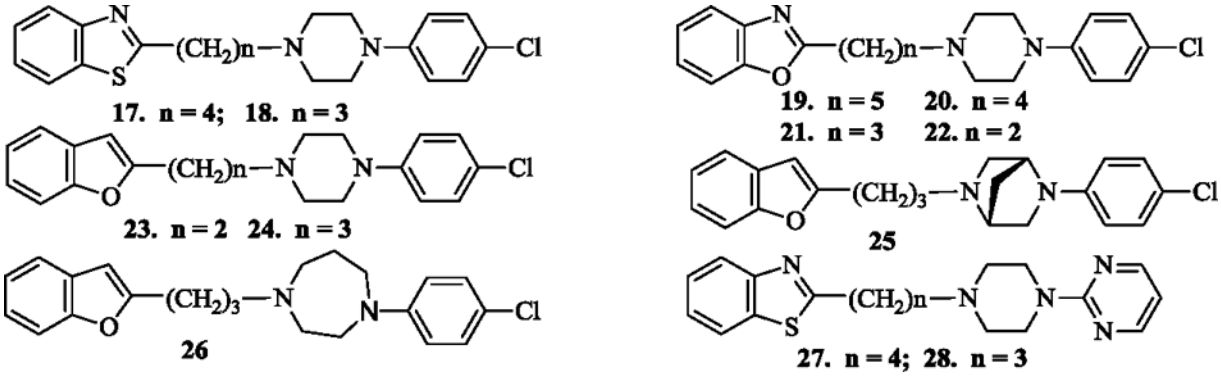


Chart 4.
Benzothiazole, Benzoxazole and Benzofuran Analogs

Table 1

Evaluation of binding affinities of 4-chlorophenyl analogs (Chart 2) at the Dopamine Receptor subtypes

Compd	Ki (pKi)±SEM values in nM at the Dopamine Receptor Subtypes						
	D_1	D_2	D_3	D_4	D_5	D_2/D_4	D_3/D_4
1.	ND	253.3±38.9	403.9±66	17.5±2.0	ND	14.5	23.1
2.	MT	635(6.2±0.05)	1340(5.87±0.06)	13(7.89±0.05)	MT	48.8	103
3.	589±56	284±21	261±23	7.8±0.4	1758±212	36.4	33.5
4.	52±3	211±22	422±41	8.7±0.5	2808±325	24.3	48.5
5.	135±8	390±34	885±65	6.9±0.3	2684±223	56.5	128
6.	ND	447(6.35±0.07)	726(6.1±0.1)	5.6 (8.26±0.03)	ND	79.8	130
7.	126±9	41.0±4.0	696±50.0	9.5±0.3	867±49	4.3	73.3
8.	641(6.19±0.06)	1543(5.81±0.08)	2477(5.6±0.1)	6.1(8.21±0.05)	MT	253	406
9.	1701 (6.41±0.07)	>10,000	4534 (5.34±0.08)	36.0 (7.45±0.04)	MT	>277	126
10.	837±92	1417±138	2772±271	13.0±1.0	MT	109	213

MT = Missed primary assay threshold of 50% inhibition; ND = Not determined

Table 2

Evaluation of the binding affinities of heteroaryl piperazine analogs (Chart 3) at the DAR Subtypes

Compd	Ki (pKi)±SEM values in nM at the Dopamine Receptor Subtypes						
	D_1	D_2	D_3	D_4	D_5	D_2/D_4	D_3/D_4
11.	238±24.0	124±10.0	86±4.0	3.5±0.2	1451±133	35.4	24.6
12.	265±24.0	183±21.0	160±16.0	5.7±0.3	3223±295	32.1	28.1
13.	180±14.0	186±16.0	229±20.0	1.8±0.1	2392±252	103	127
14.	2344 (5.63±0.09)	1092 (5.96±0.09)	355 (6.45±0.05)	12 (7.9±0.1)	>10,000	91.0	29.6
15a.	260 (6.58±0.07)	1046 (5.98±0.07)	187 (6.7±0.1)	1.1 (8.96±0.08)	2576 (5.6±0.1)	951	170
15b.	222 (6.65±0.07)	1106 (6±0.1)	170 (6.8±0.1)	20.0 (7.71±0.06)	2159 (5.7±0.1)	55.3	8.5
15c.	521 (6.28±0.08)	1861 (5.7±0.1)	156 (6.8±0.1)	23.0 (7.64±0.06)	3264 (5.5±0.1)	80.9	6.8
16a.	259±12.0	636±66.0	778±63.0	4.2±0.1	MT	151	185
16b.	495±33.0	424±25.0	68.0±10.0	21.0±1.0	1370±114	20.1	3.2
16c.	134±7.0	269±17.0	262±17.0	6.2±0.3	3812±409	43.4	42.3

MT = Missed primary assay threshold of 50% inhibition; ND = Not determined

Table 3

Evaluation of the binding affinities of benzothiazole, benzoxazole and benzofuran analogs (Chart 4) at the DAR subtypes

Compound	Ki (pKi)±SEM values in nM at the Dopamine Receptor Subtypes						
	D_1	D_2	D_3	D_4	D_5	D_2/D_4	D_3/D_4
17.	1897±364	219±12.0	31±4.0	4.0±0.0	ND	54.8	7.8
18.	1273±123	2321±314	214±47	31±40	>10,000	74.9	6.9
19.	7939±592	3108±1071	119±25	240±36.8	>10,000	13.0	0.5
20.	MT	MT	33±6	30.6±6.3	MT	>327	1.1
21.	1172±76	3962±501	180±31	145±18	>10,000	27.3	1.5
22.	MT	MT	850±126	1409±227	MT	>7.1	0.6
23.	MT	MT	MT	282.9	MT	>35.5-	>35.5
24.	1408±242	1979±291	2290±423	78.9±5.7	MT	25.1	29.0
25.	MT	MT	670.7±118.4	2397±294	MT	>4.2-	0.3
26.	274±24	2507±208	1462±133	185±11	1522±191	13.5	7.9
27.	MT	26.5±4.5	100 (7±0.06)	0.84±0.09	MT	31.5	119
28.	MT	MT	MT	3.9 (8.41±0.04)	MT	>2564	>2564
FAC 113[a]	ND	3200±58	5000±121	3.1±0.3	ND		
L-745870[b]	ND	960	2300	0.43	ND		

MT = Missed primary assay threshold of 50% inhibition; ND = Not determined.

[a] Ref 20;

[b] Ref 21.

Table 4

Binding Affinity at Other Relevant CNS Receptors, Ki in nM (pKi) ±SEM

Compd	$5HT_{1A}$	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_6$	$5HT_7$	H_1
28.	40.0 (7.39±0.04)	283 (6.55±0.03)	47.0 (7.33±0.05)	280 (6.55±0.04)	MT	MT	342 (6.47±0.06)	730 (6.14±0.06)

Compd	α_{1A}	α_{2A}	α_{2C}	M_1	M_3	DAT	NET	SERT	Sigma 1	Sigma 2
28.	259 (6.59±0.08)	844 (6.07±0.04)	126 (6.9±0.04)	MT	MT	MT	2773 (5.56±0.06)	MT	350 (6.46±0.05)	162 (6.79±0.08)

MT = Missed primary assay threshold of 50% inhibition