Virtual Screening of Natural Metabolites as Candidate Drugs for Rheumatoid Arthritis

Joseph Christina Rosy, Ashok Bala M, Krishnan Sundar*

Abstract*: *Inflammation is a complex biological response to reject and heal any harmful stimuli created by pathogens, damaged cells or irritants. One of the more prevalent inflammatory disease found in 0.5-1.0% of the world's population is Rheumatoid arthritis (RA). RA is an autoimmune disease affecting the synovia. The actual reason for this disease is still unknown and is more complex to study. So, the drugs which are commercialized acts only to reduce the outcome of the disease, pain, by inhibiting the vital enzymes responsible for the synthesis of inflammatory mediators called prostaglandins. Cyclooxygenase- I and Cyclooxygenase- II are the commonly targeted enzymes by the current drugs in market. These drugs are reported to affect the normal physiological functions of various organs leading to side effects. PGE2 is the major prostaglandin involved in this disorder and found abundant in the affected synovia. mPGES- I is a membrane protein involved in the biosynthesis of PGE2 which has been reported as a novel drug target to treat RA. Though synthesized chemical compounds have higher anti-inflammatory activity; they are reported to possess a number of side effects. Thus a library of natural compounds are collected and screened virtually as mPGES-1 inhibitors using Autodock 4.2.

***Keywords*: inflammation, mPGES-1, prostaglandin rheumatoid arthritis**

I. INTRODUCTION

Inflammation, the first protective response of the body to any injury or infection, is a complex process involving many types of cells to initiate the healing process. Arthritis is a joint disorder mainly due to inflammation in one or more joints. Though there are many types arthritis that have been reported worldwide, osteoarthritis and rheumatoid arthritis are the most prevalent types. Osteoarthritis is due to the wear and tear of cartilage tissues, over usage of joints and is associated with aging.

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which the self-antigens are recognized as foreign leading to the destruction and inflammation of the synovial joints. Like other autoimmune diseases it arises from a variable combination of environmental factors, genetic susceptibility and inappropriate activation of immune responses but still the exact reason for rheumatoid arthritis is unknown. The synovial joints of RA patients were found to be infiltrated by monocytes/macrophages, T cells and B cells. These cells releases cytokines such as interleukins 1 and 17, and tumor necrosis factor α, that play an important role in the production of prostaglandins [1].

Prostaglandins are small pro-inflammatory lipid mediators derived from arachidonic acid and are involved in numerous biological functions. Cyclooxygenase (COX) plays a key role in prostaglandin synthesis by converting arachidonic acid to prostaglandin H2 and then to prostaglandins [2]. Cyclooxygenase exist in two forms: the constitutive, COX-1 and the induced, COX-2. The non-steroidal anti-inflammatory drugs (NSAID) used to reduce inflammation inhibit both COX-1 and COX-2 through which prostaglandins synthesis is arrested. Selective COX-2 inhibitors were later developed to overcome the side effects due to NSAID [3].

Prostaglandin E2 (PGE2), a key mediator of inflammatory response, is abundant in the synovial fluid of rheumatoid arthritis patients [4]. Prostaglandin E synthase (PGES) catalyzes the final step of PGE2 biosynthesis. Three isoforms of PGES, cytosolic PGES (cPGES), microsomal PGES-1 (mPGES-1) and mPGES-2 are characterized by researchers. mPGES-1 is a glutathione dependent enzyme which shows coordinated induction with COX-2 under inflammatory conditions. Although cPGES is also a glutathione dependent enzyme, it is expressed in most of the tissues under normal conditions [5] However, mPGES-2 is not dependent on glutathione for its activity and is coupled to both COX-1 and COX-2 for PGE2 production [2]. mPGES is constitutively expressed and its activity is not significantly increased during inflammation, similar to cPGES. Hence, glutathione dependent mPGES-1could be considered as a promising therapeutic target for inflammatory diseases [6].

Drug designing has become an important part in the field of medicinal chemistry in recent years and *in silico* approaches are being used to predict the binding of small molecules to known target structure. There is a wide range of software packages available to predict the binding through molecular docking. AutoDock 4.2 is one such software which uses Lamarckian Genetic Algorithm as a search function. This algorithm is a hybrid genetic algorithm that uses a parameterized free-energy scoring function to estimate the binding energy. In this study, using AutoDock 4.2, a number of natural metabolites of marine and plants are screened to predict the compound that shows higher

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affinity towards mPGES-1, thereby inhibiting the synthesis of induced PGE2.

II. MATERIALS AND METHODS

A. Retrieval of protein structure and ligands

The three dimensional structure of microsomal prostaglandin E synthase-1 (mPGES-1) was collected from Protein Data Bank (www.rcsb.org).

Forty four marine compounds were selected from literature and their structures were collected from databases. A total of 76 plant compounds of *Aloe vera*, *Withania somnifera*, *Morinda citrifolia* collected from Dr. Dukes Phytochemical and Ethnobotanical Database were also used for the screening.

B. Computational Details

3GB RAM and Intel Core 2 duo E7500 processor (2.93GHz). Molecular docking studies were performed with Autodock 4.2 running in Debian version 9 operating system. Result analysis was performed with Discovery Studio Visualizer client 4.0

C. Protein and Ligand Preparation

A three dimensional structure of the protein molecule was downloaded from the Protein Data Bank (PDB) and it was prepared for docking by removing water and other heteroatoms, adding hydrogen bonds and charges using AutoDock 4.2.

A desired number of small compounds were obtained from various databases and they have been prepared by finding the root atom, a rigid portion of a ligand to which all other atoms are connected by non-rotatable bonds using AutoDock 4.2

To the prepared protein molecule a grid box was formed which sets the location and extend of the three dimensional area to be searched during the docking experiment

D. Molecular Docking Studies

The protein and all the 120 ligands were prepared for docking using the graphical user interface of Autodock tools. The preparation involved adding all hydrogen atoms to the proteins, which is a step necessary for calculation of partial atomic charges. Water molecules and heteroatoms were removed from the protein molecule [7]. By using the graphical user interface of Autodock tools, a 3D grid box was generated to embed the protein and, Grid parameters were set. Grid maps were calculated by running Autogrid 4. Docking parameters were set by the docking wizard of Autodock Tools [8]. Conformation search was performed using Lamarckian Genetic Algorithm which runs for 100 cycles [9]. The binding energies for each conformation of the ligand with the proteins were determined by running Autodock 4 [10].

E. Analysis of Docking

Analysis of docking was performed by using the graphical user interface of AutoDock Tools and Discovery Studio Client 4.0. Binding energies of each conformation of docked compounds were noted and the best conformation was chosen based on the binding energy and number of hydrogen bonds that they form with the protein. Various types of interactions between ligand and receptor such as hydrogen bonds, hydrophobic interactions, Van der Waals and electrostatic interactions were visualized

III. RESULTS

A. Retrieval of protein structure and ligands

A three dimensional protein structure of mPGES-1 with 1.16Å resolution was downloaded from the Protein Data Bank (PDB ID: 4AL0) (Figure 1). The list of ligands taken for this study is listed in Table 2a-2d. Asn74, Glu77, His113, TYR117, Arg126, Ser127 are the active site amino acids interacting with the substrate glutathione (Fig1b)

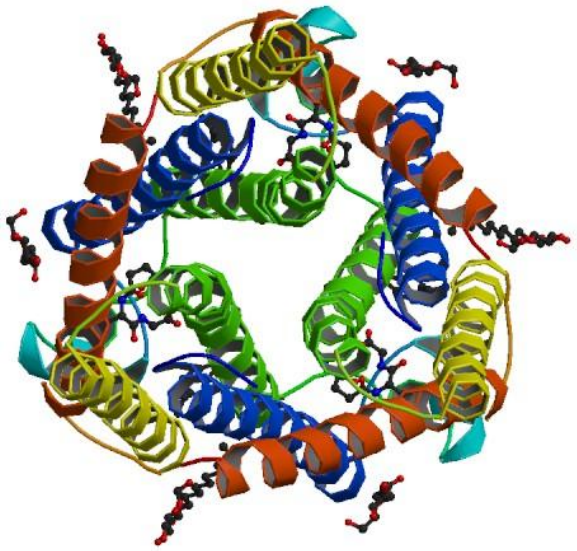

Figure 1a: Structure of mPGES-1.

B. Analysis of Docking

Initial studies were done with glutathione, the substrate for mPGES-1; which interacts with active site amino acids with a binding energy of -6.06 Kcal/mol. The interaction between glutathione and aloetic acid with the active site region of mPGES-1 is represented in the Figure 2.

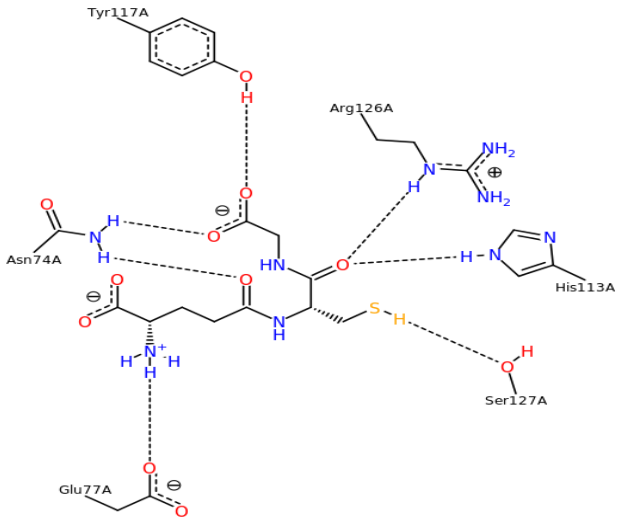

Figure 1b: Active site amino acids interacting with Glutathione

Then, 120 marine and plant metabolites were docked at the active site of mPGES-1 to find their interaction as mPGES-1 inhibitor. The compounds with a binding energy of < -6.06 kcal/mol were considered as strong binders and those with a binding energy of >-6.06 kcal/mol were considered as weak binders. Compounds exhibited positive values were concluded as non-binders. The number of strong, weak and non-binders from each source are represented in the Figure 3.

Table2a: List of marine compounds docked with mPGES-1

Isogeoditin A	Actinomadura Xanthone
Cytonyc Acid B	Isoaptamine
Certonardosterol Q6	Amphidinolide X
Steongylophorin 26	Aplysinopsin
Certonardosterol N1	Caulibugulone F
Laurenditerpenol	Isogranulatimide
Neoamphimedine	Isogeoditin B
Certonardosterol D2	Cribrostatin 6
Certonardosterol C2	Sarcodictyin A
Dictyostatin 1	Peloruside
Certonardosterol A2	Plakinamine K
L- Cadinene	Plakortide N
Iricinastatin A	Cytonyc Acid A
Certonardosterol D	Renieramycin J
Certonardosterol E3	Jaspine B
Ophiobolin A	Dolastatin 15
Certonardosterol E2	Mycalazal 8
ertonardosterol D3	Neohalichondramide
Plakorstatin 1	Phakellistatin 11
Smenospongorine	Microcinonamide A
Lamellarin D	Microcinonamide B
Andavadonic Acid	Tasiamide B

Table 2b: List of compounds from *W. somnifera* docked with mPGES-1

Withanolide L	Quercetin
Withanone	Scopoletin
Withanolide D	Campesterol
Sominone	2,3 Dehydrosomnifericin
Withanolide F	Isopelletierine
Daucosterol	Hydroxyproline
Quresimine A	Pseudotropine
Quinic Acid	Tropanol
Quresimine B	Bellaradine
2,3 Dihydrowithaferin A	Hentriacontane

Table 2c: List of compounds from *A. vera* docked with mPGES-1

Aloetic Acid	Arabinose
Rhein	Aloenin
Aloesin	Stearic Acid
Aloesone	

Table2d: List of compounds from *M. citrifolia* docked with mPGES-1

Rubian	Ethyldecamoate
Asperulosidic acid	2 methyl propanoiic acid
Lucidin	2 methyl butanoic acid
Rubichloric acid	Myristic acid
Deacetyl asperulosidic acid	Butyric acid
Ursolic acid	Methyloleate
Adipic acid	8,11,14, eicosatrienoic acid
Phycion	Eugenol
Alizarin	Eliaidic acid
Biochanin A	z-6 dodeceno gamma lactone
Scopoletin 1	Methyloctanoate
beta sitosterol	Ethylpalmitate
Gentisic acid	Benzyl alcohol
Nordamnacanthal	Ethylcaprylate
Damnacanthal	Methyldecannoate
Blumenol A	Alkamid
Niacin	Linoleic acid
Nonanoic acid	acetoin
2 methyl hexanoate	2 heptanone
heptanoic acid	Methylpalmitate
Octanoic acid	1- butanol
Undecylic acid	Hexanol
Benzonic acid	Methylelaidate
Hexanoic acid	beta caroene
2 ethylhexanoate	

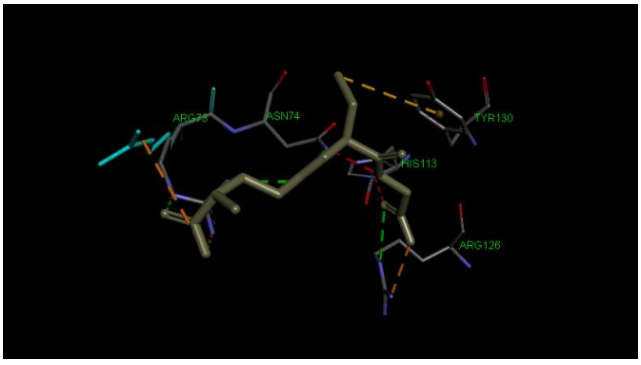

Figure 2: Interaction of mPGES with its natural substrate, glutathione

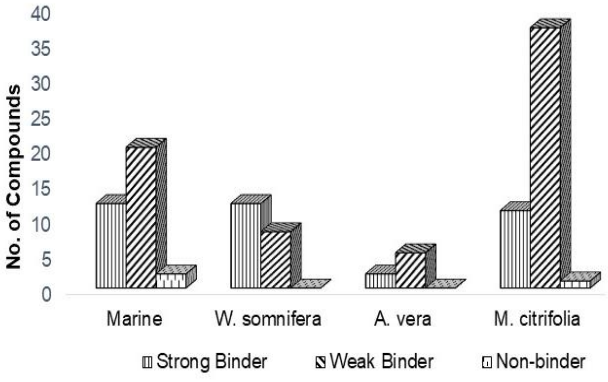

Figure 3: Analysis of binding of selected compounds with the drug target; mPGES-1

Aloetic acid, Rhein, Withanolide L, Rubian, and Withanone are the top 5 binders of mPGES-1; their source and binding energies are listed in the Table 3. The interaction of these top 5 binders with the amino acids of mPGES-1 is given in the Figures 4 and 5 and the amino acids of mPGES-1 that are involved in the interaction with the top five binders are provided in the Table 4.

Table 3: Top Five Binders of mPGES-1

LIGAND NAME	SOURCE	BINDING ENERGY (Kcal/mol)
Aloetic acid	*Aloe vera*	-8.76
Rhein	*Aloe vera*	-8.15
Withanolide L	*Withania somifera*	-8.02
Rubian	*Morinda citrifolia*	-7.79
Withanone	*Withania somifera*	-7.75

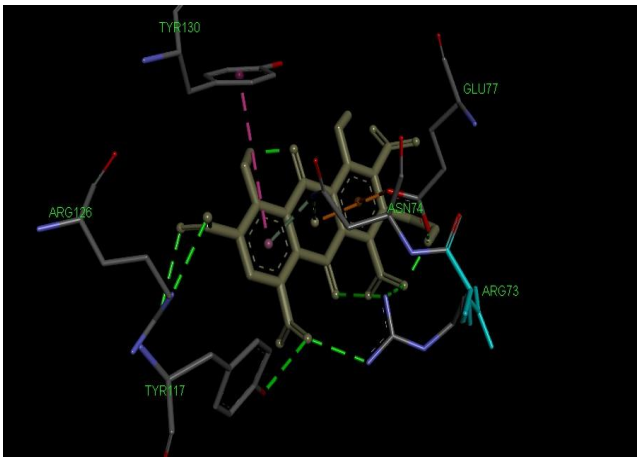

Figure 4: Interaction of aloetic acid with mPGES-1

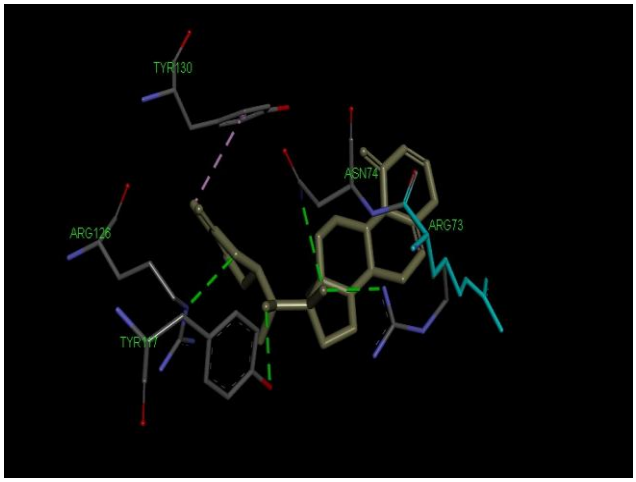

Figure 5: Interaction of withanolide L with mPGES-1

Table 4: Amino acids of mPGES-1 involved in interaction with top five binders

Glutathione (substrate)	Aloetic acid	Rhein	Withanolide L	Rubian	Withanone
Asn74	Arg73	Arg73	Arg73	Arg73	Arg73
Glu77	Asn74	Asn74	Asn74	Asn74	Glu77
His113	Glu77	His 113	Try117	Glu77	His 113
Tyr 117	Try117	Ser 127	Arg126	Pro 81	Arg126
Arg126	Arg126	Arg126	Try130	Arg126	Try130
Ser127	Try130	Thr131			

IV. DISCUSSION

In recent days natural products are extensively used in the development of drugs due to their availability and biological activity [11]. In the current study, 120 natural compounds of marine and plant sources were docked to find their interaction with the active site region of mPGES-1 so that they can be further tested as a lead molecule for the inhibition of mPGES-1 activity.

Top five compounds that exhibit higher binding affinity toward the mPGES-1 are listed with their binding energies in the Table 3. All these five compounds have their origin from plants indicating that plant compounds have greater ability to bind with mPGES-1 than the marine compounds. Although many marine compounds have been studied in the past few decades for their potent biological activity [12], they exhibited a weak binding ability toward mPGES-1 than the plant compounds.

The top two binders aloetic acid and rhein are from *A. vera*, with a binding energy of -8.76 and -8.15 Kcal/mol respectively which is considerably lower than glutathione. Also they interact with 4 out of 6 active site amino acids. Biological activity of aloetic acid has not been reported yet, so this compound can be studied further for expressing its anti-inflammatory effect. Antiproliferation studies on anthraquinone derivatives indicated that rhein has the ability to inhibit expression of certain genes responsible for breast cancer [13].

Withanolide L and withanone are the other two top binders of mPGES-1 obtained from *W. somnifera*, exhibiting a binding energy of -8.02 and -7.75 Kcal/mol respectively. Withanone is a compound derived from leaves of *W. somnifera*. Incidentally, this compound has already been suggested as a potential candidate molecule for cancer through docking studies [14]. Rubian is obtained from the bark of *M. citrifolia*, a plant reported to have a broad range of therapeutic effects [15]. Rubian exhibited a binding energy of -7.79 Kcal/mol.

The results obtained from the present study clearly indicate that the natural metabolites show a higher potential in the development of new drugs. Aloetic acid, rhein, withanolide L, rubian and withanolide D are the top five binders obtained as a result displays a better binding with the mPGES-1 than its original substrate, glutathione, leaving a hint that they can be used as a possible drug for the inhibition of mPGES-1. Since these five compounds

have their origin from plants, they can be easily synthesized. Natural compounds are already been reported to have high biological activity so, these compounds can be further utilized for *in vivo* studies and can be tested as a potential drug compound for rheumatoid arthritis and other inflammatory disorders.

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