

Molecular Interaction Dynamic of Natural Alkaloids From *Strychnos* sp. With Murine Double Minute Type 2 (MDM-2) – p53 Protein

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Abstract: MDM-2 functions as a negative regulator of the p53 tumor suppressor protein, playing a critical role in cancer development and proliferation. Reactivation of p53 through MDM-2 inhibition represents a promising therapeutic strategy for combating cancer progression. This study investigated the potential inhibitory activity of *Strychnos* species alkaloids against MDM-2 and assessed their ADMET profiles using *in silico* approaches. Molecular docking was performed using AutoDock 4.2, followed by molecular dynamics simulations in GROMACS to evaluate binding stability. Computational analyses revealed that Strychnopentamine and Leuconicine A demonstrated superior binding affinity to MDM-2 compared to the established inhibitor Nutlin-3. Both compounds exhibited favorable pharmacokinetic profiles, though Leuconicine A showed predicted hepatotoxicity and mutagenicity risks. Molecular dynamics simulations confirmed the stability of the Strychnopentamine-MDM-2 complex over 50 nanoseconds, with binding characteristics comparable to Nutlin-3. These computational findings identify Strychnopentamine as a promising lead candidate for MDM-2 inhibition, though experimental validation through *in vitro* and *in vivo* studies is required to confirm these predictions and establish therapeutic potential.

Keywords: *Strychnos* sp.; alkaloids; MDM2-p53; dynamic interactions; ADMET prediction.

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1. Introduction

Cancer remains a major global health challenge, with approximately 14 million new cases and 8.2 million deaths reported in 2021 [1]. The disease is characterized by uncontrolled

cellular proliferation, driven primarily by DNA damage and genetic mutations that disrupt normal cell division [2–4]. Despite advances in cancer therapeutics, treatment outcomes remain suboptimal due to significant drawbacks including severe side effects, limited efficacy, and high mortality rates [5–8]. This underscores the critical need for novel anti-cancer agents with improved safety profiles and reduced toxicity. Cancer-targeted therapy has emerged as a rational approach, focusing on molecules that selectively inhibit specific targets within cancer cell pathways [9,10].

Murine double minute 2 (MDM2) represents a promising molecular target in cancer therapy due to its essential role in tumor development and proliferation [11]. MDM2 functions as a negative regulator of the p53 tumor suppressor protein by binding to its transactivation domain, thereby inhibiting p53-mediated transcriptional activity [12]. Consequently, molecules that disrupt the MDM2-p53 interaction have significant potential as anticancer therapeutics [13].

Strychnos species have shown notable cytotoxic and anticancer activity against various cancer cell lines [14–16]. These biological properties are primarily attributed to alkaloids, the predominant secondary metabolites in Strychnos plants [17,18]. However, despite evidence of their anti-cancer potential, the underlying mechanisms of action remain poorly understood. Specifically, the anticancer potential of Strychnos alkaloids via MDM2 inhibition has not been investigated.

Therefore, this research was conducted to evaluate the potential activity of Strychnos alkaloids against MDM2 by characterizing their molecular interactions and assessing their pharmacokinetic and toxicological profiles using *in silico* approaches.

2. Materials and Methods

2.1. Materials.

This *in silico* computational study was conducted on a dedicated workstation running Ubuntu 20.04 LTS. The system featured an Intel® Core™ i9-12900KF processor (24 CPU at 3.6 GHz), 32 GB RAM, and an NVIDIA RTX 4060 GPU with 16 GB VRAM. AutoDock 4.2 was used for molecular docking, while the GROMACS package was utilized for molecular dynamics. BioVIA Discovery Studio Visualizer was used for protein and ligand preparation and molecular interaction visualization.

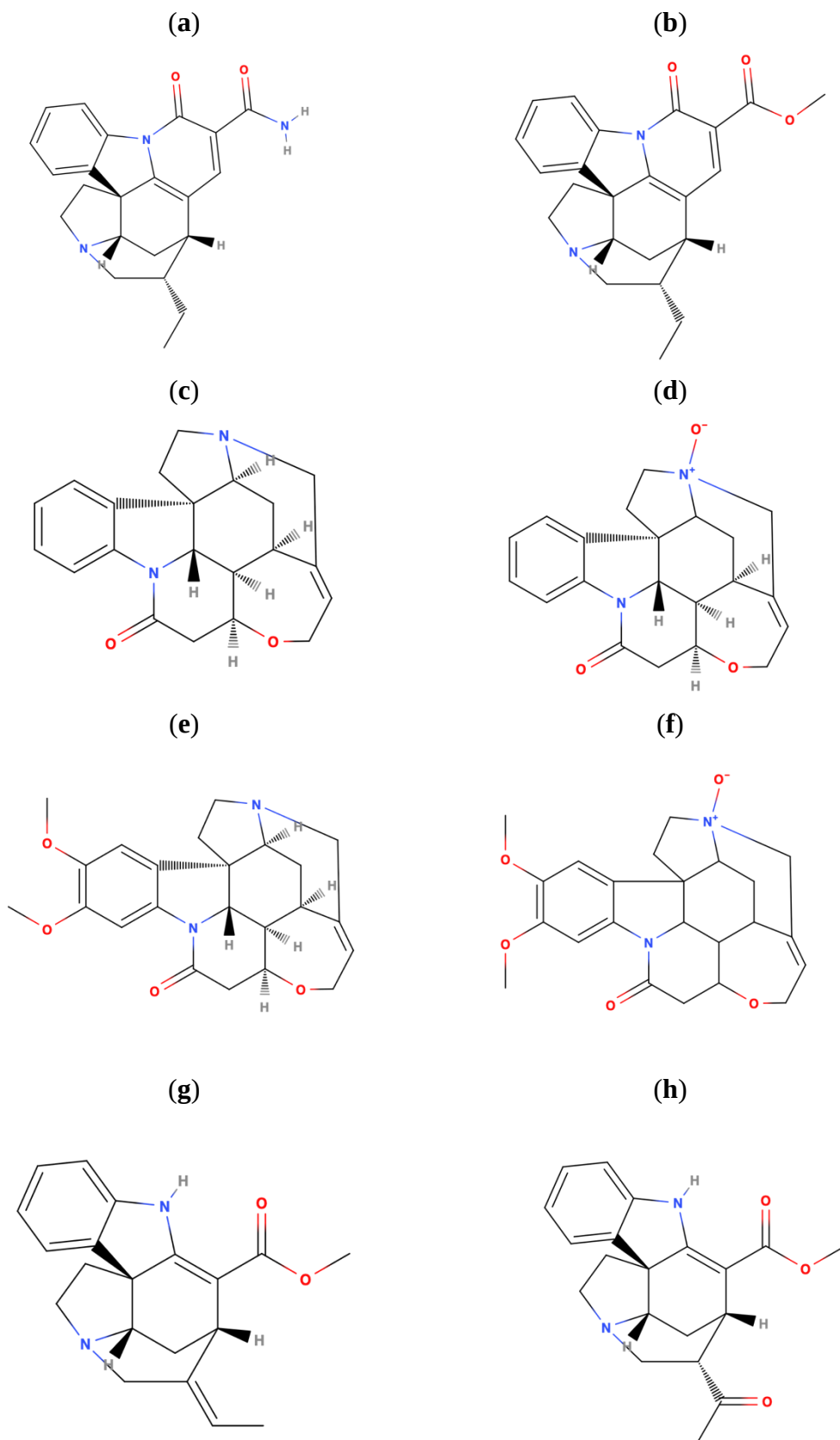
2.2. Protein and ligand preparation.

The co-crystal X-ray diffraction structure of MDM2-p53 was retrieved from the Protein Data Bank database (rcsb.org) with PDB ID: 4IPF. The macromolecules were prepared by removing all unnecessary components in the BioVIA Discovery Studio visualizer and saving them in PDB format. On the other hand, ten *Strychnos sp.* alkaloids (Figure 1) were used, and their 3D structures were downloaded from the PubChem database.

2.3. Molecular docking study.

Molecular docking was performed using AutoDock 4.2 with the AutoDock Tools interface (The Scripps Research Institute). Prior to docking the test compounds, method validation was conducted by re-docking the native ligand into the binding site. The re-docked ligand superimposed well with the original crystallographic pose, yielding an RMSD value of

1.77 Å, which confirms the reliability of the docking protocol (RMSD < 2.0 Å is generally considered acceptable for validation).



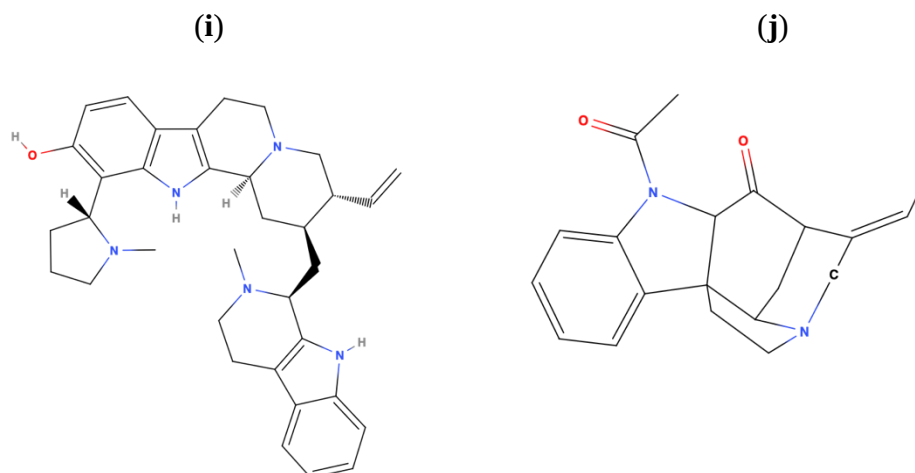


Figure 1. Two-dimensional structure of *Strychnos* sp. alkaloids. (a) Leuconicine A; (b) Leuconicine B; (c) Strychnine; (d) Strychnine N-oxide; (e) Brucine; (f) Brucine N-oxide; (g) Akuammicin; (h) Alstolucine; (i) Strychnopentamine; (j) Strychnopivotine.

The native ligand binding site was defined as the active site for docking simulations, with grid center coordinates set as summarized in Table 1. Docking parameters are summarized in Table 1. The Lamarckian genetic algorithm was employed for conformational search with 100 runs per ligand. Results were interpreted and visualized using BioVIVA Discovery Studio Visualizer to analyze molecular interactions between ligands and key amino acid residues.

Table 1. Molecular docking parameters.

Parameter	Value
Grid center (x, y, z)	13.556, 9.362, 37.838
Grid dimensions	40 × 40 × 40 points
Grid spacing	0.375 Å
Algorithm	Lamarckian genetic algorithm
Number of runs	100
Validation RMSD	1.85 Å

2.4. Molecular dynamic simulation.

Molecular dynamics simulations were conducted using GROMACS to evaluate protein-ligand complex stability under physiological conditions. Each complex was solvated in an octahedral TIP3P water box with sodium and chloride ions added for system neutralization and physiological ionic strength. Following energy minimization and equilibration phases, production simulations were run for 50 nanoseconds with a 2 fs time step. This duration was selected based on preliminary analyses confirming RMSD equilibration within 20 ns, providing adequate sampling for binding stability assessment. Each simulation was performed in triplicate to ensure reproducibility. Trajectory analysis examined root mean square deviation, root mean square fluctuation, radius of gyration, and solvent-accessible surface area to characterize structural stability, flexibility, compactness, and surface interactions. All simulation parameters are detailed in Table 2, and analyses were performed using GROMACS analytical tools with visualization in UCSF Chimera and Biovia Discovery Studio.

Table 2. Molecular dynamics simulation parameters.

Parameter	Specification
Force field (protein)	CHARMM36m
Force field (ligand)	CHARMM General Force Field
Water model	TIP3P
Box type	Octahedral
Box distance	1.0 nm from protein edge

Parameter	Specification
Ion concentration	0.15 M NaCl
Energy minimization	Steepest descent ($F_{\max} < 1000$ kJ/mol/nm)
NVT equilibration	100 ps at 310 K
NPT equilibration	100 ps at 310 K and 1 atm
Thermostat	Velocity rescaling ($\tau = 0.1$ ps)
Barostat	Parrinello-Rahman ($\tau = 2.0$ ps)
Electrostatics	Particle Mesh Ewald (cutoff: 1.2 nm)
Production time	50 ns
Time step	2 fs
Frame interval	10 ps

2.5. Pharmacokinetics and toxicological assessment.

The pharmacokinetics and toxicological aspects of *Strychnos* sp. alkaloids were assessed by an *in silico* approach using the pkCSM prediction server (<https://biosig.lab.uq.edu.au/pkcsml>). The assessment was carried out by inputting the molecule's canonical SMILES obtained from the PubChem database into the SMILES string column on the pkCSM web server. The assessment covered key ADMET parameters, including intestinal absorption, blood-brain barrier permeability, cytochrome P450 interactions, clearance rates, and toxicity endpoints such as hepatotoxicity, mutagenicity, and acute toxicity (LD_{50}).

3. Results and Discussion

3.1. Drug-likeness assessment of *Strychnos* alkaloids.

The drug-likeness properties of ten *Strychnos* species alkaloids were evaluated using Lipinski's Rule of Five (RO5) as the assessment framework. Nine of the ten alkaloids satisfied all RO5 criteria, demonstrating favorable pharmaceutical characteristics as summarized in Table 3. Strychnopentamine was the sole exception, exceeding the molecular weight threshold at 549.7 g/mol compared to the 500 g/mol limit, though it met all remaining RO5 parameters, including hydrogen bond donor and acceptor counts and lipophilicity requirements. Lipinski's Rule of Five serves as a fundamental screening tool in drug discovery, enabling identification of lead compounds with optimal pharmacological properties and drug-like characteristics [19]. Compounds that meet RO5 criteria typically show higher success rates during clinical development, resulting in reduced attrition and an increased likelihood of market approval [20]. This preliminary assessment indicates that the majority of *Strychnos* alkaloids possess promising pharmaceutical potential warranting further investigation.

Table 3. Chemical properties of *Strychnos* sp. alkaloids.

Bioactives	PubChem CID	MW	H donor	H acceptor	Log P
Strychnine	441071	334.4	0	3	1.9
Strychnine N-oxide	73393	350.4	0	3	1.4
Brucine	442021	394.5	0	5	1
Brucine N-oxide	161215	410.5	0	5	0.4
Akuammicine	10314057	322.4	1	4	2.4
Alstolucine B	11002245	338.4	1	5	1.6
Leuconicine A	44626771	361.4	1	3	2.3
Leuconicine B	46881102	376.4	0	4	2.7
Strychnopentamine	6712640	549.7	3	4	5.4
Strychnopivotine	102090449	322.4	0	3	1.6

3.2. Molecular docking study.

Molecular docking simulations were conducted to evaluate the binding affinity of *Strychnos* species alkaloids with the MDM2-p53 protein complex using a rigorously validated computational protocol (Figure 2). The assessment used free binding energy and inhibition constant (K_i) values to quantify compound-target interaction strength, with superior binding affinity indicated by lower values than the established reference compound Nutlin-3 [21].

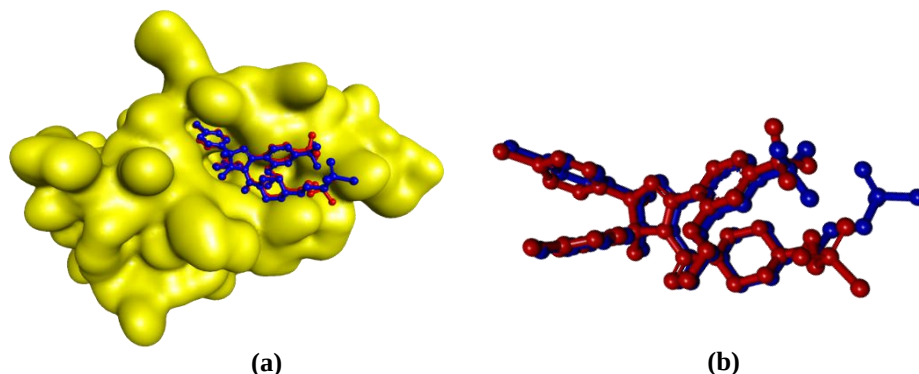


Figure 2. Results of validation methods. (a) native ligand fits in the MDM2-p53 binding pocket; (b) Superimposed structure of re-docked ligand (red) and original native ligand (blue).

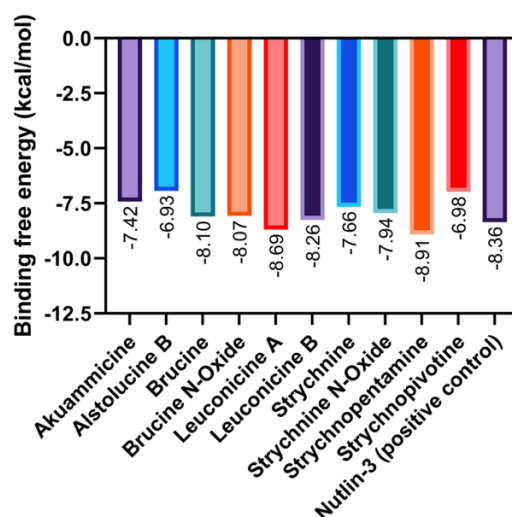


Figure 3. Docking score of *Strychnos* sp. alkaloids against MDM2-p53.

The computational analysis identified two *Strychnos* alkaloids demonstrating enhanced binding affinity relative to Nutlin-3. As presented in Figure 3 and Table 4, Strychnopentamine exhibited the most favorable binding profile with a free binding energy of -8.91 kcal/mol and K_i of 0.29 μM , followed by Leuconicine A at -8.69 kcal/mol and K_i of 0.43 μM . Both compounds surpassed Nutlin-3, which recorded a binding energy of -8.36 kcal/mol and K_i of 0.75 μM . The inverse relationship between binding energy and inhibition constants validated the computational model's consistency and reliability.

Table 4. Estimated inhibition constant (K_i) of *Strychnos* sp. alkaloids against MDM2.

Ligands	K_i (μM)
Akuammicine	3.63
Alstolucine B	8.39
Brucine	1.16
Brucine N-Oxide	1.22
Leuconicine A	0.43
Leuconicine B	0.87
Strychnine	2.43

Ligands	K _i (μM)
Strychnine N-Oxide	1.52
Strychnopentamine	0.29
Strychnopivotine	7.6
Nutlin-3	0.75

The molecular interaction analysis reveals distinct binding mechanisms that explain the superior binding affinity of Strychnopentamine and Leuconicine A compared with the reference compound Nutlin-3. These compounds demonstrate unique interaction patterns with critical amino acid residues within the MDM2-p53 binding site, providing molecular-level insight into their enhanced therapeutic potential. The comparative analysis, presented in Figures 4 and 5, identified several key differences in binding mechanisms that contribute to the enhanced performance of the Strychnos alkaloids.

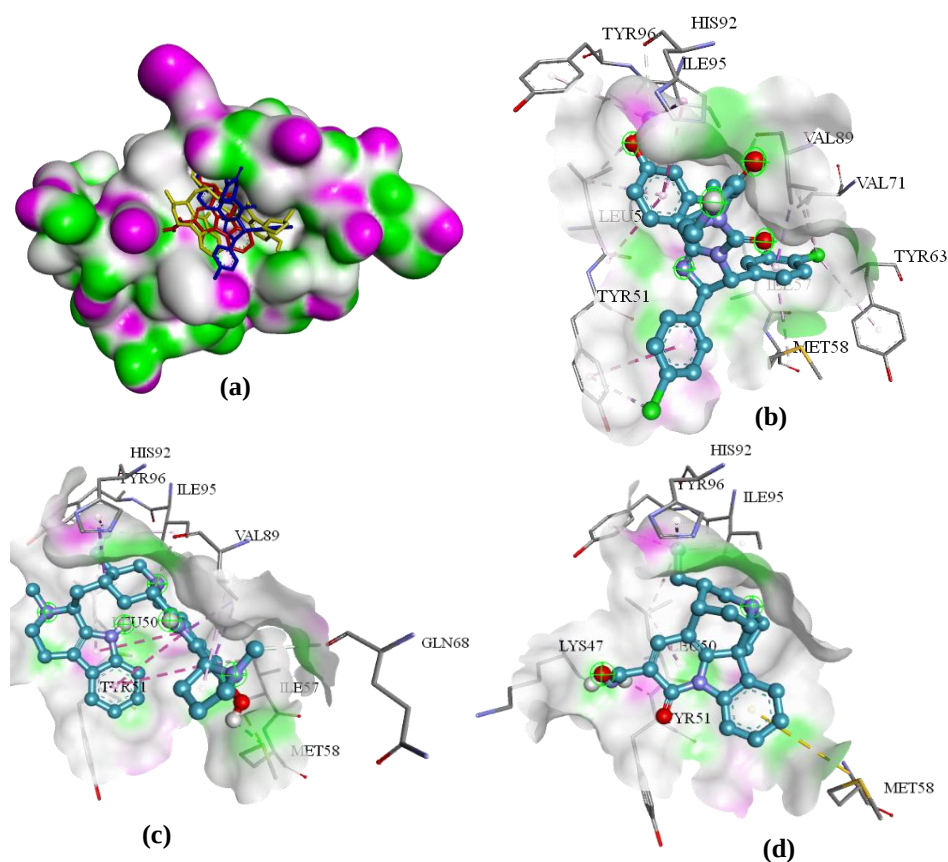


Figure 4. Representative molecular interaction of the best *Strychnos* sp. alkaloid with MDM2-p53. (a) Overlay structure of the two best *Strychnos* sp. alkaloids and Nutlin-3 fit in the MDM2-p53 active site; interactions of (b) Nutlin-3, (c) Strychnopentamine, (d) Leuconicine A with MDM2-p53.

Both Strychnopentamine and Leuconicine A establish Pi-Sulfur interactions with Met 58, a binding mode that differs significantly from Nutlin-3's Pi-Alkyl interaction with the same residue. This alternative interaction pattern appears to contribute to improved binding stability and may explain the superior binding energies observed for these natural compounds. The Pi-Sulfur interaction represents a particularly favorable binding mode, as sulfur-aromatic interactions are known to provide enhanced binding stability through favorable van der Waals contacts and partial charge interactions. This interaction type typically shows greater binding specificity and strength than conventional Pi-Alkyl interactions, which may explain the improved performance metrics observed in the computational analysis. Strychnopentamine

demonstrates additional binding advantages through the formation of two distinct Pi-Sigma interactions with His 92 and Tyr 51.

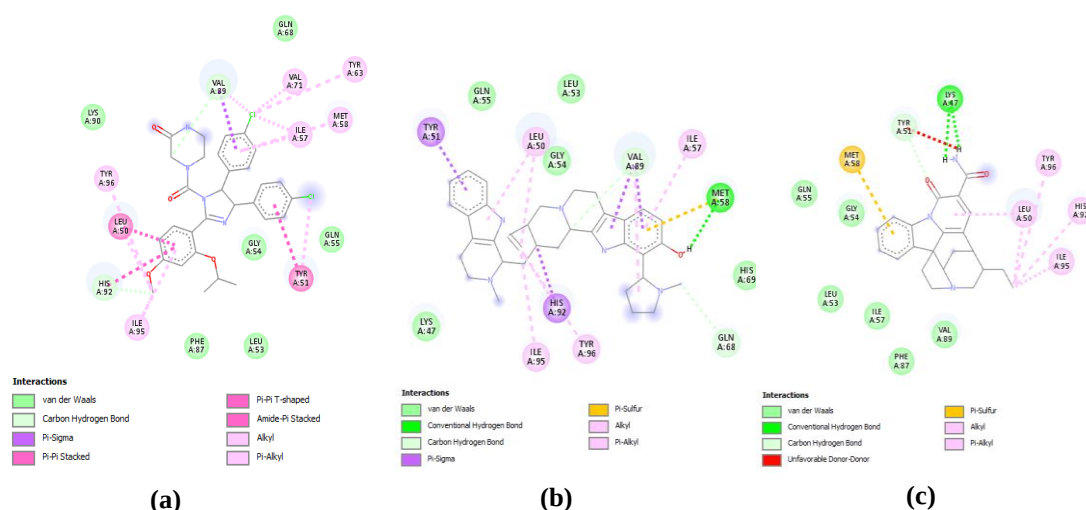


Figure 5. 2D molecular interactions of (a) Nutlin-3, (b) Strychnopentamine, and (c) Leuconicine A with MDM2-p53 amino acid residues.

These interactions are absent in both Nutlin-3 and Leuconicine A, providing a molecular basis for Strychnopentamine's superior binding affinity profile. Pi-sigma interactions are high-energy binding modes that contribute significantly to overall binding strength through favorable orbital overlap and charge-distribution effects. The presence of multiple complementary interaction modes suggests that Strychnopentamine achieves optimal geometric complementarity with the MDM2 binding site. This enhanced molecular recognition likely contributes to improved binding affinity and increased selectivity, which are essential for therapeutic efficacy and reduced off-target effects. The detailed molecular interactions are summarized in Table 5.

Table 5. Summary of direct molecular interactions of *Strychnos* sp. alkaloids with MDM2.

Ligands	Hydrogen bond interactions	Non-hydrogen bond interactions
Akuammicine	Lys 51; Lys 47	Val 89; His 92; Leu 50; Ile 95
Alstolucine B	His 92; Llys 47	Gly 54
Brucine	Gln 55; Val 89	Tyr 51; Gly 54; Leu 50; His 92; Met 58
Brucine N-Oxide	Gln 55; Val 89	Tyr 51; Gly 54; Met 58
Leuconicine A	Lys 47; Tyr 51	Met 58; Leu 50; Tyr 96; His 92; Ile 95
Leuconicine B	Lys 47; Tyr 51	Leu 50; Tyr 96; His 92; Ile 95
Strychnine	-	Leu 50; His 92; Ile 95
Strychnine N-Oxide	Val 89	Gly 54
Strychnopentamine	Gln 68; Va; 89; Met 58	Tyr 51; Leu 50; Ile 57; Val 89; Met 58; His 92; Tyr 96; Ile 95
Strychnopivotine	-	Leu 50; His 92; tyr 96; Met 58
Nutlin-3	His 92; Val 89	Tyr 96; Leu 50; Ile 95; Tyr 51; Ile 57; Met 58; Tyr 63; Val 71

3.3. Molecular dynamic simulation.

We conducted molecular dynamics simulations for 50 ns to evaluate the dynamic properties and binding characteristics of Strychnopentamine and Leuconicine A in complex with MDM2. The analysis examined molecular flexibility, interaction stability, and conformational dynamics to provide deeper insight into these alkaloids' binding mechanisms.

Root mean square deviation (RMSD) analysis (Figure 6A) provided compelling evidence for the enhanced stability of Strychnopentamine throughout the simulation period. The compound maintained remarkably consistent RMSD values that closely mirrored the

control standard, demonstrating minimal structural perturbation during the binding process. In contrast, Leuconicine A exhibited considerably greater conformational flexibility, with notable instability manifesting particularly around the 40-nanosecond time point, where significant deviations from baseline values were observed. This difference suggests fundamental differences in the binding modes and interaction strengths of the two compounds. Root mean square fluctuation (RMSF) analysis (Figure 6B) further corroborated these findings by revealing distinct residue-level interaction patterns. Strychnopentamine demonstrated consistent engagement with specific amino acid residues within the MDM2-p53 binding pocket, maintaining stable interactions throughout the simulation with minimal variation in binding orientation. This behavior closely resembled that of Nutlin-3, indicating comparable binding mechanisms and interaction profiles. Conversely, Leuconicine A showed more variable interaction patterns, suggesting less stable binding configurations and potential dissociation events during the simulation.

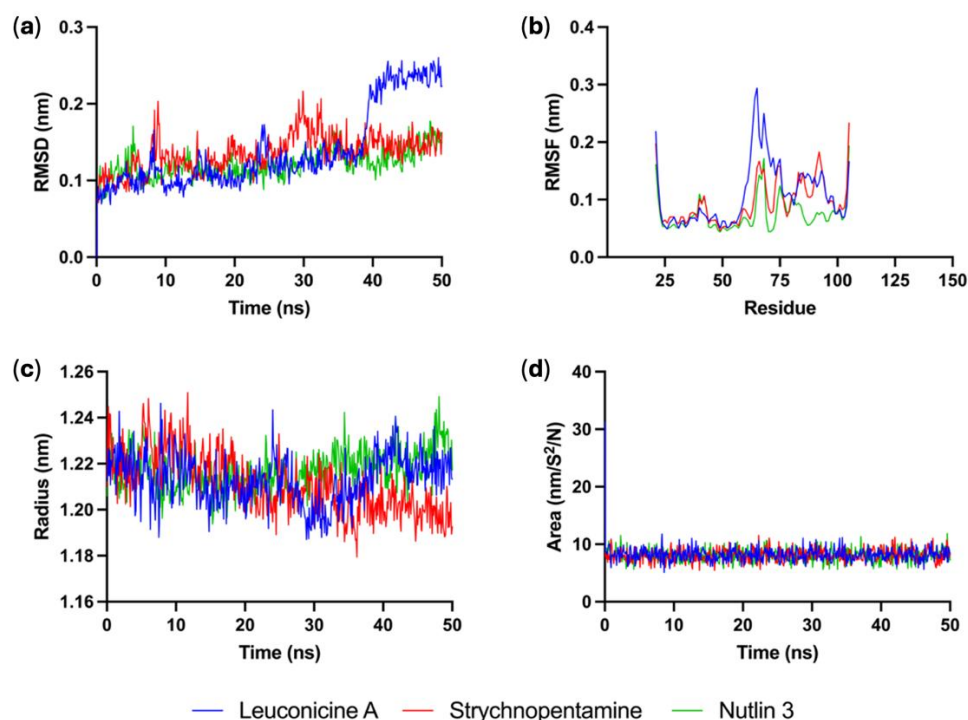


Figure 6. Molecular dynamics simulation results over 50 ns. (a) RMSD; (b) RMSF; (c) Radius of gyration; (d) SASA.

The radius of gyration analysis (Figure 6C) provided additional insights into the structural impact of compound binding on the target protein. Strychnopentamine demonstrated exceptional capability in modulating MDM2-p53 structural integrity, achieving more pronounced compaction effects than both Leuconicine A and the reference compound Nutlin-3. The consistent downward trend in radius of gyration values throughout the simulation period indicates sustained structural perturbation, which may contribute to enhanced therapeutic efficacy through disruption of the MDM2 interaction interface. Solvent-accessible surface area (SASA) calculations (Figure S6D) revealed parallels between the compounds, with Strychnopentamine and Leuconicine A showing surface interaction profiles comparable to Nutlin-3. This similarity suggests similar binding-pocket occupancy and comparable steric interactions with the active-site architecture. However, despite these similarities in surface accessibility, Strychnopentamine's overall dynamic performance clearly favored It, reinforcing

the molecular docking predictions and establishing it as the most promising candidate for further investigation and potential therapeutic development.

3.4. Pharmacokinetics and toxicological assessment.

The ADMET evaluation established comprehensive pharmacokinetic and toxicological profiles for *Strychnos* species alkaloids to assess their suitability for oral drug development. All interpretations were based on the established pkCSM computational framework [22].

The pharmacokinetic screening demonstrates generally favorable absorption profiles across all examined alkaloids (Table 6). Most compounds showed Caco-2 permeability values above 0.90 and human intestinal absorption above 80%, classifying them as highly absorbed per established classification systems [23]. Strychnopentamine presented a notable exception with a Caco-2 value of 0.472, though its human intestinal absorption of 87.329% confirms adequate bioavailability. All alkaloids demonstrated low skin permeability ($\log K_p > -2.5$), suggesting minimal transdermal absorption potential. P-glycoprotein interactions varied, with compounds acting as substrates or inhibitors, requiring careful consideration during clinical development because these interactions can significantly affect pharmacokinetic profiles when co-administered with other medications [24].

Table 6. Absorption profile of *Strychnos* sp. alkaloids.

Compounds	Water Solubility (log mol/L)	Caco2	HIA (%)	Skin permeability (log K_p)	Pgp-substrate	Pgp1-inhibitor	Pgp2-inhibitor
Akuammicine	-3.738	1.747	96.078	-2.945	Yes	No	No
Alstolucine B	-3.488	1.266	97.990	-2.978	Yes	No	No
Brucine	-2.507	1.143	96.125	-3.426	Yes	Yes	Yes
Brucine N-Oxide	-3.761	0.961	99.768	-3.288	No	Yes	No
Leuconicine A	-3.390	0.943	98.438	-3.185	Yes	Yes	Yes
Leuconicine B	-3.631	1.266	99.929	-3.006	Yes	Yes	Yes
Strychnine	-3.445	1.560	99.843	-3.255	Yes	Yes	No
Strychnine N-Oxide	-3.395	1.205	100.000	-3.082	No	Yes	No
Strychnopentamine	-2.941	0.472	87.329	-2.735	Yes	Yes	Yes
Strychnopivotine	-3.116	1.779	98.451	-3.299	Yes	No	No

Distribution analysis (Table 7) revealed that most *Strychnos* alkaloids possess a high volume of distribution ($VD_{ss} > 0.45 \log L/kg$), except Brucine N-oxide, which showed moderate distribution ($0.380 \log L/kg$), indicating extensive tissue penetration for most compounds [25]. Strychnopentamine demonstrated the most favorable overall distribution profile, combining the highest volume of distribution ($1.766 L/kg$) with substantial unbound fraction ($F_u = 0.242$), positive blood-brain barrier permeability ($\log BB = 0.3$), and favorable CNS permeability (-1.5). All alkaloids exhibited high plasma protein binding as indicated by their small fraction unbound values. Several alkaloids, including Akuammicine, Leuconicine B, Strychnopentamine, and Strychnopivotine, demonstrated the ability to cross the blood-brain barrier and penetrate the CNS ($\log BB \geq 0.3$ and $\log PS \geq -2.0$). In contrast, N-oxide derivatives (Brucine N-Oxide and Strychnine N-Oxide) displayed significantly compromised distribution properties with lower VD_{ss} values and very low unbound fractions (0.086 and 0.089 , respectively), likely limiting their therapeutic potential.

Table 7. Distribution profile of *strychnos* sp. alkaloids.

Compounds	Human VD_{ss} (log L/kg)	Fraction unbound (F_u)	BBB permeability (log BB)	CNS permeability (log PS)
Akuammicine	1.115	0.254	0.3	-1.6

Compounds	Human VDss (log L/kg)	Fraction unbound (Fu)	BBB permeability (log BB)	CNS permeability (log PS)
Alstolucine B	0.791	0.275	-0.2	-2.3
Brucine	1.000	0.295	-0.1	-2.4
Brucine N-Oxide	0.380	0.086	-0.5	-2.4
Leuconicine A	1.572	0.253	0.2	-2.2
Leuconicine B	1.463	0.221	0.5	-2.0
Strychnine	1.160	0.168	0.2	-2.2
Strychnine N-Oxide	0.551	0.089	-0.1	-2.1
Strychnopentamine	1.766	0.242	0.3	-1.5
Strychnopivotine	1.057	0.256	0.3	-2.0

Metabolism profiling assessed interactions with cytochrome P450 (CYP450) enzymes, which are critical for drug metabolism in the liver (Table 8). Induction or inhibition of CYP450 isoforms may alter therapeutic effects and toxicity [26,27]. Brucine and Strychnopentamine were predicted to act as CYP2D6 substrates, while Akuammicine, Alstolucine B, Leuconicine B, and Strychnopivotine function as CYP2D6 inhibitors. All alkaloids were predicted to be CYP3A4 substrates without inhibiting CYP3A4 or CYP2C9. Strychnopentamine uniquely demonstrated CYP1A2 and CYP2C19 inhibition potential, alongside Strychnopivotine, which also inhibits CYP2C19.

Table 8. Metabolic profile of *Strychnos* sp. alkaloids.

Compounds	CYP2D6 Substrate	CYP2D6 Inhibitor	CYP3A4 Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP3A4 Inhibitor
Akuammicine	No	Yes	Yes	No	No	No	No
Alstolucine B	No	Yes	Yes	No	No	No	No
Brucine	Yes	No	Yes	No	No	No	No
Brucine N-Oxide	No	No	Yes	No	No	No	No
Leuconicine A	No	No	Yes	No	No	No	No
Leuconicine B	No	Yes	Yes	No	No	No	No
Strychnine	No	No	Yes	No	No	No	No
Strychnine N-Oxide	No	No	Yes	No	Yes	No	No
Strychnopentamine	Yes	No	Yes	Yes	Yes	No	No
Strychnopivotine	No	Yes	Yes	No	No	No	No

Excretion profiling revealed that all *Strychnos* alkaloids possess high total clearance ($CL_{tot} > 1$ mL/min/kg), ranging from 5.395 to 9.908 mL/min/kg (Table 9), which affects drug bioavailability and half-life [28–30]. Additionally, all alkaloids were predicted to act as renal organic cation transporter 2 (OCT2) substrates, raising potential risk of drug interactions when co-administered with OCT2 inhibitors [31].

Table 9. Excretion profile of *Strychnos* sp. alkaloids.

Compounds	Total clearance		Renal OCT2 substrate
	log mL/min/kg	mL/min/kg	
Akuammicine	0.951	8.933	Yes
Alstolucine B	0.933	8.570	Yes
Brucine	0.996	9.908	Yes
Brucine N-Oxide	0.359	2.286	Yes
Leuconicine A	0.856	7.178	Yes
Leuconicine B	0.876	7.516	Yes
Strychnine	0.956	9.036	Yes
Strychnine N-Oxide	0.474	2.979	Yes
Strychnopentamine	0.959	9.099	Yes
Strychnopivotine	0.732	5.395	Yes

Toxicity assessment examined lethal dose 50 (LD50), mutagenicity (AMES toxicity), and hepatotoxicity (Table 10). *Strychnos* alkaloids exhibited LD50 values ranging from 2.449 to 3.207 mol/kg, corresponding to 944.31 to 1630.41 mg/kg body weight when adjusted for molecular weight. According to Loomis [32], compounds with LD50 of 500 to 5000 mg/kg

body weight are considered less toxic. However, important safety concerns were identified for specific compounds. Leuconicine A was predicted to exhibit both mutagenicity and hepatotoxicity, while Brucine and Leuconicine B demonstrated hepatotoxicity alone. These toxicological predictions warrant careful consideration and represent potential limitations for therapeutic development of these specific alkaloids. Strychnopentamine, despite its superior binding and dynamic profiles, showed no predicted mutagenicity or hepatotoxicity, further supporting its designation as the lead candidate. Nevertheless, these computational predictions require experimental validation, and potential hepatotoxic and mutagenic risks associated with Leuconicine derivatives must be thoroughly evaluated in preclinical studies before considering clinical translation.

Table 10. Toxicity profile of *Strychnos* sp. alkaloids.

Compounds	LD ₅₀ (mol/kg)	AMES toxicity	Hepatotoxicity
Akuammicine	2.929	No	No
Alstolucine B	2.964	No	No
Brucine	2.884	No	Yes
Brucine N-Oxide	2.512	No	No
Leuconicine A	2.990	Yes	Yes
Leuconicine B	3.207	No	Yes
Strychnine	2.798	No	No
Strychnine N-Oxide	2.449	No	No
Strychnopentamine	2.499	No	No
Strychnopivotine	2.966	No	No

4. Conclusions

This comprehensive study evaluated ten *Strychnos* species alkaloids for their therapeutic potential as MDM2 inhibitors through molecular docking analysis and ADMET profiling. The investigation identified two lead compounds with superior binding characteristics compared to the established reference standard Nutlin-3. Strychnopentamine demonstrated the highest binding affinity with a free binding energy of -8.91 kcal/mol and Ki value of 0.29 μ M, while Leuconicine A achieved -8.69 kcal/mol binding energy and a 0.43 μ M Ki value, both surpassing Nutlin-3's performance of -8.36 kcal/mol and 0.75 μ M, respectively. The enhanced binding profiles result from distinct molecular interaction patterns, including unique Pi-Sulfur interactions with Met 58 and additional Pi-Sigma interactions with His 92 and Tyr 51 for Strychnopentamine. These interactions are absent in the reference compound, explaining its superior binding characteristics. ADMET evaluation revealed favorable pharmacokinetic properties across the alkaloid series, with nine of ten compounds meeting Lipinski's Rule of Five criteria. The compounds demonstrated excellent absorption profiles, with human intestinal absorption values exceeding 87% for most alkaloids. Distribution analysis indicated extensive tissue penetration capabilities, with several compounds showing blood-brain barrier permeability suitable for central nervous system applications. However, critical safety concerns emerged during toxicological assessment. Leuconicine A presents significant development challenges because of predicted mutagenic potential and hepatotoxicity risk, while Leuconicine B raises hepatotoxicity concerns. In contrast, most alkaloids showed acceptable acute toxicity profiles with lethal dose values ranging from 944-1630 mg/kg, classified as relatively non-toxic. Based on this comprehensive evaluation, Strychnopentamine emerges as the primary development candidate, combining superior binding affinity, favorable pharmacokinetics, and an acceptable safety profile. This compound represents the optimal balance of therapeutic potential and development feasibility for advancing to preclinical studies.

Author Contributions

Conceptualization, A.S. and M.I.A.; methodology, A.S.; software, A.S.; validation, A.S., G.S., and M.I.A.; formal analysis, A.S. and R.A.; investigation, A.S., I.H., and R.A.; resources, N.H.; data curation, E.K. and S.S.; writing—original draft preparation, A.S. and N.H.; writing—review and editing, A.S., M.I.A., G.S., R.A., I.H. N.H., E.K. and S.S.; visualization, A.S.; supervision, A.S. and M.I.A.; project administration, N.H.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

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Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

Artificial Intelligence Disclosure

Artificial intelligence-based tools were used solely for language editing and stylistic refinement of the manuscript. The authors remain fully responsible for the content of this work.

Abbreviations

Abbreviation	Definition
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
BBB	Blood-Brain Barrier
BW	Body Weight
CNS	Central Nervous System
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid
GROMACS	Groningen Molecular Simulation
LD50	Lethal Dose Fifty Percent
MDM2	Murine Double Minute Type 2

PDB	Protein Data Bank
OCT2	Organic Cation Transporter Type 2
RMSD	Root Mean Score Deviation
RMSF	Root Mean Square Fluctuation
RO5	Rule of Five
SASA	Solvent Accessible Surface Area
SMILES	Simplified Molecular-Input Line-Entry System
t _{1/2}	Half-life
VD	Volume of Distribution
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity

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