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3-Aryl-2-carbomethoxybicyclo[3.2.1]oct-2-enes Inhibit WIN 35,428 Binding Potently and Selectively at the Dopamine Transporter

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Abstract—The search for medications for cocaine abuse has focused upon the design of potential cocaine antagonists or cocaine substitutes which interact at the dopamine transporter of mammalian systems. This manuscript describes the synthesis and biological evaluation of 8-substituted 2-carbomethoxy-3-arylbicyclo[3.2.1]oct-2-enes. These compounds prove potent and selective inhibitors of the dopamine transporter. Their selectivity results primarily from a reduced inhibitory potency toward the serotonin transporter. This work supports the notion that the orientation of the 3-aryl ring in the bicyclo[3.2.1]octane system affects the interaction of these molecules with the serotonin transporter far more markedly than it affects the interaction with the dopamine transporter. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The dopamine transporter (DAT), present in fibers of mammalian neurons, has been the focus of considerable research. It is at this site that cocaine is understood to exert its pharmacological activity by inhibition of reuptake of endogenous dopamine.[1–7] The DAT also provides a measure of certain neurological diseases. For example, in normal human aging the concentration of this transporter decreases about 5–7% for each decade of life.[8,9] In Parkinson's disease the transporter is considerably depleted and consequently a measure of the DAT concentration can provide a diagnostic assessment of the progression of the disease.[10,11] Although no medication currently exists which can reverse this disease, quantification of the DAT will provide an excellent marker for the success of such agents as they are developed in the future.[12–17]

In the search for medications for cocaine abuse, the DAT has provided a focus for the design of potential cocaine antagonists or cocaine substitutes.[7,18–26] Our studies have concentrated on the development of certain bicyclo[3.2.1]octanes, congeners of the prototypical cocaine-like tropane, WIN 35,428.[19,27–33] During the course of these studies we have learned that the specific presence of nitrogen at the 8-position of these tropanes is not a prerequisite for binding to the DAT and that replacement with either an oxygen[30] or even a carbon[19] can lead to potent compounds.[31] Furthermore, we[27] and others,[18,20] have learned that substitution on the aromatic ring of such compounds plays a considerable role in the binding potency of these compounds to the DAT and can affect their relative potency with respect to inhibition of the SERT.[20,34] Finally, studies have also shown that the orientation of the 3-aryl ring in either the α- or β-configuration can affect selectivity of these compounds for the DAT versus the SERT.[19,35] This latter observation prompted us to explore whether the orientation of the aromatic ring in a more planar relationship to the tropane skeleton could further enhance biological selectivity. This approach was particularly attractive since these unsaturated compounds are readily available on route to the more classical saturated bicyclo[3.2.1]octanes.

In this work we present the synthesis and binding constants (DAT and SERT) for three series of

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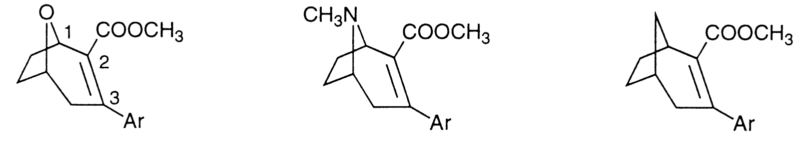
bicyclo[3.2.1]oct-2-enes (Fig. 1). We report that a number of these 2,3-enes manifest substantial binding potency at the DAT and relatively poor potency at the SERT. This results in extremely selective DAT inhibitors.

Results

Chemistry

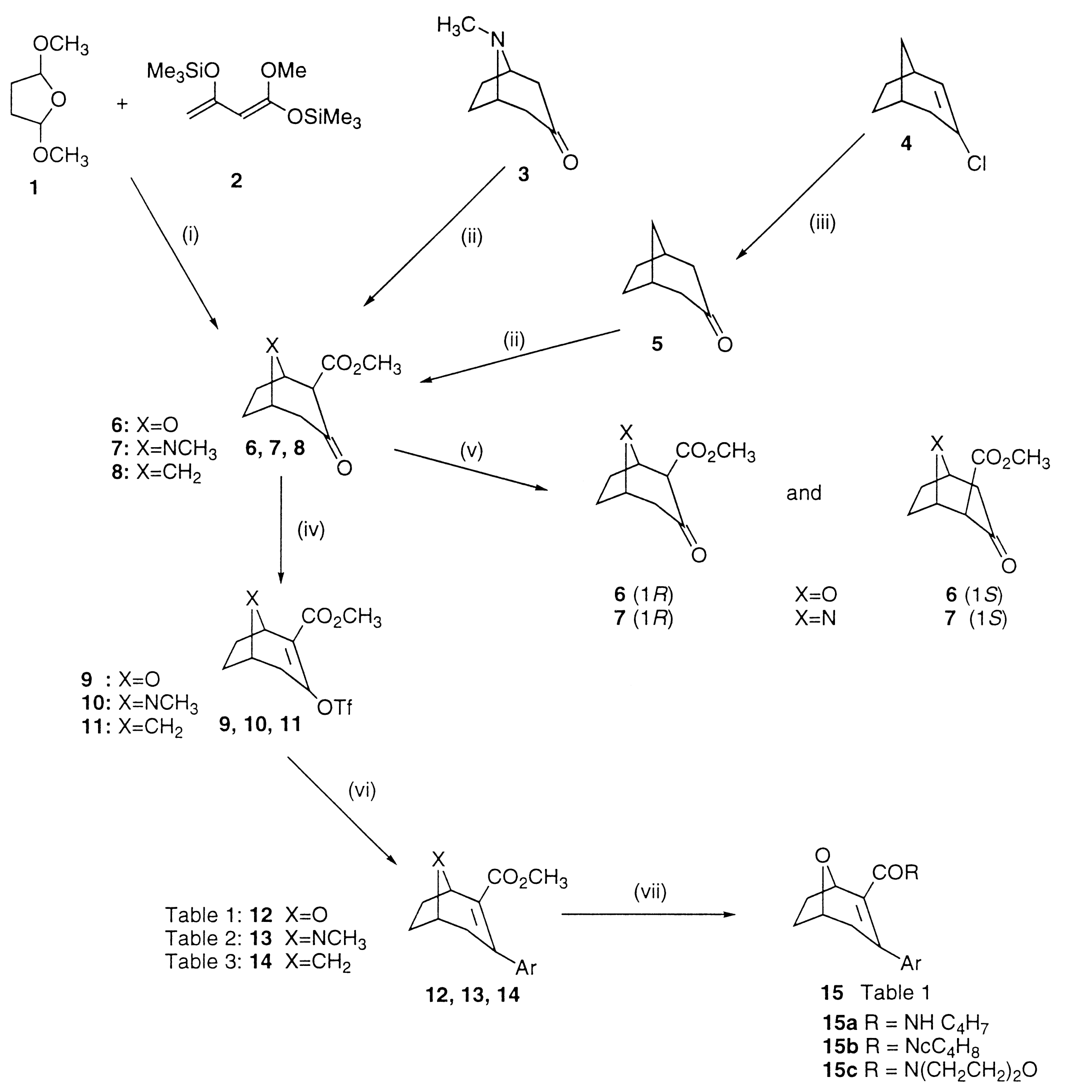
The compounds of this study are 2,3-disubstituted-bicyclo[3.2.1]oct-2-enes. Their synthesis is presented in Scheme 1.

We have previously reported the syntheses of the three critical intermediate keto esters, **6**,[30] **7**[28] and **8**.[19] In summary, **6** had previously been prepared by the reaction of 2,5-dimethoxytetrahydrofuran (**1**) with 1,3-bis-(trimethylsiloxy)-1-methoxybuta-1,3-diene (**2**).[36,37] Compound **7** was readily obtained from tropinone **3** via introduction of the C2-methyl ester followed by enantiomeric resolution of the tartrate salts to obtain both the **7**-1*R* and **7**-1*S* enantiomers.[28,38,39] Compound **8** was obtained from 3-chlorobicyclo[3.2.1]oct-2-ene (**4**) upon treatment with sulfuric acid[40] and subsequent introduction of the 2-carbomethoxy group by reaction with methyl cyanoformate in the presence of lithium diisopropyl amide.



8-Oxa Compounds 8-Aza Compounds 8-Carba Compounds

Figure 1.



Scheme 1. Synthesis of 3-aryl-8-heterobicyclo[3.2.1]oct-2-enes. Reagents: (i) $TiCl_4$, (ii) LDA, $CNCO_2CH_3$, (iii) H_2SO_4, (iv) $NaN(TMS)_2$, $PhNTf_2$, (v) (*S*)-(−)-camphanic chloride,(vi) $ArB(OH)_2$, $Pd_2(dba)_3$, (vii) $(COCl)_2$, RHNR.

Enantiomeric purity of the 1*R* and 1*S* isomers of **6** and **7** was confirmed by formation of their enol camphanates upon reaction of the enolates with (*S*)-(−)-camphanic chloride. The methyls of the resultant camphanate diastereomers had sufficiently different chemical shifts to enable their use in ^{1}H NMR spectroscopy to quantify enantiomeric excess.[30]

The ketoesters **6**, **7** and **8** were treated with *N*-phenyl-bis(trifluoromethanesulfonyl)amine and sodium bis(trimethylsilyl) amide in tetrahydrofuran[30] to obtain the enol triflates **9, 10, 11** in good yield. The enol triflates were then coupled[41] with the appropriate arylboronic acids in diethoxymethane in the presence of lithium chloride, sodium carbonate and tris(dibenzylideneacetone) dipalladium(0)[30,35] to provide the desired aryl octenes **12** (Table 2), **13** (Table 3) and **14** (Table 4). Compounds **15** (Table 2) were obtained upon hydrolysis of **12**, formation of the acid chloride, and subsequent reaction with the appropriate amines. It is interesting that while hydrolysis of saturated 8-azatropanes can generally be accomplished by simple reflux in dioxane water mixtures, this is not a sufficient means for these unsaturated enes (**12**, **13** and **14**). Indeed, LiOH hydrolysis was required in these cases.

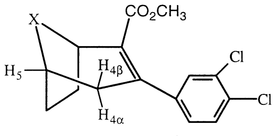
Structural characterization of these compounds is readily accomplished by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR is particularly diagnostic as exemplified for the series of 3-(3,4-dichlorophenyl)bicyclo[3.2.1]-octenes and enol triflates in Table 1. Thus the $H_{4\alpha}$ and $H_{4\beta}$ protons consistently appear at about δ 2.0–2.1 as a gem coupled doublet (J = 17.9–18.7 Hz), and δ 2.6–3.0 as a double doublet showing both the gem coupling (J = 17.9–18.9 Hz) as well as the coupling to the H_5 bridgehead proton (J = 4.4–4.9 Hz), respectively. Only in the case of **11** is the $H_{4\alpha}$ to H_5 coupling seen (J = 1.9 Hz).

It is interesting to note that the coupling constants measured between H_5 and each of $H_{4\alpha}$ and $H_{4\beta}$ are similar throughout these compounds. Calculation of the dihedral angles by means of the Karplus equation indicates that H_5–C5–C4–$H_{4\alpha}$ is about 85–90° and H_5–C5–C4–$H_{4\beta}$ is about 40–45°. This implies that the five carbon centers at C1–C2–C3–C4–C5 are almost coplanar, thus resulting in a flattened ring system.

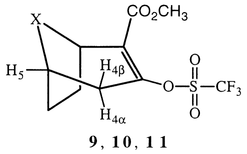
Biology

The affinities of a series of bicyclo[3.2.1]oct-2-ene analogues for the dopamine and serotonin transporters were determined in competition studies using [^{3}H]3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN 35,428 or [^{3}H]CFT) to label the dopamine transporter[5] and [^{3}H]citalopram to label the serotonin transporter.[31] Each compound was tested 2–5 times and each assay conducted in a different brain. Binding data (IC_{50}) for the 8-oxabicyclo[3.2.1]oct-2-enes are presented in Table 2. Data for the 8-azabicyclo[3.2.1]oct-2-enes are presented in Table 3, and data for the bicyclo[3.2.1]oct-2-enes are shown in Table 4. Studies were conducted in monkey striatum because these compounds are part of an ongoing investigation of structure–activity relationships at the DAT in this tissue.[5,7,27,28] Hence, meaningful comparisons with an extensive data base can be made. IC_{50} values are reported as the assay concentration of [^{3}H]WIN 35,428 and [^{3}H]citalopram were below K_d values. Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [^{3}H]WIN 35,428 and [^{3}H]citalopram binding in a concentration-dependent manner. The 8-azabicyclo[3.2.1]octenes (Table 3) are all enantiomerically pure. The 8-oxabicyclo[3.2.1]octenes, with the exception of O-1059 and O-1108 (Table 2), and the bicyclo[3.2.1]octenes (Table 4) are racemic. Consequently, comparison of binding data has to be made with care since the DAT is a stereoselective uptake mechanism and consequently one enantiomer (generally the 1*R*) is considerably more potent than the other.

Table 1. ^{1}H NMR data for representative enol triflates **9**, **10**, **11** and 2,3-enes **12g**, **13d**, **14c**[a]



12g, 13d, 14c

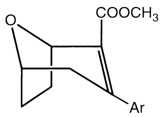


9, 10, 11

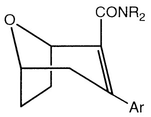
X	Compound	H_1	H_5	$H_{4\beta}$	$H_{4\alpha}$	$COOCH_3$
O	**12g**	5.00 (d)[b]	4.64 (t)	2.91 (J = 4.9, 18.7)[e]	2.06 (J = 18.7)	3.55
NCH_3	**13d**	3.86 (d)	3.36 (t)	2.76 (J = 4.7, 18.9)	1.97 (J = 18.4)	3.52
CH_2	**14c**	3.00 (t)[c]	2.45 (m)[d]	2.63 (J = 4.7, 18.7)	2.14 (J = 18.7)	3.49
O	**9**	5.04 (d)	4.69 (t)	3.00 (J = 4.7, 17.9)	2.13 (J = 17.9)	3.82
NCH_3	**10**	3.93 (d)	3.42 (t)	2.84 (J = 4.4, 18.7)	1.97 (J = 18.4)	3.81
CH_2	**11**	3.10 (t)	2.51 (m)	2.70 (J = 4.7, 18.4)	2.17 (J = 1.9, 18.1)	3.81

[a] Resonance positions in ppm from TMS.
[b] d = Doublet.
[c] t = Triplet.
[d] m = Multiplet.
[e] Coupling (J) in Hz.

Table 2. Inhibition of [^{3}H]WIN 35,428 binding to the dopamine transporter and [^{3}H]citalopram binding to the serotonin transporter by 8-oxabicyclo[3.2.1]octenes in cynomolgus monkey caudate-putamen



12



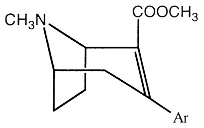
15

IC$_{50}$ (nM)

Ar	R_2	No.	Compound		Dopamine transporter [^{3}H]WIN 35,428	Serotonin transporter [^{3}H]citalopram	Selectivity SERT/DAT
C_6H_5		**12a**	(1*R*/*S*)	O-1141	> 10,000	> 10,000	—
4-FC_6H_4		**12b**	(1*R*/*S*)	O-1132	2730	> 50,000	19
4-ClC_6H_4		**12c**	(1*R*/*S*)	O-1134	238	> 60,000	278
4-BrC_6H_4		**12d**	(1*R*/*S*)	O-1155	62	> 30,000	632
4-IC_6H_4		**12e**	(1*R*/*S*)	O-1165	67.9	4,820	71
3,4-$Cl_2C_6H_3$		**12f**	(1*R*/*S*)	O-1014	12.3	1960	159
3,4-$Cl_2C_6H_3$		**12g**	(1*R*)	O-1059	4.6	2120	461
3,4-$Cl_2C_6H_3$		**12h**	(1*S*)	O-1108	58.2	> 10,000	803
4-Cl-3-FC_6H_3		**12i**	(1*R*/*S*)	O-1143	72	5300	74
3-ClC_6H_4		**12j**	(1*R*/*S*)	O-1614	1280	> 10,000	8
1-Naphthyl		**12k**	(1*R*/*S*)	O-1170	1720	> 40,000	25
2-Naphthyl		**12l**	(1*R*/*S*)	O-1140	19.7	704	36
2-Anthracenyl		**12m**	(1*R*/*S*)	O-1537	> 40,000	> 10,000	0.2
Benzofuran		**12n**	(1*R*/*S*)	O-1147	413	> 20,000	63
3,4-$(OCH_2O)C_6H_3$		**12o**	(1*R*/*S*)	O-1611	> 4000	> 2000	—
4-$CHOC_6H_4$		**12p**	(1*R*/*S*)	O-1497	> 10,000	> 10,000	—
3,4-$Cl_2C_6H_3$	cC_4H_7[a]	**15a**	(1*R*/*S*)	O-1169	2890	> 10,000	3
3,4-$Cl_2C_6H_3$	cC_4H_8[b]	**15b**	(1*R*/*S*)	O-1178	436	> 10,000	42
3,4-$Cl_2C_6H_3$	cC_4H_8O[c]	**15c**	(1*R*/*S*)	O-1179	324	> 50,000	154

[a] Cyclobutyl.
[b] Piperidinyl.
[c] Morpholinyl.

Table 3. Inhibition of [^{3}H]WIN 35,428 binding to the dopamine transporter and [^{3}H]citalopram binding to the serotonin transporter by 8-azabicyclo[3.2.1]octenes in cynomolgus monkey caudate-putamen



13

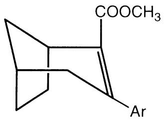
Ar	No.	Compound **13**		Dopamine transporter [^{3}H]WIN 35,428	Serotonin transporter [^{3}H]citalopram	Selectivity SERT/DAT
C_6H_5	**a**	(1*R*)	O-1449	2590	> 20,000	11
4-FC_6H_4	**b**	(1*R*)	O-1104	408	7990	20
4-FC_6H_4	**c**	(1*S*)	O-1119	1590	> 20,000	18
3,4-$Cl_2C_6H_3$	**d**	(1*R*)	O-1109	1.16	867	747
3,4-$Cl_2C_6H_3$	**e**	(1*S*)	O-1120	490	1920	4
2-Naphthyl	**f**	(1*R*)	O-1173	2.9	109	38
3,4-$Cl_2C_6H_3$; NH	**g**	(1*R*)	O-1130	0.99	53.6	54
4-FC_6H_4; NH	**h**	(1*R*)	O-1131	740	829	1

Discussion

Structure–activity relationships (SAR)

The SAR in the three 2,3-ene families is surprisingly similar. Thus, the 3-unsubstituted phenyl compounds are all poor inhibitors of both the DAT and SERT. In contrast, the 3-(3,4-dichlorophenyl) analogues (**12g**, **13d**, **14c**) are all potent inhibitors of the DAT (IC_{50} = 1.1–7.1 nM). The order of potency for the three classes of 3-(substituted-aryl) compounds at the DAT is similar and can be summarized as 3,4-Cl_2 > 3-(2-naphthyl) > F > H. Within the 8-oxabicyclo[3.2.1]octenes, in which a larger series was made, the rank order can be extended as follows: 3,4-Cl_2 > 3-(2-naphthyl) > I = Br > Cl > F > H. Also, the 8-oxa-3-(2-naphthyl) (**12l**) is considerably more potent than the 8-oxa-3-(1-naphthyl) (**12k**) (Table 2).[19] Davies[20] has reported substantial potency for an 8-aza-3-(2-naphthyl)-2-ethylketo tropane

Table 4. Inhibition of [^{3}H]WIN 35,428 binding to the dopamine transporter and [^{3}H]citalopram binding to the serotonin transporter by bicyclo-[3.2.1]octenes in cynomolgus monkey caudate-putamen



14

Ar	No.	Compound **14**		Dopamine transporter [^{3}H]WIN 35,428	Serotonin transporter [^{3}H]citalopram	Selectivity SERT/DAT
C_6H_5	**a**	(1*R*/*S*)	O-1443	3820	> 10,000	1
4-FC_6H_4	**b**	(1*R*/*S*)	O-1436	390	> 30,000	77
3,4-$Cl_2C_6H_3$	**c**	(1*R*/*S*)	O-1231	7.1	5160	726
2-Naphthyl	**d**	(1*R*/*S*)	O-1482	11.0	1310	119

and this is now confirmed for the 8-oxa-2-carbomethoxy tropane **12l**, the 8-aza-2-carbomethoxy tropane **13f** (Table 3) and for the 8-carbacycle **14d** (Table 4). Most significantly, these 2,3-unsaturated compounds are quite selective for the DAT compared with the SERT. For all three series, the 3-(3,4-dichlorophenyl) analogues were highly selective (**12g**, **13d**, **14c**) (460- to 1000-fold selectivity). Within the oxabicyclooctenes, the 4-bromophenyl (**12d**; 632-fold) is next most selective followed by the 4-chlorophenyl (**12c**; 278-fold) and 4-iodo (**12e**; 71-fold). The 3-(2-naphthyl) compounds, (**12l**, **13f**, **14e**) while potent, are less selective than their 3,4-dichlorophenyl counterparts. This is because the 3,4-dichlorophenyl analogues manifest considerably reduced affinity at the SERT compared with the 2-naphthyl analogues. The 3-(2-benzofuranyl) **12n** has $IC_{50} = 413$ nM which lies between that of the 3-(1-naphthyl) analogue **12k** ($IC_{50} = 1720$ nM) and the 3-(2-naphthyl) analogue **12l** ($IC_{50} = 19.7$ nM).

In stark contrast to the marked stereoselective binding of the 8-azatropenes to the DAT, stereoselective binding of the 8-oxatropenes is much less pronounced. Thus, while the 1*R*-8-aza-compound **13d** has an $IC_{50} =$ 1.16 nM, and its 1*S*-enantiomer **13e** manifests only 490 nM, affinity of the 1*R*-8-oxa compound **12g** is 4.6 nM whereas the affinity of its 1*S* enantiomer **12h** is 58.2 nM. Surprisingly, this 1*S* enantiomer **12h** is almost twice as potent as (−)-cocaine, notwithstanding the fact that it is of opposite stereochemistry. Biostereoselectivity is yet to be determined for the carbacycles **14**.

The influence of aromatic bulk at the C3 position is also evident. As shown in the 8-oxa series (Table 2), the affinity of 3-(1-naphthyl) is 1720 nM (**12k**) whereas the 3-(2-naphthyl) (**12l**) is 100 times more potent. Although aromatic bulk in the 3-(2)-position can increase affinity, limitations to this principal were observed with 3-(2-anthracenyl) (**12m**) which displayed an $IC_{50} = > 40{,}000$. Therefore, it is clear that excess steric bulk (2-anthracenyl), and bulk that lies transverse to the tropane skeleton as in the 3-(1-naphthyl), is deleterious to binding. This might imply that the cavity on the DAT that accepts the aromatic ring is less than about 7.3 Å in depth ('length' of an anthracenyl system), and less than about 4.2 Å in width ('width' of a naphthyl connected at C1). However, Carroll has recently reported compounds[42] in which two C3 aromatic rings, linked by an intervening carbon chain, manifest substantial DAT inhibitory potency.

The 8-oxa enes prove intolerant of amide functionality at position C2. Thus **15a**, **15b** and **15c** are of reduced potency and so differ from their nitrogen counterparts which show a marked tolerance for amide functionality in the C2 position.[43]

Within the 8-aza series, the *N*-methyl, **13d**, was 14 times less potent at the SERT than the *N*-unsubstituted compound **13g** (Table 3). This result parallels data observed for the saturated tropanes.[27,44]

The dopamine transporter

The dopamine transporter provides a channel through which dopamine is passed, presumably subsequent to 'recognition' by the macromolecule. There are diverse DAT inhibitors which are substantially different in molecular shape, size and functionality and which bind potently to the DAT. Therefore, these ligands are 'recognized' by the transporter. However, most are not substrates and, therefore, the action of recognition and transport must require different processes. The attributes that govern transport are unknown but may involve conformational changes induced by one ligand but not by another. With respect to binding, however, there is increasing evidence that these diverse ligands bind at different sites on the DAT.[29,45,46] We refer to the actual site on the DAT at which a specific array of amino acids is involved in intermolecular binding between the ligand and the protein as the ligand acceptor site. We postulate that there are a number of such ligand acceptor sites within the DAT and that binding at any one of these acceptor sites can cause inhibition of dopamine reuptake.[30,47]

In the three examples presented in this manuscript (*X* = *N*, *O* and *C*), although the relative order of potency of the various aryl substituted compounds remains similar, the potencies differ quite markedly, especially for the weaker compounds. This may imply that these families occupy different ligand acceptor sites on the DAT. If these acceptor sites are at different depths within the channel formed by the membrane-surrounded DAT, then it is conceivable that, presuming no conformational change has been affected, those that bind deeper within the channel will not necessarily inhibit the binding of those that bind more shallowly. This implies that all ligands that bind within the DAT will inhibit reuptake of dopamine but may show differences in their binding constants with respect to comparison with other non-substrate DAT ligands. This concept is currently under investigation in our laboratories.

The selectivity exhibited by the unsaturated compounds has not been presented prior to this report, however, it is extremely important from a number of perspectives. In the search for potential medications from this class of compounds, these unsaturated compounds are the precursors of the classical 3-aryltropanes and are, therefore, synthetically more readily accessible. Further, they cannot undergo epimerization in the C2-position as can the 2β,3β tropanes, nor is there the substantial yield loss which results from reduction of these 2,3-enes to provide the classical 2β, 3β tropanes. Finally, their biological half life may be anticipated to be increased compared with that of the saturated analogues as a consequence of the relatively more stable conjugated methyl ester. Consequently these unsaturated compounds offer considerable advantages for the development of clinically useful compounds.

Conclusion

In this work it has been demonstrated that appropriate orientation of the 3-aryl ring in the bicyclo[3.2.1]octane system is critical for potent interaction with the SERT. Thus, the topology of the ligand dictates its ability to occupy the acceptor site on the SERT.[30] The striking selectivity for the DAT manifested by the bicyclo-[3.2.1]oct-2,3-enes suggests that the DAT is considerably more flexible with respect to binding than is the SERT. It is possible that there are a number of tropane ligand acceptor sites[30] on the DAT and very few, or even only a single tropane acceptor site on the SERT.

Experimental

^{1}H NMR spectra were recorded on a JEOL 300 NMR spectrometer. TMS was used as an internal standard. Melting points are uncorrected and were measured on a Gallenkamp melting point apparatus. Thin layer chromatography (TLC) was carried out on Baker Si250F plates. Visualization was accomplished with either UV exposure or treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker Silica Gel 40 mM. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. All reactions were conducted under an inert (N_2) atmosphere. [^{3}H]WIN 35,428 (2β-carbomethoxy-3α-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 79.4–87.0 Ci/mmol) and [^{3}H]citalopram (86.8 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA). A Beckman 1801 scintillation counter was used for scintillation spectrometry. 0.1% Bovine serum albumin was purchased from Sigma Chemicals. (*R*)-(−)-Cocaine hydrochloride for the pharmacological studies was donated by the National Institute on Drug Abuse [NIDA]. Fluoxetine was donated by E. Lilly & Co.

General synthetic procedures

General procedure for the synthesis of enol triflates 9, 10, 11 (*1R, 1S*)-2-carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-8-oxabicyclo[3.2.1]-2-octene (9). Sodium bis-(trimethylsilyl)amide (1.0 M solution in THF, 45 mL) was added dropwise to a solution of 2-carbomethoxy-8-oxabicyclo[3.2.1]octanone, **6** (7.12 g, 38.65 mmol) in THF (100 mL) at −78 °C under nitrogen. After stirring for 30 min, *N*-phenyltrifluoromethanesulfonimide (15.2 g, 42.5 mmol) was added as a solid at −78 °C. The reaction was allowed to warm to room temperature and was then stirred overnight. The volatile compounds were then removed on a rotary evaporator. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with H_2O (100 mL) and brine (100 mL). The dried ($MgSO_4$) CH_2Cl_2 layer was concentrated to dryness on a rotary evaporator. The residue was purified by flash chromatography (eluent: 5–10% EtOAc in hexanes) to afford 9.62 g of **9**. Pale yellow oil; yield: 79%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.74 (m, 1H), 2.0–2.3 (m, 4H), 3.00 (dd, $J = 4.65$, 17.85 Hz, 3H), 3.82 (s, 3H), 4.69 (m, 1H), 5.04 (d, $J = 4.95$ Hz, 1H).

(*1R*)-*N*-Methyl-2-carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-8-azabicyclo[3.2.1]-2-octene (10). Pale yellow oil; yield: 78%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.58 (m, 1H), 1.97 (m, 2H), 2.1–2.2 (m, 2H), 2.39 (s, 3H, $NC\underline{H}_3$), 2.84 (dd, $J = 4$, 19 Hz, 1H, $\underline{H}$-4β), 3.42 (t, $J = 6$ Hz, 1H, $\underline{H}$-5), 3.8 (s, 3H, $CO_2C\underline{H}_3$), 3.93 (d, $J = 5$ Hz, 1H, $\underline{H}$-1).

(*1R,1S*)-2-Carbomethoxy-3-{[(trifluoromethyl)sulfonyl]-oxy}bicyclo[3.2.1]-2-octene (11). Clear oil; yield: 69%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41–1.7 (m, 3H), 1.8–2.05 (m, 3H), 2.17 (dd, $J = 1.9$ and 18 Hz, 1H, $\underline{H}$-4α), 2.51 (m, 1H, $\underline{H}$-5), 2.70 (dd, $J = 4.7$, 18 Hz, 1H, $\underline{H}$-4β), 3.10 (t, $J = 3.8$ Hz, 1H, $\underline{H}$-1), 3.81 (s, 3H, $CO_2C\underline{H}_3$).

General procedure for the synthesis of the 2-octenes, 12, 13, 14 (*1R,1S*)-2-carbomethoxy-3-phenyl-8-oxabicyclo[3.2.1]-2-octene (12a). 2-Carbomethoxy-3-{[(trifluoromethyl)-sulfonyl]oxy}-8-oxabicyclo[3.2.1]-2-octene, **9** (2.0 g, 6.3 mmol), phenyl boronic acid (1.02 g, 8.36 mmol), diethoxymethane (20 mL), LiCl (578 mg, 13.6 mmol), tris-(dibenzylideneacetone)dipalladium(0) (247 mg, 0.25 mmol) and Na_2CO_3 (2 M solution, 6.1 mL) were combined and heated at reflux for 1 h. The mixture was cooled to room temperature, filtered through Celite and washed with ether (100 mL). The mixture was made basic with NH_4OH and washed with brine. The dried ($MgSO_4$) ether layer was concentrated to dryness. The residue was then purified by flash chromatography (eluent: 10% EtOAc in hexanes). This afforded 1.28 g (82%) of a light brown viscous oil (**12a**): R_f 0.26 (20% EtOAc in hexanes);

^{1}H NMR (CDCl$_3$, 100 MHz): δ 7.1–7.5 (m, 5H), 4.95–5.1 (m, 1H), 4.55–4.75 (m, 1H), 3.52 (s, 3H), 2.95 (dd, 1H, *J* = 5, 18 Hz), 1.7–2.2 (m, 5H). Anal. ($C_{15}H_{16}O_3$) C, H.

(*1R,1S*)-2-Carbomethoxy-3-(4-fluorophenyl)-8-oxabicyclo-[3.2.1]-2-octene (12b). Compound **12b** was prepared from **9** with 4-fluorophenylboronic acid as described above. A light brown viscous oil was obtained (88%): R_f 0.19 (20% EtOAc in hexanes); ^{1}H NMR (CDCl$_3$, 100 MHz): δ 7.0–7.2 (m, 4H), 4.95–5.05 (m, 1H), 4.55–4.75 (m, 1H), 3.52 (s, 3H), 2.95 (dd, 1H, *J* = 5, 18 Hz), 1.7–2.3 (m, 5H). Anal. ($C_{15}H_{15}O_3F$) C, H.

(*1R,1S*)-2-Carbomethoxy-3-(3-chloro-4-fluorophenyl)-8-oxabicyclo[3.2.1]-2-octene (12i). Compound **12i** was prepared from **9** with 3-chloro-4-fluorophenylboronic acid as described above. An off-white solid was obtained (96%): R_f 0.25 (EtOAc in hexanes); mp 75–76 °C; ^{1}H NMR (CDCl$_3$, 100 MHz): δ 1.5–2.4 (m, 5H), 2.9 (dd, *J* = 5, 20 Hz, 1H, *H*-4β), 3.56 (s, 3H, OC*H*3), 4.55–4.80 (m, 1H, *H*-5), 5.0 (m, 1H, *H*-1), 6.9–7.4 (m, 3H). Anal. ($C_{15}H_{14}O_3FCl$) C, H, Cl.

(*1R,1S*)-2-Carbomethoxy-3-(3-chlorophenyl)-8-oxabicyclo-[3.2.1]-2-octene (12j). Compound **12j** was prepared from **9** with 3-chlorophenylboronic acid as described above. A light yellow oil was obtained (78%): R_f 0.43 (30% EtOAc in hexanes); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.76 (m, 1H), 2.04–2.26 (m, 4H), 2.92 (dd, 1H, *J* = 18 Hz); 3.52 (s, 3H), 4.63 (m, 1H), 5.00 (m, 1H), 6.90 (m, 1H), 7.08 (s, 1H), 7.25 (m, 2H). Anal. ($C_{15}H_{15}ClO_3$) C, H, Cl.

(*1R,1S*)-2-Carbomethoxy-3-(2-anthracenyl)-8-oxabicyclo-[3.2.1]-2-octene (12m). Compound **12m** was prepared from **9** with 2-anthracenylboronic acid as described above. A yellow solid was obtained (33%): R_f 0.39 (30% EtOAc in hexanes); mp 185.5–186.5 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.84 (m, 1H), 2.14–2.32 (m, 4H), 2.08 (dd, 1H, *J* = 18 Hz); 3.46 (s, 3H), 4.71 (m, 1H), 5.07 (m, 1H), 7.23 (m, 1H), 7.46 (m, 2H), 7.73 (s, 1H), 7.97 (m, 3H), 8.38 (m, 2H). Anal. ($C_{23}H_{20}O_3$) C, H.

(*1R,1S*)-2-Carbomethoxy-3-(3,4-methylenedioxyphenyl)-8-oxabicyclo[3.2.1]-2-octene (12o). Compound **12o** was prepared from **9** with 3,4-methylenedioxyphenylboronic acid as described above. An off-white solid was obtained (78%): R_f 0.37 (30% EtOAc in hexanes); mp 106.2–107.2 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.75 (m, 1H), 2.04–2.26 (m, 4H), 2.91 (dd, 1H, *J* = 18 Hz); 3.56 (s, 3H), 4.62 (m, 1H), 4.97 (m, 1H), 5.95 (s, 2H), 6.59 (m, 2H), 6.75 (m, 1H). Anal. ($C_{16}H_{16}O_5$) C, H.

2-Anthracenylboronic acid was prepared from 2-iodoanthracene and triisopropylborate according to a literature procedure.[41] A yellow solid was obtained (34%): ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.36 (s, 2H), 7.50 (m, 2H), 7.91 (m, 1H), 8.04 (m, 2H), 8.51 (s, 1H), 8.56 (s, 1H), 8.64 (s, 1H).

2-Iodoanthracene was prepared from 2-aminoanthracene according to a literature procedure.[48] A white solid was obtained (7%): ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.49 (m, 2H), 7.69 (m, 2H), 7.98 (m, 2H), 8.29 (s, 1H), 8.38 (s, 1H), 8.42 (s, 1H).

(*1R,1S*)-2-Carbomethoxy-3-(4-formylphenyl)-8-oxabicyclo[3.2.1]-2-octene (12p). Compound **12p** was prepared from **9** with 4-formylphenylboronic acid as described above. A white solid was obtained (25%): R_f 0.22 (30% EtOAc in hexanes); mp 93.8–94.8 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.8 (m, 1H), 2.0–2.3 (m, 4H), 2.95 (dd, *J* = 5.2, 18 Hz, 1H, *H*-4β), 3.50 (s, 3H, OC*H*$_3$), 4.66 (m, 1H, *H*-5), 5.0 (d, *J* = 5.2 Hz, 1H, *H*-1), 7.27 (d, *J* = 4.7 Hz, 2H) 7.86 (d, *J* = 4.7 Hz, 2H). Anal. ($C_{16}H_{16}O_4$) C, H.

(*1R*)-*N*-Methyl-2-carbomethoxy-3-phenyl-8-azabicyclo-[3.2.1]-2-octene (13a). Compound **13a** was prepared from **10**(*1R*) with phenylboronic acid as described above. A yellow oil was obtained (25%). R_f 0.47 (5% Et$_3$N in EtOAc). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.63 (m, 1H), 1.9–2.3 (m, 3H), 2.03 (d, *J* = 18 Hz, 1H, *H*-4α), 2.47 (s, 3H, NC*H*$_3$), 2.76 (dd, *J* = 4.7, 18 Hz, 1H, *H*-4β), 3.34 (t, 1H, *H*-5), 3.46 (s, 3H, OC*H*$_3$), 3.84 (d, *J* = 5.5 Hz 1H, *H*-1), 7.1–7.5 (m, 2H) 7.22–7.34 (m, 3H). Anal. ($C_{16}H_{19}$ $NO_2 \cdot 1/10\ H_2O$) C, H, N.

(*1R*)-*N*-Methyl-2-carbomethoxy-3-(4-fluorophenyl)-8-azabicyclo[3.2.1]-2-octene (13b). Compound **13b** was prepared from **10**(*1R*) with 4-fluorophenylboronic acid as described above. A yellow oil was obtained (72%). R_f 0.60 (10% Et$_3$N in ether). ^{1}H NMR (CDCl$_3$, 100 MHz): δ 1.63–2.3 (m, 5H), 2.45 (s, 3H, NC*H*$_3$), 2.6–2.9 (m, 1H), 3.35 (m, 1H), 3.50 (s, 3H, OC*H*$_3$), 3.85 (m, 1H), 6.9–7.2 (m, 4H). Anal. ($C_{16}H_{18}$ $NO_2F \cdot 1/6\ H_2O$) C, H, N.

(*1S*)-*N*-Methyl-2-carbomethoxy-3-(4-fluorophenyl)-8-azabicyclo[3.2.1]-2-octene (13c). Compound **13c** was prepared from **10**(*1S*) with 4-fluorophenylboronic acid as described above. A yellow oil was obtained (43%). R_f 0.49 (10% Et$_3$N in ethyl acetate). ^{1}H NMR (CDCl$_3$, 100 MHz): δ 1.63–2.3 (m, 5H), 2.45 (s, 3H, NC*H*$_3$), 2.6–2.9 (m, 1H), 3.35 (m, 1H), 3.50 (s, 3H, OC*H*$_3$), 3.85 (m, 1H), 6.9–7.2 (m, 4H). Anal. ($C_{16}H_{18}$ $NO_2F \cdot 1/3\ H_2O$) C, H, N.

(*1R*)-*N*-Methyl-2-carbomethoxy-3-(3,4-dichlorophenyl)-8-azabicyclo[3.2.1]-2-octene (13d). Compound **13d** was prepared from **10**(*1R*) with 3,4-dichlorophenylboronic acid as described above. A yellow oil was obtained (83%). R_f 0.56 (10% Et$_3$N in ethyl acetate). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.61 (m, 1H), 1.9–2.05 (m, 2H), 2.1–2.3 (m, 2H), 2.43 (s, 3H, NC*H*$_3$), 2.76 (dd, *J* = 19, 4.7 Hz, 1H), 3.36 (t, *J* = 4.9 Hz, 1H), 3.52 (s, 3H, OC*H*$_3$), 3.86 (d, *J* = 5.5 Hz, 1H), 6.96 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.2 (d, *J* = 2.2 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H). Anal. ($C_{16}H_{17}NO_2Cl_2$) C, H, N.

(*1S*)-*N*-Methyl-2-carbomethoxy-3-(3,4-dichlorophenyl)-8-azabicyclo[3.2.1]-2-octene (13e). Compound **13e** was prepared from **10**(*1S*) with 3,4-dichlorophenylboronic acid as described above. A yellow oil was obtained (69%). R_f 0.56 (10% Et$_3$N in ethyl acetate). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.61 (m, 1H), 1.9–2.05 (m, 2H), 2.1–2.3 (m, 2H), 2.43 (s, 3H, NC*H*$_3$), 2.76 (dd, *J* = 19, 4.7 Hz, 1H), 3.36 (t, $J = 4.9$ Hz, 1H), 3.52 (s, 3H, OC$\underline{H}_3$), 3.86 (d, $J = 5.5$ Hz, 1H), 6.96 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.2 (d, $J = 2.2$ Hz, 1H), 7.37 (d, $J = 8.2$ Hz, 1H). Anal. ($C_{16}H_{17}NO_2Cl_2$) C, H, N.

(*1R*)-*N*-Methyl-2-carbomethoxy-3-(2-naphthyl)-8-azabicyclo[3.2.1]-2-octene (13f). Compound **13f** was prepared from **10**(*1R*) with 2-naphthylboronic acid as described above. A yellow oil was obtained (51%). R_f 0.48 (10% Et_3N in ethyl acetate). ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.3–3.6 (m, 7H), 2.5 (s, 3H, NC$\underline{H}_3$), 3.45 (s, 3H, OC$\underline{H}_3$), 3.8–4.0 (m, 1H), 7.2–8.0 (m, 7H). Anal. ($C_{20}H_{21}NO_2 \cdot 1/2\ H_2O$) C, H, N.

(*1R*)-2-Carbomethoxy-3-(3,4-dichlorophenyl)-8-norazabicyclo[3.2.1]-2-octene (13g). 2-Methoxycarbonyl-3-(3,4-dichlorophenyl)tropene **13d** (200 mg, 0.61 mmol) was combined with 1-chloroethyl chloroformate (4 mL) and the resulting solution was brought to reflux for 2 h. The excess chloroformate was removed in vacuo and the residue was brought to reflux in methanol (30 mL) for 45 min. The methanol was then removed in vacuo and the residue was partitioned in $CHCl_3/NaHCO_3/Na_2CO_3$(aqueous) (pH = 9). The aqueous layer was extracted with $CHCl_3$ (3×10 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated. The residue was chromatographed (eluent: 5–15% Et_3N in EtOAc). Like fractions were combined to yield a yellow oil (50%). R_f 0.3 (10% Et_3N in EtOAc); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.50–2.3 (m, 5H), 2.5–2.9 (m, 1H), 3.53 (s, 3H), 3.8 (m, 1H), 4.2 (m, 1H), 6.9 (dd, 1H), 7.2 (dd, 1H), 7.4 (d, 1H). Anal. ($C_{15}H_{15}NO_2Cl_2 \cdot 1/5\ H_2O$) C, H, N.

(*1R*)-2-Carbomethoxy-3-(4-fluorophenyl)-8-norazabicyclo-[3.2.1]-2-octene (13h). Compound **13h** was prepared from **13b** as described above. A yellow oil was obtained (68%). R_f 0.7 (10% MeOH in hexanes + 0.5% NH_4OH); mp 67–68 °C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.0 (m, 6H), 3.5 (s, 3H), 3.8 (m, 1H), 4.2 (m, 1H), 6.8–7.2 (m, 4H). Anal. ($C_{15}H_{16}NO_2F$) C, H, N.

(*1R,1S*)-2-Carbomethoxy-3-phenylbicyclo[3.2.1]-2-octene (14a). Compound **14a** was prepared from **11** with phenylboronic acid as described above. An oil was obtained (75%): R_f 0.5 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.52–1.66 (m, 2H), 1.73 (d, $J = 11$ Hz, 1H), 1.79–2.06 (m, 3H), 2.23 (d, $J = 18.7$ Hz, 1H), 2.44–2.47 (m, 1H), 2.66–2.74 (m, 1H), 2.99–3.02 (m, 1H), 3.43 (s, 3H), 7.07–7.10 (m, 2H), 7.20–7.32 (m, 3H). Anal. ($C_{16}H_{18}O_2$) C, H.

(*1R,1S*)-2-Carbomethoxy-3-(4-fluorophenyl)-bicyclo[3.2.1]-2-octene (14b). Compound **14b** was prepared from **11** with 4-fluorophenylboronic acid as described above. An oil was obtained (73%): R_f 0.5 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.50–2.04 (m, 6H), 2.20 (d, $J = 18.7$ Hz, 1H), 2.44–2.48 (m, 1H), 2.63–2.71 (m, 1H), 3.00 (t, $J = 4.5$ Hz, 1H), 3.46 (s, 3H), 6.94–7.08 (m, 4H). Anal. ($C_{16}H_{17}O_2$ F) C, H.

(*1R,1S*)-2-Carbomethoxy-3-(3,4-dichlorophenyl)-bicyclo-[3.2.1]-2-octene (14c). Compound **14c** was prepared from **11** with 3,4-dichlorophenylboronic acid as described above. An oil was obtained (69%): R_f 0.5 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.48–1.70 (m, 3H), 1.76–2.03 (m, 3H), 2.14 (d, $J = 19$ Hz, 1H), 2.40–2.65 (m, 1H), 2.60–2.67 (m, 1H), 2.99–3.02 (m, 1H), 3.49 (s, 3H), 6.90 (dd, $J = 8$, 2 Hz, 1H), 7.17 (d, $J = 2$ Hz, 1H), 7.34 (d, $J = 8$ Hz, 1H). Anal. ($C_{16}H_{16}O_2Cl_2$) C, H, Cl.

(*1R,1S*)-2-Carbomethoxy-3-(2-naphthyl)-bicyclo[3.2.1]-2-octene (14d). Compound **14d** was prepared from **11** with 2-naphthylboronic acid as described above. An oil was obtained (54%): R_f 0.41 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.63–1.74 (m, 2H), 1.82–2.17 (m, 4H), 2.38 (d, $J = 18.7$ Hz, 1H), 2.52–2.55 (m, 1H), 2.79–2.87 (m, 1H), 3.14 (t, $J = 4.5$ Hz, 1H), 3.44 (s, 3H), 7.25–7.29 (m, 1H), 7.47–7.50 (m, 2H), 7.62 (d, $J = 1.4$ Hz, 1H), 7.80–7.86 (m, 3H). Anal. ($C_{20}H_{20}O_2$) C, H.

General procedure for synthesis of the 2-amide-2-octenes, 15a, b and c. To a solution of 2-carbomethoxy-3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2-ene **12f** (285 mg, 0.91 mmol) in THF/MeOH (2 mL/0.67 mL) was added a solution of lithium hydroxide (0.67 mL of a 4.8 M solution) and the resulting solution was stirred at room temperature overnight. TLC (20% ethyl acetate in hexanes with 0.5% acetic acid) showed completion of hydrolysis. Water and ether were then added and the layers separated. The aqueous phase was then acidified with 1 M HCl and extracted with ether. The ether layer was washed with brine and dried over $MgSO_4$. Concentration gave the crude product (258 mg) which was purified by column chromatography (SiO_2, 1 g, eluent: 50% ethyl acetate in hexanes with 1% acetic acid) and gave 128 mg (47%) of the acid which was used immediately for the next step.

The pure acid was taken up in CH_2Cl_2 (2 mL) and treated with oxalyl chloride (0.058 mL) and a drop of DMF. After stirring at room temperature for 1 h the volatile compounds were evaporated and the residue pumped for 4 h. The acid chloride was then dissolved in CH_2Cl_2 (2 mL) and then the required amine added (4 equivalents) neat and the solution stirred overnight. The crude amide was purified by chromatography (30–50% ethyl acetate in hexanes) and gave:

(*1R,S*)-*N*-Cyclobutyl-2-carboxamido-3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]-2-octene (15a). As a white solid. Yield = 72%. R_f 0.50 (ethyl acetate), mp 174–176 °C; ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.2–2.4 (m, 11H), 3.0 (dd, $J = 5$, 17 Hz, 1H, $\underline{H}$-4β), 4.1–4.5 (m, 1H, C($\underline{H}$)-N-), 4.6–4.8 (m, 1H, $\underline{H}$-5), 4.85 (d, $J = 5$ Hz, 1H, $\underline{H}$-1), 5.08 (br d, $J = 7$ Hz, 1H, N$\underline{H}$), 7.1 (dd, $J = 10$, 2 Hz, 1H), 7.35 (d, $J = 2.4$ Hz, 1H), 7.46 (d, $J = 10$ Hz, 1H). Anal. ($C_{18}H_{19}NO_2Cl_2$) C, H, N, Cl.

(*1R,S*)-3-(3,4-Dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2-ene-2-carboxylic acid pyrrolidine amide (15b). As a white solid (90%). R_f 0.30 (ethyl acetate), mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.4–2.5 (m, 9H), 2.7–3.5 (m, 5H), 4.55–4.80 (m, 2H), 7.15 (dd, 1H), 7.36 (d, 1H), 7.45 (d, 1H). Anal. ($C_{18}H_{19}NO_2Cl_2$) C, H, N, Cl.

(*1R,S*)-3-(3,4-Dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2-ene-2-carboxylic acid morpholine amide (15c). As a white solid (60%). R_f 0.32 (ethyl acetate), mp 141–143 °C; ^{1}H NMR (CDCl$_3$, 100 MHz): δ 1.6–3.7 (m, 14H), 4.3–4.8 (m, 2H), 7.12 (dd, 1H), 7.36 (d, 1H), 7.4 (d, 1H). Anal. ($C_{18}H_{19}NO_3Cl_2$) C, H, N, Cl.

Tissue sources and preparation

Brain tissue from adult male and female cynomolgus monkeys (*Macaca fascicularis*) was stored at −85 °C in the primate brain bank at the New England Regional Primate Research Center. The caudate-putamen was dissected from coronal slices and yielded 1.4 ± 0.4 g tissue. Membranes were prepared as described previously. Briefly, the caudate-putamen was homogenized in 10 volumes (w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4 at 4 °C) and centrifuged at 38,000×*g* for 20 min in the cold. The resulting pellet was suspended in 40 volumes of buffer, and the entire procedure was repeated twice. The membrane suspension (25 mg original wet weight of tissue/mL) was diluted to 12 mL/mL for [^{3}H]WIN 35,428 or [^{3}H]citalopram assay in buffer just before assay and was dispersed with a Brinkmann Polytron homogenizer (setting #5) for 15 s. All experiments were conducted in triplicate and each experiment was repeated in each of 2–3 preparations from individual brains.

Dopamine transporter assay

The dopamine transporter was labeled with [^{3}H]WIN 35,428 ([^{3}H]CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 81–84 Ci/mmol, DuPont-NEN). The affinity of [^{3}H]WIN 35,428 for the dopamine transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]WIN 35,428 and a range of concentration of unlabeled WIN 35,428. The assay tubes received, in Tris–HCl buffer (50 mM, pH 7.4 at 0–4 °C; NaCl 100 mM), the following constituents at a final assay concentration: WIN35,428, 0.2 mL (1 pM, 100 or 300 nM), [^{3}H]WIN 35,428 (0.3 nM); membrane preparation 0.2 mL (4 mg original wet weight of tissue/mL). The 2 h incubation (0–4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters pre-soaked in 0.1% bovine serum albumin (Sigma Chem. Co.). The filters were washed twice with 5 mL Tris.HCl buffer (50 mM), incubated overnight at 0–4 °C in scintillation fluor (Beckman Ready-Value, 5 mL) and radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). Cpm were converted to dpm following determination of counting efficiency (> 45%) of each vial by external standardization. Total binding was defined as [^{3}H]WIN 35,428 bound in the presence of ineffective concentrations of unlabeled WIN 35,428 (1 or 10 pM). Non-specific binding was defined as [^{3}H]WIN 35,428 bound in the presence of an excess (30 μM) of (−)-cocaine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]WIN 35,428 binding sites were conducted using procedures similar to those outlined above. Stock solutions of water-soluble drugs were dissolved in water or buffer and stock solutions of other drugs were made in a range of ethanol/HCl solutions. Several of the drugs were sonicated to promote solubility. The stock solutions were diluted serially in the assay buffer and added (0.2 mL) to the assay medium as described above. IC$_{50}$ values were computed by the EBDA computer program and are the means of experiments conducted in triplicate.

Serotonin transporter assay

The serotonin transporter was assayed in caudate-putamen membranes using conditions similar to those for the dopamine transporter. The affinity of [^{3}H]citalopram (spec. act.: 82 Ci/mmol, DuPont-NEN) for the serotonin transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]citalopram and a range of concentrations of unlabeled citalopram. The assay tubes received, in Tris–HCl buffer (50 mM, pH 7.4 at 0–4 °C; NaCl 100 mM), the following constituents at a final assay concentration: citalopram, 0.2 mL (1 pM, 100 or 300 nM), [^{3}H]citalopram (1 nM); membrane preparation 0.2 mL (4 mg original wet weight of tissue/mL). The 2 h incubation (0–4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters pre-soaked in 0.1% polyethyleneimine. The filters were washed twice with 5 mL Tris–HCl buffer (50 mM), incubated overnight at 0–4 °C in scintillation fluor (Beckman Ready-Value, 5 mL) and radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). Cpm were converted to dpm following determination of counting efficiency (> 45%) of each vial by external standardization. Total binding was defined as [^{3}H]citalopram bound in the presence of ineffective concentrations of unlabeled citalopram (1 or 10 pM). Non-specific binding was defined as [^{3}H]citalopram bound in the presence of an excess (10 μM) of fluoxetine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]citalopram binding sites were conducted using procedures similar to those outlined above. IC$_{50}$ values were computed by the EBDA computer program and are the means of experiments conducted in triplicate.

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References

1. Kennedy, L. T.; Hanbauer, I. *J. Neurochem.* **1983**, *34*, 1137.
2. Schoemaker, H.; Pimoule, C.; Arbilla, S.; Scatton, B.; Javoy-Agid, F.; Langer, S. Z. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *329*, 227.
3. Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. *Biochem. Pharmacol.* **1986**, *35*, 1123.

4. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. *Science* **1987**, *237*, 1219.
5. Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. *J. Pharmacol. Exp. Ther*. **1989**, *251*, 131.
6. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. *Trends Neurosci*. **1991**, *14*, 299.
7. Madras, B. K.; Kamien, J. B.; Fahey, M.; Canfield, D.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. *Pharmacol. Biochem. Behav*. **1990**, *35*, 949.
8. Van Dyck, C.; Seibyl, J.; Malison, R.; Laruelle, M.; Wallace, E.; Zoghbi, S.; Zea-Ponce, Y.; Baldwin, R.; Charney, D.; Hoffer, P. *J. Nucl. Med*. **1995**, *36*, 1175.
9. Volkow, N. D.; Ding, Y. S.; Fowler, J. S.; Wang, G. J.; Logan, J.; Gatley, S. J.; Hitzemann, R.; Smith, G.; Fields, S. D.; Gur, R. J. *Nucl. Med* **1996**, *37*, 554.
10. Kaufman, M. J.; Madras, B. K. *Synapse* **1991**, *9*, 43.
11. Morgan, G. F.; Nowotnik, D. P. *Drug News Perspect*. **1999**, *12*, 137.
12. Elmaleh, D. R.; Madras, B. K.; Shoup, T. M.; Byron, C.; Hanson, R. N.; Liang, A. Y.; Meltzer, P. C.; Fischman, A. J. *J. Nucl. Chem*. **1996**, 1197.
13. Fischman, A. J.; Bonab, A. A.; Babich, J. W.; Alpert, N. M.; Elmaleh, D. R.; Barrow, S. A.; Graham, W.; Meltzer, P. C.; Hanson, R. N.; Madras, B. K. *Neuroscience-Net* **1997**, *1*, 10.
14. Madras, B. K.; Jones, A. G.; Mahmood, A.; Zimmerman, R. E.; Garada, B.; Holman, B. L.; Davison, A.; Blundell, P.; Meltzer, P. C. *Synapse* **1996**, *22*, 239.
15. Meltzer, P. C.; Blundell, P.; Jones, A. G.; Mahmood, A.; Garada, B.; Zimmerman, R. E.; Davison, A.; Holman, B. L.; Madras, B. K. *J. Med. Chem*. **1997**, *40*, 1835.
16. Fischman, A. J.; Babich, J. W.; Elmaleh, D. R.; Barrow, S. A.; Meltzer, P. C.; Hanson, R. N.; Madras, B. K. *J. Nucl. Med*. **1997**, *38*, 144.
17. Fischman, A. J.; Babich, J. W.; Elmaleh, D. R.; Meltzer, P. C.; Barrow, S. A.; Madras, B. K. *J. Nucl. Chem*. **1995**, *36*, 87.
18. Carroll, F. I.; Lewin, A. H.; Kuhar, M. *J. Med. Chem. Res*. **1998**, *8*, 59.
19. Meltzer, P. C.; Blundell, P.; Madras, B. K. *Med. Chem. Res*. **1998**, *8*, 12.
20. Davies, H. M. L.; Kuhn, L. A.; Thornley, C.; Matasi, J. J.; Sexton, T.; Childers, S. R. *J. Med. Chem*. **1996**, *39*, 2554.
21. Lomenzo, S. A.; Izenwasser, S.; Katz, J. L.; Trudell, M. L. *Med. Chem. Res*. **1998**, *8*, 35.
22. Deutsch, H. M. *Med. Chem. Res*. **1998**, *8*, 91.
23. Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; George, C.; Katz, J. L. *J. Med. Chem*. **1995**, *38*, 3933.
24. Prakash, K. R. C.; Araldi, G. L.; Smith, M. P.; Zhang, M.; Johnson, K. M.; Kozikowski, A. P. *Med. Chem. Res*. **1998**, *8*, 43.
25. Zhang, Y. *Med. Chem. Res*. **1998**, *8*, 66.
26. Houlihan, W. J.; Boja, J. W.; Kopajtic, T. A.; Kuhar, M. J.; Degrado, S. J.; Toledo, L. *Med. Chem. Res*. **1998**, *8*, 77.
27. Meltzer, P. C.; Liang, A. Y.; Brownell, A.-L.; Elmaleh, D. R.; Madras, B. K. *J. Med. Chem*. **1993**, *36*, 855.
28. Meltzer, P. C.; Liang, A. Y.; Madras, B. K. *J. Med. Chem*. **1994**, *37*, 2001.
29. Meltzer, P. C.; Liang, A. Y.; Madras, B. K. *J. Med. Chem*. **1996**, *39*, 371.
30. Meltzer, P. C.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Chen, Z.; George, C.; Madras, B. K. *J. Med. Chem*. **1997**, *40*, 2661.
31. Madras, B. K.; Pristupa, Z. B.; Niznik, H. B.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Meltzer, P. C. *Synapse* **1996**, *24*, 340.
32. Madras, B. K.; Kaufman, M. J. *Synapse* **1994**, *18*, 261.
33. Madras, B. K.; Fahey, M. A.; Kaufman, M. J.; Spealman, R. D.; Schumacher, J.; Isacson, O.; Brownell, A.-L.; Brownell, G. L.; Elmaleh, D. R. *Soc. Neurosci. Abstr*. **1991**, *17*, 190.
34. Blough, B. E.; Abraham, P.; Mills, C. A.; Lewin, A. H.; Boja, J. W.; Scheffel, U.; Kuhar, M. J.; Boja, J. W.; Carroll, F. I. *J. Med. Chem*. **1997**, *40*, 3861.
35. Keverline, K. I.; Abraham, P.; Lewin, A. H.; Carroll, F. I. *Tetrahedron Lett*. **1995**, *36*, 3099.
36. Danishefsky, S.; Kitahara, T. *J. Am. Chem. Soc*. **1974**, *96*, 7807.
37. Chan, T.-H.; Brownbridge, P. *J. Am. Chem. Soc*. **1980**, *102*, 3534.
38. Findlay, S. P. *J. Org. Chem*. **1957**, *22*, 1385.
39. Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1991**, *34*, 883.
40. Jefford, C. W.; Gunsher, J.; Hill, D. T.; Brun, P.; Le Gras, J.; Waegell, B. *Organic Synthesis* **1988**, Coll. Vol. VI, 142.
41. Oh-e, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem*. **1993**, *58*, 2201.
42. Carroll, I. Communication, CPDD, Acapulco, Mexico, 1999.
43. Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1995**, *38*, 379.
44. Carroll, F. I.; Mascarella, S. W.; Kuzemko, M. A.; Gao, Y.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1994**, *37*, 2865.
45. Newman, A. H. *Med. Chem. Res*. **1998**, *8*, 1.
46. Rothman, R. B.; Becketts, K. M.; Radesca, L. R.; de Costa, B. R.; Rice, K. C.; Carroll, F. I.; Dersch, C. M. *Life Sciences* **1993**, *53*, 267.
47. Miller, G. M.; Gracz, I. M.; Goulet, M.; Yang, H.; Meltzer, P. C.; Hoffman, B. J.; Madras, B. K. *Soc. Neurosci. Abst*., in press.
48. Bachmann, W. E.; Boatner, C. H. *J. Amer. Chem. Soc*. **1936**, *58*, 2194.