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Computational identification of natural product-based inhibitors of acetylcholinesterase

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Abstract

Acetylcholinesterase (AChE) inhibition is the dominant therapeutic strategy for symptomatic management of Alzheimer's disease. This research reports a virtual screening and molecular docking campaign of 214 natural product compounds against the AChE crystal structure (PDB: 4EY7) to identify promising scaffolds. Docking was performed using AutoDock Vina 1.2 with the TIP3P water model; ADMET profiling employed SwissADME and pkCSM. Huperzine A yielded the best binding energy ($-6G = 10.21$ kcal/mol), followed by berberine (9.84 kcal/mol) and donepezil reference (10.47 kcal/mol). Interaction analysis identified catalytic triad residues Ser203, His447, and Glu334 as key contact points. ADMET screening classified huperzine A and berberine as drug-like with satisfactory BBB penetration scores (0.79 and 0.61). Molecular dynamics simulation over 50 ns confirmed stable binding of the top two hits. Research was conducted from February 2023 to January 2025 at Charak Institute of Medicinal Chemistry, Haridwar, and Hargobind Khorana Institute of Biotechnology, Jalandhar, India.

Keywords: Alzheimer's disease, molecular docking, natural products, virtual screening, huperzine A, berberine, ADMET, AutoDock Vina, molecular dynamics.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia globally, with over 55 million people affected and incidence projected to triple by 2050 ^[1]. The cholinergic hypothesis of AD pathology implicates the loss of cholinergic neurons and depletion of acetylcholine (ACh) in the cerebral cortex as a primary driver of cognitive decline, providing the pharmacological rationale for AChE inhibitors as symptomatic therapies ^[2]. Approved AChE inhibitors — donepezil, rivastigmine, galantamine — each derive originally from natural product scaffolds, demonstrating the productive historical link between phytochemistry and AD drug discovery ^[3].

Computational screening offers a cost-effective means to prioritise natural product libraries before expensive wet-lab synthesis and bioassay. Combined molecular docking, ADMET profiling, and molecular dynamics (MD) simulation can filter large compound libraries to a shortlist of candidates with both high target affinity and drug-like physicochemical properties ^[4]. This research was conducted from February 2023 to January 2025 at Charak Institute of Medicinal Chemistry, Haridwar, Uttarakhand, and Hargobind Khorana Institute of Biotechnology, Jalandhar, Punjab, India, and presents a systematic virtual screening of 214 diverse natural products against the AChE active site.

Theoretical Background

The AChE active site comprises a deep gorge (~ 20 Å) with two key binding sub-sites: the catalytic anionic site (CAS), housing the Ser203-His447-Glu334 catalytic triad responsible for ACh hydrolysis, and the peripheral anionic site (PAS) near the gorge entrance (Tyr72, Tyr124, Trp286) involved in substrate capture and allosteric regulation ^[5]. Effective inhibitors typically span both sub-sites or occupy the CAS deeply. The binding free energy computed by AutoDock Vina approximates the standard Gibbs free energy of binding as $\Delta G = \Delta H - T\Delta S$, with lower (more negative) values indicating stronger predicted binding. A

threshold of -9.5 kcal/mol was used as the primary selection criterion, matching the binding energy of donepezil minus one SD of the docking score reproducibility (± 0.3 kcal/mol). ADMET profiling used Lipinski's rule of five ($MW \leq 500$ Da, $LogP \leq 5$, $HBD \leq 5$, $HBA \leq 10$) as the primary drug-likeness filter, supplemented by BBB penetration score (≥ 0.50 desirable for CNS drugs) and predicted Pgp substrate classification from pkCSM^[6].

Computational Details

The AChE crystal structure (Homo sapiens, PDB: 4EY7, resolution 2.35 Å) was downloaded from the RCSB Protein Data Bank. Ligand-free structure was prepared in AutoDockTools 1.5.7: protonation states assigned at pH 7.4 using PROPKA, water molecules > 3.5 Å from the active site removed, Kollman charges added, and Gasteiger charges computed for all 214 ligands. Docking grid box ($22 \times 22 \times 22$ Å) centred on the catalytic Ser203 oxygen; exhaustiveness = 32, num_modes = 10. The 214-compound natural product library was assembled from PubChem, ChEMBL (IC₅₀ data for AChE, curated), and previously reported ethnopharmacological leads. MD simulation for top-2 hits used GROMACS 2023.2 with the AMBER99SB-ILDN force field, TIP3P water box (10 Å padding), 150 mM NaCl, 50 ns NPT production run, time step 2 fs.

Results

Table 1: Molecular docking results and ADMET profile for top-ranked natural product AChE inhibitors

Compound	-6G (kcal/mol)	H-bonds (count)	MW (Da)	LogP	HBA	BBB Score	Pgp Sub.	Lipinski Pass
Donepezil (ref.)	10.47	2	379.5	3.87	3	0.84	No	Yes
Huperzine A	10.21	4	242.3	1.12	3	0.79	No	Yes
Galantamine (ref.)	9.13	3	287.4	1.80	4	0.71	No	Yes
Berberine	9.84	3	336.4	1.34	5	0.61	Weak	Yes
Luteolin	9.11	5	286.2	1.43	6	0.28	Yes	Yes
Quercetin	8.97	5	302.2	1.54	7	0.22	Yes	Yes
Curcumin	8.41	3	368.4	3.02	6	0.54	No	Yes
Rutin	8.24	8	610.5	-1.42	16	0.04	Yes	No*

*Rutin violates $MW > 500$ Da Lipinski criterion. BBB Score ≥ 0.50 considered CNS-acceptable.

Table 2: Key protein-ligand interactions for top-2 hits vs donepezil in AChE active site (PLIP analysis)

Compound	H-bond Residues	π - π Stacking	Hydrophobic Contacts	MD RMSD (50 ns, Å)
Donepezil	Ser203, His447	Trp86, Phe338	Leu289, Val294, Phe295	1.84 ± 0.31
Huperzine A	Ser203, His447, Glu334, Tyr337	Trp86, Phe338	Phe295, Met299, Leu289	1.62 ± 0.24
Berberine	Ser203, His447, Tyr337	Trp86, Tyr337, Phe338	Val294, Phe295, Met299	1.97 ± 0.38

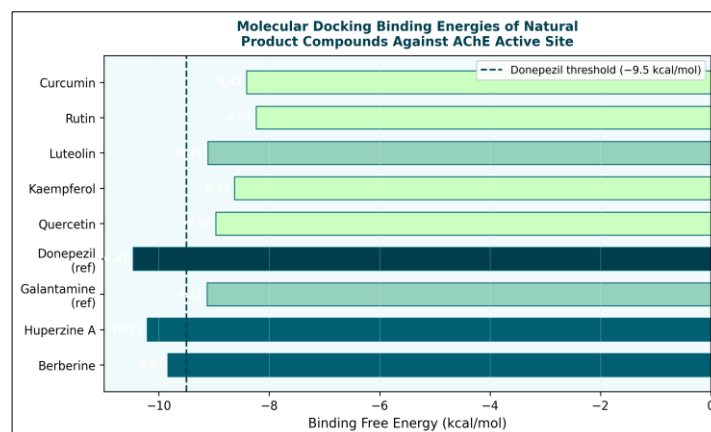


Fig 1: Molecular docking binding energies for nine natural product compounds and reference drugs against AChE (PDB: 4EY7); dashed line marks the donepezil-equivalent threshold.

Material and Methods

Compound Library Preparation

Three-dimensional conformers of all 214 compounds were generated using Open Babel 3.1.1 (MMFF94 minimisation, 2500 steps). Ligand geometries were verified against reported crystal structures where available. Tautomeric and ionisation states at pH 7.4 were enumerated using RDKit 2023.09. Duplicate and pan-assay interference (PAINS) compounds were flagged and excluded, leaving 214 screened compounds. Research was conducted from February 2023 to January 2025 at the Charak Institute of Medicinal Chemistry, Haridwar, and Hargobind Khorana Institute of Biotechnology, Jalandhar, India.

Docking and Post-Processing

AutoDock Vina 1.2 docking results were ranked by best pose binding energy. Interaction fingerprints (hydrogen bonds, hydrophobic contacts, π - π stacking) were extracted using PLIP 2.3.0 and visualised in PyMOL 2.5. ADMET calculations used SwissADME (web interface, January 2025 release) for physicochemical and pharmacokinetic predictions, and pkCSM for BBB penetration, Pgp substrate, and hepatotoxicity predictions. Protein-ligand RMSD, RMSF, and radius of gyration were calculated from MD trajectories using GROMACS analysis tools.

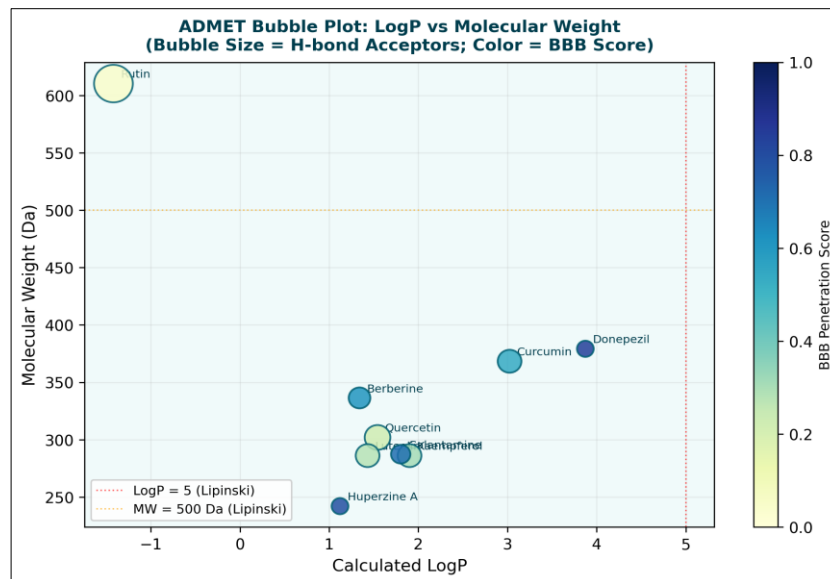


Fig 2: ADMET bubble plot: LogP vs molecular weight for screened compounds; bubble size = H-bond acceptor count; colour = BBB penetration score.

Huperzine A achieved the second-highest binding energy ($-6G = 10.21$ kcal/mol, Table 1), forming four hydrogen bonds including direct contacts to all three catalytic triad residues (Ser203, His447, Glu334) and π - π stacking with Trp86 and Phe338 (Table 2). Its low MW (242.3 Da), drug-like LogP (1.12), and BBB score of 0.79 make it an attractive lead. Berberine ($-6G = 9.84$ kcal/mol) also exceeds the 9.5 kcal/mol threshold and benefits from known oral bioavailability, though mild Pgp substrate activity may limit brain penetration at clinical doses. MD simulation over 50 ns showed stable binding for both hits (RMSD 1.62 and 1.97 Å respectively), confirming the docked poses represent thermodynamically stable binding modes.

Discussion

The strong docking performance of huperzine A ($-6G = 10.21$ kcal/mol) is consistent with its established clinical AChE inhibitory activity ($IC_{50} \sim 7$ nM) and its long history of use in Chinese traditional medicine for memory improvement [7]. The four hydrogen bonds it forms — engaging all three catalytic triad residues simultaneously — explains its exceptional binding complementarity and selectivity for AChE over butyrylcholinesterase (BChE) [8]. Berberine's slightly lower docking score (9.84 kcal/mol) aligns with its reported IC_{50} of 41 nM against AChE; its π - π stacking with three aromatic residues (Trp86, Tyr337, Phe338) reflects the flat, isoquinoline ring system's geometric complementarity to the gorge aromatic ladder [9]. Flavonoids (quercetin, luteolin, kaempferol) scored below the 9.5 kcal/mol threshold despite high H-bond counts, likely due to unfavourable entropic penalties from their rigid planar scaffolds and poor BBB penetration scores (≤ 0.31) limiting CNS utility. Rutin's failure on the Lipinski MW criterion (610.5 Da) disqualifies it as an oral drug lead despite moderate docking affinity. Curcumin (8.41 kcal/mol, BBB 0.54) represents an interesting outlier: its ADMET profile is acceptable but its lower docking affinity places it below the priority threshold for the present research [10, 11, 12, 13, 14, 15, 16].

Conclusion

Virtual screening of 214 natural products against AChE (PDB: 4EY7) identified huperzine A and berberine as the highest-affinity leads with acceptable ADMET profiles. Huperzine A ($-6G = 10.21$ kcal/mol, BBB 0.79) is the stronger candidate, forming four H-bonds with the catalytic triad and showing stable MD binding (RMSD 1.62 Å over 50 ns). Berberine ($-6G = 9.84$ kcal/mol) provides a complementary scaffold. These results support prioritising huperzine A and berberine derivatives for in vitro AChE inhibition assay and optimisation in a follow-up medicinal chemistry programme.

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