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RESEARCH ARTICLE

STRUCTURE-BASED VIRTUAL SCREENING FOR IDENTIFICATION OF NOVEL INHIBITORS AGAINST BACE1 FROM SELECTIVE MEDICINAL PLANT COMPOUNDS

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ABSTRACT

Every Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, which is characterized by amyloid β peptide deposition in the brain. Amyloid β peptide (Aβ), the major component of amyloid plaques is generated by the sequential processing of a larger protein called the Amyloid Precursor Protein (APP) by β-amyloid cleaving enzyme (BACE-1). Since BACE-1 is an important therapeutic target for AD, we have applied a computer assisted methodology unifying molecular docking studies to identify potent inhibitors against BACE-1 from 9 selective medicinal plants. These 9 selective medicinal plants compounds retrieve from TCM database were docked into the active site of BACE-1. In our present molecular docking studies provided critical information on protein ligand interactions that revealed imminent information on chemical features essential to inhibit BACE-1. There are 16 compounds selected from the selective medicinal plants possess the best scoring function. For further validation induce fit docking studies was performed on 616 compounds which result shows that, from 16 compounds there are five hits compounds have the best binds scores and binding energy compare to native compound from BACE1. Furthermore, these findings strongly suggest that these five lead compounds could serve as building blocks for designing drug like molecules to treat AD.

INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, which is the most common form of dementia that affects 4-8% of the elderly population worldwide. In 2010, there were 35.6 million peoples living with dementia, notably Alzheimer's disease patients. Experts say the number of Alzheimer's cases will likely double during the next 20 years to 65.7 million in 2030 and to more than 115 million cases in 2050. Furthermore, the total cost of dementia, including Alzheimer's disease is $605 billion in 2010, according to a report issued by the World Alzheimer's Association. AD is characterized by the progressive accumulation of extracellular amyloid plaques, intracellular neurofibrillary tangles and neuronal loss leading to memory deficits and dementia. β-Secretase (BACE1) is a type I transmembrane aspartyl protease that cleaves Amyloid Precursor Protein (APP) to produce two major components: secreted ectodomain sAPPβ and the C-terminal fragment CTF99 (Vassar, 2004). CTF99 is further processed by γ-secretase to produce Aβ 42 peptides, which accumulate to form amyloid plaques in neuronal region to causes neuronal losses (Sambamurti *et al*., 2002). BACE1-deficient mice generated from multiple groups were viable, and they showed robust reduction in neuronal Aβ production and amyloid plaque deposition. In contrast, mice over expressing human BACE1 showed an increase in brain sAPPβ, CTFβ, and Aβ levels, suggesting enhanced amyloid plaque deposition and also inhibition of BACE1 activity can reduce Aβ production (Sethu Sankaranarayanan *et al*., 2008). The ultimate evidence supporting BACE1 as a superior AD drug target is the finding that BACE1 knock-out mice do not produce Aβ (Luo Y *et al*., 2001 and Cai H *et al*., 2001). Moreover BACE1-null mice are fertile and exhibit relatively mild phenotypes such as hypomyelination (Hu X *et al*., 2006 and Willem M *et al*., 2006) and schizophrenia-like behaviors (Savonenko AV *et al*., 2008; XiaoyangLuo and Riqiang Yan, 2010). Therefore, BACE1 is a better drug target for AD because the BACE1 inhibition should cause less serious biological dysfunction in the cell. So, discovering and developing potent BACE1 inhibitors is the best treatment for AD. Several strategies of drug discovery have been explored in the search for potent BACE1 inhibitors. In this study, we analyzed the inhibitory potential of 616 compounds form 9 selective plant compounds using structure based virtual screening. The 9 selective plants are *Celastrus orbiculatus Thunb, Centella asiatica, Curcuma longa, Ginkgo biloba, Panax ginseng, Panax notoginseng, Rosmarinus officinalis, Salvia miltiorrhiza, Terminalia chebula*. These 9 medicinal plants are selected from literature based on the memory enhancing properties of plant and compounds download from TCM database.

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MATERIALS AND METHODS

Protein preparation

Protein has been prepared by using Protein Preparation Wizard in Maestro. The BACE 1 (PDB ID: 2QK5) was retrieved from Protein Data Bank and the chain A was treated to add missing hydrogen's, assign proper bond orders, treat metals and delete water molecules beyond the 5Å from the heterogeneous groups. The hydrogen bonds were optimized using sample orientations. Finally the protein structure was minimized to the default Root Mean Square Deviation (RMSD) value of 0.30Å.

Ligand preparation

The dataset of 616 compounds are used in our molecular docking studies were derived from 9 selected medicinal plants from TCM database (http://tcm.cmu.edu.tw/). The ligands were processed with the LigPrep program to assign the suitable protonation states at physiological pH= 7.2±0.2 [22]. Conformer generation was carried out with the ConfGen torsional sampling by using OPLS_2005 force field. The van der Walls radii were scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties.

Receptor Grid Generation

The grid was defined around the co-crystallized ligand (PDB ID: 2QK5). The atoms were scaled by van der Walls radii of 1.0Å with the partial atomic charge less than 0.25 defaults. The functionally important sites were defined as an enclosing box at the centroid of the workspace (co-crystallized) ligand was allowed to dock into the active site. No constraints were defined.

Virtual screening

All molecules of selected plant compounds were docked into the binding site of the receptor (PDB ID: 2QK5) using Grid-Based Ligand Docking With Energetics (Glide) software from Schrodinger (Halgren *et al*., 2004; Friesner *et al*., 2004). The virtual screening of selected medicinal plant compounds with prepared protein was performed with OPLS2001 force field using Virtual Screening Workflow (VSW) module of the Schrodinger Suite. VSW uses glide docking to rank the best compound which utilizes the scoring functions, High Throughput Virtual Screening (HTVS), Standard Precision (SP) and Extra Precision (XP). HTVS and SP modes are used for screen large set of ligands and XP docking is more accurate than the above two methods. It uses the ligand poses that have a high score from SP docking. The XP Glide Score scoring function was used to order the best ranked compounds and the specific interactions like pi-cation and pi-pi stacking were analyzed using XP visualizer in Glide module.

Induced-Fit Docking

The protein structure of BACE1 is applied with the induced-fit docking (IFD) method in the Schrodinger software suite (27). All ligands were prepared using LigPrep and were optimized with the OPLS force field in the Macro Model module in Schrodinger (27). Ligands were docked to the rigid protein using the soften-potential docking in the Glide program with the vander Waals radii scaling of 0.8 for the proteins. The resulting top 20 poses of ligands were used to the protein plasticity using the Prime program in the Schrodinger suite. Residues having at least one atom within 5A˚ of any of the 20 ligand poses were subject to a conformational search and minimization while residues outside the zone were held fixed. In this way, the flexibility of proteins was taken into account

RESULTS AND DISCUSSION

To investigate the detailed intermolecular interactions between the ligand and the BACE1 protein, an automated docking program Glide was used. In this present study, the three-dimensional structure information on the BACE1 was taken from the PDB. Further, we selected 9 medicinal plants for BACE1 docking studies, based on the memory enhancing properties. From 9 selective medicinal plants we retrieved 616 compounds from TCM compound database, which we used for Virtual Screening for finding new potent BACE1 protein inhibitors. The Virtual Screening Workflow option HTVS (High Throughput Virtual Screening), SP (Standard Precision), Glide XP (extra precision) and IFD (Induced fit docking) was then performed.

Virtual screening for BACE1

High-Throughput Virtual Screening (HTVS) is a computational technique to find potent small molecules against protein targets of BACE1. Various parameters such as Glide score, Glide energy and hydrogen bond interactions are used to assess which conformation or binding site orientation is best complement in the protein-binding site. Two main aspects were taken into account to assess the quality of docking methods: (i) Docking accuracy, which identifies the true binding mode of the ligand to the target protein, and (ii) Screening enrichment, which is a measurement of correlation between docking method and true binding ligands rather than random compound selection. Native ligand from BACE1 (PDB ID: 2QK5*)* in PDB and 616 compounds of 9 selective medicinal plants from TCM database were used in virtual screening. As explained in materials and methods we calculated our docking calculations in HTVS first, SP second and then XP mode. We filtered out 58 compounds from the HTVS process against the target BACE1 protein. In the next stage, compounds filtered based on good Glide score and good Glide energy, hydrogen bond interactions by using Glide SP and XP docking screening. From XP docking results we selected 16 hits compounds with best glide score, glide energy and hydrogen bond interactions.

Induce fit docking analysis

Docking studies were performed to gain more detailed insight into the structural basis of the binding of our 16 compounds with BACE1. As our method incorporates protein flexibility, it was important that the docking method used accounts for both ligand and receptor flexibility. We chose a protein-ligand docking method which combines rigid receptor docking (Glide) with protein structure prediction (Prime) in an induced fit docking (IFD) protocol. It was encouraging to see that, among the 16 hits compounds we identified three hits

Table 1. The result of docking studies BACE-1 with natural compounds

Entry ID	Compound Name	Medicinal Plant Name	Glide gscore	Glide energy	Hydrogen bond interaction	Distance
1	isochlorogenic acid	Centella asiatica	-14.716226	-81.566793	Lys 382 N-H···O	.979
					Glu 326 O···H-O	3.343
					Asn 294 N-H···O	3.324
					Ser 386 O···H-O	2.884
					Arg 296 N-H···O	3.108
					Gln 134 O···H-O	2.725
					Thr 133 O-H···O	4.050
					Gly 291 O···H-O	2.702
					Lys 168 O-H···O	
2	benzyl, alcohol, xylosyl(1-6)glucoside	*Panax ginseng*	-13.225116	-73.623927	Gly 95 O-H···O	3.154
					Tyr 259 O-H···O	3.646
					Asp 289 O-H···O	2.758
					Phe 169 O-H···O	3.387
					Lys 168 N-H···O	3.190
					Arg 296 N-H···O	3.137
					Arg 296 N-H···O	3.098
					Glu 326 O-H···O	3.080
					Thr 293 O-H···O	2.473
					Gln 134 O-H···O	2.722
					Ser 386 O-H···O	2.605
					Asn 294 N-H···O	2.835
3	benzyl-beta-primeveroside	*Panax ginseng*	-14.141733	-70.104241	Arg 189 N-H···O	3.236
					Ser 96 O-H···O	3.547
					Ile 187 O-H···O	3.145
					Ile 187 O-H···O	3.095
					Thr 390 O-H···O	3.317
					Thr 133 O-H···O	3.556
					Arg 296 N-H···O	3.062
					Phe 169 O-H···O	3.069
					Gly 72 O-H···O	2.878
					Thr 292 O-H···O	2.996
4	Kaempferol-O-rhamnosylhexoside	Ginkgo Biloba	-13.916	-88.275007	Thr 133 O-H···O	2.236
					Gln 134 O···H-O	2.547
					Asp 289 O-H···O	2.145
					Ser290 N-H…O	3.195
					Phe 169 O-H···O	2.217
					Gly 72 N-H…O	2.521
5	quercetin 3-O-rhamnopyranosyl	Ginkgo Biloba	-15.01099	-95.831442	Arg 296 N-H···O	2.736
					Thr 390 O-H···O	2.847
					Thr 133 O-H···O	2.645
					Ser 96 O-H···O	2.395
					Lys187 N-H…O	2.417
					Tyr 259 O-H···O	2.721
					Pro131 N-H…O	2.921
6	Native compound (PDB ID: 2QK5)	pyrrolidines	-11.221124	-80.8739	Thr 133 O-H···O	3.788
					Gly 291 O-H···O	2.774
					Asp 289 O-H···O	2.760
					Thr 293 N-H···O	3.178
					Asp 93 O-H···O	2.541

Table 2. ADME Properties of BACE1 inhibitors

Entry ID	Title	Donor HB	Accpt HB	PSA	#stars	QP Log BB	QP log HERG	QPP Caco	QPP MDCK	QP polrz	Mol MW	Human Oral Absorption	Rule Of Five
1	isochlorogenic acid	7	11.45	240.733	4	-4.836	-4.28	0.261	0.084	43.68	516.457	1	3
2	benzyl,alcohol,xylosyl(1-6)glucoside	6	17	158.078	0	-2.47	-5.36	62.479	24.699	34.9	402.397	2	1
3	Benzyl-beta-primeveroside	6	17	158.272	0	-2.406	-5.223	64.871	25.723	34.872	402.397	2	1
4	Kaempferol-O-rhamnosylhexoside	8	19.8	240.546	7	-4.297	-6.262	3.413	1.066	51.429	594.525	1	3
5	quercetin 3-O-rhamnopyranosyl	9	20.55	263.708	9	-5.303	-6.329	0.731	0.202	51.073	610.524	1	3

compounds have the best potent for inhibiting BACE1 protein. Further, detailed analysis of the top three docking hits in terms of their structures and Ligand–receptor interactions were carried out. The best three compounds out of screened and their corresponding chemical and its medicinal plant names are: compound-1 (isochlorogenic acid) from *Centella asiatica*, compound-2 (benzyl, alcohol, xylosyl(1-6)glucoside) from *Panax ginseng* and compound-3 (benzyl-beta-primeveroside) from *Panax ginseng*, compound-4 (Kaempferol-O-rhamnosylhexoside) from Ginkgo Biloba, compound-5 (quercetin 3-O-rhamnopyranosyl) from Ginkgo Biloba. The binding modes of three hits compounds and their interacting

residues are shown in Fig. A–E. Furthermore, Glide score and Glide energy of the three hits and original ligand are showed in Table 1.

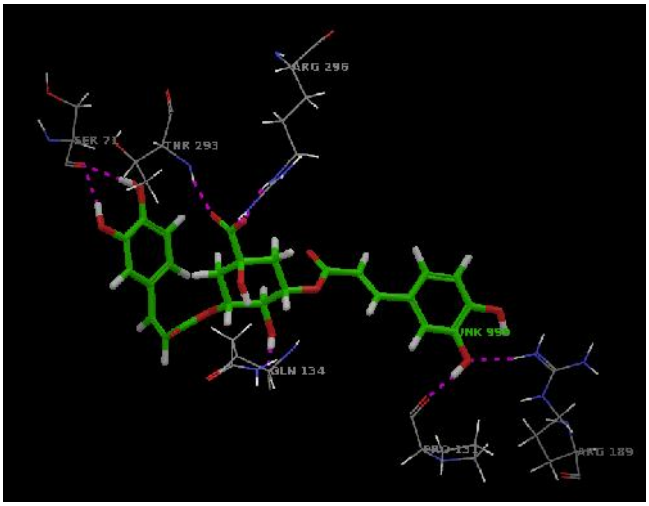

Figure A. BACE-1 binding with isochlorogenic acid

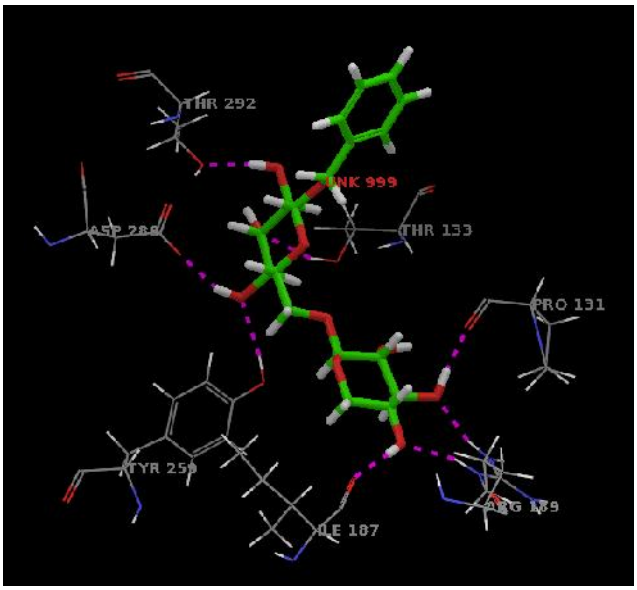

Figure B. BACE-1 binding with benzyl,alcohol,xylosyl (1-6)glucoside

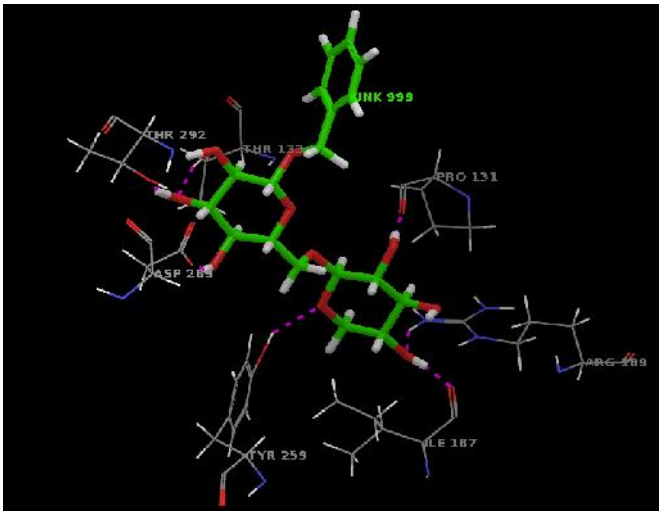

Figure C. BACE-1 binding with benzyl-beta-primeveroside

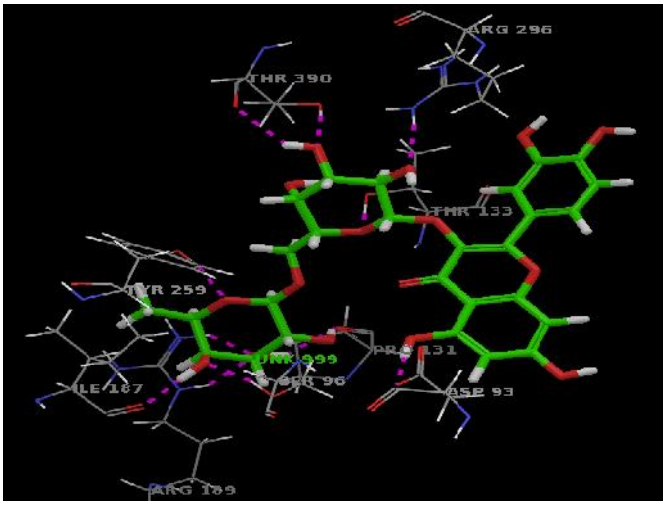

Figure D. quercetin 3-O-rhamnopyranosyl

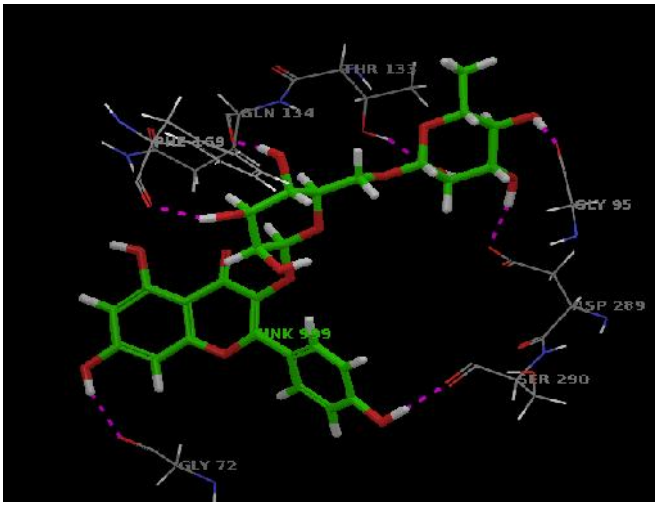

Figure E. Kaempferol-O-rhamnosylhexoside

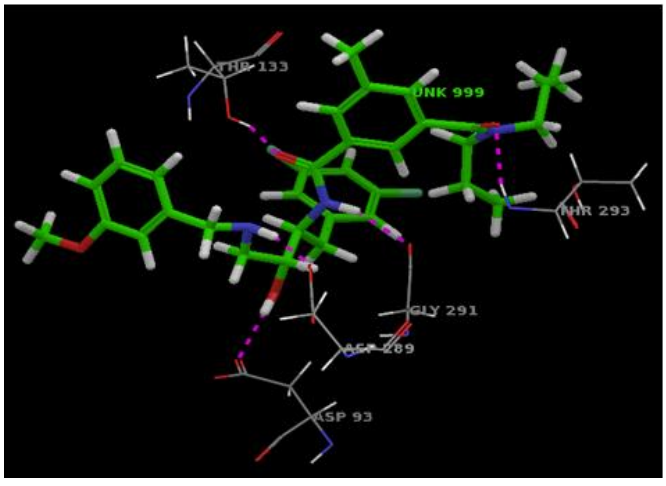

Figure F. Native compound

Conclusions

Structure-based virtual screening was carried out to identify novel BACE1 inhibitors using Glide software. Nearly 616 compounds of 9 selective medicinal plants from TCM database were docked and ranked by consensus score. Initially, we filtered out 58 compounds from the HTVS process against the target BACE1 based on the glide score and glide energy. After

58 compounds were selected for SP and XP docking using BACE1 protein and we identified 16 hits compounds. Furthermore, among the 16 hits we identified five compounds have the most potent and best BACE1 inhibitors using induce fit docking analysis. Furthermore, these five best hits compared with the native ligand which results shows that five best compounds have best binds score binding energy, hydrogen bond interaction and compare with original ligand. The novel, potent, and selective BACE1 inhibitors reported here will contribute to the development of new therapeutic approaches for the treatment of Alzheimer's disease. Figure A represents the binding mode of compound-1 (isochlorogenic acid) with BACE1. The binding mode of this compound at the active site of BACE1 formed hydrogen bond with the amino acids such as Lys 382 Glu 326, Asn 294, Ser 386, Arg 296, Gln 134, Thr 133, Gly 291, Lys 168. The length of hydrogen bond between ligand and BACE1 protein are showed in Table1.

Figure B represents the binding mode of compound-2 (floralginsenoside N) with BACE1. The binding mode of this compound at the active site of BACE1 formed hydrogen bond with the amino acids such as Gly 95, Tyr 259, Asp 289, Phe 169, Lys 168, Arg 296, Arg 296, Glu 326, Thr 293, Gln 134, Ser 386 and Asn 294. The length of hydrogen bond between ligand and BACE1 protein are showed in Table1. Figure C represents the binding mode of compound-3 (quercetin-3-O-rhamnoside-7-O-(6-feruloylgluco-(1¡V3)-rhamnoside)) with BACE1. The binding mode of this compound at the active site of BACE1 formed hydrogen bond with the amino acids such as Arg 189, Ser 96, Ile 187, Thr 390, Thr 133, Arg 296, Phe 169, Gly 72 and Thr 292. The length of hydrogen bond between ligand and BACE1 protein are showed in Table1. Figure D represents the binding mode of compound-4 (Original ligand (PDB ID: 2QK5)) with BACE1. The binding mode of this compound at the active site of BACE1 formed hydrogen bond with the amino acids such as Thr 133, Gly 291, Asp 289, Thr 293 and Asp 93 O-H¨¨O. The length of hydrogen bond between ligand and BACE1 protein are showed in Table1.

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