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Dihydropyrazole and dihydropyrrole structures based design of Kif15 inhibitors as novel therapeutic agents for cancer

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ABSTRACT

Mitotic Kinesin motors, Eg5 and Kif15, have recently emerged as good targets for cancer as they play an inevitable role during mitosis. But, most of the Eg5 inhibitors were found ineffective when the cancer cells develop resistance to them by escalating the expression of Kif15 as alternative to Eg5. Therefore, the drugs that target Kif15 became necessary to be used either as a single or in combination with Eg5 inhibitors. The present study used 39 dihydropyrazole and 13 dihydropyrrole derivatives that were having *in vitro* inhibitory potential against kinesin motors to develop a common pharmacophore hypothesis AHRR and atom-based QSAR model. The model was used for virtual screening of ZINC database and the resultant hits were docked against Kif15. The four drug candidates with high docking score were examined for their activity and pharmacokinetic behaviour. Based on the results these drugs could be considered as lead candidates in further drug development for cancer.

1. Introduction

Microtubules and tubulins have been important functional targets in cancer chemotherapy as evidenced from many of the anticancer drugs. But most of them elicit neurotoxicity and neuropathy as tubulins are also involved in non-mitotic function like neuro-transportation (Canta et al., 2009). Mitotic motors, which recently emerged as anticancer targets, have improved therapeutic index than tubulin since they are expressed only in dividing cells and not in differentiated cells (Huszar et al., 2009). Kinesin is a microtubule-dependent motor protein implicated in intracellular trafficking and cell division (Sheetz, 1996; Sharp et al., 2000; Hirokawa et al., 2009). A few of them play a vital role in the formation of bipolar spindles and separation of duplicated centrosomes during prometaphase of mitosis. They drive their motility on microtubule tracks with ATP kinase activity in which chemical energy is transformed to mechanical energy (Miki et al., 2005). Their plus-end-directed motility on one microtubule slides antiparallel microtubules apart resulting in centrosomes separation during mitosis (Blangy et al., 1995).

Eg5 and Kif15, belonging to the family of Kinesin-5 and Kinesin-12 respectively, are the two N-terminal plus-end-directed motors involved in cross-linking and sliding of mitotic spindles (Tanenbaum and Medema, 2010). Usually Kif15 takes over the role of Eg5 when it is inhibited as both of them have got similar functional role during mitosis. Monastrol, terpendole E, S-trityl-L-cysteine and ispinesib are some of the drugs that have shown inhibitory potential against human Eg5 (Mayer et al., 1999; Nakazawa et al., 2003; DeBonis et al., 2004; Bergnes et al., 2005; Sorbera et al., 2006). But all of them failed in clinic as the cells developed resistance to Eg5 inhibitors by over-expressing Kif15 which substituted the role of Eg5 (Tanenbaum et al., 2009; Jones et al., 2013). Hence, co-inhibition of Eg5 and Kif15 will be a vital therapeutic approach and the development of Kif15 inhibitors will be of great value.

Kif15 is involved in sliding of antiparallel microtubules through plus-end-directed motility on one microtubule and static interaction with other microtubule *via* nuclear protein TPX2 (Wittmann et al., 2000). Hence, Kif15 can be used as a potential anti-mitotic drug target for cancer and the inhibition of its function is tractable approach for drug development. So far, not much research has been done in this area to develop human Kif15 inhibitors. Therefore, identification of Kif15 inhibitors to be used in cancer treatments in combination with Eg5 inhibitors could be valuable. Keeping these facts in mind, structure-activity relationship studies were conducted on some already known mitotic Kinesin inhibitors to develop lead molecules as Kif15 inhibitors.

In this study, dihydropyrazole and dihydropyrrole derivatives were used to generate pharmacophore hypothesis and atom-based three dimensional quantitative structure-activity relationship (3D-QSAR) model to decipher the relationship between their structural

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information and biological activity, in view of providing insights into the development of Kif15 inhibitors with high anticancer activity. The 3D-QSAR model was used for virtual screening of drugs in ZINC database. The resultant hits were docked with human Kif15 protein to analyse the mode of binding, position and orientation. The compounds which scored high in docking were evaluated for their activities and pharmacokinetic properties. The putative molecules obtained in this study can be tested against Kif15 *in vitro* and developed as anticancer drugs in future.

2. Materials and methods

2.1. Dataset

A dataset of 39 dihydropyrazole and 13 dihydropyrrole derivatives with *in vitro* inhibitory activity against Kinesin Spindle Protein was used for the present study (Cox et al., 2006; Fraley et al., 2006; Roecker et al., 2007). *In vitro* inhibitory concentrations of these molecules against Kinesin expressed in IC_{50} was converted into pIC_{50} [$-\log(IC_{50})$] as activity and was used as dependent variable in the analysis.

2.2. Preparation of ligands

2D structures of all the 52 ligands were sketched by Marvin Sketch v5.12.1, ChemAxon (http://www.chemaxon.com). The basic structures of all the ligands are shown in Fig. 1 and various substituents with their *in vitro* IC_{50} values are listed in Table 1. In order to develop a pharmacophore model, each structure needs to be in all-atom 3D structures and low energy conformations should be generated. Using VLife Molecular Design Suite (VLife Sciences Technologies Pvt. Ltd., Pune, India) the structures of all the molecules were converted from 2D to 3D, added hydrogen atoms to ensure that the structure is an all-atom structure, and energetically optimized using Merck Molecular force field, distance dependent function, and energy gradient of 0.01 kcal/mol.

2.3. Mapping of pharmacophores

Phase v4.2 module of academic free version of Maestro v10.1 (Schrödinger, LLC, New York, NY, 2015) was used for pharmacophore generation and atom-based 3D-QSAR model building. Phase can identify common pharmacophore features which are essential for biological activity from a given set of molecules with high affinity for a particular protein target using fine-grained conformational sampling and a range of scoring techniques (Klebe et al., 1994; Dixon et al., 2006). The major pharmacophore features are H-bond acceptor (A), H- bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P), and aromatic ring (R). Pharmacophore mapping process in Phase involves creating pharmacophore sites from a set of above mentioned features, finding common pharmacophores and scoring the hypotheses. All the 52 molecules were assigned as either active or inactive based on a given activity threshold value. The threshold value given in this study was 7 for active ligands and 6.5 for inactive ligands based on their pIC_{50} values. Active ligands were considered for the generation of pharmacophore hypotheses by varying the number of matching molecules and active sites. Common pharmacopohore hypotheses (CPHs) were generated with minimum of three and maximum of six active sites which are common to all the molecules. CPHs were scored and ranked based on vector, site, volume, selectivity, number of ligands matched, relative conformational energy and activity. Based on a thorough analysis of the scores and alignment of ligands, a best CPH was selected for further studies.

2.4. Building 3D-QSAR model

Development of QSAR model is necessary to accurately predict the biological activity of new compounds towards the target protein. Phase provides the means to combine selected CPH with known activity of the ligands to create a 3D-QSAR model that identifies overall aspects of molecular structure that contribute to the activity of the ligand (Dixon et al., 2006). Out of the two options of QSAR model building, atom-based and pharmacophore-based, atom-based model was selected in this study. In atom-based QSAR model the structural components of the ligands are represented by van der Waals models of the atoms where each atom is treated as a sphere. Atoms are grouped into six classes based on their common spatial occurrence: hydrogens bonded to N, O, P, S as Hydrogen-bond donor (D), C, H—C, Cl, Br, F, I as Hydrophobic or nonpolar (H), atoms with formal negative charge as Negative ionic (N), atoms with formal positive charge as Positive ionic (P), N, O, hydrogen bond acceptors as Electron-withdrawing (W) and all other types of atoms as Miscellaneous (X).

The ligands were divided into groups of training set and test set with 70% of the total molecules being in the training set and 30% in the test set. The QSAR model was developed by placing the atoms of the ligands in training set into a cubic grid where the atoms might occupy one or more cubes. Each ligand is represented by a set of bit values that indicate the occupancy of atoms of each class and this value is used as independent variable in the model development. The present study used 37 molecules in the training set with their experimental activity as dependent variable to generate the model by setting the grid space of 1 Å. Hydrogen-bond donor, Hydrophobic or nonpolar, Electron-withdrawing and Miscellaneous atomic features of the ligands were used as independent variables. Regression analysis of the model was done by developing a series of models with increasing number of PLS factors upto 4 as the accuracy of the model increases with increasing number of PLS factors until standard deviation of the regression is approximately equal to the experimental error. The most accurate QSAR model was selected and validated by predicting the activity of 15 test set molecules. The correlation between predicted activity and experimental activity was assessed based on squared correlation coefficient (R^2) and variance ratio (F)

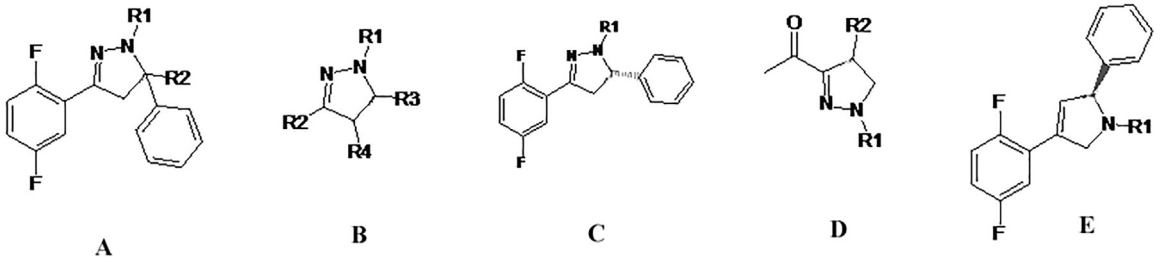

Fig. 1. Basic 2D structure of (A,B,C&,D) dihydropyrazoles and (E) dihydropyrroles.