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Novel (Bisarylmethoxy)butylpiperidine Analogues as Neurotransmitter Transporter Inhibitors with Activity at Dopamine Receptor Sites

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Abstract—A series of (bisarylmethoxy)butylpiperidine derivatives was prepared and evaluated in vitro and in vivo to determine the structural requirements necessary for dual activity at the DAT and DA/5-HT receptor sites. These hybrid ligands, constructed by combining pharmacophores specific for the DAT and DA/5-HT receptors, could be useful drugs for treating cocaine addiction by assisting cocaine addicts in maintaining abstinence. The series was evaluated in vitro for DAT and DA/5-HT receptor activity and then selected compounds were tested in vivo for their effects on cocaine-induced hyperlocomotor activity (LMA). The majority of the new compounds demonstrated high to moderate affinity (4–191 nM) for the DAT with 4-hydroxy-4-phenylpiperidine analogues **14** and **15** possessing the greatest affinity. Compounds **15** and **22** exhibited the highest ratio of reuptake inhibition-to-binding (discrimination ratio, DR), 111 and 323, respectively. These derivatives had modest affinity and antagonistic activity for dopamine D_2/D_3 receptors. Compounds **9** and **15** (DR = 0.9 and 111, respectively) stimulated locomotor activity, whereas the other compounds suppressed this response. All compounds tested except for **17** and **21** attenuated cocaine-induced hyperlocomotion.

Introduction

Cocaine addiction represents one of the major public health problems in American society today.[1] Efforts have been directed toward identifying and developing new therapeutic agents that can mediate cocaine's acute behavior and reinforcing effects;[2] however, at present there are no effective long-term pharmacotherapy that can lessen the abuse of cocaine.

Cocaine is pharmacologically a nonselective drug interacting in the central nervous system at the dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET) sites by inhibiting reuptake of the dopamine (DA), serotonin (5-HT), and norephrine (NE) neurotransmitters, respectively. Substantial evidence indicates that the DAT is a critical recognition site that contributes significantly to the reinforcing properties of cocaine.[3,4] Hence, therapeutic drug development has focused on designing agents that target the DAT.

Dopamine agonists and antagonists as medications for cocaine abuse have been evaluated and extensively reviewed.[5] Although the behavioral effects of cocaine are not consistently altered by selective dopamine antagonists,[6] many studies have reported their mediation of the reinforcing effects of cocaine.[7] The dopamine D_3 receptor has been of particular interest because of its relatively restricted localization within the limbic system compared with the dopamine D_2 receptor and due to its role as a possible target for treatment of schizophrenia and drug abuse.[8] A recent report of selective inhibition of cocaine-seeking behavior by a partial dopamine D_3 receptor agonist suggested that this receptor is an important target for the development of medications for cocaine abuse.[9]

Comparison of IC_{50} values for inhibition of [^{3}H]DA reuptake and K_i values for DAT ligand-binding affinity (e.g., [^{125}I]RTI-55) has been suggested as a method for evaluating cocaine antagonists or partial agonists in

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vitro.[10,11] In this context, important cocaine antagonists would display high discrimination ratios (ratio of reuptake inhibition-to-binding, DR) under comparable experimental conditions.[10] Although DR values from in vitro assays have not always been predictive of in vivo activities such as locomotor activation or depression, drug discrimination or substitution for cocaine and anti-cocaine activity, they can serve as an initial in vitro screening process.[11] While a pure cocaine antagonist at the DAT may be of interest in preventing the reinforcing effects of cocaine, a compound with some residual DA reuptake inhibition may have greater potential for the treatment of cocaine abuse. Such agents may serve to provide some relief from cocaine craving and alleviate the withdrawal symptoms encountered during initial stages of abstinence.

Various DAT ligands have been proposed as potential therapeutic agents for cocaine abuse including cocaine analogues[12] and disubstituted piperazine (GBR) analogues.[13,14] GBR analogues are particularly interesting because their pharmacologic properties include slow dissociation from the DAT, high transporter selectivity, and low intrinsic activities.[15] Oxygenated GBR analogues have been evaluated as DAT-specific, potentially long acting, anti-cocaine therapeutics for cocaine addiction.[16] Although the behavioral profile of GBR 12909 (**1**, Figure 1) was shown to be different from that of cocaine, this compound was reliably self-administered in nonhuman primates and fully substituted for cocaine in drug discrimination studies.[17–20] Such studies indicate that GBR 12909 might have the potential for abuse in humans, albeit to a lesser extent compared to cocaine. Importantly, when the dopamine D2-selective antagonist, spiperon, was used to pretreat monkeys, the self-administration of GBR 12909 was attenuated.[18] In related studies, the 5-HT agonist, quipazine, reliably attenuated the behavioral-stimulus effects of GBR 12909.[20] Therefore, assuming actions at the DAT are responsible for the behavioral effects of GBR 12909, it is possible that combining strong DAT binding properties and dopamine antagonistic and/or 5-HT agonistic properties in one molecule may offer a more effective treatment with less abuse liability potential than GBR 12909 and fewer side-effects observed from dopamine D2, D3 antagonist monotherapy. Although our explanation is speculative without previous neurobehavioral data from dual activity ligands, such compounds should block the binding of cocaine to the DAT with weak dopamine reuptake inhibition. Such residual dopamine uptake inhibiting properties will then be attenuated by activities at the dopamine receptor site(s) as antagonist and/or 5-HT agonist. Therefore, abuse liability that may arise from the activity of dopamine reuptake inhibition can be minimized.

As part of our program to develop potent DAT inhibitors, we have investigated numerous GBR analogues to identify structural features that confer high binding affinity for the DAT but weaker potency for inhibition of DA reuptake.[21,22] By extending our studies to measurements of DA/5-HT receptor activity, we have anticipated that some agents might influence the dopa-

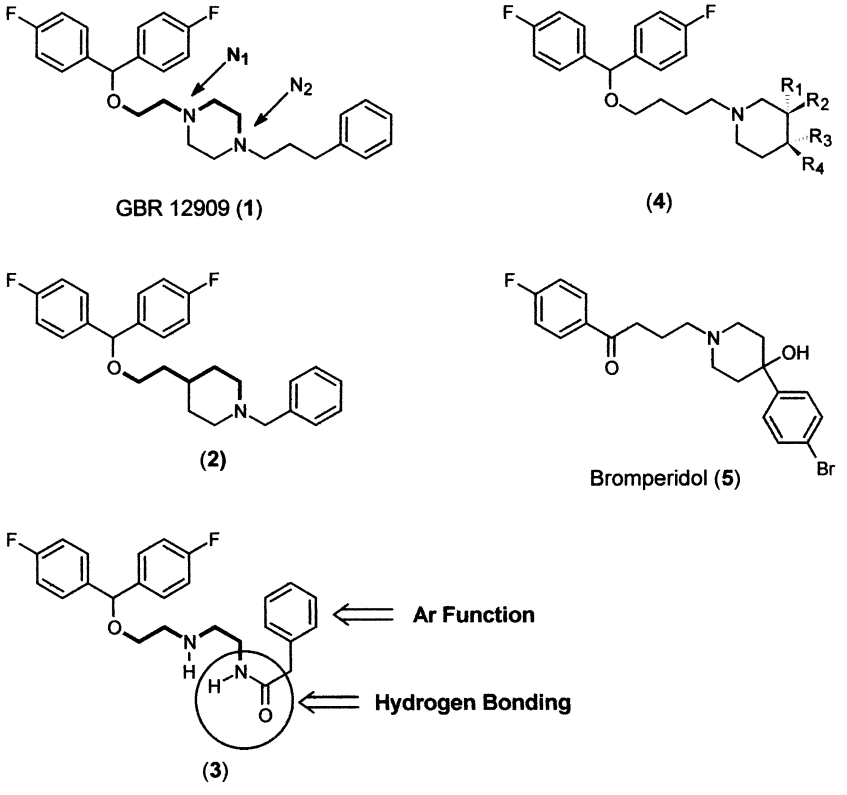


Figure 1.

minergic system in novel ways. In the current study, we used a hybrid drug approach which combines pharmacophores specific for the DAT and DA/5-HT receptors.

Structure–activity relationship (SAR) studies have demonstrated that the (bisarylmethoxy)ethyl and diamine moieties of GBR (**1**) are essential pharmacophores for retaining favorable activities at the DAT.[13,14,22] Also, at least one nitrogen atom (**2**) attached to the benzyl moiety is required.[14,23] Previously, the rigid piperazine ring of GBR (**1**) had been substituted with conformationally flexible polymethylene diamines and amine-amide moieties (**3**) without compromising biologic activities.[22] Tolerance of the amide function at the N2 nitrogen atom was interpreted as due to the possible presence of strong hydrogen-bonding capabilities at binding site(s) on the DAT protein. These acyclic analogues have provided a basis for the design of novel probes that demonstrate selective and high affinity binding to the DAT and weak potency for DA reuptake inhibition. In order to develop compounds with a mixed pharmacologic profile containing both DAT activity (binding/reuptake inhibition) and dopamine antagonistic/5-HT agonistic properties, we incorporated structural moieties believed to induce dopamine D_2/D_3 antagonism such as 4-aryl-4-piperidinol found in bromperidol and haloperidol and 5-HT activity found in arylpiperazine.[24,25] Compound selection was also based on H-bonding and aryl functionality as elucidated in our previous findings with amine–amide analogues.[26] Ahmad et al.[27] reported benzhydroxyalkylpiperidine derivatives that were non-selective reuptake inhibitors of DA, 5-HT, and norepenephrine. As shown in the general structure **4**, several important aspects of SAR were identified, including distance between the diaryl and piperidine moieties (*n*, distance as shown by the bold line in **1**–**3**) and the nature of the substituents (R_1–R_4) on the piperidine ring. A specific three-dimensional arrangement of these groups (distance, basic nitrogen, hydrogen bonding, and Ar function) was deduced to test the hypothesis concerning which chemical groups (H-bonding, Ar function, and distance) are important for a particular biologic activity and what is the transporter-bound conformation of these groups. The results of our work, together with that of other investigators, led to the preparation of several arylpiperidine derivatives. In this study, we report the preparation and pharmacologic evaluation of a series of (bisarylmethoxy)butylpiperidine derivatives having mixed activities at the DAT and DA/5-HT receptor sites.

Results and Discussion

Chemistry

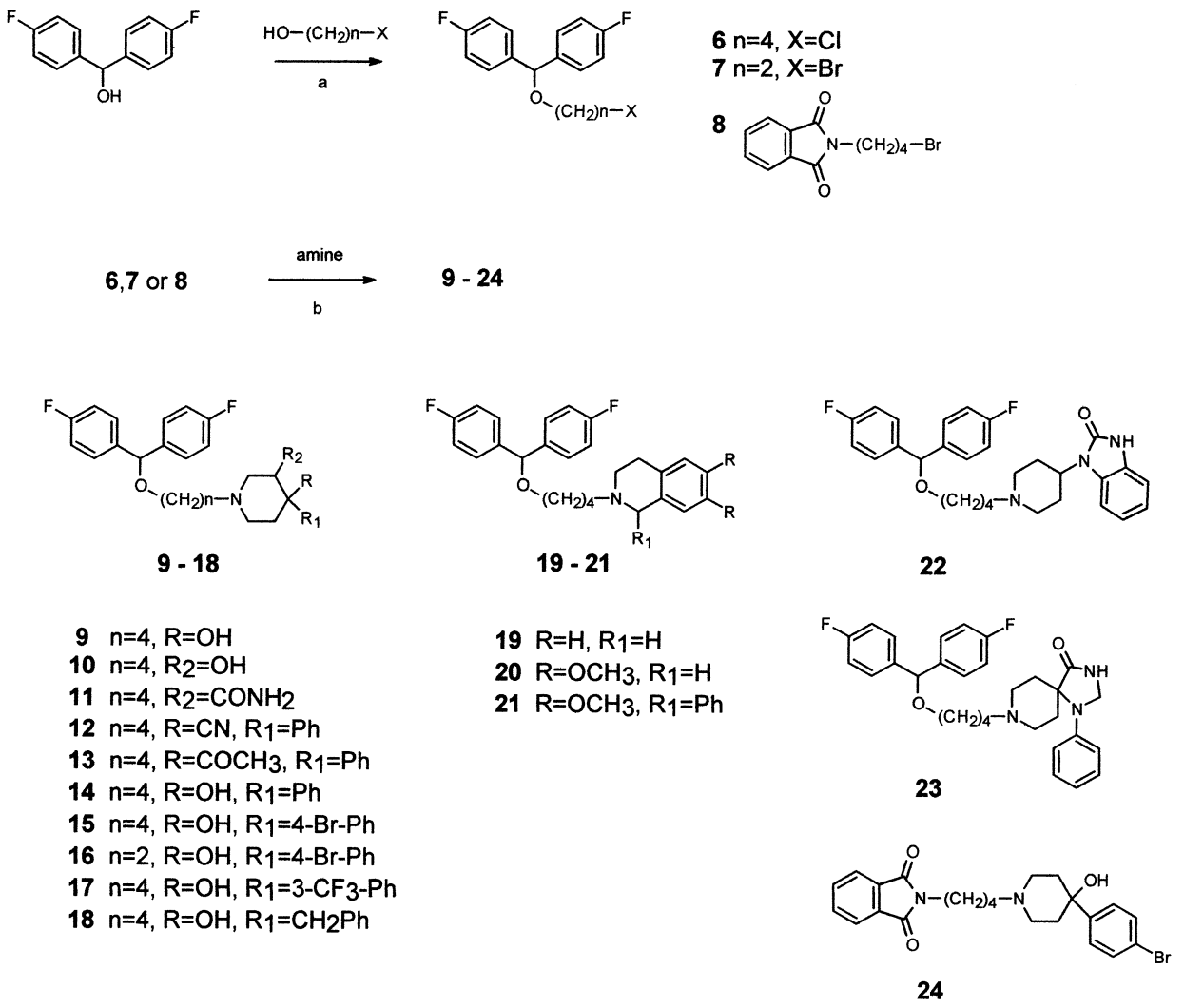
The preparation of the GBR analogues is depicted in Scheme 1. The requisite starting materials **6** and **7**[22] were prepared according to literature procedures with or without modifications, and **8** was purchased. The *N*-alkylated target compounds were prepared in good yields (73–87%) by alkylation of the appropriate amine with alkylating agents (**6**–**8**) in the presence of K_2CO_3 as base and KI as catalyst. The final products were converted into oxalate salts for elemental analysis and biologic evaluation.

In vitro characterization

This research was designed to develop new agents with higher DAT affinity and low potency for DA reuptake inhibition by selective modification of the substituents on a piperidine ring template structure (**4**). In order to find new lead compound(s) for potential cocaine antagonists, we focused on the introduction of new moieties for hydrogen bonding and aromatic function. Such modifications are presumed to be critical structural determinants for dual activity at DAT and DA/5-HT receptor sites. A new series of (bisarylmethoxy) alkylpiperidine analogues was prepared and characterized. The analogues were evaluated for their ability to displace [^{125}I]RTI-55, a cocaine analogue (K_i values in Table 1) as well as for potency of reuptake inhibition of DA, 5-HT and NE (IC_{50} values in Table 2) with HEK 293 cells expressing cDNA for human dopamine transporter (hDAT), human serotonin transporter (hSERT) or human norepinephrine transporter (hNET). In addition, some compounds were evaluated for their ability to bind and function at DA and 5-HT receptor sites (Table 3). Our initial targets were dopamine D2 and D3 receptors since the target compounds contain pharmacophores for dopamine receptors as well as 5-HT receptors as non-selective antagonists (mostly dopamine D2 and D3). However, it seems reasonable to test all dopamine receptor subtypes to get a wider view of ligand effects on dopaminergic and serotonergic systems, especially regarding the interpretation of locomotor activities. The final products were submitted to the National Institute on Drug Abuse, Cocaine Treatment Development Program (CTDP) for pharmacologic screening (Table 4).

Neurotransporter activities[28]

Substitution on the piperidine ring by –OH and $–CONH_2$ groups (**9**–**11**) without an aromatic group produced moderate binding activity (K_i = 91–114 nM) with low selectivity at the DAT (DR = 0.6–1.3). The introduction of an acetyl group and aromatic function at the 4-position of the piperidine ring (**13**) resulted in a compound that retained significant binding activity at the DAT with a DR of 48. Substitution of $–COCH_3$ with –CN (**12**, non-H-bonding group) decreased activities dramatically. Further modification of the acetyl group to a hydroxyl group (**14**) caused a marked increase in DAT affinity with reduced selectivity at the SERT (6–3) and decreased DR (48–15). When a large halogen atom (Br, **15**) was introduced at the 4-position of the aromatic ring, selectivity and potency of binding at the DAT were improved (**15** vs **14**) and a significant increase in DR (15–111) was achieved. A change in alkyl chain length (*n* = 4–2, **15** vs **16**) decreased activities at the DAT (K_i = 4–191 nM, DR = 111–37). Substitution of the bisarylmethoxy group with the phthalimide group (**24** vs **14**) caused loss of activities in some cases. Introduction of the $–CF_3$ group at the 3-position of the aromatic group (**14**–**17**) resulted in weakly selective activities at



Scheme 1. (a) concd H_2SO_4, $PhCH_3$; (b) K_2CO_3, KI, DMF, 60–70 °C.

the NET site ($K_i = 10$ nM, $IC_{50} = 780$ nM). When an aryl group was attached to the piperidine by a CH_2 linkage (benzyl group, **18**), selective binding at the DAT was maintained but the selectivity of reuptake inhibition was shifted to the NET site from the DAT site (**14**, **15** vs **18**). When the aromatic group was attached directly to the piperidine ring (**19**, **20**) without an H-bonding group, the binding was strongest at the SERT site ($K_i = 18$ and 25 nM, respectively) while selective reuptake inhibition of NE was maintained. Additional aromatic groups on the piperidine ring (**20**–**21**) were detrimental and resulted in loss of activity at the DAT. Compounds **22** and **23** contained both H-bonding (as amide) and Ar groups but the activities at the transporters were different for each compound. Compound **22** represents the strongest binding ligand at the DAT ($K_i = 1$ nM) and has the highest DR value (323). From this limited data set, it is apparent that the potency in the DAT binding assay and reuptake inhibition are relatively dependent on substitution and position on the piperidine ring.

DA/5-HT receptor activities[29]

Compounds **14**–**17**, **22** and **23** were evaluated for their ability to bind and function at DA and 5-HT receptor sites which is based on the fact that 4-hydroxy-4-arylpiperidines are the basic pharmacophore for activity at DA receptor sites. The potency and selectivity of the selected target compounds for DA and 5-HT receptors were evaluated using ligand displacement assays (see Table 3 for radioligands and cell types used). D_2 and D_3 antagonistic activities were assessed in vitro by the ability of the compounds to block [^{3}H]thymidine incorporation (inhibition of mitogenesis) induced by 10 nM quinpirole in CHO_p-D_2 and D_3 cells (Table 3). Most compounds evaluated had weak to moderate affinity and antagonistic activity at D_2 and/or D_3 sites. (Bisarylmethoxy)-butylpiperidines (**4**) are nonselective neurotransmitter inhibitors,[27] and bromperidol (**5**) is a potent nonselective dopamine D_2/D_3 receptor antagonist.[30] The combination of both structural elements in **15** provided a compound that was both a very potent DAT binder (DR value of 111) and a moderate dopamine D_2/D_3 receptor antagonist.

Table 1. Binding affinities and selectivities of bisarylmethoxybutylpiperidine derivatives at the DA, 5-HT, and NE transporters labeled with [^{125}I]RTI-55

Compound	Binding ($K_i \pm$SD [nM])			Ratios	
	DAT	SERT	NET	SERT/DAT	NET/DAT
9	114±27[a]	423±98[a]	610±130[a]	3.7	5.4
10	91±26	437±66	611 ±76	4.8	6.7
11	94±42	132±26	578±52	1.4	6.1
12	135±34	311±58	368 ±14	2.3	2.7
13	21.7±8.0	126±45	114±4.1	5.8	5.3
14	6.4±1.2	17.9 ±8.8	54±16	2.8	8.4
15	3.9±1.8	38±10	250±110	9.7	64.1
16	191±78	590±180	1240±310	3.1	6.5
17	15.8±6.1	57±16	10±1.9	3.6	0.6
18	41±12	62±27	175±47	1.5	4.3
19	66±20	18.4±4.1	260±41	0.3	3.9
20	73±33	25.3±7.2	371±35	0.4	5.1
21	375±90	3000±1100	4600±1800	8	12.3
22	1.32±0.49	3.8±1.9	22.2±3.6	2.9	16.8
23	125±22	192±95	356±36	1.5	2.9
24	>10 μM	1590±43	>10 μM	<0.2	1
GBR 12909	27±8	186±30	163±39	6.9	6.0
Cocaine[b]	915±214	419±12	600±216		
Cocaine[c]	258±23	343±31	1740±180		
Cocaine[d]	573±54	402±62	2040±240		
Cocaine[e]	350±45	260±9.4	1610±300		
Cocaine[f]	271±65	217±23	1730±280		
Cocaine[g]	621±45	496±37	1400±320		

[a]Results are average±SEM of three independent experiments assayed in triplicate.
[b]Cocaine as reference for GBR 12909.
[c]Cocaine as reference for **9**, **10**, **14**, **16**, **22**.
[d]Cocaine as reference for **11**, **24**.
[e]Cocaine as reference for **12**, **17**, **19**.
[f]Cocaine as reference for **13**–**15**.
[g]Cocaine as reference for **18, 20, 21, 23**.

Table 2. DA, 5-HT and NE reuptake inhibition and ratios of reuptake inhibition to binding of (bisarylmethoxy)butylpiperidine/piperazine derivatives at the DA transporters

Compound	Reuptake inhibition ($IC_{50} \pm$SD [nM])			Discrimination ratios
	[^{3}H]DA	[^{3}H]5-HT	[^{3}H]NE	[^{3}H]DA reuptake inhibition/DAT binding
9	101±11[a]	314±71[a]	143±50[a]	0.9
10	114±28	860±180	310±110	1.3
11	56±16	203±78	158±60	0.6
12	415±87	2030±190	393±30	3.1
13	1030±330	>10 μM	2250±950	47.5
14	96±11	136±41	179±47	15.0
15	431±88	3070±830	1870±670	110.5
16	7080±510	>10 μM	>10 μM	37.1
17	1000±160	3800±1300	780±310	63.3
18	184±47	570±110	30.7±8.3	4.5
19	1750±730	2100±400	1410±300	26.5
20	440±120	1700±390	159±21	6.0
21	3100±1400	3070±740	>10 μM	8.3
22	426±54	570±210	132±11	323
23	3490±220	111±31	64±19	27.9
24	>10 μM	361±68	>10 μM	1
GBR 12909	246±142	584±237	532±183	9.1
Cocaine[b]	152±24	425±114	445±205	
Cocaine[c]	276±22	301±53	264±57	
Cocaine[d]	237±41	348±66	190±38	
Cocaine[e]	417±35	405±39	239±26	
Cocaine[f]	278±53	189±31	209±36	

[a]Results are average±SEM of three independent experiments assayed in triplicate.
[b]Cocaine as reference for GBR 12909.
[c]Cocaine as reference for **9**, **10**, **14**, **16**, **22**.
[d]Cocaine as reference for **11**, **24**.
[e]Cocaine as reference for **12**, **17**, **19**.
[f]Cocaine as reference for **13**–**15**.

Table 3. Receptor binding profile and effects of selected target compounds **14**–**17**, **22**, and **23**

Receptor	Affinity ($K_i \pm$SD [nM])[a] Antagonist activity (IC_{50} [nM])[b]					
	14	**15**	**16**	**17**	**22**	**23**
D_1	2460±38.1[c]	3050±1440	172±10.3	1570±523	1640±527	1890±763
D_2	22.6±0.5[a]	27.6±0.8	51.5±14.6	37.4±5.0	17.0±6.17	18.8±4.32
	136±1.3[b]	90.6±40.3	42.3±13.6	70.1±13.0	48.7±2.67	67.8±1.22
D_3	239±121[a]	56.8±34.1	143±5.1	65.2±20.9	15.8±1.08	33.0±13.8
	52.0±4.8[b]	91.3±28.8	174±50.2	85.9±30.6	87.4±17.0	77.3±6.26
5-HT_{1A}	1400±247	2870±693	>10 μM	1130±37.5	857±123	682±77.7
5-HT_{2A}	566±45.5	580±44.4	639±107	621±83.3	580±58.1	241±64.7
5-HT_{2C}	5870±4140	5790±419	4300±411	6390±1760	3100±235	>10 μM
5-HT_3	2470±46.6	5270±594	nt[d]	nt	nt	nt

[a]Binding assay receptors/radioligands: D_1 (human cloned receptors in LHD_1 cells/[^{3}H]SCH 23,390); D_2 and D_3 (human cloned receptors in CHO_p cells/[^{3}H]YM-09151–2); 5-HT_{1A} (human cloned receptors in HA7 cells/[^{3}H]8-OH-DPAT); 5-HT_{2A} (rat receptors in NIH-3T3-GF6 cells/[^{3}H]ketanserin); 5-HT_{2C} (rat receptors in NIH-3T3-Pϕ cells/[^{3}H]mesulergine); 5-HT_3 (rat/mouse hybrid receptors in NG108–15 cells/[^{3}H]GR65630).
[b]D_2, D_3 antagonist assays: CHO_p cells (human receptor), [^{3}H]thymidine incorporation (inhibition of mitogenesis), quinpirole as internal standard (ED_{50} range 6.5–57 nM for D_2 receptor, ED_{50} range 2.8–25 nM for D_3 receptor).
[c]All values represent the mean of at least two determinations.
[d]nt = Not tested.

Thus, the 4-hydroxy-4-phenylpiperidine moiety provides additional binding to the DAT. Analogues **14**–**16** resemble the antipsychotic bromperidol and were found to be moderate affinity DA antagonists. Addition of aryl and hydroxyl groups at the 4-position of the piperidine ring provided a desirable combination of DAT and DA receptor activity. The 4-aryl-4-piperidinol analogues **14** and **15** demonstrated very weak binding at the D_1 and 5-HT receptor sites. Analogues **17**, **22**, and **23**, each containing a partial structure of a DA antagonist (**17**, trifluperidol; **22**, pimozide; **23**, spiperone), also showed moderate activity at the D_2/D_3 receptor sites. Most compounds had selective and weak activity at the 5-HT_{2A} receptor (K_i = 241–639 nM). As expected, the bulky bisaryl group was not well tolerated in terms of DA activities.

In vivo locomotor activity

Based on their in vitro activities at the DAT, dopamine D_2/D_3 receptors and DR values, compounds **15**–**17** (high DR) and **9**, **18**, **21** (low DR) were selected for preliminary behavioral screening which involved testing alone and in combination with cocaine for their effect on locomotor activity (LMA) in mice. Likewise, the effects of the dopaminergic antagonists (**14**–**17**, and **23**) on cocaine-induced hyperactivity were studied. Compound **22** was not studied even though it showed a high DR value at the DAT and SERT because NE reuptake inhibition was strong.[31] As summarized in Table 4, compounds **9** and **15** produced a significant increase in spontaneous LMA with low ED_{50} compared with GBR 12909. Significant suppression of spontaneous LMA was demonstrated by all the compounds except **9** and **15**. Compound **15** produced a biphasic effect on LMA; activity was increased with low doses and decreased with high doses. Biphasic effects on spontaneous LMA by dopamine D_2/D_3 dopaminergic antagonistic ligands have been previously demonstrated with other compounds.[32] Since this ligand possesses activities at the DAT and is a moderate dopamine D_2/D_3 antagonist, its effects on LMA are probably the result of activities at both DAT and DA receptor sites. Maximal stimulatory effect on spontaneous LMA by **9** and **15** is only 40–80% of that achieved with cocaine as shown by the ratio of ME/CME in Table 4. Attenuation of cocaine-induced LMA (20 mg/kg cocaine) was demonstrated by most of the compounds. Only compounds **17** and **21** failed to attenuate the hyperlocomotion induced by cocaine. Compound **24** showed very weak receptor activities (2.4 μM for D_2, 1.8 μM for D_3, data not shown) and weak 5-HT reuptake inhibition (361 nM, Table 2). No activity at the DAT and NET sites was identified; however, hyper LMA caused by cocaine was attenuated. Correlations between DR values and ED_{50} or AD_{50} were not found, although other factors such as potency as D_2/D_3 antagonists and pharmacokinetics (uptake rate into brain) might be critical for the potency in LMA studies.

Table 4. Effect of compounds **9**, **13**, **15**–**18**, **21**, **23** and **24** on locomotor activity in mouse

Compound	ED_{50} (mg/kg)[a] ID_{50} (mg/kg)[b]	ME/CME[c]	AD_{50} (mg/kg)[d]
9	6.6/S[a]	0.78	77.6
13	170/I[b]		109
15	2.42/S[a,e]	0.38	15.8
16	14.1/I[b]		13.8
17	95.7/I[b]		No attenuation
18	50.7/I[b]		16.4
21	132/I[b]		No attenuation
23	49.2/I[b]		45.7
24	82.8/I[b]		40.1
GBR 12909	6.24/S[a]	1.22	Biphasic[f]

[a]ED_{50} = dose producing 1/2 maximal stimulant activity, S = stimulation.
[b]ID_{50} = dose-producing 1/2 maximal depressant activity, where maximal depression = 0 counts/30 min, I = inhibition.
[c]Maximal effect (ME)/cocaine maximal effect (CME).
[d]AD_{50} = dose attenuating cocaine-induced stimulation by 50%, ligand–cocaine interaction study.
[e]Ligand study, see text for details; in general low doses produce stimulation and high doses inhibition.
[f]10 mg/kg enhanced and 50 mg/kg depressed 30–60 min postinjection.

It must be noted that ID_{50} is similar to ED_{50} (LMA increase or decrease) and a high ID_{50} value means low intrinsic activities of LMA inhibition for the compound alone, whether as a weak DA receptor antagonist or with other unknown activities. But if a compound also has a low AD_{50}, this means a strong counter action against cocaine at low dosage. Such activities can be explained with other in vitro activities and with other pharmacophysical properties of the ligand. High ID_{50} and low AD_{50} values (discrimination and self-administration) are currently important criteria for the selection of ligands for in vivo testing by NIDA.

Biphasic effects on spontaneous LMA by D_2/D_3 dopaminergic antagonistic ligands have been previously demonstrated with other compounds. Activities at the D_2/D_3 autoreceptors might explain some of possible underlying mechanisms of biphasic activities. Since these ligands possess activities at the DAT and are moderate inhibitors of DA reuptake, their effects on LMA are probably the result of activities at both DAT and DA receptor sites. The biphasic activity as stimulant or inhibitant cannot be explained with only in vitro receptor activities and DAT activities because the other sites also can modulate dopaminergic activities. However, the time course LMA studies will clearly demonstrate whether such compounds have beneficial long-term effects or not which also can explain pharmacokinetic factors.

Conclusions

The introduction of hydroxyl and/or aromatic substituents to the piperidine ring of template structure **4** produced retention of transporter affinity and DA reuptake inhibition. Such modification resulted in DA receptor affinity and selectivity when compared with 5-HT receptors. These data give insight into possible structural similarities between ligand-binding sites on the DAT and DA receptors. The current studies have provided additional information on pharmacophores that are responsible for selective binding and potency of reuptake inhibition at the DAT and on activities at the DA/5-HT receptor sites. Although correlation between in vitro DR values and in vivo potency in LMA activities was not observed, these new analogues inconjuction with the results of the LMA studies may provide a basis for the design of other ligands with strong binding to the DAT, low potency inhibition of DA reuptake, and binding to DA/5-HT receptor sites.

Experimental

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses, performed by Atlantic Microlabs, Atlanta, GA, were within 0.4% of calculated values. ^{1}H NMR spectra were recorded on a Varian XL-500 spectrometer. In general, $CDCl_3$ was used for the free bases and DMSO-d_6 for salts. Thin layer chromatography (TLC) was performed on 250-μm-thick silica gel plates or alumina precoated plates (Whatman, AL SIL G/UV or J.T. Baker, Baker-flex, SILICA GEL IB-F) containing fluorescent indicator (2×8 cm). Column chromatography was performed on silica gel (Baker, 40 μm Flash chromatography). Fractions were analyzed using TLC and compounds were visualized with ninhydrin (0.5 g/100 mL methanol) or iodine vapor for primary and secondary amine(s). Free bases were dissolved in ethyl acetate and/or diethyl ether, filtered, and precipitated by addition of a solution of oxalic acid. The resulting solids were collected by filtration and recrystallized.

Synthetic procedures

1-[bis(4-Fluorophenyl)methoxy]-4-chlorobutane (6). A mixture of 4-chloro-1-butanol (6.51 g, 60 mmol), 1 mL concentrated sulfuric acid, and 4,4′-difluorobenzhydrol (2.2 g, 10 mmol) in 50 mL toluene was heated at reflux for 12 h. The reaction mixture was cooled, washed successively with saturated sodium bicarbonate solution (50 mL) and water (50 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvent was removed by rotary evaporation. The resultant brown oil was purified on a silica gel column. Elution with 2% ethyl acetate–hexane afforded 2.33 g (7.5 mmol, 75%) of the product as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.71–1.80 (2H, m), 1.84–1.93 (2H, m), 3.43 (2H, t, $J=6.0$), 3.52 (2H, t, $J=6.4$), 5.30 (1H, s) 6.95–7.02 (4H, m), 7.24–7.29 (4H, m).

1-[bis(4-Fluorophenyl)methoxy]-2-bromoethane (7). As described for **6**, **7** was prepared from 2-bromo-1-ethanol and 4,4′-difluorobenzhydrol.

***N*-(4-Bromobutyl)-phthalimide (8).** This compound was purchased from Sigma Chemical Company.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-hydroxypiperidine (9). 4-Hydroxypiperidine (1.2 g, 5 mmol) was dissolved in 20 mL anhydrous DMF and stirred with K_2CO_3 powder (2.76 g, 20 mmol) and KI (100 mg, 0.6 mmol) for 0.5 h. To this turbid solution, **6** (1.55 g, 5 mmol) in 10 mL DMF was added dropwise. The reaction mixture was stirred for 72 h at 60–70 °C. The turbid reaction mixture was poured into 200 mL ethyl acetate, washed with saturated sodium chloride solution (60 mL×3), dried over magnesium sulfate, and evaporated to dryness. The crude oil was applied to a silica gel column for purification. Elution with $CHCl_3$/MeOH (98:2) afforded the desired alkylated product **9** as a colorless oil (87% yield). ^{1}H NMR ($CDCl_3$): δ 1.54–1.66 (6H, m), 1.86–1.89 (2H, m), 2.09 (2H, t, $J=10.0$), 2.31 (3H, t, $J=7.5$ overlap with br s), 2.74–2.76 (2H, m), 3.42 (2H, t, $J=6.3$), 3.66 (1H, heptet, $J=4.5$), 5.28 (1H, s), 6.98–7.02 (4H, m), 7.25–7.29 (4H, m); mp (oxalate salt): 117–120 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-3-hydroxypiperidine (10). As described for **9**, **10** was prepared from **6** and 3-hydroxypiperidine as a colorless oil (87% yield). ^{1}H NMR ($CDCl_3$): δ 1.48–1.67 (7H, m), 1.76–1.79 (1H, m), 2.26 (1H, br s), 2.33 (2H, t, $J=7.3$), 2.41–2.45 (4H, m), 3.42 (2H, t, $J=6.3$), 3.80 (1H, br s), 5.28 (1H, s),

6.99–7.03 (4H, m), 7.26–7.29 (4H, m); mp (oxalate salt): 106–109 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-3-piperidine carboxamide (11). As described for compound **9**, **11** was prepared from **6** and nipecotamide (Sigma Chemical Company) as a colorless oil (78% yield). ^{1}H NMR ($CDCl_3$): δ 1.48–1.67 (7H, m), 1.76–1.79 (1H, m), 2.26 (1H, br s), 2.33 (2H, t, J = 7.3), 2.41–2.45 (4H, m), 3.42 (2H, t, J = 6.3), 3.80 (1H, br s), 5.28 (1H, s), 6.99–7.03 (4H, m), 7.26–7.29 (4H, m); mp (oxalate salt): 143–146 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-phenylpiperidine-4-carbonitrile (12). As described for compound **9**, **12** was prepared from **6** and 4-cyano-4-phenylpiperidine hydrochloride as a colorless oil (83% yield). ^{1}H NMR ($CDCl_3$): δ 1.60–1.70 (4H, m), 2.05–2.12 (4H, m), 2.42–2.48 (4H, m), 2.99 (2H, br d, J = 12.5), 3.44 (2H, t, J = 6.5), 5.29 (1H, s), 6.96–7.00 (4H, m), 7.27–7.30 (5H, m), 7.35–7.38 (2H, m), 7.48–7.50 (2H, m); mp (oxalate salt): 176–178 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-acetyl-4-phenylpiperidine (13). As described for **9**, **13** was prepared from **6** and 4-acetyl-4-phenylpiperidine hydrochloride as a colorless oil (73% yield). ^{1}H NMR ($CDCl_3$): δ 1.56–1.68 (4H, m), 1.90 (3H, s), 2.06 (2H, t d, J = 11.8, 2.7), 2.21 (2H, br t, J = 10.8), 2.30 (2H, t, J = 7.1), 2.46 (2H, br d, J = 11.7), 2.71 (2H, br d, J = 10.5), 3.42 (2H, t, J = 5.9), 5.28 (1H, s), 6.96–7.02 (4H, m), 7.25–7.37 (9H, m); mp (oxalate salt): 138–140 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-phenylpiperidin-4-ol (14). As described for **9**, **14** was prepared from **6** and 4-phenyl-4-piperidinol as a colorless oil (79% yield). ^{1}H NMR ($CDCl_3$): δ 1.58–1.73 (6H, m), 2.13 (2H, t d, J = 13.0, 4.5), 2.37 (2H, t, J = 7.2), 2.43 (2H, br d, J = 11.8), 2.76 (2H, br d, J = 11.1), 3.42 (2H, t, J = 5.6), 5.28 (1H, s), 6.96–7.03 (4H, m), 7.22–7.34 (7H, m), 7.49 (2H, d, J = 7.9); mp (oxalate salt): 168–170 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-(4-bromophenyl)-4-piperidinol (15). As described for **9**, **15** was prepared from **6** and 4-(4-bromophenyl)-4-piperidinol as a colorless oil (80% yield). ^{1}H NMR ($CDCl_3$): δ 1.60–1.73 (6H, m), 2.12 (2H, t d, J = 12.8, 2.9), 2.39–2.45 (4H, m), 2.80 (2H, br d, J = 10.9), 3.43 (2H, t, J = 5.4), 5.29 (1H, s), 6.97–7.03 (4H, m), 7.26–7.34 (4H, m), 7.37 (2H, d, J = 8.7), 7.46 (2H, d, J = 8.4); mp (oxalate salt): 156–158 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]ethyl]-4-(4-bromophenyl)-4-piperidinol (16). As described for **9**, **16** was prepared from **7** and 4-(4-bromophenyl)-4-piperidinol as a colorless oil (76% yield). ^{1}H NMR ($CDCl_3$): δ 1.67 (2H, br d, J = 12.0), 2.07 (2H, t d, J = 12.1, 4.0), 2.14 (1H, br s), 2.52 (2H, t d, J = 11.8, 2.5), 2.71 (2H, t, J = 5.8), 2.78 (2H, br d, J = 12.0), 3.57 (2H, t, J = 6.0), 5.32 (1H, s), 6.97–7.02 (4H, m), 7.26–7.29 (4H, m), 7.35 (2H, d, J = 8.5), 7.44 (2H, d, J = 8.5); mp (oxalate salt): 148–151 °C.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-(3-trifluoromethylphenyl)-4-piperidinol (17). As described for **9**, **17** was prepared from **6** and 4-[3-(trifluoromethyl)phenyl]-4-piperidinol as a colorless oil (81% yield). ^{1}H NMR ($CDCl_3$): δ 1.56–1.67 (4H, m), 1.71 (2H, d, J = 12.0), 2.13 (2H, t t, J = 13.1, 4.0), 2.36–2.45 (4H, m), 2.79 (2H, br d, J = 11.0), 3.42 (2H, t, J = 6.3), 5.29 (1H, s), 6.97–7.01 (4H, m), 7.26–7.30 (4H, m), 7.42 (1H, t, J = 7.8), 7.48 (1H, d, J = 7.0), 7.67 (1H, d, J = 8.0), 7.83 (1H, s); mp (oxalate salt): 148–150 °C.

4-Benzyl-1-[4-[bis(4-fluorophenyl)methoxy]butyl]-4-piperidinol (18). As described for **9**, **18** was prepared from **6** and 4-benzyl-4-hydroxypiperidine as a colorless oil (73% yield). ^{1}H NMR ($CDCl_3$): δ 1.21 (1H, br s), 1.51 (2H, br d, J = 12.5), 1.56–1.66 (4H, m), 1.74 (2H, t d, J = 12.9, 3.8), 2.26 (2H, br t, J = 11.0), 2.35 (2H, t, J = 7.3), 2.65 (2H, br d, J = 11.5), 2.75 (2H, s), 3.42 (2H, t, J = 5.8), 5.28 (1H, s), 6.97–7.02 (4H, m), 7.19–7.21 (2H, m), 7.23–7.32 (7H, m); mp (oxalate salt): 136–138 °C.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-1,2,3,4-tetrahydroisoquinoline (19). As described for **9**, **19** was prepared from **8** and 1,2,3,4-tetrahydroisoquinoline as a slightly yellow oil (79% yield). ^{1}H NMR ($CDCl_3$): δ 1.68–1.71 (4H, m), 2.50 (2H, br t, J = 7.0), 2.69 (2H, t, J = 5.8), 2.88 (2H, t, J = 5.8), 3.45 (2H, br t, J = 5.5), 3.60 (2H, s), 5.28 (1H, s), 6.97–7.00 (5H, m), 7.06–7.11 (3H, m), 7.26–7.28 (4H, m); mp (oxalate salt): 135–137 °C.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (20). As described for **9**, **20** was prepared from **8** and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride as a colorless oil (81% yield). ^{1}H NMR ($CDCl_3$): δ 1.69–1.71 (4H, m), 2.50 (2H, br t, J = 6.8), 2.69 (2H, t, J = 5.8), 2.81 (2H, t, J = 5.8), 3.45 (2H, t, J = 5.8), 3.53 (2H, s), 3.83 (3H, s), 3.84 (3H, s), 5.30 (1H, s), 6.50 (1H, s), 6.59 (1H, s), 6.98–7.02 (4H, m), 7.26–7.30 (4H, m); mp (oxalate salt): 88–102 °C.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (21). As described for **9**, **21** was prepared from **6** and 6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline hydrochloride as a colorless oil (75% yield). ^{1}H NMR ($CDCl_3$): δ 1.48–1.63 (4H, m), 2.28–2.33 (1H, m), 2.49–2.56 (2H, m), 2.75 (1H, d t, J = 16.5, 4.7), 2.93–2.99 (1H, m), 3.14 (1H, d t, J = 12.0, 5.3), 3.27–3.34 (2H, m), 3.58 (3H, s), 3.84 (3H, s), 4.46 (1H, s), 5.20 (1H, s), 6.16 (1H, s), 6.60 (1H, s), 6.96–7.00 (4H, m), 7.20–7.27 (9H, m); mp (oxalate salt): 115–118 °C.

1-[1-[4-[bis(4-Fluorophenyl)methoxy]butyl]piperidin-4-yl]-1,3-dihydrobenzoimidazol-2-one (22). As described for **9**, **22** was prepared from **6** and 4-(2-keto-1-benzimidazolinyl)-piperidine as a colorless oil (80% yield). ^{1}H NMR ($CDCl_3$): δ 1.64–1.70 (4H, m), 1.82 (2H, br d, J = 10.0), 2.13 (2H, t, J = 11.5), 2.42 (2H, t, J = 7.0), 2.50 (2H, q d, J = 12.7, 3.4), 3.10 (2H, br d, J = 11.0), 3.45 (2H, t, J = 5.8), 4.40 (1H, t t, J = 12.3, 4.0), 5.30 (1H, s), 6.98–7.04 (6H, m), 7.12–7.13 (1H, m), 7.53–7.30 (5H, m), 11.09 (1H, br s); mp (oxalate salt): 225–227 °C.

8-[4-[bis(4-Fluorophenyl)methoxy]butyl]-1-phenyl-1,3,8-triazaspiro-[4,5]decan-4-one (23). As described for **9**, **23** was prepared from **6** and 1-phenyl-1,3,8-triazaspiro-[4,5]decan-4-one as a colorless oil (79% yield). ^{1}H NMR

($CDCl_3$): δ 1.59–1.70 (4H, m), 1.72 (2H, br d, $J=13.5$), 2.43 (2H, t, $J=7.5$), 2.66 (2H, t d, $J=12.7$, 5.5), 2.75–2.84 (4H, m), 3.44 (2H, t, $J=6.3$), 4.72 (2H, s), 5.29 (1H, s), 6.85 (1H, t, $J=7.3$), 6.91 (2H, d, $J=8.0$), 6.97–7.02 (4H, m), 7.25–7.30 (6H, m), 8.08 (1H, s); mp (oxalate salt): 106–110 °C.

2-[4-[4-[4-Bromophenyl]-4-hydroxypiperidin-1-yl]-butyl]-isoindole-1,3-dione (24). As described for **9**, **24** was prepared from **8** and 4-(4-bromophenyl)-4-piperidinol as a white solid (80% yield). ^{1}H NMR (DMSO-d_6): δ 1.47 (2H, p, $J=7.3$), 1.55 (2H, br d, $J=12.5$), 1.64 (2H, p, $J=7.3$), 1.85 (2H, t d, $J=12.8$, 3.8), 2.31–2.36 (4H, m), 2.61 (2H, br d, $J=10.5$), 3.42 (1H, br s), 3.61 (2H, t, $J=7.0$), 7.42 (2H, d, $J=9.0$), 7.48 (2H, d, $J=9.0$), 7.83–7.88 (4H, m); mp (oxalate salt): 223–225 °C.

Biologic methods

In vitro binding and reuptake inhibition assays. In vitro assays for [^{125}I]RTI-55-binding inhibition and [^{3}H]neurotransmitter-uptake inhibition using HEK 293 cells expressing recombinant biogenic amine transporters were carried out according to the procedures described previously.[22,28]

In vitro receptor binding and functional biochemical assays.[29] **Binding assay for the D_1 receptor** LHD_1 cells (human receptor) were grown to confluence on 100×20 mm plates in DMEM containing 10% fetal calf serum, 0.05% penicillin-streptomycin, and 400 μg/mL G418 sulfate. The cells were scraped from the plates and centrifuged at 500*g* for 5 min. The pellet was homogenized in 50 mM Tris–HCl (pH 7.7) by polytron, centrifuged at 27,000*g*, and resuspended at 10 mg protein/mL in the same buffer. The homogenate was then stored at −70 °C in 1-mL aliquots. When needed, the thawed cells were washed once and resuspended at 5 mg protein/80 mL 50 mM Tris–HCl, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4). The assay was performed in triplicate in 96-well plates. To wells containing 100 μL test drug or buffer and 100 μL [^{3}H]SCH 23,390 (0.18 nM final concentration), 0.8 mL cell homogenate (0.05 mg protein/well) was added, and the plates were incubated at 25 °C for 60 min. SCH 23,390 at 1 μM was used to determine nonspecific binding.

Binding assay for the D_2 and D_3 receptors. CHOp cells (human receptors) were grown to confluence on 100×20 mm plates in α minimum essential medium (αMEM), containing 10% fetal calf serum, 0.05% penicillin–streptomycin, and 600 μg/mL G418 sulfate. The cells were scraped from the plates and centrifuged at 500*g* for 5 min. The pellet was homogenized by polytron in 50 mM Tris–HCl (pH 7.7) and centrifuged at 27,000*g* for 12 min. The pellet was resuspended in 50 mM Tris to contain D_2 at 5 mg protein/mL or D_3 at 1 mg protein/mL and stored at −70 °C in 1-mL aliquots. On the day of the experiment, CHOp-D_2 or CHOp-D_3 cells were thawed, resuspended in 50 mM Tris, and centrifuged at 27,000*g* for 12 min. The pellet was resuspended at 5 mg protein/80 mL for D_2 and 1 mg protein/80 mL for D_3 in 50 mM Tris, containing 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, and 1 mM EDTA (pH 7.4). Then 0.8 mL cell homogenate (0.05 and 0.01 mg protein/well for D_2 and D_3, respectively) was added to wells containing 100 μL test drug or buffer and 100 μL [^{3}H]YM-09151–2 (0.21 nM final concentration). Chlorpromazine at 1 μM was used to determine nonspecific binding. The plates were incubated at 25 °C for 60 min. The incubation was terminated by rapid filtration through Whatman GF/B filter paper soaked in 0.1% polyethylenimine (PEI) on a Brandel cell harvester. The filters were washed three times with ice-cold 50 mM Tris–HCl (pH 7.7) and soaked overnight in scintillation cocktail before counting for 2 min on a Beckman LS 6000 scintillation counter.

Binding assay for the 5-HT_{1A} receptor. HA7 cells (human receptor) were grown and harvested as above. The pellet was homogenized in 50 mM Tris–HCl (pH 7.7) by polytron, centrifuged at 27,000*g*, and resuspended at 10 mg protein/mL in the same buffer. The homogenate was then stored at −70 °C in 1-mL aliquots. When needed, the thawed cells were washed once and resuspended at 10 mg protein/80 mL in 25 mM Tris–HCl, containing 100 μM ascorbic acid and 10 μM nialamide (pH 7.4). The assay was performed in triplicate in 96-well plates. To each well, 100 μL [^{3}H]8-OH-DPAT (0.5 nM final concentration), 100 μL test compound or buffer, and 0.8 mL cell homogenate (0.1 mg protein/well) were added by a Tomtec Quadra 96. Dihydroergotamine at 1 μM was used to determine nonspecific binding. The plates were incubated at 25 °C for 60 min. The incubation was terminated by rapid filtration through glass-fiber filter paper on a Tomtec cell harvester. The filters were washed four times with ice-cold 50 mM Tris–HCl (pH 7.7), dried overnight, and mixed with 10 mL scintillation cocktail before counting for 2 min on a Wallac Betaplate 1205 liquid scintillation counter.

Binding assay for the 5-HT_{2A} receptor. NIH-3T3-GF6 cells (rat receptor) were grown as above. On the day of the experiment, the cells were thawed, resuspended in 50 mM Tris–HCl, and centrifuged at 27,000*g* for 12 min. The pellet was resuspended at 1 mg protein/80 mL 25 mM Tris–HCl (pH 7.7) and 0.8 mL cell homogenate (0.01 mg protein/well) was added to wells containing 100 μL test drug or buffer and 100 μL [^{3}H]ketanserin (0.40 nM final concentration). Ketanserin at 1 μM was used to determine nonspecific binding. The plates were incubated at 25 °C for 60 min and processed as above for the 5-HT_{1A} receptor.

Binding assay for the 5-HT_{2C} receptor. NIH-3T3-Pϕ cells (rat receptor) were grown as above. The final pellet was resuspended at 3 mg protein/80 mL 50 mM Tris–HCl, containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% ascorbic acid (pH 7.7). Wells with 100 μL test drug or buffer, 100 μL [^{3}H]mesulergine (0.4 nM final concentration), and 0.8 mL cell homogenate (0.03 mg protein/well) were incubated at 25 °C for 60 min. Mesulergine at 10 μM was used to determine nonspecific binding. After incubation, the plates were processed as above for the 5-HT_{1A} receptor.

Binding assay for the 5-HT$_3$ receptor. NG108–15 cells (rat/mouse hybrid) were grown to confluence on 100×20 mm plates in DMEM with HAT supplement and 10% fetal calf serum. The cells were washed from the plates, centrifuged, homogenized as described above, and stored at −70 °C in aliquots of 15 plates/4 mL. When needed, the thawed cells were washed once and resuspended at 15 plates/20 mL 25 mM Tris–HCl (pH 7.7). The assay was performed by adding 50 μL test drug or buffer, 50 μL [^{3}H]GR65630 (1.6 nM final concentration), and 0.4 mL cell homogenate (0.13 mg protein/tube) to each tube. The tubes were then incubated at 25 °C for 45 min. Zacopride at 1 μM was used to determine nonspecific binding. The incubation was terminated by rapid filtration through Whatman GF/B filter paper soaked in 0.1% polyethylenimine (PEI) on a Brandel cell harvester. The filters were washed three times with ice-cold 50 mM Tris–HCl (pH 7.7) and soaked overnight in scintillation cocktail before counting for 2 min on a Beckman LS 6000 scintillation counter.

D$_2$ and D$_3$ functional mitogenesis assay. To measure D$_2$ and D$_3$ inhibition of quinpirole stimulation of mitogenesis (antagonist assay), CHOp cells (human receptor) were seeded in 96-well plates at 5000 cells/well. The cells were incubated at 37 °C in αMEM with 10% FBS, 0.05% penicillin–streptomycin, and 200 μg/mL G418 sulfate. After 48 h, the wells were rinsed twice with 100 μL serum-free α-MEM and incubated for 24 h at 37 °C in serum-free α-MEM. The medium was then removed and replaced with 90 μL serum-free α-MEM and 10 μL drug in sterile water plus quinpirole. After another 24 h incubation at 37 °C, 0.25 μCi [^{3}H]thymidine was added to each well. The cells were incubated for 2 h at 37 °C. Then, 10 μL/well 10X trypsin (trypsin–EDTA solution: 5 g trypsin in 20 mL) was added to release the cells, and the plates were filtered using a Tomtec cell harvester. The filters were washed four times with deionized water, dried overnight, and mixed with 10 mL scintillation cocktail before counting for 2 min on a Wallac Betaplate 1205 liquid scintillation counter. Quinpirole was run on every plate as an internal standard.

Locomotor activity[22]

This study was conducted using 16 Digiscan locomotor activity testing chambers (40.5×40.5×30.5 cm). Panels of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. Separate groups of eight nonhabituated male Swiss–Webster mice were injected via the intraperitoneal route with either vehicle (methylcellulose, saline, or distilled water) or compound (3, 10, 30, or 100 mg/kg), 20 min prior to locomotor activity testing. Just prior to placement in the apparatus, all mice received a saline injection intraperitoneally. In all studies, horizontal activity (interruption of 1 photocell beam) was measured for 1 h within 10-min periods. Testing was conducted with one mouse per activity chamber. For the cocaine/compound interaction study, 20 min following administration of vehicle or compound intraperitoneally (3, 10, 30, or 100 mg/kg), groups of eight nonhabituated male Swiss–Webster mice were injected with either 0.9% saline or 20 mg/kg cocaine intraperitoneally and placed in the Digiscan apparatus for a 1-h session.

Maximal effects of cocaine and stimulant test compound (independent studies of cocaine and test compound). One 30-min time period in which maximal effects of cocaine/test compound were evident at most doses was selected. The mean (±SEM) maximal stimulant activity (total counts in the 30-min period divided by 3) was plotted versus dose. A $\log_{10}$ transformation of the 30-min period average counts for individual subjects was performed to homogenize variances for subsequent analyses. ANOVA was used and each dose of cocaine/test compound (specified a priori) was contrasted to saline (the vehicle) to determine significant ($P<0.05$) dose effects. The 30-min period average counts across subjects was fit to a function of $\log_{10}$ dose using least-squares curve-fitting analyses (i.e., TableCurve software from Jandel). The maximum effect from the resultant dose–response curve was estimated. A test compound maximal effect/cocaine maximum effect (ME/CME) ratio was calculated and the ED$_{50}$ (dose that produces 1/2 maximal stimulant activity) was determined from a linear regression analysis of the ascending portion (up to the dose that produced a maximal effect) of the curve. The mean maximal effect for cocaine was calculated for each mouse.

Maximal effects of depressant test compound (studies of test compound alone). A 30-min time period was selected in which cocaine (20 mg/kg) produced its maximal effects as determined from the studies with cocaine alone. The mean (±SEM) activity (total counts in the 30-min period divided by 3) was plotted versus dose. A $\log_{10}$ transformation of the 30-min period average counts for individual subjects was performed to homogenize variances for subsequent analyses. ANOVA was used and each dose of the test compound (specified a priori) was contrasted to the vehicle to determine significant ($P<0.05$) dose effects. A linear least-squares regression analysis was conducted and the 30-min period average counts across subjects were regressed over the descending potion of the curve against the $\log_{10}$ dose of the test compound. The ID$_{50}$ (dose that produces 1/2 maximal depressant activity where maximum depression = 0) was calculated from the linear regression analysis.

Maximal effects of test compound–cocaine interaction studies. A 30-min time period was selected in which cocaine (20 mg/kg) produced its maximal activity as determined from the studies with cocaine alone. The mean (±SEM) maximal activity (total counts in the 30-min period divided by 3) for vehicle, vehicle pretreatment plus cocaine (20 mg/kg), and test compound pretreatment plus cocaine (20 mg/kg) were plotted in a histogram. A $\log_{10}$ transformation of the 30-min period average counts for individual subjects was performed to homogenize variances for subsequent analyses. ANOVA was used and vehicle and each dose of the test compound plus cocaine to cocaine alone (specified a priori) were contrasted to determine significant ($P<0.05$) dose effects. A linear least-squares regression analysis was performed and the 30-min period average

counts across subjects were regressed over the descending portion of the curve against the $\log_{10}$ dose of the test compound. The AD_{50} (dose that attenuates cocaine-induced stimulation by 50%) was calculated from the linear regression analysis.

Elemental Analysis

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-hydroxypiperidine (9). Anal. calcd for $C_{24}H_{31}NO_7F_2$: C, 59.62; H, 6.46; N, 2.90. Found: C, 60.12; H, 6.08; N, 2.84.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-3-hydroxypiperidine (10). Anal. calcd for $C_{24}H_{29}NO_6F_2$: C, 61.93; H, 6.28; N, 3.01. Found: C, 61.84; H, 6.23; N, 2.99.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-3-piperidine carboxamide (11). Anal. calcd for $C_{25}H_{32}N_2O_7F_2$: C, 58.82; H, 6.32; N, 5.49. Found: C, 59.02; H, 5.96; N, 5.29.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-phenylpiperidine-4-carbonitrile (12). Anal. calcd for ($C_{31}H_{32}N_2O_5F_2$): C, 67.62; H, 5.86; N, 5.09. Found: C, 67.53; H, 5.91; N, 5.05.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-acetyl-4-phenylpiperidine (13). Anal. calcd for $C_{32}H_{35}NO_6F_2$: C, 67.71; H, 6.22; N, 2.47. Found: C, 67.86; H, 6.32; N, 2.50.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-phenylpiperidin-4-ol (14). Anal. calcd for $C_{30}H_{33}NO_6F_2$: C, 66.53; H, 6.14; N, 2.59. Found: C, 66.66; H, 6.25; N, 2.59.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-(4-bromophenyl)-4-piperidinol (15). Anal. calcd for $C_{30}H_{32}BrNO_6F_2$: C, 58.07; H, 5.20; N, 2.26. Found: C, 58.09; H, 5.28; N, 2.24.

1-[4-[bis(4-Fluorophenyl)methoxy]ethyl]-4-(4-bromophenyl)-4-piperidinol (16). Anal. calcd for $C_{28}H_{28}BrNO_6F_2$: C, 56.77; H, 4.76; N, 2.36. Found: C, 56.00; H, 4.96; N, 2.43.

1-[4-[bis(4-Fluorophenyl)methoxy]butyl]-4-(3-trifluoromethylphenyl)-4-piperidinol (17). Anal. calcd for $C_{31}H_{32}NO_6F_5$: C, 61.08; H, 5.29; N, 2.30. Found: C, 60.96; H, 5.31; N, 2.26.

4-Benzyl-1-[4-[bis(4-fluorophenyl)methoxy]butyl]-4-piperidinol (18). Anal. calcd for $C_{32}H_{42}NO_8F_2$: C, 61.62; H, 6.95; N, 2.25. Found: C, 61.42; H, 5.82; N, 2.14.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-1,2,3,4-tetrahydroisoquinoline (19). Anal. calcd for $C_{28}H_{29}NO_5F_2$: C, 67.59; H, 5.88; N, 2.82. Found: C, 67.54; H, 5.86; N, 2.79.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-6,7-dimethoxy-1,2,3,4- tetrahydroisoquinoline (20). Anal. calcd for $C_{30}H_{33}NO_7F_2$: C, 64.62; H, 5.97; N, 2.51. Found: C, 64.96; H, 5.88; N, 2.24.

2-[4-[bis(4-Fluorophenyl)methoxy]butyl]-6,7-dimethoxy-1-phenyl-1,2,3,4- tetrahydroisoquinoline (21). Anal. calcd for $C_{36}H_{37}NO_7F_2$: C, 68.23; H, 5.89; N, 2.21. Found: C, 67.69; H, 6.02; N, 2.14.

1-[1-[4-[bis(4-Fluorophenyl)methoxy]butyl]piperidin-4-yl]-1,3-dihydrobenzoimidazol-2-one (22). Anal. calcd for $C_{31}H_{33}N_3O_6F_2$: C, 64.02; H, 5.72; N, 7.22. Found: C, 63.93; H, 5.67; N, 7.19.

8-[4-[bis(4-Fluorophenyl)methoxy]butyl]-1-phenyl-1,3,8-triazaspiro.[4,5]decan-4-one (23) Anal. calcd for $C_{32}H_{35}N_3O_6F_2$: C, 64.53; H, 5.92; N, 7.05. Found: C, 63.75; H, 6.01; N, 6.86.

2-[4-[4-[4-Bromophenyl]-4-hydroxypiperidin-1-yl]-butyl]-isoindole-1,3-dione (24). Anal. calcd for $C_{25}H_{27}BrN_2O_7$: C, 54.85; H, 4.97; N, 5.12. Found: C, 54.33; H, 4.49; N, 5.24.

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