

In silico study of potential alkaloid compounds in Kratom Leaves (*Mitragyna speciosa*) as CNS stimulant

Anggi Nur Prasetyo^{1*}, Sentot Purwandi¹, Sri Rahayu Dwi Purnaningtyas¹, Luluk Aniqoh Meliana Putri¹, Devita Riafinola Andaririt¹

¹Faculty of Pharmacy, Universitas STRADA Indonesia, Kediri, Indonesia

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Abstract: Dysfunction of Central Nervous System (CNS) is often associated with various neurological and psychiatric disorders. The use of stimulant can improve CNS function. Kratom leaves (*Mitragyna speciosa*) are predicted to have potential as CNS stimulant candidate through the inhibition of dopamine transporter (DAT) receptor. The objective of this study is to determine potential of alkaloid compounds contained in kratom leaves as CNS stimulant using in silico approach. This study was conducted in silico through the prediction of physicochemical properties (SwissADME), pharmacokinetic profiles (pkCSM), and molecular docking. The target protein used for DAT receptor was SLC6A3 with PDB ID: 8Y2C and 9EO4. The results showed that five test compounds met Lipinski's Rule of Five. Pharmacokinetic predictions show that five test compounds have good pharmacokinetic profiles, with favorable absorption and distribution, although some compounds have potential to cause drug interactions and certain toxicities. *Mitragynine*, *Paynantheine*, *Speciogynine*, and *Speciociliatine*, have potential as CNS stimulants by inhibiting the DAT receptor, with binding energies ranging from -6,2 to -8,3 kcal/mol. Four of the five alkaloids in kratom leaves are predicted to have potential as CNS stimulants by inhibiting DAT receptors in silico.

Keywords: Stimulant; DAT; Kratom (*Mitragyna speciosa*); In Silico; Docking.

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Introduction

Central Nervous System (CNS) plays a crucial role in human health and function (Nasution et al., 2025). The central nervous system is responsible for storing information and controlling all bodily activities (Rahmi et al., 2018). Dysfunction of CNS is often associated with various neurological and psychiatric disorders. According to World Health Organization (WHO) 2021, neurological disorders affect more than 3 billion people worldwide, with 6.8 million deaths annually. One way to improve CNS function is through the use of stimulant medications.

Central Nervous System stimulant is drugs capable of stimulating the cerebral cortex and spinal cord, with dopamine transporter (DAT) receptors serving as the primary targets of these medications (Nepal et al., 2023; Reith et al., 2022). By targeting DAT receptors, they can disrupt or inhibit dopamine transport and thereby disrupt dopaminergic signaling (Newman et al., 2021).

Indonesia is a developing country with a wide variety of medicinal plants that can be used to treat various diseases. The country ranks second globally in terms of natural resources, with approximately 30,000 plant species, representing 75% of the world's plant species (Suliasih & Mun'im, 2022). One plant with

Email: anggiprasetyo984@gmail.com (*Corresponding Author)

potential for research as Central Nervous System (CNS) stimulant is Kratom (*Mitragyna speciosa*). In Thailand, kratom leaves have been used to produce stimulant effects similar to opium and coca, which can reduce fatigue, enhance tolerance to sunshine, and alleviate psychiatric disorders such as anxiety and sleep disturbances (Prasetio, 2020).

The active chemical compounds found in kratom leaves and stems include alkaloids, steroids, quinones, saponins, and tannins (Sofia et al., 2022). The kratom plant contains more than 40 types of alkaloids, primarily *Mitragynine* (66.2%) and its derivatives, *Paynantheine* (8.6%), *Speciogynine* (6.6%), *7-Hydroxymitragynine* (2%), and *Speciociliatine* (0.8%) (Firmansyah et al., 2021). *Mitragynine* and *7-Hydroxymitragynine* are the primary alkaloids in kratom leaves, accounting for 1–2% of the dry leaf weight and constituting up to two-thirds of the total alkaloids (Kruegel & Grundmann, 2018).

In silico studies are computational approaches used in drug discovery and development, offering a cost-effective and time-efficient alternative to traditional methods (Al-Mohaya et al., 2024). In this method, binding energy, RMSD (Root Mean Square Deviation), interactions, and the types of amino acid residue bonds formed with enzymes/proteins will be examined (Dastmalchi et al., 2016). Thus, this study aims to determine the potential of alkaloid compounds contained in kratom leaves (*Mitragyna speciosa*) as CNS stimulants using an in silico approach.

Materials and Methods

Instrument and Material

The instruments used in this study were a set of laptop with specifications Intel® Core™ i3-4005U 1.70 GHz processor RAM 4.00 GB and Windows 10 64-bit, and also Software PyRx 0.8 version for docking, PyMOL for validation, and Biovia Discovery Studio 2024 for preparation and visualization. In addition, SwissADME website (<http://www.swissadme.ch/>) is used to predict physicochemical properties, and pkCSM (<https://biosig.lab.uq.edu.au/pkcsm/prediction>) is used to predict the pharmacokinetic properties of compounds.

The data used in this study were .sdf files or ligands for each of the test compounds, namely *7-Hydroxymitragynine*, *Mitragynine*, *Paynantheine*, *Speciogynine*, *Speciociliatine*, and comparison compound (Cocaine), downloaded from PubChem (www.pubchem.ncbi.nlm.nih.gov). Then, .pdb files or 3D structures of the SLC6A3 target protein were downloaded from (www.rcsb.org).

Prediction Physicochemical Properties

The test compounds were obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in the form of SMILES codes. These SMILES codes were then copied and pasted into the SwissADME website (<http://www.swissadme.ch/>) to predict physicochemical properties using the features available on that site (Muslikh et al., 2025).

Prediction Pharmacokinetic Properties

The test compounds were obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in the form of SMILES codes. These SMILES codes were then copied and pasted into the pkCSM website (<https://biosig.lab.uq.edu.au/pkcsm/prediction>). The program automatically calculates the predicted values for the selected parameters (Herdini, 2023).

Ligand and Protein Preparation

The preparation was performed by removing water molecules or H₂O ("Scripts" → Selection → Select Water Molecules → checkbox), separating the natural ligand structures from their proteins ("Scripts" → Selection → Select Ligands → checkbox), and adding polar hydrogen groups ("Chemistry" → Hydrogens → Add Polar) using Biovia Discovery Studio 2024, then saved in Protein Data Bank (.pdb) format.

The test and comparison ligands were downloaded from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>) in .sdf file format in 3D Conformer, then the ligands were prepared using Biovia Discovery Studio 2024 by saving the file (Save As → .pdb). The prepared ligands were saved in .pdb format (Makisake et al., 2022).

Molecular Docking

Molecular docking was performed using PyRx 0.8 by selecting the "Start" menu, entering ligand data through the "ligands" menu and protein data through the "macromolecules" menu, then selecting the ligand-protein pair to be docked. To determine where the ligand will bind to the protein, use the "Forward" option in the bottom right corner. The first step in the docking process is to select the "Run Vina" menu. The molecular docking process was performed using a gridbox and energy minimization parameters based on the validation results. Docking results are saved in *.csv, which displays binding energy values, and *.pdb, which displays a visualization of protein-ligand docking, including the types of bonds formed and amino acids that serve as binding sites (Mastura et al., 2020).

Validation and RMSD Value Assignment

Validation of docking results was performed using PyMOL software by comparing native ligand structure (*Cocaine*) with redocking result structure, using the following command-line syntax: “align native_ligand, redocking_result, cycles=0, transform=0”. RMSD (Root Mean Square Deviation) is the result of comparative data analysis (Mardianingrum et al., 2021). The RMSD is calculated based on the distance between corresponding atom pairs and then averaged across all compared atoms. A docking method is considered validated if the obtained RMSD value is <2 Å (Saputra et al., 2024).

Visualization Docking Result

Using Biovia Discovery Studio 2024, both 2D and 3D visualizations are performed by importing target protein data and docking results in *.pdb format. The types of binding interactions formed and the amino acids that ultimately serve as binding sites will be visible. The *.jpg format is used to save the visualization results (Mastura et al., 2020).

Data Analysis

Analysis of docking results to identify interactions between the receptor and the ligand. A lower binding energy value indicates a stronger affinity between the ligand and the receptor (Tahir, 2024). The RMSD value is considered stable and valid if it is less than 2 Å (Saputra et al., 2024).

Result and Discussion

Physicochemical Properties

Predictions of physicochemical properties were made using the SwissADME website by entering the SMILES code of the test compound. Parameters included in Lipinski's Rule of Five (Ro5) include MW, log P, HBD, and HBA (Az-Zahra et al., 2022).

Table 1. Predicted Physicochemical Properties

	Lipinski's Ro5	7-OH	MG	PN	SG	SC
MW	< 500	414,5	398,5	396,5	398,5	398,5
HBA	< 10	7	5	5	5	5
HBD	< 5	1	1	1	1	1
LogP	< 5	3,87	3,77	3,64	3,74	3,73
Complies with Lipinski's Ro5	Yes	Yes	Yes	Yes	Yes	Yes

Notes: MW = Molecular Weight; HBA = Hydrogen Bond Acceptor; HBD = Hydrogen Bond Donor; LogP = Lipophilicity; 7-OH = 7-Hydroxymitragynine; MG = Mitragynine; PN = Paynantheine; SG = Speciogynine; and SC = Speciociatine.

Based on the results of the analysis using Lipinski's Rule of Five, all five alkaloid compounds in

the test kratom leaves, namely 7-Hydroxymitragynine, Mitragynine, Paynantheine, Speciogynine, and Speciociatine met Lipinski's Rule of Five (Table 1). This reflects the molecules' tendency to form hydrogen bonds, either directly or indirectly, thereby exhibiting good compound permeability via passive diffusion mechanisms through the lipid bilayer of the target, making them potential candidates for development as oral drugs (Lipinski et al., 1997 in Wulandari et al., 2023).

Pharmacokinetic Properties

Pharmacokinetic properties were predicted using the pkCSM website by entering the SMILES code of the test compound. The parameters analyzed were Absorption, Distribution, Metabolism, Excretion, and Toxicity (Table 2).

Table 2. Predicted Pharmacokinetic Properties

Prop	Model Name	7-OH	MG	PN	SG	SC	Unit
Absorption	Water solubility (> -6)	-3,91	-4,18	-4,16	-4,18	-4,18	log mol/L
	Caco2 permeability (> 0,90)	1,04	0,91	1,14	0,91	0,91	log Papp in 10-6 cm/s
	Human Intestinal absorption (> 80%)	93,5	92,9	93,9	92,9	92,9	% Absorbed
	Skin Permeability (< -2,5)	-3,46	-3,19	-2,97	-3,19	-3,19	log Kp
	P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	(Yes/No)
	P-glycoprotein I inhibitor	Yes	Yes	Yes	Yes	Yes	(Yes/No)
Distribution	P-glycoprotein II inhibitor	No	Yes	Yes	Yes	Yes	(Yes/No)
	VDss Human (> -0,15)	0,75	1,14	0,92	1,14	1,14	log L/kg
	Fraction unbound (human)	0,32	0,19	0,31	0,19	0,19	Fu
	BBB permeability (> 0,3)	-0,21	0,35	-0,20	0,35	0,35	log BB
	CNS permeability (> -2)	-2,47	-2,27	-2,22	-2,27	-2,27	log PS
	CYP2D6 substrate	No	No	No	No	No	(Yes/No)
Metabolism	CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes	(Yes/No)
	CYP1A2 inhibitor	No	No	No	No	No	(Yes/No)

Prop	Model Name	7-OH	MG	PN	SG	SC	Unit
	CYP2C19 inhibitor	No	No	No	No	No	(Yes/No)
	CYP2C9 inhibitor	No	No	No	No	No	(Yes/No)
	CYP2D6 inhibitor	No	Yes	Yes	Yes	Yes	(Yes/No)
	CYP3A4 inhibitor	No	Yes	No	Yes	Yes	(Yes/No)
Excretion	Total Clearance	0,78	0,86	0,97	0,86	0,86	log ml/min/kg
	Renal OCT2 substrate	Yes	Yes	No	Yes	Yes	(Yes/No)
Toxicity	AMES toxicity	No	No	No	No	No	(Yes/No)
	Max. tolerated dose (human)	-0,47	-0,45	-0,54	-0,45	-0,45	log mg/kg/day
	hERG I inhibitor	No	No	No	No	No	(Yes/No)
	hERG II inhibitor	No	Yes	Yes	Yes	Yes	(Yes/No)
	Oral Rat Acute Toxicity (LD50)	2,96	3,16	2,88	3,16	3,16	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	1,58	0,31	0,46	0,31	0,31	log mg/kg_bw/day
	Hepatotoxicity	Yes	Yes	Yes	Yes	Yes	(Yes/No)
	Skin Sensitisation	No	No	No	No	No	(Yes/No)
	T.Pyriformis toxicity	0,50	0,78	0,85	0,78	0,78	log ug/L
	Minnow toxicity	0,91	-0,76	-0,68	-0,76	-0,76	log Mm

Notes: 7-OH = 7-Hydroxymitragynine; MG = Mitragynine; PN = Paynantheine; SG = Speciogynine; and SC = Speciociliatine.

The absorption analysis showed that all five compounds have good absorption profiles, as indicated by high intestinal absorption values (>90%) and Caco2 permeability values (>0,90), reflecting effective absorption and intestinal membrane permeability (S. W. Sari *et al.*, 2023; Nursanti, 2023). All compounds also exhibited good water solubility (log S > -6) (Pires *et al.*, 2015 in Inayah *et al.*, 2024). However, their negative skin permeability (log Kp < -2.5) indicates low skin penetration, suggesting limited suitability for transdermal administration (Sari *et al.*, 2023).

The distribution analysis showed that all five compounds have high VDss values (> 0,45), indicating good distribution into body tissues (Nursanti, 2021; Pires *et al.*, 2015 in Wibisono & Martino, 2023). 7-Hydroxymitragynine and Paynantheine exhibited higher unbound fraction values than the other compounds, suggesting a greater proportion of drug available to

interact with receptors (Watanabe *et al.*, 2018 in Nofianti *et al.*, 2024). Mitragynine, Speciogynine, and Speciociliatine showed BBB permeability values > 0,3, indicating easy penetration of the blood-brain barrier, whereas 7-Hydroxymitragynine and Paynantheine had negative values (Pires *et al.*, 2015 in Inayah *et al.*, 2024). In addition, all compounds had CNS permeability values below -2, indicating low penetration into the central nervous system (Pires *et al.*, 2015 in Inayah *et al.*, 2024). These properties indicate its potential as a candidate compound for the development of therapies targeting the central nervous system, including neurodegenerative diseases or central nervous system disorders (Darianudin *et al.*, 2026). However, the low predicted CNS permeability suggests the need for further verification through experimental research.

The metabolic profiling analysis showed that all five compounds are predicted to be CYP3A4 substrates rather than CYP2D6 substrates, indicating that CYP3A4 is the primary enzyme involved in their metabolism (Esteves *et al.*, 2021; Inayah *et al.*, 2024). None of the compounds were predicted to inhibit CYP1A2, CYP2C19, or CYP2C9, suggesting a low risk of interactions through these enzymes. However, Mitragynine, Speciogynine, and Speciociliatine are predicted to inhibit CYP2D6 and CYP3A4, indicating a greater potential for drug interactions. Both of these enzymes play a key role in drug metabolism in the liver. This interaction can lead to potential drug-drug interactions if the compound is taken concurrently with other medications that are also metabolized by the same enzymes, such as certain antidepressants, opioids, or anticoagulants (Wilapangga *et al.*, 2025).

In the analysis of excretion profile results, the total clearance values of the five compounds were not significantly different. Paynantheine had the highest value, meaning the drug is eliminated more rapidly (with a shorter duration of action) compared to the other four compounds. The higher the total clearance value, the faster the concentration of a drug compound decreases in the blood (Follman & Morris, 2018 in Inayah *et al.*, 2024). Of the five compounds, only paynantheine is not affected by the renal transporter protein OCT2, therefore, it can be predicted that this compound is not an OCT2 substrate that can influence excretion via urine (Pramudiyawati *et al.*, 2024).

The toxicity analysis showed that none of the five compounds were predicted to be mutagenic in the AMES test, and only 7-Hydroxymitragynine was not an hERG II inhibitor, indicating no effect on hERG II ion channels associated with cardiac arrhythmia (Pramudiyawati *et al.*, 2024). All compounds were predicted to be hepatotoxic but non-sensitizing to the skin. Hepatotoxicity is a condition characterized by liver damage. Kratom use likely does not directly cause

significant liver damage, although the risk remains, particularly with long-term use or high doses (Wilapangga et al., 2025). Their MTD values were relatively similar, indicating comparable dose tolerance. Based on LD50 values, *Mitragynine*, *Speciogynine*, and *Speciociliatine* showed lower acute toxicity, although their lower LOAEL values suggest a greater potential for long-term toxic effects (Pires et al., 2015). All compounds also exhibited high toxicity toward *Tetrahymena pyriformis* but low toxicity toward freshwater fish (minnow), indicating relatively low environmental risk (Pires et al., 2015 in Wilapangga & Arif, 2025).

Preparation of Target Proteins

The preparation was performed using Biovia Discovery Studio 2024 software, with target proteins downloaded from RCSB Protein Data Bank's website. The results of the preparation were saved in Protein Data Bank file format (.pdb) (Figure 1).

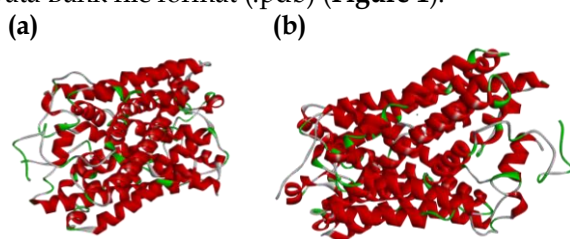


Figure 1. SLC6A3 Protein Preparation Results: (a) 8Y2C; (b) 9EO4.

Molecular Docking

Molecular docking was performed using PyRx 0.8 software. The docking results will display the binding energy values, which will be validated using PyMOL software; the RMSD (Root Mean Square Deviation) value is the result of this validation. Before validation, the center of the gridbox is first determined based on the center of the original ligand coordinates, and the gridbox size is determined based on the size of the binding site and the size of the test ligand. The center of the gridbox and its dimensions are listed in Table 3 and 4.

Table 3. Gridbox Center Settings

Gridbox Center			
Macromolecule	X	Y	Z
8Y2C	107.338	108.205	107.9130
9EO4	129.799	126.518	124.5005

Tabel 4. Gridbox Dimension Settings

Gridbox Dimension (Å)			
Macromolecule	X	Y	Z
8Y2C	63.3866	55.6699	76.1140
9EO4	72.0797	53.6417	66.0474

Visualization is then performed to examine the types of bond interactions and the amino acid residues

involved. The bond between the ligand and the receptor becomes more stable and exhibits stronger activity when it has a lower binding energy, and vice versa (Purnaningtyas & Andaririt, 2025). The RMSD value is considered stable and valid if it is less than 2 Å (Saputra et al., 2024). The similarity in amino acid residues between test compound and comparison compound indicates the presence of similar types of interactions, as can be observed from the visualization results (Oktaviani et al., 2024). However, this similarity in binding interactions does not necessarily imply an identical pharmacological mechanism, as further experimental research is needed to determine whether the test compound inhibits dopamine reuptake through a mechanism similar to that of *Cocaine* as comparator (Nielsen et al., 2024).

Table 5. Binding Energy, RMSD, and Amino Acid Residue for 8Y2C

Compounds	Binding energy (kcal/mol)	RMSD (Å)	Amino Acid Residue
<i>Cocaine</i> (Comparator)	-8,4	1,767	SER422, ASP421, TYR156, GLY425, GLY426, LEU80, ASP79, PHE326, VAL328
<i>7-Hydroxymitragynine</i>	-7,6	2,297	-
<i>Mitragynine</i>	-8,1	0,615	ASP421, TYR156, GLY426, GLY425, ASP79, PHE326, VAL328
<i>Paynantheine</i>	-7,9	0,796	TYR156, ASP79, GLY426, PHE326, VAL328
<i>Speciogynine</i>	-8,3	0,783	TYR156, GLY426, ASP79, VAL328, PHE326,
<i>Speciociliatine</i>	-7,4	0,957	SER422, TYR156, GLY426, ASP79, LEU80, VAL328

Table 5 presents the results of molecular docking against the 8Y2C protein structure, using *Cocaine* as a comparison compound, which has a binding energy of -8.4 kcal/mol with an RMSD of 1.767 Å, and interacts with key residues such as ASP79 and TYR156, which are known to play a crucial role in the inhibition mechanism through the stabilization of the outward-open conformation (Nielsen et al., 2024). Among the five test compounds, *Speciogynine* has the binding energy value closest to that of *Cocaine*, followed by *Mitragynine*. Other compounds such as *Paynantheine* and *Speciociliatine* exhibit lower energy, while *7-Hydroxymitragynine* does not yield a valid ligand pose and was therefore not

analyzed further, as indicated by an RMSD value exceeding 2 Å (Amrulloh et al., 2023). This is caused by a lack of physical and chemical constraints in the model, which can lead to issues such as unrealistic bond lengths, geometric errors, and protein-ligand clashes (Buttenschoen et al., 2024). Based on amino acid residue similarity, the compounds *Mitragynine*, *Paynantheine*, *Speciogynine*, and *Speciociliatine* exhibit similar interactions with *Cocaine* at key residues, particularly ASP79 and TYR156, which are important components of the orthosteric site (S1) (Nielsen et al., 2024). However, *Speciogynine* is superior because its binding energy is nearly identical to that of *Cocaine*, indicating a very similar strength of interaction. This suggests that *Speciogynine* is likely capable of occupying the binding pocket with an orientation and affinity similar to *Cocaine*, thereby potentially stabilizing the transporter in the outward-open conformation more strongly (Hospital et al., 2024). The binding pocket is a protein surface that forms a cavity or depression, typically surrounded by amino acid residues that form the active site in an enzyme or the binding site on a transmembrane receptor (Scott et al., 2022). Meanwhile, *Mitragynine* also exhibits high residue similarity (Figure 2 and 3) and even a smaller RMSD (indicating a more stable conformation), but its interaction strength is slightly weaker compared to *Speciogynine*, as well as *Paynantheine* and *Speciociliatine*.

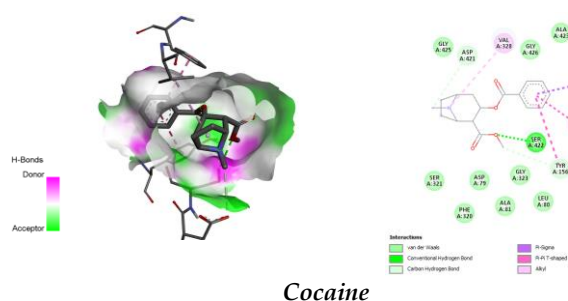


Figure 2. 3D and 2D Visualizations of *Cocaine* Binding to SLC6A3 (8Y2C code)

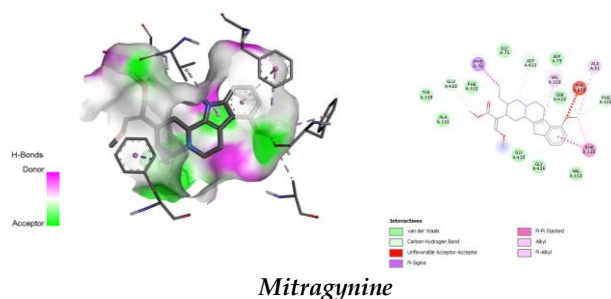


Figure 3. 3D and 2D Visualizations of *Mitragynine* Binding to SLC6A3 (8Y2C code)

Table 6. Binding Energy, RMSD, and Amino Acid Residue for 9EO4

Compounds	Binding energy (kcal/mol)	RMSD (Å)	Amino Acid Residue
<i>Cocaine</i> (Comparator)	-9,2	1,145	TYR156
7-Hydroxy-mitragynine	-8,0	2,319	-
<i>Mitragynine</i>	-7,4	0,617	-
<i>Paynantheine</i>	-7,6	0,580	-
<i>Speciogynine</i>	-8,3	0,933	TYR156
<i>Speciociliatine</i>	-6,2	0,832	-

Table 6 presents the results of molecular docking against the 9EO4 protein code, using *Cocaine* as the comparison compound, with a binding energy of -9.2 kcal/mol and an RMSD of 1.145 Å. All five test compounds have binding energy values greater than the comparison. This indicates that these five compounds have a binding energy that is slightly lower or even weaker toward the target protein SLC6A3 (structure code 9EO4) compared to *Cocaine*. Furthermore, only one compound, *Speciogynine*, shares a single amino acid residue with the comparison compound (Figure 4 & 5), this suggests that *Speciogynine* has the potential to interact with DAT through a binding mode comparable to that of *Cocaine*, specifically through interactions with residues at the S1 binding site. However, the similarity in these interaction models does not directly indicate the presence of the same pharmacological mechanism; therefore, further experimental research is needed to confirm whether *Speciogynine* can inhibit dopamine reuptake through a mechanism similar to that of *Cocaine* (Nielsen et al., 2024).

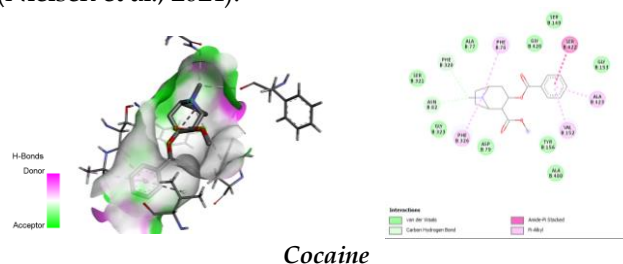


Figure 4. 3D and 2D Visualizations of *Cocaine* Binding to SLC6A3 (9EO4 code)

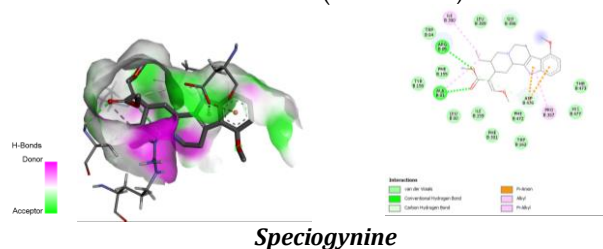


Figure 5. 3D and 2D Visualizations of *Speciogynine* Binding to SLC6A3 (9EO4 code)

Based on analysis of molecular docking results above, *Mitragynine*, *Paynantheine*, *Speciogynine*, and *Speciociliatine* are predicted to have potential as CNS stimulants based on their binding energy values. While *7-Hydroxymitragynine* did not produce a valid ligand pose and therefore could not be analyzed further, as indicated by an RMSD value greater than 2 Å (Amrulloh et al., 2023); consequently, *7-Hydroxymitragynine* does not have the potential to act as a CNS stimulant through DAT receptor inhibition. In the 8Y2C protein, *Mitragynine* exhibits the best balance between binding energy, which is close to that of cocaine, and the number of amino acid residues most similar to those of cocaine; thus, it has the potential to possess a comparable binding site. However, further experimental research is needed to confirm whether this compound inhibits dopamine reuptake through a mechanism similar to that of *Cocaine*, not only acting as a competitive inhibitor but also disrupting the sodium (Na) ion system and rigidly locking the transporter in an outward-open conformation, thereby completely halting the transport cycle (Hospital et al., 2024). Meanwhile, in 9EO4, *Speciogynine* is the only compound that shows amino acid similarity to *Cocaine*, albeit to a limited extent; however, this is supported by a sufficiently close binding energy, suggesting it may possess a binding site comparable to that of *Cocaine*, albeit weakly.

Conclusion

Four of five test compounds, namely *Mitragynine*, *Paynantheine*, *Speciogynine*, and *Speciociliatine*, are predicted to have potential as CNS stimulants by inhibiting DAT receptors in silico, with binding energies ranging from -6,2 to -8,3 kcal/mol, where *Mitragynine* and *Speciogynine* exhibit more competitive binding energy and high amino acid sequence similarity with *Cocaine* as comparator. Therefore, the alkaloids in kratom leaves may have similar binding sites and could be further developed as candidates for CNS stimulants through experimental research.

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