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Pharmacophore modeling, virtual screening, docking and *in silico* ADMET analysis of protein kinase B (PKB β) inhibitors

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ABSTRACT

Protein kinase B (PKB) is a key mediator of proliferation and survival pathways that are critical for cancer growth. Therefore, inhibitors of PKB are useful agents for the treatment of cancer. Herein, we describe pharmacophore-based virtual screening combined with docking study as a rational strategy for identification of novel hits or leads. Pharmacophore models of PKB β inhibitors were established using the DISCOtech and refined with GASP from compounds with IC_{50} values ranging from 2.2 to 246 nM. The best pharmacophore model consists of one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD) site and two hydrophobic (HY) features. The pharmacophore models were validated through receiver operating characteristic (ROC) and Güner-Henry (GH) scoring methods indicated that the model-3 was statistically valuable and reliable in identifying PKB β inhibitors. Pharmacophore model as a 3D search query was searched against NCI database. Several compounds with different structures (scaffolds) were retrieved as hits. Molecules with a Q_{fit} value of more than 95 and three other known inhibitors were docked in the active site of PKB to further explore the binding mode of these compounds. Finally *in silico* pharmacokinetic and toxicities were predicted for active hit molecules. The hits reported here showed good potential to be PKB β inhibitors.

1. Introduction

The phosphoinositide 3-kinase (PI3-K) family of enzymes has a central function in regulating variety of cellular processes as metabolism, cell growth, proliferation, apoptosis and chemotaxis [1]. The serine/threonine protein kinase B (PKB, also known as Akt) is a central point in PI3-K cascade. PKB phosphorylate a plethora of substrates and coupling PI3-K with the mammalian target of rapamycin (mTOR). PKB is a key downstream component in the PI3-K signaling pathway [2] and has an ability to phosphorylate and change the activity of many critical metabolic targets. There are three human isoforms of PKB known as PKB-α, -β, -γ or Akt-1, -2, -3. These three isoforms of PKB shares a common structure made up of three domains: the N-terminal pleckstrin homology (PH) domain binds phosphatidylinositol-3,4,5-triphosphate ($PI(3,4,5)P_3$) and, is essential in the activation of the enzyme, a catalytic kinase domain related to other AGC (protein kinase A, protein kinase G and protein kinase C) family kinases, contains a classical kinase ATP-binding site and a hydrophobic motif (HM) at the C-terminal, which forms a docking site for phosphoinositide-dependent kinase 1 (PDK1) [3]. Activation of PI3-K upon binding of ligand to receptor tyrosine kinase (RTK) at the cell surface produces $PI(3,4,5)P_3$. Binding of PKB to $PI(3,4,5)P_3$ through the PH domain of the enzyme promotes activation of the kinase by phosphorylation on Ser473 and Thr308 [4,5]. Activated PKB inhibits apoptosis and stimulates cell cycle progression by phosphorylating numerous targets in various cell types, including cancer cells. PKB is the most significant mediator of the PI3-K signaling cascade and is localized to the membrane by interactions between its PH domain and $PI(3,4,5)P_3$. Upstream RTK, the lipid kinase PI3-K and PKB itself, particularly the β-isoform, are commonly amplified or mutated at the genetic level, over-expressed, or over-activated in human tumors particularly in breast, ovarian and cervical carcinoma [6]. The lipid phosphatase PTEN (phosphatase and tensin homolog) is a negative regulator of PKB that dephosphorylates $PI(3,4,5)P_3$ and deactivates PKB. Over-expression of PKB as a result in inactivation of tumor suppressor PTEN has been correlated with an increasing number of human cancers [7]. Deletion of PTEN is frequently observed in human tumors, especially glioblastoma, endometrial, and prostate cancers [8,1]. The prevalence of PKB activity is the hallmark of several aggressive malignancies, as a result, PKB has emerged as an attractive target for the development of novel anticancer therapeutics [9]. The development of inhibitors of PKB as

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small molecule is a valuable approach for anticancer drug discovery.

There are three strategies can be applied for the development of novel PKB inhibitors, targeting toward pleckstrin homology (PH) domain [10], ATP-binding kinase domain [11], or and hinge-region domain [12]. In contrast to the above strategies, there has been increased interest in developing PKB inhibitors that specifically target the substrate binding groove adjacent to the ATP-binding site. Several publications in this area reported many different classes of ATP-competitive small molecule inhibitors of PKB [13–17]. Poor oral bioavailability and short half-life in animals are the main problem of this kind of compounds (GSK690693) as a clinical agent [18]. However, only limited number of compounds entered into early phase of clinical trials [19]. Therefore, challenges remain for the development of selective ATP-competitive inhibitors of PKB as a suitable drug candidate. Pharmacophore mapping, virtual screening and docking are the rational methods for identification of novel hits or leads with diverse chemical scaffolds [20–24]. A pharmacophore can be considered as the ensemble of steric and electrostatic features of different compounds which are necessary to ensure optimal supramolecular interactions with a specific biological target structure and to trigger or to block its biological response. Molecular docking is widely used to suggest the binding modes of protein inhibitors. *In silico* pharmacokinetic and toxicity prediction play an important role in drug discovery and development because ADMET (absorption, distribution, metabolism and excretion and toxicity) is a major cause of failure of drug candidates during late phases of drug development [25]. The present study was undertaken to explore key structural requirements for PKB β inhibition utilizing combination of the above modeling methods for the design of novel, potent and selective PKB β inhibitors.

2. Materials and methods

2.1. Dataset

The available data set of 73 compounds (Table A under Supplementary Materials) were obtained from the literature [26–28], consisted of 4-amino-1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidine-4-carboxamides (41 compounds), [26] 4-(4-aminopiperidin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidines (23 compounds) [27] and 6-phenylpurine (9 compounds) [28]. PKB β IC_{50} values were measured [26–28] by inhibition of PKB β kinase activity in a radiometric filter binding assay which employed similar experimental conditions and procedures.

2.2. Computational details

The pharmacophore mapping, database searching and molecular docking study were performed using the SYBYL X 1.2 software from Tripos Inc., St. Louis, MO, USA [29]. All the compounds were sketched using SKETCH function of SYBYL. Partial atomic charges were calculated by the Gasteiger–Huckel method and energy minimizations were performed using the Tripos force field [30] with a distance-dependent dielectric and the Powell conjugate gradient algorithm convergence criterion of 0.01 kcal/mol Å [31].

2.3. Generation of pharmacophore models

The first step in building a pharmacophore model from a set of ligands is the alignment of the molecules, including position, rotation and conformation. Twelve molecules (Fig. 1) were selected to generate 10 pharmacophore models using DISCOtech, based on chemical diversity. The genetic algorithm similarity program (GASP) was used to refine the generated model (4 models) which uses a genetic algorithm to identify common feature of different molecules. A pharmacophore contains essential features like hydrogen bond donor, hydrogen bond acceptor, hydrophobic, etc. Chemical structures of molecules used in pharmacophore mapping are shown in Fig. 1. All the parameters were kept as default except population size (125), mutation weight (96), fitness increment (0.02) and number of alignment (04) [32].

2.4. Validation of the pharmacophore model

In order to evaluate the reliability and accuracy of the generated 3D pharmacophore models, model 1–4 were validated by receiver operating characteristic (ROC) and Güner-Henry (GH) scoring methods.

2.4.1. Receiver operating characteristic (ROC) analysis

Models 1–4 were validated by assessing their ability to selectively capture diverse PKB β inhibitors from a large list of decoys (molecules which are presumably inactive against the examined target) employing ROC analysis [33]. Therefore, it was necessary to prepare valid evaluation structural database (testing set) that contains an appropriate list of decoy compounds in combination with diverse list of known active compounds. Active testing compounds were defined as those possessing PKB β (IC_{50} values) ranging from 2.5 nm to 250 nm. The testing set included 33 active compounds along with their 20 conformers generated using a genetic algorithm-based global optimizer to find the low energy conformations of a molecule. A total of 660 conformers of 33 compounds constitute a test set. The ROC curve is a function of Sp *versus* the Se, and the area under the ROC curve (AUC) value is the important way of measuring the performance of the test.

$$\mathrm{Se} = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}}$$

where TP is the number of true positive compounds, FN is the number of false negative compounds.

$$\mathrm{Sp} = \frac{\mathrm{FP}}{\mathrm{TN} + \mathrm{FP}}$$

where TN is the number of true negative compounds, FP is the number of false positive compounds.

The ROC curve is a graphical plot of the true positive rate (Se) *versus* false positive rate (1 − Sp), and the area under the ROC curve (AUC) is the important way of measuring the performance of the test.

$$\mathrm{AUC} = \sum_{X=2}^{N} \mathrm{Se}(x)[(1-\mathrm{Sp})(x) - (1-\mathrm{Sp})(x-1)],$$

where Se(x) is the percent of the true positives *versus* the total positives at rank position x, (1 − Sp)(x) is the percent of the false positives *versus* the total negatives at rank position x.

2.4.2. Güner-Henry (GH) scoring method

The GH score has been successfully applied to quantify model selectivity (best model), accuracy of hits and the recall of actives from a molecule dataset consisting of known actives and inactives. GH scoring methodology has been successfully applied for quantification of model selectivity and coverage of activity space from database mining [34] and for the evaluation of the effectiveness of similarity search in databases containing both structural and biological activity data [35]. The GH score contains a coefficient to penalize excessive hit list size and, when evaluating hit lists, is calibrated by weighting the score with respect to the yield and coverage. The GH score ranges from 0, which indicates the null model, to 1, which indicates the ideal model (*i.e.*, containing all of, and