Chapter VI

Summary and Conclusions

Chapter overview

This chapter summarizes the features which enlighten the research investigations.
6. Summary and Conclusions

- The efficiency of the inverse docking approach towards the prediction of protein targets was notably enhanced by combining various ligand-based modeling methods.

- The pharmacophore-similarity matrix was effectively applied to envisage the antimalarial behavior of the pharmacophoric groups present in the molecular dataset.

- We modeled flavone derivatives with inhibitory activities against three catalysts viz. FabZ, FabI and FabG of the fatty acid biosynthetic pathway. The rationale for selecting this dataset is based on the observation that the application of the inverse docking approach in recognizing possible hits is limited for closely-associated proteins. The integration of receptor- and ligand-centric methods effectively distinguished the binding patterns of the ligands against each enzyme.

- The docking energy of the ligands such as (-)-epigallocatechin gallate, (-)-gallocatechin gallate, (-)-catechin gallate and (-)-epicatechin gallate, were found to be very close to each other owing to the chemical similarities of the identical flavone scaffold as well as its methyl and hydroxyl groups attached at ring structures. The analysis of spatial binding patterns of the Fab enzymes active sites revealed the similar mapping of features including π/aromatic (1), H bond acceptor (2) centers, hydrophobic/aliphatic (2), and. The uniqueness of these patterns were attributed to the presence of the H bond acceptor or donor (DAC) encoded by Ser199 of FabG, His98 of FabZ and absence in FabI enzyme- pseudocenter map.

- Statistically significant and enzyme-specific molecular field point-based QSAR models were developed to study the molecular properties of flavones dataset and extended to predict the inhibitory activities of the external test set phytochemicals having antimalarial potential. Further, a machine learning model was developed with electrostatic and steric field descriptors along with shape and electrostatic field potentials to calculate activities. Finally, the best scoring molecules were selected by comparing the rank list obtained.
from docking, QSAR and machine learned models. Phytochemicals such as Citflavanone, Lupinifolin and Obovatin possessed better affinities against FabG and FabI enzymes whereas Cynaroside and Lonchocarpol A constituted better selectivity in interacting with FabZ enzymes.

- Since the docking, QSAR and machine learned models used were different in its background principles and calculations, the success rate of selecting compounds from the inverse docking task was improved.

- An inverse docking study was performed to study the *Candida albicans* protein targets using 7,8-dialkyl-1,3-diaminopyrrolo-[3,2-f] quinazolines having inhibitory activities against dihydrofolate reductase (CaDHFR) enzyme. This study was focused on the retrieval of protein targets from the protein structural data using the receptor-specific ligand binding properties in the receptor cavity and its compliance with complementary ligand molecular properties. The basis for selecting this dataset was made to overcome the inadequacies in empirical scoring functions of docking programs to effectively prioritize the protein targets in the top 10 to 20% cluster.

- The molecular field-based QSAR model was developed based on the selected quinazolines dataset and its activities against CaDHFR enzyme. The mapped descriptors in the QSAR models signified the preferential substitution of bulky chemical groups shorter than 2 carbon length with electronegative properties in relation to ethyl group at R1 site. Additionally, the R2 site should have a substitution with steric group less complex than t-Bu to develop potent CaDHFR inhibitors. This descriptor details was used to design probe molecules and subsequently docked with the protein cavity database of *C. albicans* to produce scaled energy profile for each protein entry in the database.

- The pharmacophore features present in the quinazolines dataset were perceived to study pharmacophore-based energy factor. This energy factor was clustered with scaled energy profile of protein entry using $k$ nearest neighbor approach ($k$NN) and successfully retrieved the experimental protein target, CaDHFR in the top cluster.
The other probable *C. albicans* protein targets were alpha subunit of mRNA capping enzyme, N-myristoyltransferase and phosphoacetylglucosamine mutase. A detailed analysis of these targets showed that they possessed similar binding characteristics and cavity environment. The study demonstrated the importance of recognizing probable protein targets from a panel of protein structures based on the knowledge of probe-based scaled energy profile and docking energy with the requirement of complementary receptor and ligand binding components.

The retrieval of protein targets from the inverse docking experiment was carried out in the Spinach protein structural data by the combination of ligand similarity approach. In this study, we had chosen the hormone kinetin which promotes seed germination and its precise molecular mechanism is still unknown. The rank list obtained from inverse docking and ligand similarity approaches were compared to identify the most probable protein target of kinetin.

The inverse docking study based on the docking of kinetin against a panel of Spinach proteins prioritized three targets with lowest docking score such as Chitinase, Ferredoxin-NADP$^+$ reductase and Ketol-acid reductoisomerase. The ligand similarity search using kinetin against a database of co-crystallized (bound) ligands of PDB database selected five targets viz. Superoxide dismutase [Cu-Zn], NADPH-ferredoxin reductase, Enoyl-(acyl-carrier protein) reductase, Photosystem Q(B) protein and Chitinase. The comparison of rank list of these approaches indicated Chitinase and Ferredoxin-NADP$^+$ reductase as the most protein targets having significant binding affinity. The selection of Ferredoxin-NADP$^+$ reductase as the most probable target was precluded due to the unavailability of experimental binding characteristics of Ferredoxin-NADP$^+$ reductase.

Chitinase was considered as the most probable protein target of kinetin due to the presence of experimental data that explained the interaction of kinetin. Additionally, the inverse docking and ligand similarity search approaches signified the lowest energy dock pose of chitinase-kinetin complex which revealed the interaction of active site amino acids and an equal contribution of van der Waals and electrostatic interactions with 2 H
bonds. It is also observed kinetin similarity with caffeine bound with *Gliocladium roseum* chitinase protein (PDB entry: 3G6M). This compound selection task was also supported by the experimental structure of Yeast chitinase 1 in complex with kinetin (PDB entry: 2UY5).

- An *in vitro* study focused on the effect of kinetin in Spinach seed germination was carried out which correlated with the available experimental evidences that kinetin affects the seed viability and vigourness. A very low concentration of kinetin (0.5 mg/l) exhibited an insignificant effect compared to control in inducing seed germination process. Moreover, the higher levels of kinetin (>0.5 mg/l) possessed antagonist seedling activity. It is anticipated that kinetin may have a molecular interaction with prioritized protein targets synthesized during the seed germination process and reduced growth. Hence, it can be concluded that kinetin may not be a suitable hormone for enhancing Spinach seed germination *in vitro*.

- A novel method of pharmacophore-similarity-based QSAR was built up which applied pharmacophoric (topologically situated atom-pairs) descriptors calculated from fragment molecules and explored the function of principle component analysis (PCA) for factor score calculation. The QSAR modeling was inspired by group-specific biological activity predictions and fragment-based QSAR modeling in which the neural networks to estimate site-specific biological activities were built up by utilized the factor scores as independent variables.

- Studied on 3-hydroxypyridinones fragmented derivatives having antimalarial activities, the activity contribution chart for R1 site showed that the antimalarial activity was influenced by an aliphatic linker having piperidine moiety. the ethylpiperidine and ethylmorpholine groups strongly incremented the activity at the R2 site. This observation was complied with the bis- and tris-chelating podants coordination model that explained 3-hydroxypyridinones potency as iron chelators.
• The application of PS-QSAR model to external test set indicated that at R2 site the substitution of H bond acceptor is preferred with steric properties using more than 4 interconnected atoms. The substitution of steric group with an aromatic group containing N atom at R1 site is preferred.

• Present methodology can be used to predict the contribution rate of biological activities of the sub-structures present in the molecular dataset.