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Research Article

MOLECULAR DOCKING STUDIES ON SELECTED PHYTOCOMPOUNDS FROM DIFFERENT Andrographis sp AGAINST PPAR-γ and C/EBP-α RECEPTORS FOR TYPE-2-DIABETES

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ABSTRACT

Diabetes mellitus is a prevalent disease affecting the citizens of both developed and developing countries. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. The present work deals with the analysis of binding mechanism of 11 selected natural compounds from different Andrographis species against the novel targets for type T2D namely C/EBP-α and PPAR-γ compared with Rosiglitazone (standard compound) using GOLD software. The results revealed that most of the selected herbal lead compounds were effective targets against the receptors. These compounds showed favorable interactions with the amino acid residues thereby substantiating their proven efficacy as anti-diabetic compounds. The resulting data of receptor-ligand interactions demonstrates that in silico screening method is highly efficient for identifying potential lead compounds against major disorders/diseases.

Keywords: PPAR-γ,C/EBP-α,Andrographis sp,type 2 diabetes,,3T3-L1 cells

INTRODUCTION

Type 2 diabetes (T2D) poses a major health problem globally, especially in many developing countries[1].It is brought about when the cells in the muscles, liver, and fat tissues fail to utilize insulin effectively. Human body has to maintain the blood glucose level at a very narrow range, which is done with insulin and glucagon [2]. The function of glucagon is causing the liver to release glucose from its cells into the blood, for the production of energy. The worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% in 2025[3]. Currently available therapies for T2D include antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and thiazolidindione .Due to the side effects associated with the oral hypoglycemic agents there is a growing interest in herbal remedies[4].

Carbohydrate metabolism and differentiation of 3T3-L1 adipocytes are associated with diabetes. Peroxisome proliferators activated receptor gamma (PPAR-γ) and the CCAAT/enhancer binding protein family (C/EBP-α, β, and δ) are critical factors in 3T3-L1preadipocyte differentiation [5]. There has been an extensive research focused on PPAR-γ belonging to nuclear receptor family and C/EBP-α CAAT enhancer binding proteins which are ligand-activated transcription factors. PPAR-γ and C/EBP-α is expressed most abundantly in adipose tissue and mediates the antidiabetic activity of the insulin-sensitizing drugs belonging to the thiazolidindione [6]. This key transcriptional factor plays a pivotal role in regulating adipogenesis, insulin sensitivity and glucose homeostasis[7].

A drug molecule is triggered when the binding of small molecule to the receptor protein is perfectly done. Such protein-ligand interaction is comparable to the lock-and-key principle, in which the lock encodes the protein and the key is ensembled with the ligand. The major driving force for binding appears to be hydrophobic interaction whose specificity is however controlled by hydrogen bonding interactions[8]. Therefore, in the present study the species Andrographis is said to have antidiabetic property[9].Hence phytocompounds from this species were selected and further investigated for its binding efficiency to evaluate the best fit molecule using GOLD (Genetic Optimization of Ligand Docking).

MATERIALS AND METHODS

Structure of PPAR-γ

The X-Ray crystallographic structure of PPAR-γ was obtained from the Protein Data Bank (PDB). The PDB ID 3DZY corresponds to the crystal structure of the receptor. The structure of PPAR-γ is composed of six polypeptides chains with 467 amino acids. We have employed the GOLD software to screen the activity of the 13 compounds against the receptor 3DZY. The consensus scoring and ranking was used to determine the results of Molecular screening (Figure: 1).

Figure 1: 3D structure of PPAR-γ

Structure of C/EBP-α

The C/EBP-α protein sequence was retrieved from the NCBI protein database and the corresponding three dimensional structures were not resolved experimentally in the PDB database. Hence MODELLER 9.10 was used to predict the three dimensional structure of human C/EBP-α A with Mouse C/EBP-α A as the template (PDB ID 1NWQ with a resolution of 2.80 Angstroms). The Ramachandran plot evaluation using PROCHECK shows that the structure has 92.7% of residues in the core region, thus proving a stable model. This model was taken further for docking studies (Figure: 2).

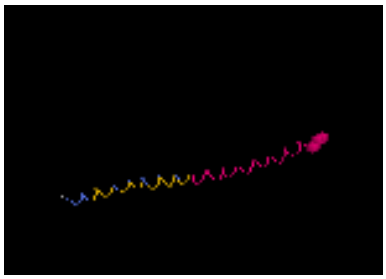

Figure 2: 3D structure of C/EBP-α

Ligand binding site of PPAR-γ

The active site of the receptor 3DZY was determined using the Q-Site Finder. (The active site of the protein includes Tyr 222, Phe 226, Pro 227, Leu 228, Thr 229, Lys 230, Gly 284, Cys 285, Gln 286, Arg 288, Ser 289, Glu 291, Ala 292, Ile 296, Met 329, Leu 330, Ser 332 and Leu 333.

Ligand binding site of C/EBP-α

The active site of the Model receptor was determined using the Q-site finder. The active site of the Model protein includes Met218, His219, Pro198, Aka197, His200, Leu201, Thr217, Ala202, Gln215, Leu220, Gln221, Pro197, Pro196 and His195.

Phytocompounds selection

The selected phytocompounds were selected from the various literatures of Andrographis species [10, 11, 12, 13, 14, 15, 16]). The 2D structure of the selected compounds was drawn using ACD Chemsketch.The structures were then converted to 3D; their geometries were optimized and saved in "MDL mol file" format.

GOLD docking simulations

Automated docking studies were performed using the genetic algorithm GOLD (Version 3.2 CCDC, Cambridge, UK)[17]. The algorithm had been previously validated and successfully tested on a data set of over 300 complexes extracted from the PDB [18].All the selected compounds from different Andrographis species under study were docked in to the binding site of the receptor (PDB ID: 3DZY and modeled C/EBP-α) using GOLD [19]. The GOLD program uses a genetic algorithm (GA) to explore the full range of ligand conformational flexibility and the rotational flexibility of selected receptor hydrogen's. Grid was prepared for the protein with the center and the size of the bounding box set on 10 Å. The coordinates of the enclosing box (x = 121 Å; y = 87 Å; z = 45 Å) were defined starting from the set of active site residues. During docking process, a maximum of 10 different conformations was considered for the drug. The conformer with highest binding score was used for further analysis [20].

RESULTS AND DISCUSSIONS

The Molecular Docking analysis of the selected natural compounds from different Andrographis speices and the receptors PPAR-γ and C/EBP-α which are involved in the regulation of insulin resistance in T2D has been performed. The inhibiting susceptibility of the compounds was evaluated using their GOLD scores generated by the GOLD software. The best docking solutions GOLD score for each compound was considered. The GOLD software resulted in identifying the best compound that interacts with the receptor. The results were evaluated based on the binding compatibility i.e. Docked energy in kcal/mol (fitness).

Binding modes and interactions

The active site of PPAR-γ and C/EBP-α offers different binding modes for the compounds as they are strongly dependent on the attached substituent [21]. The receptors bound ligand was docked deeply within the binding pocket region forming the interactions. The 18 amino acid residues from PPAR-γ and 14 amino acid residues from C/EBP-α provide a cavity for the active herbal compounds to interact with the receptor. The active compound 5-Hydroxy-7,8-dimethoxyflavanone (Figure:3) binds with the receptor C/EBP-α with the highest GOLD Score of 34.65 (Table:1) and with the receptor PPAR-γ with the highest GOLD score of 39.04 (Table:2) (Figure:4) comparatively the active compounds 5,7,2,3,4-pentamethoxyflavone (Figure : 5) (Table:1), Dihydroskullcapflavone (Figure:6) (Table:1) , binds with the C/EBP-α receptor with positive GOLD score . The active compound 17, 19, 20-Trihydroxy-5Beta, 8α H, 9beta h, 10α-labd-13-en-16, 15-olactone (Figure:7) was found to bind with PPAR-γ with a highest score of 65.66 (Table:2) but it is seen that 17, 19, 20-trihydroxy-5beta, 8α H, 9beta h, 10α-labd-13-en-16, 15-olactone binds with C/EBP-α with a very poor score of 15.02 (Table:1). Rosiglitazone, the standard compound from also shows a significant binding affinity with a GOLD score of 31.99 towards C/EBP-α (Table: 1) and GOLD score of 45.91 towards PPAR-γ (Table: 2) (Figure: 8).

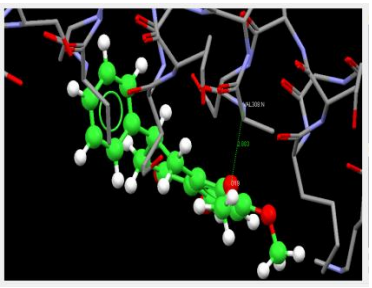

Figure 3: Interactions between C/EBP-α and 5-Hydroxy-7,8-Dimethoxyflavanone

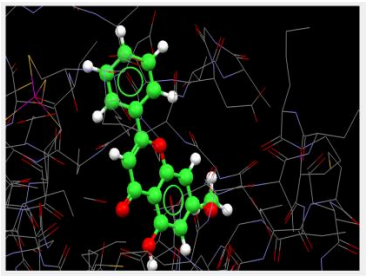

Figure 4: Interactions between PPAR-γ and 5-Hydroxy-7,8-Dimethoxyflavanone

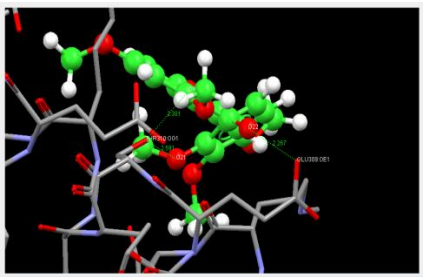

Figure 5: Interactions between C/EBP-α and 5,7,2,3,4-pentamethoxyflavone

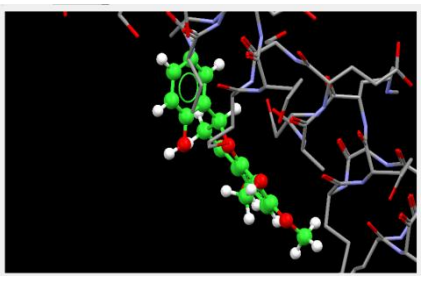

Figure 6: Interactions between C/EBP-α and Dihydroskullcapflavone

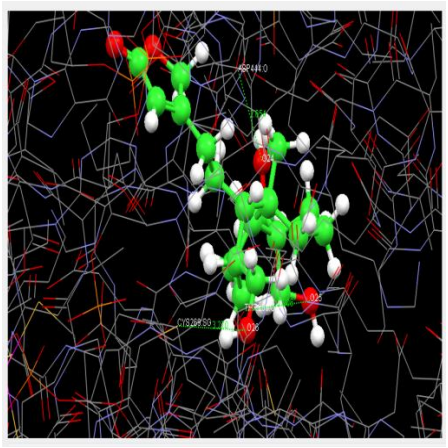

Figure 7: Interactions between PPAR-γ and 17,19,20-Trihydroxy-5beta , 8alpha H, 9beta H,10alpha-Labd-13-En-16,15-Olactone

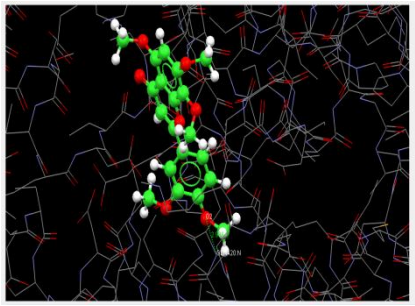

Figure 8: Interactions between PPAR-γ and Rosiglitazone

From the analysis of the H-bond formations between the selected active compounds and the C/EBP-α receptor, 5-Hydroxy-7, 8-Dimethoxyflavanone form one H-bond whereas 5, 7, 2, 3, 4-pentamethoxyflavone forms four H-bonds with PPAR-γ receptor. From the analysis it is evident that 5-hydroxy-7, 8-dimethoxyflavanone and 5, 7, 2, 3, 4-pentamethoxyflavone exhibits a better antidiabetic property when compared to the other selected compounds in comparison with standard compound (Rosiglitazone).

Table 1: Docking results of 11 selected compound from Andrographis sp compared with Roziglitazone (Standard compound) with the receptor C/EBP-α

Ligand Name/Plant name	Atom in Protein	Atom in Ligand	H-bond Distance	Score
5,7,2,3,4-PENTAMETHOXYFLAVONE/Andrographis lineata	GLU309:OE1	O22	2.267	31.9
	THR310:OG1	O21	2.591	
	THR310:OG1	O9	2.381	
2-HYDROXY-2,4,6-TRI METHOXYCHALCONE/Andrographis serphyllifolia	NO H BONDS			28.41
DIHYDROSKULLCAPFLAVONE I/Andrographils serphyllifolia	NO H BONDS			34.45
17,19,20-TRIHYDROXY-5BETA, 8ALPHA H, 9BETA H,10ALPHA-LABD-13-EN-16,15-OLACTONE/Andrographis lineata	NO H BONDS			-15.02
5-HYDROXY-7,8-DIMETHOXYFLAVANONE-/Andrographis lineata	VAL308.N	O19	2.883	34.65
5-HYDROXY-7,8,2,3,4-PENTAMETHOXYFLAVONE/Andrographis lineata	THR310:OG1	O9	2.514	32.11
	THR310:OG1	O19	2.727	
	THR310:OG1	O22	2.915	
5,2-DIHYDROXY-7-METHOXYFLAVANONE-/Andrographis paniculata	NO H BONDS			29.28
5,2-DIHYDROXY-7,8-DIMETHOXYFLAVONE-/Andrographis alata	THR310:OG1	H1	2.326	29.89
	THR310:OG1	O9	2.349	
	THR310:OG1	O22	2644	
5,2-DIHYDROXY-7-METHOXYFLAVONE/Andrographis echioides	THR310:OG10	O9	2.393	28.94
5,2-DIHYDROXY-7-METHOXYFLAVONE 2-O-BETA-D GLUCOPYRANOSIDE/Andrographis elongata	THR310:OG10	O9	2.638	33.39
	THR310:OG10	O19	2.988	
	ASN307:O	O31	2.494	
DIMETHYL 3,3',4,4'-TETRAHYDROXY- δ-TRUXINATE/Andrographis lineata	THR310:OG1	O7	2.818	27.35
	ASN307:OD1	C13	2.754	
	ASN307:CB	C13	2.974	
ROSIGLITAZONE (STANDARD COMPUOUND)	NO HBONDS			31.99

Table2: Docking results of 11 selected compound from Andrographis sp compared with Roziglitazone (Standard compound) with the receptor PPAR-γ

Ligand Name/Plant Name	Atom in Protein	Atom in Ligand	H-bond Distance	Score
5,7,2,3,4-PENTAMETHOXYFLAVONE/ Andrographis lineata	THR266:OG1	H30	2.76	47.34
	THR266:N	O18	3.035	
	THR266:OG1	O18	2.804	
	THR266:OG1	O22	2.632	
2-HYDROXY-2,4,6-TRI METHOXYCHALCONE/ Andrographis serphyllifolia	LYS440:O	O18	2.543	40.68
	THR445:N	O18	2.438	
	THR445:OG1	O20	2.533	
	MET452:SD	O20	2.991	
DIHYDROSKULLCAPFLAVONE I/ Andrographils serphyllifolia	THR266:OG1	O19	2.427	40.2
	CYS269:SG	O22	2.629	
	THR445:N	O20	2.973	
	PHE439:O	O18	2.073	
17,19,20-TRIHYDROXY-5BETA, 8ALPHA H, 9BETA H,10ALPHA-	CYS269:SG	O26	3.27	65.66

LABD-13-EN-16,15-OLACTONE/ Andrographis lineata	THR266:OG1	O25	3.906	
	ASP444:O	O24	2.654	
5-HYDROXY-7,8-DIMETHOXYFLAVANONE/ Andrographis lineata	THR445:N	O18	2.879	39.04
	PHE439:O	O26	2.126	
	PHE437:OE	O19	2.715	
5-HYDROXY-7,8,2,3,4-PENTAMETHOXYFLAVONE/ Andrographis lineata	CYS269:SG	O18	2.705	32.98
	THR445:N	O20	2.334	
5,2-DIHYDROXY-7-METHOXYFLAVANONE-/Andrographis paniculata	LYS440:O	O21	2.521	
	MET452:SD	O23	2.56	
	MET452:SD	O22	2.56	
5,2-DIHYDROXY-7-METHOXYFLAVANONE/-/Andrographis paniculata	CYS269:SG	O17	2.899	38.98
5,2-DIHYDROXY-7,8-DIMETHOXYFLAVONE/-/Andrographis alata	CYS269:SG	O22	2.885	43.53
5,2-DIHYDROXY-7-METHOXYFLAVONE/ Andrographis echioides		NO HBONDS		43.8
5,2-DIHYDROXY-7-METHOXYFLAVONE 2-O-BETA-D GLUCOPYRANOSIDE/Andrographis elongata		NO HBONDS		37.6
DIMETHYL 3,3',4,4'-TETRAHYDROXYL- δ-TRUXINATE/ Andrographis lineata	SER382:OG	C19	2.141	34.28
	LEU419:O	O18	2.986	
ROZIGLITAZONE (STANDARD COMPOUND)	ASP381:OD2	O18	3.033	45.91
	SER382:N	O15	2.692	

CONCLUSION

Computer aided drug discovery is an emerging and effective alternative for identification of novel therapeutic molecules. In the present study the selected 11 compounds from different Andrgraphis sp has been docked with the two promising targets (PPAR-γ and C/EBP-α) for T2Dtype. The interaction of the receptor and inhibitors were analyzed using GOLD and the best interacting inhibitor were screened. The receptors C/EBP-α and PPAR-γ involved in the regulation of insulin resistance in type 2 diabetics interacts with 5-hydroxy-7,8-dimethoxyflavanone and 17,19,20-trihydroxy-5beta , 8α H, 9beta H,10α-labd-13-En-16,15-olactone respectively with maximum fitness score. This study is also helpful for pharmaceutical sectors as computer aided screening would reduce the complexities involved in the discovery and development of new lead molecules.

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