Synthesis, Ligand Binding, QSAR, and CoMFA Study of 3β-(p-Substituted phenyl)tropane-2β-carboxylic Acid Methyl Esters

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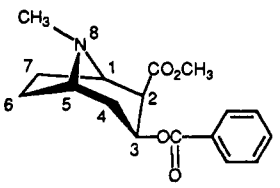
A series of 3β-(p-substituted phenyl)tropane-2β-carboxylic acid methyl esters (**2**) were synthesized and found to possess high affinity for the cocaine binding site in rat striatum. The p-chloro (**2c**) and p-iodo (**2n**) compounds, which were the most potent analogues prepared, were found to be 85 and 78 times more potent than (-)-cocaine. The p-bromo (**2m**) and p-methyl (**2d**) were also 56 and 60 times more potent than cocaine. QSAR and CoMFA studies were conducted to correlate binding affinity of the cocaine analogues with their structural features. Whereas the QSAR study gave relatively low correlations, the CoMFA study gave a correlation with high predictive value.

Several studies have shown that cocaine (**1**) binds to the dopamine transporter and inhibits dopamine transport.[1] In addition, drugs that are potent in self-administration studies are also potent inhibitors of binding at the transport sites for dopamine, whereas compounds that are weak in self-administration studies are correspondingly weak inhibitors of the binding site.[1] For example, Ritz and co-workers[2] showed that the relative potency of several compounds to displace [^{3}H]mazindol binding to the transporter from rat striatum was correlated to drug self-administration studies in nonhuman primates. Similarly, Spealman and co-workers found a good correlation between displacement of [^{3}H]cocaine binding to the transporter and drug self-administration behavior in squirrel monkeys.[3] The most potent compounds in binding and behavioral studies reported from both investigations were 3β-phenyltropane-2β-carboxylic acid methyl ester (**2a**, WIN-35,065-2) and 3β-(p-fluorophenyl)tropane-2β-carboxylic acid methyl ester (**2b**, WIN-35,428), the so-called "WIN compounds" reported by Clarke and co-workers.[4]



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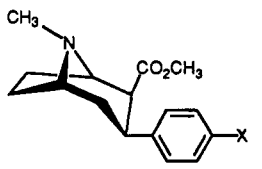

2a X = H
b X = F
c X = Cl
d X = CH_3

Only a limited number of cocaine analogues **2** have been available to study the structural requirements for binding to the dopamine transporter and for cocaine-like reinforcing properties. Moreover, no model has been developed to correlate the structural features and activity of the cocaine analogues **2**. As part of a research program aimed at the identification of the key structural features of (-)-cocaine related to its receptor binding and pharmacologic activity, we have synthesized and measured the binding affinity of several new 3β-(p-substituted phenyl)tropane-2β-carboxylic acid methyl esters.[5] In order to correlate the binding data with structural features and to take the first step in the development of a pharmacophore model for the cocaine binding site(s), we have conducted classical quantitative structure–activity relationship (QSAR) and comparative molecular field analysis (CoMFA) studies of the data.

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Results

Chemistry. Clarke and co-workers[4] reported that the addition of phenylmagnesium bromide to anhydroecgonine methyl ester (**3**) at -20 °C in ethyl ether gave a 75% yield of a 1:3 mixture of **2a** and 3β-phenyltropane-2α-carboxylic acid methyl ester (**4**) contaminated with a small amount of the 2β and 2α isomers of 3β-phenyl-2-benzoyltropane (**5**) (see Scheme I). The mixture of **2a** and **4** were separated from **5** by distillation. Chromatography on silica gel provided pure **2a** and **4**. We have repeated this reaction and found that compound **5** was not formed if the reaction is conducted at -40 °C using exactly 2 equiv of phenylmagnesium bromide. More importantly, quenching of the reaction mixture with 2 equiv of trifluoroacetic acid at -78 °C results in a 79% yield of a 1.6:1 mixture of **2a** and **4**. The pure β- and α-isomers were separated by chromatography on silica gel, eluting with a 9:1 mixture of ethyl ether and triethylamine. Starting with the appropriate arylmagnesium halide and using similar conditions, the 3β-(p-substituted phenyl)tropane-2β-carboxylic acid methyl esters (**2c–f**) and the thiophenyl analogue **2g** were obtained. The yields obtained and the properties of the compounds are listed in Table I. The 2α-isomers were not isolated. Compound **2f** is a known compound which was prepared in 1.2% yield by Clarke and co-workers.[4,6] Note that we obtained a 10% yield of **2f** using the modified procedure.

(1) Kuhar, M. J.; Ritz, M. C.; Sharkey, J. Cocaine Receptors on Dopamine Transporters Mediate Cocaine-reinforced Behavior. In *Mechanisms of Cocaine and Toxicity*; Clouet, D.; Asghar, K.; Brown, R., Eds.; NIDA Research Monograph, 1988; pp 14–22.

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(6) Clark and co-workers (ref 4) isolated **2f** as its naphthalene-1,5-disulfonate salt.

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Scheme I

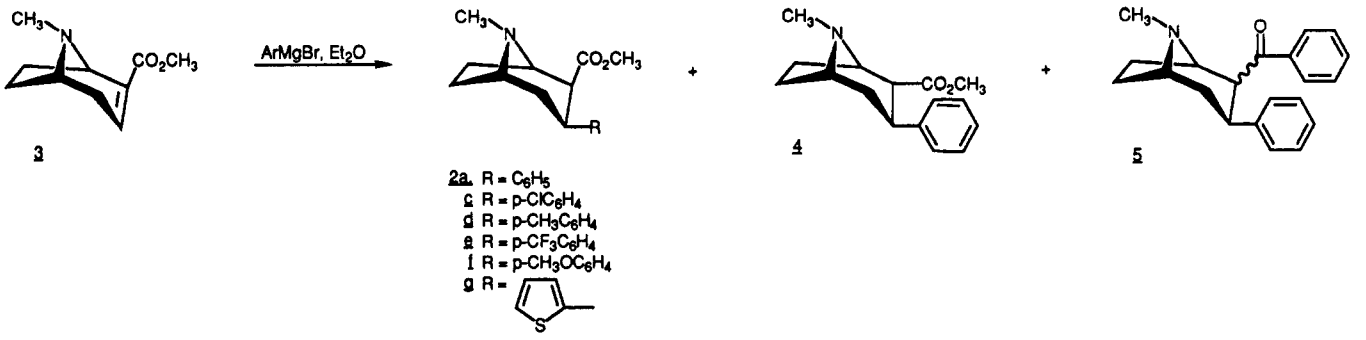
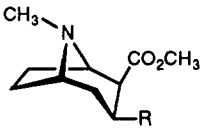


Table I. 3-Substituted Tropane-2-carboxylic Acid Methyl Esters (2)



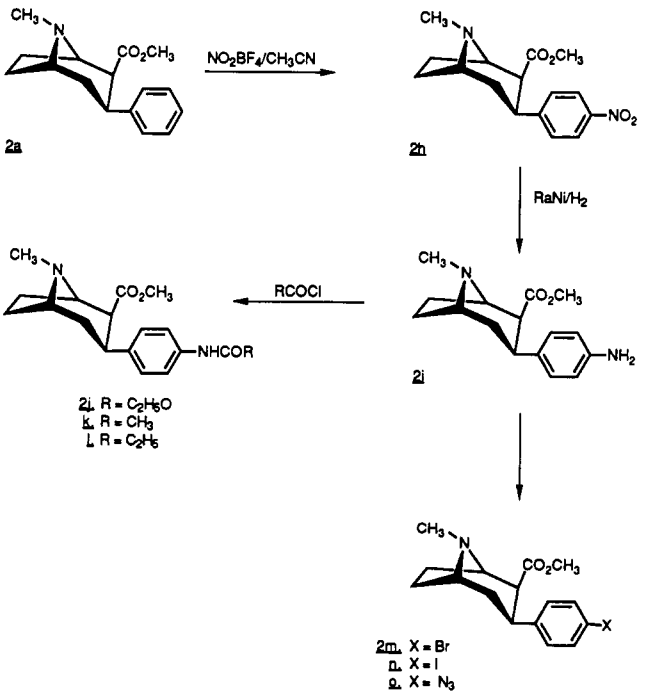
compd 2	R	mol formula[a]	yield (%)[b]	mp, °C	optical rotation $[\alpha]^{23}_D$ (c, MeOH)	^{1}H NMR δ (CH_3OH-d_4) NCH$_3$	OCH$_3$	H-2	H-3	other	aromatics
a	C_6H_5	$C_{20}H_{27}NO_8$	48	189–190	-92.9 (1.0)						
c	p-ClC_6H_4	$C_{20}H_{26}ClNO_8 \cdot 0.5H_2O$	42	128	-95.0 (0.2)	2.21 (s)	3.55 (s)	2.93 (m)	2.93 (m)		7.20/(m)
d	p-$CH_3C_6H_4$	$C_{21}H_{29}NO_8 \cdot 0.5H_2O$	33	132	-143.3 (0.18)	2.27 (s)	3.48 (s)			2.21 (s)[c]	7.11/(m)
e	p-$CF_3C_6H_4$	$C_{21}H_{26}NO_8 \cdot 0.5H_2O$	30	109–111	-77.2 (0.092)	2.23 (s)	3.50 (s)	2.99 (m)	3.04 (m)		7.30, 7.55
f[d]	p-$CH_3OC_6H_4$	$C_{21}H_{29}NO_9 \cdot H_2O$	11	66–67	-50.0 (0.092)	2.21 (s)	3.71 (s)	2.92 (m)	3.01 (m)	3.40 (s)[e]	6.72, 7.15
g		$C_{18}H_{25}NO_8S$	40	146	-90.0 (0.26)	2.21 (s)	3.52 (s)	2.81 (m)	3.17 (m)		6.71, 7.19

[a] All compounds are tartrate salts. [b] The salts were recrystallized from a methanol and ether mixture. [c] C-CH_3. [d] Clarke and co-workers (ref 4) reported a 1.2% yield of 2f. [e] OCH_3.

Kline et al.[7] recently reported that the nitration of **2a** using nitric acid gave the nitro compound **2h** along with a dinitro analogue. These authors also reported that catalytic reduction of **2h** using PtO_2 gave **2i**. The nitro analogue **2h** was isolated as the free base, the amino compound **2i** as the dihydrobromide salt. We found that nitration of **2a** under milder conditions with nitronium tetrafluoroborate in acetonitrile gave only the *p*-nitro analogue **2h** (Scheme II). Catalytic reduction of **2h** using Raney nickel catalyst yielded the *p*-amino analogue **2i**. Compounds **2h** and **2i** were characterized as their mono- and dihydrochloride salts. Acylation of **2i** with ethyl chloroformate, acetic anhydride, or propionic anhydride gave **2j–l**, respectively. Diazotization of **2i** followed by treatment with cuprous bromide, methylene iodide, or sodium azide gave **2m–o**, respectively.

Biological. Table II summarizes the IC_{50} and log $1/IC_{50}$ values for cocaine and 15 cocaine analogues for their ability to inhibit [^{3}H]-**2b**[5,8] binding to rat striatal membranes. With the exception of two compounds (**2g** and **2j**), all cocaine analogues had lower IC_{50}'s than cocaine. Moreover, eight of the analogues (**2c–f**, **2h**, and **2m–o**) were more potent than **2b**, the most potent compound reported before this study. The *p*-chloro and *p*-iodo analogues **2c** and **2n**, respectively, which are 85 and 78 times more potent than cocaine in inhibiting [^{3}H]-**2b** binding were the most potent analogues from this study. In addition, the *p*-bromo and *p*-methyl analogues **2m** and **2d**, respectively, were 56 and 60 times more potent than cocaine.

Scheme II



QSAR and CoMFA. Using conventional QSAR analysis,[9-11] the correlation between the binding affinity at the

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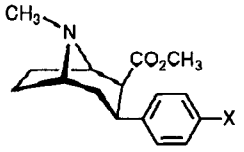
(8) Madras, B. K.; Spealman, R. D.; Fahey, M. A.; Neumeyer, J. L.; Saha, J. K.; Milius, R. A. Cocaine Receptors labeled by [^{3}H]2β-Carbomethoxy(4-fluorophenyl)tropane. *Mol. Pharmacol.* **1989**, *36*, 518–524.

Table II. Potencies of Cocaine and Related Compounds in Inhibiting Binding of 0.5 nM [^{3}H]-**2b**[a]



compound	X	IC_{50} (nM, mean ± SE)	log $1/IC_{50}$
(−)-cocaine	-	102 ● 12	-
2a	H	23.0 ± 5.0	−1.36
2b	F	15.7 ± 1.4	−1.20
2c	Cl	1.17 ± 0.10	−0.0682
2d	CH_3	1.71 ± 0.30	−0.233
2e	CF_3	13.1 ● 2.2	−1.12
2f	CH_3O	8.14 ± 1.3	−0.911
2g	*b*	257 ● 19	-
2h	NO_2	10.1 ● 0.10	−1.00
2i	NH_2	24.8 ± 1.3	−1.40
2j	C_2H_5OCONH	316 ± 48	−2.50
2k	CH_3CONH	64.2 ± 2.6	−1.81
2l	C_2H_5CONH	68.0 ± 2.7	−1.83
2m	Br	1.81 ± 0.30	−0.258
2n	I	1.26 ± 0.04	−0.100
2o	N_3	2.12 ± 0.1	−0.326

[a] All values are the mean of four to five experiments performed in triplicate. [b] 3β-(2-Thiopheneyl)tropane-2β-carboxylic acid methyl ester.

cocaine receptor and the physicochemical parameters of cocaine analogues as descriptors was determined by a partial least square (PLS) method in the SYBYL QSAR module.[12] PLS is a new regression technique[13] to solve the linear expression and produces results identical to multiple regression when applied to the same data set. The information from the PLS regression is directly comparable to the CoMFA (to be discussed later).

The molecular refractivity MR used as a steric index of the substituent and the Verloop Stermol multidimensional parameters were taken from the literature.[10,11,15] The hydrophobic parameter π of Hansch was also taken from the literature[11] or calculated from CLOGP and CMR.[14] Electronic effects of substituents were determined by using Hammett parameter σ, field (F), and resonance (R) parameters.[10,11] Twelve of the compounds shown in Table II (all compounds except **2f**, **2g**, and **2o**) were subjected to a PLS regression using the physicochemical parameters above. The selected analogues span a binding affinity range of 2.4 log units. Compound **2c** (log $1/IC_{50}$ = −0.068) was the most potent, and compound **2j** (log $1/IC_{50}$ = −2.50) was the least potent. Recognizing that too many combinations of descriptor variables with this small data set would run a high risk of error in terms of significant correlation coefficient, only correlations derived from a small number of variables were considered. Using this criterion, the PLS regression equations of combinations of two or three parameters for the analogues listed in Table II were calculated. The number of cross-validation groups was set to be equal to the number of the compounds (12) to establish the optimal number of components. This value was then used in the final calculation without cross-validation to give the QSAR equation. The resultant correlation equations are summarized in Table III.

CoMFA was also used to correlate binding affinity with the structural features of the cocaine analogues.[12,16] Structures for cocaine and the analogues **2a–f** and **2h–o** were constructed for the CoMFA[16] analyses using SYBYL software.[12] A computer representation of (−)-cocaine was constructed from the reported X-ray coordinates.[17] The analogues **2** were constructed from the (−)-cocaine structure using molecular molecule building and editing procedures. The known analogue **2f**[4] and azide **2o**, which was prepared as a potential photoaffinity ligand, were not included in the analysis and were reserved to test the predictability of the model. Due to the uncertain alignment of **2g**, this compound was also excluded from the analysis. Each starting structure was energy-minimized using MAXIMIN2 force field[12] followed by a conformational search around all rotatable bonds in the C-2 and C-3 substituents using an increment of 10°. The resulting global minimum was again optimized by MAXIMIN2 and used as the initial structure for a fully geometry optimization using AM1[18] semiempirical quantum mechanics calculations. The final AM1 geometry and charges were used in the CoMFA study. Even though the minimum-energy conformations of the cocaine analogues might not be the biologically active conformations, they are a reasonable starting point for comparative purposes and are informative.

After minimization, all the cocaine analogues were aligned by least-squares fitting of the six aromatic ring carbons and the C-3 of the tropane ring of each structure to the same atoms of compound **2a** using the FIT option of SYBYL.[12] The global minimum and aligned structures of all the compounds except **2f** and **2o** were subjected to CoMFA. Based on the molecular volume of the structures, a three-dimensional region was generated using the automatic mode of the QSAR option of SYBYL.[12] This assured that every grid in all directions protruded at least 4 Å beyond the shape of each molecule and was also separated by 2 Å along the lattice. Both steric (van der Waals) and electrostatic (coulombic) fields were probed with an sp^3 carbon possessing a +1.0 charge. The linear expression of CoMFA was calculated using the PLS method. In order to determine how well the model predicts data, each predictive value was cross-validated using five components, resulting in a determination of the optimal number of components. The statistical evaluation is shown in Table IV. The statistical parameters improved with use of up to four components, but deteriorated with five components. Since r^2 for the four component model was less than 5% different from that for the three component model, the

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(16) Cramer, R. D.; Patterson, D. E.; Bunce, J. D. Comparative Molecular Field Analysis (CoMFA). 1. Effect of Shape on Binding at Steroid to Carrier Proteins. *J. Am. Chem. Soc.* **1988**, *110*, 5959–5967.
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Table III. Selected Correlations of log $(1/IC_{50})$ against Physiochemical Parameters of 12 Cocaine Analogues[a]

regression equation	r^2	standard error of estimate	F values
$\log (1/IC_{50}) = -1.55 + (0.664)\pi$	0.409	0.628	6.93
$\log (1/IC_{50}) = -1.393 + (0.716)\pi + (0.435)\pi$	0.518	0.568	10.7
$\log (1/IC_{50}) = -1.174 + (0.463)\sigma + (0.492)\pi$	0.366	0.650	5.76
$\log (1/IC_{50}) = -2.525 + (0.422)\pi + (0.864)B_1$	0.450	0.606	8.20
$\log (1/IC_{50}) = -0.716 + (0.592)\pi - (0.470)$MR	0.550	0.549	12.2
$\log (1/IC_{50}) = -1.427 + (0.550)\pi + (1.101)F$	0.486	0.586	9.44
$\log (1/IC_{50}) = -1.706 + (0.536)\pi + (0.371)B_2$	0.359	0.655	5.60
$\log (1/IC_{50}) = -0.310 + (0.543)\pi - (0.322)B_4$	0.713	0.438	24.9
$\log (1/IC_{50}) = -0.532 + (1.074)F - (0.319)B_4$	0.517	0.568	10.7
$\log (1/IC_{50}) = -1.632 + (0.578)\pi + (0.352)\pi^2 + (1.157)F$	0.572	0.535	13.4
$\log (1/IC_{50}) = -1.477 + (0.588)\pi + (1.177)F - (0.176)R$	0.523	0.565	10.9

[a] π = hydrophobic parameter; MR = molecular refractivity of substituent; L, B_1–B_4 = Verloop Stermol multidimensional parameters; σ = Hammett para-directing parameter; F = field parameter; R = resonance parameter.

Table IV. CoMFA Analysis: Search for the Optimal Number of Components (12 Compounds, 12 Cross-Validation Groups)

	number of components				
	comp1	comp2	comp3	comp4	comp5
s	0.615	0.612	0.616	0.640	0.708
r^2	0.434	0.495	0.545	0.571	0.550
F	0.919	1.178	1.439	1.598	1.468
prob of $r^2 = 0$	0.527	0.417	0.332	0.291	0.324

Table V. Statistic Results of CaMFA Analysis with Zero Cross-Validation

s	0.212
r^2	0.946
F	47.0
prob of $r^2 = 0$	0.000

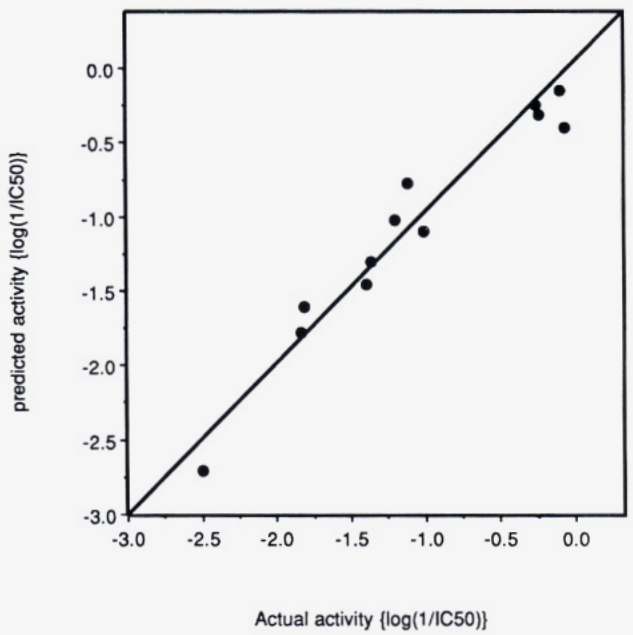

Figure 1. Predicted vs experimentally measured binding affinities to the dopamine transporter for 12 cocaine analogues.

latter was used for the final calculation. The final model obtained without cross-validation showed an $r^2 = 0.946$ and a standard error of estimate of 0.212 (Table V). Figure 1 shows a plot of the actual vs predicted binding affinities (Table II gives experimental values). Note that $r^2 \simeq 0.90$–1.0 indicates excellent predictive ability.[16] The relative contribution of steric and electrostatic potential to the CoMFA regression equation for binding affinity (log $1/IC_{50}$) to the dopamine transporter was found to be 62 and 38% steric and electrostatic, respectively. The standard deviation coefficient contour maps, derived from the final model, are used to display the CoMFA contributions of steric (Figure 2A) and electrostatic potential (Figure 2B). These contours show where the changes in fields are best associated with changes in binding affinity. The interactions are color coded with the red region associated with an increase in binding affinity and the green area a decrease. To aid visualization, highly potent **2c** and the relatively weak binding **2j** were overlayed with the contour maps. In addition, the van der Waals volume of the *p*-chloro group of **2c** is shown as yellow dots.

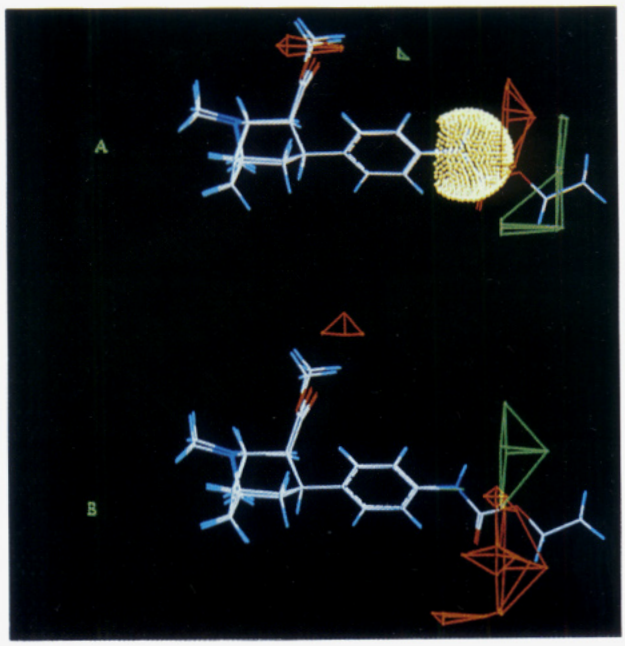

Figure 2. (A) View of the major steric fractions of the CoMFA contour map. Red and green contours show regions of lower (the standard deviation coefficient is greater than +0.05) and higher (less than −0.05) steric interactions, respectively. Molecules displayed are an overlay of cocaine analogues **2c** and **2j**. (B) View of the major electrostatic fractions of the CoMFA contour map. Red and green contours show regions of positive (the standard deviation coefficient is greater than +0.02) and negative (less than −0.02) electrostatic interactions, respectively. Molecules displayed are an overlay of cocaine analogues **2c** and **2j**.

The CoMFA model was used to make predictions for the binding affinity of analogues **2f** and **2o** that were not included in the CoMFA study. Structures for **2f** and **2o** were constructed, aligned as above, and added to the CoMFA QSAR table to calculate the predicted value. The predicted IC_{50} values for **2f** and **2o** were 7.6 and 10.6 nM, respectively.

Discussion

It has been known since the work of Clarke et al.[4] that replacement of the benzoyloxy functionality at the 3-

positions of cocaine by a phenyl moiety leads to substantially enhanced biological activity. Moreover, it has been shown by us[5] and others[7] that substitution of the phenyl ring has a marked effect on binding affinity to the dopamine transporter.

We had also found[19] that the cocaine binding site is both enantio- and stereoselective. Thus, the seven possible stereoisomers of (−)-cocaine including (+)-cocaine showed potencies for inhibiting binding of [^{3}H]-**2b** ranging from 1/600th to about 1/60th of that of cocaine.[19] A parallel effect has been reported for the "WIN" series of compounds. Specifically, **2a**, which possesses the same absolute stereochemistry as cocaine, was more than 1400 times as potent in inhibiting [^{3}H]mazindol binding than its enantiomer and its 2α-isomer **4**.[2]

Based on these observations, the investigation of additional 3β-(*p*-substituted phenyl)tropane-2β-carboxylic acid methyl esters retaining the absolute stereochemistry of (−)-cocaine, was undertaken. The para substituent was systematically varied with respect to size, electronic character, and hydrophobic properties. Analysis of binding results (Table II) does not reveal any apparent correlation. Substitution with electron-withdrawing (F, Cl, Br, I, NO_2), electron-donating (CH_3O), and neutral (CH_3) groups gave analogues with good to excellent potency. Replacement of the protons of the *p*-CH_3 analogue (**2d**) with fluorines resulted in a less potent compound (**2e**). Relatively small (Cl, CH_3) as well as large (Br, I) substituents gave potent compounds. Changing the substituent to an amino (**2i**) or acylated amino group (**2j–l**) led to a marked drop in potency. Replacement of the phenyl ring with an isosteric 2-thiopheneyl group gave a much less potent analogue (**2g**).

It is interesting to note that **2h** and **2i** were reported by Kline and co-workers[7] to be 14 and 8 times less potent than observed by us. This could be due to their use of bovine striatal membranes, [^{3}H]cocaine as the radioligand, different salts of **2h** and **2i**, or to some other unknown factor.

Since the inhibition of [^{3}H]-**2b** binding is clearly strongly dependent on the para substituent of the 3β-(*p*-substituent phenyl)tropane-2β-carboxylic acid methyl ester analogues **2**, a QSAR analysis was undertaken with the hope of finding a correlation between the binding affinity of the analogues and the physicochemical parameter(s) of the substituents. The equations along with the r^2 and *s* and *F* values are shown in Table III. The relatively low r^2 values and high *s* values show that none of the correlation equations show high intercorrelation among the 12 compounds.

CoMFA[12,16] was much more successful in the correlation of the structural features of the cocaine analogues **2** with their binding affinity than was the classical QSAR analysis. The value (0.946) of the CoMFA r^2 indicates satisfactory agreement between observed and predicted IC_{50} values. It suggests that the CoMFA sampling of the steric and electrostatic interactions of cocaine analogues may be capable of providing useful information about possible ligand–receptor interactions. Thus, the model provides satisfactory steric (shape) and electrostatic (electronic) specifications for the portion of the receptor binding site of the dopamine transporter which accommodates or interacts with the 3β-(*p*-substituted phenyl) substituents of **2a**. The steric contour map (Figure 2A) resulting from the CoMFA exhibits a major, unfavorable region, which is coded in green color. All the amide analogues (**2j–l**) place column in this region and all exhibit dramatically decreased affinities. However, in the vicinity of sterically unfavorable green region, another set of contours coded in red was observed as a positive contribution of affinity binding to the receptor. This area is located near most of the smaller substituents and apparently favors the more lipophilic groups (F, Cl, Br, I, and CH_3). The more polar functional groups such as amino and nitro are also accessible to this region but only showed moderate affinity. Analogues with a large bulky side chain with lipophilic character comparable to the small hydrophobic groups (i.e., ethoxycarbamate vs F or H) show greatly reduced potency. These results imply that receptor binding interactions can only accommodate steric bulk within a certain range, and beyond this point, hydrophobic groups do not enhance potency.

The electrostatic contour map shown in Figure 2B has a positive coefficient that is located at the lower part of the carbonyl oxygen groups of the amide. It is not clear whether the orientation of negative electrostatic potential of the carbonyl oxygen is sufficient to cause positive interaction with this region. Further modification without the steric bulk area of the amide function is needed in order to determine its electrostatic contribution (e.g., *N*-formyl substitution).

One advantage of CoMFA is the predictive potential of the model. Several successful studies suggest that CoMFA models give predicted values close to experimental results.[16] In order to test our model, the binding affinities of compounds **2f** and **2o**, which had been deliberately excluded from the CoMFA study, were used to evaluate the model's predictive ability. The predicted IC_{50} values of **2f** and **2o** are 7.6 and 10.6 nM, which is reasonably close to the experimental values of 8.1 and 2.1 nM, respectively.

It has been established that both the nitrogen and the aromatic ring of cocaine analogues are essential features of the pharmacophore for the cocaine binding site of the dopamine transporter.[2] The distance between the nitrogen and the centroid of the aromatic ring of **2a** and **1** are 5.6 and 7.7 Å, respectively. The binding affinity of **2a** is 4.4 times greater than that of **1**, suggesting that the aromatic ring of **2a** lies in a more favorable binding region. The present study has shown that the binding of **2a** is enhanced 20-fold by the addition of a *p*-chloro substituent to the aromatic ring of **2a**. It is of interest to determine if the CoMFA model can explain the difference in binding affinity between **1**, **2a**, and **2c**. An overlay of (−)-cocaine and **2c**, fitted to each other by the atoms of the tropane ring, along with the steric and electrostatic CoMFA coefficient contour map, are shown in parts A and B, respectively, of Figure 3. The 3, 4, and 5 carbons and the 3, 4, and 5 protons of the aromatic ring of (−)-cocaine are located closer to the negative and positive contour region of both maps than the *p*-chloro substituent of **2a**. If the negative region is more critical to binding than the positive region, the weaker binding of cocaine could be due to steric or electrostatic interaction in the region of negative interaction. In this regard, it is interesting to note that all substituted aromatic analogues of cocaine (sterically larger group) reported to date have shown weaker binding than (−)-cocaine.[2]

This study has provided valuable information concerning the structural requirements of the aromatic ring at C-3

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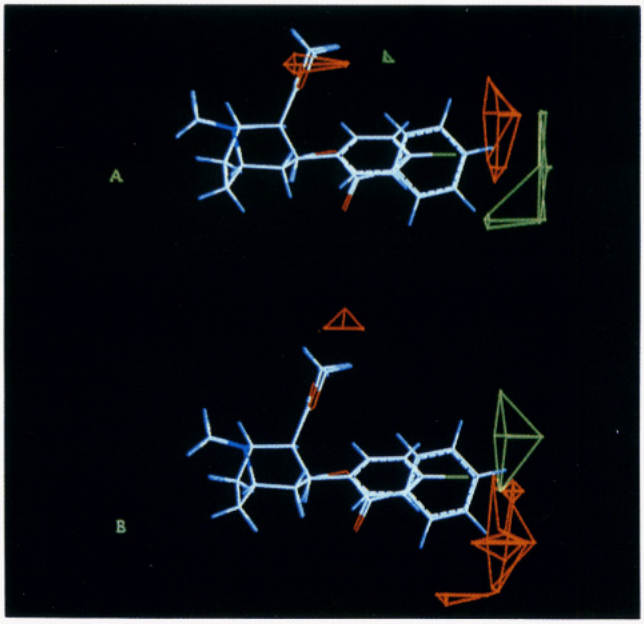

Figure 3. The overlay of the structures of (−)-cocaine and its congener **2c** and (A) the steric CoMFA contour map and (B) the electrostatic CoMFA contour map.

of cocaine and its analogues. Studies are in progress to further characterize this region as well as other regions of the cocaine structure.

Conclusions

A SAR, QSAR, and CoMFA study of a limited series of 3β-(*p*-substituted phenyl)tropane-2β-carboxylic acids methyl esters (**2**) has been described. In conclusion: (1) a good yield synthesis of **2** that provides much higher stereoselectivity for the 2β-isomer than previously reported procedures[4] has been developed; (2) highly potent inhibitors for the dopamine transport at the cocaine binding site were obtained by modification of **2a**, e.g., the *p*-chloro and *p*-iodo analogues **2c** and **2n** with potencies 85 and 78 times greater than that of (−)-cocaine; (3) a CoMFA model developed from 12 of the cocaine analogues **2** provided (a) a correlation with high predictive value, (b) a reasonable explanation for the relative potency of (−)-cocaine and the cocaine analogues **2**, and (c) information about topography of the receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. High-resolution mass spectra were obtained on a VG Analytical ZAB E spectrometer. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates eluted with $CHCl_3$–MeOH–concentrated NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. Microanalyses were carried out by Atlantic Microlab, Inc. [^{3}H]-**2b**, (3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester) with specific activity 83.1 Ci/mmol was purchased from Dupont-New England Nuclear (Boston, MA).

3β-Phenyltropane-2β-carboxylic Acid Methyl Ester (2a). A solution of (*R*)-(−)-anhydroecgonine methyl ester[4] (9.15 g, 0.051 mol) in freshly distilled Et_2O (100 mL) was added dropwise to a stirred solution of phenylmagnesium bromide (34 mL, 3 M solution, 0.102 mol) in dry Et_2O (800 mL) at −40 °C under an argon atmosphere. The mixture was stirred for 2.5 h at that temperature, cooled to −78 °C, and treated with TFA (11.6 g, 0.102 mol) in dry Et_2O (50 mL) over a period of 5 min. The mixture was allowed to warm to 0 °C and was diluted with water (300 mL). The aqueous phase was acidified to approximately pH 1 with concentrated HCl, and the Et_2O layer was separated. The aqueous layer was basified with concentrated NH_4OH at 0 °C and was extracted with Et_2O (4 × 200 mL). The dried ether solution (Na_2SO_4) was evaporated to give a yellow oil (11.05 g). The crude product was placed on a silica gel (200 g) flash column and was eluted with Et_2O–Et_3N (9:1).

The fractions containing the first component were pooled and evaporated to dryness to give 5.30 g (48%) of **2a**. This was converted to the tartaric acid salt in MeOH and was crystallized by adding Et_2O to the cloud point to give 7.5 g (45%) of **2a** tartrate: mp 189–190 °C, $[\alpha]^{23}_D$ −92.9° (*c* 2.0, MeOH).

The fractions with the second component were combined and evaporated to give 3.5 g (31%) of **4** (α-isomer). The pentane crystallized sample had mp 70–71 °C (lit.[4] mp 70–71 °C); $[\alpha]^{23}_D$ +4.56° (*c* 1.98, $CHCl_3$) (lit.[4] $[\alpha]^{25}_D$ +4.6° (*c* 2.0, $CHCl_3$).

Continued elution gave 1.33 g (14%) of the starting material anhydroecognine methyl ester (**3**).

3β-(*p*-Substituted phenyl)tropane-2β-carboxylic Acid Methyl Esters (2c–g). Compounds **2c–f** were prepared by a procedure similar to that described for **2a**. Compound **2g** was prepared by a procedure analogous to that reported by Clarke and co-workers.[4] The yields and properties of the compounds, characterized as their tartrate salts, are given in Table I.

3β-(4-Nitrophenyl)tropane-2β-carboxylic Acid Methyl Ester (2h). To a solution of **2a** (2.01 g, 0.008 mol) in dry MeCN (75 mL) at 0 °C under nitrogen was added solid nitronium tetrafluoroborate (1.50 g, 0.011 mol) in one portion. The reaction mixture was stirred at 0 °C for 8 h. The mixture was cooled to −25 °C, and ice (2 g) was added. After standing at this temperature for 8 h, the mixture was filtered cold. The filtrate was concentrated on a rotary evaporator, and the residue was purified by column chromatography on a silica gel column, eluting with pentane–Et_2O–$(iPr)_2NH$ (67:30:3). Removal of the solvent gave pure **2h** (2.0 g, 84% yield) as an oil (lit.[7] mp 95–97 °C): ^{1}H NMR ($CDCl_3$) δ 1.66 (m, 2, H-6 endo, H-7 endo), 2.19 (m, 2, H-6 exo, H-7 exo), 2.23 (s, 3, NCH_3), 2.66 (m, 1, H-4 endo), 3.05 (m, 2, H-2, H-4 exo), 3.44 (m, 1, H-3), 3.50 (s, 3, CO_2CH_3), 3.64 (m, 2, H-1, H-5), 7.40, 8.11 (AB q, 4, C_6H_4, J_{AB} = 7.50 Hz).

The hydrochloride salt of **2h** was prepared by standard methods and recrystallized from MeOH–Et_2O. Product obtained is a low melting foam: $[a]^{24}_D$ −69.18° (*c* 0.31, CH_3OH). Anal. ($C_{16}H_{21}ClN_2O_4 \cdot 1.5H_2O$): C, H, N.

3β-(4-Aminophenyl)tropane-2β-carboxylic Acid Methyl Ester (2i). Ra-Ni (400 mg) was washed several times with dry MeOH (20 mL) on a sintered glass funnel and then transferred into a round-bottom flask containing dry MeOH (20 mL). A solution of **2h** (290 mg, 0.95 mmol) in dry MeOH (5 mL) was added to the catalyst and stirred under a hydrogen atmosphere overnight at room temperature. The catalyst was removed by filtration through a Celite pad in a sintered-glass funnel. Removal of the solvent on a rotary evaporator gave crude product, which was purified by column chromatography on silica gel eluting with $CHCl_3$–MeOH–concentrated NH_4OH (80:18:2). Removal of the solvent from the combined pure fraction gave **2i** (200 mg, 77%); ^{1}H NMR ($CDCl_3$) δ 1.68–1.92 (m, 4, H-6, H-7), 2.18 (m, 2), 2.22 (s, 3, NCH_3), 2.54 (m, 1), 2.85 (m, 2), 3.33 (m, 1, H-1), 3.50 (s, 3, $COOCH_3$), 3.51 (m, 1, H-5), 6.60, 7.05 (AB q, 4, ArH).

The hydrochloride salt was prepared by standard methods and recrystallized from iPrOH–MeOH: mp 205–207 °C dec (lit.[7] mp 220–223 °C for the hydrobromide salt); $[\alpha]^{24}_D$ −73.24° (*c* 0.228, MeOH). Anal. ($C_{16}H_{24}Cl_2N_2O_2 \cdot H_2O$): C, H, N, Cl.

3β-(4-Acetamidophenyl)tropane-2β-carboxylic Acid Methyl Ester (2k). To a solution of **2i** (70 mg, 0.255 mmol) in glacial HOAc (2 mL) were added Ac_2O (0.043 mL) and zinc dust (catalytic amount). The reaction mixture was refluxed under nitrogen for 0.5 h and then cooled to room temperature. The white residue was removed by filtration, and the solvent was removed from the filtrate on a rotary evaporator. The residue was dissolved in $CHCl_3$ (25 mL). The solution was washed with a saturated solution of K_2CO_3 (2 × 5 mL), and the organic layer was dried over anhydrous Na_2CO_3. Filtration and removal of the solvent gave crude **2k**, which was purified by column chromatography on silica gel, eluting first with $CHCl_3$–MeOH (95:5) to remove the less polar impurities and then with $CHCl_3$–MeOH–NH_4OH (80:18:2). Removal of the solvent from the combined pure

fractions gave **2k** (61 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 1.62–1.81 (m, 2, H-6 endo, H-7 endo), 2.08 (s, 3, $COCH_3$), 2.21 (s, 3, NCH_3), 2.51 (m, 1, H-4 exo), 2.92 (m, 2, H-2, H-3), 3.30–3.61 (m, 6 H, CO_2CH_3, H-1, H-5, NH), 7.21 (AB q, 4, C_6H_4).

The tartrate salt of **2k** was prepared by standard methods and recrystallized from MeOH–Et_2O: mp 100 °C; $[\alpha]^{24}_D$ –82° (*c* 0.1, MeOH). Anal. ($C_{22}H_{30}N_2O_9 \cdot H_2O$): C, H; N: calcd 5.78, found 5.26.

3β-(4-Ethoxycarboxamidophenyl)tropane-2β-carboxylic Acid Methyl Ester (2j). To a stirred solution of **2i** (200 mg, 0.728 mmol) in dry CH_2Cl_2 (5 mL) were added Et_3N (0.15 mL) and ethyl chloroformate at 0 °C under nitrogen. After 3 h, the solution was diluted with 30 mL of CH_2Cl_2, washed with saturated K_2CO_3 solution (2 × 5 mL) and water (2 × 5 mL), and dried over anhydrous Na_2CO_3. Filtration and removal of the solvent gave the crude product, which was purified by column chromatography on silica gel, eluting with $CHCl_3$–MeOH (9:1). Removal of the solvent from the combined pure fractions gave **2j** (146 mg, 58% yield): ^{1}H NMR (CDCl3) δ 1.32 (t, 3, CH_3), 1.55–1.79 (m, 2, H-6 endo, H-7 endo), 2.01–2.19 (m, 2, H-6 exo, H-7 exo), 2.21 (s, 3, NCH_3), 2.55 (m, 1, H-4 endo), 2.98 (m, 2, H-4 exo, H-2), 3.35 (m, 1, H-5), 3.48 (s, 3, CO_2CH_3), 3.54 (m, 2, H-3, H-1), 4.21 (q, 2, CH_2), 6.71 (br, 1, NH), 7.23 (m, 4, C_6H_4).

The tartrate salt was prepared by standard methods and recrystallized from MeOH–Et_2O: 87–88 °C dec; [α[$^{24}_D$ –82.9° (*c* 0.14, MeOH). Anal. ($C_{23}H_{32}N_2O_{10} \cdot H_2O$): C, H,; N: calcd 5.44, found 4.84.

3β-(4-Propionylaminophenyl)tropane-2β-carboxylic Acid Methyl Ester (2l). To a solution of **2i** (120 mg, 0.437 mmol) in propionic acid (2 mL) was added propionic anhydride (0.1 mL) from a syringe. A catalytic amount of zinc dust was added to the solution, which was heated under reflux for 0.5 h. The solution was cooled to room temperature, the white precipitate that formed was removed by filtration, and the filtrate was concentrated on a rotary evaporator. The crude residue was dissolved in 25 mL of $CHCl_3$, washed with saturated K_2CO_3 (2 × 5 mL), and dried over anhydrous Na_2CO_3. Filtration and removal of the solvent gave crude **2l**, which was purified by column chromatography on silica gel column, eluting first with $CHCl_3$–MeOH (95:5) to remove the less polar impurities and then with $CHCl_3$–MeOH–NH_4OH (80:18:2). Removal of the solvent from the combined pure fraction gave **2l** (110 mg, 76% yield): ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3, CH_3), 1.31–1.79 (m, 4, 2 H-6, 2 H-7), 2.01 (m, 1, H-4 endo), 2.19 (s, 3, NCH_3), 2.25 (m, 1, H-4 exo), 2.91 (m, 2, H-2, H-3), 3.30 (m, 1, H-5), 3.41 (s, 3, CO_2CH_3), 3.55 (m, 1, H-1), 3.71 (q, 2, CH_2), 7.25 (AB q, 4, C_6H_4).

The tartrate salt was prepared by standard methods and recrystallized from MeOH–Et_2O: mp 90 °C dec; $[\alpha]^{24}_D$ –80.26 (*c* 0.106, MeOH). Anal. ($C_{23}H_{32}N_2O_9 \cdot 1.5H_2O$): C, H; N: calcd 5.51, found 5.01.

3β-(4-Azidophenyl)tropane-2β-carboxylic Acid Methyl Ester (2o). To a stirred solution of **2i** (53 mg, 0.193 mmol) in HOAc (1 mL, 3 M solution) at 0 °C was added sodium nitrite (18.66 mg, 0.27 mmol) in water (0.5 mL). After 0.5 h, a solution of sodium azide (18.19 mg, 0.279 mmol) in water (0.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature for another 0.5 h. The solvent was removed on a rotary evaporator, the residue was dissolved in 30 mL of $CHCl_3$, and the solution was dried over anhydrous Na_2CO_3. Filtration and removal of the solvent gave crude **2o**, which was purified by column chromatography on silica gel eluting with $CHCl_3$–MeOH (95:5). Removal of the solvent from the combined pure fractions gave **2o** (34.5 mg, 60%): IR 2140 (N_3), 1750 cm^{-1} (ester); ^{1}H NMR ($CDCl_3$) δ 1.51–1.78 (m, 2, H-6 endo, H-7 endo), 1.98–2.01 (m, 2, H-6 exo, H-7 exo), 2.19 (s, 3, NCH_3), 2.21 (m, 1, H-4 endo), 2.58 (m, 1, H-4 exo), 2.74 (m, 2, H-2, H-3), 3.31 (m, 1, H-5), 3.46 (s, 3, CO_2CH_3), 3.58 (m, 1, H-1), 6.90, 7.25 (AB q, 4, C_6H_4).

The tartrate salt of **2o** was prepared by standard methods and recrystallized from MeOH–Et_2O: mp 80 °C dec; $[\alpha]^{24}_D$ –81.5° (*c* 0.092, MeOH). Anal. ($C_{20}H_{26}N_4O_8 \cdot 3.5H_2O$): C, H, N.

3β-(4-Bromophenyl)tropane-2β-carboxylic Acid Methyl Ester (2m). An ice-cold solution of **2i** (100 mg, 0.364 mmol) in aqueous HOAc (2 mL, 3 M solution) was treated with a solution of sodium nitrite (35.21 mg, 0.51 mmol) in water (0.5 mL). The reaction mixture was stirred at 0 °C for 0.5 h. A solution of freshly prepared CuBr (1.12 equiv) in water (2 mL) was added to the diazonium salt at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature overnight. The solvent was removed on a rotary evaporator, the crude residue was dissolved in 50 mL of $CHCl_3$, and the solution was dried over anhydrous Na_2CO_3 overnight. Filtration and removal of the solvent gave the crude product, which was purified by column chromatography on silica gel column eluting with $CHCl_3$–MeOH (95:5). Removal of the solvent from the combined pure fractions gave **2m** (20 mg, 16%): ^{1}H NMR ($CDCl_3$) δ 1.41–1.78 (m, 2, H-6 endo, H-7 endo), 1.99–2.12 (m, 2, H-6 exo, H-7 exo), 2.21 (s, 3, NCH_3), 2.23 (m, 1, H-4 endo), 2.82 (m, 2, H-4 exo, H-2), 3.21–3.31 (m, 2, H-5, H-3), 3.46 (s, 3, CO_2CH_3), 3.52 (m, 1, H-1), 7.25 (AB q, 4, C_6H_4).

The tartrate salt was prepared by standard methods and recrystallized from MeOH–Et_2O: mp 122 °C dec; $[\alpha]^{24}_D$ –95.6° (*c* 0.09, MeOH). Anal. ($C_{20}H_{26}BrNO_8$): C, H, N.

3β-(4-Iodophenyl)tropane-2β-carboxylic Acid Methyl Ester (2n). To a stirred mixture of **2i** (140 mg, 0.510 mmol) and diiodomethane (5 mL) under an atmosphere of nitrogen was added isoamylnitrite (0.129 mL, 0.960 mmol) from a syringe. The reaction mixture was stirred at room temperature for 1 h under nitrogen. The color of the solution turned golden red. The reaction mixture was heated at 55 °C for 3 h under nitrogen. After cooling the solution at room temperature, the solvent was removed on a rotary evaporator under reduced pressure. The residue was chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH (95:5). Removal of the solvent from the combined pure fractions gave **2n** (147.4 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 1.40–1.81 (m, 2, H-6 endo, H-7 endo), 1.96–2.01 (m, 2, H-6 exo, H-7 exo), 2.21 (s, 3, NCH_3), 2.22 (m, 1, H-4 endo), 2.52 (m, 1, H-4 exo), 2.75 (m, 2, H-2, H-3), 3.32 (m, 1, H-5), 3.48 (s, 3, CO_2CH_3), 3.59 (m, 1, H-1), 6.95, 7.61 (AB q, 4, C_6H_4).

The tartrate salt was prepared by standard methods and recrystallized from MeOH–Et_2O: mp 72–74 °C dec; $[\alpha]^{24}_D$ –73.8° (*c* 0.32, MeOH). Anal. ($C_{20}H_{26}INO_8$): C, H, N.

Biological. [^{3}H]-2b Radioligand Binding. Rat striata from male Sprague–Dawley rats (250–350 g) were rapidly dissected, frozen, and stored at –70 °C until used. The frozen rat striata were homogenized in 20 volumes of 10 mM phosphate buffer (pH 7.4) containing 0.32 M sucrose using a polytron (setting 6) for 10 s. The homogeneate was centrifuged for 10 min at 40000*g*, the resulting pellet was washed in buffer, recentrifuged, and resuspended to a tissue concentration of 1.0 mg/mL. Binding assays were carried out in a total volume of 0.5 mL containing 0.5 nM [^{3}H]-**2b**. The suspensions were incubated for 2 h on ice. Incubations were terminated by filtration with three 5-mL washes through Whatman GF/B filters previously soaked in 0.05% polyethylenimine. Radioactivity was counted in 5 mL of scintillation cocktail at an efficiency of 50–55%. Nonspecific binding of [^{3}H]-**2b** was defined by the presence of 30 μM (–)-cocaine. Under these conditions, nonspecific binding was approximately 5–8% of total binding. IC_{50} values were determined from competition curves of 10–12 points utilizing the curve fitting program EBDA.[21] Mean values and standard errors were collected from 3–4 assays for each test drug.

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Supplementary Material Available: Table listing the physicochemical parameters used in the QSAR analysis (1 page). Ordering information is given on any current masthead page.

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