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Common Pharmacophore Model and 3D-QSAR Analysis of Two Different Tyrphostin Families

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Abstract: In the present study we investigated two groups of small molecular tyrosine kinase phosphorylation inhibitors (tyrphostins) with quite different structures (19 compounds of the benzylidene malononitrile famliy and 13 compounds of the 3-substituted indolin-2-ones family). With the aid of a pharmacophore analysis method (CATALYST), a common three-dimensional pharmacophore model to these two kinds of molecules has been discovered. A better 3D-QSAR analysis based on the generated pharmacophore model was conducted (correlate coeffcient R=0.956) and the model shows very good predictive ability.

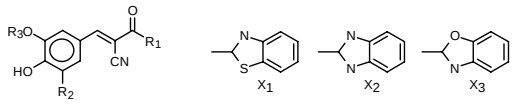
Keywords: Pharmacophore model, 3D-QSAR, tyrphostin.

Receptor tyrosine kinases (RTKs) have been known to play a crucial role in the cellular signal transduction pathways. The HER2 proto-oncogene codes for a kind of RTK which has homology to the epidermal growth factor (EGF) receptor (HER1). Amplification of the HER2 gene has been found in about 30% of primary breast, ovary, and gastric carcinomas[1]. Survival rates and tumor aggressiveness can be directly correlated to the level of HER2 expression. In this regard, regulating the celluar signal transduction *via* inhibition of HER2 has been considered a promising way of controlling malignant tumors. Recently, two groups of small molecules have been found to possess high inhibitory activity to HER2.

Pharmacophore research is a useful method to uncover a set of features that is common to a series of active molecules. The arrangement of these common features in 3D space may significantly affect the molecular activities. But the spacial relationships of the features are unable to consider in normal QSAR analysis, especially to those molecules belong to several different classes. However, how to superimpose those flexible molecules and extract the common features is still a problem.

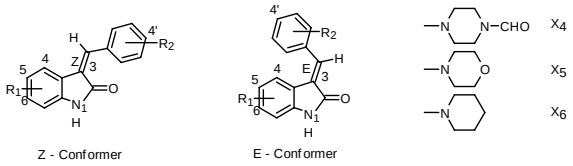
In this study we generate a 3D pharmacophore model common to the two molecule groups and regress it in a 3D QSAR analysis within the 31 molecules. In regression the pharmacophore model shows a perfect correlation coefficient of 0.956. The estimate ability of the model is also satisfactory.

Table 1. Structures and activities (means±S.E.,n=3) of Benzylidene malononitriles



No.	R_1	R_2	R_3	IC_{50}, uM
1	-NH-Ph	-H	-H	45±4.3
2	-NH-CH_2-Ph	-H	-H	12.1±2.2
3	-NH-$(CH_2)_2$-Ph	-H	-H	9.4±0.4
4	-NH-$(CH_2)_3$-Ph	-H	-H	33±5
5	-NH-$(CH_2)_4$-Ph	-H	-H	22±6
6	-Ph	-H	-H	20±3.5
7	-NH-CH_2-(4'-OH)-Ph	-H	-H	2.9±0.3
8	-NH-(4'-Cl)-Ph	-H	-H	62±7
9	-NH-(2',4'-di-OMe)-Ph	-H	-H	20±1.5
10	-NH-(2',6'-di-Me)-Ph	-H	-H	44±13
11	-NH-(2',4',6'-tri-Me)-Ph	-H	-H	21±6
12	-NH-cyclo-C_6H_{11}	-H	-H	19±3
13	-NH_2	-CH_2-S-Ph	-Me	0.13±0.007
14	-NH_2	-CH_2-S-(2'-COOH)-Ph	-Me	0.45±0.07
15	-NH_2	-CH_2-S-(4'-Me)-Ph	-Me	1.65±0.19
16	-NH_2	-CH_2-S-CH_2-Ph	-Me	0.2±0.03
17	-NH_2	-CH_2-S-X_1	-Me	0.35±0.07
18	-NH_2	-CH_2-S-X_2	-Me	1.5±0.14
19	-NH_2	-CH_2-S-X_3	-Me	6.1±0.1

Table 2. Structures and activities (no S.E.) of 3-Substituted indolin-2-ones



No.	Conformer	R_1	R_2	IC_{50}, uM
20	E	H	4'-X_4	90.2
21	Z	4-Me	4'-X_5	66.6
22	E	H	4'-X_6	92.6
23	E	H	3',5'-di-CMe_3, 4'-OH	64.8
24	E	5-Cl	3',5'-di-$CHMe_2$, 4'-OH	8.2
25	E	H	3'-CMe_3, 4'-OMe, 5'-Br	19.0
26	Z	5-Cl	3'-CMe_3, 4'-OMe	16.2
27	E	H	4'-$CHMe_2$	16.9
28	E	H	3',5'-di-$CHMe_2$, 4'-OH	7.0
29	E	H	3'-$CHMe_2$, 4'-OMe	13.2
30	Z	1-Me	4'-Br	22.5
31	Z	5-Cl	3'-$CHMe_2$, 4'-OMe, 5'-Br	15.2

Materials and Methods

The active molecules used to generate pharmacophore model fall into 2 structural

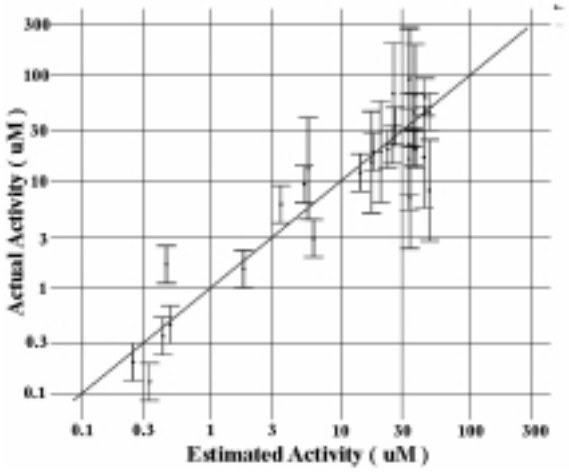
categories. One is benzylidene malononitrile family[2] and the other is 3-substituted indolin-2-ones[3] family. Their structures and bioactivities are listed in **Table 1** and **Table 2**.

Each compound was built in the CATALYST[4] View Compound Work Bench and optimized by using a forcefield derived from CHARMm with all force field components after the quadratic term truncated, and the dihedral expansion generally expressed as a single term.The Lennard-Jones expression was used for Van der Waals interactions.

The proper "active conformers" need to overlay and extract common features.are often unavailable for many bioactive molecules, so an alternative way must be found. Simplely using lowest-energy conformer is unsuitable because the molecule is flexible and the active conformer is often different from the lowest-energy conformer. Generally, the energy of the active conformer will not be 20 kcal/mol higher than the global minimum energy. Thus the molecular flexibility is taken into account by considering each compound as a collection of conformers representing different areas of the conformational space accessible to the molecule within 20 kcal/mol. The maximum number of conformers obtained in the conformational analysis is 194 for compound **3**.

The bioactivity data come from two different sources thus may not coincide with each other. And it is well known that the IC_{50} value itself can have a proper deviation to some extent. For the two reasons the parameter "uncertainty" is defined as a ratio of the reported value to the minimum and maximum possible values. And an IC_{50} = 30 (uM) with uncertainty 3 means from 30*1/3 to 30*3 (or from 10uM to 90uM) are all possible activity values. Because the activity values of the benzylidene malononitril family are mean values of 3 experiments, we set their uncertainty as 1.5 instead of the default value 3 which is set to the 3-Substituted Indolin-2-ones group.

Figure 1. Correlation line of estimated activity *vs* actual activity. (R=0.956)



Totally 10 pharmacophore models are generated using the CatHypo module of CATALYST on a SGI O2 workstation (R5000). Pair-wise root-mean-square fits are performed on all 10 pharmacophore model pairs and their similarity are evaluated and

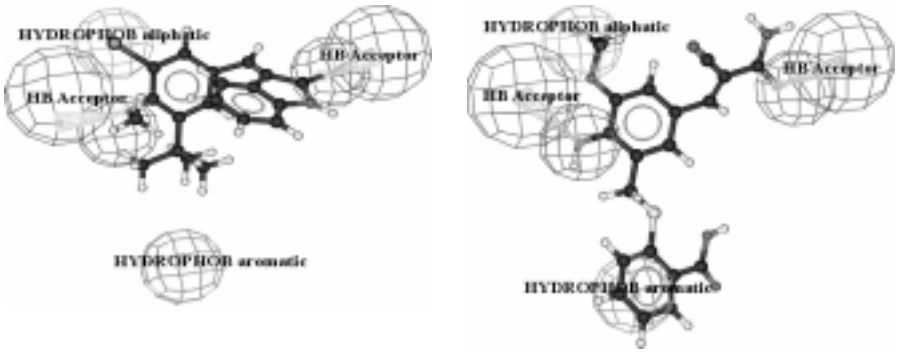
fed into a cluster algorithm. From the cluster information we picked out the most representive pharmacophore model and performed 3D-QSAR analysis.

Results and Discussion

The generated pharmacophore model consists of two hydrogen bond acceptors, one aliphatic hydrophobic group and one aromatic hydrophobic group.

All conformers of each compound are superimposed with the pharmacophore model and from the fitness degrees an activity group is gained. With the 31 groups of estimated activities we can generate the best regression from the viewpoint of statistics. Thus the adopted conformer may not be the lowest-energy conformer but may be the most possible "active conformer". So we can say the 3D QSAR regress analysis conduced takes all compounds' conformational spaces into account and reflects the molecular flexibility. The final correlated coefficent R=0.956 (See **Figure 1**).

Figure 2. Superimposition of compound 25 (Estm. 20uM, Actu.19uM, left) and 14 (Estm. 0.49uM, Actu. 0.45±0.07uM, right) with the pharmacophore model.



The common pharmacophore model superimposed very well with the two families of molecules having quite different core structures. **Figure 2** shows the fit modes of two representative compounds each of both family. The conformers adopted in the regression (the most possible active conformers) of compound 25 and 14 show the energy of 8.00 kcal/mol and 2.37 kcal/mol (relative to global lowest-energy conformer) respectively.

Acknowledgments

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