STUDY MOLECULES DOCKING OF ALKALOIDS IN KRATOM ON SEROTONIN TRANSPORTER (SERT), NOREPINEPHRINE TRANSPORTER (NET), AND MONOAMINE OXIDASE (MAO)

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Abstract

Kratom (*Mitragyna speciosa* Korth) is a tropical plant originating from Southeast Asia that predominantly contains alkaloid compounds and can potentially maintain levels of monoamine compounds in the body to treat depression. The study aimed to examine the potential of 8 alkaloid compounds in kratom as antidepressants towards four target proteins: Serotonin Transporter (SERT), Dopamine Transporter (DOPAT), Leucine Transporter (LEUT), and Monoamine Oxidase (MAO) via molecular docking. The Pyrx program is used with exhaustiveness 106 as the protocol, and the grid is adapted to the active site of each receptor. The affinity values of the alkaloid compounds in kratom are mitragynine, 7-hydroxy mitragynine, speciociliatine, paynantheine, speciogynine, corynantheidine, mitraciliatine, and 9-hydroxycorynantheidine, for MAO were -7.1, -6.1, -5.7, -6.7, -5.7, -7.7, -5.7, and -5.7 kcal/ mole. All compounds bind to amino acid residues in the target protein through hydrogen and pi (π) bonds. All the tested alkaloid compounds have the potential to be re-uptake inhibitors SERT, DOPAT, LEUT, and Monoamine Oxidase (MAO).

Keywords: *alkaloid, depression, docking study, MAO, NET, SERT*

Introduction

Depression is a disorder of the function of monoamines that causes deficient levels of serotonin, norepinephrine, and dopamine in the body, which are the basis of the cause of depression. Depression can cause a decrease in work productivity, psychotropic or narcotic dependence, and disruption in social relationships; the worst impact is suicide (Woo & Postolache, 2008). Based on a previous study regarding the prevalence of depression in Indonesia, it is known that it was 6.2% in 2013 and increased to 9.8% in 2018, with more than 19 million sufferers (Arunpongpaisal et al., 2022). Protein serotonin transporters (SERT), norepinephrine transporters (NET), and monoamine oxidase (MAO) are all involved in the pathophysiology of depression. Targets for the re-absorption of monoamine substances like serotonin, norepinephrine, and dopamine into the presynaptic neuron include serotonin transporters (SERT), norepinephrine transporters (NET), and monoamine oxidases (MAO). Therefore, these three proteins must be inhibited so that reuptake does not occur by presynaptic neurons, and their levels are maintained by using antidepressants (Andrade & Rao, 2010). Alkaloid compounds in kratom have activity as an antidepressant with a depressant effect. Antinociception is mediated through the serotonergic system and increases the release of norepinephrine and serotonin via monoaminergic neurons

(Meireles et al., 2019; Novindriani et al., 2021; Prevete et al., 2023).

One of the medicinal plants has been used for various therapies and diseases, namely kratom (*Mitragyna speciosa* Korth.). The main components of Kratom leaves are indole alkaloids. The alkaloid compounds in question are mitragynine and 7-hydroxy mitragynine. The compound 7-hydroxy mitragynine has analgesic and affinity effects that are high on opioid receptors (Kruegel et al., 2019).

Researchers have found that. The alkaloid 7-hydroxy mitragynine is more effective than morphine, even after oral application. The effect is 13 times stronger than morphine. The effect of 7-hydroxy mitragynine with the ileum (distal small intestine) guinea-pig, it was found that 7-hydroxy mitragynine acts on nerve endings and inhibits the release of neurotransmitters (Matsumoto et al., 2005)

Molecular docking is an instrument for predicting the activity of compounds against receptors using affinity values, thereby saving energy, time, and costs (Meng et al., 2011; Wijianto et al., 2020). Molecular anchoring studies of alkaloid compounds in Kratom against serotonin transporters (SERT), norepinephrine transporters (NET), and monoamine oxidase (MAO) are still minimal, so it has the potential to become an opportunity in the development of new drugs to treat depression.

Research Methods

Hardware

The instruments used in this study were the Asus X441S Intel (R) Celeron (R), 128GB SSD, RAM 2, and GPU Intel Dual-Core N3060.

Software

The applications used in this research are Pyrx, Discovery Studio 2016 Client, AutoDockTool (Version 1.5.6), Pymol (Version 2.0), ChemOffice 2D (Version 19.0), and ChemOffice 3D (Version 19.0).

Materials

The materials used in this research were the three-dimensional structures of the ligands of mitragynine, 7-hydroxy mitragynine, speciociliatine, paynantheine, speciogynine, corynantheidine, mitraciliatine, and 9-hydroxycorynantheidine. Positive controls used were citalopram, duloxetine, nortriptyline, and tranylcypromine in sdf format; the 3D structures of serotonin transporter proteins (PDBID: 6M38), norepinephrine transporter proteins (PDBID: 2A65, 4M48) (Penmatsa et al., 2013; Pidathala et al., 2021; Yamashita et al., 2005), and monoamine oxidase (PDBID: 2BYB) (De Colibus et al., 2005) and their respective natural ligands were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/) in pdb format.

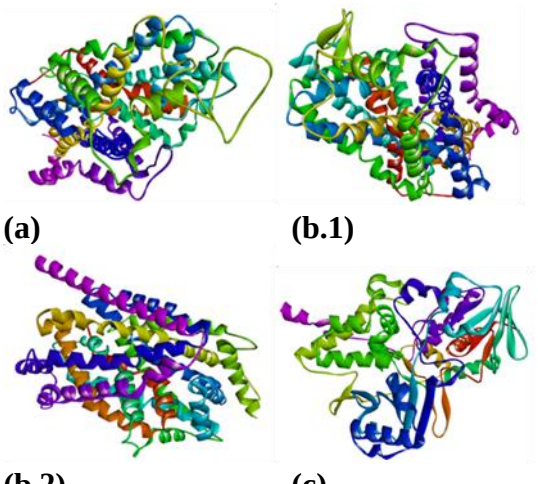
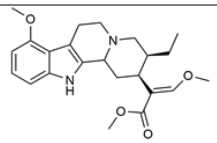
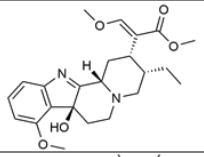
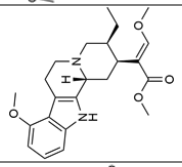
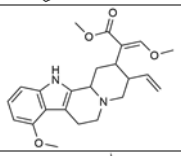
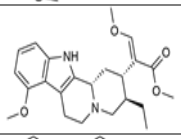
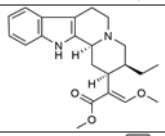
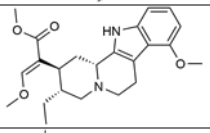
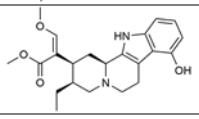


Figure 1. Crystal structure of protein serotonin transporters (SERT) (a); norepinephrine transporters toward dopamine transporter (DOPAT) (b.1); leucine transporter (LEUT)(b2); and monoamine oxidase (MAO)(c).

Table 1. Main Alkaloid Structure in Kratom

Compound	Chemical Structure
Mitragynine	
7-Hydroxymitraginine	
Speciociliatine	
Paynantheine	
Speciogynine	
Corynantheidine	
Mitraciliatine	
9-hydroxycorynantheidine	

Procedure

1) Ligand preparation
   The structures of 8 alkaloid compounds and the positive control were prepared using ChemOffice software (version 19.0) to obtain a 2-dimensional structure. Ligand energy minimization, performed using AMBER functional forms, has been very popular for protein simulations. The ligand is protonated, and a stable tautomer is also obtained using the Chem3D program (Version 19.0). The parameters observed were the log value of octanol-air conversion (LogP) and molecular weight (BM) (Grinter & Zou, 2014; Morris et al., 2009).
2) Protein preparation
   Serotonin Transporter (SERT), Norepinephrine Transporter (NET), and Monoamine Oxidase (MAO) proteins were downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) in pdb format, prepared using the program AutoDockTool (Version 1.5.6) to remove water residues, remove atomic

side chains in protein data, precisely only polar, by doing geometry optimization and energy minimization first, then changing the protein data format from "pdb" to "pdbqt" after adding a hydrogen molecule to the protein compound, then determining the coordinates of the location of the ligand attachment to the protein to be tested (Morris et al., 2009).

3) Native ligand preparation
   The preparation uses the DiscoveryStudio 2016 program to obtain native ligands from the Protein Serotonin Transporter (SERT), Norepinephrine Transporter (NET), and Monoamine Oxidase (MAO) downloaded from the protein data bank by separating the receptors and all unused molecules to leave one small molecule ligand, which is then used as method validation (Morris et al., 2009).
4) RMSD validation
   RMSD validation is a comparison stage between the native ligand and the test ligand, which will be visually tested against the receptor experimentally with overlapping ligand positions from the docking process. The Root Mean Square Deviation (RMSD) value is the basis for determining this validation. The method used is said to be valid if the RMSD value obtained is <2Å (Morris et al., 2009)
5) Docking procedure
   The docking process of the test ligands with Protein Serotonin Transporter (SERT), Norepinephrine Transporter (NET), and Monoamine Oxidase (MAO) uses the Vina Wizard program, which is integrated into Pyrx software. The results to be obtained are in the form of affinity values and the binding of ligands to target proteins or receptors (Morris et al., 2009).
6) Data analysis
   Data analysis was performed by determining the value of the physicochemical properties (LogP and BM) of the anthocyanin-derived compounds and then analyzing them by Lipinski's five laws to predict the absorption and permeability properties of the compounds as seen from the amino acids involved in the drug-receptor interaction process. The smaller the affinity value from the docking of a compound, the more stable the drug-receptor interaction will be, and it is predicted to have a higher biological affinity (Lipinski, 2004; Tijjani et al., 2022).

Results and Discussion

Analysis of the physico-chemical properties of ligands

The structure of the alkaloid compounds in kratom and the positive drug control can be seen using the two-dimensional ChemDraw application and continued with the analysis on the "pkCSM" web. The results of the analysis of the physicochemical properties of compounds based on structure to see the number of H donors (NH and OH) and H acceptors (N and O), molecular weight, and log P (Table 2).

Table 2. Physicochemical Properties of the Ligan

Compound Name	Molecular Formula	Molecular Weight	Log P	Number of H-bond donors	Number of H-bond acceptors
Mitragynine	$C_{23}H_{30}N_2O_4$	398.503	3.8251	1	5
7-Hydroxymitraginine	$C_{23}H_{30}N_2O_5$	414.502	2.7926	1	7
Speciociliatine	$C_{23}H_{30}N_2O_4$	398.503	3.8251	1	5
Paynantheine	$C_{23}H_{28}N_2O_4$	396.487	3.6011	1	5
Specioginine	$C_{23}H_{30}N_2O_4$	398.503	3.8251	1	5
Corynantheidine	$C_{22}H_{28}N_2O_3$	368.477	3.8165	1	4
Mitraciliatine	$C_{23}H_{30}N_2O_4$	398.503	3.8251	1	5
9-Hydroxycorynantheidine	$C_{22}H_{28}N_2O_4$	384.476	3.5221	2	5
Citalopram	$C_{20}H_{21}FN_2O$	324.399	3.81298	0	3
Duloxetine	$C_{18}H_{19}NOS$	297.423	4.6309	1	3
Tranylcypromine	$C_9H_{11}N$	133.194	.5012	1	1

According to the Lipinski rule, the H donor load should not be more than five, and the H acceptor should not be more than 10 to have good permeability. According to the Lipinski rule, the molecular weight of a compound that can penetrate a biological membrane is not more than 500 g/mol. A more than 500 g/mol molecular weight cannot diffuse through the cell membrane (Dougherty et al., 2019; Yang & Hinner, 2015).

A negative log P value is also not good because the molecule cannot pass through the lipid bilayer membrane. The log P value describes the compound's ability to dissolve in biological membrane fluids. The greater the log P value, the more hydrophobic the molecule (Pires et al., 2015). Molecules that are too hydrophobic tend to have a high level of toxicity because they are retained longer in the lipid bilayer and are distributed more widely in the body so that the bond selectivity becomes reduced for the target protein (Bogdanov et al., 2008). From Table 2, it is known that the test compounds and positive controls have fulfilled all the requirements of Lipinski's law.

Ligand Toxicity Test

Alkaloid compounds in kratom will be tested for toxicity using the Protox website to predict toxicity related to the chemical structure of the tested compound (Banerjee et al., 2018). The results of the toxicity of the test compounds can be seen in Tables 3–4.

Table 3. Toxicity Prediction Result

Compound	LD_{50}Mg/Kg	Toxicity Class Prediction
Mitragynine	300	3
7-Hydroxymitragynine	1000	4
Corynantheidine	300	3
Mitraciliatine	300	3
Paynantheine	300	3
Speciociliatine	300	3
Speciogynine	300	3
9-Hydroxycorynantheidine	300	3

Based on the results of the prediction of toxicity in Table 3 using Protox, data was obtained where the compounds mitragynine, corynantheidine,

mitraciliatine, paynantheine, speciociliatine, speciogynine, and 9-hydroxycorynantheidine were in class 3 in predicting toxicity, while compounds 7-hydroxymitraginin were in class 4. In the explanation from Protox Web Server in toxicity prediction, it was explained that the smaller the number or number (toxicity prediction class), the more toxic the prediction of a compound; conversely, the larger the number or number (toxicity prediction class), the safer a compound is (Banerjee et al., 2018).

Based on the results of drug target toxicity predictions in Table 4, it was determined that the corynantheidine compound did not have a virtually carcinogenic effect. In contrast, the other seven alkaloid compounds in kratom had a virtually carcinogenic effect. However, based on information from the Protox website, in terms of probability, a toxicity test is said to be valid if the probability value is > 70% (0.7). Hence, the compounds mitragynine, 7-hydroxy mitragynine, mitraciliatine, paynanhteine, speciociliatine, speciogynine, and 9-hydroxycorinanteidin are invalid because the probability value is <70% or <0.7. With this, it can be concluded that the eight alkaloid compounds in kratom are safe to use (Banerjee et al., 2018).

Table 4. Target Organ Toxicity with Test Ligands

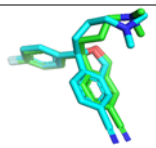
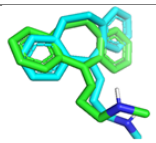
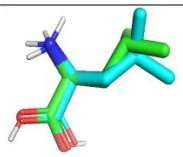
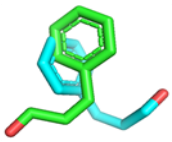
Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Mitragynine	no	yes	no	No	no
7-Hydroxyimitragynine	no	yes	no	No	no
Corynantheidine	no	no	no	No	no
Mitraciliatine	no	yes	no	No	no
Paynantheine	no	yes	no	No	no
Speciociliatine	no	yes	no	No	no
Speciogynine	no	yes	no	No	no
9-Hydroxycorynantheidine	no	yes	no	No	no

RMSD validation

The redocking results showed the Root Mean Square Deviation (RMSD) value for the natural ligand of citalopram was 1.61 Å, the ligand for nortriptyline was 0.513 Å, the leucine ligand was 1.521 Å, and the ligand tranilcypromin was 0.992 Å. The validation results for the position of the original ligand and the copy ligand can be seen in Table 5.

The validation results of Root Mean Square Deviation (RMSD) show that the position of the atoms in the redocking ligand (copied ligand) is not much different from the position of the crystallographic ligand (original ligand). The magnitude of the RMSD value and the overlapping of the ligands from the crystallography results and the ligands from the redocking results showed an RMSD value of < 2 Å. Other studies support that the smaller the error of the calculation results, the more accurate the calculation, and the protein is said to be valid and can be used in the docking process for the test ligands (Anggraeni et al., 2022; Budiarto et al., 2023; Dyas et al., 2023; Sururi et al., 2022; Wijianto et al., 2019).

Table 5. Overlay of Original Ligand Positions (green) and Copy Ligands (blue)

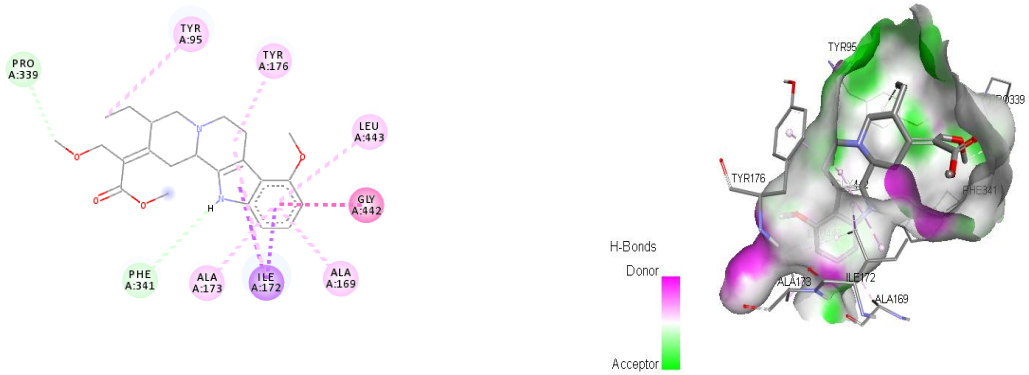
Compound	RMSD visualization
Citalopram	
Nortriptilin	
Leusin	
Tranilsipromin	

Docking and results analysis

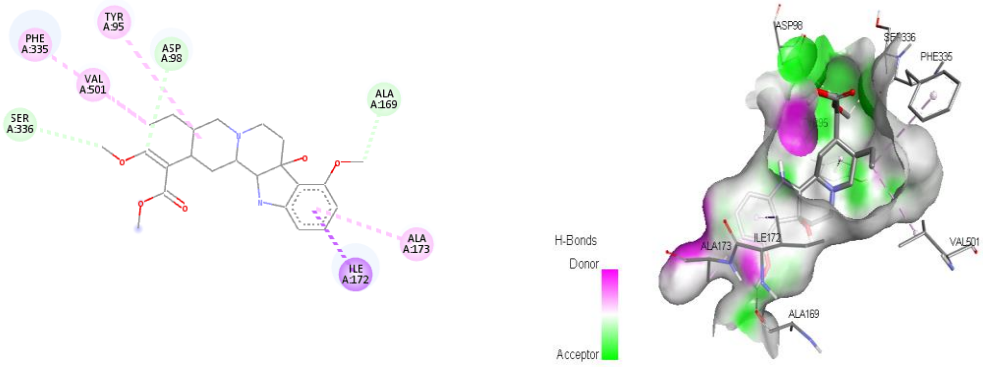
The docking process was carried out using the Pyrx application, which is based on Vina, with the parameters of the docking results being the value of bond-free energy (ΔG) and Root Mean Square Deviation (RMSD). A small Gibbs free energy value indicates that the conformation formed is stable, while a considerable Gibbs free energy value indicates an unstable complex-formed (Ahmad, 2022; Khan et al., 2019).

The optimized receptors and ligands are entered into the pyrx program. A grid box is set as the place or coordinates for the ligand to interact with the target enzyme or receptor, which is visualized in the form of a cube (Budiarto et al., 2023; Dyas et al., 2023; Feinstein & Brylinski, 2015). The grid box setting is done by adjusting the coordinates of the active sites of the receptor's natural ligands. The critical point in this study is the similarity of amino acid residues with the positive control (right pose) compared to the affinity value because the affinity value only determines the stability of a compound in the receptor (Wijianto et al., 2020). Mitracilitiatin has the best resemblance to the best amino acid residues in SERT. In the compound 7-hydroxymitraginin, spesioginin 9-hydroxycorinanteidin has the best similarity of amino acid residues in NET via Dopamine Transporter (DOPAT); however, 7-hydroxymitraginin has advantages both in terms of right pose and affinity value for DOPAT, while for Leucine Transporter (LEUT), Mitracililatin, speciociliatin and spesioginin have the best similarities in amino acid residues and affinity values. In Monoamine Oxidase (MAO), paynantein has the best amino acid residue similarities. The visualization of study docking results can be seen in Figures 2–5.

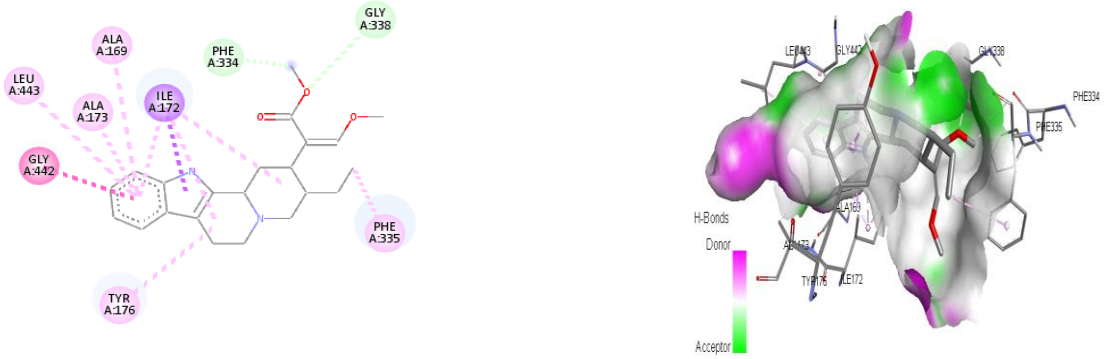
Citalopram (Positive Control)



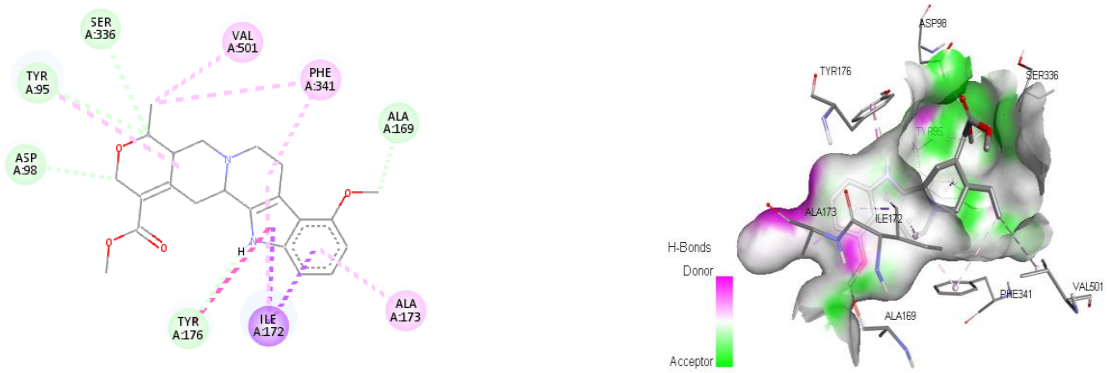
Mitragynine



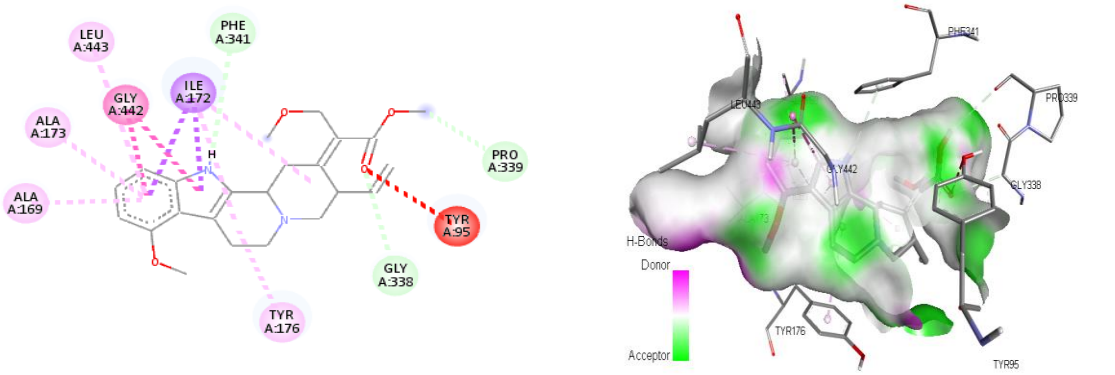
7-Hydroxymitragynine



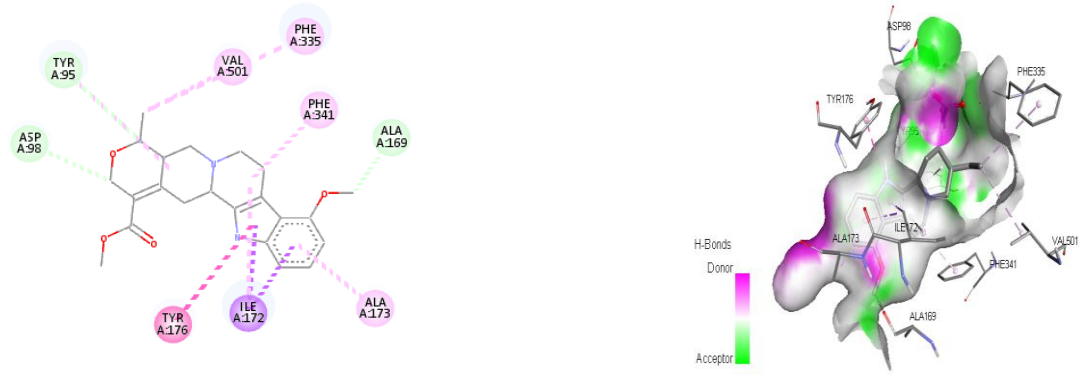
Corynantheidine



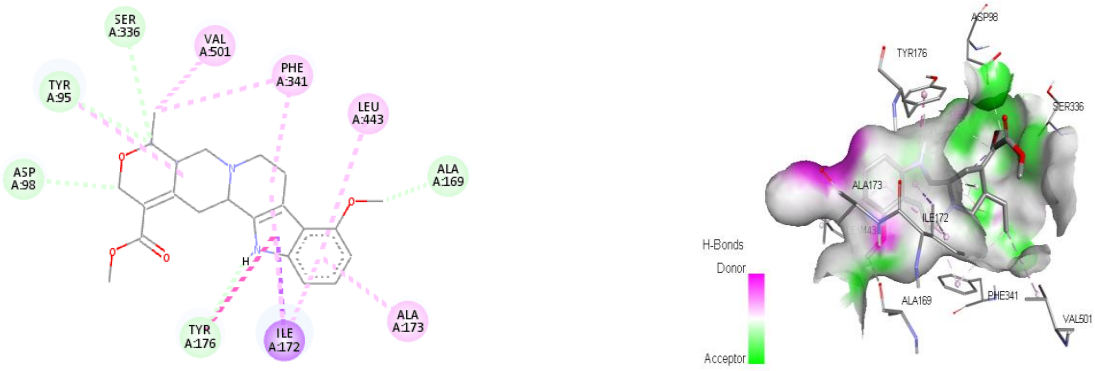
Mitraciliatine



Paynantheine



Speciociliatine



Speciogynine

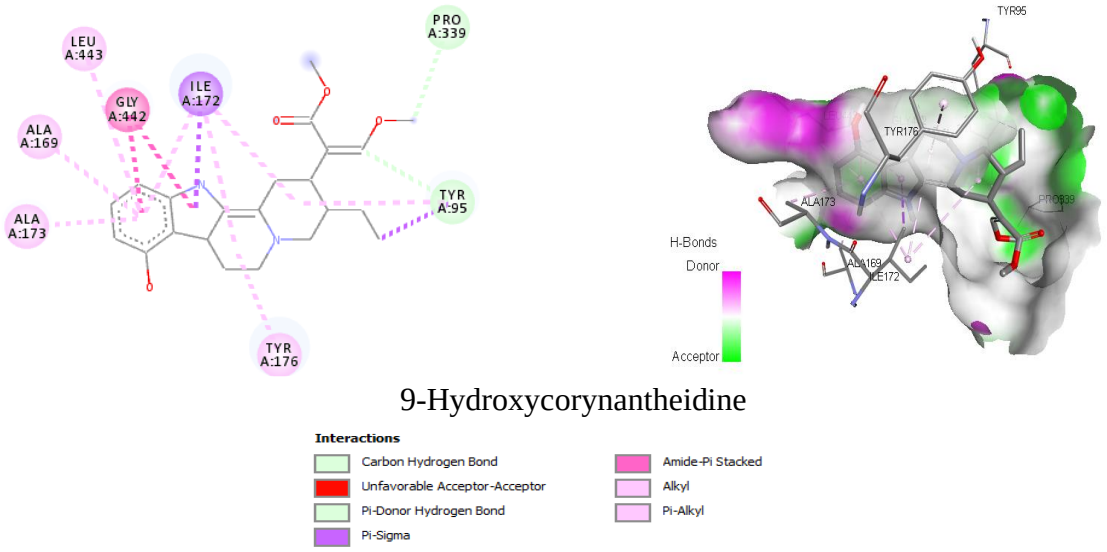
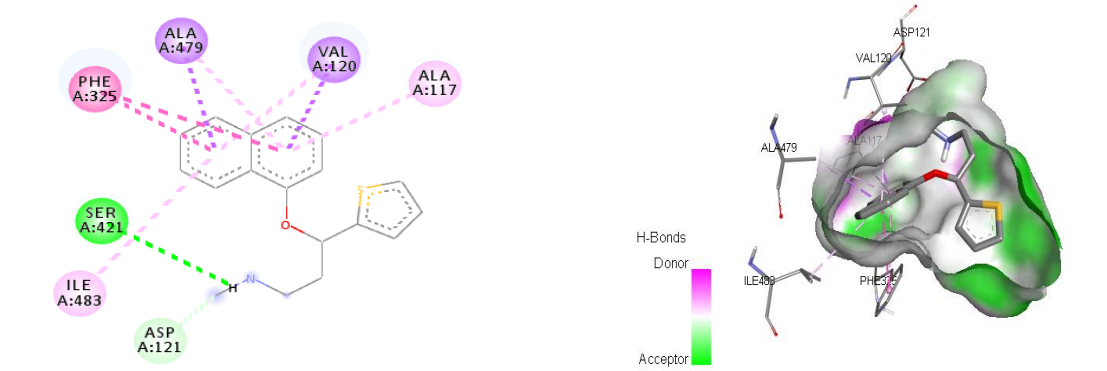
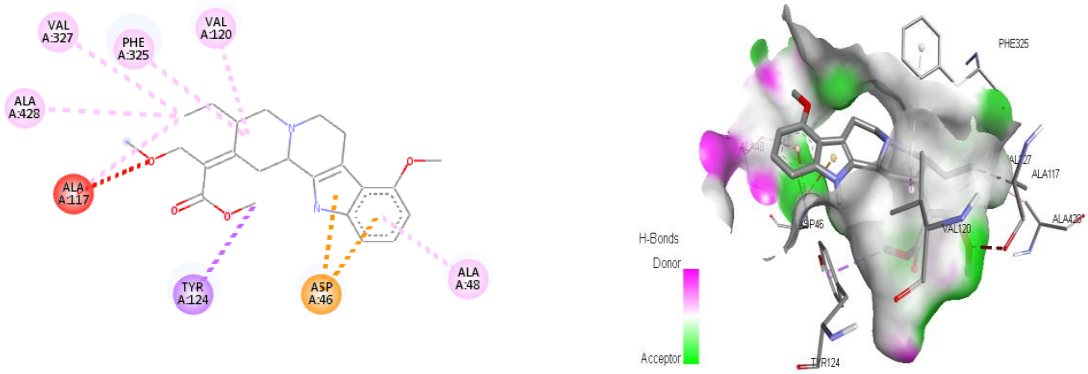
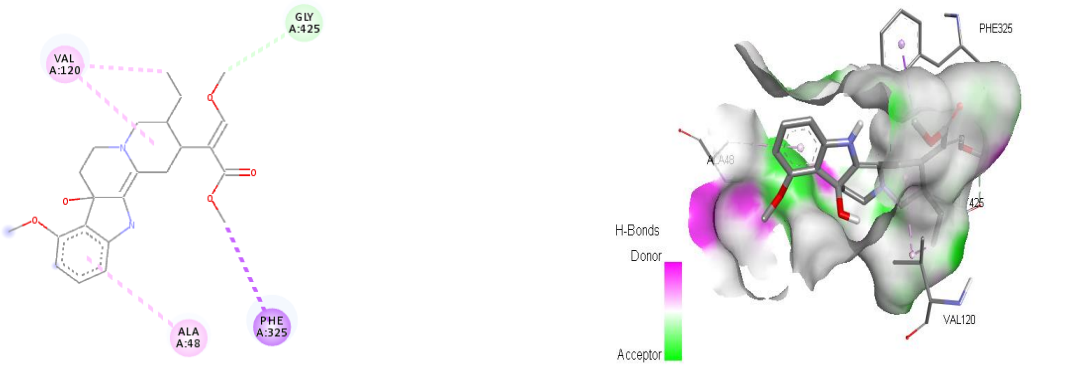


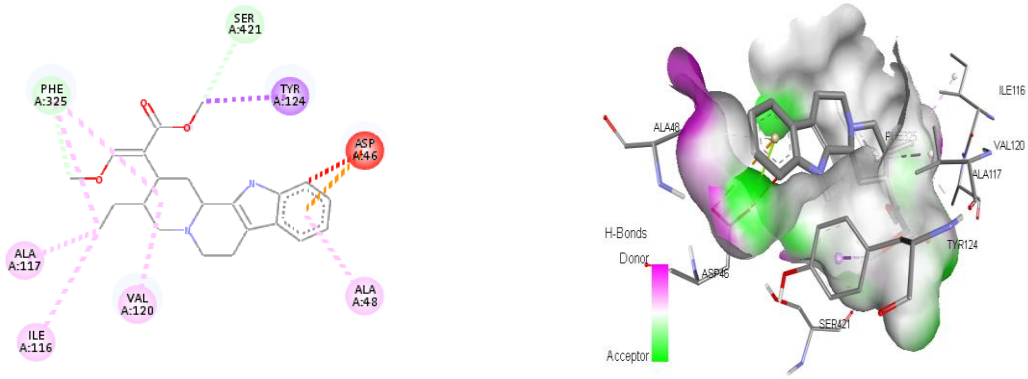
Figure 2. Visualization 2-D and 3-D docking study in Serotonin Transporter Protein (SERT)



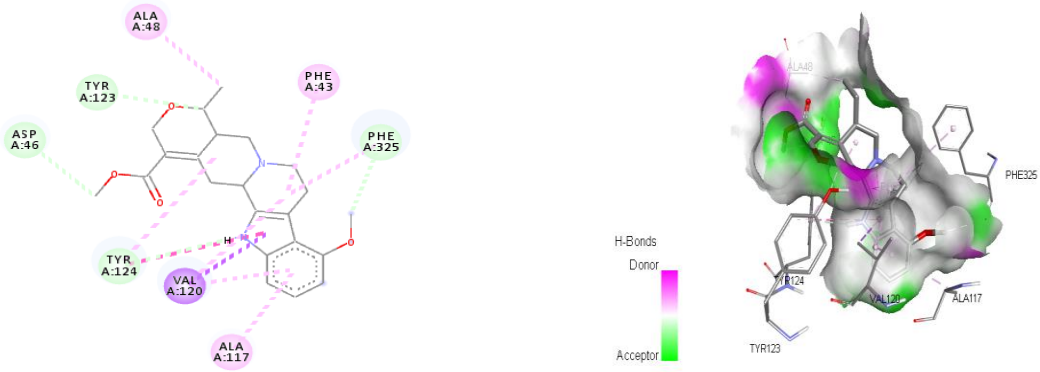




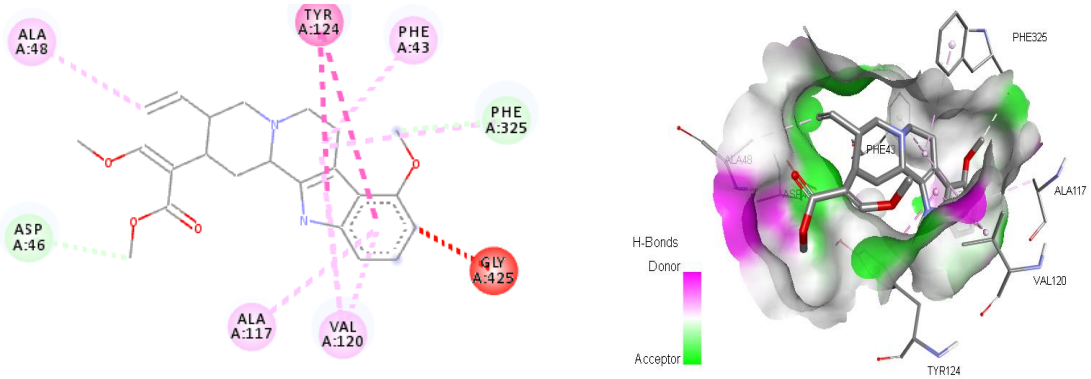
7-Hydroxymitragynine



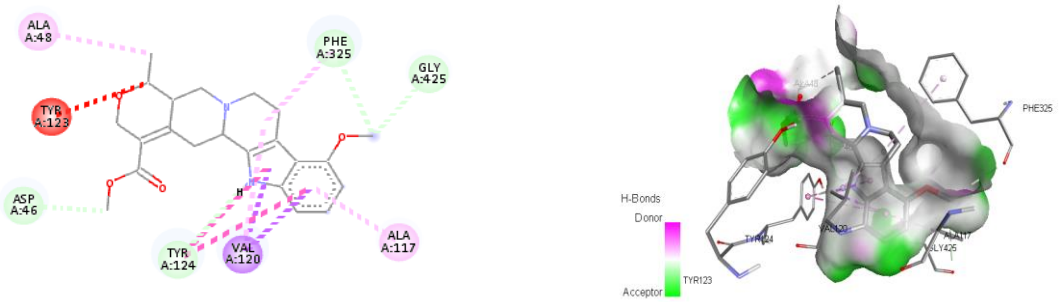
Corynantheidine



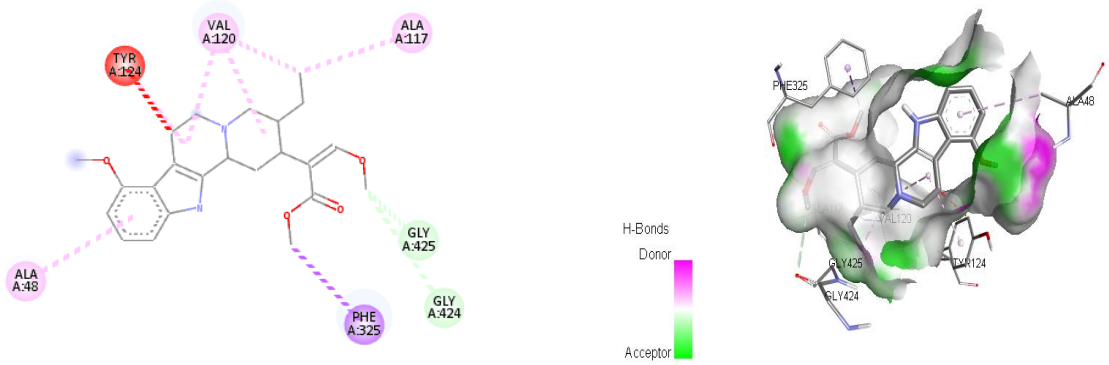
Mitraciliatine



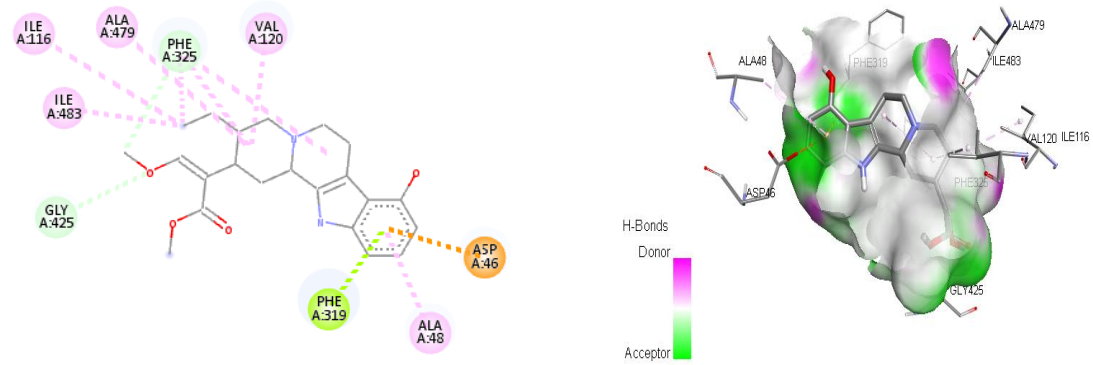
Paynantheine



Speciociliatine

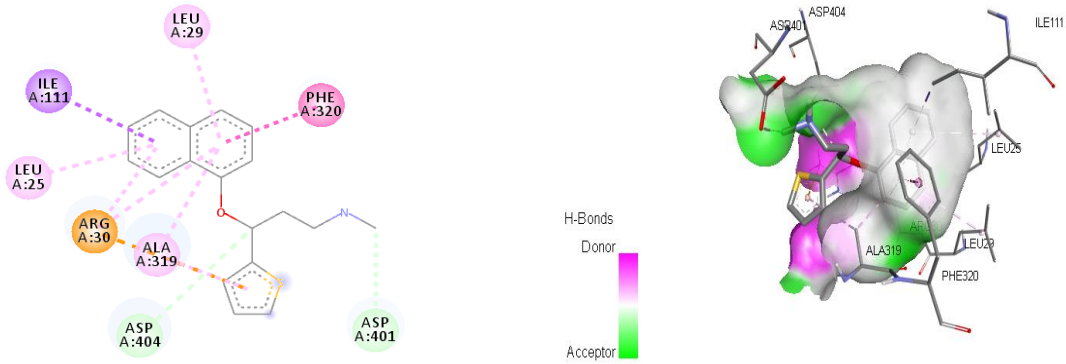


Speciogynine

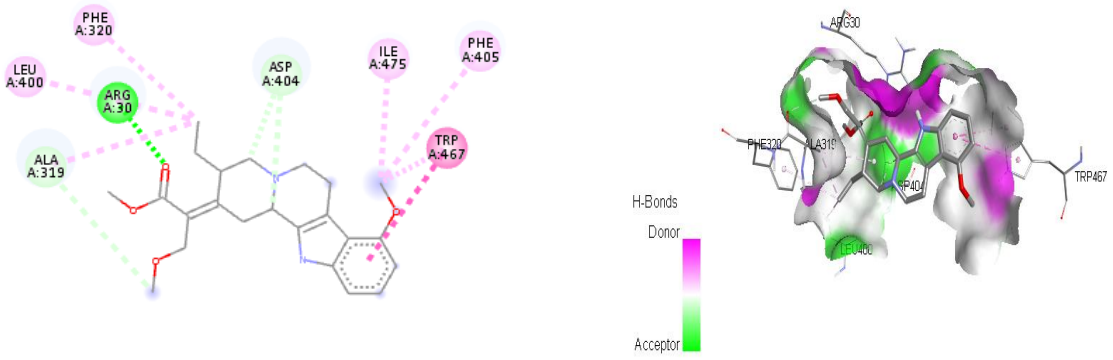


9-Hydroxycorynantheidine

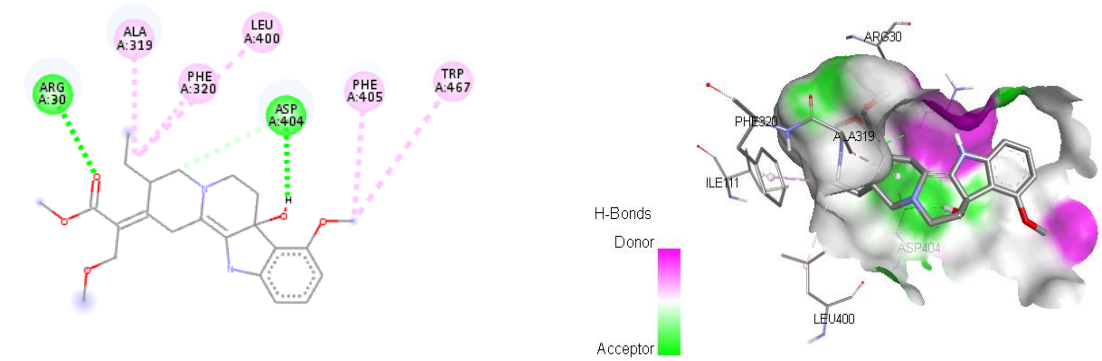
Figure 3. Visualization 2D and 3D of study docking in Dopamine Transporter Protein (DOPAT)



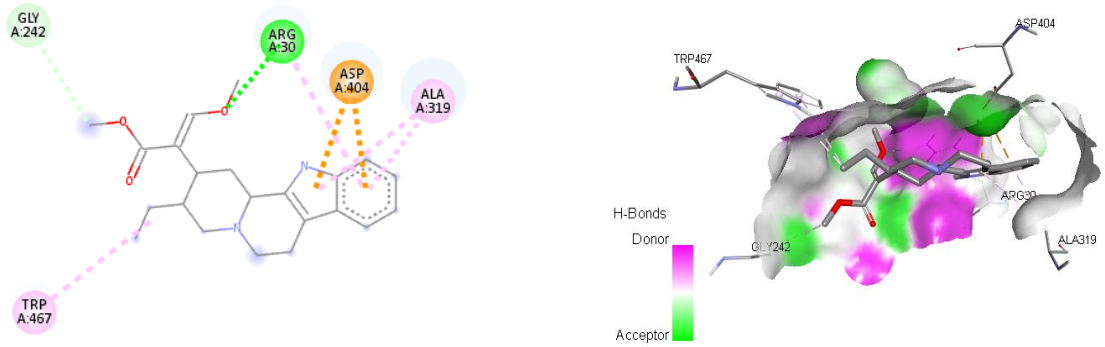
Duloxetine (Positive Control)



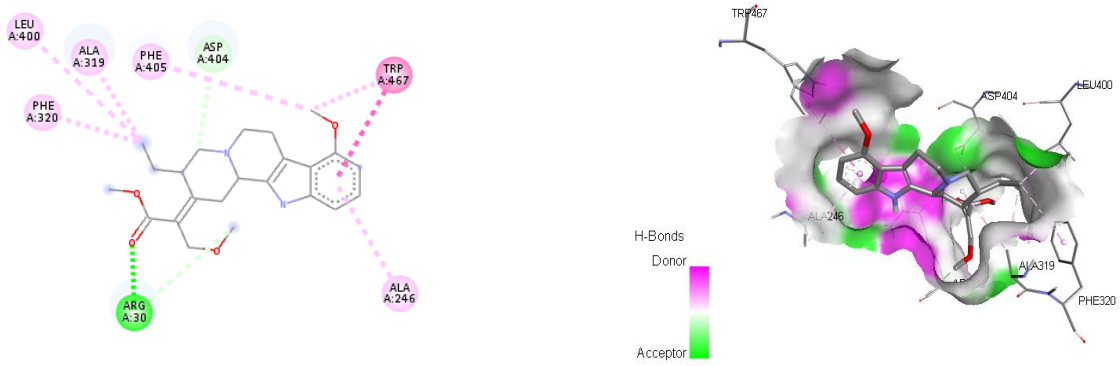
Mitragynine



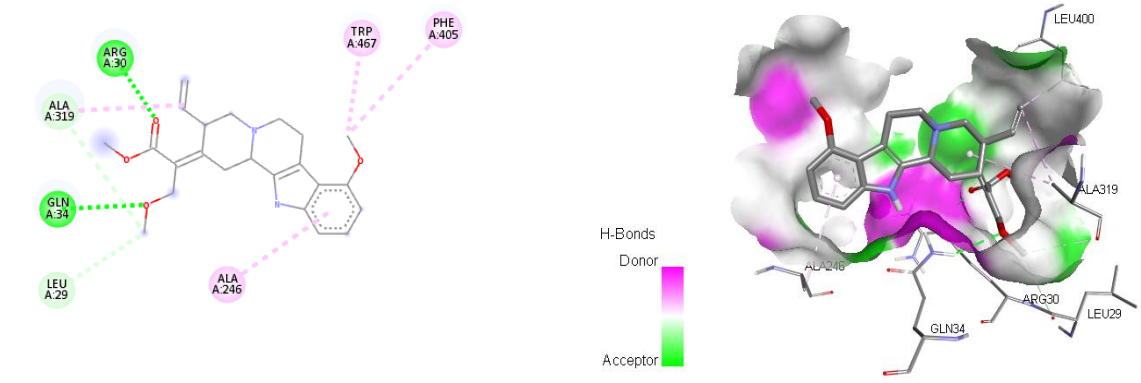
7-Hydroxymitragynine



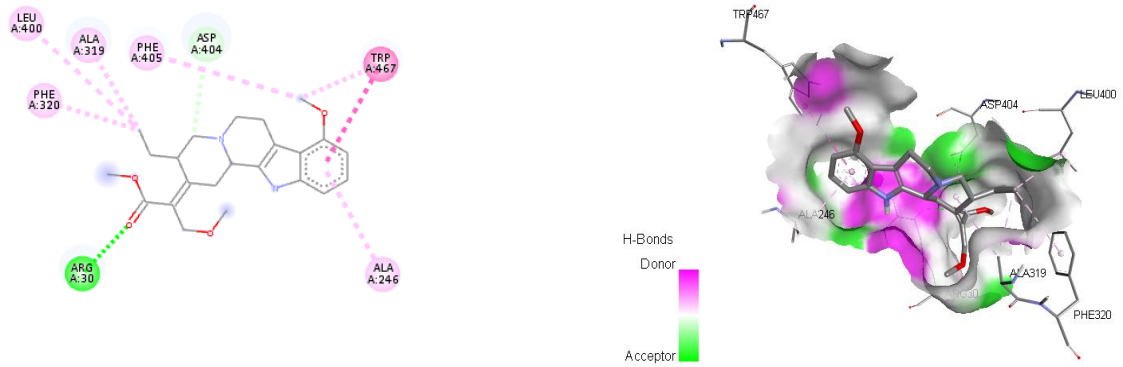
Corynantheidine



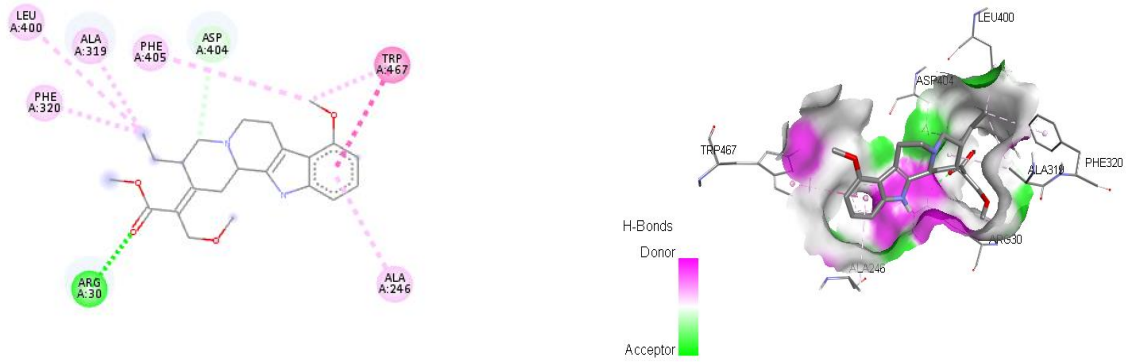
Mitraciliatine



Paynantheine



Speciociliatine



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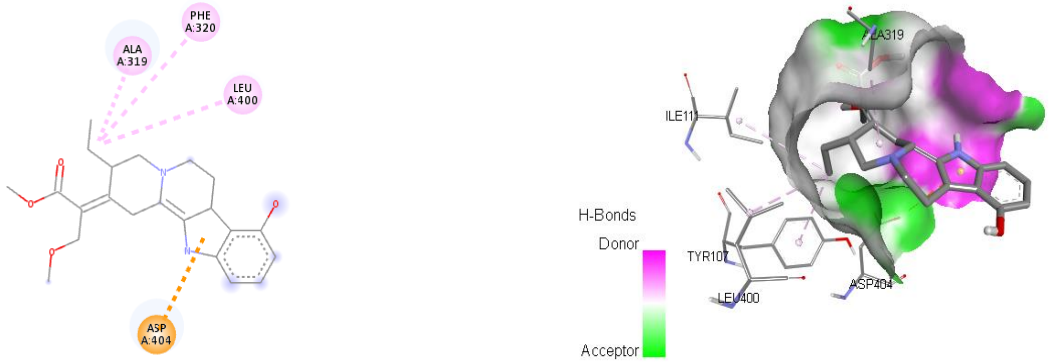


9-Hydroxycorynantheidine

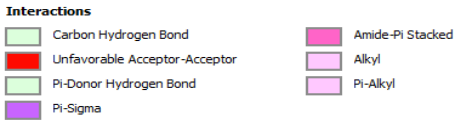
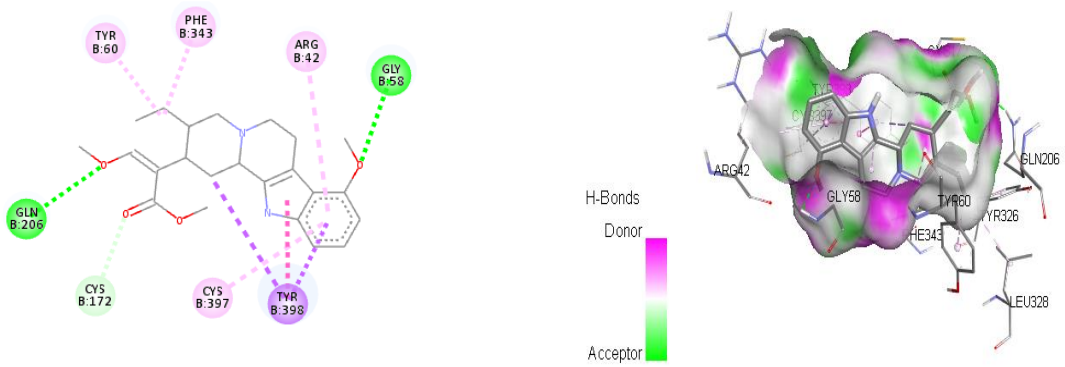
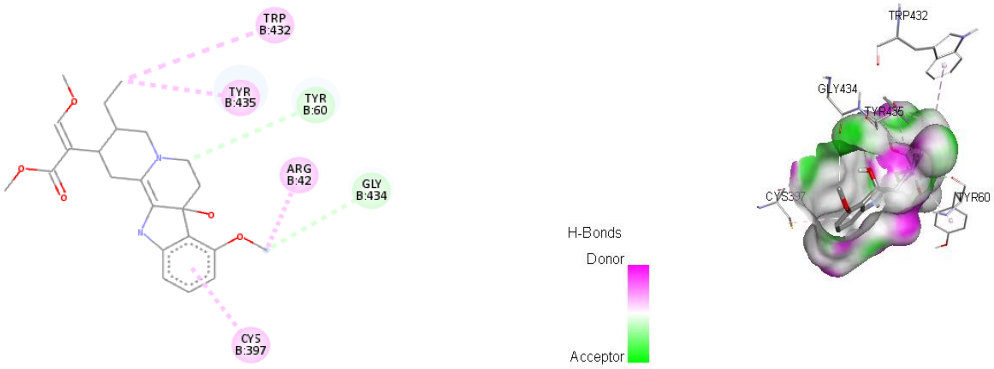


Figure 4. Visualization of study docking in Leucine Transporter (LEUT)

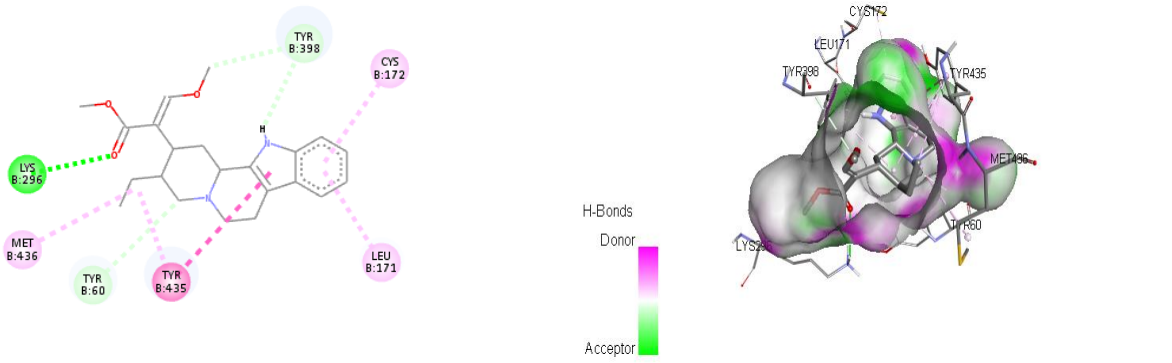
Tranylcypromine (Positive Control)



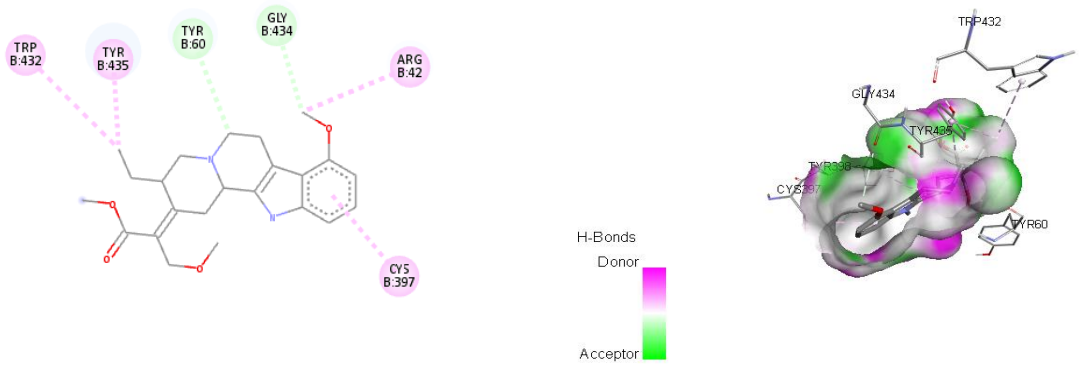
Mitragynine



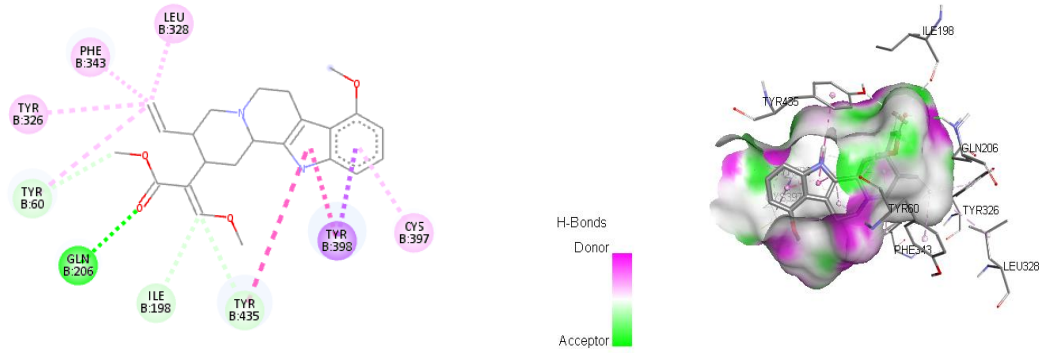
7-Hydroxymitragynine



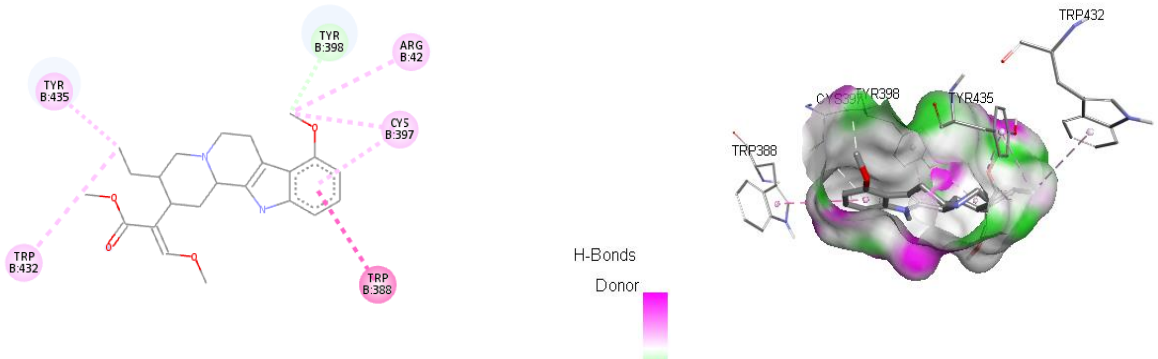
Corynantheidine



Mitraciliatine



Paynantheine



Speciociliatine

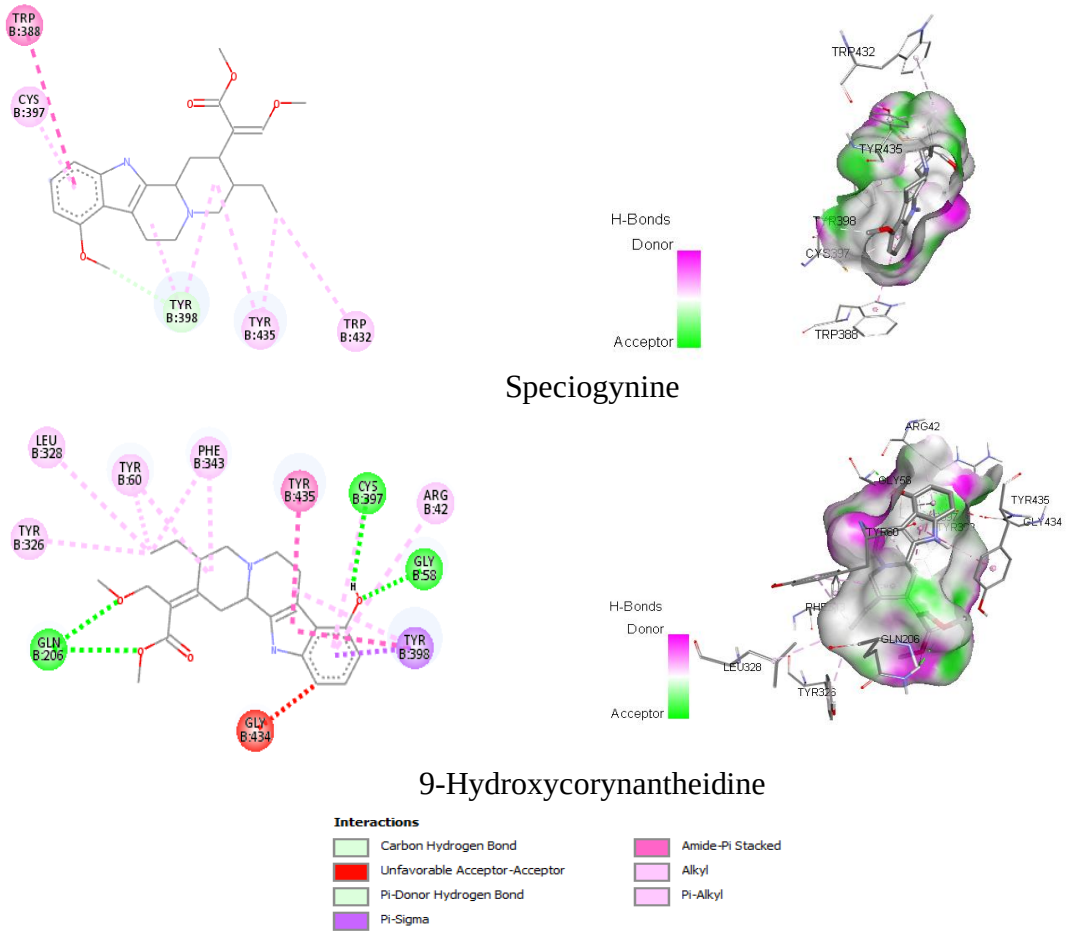


Figure 5. Visualization 2D and 3D docking study in Monoamine Oxidase (MAO)

The next step is to check the interaction relationship of the test ligand to the receptor using Discovery Studio. This aims to see the similarity of amino acid residues between the test ligand and the drug control. The interaction of drug control with Serotonin Transporter (SERT) binds H-donor to the amino acids proline (PRO) and phenylalanine (PHE). It involves alkyl bonds to the amino acid alanine (ALA). In Dopamine Transporter (DopaT), it binds H-donor to aspartic acid (ASP) and involves pi-orbital bonds on the amino acids phenylalanine (PHE), alanine (ALA), valine (VAL), and isoleucine (ILE). The Leucine Transporter (LeuT) binds H-Donor to aspartic acid (ASP). It involves Pi-Orbitals bonds to the amino acids phenylalanine (PHE), alanine (ALA), leucine (LEU), and arginine (ARG). In Monoamine Oxidase (MAO), it binds H-donor to the amino acids serine (SER) and tyrosine (TYR) and involves Pi-orbital bonds to the amino acid tyrosine (TYR). Based on the amino acid residues present in the eight alkaloid compounds in kratom, it can be concluded that the ligands tested for the alkaloid compounds in kratom interact with amino acid residues similar to those of the drug control.

From the visualization of the bond interaction between the ligand and the receptor using DiscoveryStudio, corynantheidine has the highest number of bonds with nine amino acid residues that enter the active site of Serotonin Transporter (SERT), 7-hydroxymitraginine has the highest number with eight types of

amino acid residue bonds that enter the active site of Norepinephrine Transporter via Dopamine Transporter (DopaT), Mitraciliatine, Speciociliatine, and Speciogynine have the highest number with eight amino acid residue bonds entering the active site of Norepinephrine Transporter via Leucine Transporter (LeuT), and 9-Hydroxychorinanteidin has the highest number with eleven amino acid residue bonds entering the active side of Monoamine Oxidase (MAO). The eight alkaloid compounds in kratom can meet pharmacodynamic parameters and are predicted to be antidepressant candidates through the Serotonin Transporter (SERT), Norepinephrine Transporter (NET), and Monoamine Oxidase (MAO). Based on in silico testing that has been carried out using eight alkaloid compounds in kratom, the best interactions were shown in Mitraciliatine compounds towards Serotonin Transporter (SERT) proteins compared to Norepinephrine Transporter (NET) and Monoamine Oxidase (MAO).

Conclusion

The lowest affinity value (docking score) was shown by the test ligand corynantheidine of -8.6 kcal/mol at the Serotonin Transporter (SERT) receptor. For Norepinephrine Transporter via Dopamine Transporter (DopaT), the lowest affinity value was shown for the 7-hydroxy mitragynine test ligand of -7.7 kcal/mol, while for Leucine Transporter (LeuT), the lowest affinity value was shown for Mitracilillatin, Speciociliatine, and Speciogynine test ligands. Of -7.4 kcal/mol. For Monoamine Oxidase (MAO), the lowest affinity value was shown by corynantheidine at -7.7 kcal/mol. The lowest affinity value indicates the binding affinity energy between the protein and the ligand. The lower the docking score, the lower the affinity energy, and the higher the stability of protein and ligand bonds, the more likely it will have good activity.

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