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Screening of Natural Compounds as Matrix Metalloproteinase and Aldose Reductase Inhibitors: Drug Design for Diabetic Retinopathy

[1]M.Syed Ali Fathima, [2]R.V.Ramalakshmi, [3]A.Santhanalakshmi, [4]Sonagunalan

Abstract: *Diabetic retinopathy (DR) is a micro-vascular complication of diabetes and one of the leading causes of blindness. Two of the possible candidate PROTEINS contributing to the development of diabetic retinopathy are aldose reductase (AR) and Matrix metalloproteinase-2 (MMP2). In the current study plant derived medicinal compounds and chemical compounds are studied by Docking analysis that are carried out using Maestro (10.2) (Schrodinger suite).The screened compounds were found to possess good binding affinity with these proteins and hence are considered diabetic retinopathy inhibitors. In this study, Calebin, Isolated from curcuminoid Plant of turmeric (*Curcuma longa*) was found to have high Binding Affinity and was proved to be the naturally available novel inhibitor of Aldose reductase. Acarbose was found to have high Binding Affinity to the Matrix metalloproteinase-2 drug target.From this study it is concluded that these natural compounds were found to have good binding affinity with these target proteins and considered to be effective drug targets for treatment of diabetic retinopathy (DR).*

Keywords: *Diabetic retinopathy, Aldose reductase, Matrix metalloproteinase, Schrodinger, Calebin, Acarbose.*

I. Introduction

Diabetic retinopathy (DR) is one of the most common micro-vascular complications of diabetes and one of the leading causes of blindness worldwide. The prevalence of DR increases with the duration of diabetes, and nearly all patients with type I diabetes and more than 60% with type II diabetes have some degree of retinopathy after 20 years. Chronic hyperglycemia is believed to be the primary pathogenic factor for inducing damage to retinal cells. However, the mechanisms that lead to DR are not fully understood. DR is characterized by micro aneurysms, inter-retinal edema, haemorrhages, exudates (hard exudates) and intraocular pathological neovascularization. Laser photocoagulation therapy is the most common treatment modality for DR. However, this therapy may damage neural tissue resulting in the deterioration of vision. Therefore, development of new therapeutic strategies for the treatment of excessive retinal vasopermeability and angiogenic changes are the basis for further research focus. [1]

The possible candidate genes contributing to the development of diabetic retinopathy are genes for Aldose reductase (AR), nitric oxide synthase (NOS), receptor for advanced glycation end products (RAGE), angiotensin converting enzyme (ACE), human leucocyte antigen (HLA) and vascular endothelial growth factors (VEGF). The other names of Aldose reductase gene are aldo-keto reductase family 1, member b1; akr1b1. The alternative titles or symbols for the gene are Aldose reductase; ar, aldehyde reductase 1; aldr1 and its gene map locus is 7q35. Human ALR2 gene, the gene encoding Aldose reductase has been localized to chromosome 7q35 and consists of 10 exons extending over 18 kb of DNA. [3] There is growing evidence to implicate ALR2 in the pathogenesis of diabetic micro vascular disease. Aldose reductase (AR; EC 1.1.1.21) is also present in the lens, retina, Schwann cells of peripheral nerves, placenta and red blood cells. The abnormal expression and activity of this enzyme seems to play an important role in diabetic complications. [3]

In the pathogenesis of diabetic retinopathy, retinal mitochondria become dysfunctional, their DNA is damaged, and capillary cells undergo accelerated apoptosis. Matrix metalloproteinase- 2 (MMP2) (gelatinase A) becomes activated and pro-apoptotic, and the therapies that inhibit the development of diabetic retinopathy alleviate MMP2 activation. The authors sought to elucidate the possible mechanism by which activated MMP2 contributes to mitochondrial dysfunction. [4] Primary function is degradation of proteins in the extracellular Matrix. It proteolytically digests gelatin (denatured collagen), and type IV, V, VII, and IX, X collagen. Physiologically, MMP-2 in coordination with other MMPs, play a role in normal tissue remodeling events such as embryonic development, angiogenesis, ovulation, mammary gland involution and wound healing. MMP2 is also involved in osteoblastic bone formation and/or inhibits osteoclastic bone resorption.

Molecular docking is a key to structural molecular biology and computer assisted drug Design. Finding chemical structures with feasible physiological activities is an area driven by medical and pharmaceutical research through drug discovery. Molecular docking tries to predict the structure of the intermolecular complex formed between two or more constituent molecules. The goal of ligand protein docking is to predict the predominant binding model (s) of a ligand with a protein of known three- dimensional structure. The main idea

is to dock a database of potential metabolites into a protein's binding site and then rank them based of their calculated binding affinities. Due to approximation of the stimulations, the calculated binding affinity, by itself is used to identify a protein cognate ligand amongst a crowd of candidates, and this approach can still be useful to experimentalists by narrowing down the number of molecules that need to be tested in vitro conditions. [5]

The present study was based on screening of compounds using plant derived medicinal compounds and chemical compounds. Docking analysis was carried out using Maestro (10.2) (Schrodinger suit) between these screened compounds and envelope protein targets viz. MMP2 and ALR. The screened compounds are diabetic retinopathy inhibitors which found to possess good binding affinity with these proteins and considered to be effective drug targets for treatment of diabetic retinopathy.

II. Materials And Methods

Proteins:

The Protein Data Bank (PDB) (www.rcsb.org) is a worldwide repository for processing and distribution of 3D biological macromolecular structure data. The crystal structures of the two proteins were downloaded from Protein Data Bank.

Protein name	**PDB entry**
a) Matrix metalloproteinase (MMP2)	1CK7
b) Aldose reductase protein(ALR2)	1ADS

Ligand:

The natural compounds are collected from plant sources. The plant derived natural compounds are collected from activity of plant compounds against Diabetic retinopathy disease. Compounds are considered as natural ligands, naturally available bio drugs and synthetic compounds were also considered.

Molecular docking:

Molecular docking analysis was performed using Maestro, version 10.4, Schrodinger suite. The following are the steps required for docking process.

Preparation of proteins:

The MMP2 protein under classification of Hydrolase and another protein Aldose reductase protein also under classification is oxidoreductase respectively. The natural ligands are present in both the proteins of MMP2 and ALR2 protein.

Using the protein preparation wizard of Maestro provided by Schrodinger, the MMP2 and ALR proteins prepared and subjected for docking studies. Both the proteins are consists of water molecules, co factor and metal ions. Docking process requires bond orders, ionization states etc. to be properly assigned. In order to obtain assigned protein process was carried out in each protein respectively. The water and hetero atoms present the structure were deleted. Optimization is carried out to optimize the protein hydrogen bond which greatly decrease the protein preprocess preparation of time. Minimization was done to allow hydrogen bonds to be freely minimized and it helps sufficient, heavy atoms movement to relax strained bonds and angel.

Preparation of ligand:

Totally two hundred compounds were sketched using Maestro and ligand preparation was done with ligprep, Implicit atoms were added to the compounds and the geometrics were optimized by macromodel. Epic program (version 2.1), was used for generation of ionization states of the compounds in the pH range of 7±2. After ligprep finished, ionization of compounds were generated.

Evaluating for ADME: Qikprop

Qikprop is a quick, accurate, easy to use absorption, description, metabolism and excretion (ADME). It is a prediction program present in Schrodinger suit. In silico ADME properties and structural descriptors were predicted using the program Qikprop. (version 4.4)

Molecular docking:

Schrodinger implements docking studies using Glide. Glide runs the docking in two steps,

1. Receptor grid generation
2. Ligand docking

Receptor grid generation:

Binding site analysis was carried out with the sitemap program and the receptor grids were generated for corresponding binding sites of the proteins. Both Matrix metalloproteinase protein and Aldose reductase proteins are done to form the receptor grid. The grids are defined with the dimensions of 10Å in all the X, Y, and Z axis, selecting the centroid of the binding site as the centre of the grid box. Other parameters such as Vander Waals radii scaling factor and partial charge cutoff were set as default, 1.0 and 0.25 respectively for grid generation.

Ligand docking:

Ligand docking was performed using XP mode (Extra precision) of Glide program. The docking results were viewed using glide XP visualize. Using XP visualize hydrogen, hydrophobic, electrostatic interactions can be visualized.

Analysis and visualization of docking results:

After docking, top ranked compounds were arranged based on the Glide Score. Lower Glide Score represents more favorable binding. Hydrogen bond interactions and hydrophobic interactions of the best poses were visualized and interpreted using XP visualizer.

III. Result And Discussion:

Proteins:

The crystal structure of Matrix metalloproteinase 2 and Aldose reductase proteins were downloaded from the protein data bank.

Molecular Docking:

Molecular docking analysis was performed using Maestro, version 10.2, Schrodinger suit. The steps involved in docking process are as follows.

Preparation of proteins:

To obtain the assigned crystal structure of Matrix metalloproteinase and Aldose reductase, the processing of these protein preparations was carried out using the protein preparation wizard of Maestro (version 10.2) provided by Schrodinger. The Aldose reductase protein (1ADS) consist chains and residue NAP 350. Matrix metelloprotinase 2(1CK7) have four chains, from which chain A was chosen with the residue name Zn (990), Zn (991), and this protein possesses water residues. All water residues were deleted because it has influence on the binding affinity to the ligands. The proper bond order of the protein was assigned; all hetero atoms and other unwanted chemical moieties were deleted during protein preparation. Optimizations of the protein hydrogen bond was carried out which greatly decreases the protein preprocess preparation time. Minimization of hydrogen bonds was done to facilitate sufficient heavy atoms movement to relax strained bonds and angle.

Preparation of ligands

The ligands such as natural and synthetic compounds for Aldose reductase protein (1ADS) and Matrix metalloproteinase 2 (1CK7) proteins were prepared using Ligprep (version 3.4). After ligands preparation implicit hydrogen atoms were added to the ligands and the Geometries were optimized. Number of ionization states was generated for 200 ligands by Epik program (version 5.6).

Qikprop

The absorption, distribution, metabolism and excretion (ADME) properties of all natural and synthetic compound for Matrix metalloproteinase 2(1CK7) and Aldose reductase (1ADS) proteins are predicted using Qikprop (version 4.4). Almost all the compounds for Matrix metalloproteinase 2 (1CK7) and Aldose reductase (1ADS) proteins were predicted to have very good ADME properties.

Receptor grid generation:

Docking studies are performed using Glide, version (6.7). Both Matrix metalloproteinase 2 (1CK7) and Aldose reductase proteins have natural ligands so protein site mapping (version 3.5) is not done to form the receptor grid. These proteins have natural ligands and hence site mapping is not done to form the receptor grid. These receptor grids act as the site for these respective proteins.

Ligand Docking

To confirm the validity of the results, docking was performed with two structures of AR and MMP2 (PDB IDs: 1ADS and 1CK7). All ligand conformers were flexibly docked into the selected AR X-ray crystal structures. The highest scoring compounds were subjected to XP docking. After XP analysis, the best interacting compounds were ranked based on Glide Score and the best pose of the ligand was chosen. Out of the 200 compounds selected initially, Calebin, Hesperidin, Curcumin and Acarbose, Demethoxy curcumin, Gingerenone A, Showed high glide scores (**Table 1**) for AR and MMP2 respectively. The chemical structures of these lead compounds are shown in (**fig.1**)

S. No.	Targets	PDBID	Ligands	XPGlideScore(Kcal/mol)
1.	AR	1ADS	Calenin	-12.94
2.	AR	1ADS	Hesperidin	-12.3
3.	AR	1ADS	Curcumin	-12.23
4.	MMP2	1CK7	Acarbose	-10.06
5.	MMP2	1CK7	Demethoxy curcumin	-9.44
6.	MMP2	1CK7	Gingerenone A	-9.25

Table 1: Glide Score from XP docking of various phyto compounds and drugs on AR and MMP2 targets

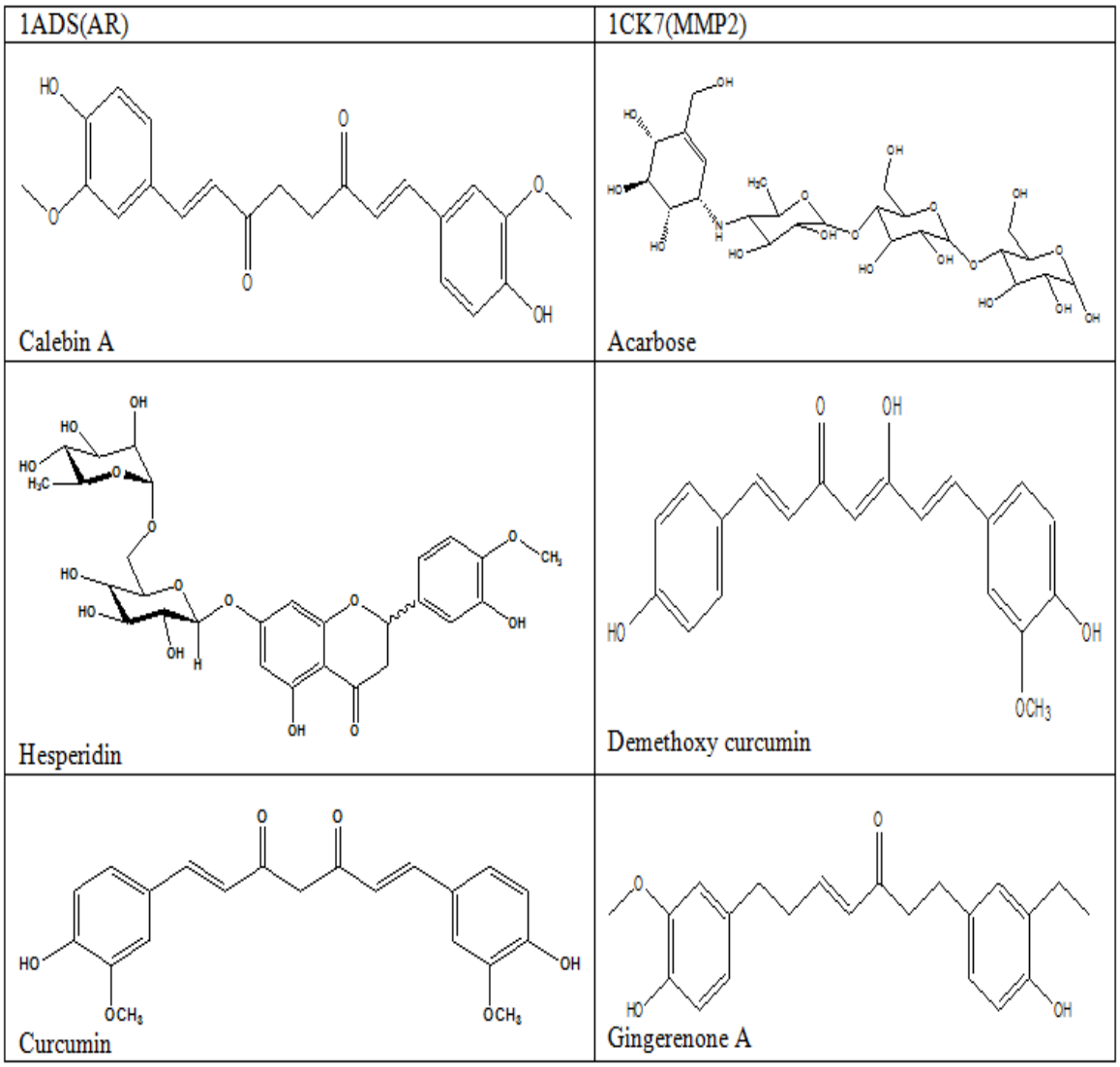
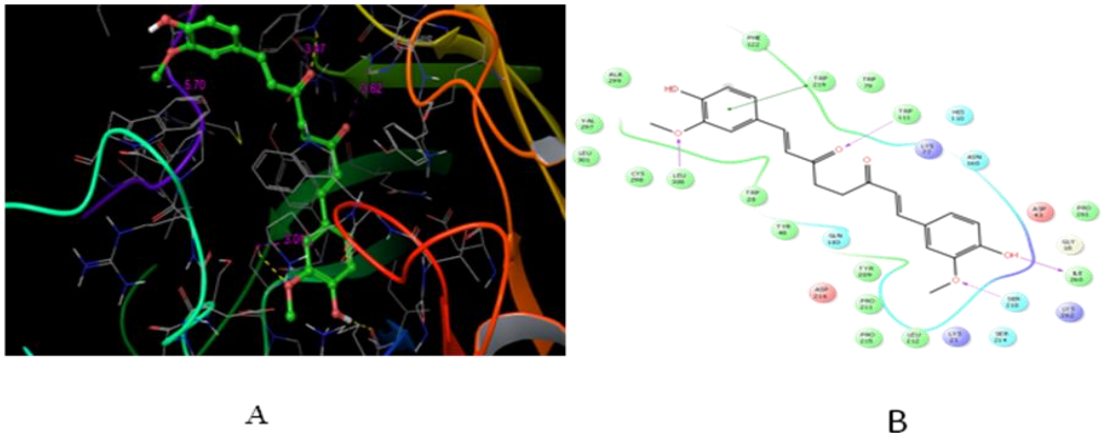


Figure 1: Chemical structures of lead molecules

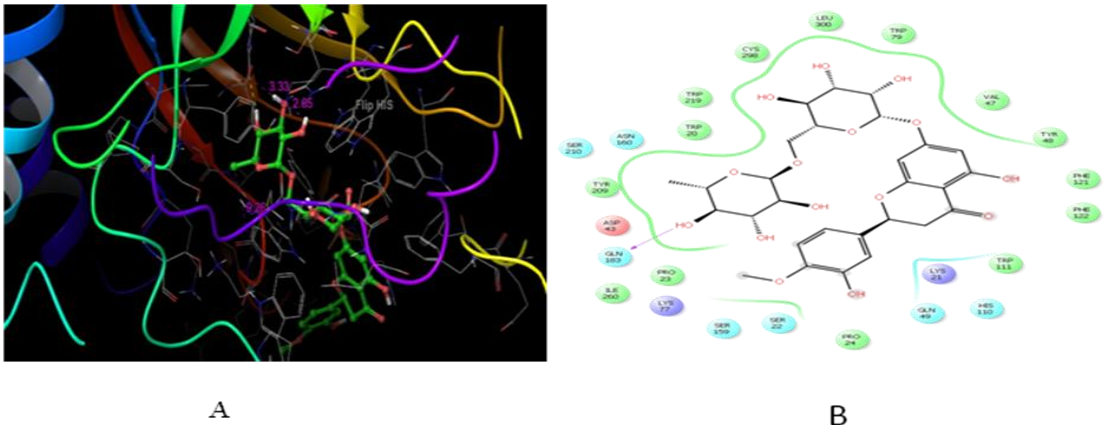
The docking process was made between natural compounds and Aldose reductase (1ADS) protein. Calebin-A is a novel curcuminoid isolated from turmeric (*Curcuma longa*) is common species belonging to the ginger family (Zingiberaceae) plant natural derivatives has become the first hit with a glide score of -12.94. Calebin-A was found to be novel inhibitor for Aldose reductase (1ADS) protein.



A B

Figure 2: (A). Hydrogen Interactions diagram between Active site residues of Aldose reductase(1ADS) protein and calebin. (B).Ligand interaction diagram of lead compound calebin A with AR (PDB ID: 1ADS)

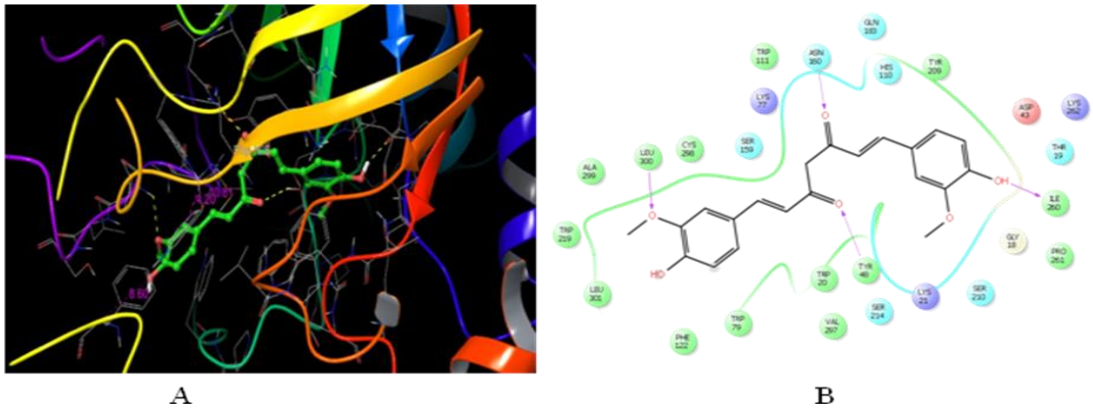
Hesperidin is a flavonoide compound abundant in citrus fruits and isolated from the ordinary orange *Citrus aurantium* L. and the family *Rutaceae*. A plant natural derivative has become the Second hit with a glide score of -12.33. Hesperidin was found to be good inhibitor against Aldose reductase (1ADS) protein.



A B

Figure 3: (A) Hydrogen Interactions diagram between Active site residues of Aldose reductase (1ADS) protein and Hesperidin (B) Ligand interaction diagram of lead compound Hesperidin with AR (PDB ID: 1ADS).

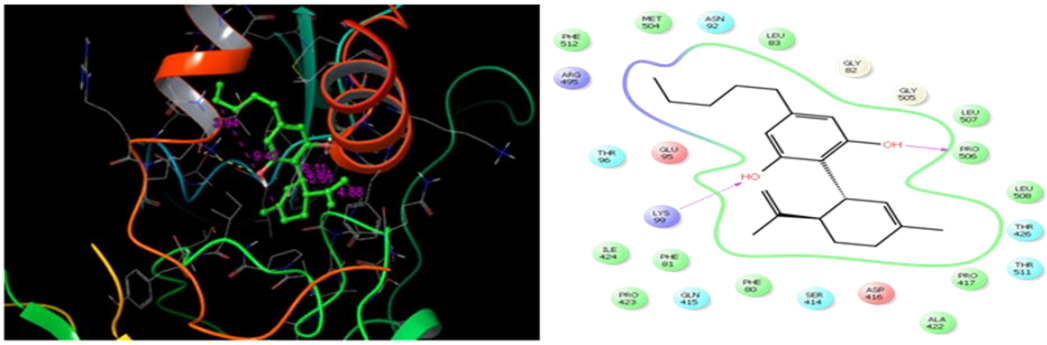
Curcumin is a Flavanoids compound which was isolated from Turmeric *(Curcuma longa)* is a common species belonging to the ginger family (zingiberaceae) plant natural derivatives has become the third hit with a glide score of -12.23. Curcumin against to Aldose reductase (1ADS) protein, Interactions were observed between the protein and ligands.



A B

Figure 4: (A) Hydrogen Interactions diagram of AR (PDB ID: 1ADS) protein with curcumin Interaction of active site residues, (B) Ligand interaction diagram of lead compound curcumin with Aldose reductase protein.

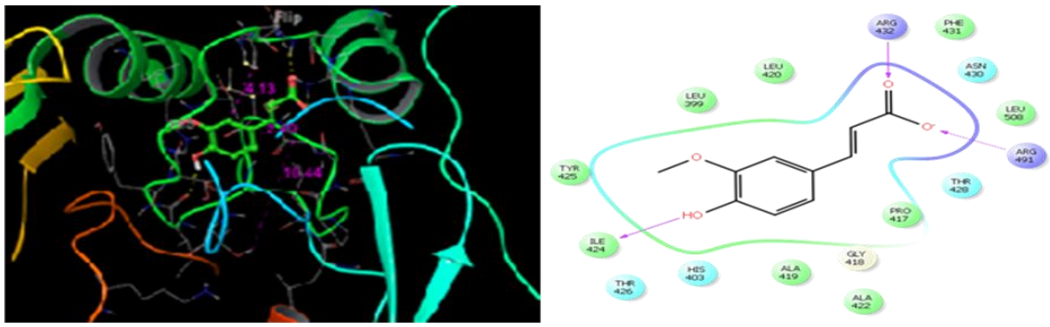
Then the docking process was made between natural compounds with Matrix metalloproteinase -2 (1CK7) proteins. Acarbose is a starch blocker, and inhibits alpha glucosidase, an intestinal enzyme that releases glucose from larger carbohydrates. It is composed of an acarviosin moiety with a maltose at the reducing terminus. It has become the first hit with a glide score of -10.06.



A B

Figure 5: (A) Hydrogen Interactions diagram of acarbose with MMP-2(1CK7) protein Interaction of active site residues, (B) Ligand interaction diagram of lead compound acarbose with MMP-2(1CK7) protein

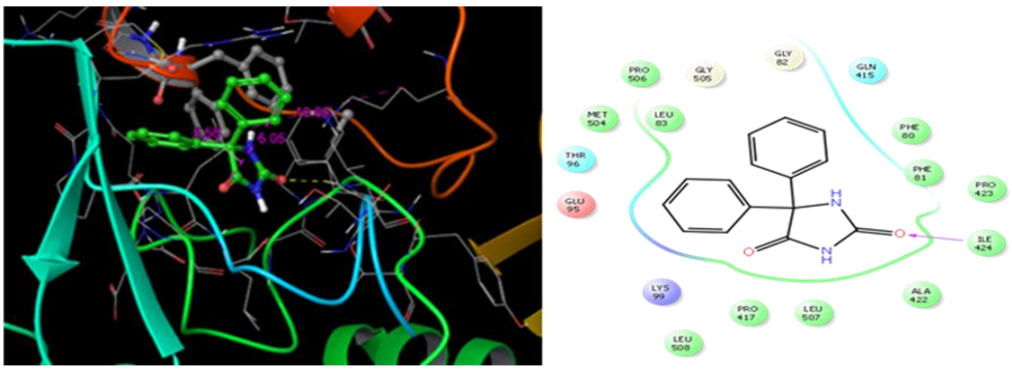
Demethoxy curcumin is a Flavanoids compound which was isolated from Turmeric *(Curcuma longa)* is a common species belonging to the ginger family (zingiberaceae) plant natural derivatives has become the second hit with a glide score of -9.44. Demethoxycurcumin against to Matrix metalloprotenase -2 (1CK7) proteins, Interactions were observed between the protein and ligands.



A B

Figure 6: (A) Hydrogen Interactions of Demethoxy curcumin with MMP-2(1CK7) protein (B) Ligand interaction diagram of lead compound of Demethoxy curcumin with MMP-2(1CK7) protein.

Gingerenone A is a diarylheptanoids seen in the rhizome of plant *Zingiber officinalae.* Is a common species belonging to the ginger family (zingiberaceae) plant natural derivatives has become the third hit with a glide score of -9.25. Gingerenone A against to Matrix metalloproteinase-2 (1CK7) protein



A B

Figure 7: (A) Hydrogen Interactions diagram of gingerenone A with MMP-2(PDB ID: 1CK7). (B) Ligand interaction diagram of gingerenone A with MMP-2

The results of screening exhibits natural compounds have best inhibitory effect on Aldose reductase (1ADS) with their gliding scores. For Structural, among 200 compounds screened the first hits were calebin A and Acarbose derivatives has proved to be and best hits for inhibiting the antigenic activity of Aldose reductase(1ADS) and MMP2(1CK7) proteins. The other natural compounds hits such as Gingerenone B, gingerenone C, Laricresinol, Demethioxy curcumin, chlorogenic acid, Quercetin, canabidiol, anisodamine, vanillic acid, syringic acid, cinnamaldehyde, cinnamic acid, cinnmyl alcohol, Beta-ocimene, cis-betaocimene, berberine, sorbinil, ferullic acid, camphor and chemical compounds hits such as Phlorizin, Glimperide, Vidagliptin, Zonisamide, con=(E)-cniferyl, Tolerestat, phenytoin, Gabapentin have good binding affinity to both AR(1ADS) and MMP-2(1CK7) proteins. Screening process of these compounds was done by using Maestro (Version 10.2), a Schrodinger suite. Plant based Natural compounds bind to the active sites of MMP-2(1CK7) and AR (1ADS) Proteins exhibits, higher binding affinity with the drug target.

IV. Conclusion

Matrix metalloproteinase-2 (MMP-2) and Aldose reductase are involved in the diabetic retinopathy infection and can be considered as the best drug targets of diabetic retinopathy disease. A docking model for these proteins have been developed with Glide 6.7, an automated docking program, successfully reproduced the binding mode of crystal structures of Matrix metalloproteinase-2(MMP-2) and Aldose reductase inhibitors. Various medicinal plant and animal natural compounds and synthetic compounds have been screened in order to find out the best leads. From this study it was concluded that, Calebin has High Binding Affinity which was Isolated from curcuminoid Plant of turmeric (*Curcuma longa*) was proved to be the naturally available novel inhibitors for Aldose reductase. Acarbose has high Binding Affinity for Matrix metalloproteinase-2 protein and was considered as the best leads for Matrix metallo protein drug targets.

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