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Research Article

In-silico Characterization of a Novel Bioactive Compound derived from *Psidium guajava* 4-[5-(Pyridin-4-yl)-1,2,4-oxadiazol-3-yl]-1,2,5-oxadiazol-3-amine: A Potential Inhibitor for Targeting Signaling Proteins involved in Diabetes Development

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ABSTRACT

Pharmacological interventions for diabetes predominantly involve chemically synthesized compounds, often causing undesirable side effects. This has led to a growing interest in plant-based therapeutic alternatives. Technological advancements have facilitated the discovery of bioactive phytochemicals with medicinal properties. This study employs molecular docking analysis to assess the antidiabetic potential of a naturally derived compound, 4-[5-(Pyridin-4-yl)-1,2,4-oxadiazol-3-yl]-1,2,5-oxadiazol-3-amine (POA), obtained from *Psidium guajava* leaf extract. The evaluation focuses on its inhibitory action against four human proteins 11 β -HSD1 (PDB: 4K1L), GFAT (PDB ID: 2ZJ4), SIRT6 (PDB ID: 3K35) and aldose reductase (PDB ID: 3G5E) associated with diabetes. Physicochemical, pharmacokinetic, and ADMET profiles were computed using online web servers molinspiration, ADMETLAB 2.0, and SWISSADME. POA demonstrated superior binding affinity (in Kcal/mol) -8.0, -7.5, -8.9 and -9.5, respectively) compared to the widely used diabetic drug metformin -5.4, -6.0, -5.4 and -7.2 with these receptor proteins. Based on molecular docking studies and pharmacokinetics/ADMET profiles, POA may act as a multitargeted, less harmful, and more efficacious medication for type 2 diabetes mellitus (T2DM) compared to metformin.

Introduction

Diabetes stands as the third most frequent disease, following cancer and heart-related disease as reported by IDF.[1] The global burden of diabetes is underscored by WHO statistics, revealing that 415 million individuals were diabetic in 2015, with projections estimating 693 million cases by 2045.[1,2] Diabetes encompasses various classifications, including type T1DM and T2DM, with T2DM accounting for over 90% of cases.[3] Characterized by insulin resistance and dysfunctional pancreatic cells, diabetes induces long-term metabolic disturbances affecting sugar, fat, and protein metabolism, leading to complications such as nephropathy, cardiovascular diseases, skin issues, and other problems.[4] Type 2 diabetes mellitus (T2DM) impacts individuals of all ages, marked by elevated blood glucose due to poor secretion of insulin.[5,6] The fundamental metabolic challenge associated with diabetes involves a relative deficiency in the secretion of insulin from β-cells in the pancreas and insulin resistance in specified tissues, disrupting the body's energy fuel homeostasis.[7]

The intricate web of cellular pathways associated in diabetes involve proteins such as 11β-HSD1, dipeptidyl peptidase IV, interleukin 1 beta, GFAT, PPAR-gamma, pyruvate dehydrogenase kinase, and other insulin receptors identified as a key regulator.[5] Various therapeutic interventions exist for diabetes management such as precautions in diet, physical activities, and

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antidiabetic medications. Concerns regarding serious side effects and disputed effectiveness have prompted a shift towards complementary and alternative therapies. Additionally, the exploration of food-derived phytoconstituents has gained much attention. Recent investigations into phytochemicals, such as abscisic acid, a phytochemical derived from okra, showed this paradigm shift. Molecular docking study of abscisic acid with nine selected human proteins (PDB ID: 4K1L, 3G5E, 4IXC, 3F7Z, 2ZJ4, 4MP2, 3DZY, 3K35, 1IR3) revealed that this may be a good option to develop an efficient antidiabetic drug.[8] Antidiabetic effects of fenugreek through integrated molecular docking, molecular dynamics, and network pharmacology studies revealed that it has antidiabetic properties through blocking the inflammatory signaling pathway, lowering the expression of inflammatory factors, and shielding islet cells, peripheral nerves, and the vascular endothelium against inflammatory cytokines.[9] In this context, *Psidium guajava* (guava) belongs to the family Myrtaceae, phylum Magnoliophyta, class Magnoliopsida, and height range of 5 to 10 meters, has gained attention for its historical use in Nigeria to treat typhoid fever.[10-15] Molecular docking studies of *P. guajava* deduced bioactive compounds with proteins involved in pancreatic cancer have also been carried out, a significant binding affinity (-9.6 Kcal/ mol) indicates that, with pharmacokinetics and toxicity level optimization, it may be used in pancreatic cancer treatment.[16] Molecular docking approaches and ADMET biographies of compounds and phyto- ingredients of *P. guajava* leaf extract were comprehensively probed for DNA gyrase subunits A(Gyr A) from *S. Typhi*. Results showed that only 2- hydroxy-cyclopentadecanone is reported to be for supereminent optimization due to its positive ADMET outlook.[17] Moreover, *P. guajava* seed extract has the capability to offset the seditious responses that are caused by indomethacin, as substantiated by its antiulcer effect.[18] At the moment, a more stringent *P. guajava* phytocompounds have proved *in-silico* anti-quorum sensing activities against *Salmonella enterica serovar Typhi*, and it was suggested that these compounds are more effective than ciprofloxacin, the conventional drug used to treat typhoid fever.[19] The antihyperglycemic, antihyperlipidemic, antiobesity potential and possible use in diabetes-associated disorders like high blood pressure and malfunction of kidney, all have been explored *in-vitro* investigations of guava leaves and fruits.[20] *P.guajava* extract treatment improved blood glucose lowering after a glucose load and lessened damage to the pancreatic islets in diabetic animals.[21] Antidiabetic potential of many medicinal plants such as *Allium sativum, T. foenum-greacum* and *Ficus bengalensis* has been explored.[22-25] More recently, bioactive compounds from *Trignonella foenum-graecum* have been identified to treat diabetes by *in-silico* studies involving molecular docking approach and ADMET predictions[26] and in the management of T2DM, the relevance of Indian traditional tisanes have also been explored.[27] These *In-silico* studies in the identification of potent phytochemicals in other complications such as cardiovascular disorders,[28] targeting COVID-19[29,30] and in cancer treatment[31] proved an accurate, fast and cost-effective method. Thus, advancements in computational research have enabled *in-silico* methods to provide revolutionary advantages for regulatory requirements and safety profile assessment in the pharmaceutical industry. In view of above research, this paper focuses on *in-silico* molecular docking study, of a specific compound derived from *Psidium guajava* leaves, namely POA, and its potential interactions with four key proteins associated with diabetes, namely 11 β -HSD1 (PDB: 4K1L), GFAT (PDB ID: 2ZJ4), SIRT6 (PDB ID: 3K35) and aldose reductase (PDB ID: 3G5E). This study aims to unravel POA's molecular and atomic interactions with studied receptor proteins. We have also calculated the pharmacokinetics and druglikeness/ADMET profiling of POA which explored it as a potential therapeutic agent for multitargeted diabetes management in comparison to metformin.

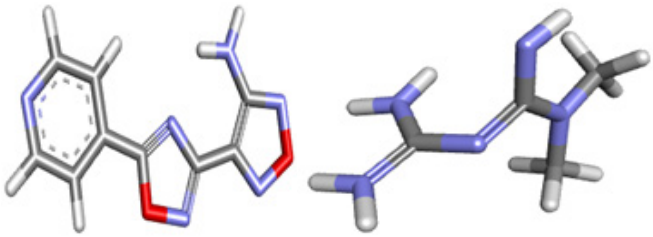
Materials and Methods

Proteins and Ligands Retrieval

The human proteins associated with diabetes mellitus (PDB ID: 4K1, 2ZJ4, 3K35, 3G5E) were downloaded from the RCSB Protein Data Bank[32] in pdb format. Subsequently, the proteins underwent preparation using Autodock Tools-1.5.7.[33] involving the removal of previously docked ligands and associated water molecules, inclusion of polar hydrogens and the incorporation of Kollman charges. The finalized protein structures saved in PDBQT format. Next, the ligand molecule POA (Pubchem CID 12007474, Fig. 1a) and metformin [a standard drug for T2DM Mellitus (Pubchem CID 4091, Fig. 1b)] were obtained from PubChem Data Bank[34] in 3D.sdf format. These ligand and Metformin structures were then converted into .pdb format using BIOVIA Discovery Studio Visualizer software.[35] The pdb-formatted ligand and drug molecules were further transformed into PDBQT format utilizing Autodock Tools-1.5.7.

Molecular Docking

The docking of proteins and ligand were executed by utilizing the AutoDock Vina version 1.1.2 software suite.[36] Flexible Blind docking was performed applying gridbox prepared to cover the whole protein having a value for exhaustiveness of ten. To ascertain the free energy (ΔG) that specifies the affinity scoring, the involvement of intra-molecular H- bonds as well as other interactions with docked complexes of protein and ligand was taken into account. After docking, best pose (with zero lower and upper rmsd value) have been taken for further analysis. Using the BIOVIA DS software, the docked complexes of



(a) (b)

Fig. 1: a) Structure of ligand POA and b) Metformin (Drug molecule)

protein and ligand were generated and for the analysis of binding patterns of these complexes, 2D and 3D illustration were drawn.

Computation of Inhibition Constant

The molecular docking analysis predicts the inhibition constant (Ki), which is utilized to evaluate the efficacy of the interaction. It also considers changes in hydrogen bonds formed with the protein's active site residues and predicted binding energies. The inhibition constant, or Ki value of the docked enzyme- inhibitor complex' is the dissociation constant (Kd). Lower dissociation probability and hence higher inhibition are associated with smaller Ki values. The formula Ki =exp(ΔG/(RT) is used to calculate it, in which temperature T (=298.15 K), gas constant R (=1.987 Kcal/K/mol), and ΔG is the free energy of binding.[37]

Pharmacodynamics of Ligand POA and Metformin

The ligand molecule POA and metformin (Standard dug for T2DM) were analyzed by molinspiration[38], an online screening server. SMILES of these two molecules were used to generate a 3D structure and .mol file was used for the calculation of molecular properties and bioavailability scores. A higher bioactivity score signifies an increased likelihood of the molecule's activity.

Pharmacokinetic and Drug-likeness Assessment of the Compound POA

The evaluation of a potential drug candidate relies significantly on its pharmacokinetic profile and toxicity. In the initial stages of computer added drug design (CADD), ADMET of drug molecules have been admitted as crucial factors. The SwissADME[39] and ADMETLAB2.0[40] online tools were used to evaluate the pharmacokinetics and likeness to be a drug of these molecules. The SMILES format of the molecules were used to generate 2D structure files in these tools. Several parameters were scrutinized to assess the ADMET properties of the molecules. Essential considerations for a drug molecule encompass pharmacokinetic parameters like P-glycoprotein, human intestinal absorption (HIA), drug-likeness predictions based on Lipinski, Ghose, and Veber criteria and BBB penetration. Evaluation of drug-likeness and the likelihood of bioactivity involved the examination of crucial parameters, including MW, LogP, number of HBA, and HBD, in accordance with Lipinski's "Rule of 5".[41] Lipinski's criteria indicate that most "drug-like" compounds adhere to conditions such as MW) ≤ 500, HBA ≤ 10, HBD ≤ 5 and logP ≤ 5. Violation of multiple principles may lead to potential issues with bioavailability. Descriptors such as HIA, bioavailability, CaCo-2 monolayer permeability, and BBB penetration are elucidated by ideal parameters like LogP and TPSA. These parameters play a pivotal role in predicting the drug-like qualities of a molecule.

Results and Discussion

Virtual screening, CADD is a quick, affordable, and reliable method for finding a novel drug molecule and a possible druggable receptor target. This study applied virtual screening through molecular docking to search for a promising candidate for T2DM. Proteins that serve as vital regulators in multiple biosynthetic pathways in T2DM, four human proteins with PDB IDs 4K1L, 2ZJ4, 3K35 and 3G5E were selected. The probable molecular docking interactions of the phytochemical POA, with these four proteins were investigated and results have been compared with well-known drug metformin. Binding energy of compound POA with these proteins was found to be -8.0 , -7.5, -8.9, and -9.5 (in Kcal/mol), respectively (Table 1). The binding affinity of the inhibitor was used to correlate and investigate with the corresponding receptor. Affinity of a ligand for the target receptor will generally increase with decreasing binding energy. Consequently, the ligand exhibiting the highest affinity can be considered as a potential subject for further investigation.

Molecular Binding Pattern of POA and Metformin with 11 β -HSD1 (PDB: 4K1L)

The 11 β-HSD1 enzyme catalyzes intracellular conversion of inert cortisone to active cortisol metabolically by employing NADPH as a co-factor.[42-45] By activating genes related to production of glucose in the liver, cortisol raises the amount of glucose produced by the body. Inhibiting this enzyme may offer a good treatment for type 2 diabetes by adjusting cortisone/cortisol levels.[46-49] An analysis by PLIP[50] online server for active site amino acids of chain A of 11 β-HSD1 enzyme (4K1L) with crystal structure of NDP (NADP) molecule exhibits hydrogen bonding with GLY41, SER43, LYS44, ILE46, ARG66, SER67, THR92, MET93, GLU94, ASN119, ILE121, TYR147, LYS187, ILE218, THR220, THR222 including other water and salt bridges. To investigate protein-ligand interaction, we performed blind docking by setting grid box over the whole protein. Interestingly in this analysis, we found that active site residues viz A:ARG66, A:GLU94 and A:ILE121 of the human 11 β -HSD1 enzyme strongly interact with our ligand POA by making three hydrogen bonds of shorter bond lengths of 2.72348 A^0, 2.98204 A^0 and 2.80218 A^0 (Table 1 and Fig. 2a, 2b) and having a better affinity and lower

inhibition constant (-8.0 Kcal/mole and 1.36 µM). In spite of these H-bonds it also binds with 8 other types of bonds (Pi-Cation, Pi-Sigma and Pi-Alkyl of Electrostatic and Hydrophobic nature) with A:ARG66, A:VAL142, A:ALA65, A:ARG66, and A:LYS44 (Fig. 2a, 2b). These bonds may help in stabilizing ligand. On comparison of interaction of ligand POA and metformin it is found that it binds with lower binding affinity and inhibition constant (-5.4 Kcal/mol and 10.99 µM) only with two H-bond with residue A:ASN119 *via* atoms H19 and H20 of bond lengths 2.93342 and 2.42113 A° (Table 1 and Fig. 2c, 2d). On the basis of binding patterns and this analysis it may be concluded that ligand POA may be a better natural potent inhibitor for human 11 β-HSD1 enzyme to treat T2DM mellitus and hyperlipidemia in comparison to metformin.

Molecular Binding Pattern of POA and Metformin with GFAT (PDB: 2ZJ4)

GFAT, a rate-limiting enzyme, is an essential regulator of the hexosamine biosynthesis pathway in T2DM.[51-53] This pathway is considered as a sensor for nutrition of cells by which peripheral insulin resistance is induced hyperglycemia.[54, 55] Studies show that human GFAT hyperactivity is linked to insulin resistance, making it a viable treatment option for type 2 diabetes.[56] The X-ray analysis reveals that the ligand AGP with GFAT forms H-bonds with THR375, SER376, SER420, GLN421, SER422, and THR425.[51] In this analysis, it was observed that residues A:THR375, A:THR425, A:SER422 and A:LYS675 of GFAT enzyme strongly interact with ligand POA by making four hydrogen bonds of shorter bond lengths of 2.35973, 1.86700, 3.08100 and 2.78462 A^0 (Table 1) and having a better affinity and inhibition constant (-7.5 Kcal/mole and 3.17 µM). In spite of these H-Bonds it also binds with 5 other types of bonds (Pi-Anion, Pi-Donor H-Bond, Pi-Alkyl, Pi-Alkyl and unfavorable donor-donor of electrostatic, H-bond and hydrophobic nature) with A:GLU560, A:SER376, A:LEU556, A:LEU673 and A:GLN421 (Fig.3a, 3b). These bonds may help stabilize the ligand. Interaction of Metformin shows that it also binds with lower binding affinity and inhibition constant (-6.0 Kcal/mol and 39.95 µM) *via* four H-bond with residue A:THR425,

Table 1: Binding affinities and inhibition constants (Inhb. Const.) of ligand POA and metformin with receptor proteins

S. No.	*Protein (PDB ID*	*Ligand and drug molecule*	*Binding affinity (ΔG) (kcal/mol)*	*Pred, Inhb. Const. Ki (µM)*	*No. of H-bonds*	*H-bonded residues*	*H-bond length (A^0)*	*No. of other types of bonds with protein*	**Other types of bonds within ligand-protein complex*
1	11 β -HSD1 (4K1L) Protein Chains (A, B, C, D)	POA	-8.0	1.36	3	A:ARG66 A:GLU94 A:ILE121	2.72348 2.98204 2.80218	8*	Electrostatic, Other, Hydrophobic, Pi-Alkyl, Unfav. Donor-Donor, Pi-Anion, Pi- Sigma, Pi- Cation , Pi-Pi Stacked, Pi-Pi T-Shaped
		Metformin	-5.4	10.99	2	A:ASN119 (H19) A:ASN119 (H20)	2.93342 2.42113	1 (Unfavorable Donor-Donor bond)	
2	GFAT (2ZJ4) Protein Chains (A)	POA	-7.5	3.17	4	A:THR375 A:THR425 A:SER422 A:LYS675	2.35973 1.86700 3.08100 2.78462	5*	
		Metformin	-6.0	39.95	4	A:THR425 A:LYS675 A:SER420 A:SER422	2.66148 2.74697 2.78283 2.45687	1 (Carbon-Hydrogen bond)	
3	SIRT6 (3K35) Protein Chains (A, B, C, D, E,F)	POA	-8.9	0.29	4	A:ARG63 A:GLN111 A:HIS131 A:LEU215	2.26865 2.75947 2.36165 2.42269	5*	
		Metformin	-5.4	10.99	2	A:GLN240 A:ASN238	3.07921 1.93053	4 Electrostatic (Attractive Charge) Carbon-Hydrogen bond)	
4	Aldose reductase (3G5E) Protein Chains (A)	POA	-9.5	0.10	3	A:SER210 A:ILE260 A:ILE260	3.16077 2.26822 2.56767	9*	
		Metformin	-7.2	5.27	4	A:ASP43 A:SER210 A:TYR48 A:GLN183	2.40854 2.30853 2.28415 2.02021	1 (Salt Bridge)	

Note: *no. of other types of bonds of POA with Protein molecules

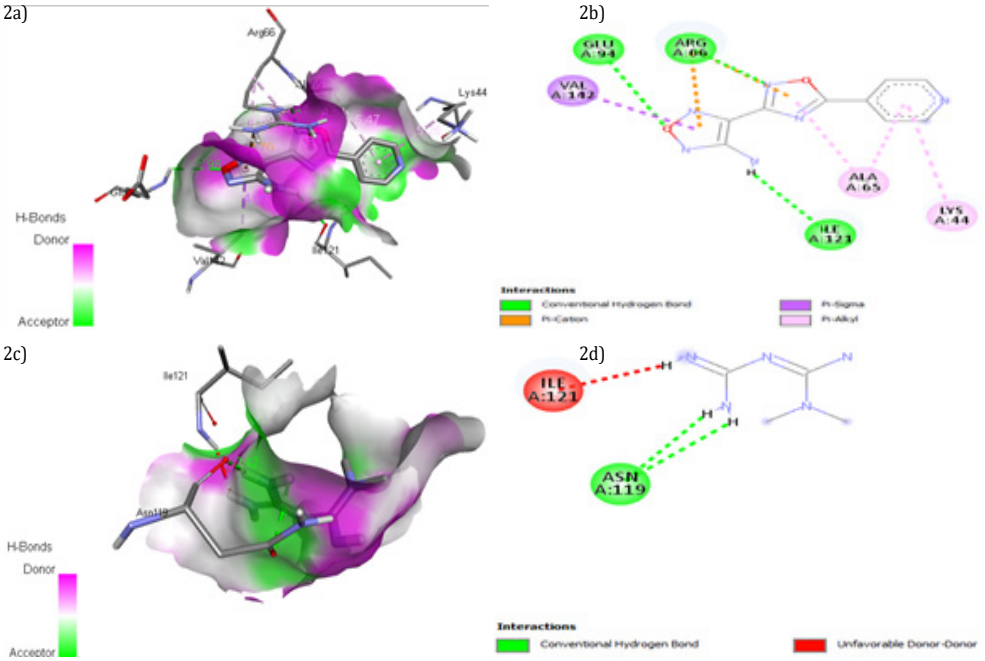


Fig 2: Molecular binding of POA and metformin with 11 β -HSD1 (PDB ID- 4K1L). (2a) Interaction of POA with residues along with H-bonds donor and acceptor regions. (2b) 2D plot with POA (2c) Interaction of metformin with residues along with H-bonds donor and acceptor regions. (2d) 2D plot with metformin

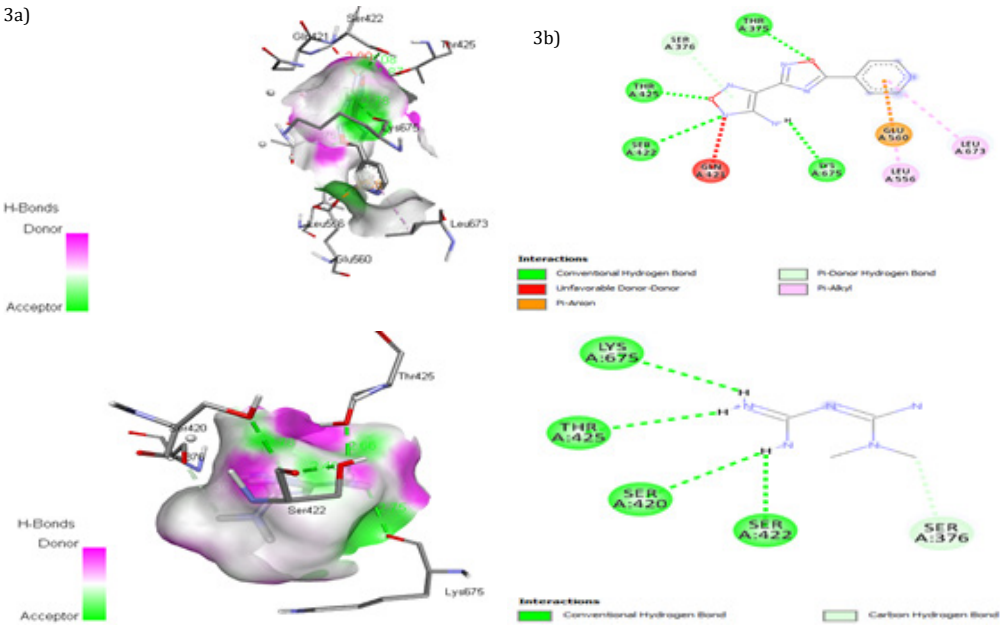


Fig. 3: Significant molecular bonding of POA and metformin with GFAT (PDB: 2ZJ4). (3a) Interaction of POA with residues along with H-bonds donor and acceptor regions. (3b) 2D plot POA (3c) Interaction of metformin with residues along with H-bonds donor and acceptor regions. (3d) 2D plot with metformin

A:LYS675, A:SER420 and A:SER422 of bond lengths 2.66148, 2.74697, 2.78283 and 2.45687 A^0. One Carbon Hydrogen Bond is also formed with residue A:SER376 of bond length 3.39161 A^0 (Table 1and Fig. 3c,3d). As a result, ligand POA may also act a potent inhibitor for GFAT enzyme to treat T2DM mellitus, comparatively metformin.

Molecular Binding Pattern of POA and Metformin with SIRT6 (PDB: 3K35)

Prominent among the mammalian sirtuins (SIRT1–7), In a number of functions of cells, it is found that SIRT6 plays an pivotal role, including the preservation of glucose homeostasis and DNA repair.[57-59] In order to regulate

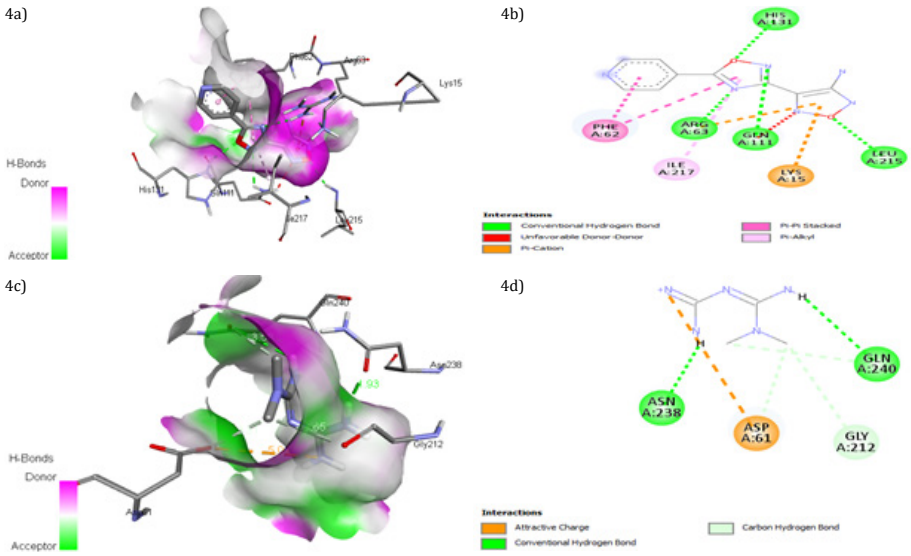


Fig. 4: Molecular binding of POA with human SIRT6 (PDB: 3K35). (4a) Interaction of POA with residues along with H-bonds donor and acceptor regions. (4b) 2D plot with POA (4c) Interaction of Metformin with residues along with H-bonds donor and acceptor regions. (4d) 2D plot with metformin

the cellular activity of different proteins, SIRT6 is a NAD+-dependent deacetylase.[60] Binding pocket within crystal structure of SIRT6 (complexed with ADP-ribose) exhibits GLN111, HIS131, ILE217, PHE62 and other residues bound with hydrogen binding.[57] Our investigation revealed that residues (A:ARG63, A:GLN111, A:HIS131 and A:LEU215) of this protein make four H-bonds with POA of bond length 2.26865, 2.75947, 2.36165 and 2.42269 A^0 (Table 1 and Fig. 4a, 4b). Interestingly, six other residues of SIRT6 viz. LYS15, PHE62, GLN111, ARG63 and ILE217 of chain A interact *via* electrostatic Pi-Cation, hydrophobic Pi-Pi stacked and one is hydrophobic Pi-Alkyl of bond length 4.22624, 3.61638, 4.03652, 4.8009 and 5.10158 A^0 with POA (Table 1 and Fig. 4a, 4b). These bonds may help stabilize ligand molecule, consequently providing higher affinity and lower inhibition constant (-8.9 Kcal/mol and 0.29 µM). Interaction of metformin shows that it only binds two H-bond with residue A:GLN240 and A:ASN238A of bond length 3.07921 and 1.93053 A°. Besides these bonds one electrostatic bond with A:GLN240 residue of bond length 3.07921 A^0 and three carbon hydrogen bond with A:ASP61, A:GLY212 and A:GLY212 residues are formed of higher bond length 3.30681, 3.65038 and 3.5812 A^0 (Table 1 and Fig. 4c,4d). This poor bonding exhibits lower binding affinity and higher inhibition constant (-5.4 Kcal/mol and 10.99 µM, Table 1) to metformin, showing poor inhibitor to SIRT6 enzyme compared to POA.

Molecular Binding Pattern of POA and Metformin with Aldose Reductase (PDB ID- 3G5E)

In the polyol pathway, aldose reductase limits the rate of reaction. With NADPH acting as a co-factor, excess D-glucose is transformed into D-sorbitol.[61] It is essential in the management of diabetic microvascular complications.[62] The metabolism of lipids also involves aldose reductase. An analysis by PLIP online serve for active site residues of chain A of aldose reductase (3G5E) with crystal structure of NDP (NADP) molecule exhibits hydrogen bonding with THR19, TRP20, ASP43, SER159, ASN160, GLN103, SER210, LEU212, GLY213, SER214, ASP216, LYS262, SER263, VAL264, THR265, GLU271, ASN272 including other π-Stacking, π-Cation Interactions and salt bridges. Our docking analysis exhibit a good binding energy (ΔG = -9.5 Kcal/mol, and Ki=0.10 µM Table 1) of POA with this protein forming three hydrogen bonds (out of two) forming with residues A:ILE260 (of bond length 2.26822 and 2.56767 A^0) and one H- bond with residue A:SER210 of bond length 3.16077 A^0. Besides these bonds it binds with eight other bonds with the residues A:LYS21, A:TYR48, A:CYS298, A:TYR209, A:TRP20, A:LYS262 and A:CYS298 viz., pi-cation, pi-donor hydrogen bond, pi-sulfur, two pi-pi stacked, pi-pi T-shaped and two pi-alkyl bond of bond lengths 4.29218, 3.72377, 4.76028, 3.67477, 5.07281, 5.53993, 4.94189, 5.48867 A^0 (Table 1 and Fig. 5 a and b). These interactions with the receptor molecule more stabilize ligand POA. It may be concluded that a very good binding affinity is due to pi-sulfur bond. Interaction of metformin shows that it binds with four H-bond with residues A: SER210, A:TYR48, A:GLN183 and A:TYR48 of bond lengths 2.40854, 2.30853, 2.28415 and 2.02021 A^0. Besides these bonds, one salt bridge with A: ASP43 residue of bond length 2.5929 A^0 is also formed (Table 1 and Fig. 5c, 5d). Binding affinity and higher inhibition constant of metformin (-7.2 Kcal/mol and 5.27 µM, Table 1) are improved due to more number of H-bonds but are less than POA molecule, showing it, a comparatively poor inhibitor to this enzyme than POA.

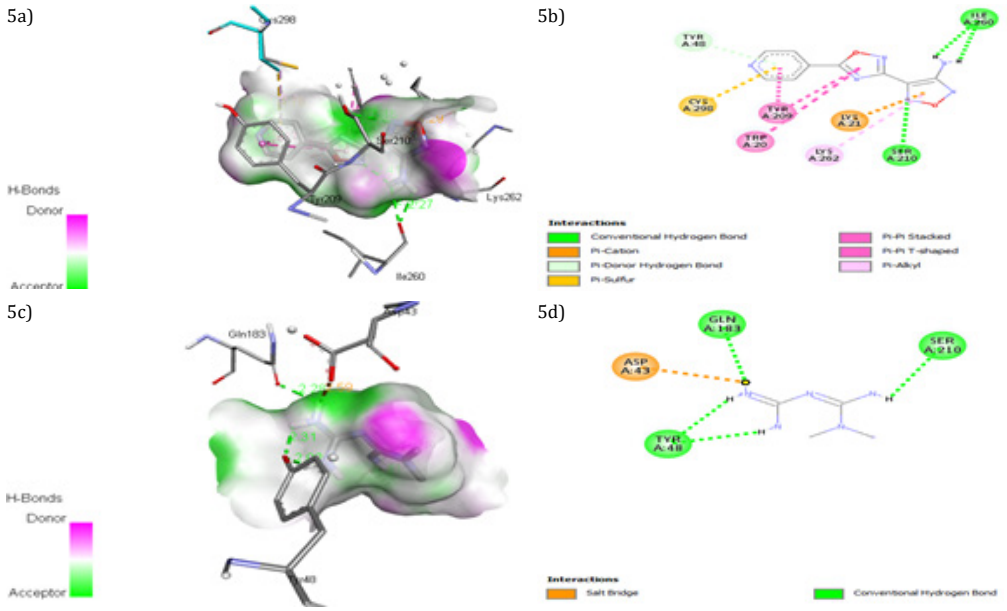


Fig. 5: Molecular binding of POA with 3G5E. (5a) POA interaction with residues, H-bonds donor, and acceptor regions. (5b) 2D plot with POA (5c) Interaction of Metformin with residues, H-bonds donor, and acceptor regions. (5d) 2D plot with metformin

Pharmacodynamics of POA Ligand and Metformin

The molinspiration bioactivity score (v22.08 beta) and molecular properties have been calculated for ligand POA and metformin and presented (in Table 2) for different parameters. On comparison, it is seen that the bioactivity score for each parameter of POA is much higher (less negative) than that of metformin. Thus it may be concluded that POA may serve as a leading inhibitor for these receptors than metformin.

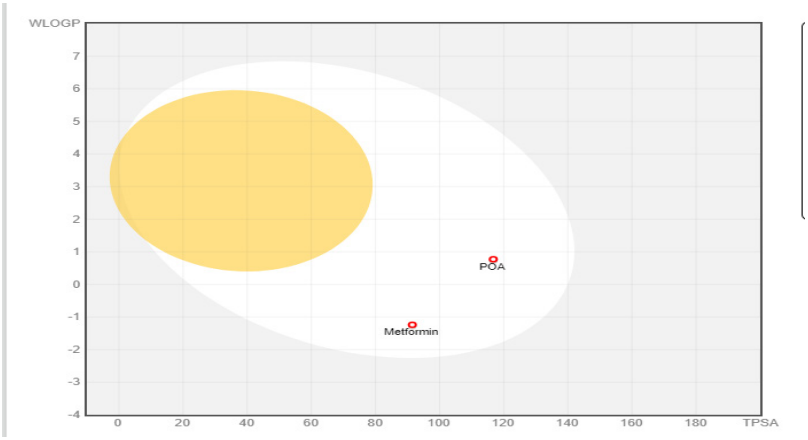
Pharmacokinetics and ADMET Assessment of POA and Metformin

The pharmacokinetics and drug-likeness parameters are presented in Table 3. POA and metformin both showed high human intestinal absorption (HIA: in Fig. 6a both lie in white portion of the boiled egg) and very low BBB permeability. Drug absorption in the human gut is modeled using the CaCo-2 permeability, which is higher (-4.489) for POA than metformin (-5.745). Thus POA is more suitable for oral dosing than metformin. Excretion (CL value) is high for POA than metformin, showing a better efflux. Drug-likeness prediction was also performed and Lipinski rule, GSK Rule, Pfizer rule and golden triangle rule were accepted by POA but in the case of metformin, the golden triangle rule was rejected. Hydrogen bonding potential and molecule bioavailability have a strong correlation with the TPSA value. The investigated compound POA's TPSA value of 116.75 A02 was observed to be significantly below the 140 A^{02} limit. Meanwhile, POA has molecular, physicochemical and ADMET characteristics within the range of upper and lower predicted values (Table 3 and Fig. 6, a, b, c). It has been concluded that POA may be a better drug molecule for T2DM in comparison to metformin.

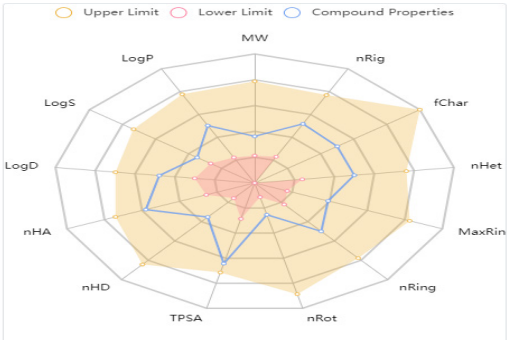
Table 2: Predicted bioactivity score and molecular properties of POA and metformin

S. No.	*Parameters*	*Bioactivity score*	
		POA	*Metformin*
1	G.P.C.R. ligand	-0.63	-1.61
2	Ion channel mod.	-0.45	-0.93
3	Kinase inhib.	-0.05	-2.83
4	Nuclear receptor	-1.54	-3.21
5	Protease inhib.	-0.97	-1.39
6	Enzyme inhib.	-0.35	-1.23
		Molecular properties	
		POA	*Metformin*
S.No.	Parameters		
1	LogP	0.3	-1.26
2	TPSA	116.76	91.50
3	natoms	17	9
4	MW	230.19	129.17
5	nON (HBA)	8	5
6	nOHNH (HBD)	2	5
7	nviolations	0	0
8	Nrotb	2	2
9	volume	180.50	126.68

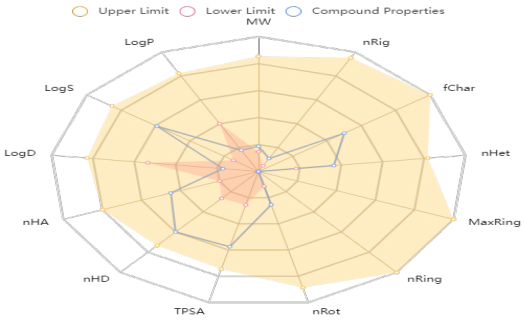
Additionally, docking analysis showed that aldose reductase (3G5E) protein revealed the best binding with POA (-9.5 Kcal/mol) and better inhibition constant (Ki), followed by SIRT6 (-8.9), 11β-HSD1 (-8.0) and GFAT (-7.5). On comparison of binding affinities of the molecule and metformin with different target proteins (Table 1, Fig. 7)



6a)



6b)



6c)

Fig. 6: (**a**) BOILED-Egg plot showing BBB penetration and HIA of POA and metformin molecules. (**b**) Radar graph* for POA (**c**) Radar graph* for metformin, *shows upper and lower range of various physicochemical and molecular properties with predicted values

Table 3: Pharmacokinetics, ADMET, and drug-likeness of POA and metformin*

Absorption	*Pred. value*	*Distribution*	*Pred. value*	*Metabolism*	*Pred. value*	*Excretion and Toxicity*	*Pred. value*
Water solubility (Log S)	-3.214 (-1.163)	Volume distribution (VD)	0.812 (1.083)	CYP2D6 and CYP3A4 subst.	No	Total drug clearance log (CLtot)	5.899 mL/min/kg (3.531)
Lipid solubility (Log P)	1.515 (-1.584)	Plasma protein binding (PPB)	72.27% (5.598%)	CYP2D6 inhib.	No	AMES toxicity, hERG I & II inhibitor	0.147 0.019
$CaCo_2$ Permeability	-4.489 (-5.745)	The fraction unbound in blood plasmas (Fu)	30.60% (76.538%)	CYP3A4 inhib.	No		
Log Kp skin permeability	-7.77 cm/s (-7.99) cm/s	BBB permeability	No	CYP1A2 inhib.	No		
HIA	High	--		CYP2C19 inhib.	No		
P-glycoprot. substrate, P-glycoprot. I & II inhibitor	0.144 0.001			CYP2C9 inhib.	No		
Gastrointestinal absorption	High						

*values in parentheses

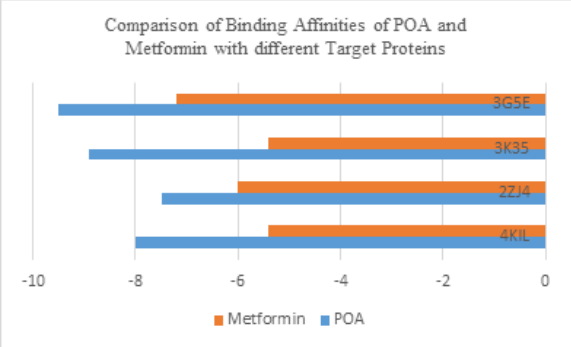


Fig. 7: Comparison of binding affinities of POA and metformin with different target proteins

revealed that POA may be a better multitargeted potent inhibitor to treat T2DM subject to successful clinical trials.

Conclusion

Present investigation related to the antidiabetic properties POA, a naturally derived compound from the leaf extract of *Psidium guajava* has revealed promising findings. Through meticulous molecular docking studies, It has been established the compound's adept binding affinity with four key proteins associated with diabetes mellitus—aldose Reductase, SIRT6, 11β-HSD1, and GFAT. Notably, the superior binding affinity of the compound, particularly with aldose reductase, positions it as a potential multitargeted inhibitor for type 2 diabetes mellitus (T2DM) treatment, outperforming the widely used metformin. The robust interaction patterns observed during docking studies and the strategic engagement of essential residues within the catalytic cavities of enzymes or active sites of proteins underscore the compound's potential therapeutic efficacy. A comprehensive analysis of pharmacokinetic and pharmacodynamic features, adhering to established drug-likeness guidelines, has further substantiated the compound's suitability as a medication for T2DM. The absence of detrimental side effects, coupled with molecular and physicochemical characteristics falling within specified bounds, positions the compound as an ideal candidate for further drug development. In light of these findings, POA emerges as a promising avenue for the development of a potent and well-tolerated medication for diabetes mellitus. Beyond its pharmaceutical potential, this naturally derived compound also holds promise for integration into functional foods and nutraceuticals, offering a multifaceted approach to addressing the global burden of diabetes and promoting overall health. Further research with clinical investigations is warranted to confirm and advance these encouraging outcomes toward practical therapeutic applications.

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