

Molecular Docking and Molecular dynamic Analysis: Interaction Studies of Natural Compounds derived from *Inula Viscosa* and Synthetic to Anti-inflammatory Targets

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ABSTRACT

Novel compounds with significant medicinal properties have gained much interest in therapeutic approaches for treating various inflammatory disorders. Different synthetic and natural inhibitors were collected from the literature and docked against (5-LOX, PLA2) receptors using molecular docking. The compounds' binding affinity were calculated after minimizing the interaction within the binding pockets of the macromolecules (5-LOX, PLA2) receptors via molecular docking. In this work, we use molecular docking methods to identify the ligand which has the best interaction energy with the fourth macromolecules among synthetic and natural products extracted from *Inula Viscosa* and also descript binding affinity in purpose to design new inhibitor ligands using molecular docking. The result shows that ligands (Sulfasalazine, 3-O-Acetylpadmatin), (3-Acetyl-7-O-methylaromadendrin, Silibinin), best inhibited 5-LOX and PLA2 respectively. This latter leads to same conclusion with experimentation inhibition study and gives a good model as initiating molecules for anti-inflammatory drug design.

Keywords: Inflammation; Phospholipase A2; 5-Lipoxygenase; *Inula Viscosa*; Molecular docking; Molecular dynamic.

1. Introduction

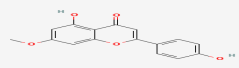
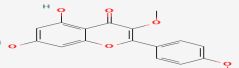
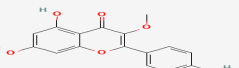
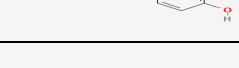
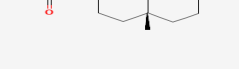
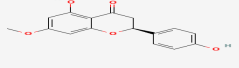
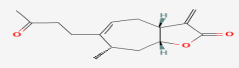
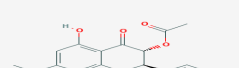
The Asteraceae family comprises a large number of flowering and usually aromatic plants, mostly growing in tropics and in cold arctic or alpine regions. It includes about 620 genera, and 23,600 species, of which *Inula* is one of the most famous. The *Inula* genus comprises more than one hundred species [1], mainly distributed in Europe, Africa and Asia. *Inula viscosa* commonly known as “Trehla or Magramane,” is a widespread plant in the Mediterranean region. *Inula Viscosa* has been used traditionally as a remedy for wounds, skin diseases, rheumatic pains, lung disorders, diabetes, gastroduodenal problems, hypertension, cancer, bronchitis, tuberculosis and infertility [1-9]. It has been widely used for therapeutic purposes in folk medicine as anthelmintic, antipyretic, antiseptic, anti-inflammatory, antiphlogistic, balsamic, expectorant, anti-scabies, diuretic, and anti-anemic and muscle relaxant [2-10]. Many biologically active compounds have been identified from *I. viscosa* such as guaianolides, sesquiterpenes, sesquiterpene acids, triterpenoids, azulenes, lactones, flavonoids, cinnamic acid and essential oils [1, 10-16]. Cytotoxic [8, 14, 16-21], antimicrobial [9, 18, 21-25], antioxidant [7, 24-28], antihypertensive [29], hypoglycemic [5], hypolipidemic [5], abortifacient and anti-implantation [4] activities of *Inula Viscosa* have been investigated with experimental studies in the literature.

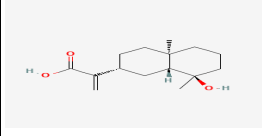
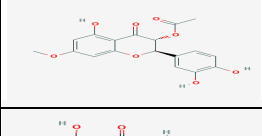
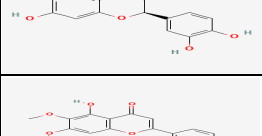
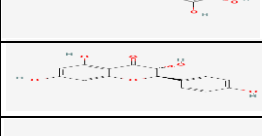
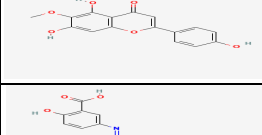
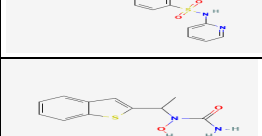
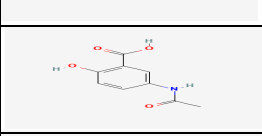
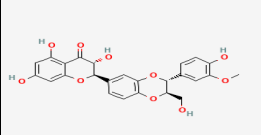
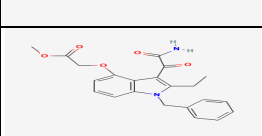
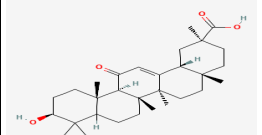
Inflammation is the body's natural defensive response to a variety of internal and external stimuli [30]. Long-term chronic inflammation is linked to the development and progression of autoimmune diseases, cancer, cardiovascular and neurological disorders. Overproduction of mediators of the arachidonic acid (AA) cascade, particularly those of the Phospholipase 2 (PLA₂) and lipoxygenase (LOX) pathways, causes major inflammatory diseases in humans [31]. The enzyme 5-lipoxygenase (5-LOX) catalyzes the oxidation of arachidonic acid, which in turn reacts with eicosapentanoic acid, resulting in the formation of leukotrienes, the chemical mediators of inflammation [32]. Phospholipase A₂ (PLA₂) enzymes are required to increase the level of arachidonic acid for metabolism and biosynthesis of eicosanoid under physiological condition as well as in inflammatory cell activation [33].

Considering the enormity of research reports on the inflammatory and breast cancer effects of natural inhibitors (molecules of *Inula viscosa*) and the importance of 5-LOX, PLA₂ as a therapeutic target in inflammation, we aimed to carry out an in-silico evaluation of

compounds derived from *Inula viscosa* against (5-LOX and PLA2) enzymes and synthetics (sulfasalazine, zileuton, amino benzoic acid, silibinin, varsapladib, Glycyrrhetic acid). By molecular docking we can study the complex (enzyme/inhibitor) formation and consequently delay its progression, in order to determine interaction of the complex (Natural and Synthetic) to the enzyme. The selected natural and synthetic inhibitors and synthetic [34-42] are given in Table 1. Our results will contribute in the design of new active molecules as 5LOX and PLA2 inhibitors, that can be synthesized basing on the molecular structures of bioactive compounds of *Inula viscosa*.

Table1. Synthetic and Natural for 5LOX, PLA2

ligand	IUPAC name	Structure	Cid
Genkwanin	5-hydroxy-2-(4-hydroxyphenyl)-7-methoxychromen-4-one		5281617
Isokaempferide	5,7-dihydroxy-2-(4-hydroxyphenyl)-3-methoxychromen-4-one		5280862
3-O-Methylquercetin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one		5280681
Padmatin	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-methoxy-2,3-dihydrochromen-4-one		12313901
Dihydrokaempferol	3,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one		662
Isocostic acid	2-[(2R,4aR)-4a,8-dimethyl-2,3,4,5,6,7-hexahydro-1H-naphthalen-2-yl]prop-2-enoic acid		10922464
Sakuranetin	(2S)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydrochromen-4-one		73571
Tomentosine	(3aR,7S,8aR)-7-methyl-3-methylidene-6-(3-oxobutyl)-4,7,8,8a-tetrahydro-3aH-cyclohepta[b]furan-2-one		155173
3-Acetyl-7-O-methylaromadendrin	(2R,3R)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4-oxo-2,3-dihydrochromen-3-yl] acetate		15139424
Inuviscolide	(3aS,5aS,8R,8aR,9aR)-8-hydroxy-8-methyl-1,5-dimethylidene-3a,4,5a,6,7,8a,9,9a-octahydroazuleno[6,5-		176489

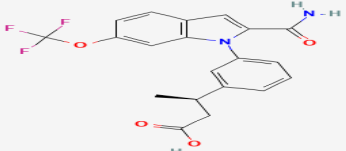
	b]furan-2-one		
Illicic Acid	2-[(2R,4aR,8R,8aR)-8-hydroxy-4a,8-dimethyl-1,2,3,4,5,6,7,8a-octahydronaphthalen-2-yl]prop-2-enoic acid		11876195
3-O-Acetylpadmatin	[(2R,3R)-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-2,3-dihydrochromen-3-yl] acetate		10406203
Taxifolin	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one		439533
Nepetin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-methoxychromen-4-one		5317284
Aromadendrin	(2R,3R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one		122850
Hispidulin	5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methoxychromen-4-one		5281628
Sulfasalazine	2-hydroxy-5-[[4-(pyridin-2-yl)sulfamoyl]phenyl]diazenyl]benzoic acid		5339
Zileuton	1-[1-(1-benzothiophen-2-yl)ethyl]-1-hydroxyurea		60490
Aminobenzoic acid	5-acetamido-2-hydroxybenzoic acid		65512
Silibinin	2R,3R)-3,5,7-trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one		31553
Varspladib	Methyl2-(1-benzyl-2-ethyl-3-oxamoylindol-4-yl) oxyacetate		9886917
Glycyrrhetic acid	(2S,4aS,6aR,6aS,6bR,10S,12aS,14bR)-10-hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-3,4,5,6,6a,7,8,8a,10,11,12,14b-dodecahydro-1H-picene-2-carboxylic acid		9897771

2. Materials and methods

2.1 Target receptor protein

The enzymes structures were downloaded from the Protein Data Bank archive-information database (www.rcsb.org/pdb), (5LOX) (PDS ID: 6NCF), (PLA2) (PDS ID: 6G5J), [43]. Table 2 presented the target macromolecules and each of the native ligand. The protein structures were prepared using MOE (Molecular Operating Environment) software [44] to remove all non-receptor atom including water, ion, and miscellaneous compounds. The obtained structures then were saved as pdb file.

Table 2. Target macromolecules, pdb codes, native ligands, and ligand structures used in the docking study

Target macromolecule (pdb code)	Ligand	Structure
(5LOX) (PDS ID: 6NCF)	(3 α , 8 α , 17 α , 18 α)-3-(acetyloxy)-11-oxours-12-en-23-oic acid	
(PLA2) (PDS ID: 6G5J)	(3~{R})-3-[3-[2-aminocarbonyl-6-(trifluoromethyloxy)indol-1-yl]phenyl]butanoic acid	

2.2. Preparation of ligands

The 16 natural compounds from *Inula Viscosa* were used collectively as ligands in this study. The three-dimensional structures of ligands were downloaded from the PubChem database and were later optimized using density functional theory (DFT) level using Becks's three-parameter Lee–Yang–parr hybrid functional (B3LYP) at 6-31G basis set [45] to simulate chemical structures of the molecules at their minimum potential energy or most stable state in a real chemical system before subjecting them to the molecular docking process. The structure then was executed an MMFF94X energy minimization force field implanted in MOE program and AM1 [46] Hamiltonian. These obtained conformations were used as starting conformations to perform docking analysis. The physico-chemical properties of the ligands are reported in Table 3. Lipinski's rule [47] which is a valuable tool for selecting the good candidates by predicting drug-like properties was also verified for all our ligands. In Figure 3 the enzyme active site with the co-crystallisation molecule is presented,

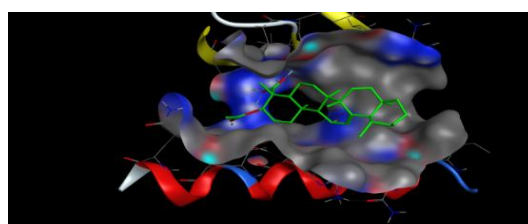
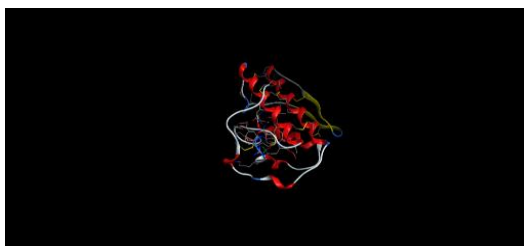
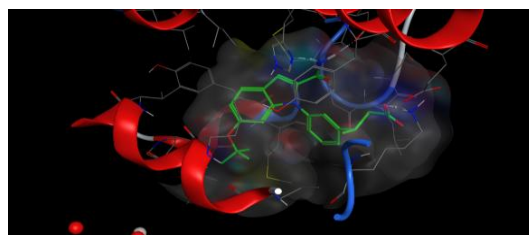


Figure.1.a. Simplified model of enzyme 6NCF.**Figure.1.b.** Active site enzyme isolated - 6NCF.**Figure.1.c.** Simplified model of enzyme 6G5J.**Figure.1.d.** Active site enzyme isolated 6G5J**Table 3.** Ligands properties (Weight: Molecular weight, TPSA ($\text{\AA}^2$): Polar surface area, logP: Octanol-water partition coefficient, logS: aqueous solubility, H- donor: Number of H- bonds donors, H acceptor: Number of H- bonds acceptors,

Ligand	Weight	H donor	H acceptor	Toxicity	TPSA	Log P	Log S
Genkwanin	284.26	2	5	NO	76	1.88	-4.02
Isokaempferide	300.26	3	6	NO	96.2	1.77	-3.70
3-O-Methylquercetin	316.26	4	7	NO	116	1.47	-3.33
Padmatin	318.28	4	7	NO	116	0.78	-2.26
Dihydrokaempferol	288.25	4	6	NO	107	1,58	-2,37
Isocostic acid	234.33	1	2	NO	37.3	3,93	-4,03
Sakuranetin	286.28	2	5	NO	76	2.91	-2.78
Tomentosin	248.32	0	3	NO	43.4	2,81	-1,66
3-Acetyl-7-O-methylaromadendrin	344.3	2	7	NO	102	2,45	-3,4
Inuviscolide	248.2	1	3	NO	46.5	2,21	-1,97
Illicic acid	252.35	2	3	NO	57.53	2,98	-3,09
3-O-Acetylpadmatin	360.3	3	8	NO	122.3	2.16	-3.04
Taxifolin	304.25	5	7	NO	107	1.28	-2.01
Nepetin	316.26	4	7	NO	116	2.13	-3.15
Aromadendrin	288.25	5	7	NO	107	1.58	-2.37
Hispidulin	300.26	3	6	NO	96.2	1.6	-3.7
Sulfasalazine	398.4	3	9	YES	150	3.70	-3.60
Zileuton	236.29	2	3	YES	94.8	2.83	-3.26
Aminobenzoic acid	195.17	3	4	NO	86.6	1.05	1.20
Silibinin	482.4	5	10	NO	155	2.55	-4.23

Varspladib	394.4	1	5	NO	101	2.74	-4.89
Glycyrrhetic acid	470.7	2	4	NO	74.6	6.41	-7.5

The above table reflects the fact that our ligands are non-toxic and can present biological activities

2.3. Molecular dynamic

to study the structural and dynamic behavior of biomolecules at the atomic level [48-49]. By solving Newton's equations of motion for all atoms in a system, MD enables exploration of conformational landscapes, molecular flexibility, and time-dependent interactions, complementing static structural data from X-ray crystallography or docking studies [50-51]. MD simulations provide valuable insights into protein stability, ligand binding modes, hydrogen bonding patterns, and dynamic fluctuations, all of which are essential for understanding molecular recognition and rational drug design. The integration of high-performance engines such as OpenMM with trajectory analysis tools like MDAnalysis facilitates efficient processing and extraction of key structural descriptors, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, and interatomic distances [50-51].

3. RESULTS AND DISCUSSION

3.1. Molecular docking

The molecular docking analysis was performed using MOE program with 16 compounds, including flavonoids and sesquiterpenes. First, the validation method was conducted to ensure the capability of docking machine. Figure 2 represented the validation result of docking protocol. All of the redocking ligands showed similar conformation with the native redocking ligands, and the RMSD values were ≤ 2.0 Å

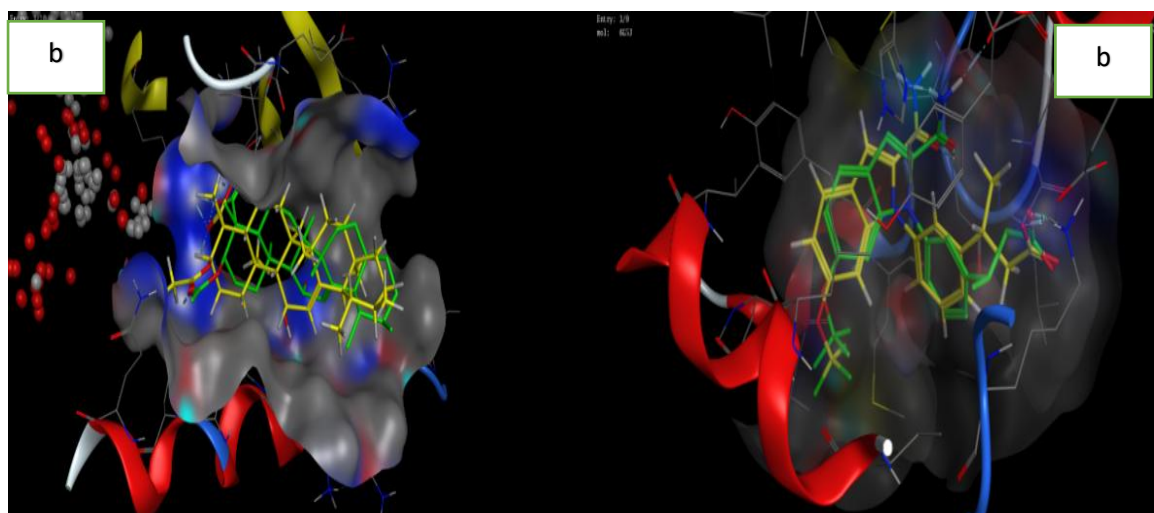


Figure 2. Overlay of the native ligands (green) and redocking conformations (yellow). (a: 5LOX; b: PLA2)

Each natural compound then was docked into each of four different targets. The lowest energy docked conformation of the best cluster was selected and analyzed. Table 4 summarizes the docking study results, presented as binding energy

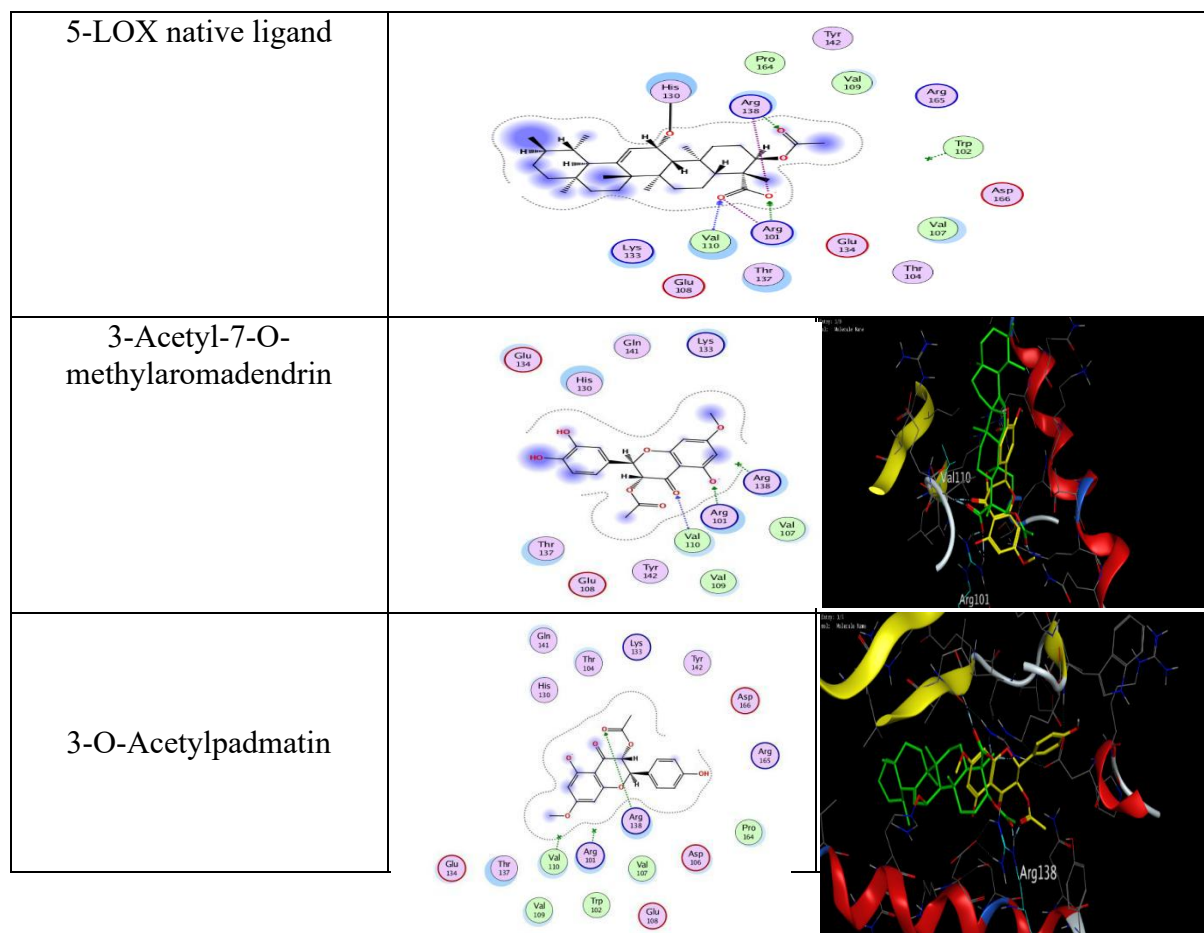
Table 4. Binding energy values of natural compounds docked to target macromolecules.

Compound	Target macromolecules; binding energy (kcal/mole)	
	5LOX	PLA2
Native ligand	-6.46469069	-7.55313253
Genkwanin	-5.36936998	-5.74437666
Isokaempferide	-5.44390202	-6.09148407
3-O-Methylquercetin	-5.61817122	-6.17404699
Padmatin	-5.6632185	-6.06801081
Dihydrokaempferol	-5.27355385	-5.62368393
Isocostic acid	-5.13338375	-5.21947336
Sakuranetin	-5.4700532	-5.73192453
Tomentosin	-5.0698905	-5.96263599
3-Acetyl-7-O-methylaromadendrin	-6.24344778	-6.27365208
Inuviscolide	-4.79326773	-6.11191416
Illicic acid	-5.27381325	-5.16497707
3-O-Acetylpadmatin	-6.30094481	-6.2406683

Taxifolin	-5.31556988	-6.12258577
Nepetin	-5.36493778	-6.19818926
Aromadendrin	-5.19482803	-5.91778088
Hispidulin	-5.22355223	-6.0497179
Sulfasalazine	-5.56916523	-
Zileuton	-4.56909513	-
Aminobenzoic acid	-4.7137022	-
Silibinin	-	-7.33975315
Varspladib	-	-7.22810411
18B Glycyrrhetic acid	-	-3.54883337

The molecular docking results and the interactions of the 2 best naturals and synthetics inhibitors ligands for each docked to target macromolecules are shown in Table 5;6 and Figures 3; 4. Residues are marked with their amino acid code of 3 letters, and job classification [52-53]. If there are multiple channels in the system, the positions are prefixed by the letters of the alphabet. Interactions between 2.5 Å and 3.1 Å are considered high and those between 3.1 Å and 3.55 Å are average. Greater than 3.55 Å interactions are weak [54].

3.1. Interaction to 5LOX



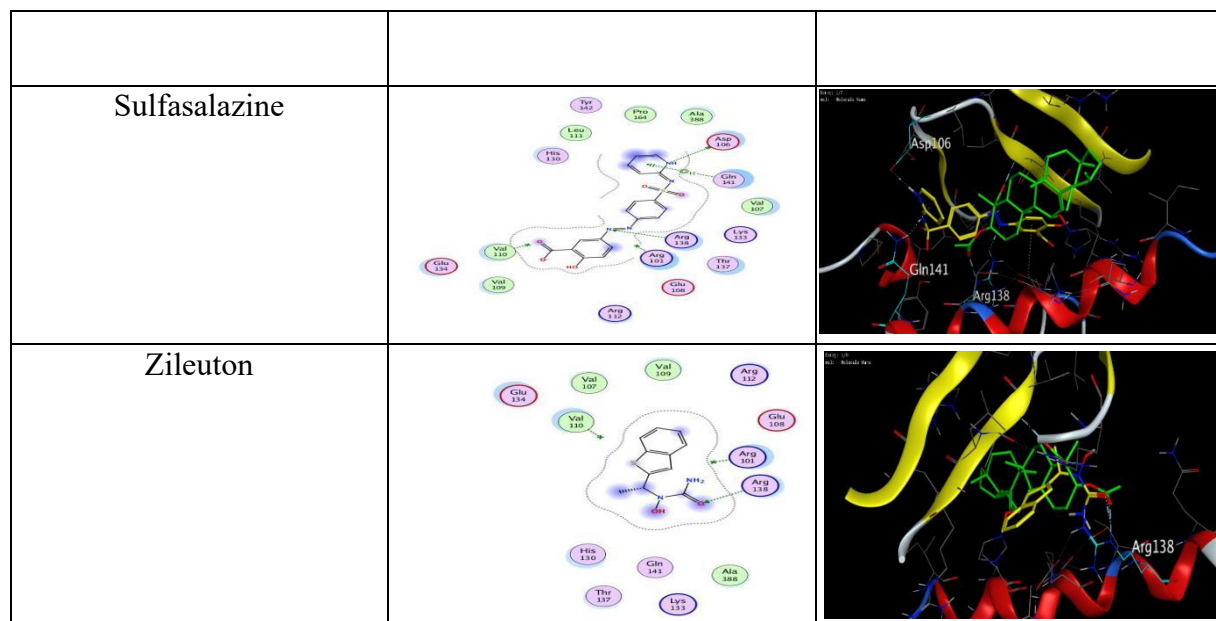


Figure 3. Model interaction of 5LOX native ligand, 3-Acetyl-7-O-methylaromadendrin, and 3-O-Acetylpadmatin, Sulfasalazine, Zileuton

Ligands	Receptor	Interactions	Distances (Å)	Energies (Kcal/mol)
7FA	NH1 ARG 101	H-acceptor	2.81	-7.3
	NH2 ARG 101	H-acceptor	2.56	-7.3
	N VAL 110	H-acceptor	2.61	-7.2
	NE ARG 138	H-acceptor	2.67	-5.5
	NH1 ARG 101	ionic	2.81	-5.9
	NH2 ARG 101	ionic	2.56	-8.2
	NH2 ARG 138	ionic	3.78	-1.0
	NH2 ARG 101	ionic	3.42	-2.2
3-Acetyl-7-O-methylaromadendrin	NE ARG 138	H-acceptor	3.09	-4.2
3-O-Acetylpadmatin	N VAL 110	H-acceptor	2.98	-3.4
	NH1 ARG 101	H-acceptor	3.26	-0.9
	NH1 ARG 101	Ionic	3.26	-2.9
	NH2 ARG 101	ionic	3.42	-2.2
Sulfasalazine	OD1 ASP 106	H-donor	3.47	-1.3
	NH2 ARG 138	H-acceptor	2.94	-2.3
	NE2 GLN 141	pi-H	3.54	-0.6
				-0.6
Zileuton	NE ARG 138	H-acceptor	3.12	-3.1

Table 5. Interactions patterns observed in the determined complexes between the ligands and the corresponding amino acid in 5LOX

The molecular docking analysis revealed distinct binding modes and interaction profiles for the studied ligands within the 5-lipoxygenase (5-LOX) binding pocket. The stability of these complexes is primarily driven by a combination of hydrogen bonding, ionic interactions, and hydrophobic contacts, with binding energies ranging from -0.6 to -8.2 kcal/mol.

The results in Table 4 show that the complex formed with 3-O-Acetylpadmatin gives the highest score (-6.3009 Kcal/mol), i.e. the most stable complex. The second stable complex is given with the 3-Acetyl-7-O-methylaromadendrin ligand (-6.2434 Kcal/mol).

Basing on the obtained results, we conclude that the reference ligand (Fig.3, Table.5) 7FA exhibited the most robust interaction profile, characterized by multiple high-energy contacts. It forms a dense network of hydrogen bonds and ionic interactions with ARG 101, VAL 110, and ARG 138.

Hydrogen Bonding: Significant H-acceptor interactions were observed with ARG 101 (2.56–2.81 Å) and VAL 110 (2.61 Å), contributing to strong stabilization energies (up to -7.3 kcal/mol).

Ionic Interactions: The presence of multiple ionic contacts with ARG 101 and ARG 138 further reinforces the complex, with the strongest ionic interaction reaching -8.2 kcal/mol at 2.56 Å. This suggests that 7FA may act as a potent inhibitor by effectively anchoring itself within the catalytic or regulatory domains of 5-LOX.

Flavonoid Derivatives: 3-Acetyl-7-O-methylaromadendrin and 3-O-Acetylpadmatin

These ligands show moderate binding affinities, primarily mediated by interactions with the arginine-rich regions of the receptor.

3-Acetyl-7-O-methylaromadendrin interacts specifically with ARG 138 via a hydrogen bond (3.09 Å, -4.2 kcal/mol), indicating a more localized binding mode compared to 7FA.

3-O-Acetylpadmatin utilizes both VAL 110 and ARG 101 for stabilization. Its binding is characterized by a mix of H-bonding (-3.4 kcal/mol) and ionic interactions (-2.9 kcal/mol), suggesting a balanced but less intense binding profile than 7FA.

The complex formed with Sulfasalazine has the lowest energy (-5.56916523 Kcal/mol) and is more active than complex formed with Aminobenzoic acid (-4.7137022 Kcal/mol) which is more active than complex formed with Zileuton (-4.7137022 Kcal/mole).

Sulfasalazine and Zileuton: Diverse Interaction Modes

Sulfasalazine demonstrates a unique interaction pattern involving ASP 106 (H-donor), ARG 138 (H-acceptor), and GLN 141 (pi-H interaction). The pi-H interaction with GLN 141 (-0.6

kcal/mol) highlights the role of aromatic features in its binding, although its overall energy profile is relatively modest.

Zileuton, a known 5-LOX inhibitor, shows a targeted H-acceptor interaction with ARG 138 (3.12 Å, -3.1 kcal/mol). This consistent involvement of ARG 138 across multiple ligands suggests it is a critical residue for ligand recognition and stabilization in the 5-LOX binding site.

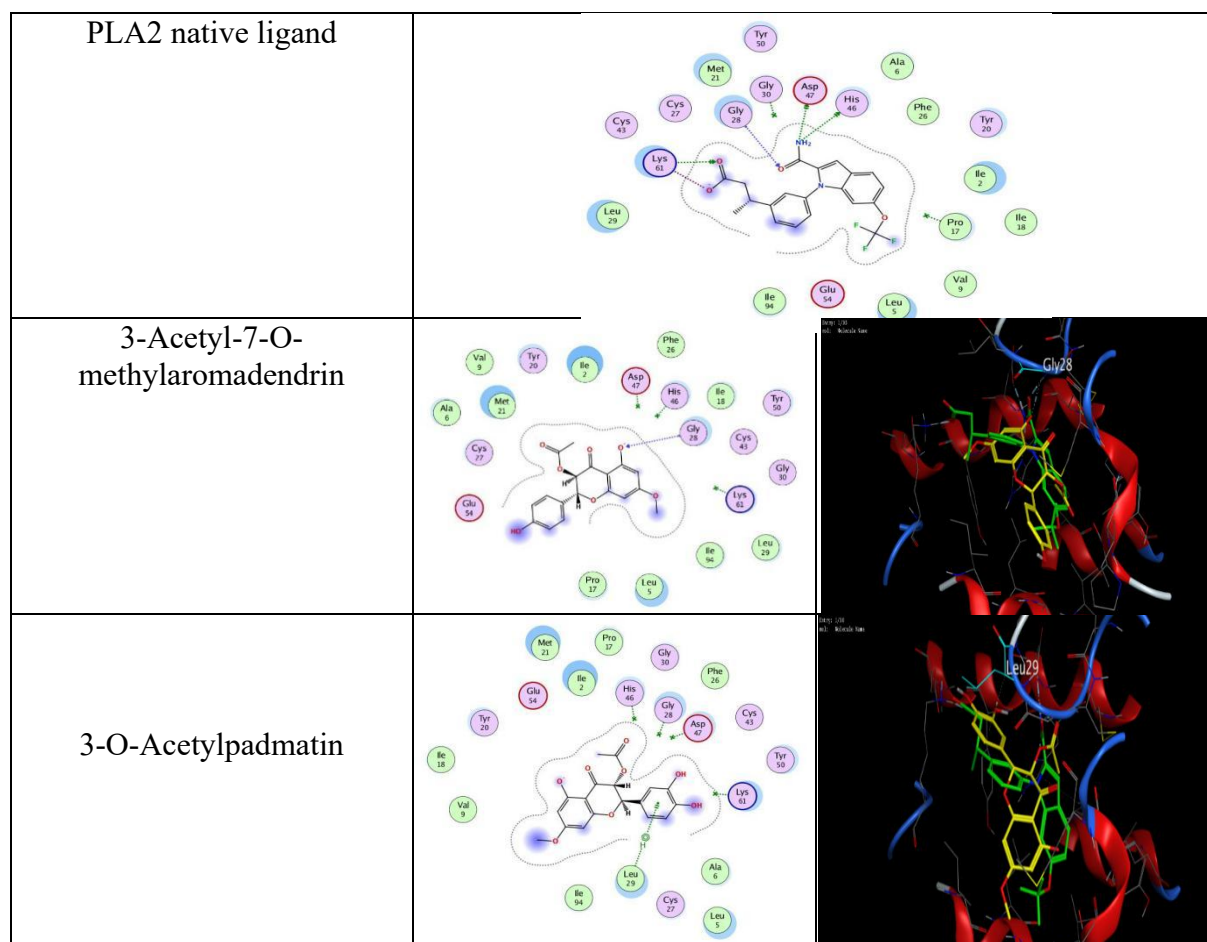
The inhibitor of the co-crystallisation 2(3 α , 8 α , 17 α , 18 α)-3-(acetyloxy)-11-oxours-12-en-23-oic acid C₃₂ H₄₈ O₅ (6NCF).

Energy (Sulfasalazine -5.56916523 Kcal/mol < Aminobenzoic acid -4.7137022 Kcal/mol)

Energy 3-Acetyl-7-O-methylaromadendrin -6.2434 Kcal/mol) < (3-O-Acetylpadmatin -6.3009 Kcal/mol)

3-O-Acetylpadmatin and Sulfasalazine would be the best to slow down the evolution of studied pathology (inflammation).

3.2. Interaction to PLA2



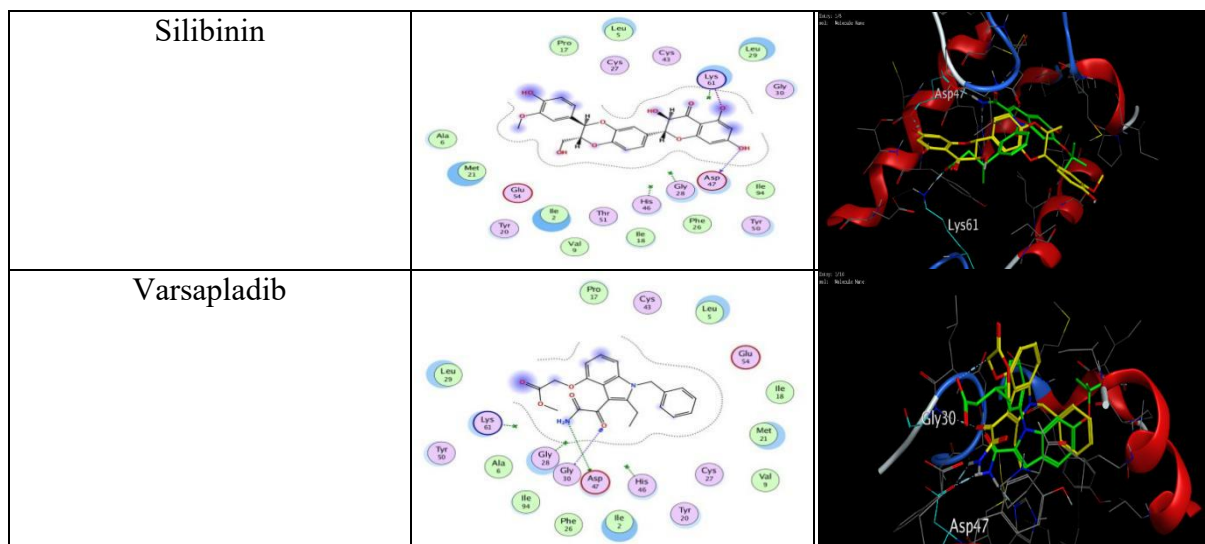


Figure 4. Model interaction of PLA2 native ligand, 3-Acetyl-7-O-methylaromadendrin, and 3-O-Acetylpadmatin, Silibinin, Varsapladib

Ligands	Receptor	Interactions	Distances (Å)	Energies (Kcal/mol)
EM8	ND1 HIS 46 (B)	H-donor	2.93	-5.4
	OD1 ASP 47(B)	H-donor	2.71	-6.9
	NZ LYS 61(B)	H-acceptor	2.48	-8.3
	N GLY 28 (B)	H-acceptor	2.74	-4.3
	NZ LYS 61(B)	ionic	3.50	-1.9
	NZ LYS 61(B)	ionic	2.48	-9.1
3-Acetyl-7-O-methylaromadendrin	N GLY 28(B)	H-acceptor	3.24	-1.6
3-O-Acetylpadmatin	CA LEU 29 (B)	pi-H	4.46	-0.7
Silibinin	O ASP 47 (B)	H-donor	3.02	-1.1
	NZ LYS 61 (B)	ionic	3.65	-1.4
Varsapladib	OD1 ASP 47(B)	H-donor	2.90	-5.0
	N GLY 30(B)	H-acceptor	3.23	-1.6

Table 6. Interactions patterns observed in the determined complexes between the ligands and the corresponding amino acid in PLA2

The molecular docking analysis of various ligands with Phospholipase A2 (PLA2) revealed diverse interaction profiles, highlighting key residues involved in ligand binding and stabilization. These interactions, encompassing hydrogen bonding, ionic interactions, and pi-H stacking, contribute to the overall binding affinity and potential inhibitory mechanisms of the ligands.

EM8 demonstrates a highly potent binding mode within the PLA2 active site, characterized by a multitude of strong interactions. This ligand engages with several critical residues, including HIS 46 (B), ASP 47 (B), LYS 61 (B), and GLY 28 (B).

Hydrogen Bonding: EM8 forms multiple hydrogen bonds, notably with HIS 46 (B) (H-donor, 2.93 Å, -5.4 kcal/mol), ASP 47 (B) (H-donor, 2.71 Å, -6.9 kcal/mol), LYS 61 (B) (H-acceptor, 2.48 Å, -8.3 kcal/mol), and GLY 28 (B) (H-acceptor, 2.74 Å, -4.3 kcal/mol). The H-acceptor interaction with LYS 61 (B) exhibits the strongest energy contribution among the hydrogen bonds, suggesting its crucial role in anchoring EM8.

Ionic Interactions: The ligand also establishes significant ionic interactions with LYS 61 (B) at distances of 3.50 Å (-1.9 kcal/mol) and 2.48 Å (-9.1 kcal/mol). The latter ionic interaction is the most energetically favorable interaction observed for EM8, indicating that LYS 61 (B) is a primary determinant of EM8's high binding affinity.

Flavonoid Derivatives: 3-Acetyl-7-O-methylaromadendrin and 3-O-Acetylpadmatin

It has been observed that two complex formed with, 3-Acetyl-7-O-methylaromadendrin and 3-O-Acetylpadmatin showed better binding with score of -6.2736 and -6.2406 Kcal/mol respectively.

These natural product derivatives exhibit more focused interaction patterns with PLA2.

3-Acetyl-7-O-methylaromadendrin primarily engages with GLY 28 (B) through an H-acceptor interaction (3.24 Å, -1.6 kcal/mol). This suggests a less extensive binding footprint compared to EM8.

3-O-Acetylpadmatin forms a pi-H interaction with LEU 29 (B) (4.46 Å, -0.7 kcal/mol). While this interaction is weaker in terms of energy, it indicates the involvement of hydrophobic or aromatic stacking, which can be important for overall ligand stability and specificity. Details of these bound structures are given in Table 6 and Fig.4.

The complex formed with Silibinin has the lowest energy (-7.33975315Kcal/mol) and is more active than complex formed with Varspladib (-7.22810411Kcal/mol).

Silibinin demonstrates a combination of hydrogen bonding and ionic interactions:

It forms an H-donor interaction with ASP 47 (B) (3.02 Å, -1.1 kcal/mol).

An ionic interaction is observed with LYS 61 (B) (3.65 Å, -1.4 kcal/mol). These interactions highlight the importance of both acidic and basic residues in its binding to PLA2.

Varspladib interacts with PLA2 through two distinct hydrogen bonds:

An H-donor interaction with ASP 47 (B) (2.90 Å, -5.0 kcal/mol).

An H-acceptor interaction with GLY 30 (B) (3.23 Å, -1.6 kcal/mol). The interaction with ASP 47 (B) is particularly strong, suggesting this residue plays a significant role in Varspladib's binding mechanism.

The inhibitor of the co-cristalisation **3~{R})-3-[3-[2-aminocarbonyl-6-(trifluoromethoxy) indol-1-yl] phenyl] butanoic acid** C₂₀ H₁₇ F₃ N₂ O₄ (6G5J)).

Energy (Silibinin -7.33975315Kcal/mol< VersapladiB -7.22810411Kcal/mol)

Energy 3-O-Acetylpadmatin -6.2406 Kcal/mol) < (3-Acetyl-7-O-methylaromadendrin - 6.2736 Kcal/mol

3-Acetyl-7-O-methylaromadendrin and Silibinin would be the best to slow down the evolution of studied pathology (inflammation).

3.3. MD stability

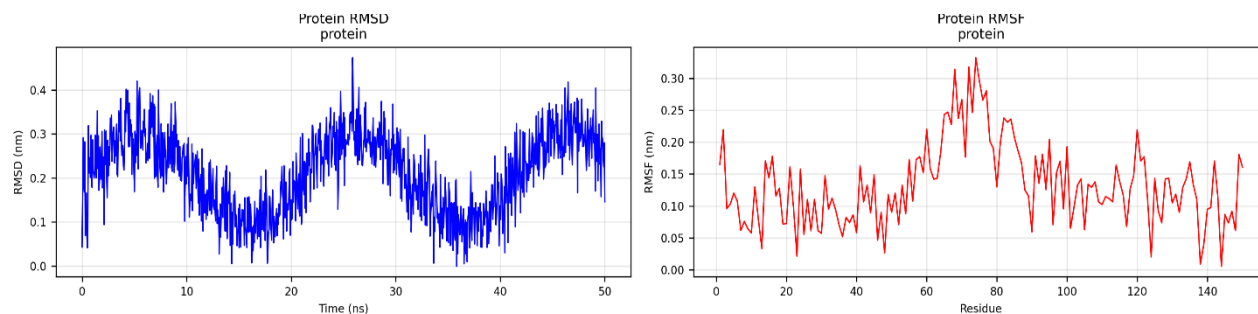


Figure. 5. RMSD and RMSF of 5-LOX

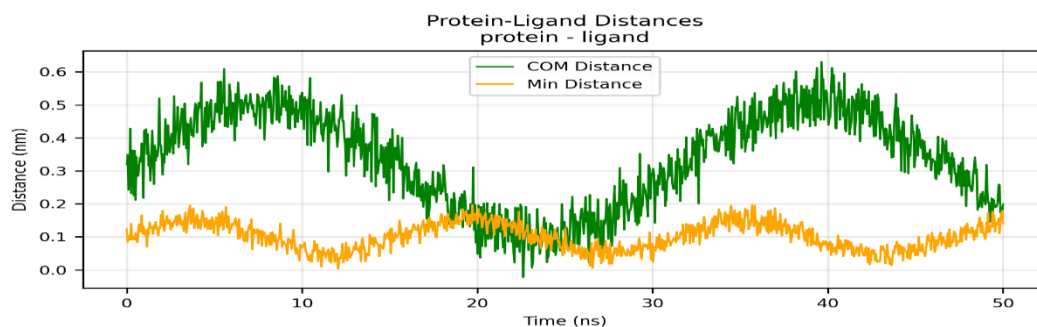


Figure. 6. Complex 5-LOX- 3-O-Acetylpadmatin distances

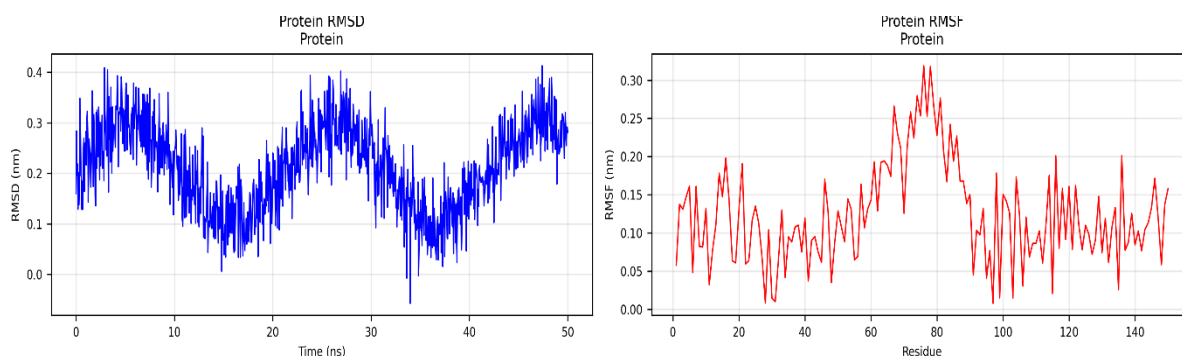


Figure. 7. RMSD and RMSF of PLA2

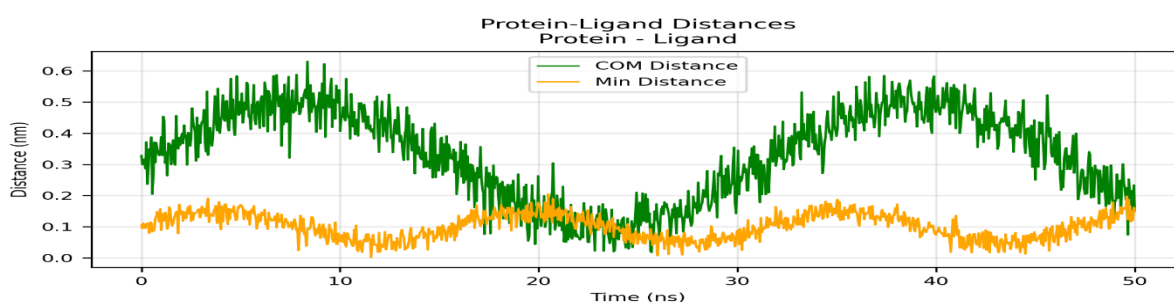


Figure. 8. Complex PLA2 -3-Acetyl-7-O-methylaromadendrin distances

A 50 ns molecular dynamics simulation of Arachidonate 5-lipoxygenase (5-LOX) complexed with 3-O-Acetylpadmatin and Phospholipase A2 (PLA2) complexed with 3-Acetyl-7-O-methylaromadendrin demonstrated stable structural behavior and persistent ligand binding throughout the trajectories. In both systems, backbone RMSD stabilized around ~ 0.21 nm without abrupt deviations, indicating that ligand binding did not induce significant conformational destabilization of the enzymes, while residue-level fluctuations (RMSF ~ 0.125 – 0.13 nm) remained moderate, with slightly higher flexibility observed in localized loop regions (residues ~ 60 – 80 for 5-LOX and ~ 70 – 85 for PLA2), whereas key active-site residues remained structurally stable. Center-of-mass and minimum distance analyses confirmed continuous ligand retention within the catalytic pockets, with no dissociation events. Both complexes maintained an average of ~ 5 hydrogen bonds, with highly occupied interactions (>70 – 85%) and several stable intermolecular contacts, highlighting strong polar and hydrophobic contributions to binding. Principal component analysis revealed compact conformational distributions with limited large-scale collective motions, suggesting restricted flexibility and dynamic convergence upon ligand binding. Overall, these results indicate that 3-O-Acetylpadmatin and 3-Acetyl-7-O-methylaromadendrin form dynamically stable and

energetically favorable complexes with 5-LOX and PLA2, respectively, supporting their potential roles as promising inhibitors of leukotriene biosynthesis and related enzymatic activity.

4. Conclusion

The present study evaluated the inhibitory potential of natural ligands from *Inula viscosa* and synthetic compounds against 5-LOX and PLA2 using computational approaches, including molecular docking and 50 ns molecular dynamics simulations. Molecular docking revealed that Sulfasalazine exhibited the highest binding affinity with 5-LOX, while among natural compounds, 3-O-Acetylpadmatin showed the most favorable interactions, suggesting its potential as a natural inhibitor. For PLA2, 3-Acetyl-7-O-methylaromadendrin and Silibinin were identified as the most promising natural leads. All natural compounds complied with Lipinski's rules for oral drug administration. Molecular dynamics analyses confirmed the dynamic stability of these complexes, with backbone RMSD values stabilizing around 0.21 nm, moderate residue flexibility (RMSF ~0.125–0.13 nm), and persistent ligand retention within the catalytic pockets. On average, ~5 hydrogen bonds and multiple stable intermolecular contacts were maintained, indicating strong polar and hydrophobic contributions to binding, while principal component analysis revealed compact conformational distributions with limited large-scale motions. Overall, these results suggest that natural inhibitors not only form energetically favorable and dynamically stable complexes but also may exhibit better inhibitory activity than synthetic compounds, supporting their potential as safer and efficient therapeutic agents for the treatment of inflammation.

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