Molecular docking and Structure-Activity Relationship Studies on Benzothiazole based non-peptidic BACE-1 inhibitors

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A similarity search on the structural analogs of an inhibitor of BACE-1 with IC_{50} 2.8 μM, which contained a P1 benzothiazole group together with a triazine ring linked by a secondary amine group, was described in this letter and some more potent inhibitors against BACE-1 were identified. The most potent compound **5** (IC_{50} = 0.12 μM) increases the inhibitory potency by 24 folds. Our results suggest that a pyrrolidinyl side group at the P3' and P4' of the inhibitors are favored for strong inhibition and a small aromatic group at the P4 position is also essential to the potency.

Keywords: BACE-1; Virtual screening; Bioassay; FRET

Alzheimer's disease (AD) is a progressive neurodegenerative condition of the brain and represents the most common form of dementia[1]. AD patients show gradual loss of memory and cognitive ability. Today, it is affecting over 24 million in the world and the number is predicted to reach 80 million by 2040[2, 3]. Currently, there is no treatment available for AD and medical therapies are usually limited to acetylcholinesterase inhibitors and NMDA antagonists[4, 5]. However, both of the two only marginally reduce the symptoms of AD but do no cure it thoroughly[6]. One of the major pathological hallmarks of AD is the deposition of the amyloid β peptide ($A\beta_{40, 42}$) to form extracellular plaques in AD brain tissues[7]. Aβ peptides are derived from the sequential cleavage on amyloid precursor protein (APP) by two aspartic acid proteases, namely β-secretase and γ-secretase[8, 9]. Although the cause of AD is not fully known to us, evidence is accumulating to highlight the important role of the Aβ in the pathogenesis of AD[1, 10, 11]. Hence, inhibitors of either protease offer alluring potential disease-ameliorating candidates in the AD drug discovery campaign. Since the β-secretase (also called BACE-1 or β-site of APP cleaving enzyme) mediated cleavage of the APP is the first step in the generation of the Aβ, inhibition of BACE-1 is considered a more prominent target for treating AD[1]. Furthermore, the possibility of interruption of the Notch pathway by γ-secretase inhibitors renders γ-secretase a less attractive drug target[4]. Over the past decade, many BACE-1 inhibitors have been reported and these inhibitors are mainly divided into two classes: peptidomimetic and non-peptidomimetic inhibitors[12-14]. The peptidomimetic inhibitors were the analogs of the natural substrates of BACE-1 and these included hydroxyethylenes, statines, norstatines, hydroxyethylamines. Among them, the first potent inhibitor was the OM99-2 and it was co-crystallised with the BACE-1 for resolving the 3-dimensional structure of the enzyme-inhibitor complex. From its crystal structure, the nature of the sub-pockets in the BACE-1 active site was

revealed and eight such pockets were known to be involved. Xiao et al employed a combinatorial chemistry approach to develop a homostatine based inhibitor which had an IC_{50} value of 143 nM in an enzymatic assay[15] and the Shering-Plough Corp presented a hydroxyethylamine based inhibitor with an IC_{50} of 4 nM[16]. Although peptidomimetic inhibitors showed potent activity against BACE-1, their relatively large molecular size, low metabolic stability and poor bioavailability render their development into therapeutic drug candidates difficult. On the other hand, a great deal of effort has been paid off to discover non-peptidic, organic compounds with better pharmacokinetic properties as drug leads. Some non-peptide compounds were identified as potent inhibitors of BACE-1. For a more detailed review on the non-peptidic inhibitors of BACE-1, refer to reference 4.

Previously we reported several non-peptidic inhibitors and to one of these inhibitors, the compound **1**[17], based on the skeleton of which we performed a similarity search in the SPECS (www.specs.net) database and found some structurally similar compounds. Most of those compounds were tested to be active to inhibit BACE-1. Herein, the discovery, enzyme inhibition assays, and molecular docking of these analogs are outlined.

Figure 1 summarises the whole process from our previous virtual screening leading to the present study. A 2-D similarity search was performed at the SPECS vendor's website by keeping the scaffold shown in Table 1 and a cutoff similarity index was set to 70%. All the compounds were built using Sybyl 8.0 (Tripos associate inc., St. Louis, MO, USA) on an SGI machine. Gasteiger-Marsili charges were assigned to the molecules and the initially built structures were subjected to energy minimization using the Powell method by applying the termination gradient of 0.05kcal/mol for 100 maximum iterations. The crystal structure of BACE-1 complexed with an inhibitor OM00-3 (PDB entry: 1M4H) resolved at 2.1Å was extracted from Brookhaven

Protein Data Bank (PDB) (www.rcsb.org/pdb). All hydrogen atoms were added and the water molecules co-crystallised with the protein were removed from the original structure using Sybyl. The modified crystal structure of BACE-1 was subjected for molecular docking using GOLD 3.0 software (CCDC, Cambridge, U.K.). Docking of all the ligands was performed with the default docking settings. The active site radius was set at a distance of 12Å from the atom number 1846 of the Asp228, which is one of the key catalytic amino acid residues in the aspartyl protease. The GoldScore fitness function was applied. The population size was set to 100. The selection pressure was set to 1.1 and the number of operations was set at 100000. The Migration, mutation and cross over parameters were set to 10, 95, and 95 respectively. For shortening the docking speed, 10 conformations were applied for each ligand and the docking was allowed to stop when the RMSD value was within 1.5 Å between the top 3 solutions of each ligand. BACE-1 inhibition assays were carried out using the Fluorescense Resonance Energy Transfer (FRET) assay kits according to the protocol described previously[17].

Table 1 lists the structures and inhibition results (IC_{50}) of the 22 tested compounds. Except for **14**, **17**, **18**, **20**, **21**, **22**, all other compounds inhibited over 50% at the initially tested concentration of 50 μM. All the compounds mainly diverse in the R and X groups as labeled in Table 1. To elucidate the relationship between the binding modes of these inhibitors and their activities, molecular docking was carried out and the best scored conformation of each compound was aligned in the active binding site of BACE-1 (Figure 2). As docking algorithms are generally believed to produce good binding poses but lack accuracy in ranking these poses[18] , to clarify the suitability of selecting the top scored docking pose for structural alignment, we further performed a comparison by calculating the RMSD values of the rest of the poses produced by GOLD with reference to the selected one. The data from the comparison is available

in Table 1 of the supplementary data. The RMSD values were lower than 2.0 Å for nearly 90% of the compounds, indicating that it was reasonable for aligning the top scored conformation for further analysis. On a whole, the central benzothiazole sit in the region between S1 and S2 where the catalytic aspartic acid residues are present. To the prime side of the active site, the interaction was extended to the S3' and S4' sub-pockets via the two R groups on the 4- and 6- positions of the triazine ring. Structurally, there were 3 major types of R groups: pyrrolidinyl, piperidinyl, and morpholinyl. Interestingly, being same at the P1, the difference in P3' and P4' as characterized by the changes among the above three R groups rendered the inhibitors' potencies change dramatically. The pyrrolidinyl compounds **2-8** performed best in the inhibition studies. Compounds **9-12** bearing the piperidinyl at the same positions didn't show comparable potencies but had moderate inhibition against BACE-1. It was noticed that compound **9** which shares high similarity to **5** with the exception at the R groups dropped its potency by almost 40% at 50 μM. The steric factor could be an important factor conferring the lower activity of the piperidinyl series as the steric confliction resulted in unfavorable accommodation of the relatively larger rings at the S3' and S4' pockets where bulky amino acids such as Tyr71, Tyr197, Tyr198, Pro70, Ile126, Arg128, Glu125 are present. Another interesting observation was that the prime side of the active site of BACE-1 is electrostatically more negative. Hence the polarity present in the piperidinyl might not be tolerated well. As opposed to the above two classes, the morpholinyl containing compounds abolished the potencies significantly (as seen in **13-23**). Among the pyrrolidinyl series, of the particular interest was that compounds **5** and **7** emerged as the two most potent inhibitors among all. **5** which has a phenyl acetamide group at the P4 position (IC_{50} of 0.12 μM) was twenty-fold more potent than the starting compound **1**. Comparing compounds **3**, **5** and **6**, a shorter length of side-chain at the X was preferred due to a two-fold difference in

inhibition potency. However, this was not observed for compound **2**. The lower activity of compound **2** could be the lack of the NH linker as compared to others in the same series. Compound **7** with a furylmethyl acetamide increased potency by five-fold as compared to **1**. Thus, a small aromatic group linked to the acetamide in the pyrrolidinyl series appeared to be unique to the improvement of the potency. Meanwhile, it is worth noting that the 'X' side chain of **7** is of comparable length to compound **3**, yet IC_{50} of the former was 2.5-fold lower. The polarity conferred by the furyl moity in **7** might be preferred for accommodating in the S4 sub-pocket near which the Thr 232 is present. Figure 3 shows the detailed docked binding conformation of the two most potent compounds: **5** and **7** in BACE-1. Notably, several hydrogen bond formations between both compounds and the active site residues were observed from docking. The linker NH between the benzothiazole and triazine was noticed to form a hydrogen bond with the OD2 oxygen of Asp 32. This H-bond is considered as indispensible to the strong binding of the target enzyme as previously described elsewhere [4, 19]. The formation of such an H-bond could possibly explain why this group of compounds appeared to show inhibitory activities against BACE-1. Another H-bond was also potentially predicted to be present between the nitrogen 3 of triazine and the hydroxyl hydrogen of Tyr 198. In addition, there was a third hydrogen-bond between the hydrogen atom from the acetamide and the carbonyl oxygen of Thr 232 from docking simulation. The two pyrrolidinyls on the triazine were in close contact with Tyr 198 and Tyr 71 respectively and contributed to hydrophobic interactions within the region. Similarly, compound **7** almost "cloned" the interaction pattern of **2** with the active site of BACE-1. The same H-bond was noticed from the linker NH and OD2 of Asp 32 whereas the acetamide hydrogen formed a hydrogen bond with the carbonyl oxygen of Gly 230 This H-Bond is also

observed for other inhibitors of BACE-1[19]. Unlike the more solvent exposed P4 phenyl ring in the compound **2**, the furyl ring in **7** was deeply docked into the S4 pocket of BACE-1.

2 had an IC_{50} which was 10-fold less potent than that of **3**, the presence of the NH group in **3** could possibly form the H-bond with the carbonyl oxygen of Thr 232. In Mok's paper describing a series of non-peptide inhibitors from virtual screening[20], the amide H of Thr 232 was involved in a key hydrogen bond contributing to the binding of the inhibitor. The similar hydrogen bond involving Thr 232 was also reported by Congreve et al. Compounds **6** and **8** showed similar potencies.

In summary, a similarity search followed by bioassays on the structural analogs of a known inhibitor of BACE-1 rapidly prioritised more potent inhibitors of the target enzyme. Our results revealed that the pyrrolidinyls at the P3' and P4' positions of the inhibitors are more favored than the piperidinyls and morpholinyls. Among the pyrrolidinyl series compounds, a phenyl acetamide at P4 led to the best potency against BACE-1. The SAR study provided invaluable insight into the important chemical information contributing to the strong potency of BACE-1 inhibitors. Furthermore, our study provided a new direction to the fragment based drug discovery which is an emerging niche approach in the development of novel drugs by searching and linking small fragments together to achieve the best efficacy for the ligands. 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.

Supplementary data

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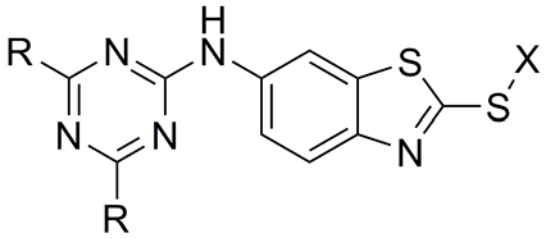
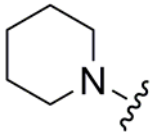
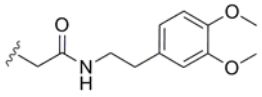
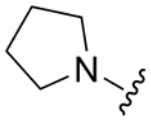
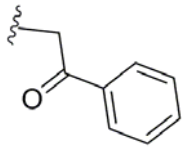
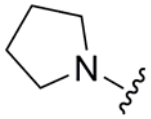
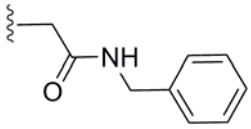
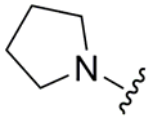
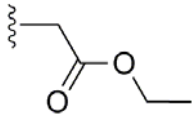
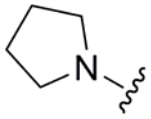
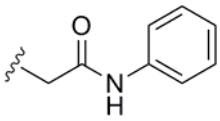
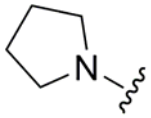
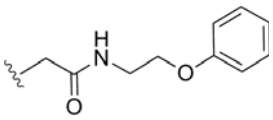
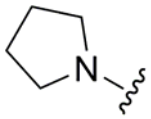
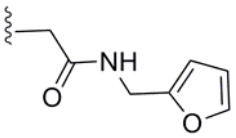
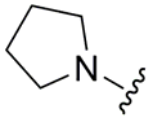
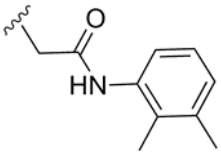
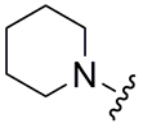
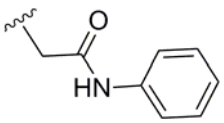
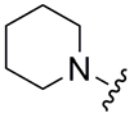
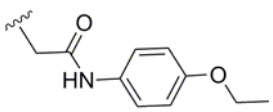
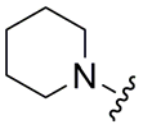
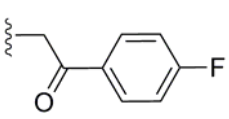
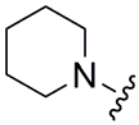
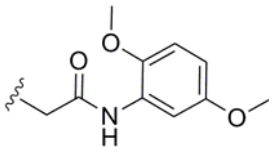
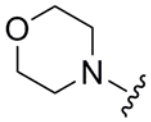
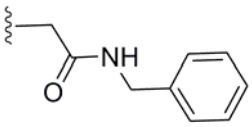
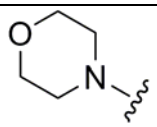
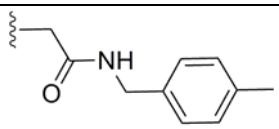
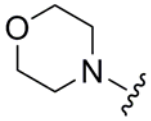
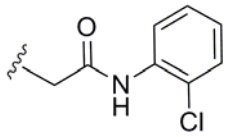
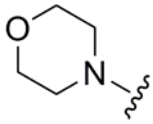
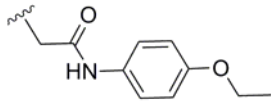
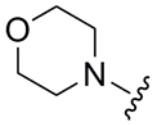
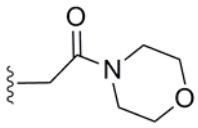
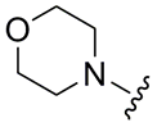
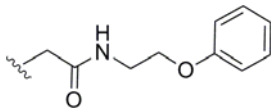
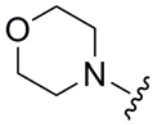
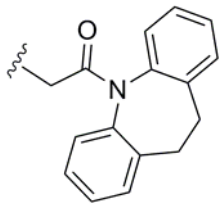
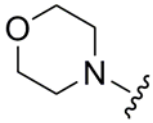
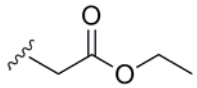
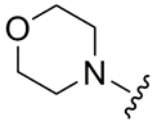
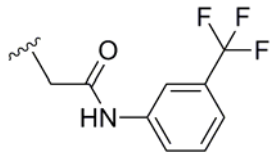
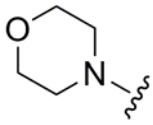
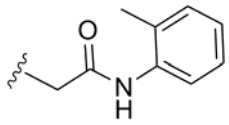
Table

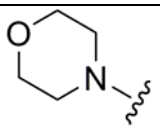
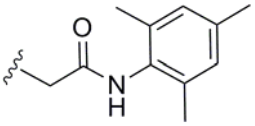
Table 1. SAR of Substitution patterns of R and X.



Compound	R	X	%inhibition at 50 μM[a]	IC_{50} (μM)
1[b]			80	2.8
2			93	~20
3[b]			83	1.7
4[b]			100	1.77

5 [b]			100	0.12
6 [b]			88	3.0
7 [b]			87	0.69
8 [b]			100	2.83
9			58	>50
10 [b]			77	17.2
11 [b]			80	~20
12 [b]			73	~18
13			50	~50

14			0	N.D.
15			50	~50
16			100	>50
17			16	N.D.
18			0	N.D.
19 [b]			100	14.7
20			42	N.D.
21			0.0	N.D.
22			0.5	N.D.

23			82	>50

[a]Values are means of duplicate measurements.

[b]Values are expressed as mean of duplicate in one experiment.

N.D = Not Determined.

Figure Legends

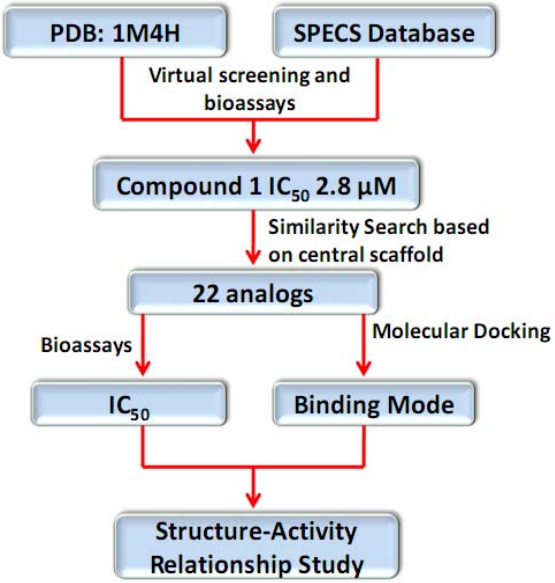


Figure 1. Schematic summary of the whole process from previous virtual screening leading to the present study.

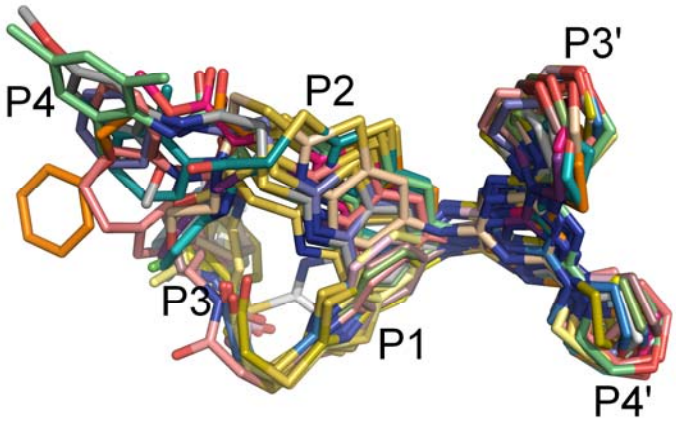
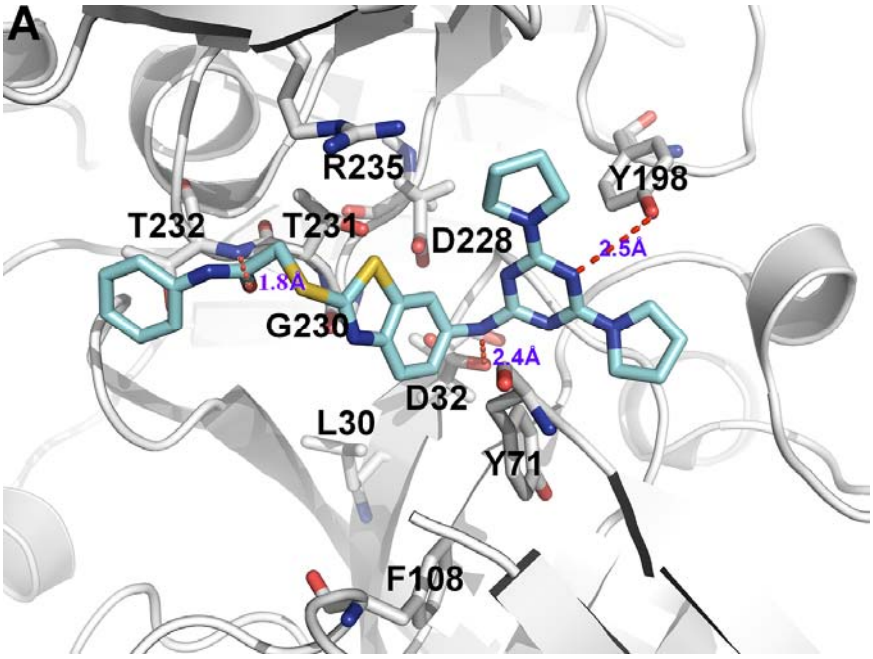


Figure 2. Alignment of 22 analogs of compound **1** in the BACE-1 active site.



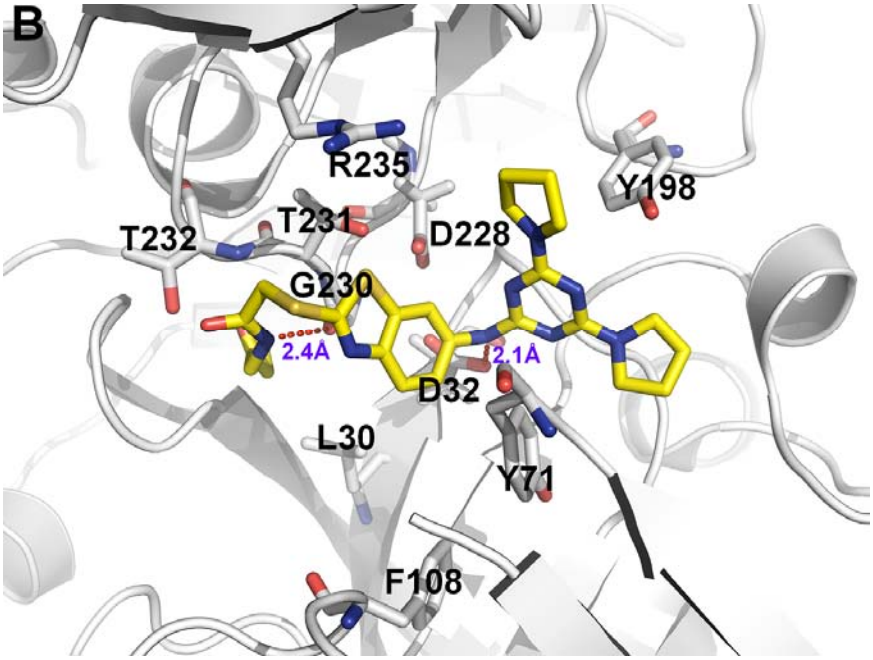


Figure 3. Cartoon representation of BACE-1 active site. Key residues are shown in stick and colored by atom type. A) Molecular docking pose of compound **5**. B) Molecular docking pose of compound **7**. Potential hydrogen bonds are depicted in dot lines and distances are labeled. The binding mode was derived from GOLD and the picture was generated by Pymol software.