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Computational Investigation of Pkcβ Inhibitors for the Treatment of Diabetic Retinopathy

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Abstract:
Diabetic Retinopathy (DR) is one of the attenuating complications of diabetes mellitus. The key gene responsible for causing diabetic retinopathy is protein kinase C beta (PKCβ). Protein kinase C is a family of protein kinase enzymes which are involved in controlling the function of other proteins through phosphorylation mechanism and plays a crucial role in signal transduction mechanisms. Among all the PKC isoenzymes, PKCβ could be a significant isoenzyme involved in vascular dysfunction during hyperglycemia. Studies show that oral administration of PKCβ inhibitor Ruboxistaurin (LY333531), decreases vessel permeability and improves retinal condition. Thus compounds that decrease the PKCβ activation would be helpful in the treatment of diabetic retinopathy. The compounds similar to Ruboxistaurin are taken from Super Target database and docking analysis was performed. Maleimide derivative 3 showed highest binding affinities compared to Ruboxistaurin and so we advise that compound may be utilized in the treatment of diabetic retinopathy.

Keywords: PKCβ, Ruboxistaurin, Diacylglycerol (DAG), Diabetic retinopathy (DR)

Background:
Diabetes mellitus is a metabolic disorder in which blood glucose levels are increased due to insulin deficiency or insulin resistance. Type 1 diabetes results from body's failure to produce insulin which leads to insulin deficiency. In type 2 diabetes, cells in the body do not react to insulin (insulin resistance). One of the complications of diabetes is diabetic retinopathy which causes injury to tissue layer and eventually leads to vision loss.DR is clinically characterized by micro vascular dysfunction, with retinal vessels basement membrane thickening, loss of pericytes and endothelial cells, blood retinal barrier breakdown, capillary non perfusion, cotton wool spots formation and neo vascularisation **[1].** Based on the methods like clustalw and phylogenetic tree construction several proteins involved in the pathogenesis of diabetic retinopathy are identified **[2, 3, 4]** and shows that BDNF **[5],** aldose reductase, nitric oxide synthase has role in diabetes and its complications **[3].** On scrutinizing we tend to take PKCβ and its role in diabetic retinopathy. In hypoglycemic conditions, DAG-PKC pathway plays a major role by which increase within the levels of DAG leads to PKC activation. DAG is derived from the hydrolysis of phosphatidylinositol 4-5 bisphosphate, by a membrane bound enzyme phospholipase C - (PLC) **[6].** DAG–PKC pathway can also be activated by hyperglycemia induced oxidants such as H2O2 which are known to activate PKC either directly or by increasing DAG production **[7, 8].** In early stages increase in DAG may activate PKC and in advanced stages, when VEGF levels are elevated PKC plays an important role in modulation of VEGF action and as a stimulator of VEGF expression. PKC activation induced by hyperglycemia could alter the expression of various growth factors like VEGF that induces retinal vessel permeability. PKCβ activation affects VEGF expression through the mRNA-stabilizing human embryonic lethal abnormal vision (ELAV) protein, HuR , in the retina **[9].** Previous work additionally explains that in order to test the linearity of the multiple sequences at a time for a

number of proteins general regression model technique algorithm (GRMT1) can be used and high accuracy sequence clustering can also be done by using a clustering algorithm **[10].** Inhibition of PKCβ leads to prevention of glucose induced increase in VEGF expression **[11, 12] (Figure 1).** Hence oral administration of PKCβ inhibitor LY333531 will forestall or reverse blood retinal barrier breakdown by inhibiting VEGF expression. Ruboxistaurin or LY333531 could be a competitive inhibitor that acts by interacting with the ATP binding site **[13, 14]** which shows selectivity towards PKC and hence found to be an important therapeutic agent for diabetic retinopathy.

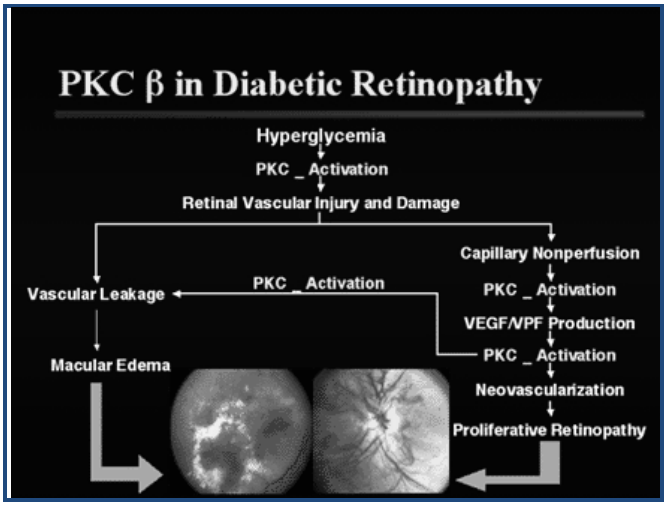

Figure 1: Role of PKCβ in Diabetic Retinopathy.

Methodology:

Preparation of proteins and ligands

The three dimensional structure of Protein kinase C beta receptor of homosapiens was retrieved from the Protein Databank (PDB: Id 1A25). The list of compounds similar to potent PKCβ inhibitor Ruboxistaurin were obtained from Super Target Database. The 3D SDF format of ligand molecules were obtained from NCBI Pubchem.

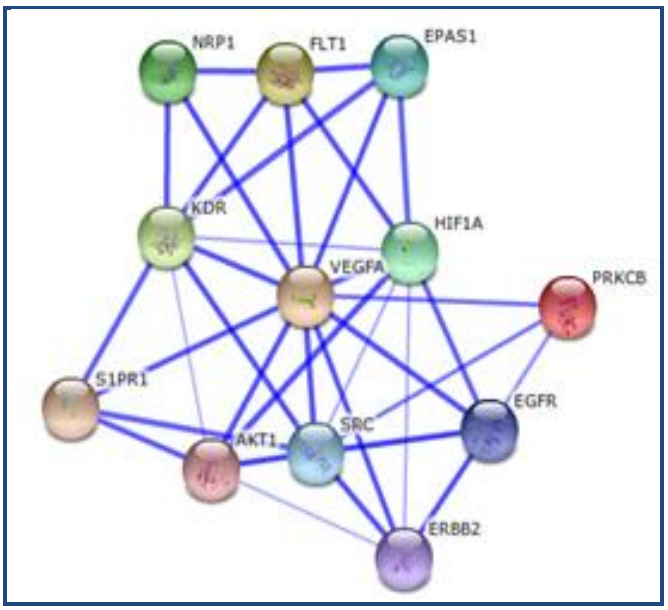

Figure 2: Protein interaction network obtained from string database

Ligand molecules

Based on the Lipinski five rules, the ligand molecules which are similar to Ruboxistaurin were taken from Super Target Database. The ligand molecule which obeys Lipinski rule were taken and used for docking studies is 20.

Energy minimization

Energy minimization studies were carried for both protein and ligand molecules by using SYBYL software by applying tripos force field, gasteiger- huckel charges were calculated.

Protein networking prediction

The protein interaction network for PKCβ and VEGF was obtained from String 9.05 database **[15] (Figure 2**).

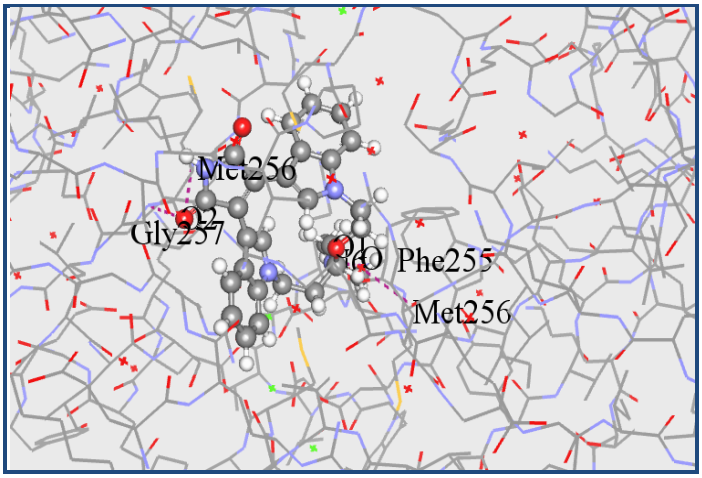

Figure 3: Docking conformations showing the interaction of Ruboxistaurin to PKCβ. Aminoacid residues showing bonded and non bonded interactions are represented in Black. Ligand molecules are shown in ball and stick representation.

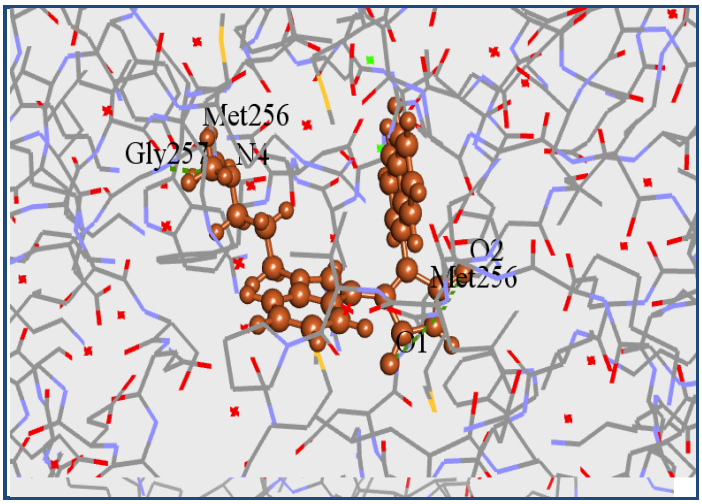

Figure 4: Docking conformations showing the interaction of Maleimide derivative 3 to PKCβ. Aminoacid residues showing bonded and non bonded interactions are represented in Black. Ligand molecules are shown in ball and stick representation.

Docking studies

Molecular docking studies of protein and receptor was carried out by using software AUTODOCK 4 **[16],** in which hydrogen's, kollman charges and gasteiger charges were added. Grid file was generated, and docking was performed by creating an

initial population of 150 individuals, Lamarckian genetic algorithm (LGA) was implemented with a maximum of 2500000 energy evaluations and maximum number of generations was 27000. This gives the best ten binding orientations of ligand molecules to its receptor.

Screening of lead molecule for its activity and drug likeliness properties

The molecular properties of ligands like log P, number of hydrogen bonds, number of atoms, molecular weight were obtained using molinspiration program **Table 1 (see supplementary material).** This tool also provides number of violations which mean that there is a deviation from Lipinski 5 rule. Those compounds having more than one violation were eliminated and remaining compounds were used for docking studies i.e., CHEMBL315357, CHEMBL91959, CHEMBL328229, CHEMBL321529, CHEMBL103055, CHEMBL421217, CHEMBL130774, CHEMBL336179, CHEMBL321315, CHEMBL105477.

Results & Discussions:

Interaction of ligands

Docking results between PKCβ and maleimide derivative 3 found to be good as it was giving lowest docking energy (-9.36kcal/mol) than ruboxistaurin (-8.61kcal/mol) **Table 2 (see supplementary material) (Figure 3 & Figure 4).** By analyzing the docked conformations, interaction of PKCβ—ruboxistaurin (reference ligand) showed formation of hydrogen bond between oxygen atom at Met256 of PKCβ and hydrogen at 51st position of ligand molecule **Table 3 (see supplementary material).** The non hydrogen bonds include interaction between Nitrogen at Met256—with oxygen at 2nd position and additionally with oxygen at 1st position; Nitrogen at Gly257—oxygen at 2nd position; Nitrogen at Phe255— nitrogen at 6th position. Interaction between PKCβ—maleimide derivative 3, showed non hydrogen bond interactions between Nitrogen at Met256 of PKCβ—nitrogen at 4th position and also with oxygen at 1st and 2nd position of maleimide derivative 3; Nitrogen at Gly257 – nitrogen at 4th. Binding energy value of Bisindolylmaleimide I from Autodock was found to be (-9.14kcal/mol). Interaction between PKCβ—Bisindolylmaleimide I, showed hydrogen bond between oxygen at Phe255 of PKC β and hydrogen at 45th position of ligand molecule. The non bonded interactions are between nitrogen at Gly257—oxygen at 2nd position; amine at Arg159—oxygen at 1st position. The binding energy of CHEMBL316239 from Autodock was (-9.12kcal/mol).-interaction between PKCβ CHEMBL316239, showed hydrogen bond formation between oxygen at Asn253—hydrogen at 57th position. The non bonded interactions between nitrogen at Phe 255—oxygen at 3rd position; nitrogen at Met 256—oxygen at 1st position, oxygen at 3rd position and also with nitrogen at 7th position; nitrogen at Gly257—oxygen at 1st position. The binding energy value of CHEMBL311543 was (-8.88kcal/mol) Interaction between PKCβ- CHEMBL311543, showed non bonded interactions between amine at Arg159of PKCβ—nitrogen at 7th position of ligand molecule.

Conclusion:

Of all the molecules obtained from the database, maleimide derivative 3, showed highest binding affinity than Ruboxistaurin by forming bonded and non bonded interactions towards PKCβ. We therefore suggest that maleimide derivative 3 is a potent inhibitor of PKCβ and may be useful in treatment of diabetic retinopathy. The potent inhibition can be discovered through in vivo and in vitro studies.

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Supplementary material:

Table 1: Molinspiration analysis of ligand molecules.

No	Compound	Milogp	molecular polar surface area	Num of atoms	molecular weigh t	number of H-bond accept ors	Number of H-bond donors	Num of violations	Number of rotatable bonds	Volume
1	CHEMBL311543	3.49	81.062	34.0	454.53	7	2	0	2	409.057
2	CHEMBL419866	4.13	72.273	37.0	494.595	7	1	0	2	449.243
3	CHEMBL316239	2.51	95.058	33.0	440.503	7	3	0	1	391.383
4	ZINC03825435	3.07	89.263	33.0	441.487	7	2	0	1	388.112
5	CHEMBL432130	3.73	72.273	35.0	468.557	7	1	0	2	426.000
6	CHEMBL131035	3.24	78.269	33.0	441.487	7	1	0	0	389.053
7	CHEMBL294120	3.34	89.263	34.0	455.514	7	2	0	1	404.913
8	Bisindolylmaleimidee I	3.83	73.896	31.0	412.493	6	2	0	6	377.044
9	maleimide derivative, 3	4.84	58.105	32.0	423.516	5	1	0	6	392.059

Table 2: Docking energies of different ligand molecules.

No	Name of the ligands	Docking energies	Bonded interactions
	Ruboxistaurin (Reference)	-8.61	Met256,Gly257
2	maleimide derivative, 3	-9.36	Met256,Gly257
3	Bisindolylmaleimide I	-9.14	Arg159,Gly257,Phe255
4	CHEMBL316239	-9.12	Phe255,Met256,Gly257
5	CHEMBL311543	-8.88	Arg159

Table 3: bonded and non bonded interactions between receptor and ligand molecules.

PKCβ→ruboxistaurin	PKCβ→ maleimide derivative 3	PKCβ→ Bisindolylmaleimide I	PKCβ→ CHEMBL316239	PKCβ→ CHEMBL311543
Met256:N→O2	Met256:N→N4	Arg159:N→O1	Phe255:N→O3	Arg159:NH1→N7
Met256:N→O1	Met256:N→O2	Arg159:NH1→O1	Met256:N→O3	Arg159:NH2→N7
Gly257:N→O2	Gly257:N→N4	Arg159:NH2→O1	Met256:N→N7	
Phe255:N→N6	Met256:N→O1	Gly257:N→O2	Met256:N→O1	
Met256: O→H51		Phe255: O→H45	Gly257:N→O1	