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3D-QSAR study of 8-azabicyclo[3.2.1] octane analogs antagonists of cholinergic receptor

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ABSTRACT

3D-QSAR models of Comparative of Molecular Field Analysis (CoMFA) and Comparative of Molecular Similarities Indices Analysis (CoMSIA) of 20 8-azabicyclo[3.2.1] octane (potent muscarinic receptor blocker) was performed. These benztropine analogs were optimized using ligand based alignment method. The conventional ligand-based 3D-QSAR studies were performed based on the low energy conformations employing database alignment rule. The ligand-based model gave q^2 value 0.819 and 0.810 and r^2 value 0.991 and 0.988 for CoMFA and CoMSIA, respectively, and the predictive ability of the model was validated. Results indicate that the CoMFA and CoMSIA models could be reliable model which may be used in the design of novel muscarinic antagonists as leads.

The 8-azabicyclo[3.2.1] octane ring system, found in atropine, scopolamine, and cocaine, is also the ring system of the synthetic novel analogs of benztropine. Such analogs have been developed that have high affinity for the dopamine transporter. Although these compounds bind to the dopamine in vitro, their behavioral effects are generally different from those of the typical dopamine uptake inhibitors, for which cocaine is a prototype. Many of these compounds have relatively high affinity for Muscarinic receptor as well as the dopamine transporter and it has been suggested that those other actions may interfere with the cocaine- like behavioral effects of benztropine (BZT) analogs.[1] 3D-QSAR studies may be used for the design of muscarinic antagonists. Lead optimization is envisaged with the help of CoMFA and CoMSIA techniques for obtaining a robust model for potent antimuscarinic agents.

A series of 20 8-azabicyclo[3.2.1] octane analogs, in particular the benztropine analogs[1,2] were selected for 3D-QSAR study. In vitro K_i values (binding parameter of the muscarinic receptor antagonist) were converted to pK_i ($-\log k_i$) value. The total set of compounds was randomly divided into the training set (15 compounds) and test set (5 compounds, labeled with *). The three-dimensional structures of benztropine analogs were constructed by using SYBYL program version 7.1 on a Silicon Graphic workstation.[3] Energy minimizations were performed using the tripos force field with a distance dependent dielectric and conjugate gradient method. The convergence criterion was 0.01 kcal/mol Å. The Gasteiger–Huckel charges were assigned.

The most crucial input for CoMFA is the alignment of the molecules.[4] The template molecule is the most bioactive compound (PMTR.Et), which was taken from the training set shown in Figure 1a and was used as the reference atoms to align all the molecules using the 'DATABASE ALIGNMENT' option in SYBYL 7.1.[5] The aligned molecules are shown in Figure 1b.

The statistical results obtained from standard CoMFA models constructed with steric and electrostatic fields are summarized in Table 5. The q^2, SEE, r^2, F and s values were computed as defined in SYBYL. The optimum number of components (5) was determined by SAMPLS analysis implemented in SYBYL 7.1 with an LOO cross-validated q^2 of 0.819, which indicated a good predictive capacity of the model ($q^2 > 0.5$). A high correlation coefficient (r^2) of 0.991 for the non-cross-validated final model showed the self consistency of the model ($r^2 > 0.9$). In both steric and electrostatic field contributions, the former accounts for 0.630, while the latter contributes 0.370, indicating that the steric factors nearly contribute the more to the binding affinities.[6]

As stated above, CoMSIA not only offered steric and electrostatic field information as CoMFA but also gave hydrophobic, hydrogen bond donor and acceptor field information, whereas these three factors are always important to the binding affinities. Thus different combination of the three fields with steric and electrostatic fields may result in different models. So, in this paper model was built by varying these five fields (Table 5). The optimum component of each model was determined by SAMPLS analysis implemented in SYBYL 7.1 with maximum q^2 value. The best 10-component model with the maximum q^2 value consisted of all the five fields as the descriptors, which gave the highest cross-validated coefficient q^2 of 0.810, thereby indicating the most powerful

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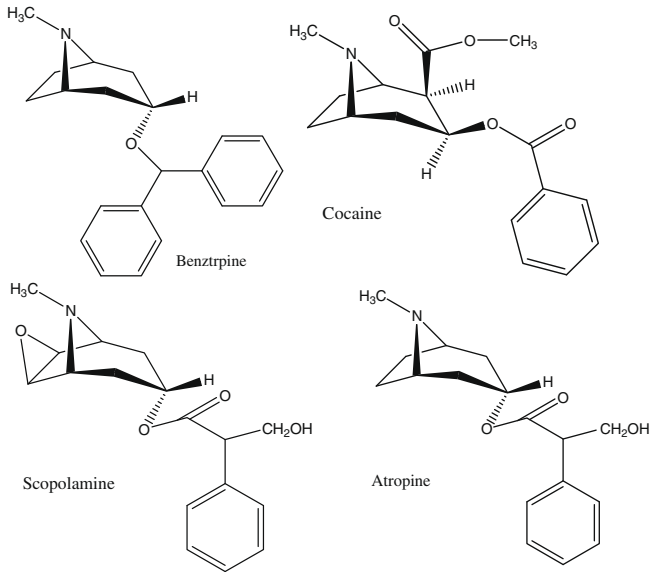
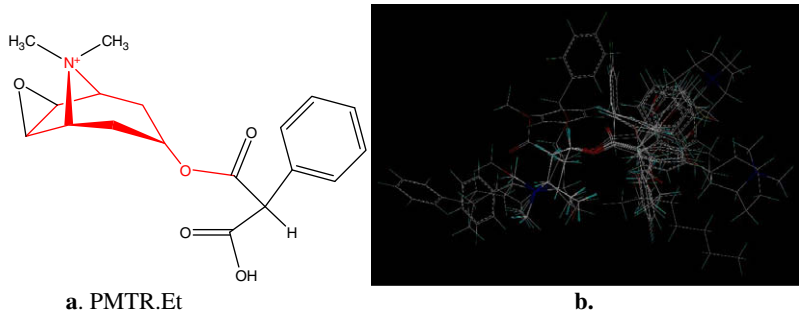


predictive capacity. The highest conventional correlation coefficient r^2 of 0.988 was also obtained for this model, showing the strong internal consistency and therefore, this model was chosen for the final analysis. The standard error of estimate and the *F*-test values of this model were 0.531 and 199.654, respectively.[7]

In order to test the predictive power of the CoMFA and CoMSIA models obtained by the training set, other 5 inhibitors (Table 4) that were not included in the training set were used as a test set. Table 4 summarizes the predicted results obtained from the CoMFA and CoMSIA models. The plots of predicted versus actual binding affinities for the test set inhibitors are shown in Figure 4a and b, which represent models based on CoMFA and CoMSIA, respectively. By comparison of the experimentally observed and theoretically predicted pK_i values of antagonist activity of a series of 8-azabicyclo[3.2.1] octane containing derivatives, it can be seen that both CoMFA and CoMSIA models performed well in the predicition of the activities of the test inhibitors. In almost all the cases, the predicted values were close to the observed pK_i values, deviating by less than 1 logarithm unit. Especially for Atropine, both CoMFA and CoMSIA models gave ideally predictive values, both of which are less than 0.2 logarithm units. The predicted correlation coefficients (r^2 predicted) are 0.993 for the CoMFA model and 0.981 for the CoMSIA model. All results including statistical data from this predictive validation analysis further confirmed the robustness of the obtained models in terms of their predictive abilities. There was no significant difference between the CoMFA and CoMSIA models for this predictive validation test.

Table 5 shows that the steric fields and electrostatic fields didn't gave the same contribution, accounting for 0.630 and 0.370, respectively, which suggests that steric fields are easy in explaining the inhibition potency of these molecules and the generated CoMFA models explain well the variations between molecules having differences in steric and electrostatic interactions. Figure 2a shows the steric contour map for the CoMFA models with the highly active inhibitor Atropine (pK_i 9.34) as a reference. Three regions at P1 and P2 positions of atropine have been identified with green polyhedra, which indicate that bulky substituents at these positions may improve the activities. The aromatic moiety at P1 position of atropine was surrounded by two green pleths lying side by side of the aromatic ring, respectively. So addition of a bulky group at this position is favorable to the binding affinities. This is indeed the case for Table 1, and Table 2 compounds. Sterically favored cyclohexyl group at P1 position of AHN1-055, AHN2-005 increased the pK_i value; however, while it was substituted by a comparatively smaller methyl group at P2 position (compound ANH2-003), the pK_i value was decreased to 1.9088. Compounds JHW 005, JHW 007 and cocaine are also account for such results. PMTR.Et, PMTR·Hx with the alkyal group at P1 position substituted by a hydrogen atom, gave a decrease pK_i value, but when the same amino group was substituted by bulky cyclohexyl group (compound PMTR·cHx), the pK_i value was increased to 8.202.Beside the benzyl group at P2 position of the reference molecule atropine, a green polyhedron appears, which means that sterically favored substituents will improve the biological activities of muscarinic antagonists/inhibitors. The presence of a bulky group butylphenyl at P2 position of GA 1-003 showed a higher binding affinity (pK_i 3.1066), while the smaller group benzyl at P2 position of AHN2-003 decreased the pK_i value to 1.9088. For compounds cocaine and benztropine the former bearing an additional bulky group on benzyl group at P2 position exhibited more potent activity than the latter. However, the CoMFA models did not give any isopleth at P3 position in the contour map.

Electrostatic fields based on the PLS analysis of the CoMFA models are shown in Figure 2b with Atropine as a reference. A large red isopleth above the benzine ring of P1 position represents an area where a negative GH charge is favored. Alkyl group at P1 position of AHN 2-003, JHW 007, JHW 005, bearing positive GH charges on the C atom, decreases the activity (pK_i 0.3982,0.0676, 0.1018). In case of PMTR·TR, PSTR·TR, PMTR·MeSOMe, PMTR·Hx have no attachment of any negatively favoured group to the benzine ring so decrease in activity, while benzyloxy group at P1 position of JHW 025, AHN 1-055, AHN 2-005, GA 1-003 having negative charges group on the C atom and benzyl ring, increases the activity (Table 3). At the same time, positively charged nitrogen atom on the cyclohexane is necessary for a blue favorable isopleth in proximity to this area. Nitrogen atoms in such position usually can form H-bonds with amino acid residues of proteasome. One blue polyhedra encompassing the tropain ring indicate positively



a. PMTR.Et **b.**

Figure 1. Alignment of the training set: **a** is the template structure (common fragment in red), **b** is the alignment of the training set for ligand-based model.

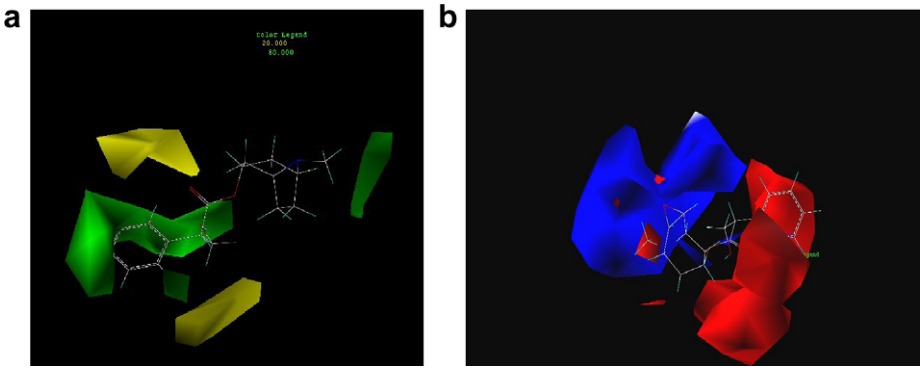


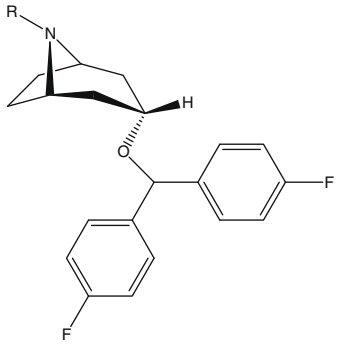
Figure 2. Standard coefficient contour maps of final CoMFA analysis with grid spacing in combination with atropine. (a) Steric contour map. Green contours refer to sterically favored regions. (b) Electrostatic contour map. Blue contours (35% contribution) refer to regions where positively charged substituents are favored; red contours (65% contribution) indicate regions where negatively charged substituents are favored.

charged groups, such as hydrogen atoms, which are beneficial to the activity. A large region of red contour surrounding atoms of benzene suggests that negatively charged oxygen atoms are necessary to increase activity. At P2 position, a red polyhedron lying below tropain group indicates substituents with GH negative charges may improve the binding affinities. It is noteworthy to point out that at P3 position, positively charged groups linked with methyl and other electropositive group such as $-(CH_3)_2{}^+I^-$ are beneficial to high activity.

CoMSIA calculates both steric and electrostatic fields, as in CoMFA, but additionally uses hydrophobic, hydrogen-bond donor and acceptor fields. Since a Gaussian function is used to determine the distance dependence, therefore, the similarity indices can also be calculated at grid points inside the molecule, not just outside, as with CoMFA.

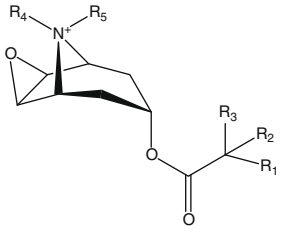
Figure 3 provides graphical representations of the CoMSIA models. To aid in visualization, the highly active inhibitor Atropine was overlaid in the maps once again. Figure 3a shows the CoMSIA steric field contour map. Green isopleths indicate regions where more steric substituents will enhance the activities. Just as discussed in CoMFA contour maps, benzyl group of P2 position is surrounded by a large green polyhedron, which suggests bulky substituents, such as 2-naphthyl methylene and 4-(*p*-benzoxyl)-benzyl groups (for example PCMS-2, PCPA·Me, AHN1-055, AHN2-00, GA-1-003, JHW 025, PMTR·cHx) are favored. A small green polyhedron nearer to *tert*-nitrogen group indicates bulky groups instead of small groups attaching on the nitrogen are preferred to this region. As in CoMFA field information, the CoMSIA models also did not give any isopleths at P3 position in the contour map. In the electrostatic CoMSIA plots (Fig. 3b), it nearly gave the same results as in CoMFA. At P1 position, a red isopleth lying above the carbon atom of benzene ring indicates the important role of such a negative atom.

Table 1



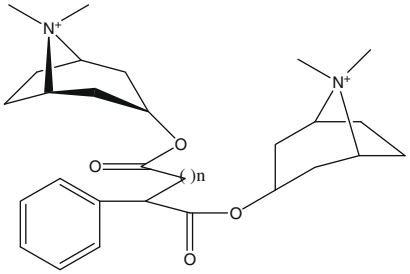
Compound code	R
AHN 1-055	$-CH_3$
AHN 2-003	–H
AHN 2-005	–Allyl
JHW 007	–Butyl
JHW 005	–Benzyl
GA 1-003	–Butylphenyl
JHW 025	$-(CH_3)_2{}^+I^-$

Table 2



Compound code	R_1	R_2	R_3	R_4	R_5
PCMS-1	Ph	Cyclopentyl	–COO-ethyl	$-CH_3$	$-CH_3$
PCMS-2	Ph	Cyclopentyl	–COO-methyl	$-CH_3$	$-CH_3$
PCPA-Me	Ph	Cyclopentyl	–H	$-CH_3$	–
PMTR·cHx	Ph	–H	–COO-cyclohexyl	$-CH_3$	$-CH_3$
PMTR.Et	Ph	–H	–COO-ethyl	$-CH_3$	$-CH_3$
PMTR·Hx	Ph	–H	–COO-*n*-hexyl	$-CH_3$	$-CH_3$
PMTR.Me.SOMe	Ph	–H	$-COOCH_2$ $SOCH_3$	$-CH_3$	$-CH_3$

Table 3



Compound code	*n*
PMTR·TR	1
PSTR·TR	2

Table 4
Experimental and predicted pK_i, residuals by CoMFA and CoMSIA models in the training and test set (with *) with ligand-based method

Compound code	pK_i Exp.	CoMFA		CoMSIA	
		PV[a]	RV[b]	PV	RV
Atropine*	9.34	9.154	0.186	9.20495	0.13505
Scopolamine*	8.95	9.1783	−0.2283	9.22039	−0.27038
PMTR.Et	8.32	8.291	0.029	8.34829	−0.02829
PMTR·cHx	8.01	8.202	−0.192	7.92805	0.08195
PMTR·Hx	7.33	7.3195	0.0105	7.49657	−0.16657
PCMS-1	7.98	7.999	−0.019	8.12142	−0.14142
PCMS-2	7.28	7.4185	−0.1385	7.19363	0.086375
PCPA·Me	7.14	7.1752	−0.0352	6.50921	0.820788
Cocaine	4.79	4.881	−0.091	5.26139	−0.47139
Benztropine	0.33	0.7269	−0.4049	1.46654	−1.14654
AHN1-055*	1.06	1.5183	−0.4543	1.40059	−0.34059
AHN2-003*	2.31	1.9088	0.3982	1.69599	0.554009
AHN2-005*	2.25	2.314	−0.064	1.34657	0.28657
JHW 007	2.6	2.5324	0.0676	2.17002	0.429981
JHW 005	3.01	2.9082	0.1018	2.96824	0.04176
GA-1-003	2.75	3.1066	−0.3567	2.99016	−0.24016
JHW 025	1.07	1.1902	−0.1192	1.0452	0.0258
PMTR·TR	7.18	6.9473	0.2327	6.97511	0.20489
PSTR·TR	6.5	6.1824	0.3176	6.59733	−0.09732
PMTR·MeSOMe	7.25	7.0477	0.2023	7.4099	−0.1599

[a] Predictive values.
[b] Residual values.
* Test set molecule.

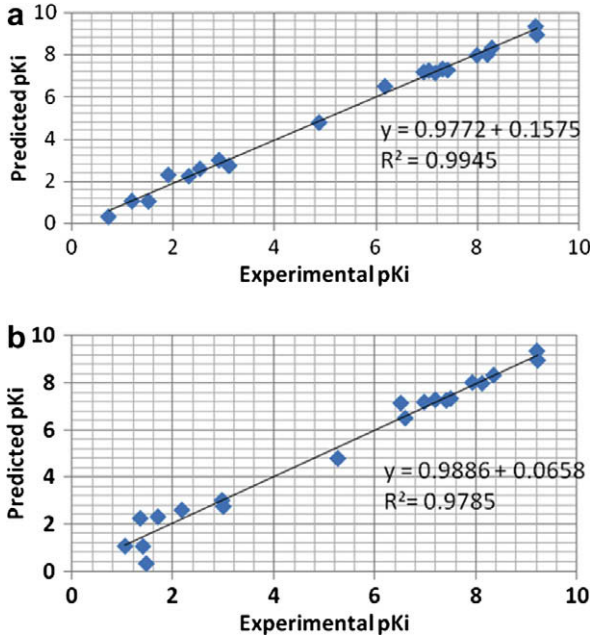


Figure 4. Predicted versus actual binding affinities for the 20 inhibitors in the set for (a) CoMFA and (b) CoMSIA models. The predicted correlation coefficients (r^2 pred.) are 0.993 for the CoMFA model and 0.981 for the CoMSIA model.

Large red polyhedra embedded in phenyl ring of P2 position and end to tropane ring denote regions of preferential negatively charged substituents. Blue isopleths around the tropane ring indicate that groups with positive charges are favored, which is consistent with the results of CoMFA contour map. The hydrophobic field is presented in Figure 3c. White and yellow contours highlight areas where hydrophilic and hydrophobic properties are preferred, respectively. Table 5 shows that hydrophobic field made the larger contribution to the CoMSIA QSAR models, which suggests that among the descriptors considered, the hydrophobicity of inhibitors is the most important factor affecting the binding affinities. Hydrophobic residues, which can effectively interact with inhibitors through hydrophobic interaction. Three large yellow polyhedra surrounding P1, P2 positions and *tert*-nitrogen group indicate that hydrophobic groups are beneficial to enhance the activity. The white isopleths around the hydrogen atoms of benzene ring reveal the necessity of the hydrophilic hydrogen atoms on the aromatic ring to the activities. The graphical interpretation of the hydrogen-bond donor interaction in the CoMSIA model is represented in Figure 3c. It highlights areas beyond the molecules where putative hydrogen-bond acceptor groups in the enzyme can form H-bonds with molecule thereby influencing binding affinities. The

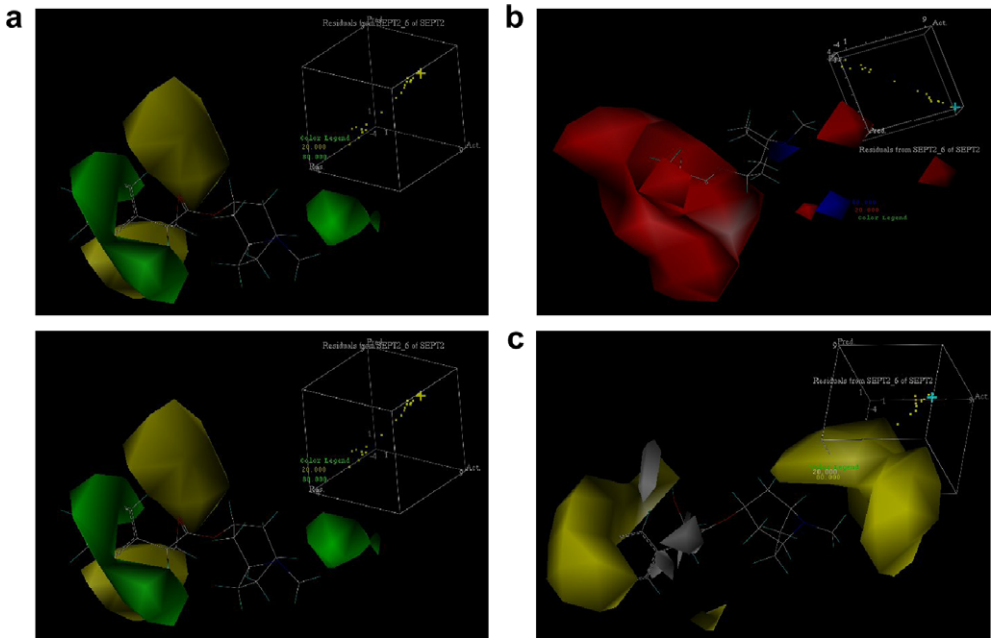


Figure 3. CoMSIA contour maps in combination with atropine: (a) the steric field distribute ion; (b) the electrostatic field distribution; (c) the hydrophobic distribution; positive potential favored areas in negative potential favored areas in red. Hydrophobic favored areas in yellow, hydrophilic favored areas in white. Sterically favored areas in green, sterically disfavored areas in yellow.

Table 5
Summary of CoMFA and CoMSIA model results

Components	Ligand-based model	
	CoMFA	CoMSIA
q^2	0.819	0.81
r^2	0.991	0.988
n	5	5
F-Values	310.57	119.65
SEE	0.332	0.531
r^2 Predicted	0.993	0.981
Field contribution		
Steric	0.63	0.115
Electrostatic	0.37	0.27
Hydrophobic	—	0.212
Donor	—	0.195
Accepter	—	0.208

q^2, LOO cross-validated correlation coefficient; r^2, non-cross-validated correlation coefficient; n, number of components used in the PLS analysis; SEE, standard error estimate; F value, F-statistic for the analysis.

cyan contours represent locations where a hydrogen-bond acceptor on the receptor will improve activity, that is, hydrogen-bond donors in the ligand directing to these regions are favorable. For example, there are two cyan areas: one surrounds the hydrogen atoms of NH group attached to the tropane moiety, which can form hydrogen bond with residues of muscarinic receptor, the other is beside the hydrogen atom of benzene of between P2 and P3 positions, which indicates the necessity of the hydrogen atoms at these positions for high activities. However, due to the methylation of the N atoms in compounds the binding affinities (pK_i value) increase greatly. Figure 3c indicates areas where hydrogen-bond acceptors in the ligand promote or decrease binding affinities. Proton acceptors in the ligand directing to white regions increase the binding affinities. A red polyhedron near to the carbon atom of the benzene ring at P1 position of the reference molecule suggests that the subunit bearing hydrogen-bond donors will increase activity, which is consistent with the results discussed in the electrostatic fields of CoMFA and CoMSIA contour maps. Furthermore, large red isopleths encompassing two oxygen atoms of the carbonyls indicate hydrogen-bond acceptors in the ligands promote activity. The two oxygen atoms of aropine formed H-bonds to the cholinergic receptor. The CoMFA and CoMSIA contour maps offered enough information for us to understand the binding mode between the inhibitors and the muscarinic receptor. Based on the analysis, several structurally novel compounds were designed and synthesized. The biologically assessed experiments indicated that the constructed models were reliable enough to be applied both in the rational design and library screening.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.164.

References and notes

1. Jonathan, L. K.; Theresa, A. K.; Gregory, E. A.; Amy, H. N. *J. Pharmacol. Exp. Therapeutics* **2004**, *309*, 650.
2. Huang, F.; Buchwald, P.; Browne, C. E.; Farag, H. H.; Wu, W. M.; Ji, F.; Hochhaus, G.; Bodor, N. *AAPS Pharm. Sci.* **2001**, *3*, 1. article 30.
3. *QSAR and Advanced CoMFA® Manual, SYBYL 7.1*; *Tripos Inc.*: 1699 S Hanley Rd., St. Louis, MO 631444, USA.
4. Amit, N. et al *Bioorg. Med. Chem.* **2007**, *15*, 626.
5. Ulf, N. *Perspect. Drug Discovery Des.* **1998**, *12-14*, 25.
6. Wang, R.; Gao, Y.; Liu, L.; Lai, L. *J. Mol. Mod.* **1998**, *4*, 276.
7. Avila, C. M. et al *Bioorg. Med. Chem.* **2006**, *14*, 6874.