Heteroaromatic Analogs of 1-[2-(Diphenylmethoxy)ethyl]- and 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazines (GBR 12935 and GBR 12909) as High-Affinity Dopamine Reuptake Inhibitors

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A new series of heteroaromatic GBR 12935 [1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine] (**1**) and GBR 12909 [1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)-piperazine] (**2**) analogs was synthesized and evaluated as dopamine transporter (DAT) ligands. Analogs **5**−**16**, in which the benzene ring in the phenylpropyl side chain of the GBR molecule had been replaced with a thiophene, furan, or pyridine ring, exhibited high affinity and selectivity for the DAT vs serotonin transporter (SERT) and stimulated locomotor activity in rats in a manner similar to the parent compound **2**. In cocaine and food self-administration studies in rhesus monkeys, both thiophene-containing (**6** and **8**) and pyridine-containing (**14** and **16**) derivatives displayed potency comparable to **2** in decreasing the cocaine-maintained responding at the doses tested (0.3, 1.7, and 3 mg/kg). However, these compounds did not produce the degree of separation between food- and cocaine-maintained responding that was seen with **2**. Among the bicyclic fused-ring congeners **17**−**38**, the indole-containing analog of **2**, **22**, showed the greatest affinity for binding to the DAT, with $IC_{50} = 0.7$ nM, whereas the corresponding indole-containing derivative of **1**, **21**, displayed the highest selectivity (over 600-fold) at this site vs the SERT site.

Introduction

Cocaine is one of the most abused drugs known. Its abuse continues to have a devastating effect on public health worldwide and plays a major role in the rapid spread of acquired immune deficiency syndrome and drug-resistant tuberculosis,[1] which results largely from needle sharing, sex for drugs, a common practice of cocaine base (crack) smoking, and related activities in shooting galleries, crack houses, and similar establishments.[1,2] Consequently, the development of safe and effective medications for the treatment of cocaine addiction is of national and worldwide interest. A number of multidisciplinary studies are currently attempting to elucidate the cocaine mechanism of action in the brain and its behavioral consequences.[3−6] It has been hypothesized that a primary modulator of the reinforcing effects of cocaine is the mesolimbic dopamine system.[4,7,8] The specific interaction of cocaine with its receptor [dopamine (DA) transporter (DAT) proteins] results in blocking reuptake of DA into dopaminergic neurons and increasing dopaminergic neurotransmission.[8] The elevated levels of dopamine in the synapse interact with dopamine receptors on the postsynaptic neurons, which results in a broad spectrum of reinforcing and stimulating effects.[9] An obvious target for research aimed at the treatment of cocaine abuse is the development of agents which are potent and selective ligands for the DAT and which may reduce the effects of cocaine.[10,11]

The development of high-affinity, slowly dissociating, low-intrinsic activity cocaine receptor agonists constitutes one approach for the treatment of cocaine addiction.[10,12,13] The disubstituted piperazines[14] including GBR 12935 [1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine] (**1**) and GBR 12909 [1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine] (**2**) were among the first agents with high affinity and selectivity at the DA reuptake site.[15,16] Compound **2** exhibits a neurochemical and behavioral profile that renders it a promising candidate as a cocaine abuse therapeutic agent. It is a slowly dissociating DAT ligand with slow onset, long duration of action and lower *in vivo* efficacy as a motor stimulant in rats compared to cocaine.[17] Compound **2** was shown to attenuate extracellular DA levels elevated by cocaine in rat striatum and nucleus accumbens in microdialysis experiments.[18,19] Recent experiments in cocaine and food self-administration studies revealed that **2** decreases the cocaine-maintained responding without decreasing normal food-maintained responding in rhesus monkeys.[20,21] It has also been shown that humans administered an oral dose of **2** report a sedative rather than a stimulant effect.[22] Because of these properties, we selected **1**, **2**, and their double-bond-containing derivatives GBR 12783 [1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl-2-propenyl)-piperazine][23] (**3**) and GBR 13069 [1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenyl-2-propenyl)piperazine][24] (**4**), respectively, as templates to develop novel ligands for the DAT as potential agents for the treatment of cocaine abuse.

In earlier studies it was shown that a significant increase in affinity and selectivity at the DAT site could be attained by modifying the central piperazine ring of the GBR molecule.[25−28] Additionally, the diamine-modified ligands displayed an *in vivo* pharmacological

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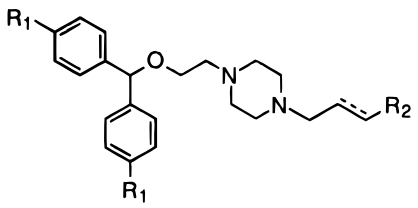
Chart 1



Compound	R_1	R_2	Bond
1 (GBR 12935)	H	Phenyl	Single
2 (GBR 12909)	F	Phenyl	Single
3 (GBR 12783)	H	Phenyl	Double
4 (GBR 13069)	F	Phenyl	Double
5	H	2-Thienyl	Single
6	F	2-Thienyl	Single
7	H	2-Thienyl	Double
8	F	2-Thienyl	Double
9	H	2-Furyl	Single
10	F	2-Furyl	Single
11	H	2-Furyl	Double
12	F	2-Furyl	Double
13	H	3-Pyridyl	Single
14	F	3-Pyridyl	Single
15	H	3-Pyridyl	Double
16	F	3-Pyridyl	Double

profile different from that of **1** and **2**.[25,28] While it has been previously established that the (diphenylmethoxy)-ethyl moiety is a very important pharmacophore for effective binding to the DAT and possibly to the [^{3}H]-cocaine-binding site, very few modifications to the phenylpropyl portion of the GBR structure have been reported.[29,30] Replacement of a benzene ring with a heteroaromatic isostere has been a very useful and well-documented modification in medicinal chemistry. This has been applied successfully in the context of nonopioid analgesics, among other studies.[31,32] We have employed this bioisosteric concept to a new series of GBR-related ligands, in which the benzene ring in the phenylpropyl chain was replaced with either thiophene, furan, or pyridine (Chart 1, compounds **5**−**16**). Substitution of the phenylpropyl chain of the GBR molecule with aromatic and heteroaromatic, five- and six-membered fused-ring systems afforded a series of structurally restricted analogs (Chart 2, compounds **17**−**38**). Herein, we report the binding affinity of these ligands to the DA and serotonin (5-HT) transporters (SERT) and their ability to inhibit the reuptake of DA and 5-HT. Preliminary locomotor activity studies in rats along with cocaine and food self-administration studies in monkeys were used to evaluate heteroaromatic analogs of **1** and **2** as potential agents for the treatment of cocaine abuse. Portions of this work have been reported in preliminary communications.[33,34]

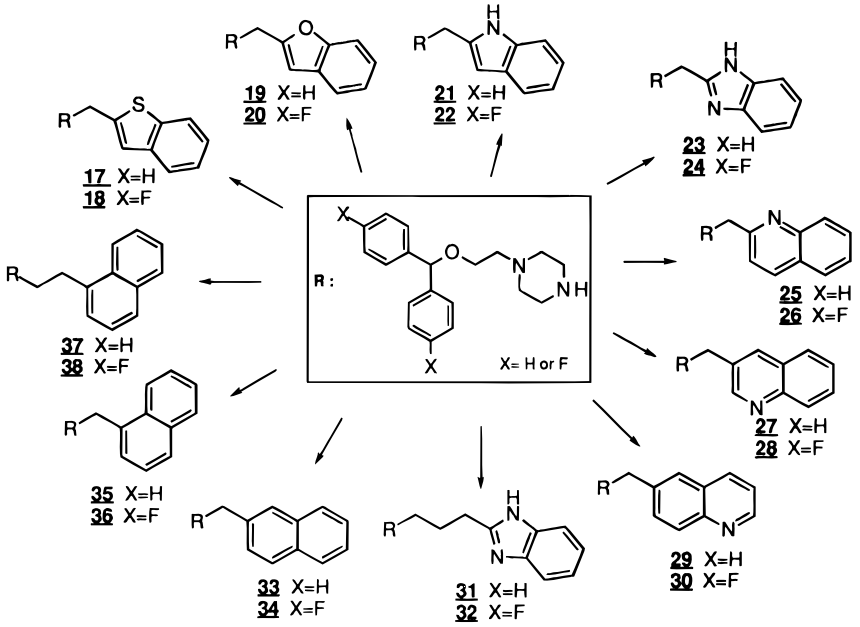
Chemistry

The monosubstituted piperazines 1-[2-(diphenylmethoxy)ethyl]piperazine or 1-[2-[bis(4-fluorophenyl)-methoxy]ethyl]piperazine were synthesized as previously described[14] by a two-step sequence: reaction of commercially available suitably substituted benzhydrol with 2-chloroethanol in the presence of sulfuric acid and subsequent reaction of the resulting alkyl chloride with excess piperazine (Scheme 1). These monosubstituted amines were then either coupled with the appropriate heteroaromatic carboxylic acid using dicyclohexylcarbodiimide (DCC) (method A) or treated with a suitable acid chloride under Schotten−Baumann conditions (method E). The resulting intermediate amides were subsequently reduced with aluminum hydride[35] (method B) to yield the target amines **4**, **7**, **8**, **11**, **12**, **14**−**22**, and **25**−**38**. Hydrogenation of **7**, **8**, **11**, and **12** using 10% Pd/C in methanol (method C) afforded saturated GBR analogs **5**, **6**, **9**, and **10**, respectively. Compound **13** was synthesized by K_2CO_3-catalyzed coupling of 1-[2-(diphenylmethoxy)ethyl]piperazine with 3-(3-chloropropyl)-pyridine in DMF in the presence of KI (method D). Compounds **23** and **24** were obtained from 2-(chloromethyl)benzimidazole and 1-[2-(diphenylmethoxy)ethyl]-piperazine or 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-piperazine, respectively, following the same procedure (method D). Benzo[*b*]thiophene-2-carboxylic acid, which was used for the synthesis of **17** and **18**, was obtained by deprotonation of benzo[*b*]thiophene with *n*-BuLi in tetrahydrofuran followed by the addition of excess dry ice (method F). All final amines were purified and crystallized as salts with the indicated organic or inorganic acids (Tables 1 and 2).

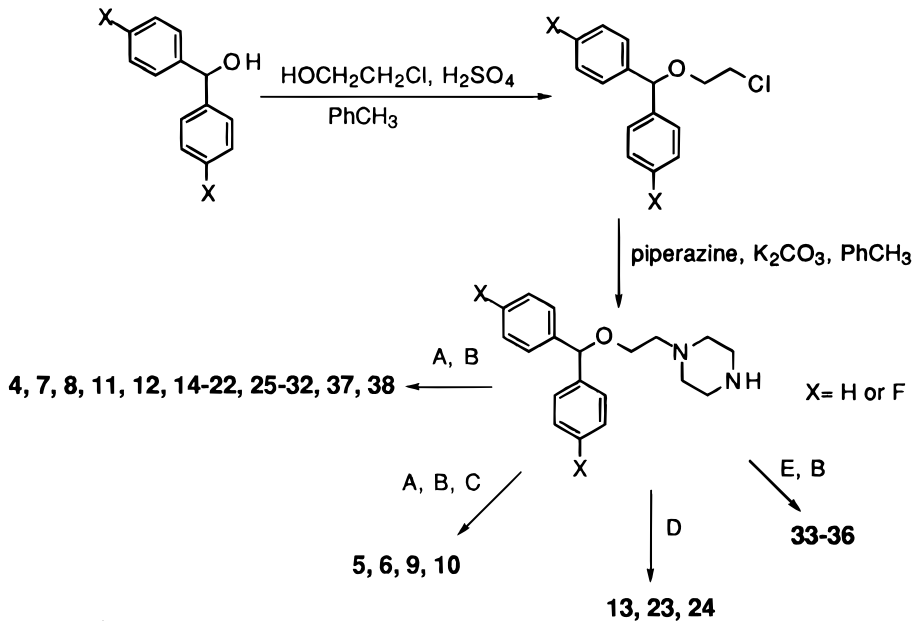
Results and Discussion

The binding data indicate that replacement of the phenylpropyl side chain in **1**−**4** with a variety of heteroaromatic-containing moieties resulted in the creation of a series of potent inhibitors of dopamine reuptake with high affinity and selectivity at the DAT (Tables 3 and 4). Among the bioisosteric analogs **5**−**16** (Table 3), the furan-containing derivative of **4**, piperazine **12**, exhibited the highest affinity for the DAT (IC_{50} = 1.8 nM), while the furan-containing analog of **3**, compound **11**, was the most selective ligand (367- and 281-fold more potent for the binding to the DAT vs SERT and inhibition of DA vs 5-HT reuptake, respectively). While the pyridine-containing derivatives **13**−**16** displayed slightly reduced potency for binding to the DAT and inhibition of DA reuptake with respect to the furan and thiophene analogs, they retained the selectivity at the DA vs 5-HT reuptake site. Generally, the bis-(4-fluoro)-substituted compounds containing an unsaturated side chain (*i.e.*, lead compound **4** and its thiophene-, furan-, or pyridine-containing analogs **8**, **12**, and **16**, respectively) exhibited slightly higher affinity at the DAT than their desfluoro and saturated analogs in all four subgroups of ligands: **1**−**4**, **5**−**8**, **9**−**12**, and **13**−**16**, respectively. However, this relationship was not the same with respect to their ability to inhibit DA reuptake. Interestingly, compound **4**, which exhibited the highest affinity for binding to the DAT among all

Chart 2



Scheme 1[a]



[a] (A) Appropriate heteroaromatic carboxylic acid, DCC, CH_2Cl_2/THF; (B) aluminum hydride, THF; (C) H_2, 10% Pd/C, MeOH; (D) 3-(3-chloropropyl)pyridine or 2-(chloromethyl)benzimidazole, K_2CO_3, KI, DMF; (E) 1- or 2-naphthoyl chloride, aq $NaHCO_3$, $CHCl_3$.

the ligands in Table 3, also displayed a high ratio (12.2-fold) of the IC_{50} for inhibition of dopamine reuptake versus binding to the DAT. The thiophene and furan derivatives **8** and **12**, respectively, also exhibited an improved ratio of 5.9 and 4, respectively (Table 3), in comparison to the parent **2** with a ratio of 2. However, the biological significance of relatively small differences among the ratios of compounds is not clear.

It is interesting to note that in the second group of fused-rings ligands, the ratio was always higher for the bis(4-fluoro)-substituted compounds than for their des-fluoro analogs (Table 4). This ratio was significantly improved for several new analogs, *i.e.*, the 2-indolyl, 1-naphthyl, or 2-benzo[*b*]thienyl derivative **22**, **36**, or **18**, respectively. Our interest in the synthesis of compound **18** was spurred by recent studies,[36] in which a series of piperazines with structural and biochemical similarities to the GBR- and BTCP-type [*N*-[1-(2-benzo-[*b*]thienyl)cyclohexyl]piperidine] of DAT ligands was synthesized and tested at the DA transport site. Surprisingly, 1-[1-(2-benzo[*b*]thienyl)cyclohexyl]-4-[1-[2-(diphenylmethoxy)ethyl]piperazine, which is a hybrid between BTCP and **3** (Chart 3), exhibited a relatively low, micromolar affinity for the binding to the DAT labeled with [^{3}H]BTCP. We synthesized new GBR and BTCP hybrids, compounds **17** and **18**, which lack the bulky spirocyclohexyl residue of BTCP. The preliminary molecular modeling experiments performed with the Quanta program provided a good structural fit of BTCP, **4**, and **18** (data not shown). The results from *in vitro* studies, using [^{125}I]RTI-55 as an agent labeling the DAT, confirmed this close fit, with **18**, the benzothiophene analog of **2**, displaying 4.1 nM affinity at this site and

Table 1. Reaction Conditions of the Intermediate Amide Reduction (Method B) and Physical Properties of the Final Amines

target amine	excess reducing agent (equiv)	reduction time	reduction temp (°C)	salt[a]	mp (°C)	crystn solvent	yield[b] (%)
7	2	10 min	rt	2 maleate	179−180	MeOH	80
8	2	10 min	rt	2 maleate	188−189	MeOH	83
				2 HCl	204−205 dec	2-PrOH	
11	2	10 min	rt	2 maleate	176−177	MeOH	65
12	2	10 min	rt	2 maleate	168−170	MeOH	71
				2 HCl	189−190 dec	2-PrOH	
13[c]				2 maleate	148−149	MeOH	64
14[d]	2	10 min	rt	2 maleate	149−150	MeOH	76
15	2	10 min	rt	2 maleate	157−158	MeOH	54
16	1.3	1 h	0	2 maleate	179−180	MeOH	52
17	5	30 min	rt	2 maleate	199−200	MeOH	68
18	3	5 min	rt	2 maleate	193−194	MeOH	74
19	5	30 min	rt	2 maleate	192−193	MeOH	82
20	3	10 min	rt	2 maleate	186−188	MeOH	79
21	5	30 min	rt	2 maleate	192−194	MeOH	65
22	2	10 min	rt	2 maleate	191.5−192	MeOH	84
23[c]				2.5 oxalate	178−179	MeOH	80
24[c]				2 maleate	170−171	2-PrOH:MeOH (4:1)	87
25	1.5	15 min	−5−0	2 maleate	183−184 dec	MeOH	15[e]
26	3	10 min	0	2 maleate	180−180.5	MeOH	25[e]
27	5	30 min	rt	2 maleate	170−172	MeOH	18[e]
28	2	5 min	rt	2 maleate	190.5−191	MeOH	55
29	5	1 h	rt	2 maleate	198−200	MeOH	43
30	1.5	15 min	rt	2 maleate	190−191	MeOH	83
31	4	30 min	rt	3 maleate	196−198	MeOH	62
32	4	10 min	rt	3 maleate	193−194	MeOH	65
33	5	50 min	rt	2 maleate	203−204	MeOH	76
34	3	12 h	rt	2 maleate	194−196	MeOH	87
35	5	50 min	rt	2 maleate	170−171	MeOH	66
36	3	12 h	rt	2 maleate	171−172	MeOH	73
37	5	1 h	rt	2 maleate	181−182	MeOH	65
38	3	12 h	rt	2 maleate	180−182	MeOH	54

[a] The CI mass spectra of all the final amines and precursor amides contained predicted (M + 1) peaks. All compounds gave CHN analysis within ±0.4 of calculated value. [b] Total yield of two steps. [c] Synthesized according to method D. [d] Compound **14** was synthesized by DCC condensation of starting piperazine and hydrogenated 3-pyridineacrylic acid (Table 2) followed by the reduction of the resulting amide. [e] Due to very low yield of amide reduction.

Table 2. Hydrogenation Conditions (Method C) and Physical Properties of the Final Compounds

target compd	reactn solvent	hydrogen pressure	reaction time	salt[a]	crystn solvent	mp (°C)	yield (%)
5	MeOH	50 psi	12 h	2 maleate	MeOH	178−180	94
6	MeOH	45 psi	36 h	2 maleate	MeOH	183−185	96
				2 HCl	MeOH:2-PrOH (1:3)	214−215 dec	
9	MeOH	atm	30 min	2 maleate	MeOH	174−175	82
10	MeOH	atm	40 min	2 maleate	MeOH	172−173	97
				2 HCl	2-PrOH	207−208 dec	
3-(3-pyridyl)propionic acid	MeOH/THF	45 psi	24 h		MeOH	147−150	97

[a] All compounds gave CHN analysis within ±0.4 of calculated value.

17, the derivative of **1**, showing 184-fold selectivity at the DAT vs 5-HT site.

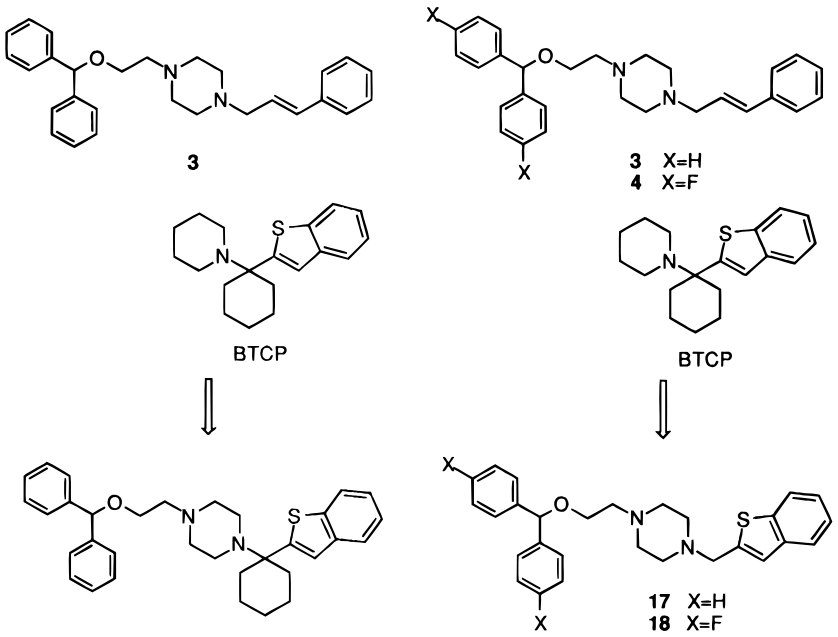
In general, compounds containing a five-membered aromatic ring fused with the benzene ring (**17**−**24**) were designed to make **1** and **2** more rigid and to retain the favorable three-atom linkage between the nitrogen atom of piperazine and the phenyl ring in the phenylpropyl side chain of the typical GBR structure. Most of the five-membered aromatic ring-containing compounds exhibited the highest selectivity for both DAT (vs SERT) binding and DA (vs 5-HT) reuptake inhibition among all congeners belonging to the series of fused-ring GBR-related ligands (Table 4). The indole derivative **22**, an analog of **2**, was the most potent ligand for the binding to the DAT with $IC_{50} = 0.7$ nM, whereas **21,** the indole derivative of **1**, displayed the highest DAT/SERT binding selectivity (over 600-fold) at this site. As seen in previous GBR series, the bis(4-fluoro) analogs were more potent and the desfluoro congeners more selective for the DAT vs SERT, mainly because of their significantly lower affinity at the 5-HT transport site. Furthermore, the combination of fused rings with only one heteroatom placed in the 2-position of the five-membered ring (compounds **17**−**22**) appeared to be more favorable in terms of selectivity of the new ligands at the DA transport site. The incorporation of an additional nitrogen atom in the five-membered ring (benzimidazole derivatives **23** and **24**, respectively) resulted in the decrease of both affinity and selectivity at the DA transport site (compare **23** and **24** vs **21** and **22**, respectively). The increase of the number of atoms between the piperazine nitrogen and heteroaromatic ring (*i.e.*, benzimidazopropyl analogs **31** and **32** vs their benzimidazomethyl congeners **23** and **24**, respectively) further decreased the selectivity of these ligands at the DA uptake site, again, mainly because of their improved

Table 3. Binding Affinities at the DA and 5-HT Transporters Labeled with [^{125}I]RTI-55 and σ Sites Labeled with [^{3}H]-(+)-Pentazocine and Affinities for DA and 5-HT Reuptake Inhibition of Heteroaromatic GBR Analogs **5**−**16** (IC_{50} ± SD, nM)[a]

	binding		reuptake			ratios		
ligand	DAT	SERT	[^{3}H]DA	[^{3}H]-5-HT	σ binding	binding SERT/DA	reuptake 5-HT/DA	reuptake/ binding (DA)
1 (GBR 12935)	3.7 ± 0.3	623 ± 13	3.7 ± 0.4	298 ± 29	17 ± 2	168	78	1
2 (GBR 12909)	3.7 ± 0.4	126 ± 5	7.3 ± 0.2	73 ± 2	43 ± 1	34	10	2
4	0.9 ± 0.1	135 ± 7	11 ± 0.6	576 ± 32	51 ± 1	158	51	12.2
5	5.2 ± 0.3	842 ± 30	9.7 ± 0.2	1990 ± 58	n/d	162	205	1.9
6	3.3 ± 0.1	105 ± 2	6.1 ± 0.7	335 ± 17	38 ± 1	32	55	1.8
7	6.4 ± 0.3	1170 ± 31	10 ± 0.7	2020 ± 141	n/d	184	200	1.6
8	2.2 ± 0.1	88 ± 2	13 ± 1.4	374 ± 17	41 ± 0.9	40	29	5.9
9	6.5 ± 0.2	1520 ± 47	8.5 ± 0.5	2550 ± 87	n/d	234	300	1.3
10	5.9 ± 0.3	204 ± 7	7.9 ± 0.5	412 ± 9	62 ± 2	35	52	1.3
11	5.0 ± 0.3	1840 ± 59	9.6 ± 0.3	2700 ± 136	n/d	367	281	1.9
12	1.8 ± 0.3	109 ± 4	7.2 ± 0.4	442 ± 23	43 ± 3	62	61	4
13	78 ± 4	2420 ± 65	70 ± 6	3700 ± 148	n/d	31	53	0.9
14	16 ± 0.2	2800 ± 139	20 ± 0.8	6520 ± 293	79 ± 2	175	321	1.3
15	44 ± 3	2670 ± 66	64 ± 2	3620 ± 179	n/d	61	57	1.5
16	13.6 ± 0.2	334 ± 12	14.5 ± 1.9	666 ± 21	59 ± 3	25	46	1.1

[a] The IC_{50} values of the test agents were determined in the above assays as described in Biological Methods.

Chart 3



affinity at the serotonin uptake site. Interestingly, compounds containing a quinoline ring, which was attached to the rest of the molecule in different positions such as 2, 3, and 6 (**25**−**30**), displayed a broad range of affinities for binding to the DAT. It is intriguing that whereas the 2-indole congener **22** was one of the most selective ligands at the DA site, its 2-quinoline analog **26** exhibited a comparably high affinity at both DA and 5-HT sites. Since the only structural difference between **22** and **26** was the additional methine atom in the ring of quinoline-containing analog **26**, the difference in the affinity at both sites of these two ligands may be explained by either the different electronic character of their nitrogen atoms or the presence of a hydrogen-bonding donor in analog **22**.

The ability for hydrogen bonding in the drug−receptor interaction may also explain the difference in binding properties of all the quinoline analogs (**25**−**30**), in which the quinoline ring is bound in different positions *i.e.*, 2, 3, or 6. In the series of naphthalene analogs **33**−**38**, the two 2-naphthalenemethyl derivatives **33** and **34** were the most selective compounds for both binding to the DAT and inhibition of DA reuptake. In contrast, their isomers, **35** and **36**, with naphthalene substituted in the 1-position lost selectivity at this site. In fact, they were more potent in inhibition of 5-HT than DA reuptake. However, the addition of another carbon atom between the piperazine nitrogen atom and the phenyl ring of the 1-naphthalene moiety resulted in at least a 10-fold improvement in the affinity of 1-naphthalene GBR analogs for the inhibition of DA reuptake (compare **35** and **36** vs **37** and **38**, respectively). In this small series of naphthalene analogs, compound **36** was especially interesting with its much higher affinity for the binding to the DAT than for the inhibition of DA reuptake (ratio of reuptake to binding = 10.1, Table 4). Generally, this series of conformationally restricted GBR-type ligands may serve as excellent templates for computer-assisted molecular modeling studies in the GBR series, which we have already initiated.

As noted above, the ratio of DA reuptake inhibition to binding inhibition was moderately high for several

Table 4. Binding Affinities at the DA and 5-HT Transporters Labeled with [^{125}I]RTI-55 and DA and 5-HT Reuptake Inhibition of Fused-Ring GBR Analogs **17**−**38** (IC_{50} ± SD, nM)[a]

	binding		reuptake		ratios		
ligand	DAT	SERT	[^{3}H]DA	[^{3}H]-5-HT	binding 5-HT/DA	reuptake 5-HT/DA	reuptake/ binding (DA)
17	18 ± 1	2420 ± 109	19 ± 1	3520 ± 289	131	184	1.1
18	4.1 ± 1.1	495 ± 18	34 ± 2	1230 ± 40	121	36	8.3
19	17 ± 0.5	1890 ± 48	22 ± 0.7	3040 ± 213	110	136	1.3
20	6.4 ± 0.2	286 ± 10	18.6 ± 0.6	767 ± 27	45	41	2.9
21	1.1 ± 0.1	668 ± 39	8.8 ± 0.7	2120 ± 166	619	241	8
22	0.7 ± 0.1	119 ± 5	13 ± 0.2	506 ± 23	163	39	18.6
23	46 ± 1	1884 ± 72	37 ± 2	4076 ± 221	41	110	0.8
24	15 ± 0.2	256 ± 7	20 ± 0.8	797 ± 43	17	40	1.3
25	199 ± 5	1990 ± 5	192 ± 8	4120 ± 212	10	21	1
26	56 ± 1	51 ± 16	106 ± 12	339 ± 31	<1	3	1.9
27	72 ± 2	1160 ± 27	111 ± 3	3040 ± 252	16	27	1.5
28	16 ± 3	485 ± 16	74 ± 3	851 ± 36	30	12	4.6
29	190 ± 6	845 ± 15	140 ± 4	1640 ± 58	4	12	0.7
30	62 ± 2	551 ± 21	73 ± 3	1040 ± 46	9	14	1.2
31	23 ± 0.5	309 ± 9	17 ± 0.7	627 ± 12	13	39	0.7
32	2.5 ± 0.1	28 ± 2	8.1 ± 0.3	74 ± 4	11	9	3.2
33	43 ± 2	903 ± 47	32 ± 0.6	926 ± 33	21	30	0.7
34	8.0 ± 0.3	312 ± 15	30 ± 1	588 ± 39	39	20	3.8
35	114 ± 5	336 ± 22	406 ± 11	83 ± 5	3	<1	3.6
36	31 ± 1	243 ± 6	312 ± 19	257 ± 12	8	<1	10.1
37	92 ± 13	462 ± 17	42 ± 0.9	578 ± 17	5	14	0.5
38	7.8 ± 0.2	46 ± 1	25 ± 0.8	119 ± 4	6	5	3.2

[a] The IC_{50} values of the test agents were determined in the above assays as described in Biological Methods.

Table 5. IC_{50} Values of Selected Compounds for Inhibition of [^{3}H]DA Uptake and [^{125}I]RTI-55 Binding in Nonstandard Conditions (IC_{50} ± SD, nM)[a]

congener	[^{3}H]DA uptake	[^{125}I]RTI-55 binding	standard ratio uptake/ binding[b]	nonstandard ratio uptake/ binding[c]
2	1.75 ± 0.14	2.63 ± 0.14	2.0	0.66
4	1.82 ± 0.12	2.07 ± 0.12	12.2	0.88
19	31.0 ± 1.1	43.6 ± 1.1	1.3	0.71
20	12.3 ± 0.3	17.8 ± 0.4	2.9	0.69
21	2.62 ± 0.04	2.19 ± 0.21	8.0	1.19
22	1.86 ± 0.05	2.94 ± 0.27	18.6	0.63

[a] Each value is the mean ± SD of three independent experiments. [b] Standard binding conditions have been described.[38] Briefly, assays were conducted using brain membranes (not synaptosomes) and 55.2 mM sodium phosphate buffer, pH 7.4, for 18−24 h at 4 °C. [c] Nonstandard binding conditions have been described,[37] and details are included in the Experimental Section.

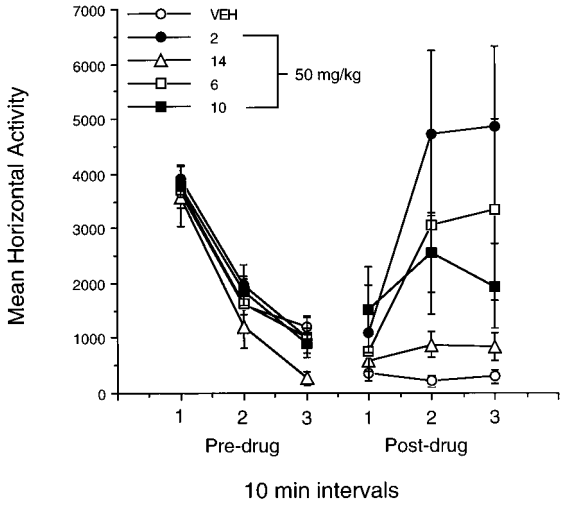


Figure 1. Effects of several heteroaromatic analogs of **2** on horizontal locomotor activity in rats following ip injection (50 mg/kg). Repeated measure analysis of variance revealed a significant drug−time interaction ($F = 2.2$, $p < 0.5$). *Post hoc* comparisons indicated that locomotor activity was increased significantly relative to the vehicle only after treatment with compounds **2**, **6**, and **14** ($p < 0.5$); $n = 6$ in each group.

congeners in this new series of GBR ligands. To determine the influence of assay procedures on the magnitude of this ratio, the IC_{50} values of selected agents for inhibition of [^{3}H]DA uptake and for inhibition of [^{125}I]RTI-55 were measured under identical conditions at the same time. Thus, as described in an earlier publication,[37] [^{125}I]RTI-55 binding was conducted under "uptake" conditions. The data (Table 5) showed that conducting the binding assay under "uptake" conditions eliminated the high uptake/binding ratios which were apparent under "standard" assay[38] conditions. These data suggest that investigators who utilize the uptake/binding ratio to detect a putative cocaine antagonist should conduct both assays using exactly the same assay conditions.

As a continuation of our previous interest[28] in the affinity of DA reuptake inhibitors for binding to σ receptors, several new ligands, all analogs of **2** and **4** (*i.e.*, **6**, **8**, **10**, **12**, **14**, and **16**), were tested at the σ-1 site (Table 3). All of these compounds retained an affinity for displacement of [^{3}H]-(+)-pentazocine[39] at this site (IC_{50} = 38−79 nM) comparable to the parent compounds (IC_{50} = 43 and 51 nM for **2** and **4**, respectively). These derivatives may constitute a novel group of heteroaromatic congeners in the piperazine class of σ ligands.

Preliminary experiments *in vivo* revealed that the new analogs of **2** containing the simple heteroaromatic rings thiophene, furan, and pyridine (*i.e.*, **6**, **8**, **10**, **12**, **14**, and **16**, respectively) stimulated the locomotor activity in rats following ip injection. This stimulating effect of a dose of 50 mg/kg for each analog is shown in Figures 1 and 2. However, the effect of the saturated compounds **6**, **10**, and **14** seemed to be slightly attenuated in comparison with **2** (Figure 1). Nonetheless, these were the first modified GBR derivatives displaying the behavioral profile similar to the parent compounds. On the basis of these results, the thiophene and pyridine derivatives **6**, **8**, **14**, and **16** were further tested in cocaine and food self-administration studies in rhesus

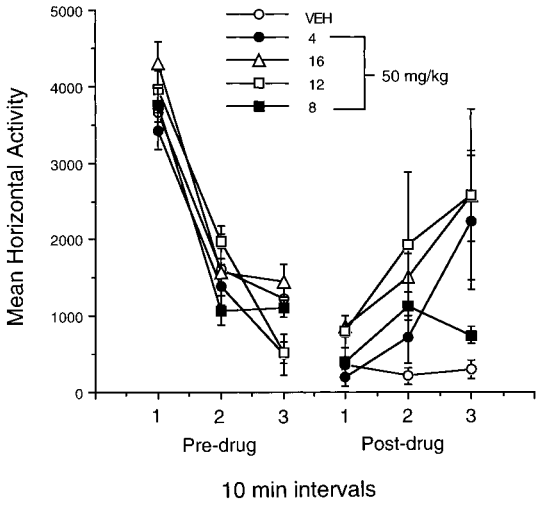


Figure 2. Effects of heteroaromatic unsaturated congeners, analogs of **4**, on horizontal locomotor activity in rats following ip injection (50 mg/kg). Repeated measure analysis of variance revealed a significant drug−time interaction ($F = 2.4$, $p < 0.5$). *Post hoc* comparisons indicated that locomotor activity was increased significantly relative to the vehicle only after treatment with compounds **4**, **12**, and **16** ($p < 0.5$); $n = 6$ in each group.

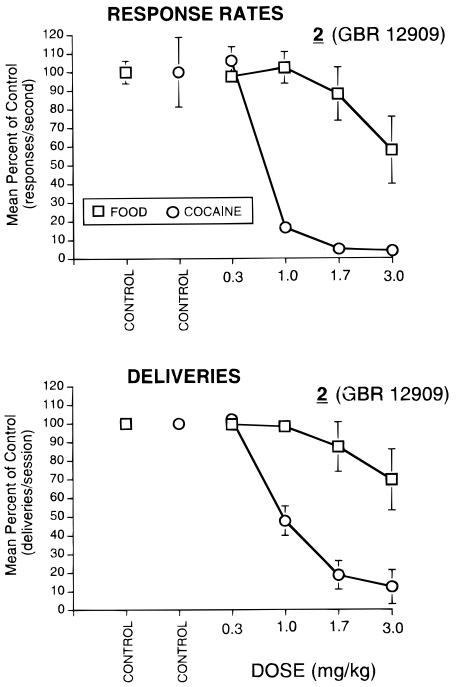


Figure 3. Effects of single doses of **2** on rates of responding (top panel)[20,21] and number of food and cocaine deliveries (bottom panel) maintained under a multiple fixed ratio (FR) food (□), FR cocaine (○, 10 μg/kg/injection) schedule. Drug effects are expressed as the mean ($n = 8$) percentage of individual control as a function of the dose (mg/kg) of GBR 12909, with variability expressed as the standard error of the mean (SEM). Control variability is expressed as a coefficient of variation (±SD, as a percentage of control).

monkeys and the results compared to the parent drug **2**. Furan derivatives **10** and **12**, however, were eliminated from these studies due to the adverse side effects they caused in monkeys.

The effects of **2** on the average rates of responding are shown in the top panel of Figure 3. In general, **2** decreased both the food and cocaine response rates in a dose-dependent manner, but the slopes of the curves

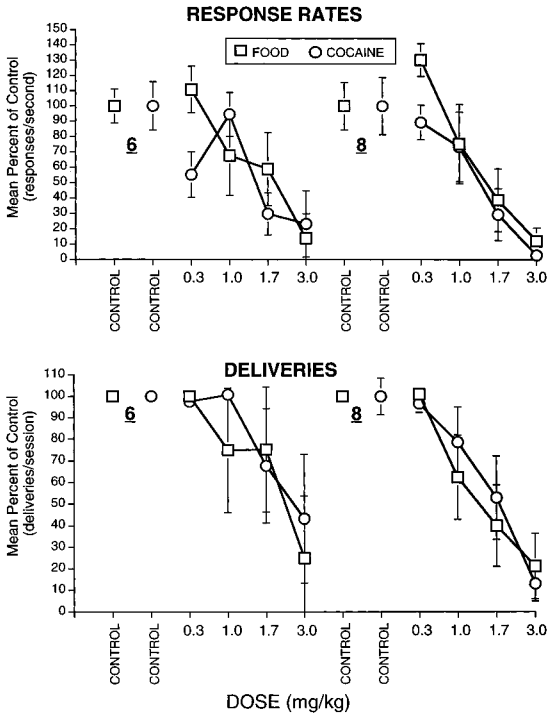


Figure 4. Effects of cumulative doses of **6** and **8** on rates of responding (top panel) and number of food and cocaine deliveries (bottom panel) maintained under a multiple FR food (□), FR cocaine (○, 10 μg/kg/injection) schedule. Drug effects are expressed as the mean ($n = 4$ for each compound) percentage of individual control as a function of the dose (mg/kg), with variability expressed as the standard error of the mean (SEM). Control variability is expressed as a coefficient of variation (±SD, as a percentage of control).

differed for each. At 1.0 mg/kg **2** had no effect on food-maintained responding but decreased cocaine-maintained responding to 16% of the control. At the highest dose tested (3.0 mg/kg) **2** decreased food-maintained responding to 57% of control, and cocaine-maintained responding to 4% of control. The bottom panel of Figure 3 shows a similar effect of **2** on food and cocaine intake. At 1.0 mg/kg **2** had no effect on food intake but decreased cocaine intake to 47% of control. At the highest dose (3.0 mg/kg) tested **2** decreased food intake to 70% of control and cocaine intake to 12% of control.

The effects of **6** and **8** on the rates of responding are shown in the top panel of Figure 4. In general, **6** decreased both food- and cocaine-maintained responding in a dose-dependent manner, although the cocaine-maintained responding was not affected by 1.0 mg/kg. Compound **8** decreased both response rate measures in a dose-dependent manner, although the lowest dose (0.3 mg/kg) increased the rates of food-maintained responding. The effects of **6** and **8** on the average number of food and cocaine deliveries (Figure 4, bottom panel) were similar to those seen in the response rate data; both compounds decreased food and cocaine intake in a dose-related manner.

The effects **14** and **16** on the rates of responding are shown in the top panel of Figure 5. Compound **14** slightly increased food-maintained responding at a dose of 1 mg/kg and decreased both response rate measures at higher doses in a dose-dependent manner. However, cocaine-maintained responding was decreased to a larger degree than food-maintained responding. Congener **16** decreased both food and cocaine rates of

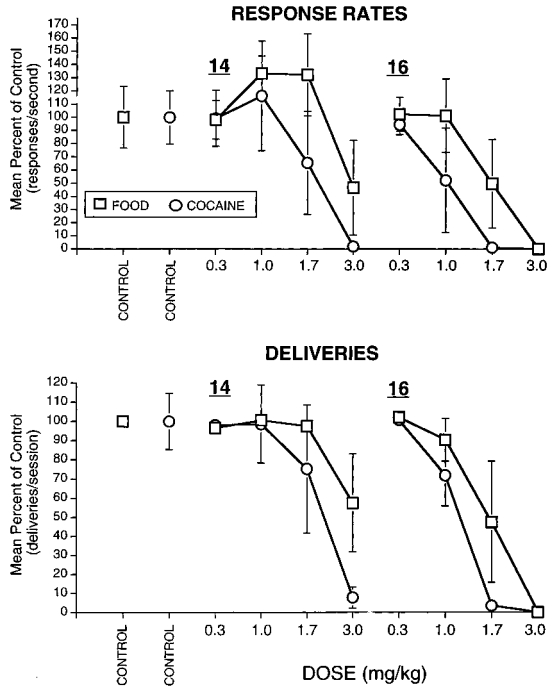


Figure 5. Effects of cumulative doses of **14** and **16** on rates of responding (top panel) and number of food and cocaine deliveries (bottom panel) maintained under a multiple FR food (□), FR cocaine (○, 10 μg/kg/injection) schedule. Drug effects are expressed as the mean ($n = 4$) percentage of individual control as a function of the dose (mg/kg), with variability expressed as the standard error of the mean (SEM). Control variability is expressed as a coefficient of variation (±SD, as a percentage of control).

responding in a dose-dependent manner with a greater effect on cocaine-maintained responding. The effects of **14** and **16** on the average number of food and cocaine deliveries (Figure 5, bottom panel) are similar to those seen in the response rate data; both compounds decreased cocaine intake more than food intake.

These results show that the thiophene- and pyridine-containing GBR analogs decreased the rates of cocaine-maintained responding and the number of cocaine deliveries in a dose-dependent manner. However, all of these agents also affected the food-maintained responding. In these studies, only **2** clearly decreased the rates of cocaine-maintained responding and the number of cocaine deliveries at the 1.0 and 1.7 mg/kg doses without affecting the rates of food-maintained responding or the number of food deliveries. This selective effect was still observed at the highest dose (3.0 mg/kg) of **2**. However, the rates of food-maintained responding and the number of food deliveries were also slightly decreased. All the new analogs tested, both the thiophene (**6** and **8**) and pyridine (**14** and **16**) derivatives, exhibited a comparable potency to the parent compound in decreasing the cocaine self-administration in monkeys at most doses tested. Both pyridine-containing congeners **14** and **16** were more selective than **6** and **8** in decreasing cocaine- vs food-maintained responding. However, the degree of separation between the food and cocaine curves was still smaller than that seen with **2**.

Conclusions

The heteroaromatic substitution in the GBR structure resulted in retention of high affinity of the new compounds for binding to the DAT. Some of these ligands were more selective than the lead compounds at this site vs the 5-HT reuptake site. However, several congeners from quinoline- and naphthalene-containing series were more potent for binding to the SERT than DAT or more potent at inhibiting 5-HT rather than DA reuptake. The series of conformationally restricted ring-fused analogs provides an interesting set of ligands for molecular modeling studies in order to further establish the electronic and sterical requirements for the high affinity and selectivity of GBR-related congeners at the DA reuptake site. Compounds substituted with simple heteroaromatics such as thiophene, furan, or pyridine stimulated locomotor activity in rats following ip injection. These compounds (thiophene and pyridine derivatives) were also potent but less selective in decreasing cocaine-maintained versus food-maintained responding in monkeys. Overall, these results suggest that the development of a partial agonist, or a series of partial agonists, GBR-type of compounds with high affinity and selectivity at the DAT may offer a suitable approach toward the development of potential medications for the treatment of cocaine abuse and addiction. Further *in vivo* experiments are being conducted to study the effects of novel ring-fused analogs of **1** and **2** on both locomotor activity in rats and cocaine and food self-administration in monkeys.

Experimental Section

1. Chemical Methods. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. ^{1}H NMR spectra of the free bases were recorded in $CDCl_3$ using a Varian XL-300 spectrometer. Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a TMS internal standard. Thin layer chromatography (TLC) was performed using 250 μm Analtech GHLF silica gel plates. The TLC systems employed are as follows: $CHCl_3$:MeOH = 19:1, $CHCl_3$:MeOH = 9:1, and $CHCl_3$:MeOH:NH_4OH = 90:9:1. No attempt was made to optimize the yields reported.

Method A: General Procedure of DCC-Catalyzed Condensation. To a stirred solution (or suspension) of the appropriate heteroaromatic acid (2−2.5 equiv) in CH_2Cl_2 or in a mixture of CH_2Cl_2 and THF was added a solution of DCC (2−2.5 equiv) in CH_2Cl_2. After 30 min of stirring at room temperature, a solution of 1-[2-(diphenylmethoxy)ethyl]piperazine or 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]piperazine (1 equiv) in CH_2Cl_2 was first added followed by pyridine (a few drops), and the mixture was stirred until the reaction was complete (TLC, typically 24 h). The mixture was filtered through a pad of Celite, the solvent removed at reduced pressure, and the reaction mixture worked up according to one of the following procedures: (1) The crude product was chromatographed (silica gel, flash column). Dicyclohexylurea, a side product of the reaction, was eluted with ethyl acetate, and then the eluent was switched to a mixture of CH_2Cl_2 and MeOH (100:1) yielding the desired amide as an oily and colorless material. (2). The crude product was dissolved in chloroform, and this solution was treated (three times) with 10% aqueous citric acid. The layers were separated, and the acidic phase was basified with 15% aqueous NaOH and the resulting solution extracted with CH_2Cl_2 to afford the amide which was then used in the next step without further purification.

Method B: Amide Reduction—General Procedure. A 1 M solution of aluminum hydride in THF[35] was added dropwise to a solution of an amide in THF and stirred under nitrogen at the indicated temperature until the reduction was completed by TLC analysis (Table 1). Excess aluminum

hydride was decomposed with cooled 15% NaOH, and the reaction mixture was extracted with Et_2O. The organic layer was then dried over Na_2SO_4 and the solvent evaporated at reduced pressure to yield the final amine as an oily product, which was purified by flash chromatography (silica gel) and/or converted to a salt of the indicated acid and recrystallized from the appropriate solvent (Table 1).

Method C: Hydrogenation. A solution of the unsaturated amine in methanol or in a mixture of methanol and THF (1:1) was hydrogenated in the presence of 10% Pd/C at room temperature until the reaction was completed (TLC; Table 2). The reaction mixture was then filtered through a pad of Celite, to remove the catalyst. The catalyst was washed with methanol, and the solvents were evaporated to yield an oily product which was transformed into a salt of the indicated acid from the appropriate solvent (Table 2).

Method D. Benzimidazo analogs **23** and **24** were synthesized by stirring a mixture of 2-(chloromethyl)benzimidazole (1 equiv), K_2CO_3 (2 equiv), KI (2 equiv), and 1-[2-(diphenylmethoxy)ethyl]piperazine or 1-[2-[bis(4-fluorophenyl)methoxy]-ethyl]piperazine (1 equiv), respectively, in DMF at 60 °C overnight. The reaction mixture was then cooled, poured into ice−water, and extracted with ethyl acetate. The crude, oily amine was chromatographed (silica gel column, CH_2Cl_2:MeOH = 100:1) and transformed into the salt of the indicated acid (Table 1). Analog **13** was synthesized following the same procedure from 1-[2-(diphenylmethoxy)ethyl]piperazine and 3-(3-chloropropyl)pyridine (Table 1).

Method E: Schotten−Baumann Condensation. To a vigorously stirred two-phase mixture of a saturated aqueous solution of sodium bicarbonate and a chloroform solution of 1-[2-(diphenylmethoxy)ethyl]piperazine [or 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]piperazine] was added the appropriate acid chloride (1.1 equiv). The reaction was monitored by TLC. After the reaction was complete, the layers were separated and the aqueous phase was discarded. The resulting organic solution was dried over Na_2SO_4 and chloroform evaporated under reduced pressure to yield the amide which was used in the next step without further purification.

Method F: Benzo[*b*]thiophene-2-carboxylic Acid. A 2.5 M solution of *n*-BuLi in hexanes (7.6 mL, 1.2 equiv) was added to a solution of benzo[*b*]thiophene (16 mmol) in dry THF (15 mL) at −20 °C. The reaction mixture was stirred at this temperature for 10 min. Dry ice was then added and reacted spontaneously. The reaction mixture was allowed to warm up to room temperature, stirred for 2 h, and poured into ice−water. The layers were separated, and the organic phase was washed with water (2×). The aqueous extracts were combined and treated with concentrated HCl to pH 2−3. The pale yellow solid precipitated out of solution was collected on a filter affording benzo[*b*]thiophene-2-carboxylic acid (77% yield): mp >220 °C dec.

^{1}H NMR ($CDCl_3$) data for compounds **4**−**38** are as follows:

4: δ 2.56 (br s, 8H), 2.68 (t, 2H, J = 5.9 Hz), 3.15 (d, 2H, J = 6.9 Hz), 3.56 (t, 2H, J = 5.9 Hz), 5.33 (s, 1H), 6.27 (dt, 1H, J = 6.8, 15.6 Hz, vinyl), 6.52 (d, 1H, J = 15.6 Hz, vinyl), 7.00 (t, 4H, J = 8.8 Hz), 7.23−7.39 (m, 9H).

5: δ 1.81−1.89 (m, 2H), 2.38 (t, 2H, J = 6.4 Hz), 2.47 (m, 4H, -N-CH_2-CH_2-N-), 2.54 (m, 4H, -N-CH_2CH_2-N-), 2.68 (t, 2H, J = 5.9 Hz), 2.85 (t, 2H, J = 7.2 Hz), 3.60 (t, 2H, J = 6.0 Hz), 5.37 (s, 1H, Ph_2C-H), 6.77−6.78 (m, 1H, thienyl), 6.89−6.92 (m, 1H, thienyl), 7.09−7.11 (m, 1H, thienyl), 7.20−7.35 (m, 10H, 2Ph).

6: δ 1.82−1.92 (m, 2H), 2.39 (t, 2H, J = 7.8 Hz), 2.44−2.58 (m, 4H), 2.67 (t, 2H, J = 5.9 Hz), 2.86 (t, 2H, J = 7.9 Hz), 3.54−3.58 (m, 2H), 5.34 (s, 1H, Ph_2C-H), 6.77−6.78 (m, 1H, thienyl), 6.79 (br s, 1H, thienyl), 6.91 (dd, 1H, J = 2.9, 4.9 Hz, thienyl), 7.01 (t, 4H, J = 8.8 Hz), 7.11 (d, 2H, J = 3.9 Hz), 7.25−7.30 (m, 4H).

7: δ 2.54 (m, 8H, -N-CH_2CH_2-N-), 2.69 (t, 2H, J = 6.0 Hz), 3.11 (d, 2H, J = 6.8 Hz), 3.60 (t, 2H, J = 5.9 Hz), 5.37 (s, 1H), 6.04−6.14 (m, 1H), 6.61−6.67 (d, 1H, J = 15.6 Hz, vinyl), 6.94 (m, 1H, thienyl), 7.12−7.14 (m, 1H, thienyl), 7.20−7.35 (m, 11H, 2Ph, 1 thienyl).

8: δ 2.55 (br s, 8H), 2.68 (t, 2H, J = 5.9 Hz), 3.11 (d, 2H, J = 6.8 Hz), 3.56 (t, 2H, J = 5.9 Hz), 5.34 (s, 1H), 6.10 (dt, 1H, J = 6.8, 15.6 Hz), 6.65 (d, 1H, J = 15.6 Hz), 6.94−6.96 (m, 1H), 7.01 (t, 4H, J = 8.8 Hz), 7.14 (d, 1H, J = 4.9 Hz), 7.25−7.30 (m, 5H).

9: δ 1.77−1.87 (m, 2H), 2.38 (t, 2H, J = 7.5 Hz), 2.48 (m, 4H, -N-CH_2-), 2.55 (m, 4H, -N-CH_2-), 2.64 (t, 2H, J = 7.5 Hz), 2.68 (t, 2H, J = 6.0 Hz), 3.60 (t, 2H, J = 6.0 Hz), 5.37 (s, 1H), 5.97 (m, 1H, furyl), 6.26 (m, 1H, furyl), 7.12−7.35 (m, 11H, 2Ph, 1 furyl).

10: δ 1.82 (quintet, J = 7.5 Hz, 2H), 2.37 (t, 2H, J = 7.6 Hz), 2.47−2.68 (m, 12H), 3.56 (t, 2H, J = 6.1 Hz), 5.34 (s, 1H), 5.98 (d, 1H, J = 3.0 Hz), 6.27 (d, 1H, J = 2.5 Hz), 6.97−7.03 (m, 4H), 7.25−7.29 (m, 5H).

11: δ 2.54 (m, 8H, -N-CH_2CH_2-N-), 2.69 (t, 2H, J = 6.0 Hz), 3.11 (d, 2H, J = 6.8 Hz), 3.60 (t, 2H, J = 6.1 Hz), 5.37 (s, 1H, Ph_2C-H), 6.15−6.23 (m, 1H), 6.32−6.38 (m, 2H), 7.20−7.35 (m, 12H).

12: δ 2.55 (br s, 8H), 2.68 (t, 2H, J = 6.0 Hz), 3.12 (d, 2H, J = 6.7 Hz), 3.56 (t, 2H, J = 6.0 Hz), 5.35 (s, 1H), 6.15−6.23 (m, 2H), 6.33−6.38 (m, 2H), 7.00 (t, J = 8.6 Hz, 4H), 7.25−7.33 (m, 5H).

13: δ 1.76−1.83 (m, 2H), 2.35 (t, 2H, J = 7.4 Hz), 2.46 (m, 4H), 2.55 (m, 4H), 2.64 (t, 2H, J = 7.6 Hz), 2.68 (t, 2H, J = 6.0 Hz), 3.60 (t, 2H, J = 6.0 Hz), 5.38 (s, 1H, Ph_2C-H), 7.19 (m, 1H, pyridyl), 7.22−7.35 (m, 10H, 2Ph), 7.48−7.51 (m, 1H, pyridyl), 8.42−8.44 (m, 2H, pyridyl).

14: δ 1.81 (quintet, J = 7.6 Hz, 2H), 2.35 (t, 2H, J = 7.4 Hz), 2.45−2.53 (m, 8H), 2.61−2.68 (m, 4H), 3.56 (t, 2H, J = 6.0 Hz), 5.33 (s, 1H), 7.00 (t, 4H, J = 8.7 Hz), 7.20 (dd, 1H, J = 4.8, 7.8 Hz), 7.25−7.30 (m, 5H), 7.50 (d, 1H, J = 7.8 Hz), 8.43−8.45 (m, 1H).

15: δ 2.57 (m, 8H, -N-CH_2CH_2-N-), 2.70 (t, 2H, J = 6.0 Hz), 3.17 (d, 2H, J = 6.3 Hz), 3.60 (t, 2H, J = 5.9 Hz), 5.36 (s, 1H, Ph_2C-H), 6.32−6.40 (m, 1H, vinyl), 6.49−6.55 (d, 1H, J = 16.2 Hz, vinyl), 7.21−7.35 (m, 11H, 2Ph, 1 pyridyl), 7.70 (m, 1H, pyridyl), 8.46 (m, 1H, pyridyl), 8.58 (m, 1H, pyridyl).

16: δ 2.56 (br s, 8H), 2.68 (t, 2H, J = 6.1 Hz), 3.17 (d, 2H, J = 6.8 Hz), 3.56 (t, 2H, J = 5.9 Hz), 5.33 (s, 1H), 6.34 (dt, 1H, J = 6.6, 15.9 Hz), 6.52 (d, 1H, J = 15.9 Hz), 7.00 (t, J = 8.6 Hz, 4H), 7.21−7.30 (m, 5H, 2Ph, 1 pyridyl), 7.67−7.70 (m, 1H), 8.45−8.47 (m, 1H), 8.58 (br s, 1H).

17: δ 2.57 (m, 8H, -N-CH_2CH_2-N-), 2.69 (t, 2H, J = 6.0 Hz), 3.60 (t, 2H, J = 6.0 Hz), 3.78 (s, 2H), 5.37 (s, 1H, Ph_2C-H), 7.14 (s, 1H, Ar), 7.22−7.35 (m, 12H, 2Ph, 2Ar), 7.67 (m, 1H, Ar), 7.78 (m, 1H, Ar).

18: δ 2.56 (br s, 8H), 2.67 (t, 2H, J = 6.0 Hz), 3.56 (t, 2H, J = 6.0 Hz), 3.78 (s, 2H), 5.31 (s, 1H), 7.00 (t, 4H, J = 8.7 Hz), 7.14 (s, 1H), 7.24−7.32 (m, 6H), 7.68 (d, 1H, J = 6.8 Hz), 7.79 (d, 1H, J = 8.7 Hz).

19: δ 2.60 (m, 8H, -N-CH_2CH_2-N-), 2.71 (t, 2H, J = 5.7 Hz), 3.60 (t, 2H, J = 5.9 Hz), 3.69 (s, 2H), 5.36 (s, 1H, Ph_2C-H), 6.59 (s, 1H, Ar), 7.20−7.34 (m, 12H, 2Ph, 2Ar), 7.46−7.53 (m, 2H, Ar).

20: δ 2.58 (br s, 8H), 2.67 (t, 2H, J = 6.0 Hz), 3.55 (t, 2H, J = 6.0 Hz), 3.69 (s, 2H), 5.32 (s, 1H), 6.59 (s, 1H), 6.99 (t, 4H, J = 8.6 Hz), 7.20−7.28 (m, 6H), 7.46−7.53 (m, 2H).

21: δ 2.52 (m, 8H, -N-CH_2CH_2-N-), 2.69 (t, 2H, J = 6.0 Hz), 3.59 (t, 2H, J = 6.0 Hz), 3.65 (s, 2H), 5.37 (s, 1H, Ph_2C-H), 7.04−7.17 (m, 4H, indole), 7.21−7.34 (m, 10H, 2Ph), 7.54 (d, 1H, J = 7.8 Hz, indole), 8.48 (br s, 1H, indole NH).

22: δ 2.52 (br s, 8H), 2.67 (t, 2H, J = 6.0 Hz), 3.56 (t, 2H, J = 6.0 Hz), 3.65 (s, 2H), 5.33 (s, 1H), 6.35 (s, 1H), 7.00 (t, 4H, J = 8.7 Hz), 7.05−7.17 (m, 2H), 7.24−7.29 (m, 4H), 7.34 (d, 1H, J = 7.9 Hz), 7.55 (d, 1H, J = 7.7 Hz), 8.54 (br s, 1H, indole NH).

23: δ 2.57 (m, 8H, -N-CH_2CH_2-N-), 2.68 (t, 2H, J = 6.0 Hz), 3.59 (t, 2H, J = 5.9 Hz), 3.80 (s, 2H), 5.36 (s, 1H, Ph_2C-H), 7.20−7.34 (m, 12H, 2Ph, 2Ar), 7.41 (br s, 1H, Ar), 7.72 (br s, 1H, Ar), 9.86 (br s, 1H, N-H).

24: δ 2.62 (br s, 8H), 2.70 (t, 2H, J = 5.9 Hz), 3.59 (t, 2H, J = 5.9 Hz), 3.85 (s, 2H), 5.35 (s, 1H), 6.99−7.04 (m, 4H), 7.23−7.30 (m, 6H), 7.59 (br s, 2H), 9.81 (br s, 1H, N-H).

25: δ 2.58 (br s, 8H, -N-CH_2CH_2-N-), 2.69 (t, 2H, J = 6.1 Hz), 3.60 (t, 2H, J = 6.1 Hz), 3.84 (s, 2H), 5.37 (s, 1H, Ph_2C-H), 7.20−7.35 (m, 10H), 7.49−7.54 (m, 1H), 7.62−7.72 (m, 2H), 7.78−7.81 (m, 1H), 8.07 (d, 1H, J = 8.5 Hz), 8.12 (d, 1H, J = 8.6 Hz).

26: δ 2.61 (br s, 8H), 2.70 (t, 2H, *J* = 5.9 Hz), 3.59 (t, 2H, *J* = 5.9 Hz), 3.86 (s, 2H), 5.35 (s, 1H), 7.01 (t, 4H, *J* = 8.7 Hz), 7.28−7.31 (m, 4H), 7.53 (t, 1H, *J* = 7.3 Hz), 7.64 (d, 1H, *J* = 8.3 Hz), 7.71 (t, 1H, *J* = 6.9 Hz), 7.82 (d, 1H, *J* = 7.7 Hz), 8.09 (d, 1H, *J* = 8.5 Hz), 8.14 (d, 1H, *J* = 8.5 Hz).

27: δ 2.54 (m, 8H, -N-CH_2CH_2-N-), 2.70 (t, 2H, *J* = 6.1 Hz), 3.60 (t, 2H, *J* = 6.0 Hz), 3.69 (s, 2H), 5.36 (s, 1H, Ph_2C-H), 7.12−7.34 (m, 10H, 2Ph), 7.54 (t, 1H, *J* = 7.3 Hz, Ar), 7.69 (t, 1H, *J* = 7.9 Hz, Ar), 7.80 (d, 1H, *J* = 8.0 Hz, Ar), 8.05−8.11 (m, 2H, Ar), 8.89 (s, 1H, Ar).

28: δ 2.54 (br s, 8H), 2.67 (t, 2H, *J* = 5.9 Hz), 3.56 (t, 2H, *J* = 6.1 Hz), 3.69 (s, 2H), 5.33 (s, 1H), 7.00 (t, 4H, *J* = 8.7 Hz), 7.24−7.29 (m, 4H), 7.54 (t, 1H, *J* = 7.2 Hz, Ar), 7.69 (m, 1H), 7.80 (d, 1H, *J* = 7.5 Hz), 8.06−8.12 (m, 2H), 8.90 (d, 1H, *J* = 2.1 Hz).

29: δ 2.55 (m, 8H, -N-CH_2CH_2-N-), 2.70 (t, 2H, *J* = 6.0 Hz), 3.60 (t, 2H, *J* = 6.0 Hz), 3.68 (s, 2H), 5.37 (s, 1H, Ph_2C-H), 7.22−7.41 (m, 10H, 2Ph), 7.73 (m, 3H, Ar), 8.04−8.14 (m, 2H, Ar), 8.88 (m, 1H, Ar).

30: δ 2.54 (br s, 8H), 2.68 (t, 2H, *J* = 6.0 Hz), 3.56 (t, 2H, *J* = 5.9 Hz), 3.68 (s, 2H), 5.33 (s, 1H), 6.99 (t, 4H, *J* = 8.6 Hz), 7.24−7.29 (m, 4H), 7.39 (q, 1H, *J* = 4.2 Hz), 7.73 (d, 3H, *J* = 7.1 Hz), 8.06 (d, 2H, *J* = 9.2 Hz), 8.13 (d, 1H, *J* = 8.6 Hz), 8.89 (d, 1H, *J* = 5.9 Hz).

31: δ 1.96−1.99 (m, 2H), 2.58−2.68 (m, 8H, -N-CH_2CH_2-N-), 2.77 (t, 2H, *J* = 5.9 Hz), 2.88 (t, 2H, *J* = 4.8 Hz), 3.10 (t, 2H, *J* = 6.1 Hz), 3.65 (t, 2H, *J* = 5.9 Hz), 5.37 (s, 1H, Ph_2C-H), 7.17−7.37 (m, 12H, 2Ph, 2 benzimidazole), 7.53−7.56 (m, 3H, benzimidazole).

32: δ 1.95−2.02 (m, 2H), 2.62 (t, 2H, *J* = 5.2 Hz), 2.68 (br s, 8H), 2.76 (t, 2H, *J* = 5.9 Hz), 3.11 (t, 2H, *J* = 6.1 Hz), 3.62 (t, 2H, *J* = 5.9 Hz), 5.36 (s, 1H, Ph_2C-H), 7.01 (t, 4H, *J* = 8.7 Hz), 7.19 (q, 2H, *J* = 3.0 Hz), 7.26−7.32 (m, 6H), 7.54 (br s, 1H).

33: δ 2.66 (m, 8H, -N-CH_2CH_2-N-), 2.73 (t, 2H, *J* = 6.0 Hz), 3.27 (t, 2H, *J* = 5.1 Hz), 3.29 (t, 2H, *J* = 5.1 Hz), 3.63 (t, 2H, *J* = 6.0 Hz), 5.39 (s, 1H, Ph_2C-H), 6.99 (1H, t, *J* = 6.0 Hz, Ar), 7.22−7.40 (m, 12H, 2Ph, 2Ar), 7.49−7.54 (m, 2H, Ar), 7.72 (d, 1H, *J* = 7.2 Hz, Ar), 7.85 (d, 1H, *J* = 9.0 Hz, Ar), 8.05 (d, 1H, *J* = 7.8 Hz).

34: δ 2.52−2.76 (m, 12H), 3.26−3.31 (m, 2H), 3.59 (t, 2H, *J* = 5.9 Hz), 5.35 (1H, s), 6.98−7.06 (m, 4H), 7.25−7.32 (m, 5H), 7.35−7.38 (m, 1H), 7.41−7.55 (m, 2H), 7.73 (d, 1H, d, *J* = 7.8 Hz), 7.86 (d, 1H, d, *J* = 7.8 Hz), 8.06 (d, 1H, *J* = 7.8 Hz).

35: δ 2.55 (m, 8H, -N-CH_2CH_2-N-), 2.70 (t, 2H, *J* = 6.0 Hz), 3.60 (t, 2H, *J* = 6.0 Hz), 3.66 (s, 2H), 5.36 (s, 1H, Ph_2C-H), 7.22−7.34 (m, 10H, 2Ph), 7.44−7.50 (m, 3H, Ar), 7.73−7.81 (m, 4H, Ar).

36: δ 2.53 (br s, 8H), 2.67 (t, 2H, *J* = 5.8 Hz), 3.56 (t, 2H, *J* = 6.0 Hz), 3.66 (s, 2H), 5.33 (s, 1H), 6.99 (t, 4H, *J* = 8.8 Hz), 7.24−7.29 (m, 5H), 7.44−7.50 (m, 3H), 7.73 (br s, 1H), 7.79−7.84 (m, 2H).

37: δ 2.53 (m, 8H, -N-CH_2CH_2-N-), 2.67 (t, 2H, *J* = 6.0 Hz), 3.59 (t, 2H, *J* = 6.0 Hz), 3.89 (s, 2H), 5.36 (s, 1H, Ph_2C-H), 7.22−7.51 (m, 14H, 2Ph, 4 naphthyl), 7.75−7.77 (m, 1H, Ar), 7.82−7.85 (m, 1H, Ar), 8.28−8.30 (m, 1H, Ar).

38: δ 2.53 (m, 8H), 2.65 (t, 2H, *J* = 6.0 Hz), 3.55 (t, 2H, *J* = 6.1 Hz), 3.90 (s, 2H), 5.32 (s, 1H), 6.99 (t, 4H, *J* = 8.7 Hz), 7.24−7.28 (m, 5H), 7.37−7.44 (m, 1H), 7.47−7.51 (m, 2H), 7.76−7.79 (m, 1H), 7.84−7.86 (m, 1H), 8.28−8.31 (m, 1H).

^{1}H NMR ($CDCl_3$) data for representative examples of precursor amides are as follows.

Precursor for amine **11**: δ 2.55 (m, 4H), 2.70 (t, 2H, *J* = 5.7 Hz), 3.61 (t, 2H, *J* = 5.9 Hz), 3.72 (m, 4H), 5.37 (s, 1H), 6.45 (m, 1H, vinyl), 6.54 (m, 1H, furyl), 6.79 (d, 1H, *J* = 15.3 Hz, vinyl), 7.24−7.33 (m, 10H, 2Ph), 7.42 (br s, 1H, furyl), 7.48 (br s, 1H, furyl).

Precursor for amine **29**: δ 2.51 (m, 2H), 2.63 (m, 2H), 2.73 (t, 2H, *J* = 5.6 Hz), 3.47 (m, 2H), 3.62 (t, 2H, *J* = 5.4 Hz), 3.83 (m, 2H), 5.37 (s, 1H), 7.24−7.33 (m, 10H, 2Ph), 7.44−7.49 (m, 1H, quinoline), 7.70−7.73 (m, 1H, quinoline), 7.90 (br s, 1H, quinoline), 8.13−8.21 (m, 2H, quinoline), 8.97−8.98 (m, 1H, quinoline).

Precursor for amine **37**: δ 2.37 (t, 2H, *J* = 4.9 Hz), 2.51 (t, 2H, *J* = 5.1 Hz), 2.65 (t, 2H, *J* = 5.7 Hz), 3.43 (t, 2H, *J* = 5.0 Hz), 3.56 (t, 2H, *J* = 5.8 Hz), 3.70 (t, 2H, *J* = 5.0 Hz), 4.15 (s, 2H), 5.34 (s, 1H), 7.23−7.33 (m, 12H, Ar), 7.39 (m, 1H, Ar), 7.54 (m, 1H, Ar), 7.82 (m, 1H, Ar), 7.87 (m, 1H, Ar), 7.97 (m, 1H, Ar).

2. Biological Methods. A. Binding and Reuptake Inhibition Assays. Binding assays for the DAT followed published procedures and used 0.01 nM [^{125}I]RTI-55[38] (SA = 2200 Ci/mmol). Briefly, 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of drug, 100 μL of radioligand ([^{125}I]-RTI-55), and 50 μL of a "blocker" or buffer. Drugs and blockers were made up in 55.2 mM sodium phosphate buffer, pH 7.4 (BB), containing 1 mg/mL bovine serum albumin (BB/BSA). Radioligands were made up in a protease inhibitor cocktail containing 1 mg/mL BSA [BB containing chymostatin (25 μg/mL), leupeptin (25 μg/mL), EDTA (100 μM), and EGTA (100 μM)]. The samples were incubated in triplicate for 18−24 h at 4 °C (equilibrium) in a final volume of 1 mL. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl/150 mM NaCl, pH 7.4) containing 2% poly(ethylenimine).

The [^{3}H]DA and [^{3}H]-5-HT uptake assays also proceeded according to published procedures.[25] Briefly, synaptosomes were prepared by homogenization of rat caudate (for [^{3}H]DA reuptake) or whole rat brain minus cerebellum (for [^{3}H]-5-HT reuptake) in ice-cold 10% sucrose, using a Potter-Elvehjem homogenizer. After a 1000*g* centrifugation for 10 min at 4 °C, the supernatants were retained on ice. The uptake assays were initiated by the addition of 100 μL of synaptosomes to 12 × 75 mm polystyrene test tubes prefilled with 750 μL of [^{3}H]ligand (5 nM final concentration) in a Krebs-phosphate buffer (pH 7.4), which contained ascorbic acid (1 mg/mL) and pargyline (50 μM) (buffer), 100 μL of test drugs made up in buffer, and 50 μL of buffer. The nonspecific uptake of each [^{3}H]ligand was measured by incubations in the presence of 1 μM GBR 12909 ([^{3}H]DA) and 10 μM fluoxetine ([^{3}H]-5-HT). The incubations were terminated after a 15 min ([^{3}H]DA) or 30 min ([^{3}H]-5-HT) incubation at 25 °C by adding 4 mL of wash buffer (10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl at 25 °C) followed by rapid filtration over Whatman GF/B filters and one additional wash cycle. The Krebs-phosphate buffer contained 154.5 mM NaCl, 2.9 mM KCl, 1.1 mM $CaCl_2$, 0.83 mM $MgCl_2$, and 5 mM glucose. The tritium retained on the filters was counted, in a Taurus beta counter, after an overnight extraction into ICN Cytoscint cocktail.

Nonstandard [^{125}I]RTI-55 binding assay and [^{3}H]DA uptake assay were carried out with minor modifications of the method described above, using the Krebs-phosphate buffer detailed above.[37] Assays were initiated by the addition of 100 μL of synaptosomes to 12 × 75 mm polystyrene test tubes which were prefilled with either Krebs-phosphate buffer plus 1 mg/mL BSA or various concentrations of test drugs in Krebs-phosphate buffer plus 1 mg/mL BSA. After a 60 min preincubation at 25 °C, [^{3}H]DA uptake or [^{125}I]RTI-55 binding was initiated by the addition of 750 μL of radioligand (5 nM [^{3}H]-DA or 10 pM [^{125}I]RTI-55) in Krebs-phosphate buffer. The incubations were terminated after a 20 min incubation at 25 °C by adding 4 mL of wash buffer (10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl at 25 °C) followed by rapid filtration over Whatman GF/B filters [presoaked in 2% poly(ethylenimine)] and one additional wash cycle. Nonspecific binding and uptake were defined using 1 μM GBR 12909. Control studies indicated that specific [^{3}H]DA uptake and [^{125}I]RTI-55 binding were linear with time up to 30 min and directly proportional to the protein concentration range used here.[37]

For the [^{3}H]-(+)-pentazocine (σ) binding assay large batches of frozen membranes were prepared from frozen guinea pig brains (Pel Freeze) as previously described.[40] The [^{3}H]-(+)-pentazocine binding assay proceeded with minor modifications of previously published procedures.[41] Briefly, incubations proceeded for 4−6 h at 25 °C (steady state) in 5 mM Tris-HCl, pH 8.0, with a protease inhibitor cocktail [chymostatin (10 μg/mL), leupeptin (10 μg/mL), EDTA (10 μM), EGTA (10 μM)]. The incubations were terminated by rapid filtration over Whatman GF/B filters presoaked in 1% poly(ethylenimine) followed by two 4 mL washes with ice-cold 5 mM Tris-HCl, pH 8.0. The tritium on the filters was measured after an

overnight extraction in 5 mL of ICN Cytoscint cocktail, using standard liquid scintillation counting methods. Nonspecific binding was determined by incubations in the presence of 10 μM DTG.

For the structure−activity study, initial experiments were conducted to determine the appropriate concentration range of each test agent at each binding site. After this, eight-point inhibition curves, ranging from 90% to 10% of control, were generated. The data of two separate experiments were pooled and fit, using the nonlinear least-squares curve-fitting language MLAB-PC[42] (Civilized Software, Bethesda, MD), to the two-parameter logistic equation[41] for the best-fit estimates of the IC_{50} and slope factor. The data are reported as IC_{50} values. As reported elsewhere,[25] the K_m values for [^{3}H]-5-HT and [^{3}H]-DA reuptake are 17.4 ± 0.8 and 38.3 ± 1.6 nM, respectively. The K_d values for [^{3}H]GBR 12935, [^{125}I]RTI-55 binding, and [^{3}H]-(+)-pentazocine are 1.35 ± 0.14, 0.91 ± 0.04, and 4.9 ± 0.1 nM, respectively.[38,43,44] The sources of radioligands and reagents are published.[25,38,43,44]

B. Locomotor Activity in Rats. Subjects. Male Sprague−Dawley rats weighting 250−300 g were group housed and maintained on a 12 h light−dark cycle (lights on 0600−1800 h) with food and water available *ad libitum* in the home cage. All the animals were adapted to the vivarium conditions for at least 1 week before experimentation was begun. Behavioral testing was always performed between 1000 and 1700 h.

Apparatus. Locomotor activity was assessed in photocell activity monitors (Omnitech Electronics, Columbus, OH) which were constructed from clear Plexiglas (30.5 cm high × 42 cm long). The activity monitors were enclosed in sound-attenuating compartments equipped with a 15 W fluorescent light, a ventilating fan that also provides masking noise, and a one-way mirror (21 × 21 cm) mounted in the door, to allow visual observation of the animals during testing. A series of 16 equally spaced infrared photocell detectors were located along two adjacent walls of the chamber 4 cm from the floor surface. Interruptions of the infrared light source by the animals were recorded and stored by an IBM AT computer.

Drugs. The drugs were dissolved in a solution comprised of 25% propylene glycol and 75% sterile water in a concentration of 30 mg/mL. It was necessary to slightly heat some of the drug solutions. Drugs were injected in a volume of 1 mL/kg.

Procedure. All rats were placed in the locomotor activity chambers for 30 min. Data were collected in 10 min intervals. The animals were then removed from the apparatus, injected with the appropriate drugs or vehicle, and returned to the activity monitors for an additional 60 min. Data were collected again in 10 min intervals.

C. Self-Administration Studies in Monkeys. Eight adult, male rhesus monkeys (*Macaca mulatta*), maintained at 90% of their *ad libitum* weight, were used as subjects (4 animals/group). All monkeys were surgically implanted with a subcutaneous vascular access port system, according to the described method,[45] and trained to press a lever for food pellets and intravenous cocaine injections.[20,21] After lever pressing was established, responding was maintained under a multiple fixed ratio 30−fixed ratio 30 schedule of reinforcement for food and cocaine, respectively, in a manner described previously[21] but with the following schedule modifications to allow for cumulative dosing of the GBR analogs. A daily session consisted of eight components (four food and four drug components) with a 15 min time out (TO) period preceding each food component and a 5 min TO period preceding each drug component. These TO periods were signaled by turning off all stimulus lamps. During TO periods 1−4 preceding the food components, a GBR analog was infused via an iv line. The sequence of doses during TO periods 1−4 was 0.3, 0.7, 0.7, and 1.3 mg/kg, respectively, allowing for a cumulative dose of 3.0 mg/kg being delivered by the end of the fourth TO period. The GBR analog was delivered in a volume proportional to the weight of the monkey (3.0 cc of solution over a 3.0 s period, per 10 kg of weight). During the TO periods preceding the drug components, there were no scheduled consequences for responding. Completion of 30 lever presses in the presence of blue stimulus lamps was followed by a food pellet delivery, and completion of 30 lever presses in the presence of red stimulus lamps was followed by a drug delivery in a volume proportional to the weight of the monkey (0.75 cc of solution over a 2.7 s period, per 10 kg of weight). Each FR-30 requirement was constrained by a 60 s limited hold (reinforcement delivery was contingent upon the emission of 30 responses within 60 s from the last reinforcer delivery), and each reinforcer delivery was followed by 3 s of darkness during which there were no scheduled consequences for lever pressing. Within each component a maximum of 10 reinforcers could be delivered. Thus, a component consisted of either 10 FR-30s, 10 limited holds, or any combination of both to total 10. This sequence of events was repeated four times during all sessions. At the beginning of each of the four 15 min TO periods, increasing doses of the GBR analogs were delivered, namely, 0.3, 0.7, 0.7, and 1.3 mg/kg, culminating in a cumulative dose sequence of 0.3, 1.0, 1.7, and 3.0 mg/kg.

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