Structure−Activity Relationships at the Monoamine Transporters and σ Receptors for a Novel Series of 9-[3-(*cis*-3,5-Dimethyl-1-piperazinyl)-propyl]carbazole (Rimcazole) Analogues

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9-[3-(*cis*-3,5-Dimethyl-1-piperazinyl)propyl]carbazole (rimcazole) has been characterized as a σ receptor antagonist that binds to the dopamine transporter with moderate affinity (K_i = 224 nM). Although the binding affinities at the dopamine transporter of rimcazole and cocaine are comparable, rimcazole only depressed locomotor activity in mice and antagonized the stimulant effects produced by cocaine. The neurochemical mechanisms underlying the attenuation of cocaine's effects are not understood, although interaction at a low affinity site/state of the dopamine transporter has been suggested. To explore further this class of compounds, a series of rimcazole analogues was designed and synthesized. Displacement of [^{3}H]WIN 35,428 binding at the dopamine transporter in rat caudate-putamen revealed that aromatic substitutions on rimcazole were not well tolerated, generally, with significant reductions in affinity for the 3,6-dibromo (**5**; K_i = 3890 nM), 1,3,6-tribromo (**6**; K_i = 30300 nM), 3-amino (**8**; K_i = 2400 nM), and 3,6-dinitro (**9**; K_i = 174000 nM) analogues. The *N*-phenylpropyl group was the only terminal piperazine nitrogen substituent that retained moderate affinity at the dopamine transporter (**11**; K_i = 263 nM). Analogues in which the carbazole ring was replaced with a freely rotating diphenylamine moiety were also prepared. Although the diphenylamino analogue in which the terminal piperazine nitrogen was unsubstituted, as in rimcazole, demonstrated relatively low binding affinity at the dopamine transporter (**24**; K_i = 813 nM), the *N*-phenylpropyl analogue was found to have the highest affinity for the dopamine transporter within the series (**25**; K_i = 61.0 nM). All of the analogues that had affinity for the dopamine transporter inhibited [^{3}H]-dopamine uptake in synaptosomes, and potencies for these two effects showed a positive correlation (r^2 = 0.7731, p = 0.0018). Several of the analogues displaced [^{3}H]paroxetine from serotonin transporters with moderate to high affinity, with the *N*-phenylpropyl derivative (**11**) having the highest affinity (K_i = 44.5 nM). In contrast, none of the analogues recognized the norepinephrine transporter with an affinity of <1.3 μM. Binding affinities for σ_1 and σ_2 receptors were also determined, and several of the compounds were more potent than rimcazole with affinities ranging from 97 nM to >6 μM at σ_1 sites and 145 to 1990 nM at σ_2 sites. The compound with the highest affinity (**25**) at σ_1 sites was also the compound with highest affinity at the dopamine transporter. These novel rimcazole analogues may provide important tools with which to characterize the relationship between the low affinity site or state of the dopamine transporter, σ receptors, and their potential roles in modulating cocaine's psychostimulant actions.

Rimcazole (9-[3-(cis 3,5-dimethyl-1-piperazinyl)propyl]carbazole; **1**) has been reported to decrease locomotor activity in mice and, when administered in combination with cocaine (**2**), to attenuate the cocaine-induced stimulation of activity (Chart 1).[1] Moreover, the sensitization that develops to repeat exposures to cocaine can be attenuated by rimcazole.[2] Thus rimcazole has a behavioral profile that sharply contrasts with that of cocaine and may function to antagonize at least some of the behavioral effects of cocaine. When these studies were reported, rimcazole was primarily known as an "atypical antipsychotic" and a moderately potent σ receptor antagonist.[3−5] The attenuation of cocaine-induced behavior was then attributed to rimcazole's σ antagonist properties. Although the pharmacological significance of σ receptors has not been determined, there are a number of reports in the literature suggesting a possible relationship between σ receptors and the dopamine transporter. For example, cocaine has been reported to bind with low affinity to σ receptors labeled with [^{3}H]haloperidol in rats.[6] Moreover, the high affinity binding of many of the aryl 1,4-dialkyl(en)yl piperazine series of ligands (e.g., GBR 12909, **3**)[7] to both σ sites and the dopamine transporter suggests an overlap in structural requirements for binding to these sites.[8−10] In addition, the photoaffinity label [^{125}I]iodo-azidoco-

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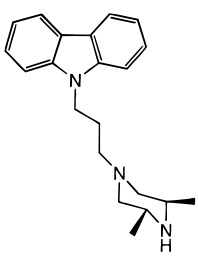
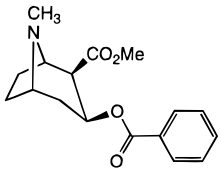
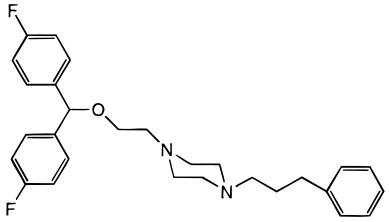
Chart 1. Chemical Structures of Dopamine Uptake Inhibitors



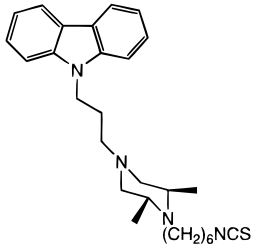
Rimcazole; 1



Cocaine; 2



GBR 12909; 3



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caine was reported to recognize both high and low affinity cocaine binding sites on the dopamine transporter, but when photoactivated this ligand selectively labeled a polypeptide with the pharmacology of a σ receptor.[11] Recently, σ ligands related to arylethylenediamines have been shown to attenuate the locomotor and toxic effects of cocaine.[12,13] The mechanism of these effects appears to involve specific antagonism at σ receptors. These studies, taken together, suggest a structural and potentially functional relationship between σ sites and the cocaine binding site on the dopamine transporter.

A variety of σ ligands have also been tested for their ability to inhibit [^{3}H]WIN 35,428 binding to the dopamine transporter and also their potency in inhibiting dopamine uptake.[14] All of the σ ligands inhibited [^{3}H]-WIN 35,428 binding in a dose-dependent and monophasic manner, in contrast to cocaine and many cocaine analogues, which recognize both high and low affinity binding sites.[15,16] Of the σ ligands studied, rimcazole demonstrated the highest affinity for the dopamine transporter ($K_i = 103$ nM).[14] Further, rimcazole showed a significantly lower potency for inhibiting dopamine uptake ($IC_{50} = 4.22$ μM), compared to its binding affinity, than any of the other ligands evaluated or any other dopamine transporter ligands reported to date.[14] This ratio of dopamine uptake inhibition (IC_{50})/dopamine transporter binding affinity (K_i) for rimcazole suggested a 40-fold difference in potency for the inhibition of dopamine uptake as compared to rimcazole's binding affinity for the dopamine transporter. The significance of this ratio in terms of in vivo function and behavior has not been established. Nevertheless, as rimcazole does not produce cocaine-like psychomotor stimulant effects and has been reported to attenuate the locomotor stimulant effects of cocaine,[1] a novel action at the dopamine transporter was suggested.[14] This novel neurochemical and behavioral profile suggested that rimcazole would be a suitable candidate for further exploration and chemical modification. We were particularly interested in determining if rimcazole and its analogues would have structure−activity relationships resembling any of the other well-known dopamine transporter ligands. In particular, rimcazole shares structural similarities with GBR 12909 (**3**) which has well-described structure−activity relationships at both the dopamine transporter and σ receptors.[9,10] In addition, it was anticipated that with a more extensive series of compounds to evaluate, the potential mechanistic roles of the dopamine transporter and/or σ sites in the behavioral actions of these compounds might be elucidated.

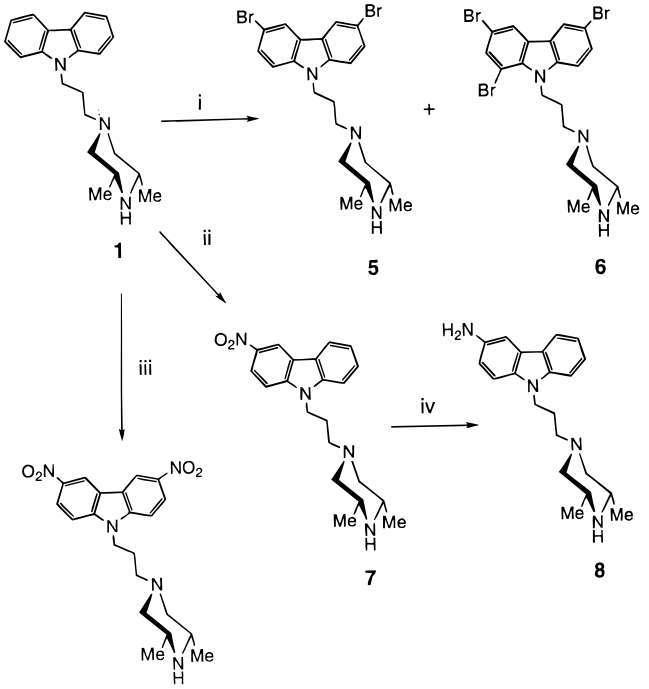
Recently, we published a series of isothiocyanato analogues of rimcazole of which one compound (**4**) demonstrated high affinity, irreversible binding to the dopamine transporter.[17] This study suggested that the rimcazole isothiocyanate analogue may be irreversibly binding to a low affinity site or state of the dopamine transporter.[17] On the basis of rimcazole's lack of cocaine-like actions we have hypothesized that this class of drugs may be binding to a low affinity site or state of the dopamine transporter that is distinct from cocaine's high affinity binding site. In support of this concept, recent studies have suggested that the locomotor stimulant[18] and subjective effects[19] of cocaine and numerous dopamine uptake inhibitors are positively related to interactions at the high affinity binding site on the dopamine transporter. Hence, the low affinity site may not play a primary role in the actions of cocaine and may provide a target for drugs with therapeutic potential in the treatment of cocaine abuse. Thus, in the present investigation, a structure−activity relationship study of the binding site on the dopamine transporter at which rimcazole and structurally related dopamine uptake inhibitors may interact was undertaken. In this pursuit, we considered the carbazole ring and secondary nitrogen of the piperazine ring to be amenable to considerable structural modification. Carbazole ring substituted analogues and analogues in which the structurally rigid carbazole was replaced with the more flexible, untethered diphenylamine moiety were prepared. In addition, selected N-substituted analogues with either the carbazole or diphenylamine moiety at the opposite terminus were synthesized.

Evaluation of the rimcazole analogues at the dopamine transporter and comparison of the structure−activity relationships to those determined at the σ_1 and σ_2 receptors were of primary interest. However, to assess their selectivity, an evaluation of these ligands at the other monoamine transporters (serotonin and norepinephrine) was undertaken. Finally, it was our aim to compare the rimcazole analogues with other existing classes of dopamine uptake inhibitors that share structural features of rimcazole, such as the aryl 1,4-dialkyl-(en)yl piperazine series of compounds. In this way comparisons of the structural, binding, and behavioral profiles of these different classes of compounds could be undertaken, and conclusions regarding the neurochemical bases of these drugs' actions might be ascertained.

Chemistry

Initial synthetic effort was directed at preparing aryl ring substituted analogues of rimcazole (**1**). Both the 3,6-

Scheme 1[a]



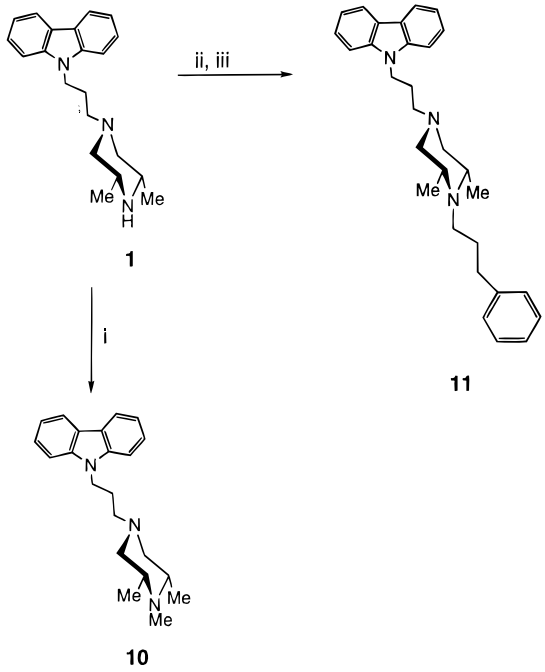
[a] Reagents and conditions: (i) Br_2, HBr/HOAc; (ii) HNO_3/HOAc; (iii) HNO_3/H_2SO_4; (iv) H_2, 10% Pd/C.

dibromo (**5**) and 1,3,6-tribromo (**6**) analogues could be obtained by treating **1** with bromine in aqueous hydrogen bromide/acetic acid (Scheme 1). The dibromo analogue (**5**) was formed in the greater amount (58%), but column chromatography, followed by recrystallization, resulted in the isolation of the tribromo analogue (**6**) in 26% yield.

To gain insight into the effects of highly polar aromatic substitutions, we targeted the mononitro (**7**) and dinitro (**9**) analogues and their reduced counterparts, monoamino (**8**) and the diamino analogues. Rimcazole (**1**) could be nitrated by modification of the method used for the nitration of imipramine.[20] We previously described the monosubstituted 3-NO_2 (**7**) and 3-NH_2 (**8**) ligands[17] but did not report the synthesis or pharmacological evaluation of these compounds. Thus **1** was treated with 2.4 equiv of nitric acid in acetic acid, to give a quantitative yield of **7** (Scheme 1). By using a stronger acid, in this case sulfuric acid, the 3,6-dinitro derivative (**9**) could be obtained, in 38% yield. Catalytic hydrogenation (10% Pd/C) of **7** gave the amine **8** in quantitative yield. However, reaction of the dinitro intermediate **9** under identical conditions resulted in the extremely polar diamino compound that could not be purified sufficiently for testing by either chromatography or recrystallization.

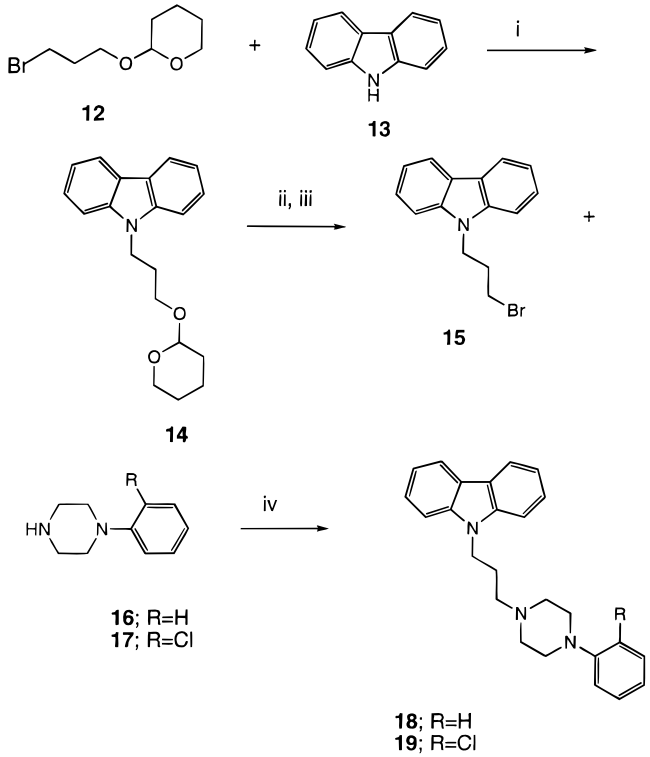
In a continuation of previous studies[17] investigating the effects of modification at the terminal piperazine nitrogen of rimcazole (**3**) by addition of methyl, aryl, and 3-phenylpropyl moieties was undertaken. Compound **10** was previously described[17] but was included in this study for additional pharmacological testing. Compound **11** was designed based on the structurally similar dopamine uptake inhibitor GBR 12909 (**3**). Acylation of **1** with 3-phenylpropionyl chloride to give the amide intermediate was followed by reduction with LAH to give **11**, as depicted in Scheme 2. In Scheme 3, the 2,6-dimethylpiperazine of **1** was replaced with aryl-piperazines. 3-Bromopropanol was reacted with dihydropyran to give the tetrahydropyran (THP)-protected alcohol **12** which was reacted with carbazole (**13**) under the basic conditions of sodium hydride in the presence of 1,4,7,-10,13-pentaoxacyclopentadecane (15-crown-5) to yield compound **14**. Deprotection under aqueous acid condi-

Scheme 2[a]



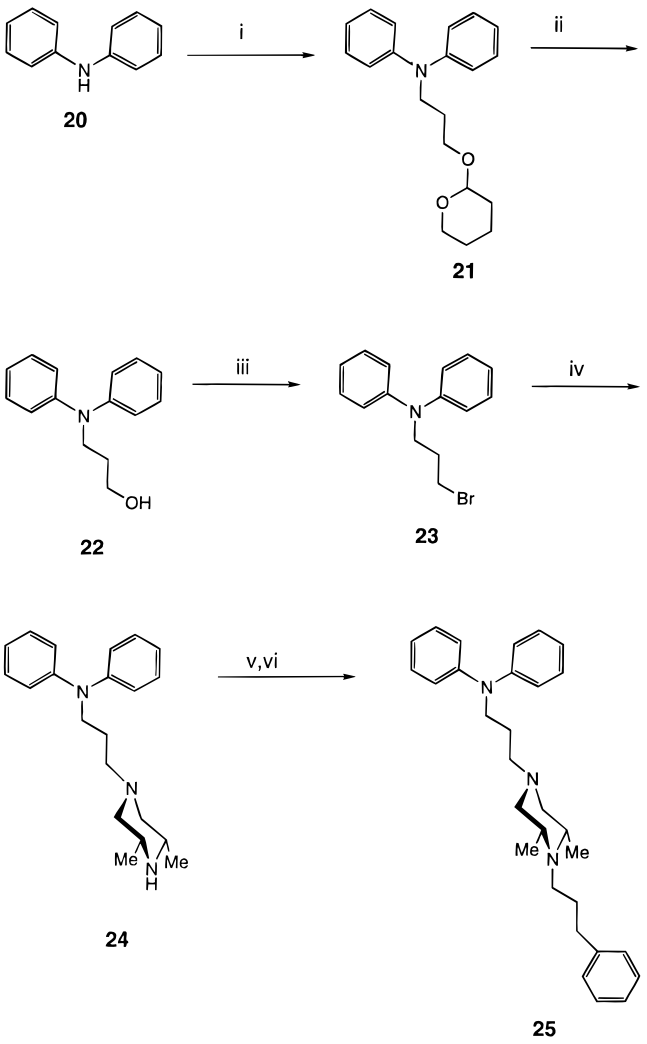
[a] Reagents and conditions: (i) CH_2O, $NaBH_3CN$; (ii) 3-phenylpropionylchloride; (iii) LAH.

Scheme 3[a]



[a] Reagents and conditions: (i) NaH, 15-crown-5; (ii) HOAc/H_2O; (iii) Ph_3P, CBr_4; (iv) K_2CO_3.

Scheme 4[a]



[a] Reagents and conditions: (i) **12**, NaH, 15-crown-5; (ii) HOAc/H_2O; (iii) Ph_3P, CBr_4; (iv) 2,6-dimethylpiperazine, K_2CO_3; (v) 3-phenylpropionylchloride; (vi) LAH.

tions followed by treatment with carbon tetrabromide and triphenylphosphine gave the alkylbromide **15**. Alkylation with the aryl piperazines **16** and **17** gave the products **18** and **19**, respectively.

Compound **24** was chosen as the target for studying the nature of the aromatic moiety of rimcazole by replacing the planar carbazole ring system with the freely rotating diphenylamine function. Diphenylamine (**20**) was alkylated with the THP-protected 1-bromopropanol (**12**) using sodium hydride in the presence of 15-crown-5, as the base, to yield **21** (Scheme 4). As described above, deprotection with aqueous acid to yield the alcohol (**22**), followed by treatment with triphenylphosphine and carbon tetrabromide, gave the alkylbromide (**23**) in good yield (97% over two steps). Alkylation of **21** with 2,6-dimethylpiperazine gave **24** in 41% yield. Acylation of the amine (**24**) with 3-phenylpropionyl chloride followed by reduction with LAH yielded the *N*-3-phenylpropyl substituted analogue **25** in 70% yield, over two steps (Scheme 4).

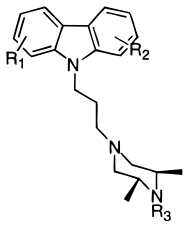
Structure−Activity Relationships

The binding affinities of rimcazole (**1**) and its analogues for all three monoamine transporters and for inhibition of [^{3}H]dopamine uptake were compared to cocaine (**2**) and GBR 12909 (**3**, Table 1). In general, chemical modification to the rimcazole structure resulted in a decrease in binding affinity at the dopamine transporter. The 3-NO_2 analogue, **7**, was slightly more potent (K_i = 109 nM) than rimcazole, **1**, (K_i = 224 nM), but reduction to the 3-NH_2 analogue, **8**, significantly decreased binding affinity (K_i = 2400 nM). Addition of a second nitro group (**9**) eliminated binding at the dopamine transporter (K_i = 174 μM) as did di- or tribromination (**5** and **6**; K_i = 3890 and 30300 nM, respectively). N-Methylation (**10**) decreased binding affinity by 2-fold, and replacement of the methyl group with an aryl ring eliminated binding altogether (**18** and **19**, K_i = 4.9 and 113 μM, respectively). It should be noted that in the N-aryl substituted analogues, **18** and **19**, the 2,6-dimethyl substituents on the piperazine ring are eliminated, and this may have contributed to the loss of binding affinity. However, for GBR 12909 (**3**; K_i = 12.0 nM), these pendant methyl groups are not present on the piperazine ring, suggesting that these groups are unnecessary for high affinity binding at the dopamine transporter. When the phenyl ring was extended away from the piperazine terminal nitrogen by a propyl chain, as seen for GBR 12909 (**3**), binding affinity was somewhat restored (**11**, K_i = 263 nM). This trend was also observed with the isothiocyanate moiety in the series of irreversible ligands based on rimcazole (**1**), reported previously.[17]

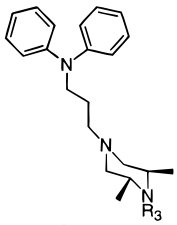
When the carbazole ring was replaced with freely rotating diphenylamine group, binding affinity was reduced by 4-fold (**24**, K_i = 813 nM). However, as in the carbazole series, addition of a 3-phenylpropyl substituent at the terminal piperazine nitrogen increased binding affinity, resulting in the most potent compound in this series, **25** (K_i = 61.0 nM). The structure of this compound most closely resembles that of GBR 12909 (**3**, K_i = 12.0 nM), suggesting that the nitrogen in the diphenylamine function of **25** may be serving as a spacer between the aryl rings and the piperazine functionality as has been reported for the nonterminal piperazine nitrogen in the aryl 1,4-dialkyl(en)yl piperidine series of compounds.[21]

Rimcazole (**1**) and most of the analogues that demonstrated moderate to low binding affinities for the dopamine transporter were evaluated for inhibition of [^{3}H]dopamine uptake in synaptosomes. Although we had previously reported that rimcazole demonstrated relatively poor potency for inhibition of dopamine uptake in a chopped tissue preparation (IC_{50} = 4.22 μM),[14] in the synaptosome preparation its potency was more comparable to its binding affinity at the dopamine transporter (IC_{50} = 140 nM). This experience in our own laboratory further underscores the caution that must be taken in comparing binding affinities and inhibition of dopamine uptake potencies in assays that are conducted under differing conditions, as we and others have discussed previously.[22−24] In this case, simply the difference of chopped tissue versus a synaptosome preparation yielded significantly different results (IC_{50} = 4.22 μM vs 140 nM) which changed a ratio of dopamine uptake inhibition to binding affinity at the dopamine transporter from ~40 to 1. Likewise, using the synaptosome preparation, each of the rimcazole analogues

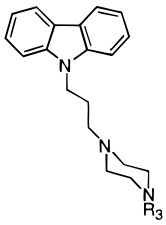
Table 1. Binding Affinities of Rimcazole Analogues at the Dopamine, Serotonin, and Norepinephrine Transporters and Inhibition of Dopamine Uptake



GROUP A



GROUP B



GROUP C

compound	substituents group, R_1, R_2, R_3	[^{3}H]WIN 35,428, DAT K_i, nM ± SEM[a]	[^{3}H]DA uptake inhibition IC_{50}, nM ± SEM	[^{3}H]paroxetine, 5HTT K_i, nM ± SEM[a]	[^{3}H]nisoxetine, NET K_i, nM ± SEM[a]
25	B, -, -, phenylpropyl	61.0 ± 6.1	36.6 ± 4.9	219 ± 32	3640 ± 440
7	A, 3-NO_2, H, H	109 ± 7	111 ± 22	1170 ± 100	4580 ± 1200
1; rimcazole	A, H, H, H	224 ± 16[d]	140 ± 5	825 ± 111	2160 ± 300
11	A, H, H, phenylpropyl	263 ± 34	639 ± 70.3	44.5 ± 6.6	2490 ± 310
10	A, H, H, CH_3	436 ± 44	472 ± 44	373 ± 14	9370 ± 1840
24	B, -, -, H	813 ± 22	1060 ± 297	13100 ± 1410	6810 ± 600
8	A, 3-NH_2, H, H	2400 ± 144	[b]nd	2100 ± 280	13900 ± 4400
5	A, 3-Br, 6-Br, H	3890 ± 506	[b]nd	1130 ± 147	1300 ± 150
18	C, -, -, phenyl	4930 ± 444	1820 ± 239	[c]IA	4120 ± 920
9	A, 3-NO_2, 6-NO_2, H	174000 ± 24400	3670 ± 145	1850 ± 290	3660 ± 470
6	A, 1,3-diBr, 6-Br, H	30300 ± 2420	[b]nd	1610 ± 250	8540 ± 2910
19	C, -, -, 2-Cl-phenyl	112700 ± 1130	2770 ± 403	[c]IA	[c]IA
2; cocaine		187 ± 19	205 ± 13	172 ± 15	3300 ± 170
3; GBR 12909		12.0 ± 1.9	2.30 ± 0.14	24.1 ± 2.3	1270 ± 190

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. [b] nd = not determined. [c] IA = inactive. [d] K_i value obtained in the present study was 2-fold higher than the previously reported value.[14]

inhibited dopamine uptake with a potency essentially equal to its binding affinity at the dopamine transporter. There was a direct relation between potency for dopamine uptake inhibition and affinity in displacement of [^{3}H]WIN 35,428 with a significant correlation between log transforms of K_i and IC_{50} values ($r^2 = 0.7731$, $p = 0.0018$).

At the serotonin transporter, most of these rimcazole analogues demonstrated moderate affinities, with rimcazole binding with a K_i of 825 nM. In general, chemical modifications to the rimcazole structure were better tolerated than at the dopamine transporter. Some differences were dramatic. For example, 3,6-dinitro substitution in compound **9** virtually eliminated binding at the dopamine transporter (K_i = 174 μM), whereas only a 2-fold decrease in affinity was observed at the serotonin transporter (K_i = 1850 nM) as compared to the parent drug, **1**. Also di- and tribromination in compounds **5** and **6** showed a similar effect, where the serotonin transporter was far more tolerant of these aromatic substitutions (K_i = 1130 and 1610 nM, respectively) than the dopamine transporter. Interestingly the *N*-3-phenylpropyl analogue, **11**, demonstrated relatively high affinity for the serotonin transporter (K_i = 44.5 nM); it was slightly less potent than GBR 12909 (K_i = 24.1 nM), whereas a 4-fold decrease in affinity was observed when the carbazole ring was replaced by the diphenylamine function (**25**, K_i = 219 nM). These structure−activity relationships are in contrast to those seen at the dopamine transporter and provide clues to separability within this class of compounds. Underscoring the different structure−activity relationships for binding to the dopamine and serotonin transporters is that there was not a significant correlation among log K_i values in these two effects ($r^2 = 0.3187$; $p = 0.06$). None of the analogues demonstrated appreciable affinity at the norepinephrine transporter (all K_i values were >1.3 μM). As demonstrated with the serotonin transporter, no significant correlation among log K_i values for binding to the dopamine and norepinephrine transporters was apparent ($r^2 = 0.2006$; $p = 0.14$).

Table 2. Binding Affinities of Rimcazole Analogues at σ_1 and σ_2 Receptors

compound	[^{3}H]pentazocine, σ_1 K_i, nM ± SEM[a]	[^{3}H]DTG, σ_2 K_i, nM ± SEM[a]
25	97.2 ± 14.0	183 ± 8
11	104.1 ± 0.4	145 ± 18
24	138 ± 2	351 ± 44
10	552 ± 110	230 ± 20
7	596 ± 66	193 ± 33
1; rimcazole	908 ± 99	302 ± 37
5	4420 ± 27	675 ± 58
8	5720 ± 440	1660 ± 200
6	>6000	1990 ± 44
3; GBR 12909	318 ± 18	116 ± 13

[a] K_i values are the averages of 2−3 experiments, ±SEM. Each experiment was carried out in duplicate.

Since rimcazole was first described as a σ ligand and often as a σ antagonist,[3−5] binding affinities at σ_1 and σ_2 receptors were obtained for the analogues and compared to the parent drug as well as GBR 12909 (**3**, Table 2). At σ_1 receptors, rimcazole (**1**) demonstrated a K_i of 908 nM. Binding affinities were improved slightly (~2-fold) for compounds **7** and **10**. A significant improvement in binding affinity was only achieved either by replacing the carbazole with the diphenylamine moiety (**24** and **25**, K_i = 138 and 97 nM, respectively) or by adding the 3-phenylpropyl group at the terminal piperazine nitrogen (**11** and **25**, K_i = 104 and 97 nM, respectively). In comparison, GBR 12909 demonstrated a binding affinity of 318 nM at σ_1 sites. The other rimcazole analogues that were evaluated were essentially inactive at σ_1 sites.

At σ_2 receptors, rimcazole demonstrated a K_i of 302 nM. The range of K_i values from most potent (**11**, K_i =

145 nM) to least potent (**6**, K_i = 1990 nM) was only ~11-fold. In essence, most of the analogues, regardless of chemical modification, had very similar binding affinities as compared to the parent drug. GBR 12909 (**3**) was slightly more potent at σ_2 sites (K_i = 116 nM) than any of the rimcazole analogues. Interestingly, affinities at σ_1 (r^2 = 0.5991; p = 0.0144) and at σ_2 (r^2 = 0.8023; p = 0.0011) were, in fact, significantly related to affinities at the dopamine transporter, adding to the linkages between σ receptors and the dopamine transporter that have been described above.

Summary

In summary, a series of rimcazole analogues have been prepared where the effects of chemical modification to the carbazole ring system and the terminal piperazine nitrogen substituent were determined. The compounds were evaluated for binding affinities at dopamine, serotonin, and norepinephrine transporters as well as at σ_1 and σ_2 receptors. In addition, compounds were evaluated for inhibition of [^{3}H]dopamine uptake, and these data were positively correlated to their binding affinities at the dopamine transporter. Structure−activity relationships demonstrated that most aromatic substitutions in the carbazole ring system decreased binding affinity at the dopamine transporter. When the carbazole ring system was replaced with the freely rotating diphenylamine function, binding affinity was slightly decreased. However, when this compound was further modified with a phenylpropyl group at the terminal piperazine nitrogen, the most potent compound (**25**) in the series resulted. Structure−activity relationships at the serotonin transporter were significantly different from those observed at the dopamine transporter in that modifications of the carbazole ring system were better tolerated. In addition, the carbazole ring was favored over the diphenylamine system in the *N*-phenylpropyl substituted analogues, with the most potent analogue being compound **11**. None of the compounds recognized the norepinephrine transporter with affinities of <1.3 μM.

Although a clear structure−activity relationship profile between the dopamine transporter and σ_1 and σ_2 receptors is not apparent, the positive correlations in binding affinities, as well as previously reported overlaps in actions of a variety of compounds at these sites, are intriguing. Further examination of the relationship between the dopamine transporter and σ sites and their roles in modulating the behavioral actions of this class of compounds is thus being pursued.

In addition, behavioral evaluation of the most provocative analogues, **11** and **25**, is currently being conducted. We are interested in determining whether these rimcazole analogues demonstrate a cocaine-like profile, as does GBR 12909, or are different, as is the parent compound rimcazole. Preliminary results in behavioral studies suggest that rimcazole (**1**) and its analogues **11** and **25** do not demonstrate cocaine-like behavioral effects. These differences in actions, in comparison to cocaine, may suggest alternative interactions at the dopamine transporter, on a molecular level.[25,26] Further investigation into the possibility that interactions at a low affinity site/state of the dopamine transporter are responsible for the lack of cocaine-like behavioral profiles for these compounds is underway.

Experimental Section

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR data were recorded on a Bruker (Billerica, Mass) AC-300 instrument. Samples were dissolved in an appropriate deuterated solvent. Proton chemical shifts are reported as parts per million (δ) relative to tetramethylsilane (Me_4Si; 0.00 ppm) which was used as an internal standard. Carbon chemical shift values (δ) are reported in parts per million (ppm) relative to deuterated chloroform ($CDCl_3$; 77.0 ppm). Mass spectra were recorded on a Hewlett-Packard (Palo Alto, CA) 5971A mass selective ion detector in the electron-impact mode with sample introduction via a HP-5890 series II gas chromatograph fitted with an HP-1 (cross-linked methyl silicone gum) 25 m × 0.2 mm i.d., 50 μm film thickness. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 °C, respectively. The initial oven temperature was 100 °C, held for 3.0 min, programmed to 295 °C at 15.0 °C/min, and maintained at 295 °C for 10.0 min. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR. Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and agree within 0.4% of calculated values. All column chromatography was performed in flash-grade silica gel (230−400 mesh, 60 Å). All chemicals and reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis, Inc.

3,6-Dibromo-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)-propyl]carbazole Hydrochloride (5) and 1,3,6-Tribromo-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]carbazole Hydrochloride (6). To a solution of rimcazole (**1**) in glacial AcOH (8 mL/mmol) were added H_2O (7 mL/mmol), Br_2 (14 drops/mmol), and aqueous HBr (4 drops of 48%/mmol). The solution was stirred for 72 h at room temperature, before being made basic with $NaHCO_3$ and extracting the organics into $CHCl_3$. Drying (Na_2SO_4) and evaporation gave the crude product. Column chromatography (5% $CHCl_3$/MeOH/NH_4OH) gave both compounds **5** (58%) at R_f 0.58 (10% $CHCl_3$/MeOH/NH_4OH) and **6** (26%) at R_f 0.62 (10% $CHCl_3$/MeOH/NH_4OH). The HCl salts were formed by dissolving the respective free bases in methanolic HCl. Recrystallization of the salts in MeOH gave the products as white crystals. Compound **5:** mp >250 °C; IR ($CHCl_3$) 732, 799 (Ph) cm^{-1}; ^{1}H NMR δ 1.02 (6H, d, J = 6.5 Hz, 2 × CH_3), 2.63 (2H, dd, J = 11.1 and 1.9 Hz, 2 × CH), 2.94 (2H, m, 2 × CH), 4.36 (2H, t, J = 6.5 Hz, CH_2N), 7.35 (2H, d, J = 8.8 Hz, H-1 aryl), 7.49 (2H, dd, J = 8.8 and 2.1 Hz, H-2 aryl), 8.13 (2H, d, J = 2.0 Hz, H-4 of aryl); ^{13}C NMR δ 20.0, 26.1, 41.0, 50.5, 55.0, 61.5, 110.2, 112.4, 124.8, 125.0, 128.2, 139.9; CI M+1 480. Anal. ($C_{21}H_{25}N_3Br_2$·2HCl), C, H, N. Compound **6**: mp >250 °C; IR ($CHCl_3$) 754 cm^{-1}; ^{1}H NMR δ 1.04 (6H, d, J = 6.4 Hz, 2 × CH_3), 2.69 (2H, d, J = 9.1 Hz, 2 × CH), 2.89 (2H, m, 2 × CH), 4.73 (2H, t, J = 7.2 Hz, CH_2N), 7.41 (1H, d, J = 8.8 Hz, *o*-H on mono-Br ring), 7.56 (1H, d, J = 8.8 and 1.8 Hz, *m*-H on mono-Br ring), 7.73 (1H, d, J = 1.8 Hz, *m*-H on mono-Br ring), 8.07 (1H, d, J = 1.9 Hz, *m*-H on di-Br ring), 8.09 (1H, d, J = 1.9 Hz, *m*-H on di-Br ring); ^{13}C NMR δ 19.7, 28.1, 42.5, 50.5, 55.5, 60.5, 103.3, 111.8, 112.1, 113.3, 122.4, 123.53 126.8, 129.5, 133.6, 140.1. Anal. ($C_{21}H_{24}N_3Br_3$.2HCl·0.5H_2O), C, H, N.

3-Nitro-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]carbazole Hydrochloride (7). Rimcazole (**1**, 1.7 g, 5.3 mmol) was dissolved in glacial AcOH (39 mL), and 70% HNO_3 was added dropwise (0.8 mL).[20] A yellow precipitate started to form immediately, and the solution was stirred overnight. The mixture was then diluted with H_2O (75 mL), and then 37% HCl was added (2.8 mL). The aqueous layer was washed with ether (2 × 30 mL) and then basified with NH_4OH. The product was extracted into $CHCl_3$ (3 × 30 mL), dried (Na_2SO_4), and evaporated to yield a red oil. The product was purified by column chromatography (7% $CHCl_3$/MeOH/NH_4OH) to give 1.59 g (82%) as a yellow solid. Formation of the HCl salt and

recrystallization from 2-PrOH yielded yellow crystals (55%): mp >250 °C; R_f 0.43 (10% $CHCl_3$/MeOH/NH_4OH); ^1H NMR δ 1.08 (6H, d, 2 × CH_3), 2.70 (2H, d), 2.93 (2H, m), 4.44 (2H, t, CH_2N), 7.34 (1H, m), 7.53 (3H, m), 8.18 (1H, d, H *m* to NO_2), 8.37 (1H, m, H *o* to NO_2), 9.00 (1H, s, H *o* to NO_2); FAB 367 (M^++1). Anal. ($C_{21}H_{26}N_4O_2$·HCl). C, H, N.

3-Amino-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]-carbazole Hydrochloride (8). Compound **7** (900 mg, 2.46 mmol) was dissolved in MeOH (60 mL) and reduced under a H_2 atmosphere (35 psi) with a 10% Pd/C (150 mg) catalyst for 45 min. The mixture was filtered through Celite and evaporated to yield the product as a brown foam. Purification by column chromatography (5−10% $CHCl_3$/MeOH/NH_4OH) gave 550 mg (67%) as a cream colored solid: R_f 0.25 (10% $CHCl_3$/MeOH/NH_4OH). The HCl salt was formed by dissolving **8** in methanolic HCl and then recrystallized from MeOH/ether: mp >250 °C; IR ($CHCl_3$) 3424, 3354, 3213 (NH) cm^{-1}; ^1H NMR δ 1.04 (6H, d, J = 6.4 Hz, 2 × CH_3), 2.72 (2H, d, J = 9.1 Hz, 2 × CH), 2.95 (2H, m, 2 × CH), 4.32 (2H, t, J = 6.7 Hz, CH_2N), 6.90 (1H, dd, J = 6.6 and 2.1 Hz, aryl-H), 7.15 (1H, m, aryl-H), 7.28 (1H, d, J = 8.9 Hz, aryl-H), 7.40 (2H, m, aryl-Hs), 7.43 (1H, d, J = 2.1 Hz, aryl-H), 7.98 (1H, d, J = 7.7 Hz, aryl-H); ^{13}C NMR δ 19.9, 26.0, 40.0, 50.6, 55.2, 60.6, 106.2, 108.8, 109.4, 115.5, 118.1, 120.3, 125.4. Anal. ($C_{21}H_{28}N_4$·2HCl), C, H, N.

3,6-Dinitro-9-[3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl]-carbazole Hydrochloride (9). Rimcazole (**1**; 0.31 g, 0.97 mmol) was dissolved in sulfuric acid (5 mL) and H_2O (4 mL) and cooled in an ice bath.[20] Nitric acid (0.31 mL) was then added slowly at which point the solution turned black. The reaction mixture was allowed to warm to room temperature and stirring was continued for 45 min. The solution was then carefully basified using 10% NH_4OH and the product extracted into 5% MeOH in $CHCl_3$ (2 × 30 mL). The organic layers were then washed with brine (1 × 30 mL), dried (Na_2SO_4), and evaporated to leave a dark gold foam (0.4 g). Column chromatography (6% $CHCl_3$/MeOH/NH_4OH) yielded the purified product as a yellow solid. The HCl salt was formed by dissolving the free base in MeOH and adding methanolic HCl to pH 2. Recrystallization was carried out in H_2O/2-PrOH (180 mg, 38%); R_f 0.33; mp >250 °C; ^1H NMR δ 1.06 (6H, d, J = 6.4 Hz, 2 × CH_3), 4.54 (2H, t, J = 6.7 Hz, NCH_2), 7.66 (2H, d, J = 9.2, 2 × *m*-H), 8.47 (2H, dd, J = 9.2, 2.2 Hz, 2 × *o*-H), 9.09 (2H, d, J = 2.2 Hz, 2 × *o*-H); ^{13}C NMR δ 19.3, 25.3, 41.3, 50.4, 54.0, 50.0, 109.9, 117.5, 122.4, 122.5, 141.7, 144.8; EI 412 (M^++1). Anal. ($C_{21}H_{25}N_5O_4$·2HCl·0.5H_2O) C, H, N.

9-[3-(*cis*-3,5-Dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]carbazole Hydrobromide (11). Rimcazole (**3**) was treated in the same manner as is described for compound **24** to give the intermediate amide (81%); R_f 0.95 (10% $CHCl_3$/MeOH/NH_4OH); IR ($CDCl_3$) 1725 cm^{-1}; NMR ($CDCl_3$) δ 1.41 (d, 6H), 1.97 (m, 4H), 2.28 (t, 2H), 2.68 (m, 4H), 2.97 (m, 2H), 4.42 (t, 2H), 7.25 (m, 7H), 7.43 (d, 4H), 8.14 (d, 2H). Reduction of the amide with lithium aluminum hydride was conducted in a manner identical to that described for compound **25** to yield, after chromatography, 81% of the product. The HBr salt was prepared by dissolving the free base in methanolic HBr and recrystallizing from THF/ether: mp 179−181 °C; R_f 0.23; IR ($CHCl_3$) 749, 724, 699 (Ph) cm^{-1}; ^1H NMR δ 0.98 (6H, d, J = 6.2 Hz, 2 × CH_3), 4.37(2H, t, J = 6.6 Hz, CH_2N), 7.17−7.32 (9H, m, aryl-Hs), 7.45 (2H, m, aryl-Hs), 8.09 (2H, d, J = 7.7 Hz, aryl-Hs); ^{13}C NMR δ 18.0, 24.4, 25.9, 33.8, 40.6, 47.5, 53.6, 54.8, 61.3, 108.8, 118.7, 120.3, 122.8, 125.5, 125.8, 128.3, 128.3, 140.5, 142.0. Anal. ($C_{30}H_{37}N_3$·HBr), C, H, N.

1-Bromo-3-propyltetrahydropyranol (12). To a solution of 3-bromo-1-propanol (9.08 g, 65 mmol) in $CHCl_3$ (150 mL) were added 3,4-dihydro-2*H*-pyran (5.45 g, 65 mmol) and a catalytic amount of toluenesulfonic acid (100 mg) and the mixture was allowed to stir at room temperature for 2 h. The reaction mixture was washed with H_2O (2 × 100 mL) and brine (2 × 50 mL) and evaporated under reduced pressure to give 13.7 g (94%) of the product as a clear oil.

9-(1-Tetrahydropyranol-3-propyl)carbazole (14). Carbazole (4.85 g, 30 mmol) was dissolved in toluene (150 mL) followed by addition of NaH (1.45 g: 60 mmol) and 15-crown-5 (1 mL, 5 mmol). After the mixture was stirred for 20 min at room temperature, compound **12** (5.45 g, 25 mmol) was added, and the mixture was warmed to reflux for 4 days. The reaction was then cooled to room temperature and quenched with H_2O. The organics were extracted with $CHCl_3$ (2 × 150 mL), dried (Na_2SO_4), and evaporated to yield the crude product as a red oil. Column chromatography (7% EtOAc in petroleum ether 35−60) gave 6.3 g (85%) of the product as a clear oil: ^1H NMR δ 1.52−1.89 (6H, m), 2.12 (2H, m), 3.26 (1H, m), 3.42 (1H, m), 3.81 (2H, t, J = 7.3 Hz), 4.48 (3H, m), 7.22 (2H, m), 7.42 (4H, m), 8.08 (2H, d, J = 7.5 Hz); EI 309 (M+), 180 (M+, CH_2CH_2-OTHP).

9-(1-Bromo-3-propyl)carbazole (15). Deprotection of **14** was effected by dissolving **14** (6.3 g, 20 mmol) in an AcOH: THF:H_2O (75 mL:30 mL:15 mL) mixture and warming to 80 °C for 3 h. After this time, the solution was allowed to cool and then was basified with NH_4OH, and the organics were extracted into $CHCl_3$ (2 × 30 mL). The organic solution was dried (Na_2SO_4) and evaporated to leave an orange oil that was homogeneous by TLC and was used in the next step without further purification. The deprotected alcohol (5.5 g, 24 mmol) was dissolved in acetonitrile (70 mL), and triphenylphosphine (9.7 g, 37 mmol) was added to the solution, followed by carbon tetrabromide (12.4 g, 37 mmol). The solution was stirred at room temperature for 2 h before basifying with 15% NaOH (100 mL) and extracting the organics into ether (3 × 50 mL). The combined organic fractions were washed with brine (1 × 100 mL), dried (Na_2SO_4), and evaporated to give the crude product as a red semisolid. The product was dissolved in ether, and residual triphenylphosphine oxide was removed by filtration to give 11.3 g of crude product. Column chromatography (10% EtOAc in hexane) yielded 7.1 g (99%) of a light green oil: ^1H NMR δ 2.38 (2H, M, CH_2), 3.37 (2H, t, J = 7.0 Hz, NCH_2), 4.49 (2H, t, J = 7.2 Hz, $BrCH_2$), 7.27 (2H, m), 7.47 (4H), m), 8.07 (2H, d, J = 7.6 Hz); EI 287, 289 (M+, M+2), 180 ($M^+-CH_2CH_2Br$).

9-[(4-Phenyl-1-piperazinyl)propyl]carbazole Hydrobromide (18). Compound **15** (1.4 g, 4.9 mmol) was dissolved in a mixture of DMF (40 mL) and H_2O (2 mL) and then 1-phenylpiperazine hydrochloride (**16**; 0.75 mL, 4.9 mmol) was added, along with potassium carbonate (2.7 g, 20 mmol). The solution was then warmed to 80 °C for 2h, by which time no **15** remained, by TLC. The mixture was diluted with H_2O (100 mL), and the organics were extracted into ether/ethyl acetate (2:1, 3 × 100 mL). The combined organic fraction was washed with H_2O (5 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 1.34 g (74%) as an oil. Column chromatography (5% $CHCl_3$/MeOH/NH_4OH) gave the solid free base (540 mg) which was converted to the HBr salt by adding saturated HBr in MeOH to a methanolic solution of the free base, to pH 2. Recrystallization from MeOH/ether gave the HBr salt as fine needles: mp >250 °C; R_f 0.05 (10% EtOAc:petroleum ether); ^1H NMR δ 2.08 (2H, m), 2.32 (2H, t, J = 7.3 Hz), 2.56 (2H, m), 3.22 (2H, m), 4.40 (2H, t, J = 6.7 Hz), 6.90 (3H, m), 7.25 (4H, m), 8.10 (2H, d, J = 7.6 Hz); ^{13}C NMR δ 25.8, 40.4, 49.1, 53.0, 54.8, 108.7, 115.9, 118.7, 119.6, 120.2, 122.7, 125.4, 129.0, 140.4, 151.2; EI 369 (M^+). Anal. ($C_{25}H_{27}N_3$ HBr) C, H, N.

9-[(4-[1-Chlorophenyl]-1-piperazinyl)propyl]carbazole Hydrobromide (19). Compound **19** was prepared as described for compound **18**, using 3-chlorophenyl-1-piperazine hydrochloride (**17**) to give 1.5 g (76%) crude product. Column chromatography (5% $CHCl_3$/MeOH/NH_4OH) gave the free base as an orange oil (820 mg) which was converted to the HBr salt as described for compound **18**; mp 230 °C; R_f 0.1 (10% EtOAc:petroleum ether); ^1H NMR δ 2.08 (2H, m), 2.37 (2H, t, J = 7.3 Hz), 2.55 (4H, m), 4.41 (2H, t, J = 6.6 Hz), 6.08 (1H, m), 7.05 (1H, m), 7.22 (3H, m), 7.34−7.50 (4H, m), 8.10 (2H, d, J = 7.6 Hz). Anal. ($C_{25}H_{26}N_3ClHBr$) C, H, N.

1-Tetrahydropyranol-3-propyldiphenylamine (21). Diphenylamine (**20**; 2.70 g, 16 mmol) was dissolved in toluene (100 mL) before adding NaH (0.8 g, 33 mmol) and 15-crown-5 (3.2 mL, 16 mmol). After 20 min at room temperature,

compound **12** (3.0 g, 13 mmol) was added and the mixture warmed to 90 °C for 4 h. The reaction was then cooled to room temperature and then quenched with H_2O. The organics were extracted with $CHCl_3$ (2 × 100 mL), dried (Na_2SO_4), and evaporated to yield a red oil. Column chromatography (3% EtOAc in petroleum ether 35−60) gave 2.0 g (48%) of the product as an oil: R_f 0.8 (5% EtOAc in petroleum ether 35−60); ^{1}H NMR δ 1.5−1.9 (10H, m), 3.43 (2H, m), 3.82 (4H, m), 4.48 (1H, t, J = 4.9 Hz), 6.91 (2H, t, J = 7.5 Hz), 7.04 (4H, d, J = 7.6 Hz), 7.28 (4H, t, J = 7.5 Hz); EI 311 (M^+), 182 ($M^+-CH_2CH_2OTHP$).

1-Diphenylamino-3-propanol (22). Compound **21** (2.0 g: 6.4 mmol) was dissolved in a AcOH:THF:H_2O (25 mL:13 mL:7 mL) mixture and warmed to 85 °C for 2.5 h. After this time, the solution was allowed to cool and then basified with NH_4OH and the organics were extracted into $CHCl_3$ (2 × 30 mL). The organic solution was dried (Na_2SO_4) and evaporated to leave an orange oil, 1.4 g (100%), that was homogeneous by TLC and was used in the next step without further purification: R_f 0.15 (5% EtOAc in petroleum ether 35−60); IR ($CHCl_3$) 3398 (OH) cm^{-1}; ^{1}H NMR δ 1.85 (2H, m), 3.72 (2H, t, J = 7.1 Hz), 3.81 (2H, t, 7.1 Hz), 6.93 (2H, t, 7.4 Hz), 6.99 (4H, d, J = 7.5 Hz), 7.26 (2H, t, 7.4 Hz); EI 227 (M^+), 182 ($M^+-CH_2CH_2OH$).

1-Bromo-3-propyldiphenylamine (23). The deprotected alcohol **22** (2.0 g, 8.8 mmol) was dissolved in acetonitrile (30 mL), and triphenylphosphine (3.5 g, 13.3 mmol) was added to the solution, followed by carbon tetrabromide (4.4 g, 13.3 mmol). The solution was stirred at room temperature for 1 h before basifying with 15% NaOH (30 mL) and extracting the organics into ether (3 × 30 mL). The solution was dried (Na_2SO_4) and evaporated to give the crude product as a red oil. Column chromatography (10% EtOAc in hexane) yielded 2.5 g (97%) of a light green oil: R_f 0.95 (10% EtOAc in hexane); IR ($CHCl_3$) 1249 (C−Br) cm^{-1}; ^{1}H NMR δ 2.24 (2H, m), 3.43 2H, t, J = 7.0 Hz), 3.89 (2H, t, J = 7.0 Hz), 7.08 (6H, m), 7.28 (4H, t, J = 7.5 Hz); EI 290 (M^+), 182 ($M^+-CH_2CH_2Br$).

[3-(*cis*-3,5-Dimethyl-1-piperazinyl)propyl]diphenylamine Oxalate (24). Compound **23** (0.35 g: 1.21 mmol) was dissolved in a mixture of DMF (10 mL) and H_2O (0.5 mL) and then 2,6-dimethylpiperazine (0.14 g, 1.21 mmol) was added, along with K_2CO_3 (0.67 g, 4.84 mmol). The solution was then warmed to 80 °C for 2h, at which time no **23** remained, by TLC. The mixture was diluted with H_2O (30 mL), and the organics were extracted into CH_2Cl_2 (3 × 30 mL). Drying (Na_2SO_4), followed by evaporation, yielded an oil that contained large amounts of DMF. Column chromatography (5% $CHCl_3$/MeOH/NH_4OH) yielded 190 mg (41%) of **24** as an oily solid: R_f 0.5 (10% $CHCl_3$/MeOH/NH_4OH). The oxalate salt of **24** was formed by warming a solution of the free base with 2 equiv of oxalic acid in EtOH. The salt was then recrystallized from 2-PrOH: mp 157−159 °C; IR ($CHCl_3$) 3401 (NH), 748, 695 (Ph) cm^{-1}; ^{1}H NMR δ 1.03(6H, d, J = 6.3 Hz, 2 × CH_3), 2.74 (2H, d, J = 9.5 Hz, 2 × CH of piperazine), 2.92 (2H, m, 2 × CH of piperazine), 3.79 (2H, t, J = 7.2 Hz, CH_2N), 6.95 (2H, dd, J = 7.3 Hz, 2 × *p*-H), 7.02 (4H, d, J = 7.6 Hz, 4 × *o*-H), 7.27 (4H, t, J = 7.5 Hz, 4 × *m*-H); ^{13}C NMR δ 19.5, 24.6, 50.0, 50.7, 55.4, 60.3, 120.8, 121.0, 129.1, 147.9; EI 323 (M^+). Anal. ($C_{21}H_{29}N_3$·1.5oxalate) C, H, N.

[3-(*cis*-3,5-Dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]diphenylamine Hydrochloride (25). Hydrocinnamic acid (0.52 g, 3.5 mmol) was dissolved in thionyl chloride (9 mL, 123 mmol) and the solution warmed to 50 °C for 90 min. The excess thionyl chloride was then removed in vacuo, and the residue (3-phenylpropionyl chloride) was taken up in toluene (10 mL) and added to a solution of **24** (0.70 g, 2.12 mmol) in toluene (10 mL). The solution was heated to reflux overnight, then washed with a 20% Na_2CO_3 solution (1 × 15 mL) and then brine (1 × 15 mL), and finally dried (Na_2SO_4). Evaporation of the solvent yielded the crude amide as a dark oil that was purified by column chromatography (5% $CHCl_3$/MeOH/NH_4OH) to give 0.8 g (81%) of the amide as a light red oil: IR ($CHCl_3$) 1725 (CO) cm^{-1}. The intermediate amide (0.80 g, 1.75 mmol) was dissolved in THF (5 mL) and added to a cooled (0 °C) mixture of lithium aluminum hydride (0.14 g, 3.69 mmol) in THF (5 mL). The mixture was then stirred at room temperature overnight before quenching with solid Rochelle salt. After being stirred for a further 15 min, the mixture was diluted with H_2O (15 mL) and the organics were extracted into ethyl acetate (3 × 15 mL). This solution was dried (Na_2SO_4) and evaporated to yield an orange oil. Column chromatography (5% $CHCl_3$/MeOH/NH_4OH) gave 0.67 g (86%) as a light yellow oil. The HCl salt was formed by dissolving the free base in methanolic HCl and then recrystallized from methanol/ether: mp 195 °C; R_f 0.15 (4% $CHCl_3$/MeOH/NH_4OH); IR ($CHCl_3$) 44, 697 (Ph) cm^{-1}; ^{1}H NMR δ 0.98 (6H, d, J = 6.2 Hz, 2 × CH_3), 3.73 (2H, t, J = 7.3 Hz, CH_2N), 6.93 (2H, t, J = 7.3 Hz, 2 × H-4), 7.02 (4H, d, J = 7.6 Hz, 2 × H-2 and 2 × H-6), 7.16−7.31 (9H, m, aryl-Hs); ^{13}C NMR δ 17.8, 24.1, 25.0, 33.9, 47.3, 49.9, 53.4, 55.1, 61.3, 120.8, 121.0, 125.7, 128.2, 129.1, 142.1, 148.3. Anal. ($C_{30}H_{39}N_3$·2HCl·0.5H_2O) C, H, N.

Pharmacology. 1. Chemicals. Chemicals and reagents were obtained from the following sources: [^{3}H]WIN 35,428 (2-β-carbomethoxy-3-β-(4-flurophenyl)tropane 1,5-naphthalene disulfonate; specific activity 84.5 Ci/mmol) from New England Nuclear (NEN, Boston, MA); cocaine hydrochloride from Sigma Chemical Co. (St. Louis, MO); [^{3}H]paroxetine (specific activity 17 Ci/mmol, NEN); [^{3}H]nisoxetine (specific activity 86 Ci/mmol, NEN); [^{3}H]dopamine (specific activity 50 Ci/mmol, Amersham); [^{3}H](+)-pentazocine (specific activity 51.7 Ci/mmol) was synthesized as described previously;[27] [^{3}H]DTG (specific activity 35.2 Ci/mmol, NEN).

2. Dopamine Transporter Binding Assay. Male Sprague−Dawley rats (200−250 g, Taconic, Germantown, NY) were decapitated, and their brains were removed to an ice-cooled dish for dissection of the caudate-putamen. The tissue was homogenized in 30 volumes of ice-cold modified Krebs-HEPES buffer (15 mM HEPES, 127 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 10 mM glucose, pH adjusted to 7.4) using a Teflon/glass homogenizer and centrifuged at 20000*g* for 10 min at 4 °C. The resulting pellet was then washed two more times by resuspension in ice-cold buffer and centrifugation at 20000*g* for 10 min at 4 °C. Fresh homogenates were used in all experiments.

Binding assays were conducted in modified Krebs-HEPES buffer on ice, essentially as previously described.[16] The total volume in each tube was 0.5 mL, and the final concentration of membrane after all additions was approximately 0.3% (w/v) corresponding to 150−300 μg of protein/sample. Increasing concentrations of the drug being tested were added to triplicate samples of membrane suspension. Five minutes later, [^{3}H]WIN 35,428 (final concentration 1.5 nM) was added, and the incubation was continued for 1 h on ice. The incubation was terminated by the addition of 3 mL of ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% BSA in water to reduce nonspecific binding) using a Brandel Cell Harvester (Gaithersburg, MD). After filtration, the filters were washed with three additional 3 mL washes and transferred to scintillation vials. Absolute ethanol (0.5 mL) and Beckman Ready Value scintillation cocktail (2.75 mL) were added to the vials which were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding per sample and approximately 250 dpm nonspecific binding. Nonspecific binding was defined as binding in the presence of 100 μM cocaine. K_i values were derived from 14 point competition assays using increasing concentrations of unlabeled compounds (0.05 nM to 100 μM) against 1.5 nM [^{3}H]-WIN 35,428. Displacement data were analyzed by the use of the nonlinear least squares curve-fitting computer program LIGAND.[28]

3. Serotonin Transporter Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs) were removed, and the midbrain was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 50000*g* for 10

min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 15 mg/mL. Ligand binding experiments were conducted in assay tubes containing 4.0 mL of buffer for 90 min at room temperature. Each tube contained 0.2 nM [^{3}H]paroxetine and 1.5 mg of midbrain tissue (original wet weight). Nonspecific binding was determined using 1 μM citalopram. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, Maryland). The filters were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Data were analyzed by using GraphPad Prism software (San Diego, CA).

4. Norepinephrine Transporter Binding Assay. Brains from male Sprague−Dawley rats weighing 200−225 g (Taconic Labs) were removed, and the frontal cortex was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron (setting 6 for 20 s), and were centrifuged at 50000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 80 mg/mL. Ligand binding experiments were conducted in assay tubes containing 0.5 mL of buffer for 60 min at 0−4 °C. Each tube contained 0.5 nM [^{3}H]Nisoxetine and 8 mg of frontal cortex tissue (original wet weight). Nonspecific binding was determined using 1 μM desipramine. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 mL) was added, and the vials were counted using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Data were analyzed by using GraphPad Prism software (San Diego, CA).

5. σ Receptor Binding Assays. σ_1 Receptors were labeled as described previously, using the σ_1-selective probe [^{3}H](+)-pentazocine and guinea pig brain membranes.[27] Guinea pig brain membranes (350−500 μg of membrane protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine in a total volume of 0.5 mL of 50 mM Tris-HCl, pH 8.0. Incubations were carried out for 120 min at 25 °C. Nonspecific binding was determined in the presence of 10 μM unlabeled haloperidol. Assays were terminated by dilution with 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and vacuum filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25 °C prior to use. Filters were counted in CytoScint cocktail (ICN, Costa Mesa, CA) after an overnight extraction of counts. Membranes were prepared from frozen guinea pig brains (minus cerebella) as previously described.[27] IC_{50} values were determined using the iterative curve-fitting program GraphPAD InPlot (San Diego, CA). IC_{50} values were then converted to apparent K_i values using the Cheng−Prusoff equation, and radioligand K_d values were determined previously.[27,29]

σ_2 Receptors were labeled as previously described using rat liver membranes, a rich source of σ_2 sites, and [^{3}H]1,3-di-*o*-tolylguanidine ([^{3}H]DTG) in the presence of 1 μM dextrallorphan to mask σ_1 receptors.[29] Assays were performed in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 160 μg of membrane protein and 5 nM radioligand. Assays included 1 μM dextrallorphan to mask σ_1 binding. Nonspecific binding was determined in the presence of 10 μM haloperidol. All other manipulations were as described above for the σ_1 receptor assay. Rat liver membranes were prepared from the livers of male Sprague−Dawley rats as previously described.[29]

6. [^{3}H]Dopamine Uptake Assay. Rats were sacrificed by decapitation and their brains removed to an ice-cooled dish for dissection of the caudate-putamen. [^{3}H]Dopamine uptake was measured in a synaptosomal preparation as described previously.[30] Briefly, the tissue was homogenized in ice-cold buffer (320 mM sucrose, 5 mM HEPES salt, pH 7.4) followed by centrifugation at 1000*g* for 10 min at 4 °C. The supernatant was saved and spun in a centrifuge at 10000*g* for 20 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in cold modified Krebs-HEPES buffer (see above for binding).

The synaptosomal tissue preparation was incubated in buffer in glass test tubes at 37 °C to which either the drug being tested or no drug was added, as appropriate. After a 5 min incubation period in the presence of drug, [^{3}H]dopamine (final concentration 15 nM) was added to each tube. After 5 min, the incubation was terminated by the addition of 2 mL of ice-cold buffer to each tube and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% polyethylenimine in water) using a Brandel cell harvester (Gaithersburg, MD). After filtration, the filters were washed with three additional 2 mL washes and transferred to scintillation vials. Methanol (1 mL) and 0.2 M HCl (2 mL) were added, and the vials were then heated at 70 °C for 2 h to extract the accumulated [^{3}H]dopamine. After cooling, 10 mL of Ready Value scintillation cocktail were added, and radioactivity was determined by liquid scintillation spectrometry at an efficiency of approximately 30%. The reported values represent specific uptake from which nonspecific uptake was subtracted (defined as uptake in the presence of 100 μM cocaine). Data were analyzed using the nonlinear regression analysis of GraphPad Prism software (San Diego, CA).

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