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Activity and QSAR study of baogongteng A and its derivatives as muscarinic agonists

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Abstract—Baogongteng A (BGT-A), a naturally occurring tropane muscarinic agonist isolated from Chinese medicinal plant, exhibits a bioactive effect different from those of many tropane alkaloids that are muscarinic antagonists. A series of racemic derivatives of BGT-A was synthesized to study the structure–activity relationships (SAR). To explore further the SAR in this series and to ultimately design muscarinic agonists for drug development, a Comparative Molecular Field Analysis (CoMFA) was performed. The values of the leave-one-out cross-validated correlation coefficient q^2 and the conventional correlation coefficient r^2 for the model are 0.613 and 0.965, respectively. The regression analysis of the data indicated that the steric effect of N-substituted group on tropane of analyzed compounds critically affected the agonistic activity to muscarinic receptors.

Muscarinic receptors play an important role in cholinergic transmission, mediating most of the excitatory and inhibitory effects of acetylcholine in the central and peripheral nervous systems. Many tropane alkaloids (such as atropine and scopolamine, Fig. 1) are potent muscarinic antagonists, but BGT-A (**1**, Table 1) isolated for the first time from the stem of a Chinese medicinal plant baogongteng (*Erycibe obtusifolia* Benth) is a muscarinic agonist and was used as a myotic agent in clinics.[1] This unique bioactivity to muscarinic receptors in the family of tropane alkaloids makes it interesting to reveal the special interaction mechanism of BGT-A with the muscarinic receptors. Review of the therapeutic potential of BGT-A, the limited amounts of BGT-A available from the natural resources, and the low efficiency of total synthesis of BGT-A[2] investigated a series of racemic derivatives of BGT-A for bioactive screening.[3,4] The bioassay result shows that some of these compounds have certain agonistic activity to muscarinic receptors. The preliminary SAR of BGT-A and its derivatives was acquired.[5]

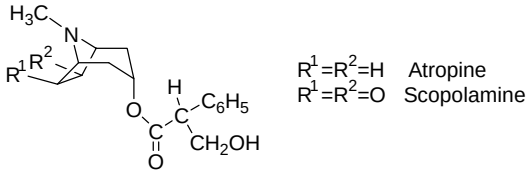

Figure 1. The structures of atropine and scopolamine.

The ligand–receptor interaction model can be constructed by docking for the known 3D structure receptor.[6] Muscarinic receptors, the guanine nucleotide binding protein-coupled receptors (GPCR), are members of a superfamily diverse group of receptors. At present, their crystal structures measured by X-ray diffraction have not been reported. To obtain a theoretical 3D-model of receptors, the homology modeling can be applied. But, it is a complicated work to construct an entire 3D structure for muscarinic receptors by homology modeling because of their relatively low homology with the putative template, bovine rhodopsin in the GPCR

Keywords: CoMFA; Baogongteng A; Muscarinic receptors; Agonistic.
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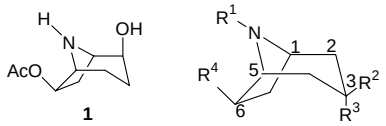
Table 1. Structures of BGT-A and its derivatives



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Compounds	R^1	R^2	R^3	R^4
2	CH_3	H	H	AcO
3	CH_3	H	AcO	AcO
4	CH_3	H	*p*-CH_3PhCOO	AcO
5	CH_3	H	*p*-CH_3PhSO_2O	AcO
6	CH_3	Cl	H	AcO
7	CH_3	O	O	AcO
8	CH_3	O	O	HO
9	CH_3	H	AcO	H
10	CH_3	H	$CH_3(CH_2)_2COO$	AcO
11	CH_3	H	$CH_3(CH_2)_4COO$	AcO
12	CH_3	H	$CH_3(CH_2)_5COO$	AcO
13	CH_3	H	$CH_3(CH_2)_6COO$	AcO
14	CH_3	H	$CH_3(CH_2)_8COO$	AcO
15	CH_3	H	CH_3CH_2COO	CH_3CH_2COO
16	CH_3	H	$CH_3(CH_2)_4COO$	$CH_3(CH_2)_4COO$
17	H	H	H	AcO
18	H	H	PhCH=CHCOO	AcO
19	CH_3	H	*p*-$CH_3OPhCOO$	AcO
20	CH_3	H	PhCH=CHCOO	AcO
21	CH_3	H	$PhSO_2O$	AcO
22	CH_3	H	*m*-CH_3PhSO_2O	AcO
23	CH_3	H	*p*-OCH_3PhSO_2O	AcO
24	CH_3	H	*p*-NO_2PhSO_2O	AcO

family, and too long sequences of amino acids.[7] In that case, it is difficult to search the binding site of muscarinic receptors for BGT-A and its active derivatives by docking. CoMFA[8] is usually applied as an effective method to build the model of ligand–receptor interaction and provide the information necessary for the understanding of structural requirement to activity.[9] Up to date, quantitative structure–activity relationship (QSAR) studies on the agonistic tropane ligands to muscarinic receptors have not been reported. In this paper, we carried out the CoMFA study of BGT-A and its agonistic active derivatives, which were prepared and tested biologically in our laboratory. The predictive 3D-QSAR model established by analyzing these targetively selected compounds could gain insight into the influence of their steric and electrostatic properties on the activity, and intensify our understanding on previous SAR studies of BGT-A and its derivatives. The novel compounds with higher agonistic activity could be designed by the results of CoMFA analyses and prepared conveniently.

Guinea pigs (250–350 g) of either sex provided by Experimental Animal Center of Shanghai Second Medical University were killed by a blow to the head and exsanguinated. The ileal longitudinal muscle was rapidly taken and gently cleaned off adhering connective tissue in a prewarmed (37 °C) and oxygenated (95%O_2 + 5%CO_2) medium of the Krebs solution (NaCl 6.6 g, $CaCl_2$ 0.28 g, KCl 0.35 g, $MgSO_4{\cdot}7H_2O$ 0.294 g, KH_2PO_4 0.162 g, $NaHCO_3$ 2.1 g, and glucose 2.0 g in 1000 ml distilled water). Prepared strips of ileal longitudinal muscle (1.5 cm) were transferred into 10 ml organ baths, loaded with 500 mg tension, and then allowed to equilibrate for 30 min and the bath fluid was changed every 10 min. Contractions were recorded isotonically with an electromechanical transducer connected to Bridge amplifier and Powerlab system recorder (Powerlab 8sp, ADInstrument). Dose–response curves to carbachol (79H0110, Sigma corporation) were drawn by cumulative addition of the agonist. The concentration of tested compounds in the organ bath was increased approximately 3-fold at each step, when the maximal and steady response to the previous addition had been attained. Dose–response curves were repeatedly established until constant responses were obtained, allowing 30 min between each curve. BGT-A and its derivatives were tested in the same protocol as that described for carbachol. Effects were expressed as percentage of the maximum effect induced by carbachol. $-\log EC_{50}$ (pEC_{50}) values were determined graphically. All data represented average values for three tests and analyzed by means of the *t* test. A value of $p < 0.05$ was considered significant.

Twenty-four target compounds with the tropane ring, whose structures and associated agonistic activity (expressed as pEC_{50}) to muscarinic receptors in ileal longitudinal muscle of guinea pigs are given in Tables 1 and 3, respectively, were divided into training set (compounds in Table 1 minus **5**, **9**, and **22**–**24**) and test set (**5**, **9**, and **22**–**24**). The CoMFA study described here was performed on a Silicon Graphics workstation using Sybyl (version 6.9).[10] The structures of entire sets of BGT-A and its derivatives were drawn by the SYBYL/SKETCH procedure. Although the lowest-energy conformation may not necessarily be the active conformation, the use of a reasonable low-energy conformation in the alignment is a useful starting point for statistical analysis. Structural energy minimization of each compound was performed by using the standard Tripos molecular mechanics force field with a 0.05 kcal/mol energy gradient convergence criterion and a distance-dependent dielectric constant (max iteration = 3000). Charges were calculated by the Gasteiger–Hückel method in the software. The alignment rule selected in CoMFA is often critical to the analytical result. Two alignment rules were used in our study for the superimposition of the target compounds (Fig. 2). In alignment 1 (Model A), the non-hydrogen atoms of the rigid tropane ring were selected as the fitting centers because the skeleton is almost unchanged. According to the previous SAR studies of BGT-A and its derivatives, their pharmacophore atoms are N-C-C-O. Thus, in alignment 2 (Model B), target compounds, except for **9**, were aligned by fitting together N-C5-C6-O. For the two alignments, all aligned compounds were superimposed using an atom-by-atom least square. **13** was selected as template in the SYBYL Fit option due to its highest

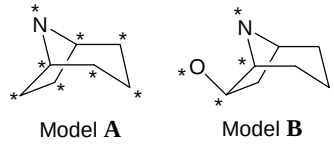

Figure 2. Two models for alignments.

activity. In the CoMFA analysis, the aligned molecules were put into a 3D grid with a distance-dependent constant spacing of 2Å, and sp^3 carbon probe atom with +1 charge was used to estimate the steric and electrostatic fields. The energetic cutoff for both fields was 30 kcal/mol. Regression analysis of the resulting matrix was performed by partial least-squares (PLS) linear regression for the training set. The cross-validated correlation coefficient (q^2) of the training set determined by a leave-one-out (LOO) was applied for the validation of the CoMFA model, following the general understanding of this method, though calculating cross-validated values for predicted pEC_{50} of each compound in the training set will be more accurate. The optimal components (N) is employed to do no validated PLS analysis to obtain the conventional correlation coefficient (r^2). The compounds of test set were utilized for external model validation.

The analytical results of the two CoMFA models are given in Table 2. The predicted values of pEC_{50} to muscarinic receptors and the residuals of the analyzed compounds are listed in Table 3. The optimum values of cross-validated r^2 for six components of the training set in the two models were almost identical, 0.607 and 0.613. In the case of alignment 1 (Model A), standard error and F value were 0.408 and 48.29. In the case of alignment 2 (Model B), they were 0.385 and 54.40, respectively. So, the following discussion only refers to the model B, as it has lower standard error and higher F value. In this analysis, the analyzed results have a 96.5% fitness compared to the biological test results ($r^2 = 0.965$). The relative contributions of steric and electrostatic fields were 73.3 and 26.7%, respectively, indicating that the agonistic activity to muscarinic receptors largely depended on the steric field. Figure 3 shows the plot of the observed activity versus the predicted one for the training set of model B. The resultant CoMFA model had a fair predictive ability.

Table 2. CoMFA analytical results about BGT-A and its derivatives

	Model A	Model B
Cross-validated r^2 (q^2)	0.607	0.613
Standard error of estimate	0.408	0.385
Conventional r^2	0.960	0.965
Optimal component	6	6
F value	48.29	54.40
Relative steric contribution	0.727	0.733
Relative electrostatic contribution	0.273	0.267

The CoMFA steric and electrostatic fields based on PLS analysis are represented as 3D contour maps in Figures 4 and 5, using the training set as reference structures. In Figure 4, the green regions indicate areas where steric bulk enhances biological activity, while the yellow regions indicate that the less steric bulk is favored to enhance activity. In Figure 5, the blue regions indicate areas where electropositive groups enhance biological activity, while the red regions electronegative groups that enhance activity. The yellow and red polyhedrons near N position in tropane ring indicate that substitution of less bulky and electron-donating group will improve the agonistic activity. According to our previous studies, different groups in tropane N, an important

Table 3. Observed and predicted agonistic activities of target compounds to muscarinic receptors by CoMFA

Compounds	pEC_{50} (Obs.)	Model A		Model B	
		pEC_{50} (Pred.)	Res.(A)	pEC_{50} (Pred.)	Res.(B)
Training set					
1	7.10	7.52	−0.42	7.50	−0.40
2	4.61	5.22	−0.61	5.17	−0.56
3	5.35	5.05	0.30	5.16	0.19
4	5.36	5.15	0.21	5.22	0.14
6	4.94	4.75	0.19	4.78	0.16
7	4.83	4.62	0.21	4.66	0.17
8	3.39	3.66	−0.27	3.55	−0.16
10	5.30	5.54	−0.24	5.54	−0.24
11	8.19	8.07	0.12	8.14	0.05
12	7.57	8.09	−0.52	8.10	−0.53
13	8.76	8.59	0.17	8.56	0.20
14	6.58	6.35	0.23	6.33	0.25
15	4.37	4.25	0.12	4.32	0.05
16	8.32	8.34	−0.02	8.26	0.06
17	8.21	7.40	0.81	7.44	0.77
18	5.70	5.90	−0.20	5.95	−0.25
19	4.52	4.63	−0.11	4.62	−0.10
20	3.93	3.84	0.09	3.71	0.22
21	7.30	7.40	−0.10	7.34	−0.04
Test set					
5	6.98	7.09	−0.13	6.97	0.01
9	4.10	4.35	−0.24		
22	7.05	6.87	−0.18	6.88	0.17
23	7.03	7.14	−0.11	7.20	−0.17
24	7.35	7.06	0.29	7.08	0.27

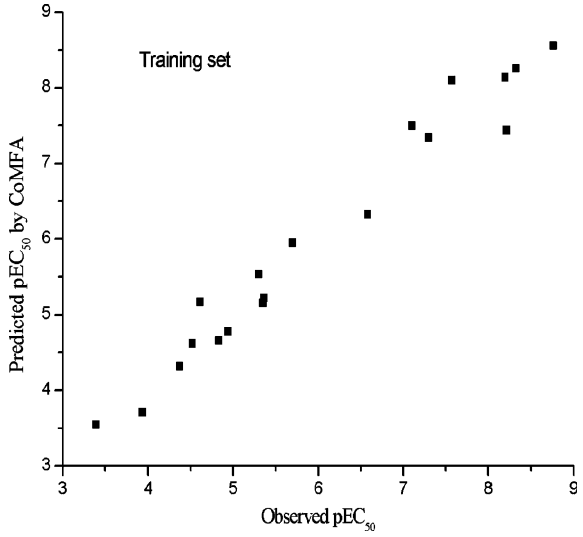

Figure 3. Predicted versus observed agonistic activity for the training set derived from the CoMFA model B.

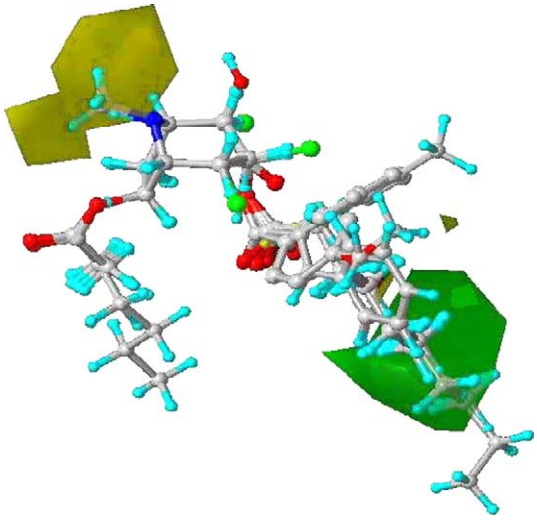

Figure 4. CoMFA SD*coefficient steric contour plots; green represents regions where steric bulk is predicted to increase activity, and yellow represents regions where an increase of steric bulk is predicted to decrease activity.

pharmacophore atom, influence critically the agonistic activity to muscarinic receptors.[5] Compared with H, the methyl is an electron-donating, but a more bulky, group. H replaced by methyl in tropane N may result in sterical inhibition between the N atom and the binding site of muscarinic receptors, though it is beneficial to the agonistic activity in electrostatic field. Considering that the steric contribution to the agonistic activity to muscarinic receptors was 73.3% from the CoMFA analysis, we speculated that the steric factor in tropane N might dominantly control the agonistic activity of target compounds to muscarinic receptors. **17** and **18** (pEC_{50} = 8.21 and 5.70), two derivatives with the least bulk atom H in tropane N, have higher values of pEC_{50} than those of **2** and **20** (pEC_{50} = 4.61 and 3.93), respectively, which have methyl substitution in the N. Also, in our previous works, we found that when H

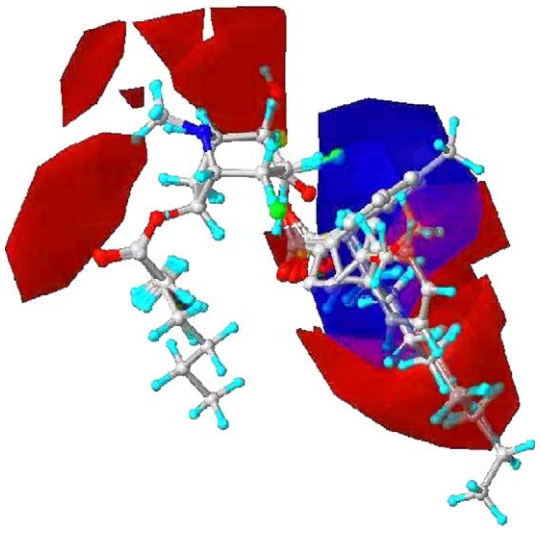

Figure 5. CoMFA SD*coefficient Electrostatic contour plots; red contours represent regions where high electron density (negative charge) is expected to increase activity, and blue contours represent regions where low electron density (partial positive charge) is predicted to increase activity.

linked at N was replaced with certain steric bulky groups no matter which donates or withdraws electrons, no or less bioactivity to muscarinic receptors was observed. Another large green area in the steric contour map indicates areas where steric bulk enhances activity. Three red polyhedrons near the carbonyl oxygen in C-6, C-3α acyl, and C-2β positions suggest that electron-rich groups are beneficial to the agonistic activity. One blue contour surrounding the C-3β position in the electrostatic contour map indicates that positively charged substituents are favorable to increase the activity. Both red and blue regions surrounding the substitution groups of C-3α position imply that the substitution effect should be complicated. To our knowledge, the *O atom in model B interacted with the positive charge part of muscarinic receptors by donating electrons and the torsion angle of N-C5-C6-O was very important to the agonistic activity. It was found that different C-3 substituted groups in these bioactive compounds contributed less steric effect to the torsion angle from the superimpositions because of the rigid skeleton of the tropane ring, but contributed to an obvious difference of chemical shift of 6-H in their ^{1}H NMR.[11–13] We deduced that the C-3 substituted group may have a potent effect on the electron density distribution of *O, which influenced the agonistic effect.

We have constructed the CoMFA model for BGT-A and its analogs as muscarinic agonists. The 3D-QSAR model gave good statistical results in terms of q^2 and r^2 values, and proved to have a relativly good predictive ability. The contour diagrams obtained for the CoMFA field contribution can account for the agonistic activity trend among the analyzed molecules. It was found that less steric bulky and electron-donating atom or group substituted in tropane N would improve the agonistic activity, but the steric effect in the N position dominantly controlled the interaction between muscarinic receptor and BGT-A derivatives. The C-3-substituted group

may contribute to the electron density distribution of active *O, which greatly influenced the agonistic activity to muscarinic receptors. Further QSAR studies are needed to elucidate this point. This model could provide a solid basis for designing novel molecules with higher activity.

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