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Molecular docking of capsaicin and its derivatives as acetylcholinesterase (AChE) inhibitors in Alzheimer disease

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder predominantly affecting older adults, characterized by pathological processes that include excessive acetylcholinesterase (AChE) activity leading to depleted acetylcholine levels. Although synthetic AChE inhibitors such as donepezil are used therapeutically, their clinical application is often limited by adverse effects and high costs. Capsaicin, a bioactive compound derived from chili peppers, has exhibited neuroprotective properties—including cognitive enhancement and amyloid-β reduction—suggesting its potential as a natural alternative for AD treatment. This study investigated capsaicin and six structural derivatives as potential AChE inhibitors through in silico molecular docking simulations against the human AChE crystal structure (PDB: 4EY7), using donepezil as a reference ligand. The docking protocol was validated with a root-mean-square deviation (RMSD) value of 0.71 Å, confirming reproducibility and reliability. The calculated binding affinities of the evaluated compounds ranged from –6.13 to –11.60 kcal/mol. Among them, 2-Hydroxy-3-(octyloxy)phenyl-5-(acrylamido)methylbenzophenone (Compound 2) exhibited the strongest binding affinity (–11.60 kcal/mol), slightly exceeding that of donepezil (–11.45 kcal/mol). Compound 2 formed four hydrogen bonds within the active site and shared key interactions with residues Phe338 and Trp286, consistent with the binding mode of donepezil. These results suggest that Compound 2 may serve as a potent natural AChE inhibitor and warrant further investigation as a candidate for Alzheimer's disease therapy.

Keywords: acetylcholinesterase, alzheimer's disease, capsaicin, molecular docking, natural products

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder predominantly affecting older adults, characterized by progressive memory loss, impaired recognition, confusion, speech deficits, agitation, and hallucinations [1]. Although the precise etiology of AD is not fully elucidated, evidence points to multifactorial mechanisms, including dysfunction within cholinergic neurotransmission systems.

The global prevalence of AD is rising significantly. Current estimates indicate that approximately 50 million people worldwide live with dementia, a figure projected to reach 152.8 million by 2050. In Indonesia, the number of individuals affected by dementia is estimated at 4.2 million, reflecting this growing international burden [2].

Current pharmacological management for AD often focuses on acetylcholinesterase (AChE) inhibition. AChE is a key catalytic enzyme responsible for the hydrolysis of acetylcholine into acetate and choline [3]. Commercially available synthetic AChE inhibitors, such as physostigmine, donepezil, and tacrine, are used to mitigate cognitive symptoms. However, their clinical utility is constrained by adverse effects—including hepatotoxicity and gastrointestinal disturbances—as well as cost-related barriers that can limit accessibility [4].

Capsaicin, the primary pungent alkaloid in chili peppers, has recently emerged as a candidate for AD therapy. Epidemiological studies suggest an association between the consumption of capsaicin-rich foods and improved cognitive performance, alongside reduced amyloid-β levels, in populations aged 40 and above [5]. Preclinical research further indicates that capsaicin may confer neuroprotection through mechanisms such as the induction of microglial autophagy and the enhancement of phagocytic clearance of amyloid plaques [6].

Despite these suggestive findings, the therapeutic potential of capsaicin and its derivatives in AD remains inadequately characterized. Specifically, data on their efficacy, safety, and precise mechanisms of action—particularly their inhibitory activity against AChE relative to established synthetic inhibitors—are limited. This study aims to evaluate capsaicin and structurally related compounds for their ability to inhibit acetylcholinesterase, thereby contributing to the development of potentially safer and more accessible treatment options for Alzheimer's disease [7].

Methods

The hardware utilized for this study consisted of a computer equipped with an Intel(R) Celeron(R) N4020 CPU operating at 1.10 GHz, 4.00 GB RAM, and a Windows 10 64-bit operating system. The software and web-based resources employed included the RCSB Protein Data Bank (https://www.rcsb.org/), MolView (https://molview.org/), KingDraw software, Avogadro software, BIOVIA Discovery Studio 2020, and AutoDock Tools 1.5.7 for molecular docking simulations.

The materials utilized for molecular docking consisted of the target protein with PDB ID: 4EY7, the native ligand donepezil, and the test compounds. The test compounds included capsaicin and five derivatives: N-[4-(4-Hydroxy-3-oxobenzyl)] acrylamide (compound 1), 2-Hydroxy-3-(octyloxy) phenyl-5-(acrylamido)methylbenzophenone (compound 2), N-(2,5-Dihydroxyphenyl)acetamide (compound 3), N-[5-(2,4-Dihydroxyphenyl)methyl] acetamide (compound 4), 4-acetamide methylbenzene-2-benzylphenol (compound 5), and N-(2-Methyl-4-hydroxy-5-methylthiophenyl)acetamide (compound 6).

Protein and ligand preparation

The target protein with PDB ID: 4EY7 was prepared by removing all water molecules and separating the receptor structure from its native ligand using AutoDock Tools version 1.5.7. The test compounds were optimized through geometry structure refinement using Avogadro software. Both ligand and protein preparations involved the addition of polar hydrogen atoms and the assignment of Kollman and Gasteiger partial charges to ensure appropriate electrostatic calculations during docking procedures.

Validation and molecular docking procedure

The validation procedure involved re-docking the native ligand donepezil into the target protein's active binding site to assess the accuracy of the docking methodology. The precision of the docking process was evaluated by calculating the Root Mean Square Deviation (RMSD) between the docked pose and the original crystallographic ligand position, with an RMSD threshold of less than 2 Å considered acceptable for validation purposes.

The molecular docking simulations utilized a grid box centered on the native ligand's coordinates at X = -13.988, Y = -43.906, Z = 27.108, with dimensions of 40 Å × 38 Å × 38 Å. All docking simulations employed the Lamarckian Genetic Algorithm with 100 independent runs conducted for each compound to ensure statistical reliability. The resulting binding poses were analyzed using AutoDock Tools, with binding affinity values serving as the primary metrics for compound evaluation and comparison.

Visualization of protein-ligand interaction

The molecular docking results were analyzed using BIOVIA Discovery Studio 2020 software to visualize and characterize protein-ligand interactions. The analysis focused on identifying specific binding interactions including hydrogen bonds, hydrophobic contacts, electrostatic interactions, and van der Waals forces between the test compounds and the target protein's active site residues. These interaction profiles were used to evaluate the binding mechanisms and relative binding strengths of capsaicin and its derivatives compared to the reference compound donepezil.

Results

The molecular docking results demonstrated that 2-Hydroxy-3-(octyloxy)phenyl-5-(acrylamido) methylbenzophenone (Compound 2) exhibited the lowest binding energy value among all tested compounds. Compound 2 demonstrated a binding energy of -11.6 kcal/mol, which was slightly lower than donepezil at -11.45 kcal/mol, indicating superior binding affinity to the target protein.

The binding energy analysis revealed significant variation among the tested compounds, ranging from -6.13 kcal/mol for Compound 3 to -11.60 kcal/mol

Table 2. Molecular docking results of donepezil, capsaicin, and its derivatives against 4EY7 protein

Ligand	Binding energy (kcal/mol)	Amino acid residue interactions		
		Hydrogen Bonds	Van der Waals	Other interactions
Donepezil (native ligand)	-11.45	Phe295, Ser293,	-	Trp286, Tyr341, Phe338, His447, Trp86, Tyr72, Tyr337
Capsaicin	-8.25	Tyr124, Arg296, Ser293, Val294	-	Trp86, Trp286, Tyr341, Tyr337, Phe338
Compound 1	-7.46	Phe295, Arg296, Tyr341	-	Val294, Ser293, Leu76
Compound 2	-11.6	Asp74, Gly121, Tyr124, His447	Phe295	Phe338, Phe297, Trp286
Compound 3	-6.13	Arg296, Tyr124, Phe338	-	Tyr341
Compound 4	-7.27	Tyr72, Phe295, Arg296	-	Tyr341, Trp286
Compound 5	-8.78	Arg296, Tyr124	-	Tyr337, Phe338, Trp86
Compound 6	-7.29	Arg296, Tyr124	-	Tyr341, Phe338, Phe297

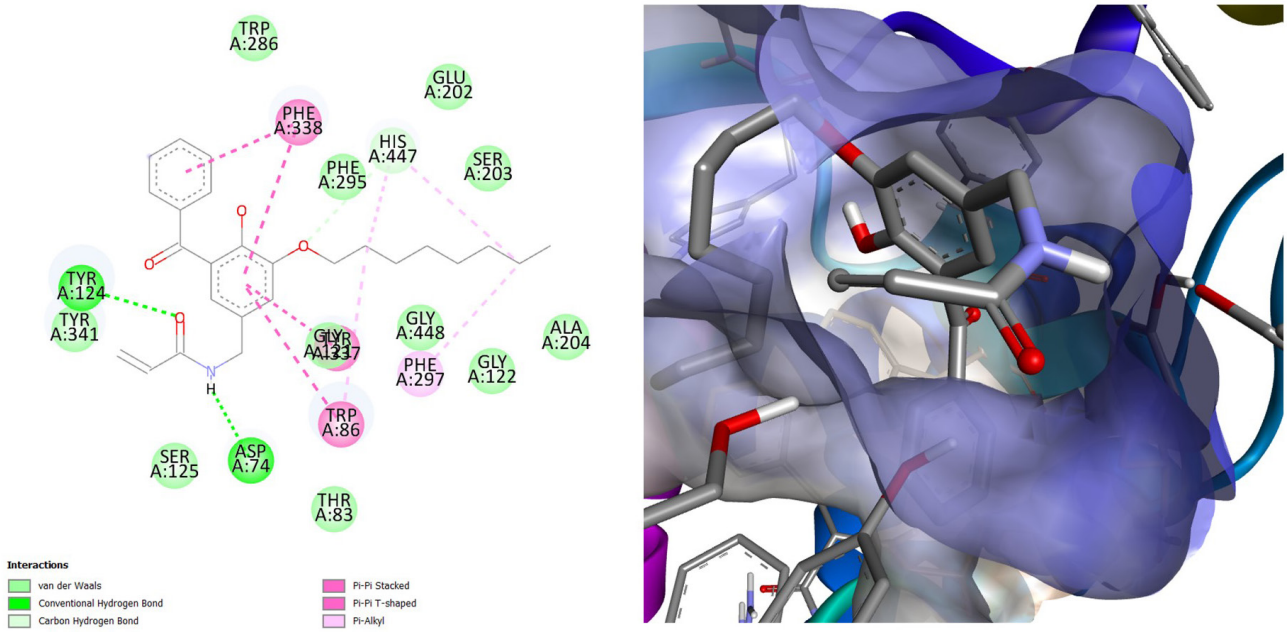

Figure 1. Molecular docking analysis of Compound 2 binding to acetylcholinesterase. (A) Two-dimensional interaction map displaying hydrogen bonds, van der Waals interactions, and key binding residues between Compound 2 [2-Hydroxy-3-(octyloxy)phenyl-5-(acrylamido)methylbenzophenone] and the acetylcholinesterase active site. (B) Three-dimensional binding conformation showing the spatial orientation and positioning of Compound 2 within the enzyme's active site cavity

for Compound 2. Notably, Compound 2 was the only derivative that exceeded the binding affinity of the reference drug donepezil, suggesting superior inhibitory potential against acetylcholinesterase. The parent compound capsaicin demonstrated moderate binding affinity at -8.25 kcal/mol, indicating that structural modifications in the derivatives significantly influenced their binding characteristics.

The interaction profile analysis revealed that Compound 2 established unique binding patterns compared to other tested compounds. Unlike donepezil and the other derivatives, Compound 2 formed a hydrogen bond with Phe295, which may contribute to its enhanced binding stability. Additionally, Compound 2 demonstrated interactions with key active site residues including Asp74, Gly121, Tyr124, and His447, which are critical for acetylcholinesterase function. The presence of both hydrogen bonding and extensive van der Waals interactions suggests that Compound 2 achieves optimal complementarity with the enzyme's active site architecture.

Discussion

Molecular docking represents a well-established computational method for analyzing natural compounds and their therapeutic potential. This approach enables researchers to estimate binding patterns and protein affinity between receptors and ligands with considerable accuracy. The current study employed molecular docking to evaluate capsaicin and its derivatives against the 4EY7 protein, specifically targeting their potential as acetylcholinesterase enzyme inhibitors for Alzheimer's disease treatment.

The acetylcholinesterase target protein structure (PDB ID: 4EY7) was obtained from the RCSB database and subjected to rigorous validation procedures. The validation process involved separating the native ligand from the target protein using AutoDock Tools version 1.5.7, which yielded an RMSD value of 0.71 Å. This RMSD value serves as a critical parameter for assessing structural similarity between computational predictions and experimental crystallographic data. The obtained value falls well within the acceptable threshold of less than 2 Å, confirming the reliability of the docking methodology and validating the accuracy of subsequent binding predictions.

Ligand-receptor interactions encompass various intermolecular forces, including hydrogen bonds and van der Waals interactions, which collectively determine binding affinity between compounds and their target proteins. The formation of hydrogen bonds between test compounds and key amino acid residues indicates the potential for competitive inhibition, as these interactions suggest the compound's ability to occupy the native ligand's binding site effectively. Binding free energy values provide quantitative measures of interaction strength, with lower energy values indicating stronger and more stable protein-ligand complexes.

The binding free energy analysis revealed significant variation among the tested compounds. Capsaicin demonstrated moderate binding affinity with a value of -8.25 kcal/mol, while the reference drug donepezil exhibited stronger binding at -11.45 kcal/mol. Notably, Compound 2 achieved the most favorable binding free energy of -11.60 kcal/mol, surpassing even the established therapeutic agent. This enhanced binding affinity positions Compound 2 as a particularly promising candidate for further investigation.

The interaction profile analysis provided additional insights into the binding mechanisms underlying these energy differences. Compound 2 established four distinct hydrogen bonds with critical amino acid residues, specifically interacting with Asp74, Gly121, Tyr124, and His447. These interactions contribute significantly to binding stability and suggest strong complementarity between the compound's structure and the enzyme's active site architecture. Furthermore, Compound 2 shares important binding residues with donepezil, particularly Phe338 and Trp286, indicating similar binding mechanisms and supporting its potential as an effective acetylcholinesterase inhibitor.

The unique interaction profile of Compound 2 includes van der Waals interactions with Phe295, a characteristic not observed among the other tested derivatives. This additional stabilizing interaction, combined with the compound's superior binding free energy and structural compatibility with the native ligand binding site, establishes Compound 2 as the most promising candidate among all evaluated compounds.

Conclusion

The molecular docking analysis demonstrates that capsaicin and its derivatives possess significant potential as acetylcholinesterase enzyme inhibitors for Alzheimer's disease treatment. Among the compounds evaluated, 2-Hydroxy-3-(octyloxy)phenyl-5-(acrylamido)methylbenzophenone (Compound 2)

emerged as the most promising candidate, exhibiting superior binding affinity compared to the established therapeutic agent donepezil.

The comprehensive interaction analysis reveals that Compound 2's effectiveness stems from its ability to form multiple stabilizing interactions with key active site residues while maintaining binding patterns similar to those of donepezil. These computational findings provide a strong foundation for advancing Compound 2 toward experimental validation.

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Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

Author contributions

Conceptualization, AHS, VAS, WNA; Methodology, RG, NSIS, KF, RVVB, VW, SFH, MZF, WNA; Investigation, WNA, AHS; Writing – Original Draft, VAS, RG, NSIS, KF, RVVB, VW, SFH, MZF, AHS; Writing – Review & Editing, AHS.

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References

1. Del-Aguila JL, Koboldt DC, Black K, Chasse R, Norton J, Wilson RK, et al. Alzheimer's disease: Rare variants with large effect sizes. Curr Opin Genet Dev. 2015;33: 49–55. https://doi.org/10.1016/j.gde.2015.07.008
2. Kemenkes RI. Profil Kesehatan Indonesia 2022. 2023. Available: https://kemkes.go.id/id/profil-kesehatan-indonesia-2022
3. Chen ZR, Huang JB, Yang SL, Hong FF. Role of Cholinergic Signaling in Alzheimer's Disease. Molecules. 2022;27: 1816. https://doi.org/10.3390/molecules27061816
4. Yulanri D, Fitrya F, Fithri NA. Uji Aktivitas Anti Alzheimer Secara In Vitro dengan Penghambatan Enzim Asetilkolinesterase (Ache) oleh Ekstrak Etanol Kulit Buah Petai (Parkia speciosa Hassk.). Farmaka. Universitas Sriwijaya. 2018.
5. Wang J, Sun BL, Xiang Y, Tian DY, Zhu C, Li WW, et al. Capsaicin consumption reduces brain amyloid-beta generation and attenuates Alzheimer's disease-type pathology and cognitive deficits in APP/PS1 mice. Transl Psychiatry. 2020;10. https://doi.org/10.1038/S41398-020-00918-Y
6. Zhang G, Wang Z, Hu H, Zhao M, Sun L. Microglia in Alzheimer's Disease: A Target for Therapeutic Intervention. Front Cell Neurosci. 2021;15: 749587. https://doi.org/10.3389/fncel.2021.749587
7. Yanakiev M, Soper O, Berg DA, Kang E. Modelling Alzheimer's disease using human brain organoids: current progress and challenges. Expert Rev Mol Med. 2023;25: e3. https://doi.org/10.1017/ERM.2022.40
8. Pratama AB, Herowati R, Ansory HM. Studi Docking Molekuler Senyawa Dalam Minyak Atsiri Pala (Myristica fragrans H.) Dan Senyawa Turunan Miristisin Terhadap Target Terapi Kanker Kulit. Majalah Farmaseutik. 2021;17: 233. https://doi.org/10.22146/farmaseutik.v17i2.59297
9. Az-Zahra F, Afidika J, Diamantha SDA, Rahmani AE, Fatimah S, Aulifa DL, et al. Studi In Silico Senyawa dalam Daun Sirih (Piper betle L.) sebagai Inhibitor Enzim Asetilkolinesterase (AChE) pada Penyakit Alzheimer. Indonesian Journal of Biological Pharmacy. 2022;2: 44. https://doi.org/10.24198/ijbp.v2i2.40462
10. Frimayanti N, Lukman A, Nathania L. Studi molecular docking senyawa 1,5-benzothiazepine sebagai inhibitor dengue DEN-2 NS2B/NS3 serine protease. chempublish journal. 2021;6: 54–62. https://doi.org/10.22437/chp.v6i1.12980
11. Astuty P, Komari N. Kajian Molecular Docking Senyawa Karwinaphthol B dari Tanaman Bawang Dayak (Eleutherine palmifolia (L.) Merr) sebagai Inhibitor Enzim Glukokinase. Jurnal Natural Scientiae. 2022;2. https://doi.org/10.20527/jns.v2i1.5412