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(−)-3β-Substituted Ecgonine Methyl Esters as Inhibitors for Cocaine Binding and Dopamine Uptake

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Ten 3β-ecgonine analogues were synthesized and characterized by ^{1}H and ^{13}C NMR, MS, and elemental analysis. The compounds were synthesized as (−)-stereoisomers from (−)-cocaine. These compounds were assessed for their ability to inhibit [^{3}H]cocaine binding to rat striatal tissue and to inhibit [^{3}H]DA uptake into rat striatal synaptosomes. In this series of compounds, the length of the spacer between the aryl group and the tropane skeleton ranged from 1 to 4 bond distances, and conformational flexibility of the linkage and orientation of the aryl ring system were controlled by various types of linkages. The most potent of the analogues was methyl-(1*R*-2-*exo*-3-*exo*)-8-methyl-3-(β-styrenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate. One of the less potent compounds was found to inhibit [^{3}H]cocaine binding and [^{3}H]DA uptake with significantly different IC_{50} values, in contrast to 14 other 3β-substituted analogues. Molecular modeling and CoMFA analysis were used to obtain a rigorous structure−function relationship for the studied compounds. The results showed that the potencies of these 3β-substituted ecgonine methyl esters were dominated by steric effects and were acutely sensitive to the distance between the aryl ring and the tropane skeleton and to the orientation of the aryl ring system relative to the tropane skeleton. The current study provides a clearer picture of the shape and size of the putative hydrophobic binding pocket for the 3β substituent at the cocaine receptor as well as emphasizing the importance of a drug's free energy of solvation in obtaining structure−activity relationships.

Introduction

For many years, the dopamine transporter has been a target of numerous mood-altering drugs.[1,2] Among them, cocaine is one of the most powerful reinforcers and a popular drug of abuse. Currently, the addictive properties of cocaine are believed to result from its blockage of dopamine (DA) uptake in nerve endings and activation of reward mechanisms.[3−16] The cocaine receptors are identified by binding of [^{3}H]cocaine, cocaine analogues, or other [^{3}H]DA transporter inhibitors (e.g. [^{3}H]mazindol)[17−19] and by inhibition of transport of [^{3}H]DA into synaptosomes or cell culture.[20−22] However, mutagenesis[23,24] and pharmacokinetic studies[25−29] indicate that dopamine and cocaine (and cocaine analogues) do not share consensus recognition sites on the DA transporter. This discrepancy highlights the possibility of designing cocaine antagonists which suppress cocaine action without affecting dopamine transport.

Efforts to isolate and characterize the cocaine receptor by normal biochemical methods and modern molecular biological techniques have been limited to a single report.[30] Structure−function studies of the cocaine receptor, therefore, have focused on structure−activity relationship (SAR) investigations. During the past few years, substantial structural information has been obtained by SAR work. In particular, reports of Carroll and co-workers have shown that the receptor can accommodate a rather bulky group at the 2-position of the tropane ring without affecting the binding potencies of the analogues.[31−33] Kozikowski and co-workers recently investigated the necessity of a hydrogen bond between the 2-position ester functional group of cocaine and the receptor. Their results indicated that hydrogen bonding between analogues and receptor was not essential.[34−36] This was evidenced by the fact that replacement of the 2β-carboxyl ester function group with a vinyl or ethyl group did not lead to significant loss of binding affinity.[37,38] A number of modifications at the nitrogen have been conducted. Reith et al.[39] and Madras et al.[40] reported that either removal of the *N*-methyl group from cocaine and WIN series compounds or replacement with larger groups produced analogues which retained relatively strong potencies. Stoelwinder et al.[41] revealed a drastic loss of potency when the electron density in the vicinity of the nitrogen atom was decreased. More recently, on the basis of the observation that some N-sulfonylated analogues maintain potency in inhibiting binding and uptake, Kozikowski et al.[30] suggested that binding to the DA transporter may not require protonation of the nitrogen atom in the analogue.

One other important area of cocaine SAR studies is modification at the 3β-position. In 1973, Clarke and coworkers reported two highly potent compounds, WIN 32 065-2 and WIN 35 428, in which a phenyl ring was directly attached to the tropane skeleton.[42] This work has been extended in the past five years not only by varying substituents[33,43,44] but also by efforts to rationalize the structure−activity relationship. Several of the para-substituted analogues reported by Carroll et al. were up to 10-fold more potent than the correspond-

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ing mono meta-substituted analogues. By increasing the distance of the phenyl ring from the tropane skeleton via a carbamoyl linkage, Kline and co-workers[45,46] were able to obtain another group of potent 3β-substituted cocaine analogues. These compounds had affinities close to cocaine with a few exceptions. The binding affinity of [^{3}H]cocaine and the inhibition of [^{3}H]-DA uptake were well correlated,[46] suggesting that those carbamate analogues bind to the dopamine transporter at the same sites as cocaine. Davies et al.[47] in 1993 reported several novel 2,3-substituted cocaine analogues in which replacement of the 2β-carbomethoxyl ester group with ketones had only minor effects on binding affinity. However, replacement of the phenyl ring at the 3β-position with an alkyl group was detrimental to activity, whereas a 3-(2-naphthyl) group results in a significant enhancement of activity.[47] These compounds demonstrate the importance of the large aryl ring for high affinity. Furthermore, the fact that 3-(2-naphthyl) was 50-fold more potent than the 3-(1-naphthyl) analogue indicated that the orientation of the aryl group in the analogues is also important for binding.[47] The importance of an aryl functional group at the 3β-position strongly indicated the presence of a hydrophobic binding site at the cocaine receptor. CoMFA studies by Carroll et al.[44−48] on 3β-(substituted phenyl)tropane-2β-carboxylic acid methyl esters showed that substitutions with increased steric bulk slightly above the 4′-position of the phenyl ring increased potency while increased steric bulk below the 4′-position decreased potency. It was also seen that there was a limit to the size of the 3β-substituent as compounds with the bulkiest para substitutions on the phenyl ring displaying the lowest activities. In addition, it was found that the electrostatic properties of the 3β-substituent may play a role in cocaine binding.[48] The importance of the 3β-substituent to cocaine binding was further emphasized by Srivastava and Crippen.[49] Three-dimensional Voronoi site modeling indicated that the 3β-substituent had the most significant effect on binding to the cocaine receptor as compared to the 2β and tropane nitrogen substituents. These studies, emphasizing the importance of the 3β-substituent in cocaine binding, made it attractive to further elucidate the steric and electrostatic properties at the 3β-position that are important for cocaine receptor binding.

Traditionally, changes in chemical structure with respect to experimental pharmacological properties are interpreted solely based on interactions with the receptor. However, the properties of compounds when they are not bound to the receptor can also have a strong influence on experimentally measured binding affinities.[50] Recent work in this laboratory has developed a model that includes the conformational flexibility, solvation free energy, and pK_a contributions of ligands to their binding affinity.[51] In this approach the alteration of chemical structure is studied with respect to changes in free energies of solvation, the pK_a of the tropane nitrogen, and the conformational flexibility along with changes in drug−receptor interactions when making structure−function interpretation of experimental results.

In our continuing efforts to investigate the SAR of cocaine, we hereby present synthesis and evaluation of a series of 3β-modified cocaine analogues which may be helpful in concatenating previous results from our laboratory with those from other research groups in describing the topology of the putative hydrophobic binding pocket at the 3β-position of the cocaine receptor.

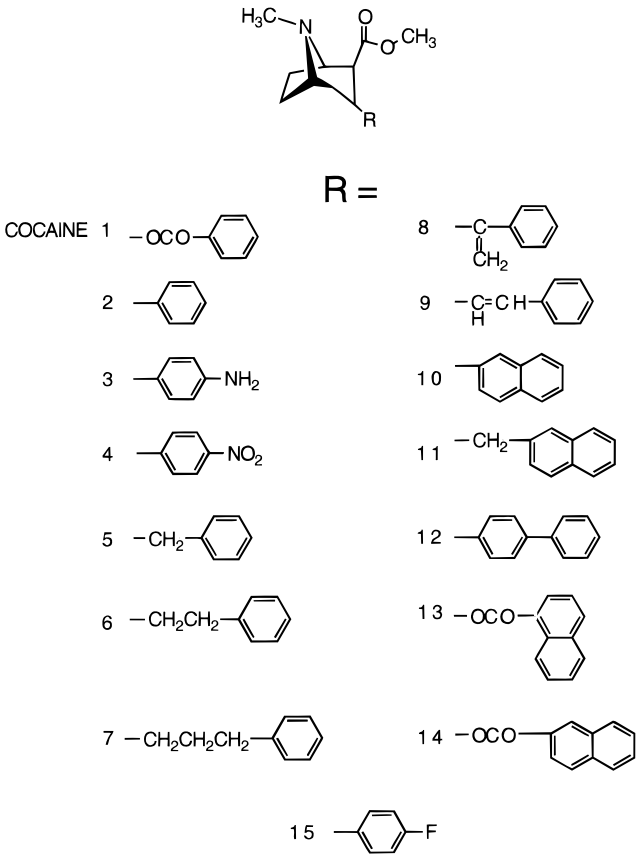
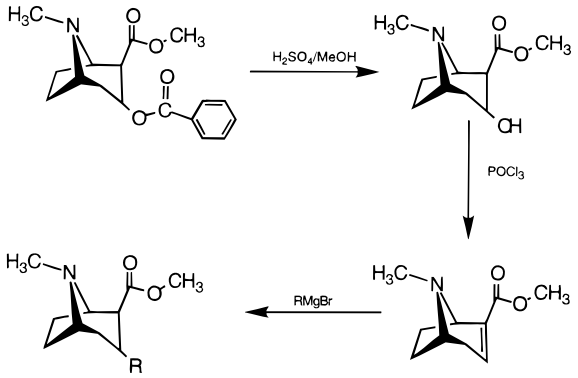


Figure 1. Cocaine and 3β-substituted cocaine analogues that make up the analysis set for the CoMFA analysis.

Scheme 1



Methods

Chemistry. The synthesis of the 3β-substituted analogues (**5**−**14** in Figure 1), which were tested for cocaine binding and DA uptake, is outlined in Scheme 1.

The different R groups can be seen in structures **5**−**14** in Figure 1. (−)-Ecgonine methyl ester and (−)-anhydroecgonine methyl ester were obtained from (−)-cocaine following the procedures of Clarke et al.[42] Compounds **5**−**12** were prepared along with arylmagnesium bromides at −40 °C. Aryl carbonyl chlorides were coupled with (−)-ecgonine methyl ester to afford the 3β-carboxy analogues **13**−**14.** Purification of product was by either preparative thin layer chromatography (TLC) or flash chromatography.

The stereochemistry for each compound was deduced by ^{1}H NMR. The critical diagnostic ^{1}H NMR parameter which differentiated the two isomers resulting from the Grignard reactions was the vicinal proton coupling constants J_{12} and J_{23}. The measured J_{12} values were 1.3−4.1 for the β-isomers and were close to zero for the α-isomers. The J_{23} values ranged from 4.6 to 8.6 for the β-isomers and 10−11 for the α-isomers. According to the Karplus relationship,[52] these coupling constants represent the dihedral angle H3−C3−C2−H2 to be between 30° and 60° for the β-isomers and between 150° and 180° for the α-isomers. Therefore, the observed vicinal proton coupling constants J_{12} and J_{23} were in good agreement with their theoretical values. Another diagnostic for the stereochemical assignment was the appearance of the *N*-methyl resonance at 2.15−2.27, about 0.2 units down field from that of the α-isomers. These observations are consistent with previous NMR studies on cocaine and WIN analogues concerning the stereochemistry of the compounds used in the present study.[43,53]

With the exception of compound **14**, all sythesized compounds were available in sufficient quantity for specific rotation to be determined. Since the synthetic protocol for **14** was the same as that for all others made, retention of stereochemical integrity is expected for **14**, although this could not be absolutely determined.

Pharmacology. Compounds **5**−**14** were tested for their ability to inhibit both [^{3}H]cocaine binding and [^{3}H]DA uptake. These two radiolabeled ligands are well-known cocaine binding site and DA uptake site probes and are commonly used to identify the cocaine receptor.[17−22] The potencies of the new 3β-substituted analogues to inhibit the binding of [^{3}H]cocaine to rat striatal P_2 membranes is a measure of the affinity of the cocaine receptor for the new drug, whereas their potencies to inhibit [^{3}H]DA uptake into striatal synaptosomes is a measure of their efficacy in blocking uptake. Both the binding and uptake assays were performed as previously described.[45,54] Briefly, rat striatal were homogenized in 0.25 M sucrose, 10 mM Na_2HPO_4, pH 7.4, using a glass Potter−Elvehjem homogenizer. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was recentrifuged at 48000*g* for 20 min. The final pellet (P_2) was suspended in 40 volumes of buffer to yield a protein concentration of approximately 1 mg/mL. Binding of [^{3}H]cocaine (specific activity 30 Ci/mmol, NEN) to the crude synaptosomal membranes and [^{3}H]dopamine (specific activity 37 Ci/mmol, NEN) uptake into the synaptosomes were measured by a filtration assay using a Skatron 96-cell harvester and thin layer scintillation counting as described.[55] The concentration of the test drugs ranged from 0.01 nM to 100 μM. These concentrations displaced specific [^{3}H]cocaine binding or [^{3}H]DA uptake from 2 to 98%. Nonspecific binding and uptake were below 5% of the total uptake. The IC_{50} values for inhibition of cocaine binding and DA uptake were determined by transformation of the competitive inhibition data to log−logit coordinates which yield better estimates of IC_{50}.

Computations. Quantum mechanical calculations were performed using Austin Model 1 (AM1) semiempirical calculations with the program AMSOL.[56−58] Molecular mechanics calculations were performed using version 6.2 of the SYBYL program and force field (Tripos Inc.). Calculations were performed on a R4000 Indigo Workstation (32 MB memory, 1 GB disk). All compounds were assumed to be in the neutral form. Energy minima for compounds with multiple rotatable bonds were determined in a multistep process. The compounds were first analyzed by the GRIDSEARCH feature in SYBYL which allows for specified dihedral angles to be rotated in set increments and fixed at a selected value. At each selected value the remainder of the compound is minimized and the energy is calculated. Compounds were minimized for 200 iterations or until the gradient norm was less than 0.001 kcal/Å with a nonbonded cutoff of 99 Å and a constant dielectric function. All rotatable bonds at both the 2β- and 3β-positions were rotated from 0 to 360° in 30° increments for all compounds except for compounds **6**, **7**, **13**, and **14** (Figure 1). These compounds all contain at least four rotatable bonds. It was thus necessary to reduce the number of conformations to be evaluated while still adequately sampling the energy surface of the compound. To do this, dihedrals were rotated in 60° increments. In addition, the phenyl ring in compounds **6** and **7** were evaluated only from 0 to 180° in 60° increments due to the structural symmetry of the ring. This CPU time-saving procedure was tested against the more expensive evaluation (0−360° in 30° increments) for compound **6**. The same five conformations were identified as the lowest energy structures.

The five lowest energy structures identified via the GRIDSEARCH analysis were then more rigorously analyzed via AM1 calculations. Each structure was initially minimized to a gradient norm of less than 1.0 kcal/Å while fixing all dihedrals to the values determined by GRIDSEARCH. These structures were then fully relaxed and minimized using AM1 until the gradient norm was less than 0.1 kcal/Å. The PRECISE keyword was used in all minimizations to allow for a more rigorous determination of the minimum energy conformer. The lowest energy structure was then chosen for future analysis, unless noted. Free energies in solvation were calculated using the AM1−SM2 model in AMSOL with the gas-phase-optimized geometry (AMSOL lSCF option). Geometry optimization was not done in the solvent model due to high computational costs (∼120 CPU hours versus ∼5 CPU minutes for the gas-phase optimization of cocaine) and previous studies showing the geometry of cocaine to be the same when optimized in both the presence of solvent or in the gas phase.[51] The free energy of solvation was obtained by taking the difference between the total energy in the presence of solvent and in the gas phase.[59]

Validation of the applied conformational searching and optimization procedure was performed by comparing results for the cocaine and WIN compounds from the GRIDSEARCH method with those from a more intensive AMSOL process in which each dihedral was rotated from 0 to 360° in 30° increments and minimized with the AM1 level of theory.[51,60] The minimum energy conformation predicted by the GRIDSEARCH procedure was in agreement with that of the more rigorous and computationally expensive AMSOL-based procedure.

CoMFA[61] was performed on 15 β-substituted cocaine analogues for which experimental data for both cocaine binding and DA uptake was available. In this study logarithms of the potency ratios (*R*) for cocaine binding and DA uptake, as reported in Table 1, were used as biological observables. Potency ratios are defined as the $IC_{50\ cocaine}/IC_{50\ compound}$.[62] On the basis of this definition, compounds with positive log potency ratios are more potent than cocaine. Conversely, compounds with negative log potency ratios are less potent than cocaine. Since compounds with large cocaine binding and low DA uptake values could be lead compounds for the development of cocaine antagonists, a cocaine binding − DA uptake difference term was included in Table 1. This allows for the easy identification of compounds with the potential to be cocaine antagonists. In addition, biological observables were obtained in which energetic contributions from solvation effects were removed from the cocaine binding and DA uptake values based on the AM1−SM2 solvation data (see Appendix, Supporting Information). These "receptor binding" values are also listed in Table 1 as receptor binding cocaine (RB_{coc}) and receptor binding dopamine uptake (RB_{DU}). Removal of solvation effects from the overall binding was performed to investigate if interactions with the receptor alone may yield a different structure−function relationship as compared to standard binding data in which both solvation effects and direct receptor interactions contribute to the experimentally measured activities. It should be noted $RB_{coc} - RB_{DU}$ terms will be identical to the cocaine binding − DA uptake values due to the same free energy of solvation being used to correct both the cocaine binding and DA uptake data. Free energies of solvation data was also used directly in combination with CoMFA to correlate the free energies of solvation with the biological activity and binding data.

Table 1. Log Potency Ratios and Free Energies of Solvation of the 3β-Substituted Cocaine Analogues Used in the CoMFA Study[a]

compd	3β-substituent	cocaine binding	DA uptake	cocaine binding − DA uptake	ΔG_{solv}	receptor binding	
						cocaine	DA
1	OCOPh	0	0	0	−4.51	0	0
2	Ph	0.53	0.66	−0.13	−3.40	−0.28	−0.15
3[b]	Ph-*p*-NH_2	−0.14	0.18	−0.32	−8.22	2.58	2.90
4[b]	Ph-*p*-NO_2	0.006	0.14	−0.134	−6.52	1.48	1.61
5	CH_2Ph	−0.94	−0.69	−0.25	−2.07	−2.73	−2.4
6	CH_2CH_2Ph	1.01	0.47	0.54	−2.08	−0.77	−1.31
7	$CH_2CH_2CH_2$Ph	−0.53	−1.11	0.58	−2.72	−1.84	−2.42
8	C(Ph)=CH_2	0.15	0.18	−0.03	−3.18	−0.83	−0.80
9[c]	CH=CHPh	1.68	1.55	0.13	−2.87	0.48	0.35
10	2-naphthyl	1.48	1.77	−0.29	−4.76	1.66	1.95
11	CH_2-2-naphthyl	−2.90	−0.81	−2.09	−3.16	−3.89	−1.80
12	1,4-biphenyl	0.99	0.85	0.14	−4.39	0.90	0.76
13	1-naphthoyloxy	−0.87	−0.76	−0.11	−5.71	0.01	0.12
14	2-naphthoyloxy	−0.51	−0.59	0.08	−5.30	0.07	−0.01
15[d]	Ph-*p*-F	0.59	0.94	−0.35	−3.04	−0.49	−0.14

[a] Potency ratios determined as presented in the Methods. [b] Cocaine binding and DA uptake data from Kline et al.[65] [c] Compound **9** was assumed to be in the trans conformation (see text). [d] Cocaine binding data obtained from Milius et al.[66] and DA uptake data obtained from Meltzer et al.[43]

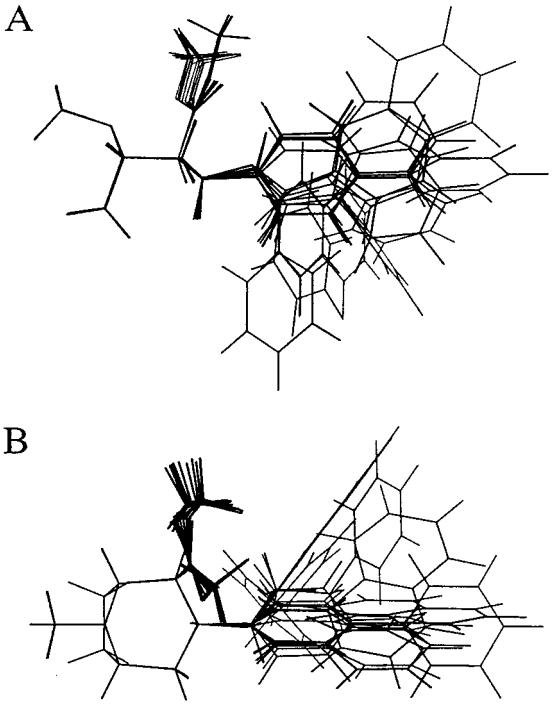


Figure 2. The 15-compound analysis set aligned with respect to the non-hydrogen atoms in the tropane ring. The top and bottom are two orthogonal views of the compounds.

The gas-phase-minimized structures and partial atomic charges from the AM1 calculations were used in the CoMFA analysis. CoMFA first involved an alignment procedure. Compounds were aligned with respect to all non-hydrogen atoms except the 3-position carbon in the tropane ring. These atoms were chosen as the template because they are conserved across all compounds in the analysis. The aligned structures are shown in Figure 2. When the alignment and CoMFA calculations were complete, standard partial least squares (PLS) analysis was performed on the structures to correlate the steric and electrostatic properties of each molecule with its biological activity and binding data. Separate CoMFA were performed for the cocaine binding and DA uptake data. The leave-one-out option was used in the PLS runs and the optimum number of components were extracted when the cross-validated r^2 (q^2) stopped increasing. Parameters used in the CoMFA analysis were varied to maximize the q^2 and minimize the standard error of prediction. This was done by systematically changing the following parameters. Use of the steric and/or electrostatic fields, energy cutoffs, the type of dielectric function used, grid size, column filtering, and probe positioning.[63] Table 2 shows the various CoMFA runs performed with the cocaine binding data for optimization of q^2 and the standard error of prediction. The changes in q^2 and the standard error of prediction can be seen as the parameters were altered. It was determined that q^2 was maximized and the standard error of prediction was minimized when the energy cutoff was 175 kcal/mol for the steric term and 50 kcal/mol for the electrostatic term, a constant ($1/r$) dielectric function was used, the probe atom position was moved by shifting the CoMFA region −0.5 Å in the *Y* direction, the column filtering was set at 8 kcal/mol, and the step size was 3 Å.

To further optimize q^2 and minimize the standard error of prediction as well as perform an additional check on the conformation of the analogues used in the CoMFA study, different conformations of the compounds in the analysis set were substituted into the CoMFA analysis. Compounds which had their activities poorly predicted by CoMFA using their global minima conformation were replaced with the second lowest energy conformation from the five structures determined by the GRIDSEARCH procedure and optimized with AMSOL. These replacements were done in a stepwise fashion as only the compound with the largest error of prediction was replaced from one CoMFA to the next. This replacement process continued until the q^2 and standard error of prediction reached an optimum value. The first replacement was done with compound **3**. Subsequent modifications to the analysis set included using different conformations of compounds **10**, **4**, **8**, and **15**. The greatest improvement of q^2 and standard error of prediction occurred when alternate conformations of compounds **8**, **10**, and **15** were used in the analysis set. The final analysis set included alternate conformations of compounds **8**, **10**, and **15** and the minimum-energy conformations of the other compounds as determined by the GRIDSEARCH procedure. The difference between the energies of the minimum energy conformation and the alternate conformation used in the CoMFA analysis for the three compounds was 0.96, 0.65, and 1.71 kcal/mol for compounds **8**, **10**, and **15**, respectively.

Results and Discussion

Table 3 shows the IC_{50} values for the compounds synthesized in the study. These IC_{50} values were used to calculate potency ratios which are reported in Table 1 along with potency ratios of previously synthesized compounds. As seen in Table 1, compounds **9** (1.68), **10** (1.48), and **6** (1.01) had the highest potency ratios for cocaine binding as compared to cocaine (0). Compound **9** was assumed to be in the trans configuration

Table 2. CoMFA Parameter Optimization to Maximize q^2 and Minimize the Standard Error of Prediction Based on the Cocaine Binding Data

fields[a]	energy cutoffs (kcal/mol)[b]	dielec funct[c]	CoMFA region[d]	min sigma (kcal/mol)[e]	step size (Å)[f]	no. of compts[g]	se[h]	q^2 [i]
both	30/30	$1/r^2$	*	*	*	1	0.790	0.160
elec	30	$1/r^2$	*	*	*	1	0.748	0.028
steric	30	$1/r^2$	*	*	*	1	0.794	0.145
			Vary Dielectric Function					
both	30/30	1/r	*	*	*	1	0.777	0.163
			Energy Cutoff					
both	50/30	1/r	*	*	*	1	0.785	0.206
both	100/30	1/r	*	*	*	1	0.762	0.245
both	175/30	1/r	*	*	*	1	0.745	0.268
both	250/30	1/r	*	*	*	1	0.745	0.244
both	175/50	1/r	*	*	*	1	0.744	0.269
both	175/100	1/r	*	*	*	1	0.744	0.269
			Minimum σ					
both	175/50	1/r	*	1.0	*	1	0.744	0.250
both	175/50	1/r	*	3.0	*	1	0.745	0.291
both	175/50	1/r	*	5.0	*	1	0.745	0.315
both	175/50	1/r	*	6.0	*	1	0.745	0.319
both	175/50	1/r	*	7.0	*	1	0.745	0.337
both	175/50	1/r	*	8.0	*	1	0.745	0.350
both	175/50	1/r	*	9.0	*	1	0.745	0.304
			Grid Step Size					
both	175/50	1/r	*	8	1	1	0.762	0.280
both	175/50	1/r	*	8	3	2	0.545	0.368
both	175/50	1/r	*	8	4	1	0.729	0.081
			Vary Probe Atom Position by Moving the CoMFA Region					
both	175/50	1/r	−0.5x	8	3	1	0.704	0.199
both	175/50	1/r	0.5x	8	3	2	0.563	0.356
both	175/50	1/r	−0.5y	8	3	2	0.507	0.452
both	175/50	1/r	0.5y	8	3	2	0.643	0.259
both	175/50	1/r	−1.0y	8	3		negative	
both	175/50	1/r	−0.5z	8	3	3	0.337	0.331
both	175/50	1/r	0.5z	8	3	2	0.585	0.029
both	175/50	1/r	−0.5xyz	8	3	1	0.766	0.200
both	175/50	1/r	0.5xyz	8	3	2	0.645	0.306

[a] Including steric and/or electrostatic fields in the CoMFA analysis. [b] Energy contributions of steric/electrostatic effects. [c] Dielectric function is either constant (1/r) or distance dependent ($1/r^2$). [d] CoMFA region was set at default placement unless otherwise specified. [e] min σ is 2.0 kcal/mol unless otherwise specified. [f] Grid size was always 2 Å unless otherwise specified. [g] Optimal number of components in the PLS analysis. [h] Standard error of prediction. [i] Cross-validated correlation coefficient.

Table 3. IC_{50} Values of the 3β-Substituted Analogues in the Inhibition of Cocaine Binding and Dopamine Uptake Determined in the Present Study

compd	3β-substituent	[^{3}H]cocaine[a]	[^{3}H]DA[b]
1	OCOPh	101 ± 26	209 ± 20
5	CH_2Ph	885 ± 18	1020 ± 52
6	CH_2CH_2Ph	9.94 ± 0.33	70.5 ± 1.0
7	$CH_2CH_2CH_2PH$	344 ± 12	2680 ± 190
8	$CH(Ph)=CH_2$	71.6 ± 0.7	138 ± 9
9[c]	CH=CHPh	2.10 ± 0.04	5.88 ± 0.09
10	2-naphthyl	3.32 ± 0.08	3.53 ± 0.09
11	methyl-2-naphthyl	81000 ± 800	1350 ± 30
12	1,4-biphenyl	10.3 ± 2.6	29.4 ± 3.8
13	1-naphthoyloxy	742 ± 48	1190 ± 90
14	2-naphthoyloxy	327 ± 63	813 ± 21

[a] [^{3}H]cocaine is in nM. [b] [^{3}H] DA is in nM. [c] Compound **9** was assumed to be in the trans configuration (see text).

for the calculations (see below). Compounds **11** (−2.90) and **5** (−0.94) had the lowest potency ratios for cocaine binding. With respect to DA uptake, compounds **10** (1.77) and **9** (1.55) had the highest potency ratios while compounds **7** (−1.11) and **11** (−0.81) had the lowest values.

Table 1 illustrates the influence of solvation effects towards binding of the 15 analogues. Calculated direct receptor binding data for binding at the cocaine receptor (RB_{coc}) and inhibition of DA uptake (RB_{DU}) were obtained based on the model presented in the Appendix (Supporting Information). As may be seen, inclusion of solvation effects leads to both increases and decreases in biological activities. Compounds such as **3** and **4** had poor cocaine binding (−0.14 and 0.006) but high RB_{coc} (2.58 and 1.48). The relatively large negative free energies of solvation of these compounds as compared to, for example, compound **2** make them more difficult to desolvate as compared to compound **2**, resulting in a lower measured cocaine binding. Once the solvation contributions are removed, these compounds are indicated to bind strongly to the receptor, as evidenced by the high RB_{coc}. Conversely, compound **6** had a high cocaine binding log potency ratio (1.01), indicating it to be more favorably bound than cocaine. Once the ΔG_{solv} contribution of −2.08 kcal/mol is taken into account, it is predicted that the direct receptor binding of compound **6** (RB_{coc} = −0.77) is much less favorable than cocaine. Similarly, compound **9** also displays much higher cocaine binding than RB_{coc}, 1.68 and 0.48, respectively, due to a less favorable ΔG_{solv} as compared to cocaine. Note that compounds **12** and **10** still maintain significant biological activity even though they have ΔG_{solv} values less than −4.0 kcal/mol.

While it should be emphasized that separation of solvation and receptor binding contributions as performed in the present study represents a simplified

Table 4. CoMFA Analysis of the 15-Compound Analysis Set

run[a]	q^2 [b]	r^2 [c]	number of components	percent contribution steric	percent contribution electrostatic	ΔG_{solv}
Steric and Electrostatic Fields						
cocaine binding	0.666	0.996	5	70	30	
dopamine uptake	0.375	0.829	2	65	35	
cocaine binding − dopamine uptake			negative			
Steric and Electrostatic Fields, and ΔG_{solv}						
cocaine binding	0.694	0.996	5	58	34	8
dopamine uptake	0.477	0.942	3	53	38	9
cocaine binding − dopamine uptake			negative			
Steric and Electrostatic Fields (Receptor Binding Data)						
cocaine binding	0.585	0.834	2	85	15	
dopamine uptake	0.498	0.882	2	77	23	

[a] Data used in the CoMFA analysis. [b] Cross-validated correlation coefficient. [c] Correlation coefficient. [d] Percent contributions of the steric, electrostatic and ΔG_{solv} terms to the CoMFA model.

model of events occurring in the binding and uptake experiments, it presents the opportunity to focus more on direct interactions between the compounds and the cocaine receptor. As stated above, the measured cocaine binding of **3** shows it to be the tenth most potent compound. Removal of solvation contributions, however, leads to **3** being the most potent compound. The magnitude of this change emphasizes that significantly different conclusions may be drawn based on SAR approaches using experimental data directly versus solvation-corrected receptor binding data as the biological observable.

In the present study the trans isomer was selected for compound **9**. The NMR data (see below) showed the presence of conformational heterogeneity for the tropane methyl and the 2β terminal methyl. This was intially interpreted as being due to the presence of the cis and trans isomers. Computational analysis and model building, however, showed that the cis isomer was sterically unfavorable. Optimization at the AM1 level yielded energies of 100.2 and −45.1 kcal/mol for the cis and trans isomers, respectively, indicating the cis configuration to be highly unfavorable. Analysis of the five trans minimum energy structures revealed two distinct conformations for the 2β-methyl ester group, with C3−C2−C10−O11 (C10 and O11 are the 2β-carbonyl carbon and oxygen atoms, respectively) dihedral angles of −13 and 158°. The total calculated free energies of the conformers, including solvation contributions, were −48.0 and −47.3 kcal/mol, suggesting both conformations to be similarly populated. Thus, the multiple peaks in the NMR data were reinterpreted as being due to conformational heterogeneity at the 2β-position. Therefore, the pharmacological data is assumed to be associated solely with the trans isomer.

To obtain a more detailed structure−function relationship with respect to substitutions at the 3β-position CoMFA analysis was performed on the 15 compounds listed in Table 1. Table 2 illustrates the importance of including both steric and electrostatic contributions in the CoMFA model as seen by the larger q^2 (0.160) when both fields are used as compared to using only the steric or electrostatic field (0.145 and 0.028, respectively). It was thus determined that future analysis would be performed with both the steric and electrostatic fields being used.

Presented in Table 4 is the CoMFA analysis on the 15 compounds using the optimal $q^2 = 0.452$ model in Table 2. Prior to the final analysis, alternate conformations of selected compounds were tested (see Methods) yielding validated q^2 values of 0.666 and 0.375 for cocaine binding and DA uptake, respectively. Analysis of the cocaine binding − DA uptake term leads to a negative q^2. Inclusion of the ΔG_{solv} of the compounds in the analysis raised the q^2 value to 0.694 and 0.477 for cocaine binding and DA uptake, respectively while the q^2 for the cocaine binding − DA uptake term was still negative. As an alternative to the use of the ΔG_{solv} data directly the receptor binding data, RB_{coc} and RB_{DU}, were used in the analysis. The q^2 for cocaine binding was decreased to 0.585, lower than when the ΔG_{solv} term was included; however, the q^2 for the DA uptake data increased further to 0.498. Thus, including contributions from solvation properties into the CoMFA leads to improved q^2 values, although the results depend on the method used.

The cocaine binding − DA uptake difference term was used as the biological observable in the CoMFA analysis to investigate structural features of the studied compounds that may distinguish between cocaine binding and DA uptake. All cocaine binding − DA uptake differences were calculated to be small positive or negative values. This indicates that separation of the two activities based on the structural properties of 3β substituents would be difficult. Contradicting this is compound **11** which favors DA uptake over cocaine binding. Compound **11** displayed an exceptionally large absolute cocaine binding − DA uptake value (−2.09) which was close to 4 times larger than the next largest difference (compound **7**, 0.58). Compound **11** had a potency ratio for cocaine binding that was substantially lower than any other compound in the analysis set; it also had the second lowest potency ratio for DA uptake (−0.81). Figure 3 shows that **11** has a unique positioning and size of its 3β-substituent. The 3β-substituent of **11** protrudes sharply toward the 2β substituent. In addition, when compared to other compounds with the same 3β-substituent positioning (see Figure 3, compound **5** and Figure 4, compound **8**), the 3β-substituent of **11** is longer and extends more toward the 2β-substituent than the others. This large absolute cocaine binding − DA uptake value suggests the potential for identifying structural properties that may contribute to the blockade of cocaine binding without affecting DA uptake and therefore have potential as therapeutic agents. Both biological activity and binding, however, are low (see Table 1), indicating this compound to be a poor lead for potential therapeutic agents.

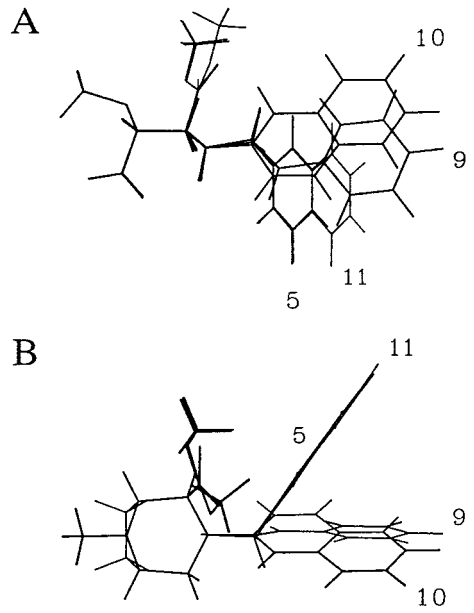


Figure 3. The two compounds with the highest cocaine receptor binding, compounds **9** and **10**, and two compounds with the lowest cocaine receptor binding, compounds **5** and **11**. The top and bottom are two orthogonal views of the compounds.

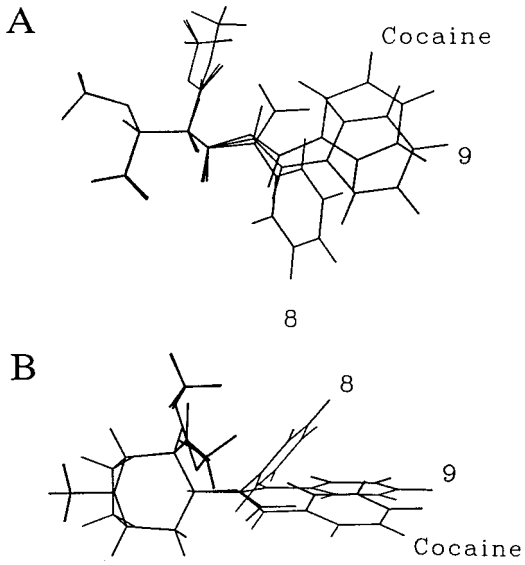


Figure 4. Cocaine, compounds **8**, and **9**. The top and bottom are two orthogonal views of the compounds.

Further inspection of Table 4 reveals that steric terms provide the largest contribution to the CoMFA models. It is also seen that electrostatics play a small but significant role in cocaine binding and DA uptake. The involvement of electrostatic interactions in cocaine binding were also seen in CoMFA studies performed by Carroll et al.[48] The fact that the electrostatic contributions are significant indicates that hydrogen bonding or other electrostatic based interactions may occur between the 3β-substituent and the receptor. This is consistent with the presence of hydrogen bond acceptors on the 3β-substituent of cocaine and compounds **13**, and **14**. Clearly, the steric term dominates, supporting the previously proposed role of hydrophobic interactions at the 3β-substituent binding site on the cocaine receptor.[49]

Analysis of results from the CoMFA study was performed using the CoMFA steric maps from the steric/electrostatic/free energy of solvation analysis (not shown). The CoMFA maps indicated unfavorable steric contributions to cocaine receptor binding if the 3β-substituent extends too far from the tropane ring. Furthermore, the contribution was more unfavorable as the 3β-substituent extended below the plane of the tropane ring and toward the 2β-substituent. Favorable steric contributions, however, were seen as the 3β-substituent extended above the plane of the tropane ring.

To gain more insight into the spatial requirements of the 3β-substituent, analysis of the modeled structures is presented in Figures 2−10. In Figure 2 two orthogonal views of all 15 compounds used in the study are presented. These show that a wide variety of conformational space in the vicinity of the 3β-substituent was sampled. The only region not well sampled was the region on the side opposite the 2β-substituent.

Figure 3 shows the two compounds with the highest cocaine binding (**9** and **10**) along with the two compounds with the lowest affinity for the cocaine receptor (**5** and **11**). It is clear that there is a binding pocket into which the 3β-substituent fits. The 3β-substituent of compounds **9** and **10** extend directly out of the tropane skeleton parallel with the vector defined by the bond between the 3β-carbon of the tropane ring and the first carbon of the substituent (CC vector). The 3β-substituents of **5** and **11** deviate significantly from being parallel with the CC vector, occupying conformational space below the plane of the tropane skeleton (Figure 3a) and toward the 2β-substituent (Figure 3b). Examination of the solvation-corrected receptor binding values (Table 1) supports these conclusions. Compounds **5** and **11** are still the least potent while **9** and **10** have potencies greater than that of cocaine.

Figure 4 shows cocaine with compounds **8** and **9**. The importance of correct 3β-positioning is seen once again in the high affinity of **9** at the cocaine receptor (1.68). Cocaine also has the correct 3β-positioning, but its affinity at the cocaine receptor is not as high (0.00). This is due to the more favorable ΔG_{solv} of cocaine as compared to **9** (−4.51 and −2.87 kcal/mol, respectively). Compound **8** does not have the correct 3β-positioning. Compound **8**, however, has a higher measured affinity (0.15) than cocaine. This is explained by the less negative ΔG_{solv} of **8** as compared to cocaine (−3.18 vs −4.51 kcal/mol, respectively). In accord with the incorrect 3β-positioning, **8** has a lower RB_{coc} (−0.83) than cocaine, consistent with the importance of the proposed correct orientation of the 3β substituent.

Figure 5 shows the WIN compounds. They all have the correct 3β-alignment, but compounds **3** and **4** have lower affinities (−0.14 and 0.006, respectively) than the unsubstituted WIN compound (0.53). This is due to the more favorable solubility of compounds **3** and **4** (−8.22 and −6.52 kcal/mol, respectively) as compared to the unsubstituted WIN compound (−3.40 kcal/mol). Removal of the solvation contributions yields RB_{coc} values for WIN, **3**, and **4** of −0.28, 2.58, and 1.48, respectively. Compound **15** has the highest affinity (0.59) of all the WIN compounds. Due to its small negative ΔG_{solv} (−3.04), however, it has the lowest RB_{coc} (−0.49). The higher RB_{coc} of **3** and **4** may be associated with the NO_2 and NH_2 substituents facilitating tighter binding through electrostatic contacts with polar residues within the

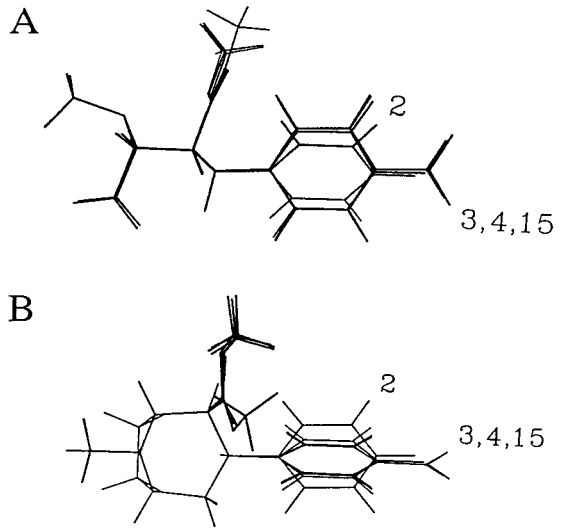


Figure 5. WIN and the WIN analogues, compounds **3**, **4**, and **15**. The top and bottom are two orthogonal views of the compounds. The 3β-substituent of the WIN analogues overlap each other.

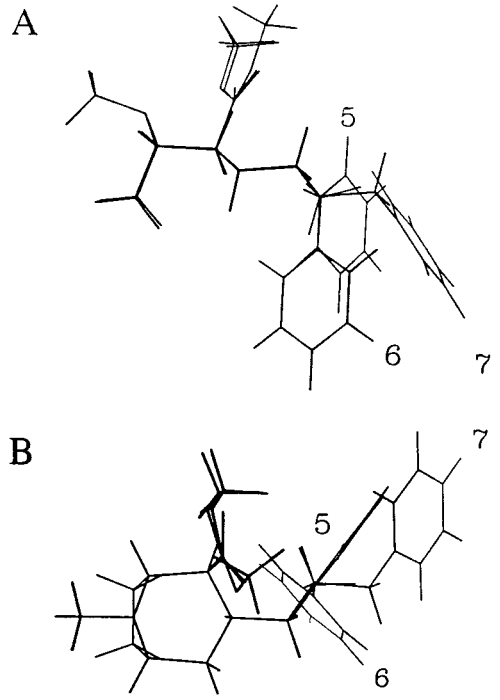


Figure 6. Compounds with different numbers of carbon atoms between the tropane ring and the phenyl ring: **5**, **6**, and **7**. The top and bottom are two orthogonal views of the compounds.

binding site, although the *p*-fluoro substituent of **15** could do this as well. Another explanation suggests that the NO_2 and NH_2 groups make the 3β-substituent larger. If the cocaine binding site is nonpolar, these polar substituents may improve binding by better filling the binding site on the receptor and thereby excluding water molecules. The simplicity of the model used for the separation of solvation effects from direct receptor binding and these two compounds being two of the three compounds with polar groups in this region of the 3β-substituent limit further interpretation of these results.

Figure 6 shows compounds with a $(CH_2)_n$ (n = 1, 2, or 3) bridge between the tropane ring 3β-position and a phenyl ring. These compounds all have similar ΔG_{solv}, but their activities at the cocaine receptor differ markedly. The n = 1, compound **5**, and the n = 3, compound **7**, have low activities (−0.94 and −0.53). The n = 2, compound **6**, exhibits higher activity (1.01). The 3β-phenyl group of **5** cannot assume the proposed correct orientation due to only a single methylene group between the 3β-carbon and the phenyl ring. The phenyl ring of **6** also does not occupy the correct region of conformational space even though its measured activity is high. Compound **7**, with its low activity, is consistent with the model for the 3β-position since its phenyl group does not occupy the proposed active space. Thus, compound **6** is inconsistent with the working model.

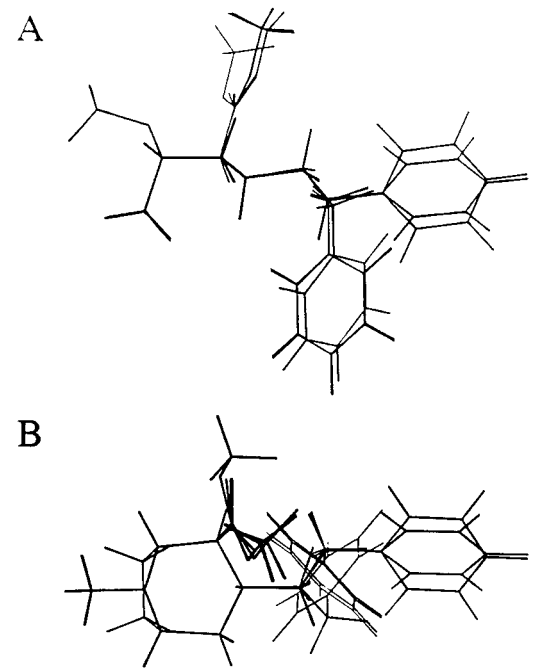


Figure 7. The five lowest energy conformations of **6** as determined by the GRIDSEARCH procedure. The top and bottom are two orthogonal views of the compounds. Note that two conformers are almost identical.

Analysis of all low-energy conformers of **6** and **7** was performed to further investigate this inconsistency. All five conformers for **6** and **7** are shown in Figures 7 and 8, respectively. With compound **6** the phenyl moiety of two of the five conformers occupies the proposed active region of conformational space, however, none of the compound **7** structures assume the proposed correct 3β-positioning. Thus it is proposed that the phenyl group of **6** can occupy the proposed active region of conformational space, leading to its higher activity as compared to **5** and **7**. The receptor binding values are consistent with this model.

Comparison of the RB_{coc} values for **5**, **6**, and **7** with that of cocaine shows them to be less tightly bound. This may be attributed to enhanced flexibility of the 3β-substituents of these compounds as compared to cocaine. The enhanced flexibilty would lower the probability of these compounds occupying the correct region of conformational space and increase the entropy loss upon binding, both of which will lead to decreased binding affinities.

Figure 9 shows three more compounds which further illustrate the requirements of the 3β-position. Com-

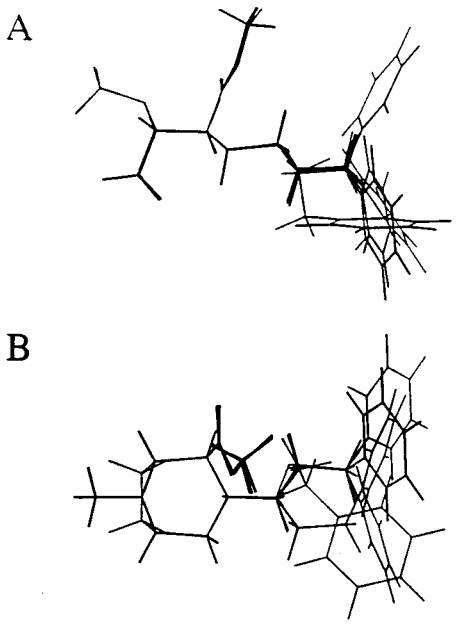


Figure 8. The five lowest energy conformations of **7** as determined by the GRIDSEARCH procedure. The top and bottom are two orthogonal views of the compounds.

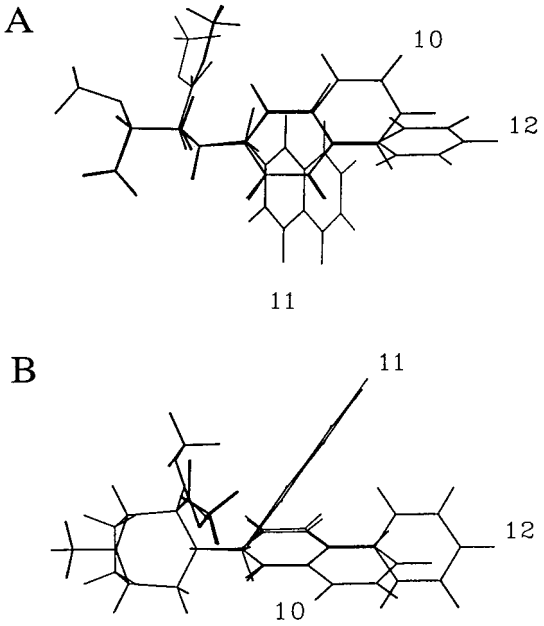


Figure 9. Compounds **10**, **11**, and **12**. The top and bottom are two orthogonal views of the compounds.

pound **12** has the correct 3β-positioning and has similar, though slightly lower, cocaine binding activity, 0.99, as **10**, 1.48. The receptor binding values for these two compounds are also similar. Figure 9 shows the 3β-substituent of compound **12** to extend further from the tropane ring than the 3β-substituent for **10**. On the basis of the longer length it would be expected that **12** would better fill the hydrophobic pocket and have a greater activity. Since its activity is slightly lower than that of **10**, it suggests that the biphenyl substituent is defining the length of the binding pocket. The 3β-substituent of compound **12** is 9.7 Å long while the 3β-substituent of compound **10** is 7.6 Å long. Thus, the optimal length of the 3β-substituent binding site is suggested to be approximately 9 Å. Analysis of the 3β-

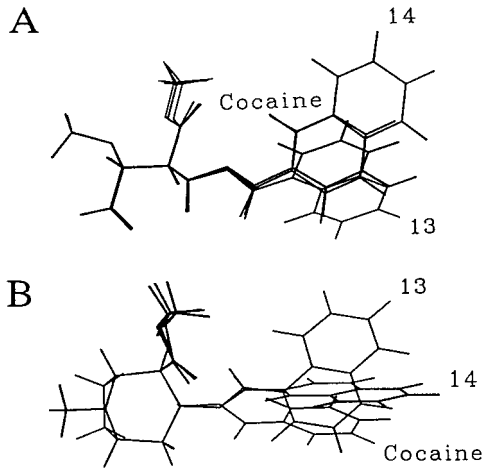


Figure 10. Cocaine, compound **13**, and **14**. The top and bottom are two orthogonal views of the compounds.

substituent in the other compounds that have the proper alignment of the 3β-substituent supports this estimate. In cocaine the length is 7.5 Å and in **9** it is 7.7 Å. For the compounds with high RB_{coc} values, compounds **3** and **4** have lengths of 6.3 and 6.5 Å respectively.

Figure 10 shows cocaine with compounds **13** and **14**. These compounds have an ester group between the 3β-position and the 3β-aryl group. Once again it is seen that incorrect positioning of the 3β-substituent can lower the cocaine receptor binding of a compound. The 3β-substituent of **14** protrudes above the CC vector, and the 3β-substituent of **13** extends sharply towards the 2β-side of the CC vector. As a result of the incorrect 3β-substituent positioning compounds **13** and **14** have low activities (−0.87 and −0.5 l, respectively), although their receptor binding values are similar to cocaine.

Analysis of Figures 3 and 9 also allows for suggestions to be made concerning the width of the 3β-substituent binding site. Figures 3a and 9a show the 3β-substituents of **10** and **12** to lie even with or slightly above the CC vector, while the 3β-substituent of **9** lies slightly below the vector and has a lower RB_{coc} value. Analysis of Figures 3b and 9b show the 3β-substituents of **10** and **12** to lie even with or slightly to the side of the CC vector away from the 2β-substituent. The apparent sensitivity of binding to the relative position of the 3β-substituent indicates the binding site is rather narrow, probably only slightly greater in diameter than required for a phenyl ring.

On the basis of the presented results, we suggest the following geometry of the 3β-substituent binding site. It is a barrel shaped region with a diameter slightly greater than the width of a phenyl ring and 9 Å in length. The orientation is such that the barrel is even with or shifted slightly above the CC vector, toward the bridging nitrogen side of the tropane ring and even with or slightly to the side of the CC vector away from the 2β-substituent. The WIN type compounds, **2**, **3**, **4**, and **15**, fit well into this barrel, due to the phenyl ring being only one bond away from the tropane ring. Further support for the proposed geometry of the 3β-substituent binding site are results showing para-substituted WIN analogues to be typically 10-fold more potent than meta-substituted analogues.[48] It is expected that meta substituents will protrude out of the proposed barrel,

leading to their lowered activities. Work by Carroll et al.[48] also shows that the binding site can accommodate meta substitutions on the phenyl ring as many meta−para-disubstituted compounds display lower IC_{50} values than their para-substituted counterparts. This indicates that the binding site may undergo subtle conformational rearrangements upon ligand binding. In addition, WIN analogues substituted at the para position with either chloro or a methyl group are approximately 10-fold more potent than WIN or compounds **3** and **4**,[44] suggesting the hydrophobic nature of the binding pocket away from the tropane ring.

Comparison with previous CoMFA studies shows that the results presented here add to our understanding of the importance and positioning of the 3β-substituent in cocaine receptor binding. Carroll et al.[48] found that 3β-substitutions with increased steric bulk slightly above the 4′-position of the phenyl ring increased potency while increased steric bulk below the 4′-position decreased potency. Furthermore, they found electrostatics accounted for 27% of the cocaine receptor binding. Work by Srivastava et al.[49] described the importance of the 3β-substituent to cocaine receptor binding. In addition, it was shown that the dominant contribution to binding was hydrophobic interactions.[49] These results are consistent with the observations in the present study.

Conclusion

The presented binding data and the computational analysis give insight into the role of solubility and the positioning of the 3β-substituents in cocaine binding and inhibition of DA uptake. Inclusion of solvation free energies improved the predictability of the CoMFA model for cocaine binding and DA uptake. This is consistent with previous studies on cocaine, WIN, and the WIN vinyl analogue that indicated solvation to contribute to the measured activities of those compounds.[51] Structural analysis of cocaine and the 3β-substituted cocaine analogues describes a binding pocket extending in a linear fashion away from the tropane ring into which the 3β-substituent must fit during binding. This binding pocket is indicated to be slightly greater in diameter than a phenyl ring and approximately 9 Å in length. It is such that the barrel is even with or shifted slightly above the CC vector, on the bridging nitrogen side of the tropane ring and parallel to or slightly to the side of the CC vector opposite the 2β-substituent.

The present study indicates that more favorable aqueous solubility contributes to a decrease in experimental binding values due to the additional energy required to desolvate the analogues as they interact with the cocaine binding site. Taking these contributions into account has allowed for a model of the conformational requirements of the 3β-substituents to be elucidated in the present study. Theoretical receptor binding values, which eliminate solvation contributions from experimentally determined activities, represent an alternate method for taking into account solvation effects when performing SAR studies. While simplifications in the receptor binding model and direct use of free energies of solvation are evident, the present work indicates that accounting for solvation can aid in the interpretation of structure−activity relationships versus using only experimentally measured binding data.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. NMR spectra were recorded on a GE QE-300 spectrometer with the free base form of the compounds except where noted. Spectra were obtained in $CDCl_3$ with TMS as an internal standard. Mass spectra were performed with a Hewlett-Packard MSD spectrometer. The free bases were dissolved in MeOH and introduced into the mass spectrometer via a Hewlett-Packard gas chromatography apparatus equipped with a 15 m (0.25 mm i.d.) methylsilicone gum GC capillary column (HP-1). Elemental analyses were performed by Micro Analysis Inc. and agreed to within 0.4% of the calculated values. Chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI 53233; Sigma Chemical Co., St. Louis, MO 63178; Lancaster Synthesis Ltd., Windham, NH 03087; Fisher Scientific, Pittsburgh, PA 15219; National Institute on Drug Abuse (NIDA), Rockville, MD 20857; New England Nuclear, Boston, MA 02118.

General Method for 3β-Aryltropanecarboxylic Methyl Esters. The procedures for preparing 3-aryltropanecarboxylic methyl esters were based on the early work by Clark et al.[42] and adapted by procedures of Carroll et al.[44] and Meltzer et al.[43] The main reaction was to couple anhydroecgonine methyl ester with an appropriate aryl Grignard reagent. The general terms of the reaction are described here followed by the analytical data and additional details for specific compound under the compound headings. A 1.0 M solution of the Grignard reagent in Et_2O was stirred under a N_2 atmosphere at low temperature (−35 to −45 °C). To the Grignard reagent was introduced a solution of 0.5 equiv anhydroecgonine methyl ester in Et_2O at such a rate that the temperature did not rise more than 2 °C. The slurry was efficiently stirred at this temperature for 2 h, and the temperature was cooled down to −70 °C when a solution of trifluroacetic acid (2 equiv) in Et_2O was used to quench the reaction. The mixture was allowed to warm to 0 °C, diluted with water, and acidified with concentrated HCl to maintain pH 1−2. The Et_2O layer and the aqueous layer were separated. The aqueous portion was neutralized with concentrated NH_4OH to pH 8−9 in an ice bath and was extracted with Et_2O (4 times). The Et_2O was dried with Na_2SO_4 and evaporated to give a yellow oil.

The crude product was chromatographed on silica gel (40g/g of compound) with Et_3N−ethyl acetate−hexane (5:35:60) for elution. The first eluent was the 2β-epimer, followed by the 2α-epimer, then anhydroecgonine methyl ester and sometimes a small amount of the 1,2-adduct. Removal of the solvent from the first eluent gave the desired 2β-epimer as a yellow oil. It was converted to a hydrochloride salt, except where noted, in MeOH and crystallized from Et_2O/MeOH.

General Method for 3-(Aryloxy)tropanecarboxylic Methyl Esters. To a 0.1 M solution of ecgnonine methyl ester stirred with 1 equiv of K_2CO_3 in toluene was added the appropriate amount of arylcarbonyl chloride over 5 min. The mixture was refluxed for 30 min and allowed to cool to room temperature. After filtration, solvent was removed to give a yellow oil. The crude product was crystallized from ethyl acetate/pentane. The compounds were converted to the hydrochloride salt before biological testing.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-8-azabicyclo[3.2.1]-octane-2-carboxylate (Ecgonine Methyl Ester). (−)-Cocaine hydrochloride (10.0 g, 0.029 mol) was dissolved in 200 mL of MeOH, combined with 20 mL concentrated H_2SO_4, and refluxed overnight. The reaction mixture was allowed to cool to room temperature. Excess solvent was evaporated under reduced pressure. The residual yellow oil was taken up in a small amount of water and neutralized with saturated Na_2CO_3 solution at 0 °C. The product was extracted into 4 × 50 mL portions of Et_2O. The combined Et_2O extracts were dried with Na_2SO_4, filtered, and evaporated to dryness. The resulting yellow oil was used in the following reaction without

further purification. If a pure product was desired, the crude product was readily purified by flash chromatography or vacuum distillation, depending on the scale of the reaction (yield >98%). Analytical data are consistent with that reported in the literature.[42,44]

(−)-Anhydroecgonine Methyl Ester. $POCl_3$ (25 mL) was added to ecgonine methyl ester (5.0 g), and the mixture was heated at reflux for 1 h. Excess $POCl_3$ was then removed by evaporation under reduced pressure. The residue was chilled in a dry ice−acetone bath, and 20 mL of MeOH was added. The mixture was then allowed to warm to room temperature, and the MeOH was removed. To the residue was added 10 mL of water followed by an appropriate amount of concentrated NH_4OH to produce a pH of approximately 8. The aqueous mixture was extracted with methylene chloride (3 × 30 mL), the combined organic portions were dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The crude product was flash chromatographed on a silica gel column with Et_2O−Et_3N (9:1) as eluent. The first fraction was collected and concentrated to dryness to afford a clear oil (1.43 g). The overall yield from cocaine hydrochloride was 72%. Analytical data are consistent with those reported in the literature.[42,44]

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-benzyl-8-azabicyclo-[3.2.1]octane-2-carboxylate (5). Starting with benzyl bromide and anhydroecgonine methyl ester, the standard procedure gave 13 as a clear oil (47%): ^{1}H NMR δ 1.44−1.47 (m, 2H), 1.92−2.04 (m, 4H), 2.15 (s, 3H, NCH_3), 2.71−2.90 (m, 2H, CH_2), 3.17−3.20 (m, 1H), 3.40−3.42 (m, 1H), 3.70 (s, 3H, OCH_3), 3.75 (m, 1H), 7.09−7.37 (m, 5H); MS *m*/*z* (relative intensity) 273.37 (M^+, 100), 258 (1), 242 (30); $[\alpha]^{25}_D = -25.09°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(2-phenylethyl)-8-azabicyclo[3.2.1]octane-2-carboxylate Hydrochloride (6). Compound **6** was synthesized from phenylethyl bromide and anhydroecgonine methyl ester following the above procedure. The oil was converted to a hydrochloride salt with MeOH and Et_2O/HCl to give small white crystals (30%): mp 163−165 °C; ^{1}H NMR δ 1.44−1.47 (m, 2H), 1.77−2.04 (m, 5H), 2.16 (s, 3H, NCH_3), 2.32 (s, 1H), 2.44−2.46 (t, 1H), 2.50−2.58 (m, 2H), 3.18−3.19 (d, 1H), 3.42−3.44 (dd, 1H, J_{12} = 1.76 Hz, J = 6.11 Hz), 3.66 (s, 1H), 3.68 (s, OCH_3), 7.14−7.32 (m, 5H); MS *m*/*z* (relative intensity) 287.40 (M^+, 100), 273 (1), 256 (20); $[\alpha]^{25}_D = -57.5°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(3-phenylpropyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (7). Starting with 3-phenylpropyl bromide and anhydroecgonine methyl ester, the final product, **7**, was separated from the epimer and the starting material as a clear oil (28%): ^{1}H NMR δ 1.39−1.60 (m, 2H), 1.87−2.00 (m, 5H), 2.16 (s, 3H, NCH_3), 2.40−2.43 (t, 1H), 2.54−2.56 (t, 2H), 3.17−3.18 (m, 1H), 3.42−3.44 (dd, 1H, J_{12} = 1.28 Hz, J_{23} = 4.57 Hz), 3.66 (s, 1H), 3.68 (s, 3H, OCH_3), 7.13−7.29 (m, 5H); MS *m*/*z* (relative intensity) 301.20 (M^+, 100), 270.10 (25), 242.10 (10); $[\alpha]^{25}_D = -57.26°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(α-styrenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate Hydrochloride (8). The formation of the Grignard reagent took 45 min in the dark. Final product was purified as a clear oil (20%). It was converted to hydrochloride salt in MeOH and crystallized from Et_2O/MeOH: mp 128−130 °C; ^{1}H NMR δ 1.58−1.67 (m, 3H), 2.07−2.17 (m, 2H), 2.18 (s, 3H, NCH_3), 2.30−2.38 (dt, 1H), 2.44−2.46 (t, 1H), 2.85−2.90 (m, 1H, J_{12} = 1.95 Hz, J_{23} = 4.52 Hz), 3.33−3.34 (d, 1H), 3.42−3.44 (s, 1H), 3.53 (s, 3H, OCH_3), 5.07−5.08 (d, 1H, J_{gem} = 1.59), 5.14−5.15 (d, 1H, J_{gem} = 1.47 Hz), 7.20−7.31 (m, 5H); MS *m*/*z* (relative intensity) 285.20 (M, 100), 270.15 (2), 254.20 (10); $[\alpha]^{25}_D = -15.88°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(*cis,trans*-β-styrenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate Hydrochloride (9). The Grignard reaction was carried out with *cis,trans*-β-bromostyrene in the dark for 45 min. The final oil products were collected and converted to a HCl salt to afford white needles (15%): mp 183−184 °C; ^{1}H NMR δ 1.47−1.67 (m, 3H), 1.97−2.00 (m, 2H), 2.16−2.19 (two s, 3H, NCH_3, relative intensity 1:1), 2.55 (m, 1H), 2.92−3.05 (m, 0.5H), 3.18−3.22 (m, 1H), 3.46−3.49 (d, 1H), 3.68−3.71 (two s, 3H, OCH_3, relative intensity 1:1), 6.00−6.60 (m, 2H), 7.15−7.36 (m, 5H); MS *m*/*z* (relative intensity) 285.15 (M^+, 100), 270.20 (2), 254.15 (10); $[\alpha]^{25}_D = -78.96°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(2-naphthyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (10). The reaction was initiated with 2-bromonaphthalene and magnesium chips in Et_2O. It took 24 h for the Grignard reaction to go to completion. The final β-epimer was formed in a 13% yield, and an additional eluent of the α-epimer (1.3%) was also collected along with 22% starting anhydroecognine methyl ester. This led to a 14.3% yield of compound **10**: ^{1}H NMR δ 1.59−1.82 (m, 3H), 2.08−2.20 (m, 2H), 2.23 (s, 3H, NCH_3), 2.68−2.76 (td, 1H), 3.00−3.03 (t, 1H), 3.10−3.18 (m, 1H), 3.39−3.41 (t, 1H), 3.43 (s, 3H, OCH_3), 3.56−3.59 (dd, 1H, J_{12} = 2.97 Hz, J_{23} = 6.38 Hz), 7.34−7.77 (m, 7H); MS *m*/*z* (relative intensity) 309.10 (M^+, 50), 250.20 (20), 165.05 (20), 152.00 (10), 83.10 (100), 82.10 (100); $[\alpha]^{25}_D = -94.19°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(2-naphthylmethyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (11). Completion of the Grignard reaction took 24 h at room temperature. Compound **11** was formed as a clear liquid (26%) after purification by TLC: ^{1}H NMR δ 1.36−1.40 (m, 3H), 1.90−2.00 (m, 4H), 2.07 (s, 3H, NCH_3), 2.15−2.17 (t, 1H), 2.80−2.96 (m, 2H), 3.09 (t, 1H), 3.31−3.33 (m, 1H), 3.63 (s, 3H, OCH_3), 3.75 (m, 1H), 7.16−7.72 (m, 7H); MS *m*/*z* (relative intensity) 323.40 (M^+, 50), 96.20 (15), 82.15 (50); $[\alpha]^{25}_D = -43.76°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(1-biphenyl)-8-azabicyclo[3.2.1]octane-2carboxylate (12). 4-Bromobiphenyl (2.42 g, 2 equiv) and magnesium chips (0.25 g) in 20 mL of ether were stirred for 16 h to allow for the formation of Grignard reagent. The final β-epimer was formed in 13% yield, and an additional eluent of the α-epimer (1.3%) was also collected along with 22% starting material anhydroecogonine methyl ester: ^{1}H NMR δ 1.63−1.82 (m, 3H), 2.12−2.23 (m, 2H), 2.28 (s, 3H, NCH_3), 2.64−2.72 (t, 1H), 2.99−3.00 (d, 1H), 3.04−3.10 (dd, 1H, J_{12} = 2.43 Hz, J_{23} = 6.01 Hz), 3.41−3.42 (m, 1H), 3.55 (s, 3H, OCH_3), 3.57−3.62 (m, 1H), 7.35−7.70 (m, 9H); MS *m*/*z* (relative intensity) 335.40 (M^+, 100); $[\alpha]^{25}_D = -103°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(1-naphthoylexy)-8-azabicyclo[3.2.1]octane-2-carboxylate (13). 13 was synthesized from ecgonine methyl ester and the 1-naphthoyl chloride and was formed as white crystals (70%): mp 109−110 °C; ^{1}H NMR δ 1.50−1.82 (m, 3H), 1.94−2.19 (m, 2H), 2.27 (s, 3H, NCH_3), 2.47−2.56 (td, 1H), 3.12−3.15 (t, 1H), 3.31−3.32 (d, 1H), 3.59 (m, 1H), 3.72 (s, 3H, OCH_3), 3.72−3.74 (dd, 1H), 7.48−8.89 (m, 7H); MS *m*/*z* (relative intensity) 303.10 (100), 272.10 (30); $[\alpha]^{25}_D = -76.05°$ (MeOH). Anal. C, H, N.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-3-(2-naphthoylexy)-8-azabicyclo[3.2.1]octane-2-carboxylate (14). 14 was obtained as white needles (69%): mp = 82−83 °C; ^{1}H NMR δ 1.90−1.94 (m, 1H), 2.14−2.18 (m, 1H), 2.23 (s, 3H, NCH_3), 2.47−2.51 (td, lH), 3.07−3.09 (t, 1H), 3.31−3.32 (d, 1H), 3.58 (m, 1H), 3.73 (s, 3H, OCH_3), 3.72−3.76 (m, 1H), 7.50−8.59 (m, 7H); MS *m*/*z* (relative intensity) 303.10 (100), 272.10 (30). Anal. C, H, N.

Biology. Male Sprague−Dawley rats weighing between 200 and 250 g were used in the experiments. All tissue samples were prepared with striatum dissected from rat brain. The dissected tissue included caudate, globus pallidus, putamen, and nucleus accumbens. All experiments used fresh tissue, and all subsequent steps for membrane preparation were conducted at 0−4 °C.

For [^{3}H]cocaine binding experiments, the striatal tissue was homogenized in ice-cold 10 mM Na_2HPO_4 buffer containing 0.25 M sucrose, pH = 7.4, using a glass Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was centrifuged at 48000*g* for 20 min. The pellet was resuspended in the same buffer with a P-10 Kinematica Polytron homog-

enizer (setting 7) for 20 s. The homogenate was centrifuged at 48000*g* for 20 min. The resulting pellet was resuspended in 40 volumes (vol/wt) of the same buffer to yield a tissue solution with approximate protein concentration of 1 mg/mL. The Lowry et al.[64] method was utilized to determine all final protein concentrations.

The membrane synaptosomal preparations utilized to assay [^{3}H]DA uptake were prepared as described by Richelson and Pfenning[6] with slight modifications. Striatal tissue was homogenized in ice-cold 0.32 M sucrose, 11 mM glucose (pH = 7.4) buffer using a glass Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 1000*g* in a Beckman centrifuge, and then the supernatant was decanted and centrifuged for 20 min at 48000*g*. The resultant P2 pellet was washed by centrifugation in the same buffer, and the final pellet was resuspended in 20 volumes (vol/wt) of the same solution.

[^{3}H]Cocaine Binding. Binding of [^{3}H]cocaine (1-benzoyl-3,4-3H(N), 29.7 Ci/mmol; New England Nuclear, Boston, MA) to rat striatal membranes was measured by filtration assay using the thin layer liquid scintillation method as described by Raymon and Eldefrawi.[55] Binding assays were conducted using six to eight concentrations of each drug, ranging from 0.01 nM to 100 μM. In general, the concentrations used displaced specific [^{3}H]cocaine binding from 10% to 90%. Each assay sample contained membrane preparation (25 μL, approximately 50 μg of protein), the drug being tested (2.5 μL) in dimethyl sulfoxide (DMSO), [^{3}H]cocaine (25 μL, 2 nM) and buffer to reach a final volume of 250 μL in 96-well microtiter plates. Nonspecific binding was defined as that measured in the presence of 100 μM unlabeled cocaine. After addition of the [^{3}H]cocaine, sample was incubated for 30 min at the end of which time the reaction was terminated by vacuum filtration through Skatron filter mats which had been presoaked in 0.05% polyethyleneimine using a Skatron 96-cell harvester and 55 wash, 55 dry cycles. The radioactivity remaining on the filter mats was counted in a Beta Plate (1205) liquid scintillation spectrometer (Wallac) as described by Raymon and Eldefrawi.[55]

[^{3}H]Dopamine Uptake. Uptake of [^{3}H]dopamine [(dihdroxyphenyl)ethylamine-3,4-T_2, 30 Ci/mmol] into rat striatal synaptosomes was measured at room temperature by filtration assay, following a previously described method,[11] with the following modifications. Striatal synaptosomes (50 μL,approximately 100 μg of protein) were preincubated with the drugs (5 μL, in DMSO and solvent, concentration range 0.01 nm to 00 μM) for 10 min, and buffer was added to reach a final volume of 250 μL in a microtiter plate. After preincubation, [^{3}H]dopamine (4 nM) was then added, and the samples were incubated for 5 min. Uptake was terminated by vacuum filtration using the Skatron 96-cell harvester and radioactivity retained on the filter mat counted as described above. Nonspecific uptake, which was typically between 3 and 5% of the total uptake, was measured at room temperature using 100 μM unlabeled cocaine. The buffer used for these samples contained choline which had been substituted equimolar for sodium. This substitution defined sodium-dependent, cocaine-sensitive [^{3}H]DA uptake.

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Supporting Information Available: An appendix that describes the model for separation of experimentally measured binding values into free energy of solvation and receptor binding contributions (4 pages). Ordering information is given on any current masthead page.

References

(1) Horn, A. S. *The Neurobiology of Dopamine*; Academic Press: New York, 1979.
(2) Horn, A. S. Dopamine uptake: A review of progress in the last decade. *Prog. Neurobiol.* **1990**, *34*, 387−400.
(3) Wise, R. A. Catecholamine theories of reward: A critical review. *Brain Res.* **1978**, *152*, 215−247.
(4) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **1991**, *14*, 299−302.
(5) Kuhar, M. J. Molecular pharmacology of cocaine: A dopamine hypothesis and its implications. *Ciba Foundation Symposium* **1992**, *166*, 81−95.
(6) Richelson, E.; Pfenning, M. Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: Most antidepressants selectively block norepinephrine uptake. *Eur. J. Pharmacol.* **1984**, *104*, 277−286.
(7) Phillips, A. G. Brain reward circuitry: A case for separate systems. *Brain Res. Bull.* **1984**, *12*, 195−201.
(8) Goeders, N. C.; Smith, J. E. Reinforcing properties of cocaine in medial prefrontal cortex: Primary action on presynaptic dopaminergic terminals. *Pharmacol. Biochem. Behav.* **1986**, *25*, 191−199.
(9) Dewit, H.; Wise, R. A. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide but not with the noradrenergic blockers phentolamine and phenoxybenzamine *Can. J. Psychol.* **1977**, *31*, 195−203.
(10) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **1987**, *237*, 1219−1223.
(11) Calligaro, D. O.; Eldefrawi, M. E. High affinity stereospecific binding of [^{3}H] cocaine in striatum and its relationship to the dopamine transporter. *Membr. Biochem.* **1988**, *7*, 87−106.
(12) Roberts, D. C. S.; Corcoran, M. E.; Fibiger, H. C. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol. Biochem. Behav.* **1977**, *6*, 615−620.
(13) Roberts, D. C. S.; Kobb, G. F.; Klonoff, P.; Fibiger, H. C. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav.* **1980**, *12*, 781−787.
(14) Roberts, D. C. S.; Kobb, G. F. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol. Biochem. Behav.* **1982**, *17*, 901−04.
(15) Martin-Iverson, M. T.; Szostak, C.; Fibiger, H. C. 6-hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. *Psycopharmacology* **1986**, *88*, 310−14.
(16) Berger, P.; Gawin, F. H.; Kosten, T. R. Treatment of cocaine abuse with mazindol. *Lancet* **1989**, *No. 1*, 283.
(17) Calligaro, D. O.; Eldefrawi, M. E. Central and peripheral cocaine receptors. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 61−68.
(18) Boja, J. W.; Kuhar, M. J. [^{3}H] cocaine binding and inhibition of [^{3}H] dopamine uptake is similar in both the rat striatum and nucleus accumbens. *Eur. J. Pharmacol.* **1989**, *173*, 215−17.
(19) Boja, J. W.; Carrol, F. I.; Rahman, M. I.; Phillip, A.; Lewin, A. H.; Kuhar, M. J. New, potent cocaine analogs: Ligand binding and transport studies in rat striatum. *Eur. J. Pharmacol.* **1990**, *184*, 329−332.
(20) Ritz, M. C.; Cone, E. J.; Kuhar, M. J. Cocaine inhibition of ligand binding at DA, NE, and serotonin transporters: A structure-activity relationship study. *Life Sci.* **1990**, *46*, 635−645.
(21) Boja, J. W.; McNeil, R. M.; Lewin, A. H.; Abraham, P.; Carroll, F. I.; Kuhar, M. J. Selective dopamine transporter inhibition by cocaine analogs. *Neuroport* **1992**, *3*, 984−86.
(22) Shimada, S.; Kitayama, S.; Lin, C.; Patel, A.; Nanthakuma, E.; Gregor, P.; Kuhar, M. J.; Uhl, G. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* **1991**, *254*, 576−78.
(23) Kitayama, S.; Shimada, S.; Xu, H.; Markham, L.; Donovan, D. M.; Uhl, G. R. Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc. Natl. Acad. Sci., U.S.A.* **1992**, *89*, 7782−7785.
(24) Neve, K. A.; Cox, B. A.; Henningsen, R. A.; Spanoyannis, A.; Neve, R. Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenyl cyclase. *Mol. Pharmacol.* **1991**, *39*, 733−739.
(25) Zimanyi, I.; Lajtha, A.; Reith, M. E. A.;. Comparison of characteristics of dopamine uptake and mazindol binding in mouse striatum. *Naunyn-Schmeideberg's Arch. Pharmacol.* **1989**, *340*, 626−632.
(26) Anderson, P. H. The dopamine uptake inhibitor GBR 12909: Selectivity and molecular mechanism of action. *Eur. J. Pharmacol.* **1989**, 166, 493−504.
(27) Krueger, B. K. Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. *J. Neurochem.* **1990**, *55*, 260−267.

(28) Madras, B. K; Fahey, M. A.; Berman, J.; Canfield, D. R.; Spealman, R. D. Effects of cocaine and related drugs in nonhuman primate; I. [^{3}H] cocaine binding sites in caudate-putamen. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 131−141.
(29) Spealman, R. D.; Madras, B. K.; Berman, J. Effects of cocaine and related drugs in nonhuman prmates. II. stimulant effects on schedule-controlled behavior. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 142−149.
(30) Raymon, L. P.; Eldefrawi, M. E. Partial purification of cocaine receptors from rat brain, Ph.D. Thesis, University of Maryland at Baltimore, 1994.
(31) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. Synthesis and ligand binding of cocaine isomers at the cocaine receptor. *J. Med. Chem.* **1991**, *34*, 883−886.
(32) Lewin, A. H.; Gao, Y.; Abraham, P.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. 2β-substituted analogues of cocaine. Synthesis and inhibition of binding to the cocaine receptor. *J. Med. Chem.* **1992**, *35*, 135−140.
(33) Carroll, F. I.; Lewin, A. H.; Kuhar, M. J.; Boja, J. W. Cocaine receptor: A structure−activity relationship study. *NIDA Research Monograph Series* **1993**, *134*, 229−237.
(34) Kozikowski, Alan P.; Roberti, Marinella; Xiang, Li; Bergmann, John S.; Callahan, Patrick M.; Cunningham, Kathryn A.; Johnson, Kenneth M. Structure-activity relationship studies of cocaine: Replacement of the C-2 ester group by vinyl argues against H-bonding and provides an esterase-resistant, high-affinity cocaine analog. J. *Med. Chem.* **1992**, *35*, 4764−4766.
(35) Kozikowski, Alan P.; Xiang, Li; Tanaka, Junji; Bergmann, John S.; Johnson, Kenneth M. Use of nitrile oxide cycloaddition (NOC) chemistry in the synthesis of cocaine analogs; mazindol binding and dopamine uptake studies. *Med. Chem. Res.* **1991**, *1*, 312−321.
(36) Kozikowski, A. P.; Roberti, M.; Johnson, K. M.; Bergmann, J. S.; Ball, R. G. SAR of cocaine: Further exploration of structural variations at the C-2 center provides compounds of subnanomolar binding potency. *Bioorg. Med. Chem. Lett.* **1993**, *3* (6), 1327−1332.
(37) Kozikowski, A. P.; Eddine Saiah, M. K.; Johnson, K. M; Bergmann, J. S. Chemistry and biology of the 2β-alkyl-3β-phenyl analogues of cocaine: Subnanomolar affinity ligands suggest a new pharmacophore model at the C-2 position. J. *Med. Chem.* **1995**, *38*, 3086−3093.
(38) Kelkar, S. V.; Izenwasser, S.; Katz, J. L.; Klein C. L.; Zhu, N.; Trudell, M. L. Synthesis, cocaine receptor affinity, and dopamine uptake inhibition of several new 2β-substituted 3β-phenyltropanes. *J. Med. Chem.* **1994**, *37*, 3875−3877.
(39) Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. *Biochem. Pharmacol.* **1986**, *35*, 1123−1129.
(40) Madras, B. K.; Kamien, J. B.; Fahey, M. A.; Canfield, D. R.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. N-modified fluorophenyltropane analogs of cocaine with high affinity for cocaine receptors. *Pharmacol. Biochem. Behav.* **1990**, *35*, 949−953.
(41) Stoelwinder, J.; Roberti, M.; Kozikowski, A. P.; Johnson, K. M.; Bergmann, J. S. Differential binding and dopamine uptake activity of cocaine analogs modified at nitrogen. *Bioorg. Med. Chem. Lett.* **1994**, *4* (2), 303−308.
(42) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cuminskey, W. R.; Bogado, E. F. Compounds affecting the central nervous system. 4. 3β-phenyltropane-2-carboxylic esters and analogs. *J. Med. Chem.* **1973**, *16*, 1260−1267.
(43) Meltzer, P. C.; Liang, A. Y.; Browell, A. L.; Elmaleh, D. R.; Madras, B. K. Substituted 3-phenyltropane analogs of cocaine: Synthesis, inhibition of binding at cocaine recognition sites, and positron emission topography imaging. *J. Med. Chem.* **1993**, *36*, 855−862.
(44) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W., Kuhar, M. J. Synthesis, ligand binding, QSAR, and CoMFA study of 3β-(p-substituted phenyl)-tropane-2β-carboxylic acid methyl esters. *J. Med. Chem.* **1991**, *34*, 2719−2725.
(45) Kline, R. H., Jr.; Wright, J.; Eshleman, A.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3b-carbamoylecgonine methyl ester analogues as inhibitors of cocaine binding and dopamine uptake. *J. Med. Chem.* **1991**, *34*, 702−705.
(46) Kline, R. H., Jr. The synthesis and pharmacolgical activity of 3β-arylecgonine methyl ester and 3β-carbamoyloxyecgonine methyl ester analogs: Probes for the characterization of the dopaminergic cocaine receptor; Ph.D. Thesis, University of Maryland at Baltimore, Baltimore, 1991.
(47) Davies, H. M. L.; Saikai, E.; Sexton, T., Childers, S. R. Novel 2-substituted cocaine analogs: Binding properties at dopamine transport sites in rat striatum. *Eur. J. Pharmacol., Mol. Pharmacol. Section.* **1993**, *244*, 93−97.
(48) Carroll, F. I.; Mascarella, S. W.; Kuzemko, M. A.; Gao, Y.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, ligand binding, and QSAR (CoMFA and classical) study of 3β-(3′-substituted phenyl)-, 3β-(4′-substituted phenyl)-, and 3β-(3′4′-disubstituted phenyl) tropane-2β-carboxylic acid methyl esters. *J. Med. Chem.* **1994**, *37*, 2865−2873.
(49) Srivastava, S.; Cripen, G. M. Analysis of cocaine receptor site ligand binding by three-dimensional Voronoi site modeling approach. *J. Med. Chem.* **1993**, *36*, 3572−3579.
(50) Pettitt, B. M.; Karplus, M. *Molecular Graphics and Drug Design*; Elsevier Science Publishers: New York, 1986.
(51) Yang, B.; Wright, J.; Eldefrawi, M. E.; Pou, S.; MacKerell, A. D., Jr. Conformational, aqueous solvation, and pKa contributions to the binding and activity of cocaine, WIN 32 065−2, and the WIN vinyl analog. *J. Am. Chem. Soc.* **1994**, *116*, 8722−8732.
(52) Karplus, M. Interpretation of the electron-spin resonance spectrum of the methyl radical. *J. Phys. Chem.* **1959**, *30*, 11−15.
(53) Carroll, F. I.; Coleman, M. L.; Lewin, A. H. Synthesis and conformational analysis of isomeric cocaines: A proton and carbon-13 nuclear magnetic resonance study. *J. Org. Chem.* **1982**, *47*, 13−19.
(54) Kline, R. H. Jr.; Eshleman, A. J.; Wright, J.; Eldefrawi, M. E. Synthesis of substituted 3β-carbamoylecgonine methyl ester analogs: Irreversible and photoaffinity ligands for the cocaine receptor/dopamine transporter. *J. Med. Chem.* **1994**, *37*, 2249−2252.
(55) Raymon, L. P.; Mahran, L. G.; Eldefrawi, M. E. The layer scintillation counting in drug-receptor binding. *Anal. Lett.* **1994**, *27*, 907−918.
(56) Cramer, C. J.; Truhlar, D. G. An SCF solvation model for the hydrophobic effect and absolute free energies of aqueous solvation. *Science* **1992**, *256*, 213−217.
(57) Cramer, C. J.; Truhlar, D. G. AM2-SM2 and PM3-SM3 parameterized SCF solvation models for free energies in aqueous solution. *J. Comput.-Aided Mol. Des.* **1992**, *6*, 629−666.
(58) Dewar, M.; Zoebisch, E.; Healy, E.; Stewart, J. AM1: A new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.
(59) Cramer, C. J.; Truhlar, D. G. Molecular orbital theory calculations of aqueous solvation effects on chemical equilibria. *J. Am. Chem. Soc.* **1991**, *113*, 8552−8555.
(60) Yang, B. Structure-activity relationship of cocaine analogs: synthesis of 3β-substituted analogs and computer-aided prediction of the pharmacological activity of cocaine analogs; Ph.D. Thesis, University of Maryland at Baltimore, Baltimore, 1994.
(61) Cramer, R. D. III; Patterson, D. E.; Bunce, J. D. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of seriods to carrier proteins. *J. Am. Chem. Soc.* **1988**, *110*, 5959−5967.
(62) MacKerell, A. D., Jr.; Hemsen, A.; Lacroix, J. S.; Lundberg, J. M. Analysis of structure-function relationships of neuropeptide Y using molecular dynamics simulations and pharmacolgical activity and binding measurements. *Reg. Pept.* **1989**, *25*, 295−313.
(63) Brusniak, M. Y. K.; Pearlman, R. S.; Neve, K. M.; Wilcox, R. E. Comparative molecular field analysis-based prediction of drug affinities at recombinant D1A dopamine receptors. *J. Med. Chem.* **1996**, *39*, 850−859.
(64) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. L. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(65) Kline, R. H., Jr.; Wright, J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-arylecgonine analogues as inhibitors of cocaine binding and dopamine uptake. *J. Med. Chem.* **1990**, *33*, 2024−2027.
(66) Milius, R. A.; Saha, J. K.; Madras, B. K.; Neumeyer, J. L. Synthesis and receptor binding of N-substituted tropane derivatives. High-affinity ligands for the cocaine receptor. *J. Med. Chem.* **1991**, *34*, 1728−1731.

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