2011 International Conference on Biology, Environment and Chemistry
IPCBEE vol.24(2011) © (2011)IACSIT Press, Singapoore

COMPARATIVE DOCKING ANALYSIS OF PHOSPHOLIPASE A_2 WITH VARIOUS COMPOUNDS ISOLATED FROM *PSEUDARTHRIA VISCIDA*

SURIYAVATHANA MUTHUKRISHNAN[1] ,RAJAN THINAKARAN[2]

[1]Department of biochemistry, periyar university, salem, Tamilnadu, India.

[2]Department of biochemistry, muthayamal college of arts and science, rasipuram, Tamilnadu, India.

Abstract. Phospholipase A_2 (PLA_2) plays a major role in the formation of inflammatory mediators. This enzyme catalyses the Sn-2 hydrolysis of phospholipids liberating free fatty acids predominantly arachidonic acid, and lysophospholipids. These products can have biological actions (or) be further metabolized to form a variety of proinflammatory lipid mediators including prostaglandins, leukotrienes (or) platelet-activating factor and thus the inhibition Phospholipase A_2 by pharmacological agents should have led to an anti-inflammatory effect. Plants serve as sources of compounds that act a potential therapeutic agent for treatment of various diseases. In present study, an attempt was made to inhibit Phorpholipase A2 by 13 different compounds reported from the root of medicinal plant *Pseudarthria viscida.* Our report can be used to develop new inhibitors with better binding affinities towards the protein Phospholipase A_2. For the binding analysis the catalytic subunit of the Phospholipase A_2 was taken for study

Keywords: Phospholipase A_2, *Pseudarthria viscida.*

1. Introduction

In silico molecular docking in one of the most powerful techniques to discover novel ligand for proteins of known structure and thus play key role in structure based drug .Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the protein. Hence in this present work we have carried out *in silico* molecules docking to analyze the binding properties of the enzyme Phospholipase A_2 with 13 different compounds reported from root of *Pseudarthria viscida.*

1.1. Compounds identified from *Pseudarthria viscida* root

The presence of compounds like 3-O-Methyl-d-glucose, Butane-1,1 Diethoxy-3-methyl, d-Mannitol-1-decyl sulfonyl, n-Hexadecanoicacid, Oleic acid, Oxirane tetra decyl, Tetradecanoic acid, Undecanoic acid was identified by GC-MS study. By HPLC analysis, the existence of phenolic compounds such as Rutin, Quercetin, Gallic acid, Ferulic acid and caffeic acid was characterized. So in total 13 compounds identified in the root of *Pseudarthria viscida* was taken for binding analysis with Phospholipase A2

2. MATERIALS AND METHODS

2.1. Ligand preparation

The three dimensional structures of compounds taken for binding analysis were downloaded in .sdf format from PubChem database. Hydrogen bonds were added and the energy was minimized using CHARMm force field. Lipinski properties such as Molecular weight, XLog P , number of hydrogen bond donors and acceptors for the compounds were obtained from PubChem (shown in Table: 1)

2.2. Protein preparation

The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is Phospholipase A2 for our consideration. The pdb Id is 1 POE and an resolution factor is 2.10 A˚ and the method of incorporation is X-ray diffraction method. The ligand and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were connected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMm Force field.

2.3. Docking studies

The active site of the protein was first identified and it is defined as the binding site. The binding sites were defined based on the ligand present in the PDB file which was followed by site sphere definition. Here site 1 was chosen as the binding site. Dockscore were used to estimate the ligand binding energies. For accurate docking of ligands into protein active sites, the docking method used in this study is LigandFit. This method employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions. A shape comparison filter is combined with a Monte Carlo conformational search for generating ligand posses consistent with the active site shape. Candidate poses are minimized in the context of the active site using a grid based method for evaluating protein-ligand interaction energies. The method appears quite promising and reproducing. Thus docking analysis of the compounds reported from *Pseudarthria viscida* root with Phospholipase A2 was carried out by ligand Fit of Discovery studio (version 2.1, Accelery software Inc.). The software allows us to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. The collection of 13 compounds and Phospholipase A2 complexes was identified via docking and their relative stabilities were evaluated using their binding affinities.

3. RESULTS AND DISCUSSION

In the field of computer based drug design, Molecular Docking holds great importance. Because of this ligands for the receptor of known structure were designed and their interaction energies were calculated using the scoring function [4]. Uses of CADD in developing specific drugs for many diseases were reported. The notable example which can serve as a proof of principle of the *in silico* approach involves a Type I TGF β receptors kinase inhibitor. The same molecule (HTS-466284/Ly364947), a 27nM inhibitor, was discovered independently using virtual screening by Biogen IDEC[5] and traditional enzyme and cell based high-throughput screening[6]. It is estimated that docking programs currently dock 70-80% of ligands correctly.

3.1. Hydrogen bond details

A close view of the binding interaction of Phospholipase A_2 with 13 compounds dentified from *Pseudarthria viscida* was shown in Fig 1. Ligand is coloured in green where as amino acids involves in hydrogen bonding were shown in blue colour. The green dotted line denotes the hydrogen bond. The number of hydrogen bond formed and amino acid residues involved in forming hydrogen bonds were presented in Table: 2. Hydrogen bond formation makes important contributions to the interactions between ligand and the enzyme. Here a maximum of 7 hydrogen bonds formed between the protein and the ligand 3-0-Methyl-d-glucose followed by six hydrogen bonds were formed between the enzyme and the ligand Rutin and Gallic acid. Thus the concept of protein –ligand interaction helps in analyzing the binding properties of the protein Phospholipase A_2 with its inhibitors. The study report also concludes that the residue Gly29, His47, Lys62, Asp48 plays an important role in binding mechanism.

3.2. Docking score

As a result of docking there were 10 different conformations were generated for all the 13 compounds reported from *Pseudarthria viscida*. But only for top ranked docked complex the scores were copied from the table browser view of Discovery studio for binding affinity analysis. The determination of the ligand binding affinity was calculated using the shape – based interaction energies of the ligand with the protein. Larger score value indicates better ligand-binding affinity.

When the enzymes docked to the compounds the scores obtained were shown in the Table:1.The compounds D-Mannitol,1-decyl sulfonyl (92.138), Rutin (82.323),Oxirane(75.14),oleic acid (71.79), and n-Hexadecanoic acid (68.58) ranked accordingly to their dock scores which includes the all binding affinities of the protein-ligand interaction. Screening of the compounds with respect to their dockscore and drug likeness would results in better lead identification. Here the D-Mannitol, 1-decyl sulfonyl had scored highest dockscore and satisfies all Lipinski's parameter. So it is known from this study that compound D-Mannitol, 1-decyl sulfonyl is considered to be potential inhibitor towards the inflammation causing enzyme Phospholipase A_2

3.3. Validation of the docking protocol

To ensure that the ligand orientation obtained from the docking studies was likely to represent valid and reasonable binding modes of the inhibitors, the ligand Fit program docking parameters had to be first validated for the crystal structure's active site (PDB id 1 POE). Protein utilities and health protocol of Discovery's studio was used to find out the active site contains amino acids such as His6, Try21, Gly29, Gly31, Cys44, His47, Asp48, Lys62 etc. Results of docking showed that the LigandFit determined the optimal of the docking inhibitor, exactly to these active sites.

Here top ranked ligands were taken for binding affinity studies. The validation process consisted of two parts

1. Hydrogen bond details of the top –ranked docked pose.3

2. Prediction of binding energy between the docked ligand and the enzyme using various score calculated using Discovery studio

4. REFERENCES

[1] Pruzanski W and Vadas P (1991).Phospholipase A2- A mediator between proximal and distal effectors of inflammation. Immunology today.12; 143-146

[2] Warrier PS(1994).Indian medicinal plants, 1st edition Orient Longman private limited, New Delhi.

[3] Brooijmans N and kuntz ID(2003).Molecular recognition and docking algorithms0.Ann Rev Biophys Biomol struct ,32:335-373

[4] Irwin JJ, Lorber DM,Mc Govern SL, Wei B, Shoichet Bk (2002). Computational Nanoscience and Nanotechnology,ISBN 0 -9708275-6-3.

[5] Singh J, Chuaqui CE, Boriack-Sjodin PA, Lee WC, Pontz T, *et al.* (2003). Successful shape-based virtual screening: the discovery of a potent inhibitor of the type I TGFbeta receptor kinase (TbetaRI). Bioorg Med Chem Lett, 13:4355-4359

[6] Sawyer JS, Anderson BD, Beight DW, Campbell RM, Jones ML, *et al*. (2003). Synthesis and activity of new aryl- and heteroaryl-substituted pyrazole inhibitors of the transforming growth factor-beta type I receptor kinase domain. J Med Chem, 46: 3953-3956

TABLE: 1NUMBER HYDROGEN BOND FORMED AND AMINO ACIDS RESIDUES INVOLVED IN FORMING HYDROGEN BONDS

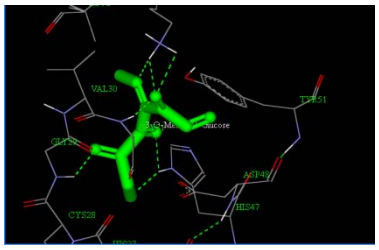
S.No	Ligand	Amino acid involved in H-bonding	Number of hydrogen bond
1	3-O-Methyl-d-glucose	Gly29,Gly31,HIS47, Lys 62	7
2	Butane-1,1- diethoxy-3-methyl	Lys 62	2
3	d-Mannitol-1- Decyl sulfonyl	Gly31, Lys62	2
4	n-Hexadecanoic acid	Gly31	1

5	Oleic acid	Lys62	2
6	Oxirane tetra decyl	Lys62	2
7	Tetradecanoic acid	Gly31, Lys62	2
8	Undecanoic acid	Lys 62	3
9	Rutin	His6, His47, Lys62, Asp48, Tyr21	1
10	Quercetin	Gly31, Lys62	6
11	Gallic acid	Gly29, His47, Lys62, Cys44, Asp48	6
12	Ferrulic acid	Lys62	2
13	Caffeic acid	Lys62	1

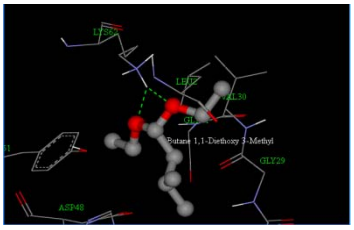
TABLE: 2 Lipinski's parameter for the compounds identified in Pseudarthria viscida and DOCKSCORE

S.No	**Ligand**	**Mol. Wt**	**X logp**	**H-Bond donor**	**H- Bond acceptor**	**DOCK SCORE**
1	3-O-Methyl-d-glucose	194.18246	-2.9	4	6	51.113
2	Butane -1,1- diethoxy-3-methyl	1602539	2.5	0	2	52.68
3	d-Mannitol-1- decyl sulfonylS	370.50	0.9	5	7	92.138
4	n-Hexadecanoic acid	256.42	6.4	1	2	68.582
5	Oleic acid	282.46	6.5	1	2	71.799
6	Oxirane tetra decyl	240.42	7.3	0	1	75.14
7	Tetradecanoic acid	228.37	5.3	1	2	64.178
8	Undecanoic acid	186.29	3.7	1	2	73.62
9	Rutin	610.5175	-1.3	10	16	106.01
10	Quercetin	302.2357	1.5	5	7	101.6
11	Gallic acid	610.5175	-1.3	10	16	34.195
12	Ferrulic acid	194.18	1.5	2	4	50.494
13	Caffeic acid	180.15	1.2	3	4	41.15

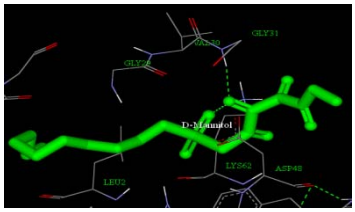
A



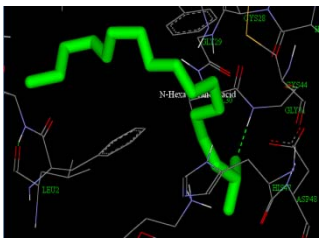
B



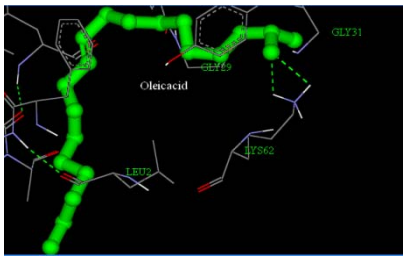
C



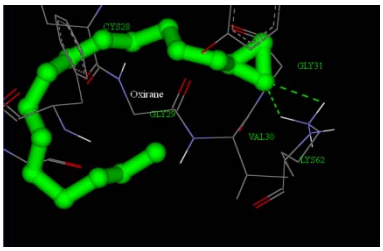
D



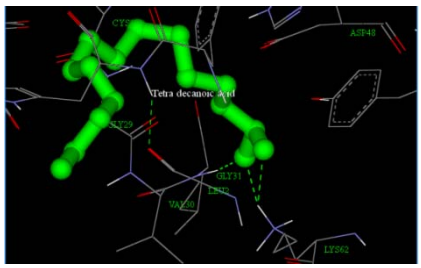
E



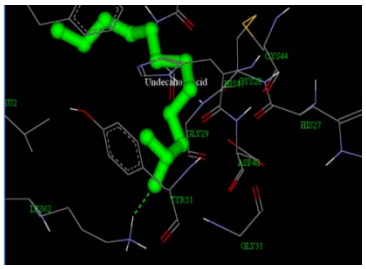
F



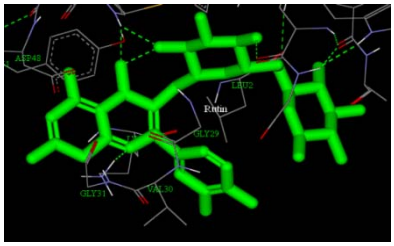
G



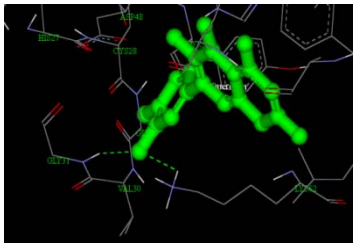
H



I

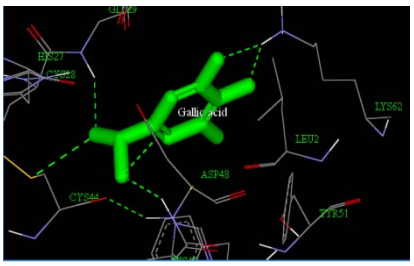


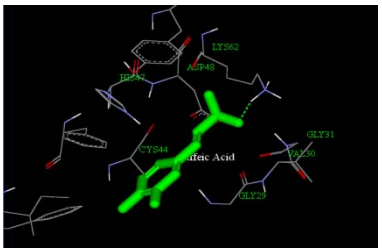
J



K

L





M

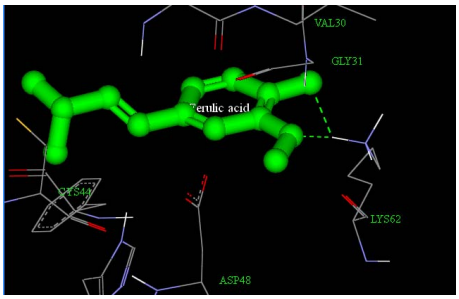


Figure 1: Summary of Docked Pose of the Compounds identified from Pseudarthria Viscida

Docked model of (A) 3-O-Methyl-d-glucose (B) Butane,1,1-diethoxy-3-methyl (C) d-Mannitol, 1-decylsulfonyl (D) n-Hexadecanoic acid (E) Oleic acid (F) Oxirane, tetradecyl (G) Tetradecanoic acid

(H) Undecanoic acid (I) Rutin (J) Quercetin (K) Gallic acid (L) Caffeic acid (M) Ferulic acid with Phospholipase A2