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Original article

Docking and 3D-QSAR (quantitative structure activity relationship) studies of flavones, the potent inhibitors of p-glycoprotein targeting the nucleotide binding domain

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ABSTRACT

In order to explore the interactions between flavones and P-gp, in silico methodologies such as docking and 3D-QSAR were performed. CoMFA and CoMSIA analyses were done using ligand based and receptor guided alignment schemes. Validation statistics include leave-one-out cross-validated R^2 (q^2), internal prediction parameter by progressive scrambling (Q^{*2}), external prediction with test set. They show that models derived from this study are quite robust. Ligand based CoMFA ($q^2 = 0.747$, $Q^{*2} = 0.639$, $r^2_{pred} = 0.802$) and CoMSIA model ($q^2 = 0.810$, $Q^{*2} = 0.676$, $r^2_{pred} = 0.785$) were developed using atom by atom matching. Receptor guided CoMFA ($q^2 = 0.712$, $Q^{*2} = 0.497$, $r^2_{pred} = 0.841$) and for CoMSIA ($q^2 = 0.805$, $Q^{*2} = 0.589$, $r^2_{pred} = 0.937$) models were developed by docking of highly active flavone into the proposed NBD (nucleotide binding domain) of P-gp. The 3D-QSAR models generated here predicted that hydrophobic and steric parameters are important for activity toward P-gp. Our studies indicate the important amino acid in NBD crucial for binding in accordance with the previous results. This site forms a hydrophobic site. Since flavonoids have potential without toxicity, we propose to inspect this hydrophobic site including Asn1043 and Asp1049 should be considered for future inhibitor design.

1. Introduction

The development of multidrug resistance (MDR) by tumor cells is a major impediment to the success of cancer chemotherapy. The multidrug resistance P-glycoprotein, multidrug resistance associated protein (MRP) and breast cancer resistance proteins (BCRP) are members of ATP binding cassette super-family [1–3]. P-glycoprotein (ABCB1), a member of ATP binding cassette super-family of transporters, is a xenobiotic efflux pump that limits intracellular drug accumulation by active extrusion of compounds out of cells. ABCB1 possess broad substrate specificity and substrates include members of many clinically important therapeutic drug classes, including anti-HIV protease inhibitors, calcium channel blockers used in the treatment of angina, hypertension, antibiotics and cancer chemotherapeutics [4,5].

P-glycoprotein is located inside the plasma membranes. The domain topology of P-gp consist of two homologous halves each consist a transmembrane domain (TMD) preceding a cytosolic nucleotide binding domain (NBD). Each TMD is composed of six transmembrane α-helix segments involved in efflux as well as in drug binding [6]. Each NBD contains three distinct motifs, Walker A, Walker B and the Signature or C motif. The Walker A motif, also known as the P-loop interacts with the phosphates of nucleotide di- and tri-phosphates. The function of Walker B sequence is less clear, but it is thought to be in Mg^{2+} coordination with the transporter and in polarizing water molecules [7]. The accurate function of Signature motif is not clear; however it is thought to be important in conducting the energy released from ATP hydrolysis to the TMD. This results in the conformational change leading to translocation of substrates across the membrane [8]. It has been proposed that Signature sequence may function as γ-phosphate sensor, detecting the presence of γ-phosphate of ATP in the opposing monomer within a dimeric NBD structure [9]. As ATP binding and hydrolysis within NBD's are vital for maintaining ABCB-1 mediated drug translocation, interruption of these processes is potentially powerful means of inhibiting the transporter activity.

Differential compounds have been shown to reverse the P-gp mediated MDR, including Verapamil [10], and Cyclosporin [11] are considered as first generation modulators and found to have side

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effects at the doses required for their clinical effectiveness [12]. The second generation modulators such as Dexverapamil and PSC 833 possess higher efficacy and lower toxicity. However, serious drug–drug interactions have been observed because of the fact that they act as substrates for cytochrome P450 3A. In case of third generation modulators (Tariquidar, Elacridar, Zosuquidar, Laniquidar) tend to possess high efficacy, low toxicity and increased selectivity [13]. However, they also failed due to toxic effects or low survival [14]. The search for modulators with low toxicity and high selectivity is still on. However, flavonoids are regarded as a new class of chemosensitizers which have the advantage of being a non-transportable inhibitor without side effects. They are reported as bifunctional modulators binding to a site partly overlap the ATP site and vicinal hydrophobic region interacting with the cytosolic domain of P-gp [15]. Hence, NBD of P-gp is an important target and in silico analysis on this target would be effective.

In silico QSAR methodologies, both 2D and 3D QSAR are proved to be powerful tools in lead optimization. It helps to identify the physicochemical properties which govern the biological activities. Comparative Molecular Field Analysis (CoMFA) [16] and Comparative Similarity Indices analysis (COMSIA) [17] allows us to map the interaction fields surrounding the structures according to their impact on given activity. However, it widely depends on the alignment of molecular structures. Literature studies suggested that numerous groups are working on flavonoids targeting P-gp. Boumendjel et al. reported 89 flavonoid derivatives composed of flavones, isoflavone, chalcones, sylbins, aurones and xanthones [18,19]. A 3D linear solvation energy model was developed by the same group (Boccard et al.) to quantify the affinity of flavonoids toward P-gp [20]. However, 3D-QSAR model by CoMFA and CoMSIA and mapping the contours inside the protein active site had proved to be effective. This prompted us to initiate the analysis to quantify the parameters crucial for high affinity ligand binding.

As, CoMFA and CoMSIA strongly depends on the alignment we took a representative set of 42 flavone derivatives [18,19]. Flavones are reported as highly active among the flavonoids with wide range of activity, which is an ideal dataset for 3D-QSAR modeling. In this study, CoMFA and COMSIA analyses were done using ligand based and receptor guided alignment schemes. Ligand based alignment was done by atom-by-atom matching, whereas receptor guided alignment was done by molecular docking. Different models were generated using combination of different fields. The results obtained from this study could be useful for the design of highly active flavones and also provide crucial insights into the Nucleotide binding domain.

2. Material and methods

All molecular modeling calculations were performed using molecular modeling programs, SYBYL 8.1, Autodock 4.0 installed on a Linux environment.

2.1. Data set

The structures of the flavone derivatives and their biological activities of forty two compounds were taken from the literature [20]. The binding affinity of each inhibitor was estimated by determining the dissociation constant K_d and converted into pK_d ($-\log K_d$) in order to use the data as dependent variable in CoMFA and CoMSIA model. Compound **13** has been ignored from the dataset to avoid the over fitting of the model, since the structure is considerably different from rest of the molecules in the dataset. The test set molecule is truly representative molecule for training set molecules. The test set molecule should cover all the biological activity which is similar to the training set molecule. The total set of compounds (41 compounds) was divided into a training set of 32 compounds and a test set of 9 compounds. The selection of test set compounds were done manually so that low, moderate, and high inhibitory activities were all represented. The structures and their activity values are displayed in Table 1.

2.2. Sequences analysis and homology modeling

Homology modeling was used to build the model for human ABCB1 NBD2 protein. The protein sequence (239 amino acids) was retrieved from Uniprot database (http://www.uniprot.org) (UniprotKB accession number: PO8183). The sequence was further used for template identification using FUGUE [21] module in SYBYL 8.1 [22], a molecular modeling package installed in Linux system. Blast search within FUGUE was utilized to select the templates. The top ranked template was aligned with the target sequence. The aligned sequence was taken for the model construction. Homology model was constructed using MODELER [23], based on the alignment obtained from FUGUE and it generates a refined 3D homology model. The generated model was validated with Ramachandran plot by PROCHECK [24]. The root mean square deviation (RMSD) between the main chain atom of the model and template was calculated and the model was used for further proceedings.

2.3. Molecular docking

The highly active compound in the dataset was docked into the binding site of NBD to provide the interaction between the ligand and the receptor. Docking was done using Autodock 4.0 software [25]. The most active compound, (compound **39**, $pK_i = 7.82$) was docked into the binding site using Autodock, which uses stochastic-search based algorithm for the generation of protein ligand complexes. Hydrogen atoms and the active torsions of ligand were assigned using AutodockTools (ADT). AutoGrid was employed to generate grid maps around the active site. The volume of the grid was set to cover the binding site with a grid-spacing interval of 0.375 Å with dimensions of $50 \times 50 \times 50$ Å. When docking was performed, some residues in the protein active site and all the torsional bonds in the ligand were set free. Lamarckian Genetic Algorithm (LGA) was employed then for conformational search with standard protocol. The job consisted of 20 runs. The initial population was 150 structures, and the maximum number of energy evaluations and generations was 25 00 000 and 27 000. The final structures were clustered and ranked according to the Autodock scoring function. The top binding mode was selected and used for receptor-guided CoMFA and CoMSIA analysis.

2.4. 3D-QSAR studies

Structural alignment is considered as one of the most crucial steps for CoMFA and COMSIA. The accuracy and reliability of CoMFA and CoMSIA models are directly dependent on the quality of the ligand alignment. In this study, ligand based and receptor guided alignment schemes were performed.

2.5. CoMFA studies

The steric and electrostatic interactions for CoMFA was calculated using Tripos force field with distance dependent-dielectric constant at all intersections in a regularly spaced (2 Å) grid taking sp^3 carbon atom was used as steric probe and a single positive charge (+1) as electrostatic probe. To improve the signal to noise ratio, the minimum sigma (column filtering) was set to 2.0 kcal/mol by omitting those lattice points whose energy