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Identification of Novel Protein Targets for Fenugreek to Treat Diabetes: A Molecular Docking Study

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ABSTRACT

Trigonella foenum-graecum has been shown to have anti-diabetic potential through a wide variety of in-vivo assays as well as by inhibiting enzymes such as alpha-glucosidase and alpha-amylase. Studies have indicated the therapeutic potential of different phytoconstituents found in *Trigonella foenum-graceum* including diosgenin trigonelline, 4-hydroxyisoleucine, leucine, and L-lysine. This study aims to find novel protein targets that these specific phytoconstituents from fenugreek can bind to, thereby helping to treat diabetes mellitus. Through multiple stages of molecular docking and analyzing the binding sites in comparison to previously reported inhibitors, a suitable and novel target protein for four of the compounds was found and the relevance to diabetes was discussed, setting up these compounds as novel inhibitors for the target proteins.

Introduction

Trigonella foenum-graecum, also known as fenugreek, is a short, annual plant from the Fabaceae family. It is found in various parts of the globe, and fenugreek leaves and seeds have been often used as a spice, condiment, and medication. In Africa, fenugreek is used as a supplement during bread preparation and the seed components of fenugreek are known to enhance the nutritional quality of the bread. In India, the leaves and seeds are utilized as flavoring and seasoning agents. In China, it is used to cure edema, and the ancient Egyptians employed fenugreek to incense the mummies[1]. Additionally, fenugreek has been recently studied for its therapeutic potential, including its antidiabetic[2], antibacterial[3], antihyperlipidemic[4], anti-angiogenic[5], and anticoagulant factors[6]. Fenugreek seeds have been the most widely studied part of the plant and many potent active compounds have been identified. These major phytoconstituents include diosgenin, a sapogenin, which are lipophilic triterpene derivatives which protect plants against microbes, fungi, and other hostile organisms[7]; trigonelline, an alkaloid[8], which is a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom; and 4-hydroxyisoleucine[9], leucine, and lysine[10], which are all amino acids. Amino acids are organic compounds that contain amino and carboxyl functional groups, along with a side chain specific to each amino acid, which are polymerized to form proteins.

Diabetes mellitus is a chronic metabolic disease that is prevalent around the world; the IDF estimated that in 2019, the global estimate of adults with diabetes was 463 million[11]. Diabetes mellitus is characterized by hyperglycemia, which is when there is too much sugar in the blood. This results in defective insulin secretion, resistance to insulin action, or both[12]. In type 1 diabetes mellitus, autoimmune destruction of pancreatic β-cells leads to a deficiency of insulin secretion, which results in metabolic derangements such as increased waist circumference, increased serum triglyceride levels, hypertension, and insulin resistance[13]. In addition, the function of pancreatic α-cells is also abnormal, so there is excessive secretion of glucagons, which would normally be suppressed by hyperglycemia. On the other hand, in type 2 diabetes mellitus, individuals have detectable levels of insulin circulating in the body. However, they often are resistant to the action of insulin, due to impaired insulin secretion by the pancreas. This then causes increased glucose production in the liver, which leads to receptor and post-receptor defects, causing insulin resistance[14]. Diabetes has been thoroughly studied, yet in spite of new medical devices and technology, it is projected that diabetes in the United States will increase by 54% to more than 54.9 million Americans between 2015 and 2030, annual deaths attributed to diabetes will climb by 38% to 385,800, and total annual medical and societal costs related to diabetes will increase 53% to more than $622 billion by 2030[15].

With these projections, finding anti-diabetic therapeutics is crucial. Fenugreek has shown to have quite a lot of anti-diabetic potential. In terms of its hypoglycemic effect, many studies have tested fenugreek generally through animal and *in vivo* studies where animals were fed with either the seeds powders or extracts for a period of time from 5 days to several weeks or with a single dose treatment regimen[16]. The results generally showed that pretreatment with fenugreek resulted in lowered blood glucose and lipid levels in the mice or rats, indicating antidiabetic potential. Additionally, current research has focused on finding effective alpha-glucosidase and alpha-amylase inhibitors. Both of these enzymes are involved in the digestion of carbohydrates. Alpha-glucosidase is a membrane-bound enzyme present in the epithelium of the small intestine, which works to facilitate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides. α-glucosidase inhibitors can retard the liberation of D-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial, or occurring after a meal, plasma glucose levels and suppression of postprandial hyperglycemia[17]. Alpha-amylase enzymes, most importantly pancreatic alpha-amylase, act as catalysts in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. Similar to alpha-glucosidase, inhibiting alpha-amylase has been shown to decrease postprandial blood glucose levels[18]. In terms of fenugreek's ability to inhibit target proteins, diosgenin was shown to inhibit both alpha-glucosidase and alpha-amylase quite significantly through *in-vitro* studies[19].

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred pose is important to predict the strength of association or binding energy between two molecules. Characterization of the binding behavior to biomolecules plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. For example, finding an inhibitor, which binds to that particular protein could prevent the action of some harmful proteins. Due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target-binding site, molecular docking is one of the most frequently used methods in structure based drug design[20]. Traditionally, multiple ligands are screened against a certain protein target to find the ligand with the best binding energy score. However, when there is not much information about the molecular mechanism of the ligands, they undergo reverse docking, a process in which multiple protein targets are screened against a certain ligand and their docking complexes are reviewed.

Methods

Reverse Docking

Our group obtained SDF files of the five phytoconstituents from fenugreek: trigonelline[21], diosgenin[22], lysine[23], leucine[24], and 4-hydroxyisoleucine[25] were from PubChem. We then individually submitted these files to ACID, a novel web server that conducts inverse docking by using AutoDock Vina, LEDOCK, PLANTS, and PSOVina for binding pose search and docks the compounds against a i9 target database containing 831 protein structures from PDB[26] covering 30 therapeutic areas[27]. After obtaining the reverse docking results for each compound, the top 30 target proteins (ranked in terms of binding energy) for each compound were selected and filtered first based on their enzyme class. The enzyme classes that our group selected were transferases, hydrolases, and oxidoreductases. The proteins from these classes were then screened for relevance to diabetes and the benefits of the protein being inhibited in relation to diabetes.

Validation with AutoDock Vina

In order to confirm the docking scores received from ACID, our group manually blind docked the target proteins that passed the screening against their respective compound using AutoDock Vina[28]. The docking score received from AutoDock Vina (ADV) was then compared with the score received from ACID.

Analysis of Binding Sites

Since blind docking was conducted, we used AutoDock Vina and Discovery Studio Visualizer[29] (DS) to identify the amino acid residues for each protein-ligand complex and we also used Discovery Studio Visualizer to identify the specific interactions for each amino acid residue. In cases where an error occurred with AutoDock Vina, PyMol[30] was used to determine the amino acid residues. In order to determine the amino acid residues through AutoDock Vina and PyMol, the output files created by manually docking and the target protein file were opened and analyzed by looking at the interactions. Similarly, the output file and protein file were opened on Discovery Studio Visualizer and the 2D interactions map was opened to identify the amino acid binding residues and the specific types of interactions. Additionally, in order to identify which proteins the ligands were actually inhibiting, we blind docked previously reported inhibitors for each target protein using AutoDock Vina and the amino acid residues from these dockings were compared to binding residues of the respective fenugreek compound. When comparing the phytoconstituents' docking to the inhibitors' docking, only conformations with the lowest RMSD values were studied.

Results

For each of the five compounds, there are three tables displaying the different results we obtained. The first table shows the top 30 target proteins based on binding energy we received from ACID. The second table depicts the ACID docking score, AutoDock Vina docking score, AutoDock Vina binding residues and the Discovery Studio binding residues for the screened and selected target proteins. The discrepancies between the ACID docking score and the AutoDock Vina score has been attributed to the fact that ACID used multiple docking softwares to come up with a docking score, and these other softwares are not accounted into the score received from AutoDock Vina. Additionally, the binding residues received from each software are very similar, but Discovery Studio was more specific in that for each amino acid residue, it also included what type of interaction was occurring between the residue and the ligand. Lastly the third table depicts the AutoDock Vina binding residues and docking score of the fenugreek compound and the reported inhibitor for each of the selected target proteins.

Table 1.1. Top 30 target proteins scored for diosgenin from ACID

Protein Code	Protein Name	Free Binding Energy (kcal/mol)	Docking Score (kcal/mol)

1NRL	Human PXR-LBD	-38.12	-11.1
5HBS	Human cellular retinol binding protein 1	-37.74	-10.95
1FCY	Human Retinoic Acid Nuclear Receptor Hrar	-37.64	-11.13
1SQN	Progesterone Receptor Ligand Binding Domain	-37.07	-11.6
1R20	Heterodimer EcR/USP	-36.82	-11.21
4UDD	Glucocorticoid Receptor	-36.66	-11.5
5HJP	LXRbeta Selective Agonists	-36.55	-11.25
4DM6	RARb LBD homodimer	-35.98	-11.05
2BZG	Thiopurine S-methyltransferase	-35.94	-11.04
3KMR	RAR alpha ligand binding domain	-35.81	-10.89
1SQB	Bovine Bc1	-35.66	-10.22
3MY0	ACVRL1 (ALK1) kinase	-35.27	-10.35
4QE6	Human FXR	-35.26	-11.27
2GL8	Retinoic acid receptor RXR-gamma	-34.78	-9.07
2P1T	Retinoid X receptor alpha	-34.71	-11.48
1CQX	Flavohemoglobin	-34.37	-10.04
3B0T	Human VDR ligand binding domain	-33.67	-10.72
2E2R	Human estrogen-related receptor gamma	-33.59	-11.07
4L8U	Human serum albumin	-33.18	-10.41
3C6K	Human spermine synthase	-33.1	-9.55
3BRT	NEMO/IKK	-33.09	-10.21
1W78	E.coli FolC	-32.44	-9.42
4QTB	Human ERK1	-32.43	-10.99
2HI4	Human Microsomal P450 1A2	-32.41	-10.71
4BVM	Peripheral membrane protein P2	-32.13	-10.96
4GQS	Human Microsomal Cytochrome P450[SEP](CYP) 2C19	-31.78	-10.19
1D2S	N-Terminal Laminin G-Like Domain of[SEP]SHBG	-31.57	-10.19
1T46	Autoinhibition and STI-571 Inhibition of[SEP]C-KIT Tyrosine Kinase	-31.56	-9.84
2Q80	Human geranylgeranyl pyrophosphate synthase bound to GGPP	-31.28	-10
4UYB	SEC14-like protein 3	-31.28	-11.15

Table 2.1. Validation of docking of selected target proteins for diosgenin

PDB Code	Name	Classification	ACID Docking	AutoDock Vina Docking	AutoDock Vina Amino Acid Residues	DS Amino Acid Residues and Interactions

			Score (kcal/mol)	Score (kcal/mol)		
4QTB	Human ERK1	Transferase/Transferase Inhibitor	-10.99	-9.2	THR288, LYS289, PHE313, THR312, ASN314, LYS317	SER265: Carbon hydrogen bond LYS317: Alkyl
2HI4	Human Microsomal P450 1A2	Oxidoreductase	-10.71	-8.2	GLN304, ASN309, GLU305, VAL308, PHE288, TYR272, VAL268, GLN265	VAL308: Pi Alkyl TYR272: Alkyl

Table 3.1 Diosgenin vs. the reported inhibitors for each target protein

PDB Code	Protein Name	Diosgenin ADV Docking Score (kcal/mol)	Diosgenin ADV Amino Acid Residues	Reported Inhibitor Name	Reported Inhibitor ADV Docking Score (kcal/mol)	Reported Inhibitor ADV Amino Acid Residues
4QTB	Human ERK1	-9.2	THR288, LYS289, PHE313, THR312, ASN314, LYS317	Ulixertinib	-9.7	TYR53, LYS71, VAL56, ASP184, GLU88, GLY186, ARG84
2HI4	Human Microsomal P450 1A2	-8.2	GLN304, ASN309, GLU305, VAL308, PHE288, TYR272, VAL268, GLN265	Asenapine	-9.0	LEU382, THR385, THR124, ALA317, LEU497

After comparing diosgenin's binding site to many reported inhibitors of each of the two target proteins 4QTB and 2HI4, human extracellular signal-regulated kinase (ERK1) and Cytochrome P450 1A2 (CYP1A2), our group found that diosgenin did not act as an inhibitor for either of the proteins. The reported inhibitor we used for ERK1 was ulixertinib31. For CYP1A2, we used asenapine32. For ERK1, diosgenin was compared to Figures 1 and 2, displaying the PyMol visualization of diosgenin's binding site and the reported inhibitors' binding sites for ERK1 and CYP1A2, respectively.

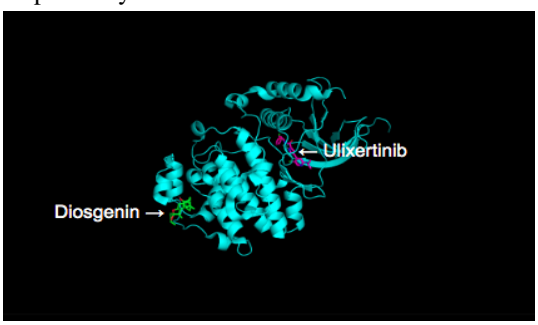


Fig 1

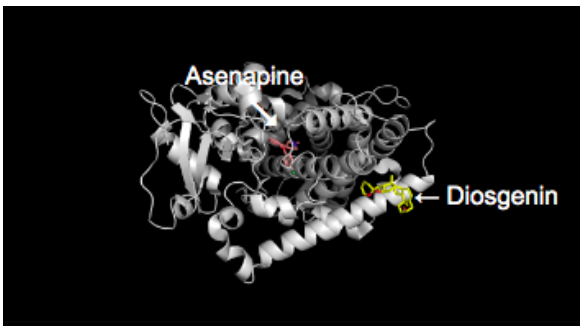


Fig 2

Figure 1 Diosgenin and ulixertinib bound to human extracellular signal-regulated kinase (ERK1)
Figure 2 Diosgenin and asenapine bound to Cytochrome P450 1A2 (CYP1A2)

Table 1.2. Top 30 target proteins scored for trigonelline from ACID

PDB Code	Protein Name	Free Binding Energy (kcal/mol)	Docking score (kcal/mol)
3L6B	Human serine racemase	-55.07	-6.56
4E1O	Human histidine decarboxylase	-53.4	-6.68
3II0	Human Glutamate oxaloacetate transaminase 1	-52.42	-6.85
3KQJ	MurA binary complex with UDP-N-acetylglucosamine	-45.79	-6.4
5HHY	Human Alanine:Glyoxylate Aminotransferase major allele	-45.22	-6.86
2O0B	Mycobacterium tuberculosis epsp synthase	-44.56	-6.24
3C8Y	Fe-only hydrogenase	-43.35	-6.59
1D4D	Flavocytochrome c fumarate reductase of shewanella putrefaciens strain mr-1	-42.7	-6.42
4USA	Aldehyde Oxidoreductase	-41.27	-6.79
2E1Q	Human Xanthine Oxidoreductase mutant	-40.83	-6.64
1OTH	Human ornithine transcarbamoylase complexed with n-phosphona-cetyl-l-ornithine	-39.46	-6.82
1FW1	Glutathione transferase zeta/maleylacetoacetate isomerase	-38.78	-6.75
4OQV	Human dihydroorotate dehydrogenase bound with DSM338	-38.49	-6.87
1SHA	Phosphotyrosine recognition domain sh2 of v-src complexed with tyrosine-phosphorylated peptides	-33.81	-6.58
2F71	Protein tyrosine phosphatase 1B with sulfamic acid inhibitors	-33.26	-6.87
2OO0	Ornithine decarboxylase	-33.15	-6.61
5KQL	LMW-PTP in complex with 2-oxo-1-phenyl-2-(phenyla-mino)ethanesulfonic acid	-33	-6.44
4ZZ1	Human gar transformylase	-31.89	-6.25
2Z98	AzoR (azoreductase)	-31.39	-6.68
2GF3	Monomeric sarcosine oxidase	-31.2	-6.75
3DDS	Glycogen phosphorylase	-30.06	-6.53
1P5J	Human Serine Dehydratase	-28.5	-6.83
3N9Z	Human CYP11A1	-27.19	-6.46
1FDR	Flavodoxin reductase	-26.57	-6.33
1PBE	P-hydroxybenzoate hydroxylase	-26.37	-6.87
5AX8	Human mitochondrial aspartate aminotransferase	-26.27	-6.74
2ZB4	Human 15-ketoprostaglandin delta-13-reductase	-25.95	-6.46
1Z6T	Apoptotic protease-activating factor 1	-25.86	-6.58
4XNH	Yeast N-terminal acetyltransferase NatE	-25.86	-6.58
1QO8	Flavocytochrome c3 fumarate reductase	-25.18	-6.43

Table 2.2 Validation of docking of selected target proteins for trigonelline

PDB Code	Protein Name	Classification	ACID Docking Score (kcal/mol)	Auto-DockVina Score (kcal/mol)	AutoDockVina Amino Acid Residues	DS Amino Acid Residues and Interactions
2E1Q	Xanthine Oxidoreductase	Oxidoreductase	-6.64	-5.8	THR262, SER347, GLY350*	LEU257: Carbon Hydrogen Bond GLY350: Van der Waals SER347: Conventional Hydrogen Bond GLU263: Conventional Hydrogen Bond THR262: Conventional Hydrogen Bond VAL259: Alkyl
2F71	Protein tyrosine phosphatase 1B	Hydrolase	-6.87	-5.4	ARG199, ARG79, LEU204	LEU204: Alkyl ARG199: Conventional Hydrogen Bond, Salt Bridge ARG79: Attractive Charge
5KQL	Low molecular weight Protein tyrosine phosphatase	Hydrolase/hydrolase inhibitor	-6.44	-5.5	LEU13, GLY14, ARG18, ILE16, TYR131	TYR131: Alkyl ILE16: Pi-Alkyl ARG18: Salt Bridge, Conventional Hydrogen Bond LEU13: Conventional Hydrogen Bond GLY14: Conventional Hyrdogen Bond ASP129: Carbon Hydrogen Bond
3DDS	Glycogen phosphorylase	Transferase	-6.53	-4.9	ASN631*	ASN631: Conventional Hydrogen Bond ASP628: Unfavorable Negative-Negative ILE170: Alkyl

* PyMol was used to identify amino acid residues

Table 3.2 Trigonelline vs. the reported inhibitors for each target protein

PDB Code	Protein Name	Trigonelline ADV Docking Score (kcal/mol)	Trigonelline ADV Amino Acid Residues	Reported Inhibitor Name	Reported Inhibitor ADV Docking Score (kcal/mol)	Reported Inhibitor ADV Amino Acid Residues
2E1Q	Xanthine Oxidoreductase	-5.8	THR262, SER347, GLY350*	Allopurinol	-6.0	GLN1195, ARG913, SER1081, ALA1080*
2F71	Protein tyrosine phosphatase 1B	-5.4	ARG199, ARG79, LEU204	Ertiprotafib	-7.8	VAL49, ASP48, ALA217, ILE219, TYR46, PHE182
5KQL	Low molecular weight Protein tyrosine phosphatase	-5.5	LEU13, GLY14, ARG18, ILE16, TYR131	2-(4-{[3-(1-Piperidinyl)propyl]amino}-2-quinolinyl)benzonitrile	-7.8	ASP129, ARG18, TYR131, GLY14, GLU50, ILE16
3DDS	Glycogen phosphorylase	-4.9	ASN631*	CP-316819	-7.8	GLU123, GLU124, LYS655*

* PyMol was used to identify amino acid residues

With trigonelline as the ligand, the target protein it inhibits is 5KQL, Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP). When trigonelline's binding site is compared to the reported inhibitor 2-(4-{[3-(1-Piperidinyl)propyl]amino}-2-quinolinyl)benzonitrile's[33] binding site, trigonelline binds to 5 out of the 6 same amino acid residues that the reported inhibitor binds to. These residues are ASP129, ARG18, TYR131, GLY14, and ILE16. Figure 3 and 4 shows the PyMol visualization of both ligands docked on to 5KQL, displaying how they have binded to the same site of the protein. Some of the interactions between the residues and trigonelline include alkyl, pi-alkyl, salt bridge, conventional hydrogen bonding and Carbon Hydrogen Bonding, showing a mix of strong polar bonds and noncovalent attractions that make it a stable complex.

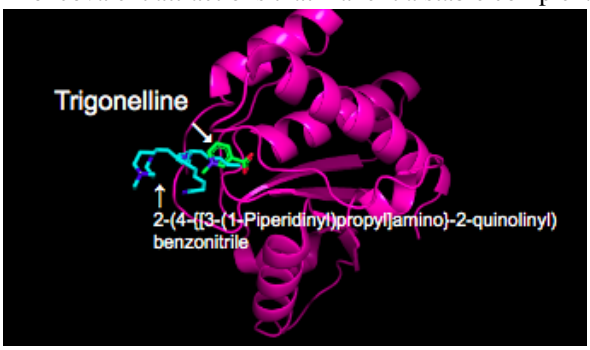


Fig 3

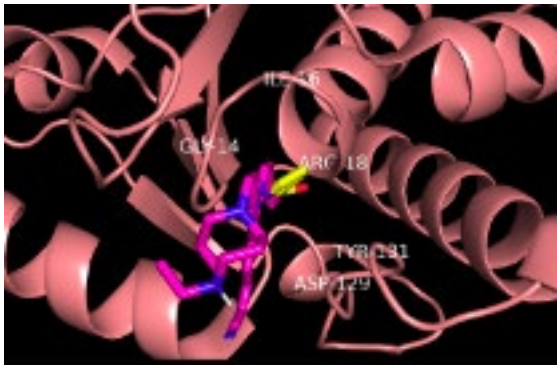


Fig 4

Figure 3 Trigonelline and 2-(4-{[3-(1-Piperidinyl)propyl]amino}-2-quinolinyl)benzonitrile bound to Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP)

Figure 4 Close-up view of binding complexes with Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP) with common binding residues labeled. The pink and blue ligand is 2-(4-{[3-(1-Piperidinyl)propyl]amino}-2-quinolinyl)benzonitrile and the yellow and red ligand is trigonelline.

Table 1.3 Top 30 target proteins scored for 4-hydroxyisoleucine from ACID

PDB Code	Name	Free Binding Energy (kcal/mol)	Docking Score (kcal/mol)
5CMM	GluK2EM LBD Dimer Assembly Complex	-35.12	-7.31
4MXU	Aspartoacylase Mutant K213E Complex	-25.87	-6.87
3T3M	Integrin Alphallbbeta3 Receptor	-23.08	-6.37
3B6R	Human Brain-type Creatine Kinase	-22.51	-6.33
1R76	Pectate Lyase from Azospirillum Irakense	-22.35	-6.13
2WAD	Penicillin-Binding Protein 2B (PBP-2B)	-21.75	-6.6
4V11	Synaptotagmin-1 with SV2A Peptide	-21.44	-6.12
4D1P	Nitric Oxide Synthases	-20.27	-6.17
1U8V	4-Hydroxybutyryl-CoA Dehydratase from Clostridium Aminobutyricum	-20.23	-6.52
2OZL	Pyruvate Dehydrogenase	-20.03	-6.8
1UNQ	Protein Kinase B	-20	-6
3IHJ	Alanine Aminotransferase 2	-19.58	-6.57
3B6R	Human Brain-type Creatine Kinase	-19.52	-6.31
5FQD	Lenalidomide-Induced CK1a degradation by the Crl4crbn Ubiquitin Ligase	-19.38	-7.32
2Y2M	Penicillin-Binding Protein 1B	-19.33	-6.52
1CQX	Flavohemoglobin from Alcaligenes Eutrophus	-19.2	-6.14
5K3S	Acetohydroxyacid Synthase	-18.93	-7.14
1BUC	Butyryl-CoA Dehydrogenase	-18.85	-6.84
3P0C	Nischarin PX-Domain	-18.81	-6.14
2F25	Sialidase Neu2 E111Q	-18.71	-6.5
1BUC	Butyryl-CoA Dehydrogenase	-18.59	-6.85
4XNH	N-Terminal Acetyltransferase NatE	-18.5	-6.5
4ZJB	C-terminal Domain of PyIRS Mutant Bound with 3-Benzothienyl-l-alanine and ATP	-18.34	-6.5
4HBU	CTX-M-15 extended-spectrum Beta-Lactamase	-18.17	-6.53
1Y93	Matrix Metalloproteinase 12	-18.06	-6.37
3VE6	Venezuelan Equine Encephalitis Virus Capsid Protein NLS and Importin Alpha	-17.95	-6.17

2V40	Human Adenylosuccinate Synthetase Isozyme 2 in Complex with GDP	-17.74	-6.31
4MR7	Extracellular Domain of Human GABA(B) receptor	-17.56	-6.54
2OUR	Phosphodiesterase 10	-17.35	-6.42
3ZOO	Cytochrome C	-17.28	-6.79

Table 2.3 Validation of docking of selected target proteins for 4-hydroxyisoleucine

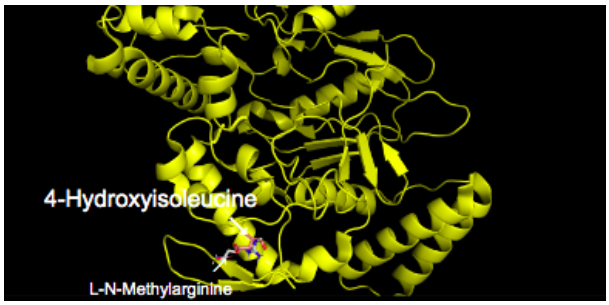
PDB Code	Name	Classification	ACID Docking Score (kcal/mol)	AutoDock Vina Docking Score (kcal/mol)	AutoDock Vina Amino Acid Residues	DS Amino Acid Residues and Interactions
3B6R	Human Brain-type Creatine Kinase	Transferase	-6.33	-4.8	VAL75, VAL72, CYS283, CYS74, GLY73, THR59, LEU201, LEU202	VAL72: Conventional Hydrogen Bond THR71: Carbon Hydrogen Bond CYS74: Conventional Hydrogen Bond LEU201: Conventional Hydrogen Bond, Unfavorable Acceptor-Acceptor
4D1P	Nitric Oxide Synthases	Oxidoreductase	-6.17	-5.9	THR80, VAL465, GLY440, CYS441, GLN435, LEU431	GLN 435: Conventional Hydrogen Bond LEU431: Conventional Hydrogen Bond GLN 462: Conventional Hydrogen Bond ASP82: Conventional Hydrogen Bond CYS441: Carbon Hydrogen Bond
3IHJ	Alanine Aminotransferase 2	Transferase	-6.57	-4.4	GLU421, ALA417, LEU435	LEU435: Conventional Hydrogen Bond (2) GLU421: Salt Bridge, Conventional Hydrogen Bond PRO434: Alkyl
1YP3	Matrix Metalloproteinase 12	Hydrolase	-6.37	-6	THR239, TYR240, LYS241, LEU214, VAL235, PHE237	THR215: Carbon Hydrogen Bond THR239: Conventional Hydrogen Bond HIS219: Attractive Charge, Unfavorable Donor-Donor VAL235: Conventional Hydrogen Bond (2)

						PHE237: Conventional Hydrogen Bond LYS201: Unfavorable Acceptor-Acceptor

Table 3.3 4-hydroxyisoleucine vs. the reported inhibitors for each target protein

PDB Code	Protein Name	4-Hydroxyisoleucine ADV Docking Score (kcal/mol)	4-Hydroxyisoleucine ADV Amino Acid Residues	Reported Inhibitor Name	Reported Inhibitor ADV Docking Score (kcal/mol)	Reported Inhibitor ADV Amino Acid Residues
3B6R	Human Brain-type Creatine Kinase	-4.8	VAL75, VAL72, CYS283, CYS74, GLY73, THR59, LEU201, LEU202	BU99006	-5.8	THR71, MET70, CYS283, VAL72, LEU201, LEU202, THR59, VAL75
4D1P	Nitric Oxide Synthases	-5.9	THR80, VAL465, GLY440, CYS441, GLN435, LEU431	L-N-Methylarginine (L-NMA)	-6.3	LYS72, SER78, ASN73, GLN462, THR80, VAL465, ASP82, GLN435, LEU431
3IHJ	Alanine Aminotransferase 2	-4.4	GLU421, ALA417, LEU435	L-cycloserine	-3.9	PRO309, LEU435, LYS418, GLU421
1YP3	Matrix Metalloproteinase 12	-6	THR239, TYR240, LYS241, LEU214, VAL235, PHE237	RXP470.1	-4.8	VAL235, LEU214

With 4-hydroxyisoleucine as the ligand, all the target proteins were inhibited in some capacity but based on docking scores, interactions, and the number of common residues between trigonelline and the reported inhibitor, the most suitable protein for 4-hydroxyisoleucine is 4D1P, Nitric Oxide Synthase. Comparing 4-hydroxyisoleucine's binding to 4D1P with the reported inhibitor L-N-Methylarginine[34] showed that 4-hydroxyisoleucine binds to 6 out of the 9 same amino acid residues as L-N-Methylarginine, with those being GLN462, THR80, VAL465, ASP82, GLN435, and LEU431. Figure 5 and 6 shows the PyMol visualization of both ligands docked on to 4D1P, displaying how they have binded to the same site of the protein. Interactions in this complex include many conventional hydrogen bonds and a Carbon Hydrogen bond and there are no unfavorable forces. These are very strong polar forces, making the attraction between the ligand and protein very stable.



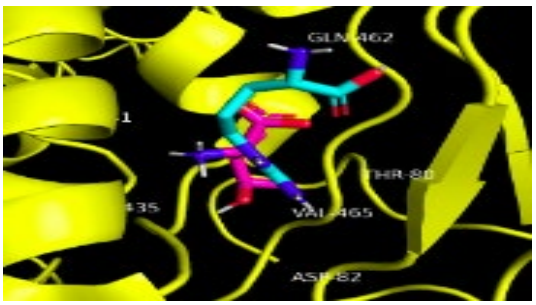


Fig 5 Fig 6

Figure 5 4-hydroxyisoleucine and L-N-Methylarginine bound to 4D1P

Figure 6 Close-up view of binding complexes with 4D1P with common binding residues labeled. The blue ligand is L-N Methylarginine and the pink ligand is 4-hydroxyisoleucine.

Table 1.4 Top 30 target proteins scored for leucine from ACID

PDB Code	Name	Free Binding Energy (kcal/mol)	Docking Score (kcal/mol)
5CMM	GluK2EM LBD dimer	-32.07	-7.13
3FV2	Human glutamate receptor: GluR5	-28.85	-7.11
1ITU	Human renal dipeptidase	-28.66	-6.51
5D6E	Human methionine aminopeptidase 2	-27.54	-6.6
4V11	Synaptotagmin-1	-26.86	-6.05
2C46	Human RNA guanylyltransferase	-25.28	-6.42
3BWY	Human 108M catechol O-methyltransfer-ase	-24.91	-7.01
3DYD	Human tyrosine aminotransferase	-24.67	-6.6
1U8V	4-Hydroxybutyryl-CoA dehydratase from clostridium aminobutyricum	-24.54	-6.55
2NZ2	Human argininosuccinate synthase	-24.3	-6.64
3T3M	Alphallbbeta3 receptor antagonist	-24.13	-6.35
4U3T	Neisseria gonorrhoeae penicillin-binding protein 2 derived from penicillin-resistant strain 6140	-23.72	-6.27
2BM0	Ribosomal elongation factor G (EF-G) Fusidic acid resistant mutant T84A	-23.58	-6.35
1KNR	L-aspartate oxidase: R386L mutant	-22.56	-6.74
2CVD	Human hematopoietic prostaglandin D synthase	-21.81	-6.28
1GYX	YdcE, a 4-Oxalocrotonate Tautomerase Homologue from Escherichia coli	-21.48	-6.49
1CQX	Flavohemoglobin from Alcaligenes eu-trophus	-20.85	-6.33

3I4A	Dimethylarginine dimethylaminohydrolase-1 (DDAH-1)	-20.84	-6.73
5K3S	Arabidopsis thaliana acetohydroxyacid synthase	-20.44	-6.89
3QFS	NADPH-Cytochrome P450 Reductase	-19.89	-6.29
3B6R	Human Brain-type Creatine Kinase	-19.87	-6.35
1UNQ	Pleckstrin Homology Domain Of Protein Kinase B/Akt	-19.85	-5.98
1FDR	Flavodoxin reductase from E. Coli	-19.19	-6.5
4RQR	Human Glutaredoxin	-19.15	-6.07
2OUR	PDE10A2 mutant D674A	-19.14	-6.33
3ODU	CXCR4 chemokine receptor	-19.14	-6.43
5F0X	Human GRP78 (70kDa heat shock protein 5 / BIP) ATPase domain	-18.77	-6.71
1NP7	Synechocystis sp. PCC6803 cryptochrome	-18.55	-6.5
1COY	Cholesterol oxidase	-18.45	-6.56
5TC4	Human mitochondrial methylenetetrahydrofolate dehydrogenase-cyclohydrolase (MTHFD2)	-18.39	-6.52

Table 2.4 Validation of docking of selected target proteins for leucine

PDB Code	Name	Classification	ACID Docking Score (kcal/ mol)	AutoDock Vina Docking Score (kcal/mol)	AutoDock Vina Amino Acid Residues	DS Amino Acid Residues and Interactions
3QFS	NADPH-Cyochrome P450 Reductase	Oxidoreductase	-6.29	-4.8	SER599, TYR607, PRO536, VAL608	HIS231: Pi-Alkyl, Salt Bridge, Positive-Positive ILE338: Alkyl HIS331: Salt Bridge PHE219: Pi-Sigma ASP262: Salt Bridge (2) ASP251: Salt Bridge GLU364: Salt Bridge GLU459: Salt Bridge (3)

5D6E	Human methionine aminopeptidase 2	Hydrolase	-6.6	-5.7	ASP262, GLU364, ILE338, ASP251, PHE219, HIS231	TYR607: Pi-Sigma SER599: Conventional Hydrogen Bond MET638: Alkyl PRO536: Alkyl VAL608: Alkyl

Table 3.4 Leucine vs. the reported inhibitors for each target protein

PDB Code	Protein Name	Leucine ADV Docking Score (kcal/mol)	Leucine ADV Amino Acid Residues	Reported Inhibitor Name	Reported Inhibitor ADV Docking Score (kcal/mol)	Reported Inhibitor ADV Amino Acid Residues
3QFS	NADPH-Cytochrome P450 Reductase	-4.8	SER599, TYR607, PRO536, VAL608	Fumagillin	-7.2	ASP262, PHE219, HIS231, LEU328, ASN329, HIS331, HIS339, TYR444, PRO443
5D6E	Human methionine aminopeptidase 2	-5.7	ASP262, GLU364, ILE338, ASP251, PHE219, HIS231	7-Ethoxyresorufin	-8.0	ARG517, TYR458, SER460, TYR459, VAL477, CYS475, TRP679

With leucine as the ligand, the target protein that it inhibits is 5D6E, Human Methionine Aminopeptidase 2 (MetAP2). When leucine's binding site is compared to the reported inhibitor fumagllin's[35] binding site, leucine binds to 4 out of the 9 amino acid residues that fumagillin binds to. These are ASP262, PHE219, HIS231, and HIS331. Figure 7 and 8 shows the PyMol visualization of both ligands docked on to 5D6E, displaying how they have binded to very similar sites of the protein. Some of the interactions between the residues and leucine include many Salt Bridges, Pi-Alkyl, Pi-Sigma and Positive-Positive interactions. The leucine-5D6E complex has a higher binding energy and docking score in comparison to 3QFS and it also has many salt bridge interactions at the binding site, which are non-covalent interactions between two ionized sites made up of a hydrogen bond and an electrostatic interaction. Therefore, these interactions make the complex with 5D6E stronger and more stable.

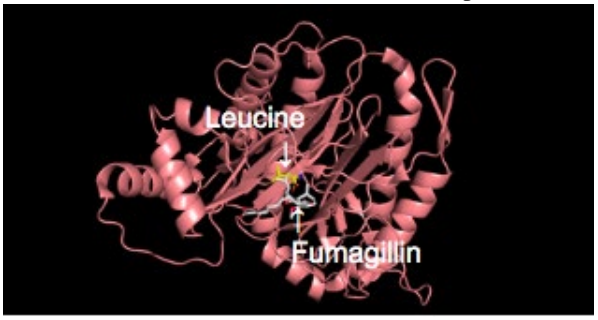


Fig 7

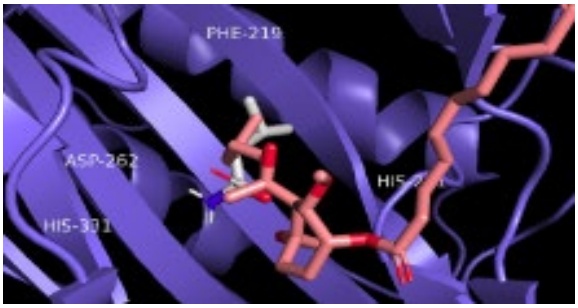


Fig 8

Figure 7 Leucine and fumagillin bound to Human Methionine Aminopeptidase 2 (MetAP2)

Figure 8 Close-up view of binding complexes with Human Methionine Aminopeptidase 2 (MetAP2) with common binding residues labeled. The light pink ligand is fumagillin and the light grey ligand is leucine.

Table 1.5 Top 30 target proteins scored for L-lysine from ACID

PDB Code	Name	Free Binding Energy (kcal/mol)	Docking Score (kcal/mol)
1S1D	Apyrase	-56.97	-6.11
3T3M	alphaIIbbeta3 Receptor Antagonist	-41.63	-6.15
1CJY	Human Cytosolic Phospholipase A2	-39.18	-5.82
2KFZ	Klenow Fragment	-30.57	-5.82
1G1T	E-selectin Lectin/EFG Domans	-29.75	-5.49
3ORH	guanidinoacetate N-methyltransferase	-27.97	-6.53
1IMB	Inositol Monophosphatase	-27.87	-6.15
1JZ8	E. Coli Beta-galactosidase	-27.34	-6.03
1KQP	NH3-Dependent NAD+ Snythetase	-25.57	-6
4IAR	5-HT1B-BRIL	-24.87	-6.33
2F25	Sialidase Neu2 E111Q Mutant	-24.02	-6.15
1QGI	Chitosanase from Bacillus Circulans	-22.85	-5.88
4HZE	human Arginase-2	-22.84	-6.02
5HIA	Human hypoxanthine-guanine phosphori-bosyltransferase	-22.27	-6.01
2AEB	human arginase I at 1.29	-21.88	-6.16
3I4A	Dimethylarginine dimethylaminohydro-lase-1 (DDAH-1)	-21.43	-6.43
3FE4	Human Carbonic Anhydrase	-21.38	-6.15
3BIU	human peptidylarginine deiminase 4	-20.82	-6.01
3GR4	Human Pyruvate Kinase M2	-19.66	-6.06
4V11	Synaptotagmin-1	-18.67	-5.96
4XCT	hydroxamate based inhibitor ARP101 (EN73)	-17.6	-6.62
3BRT	NEMO/IKK association domain structure	-17.16	-6.11
2ZC6	Penicillin-binding protein 1A (PBP 1A) acyl-enzyme complex (tebipenem) from Streptococcus pneumoniae	-17.03	-6.13
4JNI	uPA	-16.44	-6.36
4XIA	D-Xylose Isomerase	-16.35	-6.46
2QTL	FAD-containing FNR-like Module of Hu-man Methionine Synthase Reductase	-16.17	-6.1
2Y2M	Penicillin-Binding Protein (PBP-1B)	-16.17	-6.03
1LI4	Human S-adenosylhomocysteine hydro-lase	-15.83	-6.58
2CEV	Arginase from Bacillus Caldevelox	-15.79	-6.27
2Y6D	MMP7 Inhibitor	-15.77	-6.08

Table 2.5 Validation of docking of selected target proteins for L-lysine

PDB Code	Name	Classification	ACID Docking Score (kcal/mol)	AutoDock Vina Docking Score (kcal/mol)	AutoDock Vina Amino Acid Residues	DS Amino Acid Residues and Interactions
2AEB	Human Aarginase I at 1.29	Hydrolase/ Hydrolase Inhibitor	-6.16	-4.9	PRO167, PHE147, PRO157, ILE156, VAL165, ASP158	ASP158: Salt Bridge VAL165: Conventional Hydrogen Bond (2), Alkyl PHE147: Pi-Alkyl ILE156: Alkyl PRO157: Conventional Hydrogen Bond (2)
3BIU	Human Peptidylarginine Deiminase 4	Hydrolase/Hydrolase Inhibitor	-6.01	-4.3	VAL290, SER288, LEU117*	ILE121: Alkyl VAL289: Alkyl VAL290: Conventional Hydrogen Bond SER288: Conventional Hydrogen Bond (2) ASP287: Salt Bridge, Charge-Charge LEU117: Conventional Hydrogen Bond

* PyMol was used to identify amino acid residues

Table 3.5 L-lysine vs. the reported inhibitors for each target protein

PDB Code	Protein Name	L-Lysine ADV Docking Score (kcal/mol)	L-Lysine ADV Amino Acid Residues	Reported Inhibitor Name	Reported Inhibitor ADV Docking Score (kcal/mol)	Reported Inhibitor ADV Amino Acid Residues
2AEB	Human Arginase I at 1.29	-4.9	PRO167, PHE147, PRO157, ILE156, VAL165, ASP158	L-Lysine	-4.9	PRO167, PHE147, PRO157, ILE156, VAL165,

						ASP158
3BIU	Human Peptidylarginine Deiminase 4	-4.3	VAL290, SER288, LEU117*	GSK484 hydrochloride	-7.2	GLN146, SER288, VAL290*

* PyMol was used to identify amino acid residues

With L-lysine as the ligand, it actually inhibits both the target proteins, 2AEB (Human Arginase I) and 3B1U (Human Peptidylarginine Deiminase 4). However, L-lysine's inhibition of Human Arginase I has already been reported[36], so looking at L-lysine's inhibition of 3B1U will be more novel and significant. When L-lysine's binding site on 3B1U is compared to the reported inhibitor GSK484 hydrochloride's[37] binding site, L-lysine binds to 2 out of the 3 residues that GSK484 hydrochloride binds to and these residues are SER288 and VAL290. Figure 9 and 10 shows the PyMol visualization of both ligands docked on to 3B1U, displaying how they have binded to the same binding site of the protein. Some of the interactions between the residues and L-lysine include Alkyl, Conventional Hydrogen Bonds, Salt Bridge, and Charge-Charge and these polar bonds and highly attractive nonpolar interactions provide stability and allow l-lysine to remain bound to 3B1U as an inhibitor.

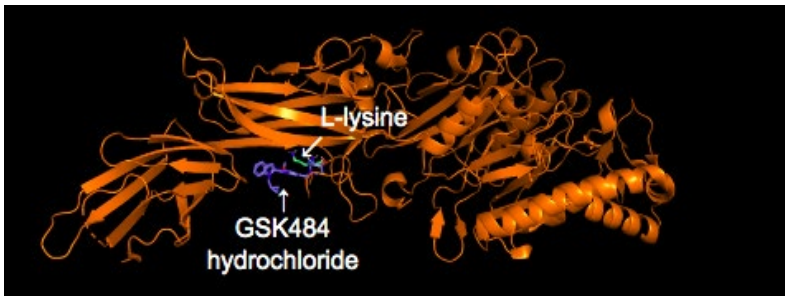


Fig 9

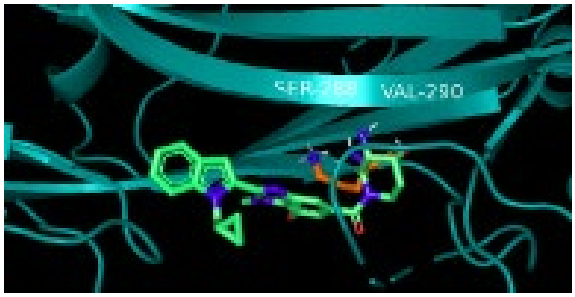


Fig 10

Figure 9 L-lysine and GSK484 hydrochloride bound to Human Peptidylarginine Deiminase 4

Figure 10 Close-up view of binding complexes with Human Peptidylarginine Deiminase 4 with common binding residues labeled. The light green ligand is GSK484 hydrochloride and the orange ligand is L-lysine.

Discussion

The goal of this project was to find novel protein targets for fenugreek that can help treat diabetes mellitus. Our results show the different target proteins that each phytochemical constituent of fenugreek, exempting diosgenin, is able to inhibit well, but it is more important to look at the significance of these inhibitions and their relevance to diabetes. Trigonelline inhibits low molecular weight Protein tyrosine phosphatase, a member of the PTP family, but is significantly less researched in its potential therapeutic ability in comparison to PTP1B[38]. LMPTP has been proposed to regulate insulin signaling through IR dephosphorylation[39]. Knockdown of LMPTP expression by antisense oligonucleotides improves the glycemic profile and decreases insulin resistance in diet-induced obese (DIO) C57BL/6 (B6) mice[40] and overexpression of catalytically inactive LMPTP in immortalized mouse fibroblasts increases insulin-induced IR tyrosine phosphorylation[41], showing that LMPTP regulates insulin signaling through its phosphatase activity. Possible future research could include a deeper study into trigonelline's inhibition of LMPTP, or synthesis of selective LMPTP inhibitors in order to increase insulin secretion in diabetic patients. 4-Hydroxyisoleucine inhibits Nitric Oxide

Synthase the best, and this protein is a key regulator of endothelial function, which increases skeletal muscle perfusion, and the surface area of capillaries to allow insulin exchange. However, this is the opposite for what is wanted. Several studies have demonstrated that a reduction of nitric oxide synthases diminishes perfusion and glucose uptake in the skeletal muscle in both humans and rodents. Hence, inhibiting nitric oxide synthases is important in improving the condition of patients with type 2 diabetes[42]. Leucine has been shown to inhibit human methionine aminopeptidase 2, an enzyme that, under normal cellular conditions, methionine aminopeptidase 2 (MetAP2) catalyzes the removal of the N-terminal methionine, the starting amino acid of a polypeptide chain, from novel proteins shortly after translation. In Type II diabetic preclinical studies, MetAP2 inhibitors have shown to produce clinically significant weight loss characteristics and reduced average blood glucose levels[43]. Leucine's high binding affinity to MetAP2 and potential as a MetAP2 inhibitor can help extend the catalog of already synthesized MetAP2 inhibitors. Lastly, in this study, it was found that L-lysine inhibited Human peptidylarginine deiminase 4, a nuclear citrullinating enzyme that is critically involved in the release of decondensed chromatin from neutrophils as neutrophil extracellular traps (NETs). NETs, together with fibrin, are involved in defense against pathogens, but the formation of NETs (NETosis) has negative effects related to many diseases, such as diabetes mellitus[44]. In parallel with increased NETosis, neutrophil PAD4 protein expression is elevated approximately 4-fold in patients with type 1 diabetes mellitus and type 2 diabetes mellitus[44]. Increased NETS levels also create many complications that are often associated with diabetes patients such as impaired wound healing, especially in relation to diabetic foot ulcers[45], and increased incidences of heart disease and thrombosis[46]. Further research on PAD4 inhibitors like L-lysine would therefore be quite beneficial for diabetic patients.

Conclusion

The phytoconstituents from fenugreek have long been studied for their anti-diabetic effects in vivo and in vitro, more specifically for their ability to inhibit enzymes such as alpha glucosidase and alpha amylase. In this study, however, we have shown that the specific compounds trigonelline, 4-hydroxyisoleucine, leucine, and L-lysine are capable of inhibiting novel protein targets that can help treat diabetes mellitus in even more ways. It is important to note that we only picked the highest score and best matching protein, but compounds like 4-hydroxyisoleucine binding to other proteins could also result in the compound's biological effects. This study only looks at their ideal mechanism of action since it was only a molecular docking study, so future studies should focus on in-vitro studies that look at these compounds' inhibition of their respective target proteins such as LMPTP. If successful, further research can also be done to create semi-synthetic drugs based on the compounds to target the specific proteins in diabetic patients.

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