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In-silico and *In-vitro* Evaluation of Vindoline as a Potential Anti-inflammatory Agent Targeting Cox-2 And Nf-κb

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Anti-inflammatory agents are essential for managing inflammation-driven diseases, with COX-2 (cyclooxygenase-2) and NF-êB (nuclear factor kappa B) serving as key molecular targets. Vindoline, a phytochemical derived from *Catharanthus roseus*, has demonstrated potential pharmacological properties, including anti-inflammatory activity. This study aims to evaluate the binding affinity and interactions of vindoline with COX-2 and NF-êB through molecular docking studies using AutoDock Vina. Diclofenac sodium was used as the reference standard. The 3D structure of vindoline was obtained from PubChem, and protein structures of COX-2 (PDB ID: 6COX) and NF-êB (PDB ID: 1NFK) were retrieved from the Protein Data Bank. Molecular docking was performed using AutoDock Vina to analyze binding affinities, and protein-ligand interactions were visualized using BIOVIA Discovery Studio 2025. ADME/T analysis was performed using SwissDock and Protox 3.0. The results demonstrated strong binding interactions of vindoline with COX-2 (-9.4 kcal/mol) and NF-êB (-7.0 kcal/mol) compared to Diclofenac Sodium (-8.0 kcal/mol and -6.4 kcal/mol, respectively). Key interactions, including hydrogen bonding and hydrophobic contacts at the active sites, supported its potential inhibitory activity. The results of egg albumin denaturation assay revealed that Vindoline (50–250 μg/mL) significantly inhibits protein denaturation and exhibits superior inhibition compared to Diclofenac Sodium. Vindoline also demonstrated high solubility and a potentially safer profile than Diclofenac Sodium. It suggests its potential as a lead compound for the development of anti-inflammatory drugs.

Keywords: ADME/T Analysis, AutoDock Vina, Cyclooxygenase-2, Egg Albumin Denaturation Assay. Molecular Docking, Nuclear Factor Kappa B, Vindoline.

Inflammation is a multifaceted biological response triggered by harmful agents such as pathogens, injured cells, or irritants.[1,2] While short-term (acute) inflammation plays a crucial role in defending the body and promoting tissue healing, [3,4] prolonged (chronic) inflammation is associated with the development of numerous health conditions, including rheumatoid arthritis, heart disease, neurodegenerative disorders, and cancer.[5,6,7] Key molecular mediators in the inflammatory cascade include cyclooxygenase-2 (COX-2) and nuclear factor kappa B (NF-êB).[8,9] COX-2, an inducible enzyme, catalyzes the conversion of arachidonic acid to pro-inflammatory prostaglandins,[10,11] while the transcription factor NF-êB plays a key role in controlling the activity of

genes that govern inflammatory responses, immune function, and cell survival.[12,13] These molecules have emerged as critical therapeutic targets for the development of anti-inflammatory drugs.

Vindoline, an alkaloid derived from *Catharanthus roseus* (commonly known as the Madagascar periwinkle), is one of the key phytoconstituents of this medicinal plant, renowned for its diverse therapeutic properties.[14,15] *Catharanthus roseus* has long been utilized in traditional medicine, and its alkaloids, such as vincristine and vinblastine, are well-established chemotherapeutic agents.[16,17] Vindoline, though less explored, has shown potential pharmacological activities, including anti-inflammatory, antioxidant, and anticancer effects.[18,19] Given its promising biological properties, investigating vindoline as a potential inhibitor of COX-2 and NF-êB offers a compelling avenue for drug discovery. Molecular docking has revolutionized drug discovery by facilitating the virtual screening of compounds and identifying key protein-ligand interactions.[20]

This study aims to evaluate the anti-inflammatory potential of vindoline through *in-silico* molecular docking studies and in-vitro study. By evaluating the binding affinities and interactions of vindoline with COX-2 and NF-êB, this research aims to provide insights into the mechanism of action of vindoline and establish its potential as a lead compound for anti-inflammatory drug development.

MATERIALS AND METHODS

Software and Tools

For *in-silico* evaluation of Vindoline as a potential anti-inflammatory agent targeting COX-2 and NF-êB the following software, tools and applications were used: PubChem, RCSB Protein Data Bank (PDB), AutoDock MGL Tools, AutoDock Vina, BIOVIA Discovery Studio 2025, SwissADME Online Tool, and Protox 3.0 Online Tool.

In-Silico Anti-Inflammatory Activity

Stepwise Procedure for Molecular Docking

Molecular docking of vindoline with 6COX (Cyclooxygenase-2, COX-2) and 1NFK (Nuclear factor-kappaB, NF-êB) involved several key steps, including protein preparation, ligand preparation, docking, and visualization.

Step 1: Preparation of Protein (Target Molecules)

The protein receptor and ligand molecules were selected based on their relevance to the study. Two proteins Cyclooxygenase-2 (COX-2) and Nuclear Factor-kappaB (NF-êB) were chosen for this study.

Retrieval of the Protein Structure

The three-dimensional structures of COX-2 (PDB ID: 6COX) and NF-êB (PDB ID: 1NFK) were retrieved from the RCSB Protein Data Bank, accessible at https://www.rcsb.org/.[21] The corresponding PDB files were downloaded and used for docking analysis (Table 1).

Remove Unnecessary Molecules and Prepare the Protein for Docking

The protein structures (6COX.pdb and 1NFK.pdb) were loaded in AutoDock Tools. Water molecules were removed (*Edit '! Delete Water*). Polar hydrogens were added (*Edit '! Hydrogens '! Add '! Polar only*). Kollman charges were assigned (*Edit '! Charges '! Add Kollman Charges*). The modified protein structures were then saved in pdbqt format as 6COX_clean.pdbqt and 1NFK_clean.pdbqt.[25,26]

Step 2: Preparation of Ligand (Vindoline)

Obtain the Ligand Structure

The 3D structures of Vindoline (PubChem ID: 260535) and Diclofenac Sodium (PubChem ID: 5018304) were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).[27] The structure was initially downloaded in SDF format and later converted to PDB format using Discovery Studio 2025 (Figure 1).

Prepare Ligand for Docking (Using AutoDock Tools)

AutoDock Tools (ADT) was used for ligand preparation. The vindoline.pdb file was loaded into the software, hydrogens were added, and Gasteiger charges were assigned. The processed structure was then converted and saved in PDBQT format as vindoline.pdbqt.

Step 3: Docking Procedure

AutoDock Vina was used for molecular docking simulations to predict the binding affinity and orientation of Vindoline and Diclofenac sodium within the active site of COX-2 and NF-êB. This software facilitates the identification of potential lead compounds, enabling the rational design of novel inhibitors with improved efficacy.[28,29,30]

Grid Box Preparation (Defining Binding Site)

The prepared protein structures (6COX_clean.pdbqt or 1NFK_clean.pdbqt) and the ligand (vindoline.pdbqt) were loaded into the software (Figure 2). The grid box was set (*Grid '! Set Grid Box*) and centered around the active site of COX-2 or NF-êB. The x, y, and z dimensions (x=47.061, y = 25.506, z = 36.889) were adjusted to encompass the binding site, and default parameters in AutoDock Vina were used for the simulations. The configuration file was then saved as config.txt.

Docking Execution

The docking simulation was carried out in the Command Prompt. The working directory containing vina.exe was accessed using the command cd C:\path\to\AutoDockVina. Docking was executed with the following command: vina.exe —receptor 6COX.pdbqt —ligand vindoline.pdbqt —config config.txt —log vina_log.txt —out docked_output.pdbqt. The configuration file (config.txt) defined docking parameters, including grid box coordinates, exhaustiveness, and center position. After completion, the vina_log.txt file was analyzed to obtain binding affinity scores.[31]

Post-Docking Analysis

The binding affinity scores (in kcal/mol) were extracted from the vina_log.txt file and protein-ligand interactions were visualized to identify hydrogen bonds and hydrophobic interactions. The binding affinity scores of

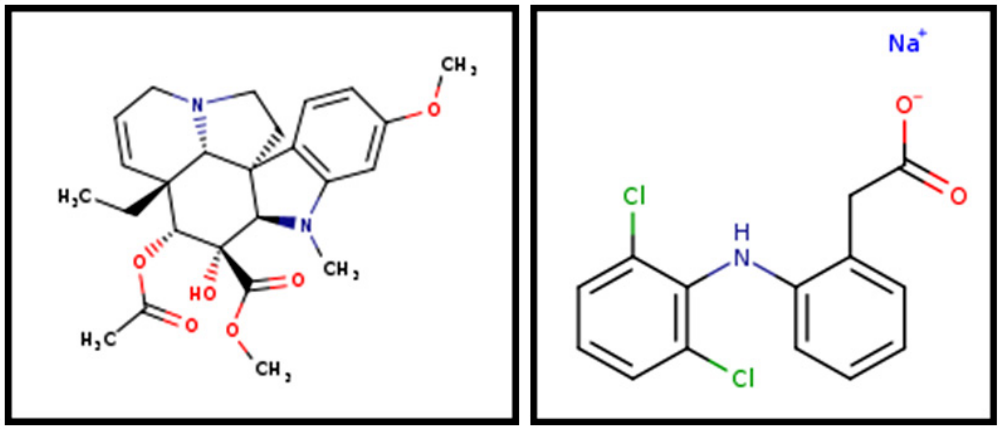

Fig. 1. 2D Structure of Vindoline and Diclofenac Sodium

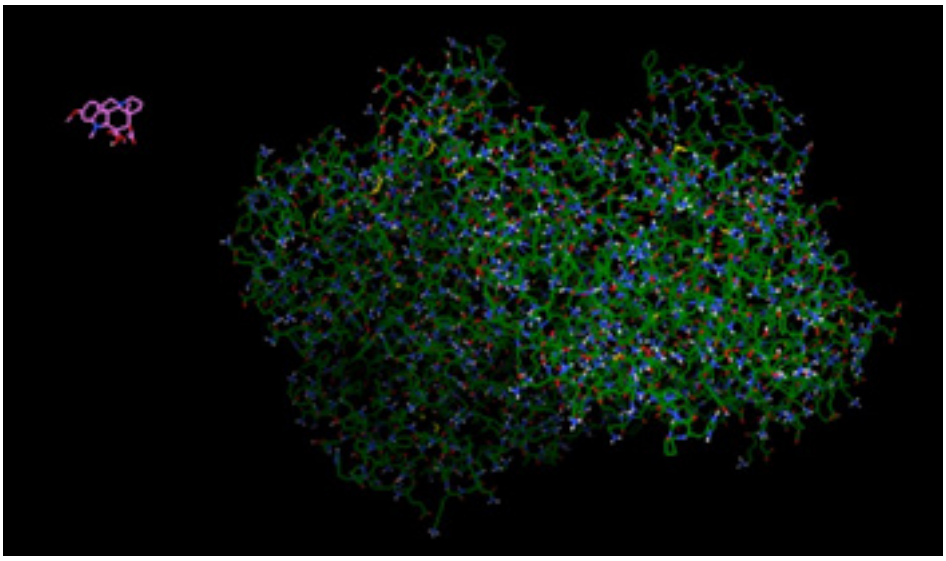

Fig. 2. Import of Protein and Ligand in AutoDock Vina

Vindoline were compared with those of the Standard Ligand (Diclofenac Sodium).

Step 4: Visualization in Discovery Studio 2025

Molecular interactions were analyzed using BIOVIA Discovery Studio 2025. The prepared protein structure (6COX_clean.pdbqt or 1NFK_clean.pdbqt) and the docked ligand (docked_output.pdbqt) were loaded into the software. Ligand binding was examined through the Ligand-protein Interactions tool. Hydrogen bonding sites were identified via Receptor-Ligand Interactions. Additionally, 2D and 3D interaction

Table 1. Target Proteins and their Respective Functions

Proteins	Protein Description	Protein Function	PDB ID
COX-2	Cyclooxygenase-2	Inducible enzyme that converts arachidonic acid into pro-inflammatory prostaglandins (e.g., PGE,), leading to pain, fever, and swelling. Upregulated in response to cytokines and stress.[22,23]	6COX
NF-êB	Nuclear factor-kappaB	A transcription factor responsible for controlling the expression of pro-inflammatory cytokines such as TNF-á, IL-1â, and IL-6, chemokines, and adhesion molecules. Activates immune responses and sustains chronic inflammation. Also induces COX-2 expression, amplifying inflammation.[24]	1NFK

Table 2. Binding Affinity of Vindoline and Diclofenac Sodium with 6COX (Cyclooxygenase-2)

Molecule Name	Binding Affinity (kcal/mol)	Hydrogen Bond Interactions (Protein Residues)	Bond Length (Aº)	Amino Acid Residue Interactions (Hydrophobic / Pi-Cation / Pi-Anion / Pi-Alkyl)
Vindoline (Test Compound)	-9.4	B: LEU224 B: GLN374 A: ASN375	3.57 3.78 3.49	A: PHE142, A: LEU145, B: LEU145, B: PHE142
Diclofenac Sodium (Standard)	-8.0	B: SER143 B: ASN144 B: GLU140 A: GLU236	3.03 3.15 2.01 1.76	A: LEU238

Table 3. Binding Affinity of Vindoline and Diclofenac Sodium with 1NFK (Nuclear factor-kappaB)

Molecule Name	Binding Affinity (kcal/mol)	Hydrogen Bond Interactions (Protein Residues)	Bond Length (Aº)	Amino Acid Residue Interactions (Hydrophobic / Pi-Cation / Pi-Anion / Pi-Alkyl)
Vindoline (Test Compound)	-7.0	A: ARG54 B: SER72 B: GLY66	3.60 2.30 3.57	B: ARG56, B: PHE53, C: DG2
Diclofenac Sodium (Standard)	-6.4	A: ASN136 A: ASN136 A: GLY66 A: LEU67	2.16 2.72 2.11 2.06	A: LYS77, A: PRO68

maps were generated. High-quality images for publication were saved.[32]

ADME/T Analysis

The ADME analysis was performed using the SwissADME online server. Lipinski's rule of five, ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity), Drug-likeness properties, pharmacokinetics, and physicochemical properties of Vindoline and Standard Diclofenac Sodium were evaluated. Toxicity data for both drugs were obtained from the Protox 3.0 online server.[33,34]

***In-Vitro* Anti-Inflammatory Activity**

Egg Albumin Denaturation Assay

This assay evaluates the anti-inflammatory potential of test compounds by determining their ability to prevent or inhibit the denaturation of egg albumin under specific conditions. Inflammation can cause denaturation of proteins. Denaturation is the process in which a protein loses its biological activity and undergoes structural changes.[35] In this assay, egg albumin was used as a model protein, and denaturation was induced by exposing it to extreme conditions such as heat, pH variations, or other denaturing agents. This structural alteration resulted in changes to its physical properties and a loss of functional activity.[36] Inflammation is often associated with protein denaturation, substances with anti-inflammatory properties are believed to stabilize protein structures and inhibit denaturation.[37]

Collection and Preparation of 1% Egg Albumin Solution

A fresh hen's egg was purchased from the local market, and the egg white (albumin) was carefully separated into a beaker without disturbing the yolk. To prepare a 1% egg albumin solution, 1 ml of the translucent egg white was transferred

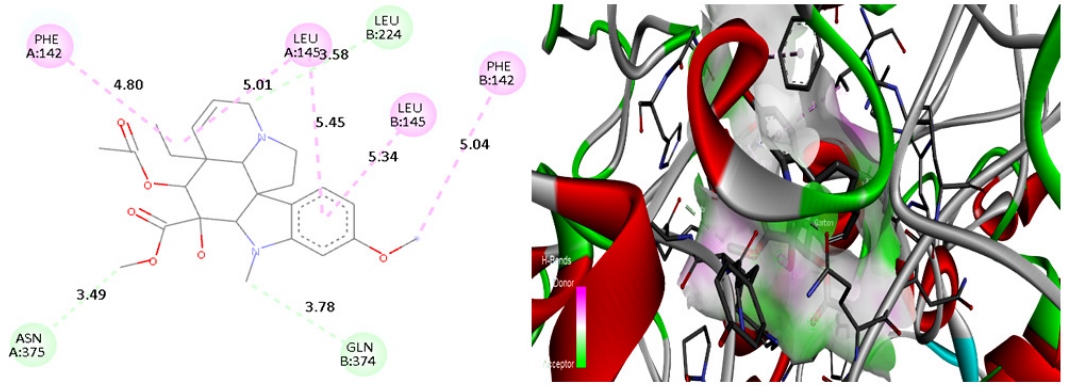

Fig. 3. Interaction of Vindoline with Target Receptor Cyclooxygenase-2 (PDB ID- 6COX)

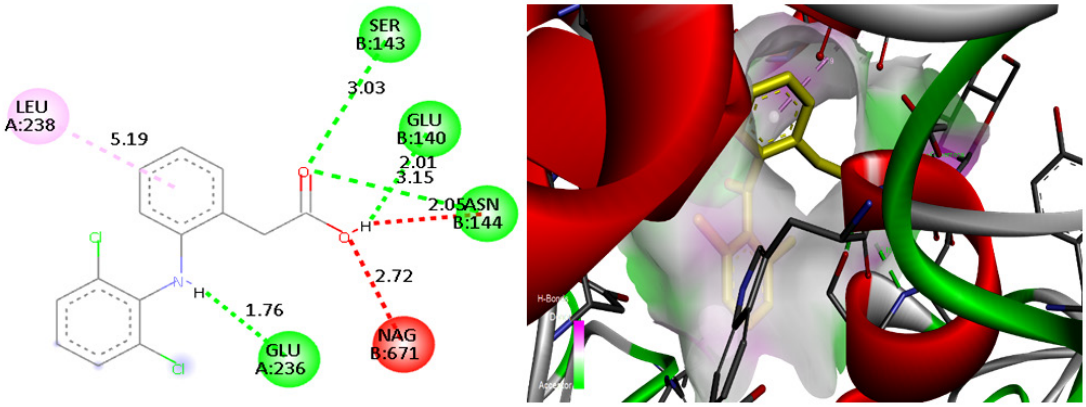

Fig. 4. Interaction of Diclofenac Sodium with Target Receptor Cyclooxygenase-2 (PDB ID- 6COX)

into a 100 ml volumetric flask. Cold-distilled water was then added to bring the volume to 100 ml. The solution was thoroughly stirred with a magnetic stirrer to ensure uniform mixing.

Procedure of Egg Albumin Assay

The anti-inflammatory activity of isolated Vindoline was evaluated in vitro by assessing its ability to inhibit egg albumin denaturation.

A reaction mixture was prepared by combining 0.2 mL of a 1% egg albumin solution with 2 ml of either Vindoline or the reference drug, diclofenac sodium, at varying concentrations ranging from 50 to 250 ìg/ml. This was followed by the addition of 2.8 ml of phosphate-buffered saline (PBS) at pH 7.4, resulting in a final volume of 5 ml. The pH was carefully adjusted to 7.4 using a minimal volume of 1N HCl. A control solution was also prepared by mixing 2 ml of triple-distilled water with 0.2 ml of the 1% egg albumin and 2.8 ml of PBS. All mixtures were incubated at 37 ± 2°C for 30 minutes and then subjected to heating in a water bath at 70 ± 2°C for 15 minutes. Once cooled, the absorbance was recorded at 660 nm using a UV/Vis spectrophotometer, with triple-distilled water serving as the blank. The percentage inhibition of protein denaturation was calculated using the following formula.

$$\% \text{ Inhibition} = (\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control}) \times 100$$

Statistical Analysis

All statistical analyses were conducted using Microsoft Excel. The results are presented as the mean ± standard error of the mean (SEM). Differences between groups were assessed using one-way ANOVA (Single Factor). P-values were

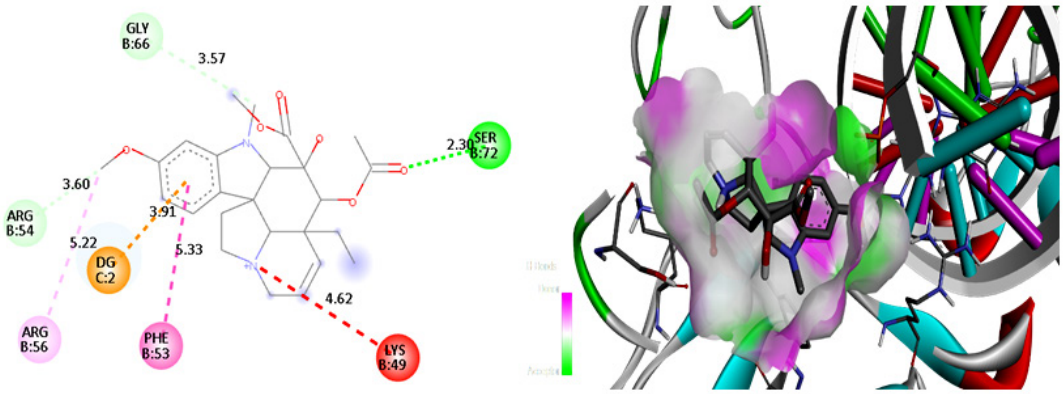

Fig. 5. Interaction of Vindoline with Target Receptor Nuclear factor-kappaB (PDB ID- 1NFK)

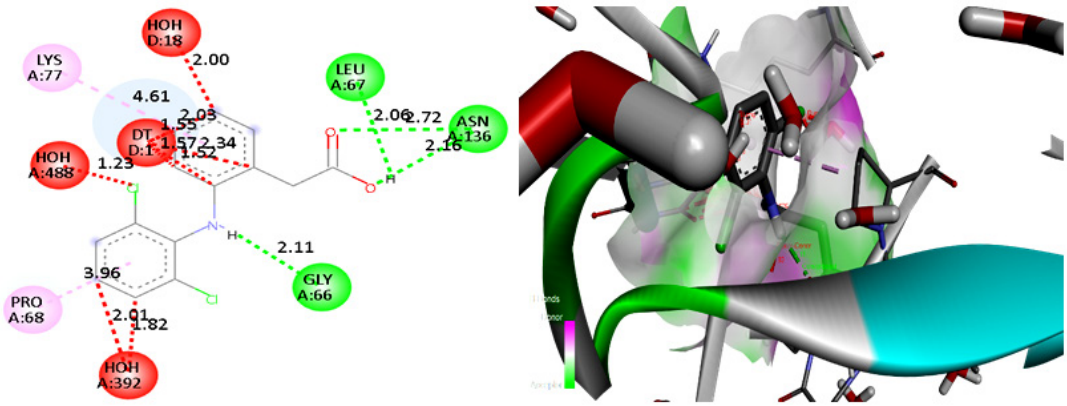

Fig. 6. Interaction of Diclofenac Sodium with Target Receptor Nuclear factor-kappaB (PDB ID- 1NFK)

Table 4. ADME Analysis of Vindoline and Diclofenac Sodium

Drug	Lipinski's Rules				Lipinski's Rule Violations	GI Absorption	BBB Permeability	P-gp Substrate	CYP3A4 Inhibitor	Bioavailability Score
	MW(<500)	Log P(≤ 5)	HBA(≤10)	HBD(≤5)						
Vindoline	456.53 g/mol	1.78	7	1	0	High	No	No	Yes	0.55
Diclofenac Sodium	318.13 g/mol	0.65	2	1	0	High	Yes	Yes	No	0.55

calculated for each comparison, and a p-value less than 0.05 was considered statistically significant.

RESULTS

The molecular docking analysis was performed to evaluate the binding affinity and interaction of Vindoline with Cyclooxygenase-2 (6COX) and Nuclear Factor-kappa B (1NFK) in comparison with Diclofenac Sodium as a standard. Against 6COX, Vindoline exhibited a higher binding affinity (-9.4 kcal/mol) than Diclofenac Sodium (-8.0 kcal/mol), indicating a stronger interaction with the target (Table 2).

Vindoline formed hydrogen bonds with residues B: LEU224 (3.57 Å), B: GLN374 (3.78 Å), and A: ASN375 (3.49 Å), along with hydrophobic interactions involving A: PHE142, A: LEU145, B: LEU145, and B: PHE142 (Figure 3).

In contrast, Diclofenac Sodium exhibited hydrogen bonding with B: SER143 (3.03 Å), B: ASN144 (3.15 Å), B: GLU140 (2.01 Å), and A: GLU236 (1.76 Å), with hydrophobic interaction involving A: LEU238 (Figure 4).

Similarly, docking results with 1NFK revealed that Vindoline had a binding affinity of -7.0 kcal/mol, which was higher than Diclofenac Sodium (-6.4 kcal/mol) (Table 3). Hydrogen bonding interactions for Vindoline were observed with A: ARG54 (3.60 Å), B: SER72 (2.30 Å), and B: GLY66 (3.57 Å), alongside hydrophobic interactions with B: ARG56, B: PHE53, and C: DG2 (Figure 5). Diclofenac Sodium, on the other hand, formed hydrogen bonds with A: ASN136 (2.16 Å, 2.72 Å), A: GLY66 (2.11 Å), and A: LEU67 (2.06 Å), with additional hydrophobic interactions with A: LYS77 and A: PRO68 (Figure 6).

The overall results suggest that Vindoline exhibits a stronger binding affinity and favorable interactions with both targets (COX-2 and NF-êB), potentially indicating its role as a promising anti-inflammatory agent. Figures 3-6 illustrate the binding interactions of Vindoline and Diclofenac Sodium with their respective target receptors.

Results of ADME/T Analysis

Vindoline and Diclofenac Sodium exhibit distinct pharmacokinetic and physicochemical properties (Table 4). Vindoline (Plant-derived

alkaloid) has a higher molecular weight (456.53 g/mol) and greater lipophilicity than Diclofenac Sodium (318.13 g/mol). Both drugs show high gastrointestinal absorption. Their metabolic profiles also vary; Vindoline primarily inhibits the CYP3A4 and CYP2D6 enzymes, whereas Diclofenac Sodium predominantly inhibits the CYP1A2 enzyme. Both compounds adhere to Lipinski's Rule of Five, indicating favorable drug-likeness, and have a similar bioavailability score of 0.55 (Figure 7 & 8). Diclofenac Sodium can cross the blood-brain barrier. Vindoline is more suitable for peripheral use as it does not cross the BBB.

The Boiled-Egg model is plotted based on WLOGP (lipophilicity) vs. TPSA (topological polar surface area). The Boiled-Egg model (Figure 9) suggests that Vindoline (Molecule 1) falls within the white region, indicating good gastrointestinal absorption but poor blood-brain barrier (BBB) permeability. While, Diclofenac Sodium (Molecule 2) is positioned in the yellow area, indicating potential BBB penetration, it is suitable for CNS-related conditions (Table 5).

Results of Egg Albumin Denaturation Assay

The ability of Vindoline to inhibit egg albumin denaturation was evaluated and compared with that of the standard drug, Diclofenac Sodium. The experiment was carried out in triplicate to ensure reliability. The outcomes, indicating the percentage inhibition of protein denaturation, are presented in Table 6.

The findings revealed that Diclofenac Sodium, used as the standard, exhibited protein denaturation inhibition of 34.78%, 47.82%, 56.52%, 60.86%, and 69.56% at concentrations of 50, 100, 150, 200, and 250 ìg/ml, respectively. In comparison, Vindoline demonstrated inhibition values of 43.47%, 52.17%, 65.21%, 69.56%, and 78.26% at the same concentration levels. The highest inhibitory effects were recorded at 250 ìg/ml, where Vindoline and Diclofenac Sodium showed inhibition percentages of 78.26% and

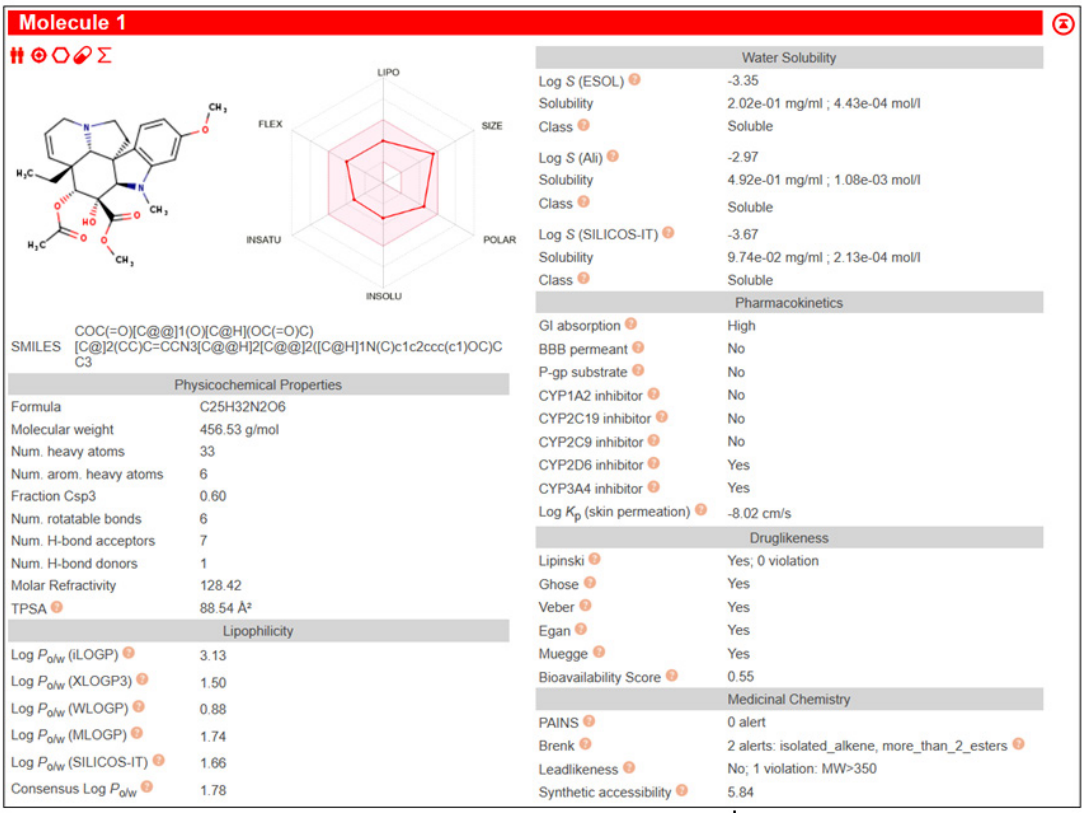

Fig. 7. ADME Analysis of Vindoline

69.56%, respectively. These results suggest that Vindoline exhibits greater anti-inflammatory activity than the standard drug at the highest tested concentration. A graphical representation comparing the percentage inhibition of protein denaturation by Vindoline and Diclofenac Sodium is provided in Figure 10.

DISCUSSION

Inflammation is a natural defense mechanism, but when it becomes uncontrolled, it can contribute to the development of chronic diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) like Diclofenac Sodium are commonly used to manage inflammation, but their potential side effects and toxicity raise concerns, prompting the need for safer alternatives. This study investigated the anti-inflammatory potential of Vindoline, a naturally occurring alkaloid. We compared its effectiveness with that of Diclofenac Sodium by analyzing their molecular interactions with key inflammatory targets, COX-2 and NF-êB and its effect on HRBC membrane lysis.

Our findings revealed that Vindoline binds more strongly to both COX-2 (-9.4) and NF-êB (-7.0) than Diclofenac Sodium (-8.0 and -6.4, respectively), suggesting it may be more effective in inhibiting these inflammation-related proteins.

Protein denaturation refers to the loss of a protein's secondary and tertiary structure, often resulting in the loss of its biological activity. This process is widely recognized as an indicator of inflammation. When proteins become denatured, they can give rise to autoantigens, which may trigger inflammatory responses and contribute to conditions such as rheumatoid arthritis, cancer, and diabetes. Therefore, inhibiting protein denaturation is considered a potential strategy for controlling inflammation.[39] The egg albumin denaturation method offers a cost-effective approach to evaluating the anti-inflammatory potential of isolated compounds. In this assay, proteins are denatured by exposing them to high temperatures.

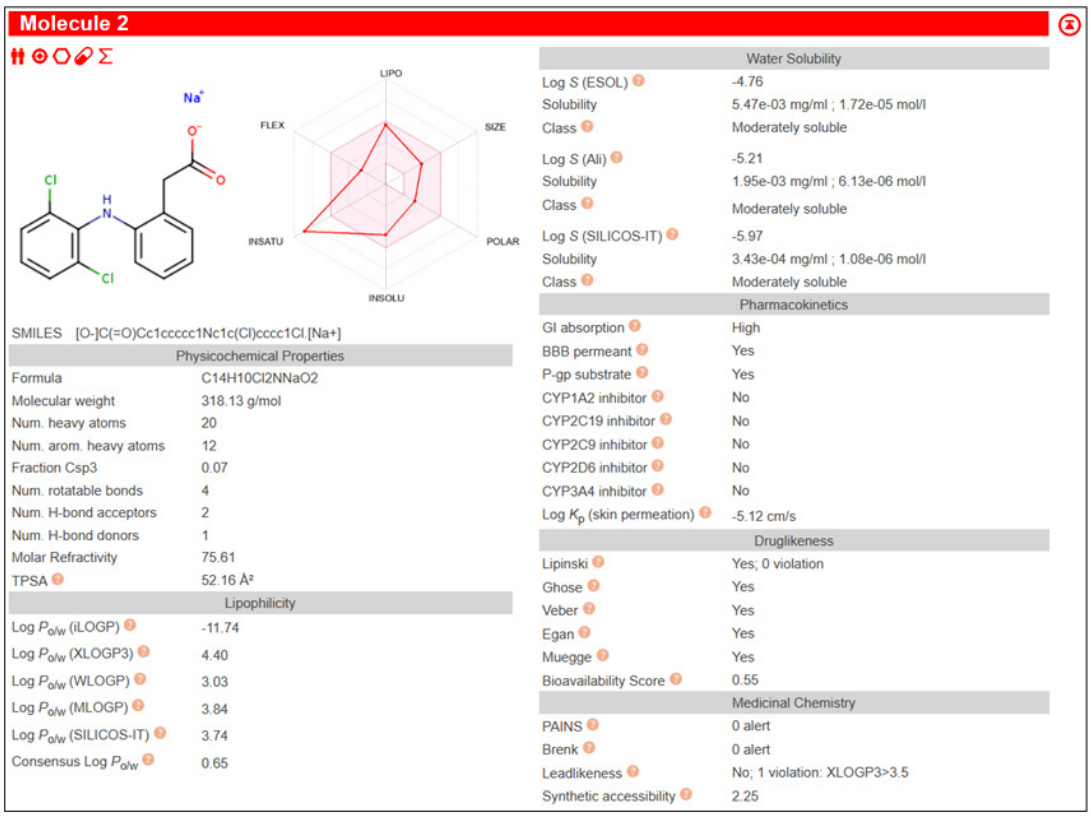

Fig. 8. ADME Analysis of Diclofenac Sodium

The results obtained demonstrated that Vindoline can statistically significantly ($p<0.05$) inhibit protein denaturation. Standard drug Diclofenac sodium showed 34.78, 47.82, 56.52, 60.86 and 69.56% inhibition of protein denaturation at 50, 100, 150, 200 and 250 µg/ml of sample concentration whereas Vindoline showed 43.47, 52.17, 65.21, 69.56 and 78.26 % at 50, 100, 150, 200 and 250 µg/ml of sample concentration in the concentration dependent manner. This study demonstrates that the percentage inhibition of protein denaturation was significantly increased with Vindoline compared to the standard drug diclofenac sodium. The highest level of inhibition was recorded at a sample concentration of 250 ìg/ml. According to the results of the egg albumin denaturation assay, Vindoline was found to be more effective in curing inflammation, exhibiting the highest % inhibition of protein denaturation compared to the conventional standard drug Diclofenac Sodium.

Table 5. Toxicity Prediction Data of the Vindoline and Diclofenac Sodium as per Protox server Results

Drug	Vindoline	Diclofenac Sodium
Predicted LD_{50} (mg/kg)	150mg/kg	53mg/kg
Predicted Toxicity Class	3	3
Average Similarity %	43.13%	98.59%
Prediction Accuracy	54.26%	72.9%
Hepatotoxicity	Inactive	Active
Carcinogenicity	Active	Inactive
Immunotoxicity	Active	Inactive
Neurotoxicity	Active	Active
Nephrotoxicity	Active	Active
Respiratory Toxicity	Active	Active
Cardiotoxicity	Inactive	Inactive
Mutagenicity	Inactive	Inactive
Cytotoxicity	Inactive	Inactive

Additionally, Vindoline demonstrated better water solubility (ESOL: -3.35) compared to Diclofenac Sodium (ESOL: -4.76), which could lead to better absorption and bioavailability—an important factor in drug effectiveness.

One significant advantage of Vindoline is that it does not cross the blood-brain barrier (BBB), making it a safer choice for treating peripheral inflammation without the risk of central nervous system (CNS) side effects. In contrast, Diclofenac Sodium can penetrate the BBB, which

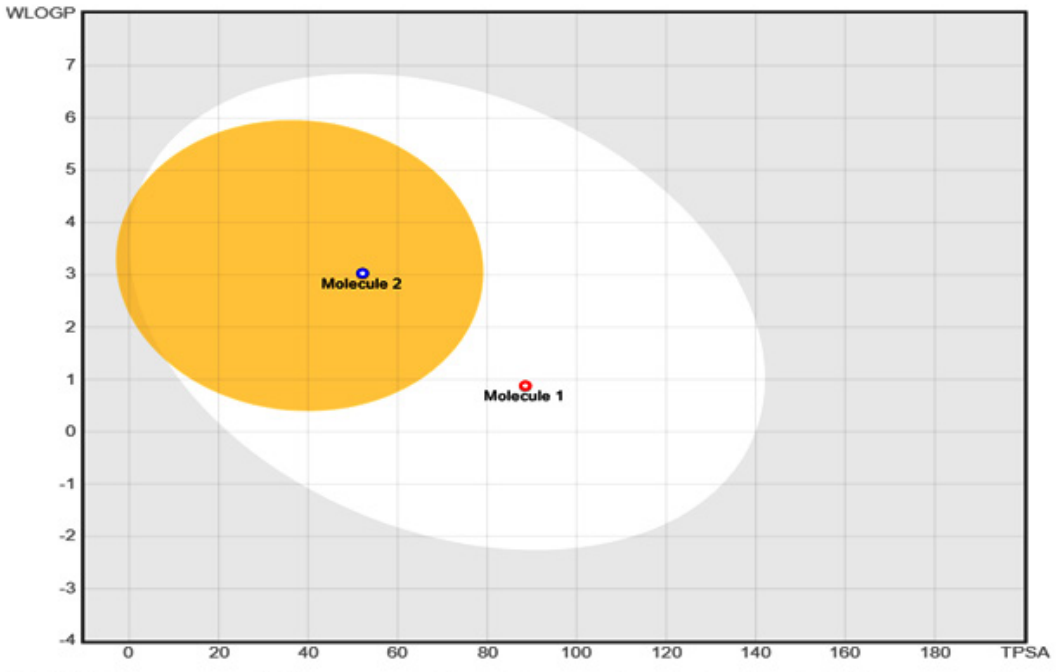

Fig. 9. BOILED-Egg model representing the gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeability of Molecule 1 (Vindoline) and Molecule 2 (Diclofenac Sodium)

may contribute to neurological side effects over long-term use. In terms of toxicity, while both drugs exhibit certain risks, Vindoline has a higher LD_{50} (150 mg/kg) than Diclofenac Sodium (53 mg/kg), indicating a lower acute toxicity. However, while Diclofenac Sodium is primarily associated with liver toxicity, Vindoline carries potential neurotoxic, nephrotoxic, and immunotoxic risks, highlighting the need for further safety studies.

Overall, Vindoline shows promise as a potential anti-inflammatory agent with strong molecular interactions, improved solubility, and a potentially better safety profile than Diclofenac Sodium. Its lack of BBB permeability makes it a safer option for managing inflammation outside the CNS. However, further in-depth studies, including in vitro and in vivo research, are needed to fully evaluate its efficacy, pharmacokinetics, and long-

Table 6. Effect of Vindoline & Diclofenac Sodium on Egg Albumin Denaturation

S. No.	Treatment Group	Concentration (µg/ml)	Absorbance recorded at 660nm	% Inhibition on Egg Albumin Denaturation ± SEM
1	Control	0.023	-	
2	Standard	50	0.015	34.78±0.141
	(Diclofenac Sodium)	100	0.012	47.82±0.292
		150	0.010	56.52±0.413
		200	0.009	60.86±0.219
		250	0.007	69.56±0.269
3	Test (Vindoline)	50	0.013	43.47±0.265
		100	0.011	52.17±0.467
		150	0.008	65.21±0.561
		200	0.007	69.56±0.338
		250	0.005	78.26±0.057

All values are expressed in terms of Mean of % Inhibition ± SEM and are found to be significant when compared to standard $p<0.05$

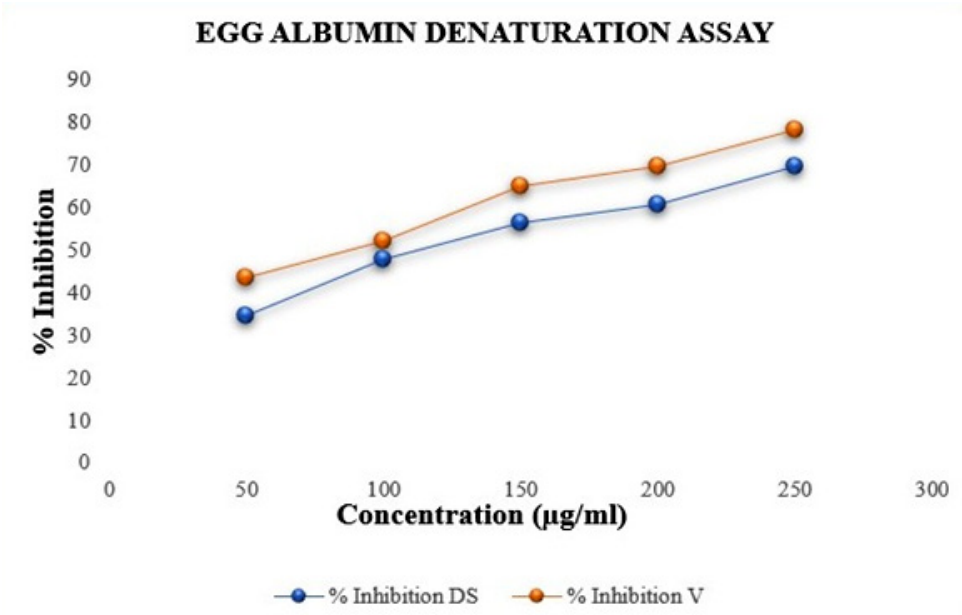

Fig. 10. Effect of Vindoline and Diclofenac Sodium on HRBC Membrane Stabilization

term safety before it can be considered for clinical use.

CONCLUSION

Vindoline exhibits stronger binding affinity to COX-2 and NF-êB, and a better percentage of inhibition of protein denaturation indicating superior anti-inflammatory potential compared to diclofenac sodium. Its higher solubility enhances bioavailability, and its inability to cross the BBB minimizes CNS-related side effects, making it more suitable for non-CNS inflammation. With an optimal LogP (~1.8) and a higher LD50 (150 mg/kg), Vindoline demonstrates better permeability and lower toxicity, lacking hepatotoxicity, a significant concern with diclofenac sodium. Given its improved efficacy, solubility, safety profile, and reduced systemic risks, Vindoline emerges as a promising candidate for anti-inflammatory therapy.

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Data availability statement

This statement does not apply to this article.

Ethics statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed consent statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical trial registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author's contribution

Dr. Preeti Chaudhary and Akash Kumavat performed molecular docking and preparation of the manuscript; Dr. Pritam Khandave visualized the docking results; Dr. Rupesh Pingale provided the facilities, expertise, and software to perform all computational studies; Sakshi Singh and Omkar Dudhal performed ADME analysis; Sonali Korlekar and Shubham Tivlekar performed toxicity studies.

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