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FULL PAPER

In silico Study of the Antichagasic Activity of Aromatic Compounds

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Abstract:

Chagas disease is a parasitic tropical disease caused by *Trypanosoma cruzi* and transmissible to humans and other mammals by insects of the Triatominae subfamily. Due to the negligence of the pharmaceutical industry in relation to the development of new anti-drugs and the existing drugs have low efficacy and many side effects, the development of new research in the area is extremely relevant. Thus, the objective of this work was to relate the biological activity of glyceraldehyde 3-phosphate dehydrogenase enzyme inhibitors with molecular descriptors such as partition coefficient (LogP), water solubility (LogS), HOMO-LUMO frontier orbitals and potential of ionization. The descriptors were calculated machine learning techniques and semi-empirical calculations using the PM7 method. A molecular coupling simulation was also performed aiming at a better understanding of the interaction of the compounds with the active site of the enzymatic target. The compounds were found to have attractive interaction energy with the enzyme and to provide adequate solubility for good pharmacokinetics. It was also observed a relation of the pharmacological activity of some compounds with the energy of the LUMO orbital.

Keywords: Chagas disease; molecular docking; molecular modeling; aromatic compound

1. Introduction

Chagas disease is an infectious process caused by the protozoan *Trypanosoma cruzi*, transmitted to humans by triatomine insects commonly known as "barbeiro" [1]. This pathology has spread throughout the world due to the migration of people from endemic to non-endemic regions. In addition to transmission by the insect, there are other means of transmission of Chagas' disease, such as blood transfusion, congenital transmission, accidental transplantation of organs, as well as oral and sexual transmission [2].

Chagas disease has been neglected by the pharmaceutical industry because it represents a low-profit market [3].

Most of natural and synthetic compounds that present high pharmacological activity against T. cruzi target glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme responsible for the conversion of glyceraldehyde-3-phosphate to 1,3 -diphosphoglycerate. The infective forms of T. cruzi are dependent on the glycolytic pathway, which makes the GAPDH enzyme a promising target for the creation of antichagasic drugs since the inhibition of GAPDH will cause the inhibition of the T. cruzi glycolytic pathway [4, 5].

Several natural compounds, such as the flavonoids tiliroside [6, 7], 7-hydroxy-4',6-dimethoxyisoflavone [8], 3',4',5',5,7-pentamethoxyflavone [9], quercetin, guajaverin [6] and isosakuranetin [10], besides chalepin and other synthetic derivatives of coumarin [6, 11] and the xanthonoid mangiferin [11] have shown promising results for the inhibition of the GAPDH enzyme (Figure 1).

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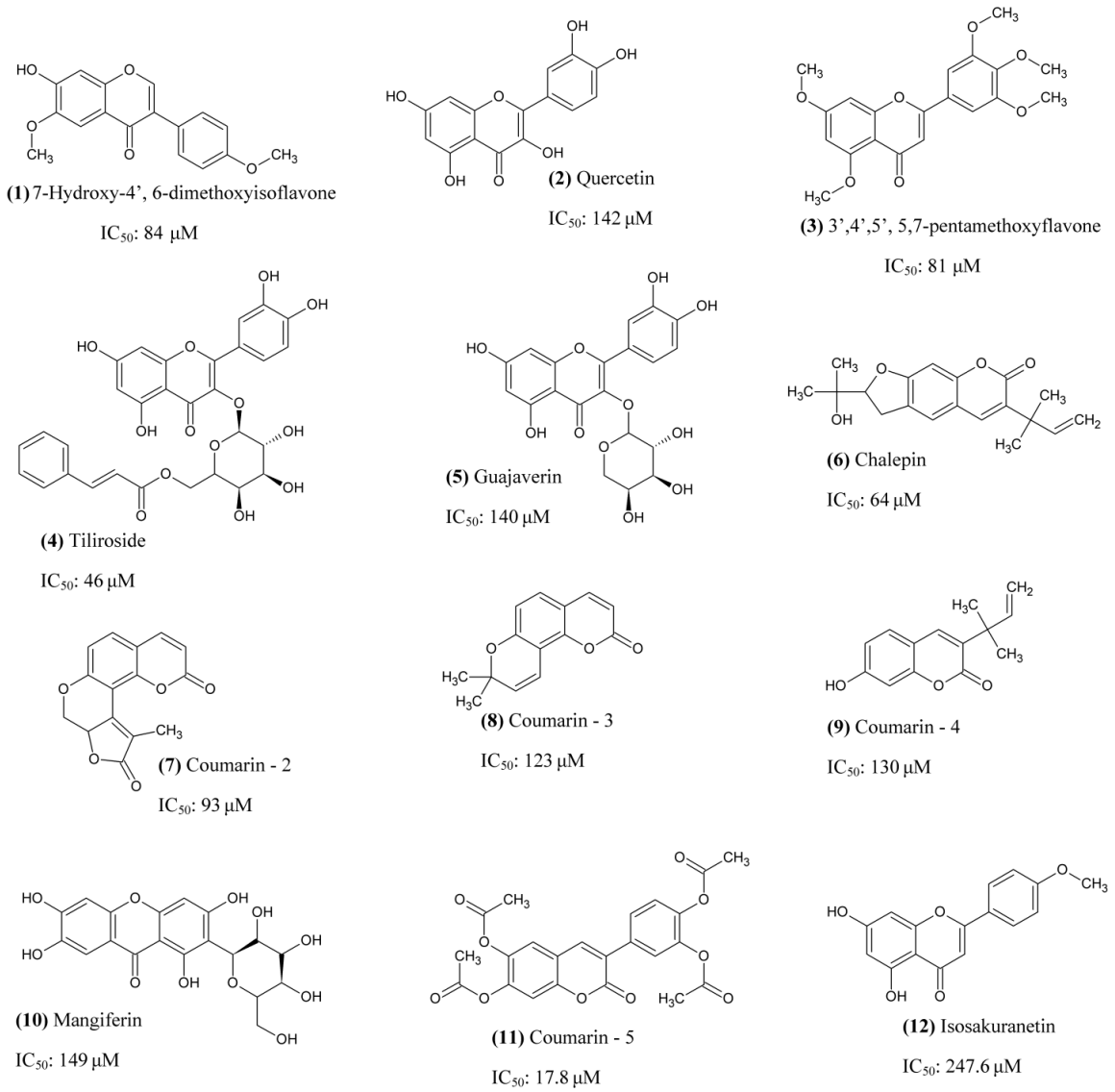

Figure 1. Glyceraldehyde 3-phosphate dehydrogenase inhibitors involved in the study.

Flavonoids, xanthonoids, and coumarins are natural compounds that occur in several foods of plant origin and present two nuclei that form heterocyclic in some classes [12]. These compounds have received much attention from the scientific community, including in the area of molecular modeling, not only for their anti-chagasic effect but also due to several other pharmacological activities, such as antioxidant, antimicrobial, antithrombotic and anti-inflammatory, among others [13- 18].

Molecular modeling studies involving compounds with biological activity against the enzyme glyceraldehyde 3-phosphate dehydrogenase from T. cruzi contribute to a mechanistic proposal of the interaction of these compounds with the enzyme. The relevance of the present study lies in the understanding of the binder-enzyme interaction, which will enable future screening for antiparasitic substances with an inhibitory potential for the T. cruzi Glyceride-3-phosphate dehydrogenase enzyme

Therefore, in the current study to investigate flavonoids as inhibitors of GAPDH enzyme of T. cruzi, we applied a combination of docking studies and calculations of frontier orbitals, ionization potential, logP and logS descriptors of the selected flavonoids to verify the interaction energy of the compounds in complex with the enzyme by molecular docking.

2. Results and Discussion

2.1 Molecular Descriptors

The present study sought to investigate the solubility of compounds **1-12** in the aqueous and lipophilic equilibrium phase since the solubility of the compounds is of great importance for their pharmacological activity because the drugs need to cross the biological barrier Lipophilic [19].

About the octanol/water partition coefficient (LogP) calculated by *ALOGPS 2.1*, the values calculated for compounds **1-12** can be visualized in **Table 1**. Guajaverin and quercetin present the lowest logP value, suggesting that even such compounds are more soluble in organic solvents, they have less efficiency in the permeability to hydrophobic biological barriers, when compared to other target compounds of the present study.

Table 1. LogP and logS values of the compounds calculated by ALOPS 2.1.

Compound	LogS (calc)	LogP (calc)	LogP (exp)
7-Hydroxy-4', 6-dimethoxyisoflavone	-3.85	2.71	-
3',4',5', 5,7-pentamethoxyflavone	-4.67	3.03	-
Quercetin	-3.06	1.81	1.82 [20]
Guajaverin	-2.50	0.70	-
Tiliroside	-3.52	2.97	2.71 [20]
Chalepin	-4.15	3.54	-
Coumarin-2	-2.80	1.62	-
Coumarin-3	-3.81	3.17	-
Coumarin-4	-2.86	3.51	-
Coumarin-5	-4.89	2.79	-
Mangiferin	-1.92	-0.09	-
Isosakuranetin	-3.51	2.95	-

Calculations of water solubility (LogS) show that coumarin-5 has the lowest LogS value (less water soluble), while chalepin coumarin has the highest value, in accordance with logP calculations.

The results show that all the compounds involved in this work have an adequate solubility for bioavailability because it can be said that compounds with logS values between -1 and -5 present hydrophilicity required for aqueous solubility and lipophilicity to interact with hydrophobic surfaces [21].

According to the results, compounds **1-12** have adequate solubility to meet pharmacokinetic requirements, presenting sufficient hydrophilicity to interact with biological fluids (plasma) and lipophilicity suitable to interact with hydrophobic (cell membrane) surfaces.

The frontier molecular orbitals (HOMO and LUMO) were calculated by the PM7 semi-empirical quantum method to predict the electronic features of compounds **1-12**. HOMO and LUMO energies are used as indexes of chemical reactivity and are commonly correlated with other indices, such as electron affinity and ionization potential [22, 23]. Table 2 shows the energies of the frontier orbitals for compounds **1-12**.

It can be observed that mangierin and triliroside, the compounds with the highest inhibitory activity of GAPDH enzyme, they have low LUMO orbital energy, which indicates that its stability to the active site can occur by the interaction of the LUMO of the mangiferin and triliroside with HOMO orbitals from the enzyme. It is also observed that the coumarin-2 compound has low energy in the LUMO orbital, and although it does not have the lowest value of IC_{50}, it presented good inhibitory activity of the enzyme, reinforcing the contribution of the energy of the orbital to the interaction.

Table 2 also shows the molecular weight and ionization potential values of compounds **1-12**. Low ionization potential values for active compounds may indicate possible mechanisms of charge transfer in the interaction of the ligand within the receptor and may also indicate that the ionic form of the substance is the one with biological activity. Regarding observed in **Table 2**, guajaverin, the compound with the lowest inhibition performance among those included in the present study was the one with the highest energy value of ionization potential.

2.2 Molecular Docking

Table 3 shows the results of the docking study between compounds **1-12** with the enzyme glyceraldehyde 3-phosphate dehydrogenase. In addition to binding energy, **Table 2** also shows details of the hydrogen interactions occurring between the ligands and the macromolecule [24].

According to the results, compounds **1-12** interacted with the enzyme glyceraldehyde 3-phosphate dehydrogenase attractively, and the compounds coumarin-5, coumarin-2, tiliroside,

and chalepin, have lower interaction energy, showing to be more stable in complexes with the active site of the enzyme (GADPH).

Table 2. Descriptors used in analysis.

Compound	IC_{50} μM (exp)	E_{HOMO} eV (calc)	E_{LUMO} eV (calc)	$\Delta E_{LUMO\ HOMO}$	Molecular weight	Ionization potential eV
angiferin	17.8	-9.251	-1.253	7.998	422.344	9.251
Tiliroside	46	-9.150	-1.230	7.920	594.527	9.150
Chalepin	64	-8.949	-0.912	8.037	314.380	8.948
3',4',5', 5,7-pentamethoxyflavone	81	-8.808	-0.696	8.112	372.373	8.499
7-Hydroxy-4', 6-dimethoxyisoflavone	84	-8.604	-0.728	7.877	298.294	8.604
Coumarin-2	93	-9.436	-1.480	7.956	270.241	9.436
Coumarin-3	123	-8.889	-0.983	7.906	228.247	8.889
Coumarin-4	130	-9.185	-0.968	8.217	230.262	9.184
Guajaverin	140	-9.528	-1.087	8.441	434.355	9.529
Quercetin	142	-9.089	-1.114	7.975	302.239	9.090
Coumarin-5	149	-8.886	-1.228	7.658	454.389	8.886
Isosakuranetin	247.6	-9.125	-0.337	8.788	286.283	9.125

Table 3. The result of the docking study of the compounds with the enzyme glyceraldehyde 3-phosphate dehydrogenase.

Compound	Docking Free Energy (kcal/mol)	Donor H-bond	Acceptor H-bond	Distance H-bond (Å)
7-Hydroxy-4', 6-dimethoxyisoflavone	-6.69	SER 247 C: H	LIG: O	2.3
3',4',5', 5,7-pentamethoxyflavone	-7.13	CYS 166 C: H	LIG: O	2.25
		CYS 166 C: H	LIG: O	2.10
		SER 247 C: H	LIG: O	2.14
Quercetin	-5.62	LIG: H	THR 197 C: O	2.16
		LIG: H	THR 167 C: O	1.92
		CYS 166 C: H	LIG: O	2.04
		SER 247 C: H	LIG: O	1.87
Guajaverin	-6.17	CYS 166 C: H	LIG: O	1.90
		SER 247 C: H	LIG: O	2.07
		ASN 335 C: H	LIG: O	1.91
Tiliroside	-7.29	LIG: H	THR 199 C: O	2.17
		CYS 166 C: H	LIG: O	2.05
		SER 247 C: H	LIG: O	2.07
		ASN 335 C: H	LIG: O	1.91
Chalepin	-7.21	CYS 166 C: H	LIG: O	1.91
Coumarin-2	-7.83	THR 167 C: H	LIG: O	1.83
Coumarin-3	-6.66	NA	NA	NA
Coumarin-4	-6.03	LIG: H	THR 226	1.99
Coumarin-5	-8.51	SER 247 C: H	LIG: O	1.71
Mangiferin		LIG: H	THR 226 C: O	1.84
		LIG: H	SER 110 C: O	1.69
		THR 226 C: H	LIG: O	1.91
		LIG: H	THR 226 C: O	1.95
		LIH: H	SER 134 C: O	1.96
Isosakuranetin	-6.69	SER 247 C: H	LIG: O	1.74
		LIG: H	THR 197	2.19
		ARG 249 C: H	LIG: O	2.13

LIG: Ligand; CYS: cysteine; SER: serine; ASN: asparagine; THR: threonine; NA: Not Apply.

Figure 2 shows the more stable conformation of compounds **1-12** at the site of action of the GADPH enzyme.

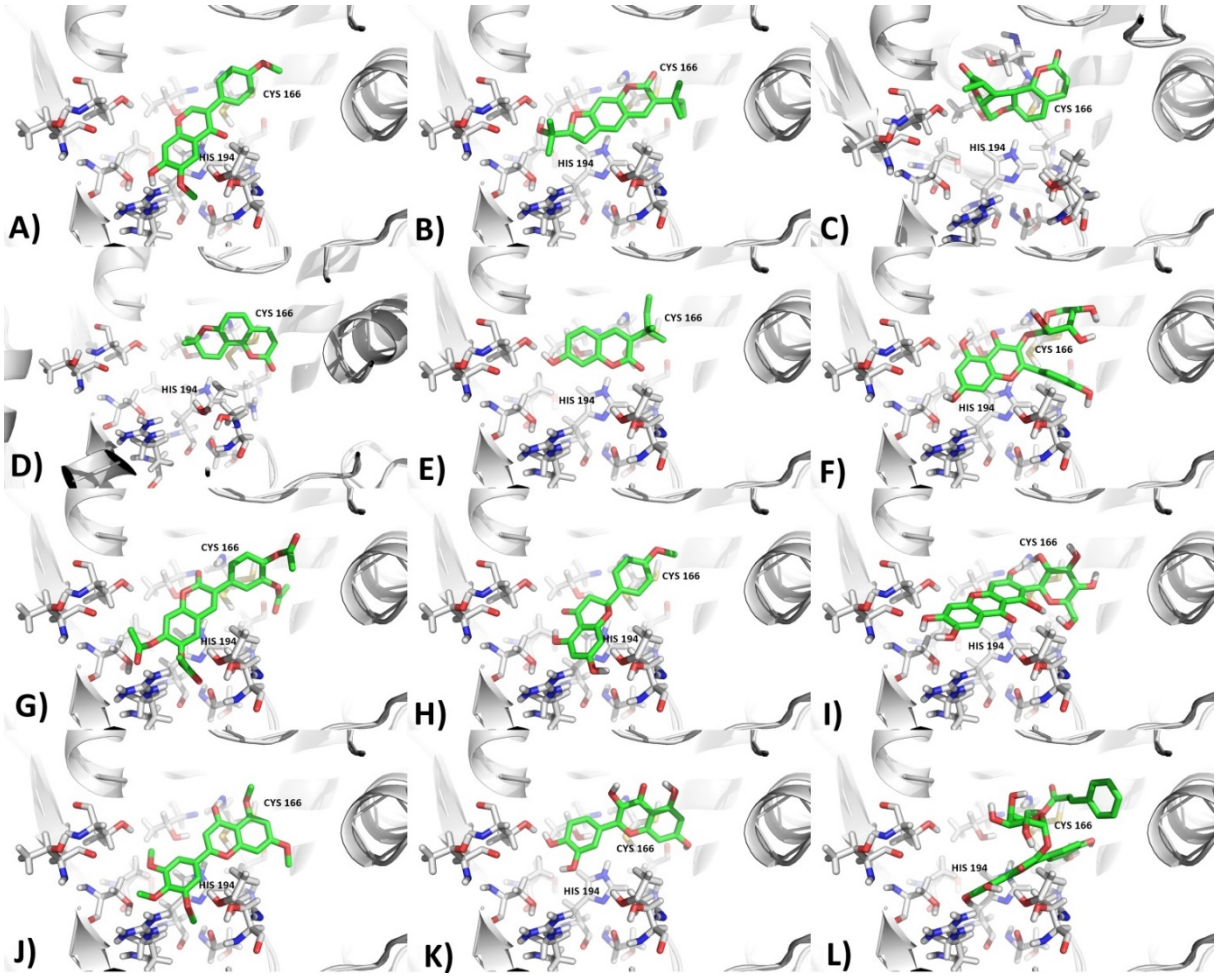

Figure 2. Compounds in the site of action of the enzyme GAPDH. A) 7-Hydroxy-4', 6-dimethoxyisoflavone; B) chalepin; C) coumarin 2; D) coumarin 3; E) coumarin 4; F) guajaverin; G) coumarin 5; H) isosakuranetin; I) mangiferin; J)3',4',5', 5,7-pentamethoxyflavone; K) quercetin; L) tiliroside.

Figure 2 shows that all compounds interact with the amino acids HIS 194 and CYS 166, which are essential for catalytic activity of the enzyme since this activity involves the nucleophilic attack of catalytic cysteine (CYS 166) on the substrate. The HIS 194 is responsible for the activation of CYS 166 and also for the formation of the tetrahedral intermediate, which will later decompose by transferring a hydride to the NAD + cofactor, forming a highly energetic thioester. After phosphorylation, the esther will release the product of the enzymatic catalysis, that is, 1,3-bisphosphoglycerate. This result suggests that all rings present in the studied compounds, as well as their polar groups, are significant for the pharmacological activity of these compounds [25].

2.3 Validation of the Methodology

The redocking presented value of RMSD = 0.83 Å, considering the most stable pose of the densest cluster. This result is considered satisfactory when the RMSD (which measures the deviation) between the best pose and the ligand complexed crystallographic is less than 2.0 Å [26]. Thus, the value displayed at this step validates the conditions used for the present docking study.

3. Material and Methods

Compounds **1-12** (**Figure 1**) were selected as ligands since they are reported in the literature as inhibitors of T. cruzi GAPDH enzyme.

3.1 Calculation of molecular descriptors

All structures of the ligands were prepared with *ChemSketch 11.0*. Molecular structure optimization and calculations of the frontier molecular orbitals, in addition to the ionization potential of the compounds, were performed by quantum mechanics using the semi-empirical method PM7 [27], with *MOPAC7* software [28].

The *ALOGPS 2.1* software was used for calculations of the partition coefficient (log P), and water solubility (log S) of the compounds [28] *ALOGPS 2.1* predicts the partition coefficient (log P) and water solubility (log S) of the compounds [29]. The *ALOGPS* was built on the *Associated Neural Network* (ASNN), which is a machine learning algorithm that combines neural network with k-neighbors [30]. 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 log P prediction presents an RMS value of 0.35 and mean standard error S = 0.26 [31, 32]. 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 [33].

3.2 Molecular Docking Study

The crystallographic structure of the enzymatic target GAPDH was obtained from the Protein Data Bank database [PDB ID: 1K3T] [34]. The enzyme was elucidated by X-ray crystallography, with a resolution of 1.95 Å. Gasteiger loads and polar hydrogens required for power calculations were added considering the target structure, with the water molecules removed. The non-polar hydrogens of the ligands were suppressed, and the rotational bonds of each ligand were automatically set.

AutoDock 4.0 software [35] was used as the choice to conduct the studies in the GAPDH target. The *AutoDock* Tools module was used to prepare and analyze the computational simulations. *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 (target site). The *AutoGrid 4.0* software was used to generate the maps for the ligands. The box was positioned in the catalytic region of the enzyme. The Lamarckian Genetic algorithm (GA-LS) was chosen to search for the best conformations [36]. The LGA combines an efficient generalized search from locations far from the minimum, which is performed by the genetic algorithm. However, the genetic algorithm is inefficient for near local search to the minimum. For this reason, we use a stochastic search method associated with a deterministic minimization method [37]. LGA begins the search for the random construction of an initial population of some individuals determined by the operator, each being symbolized by a chromosome. The projection of the initial population is followed by successive generations of routes until the maximum number of generations or the maximum number of energy evaluations is obtained [35, 38]. 100 runs were performed for each ligand (genetic algorithm with local search). The initial population was defined as 150, and the search process occurred through random initial conformations. The maximum value of energy assessments chosen was 25,000,000, while the maximum number of generations was set to 27,000, as well as the number of elitism was maintained at 1. The rates of genetic mutation and crossover were respectively 0.02 and 0, 80. After completing the calculations, 100 different conformations were obtained and grouped into different clusters, defined by energy proximity and RMS values, according to the *AutoDock* default [35]. During the search process, the enzyme was kept rigid, while the ligands were flexible. Docking calculations were validated by redocking.

4. Conclusions

The computational study carried out in this work allowed a better view, at a molecular level, regarding the interaction of compounds 1-12 with the enzyme, showing that the compounds that have lower IC50 also have the more stable energy of the receptor of the drug. Calculations of the molecular descriptors revealed that the energy of the LUMO orbital of the compounds might be influencing the pharmacological activity of the compounds by inhibition the GAPDH enzyme. The molecular docking study revealed that the hydrophobic ring groups and the polar groups of

the compounds are essential for their biological activity since all ligands interact with the amino acid of the active site CYS 166 by hydrogen bonding and with the amino acid HIS 194 by van der Waals interaction.

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