IN SILICO RE-TARGETING: MOLECULAR DOCKING OF *PIPER LONGUM* ALKALOIDS AGAINST MITOCHONDRIAL REGULATORS OF CASPASE-INDEPENDENT CELL DEATH

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ABSTRACT

Malignant cells frequently evade conventional chemotherapeutics by acquiring inactivating mutations within canonical caspase execution cascades. Therapeutic strategies that bypass executioner caspases by driving mitochondrial outer membrane permeabilization (MOMP) directly represent a compelling counter-strategy. While Piper longum L. alkaloids—predominantly piperine, piper longumine (piplartine), and piperlonguminine—are recognized for their broad cytotoxic profiles, their structural affinities toward non-canonical mitochondrial death switches remain uncharacterized. This study executes an in silico target-reprofiling workflow evaluating key P. longum alkaloids against critical mitochondrial gating regulators: the Voltage-Dependent Anion Channel 1 (VDAC1), pro-apoptotic BAX, and BAK. Molecular docking executed via Auto Dock Vina, coupled with Swiss ADME pharmacokinetics and ProTox-II toxicity profiling, demonstrates that piperlongumine and piperine showed favourable predicted interactions with the hydrophobic BH3-binding groove of BAX and the cytosolic hydrophobic funnel of VDAC1 with favourable predicted docking scores ranging from -6.2 to -7.8 kcal/mol. These computational findings provide preliminary insights into the possible interactions of P. longum phytochemicals with mitochondrial proteins involved in caspase-independent cell death. Further experimental studies are required to confirm their biological effects..

Key Words: *Piper longum, in silico, bioactive, mitochondrial regulators.*

INTRODUCTION

The failure of classic antineoplastics is largely driven by lesions along the executioner caspase pathway, including transcriptional silencing of caspase-3, mutational ablation of caspase-9, or functional hyperactivation of Inhibitor of Apoptosis Proteins (IAPs) such as XIAP and survivin. Overcoming this therapeutic bottleneck requires engagement of caspase-independent programmed cell death, in which mitochondrial outer membrane permeabilization (MOMP) serves as the point of no return. Following MOMP, cell death-related factors, particularly Apoptosis-Inducing Factor (AIF) and Endonuclease G (EndoG),—extrude from the intermembrane space and translocate to the nucleus to execute peripheral chromatin condensation and large-scale DNA fragmentation without caspase catalysis.

The primary structural gatekeepers of MOMP are pore-forming proteins:

BAX (Bcl-2-associated X protein) and BAK (Bcl-2 antagonist/killer 1): Pro-apoptotic Bcl-2 family members whose activation and oligomerization form proteolipid toroidal pores in the outer mitochondrial membrane.

VDAC1 (Voltage-Dependent Anion Channel 1): An important channel protein of the outer mitochondrial membrane involved in the exchange of metabolites and regulation of mitochondrial function.

Piper longum L. (Piperaceae) contains a dense repertoire of bioactive alkamides, primarily piperine, piperlongumine (piplartine), and piperlonguminine. Although these compounds have been documented to trigger loss of mitochondrial membrane potential ($\Delta\Psi_m$) and ROS-mediated cytotoxicity *in vitro*, molecular docking investigations have almost exclusively focused on generic oncogenic kinases, topoisomerases, or tubulin. This study applies an *in silico* reverse-docking pipeline using accessible bioinformatics tools to quantify the direct binding mechanics between *P. longum* alkaloids and the mitochondrial execution machinery governing caspase-independent death.

METHODOLOGY

Ligand Retrieval and Energy Minimization

Three-dimensional coordinates for *Piper longum* alkaloids were downloaded from NCBI PubChem in SDF format:

- **Piperine** (PubChem CID: 638024; formula: $C_{17}H_{19}NO_3$)
- **Piperlongumine / Piplartine** (PubChem CID: 637858; formula: $C_{17}H_{19}NO_5$)
- **Piperlonguminine** (PubChem CID: 5320621; formula: $C_{16}H_{19}NO_3$)

Ligands were imported into Open Babel within PyRx, converted to .pdbqt format, assigned Gasteiger partial charges, and subjected to energy minimization using the Universal Force Field (UFF) with a conjugate gradient algorithm (1,000 steps; convergence criterion 10^{-4} kcal/mol).

Receptor Retrieval and Refinement

High-resolution crystal structures for target proteins were retrieved from the RCSB Protein Data Bank:

- BAX: PDB ID 1F16 (Solution structure of monomeric BAX, resolution: NMR)
- BAK: PDB ID 2YV6 (Crystal structure of the pro-apoptotic BAK core, resolution: 2.00 Å)
- VDAC1: PDB ID 3EMN (Crystal structure of human VDAC1 at 2.30 Å resolution)

Structure preparation in BIOVIA Discovery Studio and AutoDockTools (v1.5.6) involved removing crystallographic water molecules, native non-covalent co-factors, and alternate conformations. Polar hydrogens were added, and Kollman united-atom charges were assigned.

Grid Generation and Molecular Docking Execution

Molecular docking was executed using AutoDock Vina (v1.1.2) with an exhaustiveness setting of 8. Grid bounding boxes were dimensioned to fully enclose the functional regulatory pockets:

- BAX: PDB ID 1F16 (solution NMR structure of human BAX).
- BAK: PDB ID 2YV6 (crystal structure of human BAK, resolution 2.50 Å).
- VDAC1: PDB ID 3EMN (crystal structure of mouse VDAC1, resolution 2.30 Å).

Pharmacokinetics, Drug-Likeness, and In Silico Toxicity

Drug-likeness and physicochemical properties were computed via Swiss ADME (Swiss Institute of Bioinformatics), calculating molecular weight (M_W), topological polar surface area (TPSA), lipophilicity (Log $P_{o/w}$), and adherence to Lipinski's Rule of Five. Toxicity endpoints, including predicted oral LD_{50} and toxicological classes, were derived from the ProTox-II server.

RESULTS & DISCUSSION

Free Binding Affinities and Target Selectivity

AutoDock Vina scoring energies demonstrated negative free binding energies (ΔG) across all ligand-receptor pairs, indicating favourable predicted interactions between the ligands and target proteins.

Target Receptor	PDB ID	Piperlongumine (ΔG, kcal/mol)	Piperine (ΔG, kcal/mol)	Piperlonguminine (ΔG, kcal/mol)	Standard Reference Ligand (ΔG, kcal/mol)
BAX (Trigger Site)	1F16	-7.3	-6.8	-6.5	Navitoclax/ABT-263 (-8.6)
BAK (BH3 Groove)	2YV6	-6.2	-6.4	-6.0	Obatoclax (-7.1)
VDAC1 (Pore Funnel)	3EMN	-7.8	-7.5	-6.9	DIDS inhibitor (-7.2)

Binding Affinity Hierarchy for VDAC1 (3EMN):

Piperlongumine (-7.8 kcal/mol) > Piperine (-7.5 kcal/mol) > DIDS Reference (-7.2 kcal/mol)

Both piperlongumine and piperine surpassed the computational binding threshold of the standard reference VDAC1 inhibitor (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; DIDS), suggesting favourable predicted interactions with the VDAC1 channel.

Molecular Interactions at Critical Active Sites

BAX Interaction Complex (1F16)

Piperlongumine docked stably into the hydrophobic cleft formed by α-helices 1 and 6, a region responsible for allosteric conformational shifts preceding BAX insertion into the outer mitochondrial membrane:

- Conventional Hydrogen Bonds: Formed between the oxygen atom of the 5,6-dihydropyridin-2-one ring and Lys21 (2.42 Å), with secondary contact to Thr182.
- Hydrophobic Contacts: The 3,4,5-trimethoxyphenyl moiety forms extensive alkyl and π-alkyl interactions with Leu70, Met74, and Phe105.
- Significance: Direct binding in this groove destabilizes the auto-inhibited monomeric fold of BAX, which may be relevant to the structural changes associated with BAX activation. However, this possibility requires further experimental confirmation.

VDAC1 Channel Blockade and Destabilization (3EMN)

The N-terminal α-helix of VDAC1 lines the interior pore wall, regulating channel voltage gating:

- Piperlongumine Binding: Anchors into the central lumen via dual hydrogen bonds with Lys12 (2.18 Å) and Asn207 (2.85 Å). Strong π-sigma interactions were noted against Phe99.
- Piperine Binding: Projects its 1,3-benzodioxole scaffold into a sub-pocket surrounded by Leu10, Gly11, and Thr51, establishing favourable hydrophobic packing and electrostatic interactions with Arg15.
- Significance: The predicted binding interactions suggest that these compounds may influence the function of VDAC1. However, their actual effects on channel activity, mitochondrial membrane integrity and the release of cell death-related proteins need to be investigated experimentally.

Parameter / Endpoint	Filter / Unit	Piperlongumine	Piperine	Piperlonguminine
Molecular Weight	< 500 g/mol	317.34 g/mol	285.34 g/mol	273.33 g/mol
H-Bond Acceptors / Donors	≤ 10 / ≤ 5	5 / 0	3 / 0	3 / 1
Rotatable Bonds	≤ 10	5	4	4
Log $P_{o/w}$ (Consensus)	< 5.0	1.86	3.15	2.82
Topological Polar Surface Area (TPSA)	< 140 Å^2	55.84 Å^2	38.77 Å^2	47.56 Å^2
GI Absorption	High / Low	High	High	High
Blood-Brain Barrier (BBB)	Permeant?	Yes	Yes	Yes
Lipinski Violations	Max 1	0 (Fully Compliant)	0 (Fully Compliant)	0 (Fully Compliant)
Predicted Oral LD_{50}	mg/kg	1,250 mg/kg	514 mg/kg	1,600 mg/kg
Toxicity Class (ProTox-II)	Class 1–6	Class 4 (Harmful if swallowed)	Class 4 (Harmful if swallowed)	Class 4 (Harmful if swallowed)

All three compounds satisfy Lipinski's Rule of Five based on the predicted physicochemical properties presented in the table.

CONCLUSIONS

This in silico study evaluated the possible interactions of selected *Piper longum* phytoconstituents with three mitochondrial proteins: BAX, BAK and VDAC1. The molecular docking results showed favourable predicted interactions, particularly for piperlongumine and piperine. The ADMET analysis also provided preliminary information about the drug-likeness and predicted toxicity of these compounds. These findings suggest that *Piper longum* phytochemicals may be worth further investigation in relation to mitochondrial cell death pathways. However, experimental studies are necessary to confirm their interactions with these proteins and their possible role in caspase-independent cell death.

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