Section A-Research Paper
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Research Article

In silico screening of Physostigmine and Harmaline as potential DPP-IV inhibitors

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Abstract
In the current study, we have examined the potential of Physostigmine and Harmaline, two natural alkaloids of significant importance, as inhibitors of DPP-IV. 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 inhibitory potential of these compounds with the DPP-IV enzyme. The observation revealed that both compounds exhibited the ability to form conventional hydrogen bonds with the DPP-IV enzyme, suggesting their potential as inhibitors of the enzyme. By employing the strategy of synthesizing diverse semisynthetic derivatives, it is plausible to enhance the efficacy of DPP-IV inhibitors. Given that both of these molecules exhibit a range of drug-like properties, it is reasonable to consider their potential for further development.
Keywords: *In silico* screening; Physostigmine; Harmaline; ADMET; DPP-IV; T2DM

1. Introduction

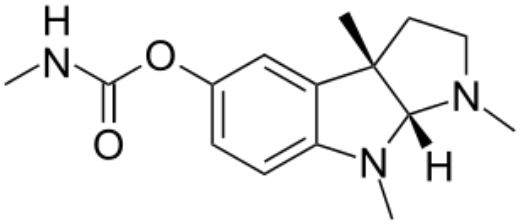
Physostigmine, a pharmacological agent classified as a cholinesterase inhibitor, has been widely employed in the medical field for the treatment of glaucoma as well as anticholinergic toxicity. Physostigmine, a pharmacological compound, exerts its inhibitory effects on acetylcholinesterase (AChE), an enzyme crucial for the degradation of acetylcholine (ACh) following its release and subsequent utilisation. Physostigmine exerts its effects on the metabolism of acetylcholine, leading to an indirect stimulation of both nicotinic and muscarinic receptors. This occurs as a result of the subsequent augmentation in the synaptic availability of acetylcholine(Arens & Kearney, 2019; Batiha et al., 2020; Kunzler & Erickson, 2021).

Harmaline, classified as a fluorescent indole alkaloid, belongs to the group of harmala alkaloids and beta-carbolines. The substance in question can be identified as the partially

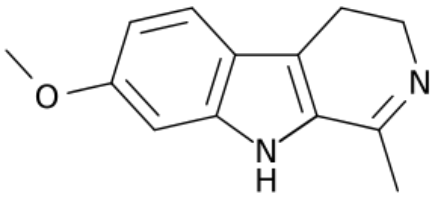
hydrogenated variant of harmine. Harmaline, a naturally occurring compound, can be found in several plant species, including Peganum harmala, commonly known as Syrian rue. Another notable source of harmaline is Banisteriopsis caapi, a plant traditionally used in the preparation of the hallucinogenic beverage known as ayahuasca. The Syrian rue seeds contain harmala alkaloids, which are present at a concentration of 3% by dry weight. These alkaloids can be extracted from the seeds(Bello et al., 2021; Hamid et al., 2017; Moloudizargari et al., 2013).

Dipeptidyl peptidase-IV (DPP-IV) inhibitors represent a pharmacological category of prescription medications employed in conjunction with dietary modifications and physical activity to regulate elevated blood glucose levels in individuals diagnosed with type 2 diabetes mellitus. The DPP-4 inhibitor class encompasses several medications, namely sitagliptin, saxagliptin, linagliptin, and alogliptin. Single-ingredient products, as well as combination formulations with other diabetes medications like metformin, are readily accessible to individuals seeking treatment for diabetes. DPP-IV inhibitors exert their pharmacological effect by facilitating postprandial insulin secretion, thereby reducing blood glucose levels. Insulin, a vital hormone, plays a crucial role in facilitating the transportation of glucose from the bloodstream into various tissues within the body. This process enables the utilization of glucose as an energy source, thereby contributing to the maintenance of stable blood sugar levels(Baig et al., 2022; Husain et al., 2022; A. Khan et al., 2021; Mohammad et al., 2022).

In the current study, we have examined the potential of Physostigmine and Harmaline, two natural alkaloids of significant importance, as inhibitors of DPP-IV. 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 inhibitory potential of these compounds with the DPP-IV enzyme. The structures of Physostigmine and Harmaline are depicted in Figure 1.



Physostigmine



Harmaline

Figure 1. The structures of Physostigmine and Harmaline

2. Material and Methods

2.1 Pharmacokinetics predictions

The Lipinski rule of five and the pharmacokinetic (ADME) characteristics of molecules were investigated using PubChem(Kim et al., 2021), molinspiration(“Molinspiration

Cheminformatics," 2006), and SwissADME(Daina et al., 2017) 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/)(Xiong et al., 2021).

2.2 Molecular docking studies

In order to further optimization, the molecules were subjected for binding affinity studies with DPP-IV enzyme. The Autodock vina 1.1.2 with PyRx Virtual Screening Tool 0.8 software of the Chimera version 1.10.2(Dallakyan & Olson, 2015) and the Biovia Discovery studio was used to perform molecular docking(Accelrys Software, 2012). The structures of Ricinine and Arecoline and native ligand were drawn using ChemDraw Ultra 8.0 version and saved in mol file format. The energy minimization was executed by Universal Force Field (UFF) in PyRx software(Rappé et al., 1992). The crystal structure of the human DPP-IV in complex with a cyclohexalamine inhibitor (PDB ID: 2P8S) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). The 3D ribbon view of DPP-IV in complex with native ligand is illustrated in Fig. 2. The binding mode and binding affinity of native ligand was used to validate the results of designed derivatives. With an exhaustiveness value of 8, the three-dimensional grid box (size_x = 62.5455580638A^{o}, size_y = 68.1442437431A^{o}, size_z = 64.3386815524A^{o}) was modified for molecular docking simulations. The complete molecular docking approach was carried out in accordance with the methods outlined by S. L. Khan *et al.*(Chaudhari et al., 2020; Khan, Sharuk L; Siddiui, 2020; S. Khan et al., 2021; S. L. Khan et al., 2020, 2021; Siddiqui et al., 2021).

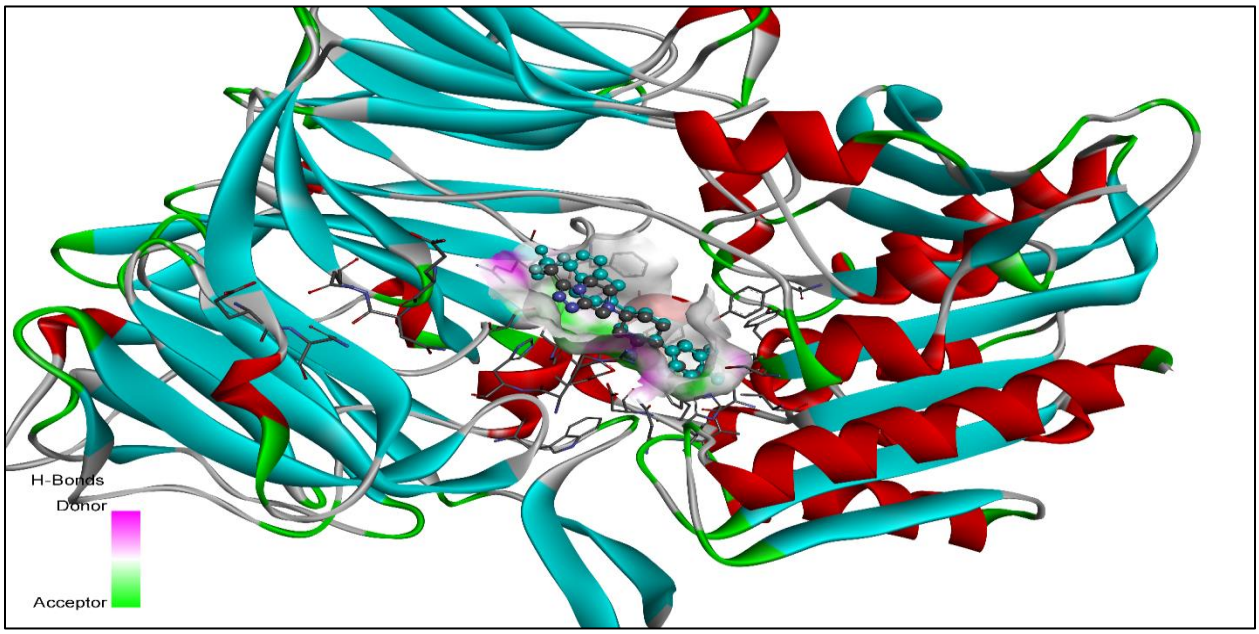


Figure 2. The 3D ribbon view of DPP-IV in complex with cyclohexalamine inhibitor (native ligand)

3. Results and Discussion

3.1 Pre-ADMET Analysis

Table 1 presents a comprehensive compilation of the physicochemical properties exhibited by various molecules. In the context of physicochemical analysis, it is observed that the values of all the molecules under investigation fall within the acceptable range. The lipophilicity of the drug plays a crucial role in various pharmacokinetic processes, including solubility, absorption, membrane permeability, plasma protein binding, distribution, and tissue penetration. This lipophilicity is closely associated with the logarithm of the partition coefficient (logP) and the logarithm of the aqueous solubility (logS) values. The inclusion of logP and logS as components of the Lipinski rule of five was necessitated by the importance of the drug's lipophilicity. In the current study, it was observed that all the parameters examined fell within the acceptable range. Furthermore, these parameters exhibited optimal oral bioavailability, suggesting that they have the potential to be formulated for oral administration(Lobo, 2020; Waring, 2010).

Table 2 showcases the inherent drug-likeness properties exhibited by molecules. Various parameters, including QED, NPscore, Lipinski rule, Pfizer rule, GSK rule, Golden Triangle, and Chelator rule, were computed in this study. The majority of the compounds exhibited a favorable range of quantitative estimate of drug-likeness (QED)(Bickerton et al., 2012; Kosugi & Ohue, 2021). The natural product-likeness score, commonly referred to as the NPscore, is typically observed to exhibit a numerical range spanning from -5 to 5. In the event that the score is elevated, it is plausible to infer that the molecule under consideration possesses a heightened probability of being classified as a natural product (NP)(Ertl et al., 2008; Menke et al., 2021). Both of the molecules exhibited properties similar to those of NPs. Both of the compounds under investigation exhibit compliance with the GSK rule and the Golden Triangle rule, suggesting that they may possess a more advantageous ADMET profile.

The absorption parameters of the molecules are presented in Table 3. The utilization of the human colon epithelial cancer cell line, Caco-2, serves as a representative model for investigating the absorption of medications within the human digestive tract. The optimal Caco-2 permeability is achieved when the value exceeds -5.15 Log unit. It is fortunate that Harmaline exhibited optimal Caco-2 permeability in this regard(Lee et al., 2017). Both Physostigmine and Harmaline have been found to exhibit P-glycoprotein (Pgp) substrate activity. Physostigmine exhibited a level of inhibitory activity in the context of human intestinal absorption that can be classified as moderate. The bioavailability of the molecules, specifically F20% and F30%, was observed to fall within the acceptable range of values.

Table 4 presents a comprehensive depiction of the distribution and metabolism profile of molecules. Plasma protein binding (PPB), which refers to the extent to which drugs bind to proteins in the bloodstream, is an important pharmacokinetic parameter to consider. In the case

of drugs with high protein binding, there is a potential concern regarding their therapeutic index, which is a measure of the drug's safety and efficacy. It is worth noting that both of the compounds under investigation in this study exhibited plasma protein binding levels below 90%. The volume distribution (VD) of all the molecules analyzed in this study fell within the acceptable range, as defined by the optimal range of 0.04-20L/kg. Both of the molecules exhibited a high potential for penetrating the blood-brain barrier (BBB). In the current study, it was observed that both molecules exhibited potential inhibitory effects on CYP enzymes(Xiong et al., 2021).

The tabulation of molecules' excretion and toxicity profile is presented in Table 5. Both of the molecules exhibited a moderate level of clearance (CL). According to the established criteria, a high rate of renal filtration is defined as being greater than 15 mL/min/kg. A moderate rate falls within the range of 5-15 mL/min/kg, while a low rate is characterized as being less than 5 mL/min/kg. All of the molecules under investigation demonstrated a relatively brief half-life, with a recorded value of less than three hours ($T_{1/2}$, <3h). The toxicity profile of the molecules exhibited favorable characteristics, with a significant proportion of the observed values falling within the acceptable range(Xiong et al., 2021). Table 6 showcases the environmental toxicity profile of the designed molecules, which includes the bioconcentration factors, IGC50, LC50FM, and LC50DM. The molecules exhibited an environmental toxicity profile that was found to be optimal and fell within the acceptable range.

Table 1. Physicochemical properties calculated for molecules

Code	Physicochemical Properties							
	Molecular Weight	Volume	nHA	nHD	nRot	TPSA	logS	logP
NL	419.150	363.865	5	2	3	59.970	-1.211	1.409
Physostigmine	275.160	282.352	5	1	3	44.810	-3.207	1.616
Harmaline	214.110	225.336	3	1	1	37.380	-3.268	2.461

Table 2. Drug-likeness properties of molecules

Code	Medicinal Chemistry						
	QED	NPscore	Lipinski Rule	Pfizer Rule	GSK Rule	Golden Triangle	Chelator Rule
NL	0.600	-0.766	Accepted	Accepted	Rejected	Accepted	0 alert
Physostigmine	0.848	1.146	Accepted	Accepted	Accepted	Accepted	0
Harmaline	0.778	0.723	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	-5.036	1.5e-05	---	---	---	---	---
Physostigmine	-4.831	2e-05	---	+	+++	+++	+++
Harmaline	-5.123	1.7e-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	54.755	3.838	--	44.865	---	---	--	++	--	--	++	+	++	-
Physostigmine	37.675	1.835	+++	78.253	--	--	---	+++	---	--	++	++	---	--
Harmaline	86.413	1.473	+++	12.601	+++	+++	--	++	---	+++	++	+++	---	-

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	7.250	0.279	+++	-	---	+	+++	--	+	---	---	++
Physostigmine	6.621	0.643	---	---	--	+++	+++	-	--	---	---	++
Harmaline	8.992	0.334	-	--	---	+++	+++	++	--	--	++	+++

Table 6. Environmental toxicity profile of molecules

Code	Environmental toxicity			
	Bioconcentration Factors	IGC50	LC50FM	LC50DM
NL	1.962	2.751	3.581	7.350

Physostigmine	0.261	2.112	4.518	5.004
Harmaline	0.825	3.407	3.827	5.693

3.2 Molecular Docking Studies

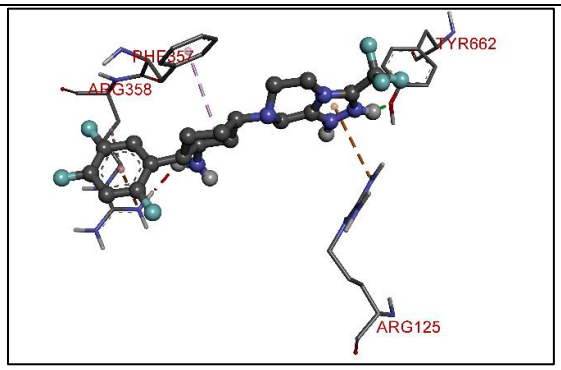
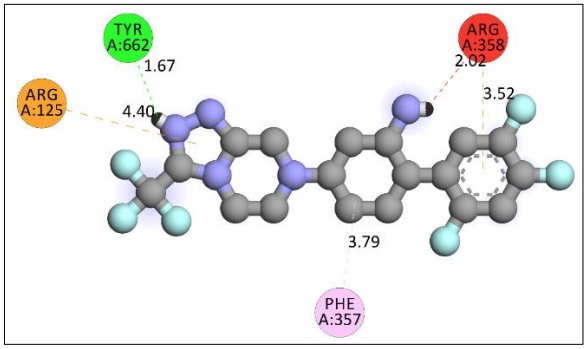
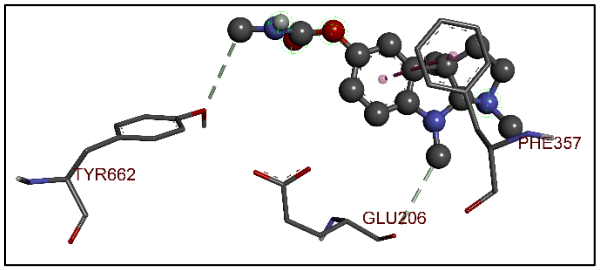
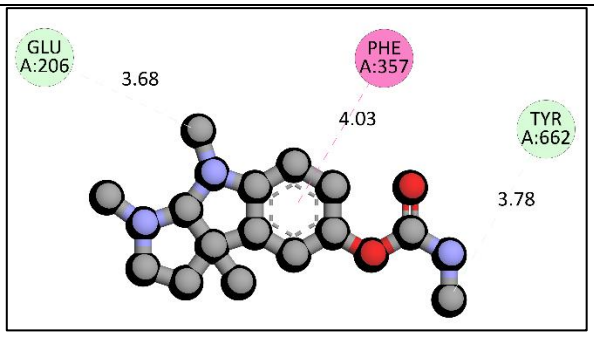
The docking interactions of molecules are tabulated in Table 7 and the docking poses are exemplified in Table 8. The binding affinities of all the docked compounds have been compared with the binding mode of native ligand present in the crystal structure of DPP-IV enzyme (PDB ID: 2P8S). Native ligand exhibited -9.1 kcal/mol binding affinity with enzyme and formed only one conventional hydrogen bond with Ty662. It has developed two electrostatic (Pi-cation) bonds with Arg125 and Arg358. It has exhibited hydrophobic (Pi-alkyl) bonds with Arg358 and Phe357. Physostigmine exhibited -6.9 kcal/mol binding affinity with enzyme and formed two carbon-hydrogen bonds with Glu206 and Tyr662. It has formed Pi-Pi stacked bond with Phe357. Harmaline demonstrated -6.7 kcal/mol docking score and formed two hydrogen bonds (one conventional and one carbon-hydrogen) with Glu206 and Val207. It has developed many hydrophobic (Pi-Pi stacked, alkyl and Pi-alkyl) interactions with Phe357, Tyr666, and Arg358. The observation revealed that both compounds exhibited the ability to form conventional hydrogen bonds with the DPP-IV enzyme, suggesting their potential as inhibitors of the enzyme. By employing the strategy of synthesizing diverse semisynthetic derivatives, it is plausible to enhance the efficacy of DPP-IV inhibitors. Given that both of these molecules exhibit a range of drug-like properties, it is reasonable to consider their potential for further development.

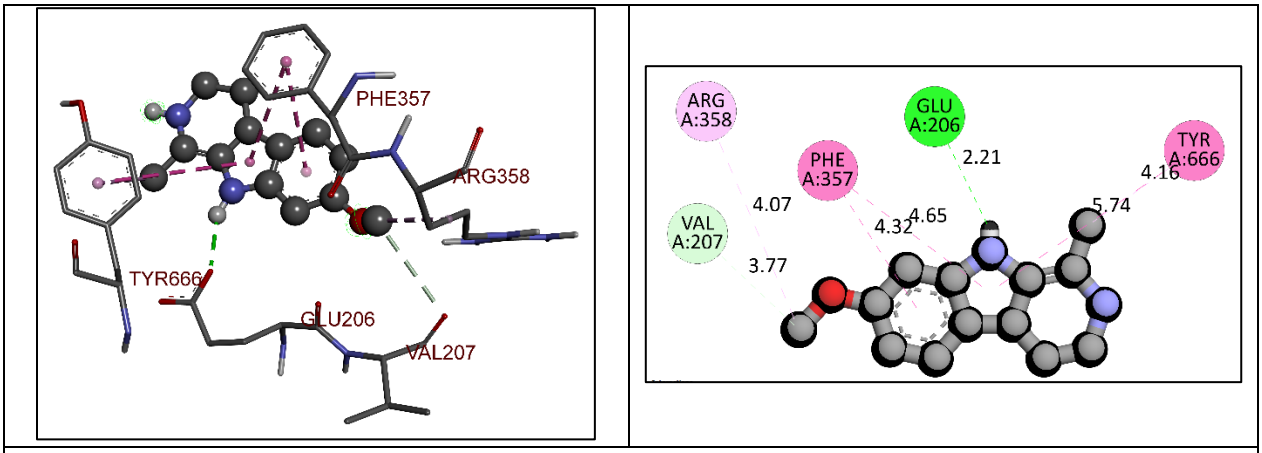
Table 7. The binding interactions of molecules with DPP-IV enzyme

Active amino residues	Bond length (A^0)	Bond type	Bond category	Binding affinity (kcal/mol)
Native ligand				
TYR662	1.66907	Hydrogen Bond	Conventional Hydrogen Bond	-9.1
ARG125	4.39768	Electrostatic	Pi-Cation	
ARG358	3.52293			
ARG358	5.41244	Hydrophobic	Pi-Alkyl	
PHE357	3.79334			
Physostigmine				
GLU206	3.67688	Hydrogen Bond	Carbon Hydrogen Bond	-6.9
TYR662	3.78141			
PHE357	4.02794	Hydrophobic	Pi-Pi Stacked	
Harmaline				
GLU206	2.21072	Hydrogen Bond	Conventional Hydrogen Bond	-6.7

VAL207	3.76985		Carbon Hydrogen Bond	
PHE357	4.65037	Hydrophobic	Pi-Pi Stacked	
TYR666	5.737			
PHE357	4.31712			
ARG358	4.06538		Alkyl	
TYR666	4.15528		Pi-Alkyl	

Table 8. The docking poses of molecules

3D-docking poses	2D-docking poses
	
Native ligand	
	
Physostigmine	



Harmaline

Conclusion

In the current study, we have examined the potential of Physostigmine and Harmaline, two natural alkaloids of significant importance, as inhibitors of DPP-IV. It was observed that all the parameters examined fell within the acceptable range. Furthermore, these parameters exhibited optimal oral bioavailability, suggesting that they have the potential to be formulated for oral administration. Both of the molecules exhibited properties similar to those of NPs. Both of the compounds under investigation exhibit compliance with the GSK rule and the Golden Triangle rule, suggesting that they may possess a more advantageous ADMET profile. Both of the molecules exhibited a high potential for penetrating the blood-brain barrier (BBB). The toxicity profile of the molecules exhibited favorable characteristics, with a significant proportion of the observed values falling within the acceptable range. The observation revealed that both compounds exhibited the ability to form conventional hydrogen bonds with the DPP-IV enzyme, suggesting their potential as inhibitors of the enzyme. By employing the strategy of synthesizing diverse semisynthetic derivatives, it is plausible to enhance the efficacy of DPP-IV inhibitors. Given that both of these molecules exhibit a range of drug-like properties, it is reasonable to consider their potential for further development.

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