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ORIGINAL ARTICLE

# Screening of Potential Inhibitors against Flotillin-1 as Therapeutics for Alzheimer's Disease

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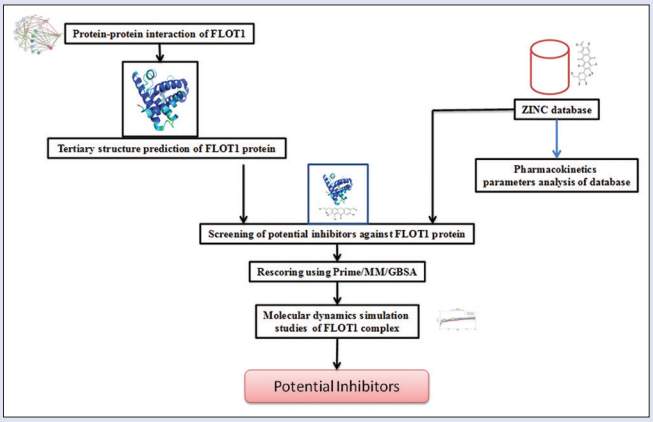
## ABSTRACT

**Background:** Alzheimer's disease (AD) is one of the most common neurological disorders occurring in older people. So far, no specific drug is available for the disease, and only palliative medicines are available for the patients. Accumulation of amyloid beta (Aβ) peptides is considered to play a crucial role in the generation of the disease. Aβ peptide is generated by the proteolysis of amyloid precursor protein by two distinct proteases β and γ-secretase. Flotillin-1 (FLOT1) directly binds to the cytoplasmic tail of β-secretase and affects the sorting and recycling of the enzyme. Increased expression of FLOT1 has been correlated with the progression of AD, while FLOT1 knockdown causes a reduction in Aβ production. Thus, FLOT1 is an attractive therapeutic target for the suppression of beta-secretase 1 (*BACE-1*). **Objective:** This study aims to screen the potential inhibitors against FLOT1 as therapeutics for AD. **Materials and Methodology:** In this work, protein–protein interactions, tertiary structure prediction, molecular docking, and molecular dynamics (MD) simulation were performed. **Results:** Tertiary structure prediction of FLOT1 and screening inhibitors against it helped in finding key molecules with potential therapeutic properties. Protein–protein interaction study of FLOT1 deciphered the interactors playing key role in AD. Pharmacokinetic parameters were quantified for each potential inhibitor. The results of MD simulation analysis revealed that the ZINC67911837 had better inhibitory activities with FLOT1. **Conclusion:** The analysis suggests that the ZINC67911837 compound could be a novel potential inhibitor of FLOT1 to modulate *BACE-1* activity and used as a therapeutic agent for the treatment of AD. This study facilitates the initiation of the natural drug discovery process for the treatment of AD patients.

**Key words:** Alzheimer's disease, drug designing, inhibitors, molecular dynamics simulation, protein–protein interaction, therapeutics

## SUMMARY

- This study was performed to screen potential inhibitors against flotillin-1 (FLOT1) as therapeutics for Alzheimer's disease (AD). *In silico* studies were involved to screen out the natural compounds against the predicted tertiary structure of FLOT1 by using template-based modeling, protein–protein interactions, molecular docking, MM-generalized-born surface area analysis, and MD simulation approaches. The analysis suggests that the ZINC67911837 compound could be used as a novel potent inhibitor for the treatment of AD. This study facilitates the initiation of the natural drug discovery process for the treatment of AD patients.




**Abbreviations used:** Aβ: Amyloid beta; APP: Amyloid precursor protein; BACE-1: Beta-secretase 1; NFTs: Neurofibrillary tangles; TRK/MAPK: Tyrosine kinase receptor (mitogen-activated protein kinase); NCBI: National Centre for Biotechnology Information; PSI-BLAST: Position-specific Iterated BLAST; OPLS: Optimized potential for liquid simulations; ADMET: Absorption, distribution, metabolism, excretion, and toxicity; GBSA: Generalized-born surface area; MD: Molecular dynamics; NPT: Constant number (N), pressure (P), and temperature (T); NVT: Constant number (N), volume (V), and temperature (T); RMSD: Root mean square deviation; Rg: Radius of gyration; RMSF: Root mean square fluctuation; KEGG: Kyoto Encyclopaedia of Genes and Genomes; CNS: Central nervous system; BLAST: Basic Local Alignment Search Tool; FLOT1: Flotillin-1.

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## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that occurs in old age.[1] Deposition of amyloid beta (Aβ) and neurofibrillary tangles (NFTs) are the main cause of neuronal cell death in AD. The mechanism of disease progression is still not clear, and treatment options for AD are limited.[2] *FLOT 1* gene encodes a protein flotillin 1 (FLOT1) which is a caveolae-associated integral membrane protein.[3] Caveolae are involved in functions such as vesicular trafficking and signal transduction. Some studies suggested that FLOT1 protein plays an important role in beta-amyloid and NFTs formation pathways.[4] FLOTs are associated with the inner leaflet of the plasma membrane in the form of palmitoylated and myristoylated proteins with approximately 48kDa molecular weight. FLOT1 and FLOT2 are two different forms of the protein that lead to the formation of microdomains with a defined size. The stability of FLOT1 is dependent on FLOT12 protein or reggie proteins that were discovered as regeneration proteins in axons of goldfish retinal ganglion cells. It belongs



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to stomatin, prohibitin, FLOT, and HF1K/C protein family.[5] FLOT1 has a palmitoylation site at cysteine 34, which has an important role in plasma membrane localization. Some studies reported that in mammalian hippocampal neurons, FLOTs play a key role in differentiation as their downregulation results in the failure of neurons to differentiate and regulate tyrosine kinase receptor/mitogen-activated protein signaling.[6-9] Other studies report that FLOT1 plays a crucial role in the pathogenesis of neurodegenerative diseases such as Parkinson's disease (PD), prion disease, and AD. In the case of AD, Aβ was found to be accumulated in FLOT1 positive endocytic vesicles.[10] Cytoplasmic domains of the cargo proteins contain sorting signals that regulate the sorting of transmembrane proteins. A short cytoplasmic tail consisting of 23 amino acids in beta-secretase 1 (*BACE-1)* also contains a sorting signal of an acidic cluster, di-leucine type.[11] The sorting of *BACE-1* involves the acidic cluster, di-leucine-type motif binding to the members of Golgi-localized γ ear-containing ARF-binding protein family.[12] FLOT1 binds to the di-leucine motif of the cytoplasmic tail of *BACE-1* and competes with GGA2, influencing the endosomal sorting of *BACE-1,* which inhibits amyloidogenic processing of amyloid precursor protein (APP). FLOT1 appears to be important for the cellular targeting of *BACE-1*. In addition, studies observed the overexpression of FLOT1 in AD patients, patients with Down syndrome, and non-demented patients.[13-15] Structure-based drug designing is a popular approach to screen out the potential inhibitors against a target to find therapeutics against a disease. This study reveals the identification and validation of novel FLOT1 inhibitors using protein–protein interaction, homology modeling, high-throughput virtual screening, molecular docking, MM-generalized-born surface area (GBSA), and molecular dynamics (MD) simulation approaches.

## MATERIALS AND METHODOLOGY

### Protein–protein interaction of flotillin-1

STRING database was used to find out the interacting partners of FLOT1 with the help of physical and functional association. Flotillin-1 keyword was used to search protein–protein interactions against *Homo sapiens*.[16]

### Retrieval of protein sequences and tertiary structure prediction

The FLOT1 sequence was obtained from NCBI (CAG33227.1 Flotillin-1 [*H. sapiens*]) in fasta format. Position-specific Iterated-Basic Local Alignment Search Tool[17] was used to find out the homologous sequences. 1WIN, 3OND, 3WDQ, and 5YFP templates were selected on the basis of identity for prediction of the tertiary structure of FLOT1. Modeller tool 9.15 (University of California, San Francisco, Accelrys)[18] was used to perform tertiary structure prediction, and the PROCHECK server[19] was used to assess the quality of the predicted structure.

### Protein preparation

The most important process before docking, Protein Preparation Wizard module (Schrödinger Suite 2019-2), was used for the preparation of modeled protein. All water and HETATOM were removed, and hydrogen atoms were incorporated into the protein structure. Optimized Potential for Liquid Simulations (OPLS) 2005 force field was used to minimize protein structure energy to reduce steric clashes.[20]

### Prediction of binding sites and grid generation for flotillin-1 protein

SiteMap module v5.0.011 (SiteMap, Schrödinger, LLC, NY, USA, 2019) was used to find out the best active site for the target protein.[21] Top-ranked potential receptor binding sites were identified at the default parameters. The generated binding site pocket was subjected to grid generation by using a receptor grid generation module.

### Screening of potential inhibitors against flotillin-1 protein

Zinc database was used to retrieve natural compounds, which contains 25,440 entries.[22] The world's largest Traditional Chinese Medicine Database was used to screen inhibitors against FLOT1 protein. LigPrep version 49011 module (Schrödinger Release 2019-2: LigPrep, Schrödinger, LLC, NY, USA, 2019) tool was used for the preparation of ligands at the default parameters.[23] Virtual screening of libraries with FLOT1 was performed by GLIDE module version 82011 (Glide, Schrödinger, LLC, NY, USA, 2019) with high-throughput virtual screening), extra precision, and standard precision.[24,25] After the screening, the ligand–receptor complex was subjected to analysis by the MM-GBSA approach.

### Pharmacokinetics parameters analysis of database

Qikprop tool (QikProp, Schrödinger, LLC, NY, USA, 2019) was used to analyze pharmacokinetic parameters such as absorption, distribution, metabolism, excretion, and toxicity of each compound at default parameters.[26] Many physical descriptors were calculated for each ligand for their druggability.

### Rescoring using prime/MM/generalized-born surface area

Prime MMGBSA v3.000 (Prime MMGBSA, Schrödinger, LLC, NY, USA, 2019) tool was used for protein–ligand binding free energy calculations for each docked pose using OPLS 2005 force filed in GBSA continuum solvent model.[27,28]

$\Delta G_{bind}$ was calculated by the following equation:

$\Delta G_{bind} = E_{Complex}$ (minimized) $- E_{ligand}$ [unbound, minimized] + $E_{Receptor}$ [unbound, minimized]).

Where $\Delta G_{bind}$ = relative free energy (including ligand and receptor strain energy), $E_{complex}$ (minimized) = MMGBSA energy of minimized complex, $E_{Ligand}$ (unbound, minimized) = MMGBSA energy of the ligand after removing it from the complex and allowing it to relax, $E_{receptor}$ (unbound, minimized) = MMGBSA energy of protein after excluding it from the ligand.

### Molecular dynamics simulation studies of flotillin-1 complex

MD simulations were performed using the GROMACS (v. 5.1.2) (University of Groningen, KTH Royal Institute of Technology, Uppsala universitet) using the Amber force field.[29,30] PDB2gmx program was used to assign protons in complex protein to generate topology files. After that, the TIP3P water model with the triclinic box was used to build a simulation box. Counterions (Na and Cl) were also added to neutralize the system, and the steepest descent integrator was used for energy minimization of each complex.[31] NPT (Constant number, pressure, and temperature) and (NVT) constant number, volume, and temperature conditions were applied for the equilibration of the system at 300 K for 100 ps.[32] Berendsen weak-coupling method and Lennard–Jones potentials were used for maintaining the temperature and pressure of each system.[33] Root mean square deviation (RMSD), radius of gyration, and root mean square fluctuation (RMSF) were used to study the stability of the structure, dynamics behavior of residues, and levels of compaction for top three complexes ZINC31158144, ZINC67911837, and ZINC14437962 with FLOT1. The production simulation was performed for 20 ns. Xmgrace (Grace Development Team, 3309 Fairmont Drive Nashville) was employed for trajectory analysis and visualization.[34]

## RESULTS AND DISCUSSION

### Protein–protein interaction of flotillin-1

Network stats were observed for each node [Table 1], and many types of interactions were found such as molecular function, cellular

component, biological process, Kyoto Encyclopaedia of Genes and genomes pathways, and protein domains for the FLOT1 protein. The most significant interacting partners such as CTNNB1, SNAI2, SLC6A3, CTNND1, FER, FYN, CAV1, FLOT2, CDH1, LRFN3, SLC6A3, SORBS1, PTOV1, SVIL, NGB, APP, *BACE-1*, and PSEN1 were found with respect to FLOT1 [Figure 1] and [Table 2]. Further information about protein–protein interactions is provided in Supplementary Files 1 and 2.

## Tertiary structure prediction of flotillin-1

Three modeled structures were obtained after three-dimensional structure prediction. On the basis of Ramachandran plot analysis, structure no. 2 which had the highest most favored regions (80.3%), additional allowed regions (13.4%), minimum disallowed regions (2.1%), and generously allowed regions (4.2%) was selected for further study [Table 3 and Figure 2].

## Active site prediction of flotillin-1

The top five active sites were obtained on the basis of SiteScore, size, $D_{score}$, and volume [Table 4]. The first active site pocket was chosen as a best binding site on the basis of the highest site score, size, and volume.

## Screening of potential inhibitors against flotillin-1

After applying different docking protocols, the top five binding affinities of FLOT1 against different compounds such as ZINC31158144, ZINC67911837, ZINC67911840, ZINC04096945, and ZINC14437962 were observed in terms of docking score, glide score, and glide energy. For these compounds, different negative values were obtained such as −7.89936, −7.87492, −7.70318, −7.60791, and −7.56147, respectively [Table 5]. Interactions of residue with the different compounds are shown in Figure 3a-e and Table 6.

## Assessment of pharmacokinetic parameters for compounds

Druggability of compounds was studied on the basis of parameters including central nervous system (CNS), molecular weight, donor hydrogen bond, acceptor hydrogen bond, predicted octanol/water partition coefficient, percent human oral absorption, and Lipinski's Rule of five [Table 7].

## Rescoring using Prime/MM/generalized-born surface area

Fundamental energy was obtained for receptor, ligands, and receptor–ligand complex. On the basis of many types of energies (Coulomb, covalent, Van der Waals, lipophilic, generalized born electrostatic solvation, total energy, H-bonding correction, pi-pi packing correction, and self-contact correction), MM combined with Poisson–Boltzmann or generalized born and surface area (MMGBSA) dG Bind was calculated for rescoring each docked complex. Three compounds (ZINC31158144, ZINC67911837, and ZINC14437962 with energies −39.3837, −37.7105, and −32.9286, respectively) had better binding affinity value in comparison to ZINC67911840 and ZINC04096945 [Table 5]. MD or Monte Carlo simulation approaches could be an alternative method to identify binding affinities with the calculation of interacting energies, but some research has suggested that MMGBSA and linear interaction energy approach are widely used to calculate binding affinities.[27,35]

## Molecular simulation studies of flotillin-1 complex

RMSD, RMSF, and Rg values were calculated for the top three docked complexes (ZINC14437962, ZINC67911837, and ZINC31158144 with FLOT1) to explore more information about structural stability and binding affinity. Analysis of RMSD of ZINC67911837 clearly reveals that the system was stable between 15,000 and 20,000 ps. In the case of Rg and RMSF values, ZINC67911837

**Table 1:** Statistics of protein-protein interaction network of flotillin-1 (Ramachandran plot statistics)

| Network statistics | |
|---|---|
| **Descriptions** | **Values** |
| Number of nodes | 21 |
| Number of edges | 62 |
| Average node degree | 5.9 |
| Average local clustering coefficient | 0.738 |
| Expected number of edges | 29 |
| PPI enrichment *P* value | 6.26E-08 |

PPI: Protein–protein interaction

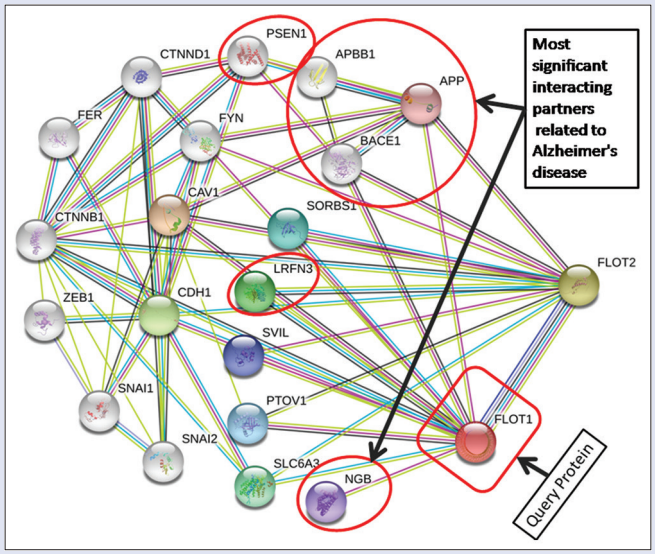



**Figure 1:** Protein–protein interaction network of flotillin-1 predicted by STRING database. In the network query, protein showing the interacting partners of flotillin-1. The red circle and the rectangular line showing the most significant interacting partners of flotillin-1 which are related to Alzheimer's disease and involved in their pathogenesis

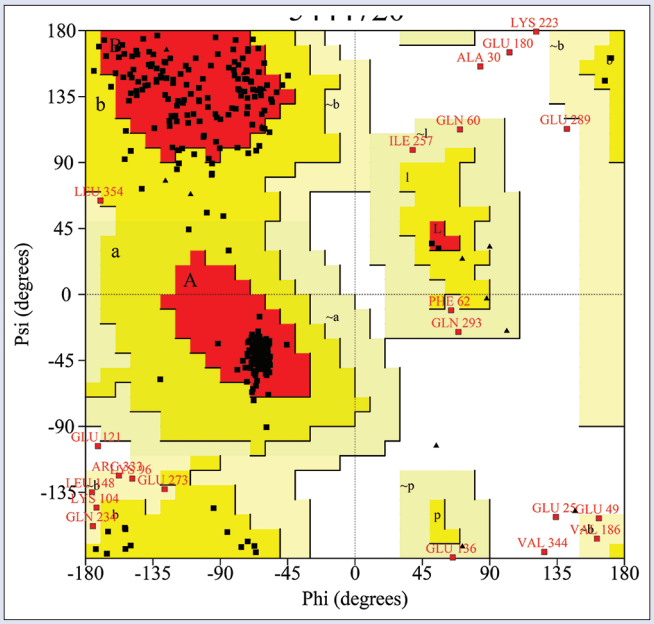



**Figure 2:** Ramachandran plot showing phi and psi angle relationship of residues in flotillin-1

**Table 2:** Representation of interacting genes with their description and score

| Query protein | | |
|---|---|---|
| FLOT1 | FLOT1 may act as a scaffolding protein within caveolar membranes, functionally participating in the formation of caveolae or caveolae-like vesicles; FLOT (427 aa) | |
| **Predicted functional partners** | | |
| **Name** | **Description** | **Score** |
| CAV1 | Caveolin-1 may act as a scaffolding protein within caveolar membranes. Interacts directly with G-protein alpha subunits and can functionally regulate their activity (By similarity). Involves in the co-stimulatory signal essential for the TCR-mediated T-cell activation. Its binding to DPP4 induces T-cell proliferation and NF-kappa-B activation in a TCR/CD3-dependent manner. Recruits CTNNB1 to caveolar membranes and may regulate CTNNB1-mediated signaling through the Wnt pathway. Negatively regulates TGFB1-mediated activation of SMAD2/3 by mediating the internal internalization of TGFBR1 from membrane rafts leading to its subsequent degradation . (178 aa) | 0.975 |
| FLOT-2 | FLOT-2 may act as a scaffolding protein within caveolar membranes, functionally participating in the formation of caveolae or caveolae-like vesicles. May be involved in epidermal cell adhesion and epidermal structure and function; belongs to the band 7/mec-2 family. FLOT subfamily (428 aa) | 0.968 |
| CDH1 | Cadherin-1: cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility, and proliferation of epithelial cells. Has a potent invasive suppressor role. It is a ligand for integrin alpha-E/beta-7 (882 aa) | 0.926 |
| LRFN3 | Leucine-rich repeat and fibronectin Type-III domain-containing protein 3: cell adhesion molecule that mediates homophilic cell-cell adhesion in a Ca (2+)-independent manner. Promotes neurite outgrowth in hippocampal neurons (By similarity); fibronectin Type III domain-containing (628 aa) | 0.925 |
| SLC6A3 | Sodium-dependent dopamine transporter: amine transporter. Terminates the action of dopamine by its high-affinity sodium-dependent reuptake into presynaptic terminals; solute carriers (620 aa) | 0.919 |
| SORBS1 | Sorbin and SH3 domain-containing protein 1: It plays a role in tyrosine phosphorylation of CBL by linking CBL to the insulin receptor. Required for insulin-stimulated glucose transport. Involved in the formation of actin stress fibers and focal adhesions (by similarity) (1292 aa) | 0.886 |
| PTOV1 | Prostate tumor - overexpressed gene 1 protein; may activate transcription. Required for nuclear translocation of FLOT-1. Promotes cell proliferation (416 aa) | 0.877 |
| SVIL | Supervillin; isoform 1 - forms a high-affinity link between the actin cytoskeleton and the membrane. It is among the first costameric proteins to assemble during myogenesis, and it contributes to myogenic membrane structure and differentiation. Appears to be involved in myosin II assembly. May modulate myosin II regulation through MLCK during cell spreading, an initial step in cell migration. May play a role in invadopodial function; Belongs to the villin/gelsolin family (2214 aa) | 0.810 |
| NGB | Neuroglobin: It is involved in oxygen transport in the brain. Hexacoordinate globin displaying competitive binding of oxygen or the distal His residue to the iron atom. Not capable of penetrating cell membranes. The deoxygenated form exhibits nitrite reductase activity, inhibiting cellular respiration via NO-binding to cytochrome c oxidase. Involved in neuroprotection during oxidative stress. May exert its anti-apoptotic activity by acting to reset the trigger level of mitochondrial cytochrome c release necessary to commit the cells to apoptosis (151 aa) | 0.787 |
| APP | Amyloid-beta A4 protein; N-APP binds TNFRSF21 triggering caspase activation and degeneration of both neuronal cell bodies (via caspase-3) and axons (via caspase-6); Endogenous ligands (770 aa) | 0.774 |

TCR: T-cell receptor; APP: Amyloid precursor protein; FLOT: Flotillin

**Table 3:** Ramachandran plot statistics of flotillin-1

| Ramachandran plot statistics | Residue | Percentage |
|---|---|---|
| Residues in most favored regions (A, B, L) | 269 | 80.3 |
| Residues in additional allowed regions (a, b, l, p) | 45 | 13.4 |
| Residues in generously allowed regions (~a, ~b, ~l, ~p) | 14 | 4.2 |
| Residues in disallowed regions | 7 | 2.1 |
| Number of nonglycine and nonproline residues | 335 | 100.0 |
| Number of end-residues (excl. Gly and Pro) | 2 | |
| Number of glycine residues (shown as triangles) | 15 | |
| Number of proline residues | 5 | |
| Total number of residues | 357 | |

**Table 4:** Details of the top five predicted active sites of flotillin-1 protein

| Site score | Size | $D_{score}$ | Volume |
|---|---|---|---|
| 1.043 | 368 | 0.958 | 755.97 |
| 1.034 | 164 | 0.971 | 626.32 |
| 0.932 | 96 | 0.891 | 225.01 |
| 0.793 | 59 | 0.737 | 132.74 |
| 0.768 | 50 | 0.698 | 160.87 |

showed stability during the overall simulation time. Evidently, MD simulation suggests that the ZINC67911837 complex was stable than ZINC31158144 and ZINC14437962. The values of RMSD, RMSF, and Rg for each complex are shown in Figures 4-6.

FLOT1 appears to have a strong relationship with other proteins involved in cell death, cleavage of APP, degradation of neuron cells, the formation of NFTs, and Aβ processing [Figure 7]. After finding the best docking score of the top five compounds, they were subjected

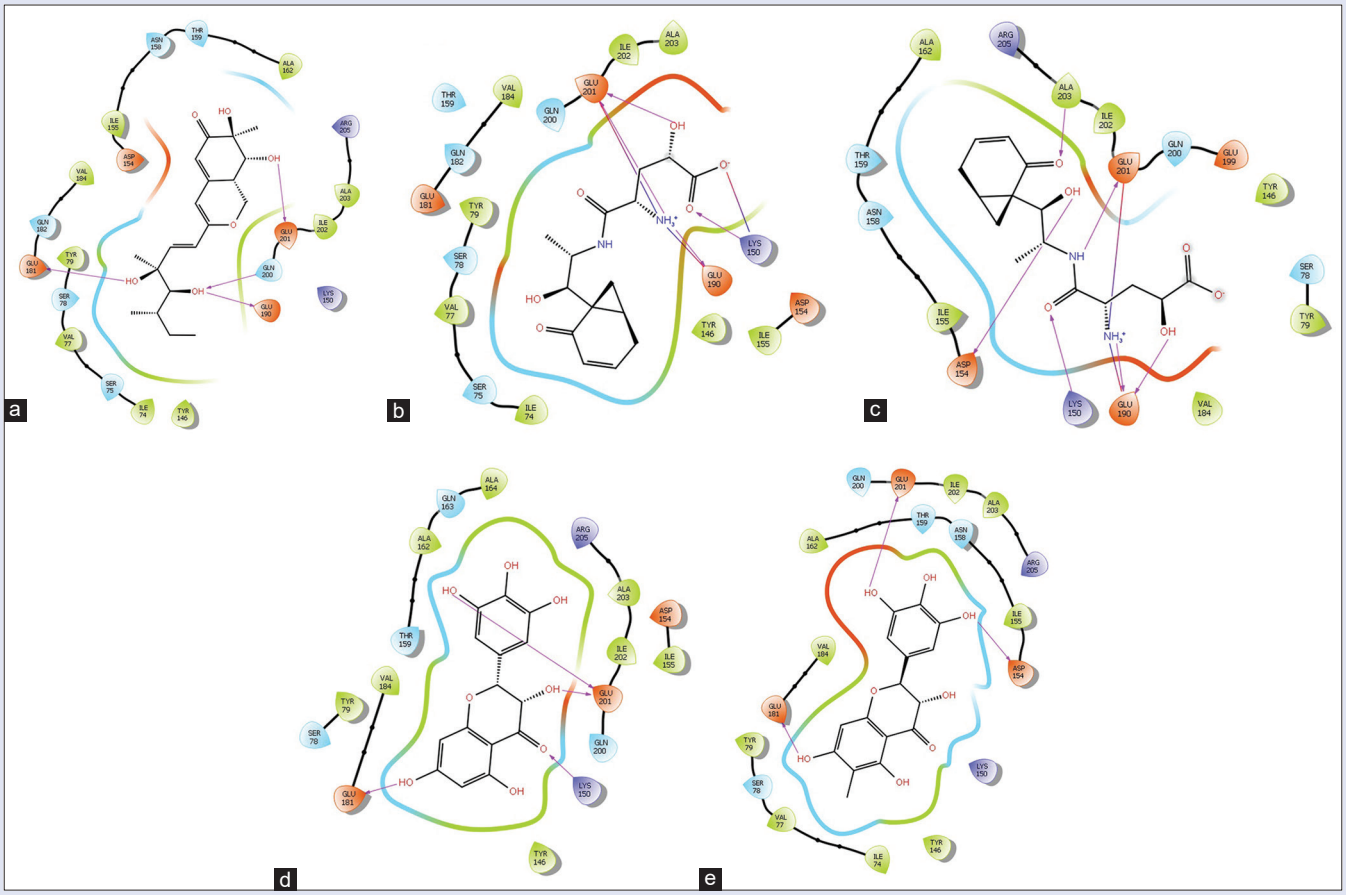



**Figure 3:** Ligand–protein interactions of flotillin-1 with their residues. (a) ZINC31158144, (b) ZINC67911837, (c) ZINC67911840, (d) ZINC04096945, (e) ZINC14437962

**Table 5:** Top five best binding affinity of flotillin-1 and compounds with their docking score, glide Gscore, glide energy, and MMGBSA dG Bind

| Title | Docking Score | Glide Gscore | Glide Energy | MMGBSA dG Bind |
|---|---|---|---|---|
| ZINC31158144 | −7.89936 | −7.89936 | −52.3754 | −39.3837 |
| ZINC67911837 | −7.78032 | −7.87492 | −46.6196 | −37.7105 |
| ZINC67911840 | −7.60858 | −7.70318 | −50.0029 | −26.919 |
| ZINC04096945 | −7.57361 | −7.60791 | −46.9165 | −27.9404 |
| ZINC14437962 | −7.53447 | −7.56147 | −50.8666 | −32.9286 |

MMGBSA: MM combined with Poisson–Boltzmann or generalized born and surface area

**Table 6:** The top five best binding affinity of the flotillin-1 and compounds with their interacting residues

| Complex | Interacting residues |
|---|---|
| ZINC31158144 | Asn158, Thr159, Ala162, Ile155, Asp154, Val184, Gln182, Glu181, Tyr79, Ser78, Val77, Ser75, Ile74, Tyr146, Arg205, Ala203, Ile202, Glu201, Gln200, Glu190, Lys150 |
| ZINC67911837 | Ala164, Gln163, Ala162, Thr159, Val184, Glu181, Tyr79, Ser78, Arg205, Ala203, Ile202, Glu201, Gln200, Lys150, Tyr146, Asp154, Ile155 |
| ZINC67911840 | Ala162, Thr159, Asn158, Ile155, Asp154, Arg205, Ala205, Ile202, Glu201, Gln200, Glu199, Tyr146, Ser78, Tyr79, Lys150, Glu190, Val184 |
| ZINC04096945 | Ala164, Gln163, Ala162, Thr159, Val184, Glu181, Tyr79, Ser78, Tyr146, Lys150, Gln200, Ile202, Ala203, Arg205, Asp154, Ile155 |
| ZINC14437962 | Arg205, Ala203, Ile202, Glu201, Gln200, Ala162, Thr159, Asn158, Ile155, Asp154, Val184, Glu181, Tyr79, Ser78, Val77, Ile74, Tyr146, Lys150, Ile155, Asp154 |

to Prime MMGBSA (free energy calculation of receptor–ligand complex) for the calculation of binding affinity of the complex. After rescoring of the binding energy of compounds, ZINC31158144, ZINC67911837, and ZINC14437962 showed the highest MMGBSA dG bind score at −39.3837, −37.37105, and −32.9286, respectively. Further, MD simulations of the top three docked complexes were performed to observe more information about structural stability and their binding affinity. In summary, MD simulation suggests that the ZINC67911837 complex was stable than ZINC31158144 and ZINC14437962. From the above results, the ZINC67911837

**Table 7:** Calculation of descriptors for the top five compounds with their permissible ranges

| Title | CNS | mol MW | Donor HB | Accept HB | QPlogPo/w | Percentage of human oral absorption | Rule of five |
|---|---|---|---|---|---|---|---|
| ZINC31158144 | −2 | 352.427 | 4 | 7.65 | 14.279 | 82.574 | 0 |
| ZINC67911837 | −2 | 326.349 | 5 | 9.9 | 19.593 | 3.99 | 1 |
| ZINC67911840 | −2 | 326.349 | 5 | 9.9 | 20.429 | 0 | 1 |
| ZINC04096945 | −2 | 320.255 | 5 | 7.2 | 17.502 | 27.043 | 1 |
| ZINC14437962 | −2 | 334.282 | 5 | 7.2 | 17.19 | 32.864 | 1 |

Permissible ranges for different parameters: Solute molecular weight (130.0/725.0); Donor HBs (0.0/6.0); Acceptor HBs (2.0/20.0); Percentage of human oral absorption (±20%) (<25%: poor), (>80%: high); Lipinski rule of 5 - (maximum=4); Predicted CNS activity (-- to ++) - −2 (inactive), +2 (active). QPlog Po/w: 2.0–6.5. QPlog Po/w: Predicted octanol/water partition coefficient; CNS: Central nervous system; HBs: Hydrogen bonds; MW: Molecular weight

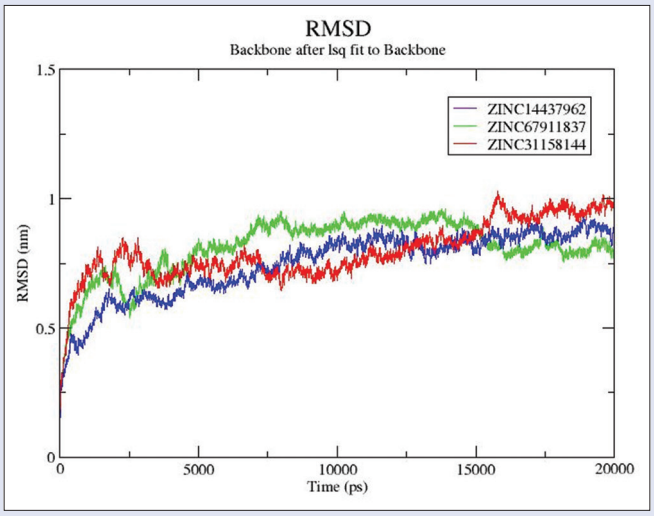



**Figure 4:** Root mean square deviation of flotillin-1 with natural compounds. Blue, green, and red colors show the time evolution of ZINC14437962, ZINC67911837, and ZINC31158144 with flotillin-1 complex, respectively

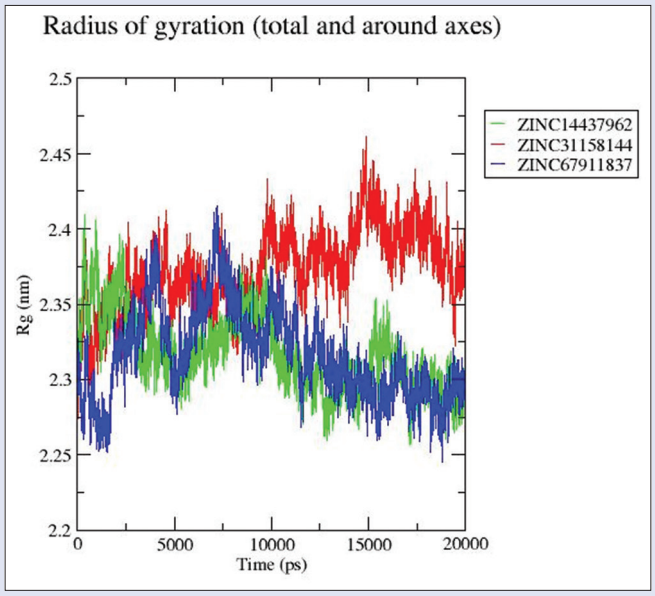



**Figure 5:** Radius of gyration of flotillin-1 with natural compounds. Green, red, and blue colors show the time evolution of ZINC14437962, ZINC31158144, and ZINC67911837 with flotillin-1 complex, respectively

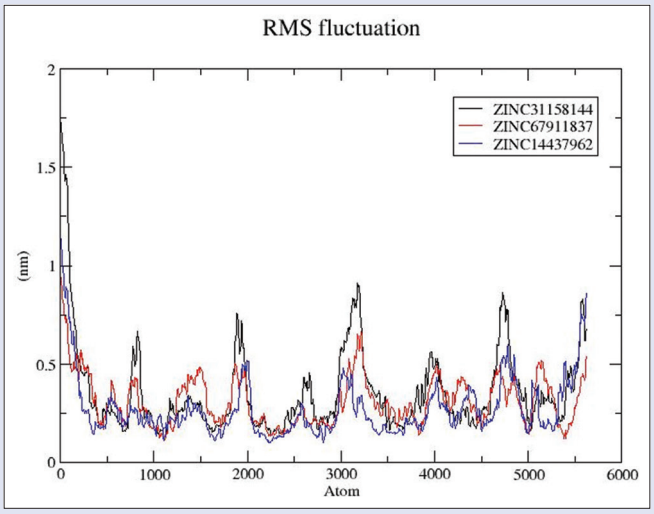



**Figure 6:** RMSF fluctuations of the flotillin-1 complex with natural compounds. Black, red, and blue colors show the time evolution of ZINC31158144, ZINC67911837, and ZINC14437962 with flotillin-1 complex, respectively

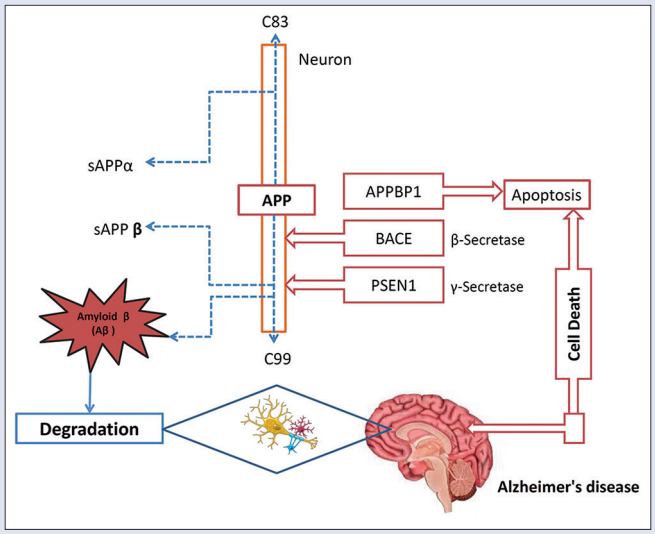
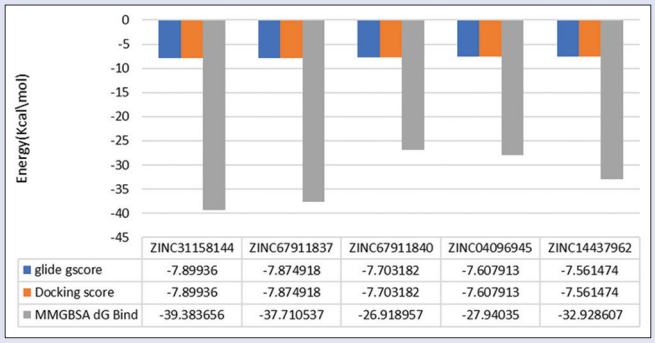



**Figure 7:** Flotillin-1 integrators such as amyloid precursor protein, beta-secretase, PSEN1, and APBP1 have shown strong interactions with each other. APBP1 is responsible for cell death via apoptosis while γ-secretase and beta-secretase (BACE 1) are responsible for cleavage of amyloid precursor protein which triggers the formation of β-amyloid that is responsible for degradation of neurons cell

compound could be used as a novel potent inhibitor of FLOT1 through which *BACE-1* activity may be suppressed, which will work as a therapeutic against AD [Figure 8].




| | ZINC31158144 | ZINC67911837 | ZINC67911840 | ZINC04096945 | ZINC14437962 |
|---|---|---|---|---|---|
| glide gscore | -7.89936 | -7.874918 | -7.703182 | -7.607913 | -7.561474 |
| Docking score | -7.89936 | -7.874918 | -7.703182 | -7.607913 | -7.561474 |
| MMGBSA dG Bind | -39.383656 | -37.710537 | -26.918957 | -27.94035 | -32.928607 |

**Figure 8:** Binding affinity score of natural compounds with flotillin-1 protein

## CONCLUSION

AD is a complex neurological disorder in older people, which requires long-term medication. So far, unavailability of specific drugs against AD has triggered an urgent thirst for the identification of novel drug targets and their validation. FLOT1 is one of the potential therapeutic targets for the disease; considering its role in Aβ generation and its strong relation with other proteins in Aβ generation pathways, the protein's functions need to be explored. There is a need for safe and effective drugs with minimal side effects to treat AD. Inhibiting the neurofibrillary deposition of Aβ plaques is the main goal of most of the drugs. In comparison to *BACE-1*, FLOT1 appears to be a safer and effective therapeutic target. The inhibitors identified in this study provide a basis for further exploration and investigations.

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## Conflicts of interest

There are no conflicts of interest.

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