The v600e mutation of b raf biology essay

Science, Biology

Yan Lia,*,†, Chunxiao Hana,†, Jinghui Wanga, Shuwei Zhanga, Guohui Lib,*, Ling Yangca Department of Materials Science and Chemical Engineering, Dalian University of Technology, Dalian, Liaoning, 116023, China. b Laboratory of Molecular Modeling and Design, State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China. c Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Graduate School of the Chinese Academy of Sciences, Dalian, Liaoning, 116023, China.† These authors contributed equally to this work.* Author to whom correspondence should be addressed; E-Mail: yanli@dlut. edu. cn; Tel.: +86-411-84986062; Fax: +86-411-84986063. ABSTRACT: The V600E mutation of B-Raf is closely related with various human cancers. Presently, both ligand-based and receptor-based three-dimensional quantitative structure-activity relationship (3D-QSAR) modelings were performed on the 107 pyrazolopyrimidine and pyrazolopyridine-based inhibitors of B-RafV600E kinase by using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The obtained best model with Q2 of 0. 504 and R2ncv of 0. 960 is statistically reliable to predict the inhibitors' activity, which is demonstrated by a test set with R2pred of 0. 872. Besides, 3D contour maps, molecular docking and molecular dynamics (MD) were also carried out and the results correlate well with each other. Finally, we obtained five conclusions which can be a guidance to the development of novel B-RafV600E kinase inhibitors. Keywords: B-RafV600E kinase; pyrazolopyrimidine and pyrazolopyridine-based inhibitors; 3D-QSAR; docking; MD

1. Introduction

Rapidly Accelerated Fibrosarcoma, short for Raf, is the receptor tyrosine kinase effector which has serine/threonine kinase activities [1, 2, 3, 4]. It makes a central contribution to the RAS-RAF-MEK-ERK (MAPK) pathway which transduces signals from membrane-based receptors to the nucleus to mediate cell proliferation, differentiation and survival [5, 6]. Numerous cancers are related to the constitutive activation of the above signaling pathway [7, 8]. B-Raf is one of the isoforms of the Raf kinase family and plays a primary role in the activation of MEK [9, 10]. In about 7% of human cancers, the mutation of B-Raf has been detected, which most remarkably associates with the melanoma cancer (50%-70%) compared with ovarian cancer (~35%), thyroid cancer (~30%) and colorectal cancer (~10%) [11, 12]. Among all the mutations, a valine acid substituted by a glutamic acid at residue 600 is found in more than 90% of the mutated B-Raf kinases, resulting in a 500 times higher in B-RafV600E kinase activity than B-RafWT in vivo [11, 12, 13]. Consequently, it is of great importance to develop efficient inhibitors of the B-RafV600E kinase acted as a therapeutic target of human cancers [12, 13, 14, 15, 16]. According to the different conformations of the interaction between B-Raf kinase and its inhibitors, the inhibitors can be primarily categorized into two types: Type I targets the ATP binding site in the kinase's active conformation while type II occupies another binding site close to the ATP region in the inactive conformation [17, 18]. The former type kinase inhibitors bind in and around the adenine region which is occupied by the adenine of ATP and don't need a " DFG-out" conformation during the binding process [18]. The latter type kinase inhibitors occupy the

allosteric site which is a hydrophobic site adjacent to the ATP region created by the DFG-out conformation of the activation loop [18, 19]. Sorafenib is the first approved B-Raf kinase inhibitor by the European Medicines Agency and the Food and Drug Administration (FDA) which belongs to type II [10, 20]. Although Sorafenib has shown potent inhibition to the B-Raf kinase, it is not selective with inhibitory activity against other kinases including VEGFR2, VEGFR3, FLT-3, PDGFR and c-KIT [21, 22, 23, 24]. What's more, it fails to inhibit the mutation of B-Raf kinase in advance melanoma patients because of the uncorrelation between the B-Raf mutation status and the clinical response to sorafenib [21, 25, 26, 27]. PLX4032, another B-Raf kinase inhibitor belonging to type I, has just been approved by FDA in August 2011 [21]. In order to get novel potent B-Raf kinase inhibitors, some studies have been focused on the approved PLX4032. Recently, on the basis of the structure of PLX4032, Steve Wenglowsky et al. reported a series of pyrazolopyrimidine and pyrazolopyridine derivatives inhibiting B-RafV600E kinase that belong to type I [6, 28, 29, 30]. The in vivo efficacy and preliminary safety profiles of some compounds are so good that they were selected for the preclinical test [6]. Considering the time-consuming process of the conventional drug discovery followed by a high investment, it is very useful and important to introduce computer-aided drug design (CADD) approaches to accelerate the process [12, 31]. Based on computational chemistry, CADD is applied to provide estimate properties of the drug candidates which will influence their binding affinity and design new drugs [32]. In the last few years, some relative CADD research studies have already been carried out to develop B-Raf kinase inhibitors. In 2008, Filip

Fratev et al. studied a series of disubstituted pyrazine scaffold inhibitors to analyze the conformational stability of the unbounded B-Raf kinases and disclosed a unique salt bridge network in these kinases [8]. In 2010, Jae Yoon Chung et al. performed research on 37 pyrazole-based derivatives targeting B-Raf kinase to identify the relationship between inhibitory activity and receptor-ligand interactions [33]. Furthermore, Yong Ai et al. investigated a series of pyridopyrazinones served as potent inhibitors to B-Raf kinase to reveal the structural requirements for the inhibitory activity and to explore the binding mode between the kinase and the ligand [34]. In 2011, Ying Yang et al. studied both the key structural requirements and the binding mode between the pyridoimidazolone derivatives and B-Raf kinase [17]. Moreover, Julio Caballero et al. used in silico methods to explain the differences in B-Raf inhibitory activities when pyrazole derivatives were substituted by various hydroxy substituted cycloalkyl groups at N1 position [35]. Despite the above CADD studies on B-Raf kinase inhibitors, few people tries to focus on the in silico research of the pyrazolopyrimidine and pyrazolopyridine-based inhibitors of B-RafV600E kinase. Thus in the present work, a series of the pyrazolopyrimidine and pyrazolopyridine-based compounds were taken to build 3D-QSAR models by using the comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) approaches [36, 37, 38]. The contour maps obtained from the optimal model can disclose the relationship between the key structural requirements and the biological activity of the inhibitor [39]. Moreover, a test set of 25 free compounds was employed to demonstrate the predictive ability of the model from the external. In addition, an in silico method integrating molecular

docking together with MD was also carried out in order to better understand the potential binding modes of these B-RafV600E inhibitors. The developed results will be helpful to modify and design novel B-RafV600E inhibitors possessing a high potency.

2. Materials and methods

2. 1. Dataset and biological activityDiscarding those compounds with unspecified activity, a total of 107 pyrazolopyrimidine and pyrazolopyridine-based derivatives [6, 28, 29, 30] were adopted as a dataset to establish models. All the compounds with inhibitory potency tested on human B-Raf kinase in vitro were obtained from the same research team, and their half maximal inhibitory concentration(IC50) values were converted into corresponding pIC50 (-logIC50) values (ranging from 5. 41 to 9. 70) which were used as dependent variables in the subsequent QSAR studies. The 10 representative compounds with structures and IC50 values are shown in Table 1. In order to validate the accuracy of the established QSAR models, the compounds were divided into training (82 molecules) and test (25 molecules) sets in approximately a ratio of 4: 1, separately. For the selection of the test set, the diversified structure of the compounds and their averagely distributed activity values in the whole dataset should be considered as two crucial principles. Tables S1-S5 (supporting information) show the structures and the IC50 values of all the chemicals.

[Table 1]

2. 2. Molecular modeling and alignmentIn order to build the most accurately predictive 3D-QSAR model, two totally different alignment methods were

performed on the 107 inhibitors. In the ligand-based alignment instantly the first method, compound 44 which possesses the highest biological activity was selected as the template to fit the common substructure (Fig. 1A, shown in blue bold) of the remaining compounds. In the receptor-based alignment instantly the second method, the various conformations of the 107 compounds were firstly got from molecular docking and then, were subjected to the process of the ligand-based alignment method. Both of the two models' alignment results are shown in Fig. 1B and 1C.

[Fig. 1]

Presently, all the three-dimensional structure's building and molecular modeling studies were carried out with SYBYL 6. 9 package (Tripos Associates, St. Louis, MO, U. S.). Besides, partial atomic charges were figured out by the Gasteiger-Huckel method [40] while energy minimizations were calculated by using tripos force field [41]. Furthermore, the Powell conjugate gradient method with convergence criterion set as 0. 05 kcal/mol Å was adopted. 2. 3. CoMFA and CoMSIA calculationsIn order to predict the relationship between the structural requirements and biological activity of these compounds [42], both CoMFA and CoMSIA techniques were applied to build 3D-QSAR models. In CoMFA calculations, the aligned compounds were placed in a 3D grid box of 2 Å in the x, y and z directions. Meanwhile, an sp3 carbon probe atom with a charge of +1. 0 was used to calculate the steric and electrostatic field energies at various lattice points. To improve the ratio of signal to noise [43], the default energy cut-off value was set to 30 kcal/mol. As a result, any grid points whose energy variation was under this threshold would be replaced by 30 kcal/mol. In CoMSIA calculations, apart

from the steric and electrostatic descriptors, hydrophobic, hydrogen-bond donor and hydrogen-bond acceptor fields were added to stress the relevance between the important structural features of the aligned compounds and their biological activities [44]. Similar to the CoMFA process, the 3D lattice box with a grid spacing of 2 Å and the probe atom Csp3+ with a +1. 0 charge were employed. But a different shape of the Gaussian function was applied to calculate the distance from the probe atom to each molecule atom. CoMSIA similarity indices (AF) for a molecule j with atom i at a grid point q are illustrated by the same equation in our previous work [45]. 2. 4. PLS analysis and validationsTo derive the 3D-QSAR models, partial least square (PLS) linear regression was adopted to correlate the pIC50 values (the dependent variables) with the CoMFA or CoMSIA values (the independent variables). Firstly, the predictive value of each compound in the models was calculated by the leave-one-out (LOO) methodology. The cross-validated coefficient Q2 is calculated by Eq. (1) :(1)where the Yp, Yo and Ym are predicted, observed and mean values of the pIC50, respectively for the training set [44]. Secondly, the best value for the components field obtained from the first stage with highest Q2 and lowest standard error of prediction (SEP) was used to perform the no validation stage. This process is evaluated by the Pearson coefficient (R2ncv), standard error of estimate (SEE) and F values. Finally, the test set was employed to assess the models' ability to predict the biological activity of these compounds. Our previous work describes the equation used to calculate the predictive R2pred [45]. 2. 5. Molecular dockingIn order to both reveal the binding interactions and identify the appropriate conformations between B-Raf kinase and its

pyrazolopyrimidine and pyrazolopyridine-based inhibitors, molecular docking was implemented by using the Surflex-dock module in Sybyl-X 1. 1. In our present work, the X-ray crystal structure (PDB ID: 3TV6) [6] of human B-Raf kinase with a high resolution of 3. 30 Å was obtained from the Protein Data Bank (http://www. rcsb. org/pdb/home/home. do) [46]. Initially, the hetero atoms such as the ligand were eliminated from the protein while polar hydrogens were added. Then, the protomol, into which the ligands are placed by calculating the potentially typical binding site, was automatically generated by adjusting two significant parameters, namely protomol_bloat and protomol_threshold. The former affects the volume while the latter indicates how much the protomol can be buried in the protein. All the other parameters maintained the default values in the software. Finally, all the 107 inhibitors were docked into the binding pocket during which each compound got 20 possible conformations with different docking scores. 2. 6. Molecular dynamics simulationsBased on the docked conformation of compound 44, AMBER 10 package [47] was adopted to perform the MD simulations to get a reasonable structure. The ligand parameters and charges were set by using the general atom force field (GAFF) [48] and the AMI-BCC method [49] while the protein parameters were depicted by the standard AMBER force field for bioorganic systems (ff99SB) [50]. Besides, the initial structure was put into a rectangular box full of TIP3P water that extends at least 10 Å from the solute to each face of the box and was neutralized by the sufficient chloridion ions. The process of the simulation is depicted as follows: Firstly, the full system was gradually energy minimized by 2500 steepest descent steps with following 2500 conjugate-gradient steps. Secondly, when the temperature

and pressure of the system respectively rose to 300 K and 1 atm at a constant force of 2. 0 kcal/mol Å-2, a 50 ps pressure-constant period and a 500 ps equilibration were carried out in the NPT ensemble. Finally, the production phase was run for 5 ns with a 2 fs time step under the periodic boundary conditions.

3. Results and discussion

3. 1. 3D-QSAR statistical resultsTo further study what kind of structural alignment of the compounds was better in helping build a successful 3D-QSAR model, two alignment methods, namely the ligand-based alignment and the receptor-based alignment were attempted in this study. The statistical parameters of the obtained models using the same training set are listed in Table 2. Due to the normal criterion that a Q2 > 0. 5 is considered to be statistically important to prove the high predictive ability of a 3D-QSAR model [51], the optimal receptor-based models failed completely with Q2 of 0. 175 and 0. 198 respectively obtained for CoMFA and CoMSIA modeling. So was the optimal ligand-based CoMFA model whose Q2 is only as 0. 393. As a result, the analysis of the ligand-based CoMSIA model was focused on from now on.

[Table 2]

In CoMSIA modeling, to avoid the risk of leaving out some optimal ones [52], 31 models got from different combinations of the five field descriptors using the same data set were tried. Eventually, the best CoMSIA model was established by using the steric, the electrostatic and hydrophobic fields. It gives a Q2 value of 0. 504 with 10 components calculated from the PLS

analysis, as well as a SEE of 0. 190 and an F values equaling to 172. 555. All these significant statistical parameters indicate a great internal predictive ability of the model. Meanwhile, a high R2ncv of 0. 960 reveals its self-consistency as well. As to the field contribution, the steric field occupies 0. 279 while the electrostatic field accounts for 0. 253, indicating an almost equal influence on affecting the biological activity of these inhibitors. Besides, the proportion of the hydrophobic field is 0. 467 which is much bigger than the other two fields, suggesting its key role in building the CoMSIA model. Apart from the internal prediction, an external examination of the predictive power of the model was further carried out using a test set of 25 compounds. A high predictive coefficient R2pred of 0. 872 was achieved, validating the efficacy of the model. Fig. 2 shows the correlation plot of the observed versus the predicted pIC50 values for the training (filled blue rhombus) and test (filled pink circle) molecules. Clearly, the whole points are uniformly distributed above and below the regression line while the predicted pIC50 values are closer to the observed ones. Both of them illustrate the reliability of the model to predict the variation between the chemical structure modification and its biological activity.

[Fig. 2]

3. 2. 3D-QSAR contour mapsTo predict how the significant regions affect the biological activity of these inhibitors with some variations in the steric, electrostatic and hydrophobic fields, the coefficient contour maps of the best ligand-based CoMSIA model were generated. They were calculated using the fields standard deviation at each grid point and the coefficient from the PLS analysis (StDev*Coeff) [53]. To help in visualization, the most potent

compound 44 is selected as the representative ligand in the contour maps (Fig. 3).

[Fig. 3]

In the steric field contour map (Fig. 3A), green (sterically favorable) and yellow (sterically unfavorable) contour maps account for 85% and 15% contributions, respectively. A large green polyhedron close to position 10 shows that a bulky substituent is favorable to the inhibitory activities. This is demonstrated by the fact that compound 85 has a higher activity than any other compounds with relatively small substituents, including compounds 82, 83 and 84. Another large green polyhedron near position 3 elucidates the same effect of bulky groups to the biological activity. This consists with the fact that compound 50 is more potent than compound 34. In addition, a medium-sized green polyhedron located around position 23 indicates that the inhibitory activities benefit from bulky groups. The evidence can be found by comparing compound 38 (position 23 = -F) and compound 37 (with no substituent at position 23). A large yellow polyhedron near position 19 suggests that too large substituents are disfavorable to the biological activity. For instance, the addition of heterocycle at position 19 like compounds 43, 41 and 29 decreases the activity when compared to compound 1 with a long chain group at the same position. Besides, the existed yellow block at position 5 leads to the downgrade biological activity of compound 86 in comparison with compound 84. There is an identical effect with the medium-sized yellow block observed near position 11 to the inhibitory activities. This is validated by the fact that compound 107 with a bulkier group exhibits lower activity than compound 1. In the electrostatic

contour map (Fig. 3B), blue (electropositive groups favorable) and red (electronegative groups favorable) contour maps represent 85% and 15% contributions, respectively. A blue polyhedron observed partially encompassing position 20 indicates that the substituents should be electropositive to enhance the inhibitory activities. This is exemplified by compound 36 with a higher activity substituted by a phenyl when compared with compound 37 substituted by 2-F-phenyl. It is also the same with the blue block in the vicinity between positions 8 and 10. Additionally, the contour plot of the blue polyhedron close to the phenyl ring C suggests a favor for positive group around this region. This may explain why compound 12 is more active than compound 16. A red polyhedron near position 11 represents an area where high electron density is favorable for the active site, such as the order of biological activity for the following compounds: 107 > 84 > 83 > 82. Comparison of the activities of 32 vs 78, 33 vs 79, 34 vs 80, and 35 vs 81 validates that a negative substituent (N) is favored at position 2 where neighbored by a red contour plot. A large and a subtle red polyhedrons shown in Fig. 3B are a little far away from the template compound, which will be discussed in section 3. 3. Fig. 3C shows the hydrophobic contour map, where the yellow (hydrophobic favorable) and white (hydrophilic favorable) contour maps account for 85% and 15% contributions, respectively. Two medium-sized yellow polyhedrons around the phenyl ring C suggest that hydrophobic substituents like -F, -Cl are beneficial to the improvement of inhibitory activity. This is well illustrated by the order of activity for these compounds: 14 > 13 > 12. Also in the case that compound 9 is more potent than compound 11. Both a large and a small

white polyhedrons are observed close to ring D showing that the addition of a hydrophilic group is favored in this region. Compound 24 with a higher activity than compound 40 validates the fact. The effect of another white block besides position 11 can be demonstrated by the decrease in potency of compound 107 compared with compound 57. A medium-sized yellow polyhedron and a small white polyhedron will be analyzed in section 3. 3 due to their far distance to the representative compound. 3. 3. Docking resultsFig. 4 shows the binding pocket we built. Again, the highest docking score's conformation of compound 44 is shown superimposed as a template to aid in visualization. Six hydrogen bonds were generated to fasten the ligand in the binding site (Fig. 4A). The oxygen atom of the carbonyl from Gln530 acts as a H-bond acceptor to produce a hydrogen bond with the NH group at position 1 (-O... HN, 2. 74 Å, 140. 7°). The N atoms at positions 2 and 6, serving as hydrogen bond acceptors, form H-bonds with the NH of Cys532 (-N... HN, 2. 97 Å, 159. 3°)and OH of Thr529 (-N... HO, 3. 03 Å, 136. 9°), respectively. The oxygen atom of the carbonyl at position 11 also forms a H-bond with the backbone NH of Lys483 (-O... HN, 2. 87 Å, 143. 4°). Besides, one of the sulfonyl oxygens at position 19 accepts a H-bond from the side chain NH of Asp594 (-O... HN, 2. 17 Å, 153. 9°) while the other oxygen at the same position forms a H-bond with the backbone NH of Gly596 (-O... HN, 2. 99 Å, 146. 5°).

[Fig. 4]

As seen from Fig. 4, the binding pocket is composed of 24 amino acid residues, including Ile463, Val471, Ala481, Val482, Lys483, Glu501, Leu505, Thr508, Ile513, Leu514, Leu515, Phe516, Ile527, Val528, Thr529, Gln530,

Trp531, Cys532, Phe583, Gly593, Asp594, Phe595, Gly596, Leu597. This finding corresponds well to the previous hydrophobic contour maps. The medium-sized yellow contour (Fig. 3C) far away from the template is probably generated by hydrophobic residue Ile463. So is the subtle white contour due to hydrophilic residue Thr529. Some hydrophobic residues are observed around the phenyl ring C including the Val471, Ala481 and Phe595. It can be well validated by the previous yellow contour results. The presence of hydrophilic residue Asp594 located close to position 11 while Thr508, Thr529 and Gly593 around ring D correlate well with the white blocks in these areas, indicating that the inhibitor will be more potent with a hydrophilic substituent here. There are rare amino acid residues besides positions 3 and 10 suggesting that large substituents are beneficial to these regions. This is in agreement with two large green polyhedrons of the previous CoMSIA steric contour map (Fig. 3A). In addition, there is sufficient room from position 23 to the edge of the docking pocket, also indicating the positive effect of bulky groups to the inhibitory activity. However, too large substituents would probably bring about steric clash with amino acid residues Leu515 and Phe516, which can be demonstrated by the presence of the medium-sized green contour map nearby (Fig. 3A). The existence of amino acid residues Val471 and Lys483 close to position 11 may also result in the same steric clash when introduced in a larger substituent at this position, consisting with the presence of a yellow contour near position 11 (Fig. 3A). Obviously, it is observed that a great number of amino acid residues located around positions 5 and 19 lead to no vacant space. As a result, a smaller substitutional group is favorable to the improvement of

biological activity, corresponding to the yellow contours appearing close to positions 5 and 19 in Fig. 3A. The large red contour (Fig. 3B) observed a little far away from the template compound is potentially related to Asp594 nearby shown in Fig. 4A. So is another subtle red contour connected with Glu501. These analyses explain why the introduction of electronegative groups in regions encompassed by red contours has nothing to do with the improvement of the inhibitory activity. In conclusions, the above docking analyses are in well consistence with the previous 3D-QSAR contour maps, suggesting the reliability and reasonableness of the optimal CoMSIA model. 3. 4. Molecular dynamics analysisIn order to further investigate the conformation of the ligand binding to the active site of the receptor, a 5 ns molecular dynamics simulation was carried out on the basis of the docked complex including 3TV6 B-Raf kinase and the most potent compound 44. Fig. 5A shows the root-mean-square deviation (RMSD) of the trajectory that depicts the initial structure ranging from 0. 68 to 1. 78 Å. It is obvious that the RMSD of the complex reaches around 1. 39 Å and sustains the value throughout the whole simulation process after 4. 2 ns, indicating the metastable conformation for the structure of the docked complex. Besides, Fig. 5B depicts a superposition between the average structure for the last 1 ns and the original docked structure. Both of them locate in the same binding site without any significant difference between the docked structure and the average structure, which verifies the rationality of the docking model. The only difference is that only two hydrogen bonds (the H-bonds at positions 1 and 2) formed between the ligand and the amino acid residues are left after MD simulation, which might result in the deviation of ring D.

This can be explained by the fact that the protein is rigid in the docking method while it is flexible in the MD simulation, much closer to physiological environment.

[Fig. 5]

Conclusions

For the first time, we tries to integrate 3D-QSAR, molecular docking and molecular dynamics together into the research of the pyrazolopyrimidine- and pyrazolopyridine-based inhibitors of B-RafV600E kinase. Based on the 107 pyrazolopyrimidine and pyrazolopyridine derivatives, CoMFA and CoMSIA methods have been adopted to build the ligand-based and receptor-based 3D-QSAR models. The obtained best ligand-based CoMSIA model possesses a high Q2, R2ncv and R2pred, indicating the statistically predictable ability of this model. Besides, a good consistency among the optimal 3D-QSAR model, the docking analysis and MD simulation validates the robustness of the model. Furthermore, the contour maps of the optimal 3D-QSAR model disclose the relationship between the structural requirements and inhibitory activity. In summary, our conclusions are described as follows with compound 44 as a reference:(1) Bulky substituents at position-3, -10 and -23 can improve the inhibitory activity but impair the activity at position-5, -11 and -19.(2) Electropositive groups at position-8, -10, -20 and ring C are helpful to enhance the potency of B-RafV600E inhibitors while electronegative groups at position-2 and -11 have the same effect.(3) Hydrophobic substituents at ring C are beneficial to improve the biological activity while hydrophilic substituents at position-11 and ring D are good for the activity.(4) The binding pocket between the ligand and the receptor is

composed by the Ile463, Val471, Ala481, Val482, Lys483, Glu501, Leu505, Thr508, Ile513, Leu514, Leu515, Phe516, Ile527, Val528, Thr529, Gln530, Trp531, Cys532, Phe583, Gly593, Asp594, Phe595, Gly596, Leu597.(5) One H-bond is formed between the NH group at position-1 and the oxygen atom of the carbonyl from Gln530, the other is produced by the N atom at position-2 with the NH group from Cys532. All in all, the studies obtained from the above 3D-QSAR, molecular docking and molecular dynamics may provide useful clues to help modify and design novel B-RafV600E inhibitors.

Acknowledgements

The research is supported by the high-performance computing platform of Northwest A&F University, with financial support given by the National Natural Science Foundation of China (Grant No. 11201049), the National High Technology Research and Development Program (" 863") of China (No. 2009AA02Z205). GH Li also appreciates the supports from the National Natural Science Foundation of China (31070641), the National Key Basic Research Development Program (2012CB721000) and " Hundreds Talents Program" of the Chinese Academy of Sciences.