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Pharmacophore modeling studies of type I and type II kinase inhibitors of Tie2

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ABSTRACT

In this study, chemical feature based pharmacophore models of type I and type II kinase inhibitors of Tie2 have been developed with the aid of HipHop and HypoRefine modules within Catalyst program package. The best HipHop pharmacophore model Hypo1_I for type I kinase inhibitors contains one hydrogen-bond acceptor, one hydrogen-bond donor, one general hydrophobic, one hydrophobic aromatic, and one ring aromatic feature. And the best HypoRefine model Hypo1_II for type II kinase inhibitors, which was characterized by the best correlation coefficient (0.976032) and the lowest RMSD (0.74204), consists of two hydrogen-bond donors, one hydrophobic aromatic, and two general hydrophobic features, as well as two excluded volumes. These pharmacophore models have been validated by using either or both test set and cross validation methods, which shows that both the Hypo1_I and Hypo1_II have a good predictive ability. The space arrangements of the pharmacophore features in Hypo1_II are consistent with the locations of the three portions making up a typical type II kinase inhibitor, namely, the portion occupying the ATP binding region (ATP-binding-region portion, AP), that occupying the hydrophobic region (hydrophobic-region portion, HP), and that linking AP and HP (bridge portion, BP). Our study also reveals that the ATP-binding-region portion of the type II kinase inhibitors plays an important role to the bioactivity of the type II kinase inhibitors. Structural modifications on this portion should be helpful to further improve the inhibitory potency of type II kinase inhibitors.

1. Introduction

Angiogenesis, the formation of new blood vessels from preexisting vasculature, is one of the critical conditions for the growth of solid tumors. Thus anti-angiogenesis is believed to be a promising anticancer therapy approach [1,2]. The most famous example is destroying the vascular endothelial growth factor (VEGF)/KDR (VEGFR-2) signaling cascade, by, typically, functional inhibition of VEGFR-2 [3]. For this purpose, several small molecular drugs that inhibiting VEGFR-2, including sunitinib (Sutent) and sorafenib (Nexavar), have been approved to enter the market by FDA [4]. A large amount of compounds targeting VEGFR-2 are under clinical trials or preclinical studies [5]. In addition to the well studied VEGF/VEGFR-2 channel, there is another important one, namely, angiopoietins/Tie2 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2), which has also been well proved to be involved in the angiogenesis [6].

Tie2 has been found to be overexpressed in many tumors, including mammary adenocarcinoma, thyroid carcinoma, bronchogenic carcinoma, and prostatic carcinoma [7–10]. Functional inhibition of Tie2 has been shown to lead a significant obstruction of tumor angiogenesis, thereby stop the tumor growth [11,12]. Therefore, Tie2 has become a very promising target for anti-angiogenesis therapy of tumors. Currently, some academic institutions and pharmaceutical companies have been involved in the development of Tie2 inhibitors. As far as we know, more than two hundred small molecule inhibitors against Tie2 have been reported publicly at present. At least two compounds, namely, ARRY-614 (Array Biopharma. Inc.) and CP-547632 (Pfizer), have entered the clinical trials. All of these provide a solid basis for elucidating the structure–activity relationship (SAR) of these diverse compounds, and facilitate the identification of new Tie2 inhibitors.

In order to achieve this objective, we collected all the Tie2 inhibitors publicly available. A total of 263 compounds were obtained [13–21]. From these compounds a quantitative pharmacophore model was developed with the aid of HypoRefine module within Catalyst [22]. Unfortunately the established model has almost no any predictive ability to the external compounds of the training set. A careful analysis shows that the 263 inhibitors belong to two distinct types of inhibitors, i.e. type I and type II kinase inhibitors, which bind in different regions of the catalytic domain of Tie2. A comprehensive structural analysis of type I and type II kinase inhibitors has been carried out by Liu et al., in which they made use of the crystal structural information of various kinds of

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kinase-ligand complexes [23,24]. For type I kinase inhibitors, they just bind in and around the region originally occupied by ATP, which are typically ATP competitive inhibitors. For type II kinase inhibitors, however, besides the ATP binding region (the corresponding portion of ligand occupying this region is called ATP-binding-region portion, shortened as AP), they need to occupy a hydrophobic site that is directly adjacent to the ATP binding pocket and is usually called allosteric site (the corresponding portion of ligand occupying this region is called hydrophobic-region portion, shortened as HP), which presents just in the inactive conformation of kinase. Furthermore, there is a bridge portion (shortened as BP, see Fig. 1) linking the AP and HP, which is usually an amide or urea. BP usually forms hydrogen-bond pair with the conserved residues in the allosteric site: one hydrogen bond with the side chain of a conserved glutamic acid in the αC-helix and the other with the backbone amide of aspartic acid in the DFG motif (see Fig. 1).

Preceding experience taught us that two types of pharmacophore models rather than a single type of model should be used in order to correctly describe the structure–activity relationship of all the Tie2 kinase inhibitors. Thus, in this account, we shall develop two types of pharmacophore models: one for the type I kinase inhibitors and the other one for the type II kinase inhibitors. It is expected that these established models should be capable of correctly illustrating the structure–activity relationship of Tie2 kinase inhibitors, as a result, be helpful to the identification of novel Tie2 inhibitors. This study provides a good example that a bad quantitative structure–activity relationship (QSAR) model might stem from ignoring different action mechanisms of the small molecules with their target protein. Developing multiple models, one of which corresponds to a different action mechanism, is an appropriate way to overcome this type of problem.

2. Materials and methods

2.1. Pharmacophore modeling

All the pharmacophore modeling calculations were carried out by using the CATALYST 4.11 software package (Accelrys, San Diego, USA) on IBM graphic workstation. The HipHop and HypoRefine modules within Catalyst were used for the constructions of qualitative and quantitative models, respectively [22,25].

For the type I kinase inhibitors of Tie2, just qualitative pharmacophore models were developed since the collected type I kinase inhibitors do not bear sufficient diversity in bioactivity and chemical structure. Four most active type I inhibitors were selected to form a training set. Chemical structures as well as IC_{50} values of these inhibitors are given in Chart 1 (A1–A4). Compound A1 was considered as 'reference compound' specifying a 'principal' value of 2 and a 'MaxOmitFeat' value of 0, meaning its structure and conformation would have the strongest influence in the model building phase. The 'principal' and 'MaxOmitFeat' values were set to 1 and 0 respectively for the remaining compounds in the training set. The initial features, which were specified based on an overview of all the training set molecules, include hydrogen-bond acceptor (A), hydrogen-bond donor (D), general hydrophobic (Z), hydrophobic aliphatic (Y), hydrophobic aromatic (X), and aromatic ring (R).

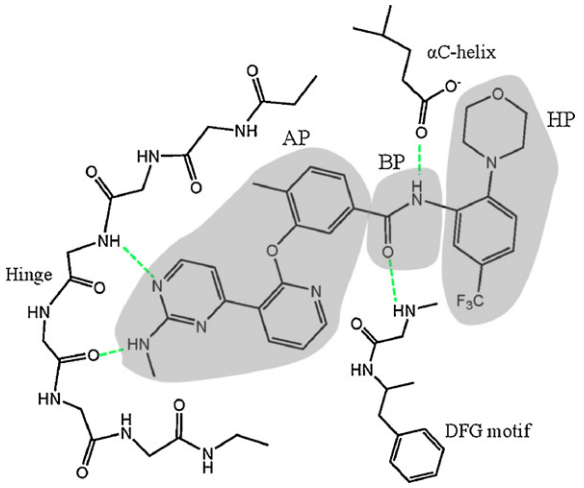

Fig. 1. Schematic representation of the structural characteristics necessary for type II kinase inhibitors. AP, ATP-binding-region portion, HP, hydrophobic-region portion, BP, bridge portion linking AP and HP.

A pre-survey to the type II kinase inhibitors of Tie2 shows that these inhibitors bear satisfactory diversity in both bioactivity and chemical structure. Thus quantitative HypoRefine pharmacophore modeling was carried out for the type II kinase inhibitors. Twenty type II inhibitors with sufficient diversity in bioactivity and structure were selected to form a training set. The IC_{50} values of the selected twenty inhibitors span a range of four orders of magnitude (from 0.001 μM to 4.680 μM). Chemical structures as well as experimental IC_{50} values of the training set molecules are given in

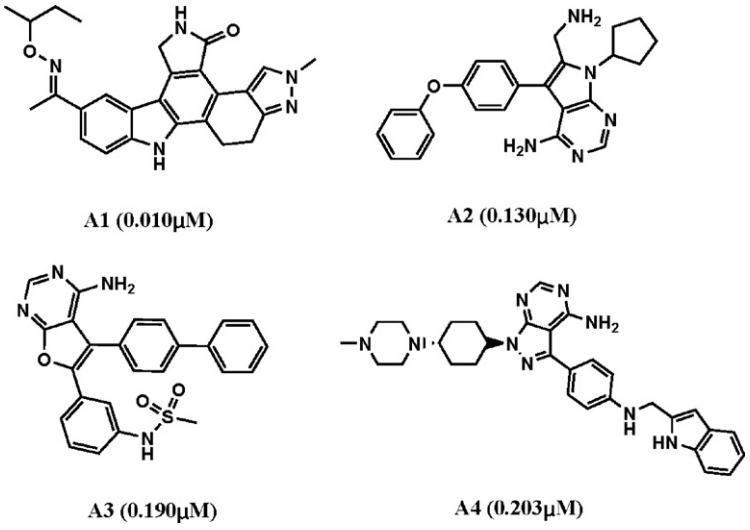

Chart 1. Chemical structures of type I Tie2 kinase inhibitors in the training set together with their biological activity data (IC_{50} values, μM) for HipHop run.

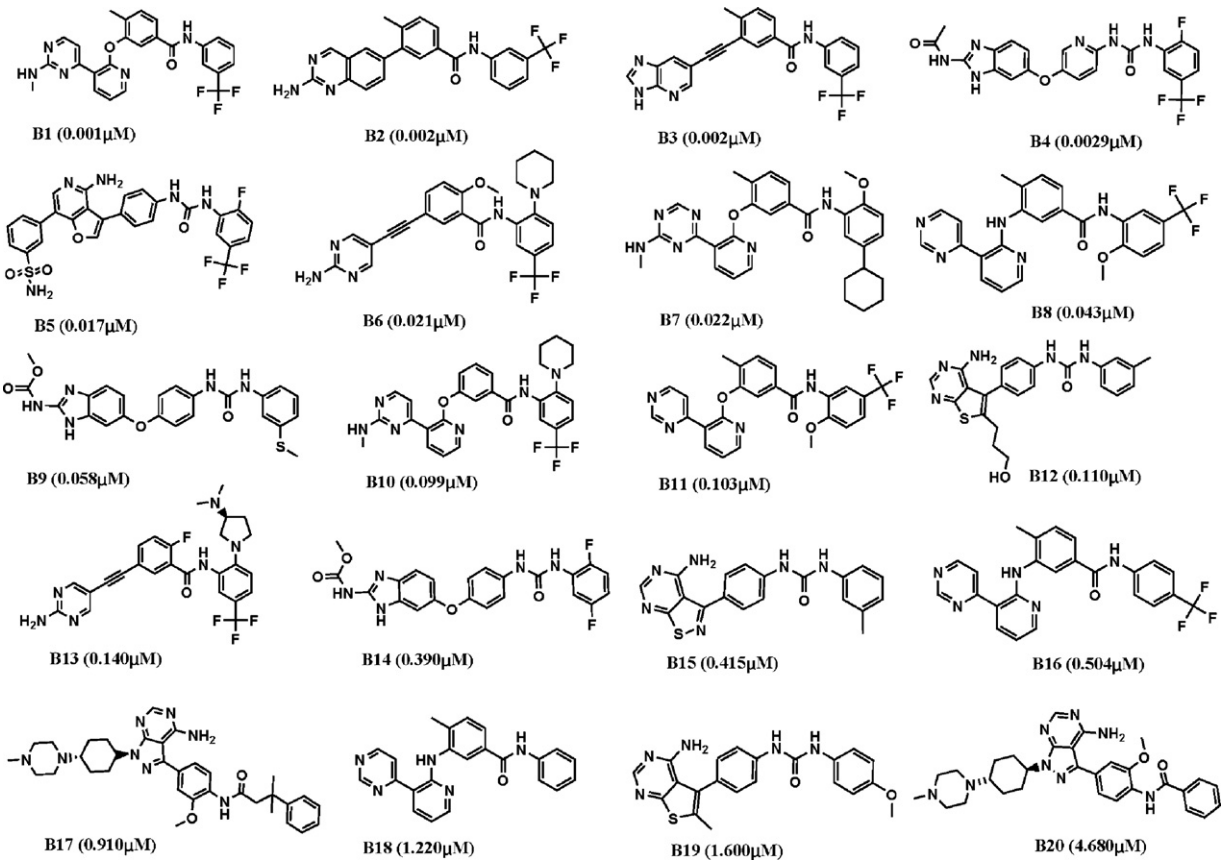

Chart 2. Chemical structures of type II Tie2 kinase inhibitors in training set (B1–B20) together with their biological activity data (IC_{50} values, μM) for HypoRefine run.

Chart 2 (B1–B20). Before the HypoRefine run, HipHop modeling was first performed which purpose was to identify the common pharmacophore features necessary for the active type II Tie2 inhibitors [22]. Following the information provided in the HipHop run, five kinds of features including hydrogen-bond acceptor (A), hydrogen-bond donor (D), general hydrophobic (Z), hydrophobic aliphatic (Y), and hydrophobic aromatic (X) were chosen as the initial pharmacophore features in HypoRefine run. The value for 'excluded volume' was set to 2 to consider the steric effect.

All molecules were built in 2D/3D Visualizer in Catalyst software package and all of the structures were minimized using the CHARMM-like force field implemented in the program [26]. A series of energetically reasonable conformational models which represent the flexibility of each compound were generated within the Catalyst catConf module using the Poling Algorithm [27]. We chose a maximum number of 250 conformers, the 'best conformational analysis' method, and an energy threshold of 20 kcal/mol above the global energy minimum for conformation searching.

2.2. Pharmacophore model evaluation

For the qualitative pharmacophore model of type I inhibitors, test set method was used for validation. The test set used here contains a total of 110 type I inhibitors, given in Table S1 (TS_I, see Supplementary material). For the validation of quantitative pharmacophore model of type II inhibitors, both test set and cross validation methods were used [28]. For the use of test set method, 129 compounds, containing all the collected type II inhibitors except the twenty compounds (B1, B2, . . ., B20) in the training set, were selected to form the test set, TS_II, shown in Table S2 (see Supplementary material). The cross validation was performed by using CatScramble program within CATALYST [29]. The CatScramble strategy tries to scramble the experimental activities in the training set randomly, and the resulting training sets are used for HypoRefine run. The confidence level was set to 95%. Thereby CatScramble program generated 19 random spreadsheets to construct hypotheses using exactly the same conditions as used in generating the original pharmacophore hypotheses.

3. Results

3.1. HipHop pharmacophore model of type I kinase inhibitors of Tie2

Among all the collected Tie2 inhibitors, a total of 114 compounds are found to belong to type I inhibitors. The lack of highly active compounds as well as limited structural diversity make it not suitable for using these compounds to create a quantitative pharmacophore model. Thus, qualitative HipHop pharmacophore modeling was performed based on the four most active type I inhibitors (A1–A4, Chart 1).

The HipHop run exported ranked top 10 hypotheses. And the best hypothesis (referred to as Hypo1_I, see Fig. 2A), which has the highest ranking score 49.3298, contains one hydrogen-bond acceptor, one hydrogen-bond donor, one general hydrophobic, one hydrophobic aromatic, and one ring aromatic feature. Fig. 2B presents the Hypo1_I aligned with the most active compound A1.

The test set TS_I (Table S1, see Supplementary material), which contains all the collected type I inhibitors except compound A1–A4, was used to validate the HipHop model Hypo1_I. Each compound in TS_I was mapped on the Hypo1_I. Table S1 (see Supplementary material) presents the calculated fit value for each compound. In general, a compound with higher activity corresponds to a higher fit value. Further all inhibitors were classified into three categories, i.e. highly active (<0.1 μM), moderately

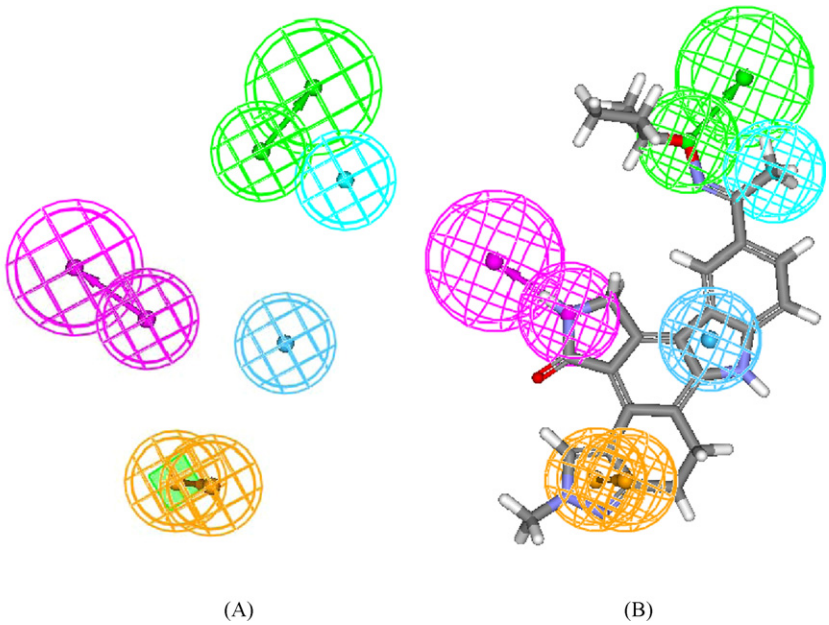

Fig. 2. Pharmacophore model of type I Tie2 kinase inhibitors generated by HipHop. (A) The best ranking HipHop model Hypo1_I. (B) Hypo1_I aligned with the most active compound A1 (IC_{50} = 0.010 μM). These features are color coded with green, hydrogen-bond acceptor, magenta, hydrogen-bond donor, cyan, general hydrophobic feature, dark blue, hydrophobic aromatic feature, orange, aromatic ring.

active (0.1 μM–1 μM) and low active (>1 μM). Fig. 3 presents the distribution of fit values for the three category compounds. Obviously, for the highly active category, the highest peak occurs at the point of "Fix-Value = 4.9", and the fit values of 77% compounds are in the range of 4.5 and 5. For the moderately active category, the highest peak corresponds to the point at "Fix-Value = 3.7" and 59% compounds in this category have a fit value between 3.1 and 4.1. For the low active category, the highest peak locates at the point of "Fit-Value = 2.9", and the fit values of 64% compounds in this category are between 2.5 and 3.1. These indicate that compounds with higher inhibitory potency afford larger fit values and vice versa in general. Therefore, it is obvious that Hypo1_I is capable of qualitatively distinguishing between highly, moderately and low activity compounds.

3.2. Pharmacophore models of type II inhibitors of Tie2

HipHop Pharmacophore model. Before performing quantitative pharmacophore modeling for the type II kinase inhibitors, qualitative HipHop models were first generated based on the four most-active compounds in the training set (B1–B4, Chart 2), whose purpose was to identify common chemical features necessary for potent type II kinase inhibitors. The best HipHop pharmacophore model generated is presented in Fig. 4A. Fig. 4B is the mapping of the best HipHop model with one of the most active compound B2 in the training set. The best hypothesis contains six features: two hydrogen-bond donors, one general hydrophobic, one hydrophobic aliphatic, and two hydrophobic aromatic features.

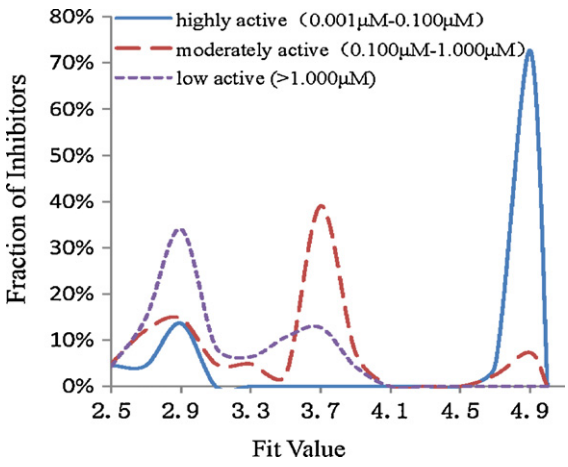

Fig. 3. Distributions of the fit values of the test set (TS_I) compounds in the three categories (highly active, moderately active, and low active).

HypoRefine Pharmacophore model. The HipHop pharmacophore model has indicated the importance of four pharmacophore features for potent type II kinase inhibitors, including hydrogen-bond donor, general hydrophobic, hydrophobic aliphatic, and hydrophobic aromatic feature. Thus these four types of pharmacophore features were selected as the initial pharmacophore features in the HypoRefine run. In addition, the hydrogen bonding acceptor was also included since it exists in most of the known type II kinase inhibitors. The training set used here contains twenty compounds (B1–B20, Chart 2).

The top 10 hypotheses as well as their statistical parameters obtained in the HypoRefine run are shown in Table 1. The best pharmacophore model (Hypo1_II, Fig. 5A) was characterized by the lowest total cost value (81.3321), the highest cost difference (92.9119), the lowest RMSD (0.74204), and the best correlation coefficient (0.976032). The fixed cost and null cost are 75.7595 and 174.244 bits, respectively. Hypo1_II contains five features, including two hydrogen bond donors, one hydrophobic aromatic and two general hydrophobic features. Two excluded volumes are also included in Hypo1_II. The 3D space and distance constraints of these pharmacophore features are shown in Fig. 5B. Fig. 5C and D presents the Hypo1_II aligned with one of the most active compound B2 (IC_{50} = 0.002 μM) and one of the least active compound B19 (IC_{50} = 1.600 μM) in the training set. Clearly, all hypothesis features are mapped very well with the corresponding chemical functional groups on compound B2, while two hypothesis features, i.e. the two hydrogen-bond donors, are not mapped well or completely not mapped with any functional group on compound B19. These reflect the validity of the pharmacophore model Hypo1_II to some extent.

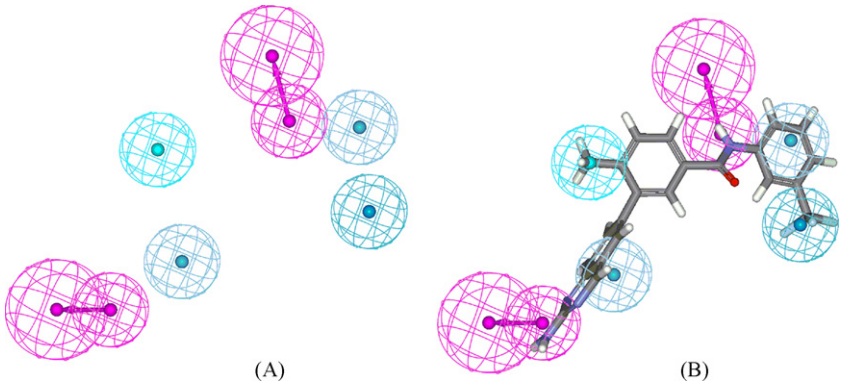

Fig. 4. Pharmacophore model of type II Tie2 kinase inhibitors generated by HipHop. (A) The best ranking HipHop model. (B) The best ranking HipHop model aligned with one of the most active compound B2 (IC_{50} = 0.002 μM). The features are color coded with magenta, hydrogen-bond donor, cyan, general hydrophobic feature, light blue, hydrophobic aromatic feature, blue, hydrophobic aliphatic feature.

Table 1
Statistical parameters of the top 10 hypotheses of type II Tie2 kinase inhibitors generated by HypoRefine program.

Hypo name	Total cost	Cost diff.[a]	RMSD	Correlation (*r*)	Features[b]
Hypo1_II	81.3321	92.9119	0.74204	0.976032	$DDXZZE_2$
Hypo2_II	81.7573	92.4867	0.77271	0.973972	$DDZZZE_2$
Hypo3_II	85.7067	88.5373	0.982633	0.957597	$DDYZZE_2$
Hypo4_II	85.7595	88.4845	0.983302	0.957544	$DDXYZE_2$
Hypo5_II	87.424	86.82	1.0791	0.94857	$DDYZE_1$
Hypo6_II	90.6067	83.6373	1.21216	0.934676	$DDXZE_2$
Hypo7_II	91.2449	82.9991	1.18755	0.937917	DDYZ
Hypo8_II	91.3418	82.9022	1.2459	0.930817	$DXXYZE_1$
Hypo9_II	91.8289	82.4151	1.26507	0.92859	$DXXXYE_1$
Hypo10_II	92.7289	81.5151	1.21805	0.934975	DDXY

[a] (Null cost-total cost), null cost = 174.244, fixed cost = 75.7595, configuration cost = 16.5736. All cost values are in bits.
[b] D, X, Y, Z and E present hydrogen-bond donor, hydrophobic aromatic, hydrophobic aliphatic, general hydrophobic, and excluded volume, respectively.

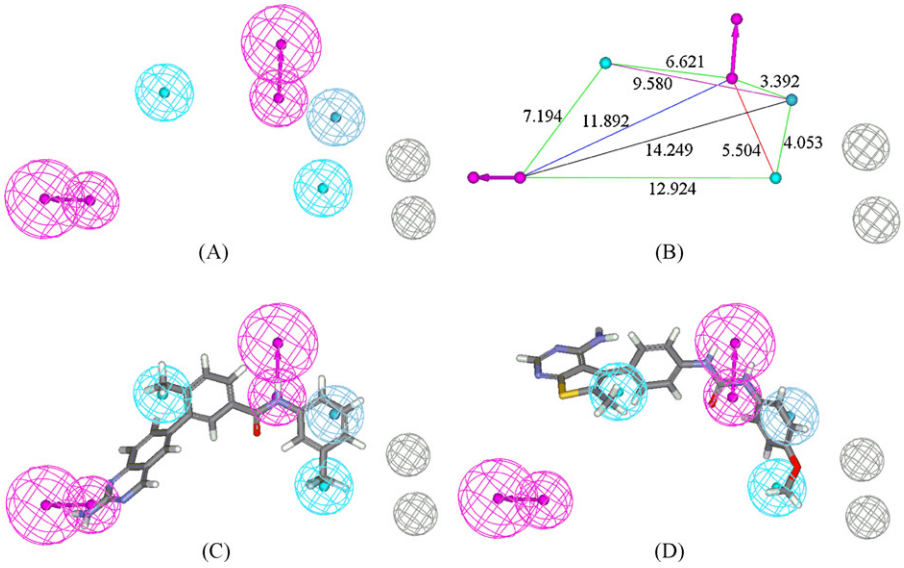

Fig. 5. Pharmacophore models of type II Tie2 kinase inhibitors generated by HypoRefine. (A) The best HypoRefine model Hypo1_II. (B) 3D spatial relationship and geometric parameters of Hypo1_II. (C) Hypo1_II mapping with one of the most active compound B2 (IC_{50} = 0.002 μM). (D) Hypo1_II mapping with one of the least active compound B19 (IC_{50} = 1.600 μM). These chemical features are color coded with magenta, hydrogen-bond donor, cyan, general hydrophobic feature, light blue, hydrophobic aromatic feature, grey, excluded volume.

The predictive ability of Hypo1_II for the training set was then examined. The estimated inhibitory activities together with experimental IC_{50} values for the training set compounds are given in Table 2. From Table 2, one can see that the estimated IC_{50} value is very close to the corresponding experimental IC_{50} value. And the error values, defined as the ratio between experimental activity and estimated activity, are all less than 3, further demonstrating a remarkable consistency between estimated and experimental IC_{50} values.

Validation of Hypo1_II. A good pharmacophore is not only able to predict the activity of the training set compounds correctly, but also capable to predict the activity of external compounds to the

Table 2
Experimental and estimated (by Hypo1_II) IC_{50} values (μM) together with the error values (defined as the ratio between experimental activity and estimated activity) of the training set compounds B1–B20.

Mol number	Exptl.[a]	Pred.[b]	Error[c]	Mol number	Exptl.[a]	Pred.[b]	Error[c]
B1	0.001	0.00068	−1.5	B11	0.100	0.035	−2.9
B2	0.002	0.0019	−1	B12	0.110	0.059	−1.9
B3	0.002	0.0061	+3	B13	0.140	0.390	+2.8
B4	0.0029	0.0037	+1.3	B14	0.390	0.470	+1.2
B5	0.017	0.022	+1.3	B15	0.420	0.300	−1.4
B6	0.021	0.027	+1.3	B16	0.500	0.620	+1.2
B7	0.022	0.026	+1.2	B17	0.910	0.740	−1.2
B8	0.043	0.049	+1.1	B18	1.200	0.710	−1.7
B9	0.058	0.075	+1.3	B19	1.600	1.100	−1.4
B10	0.099	0.058	−1.7	B20	4.700	6.200	+1.3

[a] Exptl. = experimental activity (IC_{50} values, μM).
[b] Pred. = predicted activity (IC_{50} values, μM).
[c] The negative value indicates that the experimental IC_{50} is higher than the predicted IC_{50}.

training set. Thus an independent test set, TS_II, containing 129 compounds (Table S2, see Supplementary material), was used to validate the Hypo1_II.

Table S2 (see Supplementary material) gives the experimental and estimated IC_{50} values of the test set compounds. Most of the error values are less than 5, and just 8 compounds have error values larger than 10. Further, a regression analysis of the experimental and predicted inhibitory activity values for the test set compounds gives a fairly good correlation coefficient of 0.764542 (see Fig. 6), indicating a good predictive ability.

Fischer randomization test was used to further evaluate the statistical relevance of Hypo1_II. Since a confidence level of 95% was chosen in this study, a total of 19 random spreadsheets were created for generating pharmacophore hypotheses. The total costs of pharmacophore models obtained in the 19 HypoRefine runs as well as the original HypoRefine run are presented in Fig. 7. From Fig. 7, one can see that the original hypothesis is far more superior to those of the 19 random hypotheses generated. These results provide confidence on our pharmacophore model.

4. Discussion

Type I kinase inhibitors, according to their definition, just occupy the ATP binding pocket. This type of inhibitors typically form 1–3 hydrogen bonds with the kinase hinge residues that link the N- and C-terminal kinase domains; these hydrogen bonds mimic those normally formed by the exocyclic amino group of adenine. Clearly, the hydrogen bond donor in Hypo1_I (see Fig. 2) should be responsible for this type of hydrogen bonds.

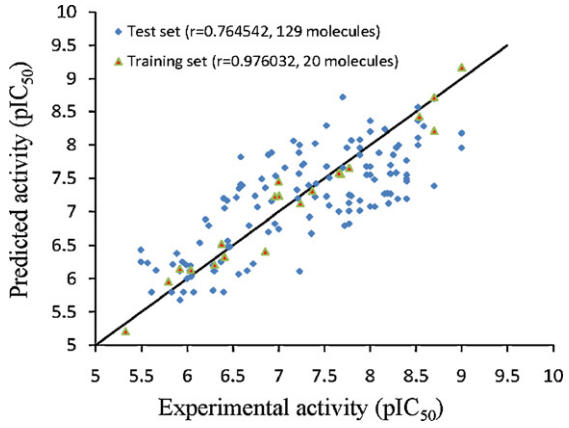

Fig. 6. Plot of the correlation (r) between the experimental activity and the predicted activity by Hypo1_II for test set (TS_II) molecules (in blue) and training set molecules (in red).

As it has been mentioned before that a typical type II kinase inhibitor is composed of three portions: AP (occupying the ATP-binding-region), HP (occupying the hydrophobic region), and BP (linking the AP and HP). And the BP usually forms hydrogen-bond pair with the conserved residues in the hydrophobic region: one hydrogen bond with the side chain of a conserved glutamic acid in the αC-helix and the other with the backbone amide of aspartic acid in the DFG motif. The three portions of type II kinase inhibitors as well as their representative chemical features can be easily identified in the pharmacophore model Hypo1_II of the type II kinase inhibitors (see Fig. 8A). Obviously one hydrogen bond donor and one hydrophobic feature are located in the ATP-binding region. In the allosteric site, there are one hydrophobic aromatic and one general hydrophobic feature. One hydrogen bond donor is in the bridge region. This space arrangement of the pharmacophore features completely satisfies the requirement of the kinase binding site (see Fig. 1). Further, a careful analysis of the Hypo1_II mapping with the test set compounds reveals that those pharmacophore features in the allosteric site and bridge region can be mapped very well with almost all the type II kinase inhibitors. However, the pharmacophore features in the ATP-binding region are not mapped well in some cases (see Fig. 8B). And those compounds with a bad mapping with the pharmacophore features in the ATP-binding region generally have a lower inhibitory potency. Whereas, the compounds with a very good mapping with the pharmacophore features in the ATP-binding region generally have a higher

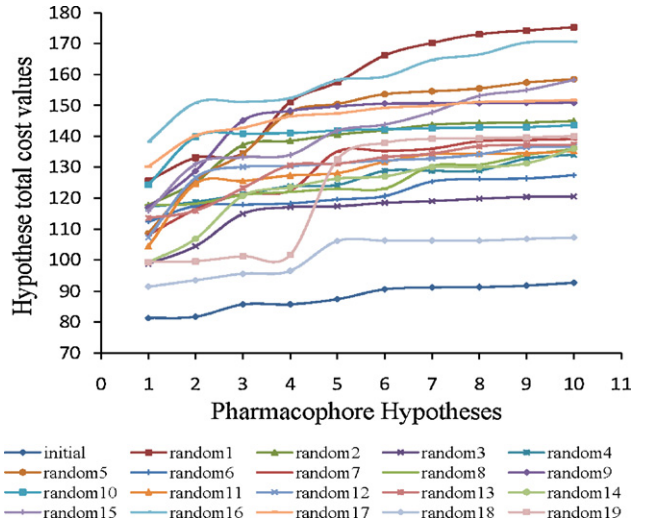

Fig. 7. The difference in total cost of hypotheses between the initial spreadsheet and 19 random spreadsheets after CatScramble run.

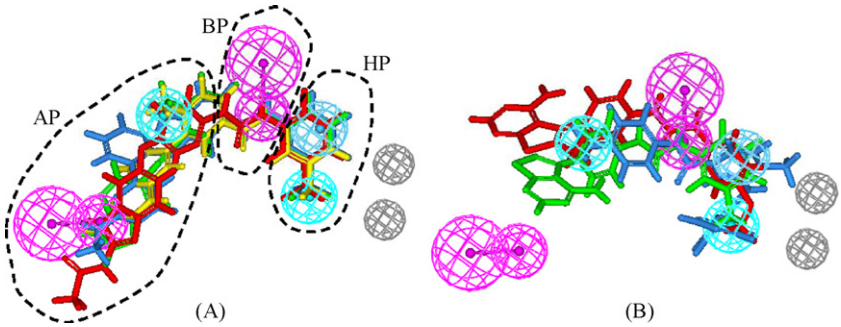

Fig. 8. (A) Hypo1_II mapped with highly active training set compounds B1 (blue), B2 (yellow), B3 (green), and B4 (red). (B) Hypo1_II mapped with low active training set compounds B15 (green), B16 (blue), and B19 (red). These chemical features are color coded with magenta, hydrogen-bond donor, cyan, general hydrophobic feature, light blue, hydrophobic aromatic feature, grey, excluded volume. AP, ATP-binging-region potion, HP, hydrophobic-region portion, BP, bridge porting linking AP and HP.

inhibitory potency (see Fig. 8A). These suggest that the AP part of the type II kinase inhibitors plays an important role to the bioactivity of the type II kinase inhibitors. And structural modifications on this part should be helpful to further improve the inhibitory potency of type II kinase inhibitors.

5. Conclusions

In this study, chemical feature based pharmacophore modeling of type I and type II kinase inhibitors of Tie2 have been carried out by using HipHop and HypoRefine modules within Catalyst program package. For type I kinase inhibitors, just qualitative pharmacophore modeling has been performed due to the lack of highly active compounds and limited structural diversity of the known type I inhibitors. The best hypothesis Hypo1_I contains one hydrogen-bond acceptor, one hydrogen-bond donor, one general hydrophobic, one hydrophobic aromatic, and one ring aromatic feature. For type II kinse inhibitors, a HipHop model was first created for the purpose of identifying the common pharmacophore features necessary for potent type II kinase inhibitors. Following the information provided by the HipHop model of type II kinase inhibitors, the quantitative pharmacophore modeling was carried out with the use of HypoRefine program. The best HypoRefine model, Hypo1_II, which was characterized by the highest cost difference (92.9119), the lowest RMSD (0.74204), and the best correlation coefficient (0.976032), consists of two hydrogen-bond donors, one hydrophobic aromatic, and two general hydrophobic features, as well as two excluded volumes. Both test set and cross validation methods have been used to validate the pharmacophore model, Hypo1_II. Results obtained by using the test set method show a fairly good correlation between the experimental and predicted IC_{50} values, indicating a good predictive ability. The statistical confidence of Hypo1_II has also been confirmed by using CatScramble program within CATALYST. Further, the space arrangement of the pharmacophore features in Hypo1_II is consistent with the locations of the three typical portions making up a type II kinase inhibitor, namely, the portion occupying the ATP binding region (ATP-binding-region portion, AP), that occupying the hydrophobic region (hydrophobic-region portion, HP), and that linking AP and HP (bridge portion, BP). Analysis of the pharmacophore model (Hypo1_II) mapped with the test set (TS_II) compounds reveals that the ATP-binding-region portion of the type II kinase inhibitors plays an important role to the bioactivity of the type II kinase inhibitors. Structural modifications on this portion should be helpful to further improve the inhibitory potency of type II kinase inhibitors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2008.11.008.

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