Research Article

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DOCKING STUDY AND DESIGNING OF ANTIASTHMATIC DRUG USING sPLA2 AS A THERAPEUTIC TARGET PROTEIN

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Abstract

Asthma is a complex disease of the respiratory tract characterized by allergen-induced breathlessness that is, orchestrated by the interaction of immune, inflammatory, and resident lung cells which is controlled by number of target proteins. In this work sPLA2 is selected as potential target as it is expressed at the highest levels in airways of asthmatics. Attempt has been made to design a drug against this target protein. The homology modeling of sPLA2 protein, ligand designing and drug validation studies done by using bioinformatics tools and softwares. The result indicates that the designed ligand molecule 2(R) – 2 hydroxyl – 3 [(3-vinylbenzyl) oxy] propanamide possess non mutagenic, non tumerogenic, non irritant properties and could be a promising drug target for the disease asthma showing valid drug likeness score and negative gibbs free energy.

Keywords: sPLA2, Asthma, Drug Design, Homology Modelling, Docking

Introduction

Asthma is a chronic inflammatory disease of the respiratory system that affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15—20 years. In this disease the airway occasionally constricts, becomes inflamed, and is lined with excessive amounts of mucus, often in response to one or more triggers. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning. These episodes may be triggered by exposure to an environmental stimulant (allergen), cold air, warm air, moist air, exercise or exertion, or emotional stress[1]. Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic). Genetic mapping studies have demonstrated that one or more asthma susceptibility genes are located on chromosome 5q31-q33 in humans[2, 3]. This chromosomal region contains a cytokine gene cluster that may potentially play a role in airway inflammation associated with atopic asthma. Interleukin (IL)-9, a Th2-type cytokine, is one of these cytokines and has been suggested as a candidate gene for asthma[4, 5]. In the recent studies, a relationship between airway inflammation, Uteroglobin-related protein 1 (UGRP1) expression, (a secretory protein of ~10 kDa that is highly expressed in epithelial cells of airways i.e. the trachea, bronchus, and bronchioles [6]) and interleukin-9 (IL-9), an asthma candidate gene, was evaluated by using a murine model of allergic

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bronchial asthma. These studies showed that the UGRP1 is down regulated in inflamed airways, such as allergic asthmatics, and IL-9 might be an important mediator for modulating UGRP1 expression. Although the functional roles of UGRP1 in airway physiology are not understood. However, the following three lines of evidence suggest a possible involvement of UGRP1 in the pathogenesis of asthma: *i*) high levels of expression in lungs, *ii*) amino acid sequence similarities to uteroglobin (also called Clara cell secretory protein; CCSP), which is known as a regulator of airway inflammation[7,8], and *iii*) chromosomal location of the human *UGRP1* gene at 5q31-q32 [9], the region that has been assigned as one of asthma susceptibility loci[10]. Soluble phospholipase A_2 ($sPLA_2$) was suggested as a target protein for uteroglobin/CCSP that can inhibit $sPLA_2$ activity, resulting in an anti-inflammatory effect. As, UGRP1 has sequence and functional similarity to the uteroglobin/CCSP that is also secreted by the epithelial cells in airways. Therefore, it is possible that UGRP1 is able to inhibit $sPLA_2$ activity. Recent studies proved that sPLA2 groups V and X were differentially expressed in asthmatic subjects compared to non-asthmatic controls. Of the two sPLA2 enzymes, only sPLA2-X was over expressed in asthmatics relative to controls[11]. Thus, sPLA2s are attractive therapeutic targets because these enzymes may be preferentially involved in the production of pro-inflammatory AA metabolites that are key for asthma immunopathogenesis[12]**.** In this study a drug has been designed against the sPLA2 as a target protein using *in silico* approach.

Methodology

Homology modelling and model optimization

The three dimensional structure of selected sPLA2 protein was not available in PDB Database (www.rcsb.org/pdb), hence, it is important to determine its three dimensional structure. Sequence of sPLA2 protein was retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/) with ID: NP_003552 and performed NCBI BLAST (http://blast.ncbi.nlm .nih.gov/Blast.cgi) for homology searching. Chain A Carboxylic Ester Hydrolase protein (PDB ID: 1LE6 A) was found to be much similar and used as template for homology modeling. The modeling was carried out using MODELLER 9V7 (http://www.salilab.org/modeller/). The target and template sequences were aligned and used to generate 3D structure of sPLA2 protein. Generated model was then undergo the process of loop refinement, model optimization and evaluation using Ramachandran plot and PROCHECK[13] Program of Structural Analysis and Verification Server (SAVES) for the protein stability. After final analysis and validation process the best model was selected with maximum core value and zero bad contacts.

Active site prediction

After the selection of final protein model, the possible active sites were identified using LIGSITE[14].

Virtual screening and designing of ligand molecule

Lead molecule was screened on the basis of already existing drugs of asthma and ligand molecules were generated from lead molecule using LIGBUILDER[15].

Optimization and docking of ligand molecule

The generated ligand molecules were analyzed for drug relevant properties using online tools named OSIRIS property explorer, Molsoft LLC, and Molinspiration cheminformatics and finally better quality ligand molecule was taken for further docking study. The docking of ligand molecule to the active site of the sPLA2 protein was performed using QUANTUM 3.3 software[16] to find out the value of Gibbs free energy.

Result and discussion

The sPLA2 protein sequence was retrieved from NCBI database and was found to contain 163 amino acids. The similarity searching was performed by NCBI BLAST against PDB, the protein from 43-165 sequence was found to be 100% homologous to 1-123 amino acids of chain A Carboxylic Ester Hydrolase protein, which was taken as the template for homology modeling. The models which were generated using MODELLER 9V7, often contain unfavourable bond length, bond angles and bad contacts. Therefore it was essential to minimize the energy to regularise local bond and angle as well as to remove bad contacts. These models was optimized and verified by Ramachandran plot with the help of Swiss pdb viewer (energy minimization and build

loop formation) and then by PROCHECK program. The final model **(Figure 1)** selected was having 97.1% of the residues in the core region with zero bad contacts. **(Table 1a and 1b)**.

The total energy of model protein was found to be -4614.343Kj/mol initially, but after energy minimization it came down to -5180.187 Kj/mol.

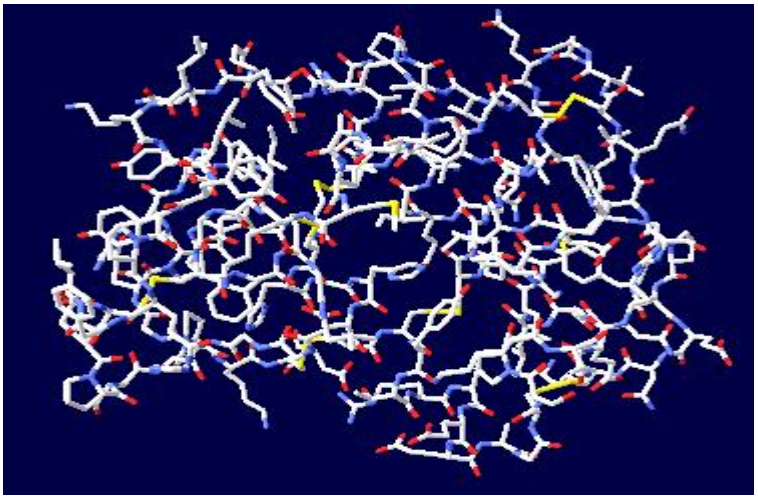


Figure 01: Selected Modeled sPLA2 protein structure

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
|                                                                             |
|  /var/www/html/Services/SAVS/jobs/3505992/apdb.pdb   2.0       123 residues |
|                                                                             |
|  Ramachandran plot:   97.1% core    2.9% allow    0.0% gener    0.0% disall |
|                                                                             |
|  All Ramachandrans:    0 labelled residues (out of 121)                     |
|  Chi1-chi2 plots:      0 labelled residues (out of  82)                     |
|                                                                             |
|  Main-chain params:    6 better     0 inside      0 worse                   |
|  Side-chain params:    5 better     0 inside      0 worse                   |
|                                                                             |
+| Residue properties: Max.deviation:     5.9             Bad contacts:    2  |
+|                     Bond len/angle:    3.5    Morris et al class:  1  1  2  |
|                                                                             |
|  G-factors          Dihedrals:   0.13  Covalent:  -0.05    Overall:   0.06  |
|                                                                             |
|  M/c bond lengths: 99.5% within limits   0.5% highlighted                   |
|  M/c bond angles:  95.8% within limits   4.2% highlighted                   |
|  Planar groups:   100.0% within limits   0.0% highlighted                   |
|                                                                             |
 +-----------------------------------------------------------------------------+
```

Table 1a: Procheck summary of the selected model of sPLA2 protein before energy minimization and loop build formation

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
|                                                                            |
| /var/www/html/Services/SAVS/jobs/904995/apdb.pdb   2.0        123 residues |
|                                                                            |
| Ramachandran plot:   97.1% core    2.9% allow    0.0% gener    0.0% disall |
|                                                                            |
| All Ramachandrans:    0 labelled residues (out of 121)                     |
| Chi1-chi2 plots:      0 labelled residues (out of  82)                     |
|                                                                            |
| Main-chain params:    6 better     0 inside      0 worse                   |
| Side-chain params:    5 better     0 inside      0 worse                   |
|                                                                            |
| Residue properties: Max.deviation:     5.0             Bad contacts:    0  |
|                     Bond len/angle:    3.2    Morris et al class:  1  1  2 |
|                                                                            |
| G-factors           Dihedrals:   0.07  Covalent:   0.34    Overall:   0.18 |
|                                                                            |
| M/c bond lengths:100.0% within limits   0.0% highlighted                   |
| M/c bond angles:  98.7% within limits   1.3% highlighted                   |
| Planar groups:    73.3% within limits  26.7% highlighted       1 off graph  |
|                                                                            |
+----------------------------------------------------------------------------+
```

Table 1b: Procheck summary of the selected model of sPLA2 protein after energy minimization and loop build formation.

For the final model of sPLA2 protein, the active sites were identified using LIGSITE. Three possible sites were obtained and PKT 138 was chosen as the most biologically favorable site for the docking study **(Figure 2)**. The minimum distance between the residue to the active site position was identified which is important to dock lead molecule in the active site position of the target molecule.

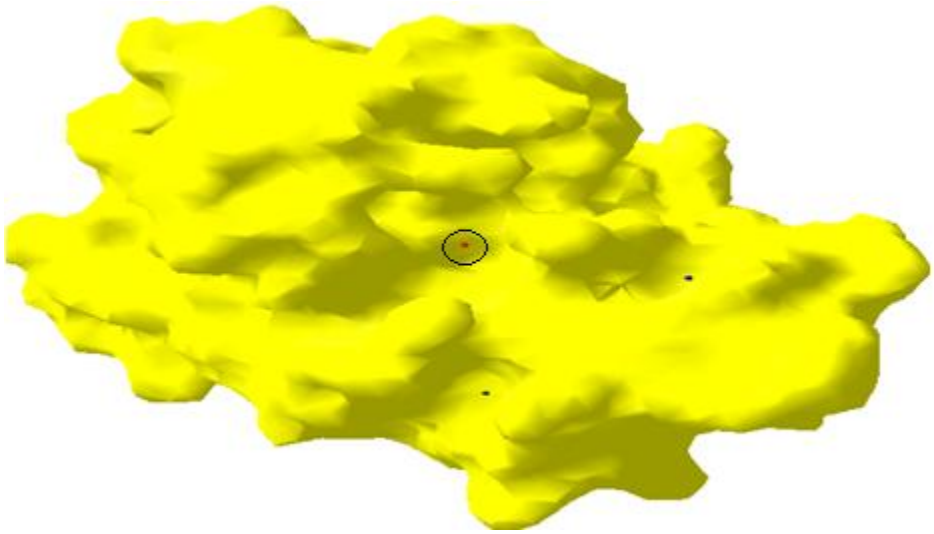
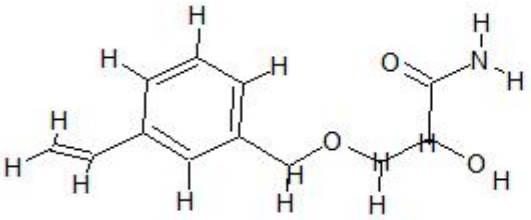


Figure 02: sPLA2 protein structure showing three active sites. Most favorable active site is red in colour.

Now on the basis of already existing drugs of asthma, Pyrrolidine was chosen as a lead molecule. After this, the rigid docking was performed using HEX 4.5 in which the lead molecule was docked with the target molecule to the minimum distance residue to the active site position. Using the coordinates information of the lead molecule, the ligand molecules were generated using LIGBUILDER. Out of generated molecules, the best ligand molecule was selected on the basis of parachor value and drug likeness properties. As parachor value is an additive and constitutive molecular parameter which appears

to be important in the passage of the drug molecule from the site of administration or synthesis to the site of action.

The selected ligand molecule named as 2(R) – 2 hydroxyl – 3 [(3-vinylbenzyl) oxy] propanamide shows 496.2 cm^3 parachor value **(Figure 3)** and has lipophilic behavior according to Log P value (0.4) **(Figure 4)** and is found to be hydrophobic in nature which is a major determinant of drug likeness. On the other hand, the ligand molecule is also following physiochemical and biological parameters of Lipinski's rule of five which states that in general, an orally active drug should have not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight from 160-480, lop P less than 5, molar refractivity from 40-130.

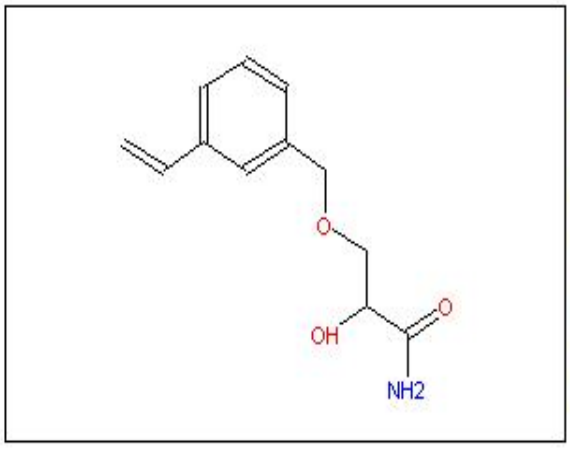


O=C(N)C(O)COCc1cc(ccc1)\C=C

Molecular Formula	= $C_{12}H_{15}NO_3$
Formula Weight	= 221.2524
Composition	= C(65.14%) H(6.83%)
Molar Refractivity	= 62.79 ± 0.3 cm^3
Molar Volume	= 186.2 ± 3.0 cm^3
Parachor	= 496.2 ± 4.0 cm^3
Index of Refraction	= 1.589 ± 0.02
Surface Tension	= 50.4 ± 3.0 dyne/cm
Density	= 1.187 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 24.89 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 221.105193 Da
Nominal Mass	= 221 Da
Average Mass	= 221.25672 Da

(2*R*)-2-hydroxy-3-[(3-vinylbenzyl)oxy]propanamide

Figure 03: Structure of the ligand molecule with their physiochemical properties.



Molecular formula: C12 H15 N O3
Molecular weight: 221.11
Number of HBA: 3
Number of HBD: 3
MolLogP : 0.17
MolLogS : -2.73 (in Log(moles/L)) 412.10 (in mg/L)
MolPSA : 59.65 A^2
MolVol : 228.76 A^3
Number of stereo centers: 1

Figure 04: Diagram showing Molecular properties of the ligand molecule.

The data given in **Figure 5** shows drug likeness score -1.06 which falls in the suitable range of -12 to 8 which shows the overall potential of the selected ligand molecule to qualify for a drug.

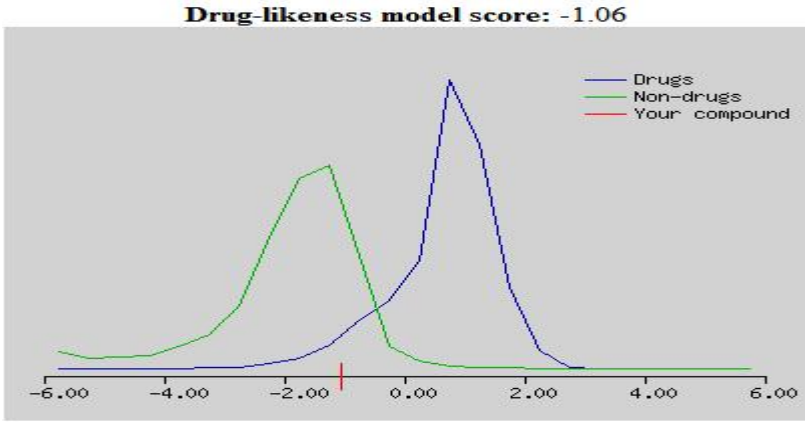


Figure 05: Diagram showing drug Likeness Score of the Ligand molecule

To understand the interaction between target protein and ligand molecule **(Figure 6)**, flexible docking was performed using QUANTUM 3.3. The ligand molecule was attached at the suitable active site of the protein molecule showing -20.19 Kj Gibbs free energy which is another major criterion for the selection of a drug molecule as negative and low docking energy indicate strong favourable bonds indicating ligand molecule in its most favourable conformation.

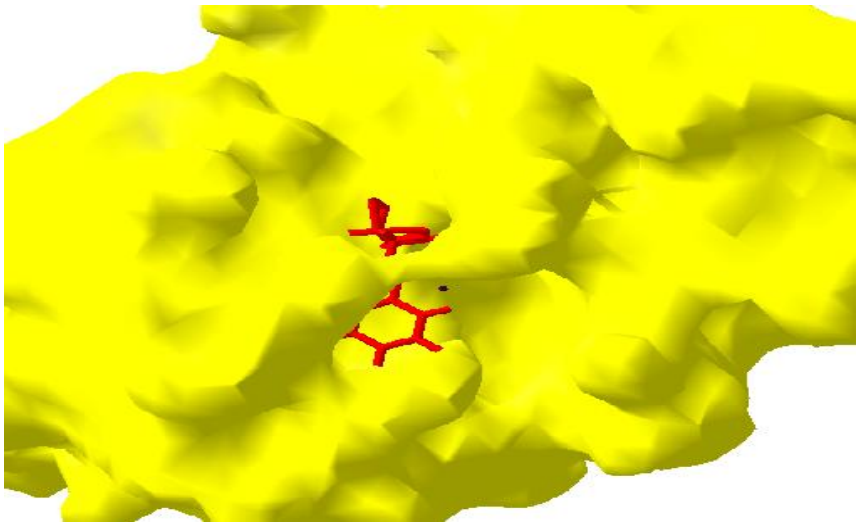


Figure 06: View of molecular surface of sPLA2 protein docked with Ligand molecule (red).

Conclusion

sPLA2 protein is supposed to be most potent drug target for asthma disease. In this work, an attempt has been made to design a three dimensional structure of sPLA2 protein on the basis of homology modeling using MODELLER 8V1 software. The most reliable and stable structure was selected by energy minimization and loop build formation using Ramachandran plot and PROCHECK program. The ligand molecule was designed by LIGBUILDER and assessed by Parachor value and ADMET properties of drug. Docking of this ligand molecule with target protein shows negative Gibbs free energy which is a favourable condition for solubility in aqueous carrier solution. As this drug molecule shows good drug likeness and drug score with no violation to the Lipinski's rule of five, therefore, it could be treated as potential drug for treating asthma disease.

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