Research Article

Design and Molecular Screening of Various Compounds by Molecular Docking as BACE-1 Inhibitors

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Alzheimer's disease is a neurodegenerative disorder and does not have a complete cure till date. Various molecules are in clinical research and are in the pipeline to target major disease-causing agents. Beta Secretase Amyloid Cleaving Enzyme, or BACE-1, also known as β-secretase, is one of the major drug targets for the treatment of Alzheimer's disease. Molecular docking was performed with modified compounds derived from flavonoids (Quercetin, Myricetin & Baicalein), ferulic acid, and donepezil with the BACE-1 protein. The key residues of the active site of BACE-1 are Asp228, Thr232 at the S3 pocket, Tyr71 and Thr72 of the β-hairpin flap, and Gly11 at loop 10s. On the basis of docking score, alignment with Lipinski's rule, and toxicity, it was estimated that derivatives of Baicalein (b17, b39), Myricetin (T25, T21), and Quercetin (SP27, SP32) exhibit better results than their parent compounds. The molecules reach the active site of the BACE-1 gorge and clearly indicate that natural products could be a major breakthrough in Alzheimer's disease study.

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1. Introduction

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder manifested by progressive impairment of activities of daily living, cognitive and memory deterioration, and a variety of neuropsychiatric symptoms and disturbances [1][2]. According to the Dementia India report (2010), about fifteen crores of rupees is being spent by the government, and the figures would be doubled by 2030. It is estimated that a total of 15 million people will be affected by AD in India by 2030.

Understanding the cause of AD is essential for the management of the disease. Individuals who suffer from Alzheimer's have numerous Senile Plaques (SPs) and Neuro Fibrillary Tangles (NFTs) characterized by neural cell loss and vascular damage [3]. β-Amyloid (a protein fragment) that builds the connection between the nerve cells is the cause of the above condition.

β- Amyloid hypothesis: - The β-amyloid is a by-product of the protein Amyloid Precursor Protein (APP), whose function is believed to be involved in neuronal degradation [4][5][6]. On the surface of neurons, there is a transmembrane protein called the Amyloid Precursor Protein (APP), which is cleaved by a series of enzymes called secretase. In normal physiology, α-secretase cleaves APP to give soluble α-APP and an Amyloid-β unit [7].

The Aβ unit is cleaved by γ-secretase to give Aβ-40, which contains 40 amino acid residues. In the case of Alzheimer's disease, instead of α-secretase, an abnormal β-secretase cleaves APP followed by γ-secretase and produces Amyloid-β protein, and tangles are fibres of tau protein that build inside the cells [8][9]. In order to explain the pathophysiology, the **β-amyloid hypothesis** has been discussed **(Fig.1).**

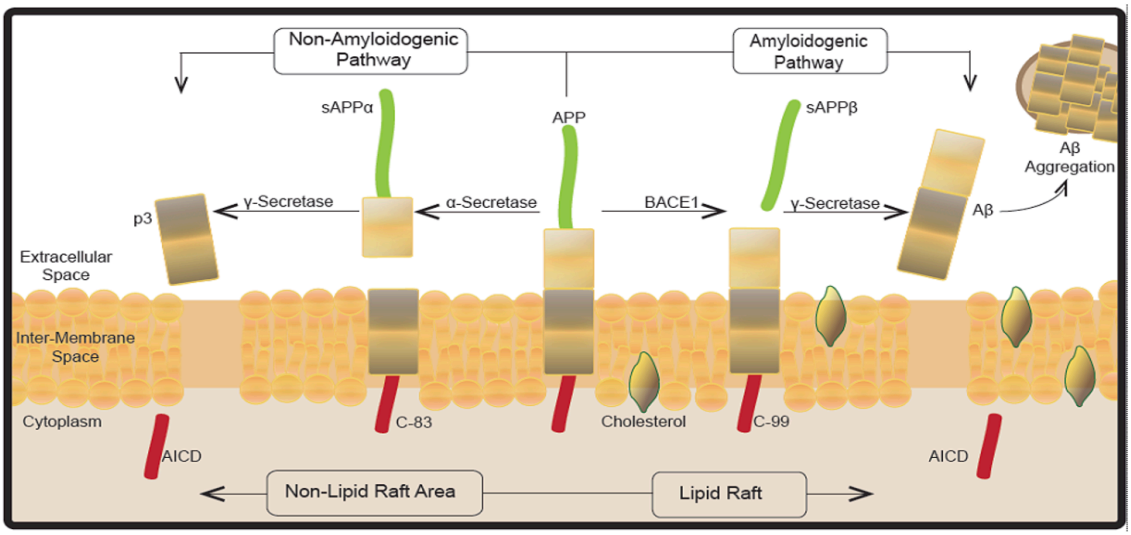

Fig. 1. Amyloid Cascade Hypothesis

Amyloid β plaques and tangles [10] tend to develop in a predictable pattern and in far more numbers in the brain areas that are important for memory in persons suffering from Alzheimer's disease. The

pathophysiology of this disease is related to the injury and death of neurons starting from the hippocampus that ultimately extends to the entire region of the brain.

2. Literature Review

2.1. Review on major protein target (BACE-1)

Alzheimer's disease is thought to be caused by the abnormal build-up of proteins in and around brain cells. One of the proteins involved is called amyloid β, deposits of which form plaques around brain cells.

According to the amyloid hypothesis, Aβ accumulation is a basic cause of AD. In Alzheimer's brain physiology, APP is cleaved by β and γ secretase enzymes, yielding 40 soluble amino acid peptides. But in the case of AD, a two-step proteolytic process is initiated by the **Beta-site Amyloid Precursor Protein Cleaving Enzyme or BACE-1 (β-secretase)**, followed by **γ-secretase**, yielding a 42 insoluble amino acid peptide called amyloid-β (Aβ), and consequently forming β-amyloid plaques**. β-Secretase (BACE-1) is the first protein that acts on the amyloid precursor protein (APP) in the production of amyloid-β (Aβ). The BACE-1 enzyme has long been observed as an important therapeutic target for AD in the development of inhibitor drugs for the reduction of Aβ (Fig. 2).** Presently, β-secretase is a major drug target for AD, and the development of its inhibitor drugs is being pursued in many research laboratories around the world [11][12][13][14].

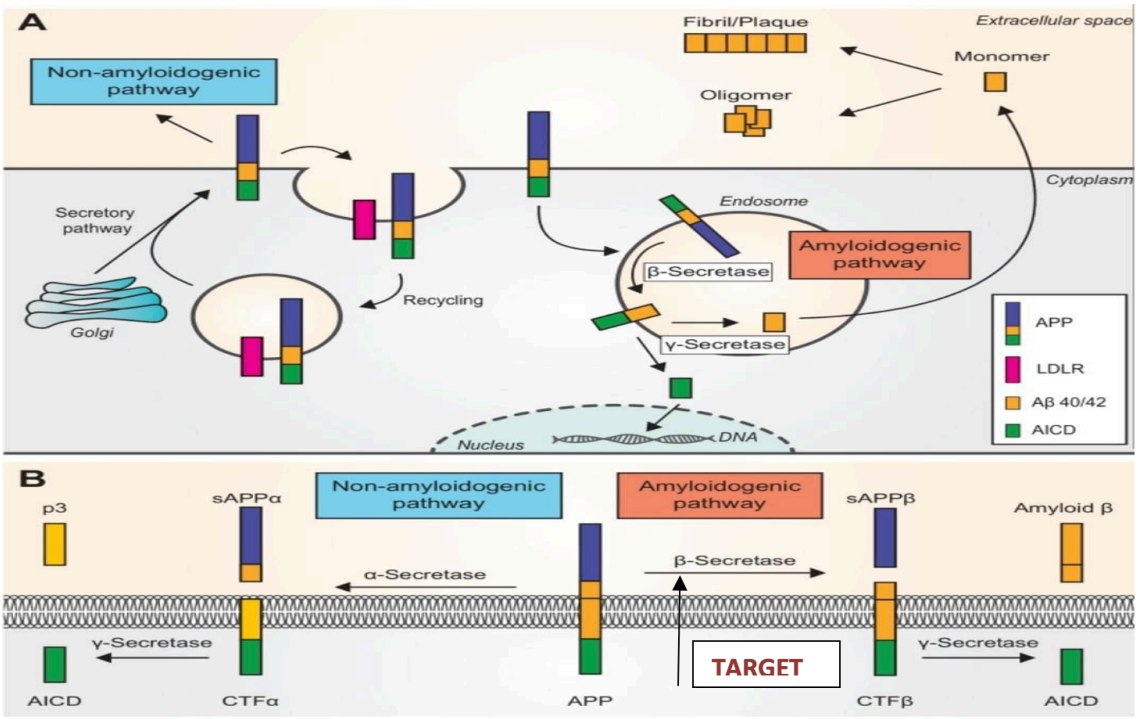

Fig. 2. Target identification from the Amyloid β pathway

Due to its evident rate limiting function, BACE-1 seems to be a prime target to prevent Aβ generation in AD. BACE-1, or Beta-site Amyloid Precursor Protein Cleaving Enzyme, consists of a **501-amino acid sequence** and bears a transmembrane aspartic protease. BACE-1 has two aspartic protease active site regions from residues 93-96 and from residues 289-292. **BACE-1 (www.rcsb.org, PDB ID: 2ZHT)** has an N-terminal end, a C-terminal end, and the active site cleft is located between the N- and C-terminal lobes. The active site is partially shielded by an **anti-parallel hairpin loop** known as **"flap,"** which controls substrate access and proteolytic specificity. The binding site of BACE-1, which interacts with key residue **Asp228**, establishes a force of interaction with **Thr232 at the S3 pocket,** interacts with **Tyr71** and **Thr72** of the **β-hairpin flap**, and stabilizes the binding by hydrogen bonding with **Gly11 at loop 10s.** To complement this, **Ile126 and Arg128** are essential for interaction and are located near **loop 113s,** present opposite to that of **loop 10s (Fig. 3)** [11][13][14][15].

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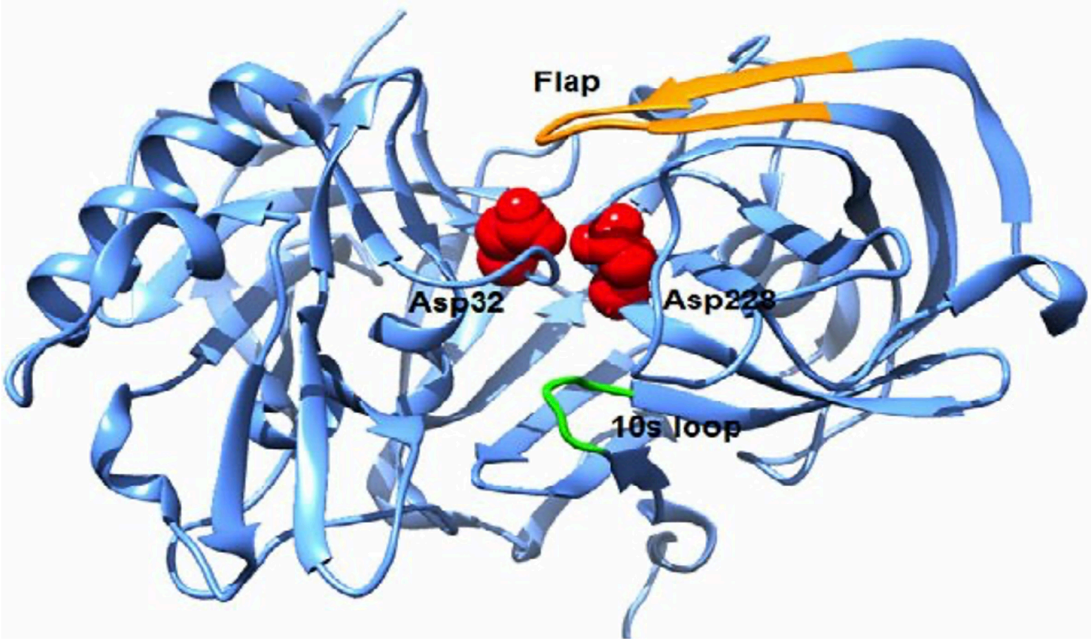

Fig. 3. Active site of BACE-1 protein

Apart from these, various therapeutic strategies involving natural products have been studied to develop new molecules via drug design. It has been found that various natural products could be major agents for inhibiting beta-amyloid plaques, serving as diagnostic tools for AD senile plaques, and acting as BACE-1 inhibitors. Currently, many therapeutic strategies are being explored to treat AD; the US-FDA approved drugs number only five, of which four, **Donepezil, Rivastigmine, Tacrine, and Galantamine, are Cholinesterase inhibitors, and one, Memantine, is an NMDA antagonist** [16].

2.2. Review on drugs under clinical trial against BACE-1 inhibitors

Various **BACE-1 inhibitors** have entered clinical trials in recent years. Lilly inhibitors **LY2886721** and **LY3202626** failed in phase 2 trials due to hepatotoxicity and impaired cognitive performance, respectively. Five additional **BACE-1 inhibitors** like **Lanabecestat, Atabecestat, Verubecestat, Elenbacestat, and Umibecestat (Fig. 4)** have **failed phase 3 clinical trials** due to **lack of cognitive performance** or **associated side effects. Lanabecestat (NCT02783573)** was discontinued as it **failed to reduce** cognition. Similarly, **Atabecestat (NCT02569398)** was discontinued after a **trial in preclinical AD** due to recurrent hepatotoxicity and worse cognitive outcomes. **Verubecestat (NCT01953601) failed** to **decrease** the cognitive decline in **mild-to-moderate AD** patients. **CNP520 or Umibecestat (NCT03131453)** was initially found to be safe and in early phase 1 and 2 clinical trials until **worse**

cognitive decline performance and significant **weight loss** were observed in **phase 3 clinical** trials. **Elenbecestat (NCT02956486)** was discontinued after some safety concerns were found. The **BAN2401** is targeted against **BACE-1** also, and trials to study its cognitive decline are being adopted. No **BACE-1** inhibitors are being actively investigated in clinical trials [6][15][17][18].

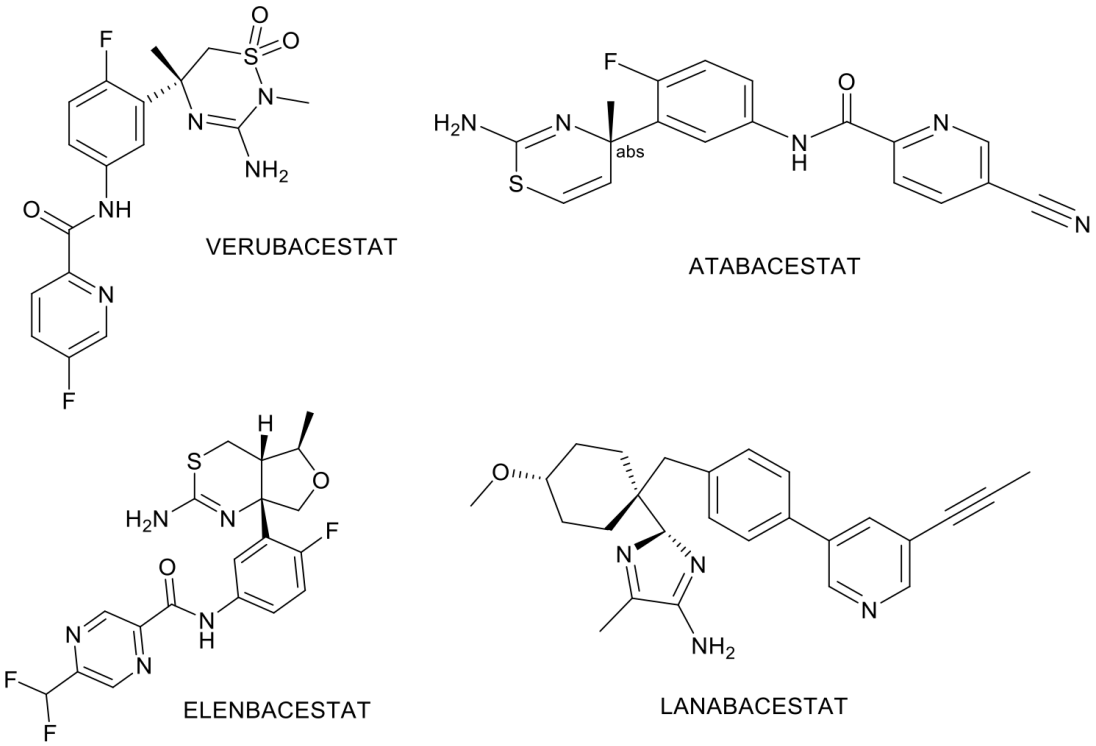

Fig. 4. Drugs under clinical trial against BACE-1

2.3. Review on drugs against Alzheimer's Disease (Donepezil)

The drug most extensively researched and useful in all stages of AD is **Donepezil.** Donepezil hydrochloride is an acetylcholinesterase inhibitor used in mild, moderate, and severe Alzheimer's disease. Studies were then focused on finding a new type of **acetylcholinesterase (AChE)** inhibitor that would overcome the disadvantages of these two compounds. Donepezil hydrochloride is the second drug approved by the **USFDA** for the treatment of mild to moderate Alzheimer's disease. It is a new class of acetylcholinesterase inhibitor having an **N-Benzyl piperidine** and an **indanone moiety (Fig. 5)** which shows longer and more selective action. **The thiazolo-pyrimidine derivatives have a wide range of biological activities such as calcium channel blocking, antimalarial, and antitubercular,**

acetylcholinesterase inhibitory, glutamate receptor antagonistic,5-HT2a receptor antagonistic, and anticancer activities [16].

On site 3 of the benzene ring, as mentioned in the above structure, modification in that part can lead to a strong acetylcholinesterase inhibitor. The results of the enzyme inhibitions exhibit that electron-withdrawing groups like **Cl, F can render the best effect** at the ortho and para positions of the phenyl ring. **Introduction of the basic nitrogen to the molecule, which will be protonated at physiological pH, will provide an additional ionic π interaction within the catalytic site of acetylcholinesterase and lead to a more potent inhibitor** [19][20].

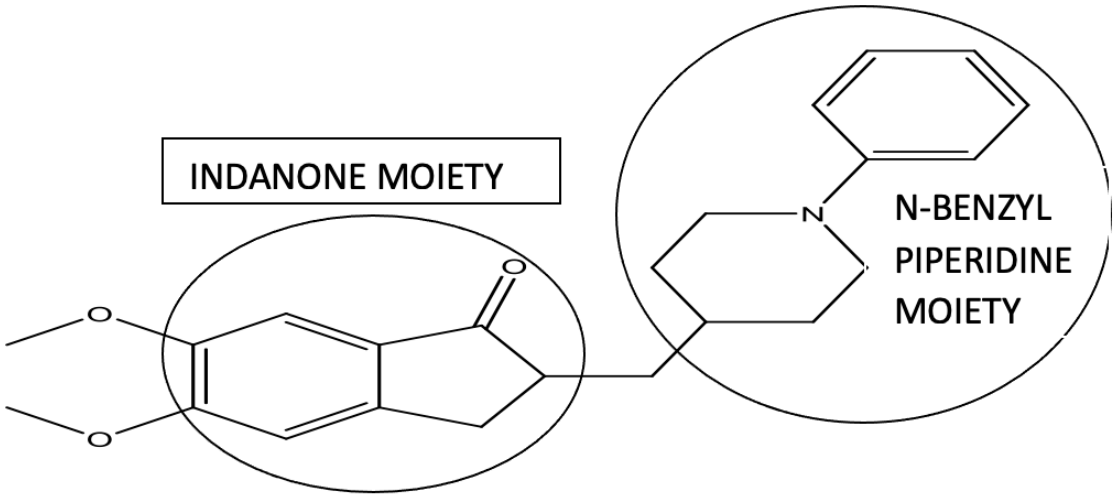

Fig. 5. Donepezil structure elucidating the major moieties

2.4. *Review on natural products against Alzheimer's disease*

Greater trial failures highlight the need for different approaches to AD therapy. Till date, **amyloid-based therapeutics** appears to be **ineffective** in modifying the disease course for AD. Future clinical trial efforts should instead **focus** on applying **natural products** to anti-amyloid treatment strategies for the **preclinical disease (**the earlier the better).

Natural compounds are an emerging approach for AD therapy. During the 90s, several other compounds were studied in clinical trials for AD therapy. Many natural compounds have been reported to effectively modulate Beta-site Amyloid precursor protein-Cleaving Enzyme 1 (BACE-1). Patients suffering from the disease are characterised by reduced cognitive function, progressive deterioration

of memory and neuronal damage, and changes in mood and behaviour. Inhibition of BACE1 by natural products has rendered promising results in AD therapies. Various flavonoids (Galangin, Myricetin, Baicalein, Quercetin), alkaloids (berberine), terpenes, curcumin, ferulic acid, etc., may exhibit potential BACE1 inhibition [5].

2.4.1. Flavonoids (Quercetin)

The flavonoids like quercetin, myricetin, and baicalein contain considerable pharmacological effects (Fig. 6). Quercetin has demonstrated antioxidant and therapeutic potential against Alzheimer's disease (AD). **Quercetin inhibits amyloidogenesis and amyloid β accumulation, thereby reducing amyloid plaque levels in AD-associated mouse models** [5][21].

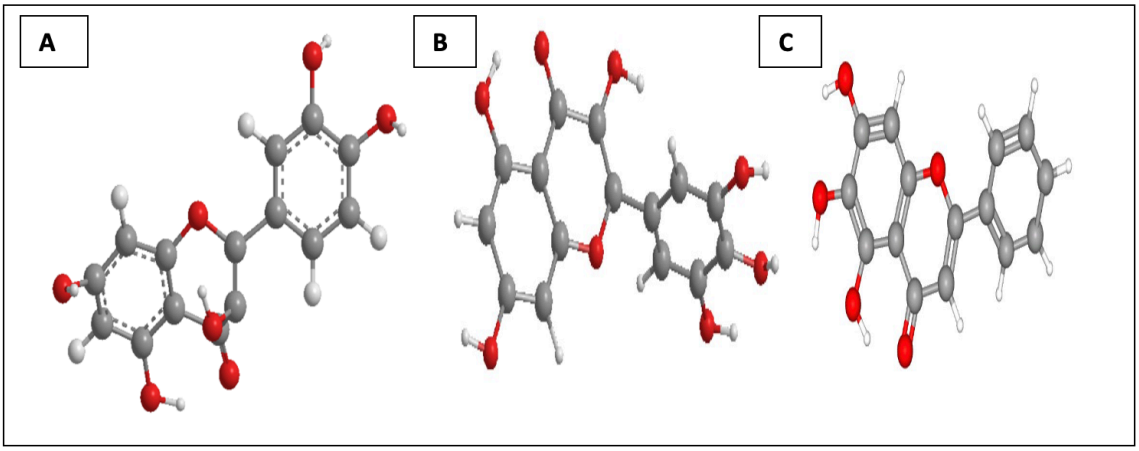

Fig. 6. 3D structure representation of flavonoids, A. quercetin, B. myricetin, and C. baicalein

2.4.2. Flavonoids (Myricetin)

Myricetin (3,3′,4′5,5′,7-hexahydroxylflavone) is a common natural flavonoid found in many fruits, vegetables, and herbs. Myricetin has two aromatic rings A and B in its structure that are combined by a three-carbon chain forming a cyclic ring C, and reports suggest that the presence of more hydroxyl groups makes it a potent antioxidant. **Myricetin is an ideal iron ion chelating agent**, thereby able to reduce brain iron ion content by the inhibition of transferrin receptor 1 expression, the increase of the activity of antioxidant enzymes, and the reduction of lipid peroxidation, thus significantly reversing the **cognitive dysfunction in mice caused by scopolamine** [5][22]. Shimmyo et al. have reported that

myricetin has dual activity, as **it can directly inhibit BACE1 activity** without affecting protein expression and **showed activation of α-secretase** in a cell-free enzyme activity assay.

2.4.3. Flavonoids (Baicalein)

Baicalein (5,6,7-trihydroxyflavone) is a flavone, originally isolated from the roots of Scutellaria baicalensis and Scutellaria lateriflora. It has a wide range of roles as an antioxidant, hormone antagonist, radical scavenger, anti-inflammatory, anti-microbial, neuroprotective, apoptosis inducer, etc. [5][23]. As a flavonoid with two pro-hydroxyl groups, baicalein exhibits strong antioxidant activity by direct scavenging of hydroxyl and superoxide radicals. The position and availability of hydroxyl groups are essential for radical scavenging activity. The presence of an ortho-dihydroxy group or catechol structure in the A ring is essential for an intrinsic antioxidant property. The neuroprotective effect of baicalein may be due to the increase in the number of dopaminergic neurons and is caused by anti-apoptotic mechanisms of baicalein [23].

2.5. Ferulic acid

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is a widely distributed constituent of plants. Ferulic acid (FA) is an **antioxidant** naturally present in plant cell walls with anti-inflammatory activities, and it is able to **act as a free radical scavenger** [24].

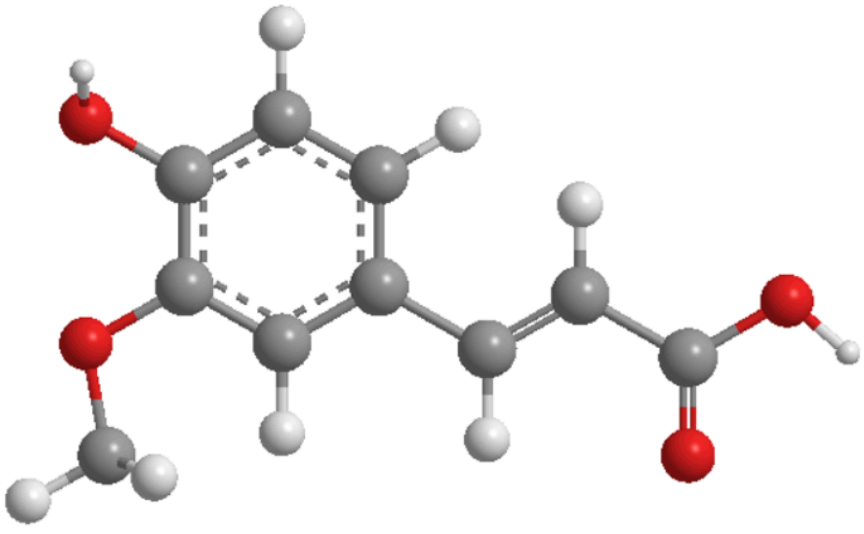

Fig. 7. 3D representation of ferulic acid structure

3. Aims & Objectives

Based on the above literature study and etiology of the disease, **BACE-1 enzyme** is selected as an important **therapeutic target for AD** in the development of inhibitor drugs for reduction of Aβ. **Small molecules** that may be inhibitors are designed to be **complementary** to the target binding site. *In silico* drug design of various natural products like **flavonoids (myricetin, baicalein, quercetin), ferulic acid, and donepezil (here taken as standard)** was chosen as the lead molecule because they form hydrogen bonds and π- π interactions with our target. **Various libraries** of lead compounds have been designed by **modification of the above scaffolds** (structure-based drug design or SBDD) based on their structure-activity relationships to monitor the best-suited lead that becomes a **suitable target** for the receptor. Screening the designed leads for their **drug likeness, *in silico* toxicity and absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies**, and **docking scores (obtained from *in silico* docking)**, and a candidate drug which binds with high affinity and high selectivity (also known as **docking and virtual screening)**, are predicted to show similar actions in our body, and a **best match between the receptor and ligand is found**. The lead molecule with **no toxicity** is selected as a result of screening of a library of compounds **with the best docking scores, ligand-receptor interactions, and their ability to reach the active site of the enzyme** and was chosen as our future lead molecule. **Virtual screening** is a fast and cost-effective method that generates leads for drug design. It helps to become oriented with the **AutoDock Vina** user interface and some other tools. Thus, the **leads, which involve the prediction of ligand conformation and orientation within a binding site** of the BACE-1 protein, may be leads for further drug design on Alzheimer's disease.

4. Materials and Methods

4.1. Materials

ChemDraw Ultra 12.0 (CambridgeSoft, 100 Cambridge Park Drive, Cambridge), AutoDock Vina (The Scripps Research Institute, La Jolla, California), Cheminformatics tool eLEA3D (https://chemoinfo.ipmc.cnrs.fr/LEA3D/index.html), Discovery Studio Visualizer software, Molinspiration (https://www.molinspiration.com/cgi-bin/properties), Discovery Studio Visualizer software, admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), and ChemMine Tools.

Molecular docking

Docking enables us to understand the *in silico* drug-receptor interactions. The ligand or a designed molecule is targeted to a specific protein to form a non-covalent bond of a stable ligand-protein complex. The information obtained from the docking suggests the **docking score, free energy, and stability of complexes**. Nowadays, docking enables us to predict the tentative binding parameters of the ligand-receptor complex.

Construction of a chemical library of compounds

The traditional approach in drug discovery included utilizing the **ancient knowledge** of chemicals, **plants**, materials, and things **for a particular target**, mostly guided by trial-and-error methods. Larger compounds **(approximately 20)** have completed **randomized control trials**, **large phase 3**, **double-blind**, and **in cohorts** of patients at various stages of AD. None have demonstrated any efficacy in **slowing cognitive decline** or improving the disease globally. Thus, ancient knowledge of **Donepezil (Standard)**, **Quercetin**, **Myricetin**, **Baicalein**, and **Ferulic acid** was chosen as probable scaffolds which are in study against Alzheimer's disease. The target selected is **BACE-I or β-secretase enzyme**, which is the **rate-limiting step** in the **amyloidogenic pathway**.

Further, the **refinements of lead compounds** are accomplished by the laboratory techniques which have a **tropism towards drug action** with fewer side effects.

4.2.1.1. Chemical modification of the structure of Donepezil (Standard)

The structural activity relationship of Donepezil, site 3 of the benzene ring, leads to a strong acetylcholinesterase inhibitor. The introduction of the basic nitrogen to the molecule, which will be protonated at physiological pH, will provide an additional ionic π interaction within the catalytic site of acetylcholinesterase and lead to a more potent inhibitor. The π-π interactions play an important role, giving the ligand – acetylcholinesterase complexes high stability and, at the same time, improving the recognition process between this enzyme and the target molecules [25][26].

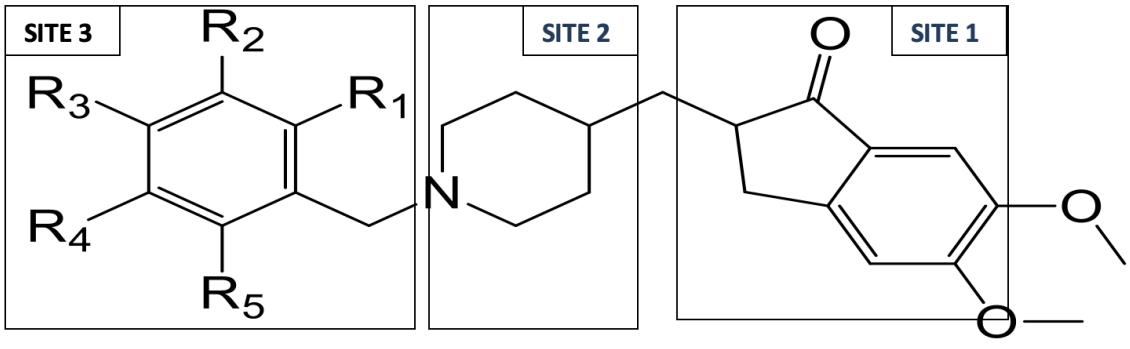

Fig. 9. Mention what is Site 1, Site 2 and Site 3 in the above figure

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MOL.	R1	R2	R3	R4	R5	R6	Docking Score Kcal/mol
Ss1	N-CH3	OH	OH	OH	OH	OH	-7.5
Ss2	OH	N-CH3	OH	OH	OH	OH	-7.3
Ss3	OH	OH	N-CH3	OH	OH	OH	-7.5
Ss4	OH	OH	N-CH3	OH	OH	OH	-7.7
Ss5	OH	F	N-CH3	OH	OH	OH	-7.9
Ss6	OH	N-CH3	F	OH	OH	OH	-7.6
Ss7	OH	Cl	N-CH3	OH	OH	OH	-7.9
Ss8	Cl	OH	N-CH3	OH	OH	OH	-7.4
Ss9	Cl	OH	N-CH3	OH	Cl	OH	-7.9
Ss10	Cl	N-CH3	OH	OH	OH	OH	-7.8
Ss11	OH	N-CH3	Cl	OH	OH	OH	-7.5
Ss12	N	Cl	OH	OH	Cl	OH	-7.9
Ss13	OH	N-CH3	I	OH	OH	OH	-7.5
Ss14	OH	I	N-CH3	OH	OH	OH	-7.7
Ss15	**I**	**N**	**OH**	**OH**	**I**	**OH**	**-8.4**
Ss16	NH_2	F	OH	OH	OH	OH	-7.5
Ss17	F	OH	NH_2	OH	F	OH	-7.7
Ss18	F	NH_2	OH	OH	F	OH	-7.8
Ss19	OH	F	NH_2	OH	F	OH	-8.3
Ss20	**Cl**	NH_2	**OH**	**OH**	**Cl**	**OH**	**-8.4**
Ss21	Br	OH	NH_2	OH	OH	OH	-7.0
Ss22	I	OH	NH_2	OH	OH	OH	-7.6
Ss23	OH	NH_2	I	OH	NH_2	OH	-7.1

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MOL.	R1	R2	R3	R4	R5	R6	Docking Score Kcal/mol
2224	I	NH_2	OH	OH	OH	NH_2	-7.7
Ss25	OH	I	NH_2	OH	I	OH	-6.9
Ss26	I	OH	NH_2	OH	I	OH	-7.2
Ss27	NH_2	OH	CH3	OH	OH	OH	-7.5

4.2.1.2. Chemical modification of the structure of Ferulic acid

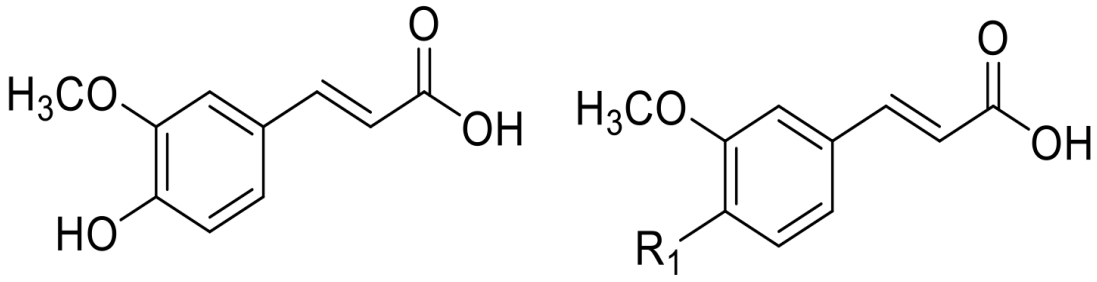

Fig. 10. Structure of Ferulic acid (left) and chemical modification in R_1 of Ferulic acid

The structure-activity relationship of ferulic acid involves modification of various groups like hydroxyl and methoxy group substitution in Ring-A (phenyl ring). Substitution in the meta as well as para position of the aryl ring is important for some inhibitory activities[12][24]. The -OCH_3 group attached at C-3 of the phenyl ring and the carboxylic acid (-COOH) group act as anchors of ferulic acid, by which it binds to the lipid bilayer**; thus, the only region of modification is the –OH group in C-3 of the phenyl ring (marked as R_1 in Fig. 10).** The docking scores and the molecules designed are represented in **Table 1.**

MOLECULES	R1	Nomenclature	Docking Score Kcal/mol
SM1	-Cl	(E)-3-(4-chloro-3-methoxyphenyl)acrylic acid	-6.3
SM2	-F	(E)-3-(4-fluoro-3-methoxyphenyl)acrylic acid	-6.9
SM3	-I	(E)-3-(4-iodo-3-methoxyphenyl)acrylic acid	-5.2
SM4	$-NH_2$	(E)-3-(4-amino-3-methoxyphenyl)acrylic acid	-5.2
SM5	$-NO_2$	(E)-3-(3-methoxy-4-nitrophenyl)acrylic acid	-6.8
SM6	-CN	(E)-3-(4-cyano-3-methoxyphenyl)acrylic acid	-5.8
SM7	$-CH_3$	(E)-3-(3-methoxy-4-methylphenyl)acrylic acid	-6.9
SM8	$-CH_2CH_3$	(E)-3-(4-ethyl-3-methoxyphenyl)acrylic acid	-5.6
SM9	$-CH_2CH_2CH_3$	(E)-3-(3-methoxy-4-propylphenyl)acrylic acid	-5.7
SM10	$-CH_2CH_2CH_2CH3$	(E)-3-(4-butyl-3-methoxyphenyl)acrylic acid	-5.9
SM11	$-CH_2Cl$	(E)-4-(4-(chloromethyl)-3-methoxyphenyl)-but-3-en-2-one	-5.5
SM12	$-CH_2CH_2Cl$	(E)-3-(4-(2-chloroethyl)-3-methoxyphenyl)acrylic acid	-7.1
SM13	$-CH_2CH_2CH_2Cl$	(E)-3-(4-(3-chloropropyl)-3-methoxyphenyl)acrylic acid	-5.8
SM14	$-CH_2F$	(E)-3-(4-(fluoromethyl)-3-methoxyphenyl)acrylic acid	-5.4
SM15	$-CH_2CH_2F$	(E)-3-(4-(2-fluoroethyl)-3-methoxyphenyl)acrylic acid	-6.5
SM16	$-CH2CH_2CH_2F$	(E)-3-(4-(3-fluoropropyl)-3-methoxyphenyl)acrylic acid	-6.8

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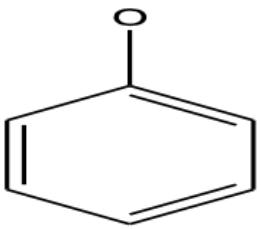
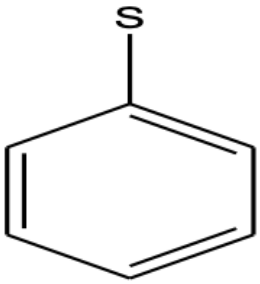
MOLECULES	R1	Nomenclature	Docking Score Kcal/mol
SM17		(E)-3-(3-methoxy-4-phenoxyphenyl)acrylic acid	-7.5
SM18		(E)-3-(3-methoxy-4-(phenylthio)phenyl)acrylic acid	-7.5

Table 1. Modification in R_1 and the representation of Docking score

4.2.1.3. Chemical modification of the structure of Myricetin

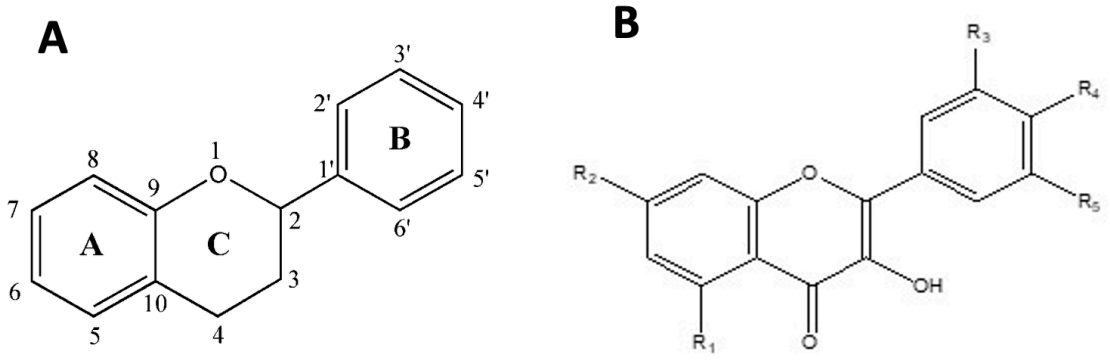


Fig. 11. A. Structure of Myricetin, B. Chemical modification in R_1, R_2, R_3, R_4, R_5 of Myricetin

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The Myricetin structure includes Ring A and Ring C, both referred to as Chroman. -OH groups are attached at the C-5 & C-7 positions of Ring A, and a Ring B (phenyl) is attached at C-2. An OH group is present at C-3, C-4, or C-5, and phenolic OH groups are attached at various positions in the structure. The structure-activity relationship refers to di-substitution or tri-substitution in Ring B and rare substitution at C-2 & C-6 (OH). The hydroxyl groups are free, etherified, or engaged in glycosidic linkage, isoprenylation (C_5H_8), or methylation (CH_3) at C-5 and C-7. A double bond is seen between C-2 & C-3, and a carbonyl group is at C-4 [22]. The docking scores and the molecules designed are represented in **Table 2.**

MOL.	R1	R2	R3	R4	R5	DOCKING SCORE Kcal/mol
T1	**F**	OH	OH	OH	OH	-7.6
T2	**Cl**	OH	OH	OH	OH	-6.8
T3	**NH_2**	OH	OH	OH	OH	-7.1
T4	**CH_3**	OH	OH	OH	OH	-7.1
T5	OH	**F**	OH	OH	OH	-7.1
T6	OH	**Cl**	OH	OH	OH	-7.1
T7	OH	**NH_2**	OH	OH	OH	-6.6
T8	OH	**CH_3**	OH	OH	OH	-7.2
T9	OH	OH	OH	OH	**F**	-8.2
T10	OH	OH	OH	OH	**Cl**	-8.0
T11	OH	OH	OH	OH	**NH_2**	-7.8
T12	OH	OH	OH	OH	**CH_3**	-6.9
T13	OH	OH	OH	**F**	OH	-7.2
T14	OH	OH	OH	**Cl**	OH	-7.2
T15	OH	OH	OH	**NH_2**	OH	-7.0
T16	OH	OH	OH	**CH_3**	OH	-7.2
T17	OH	OH	**F**	OH	OH	-7.2
T18	OH	OH	**Cl**	OH	OH	-7.2
T19	OH	OH	**NH_2**	OH	OH	-7.8
T20	OH	OH	**CH_3**	OH	OH	-7.2
T21	**OH**	**OH**	**F**	**F**	**OH**	**-8.4**
T22	OH	OH	**Cl**	**Cl**	OH	-7.4
T23	OH	OH	OH	**F**	**F**	-7.3

MOL.	R1	R2	R3	R4	R5	DOCKING SCORE Kcal/mol
T24	OH	OH	OH	**Cl**	**Cl**	-7.1
T25	**OH**	**OH**	**F**	**F**	**F**	**-8.6**
T26	OH	OH	**Cl**	**Cl**	**Cl**	-7.4

Table 2. Modification in various regions and their representation of Docking score

4.2.1.4. Chemical modification of the structure of Quercetin

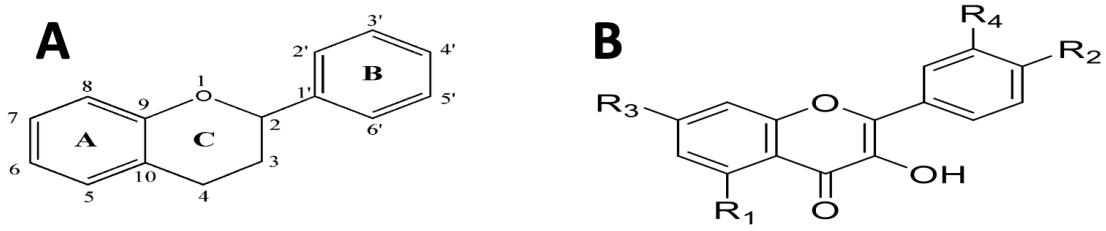
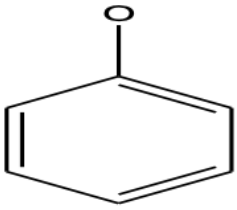
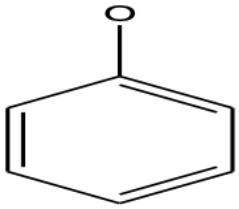


Fig. 12. A. Structure of Quercetin, B. Chemical modification in R_1, R_2, R_3, R_4 of Quercetin

doi.org/10.32388/9OCR7D

MOL.	R1	R2	R3	R4	DOCKING SCORE Kcal/mol
SP1	CH_3	OH	OH	OH	-7.8
SP2	C_2H_5	OH	OH	OH	-7.4
SP3	$CH_2CH_2CH_3$	OH	OH	OH	-6.9
SP4	Cl	OH	OH	OH	-7.0
SP5	CH_2Cl	OH	OH	OH	-7.2
SP6		OH	OH	OH	-7.6
SP7	SH	OH	OH	OH	-7.1
SP8	F	OH	OH	OH	-7.8
SP9	CN	OH	OH	OH	-7.1
SP10	OH	CH3	OH	OH	-7.1
SP11	OH	C2H5	OH	OH	-7.1
SP12	OH	CH2CH2CH3	OH	OH	-7.2
SP13	OH	Cl	OH	OH	-7.3
SP14	OH	CH2Cl	OH	OH	-7.4
SP15	OH		OH	OH	-7.8
SP16	OH	SH	OH	OH	-7.2

doi.org/10.32388/9OCR7D

MOL.	R1	R2	R3	R4	DOCKING SCORE Kcal/mol
SP17	OH	F	OH	OH	-7.2
SP18	OH	CN	OH	OH	-7.8
SP19	OH	OH	CH_3	OH	-6.9
SP20	OH	OH	C_2H_5	OH	-6.9
SP21	OH	OH	$CH_2CH_2CH_3$	OH	-7.0
SP22	OH	OH	Cl	OH	-6.8
SP23	OH	OH	CH_2Cl	OH	-7.9
SP24	OH	OH		OH	-7.8
SP25	OH	OH	SH	OH	-6.6
SP26	OH	OH	F	OH	-7.0
SP27	**OH**	**OH**	**CN**	**OH**	**-8.1**
SP28	OH	OH	OH	CH_3	-7.7
SP29	OH	OH	OH	C_2H_5	-7.4
SP30	OH	OH	OH	$CH_2CH_2CH_3$	-7.4
SP31	OH	OH	OH	Cl	-7.4
SP32	**OH**	**OH**	**OH**	**CH_2Cl**	**-7.9**
SP33	OH	OH	OH		-7.8
SP34	OH	OH	OH	SH	-6.8
SP35	OH	OH	OH	F	-7.7
SP36	OH	OH	OH	CN	-7.5
SP37	OH	OH	CN	CN	-7.9
SP38	CN	OH	CH_2Cl	OH	-7.4
SP39	OH	F	OH	F	-7.7

doi.org/10.32388/9OCR7D

Table 3. Modification in various regions and their representation of docking score

4.2.1.5. Chemical modification of the structure of Baicalein

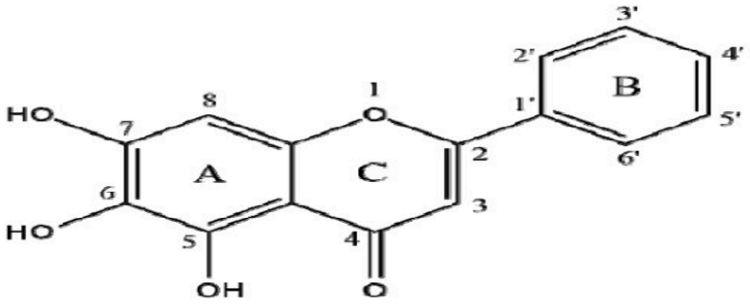


Fig. 13. Structure of Baicalein

The structure of Baicalein consists of Ring A, B, and Ring C, of which Ring A and C are chroman rings and the phenyl ring, also referred to as Ring B. After analyzing various articles, its structure-activity relationship suggested that the 5,6,7-trihydroxyl substituent and 4-carboxyl group were important for the minimum activity of inhibition. Ring B & C are not essential for activity, but they enhance activity effectively. A 4' substitution on the B ring may increase or decrease the activity [23], but multiple substitutions are detrimental to the activity. Due to steric hindrance, a substitution in the 8th position of ring A reduces the potency or potent inhibitory activity. The conversion of flavone into a flavanone (C2-C3) double bond structure in the C-ring improved water solubility. The C-6 hydroxyl group is important, and it is involved in the partial inhibition of the BACE 1 group. Different substituents connected to B increase radical scavenging activity and various other activities [5].

The chemical modifications are divided into various clusters, such as (as in Fig. 14)

- Cluster I- Modification in Ring B (saturation of Ring B) with substitution at the R1 position
- Cluster II- Same modification as Cluster I with removal of the C2-C3 double bond in Ring C
- Cluster III- Locking figure-2 at the 3' position with tert-butyl and then having a substituent at R-1
- Cluster IV: Simple mono-substitution in Ring B at R1
- Cluster V: Multiple substitutions at different positions of Baicalein at R1, R2, R3, & R4

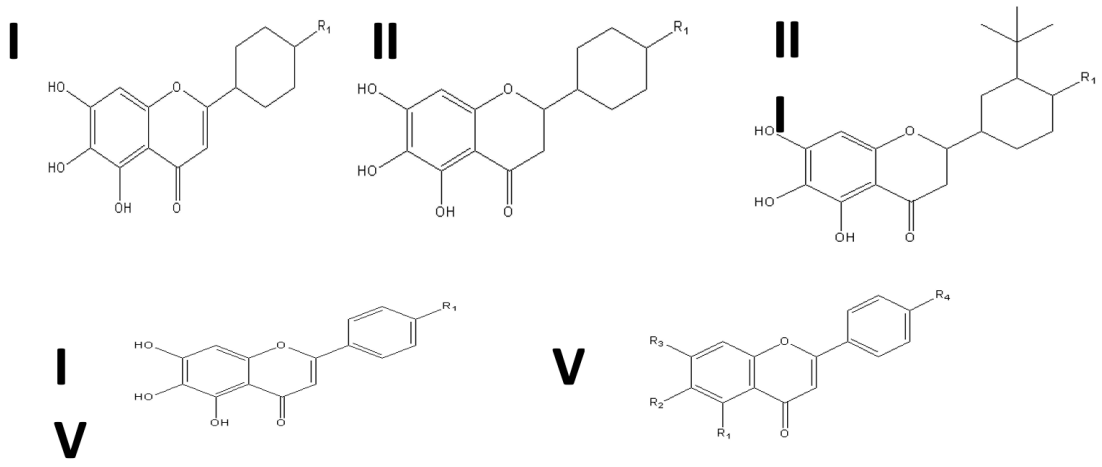


Fig. 14. A. Structure cluster modifications of I, II, III, IV, V of Baicalein

doi.org/10.32388/9OCR7D

Sl no. (cluster)	Nomenclature	R_1	Docking Score Kcal/mol
b1 (I)	2-cyclohexyl-5,6,7-trihydroxy-4H-chromen-4-one	H	-8.3
b2 (I)	4-(5,6,7-trihydroxy-4-oxo-4H-chromen-2-yl) cyclohexane carboxylic acid	COOH	-7.4
b3 (I)	5,6,7-trihydroxy-2-(4-hydroxycyclohexyl)-4H-chromen-4-one	OH	-7.6
b4 (I)	4-(5,6,7-trihydroxy-4-oxo-4H-chromen-2-yl) cyclohexane carboxamide	CONH2	-7.6
b5 (I)	5,6,7-trihydroxy-2-(4-methylcyclohexyl)-4H-chromen-4-one	CH3	-8.4
b6 (II)	2-cyclohexyl-2,3-dihydro-5,6,7-trihydroxychromen-4-one	H	-7.7
b7 (II)	4-(3,4-dihydro-5,6,7-trihydroxy-4-oxo-2H-chromen-2-yl) cyclohexane carboxylic acid	COOH	-7.1
b8 (II)	2,3-dihydro-5,6,7-trihydroxy-2-(4-hydroxycyclohexyl) chromen-4-one	OH	-7.9
b9 (II)	4-(3,4-dihydro-5,6,7-trihydroxy-4-oxo-2H-chromen-2-yl) cyclohexane carboxamide	CONH2	-7.5
b10 (II)	2,3-dihydro-5,6,7-trihydroxy-2-(4-methylcyclohexyl) chromen-4-one	CH3	-8.0
b11(III)	2-(3-tert-butylcyclohexyl)-2,3-dihydro-5,6,7-trihydroxychromen-4-one	H	-7.9
b12(III)	2-tert-butyl-4-(3,4-dihydro-5,6,7-trihydroxy-4-oxo-2H-chromen-2-yl) cyclohexane carboxylic acid	COOH	-7.9
b13 (III)	2-(3-tert-butyl-4-hydroxycyclohexyl)-2,3-dihydro-5,6,7-trihydroxychromen-4-one	OH	-8.5
b14 (III)	2-tert-butyl-4-(3,4-dihydro-5,6,7-trihydroxy-4-oxo-2H-chromen-2-yl) cyclohexane carboxamide	CONH2	-8.6

doi.org/10.32388/9OCR7D

Sl no. (cluster)	Nomenclature	R_1	Docking Score Kcal/mol
b15 (IV)	5,6,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	OH	-8.7
b16 (IV)	4-(5,6,7-trihydroxy-4-oxo-4H-chromen-2-yl) benzoic acid	COOH	-9.4
b17(IV)	**4-(5,6,7-trihydroxy-4-oxo-4H-chromen-2-yl) benzamide**	**CONH2**	**-9.5**
b18(IV)	5,6,7-trihydroxy-2-(4-nitrophenyl)-4H-chromen-4-one	NO2	-8.3
b19(IV)	2-(4-aminophenyl)-5,6,7-trihydroxy-4H-chromen-4-one	NH2	-8.7
b20(IV)	5,6,7-trihydroxy-2-p-tolyl-4H-chromen-4-one	CH3	-8.9
b21(IV)	2-(4-fluorophenyl)-5,6,7-trihydroxy-4H-chromen-4-one	F	-8.5
b22(IV)	(4'-benzyl)-baicalein	Benzene	-9.2
b23(IV)	(4'-carbonyl benzene)-baicalein	Carbonyl-benzene	-8.8
b24(IV)	(4'-alpha glucopyranose)-baicalein	Alpha-glucopyranose	-9.2
b25(IV)	(4'-alpha fructofuranose)-baicalein	Alpha –fructo furanose	-8.7
b26(IV)	2-(4-(5,6,7-trihydroxy-4-oxo-4H-chromen-2-yl) benzamido) acetic acid	Glycine	-8.7

Table 4. Modification in various regions and their representation of docking score

doi.org/10.32388/9OCR7D

Sl No. (cluster)	Nomenclature	R1	R2	R3	R4	Docking score **Kcal/mol**
b27 (V)	5-fluro-6,7-dihydroxy-2-phenyl-4Hchromen-4-one	-F	-OH	-OH	-----	-8.8
b28 (V)	6-fluro-5,7-dihydroxy-2-phenyl-4H-chromen-4-one	-OH	-F	-OH	-----	-8.2
b29 (V)	5,6-difluro-7-hydroxy-2-phenyl-4Hchromen-4-one	-F	-F	-OH	-----	-8.3
b30 (V)	6,7-difluro-5-hydroxy-2-phenyl-4H-chromen-4-one	-OH	-F	-F	-----	-8.2
b31 (V)	6,7-diamino-5-hydroxy-2-phenyl-4H-chromen-4-one	-OH	-NH2	-NH2	-----	-8.5
b32 (V)	5-hydroxy-6,7dimercapto-2-phenyl-4H-chromen-4-one	-OH	-SH	-SH	-----	-7.4
b33 (V)	4-(5,6,7-trifluro-4-oxo-4H-chromen-2yl) benzoic acid	-F	-F	-F	-COOH	-8.9
b34 (V)	4-(5,6-difluro-7-hydroxy-4-oxo-4H-chromen-2-yl) benzoic acid	-F	-F	-OH	-COOH	-8.9
b35 (V)	4-(5-hydroxy-6,7-dimercapto-4H-chromen-2-yl) benzoic acid	-OH	-SH	-SH	-COOH	-8.1
b36 (V)	7-hydroxy-5,6-dimercapto-2-phenyl-4H-chromen-4-one	-SH	-SH	-OH	-----	-7.5
b37 (V)	7-amino-5,6dihydroxy-2-phenyl-4H-chromen-4-one	-OH	-OH	-NH2	-----	-8.6
b38 (V)	6,7-diamino-5-hydroxy-2-phenyl-4H-chromen-4-one	-OH	-NH2	-NH2	-----	-8.5
b39 (V)	**4-(5-fluro-6,7-dihydroxy-4-oxo-4H-chromen-2-yl) benzoic acid**	**-F**	**-OH**	**-OH**	**-COOH**	**-9.5**

doi.org/10.32388/9OCR7D

b40 (V)	4-(6,7-difluro-5-hydroxy-4-oxo-4H-chromen-2-yl) benzoic acid.	-OH	-F	-F	-COOH	-9.2

4.2.2. Determination of bioactivity of the prepared ligand: -

Ligands were prepared by using ChemDraw 2D software, and the bioactivity score and the chemical characteristic features of the ligands (Lipinski's rule) were checked by using the SwissADME website with the SMILES of the ligand through ChemSketch software. The ligand molecules that fulfilled the requirements of Lipinski's rule were considered for further study.

4.2.3. Identification of the active site of the receptor: -

BACE-1 (www.rcsb.org, PDB ID: 2ZHT), (www.rcsb.org, PDB ID: 2WJO) has an N-terminal end, a C-terminal end, and the active site cleft is located between the N- and C-terminal lobes. The active site is partially shielded by an **anti-parallel hairpin loop** known as **"flap,"** which controls substrate access and proteolytic specificity. The binding site of BACE-1, which interacts with key residue **Asp228**, establishes a force of interaction with Thr232 at the S3 pocket, interacts with **Tyr71** and **Thr72** of the **β-hairpin flap**, and stabilizes the binding by hydrogen bonding with **Gly11 at loop 10s.** To complement this, **Ile126 and Arg128** are essential for interaction and are located near **loop 113s**, present opposite to that of **loop 10s (as in Fig. 3).**

4.2.4. Retrieving Required Ligand and Receptor.pdb files from major databases:

Receptor or **BACE-1 (www.rcsb.org, PDB ID: 2ZHT) was retrieved**, and the small molecules, referred to as **ligands** were prepared in **PDBQT** format as **receptor.pdbqt**, and **ligand.pdbqt** for protein and ligand, respectively. The ligand and protein refinement by **AutoDock** software was executed by removing all water molecules from the protein, adding polar hydrogens and Kollman charge. The **grid box** enclosing the receptor and ligand, having dimensions **[center_X = 64.910; center_Y = 46.971; center_Z = -0.378]**, and then the modified protein along with the grid box, was saved as .pdbqt format inside the operating system. The dock command was attempted in the **command prompt**, and the docking output was saved inside the operating system as **output.pdbqt.** After getting the docking output of each molecule, the receptor – ligand interaction was checked by using **Discovery Studio**

software, and the interactions (electrostatic, van der Waals forces, hydrogen bond, etc.) were determined by a 2D picture of the **interaction.**

5. Results and Discussions

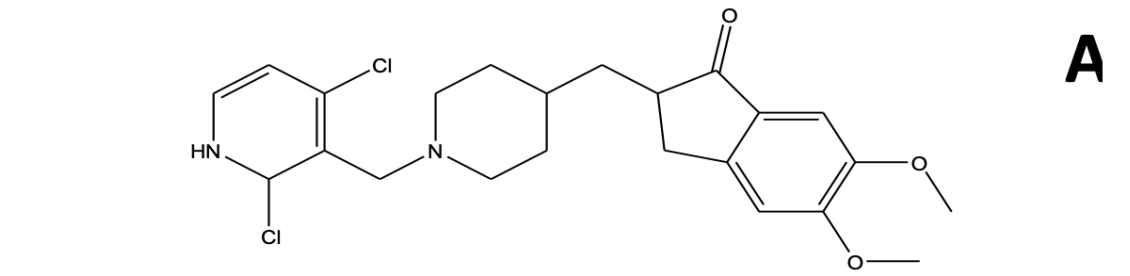
All the molecules were docked with the crystal structure of human **BACE-1 (www.rcsb.org, PDB ID: 2ZHT & 2WJO)**.

5.1. Docking Results and Interactions of Standard Donepezil

5.2. Docking Results and Interactions of derivatives of Donepezil

The ligands exhibiting a cutoff score of 8.3 and above was selected. The best ligands selected from Donepezil derivatives are **SS20 (Docking score 8.4) and SS15 (Docking score 8.4). Our ligand SS15 forms van der Waals force** with **Thr 232 and pi-donor hydrogen bond with Gly 11. However, SS20** forms a considerable **carbon-hydrogen bond** at **Thr 232 and van der Waals interaction with Gly 11.** The two designed ligands have a **good effective docking score, and** both exhibit interactions at the **loop 10s and β-hairpin flap,** thus could be essential for BACE-I stabilization and also signifies that they could reach the active site of the receptor **(Fig. 15 & Fig. 16).**

SS20:



2-((1-((2,4-dichloro-1,2-dihydropyridin-3-yl)methyl)piperidin-4-yl)methyl)-2,3-dihydro-5,6-dimethoxyinden 1-one

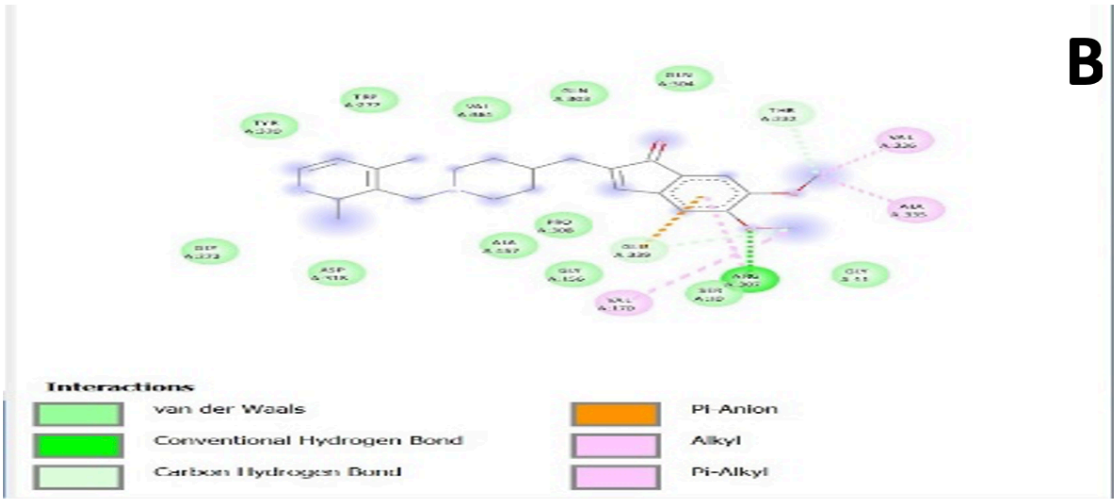


Fig. 15. A. Selected Donepezil derivative, SS20 B. Ligand-receptor interaction of SS20

SS15:

doi.org/10.32388/9OCR7D

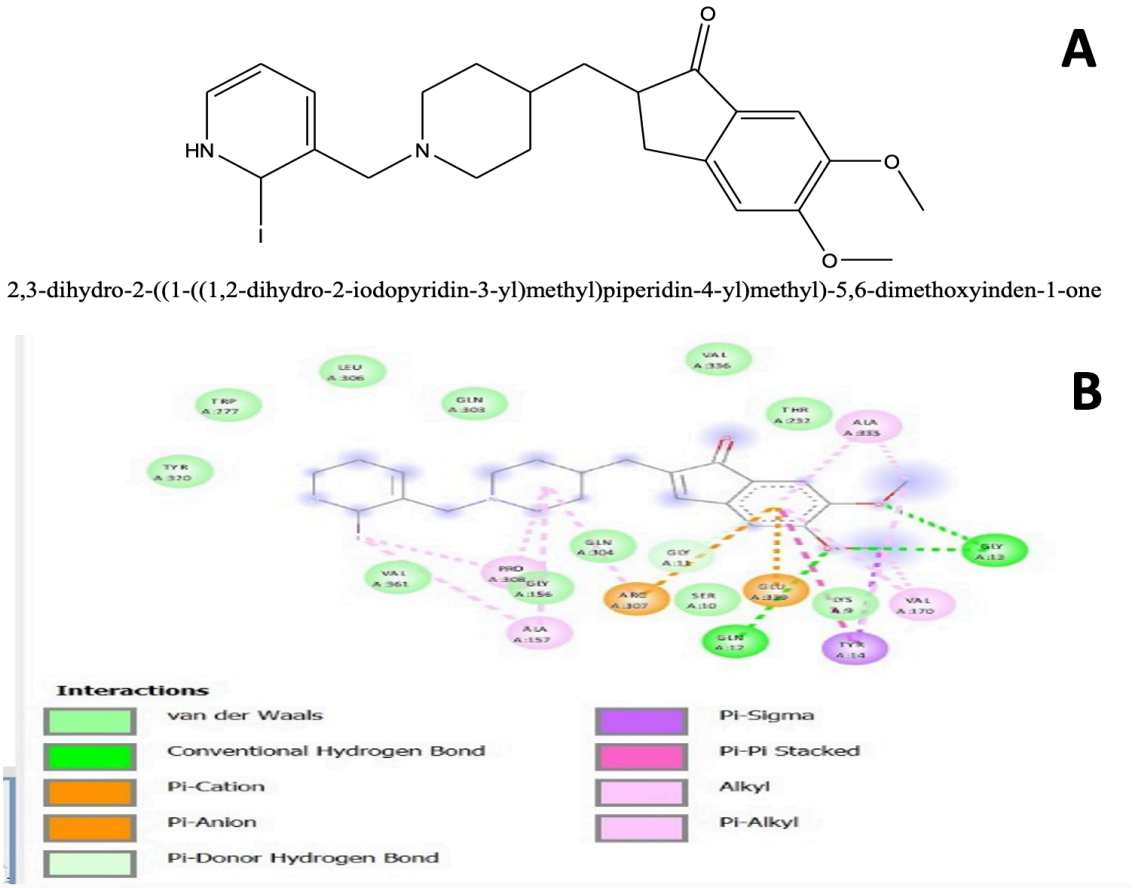


Fig. 16. A. Selected Donepezil derivative, SS15 B. Ligand-receptor interaction of SS15

5.3. Docking Results and Interactions of Quercetin

The BACE-I protein consists of **three important regions, catalytic aspartic site, flap region, and the 10s loop region (Fig. 3). Our ligand SP32 reaches all three active sites, and a comparable docking score of -7.9** is achieved from molecular screening. The molecules **form van der Waals forces** with **aspartic residues Asp 32 and Asp 228 (aspartate active site).** The molecule also forms conventional hydrogen bonds with **Arg 128** which is responsible for **interaction in loop 113s, opposite to loop 10s (important for stabilization).** The molecule also reaches the **flap active site Tyr 71** which forms the **β-hairpin flap and is responsible for stabilization of the BACE-I enzyme.** The ligand exhibits **van der Waals interactions** with **Thr 231,** located near the S3 pocket. **Thus, SP32** could be our **chosen ligand** as compared to **SP27,** which also has a considerable carbon-hydrogen bond at **Gly 11 and van der Waals interactions with Thr 232.**

doi.org/10.32388/9OCR7D

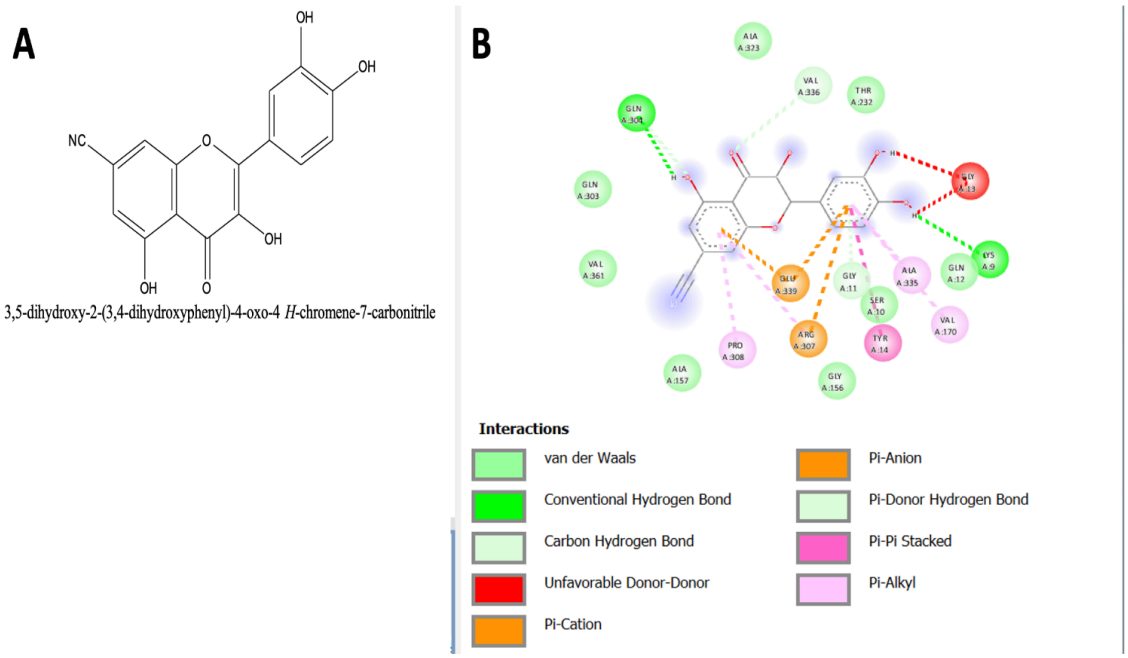


Fig. 17. A. Selected Quercetin derivative, SP 27 B. Ligand receptor interaction of SP27

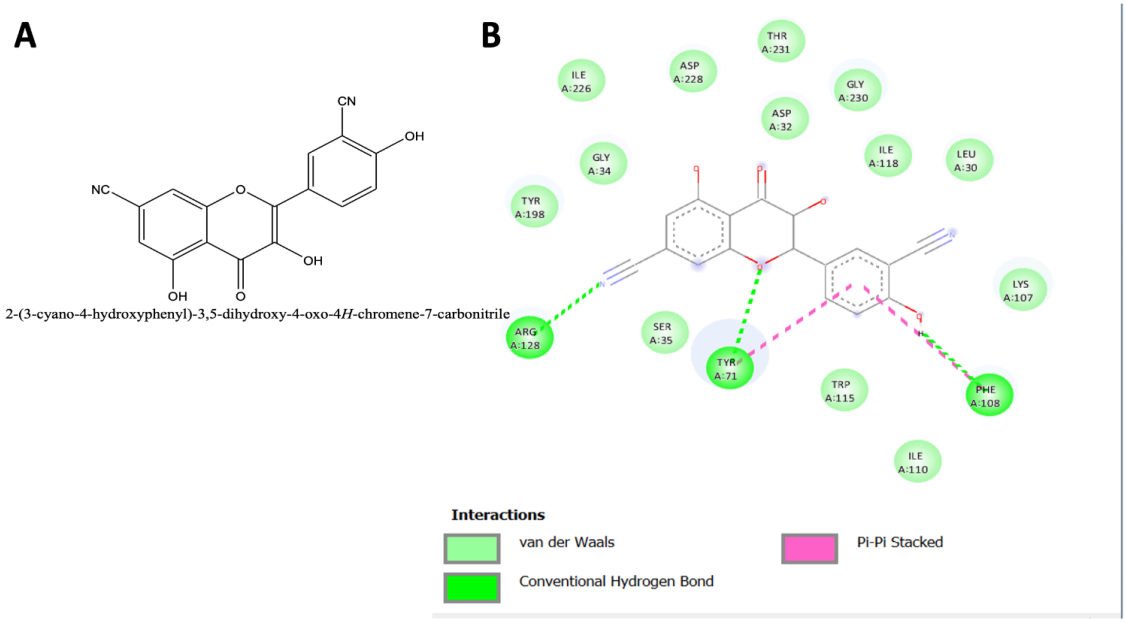


Fig. 18. A. Selected Quercetin derivative, SP32 B. Ligand receptor interaction of SP32

doi.org/10.32388/9OCR7D

5.4. Docking Results and Interactions of Myricetin

The two ligands were selected on the basis of their docking scores, **T21 (Docking score -8.4) and T25 (Docking score -8.6).** However, they exhibit considerable interaction with T25 and T21. Our ligand, **T25, reaches the S3 active site at Thr 232** (forms van der Waals interactions with T25); similarly, both **T21 and T25 reach important regions of the 10s loop or Gly 11.** The glycine residue in the 11th position of the BACE-I protein forms hydrogen bonds with the formed substrate and leads to stabilization of the 10s loop. The interaction of BACE-I is stabilized and balanced through the interaction with loop 10s. T25 thus stabilizes the BACE-I protein by interacting with loop 10s. It also targets the S3 pocket, which is also an important area of target for drug design. **The interactions of T25 with both loop 10s and S3 pocket enable BACE-I to change into open and closed conformers.** Compounds which can **block the potential transition of the open and closed conformers** could be good **BACE-I inhibitors; thus, T25 could be our chosen ligand.**

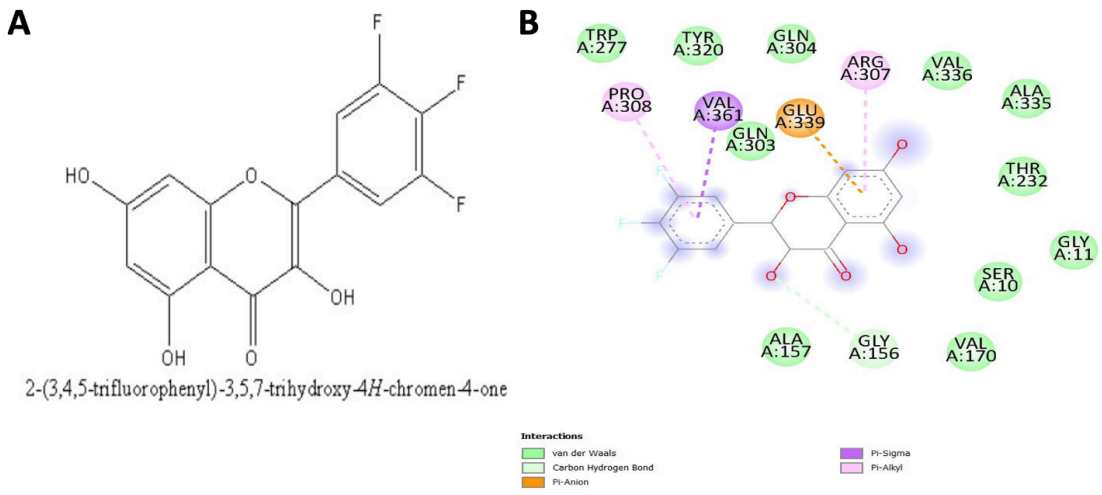


Fig. 19. A. Selected derivative of Myricetin T25 B. Ligand-receptor interaction of T25

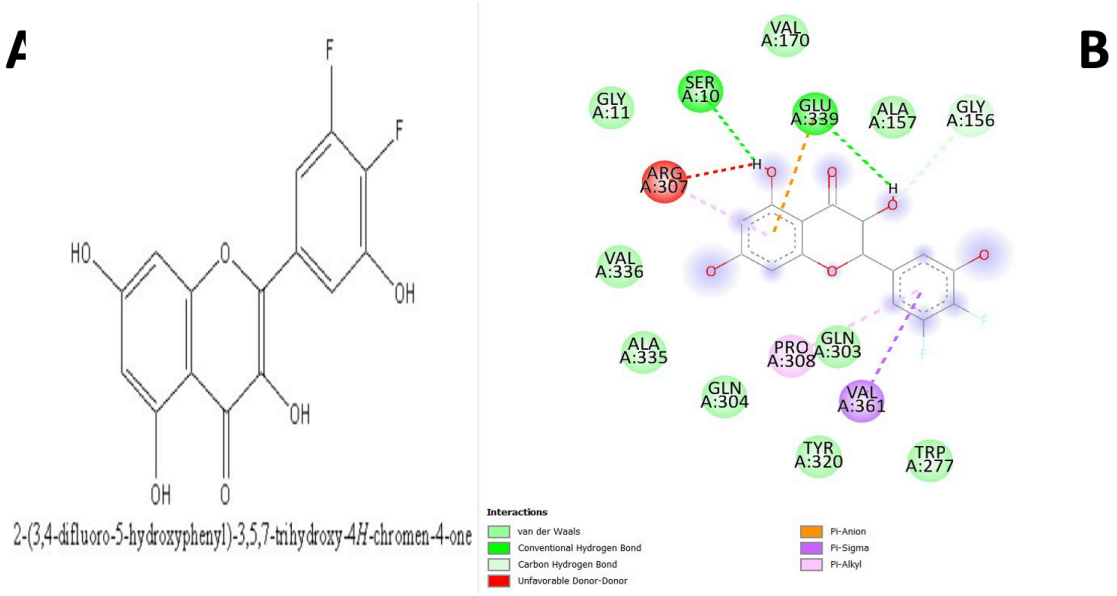


Fig. 20. A. Selected derivative of Myricetin T21 B. Ligand-receptor interaction of T21

5.5. Docking Results and Interactions of Baicalein

Cluster I-IV of Baicalein structure was focused on Ring B & Ring C modification, and Cluster V concentrated on Ring A modification of Baicalein [27].

In cluster I, saturation of Ring B reduced the docking score; planarity of the ring is useful. Further, we added a substituent in the 4' position to analyze the effect of the substituent on this modified structure. Electron-withdrawing groups like COOH and $CONH_2$ further reduced the docking score, which can be due to the bulkiness of the group or their –I effect. Electron-donating groups like –OH did not bring a considerable change.

In cluster II, the saturated double bond in Ring A and C did not make a major difference in the binding affinity; thus, the chroman ring is essential and detrimental for activity.

In cluster III, a tert-butyl group introduced in Ring B improved the affinity but not considerably higher.

In cluster IV, mono-substitution at the 4'-position of Ring B increases the binding scores in the structure of baicalein. Strong EWGs like -NO_2 and -F reduced the activity of baicalein, while EWGs like -COOH and -$CONH_2$ improved the docking score.

doi.org/10.32388/9OCR7D

Cluster V focused on the A-ring, mainly analyzing the importance of tri-hydroxyl groups in activity as a BACE1 inhibitor.

After docking baicalein, we concluded **(i) the planarity of the baicalein structure contributes to its BACE1 inhibitory property; (ii) COOH, $CONH_2$, and benzene increases the BACE1 activity, probably due to a conjugation effect; (iii) 6-OH is important** and its substitution with isosteres also decreases the BACE1 activity; this is probably due to its interaction with **isoleucine amino acid** at the binding site, which appears to be essential for activity.

The selected ligand, b17 from cluster IV, and b39 from cluster V, was selected as our ligand with the highest docking score of -9.5. Both ligands exhibited van der Waals interactions with **Tyr 71 of the β-hairpin flap**, and a conventional hydrogen bond was observed with **Ile 126**, which is located **near loop 113s**, also located opposite to that of loop 10s. **4-(5-fluoro-6,7-dihydroxy-4-oxo-4H-chromen-2-yl) benzoic acid, or b39,** has a fluoro group substitution with isosteres at the R1 position and is essential for activity. **The flap region and the loop region are essential for the stabilization of the BACE-I protein**, and the inhibitor must reach the active site. **Our ligand happens to reach the receptor with good binding affinity or score; hence, it could be our chosen ligand.**

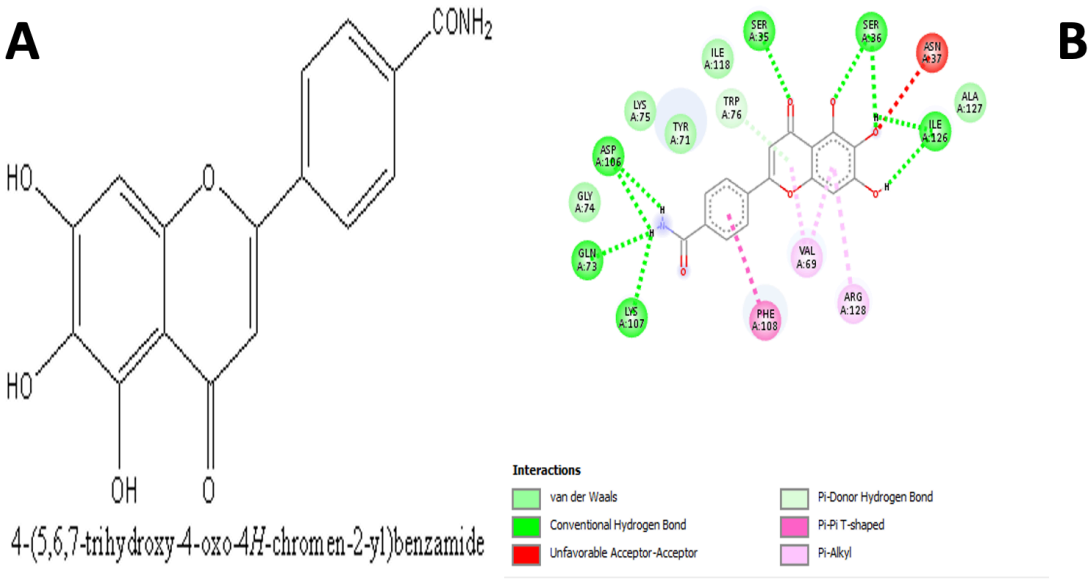


Fig. 20. A. Selected derivative of baicalein, b17. B. Ligand-receptor interaction of b17

doi.org/10.32388/9OCR7D

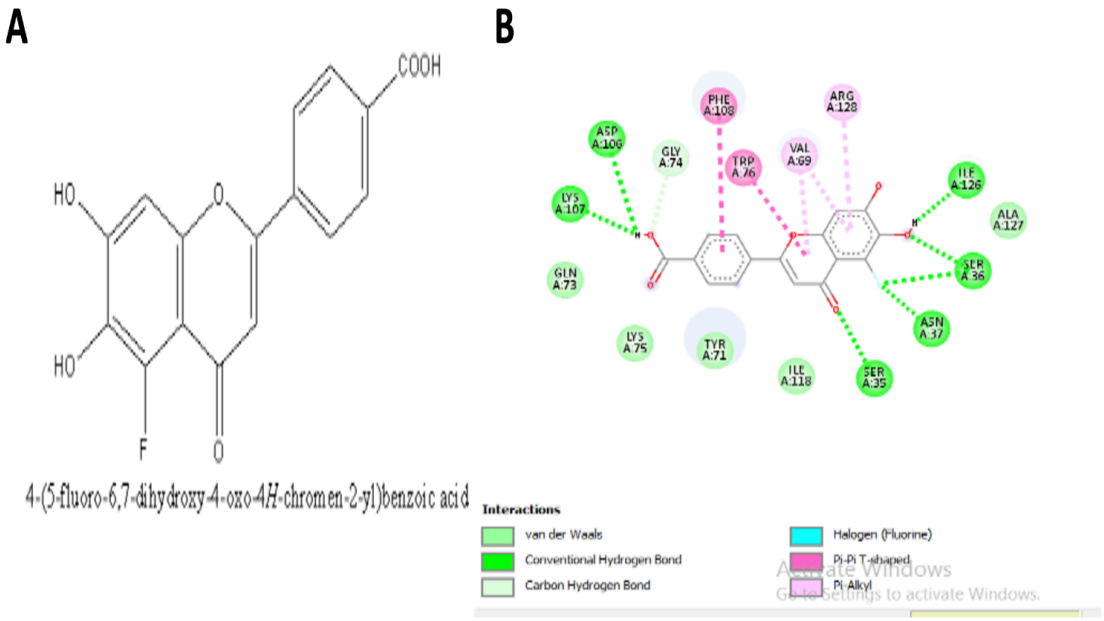


Fig. 20. A. Selected derivative of baicalein, b39. B. Ligand-receptor interaction of b39

5.6. Docking Results and Interactions of Ferulic Acid

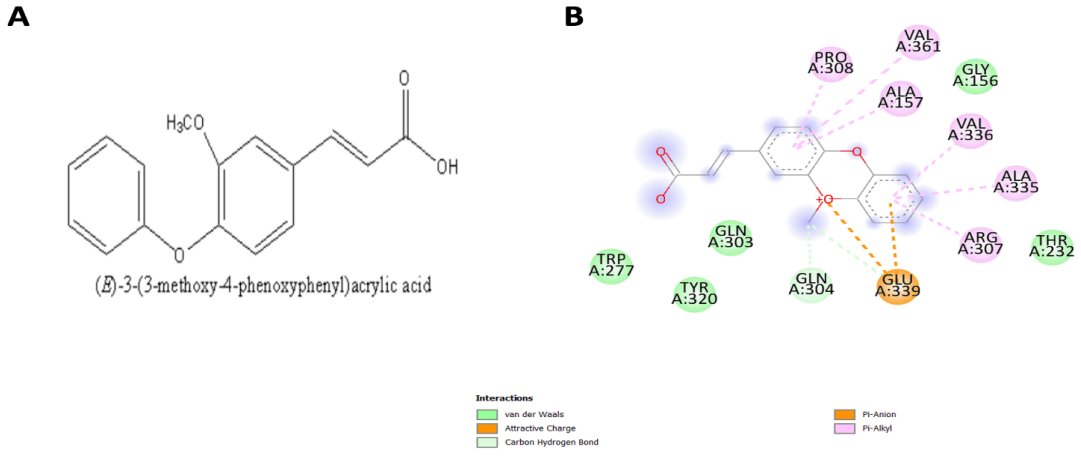


Fig. 21. A. Selected derivative of ferulic acid, SM17. B. Ligand-receptor interaction of SM17

doi.org/10.32388/9OCR7D

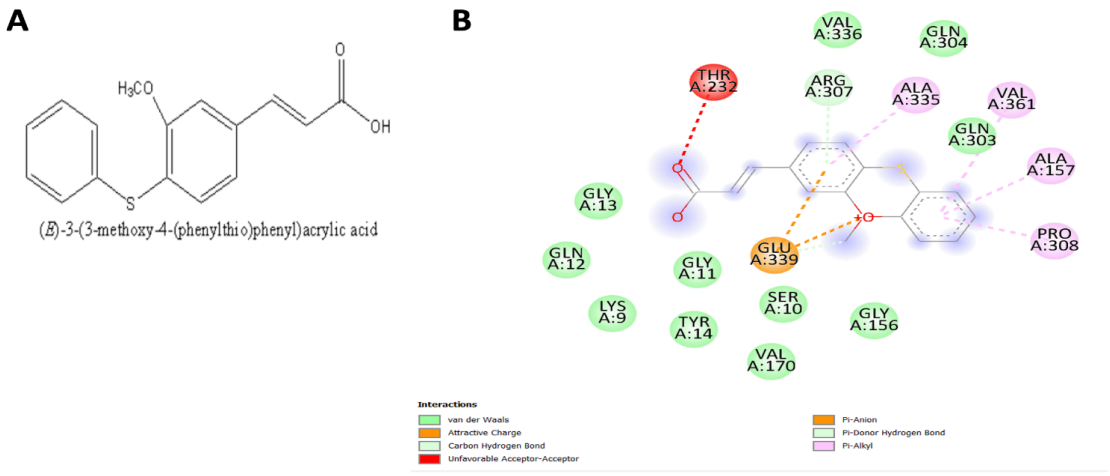


Fig. 22. A. Selected derivative of Ferulic acid SM18 B. Ligand receptor interaction of SM18

The two ligand derivatives selected on the basis of their docking scores are **SM17** and **SM18 (Docking score -7.5 for both)**. However, **SM18** could reach the active site more effectively than SM17. The ligand **SM18** happens to exhibit an unfavourable acceptor-acceptor bond with **Thr 232, which is located at the S3 pocket of BACE-I (active site key residue).** The ligand also forms **van der Waals forces with Gly 11 at loop 10s (Fig. 22).** Thus, it **stabilizes the loop 10s and balances the interaction with the BACE-1 substrate.** The stabilization of the BACE-I structure depends on the flap and the 10s loop. **Our designed ligand happens to reach both the active sites.** In an open 10s loop conformation, the flap active site has an affinity to bind together, but if our ligand reaches these regions, the stabilization of BACE-I could be of major importance.

Evaluation of drug likeliness of all the **10** screened compounds was observed on the MOLINSPIRATION server. Among the above screened compounds, all the compounds confirmed drug-like properties after passing Lipinski's rule of five. Most of the drugs belong to class 5, which may be harmful if swallowed; however, SS20 and SS15 get toxic if swallowed, and SM17 and SM18 also may be harmful, although **(Table 5)**. Thus, all the predicted scaffolds could be future ligands for Alzheimer's disease research. We observe that the flavonoids are not so harmful as compared to the derivatives of **Donepezil (SS20 and SS15)** and Ferulic acid **(SM17 and SM18)**. However, selected ligands of Donepezil and ferulic acid show greater binding scores and comparable drug-receptor interactions. **The flavonoid derivatives of Quercetin (SM27 and SM32), Myricetin (T21 and T25), and Baicalein (B17**

and B39) could reach the active site of BACE-I effectively with higher docking scores than the standard **Donepezil (-7.9 kcal/mol).**

Ligand code	Predicted toxicity	LogP	H-bond donor	H-bond acceptor	Molecular weight (gm/mol)	Docking Score Kcal/mol
SS20	CLASS 3	4.6	2	6	314.29	-8.4
SS15	CLASS 3	4.23	4	7	378.33	-8.4
T21	**CLASS 5**	**2.56**	**4**	**8**	**322.22**	**-8.4**
T25	**CLASS 5**	**2.99**	**3**	**8**	**324.21**	**-8.6**
SP27	**CLASS 5**	**2.15**	**4**	**7**	**311.25**	**-8.1**
SP32	**CLASS 5**	**2.32**	**3**	**7**	**320.26**	**-7.9**
b17	**CLASS 5**	**1.50**				**-9.5**
b39	**CLASS 5**	**2.98**				**-9.5**
SM17	CLASS 4	3.57	1	4	270.28	-7.5
SM18	**CLASS 4**	**3.94**	**1**	**3**	**286.35**	**-7.8**
Donepezil						**-7.9**

Table 5. Drug likeliness and Toxicity list for the screened compounds

CLASS 1, CLASS 2: Fatal if swallowed; CLASS3 Toxic if swallowed; CLASS 4:Harmful if swallowed; CLASS 5: May be harmful; CLASS 6- Non-toxic

6. Conclusion

In this study, we have undertaken a **few ligand designs** as **inhibitors** of **BACE-I** but also investigated the detailed **binding interactions** and ***in silico* toxicity studies** against those target proteins. Currently, the designed compounds have **revealed promising anti-Alzheimer's activity** *in silico.*

Quercetin derivative ligands like **SP32, SP27** reached all the active regions of BACE-I, i.e., **flap region, catalytic aspartase, and 10s loop, with significant docking scores of -7.9 and -8.1 kcal/mol. Myricetin derivatives ligands (T21 and T25) reached the 10s loop and the S3 pocket, which is essential for BACE-I stabilization. They exhibited comparable docking scores to the standard, i.e., -8.4 kcal/mol. Baicalein derivatives ligands, viz., b17 and b39, interacted with the β-hairpin flap and loop 113s of BACE-I. The best docking score was obtained for Baicalein derivatives, viz., -9.5 kcal/mol (docked with PDB ID 2wjo). Ferulic acid derivative, namely SM-18 (docking score -7.9 kcal/mol), could also reach the Thr 232, or S3 pocket, or active site key residue and loop 10s. Donepezil derivatives could be modified further to get comparable binding and reduced toxicity.**

Our prepared derivatives were checked in ChemMine Tools for any similarity search in our library, but it was found that no search was found in the ChEMBL Fingerprint search or chemical database.

However, **BACE-1 inhibitors** like **Lanabecestat, Atabecestat, Verubecestat, Elenbacestat, and Umibecestat (Fig. 4)** have **failed phase 3 clinical trials** due to **associated side effects. Natural compounds are an emerging approach for AD therapy.** During the 90s, several other compounds were studied in clinical trials for AD therapy. Our work **may explore** some newer avenues in the work-field of **designing anti-protein fibrillators,** which might be used as **future leads** for developing candidate drugs.

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Declarations

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