Original Article

Investigation Of Papaverine And Laudanosine As Alpha Amylase Inhibitors For The Treatment Of Diabetes Mellitus

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Abstract

The present study was designed to specifically investigate the inhibitory effects of two naturally occurring compounds, Papaverine and Laudanosine, on alpha amylase activity. A comprehensive evaluation of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of the compounds was undertaken. Following the aforementioned procedures, a series of molecular docking investigations were conducted in order to assess the binding affinity capabilities of the aforementioned compounds towards the alpha amylase enzyme. From in silico ADMET analysis it was concluded that, these compounds possess drug-likeness properties. From molecular docking it can be concluded that Laudanosine is having very less binding affinity with alpha amylase and it is forming very less stable complex with it whereas, Papaverine significantly having very good binding affinity and forming more stable complex with alpha amylase. Papaverine can be developed further as potential alpha amylase inhibitor for the treatment of diabetes mellitus. Furthermore, it is imperative to generate a substantial amount of high-quality data through the utilization of a wider range of in vitro and in vivo models.

Keywords: Molecular docking; Papaverine; Laudanosine; ADMET; Alpha amylase; T2DM

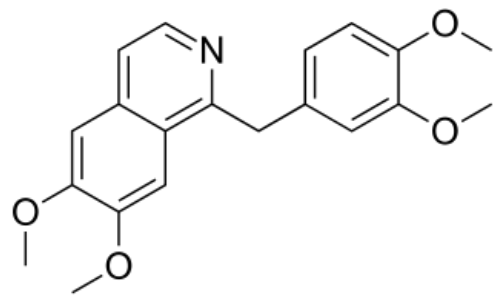
1. Introduction

The global population of individuals diagnosed with diabetes is experiencing a concerning increase in mortality rates and deteriorating health conditions. Consequently, there is an urgent need to prioritize the development of a highly efficacious therapeutic intervention. The increasing popularity of traditional medicine among individuals with diabetes can be attributed to its perceived effectiveness and safety. The presence of alkaloids, flavonoids, and saponins within the plant species under investigation has been identified as the potential underlying mechanism responsible for the anticipated therapeutic effect in individuals with diabetes mellitus. It has been observed that a single plant has the potential to possess multiple phytochemical components. Consequently, the combination of various plants or herbs has been found to yield a notable pharmacological effect with enhanced efficiency. If the holistic approach proves to be efficacious, there is a possibility that it could result in the development of a product that is both safer and more tolerable for individuals[1–6].

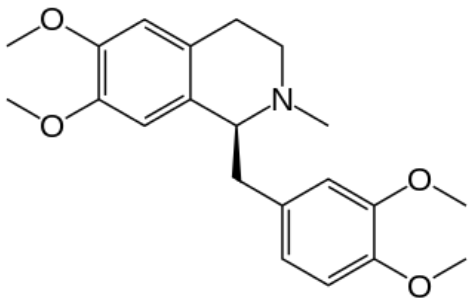
The process of oligosaccharide and monosaccharide glucose formation involves the enzymatic action of α-amylase, which catalyzes the cleavage of α-D-(1,4) glycosidic bonds present in carbohydrates. The enzyme α-glucosidase is responsible for the hydrolysis of oligosaccharides, resulting in the breakdown of these complex carbohydrates into individual glucose monosaccharide units. Consequently, individuals who consume a substantial amount of carbohydrate-rich foods may consider the utilization of enzyme inhibitors as a means to maintain stable blood sugar levels[7]. Given the prevailing distribution of diabetics, with approximately 80% residing in low and middle-income nations, it is noteworthy to highlight the exorbitant cost associated with the procurement of these medications.

Numerous endeavors have been undertaken thus far in the quest to ascertain inhibitors of α-amylase and α-glucosidase from a diverse range of sources, including plants, bacteria, marine algae, and fungus. The predominant research emphasis has been placed on the investigation of crude extracts, whether organic or aqueous in nature. However, a limited subset of studies has also examined the pure substances in isolation[8,9].

Therefore in present study, we have selected two natural compounds i.e. Papaverine and Laudanosine to investigate their alpha amylase inhibitory potential. An extensive analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds was conducted. Subsequently, molecular docking studies were performed to investigate the binding affinity potential of these compounds with the alpha amylase enzyme. The structures of Papaverine and Laudanosine are depicted in Figure 1.



Papaverine



Laudanosine

Figure 1. The structures of Papaverine and Laudanosine

2. Material and Methods

2.1 Pharmacokinetics predictions

The Lipinski rule of five and the pharmacokinetic (ADME) characteristics of molecules were investigated using PubChem[10], molinspiration[11], and SwissADME[12] servers. ADMETlab 2.0 is a totally revamped version of the AMDETlab web server, which is commonly used for predicting the pharmacokinetics and toxic characteristics of various compounds (https://admetmesh.scbdd.com/)[13].

2.2 Molecular docking studies

Molecular docking was performed on Lenovo ThinkPad with 64-bit operating system, Processor: Intel(R) Core(TM) i5-4300M CPU @2.60 GHz 2.59 GHz, RAM: 4GB by using PyRx-Virtual Screening Tool.

Ligand Preparation

The structure of Papaverine and Laudanosine, represented as an SDF File, was drafted using ChemDraw Ultra version 12.0, and the structures of the naturally occurring ligands were obtained from the PubChem database maintained by the US National Library of Medicine (https://pubchem.ncbi.nlm.nih.gov/). Structures then imported into PyRx 0.8 using open bable tool and energy minimization (optimization) was performed by considering fundamental parameters based on the element, its hybridization, and connectivity i.e. by Universal Force Field (UFF)[14]. This ligands was then converted to AutoDock Ligand format (pdbqt).

Target Preparation

The RCSB Protein Data Bank was consulted in order to get the enzymes' three-dimensional crystal structures(https://www.rcsb.org/). 3D ribbon view of selected enzymes with native ligand in the cavity are illustrated

in Figure 2. The viral protein structure was optimized, purified and prepared for docking with the help of Discovery Studio Visualizer 2019 by removing unwanted water molecules, bound ligands from protein structure and saved again in pdb file format to the same folder.

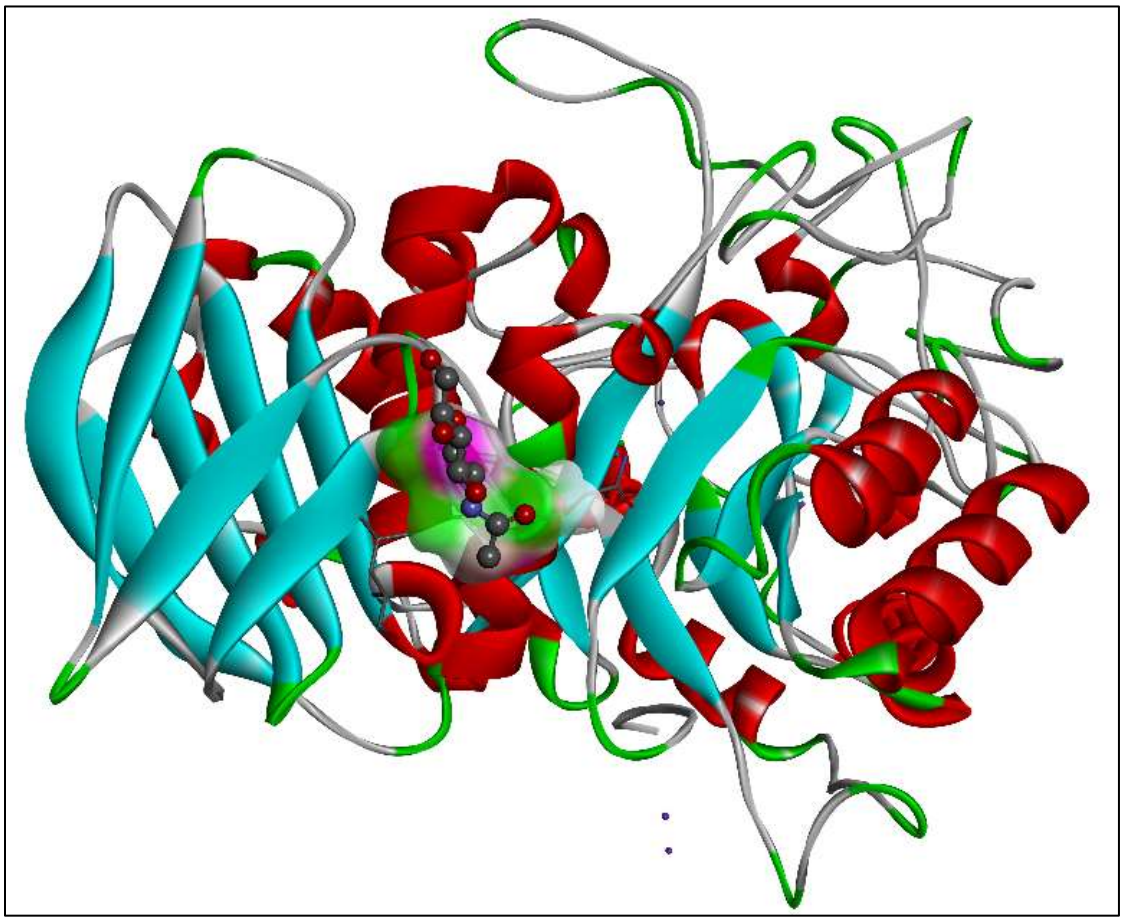


Figure 2. 3D ribbon view of alpha amylase with native ligand in the cavity (PDB ID: 3BAX)

Molecular Docking

The purified target files were loaded to docking software PyRx 0.8 using load molecule option from the file toolbar. Chain-A was used to perform the docking as it contains the active amino acid residues. The receptor structure then converted to Autodock macromolecule (pdbqt format) by using right click option. Binding affinity studies were performed by using Vina Wizard Tool in PyRx 0.8. For molecular docking, the three-dimensional grid box of known size (Alpha amylase, size_x = 55.5421 A^0, size_y = 58.2603A^0, size_z = 39.9963A^0) was adjusted (to define area for interactions) with exhaustiveness value of 8. After selecting molecules, the active cavity was selected to define the cavity with the help of Toggle Selection Spheres option given in PyRx. To occupy all the active binding sites and essential residues, the grid box was aligned properly. All the ligands and target enzymes then subjected for docking to get the finding affinity with each other's.

Identification of Cavity and Active Amino Acid Residues

The active amino acid residues in the protein were identified and noted using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287). The selection of the amino acids in the active site was used to analyze the grid box and to define cavity. All the docking poses, ligand and protein interactions were studies by importing output files into Discovery Studio which enables us to identify the types of interactions. Discovery Studio is an offline life sciences software that offers tools to study drug receptor interaction, docking poses visualization and macromolecule

preparations. The complete molecular docking technique, including identifying cavity and active amino acid residues, was carried out using the strategy described by Khan et al.[15–23].

3. Results and Discussion

3.1 Pre-ADMET Analysis

Table 1 presents the tabulated physicochemical characteristics of molecules. In the context of physicochemical examination, the observed values of all the molecules fall within the permitted range. The addition of logP and logS as a component of the Lipinski rule of five was necessary by the importance of the drug's lipophilicity. In the current study, all of these characteristics were found to fall within the permissible range and demonstrated optimal oral bioavailability. This suggests that they have the potential to be formulated for administration through the oral route[24,25]. Table 2 provides a demonstration of the drug-likeness characteristics shown by several compounds. Various parameters, including QED, NPscore, Lipinski rule, Pfizer rule, GSK rule, Golden Triangle, and Chelator rule, were computed. The natural product-likeness score, often referred to as the NPscore, typically ranges from -5 to 5. A higher score indicates an increased probability that the molecule under consideration is an NP[26,27]. All of the molecules exhibited characteristics resembling those of nonpolar compounds. Both of the drugs demonstrate compliance with the GSK rule and the Golden Triangle rule, which suggests that they may possess a more advantageous ADMET profile.

The absorbance characteristics of the compounds are presented in Table 3. The optimal Caco-2 permeability is achieved when the value exceeds -5.15 Log unit. Regrettably, none of the molecules exhibited the desired level of Caco-2 permeability[28]. Both compounds exhibited action as P-glycoprotein (Pgp) substrates. All of the proposed compounds exhibited a modest level of inhibition in terms of human intestinal absorption (HIA). The bioavailability of the compounds at F20% and F30% fell within the permissible range of values.

Table 4 illustrates the distribution and metabolic characteristics of the compounds. Plasma protein binding (PPB), which refers to the extent to which medications bind to proteins in the blood, is an important factor to consider in pharmacology. Medications that exhibit high levels of protein binding may have a narrow therapeutic index, meaning that the difference between a safe and effective dose and a toxic dose is quite small. In the case of both Papaverine and Laudanosine, their PPB values were found to be less than 90%. The volume distribution (VD) of all the molecules was within the permissible range of 0.04-20L/kg. Both of the compounds exhibited a moderate capacity for penetrating the blood-brain barrier (BBB). Both compounds exhibited potential for inhibiting CYP enzymes[13].

Table 5 presents a comprehensive overview of the excretion and toxicity characteristics of many compounds. Both of the compounds exhibited a moderate rate of clearance. All of the molecules had a brief duration of half-life. The compounds had a favorable toxicity profile, with several of the values falling inside the acceptable range[13]. Table 6 presents the environmental toxicity profile of the proposed compounds, including the bioconcentration factors, IGC50, LC50FM, and LC50DM. The compounds exhibited an environmental toxicity profile that was optimal and fell within the permissible range.

Table 1. Physicochemical properties calculated for molecules

Code	Physicochemical Properties							
	Molecular Weight	Volume	nHA	nHD	nRot	TPSA	logS	logP
NL	221.090	199.470	7	5	3	119.250	-0.151	-1.931
Papaverine	339.150	353.873	5	0	6	49.810	-4.047	2.889
Laudanosine	357.190	376.442	5	0	6	40.160	-2.785	2.589

Table 2. Drug-likeness properties of molecules

Code	Medicinal Chemistry						
	QED	NPscore	Lipinski Rule	Pfizer Rule	GSK Rule	Golden Triangle	Chelator Rule
NL	0.337	2.019	Accepted	Accepted	Accepted	Accepted	0 alert
Papaverine	0.682	0.116	Accepted	Accepted	Accepted	Accepted	0
Laudanosine	0.791	0.610	Accepted	Accepted	Accepted	Accepted	0

Table 3. An absorption parameters of molecules

Code	Absorption						
	Caco-2 Permeability	MDCK Permeability	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%
NL	-5.327	9.6e-05	---	++	++	--	+++
Papaverine	-4.859	2.9e-05	---	+++	---	---	-
Laudanosine	-4.682	3.1e-05	+++	+++	---	-	++

Table 4: Distribution and metabolism profile of molecules

Code	Distribution				Metabolism									
	PPB (%)	VD	BBB Penetration	Fu	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
					Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
NL	12.810	0.396	--	87.721	---	---	---	---	---	---	---	---	---	---
Papaverine	83.807	1.002	---	5.039	--	+++	++	+++	++	++	---	+++	+++	+++
Laudanosine	72.779	1.691	+++	13.342	---	+++	--	+++	--	+++	--	+++	--	+++

Table 5. Excretion and toxicity profile of molecules

Code	Excretion		Toxicity									
	CL	T1/2	H-HT	DILI	AMES Toxicity	Rat Oral Acute Toxicity	FDA MDD	Skin Sensitization	Carcinogencity	Eye Corrosion	Eye Irritation	Respiratory Toxicity
NL	1.803	0.819	-	-	+	---	---	---	---	---	---	---
Papaverine	10.340	0.779	+	+++	-	--	--	-	--	---	---	++
Laudanosine	10.079	0.804	--	++	--	+	++	-	---	---	---	+++

Table 6. Environmental toxicity profile of molecules

Code	Environmental toxicity			
	Bioconcentration Factors	IGC50	LC50FM	LC50DM
NL	0.271	0.522	0.918	1.100
Papaverine	2.736	4.155	5.183	6.727
Laudanosine	2.617	4.287	5.130	6.850

3.2 Molecular Docking Studies

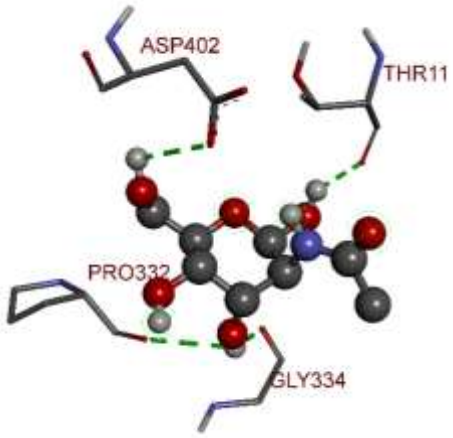
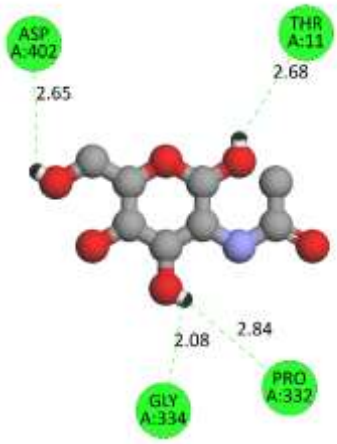
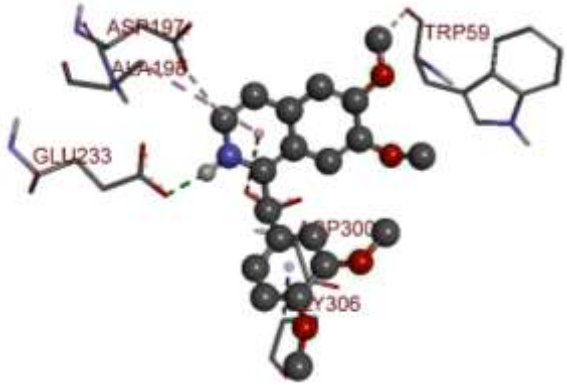
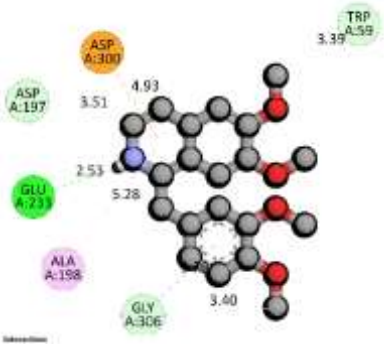
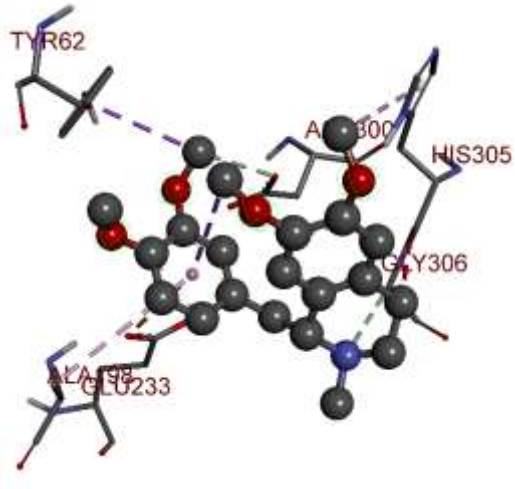
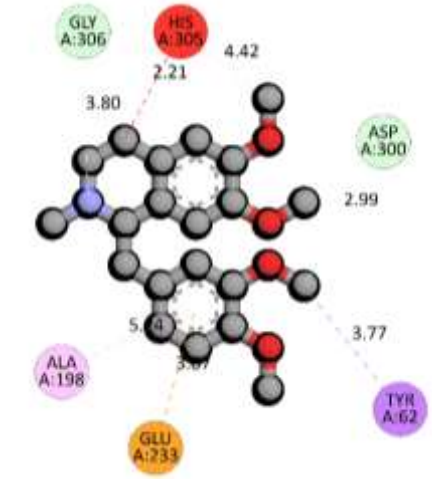
Molecular docking is a computer tool that enables us to digitally screen molecules to assess the ligand's preliminary activity potential against biological targets. This may be accomplished by determining the ligand's affinity for the target. The docking interactions of molecules are tabulated in Table 7 and the docking poses are exemplified in Table 8. The binding affinities of all the docked compounds have been compared with the binding mode of native ligand present in the crystal structure of alpha amylase (PDB ID: 3BAX).

The NL (3bax) exhibited -5.7 kcal/mol binding affinity and formed four conventional hydrogen bonds with Thr11, Pro332, Gly334 and Asp402. Papaverine exhibited -5.4 kcal/mol of binding affinity and formed one hydrogen bonds one conventional and three carbon hydrogen bonds with Glu233, Asp197, Gly306 and Trp59. It also showed interactions electrostatic and hydrophobic (Pi-Anion, Pi-Sigma and Pi-Alkyl) with Asp300, Gly306 and Ala198. Laudanosine displayed -2.4 kcal/mol of binding affinity and formed two carbon hydrogen bonds with Asp300 and Gly306. It also formed interactions both electrostatic and hydrophobic (Pi-Anion, Pi-Sigma and Pi-Alkyl) with Glu233, Tyr62, Ala198 and His305. From molecular docking it can be concluded that Laudanosine is having very less binding affinity with alpha amylase and it is forming very less stable complex with it whereas, Papaverine significantly having very good binding affinity and forming more stable complex with alpha amylase. Papaverine can be developed further as potential alpha amylase inhibitor for the treatment of diabetes mellitus.

Table 7. The binding interactions of molecules with alpha amylase

Active amino acid residues	Bond Length	Bond Type	Bond Category	Ligand energy	Docking score
NL (alpha amylase, 3BAX)					
THR11	2.6828	Hydrogen Bond	Conventional Hydrogen Bond	80.7	-5.7
PRO332	2.83876				
GLY334	2.07787				
ASP402	2.64586				
Papaverine					
GLU233	2.52906	Hydrogen Bond	Conventional Hydrogen Bond	400.35	-5.4
ASP197	3.50713		Carbon Hydrogen Bond		
GLY306	3.40447				
TRP59	3.39431				
ASP300	4.93248	Electrostatic	Pi-Anion		
GLY306	3.79435	Hydrophobic	Pi-Sigma		
ALA198	5.27684		Pi-Alkyl		
Laudanosine					
ASP300	2.98863	Hydrogen Bond	Carbon Hydrogen Bond	405.37	-2.4
GLY306	3.79778				
GLU233	3.8744	Electrostatic	Pi-Anion		
TYR62	3.77367	Hydrophobic	Pi-Sigma		
	3.90432				
ALA198	5.24459		Pi-Alkyl		
HIS305	4.42076				

Table 8. The docking poses of molecules

3D-docking poses	2D-docking poses
	
Native ligand	
	
Papaverine	
	
Laudanosine	

Conclusion

In the current investigation, a deliberate choice was made to examine the alpha amylase inhibitory capabilities of two naturally occurring compounds, namely Papaverine and Laudanosine. A comprehensive evaluation of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of the compounds was undertaken. Following the aforementioned procedures, a series of molecular docking investigations were conducted in order to assess the binding affinity capabilities of the aforementioned compounds towards the alpha amylase enzyme. From in silico ADMET analysis it was concluded that, these compounds possess drug-likeness properties. From molecular docking it can be concluded that Laudanosine is having very less binding affinity with alpha amylase and it is forming very less stable complex with it whereas, Papaverine significantly having very good binding affinity and forming more stable complex with alpha amylase. Papaverine can be developed further as potential alpha amylase inhibitor for the treatment of diabetes mellitus. Moreover, extensive quality data is need to be generated using more in vitro and in vivo models.

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