



# Fragment based *de novo* design and ADME/T analysis of dual binding site acetylcholinesterase inhibitors for Alzheimer's disease

Shikhar Gupta, and C. Gopi Mohan\*

Department of Pharmacoinformatics  
National Institute of Pharmaceutical Education and Research (NIPER)  
Sector 67, S.A.S Nagar, Mohali, Punjab-160062, INDIA  
cmohan@niper.ac.in, cgopimohan@yahoo.com



## INTRODUCTION

- Alzheimer's disease (AD) is a chronic, irreversible and progressive neurodegenerative disorder with both genetic and non-genetic causes.
- Symptomatic treatment of AD involves use of Acetylcholinesterase (AChE) inhibitors.
- AChE can accelerate the assembly of A $\beta$  fibril formation by interaction through the peripheral anionic site (PAS).
- It has initiated a great interest towards the design and development of dual binding site inhibitors of both catalytic site (CS) consisting of the catalytic triad (S200, H440 and E327) together with W84 and F330 and PAS including Y70, Y121 and W279 of AChE enzyme.
- Fragment based drug discovery has gathered considerable momentum over the past few years.
- The geometry of the active-site gorge of AChE, with CS and PAS separated by 14 Å, makes it a particularly suitable target for applying fragment based approach.
- Hence, fragment based approach is best suited for designing of dual binding site AChE inhibitors (AChEIs).

## OBJECTIVES

- Selection and dissection of 20 diverse co-crystallized AChE inhibitors for fragment generation.
- Generation of new dual binding site AChEIs by fragment-based *de novo* design.
- AChEIs multi parameter optimization (AChEIs MPO) and Toxicity filtering of new dual binding site AChEIs to discard unwanted compounds.
- Molecular docking study to further prioritize the hits and obtained their binding conformation.
- Synthetic accessibility prediction of new dual binding site AChEIs.

## METHODOLOGY

- We have collected 20 diverse co-crystallized AChE inhibitors from protein data bank (PDB).
- We have used 31 fragments as seed structure, resulting from the dissection of 20 co-crystallized AChE inhibitors.

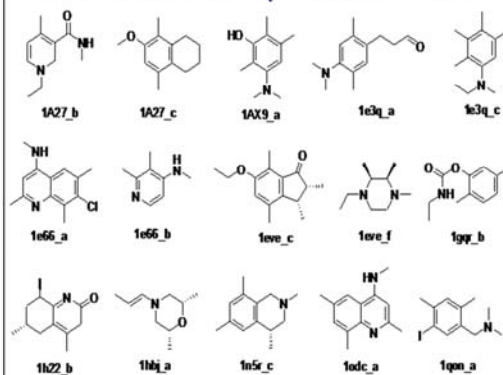


Fig. 1: Chemical Structures of some of the seeds used in the present study and initial 4 letters represent the PDB id of the AChEIs from which they obtained.

- The physicochemical properties of 31 seeds were calculated from Discovery Studio2.5 (DS 2.5), and Schrödinger software.
- Binding pocket of the active site was analyzed by using the POCKET module of Lig Builder software. The co-crystallized structure of Bis(7)Tacrine (PDB id: 2CKM) was used to define the pocket and a grid of 10 Å was defined.
- Dual binding site AChEIs were constructed by GROW module, from a seed structure through Lig Builder software, which were collected and PROCESS module were used for extracting the desired compounds.
- Generated compounds were filtered by AChEIs MPO approach.
- DEREK software was then used to filter the hits obtained from the above filters and access the toxicity of generated AChEIs.
- Molecular docking analysis was carried out using FlexX 3.1.1 software (BioSolve IT GmbH).
- Crystal structure of AChE complexed with donepezil (PDB id: IEVE) was chosen as reference protein with the active site having a radius of 10Å for docking study.
- SYLVIA software was used to access the synthetic feasibility of new AChEIs.

## RESULTS AND DISCUSSION

- All fragment seeds possessed properties consistent with the "Astex Rule of 3" (Number of hydrogen-bond donors  $\leq 3$ , Number of hydrogen bond acceptors  $\leq 3$ , and  $\log P \leq 3$ ).

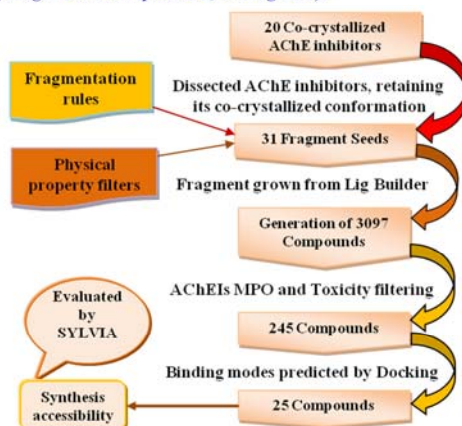


Fig. 2: Flow chart showing fragment based *de novo* designing strategy used in the present study.

- In addition, molecular weight (MW) was  $\leq 250$ , number of rotatable bonds was on average  $\leq 3$  and polar surface area was (PSA  $\leq 60 \text{ \AA}^2$ ) respectively.
- Based on 31 seeds, Lig Builder generated 3097 compounds.
- The generated 3097 compounds were filtered by our developed AChEIs MPO approach.
- AChEIs MPO approach suggested an optimum value for the seven physicochemical properties to be in the following range (i)  $\log P \leq 5$ ; (ii)  $\log D \leq 5$ ; (iii)  $350 \leq MW \leq 550$ ; (iv)  $PSA \leq 80 \text{ \AA}^2$ ; (v)  $0 \leq CNS \leq 2$ ; (vi)  $HBD \leq 2$ ; and (vii)  $3 \leq HBA \leq 6$  for prominent AChEIs.
- The sequential filtering by considering the above seven MPO reduced the hits to 245 compounds.

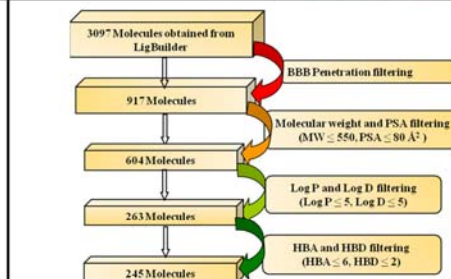


Fig. 3: Flow chart showing sequential AChEi MPO filtering strategy.

- These 245 compounds were further docked into the active site of AChE by FlexX and toxicity was predicted through DEREK software.

- Finally, we got 25 leads showing good docking score in AChE and no major toxicity as predicted by DEREK.

Table 1: Structure and physicochemical properties of the top 3 hits obtained by FBDD.

S.No.	Structure	ALogP	MW	HBA	HBD	PSA	CNS	LogD
1.		3.39	438.6	3	2	62.9	1	3.37
2.		4.78	489.6	4	2	70.6	2	3.93
3.		4.98	505.6	4	0	71.5	1	4.98

- These 25 leads were further evaluated for its synthetic accessibilities by the SYLVIA program.

## CONCLUSIONS

- Herein, we proposed a novel and comprehensive workflow of FBDD of dual binding site AChE inhibitors.
- The dissection of inhibitors of the same protein target is an interesting approach to select the best fragments for FBDD.
- Fragments were generated from 20 diverse co-crystallized AChE inhibitors according to the retrosynthetic combinatorial analysis procedure (RECAP).
- Also, we report our efforts to develop a AChE inhibitors multi parameter optimization (AChEIs MPO) approach. It does not focus on single end points or hard cutoffs.
- Molecular docking used as an additional tool for prediction of the binding conformations of new AChEIs.
- Finally, FBDD, AChEIs MPO approach followed by docking analysis strategy resulted 25 dual binding site AChEIs with all favorable drug-like properties.

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## ACKNOWLEDGEMENT

C. Gopi Mohan acknowledges the Department of Biotechnology (FD-Dy. No. 102/IFD/SAN/884/2006-2007) New Delhi, India for financial support of BIOINFARM project. Shikhar Gupta is recipient of Senior Research Fellowship from CSIR, India.