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Research Article

IDENTIFICATION OF DUAL AGONISTIC NOVEL LIGANDS FOR INSULIN RECEPTOR AND PPARγ THROUGH MOLECULAR DOCKING

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ABSTRACT

Diabetes mellitus (DM) is a progressive disease characterized by insulin deficiency and insulin resistance. The targets insulin receptor and PPARγ associated protein [PDB: 1IRK, 3KDU] were obtained from protein data bank. Chemsketch 12.0 software was used to draw the three dimensional structure of the phytocompounds. The drug likenesses of the compounds were evaluated by checking the Lipinski and ADMET properties by using Accord for Excel. Among the 11 compounds, eight compounds were satisfied and 4 were not satisfied the Lipinski properties. In the prediction of ADMET [Absorption, Distribution, Excretion, Metabolism, Toxicology] properties for the chosen compounds, catechin, costunolide, eremanthin, saponin were found to be toxic. After screening 4 ligands namely novel gymnemic diacetate, novel gymnemic triacetate, novel dihydroxy gymnemic triacetate, gallic acid were tested, through molecular docking interactions using Discovery Studio 2.1 version. All the 4 compounds interacted with insulin receptor and were predicted to promote the insulin signaling pathway. At the same time only 2 compounds interacted with PPARγ and were predicted to promote PPAR signaling pathway. Hence, these 2 novel compounds namely gymnemic diacetate and gymnemic triacetate were identified as potent medicinal compounds as dual agonistic ligands for insulin receptor and PPARγ.

Keywords: Diabetes mellitus, Insulin receptor, PPARγ, Dual agonists, Novel gymnemic diacetate and Novel gymnemic triacetate.

INTRODUCTION

Diabetes mellitus (DM) is a multisystem disease with both biochemical and anatomical consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin due to the decreased insulin secretion from β- cells (Type-1), and insulin resistances (Type-2) in the peripheral tissues[1]. The symptoms of the diabetes patients are having blurred vision, unusual thirst, frequent urination, slow-healing cuts, unexplained tiredness, rapid weight loss (Type-1 diabetes), erectile dysfunction, numbness or tingling in hands or feet[2].

The broad scope of anti-diabetic therapy is to restrict blood glucose control, by controlling fasting glucose levels and by controlling elevations in postprandial blood glucose[3] in which it adapts some definite mechanism. Biguanides, sulphonylureas and thiozolidinediones became available for treatment of type- 2 diabetes and have been effective hypoglycemic agents. Insulin injections are used to manage type-1 diabetes. However they cause some side effects including hypoglycaemia, anorexia nervosa, liver atrophy, metallic taste, gastro-intestinal discomfort and nausea, insulin allergy, insulin resistance and insulin neuropathy. Therefore, finding other anti-diabetes agents, especially those made from natural sources is an important goal for diabetes researchers. Despite the great interest in the development of new drugs to revert the burden of complications associated with this disease and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies only a few of them have been tested in humans[4-8]. Traditional medicine plays an important role in the health care of human population where 80% of the world population depends on herbal medication[9]. Hence it is more essential to develop more targeted and effective ligands against diabetes.

Generally, the oral hypoglycemic agents are agonists of Peroxisome Proliferator Activated Receptors gamma (PPARγ) and work by enhancing insulin action thus promoting glucose utilization in peripheral tissues and have no effect on insulin secretion[10]. At the same time, insulin that given through the intravenous injection, binds to transmembrane receptors located in insulin sensitive tissues. This binding of insulin receptor with insulin on the outer surface of the plasma membrane initiates a phosphorylation cascade leading to activation of proteins and induction of gene expression promoting anabolic cellular processes[11]. Thus, these two receptors are chosen for the present study as the targets to develop dual agonistic ligands through molecular docking.

MATERIALS AND METHODS

Medicinal compounds

We followed the bioassay guided fractionation for the isolation of the phytocompounds. The compounds are saponin (*Eugenia jambolana)*; catechin (*Cassia fistula)* possessing antidiabetic activity[12]; costunolide (*Costus speciosus)* possessing antidiabetic activity[13] and antioxidant activity[14]; novel dihydroxy gymnemic triacetate possessing antidiabetic activity[15], novel gymnemic diacetate and novel gymnemic triacetate (*Gymnema sylvestre*); gallic acid (*Terminalia bellerica)* possessing antidiabetic activity[16]; polysaccharide (*Tinospora cordifolia*); terpenoid possessing antimicrobial activity[17] and lupeol (*Elephantopus scaber)*. The structures of these compounds are elucidated using NMR studies.

Receptor structure

We used the insulin receptor and PPAR gamma associated protein structure with PDB ID: 1IRK, 3KDU from protein databank (PDB) for this study.

Software and tools

We used ACD/Chemsketch to draw molecular structures and calculate chemical properties. The ADMET (Absorbtion, Distribution, Excretion, Metabolism, Toxicology) properties were calculated for the phytocompounds using Accord excel for excel an Accelry's product. The docking module in Discovery Studio an Accelrys Software Inc (2.1) is used for docking studies.

RESULTS

The NMR structure and 3D structure of the phytocompounds namely a) catechin, b) polysaccharide, c)costunolide, d) eremanthin, e) gymnemicdiacetate, f) gymnemic triacetate, g) dihydroxy gymnemic triacetate, h) novel saponin, i) lupeol, j) gallic acid, k) terpenoid are shown in figs:1&2 respectively.

Table-1 describes the Lipinski properties of the chosen compounds evaluated by Accord Excel 6.1. The compounds catechin, costunolide, eremanthin, gymnemic diacetate, gymnemic triacetate, dihydroxy gymnemic triacetate, gallic acid are satisfying the Lipinski properties. Whereas the compounds Polysaccharide, novel saponin, lupeol, terpenoid are not satisfying the Lipinski properties.Table-2 depicts the ADMET [Absorption, Distribution, Excretion, Metabolism, Toxicology] properties for the chosen compounds where, catechin, costunolide, eremanthin, saponin has a value 1 value indicates the

toxic effect. And the other compounds have level 0 represent non toxic effect. Hence, the compounds namely novel gymnemic diacetate, novel gymnemic triacetate, novel dihydroxy gymnemic triacetate, gallic acid were considered for the docking studies.

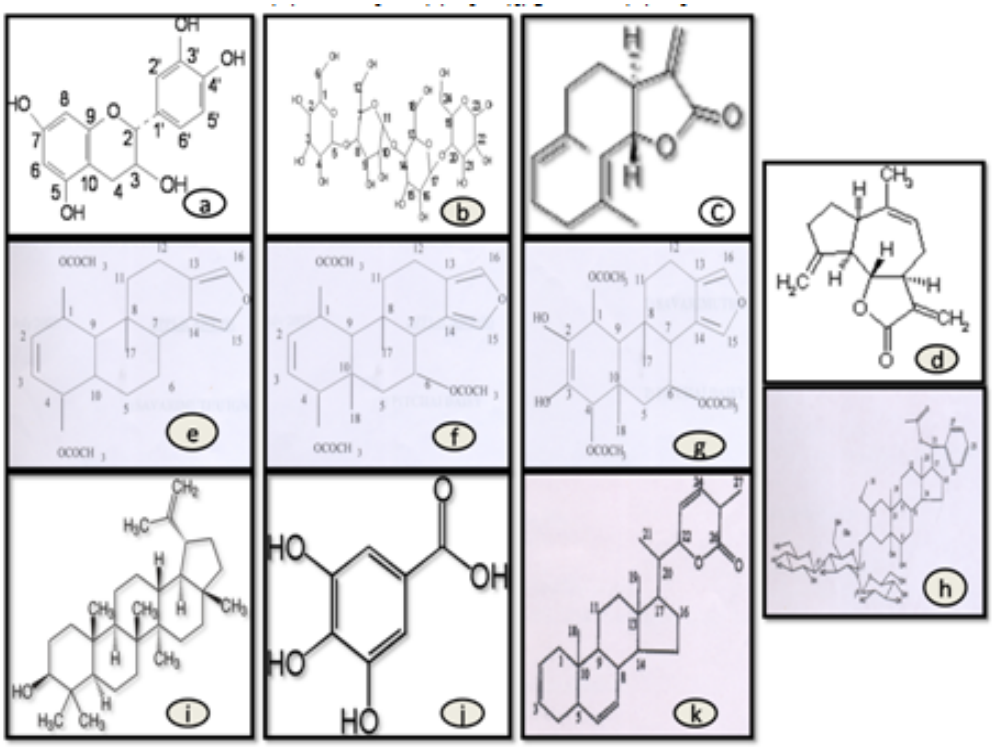

Fig. 1: The NMR structure of the phytoconstituents namely a) catechin, b) polysaccharide, c) costunolide, d) eremanthin, e) gymnemic diacetate, f) gymnemic triacetate, g) dihydroxy gymnemic triacetate, h) novel saponin, i) lupeol, j) gallic acid, k) terpenoids.

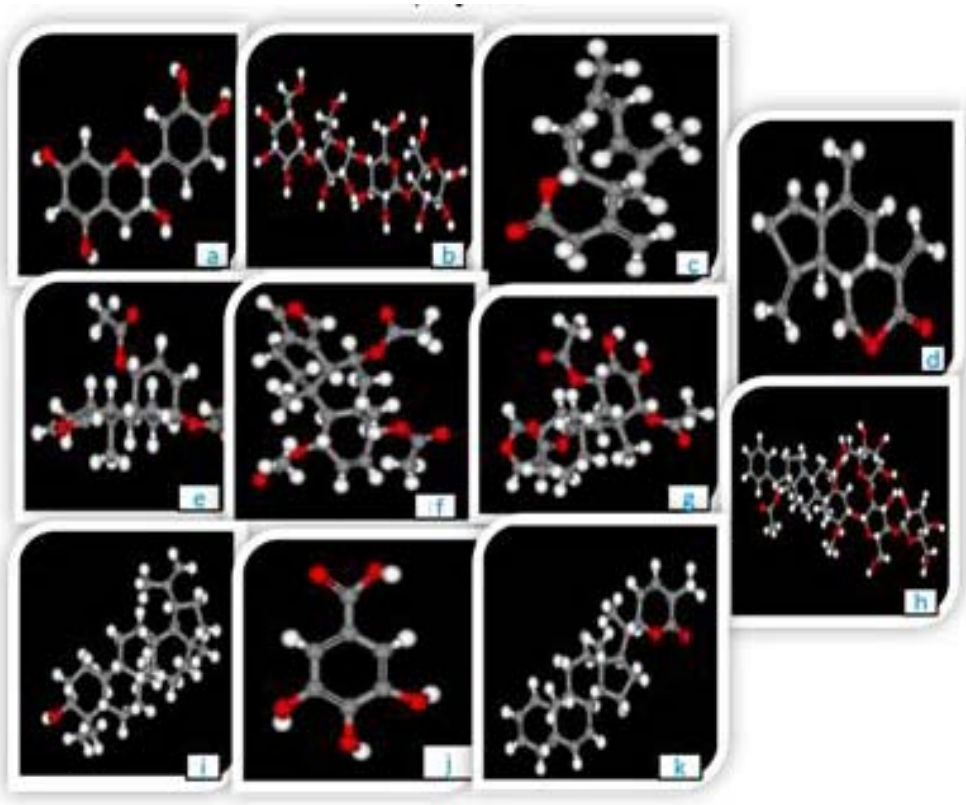

Fig. 2: the three dimensional structures of phytoconstituent namely a) catechin, b) polysacceride, c) costunolide, d) eremanthin, e) gymnemic diacetate, f) gymnemic triacetate, g) dihydroxy gymnemic triacetate, h) novel saponin, i) lupeol, j) gallic acid, k) terpenoids.

Table 1: Lipinski properties of phytocompounds

S. No.	Compound	Hydrogen bond donors (<=5)	Hydrogen bond acceptors (<=10)	Molecular weight (<=500) [g/mol]	Alog P (<=5)
1	Catechin	5	6	290.29	2.1127
2	Polysacchride	3	11	454.50912	-1.5
3	Costunolide	0	0	232.3181	3.3
4	Eremanthin	0	2	230.30222	2.6
5	Gymnemic diacetate	0	5	358.47	3.102
6	Gymnemic triacetate	0	7	430.54	2.7509
7	Dihydroxy Gymnemic triacetate	2	9	462.54	0.7009
8	Saponin	12	21	973.191	-0.187103
9	Lupeol	1	1	426.801	8.0281
10	Gallic acid	4	5	170.11954	0.7
11	Terpenoid	0	2	394.65	6.3443

Table 2: ADMET properties of phytocompounds

S. No.	Compound Name	Aqueous Solubility	BBB Penetration	Cyt p450	Hepa Toxicity	HIA	Protein Binding Level
1	Catechin	2	1	0	1	0	1
2	Polysaccharide	1	1	0	0	3	0
3	Costunolide	2	1	0	1	0	1
4	Eremanthine	2	1	0	1	0	0
5	Gymnemic diacetate	2	3	0	0	1	0
6	Gymnemic triacetate	2	3	0	0	1	0
7	Dihydroxy Gymnemic triacetate	3	1	0	0	0	0
8	Novel saponin	2	4	0	1	3	0
9	Lupeol	0	4	0	0	3	2
10	Gallic acid	4	3	0	0	0	0
11	Terpenoid	1	1	0	0	1	0

The protein structure of the two receptors namely insulin receptor and PPARγ retrieved from protein data bank (PDB) are shown in fig (3)A and B. Then the retrieved proteins were subjected to energy minimization using CHARM force filed followed by defining sphere. 1IRK receptor with defined sphere in red colour and binding site in green colour are shown in fig(3)C, PPARγ with defined sphere in red colour and binding site in green colour are shown in (3)D.

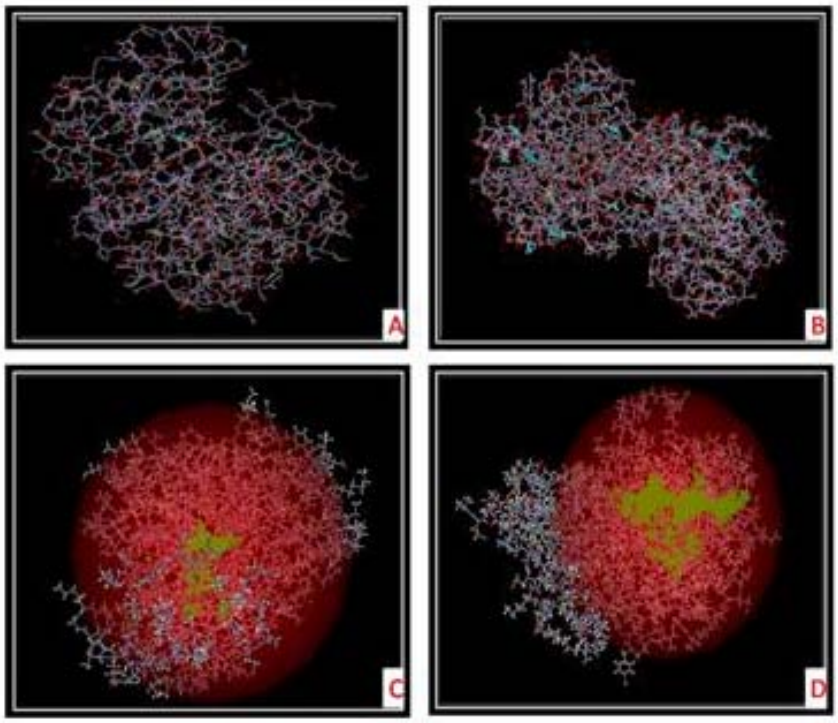

Fig. 3: PDB structure of the a) IIRK receptor b) 3KDU receptor c) IIRK receptor with defined sphere in red colour and binding site in green colour d) 3KDU receptor with defined sphere in red colour and binding site in green colour.

While docking all the 4 compounds were made interactions with 1IRK receptor. The docking interaction between the 1IRK receptor with the compounds namely gymnemic diacetate, gymnemic triacetate, dihydroxy gymnemic triacetate, gallic acid are presented in fig:4 a,b,c & d respectively. But, of the four compounds, only novel gymnemic diacetate, novel gymnemic triacetate were docked with 3KDU and the docked results are presented in fig: 5a & b respectively. In these results, the aminoacids were displayed in pink colour sticks**.** The ligands are represented in ash colour. The hydrogen bonds are denoted in green dot lines**,** and bumps are denoted in pink dot lines.

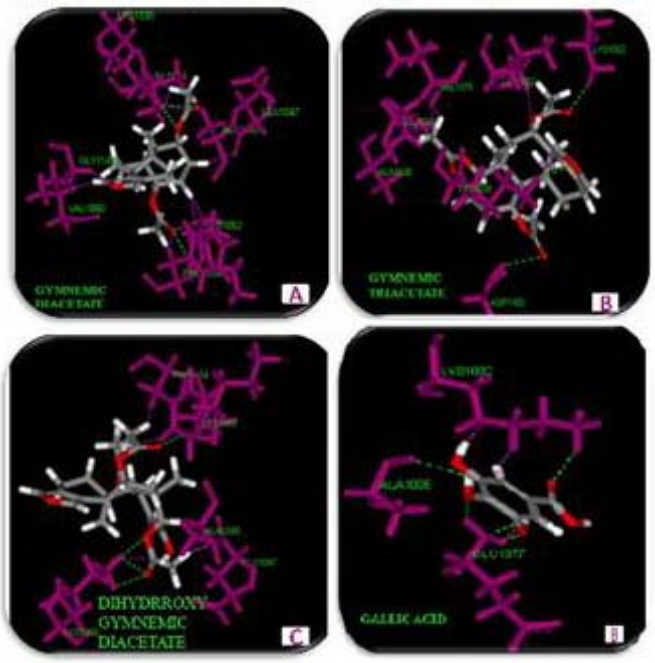

Fig. 4: The docking interaction between the IIRK receptor with the compounds a) gymnemic diacetate, b) gymnemic triacetate, c) dihydroxy gymnemic triacetate, d) gallic acid.

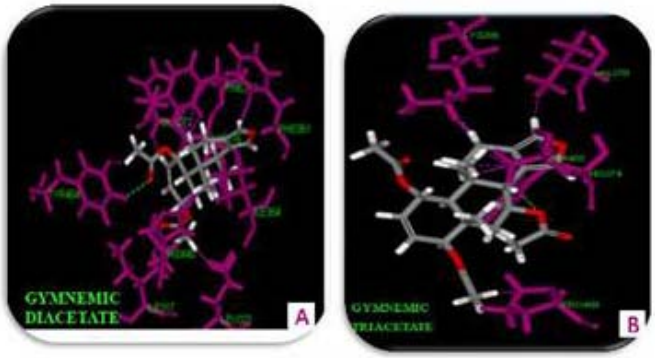

Fig. 4: The docking interaction between the IIRK receptor with the compounds a) novel gymnemic diacetate, b) novel gymnemic triacetate.

The details of docking interaction between compounds and the receptor including docking poses**,** docking scores and residues involved ligand receptor hydrogen bonds are presented in Table-3**.**

DISCUSSION

In-silico drug screening is an effective alternative for identification of lead compounds. According to Ganguly *et al***.,** (2010)[18] lead compounds could be identified and tested using molecular docking for their effectiveness against major molecules of interest for diabetes diseases**.** A variety of computational factors are used to identify novel compounds. The most well known factor is the "Lipinski's rule of five".

Christopher A. Lipinski formulated Lipinski's rule of five to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans[19]. The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties. In connection to these contexts, it is very much clear from table -1 that phytocompounds including catechin, costunolide, eremanthin, dihydroxy gymnemic triacetate, gymnemic diacetate, gymnemic triacetate and gallic acid are satisfying the Lipinski properties.

One of the most daunting steps for a drug candidate is having favorable ADMET characteristics. Prentis *et al***.,** (1988)[20] suggested that optimizing these properties during early drug discovery is crucial for reducing ADMET problems later in the development process. Such early identification helps to make the research process more efficient and cost-effective to eliminate compounds with unfavorable ADMET characteristics early on, and evaluate proposed structural refinements that are designed to improve ADMET properties, prior to resource expenditure on synthesis.

Though, ADMET properties include models for intestinal absorption, aqueous solubility, blood brain barrier penetration, plasma protein binding, cytochrome P_{450} 2D6 inhibition, and hepatotoxicity, hepatotoxicity is the major model to be tested as toxicity is responsible for many compounds failing to reach the market and for the withdrawal of a significant number of compounds from the market once they have been approved[21]. In the present study catechin, costunolide, eremanthin, saponin has 1 value indicates the toxic effect. And the other compounds have level 0 represent non toxic effect. Thus, novel gymnemic diacetate, novel gymnemic triacetate, novel dihydroxy gymnemic triacetate, gallic acid have satisfied both the Lipinski's rule of five and ADMET, achieving the status of 'oral drug-likeness' and are chosen for docking against the receptors.

Table 3: Results of drug-receptor interactions

S. No.	Compound name	Docking poses Absolute energy	Docking score			Ligand-receptor hydrogen bond				
			Lib dock score	Total no.of hydrogen bonds	No. of contacts	Amino acid	Position	Atom in amino acid	Atom in Ligand	Bond length
1	Gymnemic Diacetate 1IRK	19.875	113.0	3	17	LYS	1052	NH2	025	1.537
						PHE	1054	NH	025	2.170
						LYS	1030	HZ1	019	1.129
	Gymnemic diacetate 1ZGY	90.096	105.9	1	6	TYR	464	HH	O25	2.434
2	Gymnemic Triacetate 1IRK	17.74	123.2	3	5	LYS	1030	HZ1	016	2.378
						LYS	1052	NH2	024	2.131
						ASP	1150	NH	027	2.490
	Gymnemic Triacetate 1ZGY	9.893	87.54	1	11	HIS	274	HD1	O22	2.887
3	Dihydroxy Gymnemic Triacetate 1IRK	107.981	85.04	4	14	CYS	42	H61	032	2.244
						ARG	46		0.30	1.768
						HH	21		0.30	2.384
						HH	21			2.126
4	GallicAcid 1IRK	54.657	111.7	3	32	CYS	42	N	H21	2.431
						CY	-	N	H109	1.763
							6	C	H24	1.690

The screened ligands are then tested using molecular docking. Virupakshaiah *et al.,* 2007[22], defined that docking is the process of fitting together of two molecules in 3-dimensional space. Pyne and Gayathiri (2005)[23] suggested that, if the molecules in nature have a tendency to be found in their low energy form, then the final configuration should also be of low energy. Understanding these properties is crucial in rationale design of potent drugs. Hence, docking allows the scientist to virtually screen the compounds and predicts the strongest binders based on various scoring function.

Fantin *et al*.**,** (1988)[24] reported that on the molecular level insulin receptors play key roles in the complex hepatic metabolic responses. Insulin receptors are unique docking molecules whose actions are very tightly regulated by the phosphorlytion at various sites. In this context, all the 4 compounds screened for the docking studies were docked with 1IRK with hydrogen bonds and other interactions producing lower energy value with high libdock score. Here, it is wise to recall the report of Taniguchi *et al*.**, (**2006)[25] which states that insulin receptor spans the cell membrane and functions as a receptor tyrosine kinase and hence, the binding of the ligands to the receptor on the outer surface of the plasma membrane could be involving in the initiation of phosphorylation cascade which could lead to the activation of proteins and induction of gene expression and could cause the up regulation Glucose-4 promoting glucose utilization.

PPARγ plays a critical role in glucose homeostasis and is the molecular target of a class of insulin-sensitizing drugs. In the present study the novel gymnemic diacetate and novel gymnemic triacetate, are interacted with PPARγ with low docking energy, and high lipdock score as well as hydrogen bonding. This shows that these compounds must possess an activating effect on PPARγ. As Walker *et al*., (1998)[10] stated, these 2 compounds could be the agonists for PPARγ and work by enhancing insulin action and promoting glucose utilization in peripheral tissues and lead to insulin sensitivity. According Florian *et al*.**,** (2006)[26] PPARγ ligands are widely used for the treatment of type -2 diabetes. So, these 2 compounds could also be consider as PPARγ ligands and could be developed in to drugs for the treatment of type-2 diabetes**.**

The docking interaction of the phytocompounds namely novel gymnemic diacetate and novel gymnemic triacetate with both insulin receptor and PPARγ indicates that these 2 compounds could posses an efficacy to promote both insulin receptor and PPARγ insulin signaling cascade. This confirms that these 2 compounds could be considered for drug design and development for the treatment of both type-1 and type-2 diabetes after *in- vitro*, *in- vivo* and clinical studies.

CONCLUSION

All the 4 compounds screened for docking analysis, are docked with insulin receptor and could be able promote the insulin signaling pathway. At the same time only 2 compounds are docked with PPARγ and could be able to promote PPAR signaling pathway. Hence it is clear that these 2 compounds could phosphorlyate both the insulin receptor and PPARγ there by providing sites for a multitude signaling molecules essential for diversification and modulation of insulin action as well as insulin sensitivity. Hence we could conclude that, these 2 compounds namely novel gymnemic diacetate and novel gymnemic triacetate, could be developed into the potent oral drugs for the treatment of diabetes**.**

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