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

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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-β (Aβ), formed by the continuously proteolytic processing of β-amyloid precursor protein (APP) by ß and γ-secretase [7, 8], plays a central role in the pathogenesis of AD. Recent evidence indicated certain links between Aβ and AChE [9]. On one hand, the presence of Aβ increases AChE activity, and on the other hand, AChE could form a complex with Aβ, which changes the conformation of Aβ and then promotes the aggregation Aβ. Thus, simultaneously inhibition of AChE and β-secretase (BACE 1) not only reduces Aβ generation and hydrolysis of ACh but also weakens the interaction between ACh and Aβ.

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 various insults, such as Aβ. It also acts as free radical scavengers and neuronal cell protectors to oxidative damage [14]. Natural flavonoids are well known anti-oxidants. In addition, various studies have reported the defensive effects of natural phenol, counting flavonoids and flavones, against various insults, such as Aβ. 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 β-Secretase BACE (PDB: 3J6A) 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 (Schrödinger, 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.

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 archetypical 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) benzoyl)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) benzoyl[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.
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 β-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 β-Secretase BACE protein has been analyzed from the G-score, GLIDE energy and H-bonds.
To study the molecular basis of interaction and affinity of binding of flavones fused thiazole analogues to Acetyl Cholinesterase and β-Secretase BACE protein, all the ligands were docked into the active site of Acetyl Cholinesterase and β-Secretase BACE. The docking result of these ligands is given in table 1. The interaction energy includes van der Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding were calculated for each minimized complex. The docking score using GLIDE varied from -0.16 to -7.5 against acetyl cholinesterase and -1.6 to -9.3 for BACE. The GLIDE Score for, a standard Donepezil, Galantamine, Rivastigmine and Tacrine docked with Acetyl cholinesterase was -4.97, -5.1, -2.64 & -4.3 and with BACE -5.8, -5.4, -3.04 & -6.1.

This proves that flavones fused thiazole analogues could be potential drugs for Anti-Alzheimer drug development. The GLIDE score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specific conformation of the protein receptor. Generally speaking, for low GLIDE score, good ligand affinity to the receptor may be expected. 1-(5-［3,5,6,7,8-pentahydroxy-4-oxo-4H-chromen-2-yl]benzo[d]thiazol-2-yl)-3-(2-oxobutyl)urea (PS122) showed the best the inhibition for the Acetyl Cholinesterase with -7.5 glide score and β-Secretase BACE with -9.3 glide score protein receptor. We found a very good agreement between the localization of the inhibitor upon docking and from the crystal structure of the protein. Conformational analysis of different docked complexes also shows that residues GLU 313, ASN 533 and ARG 247 for Acetylcholinesterase and GLY 59, TYR 246 & THR 120 for BACE play important role in this receptor’s activity. Docking studies performed by GLIDE has confirmed that above inhibitors fit into the binding pocket of the Acetyl cholinesterase and BACE receptor. From the results, we may observe that for successful docking, intermolecular hydrogen bonding and lipophilic interactions between the ligand and the receptor are very important. The main reason for the increase in GLIDE score is due to the penalties for close intra-ligand contacts.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Glide Score against AChE</th>
<th>Glide Score against BACE</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>PS122</td>
<td>-7.5</td>
<td>-9.3</td>
</tr>
<tr>
<td>2.</td>
<td>Donepezil</td>
<td>-4.97</td>
<td>-5.8</td>
</tr>
<tr>
<td>3.</td>
<td>Galantamine</td>
<td>-5.1</td>
<td>-5.4</td>
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<tr>
<td>4.</td>
<td>Tacrine</td>
<td>-4.3</td>
<td>-6.1</td>
</tr>
<tr>
<td>5.</td>
<td>Rivastigmine</td>
<td>-2.64</td>
<td>-3.04</td>
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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 hydroxyl 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.

ACKNOWLEDGEMENT

The Author P.Parasuraman is grateful to University Grants Commission, New Delhi for providing UGC-BSR fellowship.

REFERENCE