Novel Non-peptide β-secretase Inhibitors Derived from Structure-Based Virtual Screening and Bioassay

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This letter describes an efficient approach by integrating virtual screening with bioassay technology for finding small organic inhibitors targeting β-secretase (BACE-1). 15 hits with inhibitory potencies ranging from 2.8 to 118 μM (IC_{50}) against β-secretase were successfully identified. Compound **12** with IC_{50} of 2.8 μM is the most potent hit against BACE-1. Docking simulation from GOLD 3.0 suggests putative binding mode of **12** in BACE-1 and potential key pharmacophore groups for further designing of non-peptide compounds as more powerful inhibitors against BACE-1.

Keywords: β-secretase; Virtual screening; Bioassay; FRET

Alzheimer's disease (AD), a neurodegenerative disorder, is the most common form of dementia and accounts for two thirds of all cases. The disease is getting worse and worse as the population of aging people is getting higher worldwide. Today it is the sixth-leading cause of death in the United States and has become a major social and economic burden for both the society and family[1]. Cure for this disease is currently unavailable although extensive research has been focused on the development of therapeutic approaches. Thus, investigations on new drug discovery and development for AD are of urgent necessity. AD is pathologically characterized by the presence of intracellular neurofibrillary tangles and extracellular senile plaques in the brain[2-4]. The major components of the plaques remained unknown until a small peptide termed beta amyloid (Aβ) was purified from neuritic plaques[5]. This peptide consists of 39-43 residues that are endo-proteolytically derived from a transmembrane amyloid precursor glycoprotein (APP)[6]. Following the discovery and report of Aβ, a dominant hallmark of pathogenesis known as amyloid cascade hypothesis was developed to propose that the overproduction and aggregation of a 42-amino acid form of Aβ is followed by its deposition in the plaques in the brain[7-9]. The endoproteolytic cleavage of the APP to get Aβ involves the sequential actions of two proteases, the β-secretase (BACE-1, hereinafter) and γ-secretase[10]. Therefore, BACE-1 has become an attractive therapeutic target and its inhibitors are potential drug candidates for the treatment of AD. In the past decade, the major effort in designing BACE-1 inhibitors was the production of transition state isosteres such as hydroxyethylamines, reduced amides, statine-based peptidometic inhibitors countering the catalytic aspartyl groups.

However, no non-peptidic inhibitors were reported and none of the already reported BACE-1 inhibitors has been marketed as efficient drug so far due to the complication by the requirement for central nervous system penetration[11, 12]. Hence, identification of novel small non-peptide inhibitors is necessary to make the pharmacokinetic properties of chemicals more favorable for further development and enlarge the space of drug lead discovery as well as to bring the leads into pre-clinical and clinical trials.

While peptidomimetic transition state isostere based inhibitors, such as statine, homostatine, norstatine, and hydroxyethylamine, have dominated the major effort in the design of potent inhibitors of human BACE-1. Li's group employed a combinatorial chemistry approach to develop homostatine based inhibitor which had an IC_{50} value of 143 nM in an enzymatic assay[13] and Shering-Plough Corp presented a hydroxyethylamine based inhibitor with an IC_{50} of 4 nM[14]. Only till recently, some non-peptide compounds were identified as inhibitors of BACE-1. Astex researchers highlighted their work in discovering aminopyridine and cyclic amidine classes as BACE-1 inhibitors[15]. Barrow et al reported the identification of spiropiperidine inhibitor template for BACE-1[16]. Although the inhibitors from others have been demonstrated potent in enzymatic assays, this has not discouraged us from exploring new BACE-1 inhibitors with alternative structural scaffolds. These inhibitors seldom enter the brain due to their unfavorable physicochemical properties, such as their high polar surface areas and high number of H-bond donors and acceptors as they are peptides in nature. Therefore, identifying selective nonpeptidic BACE-1 inhibitors

with ideal hydrophobicity for CNS penetration and good pharmacokinetic properties would be demanding.

To discover novel small molecule inhibitors with new chemical skeleton as potential drug leads, we applied a receptor-based virtual screening approach to search the compound database Specs (www.specs.net) containing ~280,000 chemicals and identified 42 hit compounds. All calculations were performed on IBM cluster equipped with 64 processors. Crystal structure of BACE-1 complexed with an inhibitor OM00-3 (PDB entry: 1M4H) resolved at 2.1Å[17] was extracted from Brookhaven Protein Data Bank (PDB) (www.rcsb.org/pdb). Hydrogen atoms were added and water molecules co-crystallized with the protein were removed from the original structure using Sybyl 8.0 (Tripos associate inc., St. Louis, MO, USA). The modified crystal structure of BACE-1 was used as the target for virtual screening on commercial chemical databases Specs by using GOLD 3.0 software (CCDC, Cambridge, U.K.). Chemical database Specs was edited from its original sdf file format to mol2 format. The default parameters in GOLD 3.0 were used. The active site radius is 15Å from atom 1846 OD2 of Asp228, which is one of the key amino acid residues in the aspartyl protease. The GoldScore fitness function was applied and top 3000 molecules with the highest GOLDscores from initial virtual screening were then re-submitted for multiple docking of 10 conformations for each ligand. Finally, the top 1000 hits were selected for further visual inspection of their binding conformation and geometrical matching quality with the active sites of BACE-1. Based on the predicted putative H-bonds formed by the hits and active site residues

of BACE-1, the potential hydrophobic and aromatic-aromatic interactions, as well as predicted clogP values of 4~6 for blood brain barrier, 42 compounds among the top 1000 hits were selected for biological assays. Among them, 15 new potential BACE-1 inhibitors (Shown in Table 1) were discovered to be active through bioassay with FRET technology[18], demonstrating that the applied approach is a highly efficient way to discover active compounds with new scaffold different from current peptidic BACE-1 inhibitors. In this study, nearly one-third of the compounds (12/42) demonstrated their inhibitory potencies of greater than 50% of BACE-1 at 100 μM and the most active compound **12** identified from this work has an IC_{50} value of 2.8 μM. Although it is weaker than the positive control, a statine-based peptide with an IC_{50} value of 120 nM from our test (reference IC_{50} value is 30 nM) [19], it is novel in terms of its organic structure with smaller molecular weight that makes it possible to penetrate the brain barrier. The generation of several different structural scaffolds as novel pharmacophores of BACE-1 inhibitors implies the possibility and importance of the fast, economic computer-assisted approach in modern drug discovery and design.

From our docking study, all 15 inhibitors were proposed to bind with BACE-1 within the enzyme active pocket. Due to the fact that BACE-1 consists of more sub-pockets (S4'-S4) than other aspartyl proteases in its active site, it is expected that inhibitors capable of interacting with more sub-sites could lead to stronger inhibitory effect. Such expectation is consistent with our bioassay results, as exemplified by inhibitory differences among all inhibitors. Compounds **12** and **13** with higher activity

almost occupy the whole active pocket of BACE-1 while all the rest bind with BACE-1 mainly via S3-S3' interaction. Notably, **12** exhibited low micromolar potency against BACE-1 in the FRET assay. As shown in Figure 1, molecular docking derived from GOLD suggested a reasonable binding mode of compound **12** in BACE-1. Being the central "bridge" of **12**, the benzothiazole ring occupies S1 sub-pocket, making aromatic-aromatic interaction with Tyr71. The linker sulfur group fits the small, shallow S3 and S4 pocket is occupied by di-methoxy phenyl group, contributing to hydrophobic and Van der Waals force within the site. The right (prime) side of the active site is mainly occupied by thiazine together with two piperidine groups on it. Several hydrogen bond interactions were observed from the docking simulation (Figure 2). Among them the most important interaction is the H-bond formed between the linker NH with oxygen in Asp228, thereby mimicking the isostere warheads in previously reported synthetically optimized inhibitors. Besides, Gly34 and Tyr198 also form two hydrogen bonds with the triazine and piperidine in **12** respectively. Interestingly, a similar molecule **13** with IC_{50} of 10.2 μM was also identified from the virtual screening. However, the change from dimethoxyphenyl group to fluorobenzne in **13** rendered the activity lose by 5 folds. Hence, the Van der Waals interactions between the Ala231 to the methoxy group on the P4 phenyl ring in the inhibitors might be essential for strong inhibition of the enzymatic activity. As little is known about the use of compound **12** elsewhere before, the chemical scaffold in **12** might represent a new class for further drug lead optimization targeting BACE-1.

Albeit compound **12** is a novel moderate inhibitor of BACE-1, further search of compounds bearing important pharmacophores in **12** will be continued. Furthermore, implementation of multiple scoring functions in preliminary computational predictions of potential hits would contribute to better enrichment rates during virtual screening and molecular docking process. Recently, Vijayan et al reported a hybrid structure-based virtual screening for identification of several prospective BACE-1 inhibitors and the study ensured the superiority of the modified methodology over conventional docking methods in yielding higher enrichment rates[20].

In summary, structure-based virtual screening in combination with bioassay resulted in identification of multiple novel non-peptide inhibitors of human BACE-1. This method provided an efficient and high hit-rate approach for inhibitor discovery against BACE-1. The inhibitors reported herein are mostly hydrophobic in nature with moderate molecular sizes (~500-600 Da). Therefore, they are possibly developed to be penetrants of blood brain barrier and able to achieve the terminal effect of addressing the underlying neuropathology. The most potent molecule, compound **12** has a benzothiazole ring which docks into the S1 pocket of the enzyme and spans the interaction through almost all the sub-sites of BACE-1. The docking pose of compound **12** in the active site of BACE-1 is useful in guiding lead optimization and structure-activity relationships study in future. Encouraged by current knowledge, our effort in optimizing present sub-micromolar hits into more potent nanomolar leads will be continued based on the molecular clues from this research.

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18. BACE-1 inhibition assays were carried out using a Fluorescense Resonance Energy Transfer(FRET) assay kit purchased from invitrogen in the 96-well black flat-bottomed microplate with a final volume of 100 µL/well containing a final concentration of 5% DMSO. The assays were run at room temperature for 1 hour under the following conditions: BACE-1 in 50 mM Tris (pH 7.5), 10% glycerol (0.3 unit/mL) was incubated with DMSO dissolved compounds before the initiation of the reaction by adding FRET peptide substrate Rh-EVNLDAEFK-Quencher (250 nM). The reaction kinetics was monitored on a TECAN spectrofluorometer with excitation and emission wavelengths at 545nm and 585nm respectively. Mean kinetic rate was measured in RFU/min and the results were fit into GraphPad Prism5 for IC_{50} calculation. The compounds were initially tested for inhibition of BACE-1 at 100 µM. The IC_{50} values were then determined on all the compounds that displayed 50% or higher inhibition rate in the initial assay. All the IC_{50} measurements were done in duplicate. A statin peptide (H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Sta-Val-Ala-Glu-Phe-OH) derivative, was obtained from AnaSpec (San Jose) and incorporated in the assay as a positive control and the negative control well included DMSO for the replacement of the test compound.

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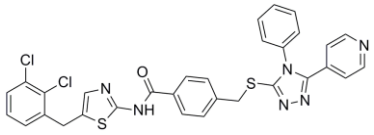
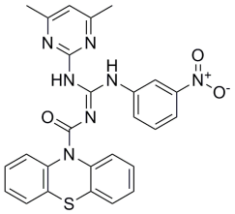
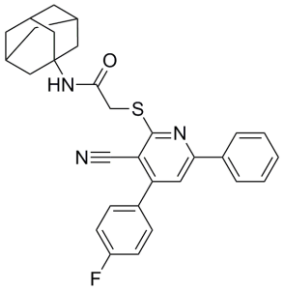
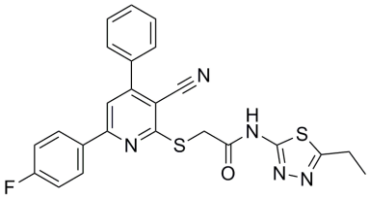
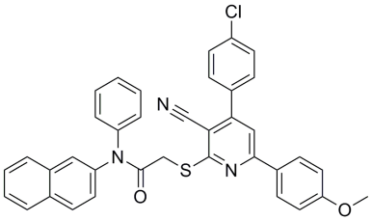
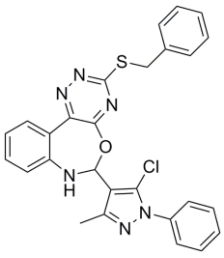
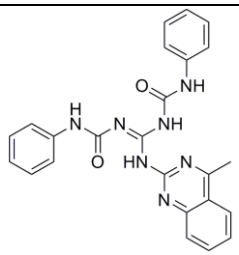
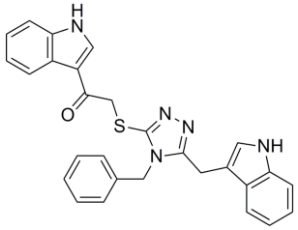
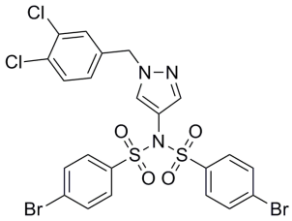
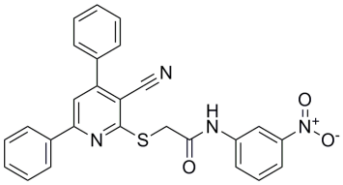
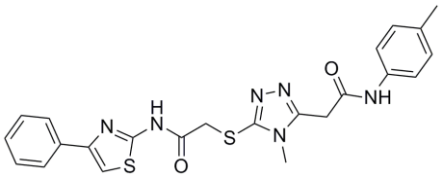
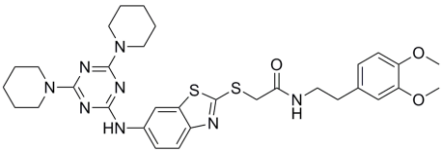
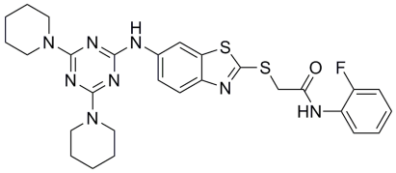
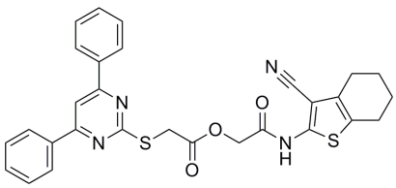
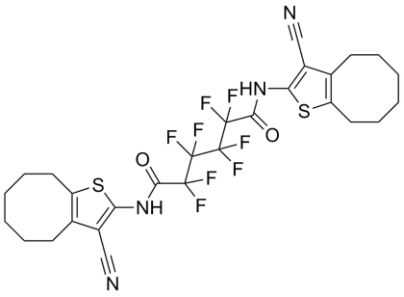
TABLE

Table 1. Inhibition of β-secretase by compounds selected from virtual screening

Compound	Structure	%inhibition at 100 μM	IC_{50} (μM)[a]
1[b]		78	>100
2		80	28.6 (±1.5)
3[b]		45	118
4		80	50 (±2.6)
5		68	50 (±4.0)
6[b]		48	100

7		100	3 (±1.2)
8[b]		75	100
9		50	90 (±25.1)
10		100	20 (±1.4)
11		83	21 (±1.5)
12		80	2.8 (±1.2)
13		90	10.2 (±1.1)
14		75	34.5 (±3.2)

15		87	12.2 (±1.1)
16[c]	H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Sta-Val-Ala-Glu-Phe-OH	100	0.12(± 0.012)

[a]IC_{50} values are means of two experiments. S.D. values are given in parentheses. [b] IC_{50} values of compound **1**, **3**, **6**, and **8** were estimated. [c]The positive control in the assay demonstrated an IC_{50} value of 120 (± 1.2) nM.

Figure Legends

Figure 1. Molecular docking derived binding pose of compound **12** in the active site (surface representation) of BACE-1. Inhibitor is colored by atom type. Two residues, Thr 72 and Gln 73, were deleted for a whole view of active site. Surfaces of catalytic aspartic acids 32 and 228 are colored in red. S4-S4' sub-sites of BACE-1 are labeled in black. The binding mode was derived from GOLD and the picture was generated by InsightII software (Accelrys).

Figure 2. A representation of docking simulated binding mode of compound **12** bound in the active site of BACE-1. Hydrogen bonds are represented by dotted lines. This figure was generated by ChemDraw 8.0. BACE-1 sub-pockets are labeled in "S" and corresponding chemical moieties in **12** are labeled in "P".