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Molecular docking analysis of modified gedunin from neem with snake venom enzymes

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Abstract:
Snakebites are a problem due to the increasing number of deaths and permanent disabilities. There is currently a shortage of antidotes for snakebite. The existing antibody antidote, produced from horse/sheep plasma/sera is expensive, species-dependent, and causes fatal side effects. Therefore, it is of interest use of natural flavonoid named gedunin from the *Azadirachta indica* (Neem) plant species to combat snakebites. Thus, we show the molecular docking analysis of gedunin (C26H31N2O6F) with enzymes (common in snake species) such as 5-nucleotidase, acetyl cholinesterase, L-aao, metalloproteinase, serine, thrombin and phospholipase A2. The modified gedunin in the enzyme pocket showed improved pharmacological properties for further consideration in combating snakebites.

Keywords: Gedunin, Inhibitors, Snake venom enzymes, plant metabolite

Background:
Snakebite has become a generally neglected public health issue, responsible for more than 137,880 deaths and poisoning around two million people annually worldwide. Women, children, the poor, and farmers are at a higher risk with weak and sparse medical resources such as anti-serum **[5].** The side effects of antiserum are serum sickness or delayed hypersensitivity and local tissue damage due to non-immunoglobulin proteins in the antiserum **[3].** However, plant extracts have been effective against snake venom since ancient times and retain growing interest due to abundance and safety **[11].** A study suggests that *Azadirachta indica* have compounds such as steroids, alkaloids, triterpenoid, tetraterpenoid, tannins, phenols, pterocarpans, and glycosides effective against snake venom by neutralizing multiple toxins and enzymes (hydrolases, proteases, phospholipase, ATPase, transaminase, Nucleotidase) poison **[13].** Gedunin is a tetra nortriterpenoid isolated from the neem tree (*Azadirachta indica*, Meliaceae) used in traditional medicine to treat malaria and other infectious diseases. Moreover, Gedunin from Neem has shown anti-proliferative activity against various cancer cell lines, including prostrate, colon, and ovarian cancers. Gedunin is a robust and thiol-reactive electrophile that activates the heat shock response **[13]**. Therefore, it is of interest to show the molecular docking analysis of a natural flavonoid named gedunin from the *Azadirachta indica* (Neem) plant species with enzymes (common in snake species) such as 5-nucleotidase, acetyl cholinesterase, L-aao, metalloproteinase, serine, thrombin and phospholipase A2. The modified gedunin in the enzyme pocket showed improved pharmacological properties for further consideration in combating snakebites.

Materials and Methods:
The ICM method:
The ICM software was used to perform flexible ligand docking with map grid calculated for the enzyme active site pocket. The Monte Carlo method used in the ICM follows a procedure **[5]** where random movement for the conformational variable of the ligand in the enzyme pocket is possible **[3].** Calculation of the

desolvation energy followed by selecting the minimized conformation using the Metropolis method was completed. The user-defined multiplier was kept at three according to the number of ligand rotational bonds. The calculated grid maps for hydrogen bonds, van der Waals bonds, electrostatic and hydrophobic potentials reduced the time required for ligand sampling. This generated 0.5 Å grid spacing maps at the ligand-binding site. The global optimization of the energy carried out requires a high dimensionality is reduced in the ICM by assigning internal coordinates to each atom **[14].**

Ligand preparation:

The ligand was developed using the ICM object by removing water followed by optimization of hydrogen, his-pro-asn-gln-cys. This conformational analysis was completed outside the pocket of the enzyme (receptor). The modified form of Gedunin (C26H31N2O6F) is the ligand for the study (**Figures 1 and 2).**

Table 1: Binding energy of Inhibitor [C26H31N2O6F]–receptor enzyme complex.

Snake Venom Enzymes	Inhibitor [C26H31N2O6F] Binding Energy [Kcal/mol]
5` Nucleotidase	-9.3
Acetyl cholinesterase	-8.4
L - AAO	-14.8
Metalloproteinase	-10.6
Phospholipase A2	-9.9
Thrombin like hydrolase	-9.6

Receptor preparation:

The PDB structure coordinates for 5-nucleotidase, acetyl cholinesterase; L-aao, metalloproteinase, serine, thrombin and phospholipase A2 were downloaded from RCSB PDB and processed using the ICM-Pro Molsoft-Software **[9][12]**

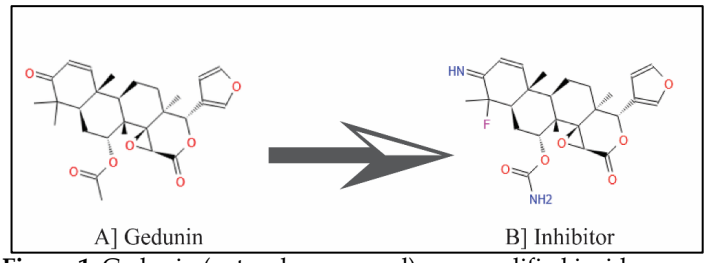

Figure 1: Gedunin (natural compound) was modified inside venom enzyme pockets at 3 positions, which includes methyl substitution using ICM Mol soft software.

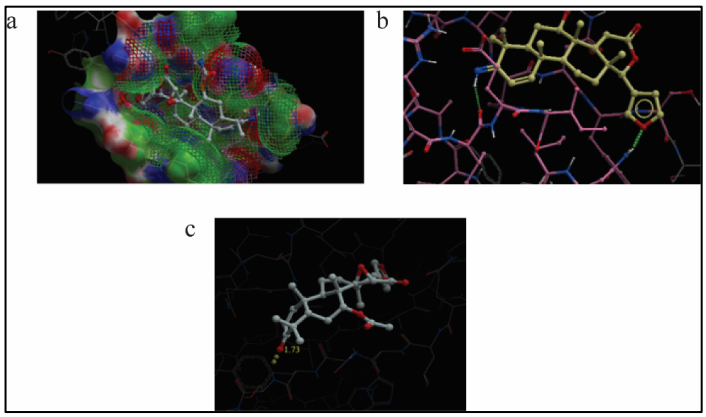

Figure 2: (a) Docked pose of inhibitor [C26H31N2O6F] inside the active site of venom enzyme 5'Nucleotidase; (b) 3D view of the interaction between Gedunin and neighboring amino acids of 5' Nucleotidase; (c) 3D view of the change in the interaction between inhibitor [C26H31N2O6F][after modification] and neighboring amino acids of 5' Nucleotidase.

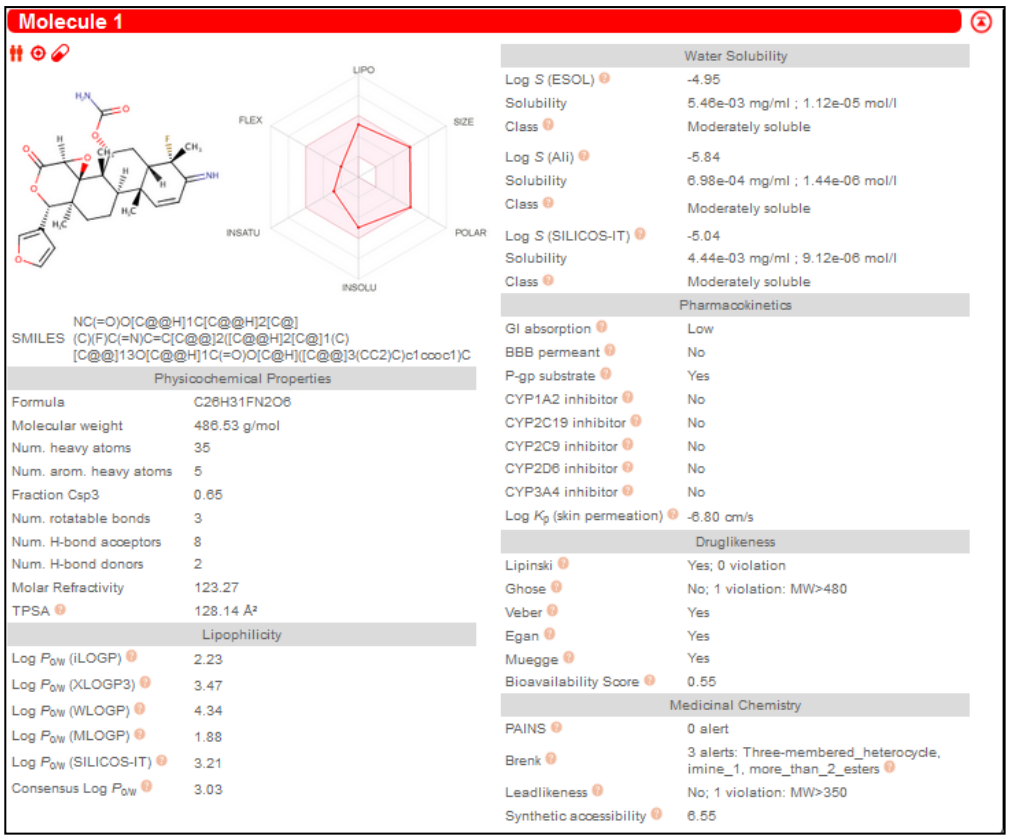

Figure 3: The ADME Computed parameters predicted using SwissADME Tool **[15].** The pink region is depicting the optimal range of each property. Physiochemical properties, Lipophilicity, Pharmacokinetics, Water solubility, Drug-likeness and Medical chemistry are determining factors for Drug's ADME.

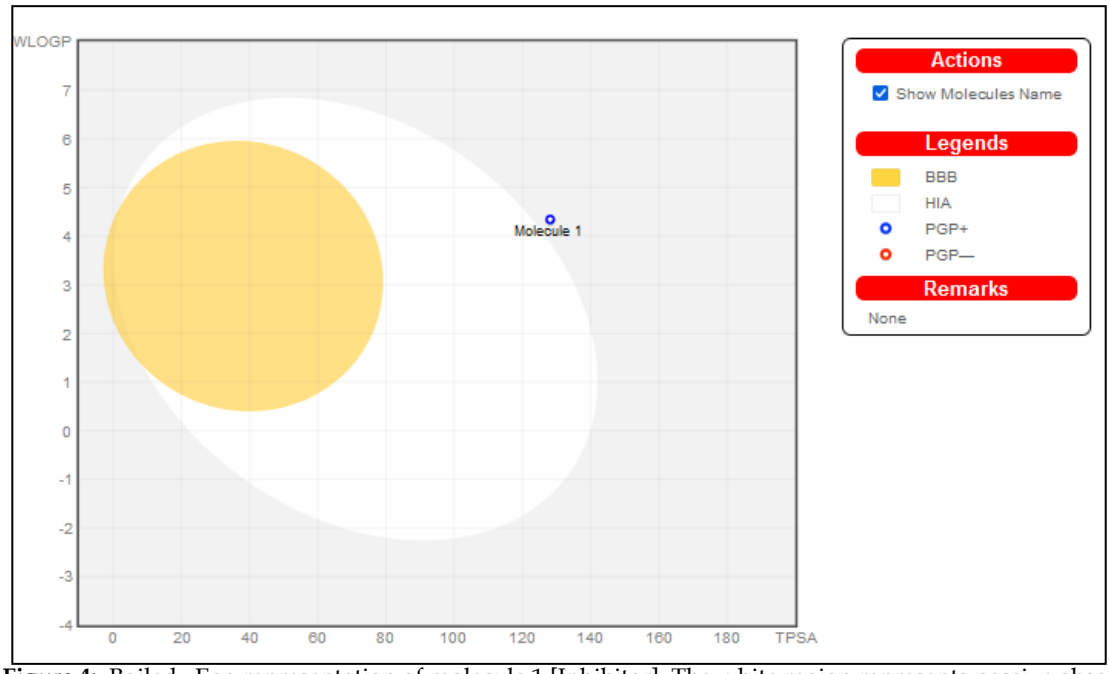

Figure 4: Boiled –Egg representation of molecule 1 [Inhibitor]. The white region represents passive absorption of by GI tract; yellow [yolk] region depicts brain penetration probability. Grey region is non-BBB permeant and low GI absorption. Molecules with a round blue colour circle or red colour circle are for P-gp [+] and P-gp [–] category respectively.

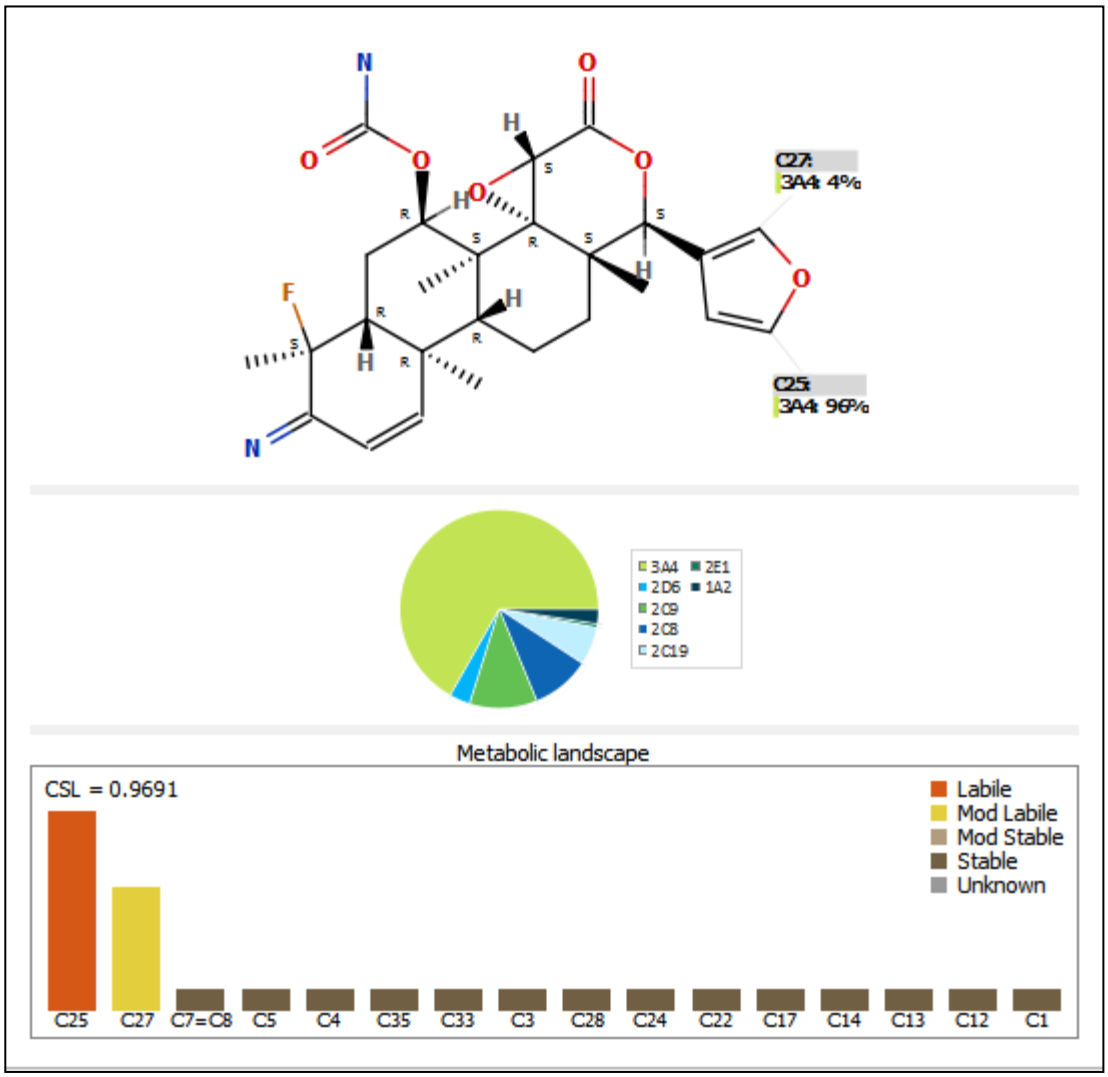

Figure 5: P450 Isoform type for substrate inhibitor [C26H31N2O6F] is majorly 3A4 [Green region in pie chart]. The red bar in Metabolic landscape represents most liable [96%] component of inhibitor i.e. C25 by 3A4 decomposition. The yellow bar represents C27 as moderate labiality [4%]

Table 2: HBond:- Hydrogen Bond energy, VwInt:- The Vander Waals Interaction Energy [sum of gc and gh Vander Waals], Hphob:- Hydrophobic Energy in exposing a surface to the water, N flex:- Number of Rotatable torsions, Eintl:- Internal Conformational Energy of the ligand, Dsolv:- The desolvation of exposed H-bond donors and acceptors, SolEl:- The solvation electrostatics energy change upon binding, **Pmf Score:-** mean force score of ligand-receptor interaction strength.[lower the score, better the strength], **DTSsc:** loss of entropy by the rotatable protein side-chains.

Enzyme name	Docked score	HBond	Hphob	VwInt	Eintl	Dsolv	SolEl	Pmf Score	DTSsc
Acetyl cholinesterase	-14.98	-3.33	-3.63	-18.74	1.275	12.71	4.75	-51.33	0.93
5÷ Nucleotidase	-7.035	-1.748	-4.743	-19.32	1.61	17.32	7.156	-29.36	1.569
Metalloproteinase	-9.993	-1.832	-4.864	-19.5	2.121	14.6	6.032	-85.76	1.083

Table 3: Illustration of Receptor pockets/ligand interaction site's pocket volume, area, Aromaticity, Hydrophobicity and druggness probability. Buriedness of pocket ranges from 0 to 1 [open to completely buried].

Enzyme	Pocket	Volume	Area	Hydrophobicity	Buriedness	Aromaticity	DLID prob*	Non-sphericity
		[A~]	[A~]					
Acetyl cholinesterase	1	239.25	299.36	0.561896	0.739845	0.10058	0.03	1.606221
Metalloproteinase	1	239.5	252.9	0.5673	0.745	0.1038	1	1.35
5÷ Nucleotidase	1	164.9	198.2	0.429	0.6769	0	0.71	1.363

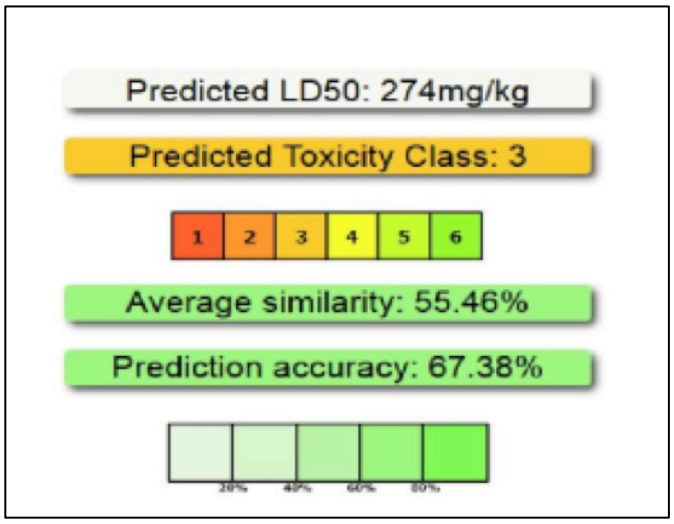

Figure 6: Oral Toxicity prediction **[14]** of the inhibitor [C26H31N2O6F]

Table 4: Depicting the physical properties of the Inhibitor [C26H31N2O6F] molecule using ICM-Mol soft.

Molecular weight	486.2166
HBA	9
HBD	3
ROtB	3
Drug likeliness	-0.0341
Mol Area	466.626
Mol HF	-216.73
Mol half-life	1.62
Mol Log S	-5.09
Groups	Furan, Ester, Aldmine, Ether, Oxirane, Halo

Table 5: P450 Isoform Classification of Inhibitor [C26H31N2O6F]

	Isoform Type	Probability value
Majorly belongs to 3A4 Isoform	1A2	0.024
	2C19	0.062
	2C8	0.096
	2C9	0.11
	2D6	0.034
	2.00E+01	0.006
	3A4	0.668
P450_3A4_Sites	C1 2.65034e-5 stable	
	C3 1.68453e-6 stable	
	C4 1.28204e-6 stable	
	C5 7.96064e-7 stable	
	C12 2.7001e-5 stable	
	C13 7.07775e-7 stable	
	C14 6.99382e-7 stable	
	C17 7.58538e-8 stable	
	C22 0.000948583 stable	
	C24 0.000201541 stable	
	C25 96.0074 labile	
	C27 3.98283 mod labile	
	C28 1.85384e-6 stable	
	C33 0.00156411 stable	
	C35 5.51779e-5 stable	
	C7=C8 0.00692835 stable	
P450_3A4_CSL		0.9691
P450_3A4_CSL_Uncertainty		0.04868

Table 6: ADME Properties: Probability scoring profile of inhibitor [C26H31N2O6F]. (a) Inhibitor is Non-CNS and non-BBB permeable and has a low score for 2C9_pKi. (b) Inhibitor with least probability score.

Profile Name	Score	Standard Deviation	Desired value [According to Lipinski rule of five]
Intravenous CNS Scoring Profile Score	0.07932	0.1037	NA
Intravenous Non-CNS Scoring Profile Score	0.1906	0.1894	NA
Lipinski Rule of Five Score	1	0.000846	NA
Oral CNS Scoring Profile Score	0.04286	0.08281	NA
Oral Non-CNS Scoring Profile Score	0.103	0.1619	NA
BBB log brain blood	-0.8012	Inf	-0.2 to 1
Log S	0.1163	1.033	>2
logS_pH7_4	0.1163	1.033	NA
Log P	2.831	0.4351	0 -3
Log D	2.831	0.4351	0 -3
2C9_pKi	5.304	Inf	<6
hERG_pIC50	4.193	0.9567	<6.3
Mol .weight	486.5	0	<500 DA
HBD	2	0	0 — 5
HBA	8	0	0 — 10
TPSA	128.1	0	<140 A^2
Flexibility	0.075	0	NA
Rotatable bonds	3	0	0 -9

Property	Category	Probability	Desired value
BBB category		0.74	-
HIA category	- +	0.863636	+
P _gp Inhibitor	No	0.53	NO
2D6_affinity_category	Very high	0.5625	<6
PPB90_category	Low	0.58	Low

Molecular docking:

Molecular docking analysis of snake venom enzymes with modified gedunin from neem was completed using ICM-Pro Molsoft-Software **(Tables 1, 2, 3).** The ICM score is the sum of the ligand-target Vander-Waals interactions and the internal force field energy of the ligand (DE IntFF 5), hydrogen bond

interactions (DE HBond Tor), free energy changes due to conformational energy loss with ligand binding (TDS), solvation of electrostatic energy with ligand binding (DE HBDesol), hydrophobic free energy generation (DE HPhobSolEl), hydrogen bond interactions (DE HBond Tor), hydrogen bridge donor-acceptor desolvation energy as described elsewhere **[6].**

Pharmacokinetics Analysis :

ADMET (absorption, distribution, metabolism, excretion, toxicity) properties were calculated using SwissADME (**Figure 3)** as described elsewhere **[16].**

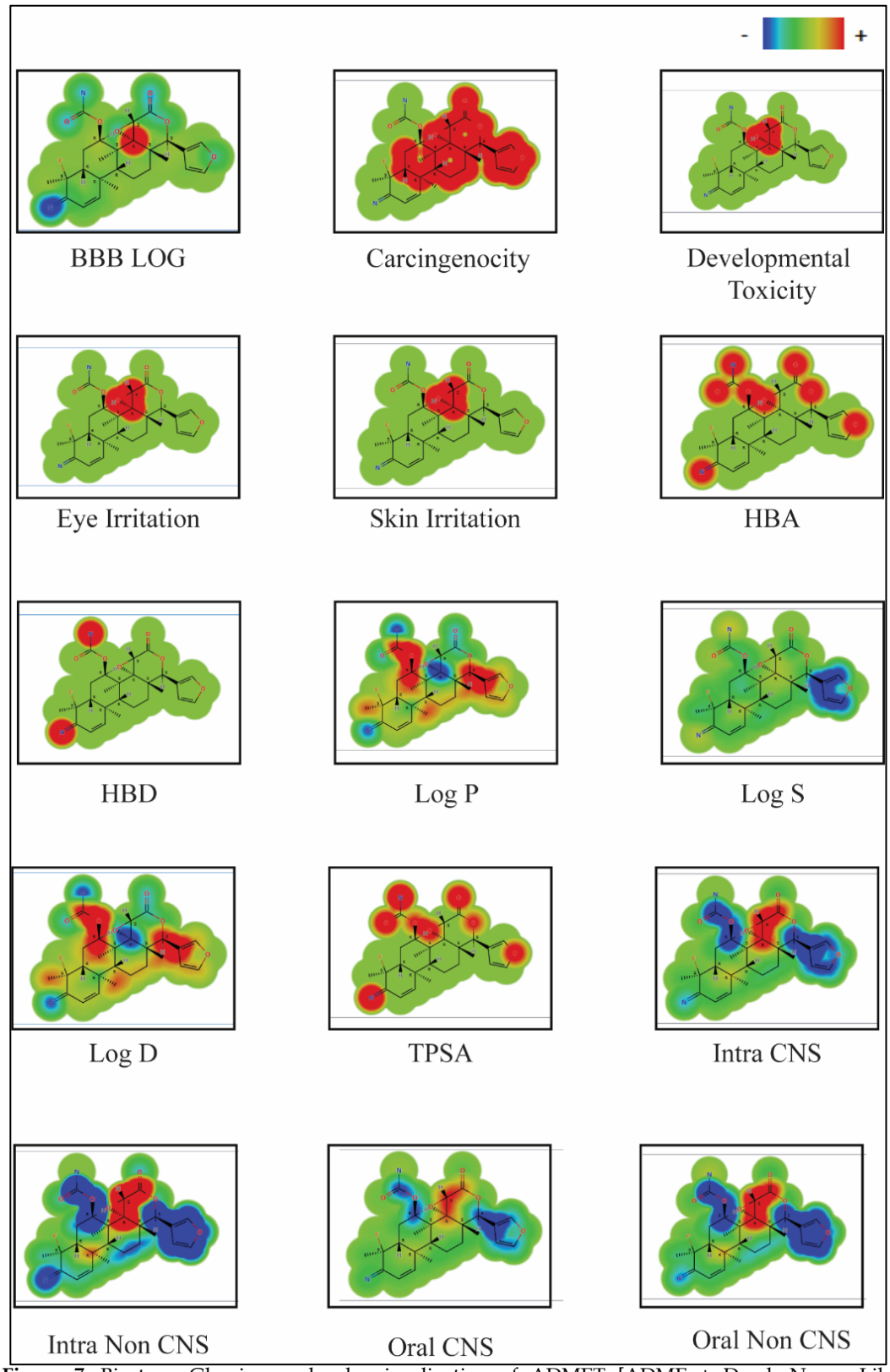

Figure 7: Biostere: Glowing molecule visualization of ADMET [ADME + Derek Nexus Likelihood] properties ofthe inhibitor [C26H31N2O6F]. Red region is increasing the predicted value, blue region is decreasing the predicted value whereas green region does not affect.

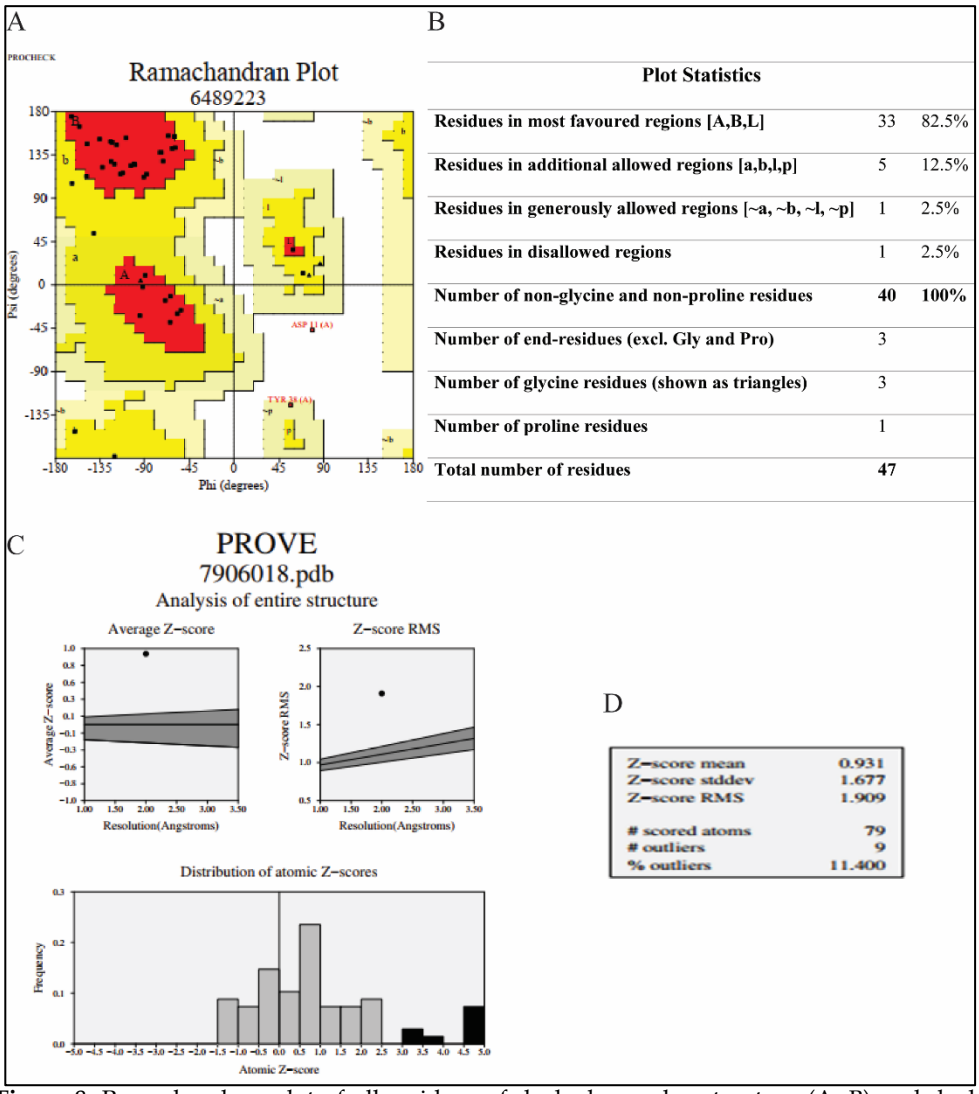

Figure 8: Ramachandran plot of all residues of docked complex structure (A, B) and docked complex structure analysis by PROVE server (C).

Table 7: Toxicity prediction profiling of inhibitor [C26H31N2O6F] using Derek-Nexus Likelihood from Stardrop. Plausible reports support the proposition that the inhibitor can cause skin and eye irritation. Carcinogenicity profile is equivocal of proposition for and against of inhibitor.

Property			
No Report	Plausible	Equivocal	
Photo — allergenicity	Skin sensitisation		Carcinogenicity
Occupational asthma	Developmental toxicity		
Respiratory sensitisation	Hepatotoxicity		
Splenotoxicity Probability	Skin irritation		
Teratogenicity	Eye irritation		
Testicular toxicity			
Adrenal gland toxicity			
Thyroid toxicity			
Ocular toxicity			
Pulmonary toxicity			

Result & Discussion:

The modified gedunin compound shown in **Figure 1** is the ligand. The modified gedunin using ICM-Pro was selected after lowering the steric score and the ICM score is shown in **Figure 2**. The modified gedunin showed an increased number of hydrogen and non-covalent bonds with low steric hindrance allowing easy binding of inhibitor inside the pocket of the enzyme. The results of the molecular docking **[2]** for the compound are given in **Table 1**. The lowest score shows effective binding of enzyme and inhibitor with the Hp score calculated as the difference between the conformation of the free ligand and the hydrophobic interaction energy found as -3.63, -4.743, -4.864 for acetyl cholinesterase, 5'nucleotidase, and metalloproteinase, respectively. It implies that 5'nucleotidase and metalloproteinase have the least hydrophobic interactions with the compound.

Table 2 shows the evaluation function for ligand enzyme complexes based on intermolecular interactions with hydrogen bond energy, van der Waals interaction energy (sum of gc and gh Vander Waals), hydrophobic energy when the surface is exposed to water, number of rotatable torsions, internal conformational energy of the ligand. Desolvation of exposed hydrogen-bond donors and acceptors is the change in the electrostatic solvation energy upon binding, mean force value of the ligand-receptor interaction strength **[14].** Acetyl cholinesterase has the least negative (preferred) ICM score -14.98 followed by -7.035, -9.993 and PMF score: -51.33 followed by -29.36, -85.76 for 5` nucleotidase or metalloproteinase, all with ICM score <-15.0 and PMF score <-37.5. 5' nucleotidase and metalloproteinase – inhibitor complex is less stable than acetyl cholinesterase–inhibitor complex. The selection of the enzyme docking sites is based on the DLID likelihood for each pocket in enzymes. The DLID probability is highest in the active site of the enzyme. **Table 3** shows the DLID, pocket buridness of enzymes. Pocket with a

high DLID score is preferred as it has high drug likeliness in enzymes, which can be easily seen in molecular dynamics. Molecular dynamics simulation using GROMACS for 50 ns (nano-second) show that the average angle between Gedunin and 5'NT enzyme is between 70-760 with density between 980-984 kg/m^3 increased due to complex formation.

The radius of gyration decreased from 1.2 to 1 after complex formation, implying tight bonding of complex. The number of hydrogen bonds between peptide-water is a maximum of 100-160, while between peptide-peptide their number is five. The binding energies decreased considerably to ~ 0 Ki/mol, which shows the stability of the structure. Initially, RMSD was 8 nm, whereas, after the complex formation, RMSD is stable. The pressure is approximately 400 Bar and RMS is between 0.2-0.7. The overall simulation analysis shows that the molecule binds to the active center of 5'NT and the complex becomes stable. The Ramachandran plot analysis of all complex residues using Prove server with a mean z score of 0.931 is shown **(Figure 8).** Lipinski's rule of five determines the biological activity of the drug **[10]**. The total absorbed mass/dose of the drug is shown for the compound in **Table 6**. **Table 6** also shows that ligand is a non-CNS drug calculated using SwissADME and medicinal chemistry analysis. **Figure 3** shows the drug probability for the ligand.

Figure 4 shows a boiled egg diagram, with the yellow (egg yolk) region for the likelihood of brain penetration and the white region for passive absorption through the gastrointestinal tract. **Table 5** shows that the ligand has broad substrate specificity and primarily through the phase 1 enzyme CYP Isoform 3A4 with a probability of 0.9691. **Table 5** shows a probability of 0.66 according for isoform 3A4. The metabolism landscape shown in **Figure 5** shows the selectivity of the ligand to isoform 3A4. **Figure 5** shows that C25 and C27 are more labile than the rest of the ligand sites. The binding features for 2C9_Pki with hERG pic50 are shown in **Table 6**. **Table 7** shows the Derek nexus probability **[16]** for the ligand with toxicity data **[2, 15]**. The oral toxicity prediction is shown in **Figure 6** for Protox II, and the LD50 with 274 mg/kg. **Figure 7** shows the bioactivity of the R groups in the ligand using the luminous molecular property of Stardrop Software. Thus, these data help to describe the molecular docking analysis of gedunin (C26H31N2O6F) with enzymes (common in snake species) such as 5-nucleotidase, acetyl cholinesterase, L-aao, metalloproteinase, serine, thrombin and phospholipase A2 towards in combating snakebites.

Conclusion:

We show the molecular docking analysis of gedunin (C26H31N2O6F) with enzymes (common in snake species) such as 5-nucleotidase, acetyl cholinesterase, L-aao, metalloproteinase, serine, thrombin and phospholipase A2. The modified gedunin in the enzyme pocket improves the pharmacological properties for further consideration in combating snakebites.

Abbreviations:

PMF – peptide mass fingerprinting
LD50 – lethal dose 50
ADME – absorption, distribution, metabolism, and excretion
DLID – drug-like density
5'NT – 5'nucleotidase
RMSD – root mean square deviation
RMS – root mean square
CNS – center nervous system
TPSA – total polar surface area
CYP - cytochrome

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Conflict of interest:

The authors declare no conflicts of interest.

Author contributions:

Priya Dagar and Abha Mishra conceived the idea. Priya Dagar performed the computations. Abha Mishra verified the analytical methods and helped with the development of the manuscript.

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