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In silico study of the natural compounds inhibiting angiotensin converting enzyme II

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Abstract: Hypertension is a health problem of high prevalence worldwide. Because it is an important cardiovascular risk factor, the development of new drugs that are more effective and with fewer side effects is extremely important. Recent studies have shown that several natural compounds have good antihypertensive activity by inhibiting the angiotensin II converting enzyme (ACE), which makes them good candidates the prototype for the development of new drugs. Based on this perspective, this work proposes to evaluate the solubility (partition coefficient and water solubility) of the natural compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside, through the software ALOGPS 2.1, and observe their interaction with the ACE, through molecular docking, with the software Autodock 4.2, aiming to corroborate the experimental data widely described in the literature. it was observed that all the compounds involved in the study had adequate partition coefficient and water solubility to interact with aqueous (biological fluids) and liposoluble (plasma membrane) surfaces. It was also observed, through the molecular docking study, that all the compounds interacted attractively with the active site of the enzyme, forming intermolecular interactions with the amino acids of the site and with the zinc ion, which is of extreme importance for the enzyme to convert angiotensin I in angiotensin II. Among the compounds involved in the study, epicatechin 3-O-gallate showed the most stable interaction with the active site, with energy at -8.02 kcal / mol. The theoretical results developed in this work allowed a better view, at a molecular level, of the interactions between several natural compounds with the active site of ACE. It can be observed that the polar groups of the compounds are of extreme importance for the interaction of the zinc ion and for its biological activities.

Keywords: Hypertension, molecular docking, molecular modeling, natural compound

1. Introduction:

Hypertension is one of the main health problems in the world, besides being considered a serious risk factor for cardiovascular diseases and one of the causes of the reduction of the quality and life expectancy of individuals [1].

Currently in the pharmaceutical market there is a wide range of antihypertensives with varied mechanisms of action. Among these, a class that has gained prominence are the angiotensin converting enzyme (ACE) inhibitors [2]. Despite the large amount of active compounds on the market, it is still necessary to search for new substances, which are more effective and have fewer adverse effects.

Recent research has shown that a number of natural compounds have antihypertensive activity by inhibiting ACE [3], which makes them good prototype candidates for the synthesis of new antihypertensives. Although the amount of naturally occurring drugs is declining while the advance in molecular synthesis increases, there is still a lot to be analyzed in molecules already isolated [4], because from these structures, new substances can be synthesized.

In the development of a drug, it is necessary to take into account the pharmacokinetic characteristics of the substance, besides pharmacodynamics, because, if the substance does not arrive at the appropriate place of action, the activity is compromised [5]. Among the several methods used for the development of new drugs, molecular modeling has been gaining strength over time, since it has tools that contribute satisfactorily with corroboration of experimental studies to evaluate the pharmacokinetic and pharmacodynamic aspects of the compounds.

Based on this context, this work aims to evaluate, through a molecular modeling study, the solubility of natural compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside, as well as to verify the interaction of these compounds with ACE by molecular docking in order to corroborate with experimental studies.

2. Materials and Methods:

2.1 Molecular Docking Study

The molecular docking was run using AutoDock 4.0 software [6]. The crystallographic structure of the human testicular angiotensin converting enzyme (ACE) was obtained from the Protein Data Bank database [PDB ID: 1UZE] [7]. This enzyme was elucidated by X - ray crystallography, with a resolution of 1.82 Å. A set of five natural molecules, found in plants, was chosen for the study, according to reports in the literature on inhibition of angiotensin converting enzyme. The molecules were: oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside. Ligands were obtained through the Pubchem database. The AutoDock Tools module was used to prepare and analyze the computational simulations. Gasteiger loads and polar hydrogens required for power calculations were added considering the target structure, with the water molecules removed. Gasteiger charges were also assigned to the ligands, with non-polar hydrogens being suppressed. AutoDock requires pre-calculated three-dimensional maps arranged in a box composed of a three-dimensional grid of points in a region defined in the macromolecule. The AutoGrid 4.0 program was used to generate the maps for the ligands. The box was positioned in the catalytic region of the enzyme with dimensions in the X-, Y- and Z- axis were, respectively, 66 Å 68 Å 74 Å with spacing of 0.375 Å. The Lamarckian Genetic algorithm (GA-LS) [8] was chosen to search for the best conformations with 100 runs for each ligand (genetic algorithm with local search). During the search process, the enzyme was held rigid, while the ligands were kept flexible. The initial population was defined as 150 and the search process occurred through random initial conformations. The maximum value of energy ratings chosen was 25,000,000. The maximum number of generations was 27,000. The number of elitism chosen was 1. Gene and crossover mutation rates were respectively defined as 0.02 and 0.80. At the end of the calculations, 100 different poses were obtained and grouped into

different clusters, defined by energy proximity and RMS values (Root Mean Square deviation), according to the AutoDock default. The validation of the methodology used was done through the redocking technique.

2.2 Solubility study

The software ALOPS 2.1 [9], which is based on machine learning calculations by neural networks, was used for the partition coefficient (Log P) and water solubility (Log S) calculations of the compounds. The ALOGPS was built on the Associated Neural Network (ASNN). The system implemented in ALOGPS for log P calculations was developed with 12908 molecules from the PHYSPROP database, using 75 E-state indices. Sixty-four neural networks were enabled using 50% of molecules chosen by coincidence from the whole set. The accuracy of the prediction log P presents an RMS value of 0.35 and mean standard error S = 0.26 [10-11]. For the calculation of water solubility, ALOGPS was developed using 1291 molecules. The accuracy of the log S prediction presents RMS = 0.49 and mean standard error S = 0.38 [12].

3. Results and discussion:

3.1 Solubility study

The present study investigated the solubility of the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside in the middle of the phase of aqueous and lipophilic equilibrium, since it is known the great importance that the solubility of the molecules has in relation to their pharmacological activity , this fact is due to the need of the compound to cross the lipophilic barrier and to interact with biological fluids [13].

The partition coefficient (log P) and water solubility (log S) obtained for the chemical compounds target of this study can be visualized in **Table 1**.

Table 1. Results of log P and log S of substances calculated by ALOPS 2.1

Compound	Log S (calc)	Log P (calc)
Oleroupein	-2.86	0.63
Guanosine	-1,26	-1.61
Epicatechin 3-O-gallate	-3.80	2.38
Mirtilin	-2.75	0.58
Ligrostroside	-2.55	0.77

Analyzing the values calculated by ALOPS 2.1 software (**Table 1**) we can observe that the epicatechin 3-O-gallate molecule has the lowest log S (less water soluble), while guanosine presented the highest value, representing greater solubility in water. It is observed that log P with the highest value was that of epicantequina3-O-gallate, in agreement with the values of log S.

The calculations show that all molecules proposed in this work have sufficient solubility to cross hydrophobic barriers and interact with aqueous fluids, since compounds with log S values between -1 and -5 present satisfactory hydrophilicity for aqueous solubility and lipophilicity to interact with hydrophobic surfaces [14]. Molecules having log S values

above -1 are very polar and have difficulty transposing into hydrophobic surfaces [14].

3.2 Molecular Docking

Table 2 shows the results obtained through the docking study between the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside with the angiotensin-converting enzyme (ACE).

Table 2. Value of the molecular docking energy of the compounds against the ACE

Compound	Docking Free Energy (kcal/mol)	Vdw, Hydrogen Bond and Solubility Interaction Energy (kcal/mol)	Eletrostatic Energy (kcal/mol)	Torsional Energy (kcal/mol)
Oleroupein	-6,16	-10,76	-0,47	5,07
Guanosine	-6,77	-8,39	-0,17	1.79
Epicatechin 3-O-gallate	-8,02	-10,69	-0,61	3.28
Mirtilin	-5,37	-8,29	-0,96	3,88
Ligrostroside	-6,56	-10,95	-0,38	4,77

Table 2 shows that all compounds involved in this study interacted with the angiotensin converting enzyme (ACE) in an attractive manner, and the compound 3-O-gallate epicatechin obtained lower interaction energy, being shown to be more stable in complex with the site of the macromolecule. It can also be observed that the ligand and oleroupein compounds obtained van der waals interaction, hydrogen bonding and solvation energies as satisfactory as the epicatechin 3-O-gallate, but with higher torsional energies, directly affecting the free energy of the docking .

Figure 1 shows the more stable conformation of the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin, ligstroside at the active site of the angiotensin converting enzyme (ACE). It can be observed that in addition to interacting with the amino acids of the active site, all compounds studied interact ion-dipole with the zinc ion (Zn ++). This fact is of great importance because the enzyme requires this component for the conversion of angiotensin I to angiotensin II, that is, with the occupation of the active site and interaction with Zn ++, the enzyme is unable to convert substrate into the final product.

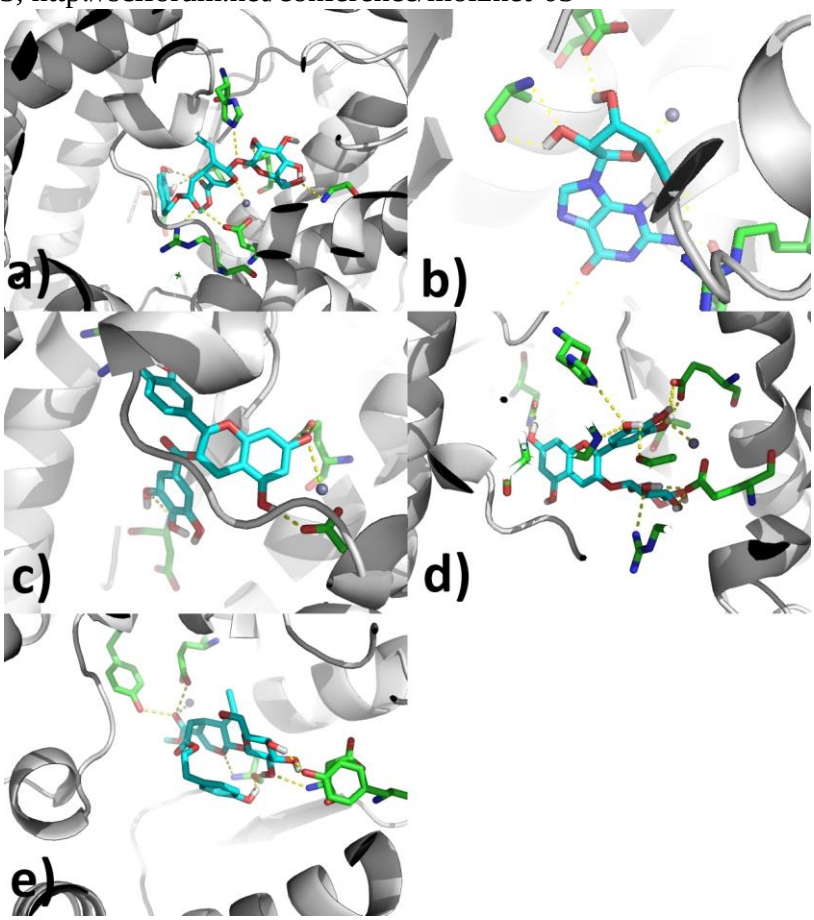

Figure 1: more stable conformations of the complex compounds/enzyme. a) Oleroupin; b) Guanosine; c) Epicatechin 3-O-gallate; d) Mirtilin; e) Ligstroside

Table 3. Description of the hydrogen bonds formed between ligands and active site of the enzyme

Compound	Number of H-bonds (n)	Aceptor H-bond	Donor – L-H	Distance H-bond (Å)
Oleroupein	2	ALA 356	LIG – O	1.45
		LIG – H	GLU 384	1.89
Guanosine	5	LIG – H	ALA 356	2.23
		LIG – H	GLU 411	2.15
		LIG – H	GLU 411	1.34
		ARG 522	LIG – O	2.10
		ALA 356	LIG – O	1.65
Epicatechin 3-O-gallate	5	LIG - H	ASN 70	1.62
		LIG - H	GLU 411	1.55
		LIG - H	ASN 70	1.67
		LIG - H	GLU 384	2.3
		ASP 358 - H	LIG – O	1.89
Mirtilin	4	LIG – H	ASN 70	1.34
		LIG – H	GLU 411	1.89
		LIG – H	GLU 384	2.10
		HIS 513	LIG - O	1.67
Ligrostroside	7	ASP 358	LIG – O	1.78
		SER 355	LIG – O	1.99
		HIS 513	LIG – O	1.76
		LIG – H	TYR 523	2.4
		LIG – H	ASN 70	1.32
		LIG – H	SER 516	1.56
		LIG – H	ASN 70	2.15

In **Table 3** the main characteristics of the hydrogen bonds formed between the binding compounds and the active site amino acids of the enzyme can be observed.

Ligands interacted through hydrogen bonds with similar active site amino acids, such as ASN 70, ASP 358, ALA 356, and others. This shows the importance of the polar groups of these compounds for the interaction with the active site of the enzyme, because in addition to the large number of hydrogen bonds, all interact with the zinc ion, as already mentioned above.

4. Conclusion

The computational study carried out in this work allowed a better view, at a molecular level, regarding the interaction of compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside with the enzyme, showing that the compounds can be used as a prototype for synthesis of new ACE inhibitors.

5. References

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