Novel 3-Aminomethyl- and 4-Aminopiperidine Analogues of 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazines: Synthesis and Evaluation as Dopamine Transporter Ligands

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We have undertaken a program to develop cocaine antagonists based on the premise that such compounds should block cocaine binding but permit reuptake of dopamine at the dopamine transporter (DAT). To evaluate the structural features of potential cocaine antagonists, 3-aminomethylpiperidine and 4-aminopiperidine moieties were incorporated at the central bridge region (piperazine ring) of GBR 12935. The compounds were assayed as inhibitors of [^{125}I]RTI-55 binding at the DAT and monoamine transport. The results indicated that most of the new compounds preferentially inhibited norepinephrine reuptake by its transporter (NET) but in some cases retained binding selectivity for the DAT. In general, the binding selectivity and potency of [^{3}H]NE reuptake inhibition were very sensitive to modifications of the central bridge diamine moiety (position of two basic nitrogen atoms). Compound **6** exhibited the highest ratio (14-fold) of DA reuptake inhibition to RTI-55 binding inhibition at the DAT; however, in an in vitro assay of cocaine antagonism, this compound failed to reduce inhibition of [^{3}H]DA uptake by cocaine. These results demonstrated that separation of biological activities into the binding and reuptake inhibition can be achieved by alterations in the internal diamine component of GBR 12935, but additional modifications are necessary before these agents constitute lead compounds for development as cocaine antagonists.

Introduction

Because of its powerful reinforcing properties and stimulant effect, cocaine is one of the most widely abused drugs. The consequences of cocaine abuse have had profound negative effects on public health and safety.[1−5] The reinforcing properties and other behavioral effects of cocaine have been attributed to its action on dopaminergic neurons (dopamine hypothesis).[1,6] However, the actions of cocaine are believed to include the blockade of reuptake of a variety of neurotransmitters, including dopamine (DA), serotonin (5-HT), and norepinephrine (NE).[1−5] As a result, many research efforts have been directed to the synthesis of analogues and the development of structure−activity relationships (SAR) focused on elucidating the nature of cocaine binding at these sites.[7−10] Although it has been shown that the stimulant activities of cocaine and cocaine-like compounds are directly related to their in vitro binding affinity for the dopamine transporter (DAT),[11] it has been suggested that cocaine antagonists may be structurally dissimilar to cocaine.[11]

The development of cocaine antagonists[12] has been of considerable interest because of their potential use in the treatment of cocaine addiction. Among these, the GBR-type compounds[13] have been extensively studied because of their unique binding properties in vitro and in vivo at DAT sites in the CNS.[14−18] Unfortunately, these compounds are highly lipophilic and bind to nonspecific "piperazine acceptor" sites.[19,20] Other studies, focused on analogues that incorporate modifications of the central diamine and aralkyl moieties,[21−23] established that a two-carbon distance appeared to be optimal for DAT activity. Although these studies made some correlations between ligand structure and inhibition of DAT binding, the key question remained unanswered, i.e., Is it possible to dissociate binding and reuptake inhibition by modifying the chemical structure of GBR compounds? Separation of these two activities is an important issue because the ability to inhibit cocaine binding without significantly reducing dopamine reuptake may constitute the basis for a functional cocaine antagonist.[24]

In an effort to develop GBR analogues which have a dissociation of binding and reuptake inhibition activities, we focused on probing the role of the central diamine unit.[25,26] Our primary objective was to identify structural features which confer a strong binding activity at the DAT but have a weaker effect on DA reuptake. As a first step in evaluating the positional/conformational requirements for each nitrogen atom in the ring (positional flexibility for activity and selectivity), piperidine-based GBR analogues with flexible amine side chains at the 3-position were selected (Figure 1). By making substitutions with head ((bisphenylmethoxy)-ethyl) and tail (phenylpropyl) groups at both sides of the central ring, the resulting analogues were expected to have limited and selective flexibility at the N1/N2

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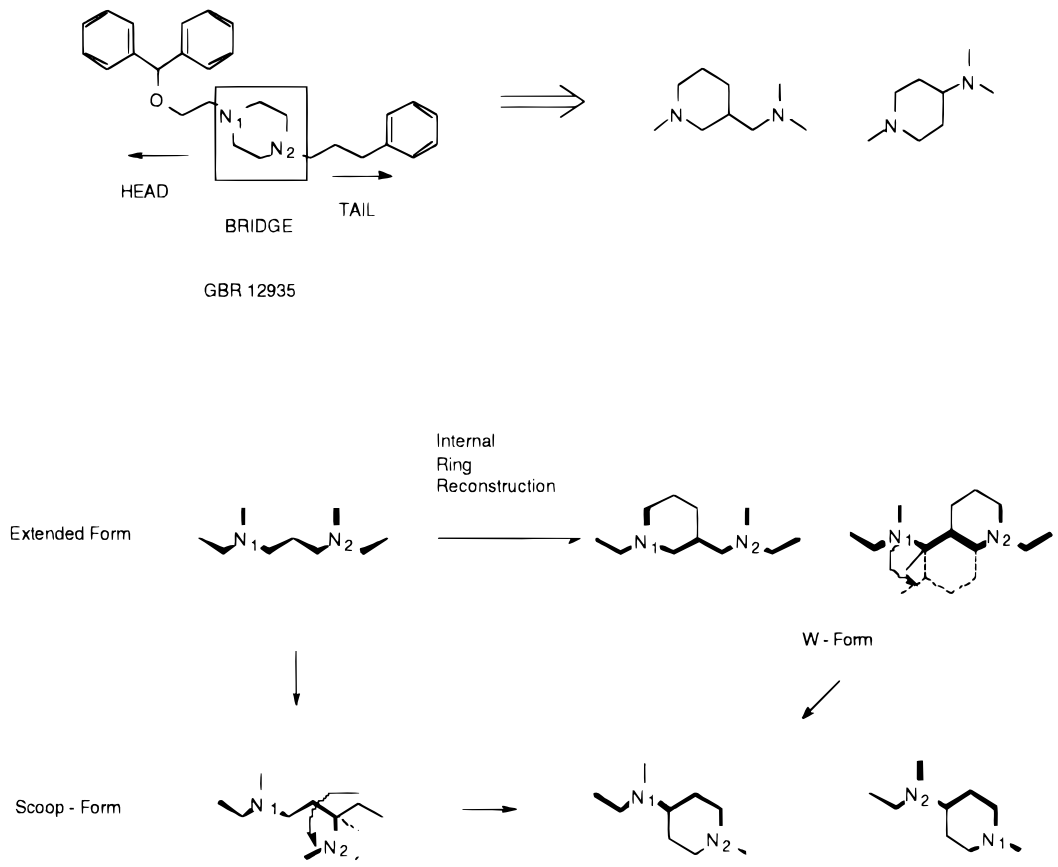


Figure 1. Design strategy for the GBR analogues prepared in this study.

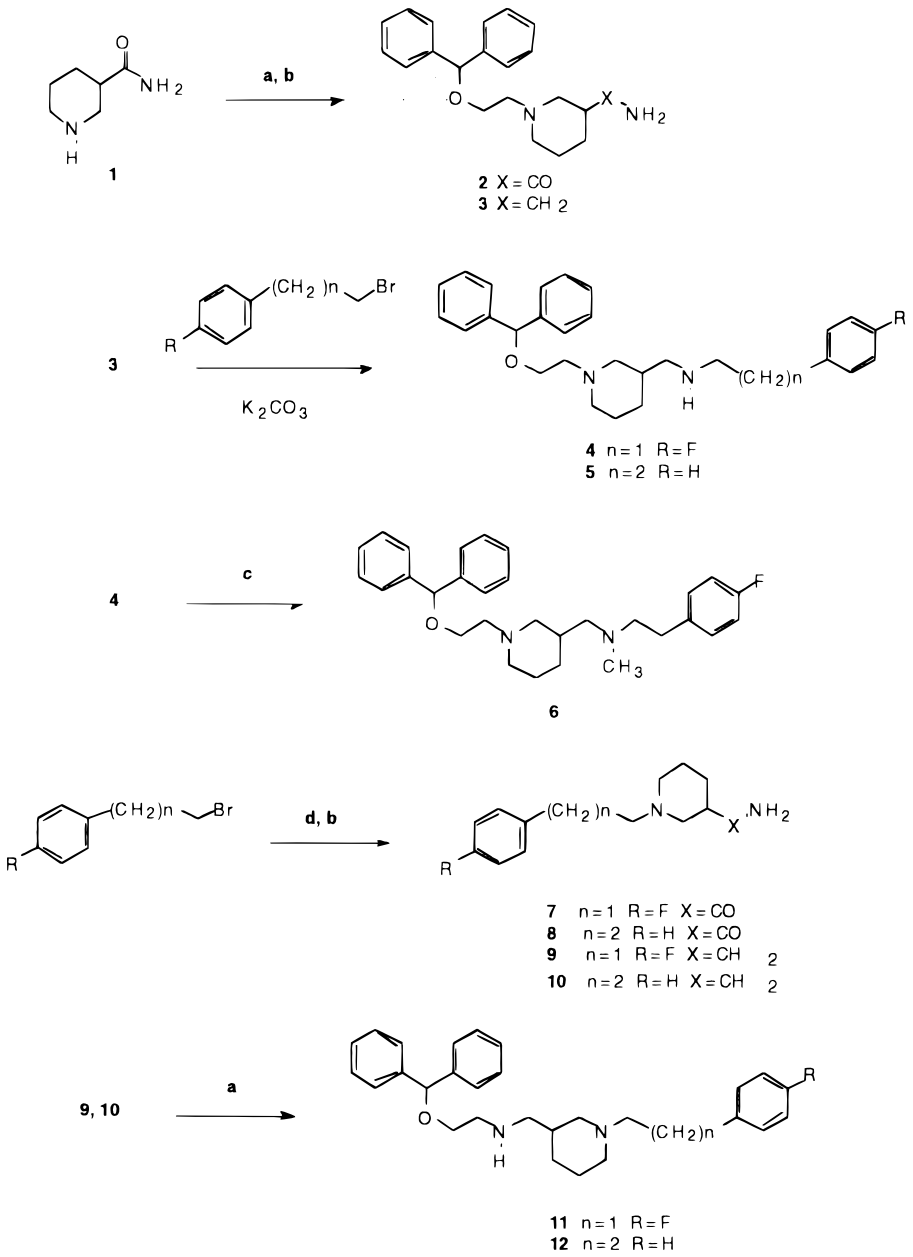
nitrogen atoms. The 4-aminopiperidine ring was chosen as a diamine substitution of the piperazine ring in the central portion of the GBR structure. Although the amine at the exocyclic chain of the piperidine ring is more conformationally restricted than that of 3-aminomethylpiperidine, it is more flexible than the piperazine ring. The spacial orientation of each nitrogen atom of the piperidine analogues containing a fixed three-carbon unit (distance from N1 to N2) is to be evaluated. The extended form will provide a 3-aminomethylpiperidine ring core whereas the side-chain-rotated form will result in a more conformationally restrained 4-aminopiperidine ring structure. Two series of semi-rigid piperidine ring analogues with exocyclic amines, which are expected to have low binding activity at the "piperazine acceptor" site,[27] were synthesized and evaluated for in vitro binding and reuptake inhibition at dopamine, serotonin, and norepinephrine transporters (DAT, SERT, and NET).

Chemistry

The synthetic methods for preparation of 3-aminomethylpiperidine ring analogues of GBR 12935 are illustrated in Scheme 1. Nipecotamide **1** was used as starting material in the synthesis of compounds **4**−**6**, **11**, and **12**. The N-alkylated amide intermediates **2**, **7**, and **8** were prepared in good yields (88%, 79%, and 85%, respectively) by alkylation of the nipecotamide with head or tail alkylating agents such as (bisphenylmethoxy)ethyl bromide, 3-phenyl-1-bromopropane, or 4-fluorophenethyl bromide in the presence of K_2CO_3. The resulting amides **2**, **7**, and **8** were reduced with lithium aluminum hydride to give primary amine intermediates **3**, **9**, and **10** in good yields (84%, 80%, and 79%, respectively). Monoalkylation of these primary amines **3**, **9**, and **10** with appropriate head or tail alkylating agents produced the target GBR analogues **4**, **5**, **11**, and **12**. Synthesis of N-methylated piperidine analogue **6** was achieved using formaldehyde sodium cyanoborohydride (80% yield).[28]

The ^{1}H NMR spectrum of N-alkylated nipectotamide **2** in the synthesis of 3- aminomethylpiperidine GBR-type analogues showed some unexpected chemical shift and splitting patterns. Interestingly, these patterns resulted from the internal hydrogen bonding between the amide proton and the basic tertiary amine. This hydrogen bonding appeared to stabilize structures that are relatively unfavorable on steric and energetic grounds. ^{1}H NMR spectroscopy aided in assigning the ring structure in these N-alkylated nipecotamides. The key feature was a significant difference in the absorption of two protons in the $-CONH_2$ group. Intramolecular hydrogen bonding led to a specific downfield shift and broadening of the peak corresponding to one of the amide protons. This effect was also reflected by difference in chemical shift, peak shapes, and coupling constants for the H*c* proton adjacent to H*a*/H*b* and H*d*/H*e* proton pairs. In the absence of internal hydrogen bonding, axial and equatorial configurations are preferred for the H*c* proton and the amide functionality, respectively. This is due to steric hindrance between the amide and ring moieties. With the H*c* proton in an axial position, coupling to the H*a* proton is expected to produce a triplet with 2 large *J* values, which arise from

Scheme 1[a]



[a] (a) $(Ph)_2CHOCH_2CH_2Br$, K_2CO_3, DMF; (b) LAH, THF; (c) HCHO, $NaCNBH_3$; (d) **1**, K_2CO_3, DMF.

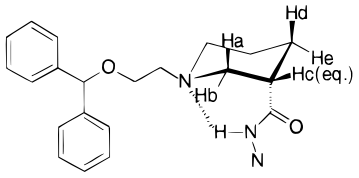
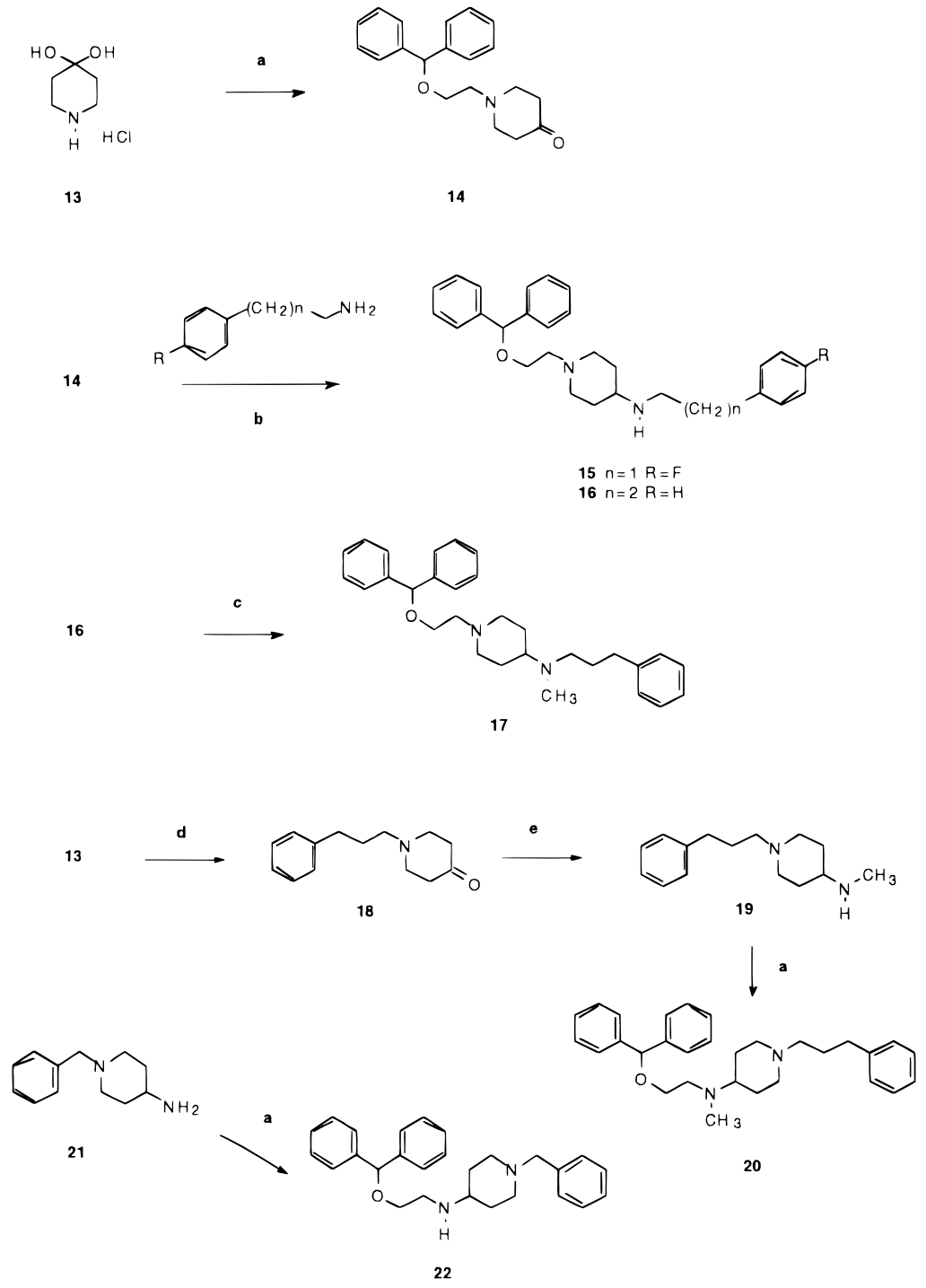


Figure 2. Proposed structure for intermediate **5** illustrating internal hydrogen bonding.

geminal and ax-ax couplings, and a doublet of doublets with 1 large and 1 small *J* value from ax-ax and ax-eq couplings. In contrast, the measured ^{1}H NMR spectrum of the H*a* proton revealed a doublet of doublets with 1 large and 1 small *J* value from geminal and ax-eq couplings. These findings are consistent with the internal hydrogen bonding depicted in Figure 2.

Preparation of the 4-aminopiperidine ring analogues of GBR 12935, Scheme 2, used 4-piperidone monohydrate HCl and *N*-benzyl-4-aminopiperidine as the starting materials in the synthesis of **15**−**17**, **20**, and **22**. N-Alkylation of 4-piperidone monohydrate HCl with 2-bromoethyl diphenylmethyl ether or 1-bromo-3-phenylpropane proceeded in good yield (92%, 88%) to give ketone intermediates **14** and **18**, respectively. The alkylated ketone intermediates **14** and **18** underwent reductive amination with the appropriate primary amine.[29] Reductive aminations of ketone intermediate **14** were performed using a 4-fold excess of the amines, 3-phenyl-1-propylamine, or 4-fluorophenethylamine, and gave products **15** and **16** in 38% and 25% yields, respectively. Introduction of the methyl group into the secondary amine **16** using a formaldehyde sodium cyanoborohydride system gave tertiary amine **17** in moderate yield (54%).[25] As an alternate route to improve yields, an indirect pathway was developed using reductive amination with methyl amine followed by alkylation with tail-aralkyl halides. Reductive amination of ketone

Scheme 2[a]



[a] (a) $(Ph)_2CHOCH_2CH_2Br$, K_2CO_3, DMF; (b) $NaCNBH_3$, ethanolic HCl, molecular sieve; (c) HCHO, $NaCNBH_3$; (d) $PhCH_2CH_2CH_2Br$, K_2CO_3, DMF; (e) CH_3NH, $NaCNBH_3$.

intermediate **18** proceeded smoothly in good yield (80%), and the subsequent N-alkylation with (bisphenylmethoxy)ethyl bromide yielded target compound **20** in a high yield (93%). Monoalkylation of the *N*-benzyl-4-aminopiperidine (5 equiv) with (bisphenylmethoxy)ethyl bromide yielded GBR analogue **22** in moderate yield (70%).

Biological Results and Discussion

Biological evaluation of these compounds was performed with HEK 293 cells expressing cDNA for human dopamine transporters (hDAT), human serotonin transporters (hSERT), or human norepinephrine transporters (hNET). All compounds were tested for ability to displace [^{125}I]RTI and inhibit uptake of [^{3}H]DA, [^{3}H]5-HT, and [^{3}H]NE. The results of the in vitro biological assays are shown in Tables 1 and 2. In the 3-aminomethylpiperidine series, compounds **4**, **5**, and **6**, in which the head group (bisphenylmethoxy ethyl moiety) is linked directly to the piperidine ring, demonstrated high affinity at the DAT (K_i = 0.070−0.090 μM) and modest to significant affinity for the other transporters as well (SERT K_i = 0.300−0.800 μM, NET K_i = 0.040−0.190

Table 1. Binding Affinities and Selectivities of GBR Analogues at the DA (DAT), 5-HT (SERT), and NE (NET) Transporters Measured by Inhibition of [^{125}I]RTI-55 Binding ($K_i \pm$ SD, μM)[a]

	binding ($K_i \pm$ SD, μM)			selectivity ratio	
compound	DAT	SERT	NET	SERT/DAT	NET/DAT
4	0.087 ± 0.009	0.89 ± 0.37	0.190 ± 0.098	10.2	2.1
5	0.070 ± 0.027	0.32 ± 0.10	0.041 ± 0.015	4.5	0.6
6	0.090 ± 0.013	0.53 ± 0.22	0.110 ± 0.029	5.9	1.3
11	0.100 ± 0.030	0.35 ± 0.16	0.150 ± 0.043	3.3	1.5
12	0.018 ± 0.002	0.21 ± 0.055	0.023 ± 0.003	11.9	1.3
15	0.110 ± 0.014	0.220 ± 0.110	0.079 ± 0.038	2.0	0.7
16	0.160 ± 0.044	0.055 ± 0.011	0.120 ± 0.017	0.4	0.8
17	0.340 ± 0.140	0.087 ± 0.023	0.042 ± 0.011	0.3	0.1
20	0.030 ± 0.007	1.40 ± 0.27	0.029 ± 0.009	47.1	1.0
22	0.083 ± 0.028	2.16 ± 0.33	0.180 ± 0.081	26	2.1
GBR 12935	0.073 ± 0.039	2.09 ± 0.76	0.630 ± 0.059	28.6	8.6
cocaine	0.760 ± 0.093	0.390 ± 0.088	1.77 ± 0.37		
cocaine[b]	0.960 ± 0.190	0.490 ± 0.067	1.58 ± 0.26		
cocaine[c]	0.370 ± 0.033	0.400 ± 0.097	1.60 ± 0.23		

[a] The K_i values for the test ligands were determined with assays described in the Methods section. Results are mean ± SD for three independent experiments assayed in triplicate. [b] Cocaine as reference for **15**, **17**, **20**, and **22**. [c] Cocaine as reference for GBR 12935.

Table 2. DA, 5-HT, and NE Reuptake Inhibition and Ratios of Reuptake to Binding of GBR Analogues at DA and 5-HT Transporters ($IC_{50} \pm$ SD, μM)[a]

	reuptake inhibition ($IC_{50} \pm$ SD, μM)			discrimination ratio	
compound	[^{3}H]DA	[^{3}H]5-HT	[^{3}H]NE	[^{3}H]DA reuptake/ DAT binding	[^{3}H]5-HT reuptake/ SERT binding
4	0.364 ± 0.069	1.83 ± 0.58	0.190 ± 0.003	4.2	2.1
5	0.105 ± 0.018	0.770 ± 0.190	0.048 ± 0.012	1.5	2.5
6	1.26 ± 0.34	2.13 ± 0.47	0.350 ± 0.079	14.0	4.0
11	0.500 ± 0.059	1.13 ± 0.20	0.200 ± 0.060	4.8	3.3
12	0.150 ± 0.029	0.820 ± 0.160	0.097 ± 0.030	8.1	3.8
15	0.320 ± 0.130	0.470 ± 0.190	0.170 ± 0.055	2.9	2.1
16	0.390 ± 0.009	0.390 ± 0.012	0.230 ± 0.072	2.5	7.1
17	0.210 ± 0.021	0.310 ± 0.120	0.180 ± 0.069	0.6	3.6
20	0.330 ± 0.120	1.91 ± 0.71	0.200 ± 0.037	11.1	1.4
22	0.270 ± 0.070	1.86 ± 0.59	0.087 ± 0.010	3.2	0.9
GBR 12935	0.018 ± 0.003	3.71 ± 1.43	0.165 ± 0.017	0.25	1.8
cocaine	0.190 ± 0.021	0.340 ± 0.078	0.230 ± 0.041		
cocaine[b]	0.220 ± 0.029	0.220 ± 0.036	0.220 ± 0.060		

[a] IC_{50} values for the test ligands were determined with assays described in Methods. Results are mean ± SD of three independent experiments assayed in triplicate. [b] Cocaine as reference for **9**.

μM). This phenomenon may be related to control of ligand recognition and neurotransmitter reuptake by different structural regions of transporter molecules. Compound **5**, which possesses a three-carbon spacer between the N2 nitrogen and tail benzene ring, had approximately 2-fold stronger binding to the NET compared to the DAT. In contrast, its regioisomer, compound **12**, retained selectivity for the DAT as well as potent reuptake inhibition at the NET. The piperidine ring GBR derivatives, 3-aminomethylpiperidines **5** and **12**, represented an interesting pair of regioisomers. These compounds demonstrated a 4-fold difference in binding affinity and a 2-fold difference in [^{3}H]DA reuptake inhibition at the DAT. The other regioisomer pair **4** and **11** showed little selectivity among the three transporters. Compound **12**, in which the piperidine ring was directly linked to the tail-aralkyl group, possessed the strongest binding affinity at the DAT ($K_i = 0.018$ μM) and better binding selectivity at the DAT compared to its regioisomer **5**. Replacement of the *p*-fluorophenethyl moiety with a phenylpropyl moiety improved slightly the binding activity at the DAT, but significantly enhanced activity at the NET site (compound **5**). Methylation of N2 (compounds **4** to **6**) did not improve binding affinity to the DAT but decreased potency for inhibition of [^{3}H]DA reuptake (**4**, $IC_{50} = 0.36$ μM and **6**, $IC_{50} = 1.26$ μM). Of particular note, one member of this series, compound **6**, exhibited the highest discrimination ratio (14-fold) of DA uptake inhibition to binding inhibition, reduced selectivity for the NET, and moderate affinity for the DAT.

The conformationally more rigid 4-aminopiperidine GBR analogue **16** demonstrated 2- and 3-fold stronger binding at the SERT compared to the NET and DAT, respectively, and mild selectivity for inhibition of [^{3}H]-NE reuptake (2-fold vs [^{3}H]DA and [^{3}H]5-HT) was maintained. This higher binding affinity at the SERT was shifted to the NET and less so to the DAT when the phenylpropyl group was replaced with a 4-fluorophenyl moiety (**16** to **15**). When the N2 secondary amine of analogue **16** was methylated, binding affinity at the NET site was improved ~3-fold; however, DAT and SERT binding were reduced. Compound **20**, the regioisomer of **17**, demonstrated strong binding affinity at the DAT and NET but very weak binding at the SERT (16-fold lower). Interestingly, this regioisomer pair showed an improved discrimination ratio for reuptake to binding at the DAT (0.6 to 11.1). This was also observed in the 3-aminomethylpiperidine series (**5** vs **12**). Replacement of phenylpropyl group by a benzyl moiety in compound **22** resulted in retention of transporter binding which supports findings by Dutta et al.[30]

However, selectivity for inhibition of [^{3}H]NE reuptake and selective binding at the DAT (2-fold vs NET site) were maintained.

Although the contribution of positional flexibility of each amino group to binding could not be determined, several interesting structural features responsible for biological activities were identified. The piperidine ring analogues with a branched basic amine side chain demonstrated the importance of the central bridge ring for modulating the relationship between binding affinity and potency of reuptake inhibition at all three transporter sites. A propylene moiety between two basic nitrogen atoms appears to be the optimal linkage for inhibition of [^{3}H]NE reuptake. However, binding properties were altered by the position of each nitrogen atom in the ring structure.

Based upon its apparent selectivity for inhibition of DA reuptake vs DAT binding ($IC_{50}/K_i = 14.0$), compound **6** was selected for further evaluation using an in vitro cocaine antagonism assay. The ability of this compound to reduce the effectiveness of cocaine to inhibit the reuptake of dopamine was determined at a concentration ranging from 0.010−0.100 μM. Unfortunately, none of the concentrations of drug reduced the inhibition of [^{3}H]DA uptake by cocaine. These data suggest that uptake-to-binding ratios derived from cells transfected with recombinant transporters must be interpreted with caution when used to guide the development of possible cocaine antagonists.[14,31] Interactions of ligands at binding site(s) in the recombinant transporters may be different from those present in rat synaptosomes which may also differ from human transporters in vivo.

Conclusions

A series of GBR 12935 analogues were synthesized to investigate substitution effects with bioisosteric diamine units on cocaine binding and neurotransmitter reuptake. The results demonstrated that substantial modifications can be made without attenuating effects at the DAT. Even though a comprehensive model for explaining the contribution of flexibility at each nitrogen atom was not established, a degree of separation between cocaine binding and dopamine reuptake inhibition was achieved. Although compound **6** demonstrated a high ratio (14-fold) of reuptake inhibition to binding, this compound did not demonstrate cocaine antagonist effects in vitro. Nevertheless, our results may provide directions for the design of newer GBR analogues which may have stronger binding at cocaine recognition sites but much weaker inhibition of DA reuptake.

Experimental Section

General Chemical Methods. All chemicals and solvents were obtained from commercial suppliers and were used without further purification. In general, all reactions were performed under atmospheric conditions and at ambient temperature unless otherwise noted. High boiling point solvents (DMF, DMSO, etc.) that are water soluble were removed from the reaction mixture by first pouring into ethyl acetate and/or diethyl ether followed by washing with a saturated NaCl solution. All organic extracts were dried over $MgSO_4$ or Na_2SO_4 except where otherwise noted. Volatile solvents were removed by rotary evaporation under reduced pressure. Melting points were determined on a Thomas-Hoover melting point apparatus or an electrothermal melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, for carbon, hydrogen, and nitrogen and were within ±0.4% theoretical values unless noted otherwise. ^{1}H and ^{13}C NMR spectra were determined on a Varian XL-300 spectrometer. Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a TMS internal reference standard. In general, $CDCl_3$ was used for the free bases and DMSO-d_6 was used for salts. Coupling constants (J values) were given in hertz. Thin layer chromatography (TLC) was performed on 250 μm thickness 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) developed with appropriate solvent mixtures. Column separations were performed on silica gel (Baker, 40 μm flash chromatography). The collected fractions were analyzed using TLC and visualized by ninhydrin (0.5 g in 100 mL of methanol) for primary and secondary amine(s), ultraviolet light, or iodine vapor. For salt formation, free bases were dried and dissolved in ethyl acetate or/and diethyl ether and filtered. Oxalic acid solution in same solvent or HCl in ether was added to the filtrates until no further turbidity or precipitation occurred. The resultant solids were collected by filtration and recrystallized from appropriate solvents. Unfilterable gels were evaporated to dryness and recrystallized.

Synthetic Chemical Methods. *N*-[2-(Bisphenylmethoxy)ethyl]-3-piperidinecarboxamide (2). A suspension of nipecotamide (1.24 g, 9.67 mmol), 1-(bisphenylmethoxy)-2-bromoethane (2.56 g, 8.79 mmol), and K_2CO_3 powder (2.43 g, 17.6 mmol) in anhydrous DMF (20 mL) was stirred at ambient temperature for 48 h. The reaction mixture was diluted with 200 mL of ethyl acetate and washed with 60 mL of saturated NaCl solution (×3), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$:MeOH:Et_3N (96:4:0.4) as eluent, to give pure product **2** as a colorless oil (88%) that gradually solidified on cooling. Mp: (HCl salt): 223−224 °C; (free base): 94−97 °C. ^{1}H NMR ($CDCl_3$): δ 1.49−1.61 (2H, m), 1.68−1.81 (1H, m), 1.95 (1H, br d, J = 9.6), 2.15 (1H, br t, J = 10.7), 2.28 (1H, dd, J = 2.6, 11.7), 2.47 (1H, m), 2.54 (1H, ddd, J = 4.4, 6.0, 13.3), 2.67 (1H, ddd, J = 4.6, 6.7, 13.3), 2.80 (1H, br d, J = 10.5), 3.02 (1 H, br d, J = 11.0), 3.50−3.62 (2H, m), 5.31 (1H, br s), 5.34 (1H, s), 7.20−7.40 (10H, m), 8.26 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 22.6, 26.8, 41.4, 54.0, 54.5, 57.5, 65.7, 83.9, 84.0, 126.8, 127.5, 128.4, 142.0, 177.9.

***N*-(4-Fluorophenylethy)-3-piperidinecarboxamide (7)** was prepared as described for **2** to give the title compound **7** (2.13 g, 8.5 mmol, 85%) as a sligthly yellow solid. Mp (free base): 91−93 °C. ^{1}H NMR ($CDCl_3$): δ 1.55−1.79 (3H, m), 1.83−1.90 (1 H, m), 2.19 (1 H, br t, J = 9.5), 2.39 (1H, br d, J = 11.1), 2.48 (1H, apparent br quintet, J = 4.2), 2.52−2.83 (6H, m), 5.38 (1H, br s), 6.94−7.00 (2H, m), 7.11−7.27 (2H, m), 7.53 (1 H, br s). ^{13}C NMR ($CDCl_3$): δ 22.6, 26.9, 32.3, 41.7, 53.4, 55.2, 59.9, 115.0, 115.3, 129.9, 130.0, 135.7,135.7, 177.6.

***N*-(3-Phenylpropyl)-3-piperidinecarboxamide (8)** was prepared as described for **2** to give the title compound **8** (1.94 g, 7.9 mmol, 79%) as a slightly yellow solid. Mp (free base): 88−91 °C; (HCl salt): hygroscopic. ^{1}H NMR ($CDCl_3$): δ 1.54−1.67 (2H, m), 1.69−1.89 (4H, m), 2.17 (1H, br t, J = 10.4), 2.35 (3H, t overlapping with br d peak, J = 7.5), 2.48 (1H, apparent br quintet, J = 4.2), 2.61 (3H, t, overlapping with br d peak, J = 7.6), 2.80 (1H, br d, J = 8.3), 6.04 (1H, br s), 7.15−7.21 (3H, m), 7.26−7.30 (2H, m), 7.80 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 22.8, 26.9, 28.4, 33.7, 41.80, 53.7, 55.3, 58.2, 125.80, 128.2, 128.3, 141.8, 178.0.

3-(Aminomethyl)-1-[2-(bisphenylmethoxy)ethyl]piperidine (3). A solution of the amide **2**, *N*-[2-(bisphenylmethoxy)-ethyl]-3-piperidinecarboxamide (2.53 g, 7,48 mmol), in 20 mL of THF was added dropwise to a 1.0 M solution of $LiAlH_4$ (30 mL, 30 mmol) in THF, and the mixture stirred for 24 h at room temperature. After careful sequential addition of water, NaOH solution, and again water, the mixture was filtered and the resultant filter cake was re-extracted with EtOAc (60 mL × 2). The combined organic filtrate and extracts were dried, filtered, and evaporated to give an oil. This crude product was

purified by column chromatography with silica gel using $CHCl_3$:MeOH:NH_4OH (9:1:0.1) to give 2.05 g (6.3 mmol, 84%) of pure clear oil. Mp (HCl salt): hygroscopic; (oxalate salt): hygroscopic. ^{1}H NMR ($CDCl_3$): δ 0.89 (1 H, qd, J = 4.1, 11.5), 1.53−1.81 (5H, m), 2.02 (1H, td, J = 3.1, 11.1), 2.55 (2H, d, J = 6.4), 2.67 (4H, t overlapping with br s peak, J = 6.2), 2.84 (1 H, br d, J = 11.3), 2.93 (1 H, br d, J = 9.3), 3.61 (2H, t, J = 6.1), 5.38 (1H, s), 7.20−7.35 (10H, m). ^{13}C NMR ($CDCl_3$): δ 24.9, 28.3, 38.8, 45.9, 54.6, 58.3, 58.4, 66.9, 83.8, 127.0, 127.4, 128.3, 142.1.

3-(Aminomethyl)-1-[2-(4-fluorophenyl)ethyl]piperidine (9) was prepared as described for **3** to give the title compound **9** (1.20 g, 5.1 mmol, 79%) as a slightly yellow oil. Mp (oxalate salt): 60−70 °C broad range. ^{1}H NMR ($CDCl_3$): δ 0.92 (1H, qd, J = 4.1, 11.7), 1.39 (2H, br s), 1.54−1.81 (5H, m), 1.97 (1H, td, J = 3.0, 11.3), 2.52−2.60 (4H, m), 2.76−2.81 (2H, m), 2.92 (1H, br d, J = 11.1), 3.02 (1H, br d, J = 8.8), 6.93−6.99 (2H, m), 7.13−7.17 (2H, m). ^{13}C NMR ($CDCl_3$): δ 25.1, 28.6, 32.7, 39.7, 46.4, 54.4, 58.1, 61.1, 114.9, 115.2, 129.9, 130.0, 136.1, 136.1.

3-(Aminomethyl)-1-(3-phenylpropyl)piperidine (10) was prepared as described for **3** to give the title compound **10** (1.39 g, 6.0 mmol, 80%) as a slightly yellow oil. Mp (bis-oxalate salt): 43−48 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (1H, qd, J = 4.3, 11.7), 1.50−1.90 (10H, m), 2.35 (2H, t, J = 7.8), 2.55 (2H, d, J = 5.5), 2.61 (2H, t, J = 7.7), 2.84 (1H, br d, J = 11.1), 2.92 (1H, br d, J = 7.2), 7.14−7.19 (3H, m), 7.24−7.29 (2H, m). ^{13}C NMR ($CDCl_3$): δ 25.0, 28.5, 28.6, 33.7, 39.6, 46.3, 54.3, 58.1, 58.5, 125.6, 128.1, 128.2, 142.1.

1-[2-(Bisphenylmethoxy)ethyl]-3-[[(*N*-4-fluorophenylethyl)amino]methyl]piperidine (4) was prepared as described for **2** to give the title compound **4** (0.33 g, 0.74 mmol, 60%) as a colorless oil. Mp (bis-oxalate salt): 200.5−202.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.83−0.93 (1H, m), 1.48−1.79 (6H, m), 2.00 (1H, td, J = 2.9, 11.1), 2.47 (2H, d, J = 5.9), 2.66 (2H, t, J = 6.1), 2.70−2.82 (4H, m), 2.85 (1H, br d, J = 12.2), 2.91 (1H, br d, J = 7.3), 3.59 (2H, t, J = 6.2), 5.37 (1H, s), 6.93−6.99 (2H, m), 7.10−7.15 (2H, m), 7.20−7.36 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.1, 29.0, 35.4, 36.4, 51.3, 53.9, 54.7, 58.3, 59.1, 67.0, 83.8, 83.9, 115.0, 115.3, 127.0, 127.1, 127.4, 128.3, 129.9, 130.0, 135.6, 135.7, 142.2. Anal. ($C_{33}H_{39}N_2O_9F$) C, H, N.

1-[2-(Bisphenylmethoxy)ethyl]-3-[[(*N*-3-phenylpropyl)amino]methyl]piperidine (5) was prepared as described for **2** to give the title compound **5** (0.23 g, 0.52 mmol, 61%) as a colorless oil with the exception that an amine to alkyl halogen ratio of 3:1 was employed. Mp (bis-oxalate salt): 147−150 °C. ^{1}H NMR ($CDCl_3$): δ 0.90−1.01 (1H, m), 1.53−1.89 (8H, m), 2.08 (1H, td, J = 2.5, 10.8), 2.50 (2H, d, J = 6.2), 2.60 (2H, t, J = 7.3), 2.61 (2H, t, J = 7.7), 2.67 (2H, t, J = 6.1), 2.82 (1 H, br d, J = 11.3), 2.91 (1H, br d, J = 9.3), 3.60 (2H, t, J = 5.9), 5.37 (1H, s), 7.15−7.36 (15H, m). ^{13}C NMR ($CDCl_3$): δ 24.8, 28.8, 31.1, 33.5, 35.8, 49.4, 53.8, 54.7, 58.3, 58.9, 66.9, 83.9, 125.8, 127.0, 127.4, 128.3, 141.9, 142.2. Anal. ($C_{34}H_{42}N_2O_9\cdot{}^1/_2H_2O$) C, H, N.

1-[2-(4-Fluorophenyl)ethyl]-3-[[*N*-[2-(bisphenylmethoxy)ethyl]amino]methyl]piperidine (11) was prepared as described for **2** to give the title compound **11** (0.49 g, 1.1 mmol, 69%) as a colorless oil. Mp (oxalate salt): 209−211 °C, ^{1}H NMR ($CDCl_3$): δ 0.93 (1H, qd, J = 3.6, 11.5), 1.59−1.83 (6H, m), 1.97 (1H, td, J = 3.1, 11.2), 2.48−2.51 (2H, m), 2.53−2.56 (2H, m), 2.75−2.78 (2H, m), 2.82 (2H, td, J = 1.7, 5.2), 2.91 (1H, br d, J = 11.3), 3.03 (1H, br d, J = 8.9), 3.59 (2H, t, J = 5.2), 5.37 (1H,s), 6.92−6.98 (2H, m), 7.11−7.16 (2H, m), 7.20−7.35 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.2, 29.2, 32.7, 36.5, 49.5, 53.9, 54.4, 58.6, 61.1, 68.3, 83.8, 83.8, 114.9, 115.1, 126.9, 127.4, 128.3, 129.9, 130.0, 136.1, 136.1, 142.2. Anal. ($C_{33}H_{39}N_2O_9F$) C, H, N.

1-(3-Phenylpropyl)-3-[[*N*-[2-(bisphenylmethoxy)ethyl]amino]methyl]piperidine (12) was prepared as described for **2** to give the title compound **12** (0.55 g, 1.24 mmol, 70%) as a colorless oil. Mp (oxalate salt): 187−189 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (1H, qd, J = 4.3, 11.8), 1.55−1.91 (9H, m), 2.35 (2H, t, J = 7.8), 2.46 (2H, dd, J = 2.8, 6.4), 2.61 (2H, t, J = 7.7), 2.81 (3H, dd, overlapping with br d peak, J = 4.6, 6.1), 2.94 (1H, br d, J = 10.4), 3.57 (2H, t, J = 5.2), 5.36 (1H, s), 7.17−7.34 (15H, m). ^{13}C NMR ($CDCl_3$): δ 25.2, 28.6, 29.3, 33.9, 36.5, 49.5, 54.0, 54.4, 58.7, 58.8, 68.3, 83.8, 125.7, 126.9, 127.4, 128.2, 128.4, 142.2, 142.2. Anal. ($C_{34}H_{42}N_2O_9\cdot{}^1/_2H_2O$) C, H, N.

1-[2-(Bisphenylmethoxy)ethyl]-3-[[*N*-methyl-*N*-[2-(4-fluorophenyl)ethyl]amino]methyl]piperidine (6). To a stirred solution of 1-[2-(bisphenylmethoxy)ethyl]-3-[[*N*-[2-(4-fluorophenyl)ethyl]amino]methyl]piperidine (0.45 g, 1 mmol) **4** and 37% aqueous formaldehyde (0.4 mL, 5 mmol) in 6 mL of isopropanol was added sodium cyanoborohydride (0.126 g, 2 mmol). The resultant turbid reaction mixture was stirred for 24 h at room temperature. The volatiles were removed by rotary evaporation, 100 mL of ethyl acetate was added, and the organic layer was washed with water (30 mL × 3). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness to give the crude product which was purified by column chromatography using silica gel and the appropriate eluent to give pure product as an oil. Mp (oxalate salt): 149−151 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (1H, qd, J = 4.3, 11.8), 1.55−1.83 (5H, m), 2.01 (1H, td, J = 2.6, 11.0), 2.19 (2H, d, J = 6.7), 2.25 (3H, s), 2.45−2.73 (6H, m), 2.92 (1 H, br d, J = 11.3), 2.98 (1 H, br d, J = 11.6), 3.62 (2H, t, J = 6.1), 5.39 (1H, s), 6.92−6.98 (2H, m), 7.10−7.16 (2H, m), 7.21−7.37 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.0, 29.3, 32.7, 34.1, 42.8, 54.7, 58.3, 59.3, 59.9, 62.1, 66.7, 84.0, 114.8, 115.1, 127.0, 127.0, 127.4, 128.2, 128.3, 130.0, 130.1, 136.1, 136.2, 142.1. Anal. ($C_{34}H_{41}N_2O_9F$) C, H, N.

1-[2-(Bisphenylmethoxy)ethyl]piperidine-4-one (14) was prepared as described for **2** to give the title compound **14** (1.20 g, 5.1 mmol, 79%) as a pale yellow oil (2.85 g, 9.2 mmol, 92%). Mp (oxalate salt): 135−137 °C. ^{1}H NMR ($CDCl_3$): δ 2.43 (4H, t, J = 6.2), 2.78−2.84 (6H, apparent q, J = 6.7), 3.63 (2H, t, J = 5.8), 5.38 (1H, s), 7.21−7.37 (10H, m). ^{13}C NMR ($CDCl_3$): δ 41.2, 53.4, 56.7, 67.3, 83.9, 84.0, 126.9, 127.4, 128.3, 142.0, 209.0.

1-(3-Phenylpropyl)piperidine-4-one (18) was prepared as described for **2** to give the title compound **18** (3.82 g, 17.6 mmol, 88%) as a pale yellow oil. Mp (oxalate salt): 149−153 °C. ^{1}H NMR ($CDCl_3$): δ 1.85 (2H, quintet, J = 7.5), 2.42−2.49 (6H, m), 2.67 (2H, t, J = 7.6), 2.72 (4H, t, J = 6.0), 7.16−7.20 (3H, m), 7.26−7.30 (2H, m). ^{13}C NMR ($CDCl_3$): δ 29.0, 33.4, 41.1, 53.9, 56.5, 125.7, 128.2, 128.3, 141.8, 209.0.

1-[2-(Bisphenylmethoxy)ethyl]-4-[*N*-(3-phenylpropyl)amino]piperidine (16). To a solution of 3-phenyl-1-propylamine (5.40 g, 40 mmol) and 20 g of 3 Å molecular sieves in 100 mL of iPrOH was added 1.0 M HCl−ethanol dropwise to adjust pH up to 8. This was followed by the addition of 1-[2-(bisphenylmethoxy)ethyl]-4-piperidone (2.53 g, 8.2 mmol) and $NaBH_3CN$ (0.63 g, 10 mmol). The resulting solution was stirred for 72 h, diluted with 100 mL of MeOH, filtered, and evaporated to give a crude oil. The crude oil was dissolved in 100 mL of ethyl acetate, washed sequentially with 30 mL of 0.1 N NaOH (×3) and saturated NaCl, dried, and evaporated to yield an oil that was purified by column chromatography. The isolated pure compound **16** (1.34 g, 3.1 mmol, 38%) was treated with oxalic acid in ethyl acetate or ether to give bis-oxalate salt which was recrystallized from iPrOH−H_2O. Mp (oxalate salt): 227−230 °C, ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, qd overlapping with br s peak, J = 3.7, 11.9), 1.76−1.86 (4H, quintet overlapping with br d peak, J = 7.4), 2.07 (2H, td, J = 2.2, 11.6), 2.41 (1 H, tt, J = 4.1, 10.6), 2.62−2.68 (6H, m), 2.88 (2H, br d, J = 11.9), 3.58 (2H, t, J = 6.2), 5.38, (1H, s), 7.17−7.35 (15H, m). ^{13}C NMR ($CDCl_3$): δ 31.9, 32.8, 33.7, 46.2, 53.0, 54.8, 57.9, 67.1, 83.8, 125.7, 127.0, 127.3, 128.3, 142.3. Anal. ($C_{33}H_{40}N_2O_9$) C, H, N.

1-[2-(Bisphenylmethoxy)ethyl]-4-[*N*-[2-(4-fluorophenyl)ethyl]aminolpiperidine (15) was prepared as described for **16** to give the title compound **15** (0.16 g, 0.37 mmol, 25%) as a slightly yellow oil. Mp (oxalate): 235−237 °C, ^{1}H NMR ($CDCl_3$): δ 1.44 (2H, qd, J = 3.5, 10.4), 1.88 (2H, br d, J = 9.9), 2.21 (2H, br t, J = 10.9), 2.56 (1H, tt, J = 3.9, 10.1), 2.72 (2H, t, J = 5.9), 2.76−2.81 (2H, m), 2.85−2.90 (2H, m), 2.96 (2H, br d, J = 12.2), 3.60 (2H, t, J = 5.9), 5.38 (1H, s), 6.94−

6.96 (2H, m), 7.13−7.18 (2H, m), 7.20−7.35 (10H, m). ^{13}C NMR ($CDCl_3$): δ 31.6, 35.2, 47.9, 52.4, 57.6, 57.6, 66.5, 84.0, 115.1, 115.4, 126.9, 127.5, 128.4, 130.0, 130.0, 135.1, 135.1, 142.0. Anal. ($C_{32}H_{37}N_2O_9F\cdot{}^1/_2H_2O$) C, H, N.

1-[2-(Bisphenylmethoxy)ethyl]-4-[*N*-methyl-*N*-(3-phenylpropyl)amino]piperidine (17) was prepared as described for **6** to give the title compound **17** (0.25 g, 0.56 mmol, 54%) as a clear colorless oil. Mp (bis-oxalate salt): 201−203 °C. ^{1}H NMR ($CDCl_3$): δ 1.56 (2H, qd, J = 3.5, 12.0), 1.70 (2H, br d, J = 1 1.3), 1.80 (2H, quintet, J = 7.7), 2.03 (2H, td, J = 2.1, 11.7), 2.26 (3H, s), 2.38 (1H, tt, J = 3.8, 1.5), 2.48 (2H, t, J = 7.6), 2.61 (2H, t, J = 8.0), 2.66 (2H, t, J = 6.2), 2.99 (2H, br d, J = 11), 3.58 (2H, t, J = 6.1), 5.36 (1H, s), 7.17−7.36 (15H, m). Anal. ($C_{34}H_{42}N_2O_9$) C, H, N.

1-(3-Phenylpropyl)-4-methylaminopiperidine (19) was prepared as described for **6** to give the title compound **19** (2.05g, 8.82 mmol, 80%) as a slightly yellow oil without pH adjustment step. Mp (bis-oxalate salt): 189−191 °C. ^{1}H NMR ($CDCl_3$): δ 1.56 (2H, qd, J = 3.8, 10.9), 1.77 (2H, quintet, J = 7.7), 1.95 (2H, br d, J = 11.5), 2.10 (2H, br t, J = 11.0), 2.40 (2H, t, J = 7.7), 2.46 (3H, s), 2.56 (2H, t, J = 7.6), 2.68 (1H, tt, J = 4.0, 10.6), 2.95 (2H, br d, J = 12.5), 7.09−7.13 (3H, m), 7.18−7.23 (2H, m). ^{13}C NMR ($CDCl_3$): δ 28.0, 29.2, 31.5, 33.3, 51.3, 55.9, 57.3, 125.9, 128.4, 141.4.

1-(3-Phenylpropyl)-4-[*N*-methyl-*N*-[2-(bisphenylmethoxy)ethyl]amino]piperidine (20) was prepared as described for **2** to give the title compound **20** (1.95 g, 4.41 mmol, 93%) as a pale yellow oil. Mp (bis-oxalate salt): 222−225 °C. ^{1}H NMR ($CDCl_3$): δ 1.55 (2H, qd, J = 3.5, 12.1), 1.70−1.90 (6H, m), 2.28 (3H, s), 2.32 (2H, t, J = 7.7), 2.38 (1 H, tt, J = 3.8, 11.5), 2.61 (2H, t, J = 7.7), 2.75 (2H, t, J = 6.3), 2.95 (2H, br d, J = 11.7), 3.54 (2H, t, J = 6.2), 5.36 (1H, s), 7.16−7.36 (15H, m). ^{13}C NMR ($CDCl_3$): δ 27.8, 28.8, 33.8, 38.6, 53.2, 53.3, 58.0, 61.3, 68.0, 83.8, 83.8, 125.6, 126.9, 127.3, 128.2, 128.2, 128.3, 142.1, 142.3. Anal. ($C_{34}H_{42}N_2O_9$) C, H, N.

1-Benzyl-4-[2-(bisphenylmethoxy)ethyl]aminopiperidine (22) was prepared as described for **2** to give the title compound **22** (0.83 g, 2.07 mmol, 70%) as a sligthly yellow oil. Mp (bis-oxalate salt): >240 °C, ^{1}H NMR ($CDCl_3$): δ 1.40 (2H, qd, J = 3.2, 12.2), 1.64 (1H, br s), 1.82 (2H, br d, J = 12.5), 2.00 (2H, td, J = 2.3, 11.6), 2.44 (1H, tt, J = 4.1, 10.5), 2.85 (4H, t overlapping with br d peak, J = 5.3), 3.49 (2H, s), 3.58 (2H, t, J = 5.3), 5.36 (1H, s), 7.22−7.34 (15H, m). ^{13}C NMR ($CDCl_3$): δ 32.7, 46.3, 52.4, 54.7, 63.0, 68.7, 83.9, 126.9,126.9, 127.4, 128.1, 128.3, 129.1, 142.2. Anal. ($C_{31}H_{36}N_2O_9$) C, H, N.

Biological Methods. [^{125}I]RTI-55 Binding Assay. All drugs were prepared as 10 mM stock solutions in DMSO. The final DMSO concentration in the assay was 0.01%. Pipetting was performed with a Biomek 2000 robotic workstation. HEK 293 cells expressing hDAT, hSERT, or hNET were grown on 150 mm diameter tissue culture dishes. The medium was poured off, the cells were washed with 10 mL of phosphate buffered saline, and 10 mL of lysis buffer (2 mM HEPES, 1 mM EDTA) was added. After 10 min, the cells were scraped from the plates, poured into centrifuge tubes, and centrifuged for 20 min at 30000*g*. The supernatant was removed, and the pellet was resuspended in 0.32 M sucrose and dispersed with a Polytron at setting 7 for 10 s. Each assay contained 50 μL of membrane preparation (approximately 15 μg of protein), 25 μL of drug, and 25 μL of [^{125}I]RTI-55 (40−80 pM final concentration) in a final volume of 250 μL Krebs HEPES buffer, which was used as the assay diluent. The membranes were preincubated with drug for 10 min prior to addition of [^{125}I]RTI-55. The assay tubes were incubated for 90 min at room temperature in the dark, and incubation was terminated by filtration onto GF/C filters using a Tom-tech harvester. Scintillation fluid was added to each square, and radioactivity remaining on the filter was measured using a Wallac μ- or β-plate reader. Competition experiments were conducted in duplicate. The results were analyzed using GraphPAD Prism, and IC_{50} values were converted to K_i's using the Cheng−Prusoff equation. Additional details about this assay have been published elsewhere.[32,33]

[^{3}H]Neurotransmitter Uptake for HEK 293 Cells Expressing Recombinant Biogenic Amine Transporters. HEK 293 cells expressing hDAT, hSERT, or hNET were plated on 150 mm dishes and grown until confluent. The medium was removed, and the cells were washed twice at room temperature with phosphate buffered saline (PBS). Following addition of PBS (3 mL), the plates were placed in a 25 °C water bath for 5 min. The cells were gently scraped and then triturated with a pipette. One plate provided sufficient cells for 48 measurements, and cells from multiple plates were combined. Krebs HEPES buffer (350 μL) and drugs (50 μL) were added to 1.0 mL assay vials and placed in a 25 °C water bath. Aliquots of cell suspension (50 μL) were added, incubation was continued for 10 min, and [^{3}H]DA, [^{3}H]5HT, or [^{3}H]NE (50 μL, 0.020 μM final concentration) was added. After an additional 10 min of incubation, the reaction was terminated by filtration onto GF/C filters using a Tom-tech harvester using filters presoaked in 0.05% polyethyleneimine. Scintillation fluid was added to each square, and radioactivity remaining on the filter was measured using a Wallac μ- or β-plate reader. All assays were performed in triplicate with six drug concentrations. The results were analyzed using GraphPAD Prism. Additional details about this assay have been published elsewhere.[32,33]

Determination of Cocaine Antagonism of [^{3}H]Dopamine Uptake in HEK293 Cells. HEK 293 cells expressing hDAT were grown on 150 mm plates to 100% confluence. The media was removed, and the cells were washed twice with phosphate buffered saline (PBS, pH 7.4) at room temperature. Following addition of 3 mL of Krebs Hepes buffer (pH 7.4), the plates were warmed in a 25 °C water bath for 5 min and the cells were gently scraped and triturated with a pipette. Cells from multiple plates were combined, and four plates provided sufficient cells for the 144 assay wells which were required to generate four cocaine dose−response curves. The experimental conditions for the four curves included a control curve to determine the effect of 0.01% DMSO and three cocaine dose−response curves in the presence of one of three selected concentrations of the test compound, which centered around the K_i determined in the [^{125}I]RTI-55 binding assay. The assay was conducted in 96 1 mL vials. Krebs HEPES (300 μL) and cocaine, mazindol, or buffer (50 μL) were added to each vial in triplicate. Nine concentrations of cocaine ranging from 0.01 to 10 μM were used. Vehicle or one of three concentrations of test compound were added to the vials, and the mixtures were placed in a 25 °C water bath. Specific uptake was defined as the difference in uptake observed in the presence and absence of 5 μM mazindol. Cells (50 μL) were added and preincubated with the test compound for 10 min. The assay was initiated by the addition of [^{3}H]dopamine (50 μL, 0.02 μM final concentration) and terminated after 10 min by filtration through Whatman GF/C filters that were presoaked in 0.05% polyethylenimine. Cocaine IC_{50} values were calculated using GraphPAD Prism software. Three to four independent determinations were performed for each dose−response curve. Statistical analysis of curves generated in the presence or absence of test compound was performed using two-way ANOVA. Cocaine IC_{50} values were compared using one-way ANOVA or *t*-tests, as appropriate. Additional details about this assay have been published elsewhere.[32,33]

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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