

Alzheimer's disease Multi-Target Directed Inhibitor Design Using Sequential Virtual Screening Techniques

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Alzheimer's disease (AD) is a complex neurodegenerative disorder with multifactorial syndrome of the central nervous system among elderly people. Several cholinesterase inhibitors are nowadays utilized only for symptomatic treatment of AD. Hence, existing pharmacological approaches with one-compound-one-target are limited in their ability to modify the pathology of AD. Multi-target directed (MTD) drugs have been found effective in controlling neurodegenerative diseases. Thus, various dual inhibitors of acetylcholinesterase (AChE) and α -secretase (BACE-1) were designed based on the multi-target-directed ligands strategy. We have developed an *in silico* strategy to screen compounds for AChE and BACE-1 dual inhibition. Eight new MTD ligands were identified using sequential virtual screening techniques, which include pharmacophore model development of known AChE and BACE-1 inhibitors; Virtual screening of different small molecule databases followed by molecular docking at the active site of both these enzymes. Compound 2 was found to be most promising hit and could be further explored for experimental analysis. Our present strategy for identification of AChE and BACE-1 dual inhibitors might be one of the promising direction to discover better leads for the treatment of Alzheimer's disease.

Keywords: Clustering, GFA, QSAR, AChE, ADMET, Screening

Introduction

It is well proven that AChE is the most promising target for symptomatic treatment of Alzheimer's disease (AD), while BACE-1 is an attractive therapeutic target due to its intervention in A β formation for the pathogenesis. Thus, ligands showing both AChE and BACE-1 enzyme inhibitory activity should alleviate clinical symptom in short-term therapy, and also will have an effect by preventing the formation of A β , thereby slowing the progression of disease.

In light of this, an alternative strategy, based on the assumption that a single compound may be able to bind multiple targets, is now emerging, and leading to the shift from single to multi-target-directed (MTD) ligands that are more adequate to face the disease complexity.

Our work was focused on to design MTD ligands showing both AChE and BACE-1 enzyme inhibitory activity by employing pharmacophore modeling and sequential virtual screening (VS) techniques. The screened virtual hits were then validated using molecular docking analysis at the active site of both these enzymes. We made an attempt to identify the hypothetical 3D ligand based pharmacophore model by using common feature hypothesis generation approach: HipHop module in Catalyst software implemented in Accelrys Discovery Studio program. For generating

common feature chemical hypothesis different series of compounds should be present in the training set, so that our generated model could be able to identify active ligands belonging to diverse chemical classes.

On therapeutic fronts, anti-AD drugs should have good oral bioavailability and CNS penetration. The most commonly used descriptor for *in silico* prediction of CNS penetration is log-BB, which is defined as the ratio of the steady-state concentrations of a compound in the brain to that in the blood ($\log\text{-BB} = \log [\text{brain}] / [\text{blood}]$)¹. Thus log-BB filter was applied to filter out non-CNS penetrate compounds. Our strategy starts from preparing ligand based pharmacophore models for both AChE and BACE-1 followed by (i) sequential VS of the three different databases; (ii) application of drug likeness and log-BB filter and (iii) docking analysis on both AChE and BACE-1 enzyme.

Development of Pharmacophore models for AChE inhibitors and BACE-1 inhibitors

Review of literature showed that bis- and bi-functional inhibitors acting at catalytic site and PAS are more potent, and also decreases the A β peptide aggregating effect². So, such types of ligands from PDB were chosen to generate HipHop pharmacophore models. 9 co-crystallized ligands were extracted from the PDB, and common feature pharmacophore models were generated using these AChE ligands.

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The resultant pharmacophore models are ranked as they are built. The ranking is a measure of how well the compounds map onto the proposed pharmacophores. Our best hypotheses Hypo-1 consisting of three different chemical features with distances between them given in Å units were selected. These include, ring aromatic (RA), ring hydrophobe (RH) and hydrophobe (HP), which is pictorially depicted in Fig. 1 respectively. Hypo-1 was validated using external data set 285 compounds from binding database.

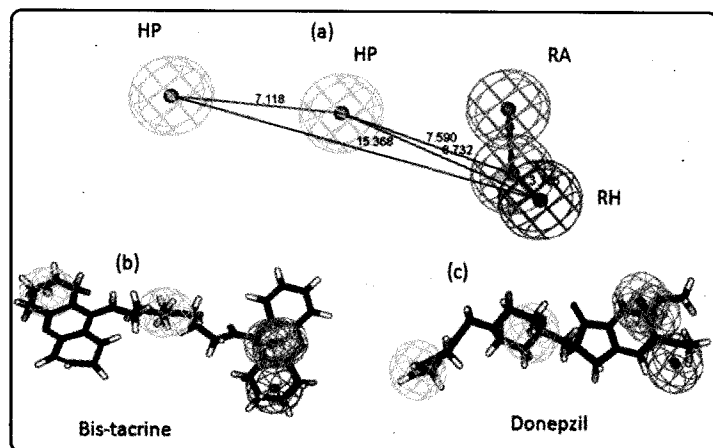


Fig. 1 : Different pharmacophoric features of Hypo-1 (a) with distances between them in Å. ring aromatic (RA), ring hydrophobe (RH) and hydrophobe (HP). Mapping of the pharmacophoric features to Bis-Tacrine (b) and Donepezil (c) compound.

The dataset consisting of 285 compounds were divided into three classes: active, moderately active and least active or inactive based upon their IC_{50} values. Activity range (0-500) nM were considered for active compounds, (501-5000) nM for moderately active and above 5000 nM for least active or inactive compounds. Hypo-1 screened more number of active and moderately active compounds. It also screened lesser number of inactive compounds from this binding database, and is presented in Table 1.

Table 1 : Screening of validation of dataset by Hypo-1 and Hypo-2 generated for AChE inhibitors

Pharmacophore	Active (226)	Moderately active (37)	Least active/ Inactive (22)
Hypo-1	200	18	4
Hypo-2	181	17	5

Another supporting factor in favor of Hypo-1 is aromatic ring feature (R) in the hypothesis (Fig. 1). AChE enzyme contains several aromatic residues in the active site, including Trp-84 in the catalytic site and Trp-297 in the PAS, which are capable of making p-p stacking interaction with the aromatic moieties. So we finally selected Hypo-1 for the VS of three different databases (Specs, NCI and IB Screen).

Pharmacophore model for BACE-1 inhibitors were generated in similar manner as for AChE inhibitors. Ligands were extracted from protein crystal structures co-crystallized with the inhibitors available in PDB. The best ranked hypothesis Hypo-1 consists of three different chemical features with distances between them given in Å units. These include, hydrogen bond acceptor (HA), ring aromatic (RA), and positively ionizable (PI) chemical features, and is pictorially depicted in Fig. 2 respectively.

Table 2 : Screening of validation of dataset by Hypo-1 and Hypo-2 generated for BACE-1 inhibitors.

Pharmacophore (Hypothesis)	Active (144)	Moderately active (64)	Least active/ Inactive (79)
Hypo-1	133	35	13
Hypo-2	129	30	17

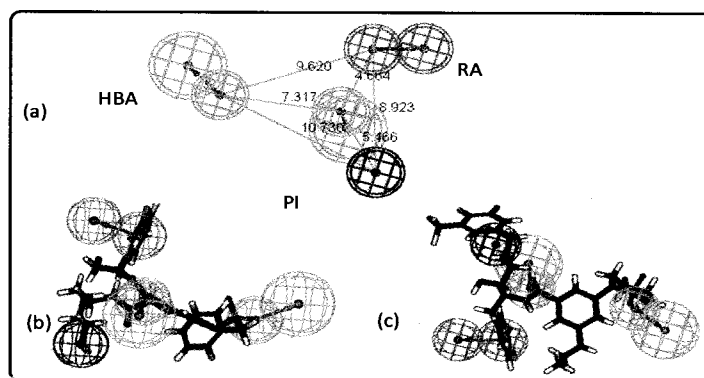


Fig. 2 : Different pharmacophoric features of Hypo-1 (a) with distances between them in Å: hydrogen bond acceptor (HBA), ring aromatic (RA), positively ionizable (PI). Mapping of the pharmacophoric features to inhibitors extracted from PDB ID 1tqf (b) and 2vnm (c) respectively.

For the validation of pharmacophore model for BACE-1, we took dataset of 287 compounds from binding database and divided the compounds into three different categories based upon their IC_{50} values. Hypo-1 screened highest number of actives and least number of inactives among other hypothesis, and is presented in Table 2 respectively. Thus we used Hypo-1 for VS of three different databases (Specs, NCI and IB Screen).

Sequential Virtual Screening

The strategy adopted for sequential virtual screening (VS) is depicted pictorially in Fig. 3. We have used three different small compound databases: Specs, NCI and IB Screen. The number of compounds present in these databases is shown in parentheses (Fig. 3).

The first step of VS was performed using AChE pharmacophore model, which filtered 9108 compounds from Specs database, 12,376 compounds from NCI database and 4,437 compounds from IB Screen database.

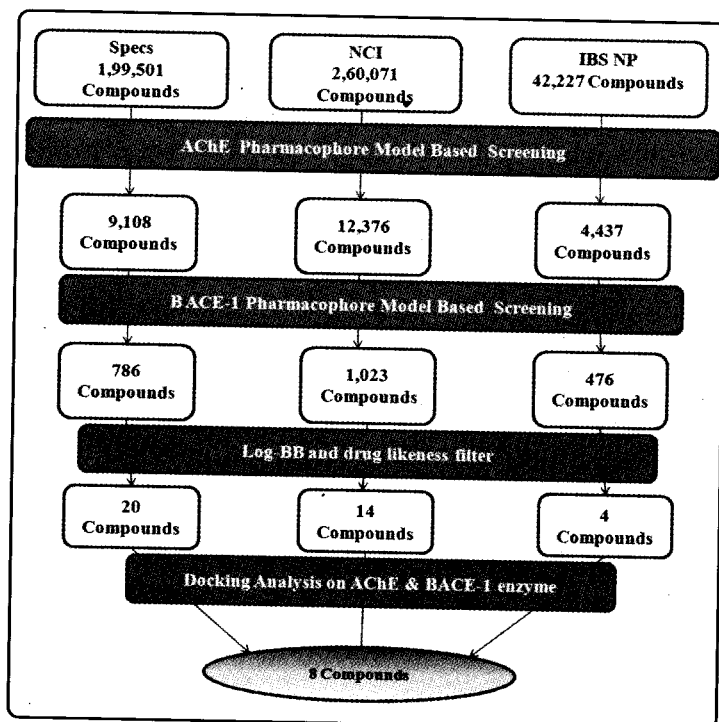


Fig. 3 : Different steps adopted in sequential virtual screening strategy.

Second step in VS involve filtering using BACE-1 pharmacophore model leading to 788 compounds (Specs), 1023 compounds (NCI) and 476 compounds (IB screen) respectively. The idea behind these two different AChE and BACE-1 pharmacophore model based filtering enabled us to identify MTD ligands from three different databases.

Third step involve filtering using the drug likeness and Log-BB value. Molecular descriptors corresponding to Lipinski's rule of 5 were calculated and any compound that violated more than 2 descriptors was rejected. Limit on the molecular weight of compounds was extended to 550 Da, instead of the upper limit of 500 Da, as suggested by Lipinski³. The model for blood-brain-barrier (BBB) penetration in Discovery studio software classifies compound as very high penetrants ($\log\text{-BB } e'' > 0.7$), high penetrants ($0 < \log\text{-BB} < 0.7$), medium penetrants ($-0.52 < \log\text{-BB} < 0$) and low penetrants ($\log\text{-BB } d'' < -0.52$)⁴. The filtered compounds include: 20 compounds (Specs), 14 compounds (NCI) and 4 compounds (IB Screen) from the databases. Last step in VS involve the molecular docking of these 38 compounds at the active site of the AChE and BACE-1 enzymes and ranking the compounds on the basis of docking score. Finally, 8 lead compounds were identified showing good docking scores on both the enzymes and also having satisfactory ADMET properties.

Molecular Docking Analysis

Flexible molecular docking simulations were performed with GOLD software, which uses a genetic algorithm⁵. For docking analysis, *Torpedo californica* AChE enzyme which is in complex with the Aricept ligand (PDB ID: 1eve, resolution 2.5 Å) was chosen, while for the BACE-1 enzyme (PDB ID: 2b8l, resolution 1.7 Å) which is in complex with the isophthalic-type Merck's inhibitor was used. Default setting was used in both the docking procedures and GOLD fitness score was used for compound prioritization obtained from VS hits. On the basis of the docking score and ligand protein interactions, eight final hits were selected which could be potent inhibitors of both AChE and BACE-1 enzyme. Compounds were evaluated based on the following criteria: 1) Potential for making bonding interactions with the active site residues, 2) Mode of binding at the active site with the available experimental crystal structures. Finally, five compounds from the Specs database, two compounds from the NCI database and one from the IB screen NP database were selected respectively.

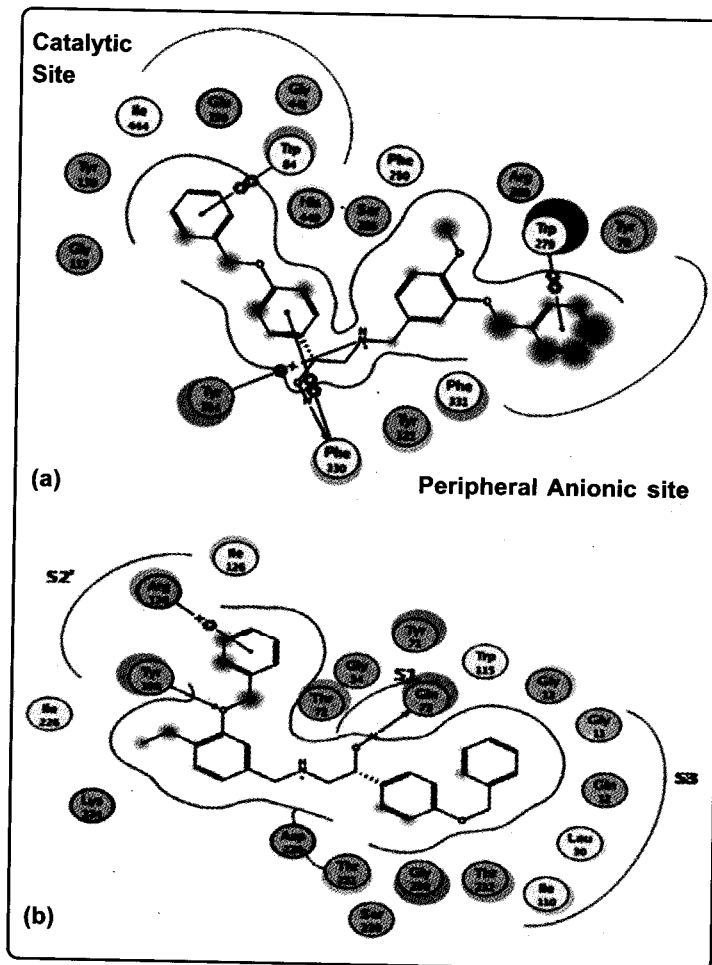


Fig. 4 : Binding site interactions of Compound 2 in the active site cavity of AChE enzyme (PDB code: 1eve) (a), and BACE-1 enzyme (PDB code: 2b81) (b). Amino acid residues are indicated using three-letters code. Only some of them are shown for simplicity.

These ligands were interacting with both the catalytic site and PAS of AChE. Docking pose of compound **2** with respect to the AChE and BACE-1 enzyme is depicted in Fig. 4(a, b). The binding orientation of compound **2** in AChE enzyme was comparable to Aricept (PDB code: 1eve) and making p-p interactions with the indole rings of Trp-84 and Trp-279 (Fig. 4a). In the middle of the gorge site the ligand is interacting with the active site by p-cation and p-p interactions with Tyr-334 and Phe-330 amino acid residues respectively.

Several studies suggested Phe-330 may serve as an additional quaternary binding site, midway down the gorge, between the PAS and the anionic subsite at the active site⁶. Dual binding site AChE inhibitors of this kind might play an important role in the symptomatic treatment of AD, since the interaction at PAS may inhibit the AChE induced α -amyloid aggregation, a characteristic pathological event in AD. In case of BACE-1 enzyme the ligand is making hydrogen bonding interactions with the Gln-73 of S1 pocket (Fig. 4b). The protonated nitrogen of the ligand is able to reach the acidic environment formed by the catalytic residues Asp-32 and Asp-228; its position inside the active site resembles that of protonated nitrogen of classical hydroxyl ethylamine inhibitors. S2' pocket is occupied by the benzyl group and further reinforcement by the p-p interaction with Arg-128, and hydrogen bonding interaction with the Tyr-198 amino acid residue in BACE-1 enzyme. S3 is completely occupied by benzyloxy group, contributing to hydrophobic and Van der Waals force within the site (Fig. 4b).

Conclusions

To explore novel effective drugs for the treatment of AD, different series of dual inhibiting compounds of AChE and BACE-1 were designed and investigated. After analysis of AChE and BACE-1 enzyme crystal structures and their importance in the AD, we developed a strategy to identify novel dual inhibitors of these enzymes. Combination of pharmacophore based sequential virtual screening and molecular docking helped us to identify dual inhibitors acting on both AChE and BACE-1 enzymes. Use of log-BB and drug-likeness filter enabled us to select the compounds which can cross BBB and possess drug like properties. Our proposed computational model developed from the present study should guide in the future design of more potent and selective multi-target directed inhibitors. In addition, we believe that the present methodology of identifying novel inhibitors of different AD drug targets would complement the existing *in vitro* methods, and should reduce heavy and expensive experimental measurements.

References

1. Clark DE, *Drug Discovery Today*. 2003, 20, 8, 927-933.
2. Li W, Mak M, Jiang H, Wang Q, Pang Y, *et al.*, *Neurotherapeutics*. 2009, 6, 1, 187-201.
3. Lipinski CA, *Drug Discovery Today*. 2004, 4, 337-341.
4. Zhu Y, Xiao K, Ma L, *et al.*, *Bioorganic & Medicinal Chemistry*. 2009, 17, 4, 1600-1613.
5. Bursulaya BD, Totrov M, *et al.*, *Journal of Computer-Aided Molecular Design*. 2003, 17, 11, 755-763.
6. Kryger G, Silman I and Sussman JL, *Structure with Folding & Design*. 1999, 7, 297-307.

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