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Investigation of various N-heterocyclic substituted piperazine versions of 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol: Effect on affinity and selectivity for dopamine D3 receptor

Dennis A. Brown [a], Manoj Mishra [a], Suhong Zhang [a], Swati Biswas [a], Ingrid Parrington [b], Tamara Antonio [b], Maarten E. A. Reith [b], Aloke K. Dutta [a,*]

[a] Wayne State University, Department of Pharmaceutical Sciences, Applebaum College of Pharmacy and Health Sciences, Rm# 3128, Detroit, MI 48202, United States
[b] New York University, Department of Psychiatry, New York, NY 10016, United States

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ABSTRACT

Here we report on the design and synthesis of several heterocyclic analogues belonging to the 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol series of molecules. Compounds were subjected to [^{3}H]spiperone binding assays, carried out with HEK-293 cells expressing either D2 or D3 dopamine receptors, in order to evaluate their inhibition constant (K_i) at these receptors. Results indicate that N-substitution on the piperazine ring can accommodate various substituted indole rings. The results also show that in order to maintain high affinity and selectivity for the D3 receptor the heterocyclic ring does not need to be connected directly to the piperazine ring as the majority of compounds included here are linked either via an amide or a methylene linker to the heterocyclic moiety. The enantiomers of the most potent racemic compound **10e** exhibited differential activity with (−)-**10e** (K_i; D2 = 47.5 nM, D3 = 0.57 nM) displaying higher affinity at both D2 and D3 receptors compared to its enantiomer (+)-**10e** (K_i; D2 = 113 nM, D3 = 3.73 nM). Additionally, compound (−)-**10e** was more potent and selective for the D3 receptor compared to either 7-OH-DPAT or 5-OH-DPAT. Among the bioisosteric derivatives, the indazole derivative **10g** and benzo[*b*]thiophene derivative **10i** exhibited the highest affinity for D2 and D3 receptors. In the functional GTPγS binding study, one of the lead molecules, (−)-**15**, exhibited potent agonist activity at both D2 and D3 receptors with preferential affinity at D3.

1. Introduction

The dopamine (DA) receptor system has been aggressively targeted in pharmacotherapeutic treatments of numerous disorders, including drugs of abuse, schizophrenia, and Parkinson's disease (PD).[1,2] DA receptors, belonging to the class of G-protein coupled receptors, are found throughout the CNS and periphery. So far five subtypes of DA receptors have been identified.[3] The various receptor subtypes can be classified as being either D1-like or D2-like. These classifications are based on receptor pharmacology and function.[4–7] The D1-like receptors, which include the D1 and D5 subtypes, activate adenylate cyclase activity upon receptor activation. The D2-like receptors, which include the D2, D3, and D4 subtypes, inhibit adenylate cyclase activity. Interestingly, the D3 receptor was found to have a distribution in the brain that is different than that of the D2 receptor.[8] The highest levels of DA D3 receptor expression were found to be in the limbic region of the brain, which has been implicated in various psychiatric disorders. The D2 and D3 receptor subtypes posses 50% overall structural homology, and 75–80% in the agonist binding sites.[9,10]

DA receptor agonists have been used more extensively in the treatment of Parkinson's disease (PD) than any other type of pharmacotherapy.[11,12] PD is a progressive, neurodegenerative disorder that is characterized by the disintegration of the nigrostriatal dopaminergic pathway.[13] Recent estimates place the rate of Americans over the age of 65 affected with PD to be around 1%. The initial goal of DA agonist therapy was to overcome the numerous drawbacks to levodopa therapy, including the development of debilitating dyskinesias, and the inherent toxicity of levodopa to dopaminergic neurons.[14] Dopaminergic agonists are known to posses inherent antioxidant properties. In this regard, dopamine D3 receptor has been implicated in neuroprotective therapy for PD.[15] It has also been demonstrated from primate and rodent experiments that exposure to levodopa leads to over expression of D3 receptor in the basal ganglia and the resultant levodopa induced dyskinesia can be reduced substantially with the treatment of D3 preferring agonists.[16,17] Consequently, high affinity agonists for D3 receptor with high selectivity will be able to delineate more effectively the functional properties of the D3 receptor and its importance in various neuro-disorders.

* Corresponding author. Tel.: +1 313 577 1064; fax: +1 313 577 2033.
E-mail address: adutta@wayne.edu (A.K. Dutta).

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An intensive effort has been directed toward development of selective ligands for D3 receptor. A large number of compounds with various selectivities for the D3 receptor have been developed.[18,19] The overall sequence identity between D2 and D3 receptors is very high. Moreover, when the trans membrane domains, which are the most relevant sites of ligands interaction within the receptor, are considered then the sequence identity becomes close to greater than 80%.[10] Furthermore, both receptors share nearly identical active binding sites for agonist interaction which makes the job of developing selective agonists for D3 receptor even more challenging.[20–22] Some of the well known D3 selective agonists include ropinirole and pramipexole, and these agonists were shown to exhibit a 4–10-fold higher affinity for the D3 than D2 receptor.[23] On the other hand, numerous ligands as antagonists have been developed so far with a number of lead compounds showing high selectivity for the D3 receptor. The majority of these compounds contain a piperazine ring connected to a suitable benzamide-type moiety via variable linker size.[18,19,24] The template, I, for these compounds is shown in Figure 1. Numerous different aromatic moieties have been introduced as R substituents in the benzamide moiety with some of the substituents produced high selectivity for the D3 receptor. On the other hand only a handful number of *N*-phenyl substituents as R′ substituents on the piperazine moiety have been investigated. Some of the lead molecules derived from this template is listed in Figure 1.[18] It is important to mention here the difficulties in comparing binding data across laboratories as the conditions of binding assays vary significantly.

We have previously reported a hybrid structure approach as part of our ongoing effort to design and develop selective agonists for the DA D3 receptor.[25–27] Our hybrid approach combines agonist aminotetralin or bioisosteric equivalent fragments with arylpiperazine fragments via a linker to yield compounds that are DA D3 selective agonists and partial agonists. Our SAR studies so far have demonstrated that the hybrid analogues retain agonist property in spite of addition of piperazine fragment and most of these molecules exhibit significant selectivity for the D3 receptor. Two of our lead molecules, **D-237** and **D-264**, shown in Figure 1 exhibited high affinity and selectivity for D3 receptor in vitro binding assay and in vivo functional assays. They were also quite potent in an in vivo PD animal model, indicating their site-specific agonist activity in the CNS.[28,29] In our further quest to understand the role of piperazine ring and the effect of different N-substitutions in the piperazine ring, the current compounds were designed. In these compounds, different *N*-aromatic heterocyclic substitutions, specifically various indole substitutions, have been incorporated. One of our long-term objectives in this project is to develop a reliable pharmacophore model for these hybrid compounds with which we would be able to rationalize the observed DA D2/D3 binding affinities and selectivities of our compounds.

2. Chemistry

Scheme 1 outlines the syntheses of **9a**, **9b** and enantiomerically pure form of **9b**, ((+)-**9b** and (−)-**9b**). The starting materials for these compounds were appropriately substituted 5- and 7-methoxy-2-tetralones **1a**–**b**. These were condensed with propyl amine under reductive amination conditions to give secondary amines **2a**–**b**, which were then reacted with chloroacetyl chloride to give α-chloro amides **3a**–**b**. Enantiomeric synthesis of compound **9b** was performed by converting the racemic amine **2b** to its diastereomeric salt by using optically active synthetic resolving agent. The synthesis of this resolving agent (4-(2-chlorophenyl)-5,5-dimethyl-2-hydroxy 1,3,2-dioxaphosphorinane-2-oxide), commonly known as chlocyphos, and its resolution to derive two enantiomers

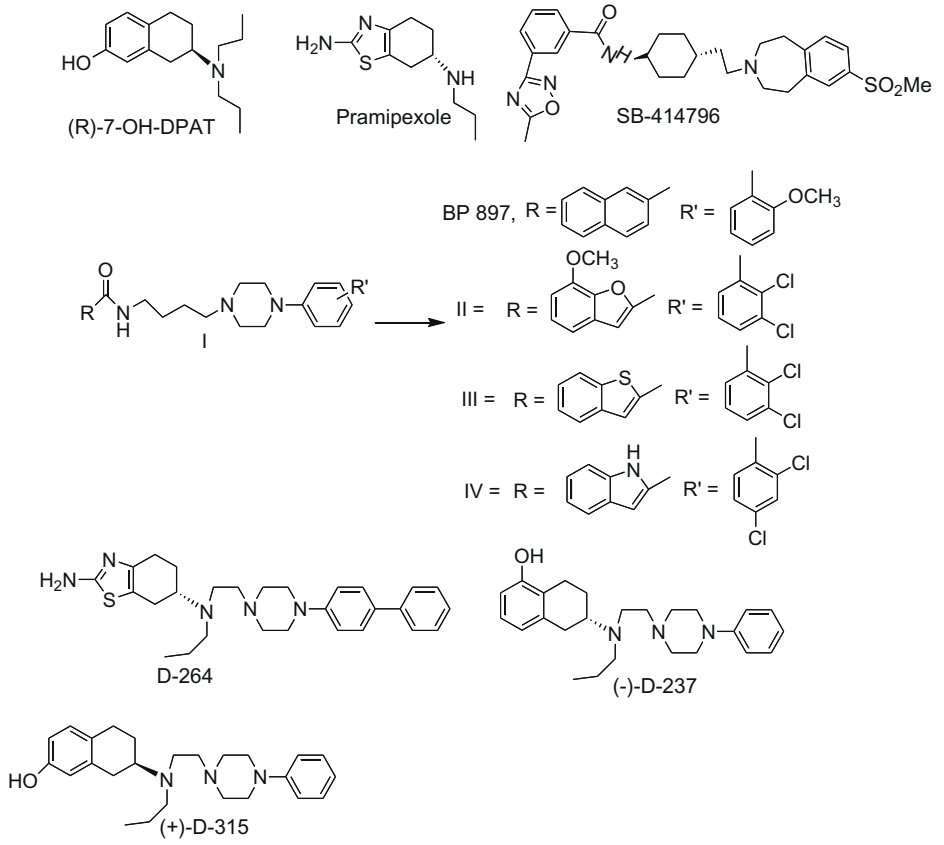
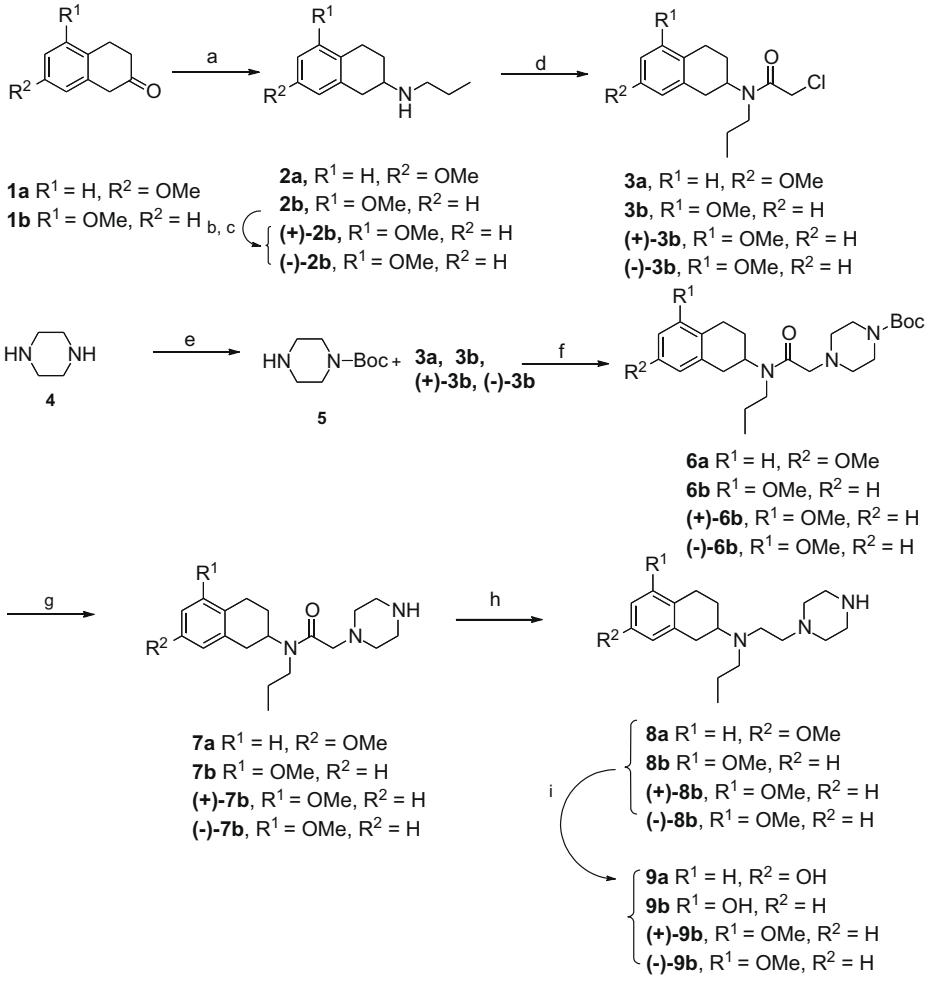


Figure 1. Molecular structure of dopamine D3 selective agonist and antagonist molecules.



Scheme 1. Reagents and conditions: (a) *n*-propyl amine, $NaCNBH_3$, AcOH, $(CH_2Cl_2)_2$; (b) (+)- or (−)-chlocyphos, ethanol, recrystallized from isopropanol; (c) 20% NaOH, rt, 2 h; (d) propionyl chloride, Et_3N, CH_2Cl_2; (e) $(Boc)_2O$, CH_2Cl_2; (f) K_2CO_3, CH_3CN, reflux; (g) CF_3COOH, CH_2Cl_2; (h) $LiAlH_4$, THF, reflux; (i) BBr_3, −78 °C, CH_2Cl_2.

was carried out by following a literature procedure and our earlier publication.[28,30] Thus, compound **2b** was resolved into the enantiomeric pure compound (**+**)-**2b** and (−)-**2b** using (+)- or (−)-chlocyphos by following the procedure reported by us earlier. N-Alkylation of amines with mono Boc-protected piperazine[31] gave amides **6a**–**b**, (**+**)-**6b** and (−)-**6b** which were then reduced using lithium aluminum hydride. Demethylation in the presence of boron tribromide afforded phenol intermediates **9a**–**b**, (**+**)-**9b**, (−)-**9b**.

Scheme 2 describes the syntheses of indoles **10a**–**j** as well as enantiomeric pure (+)-**10e** and (−)-**10e**. Secondary amines **9a**–**b**, (**+**)-**9b** and (−)-**9b** were condensed with either aldehydes under reductive amination conditions or carboxylic acids under amide coupling reactions to yield the final compounds **10a**–**j**, (**+**)-**10e** and (−)-**10e**.

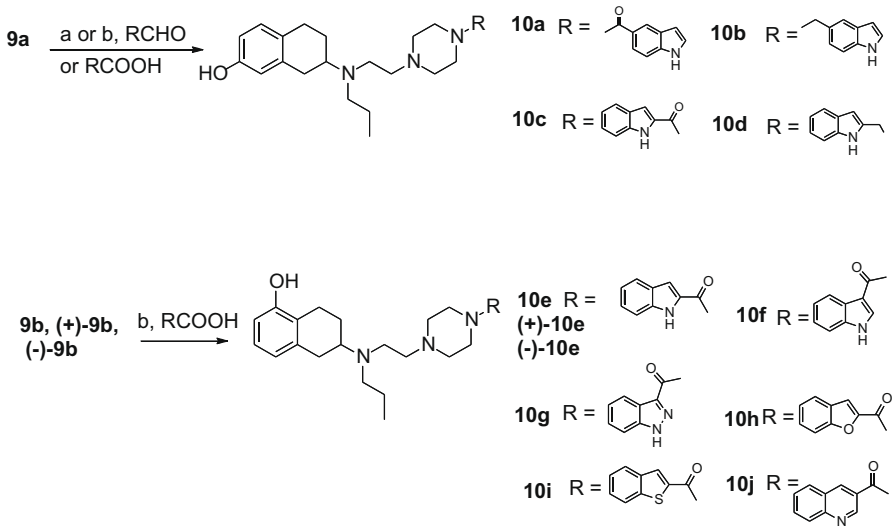
The synthesis of optically active indole (−)-**15** began with compound **5** and is shown in Scheme 3. N-Alkylation with bromoethanol followed by Swern oxidation gave aldehyde **12**. Condensation of **12** with optically active secondary amine **13**[28] afforded **14**. Deprotection followed by amide coupling with indole-5-carboxylic acid gave (−)-**15**.

Scheme 4 outlines the synthesis of indole **22**. 5-Bromoindole was protected using triisopropylsilyl chloride in the presence of NaH to provide **17**. Coupling of this compound with **5** using $PdCl_2[P(o\text{-tol})_3]_2$ and sodium *t*-butoxide in xylenes gave intermediate **18** in good yield. Next, cleavage of both amine protecting groups followed by condensation with α-chloro amide **3a** gave **21**. Amide reduction with borane and demethylation using HBr completed the synthesis of **22**.

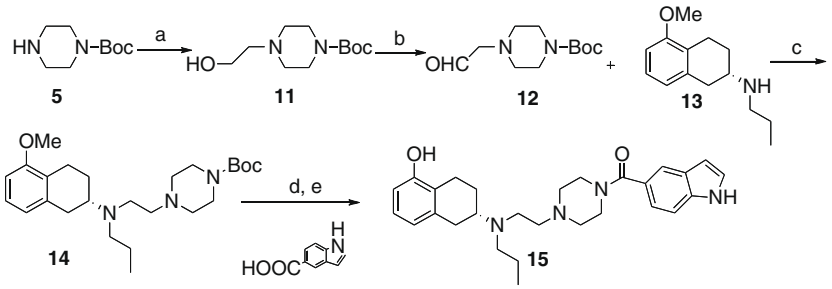
3. Results and discussion

In our current report we have designed a series of *N*-piperazine substituted novel hybrid derivatives. The majority of compounds designed represent various substituted indole derivatives with additional compounds falling in the category of bioisosteric analogues of indole.

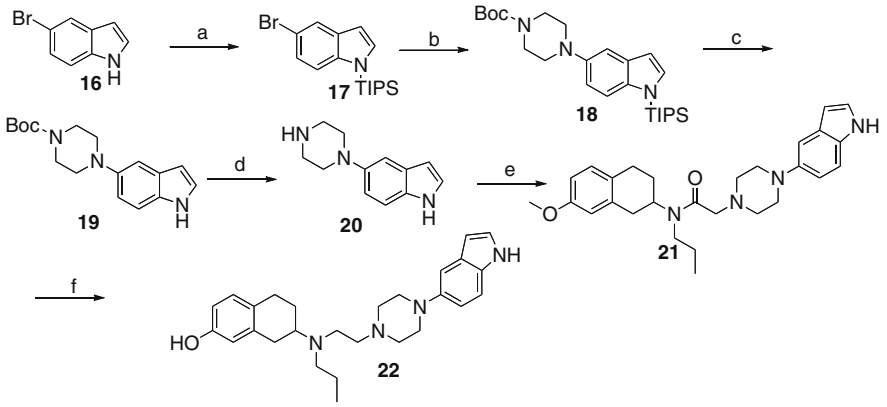
Table 1 summarizes the binding data for the indole analogs that were synthesized. Compound **10e**, which is a 5-hydroxy aminotetralin compound with a 2-substituted indole amide, proved to be twice as potent in binding at D2 receptors and almost seven times more potent at D3 receptors (K_i D2 = 51.2 nM, D3 = 0.550 nM) than its corresponding 7-OH counterpart **10c** (K_i D2 = 116 nM, D3 = 3.72 nM). The observed differences in D2 binding affinities between **10c** and **10e** correlate well with parent hybrid compounds **D-315** and **D-237** (see Table 1). However, the differences in D3 binding between **10c** and **10e** are more significant than between **D-315** and **D-237**. Thus, the selectivity of **10e** for D3 receptor is much higher than **10c** (D2/D3; 93 vs 31 for **10e** and **10c**, respectively). On the other hand, the 5-hydroxy derived 3-substituted indole-acyl derivative **10f** showed less affinity for D3 receptors compared to its 2-substituted indole **10e** counterpart (K_i; 58.9 and 3.62 nM for D2 and D3 receptors, respectively for



Scheme 2. Reagents: (a) $NaCNBH_3$, AcOH, $ClCH_2CH_2Cl$; (b) EDCI, HOBT, Et_3N, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) 2-bromoethanol, K_2CO_3, CH_3CN; (b) Swern oxidation; (c) $Na(OAc)_3BH$, acetic acid, dichloroethane; (d) aq HBr (48%), reflux, 3 h; (e) EDCI, HOBt, Et_3N.



Scheme 4. Reagents and conditions: (a) triisopropylsilyl chloride, NaH, THF; (b) **5**, $PdCl_2[P(o\text{-tol})_3]_2$, NaO*t*Bu, xylenes, reflux, overnight; (c) 1 M Bu_4NF, THF, rt, 4 h; (d) TFA, CH_2Cl_2; (e) **3a**, K_2CO_3, CH_3CN, reflux; (f) (i) BH_3, THF; (ii) HBr, reflux.

10f). The other 5-substituted indole amide derivative **10a** derived from the 7-hydroxy series, exhibited higher affinity than its **10c** counterpart in the same series (see Table 1). Compounds **15** and **10a** exhibited comparable affinity for the D3 receptor (K_i; 2.27 vs 1.67 nM, respectively) (Table 1).

Next a series of bioisosteric analogues of 5-hydroxy indole derivative was designed and synthesized. The benzo[*b*]thiophene derivative **10i** showed high affinity for D2 and D3 receptors (K_i = 76.9 and 1.69 nM for D2 and D3 receptors, respectively). On the other hand, the benzofuran derivative **10h** was two- to three-fold less potent in binding compared to **10i** (K_i = 132 and 5.23 nM for D2 and D3 receptors). The quinoline derivative **10j** exhibited similar binding potency as **10h** and was the least potent at D2 receptors in this bioisosteric series, with a K_i value of 158 nM. Interestingly, the indazole derivative **10g** exhibited the highest affinity for D2 in this current series of molecules and was also

Table 1
Affinity for cloned D_{2L} and D_3 receptors expressed in HEK cells measured by inhibition of [^{3}H]spiperone binding

Compound	K_i (nM), D_{2L} [^{3}H]spiperone	K_i (nM), D_3 [^{3}H]spiperone	D_{2L}/D_3
(±)-7-OH-DPAT	311 ± 47	6.19 ± 1.4	50.2
(−)-5-OH-DPAT	58.8 (5) ± 11.0	1.36 (4) ± 0.28	43
D-237[a]	26.0 ± 7.5	0.825 ± 0.136	31.5
D-264[b]	264 ± 40	0.92 ± 0.23	253
D-315	40.6 ± 3.6	1.77 ± 0.42	22.9
10c (**D-282**)	116 ± 12	3.72 ± 1.12	31.2
10d (**D-283**)	100 ± 1	4.82 ± 1.10	20.7
10b (**D-284**)	144 ± 5	3.87 ± 0.65	37.2
10a (**D-285**)	65.9 ± 9	1.67 ± 0.26	39.5
22 (**D-286**)	30.0 ± 4.9	2.00 ± 0.48	15.0
(−)-**15** (**D-313**)	157 ± 35	2.27 ± 0.52	69.2
10e (**D-328**)	51.2 ± 7.0	0.550 (4) ± 0.084	93.1
(−)-**10e** (**D-366**)	47.5 (5) ± 6.2	0.570 ± 0.094	83
(+)-**10e** (**D-365**)	113 (6) ± 21	3.73 (4) ± 0.56	30.2
10f (**D-329**)	58.9 ± 7.9	3.62 (5) ± 0.79	16.3
10g (**D-334**)	28.0 ± 2.4	2.83 (5) ± 0.59	9.89
10h (**D-333**)	132 ± 8	5.23 (5) ± 1.13	25.2
10i (**D-332**)	76.9 ± 9.2	1.69 (4) ± 0.41	45.5
10j (**D-331**)	158 ± 22	5.18 (5) ± 0.75	30.5

Results are means ± SEM for three to six experiments each performed in triplicate.
[a] See Ref. 28.
[b] See Ref. 29.

among the D3 compounds with highest binding potency in this bioisosteric series of compounds (K_i = 28 and 2.83 nM for D2 and D3, respectively).

5-Substituted indole derivative **10b**, with a methylene unit between the heterocycle and the piperazine fragment, showed affinity for both D2 and D3 that was half as compared to its amide counterpart **10a** (K_i; 144 and 3.87 nM for D2 and D3 receptors, respectively for **10b**). However, such a change in binding affinity was not observed between the 2-substituted similar indole compounds **10c** and **10d**. The reason for this is not fully understood. Compound **22**, in which the piperazine moiety is directly attached to the 5-position of indole, demonstrated high affinity for both D2 and D3 (K_i D2 = 30 nM, D3 = 2 nM).

The above data demonstrates that the position of attachment to the indole moiety influences the binding affinity profiles for both D2 and D3 receptors. For example, changing the point of indole attachment from the 2 position (**10e**) to the 3 position (**10f**) results in a very minor shift in K_i values for D2 receptor (51.2 nM for **10e** vs 58.9 nM for **10f**). However, the 2-substituted indole amide **10e** was sevenfold more potent than **10f** for binding interaction with D3 receptors (K_i; 0.55 vs 3.62 nM). It will be interesting to investigate in the future whether such changes in affinities can be accounted for by alteration of any electronic properties in the two substituted indoles or other factors. From a structural point of view, it seems that the role of the indole N-atom is less critical here, especially in **10e**. Our data also show that the nature of the indole group substitution coupled with the position of the hydroxyl group substitution in the aromatic ring play important roles in determining the D2/D3 binding affinities of hybrid aminotetralin arylpiperazine molecules as well as their selectivity.

As mentioned above, compound **10e** is the most potent and selective molecule in binding to D3 receptors in the current series of molecules. Synthesis of enantiomers of **10e** was carried out followed by characterization of their binding to D2/D3 receptors. As expected, (−)-**10e** exhibited higher affinity and selectivity for D2/D3 receptors than its corresponding enantiomeric counterpart (+)-**10e** (K_i; 47.5 nM and 0.57 nM vs 113 nM and 3.73 nM for D2 and D3 receptors, respectively). In this regard, (−)-**10e** exhibited higher affinity and selectivity for the D3 receptor compared to either (±)-7-OH-DPAT or (−)-5-OH-DPAT (K_i; 0.57 nM vs 6.19 and 1.36 nM; D2/D3: 83 vs 50 and 43). Compound (−)-**15** (K_i; 157 nM and 2.27 nM for D2 and D3, respectively) was selectively synthesized as our current results and previous results consistently demonstrated that in the 5-hydroxy series of hybrid compound, it is the (−)-isomeric version which exhibits the highest affinity for the D3 receptor compared to the (+)-version.[28] Another interesting aspect of the results from current SAR studies concerns the role of piperazine N-atoms in interacting with D2/D3 receptors. The piperazine N-atom connected to the aromatic moiety in compound **D-264** and **D-237** is expected to be less basic compared to the other piperazine N-atom. In the current indole amides and other heterocyclic amide derivatives this N-atom is expected to be even lesser basic in nature. However, this loss of basicity has not impacted their affinity for D2/D3 receptors, possibly indicating very little or no involvement of this N-atom in H-bonding and ionic interaction with the target receptors.

Next we evaluated one of the optically active lead compounds (−)-**15** and the reference (−)-5-OH-DPAT in the GTPγS functional assay for D2 and D3 receptors. The assay was carried out with cloned human D2 and D3 receptors expressed in CHO- and AtT-cells, respectively. The half maximal stimulation (EC_{50}) exhibited by (−)-**15** at D2 receptors was in the nanomolar range and at D3 receptors subnanomolar (EC_{50}; 10.4 and 0.14 nM for D2 and D3 receptor, respectively) whereas the compound maximally stimulated the GTPγS signal comparable to the full agonist dopamine itself, indicating full agonist activity at both D2 and D3 receptor. The functional data shows that (−)-**15** has a preferential intrinsic stimulatory effect at D3 receptors compared to D2 receptors (D2/D3 (EC_{50}) = 74, Table 2).

4. Conclusion

In this report, we have demonstrated that N-substitution on the piperazine ring can accommodate various substituted indole rings.

Table 2
Stimulation of [^{35}S]GTPγS binding to the cloned hD2 receptor expressed in CHO cells and cloned hD3 receptor expressed in AtT-20 cells

Compound	CHO-D2		AtT-D3		
	EC_{50} (nM) [^{35}S]GTPγS	$\%E_{max}$	EC_{50} (nM) [^{35}S]GTPγS	$\%E_{max}$	D2/D3
Dopamine	209 (4) ± 29	100 (definition)	8.53 ± 0.62	100 (definition)	24.5
D-264[b]	19.9 ± 0.9	119 ± 6	0.085 ± 0.016	102 ± 19	248
(−)-**D-237**[a]	2.22 ± 0.27	63.4 ± 3.5	0.121 ± 0.002	78.5 ± 9.5	18.3
(−)-**15** (**D-313**)	10.4 (4) ± 1.6	77.8 ± 0.9	0.14 ± 0.03	92.2 ± 5.8	74.3
(−)-5-OH-DPAT	41.2 ± 6.0	80.0 ± 4.4	1.23 ± 0.53	91.2 ± 1.0	33.5

EC_{50} is the concentration producing half-maximal stimulation; for each compound, maximal stimulation (E_{max}) is expressed as percent of the E_{max} observed with 1 mM (D2) or 100 μM (D3) of the full agonist DA ($\%E_{max}$). Results are means ± SEM for 3–4 experiments each performed in triplicate.
[a] See Ref. 28.
[b] See Ref. 29.

Our results have also shown that in order to maintain high affinity and selectivity for the D3 receptor, the heterocyclic ring does not need to be connected directly to the piperazine ring as the majority of compounds included here are linked either via an amide or a methylene linker. Thus, compound (−)-**10e** with an amide linker connected to the 2-position of the indole ring was among the compounds with high affinity and selectivity at the D3 receptor. Moreover, compound (−)-**10e** was more potent and selective for the D3 receptor compared to either 7-OH-DPAT or 5-OH-DPAT. As found from our previous studies on hybrid compounds, compounds belonging to 5-hydroxy series in general produced higher potency than those in the 7-hydroxy series. Among the bioisosteric derivatives, the indazole derivative **10g** and benzo[*b*]thiazole **10i** exhibited the highest affinity for D2 and D3 receptors. In the functional GTPγS studies, compound (−)-**10e** exhibited good selectivity at the D3 receptor, conforming to its binding data. Our current ongoing SAR studies will shed more light in regards to the interaction of hybrid molecules which will be used to develop a pharmacophore model for these compounds.

5. Experimental

Analytical silica gel-coated TLC plates (Silica Gel 60 F_{254}) were purchased from EM Science and were visualized with UV light or by treatment with either phosphomolybdic acid (PMA) or ninhydrin. Flash chromatography was carried out on Baker Silica Gel 40 mM. ^{1}H NMR spectra were routinely obtained on GE-300 MHz and Varian 400 MHz FT NMR. The NMR solvent used was either $CDCl_3$ or CD_3OD as indicated. TMS was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. and were within ±0.4% of the theoretical value. Optical rotations were recorded on a Perkin Elmer 241 polarimeter. [^{3}H]spiperone (15.0 Ci/mmol) and [^{35}S]GTPS (1250 Ci/mmol) were from Perkin Elmer (Boston, MA). 7-OHDPAT and (+)-butaclamol were from Sigma–Aldrich (St. Louis, MO).

5.1. Procedure A: (7-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (2a)

7-Methoxy-2-tetralone (6.21 g, 35.2 mmol) and acetic acid (5.3 ml, 105.6 mmol) were dissolved in dichloroethane (100 ml) and cooled to 0 °C. Propyl amine (5.7 ml, 70.4 mmol) was added and the mixture was stirred under a N_2 atmosphere for 30 min. $NaCNBH_3$ (5.5 g, 80.8 mmol) in anhydrous MeOH (15 ml) was then added to the mixture and allowed to stir overnight at ambient temperature. The volatiles were then evaporated and the mixture was partitioned between ethyl acetate and 1 M NaOH. The organic layer was separated, dried (Na_2SO_2), filtered, and concentrated. The crude residue was then taken up in EtOAc, at which time ethereal HCl was added, and the material evaporated to dryness. The crude salt was dissolved in a minimum volume of MeOH, and precipitated by the gradual addition of diethyl ether. The salt was collected via filtration and dried to yield 5.09 g (64%, free base) and used in the subsequent transformations. ^{1}H NMR (free base) (400 MHz, $CDCl_3$) 0.91–0.95 (t, 3H, J = 7.6 Hz), 1.38 (br s, 1H), 1.48–1.60 (m, 3H), 2.04–2.09 (m, 1H), 2.54–2.62 (m, 2H), 2.67–2.71 (t, 3H, J = 7.6 Hz), 2.88–2.92 (m, 2H), 2.97–3.04 (m, 1H), 3.81 (s, 3H), 6.60–6.61 (dd, 1H, J = 1.6 Hz), 6.65–6.78 (m, 1H), 6.95–6.98 (d, 1H, J = 8.8 Hz).

5.2. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (2b)

This compound was prepared following Procedure A from 5-methoxy 2-tetralone (6.0 g, 34.0 mmol), acetic acid (5.1 ml, 102 mmol), propyl amine (5.5 ml, 68 mmol), and $NaCNBH_3$ (5.3 g, 85 mmol) to give 4.63 g of **2b** (62%, free base). ^{1}H NMR (free base) (400 MHz, $CDCl_3$) 0.92–0.964 (t, 3H, J = 7.6 Hz), 1.39 (br s, 1H), 1.49–1.61 (m, 3H), 2.05–2.10 (m, 1H), 2.53–2.62 (m, 2H), 2.66–2.70 (t, 3H, J = 7.6 Hz), 2.87–2.94 (m, 2H), 2.98–3.03 (m, 1H), 3.81 (s, 3H), 6.65–6.67 (d, 1H, J = 8 Hz), 6.96–6.71 (d, 1H, J = 8 Hz), 7.07–7.11 (t, 1H, J = 7.2 Hz).

Compound **2b** was resolved into its enantiomerically pure form (**+**)-**2b** and (−)-**2b** following the procedure reported by us earlier.

5.3. Procedure B: 2-Chloro-*N*-(7-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-*N*-propyl-acetamide (3a)

Compound **2a** (HCl salt, 5.0 g, 19.5 mmol) and Et_3N (5.5 ml, 39.1 mmol) was stirred at 0 °C in CH_2Cl_2 (65 ml) for 15 min. Chloroacetyl chloride (1.9 ml, 23.5 mmol) was added dropwise and the resulting solution was stirred at room temperature for 20 min, at which time the reaction mixture was poured into a 1 M solution of NaOH (50 ml) and the product was extracted with dichloromethane, dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by column chromatography (Hex/EtOAc, 3:1) to give 5.5 g (95%) of **3a**. ^{1}H NMR (400 MHz, $CDCl_3$) 0.90–0.98 (m, 3H), 1.64–1.72 (m, 2H), 3.19–3.27 (m, 2H), 4.00 (s, 3H), 6.61–6.62 (dd, 1H, J = 1.6 Hz), 6.64–6.77 (m, 1H), 6.96–6.99 (d, 1H, J = 8.8 Hz).

5.4. 2-Chloro-*N*-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-*N*-propyl-acetamide (3b)

This compound was prepared from **2b** (4.63 g, 21.1 mmol), triethyl amine (5.1 ml, 42.2 mmol), and chloroacetyl chloride (1.80 ml, 25.4 mmol) by following Procedure B to give **3b** (5.8 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.90–0.98 (m, 3H), 1.64–1.72 (m, 2H), 3.19–3.27 (m, 2H), 4.00 (s, 3H), 6.61–6.68 (m, 2H), 6.96–7.04 (m, 1H).

5.5. (+)-2-Chloro-*N*-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-*N*-propyl-acetamide ((+)-3b)

This compound was prepared from (+)-**2b** (3.09 g, 14.07 mmol), triethyl amine (3.4 ml, 28.1 mmol), and chloroacetyl chloride (1.20 ml, 16.93 mmol) by following Procedure B to give (**+**)-**3b** (3.95 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.90–0.97 (m, 3H), 1.65–1.72 (m, 2H), 3.18–3.27 (m, 2H), 4.00 (s, 3H), 6.607–6.68 (m, 2H), 6.97–7.03 (m, 1H).

5.6. (−)-2-Chloro-*N*-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-*N*-propyl-acetamide ((−)-3b)

This compound was prepared from (−)-**2b** (1.98 g, 9.02 mmol), triethylamine (2.2 ml, 18 mmol), and chloroacetyl chloride (0.77 ml, 10.85 mmol) by following Procedure B to give (−)-**3b** (2.50 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.87–0.95 (m, 3H), 1.63–1.78 (m, 2H), 3.1–3.30 (m, 2H), 3.89 (s, 3H), 6.61–6.68 (m, 2H), 6.97–7.02 (m, 1H).

5.7. Procedure C: 4-{[(7-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-carbamoyl]-methyl}-piperazine-1-carboxylic acid *tert*-butyl ester (6a)

Compound **3a** (4.22 g, 14.3 mmol), **5** (4.35 g, 17.2 mmol), K_2CO_3 (3.94 g, 28.5 mmol) were refluxed in CH_3CN (100 ml) for 1 h. The solution was cooled, filtered, and concentrated. The crude material was then partitioned between EtOAc and H_2O, and the organic layer

was separated, dried (Na_2SO_4), and concentrated. The crude mixture was purified by column chromatography (EtOAc) to give 5.1 g (71%) of **6a**. ^{1}H NMR (400 MHz, $CDCl_3$) 0.86–0.90 (t, 3H, J = 8 Hz), 1.45 (s, 9H), 1.59–1.82 (m, 2H), 1.87–1.94 (m, 1H), 1.95–1.97 (m, 3H), 2.420 (br s, 2H), 2.50–2.63 (m, 2H), 2.76–2.82 (m, 1H), 2.96–3.20 (m, 3H), 3.88–3.47 (m, 4H), 3.81 (s, 3H), 6.60–6.61 (dd, 1H, J = 1.6 Hz), 6.65–6.78 (m, 1H), 6.95–6.98 (d, 1H, J = 8.8 Hz).

5.8. 4-{[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-carbamoyl]-methyl}-piperazine-1-carboxylic acid *tert*-butyl ester (6b)

This compound was prepared from **3b** (5.8 g, 19.6 mmol), **5** (5.9 g, 23.6 mmol), and K_2CO_3 (5.4 g, 41.2 mmol) according to Procedure C for to give **6b** (6.6 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.87–0.91 (t, 3H, J = 8 Hz), 1.44 (s, 9H), 1.58–1.81 (m, 2H), 1.88–1.95 (m, 1H), 1.96–1.98 (m, 3H), 2.40 (br s, 2H), 2.51–2.63 (m, 2H), 2.78–2.83 (m, 1H), 2.95–3.29 (m, 3H), 3.88–3.47 (m, 4H), 3.81 (s, 3H), 6.63–6.70 (m, 2H), 7.10–7.14 (t, 1H, J = 7.2 Hz).

5.9. (+)-4-{[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-carbamoyl]-methyl}-piperazine-1-carboxylic acid *tert*-butyl ester ((+)-6b)

This compound was prepared from (+)-**3b** (5.22 g, 17.64 mmol), **5** (5.31 g, 21.24 mmol), and K_2CO_3 (4.86 g, 37.08 mmol) according to the Procedure C for to give (+)-**6b** (5.52 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.87–0.91 (t, 3H, J = 8 Hz), 1.45 (s, 9H), 1.60–1.81 (m, 2H), 1.88–1.94 (m, 1H), 1.96–2.00 (m, 3H), 2.40 (br s, 2H), 2.51–2.65 (m, 2H), 2.78–2.82 (m, 1H), 2.99–3.28 (m, 3H), 3.39–3.47 (m, 4H), 3.81 (s, 3H), 6.63–6.70 (m, 2H), 7.10–7.13 (t, 1H, J = 7.2 Hz).

5.10. (−)-4-{[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-carbamoyl]-methyl}-piperazine-1-carboxylic acid *tert*-butyl ester ((−)-6b)

This compound was prepared from **3b** (2.23 g, 7.54 mmol), **5** (2.26 g, 9.08 mmol), and K_2CO_3 (2.08 g, 15.85 mmol) according to the Procedure C for to give **6b** (2.62 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.87–0.92 (t, 3H, J = 8 Hz), 1.44 (s, 9H), 1.58–1.82 (m, 2H), 1.88–1.97 (m, 1H), 1.96–2.00 (m, 3H), 2.41 (br s, 2H), 2.51–2.60 (m, 2H), 2.78–2.85 (m, 1H), 2.95–3.31 (m, 3H), 3.88–3.46 (m, 4H), 3.81 (s, 3H), 6.63–6.70 (m, 2H), 7.10–7.13 (t, 1H, J = 7.2 Hz).

5.11. Procedure D: *N*-(7-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-2-piperazin-1-yl-*N*-propyl-acetamide (7a)

Compound **6a** (5.1 g, 11.5 mmol) was dissolved in 40 ml of dichloromethane and 40 ml of trifluoroacetic acid was added. The mixture was stirred for 3 h, at which time the solution was concentrated to dryness, dissolved in satd $NaHCO_3$, and extracted with EtOAc. The organic layer was dried (Na_2SO_4), filtered, and concentrated to yield 3.8 g (96%) of **7a**, which was used as is in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) 0.93–0.96 (t, 3H, J = 7.6 Hz), 1.62 (m, 3H), 1.85–1.97 (m, 3H), 2.56 (m, 1H), 2.81–2.88 (m, 5H), 2.98–3.34 (m, 9H), 3.81 (s, 3H), 6.60–6.61 (dd, 1H, J = 1.6 Hz), 6.65–6.78 (m, 1H), 6.95–6.98 (d, 1H, J = 8.8 Hz).

5.12. *N*-(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-2-piperazin-1-yl-*N*-propyl-acetamide (7b)

This compound was prepared from **6b** (6.6 g, 14.8 mmol), and TFA (40 ml) by following Procedure D to give **7b** (4.8 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.92–0.964 (t, 3H, J = 7.6 Hz), 1.63 (m, 3H), 1.84–1.97 (m, 3H), 2.55 (m, 1H), 2.80–2.87 (m, 5H), 2.97–3.34 (m, 9H), 3.82 (s, 3H), 6.64–6.71 (m, 2H), 7.06–7.15 (m, 1H).

5.13. (+)-*N*-(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-2-piperazin-1-yl-*N*-propyl-acetamide ((+)-7b)

This compound was prepared from (+)-**6b** (1.65 g, 3.70 mmol), and TFA (10 ml) by following the Procedure D to give (+)-**7b** (1.22 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.92–0.97 (t, 3H, J = 7.6 Hz), 1.62–1.64 (m, 3H), 1.84–1.98 (m, 3H), 2.52–2.55 (m, 1H), 2.80–2.89 (m, 5H), 2.97–3.35 (m, 9H), 3.82 (s, 3H), 6.64–6.70 (m, 2H), 7.06–7.14 (m, 1H).

5.14. (−)-*N*-(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-2-piperazin-1-yl-*N*-propyl-acetamide ((−)-7b)

This compound was prepared from (−)-**6b** (1.38 g, 3.08 mmol), and TFA (10 ml) by following the Procedure D to give (−)-**7b** (1.02 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.91–0.97 (t, 3H, J = 7.6 Hz), 1.62–1.65 (m, 3H), 1.84–1.95 (m, 3H), 2.56 (m, 1H), 2.80–2.88 (m, 5H), 2.97–3.35 (m, 9H), 3.80 (s, 3H), 6.64–6.70 (m, 2H), 7.06–7.16 (m, 1H).

5.15. Procedure E: (7-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-(2-piperazin-1-yl-ethyl)-propyl-amine (8a)

To a suspension of $LiAlH_4$ (1.6 g, 40.5 mmol) in THF (100 ml) in an ice bath was added **7a** (3.8 g, 11.0 mmol) dissolved in a solution of THF (25 ml). After addition, the mixture was refluxed for 3 h and cooled to 0 °C. 10% NaOH was added dropwise, and the mixture stirred for 20 min, and filtered. The solution was dried (Na_2SO_4), filtered, and concentrated to give **8a** (3.21 g, 97%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) 0.91–0.96 (t, 3H, J = 7.6 Hz), 1.40–1.59 (m, 3H), 1.97–2.22 (m, 1H), 2.41–3.11 (m, 19H), 3.82 (s, 3H), 5.2 (br s, 1H), 6.61–6.62 (dd, 1H, J = 1.6 Hz), 6.66–6.79 (m, 1H), 6.96–6.99 (d, 1H, J = 8.8 Hz).

5.16. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-(2-piperazin-1-yl-ethyl)-propyl-amine (8b)

This compound was prepared from **7b** (4.8 g, 13.8 mmol) and $LiAlH_4$ (2.0 g, 55.2 mmol) by following Procedure E to give **8b** (4.2 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.92–0.96 (t, 3H, J = 7.6 Hz), 1.41–1.59 (m, 3H), 1.98–2.22 (m, 1H), 2.41–3.1 (m, 19H), 3.81 (s, 3H), 5.2 (br s, 1H), 6.58–6.60 (d, 1H, J = 8 Hz), 6.70–6.72 (d, 1H, J = 8 Hz), 7.04–7.1 (t, 1H, J = 8 Hz).

5.17. (+)-5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-(2-piperazin-1-yl-ethyl)-propyl-amine ((+)-8b)

This compound was prepared from (+)-**7b** (1.22 g, 3.51 mmol) and $LiAlH_4$ (0.510 g, 14.05 mmol) by following the Procedure E to give (+)-**8b** (1.00 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.92–0.97 (t, 3H, J = 7.6 Hz), 1.41–1.60 (m, 3H), 1.98–2.24 (m, 1H), 2.41–3.12 (m, 19H), 3.80 (s, 3H), 5.22 (br s, 1H), 6.58–6.61 (d, 1H, J = 8 Hz), 6.70–6.73 (d, 1H, J = 8 Hz), 7.04–7.11 (t, 1H, J = 8 Hz).

5.18. (−)-5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-(2-piperazin-1-yl-ethyl)-propyl-amine ((−)-8b)

This compound was prepared from (−)-**7b** (1.02 g, 2.93 mmol) and $LiAlH_4$ (0.42 g, 11.72 mmol) by following the Procedure E to give (−)-**8b** (0.90 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.92–0.98 (t,

3H, *J* = 7.6 Hz), 1.41–1.61 (m, 3H), 1.98–2.20 (m, 1H), 2.40–3.20 (m, 19H), 3.83 (s, 3H), 5.19 (br s, 1H), 6.58–6.60 (d, 1H, *J* = 8 Hz), 6.70–6.72 (d, 1H, *J* = 8 Hz), 7.04–7.12 (t, 1H, *J* = 8 Hz).

5.19. Procedure F: 7-[(2-Piperazin-1-yl-ethyl)-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-2-ol (9a)

Compound **8a** (3.21 g, 9.70 mmol) was dissolved in 120 ml of CH_2Cl_2 and cooled to -78 °C. 1 M boron tribromide (30 ml) was added dropwise and the mixture was allowed to warm to ambient temperature and was stirred overnight. Satd $NaHCO_3$ was added and the product extracted with dichloromethane, dried (Na_2SO_4), filtered, and concentrated to yield the crude product. Column chromatography (7:3:1 CH_2Cl_2/MeOH/Et_3N) afforded 2.41 g (84%) of **9a**. ^{1}H NMR (400 MHz, $CDCl_3$) 0.98–1.02 (t, 3H, 7.6 Hz), 1.27–1.31 (m, 2H), 1.70–1.82 (m, 3H), 2.25–2.28 (m, 1H), 2.58–2.06 (m, 1H), 2.73–2.78 (m, 4H), 2.97–3.09 (m, 5H), 3.16–3.20 (m, 9H), 6.45 (s, 1H), 6.53–6.55 (d, 1H, *J* = 9.2 Hz), 6.84–6.85 (d, 1H, *J* = 8.4 Hz).

5.20. 6-[(2-Piperazin-1-yl-ethyl)-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-1-ol (9b)

This compound was prepared from **8b** (4.2 g, 12.6 mmol), and boron tribromide (39 ml) following Procedure F to give **9b** (3.1 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.99–1.02 (t, 3H, 7.6 Hz), 1.28–1.30 (m, 2H), 1.71–1.83 (m, 3H), 2.26–2.29 (m, 1H), 2.57–2.06 (m, 1H), 2.72–2.77 (m, 4H), 2.97–3.07 (m, 5H), 3.16–3.21 (m, 9H), 6.58–6.59 (d, 1H, *J* = 8 Hz), 6.61–6.28 (m, 1H, *J* = 8 Hz), 6.92–6.95 (t, 1H, *J* = Hz).

5.21. (+)-6-[(2-Piperazin-1-yl-ethyl)-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-1-ol ((+)-9b)

This compound was prepared from (**+**)-**8b** (1.00 g, 3.00 mmol), and boron tribromide (10 ml) by following the Procedure F to give (**+**)-**9b** (0.78 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.99–1.02 (t, 3H, 7.6 Hz), 1.28–1.32 (m, 2H), 1.73–1.83 (m, 3H), 2.24–2.29 (m, 1H), 2.57–2.07 (m, 1H), 2.73–2.77 (m, 4H), 2.97–3.06 (m, 5H), 3.16–3.20 (m, 9H), 6.58–6.60 (d, 1H, *J* = 8 Hz), 6.59–6.28 (m, 1H, *J* = 8 Hz), 6.92–6.97 (t, 1H, *J* = Hz).

5.22. (−)-6-[(2-Piperazin-1-yl-ethyl)-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-1-ol ((−)-9b)

This compound was prepared from (−)-**8b** (0.90 g, 2.70 mmol), and boron tribromide (9 ml) by following the Procedure F to give (−)-**9b** (0.62 g, 73.3%). ^{1}H NMR (400 MHz, $CDCl_3$) 0.99–1.02 (t, 3H, 7.6 Hz), 1.28–1.30 (m, 2H), 1.71–1.82 (m, 3H), 2.24–2.29 (m, 1H), 2.57–2.08 (m, 1H), 2.72–2.77 (m, 4H), 2.97–3.09 (m, 5H), 3.16–3.23 (m, 9H), 6.58–6.59 (d, 1H, *J* = 8 Hz), 6.61–6.27 (m, 1H, *J* = 8 Hz), 6.92–6.96 (t, 1H, *J* = Hz).

5.23. Procedure G: (4-{2-[(7-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-5-yl)-methanone (10a)

Indole-5-carboxylic acid (30 mg, 0.19 mmol), EDCI (45 mg, 0.24 mmol), HOBT (31 mg, 0.24 mmol), triethylamine (31 mg, 0.32 mmol), and compound **9a** (50 mg, 0.16 mmol) were dissolved in dry CH_2Cl_2 (10 ml) and stirred at ambient temperature overnight. The mixture was poured into satd NaHCO3 and extracted with dichloromethane. The organic extract was dried (Na_2SO_4), filtered and concentrated to yield the crude product. Column chromatography afforded **10a** (26 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* = 7.2 Hz), 1.40–1.54 (m, 3H), 1.93 (d, 1H, *J* = 10.0 Hz), 2.45–2.78 (m, 14H), 2.86–2.91 (m, 1H), 3.70 (m, 4H), 6.45 (d, 1H, *J* = 2.0 Hz), 6.55–6.58 (m, 2H), 6.87 (d, 1H, *J* = 8.0 Hz), 7.21–7.24 (m, 2H), 7.33 (d, 1H, *J* = 8.4 Hz), 7.71 (s, 1H), 8.79 (1H, bs). The free base of **10a** was converted into oxalate salt. Mp 194–199 °C. Anal. [$C_{28}H_{38}N_4O{\cdot}2.0(COOH)_2$] C, H, N.

5.24. 7-({2-[4-(1*H*-Indol-5-ylmethyl)-piperazin-1-yl]-ethyl}-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-2-ol (10b)

Compound **10b** was prepared according to Procedure A using **9a** (65 mg, 0.20 mmol) and indole-5-carboxaldehyde (35 mg, 0.24 mmol) to give **10b** (29 mg, 32%) after column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, 3H, *J* = 7.2 Hz), 1.33–1.44 (m, 3H), 1.85 (d, 1H, *J* = 10.0 Hz), 2.41–2.82 (m, 19H), 3.60 (s, 2H), 6.35 (d, 1H, *J* = 2.0 Hz), 6.49–6.58 (m, 2H), 6.85 (d, 1H, *J* = 8.4 Hz), 7.14–7.20 (m, 2H), 7.31 (d, 1H, *J* = 8.4 Hz), 7.54 (s, 1H), 8.38 (br s, 1H). The free base of **10b** was converted into oxalate salt. Mp 198–203 °C. Anal. [$C_{28}H_{38}N_4O{\cdot}2.5(COOH)_2{\cdot}3H_2O$] C, H, N.

5.25. (4-{2-[(7-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-2-yl)-methanone (10c)

Compound **10c** was prepared following Procedure G using **9a** (100 mg, 0.32 mmol) and indole-2-carboxylic acid (61 mg, 0.38 mmol) to give **10c** (125 mg, 86%) after column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz,), 1.42–1.64 (m, 3H), 1.94 (br s, 1H), 2.51–2.75 (m, 14H), 2.88–2.96 (m, 1H), 3.95 (br s, 4H), 6.53 (br s, 1H), 6.59–6.61 (m, 1H), 6.75 (s, 1H), 6.90 (d, 1H, *J* = 12.0 Hz), 7.11–7.18 (m, 1H), 7.22–7.26 (m, 1H), 7.40 (d, 1H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 8.0 Hz), 9.72 (s, 1H). The free base of **10c** was converted into HCl salt. Mp 203–205 °C. Anal. [$C_{28}H_{36}N_4O_2{\cdot}2HCl{\cdot}1.2H_2O$] C, H, N.

5.26. 7-({2-[4-(1*H*-Indol-2-ylmethyl)-piperazin-1-yl]-ethyl}-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-2-ol (10d)

Compound **10d** was prepared according to Procedure A using **9a** (100 mg, 0.32 mmol) and 1*H*-indole-2-carbaldehyde (100 mg, 0.69 mmol) to give **10d** (125 mg, 85%) after column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3H, *J* = 7.2 Hz), 1.39–1.50 (m, 3H), 1.89 (br s, 1H), 2.41–2.73 (m, 18H), 2.82–2.88 (m, 1H), 3.65 (s, 2H), 6.35 (s, 1H), 6.44 (br s, 1H), 6.54–6.57 (m, 1H), 6.87 (d, 1H, *J* = 8.8 Hz), 7.04–7.08 (m, 1H),7.10–7.16 (m, 1H), 7.30 (d, 1H, *J* = 7.6 Hz), 7.54 (d, 1H, *J* = 8.0 Hz), 8.63 (s, 1H). The free base of **10d** was converted into oxalate salt. Mp 135–137 °C. Anal. [$C_{28}H_{38}N_4O{\cdot}3.0(COOH)_2{\cdot}0.3H_2O$] C, H, N.

5.27. (4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-2-yl)-methanone (10e)

Compound **10e** was prepared following Procedure G using **9b** (43 mg, 0.14 mmol) and indole-2-carboxylic acid (30 mg, 0.19 mmol) to give **10e** (32 mg, 62%) after column chromatography. ^{1}H NMR (400 MHz, CD_3OD) δ 1.02–1.06 (t, 3H, *J* = 7.2 Hz), 1.77–1.79 (m, 3H), 2.27 (m, 1H), 2.63–2.66 (br s, 4H), 3.03–3.16 (m, 5H), 3.30–3.41 (m, 3H), 3.89 (br s, 4H), 6.59–6.61 (d, 1H, *J* = 7.6 Hz), 6.63–6.65 (d, 1H, *J* = 8 Hz), 6.84 (s, 1H), 6.94–6.98 (t, 1H, *J* = 7.2 Hz), 7.04–7.08 (t, 1H, *J* = 7.2 Hz), 7.19–7.23 (t, 1H, *J* = 7.2 Hz), 7.41–7.61 (d, 1H, *J* = 8 Hz), 7.59–7.61 (d, 1H, *J* = 8 Hz). The free base of **10e** was then converted into its HCl salt. Mp 166–165 °C. Anal. [$C_{28}H_{36}N_4O_2{\cdot}3HCl$] C, H, N.

5.28. (+)-(4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-2-yl)-methanone ((+)-10e)

Compound (+)-**10e** was prepared following the Procedure G using (+)-**9b** (30 mg, 0.094 mmol) and indole-2-carboxylic acid (23 mg, 0.14 mmol) to give **10e** (18.5 mg, 51.4%) after column chromatography, $[\alpha]_D$ +22 (*c* 1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 0.88–0.92 (t, 3H, *J* = 7.6 Hz), 1.48–1.63 (m, 3H), 1.98–2.10 (m, 1H), 2.53–2.99 (m, 15H), 3.96 (br s, 4H), 6.57–6.59 (d, 1H, *J* = 7.6 Hz), 6.63–6.66 (d, 1H, *J* = 7.6 Hz), 6.78 (s, 1H), 6.96–7.03 (t, 1H, *J* = 7.6 Hz), 7.12–7.16 (t, 1H, *J* = 7.6 Hz), 7.26–7.30 (m, 1H), 7.41–7.43 (d, 1H, *J* = 8.4 Hz), 7.64–7.66 (d, 1H, *J* = 8.0 Hz). The free base of (+)-**10e** was then converted into its HCl salt. Mp 169–170 °C. Anal. [$C_{28}H_{36}N_4O_2$·2HCl] C, H, N.

5.29. (−)-(4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-2-yl)-methanone ((−)-10e)

Compound (−)-**10e** was prepared following the Procedure G using (−)-**9b** (50 mg, 0.16 mmol) and indole-2-carboxylic acid (51 mg, 0.31 mmol) to give (−)-**10e** (33 mg, 55%) after column chromatography, $[\alpha]_D$ −21.6 (*c* 1, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 0.885–0.92 (t, 3H, *J* = 7.2 Hz), 1.51–1.61 (m, 3H), 2.10 (m, 1H), 2.50–2.99 (m, 15H), 3.96 (br s, 4H), 6.58–6.65 (dd, 2H, *J* = 18.6, 6.84), 6.78 (s, 1H), 6.96–7.00 (t, 1H, *J* = 8.0 Hz), 7.12–7.16 (t, 1H, *J* = 7.2 Hz), 7.26–7.30 (t, 1H, J = 7.6 Hz), 7.41–7.43 (d, 1H, *J* = 8.4 Hz), 7.64–7.66 (d, 1H, *J* = 7.6 Hz). The free base of (−)-**10e** was then converted into its HCl salt. Mp 168–169 °C. Anal. [$C_{28}H_{36}N_4O_2$·2HCl] C, H, N.

5.30. (4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-3-yl)-methanone (10f)

Compound **10f** was prepared following Procedure G using **9b** (50 mg, 0.16 mmol) and indole-3-carboxylic acid (35 mg, 0.21 mmol) to give **10f** (38 mg, 53%) after column chromatography. ^1H NMR (400 MHz, CD_3OD) δ 1.03–1.07 (t, 3H, *J* = 7.2 Hz), 1.83–1.96 (m, 3H), 2.32 (s, 1H), 2.53–2.67 (m, 5H), 2.81–2.83 (m, 2H), 3.09–3.18 (m, 4H), 3.41–3.47 (m, 2H), 3.56–3.59 (m, 1H), 3.79 (br s, 4H), 6.61–6.63 (d, 1H, *J* = 8.4 Hz), 6.64–6.66 (d, 1H, *J* = 8.4 Hz), 6.96–6.99 (t, 1H, *J* = 7.2 Hz), 7.13–7.21 (m, 1H), 7.41–7.51 (m, 1H), 7.62–7.64 (m, 1H), 7.71–7.73 (d, 1H, *J* = 7.2 Hz), 7.83–7.84 (1H, *J* = 7.2 Hz). The free base of **10f** was converted into its HCl salt. Mp 147–151 °C. Anal. [$C_{28}H_{36}N_4O_2$·3HCl] C, H, N.

5.31. (4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indazol-3-yl)-methanone (10g)

Compound **10g** was prepared by following Procedure G, using **9b** (50 mg, 0.158 mmol) and indazole 3-carboxylic acid (45 mg, 0.277 mmol) to give **10g** (34 mg, 47%) after column chromatography. ^1H NMR (400 MHz, CD_3OD) δ 1.04–1.07 (t, 3H, *J* = 6.8 Hz), 1.83–1.93 (m, 3H), 2.31 (m, 1H), 2.62–2.70 (m, 5H), 2.81 (br s, 2H), 3.08–3.47 (m, 8H), 3.89 (br s, 2H), 4.08 (br s, 2H), 6.61–6.62 (m, 2H), 6.95–6.99 (t, 1H, *J* = 7.2 Hz), 7.22–7.26 (t, 1H, *J* = 8 Hz), 7.41–7.45 (t, 1H, *J* = 6.4 Hz), 7.57–7.59 (d, 1H, *J* = 8.8 Hz), 7.94–7.96 (d, *J* = 8 Hz). The free base of **10g** was converted in to its HCl salt. Mp 167–170 °C. Anal. [$C_{27}H_{35}N_5O_2$·3HCl·2.5H_2O] C, H, N

5.32. Benzofuran-2-yl-(4-{2-[(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-methanone (10h)

Compound **10h** was prepared following Procedure G using **9b** (214 mg, 0.674 mmol) and benzofuran-2-carboxylic acid (131 mg, 0.809 mmol) to give **10h** (108 mg, 35%) after column chromatography. ^1H NMR (400 MHz, CD_3OD) δ 0.94–0.98 (t, 3H, *J* = 7.2 Hz), 1.60–1.68 (m, 3H), 2.14–2.65 (m, 8H), 2.77–3.03 (m, 8H), 3.87 (br s, 4H), 6.55–6.60 (m 2H), 6.89–6.93 (t, 1H, *J* = 7.2 Hz), 7.29–7.33 (t, 1H, *J* = 6.4 Hz), 7.36 (s, 1H), 7.42–7.45 (t, 1H, *J* = 7.2 Hz), 7.56–7.58 (d, 1H, *J* = 8.8 Hz), 7.70–7.72 (d, 1H, *J* = 7.2 Hz). The free base of **10h** was converted in to its HCl salt. Mp decomp at 140 °C. Anal. [$C_{28}H_{35}N_3O$·2HCl·1.5H_2O] C, H, N.

5.33. Benzo[*b*]thiophen-2-yl-(4-{2-[(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-methanone (10i)

Compound **10i** was prepared following Procedure G using **9b** (175 mg, 0.551 mmol) and benzo[*b*]thiophene-2-carboxylic acid (118 mg, 0.661 mmol) to give **10i** (263 mg, 64%) after column chromatography. ^1H NMR (400 MHz, CD_3OD) 0.91–0.97 (t, 3H, *J* = 7.2 Hz), 1.53–1.68 (m, 3H), 2.50–2.63 (m, 7H), 2.78–3.02 (m, 7H), 3.77–3.79 (br s, 4H), 6.55–6.59 (t, 2H, *J* = 8 Hz), 6.89–6.93 (t, 1H, *J* = 8.4 Hz), 7.40–7.46 (m, 2H), 7.61 (s, 1H), 7.85–7.91 (m, 2H). The free base of **10i** was converted into its HCl salt. Mp 131–134 °C. Anal. [$C_{28}H_{35}N_3O_2S$·2HCl·1.5H_2O] C, H, N.

5.34. (4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-quinolin-3-yl-methanone (10j)

Compound **10j** was prepared following Procedure G using **9b** (172 mg, 0.542 mmol) and quinoline-3-carboxylic acid (112 mg, 0.650 mmol) to give **10j** (256 mg, 85%) after column chromatography. ^1H NMR (400 MHz, CD_3OD) 0.93–0.96 (t, 3H, *J* = 7.2 Hz), 1.57–1.59 (m, 3H), 2.18 (m, 2H), 2.55–3.00 (m, 14H), 3.55 (br s, 2H), 3.85 (br s, 2H), 6.53–6.58 (t, 2H, *J* = 8 Hz), 6.88–6.91 (t, 1H, *J* = 11.2 Hz), 7.81–7.72 (t, 1H, *J* = 7.6 Hz), 7.85–7.88 (t, 1H, *J* = 7.6 Hz), 8.03–8.1 (m, 2H), 8.45 (br s, 1H), 8.89 (br s, 1H). The free base of **10j** was converted into its HCl salt. Mp 142–146 °C. Anal. [$C_{29}H_{36}N_4O_2$·3HCl·2H_2O] C, H, N.

5.35. 4-(2-Hydroxy-ethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (11)

To a mixture of compound **5** (3.00 g, 16.11 mmol), potassium carbonate (2.33 g, 48.32 mmol) in acetonitrile (50 ml) was added 2-bromoethanol (3.00 g, 24.17 mmol) under nitrogen atmosphere. The mixture was refluxed for 4 h, cooled, filtered, and concentrated. The crude mixture was purified by column chromatography (EtOAc/MeOH, 9:1) to give **11** (2.76 g, 75%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.45 (t, 4H, *J* = 5.2 Hz), 2.55 (t, 2H, *J* = 5.2 Hz), 3.44 (t, 4H, *J* = 5.2 Hz), 3.62 (t, 2H, *J* = 5.2 Hz).

5.36. (*S*)-*tert*-Butyl-4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl) amino)ethyl)piperazine-1-carboxylate (14)

To a solution of oxalyl chloride (0.76 ml, 8.76 mmol) in CH_2Cl_2 (20 ml) was added DMSO (1.10 ml, 17.52 mmol) at −78 °C and stirred for 5 min. Compound **11** (1.0 g, 4.38 mmol) was added to the mixture and was kept stirring for 30 min. The reaction temperature was raised to room temperature after addition of triethylamine (3.64 ml, 26.3 mmol). The solution was quenched with

water and extracted with dichloromethane. The organic layer was dried over sodium sulfate and evaporated to afford crude **12** (0.99 g) in quantitative yield and was used in the next reaction without any further purification. Compound **12** (0.99 g, 4.34 mmol) was then reacted with **13** (0.87 g, 3.9 mmol) following Procedure A to provide **14** (1.1 g, 67%). $[\alpha]_D^{20}$ −32.43 (*c* 1.03, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.46–1.61 (m, 12H), 2.04 (br s, 1H), 2.34–2.55 (m, 8H), 2.67–3.01 (m, 7H), 3.41–3.43 (m, 4H), 3.80 (s, 3H), 6.40 (d, 1H, *J* = 8.0 Hz), 6.70 (d, 1H, *J* = 7.6 Hz), 7.08 (m, 1H).

5.37. (*S*)-(4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-(1*H*-indol-5-yl)-methanone (15)

Compound **14** (1.1 g, 3.9 mmol) was refluxed in 48% HBr (30 ml) for 2 h, at which time the solution was cooled and concentrated to dryness. The crude hydrobromic salt was basified by the addition of satd $NaHCO_3$ and the free base extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), filtered, and concentrated to give the crude phenol, which was used as is in the next reaction. The synthesis of **15** was completed by reacting this material 290 mg, 0.67 mmol) with indole-5-carboxylic acid (130 mg, 0.81 mmol) following Procedure G to give 230 mg (74%) after column chromatography. $[\alpha]_D^{20}$ −21.0 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.41–1.571 (m, 3H), 2.02 (br s, 1H), 2.48–2.80 (m, 13H), 2.87–2.95 (m, 2H), 3.63 (br s, 4H), 6.54–6.67 (m, 2H), 6.94–6.98 (m, 1H), 7.24–7.26 (m, 2H) 7.36–7.38 (m, 1H), 7.71 (s, 1H), 8.48 (s, 1H). The free base of **15** was converted into its oxalate salt. Mp 94–96 °C. Anal. [$C_{28}H_{36}N_4O_2$?2.5$(COOH)_2$] C, H, N.

5.38. 5-Bromo-1-triisopropylsilyl-*1H*-indole (17)

5-Bromoindole (1.4 g, 7.14 mmol) was dissolved in dry THF (50 ml) and cooled to 0 °C, which was followed by the addition of NaH (0.34 g, 14.28 mmol) portion wise. The reaction mixture was allowed to stir at room temperature for 1 h. The reaction mixture was again cooled to 0 °C and triisopropylsilyl chloride (1.78 g, 9.28 mmol) was added drop wise. The mixture was allowed to warm to room temperature and was stirred overnight. The solvent was evaporated under reduced pressure and triturated with ethyl acetate (100 ml). The organic layer was washed with brine (50 ml) and dried over Na_2SO_4 and evaporated. The compound was purified by flash column chromatography using pure hexanes to afford colorless oily product **17** (1.90 g, 76%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (d, 18H, *J* = 8.0 Hz), 1.63–1.71 (m, 3H), 6.56 (d, 1H, *J* = 3.2 Hz), 7.20–7.25 (m, 2H), 7.37 (d, 1H, *J* = 8.8 Hz), 7.74 (d, 1H, *J* = 2.0 Hz).

5.39. 4-(1-Triisopropylsilyl-1*H*-indole-5-yl)-piperazine-1-carboxylate (18)

A mixture of compound **17** (1.8 g, 5.10 mmol), **5** (0.86 gm, 4.59 mmol), sodium *tert*-butoxide (0.638 gm, 6.64 mmol) and $PdCl_2[P(o\text{-tol})_3]_2$ (0.20 g, 0.23 mmol, 5% wt) in 100 ml of xylenes was heated at 110 °C overnight. The reaction mixture was diluted with diethyl ether/hexanes (50:50) and filtered through a silica plug and eluted with 10% ethyl acetate in hexanes. All organic fractions were collected and evaporated in vacuo and the crude product was purified by flash column chromatography using diethyl ether/hexanes (20:80) to afford **18** (1.10 g, 52%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (d, 18H, *J* = 6.4 Hz), 1.48 (d, 9H), 1.63–1.70 (m, 3H), 3.08 (t, 4H, *J* = 4.4 Hz), 3.60 (t, 4H, *J* = 5.2 Hz), 6.53 (d, 1H, *J* = 2.4 Hz), 6.86–6.89 (dd, 1H, *J* = 2.4 Hz, *J* = 9.2 Hz), 7.13 (d, 1H, *J* = 2.0 Hz), 7.20 (d, 1H, *J* = 3.2 Hz), 7.40 (d, 1H, *J* = 9.2 Hz).

5.40. *tert*-Butyl 4-(1*H*-indole-5-yl)-piperazine-1-carboxylate (19)

To a stirring solution of compound **18** (1.10 g, 2.40 mmol) in THF (20 ml) was added tetrabutylammonium fluoride (1 M in THF, 15 ml). The reaction mixture was stirred for 3 h to complete the reaction. The solvent was evaporated in vacuo and triturated with diethyl ether (100 ml). The ether layer was washed with saturated $NaHCO_3$ (20 ml) and brine (20 ml), dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash column chromatography over silica gel using ethyl acetate/hexanes (30:70) to afford **19** (0.71 g, 95%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.07 (t, 4H, *J* = 4.8 Hz), 3.61 (t, 4H, *J* = 5.2 Hz), 6.46–6.47 (m, 1H), 6.94–6.97 (dd, 1H, *J* = 2.0 Hz, *J* = 8.8 Hz,), 7.16 (m, 2H), 7.30 (d, 1H, *J* = 8.8 Hz), 8.15 (br s, 1H).

5.41. 2-[4-(1*H*-Indol-5-yl)-piperazin-1-yl]-*N*-(7-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-*N*-propyl-acetamide (21)

To a stirring solution of compound **19** (800 mg, 2.65 mmol) in dry CH_2Cl_2 (20 ml) was added trifluoroacetic acid (20 ml). After stirring for 2 h at room temperature the solvent was removed in vacuo and the crude semi solid residue collected was triturated with ethyl acetate and filtered to get **20** (780 mg, 94%). Compound **20** (780 mg, 3.87 mmol), **3a** (1.15 g, 3.87 mmol), K_2CO_3 (474 mg, 3.44 mmol), and acetonitrile (75 ml) were refluxed for 3 h. The reaction mixture was cooled, filtered, and concentrated. The crude material was purified by column chromatography (EtOAc/MeOH, 95:5) to yield **21** (220 mg, 22%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (t, 3H, *J* = 7.2 Hz), 1.38–1.46 (m, 2H), 1.51–1.61 (m, 1H), 1.94 (d, 1H, *J* = 12.0 Hz), 2.46 (t, 2H, *J* = 7.2 Hz), 2.61–2.75 (m, 4H), 2.96–3.08 (m, 5H), 3.40 (s, 2H), 3.56–3.85 (m, 4H), 6.40 (br s, 1H), 6.47 (d, 1H, *J* = 2.0 Hz), 6.51–6.54 (dd, 1H, *J* = 2.4 Hz and 8.0 Hz), 6.82 (d, 1H, *J* = 8.0 Hz), 6.88–6.90 (dd, 1H, *J* = 2.0 Hz and 8.8 Hz), 7.09–7.11 (m, 2H), 7.25 (d, 1H, *J* = 8.8 Hz), 8.12 (br s, 1H).

5.42. 7-((2-(4-(1*H*-indol-5-yl)piperazin-1-yl)ethyl)(propyl)-amino)-5,6,7,8-tetrahydro-naphthalen-2-ol (22)

To a stirring cold solution of compound **21** (220 mg, 0.492 mmol) in dry THF was added BH_3·THF (211 mg, 2.46 mmol) and the reaction mixture was refluxed overnight. After cooling the reaction mixture to room temperature, 1 ml of methanol was added and the solvent was evaporated. The residue was dissolved in 15 ml of concd HBr and refluxed for 1 h. The solvent was evaporated and the crude product was dissolved in dichloromethane (50 ml). The organic layer was washed with saturated $NaHCO_3$ (30 ml) and dried over Na_2SO_4. The solvent was evaporated and the crude product was purified by flash column chromatography over silica gel using ethyl acetate/MeOH/Et_3N (95:5:0.2) to afford **22** (20 mg). ^1H NMR (400 MHz, CD_3OD) δ 0.95 (t, 3H, *J* = 7.6 Hz), 1.53–1.67 (m, 3H), 2.08 (d, 1H, *J* = 9.6 Hz), 2.60–2.88 (m, 14H), 3.10–3.13 (m, 5H), 6.35 (d, 1H, *J* = 2.8 Hz), 6.52–6.55 (m, 2H), 6.87 (d, 1H, *J* = 8.8 Hz), 6.91–6.94 (dd, 1H, *J* = 8.8 Hz and 2.4 Hz), 7.16–7.17 (m, 2H), 7.29 (d, 1H, *J* = 8.8 Hz). Free base converted into oxalate salt. Mp 179–183 °C.

5.43. Biological experiments: potencies at DA D2 and D3 receptors

Compounds were tested for inhibition of radioligand binding to DA receptors as described in our previous study.[29] Briefly,

membranes from human embryonic kidney (HEK) 293 cells expressing rat D2L and D3 receptors were incubated with each test compound and [^{3}H]spiperone (0.6 nM, 15 Ci/mmol, Perkin Elmer) for 1 h at 30 °C in 50 mM Tris–HCl (pH 7.4), 0.9% NaCl, and 0.025% ascorbic acid. (+)-Butaclamol (2 μM) was used to define nonspecific binding. Assays were terminated by filtration in the MACH 3-96 Tomtec harvester (Wallac, Gaithersburg, MD). IC_{50} values were estimated by nonlinear regression analysis with the logistic model in the least squares fitting program ORIGIN, and converted to inhibition constants (K_i) by the Cheng–Prusoff equation.[32] In this conversion, the K_d values for [^{3}H]spiperone binding were 0.057 nM for D2 receptors and 0.125 nM for D3 receptors.

5.44. Measurement of stimulation of dopamine D2 and D3 receptors—[^{35}S]GTPγS binding

All procedures were as described in our recent work.[29] Briefly, Chinese hamster ovary (CHO) cells expressing human D2L receptors and ATt-20 cells expressing human D3 receptors served as the source for membrane fractions. GTPγS binding assays contained test drug, DA (1 mM for D2 cells, and 100 μM for D3 cells) as indicator of binding plateau, [^{35}S]GTPγS (0.17 nM, 1,250 Ci/mmol, Perkin Elmer), and cell suspension (with GDP for final concentration in assay of 3 μM for D2 or 6 μM for D3). After incubation at room temperature in a shaking water bath for 60 min, cells were harvested by filtration and assayed for ^{35}S radioactivity. Nonspecific binding of [^{35}S]GTPγS was measured with 10 μM GTPγS and the EC_{50} (concentration producing half-maximal stimulation) of the test drug was estimated by nonlinear logarithmic fitting (logistics model) with OriginPro 7.0. The plateau binding (maximal binding stimulation) with test drug was expressed as percent of maximal binding observed with the full agonist DA ($\%E_{max}$).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.031.

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