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COMPARATIVE MOLECULAR FIELD ANALYSIS OF BENZOPYRAN-4-CARBOTHIOAMIDE POTASSIUM CHANNEL OPENERS

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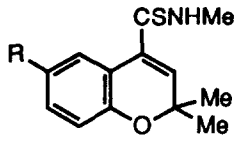
Abstract: Comparative molecular field analysis (CoMFA) was applied to 6-substituted benzopyran-4-carbothioamide potassium channel openers **1**. CoMFA suggested that the electrostatic, steric, and hydrophobic factors of the 6-substituent were correlated with the vasorelaxant activity, among which the contribution of electrostatic factor was the most important.

Potassium channel openers are potent smooth muscle relaxants and supposed to be applicable to the treatment of many kinds of disorders such as asthma, hypertension, and urinary incontinence. [1] Previously we have developed a pharmacophore model of potassium channel openers and designed new openers based on the pharmacophore model. [2a] Because these compounds have been proven to have a potent vasorelaxant activity, a series of their analogs has been designed and synthesized to gain insights into the structural requirements for potassium channel openers. [2] In the preceding paper, we have analyzed the relationship between the vasorelaxant activity and the structure of 6-substituted benzopyran-4-carbothioamides **1** quantitatively and revealed that the activity is correlated linearly with electronic parameter (σ_m) and parabolically with hydrophobic (π) and steric (L) parameters of the 6-substituents. [3] In this report, we analyze three-dimensional structure-activity relationships of benzopyran potassium channel openers **1** using the comparative molecular field analysis (CoMFA) [4] and compare the results with the findings of the quantitative structure-activity relationships (QSAR). [3]

Compounds and activity analyzed are listed in Table I. [3] A total of 13 compounds was used for this analysis. The vasorelaxant activity was determined by the effect on 30 mM KCl response in isolated rat aorta.

Three-dimensional structures of compound **1** for CoMFA were constructed by the procedure similar to that of previous study. [2a] Thus, the initial geometry was generated using molecular modeling software SYBYL [5] with the standard bond lengths and angles. These initial structures were optimized using molecular mechanics method with TRIPOS force field.[6] In order to find the candidates for local minimum conformation, a preliminary conformational search was performed by the systematic search method implemented in SYBYL system. All rotatable bonds were rotated with 5 degree increment. The thioamide group of **1** was set to fit with that of previously defined proposed active conformation of **1a** [2a] because the thioamide sulfur atoms were expected to occupy the same hydrogen bonding region interacting with the receptor in our pharmacophore model.[2a] For each candidate of local minimum, the geometry was optimized using Discover with CVFF force field. [7] Then the optimized structures were further minimized by MOPAC [8] version 6.0 devised within Insight II system. [9] PM3 hamiltonian was used in the MOPAC calculations. All calculations using MOPAC and CVFF were run on

Table I. Compounds, activities, and parameters used to derive eq. 1



1

compd.	R	observed [a] pEC_{50}	calculated [b] pEC_{50}	difference [c]	π [d]
1a	CN	7.61	7.67	-0.06	-0.57
1b	NO_2	8.87	8.89	-0.02	-0.28
1c	SO_2Ph	7.72	7.71	0.01	0.27
1d	SO_2Me	6.34	6.31	0.03	-1.63
1e	CF_3	8.69	8.66	0.03	0.88
1f	C_2F_5	7.90	7.93	-0.03	1.89
1g	OCF_3	8.39	8.40	-0.01	1.04
1h	COOMe	7.02	7.07	-0.05	-0.01
1i	Br	8.03	7.82	0.21	0.86
1j	Cl	7.43	7.54	-0.11	0.71
1k	H	5.37	5.35	0.02	0.00
1l	Et	6.49	6.55	-0.06	1.02
1m	OMe	6.38	6.34	0.04	-0.02

[a] Reference 3. Negative logarithm of the molar concentration required to relax rat aorta precontracted with 30 mM KCl by 50% of intrinsic activity. [b] calculated using eq. 1. [c] Difference between observed and calculated values. [d] Hydrophobic parameter for the R substituent.[10]

Silicon Graphics workstation IRIS 240/GTX. The lowest-energy conformer of each compound was assumed as an active conformer, in which the terminal group of 6-substituent with non-symmetrical group was located below the benzopyran ring plane (behind the benzopyran ring plane in the figure in Table I) except **1f** and **1g**. In the cases of compounds **1f** and **1g**, the corresponding conformers were not the lowest-energy but the second lowest-energy. However, since the differences in the energy were very small (0.20 and 0.07 kcal/mole [MOPAC PM3] for **1f** and **1g**, respectively), the conformations of the second ones for **1f** and **1g**, which were similar to the lowest-energy conformation of the other compounds, were used for the CoMFA. All atomic charges were calculated by CNDO/2 method using MOPAC geometry. All calculations of SYBYL system and CNDO/2 were run on Evans & Sutherland graphic workstation ESV3/33.

Proposed active conformer of compound **1a** (Fig. 1) [2a] was arbitrarily selected as a template molecule and the other compounds were superposed on **1a** by matching the corresponding atoms of the benzopyran ring system of each molecule. CoMFA calculations were performed using the QSAR option of SYBYL version 5.5. Cross-validation method [4] was used for the determination of number of the optimum components. Independent variables used were the steric interaction-energy (field) at each grid point, the electrostatic interaction-energy (field) at each grid point, and the hydrophobic parameters π and π^2 for the 6-substituent. Except where noted, default values of SYBYL system were used for partial least square (PLS) calculations and cross-validation. The grid region of 18 Å X 17 Å X 18 Å was defined around the molecules and used for the interaction-energy calculations. Grid spacing for the cross-validation and the final PLS calculations was 1 Å. Sp3 carbon was used as a probe for a steric interaction energy calculation and +1 charge for an electrostatic. In order to accelerate the

cross-validation calculation, the steric and electrostatic interaction-energy grid points which had a value of a standard deviation below 1.0 were excluded from the calculations. In the final PLS calculation, all grid points were used for the calculation.

Cross-validation suggests that the optimum number of component was 6 (cross-validated r^2 of 6 component model: 0.571). Final PLS equation (eq. 1) was derived using 6 component model. The observed and calculated activities using eq. 1 are shown in Table I. Contour maps of the coefficients of the each grid point are depicted in Fig. 1. With respect to the relative contribution of each structural parameter, the contribution of electrostatic interaction-energy to the activity was the most significant ($\pi + \pi^2$, 25.2%; steric, 31.7%; electrostatic, 43.1%).

$$pEC_{50} = 5.290 - 0.308\,\pi^2 + 0.515\,\pi + [\text{Steric}] + [\text{Electrostatic}] \quad (1)$$
$$n = 13, \quad r = 0.997, \quad s = 0.109, \quad F = 179.47, \quad \text{ideal } \pi = 0.84$$

We previously derived QSAR equation (eq. 2) for a set of 13 compounds of Table I [3]. In eq. 2, σ_m, L, and π are the Hammett electronic parameter, the STERIMOL parameter representing the length of substituent, and the hydrophobic constant, respectively, of R.

$$pEC_{50} = 4.067\,(\pm 1.352)\,\sigma_m - 0.234\,(\pm 0.224)\,L^2 + 1.756\,(\pm 1.825)\,L$$
$$- 0.347\,(\pm 0.275)\,\pi^2 + 0.791\,(\pm 0.331)\,\pi + 2.794 \quad (2)$$
$$n = 13, \quad r = 0.957, \quad s = 0.393, \quad \text{ideal } \pi = 1.14, \quad \text{ideal L} = 3.75$$

Both two equations include electronic, steric, and hydrophobic terms and give similar optimum π values. In the pharmacophore model, [2a] the area around the 6-substituent has been assumed to be a hydrogen bonding region. The electrostatic interaction-energy term in eq. 1 and σ_m in eq. 2 seem to reflect the hydrogen bond accepting character of the 6-substituents. Contour map of the electrostatic field (Fig. 1a) directly indicates the intermolecular interaction between potassium channel opener **1** and the putative receptor, increasing the activity by the interaction with +1 charge. Electronic parameter σ_m is originally an intramolecular electronic parameter. [11] But in this case, σ_m seems to reflect the intermolecular interaction between compound **1** and the receptor when considered together with the CoMFA result and the pharmacophore model. [2a] Thus it seems that the more the electron withdrawing character of R substituent increases, the more electron rich the R becomes, that is to say, the more the interaction of R with proton of the receptor increases. CoMFA indicates that there is an optimum region of steric interaction in the direction along the length of the R substituent (Fig. 1b). This is consistent with the steric effect derived from the QSAR (eq. 2). The black and clear contour region in Fig. 1b corresponds to the optimum length appeared in eq. 2. With regard to the hydrophobicity, it is not clear whether π is associated with the transportation of these compounds into the active site or with the specific hydrophobic interactions with the receptor.

In conclusion, the CoMFA showed that the vasorelaxant activity of 6-substituted benzopyran-4-carbothioamides **1** had a good correlation with the electrostatic field, steric field, and the hydrophobic parameter of the 6-substituent. The CoMFA also gave us very critical information about the interaction of the opener **1** with the receptor three-dimensionally. The results appeared to be very useful for tuning up the pharmacophore model previously developed. [2a]

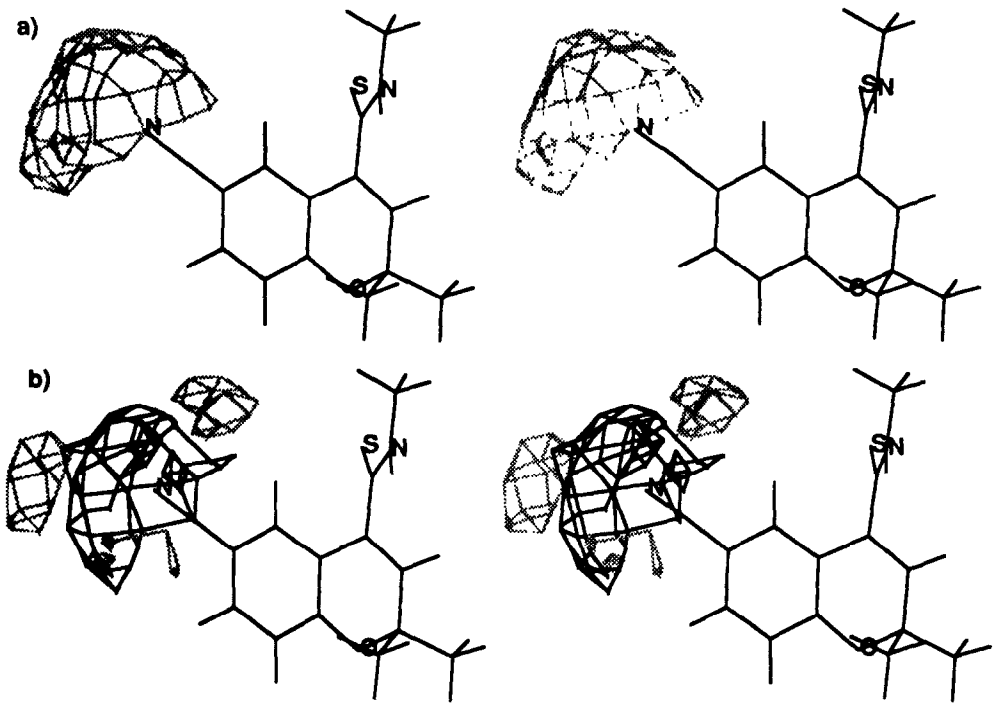


Fig. 1 a) Stereo view of the major electrostatic fractions of the CoMFA contour map. Contour shows region in which the standard deviation coefficient (the coefficient times the standard deviation of the corresponding column) is greater than 0.008. b) Stereo view of the major steric fractions of the CoMFA contour map. Black and clear contour shows region in which the standard deviation coefficient is greater than 0.008. Dim contour shows region in which the standard deviation coefficient is less than -0.008. Compound **1a** is used as a reference structure.

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