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Fragment based *de novo* Design and ADME/T Analysis of Dual Binding Site Acetylcholinesterase Inhibitors for Alzheimer's Disease

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Abstract

Fragment-based *de novo* design has been successfully carried out to identify novel dual binding site acetylcholinesterase inhibitors. Dataset consists of 18 co-crystallized inhibitors of the acetylcholinesterase enzyme from protein data bank. They were dissected into 31 chemically diverse and commercially available fragments. The newly generated compounds have been filtered through detailed ADMET analysis followed by molecular docking experiment. Finally, synthesis accessibility of the new leads was predicted by SYLVIA software. We identified 15 potential leads from the present study. These virtual screened compounds are expected to be important leads for the search of dual binding site acetylcholinesterase inhibitors and may provide invaluable insights to further understand the structural basis of catalysis and inhibition of acetylcholinesterase enzyme.

Keywords: Alzheimer's disease; acetylcholinesterase; fragment-based; Docking

Alzheimer's disease (AD), the most common form of dementia is an age-related neurodegenerative disorder characterized by progressive memory loss and other cognitive impairments (Goedert *et al*, 2006). Although the etiology of AD is unknown, histopathological hallmarks such as β-amyloid (Aβ) deposits, tau (τ) - protein aggregation, oxidative stress, and low levels of acetylcholine (ACh) seem to play significant role (Querfurth *et al*, 2010). So far, the cholinergic hypothesis is still the practical approach for treating AD. Cholinesterase inhibitors can reduce AD symptoms by inhibiting acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of ACh at the synaptic cleft (Racchi *et al*, 2004). Hence, acetylcholinesterase inhibitors (AChEIs) are widely used to alleviate symptoms of moderate AD patients (Barril *et al*, 2001). At present, several AChEIs, viz. tacrine, rivastigmine, donepezil and galanthamine have been approved by FDA for the

treatment of mild to moderate AD. On the other hand, memantine, an *N*-methyl *D*-aspartate receptor blocker was approved for the management of moderate-to-severe AD (Ibach *et al*, 2004). However, the clinical use of the AChEIs is limited primarily due to their adverse effects and modest benefits towards AD patients. Thus, novel and more effective treatment, including AChEIs, needs to be developed (Robichaud *et al*, 2006).

Recently, it has been reported that AChE accelerates Aβ aggregation which initiated a great interest towards the design and development of dual binding site inhibitors of both 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 (i.e. a dual binding site inhibitor) respectively. Moreover, it has been observed that dual binding site AChEIs are promising disease modifying AD drug candidates because they can simultaneously improve cognition and slows the Aβ aggregating activity of AChE (Reyes *et al*, 2004).

Small-molecule drug discovery has always been a struggle of attrition, but in the past few years pressure has mounted to increase efficiency at all stages of the process. A potential solution for lead identification and optimization, fragment-based drug discovery (FBDD), is becoming increasingly popular and emerged as an important methodology for the generation of high quality leads (Rees *et al*, 2004, Schulz *et al*, 2009, Zartler *et al*, 2005, Carr *et al*, 2005). In recent years, fragment based compounds have already yielded very promising results in Phase I clinical trials, including a Bcl-xL inhibitor from Abbott (ABT263) (Nienaber *et al*, 2000), a kinase inhibitor from Astex (AT9283) (Gill *et al*, 2004), a PPAR agonist from Plexxikon (PLX-204) (Card *et al*, 2005), and an HSP90 inhibitor from a collaborative efforts of Novartis and Vernalis (NVP-AUY- 922) (Jensen *et al*, 2008) respectively.

There are number of reported dual binding site AChEIs including bis(7)-tacrine the heptylene- linked tacrine dimer, which possessed both optimal AChE inhibitory potency and AChE/BuChE selectivity than tacrine (Pang *et al*, 1996), bis-huperzine B (Feng *et al*, 2005), bis-5-amino-5,6,7,8-tetrahydroquinolinone (Yu *et al*, 2008), and bis (-) nor-meptazinol (Xie *et al*, 2008) derivatives. The bis-galanthamine linked by alkylene was more potent than galanthamine in the AChE inhibition (Guillou *et al*, 2000). Also, hybrid of tacrine and huperzine A shows higher activity and selectivity for AChE enzyme compared to tacrine and huperzine A (Camps *et al*, 2000). The geometry of the active-site gorge of AChE, with CS and PAS separated by 14 Å and located at its two extremities, makes it a particularly suitable target for applying FBDD based approach (Sussman *et al*, 1991).

The main objective of the present work was to develop a novel approach of FBDD of dual binding site AChEIs that tries to fill the gap from fragment to ligand by

taking the advantage of a large number of available crystal structures of AChE enzyme with structurally diverse inhibitors. The 18 co-crystallized inhibitors of the AChE enzyme from PDB were selected and dissected into 31 chemically diverse and commercially available fragments with low molecular weight (≤ 300 Da). In the process of fragment generation by dissecting known co-crystallized AChEIs, we have retained the spatial positions and orientations of fragments for their 3D interactions with the AChE binding pocket. Hence, FBDD approaches allow for a greater coverage of chemical space and generally produce higher efficiency ligands. Its novelty lies in the fact that fragments obtained by chopping different co-crystallized inhibitors of the AChE enzyme from PDB and their bio-active conformations were used as building blocks for the generation of new dual binding site leads against AChE enzyme.

Materials and Methods

An outline of fragment based approach used in the present study is schematically depicted in Figure 1.

Fragment Generation

Compared to other *de novo* methods in FBDD, our strategy is unique to take advantage of both i.e. the interaction information from the PDB and chemical diversity for the generation of new dual binding site of AChEIs. In the first step, known structurally diverse co-crystallized AChEIs were superimposed on the bis (7)-tacrine bound co-crystal structure of AChE enzyme. Then other AChEIs were deconstructed into small fragments according to predefined fragmentation rules. The fragments that bound to the adjacent sites of the target protein were then grown to create the desired and novel compounds. AChEIs with good AChE inhibitory activities and structural diversity have fully probed and occupied almost all regions in the active site. In addition, without any artificial modifications, the generated component fragments will maintain their original spatial positions and orientations. Thus, the fragment binding conformations are more accurate than those predicted with fragment docking. We used structurally diverse 18 co-crystallized inhibitors of the AChE enzyme. AChEIs within the co-crystallized structures were superimposed onto the reference crystal structure used for the binding site generation. Therefore, the binding conformations of these AChEIs were well-aligned in the same dual binding site and extracted from the complex structures to generate molecular fragments. These were further used as seeds to generate new dual binding site AChEIs and their molecular structure are presented in Figure 2.

Compound deconstruction rules are also very important in fragment generation. Retrosynthetic combinatorial analysis procedure (RECAP) is a classical method that includes 11 fragmentation rules based on retrosynthetic chemistry knowledge (Lewell *et al*, 1998). Correct fragments were generated by dissecting the aligned

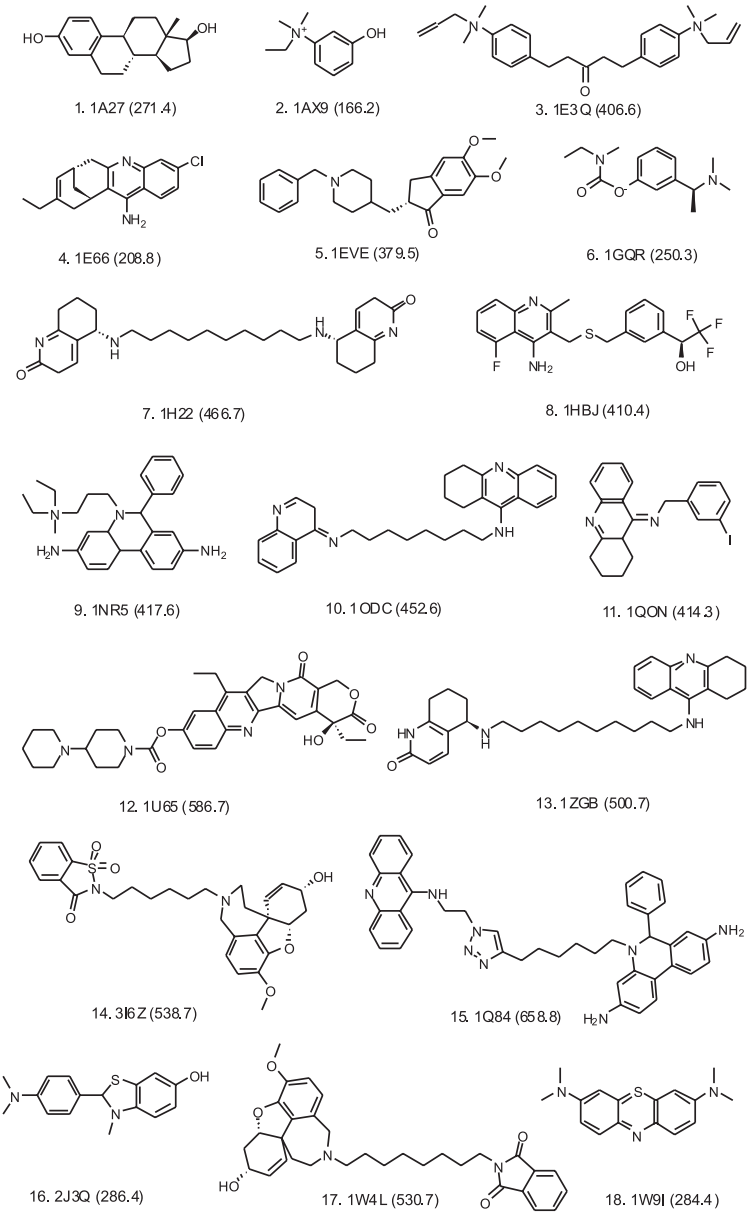

Fig. 1: Schematic representation of workflow performed in the present study.

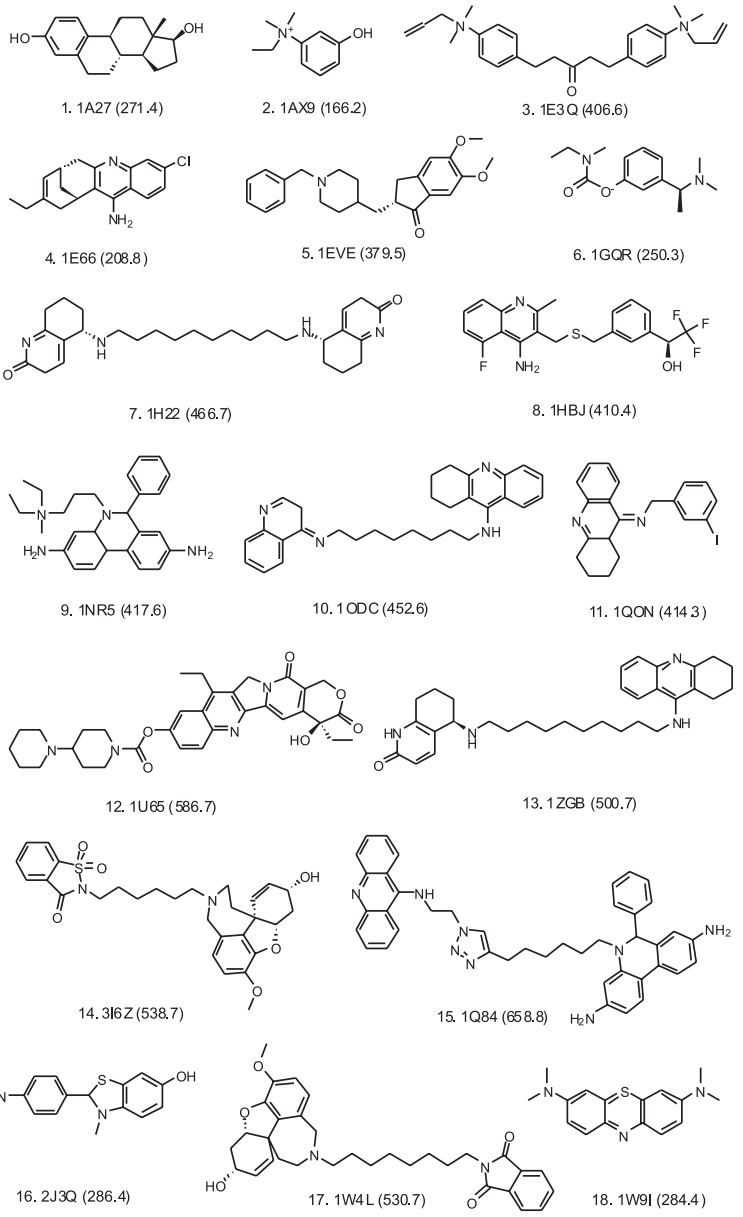

Fig. 2: Chemical structures of co-crystallized AChE inhibitors selected for defragmentation, along with their PDB ID and molecular weight (MW)

inhibitors with their binding conformations based on the RECAP procedure (Lewell *et al*, 1998). Accordingly, hydrogen bonds and ring-connecting bonds are never cleaved, and molecules with more than the defined "maximum atoms" (i.e., maximum number of atoms allowed in the molecule to be fragmented) are not

cleaved. All resultant fragments in the library are not only suitable for separate regions of the active site, but also maintain their original binding modes and conformations of the initial known inhibitors within the protein target active site.

New compounds generation

We used LigBuilder software to generate the new compounds. First, it analyzes the AChE binding pocket using the POCKET module of LigBuilder software (Wang et al, 2000). The pocket algorithm generates a pocket and the grid file which is used as an input for running GROW or LINK module to build ligand from the fragment. It also generates key interaction sites based on the key interacting atoms of the important active site residues. Along with this file a structure based pharmacophore is also generated. For pharmacophore generation the minimum distance between the features was kept at 3.5 Å, so as to avoid any steric and electrostatic clashes between protein residues and designed ligands. Maximum number of features in the pharmacophore was kept as eight, as more features will not be significant. After generating a pharmacophore map and the key site map by pocket module, next step is to build lead compounds by using these chemical features and key site information of AChE active site. To calculate the binding affinity, this program uses the SCORE algorithm. SCORE is an empirical procedure developed to estimate the binding free energy of a compound to its receptor protein when the 3D structure of the complex is known. PROCESS module ranks the compounds generated by the GROW module based on the SCORE function in the LigBuilder, which extract the desired compounds, and convert them to viewable mol2 files. LigBuilder has couple of built in chemical criteria to avoid "bad structures", such as -O-O-, -N-O-, -N-N-, and etc. Compounds meeting all these criteria will be considered for output. The new compounds were further evaluated by the ADMET filters.

ADMET filtering

We have computed the Lipinski's rule-of five and other pharmacokinetic properties of the compounds, which includes (Egan *et al*, 2004): distribution coefficient (Log D), computed aqueous solubility (Log S), polar surface area (PSA), percent human oral absorption, BBB penetration, and CNS activity using ADME module of Discovery studio DS2.5 software (Accelrys: 2.5, CA, 2006). The rule-of-five suggested that a chemical compound could be an orally active drug for humans. Toxicity profiles of the compounds were assessed using DEREK software (DEREK 10.0, U.K., 2010)**.** DEREK is a knowledge-based expert system for the qualitative prediction of toxicity. DEREK makes its predictions based on a series of rules and each rule describes the relationship between a structural feature and its associated toxicity (DEREK 10.0, U.K., 2010)**.** Further, docking analysis was performed on

the generated compounds to priorities the hits and to identify their dual binding site nature.

Molecular docking

GOLD (genetic optimization for ligand docking) program (Verdonk *et al*, 2003) was used for docking study and the GOLD score option was selected as the fitness function, denoted in short form as GOLD fitness score (GFS). The X-ray coordinates of donepezil bound to the active site of the AChE enzyme were used to define the active site region with an active site radius of 9.5 Å, by keeping all the water molecules within the active site. The annealing parameters of van der Waals and hydrogen-bonding interactions were considered within 4.0 Å and 2.5 Å, respectively, and other parameters were kept at the default setting. GOLD has shown good reproducibility of experimentally observed binding conformation of donepezil. In our study the superimposition of the docked donepezil onto the crystallographic geometry yielded RMSD of 0.55 Å which satisfies our docking protocol. Further, the best conformation of each selected compound comprises the output on the basis of the GFS score and interactions formed between the ligands and the active site. Finally promising leads were obtained by several methods including ADMET analysis, molecular docking, and ranked compounds were further evaluated for their synthesis accessibility by SYLVIA software (SYLVIA, Molecular Networks GmbH).

Synthesis accessibility prediction

In order to evaluate the synthesis feasibility of our designed leads, SYLVIA software was used (SYLVIA, Molecular Networks GmbH). SYLVIA scores compounds from 1 (easy to synthesize) to 10 (difficult to synthesize) on the basis of synthesis accessibility. Scoring function in SYLVIA includes- different structural complexity measures, retro synthetic reaction fitness and different measures of similarity to available starting material (Boda *et al*, 2007).

Results and Discussion

Fragment Generation

FBDD approach offers several advantages over conventional drug design methods as fragment libraries are more diverse and synthetic resources are used more efficiently (Congreve *et al*, 2008, Law *et al*, 2009). Eighteen representative complex structures were selected according to the structural diversity of the co-crystallized ligands (Figure 1- Inhibitors **1** to **18**). In the dissection process of inhibitors, the fragments were chosen on the basis of their resemblance to the initial lead, and their small size (<300 Da). Physicochemical analysis of the fragment seeds shows that all fragments possessed properties consistent with the ‘‘Astex Rule of 3’’

(Number of hydrogen-bond donors ≤ 3, Number of hydrogen-bond acceptors ≤ 3, and logP ≤ 3). In addition, it was noted that all fragments also possess molecular weight (MW) ≤ 300, number of rotatable bonds on average ≤ 3 and polar surface area was (PSA ≤ 60 Å^2) respectively.

We generated 31 fragments as seed structure from the dissection of 18 co-crystallized AChEIs, which were used as seed structures for the generation of new dual binding site AChEIs and the fragments were kept as simple as possible. The structure of 31 fragments are shown in Figure 3 and fragments are denoted as, for example, 1A27_a fragment a, where 1A27 is the PDB ID of the initial co-

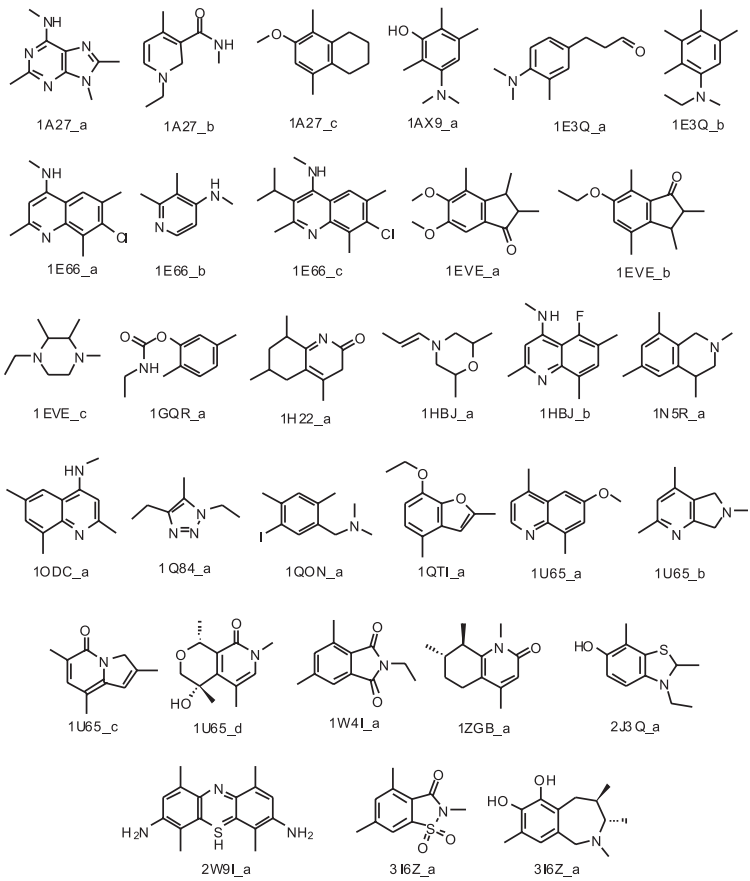

Fig. 3: Chemical structures of 31 fragments resulting from the deconstruction of the co-crystallized AChE inhibitors along with their PDB ID and fragment name

crystallized AChEI and 'a' is the serial number of the generated fragment. We have also calculated the physicochemical properties of 31 fragment seeds using DS2.5 program (Accelrys: 2.5, CA, 2006).

The bis (7)-tacrine bound co-crystal structure of AChE enzyme (PDB ID: 2CKM) was used to describe the binding pocket having a grid size of 10 Å. New AChEIs were originated from a fragment "seed" structure based on the Genetic Algorithm (GA) procedure, which runs under generational-replacement mode of LigBuilder Software (Wang *et al*, 2000). To allow the growth of fragment seed into new compounds the growing site must be defined in the seed structures. Two criteria were taken into considering while defining the site, first the growth of compound should not result in steric clashes with the amino acids in the active site and it could grow towards the PAS. Each seed allowed to grow on the selected grow points. We have selected different hydrogen atoms in all the 31 seeds as growing points. GROW module was used to generate ligands based on its inbuilt fragment library, which contains various molecular fragments as building-blocks to build ligands. This library contains nearly 60 fragments, including most of the common chemical groups and ring frameworks observed in organic compounds. We allowed the molecules to grow up to 50 generations. The compounds generated by GROW module were collected and used as an input for the PROCESS module.

New compounds generation

LigBuilder software was used to design new AChEIs by considering the structural requirements of AChE enzyme active site (Wang *et al*, 2000). We generated 3097 new compounds by the GROW module of LigBuilder from 31 fragment seeds as shown in Figure 1. For fragment growing, functional groups are added to the initial detected seed fragment, guided by fragment binding orientation. Fragment binding in the proximal regions in active site are obtained by deconstructing the known AChEIs, which might be beneficial for improving the synthetic feasibility of the new compounds. The bis(7)-tacrine bound co-crystal structure of AChE enzyme (PDB ID: 2CKM) having resolution 2.15 Å is taken as a reference structure for building the ligands (Pang *et al*, 1996). This structure is selected as a target structure because bis (7)-tacrine was the first purposely designed potent and selective dual binding site inhibitor of AChE enzyme by utilizing computer modeling of ligand docking with the target proteins. This was done to identify low affinity sites normally missed by X-ray crystallography and design bi-functional analogs capable of simultaneous binding at the CS and PAS respectively (Pang *et al*, 1996). Applying this strategy to 9-amino-1,2,3,4-tetrahydroacridine (THA) or tacrine, a drug for AD, the alkylene linked bis-THA analogs were discovered. These analogs were up to 10,000-fold more selective and 1,000-fold more potent than THA in inhibiting AChE enzyme.

ADMET filtering

ADMET (absorption, distribution, metabolism, excretion, toxicity) properties of the compounds at the early stages of the discovery are very important indicator for

selecting the compounds for further studies (Alavijeh *et al*, 2005). The physico-chemical properties linked with compounds that have good blood-brain barrier (BBB) penetration, optimum oral bioavailability, less or no toxicity and also optimum solubility are the significant filters for selecting CNS active compounds and there is a need for compounds with good pharmacokinetic properties (Klebe *et al*, 2006). After carefully analyzing the ADMET properties of 3097 compounds obtained from the FBDD strategy by LigBuilder, we finally selected 245 hits, which were not showing any toxicity or less toxicity predicted by the DEREK software (DEREK 10.0, U.K., 2010). Finally, hits having good pharmacokinetic parameters and CNS activity were carried forward for structure-based evaluation by molecular docking analysis at the dual binding site of the AChE enzyme.

Molecular Docking and Synthesis accessibility prediction

Molecular docking is a computationally intensive structure-based technique that generates and scores putative protein–ligand complexes according to their calculated binding affinities (Mizutani *et al*, 2004). It has been successfully used for identifying active compounds by filtering out those that do not fit into the binding sites. Among the available crystal structures of the AChE enzyme, docking studies were performed on the *Torpedo californica* AChE (*Tc*AChE) structure (PDB ID: 1EVE), considering that size and shape complementarity as well as the dual binding site nature of the donepezil was similar to xanthostigmine. *Tc*AChE has almost identical amino acid residues with the *human*AChE (*h*AChE) (PDB ID: 1B41) (Kryger *et al*, 2000) at both the CS and PAS, apart from the substitution of F330 (*Tc*) with Y337 (human).

In order to further priorities the retrieve 245 hits from ADMET analysis and subsequently to identify the true/ (or false) positives docking studies were performed at the dual binding site of AChE enzyme using GOLD program. Docking results were reported as the highest scoring pose for each compound, and also on the basis of their ability to form favorable interactions within the active site of AChE enzyme. Based on the GOLD fitness scoring (GFS) function finally 15 compounds were filtered as potential leads. These leads were further filtered using synthesis accessibility studies. All the hit compounds possessed the GFS higher or equal to 52 kJ/mol (GFS $\geq$ 52 kJ/mol). The hit compounds showed excellent interactions with the critical residues of PAS and CS of AChE. Different ADMET parameters of these 15 compounds and their corresponding GFS values are given in Table 1. The structures of these 15 lead compounds are presented in Figure 4.

Table 1: ADMET parameters of designed lead compounds obtained by FBDD approach

S. No.	[a]Toxicity	[b]ADMET_ BBB	[c]logS	[b]AlogP	[b]logD	[b]PSA	[c]% H_ O_Abs	GFS
1.	None	1	-4.87	4.40	4.30	55.4	100.0	64.4
2.	None	1	-4.80	4.05	4.05	79.6	100.0	70.2
3.	HERG	2	-2.48	2.90	2.87	96.3	73.7	65.9
4.	None	2	-4.43	3.04	3.04	77.6	100.0	66.2
5.	HERG	1	-4.98	4.83	4.73	71.8	100.0	72.5
6.	None	2	-4.13	3.01	3.45	56.3	82.8	58.9
7.	HERG	1	-3.93	4.44	4.44	73.6	90.6	58.1
8.	Skin sensitisation	1	-4.37	4.99	4.99	72.6	88.3	54.4
9.	None	2	-4.26	2.94	3.37	56.3	83.7	52.1
10.	None	1	-4.51	4.37	3.05	64.1	100.0	53.1
11.	HERG	1	-4.09	1	4.72	4.6	61.2	66.3
12.	Skin sensitisation	1	-6.07	2	4.24	4.2	74.1	61.2
13.	Skin sensitisation	2	-6.00	2	4.88	4.9	90.1	57.3
14.	None	2	-4.15	1	4.18	4.2	81.7	59.5
15.	None	2	-3.46	0	3.01	2.7	96.2	61.3

[a]Toxicity prediction using DEREK software;
[b]Prediction using Schördinger software;
[c]Prediction using Discovery studio ADME module software;
logS = Predicted aqueous solubility, log S on a –6.5 to 0.5;
% H_O_Abs = Percent Human Oral Absorption;
AlogP = Log of the octanol-water partition coefficient using Ghose and Crippen's method;
logD =The octanol-water partition coefficient calculated taking into account the ionization states of the compound;
PSA = van der Waals surface area of polar nitrogen and oxygen atoms.

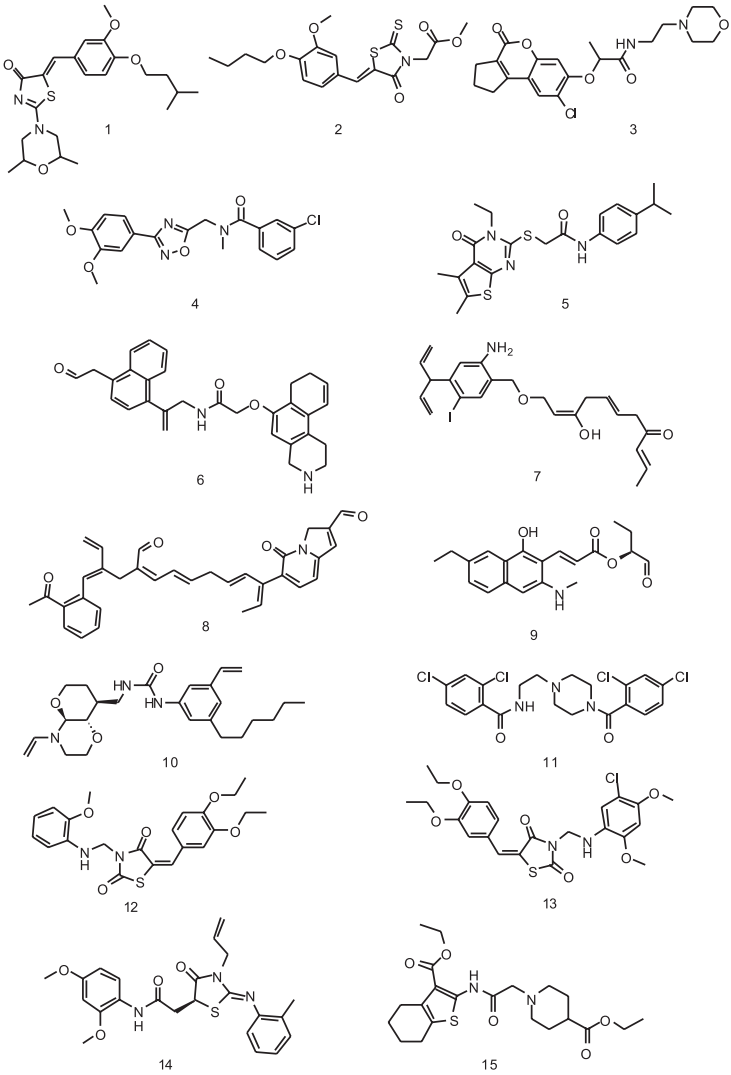

Fig. 4: Chemical structures of 15 designed compounds by fragment based *de novo* design

The synthesis feasibility of 15 prioritized newly generated AChEIs was predicted by SYLVIA software. All 15 compounds were showing SYLVIA score below 7 suggesting its better predictive synthetic feasibility. The binding orientation of the best designed compound **5** (Table 1, Figure 4) from FBDD approach, is shown in Figure 5. It showed highest GFS of 72.5 kJ/mol and formed π-π stacking interactions between the benzene ring of compound **5** and indole ring of W84 (CS) in AChE enzyme (Figure 5). This result is in agreement with the earlier reported AChE-donepezil complexes in which benzene ring of donepezil

formed π-π stacking interaction with the W84 of AChE enzyme. In addition, two water mediated hydrogen bonding interactions were also observed between (i) the carbonyl oxygen (C=O) of compound **5** and hydroxyl group of Y121 through water (WAT1157), and (ii) second water (WAT1350) mediated hydrogen bonding interaction was observed between one of the nitrogen atom of thienopyrimidine ring of compound **5** and carboxylate group of D72, in the middle of the gorge site (CS) of the AChE enzyme as shown in Figure 5.

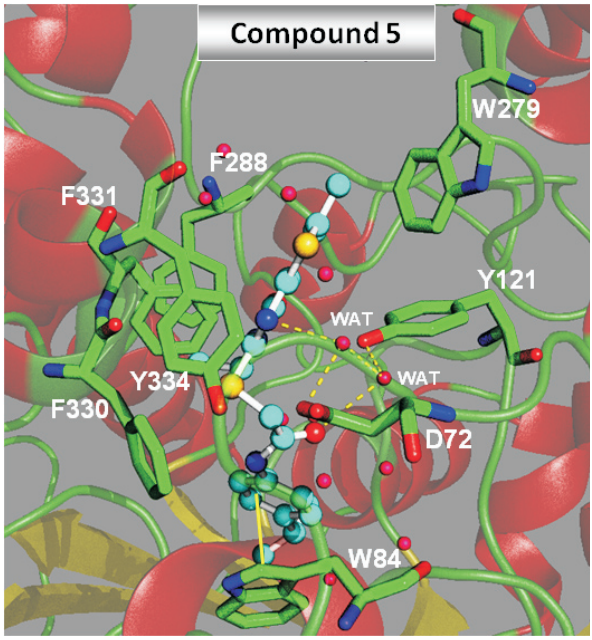

Fig. 5: Molecular docking derived binding pose of the lead compound 5 in the dual binding site (CS and PAS) of AChE enzyme. The inhibitor is shown as ball and stick model in the surface representation of the enzyme. Water compounds are shown as red dotted spheres. Hydrogen bonding is shown as dotted lines (yellow color) and π-π interaction by straight line (yellow color). GOLD software was used to derive the binding mode and the picture was generated from PyMOL software.

Conclusion

In summary, we report here a novel FBDD approach to generate new dual binding site AChEIs followed by ADMET and docking analysis. The present study revealed that the dissection of co-crystallized inhibitors is an interesting approach to select the best fragments in terms of binding efficiencies and synthetic feasibility. Rules similar to those used in RECAP were applied in fragment generation process and suitable fragments that bound simultaneously to CS and PAS of the AChE binding site were obtained to satisfy the primary requirement of this strategy.

These fragments were used as seeds to generate 3097 new dual binding site leads from the GROW module of LigBuilder. These new leads were further evaluated by ADMET and molecular docking analysis to obtained hits with good physico-chemical properties and access their interactions in the dual binding site of AChE enzyme. Finally, the synthetic feasibility of these leads was evaluated by SYLVIA software.

Our FBDD strategy identified 15 new promising leads. Moreover, these leads have all the properties required for drug-like compounds acting as AChEIs. Molecular docking study of compound **5** allowed us an in depth analysis, and interpretation of the dual binding site interactions of these inhibitors with the AChE enzyme. The effective strategy described in this work could be an invaluable tool in the discovery and optimization of potential drug like leads against specific targets.

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