MODIFICATION AND STRUCTURE EVALUATION OF CLOROGENIC ACID COMPOUNDS AS BACE1 INHIBITORS FOR THE TREATMENT OF ALZHEIMER

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ABSTRACT
Alzheimer's disease remains an unresolved health issue to this day, mainly due to the limitations and adverse effects of available medications. Alzheimer's is characterized by the accumulation of amyloid plaques formed through the action of the BACE1 enzyme. Chlorogenic acid has shown potential as an Alzheimer's drug candidate due to its diverse neurological benefits. The aim of this research is to develop chlorogenic acid as a BACE1 inhibitor by modifying its molecular structure to enhance its effectiveness. The study employs in silico methods, involving structural modifications of chlorogenic acid and assessing its activity using molecular docking. The research employs in silico approaches to modify the structure of chlorogenic acid and analyze the interactions of the resulting compounds with BACE1 through molecular docking. The modified compounds demonstrate improved potential as BACE1 inhibitors as they can directly bind to the amino acid residues ASP A:32 and ASP A:228 on BACE1. Chlorogenic acid's structure can be modified to yield novel compounds with the potential to inhibit BACE1, offering a promising solution for Alzheimer's treatment. This study provides valuable insights for the development of more effective and safer Alzheimer's drugs.

Keywords: alzheimer; BACE1 enzyme inhibitor; in silico; molecular docking

INTRODUCTION
The increase in Alzheimer's sufferers is known to continue to increase (Association, 2019), in Indonesia, there were 1.2 million cases of dementia in 2015, and this figure is expected to continue to increase to reach 3,980,000 cases in 2050 (Turana et al., 2019). Pathologically, Alzheimer's is characterized by the accumulation of amyloid beta peptides as fibrillar plaques and dissolved oligomers in brain areas. the amyloid beta peptide is initiated by BACE1 cleavage on amyloid precursor protein, the active site of BACE1 is known to be located on two aspartic acid residues (Sinha et al., 1999; Skovronsky, Moore, Milla, Doms, & Lee, 2000; Vassar et al., 1999; Vetrivel & Thinakaran, 2010). Current BACE1 inhibitors are still ineffective and have many detrimental effects, verbucestat as a BACE1 inhibitor is known not to selectively inhibit APP processing but also significantly inhibits long-term potentiation and interferes with the physiological processing of BACE nerve substrates (neuregulin and Scn2b) which are required for nerve transmission (Li, Liu, & Selkoe, 2019).

Lanabecestat, as another BACE1 inhibitor, is also known to not have a consistent dosage and has several side effects including psychiatric side effects, weight loss, and hair color changes (Wessels et al., 2020). Atabecestat as a BACE 1 inhibitor is also not better than verbucestat and Lanabecestat because it is known to have the potential to cause decreased cognition, and increased liver enzymes which indicate problems with the liver (Novak et al., 2020), Based on this background, it is necessary to search for and develop anti-Alzheimer drugs, especially BACE 1 inhibitors.Chlorogenic acid is one of the potential compounds that can be developed as a BACE1 inhibitor. Chlorogenic acid is an ester of caffeic acid and (-)quinic acid and functions as an intermediary in lignin production. Studies have proven that chlorogenic acid has the potential to restore cognitive function, suppress neuroinflammation through inhibition of pro-inflammatory cytokine production and NF-κB signaling pathways, and inhibit oxidative stress through activating the Nrf2 signaling pathway (Alam et al., 2016; Boerjan, Ralph, & Baucher, 2003; Ochiai, Saitou, Suzukamo, Osaki, & Asada, 2019). However,

chlorogenic acid is known to have the potential to cause immunotoxicity. Immunotoxicity has been described as a deleterious consequence on the local and systemic immune system (Elbouzidi et al., 2022; Gupta, 2015). This study aims to modify the structure of chlorogenic acid compounds as anti-alzheimer's by considering the potential for inhibition of BACE1 cleavage through hydrogen bonding with specific amino acids, namely ASP 228 and ASP 32, increasing the docking score, and eliminating the potential for immunotoxicity through in silico prediction.

METHOD

The research was carried out in silico, and the structure of the chlorogenic acid compounds was drawn and modified using ChemDraw Ultra 12.0, the modified results were then checked for originality in several databases. Toxicity analysis was performed using ProTox-II (https://tox-new.charite.de/protox_II/), Pharmacokinetic and bioactivity analysis was performed using Swissadme (http://www.swissadme.ch/) and Molinspiration (https://www.molinspiration.com/), an analysis of the potential for the development of compounds as drugs were carried out using Lipinski's Five rules via SCFBio (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp), while molecular Docking performed using Autodock4 and visualized using Discovery Studio 2021 Client on the 4IVT target protein downloaded from the protein data bank (https://www.rcsb.org/structure/4ivt) (Benet, Hosey, Ursu, & Oprea, 2016; Bhat & Chatterjee, 2021; Gogoi et al., 2021; Pantaleão, Fernandes, Gonçalves, Maltarollo, & Honorio, 2022; Ranjith & Ravikumar, 2019).

RESULTH AND DISCUSSION

Compound Structure Modification

The compound modification was carried out by replacing the carboxylic group in the structure of the chlorogenic acid compound with an amide group (Figure 1), substitution with an amide group is specific for targeting the amino acids ASP 228 and ASP 32 as BACE1 inhibitors. In silico studies have proven that amide groups can form hydrogen bonds with ASP 228 and ASP 32, chlorogenic acid compounds which are modified following the principle of structural similarity will produce the same reaction (Cosconati et al., 2010; Hassan et al., 2018)

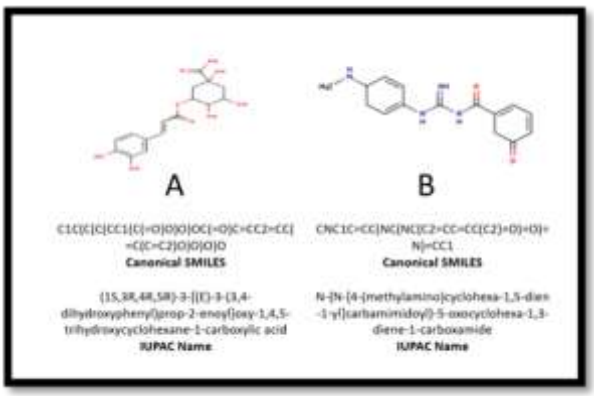

Figure 1. A. Structure of Chlorogenic Acid, B. Modified Compound Structures

Toxicity Analysis

Toxicity analysis using ProTox-II showed that the structure of the chlorogenic acid compound has the potential to cause immunotoxicity with a probability of 99%, while the structure of the modified compound shows the opposite as shown in Table 1, where the structure of the modified compound is known to have no potential for immunotoxicity.

Table 1.
Toxicity Prediction using ProTox-II

Type	Chlorogenic acid	Modified compounds
Hepatotoxicity	inactive	inactive
Carcinogenicity	inactive	inactive
Immunotoxicity	active	inactive
Mutagenicity	inactive	inactive
Cytotoxicity	inactive	inactive
Aryl hydrocarbon Receptor (AhR)	inactive	inactive
Androgen Receptor (AR)	inactive	inactive
Androgen Receptor Ligand Binding Domain (AR-LBD)	inactive	inactive
Aromatase	inactive	inactive
Estrogen Receptor Alpha (ER)	inactive	inactive
Estrogen Receptor Ligand Binding Domain (ER-LBD)	inactive	inactive
Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	inactive	Inactive
Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	inactive	Inactive
Heat shock factor response element (HSE)	inactive	Inactive
Mitochondrial Membrane Potential (MMP)	inactive	inactive
Phosphoprotein (Tumor Supressor) p53	inactive	inactive
ATPase family AAA domain-containing protein 5 (ATAD5)	inactive	inactive

Pharmacokinetic Analysis and Potential Bioactivity

Table 2.
Pharmacokinetic Predictions using Swissadme

Type	Chlorogenic acid	Modified compounds
GI absorption	Low	High
BBB permeant	No	No
P-gp substrate	No	No
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Bioavailability Score	00.11	00.55

Swissadme showed prediction results for chlorogenic acid which has a low potential for absorption through the gastrointestinal tract while the modified compounds showed the opposite results, namely the potential for better absorption in the gastrointestinal tract as shown in Table 2. The modified compounds also showed better bioavailability scores compared to acids. chlorogenic, descriptively, the bioavailability score is very significantly different with a value of 0.55 compared to chlorogenic acid which only has a value of 0.11,

the comparison of the two can also be seen in Figure 2 where the modified compound is included in the boiled egg category and chlorogenic acid is not. Drug absorption and bioavailability are important factors of pharmacokinetics. Both of these factors greatly affect the effectiveness and safety of an active substance when it is used as a drug. Both of these factors can also affect the onset, intensity, and duration of administration of a drug (Bertol & Favretto, 2023).

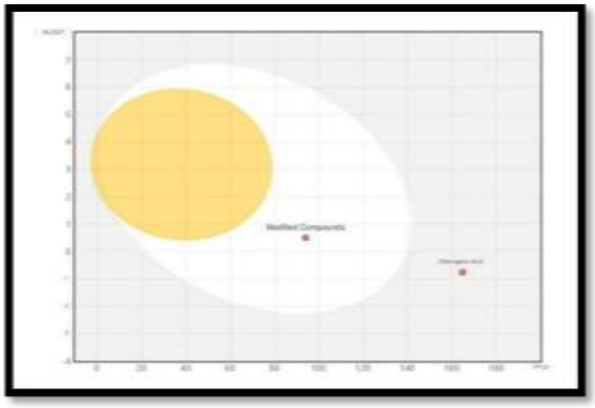

Figure 2. Boiled Egg

Bioactivity analysis by molinspiration showed that chlorogenic acid and the modified compound had the same ability as enzyme inhibitors, although it can be seen that the modified compound was still better with a score of 0.71 than chlorogenic acid which had a score of 0.62 as shown in table 3. Considering BACE1 is an enzyme that plays a role in the formation of amyloid plaques which is the cause of Alzheimer's, so chlorogenic acid and its modified compounds have the potential to inhibit the BACE1 enzyme (Hussain et al., 1999; Yan et al., 1999).

Table 3.
Bioactivity prediction using molinspiration (dark green color indicates good activity potential, light green color indicates moderate activity potential, and red color indicates no activity potential)

Type	Chlorogenic acid	Modified compounds
GPCR ligand	0.29	0.58
Ion channel modulator	0.14	0.36
Kinase inhibitor	0.00	-0.48
Nuclear receptor ligand	0.74	-0.12
Protease inhibitor	0.27	0.63
Enzyme inhibitor	0.62	0.71

Analysis of Lipinski's five rules

A rule of thumb for determining whether a chemical compound with a particular pharmacological activity also has good chemical and physical properties can be determined by Lipinski's five rules. Because most drugs taken orally are small to medium in size, Christopher A. Lipinski developed this rule in 1997 to maximize the requirements for potential active compounds as drugs (Lipinski, 2004).

Lipinski's rule of five states, orally active drugs may not violate more than one condition including a molecular mass of fewer than 500 Daltons, high lipophilicity (expressed as a LogP of less than 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and molar refraction should be between 40-130 (Lipinski et al., 2012). Chlorogenic

acid is known to have more than 5 hydrogen bonds, to be exact 6 hydrogen bonds and this makes chlorogenic acid an active compound that does not meet the lipinski five rules to be used as a drug candidate.

Table 4.
Analysis of Lipinski's five rules

Type	Chlorogenic acid	Modified compounds
Mass (Dalton)	354	286
hydrogen bond donor	6	4
hydrogen bond acceptors	9	6
LOGP	-0.64	0.51
Molar Refractivity	82.51	80.53

Molecular docking of the BACE1 Protein

Molecular docking analysis showed that chlorogenic acid binds to 5 types of amino acids with a bond energy value of -5.86 kcal/mol, however, chlorogenic acid does not bind to 2 specific amino acids in BACE1 which play a role in forming amyloid plaques. The modified compounds show different results, the modified compounds show bonds with 3 types of amino acids, the bond energy of the modified compounds is -9.69 kcal/mol, and binds specifically to ASP A:32 and ASP A:228 which are specific amino acids that play a role on amyloid plaque formation [30]. When compared with natural ligands, the types of amino acids bound by modified compounds are also bound by natural ligands as shown in Figure 2.

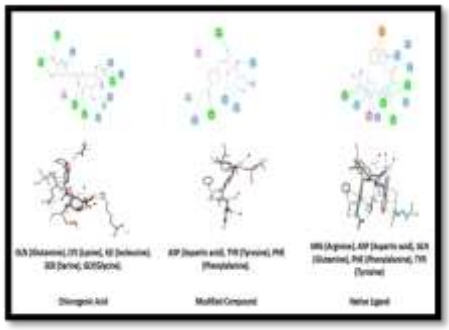

Figure 3. Ligand interaction analysis on BACE1 protein

ASP or aspartic acid (aspartic residue) is an amino acid residue that is known to coordinate the cleavage of the APP molecule, in the active site, aspartate residues (ASP A:32 and ASP A:228) cleave APP through hydrogen boding facilitated by water molecules (Zhu et al., 2010). Based on these reasons, this study carried out a molecular docking process while retaining water molecules. Docking without a water molecule was also tried, but the resulting bond was in accordance with the prediction that none of the functional groups are attached to the aspartate residue as shown in Figure 3.

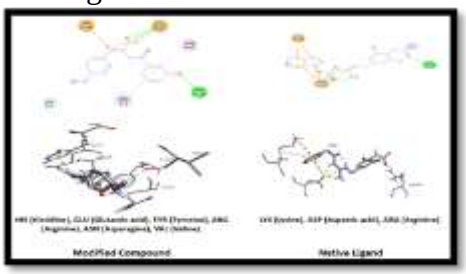

Figure 4. Ligand interaction analysis on BACE1 protein without water molecules.

The modified compounds were examined using several databases including Google Patents, Chemicalize, PubChem, ChEMBL, and ChEBI. Based on the results of the search conducted, until March 19, 2023, there were no compound structures that had similarities to the modified chlorogenic acid compound structures. Chlorogenic acid which is known to have known immunotoxicity cannot be developed as a drug, because to register an active substance as a drug one of the requirements that must be carried out is to carry out an immunotoxicity study (ICH, 2011). Therefore, modified compounds can be said to be potentially superior when comparing the toxicity possessed by both. Chlorogenic acid does show poorer absorption and bioavailability compared to the modified compounds, but these two compounds score very well on other parts because both are not inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 which are members of the cytochrome P450 superfamily enzymes.

Members of the cytochrome P450 superfamily enzymes are known to be very important for the body, especially in the process of drug metabolism. It is known that drugs that block the CYP enzyme pathway can increase the levels of other drugs metabolized through the same pathway, potentially causing the drug to stay in the body longer and increasing drug toxicity (Denison et al., 2023; Xing et al., 2023). The modified compound is known to have a binding energy of -8.51 kcal/mol in BACE1 protein which has water molecules removed, lower than the results of docking BACE1 protein which has water molecules. It is known that modified compounds and natural ligands do not bind to aspartate residues in interactions with BACE1 proteins that do not involve water molecules, based on this it is known that water molecules affect the interaction between ligands and the active site of the BACE1 protein. The structure of the modified compound and chlorogenic acid is known to have an insignificant position with the aspartate residue when the molecular docking process is carried out while retaining water molecules as shown in Figure 4.

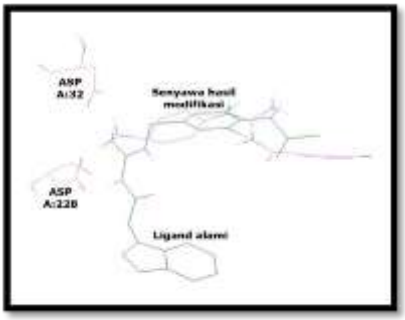

Figure 5. Ligand interaction position on BACE1 protein docked with water molecule

The modified chlorogenic acid does have potential as a BACE1 inhibitor, but this still needs to be confirmed by various other molecular docking methods including using various software for comparison. The molecular docking process also does not at all prove that the modified compound can actually be used as a BACE1 inhibitor, the synthesis, and evaluation in vitro and in vivo will prove that the predicted results of molecular docking are truly effective as BACE1 inhibitors including proving various types of toxicity.

CONCLUSION

The modified compound has the potential to be better than chlorogenic acid in terms of pharmacokinetics, bioactivity, and potential as a BACE1 inhibitor through in silico evaluation. The modified compound also complies with Lipinski's five rules, which means that the compound has the potential to be developed and get further evaluation as an Alzheimer's drug candidate with lower toxicity when compared to chlorogenic acid.

REFERENCES

Alam, M. K., Farhad, M., Chakrabarty, N., Montasir, S., Kabir, M. S. H., & Rahman, M. M. (2016). Molecular Docking for Identification of Novel Potential BACE1 Inhibitors for Alzheimer's Disease Treatment of Some Isolated Compounds from Spondias pinnata.

Association, A. (2019). Alzheimer's disease facts and figures. Alzheimer's & Dementia, 15(3), 321–387.

Bertol, E., & Favretto, D. (2023). Pharmacokinetics: Drug Absorption, Distribution, and Elimination. In Karch's Drug Abuse Handbook (pp. 133–178). CRC Press.

Bhat, V., & Chatterjee, J. (2021). The use of in silico tools for the toxicity prediction of potential inhibitors of SARS-CoV-2. Alternatives to Laboratory Animals, 49(1–2), 22–32.

Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. Annual Review of Plant Biology, 54(1), 519–546.

Cosconati, S., Forli, S., Perryman, A. L., Harris, R., Goodsell, D. S., & Olson, A. J. (2010). Virtual screening with AutoDock: theory and practice. Expert Opinion on Drug Discovery, 5(6), 597–607.

Denison, M., Ahrens, J. J., Dunbar, M. N., Warmahaye, H., Majeed, A., Turro, C., … Kodanko, J. J. (2023). Dynamic Ir (III) Photosensors for the Major Human Drug-Metabolizing Enzyme Cytochrome P450 3A4. Inorganic Chemistry.

Elbouzidi, A., Ouassou, H., Aherkou, M., Kharchoufa, L., Meskali, N., Baraich, A., … Hano, C. (2022). LC–MS/MS Phytochemical Profiling, Antioxidant Activity, and Cytotoxicity of the Ethanolic Extract of Atriplex halimus L. against Breast Cancer Cell Lines: Computational Studies and Experimental Validation. Pharmaceuticals, 15(9), 1156.

Gogoi, B., Chowdhury, P., Goswami, N., Gogoi, N., Naiya, T., Chetia, P., … Borah, P. (2021). Identification of potential plant-based inhibitor against viral proteases of SARS-CoV-2 through molecular docking, MM-PBSA binding energy calculations and molecular dynamics simulation. Molecular Diversity, 25, 1963–1977.

Gupta, R. C. (2015). Handbook of toxicology of chemical warfare agents. Academic Press.

Hassan, M., Shahzadi, S., Seo, S. Y., Alashwal, H., Zaki, N., & Moustafa, A. A. (2018). Molecular docking and dynamic simulation of AZD3293 and solanezumab effects against BACE1 to treat Alzheimer's disease. Frontiers in Computational Neuroscience, 12, 34.

Hussain, I., Powell, D., Howlett, D. R., Tew, D. G., Meek, T. D., Chapman, C., … Ryan, D. M. (1999). Identification of a novel aspartic protease (Asp 2) as β-secretase. Molecular and Cellular Neuroscience, 14(6), 419–427.

ICH, I. C. H. (2011). S6 (R1), Addendum to ICH S6 guideline: Preclinical Safety Evaluation of Biotechnology-Derived Biopharmaceuticals. Brussells, Belgium, June.

Li, S., Liu, L., & Selkoe, D. (2019). Adverse Neurologic Effect of BACE1 inhibitors in Alzheimer's disease trials. The New England Journal of Medicine, 381(4), 388.

Novak, G., Streffer, J. R., Timmers, M., Henley, D., Brashear, H. R., Bogert, J., … Tritsmans, L. (2020). Long-term safety and tolerability of atabecestat (JNJ-54861911), an oral BACE1 inhibitor, in early Alzheimer's disease spectrum patients: a randomized, double-blind, placebo-controlled study and a two-period extension study. Alzheimer's Research & Therapy, 12(1), 1–16.

Ochiai, R., Saitou, K., Suzukamo, C., Osaki, N., & Asada, T. (2019). Effect of chlorogenic acids on cognitive function in mild cognitive impairment: a randomized controlled crossover trial. Journal of Alzheimer's Disease, 72(4), 1209–1216.

Pantaleão, S. Q., Fernandes, P. O., Gonçalves, J. E., Maltarollo, V. G., & Honorio, K. M. (2022). Recent advances in the prediction of pharmacokinetics properties in drug design studies: a review. ChemMedChem, 17(1), e202100542.

Ranjith, D., & Ravikumar, C. (2019). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Ipomoea mauritiana Jacq. Journal of Pharmacognosy and Phytochemistry, 8(5), 2063–2073.

Sinha, S., Anderson, J. P., Barbour, R., Basi, G. S., Caccavello, R., Davis, D., … Hong, J. (1999). Purification and cloning of amyloid precursor protein β-secretase from human brain. Nature, 402(6761), 537–540.

Skovronsky, D. M., Moore, D. B., Milla, M. E., Doms, R. W., & Lee, V. M.-Y. (2000). Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-β precursor protein in the trans-Golgi network. Journal of Biological Chemistry, 275(4), 2568–2575.

Turana, Y., Tengkawan, J., Suswanti, I., Suharya, D. Y., Riyadina, W., & Pradono, J. (2019). Primary prevention of Alzheimer's disease in Indonesia. International Journal of Aging Research, 2(3), 40.

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., … Loeloff, R. (1999). β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science, 286(5440), 735–741.

Vetrivel, K. S., & Thinakaran, G. (2010). Membrane rafts in Alzheimer's disease beta-amyloid production. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1801(8), 860–867.

Wessels, A. M., Tariot, P. N., Zimmer, J. A., Selzler, K. J., Bragg, S. M., Andersen, S. W., … Willis, B. A. (2020). Efficacy and safety of lanabecestat for treatment of early and mild Alzheimer disease: the AMARANTH and DAYBREAK-ALZ randomized clinical trials. JAMA Neurology, 77(2), 199–209.

Xing, Y., Yu, Q., Zhou, L., Cai, W., Zhang, Y., Bi, Y., … Han, L. (2023). Cytochrome P450-mediated herb-drug interaction (HDI) of Polygonum multiflorum Thunb. based on pharmacokinetic studies and in vitro inhibition assays. Phytomedicine, 112, 154710.

Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tory, M. C., Pauley, A. M., … Buhl, A. E. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. Nature, 402(6761), 533–537.