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An Analysis of the Binding of Cocaine Analogues to the Monoamine Transporters Using Tensor Decomposition 3-D QSAR

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Dedicated to Professor B.R. Condray

Abstract—The conformation and alignment of cocaine analogues bound to the monoamine transporter proteins were explored using the tensor decomposition 3-D QSAR method. It is proposed from these calculations that the bound conformation of these ligands to the three transporter proteins has the 3β-aryl substituent in a conformation in which the aryl group is orthogonal or approximately orthogonal to the tropane ring. Based on these results, rigid and semi-rigid tropane analogues were designed, synthesized and their affinities for the monoamine transporters were determined.

Introduction

The monoamine transporter proteins[1,2] are important biopolymers involved in neurotransmission by dopamine, serotonin and norepinephrine. They are referred to here as DAT, SERT, and NET, respectively. Their function is to remove the neurotransmitters from the synaptic cleft and inhibition of this process is thought to be associated with, among other effects, the addictive properties of cocaine.[3] The transporter proteins are transmembrane proteins thought to have 12 transmembrane helices[4] and all have been cloned.[5]

The three-dimensional structures of the transporter proteins are not known. Designing ligands with high affinity and specificity for these proteins is generally based on the analysis of structure–activity data derived from published binding studies. The pioneering work of Hansch[6] provided a formalism for analyzing structure–activity data in which the data are computed from the two-dimensional structure of the ligands. This was extended by Cramer et al.[7] who developed the CoMFA method for the analysis of structure–activity data in which structural data are computed from the three-dimensional structures of bioactive compounds. Using CoMFA, a receptor-bound conformation and alignment for a reference compound in a series is assumed. A pair-wise superposition of each ligand in the series on this reference structure is carried out and field-related energies are computed at defined points on a grid about the bioactive compounds. These field probe energies, which are conformation and alignment dependent, are used as descriptors to derive three-dimensional quantitative structure–activity relationships, 3-D QSAR, using partial least squares, PLS, regression.[8]

This method has been used extensively but has the limitation of having to assume a receptor-bound conformation and alignment. CoMFA is sensitive to the alignment[9] and spatial orientation[10] assumed. Several methods of 3-D QSAR have been proposed to overcome these limitations, for example HASL[11] and 4-D QSAR.[12,13]

We have developed within the Hansch formalism[6] a method for dealing with the conformation/alignment problem, tensor decomposition 3-D QSAR.[14] It is an extension of CoMFA in that the fixed conformation/

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alignment constraints of this method are relaxed to allow the compounds under study to assume multiple conformations and alignments.[14] For each ligand in a series molecular field probe energies are computed relative to a reference in multiple, low energy conformations and alignments. The field probe energies, which are compound, conformation and alignment dependent, are third order tensors. These tensors are decomposed using three-way partial least squares, PLS, regression[15] to provide the conformations and alignments most highly correlated with the observed changes in activity. In an initial study, it predicted the bound conformation and alignment of a series of dihydrofolate reductase inhibitors.[14]

The method is outlined below in eqs (1)–(5). The array of molecular field descriptors, $\boldsymbol{X}_{lmn}$ computed for a specific compound, l, in a specified conformation m and alignment n is shown as a third order tensor in eq (1), where a, b, and c are the elements of the tensors. $\boldsymbol{X}_{lmn}$ is a three-way array in which the ways are l, m and n.

$$\boldsymbol{X}_{lmn} = \sum_{o}\sum_{p}\sum_{q} a_{lo} b_{mp} c_{nq} g_{opq} \tag{1}$$

Assuming that activity, y_l, is a function of $\boldsymbol{X}_{lmn}$ leads to eq (2) in which the summation is taken over all variables, J. The indices, o, p, q are the number of PLS components computed for each way in the three-way array. This will be discussed below.

$$y_l = \sum_{j=1}^{J} f(\boldsymbol{X}_{lmn}) = \sum_{j=1}^{J} f\left(a_{lo} b_{mp} c_{nq} g_{opq}\right) \tag{2}$$

This model can be computed by decomposing the $\boldsymbol{X}_{lmn}$ into the coefficient for compound, a_{lo}, the coefficient for conformation, b_{mp}, and the coefficient for alignment, c_{mq}. The term, g, can be interpreted as the elements of a third order tensor which transform the three-way array, $\boldsymbol{X}$, from one Cartesian coordinate system to another in the same way that factor loadings transform objects from one rectangular Cartesian coordinate system to another. The o, p, q indices then can be thought of as the number of ways that conformation and alignment can effect or influence activity for each compound.

Three-way PLS regression can be used to compute the above coefficients, which are indicators of the bound conformation and alignment. The three-way PLS model is given in eqs (3)–(5). This is very similar to the two-way PLS model in which the latent variables, t_{la} and u_{ai} are extracted from the descriptor and activity data approximately along their axes of greatest variation and which are optimally correlated. It is the regression method of choice to use with data which contain relatively low signal to noise, as is the case here. It is also stable when used for the analysis of under determined data, that is data in which the number of descriptors greatly exceeds the number of compounds. The latent variables are related through the inner relation as shown in eq (5).

$$X_{lmn} = \sum_{j=1}^{J}\left[x_{jlmn} + \sum_{a=1}^{A} t_{la} \otimes P_{amn} + e_{jlmn} \right] \tag{3}$$

$$y_{li} = y_i + \sum_{a=1}^{A} u_{la} q_{ai} + e_{li} \tag{4}$$

$$\hat{\mathrm{u}} = bt \tag{5}$$

The three-way PLS model above is similar to the two-way version with the exception that in the prediction phase of the analysis, the Kronecker product, $t_l \ldots P_{mn}$ is computed to estimate the systematic variation in the data. P_{mn} is a two-way array which contains the conformation/alignment weights. These elements are the composites of the terms, b and c, in eqs (1) and (2). An orthogonal decomposition of this two-way array would give these terms directly. However, for the purposes of this analysis it is only necessary to compute P as its largest elements contain information about the bound conformation/alignment.

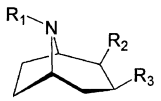
The conformational and alignments are ranked using a metric which is shown in eq (6). CAW is the fraction of the Y-variance explained by the molecular field analysis descriptors computed for each conformation/alignment set. In eq (6), Var is the Y-variance explained in component a and is summed over all components, A.

$$CAW_{mn} = \sum_{a=1}^{A} \mathrm{Var}_a * p_{mn}^2 \tag{6}$$

The p are elements of P and p_{mn}^2 are the squares of the PLS loadings for variables for conformation m and alignment n.

As used here, three-way PLS is a filter to select a bound conformation/alignment. Using genetic function analysis, GFA, with PLS, or GFA/PLS,[16] predictive models can be derived for the design of new ligands. GFA is a newly described method for model derivation that uses genetic operators, mutations and cross-links, to generate models by selecting descriptors, or sets of descriptors, which are optimal for relating the structural data to activity.[16] It was originally developed using ordinary regression as the modeling method but more recently we have integrated it with PLS as the modeling method.[16] In the final stages of model development, the analysis is rotated back into the original variable space which allows for a straight-forward interpretation of the results. This method has been incorporated into the Cerius[2] program which was used here.

As a further test, and validation, of this method a set of 89 cocaine derivatives, all 2β,3β-aryl tropane derivatives in the 1*R* configuration, with binding data to the DAT, SERT and NET, were taken from the literature.[17–19] The structures are represented by **1** where R_1 is H or CH_3 and R_2 and R_3 are freely rotating groups.



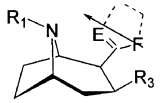
1

Both the R_2 and R_3 positions were variously substituted. The reference compound for this study has R_1 = methyl, R_2 = carbomethoxy and R_3 = phenyl. The data were from reports from the same laboratory and were obtained under the same conditions. All compounds had IC_{50} data reported for binding to the DAT, SERT, and NET, and in order to minimize experimental variation in these data, their data were determined using the same labeling ligands. These were [^{3}H]WIN 35,428, [^{3}H]paroxetine, and [^{3}H]nisoxetine, for the DAT, SERT and NET, respectively. The IC_{50} (nM) values were converted to the $-\log(IC_{50})$, or pIC_{50}, and the affinity range for the set was from a pIC_{50} of 4.0 to a pIC_{50} of 9.4. The data set was divided into a 68-compound training set and a 21-compound test set. The training set was established by selecting every fourth compound from the original set so that the test and training sets represented the complete range in activity. The training set data are available electronically on request from the author at wjdiii@uic.edu.

Molecular Modeling and Data Analysis

To explore the conformational properties of the compounds in the 68-compound training set, their structures were built using the Cerius^2 program[20] and the Universal Open Force Field.[21] The molecules were modeled in the neutral form and partial charges were applied using the Charge-Equilibration[22] method. Structures were minimized to convergence.

From results of the conformational analyses, several generalizations became apparent. All compounds had several low energy conformations within 10 kcal/mol of the minimum.



2

Many compounds in their lowest energy conformations had the aryl group orthogonal or approximately orthogonal to the tropane ring. Some, however, had the aryl group in an orientation that was approximately planar with the C3–C4 bond of the tropane ring. While the chair form of the tropane ring was generally favored by approximately 2 kcal/mol over the boat form, a number of compounds existed in their lowest energy conformation with the tropane in the boat form. On inspection of their structures, there did not seem to be any obvious relationship with substituents or activity. The conformations were also computed with the Dreiding[23] force field which conserved these low energy conformations.

The conformation of the 2β-substituent existed in an orientation in which its dipole was aligned either toward or away from the amino nitrogen. This is shown in structure **2** with the negative dipole directed toward the tropane nitrogen. The atoms E and F indicate hetero atoms corresponding to the ester group present in the reference structure and the dashed bonds indicate that these atoms may be included in a five-membered heterocyclic structure. In order to examine the effect of the orientation of the amino substituent, the analyses showed that in all cases the amino substituent is in the equatorial position as shown in **2** and that the axial conformer was at least 2 kcal/mol higher in energy than the equatorial isomer. It has been proposed that the orientation of the electron pair of the amino nitrogen effects transporter selectivity.[24] These generalizations were used to define the seven conformations used in the analysis.

Conformation 1 is defined as having the tropane ring in the chair form, the dipole moment of the C2-substituent pointing toward the amino nitrogen and the phenyl ring orthogonal to the plane of the C2–C3–C4 atoms in the tropane group.

Conformation 2 is defined as having the tropane ring in the chair form, the dipole moment of the C2-substituent pointing away from the amino nitrogen and the phenyl ring orthogonal to the plane of the C2–C3–C4 atoms in the tropane ring.

Conformation 3 is defined as having the tropane ring in the chair conformation, the dipole of the C2-substituent pointing toward the amino nitrogen and the phenyl ring in the plane of the C3–C4 bond of the tropane ring.

Conformation 4 is defined as having the tropane ring in the chair conformation, the dipole of the C2-substituent pointing away from the amino nitrogen and the phenyl ring in the plane of the C3–C4 bond of the tropane ring.

Conformation 5 is defined as having the tropane ring in the boat conformation, the dipole of the C2-substituent pointing toward the amino nitrogen and the phenyl ring orthogonal to the plane of the C2–C3–C4 atoms in the tropane ring.

Conformation 6 is defined as having the tropane ring in the boat conformation, the dipole of the C2-substituent pointing away from the amino nitrogen and the phenyl ring orthogonal to the plane of the C2–C3–C4 atoms in the tropane ring.

Conformation 7 is the lowest energy conformer of each compound. Structures of these conformations are available from the authors.

The alignment rule used involved all of the atoms of the tropane system, the carbon atoms attached to the C2- and C3-positions of the tropane ring and the atoms E

and F in structure **2**. Several other alignments using subsets of these atoms, which are present in all of the structures in the data set, were considered but they resulted in little or no difference in the molecular field descriptors generated. Each compound in the training set was aligned pair-wise with the reference compound using this alignment and the seven conformations above.

The molecular field descriptors were generated using five probes, the proton probe, the methyl probe, the hydroxide probe, the methanide probe and the methylium probe. These probes are assumed to represent functional groups in the receptor that can interact with complementary groups of the ligand. The proton probe represents a cationic site and hydrogen bond donor, the hydroxide ion an anionic site and hydrogen bond acceptor, the methanide probe represents an anionic site with steric interactions, the methylium probe represents a cationic site with steric interacations and the methyl probe represents a steric/hydrophobic site.

A (10×10×10) X cube was used with energies computed at 2 X points in the grid for each probe. A 30 kcal/mol cut-off was used and data were filtered by retaining the 10% of the descriptors with the highest variance. They were mean-centered but were not scaled. The descriptors were then exported and analyzed with three-way PLS software developed in this laboratory for this project. The activity data were analyzed as a two-way array so that a single PLS model resulted.

Three-way PLS with cross-validation indicated that a three-component model explained 74% of the *Y*-variance and 41% of the *X*-variance. The *Y*-variances explained in each component were 39, 19 and 15%, respectively, while the *X*-variances explained by each component were 18, 15 and 8%, respectively. The conformation/alignment weights, CAW, from the three-component model are in Table 1.

The two highest weighted conformations are 2 and 5. Both have the phenyl group in a conformation orthogonal or approximately orthogonal to the plane of the tropane ring. The 2β-substituent in conformation 2 has its dipole oriented away from the amino group while in conformation 5 the dipole is oriented toward the amino nitrogen. Conformations 3 and 4, with the phenyl ring in the plane of the C3,C4 bond are ranked lower as was conformation 7, the lowest energy conformer of each ligand.

Table 1. CAW from the three-way PLS analysis

Conformation	CAW
1	0.100
2	0.115
3	0.106
4	0.110
5	0.117
6	0.096
7	0.100

GFA/PLS was applied to the descriptor data of the training set ligands in the one alignment and conformation 2 using the binding data to the DAT, SERT and NET. A 3-component PLS model was used and the default settings of the Cerius[2] program were used for model development. The final PLS models were rotated into the original data space for ease of interpretation. The models, eqs (7)–(9), resulted with n the number of compounds, r^2 the correlation coefficient, and $XV{-}r^2$ the cross-validated correlation coefficient.

$$\begin{aligned}(\mathrm{DAT})\mathrm{pIC}_{50} = {} & 5.34 + 0.04(\mathrm{OH}^-/647) - 0.02 \\ & \times (\mathrm{CH}_3/633) + 0.04(\mathrm{CH}_3/457) \\ & - 0.01(\mathrm{OH}^-/576) - 0.04 \\ & \times (\mathrm{CH}_3/855) - 0.04(\mathrm{CH}_3/477) \\ & + 0.02\left(\mathrm{CH}_3^-/744\right) + 0.05 \\ & \times (\mathrm{CH}_3/367) - 0.03(\mathrm{CH}_3/669) \\ & - 0.02(\mathrm{CH}_3/587) + 0.04 \\ & \times \left(\mathrm{CH}_3^-/346\right) - 0.02\left(\mathrm{CH}_3^+/657\right) \\ & - 0.04\left(\mathrm{CH}_3^+/525\right) + 0.01 \\ & \times \left(\mathrm{CH}_3^+/744\right)(\mathrm{DAT}) \end{aligned} \quad (7)$$

$$n = 68;\quad r^2 = 0.85;\quad XV - r^2 = 0.69;\quad r^2(\text{test set}) = 0.70$$

$$\begin{aligned}(\mathrm{SERT})\mathrm{pIC}_{50} = {} & 6.06 + 0.01\left(\frac{\mathrm{CH}_3^+}{734}\right) + 0.03 \\ & \times \left(\frac{\mathrm{CH}_3}{633}\right) - 0.22\left(\frac{\mathrm{OH}^-}{687}\right) - 0.04 \\ & \times \left(\frac{\mathrm{CH}_3}{376}\right) + 0.02\left(\frac{\mathrm{CH}_3}{636}\right) - 0.02 \\ & \times \left(\frac{\mathrm{CH}_3^-}{468}\right) + 0.03\left(\frac{\mathrm{CH}_3^+}{367}\right) + 0.04 \\ & \times \left(\frac{\mathrm{CH}_3}{736}\right) - 0.03\left(\frac{\mathrm{CH}_3^-}{676}\right) - 0.03 \\ & \times \left(\frac{\mathrm{CH}_3^-}{156}\right) - 0.02\left(\frac{\mathrm{OH}^-}{564}\right) - 0.03 \\ & \times \left(\frac{\mathrm{CH}_3^-}{586}\right) - 0.03\left(\frac{\mathrm{CH}_3^-}{588}\right)(\mathrm{SERT}) \end{aligned} \quad (8)$$

$$n = 68;\quad r^2 = 0.95;\quad XV - r^2 = 0.91;\quad r^2(\text{test set}) = 0.85$$

$$\begin{aligned}(\text{NET})\text{pIC}_{50} = {} & 6.15 - 0.01\left(\text{H}^{+}/478\right) - 0.04 \\ & \times \left(\text{CH}_3^{-}/276\right) + 0.02(\text{CH}_3/735) \\ & - 0.01\left(\text{CH}_3^{-}/377\right) - 0.02 \\ & \times \left(\text{CH}_3^{+}/626\right) - 0.02\left(\text{CH}_3^{+}/756\right) \\ & - 0.02(\text{HO}^{-}/276) - 0.03 \\ & \times (\text{CH}_3/447) - 0.02\left(\text{CH}_3^{-}/678\right) \\ & + 0.02\left(\text{H}^{+}/375\right) + 0.05 \\ & \times \left(\text{CH}_3^{-}/457\right) - 0.02(\text{CH}_3/624) \\ & (\text{NET}) \end{aligned} \quad (9)$$

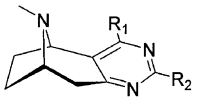
$n = 68;\ \ r^2 = 0.80;\ \ XV - r^2 = 0.61;\ \ r^2(\text{test set}) = 0.64$

All models have good statistical parameters with those for the SERT and NET somewhat better. These models, along with the results of the conformation/alignment calculations, are interpreted to mean that the same conformation is bound at all of the transporters. A comparison of the intercept terms shows that the ligands have an 8- to 10-fold higher selectivity for the NET and SERT than for the DAT. The MFA descriptors are indicated by the probe used and a number which is an indicator for its position in space around the ligands. The digits in the position indicator are the x, y and z coordinates in the box containing the ligand and the MFA descriptors are computed at the same positions for each receptor. A comparison of the descriptors shows that the (OH^-/687) term for the SERT is highly weighted indicating that the ligand interacts with a negative charge or dipole on the receptor at this position which is located such that the 2β-substituent is involved. The models for SERT and DAT include terms for the descriptor (CH_3/633) while this term does not appear in the NET equation. For the SERT the coefficient is positive indicating a hydrophobic interaction but for the DAT the coefficient is negative indicating an unfavorable steric interaction. The position coincides with substituents of the 3-position of the 3β-phenyl substituent. This suggests that a hydrophobic substituent in this position would be favorable for interaction with the SERT, unfavorable for interaction with the DAT and have no effect on binding to the NET. An unfavorable steric interaction near the 4-position of the 3β-phenyl substituent on the NET is indicated by the (CH_3/624) term in eq (9).

The three-way PLS results indicate that the aryl group in the 3β-position should be in a conformation in which the aryl group will be orthogonal or approximately orthogonal to the tropane ring when bound to all of the transporters. Further, the GFA/PLS results indicate that a hydrophobic group in the 3-position of the aryl group should be favorable for binding to the SERT and unfavorable for binding to the DAT. These results also suggest that the dipole of the 2β-substituent is oriented toward or away from the amino nitrogen. This is not explored in this report. The compounds designed below only test the hypothesis that the orientation of the 3β-aryl group is orthogonal or approximately orthogonal to the tropane ring.

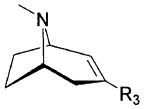
Chemistry

In order to test these results, two classes of compounds were designed and synthesized: (1) a class of rigid analogues[3] in which a pyrimidino group is fused to the 2,3-position of the tropane ring and (2) a class of semi rigid compounds[4] in which a naphthyl group is attached to the 3-position of the tropene system. These are shown as structures **3** and **4**.



3

3a; R_1=H, R_2=C_6H_5
3b; R_1=H, R_2=NH_2
3c; R_1=H, R_2=CH_3
3d; R_1=R_2=H
3e; R_1=R_2=C_6H_5

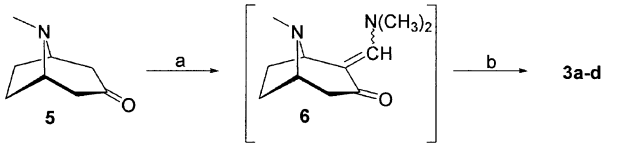


4

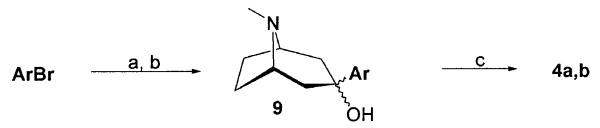
4a; R_3=1-naphthyl
4b; R_3=2-naphthyl

The structures represented by **4a**,**b** can be considered to be α,β-disubstituted styrene derivatives which are known to be semi rigid structures with restricted rotation of the aryl group about the C3-bond.[25,26] Conformational analyses, as discussed above, were carried out to explore their conformational space. The global minima and higher energy wells were found by performing a systematic energy search where each torsion bond was rotated in 30° increments. This analysis of **4a** shows that its lowest energy conformation has the naphthyl group with a torsion angle with vinylene group of 109° and the benzo group below the tropane ring. Its barrier to rotation through a planar transition state to give the conformer in which the benzo group is above the plane of the tropane ring is approximated to be about 25 kcal/mol. The former conformer is more stable by 0.11 kcal/mol. The global minimum for **4b** has a torsion angle for the naphthyl group with the vinylene group of 141° and the benzo group below the plane of the tropane ring. The low energy conformer with the benzo group above the plane of the ring is 0.24 kcal/mol higher in energy. Compound **4b** is somewhat more flexible than **4a** with a barrier to rotation through a planar transition state that is estimated to be about 6 kcal/mol. The conformational preferences for these two compounds are clearly those with their naphthyl groups in a conformation that is skewed significantly out of the plane of the tropane ring.

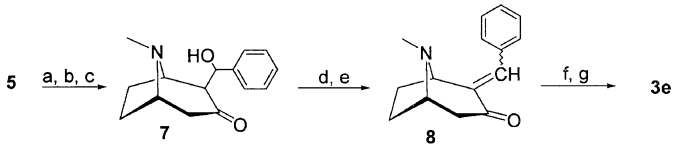
The **3a**–**d** series was prepared according to Scheme 1. The tropinone, **5**, was converted to the enaminone, **6**, with dimethyl formamide dimethylacetal, DMFDMA. The excess DMFDMA was removed in vacuo but the product, **6**, was used for further reaction without further



Scheme 1. Synthesis of pyrimidine derivatives, **3a**–**d**: (a) dimethyl formamide dimethyl acetal, reflux 24 h; (b) R_2-C(NH)NH_2, $NaOCH_3$, CH_3OH.



Scheme 3. Synthesis of tropenes, **4a**,**b**: (a) BuLi, reflux; (b) **5**, reflux; (c) TFA, reflux.



Scheme 2. Synthesis of pyrimidine derivative, **3e**: (a) LDA; (b) benzaldehyde; (c) NH_4Cl (aq); (d) $O(OCOCH_3)_2$, Et_3N; (e) SiO_2; (f) $C_6H_5C(NH)NH_2$, $NaHCO_3$; (g) 10% Pd/C, *o*-xylene.

purification. Reaction of **6** with the appropriate amidine gave the pyrimidine which was characterized by mass spectral and NMR data. The compounds were isolated as racemates and resolved using chiral HPLC. One of the diastereoisomers of **3a** was converted to the quaternary salt with methyl iodide and its absolute configuration was determined by X-ray crystallography. Its structure was found to be the (1*S*) isomer, which was found to be dextrorotatory. The optical rotations of all of the resolved isomers were obtained and it was assumed that the dextrorotatory isomers had the (1*S*) configuration and the levorotatory isomers had the (1*R*) configuration. The diphenyl compound, **3e**, was prepared according to Scheme 2.

The benzylidene **8** was prepared from the aldol **7** according to the procedure of Majewski and Lazny.[27] The benzylidene was treated with benzamidine to give a mixture of dihydropyrimidines. Aromatization was effected by refluxing the mixture with 10% Pd/C in *o*-xylene. The racemic product was not resolved.

The tropanes **4a**,**b** were prepared from **5** via the appropriate aryl lithium compound. The intermediate alcohols were treated with trifluoroacetic acid to give the racemic alkenes. This is shown in Scheme 3.

Table 2. Binding data, IC_{50} (nM), for the rigid and semirigid analogues[a]

Compd	hDAT	hSERT	hNET
(−)**3a**	58,300 (20,200)	6140 (3350)	NA
(+)**3a**	48,700 (20,100)	6030 (3400)	NA
(−)**3b**	NA[b]	NA	NA
(+)**3b**	NA	NA	NA
(−)**3c**	NA	NA	NA
(+)**3c**	NA	NA	NA
(−)**3d**	NA	NA	NA
(+)**3d**	NA	NA	NA
(+/−)**3e**	30,000 (11,200)	3650 (1700)	NA
(+/−)**4a**	561 (191)	35.3 (9.0)	9880 (41.9)
	595 (72)[c]	47.6 (9.8)[c]	9020 (3250)[c]
(+/−)**4b**	31.9 (6.1)	2.92 (1.1)	368 (194)
	51.5 (6.1)[c]	4.43 (1.7)[c]	418 (190)[c]

[a]Standard deviations are in parentheses.
[b]NA, no affinity.
[c]Tested as HCl.

Pharmacology

The binding experiments were carried out with cloned human monoamine transporter proteins, hDAT, hSERT and hNET, expressed in HEK-293 cells; conditions for hDAT and hSERT cells were as described by us previously[28] and for hNET in sodium phosphate buffer with 300 mM Na^+ and 5 mM K^+ as described by Zhu et al.[29] The labeling ligands were [^{3}H]-WIN-35,428, [^{3}H]-citalopram and [^{3}H]-nisoxetine for the hDAT, hSERT and hNET, respectively. Radioligand concentrations were appreciably below their K_d values so that IC_{50} estimates are close to their K_i values. All compounds were tested as the free bases except as noted; **4a**,**b** were also tested as the hydrochlorides. Concentrations of test compounds ranged from 10^{-11} to 10^{-3} M. The data are given in Table 2.

Discussion

With the exceptions of **3a** and **3e**, none of the rigid heterocyclic derivatives showed affinity for the transporter proteins and the affinities of **3a** and **3e** for the hDAT and hSERT were low. A comparison of the structures of **3a** with cocaine shows that the phenyl groups in both are highly coincidental with a distance from the tropane nitrogen to the 4-position of the phenyl group to be 9.08 Å in each case. In the crystal structure of the methiodide, the 2′-phenyl group is approximately orthogonal to the pyrimidine. This residual activity at the hDAT and hSERT for **3a** could result from a favorable interaction of this group in the binding pockets of these two proteins. The most potent of the pyrimidines is **3e** which has an affinity for the transporters that is approximately 2-fold that of the **3a** isomers. This indicates that there is a large area of the receptors associated with the 2-substituents that can accommodate steric bulk. The 6′-phenyl would not be expected to occupy the same site as the 2β-substituent of cocaine derivatives.

The affinities of **4a** for the hDAT and hSERT and its selectivity for the hSERT/hDAT of 12 are consistent with our calculations and substituent effects[30,31] involving the naphthyl group in the 3-position of tropane. The high affinity of **4b** for the hSERT is approximately

an order of magnitude below that reported for the most potent inhibitors of serotonin uptake.[32]

Several CoMFA studies of the binding of tropanes to the monoamine transporters have been published.[31–37] Although none of these studies resulted in the design of new compounds, Davies et al.[21] proposed that a naphthyl group in the 3β-position of tropane may fit in a narrow binding cleft which can accommodate a relatively planar aromatic group. The inactivity of the pyrimidino compounds and the high affinities of **4a** and **4b** support this hypothesis.

Our molecular field analysis also suggests that a hydrophobic interaction of the naphthyl group with the hSERT contributes to higher affinity compared to the hDAT and that an additional, favorable electrostatic interaction of the 2β-substituent contributes to hSERT selectivity. To explore these findings, additional analogues are being synthesized.

Experimental

Chemistry

Reagents were obtained from commercial sources and used as supplied. Chromatography was accomplished using 200–400 mesh, 60 Å silica gel. Thin layer chromatography utilized layer thickness 250 μm, particle size 5–17 μm, 60 Å silica gel on glass plates with UV indicator and absorption was read at 254 nm. Melting points were taken on a Thomas Hoover capillary melting point apparatus and are uncorrected. Solvents were distilled. In the case of moisture sensitive reactions, glassware was flame dried. Anhydrous tetrahydrofuran was obtained by distillation over lithium aluminum hydride and diethyl ether was distilled over sodium. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian XL-300 spectrometer and a Bruker DPX Avance 300 spectrometer. ^{1}H NMR chemical shifts are reported in ppm down field from tetramethylsilane ($\delta = 0$), J values are reported in Hz. APT, COSY, HMQC and HMBC nmr experiments were carried out on the pymidines to provide chemical shifts. Optical rotation was measured on a Perkin-Elmer 241 polarimeter at the 598 nm Na $_D$-line. Mass spectra were obtained on a Hewlett-Packard model 5989B using electrospray conditions with 50% acetonitrile/1% acetic acid, a Mat-90 spectrometer operated at 70 eV, or a Finnigan LCQ with an ion trap. Elemental analysis (C, H, N) was done by Midwest Microlabs, Indianapolis, IN, USA (within ±0.4%).

The pyrimidino compounds were resolved using chiral HPLC. The column used was Chiralpak AD with heptane/abs ethanol (95:5) or heptane/isopropyl alcohol (90:10). The separations were monitored at 220 or 250 nm and samples were collected manually. HPLC analysis of the separated enantiomers showed no other enantiomer present. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 598 nm Na $_D$-line.

A general procedure for synthesis of (2′-substituted)-2,3-dehydropyrimido[4′,5′:2,3]tropanes (3a–d)

Dimethyl formamide dimethyl acetal (13.3 mL, 100 mmol) was added to **5** (3.48 g, 25 mmol) under an atmosphere of nitrogen. The stirred mixture was gently refluxed for 2 days. The solvents were removed in vacuo and **6** was used without further purification.

A cold solution of sodium methoxide (62.5 mmol, 14.3 mL, 25 wt % solution in methanol) was added to a suspension of the appropriate amidine salt (31.25 mmol) in dry, distilled methanol (75 mL) under a nitrogen atmosphere. The crude **6** (< 25 mmol), in dry, distilled methanol (75 mL) was added, and the mixture was heated to reflux for 2 days. After cooling to room temperature, water (100 mL) was added and the low boiling solvents were removed in vacuo. The aqueous mixture was saturated with ammonium chloride and extracted with a mixture of 25% 2-propanol in chloroform (16×50 mL). The organic extracts were washed with water (2×25 mL), dried over sodium sulfate, filtered, and concentrated to give the desired product.

(2′-Phenyl)-2,3-dehydropyrimido[4′,5′:2,3]tropane (3a). This pyrimidine was prepared from **6** and benzamidine hydrochloride as described. Removal of the solvent afforded 7.00 g of a brown solid. Recrystallization from hexanes afforded **3a** as a yellow solid: yield 2.97 g (47.2%); mp 111–112 °C; R_f 0.65 (20% methanol in chloroform); 1*S* $[\alpha]_D^{22} -90°$ (*c* 0.01, methanol); 1*R* $[\alpha]_D^{22} +88°$ (*c* 0.01, methanol). ^{1}H NMR (chloroform-*d*) δ 1.66 (m, 1H, *H*6), 1.79 (m, 1H, *H*7), 2.22–2.44 (m, 2H, *H*6, *H*7), 2.38 (s, 3H, NCH_3), 2.61 (d, $J = 18.8$, 1H, *H*4), 3.32 (dd, $J = 18.8$, $J = 5.1$, 1H, *H*4), 3.56 (m, 1H, *H*5), 3.92 (d, $J = 5.64$, 1H, *H*1), 7.45 (m, 3H, Ph*H*), 8.38 (s, 1H, pyrimidine*H*), 8.40 (m, 2H, Ph*H*,); ^{13}C NMR ($CDC1_3$) δ 29.67 (*C*6), 34.91 (*C*7), 37.25 (N*C*H_3), 37.39 (*C*4), 58.48 (*C*5), 60.90 (*C*1), 128.33 (*Ph*), 128.90 (*Ph*), 130.68 (*Ph*), 132.04 (*C*2), 138.26 (*Ph*), 154.26 (pyrimidine*C*H), 163.53, 164.11; EI-MS (*m*/*z*) 251 (M^+, 31.0), 224 (11.6), 223 (73.5), 222 (100), 57 (9.08). Anal. ($C_{16}H_{17}N_3$) C, H, N.

(2′-Amino)-2,3-dehydropyrimido[4′,5′:2,3]tropane (3b). This pyrimidine was prepared from **6** and guanidine hydrochloride as described. The crude product, 3.37 g, was purified by elution over silica gel with ethyl acetate, chloroform, followed by chloroform/methanol (4:1) to give a yellow solid. It was recrystallized from ethyl acetate: yield, 1.22 g (25.6%); mp 160–164 °C; 1*S* $[\alpha]_D^{22} -91°$ (*c* 0.01, methanol); 1*R* $[\alpha]_D^{22} +84°$ (*c* 0.01, methanol); R_f 0.29 (20% methanol in chloroform). ^{1}H NMR (chloroform-*d*) δ 1.59 (m, 1H, *H*6), 1.74 (m, 1H, *H*7), 2.24–2.34 (m, 2H, *H*6, *H*7), 2.37 (s, 3H, NCH_3), 2.29 (d, 1H, *H*4), 3.09 (dd, $J = 18.4$, $J = 5.4$, 1H, *H*4), 3.50 (m, 1H, *H*5), 3.80 (d, $J = 5.6$, 1H, *H*1), 5.21 (b, 2H, NH_2), 7.92 (s, 1H, pyrimidine*H*); ^{13}C NMR ($CDC1_3$) δ 29.55 (*C*6), 34.92 (*C*7), 37.02 (*C*4), 37.16 (N*C*H_3), 58.26 (*C*5), 60.57 (*C*1), 124.45 (*C*2), 155.42 (pyrimidine*C*H), 162.36 (pyrimidine*C*NH_2), 164.89 (*C*3); EI-MS (*m*/*z*) 190 (M^+, 27.97), 162 (58.64), 161 (100), 160 (7.12), 134 (11.91). Anal. ($C_{10}H_{14}N_4$) C, H, N.

(2′-Methyl)-2,3-dehydropyrimido[4′,5′:2,3]tropane (3c). This pyrimidine was prepared from **6** and acetamidine hydrochloride as described. The crude aqueous mixture was extracted with ethyl acetate (8×50 mL). The extracts were dried over sodium sulfate, the drying agent filtered and the solvent was removed. The crude residue, 3.54 g, was purified by elution over silica gel with ethyl acetate, followed by chloroform/methanol (4:1) that afforded a white solid: yield 1.51 g (1.9%); mp 38–39.5 °C; 1*S* $[\alpha]_D^{22}$ −88° (*c* 0.01, methanol); 1*R* $[\alpha]_D^{22}$ +83° (*c* 0.01, methanol); R_f 0.51 (20% MeOH in $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.61 (m, 1H, *H*6), 1.78 (m, 1H, *H*7), 2.23–2.46 (m, 2H, *H*6, *H*7), 2.38 (s, 3H, NCH_3), 2.52 (d, *J* = 18.5, 1H, *H*4), 2.67 (s, 3H, pyrimidineCH_3), 3.24 (dd, *J* = 18.5, *J* = 4.9, 1H, *H*4), 3.57 (m, 1H, *H*5), 3.90 (d, *J* = 5.6, 1H, *H*1), 8.24 (s, 1H, pyrimidineH_3); ^{13}C NMR ($CDCl_3$) δ 26.08 (pyrimidine*C*H_3), 29.51 (*C*6), 34.75 (*C*7), 37.13 (*C*4), 37.21 (N*C*H_3), 58.32 (*C*5), 60.69 (*C*1), 130.94 (*C*2), 153.92 (pyrimidine*C*H), 163.68 (*C*3), 166.47 (pyrimidine*C*CH_3); EI-MS (*m*/*z*) 189 (M^+, 53.00), 161 (81.49), 160 (100), 133 (21.39), 82 (26.23). Anal. ($C_{11}H_{15}N_3$) C, H, N.

2,3-Dehydropyrimido[4′,5′:2,3]tropane (3d). This pyrimidine was prepared from **6** and formamidine acetate. A cold solution of sodium methoxide (80 mmol, 18.3 mL, 25 wt% solution in methanol) was added to a suspension of formamidine acetate (4.16 g, 40 mmol) in dry, distilled methanol (90 mL) under a nitrogen atmosphere. Crude, **6** (<32 mmol), in dry, distilled methanol (90 mL) was added, and the mixture was heated to reflux for 2 days. After cooling to room temperature, water (130 mL) was added and the low boiling point solvents were removed in vacuo. The aqueous mixture was saturated with ammonium chloride and extracted with mixuture of 25% 2-propanol in chloroform (16×70 mL). The organic extracts were washed with water (2×30 mL), dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by elution over silica gel with chloroform/methanol (4:1). Recrystallization from hexanes gave a white solid: yield 2.05 g (38.9%); mp 95–97 °C; 1*S* $[\alpha]_D^{22}$ +83° (*c* 0.01, methanol); 1*R* $[\alpha]_D^{22}$ −76° (*c* 0.01, methanol); R_f 0.51 (20% methanol in chloroform). ^{1}H NMR (chloroform-*d*) δ 1.65 (m, 1H, *H*6), 1.81 (m, 1H, *H*7), 2.26–2.42 (m, 2H, *H*6, *H*7), 2.38 (s, 3H, NCH_3), 2.58 (dd, *J* = 18.5, *J* = 1.1, 1H, *H*4), 3.27 (ddd, *J* = 18.63, *J* = 5.1, *J* = 1.7, 1H, *H*4), 3.57 (m, 1H, *H*5), 3.93 (d, *J* = 5.6, 1H, *H*1), 8.33 (s, 1H, pyrimidineC*H*), 8.98 (s, 1H, pyrimidineNC*H*N); ^{13}C NMR ($CDCl_3$) δ 29.48 (*C*6), 34.66 (*C*7), 37.34 (*C*4), 37.43 (N*C*H_3), 58.44 (*C*5), 60.95 (*C*1), 134.54 (*C*2), 153.74 (pyrimidine*C*H), 157.47 (pyrimidineN*C*HN), 163.90 (*C*3); EI–MS *m*/*z* 146 (100), 83 (62.54), 78 (63.71), 63 (85.37), 61 (73.42). Anal. ($C_{10}H_{13}N_3$) C, H, N.

(2′,6′ - Diphenyl) - 2,3 - dehydropyrimido[4′,5′:2,3]tropane (3e). The procedure of Majewski and Lazny[28] was used to prepare the aldol, **7**, which was converted to the benzylidene, **8**. The spectral data for both compounds were consistent with that reported.[28] The benzylidene (4.55 g, 20 mmol), was reacted with benzamidine hydrochloride (9.40 g, 60 mmol), and sodium hydrogen carbonate (5.04 g, 60 mmol) under reflux in DMF (50 mL) for 8 h. DMF was removed in vacuo and the crude product was taken up in water (100 mL). The pH of the solution was adjusted to 9 using ammonium hydroxide and the basic solution was extracted with ethyl acetate (8×50 mL). The crude extracts were dried over sodium sulfate and the solvent was evaporated to yield 3.4 g of solid. This crude extract was refluxed with 10% palladium on carbon in *o*-xylene (50 mL) for 8 h. The *o*-xylene was removed in vacuo, and the crude product was taken up in water (100 mL), the pH was adjusted to 9 using ammonium hydroxide (28%) and the basic mixture was extracted with ethyl acetate (8×50 mL). The extracts were dried over sodium sulfate and the solvent was evaporated to yield 3.3 g of solid which was purified by column chromatography by eluting with petroleum ether, followed by ethyl acetate/triethylamine (9:1) to give a yellow solid: yield 610 mg (9.3%); mp. 123–127 °C. ^{1}H NMR (chloroform-*d*) δ 1.70–185 (m, 1H), 1.90–2.05 (m, 1H), 2.30–2.60 (m, 3H), 2.46 (s, 3H), 2.95 (m, 1H), 3.65 (m, 1H), 4.45 (m, 1H), 5.45–7.30 (m, 5H), 7.60 (s, 1H); ^{13}C NMR (chloroform-*d*) δ 30.08, 35.14, 37.39, 38.19, 58.96, 59.88, 128.51, 128.76, 128.80, 129.57, 129.63, 129.79; 130.61; 138.14, 138.42, 162.59, 162.74, 164.58. ESMS *m*/*z* 328.3 $[M+H]^+$. Anal. ($C_{22}H_{21}N_3$) C, H.

Crystal structure details for the methiodide of 3a. $C_{17}H_{20}N_3^+I^-$:0.75 H_2O, M_r = 406.77, tetragonal space group $P4_32_12$, *a* = 7.1541(1), *b* = 7.1541(1), and *c* = 65.855(4) Å, V = 3370.6(2) Å3, Z = 8. *Dx* = 1.60 Mg/m^3, CuK_α radiation (λ = 1.54178 Å), μ = 14.95 mm^{-1}, *F*(000) = 1628, T = 293 K. Data were collected on a Bruker P4 automatic diffractometer with a graphite monochromator in the incident beam. There were 2214 independent data with 1811 observed (I > 2σI) giving an R_{int} of 0.030. A face-centered absorption correction was done (maximum and minimum transmission factors were 0.552 and 0.121). The structure was solved by direct methods using SHELXS.[38] The structure was refined on F^2 values using all 2214 data and 199 parameters with program SHELXL.[38] The Flack parameter (2) was −0.01[39] indicating that the experimental data was accurate enough to determine the absolute configuration of **3a**. All non-hydrogen atoms were refined with anisotropic thermal displacements and H atoms were included, at calculated positions, using a riding model. Final agreement factors were R(F) = 0.044 and wR(F2) = 0.099 for the observed data, S = 1.17 with maximum and minimum peaks in the final difference map of 0.36 and −0.83 Å. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EW, UK (deposit@ccdc.cam.ac.uk).

(IR*S*)-3-(1-naphthyl)-trop-3-ene (4a). Compound **4a** was prepared from tropin-3-one and 1-bromonaphthalene. A solution of n-butyl lithium (5 mL in 2.5 M in hexanes, 12.5 mmol) in dry, distilled diethyl ether (10 mL) was stirred under an atmosphere of nitrogen in a flame dried three-neck round bottom flask equipped with a condenser, an additional funnel, and a ground glass stopper. To this solution, 1-bromonaphthylene (13 mmol) in dry, distilled diethyl ether (5 mL) was added drop-wise

through the addition funnel at a rate to maintain a gentle reflux for one hour. Tropin-3-one (1.392 g, 10.0 mmol) in dry distilled diethyl ether (5 mL) was then added drop-wise. This mixture was refluxed for four hours. The mixture was cooled in an ice bath, and the reaction was quenched with 20 mL of ice-cold dilute hydrochloric acid (5%). The aqueous layer was extracted with diethyl ether (50 mL) to remove any acidic/neutral impurities, then made basic to pH 9–10 by the drop-wise addition of 30% ammonium hydroxide. This aqueous solution was extracted with ethyl acetate (8×25 mL), and the organic extracts were dried over sodium sulfate to give the intermediate alcohol **9**. The alcohol (785 mg, 3.0 mmol) was refluxed in a 50 mL round bottom flask in 20 mL of trifluoroacetic acid for two hours. The solvent was removed in vacuo and 10mL of ice-cold water was added. The mixture was made basic to pH 9–10 with the drop-wise addition of 30% ammonium hydroxide and extracted with ethyl acetate (8×25 mL). The organic extracts were dried over sodium sulfate. Removal of the sodium sulfate by filtration, and removal of the solvent afforded 803 mg of a crude oil. The oil was taken up in 10 mL of chloroform, and the addition of 100 mL of petroleum ether resulted in the precipitation of a pure white powder (695 mg, 2.8 mmol, 94% yield), mp. 134–137 °C; R_f=0.33 (10% triethylamine in ethyl acetate); ^{1}H-NMR (*d*-chloroform) δ 1.76–181 (m, 2H), 2.01–2.03 (m, 3H), 2.11–2.28 (m, 2H), 2.32 (s, 3H), 2.33–2.37 (m, 2H), 3.21 (m, 2H), 3.27 (m, 2H), 6.98 (t, 2H), 7.50 (t, 2H); ESMS *m*/*z* 250.1 $[M+H]^+$. Anal. ($C_{18}H_{19}N$) C, H.

(1R*S*)-3-(2-Naphthyl)-trop-3-ene (4b). The compound was prepared from tropin-3-one and 2-bromonaphthylene in the same manner as **4a**. Following the dehydration of the intermediate alcohol, the mixture was made basic to pH 9–10 with the drop-wise addition of 30% ammonium hydroxide and extracted with ethyl acetate (8×25 mL). The organic extracts were dried over sodium sulfate. Removal of the sodium sulfate by filtration, and removal of the solvent afforded 850 mg of a crude oil. The oil was purified by column chromatography by elution with 10% triethylamine in ethyl acetate over silica gel. The white solid was purified by recrystalization from ethyl acetate (691 mg, 2.8 mmol, 91%), mp. 89–91 °C; R_f=0.31 (10% triethylamine in ethyl acetate); ^{1}H-NMR (*d*-chloroform) δ 1.67–1.72 (m, 1H), 1.94–2.01 (m, 1H), 2.12–2.30 (m, 3H), 2.49 (s, 3H), 3.02–3.07 (m, 1H), 3.54–3.57 (m, 2H), 6.39–6.47 (d, 1H), 7.44–7.83 (m, 7H); ESMS *m*/*z* 250.1 $[M+H]^+$. Anal. ($C_{18}H_{19}H$) C, H.

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References and Notes

1. Reith, M. E. A.; Sershen, H.; Lajtha, A. *Life Sci*. **1980**, *27*, 1055.
2. Povlock, S. L.; Amara, S. G. In *Neurotransmitter Transporters, Structure, Function, and Regulation*; Reith, M. E. A.; Ed.; Humana: Totowa, New Jersey, 1997; p. 1.
3. Leshner, A. I. *NIDA Res. Rep*. May, 1999.
4. Schuldiner, S. In *Neurotransmitter Transporters, Structure, Function, and Regulation*; Reith, M. E. A.; Ed.; Humana: Totowa, New Jersey, 1997; p. 215.
5. Miller, J. W.; Kleven, D. T.; Domin, B. A.; Fremeau, Jr., R. T. In *Neurotransmitter Transporters, Structure, Function, and Regulation*; Reith, M. E. A.; Ed.; Humana: Totowa, New Jersey, 1997; p. 101.
6. Hansch, C. *Accts. Chem. Res*. **1969**, *2*, 232.
7. Cramer, R. D., III; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc*. **1988**, *110*, 5959.
8. Glen, W. G.; Dunn, W. J., III; Scott, D. R. *Tetrahedron Comput. Methodol*. **1989**, *2*, 349.
9. Folkers, G.; Merz, A.; Rognan, D. In *3-D-QSAR in Drug Design: Theory, Methods and Applications*; Kubinyi, H.; Ed.; ESCOM: Leiden, 1993; p. 583.
10. Cho, S. J.; Tropsha, A. *J. Med. Chem*. **1995**, *38*, 1060.
11. Dowyeko, A. M. *J. Med. Chem*. **1988**, *31*, 1396.
12. Hopfinger, A. J.; Venkatarangan, P.; Tseng, Y.-F.; Wang, S.; Duca, J. S. *Int. J. Chem*. **2000**, *3*, 38.
13. Vedani, A.; Briem, H.; Dobler, M.; Dollinger, H.; McMasters, D. R. *J. Med. Chem*. **2000**, *43*, 4416.
14. Dunn, W. J., III; Hopfinger, A. J.; Catana, C.; Duraiswami, C. *J. Med. Chem*. **1996**, *39*, 4825.
15. Lohmöller, J.-B.; Wold, H. In *Proceedings of the European Meeting of the Psychometric Society*; The University of Gronigen: The Netherlands, 1980; p. 80.
16. Rogers, D.; Dunn III, W. J. In *Genetic Algorithms in Molecular Modeling*; Devillers, J.; Ed.; Academic: London, 1996; p. 109.
17. Carroll, F. I.; Mascarella, S. W.; Kuzemko, M. A.; Gao, Y.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1994**, *37*, 2865.
18. Blough, B. E.; Abraham, P.; Lewin, A. H.; Kuhar, M. J.; Boja, J. W.; Carroll, F. I. *J. Med. Chem*. **1996**, *39*, 4027.
19. Kotian, P.; Mascarella, A. P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. *J. Med. Chem*. **1996**, *39*, 2753.
20. Anon. *Cerius*^{2}, version 3.6, Molecular Simulations, Inc.: 6985 Scranton Road, San Diego, CA 92121, USA.
21. Rappe', A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A.; Skiff, W. M. *J. Am. Chem. Soc*. **1992**, *114*, 10024.
22. Rappe', A. K.; Goddard, W. A. *J. Phys. Chem*. **1991**, *95*, 3358.
23. Smith, M. P.; Johnson, K. M.; Zhang, M.; Flippen-Anderson, J. L.; Kozikowski, A. P. *J. Am. Chem. Soc*. **1998**, *120*, 9072.
24. Mayo, S. L.; Olafson, B. D.; Goddard, W. A., III *J. Phys. Chem*. **1990**, *94*, 8897.
25. Anderson, J. E.; Hazlehurst, C. J. *J. Chem. Soc. Chem. Commun*. **1980**, 1188.
26. Anderson, J. E.; Barkel, D. J. D.; Cooksey, C. J. *Tetrahedron Lett*. **1983**, *24*, 1077.
27. Dutta, A. K.; Xu, C.; Reith, M. E. A. *J. Med. Chem*. **1998**, *41*, 3293.
28. Majewski, M.; Lazny, R. *J. Org. Chem*. **1995**, *60*, 5825.
29. Zhu, M.-Y.; Blakeley, R. D.; Apparsundaram, S.; Ordway, G. A. *J. Neurochem*. **1998**, *43*, 1547.
30. Carroll, F. I.; Howell, L. L.; Kuhar, M. J. *J. Med. Chem*. **1999**, *42*, 2721.
31. Davies, H. M. L.; Gilliatt, V.; Kuhn, L. A.; Saikali, E.; Ren, P.; Hammond, P. S.; Sexton, T.; Childers, S. R. *J. Med. Chem*. **2001**, *44*, 1509.
32. Carroll, F. I.; Gao, Y. G.; Rahman, M. A.; Bja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1991**, *34*, 2719.

33. Carroll, F. I.; Mascarella, S. W.; Kusemki, M. A.; Gao, Y. G.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem*. **1994**, *37*, 2865.
34. Muszynski, I. C.; Scapozza, L.; Kovar, K.-A.; Golkers, G. *Quant. Struct-Act. Relat*. **1999**, *18*, 342.
35. Liske, S. F.; Yang, B.; Eldefrawi, M. E.; MacKerell, A. D., Jr.; Wright, J. *J. Med. Chem*. **1998**, *41*, 864.
36. Nwman, A. H.; Izenwasser, S.; Robarge, M. J.; Kline, R. H. *J. Med. Chem*. **1999**, *42*, 3502.
37. Surname, N.; Harrison, A.; Trudell, M. L.; Klein-Stevens, C. L. *Struct. Chem*. **1999**, *8*, 91.
38. Sheldrick, G. SHELXTL-*Plus*. *Release 5.03*; Siemens Analytical X-Ray Instruments: Inc: Madison, WI, USA, 1994.
39. Flack, H. D.; Bernardinelli, G. *J. Appl. Cryst*. **2000**, *33*, 1143.