基于吡咯烷与正丁烷类衍生物 CCR5 拮抗剂的药效团模型构建

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摘要：　吡咯烷与正丁烷类 CCR5(化学趋化因子受体 5)拮抗剂可通过抑制人类免疫缺陷病毒(HIV-1)包膜蛋白与 CCR5 的相互作用而阻断病毒进入细胞. 本文使用已知拮抗剂结构和活性信息构建了一个三维药效团模型. 按照Catalyst/HypoGen 模块的要求, 选择了 25 个结构和活性均具备差异性的分子作为药效团产生的训练集. 其中训练集分子以 IC_{50} 值表示的生物活性值跨度为 0.06 到 10000 $nmol \cdot L^{-1}$. 最好的药效团模型(Hypo 1)由两个正离子化特征以及三个疏水特征组成, 训练集预测相关系数为 0.924, 均方根偏差为 1.068. 模型用于预测由 74 个分子组成的测试集化合物活性, 结果表明模型可以提供较好的活性预测结果并用于新的拮抗剂的设计.

关键词：　CCR5；　HIV-1；　药效团模型；　基于配体的药物设计
中图分类号：　O641

Pharmacophore Model Generation Based on Pyrrolidine- and Butane-derived CCR5 Antagonists

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Abstract：　A three-dimensional pharmacophore model was developed for a considerable number of pyrrolidine-based and butane-based chemokine (C-C motif) receptor 5(CCR5) antagonists, which can block the entry of human immunodeficiency virus type 1 (HIV-1) by inhibiting the interaction of HIV-1 envelope protein and CCR5. The pharmacophore model was generated using a training set consisting of 25 carefully selected antagonists with the diverse molecular architecture and bioactivity, as required by the Catalyst/HypoGen program. The activity of the training set molecules expressed in IC_{50} (half-inhibitory concentration) covered from 0.06 to 10000 $nmol \cdot L^{-1}$. The most predictive pharmacophore model (Hypo 1), consisting of two positive ionizable points and three hydrophobic groups，had a correlation of 0.924 and a root mean square of 1.068, and a cost difference of 63.67 bits between the null cost and the total cost. The model was applied in predicting the activity of 74 compounds as a test set. The results indicated that the model was able to provide clear guidelines and accurate activity prediction for novel antagonist design.

Key Words：　CCR5；　HIV-1；　Pharmacophore model；　Ligand-based drug design

　　Human immunodeficiency virus type 1 (HIV-1) infection, which eventually leads to the acquired immunodeficiency syndrome　(AIDS), was first discovered by Barre-Sinoussi and his co-workers [1,2] in 1983. At present, AIDS remains to be a lethal disease threatening human′s health, especially affecting sub-Sa-haran Africa and Southeast Asia. To inhibit the replication of HIV-1 and slow the suppression of the immune system, current therapies utilize a combination of protease and reverse transcriptase inhibitors[3]. Although the therapy can sup- press viral replication and delay the progression of AIDS, the virus is not eradi-

cated and the immune system eventually succumbs to infection[3]. Because of the long side effects of protease and reverse transcriptase inhibitors and the increasing transmission of resistant variants, it is urgent to develop novel classes of drugs, which are able to suppress the replication of HIV-1 efficiently.

Chemokine receptor CCR5, a primary co-receptor essential for the recognition of HIV and its entry into cell, has been identified as a potential novel target for the treatment of HIV-1 infection[4]. Because of the absence of any three-dimensional structure of CCR5, rational design of inhibitors against the receptor by a structure-based approach is not so feasible. Fortunately, many chemically diverse small molecules as CCR5 antagonists have been reported and some of them are in preclinical and clinical development[5]. Researches on correlating the physicochemical properties or structural features of the antagonists with their biological activities are believed to gain an insight into the interaction mechanism of CCR5 receptor and antagonists as well as providing useful clues for designing novel antiHIV-1 drugs.

The HypoGen module in Catalyst software is a predictive pharmacophore method and attempt to derive structure-activity relationship (SAR) model from a set of molecules with known structures and activity. There are many applications of HypoGen in drug discovery and toxicology research (for reference lists, see http://www.accelrsy.com). Pharmacophore models were reported by Debnath for piperidine-based and piperazine-based CCR5 antagonists developed by Schering-Plough research institute[6]. However, Merck Research Laboratories have reported several kinds of high active pyrrolidine-based and butane-based compounds as CCR5 antagonists in recent years. With abundant structure and activity information, pharmacophore models based on these compounds were developed using the Catalyst/HypoGen module. The validation of the model was done on a large test set. The aim of our work is to gain useful information in understanding the possible inhibitory mode of the CCR5 inhibitors as well as to provide clear guidelines and accurate activity prediction for novel antagonist design.

1   Materials and methods

1.1   Selection of training set

The biological activity data (CCR5 binding [^{125}I]-MIP-1α assay[7]) of 99 compounds, represented as IC_{50} (nmol·L^{-1}), were collected from the literatures[8–27]. All the compounds were developed by the Merck Research Laboratories, which confirmed the consistency of the biological assay. The compounds were divided into a training set and a test set. The training set consists of 25 compounds. The selection of the training set and the test set was based on the following rules: (a) both the training and the test sets should have structures from each class of compounds to ensure structural diversity; (b) both the training and the test sets should cover the molecular bioactivities (IC_{50}) as wide as possible. The IC_{50} value of the training set molecules covered a wide range from 0.06 to 10000 nmol·L^{-1}. The chemical structures of the training set are listed in Fig.1. The activity values are classi-

fied as follows: $IC_{50} \leqslant 10$ nmol·L^{-1} means the compounds are highly active (represented as ++); 10 nmol·L$^{-1} < IC_{50} \leqslant 1000$ nmol·L^{-1} means the compounds are moderately active (represented as +); and $IC_{50} > 1000$ nmol·L^{-1} means the compounds are inactive (represented as −). To validate our pharmacophore hypothesis, 74 compounds with available IC_{50} values were used as the test set.

1.2   Molecular modeling

Molecular modeling was performed on an IBM IntelliStation Z Pro workstation. The Catalyst 4.11 software (Accelrys Inc., San Diego, CA) was used to generate the pharmacophore models. All stereoisomeric centers in the molecules were assigned as shown in the original literatures. Conformation models for all the molecules including the training set and the test set were generated using the Catalyst/ConFirm module with the "best quality" conformational search option. A maximum of 250 conformations were generated using the Poling algorithm[28] to ensure coverage of the conformational space with an energy constraint of 41.84 kJ·mol^{-1}. As the R/S configuration directly affects the activity of the compounds, we set the parameter of the absolute stereochemistry to turn off the mirror image mapping. All the other parameters were set as defaults. An analysis of the training set by the tools of "show function mapping" showed that hydrogen bond acceptor (HA), hydrophobic (HY), ring aromatic (RA), and positive ionizable (PI) features could effectively map all the critical chemical/structural features of the molecules in the set. In the initial tests of pharmacophore generation, we found that HY, RA, and PI features dominated in the most useful model. Therefore, these three features were used to generate 10 pharmacophore hypotheses from the training set with a default uncertainty value of three.

2   Results and discussion

2.1   Pharmacophore generation

A set of 10 hypotheses has been generated using the structure and the activity information from the training set. Table 1 lists the cost values, correlation coefficients (r), root mean square deviations (RMSD), and pharmacophore features of the 10 hypotheses. The first hypothesis (Hypo 1) is selected as the best pharmacophore hypothesis with the highest cost difference (63.66), the lowest RMSD (1.068), and the best correlation coefficient (0.924). Hypo 1 comprises of two PI features and three HY points (Fig. 2).

The cost value analysis is important for evaluating the quality of the hypothesis model[29]. The cost value of a hypothesis (namely total cost, represented in bits unit) is calculated by the following equation:

$$cost = eE + wW + cC$$

where e, w, and c are the coefficients associated with the error (E), weight (W), and configuration (C) components, respectively. The other two important cost calculations are the "fixed cost" and the "null cost". The "fixed cost" represents the simplest model that the estimation perfectly fits the actual data,
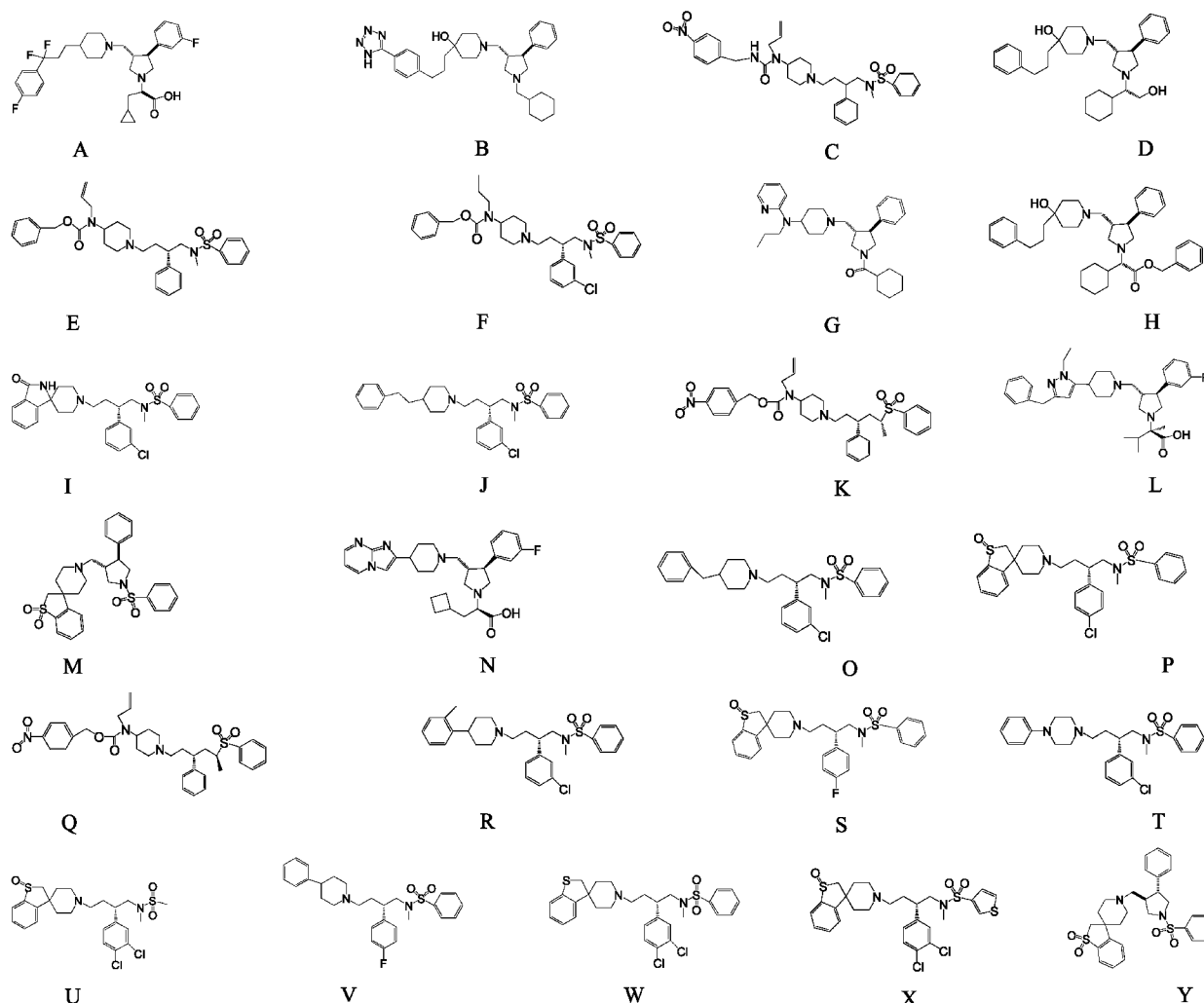
Fig.1　Chemical structures of 25 compounds in the training set

All structures were drawn using ISIS Draw 2.5 (MDL Information Systems, Inc., San Leandro, CA); A–Y represent the compound numbers.

Table 1　Results of the pharmacophore model generation with the training set data[a]

Hypothesis No.	Total cost	Error cost	RMSD	r	Features[b]
1	116.61	98.33	1.068	0.924	HY, HY, HY, PI, PI
2	123.91	107.29	1.362	0.871	HY, HY, PI, PI
3	124.55	107.88	1.379	0.868	HY, HY, PI, PI
4	125.01	107.14	1.358	0.873	HY, HY, PI, PI, RA
5	125.06	108.20	1.389	0.866	HY, HY, PI, PI, RA
6	125.06	108.20	1.389	0.866	HY, HY, PI, PI, RA
7	125.42	107.11	1.357	0.874	HY, HY, PI, PI, RA
8	125.45	108.97	1.411	0.861	HY, HY, PI, PI, RA
9	125.45	108.97	1.411	0.861	HY, HY, PI, PI, RA
10	128.75	112.52	1.508	0.839	HY, HY, PI, PI, RA

[a] null cost=180.27; fix cost=100.31; configuration=15.10; All costs are in units of bits; [b] HY: hydrophobic; RA: ring aromatic; PI: positive ionizable

whereas the null cost is the cost of a pharmacophore when the activity data of every molecule in the training set is the average value of all activities in the set and the pharmacophore has no features. A meaningful pharmacophore hypothesis may result when the difference between the two values　(null cost-fixed cost) is large. A value of 70–100 bits suggests an excellent chance (>90%) for a true correlation. At the same time, the total cost of any pharmacophore hypothesis should be close to the fixed cost to provide any useful models. As shown in Table 1, the differences between the null cost and the fixed cost and between the null cost and the total cost of the best hypothesis are 79.96 and 63.66, respectively. The cost difference is large enough and the total cost is close to the fixed cost, which implies that the correlation of the generated model was not obtained by chance.

The estimated results of the training set molecules by Hypo 1 are listed in Table 2. On the basis of the activity scale assigned and described in the materials and methods, only one highly active (++) compound E was estimated to have moderate activity (+), one inactive (−) compound X was overestimated as moderate activity (+), and one moderate active compound Q (+) was underestimated as no activity (−). It suggests that Hypo 1 has a good ability to predict the active values of the training set molecules.

Fig.3 shows the mapping of the most active compound (compound A, actual IC_{50}=0.06 nmol·L^{-1}) and the most inactive compound (compound Y, actual IC_{50}=10000 nmol·L^{-1}) from the training set to Hypo 1. The "fast fit" option was used in the map-
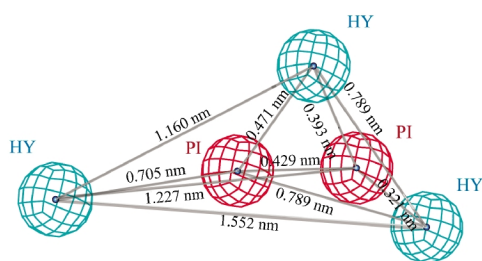
Fig.2　The best hypothesis model Hypo 1 produced by the HypoGen module of Catalyst 4.10

Pharmacophore features are color-coded with light-blue for the HY groups and red for the PI groups.

Table 2　The experiment biological data (IC$_{50}$) and the estimated activity (IC$_{50}$) of the training set molecules based on the top rank hypothesis (Hypo 1)

Compound	Fit value[a]	IC$_{50}$ (nmol·L^{-1})[b]		Activity scale[b]		Error[c]
		actual	estimated	actual	estimated	
A	8.94	0.06	0.15	++	++	+2.5
B	8.44	0.2	0.16	++	++	−1.2
C	6.58	0.75	6.9	++	++	+9.2
D	7.83	1	0.77	++	++	−1.3
E	6.62	1.5	12	++	+	+7.9
F	6.79	4	4.7	++	++	+1.2
G	6.03	12	11	+	+	−1.1
H	6.74	24	15	+	+	−1.6
I	5.43	45	170	+	+	+3.8
J	6.48	65	93	+	+	+1.4
K	6.61	110	44	+	+	−2.5
L	6.49	120	160	+	+	+1.3
M	5.47	170	650	+	+	+3.8
N	6.77	200	15	+	+	−13
O	6.03	250	180	+	+	−1.4
P	5.01	270	590	+	+	+2.2
Q	6.14	300	1400	+	−	+4.7
R	5.19	400	260	+	+	−1.5
S	5.46	570	760	+	+	+1.3
T	4.97	700	540	+	+	−1.3
U	4.53	850	560	+	+	−1.5
V	4.68	1000	530	+	+	−1.9
W	5.26	1000	550	+	+	−1.8
X	5.35	4000	680	−	+	−7.6
Y	4.53	10000	1200	−	−	−8.0

[a] Fit value indicates how well the features in the pharmacophore overlap the chemical features in the molecule; [b] The activity scale is the same as that defined in the materials and methods session; [c] + indicates that the estimated IC$_{50}$ is higher than the actual IC$_{50}$, error=estimated IC$_{50}$/actual IC$_{50}$; − indicates that the estimated IC$_{50}$ is lower than the actual IC$_{50}$, error=actual IC$_{50}$/estimated IC$_{50}$.

ping. Compound A mapped onto all the features of the model very well and the estimated activity was 0.15 nmol·L^{-1}, whereas compound Y missed a PI feature and a HY point feature with a 1200 nmol·L^{-1} predicted activity. It suggests that the positive ionizable nitrogen group and the HY group play key roles in the binding of CCR5 antagonists with the receptor.
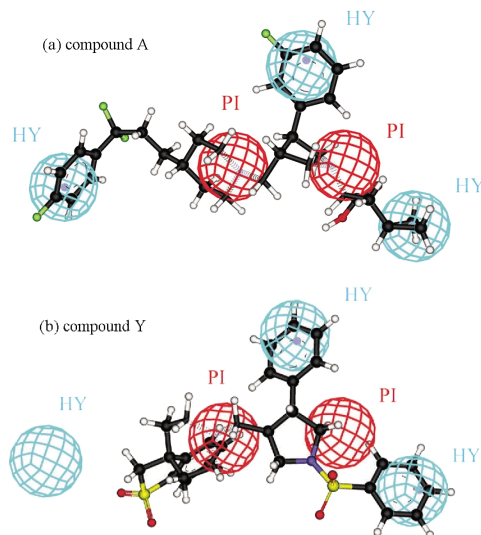

Fig.3　Mapping of the most active compound A and the most inactive compound Y from the training set onto the selected pharmacophore model (Hypo 1)

Pharmacophore features are color-coded with light-blue for the HY groups and red for the PI groups.

2.2　Validation of pharmacophores

2.2.1　Fisher test

　　The CatScramble module was applied to perform the Fisher's randomization test for assessing the quality of the pharmacophore. The activity data among the training set was randomized and then the same features and the parameters were used to

Table 3　Results from cross-validation run using CatScramble module

Validation	Total cost	Fixed cost	Error cost	RMSD	r	Configuration cost
Results for unscrambled						
	116.61	100.31	98.33	1.068	0.924	15.10
Results for scrambled						
trial_1	132.85	99.18	114.74	1.566	0.829	13.96
trial_2	157.05	97.78	139.13	2.098	0.668	12.56
trial_3	143.41	99.66	126.22	1.836	0.752	14.45
trial_4	141.77	100.60	120.85	1.715	0.793	15.39
trial_5	165.19	98.03	149.78	2.292	0.568	12.81
trial_6	133.08	97.01	119.59	1.685	0.794	11.80
trial_7	140.66	100.26	119.61	1.686	0.802	15.05
trial_8	151.19	100.35	134.53	2.009	0.690	15.14
trial_9	148.12	100.58	130.71	1.931	0.719	15.37
trial_10	144.58	98.21	129.47	1.905	0.728	13.00
trial_11	160.18	100.48	143.79	2.185	0.616	15.27
trial_12	154.20	96.55	136.69	2.051	0.690	11.33
trial_13	160.45	100.01	143.99	2.189	0.615	14.80
trial_14	157.74	96.81	143.43	2.178	0.623	11.59
trial_15	136.20	100.88	111.56	1.482	0.859	15.66
trial_16	138.87	97.92	124.70	1.802	0.760	12.70
trial_17	159.14	99.59	137.06	2.059	0.697	14.38
trial_18	121.38	98.53	104.59	1.281	0.889	13.32
trial_19	137.40	101.24	117.98	1.646	0.807	16.02

generate the pharmacophore hypothesis. In the generated pharmacophores with random data, if there are similar or better cost values, RMSD, and correlation with the original pharmacophore, the original one can be considered as generated by chance. Nineteen spreadsheets were developed to achieve a 95% confidence level. These results are reported in Table 3, and it is found that none of the generated hypotheses after randomization has a better cost value compared with the Hypo 1. Of the 19 runs, only one (trial_18) has a correlation close to 0.889, but the RMSD, cost difference, and error cost value are not as good as the original hypothesis. According to the software documentation and

the available literature, this result indicates that there is a 95% chance for Hypo 1 to represent a true correlation in the training set. The cross-validation experiments have convicted the pharmacophore model we obtained.

2.2.2 Test set prediction

The purpose of the pharmacophore model is not just to predict the activity of the training set molecules accurately. On the contract, the most important thing is its ability to predict the activity of the test set molecules. So the best pharmacophore model (Hypo 1) was applied to predict the activity of a large test set including 74 molecules (The chemical structures of the

Table 4   Experimental biological data (IC$_{50}$) and estimated activity (IC$_{50}$) of test set molecules based on the top ranked hypothesis (Hypo 1)

Compound No.	Actual IC$_{50}$ (nmol·L^{-1})	Estimated IC$_{50}$ (nmol·L^{-1})	Activity scale[a]	Estimated activity scale	Error[b]	Compound No.	Actual IC$_{50}$ (nmol·L^{-1})	Estimated IC$_{50}$ (nmol·L^{-1})	Activity scale[a]	Estimated activity scale	Error[b]
1	60	420	+	+	7.0	38	1.7	12	++	+	6.8
2	590	540	+	+	−1.1	39	0.5	2.7	++	++	5.4
3	80	91	+	+	1.1	40	0.1	0.22	++	++	2.2
4	150	890	+	+	5.9	41	0.3	0.35	++	++	1.2
5	40	540	+	+	13	42	0.9	12	++	+	14
6	70	550	+	+	7.8	43	0.5	6.1	++	++	12
7	120	560	+	+	4.7	44	4.8	0.52	++	++	−9.3
8	35	540	+	+	16	45	1.6	0.12	++	++	−14
9	10	530	++	+	53	46	5	16	++	+	3.1
10	15	390	+	+	26	47	8	14	++	+	1.8
11	200	180	+	+	−1.1	48	27	2.3	+	++	−12
12	300	620	+	+	2.1	49	0.67	0.87	++	++	1.3
13	15	320	+	+	21	50	0.29	0.48	++	++	1.6
14	50	320	+	+	6.3	51	0.73	3.7	++	++	5.1
15	5	870	++	+	170	52	1.6	4.7	++	++	3.0
16	35	560	+	+	16	53	0.23	3.1	++	++	13
17	30	540	+	+	18	54	0.6	4.6	++	++	7.7
18	200	70	+	+	−2.9	55	1	3.8	++	++	3.8
19	2	36	++	+	18	56	2.5	0.39	++	++	−6.4
20	3	40	++	+	13	57	0.84	0.41	++	++	−2.1
21	60	440	+	+	7.3	58	2.8	22	++	+	7.9
22	49	660	+	+	13	59	8.2	3500	++	−	430
23	7	0.1	++	++	−70	60	100	45	+	+	−2.2
24	100	1.1	+	++	−89	61	1	6.8	++	++	6.8
25	5	0.084	++	++	−60	62	29	24	+	+	−1.2
26	2	0.1	++	++	−19	63	0.5	0.19	++	++	−2.6
27	0.4	0.078	++	++	−5.1	64	1.2	0.81	++	++	−1.5
28	0.5	0.24	++	++	−2.1	65	1.2	10	++	++	8.7
29	4	0.19	++	++	−21	66	0.8	7.8	++	++	9.8
30	2	16	++	+	7.8	67	0.6	6.3	++	++	10
31	0.1	0.57	++	++	5.7	68	2.3	0.4	++	++	−5.7
32	36	320	+	+	8.9	69	4.2	1.1	++	++	−3.7
33	0.2	0.12	++	++	−1.6	70	4.8	3.1	++	++	−1.5
34	6	21	++	+	3.6	71	0.8	2.2	++	++	2.7
35	66	620	+	+	9.4	72	16	23	+	+	1.5
36	3.9	20	++	+	5.1	73	41	0.67	+	++	−61
37	1.8	14	++	+	8.0	74	6.9	3.8	++	++	−1.8

[a]The activity scale is the same as that defined in the Materials and Methods Section. [b]The calculation of error is the same as in Table 2.

molecules are in the supporting material, which is available freely from www.whxb.pku.edu.cn) and to investigate whether it can identify active and inactive molecules correctly. The "fast fit" option is used in all the cases. The detailed estimation values are listed in Table 4.

In 48 highly active compounds, 35 compounds are accurately classified as highly active and 13 compounds are classified as moderately active. Three of the 27 moderate molecules are overestimated as highly active. One highly active molecule is underestimated as inactive. The estimation of some compounds such as 23, 24, and 25 is not so good. This kind of compounds has a hydroxyl group on the piperidine cycle, which induced bad effect to the activity. The pharmacophore model could only give the principal features corresponding to the activity while the detailed information like the steric clashes and poor electronic contact cannot be considered by the model. It caused the limitation of the model and future work is on the way to combine the pharmacophore model and CoMFA together to give a more accurate model. Despite the limitations, the selected hypothesis showed a good ability to discriminate the active and the inactive compounds, especially in the selection of highly active compounds with a correlation coefficient (r) of 0.703 on the test set. It indicates that the model is able to select unknown compounds with the good active value and will be applied in the following design of novel compounds.

3   Conclusions

The ligand-based computational approach, pharmacophore model generation, was employed to identify molecular structure requirements as effective CCR5 antagonists. The model was generated based on a set of pyrrolidine-based and butane-based CCR5 antagonists. Twenty-five compounds were carefully selected as the training set. The final selected model is composed of three HY points and two PI features. Mapping of the most active and most inactive compounds suggests that the positive ionizable nitrogen groups and the HY groups play key roles in the binding of CCR5 antagonists with the receptor. The application of the pharmacophore model on the test set shows that it is able to discriminate the activity scale of compounds. Our model should be benefit in understanding the possible inhibitory mode of the CCR5 inhibitors and provide clear guidelines and accurate activity prediction for novel antagonist design.

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