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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2771–2775
Bioorganic & Medicinal Chemistry Letters

In silico multi-filter screening approaches for developing novel β-secretase inhibitors

Taku Fujimoto, Yasuo Matsushita, Hiroaki Gouda, Noriyuki Yamaotsu and Shuichi Hirono*

School of Pharmaceutical Sciences, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo, Japan

Received 13 November 2007; revised 11 March 2008; accepted 7 April 2008
Available online 10 April 2008

Abstract—A large database of chemical structures was screened for potential inhibitors of β-secretase was carried out using in silico multi-filter techniques. Substructure screening, computer-aided ligand docking, binding free energy calculations, and partial interaction energy analyses were performed successively to identify chemical compounds which could serve as different scaffolds from known β-secretase inhibitors for future drug design. We showed that our in silico multi-filter screening retrieved all known inhibitors from the compound database investigated, which suggests that the other compounds identified as inhibitors by this computerized screening process are potential β-secretase inhibitors.

Alzheimer's Disease (AD), the major symptom of which is dementia, is a neurodegenerative disease mainly affecting elderly people and is increasingly prevalent in a country which is rapidly progressing, especially as there is currently no effective drug therapy available. Neuritic plaque, also called senile plaque, which is the product of aggregation of β-amyloid peptides (A-β) is observed in the brains of the patients with AD and is considered to be one of the key factors in the etiology of the disease.[1–3] In the biological process generating A-β, β-secretase catalyzes the penultimate step in the plaque formation, cleaving these peptides from Amyloid Precursor Protein.[4] Accordingly, work aimed at identifying inhibitors of β-secretase has been developed rapidly because the inhibition of the generation of A-β may lead to the discovery of a potential therapeutic agent. Thus, we performed in silico multi-filter screening to discover inhibitors of novel structure which will support different structural scaffolds for future drug design than the already known inhibitors employing the procedures shown in Figure 1.

Substructure screening (First filter: 2D-query search): In this study, for the purpose of substructure screening, we first determined the major chemical substructures of ligands which are common between known inhibitors. This was performed by making a thorough investigation of the three-dimensional structures of β-secretase complexed with the known inhibitors from the Protein Data Bank (PDB ID: 1TQF, 1W51, 2B8L, 2B8V, and 2FDP). From this investigation using computer graphics, it was found that the hydroxyl groups of the inhibitors commonly formed hydrogen bonds with carboxylic group of Asp32 or Asp228 and benzene rings of all the inhibitors made hydrophobic interactions with methyl group of Thr72 and Thr231 and alkyl part of the side chain of Gln73 in β-secretase. We selected these two chemical groups as query substructures, and used UNITY-2D module of Sybyl7.3 (Tripos Inc.) to screen chemical compounds which containing these groups (hydroxyl group and benzene ring) from the database of Namiki Shoji Co. Ltd, which comprises approximately 3.5 million compounds. By applying this first filter, we found approximately 70,000 chemical compounds (first 'hits'; about 2% of the total compounds screened).

High throughput protein structure-based virtual screening (Second filter: 3D-coarse docking): The second filter was applied by screening the first 'hits' by high throughput protein structure-based virtual ligand docking. Our objective in this filter is to roughly exclude those compounds which were not expected to bind with β-secretase. In this filter, we used HTVS (High Throughput Virtual Screening) mode of GLIDE (*Schrödinger*

Keywords: Drug design; In silico screening; β-Secretase; MM/PBSA; Computational alanine scanning; Ligand docking.
* Corresponding author. E-mail: hironos@pharm.kitasato-u.ac.jp

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doi:10.1016/j.bmcl.2008.04.011

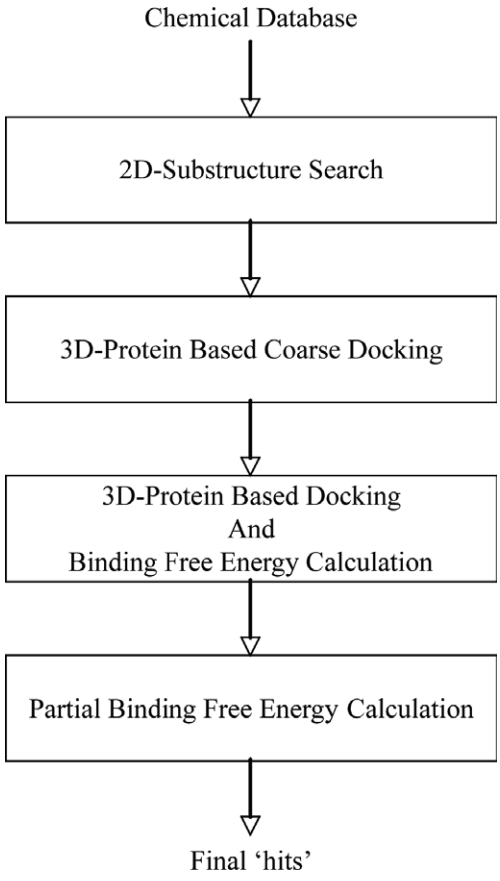

Figure 1. Flowchart of our in silico multi-filter screening approaches. A large chemical database was first screened by 2D-substructure search to obtain first 'hits'. The first 'hits' were docked into the binding site of β-secretase using High Throughput Virtual Screening (HTVS) mode of docking program GLIDE to retrieve second 'hits'. The second 'hits' were then docked using Standard Precision (SP) mode of GLIDE and their binding free energies were calculated using MM/PBSA method to select third 'hits'. Final 'hits' were determined by setting a limiting condition that partial binding free energies of the third 'hits' to Asp32 and Asp228 in the binding site of β-secretase are greater than 4 kcal/mol.

L.L.C.)[5] as a tool for virtual ligand docking. We used several conformations of β-secretase to allow for protein flexibility. To select conformations of β-secretase used for GLIDE-HTVS docking, protein structures registered in PDB were classified by root mean square deviation of active site conformations defined as amino acids within 5 Å from the ligands. The first protein structure we selected was the structure of PDB ID:2B8L[6] because the resolution of this was higher than the other crystals. The remaining protein structures were then compared with active site conformation of structure of PDB ID:2B8L. Crystal structures of which root mean square deviation was closer than 1 Å were classified into the group of PDB ID:2B8L and rejected. Subsequently, those structures with highest resolution remaining were selected. This process was reiterated until all structures were either selected or classified. We finally selected four structures (PDB ID: 1TQF,[7] 1W51,[8] 2B8L, and 2G94[9]). Missing atoms and residues of the selected four structures were added by protein structure prediction program PRIME (*Schrödinger L.L.C.*).[10] Grids used for the ligand docking were generated within 20 Å from the center of active site of these fixed structures. In order to determine the criteria score of GLIDE-HTVS-Score for this filter, we first docked ligands the binding modes of which are already known (PDB ID: 1TQF, 1W51, 2B8L, 2B8V, and 2FDP). As the differences between the lowest score and second and third from the lowest were both approximately 0.15, we set the criteria of GLIDE-HTVS-Score as −8.6, which is 0.15 lower than the lowest score we observed.

The ligand first 'hits' were then docked into the binding site of β-secretase. Figure 2 illustrates several ligands (capped sticks) docked into the binding site of β-secretase (represented by Connolly surfaces) using the HTVS mode of GLIDE. The docked ligands were ranked by GLIDE-HTVS-Score and the compounds with GLIDE-HTVS-Score lower than −8.6 were removed (second 'hits'). Concerning the evaluation of isomers here, we selected all the isomers as 'hits', in case at least one of the isomers had higher score than −8.6. In this process, we defined about 230 chemical compounds as second 'hits' (comprising 0.33% of the first 'hits').

Binding mode prediction and binding free energy calculation (Third filter: 3D standard docking): In the binding mode prediction, we used Standard Precision mode of GLIDE and MM/PBSA methods[11] to predict the binding mode of the compounds identified as second 'hits'. We used several ligands the binding modes of which were known (PDB ID: 1TQF, 1W51, 2B8L, 2B8V, and 2FDP), and the same four protein structures as GLIDE-HTVS for the purpose of establishing GLIDE-SP docking parameters. We also generated five

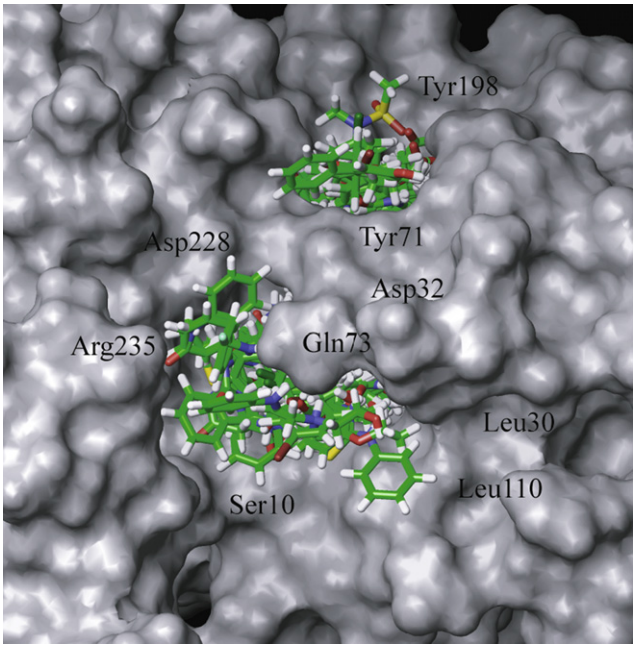

Figure 2. Illustration of several ligands (capped stick) docked into the binding site of β-secretase (Connolly surface) using HTVS mode of GLIDE. The docking poses of these ligands were evaluated and identified by GLIDE-HTVS-Score. The residues shown in this figure are some of binding site residues of β-secretase.