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RESEARCH ARTICLE

In-silico approaches in designing new drug candidates (THICAPA and POET) for alzheimer's disease

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Abstract

Background: Memory and cognitive regression are the first symptoms of Alzheimer's Disease (AD), which may progress to speech and mobility challenges which affecting around 35% of those who over the age of 80 years old. Preliminary study shows THICAPA and Palm Oil Extracted Tocotrienol (POET) is effective in reducing AD symptoms in Dorosophila melagnoster. **Objective:** The purpose of this study is to elucidate the binding interaction between THICAPA and POET towards APP and PS1 at the molecular level. **Method:** The binding of THICAPA and POET towards APP (PDB ID: 6SZF), PS1 (PDB ID: 7D8X), and their genetic mutation variations (APP variant n = 6, PS1 variant n = 200) have been studied using in-sillico molecular docking (Autodock 4.2) approaches and comparing the Binding Free Energy (BFE) of the binding interactions. **Result:** From the 416 dockings (n = 100 per docking, ∑n = 41,600), the authors revealed that all dockings had negative BFE which showed the low BFE towards both APP (E22K variant ΔG THICAPA = -6.20 kcal/mol, D23N variant ΔG POET = -7.25 kcal/mol) and PS1 (A413V variant ΔG THICAPA = -8.34 kcal/mol, L174M variant ΔG POET = -10.94 kcal/mol). **Conclusion:** THICAPA and POET showed a negative BFE with APP and PS1. Thus, the result suggesting that THICAPA and POET may be the potential drug candidates for treating AD.

Introduction

Alzheimer's Disease (AD) is a degenerative neurological disorder characterised by a progressive deterioration in cognitive and memory functions, which is subsequently accompanied by alterations in behaviour, speech, neuropsychiatric symptoms, and motor abilities (Anand *et al*., 2014; Deture & Dickson, 2019). According to the World Health Organization (WHO) report of 2022, it has been anticipated that roughly 260,000 individuals in Malaysia, including 8.5% of the older adult population, are afflicted by Dementia. According to a report by Alzheimer's Disease International in 2014, the estimated number of individuals diagnosed with AD in 2015 was 123,000. Projections indicate that this Figure is expected to rise to 261,000 by 2030 and grow to 590,000 by 2050.

The primary pathological mechanism behind AD is the development of amyloid-beta (Aβ) plaques, which subsequently leads to neuronal death. The findings from genetic investigations have indicated that mutations in the genes APP and PS1 are associated with developing a hereditary variant of AD known as familial AD (FAD). Mutation of the amyloid precursor protein (APP) and Presenilin 1 (PS1) genes has been observed, causing an extended cleavage of APP. Creating Aβ plaques is attributed to the aggregation of longer Aβ peptide synthesis and insoluble Aβ, accelerating the deposition process and promoting plaque formation.

Therefore, preventing or inhibiting Aβ aggregation is paramount in treating AD.

There is increased interest in combination medicines and innovative approaches to AD treatment due to the complexity of AD and the uneven effectiveness of single-drug therapies. The proposed therapeutic approach involves inhibiting or blocking the interaction between APP and the inhibition of PS1. In preliminary tests, two substances exhibited intriguing neuroprotective properties, namely 1,2,3,4-tetrahydroisoquinoline-3-carbonyl (THICAPA) and palm oil extract tocotrienol (POET). The chemicals were tested using *Drosophila* sp., which serves as a model organism for the research of Alzheimer's disease.

The previous findings indicate that both THICAPA (Tan *et al*., 2023) and POET (Leow *et al*., 2021) possess the capacity to decelerate cellular ageing and manifest neuroprotective properties, rendering them viable candidates for the treatment of Alzheimer's disease. Given the preliminary nature of the findings, it is imperative to conduct additional research and rigorous clinical studies to establish the safety and efficacy of THICAPA and POET as prospective therapy options for Alzheimer's disease (AD). Furthermore, this study aims to elucidate the binding interaction between THICAPA and POET towards APP and PS1 at the molecular level.

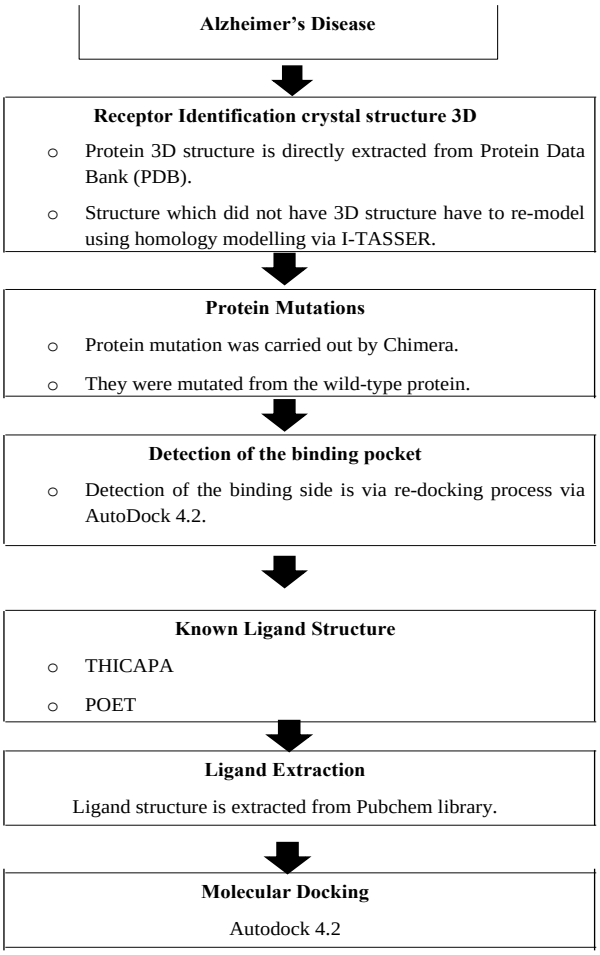

Figure 1: Research outline protocol

Methods

Design

This study utilised the Computer Aided Drug Design (CADD), such as molecular docking, to elucidate the binding interaction of THICAPA and POET towards APP and PS1 and their variant mutations. The research outline protocol is summarised in Figure 1.

Protein preparation

The native structure of APP (PDB ID: 6SZF) and PS1 (PDB ID: 7D8X) were retrieved from the Protein Data Bank (PDB), and they were mutated accordingly to their variant mutation for the docking process. The Chimera 1.14 (Pettersen *et al*., 2004) software was employed to introduce system mutations. However, only mutations involving replacement or substitution were considered in this study to maximise the consistency of the 3D model structure through all the mutations. Other mutations, such as deletion, addition, translocation, duplication, and insertion, will involve restructuring the whole protein; thus, they are not included in this study.

Molecular docking

The extracted proteins were cleaned using Biovia Discovery Studio 16.1 (BIOVIA, 2019). The proteins were then subjected to protonation and optimising the hydrogen network using the Playmolecule web server (Martínez-Rosell *et al*., 2017).

This study used three ligands: POET, THICAPA, and FTO (control). THICAPA (Figure 2) and POET (Figure 3) were sketched and subjected to energy minimisation (by MMS forcefield) using PerkinElmer Chem3D 17.1. Meanwhile, FTO was maintained in its original conformation from the crystal structure (Figure 4).

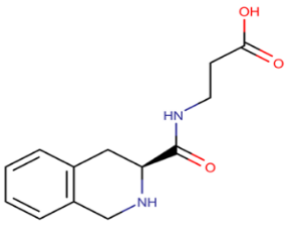

Figure 2: Chemical structure of THICAPA

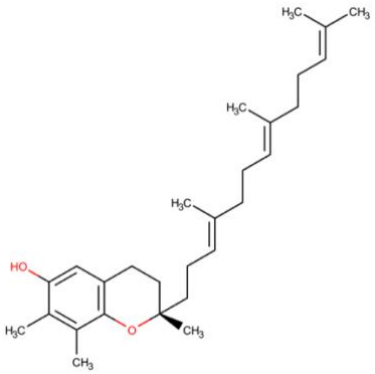

Figure 3: Chemical structure of Palm Oil Extracted Tocotrienol (POET) (γ).

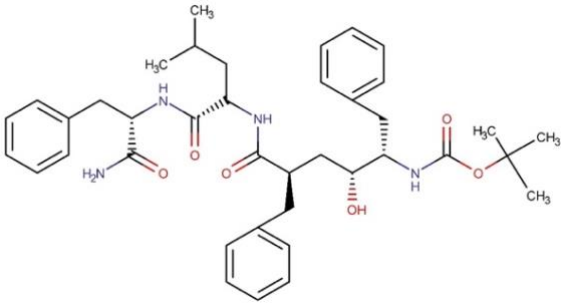

Figure 4: Chemical structure of control (FTO)

The docking parameters were validated by re-docking the control PS1 complex with the co-crystallized ligand FTO using AutoDock 4.2 (Morris *et al*., 2009). The coordinates from the re-docking process are 164.22, 174.11, and 148.43 (x, y, and z, respectively). Redocking of the Control produced RMSD ≤ 1.99 Å. An RMSD of ≤ 2.00 indicates successful and reproducible docking parameters (Gohlke *et al*., 2000; Mena-Ulecia *et al*., 2015). Thus, these parameters were applied in the docking of POET and THICAPA to the wild type and mutants of PS1.

Results

Amyloid Precursor Protein (APP)

THICAPA

Table I shows the docking result of THICAPA to the APP variants. They are E22K, D23N, E22Q, A21G, WT, and E22G (Figure 5). They are evaluated based on the low Binding Free Energy (BFE) from the docking process

Table I: The docking result of THICAPA towards APP

Mutation code	Binding free energy (kcal/mol)
E22K	-6.20
D23N	-6.06
E22Q	-5.89
A21G	-5.86
WT	-5.82
E22G	-5.65

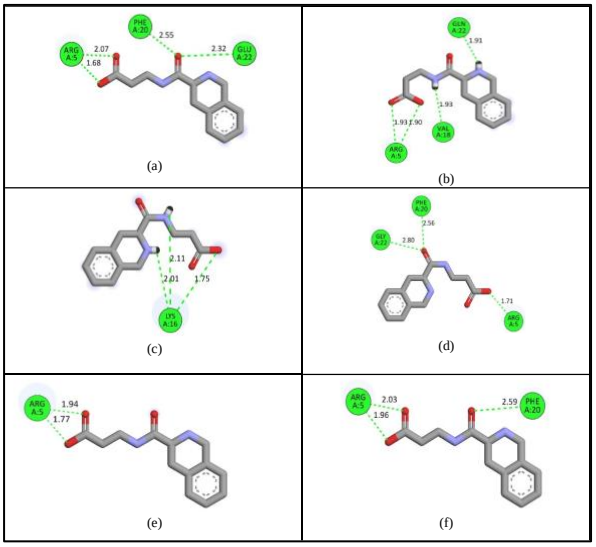

Figure 5: The 2D binding interaction of THICAPA with APP. (a)Wild-type (b) E22Q (c) E22K (d) E22G € D23N (f) A21G.

POET

Table II displayed the docking result of POET to the APP. Figure 6 showed binding interaction of D23N, E22Q, WT (wild type), E22G, A21G and E22K. They are evaluated based on the low BFE from the docking process.

Table II: The docking result of POET towards APP

Mutation code	Binding free energy (kcal/mol)
D23N	-7.25
E22Q	-7.18
WT	-6.91
E22G	-6.90
A21G	-6.65
E22K	-6.62

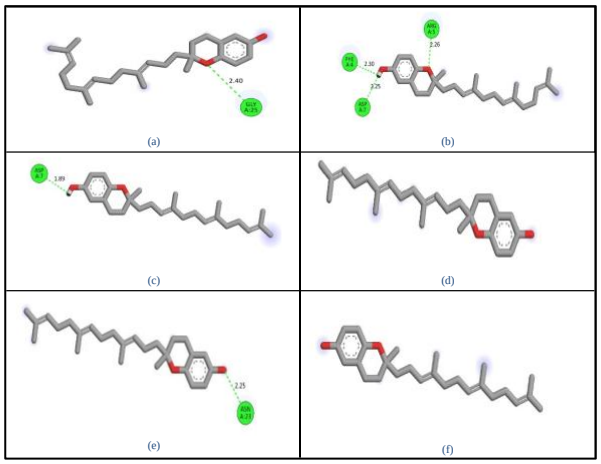

Figure 6: The 2D binding interaction of POET with APP. (a)Wild-type (b) E22Q (c) E22K (d) E22G (e) D23N (f) A21G

Presenilin 1 (PS1)

THICAPA

The docking outcomes of THICAPA with the WT and mutant types of PS1 are showed in Table III. The mutants include A431V, A434T, L282F, T116S-P117T, Y159F, A285S, L113P, G209A, I299F, and L235R (Figure 7). They were ranked based on the ten lowest FBE from 201 dockings.

Table III: The docking result of THICAPA towards PS1

Mutation code	F.B.E (kcal/mol)
WT	-8.87
L174M	-10.94
A431V	-10.93
V261F	-10.85
V261I	-10.83
L166P	-10.82
V272A	-10.79
M135S	-10.73
L173F	-10.72
S170F	-10.70
V261L	-10.70

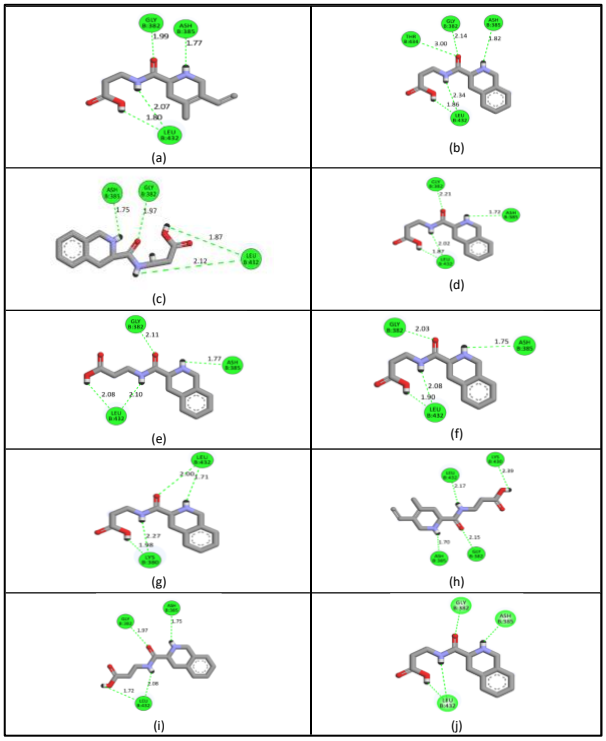

Figure 7: The 2D binding interaction of THICAPA with PS1. (a)A431V (b) A434T (c) L282F (d) T116S-P117T (e) Y159F (f) A285S (g)L113P, (h) G209A (i) I299F (j) L235R

POET

The docking result for the ten mutations with the lowest BFE when considering POET's interaction with PS1 is shown in Table IV. These encompass WT, L174M, A431V, V261F, V261I, L166P, V272A, M135S, L173F, S170F and V261L (Figure 8). They were ranked based on the ten lowest FBE from 201 dockings.

Table IV: The docking result of POET towards PS1

Mutation code	F.B.E (kcal/mol)
WT	-8.05
A431V	-8.34
A434T	-8.11
L282F	-8.10
T116S-P117T	-8.10
Y159F	-8.10
A285S	-8.09
L113P	-8.09
G209A	-8.09
I299F	-8.09
L235R	-8.09

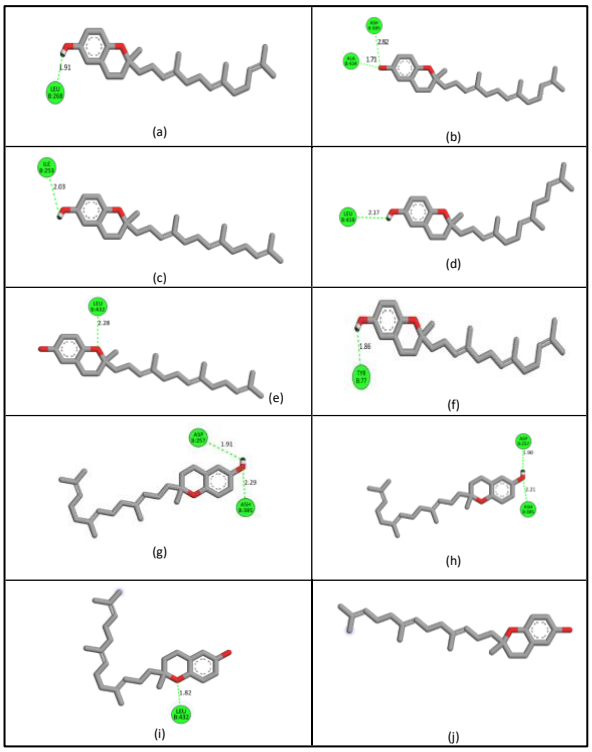

Figure 8: The 2D binding interaction of POET with PS1. (a) L174M (b) A431V (c) A261F (d) A261I (e) L166P (f)V272A, (g) M135S (h)L173F (i) S170F (j)V261L.

Discussion

The objective of the docking simulation is to provide a comprehensive understanding of the binding interaction between THICAPA and POET with PS1.

Amlyloid Precursor Protein (APP)

A novel avenue emerges by targeting the β-cleavage site of APP, offering a potential strategy to inhibit Aβ production without the risk associated with BACE1 inhibition. Monoclonal antibodies and proteins that bind to the β-cleavage site of APP have demonstrated similar approaches. APP dimerisation is vital for its processing, involving glycine-XXX-glycine (GXXXG) motifs in transmembrane helices that facilitate protein dimerisation. These motifs overlap with cholesterol-binding domains, and *in vitro* cholesterol binding to APP obstructs dimerisation.

Table I displayed the docking result of THICAPA to the APP variants. E22K has the lowest BFE at -6.2 kcal/Mol, followed by D23N (6.06 kcal/mol), E22Q (5.89 kcal/mol), A21G (-5.86 kcal/mol), WT (-5.82 kcal/mol) and E22G at -5.65 kcal/mol. These values encapsulate the strengths of the interactions between THICAPA and the different APP variants, with E22K exhibiting the most energetically favourable binding and other variants in descending order. The interaction pictures of the variants and THICAPA are displayed in Figure 5.

Table II presents the docking outcome of POET to the amyloid precursor protein (APP) and its mutations. The data demonstrates that variation D23N has the most negative BFE value, measuring -7.25 kcal/Mol. The subsequent values are as follows: E22Q at -7.18 kcal/Mol, WT (wild type) at -6.91 kcal/Mol, E22G at -6.9 kcal/Mol, A21G at -6.65 kcal/Mol, and E22K at -6.62 kcal/Mol, in that order. The aforementioned values represent the alterations in energy that occur during the process of binding, so revealing the relative potency of the interactions between POET and the different mutations found in the APP variation. The interaction pictures of the variants and THICAPA are displayed in Figure 6.

The molecular docking of THICAPA and POET to APP might give an insight into how THICAPA and POET interact with APP for drug targeting strategy. POET and THICAPA showed the negative BFE from Tables I and II. The negative BFE indicates that both ligands successfully form the stable complex with APP by finding the preferred orientation with the lowest BFE possible (Tripathi & Misra, 2017).

The binding of THICAPA and POET to APP results in structural alterations of the APP molecule. The alteration in the structure hinders the binding of APP within the catalytic region. The elucidation of this phenomenon can be enhanced by applying the theoretical frameworks of lock and key enzyme theories, as first described by Emil Fischer. According to VanEtten *et al*. (1967), the author posited that the interaction between a substrate and an enzyme can be likened to how a key fits into a lock. According to Kumar *et al*. (2020), for the enzyme to exhibit its catalytic activity, the substrate must maintain the same conformation within the binding site. The interaction of POET and THICAPA induces conformational changes in APP structure, thereby inhibiting APP cleavage by PS1.

However, despite the protein mutations, THICAPA and POET can still maintain their ability to establish binding interactions with APP and its various mutated forms. The strong hydrogen bonds that successfully formed between APP and THICAPA or POET are the key which holds the binding interactions. Although no specific coordinates or amino acids are targeted on APP, the binding of THICAPA and POET to the APP will disrupt the original structure of APP in the catalytic pocket. This binding ability underscores their potential as effective therapeutic agents across genetic contexts.

Presenilin 1 (PS1)

This study employed a cross-competition kinetic analysis to investigate the binding properties of inhibitors and ligands concerning γ-secretase. The aim was to determine whether inhibitors with non-transition state non-competitive characteristics hinder the activity of γ-secretase by binding to its catalytic site. Previous studies by Tagami *et al*. (2017) and Tian *et al*. (2003) have also identified these as non-competitive γ-secretase inhibitors. The docking procedure was initiated by utilising the initial coordinates obtained from this work, which were compared to earlier studies on the binding of inhibitors and ligands in a competitive manner (Tagami *et al*., 2017; Tian *et al*., 2003). The aforementioned coordinates were subsequently employed to dock the THICAPA and POET modules within the PS1 facility. The binding coordinates between transmembrane helix 6 (TM6) and transmembrane helix 7 (TM7) have garnered significant attention owing to their crucial role in the catalytic activity of γ-secretase.

The objective is to establish the potential binding interaction that hinders the cleavage of APP by obstructing the presenilin through the docking of THICAPA and POET. The negative values of BFE in kcal/mol were observed in Table III (PS1-THICAPA) and Table IV (PS1-POET). When a drug molecule forms a bond with a target, it liberates binding energy, reducing the overall energy of the complex due to a decrease in the binding energy (Du *et al*., 2016).

The docking results of THICAPA with both the wild-type (WT) and mutant forms of PS1 are presented in Table III, while the picture of their 3D interactions is shown in Figure 7. The identified mutant variants encompass A431V, A434T, L282F, T116S-P117T, Y159F, A285S, L113P, G209A, I299F, and L235R. Among the 201 docked variants, A431V demonstrated the most favourable binding energy of -8.34 kcal/mol, while the wild-type (WT) exhibited a binding energy of 8.05 kcal/mol. The subsequent values are as follows: -8.11 kcal/mol for the A434T mutation, -8.1 kcal/mol for the L282F mutation, -8.1 kcal/mol for the T116S-P117T mutation, -8.1 kcal/mol for the Y15F mutation, -8.09 kcal/mol for the A285S mutation, -8.09 kcal/mol for the L113P mutation, -8.09 kcal/mol for the G209A mutation, -8.09 kcal/mol for the I299F mutation, and -8.09 kcal/mol for the L235R mutation.

The docking outcome for the ten mutations exhibiting the lowest binding energy, considering the interaction between POET and PS1, is presented in Table IV. In contrast, the picture of their 3D interactions is shown in Figure 8. The variants under consideration are L174M, A431V, V261F, V261I, L166P, V272A, M135S, L173F, S170F, and V261L. Among the analysed variants, L174M exhibited the lowest binding energy of -10.94 kcal/mol. This was followed by A431V (-10.93 kcal/mol), V261F (-10.85 kcal/mol), V261I (-10.83 kcal/mol), L166P (-10.82 kcal/mol), V272A (-10.79 kcal/mol), M135S (-10.73 kcal/mol), and L173F (-10.72 kcal/mol). On the other hand, both S170F and V261L displayed the highest binding free energy (BFE) at -10.7 kcal/mol. The findings presented in this study provide insights into the binding affinities between different mutation variants and THICAPA during their interaction with PS1. The docking interactions between PS1 with THICAPA and POET are shown in Figures 7 and 8. The successful hydrogen bonds formed, making THICAPA and POET, occupy the catalytic pocket in PS1, especially at amino acids ASP 257 and ASP 385.

The diverse range of binding energies provides valuable information regarding potential disparities in binding affinities, hence providing insights into the effects of these mutations on interactions between drugs and proteins. A comprehensive comprehension of these variations is crucial for developing pharmaceuticals that target specific mutations and augment treatment approaches.

Conclusion

The findings of this study indicate that the catalytic aspartates in PS1 can potentially be obstructed by the binding of THICAPA and POET, leading to a reduction in amyloidogenic cleavage. The APP can also serve as the specific location for drug binding, as this binding event induces a conformational change in APP, leading to the catalytic site for β-secretase. The current findings of this study align with the initial investigation, indicating that THICAPA and POET exhibit efficacy in mitigating AD in *Drosophila sp*. In summary, the thorough research reported in this study enhances our understanding of the potential effectiveness of THICAPA and POET as treatments for AD, considering different genetic variants. The aforementioned observations can enhance therapeutic approaches for AD, potentially resulting in more efficient and focused intervention.

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