

Molecular Docking Study Of Secondary Metabolites From Turmeric (*Curcuma longa*) As Potential MAO-A Inhibitors For Antistress Therapy

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Abstract

Monoamine Oxidase A (MAO-A) is a key enzyme in neurotransmitter metabolism, making its inhibition a strategic approach for antistress drug development. Turmeric (Curcuma longa) secondary metabolites possess potential therapeutic properties as MAO-A inhibitors. This study aims to evaluate the potential of turmeric secondary metabolites as MAO-A inhibitors through in silico molecular docking, pharmacokinetic profiling (ADME-Tox), drug-likeness evaluation, and subsequent structural modification of the lead compound. A computational approach was employed using 70 secondary metabolites from Curcuma longa L. Molecular docking was performed via PyRx-AutoDock Vina, followed by ADME-Tox and drug-likeness evaluation using SwissADME, pkCSM, and ToxTree, while structural modification was designed using ChemDraw. Demethylcurcumin emerged as the best candidate among seven top compounds, demonstrating a favorable binding affinity of -9.3 kcal/mol and an RMSD of 1.001 Å. Structural modification yielded a derivative, (4E,6E)-1-(3,4-dihydroxyphenyl)-7-hydroxyocta-4,6-dien-1-one, which exhibited improved drug-likeness, pharmacokinetic profiles, and toxicity parameters compared to the parent compound. Demethylcurcumin shows strong potential as a natural MAO-A inhibitor, and its structural derivative provides an enhanced pharmacological and safety profile for further antistress drug development.

Keywords: ADMET, *Curcuma longa*, Demethylcurcumin, Molecular docking, Monoamine Oxidase A (MAO-A).

INTRODUCTION

Stress and anxiety disorders represent continuously increasing global mental health issues, particularly among adolescents and young adults. Data from the Global Burden of Disease indicate that the prevalence of anxiety disorders has experienced a significant increase over the past few decades. Physiologically, chronic stress triggers dysregulation within the central nervous system, where an elevated activity of the Monoamine Oxidase-A (MAO-A) enzyme plays a detrimental role. MAO-A is an outer mitochondrial membrane-bound enzyme responsible for the oxidative deamination of monoamine neurotransmitters, including serotonin, dopamine, and norepinephrine. Excessive catabolism mediated by overactive MAO-A leads to a substantial depletion of these neurotransmitters within synaptic clefts, directly contributing to the onset of depression, anxiety disorders, and chronic psychological stress. Consequently, the selective and reversible inhibition of MAO-A activity serves as a primary pharmacological target and a strategic therapeutic avenue in the development of modern antistress and antidepressant therapies.

In recent years, the exploration of natural products for neuroprotective and psychotherapeutic agents has gained immense momentum. Indonesia possesses abundant tropical biodiversity and natural resources with immense potential to be developed as novel drug candidates, one of the most prominent being turmeric (*Curcuma longa*). Turmeric has long been utilized in traditional medicine systems and contains a vast array of secondary metabolites such as curcuminoids, monoterpenoids, and sesquiterpenoids which have been widely reported to possess broad biological activities, including antioxidant, anti-inflammatory, and neuroprotective properties. Despite its traditional efficacy, evaluating the exact molecular mechanisms of dozens of individual constituents through conventional wet-lab experimentation remains exceptionally resource-intensive, time-consuming, and costly.

To overcome these limitations, Computer-Aided Drug Design (CADD) approaches specifically in silico molecular docking methodologies combined with robust pharmacokinetic (ADME) and toxicity predictions provide a highly efficient, rapid, and mechanistic strategy for evaluating the therapeutic potential of natural compounds at the molecular level. Molecular docking allows researchers to visualize and quantify the binding orientation, interaction energies, and affinity of phytochemical ligands within the active catalytic pocket of target macromolecules. Furthermore, computational ADME-Tox profiling ensures that prospective drug molecules possess acceptable drug-likeness, high oral bioavailability, favorable blood-brain barrier permeability, and minimal toxicity risks prior to synthesis. Building upon this technological framework, this study aims to comprehensively analyze the therapeutic potential of 70 secondary metabolites derived from *Curcuma longa* as target inhibitors against the MAO-A enzyme in silico, evaluate their comprehensive drug-likeness, ADME profiles, and toxicological safety parameters, and execute rational structural modifications on the selected lead candidate to obtain an optimized, safer, and highly efficacious antistress agent.

RESEARCH METHODS

This study employed an in silico descriptive-analytical approach to evaluate 70 secondary metabolites of turmeric (*Curcuma longa*) retrieved from the PubChem database against the active binding pocket of the Monoamine Oxidase-A (MAO-A) enzyme (PDB ID: 2Z5X).

The computational research was conducted using a standardized hardware configuration consisting of an HP laptop powered by an Intel® Celeron® N4120 CPU @ 1.10 GHz, 4 GB of RAM, and 238 GB of storage running on the Windows 11 operating system. The software ecosystem utilized throughout the workflow included PubChem, Protein Data Bank (PDB), PyRx-AutoDock Vina, VegaZZ, BIOVIA Discovery Studio Visualizer, ChemDraw, and PyMOL, alongside specialized in silico prediction web servers such as SwissADME, pkCSM, and Toxtree. The primary biological and chemical materials comprised the three-dimensional (3D) crystal structure of the target macromolecule Monoamine Oxidase A (MAO-A) complexed with its native co-crystal inhibitor ligand Harmine (PDB ID: 2Z5X, crystallographic resolution 2.20 Å) downloaded from the RCSB Protein Data Bank, alongside the two-dimensional (2D) and 3D structural SDF/PDB files of 70 phytochemical secondary metabolites of turmeric obtained from the PubChem database.

The detailed sequential research stages executed in this study encompass:

1. **Protein and Ligand Preparation:** The 3D crystal structure of the MAO-A target protein was retrieved and prepared by removing co-crystallized water molecules, heteroatoms, and native non-essential ligands using BIOVIA Discovery Studio. Subsequently, polar hydrogen atoms and Gasteiger partial atomic charges were incorporated into the protein structure using AutoDockTools to prepare a stable macromolecular receptor grid. The 2D and 3D structural geometries of all 70 test ligands were drawn using ChemDraw, optimized, and subjected to comprehensive energy minimization protocols using VegaZZ to achieve stable, low-energy conformers.
2. **Method Validation (Redocking):** Validation of the docking protocol was executed by redocking the native co crystal ligand (Harmine) into its original active binding site within the MAO-A protein using PyRx-AutoDock Vina. The validation metric was established by calculating the root-mean-square deviation (RMSD) between the redocked conformation and the crystallographic pose, alongside recording the binding affinity value, ensuring compliance with standard validation thresholds (≤ 2.0 Å).

3. **Molecular Docking Simulation:** High-throughput docking simulations of all 70 test ligands against the prepared MAO-A enzyme active site were conducted systematically using PyRx-AutoDock Vina. The grid box parameters (center coordinates and dimensions) were meticulously defined based on the spatial localization of the native ligand binding pocket. Post-docking visualization, conformational analysis, and mapping of non-covalent amino acid residue interactions (hydrogen bonds and hydrophobic contacts) were carried out using PyMOL and BIOVIA Discovery Studio Visualizer.
4. **Pharmacokinetic and Toxicity Evaluation:** Comprehensive drug-likeness profiling (Lipinski's Rule of Five) and ADME (Absorption, Distribution, Metabolism, Excretion) properties of the screened compounds were evaluated using the SwissADME web server. Furthermore, multi-parameter toxicological properties, including acute oral toxicity, structural alerts, and toxicological hazards, were analyzed using Toxtree (Cramer classification) and pkCSM.

Rational Structural Modification: Guided by the computational screening results, rational structural modifications were designed and modeled on the selected lead compound using ChemDraw. The objective of this modification was to fine-tune molecular weight, lipophilicity, and structural topology to enhance binding affinity, improve central nervous system penetration, eliminate metabolic liability against cytochrome P450 enzymes, and optimize safety parameters.

RESULTS AND DISCUSSION

The computational evaluation began with the validation of the molecular docking protocol through redocking the native co-crystal ligand, Harmine, into the active catalytic site of the Monoamine Oxidase-A (MAO-A) enzyme. The redocking procedure yielded a binding affinity of -8.2 kcal/mol and an RMSD value of 1.497 Å. This statistical outcome successfully satisfied international validation criteria (≤ 2.0 Å), confirming that the docking parameters, grid box dimensions, and scoring functions utilized in PyRx-AutoDock Vina were highly reliable and accurate for predicting ligand-receptor interactions in subsequent screening stages.

Following the validation phase, high-throughput molecular docking was performed on the library of 70 secondary metabolites derived from *Curcuma longa*. From this extensive virtual screening, the seven top-performing secondary metabolites exhibiting the highest binding affinities and optimal conformational stability against the MAO-A enzyme were successfully identified. The comprehensive docking scores, RMSD values, and interacting amino acid residues of these top candidates, alongside the native ligand reference, are compiled in Table 1.

Table 1. Molecular Docking Results of the 7 Best Turmeric Secondary Metabolites Against the MAO-A Enzyme

Compound Name	Binding Affinity (kcal/mol)	RMSD (Å)	Amino Acid Residues
Harmine (Ligan Natif)	-8.2	1.497	TYR A:444, TYR A:407, ILE A:180, MET A:445
Bisdemethoxycurcumin	-9.6	0,734	TYR A: 444, TYR A: 407, CYS A: 406, ARG A: 51
Dihydrocurcumin	-9.4	0.916	CYS A:406, ALA A:272, TYR A:407, TYR A:444
			GLY A:66, GLY A:50
Demethylcurcumin	-9.3	1.001	MET A:445, ILE A:180, TYR A:407, TYR A:444

Tetrahydrocurcumin	-9.3	1.41	ALA A:272, CYS A:406, TYR A:444, TYR A:407
Cyclocurcumin	-8.3	1.128	TYR A:407, TYR A:444, MET A:445, ILE A:180
Curcumin	-8.2	1.352	MET A:445, THR A:435, TYR A:444, TYR A:407
3 - Carene	-6.8	0.638	TYR A: 444, TYR A: 407, MET A: 445

A thorough examination of Table 1 reveals that Bisdemethoxycurcumin exhibited the lowest binding energy (-9.6 kcal/mol), closely followed by Dihydrocurcumin (-9.4 kcal/mol), Demethylcurcumin (-9.3 kcal/mol), and Tetrahydrocurcumin (-9.3 kcal/mol). These extraordinary binding energies suggest that curcuminoid-based secondary metabolites possess exceptional thermodynamic stability when interacting with the MAO-A active site. To understand the molecular foundation of these binding affinities, non-covalent interaction profiling was performed. The docking analysis demonstrated that these top-tier compounds interact extensively with key residues residing within the hydrophobic and catalytic cavity of MAO-A, particularly Tyr A:444 and Tyr A:407. Notably, these specific aromatic residues are identical to those anchoring the native co-crystal ligand Harmine, proving that turmeric secondary metabolites share a remarkably similar inhibitory binding mode.

Expanding the interaction analysis further, Demethylcurcumin which achieved a robust binding affinity of -9.3 kcal/mol and a low RMSD of 1.001 Å formed stable conventional hydrogen bonds and extensive hydrophobic contacts with crucial amino acid residues including Tyr A:444, Tyr A:407, Met A:445, and Ile A:180. The consistent engagement of these specific aromatic and sulfur-containing residues accounts for the high structural stability and inhibitory potential of curcuminoid variants inside the enzymatic cleft.

Subsequent comprehensive evaluation covering binding stability, Lipinski's Rule of Five compliance (drug-likeness), ADME profiles (such as high gastrointestinal absorption), and multi-parameter toxicity levels led to the selection of Demethylcurcumin as the overarching best lead candidate for further optimization. Evaluation via SwissADME confirmed that these turmeric secondary metabolites comply well with Lipinski's Rule of Five, indicating favorable oral bioavailability parameters. All top candidate compounds exhibited high gastrointestinal (GI) absorption profiles. This high permeability across the intestinal epithelial barrier, combined with optimal lipophilicity ($\text{LogP} \leq 5$), strongly supports their pharmacological potential as orally active therapeutic agents targeting the central nervous system.

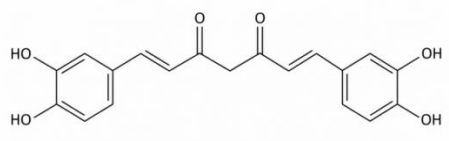
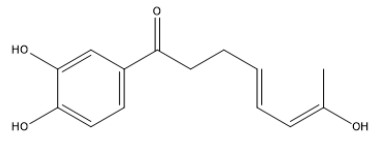
To further optimize the pharmacokinetic profile, metabolic stability, and toxicological safety of the lead compound, rational structural modification was executed on Demethylcurcumin using ChemDraw. Table 2 presents a comparative evaluation of the parent compound, the native ligand, and the newly designed modified derivative across molecular docking parameters, drug-likeness rules, ADME metrics, and toxicological endpoints.

Table 2. Evaluation Results of the Modified Compound Based on Molecular Docking, Drug-Likeness, ADME, and Toxicity

Parameter	Harmine	Demethylcurcumin	Modified Compound
Binding Affinity (kcal/mol)	-8.2	-9.3	-7.8
RMSD (Å)	1.497	1.001	1.631
Acid Amino Residues	TYR A:444 TYR A:407 ILE A:180 MET A:445	MET A:445 ILE A:180 SER A:24 THR A:435 TYR A:407 ILE A:23 TYR A:444	ILE A:180 ILE A:335 PHE A:180 CYS A:180 TYR A:407 GLY A:67

		ALA A:448 ARG A:51	
Formula	C13H12N2O	C20H18O6	C14H16O4
Bobot Molekul (g/mol) (≤ 500 Da)	212.25	354.35	248.27
H-Bond Acceptors (≤ 10)	2	6	4
H-Bond Donors (≤ 5)	1	3	3
LogP (≤ 5)	3.08	2.85	3.08
Kelarutan (LogS)	Cukup Larut	Larut	Larut
GI Absorption	High	High	High
BBB Permeant	Yes	No	Yes
P-gp Substrate	No	No	No
CYP1A2 Inhibitor	Yes	No	No
CYP2C19 Inhibitor	No	Yes	No
CYP2C9 Inhibitor	No	No	No
CYP2D6 Inhibitor	No	No	No
CYP3A4 Inhibitor	No	Yes	No
Bioavailability Score	0.55	0.55	0.55
Cramer Class	High (Class III)	High (Class III)	Low(Class I)
Genotoxicity Carcinogenicity	Structural Alert	Negative	Negative
Non-genotoxicity Carcinogenicity	Negative	Negative	Negative
AMES Toxicity	Yes	No	No
Hepatotoxicity	No	No	No
hERG I Inhibitor	No	No	No
hERG II Inhibitor	No	Yes	No

Table 3. Structural Transformation Design of Demethylcurcumin into the Modified Compound

Demethylcurcumin	Structural Modification
	
(1E,6E)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione	(4E,6E)-1-(3,4-dihydroxyphenyl)-7-hydroxyocta-4,6-dien-1-one

The structural transformation of Demethylcurcumin into the novel derivative (4E,6E)-1-(3,4-dihydroxyphenyl)-7-hydroxyocta-4,6-dien-1-one successfully optimized critical pharmacological properties. As detailed in the comparative evaluation, modifying the molecular framework reduced the molecular weight from an initial 354.35 Da down to an optimal 248.27 Da. This rational size and volume reduction significantly enhanced the pharmacokinetic profile of the molecule; specifically, computational prediction confirms that the modified compound successfully acquires the capacity to cross the blood-brain barrier (BBB permeant: Yes). Central nervous system penetration is an absolute, indispensable prerequisite for any therapeutic agent intended to manage stress, anxiety, and depression by inhibiting cerebral MAO-A.

Furthermore, metabolic liability evaluations revealed that while parent curcuminoids occasionally exhibit inhibitory effects on major hepatic cytochrome P450 isoenzymes, the newly designed derivative showed no inhibitory activity against major CYP isoenzymes (including CYP2C19 and CYP3A4). This absence of cytochrome inhibition drastically minimizes potential adverse clinical drug-drug interactions when administered alongside other medications. From a toxicological safety perspective, predictions via Toxtree demonstrated that the predicted Cramer toxicity classification improved remarkably from

Class III (High) in the parent compound to Class I (Low) in the derivative. Although the calculated binding affinity experienced a slight numerical shift to -7.8 kcal/mol, the modified compound robustly retains critical non-covalent interactions with vital amino acid residues at the active catalytic site of MAO-A. These combined computational findings successfully establish an enhanced, highly safe, and pharmacologically viable profile for the designed derivative as a promising candidate for further anti-stress drug development.

CONCLUSION

Based on comprehensive in silico evaluations, secondary metabolites derived from turmeric (*Curcuma longa*) demonstrate significant therapeutic potential as Monoamine Oxidase-A (MAO-A) enzyme inhibitors. Demethylcurcumin was successfully identified as the optimal lead candidate among the screened phytochemical library, exhibiting a favorable binding affinity of -9.3 kcal/mol and a stable RMSD of 1.001 Å. Furthermore, rational structural modification of demethylcurcumin successfully yielded the novel derivative (4E,6E)-1-(3,4-dihydroxyphenyl)-7-hydroxyocta-4,6-dien-1-one, which demonstrated marked improvements in drug-likeness parameters, enhanced pharmacokinetic profiles notably achieving blood-brain barrier permeability a minimal risk profile regarding cytochrome P450 enzyme interactions, and a safer toxicological classification improving to Cramer Class I. These computational findings collectively establish the modified structural derivative as a highly promising, safe, and effective candidate for future preclinical development as a novel anti-stress therapeutic agent.

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