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3D-QSAR analysis of a new type of acetylcholinesterase inhibitors

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Acetylcholinesterase (AChE) inhibitors are an important class of medicinal agents used for the treatment of Alzheimer's disease. A screening model of AChE inhibitor was used to evaluate the inhibition of a series of phenyl pentenone derivatives. The assay result showed that some compounds displayed higher inhibitory effects. In order to study the relationship between the bioactivities and the structures, 26 compounds with phenyl pentenone scaffold were analyzed. A 3D-QSAR model was constructed using the method of comparative molecular field analysis (CoMFA). The results of cross-validated R^2cv=0.629, non-cross-validated R^2=0.972, SE=0.331, and F=72.41 indicate that the 3D-model possesses an ability to predict the activities of new inhibitors, and the CoMFA model would be useful for the future design of new AChE inhibitors.

acetylcholinesterase (AChE), comparative molecular field analysis (CoMFA), phenyl pentenone derivatives

AChE's vital function is the hydrolytic destruction of the neurotransmitter acetylcholine (ACh), which terminates the impulse transmission at cholinergic synapses. Through activation of either ionotropic or nicotinic receptors, ACh exerts many physiological functions both in the periphery and the central nervous system (CNS)[1], e.g., smooth muscle contraction, modulation of cardiac rate and force, motor control, temperature regulation, memory and pain modulation, and so on. Inhibition of AChE leads to the accumulation of ACh and enhanced cholinergic transmission, and has long been an attractive target for drug development.

AChE inhibitors are an important class of medicinal agents useful for the treatment of Alzheimer's disease[2]. In the past ten years, several AChE inhibitors, such as donepezil hydrochloride[3,4] and huperzine A[5], have been launched into the market. They are effective in treating Alzheimer's disease.

QSAR studies of AChE inhibitors indicated[2] that hydrophobicity and the presence of an ionizable nitrogen are the pre-requisites for the inhibitors to interact with AChE. In the present study, a series of compounds with the scaffold phenyl-1′-penten-3′-one are of these features, their activities on AChE were examined. Subsequently, a 3D-QSAR model was generated using the method of comparative molecular field analysis (CoMFA) and used to predict the activities of several new compounds. It has been demonstrated that the steric and electrostatic features of some substituents significantly influence the activity.

1 Materials and methods

1.1 Agents and apparatus

Na_2HPO_4, KH_2PO_4, $NH_2OH{\cdot}HCl$, NaOH, HCl and $FeCl_3$ were purchased from Beijing Chemical Reagents Company. Acetylcholine (ACh) was purchased from Acros Organics in USA.

Fluostar galary microplate assay apparatus was purchased from BMG Company in Germany. SGI Fuel

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R6000 workstation was manufactured by SGI. Sybyl 7.0 software package was developed by Tripos Inc.

1.2 Compound samples

All the compound samples except reference compounds were provided by National Center for Pharmaceutical Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences. Huperzine A was produced in Ningbo Pharmaceutical Factory of Chinese Traditional Medicine. Donepezil hydrochloride was provided by Jinan Chenghui Shuangda Chemical Industry Limited Company.

1.3 Activity assay of AChE

The assay was performed according to the literature method[6,7]. Briefly, a mixture of 7 mmol/L acetylcholine (ACh; 20 μL) and test sample (at a given concentration; 10 μL) was added to the preformed AChE system (20 μL) in culture wells. The wells were incubated for 1 h at 37℃, treated with a mixture of 1 mol/L $NH_2OH \cdot HCl$ (35 μL) and 3.5 mol/L NaOH (35 μL), followed by addition of a solution of HCl/H_2O 1:2 (40 μL), 10% $FeCl_3$ solution (40 μL). Then, the UV/VIS absorbance of each well was measured at 530 nm. The wells without sample, and without ACh and sample, were taken as negative control and blank, respectively, and huperzine A and donepezil hydrochloride were used as reference compounds.

1.4 Data analysis

The 50% inhibitory concentration, IC_{50}, was calculated from the contrast values.

2 The structures of the compounds and their activities on AChE

In this study, 38 compounds with the scaffold of phenyl-1′-penten-3′-one (Figure 1) were examined as well as 2

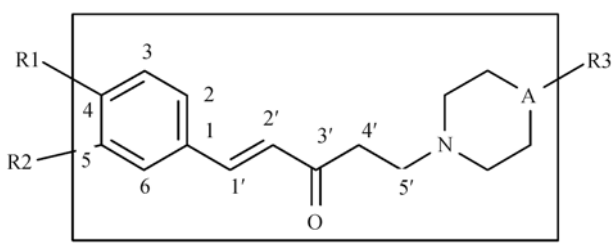

Figure 1 The structure scaffold of the compounds.

reference compounds huperzine A and donepezil hydrochloride according to the activity assay of AChE above. Most of them exhibited activities on AChE, but only a few compounds displayed strong activities (Table 1). The IC_{50} values of huperzine A and donepezil hydrochloride were 0.20 ± 0.1 μmol/L and 0.13 ± 0.04 μmol/L, respectively.

3 QSAR analyses

3.1 Determination of the active conformation and molecular modeling

Determination of the active conformation is the first key step of CoMFA[8]. Because there is no report about the X-ray crystallographic structure of AChE with an analogue of this kind of compounds, the system search approach and molecular mechanics were used in the first step of QSAR analyses to determine the minimum energy conformation of the scaffold, then the scaffold conformation was fixed. The minimum energy conformations of the side chains were searched using the same method. In the training set, compound 5 exhibited the highest activity, and was used as the structural template. The determination of the minimum energy conformations of the other 25 compounds was in agreement with that of compound 5. Their minimization procedures were set up by Powell method, energy gradient 0.05 kcal/mol, Tripos Force Field, Gasteiger-Hückel charge and 1000 max iterations.

3.2 Molecular superposition

The molecular dock program Dock was used to determine the conformation of the active compound 5 in the active pocket of AChE, then the compound and the amino acid residues within 0.8 nm around the compound in the active pocket were minimized, and then the conformation of the compound constrained by AChE was obtained, which was regarded as the active conformation interacting with AChE. This conformation should have more accordance with the requirements of electrostatic field and steric field at the active sites.

At first, the protein structure file 1EVE.pdb of AChE with ligand E2020 was downloaded from the RCSB Protein Data Bank, then the restoring conditions for E2020 were optimized by changing the parameters for Dock program, including reminimize-layer-number, bump-maximum, dielectric-factor and so on. 18532 docking conditions were obtained by the combination of these parameters and used to the dock computation of E2020. The root-mean-square deviation (RMSD) be-

Table 1 The structures of the compounds and their bioactivities from experiment and computation

Compound	R1	R2	R3	A	pIC_{50}	PA [a)]	Residue
1	−O−CH_3	−O−CH_3	−CH_2−phenyl	N	6.54	6.53	0.01
2	−OH	H	−CH_2−phenyl	N	6.10	6.34	−0.24
3	−Cl	H	−CH_2−phenyl	N	6.03	5.71	0.32
4	−O−C2H5	−O−CH3	−CH_2−phenyl	N	6.14	6.15	−0.01
5	−O−CH3	H	−CH_2−phenyl	N	6.61	6.55	0.06
6	H	−Cl	−CH_2−phenyl	N	6.03	5.89	0.14
7	−O−CH_3	−O−C_2H_5	−$CO_2C_2H_5$	N	3.72	3.93	−0.21
8	−O−$CH_2CH_2CH_3$	H	−CH_2−phenyl	N	4.19	4.01	0.18
9	−CN	H	−$CO_2C_2H_5$	N	3.03	3.45	−0.42
10	−O−CH_3	−O−CH_3	−$CO_2C_2H_5$	N	4.38	4.68	−0.30
11	−Br	H	−CH_2−phenyl	N	6.44	6.35	0.09
12	−Cl	H	−$CO_2C_2H_5$	C	4.27	4.05	0.18
13	−CN	H	−$CO_2C_2H_5$	C	5.21	5.40	0.19
14	−OH	H	−$CO_2C_2H_5$	C	3.13	3.26	−0.13
15	−O−CH_3	−O−C_2H_5	−$CO_2C_2H_5$	C	5.19	5.03	0.16
16	−O−CH_3	−OH	−$CO_2C_2H_5$	C	5.47	5.37	0.10
17	−O−C_4H_9	H	−CH_2−phenyl	C	5.42	5.75	−0.33
18	−F	H	−CH_2−phenyl	C	5.44	5.31	0.13
19	H	H	−CH_2−phenyl	C	5.01	5.33	−0.32
20	−O−CH_3	−O−CH_3	−$CO_2C_2H_5$	C	5.74	5.60	0.14
21	−OH	−O−CH_3	−$CO_2C_2H_5$	C	4.71	4.62	0.09
22	−CH_3	H	−CH_2−phenyl	C	4.62	4.87	−0.25
23	−O−C_2H_5	−O−CH_3	−CH_3	C	4.48	4.64	−0.16
24	−O−CH_3	−O−CH_3	−CH_3	C	3.83	3.55	0.28
25	−O−CH_3	H	−CH_2−phenyl	C	5.02	5.30	−0.28
26	−O−C_2H_5	−O−CH_3	−$CO_2C_2H_5$	C	5.24	5.48	−0.24
27	−O−C_2H_5	−O−CH_3	−CH_3	C	4.49	4.20	0.29
28	−NO_2	H	−CH_2−phenyl	N	4.74	4.79	−0.05
29	CF_3	H	−CH_2−phenyl	C	1.03	1.36	−0.33
30	CF_3	H	−CH_2−phenyl	N	1.37	1.72	−0.35

a) Predicted activity by CoMFA model.

tween each post-docking conformation and the crystal conformation of E2020 was calculated, and the optimal condition for E2020 (RMSD=0.228) was selected and used to dock the 26 compounds (ligands) to the active sites, and then their conformations in the active sites were obtained (Figure 2).

The post-docking conformations of these ligands were imported at AChE active sites. The minimization procedures of the ligands and the amino acid residues within 0.8 nm around the ligands were set up by MMFF94 Force Field, steepest-descending algorithm, and conjugate gradient method with energy gradient of 0.2 kJ/mol. Then the ligands were extracted from the minimized complex and loaded with MOPAC charge, and their CoMFA was studied.

3.3 CoMFA studies

CoMFA was performed with the QSAR module of SYBYL 7.0 for each combination of steric and electrostatic molecular fields, which were sampled at each point of regularly spaced grids of 1.0 and 1.5 Å. The steric and electrostatic fields were calculated using the default probe, a sp^3 carbon atom with a charge of +1. CoMFA calculates steric fields using a Lennard-Jones potential and electrostatic fields using a Coulomb potential. The calculated force fields included steric field and electrostatic field[9]. The number of the optimal principal

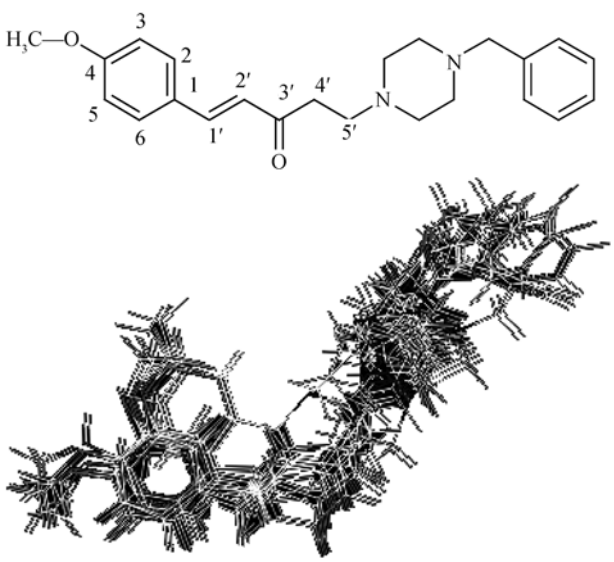

Figure 2 The superposition of AChE inhibitors.

constituents was determined by cross validation and partial least square (PLS) method. Finally, the CoMFA model was constructed. In order to increase the signal−noise ratio, 4.0 kcal/mol of Column filtering was set in PLS analysis. The contour maps of steric field and electrostatic field related with activities of the compounds were observed in the workstation by View-CoMFA in the Spreadsheet.

4 Results and discussion

4.1 CoMFA studies

The statistical parameters of the CoMFA Model are summarized as follows. Cross-validation parameter R^2_{CV}=0.629, the number of the optimal main constituents was 6. Generally, R^2_{CV}>0.5 indicates that the model has very good prediction potential. The correlation coefficient of non-cross-validated R^2=0.972, standard error SE=0.331, F=72.41. The activities of the training set compounds and the test set compounds were predicted by the CoMFA model. The results, showed in Table 1 and Figure 3, indicate that the prediction values are very similar to their experiment values.

4.2 Visual inspection of 3D contour maps generated by CoMFA

The 3D steric contour map from the best CoMFA model is shown in Figure 4. The most active derivative compound 5 is shown inside steric and electrostatic fields. Green contours and yellow contours represent the effect of steric field on the activity. Green contours indicate the regions where an increase in steric bulk will enhance activity, and yellow contours indicate the regions where an increase in steric bulk will reduce activity. The steric contour maps are localized around R1, R2, and R3 substituents, suggesting their importance for eventual steric interactions with AChE.

The electrostatic contour map from the best CoMFA model is shown in Figure 5. The most active derivative compound 5 is shown inside fields. Blue and red contours encompass the areas where an increase in positive or negative charge, respectively, is favorable for binding properties. The electrostatic contour maps are around R1, R2, R3 groups and A atom, indicating that their electrostatic features are important for electrostatic interaction with AChE.

From these contour maps, it can be observed that there are green contours and red contours around R1

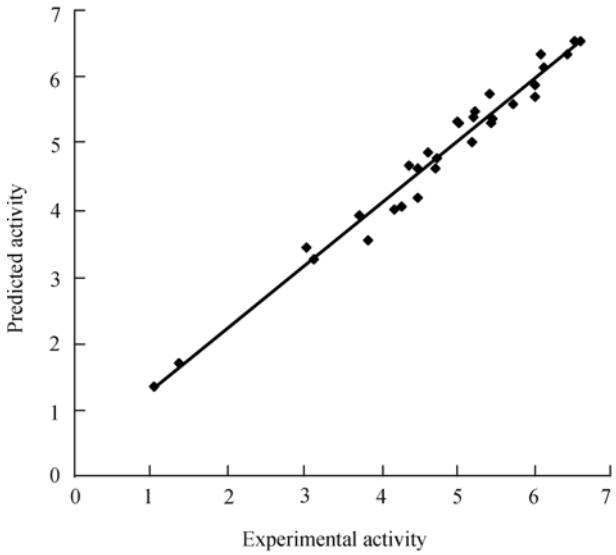

Figure 3 Predicted versus experimental pIC50 values derived from the steric/electrostatic CoMFA model of the training and test sets of phenyl pentenone derivatives.

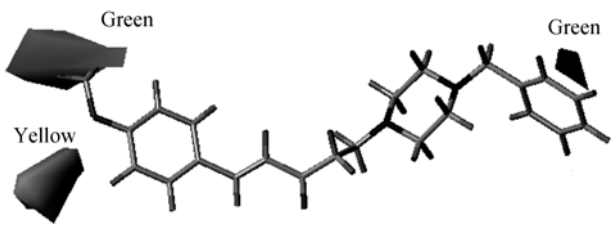

Figure 4 Steric contour map from the best CoMFA model. Compound 5 is shown inside fields. Steric contour plots: green contours indicate the regions where an increase in steric bulk will enhance affinity, whereas yellow contours indicate sterically disfavored regions.

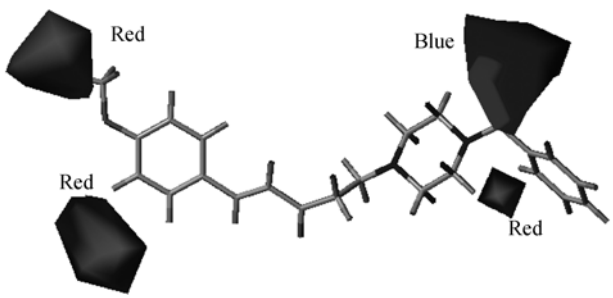

Figure 5 Electrostatic contour map from the best CoMFA model. The most active derivative compound 5 is shown inside fields. Blue and red contours encompass the areas where an increase in positive or negative charge, respectively, is favorable for binding properties.

substituent, which suggests that a larger electronegative group in R1 position favors activity. Compared with compound 20, compounds 1, 4, 5 and 12 have increased steric effects in R1 position, so the activities of these compounds were increased significantly.

There are yellow contours and red contours around R2 substituent, indicating that larger electropositive groups in R2 position disfavor the activity. Because of the larger groups in R2 substituent, we can explain why compound 1 displayed lower activity than compound 5, and why compound 8 exerted lower activity than compound 11.

Around R3 substituent, there are green contours and blue contours, revealing that larger electropositive groups in R3 position can increase the activity. Because of the large substituents in R3 position, compound 11 displayed higher activity than compound 25, and compound 23 exhibited higher activity than compound 24. Because of the electropositive groups in R3 position, compound 1 exerted higher activity than compound 1, and compound 2 displayed higher activity than compound 15, and compound 3 showed higher activity than compound 13.

Besides, around atom A, there is a red contour, suggesting that the electronegative atom in this position is more favorable for activity. Because a basic nitrogen has more electronegativity than a basic carbon, compound 5 displayed higher activity than compound 22.

5 Conclusion

In conclusion, AChE inhibitor screening model was established and applied, and the activities of a series of phenyl pentenone derivatives on AChE were evaluated. Subsequently, a 3D-QSAR model was constructed using molecular mechanics and CoMFA method. The CoMFA model not only can explain the activity results, but also has high potential for activity prediction, so it will be useful for the future new AChE inhibitor design.

The CoMFA model suggests that the AChE inhibitors with high activity should have the following features: hexa-heterocycle is piperazine, electronegative atoms or groups in R1 position favor the activity, electronegative atoms in R2 position also enhance the activity, and electropositive large groups in R3 position are favorable for the activity.

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