In silico exploration of Agrimophol and convallotoxin as potential alpha amylase inhibitors for the treatment of T2DM

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Abstract

The purpose of this research was to determine whether or not Agrimophol and Convallotoxin, two naturally occurring chemicals, suppress alpha amylase activity. The substances' ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties were analyzed in depth. In order to evaluate the compounds' binding affinity towards the alpha amylase enzyme, a series of molecular docking tests were performed after the aforementioned steps were taken. From *in silico*ADMET analysis it was concluded that, these compounds possess drug-likeness properties. Agrimophol exhibits -8.1 kcal/mol of binding affinity and formed four conventional hydrogen bonds with His201, Tyr151 and His305. It also showed electrostatic and Hydrophobic Interactions (Pi-Anion, Alkyl) with Asp300, Ile235 and Ala307.Convallotoxin displayed two conventional and one carbon hydrogen bond with Gly306 and Gln63. It also showed hydrophobic Interactions (Pi-sigma) with Tyr151. It has formed -9.8 kcal/mol binding affinity and formed very stable complex. From molecular docking it can be concluded that Agrimophol and Convallotoxin significantly having very good binding affinity and forming more stable complex with alpha amylase than native ligand. These can be developed further as potential alpha amylase inhibitor for the treatment of diabetes mellitus.

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1. Introduction

The death rates and health conditions of persons diagnosed with diabetes are seeing a worrisome escalation on a worldwide scale. Therefore, it is essential to prioritize the development of a treatment strategy that has great efficacy. The growing prevalence of traditional medicine use among patients diagnosed with diabetes may be ascribed to its perceived efficacy and safety. The possible therapeutic impact in persons with diabetes mellitus is believed to be attributed to the presence of alkaloids, flavonoids, and saponins in the plant species being studied. The phenomenon of a single plant exhibiting the presence of several phytochemical constituents has been documented. As a result, the amalgamation of different botanical specimens or herbal substances has been seen to provide a significant pharmacological impact with heightened efficacy. If the holistic approach demonstrates effectiveness, it may lead to the

creation of a product that is not only safer but also more bearable for people[1–6].

The enzymatic activity of α-amylase is involved in the synthesis of oligosaccharides and monosaccharide glucose. This process entails the catalysis of the cleavage of α-D-(1,4) glycosidic linkages found in carbohydrates. The enzyme α-glucosidase is accountable for the process of hydrolyzing oligosaccharides, leading to the decomposition of these intricate carbohydrates into separate glucose monosaccharide units. Hence, those who regularly eat a significant quantity of meals rich in carbohydrates may contemplate the use of enzyme inhibitors as a strategy to maintain consistent levels of blood glucose[7]. The distribution of individuals with diabetes is mostly concentrated in poor and middle-income countries, accounting for around 80% of the affected population. It is important to emphasize the significant financial burden involved with the acquisition of these drugs. A wide array of efforts have been made so far in the pursuit of identifying inhibitors of α-amylase and α-glucosidase from various sources, such as plants, bacteria, marine algae, and fungi. The primary focus of study has been directed towards the examination of crude extracts, regardless of whether they are organic or aqueous in composition. Nevertheless, only a restricted number of research have investigated the individual pure chemicals in isolation[8,9].

In the current investigation, two natural chemicals, namely Agrimophol and Convallotoxin, were chosen to examine their potential as inhibitors of alpha amylase. A comprehensive evaluation of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of the substances was undertaken. Following that, molecular docking experiments were conducted in order to examine the binding affinity capabilities of these compounds with the alpha amylase enzyme. Figure 1 illustrates the molecular structures of Agrimophol and Convallotoxin.
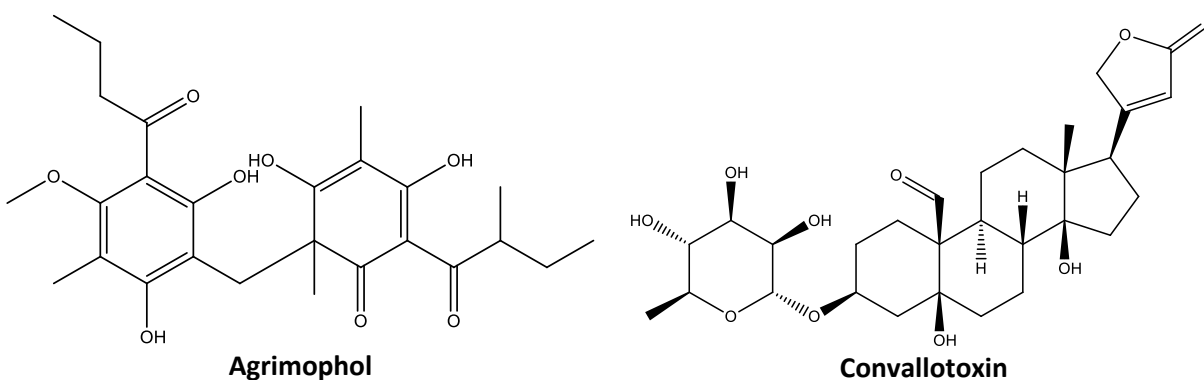
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Figure 1. The structures of **Agrimophol** and**Convallotoxin**

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 **Agrimophol** and **Convallotoxin**, 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 Figure2.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.
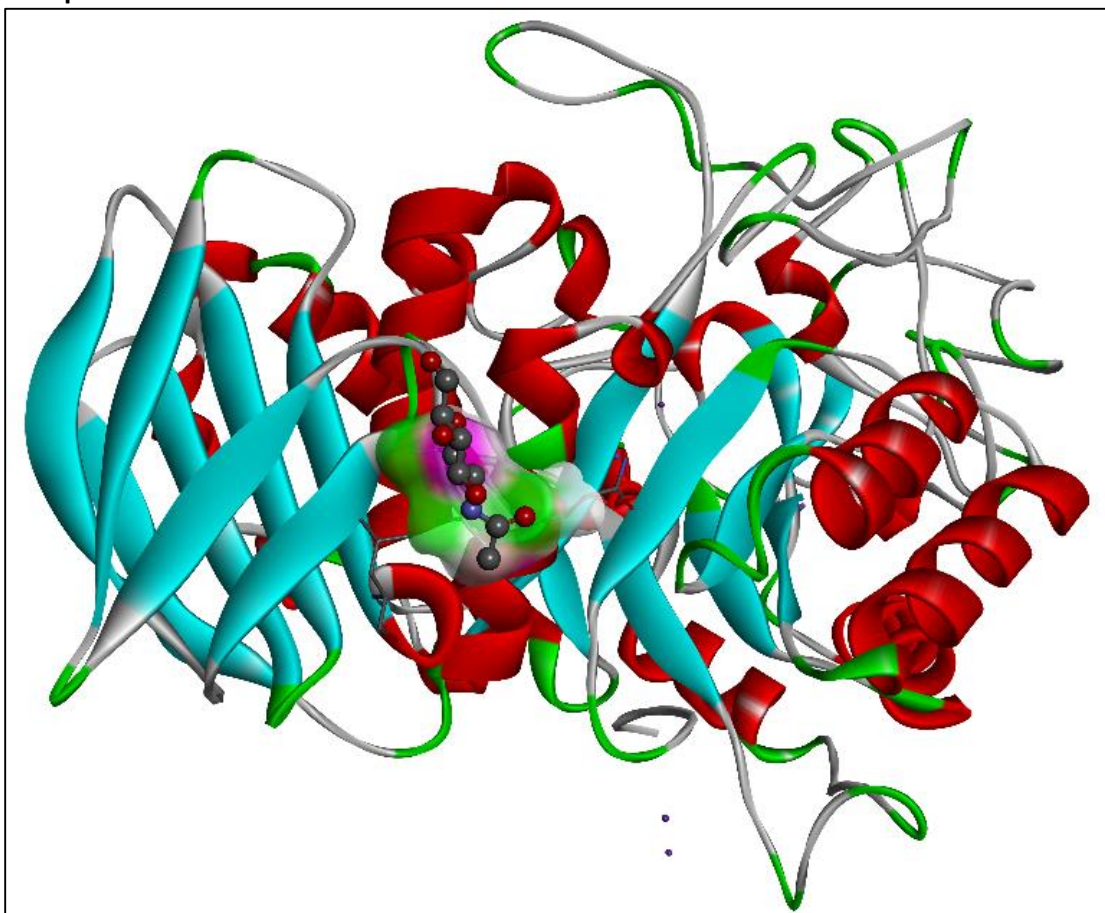
Figure 2. 3D ribbon view of alpha amylase with native ligand in the cavity (PDB ID: 3BAX)

Molecular Docking

The purified target fileswere 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 Convallotoxin, 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 low 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.

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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
Agrimophol	474.230	490.369	8	4	9	141.360	-4.000	4.782
Convallotoxin	550.280	538.794	10	5	4	162.980	-3.272	0.780

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
Agrimophol	0.296	1.670	Accepted	Accepted	Rejected	Accepted	0
Convallotoxin	0.192	3.139	Accepted	Accepted	Rejected	Rejected	0

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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	---	++	++	--	+++
Agrimophol	-4.975	1.2e-05	+	++	---	---	---
Convallotoxin	-6.375	5.2e-05	---	+++	++	+++	+++

Table 4: Distribution and metabolism profile of molecules

Code	Distribution				Metabolism									
					CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
	PPB(%)	VD	BBB Penetration	Fu	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
NL	12.810	0.396	--	87.721	---	---	---	---	---	---	---	---	---	---
Agrimophol	97.622	0.519	---	1.259	--	++	--	++	++	+	+	--	--	+
Convallotoxin	39.088	0.562	-	35.713	---	++	---	--	---	---	---	---	+	--

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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	1.803	0.819	-	-	+	---	---	---	---	---	---	---
Agrimophol	0.114	0.160	++	+++	---	--	---	--	---	---	---	-
Convallotoxin	1.405	0.523	--	---	++	+++	+++	+++	++	----	---	+++

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				
HIS305	4.42076				
Agrimophol					
	2.0175	Hydrogen Bond	Conventional Hydrogen Bond	324.06	-8.1
HIS201	2.87819				
TYR151	2.05947				
HIS305	2.53115				
ASP300	4.62948	Electrostatic	Pi-Anion		
ILE235	4.83579	Hydrophobic	Alkyl		
ALA307	3.76561				
Convallotoxin					
GLY306	2.94486	Hydrogen Bond	Conventional Hydrogen Bond	636.95	-9.8
GLN63	2.10798				
	3.59673		Carbon Hydrogen Bond		
TYR151	3.8148	Hydrophobic	Pi-Sigma		

Table 6. Environmental toxicity profile of molecules

Table 7. The binding interactions of molecules with alpha amylase

Code	Environmental toxicity			
	BioconcentrationFactors	IGC50	LC50FM	LC50DM
NL	0.271	0.522	0.918	1.100
Agrimophol	0.221	3.605	3.764	4.703
Convallotoxin	0.532	3.314	4.709	4.696

3.2 Molecular Docking Studies

Molecular docking is a computational technique that facilitates the virtual screening of molecules in order to evaluate the initial activity potential of a ligand against certain biological targets. The ligand's affinity for the target may be determined in order to achieve this. The docking interactions between molecules have been compiled and organized in Table 7, while
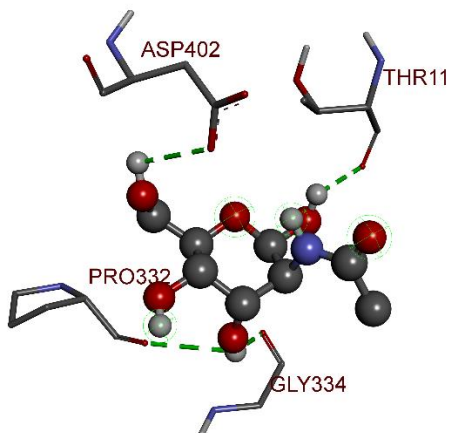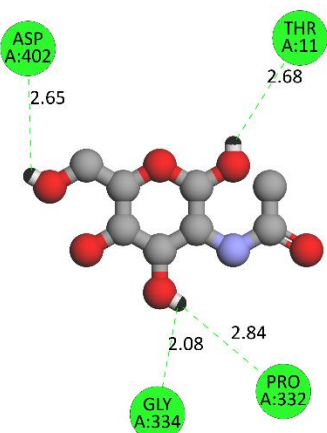
specific examples of docking postures are provided in Table 8. The binding affinities of the docked compounds were compared to the binding mode of the native ligand found in the crystal structure of alpha amylase (PDB ID: 3BAX).

The NL (3bax) exhibited -5.7 kcal/mol binding affinity and formed four conventional hydrogen bonds with Thr11, Pro332, Gly334

and Asp402.Agrimophol exhibits -8.1 kcal/mol of binding affinity and formed four conventional hydrogen bonds with His201, Tyr151 and His305. It also showed electrostatic and Hydrophobic Interactions (Pi-Anion, Alkyl) with Asp300, Ile235 and Ala307.Convallotoxin displayed two conventional and one carbon hydrogen bond with Gly306 and Gln63. It also showed hydrophobic Interactions (Pi-sigma) with Tyr151. It has formed -9.8 kcal/mol binding affinity and formed very stable complex. From molecular docking it can be concluded that Agrimophol and Convallotoxin significantly having very good binding affinity and forming more stable complex with alpha amylase than native ligand. These can be developed further as potential alpha amylase inhibitor for the treatment of diabetes mellitus.

Table 8. The docking poses of molecules

3D-docking poses	2D-docking poses

Native ligand

Agrimophol
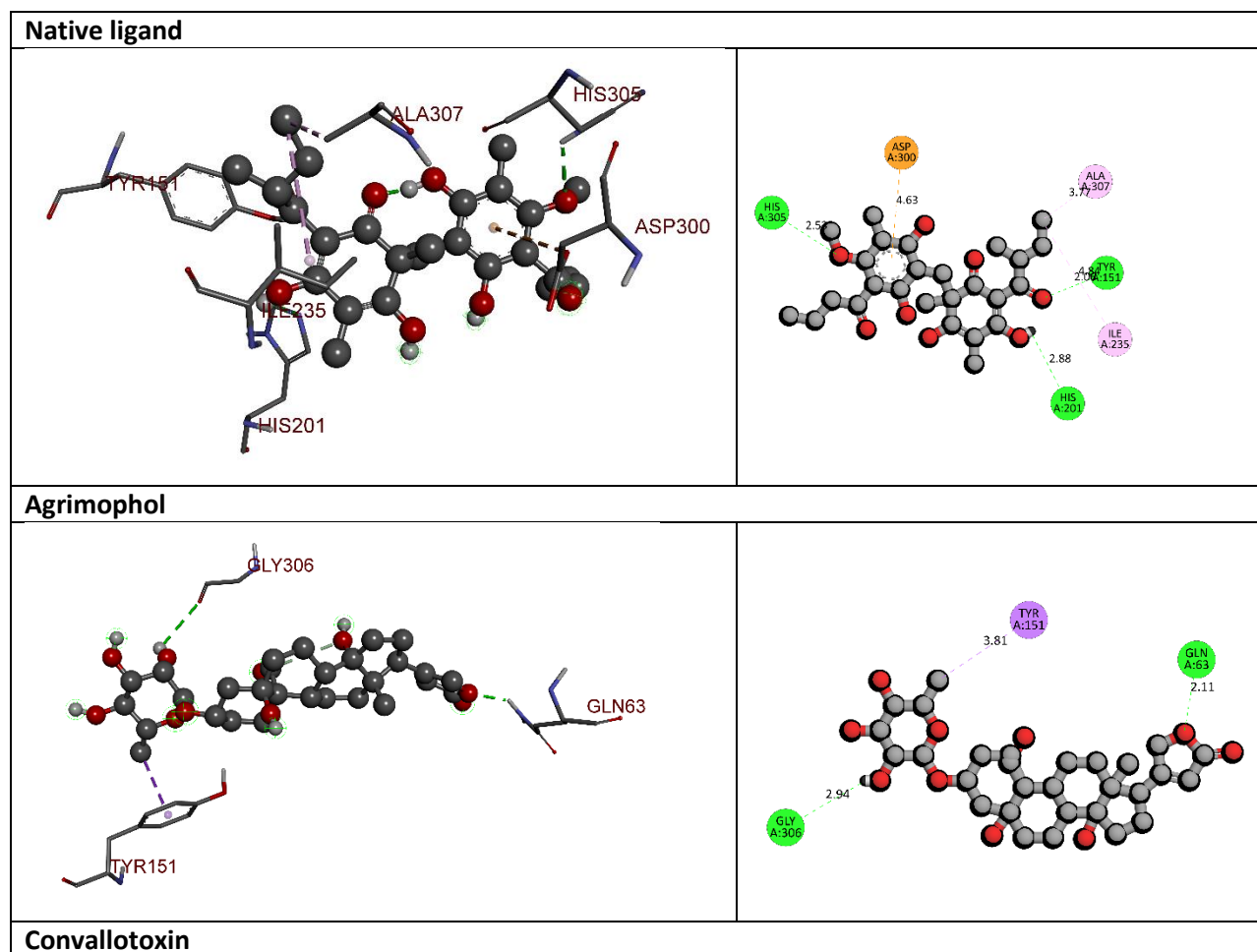
Convallotoxin

Conclusion

The present study included a conscious decision to investigate the inhibitory effects of two naturally occurring chemicals, Agrimophol and Convallotoxin, on alpha amylase. A thorough assessment was conducted to evaluate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the substances. In accordance with the aforementioned methodologies, a sequence of molecular docking analyses were performed to evaluate the binding affinity of the stated drugs towards the alpha amylase enzyme. Based on the results of in silico ADMET research, it may be inferred that these compounds exhibit drug-like characteristics. Based on the results obtained from molecular docking analysis, it can be inferred that Agrimophol and Convallotoxin exhibit a notably high binding affinity and form a more stable complex with alpha amylase compared to the natural ligand. These compounds have the potential to be further developed as alpha amylase inhibitors for the treatment of diabetic mellitus.

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