

Exploring the antihyperglycemic potential of tetrapeptides devised from AdMcl via different receptor proteins inhibition using in silico approaches

International Journal of
Immunopathology and Pharmacology
Volume 36: 1–15
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DOI: 10.1177/03946320221103120
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Abstract

Introduction: Diabetes mellitus is a heterogenous group of chronic metabolic disorders that results due to deficiency in insulin secretion and signalling. Multiple factors held responsible for onset of diabetes due to defects in glucose metabolism and cellular signalling mechanism. Over the past few years, many plant derived bioactive compounds have been recorded with increased efficacy and fewer side-effects against variety of diseases.

Methods: In the current study, molecular docking and molecular dynamics simulation approaches were employed to evaluate the tetrapeptides devised from AdMcl protein of *Momordica charantia*. Due to unavailability of appropriate template for modelling of 3D structure of AdMcl protein, I-TASSER server was employed for prediction of good quality tertiary structure. Predicted model was refined by GalaxyRefine Web and evaluated by Verify 3D, ERRAT and Ramachandran plot analysis. Next, a ready-to-dock library of fifty tetrapeptides as potent inhibitors was prepared and docked against aldose reductase (AR), protein tyrosine phosphatase 1B (PTP1B), α -glucosidase, α -amylase and glycogen synthase kinase 3-beta as receptor proteins. Molecular dynamics (MD) simulation was performed on Schrodinger's Desmond Module to check stability of the best docking complex.

Results: Top five ligands were selected against each receptor protein based on their binding pattern and docking scores. Among selected ligands (i.e. VEID, TVEV, AYAY, EEIA, ITTV, TTIT, LPSM, RGIE, TTVE and EIAR) followed all parameters in drug scanning and ADMET screening tests. The MD simulations confirmed that the best selected peptide (i.e. VEID) docked with AR and PTP1B was structurally stable.

Conclusion: In the light of overall results of all analyses employed in this study, the selected ligands could be further processed as potential hypoglycaemic drug candidates.

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Keywords

ADMET, Lipinski's rule of five, molecular docking, molecular dynamics simulation, *Momordica charantia*

Date received: 12 January 2022; accepted: 5 May 2022

Introduction

Diabetes mellitus (DM) is a group of heterogeneous chronic disorders characterized by elevated level of glucose. Among several categories of DM, the two main subtypes type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are the most common which are resulted due to progressive destruction in the pancreatic beta cells.¹ The prolonged hyperglycaemic condition in diabetes causes severe damage of many body organs including eyes, kidney, brain and heart. The defect in insulin secretion and activity causes the abnormality in carbohydrate, fats and protein metabolism.² The cumulative diabetic hyperglycaemia is associated with high risk of developing vascular, renal, neurological, immunological and retinal complications.³ According to an estimate, over 400 million people were suffering from DM by the year 2015 and the number is predicted to increase to 642 million by 2040.⁴

Plants derived natural compounds are a useful source in the management of different health-related issues and diseases. These phytochemicals have been reported as anti-carcinogenic, anti-diabetic, antioxidant, antiviral, antimicrobial and anti-inflammatory agents.^{5,6} *Momordica charantia* is commonly called as bitter melon notably known as antifungal, antibacterial, anti-inflammatory and anti-diabetic agent. Moreover, the extract of different parts of the plant contains different hypoglycaemic compounds including charantin, polypeptide-p, cucurbitane type glycosides, vicine and AdMc1 protein. Despite of many therapies, DM is still the most common chronic disorder and considered as seventh leading cause of death worldwide.⁷ In our study, we predicted the tertiary structure of AdMc1 protein and designed molecular docking-based study to explore the inhibitory patterns of different ligands devised from AdMc1 protein to target different proteins including aldose reductase (PDB ID: 4LAU), protein tyrosine phosphatase 1B (PDB ID: 2QBS), α -glucosidase (PDB ID: 5KZW), α -amylase (PDB ID: 4W93), and glycogen synthase kinase 3-beta (GSK-3 β) (PDB ID: 1Q41). Being a seed storage protein, the AdMc1 protein can be extracted from the forming seeds of unripe fruits.⁸

Aldose reductase (AR) is the major rate limiting enzyme of polyol pathway that catalyses glucose into sorbitol mediated by NADH as a cofactor and plays a critical role in diabetes complications.⁹ Under normal glucose metabolism, only minimal amount of glucose enters in polyol

pathway in non-phosphorylated form but under extreme hyperglycaemia 30% of the glucose enters into polyol pathway which turns the oxidative stress rate high in cell and tissues damage.¹⁰ The prolonged hyperglycaemic state triggered the over expression of aldose reductase results in the accumulation of sorbitol. Therefore, the need of aldose reductase inhibitors is necessary to avoid the oxidative stress in cells due to accumulation of alcoholic sorbitol to decrease the complication of diabetes. Protein tyrosine phosphatase 1B (PTP1B) plays a critical role in multiple cellular signalling pathways more specifically in the insulin signalling and glucose metabolism by dephosphorylation of insulin receptor kinase substrates.¹¹ The previous studies illustrated that elevated level of PTP1B is the leading cause of many metabolic disorders including DM and obesity. As a negative regulator of insulin signalling pathway, the PTP1B is an attractive pharmacological target for diabetes and obesity.¹²

The α -glucosidase and α -amylase are a group of primary enzymes that play critical roles in carbohydrate metabolism and glycoprotein synthesis. The α -glucosidase is an attractive target to delay the postprandial glucose absorption and digestion in DM.¹³ The α -amylase being a brush border enzyme hydrolyses the disaccharides and oligosaccharides into monosaccharides. The α -glucosidase and α -amylase inhibitors stop the degradation of sugar in hyperglycaemic state to control the T2DM.¹⁴ Glycogen synthase kinase 3-beta (GSK-3 β) is a serine/ threonine kinase and responsible for multiple functions such as glycogen metabolism, insulin signalling and neuronal functioning.¹⁵ The over-expression of GSK-3 β causes the disruption in insulin signalling pathways by inactivating various glucose reducing enzymes. Hence, the targeting of GSK-3 β is an effective method to control DM.¹⁶ The importance of plants derived natural bioactive compounds in the management of diabetes mellitus has been reported in literature.¹⁷

The current ongoing synthetic combinational drug therapies for diabetes at clinical stage are correlated with many other complications with severe side-effects. In this study, the binding and interaction patterns of plant-derived peptides have been evaluated as inhibitors of different receptor proteins involved in the events of DM. The aim of this study was to target different glucose transporters to control hyperglycaemic condition. The main purpose of this study was to check the insulin-like activity and drug ability of *M. charantia* derived tetrapeptides to report them

as anti-diabetic agents against different receptor proteins.

Material and methods

This is an in silico study in which tetrapeptides from the AdMc1 protein of *Momordica charantia* were devised and evaluated for their anti-diabetic potential. The study took 6 months and was conducted in the Molecular and Medical Genetics Lab., Department of Biochemistry, Government College University Faisalabad, Pakistan.

3D structure prediction, refinement and validation

The three-dimensional (3D) structure of AdMc1 protein from *M. charantia* was predicted using amino acid sequence of the protein. Due to absence of suitable template, the 3D structure was predicted with the help I-TASSER server,¹⁸ and refined by GalaxyRefine server.¹⁹ Verify 3D,²⁰ ERRAT and Ramachandran plot analysis,²¹ were also used to evaluate the predicted and refined tertiary structure.

Devising of tetrapeptides as ligands

Motifs are the consensus and conserved sequence patterns related to distinct properties of proteins and nucleotides. The AdMc1 protein from *M. charantia* was retrieved from NCBI's Entrez Protein database under accession number: CDG50933.1. The BLAST was performed to find homologs of AdMc1 protein to explore the conserved regions among them. The MEME suite was used to predict five motifs from ten selected homologs.^{22,23} Among all predicted motifs, 50 tetrapeptides were prepared from the most consensus regions. The chemical structures of predicted tetrapeptides were drawn using ACD/ChemSketch software and saved in MOL format.²⁴ All tetrapeptides were minimized and saved into MOE database in .mdb format for docking purpose.

Retrieval and optimization of receptor proteins

The 3D structures of aldose reductase (PDB ID: 4LAU), protein tyrosine phosphatase 1B (PDB ID: 2QBS), α -glucosidase (PDB ID: 5KZW), α -amylase (PDB ID: 4W93) and glycogen synthase kinase 3-beta (GSK-3 β) (PDB ID: 1Q41) were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>). All the receptor proteins were optimized with default parameters including removal of water molecules, addition of hydrogen atoms, energy minimization and 3D protonation in MOE software. The active sites of all the receptor proteins were predicted by MOE site finder tool with default score setting as finding the active site is the most critical step in docking experiments. The ligand database of 50 tetrapeptides was docked against each receptor protein separately. Among all

protein-ligand interactions, only top five interacted ligands were selected on the basis of best S-scores and root mean square deviation (RMSD) values.

Molecular docking

The molecular docking of plant-based tetrapeptides was conducted against five receptor proteins using MOE. A ready-to-dock library of 50 tetrapeptides was prepared from the conserved regions of AdMc1 protein of *M. charantia* and docked against the active sites of selected receptor proteins separately. The docking analysis was done using parameters as rescoring 1: London dG; retain: 10; refinement: force field; rescoring 2: London dG and retain: 10.

In silico drug scan and ADMET profiling

The drugability of selected ligands based on Lipinski's rule of five (Ro5) was evaluated using SwissADME.²⁵ According to Ro5, the potent drug candidate must have molecular mass: ≤ 500 Dalton, molar refractive index: 40–130, partition coefficient (log P): ≤ 5 , hydrogen bond donors: ≤ 5 , and hydrogen bond acceptors: ≤ 10 . The online bioinformatics tool admetSAR was used to discover the ADMET (absorption, distribution, metabolism, excretion and toxicity) attributes of the selected molecules.²⁶ Only those peptides were considered as lead drug candidates that accomplished all the ADMET models successfully.

Molecular dynamics simulation

Molecular dynamics (MD) simulation is used to check the stability of the docking complex.²⁷ The top-ranked complexes (i.e. AR-VEID and PTP-VEID) were chosen for the MD simulation study based on molecular interaction and visual examination of human AR and PTP docking findings. The MD simulation was performed on Schrodinger's Desmond Module.²⁸ To make predictions, a water-soaked solvent solution was employed. The water-soaked solvated system was created in Desmond using the System Builder tool. The TIP3P water model is being investigated for resolving the problem. The orthorhombic simulation was created using a box with periodic boundary conditions and a buffer distance of at least 10 Å from the protein's outer surface. An appropriate quantity of counter-ions was added to the system to neutralize it. The simulation box's isosmotic state was maintained by adding 0.15 M NaCl. Before the simulation's production run, a predetermined equilibration process was followed. The MD simulation was performed at a temperature of 300K and a pressure of 1.013 bar. The simulation was lasted for 200 nanoseconds, and 1000 frames were recorded to the trajectory. The MD simulation trajectory was analysed using the Simulation Interaction Diagram.

Statistical analysis

Different online tools and software were used for statistical analyses. The 3D structure was predicted using I-TASSER server and evaluated using verify 3D, ERRAT and Ramachandran plot analysis. The UCSF Chimera was used for model visualization and RMSD prediction. The MEME suite and MOE were used for the prediction of motifs and docking, respectively. The SwissADME was employed for drug scan and admetSAR was used for ADMET profiling. Schrodinger's Desmond Module was used for the simulation studies.

Results

3D structure prediction, refinement and validation

The I-TASSER server was employed to predict the 3D model of AdMc1 protein (Figure 1(a)). The GalaxyRefine

server enhanced the number of residues in the Ramachandran favoured regions from an initial 69% to 86% after refinement of the predicted model. Among five represented models by GalaxyRefine server, the model 1 was selected on the basis of best RMSD (0.577) and GDT-HA (0.9040) scores.

The structural evaluation is an important step to check the stability and accuracy of the predicted model. According to Verify 3D, 80.43% of the amino acid residues showed averaged 3D-1D score ≥ 0.2 . If at least 80% of the amino acid residues of the predicted model show ≥ 0.2 3D-1D score then the model is considered as of good quality. The ERRAT score of the predicted model was 85.1562 that indicates good quality of the refined model. Ramachandran plot was used to analyze the polypeptides backbone regions and torsion angles (psi) ψ against (phi) ϕ of amino acid residues present in the protein. The AdMc1 3D model acquired 84.8% of the residues in the Ramachandran plot

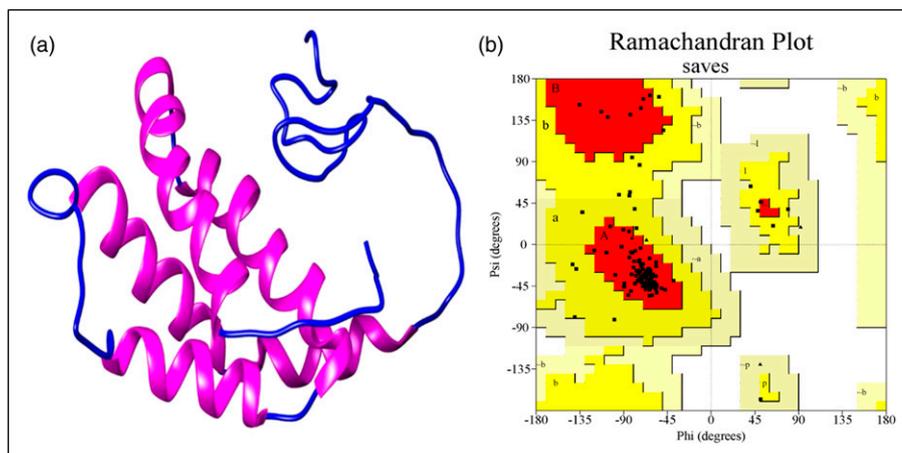


Figure 1. Prediction and evaluation of 3D structure of AdMc1 protein. (a) Predicted 3D model of AdMc1 protein by I-TASSER server; (b) Ramachandran plot analysis of best selected model. Most favoured regions are highlighted as red and allowed regions are highlighted as yellow.

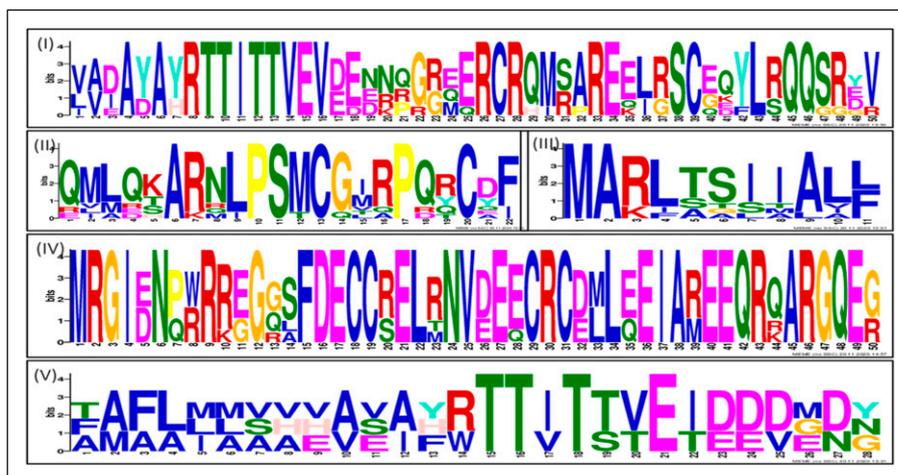


Figure 2. Predicted motifs used to devise tetrapeptides from AdMc1 of *Momordica charantia*.

favoured region (A, B, L), 15.2% in additionally allowed regions (a, b, l, p), 0.0% residues in disallowed regions (~a, ~b, ~l, ~p) and 0.0% residues belonged to generously allowed regions which signify the accuracy of the GalaxyRefine predicted model (Figure 1(b)).

Devising of tetrapeptides as ligands

The sequence of AdMc1 protein was used to search the homologs using BLAST and top ten homologs were selected on the basis of query converge. The MEME Suite was used to explore five motifs from the selected homologs (Figure 2). The most conserved regions of the predicted motifs were used to devise 50 tetrapeptides as ligand molecules.

Molecular docking

Molecular docking predicts the perfect orientation of ligand molecules to bind with the receptor molecule. This study includes ready-to-dock library of 50 tetrapeptides devised from AdMc1 protein of *M. charantia* that was docked against aldose reductase, protein tyrosine phosphatase 1B, α -glucosidase, α -amylase and glycogen synthase kinase 3-

beta (GSK-3 β) as receptor proteins using MOE software. The top hit compounds were selected on the basis of their S-scores and energy validations. The receptor proteins were selected due to their functional roles in onset of diabetes and up-regulation of glucose homeostasis.

Interaction analyses

For each receptor molecule, top five conformations were selected on the bases of S-scores, structural interactions and binding patterns (Table 1). For aldose reductase, the peptide VEID with S-score of -13.6772 showed interactions with active amino acids (i.e. Tyr48, Ala299) of the binding pocket of the receptor protein (Figure 3). The remaining four peptides (i.e. LEEI, TVEV, AYAY and EEIA) also showed good S-scores and binding interactions with active amino acids.

Protein tyrosine phosphatase 1B is an intracellular protein and emerged as a key regulator of various signalling networks. The peptide VEID with S-score of -12.9187 was found as best ligand (Figure 4) while the peptides TVEV and ITTV with S-scores of -11.2355 and -10.8165 , respectively, also interacted with major active amino acids (i.e. Arg47, Tyr46, Lys41) of the binding pocket of the

Table 1. Interactions of top five devised peptides with five receptor proteins.

Sr. No.	Ligand	Receptor	S-score	RMSD	Interactions
1	VEID	Aldose reductase	-13.68	2.07	Tyr48, Ala299
2	LEEI		-13.57	2.80	Trp111
3	TVEV		-13.16	0.90	Trp111, Tyr48, Gln49
4	AYAY		-13.08	1.71	Trp111, Ala299
5	EEIA		-13.06	1.69	Trp111
6	VEID	PTP-1B	-12.92	1.76	Arg47, Lys36, Tyr46, Ser50
7	LEEI		-12.68	1.77	Ala35
8	TVEV		-11.24	1.95	Lys41, Ala35
9	AYAY		-10.93	1.96	Lys36
10	ITTV		-10.82	2.17	Arg47, Lys36
11	LEEI	α -glucosidase	-14.76	1.27	Asp404, Try481, Asp518
12	ITTV		-13.41	1.23	Asp616, Ser676
13	TTIT		-12.93	1.36	Asp616, Asp282
14	LPSM		-12.61	1.57	Arg600
15	EEIA		-12.50	2.01	Asp616, Asp518
16	EIDD	α -amylase	-12.43	2.14	Asp300
17	TTIT		-11.95	1.28	Glu233, His201, Tyr151
18	RGIE		-11.48	1.86	Asp300, Asn298, Gln63
19	NVDE		-11.32	2.65	Asp300, Glu233, His299
20	TTVE		-11.01	2.57	Glu233
21	TTVE	GSK3- β	-17.14	1.59	Arg141, Asn64, Gln185, Thr138
22	EIAR		-17.09	1.22	Asn186, Asp200
23	AYAY		-15.59	2.72	Val135, Ile62, Lys183, Ser203
24	TTIT		-15.17	1.44	Val135, Asn64, Asn186, Arg141, Tyr134
25	LEEI		-14.80	1.83	Gln185, Ile62, Pro136, Asn186

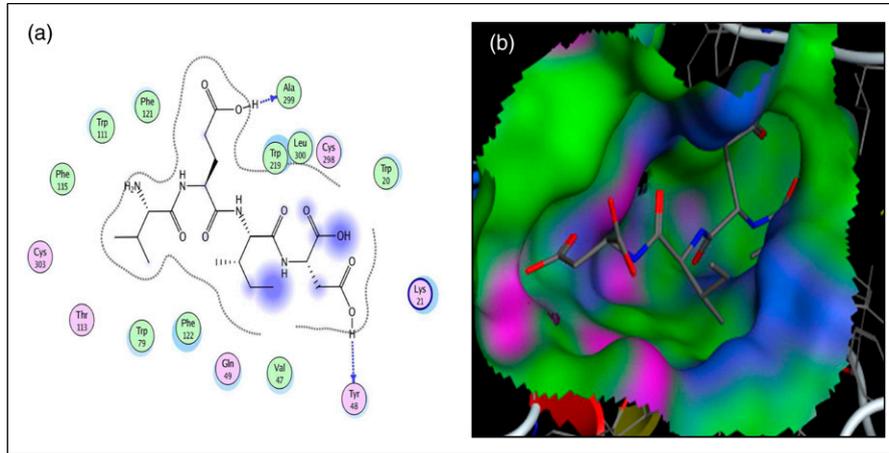


Figure 3. Interaction (a) and binding patterns (b) of VEID peptide with aldose reductase receptor.

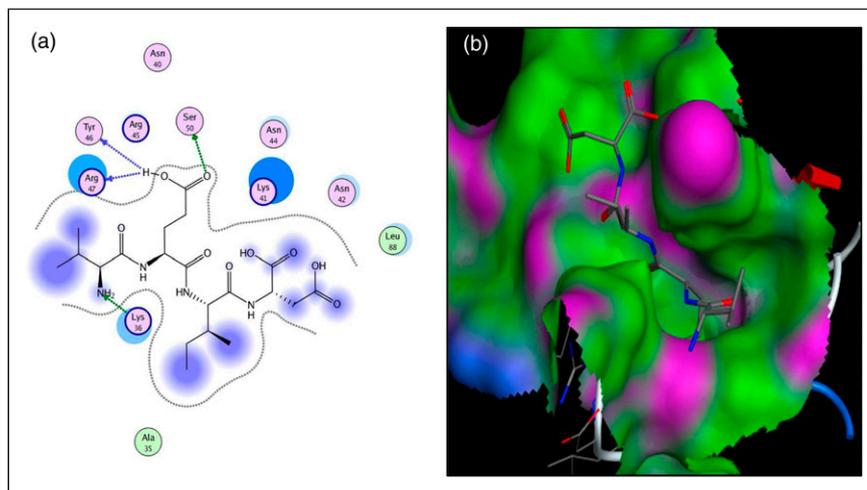


Figure 4. Interaction (a) and binding patterns (b) of VEID peptide with Protein Tyrosine Phosphatase 1B receptor.

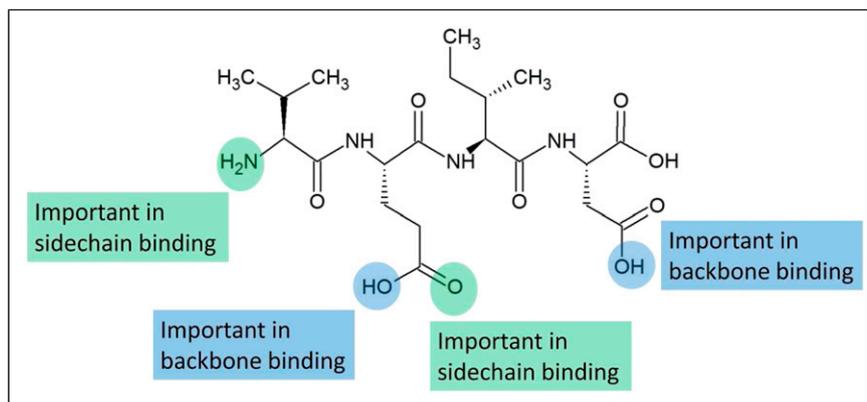


Figure 5. Structure-activity relationships of peptide VEID with receptors aldose reductase and protein tyrosine phosphatase 1B.

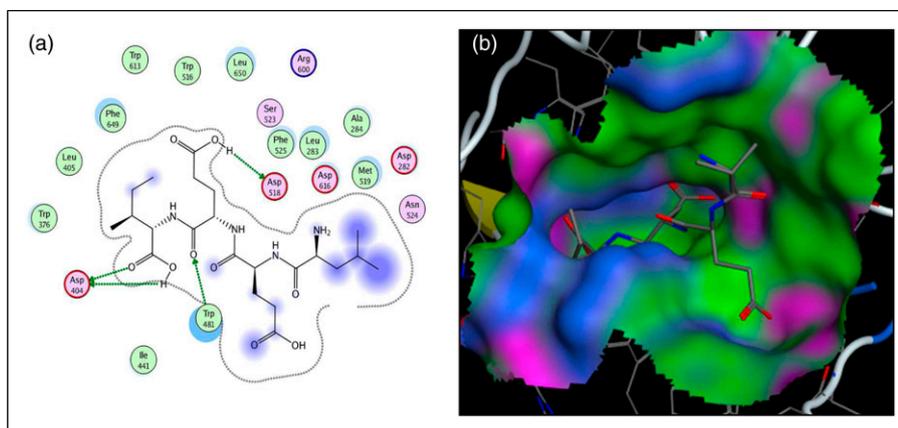


Figure 6. Interaction (a) and binding patterns (b) of LEEI peptide with α -glucosidase receptor.

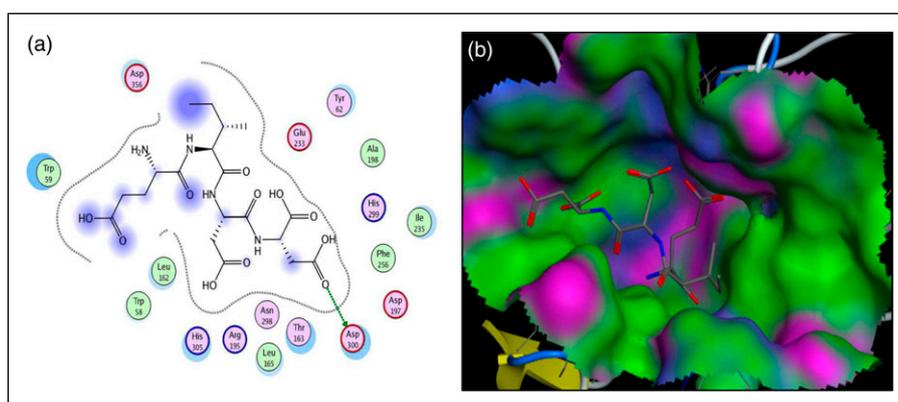


Figure 7. Interaction (a) and binding patterns (b) of EIDD peptide with α -amylase receptor.

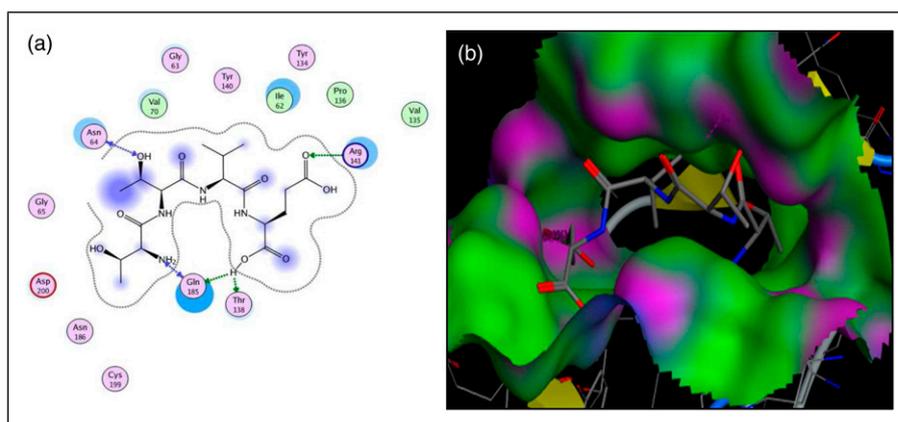


Figure 8. Interaction (a) and binding patterns (b) of TTVE peptide with GSK-3 β receptor.

receptor protein. The structure-activity relationship of VEID with receptors aldose reductase and protein tyrosine phosphatase 1B is shown in [Figure 5](#).

The next two receptor proteins α -glucosidase and α -amylase belong to the class of catalyzing enzymes and involved in carbohydrates metabolism. The active site of

α -glucosidase was comprised of Asp404, Asp518, Asp616, Arg600 amino acids and interacted with all the selected ligands with top S-score of -14.7575 by the ligand LEEI ([Figure 6](#)). All remaining ligands also showed good S-scores and interactions. Against α -amylase, the ligand EIDD with S-score of -12.4265 interacted with active

amino acid (Asp300) of the binding pocket (Figure 7). All the remaining ligands also showed excellent interactions and S-scores with other active amino acids (i.e. Asp300, Glu233, His201, Tyr151 and His299) of the active site.

Glycogen synthase kinase 3-beta (GSK-3 β) is a cellular kinase and plays a critical role in insulin signalling and glycogen metabolism. In this study, the peptide TTVE with S-score of -17.1351 showed interactions with Arg141, Asn64, Gln185 and Thr138 (Figure 8). The top five ranked ligands also showed good S-scores and binding interactions with active residues (i.e. Arg141, Gln185, Asn186, Asp200, Val135, Tyr134 and Pro136) of the receptor protein.

Drug scan and ADMET screening

The Lipinski's rule of five illustrates the drug-like behaviour of proposed drug candidates. In the current study, top five ligands against each protein were selected on the bases of S-score, ligand interactions and energy validations. Among selected ligands, the peptides VEID, TVEV, AYAY, EEIA, ITTV, TTIT, LPSM, RGIE, TTVE and EIAR violated only one rule and the peptides LEEI, EIDD and NVDE violated two rules of Ro5 (Table 2).

Evaluation of ADMET based properties of leading drug compounds are the crucial step in drug discovery. ADMET (absorption, metabolism, distribution, excretion and toxicity) are the five parameters to check the bioavailability and drugability of drug candidates. The above parameters evaluated the leading drug candidates using different threshold values. All the hit compounds for each protein

passed the threshold of drugability. The results of admetSAR suggested that all the selected ligands are non-Ames toxic and non-carcinogens (Table 3). The evaluation of ADMET profiling of each ligand revealed that all the selected ligands are tolerable and safe and therefore they could be referred as efficient drug candidates against selected receptor proteins.

Molecular dynamics simulation

Docking alone cannot provide full insight into the binding mode, stability and dynamics of proposed ligands.²⁹ Therefore, MD simulation was carried out for different nanoseconds frames based on stability point of the docked complexes with human AR and PTP proteins using Desmond module of Schrödinger. For dynamics understanding, MD simulations of the best ligand (i.e. VEID) for aldose reductase and protein tyrosine phosphatase were performed. The MD simulation revealed that VEID fits tightly into the binding pocket of human AR as simulation trajectories of both ligand and receptor are aligned. Human AR and VEID made a strong complex and showed a strong structural stability after docking as the RMSD of the complex was 0.5 Å (Figure 9(a)) throughout 200 ns MD simulation which is almost a negligible difference.

Similarly, the MD simulation RMSD trajectory of human PTP and VEID has revealed that the complex is structurally stable with RMSD value of 1.1 Å to 1.6 Å (change in RMSD is below 3 Å) throughout 200 ns time period (Figure 9(b)). The combined RMSD of the protein-ligand complex during MD simulation also remained stable throughout the simulation time frame of 200 ns as

Table 2. Drug-likeness properties of selected peptides.

Peptides	Receptor	Molecular properties ^a						
		MW (g/mol)	HBD	HBA	nrotb	Log P	A	Violations
VEID	AR/PTP	474.51	7	10	18	-0.87	115.28	1
TVEV	AR/PTP	446.50	7	9	16	-0.93	109.86	1
AYAY	AR/PTP/GSK	486.52	7	8	14	-0.17	126.30	1
EEIA	AR/GS	460.48	7	10	18	-1.34	110.47	1
ITTV	PTP/GS	432.51	7	8	15	-0.87	109.25	1
TTIT	GS/Amy/GSK	434.48	8	9	15	-2.06	105.61	1
LPSM	GS	446.56	5	7	15	-0.80	117.58	1
RGIE	Amy	473.52	9	9	20	-2.13	117.80	1
TTVE	Amy/GSK	448.47	10	8	16	-2.05	106.22	1
EIAR	GSK	487.55	9	9	20	-1.60	122.61	1
LEEI	AR/PTP/ GS/GSK	502.56	7	10	20	-0.50	124.90	2
EIDD	Amy	490.48	8	12	19	-2.10	112.25	2
NVDE	Amy	475.46	8	11	18	-2.97	108.58	2

MW: Molecular weight, HBD: Number of hydrogen bond donors; HBA: Number of hydrogen bond acceptors, nrotb: Number of rotatable bonds, log P: The logarithm of octanol/water partition coefficient, A: Molar refractivity.

^aMolecular properties were calculated using SwissADME an online tool.

Table 3. ADMET profiling of best hit compounds.

Ligand	Absorption and distribution				Metabolism										Toxicity	
	BBB	HIA	Caco-2 Permeability	PGS	PGI	ROCT	CYP3A4 substrate	CYP2C9 substrate	CYP2D6 substrate	CYP3A4 inhibition	CYP2C9 inhibition	CYP2C19 inhibition	CYP2D6 inhibition	CYP1A2 inhibition	AMES toxicity	Carcinogens
VEID	+	-	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
LEEI	+	+	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
TVEV	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
AYAY	-	+	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
EEIA	+	-	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
ITTV	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
TTTT	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
LPSM	+	-	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
EIDD	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
RGIE	+	+	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
NVDE	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
TTVE	+	-	Caco2-	NS	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC
EIAR	+	+	Caco2-	Substrate	NI	NI	NS	NS	NS	NI	NI	NI	NI	NI	NAT	NC

BBB: Blood-Brain Barrier; HIA: Human Intestinal Absorption; PGS: P-glycoprotein substrate; PGI: P-glycoprotein inhibitor; ROCT: Renal Organic Cation Transporter; NS: Non-substrate; NI: Non-inhibitor; NAT: Non-Ames toxic; NC: Non-carcinogenic.

combined trajectories did not show any notable fluctuations in trajectory or RMSD change. From this stability, it is elucidated that with respect to time this docking complex is structurally stable after making hydrogen bonds and other interactions.

The protein-ligand interaction fingerprint (PLIF) and protein-ligand contact graphs were analysed to study time-dependent changes in the interactions of the ligand with key residues of receptor proteins. The interaction histograms are showing the crucial amino acids of human AR (Figure S1(a)) and PTP (Figure S1(b)) proteins for interactions with VEID which are involved in hydrogen bonds, hydrophobic and ionic interactions, and water bridges. Complex stabilization can mainly be attributed to several

H-bond contacts and π - π interactions. The protein-ligand interaction diagram (Figure S2) indicated that VEID has made strong hydrogen bond interactions with Lys21, Asp43, Tyr48, Asn160 and Gln183 of human AR while water-mediated H-bond interactions of VEID were noted with Trp20, Tyr209 and Cys298. The π - π interactions were not found between human AR (receptor) and VEID (ligand). Notably, in case of human PTP, the Gly220 showed maximum number of interactions with VEID (ligand). In addition, protein-ligand interaction diagram has showed VEID interaction types and intensities with human PTP protein (Figure S3). The VEID docked complex has showed strong hydrogen bonds with Ser216, Ala217, Gly218, Ile219, Gly220, Arg221 and Gln266 while VEID

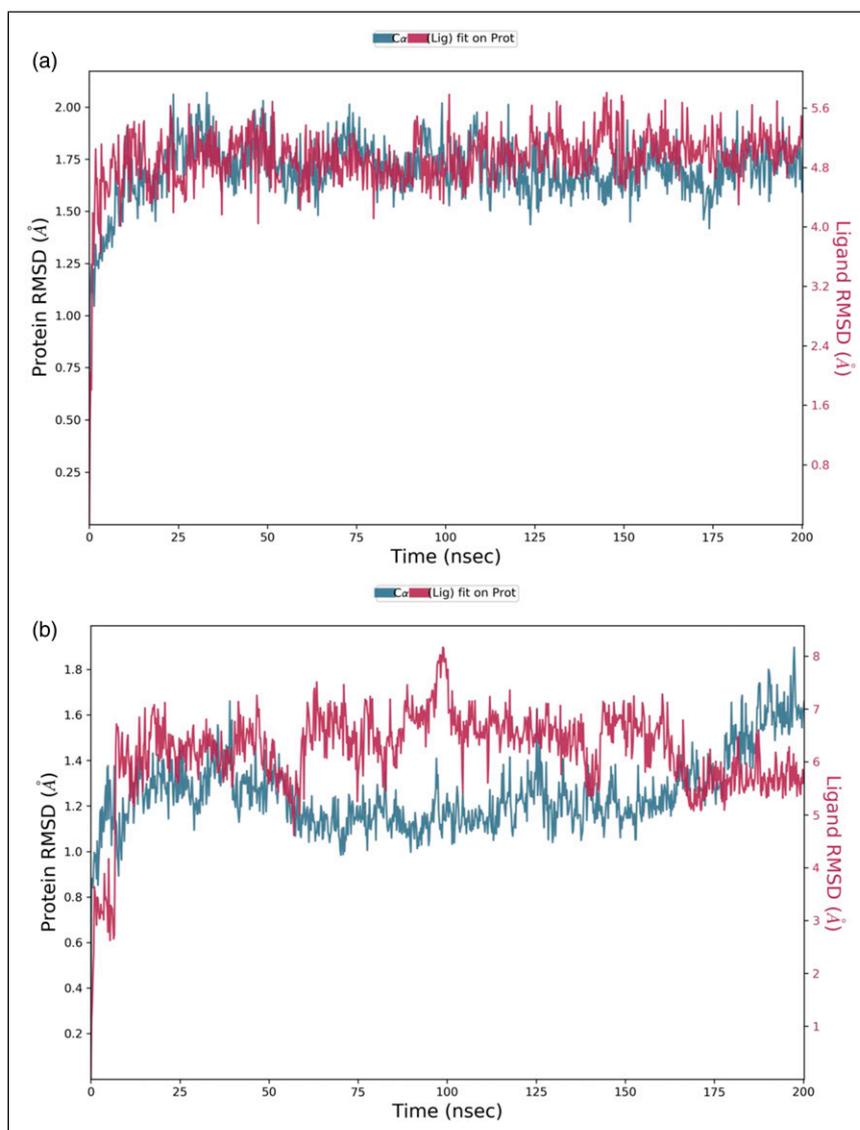


Figure 9. RMSD plots of locations of $C\alpha$ -atoms (\AA) for the initial structure and the simulation time (ns) of VEID (ligand) with (a) human aldose reductase and (b) human protein tyrosine phosphatase for the MD simulation of their interaction complex.

has showed water-mediated H-bond interactions with Lys120 and Gln262. The π - π interactions were not found between human PTP and VEID (ligand).

To monitor the human AR-VEID interactions continuity during the simulation, a plot of active site residues was plotted against trajectories frames (Figure S4). Notably, Trp20, Lys21, Asp43, Tyr48, Trp11, Asn160, Gln183 and Tyr209 have showed interactions with VEID throughout 200 ns while Trp79 and Cys298 remained in contact with VEID during most of the time. Asp21, Val47, His100 and Ala208 have showed interactions in patches during MD simulation but did not show binding consistency. In case of PTP-VEID complex, the amino acid residues Ala217, Ile219, Gly220, Arg221 and Gln262 have showed interaction consistency with VEID throughout 200 ns while Lys116, Lys120, Phe182, Gly218 and Gln266 remained in contact with VEID during most of the time but did not show binding consistency throughout 200 ns MD simulation (Figure S5).

No significant flexibility as measured by Root Mean Square Fluctuation (RMSF) was observed in the secondary structure elements (SSE) (α -helices and β -strands: Helix =

31.31%, Strand = 11.88%, and Total SSE = 43.19%) of the human AR protein model (Figure S6) with major fluctuations only in residues 210-214 (Figure 10(a)). Similarly, no significant flexibility as measured by RMSF was observed in the SSEs (α -helices and β -strand: Helix = 31.35%, Strand = 16.67%, and Total SSE = 51.02%) of the human PTP protein model (Figure S7) with no major fluctuations (Figure 10(b)).

The percentage of SSE remained close to 43% throughout the simulation period for AR-VEID complex. The amino acids that interacted with VEID did not show any major fluctuations in their RMSF values (Figure 10(c)) while VEID element number 4 has shown some fluctuations in RMSF as this element is not involved in any interaction with the human AR and therefore it will not fluctuate the structural stability of the docked complex. The amino acids of human PTP protein that interacted with the ligand VEID has showed some fluctuations in their RMSF values of elements 6 and 10 (Figure 10(d)) as these elements are not involved in making interactions with human PTP and therefore it will not affect structural stability of the PTP-VEID docked complex.

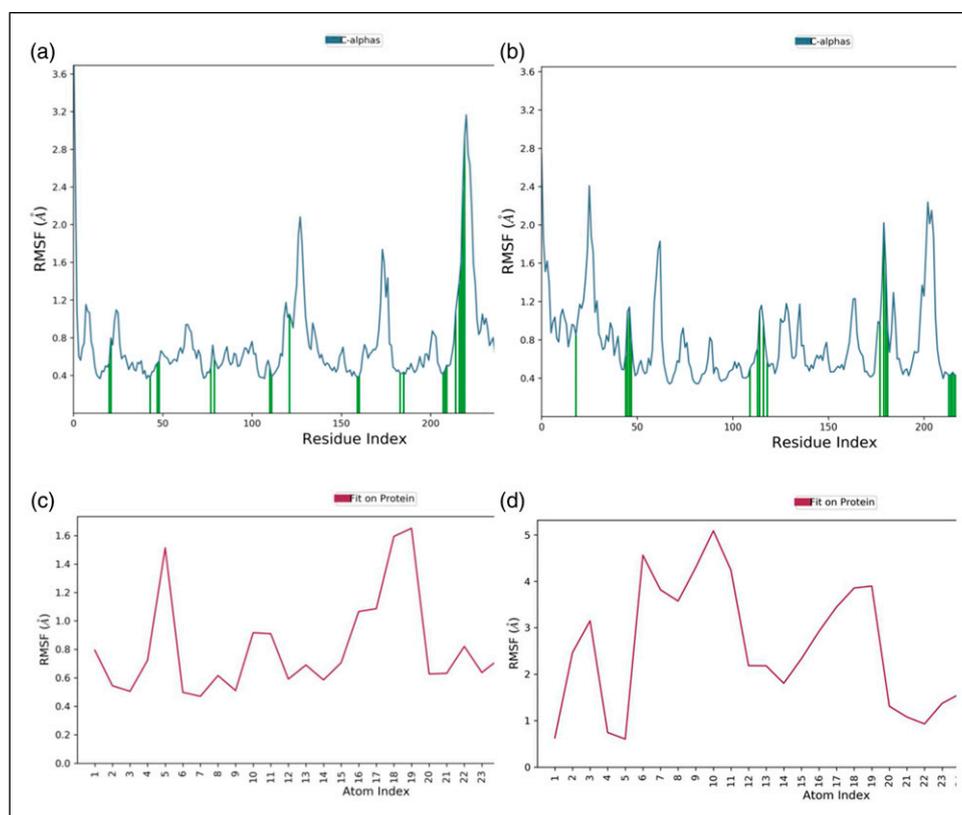


Figure 10. The Root Mean Square Fluctuation (RMSF) plot of proteins and ligands based on $C\alpha$ atoms of receptor proteins. Protein residues that interacted with the VEID are marked with green vertical bars. Protein RMSF plots of (a) human aldose reductase and (b) human protein tyrosine phosphatase; Ligand (VEID) RMSF plots for (c) human aldose reductase and (d) human protein tyrosine phosphatase.

Ligand features, including RMSD, solvent accessible surface area (SASA), the radius of the gyration (rGyr), intramolecular hydrogen bonds, molecular surface area (MolSA) and polar surface area (PSA), are reported in Figure S8 for AR-VEID complex. The rGyr is used to measure how extended a ligand is, and it is equivalent to its principal moment of inertia. The rGyr of the ligand remained in equilibrium throughout 200 ns simulation. The molecular surface area (MolSA) was calculated with probe radius which was between 420 \AA^2 to 440 \AA^2 . The solvent accessible surface area (SASA) is the surface area of a molecule accessible by a water molecule. Higher scores mean more of the molecule is sticking out into water. Lower scores mean that more of the molecule is buried in the protein. For drug applications, we want to go with the lowest score when choosing an ideal molecule to pursue. The SASA RMSD of VEID remained $100\text{--}155 \text{ \AA}^2$ which means ($\text{SASA} \leq 155 \text{ \AA}^2$) that ligand's maximum residues are within binding cavity of the human AR.

Ligand features for PTP-VEID, including the RMSD, SASA, rGyr, intramolecular hydrogen bond, MolSA and PSA are reported in Figure S9. The rGyr of the VEID remained in equilibrium throughout 200 ns simulation. The MolSA was calculated with probe radius which was between 420 and 440 \AA^2 . The RMSD of the VEID SASA remained $240\text{--}320 \text{ \AA}^2$. As polar surface area of VEID in case of interaction with human PTP is $\text{PSA} > 140 \text{ \AA}^2$ it means that the ligand has good oral and intestinal absorption to target binding residues of human PTP.

Discussion

The globally prevalence of diabetes is the alarming situation associated with high mortality and morbidity rate. Diabetes mellitus is the group of metabolic disorders associated with extreme hyperglycaemic state and insulin resistance in the body.³⁰ Many factors in human body are responsible for the onset of diabetes as a single mutational change in any metabolic protein creates hindrance in insulin signalling and glucose metabolism.¹⁷ Recently, plants derived compounds have attained great importance as anti-diabetic agents due to fewer side-effects and more safety.³¹ A vast variety of plants derived compounds have been reported with clinical perspective in the treatment of diabetes and inflammatory diseases.³¹ The use of computational biology helps scientists to explore the binding pattern and drugability of different hit compounds before the actual laboratory trial.³²

The main aim to come over the complications of diabetes is to normalize the glucose homeostasis and cellular signalling. Currently, many conventional drugs are available in the market but the outcomes are undesirable.³³ In literature, many proteins have been reported as targets for diabetes including aldose reductase, protein tyrosine

phosphatase 1B, α -glucosidase, α -amylase, glycogen synthase kinase 3-beta, DPP-IV and SGLTs.^{11,17,34,35}

In the current study, aldose reductase, protein tyrosine phosphatase 1B, α -glucosidase, α -amylase and glycogen synthase kinase 3-beta were used as targeted receptor proteins. Aldose reductase is the rate limiting enzyme of polvol pathway which reduces the D-glucose in D-sorbitol. Overexpression of aldose reductase leads toward the onset of many diseases including diabetes. Kaushik et al.,³⁶ checked the bioactive constituents of *Pinus roxburghii* against the target protein aldose reductase to treat diabetes. The bioactive compounds of *P. roxburghii* (e.g. secoisoresinol, pinoresinol and cededarin) showed the maximum value and mainly interacted with Trp111, His110, Asn160, Tyr48, Cys298, Asp216, Leu212, Asp43, Thr199 and Trp20 of the active site of receptor protein. Similarly, in the previous studies, many compounds reported as aldose reductase inhibitor shared common interactions with Try111, Tyr48 and Leu300 residues of the binding pocket.^{10,37} In the current study, Try111 and Tyr48 were also reported as main interference residues of the binding pocket.

Protein tyrosine kinase 1B is an attractive target for treating diabetes as it plays a crucial role in cellular networking and glucose uptake in the body. In previous literature studies, many scientists used pharmacophore-based drug study to evaluate the ligand-protein interactions at the active site of different models of PTP-1B. The substrate specificity of PTP-1B has been evaluated by docking with different ligands at active site.³⁸ Zhang et al.,¹¹ used pharmacophore computational approach to check the ligand-protein interaction between 16 optimized ligands and PTP-1B receptor protein. These ligands were evaluated as inhibitors of active site of PTP-1B. They showed that Lys41, Tyr46, Arg47 and Asp48 residues of the active site play a key role in enhancing the biological activity of the inhibitors and same has been reported in the current study.

Glycogen synthase kinase 3 β (GSK-3 β) plays a crucial role in non-insulin-dependent diabetes mellitus.¹⁶ GSK-3 β belongs to the super family of MAP (mitogen activated protein) proteins. GSK-3 β exists in two isoforms α and β with 97% sequence similarity. The main site of GSK-3 β in cell is cytoplasm but a limited amount also presents in mitochondria.³⁹ GSK-3 β plays a multifunctional role in cellular signalling cascade which is responsible for insulin resistance and glucose homeostasis.⁴⁰ Because of all the characteristic features and involvement of GSK-3 β in the disease, it attains much attention as target to cure many disorders such as diabetes, Alzheimer's disease and many neuronal disorders. Middha et al.,¹⁶ performed computational docking of compounds from *H. rhamnoides* and *H. salicifolia* as inhibitors against GSK-3 β as receptor protein. The compounds derived from these plants were evaluated to check their efficacy and potency against the receptor

protein. The compounds isorhamnetin-3-glucosidase and isorhamnetin-5-glucosidase, lutein D and zeaxanthin analogues showed strong bindings as inhibitors of GSK-3 β protein. The drugability of these hit compounds showed good bioavailability and absorption with no toxicity and carcinogenicity reported.

Bustanji et al.,³⁴ used curcumin and curcumin derivatives as potent GSK-3 β inhibitors. Curcumin binds with the Arg141, Lys85 residues of the binding pocket with high potency (IC₅₀ ¼ 66.3 nM) and could serve as an effective inhibitor of the GSK-3 β . Nisha et al.,¹⁵ docked and evaluated certain ligands (i.e. indirubin, hymenialdisine, meridianins, 6-bromoindirubin-3-oxime) as inhibitors of GSK-3 β . Among the four selected ligands, 6-bromoindirubin-3-oxime (6-BIO) was reported as best inhibitor on the basis of interactions, energy validations and drug screening test. The compound 6-bromoindirubin-3-oxime interacted with the active amino acids Val78, Lys85, Leu132, Ala83, Tyr134, Val135, Pro136, Leu188, Thr138, Arg141 and Asp200. Therefore, 6-BIO can be considered as a lead drug candidate against GSK-3 β .

In all the above literature studies, the reported binding residues were found same in the current study against the selected receptor proteins. So, it can be concluded that the leading peptides reported in the current study can be accessed as potent inhibitors with respect to their leading receptor proteins. Pancreatic α -amylase is the key enzyme that breaks the starch and dietary carbohydrates into simple monosaccharides which are further degraded into glucose molecules to enter into the blood stream upon the action of α -glucosidase enzyme. The α -amylase and α -glucosidase are the key enzymes in carbohydrate metabolism and glucose absorption. Thus, the inhibition of both enzymes can suppress the carbohydrate metabolism, delay the glucose absorption and reduce the blood glucose level in hyperglycaemic state.⁴¹

Hua et al.,⁴² used the green tea derivatives to check their anti-diabetic effects. Molecular docking study predicted that the amino acids Thr306, Asp352, Arg213, Glu277, Asp215, Arg442 and Phe303 of α -glucosidase and His201, Glu233, Asp197, Gln63 and Trp59 of α -amylase were found to be involved in ligand-protein interactions. Alqahtani et al.,⁴¹ used *Nuxia oppositifolia* derivatives 3-oxolupenal, katononic acid as α -amylase and α -glucosidase inhibitors. The molecular docking confirmed strong interactions of both ligands against these key enzymes. The docking study revealed Lys200, Tyr62, His101, Tyr151, Leu162, Val163, Asp197, Asp300, His305Ala198, His201, Glu233, Ile235, His299, Gly306 and Ala307 as interacting residues against α -amylase. Similarly, Arg608, Glu866, Val867, Arg585, Val588, Arg594, His717, Leu865, Met363, His584, Ser864 and Leu868 were involved in the interactions with α -glucosidase. This indicated good

efficacy of 3-oxolupenal and katononic acid as potent inhibitors.

The drugability of top 25 compounds (five hit compounds against each protein) has been evaluated on the basis of Lipinski's rule of five. The ligands (VEID, TVEV, AYAY, EEIA, ITTV, TTIT, LPSM, RGIE, TTVE and EIAR) violated only one rule meanwhile the ligands (LEEI, EIDD and NVDE) violated two rules of Lipinski rule of five. Although these ligands violated one rule but these are acceptable as drug candidates due to bioavailability and non-toxicity. All the hit compounds selected in this study passed the ADMET screening test of drugs assessment and therefore could be used as effective drug candidates to inhibit the respective receptor proteins explained in this study. The objective of this study was to evaluate plant-derived peptides as effective inhibitors against different receptor proteins which are involved in glucose metabolism and insulin secretion. The defect in insulin signalling and glycogen metabolism leads toward the severe consequences such as neural damage, hepatic damage and elevated level of oxidative stress. The drug discovery using in silico approaches is expected to find new drugs quickly which should be cheaper and effective. In spite of all these pros, the in silico approaches have some limitations because various online bioinformatics tools/software give different results for the same analyses, and therefore, one cannot fully rely on the results without wet lab investigation and validation.

Conclusion

Fifty ligands were prepared from highly conserved regions of predicted motifs of AdMc1 protein and docked against five different receptor proteins to explore anti-diabetic properties of devised peptides. Top five confirmations with best energy scores and energy validations were selected for further analyses. The docking scores, structural patterns and drugability of all hit compounds revealed the inhibitory effects of selected ligands (i.e. VEID, TVEV, AYAY, EEIA, ITTV, TTIT, LPSM, RGIE, TTVE and EIAR) against selected receptor proteins. The results of the current study showed that the selected peptides have the potential of being good inhibitors of selected targeted proteins. Moreover, the MD simulations have elucidated from RMSD trajectories, interaction histograms and contact graphs that VEID docked complexes with both human AR and PTP protein are structurally stable.

Acknowledgements

The authors would like to gratefully acknowledge Department of Biochemistry, Government College University Faisalabad for providing space and facilities to accomplish this study.

Authors' contributions

All authors contributed to the study. Research design and supervision was performed by GM. Most experiments were conducted and data were analysed by HSM and RA. Molecular dynamics simulation was performed by MZ and SAA. The draft manuscript was prepared by HSM that was proofread by GM.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethics approval

The ethical approval to conduct this study is not applicable because this is an *in silico* study and no humans or other organisms were used in this study.

Informed consent

Not applicable as no humans or other organisms were used in this study.

Data availability

The data that support the findings of this study are available from the corresponding author, Ghulam Mustafa, upon reasonable request

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Supplemental Material

Supplemental material for this article is available online.

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