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Protein-based Alignment in 3D-QSAR of Polo-Like Kinase 4 Inhibitors

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Abstract. Polo-like kinase 4 (PLK4), a mitotic kinase was regarded as a potential target for cancer therapy. Three-dimensional quantitative structure–activity relationship (3D QSAR) studies were performed on PLK4 inhibitors, based on molecular docking obtained by using GOLD and comparative molecular field analysis (CoMFA). The high leave one out (LOO) cross-validated correlation coefficient (q^2 = 0.531) reveals that the model is a useful tool for the prediction of test set as well as newly designed structures against PLK4 activity. The superimposed CoMFA models on the receptor site of PLK4 are guiding the design of potential inhibitory structures directed against PLK4 activity.

Introduction

The Polo-like kinase (PLK) family of serine/threonine kinases has an important role in regulating mitosis [1]. There are five members known to the researchers. Among PLK1, 2, 3, 4 and 5, PLK1 is the most studied one. Several PLK1 inhibitors have been taken into the clinic [2]. However, PLK4 enzyme received little attention. It has been shown that treating breast cancer cells with PLK4 RNAi stops centriole duplication and leads to cell death and furthermore normal breast cells are not affected. These findings proved PLK4 as a target for anticancer research [3].

In recent years, a number of Polo-like kinase(PLK) inhibitors have appeared in the patent and primary literatures. Among them, Peter B. Sampson, Henry W. Pauls and his colleagues synthesized a series of (1R,2S)-2-(1H-indazol-6-yl)spiro[cyclopropane-1,3’-indolin]-2’-one based skeleton derivatives as potent(Fig. 1), selective PLK4 inhibitors. Forthmore, An X-ray crystal structure of a similar analogue inhibitor in complex with PLK4 have been determined which provide important information about the interaction with the residues of the binding site [4, 5].

Recently many studies have been published in which the combination of receptor based methods and 3D QSAR. The three-dimensional structure of a target protein, along with a

docking protocol is used to guide alignment selection for comparative molecular field analysis. It is quite appealing to combine the accuracy of a receptor-based alignment with the computational efficiency of a ligand-based method. Receptor structures either experimentally resolved or obtained by homology modeling, can provide important information that is critical for an alignment in CoMFA, while QSAR can provide better prediction of binding energies.

In this study, we applied this receptor-based technique to a set of 78 PLK4 inhibitors which have been recently developed [4, 5]. In order to fast recognize the inhibitor that we discussed, the numbers of the inhibitors from the original papers have unchanged in current study. The crystal structure of the catalytic domain of PLK4 enzyme together with an automatic docking program was used to determine the molecular alignment of the ligands. The 3D QSAR model obtained by this way yielded a high correlation between the experimentally determined binding affinity and the calculated molecular interaction fields. It was shown that the receptor-based 3D QSAR yields a better prediction of the binding affinity than using an interaction energy-based model or a ligand-based 3D QSAR analysis. Encouraged by these results the receptor-based 3D QSAR model should now be used for the screening and prediction of novel inhibitors for PLK4 enzyme.

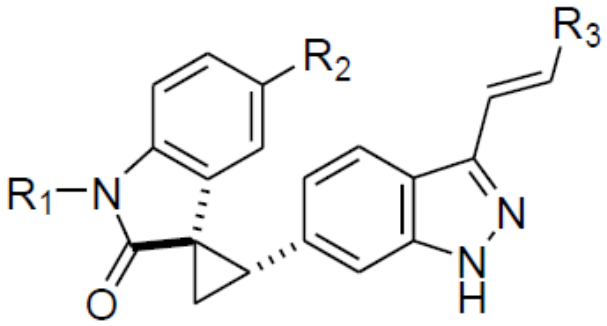

Figure 1. (1R,2S)-2-(1H-indazol-6-yl)spiro[cyclopropane-1,3'-indolin]-2'-one based skeleton.

Computational Details

Biological Data and Molecular Structures

A series of PLK4 inhibitors, published by Peter B. Sampson and Henry W. Pauls et al., were divided into a training set and a test set. The training set consists of 41 compounds and the test set, selected randomly, is comprised of 8 compounds. The PLK4 IC_{50s} were converted to pIC_{50} ($-logIC_{50}$) and used as dependent variables in the CoMFA calculations.

The 3D structures of these compounds were constructed using the molecular modeling software package SYBYL-X 2.1. Partial atomic charges were calculated by the MMFF94 method, and energy minimizations were performed using the Tripos force field with a distance-dependent dielectric and the Powell conjugate gradient algorithm (convergence criterion of 0.001 kcal/mol Å) . All compounds were generated in the protonation state under physiological condition.

Molecular Docking

To locate the appropriate binding orientations and conformations of the inhibitors interacting with PLK4, a powerful computational searching method was needed. The advanced molecular docking program GOLD (version 5.3), with a powerful genetic algorithm (GA) method for conformational search and docking programs, was employed to generate an ensemble of docked conformations. Atomic coordinates for PLK4 complex with the ligand ((1R,2S)-2-{3-[(E)-2-{4-[(dimethylamino)methyl]phenyl}ethenyl]-2H-indazol-6-yl}-5'-meth oxyspiro[cyclopropane-1,3'-indol]-2'(1'H)-one) that was used for our modeling study have been deposited in the Brookhaven Protein DataBank with a resolution of 2.4 Å (PDB ID: 4JXF) [5]. The original ligand was removed from the coordinated set of PLK4, the ligands were scored based on the fitness function 'GoldScore'. GOLD was run to save up to 10 top-ranked docking solutions for the ligands.

Structural Alignment

Steric and electrostatic interactions were calculated using the Tripos force field with a distance-dependent dielectric constant at all intersections in a regular space (2 Å) grid taking a sp^3 carbon atom as steric probe and a +1 charge as electrostatic probe. The cutoff was set to 30 kcal/mol. With standard options for scaling of variables, the regression analysis was carried out.

To form the basis for a statistical significant model, the method of partial least squares (PLS) regression was used to analyze the inhibitors by correlating variations in their biological activities with variations in their interaction fields. The optimum number of PLS components corresponding to the smallest standard error of prediction, was determined by the leave-one-out (LOO) cross-validation procedure. Using the optimal number of components, the final PLS analysis was carried out without cross-validation to generate a predictive model with a conventional correlation coefficient.

Ten conformations were obtained using GOLD for each ligand. The conformations and their alignment-the relative binding positions of the conformations in PLK4, all obtained from GOLD, were used directly in CoMFA to explore the specific contributions of electrostatic and steric effects to the molecular bioactivities. After replacing some conformations, finally, a new CoMFA model with a cross-validated q^2 value of 0.531 for 6 components was obtained by this way.

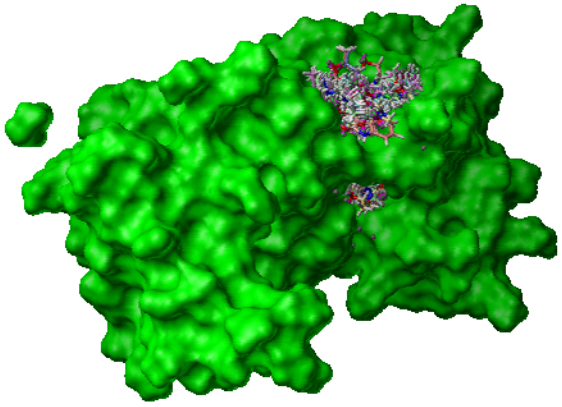

Figure 2. Superimposition of 49 inhibitors at the binding site of PLK4.

Due to the structural similarity of the analyzed data set with the co-crystallized inhibitor, it is likely that all active compounds show the same orientation at the binding pocket of the PLK4. We employed default settings of the GOLD program to end up with a consistent alignment of all 49 derivatives as described in detail in the Methods Section. 49 conformational binding modes for each ligand were generated at the active site. Moreover, it was observed that the orientations of all 49 inhibitors as extracted from the GOLD docking are quite similar as shown in Fig. 2.

Results and Discussion

The statistical values of CoMFA results are summarized below. 41 inhibitors were randomly included in a training set for constructing CoMFA models, and the remaining 8 were used as a test set for model validation. PLS analysis was carried out for the 59 binding conformations, and the results showed that a CoMFA model with a leave-one-out cross-validated q^2 of 0.531 for six components was obtained. The non-cross-validated PLS analysis with the optimum components of 6 resulted in a conventional r^2 of 0.973, F=189.874, and an estimated standard error of 0.070. The steric field descriptors explain 77.0% of the variance, while the electrostatic descriptors explain 23.0%, which indicate that steric factor in the model is predominant. The results demonstrate that the activities predicted by the constructed CoMFA model are in good agreement with the experimental data, suggesting that a reliable CoMFA model was successfully constructed.

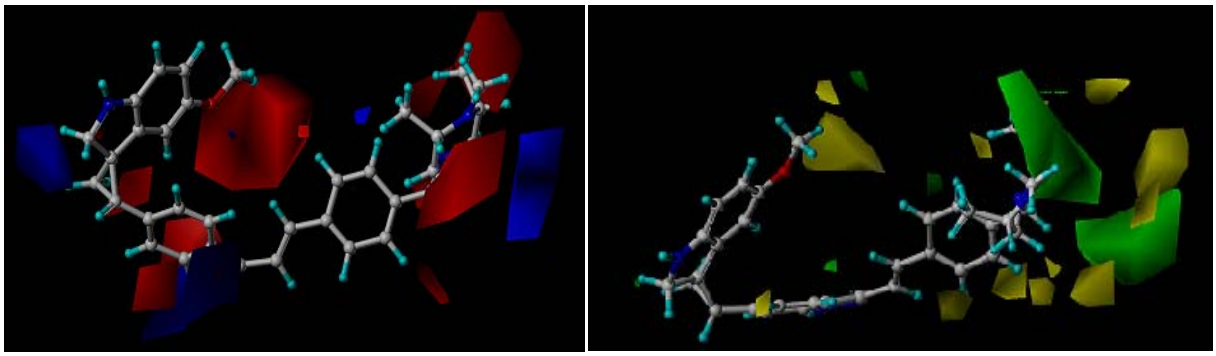

Figure 3. CoMFA electrostatic field contour and steric field contour maps in combination with inhibitor 56b.

CoMFA steric and electrostatic contours are shown in Fig. 3. The steric interaction is represented by green and yellow contours, in which green-coloured regions indicate areas where increased steric bulk is associated with enhanced activity, and yellow regions suggest areas where increased steric bulk is unfavourable. Electrostatic interaction is indicated by red and blue contours, among which blue-coloured regions show areas where more positively charged groups are favoured, and red regions highlight areas where increased negatively charged groups are favoured.

One small blue contour is observed near indazole ring of inhibitor 56b, as we all know, two strong H-bonds are formed from indazole to Glu90 and Cys92 of the most inhibitors. Natively electron-withdrawing phenyl group can make the neighbor amino more positive, so two more strong hydogen bonds may be formed to the side chain of Glu90 and Cys92 respectively. Four small red contours are found near the phenyl group of R_3 substituent, indicating that more

negatively charged groups can favourably interact with the surrounding residues. Near the tail amino group of R_3 substituent, two green contour and one blue contour are found. According to the docking study, the amino group forms a H-bond net by this region. All in all, a big and electronal R_3 substituent with a positive charged tail may impove affinity. At last, one big red contour enveloped one yellow contour are found near the R_2 substituent region, indicating that small and more electronic group may improve affinity [6-8].

Validation of the 3D-QSAR Models

The 8 randomly selected compounds 28, 30, 33, 35b, 41, 41b, 48b and 54 were used as the test set to verify the constructed CoMFA model. The predicted pIC_{50}s with the QSAR models were in good agreement with the experimental data within a statistically tolerable error range. The test results indicated that the CoMFA model would be reliable for use in new PLK4 inhibitor design.

Summary

In summary, 3D QSAR analyses have been performed on 49 compounds using molecular docking method and CoMFA. A satisfactory CoMFA model was obtained to predict the activities of test set structures. The steric and electrostatic recognition sites of the peripheral binding site as well as the superimposed CoMFA contours on the receptor sites will guide the design of novel structures which demonstrate optimal binding to and inhibition of PLK4.

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