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Structure based drug design of Pim-1 kinase followed by pharmacophore guided synthesis of quinolone-based inhibitors

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ABSTRACT

Over expression of Human phosphatidyl inositol mannoside kinases isoform 1 (Pim-1 kinase) has been reported in several leukemia and solid tumors. Our continuous interest to reveal the secrecies of the mysterious Pim-1 kinase binding pocket has led us to employ a structure based drug design procedure based on receptor-ligand pharmacophore generation protocol implemented in Discovery Studio 4.5 (DS 4.5). Subsequently, we collected 104 crystal structures of Pim-1 kinase from the Protein Data Bank (PDB) and used them to generate pharmacophores based on the anticipated co-crystallized ligand-Pim 1 kinase receptor interactions. All selected pharmacophoric features were enumerated and only those that had corresponding valuable receptor-ligand interactions were retained. This was followed by modeling all pharmacophore combinations and scoring them according to their Receiver Operating Characteristic (ROC) curve analysis parameters as well as a DS.4.5 built-in Genetic Function Algorithm (GFA) validating model. Accordingly, 111 pharmacophores resulted with acceptable ROC performances; **1XWS_2_04, 2BIK_2_06**, and **1XWS_2_06** (ROC AUC value of: 0.770, 0.743 and 0.741 respectively) were the best pharmacophores. These pharmacophores were employed to guide the synthesis of new series of 7-[(2-Carboxyethyl)amino]-1-substituted-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid and their reduced 8-amino derivatives. The synthesized compounds were later evaluated for their Pim-1 kinase inhibitory potencies. Of which the most potent illustrated an IC_{50} value of 0.29 μM against Pim-1 kinase.

1. Introduction

Human phosphatidyl inositol mannoside kinases (Pim kinases) are a family of serine/threonine kinases composed of three members; Pim-1, Pim-2 and Pim-3. Pim kinases proto oncogenes regulate several signaling pathways which are directly related to the development of numerous malignancies. The Pim-1 kinase isoform (a 313-amino acid kinase) has been specifically reported in the last two decades for its direct role in tumor genesis process in various hematological malignancies, such as lymphoma and acute myeloid leukemia.[1–3] Similarly, over expression of Pim-1 has been linked to several solid tumors such as urothelial and prostate carcinomas.[2–7] Furthermore, developing effective Pim-1 inhibitors is also important to overcome the Pim kinase promoted chemo-resistance of tumor cells through hypoxia-induced chemotherapy resistance.[4,8] Per se, the Pim-1 is considered as a very attractive target for pharmacological inhibition in cancer therapy.

The crystal structure of Pim-1 kinase adopts a two-lobe kinase fold; the N-terminal and the C-terminal. The two lobes are combined with the hinge region amino acids residues, comprising, Glutamine 121, Arginine 122, Proline 123, Glutamine 124 and Valine 126, this hinge region also extends to the Aspartate 128 and Aspartate 131 residues. Along the hinge region resides is the deep Adenosine triphosphate (ATP) binding pocket which encompasses, the phosphate binding loop lying on the top of the ATP binding pocket like a lid (P-loop: residues 46–54), the catalytic loop (C-loop: residues 166–170), and the activation loop (A-loop: residues 191–202).[1,2] Designing new selective kinase inhibitors has been always an issue due to the high degree of conservation in the protein amino acid sequence among different kinases, but the Pim-1 kinase binding pocket is known to have some distinctive features that can be exploited, for example; Pim-1 kinase binding pocket is

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considered atypical in many aspects; this is due to the presence of the Proline 123 moiety in the hinge region where adenine usually binds via two hydrogen bonds.[7,9] As well, the hydrophobic region I (BR-I) in Pim-1 kinase is distinctively different from other kinases'. So, revealing more possible binding interactions inside the binding pocket of Pim-1 kinase will open the road towards discovering more selective and specific new inhibitors against this target.

We believe that illuminating the secrecies of the mysterious Pim-1 kinase binding pocket and understanding the Pim-1 versus ligand interaction models is of great importance for determining the potential activities of novel Pim-1 kinase inhibitors and designing new ones. In our previously published research project,[7] we designed a QSAR guided ligand-based study for Pim-1 kinase enzyme, and we were able to reveal some of the steric and electronic features that represent the optimal interactions between Pim-1 kinase inhibitors and its binding pocket from the resulted QSAR successful equation. Our interest to reveal even more ambiguous points inside the binding pocket of Pim-1 kinase has encouraged us to continue our investigation through another structure based study. Subsequently, our aim of this work is to extract and interpret Pim-1 kinase co-crystallized ligands from the Protein Data Bank (PDB) focusing on X-ray structure complexes, which have a satisfactorily good resolution. A PDB complex does not only contain experimental data but also a reasonable amount of interpretation that can be done by a researcher.[10] In most cases, structure determination efforts focus on the macromolecule (Pim-1 kinase protein, for example), while in our work ligand structures were elucidated then the potential interactions between the ligand and the Pim-1 kinase binding pocket were clarified and explained.

Furthermore, the most successful pharmacophores that resulted from modeling the ligand-receptor interactions were engaged as guiding maps to design new 7-[(2-Carboxyethyl) amino]-1-alkyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid derivatives as novel inhibitors against Pim-1 kinase enzyme. The present study is aiming to explore the key structural requirements for Pim-1 kinase inhibition utilizing combination of the above modeling methods for the design of novel, selective Pim-1 kinase inhibitors.

2. Results

Receptor-ligand pharmacophore generation technique models drug–receptor interactions using information derived from co-crystallized ligand contact points with their receptors.[11] HYPOGEN detects a 3D collection of five to six chemical features that reflect the hot contact points between the co-crystallized ligand and the binding pocket in which it was originally entrenched, which delivers a relative configuration for each co-crystallized ligand consistent with their binding modes inside the receptor site. A total of 104 X-ray Pim-1 kinase crystal structure complexes were used in this study. 111 training Hypogens (pharmacophores) were generated, of which only 7 pharmacophores showed acceptable results upon validation with Receiver Operating Characteristic (ROC) analysis as well as a DS.4.5 built-in Genetic Function Algorithm (GFA) validating model. The 7 successful pharmacophores were then engaged as guiding maps to design new quinolone-based series of Pim-1 kinase inhibitors.

2.1. Literature survey and data mining

The ChEMBL data base was surveyed to collect as many X-ray crystal structure complexes for Pim-1 kinase as possible. Pim-1 kinase target **(CHEMBL2147)** revealed 104 crystal structures in the Protein Data Bank (http://www.rcsb.org/) please refer to SM-1 in Supplementary material for the names of all PDB crystal structures used. Altogether, the 104 X-ray crystal structure complexes were collected, cleaned, and then engaged in consequent modeling processes.

2.2. Generating receptor-ligand pharmacophores

After employing the receptor-ligand pharmacophore generation procedure, only 111 pharmacophores resulted in a ROC-AUC value above 0.5. Table 1 shows the PDB code of the Pim-1 kinase crystals that displayed accepted results in addition to their resolution values and the structure of their native co-crystallized ligands. Moreover, Table 1 shows a description of the enumerated ligand features during the first step of the receptor-ligand pharmacophore generation procedure, the features that matched the receptor-ligand interactions, and the number of pharmacophores generated from each crystal structure during the receptor-ligand pharmacophore generation step. Additionally, Table 2 shows a description of the 111 pharmacophores generated in addition to their validation results in the ROC analysis procedure, i.e. (Number of true positives, Number of true negatives, Number of true positives, Number of true negatives, Sensitivity and Specificity).

2.3. Assessing the Validity of the resulted pharmacophores

In each pharmacophore generation run the resulting pharmacophores were directly graded according to their ROC performance and their GFA model results.

2.3.1. Receiver Operating Characteristic (ROC) curve

ROC analysis aims towards analyzing the ability of a particular pharmacophore to sort a total list of already known actives and inactives into their correct classes. The ROC performance is usually illustrated by the area under the curve (AUC) of the corresponding ROC along with other parameters such as: No. of true positives, No. of true negatives, No. of false positives, No. of false negatives, Sensitivity and specificity.[12–14]

Table 2 shows the ROC analysis results of our selected passed pharmacophores. Fig. 1A and B show the ROC curve of the pharmacophores with best ROC results (i.e.: Hypo **1XWS-2–4** (AUC value of 77.0%), Hypo **2BIK-2–6** (AUC value of 74.3%), Hypo **1XWS-2–6** (AUC value of 74.1%), Hypo **3DCV-2–3** (AUC value of 71.1%), Hypo **3BGZ-2–3** (AUC value of 71.2%), Hypo **3DCV** (AUC value of 71.10%), **and** Hypo **2BIK-2–7** (AUC value of 70.0%)**)**.

The fair results of ROC curves are attributed to the fact that the AUC calculation method during the ROC analysis process is dependent on the method used for curve fitting. ROC-AUC does not account for occurrence or different misclassification costs coming from false-negative and false-positive analyses;[15] meaning that using the pharmacophore mapping procedure in the early steps of ROC analysis to identify the actives from the in-actives does not in some cases give the right results. For example, in this case; the pharmacophore mapping procedure in DS 4.5 doesn't take into account the Pi-anion interactions (Anion-π interactions are defined as favorable non-covalent contacts between electron-rich anions and electron-deficient aromatic systems (π-acid)). This means that some truly active Pim-1 kinase inhibitors will be falsely unmapped in the very early steps of the ROC analysis and so, all the later results will be in some way undervalued since some truly active pim-1 kinase inhibitors were excluded during the early steps of ROC analysis procedure. For this reason, this explanation encouraged us to accept the fairly good ROC analysis results and