CoMFA Study of Piperidine Analogues of Cocaine at the Dopamine Transporter: Exploring the Binding Mode of the 3α-Substituent of the Piperidine Ring Using Pharmacophore-Based Flexible Alignment

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A 3D-QSAR CoMFA study of piperidine-based analogues of cocaine with flexible 3α-substituents is described. A series of pharmacophore models were generated based on three representative compounds **1p**, **2i**, and **3c** using the Genetic Algorithm Similarity Program (GASP) method. The flexible superposition of all studied compounds was performed for each pharmacophore model using the FlexS algorithm and the three-dimensional structure of **2i** as a template. All sets of the overlaid structures with the top-ranked conformers were used for CoMFA modeling. Two best initial CoMFA models were selected and further optimized by identifying the best-fitting conformer of each compound. Compared with the initial models, the conventional correlation coefficients r^2 for the optimized models 1 and 2 were improved from 0.90 and 0.837 to 0.997 and 0.993, respectively. The leave-one-out cross-validated coefficients q^2 for the optimized models 1 and 2 were improved from 0.515 and 0.296 to 0.828 and 0.849, respectively. The results of the two CoMFA models suggest that both steric and electrostatic interactions play important roles in the binding of the 3α-substituents of the piperidine-based analogues of cocaine. The contributions from steric and electrostatic fields for model 1 were 0.621 and 0.379, respectively. The contributions from steric and electrostatic fields for model 2 were 0.493 and 0.507, respectively. The two highly predictive CoMFA models indicate that the 3α-substituent has two possible binding modes at the DAT. The CoMFA contour maps provide a visual representation of prospective binding modes of the 3α-substituent of the piperidine-based analogues of cocaine and can be used to design novel DAT inhibitors that may be useful for the treatment of cocaine abuse and certain neurological disorders.

Introduction

The monoamine transporters have been studied extensively as the targets for addiction therapy in the past decade.[1−4] It has been shown that cocaine and other abused drugs have the capability to bind to the monoamine transporters.[5−8] Substantial evidence has suggested that the dopamine transporter (DAT) is the key site of action for cocaine in the central nervous system even though the mechanisms that mediate the addictive character of cocaine are more complex.[4,5,9−12] Several series of compounds, including cocaine/tropane analogues, dialkylpiperazines, methylphenidates, and mazindols, have been synthesized and tested for their transporter-binding potencies.[4] Among these compounds, tropane- and piperidine-based cocaine analogues have been in the center of investigation since they are structurally similar to cocaine.[13−42]

A generally recognized and applied pharmacophore model for cocaine and tropane-based monoamine transporter inhibitors comprises two electrostatic interactions of the basic nitrogen and the ester group of the C-2 substituent, and one hydrophobic interaction of the C-3 aryl group.[4,5,43−45] This model has been disputed because of the finding that in certain compounds neither the basic nitrogen[46−49] nor the ester group[50] was necessary for high binding affinity and inhibition of monoamine reuptake. Instead, a hydrophobic pocket was proposed to exist in the vicinity of the C-2 carbon.[50,51] On the contrary, Crippen et al. reported that the C-2 substituent did not have a significant effect on the binding activity of cocaine analogues.[52] Carroll et al., however, provided further evidence for an electrostatic interaction at the C-2β-position in a later study.[17]

Other models proposed for the DAT binding site include a linear fashion binding pocket for the 3β-substituted tropane-based cocaine analogues[53] and a prohibited conical region about 5.5−10 Å distant from the 3α-substituted piperidine ring.[34] Noticeably, high potency at the DAT of dimeric piperidine-based esters and amides suggested that the flexible linker combining the two piperidine units was able to adjust its orientation and to avoid unfavorable interactions with the binding site.[54] All these lines of evidence suggest that the DAT binding site is much more complicated than the proposed pharmacophore models.

In an attempt to uncover the details of the DAT binding site, a number of 3D-QSAR studies were performed. Several QSAR/CoMFA[55] studies focused on phenyltropanes concluded that an increased negative electrostatic potential in the regions around the 3β-substituent of the tropane ring and the para-position of the phenyl ring favored high potency in inhibition of the monoamine transporters.[15,56,57] Recent studies of

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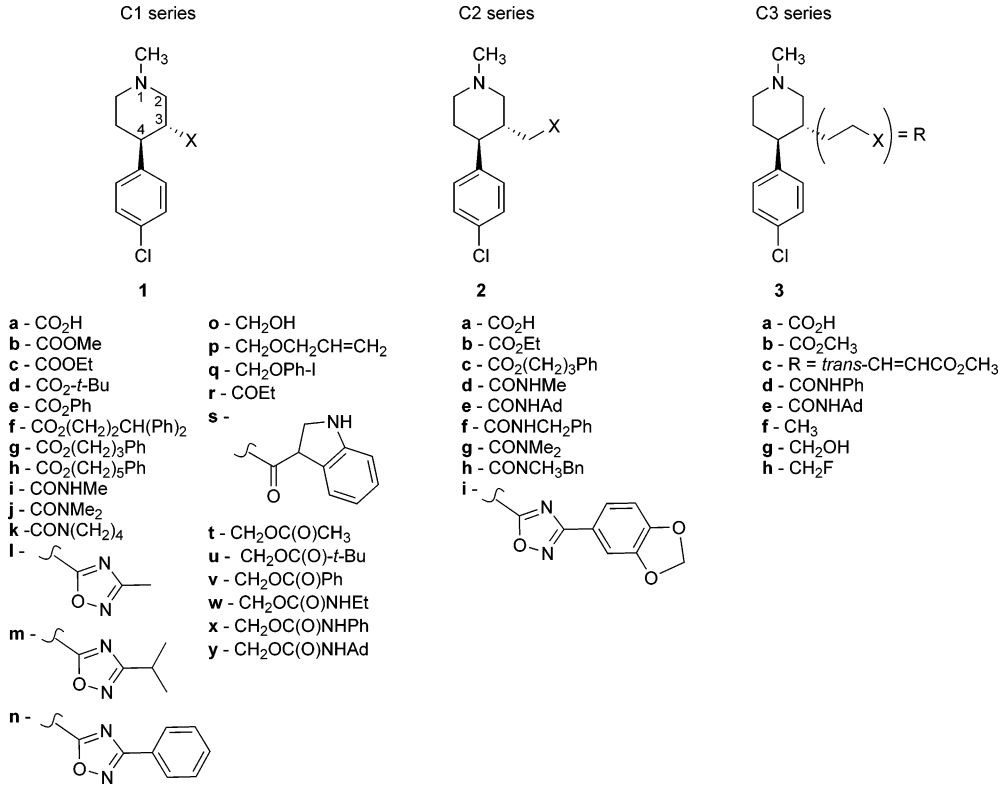

Figure 1. Three series of the piperidine-based cocaine analogues used in our CoMFA studies. The ligands were assigned into the series C1, C2, and C3 according to the number of carbon atoms between the attachment point of the 3α-substituent and its first polar atom.

aryltropanes and piperidinols suggested that the DAT and SERT have a large cavity that can accommodate bulky C-2 substituents of tropanes,[42,58] and the size of the substituents at the para-position in both phenyl rings of piperidinols is important for inhibition of DA reuptake.[59] Wright et al. studied the role of the 3β-substituent of tropanes in binding to the DAT and blocking DA reuptake.[53] Their CoMFA model indicated that the β-substituent binding site is barrel-shaped and hydrophobic interactions make a dominant contribution to the binding, which is consistent with the studies of 3α-substituted tropane analogues reported by Newman et al.[60] Newman and co-authors also studied N-substituted tropanes and concluded that the steric interaction of the N-substituent with the DAT is a principal factor for the binding affinity.[28]

To construct detailed models of the monoamine transporter binding sites that accommodate the piperidine-based ligands, we decided to create CoMFA models of the DA transporter using data accumulated for the piperidine-based ligands. To effectively overlay the flexible 3α-substituents and to identify active conformers of these molecules, a novel strategy, pharmacophore-based superposition of the fittest conformers, was developed and successfully applied. A total of 42 piperidine-based molecules with a variety of 3α-substituents were studied, and two highly predictive CoMFA models were generated and evaluated.

Materials and Methods

All molecular modeling and CoMFA studies were performed on an SGI or a Linux computer using the SYBYL 6.9 software package.[61] The structural and biological data were collected from the papers previously published by Kozikowski et al.[24,25,34,35,62] and Petukhov et al.,[36] in which all compounds were tested for their ability to inhibit high affinity uptake of [^{3}H]-DA using rat nerve endings (synaptosomes) obtained from brain regions enriched in DAT.

All DAT ligands were separated into a training set with 36 molecules and a test set with six molecules (Figure 1, Tables 1 and 2). Additionally, all compounds were assigned into three series C1, C2, and C3 according to the number of carbon atoms between the attachment point of the 3α-substituent and its first polar atom. The molecules were built and minimized to a convergence criterion of 0.05 kcal/mol·Å using the Tripos force field[63] and Gasteriger−Marsili charges.[64] A protonated piperidine nitrogen was adopted for all ligands used in this paper. The major steps of the applied computational protocol are shown in Figure 2.

Pharmacophore Hypotheses and Structure Alignment. Three molecules, **1p**, **2i**, and **3c**, were selected to represent the three series, C1, C2, and C3, respectively. The pharmacophore searching step was accomplished using the Genetic Algorithm Similarity Program (GASP),[65] which employed a genetic algorithm[66] for identifying common spatial arrangement of functional groups of different molecules. The parameters were set as default except the following: population size was 125, mutation weight was 96, fitness increment was 0.02, number of alignment was 8.

Once a pharmacophore was generated, the three-dimensional structure of one of the three representative molecules, compound **2i**, was used as a template in structure alignment for all molecules of the three series. This step was performed using an incremental construction algorithm and a scoring function based on intermolecular interactions and overlapping density functions implemented in the Flexible Superposition (FlexS) technique.[67−69] The minimum volume overlap was set at 0.6, and the number of alignments per ligand was 30. A top-ranked conformer for each compound was initially utilized in CoMFA modeling. The MOPAC charges[70] for FlexS and CoMFA modeling were calculated using the AM1 method.[71,72]

CoMFA Modeling and Optimization. The steric and electrostatic field energies were calculated using an sp^3 carbon

Table 1. Observed and CoMFA-Predicted DAT Activities of the Training Set of Ligands

	DAT activity		model 1		model 2	
compound	K_i, nM	pK_i	predicted	residual	predicted	residual
1a	173000	3.76	3.72	0.04	3.72	0.04
1b	233	6.63	6.73	−0.10	6.65	−0.02
1c	520	6.28	6.36	−0.08	6.37	−0.09
1d	318	6.50	6.52	−0.02	6.46	0.04
1f	1160	5.94	5.94	0.00	5.84	0.10
1g	1420	5.85	5.84	0.01	5.76	0.09
1h	53	7.28	7.28	0.00	7.26	0.02
1i	9900	5.00	4.98	0.02	5.02	−0.02
1j	2140	5.67	5.68	−0.01	5.67	0.00
1k	908	6.04	6.02	0.02	6.10	−0.06
1l	187	6.73	6.72	0.01	6.80	−0.07
1m	391	6.41	6.44	−0.03	6.43	−0.02
1o	497	6.30	6.33	−0.03	6.34	−0.04
1p	60	7.22	7.19	0.03	7.30	−0.08
1q	793	6.10	6.11	−0.01	6.11	−0.01
1s	257	6.59	6.59	0.00	6.62	−0.03
1t	599	6.22	6.21	0.01	6.30	−0.08
1u	272	6.57	6.60	−0.03	6.45	0.12
1v	273	6.56	6.52	0.04	6.61	−0.05
1w	181	6.74	6.77	−0.03	6.76	−0.02
1y	56	7.26	7.24	0.02	7.26	0.00
2a	3008	5.52	5.52	0.00	5.61	−0.09
2b	79	7.10	7.17	−0.07	7.13	−0.03
2c	36	7.45	7.45	0.00	7.46	−0.01
2d	926	6.03	5.96	0.07	5.96	0.07
2e	706	6.15	6.16	−0.01	6.24	−0.09
2f	270	6.57	6.69	−0.12	6.52	0.05
2g	16	7.81	7.78	0.03	7.83	−0.02
2i	44	7.36	7.31	0.05	7.40	−0.04
3a	1303	5.89	5.92	−0.03	5.87	0.02
3c	53	7.28	7.25	0.03	7.26	0.02
3d	201	6.70	6.68	0.02	6.67	0.03
3e	446	6.35	6.31	0.04	6.38	−0.03
3f	20	7.70	7.59	0.11	7.46	0.24
3g	26.4	7.58	7.61	−0.03	7.58	0.00
3h	43	7.37	7.30	0.07	7.28	0.09

Table 2. Observed and CoMFA-Predicted DAT Activities of the Test Set of Ligands

	DAT activity		model 1		model 2	
compound	K_i, nM	pK_i	predicted	residual	predicted	residual
1e	906	6.04	6.08	−0.04	5.49	0.55
1n	497	6.30	6.36	−0.06	6.51	−0.21
1r	294	6.53	6.85	−0.32	6.63	−0.10
1x	91	7.04	7.32	−0.28	6.63	0.41
2h	165	6.78	7.06	−0.28	6.90	−0.12
3b	68	7.16	7.45	−0.29	7.06	0.10

atom with a +1 charge as a probe. Principal Component Analysis (PCA)[73] and Partial Least Squares (PLS) regression[74,75] with column filtering of 2.0 kcal/mol were performed to correlate the biological data and molecular fields. Leave-One-Out cross-validation was utilized to optimize the number of principal components and to evaluate the predictive capability of models. PLS procedures without cross-validation were performed to create predictive models.

Once initial CoMFA models were generated using the top-ranked conformers of the training compounds for each pharmacophore, the optimization step was performed for the two best models by replacing the top conformer of each compound with the other alignments to identify the fittest conformer for a specific model, which was evaluated by the cross-validated correlation coefficient q^2. Those molecules with large deviations between the observed and predicted biological data in the initial CoMFA models were selected for more rigorous optimization. Multiple iterations of the above procedure were performed for all training compounds until a CoMFA model with a high cross-validated coefficient was obtained.

Results and Discussion

The overall strategy of the molecular modeling and optimization is shown in Figure 2. The discovery of active conformers and structure alignment are two critical steps in CoMFA modeling, especially for flexible compounds such as 3α-substituted piperidine-based analogues of cocaine. To generate potential conformations that the ligands may adopt, we decided to use the automated feature alignment available in GASP. The advantage of using feature alignment approaches (GASP, DISCO, HipHop, and others) comparing to scaffold superposition based on the RMS fitting, which is commonly used in CoMFA modeling, is that they provide an effective way to align flexible and diverse compounds.[65,76−78]

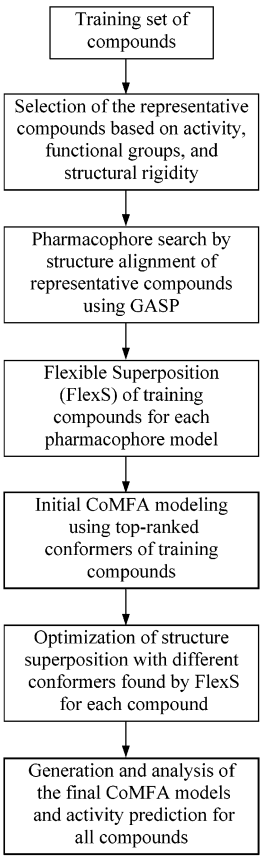

Figure 2. Flowchart of pharmacophore generation, optimization of structure superposition, and CoMFA model generation.

Since GASP can effectively generate pharmacophore models only for a limited number of ligands, three ligands (one from each series) were chosen based on the following three criteria. First, a representative molecule should be DAT-active; second, it should have meaningful functional groups, which could be used in pharmacophore search; and third, the 3α-substituents of these molecules should be relatively rigid with only a few rotatable bonds, thus limiting the number of distinct models obtained for further evaluation by CoMFA modeling. Three compounds **1p**, **2i,** and **3c** were selected from the training set to represent the three series of the piperidine-based cocaine analogues. All of these three representative compounds are highly potent and relatively rigid and contain meaningful functional groups. A total of eight pharmacophore models were generated by GASP based on these three compounds. For each pharmacophore model, all compounds from the C1, C2, and C3 series were superimposed on **2i** using the flexible superposition algorithm FlexS. The initial CoMFA models were constructed using the top-ranked conform-

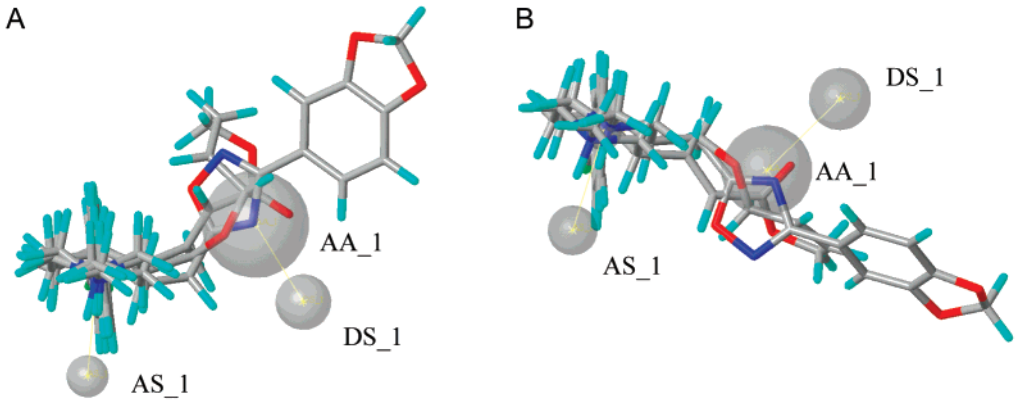

Figure 3. Pharmacophores A and B as discovered by GASP. Both pharmacophores have one hydrogen bond (H-bond) donor site (DS_1) corresponding to one acceptor atom (AA_1), and one H-bond acceptor site (AS_1) corresponding to the hydrogen atom connected to the piperidine nitrogen. The lipophilic sites corresponding to the centroid of the 4-chlorophenyl ring are in the same place in both models and are not shown for reasons of clarity.

Table 3. Summary of Statistics and Field Contributions for Models 1 and 2

	model 1		model 2	
	initial	optimized	initial	optimized
no. of training compounds	36	36	36	36
no. of test compounds	6	6	6	6
optimal no. of components	4	6	3	6
q^2	0.515	0.828	0.296	0.849
standard error of prediction	0.599	0.369	0.71	0.346
r^2	0.900	0.997	0.837	0.993
standard error of estimate	0.271	0.051	0.342	0.074
F values	70.1	1462.3	54.8	688.8
probability of $r^2 = 0$	0	0	0	0
field contributions				
steric	0.474	0.621	0.498	0.493
electrostatic	0.526	0.379	0.502	0.507

ers of the ligands in the training set for all eight pharmacophore models. Among all of the eight initial CoMFA models, only one had a cross-validated coefficient q^2 above 0.5 (model 1), and the second-best model had a q^2 value of 0.296 (model 2). Pharmacophores A and B correspond to the CoMFA models 1 and 2, respectively (Figure 3 and Table 3). The pharmacophore A differs from B by the location of the hydrogen bond donor site (DS_1). In pharmacophore A, DS_1 is on the same side of the piperidine ring as the hydrogen bond acceptor site (AS_1) whereas in pharmacophore B, DS_1 is located on the opposite side of AS_1. Both pharmacophore models have a lipophilic site corresponding to the centroid of the 4-chlorophenyl ring.

One possibility for the low predictivity of the CoMFA models is that these pharmacophores, which were derived from the three representative structures **1p**, **2i**, and **3c** by GASP, were not suitable for all molecules. On the other hand, it is also possible that the top conformers of the training compounds found by FlexS, which were initially utilized for CoMFA modeling, did not ideally fit in the pharmacophore model and hence produced large deviations between the observed and predicted biological data. Assuming that the latter problem was more likely to cause the low predictivity of the initial CoMFA models, an additional refinement of the structure alignment was performed. In the two best CoMFA models 1 and 2, the top conformer identified by FlexS was replaced with different conformers for each compound, and the overall superposition of all training compounds was reevaluated repeatedly until the final CoMFA model with high accuracy and predictive power was achieved.

The r^2 values of the two optimized models both increased to above 0.99. Surprisingly, model 2 has an even higher q^2, 0.849, compared with the q^2 for model 1, 0.828. Contributions from steric and electrostatic fields for optimized model 1 have changed to 0.621 and 0.379, respectively. Optimized model 2 has almost the same contributions from the steric and electrostatic fields as its initial model. The statistical parameters and field contributions of initial and optimized models 1 and 2 are summarized in Table 3. The predictive ability of models 1 and 2 was further evaluated by a test set of six compounds using their top conformers. The observed and CoMFA-predicted pK_i values for the training set and test set compounds are shown in Tables 1 and 2. The residuals between the predicted and observed activities of the test compounds are consistent with the standard error of prediction of the two models. The correctly predicted activities of the test compounds provide further verification of the accuracy of the two models.

The CoMFA contour maps for the optimized models 1 and 2 are illustrated using compound **2i** as the reference structure in Figure 4. The electrostatic maps show that there is one positive charge favorable (blue) area located close to the first atom of the 3α-substituent in model 1 (Figure 4a), whereas model 2 has two smaller positive charge favorable areas on both sides of the 3α-substituent (Figure 4c). Negatively charged 3α-substituents near these areas would produce a negative effect on affinity. It is consistent with the result that the carboxylic acids **2a** (C2 series) and **3a** (C3 series) are more active than **1a** (C1 series), assuming that the carboxyl groups of **1a**, **2a**, and **3a** are negatively charged under physiological conditions. For the same reason, compounds in the C1 series may, in general, be less potent than compounds in the C2 and C3 series, as the electron-rich functional groups in the former compounds are closer to these negatively charged areas in the DAT than the functional groups in C2 and C3 ligands. Both models 1 and 2 display several electron density favorable (red) regions around the 3α-substituent spanning from its third to sixth atoms, which may suggest that the DAT in this area has several hydrogen bond (H-bond) donor sites rather than only the one depicted in

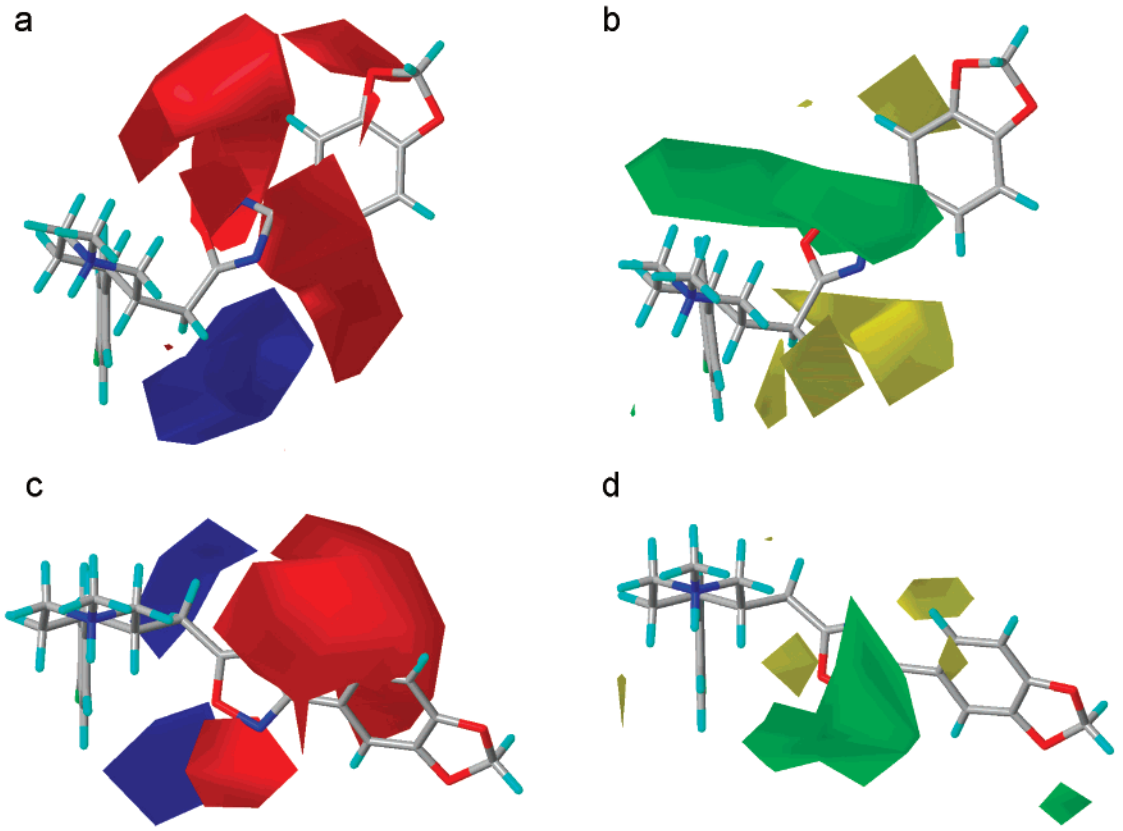

Figure 4. CoMFA contour maps of optimized model 1 and model 2. (a, b) Electrostatic and steric components for model 1. (c, d) Electrostatic and steric components for model 2. In the electrostatic contour maps, greater affinity is correlated with more positive charge near blue and more negative charge near red; in the steric contour maps, greater affinity is correlated with more bulky groups near green and less bulky groups near yellow.

the pharmacophores A and B (Figure 3). Generally, the binding affinity can be increased by introducing electron-rich atoms in this area. This is consistent with the observation that the N-monosubstituted amides **2d**−**f** and **3d**,**e** exhibit only moderate potency, whereas the N-disubstituted amide **2g** exhibits 12- to 58-fold higher activity. Similarly, the carbamates **1x**,**y** are highly active as these ligands have one additional electron-rich atom that could compensate the negative effect of the amide hydrogen observed in ligands **2d**−**f** and **3d**,**e**.

Both CoMFA models have a large steric favorable (green) area in which bulky groups increase binding affinity (Figures 4b and 4d). In both models these areas are located opposite to the corresponding H-bond donor site in pharmacophores A and B (Figure 3). The presence of the steric favorable area close to the piperidine ring in model 1 (Figure 4b) may be one of the reasons why tropane-based compounds are generally more active than piperidine-based compounds, as the additional two-carbon bridge in tropane-based ligands would be positioned close to this steric favorable area. It is also consistent with the high activity of compound **3f**, which has no polar groups in the 3α-substituent but may have favorable steric interactions with this area. Model 1 has two steric unfavorable (yellow) areas parallel to the steric favorable (green) area on the opposite side of the substituent of the reference structure **2i** (Figure 4b). The existence of the large steric unfavorable area is consistent with the prohibited area proposed earlier[34] and may be a reason for the lower activity of compounds **1m**,**n**. Model 2 displays steric unfavorable (yellow) areas in the positions similar to model 1 but with smaller sizes, which can be understood in terms of smaller contributions from the steric field for this model (Figure 4d). There are two more steric unfavorable areas in model 1 with one located about 1.5 Å away from the favorable area and the other one near the 4-chlorophenyl group. Introduction of any bulky group in these areas is expected to decrease the potency of compounds. All of the differences of the orientation of the ligands and the CoMFA steric and electrostatic fields between the models 1 and 2 indicate that both models are unique, and each may represent a distinct possible binding mode of the 3α-substituent.

Conclusions

A successful strategy of pharmacophore-based alignment of the fittest conformers was designed and applied to determine the details of the DAT binding site in proximity to the 3α-substituent of the piperidine-based analogues of cocaine. Two highly predictive and statistically significant CoMFA models were constructed. Both CoMFA models suggest that steric and electrostatic interactions play important roles in the binding of the 3α-substituent of the piperidine-based ligands at the DAT. The fact that two distinct models were obtained indicates that the 3α-substituent may adopt multiple binding modes. Overall, these findings provide guidance for the design and improvement of compounds with DAT activity, which is important for the development of a treatment of cocaine addiction and certain neurological disorders.

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