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Designing Inhibitor against Phospholipases A_2 Enzyme through *Inslico*-Molecular Docking Studies

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Abstract

Pyrazole, hydroxyimino, aldehyde and isoxazole derivatives exhibit a broad spectrum of biological activities such as antimicrobial, anti-inflammatory and antitumor activities. With growing application on their synthesis and bioactivity, chemists and biologists in recent years have considerable attention on the research of these derivatives. In the view of potential importance of these derivatives, we have crystallized few of the derivatives and its report has been published. The present study focuses on docking studies of these derivatives against Phospholipases A_2 enzyme. This enzymes has implicated as potential targets for anti-inflammatory drug design. co-crystal structure (PDB ID: 1POE) of PLA_2 deposited in Protein Data Bank has been retrieved for docking analysis. Docking studies using Schrodinger's GLIDE reveals that these derivatives shows better binding energy and score in the defined active site. These results may provide a guiding role to design a lead molecule which may reduce inflamation.

Key words: Pyrazole, Hydroxyimino, Aldehyde, Isoxazole, Anti-inflammation, Phospholipases A_2, Molecular Docking

1. Introduction

Phospholipases A_2 (PLA_2s) EC 3.1.1.4 are enzymes that release fatty acids from the second carbon group of glycerol. This particular phospholipase specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. Upon downstream modification by cyclooxygenases, arachidonic acid is modified into active compounds called eicosanoids. Eicosanoids include prostaglandins and leukotrienes, which are categorized as anti-inflammatory and inflammatory mediators[1].

PLA_2 are commonly found in mammalian tissues as well as insect and snake venom[2]. Venom from both snakes and insects is largely composed of melittin, which is a stimulant of PLA_2. Due to the increased presence and activity of PLA_2 resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site[3].

There are also prokaryotic A_2 phospholipases. Additional types of phospholipases include phospholipase A_1, phospholipase B, phospholipase C, and phospholipase D[4]

Phospholipases A_2 can be classified based on sequence homology[5]. They include several unrelated protein families with common enzymatic activity. Two most notable families are secreted and cytosolic phospholipases A_2. Other families include Ca^{2+} independent PLA_2 (iPLA2) and lipoprotein-associated PLA_2s (lp-PLA_2), also known as platelet activating factor acetylhydrolase (PAF-AH).

The extracellular forms of phospholipases A_2 or secreted phospholipases A_2 ($sPLA_2$) have been isolated from different venoms (snake, bee and wasp), mammalian tissue (including pancreas and kidney) as well as from bacteria. They require Ca^{2+} for activity. Pancreatic $sPLA_2$ serves for the initial digestion of phospholipid compounds in dietary fat. Venom phospholipases help to immobilize prey by promoting cell lysis. In mice, group III $sPLA_2$ are involved in sperm maturation[6] and group X are thought to be involved in sperm capacitation[7].

$sPLA_2$ has been shown to promote inflammation in mammals by catalyzing the first step of the arachidonic acid pathway by breaking down phospholipids, resulting in the formation of fatty acids including arachidonic

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acid. This arachidonic acid is then metabolized to form several inflammatory and thrombogenic molecules. Excess levels of $sPLA_2$ is thought to contribute to several inflammatory diseases, and has been shown to promote vascular inflammation correlating with coronary events in coronary artery disease and acute coronary syndrome[8] and possibly leading to acute respiratory distress syndrome and progression of tonsillitis in children. In mice, excess levels of $sPLA_2$ have been associated with inflammation thought to exacerbate asthma[9] and ocular surface inflammation (dry eye)[10]. Increased $sPLA_2$ activity is observed in the cerebrospinal fluid of humans with Alzheimer's disease and multiple sclerosis, and may serve as a marker of increases in permeability of the blood-cerebrospinal fluid barrier[11].

The intracellular PLA_2 or cytosolic phospholipases A_2 are Ca^{2+} dependent with different three dimensional structures compared to secreted PLA_2. They include C2 domain and large catalytic domain. These phospholipases are involved in cell signaling processes, such as inflammatory response. The produced arachidonic acid is both a signaling molecule and the precursor for other signaling molecules termed eicosanoids. These include

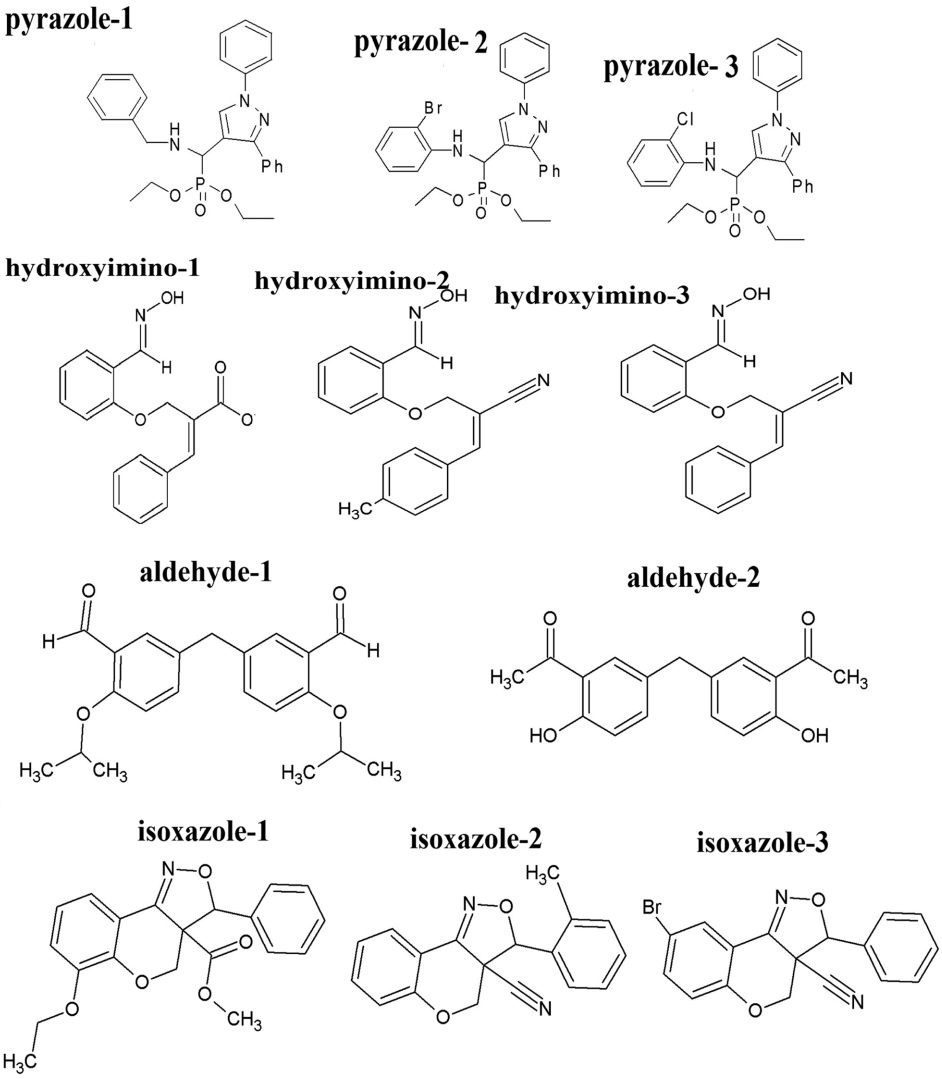

Fig. 1. Schematic representation of the X-ray crystallographically solved structures of all derivatives.

leukotrienes and prostaglandins. Some eicosanoids are synthesized from diacylglycerol, released from the lipid bilayer by phospholipase C. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) also known as platelet-activating factor acetylhydrolase (PAF-AH) is a phospholipase A_2 enzyme that in humans is encoded by the PLA_2G_7 gene[12,13]. Lp-PLA_2 is a 45-kDa protein of 441 amino acids[14].

It is one of several PAF acetylhydrolases, in the blood. It travels mainly with low-density lipoprotein (LDL). Less than 20% is associated with high-density lipoprotein HDL. It is an enzyme produced by inflammatory cells and hydrolyzes oxidized phospholipids in LDL. Lp-PLA_2 is platelet-activating factor (PAF) acetylhydrolase (EC 3.1.1.47), a secreted enzyme that catalyzes the degradation of PAF to inactive products by hydrolysis of the acetyl group at the sn-2 position, producing the biologically inactive products LYSO-PAF and acetate ["entrez gene: PLA_2G_7 phospholipase A_2, group VII].

Targeting the above protein enzymes is necessary to reduce the increasing inflammation. Hence, high throughput virtual screening (HTVS) has been carried out for pyrazole, hydroxyimino, aldehyde and isoxazole derivatives (Fig. 1) which compound have wide spectrum of biological and pharmacological properties, including antibacterial, antimalarial, anticonvulsant, and antitumor activities. Hence, we analyzed against secretory PLA_2 (PDB ID: 1POE) using Glide version 9.0 in Schrodinger. High ranked compounds have been selected on the basis of glide energy, docking score and hydrogen bond interactions from Induced Fit Docking (IFD) procedures. Comparative analysis have been carried out for the above mentioned complexes with the co-crystal structure (PDB ID: 1POE) and the results are discussed.

2. Experimental Section

2.1. Ligand Preparation

The structures of pyrazole, hydroxyimino, aldehyde, isoxazole, and indole derivatives are taken from crystallographic studies discussed in part I. The ligands were prepared for docking using LigPrep module of Schrodinger suite of tools. First, all the hydrogen atoms were added to ligand molecules as they had implicit hydrogen atoms. The bond orders of these ligands were fixed. The ionization states of the ligands were generated in the pH range of 5.0 to 9.0 using epic, as the micro-environment of the binding site can fluctuate over different pH values. Most probable tautomers and all possible stereo isomers were generated to study the activity of individual stereotypes of each ligand. In the final stage of Ligprep, ligands were minimized with OPLS-2001 force field.

2.2. Protein Preparation

Three dimensional protein structure of Phospholipase A_2 (Pdb id: 1POE) were downloaded from RCSB Protein databank. Protein preparation plays a very important role in the *in silico* docking studies as the correctness of protein structure is crucial to get the correct interactions with the ligands. The protein structure was prepared by multi-step process through the "Protein Preparation Wizard" of the Schrodinger suite. Firstly, the bond orders in the protein structure were assigned, hydrogen atoms were added and all the crystallographic water molecules were removed. The substrate Rhodamine-6G was retained for the purpose of grid generation. The structure was then subjected to single-point energy calculation using the protein modeling package, prime. The calculation was carried out using OPLS-2001 force field incorporating implicit salvation. Then protein-ligand complex was subjected to energy minimization using the Schrodinger implementation of steepest descent algorithm with OPLS-2005 force field with implicit salvation. The entire complex was minimized and minimization terminated when the root mean square deviation (RMSD) of the heavy atoms in the minimized structure realative to X-ray structure exceeds 0.3 Å.

2.3. Semi-Flexible Docking Studies

Docking studies on prepared ligands were carried out in the active site region of PLA_2 using docking program Glide[15]. The shape and properties of the receptor were represented on a grid by several set of fields that help progressively in more accurate scoring of ligands poses. The protein-ligand complexes prepared as described above were employed to build energy grids using default values of protein atom scaling (1.0) within a cubic box of dimensions 20 $Å^3$ centering the residues of the active site. In this docking, semi-flexible docking

protocols were used. The ligands being docked were kept flexible, in order to explore an arbitrary number of torsional degrees of freedom in addition to the six spatial degrees of freedom spanned by the translational and rotational parameters. The ligand poses were generated through a series of hierarchical filters that evaluated the ligand interactions with the receptor. The process of virtual screening was carried in two phases, using three different protocols i.e. standard precision (SP) and extra precision (XP) docking protocols.

2.4. Induced Fit Docking Studies

Induced fit docking studies were carried out on the selected ligands from the semi-flexible docking studies, where in induced fit models have been obtained to fit ligands in non-cognate structures. In other words the protein structure was induced to fit the ligands. For the protein model, initial docking was performed with a grid (defined using the centroid of the residues of the site) with default parameters. Twenty poses were chosen to be saved after initial Glide docking, which was carried out with the van der Waals scaling of 0.4 for both protein and ligand non-polar atoms. After obtaining initial docking poses, prime side chain and backbone refinement together with the minimization of the docked pose was carried out within a sphere of 5 Å from each pose saved. Glide re-docking was carried out in Prime refined structures having Prime energy values within 20 Kcal/mol of the lowest energy value. The RMSD values of ligand poses having best induced fit docking score were determined.

3. Results and Discussion

PLA_2 is involved in the generation of eicosanoids, which play an important role in pathophysiological processes such as in ammation, platelet aggregation, and acute hypersensitivity reactions. Modulation of PLA_2 activity is a very important pharmacological goal, and there is a great interest in developing inhibitors for

Table 1. Docking results of all derivatives with PLA_2 along with intermolecular hydrogen bond interactions with active site residues

Compound Category		Docking score	Glide evdw	Glide ecoul	Glide energy	Intermolecular H-Bonding	Distance (Å)
Co-Crystal-ligand (PDB ID: 1POE)	S.No	-7.46	-39.26	-11.6	-50.63	(LYS 62) N-H…O (GLY 31) N-H…O (GLY 29) N-H…O	2.8 3.0 2.9
Pyrazole derivatives	1	-5.77	**-54.72**	2.78	**-51.94**	(GLY 29) N-H…O	3.0
	2	-3.79	-42.54	0.43	-42.12	(HIS 47) N-H…O	2.9
	3	-6.89	**-53.72**	-0.32	**-54.03**	(GLY 29) N-H…O O-H…O (TYR 27)	3.4 2.7
Hydroxyimino derivatives	1	-4.31	-27.78	-6.23	-34.01	O-H…O (CYS 44) O-H…O (ASP 48) (GLY 31) N-H…N	2.8 2.7 3.1
	2	-7.21	-32.84	-6.92	-39.76	O-H…O (ASP 48) O-H…N (GLY 31) (HIS 47) N-H…O	2.6 3.2 2.8
	3	-5.94	-28.44	-5.91	-34.35	O-H…O (CYS 44)	2.6
Aldehyde derivatives	1	-7.21	-42.60	-3.26	-45.86	(HIS 47) N-H…O (GLY 29) N-H…O	3.0 2.6
	2	**-8.01**	-25.80	**-14.99**	-40.79	O-H…O (LEU 2) (HIS 47) N-H…O O-H…O (ASP 48)	2.7 2.6 2.6
Isoxazole derivatives	1	-5.85	-38.64	-5.20	-43.84	(GLY 29) N-H…O	2.9
	2	-6.34	-37.56	-0.39	-37.96	(GLY 29) N-H…O	3.0
	3	**-7.78**	**-40.46**	-5.15	-45.61	(HIS 47) N-H…O	3.1

this enzyme in order to treat certain chronic in ammatory conditions such as rheumatoid arthritis and asthma. To this end, a clear understanding of the interaction of PLA_2 enzymes with their ligands is required. During the last two decades computer-assisted methods have been proven to be very powerful and successful in improving our understanding of structure-activity relationships of ligands and their receptors, in particular enzyme-inhibitor complexes, and using this information to rationally modify their interaction.

Binding site of PLA_2 is composed of a "hydrophobic site" where the fatty acid tails of substrates bind, and the "catalytic site" for substrate cleavage. The "hydrophobic site" in PLA_2-IIA consists of aliphatic and aromatic residues within or close to the N-terminal helix (In PLA_2-IIA: Leu2, Phe5, Ile9, Ala17, Ala18, Tyr21, Cys28, Cys44, and Phe98). And catalytic site of PLA_2-IIA contains hydrophilic residues His47 and Asp48, which together with the catalytic Ca^{2+} are needed for the enzyme's cleavage mechanism consisting on the nucleophilic attack of a water molecule at the sn-2 acyl ester bond of diacylglycerol. Due to the fact that PLA_2 substrates interact with the catalytic Ca^{2+}, this interaction has been included in the design of inhibitors as a hot spot for improving affinity. In present study, *insilico* molecular docking studies of five types of ligands (pyrazole, hydroxyimino, aldehyde, isoxazole and indole derivatives) against human PLA_2 II (Pdb id: 1POE) has been carried out using Glide module of Schrodinger 2009. Docking results of best complex of these compounds with their key active site residues interaction are tabulated in (Table 1).

Based on docking score, glide energy and key active site interactions, ligands of pyrazole, aldehyde and isoxazole derivatives show better binding affinity compared to cocrystalised inhibitor. Among pyrazole

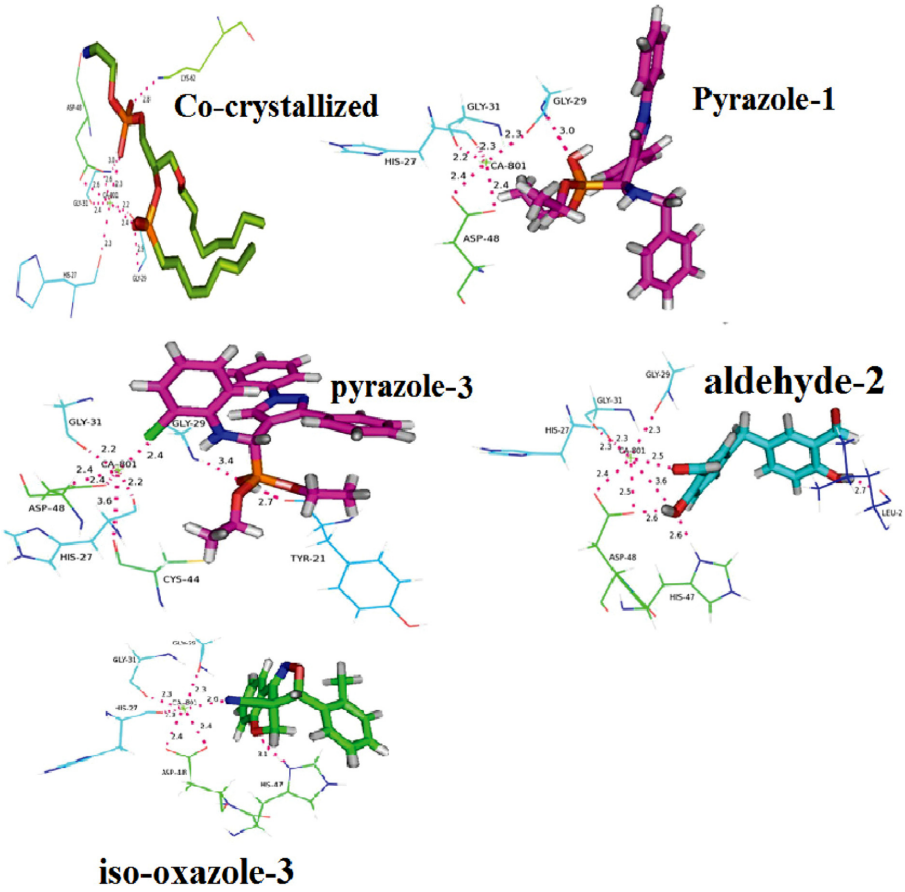

Fig. 2. Hydrogen bond interaction of best derivative with co-crystal ligand in PLA_2 active site.

derivatives, pyrazole (**1** and **3**) show better glide energy (-51.94, -54.03) and reasonable docking score (-5.77, -6.89) compared to cocrystallised inhibitor (-7.46 and -50.63). Favorable binding of these two compounds is due to van der Waals interactions (Evdw = -54.72 and -53.72) compared to cocrystallised inhibitor (Evdw = -39.26).

The binding mode of compounds are similar to cocrystallised inhibitor and exhibit strong hydrogen bond interactions with catalytic hydrophobic site Gly 29 and Tyr 27, respectively along with one Ca^{2+} coordination for inhibition of enzyme's cleavage mechanism and its interaction shown in Fig. 2. Among aldehyde derivatives, aldehyde **2** exhibits better docking score (-8.01) and rescannable glide energy (-40.79) compared with cocrystallised inhibitor (-7.46, -50.63). Favorable binding of compound is due to the higher electrostatic (-14.99) and rescannable van der Waals interaction (-25.80). Compound having three hydrogen bond interactions with catalytic residues (His 47, Asp 48 and Leu 2) and one Ca^{2+} coordination along with similar hydrophobic interactions exhibited by cocrystallised inhibitor.

Among isoxazole derivatives, isoxazole **3** shows better docking score (-7.78) and rescannable glide energy (-45.61) compared with cocrystalised inhibitor (-7.46, -50.63). favorable binding of this compound is due to van der Walls interactions (-40.46) and rescannable electrostatic interaction (-5.15) compared to co crystallized inhibitor. This compound makes one hydrogen bonding interaction with catalytic hydrophilic residue (His 47) and one Ca^{2+} ion coordination and fully surrounded with hydrophobic amino acid.

Thus, these compounds could be thought of possessing potent activity compared to already cocrystalised inhibitor from our *insilico* docking studies. For better understanding of inhibition, molecular dynamics studies are needed so that the interactions with key active site residues and thermodynamic properties, search as free energy are to be explored.

4. Conclusions

Docking analysis of all the ten derivatives with the protein targets, Phospholipase A_2 brought important interactions operating at the molecular level. Hence targeting the above proteins is necessary for the design of broad spectrum of anti-inflammation. In order to study the binding mode and enzyme-inhibitor interaction with the active site residues of the target proteins, we have compared our compound with all ready co-crystallized ligand. The above established enzyme-inhibitor complex is taken as a model for explaining our interaction the active sites of the enzyme The docking studies with the all derivatives showed that our derivatives also established a similar way of binding with the target protein which is explained by their comparable binding energy and interaction with the active site residues of the enzymes. Among all derivatives, pyrazole compounds **1** and **3** show better glide energy (-51.94, -54.03) and reasonable docking score (-5.77, -6.89) compared to cocrystallised inhibitor (-7.46 and -50.63). Thus inshort this study will be useful for the design of novel anti- inflammatory agents based on docking methods.

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References

[1] E. A. Dennis, "Diversity of group types, regulation, and function of phospholipase A_2", J. Biol. Chem., Vol. 269, pp. 13057-13060, 1994.

[2] J. P. Nicolas, Y. Lin, G. Lambeau, F. Ghomashchi, M. Lazdunski, and M. H. Gelb, "Localization of structural elements of bee venom phospholipase A_2 involved in N-type receptor binding and neurotoxicity", J. Biol. Chem., Vol. 272, pp. 7173-7181, 1997.

[3] A. Argiolas and J. J. Pisano, "Facilitation of phospholipase A_2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom", J. Biol. Chem., Vol. 258, pp. 13697-13702, 1983.

[4] D. L. Nelson, A. L. Lehninger, and M. M. Cox, "Lehninger principles of biochemistry", Macmillan, 2008.

[5] D. A. Six and E. A. Dennis, "The expanding superfamily of phospholipase A(2) enzymes: classification and characterization", Biochim. Biophys. Acta, Vol. 1488, pp. 1-19, 2000.

[6] H. Sato, Y. Taketomi, Y. Isogai, Y. Miki, K. Yamamoto, S. Masuda, T. Hosono, S. Arata, Y. Ishikawa, T. Ishii, T. Kobayashi, H. Nakanishi, K. Ikeda, R.

Taguchi, S. Hara, I. Kudo, and M. Murakami, "Group III secreted phospholipase A_2 regulates epididymal sperm maturation and fertility in mice", J. Clin. Invest., Vol. 120, pp. 1400-1414, 2010.

[7] J. Escoffier, I. Jemel, A. Tanemoto, Y. Taketomi, C. Payre, C. Coatrieux, H. Sato, K. Yamamoto, S. Masuda, K. Pernet-Gallay, V. Pierre, S. Hara, M. Murakami, M. De Waard, G. Lambeau, and C. Arnoult, "Group X phospholipase A_2 is released during sperm acrosome reaction and controls fertility outcome in mice", J. Clin. Invest., Vol. 120, pp. 1415-1428, 2010.

[8] Z. Mallat, G. Lambeau, and A. Tedgui, "Lipoprotein-associated and secreted phospholipases A_2 in cardiovascular disease: roles as biological effectors and biomarkers", Circulation, Vol. 122, pp. 2183-2200, 2010.

[9] W. R. Jr. Henderson, R. C. Oslund, J. G. Bollinger, X. Ye, Y. T. Tien, J. Xue, and M. H. Gelb, "Blockade of human group X secreted phospholipase A_2 (GX-sPLA$_2$)-induced airway inflammation and hyper responsiveness in a mouse asthma model by a selective GX-sPLA$_2$ inhibitor", J. Biol. Chem., Vol. 286, pp. 28049-28055, 2011.

[10] Y. Wei, S. P. Epstein, S. Fukuoka, N. P. Birmingham, X. M. Li, P. and A. Asbell, "sPLA$_2$-IIa amplifies ocular surface inflammation in the experimental dry eye (DE) BALB/c mouse model", Invest. Ophth. Vis. Sci., Vol. 52, pp. 4780-4788, 2011.

[11] S. Chalbot, H. Zetterberg, K. Blennow, T. Fladby, N. Andreasen, I. Grundke-Iqbal, and K. Iqbal, "Blood-cerebrospinal fluid barrier permeability in Alzheimer's disease", J. Alzheimers dis., Vol. 25, pp. 505-515, 2011.

[12] D. G. Tew, C. Southan, S. Q. Rice, M. P. Lawrence, H. Li, H. F. Boyd, K. Moores, I. S. Gloger, and C. H. Macphee, "Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins", Arterioscler. Thromb. Vasc. Biol., Vol. 16, pp. 591-599, 1996.

[13] L. W. Tjoelker, C. Wilder, C. Eberhardt, D. M. Stafforini, G. Dietsch, B. Schimpf, S. Hooper, H. Le Trong, L. S. Cousens, and G.A. Zimmerman, "Anti-inflammatory properties of a platelet-activating factor acetylhydrolase", Nature, Vol. 374, pp. 549-553, 1995.

[14] A. Zalewski and C. Macphee, "Role of lipoprotein-associated phospholipase A_2 in atherosclerosis: biology, epidemiology, and possible therapeutic target", Arterioscler. Thromb. Vasc. Biol., Vol. 25, pp. 923-931, 2005.

[15] Version, Glide. "5.5, Schrödinger." LLC, New York, NY, 716, 2009.