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Bioorganic & Medicinal Chemistry 13 (2005) 77–87
Bioorganic & Medicinal Chemistry

Synthesis and in vitro binding of *N*-phenyl piperazine analogs as potential dopamine D_3 receptor ligands

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Received 21 July 2004; revised 29 September 2004; accepted 29 September 2004
Available online 11 November 2004

Abstract—A series of *N*-(2-methoxyphenyl)piperazine and *N*-(2,3-dichlorophenyl)piperazine analogs were prepared and their affinities for dopamine D_2, D_3, and D_4 receptors were measured in vitro. Binding studies were also conducted to determine if the compounds bound to sigma (σ_1 and σ_2) and serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7) receptors. The results of the current study revealed a number of compounds (**12b**, **12c**, **12e**, and **12g**) having a high affinity for D_3 (K_i at D_3 receptors ranging from 0.3 to 0.9 nM) versus D_2 (K_i at D_2 receptors ranging from 40 to 53 nM) receptors and a log *P* value indicating that they should readily cross the blood brain barrier (log *P* = 2.6–3.5). All of the compounds evaluated in this study had a high affinity for serotonin 5-HT_{1A} receptors. These compounds may be useful as probes for studying the behavioral pharmacology of the dopamine D_3 receptor, as well as lead compounds for the development of radiotracers for studying D_3 receptor regulation in vivo with the functional imaging technique, positron emission tomography.

1. Introduction

The dopamine receptor subtypes are members of the G protein coupled receptor protein superfamily. Cloning studies have demonstrated that there are five subtypes of dopamine receptors expressed in mammalian brain. These five subtypes have been classified into two major classes, the $D_{1\text{-like}}$ and $D_{2\text{-like}}$ receptors. The $D_{1\text{-like}}$ receptor subtypes includes the D_1 (D_{1a}) and D_5 (D_{1b}) receptors. Stimulation of $D_{1\text{-like}}$ receptors results in an activation of adenylyl cyclase and an increase in the production of cAMP. The $D_{2\text{-like}}$ receptor subtypes consists of the D_2, D_3, and D_4 receptors. Agonist stimulation of $D_{2\text{-like}}$ receptors results in an inhibition of adenylyl cyclase activity, an increase in the release of arachadonic acid, and an increase in phosphatidylinositol hydrolysis.[1]

Over the past decade there has been interest in developing agents that can function as agonists, partial agonists, and antagonists of the dopamine D_3 receptor.[1,2] This interest was generated by the hypothesis that dopamine D_3 receptors may play a role in the development of a number of neurological and psychiatric disorders. Receptor autoradiography studies have shown that both D_2 and D_3 receptors are widely distributed in striatal regions of human[3] and nonhuman primate[4] brain. However, the high density of D_3 receptors in limbic regions suggests that this receptor may play an important role in the pathological abnormalities associated with many dopaminergic-based CNS disorders. Autoradiography studies have also revealed a decrease of D_3 receptors in the frontal cortex and an increase in expression in the ventral striatum of schizophrenics compared to normal individuals.[5,6] Dopamine D_3 receptors are also believed to play a role in the dyskinesias associated with Parkinson's disease.[7] For example, chronic treatment of squirrel monkeys with the neurotoxin, 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), which causes a selective destruction of the nigrostriatal dopaminergic system, results in a decline of D_3 receptors in the

Keywords: Dopamine D_3 receptors; Atypical antipsychotics.
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doi:10.1016/j.bmc.2004.09.054

caudate (motor region) but not the putamen of globus pallidus (limbic regions). However, treatment with the drug levodopa led to a restoration of D_3 receptor levels and a reversal of Parkinsonian symptoms in MPTP-treated animals.[7] Finally, the activation of dopamine D_3 receptors is currently believed to be involved in the sensitization/rewarding properties of psychostimulants, such as cocaine. Therefore, partial agonists or antagonists that can reduce the interaction of psychostimulant-induced increases in synaptic dopamine levels with the D_3 receptor may be useful in the pharmacological treatment of cocaine abuse.[1]

A number of conformationally flexible benzamide analogs displaying a high affinity and selectivity for D_3 versus D_2 receptors have been reported in recent years. Examples of this include BP 897, NGB 2904, and the structural congeners **1**–**4** (Fig. 1). A common structural feature in the conformationally flexible benzamide analogs is the *N*-2,3-dichlorophenylpiperazine ring and the four carbon spacer group separating the benzamide and the basic amino moieties.[8–12,27,28] However, the relatively high lipophilicity of these analogs indicates that they are not likely to enter the brain and block D_3 receptors in the CNS. For example, the calculated log *P* values of benzamide analogs BP 897, **1** and **2** (Fig. 1) are 4.73, 5.91, and 7.07, respectively, which are not within the range of log *P* values for compounds that readily cross the blood–brain barrier.[13]

We have recently reported a series of conformationally flexible benzamide analogs displaying a modest selectivity for D_3 versus D_2 receptors.[14] For example, the naphthamide analog **5** has a 28-fold selectivity for D_3 versus D_2 receptors. Unfortunately, the calculated log *P* value of this compound is 5.76, which is also outside the range for compounds that readily cross the blood–brain barrier. As part of a continuing effort to develop potent and selective D_3 receptor ligands for behavioral and imaging studies of the D_3 receptor in vivo, we prepared a series of structural analogs of **5** and measured their affinity for dopamine $D_{2\text{-like}}$ (D_2, D_3, D_4), serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7) and sigma (σ_1 and σ_2) receptors, as well as monoamine transporter sites (DAT, SERT, NET). The strategy chosen for the current study involved the combination of the following approaches: (a) replacement of the 2,3-dimethoxy-5-bromobenzene

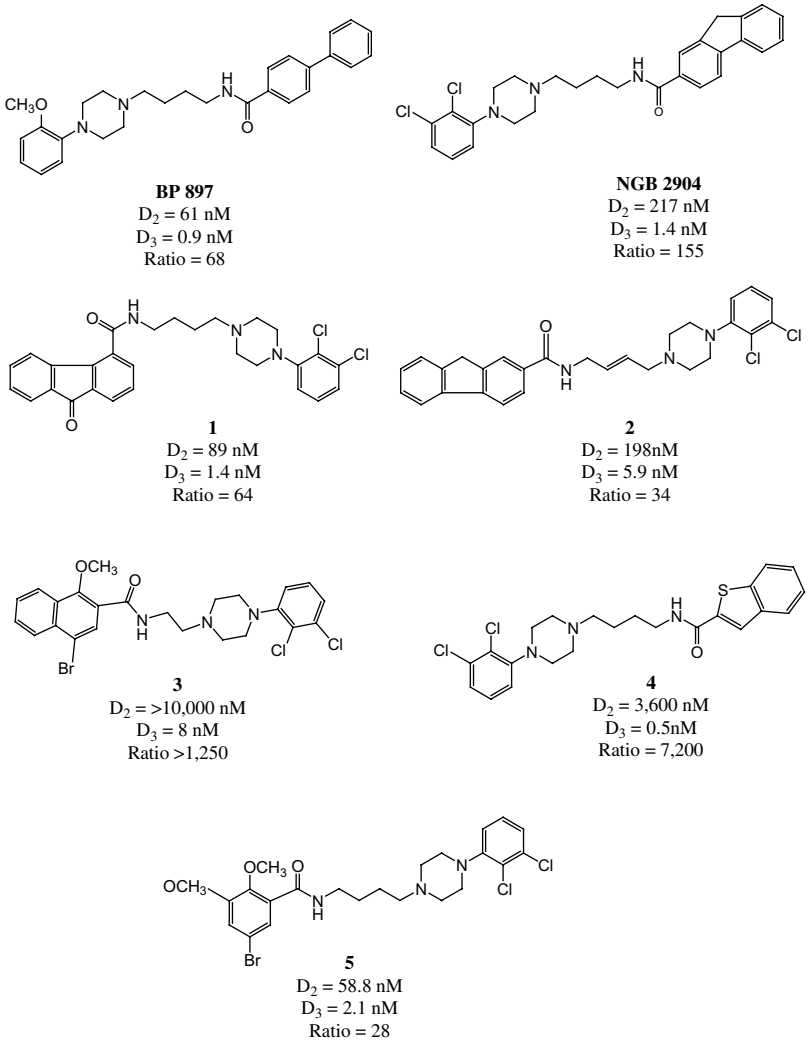


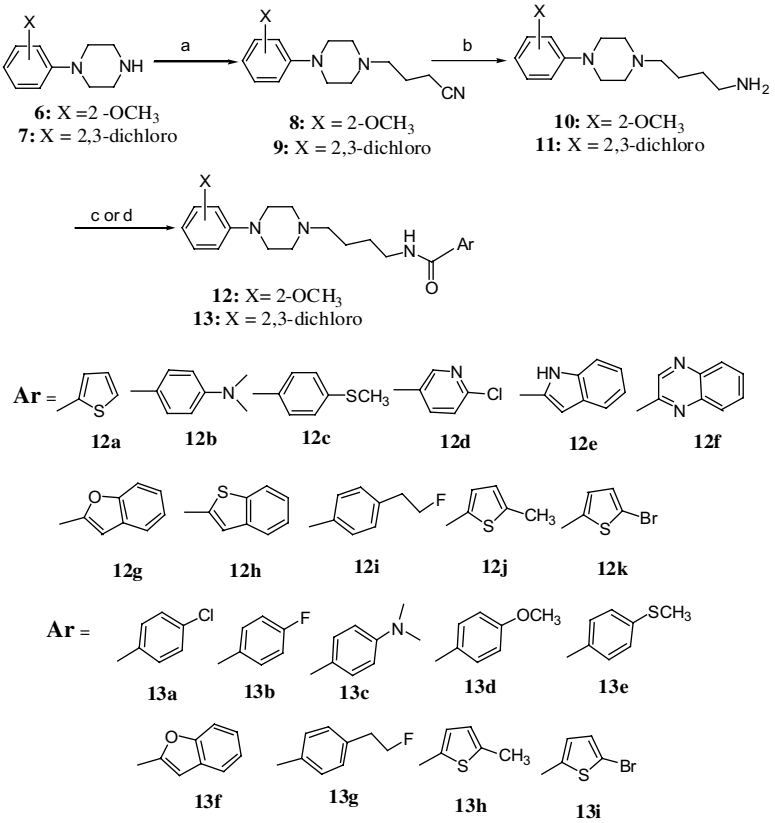
Figure 1. Structures of D_3-selective benzamide analogs reported in the literature.

ring of **5** with other aromatic or heteroaromatic moieties to explore the structure–activity relationship of the benzamide group and, (b) comparison of the *N*-(2,3-dichlorophenyl)piperazine of NGB 2904 and compounds **1**–**5** with the *N*-(2-methoxyphenyl)piperazine of BP 897 to determine the effect of this substitution on dopamine receptor affinity and calculated log *P* values. The results of this study led to the identification of a number of compounds possessing a high affinity (nM) and moderate selectivity (10- to 100-fold) for dopamine D_3 versus D_2 receptors with a log *P* value within the range needed for crossing the blood–brain barrier by passive diffusion.

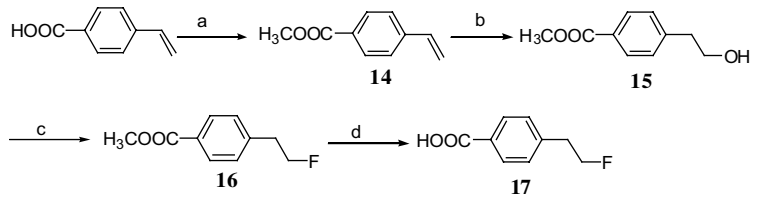
2. Results

The synthesis of the target compounds is shown in Scheme 1. Reaction of *N*-phenyl piperazine analogs **6** and **7** with bromobutyronitrile gave N-alkylated products, **8** and **9**, in 70% yield. Reduction with lithium aluminum hydride in THF gave the corresponding amines, **10** and **11**, in 90% yield. Condensation of amines **10** and **11** with the appropriate benzoic acid gave the corresponding amine analogs, **12** and **13**, in excess of 90% yield. Synthesis of 4-(2-fluoroethyl)benzoic acid, **17**, was accomplished using the reaction sequence outlined in Scheme 2.

The results of the in vitro binding assays for dopamine $D_{2\text{-like}}$ and sigma receptors are summarized in Table 1. All compounds listed in Table 1 had a high affinity (nM) for dopamine D_3 receptors and a reduced affinity for D_2 receptors. There was little difference between the *N*-(2-methoxyphenyl)piperazine and *N*-(2,3-dichlorophenyl)piperazine analogs with respect to binding affinity for D_2 and D_3 receptors (i.e., compare **12b** vs **13c**, **12c** vs **13e**, and **12g** vs **13f**). The most potent and selective D_3 analogs were compounds **12e** and **12h**,



Scheme 1. Reagents: (a) 4-bromobutyronitrile, $N(C_2H_5)_3$, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) (1) ArCOOH, $ClCOOC_2H_5$, $N(C_2H_5)_3$, (2) **10** or **11**; (d) ArCOOH, **10** or **11**, Bop-Cl, $(C_2H_5)_3$.



Scheme 2. Reagents: (a) $(CH_3)_3OBF_4$, CH_2Cl_2, $N(C_2H_5)_3$; (b) (1) BH_3/THF, (2) NaOH, 35% H_2O_2; (c) $(C_2H_5)_2NSF_3$, CH_2Cl_2; (d) (1) NaOH, CH_3OH/H_2O, (2) HCl.

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

#	K_i (nM)[a]						
	D_2[b]	D_3[c]	D_4[d]	D_2:D_3[e]	σ_1[f]	σ_2[g]	Log P[h]
12a	22.4 ± 2.8	2.6 ± 0.7	29.0 ± 6.2	9	1433 ± 193	4602 ± 1802	2.51
12b	34.4 ± 4.7	0.8 ± 0.1	896 ± 272	43	1259 ± 294	1567 ± 307	3.09
12c	31.7 ± 4.0	0.6 ± 0.1	674 ± 85	53	>10,000	2004 ± 212	3.52
12d	51.5 ± 4.0	3.2 ± 1.4	612 ± 65	16	1265 ± 132	1760 ± 1208	2.66
12e	37.4 ± 6.2	0.3 ± 0.05	476 ± 153	125	1800 ± 758	1944 ± 859	3.62
12f	54.1 ± 5.0	4.2 ± 0.7	1414 ± 489	13	7401 ± 2252	447 ± 56	2.59
12g	36.5 ± 3.9	0.9 ± 0.1	868 ± 340	41	1081 ± 193	678 ± 37	3.52
12h	21.5 ± 1.6	0.2 ± 0.04	305 ± 108	108	1443 ± 358	1426 ± 333	4.76
12i	54.5 ± 7.6	2.4 ± 0.5	804 ± 46	23	3543 ± 2500	2205 ± 259	2.94
12j	23.1 ± 6.3	2.1 ± 0.40	ND[i]	11	1095 ± 159	924 ± 142	3.32
12k	23.1 ± 6.0	1.3 ± 0.51	ND[i]	18	342 ± 73	383 ± 83	3.62
13a	27.1 ± 2.5	0.5 ± 0.11	480 ± 154	54	2278 ± 873	5589 ± 2099	5.76
13b	11.7 ± 1.9	0.3 ± 0.03	136 ± 38	39	924 ± 160	1605 ± 133	5.72
13c	25.7 ± 4.4	0.4 ± 0.08	1450 ± 686	59	>10,000	6754 ± 491	5.41
13d	11.4 ± 0.9	0.3 ± 0.06	498 ± 214	39	8659 ± 1400	7069 ± 642	5.16
13e	28.0 ± 5.3	0.5 ± 0.01	778 ± 254	56	>10,000	8422 ± 3974	5.84
13f	39.5 ± 5.0	0.6 ± 0.13	842 ± 288	65	5673 ± 151	3672 ± 368	5.85
13g	24.1 ± 3.1	1.0 ± 0.11	1227 ± 419	24	8761 ± 2578	7208 ± 3908	5.26
13h	20.3 ± 6.2	0.7 ± 0.1	ND[i]	29	1422 ± 268	3646 ± 1474	5.64
13i	14.2 ± 2.7	0.7 ± 0.1	758 ± 91	20	612 ± 177	3010 ± 619	5.94
Spiperone	0.05 ± 0.01	0.30 ± 0.06	ND[i]	6.4	ND[i]	ND[i]	—
NGB-2904	112 ± 22	2.0 ± 0.4	ND[i]	56	ND[i]	ND[i]	6.94[j]

[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 CHO cells using [^{125}I]IABN as the radioligand.
[c] K_i values for D_3 receptors were measured on human D_3 expressed in CHO cells using [^{125}I]IABN as the radioligand.
[d] K_i values for D_4 receptors were measured on human D_4 expressed in CHO cells using [^{125}I]IABN as the radioligand.
[e] K_i for D_2 receptor/K_i for D_3 receptor.
[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 Clog P.
[i] Not determined.
[j] Ref. 12.

which had subnanomolar affinity for D_3 receptors and a D_2:D_3 ratio >100. Other potent and selective compounds were **12b**, **12c**, and **12g**, which had subnanomolar D_3 affinity and D_2:D_3 ratios > 40. A noteworthy observation was the low affinity of compounds **12a–k** and **13a–i** for σ_1 and σ_2 receptors. This is in contrast to our previous observations with a series of napthamide analogs, which had a high affinity and selectivity for D_3 versus D_2 receptors, but which also had high affinity for σ_1 and σ_2 receptors.[15]

The data from the in vitro binding studies for serotonin receptors and monoamine transporters is summarized in Table 2. All compounds prepared in this study were found to have a high affinity for serotonin 5-HT_{1A} receptors. A similar observation was made with the arylpiperazine analogs reported by Murray et al.[16] As with the $D_{2\text{-like}}$ receptors, there was little difference between the *N*-(2-methoxyphenyl)piperazine and *N*-(2,3-dichlorophenyl)piperazine analogs with respect to binding affinity for the 5-HT_{1A} receptor. With the exception of **13i**, all compounds had a relatively low affinity for 5-HT_{2A} receptors. Many of the *N*-(2,3-dichlorophenyl)piperazine analogs had a modest affinity for serotonin 5-HT_{2C} receptors, which was also much higher than the corresponding *N*-(2-methoxyphenyl)piperazine congener (e.g., compare **12c** vs **13e**, **12g** vs **13f**, and **12i** vs **13g**). Compounds that were tested had a relatively low affinity for serotonin 5-HT_{5A} and 5-HT_6 receptors, as well as for the monoamine transporters, SERT, NET, and DAT (Table 2). Many of the compounds described in this study were also screened for activity at serotonin 5-HT_3, 5-HT_4, and 5-HT_7 receptors and were found to be inactive at these receptors ($K_i > 10\,\mu M$; data not shown).

Based upon the results of our competitive radioligand binding studies, a panel of seven compounds, which exhibited selectivity at D_3 receptors compared to D_2 receptors, was evaluated for intrinsic activity at D_3 dopamine receptors. Intrinsic activity was evaluated as the ability of a compound to inhibit forskolin-dependent stimulation of adenylyl cyclase activity in transfected HEK-293 cells expressing human D_3 dopamine receptors. For each experiment the test compounds were compared to the activity of the full agonist quinpirole and the antagonist haloperidol at D_2-like dopamine receptors. In this cell line, we consistently find that quinpirole (10 nM) has intrinsic activity comparable to dopamine (10 μM) and, therefore, is a full agonist at D_3 receptors (data not shown). For this cell line we generally observe a maximal inhibition that ranges from 35% to 40% for the full agonists dopamine and quinpirole. For this initial evaluation, a concentration of $\geqslant 10\times$ the K_i values

Table 2. Binding affinities for serotonin receptors and monoamine transporter sites

#	K_i (nM)[a]							
	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_{5A}	5-HT_6	SERT	NET	DAT
12a	0.7 ± 0.1	1417 ± 890	ND[b]	3352 ± 1849	ND	ND	ND	ND
12b	6.0 ± 1.3	1506 ± 283	ND	ND	ND	ND	ND	ND
12c	1.5 ± 0.2	595 ± 167	507 ± 157	ND	ND	1036 ± 263	ND	ND
12d	4.4 ± 1.0	940 ± 597	ND	ND	ND	ND	ND	ND
12e	0.87 ± 0.35	341 ± 88	202 ± 59	ND	>10,000	788 ± 143	>10,000	2312 ± 473
12f	0.29 ± 0.09	843 ± 76	1006 ± 357	4267 ± 872	ND	896 ± 255	ND	>10,000
12g	2.2 ± 0.52	886 ± 236	367 ± 116	ND	ND	692 ± 243	ND	ND
12h	0.73 ± 0.18	431 ± 66	ND	479 ± 140	>10,000	1275 ± 805	ND	2505 ± 2401
12i	5.79 ± 1.50	857 ± 114	1860 ± 788	2406 ± 666	ND	ND	ND	ND
13a	5. 0 ± 0.9	201 ± 45	88 ± 21	>10,000	1379 ± 245	644 ± 135	418 ± 148	>10,000
13b	0.23 ± 0.05	85 ± 16	32 ± 15	374 ± 135	333 ± 67	438 ± 154	156 ± 44	ND
13c	8.0 ± 1.7	154 ± 17	144 ± 14	>10,000	2626 ± 639	1004 ± 261	110 ± 28	>10,000
13d	2.67 ± 0.49	47.1 ± 16.0	71 ± 18	>10,000	4466 ± 1103	278 ± 39	168 ± 32	ND
13e	2.20 ± 0.28	66.8 ± 11.0	53 ± 14	>10,000	1760 ± 325	774 ± 232	126 ± 39	ND
13f	3.0 ± 0.6	70.9 ± 10.0	57 ± 17	>10,000	>10,000	1460 ± 432	375 ± 118	7142 ± 1839
13g	6.0 ± 1.3	52.9 ± 9.0	349 ± 57	ND	1102 ± 166	1632 ± 525	555 ± 180	795 ± 190
13i	0.63 ± 0.11	19.2 ± 4.0	85 ± 26	185 ± 58	386 ± 68	384 ± 88	108 ± 19	3472 ± 681

[a] Data provided by the Psychoactive Drug Screening Program at Case Western University [Mean ± S.D.].
[b] Not determined.

was used for each test compound to insure that >90% of the receptor sites were occupied. This panel of compounds had varying intrinsic activity at D_3 dopamine receptors (Table 3). For example, **12i** inhibited forskolin-dependent adenylyl cyclase activity in a manner analogous to that observed for quinpirole and, as such, could be classified as a full agonist (>90% of the maximum response). Compound **12b** showed low activity and could be tentatively classified as an antagonist, or a weak partial agonist (21% of maximum response).

Table 3. Intrinsic activity of selected *N*-phenyl piperazine analogs at D_3 receptors

Compound	% Inhibition	% Maximum response	Activity
None	0	0	—
Haloperidol	3 ± 3 (8)	8	Antagonist
12b	8 ± 1 (3)	21	Weak partial agonist
12d	15 ± 1 (7)	39	Partial agonist
12g	17 ± 2 (7)	44	Partial agonist
12h	21 ± 4 (5)	47	Partial agonist
12e	23 + 2 (5)	52	Partial agonist
12c	25 ± 3 (5)	65	Partial agonist
12i	35 ± 2 (5)	92	Agonist
Quinpirole	38 + 6 (8)	100	Agonist

The intrinsic activity of the compounds were evaluated for the ability to inhibit forskolin-dependent (100 μM) stimulation of adenylyl cyclase activity using a whole cell assay. The values for the percent inhibition are the reported as the actual percent of inhibition of the ^{3}H-cAMP accumulation relative to the assay performed in the absence of a test compound, minus basal activity. Values are reported as the mean values ± S.E.M. and the number in parenthesis is the number of independent experiments. The classic D_2-like dopamine receptor antagonist and agonist (haloperidol and quinpirole, respectively) were included in each assay as reference compounds. The percent maximum response is the value for the inhibition normalized to the value for the full agonist quinpirole. The concentrations for all the compounds was $\geqslant 10\times$ the K_i value of the compound at human D_3 dopamine receptors, obtained from competitive radioligand binding experiments.

The remaining five compounds appear to be partial agonists, with activity ranging from 39% to 65% of the maximum response. The intrinsic activity of these compounds did not appear to be correlated with either (a) affinity at the D_3 receptor or (b) the magnitude of D_3 receptor selectivity.

A property that was quite different between the *N*-(2-methoxyphenyl)piperazine and *N*-(2,3-dichlorophenyl)piperazine analogs was the calculated log *P* values. The difference in log *P* values between the *N*-(2-methoxyphenyl)piperazine and *N*-(2,3-dichlorophenyl)piperazine analogs was 2.32 units. Consequently, many of the *N*-(2-methoxyphenyl)piperazine analogs had log *P* values that within the range that would predict a high brain uptake, whereas all of the *N*-(2,3-dichlorophenyl)piperazine analogs had log *P* values > 5.0. These data suggests that the *N*-(2,3-dichlorophenyl)piperazine analogs may be too lipophilic to readily cross the blood–brain barrier. The compounds displaying a high D_2:D_3 ratio (>40) and reasonable log *P* value (3.0–3.6) include compounds **12b**, **12c**, **12e**, and **12g**.

3. Discussion

The goal of the current study was to prepare ligands having a high affinity and selectivity for D_3 versus D_2 receptors, and a reduced lipophilicity that will assure a high brain uptake in vivo. Although many potent and selective D_3 ligands have been reported in the literature, the majority of these ligands were found to have calculated log *P* values > 5.0, which would limit their ability to readily cross the blood–brain barrier. Previous studies with carbon-11 labeled aliphatic alcohols indicated that the log *P* range for a compound to have a high brain uptake (i.e., % brain extraction > 85% at a cerebral blood flow of 100 mL/min) to range from −0.32 to 3.2.[13] Given the structural requirements of the lead compounds for

the current study (Fig. 1), which consisted of a benzamide ring and an *N*-phenylpiperazine moiety separated by a four carbon spacer unit, it was not likely that we would prepare an analog near the low end of the log *P* range described above. However, we thought it might be possible to prepare analogs of the lead compounds that would fall within the upper limit of this log *P* range. Based on the data shown in Table 1, this goal was accomplished by: (a) replacing the *N*-(2,3-dichlorophenyl)piperazine group with an *N*-(2-methoxyphenyl)piperazine moiety and, (b) attaching a heteroatom into the benzamide aromatic ring. The most promising analogs were achieved by substituting the *para* position of the benzamide ring with either a dimethylamino group (i.e., **12b**) or a methylthio group (i.e., **12c**), or replacing the benzene ring with either a 2-indole (**12e**) or 2-benzofuran (**12g**) ring system. These compounds had D_2:D_3 selectivity ratios > 50 and log *P* values ranging from 3.1–3.6, which, based on the data presented by Dishino et al.,[13] should result in a brain extraction of 70–80%. These compounds may be useful probes in the study of the behavioral pharmacology of dopamine D_3 receptors. In addition, the presence of the 2-methoxy group of compounds **12b**, **12c**, **12e**, and **12g** indicates that the corresponding carbon-11 labeled versions of the compounds can be prepared via alkylation of the corresponding des-methyl precursor with [^{11}C]iodomethane. Therefore, [^{11}C]**12b**, [^{11}C]**12c**, [^{11}C]**12e**, and [^{11}C]**12g** may be useful radiotracers for studying the regulation of dopamine D_3 receptors in a variety of CNS disorders using the noninvasive imaging technique, PET.

In addition to having a high affinity for dopamine D_3 receptors, all of the compounds described in this report were found to have a high affinity for serotonin 5-HT_{1A} receptors. This is consistent with the pharmacological properties of the piperazine analogs described by Murray et al.[16] Serotonin 5-HT_{1A} receptors are autoreceptors located on the cell bodies of serotonergic neurons arising from the dorsal raphe nucleus.[17] 5-HT_{1A} receptors are also postsynaptic receptors affecting the activity of non-serotonergic neurons in the hippocampus and prefrontal cortex.[18] Activation of the somatodendritic autoreceptors decreases the rate of firing of serotonergic neurons, whereas activation of postsynaptic 5-HT_{1A} receptors facilitates the release of acetylcholine, noradrenaline, and dopamine.[18] Previous studies have shown that 8-OH-DPAT, a 5-HT_{1A} full agonist, reduces catalepsy produced by the typical antipsychotic, haloperidol.[19] Furthermore, the 5-HT_{1A} partial agonists, buspirone and tandospirone, have been shown to decrease the parkinsonian-like side effects in schizophrenic patients treated with antipsychotics.[20–22] Therefore, an agent functioning as either an antagonist or partial agonist at dopamine D_3 receptors and as a partial agonist at 5-HT_{1A} receptors may be useful in treating schizophrenia, while having a low tendency for causing extrapyramidal side-effects. The observation that the compounds shown in Table 3 display a range of functional activities at the D_3 receptor, varying from a full agonist (**12i**), partial agonists (**12d**, **12g**, **12h**, **12e**, and **12c**), to an antagonist (**12b**), suggests that they should be useful in evaluating D_2 versus D_3 associated behaviors, or the relative role of these two structurally homologous receptor subtypes, in a variety of CNS disorders.

Another application of the compounds described in this study would be to serve as radiotracers for imaging dopamine D_3 receptors with PET. Although the compounds described in this report were found to have a high affinity for the serotonin 5-HT_{1A} receptor, we do not expect that labeling of 5-HT_{1A} receptors would prevent dopamine D_3 receptor imaging studies in vivo. As stated above, dopamine D_3 receptors have a high density in limbic regions of the brain, such as the nucleus accumbens and olfactory tubercle, whereas 5-HT_{1A} receptors are localized in the dorsal raphe and hippocampus. Therefore, it should be possible to image D_3 receptors in the nucleus accumbens without interference from binding to 5-HT_{1A} receptors. However, the high 5-HT_{1A} affinity of these will likely prevent imaging dopamine D_3 receptors in the prefrontal cortex.

In conclusion, we have completed a structure–activity relationship study on a series of conformationally flexible benzamides with the goal of identifying potential probes for studying the behavioral pharmacology and radiotracers for imaging dopamine D_3 receptors with PET. The results of this study identified a number of compound having a high affinity and selectivity for D_3 versus D_2 receptor with a log *P* value that may result in a high uptake in brain in vivo. Studies are currently ongoing to determine the functional activity of the compounds described above at dopamine D_3 and serotonin 5-HT_{1A} receptors. We are also exploring the potential of ^{11}C-labeled versions of **12b**, **12c**, **12e**, and **12g** as radiotracers for imaging dopamine D_3 receptors in vivo with PET.

4. Experimental section

All reactions were carried out under an inert nitrogen atmosphere with dry solvents, under anhydrous conditions, unless otherwise stated. Reagents and grade solvents were used without further purification. Melting points were determined using MEL-TEMP 3.0 apparatus and uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All chemical shift values are reported in ppm (δ). Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. and the analytical results were within ±0.4% of the theoretical values for the formula given unless otherwise listed (Table 4). Binding assays for D_2, D_3, and D_4, σ_1 and σ_2 receptors were conducted as described below. All other receptor binding studies were conducted through the Psychoactive Drug Screen Program supported by the National Institutes of Mental Health (NO1MH32004).

4.1. 4-Vinyl-benzoic acid methyl ester (14)

A solution of 4-vinylbenzoic acid (2.08 g, 14.0 mmol) and trimethyloxonium tetrafluoroborate (2.60 g, 17.5 mmol) in CH_2Cl_2 (200 mL) was added triethylamine (1.56 g, 15.4 mmol) at ambient temperature. The reac-

Table 4. Elemental analysis

Compd	Formula	Calcd			Found		
		C	H	N	C	H	N
12a	$C_{20}H_{27}N_3O_2S{\cdot}2HCl{\cdot}0.5H_2O$	52.74	6.64	9.23	52.69	6.49	9.21
12b	$C_{24}H_{34}N_4O_2{\cdot}0.5H_2O$	68.7	8.41	13.35	69.04	8.38	13.37
12c	$C_{23}H_{31}N_3O_2S$	66.79	7.56	10.16	66.51	7.57	10.11
12d	$C_{21}H_{27}ClN_4O_2$	62.6	6.75	13.91	62.55	6.81	13.66
12e	$C_{24}H_{30}N_4O_2{\cdot}0.25H_2O$	70.13	7.48	13.63	70.09	7.49	13.65
12f	$C_{24}H_{29}N_5O_2$	68.71	6.97	16.69	69.01	6.9	16.7
12g	$C_{24}H_{29}N_3O_3$	70.74	7.17	10.31	70.47	7.3	10.19
12h	$C_{24}H_{29}N_3O_2S$	68.05	6.9	9.92	67.92	6.76	9.88
12i	$C_{24}H_{32}FN_3O_2$	69.71	7.8	10.16	69.66	7.79	10.1
13a	$C_{21}H_{24}Cl_3N_3O$	57.22	5.49	9.53	56.96	5.58	9.42
13b	$C_{21}H_{24}Cl_2FN_3O$	59.44	5.7	9.9	59.69	5.76	9.96
13c	$C_{23}H_{30}Cl_2N_4O$	61.47	6.73	12.47	61.44	6.73	12.36
13d	$C_{22}H_{27}Cl_2N_3O_2$	60.55	6.24	9.63	60.6	6.23	9.58
13e	$C_{22}H_{27}Cl_2N_3OS$	58.4	6.02	9.29	59.14	6.16	9.26
13f	$C_{23}H_{25}Cl_2N_3O_2$	61.89	5.65	9.41	61.89	5.62	9.39
13g	$C_{23}H_{28}Cl_2FN_3O$	61.06	6.24	9.29	61.12	6.38	9.25

tion mixture was stirred for 20 h at room temperature, then washed with saturated Na_2CO_3 (50 mL), saturated NaCl (50 mL) and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by chromatography with hexane–ether (10:1) to afford 1.72 g (76%) of **14** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 6.76 (dd, J = 17.5 Hz, J = 10.8 Hz, 1H), 5.88 (d, J = 17.1 Hz, 1H), 5.40 (d, J = 10.8 Hz), 3.93 (s, 3H).

4.2. 4-(2-Hydroxyethyl)benzoic acid methyl ester (15)

Compound **14** (1.95 g, 12.0 mmol) in 1 M BH_3 in THF (24 mL) was stirred 1 h at 0 °C, then 1 h at ambient temperature. A solution of 1 N NaOH (36 mL) was added to the reaction mixture at 0 °C, then a solution of 35% H_2O_2 (20 mL) was added. The mixture was stirred 30 min at 0 °C, then 30 min at ambient temperature. Ethyl acetate (100 mL) was added, the organic layer was separated, washed with water (50 mL), saturated Na_2CO_3 (50 mL), saturated NaCl (50 mL) and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by chromatography with hexane–ether (1:1) to afford 1.16 g (54%) of **15** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 3.92 (s, 3H), 3.91 (t, J = 6.6 Hz, 2H), 2.95 (t, J = 6.6 Hz, 2H).

4.3. 4-(2-Fluoroethyl)benzoic acid methyl ester (16)

A solution of **15** (1.16 g, 6.44 mmol) in CH_2Cl_2 (20 mL) was added DAST (1.56 g, 9.66 mmol) at 0 °C. The mixture was warmed to ambient temperature and stirred overnight, then ethyl acetate (100 mL) was added, washed with water (50 mL), saturated Na_2CO_3 (50 mL), saturated NaCl (50 mL), and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by chromatography with hexane–ether (10:1) to afford 1.06 g (91%) of **16** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 4.67 (dt, J = 47.1 Hz, J = 6.3 Hz, 2H), 3.93 (s, 3H), 3.08 (dt, J = 24.6 Hz, J = 6.3 Hz, 2H).

4.4. 4-(2-Fluoroethyl)benzoic acid (17)

A solution of **16** (174 mg, 0.96 mmol) in methanol (3 mL) and water (1 mL) was added NaOH (58 mg, 1.43 mmol) at ambient temperature. The mixture was stirred for 2 days at ambient temperature, water (5 mL) was added, extracted with ether (20 mL). The aqueous layer was acidified with HCl (1:1) to pH = 1, the white solid was filtered out to afford 160 mg (100%) of **17**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 4.68 (dt, J = 46.8 Hz, J = 6.3 Hz, 2H), 3.10 (dt, J = 24.6, J = 6.3 Hz, 2H).

4.5. 4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyronitrile (8)

A solution of 1-(2-methoxyphenyl)piperazine hydrochloride **6** (17.1 g, 75.0 mmol) and 4-bromobutyronitrile (12.2 g, 82.3 mmol) in CH_2Cl_2 (150 mL) was added triethylamine (22.7 g, 225.0 mmol) at ambient temperature. The mixture was stirred for 2 days, then CH_2Cl_2 (50 mL) was added, washed with saturated Na_2CO_3 (50 mL × 2), saturated NaCl (50 mL) and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was recrystallized from ether to afford 13.2 g (68%) of **8** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04–7.01 (m, 1H), 6.94 (m, 2H), 6.88 (m, 1H), 3.87 (s, 3H), 3.09 (m, 4H), 2.64 (m, 4H), 2.53 (t, J = 6.9 Hz, 2H), 2.46 (t, J = 6.9 Hz, 2H), 1.87 (p, J = 6.9 Hz, 2H).

4.6. 4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyamine (10)

A solution of **8** (13.0 g, 50.0 mmol) in THF (100 mL) was added to a solution of $LiAlH_4$ (2.85 g, 75.0 mmol) in THF (50 mL) in 1 h at 0 °C. The reaction mixture was stirred for 2 h and then heated to reflux for 2 h. Ice water (15 mL) was carefully added at 0 °C. The white solid was filtered and washed with THF. The filtrate was evaporated in vacuo, and the crude product was dissolved in

CH_2Cl_2 (150 mL), washed with saturated NaCl, and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by chromatography with CH_2Cl_2–MeOH–$N(C_2H_5)_3$ (10:5:0.5) to afford 11.5 g (87%) of **10** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99–6.84 (m, 4H), 3.85 (s, 3H), 3.10 (m, 4H), 2.72 (t, J = 6.9 Hz, 2H), 2.65 (m, 4H), 2.42 (t, J = 6.9 Hz, 2H), 1.60–1.45 (m, 4H).

4.7. Thiophene-2-carboxylic acid [4-[4-(2-methoxyphenyl)-piperazin-1-yl]-butyl]amide (12a)

A solution of 2-thiophenecarboxylic acid (257 mg, 2.0 mmol) in $SOCl_2$ (1.5 mL) was heated to reflux for 1 h. After evaporation of the $SOCl_2$, CH_2Cl_2 (5 mL) was added. The solution was added into a solution of **10** (440 mg, 1.67 mmol) in CH_2Cl_2 (5 mL) at 0 °C and triethylamine (1 mL) was added. The mixture was stirred overnight, then ethyl acetate (75 mL) was added, and the organic layer washed with saturated Na_2CO_3 (30 mL × 2), saturated NaCl (30 mL), and dried over Na_2SO_4. After evaporation of the organic layer in vacuo, the crude product was purified by chromatography with ether–MeOH (10:1) to afford 0.51 g (82%) of **12a** as a colorless oil; mp 212–214 °C (HCl salt). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 3.75 Hz, 1H), 7.46 (d, J = 4.95 Hz, 1H), 7.08 (t, J = 4.2 Hz, 1H), 7.01 (m, 1H), 6.94 (m, 2H), 6.88 (m, 1H), 6.49 (br, 1H), 3.88 (s, 3H), 3.49 (m, 2H), 3.12 (m, 4H), 2.70 (m, 4H), 2.51 (m, 2H), 1.68 (m, 4H). Anal. ($C_{20}H_{27}N_3O_2S$·2HCl·0.5H_2O) C, H, N.

4.8. 4-Dimethylamino-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]benzamide (12b)

A solution of 4-(dimethylamino)benzoic acid (207 mg, 1.25 mmol) in CH_2Cl_2 (5 mL) was added to ethyl chloroformate (142 mg, 1.31 mmol) at −5 °C, then triethylamine (132 mg, 1.31 mmol) was added. The reaction mixture was stirred for 15 min at −5 °C, then a solution of **10** (300 mg, 1.14 mmol) in CH_2Cl_2 (5 mL) was added. The mixture was stirred overnight at ambient temperature, then ethyl ether (75 mL) was added, washed with saturated Na_2CO_3 (25 mL × 2), saturated NaCl (25 mL), and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by chromatography with ether–MeOH (10:2) to afford 229 mg (49%) of **12b** as a white solid; mp 126–128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 9.0 Hz, 2H), 7.00 (m, 1H), 7.92 (m, 2H), 6.86 (m, 1H), 6.66 (d, J = 9.0 Hz, 2H), 6.39 (br, 1H), 3.86 (s, 3H), 3.47 (m, 2H), 3.11 (m, 4H), 3.01 (s, 6H), 2.68 (m, 4H), 2.49 (m, 2H), 1.67 (m, 4H). Anal. ($C_{24}H_{34}N_4O_2$·0.5H_2O) C, H, N.

4.9. *N*-[4-[4-(2-Methoxyphenyl)piperazin-1-yl]-butyl]-4-methylsulfanylbenzamide (12c)

Compound **12c** was prepared according to the procedure for compound **12b** except using 4-(methylthio)benzoic acid, which afford 404 mg (65%) of **12c** as a white solid; mp 153–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.4, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.98 (m, 1H), 6.92–6.85 (m, 4H), 3.86 (s, 3H), 3.48 (m, 2H), 3.10 (m, 4H), 2.73 (m, 4H), 2.54 (m, 2H), 2.48 (s, 3H). Anal. ($C_{23}H_{31}N_3O_2S$) C, H, N.

4.10. 4-Chloro-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]benzamide (12d)

Compound **12d** was prepared according to the procedure for compound **12b** except using 6-chloronicotinic acid, which afford 315 mg (52%) of **12d** as a white solid; mp 126–128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (d, J = 2.4 Hz, 1H), 8.08 (dd, J = 8.55 Hz, J = 2.7 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.22 (br, 1H), 7.03–6.84 (m, 4H), 3.85 (s, 3H), 3.49 (m, 2H), 3.03 (m, 4H), 2.66 (m, 4H), 2.49 (t, J = 6.6 Hz, 2H), 1.71 (m, 4H). Anal. ($C_{21}H_{27}ClN_4O_2$) C, H, N.

4.11. 1*H*-Indol-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]amide (12e)

A solution of indole-2-carboxylic acid (242 mg, 1.5 mmol) and **10** (395 mg, 1.5 mmol) in CH_2Cl_2 (15 mL) was added to BOP-Cl (420 mg, 1.65 mmol) at ambient temperature, then triethylamine (304 mg, 3.0 mmol) was added. The mixture was stirred overnight, then ethyl acetate (75 mL) was added, the organic layer washed with saturated Na_2CO_3 (30 mL), saturated NaCl (30 mL), and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by chromatography with EtOAc–MeOH (5:1) to afford 352 mg (58%) of **12e** as a white solid; mp 146–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.34 (br, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.16 (t, J = 7.2 Hz, 1H), 7.02 (m, 1H), 6.95 (m, 2H), 6.88 (m, 2H), 6.66 (br, 1H), 3.88 (s, 3H), 3.55 (m, 2H), 3.15 (m, 4H), 2.72 (m, 4H), 2.53 (t, J = 6.9 Hz, 2H), 1.73 (m, 4H). Anal. ($C_{24}H_{30}N_4O_2$·0.25H_2O) C, H, N.

4.12. Quinoxaline-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]amide (12f)

Compound **12f** was prepared according to the procedure for compound **12e** except using 2-quinoxaline-carboxylic acid, which afford 287 mg (45%) of **12f** as a white solid; mp 157–159 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.19 (m, 1H), 8.11 (m, 2H), 7.90–7.81 (m, 2H), 7.01–6.84 (m, 4H), 3.86 (s, 3H), 3.60 (q, J = 6.3 Hz, 2H), 3.12 (m, 4H), 2.69 (m, 4H), 2.51 (t, J = 7.2 Hz, 2H), 1.75 (m, 4H). Anal. ($C_{24}H_{29}N_5O_2$) C, H, N.

4.13. Benzofuran-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]amide (12g)

Compound **12g** was prepared according to the procedure for compound **12b** except using 2-benzofuran-carboxylic acid, which afford 402 mg (66%) of **12g** as a white solid; mp 120–122 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (m, 1H), 7.48 (m, 1H), 7.46 (m, 1H), 7.40 (m, 1H), 7.29 (m, 1H), 7.03–6.85 (m, 5H), 3.87 (s, 3H), 3.53 (q, J = 6.0 Hz, 2H), 3.14 (m, 4H), 2.71 (m, 4H), 2.50 (t, J = 6.9, 2H), 1.71 (m, 4H). Anal. ($C_{24}H_{29}N_3O_3$) C, H, N.

4.14. Benzo(*b*)thiophene-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]amide (12h)

Compound **12h** was prepared according to the procedure for compound **12b** except using thianaphthene-2-carboxylic acid, which afford 376 mg (59%) of **12h** as a white solid; mp 137–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.78 (m, 3H), 7.44–7.36 (m, 2H), 7.02–6.84 (m, 4H), 6.77 (br, 1H), 3.85 (s, 3H), 3.51 (q, J = 6.3 Hz, 2H), 3.09 (m, 4H), 2.67 (m, 4H), 2.48 (t, J = 6.9 Hz, 2H), 1.70 (m, 4H). Anal. ($C_{24}H_{29}N_3O_2S$) C, H, N.

4.15. 4-(2-Fluoroethyl)-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzamide (12i)

Compound **12i** was prepared according to the procedure for compound **12e** except using **17**, purified with ether–methanol (10:1) to afford 112 mg (54%) of **12i** as a white solid; mp 122–124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 7.04–6.85 (m, 4H), 6.73 (br, 1H), 4.62 (dt, J = 46.8 Hz, J = 6.3 Hz, 2H), 3.86 (s, 3H), 3.49 (q, J = 6.0 Hz, 2H), 3.10–2.98 (m, 6H), 2.66 (m, 4H), 2.48 (t, J = 6.9 Hz, 2H), 1.70 (m, 4H). Anal. ($C_{24}H_{32}FN_3O_2$) C, H, N.

4.16. 5-Methyl-thiophene-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]amide (12j)

Compound **12j** was prepared according to the procedure for compound **12e** except using 5-methyl-thiophene-2-carboxylic acid, which afford 230 mg (59%) of **12j** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (d, J = 3.6 Hz, 1H), 7.02–6.84 (m, 4H), 6.70 (d, J = 3.6 Hz, 1H), 6.25 (br, 1H), 3.85 (s, 3H), 3.45 (m, 2H), 3.08 (m, 4H), 2.64 (m, 4H), 2.48 (s, 3H), 2.44 (t, J = 7.2 Hz, 2H), 1.64 (m, 4H).

4.17. 5-Bromo-thiophene-2-carboxylic acid [4-[4-(2-methoxyphenyl)piperazin-1-yl]-butyl]amide (12k)

Compound **12k** was prepared according to the procedure for compound **12e** except using 5-methyl-thiophene-2-carboxylic acid, which afford 230 mg (51%) of **12k** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 4.2 Hz, 1H), 7.01–6.87 (m, 6H), 3.85 (s, 3H), 3.44 (m, 2H), 3.18 (m, 4H), 2.82 (m, 4H), 2.60 (m, 2H), 1.69 (m, 4H).

4.18. 4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyronitrile (9)

Compound **9** was prepared according to the procedure for compound **8**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (m, 3H), 6.95 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H), 3.07 (m, 4H), 2.65 (m, 4H), 2.56 (t, J = 6.9 Hz, 2H), 2.46 (t, J = 7.2 Hz, 2H), 1.88 (p, J = 6.9 Hz, 2H).

4.19. 4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butylamine (11)

Compound 11 was prepared according to the procedure for compound **10**, purified with CH_2Cl_2–MeOH–$N(C_2H_5)_3$ (10:5:1) to afford 3.65 g (90%) of **11** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (m, 2H), 6.92 (m, 1H), 3.03 (m, 4H), 2.69 (m, 2H), 2.60 (m, 4H), 2.37 (m, 2H), 1.56–1.40 (m, 4H).

4.20. 4-Chloro-*N*-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]-butyl]benzamide (13a)

Compound **13a** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 267 mg (60%) of **13a** as a white solid; mp 140–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.08–7.06 (m, 2H), 6.80 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H), 6.64 (br, 1H), 3.43–3.37 (m, 2H), 2.93 (m, 4H), 2.55 (m, 4H), 2.40 (t, J = 6.6 Hz, 2H), 1.60 (m, 4H). Anal. ($C_{21}H_{24}Cl_3N_3O$) C, H, N.

4.21. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-butyl}-4-fluorobenzamide (13b)

Compound **13b** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 279 mg (66%) of **13b** as a white solid; mp 139–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (dd, J = 8.7 Hz, J = 5.4 Hz, 2H), 7.17–7.08 (m, 4H), 6.91 (dd, J = 6.6 Hz, J = 3.0 Hz, 1H), 6.64 (br, 1H), 3.52–3.46 (m, 2H), 3.04 (m, 4H), 2.66 (m, 4H), 2.50 (t, J = 6.9 Hz, 2H), 1.69 (m, 4H). Anal. ($C_{21}H_{24}Cl_2FN_3O$) C, H, N.

4.22. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-butyl}-4-dimethylaminobenzamide (13c)

Compound **13c** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 202 mg (45%) of **13c** as a white solid; mp 161–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 9.0 Hz, 2H), 7.07–7.04 (m, 2H), 6.84 (dd, J = 6.6 Hz, J = 2.7 Hz, 1H), 6.58 (d, J = 9.0 Hz, 2H), 6.24 (br, 1H), 3.397–3.36 (m, 2H), 2.97 (m, 4H), 2.93 (s, 6H), 2.55 (m, 4H), 2.38 (t, J = 6.9 Hz, 2H), 1.58 (m, 4H). Anal. ($C_{23}H_{30}Cl_2N_4O$) C, H, N.

4.23. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-butyl}-4-methoxybenzamide (13d)

Compound **13d** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 210 mg (48%) of **13d** as a white solid; mp 142–144 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 8.7 Hz, 2H), 7.07–7.05 (m, 2H), 6.83 (d, J = 8.7 Hz, 2H), 6.82 (m, 1H), 6.42 (br, 1H), 3.75 (s, 3H), 3.42–3.36 (m, 2H), 2.96 (m, 4H), 2.55 (m, 4H), 2.39 (t, J = 6.9 Hz, 2H), 1.59 (m, 4H). Anal. ($C_{22}H_{27}Cl_2N_3O_2$) C, H, N.

4.24. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-butyl}-4-methylsulfanylbenzamide (13e)

Compound **13e** was prepared according to the procedure for compound **12e**, purified with ether–methanol (10:1) to afford 361 mg (53%) of **13e** as a white solid;

mp 155–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 7.15–7.13 (m, 2H), 6.89 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H), 6.66 (br, 1H), 3.48–3.44 (m, 2H), 3.03 (m, 4H), 2.63 (m 4H), 2.49 (s, 3H), 2.47 (t, J = 6.9 Hz, 2H), 1.67 (m, 4H). Anal. ($C_{22}H_{27}Cl_2N_3OS$) C, H, N.

4.25. Benzofuran-2-carboxylic acid {4-[4-(2,3-dichlorophenyl)piperazin-1-yl]-butyl}-amide (13f)

Compound **13f** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 317 mg (71%) of **13f** as a white solid; mp 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 7.6 Hz, 1H), 7.49–7.38 9 (m, 3H), 7.31 (d, J = 8.1 Hz, 1H), 7.16–7.13 (m, 2H), 7.02 (br, 1H), 6.94 (dd, J = 7.2 Hz, J = 2.4 Hz, 1H), 3.57–3.51 (m, 2H), 3.11 (m, 4H), 2.68 (m, 4H), 2.48 (t, J = 6.6 Hz, 2H), 1.70 (m 4H). Anal. ($C_{23}H_{25}Cl_2N_3O_2$) C, H, N.

4.26. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]-butyl}-4-(2-fluoroethyl)-benzamide (13g)

Compound **13g** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 221 mg (49%) of **13g** as a white solid; mp 136–138 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.16–7.14 (m, 2H), 6.91 (dd, J = 6.75 Hz, J = 3.0 Hz, 1H), 6.64 (br, 1H), 4.64 (dt, J = 47.1 Hz, J = 6.3 Hz, 2H), 3.53–3.46 (m, 2H), 3.05 (dt, J = 24.3 Hz, J = 6.3 Hz, 2H), 3.03 (m, 4H), 2.64 (m, 4H), 2.49 (t, J = 6.6 Hz, 2H), 1.69 (m, 4H). Anal. ($C_{23}H_{28}Cl_2FN_3O$) C, H, N.

4.27. 5-Methyl-thiophene-2-carboxylic acid {4-[4-(2,3-dichlorophenyl)piperazin-1-yl]-butyl}-amide (13i)

Compound **13i** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 68 mg (35%) of **13i** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, J = 3.6 Hz, 1H), 7.13 (m, 2H), 6.94 (m, 1H), 6.69 (d, J = 3.6 Hz, 1H), 6.35 (br, 1H), 3.44 (m, 2H), 3.07 (m, 4H), 2.67 (m, 4H), 2.48 (s, 3H), 2.47 (m, 2H), 1.65 (m, 4H).

4.28. 5-Bromo-thiophene-2-carboxylic acid {4-[4-(2,3-dichlorophenyl)piperazin-1-yl]-butyl}-amide (13k)

Compound **13k** was prepared according to the procedure for compound **12b**, purified with ether–methanol (10:1) to afford 267 mg (60%) of **13k** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, J = 3.9 Hz, 1H), 7.14 (m, 2H), 7.02 (d, J = 3.9 Hz, 1H), 6.93 (m, 1H), 6.40 (br, 1H), 3.44 (m, 2H), 3.07 (m, 4H), 2.66 (m, 4H), 2.48 (t, J = 6.6 Hz, 2H), 1.66 (m, 4H).

4.29. Dopamine receptor binding assays

A filtration binding assay was used to characterize the binding properties of membrane-associated receptors.[23] For human $D_{2\ Long}$, D_3, and D_4 dopamine receptors expressed in HEK 293 cells, membrane homogenates (50 μL) were suspended in 50 mM Tris–HCl/150 mM NaCl/10 mM EDTA buffer, pH 7.5 and incubated with 50 μL of ^{125}I-IABN[23] at 37 °C for 60 min. Non-specific binding was defined using 20 μM (+)-butaclamol. For competition experiments the radioligand concentration is generally equal to 0.5 times the K_d value and the concentration of the competitive inhibitor ranges over 5 orders of magnitude. Each competition curve was performed using two concentrations of inhibitor per decade and all assays are performed in triplicate. Binding was terminated by the addition of cold wash buffer (10 mM Tris–HCl/150 mM NaCl, pH 7.5) and filtration over a glass-fiber filter (Schleicher and Schuell No. 32). Filters were washed with 10 mL of cold buffer and the radioactivity were measured using a Packard Cobra gamma counter. Estimates of the equilibrium dissociation constant and maximum number of binding sites were obtained using unweighted non-linear regression analysis of data modeled according to the equation describing mass action binding.[24] Data from 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. Competition curves were modeled for a single site and the IC_{50} values will be converted to equilibrium dissociation constants (K_i values) using the Cheng and Prusoff[25] correction. Mean K_i values ± S.E.M are reported for at least three independent experiments.

4.30. 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.[26] Transfected HEK cells were treated with serum-free medium containing 2,8-^{3}H-adenine (ICN) and cells were incubated at 37 °C for 60 min. The media was then replaced with serum-free media containing 0.1 mM 3-isobutyl-1-methylxanthine (Sigma) and incubated for 37 °C for 10 min. Cells and drugs 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.[23,26]

4.31. σ receptor binding assays

The σ_1 receptor binding assay was conducted using guinea pig brain membrane homogenates (100 μg protein). Membrane homogenates were incubated with 3 nM [^{3}H](+)-pentazocine (31.6 Ci/mmol) in 50 mM Tris–HCl (pH 8.0) at 25 °C for either 120 or 240 min. Test compounds were dissolved in ethanol then diluted in buffer for a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris–HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber fil-

ters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL of ice-cold buffer. Non-specific binding was determined in the presence of 10 μM (+)-pentazocine. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50% for tritium.

The σ_2 receptor binding assay was conducted using rat liver membrane homogenates (35 μg of protein). Membrane homogenates were incubated with 3 nM [^{3}H]DTG (38.3 Ci/mmol) in the presence of 100 nM (+)-pentazocine to block σ_1 sites. Incubations were carried out in 50 mM Tris–HCl (pH 8.0) for 120 min at 25 °C in a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris–HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL of ice-cold buffer. Non-specific binding was determined in the presence of 5 μM DTG. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50% for tritium.

The IC_{50} values at sigma sites were generally determined in triplicate from non-linear regression of binding data as analyzed by JMP (SAS Institute; Cary, NC), using eight concentrations of each compound. K_i values were calculated using the method of Cheng–Prusoff[25] and represent mean values ± SEM. All curves were fit to a one site fit and gave Hill coefficients of 0.8–1.0. The K_d value used for [^{3}H]DTG in rat liver was 17.9 nM and was 4.8 nM for [^{3}H](+)-pentazocine in guinea pig brain.[14,15]

Acknowledgements

This research was funded by grants DA 12647 and DA 16181 awarded by the National Institute on Drug Abuse.

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