

## BALANCING ANTI-AMYLOID AND ANTI-CHOLINESTERASE CAPACITY IN A SINGLE CHEMICAL ENTITY: *INSILICO* DRUG DESIGN

<sup>1</sup>\*PAVADAI PARASURAMAN, <sup>1</sup>RAMALINGAM SURESH, <sup>2</sup>DHANARAJ PREMNATH

<sup>1</sup>Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu 608002, <sup>2</sup>Department of Bioinformatics, Karunya University, Karunya Nagar, Coimbatore, Tamil Nadu 641114, India. Email: pvpam@gmail.com

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### ABSTRACT

**Objective:** Alzheimer's disease is currently deliberation to be a complex, multi factorial syndrome, unlikely to take place from a single causal factor; instead, a number of allied biological alterations are thought to put in to its pathogenesis. This may clarify why the currently available drugs, developed according to the archetypal drug discovery paradigm of "one-molecule-one-target," have turned out to be palliative. In light of this, drug combinations that can act at different levels of the neurotoxic cascade offer new avenues in the direction of curing Alzheimer's and other neurodegenerative diseases. In similar, a new strategy is promising that of developing a single chemical entity able to amend multiple targets simultaneously. This has lead to a new paradigm in medicinal chemistry, the "multi-target-directed ligand" design strategy, which has previously been successfully exploited at both academic and industrial levels.

**Method:** Flavone fused thiazole moiety were docked against AChE and BACE enzyme Using Schrodinger software.

**Results and Conclusion:** As a case study, we report here on 1-(5-(3, 5, 6, 7, 8-pentahydroxy-4-oxo-4H-chromen-2-yl) benzo[d]thiazol-2-yl)-3-(2-oxobutyl) urea (PS122), a new molecule developed following this strategy. The *in-silico* profile of 1-(5-(3, 5, 6, 7, 8-pentahydroxy-4-oxo-4H-chromen-2-yl) benzo[d]thiazol-2-yl)-3-(2-oxobutyl) urea demonstrates the suitability of the new strategy for obtaining innovative drug candidates for the treatment of neurodegenerative diseases as it inhibit both AChE and BACE enzyme through *in-silico* studies.

**Keywords:** *InSilico*, Alzheimers disease, Flavones, Thiazole, Multi-targeted.

### INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is a complex neurodegenerative disorder [1, 2]. The fact that complexity and multiple etiologies of AD make single-target strategy difficult to shed desirable therapeutic effect makes the choice of Multi-Target-Directed Ligand (MTDL) to be a potentially more effective strategy [3]. MTDL, whose goal is to enhance efficacy and improve safety, is rationally designed to hit multiple targets for a particular disease to improve pharmacological profiles [4-6]. Until now, most drugs approved for AD treatment are AChE inhibitors, which improve the ACh level in the brain by decreasing the hydrolysis of ACh. Amyloid- $\beta$  ( $A\beta$ ), formed by the continuously proteolytic processing of  $\beta$ -amyloid precursor protein (APP) by  $\beta$  and  $\gamma$ -secretase [7, 8], plays a central role in the pathogenesis of AD. Recent evidence indicated certain links between  $A\beta$  and AChE [9]. On one hand, the presence of  $A\beta$  increases AChE activity, and on the other hand, AChE could form a complex with  $A\beta$ , which changes the conformation of  $A\beta$  and then promotes the aggregation  $A\beta$ . Thus, simultaneously inhibition of AChE and  $\beta$ -secretase (BACE 1) not only reduces  $A\beta$  generation and hydrolysis of ACh but also weakens the interaction between ACh and  $A\beta$ .

It is critical to recognize, however, that combining two or more drugs always raises the potential for greater side effects. These may include the known side effects of each drug, or completely unexpected side effects caused by interactions between the respective drugs [10]. Another therapeutic option is now emerging, based on the paradigm that a rationally designed, single molecule may possess multiple concomitant biological properties. Clearly, therapy with such a single multimodal drug would have inherent advantages over combination therapy [11]. Such therapy would prevent the challenge of administering multiple single-drug entities that could have different bioavailability, pharmacokinetics, and metabolism. Furthermore, in terms of pharmacokinetic and toxicological optimization, the clinical development of a drug able to hit multiple targets should not, in principle, be different from the development of any other single lead molecule. This therapy thus offers a far more simple approach than combination therapy. In addition, the risk of possible drug-drug interactions would be reduced and the therapeutic regimen greatly simplified, with the

prospect of enhanced patient compliance, which is a critical issue in AD care [12]. The development of an effective multimodal treatment is, however, not so clear cut. Neurodegeneration in AD is the result of a several-step process, and addressing with a single drug the molecular dynamics of disease progression is not an easy task. When designing a new chemical entity, one should keep in mind that AD has many stages of progression, and it is crucial to assess the relationship between the progression timeline and a specific molecular target [13].

Considering the above, we focused on Multi-Target-Directed Ligands by incorporating two different heterocyclic (Benzthiazole & Flavone), which have effect on AChE and BACE through molecular docking studies. Benzothiazole derivative KHG21834 is neuroprotective against the  $A\beta$ -induced degeneration of neuronal cells *in vitro* and *in vivo* [14]. Natural flavonoids are well known antioxidants. In addition, various studies have reported the defensive effects of natural polyphenol, counting flavonols and flavones, against various insults, such as  $A\beta$ . It also acts as free radical scavengers and neuronal cell protectors to oxidative damage [15].

### MATERIALS AND METHOD

#### Protein Structure Preparation

The X-ray crystal structures of Acetyl Cholinesterase (PDB: 3LII) and  $\beta$ -Secretase BACE (PDB: 3U6A) retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (Figure 1 and 2) was used in this study. Water molecules of crystallization were detached from the composite and the protein was optimized for docking using the protein preparation and refinement utility provided by Schrödinger LLC. Partial atomic charges were assigned according to the OPLS-AA force field [16].

#### Ligand structure preparation

The structures of 243 flavone fused thiazole analogues, (Figure 3) were constructed using the splinter dictionary of Maestro 9.3 (Schrodinger, LLC) using the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) force field with the steepest descent followed by curtailed Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-AA force field.



Fig. 1: X-ray crystal structure of protein Acetyl Cholinesterase (AChE) enzyme with inhibitor (PDB ID: 3LIH)

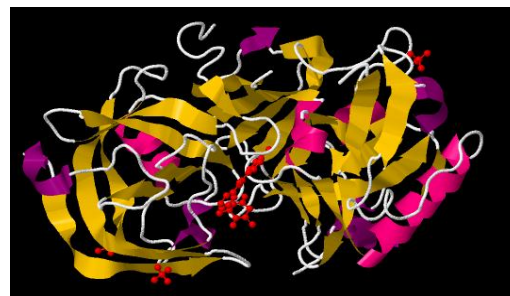
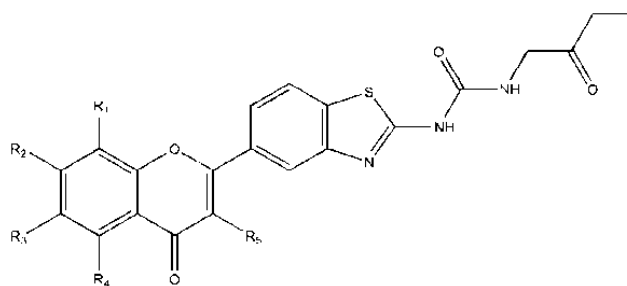


Fig. 2: X-ray crystal structure of protein  $\beta$ -Secretase BACE with inhibitor

(PDB ID: 3U6A)



Where  $R_1, R_2, R_3, R_4, R_5 = -H$  or  $-OH$  or  $-OCH_3$

Fig. 3: Designed MTDL for inhibiting both AChE & BACE

#### Docking Protocol

All docking calculations were performed using the "Extra Precision" (XP) mode of GLIDE program. The binding site, for which the various energy grids were calculated and stored, is defined in terms of two concentric cubes: the bounding box, which must contain the center of any acceptable ligand pose, and the enclosing box, which must contain all ligand atoms of an acceptable pose, with a Root Mean Square Deviation (RMSD) of less than 0.5 Å and a maximum atomic displacement of less than 1.3 Å were eliminated as redundant in order to increase diversity in the retained ligand poses.

The scale factor for van der Waals radii was applied to those atoms with absolute partial charges less than or equal to 0.15 (scale factor of 0.8) and 0.25 (scale factor of 1.0) electrons for ligand and protein, respectively. The max keep variable which sets the maximum number of poses generated during the initial phase of the docking calculation were set to 5000 and the keep best variable which sets the number of poses per ligand that enters the energy minimization was set to 1000. Energy minimization protocol includes dielectric constant of 4.0 and 1000 steps of conjugate gradient. Upon completion of each docking calculation, at most 100 poses per ligand were generated.

The best docked structure was chosen using a GLIDE score (Gscore) function. Another scoring function used by GLIDE is E-model, which itself derived from a combination of the Gscore, Coulombic, van der Waals and the strain energy of the ligand.

#### QikProp analysis

QikProp efficiently evaluates pharmaceutically relevant properties for over half a million compounds per hour, making it an indispensable lead generation and lead optimization tool. Accurate prediction of Absorption, Distribution, Metabolism, Elimination (ADME) properties prior to expensive experimental procedures, such as High Throughput Screening (HTS), can eliminate unnecessary testing on compounds that will ultimately fail; ADME prediction can also be used to

focus lead optimization efforts to enhance the desired properties of a given compound.

#### RESULTS AND DISCUSSION

##### Results from Qikprop

The ADME properties of the designed ligands were predicted using QikProp. The compounds prepared were subjected to drug-likeness filter. The criteria of the filter includes molecular weight 407- 557, number of heavy atoms 20-70, Number of hydrogen bond donors 1-5, Number of hydrogen bond acceptors 7-11. All the designed ligands conformed to the above mentioned criteria and they were evaluated for docking using GLIDE software.

##### Receptor grid generation

GLIDE receptor grid was generated to determine the size of the active site. The most probable orientation of the ligands in the binding pocket is identified and a scoring function is used to quantify the strength of the interaction a molecule can make in a particular orientation. In order to provide better correlation between good poses and good scores, the GLIDE XP precision was favored over the standard mode.

##### Validation of the docking protocol

The docking analysis was done for the ligands such with the target protein Acetyl Cholinesterase and  $\beta$ -Secretase BACE using the docking software GLIDE and the docked images are shown. The structures docked by GLIDE are generally ranked according to the GLIDE Scoring Function (more negative).

The scoring function of GLIDE docking program is presented in the G-score form. The most straightforward method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation) predicted by the object scoring function. In the present study, Extra Precision GLIDE docking procedure was validated by removing the inhibitor compound with Acetyl Cholinesterase and  $\beta$ -Secretase BACE protein has been analyzed from the G-score, GLIDE energy and H-bonds.



Fig. 8: Structure of potent lead PS122

**CONCLUSION**

Multiple, interrelated, biochemical pathways are thought to contribute to the neurodegenerative process of AD. **PS122** is a new drug candidate that was designed and docked against AChE and BACE in a deliberate attempt to find an MTDL able to interfere with different key target points of AD neurodegeneration. In conclusion, we have identified a best molecule of Poly hydroxy flavanoid fused thiazole derivative **PS122** as a single chemical entity to inhibit both AChE & BACE through docking studies. This compound **PS122** is considered as a best (lead) molecule, and we need to synthesis for further studies.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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**REFERENCE**

1. Aurn K G, Nagaswamy K, Jordan T. Recent Developments of Structure Based  $\beta$ -Secretase Inhibitors for Alzheimer's disease. *Curr Top Med Chem* 2005; 5:1609-22.
2. Tao G, Doug W H. Development of BACE1 Inhibitors for Alzheimer's disease. *Curr Med Chem* 2006; 13:1811-29.
3. Morphy R, Rankovic Z. Designing Multiple Ligands - Medicinal Chemistry Strategies and Challenges. *Curr Pharm Design* 2009; 15:587-600.
4. Bolognesi ML, Cavalli A, Valgimigli L, Bartolini M, Rosini M, Andrisano V, Recanatini M, Melchiorre C. Multi-target-directed drug design strategy: from a dual binding site acetylcholinesterase inhibitor to a trifunctional compound against Alzheimer's disease. *J Med Chem* 2007; 50:6446-49.
5. Zhu YP, Xiao K, Ma LP, Xiong B, Fu Y, Yu HP, Wang WD, Hu Y, Peng HL, Li JY, Gong Q, Chai Q, Tang XC, Zhang HY, Li J, Shen JK. Design, synthesis and biological evaluation of novel dual inhibitors of acetylcholinesterase and beta-secretase. *Bioorg Med Chem* 2009; 17:1600-13.
6. Marco-Contelles J, León R, de los Ríos C, Samadi A, Bartolini M, Andrisano V, Huertas O, Barril X, Luque FJ, Rodríguez-Franco MI, López B, López MG, García AG, Carreiras M do C, Villarroya M. Tacipyridines, the First Tacrine–Dihydropyridine Hybrids, as Multitarget-Directed Ligands for the Treatment of Alzheimer's Disease. *J Med Chem* 2009; 9:2724-32.
7. John V, Beck JP, Bienkowski MJ, Sinha S, Heinrikson RL. Human beta-secretase (BACE) and BACE inhibitors. *J Med Chem*. 2003; 46:4625-30.
8. Kornilova AY, Wolfe MS. Secretase inhibitors for Alzheimer's disease. *Annu Rep Med Chem* 2003; 38:41-50.
9. Magdolna P, János K. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochem Int* 2008; 53:103.
10. Toews ML, Bylund DB. Pharmacologic principles for combination therapy. *Proc Am Thorac Soc* 2005; 2:282–289; discussion 290–291.
11. Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, et al. Multi-target-directed ligands to combat neurodegenerative diseases. *J Med Chem* 2008; 51:347–372.
12. Small G, Dubois B. A review of compliance to treatment in Alzheimer's disease: potential benefits of a transdermal patch. *Curr Med Res Opin* 2007; 23:2705–2713.
13. Van der Schyf CJ, Mandel S, Geldenhuys WJ, Amit T, Avramovich Y, Zheng H, et al. Novel multifunctional anti-Alzheimer drugs with various CNS neurotransmitter targets and neuroprotective moieties. *Curr Alzheimer Res* 2007; 4:522–536.
14. Choi MM, Kim EA, Hahn HG, Nam KD, Yang SJ, Choi SY, Kim TU, Cho SW, Huh JW. Protective effect of benzothiazole derivative KHG21834 on amyloid-induced
15. Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H. Flavonols and flavones as BACE-1 inhibitors: Structure–activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. *Biochim Biophys Acta*. 2008; 1780:819–825.
16. Andurmila Joshi, Manoj Kumar Gadhwal, Swati Patil, Priscilla D Mello. Homology Modeling of Aryl Hydrocarbon Receptor and Docking of Agonists and Antagonists. *Int J Pharm Pharm Sci*, 2013; 5(2):76-81.