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Research Article

In silico exploration of Ricinine and Arecoline as potential DPP-IV inhibitors for the treatment of T2DM

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Abstract

In the current study, we have examined the potential DPP-IV inhibitory properties of Ricinine and Arecoline, two natural alkaloids that hold significant importance. A comprehensive analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds was conducted, followed by a subsequent investigation using molecular docking techniques to study their interactions with the DPP-IV enzyme. In the realm of physicochemical analysis, it is imperative to ascertain that the values associated with various molecules fall within the acceptable range. Ensuring that these parameters are within the acceptable range is crucial for accurate characterization and evaluation of the molecules Both of the compounds under investigation have been found to meet the GSK rule, which is a set of criteria used to assess the likelihood of a compound being orally bioavailable. Additionally, these compounds exhibit a more favourable ADMET profile, referring to their absorption, distribution, metabolism, excretion, and toxicity characteristics. The toxicity profile of the suggested molecules exhibited favourable properties, with a significant number of values falling within the acceptable range. Based on the findings obtained from molecular docking studies, it was observed that both compounds exhibited the formation of conventional hydrogen bonds with the DPP-IV enzyme. This observation suggests that these compounds possess the potential for inhibiting the enzyme's activity. By employing the strategy of synthesising diverse semisynthetic derivatives, it is plausible to enhance the efficacy of DPP-IV inhibitors. Based on the observation that both of these molecules exhibit a range of drug-likeness properties, it is reasonable to consider their potential for further development.

Keywords: Ricinine; Arecoline; Molecular docking; ADMET; DPP-IV; *In silico*

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1. Introduction

Since the year 2006, dipeptidyl peptidase-IV inhibitors, also known as DPP-IV, have been used as a potential treatment alternative for the management of type 2 diabetes mellitus. DPP-IV inhibitors are a family of small molecules that may be consumed orally, despite the fact that they come in a variety of forms. These compounds have a very precise interaction with the catalytic region of DPP-IV, but they do not in any way interfere with the protein's other well-established actions, such the effect it has on the immune system. Inhibitors of DPP-IV do not have any intrinsic qualities that reduce glucose levels. Because of this, their efficacy as therapeutic agents for diabetes is almost entirely reliant on their ability to suppress the action of DPP-IV. In turn, this inhibition exerts its impact via the protective effects that it has on the substrates that are implicated. It is possible to claim that the incretin hormone that is known as glucagon-like peptide 1 is the one that has the greatest degree of relevance among these available choices. As a result of the dependence of glucagon-like peptide 1 on glucose, the possibility of developing hypoglycemia as a side effect of using DPP-IV inhibitors is very low(Caron et al., 2017; Fadini & Avogaro, 2011). All DPP-IV inhibitors exhibit the presence of class effects, which are strongly related with the mechanism of action. These effects suggest that the inhibitor is doing its job. These outcomes include a good safety profile and tolerability, as well as the potential to promote glycaemic management. Also included in this category is the ability to reduce insulin resistance. However, it is essential to keep in mind that taking any of these drugs is connected with a slightly increased possibility of developing acute pancreatitis. The term "compound-specific effects" refers to the unique consequences that result from differences in the chemical make-up and/or pharmacokinetic properties of various substances. The customized therapeutic usage of DPP-IV inhibitors may be impacted by compound-specific effects, which may perhaps elucidate off-target adverse effects, such as hospitalization for heart failure, which are solely linked with a particular DPP-IV inhibitors. DPP-IV inhibitors, in general, have a positive therapeutic profile and exhibit both safety and effectiveness in the majority of people who have been diagnosed with type 2 diabetes mellitus(Arulmozhi & Portha, 2006; Drucker, 2003; Salvatore et al., 2007).

Therefore in present investigation we have studied the Ricinine and Arecoline, significantly important natural alkaloids as potential DPP-IV inhibitors. We have performed in

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depth ADMET analysis followed by molecular docking studies of these compounds on DPP-IV enzyme to check their binding inhibitory potential with it.

2. Material and Methods

2.1 Pharmacokinetics predictions

The Lipinski rule of five and the pharmacokinetic (ADME) characteristics of designed derivatives 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. 1. 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).

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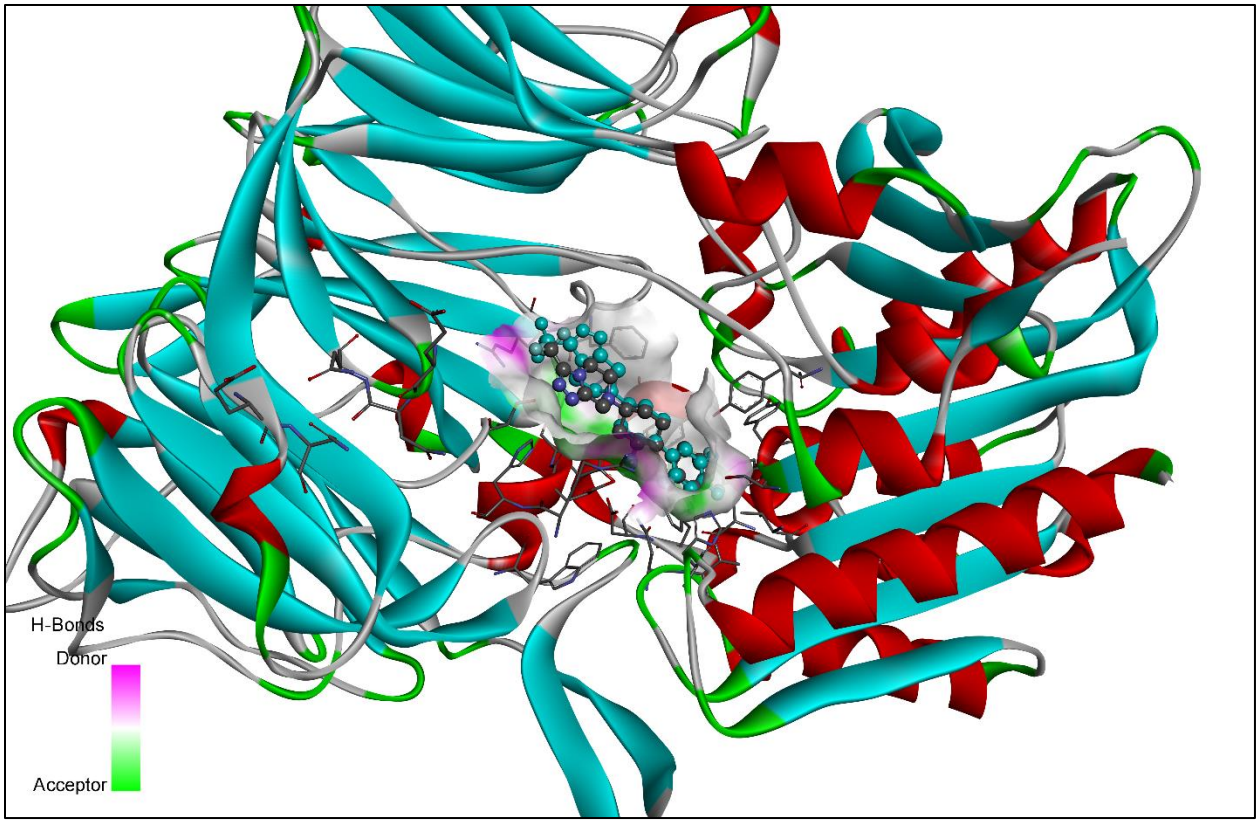

Fig. 1. The 3D ribbon view of DPP-IV in complex with cyclohexalamine inhibitor (native ligand)

3. Results and Discussion

3.1 Pre-ADMET Analysis

The physicochemical properties of molecules are tabulated in Table 1. In physicochemical analysis, values of molecules displayed within the acceptable range i.e. molecular weights, nHA, nHD, nRot, Van der Waals volume, and TPSA. The drug's lipophilicity, which is essential for solubility, absorption, membrane penetration, plasma protein binding, distribution, and tissue penetration, is directly connected to the logP and logS values. The significance of the drug's lipophilicity necessitated the inclusion of logP and logS as an element of the Lipinski rule of five. In present investigation all these parameters were within the acceptable range and displayed optimum oral bioavailability which indicates they can be developed to be delivered through oral route(Lobo, 2020; Waring, 2010).

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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
Ricinine	164.06	164.760	4	0	1	55.020	-1.428	-0.070
Arecoline	191.070	185.440	3	0	2	29.540	0.598	0.142

The drug-likeness properties of molecules are exemplified in Table 2. The different parameters such as QED, NPscore, Lipinski rule, Pfizer rule, GSK rule, Golden Triangle, and Chelator rule were calculated. Most of the compounds showed attractive range of QED(Bickerton et al., 2012; Kosugi & Ohue, 2021). Typically, the natural product-likeness score, also known as the NPscore, falls somewhere in the range of -5 to 5. If the score is higher, then there is a greater likelihood that the molecule in question is an NP(Ertl et al., 2008; Menke et al., 2021). Both the compounds satisfy the GSK rule & have a more favorable ADMET profile. The compounds satisfying the Golden Triangle rule may have a more favorable ADMET profile but unfortunately none of the molecules accepted the rule.

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
Ricinine	0.598	-0.422	Accepted	Accepted	Accepted	Rejected	0
Arecoline	0.576	0.721	Accepted	Accepted	Accepted	Rejected	0

An absorption parameters of the molecules are illustrated in Table 3. Caco-2 permeability is optimum when the value is higher than -5.15 Log unit and unfortunately none of the molecules displayed optimum Caco-2 permeability(Lee et al., 2017). It is possible to acquire a better knowledge of the process of drug efflux with the aid of MDCK-MDR1, which also draws attention to early potential problems with drug permeability. It has been discovered that the permeability of MDCK-MDR1 may, in addition to intestinal permeability, be used as an accurate predictor of the permeability of the blood brain barrier(Feng et al., 2019). None of the molecules displayed Pgp-inhibitor and Pgp-substrate activity. Both the molecules displayed moderate

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inhibitory human intestinal absorption (HIA). F20% and F30% bioavailability of the molecules were within the range of acceptable values.

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	---	---	---	---	---
Ricinine	-4.624	2.7e-05	-	-	-	-	-
Arecoline	-4.437	3.7e-05	---	--	---	--	--

The distribution and metabolism profile of molecules are depicted in Table 4. Plasma protein binding (PPB, <90%), drugs with high protein-bound may have a low therapeutic index, many of the molecules displayed PPB less than 90%. Volume distribution (VD, optimal 0.04-20L/kg) of all the molecules were within the range of acceptable limit. Both of the molecules displayed strong BBB penetration potential. In present investigation, both of the molecule showed CYP substrate potential(Xiong et al., 2021).

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	---	---	--	++	--	--	++	+	++	-
Ricinine	31.486	0.847	+++	64.77	-	+++	--	+	---	++	-	-	-	-
Arecoline	13.568	1.570	++	92.812	---	+	---	++	---	-	---	++	---	-

An excretion and toxicity profile of molecules are tabulated in Table 5. Both of the molecules displayed moderate clearance (CL, High: >15 mL/min/kg; moderate: 5-15 mL/min/kg; low: <5 mL/min/kg) rate. Both the molecules exhibited short half-life ($T_{1/2}$, <3h). Toxicity profile of the molecules suggested favorable properties and displayed many of the values were within the range (Xiong et al., 2021).

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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	+++	-	---	+	+++	--	+	---	---	++
Ricinine	6.648	0.793	+++	-	-	+++	---	--	-	+	++	+
Arecoline	11.153	0.754	--	--	+	--	+	+++	++	++	-	-

An environmental toxicity profile (Bio concentration factors, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$) of designed molecules are demonstrated in Table 6. An environmental toxicity profile of the molecules was optimum and within the acceptable range.

Table 6. Environmental toxicity profile of molecules

Code	Environmental toxicity			
	Bioconcentration Factors	IGC50	LC50FM	LC50DM
NL	1.962	2.751	3.581	7.350
Ricinine	0.481	2.364	2.894	3.790
Arecoline	0.374	2.037	2.893	3.403

3.2 Molecular Docking Studies

The docking interactions of molecules are tabulated in Table 7 and the docking poses are exemplified in Table 8.

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			
Ricinine				

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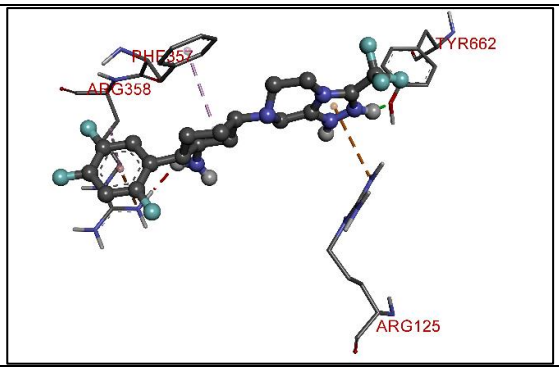
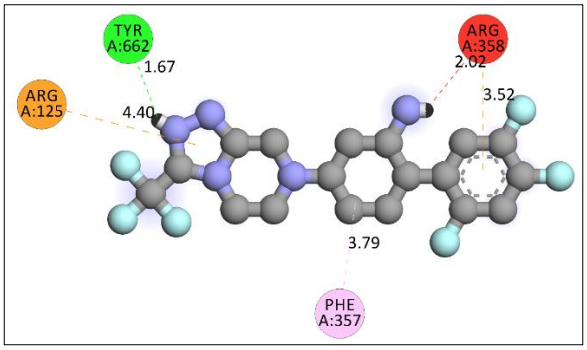
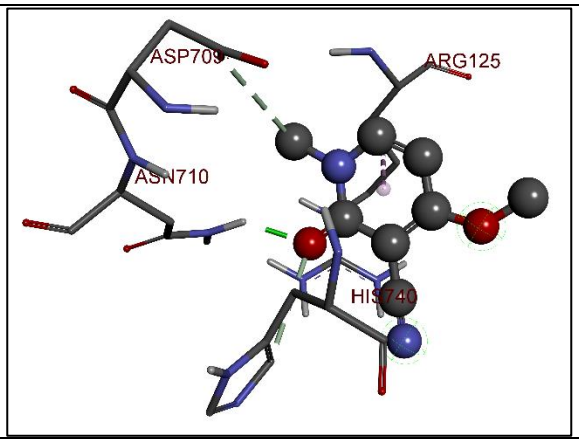
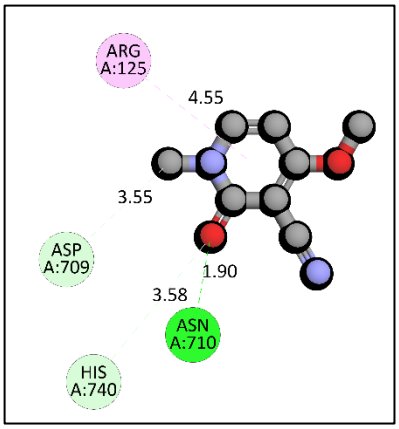
ASN710	1.89598	Hydrogen Bond	Conventional Hydrogen Bond	-5.4
ASP709	3.54976		Carbon Hydrogen Bond	
HIS740	3.58313			
ARG125	4.55045	Hydrophobic	Pi-Alkyl	
Arecoline				
ASN710	2.30204	Hydrogen Bond	Conventional Hydrogen Bond	-4.8
GLU205	3.30421		Carbon Hydrogen Bond	

Table 8. The docking poses of molecules

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

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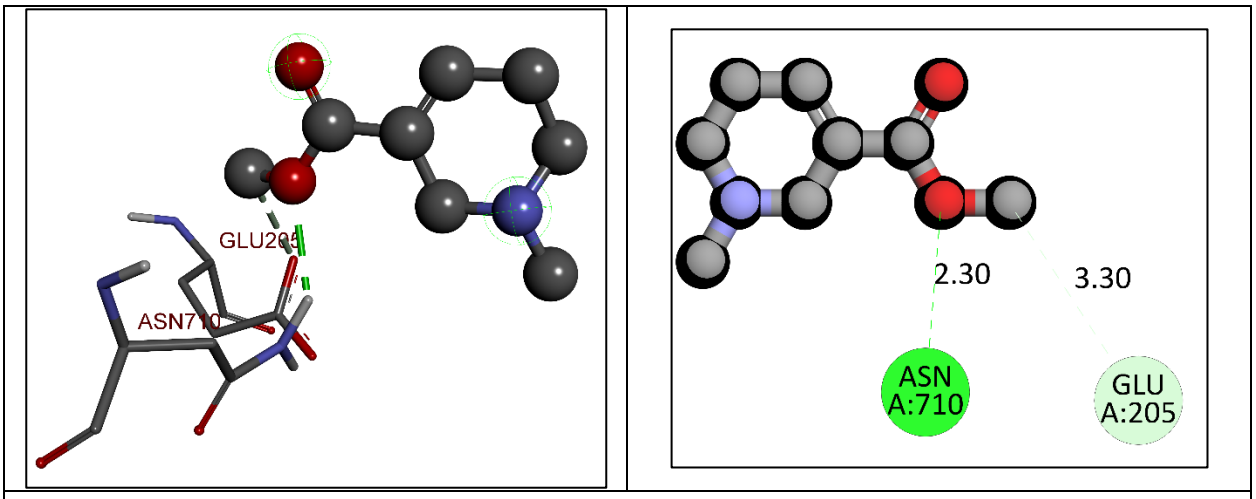

Arecoline

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. Ricinine demonstrated -5.4 kcal/mol binding affinity with DPP-IV and displayed three hydrogen bonds (one conventional and two carbon-hydrogen) with Asn710, Asp709, and His740. It has developed one Pi-alkyl bond with Arg125. Arecoline formed two hydrogen bonds (one conventional and once carbon-hydrogen) with Asn710 and Glu205. It has displayed -4.8 kcal/mol binding affinity with enzyme. It was observed that, both the compounds formed conventional hydrogen bonds with DPP-IV enzyme which indicate these having potential of enzyme inhibition. If we can optimize these molecules by preparing different semisynthetic derivatives, we can have better DPP-IV inhibitors. As both these molecules displayed most drug-likeness properties it can be developed further.

Conclusion

In the present investigation, Ricinine and Arecoline which are significantly important natural alkaloids have been studied for their potential DPP-IV inhibitory potential. In depth ADMET analysis was performed followed by molecular docking studies of these compounds on DPP-IV enzyme. In physicochemical analysis, values of molecules displayed within the acceptable range

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i.e. molecular weights, nHA, nHD, nRot, Van der Waals volume, and TPSA. Both the compounds satisfy the GSK rule & have a more favorable ADMET profile. Toxicity profile of the molecules suggested favorable properties and displayed many of the values were within the range. From molecular docking studies, it was observed that, both the compounds formed conventional hydrogen bonds with DPP-IV enzyme which indicate these having potential of enzyme inhibition. If we can optimize these molecules by preparing different semisynthetic derivatives, we can have better DPP-IV inhibitors. As both these molecules displayed most drug-likeness properties it can be developed further.

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