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Synthesis and characterization of selective dopamine D_2 receptor antagonists. 2. Azaindole, benzofuran, and benzothiophene analogs of L-741,626

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ABSTRACT

A series of indole, 7-azaindole, benzofuran, and benzothiophene compounds have been prepared and evaluated for affinity at $D_{2\text{-like}}$ dopamine receptors. These compounds share structural elements with the classical $D_{2\text{-like}}$ dopamine receptor antagonists haloperidol, *N*-methylspiperone and benperidol. Two new compounds, 4-(4-iodophenyl)-1-((4-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol (**6**) and 4-(4-iodophenyl)-1-((5-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol (**7**), were found to have high affinity to and selectivity for D_2 versus D_3 receptors. Changing the aromatic ring system from an indole to other heteroaromatic ring systems reduced the D_2 binding affinity and the D_2 versus D_3 selectivity.

1. Introduction

The neurotransmitter dopamine is synthesized by mesencephalic neurons of the substantia nigra and ventral tegmental area and by hypothalamic neurons of the arcuate and periventricular nuclei. Dopamine is present in the central nervous system and peripheral nervous system.[1] Dopamine is associated with fine movement coordination, emotion, affect, cognition and memory.[2] Regulation of dopamine plays a crucial role in mental and physical health. Abnormal activity of the dopamine system has been implicated in psychological and neurological disorders including Parkinson's disease, schizophrenia, mood disorders, addiction, attention deficit hyperactivity disorder, and Tourette syndrome.[3–10]

Dopamine receptors belong to a large superfamily of neurotransmitter and hormone receptors which are coupled to their specific effectors function via guanine nucleotide regulatory (G) proteins. Based upon genomic and cDNA cloning studies, it is thought that there are five functionally active dopamine receptor subtypes expressed in mammals. These five receptor subtypes have been classified into two major subtypes ($D_{1\text{-like}}$ and $D_{2\text{-like}}$) based on their pharmacological, biochemical, and physiological characteristics. The $D_{1\text{-like}}$ receptor subtypes consist of the D_1 (D_{1a}) and the D_5 (D_{1b}) dopamine receptors. The $D_{2\text{-like}}$ receptor subtypes are known to include several receptor subtypes and isoforms, including D_{2short} (D_{2S}), D_{2long} (D_{2L}), D_3, and D_4 receptors.[11] Agonist stimulation of $D_{1\text{-like}}$ receptors results in an activation of adenylyl cyclase with increased production of cAMP.[12] Stimulation of the $D_{2\text{-like}}$ receptors results in an inhibition of adenylyl cyclase activity, a decrease in intracellular levels of cAMP, an increase in the release of arachidonic acid and an increase in phosphatidylinositol hydrolysis.[10]

The D_2 and D_3 dopamine receptors have approximately 46% amino acid homology. In contrast, the transmembrane spanning (TMS) regions of the D_2 and D_3 receptors, which are thought to construct the ligand binding site, share 78% homology.[13] Despite their structural similarities, D_2 and D_3 receptors differ in their (a) neuroanatomical localization,[14] (b) levels of receptor expression,[15,16] (c) efficacy in response to agonist stimulation,[10] and (d) regulation and desensitization.[17] Because of the high degree of homology between D_2 and D_3 receptor binding sites, the pharmacologic properties of these two receptor subtypes are similar, and it has been difficult to obtain compounds that can bind selectively to either the D_2 or the D_3 dopamine receptor subtype.[10,15,16,18–20] Despite this difficulty, D_2 and D_3 dopamine receptor selective agonists and antagonists would be useful pharmacologic tools to precisely define the role of the two $D_{2\text{-like}}$ receptor subtypes in a variety of experimental physiological and behavioral situations, including the reinforcing and toxic properties of cocaine,[21,22] socialization, memory, and the regulation of interneuronal activity in the basal ganglia.[23]

Previously, we reported the in vitro binding properties of a series of indole derivatives based on haloperidol, *N*-methylspiperone, and benperidol (Fig. 1). Several novel analogues of L-741,626 (**1a**),

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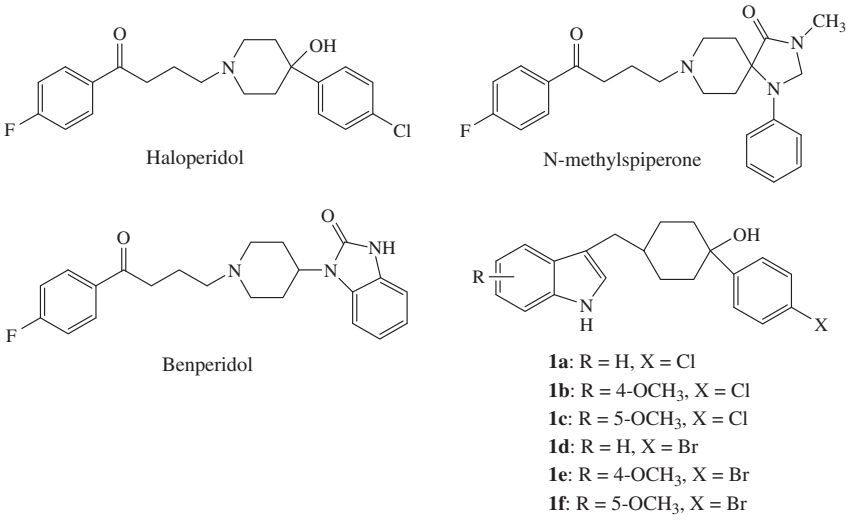
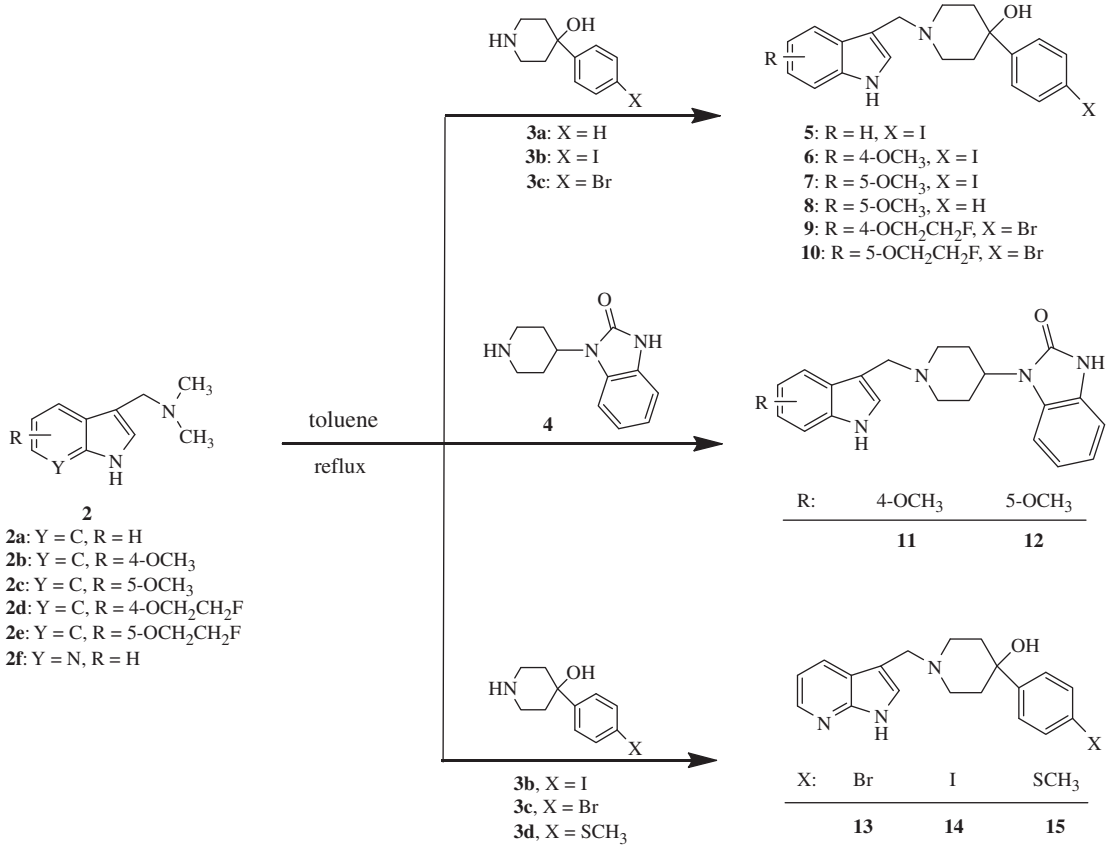


Figure 1. Structures of the lead compounds for the development of D_2-selective radiotracers.

a D_2-selective antagonist,[24] were made and shown to have selectivity for D_2 over D_3 and D_4 receptors[25,26] (Fig. 1). The current study involved the following strategies: (a) continuation of the previous study by making additional structural congeners of **1a**; (b) replacement of the indole ring with other heteroaromatic ring systems such as 7-azaindole, benzofuran, and benzothiophene; and (c) the development of fluorine-containing analogs which could serve as potential radiotracers for imaging dopamine D_2 versus D_3 receptors with positron emission tomography (PET).

2. Chemistry

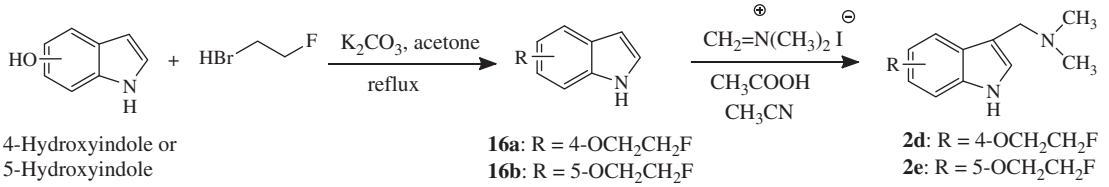
Compounds **5**–**15** were synthesized by treating the respective gramine derivatives **2a**–**f** with the appropriate pyridine derivatives **3a**–**d** and **4** in refluxing toluene (Method A, Scheme 1). Gramine and 5-methoxygramine are commercially available. The gramines **2d** and **2e** were obtained by alkylation of 4-hydroxyindole or 5-hydroxyindole with 1-bromo-2-fluoroethane, followed by reacting with *N*,*N*-dimethylmethyleneammonium iodide



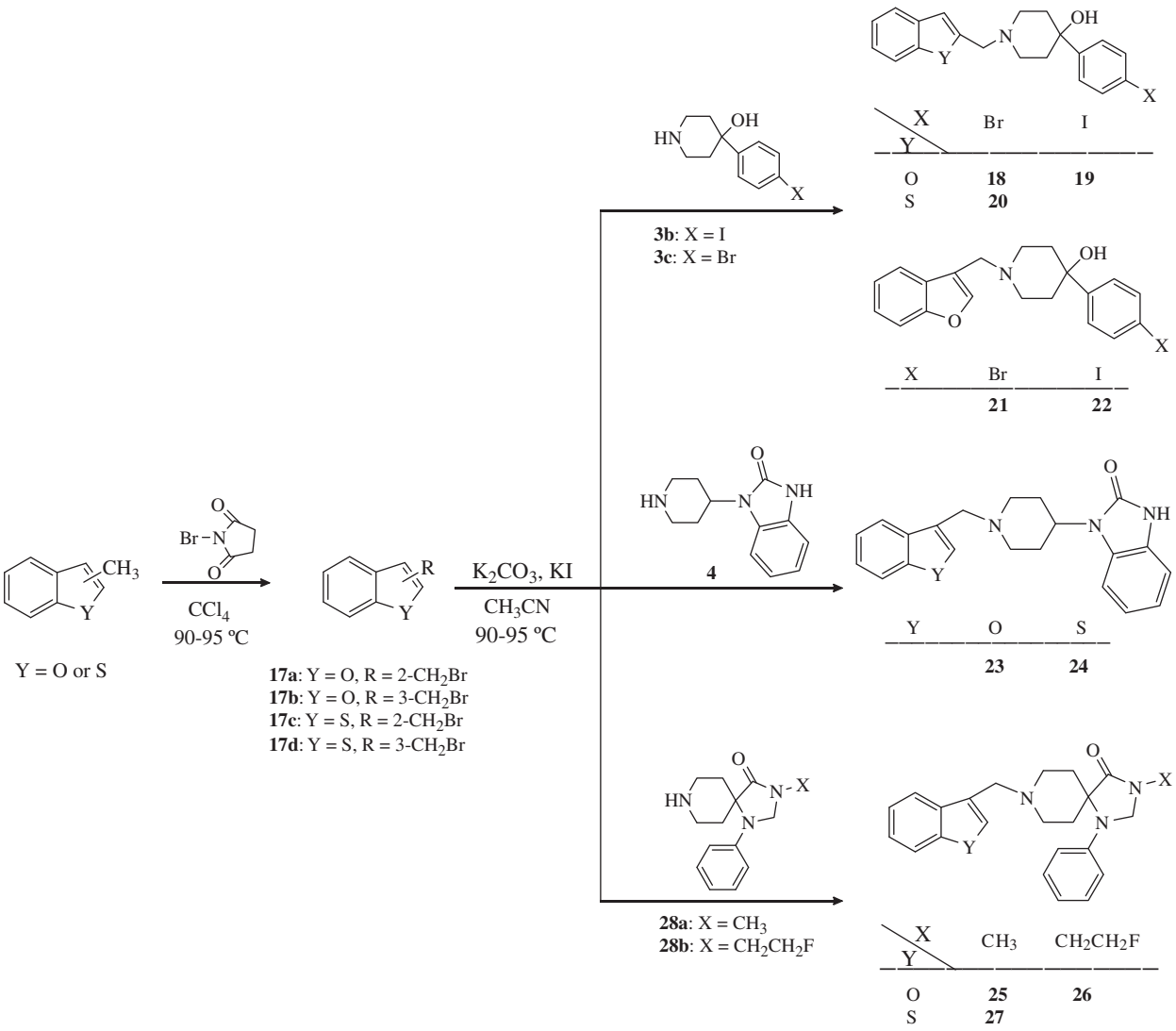
Scheme 1. Method A.

(Eschenmoser's salt) (Scheme 2). The 4-methoxygramine, **2b**, and 7-azagramine, **2f**, were synthesized by treating 4-methoxyindole and 7-azagramine with *N*,*N*-dimethylmethyleneammonium iodide, respectively.

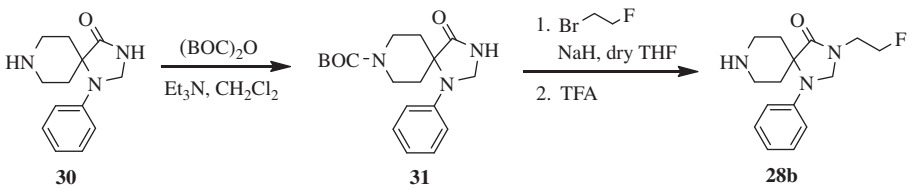
Compounds **18**–**27** were synthesized by reacting the respective bromomethylbenzofurans (**17a**–**b**) or bromomethylbenzo[*b*]thiophenes (**17c**–**d**) with the appropriate piperidines in the presence of potassium carbonate and potassium iodide in acetonitrile



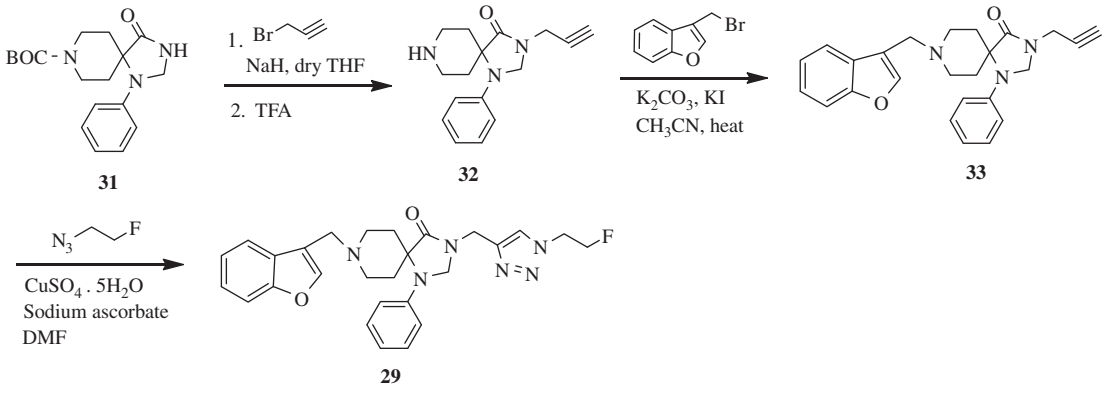
Scheme 2.



Scheme 3. Method B.



Scheme 4.



31 32 33 29

Scheme 5.

(Method B, Scheme 3). The required bromomethyl derivatives were made by refluxing benzofurans or benzo[*b*]thiophenes with *N*-bromosuccinimide in carbon tetrachloride. The piperidine **28b** was prepared from 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**30**). First, the N-protected derivative **31** was synthesized and alkylated with 1-bromo-2-fluoroethane, followed by deprotection with trifluoroacetic acid to give the required piperidine **28b** (Scheme 4). Compound **29** was synthesized via alkylation of **31** with propargyl bromide, followed by deprotection with trifluoroacetic acid to give **32**. N-alkylation of **32** with freshly prepared 3-bromomethylbenzofuran **17b**, followed by reaction with 1-azido-2-fluoroethane in the presence of sodium ascorbate and copper sulfate gave the triazole analog, **29** (Scheme 5).

3. Radioligand binding studies at dopamine receptors

Competitive radioligand binding studies were performed to determine the equilibrium dissociation constants of each compound at human D_2, D_3 and D_4 dopamine receptors (Table 1). For these studies, tissue homogenates from stably transfected

Table 1
Binding affinities for dopamine D_2/D_3 and sigma σ_1/σ_2 receptors

Compound	K_i (nM)[a]						
	D_2[b]	D_3[c]	D_4[d]	D_3/D_2[e]	σ_1[f]	σ_2[g]	Log *P*[h]
5	3.8 ± 0.8	94.4 ± 1.2	53.4 ± 15.2	25	2.00 ± 0.52	2200 ± 437	1.98
6	0.9 ± 0.1	122 ± 2.0	60.4 ± 18.7	136	881 ± 154	970 ± 178	2.60
7	0.9 ± 0.1	100 ± 13	129 ± 5.1	112	197 ± 26	3262 ± 387	2.54
8	1396 ± 317	2927 ± 198	>10,000	2.1	1527 ± 158	2761 ± 574	1.43
9	27.7 ± 6.6	849 ± 164	668 ± 22	31	3224 ± 479	9510 ± 1020	2.61
10	13.8 ± 2.5	188 ± 7.1	344 ± 49	14	227 ± 63	10,420 ± 1512	2.54
11	23.3 ± 3.1	132 ± 32	1390 ± 108	5.7	4129 ± 382	16,134 ± 2968	2.54
12	12.5 ± 2.0	68.2 ± 16	2230 ± 484	5.5	1768 ± 303	27,230 ± 5205	2.45
13	212 ± 25	2983 ± 305	620 ± 72	14	15.1 ± 2.3	1.90 ± 0.15	2.20
14	61.0 ± 4.9	1585 ± 380	634 ± 85	26	1.20 ± 0.51	5.53 ± 2.37	2.46
15	254 ± 40	4938 ± 780	937 ± 145	20	8.07 ± 2.00	7.75 ± 1.24	1.85
18	17.3 ± 0.6	31.8 ± 4.1	254 ± 5.3	1.8	2.45 ± 0.78	1398 ± 98	3.19
19	14.3 ± 1.7	49.0 ± 5.6	245 ± 11	3.4	1.41 ± 0.41	2368 ± 52	3.45
20	46.8 ± 3.0	142 ± 27	377 ± 47	3.0	2.13 ± 0.44	1964 ± 177	4.97
21	6.6 ± 1.2	10.9 ± 0.5	94.5 ± 6.4	1.7	0.59 ± 0.15	377 ± 20	3.12
22	2.4 ± 0.7	11.2 ± 1.2	40.4 ± 10.0	4.7	0.51 ± 0.13	393 ± 54	3.38
23	13.6 ± 3.6	3.0 ± 0.7	163 ± 11.9	4.5	1779 ± 256	18,054 ± 3531	3.39
24	37.8 ± 6.0	46.7 ± 4.8	72.6 ± 0.8	1.2	1879 ± 200	14,575 ± 1168	4.73
25	7.1 ± 1.3	2.1 ± 0.5	492 ± 103	0.3	708 ± 141	24,879 ± 4201	2.02
26	8.0 ± 1.9	9.3 ± 2.2	945 ± 162	1.2	28 ± 8	10,164 ± 641	2.38
27	71.4 ± 14	60.1 ± 17	352 ± 72	0.8	331 ± 87	20,248 ± 7658	3.36
29	2.0 ± 0.1	0.9 ± 0.2	978 ± 11.9	0.5	802 ± 227	22,028 ± 3297	1.46
Haloperidol	1.1 ± 0.1	12.7 ± 3.9	ND[i]	12	1.45 ± 0.33	24.2 ± 3.0	4.50
1a[j]	10.0 ± 2.5	104 ± 19	449 ± 123	10	19.3 ± 3.6	1811 ± 569	1.54
1b[j]	4.2 ± 0.4	250 ± 6	>2000	59	>5000	1246 ± 234	2.10
1c[j]	4.2 ± 0.8	204 ± 60	>3500	49	242 ± 68	1900 ± 241	2.16
1d[j]	4.8 ± 0.3	110 ± 39	652 ± 7	28	12.1 ± 2.5	2134 ± 356	1.72
1e[j]	2.3 ± 0.7	190 ± 34	840 ± 197	82	2557 ± 334	943 ± 64	2.34
1f[j]	2.5 ± 0.7	96.9 ± 6.1	700 ± 80	39	108 ± 17	2702 ± 696	2.28

[a] Mean ± SEM, K_i values were determined by at least three experiments.
[b] K_i values for D_2 receptors were measured on human $D_{2(long)}$ expressed in HEK 293 cells using [^{125}I]ABN as the radioligand.
[c] K_i values for D_3 receptors were measured on human D_3 expressed in HEK 293 cells using [^{125}I]ABN as the radioligand.
[d] K_i values for D_4 receptors were measured on human D_4 expressed in HEK 293 cells using [^{125}I]ABN as the radioligand.
[e] K_i for D_3 receptors/K_i for D_2 receptors.
[f] K_i for inhibiting the binding of [^{3}H](+)-pentazocine to guinea pig brain homogenates.
[g] K_i for inhibiting the binding of [^{3}H]DTG to rat liver homogenates.
[h] Calculated value using the program *C* log *P*.
[i] Not determined.
[j] Data from Ref. 25.

HEK 293 cells were used in conjunction with the radioligand ^{125}I-IABN. We have previously reported that the benzamide ^{125}I-IABN binds with high affinity and selectively to $D_{2\text{-like}}$ dopamine receptors, but that it binds non-selectively to D_2 versus D_3 dopamine receptor subtypes.[27]

First, a comparison of indole analogs structurally related to haloperidol was made in which a substituent was added at the 4- or 5-position of the indolyl moiety. We found that the addition of the methoxy group resulted in a fourfold increase in affinity at D_2 receptors but did not change the affinity at D_3 receptors, resulting in compounds that were 110- to 140-fold selective at D_2 receptors (**7** and **6**). The addition of 2-fluoroethoxy group resulted in a 3- to 6-fold decrease in affinity at D_2 receptors and a 2- to 8-fold decrease in affinity at D_3 receptors; thus compounds **10** and **9** resulted in no significant difference in selectivity compared to the unsubstituted analog (**1d**[25]). The halide substituents on the phenyl-piperidinol moiety are pivotal for high affinity binding at the dopamine D_2 receptors and for selectivity over D_3 receptors. The iodide analogs (**6** and **7**) have higher affinity and selectivity at D_2 versus D_3 dopamine receptors than the chloride and bromide analogs (**1b**, **1c**, **1e** and **1f**[25]). In addition, the affinity at both D_2 and D_3 dopamine receptors decreases dramatically in the unsubstituted analog, **8**. The 7-azaindole analogs (**13**–**15**) bind with 14- to 26-fold selectivity for D_2 over D_3 receptors. The benzofuran (**18**, **19**, **21**, **22**, **23**, **25**, **26** and **29**) and benzothiophene (**20**, **24**, and **27**) analogs bind non-selectively to the D_2 over D_3 dopamine receptors. Similar results for benzofuran analogs of L-741,626 were previously reported by Grundt et al.[26]

4. Adenylyl cyclase studies with $D_{2\text{-like}}$ receptors

The intrinsic efficacy of the two compounds exhibiting the greatest D_2 versus D_3 receptor subtype binding selectivity (**6** and **7**) was evaluated using a forskolin-dependent adenylyl cyclase inhibition assay (Fig. 2). The $D_{2\text{-like}}$ receptor full agonist quinpirole was used as a reference compound. At a dose of 10 nM, quinpirole administration resulted in >70% inhibition of cAMP accumulation, whereas at the same dose of compounds **6** and **7** no appreciable increase or decrease in forskolin-dependent adenylyl cyclase activation was observed. In addition, both **6** and **7** were able to attenuate the effect of quinpirole. Based upon these results, we categorize compounds **6** and **7** as neutral antagonists at D_2 dopamine receptors. These results are consistent with our previous studies on this class of methoxy substituted indole piperidines.[25]

5. Radioligand binding studies at sigma receptors

In vitro binding studies were conducted to determine the affinity of the target compounds at sigma-1 (σ_1) and sigma-2 (σ_2) receptors. The σ_1 binding studies were conducted using the σ_1-selective radioligand, [^{3}H](+)-pentazocine in guinea pig brain membranes; σ_2 sites were assayed in rat liver membranes with [^{3}H]DTG in the presence of 100 nM unlabeled (+)-pentazocine to mask σ_1 sites, or with the σ_2 selective ligand [^{3}H]RHM-1, alone.[28,29] Although haloperidol is a dopaminergic antagonist that is used clinically as a neuroleptic, its high affinity at sigma receptors has precluded its usefulness as a radioligand for in vitro or in vivo radioligand binding studies. Almost all of the compounds in this study bound with low affinity (>350 nM) at σ_2 receptors with the exception of compound **13**, which has a nanomolar (nM) affinity for σ_2 receptors and a sevenfold selectivity for σ_2 versus σ_1 receptors. The affinity at σ_1 receptors varied from 0.5 nM to >4000 nM. The 7-azaindole analogs have high affinity at both σ_1 and σ_2 receptors, whereas the benzofuran analogs **21** and **22** have a high affinity and selectivity for σ_1 versus σ_2 receptors.

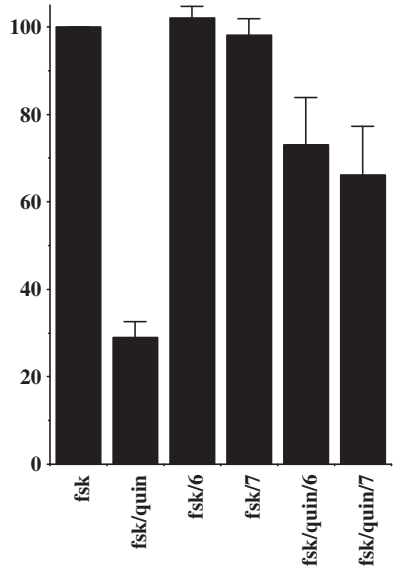


Figure 2. Evaluation of the intrinsic efficacy of compounds **6** and **7** using a Forskolin-dependent adenylyl cyclase inhibition assay. The intrinsic efficacy of compounds **6** and **7** was evaluated by determining the percent inhibition of a forskolin-dependent adenylyl cyclase assay with human D_{2long} receptors expressed in stably transfected HEK 293 cells. The extent of inhibition of the two test compounds was compared to the percent inhibition obtained using the full agonist quinpirole (quin) at a concentration of 10 nM. At this concentration of quinpirole the mean ± S.E.M. inhibition of forskolin (fsk) stimulation was 71 ± 3.6 percent. The test drugs were tested at a final concentration equal to 10 nM, either in the absence or presence of quinpirole. The bar graph represents the mean ± the S.E.M. values obtained for $n = 4$.

6. Discussion

The current study is a continuation of our effort to develop ligands having a high affinity and selectivity for D_2 versus D_3 dopamine receptors. In previous studies, we prepared a number of structural analogs of the classical $D_{2\text{-like}}$ dopamine receptor antagonist, haloperidol, having a moderate to high affinity at D_2 versus D_3 receptors.[25] We have expanded this initial structure–activity relationship (SAR) study and have identified two iodine-containing derivatives of the methoxy substituted indoles (**6** and **7**), which (a) bind at D_2 receptors with nanomolar affinity and (b) have >100-fold selectivity for human D_2 receptors compared to the human D_3 dopamine receptor subtype. These two analogs were also found to bind with low affinity at the σ_1 and σ_2 receptors. The binding profiles of **6** and **7** indicate that they are the most potent and selective D_2-antagonists reported to date. The lipophilicities (log *P*) of **6** and **7** (Table 1) also suggest that they will readily cross the blood–brain barrier and are good candidates for the development of D_2 receptor selective imaging agents for the functional imaging technique, PET. The iodo substituent on the phenyl-piperidinol moiety was found to be pivotal for high affinity binding at the dopamine D_2 receptors and for selectivity over D_3 receptors. The substitution at the 4- or 5-position on the indolyl moiety also affects the affinity binding and selectivity. The 2-fluoroethoxy substituted indoles (**9** and **10**) have lower affinity and selectivity for D_2 over D_3 compared to the methoxy substituted indoles (**1e** and **1f**[25]). However, the minor reduction in affinity in going from the methoxy group to the 2-fluoroethoxy group (compare **10** to **1f**) suggests that this may be a useful strategy for preparing a fluorine-18 radiotracer for imaging D_2 versus D_3 receptors.

We have also examined heteroatom replacements in the indole ring. The 7-azaindole analogs have lower affinity at both D_2 and D_3 dopamine receptors than the corresponding indole analogs. The benzofuran and benzothiophene analogs bind non-selectively to both D_2 and D_3 dopamine receptors. The interesting SAR at σ_1

and σ_2 receptors of **13**–**15** and **18**–**22** may provide useful information for molecular modeling studies of sigma receptors.[30,31]

7. Experimental

7.1. Chemical analysis

^{1}H NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals: br s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, s = singlet. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA and were within ±0.4% of the calculated values. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). All reactions were carried out under an inert atmosphere of nitrogen. Lipophilicity measurements of the compounds were estimated using the computational program, *C* log *P* (Advanced Chemistry Development, Inc., Toronto, Canada).

7.2. General procedure: Method A

A mixture of gramine derivatives (4.0 mmol) and appropriate amines (4.8 mmol) in toluene (15 mL) was stirred at reflux overnight. The volatile components were evaporated and the resulting residue was purified by silica gel column chromatography (dichloromethane–methanol–ammonium hydroxide, 90:10:0.5) to afford the target compounds. The oxalate salt was prepared using one equivalent of oxalic acid in ethyl acetate.

7.3. General procedure : Method B

A mixture of methylbenzofurans, or methylbenzo[*b*]thiophenes (4.0 mmol) and *N*-bromosuccinimide (NBS, 4.4 mmol) in carbon tetrachloride was heated at reflux overnight. After cooling to room temperature, the reaction mixture was filtered and evaporated in vacuo to give the corresponding bromomethyl analogs. These crude bromomethyl analogs were treated with the appropriate amine (4.0 mmol) in the presence of K_2CO_3 and KI in acetonitrile at reflux overnight. After cooling to room temperature, the reaction mixture was filtered and evaporated in vacuo. Column chromatography (5% methanol in dichloromethane) of the resulting residue gave the desired compounds. The oxalate salt was prepared using one equivalent of oxalic acid in ethyl acetate.

7.4. 1-((1*H*-Indol-3-yl)methyl)-4-(4-iodophenyl)piperidin-4-ol oxalate (5)

Method A. Yield 56% from gramine (**2a**) and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt gave **5** as an off-white powder, mp 149–150 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.16 (br s, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.14–7.24 (m, 3H), 3.82 (s, 2H), 2.90–2.94 (m, 2H), 2.47–2.55 (m, 2H), 2.08–2.18 (m, 2H), 1.69–1.74 (m, 2H). Anal. ($C_{20}H_{21}IN_2O{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.5. 4-(4-Iodophenyl)-1-((4-methoxy-1*H*-indol-3-yl)methyl)-piperidin-4-ol oxalate (6)

Method A. Yield 52% from 4-methoxygramine (**2b**) and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt gave **6** as an off-white powder, mp 129–130 °C (dec); ^{1}H NMR (free base, $CDCl_3$ + CD_3OD) δ 7.63 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.96–7.08 (m, 3H), 6.49 (d, *J* = 7.5 Hz, 1H), 4.04 (s, 2H), 3.91 (s, 3H), 2.90–2.94 (m, 2H), 2.59–2.65 (m, 2H), 2.06–2.16 (m, 2H), 1.65–1.69 (m, 2H). Anal. ($C_{21}H_{23}IN_2O_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$) C, H, N.

7.6. 4-(4-Iodophenyl)-1-((5-methoxy-1*H*-indol-3-yl)methyl)-piperidin-4-ol oxalate (7)

Method A. Yield 45% from 5-methoxygramine (**2c**) and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt gave **7** as a tan powder, mp 121–122 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.30 (br s, 1H), 7.65 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 2.3 Hz, 2H), 6.88 (dd, *J* = 9.0 and 2.3 Hz, 1H), 3.89 (s, 2H), 3.87 (s, 3H), 2.99–3.03 (m, 2H), 2.63–2.71 (m, 2H), 2.13–2.29 (m, 2H), 1.69–1.73 (m, 2H). Anal. ($C_{21}H_{23}IN_2O_2{\cdot}C_2H_2O_4{\cdot}1.25H_2O$) C, H, N.

7.7. 1-((5-Methoxy-1*H*-indol-3-yl)methyl)-4-phenylpiperidin-4-ol oxalate (8)

Method A. Yield 93% from 5-methoxygramine (**2c**) and 4-hydroxy-4phenylpiperidine (**3a**). Conversion to the oxalate salt gave **8** as an off-white powder, mp 147–148 °C (dec). ^{1}H NMR (free base, $CDCl_3$) δ 8.17 (br s, 1H), 7.48–7.52 (m, 2H), 7.32–7.37 (m, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.22 (d, *J* = 2.4 Hz, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 6.87 (dd, *J* = 8.8 and 2.4 Hz, 1H), 3.88 (s, 3H), 3.79 (s, 2H), 2.90–2.94 (m, 2H), 2.51–2.60 (m, 2H), 2.14–2.24 (m, 2H), 1.73–1.78 (m, 2H), 1.66 (br s, 1H). Anal. ($C_{21}H_{24}N_2O_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$) C, H, N.

7.8. 4-(4-Bromophenyl)-1-((4-(2-fluoroethoxy)-1*H*-indol-3-yl)-methyl)piperidin-4-ol oxalate (9)

Method A. Yield 43% from 4-(2-fluoroethoxy)gramine (**2d**) and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **9** as an off-white powder, mp 179–180 °C (dec); ^{1}H NMR (free base, $CDCl_3$ + CD_3OD) δ 7.44 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.17 (s, 1H), 7.01–7.10 (m, 2H), 6.46–6.49 (m, 1H), 4.93–4.96 (m, 1H), 4.77–4.80 (m, 1H), 4.38–4.41 (m, 1H), 4.29–4.31 (m, 1H), 4.12(s, 2H), 2.96–3.00 (m, 2H), 2.69–2.76 (m, 2H), 2.11–2.21 (m, 2H), 1.68–1.72 (m, 2H). Anal. ($C_{22}H_{24}BrFN_2O_2{\cdot}$–$C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

7.9. 4-(4-Bromophenyl)-1-((5-(2-fluoroethoxy)-1*H*-indol-3-yl)-methyl)piperidin-4-ol oxalate (10)

Method A. Yield 27% from 5-(2-fluoroethoxy)gramine (**2e**) and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **10** as a tan powder, mp 163–164 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.18 (br s, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 2H), 7.17–7.29 (m, 3H), 6.91 (dd, *J* = 8.8 and 2.5 Hz, 1H), 4.79 (dt, *J* = 47.5 and 4.1 Hz, 2H), 4.28 (dt, *J* = 28.1 and 4.1 Hz, 2H), 3.77 (s, 2H), 2.88–2.92 (m, 2H), 2.48–2.56 (m, 2H), 2.08–2.18 (m, 2H), 1.68–1.72 (m, 2H). Anal. ($C_{22}H_{24}BrFN_2O_2{\cdot}$–$C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

7.10. 4-(2-Fluoroethoxy)gramine (2d)

A mixture of 4-hydroxyindole (1 equiv), 1-bromo-2-fluoroethane (5 equiv) and potassium carbonate (4 equiv) in acetone was heated at reflux overnight. The reaction mixture was cooled down, filtered and concentrated. Purification by silica gel column chromatography (hexane–ethyl acetate, 3:1) gave 4-(2-fluoroethoxy)indole (**16a**) (99%). ^{1}H NMR ($CDCl_3$) δ 8.16 (br s, 1H),

7.04–7.13 (m, 3H), 6.69–6.71 (m, 1H), 6.52 (d, J = 7.6 Hz, 1H), 4.90–4.93 (m, 1H), 4.74–4.77 (m, 1H), 4.41–4.44 (m, 1H), 4.31–4.34 (m, 1H). A solution of **16a** (1 equiv) in acetonitrile was slowly added to a solution of *N,N*-dimethylmethyleneammonium iodide (1.2 equiv) in acetonitrile and acetic acid (2:1). The solution was stirred for 3 h and partitioned between 2-propanol and chloroform (1:3) and 10% aqueous sodium hydroxide. The aqueous layer was extracted with the same organic solvent. The organic layers were combined, dried, and evaporated to give **2d** as a tan solid (51%). This was used without further purification. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.07–7.17 (m, 3H), 6.48–6.51 (m, 1H), 4.94–4.97 (m, 1H), 4.78–4.81 (m, 1H), 4.38–4.41 (m, 1H), 4.29–4.31 (m, 1H), 4.23 (s, 2H), 2.53 (s, 6H).

7.11. 5-(2-Fluoroethoxy)gramine (2e)

Preparation according to the method described for **2d** afforded **2e** (83% from 5-hydroxyindole) as a yellow solid which was used without further purification. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.27–7.33 (m, 1H), 7.13–7.17 (m, 2H), 6.87–6.91 (m, 1H), 4.85–4.88 (m, 1H), 4.69–4.72 (m, 1H), 4.32–4.35 (m, 1H), 4.22–4.25 (m, 1H), 3.63 (s, 2H), 2.29 (s, 6H).

7.12. 1-(1-((4-Methoxy-1*H*-indol-3-yl)methyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one oxalate (11)

Method A. Yield 60% from 4-methoxygramine (**2b**) and 4-(2-keto-1-benzimidazolinyl)piperidine (**4**). Conversion to the oxalate salt gave **11** as an off-white powder, mp 209–210 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.73 (s, 1H), 8.31 (s, 1H), 6.97–7.35 (m, 7H), 6.53 (d, J = 7.6 Hz, 1H), 4.34–4.42 (m, 1H), 4.11 (s, 2H), 3.95 (s, 3H), 3.26–3.30 (m, 2H), 2.54–2.60 (m, 2H), 2.38–2.44 (m, 2H), 1.78–1.83 (m, 2H). Anal. ($C_{22}H_{24}N_4O{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.13. 1-(1-((5-Methoxy-1*H*-indol-3-yl)methyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one oxalate (12)

Method A. Yield 87% from 5-methoxygramine (**2c**) and 4-(2-keto-1-benzimidazolinyl)piperidine (**4**). Conversion to the oxalate salt gave **12** as a tan powder, mp 197–198 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 9.77 (s, 1H), 8.18 (s, 1H), 7.00–7.25 (m, 7H), 6.87 (dd, J = 8.8 and 2.4 Hz, 1H), 4.33–4.42 (m, 1H), 3.90 (s, 3H), 3.77 (s, 2H), 3.16–3.19 (m, 2H), 2.44–2.55 (m, 2H), 2.19–2.27 (m, 2H), 1.78–1.82 (m, 2H). Anal. ($C_{22}H_{24}N_4O{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.14. 1-((1*H*-Pyrrolo[2,3-*b*]pyridine-3-yl)methyl)-4-(4-bromophenyl)piperidin-4-ol oxalate (13)

Method A. Yield 75% from 7-azagramine (**2f**) and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **13** as a off-white powder, mp 208–209 °C; ^{1}H NMR (free base, $CDCl_3$ + CD_3OD) δ 8.22 (dd, J = 4.8 and 1.4 Hz, 1H), 8.11 (dd, J = 8.0 and 1.4 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.41 (s, 1H), 7.37 (d, J = 8.8 Hz, 2H), 7.13 (dd, J = 8.0 and 4.8 Hz, 1H), 3.88 (s, 2H), 2.92–2.96 (m, 2H), 2.66–2.74 (m, 2H), 2.10–2.20 (m, 2H), 1.73–1.78 (m, 2H). Anal. ($C_{19}H_{20}BrN_3O{\cdot}C_2H_2O_4{\cdot}2H_2O$) C, H, N.

7.15. 1-((1*H*-Pyrrolo[2,3-*b*]pyridine-3-yl)methyl)-4-(4-iodophenyl)piperidin-4-ol oxalate (14)

Method A. Yield 48% from 7-azagramine (**2f**) and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt gave **14** as a white powder, mp 187–188 °C; ^{1}H NMR (free base, $CDCl_3$ + CD_3OD) δ 8.26 (dd, J = 4.9 and 1.4 Hz, 1H), 8.14 (dd, J = 8.0 and 1.4 Hz, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.61 (s, 1H), 7.25 (d, J = 8.7 Hz, 2H), 7.18 (dd, J = 8.0 and 4.9 Hz, 1H), 4.17 (s, 2H), 3.00–3.22 (m, 4H), 2.30–2.42 (m, 2H), 1.78–1.83 (m, 2H). Anal. ($C_{19}H_{20}IN_3O{\cdot}C_2H_2O_4{\cdot}2.5H_2O$) C, H, N.

7.16. 1-((1*H*-Pyrrolo[2,3-*b*]pyridine-3-yl)methyl)-4-(4-(methylthio)phenyl)piperidin-4-ol oxalate (15)

Method A. Yield 55% from 7-azagramine (**2f**) and 4-(4-methylthiophenyl)-4-hydroxypiperidine (**3d**). Conversion to the oxalate salt gave **15** as a tan powder, mp 191–192 °C (dec); ^{1}H NMR (free base, $CDCl_3$ + CD_3OD) δ 8.21 (dd, J = 4.8 and 1.4 Hz, 1H), 8.10 (dd, J = 8.0 and 1.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.41 (s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.13 (dd, J = 8.0 and 4.8 Hz, 1H), 3.84 (s, 2H), 2.86–2.92 (m, 2H), 2.60–2.70 (m, 2H), 2.47 (s, 3H), 2.05–2.18 (m, 2H), 1.74–1.80 (m, 2H). Anal. ($C_{20}H_{23}N_3OS{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.17. 1-(Benzofuran-2-ylmethyl)-4-(4-bromophenyl)piperidin-4-ol oxalate (18)

Method B. Yield 16% from 2-methylbenzofuran and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **18** as an off-white powder, mp 174–175 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.49–7.54 (m, 2H), 7.47 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.18–7.29 (m, 2H), 6.63 (s, 1H), 3.76 (s, 2H), 2.87–2.91 (m, 2H), 2.52–2.61 (m, 2H), 2.14–2.24 (m, 2H), 1.69–1.75 (m, 2H). Anal. ($C_{20}H_{20}BrNO_2{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.18. 1-(Benzofuran-2-ylmethyl)-4-(4-iodophenyl)piperidin-4-ol oxalate (19)

Method B. Yield 61% from 2-methylbenzofuran and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt gave **19** as an off-white powder, mp 199–200 °C (dec). ^{1}H NMR (free base, $CDCl_3$) δ 7.67 (d, J = 8.7 Hz, 2H), 7.48–7.55 (m, 2H), 7.26 (d, J = 8.7 Hz, 2H), 7.18–7.29 (m, 2H), 6.62 (s, 1H), 3.76 (s, 2H), 2.87–2.90 (m, 2H), 2.51–2.60 (m, 2H), 2.13–2.23 (m, 2H), 1.69–1.74 (m, 2H). Anal. ($C_{20}H_{20}INO_2{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

7.19. 1-(Benzo[*b*]thiophen-2-ylmethyl)-4-(4-bromophenyl)-piperidin-4-ol oxalate (20)

Method B. Yield 69% from 2-methylbenzo[*b*]thiophene and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **20** as a white powder, mp 225–226 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.78–7.81 (m, 1H), 7.68–7.71 (m, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.27–7.34 (m, 2H), 7.17 (s, 1H), 3.86 (s, 2H), 2.87–2.91 (m, 2H), 2.49–2.58 (m, 2H), 2.10–2.20 (m, 2H), 1.69–1.75 (m, 2H). Anal. ($C_{20}H_{20}BrNOS{\cdot}C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

7.20. 1-(Benzofuran-3-ylmethyl)-4-(4-bromophenyl)piperidin-4-ol oxalate (21)

Method B. Yield 22% from 3-methylbenzofuran and 4-(4-bromophenyl)-4-hydroxypiperidine (**3c**). Conversion to the oxalate salt gave **21** as an off-white powder, mp 202–203 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.72–7.76 (m, 1H), 7.57 (s, 1H), 7.48–7.50 (m, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 7.23–7.33 (m, 2H), 3.72 (s, 2H), 2.85–2.89 (m, 2H), 2.46–2.55 (m, 2H), 2.06–2.16 (m, 2H), 1.69–1.74 (m, 2H). Anal. ($C_{20}H_{20}BrNO_2{\cdot}C_2H_2O_4$) C, H, N.

7.21. 1-(Benzofuran-3-ylmethyl)-4-(4-iodophenyl)piperidin-4-ol oxalate (22)

Method B. Yield 33% from 3-methylbenzofuran and 4-(4-iodophenyl)-4-hydroxypiperidine (**3b**). Conversion to the oxalate salt

gave **22** as a white powder, mp 198–199 °C; ^{1}H NMR (free base, $CDCl_3$) δ 7.72–7.75 (m, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.46–7.50 (m, 1H), 7.25 (d, J = 8.7 Hz, 2H), 7.24–7.33 (m, 2H), 3.72 (s, 2H), 2.84–2.88 (m, 2H), 2.45–2.54 (m, 2H), 2.05–2.16 (m, 2H), 1.68–1.74 (m, 2H). Anal. ($C_{20}H_{20}INO_2 \cdot C_2H_2O_4$) C, H, N.

7.22. 1-(1-(Benzofuran-3-ylmethyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2-(3*H*)-one oxalate (23)

Method B. Yield 22% from 3-methylbenzofuran and 4-(2-keto-1-benzimidazolinyl)piperidine (**4**). Conversion to the oxalate salt gave **23** as a white powder, mp 244–245 °C; ^{1}H NMR (free base, $CDCl_3$) δ 9.72 (s, 1H), 7.76–7.79 (m, 1H), 7.58 (s, 1H), 7.48–7.51 (m, 1H), 7.28–7.35 (m, 3H), 7.03–7.12 (m, 3H), 4.33–4.41 (m, 1H), 3.72 (s, 2H), 3.12–3.16 (m, 2H), 2.42–2.54 (m, 2H), 2.20–2.27 (m, 2H), 1.80–1.84 (m, 2H). Anal. ($C_{21}H_{21}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N.

7.23. 1-(1-(Benzo[*b*]thiophen-3-ylmethyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2-(3*H*)-one oxalate (24)

Method B. Yield 63% from 3-methylbenzo[*b*]thiophene and 4-(2-keto-1-benzimidazolinyl)piperidine (**4**). Conversion to the oxalate salt gave **24** as a pale yellow powder, mp 242–243 °C; ^{1}H NMR (free base, $CDCl_3$) δ 9.92 (s, 1H), 8.01–8.04 (m, 1H), 7.86–7.89 (m, 1H), 7.34–7.46 (m, 3H), 7.23–7.26 (m, 1H), 7.03–7.12 (m, 3H), 4.36–4.44 (m, 1H), 3.82 (s, 2H), 3.12–3.16 (m, 2H), 2.43–2.54 (m, 2H), 2.21–2.29 (m, 2H), 1.79–1.84 (m, 2H). Anal. ($C_{21}H_{21}N_3OS \cdot C_2H_2O_4$) C, H, N.

7.24. 8-(Benzofuran-3-ylmethyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (25)

Method B. Yield 34% from 3-methylbenzofuran and 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**28a**). Conversion to the oxalate salt gave **25** as an off-white powder, mp 209–210 °C; ^{1}H NMR (free base, $CDCl_3$) δ 7.81–7.84 (m, 1H), 7.57 (s, 1H), 7.46–7.49 (m, 1H), 7.27–7.33 (m, 4H), 6.82–6.92 (m, 3H), 4.66 (s, 2H), 3.72 (s, 2H), 2.99 (s, 3H), 2.87–2.90 (m, 4H), 2.65–2.73 (m, 2H), 1.62–1.67 (m, 2H). Anal. ($C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N.

7.25. 8-(Benzofuran-3-ylmethyl)-3-(2-fluoroethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (26)

Method B. Yield 31% from 3-methylbenzofuran and 3-(2-fluoroethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**28b**). Conversion to the oxalate salt gave **26** as a white powder, mp 175–176 °C; ^{1}H NMR (free base, $CDCl_3$) δ 7.80–7.83 (m, 1H), 7.56 (s, 1H), 7.45–7.49 (m, 1H), 7.27–7.33 (m, 4H), 6.84–6.94 (m, 3H), 4.79 (s, 2H), 4.64 (dt, J = 47.2 and 4.6 Hz, 2H), 3.73 (dt, J = 28.6 and 4.6 Hz, 2H), 3.71 (s, 2H), 2.80–2.89 (m, 4H), 2.62–2.73 (m, 2H), 1.65–1.70 (m, 2H). Anal. ($C_{24}H_{26}FN_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

7.26. 8-(Benzo[*b*]thiophen-3-ylmethyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (27)

Method B. Yield 37% from 3-methylbenzo[*b*]thiophene and 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**28a**). Conversion to the oxalate salt gave **27** as a white powder, mp 199–200 °C; ^{1}H NMR (free base, $CDCl_3$) δ 8.08–8.11 (m, 1H), 7.85–7.87 (m, 1H), 7.28–7.44 (m, 5H), 6.81–6.91 (m, 3H), 4.66 (s, 2H), 3.83 (s, 2H), 2.99(s, 3H), 2.88–2.91 (m, 4H), 2.65–2.76 (m, 2H), 1.62–1.66 (m, 2H). Anal. ($C_{23}H_{25}N_3OS \cdot C_2H_2O_4$) C, H, N.

7.27. 3-(2-Fluoroethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (28b)

A solution of di-*tert*-butyl dicarbonate ($(BOC)_2O$, 7.0 mmol) in dichloromethane was added in a solution of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**30**, 6.5 mmol) and triethylamine (7.0 mmol) in dichloromethane. The reaction mixture was stirred at room temperature for 3 h. After the work-up, the N-protected compound **31** was obtained. Sodium hydride (15.8 mmol) and 1-bromo-2-fluoroethane (7.0 mmol) were added in a cold solution of **31** (4.5 mmol) in anhydrous *N*,*N*-dimethylformamide. The reaction mixture was stirred at room temperature for 4 h. The obtained product was treated with trifluoroacetic acid to give **28b**, which was used without further purification.

7.28. 8-(Benzofuran-3-ylmethyl)-3-((1-(2-fluoroethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (29)

Sodium hydride (2.5 equiv) and propargyl bromide (1.5 equiv) were added into a cold solution of **31** (1.0 equiv) in anhydrous tetrahydrofuran. The reaction mixture was stirred at ambient temperature for 4 h and washed with water. Evaporation of the organic layer gave the product which was treated with trifluoroacetic acid to give **32**. Using the general method B, **33** was made from **32** and 3-methylbenzofuran. To a solution of **33** (1 equiv) and 1-azido-2-fluoroethane (2 equiv) in anhydrous *N*,*N*-dimethylformamide was added sodium ascorbate (5 equiv) and copper sulfate pentahydrate (0.5 equiv). The reaction was stirred for 3 h and TLC analysis indicated complete consumption of the reactants. Water was added and the product was extracted with ethyl acetate. The combined organic layers were washed with water, dried, and concentrated. Column chromatography (5% methanol in dichloromethane) of the resulting residue gave the product (4% overall yield from 3-methybenzofuran). Conversion to the oxalate salt gave **29** as a white powder, mp 160–161 °C; ^{1}H NMR (free base, $CDCl_3$) δ 7.80–7.83 (m, 1H), 7.69 (s, 1H), 7.56 (s, 1H), 7.46–7.49 (m, 1H), 7.24–7.33 (m, 3H), 6.81–6.91 (m, 3H), 4.85–4.88 (m, 1H), 4.76 (s, 2H), 4.71 (s, 2H), 4.69–4.72 (m, 2H), 4.60–4.63 (m, 1H), 3.72 (s, 2H), 2.82–2.88 (m, 4H), 2.62–2.73 (m, 2H), 1.61–1.65 (m, 2H). Anal. ($C_{27}H_{29}FN_6O_2 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

8. Radioligand binding and functional assays

8.1. Dopamine receptor binding assays

The method for the iodination of ^{125}I-IABN using peracetic acid has been previously described.[27] For radioligand binding studies, membrane homogenates from stably transfected HEK 293 cells expressing either the human D_2, D_3 or D_4 receptors were prepared using a polytron tissue homogenizer (Brinkman Instruments, Westbury, NY). The tissue was suspended in 50 mM Tris–HCl, 150 mM NaCl and 1 mM EDTA at pH 7.5 to approximately 5–20 μg of protein per 50 μL prior to the assay. Assays were performed in a total volume of 150 μL. Binding reactions were carried out for 60 min at 37 °C and the reaction was terminated by rapid filtration over Schleicher and Schuell No 32 glass fiber filters (Whatman plc, Maidstone, England). After washing filters with buffer, the radioactivity of the ^{125}I-labeled ligand was quantitated using a Packard Cobra gamma counter with an efficiency of 75%. Protein concentrations were determined using a BCA reagent (Pierce, Rockford, Illinois) with bovine serum albumin as the protein standard.

For competition curves using a transfected cell line expressing D_2, D_3 or D_4 dopamine receptors, experiments were performed in

triplicate with two concentrations of inhibitor per decade over at least five orders of magnitude. The concentration of the radioligand was approximately equal to the K_d values. Controls containing either no inhibitor or 2 μM (+)-butaclamol were used to define total binding and nonspecific binding, respectively. Competition data for $D_{2\text{-like}}$ dopamine receptors were modeled for a single-site fit using the TableCurve program (Jandel Scientific Software, San Rafael, California); the IC_{50} values for the competitive inhibitors were converted to K_i values using the Cheng and Prusoff corrections.[32]

8.2. Sigma receptor binding assays

Test compounds were dissolved in *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) or ethanol and then diluted in 50 mM Tris–HCl buffer, pH 7.4, containing 150 mM NaCl and 100 mM EDTA. Membrane homogenates were made from guinea pig brain for σ_1 binding assay and rat liver for σ_2 binding assay. Membrane homogenates were diluted with 50 mM Tris–HCl buffer, pH 8.0, and incubated at 25 °C in a total volume of 150 μL in 96-well plates with the radioligand and test compounds with concentrations ranging from 0.1 nM to 10 μM. After incubation was completed, the reactions were terminated by the addition of 150 μL of ice-cold wash buffer (10 mM Tris–HCl, 150 mM NaCl, pH 7.4) using a 96-channel transfer pipette (Fisher Scientific, Pittsburgh, PA), and the samples harvested and filtered rapidly through 96-well fiber glass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris–HCl buffer, pH 8.0, for 1 h. Each filter was washed three times with 200 μL of ice-cold wash buffer. A Wallac 1450 MicroBeta liquid scintillation counter (Perkin–Elmer, Boston, MA) was used to quantitate the bound radioactivity.

The σ_1 receptor binding assay was conducted using guinea pig brain membrane homogenates (~300 μg protein) and ~5 nM [^{3}H](+)-pentazocine (34.9 Ci/mmol, Perkin–Elmer, Boston, MA). The incubation time was 90 min. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol.

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg protein) and ~1 nM [^{3}H]RHM-1 (80 Ci/mmol, American Radiolabeled Chemicals Inc., St. Louis, MO) alone or ~5 nM [^{3}H]DTG (58.1 Ci/mmol, Perkin–Elmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_1 sites. The incubation time was 60 min for [^{3}H]RHM-1 and 120 min for [^{3}H]DTG. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC_{50} value). Competitive curves were best fit to a one-site fit and gave pseudo-Hill coefficients of 0.6–1.0. K_i values were calculated using the method of Cheng and Prusoff[30] and represent mean values ± SEM. The K_d value used for [^{3}H](+)-pentazocine with guinea pig brain homogenates was 7.89 nM; a K_d value of 30.73 nM was used for [^{3}H]DTG with rat liver, while 0.66 nM was used for [^{3}H]RHM-1 with rat liver.[29]

8.3. Whole cell adenylyl cyclase assay

The accumulation of ^{3}H-cyclic AMP in HEK cells was measured by a modification of the method of Shimizu et al.[33] as previously described.[27] Transfected HEK cells were treated with serum-free medium containing 2,8-^{3}H-adenine (ICN) and cells were incubated at 37 °C for 75 min. Cells and drugs diluted in serum-free media containing 0.1 mM 3-isobutyl-1-methylxanthine (Sigma) were mixed to give a final volume of 500 μL and cells were incubated for 20 min at 37 °C. The reaction was stopped by addition of 500 μL of 10% trichloroacetic acid and 1 mM cyclic AMP. After centrifugation, the supernatants were fractionated using Dowex AG1-X8 and neutral alumina to separate the ^{3}H-ATP and the ^{3}H-cyclic AMP. Individual samples were corrected for column recovery by monitoring the recovery of the cyclic AMP using spectrophotometric analysis at OD 259 nm.[27,33]

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Appendix

Elemental analyses

Compound	%C		%H		%N	
	Calc'd	Found	Calc'd	Found	Calc'd	Found
5	48.90	48.50	4.66	4.44	5.18	4.76
6	49.61	49.92	4.62	4.98	5.03	4.63
7	48.05	47.95	4.82	4.55	4.87	4.46
8	64.10	64.07	6.20	6.08	6.50	6.37
9	52.76	52.96	4.98	5.03	5.13	5.09
10	52.76	53.13	4.98	5.25	5.13	5.25
11	61.53	61.13	6.02	5.74	11.96	11.77
12	61.53	61.50	6.02	5.72	11.96	11.75
13	49.23	48.83	5.11	4.80	8.20	7.81
14	44.38	44.32	4.79	4.39	7.39	7.39
15	57.25	57.63	5.90	5.62	9.10	9.31
18	53.45	53.66	4.89	5.18	2.83	2.76
19	48.81	49.17	4.47	4.63	2.59	2.51
20	52.70	52.97	4.62	4.57	2.79	2.78
21	55.48	55.25	4.66	4.69	2.94	3.00
22	50.49	50.31	4.24	4.28	2.68	2.71
23	62.51	62.61	5.36	5.22	9.51	9.53
24	60.91	61.05	5.11	5.21	9.27	9.10
25	63.89	63.93	5.90	5.75	8.94	8.93
26	61.65	61.84	5.77	5.62	8.30	8.29
27	62.35	62.22	5.65	5.64	8.73	8.54
29	58.38	58.16	5.58	5.41	14.09	13.69

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