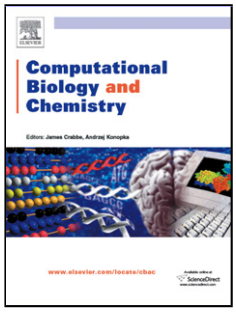

Title: Virtual Screening of B-Raf Kinase Inhibitors: A Combination of Pharmacophore Modelling, Molecular Docking, 3D-QSAR Model and Binding Free Energy Calculation Studies

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Virtual Screening of B-Raf Kinase Inhibitors: A Combination of Pharmacophore Modelling, Molecular Docking, 3D-QSAR Model and Binding Free Energy Calculation Studies

Wen Zhang[a] , Kai-Xiong Qiu[a] , Fang Yu[a] , Xiao-Guang Xie[b] , Shu-Qun Zhang[c] , Ya-Juan Chen[a,*] , Hui-Ding Xie[a,*]

[a] Department of Medicinal Chemistry, School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming, Yunnan 650500, PR China

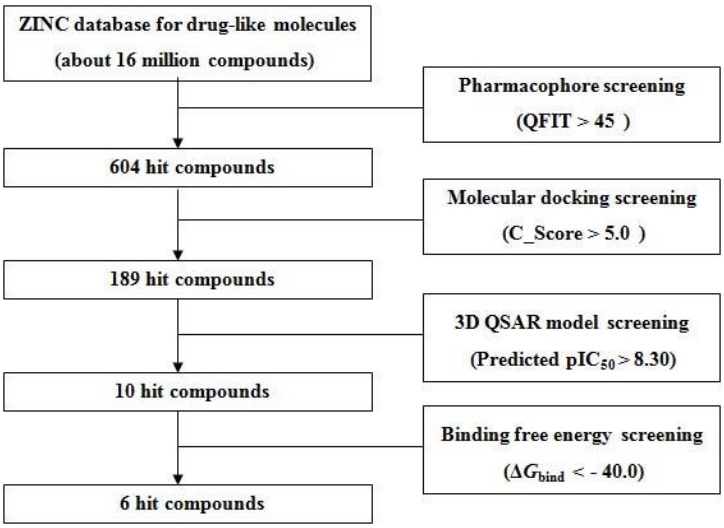
[b] Department of Chemistry, Yunnan University, Kunming, Yunnan 650091, PR China

[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, Yunnan 650201, PR China

*** Corresponding author.**

E-mail addresses: cherish_719@sina.com (W. Zhang), chenneyao16@hotmail.com (K.-X. Qiu), yufang519@163.com (F. Yu), xgxie@ynu.edu.cn (X.-G. Xie), zhangshuqun@mail.kib.ac.cn (S.-Q. Zhang), Chenyajuan0873@163.com (Y.-J. Chen), front701228.student@sina.com (H.-D. Xie)

Graphical Abstract



Highlights:

- A combined virtual screening of B-Raf inhibitors was performed.
- Five of six hit compounds show good biological activities (IC_{50} < 50μM).
- Five structurally diverse compounds are expected to develop novel B-Raf inhibitors.

Abstract

B-Raf kinase has been identified as an important target in recent cancer treatment. In order to discover structurally diverse and novel B-Raf inhibitors (BRIs), a virtual screening of BRIs against ZINC database was performed by using a combination of pharmacophore modelling, molecular docking, 3D-QSAR model and binding free energy (ΔG_{bind}) calculation studies in this work. After the virtual screening, six promising hit compounds were obtained, which were then tested for inhibitory activities of A375 cell lines. In the result, five hit compounds show good biological activities (IC_{50} < 50μM). The present method of virtual screening can be applied to find structurally diverse inhibitors, and the obtained five structurally diverse compounds are expected to develop novel BRIs.

Keywords

B-Raf kinase inhibitors; Virtual screening; Pharmacophore; Molecular docking; 3D QSAR; Binding free energy calculation

1. Introduction

MAPK pathway, which is also called Ras-Raf-MEK-ERK pathway, has been identified as a very important pathway for cell proliferation and survival because it can be activated easily in human cancers (up to 30%) (Wellbrock et al., 2004; Li et al., 2007). During the MAPK pathway, three isoforms exist for Raf kinase (A-Raf, B-Raf, and C-Raf), and B-Raf kinase has been considered as the most important activator

because the mutation of B-Raf kinase in human cancers is up to 7% (Mercer and Pritchar, 2003). In various human cancers, the mutation frequency of B-Raf kinase is different, such as melanoma (50%–70%), ovarian cancers (35%), thyroid cancers (30%), and colorectal cancers (10%) (Tuveson et al., 2003). Therefore, B-Raf kinase has been considered as an important target in recent cancer treatment (Garnett and Marais, 2004; Madhunapantula and Robertson, 2008).

There are two B-Raf kinase inhibitors (BRIs) approved by FDA (the Food and Drug Administration) and used in clinic. One is Sorafenib, which is used to treat renal cell carcinoma and hepatocellular carcinoma. Another is Vemurafenib, which is used to treat metastatic melanoma. Furthermore, some BRIs (RAF265, GSK2118436 and SB-590885) are in the clinical development. However, these inhibitors still have some major side effects and can develop drug resistance though they showed success of clinical efficiency in cancer treatments. Therefore, it is still necessary to find novel and potent BRIs (El-Nassan, 2014).

In order to discover new structurally diverse BRIs, a virtual screening of BRIs against ZINC database was performed by using a combination of pharmacophore modelling, molecular docking, 3D-QSAR model and binding free energy (ΔG_{bind}) calculation studies in the present work. Six promising hit compounds were obtained by the virtual screening, which were tested for inhibitory activities of A375 cell lines. As a result, five structurally diverse compounds show good inhibitory activities. We hope that the present method of virtual screening can be applied to discover structurally diverse inhibitors, and the obtained five compounds are expected to develop new potent BRIs.

2. Methodology

2.1 Pharmacophore hypothesis

The pharmacophore hypothesis was performed by using GALAHAD (Genetic Algorithm with Linear Assignment of Hypermolecular Alignment of Database)

module of SYBYL, which includes two main stages: firstly, the ligands are aligned to each other in internal coordinate space; secondly, the produced conformations are aligned in Cartesian space. The features, which were used to generate the pharmacophore model, include hydrogen bond acceptor atoms, hydrogen bond donor atoms and hydrophobic centers (Richmond et al., 2006; Shepphird and Clark, 2006; Andrade et al., 2008).

2.2 Molecular docking

The molecular docking process was performed by using the Surflex-Dock module of SYBYL. All the compounds were docked into the binding site of B-Raf kinase crystal structure (PDB code: 4MBJ) (Newhouse et al., 2013). Before the docking, all the water molecules were removed, the ligand was extracted, and hydrogen atoms were added to the receptor. During the docking, the protomol_threshold was set to 0.50 Å, the protomol_bloat was set to 0, and other parameters were set to default values. In this study, each conformer of all compounds was docked into the binding site 10 times and the C_score values were used to evaluate the docking analysis. In the Surflex-Dock, the structure of the receptor is rigid, and the structures of ligands are flexible.

2.3 3D QSAR studies

CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis) are two important 3D QSAR methodologies, which were performed by using QSAR module of SYBYL. In CoMFA model (Crame et al., 1988), two fields were calcualted (steric field and electrostatic fields). Lennard-Jones potential was used to calculate steric fields while Coulombic potential was used to compute electrostatic fields. A sp^3 carbon probe atom as steric probe and a +1.0 charge as an electrostatic probe were taken to calculate the probe-ligand interaction energies. The cut-off for energies was set to ±30 kcal/mol. In CoMSIA model (Klebe et al.,

1994), five fields were computed (steric field, electrostatic field, hydrophobic field, hydrogen bond donor field and hydrogen bond acceptor field). A probe of charge +1, a radius of 1, hydrophobicity and hydrogen bonding properties of +1 were used to calculate the five fields, and an attenuation factor was set to 0.3 for the Gaussian distance-dependent function.

2.4 MD Simulations

All the MD (molecular dynamic) simulations were carried out by using AMBER 12 software package (Case et al., 2012). The initial structures of all the complexes for the MD simulations were obtained from the docking results. The GAFF (general AMBER force field) was taken for ligands, and AM1-BCC method was applied to assign their partial charges (Jakalian et al., 2000). During the MD simulations, the whole system was dipped into a water box of TIP3P with a margin distance of 10 Å (Jorgensen et al., 1983), the PME (particle mesh Ewald) was adopted, and the cut-off distance of non-bonded interactions was set to 10 Å. The bonds involving hydrogen were constrained by the SHAKE algorithm (Ryckaert et al., 1977).

2.5 Binding Free Energy Calculation

The binding free energy (ΔG_{bind}) calculation of each complex was performed by using MM-GBSA (Molecular Mechanics Generalized Born Surface Area) method in AMBER 12 (Miller et al., 2012). In MM-GBSA, all the 100 snapshots of the MD simulated structure within the last 1 ns trajectory were extracted to perform ΔG_{bind} calculation, which was calculated as follows:

$$\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{receptor} + \Delta G_{ligand}) \quad (1)$$

where $\Delta G_{complex}$, $\Delta G_{receptor}$ and ΔG_{ligand} are the free energy of the complex, receptor, and ligand, respectively. The free energy (ΔG) can be obtained by the following equations:

$$\Delta G = \Delta E_{gas} + \Delta G_{sol} - T\Delta S_{gas} \quad (1)$$

$$\Delta E_{gas} = \Delta E_{ele} + \Delta E_{vdw} \quad (2)$$

$$\Delta G_{sol} = \Delta G_{GB} + \Delta G_{SA} \quad (3)$$

Where ΔE_{gas} (molecular mechanics energy in the gas phase) is composed of ΔE_{ele} (electrostatic interactions) and ΔE_{vdw} (van der Waals interactions), ΔG_{sol} (solvation free energy) consists of ΔG_{GB} (polar contribution) and ΔG_{SA} (non-polar contribution), and $T\Delta S_{gas}$ represents entropy terms.

2.6 MTT Assay

A375 cells, grown in tissue culture flasks and maintained in 5% CO_2 at 37 ℃, were used for cell culture experiments. The cells were plated onto the 96-well plates at a density 6,500 cells/well and cultured for 24 h. Then each well was added in 10 μL solution of the hit compound (obtained from the virtual screening) at different concentration (1, 10, 100μM), which were incubated for 48 h. Then each well was added in 20 μL MTT solution (0.5 mg/ml) and incubated for 4 h. After removing the upper solution, each well was added in 100 μL solvent (0.2% DMSO/chloroform), which OD (optical density) was determined at 570 nm. The inhibitions against A375 cell line were calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{OD (control group)} - \text{OD (dosing group)}}{\text{OD (control group)}} \text{ x } 100\%$$

The IC_{50} values (concentration of the inhibitor which inhibits 50% cellular growth) were calculated by using LOGIT method.

3. Results and discussion

3.1 Virtual screening

The sequential flowchart of virtual screening we performed in the present work is depicted in Fig.1, which contains pharmacophore screening, molecular docking screening, 3D-QSAR model screening and binding free energy screening.

The first step of the virtual screening is pharmacophore screening, which can increase the speed of the screening greatly. In our previous work (Xie et al., 2015), we performed pharmacophore generation based on a series of imidazopyridine BRIs by using GALAHAD method. The obtained best GALAHAD model (model_06) contains three hydrophobes, three hydrogen donor atoms and two acceptor atoms (Xie et al., 2015). The model_06 was converted into an UNITY query, which was screened against ZINC database for drug-like molecules (about 16 million compounds) (Irwin and Shoichet, 2005). During the pharmacophore screening, Lipinski's rule of five and Van der Waals bumps were used to reduce the dataset (Kothandan et al., 2013), the "3D Search" option was applied, and the QFIT (pharmacophoric match between query and the hit compound) values of hit compounds were set to more than 45 (QFIT > 45) (Xie et al., 2015). After the first step of the virtual screening, 604 hit compounds were obtained.

The second step of the virtual screening is molecular docking screening. The 604 hit compounds obtained from the pharmacophore screening were further docked into the binding site of B-Raf kinase receptor by using the Sulflex-Dock, and the C_score values of the molecular docking were set to more than 5.0 (C_score > 5.0) (Jain, 2003; Xie et al., 2015). As a result, 189 hit compounds were selected after the molecular docking screening.

The third step of the virtual screening is 3D QSAR model screening. In order to obtain the best 3D QSAR model, in our previous work (Xie et al., 2015), CoMFA and CoMSIA were performed on a series of imidazopyridine BRIs to build 3D QSAR models based on both pharmacophore and docking alignments. Finally, the CoMSIA model based on the pharmacophore alignment shows the best result (q^2= 0.621, r^2_{ncv}=0.996, r^2_{pred}=0.885), which was used to predict the pIC_{50} values of the 189 hit compounds obtained from the molecular docking screening. As a result, 10 hit

compounds with the best predicted pIC_{50} values ($pIC_{50} > 8.300$) were selected.

The last step of the virtual screening is binding free energies (ΔG_{bind}) screening, which can increase the accuracy of screening effectively. Before the ΔG_{bind} calculations, MD simulations were performed in the constant temperature and pressure with a step of 2 fs for 10 ns. The ΔG_{bind} of the 10 complexes (combined by the B-Raf kinase and the hit compound obtained from the 3D QSAR model screening) were calculated by using MM-GBSA method (Hou, et al. 2011). As a result, 6 hit compounds with low binding free energy ($\Delta G_{bind} < -40.0$ kcal·mol^{-1}) were selected. Their ZINC numbers, chemical structures, QFIT values, C_score values, predicted pIC_{50} values and binding free energies (ΔG_{bind}) are listed in Table 1.

3.2 Biological Activity Evaluation

For melanoma cancer, the frequency of mutation in B-Raf kinase is up to 70% (Tuveson et al., 2003). Therefore, the inhibitory activities against the proliferation of A375 (Human malignant melanoma) cell lines of the hit compounds were tested to evaluate their biological activities. The six hit compounds obtained from the above virtual screening were evaluated for inhibitory activities against A375 cell line by using MTT assay. The result is listed in table 2. It can be seen that five of six hit compounds show quite good inhibitory activities and possess low IC_{50} values ($IC_{50} < 50$ μM), which indicates that the present virtual screening strategy is an effective method to discover structurally diverse inhibitors.

4. Conclusion

B-Raf kinase has been proven to be an important target in recent cancer treatment. In order to find novel B-Raf inhibitors (BRIs) with diverse structure, a virtual screening of BRIs against ZINC database was carried out by using a combination of pharmacophore modelling, molecular docking, 3D-QSAR model and binding free energy (ΔG_{bind}) calculation studies in our work. Six hit compounds were obtained after the virtual screening. Their biological activities were evaluated by testing the

inhibitory activities against the proliferation of A375 cell lines. As a result, five of six hit compounds show good biological activities (IC_{50} < 50 μM). We hope that the present method of virtual screening can be applied to discover structurally diverse inhibitors, and the obtained five compounds are expected to develop new potent BRIs.

Author Contributions

Wen Zhang, Kaixiong Qiu, Fang Yu, Shuqun Zhang and Yajuan Chen performed the experiments and data treatments. Writing was done by Huiding Xie, and Yajuan Chen. The management and submission tasks were done by Huiding Xie and Xiaoguang Xie.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

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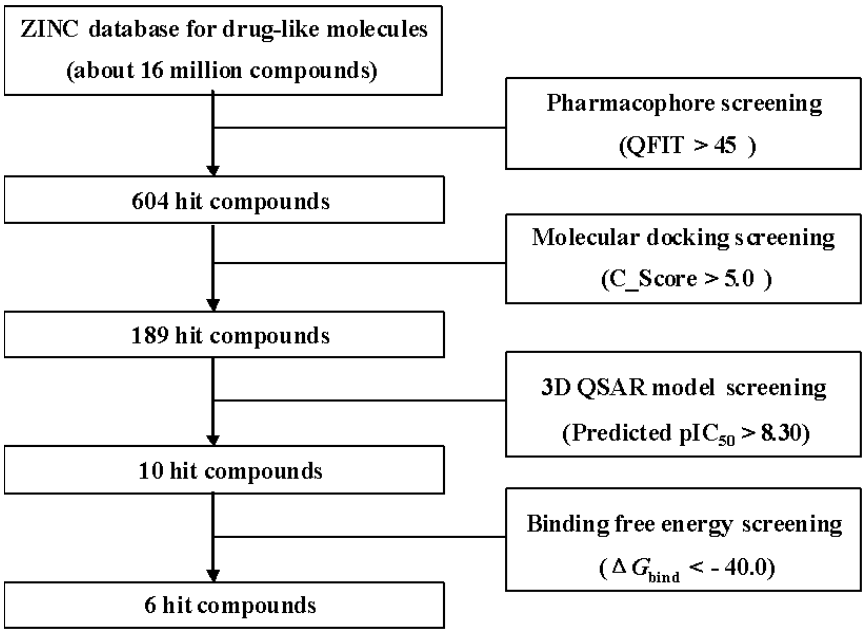


Fig.1 Virtual screening flowchart

Table 1 ZINC number, chemical structure, QFIT, C_score, predicted pIC_{50} and ΔG_{bind} of the 6 hit compounds.

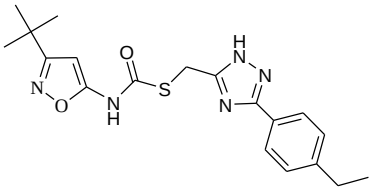
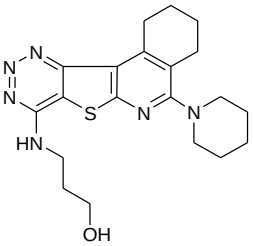
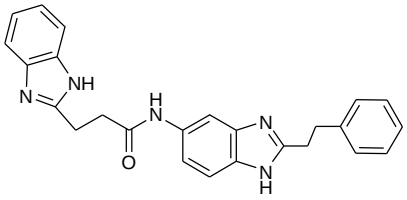
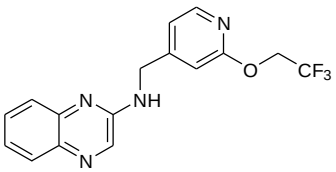
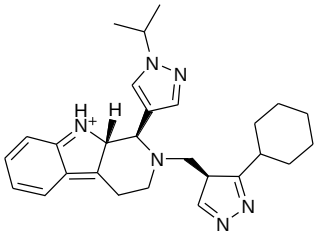
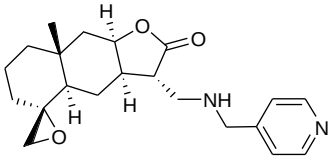
Hit compound	Structure	QFIT Value	C_Score Value	Pred. pIC_{50}	$\triangle G_{bind}$
ZINC05318650 (Hits_1)		58.66	6.11	9.575	-41.19
ZINC20677564 (Hits_2)		60.89	6.79	8.661	-45.99
ZINC15863901 (Hits_3)		46.65	8.57	8.639	-51.57
ZINC52105867 (Hits_4)		46.87	5.50	8.580	-40.64
ZINC64074547 (Hits_5)		47.74	5.02	8.438	-44.78
ZINC04000073 (Hits_6)		61.56	5.28	8.378	-43.74

Table 2 The inhibitory activities against A375 cell line of the six hit compounds.

Hit Compound	Inhibition (%)			IC_{50}(μM)
	at 1μM	at 10μM	at 100μM	
Hits_1	2.48	6.69	95.19	21.60
Hits_2	2.86	20.52	97.42	13.10
Hits_3	2.39	9.99	14.95	>100
Hits_4	3.46	15.16	93.91	17.90
Hits_5	3.63	12.47	96.92	15.05
Hits_6	7.04	11.64	76.81	40.05