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Molecular docking studies of natural and synthetic compounds against human secretory PLA_2 in therapeutic intervention of inflammatory diseases and analysis of their pharmacokinetic properties

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Literature survey reveals that there are several natural and synthetic anti-inflammatory compounds reported till date. As a therapeutic drug target, PLA_2 inhibition is preferred over other anti-inflammatory drug targets. The pro-inflammatory effects of group X $sPLA_2$ are acquired from multiple pathways. This study aims to identify the best anti-inflammatory compound among 22 compounds reported in literature using *in silico* approach. The compound ligands are subjected to docking against the target protein human $sPLA_2$ [PDB ID: 5G3M] at the active site using AutoDock 4.2.6. Based on the Δ binding free energy and hydrogen bonding interactions, it was observed that ten compounds fit at the active site. Out of these, compound 1 (14-deoxyandrographolide) was selected as the best compound. Absorption, distribution, metabolism, and excretion (ADME) properties of the ligands are analyzed using pkCSM software available online. Compound 1 exhibited the best conformational fit when compared to the co-crystal inhibitor 4-Benzylbenzamide.

Keywords: ADMET, AutoDock, Inflammation, $sPLA_2$ inhibitor

Phospholipase A_2 (PLA_2) consists of a diverse family of lipolytic enzymes which hydrolyze the sn-2 fatty acid ester bond of glycerophospholipids producing free fatty acid and lysophospholipids[1-3]. PLA_2s take part in the pathophysiological processes and release arachidonic acid from membrane phospholipids which results in the production of various pro-inflammatory lipid mediators like prostaglandins and leukotrienes[4]. Based on their biochemical features, these PLA_2s are categorized into several families-secretory PLA_2 ($sPLA_2$)[5-10], arachidonoyl-specific cytosolic PLA_2 ($cPLA_2$)[11-12], and Ca^{2+}-independent PLA_2[13]. Cyclo-oxygenase-2 (COX-2) and Lipoxygenase (LOX) are downstream enzymes to PLA_2 in the inflammatory response. There are previous studies that have reported the *in silico* analysis of natural compounds against anti-inflammatory and immune-modulatory targets[14,15]. As a therapeutic drug target PLA_2 inhibition is preferred over COX-2 and LOX[16,17]. The $sPLA_2$s (13-55 kDa) are the first PLA_2 enzymes that were reported[18].

The pro-inflammatory effects of group X $sPLA_2$ are acquired from multiple pathways including production of lysophosphatidylcholine and non-esterified fatty acids[19], ERK1/2 kinase activation, effects on activation of MAPK pathway, and increased release of arachidonicacid[20].The crystal structure of human $sPLA_2$is a homo-dimer consisting of two identical chains A and B. Figure 1 shows human $sPLA_2$ (PDB ID: 5G3M) chain A and B with 4-Benzylbenzamide (9JH) and Ca^{2+} (CA). Because of the role played by $sPLA_2$ in inflammatory diseases and various cancers, significant efforts are being made for the discovery and development of $sPLA_2$ inhibitors[17]. A number of $sPLA_2$ inhibitors have already been developed for various inflammatory and oncological diseases and also currently few are under clinical trials[17,18].

Molecular docking describes the "best-fit" orientation of a ligand that binds to a particular protein of interest. It is used for predicting the structure of the intermolecular complex. Ligand is a small molecule interacting with protein's binding sites and there are various possible mutual conformations in which binding can occur. Every mutual conformation is called binding mode[21]. To predict the affinity and activity of the small molecule, molecular docking studies are frequently carried out for the prediction of the binding orientation of small molecule drug candidates to their protein targets.

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Numerous natural and synthetic compounds have been reported to potentially inhibit inflammation[22]. In the present study, we attempt a theoretical study of a few natural and synthetic bioactive compounds by molecular docking to inhibit human $sPLA_2$ and further to analyze the ADME properties (absorption, distribution, metabolism, and excretion).

Materials and Methods

Preparation of protein molecule

The experimental structure of a novel inhibitor in complex with secreted phospholipase A2 ($sPLA_2$) (PDB ID: 5G3M)[23] was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/) as a PDB file. The co-crystal selective inhibitor 9JH was removed to derive the native target protein. The target protein $sPLA_2$is a homo-dimer consisting of two identical chains A and B. AutoDock Tools (ADT)[24] was used to prepare the protein. During protein preparation, chain B, all water molecules and hetero-molecules of 5G3M were deleted and polar hydrogen atoms and Kollman charges were added.

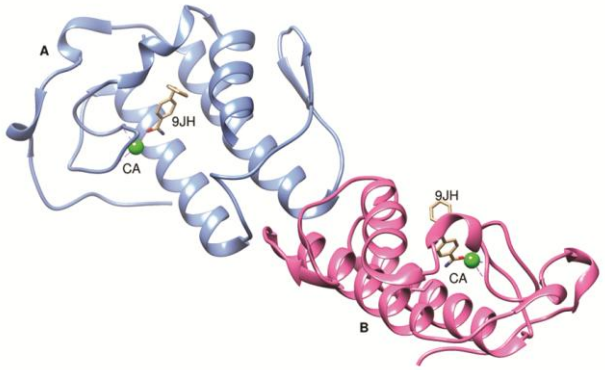

Fig. 1 — Structure of human group X $sPLA_2$ (PDB ID-5G3M) chain A and B with the inhibitor 9JH and Ca^{2+} ion (CA)

Table 1 — List of the compounds (1-22) considered for molecular docking studies

Compound	Name
1	14-deoxyandrographolide
2	Andrographolide
3	Neoandrographolide
4	Andrograpanin
5	5-hydroxy-7,8-dimethoxyflavone
6	5-hydroxy-7,8,2',5'-tetramethoxyflavone
7	5-hydroxy-7,8,2',3'-tetramethoxyflavone
8	Pentadecanoic acid, 14 methyl, methyl ester
9	Oleic acid
10	Hexadecanoic acid, 1-hydroxymethyl-1,2 ethanediyl ester
11	Corynan-17 ol 18,19 didehydro-10 methoxy-acetate ester
12	Heptadecane -9-hexyl
13	Heneicosane
14	Docosane
15	Tetracosane
16	Triacontane
17	14-deoxy-11,12-didehydroandrographolide
18	14-deoxy-14,15-didehydroandrographolide
19	Bisandrographolide
20	14-acetylandrographolide
21	19-O-acetylanhydroandrographolide
22	5-hydroxy-7,8-dimethoxyflavanone

Preparation of ligand

The structures of ligands (compounds 1-22) were obtained from literature and modeling was done by using ChemSketch (a free chemical drawing package) and further saved in the mol format (Table 1). Using Open Babel (http://openbabel.org)[25]mol file was converted into PDB format. The ligands were prepared using AutoDock Tools (ADT). In ligand preparation, non-polar hydrogen atoms were merged and Gasteiger partial charges were assigned.

Docking

To analyze the binding affinity of the ligands with the $sPLA_2$ enzyme active site residues (PDB ID: 5G3M), molecular docking study was performed using the software, AutoDock 4.2.6[24]. A grid box that covered the active site residues of the target protein was generated for obtaining the best conformational state of docking. The docking grid box size was set to 50 × 48 × 40 Å dimension with a spacing of 0.375 Å and centered at 0.138, −3.419, −2.973 of X, Y and Z coordinate. Docking was carried out using Lamarckian Genetic Algorithm (LGA).

ADME prediction

pkCSM is a quick and accurate program, freely available online for prediction of the ligands absorption, distribution, metabolism, and excretion (ADME). This web server provides an integrated freely available platform for rapid screening of multiple pharmacokinetic properties. Based on Lipinski's rule of five, ADME properties determine the drug-like activity of the ligand molecules. If ADME properties are predicted accurately prior to expensive experimental procedures, it can eliminate unnecessary testing on compounds which will ultimately fail. ADME prediction for the 22 ligands was tabulated.

Results and Discussion

The natural and synthetic compounds (compounds 1-22) reported in literature to be potential inhibitors of

inflammation are analyzed using docking studies. Prior to performing the docking studies validation of the docking protocol was done. The co-crystal inhibitor 4-Benzylbenzamide (9JH) was removed from the protein and again docked in the active site. The root mean square deviation between the predicted conformation and the X-ray crystallographic conformation was approximately 0.35 A°. This shows the ability of the docking protocol for reproducing the binding mode of the co-crystal inhibitor. The potential active site residues were derived from literature.

From AutoDock results it was observed that, out of 22 ligands only ten ligands compounds 1, 3, 4, 5, 7, 12, 17, 18, 20 and 22 docked at the binding site (Table 2). Based on the Δ binding free energy compounds 1, 4, 17, 18, 20, and 22 showed better results compared to co-crystal ligand 9JH. Further among these compounds, compound 1 showed the best interaction with the active site residues (Table 2). Figure 2 represents the Ligplot interaction of the docked complex of 9JH at the active site of the target protein and (Fig. 3) represent docked complex of compound 1 at the active site, respectively, obtained from Ligplot[+26]. ADME properties are used for determining the drug-like activity of the ligand molecules. ADME prediction can be utilized for focusing on lead optimization to enhance the desired properties of a given compound. All the compounds except compounds 9, 12, 13, 14, 15 and 16 satisfy Lipinski's rule of five and ADME properties. The ADME results of the ligand molecules are shown in (Table 3). Compound 1 showed a comparatively lower percentage of oral absorption as compared to compounds 4, 5, 6, 19 and 21, however, the other ADME property values are well within the range.

Table 2 — Molecular docking results of compounds 1, 3, 4, 5, 7, 12, 17, 18, 20, 22 and 9JH at active site of target protein 5G3M

Compound	Δ Bindingfree energy (Kcal/mol)	Interaction (D-H…O]	Distance Å
9JH	−7.52	Asp47[N-H…O]	2.72
		[N-H…O]Cys43	3.05
		Gly28[N-H…O]	2.89
1	−8.97	His46[N-H…O]	3.17
		[O-H…O]Asp47	2.67
		Gly28[O-H…O]	3.32
3	−7.85	His46[N-H…O]	2.52
		Lys61[N-H…O]	3.20
		[O-H…O]Gly28	3.25
4	−9.49	[O-H…O]Asp47	2.77
		His46[N-H…O]	3.01
5	−8.02	Lys61[N-H…O]	3.07
		Lys61[N-H…O]	2.86
		[O-H…O]Asp47	2.92
		[O-H…O]Phe26	2.84
7	−7.05	Lys61[N-H…O]	2.95
		Gly30[N-H…O]	3.34
		His46[N-H…O]	2.87
		[O-H…O]Pro17	2.76
12	−8.08	Gly28[N-H…O]	2.93
		His46[N-H…O]	2.87
17	−9.98	Lys61[N-H…O]	2.89
		Gly30[N-H…O]	3.18
		Gly28[N-H…O]	2.99
18	−9.98	Gly30[N-H…O]	3.15
		Gly28[N-H…O]	3.02
20	−10.88	Lys61[N-H…O]	2.88
		Gly30[N-H…O]	3.18
		Gly28[N-H…O]	2.95
22	−8.97	Lys61[N-H…O]	2.90
		Gly30[N-H…O]	3.13
		His46[N-H…O]	3.13

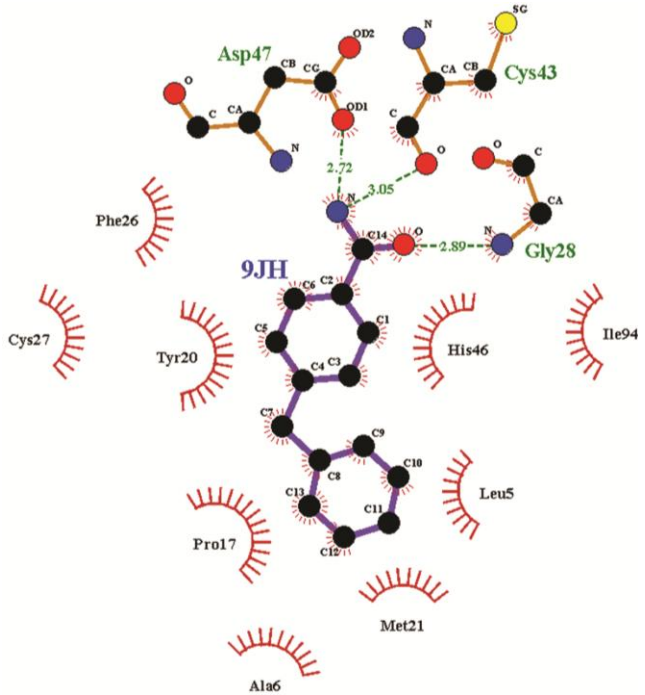

Fig. 2 — Binding interaction of 9JH with the active site residues of (PDB ID-5G3M) chain A shown using Ligplot. Dashed lines between atoms show Hydrogen bond, arc with spokes radiating towards the ligand atoms represents hydrophobic contacts, spokes radiating back show the contacted atoms

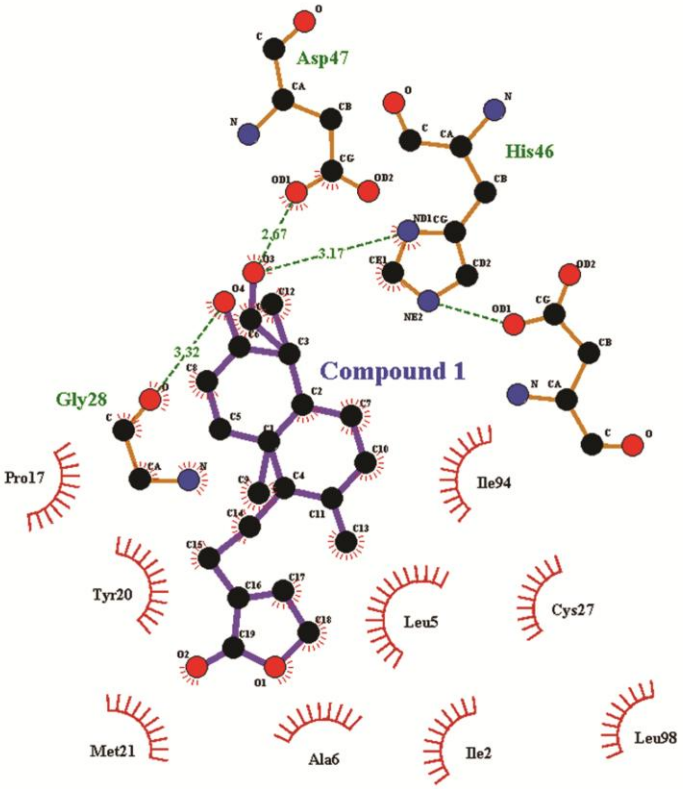

Fig. 3 — Binding interaction of compound 1 with the active site residues of (PDB ID-5G3M) chain A shown using Ligplot. Dashed lines between atoms show Hydrogen bond, arc with spokes radiating towards the ligand atoms represents hydrophobic contacts, spokes radiating back show the contacted atoms

Table 3 — pKCSM results showing ADME properties of compounds (1-22). Donor HB: estimated number of hydrogen bond donated by solute, Acceptor HB: estimated number of hydrogen bond accepted by solute, QplogPo/w: predicted octanol/water partition coefficient

Compound	Molecular weight	Donor HB (0-6)	Acceptor HB (2-10)	QplogP$_O$/w (−2 to 6.5)	Percent oral absorption (>80%-high <25%-poor)
1	334.45	2	4	3.00	93.82
2	350.45	3	5	2.00	92.43
3	480.60	4	8	1.84	47.71
4	318.46	1	3	4.02	97.28
5	298.30	1	5	3.18	94.95
6	342.35	0	6	3.50	100
7	286.24	4	6	2.28	81.96
8	256.43	0	2	5.25	93.18
9	282.47	1	1	6.11	89.26
10	284.48	0	2	6.03	92.42
11	326.44	1	3	3.67	92.56
12	324.64	0	0	9.07	90.34
13	296.58	0	0	8.44	89.47
14	310.61	0	0	8.83	89.12
15	338.66	0	0	9.61	88.43
16	422.82	0	0	11.95	86.37
17	318.33	1	4	1.26	77.12
18	318.32	1	4	1.26	77.12
19	320.30	0	5	1.00	95.32
20	374.35	1	6	1.00	75.16
21	360.36	1	5	1.53	98.03
22	268.31	2	3	4.07	90.04

Conclusion

Here, we have carried out a comparative study of several reported anti-inflammatory compounds using *in silico* approach and analyzed their Δ binding free energy, interactions at active site residues and their ADME properties. Comparative docking analysis between different compounds against the protein human group X sPLA$_2$ (PDB ID: 5G3M) reveals positive information for further research. The Δ binding free energy of 4-Benzylbenzamide (inhibitor) against 5G3M is −7.52 Kcal/mol. The Δ binding free energy of compound 1 at the binding site against 5G3M is −8.97 Kcal/mol. There are hydrogen bond interactions of the ligands toward the protein in the active site key residues. Therefore, compound 1 has high binding affinity toward the target. The Δ binding free energy and interaction with the active site residues show that the compound 1 is better than the reference drug inhibitor. On applying Lipinski's rule of five to the 22 compounds (compound 1-22), for evaluation of drug likeness (absorption, distribution, metabolism, and excretion), except compounds 9, 12, 13, 14, 15 and 16, others do not violate the rule which determines drugs pharmacological activity in the body. Thus, this study proposes that among the 22 reported compounds, the natural compound 1 (14-deoxyandrographolide) is the best anti-inflammatory agent based on docking studies and ADME properties and can be further studied for its anti-inflammatory property *in vivo*.

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Conflict of interest

All authors declare no conflict of interest.

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