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Research Article

Computational Studies of Donepezil and Acetylcholinesterase Molecular Dynamics Interactions

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ABSTRACT

Donepezil is an acetylcholinesterase (AChE) inhibitor commonly used as an anti-dementia medication for patients with Alzheimer's Disease. This research aims to determine the stability of each enantiomer *R* and *S* of donepezil as an AChE inhibitor using molecular dynamics (MD) simulations. The MD simulations were performed on a Cloud Protein Simulator (CPS) with YASARA-Structure as the main software and PyPLIF HIPPOS as the software to identify protein-ligand interactions. The MD simulations were performed in three replications of 50 ns for each enantiomer complexed to AChE. The stabilities of the AChE-donepezil complexes were analyzed by assessing the root-mean-squared deviation (RMSD) values of the AChE backbone atoms (RMSDBb) and the ligand (RMSDLigMov) during the last 5 ns of simulation time. The dynamics of the protein-ligand interactions were also analyzed during the last 5 ns. The results of these MD simulations indicate that the *S*-donepezil serves as a better AChE inhibitor compared to the *R*-donepezil.

INTRODUCTION

Alzheimer's Disease (AD) is one of the brain disorders characterized by the decline in a person's attention, memory, and personality. Cognitive function in patients with AD does not disappear suddenly but begins with a decrease in attention and memory, gradually affecting personality changes (Breijyeh and Karaman, 2020). Based on current epidemiological research data, AD affects over 50 million patients worldwide. This number is projected to double every 20 years and reach approximately 140 million by 2050 in the global population (Breijyeh and Karaman, 2020; Liang *et al*., 2021).

Basically, AD is one of the consequences of acetylcholinesterase dysfunction. In this case, acetylcholinesterase (AChE) is an enzyme that catalyzes acetylcholine (ACh) into inactive forms, namely acetate and choline. Measurement of AChE enzyme activity can reflect the accumulation of ACh in the body, where the results indicate higher AChE enzyme activity in patients with AD. Compounds that can inhibit AChE enzyme activity suggest their potential as a fundamental basis for developing drugs for the treatment of AD (Kitphati *et al*., 2012).

Donepezil is a leading compound previously discovered for treating AD and has been used in over 50 countries (Cacabelos, 2007). Compared to other conventional acetylcholinesterase inhibitors (AChEIs), donepezil is a highly selective and reversible piperidine derivative with AChEI activity, showing the best pharmacological profile in terms of cognitive enhancement (40%-58%) and side effects (6%-13%) in patients with AD (Cacabelos, 2007). So far, donepezil used for the indication of AD only provides symptomatic benefits in terms of cognitive function improvement and has not been proven to slow down or halt the progression of AD (Dou *et al*., 2018).

Donepezil is widely marketed as a racemate consisting of *R*-donepezil and *S*-donepezil (Lili *et al*., 2013). From the previous research, *R*-donepezil and *S*-donepezil may have different stability in our body. Accordingly, understanding their relative activities is crucial

in determining the therapeutic potential and efficacy of the drug (Lili *et al*., 2013). In some cases, one enantiomer of a drug may be more effective or better tolerated in certain patient populations due to genetic or physiological differences (Lu *et al.,* 2015). Therefore, more efforts are needed to further explore the functional properties of *R*-donepezil and *S*-donepezil, particularly in the context of computational-based development of targeted medicines.

The initial exploration through molecular dynamics (MD) simulations and protein-ligand interaction studies with the AChE target is expected to provide insights at the molecular level to enhance the quality of structure-based virtual screening in Alzheimer's drug discovery. There have been no previous studies revealing the essential interactions of each dimer of donepezil, both from *S*-donepezil and *R*-donepezil. To fill this gap in our knowledge of AD treatments, this research aimed to focus on investigating the essential interactions of each donepezil with AChE as the protein target during a series of 50 ns MD simulations.

METHODS

Materials and Instrumentations

The crystal structure of AChE (1E66.pdb) was obtained from Protein Data Bank (PDB) with the following link; https://www.rcsb.org/structure/1E66) and the three-dimensional (3D) structure of *R*-donepezil and *S*-donepezil were obtained from https://pubchem.ncbi.nlm.nih.gov/. The MD simulations were performed using the Protein Cloud Simulator (CPS) Server, Gold version (a private online server with 8 CPUs - Intel® Xeon® CPU E5-2680 v3 @ 2.50GHz), 7 and 16 GB RAM, with UBUNTU 18.04.6 LTS as the operating system, with YASARA-Structure 22.8.22 and PyPLIF HIPPOS as the additional software. Additionally, a personal computer (PC) with Windows 10 Pro operating system and YASARA-Structure 22.8.22 installed was used for visualization and to control the CPS. Additional plugins for YASARA-Structure for Windows namely molmod.id plugins were obtained from https://molmod.id/yasara/molmod-yasara-plugins.zip.

Procedures

The main structure of AChE with the PDB of 1E66 was inputted into YASARA-Structure 22.8.22 with SeqRes settings activated in the YASARA window. The SeqRes identification results indicate that the missing residues in the N-terminal were D, D, and H, and the missing residues in the C-terminal were A, C, D, G, E, L, S, and S. The loop building modules in YASARA were used to complete the missing residues. Then, the following commands were executed subsequently: Hydrogens were correctly added at the physiological pH setting the pH value to 7.4, and a complete clean-up (CleanAll) was performed. To perform the energy minimization experiment, a simulation cell was constructed in a cubic shape, with a distance of 5 Å around all atoms with the Force Field was set to AMBER14. The molecular docking validation was conducted 100 times with the co-crystal ligand Huprine-X (HUX) using the molmod.id plugins, with estimation docking time 1 minute per complex. Configuration optimization was performed to obtain root-mean-square deviation (RMSD) values for docking poses less than 2.0 Å in at least 95% of the re-docking simulations (Istyastono, 2021). The enantiomer structures of *R*-donepezil and *S*-donepezil were collected from PubChem. The available structures were docked 100 times onto the virtual target file using a validated docking protocol based on previous stages. The best docking results from each molecular docking were used as input for molecular dynamics simulations. Preparation settings were performed on the CPS to prepare for the MD simulations process of the AChE-donepezil complex for 50 ns at a temperature of 310 K (Istyastono and Riswanto, 2022). The MD simulation was configured with snapshots taken at every 100 ps interval. The MD simulations were done for 3 representatives of the best poses from each enantiomer. The MD simulations and interaction analyses were also conducted on 3 out of 100 re-docking simulations of the co-crystal ligand.

The stability of the AChE-ligand complexes was analyzed by calculating the RMSD values of the backbone (RMSDBb) and the RMSD of the ligand movement (RMSDLigMov) at every 5 ns. If the ΔRMSDBb and the ΔRMSDLigMov are ≤2 Å for 5 ns within the initial 10 ns of the production run, the complex is considered stable (Liu *et al*., 2017; Liu and Kokubo, 2017). The last 5 ns snapshots generated from the simulation were analyzed using PyPLIF HIPPOS (Istyastono *et al*., 2020) in the form of a bitstring and ΔG (kcal/mol) analysis. This is achieved by calculating the percentage of interactions from the bitstring that can be considered dominant, with a threshold of ≥87.8% (Riswanto *et al*., 2017). The calculation of ΔG (kcal/mol) values were performed

referring to the previous research conducted by Prasasty and Istyastono (2020).

RESULTS AND DISCUSSION

The research presented in this article demonstrated the stability of the potent inhibitor donepezil and its respective enantiomers against the acetylcholinesterase enzyme (AChE). Table 1 and Figure 1 show the RMSDbb and RMSDLigMov values of the 3 complexes of AChE-HUX, 3 complexes of AChE-*S*-donepezil complexes, and 3 complexes of AChE-*R*-donepezil complexes, respectively.

The initial AChE-*S*-donepezil complexes were the best three complexes in terms of the RMSD value in the docking simulations, while the initial AChE-*R*-donepezil were the best two complexes and another with the highest RMSD value to sample more plausible poses. Based on the results of the 100 times docking performed on *R*-donepezil to AChE, several binding poses were found that yielded RMSD values around ±9 Å. From those results, one pose was selected to examine the stability of the ligand when binding to the AChE binding pocket.

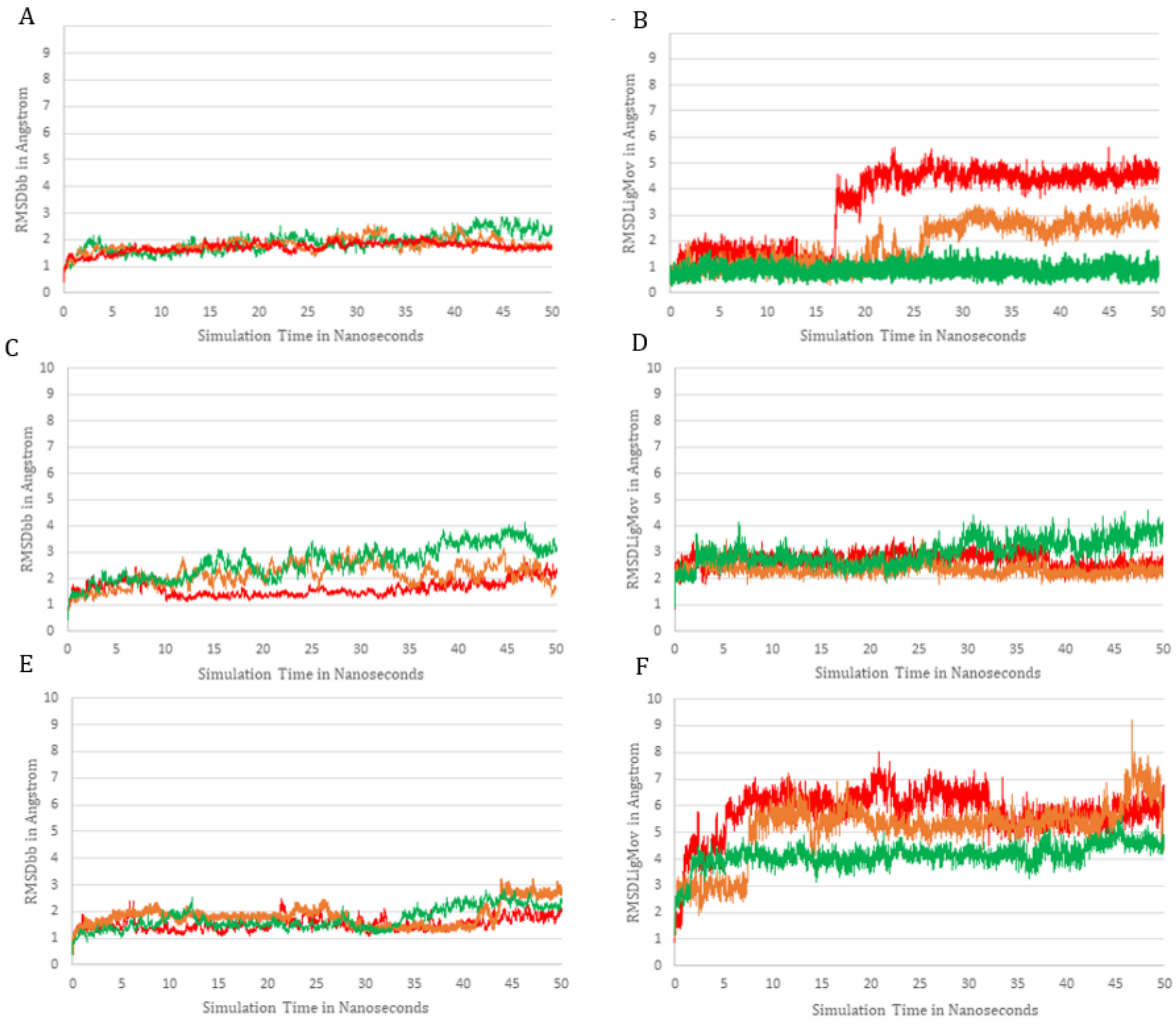

Figure 1. The time (ns) vs RMSD (Å) graph of the AChE backbone atoms (left) and ligand movement (right) was plotted for the 0-50 ns duration, comparing the molecular dynamics simulations of AChE and Huprine X (A and B), S-donepezil (C and D) and R-donepezil (E and F). As a note Huprine X 1, S-donepezil 1 and R-donepezil 1 plotted in red; Huprine X 2, S-donepezil 2 and R-donepezil 2 plotted in orange; Huprine X 3, S-donepezil 3 and R-donepezil 3 plotted in green.

Table 1. ΔRMSD back bone (ΔRMSDbb) and ligand movement (ΔRMSDLigMov) of AChE and 3 selected poses of selected ligands on the last 5 ns of simulations

MD Simulations Complex	ΔRMSDbb (Å)	ΔRMSDLig Mov (Å)
AChE-Huprine X 1	0.332	1.469
AChE-Huprine X 2	0.535	1.591
AChE-Huprine X 3	0.91	1.233
AChE- *S*-donepezil 1	0.768	1.059
AChE- *S*-donepezil 2	1.585	1.08
AChE- *S*-donepezil 3	1.384	1.875
AChE-*R*-donepezil 1	0.711	1.949
AChE-*R*-donepezil 2	0.713	4.9
AChE-*R*-donepezil 3	0.778	1.511

Table 2. The last 5 ns average ΔG (kcal/mol) values of ligands and AChE

Ligand	ΔG (kcal/mol)	
	Average (45-50 ns)	Average (all poses)
Huprine X 1	-10.688	-10.901
Huprine X 2	-10.199	
Huprine X 3	-11.816	
S-donepezil 1	-12.985	-12.093
S-donepezil 2	-12.458	
S-donepezil 3	-10.836	
R-donepezil 1	-10.990	-
R-donepezil 2	-	
R-donepezil 3	-12.629	

According to Liu *et al*., (2017), a good ΔRMSD value based on 5 ns MD simulations is ≤2.0 Å. Based on Figures 1-3 and Table 1, the ΔRMSD values of the last 5ns of all MD simulations are ≤2.0 Å. This finding indicates that the ligands could stabilize the AChE. However, based on the ΔRMSDLigMov results of the MD simulations, one of the AChE-*R*-donepezil complexes showed value of 4.9 Å. This result indicates that *R*-donepezil exhibits instability in binding to the AChE binding pocket. Interestingly, in the previously 100 times docking simulations, this complex showed a docking RMSD value of 0.1008 Å, which indicates that among the other two complexes, the AChE-*R*-donepezil complex had a highly accurate docking result but it was unstable in the MD simulations.

As mentioned in the previous paragraph, the stability of the AChE backbone atoms was assessed in this study by evaluating the RMSD deviation of the backbone atoms (ΔRMSDBb) and the ligand move (ΔRMSDLigMov) at 5 ns intervals throughout the 50 ns simulation period, following the recommendation of Istyastono and Prasasty (2020). The RMSD measures the deviation or difference between the atomic coordinates of a reference structure and a trajectory of structures obtained during a MD simulation. RMSD is particularly valuable in assessing how a molecule or system's conformation changes over time during the simulation. According to Liu *et al*. (2017), the stability of a protein-ligand complex depends on the deviation of RMSD parameter, with the condition that it should below 2 Å throughout the simulation (Liu and Kokubo, 2017).

Based on the ΔRMSDBb parameter in Figures 1A, 1C and 1E, it can be seen that all complexes are relatively stable during the 50 ns

simulation period. There are some curve movements that surpass 2 Å, but overall, they are still considered stable when the values are averaged. The backbone of a protein or molecule consists of a sequence of atoms that form the structural framework, typically including the atoms in the peptide bonds and the alpha carbon atoms. By calculating the RMSD for the backbone atoms it is possible to assess how similar or dissimilar their overall structural conformations are (Arnittali *et al*., 2019).

In Figures 1B, 1D and 1F, the stability of the ligands in the complexes is depicted based on the ΔRMSDLigMov parameter. According to the graph, the movement of the *S*-donepezil compound is sufficiently stable, since it remains within the AChE binding pocket throughout the simulation. This finding is supported by Lu *et al.* (2015), who concluded that plasma concentrations of *S*-donepezil (based on CYP2D6 polymorphisms) are significantly associated with therapeutic response. Additionally, in 51 patients, it was observed that the average plasma concentrations of *S*-donepezil were higher compared to *R*-donepezil, and the ratio of plasma *R*-donepezil to *S*-donepezil ranged from 0.34 to 0.85 (Lili *et al*., 2013). The *R*-donepezil exhibited a faster degradation rate compared to *S*-donepezil (Lili *et al*., 2013). Based on the above findings, it can be shown that *S*-donepezil is more stable than *R*-donepezil.

Table 3. Average of hydrophobic Interaction hotspot (in %) of AChE and ligands identified by PyPLIF HIPPOS from 45 to 50 ns in MDS snapshots

Interacting residue	Interaction Type	Interaction Percentage		
		Huprine X	*S*-donepezil	*R*-donepezil
Tyr70	hydrophobic	30.27%	74.91%	76.44%
Asp72	hydrophobic	1.99%	91.81%	55.42%
Glu73	hydrophobic	-	-	0.13%
Gln74	hydrophobic	-	-	3.39%
Phe75	hydrophobic	32.06%	-	-
Phe78	hydrophobic	24.75%	-	-
Trp84	hydrophobic	69.39%	99.20%	65.13%
Asn85	hydrophobic	-	0.06%	0.06%
Tyr121	hydrophobic	88.42%	57.81%	61.34%
Glu199	hydrophobic	13.57%	8.31%	-
Trp233	hydrophobic	0.06%	-	-
Trp279	hydrophobic	9.98%	0.93%	67.33%
Leu282	hydrophobic	-	-	0.19%
Asp285	hydrophobic	-	-	0.26%
Ile287	hydrophobic	-	-	4.12%
Phe288	hydrophobic	0.79%	-	-
Phe290	hydrophobic	63.87%	0.39%	2.72%
Phe330	hydrophobic	100%	100%	68.19%
Phe331	hydrophobic	95.54%	0.06%	60.47%
Leu333	hydrophobic	25.14%	11.97%	24.95%
Tyr334	hydrophobic	64.07%	100%	100%
Leu358	hydrophobic	-	-	0.13%
Trp432	hydrophobic	85.16%	47.23%	35.32%
Met436	hydrophobic	32.33%	24.01%	-
Ile439	hydrophobic	52.62%	86.82%	6.05%
His440	hydrophobic	59.94%	56.22%	13.70%
Tyr442	hydrophobic	40.31%	94.94%	32.40%
Ile444	hydrophobic	-	1.53%	-

Table 4. Average of non-hydrophobic Interaction hotspot (in %) of AChE and ligands identified by PyPLIF HIPPOS from 45 to 50 ns in MDS snapshots

Interacting residue	Interaction Type	Interaction Percentage		
		Huprine X	*S*-donepezil	*R*-donepezil
Tyr70	H-bond (as the donor)	-	-	1.92%
Tyr70	aromatic (edge-to-face)	18.23%	64.73%	27.47%
Tyr70	aromatic (face-to-face)	0.06%	21.29%	11.37%
Asp72	ionic (as the anion)	26.21%	-	3.12%
Gln74	H-bond (as the donor)	-	-	19.62%
Phe75	aromatic (edge-to-face)	0.06%	-	-
Ser81	H-bond (as the donor)	-	-	15.23%
Trp84	aromatic (edge-to-face)	49.43%	14.50%	0.66%
Trp84	aromatic (face-to-face)	64.73%	92.34%	32.93%
Asn85	H-bond (as the donor)	-	-	5.65%
Tyr121	H-bond (as the acceptor)	-	68.66%	31.13%
Tyr121	aromatic (edge-to-face)	32.86%	64.40%	13.70%
Tyr121	aromatic (face-to-face)	3.79%	2.99%	0.06%
Ser122	H-bond (as the donor)	-	0.06%	-
Ser122	H-bond (as the acceptor)	0.06%	-	-
Glu199	ionic (as the anion)	25.61%	-	-
Trp279	aromatic (edge-to-face)	-	49.63%	63.53%
Trp279	aromatic (face-to-face)	-	16.03%	53.75%
Phe290	aromatic (edge-to-face)	4.72%	1.26%	0.13%
Phe290	aromatic (face-to-face)	1.26%	5.85%	-
Phe330	aromatic (edge-to-face)	26.41%	78.57%	52.69%
Phe330	aromatic (face-to-face)	87.15%	43.51%	24.15%
Phe331	aromatic (edge-to-face)	11.17%	0.06%	30.47%
Phe331	aromatic (face-to-face)	0.13%	-	24.88%
Tyr334	H-bond (as the donor)	-	-	0.59%
Tyr334	aromatic (edge-to-face)	33.53%	37.12%	80.37%
Tyr334	aromatic (face-to-face)	2.99%	65.13%	26.81%
Trp432	H-bond (as the donor)	0.19%	-	-
Trp432	aromatic (face-to-face)	37.12%	-	-
Trp432	aromatic (edge-to-face)	14.77%	32.66%	1.72%
His440	aromatic (edge-to-face)	26.81%	-	7.65%
His440	aromatic (face-to-face)	5.38%	-	0.46%
Tyr442	H-bond (as the donor)	25.01%	12.10%	1.92%
Tyr442	aromatic (edge-to-face)	-	3.59%	0.39%
Tyr442	aromatic (face-to-face)	0.46%	-	-

The stability of the compound-protein complex can be identified through the value of ΔG (Yanuar *et al*., 2023). Table 2 presents a comparison of the average ΔG calculations from the last 5 ns snapshots of Huprine X, *S*-donepezil, and *R*-donepezil. The calculated ΔG, based on the results of MD simulations, refers to the change in Gibbs free energy during the process of molecular complex formation. The ΔG is a measure of the stability or strength of the bond between the target molecule and the ligand formed during the MD simulation (Hata *et al*., 2021).

The results (Table 2) show that the ΔG value of *S*-donepezil is the lowest, indicating that *S*-donepezil is highly stable in binding with the target protein, which is AChE. This is consistent with the previous findings that *S*-donepezil is sufficiently stable and exhibits good results in *in vitro* and *in vivo* experiments (Lili *et al*., 2013; Lu *et al*., 2015). The average ΔG for *R*-donepezil was not calculated because, according to Table 1, the ΔRMSDLigMov value for *R*-donepezil 2 is extremely high, suggesting that during the course of the MD simulations, *R*-donepezil did not find a favorable binding conformation.

The movement of the ligand within the binding pocket facilitated the exploration of the allosteric site of dipeptidyl peptidase 4 (Istyastono and Riswanto, 2022; Nongonierma *et al*., 2014). Since the binding pocket of the AChE is relatively small compared to dipeptidyl peptidase 4, PyPLIF HIPPOS in this research could not discover the allosteric site (Istyastono *et al*., 2020; Istyastono and Riswanto, 2022). Instead, PyPLIF HIPPOS could confirm the identification of the most probable active enantiomer between *S*- donepezil or *R*-donepezil. PyPLIF HIPPOS generates interaction bitstrings that consider both the amino acid's main chain and side chains. However, when identifying the key factors influencing protein-ligand binding, the emphasis has primarily been on the protein's side chain rather than the main chain (Istyastono *et al*., 2020).

Previous research conducted by (Dvir *et al*., 2010; Weise *et al*., 1990) showed that Trp84 and Phe 330 are part of the potential 'anionic' (choline) binding sites. In the context of the 'anionic' (choline) binding site, these residues are important because they help position and stabilize choline or choline-like ligands within the enzyme's active site. This positioning is critical for the catalytic function of AChE, since it allows the enzyme to efficiently hydrolyze acetylcholine and regulate neurotransmission by terminating the signal at cholinergic synapses. Identification of the inhibition on specific amino acid residues such as Trp84 and Phe330 is essential for designing drugs or inhibitors that target AChE, which can have therapeutic implications in conditions where cholinergic signaling needs to be modulated, such as in AD (Dvir *et al*., 2010).

Based on the identification results from PyPLIFF HIPPOS (Istyastono *et al*., 2020), *S*-donepezil demonstrated 99.2% hydrophobic and 92.34% aromatic (face-to-face) interactions on Trp 84 (Tables 3 and 4), according to the average percentage of interactions from the three selected ligands. This percentage significantly exceeds the threshold stated in the method, which is ≥87.8% (Riswanto *et al*., 2017). Therefore, the interaction between the amino acid Trp 84 and the *S*-donepezil compound indicates a dominant interaction. In contrast, Huprine X and *R*-donepezil only showed hydrophobic interaction percentages of 69.39% and 65.13%, respectively.

The calculation results of the percentage of interactions in Tables 3 and 4 indicate differences in the types of interactions, including both hydrophobic and non-hydrophobic interactions, between the AChE complex and the ligand. In addition to Trp 84, Phe330 also plays an active role in forming aromatic (edge-to-face) and hydrophobic interactions with percentages of calculated interactions of 78.57% and 100%, respectively. Considering the allosteric region of the AChE enzyme (Dvir *et al*., 2010), Phe330 is also one of the amino acids that can synergize with Trp 84 to form bonds in inhibiting the binding site of the AChE enzyme.

CONCLUSIONS

Molecular dynamics simulation is one of the powerful computational methods for testing the stability of a donepezil compound. *S*-donepezil has been proven to be stable in binding with AChE through *in vitro*, *in vivo*, and *in silico* studies. Through PyPLIF HIPPOS, this research shows that *S*-donepezil can bind with Tyr 84, which is one of the crucial amino acids in the AChE binding site. These findings provide further insights for researchers to establish the appropriate protocols to determine the active conformation of chiral compounds, specifically in this case, the active compounds under study which are *S*-donepezil and *R*-donepezil.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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