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Molecular modeling studies for exploring structural requirement of acetylcholinesterase inhibitors

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***Abstract*— Inhibition of the neurotransmitter acetylcholine (ACh) can control the alzheimer's disease (AD). The ACh hydrolyzes to produce choline and acetyl groups through acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the synaptic region, which play a key role in accelerating senile amyloid β-peptide (Aβ) plaque depositions, leads to generation of AD. The present study has been emphasized to explore both ligand- and structure-based QSAR and docking studies on a set of structurally diverse compounds to explore prime structural features responsible for selective binding to AChE, vis–a-vis inhibiting enzyme activity. Both the studies showed the importance of HB acceptor and donor, and hydrophobic features of the molecule for effective binding. Systematic comparisons revealed that structure-based study has advantages in efficiently identifying potent hits with structural diversity over simple ligand-based study. Structure-based QSAR study (site score = 1.006) adjudged the significance of features obtained from ligand-based QSAR model (ROC score = 0.850, sensitivity = 0.710, specificity = 0.932). Presence of electronegative groups, and acyclic and aromatic rings in the molecular scaffold depict the importance in selective AChE inhibition.**

***Keywords*— AChE, Bayesian model, docking, Site Map analysis**

I. Introduction

Deficiency in cholinergic neurotransmission is significant for the progression of Alzheimer's disease (AD). The ACh hydrolyzes to produce choline and acetyl group through the biocatalyst acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the synaptic region [1]. Inhibition of AChE enzyme can sustain the ACh depletion, which is the main strategy for the treatment of AD. In the present study, a group of compounds are considered for the molecular modeling studies to explore the structural requirements through ligand- and structure-based quantitative structure activity relationship (QSAR), site map, molecular docking studies for potential AChE inhibition.

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II. Materials and Methods

In this work, a set of structurally diverse compounds (n = 225) [2-17], having AChE inhibitory activity (IC_{50} in nM), have been considered for modeling study. The compounds were divided into training and test sets by k-means clustering. The crystal structure of the AChE enzyme (PDB code: 4EY7) [18], obtained from RCSB Protein Data Bank [19], has been used for structure-based modeling, site map and docking studies. The QSAR models were generated for understanding of steric (s), electrostatic (e), hydrophobic (p), ring aromatic (r), HB acceptor (a) and donor (d) requirement for enzyme inhibition.

A. Bayesian model

Development of Bayesian model [20, 21] helps to classify active and inactive compounds. The model was developed by the help of "Create Bayesian model" tool in Discover Studio 2.5 (DS 2.5) [22]. The 2D descriptors selected for model development were molecular function class fingerprints of maximum diameter 6 (FCFP_6), AlogP, molecular weight, number of rotatable bonds, number of rings, number of aromatic rings, number of hydrogen bond donors and acceptors, and molecular fractional polar surface area. The developed model emphasized some good fingerprints which are favourable for AchE inhibition and some bad fingerprints, the presence of which in the molecules may reduce the enzyme activity. The external predictability of the model was tested on the test set compounds.

B. Molecular docking

Molecular docking was carried out to find out the binding interaction of the small molecule at the active site of protein by using Grid-Based Ligand Docking with Glide in Schrodinger [23, 24]. Protein was prepared for experimentation using "Protein preparation wizard" module [25]. Hydrogen atoms were added, followed by energy minimization and optimization by OPLS force field. Protonation states were determined at physiological pH 7.4 using PROPKA. Water molecules around the catalytic zone upto 5 Å were kept during protein preparation and rest of the water molecules were removed. The ligand structures were prepared by the ligand preparation LigPrep module. The grid was generated according to the active site of the protein, after ligand and protein preparation. The grid-enclosing box was centered to the active sites of the corresponding 3D-structure of the receptor so as to enclose them within 3 Å from the centroid of these residues. Finally, docking calculation was performed with XP mode. Glide XP mode determines all reasonable orientation for each low-energy conformer in the designated binding site.

C. *Site map analyses*

The crystal structure of protein 4EY7 [18] was selected, and the site recognition tool 'Site Map' [26] of Maestro was run on the protein structure after extracting the ligand. Site Map applies an algorithm similar to Goodford's Grid algorithm [27] that uses interaction energies between protein residues and grid probes to generate preferable interaction sites. Sites containing more than 15 points were considered as druggable pockets. A restrictive hydrophobicity definition, a standard grid (1.0 Å), and OPLS force field were used. The physiological properties were calculated by Site Maps that, include size, volume, degree of contact, hydrophobic/hydrophilic balance, and hydrogen bonding possibilities (acceptors/donors). In addition, two scores – SiteScore and Dscore were calculated. Site-Score determines the druggability of the selected pocket. A pocket having SiteScore of 0.8 may be considered as druggable, whereas a value of more than 1.0 is indicative of the highly promising site. The Dscore parameter penalizes highly hydrophobic sites since these sites are not suitable for polar ligands. The Site Map analysis has been carried out to find out available interaction options for the ligand at the receptor binding sites.

III. Results & Discussion

A. *Bayesian modeling*

The cross-validated ROC plot (AUROCcv = 0.850) and the parameters, like specificity (0.932), sensitivity (0.710), concordance (0.840), and enrichment data (Table 1) of the developed Bayesian model (Model 1) indicate its ability to separate active and inactive compounds effectively. The five-fold cross-validation results AUCROCTest (0.759), validates the developed Bayesian model with the test set, signifies the acceptability of the model (Fig. 1). Top five favourable and unfavourable molecular features for AchE inhibitory activity were identified based on a Bayesian score using FCFP_6, shown in Fig. 2.

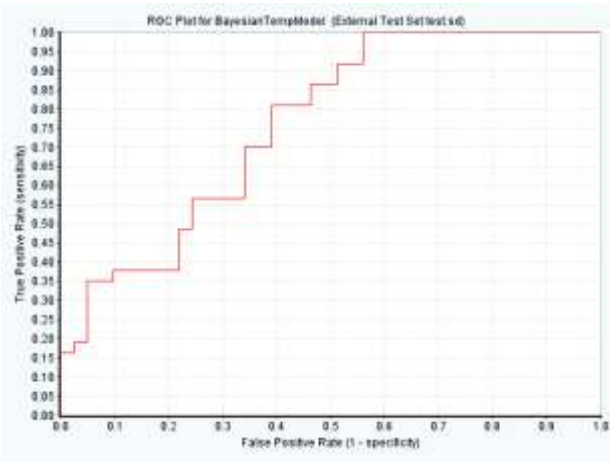

Figure 1. ROC plot for test set

Figure 2. Good and bad fragments from the study

B. *Docking interaction*

The highly active compound was docked at the active site of the crystal structure, 4EY7 [18] (Fig. 3). The catalytic amino acids (within 4 Å) include Tyr72, Tyr341, Tyr337, (polar amino acids) and Phe295, Trp86, Trp286, His447, Gly448 (non-polar amino acids). Docking results satisfied the geometric criterion for HB formation and π-π interaction. It is observed that HB- length is ≤ 2.8 Å, the minimum angle of donor ≥ 120° and acceptor ≥ 90°, and pi-interaction: bond length 4.4-5.5Å and angle 30° - 60°. The interaction study showed that carbonyl group of adjacent anthracen scaffold interacted with Phe295, and carbonyl group of the backbone chain interacted through the HB with Tyr124. The residues Trp286 and Tyr341 formed the π-π stacking interactions and Trp 86 formed the π-cation interaction. But the least active compound didn't show any interaction with Phe295 which is important for the activity.

C. *Site map analysis*

To explore structure-based QSAR study, Site Map is generated at the active site of the AChE enzyme through the docked pose of ligand. The ligand bound pocket of the protein structure showed good druggability (SiteScore: 1.006, Dscore: 1.050) score. Red color indicates HB acceptor region. Blue color indicates HB donor region and yellow color indicates hydrophobic region. Oxygen atom attached to five membered ring acts as a HB acceptor in red contour. Blue contour near electronegative atoms nitrogen and oxygen signifies the region HB donor. Alkane chains and benzene rings depict hydrophobic property for its non-polarity (Fig. 4).

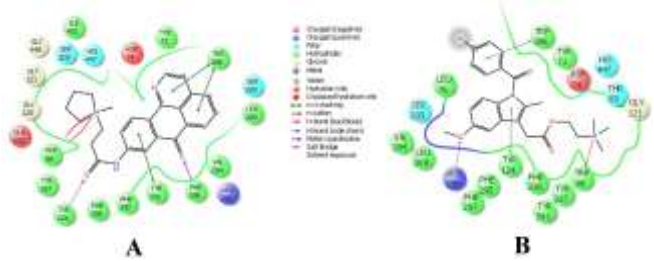

Figure 3. Docked poses of (A) most and (B) least active compounds.

Table 1. Enrichment data

Output	Category %	1%	5%	10%	25%	50%	75%	90%	95%	99%
Bayesian Model	41.33%	3.20%	12.90%	25.80%	53.20%	77.40%	91.90%	100%	100%	100%

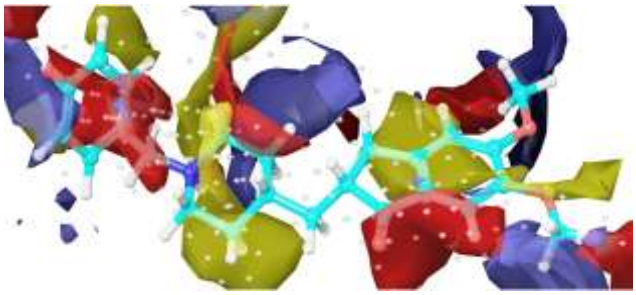

Figure 4. Site map features in AChE catalytic site. Blue: Donor, Red: acceptor and Yellow: hydrophobic features.

IV. Conclusion

In the present study, both ligand-based and structure-based QSAR studies have performed on a structurally diverse AChE inhibitors and receptor cavity. Ligand-based QSAR study provides information on some essential groups as well as fragments, and structure-based QSAR study reveals vital feature sites in the active site cavity, which are important for binding inhibition to the receptor. Systematic comparisons revealed that structure-based QSAR has advantages in efficiently identifying potent hits with structural diversity over simple ligand-based QSAR. Both types of studies confirm the importance of HB acceptor, and donor with hydrophobic features. Presence of electronegative groups, and acyclic and aromatic rings in the molecular scaffold depicts their importance in AChE inhibition.

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