CoMFA Study of Novel Phenyl Ring-Substituted 3α-(Diphenylmethoxy)tropane Analogues at the Dopamine Transporter

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A series of phenyl ring-substituted analogues of 3α-(diphenylmethoxy)tropane (benztropine) has been prepared as novel probes for the dopamine transporter. Cross-validated comparative molecular field analysis (CoMFA) models of the binding domain on the dopamine transporter were constructed using 37 geometry-optimized structures of these compounds and their corresponding binding affinities (K_i values) for the displacement of [^{3}H]WIN 35,428 or potency of [^{3}H]dopamine uptake inhibition (IC_{50} values) in rat caudate putamen tissue. The most predictive model ($q^2 = 0.78$) correlated the steric component of CoMFA to the dependent variable of [^{3}H]WIN 35,428 binding affinities. A novel series of seven phenyl ring-substituted analogues of 3α-(diphenylmethoxy)tropane was prepared, and our best molecular model was used to accurately predict their binding affinities. This study is the first to provide a CoMFA model for this class of dopamine uptake inhibitors. This model represents an advancement in the design of novel dopamine transporter ligands, based on 3α-(diphenylmethoxy)tropane, and further substantiates structure−activity relationships that have previously been proposed for this class of compounds. This CoMFA model can now be used to predict the binding affinities of novel 3α-(diphenylmethoxy)tropane analogues at the dopamine transporter and will be useful in the design of molecular probes within this class of dopamine uptake inhibitors.

Introduction

Over the past decade, a significant research effort has been directed toward the elucidation of neurochemical mechanisms underlying the reinforcing and addictive properties of cocaine. Cocaine is known to bind with moderate and equipotent affinity to all three of the monoamine neurotransmitter transporters and subsequently inhibits the reuptake of dopamine, serotonin, and norepinephrine into their respective presynaptic neurons. However, it is the inhibition of dopamine uptake that appears to play a pivotal role in cocaine's reinforcing actions.[1,2] Although recent studies in dopamine transporter knockout mice have provided evidence that this may not be the exclusive neurochemical target for these actions,[3−5] there is significant support for the further elucidation of its role in the abuse liability of cocaine and other psychostimulants. Moreover, the development of a mechanistically based cocaine-abuse pharmacotherapeutic continues to focus on drugs that selectively target the dopamine transporter.[6−8]

The development of novel dopamine uptake inhibitors, as molecular probes for the dopamine transporter as well as potential leads for the treatment of cocaine abuse, has been the focus of numerous laboratories, i.e., for review see refs 6 and 7. Although classical medicinal chemistry has primarily been implemented to design these compounds, both quantitative structure−activity relationship (QSAR) and other computer-aided drug design approaches have been developed by several laboratories as a complement. Specifically, the use of comparative molecular field analysis (CoMFA) has been described for the cocaine series of compounds by Carroll[9−11] and by MacKerell[12,13] and their colleagues. These studies primarily focused on the 3β-position of the tropane ring of cocaine and identified structural features with optimal electrostatic and steric factors as determinants of high-affinity binding at the dopamine transporter. These resulting cross-validated models have provided a high predictive capacity to assess relative steric and electrostatic contributions of substitution at the 3-position on the tropane ring. These models, in turn, allowed the inference of the binding site environment in terms of these factors and subsequently provided guidance in the design of optimally active 3-substituted cocaine analogues. These reports demonstrate the value of 3D-QSAR techniques such as CoMFA in the design of third-generation high-affinity ligands at the dopamine transporter. To date, however, the molecular models of the dopamine transporter have been based on cocaine and its analogues[9−15] providing limited descriptor sampling for ligands that bind to this protein. Nevertheless, other structural classes of dopamine uptake inhibitors may access binding domains on the dopamine transporter that are distinctive from cocaine.[16,17] Thus, molecular models derived from structural classes of dopamine transporter ligands, other than cocaine and its analogues, will undoubtedly provide further insight into structure−activity relationships and binding domain structure and function and will also provide new leads toward the development of highly selective and potent ligands for this molecular target.

We have been investigating a series of dopamine uptake inhibitors based on the tropane-based drug 3α-

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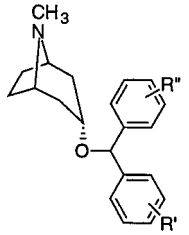
(diphenylmethoxy)tropane (benztropine). In the course of these studies, we have undertaken chemical modification of both the 3-position[18−20] and the tropane nitrogen.[21] Structure−activity relationships derived from these series of compounds demonstrated a significant divergence from the cocaine series of compounds, i.e., for review see ref 22. In addition, several studies describing the 2-carbomethoxy-substituted-3α-(diphenylmethoxy)tropanes also suggest a divergence in structure−activity relationships between these two classes of tropane-based dopamine uptake inhibitors.[23,24] These differences, coupled with a distinctive behavioral profile in animal models of cocaine abuse, have suggested that this class of dopamine uptake inhibitors may be accessing a different binding domain at the dopamine transporter as compared to cocaine.[25,26]

To identify the location and structure of the binding domain on the dopamine transporter to which this class of dopamine uptake inhibitors binds, several approaches may be taken. First, the development of structure−activity relationships provides insight into the physical and electronic environment in which the drug molecules may interact. Further, molecular tools such as irreversible ligands and radiolabeled ligands are extremely useful in identifying specific peptide residues that are covalently attached to the drug molecule and allow subsequent characterization of those sites. Since the development of these types of compounds requires the incorporation of electrophiles (i.e. NCS, N_3) or radionuclides (i.e. ^{125}I) into the pharmacophore, steric and electronic requirements for optimal binding must be determined so as to precisely position these chemical moieties to achieve both high-affinity binding and, in the case of irreversible ligands, covalent attachment. To optimize the probability of designing these molecular probes, we have initiated molecular modeling studies using the active analogue approach.[27−29] In the present study, we focused our attention on the 3-position diphenyl ether of the benztropine series of molecules to (1) provide direction in design of optimal structures for high-affinity binding at the dopamine transporter and (2) compare these models to those described for this position in the cocaine series of compounds.

We have previously described a large series of aryl ring-substituted 3α-(diphenylmethoxy)tropane analogues and described structure−activity relationships at this position.[18−20] Furthermore, we have discovered that replacing the diphenyl ether moiety with a number of aryl and arylalkyl substituents uniformly resulted in significant reduction in binding affinity at the dopamine transporter.[22] Therefore, the focus of the present study was to develop a molecular model in the 3α-diphenyl ether series that could be used to accurately predict the binding affinities of novel compounds that may prove useful for future studies of the dopamine transporter. Our current study utilized the molecular fields from the geometry-optimized structures of a series of 37 3α-(diphenylmethoxy)tropane analogues for correlation to either inhibition of [^{3}H]WIN 35,428 binding to dopamine transporters in rat caudate putamen or inhibition of [^{3}H]-dopamine reuptake in the same tissue. The results of these studies were envisioned to provide new leads toward the development of benztropine-based dopamine uptake inhibitors. In addition, a comparison of the analyses of the binding pocket could be made that has been described by others to be accessed by the cocaine series of compounds.[9−13] To this end, we describe herein the derivation of a valid CoMFA model based on a series of phenyl-ring substituted 3α-(diphenylmethoxy)tropane analogues. Subsequently, a series of seven novel compounds were synthesized and their binding affinities at the dopamine transporter were predicted. Experimental data were obtained for both binding affinity and dopamine uptake potency for these compounds and compared to the K_i values derived from the best predictive CoMFA model.

Table 1. CoMFA Training Set Compounds



compd	R′	R″	[^{3}H]WIN 35,428 binding K_i, nM (% error)[a]	[^{3}H]DAUI IC_{50}, nM[a]	compd	R′	R″	[^{3}H]WIN 35,428 binding K_i, nM (% error)[a]	[^{3}H]DAUI IC_{50}, nM[a]
8	4-F	4-F	11.8 (11)	71.0	**23**	4-OH	H	297 (13)	677
9	4-Cl	4-Cl	20.0 (14)	75.0	**24**	H	H	118 (9)	403
10	4-Br	4-Br	91.6 (13)	34.0	**25**	3-Cl	H	22.0 (7)	228
11	4-Me	4-Me	420 (7)	2540	**26**	3-CF_3	H	187 (5)	457
12	4-OMe	4-OMe	2000 (7)	2880	**27**	3,4-diCl	4-F	18.9 (14)	24.0
13	4-F	H	32.2 (10)	48.0	**28**	3,4-diCl	H	21.1 (19)	47.0
14	4-Cl	H	30.0 (12)	115	**29**	2-NH_2	H	1840 (8)	373
15	4-Br	H	37.9 (7)	29.0	**30**	2-F	H	50.0 (12)	140
16	4-Me	H	187 (5)	512	**31**	2-Me	H	309 (6)	1200
17	4-OMe	H	78.4 (8)	468	**32**	4-Br	4-F	15.2 (19)	27.2
18	4-CN	H	196 (9)	222	**33**	2-Cl	H	228 (9)	977
19	4-NO_2	H	197 (8)	219	**34**	3-F	3-F	47.4 (11)	407
20	4-Et	H	520 (8)	984	**35**	3-F	H	68.5 (12)	250
21	4-*t*-Bu	H	1920 (7)	4460	**36**	3,4-diF	H	27.9 (11)	181
22	4-CF_3	H	635 (10)	2160	**37**	3-F	4-F	23.3 (8)	139

[a] Each value represents data from at least three independent experiments, each performed in triplicate (data from refs 18−20 and 26).

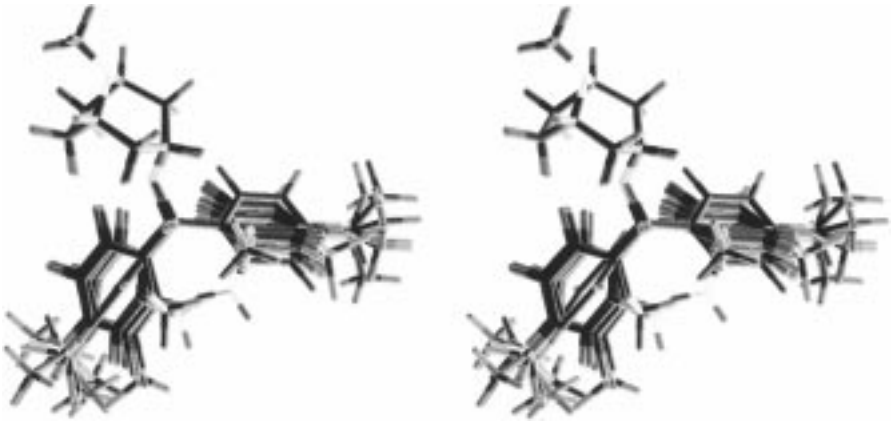


Figure 1. Stereoview of CoMFA training set. Compounds were aligned based on non-hydrogen atoms in the tropane ring.

Table 2. CoMFA Analysis Results

analysis no.	dependent data type (descriptors)	CoMFA fields (cutoff values)	probe atom (grid size)	cross-validated predictive q^2 values (no. components)	PRESS values
1	[^{3}H]WIN binding	electronic/steric (30/30)	C (sp^3) (2 Å)	0.37 (3)	0.57
2	[^{3}H]WIN binding (cLogP)	electronic/steric (30/30)	C (sp^3) (2 Å)	0.65 (4)	0.43
3	[^{3}H]WIN binding	steric (30)	C (sp^3) (1 Å)	0.67 (3)	0.40
4	[^{3}H]WIN binding (cLogP)	steric (30)	C (sp^3) (1 Å)	0.78 (4)	0.34
5	[^{3}H]DAUI	electronic/steric (30/30)	C (sp^3) (2 Å)	0.25 (2)	0.56
6	[^{3}H]DAUI (cLogP)	electronic/steric (30/30)	C (sp^3) (2 Å)	0.37 (4)	0.52
7	[^{3}H]DAUI	steric (30)	C (sp^3) (1 Å)	0.59 (5)	0.43
8	[^{3}H]DAUI (cLogP)	steric (30)	C (sp^3) (1 Å)	0.61 (6)	0.42

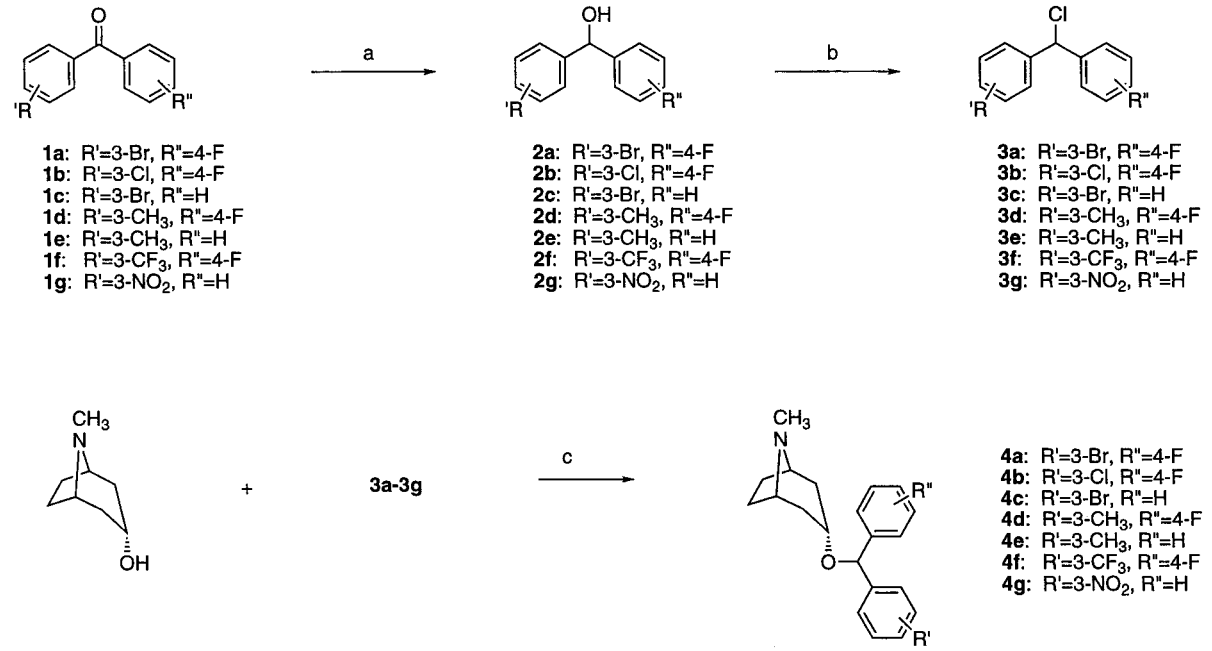
Molecular Modeling − CoMFA

Molecular modeling studies were performed using the SYBYL software package (Tripos version 6.3, R4000) installed on a Silicon Graphics IRIS Indigo XZ workstation running IRIX 5.3. Use of the algorithm CoMFA allowed the development of a 3D-QSAR model that describes the structure−activity relationships for a series of analogues.[27] Once derived, a valid CoMFA model was utilized to design more active compounds by predicting the pharmacological activities of proposed analogues. Additionally, the results from these models can be used to predict the characteristics of ligand−receptor interactions.

The structures for members of our data set were derived from the known X-ray crystal structure of 4′-chloro-3α-(diphenylmethoxy)tropane and molecular fragments and standard bond lengths and angles from the SYBYL structural library.[19] Optimized geometries and partial charges of the original X-ray structure as well as all derived structures were obtained using the AM1[30] model Hamiltonian as implemented in the MOPAC program (version 6.0) using the PRECISE convergence criteria. The compounds that were used as a training set in the CoMFA analyses are listed in Table 1. For the CoMFA studies all compounds were aligned by fitting together the non-hydrogen atoms of the tropane ring (Figure 1). As some of the compounds in the training set have a chiral benzylic center resulting from asymmetric aryl substitution, two obvious choices for alignment resulted. In addition, two regioisomers existed for each enantiomer in the 2- and 3-substituted analogues. We tested all four possible sets of alignments and determined that using equally weighted enantiomers with the substituent oriented in the space between the aryl rings gave the best model. Logarithms of 1/inhibition of [^{3}H]WIN 35,428 binding (K_i) and 1/[^{3}H]-dopamine uptake inhibition (IC_{50}) were used as the dependent variables to more evenly distribute the data. Partial least-squares (PLS) analyses were performed using steric (Lennard−Jones, cutoff range 5−30 kcal) and electrostatic (Coulombic, 30 kcal cutoff) fields either in combination or individually. Additionally, molar refractivity (CMR) and partition coefficients (cLogP) were calculated using CMR and CLOGP modules within SYBYL, respectively. Since the use of several descriptors with even moderately sized training sets may lead to artificially significant correlation coefficients, correlations were determined with standard CoMFA weighting and only one additional descriptor.

The results of the CoMFA analyses can be seen in Table 2. The most predictive model, as indicated by the highest cross-validated (leave-one-out method used) correlation ($q^2 = 0.78$), column filtering < 0.5 kcal/mol, resulted from the PLS analysis of the compounds in the training set with respect to inhibition of [^{3}H]WIN 35,-428 binding. In the final model, only the standard (30 kcal/mol cutoff) steric fields derived from the use of an sp_3-hybridized probe atom (the use of a H probe was

Scheme 1[a]



[a] (a) $NaBH_4$; (b) $SOCl_2$; (c) 160 °C.

also analyzed and found to give no improvement to the model) in a matrix of grid points spaced at 1 Å and cLogP descriptors were used as the independent variables. The seven test compounds were derived from the same AM1 geometry-optimized X-ray crystal structure as was used for the training set compounds. The optimized geometries and partial charges for these compounds were also obtained using an AM1 Hamiltonian, and the non-hydrogen atoms of their respective tropane rings were fit with the same atoms of the compounds in the training set prior to predicting binding affinities.

Chemistry

The synthesis of compounds **4a**−**4g** is depicted in Scheme 1, and they were prepared as previously described for the first series of *ortho*- and *meta*-substituted 3α-(diphenylmethoxy)tropanes.[20] In brief, commercially available substituted benzophenones **1a**−**1g** were reduced to their corresponding benzhydrols **2a**−**2g** using $NaBH_4$ in absolute ethanol or methanol. Conversion to the benzhydryl chlorides **3a**−**3g** was achieved in refluxing thionyl chloride. The excess thionyl chloride was removed in vacuo, and the benzhydryl chloride was added neat or in a minimal volume of anhydrous ether to an equimolar portion of tropane, at 160 °C. The reaction mixture was allowed to stir for 20−60 min, at this temperature. The hydrochloride salts were isolated via an ion-pairing extraction technique as previously described[19] and recrystallized to give 25−65% yield of the desired products **4a**−**4g**. Selected physical properties of these compounds are depicted in Table 3.

Pharmacology

The compounds were evaluated for displacement of [^{3}H]WIN 35,428 binding to the dopamine transporter and for inhibition of [^{3}H]dopamine uptake in rat caudate putamen tissue, as described previously.[19,20] All of the compounds monophasically displaced [^{3}H]WIN 35,428 binding with affinities of 23.4−187 nM (K_i). Inhibition of [^{3}H]dopamine uptake was determined for each of the compounds (IC_{50} = 104−795 nM). Since the most predictive model was based on the binding affinities, these data were then compared to the predicted values.

Table 3. Physical Properties of Compounds **4a**−**4g**

compd	recryst solvent	mp, °C	MS (M^+)	formula	% yield
4a	EtOAc/acetone	152−154	405	$C_{21}H_{24}NOBr_2F$	65
4b	EtOAc	155−157	359	$C_{21}H_{24}NOClF$	40
4c	EtOAc	175−177	387	$C_{21}H_{25}NOBrCl$	45
4d	EtOAc	136−138	339	$C_{22}H_{27}NOFCl$	46
4e	acetone	134−136	321	$C_{22}H_{28}NOCl$	61
4f	EtOAc	165−167	393	$C_{22}H_{24}NOClF_4$	35
4g	EtOAc	129−131	352	$C_{21}H_{25}N_2O_3Cl$	25

Results and Discussion

Molecular modeling studies, using CoMFA, were undertaken to aid in the design of novel dopamine transporter ligands as well as to provide insight into the steric and electronic nature of the binding pocket in which this series of 3α-(diphenylmethoxy)tropane analogues interacts. The CoMFA training set in which the compounds were energy-minimized and superimposed based on the non-hydrogen atoms on the tropane ring can be seen in Figure 1. Several predictive models were derived using a series of 37 phenyl ring-substituted 3α-(diphenylmethoxy)tropane analogues and either [^{3}H]-WIN 35,428 binding (K_i values) or [^{3}H]dopamine uptake inhibition (IC_{50} values) constants as the dependent variables (Table 2). CoMFA fields were generated that included either electronic and steric components or steric components alone. A 1 or 2 Å sp^3 carbon grid size was evaluated for each model. This optimal model included the additional descriptor cLogP, a calculated measure of the partition coefficient. The inclusion of additional descriptors in QSAR model development is a common tactic, since expanded sampling of descriptor space is more likely to yield a predictive model. With

Table 4. Predicted versus Actual Results of Radiolabeled Binding and Uptake Experiments

		inhibition of [^{3}H]WIN 35,428 binding K_i, nM		[^{3}H]DA uptake inhibition[a]
compd	substitution	exptl[a] (% error)	pred[b]	IC_{50} ± SEM, nM
4a	3′-Br, 4″-F	38.2 (14)	31.4	179 ± 51
4b	3′-Cl, 4″-F	23.4 (18)	25.3	123 ± 23
4c	3′-Br	27.0 (12)	47.9	104 ± 27
4d	3′-CH_3, 4″-F	30.9 (9)	39.8	108 ± 23
4e	3′-CH_3	78.0 (7)	61.0	226 ± 70
4f	3′-CF_3, 4″-F	88.1 (10)	63.7	182 ± 36
4g	3′-NO_2	187 (10)	64.7	795 ± 199

[a] Each experimental value represents data from at least three independent experiments, each performed in triplicate. [b] The most predictive model (analysis 4 in Table 3), without cross-validation, was used to predict these K_i values.

the additional descriptor, the corresponding QSAR equation becomes:

$$\text{activity}_n = \text{constant}_n + A_n(\text{steric}_{xyz}) + B_n(\text{electrostatic}_{xyz}) + \ldots + A'_n(\text{steric}_{xyz}) + B'_n(\text{steric}_{xyz}) + \ldots + C(\text{cLogP})$$

in which the activity of compound *n* is proportional to the summation of the three-dimensional steric and electrostatic terms and the cLogP value for compound *n*. In our own case, the inclusion of cLogP as an independent variable led to an improvement in our model from 0.67 to 0.78 (cf. Table 2). While steric and electrostatic considerations are likely strong determinants of the calculated partition coefficient, the improvement of our model by inclusion of this additional term indicates that it encodes structural information not exhaustively sampled by the steric and electrostatic terms calculated by CoMFA. The most predictive model in this study provided a q^2 value of 0.78 (analysis 4 in Table 2) and demonstrated that the steric component of this series is a significant determinant of binding affinity at the dopamine transporter. The best CoMFA model was then used to predict the binding affinities of a new series of phenyl ring-substituted 3α-(diphenylmethoxy)tropane analogues. Compounds **4a**−**4g** were prepared and evaluated for their binding affinity at the dopamine transporter, and these experimental values were then compared to the values predicted by the best model. As can be seen in Table 4, the final PLS analysis (non-cross-validated) closely predicted the K_i values for the test set compounds **4a**−**4f**.

The one exception in this series was the 3′-NO_2-substituted analogue whose predictive value was lower than its actual value (K_i = 64.7 nM vs 187 nM). Based on our observations[19,22] and these data, the disparity of the predicted versus the actual activity for this analogue may be due to the fact that the NO_2 substituent has significant electron-withdrawing character that may contribute negatively to its binding at the dopamine transporter. Since the most predictive model chosen considered only the steric component, electronic contribution of the NO_2 group is not considered, and this may account for the less accurate prediction of the K_i value for compound **4g**. Nevertheless, this study demonstrates that the CoMFA model described herein will be useful in predicting the binding affinities of phenyl ring-substituted 3α-(diphenylmethoxy)tropane analogues and confirmed the structure−activity relationship derived previously[18−20] which showed that small halogens (F, Cl) in the *meta* or *para* positions of one or both phenyl rings are well-tolerated at the dopamine transporter. Compounds that have sterically bulkier groups or strongly electronic groups in these positions bind with considerably lower affinity. Furthermore, compounds with substituents in the *ortho* positions likewise have lower binding affinities at the dopamine transporter, possibly due to unfavorable steric or electronic interactions with the protein. In addition, the effect these substituents may have on the torsional angle in which the phenyl rings reside may also provide a less than optimal interaction at the binding site. On the basis of these results, we have chosen to retain the optimal substituents of small halogens in the *para* and/or *meta* positions in our subsequent structure−activity studies in which the N-CH_3 group has been replaced with various aryl and alkylaryl substituents.[21] Further, it was deemed unwise to synthesize potential irreversible ligands with either the radiolabel or the electrophile at the 3-position diphenyl ether since both steric bulk and electronic properties of these substituents were predicted to contribute negatively to binding affinity at the dopamine transporter. Subsequently, we have developed a photoaffinity label that binds irreversibly and radiolabels dopamine transporters, with these substituents attached to the *N*-butylphenyl moiety.[31] Interestingly, peptide mapping studies using this photoaffinity label along with proteolytic and immunological precipitation techniques have provided further evidence to support the concept that distinctive binding domains are accessed by the 3α-(diphenylmethoxy)tropanes versus the tropane-based cocaine analogues.[32]

Currently we are employing the same methods described herein to derive molecular models for optimal N-substituents[33] and modeling the binding pocket that accommodates these substituents. Our earlier studies demonstrated that binding selectivity for the dopamine transporter versus the m_1 muscarinic receptor could not be achieved by modification in the 3-position of the benztropine molecule; substituents that improved binding affinity at the dopamine transporter generally retained high binding affinity at muscarinic receptors. Conversely, substituents that decreased binding affinity at muscarinic receptors had essentially the same effect on dopamine transporter binding. However, in the N-substituted 4′,4″-difluoro-substituted series of 3α-(diphenylmethoxy)tropane analogues, a separation in the binding affinities for the dopamine transporter and muscarinic receptors was achieved.[21] Therefore we envision that the further development of predictive models for the dopamine transporter, making use of this new series of N-substituted compounds, will enhance our capability of designing more selective ligands for the dopamine transporter. Further, the determination of whether a predictive model can be derived for these compounds at the m_1 muscarinic receptor will also prove useful in the design of third-generation molecules with improved selectivity and potency at the dopamine transporter.

Finally, previously reported CoMFA studies on the 3-position of the tropane ring of cocaine also suggest that the steric component is a predominant factor in the

binding affinities of these analogues with electrostatics playing a smaller yet significant role.[9−13] QSAR in the cocaine series of compounds reflects opposite optimal stereochemistry at the 3-position as well as a different rank order of substituents that give optimal binding at the dopamine transporter as compared to the benztropine analogues. A comparison of these QSAR investigations with our CoMFA model, along with classical structure−activity studies, further substantiates the hypothesis that the cocaine and benztropine classes of compounds interact with different binding domains. Therefore the present study demonstrates that a CoMFA model can be derived for the 3α-(diphenylmethoxy)-tropane class of dopamine uptake inhibitors and can be useful in designing novel ligands with which to further elucidate the role of the dopamine transporter in the reinforcing effects and abuse liability of psychomotor stimulants such as cocaine. Furthermore, the binding site model derived from these studies confirms differences in binding domains that are likely interacting with these tropane-based classes of molecules and underscores the possibility of developing dopamine uptake inhibitors that are structurally and pharmacologically distinctive from cocaine and hence may have therapeutic utility in the treatment of its abuse.

Experimental Methods

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker (Billerica, MA) AC-300 spectrometer. Samples were dissolved in the indicated deuterated solvents, and chemical shifts are reported as parts per million (δ) relative to tetramethylsilane (Me_4Si, 0.00 ppm) as internal standard. Chemical shifts for ^{13}C spectra were reported as relative to deuterated chloroform ($CDCl_3$, 77.0 ppm). Mass spectra were obtained using a Hewlett-Packard (Palo Alto, CA) 5971A mass selective ion detector (MSD) in the electron-impact mode. Samples were introduced into the MSD via an HP-5890 series II gas chromatograph (GC) fitted with an HP-1 (cross-linked methyl silicone gum, 25 m × 0.2 mm i.d., 50 μm film thickness) column. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 °C, respectively. The initial GC oven temperature was 100 °C, held for 3.0 min, increased to 295 °C at a rate of 15 °C/min, and held at this final temperature for 10 min. Infrared spectra were recorded with a Perkin-Elmer 1600 series FTIR spectrophotometer with KBr disks. Elemental analysis performed by Atlantic Microlab, Inc. (Norcross, GA) agreed to within 0.4% of the calculated values. The TLC solvent used was $CHCl_3/CH_3OH/NH_4OH$ (89:10:1) unless otherwise indicated. All chemicals and reagents were purchased from Lancaster Synthesis, Inc. or Aldrich Chemical Co.

General Synthetic Method for Substituted 3α-(Diphenylmethoxy)tropanes (4a−4g). Commercially available benzophenones **1a−1g** (10 mmol) were dissolved in absolute ethanol or methanol (75−100 mL) and $NaBH_4$ (10 mmol) was added. Reactions were typically allowed to proceed for 1−2 h after which time the excess alcohol was removed in vacuo. The dry white residue was taken up in H_2O (50 mL) and ether (50 mL), and after the two phases were mixed and separated, the aqueous phase was further extracted with ether (2 × 25 mL). The ether fractions were combined, washed with H_2O (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Reaction products were obtained in yields of 93−98%. Resultant benzhydrols **2a−2g** (5−10 mmol) were dissolved in $SOCl_2$ (10 mL) in an atmosphere of argon. The reaction was allowed to proceed overnight at ambient temperature. Excess $SOCl_2$ was removed in vacuo (addition of 2 small portions of dry toluene and removal in vacuo ensured removal of all $SOCl_2$). Resultant oils were determined spectroscopically to be the desired benzhydryl chlorides **3a−3g**. These benzhydryl chlorides were individually added to a tropine (5−10 mmol) melt (160 °C) over 5 min. The reactions were allowed to proceed for 10−45 min resulting in brown oils which solidified upon cooling. The crude products were dissolved in $CHCl_3$ (30 mL) and transferred to separatory funnels. Ion pairing of the desired products was carried out via extraction with 2.8N HCl (30 mL) as previously described.[19] The phases were mixed and separated and the aqueous phase was further extracted with $CHCl_3$ (2 × 25 mL). The organic fractions were combined, washed with 2.8 N HCl (2 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo resulting in light brown foams which were recrystallized to give the pure products **4a−4g** as HCl salts (25−65% yield).

Pharmacology. 1. Dopamine Transporter Binding Assay. A detailed description of the binding methods can be found in ref 34. Briefly, 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 of ice-cold modified Krebs-HEPES buffer (15 mM HEPES, 127 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 10 mM 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 μg 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; 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.

2. [^{3}H]Dopamine Uptake Assay. Rats were sacrificed by decapitation and their brains removed to an ice-cooled dish for dissection of the caudate putamen. [^{3}H]Dopamine uptake was measured in a chopped tissue preparation as described previously.[35] Briefly, the tissue was chopped into 225 μm slices on a McIllwain tissue slicer with two successive cuts at an angle of 90°. The strips of tissue were suspended in oxygenated modified Krebs-HEPES buffer (see above), which was pregassed with 95% O_2/5% CO_2 and warmed to 37 °C. After rinsing, aliquots of tissue slice suspensions were incubated in buffer in glass test tubes at 37 °C to which either the drug being tested or no drug was added, as appropriate. After a 5 min incubation period in the presence of drug, [^{3}H]dopamine (final concentration 15 nM, specific activity 50 Ci/mmol; Amersham Corp., Arlington Heights, IL) was added to each tube. After 5 min the incubation was terminated by the addition of 2 mL of ice-cold buffer to each tube and filtration under reduced pressure over glass fiber filters (presoaked in 0.1% poly(ethylenimine) in water). The filters were rinsed and

placed in scintillation vials to which 1 mL of methanol and 2 mL of 0.2 M HCl were added to extract the accumulated [^{3}H]-dopamine. Radioactivity was determined by liquid scintillation spectrometry at an efficiency of approximately 30%. The reported values represent specific uptake from which nonspecific binding to filters was subtracted.

3. Analysis of Data. Saturation and displacement data were analyzed by the use of the nonlinear least-squares curve-fitting computer program LIGAND.[36] 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. Uptake data were analyzed using standard analysis of variance and linear regression techniques.[37] IC_{50} values were calculated using the linear portion of the concentration−response curve (linear regression $p < 0.05$).

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