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Molecular Docking and Dynamics Simulation for Searching Anti-Cancer Compounds of Piperlongumine Derivatives that Have Potential as an Inhibitor Against MAO-B (Monoamine Oxidase B)

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ABSTRACT

The docking of the piperlongumine molecule and its derivatives has been carried out to find molecules that have the potential as anti-cancer. A total of 18 ligands were docked to the 2v5z protein using the autodock4 and autodock vina programs. The binding energies of piperlongumine and piperlongumine derivatives [R1 = CH3 and R2 = H] were -8.6 kcal/mol and -9.3 kcal/mol, respectively. Based on molecular dynamics simulations, the hydrogen bond interaction fraction was dominated by GLN 206 residue in both the SAG (88%) and piperlongumine derivatives ((R1=CH3, R2 = H)(93%) ligand, for this reason, this piperlongumine derivative molecule is predicted to have potential as MAO B inhibitor.

Keyword: piperlongumine, molecular docking , molecular dynamics simulations

1. INTRODUCTION

Plants have been a source of medicine for thousands of years. Species of the genus Piper are among the important medicinal plants used in various medicinal systems[1, 2]. Piper longum L. (Piperaceae), commonly known as "long pepper", is widely distributed in tropical and subtropical regions of the world, throughout the subcontinent of India, Sri Lanka, Middle East countries, and America (Zaveri, M., 2010). Piper longum (L.) contains phytochemicals of the alkaloid group, including piperine, piperidine, and other many compounds (Koko, 2010). Piperlongumine is one of the alkaloid compounds extracted from Javanese chili (Piper longum L), known to produce neuroprotective effects in the brain and exhibit various pharmacological activities such as antitumor, antidiabetic, antiangiogenic, anti-inflammatory, and analgesic properties (Zhang, L., 2019 and Wu, Y., 2014).

Monoamine oxidase B (MAO-B) is an outer-membrane-binding mitochondrial flavoenzyme that functions in the oxidative deamination of dopamine in the striatum. Inhibition of MAO-B in the brain can slow the depletion of dopamine stores and increase levels of endogenous dopamine, and exogenously produced dopamine given levodopa. Furthermore, MAO-B inhibitors may also exert a neuroprotective effect by reducing the production of potentially harmful byproducts of dopamine metabolism in the brain (Azam, F., 2012).

Two isoforms of monoamine oxidase (MAO), 1MAO A and MAO B, are present in humans and both are 60-kDa mitochondrial outer membrane-bound flavoenzymes that share 70% sequence

identity. These enzymes have differences and overlapping specificities in the oxidative deamination of neurotransmitters and amines contained in food, the development of specific reversible inhibitors has been a long-sought goal. The expression of MAO B levels in neural tissue increases 4-fold with age, resulting in increased metabolic rates of dopamine and production of higher levels of hydrogen peroxide, which is thought to play a role in the etiology of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Thus, it is important to develop specific and reversible MAO B inhibitors that could lead to clinically useful neuroprotective agents. Recent crystal structures of human MAO B in complex with several pharmacologically important inhibitors have been resolved with 1,6 Å resolution. The access channel from the protein surface to the active site of the enzyme consists of two cavities, an inlet cavity, and an active site cavity (Frantisek, 2005).

The three-dimensional (3D) complex structure formed between the drug target and the drug candidate plays an important role in structure-based drug design (SBDD), where the drug candidate molecule is designed concerning the 3D structure of the drug target. Thus, computational prediction of the structure of protein-ligand complexes, i.e. computational docking, plays an important role in SBDD. For computational docking, various procedures have been proposed that can predict the structure of protein-ligand complexes with high accuracy. To evaluate the docking software, a docking pose that gives the root mean square deviation (RMSDs) for the experimental structure less than or equal to 2.0 is considered a reasonable pose. When at least one plausible pose is obtained from computational docking, the docking protocol is considered a success. The success rate of many docking programs has been reported to be higher than 70%. However, some well-programmed software has a success rate of more than 80% (Koichi Kato, 2021) [2,3]. Although reasonable docking poses are preferably obtained as the highest-scoring poses of the ligands, the evaluation method for docking poses without experimental knowledge has not been sufficiently developed.

Generally, the docking computational program generates several candidate structures for protein-ligand complexes. For efficient in silico drug design trials, one or more candidates should be selected for the next drug design step. In many cases, known experimental results, such as activity-structure studies, can be used for candidate pose selection. If useful experimental results are not available, pose selection is made from the evaluation of the score function. While this scoring method is likely to provide an appropriate score for docking pose candidates, previous data suggest that about 50% of the highest-ranked poses can be considered structurally plausible. For this reason, classical molecular dynamics (MD) simulations are sometimes used for docking pose selection (Koichi Kato, 2021).

Drug design and computational approach is a suitable combination to search for new inhibitors against cancer. This combination can make a significant impact because it is relatively cost-effective, fast, and almost universally applicable in many areas of the target. Computational approaches can assist in drug design in various ways, such as docking and simulation.

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2. RESEARCH METHOD

2.1 Materials and tools

The material studied is the piperlongumine molecule and its derivatives are taken from (Wu, Y, 2014). The energy of the piperlongumine molecule and its derivatives was minimized through the Open Babel program into a 3-dimensional structure in pdb format, while the receptor (2v5z.pdb) downloaded from the RCSB website was directly used without optimization. The software used is autodock4 and autodock vina, LigPlot, MGLTools 1.5.7, Pymol, and Desmond while the hardware consists of a computer with an Intel Core i3 processor, 6 GB RAM, 64-bit Windows operating system.

2.2 Molecular Docking

Before molecular docking, the SAG ligand and receptor (2v5z without SAG) in pdb format were created in the pdbqt file in the MGLTools program. Grid parameter marking was performed to navigate the ligand to the active site of MAO B. The grid space was set at 0.375 Å, the central grid

box was set at x = 52,136 , y = 156.183 , z = 28,029, the number of grid points along the x, y, and z dimensions was set at 40 x 40 x 40, energy_range = 4 and exhaustiveness = 256. The docking results are then visualized with pymol and MGLTools

2.3 Molecular dynamics simulation

Molecular dynamics simulations were carried out with the Desmond program for the SAG complex, piperlongumine, and piperlongumine derivatives (substituents R1 = CH3 and R2 = H) with 2v5z receptors. All complexes were individually solvated by placing a box filled with water with a size of 10 with a single point charge (SPC) water model with periodic boundary conditions (PBC). After optimization, the system is simulated in the NPT ensemble maintained at a temperature of 300 K and pressure using the Nose-Hoover thermostatic algorithm. The analysis of the ligand-receptor interaction was carried out using a simulation interaction diagram tool. The results of the analysis in the form of RMSD and RMSF ligand-protein against the reference.

3. RESULTS AND ANALYSIS

3.1 Docking protocol validation

The validation of the docking protocol must be carried out before the docking of the molecules (ligands) is tested. Its procedure has been carried out by docking the SAG molecule (co-crystal ligand) to the 2v5z receptor (re-docking) using the vina autodock. The docked complex is superimposed on the co-crystal complex with Pymol 2.3 shown in Figure 1. which shows the superimposition with an RMSD value of 1.706 Å which is still below the RMSD tolerance < 2 Å so that the docking protocol is considered valid.

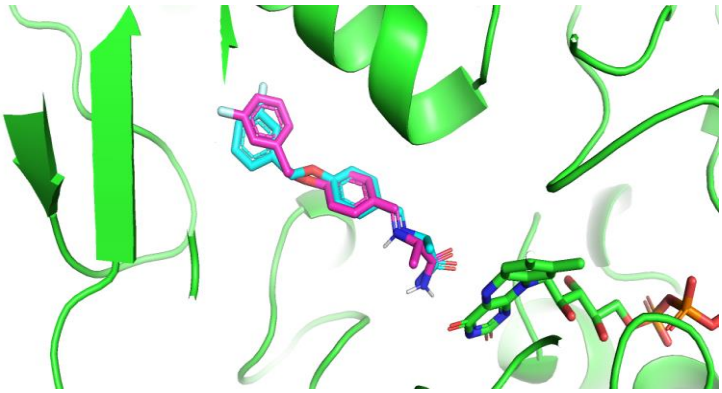


Figure 1. Superimposition (overlap) of native ligand (pink) with co-crystal ligand (tosa blue) in the active site obtained using Pymol (RMSD value = 1.706 Å)

3.2 The SAG, piperlongumine and its derivatives ligand-protein interactions and the binding energy (kcal/mol)

The binding energies of the piperlongumine ligand and its derivative-2v5z are presented in Table 1. The structure of piperlongumine with R_1 and R_2 as substituent groups is shown in **Figure** 2. The smallest binding energy was obtained for the native ligand-2v5z conformation of -10.4 kcal/mol while for the piperlongumine ligand conformation and its derivatives-2v5z were smaller than -10.4 kcal/mol. The binding energy of the piperlongumine-derived ligands with R1 = -CH3 and R2=H with the 2v5z receptor is -9.3 kcal/mol and this is close to the binding energy value of the native ligand-2v5z while the binding energy for the parent compound (piperlongumine)-2v5z is only -8.6 kcal/mol. Therefore, the ability of piperlongumine derivative molecules with substituents R_1= -CH_3 and R_2 = H is predicted to be similar to the native ligand SAG [((S)-(+)-2-[4-(Fluorobenzyloxy-Benzylamino)Propionamide]) as inhibitors.

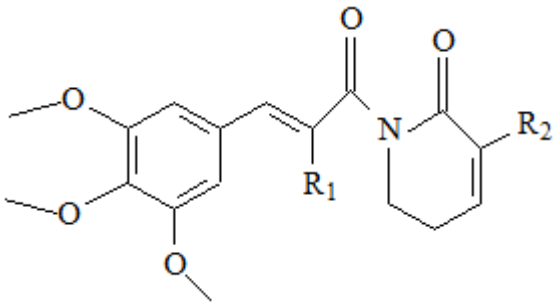


Figure 2. Structure of piperlongumine with R_1 and R_2 as substituent groups

Table 1. The binding energies, interacting amino acid residues, types of interactions for co-crystal ligands, piperlongumine, and their derivatives with 2v5z receptors

Ligands	binding energies (kcal/mol)	interacting amino acid residues	types of interactions
(S)-(+)-2-[4-(Fluorobenzyloxy-Benzylamino)Propionamide] ***(Ligand Co-crystal)***	-10.4	**TYR326, PHE343, GLN206, LEU164, ILE316, TYR60, LEU171, PHE168, TYR345**	Hydrogen bond, hydrophobic
Piperlongumine	-8.6	**TYR326, PHE343, GLN206, LEU164, TYR435, LEU171,** ILE199, TRP119, SYS172, ILE198,	hydrophobic
R_1 = metil, R_2 = H	-9.3	**TYR326, ILE316, LEU164, GLN206, PHE168, PRO104, PHE103, TRP119, ILE199, TYR396**	hydrophobic
R_1 = ethyl, R_2 = H	-8.7		
R_1 = H, R_2 = morpholine	-7.8		
R_1 = methyl, R_2 = morpholine	-7.6		
R_1 = ethyl, R_2 = morpholine	-7.6		
R_1 = propyl, R_2 = morpholine	-7.7		
R_1 = H, R_2 = Cl	-8.9		
R_1 = methyl, R_2 = Cl	-7.9		
R_1 = ethyl, R_2 = Cl	-7.0		
R_1 = propyl, R_2 = Cl	-8.5		
R_1 = butyl, R_2 = Cl	-8.5		
R_1 = H, R_2 = Br	-8.2		
R_1 = methyl, R_2 = Br	-8.9		
R_1 = ethyl, R_2 = Br	-7.1		
R_1 = propyl, R_2 = Br	-7.9		
R_1 = butyl, R_2 = Br	-7,5		

Docking of piperlongumine derivative molecules with substitution [R1 = H, R2 = Cl; R1 = -CH3, R2 = Br] has a binding energy of -8.9 kcal/mol which is still smaller than the binding energy of the parent compound, indicating a good binding conformation in this study so that these molecules are expected to have better potential as inhibitors than piperlongumine.

Figures 1 3D and 2 2D show the interaction of the SAG (Co-Crystal) ligand with several amino acid residues (TYR 326, PHE 343, GLN 206, LEU 164, ILE 316, TYR 60, LEU 171, PHE 168, TYR 345) in the site active. These interactions were also found in the interaction of piperlongumine ligands and piperlongumine derivatives (R_1 = -CH_3, R_2 = H) as shown in Figures 3 and 5 and presented in Table 4.1, allowing these ligands to an inhibitory potential such as SAG ligands. In Figure 2D, residues GLN 206 and TYR 60, and TYR 435 provide hydrogen bonding and hydrophobic interactions on the SAG ligand, respectively. Likewise, other residues provide hydrophobic interactions.

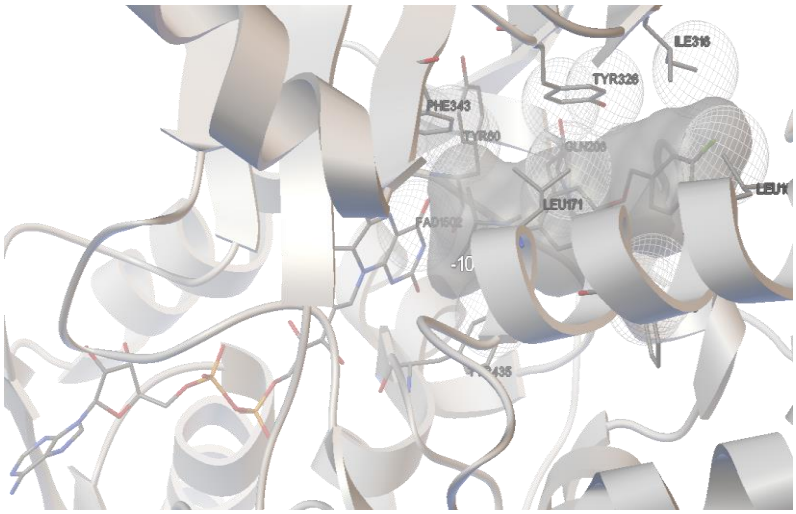


Figure 3. The SAG ligand -MAO-B 3D interaction (-10.4 kcal/mol)

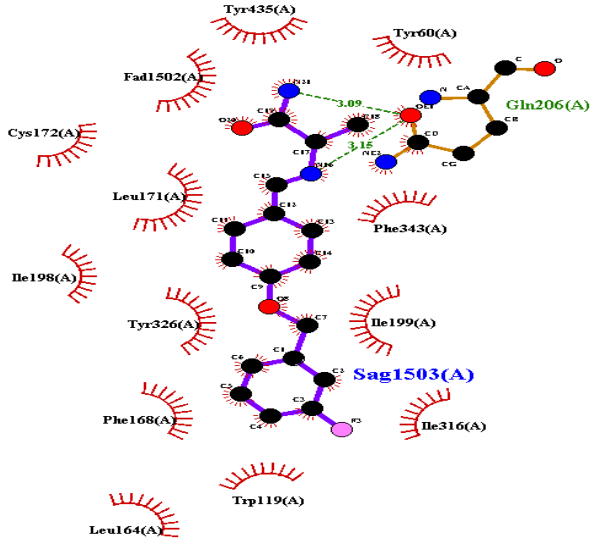


Figure 4. Ligplot 2D SAG ligand -MAO-B interaction

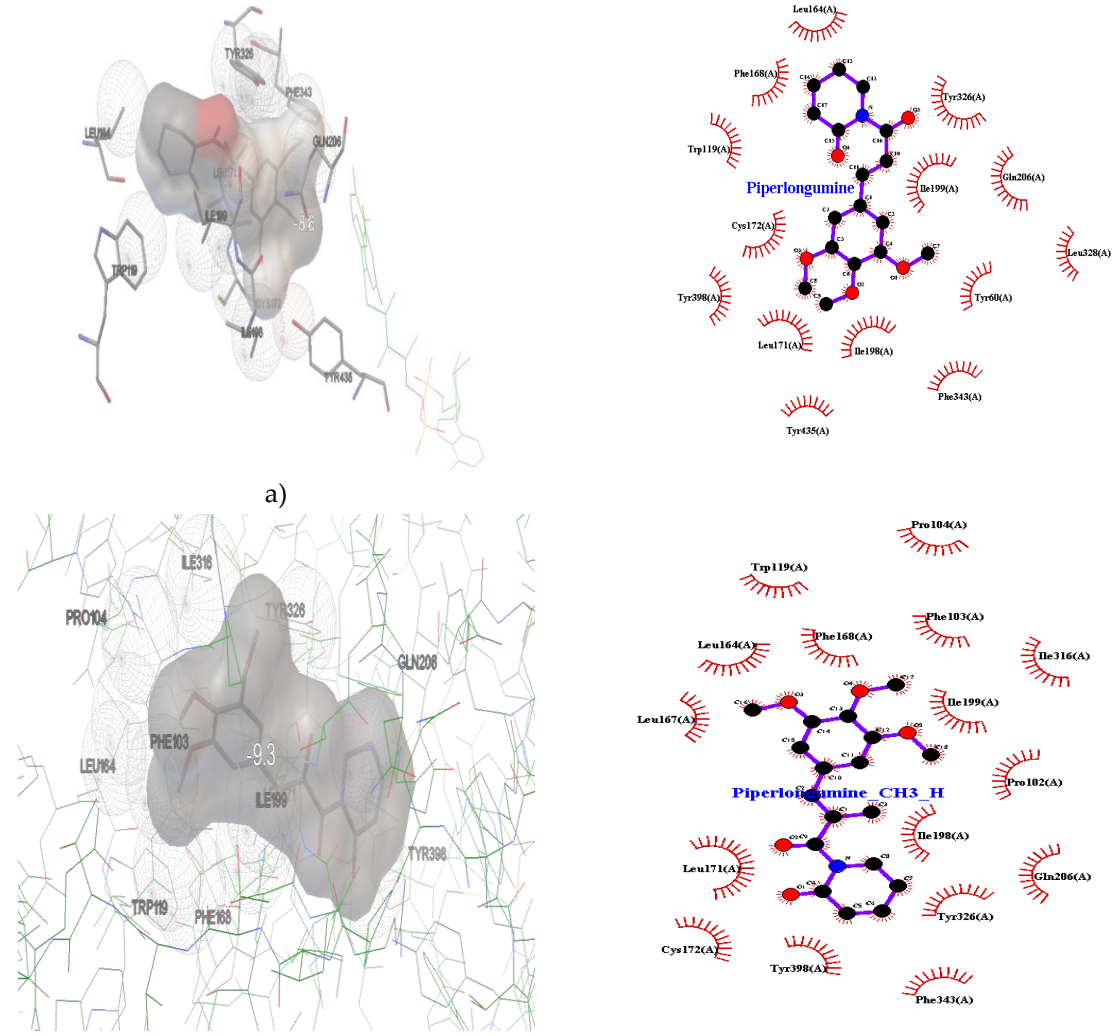


Figure 5. The 3D and 2 D interaction of a) piperlongumine; b) piperlongumine derivative (R_1=CH_3, R_2 = H) ligand with MAO-B

3.3 Molecular dynamics (MD) aspect

The Stability and dynamics properties of the complex formed from the SAG ligand, piperlongumine, and piperlongumine derivatives with substitutions R1 = -CH_3 and R2 = H with 2v5z protein cannot be known based on the results of molecular docking simulations. These properties will be known based on molecular dynamics simulations. In this study, MD simulation was carried out even though it was only 15 ns to analyze RMSD and RMSF proteins-ligand (piperlongumine derivatives with substitutions R1 = -CH_3 and R2 = H with 2v5z protein) (**Figure** 4).

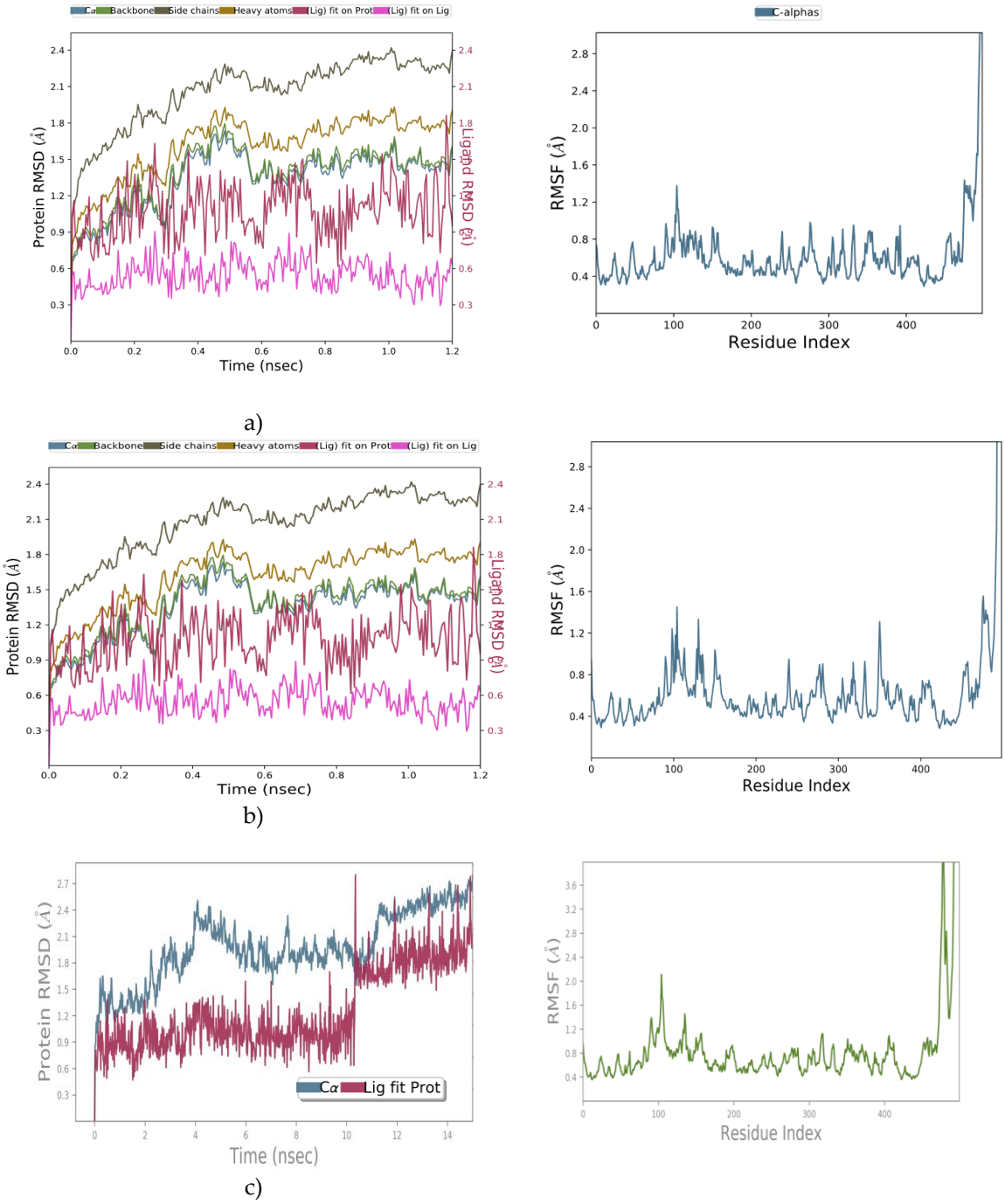


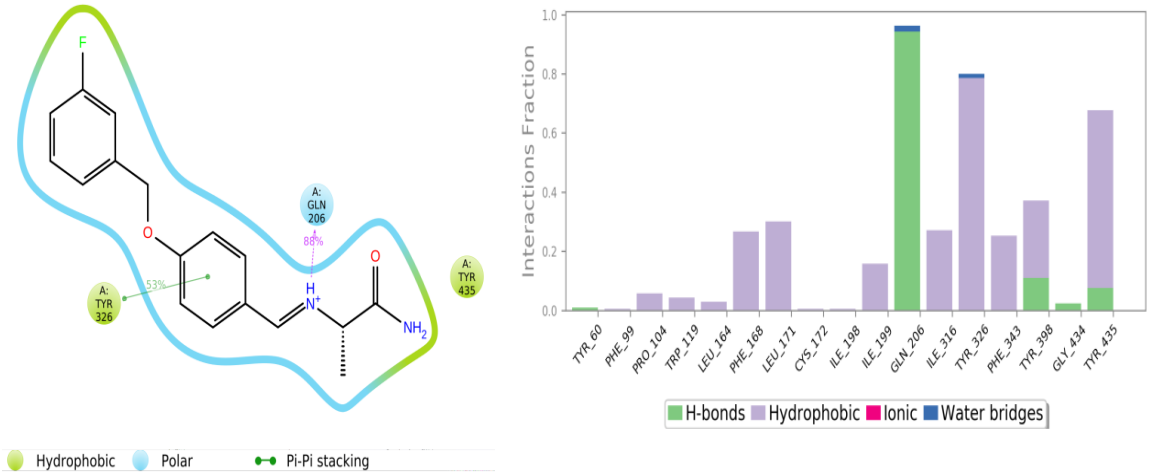
Figure 6. The RMSD and RMSF of a) SAG, b) piperlongumine, and c) piperlongumine derivative (R_1=CH_3, R_2 = H)-MAO B complex were obtained by the molecular dynamics simulations

The plot in Figure 6 shows the RMSD evolution of a protein (left Y-axis). All protein frames are first aligned on the reference frame backbone, and then the RMSD is calculated based on the atom selection. Monitoring the RMSD of the protein can give insights into its structural conformation throughout the simulation. RMSD analysis can indicate if the simulation has equilibrated, its fluctuations towards the end of the simulation are around some thermal average structure. Changes of the order of 1-3 Å are perfectly acceptable for small, globular proteins. Changes much larger than that, however, indicate that the protein is undergoing a large conformational change during the simulation. It is also important that the simulation converges, the RMSD values stabilize around a

fixed value. In the case the RMSD of the protein is still increasing or decreasing on average at the end of the simulation, then this system has not equilibrated, and so the simulation needs to be continued again. Ligand RMSD (right Y-axis) indicates how stable the ligand is with respect to the protein and its binding pocket. In the above plot, 'Lig fit Prot' shows the RMSD of a ligand when the protein-ligand complex is first aligned on the protein backbone of the reference and then the RMSD of the ligand heavy atoms is measured. If the values observed are significantly larger than the RMSD of the protein, then it is likely that the ligand has diffused away from its initial binding site.

The Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain. On this plot, peaks indicate areas of the protein that fluctuate the most during the simulation. Typically it will observe that the tails (N- and C-terminal) fluctuate more than any other part of the protein. Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions.

MD simulations provide a good understanding of the ligand-protein interactions, explain conformational stability, and good correlation with protein-ligands. The residues GLN 206, TYR 435, TYR 326, PHE 343, LEU 171, CYS 172, and ILE 199 were observed important residues that play a specific role in ligand binding to proteins (Figure. 5). The hydrogen bond interaction fraction was dominated by the GLN 206 residue (88%) in both the SAG ligand and the piperlongumine derivative ((R1=CH3, R2 = H)(93%) while the TYR 435 residue contributed to the increasing hydrogen bonding in the ligand. SAG, piperlongumine, and piperlongumine derivatives (R1=CH3, R2 = H), but smaller Pi-Pi stacking interactions were also observed, namely 53% (i), 45% (ii), and 36% (iii). This piperlongumine derivative has a high bonding affinity compared to its parent compound (piperlongumine). With the highest bond affinity of -9.3 kcal/mol among the piperlongumine derivatives, the piperlongumine derivative (R1=CH3, R2 = H) is expected to be investigated further for in-vivo or in-vitro studies, the hydrophobic interactions of residues with SAG formed with a percentage of less than 20% were PRO 104, TRP 119, LEU 164 while those with more than 20% were PHE 168, LEU 171, ILE 316, PHE 343, TYR 398 and TYR 435. In piperlongumine ligands, hydrophobic interactions of more than 20% occur in only two residues, namely LEU 171 and TYR 326 although there is an addition to the TYR 326 residue after the substitution of groups R1 = CH3 and R2 = H).



(i)

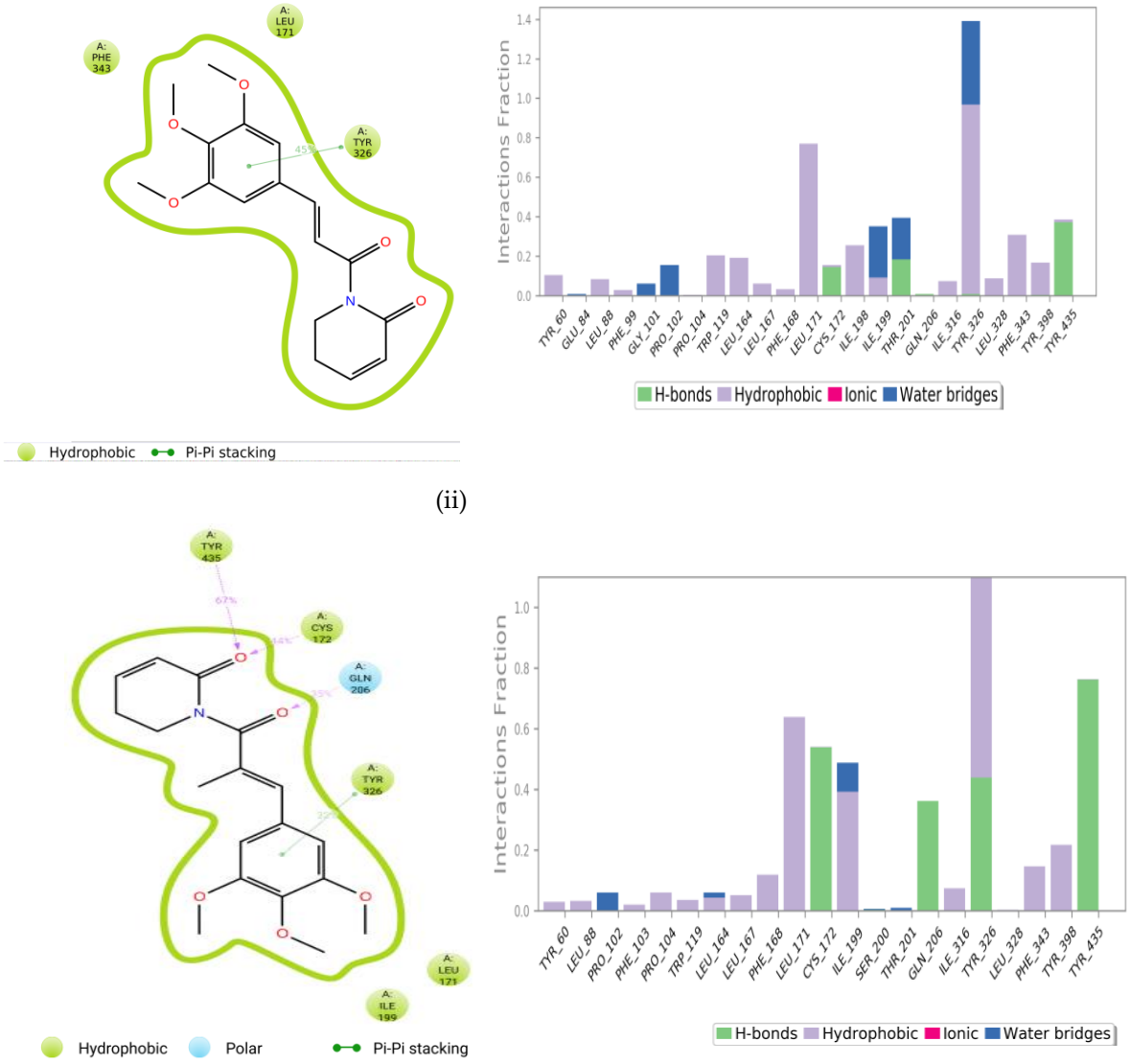


Figure 7. Protein-ligand contact plot and ligand-protein interaction residues of SAG (i), piperlongumine (ii), and piperlongumine derivative (R_1=CH_3, R_2 = H) (iii) -MAO B complex

The stability of the interaction of GLN 206 residues as a result of molecular docking on these three ligands can also be observed in the molecular dynamics simulation results. By comparing the results in Table 1 and Figure 5, it can be seen that the interaction of Hydrogen bonding GLN 206 on piperlongumine is no longer observed. This indicates a weak or unstable interaction between the piperlongumine ligand and residue GLN 206 in contrast to the piperlongumine derivatives (R1 = CH3 and R2 = H). The large H-bond fraction and the stability of hydrogen bonding interactions between the piperlongumine derivatives with R1 = CH3 and R2 = H groups provide the potential towards inhibiting MAO B, especially in the treatment of cancer.

4. CONCLUSION

Until now no drug can completely treat cancer, the use of radiotherapy in cancer treatment will damage not only cancer cells but also healthy cells, research is still ongoing to develop molecules as guides that work with potential against cancer disease. The motivation of this molecular docking study is to find natural compounds and their derivatives that are more potent to inhibit MAO B

(monoamine oxidase). A total of seventeen molecules (piperlongumine and its derivatives) have been studied through molecular docking and molecular dynamics simulations. One piperlongumine derivative with substitution groups R1 = -CH3 and R2 = H) has the smallest binding energy of -9.3 kcal/mol and a hydrogen bond interaction fraction of 93% so this ligand is predicted to have better potential as an MAO B inhibitor than the parent molecule (piperlongumine) and this study can also be the basis for the discovery of natural molecules and their derivatives that have potential as anticancer.

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