Highly Selective Chiral N-Substituted 3α-[Bis(4′-fluorophenyl)methoxy]tropane Analogues for the Dopamine Transporter: Synthesis and Comparative Molecular Field Analysis

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In a continuing effort to further characterize the role of the dopamine transporter in the pharmacological effects of cocaine, a series of chiral and achiral N-substituted analogues of 3α-[bis(4′-fluorophenyl)methoxy]tropane (**5**) has been prepared as potential selective dopamine transporter ligands. These novel compounds displaced [^{3}H]WIN 35,428 binding from the dopamine transporter in rat caudate putamen with K_i values ranging from 13.9 to 477 nM. Previously, it was reported that **5** demonstrated a significantly higher affinity for the dopamine transporter than the parent drug, 3α-(diphenylmethoxy)tropane (**3**; benztropine). However, **5** remained nonselective over muscarinic m_1 receptors (dopamine transporter, $K_i = 11.8$ nM; m_1, $K_i = 11.6$ nM) which could potentially confound the interpretation of behavioral data, for this compound and other members of this series. Thus, significant effort has been directed toward developing analogues that retain high affinity at the dopamine transporter but have decreased affinity at muscarinic sites. Recently, it was discovered that by replacing the *N*-methyl group of **5** with the phenyl-*n*-butyl substituent (**6**) retention of high binding affinity at the dopamine transporter ($K_i = 8.51$ nM) while decreasing affinity at muscarinic receptors ($K_i = 576$ nM) was achieved, resulting in 68-fold selectivity. In the present series, a further improvement in the selectivity for the dopamine transporter was accomplished, with the chiral analogue (*S*)-*N*-(2-amino-3-methyl-*n*-butyl)-3α-[bis(4′-fluorophenyl)methoxy]tropane (**10b**) showing a 136-fold selectivity for the dopamine transporter versus muscarinic m_1 receptors ($K_i = 29.5$ nM versus $K_i = 4020$ nM, respectively). In addition, a comparative molecular field analysis (CoMFA) model was derived to correlate the binding affinities of all the N-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues that we have prepared with their 3D-structural features. The best model ($q^2 = 0.746$) was used to accurately predict binding affinities of compounds in the training set and in a test set. The CoMFA coefficient contour plot for this model, which provides a visual representation of the chemical environment of the binding domain of the dopamine transporter, can now be used to design and/or predict the binding affinities of novel drugs within this class of dopamine uptake inhibitors.

Introduction

The socioeconomic costs and serious health risks associated with illicit drug use, and in particular cocaine (**1**) abuse, have accentuated the need for prevention and treatment. One approach to the development of a pharmaceutical treatment for cocaine abuse has been to focus on identifying the mechanistic correlates to cocaine's pharmacological effects. The prevailing concept that underlies this pursuit has been that as we better understand how cocaine produces its reinforcing effects that may lead to its abuse and addiction, we can potentially intervene on a molecular level, to prevent further illicit drug taking. To this end, the preponderance of research suggests that the primary mechanism by which cocaine exerts its reinforcing and psychomotor stimulant effects appears to be through the blockade of the dopamine transporter and subsequent accumulation of dopamine in the synapse.[1,2] As a result, a thorough understanding of the structure and function of the dopamine transporter and of the cocaine binding site has been deemed critical for development of drugs that may be useful in the treatment of cocaine abuse. To date, the dopamine transporter has been cloned and expressed, but its 3D-structure is unknown.[3−6] In efforts to develop a pharmacotherapeutic in absence of topological information about the dopamine transporter, considerable research has been conducted toward the elucidation of the cocaine recognition site or sites through the use of novel, high-affinity ligands.[7,8] Structure−activity relationship (SAR) studies of cocaine and 3β-phenyltropane-2β-carboxylic acid methyl ester (WIN 35,065-2; **2**) analogues have proven useful in the iden-

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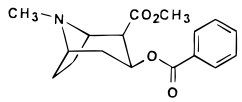
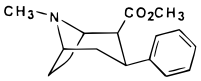
tification of structural requirements for potency and selectivity at the dopamine transporter within this class of drugs.[7]



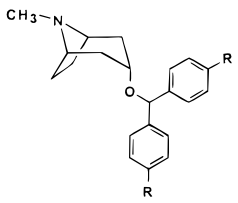
Cocaine, **1**



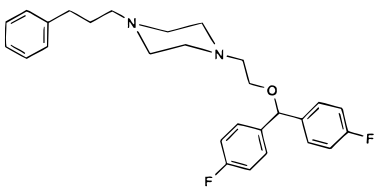
WIN 35.065-2, **2**

The generation of 3D-quantitative structure−activity relationship (3D-QSAR) models using comparative molecular field analysis (CoMFA)[9] has been explored in the 3β-phenyltropane-2β-carboxylic acid methyl ester class of dopamine uptake inhibitors.[10−14] The molecular models generated have provided predictive models of the steric and electrostatic environment of the binding site, which in turn can provide guidance in optimization of further 2- and 3-substituted tropane analogues. These reports demonstrate the usefulness of molecular modeling in the determination of binding site topology and generation of high-affinity drugs. While other structurally distinct dopamine uptake inhibitors may access binding sites that are unique,[15−17] molecular models of these compounds at the dopamine transporter have been limited to one report.[18] Thus, the need for further investigation using this technique is warranted and will undoubtedly provide additional information on the structure and function of the dopamine transporter.

One class of dopamine transporter inhibitors that our group has been investigating is based on 3α-(diphenylmethoxy)-1α*H*,5α*H*-tropane (benztropine; **3**).[19−21] Benztropine binds equipotently to cocaine at the dopamine transporter but demonstrates lower affinity than cocaine for the other monoamine transporters.[20,21] The benztropines are intriguing since they contain a tropane ring as seen in cocaine and a diphenylmethoxy moiety found in the arylpiperazine class of dopamine uptake inhibitors (e.g. GBR 12909; **4**).[21] We have previously reported on several series of phenyl ring-substituted analogues of benztropine,[22−24] of which the most potent compound was 3α-[bis(4′-fluorophenyl)methoxy]tropane (**5**). Compound **5** demonstrated significantly higher



Benztropine, **3** R=H
5 R=F



GBR 12909, **4**

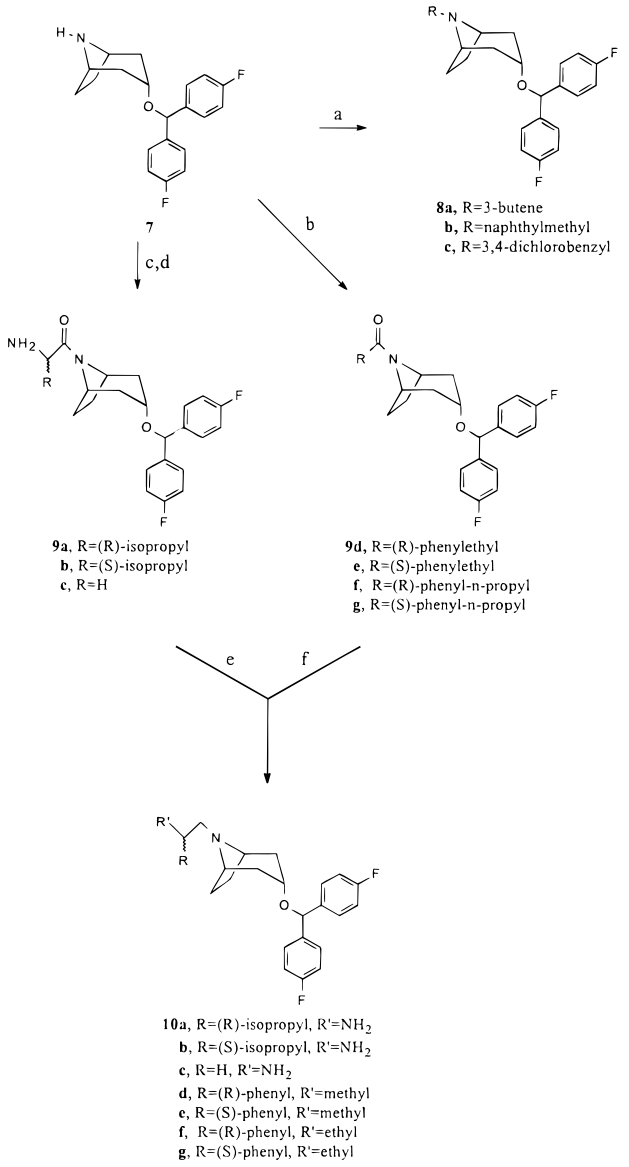
affinity for the dopamine transporter than benztropine (**3**) but retained high affinity for muscarinic receptors (dopamine transporter, $K_i = 11.8$ nM; m_1, $K_i = 11.6$ nM). Interestingly, several of these 3α-(diphenylmethoxy)-tropane analogues bound with high affinity to the dopamine transporter and potently blocked dopamine uptake but did not produce stimulation of locomotor activity or cocaine-like discriminative stimulus effects.[25,26] Structure−activity relationships derived from this series of compounds revealed a significant divergence from cocaine and related analogues at the dopamine transporter.[27] Recently a photoaffinity ligand, [^{125}I]-*N*-[*n*-butyl-4-(4‴-azido-3‴-iodophenyl)]-4′,4″-difluoro-3α-(diphenylmethoxy)tropane, has been shown to label a binding site on the dopamine transporter that is distinct from that which is labeled by the cocaine-based photoaffinity label, RTI 82 ([^{125}I]3β-(*p*-chlorophenyl)-tropane-2β-carboxylic acid 4′-azido-3′-iodophenylethyl ester).[17,28] Taken together, these studies suggest that this class of dopamine uptake inhibitors may be accessing a binding site distinct from that of cocaine. We have hypothesized that the discrepant behavioral profile of this class of dopamine uptake inhibitors may, in part, be due to interactions at a distinctive binding domain on the dopamine transporter.

In our initial studies on the 3α-(diphenylmethoxy)-tropanes we found that while certain aryl ring substitutions improved binding affinity at the dopamine transporter, high selectivity for the dopamine transporter over muscarinic m_1 receptors was not achieved.[22−24] As a result, it was possible that the antimuscarinic activity of this class of compounds might be masking stimulation of locomotor activity or cocaine-like subjective effects. Although, behavioral studies using classical muscarinic antagonists, such as atropine or scopolamine, did not support this notion, development of compounds without the muscarinic component was desirable.[26]

In an attempt to eliminate the muscarinic binding component for this class of dopamine uptake inhibitors, a series of N-substituted 3α-[bis(4′-fluorophenyl)methoxy]-tropane analogues were designed.[29] In this study, a separation of binding affinities for the dopamine transporter versus muscarinic m_1 receptors was achieved, with the most potent and selective compound being *N*-(4″-phenyl-*n*-butyl)-3α-[bis(4′-fluorophenyl)methoxy]-tropane (dopamine transporter, $K_i = 8.51$ nM; m_1, $K_i = 576$ nM).[29]

Since the 3α-[bis(4′-fluorophenyl)methoxy]tropanes do not have a chiral center, enantioselectivity in this series could not be determined. Profound enantioselectivity has previously been demonstrated in the 2-carbomethoxy-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane series of dopamine transporter ligands.[30] Thus, our reasoning for synthesizing chiral N-substituted analogues was that higher affinity and selectivity may be achieved in this structurally similar group of compounds by incorporating a chiral center in the N-substituent. Furthermore, the synthesis of the present series of chiral compounds, using commercially available chiral amino acids, provides the advantage of far simpler and higher yield chemistry than previously reported chiral analogues.[30] To this end, chiral N-substituted analogues were designed to determine whether enantioselective binding could be achieved and thus to potentially improve selectivity of these compounds at the dopamine transporter. Herein, the synthesis and evaluation for binding at the dopamine transporter and muscarinic m_1 receptors of several novel chiral N-substituted analogues of 3α-[bis(4′-fluorophenyl)methoxy]tropane are described. In addition, we also report the derivation of a highly predictive CoMFA model based on the expanded series of N-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues at the dopamine transporter.

Scheme 1[a]



[a] (a) RBr, K_2CO_3, DMF; (b) RCOOH, DCC, HOBt, Et_3N, DMF; (c) RCOCl, $CHCl_3$, H_2O, $NaHCO_3$; (d) 4-(aminomethyl)piperidine, $CHCl_3$; (e) $LiAlH_4$, THF; (f) AlH_3, THF.

Chemistry

The compounds that were used in the present study are listed in Table 1. The synthesis of the novel analogues **8a**−**c** and **10a**−**g** is depicted in Scheme 1, and Table 2 lists their chemical yields and physical properties.

Achiral analogues **8a**−**c** were prepared directly by alkylation of **7**[31] using the appropriate alkyl halide in DMF with K_2CO_3.[29] Amino acid analogues **9a**−**c** were prepared by modification of the method by Carpino et al.[32] The appropriate *N*-(9-fluorenylmethoxycarbonyl) amino acid was converted to its acid chloride by refluxing in thionyl chloride/dichloromethane. The resulting amino acid chlorides were coupled with **7** in chloroform and aqueous sodium bicarbonate. Deprotection with 4-(aminomethyl)piperidine gave the free amines **9a**−**c**. Chiral analogues **9d**−**g** were prepared using an amidation method, previously described,[29] with 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole hydrate (HOBt). The corresponding amides **9a**−**g** were reduced with AlH_3 or $LiAlH_4$ (LAH) to give compounds **10a**−**g.**

Table 1. N-Substituted 3α-[Bis(4′-fluorophenyl)methoxy]-tropanes Binding to the Dopamine Transporter (DAT) and to Muscarinic (m_1) Receptors

		K_i, nM (±SEM)[a]	
compd	N-substituent	DAT	m_1[e]
5	methyl	11.8 ± 1.3[b]	11.6 ± 0.9
6	4″-phenyl-*n*-butyl	8.51 ± 1.2[c]	575.5 ± 11
7	hydrogen	11.2 ± 1.2[c]	203 ± 17
8a	3″-butene	31.9 ± 2.8	278 ± 19
8b	naphthylmethyl	367 ± 37	1790 ± 49
8c	3‴,4‴-dichlorobenzyl	392 ± 35	3880 ± 370
10a	(*R*)-2″-amino-3″-methyl-*n*-butyl	56.4 ± 9.6	2180 ± 86
10b	(*S*)-2″-amino-3″-methyl-*n*-butyl	29.5 ± 3.5	4020 ± 590
10c	2″-aminoethyl	13.9 ± 1.7	1250 ± 140
10d	(*R*)-2″-phenyl-*n*-propyl	477 ± 53	392 ± 15
10e	(*S*)-2″-phenyl-*n*-propyl	301 ± 27	1900 ± 48
10f	(*R*)-2″-phenyl-*n*-butyl	400 ± 52	1440 ± 180
10g	(*S*)-2″-phenyl-*n*-butyl	228 ± 21	2360 ± 300
10h	4″-(4‴-nitrophenyl)-*n*-butyl	20.2 ± 2.2[c]	299 ± 20
11	4″-(4‴-aminophenyl)-*n*-butyl	29.7 ± 3.6[d]	134 ± 16
12a	*n*-butyl	24.6 ± 1.9[c]	399 ± 28
12b	allyl	29.9 ± 2.9[c]	177 ± 21
12c	cyclopropylmethyl	32.4 ± 2.9[c]	257 ± 29
12d	3″-phenyl-*n*-propyl	41.9 ± 4.6[c]	312 ± 11
12e	indol-3″-ylethyl	44.6 ± 4.9[c]	3280 ± 220
12f	2″-{[(4‴-nitrophenyl)phenyl]-methoxy}ethyl	57.0 ± 9.7[c]	460 ± 51
12g	3″-(4‴-fluorophenyl)-*n*-propyl	60.7 ± 7.3[c]	686 ± 86
12h	benzyl	82.2 ± 12[c]	1030 ± 150
12i	cinnamyl	86.4 ± 10[c]	401 ± 22
12j	4‴-fluorobenzyl	95.6 ± 9.6[c]	1540 ± 120

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. [b] Data from ref 23. [c] Data from ref 29. [d] Data from ref 28. [e] Muscarinic data analyzed with the computer program PRISM and differs from affinities previously reported by Agoston et al.[29] in which the program LIGAND was used to analyze the data.

Molecular Modeling: CoMFA

The aim of the present study was to extend the SARs of the N-substituted 3α-[bis(4′-fluorophenyl)methoxy]-tropane analogues and to derive valid CoMFA models for this series at the dopamine transporter and muscarinic m_1 receptor. Table 1 lists the K_i values for the compounds that were used in the CoMFA study.

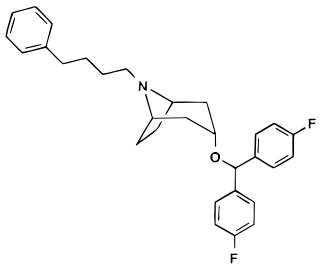
Molecular modeling studies were performed using the Sybyl (version 6.5)[33] and MOPAC (version 6.0)[34] software packages installed on a Silicon Graphics Octane workstation running IRIX 6.5. CoMFA was used for the development of the 3D-QSAR model. The dopamine transporter inhibitors considered in this work were divided into two data sets: the 'training set' (Table 1 minus **10f**, **12d**, and **12g**), on which the CoMFA analysis was actually performed, and the 'test set' (**10f**, **12d**, and **12g**) used only for validating the predictive ability of the 3D-QSAR model. Due to the relatively small number of chiral analogues in the training set, only one chiral analogue (**10f**) was chosen to be in the test set. Compounds **12d** and **12g** were chosen to be in the test set to see how well the model could predict activity for small changes in ligand structure.

The entire set of N-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues was drawn using the SKETCH option in Sybyl 6.5 starting from the X-ray coordinates of **6**. Multiple conformations were drawn for N-substituents that could adopt more than one orienta-

Table 2. Physical Properties of Compounds **8a**−**c** and **10a**−**g**[a]

compd	recryst solv	mp, °C	formula[b]	OR: $[\alpha]^{23}_D$ (*c*, MeOH)	yield,[d] %
8a	ether/IPA	181	$C_{24}H_{27}F_2NO\cdot HCl$	NA[c]	52
8b	ether/MeOH	>220	$C_{31}H_{30}F_2NO\cdot HBr$	NA	20
8c	MeOH	>220	$C_{27}H_{25}Cl_2F_2NO\cdot HBr$	NA	50
10a	ether/MeOH	182−185	$C_{25}H_{32}F_2N_2O\cdot 2HBr$	8.15 (1.08)	25
10b	ether/MeOH	180−183	$C_{25}H_{32}F_2N_2O\cdot 2HBr$	−9.61 (1.04)	40
10c	ether/MeOH	>230	$C_{22}H_{26}F_2N2O\cdot 2HBr$	NA	34
10d	amorphous solid		$C_{31}H_{31}F_2NO\cdot$fumarate$\cdot H_2O$	17.18 (1.03)	73
10e	amorphous solid		$C_{31}H_{31}F_2NO\cdot$fumarate$\cdot H_2O$	−17.29 (0.96)	66
10f	EtOAc	119−121	$C_{30}H_{33}F_2NO\cdot HCl\cdot 1/4H_2O$	16.05 (1.19)	41
10g	EtOAc	119−121	$C_{30}H_{33}F_2NO\cdot HCl\cdot 1/4H_2O$	−15.91 (1.30)	49

[a] General procedures for the synthesis of **8a**−**c** and **10a**−**g** are given in the Experimental Section. [b] All compounds were analyzed for C,H,N and the results agreed to ±0.4% with theoretical values. [c] NA, not applicable. [d] from compound **7**.



6

tion. This resulted in a number of compounds having more than one possible conformer. The conformer which gave the best q^2 (cross-validated r^2) value (highest) after energy minimization, charge assignment, and alignment was chosen for the final model. Geometries were optimized and partial charges assigned using the AM1 model Hamiltonian[35] as implemented in the MOPAC program using PRECISE convergence criteria. It is recognized that **6** may not adopt the X-ray coordinates when bound to the dopamine transporter; however, the use of a reasonable low-energy conformation as a template is a practical starting point for statistical comparisons of flexible structures within the Sybyl CoMFA model.[36] All compounds in the CoMFA study were aligned via root-mean-square fitting of the non-hydrogen atoms of the tropane ring (Figure 1).

A table was constructed of all the aligned compounds. Logarithms of 1/inhibition of [^{3}H]WIN 35,428 binding (K_i) was added as the biological observable to obtain a more evenly distributed dependent variable. The computed log *P* (cLogP) values were also determined and added to the table.

The CoMFA columns, which are descriptors of the shape of the noncovalent fields surrounding the tested molecules, were added. These analyses were performed using steric (Lennard−Jones) and electrostatic (Coulombic) fields either in equal combination or individually. Energy cutoff values of 30 kcal/mol were selected for both steric and electrostatic fields. The CoMFA region was created automatically and extended 4 Å beyond every aligned molecule in the *x*-, *y*-, and *z*-directions. The steric and electrostatic energy fields were individually calculated by a sp^3 carbon probe atom with a charge of +1 located at the intersection of grid points spaced at 2 Å within the CoMFA region.

To obtain a 3D-QSAR, partial least squares (PLS) was used to correlate selected columns within the table. The *leave-one-out* procedure was initially selected for cross-validation of the PLS statistical method to determine the optimal number of components and the predictive

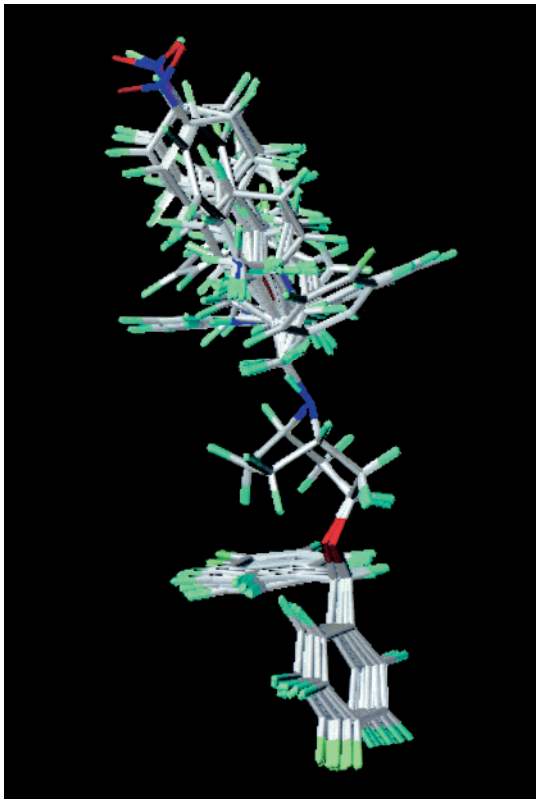


Figure 1. Alignment of the non-hydrogen atoms in the tropane ring of all 25 compounds within the CoMFA study.

value of the model. During this procedure, each compound in the 'training set' was systematically removed and its activity predicted by a model derived from the remaining compounds. Column filtering was set to 2 kcal/mol and the number of components to 10. The number of components in the cross-validated run, which corresponded to the highest q^2 value that did not increase by at least 5% from the addition of further components, was chosen as optimal. The final PLS model was derived using the optimal number of components with no validation.

Results and Discussion

All compounds were tested for their displacement of [^{3}H]WIN 35,428 from the dopamine transporter in rat caudate putamen. Muscarinic m_1 binding was evaluated by displacement of [^{3}H]pirenzipine from rat brain homogenate. The results are shown in Table 1.

The structure−activity relationships for compounds **5**−**7**, **10h**, and **12a**−**j** have been previously discussed.[28,29] To summarize, a variety of N-substitutions providing

tertiary amines, including alkyl (e.g. methyl **5**, *n*-butyl **12a**, allyl **12b**, and cyclopropylmethyl **12c**) and arylalkyl (phenylalkyl **6**, **10h**, **11**, **12d**,**g**,**h**,**j,** indol-3-ylethyl **12e**, and cinnamyl **12i**), were well-tolerated at the dopamine transporter. In general, for the phenylalkyl substituents, it was determined that increasing the length of the alkyl tether between the tropane nitrogen and the phenyl ring increased binding affinity. None of the previously reported compounds bound with high affinity to the other monoamine transporters. However, by substituting other moieties for the methyl group of **5**, muscarinic affinity was significantly decreased while generally retaining the other binding characteristics. The most potent and selective compound in this series was the *N*-(4-phenyl-*n*-butyl) analogue **6** (dopamine transporter, K_i = 8.51 nM; m_1, K_i = 576 nM)**.**

A priority in the present study was to determine the effect of chiral N-substitution of 3α-[bis(4′-fluorophenyl)-methoxy]tropane analogues on binding to the dopamine transporter and muscarinic m_1 receptors. We found that the valine-derived N-substituent, **10a** and **10b**, was well-tolerated at the dopamine transporter. The combination of structural features of **12a** and **12h** into chiral analogues **10d**−**g** resulted in loss of binding affinity at the dopamine transporter. From this limited set of chiral N-substituted analogues, it appears that the *S*-configuration is preferred slightly over the *R*-configuration. For example, analogues **10b**, **10e**, and **10g** are approximately 2-fold higher in potency at the dopamine transporter than each of their *R*-configuration stereoisomers. Moreover, since the opposite enantioselectivity is apparent at muscarinic m_1 receptors, a further separation in the binding affinities for the dopamine transporter versus muscarinic m_1 receptors was achieved. Selectivity for the dopamine transporter was increased from 68-fold in our previously most selective compound **6** to 136-fold in compound **10b** (dopamine transporter, K_i = 29.5 nM; m_1, K_i = 4020 nM) while still retaining good potency at the dopamine transporter.

The butenyl N-substituent **8a** supported the previously reported SARs with a K_i = 31.9 nM. However, aromatic substituted compounds **8b** and **8c** showed a substantial decrease in affinity (K_i = 367 and 392 nM, respectively). Amine-containing N-substituents **10a**−**c** bound with moderate to high affinity to the dopamine transporter. Comparison of these analogues with their non-amine-containing counterparts or with other N-substituted analogues of similar size and shape indicates that steric factors may be playing a greater role than electronic factors in binding of these compounds to the dopamine transporter. These findings were substantiated further using the 3D-QSAR method of analysis.

Analogues from our previous report[29] that had an N-substituent which rendered the nitrogen of the tropane ring nonbasic were not included in the CoMFA study because of their pernicious effects on the model. These ligands did not bind appreciably to the dopamine transporter (K_i > 2000 nM),[29] and this result was deemed likely due to the lack of a basic nitrogen, although this finding is in contrast to recent reports by Kozikowski,[37] Madras,[38] and Meltzer[39] which convey that a neutral tropane nitrogen or non-nitrogen bridge does not have a detrimental effect on dopamine transporter affinity in the cocaine and WIN series of tropane-based compounds. When the nitrogen in the tropane ring of a series of 2-carbomethoxy-3-(diarylmethoxy)-tropane (difluoropine) analogues was replaced with oxygen, a significant decrease in binding affinities resulted.[39] These findings support our results and further substantiate a separation between SARs in these two tropane-based classes of dopamine transporter ligands.

Table 3. Summary of CoMFA Results

optimal no. of components	4
q^2	0.746
standard error of prediction	0.297
r^2	0.983
standard error of estimate	0.077
F values	247.59
probability of $R^2 = 0$ ($n_1 = 4$, $n_2 = 17$)	0
contributions	
cLogP	0.236
steric	0.764

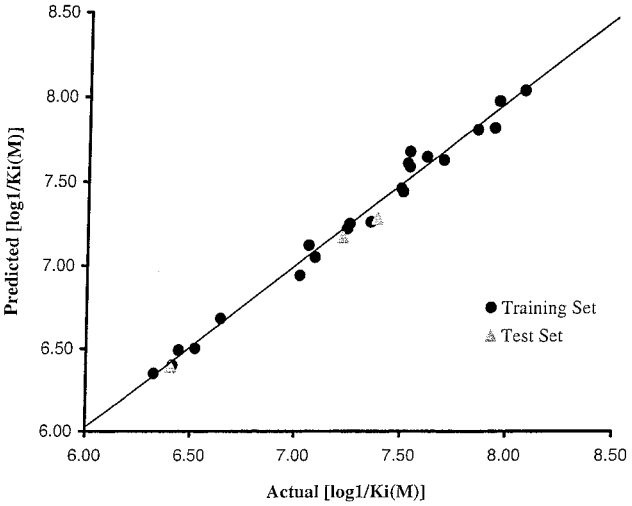


Figure 2. Plot of predicted versus measured values from non-cross-validated CoMFA model.

Several predictive models were derived using the training set mentioned earlier. The most predictive model resulted when only steric fields were considered in the CoMFA. Addition of the cLogP values to the PLS analysis resulted in an increased q^2 value in every model. The summary of the CoMFA results is shown in Table 3. The optimal number of components was determined to be 4 by the selection criteria described above. The q^2 obtained was 0.746 with a standard error of prediction of 0.297. The PLS run with no cross-validation yielded an R^2 of 0.983 with a standard error of estimate of 0.077. The relative contribution for sterics is 0.764 versus 0.236 for cLogP. From this model, the activity of each compound was predicted. These were compared to the actual values (experimentally determined) in Figure 2. Compounds **10f**, **12d**, and **12g** (test set) were used to evaluate the predictive power of this model. Table 4 shows the actual, predicted, and residual values for the training set and the test set. This model showed exceptional predictive ability with a residual standard deviation in the test set of 0.05 log unit across a range of 1.75 log units. Chiral analogue **10f** was very well-predicted with a residual value of 0.01. The ability of the model to distinguish between small structural alterations of the N-substituent is illustrated by the

Table 4. CoMFA Actual, Predicted, and Residual Activities[a]

compd	actual	predicted	residual
	Training Set		
5	7.93	7.82	0.11
6	8.07	8.04	0.03
7	7.95	7.98	−0.03
8a	7.50	7.44	0.06
8b	6.44	6.49	−0.05
8c	6.41	6.40	0.01
10a	7.25	7.25	0.00
10b	7.53	7.59	−0.06
10c	7.85	7.81	0.04
10d	6.32	6.35	−0.03
10e	6.52	6.50	0.02
10g	6.64	6.68	−0.04
10h	7.69	7.63	0.06
11	7.53	7.68	−0.15
12a	7.61	7.65	−0.04
12b	7.52	7.61	−0.09
12c	7.49	7.46	0.03
12e	7.35	7.26	0.09
12f	7.24	7.22	0.02
12h	7.09	7.05	0.04
12i	7.06	7.12	−0.06
12j	7.02	6.94	0.08
std dev			0.06
	Test Set		
10f	6.40	6.39	0.01
12f	7.38	7.28	0.10
12g	7.22	7.17	0.05
std dev			

[a] Activities are expressed as log[1/K_i(M)].

phenylpropyl analogue **12d** and the *p*-fluorophenylpropyl analogue **12g** with residual values of 0.10 and 0.05, respectively.

A graphical representation of the CoMFA model can be seen in Figure 3. The CoMFA contours corresponding to steric fields are shown together with compound **6** for visualization purposes. The contour map suggests that moving steric bulk away from the basic nitrogen of the tropane (green areas) ring is advantageous for dopamine transporter binding. Interestingly, steric bulk is disfavored (yellow areas) surrounding the 2-position of the N-substituent. This is evident from the chiral analogues **10d**−**g** which have a phenyl group protruding from the 2-position.

Attempts to derive a valid 3D-QSAR model for muscarinic m_1 binding were unsuccessful. There was no apparent correlation of structure with activity in this series of compounds. While the reasons for this are unclear at this time, it may be that the alignment of our test set is incorrect for the muscarinic m_1 receptor binding site. Further examination will be required to determine the pharmacophore at the muscarinic m_1 receptor, in this class of compounds, before proper alignment may be accomplished. Also, our attempts at eliminating affinity at the muscarinic m_1 receptor may have biased our set of compounds and resulted in a nonpredictive model. Nonetheless, we have achieved our goal of further improving selectivity for binding at the dopamine transporter versus muscarinic receptors and have determined that certain chiral N-substitutions in this series of compounds provide separation.

Conclusion

Several new chiral N-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane ligands have been synthesized and tested for dopamine transporter and muscarinic m_1 receptor binding. The binding selectivity for the dopamine transporter over the muscarinic m_1 receptor was improved with the chiral analogue **10b** showing 136-fold selectivity.

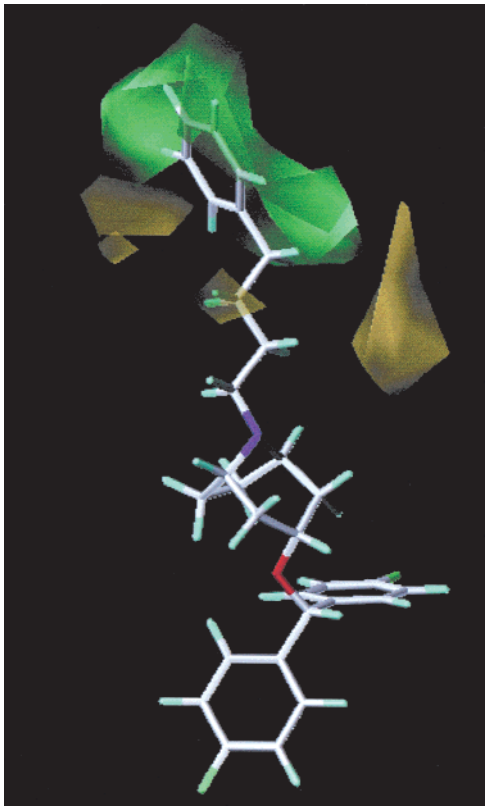


Figure 3. CoMFA steric STDEV*COEFF contour plots from the analysis model derived with no cross-validation. Compound **6** is shown inside the field. Green contours (level 75%) surround regions where steric bulk favors binding. Yellow contours (level 25%) surround regions were steric bulk disfavors binding.

A CoMFA model has been derived which accounts for the 3D-QSAR at the dopamine transporter. Good correlations were achieved between steric fields with cLogP and binding affinities. Previous CoMFA studies on tropane-based ligands also suggest that the steric component is the predominant factor in the binding affinities of these analogues with electostatics playing a smaller role.[10−14] A comparison of these 3D-QSAR models with our CoMFA models further substantiates the hypothesis that the benztropine class of compounds may be interacting with a different binding domain at the dopamine transporter as compared to cocaine and its analogues.[17] Furthermore, the information obtained from the present model, coupled with our previous CoMFA model[18] on phenyl ring-substituted benztropines, will provide leads toward mapping the topology of the dopamine transporter. Moreover, these novel molecular probes, and the structural information they provide, will undoubtedly contribute direction toward the development of a pharmacotherapeutic for cocaine abuse.

Experimental Section

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker (Billerica, Mass) AC-300 instrument. Samples were dissolved in an appropriate deu-

terated solvent ($CDCl_3$ or CD_3OD). 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. Infrared spectra were recorded as a neat film on NaCl plates with a Perkin-Elmer 1600 series FTIR. Optical rotations were obtained at the sodium D line on a Jasco DIP-370 digital polarimeter (100-mm cell). Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and agree within ±0.4% of calculated values. TLC solvent used was $CHCl_3$/MeOH/NH_4-OH, 90:10:1, unless otherwise indicated. All chemicals and reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis, Inc. unless otherwise indicated and used without further purification.

Synthesis of N-Substituted 3α-[Bis(4′-fluorophenyl)-methoxy]tropanes 8a−c. General Method. Compound **7**[29] (727 mg, 2.5 mmol) was converted to its free base form by extracting with $CHCl_3$ (3 × 10 mL) from 20% NH_4OH (20 mL), drying, evaporating and then dissolving it in 20 mL dry DMF. Anhydrous K_2CO_3 (376 mg, 2.72 mmol) and the appropriate alkyl bromide (2.7 mmol) were added, and the reaction mixture was allowed to stir at room temperature for 5 h. Inorganics were removed by suction filtration, the filter pad was washed with ether and the filtrate was poured into a separatory funnel. Extraction from H_2O (10 mL) with ether (3 × 10 mL) was followed by washing the combined organic portions with H_2O (1 × 10 mL) and drying (Na_2SO_4). Evaporation of the volatiles gave product as a clear oil. The crude free base was dissolved in a minimal volume of methanol and acidified to pH 2 with a saturated solution of methanolic HBr or HCl. Evaporation of the solvent and recrystallization gave pure product as the HBr or HCl salt (20−52% yield).

Synthesis of N-Substituted 3α-[Bis(4′-fluorophenyl)-methoxy]tropanes 10a−c. General Method. The appropriate ((9-fluorenylmethyl)oxy)carbonyl (Fmoc) amino acid chloride was prepared according to Carpino et al.[32] Compound **7**[29] (350 mg, 1.1 mmol) was converted to its free base form by extracting with $CHCl_3$ (3 × 10 mL) from 20% NH_4OH (20 mL), drying, evaporating and then dissolving it in 10 mL amylene stabilized chloroform and 10 mL of 10% sodium bicarbonate. To this biphasic mixture was added the appropriate Fmoc-protected amino acid chloride (1 mmol) dissolved in 10 mL of amylene-stabilized chloroform. After 10 min of vigorous stirring, the layers were separated and 0.5 mL of *N*-methylpiperazine was added, followed by immediate washing with 5% HCl. To the organic layer was added 5 mL of 4-(aminomethyl)-piperidine and after 40 min the organic phase was washed with brine (2 × 20 mL), phosphate buffer (0.5 M, pH = 5.5) (4 × 20 mL), and brine (1 × 20 mL), dried (Na_2SO_4), filtered, and solvent removed in vacuo. The crude amide was purified by flash chromatography using 95:5:0.5 chloroform:methanol: ammonium hydroxide (CMA). The amide was dissolved in 10 mL of anhydrous THF and added to a 30 mL suspension of LAH (143 mg, 3.76 mmol) in THF at 0 °C. The reaction was stirred at 0 °C for 5 min and then allowed to warm to room temperature for 2 h. The reaction was quenched by slow addition of a 1:1 mixture of THF and H_2O at 0 °C. The gelatinous product was dissolved in 60 mL of ether and 4 mL of 15% NaOH. After 20 min of stirring at room temperature, the mixture was filtered over a pad of sodium sulfate. The organic filtrate was evaporated to give the crude product as an oil which was purified by flash chromatography using 95: 5:0.5 CMA. The oil was dissolved in a minimal volume of methanol and acidified to pH 2 with a saturated solution of methanolic HBr. Evaporation of the solvent and recrystallization gave pure product as the HBr salt (25−40% yield).

Synthesis of N-Substituted 3α-[Bis(4′-fluorophenyl)-methoxy]tropanes 10d−g. General Method. Compound **7**[29] (663 mg, 2.0 mmol) and triethylamine (1.2 mL, 8.6 mmol) were added to a mixture of the appropriate chiral acid (2.2 mmol), dicyclohexylcarbodiimide (DCC; 453 mg, 2.2 mmol), and 1-hydroxybenzotriazole hydrate (HOBt; 297 mg, 2.2 mmol) in 50 mL dry DMF. The reaction mixture was allowed to stir for 1 h at 0 °C under an atmosphere of argon. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 48 h. After completion of the reaction (assessed by TLC), 15 mL of H_2O was added. The reaction mixture was basified by adding a few drops of concentrated NH_4OH to pH 9. The organic products were extracted with ether (3 × 30 mL) and the combined ether fractions were washed with H_2O (2 × 25 mL), dried (Na_2SO_4), filtered, and evaporated to an orange oil. Alane (AlH_3) was made by careful addition of 1.0 g 98% sulfuric acid in 2 mL anhydrous THF to 0.76 g (20 mmol) of $LiAlH_4$ in 40 mL anhydrous THF at 0 °C under an atmosphere of argon. After 15 min of stirring at room temperature, a solution of the intermediate amide in 10 mL of anhydrous THF was added dropwise to the reaction mixture under argon. After 2 h of stirring at room temperature, the reaction mixture was hydrolyzed by slow addition of a 1:1 mixture of THF and H_2O at 0 °C. The gelatinous product was dissolved in 60 mL of ether and 4 mL of 15% NaOH. After 20 min of stirring at room temperature, the mixture was filtered over a pad of sodium sulfate. The organic filtrate was evaporated to give the crude product as an oil which was purified by flash chromatography using 3:1 hexane:ethyl acetate (1% TEA). The oil was dissolved in a minimal volume of methanol and acidified to pH 2 with a saturated solution of methanolic HCl. Alternatively, the fumarate salt was produced by dissolving free base in acetone followed by addition of an equimolar amount of fumaric acid. Evaporation of the solvent and recrystallization gave pure product as the HCl or fumarate salt (41−73% yield).

Representative spectral data of 8c: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79−2.03 (m, 6H), 2.09−2.14 (m, 2H), 3.08 (br s, 2H), 3.56 (app t, J = 3.45, 2.3 Hz, 1H), 5.36 (s, 1H), 6.99 (app t, J = 8.61, 8.69 Hz, 4H), 7.17−7.37 (m, 6H), 7.47 (d, J = 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 36.4, 55.6, 58.2, 69.5, 79.2, 115.0, 115.2, 127.6, 128.2, 128.3, 129.9, 130.1, 132.0, 138.5, 140.6, 160.2, 163.5; IR (neat film NaCl plate) 1501 (ROR), 1602 (aromatic), 2800−3000 (aliphatic stretch), 3000−3200 (aromatic stretch) cm^{-1}.

Representative spectral data of 10a: $[\alpha]^{25}_D$ +8.15, CH_3-OH, c = 1.08; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (d, J = 6.8 Hz, 6H), 1.4−1.8 (m, 8H), 2.05−2.08 (m, 2H), 2.4−2.6 (m, 2H), 3.1 (d, J = 1.8 Hz, 2H), 3.5 (s, 1H), 5.36 (s, 1H), 6.96−7.02 (m, 4H), 7.24−7.29 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.99, 19.18, 25.4, 26.8, 31.7, 36.2, 36.5, 54.8, 57.1, 57.7, 60.4, 69.4, 79.2, 114.9, 115.2, 128.2, 128.3, 137.9, 160.3, 163.5.

Representative spectral data of 10f: $[\alpha]^{25}_D$ +16.05, CH_3-OH, c = 1.19; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75 (t, J = 7.35 Hz, 3H), 1.43−1.56 (m, 1H), 1.68−2.05 (m, 8H), 2.54 (br s, 3H), 3.08 (br s, 1H), 3.49 (s, 1H), 5.32 (s, 1H), 6.94−7.00 (app t, J = 8.35, 8.65 Hz, 4H), 7.16−7.30 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.6, 24.9, 25.8, 27.8, 34.6, 58.4, 59.0, 68.2, 79.6, 115.1, 115.3, 126.6, 127.7, 128.1, 128.2, 128.5, 138.0, 143.0, 160.3, 163.6.

Single-Crystal X-ray Analysis of 6. A clear rectangular 0.40 × 0.20 × 0.04 mm crystal recrystallized from acetone, $C_{30}H_{34}OF_2N^{+\cdot}$ Br, FW = 542.49, was selected for data collection. Data were collected on a computer-controlled diffractometer with an incident beam graphite monochromator (Siemens P4 with Cu Kα radiation, λ = 1.54178 Å, T = 295 K). A least-squares refinement using 30 centered reflections within 14° < 2θ < 56° gave the triclinic $P\bar{1}$ cell, a = 6.539(1), b = 7.170-(1), c = 28.181(4) Å, with V = 1311.5(3) Å^3, Z = 2, and d_{calc} = 1.374 gm/cm^3. A total of 4826 reflections were measured in the $\theta/2\theta$ mode to $2\theta_{max}$ = 115°, of which there were 4673 independent reflections. Corrections were applied for Lorentz and polarization effects. A face indexed numerical absorption correction was applied, μ = 2.439 mm^1, and maximum and minimum transmissions were 0.90 and 0.40, respectively. The structure was solved by direct methods with the aid of the program SHELXTL97.[40] The full-matrix least-squares refinement on F_o^2 varied 443 parameters including the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, CH distances set to

0.96 Å, H angles idealized, U_{iso}(H) were set to 1.2U_{eq}(C) or, if methyl, 1.5U_{eq}(C)]. The final R values for the 4072 observed reflections with $F_o > 4\sigma F_o$ were $R_1 = 0.0728$, and $wR_2 = 0.22$ for all data.[41] The final difference Fourier excursions were 0.64 and −0.52 eÅ^3.

The molecule was refined as a $P\bar{1}$ but cannot have a center due to molecular approaches of the fluorophenyl groups across the inversion center. The disordered fluorophenyl groups are at equal occupancy and can occupy the cell with a Z of 2 with different parts of the disorder across the false center with normal approaches. A refinement as a $P1$ is also disordered for both molecules and is of poor geometry. The false center is likely due to racemic twinning and stacking faults.

Pharmacology. 1. Dopamine Transporter Binding Assay. Male Sprague−Dawley rats (200−250 g; Taconic, Germantown, NY) were decapitated and their brains removed to an ice-cooled dish for dissection of the caudate putamen. The tissue was homogenized in 30 volumes 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 D-glucose, pH adjusted to 7.4) using a Brinkman polytron 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. The total volume in each tube was 0.5 mL and the final concentration of membrane after all additions was 0.5% (w/v) corresponding to 200−300 mg of protein/sample. Triplicate samples of membrane suspension were preincubated for 5 min in the presence or absence of the compound being tested. [^{3}H]WIN 35,428 (2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate; specific activity 82.4 Ci/mmol; from New England Nuclear, Boston, MA; 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). 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, defined as binding in the presence of 100 μM cocaine. Each compound was tested with concentrations ranging from 0.01 nM to 100 μM for competition against binding of [^{3}H]WIN 35,428, in three independent experiments, each performed in triplicate.

In both saturation and competition experiments, two components of [^{3}H]WIN 35,428 binding were apparent. Analysis of the data utilizing the LIGAND program revealed a high-affinity component with a K_D of 7 ± 5 nM and a B_{max} of 445 ± 338 fmol/mg protein and a low-affinity component with a K_D of 126 ± 115 nM and a B_{max} of 1995 ± 559 fmol/mg protein.

Saturation and displacement data were analyzed by the use of the nonlinear least-squares curve-fitting computer program LIGAND.[42] Data from replicate experiments were modeled together to produce a set of parameter estimates and the associated standard errors of these estimates. In each case, the model reported fit significantly better than all others according to the F test at $p < 0.05$. The K_i values reported are the dissociation constants derived for the unlabeled ligands.

2. Muscarinic m_1 Binding Assay. Whole frozen rat brains excluding cerebellum (Taconic, Germantown, NY) were thawed in ice-cold buffer (10 mM Tris-HCl, 320 mM sucrose, pH 7.4) and homogenized with a Brinkman polytron in a volume of 10 mL/g of tissue. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The resulting supernatant was then centrifuged at 10000*g* for 20 min at 4 °C. The resulting pellet was resuspended in a volume of 5 mL/g in 10 mM Tris buffer (pH 7.4).

Assays were conducted in binding buffer (10 mM Tris-HCl, 5 mM $MgCl_2$). The total volume in each tube was 0.5 mL and the final concentration of membrane after all additions was approximately 200−300 mg of protein/sample. [^{3}H]Pirenzepine (specific activity 73.9 Ci/mmol; from New England Nuclear, Boston, MA; final concentration 3 nM) was added and the incubation was continued for 1 h at 37 °C. The incubation was terminated by the addition of 5 mL of ice-cold buffer (10 mM Tris-HCl, pH 7.4) and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.5% polyethylenimine in water to reduce nonspecific binding) using a Brandel cell harvester (Gaithersburg, MD). The filters were washed with two additional 5-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 that were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 15 000 dpm total binding per sample and approximately 900 dpm nonspecific binding, defined as binding in the presence of 10 μM QNB (quinuclidinyl benzilate). Each compound was tested with concentrations ranging from 0.01 nM to 100 μM for competition against binding of [^{3}H]pirenzepine, in at least three independent experiments, each performed in triplicate. Displacement data were analyzed by use of the nonlinear curve-fitting computer program PRISM.[43]

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