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In silic study of alkaloids derived from *Catharanthus roseus* in the active site of *Trypanosoma cruzi* by molecular docking

Estudo *in silico* de alcalóides derivados da *Catharantus roseus* em sítio ativo do *Trypanossoma cruzi* via ancoragem molecular

Estudio in silico de alcalóides derivados da *Catharantus roseus* en el sitio activo de *Trypanosoma cruzi* mediante anclaje molecular

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Abstract
Chagas disease is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi*. Currently only the drugs benznidazole and nifurtimox are used in the treatment of the disease. However, in addition to adverse effects, these drugs have reduced effectiveness, especially for chronic cases of the disease. A viable alternative is the investigation of new drugs from natural products. *Catharanthus roseus* is a common plant in tropical regions and has more than 130 terpenoids of relevant scientific interest. The present work aimed to investigate natural products in the prospect of new antichagasics drugs. In silico molecular docking were performed for 21 alkaloids derived from *Catharanthus roseus* in the active site of cruzain, the main cysteine protease of Trypanosoma cruzi. The main results demonstrated the formation of enzyme complexes of considerable stability for the compounds strictosidine (ΔG = -11.23 Kcal.mol^{-1}), ajmalicine (ΔG = -9.59 Kcal.mol^{-1}) and serpentine (ΔG = - 9.28 Kcal.mol^{-1}). These results demonstrated a good enzyme inhibitory activity of cruzain. ADMET and PASS predictions showed promising results for absorption rates and bioavailability. Therefore, the investigated molecules were considered promising in the prospect of new anti-chagasic drugs.
Keywords: Trypanosoma cruzi; Catharanthus roseus; Molecular docking.

Resumo
A Doença de Chagas é uma doença tropical negligenciada causada pelo protozoário *Trypanosoma cruzi*. Atualmente apenas os fármacos benznidazol e nifurtimox são utilizados no tratamento da doença, porém, além dos efeitos adversos, estes fármacos possuem eficácia reduzida principalmente para casos crônicos da doença. Uma alternativa viável consiste na investigação de novos fármacos a partir de produtos naturais. A *Catharanthus roseus* é uma planta comum em regiões tropicais e possui mais de 130 alcaloides de relevante interesse científico. O presente trabalho teve por objetivo investigar produtos naturais na prospecção de novos fármacos antichagásicos. Estudos *in silico* de ancoragem molecular foram realizados para 21 terpenoides derivados da *Catharanthus roseus* no sítio ativo da

cruzaína, principal cisteíno protease do *Trypanosoma cruzi*. Os principais resultados demonstraram a formação de complexos enzimáticos de considerável estabilidade para os compostos estrictosidina (ΔG = -11,23 Kcal.mol^{-1}), ajmalicina (ΔG = -9,59 Kcal.mol^{-1}) e serpentina (ΔG = -9,28 Kcal.mol^{-1}). Tais resultados demonstraram uma boa atividade inibidora enzimática da cruzaína. Previsões via ADMET e PASS apresentaram resultados promissores para taxas de absorção e biodisponibilidade. Assim sendo, as moléculas investigadas foram consideradas como promissoras na prospecção de novos fármacos antichagásicos.
Palavras-chave: Trypanosoma cruzi; Catharanthus roseus; Ancoragem molecular.

Resumen
La enfermedad de Chagas es una enfermedad tropical desatendida causada por el protozoario *Trypanosoma cruzi*. Actualmente, solo los fármacos benznidazol y nifurtimox se utilizan en el tratamiento de la enfermedad, sin embargo, además de los efectos adversos, estos fármacos tienen una eficacia reducida, principalmente para los casos crónicos de la enfermedad. Una alternativa viable es la investigación de nuevos fármacos a partir de productos naturales. *Catharanthus roseus* es una planta común en las regiones tropicales y tiene más de 130 alcaloides de interés científico relevante. El presente trabajo tuvo como objetivo investigar productos naturales en la prospección de nuevos fármacos antichagásicos. Se realizaron estudios de anclaje molecular *in silico* para 21 terpenoides derivados de *Catharanthus roseus* en el sitio activo de cruzina, la principal cisteína proteasa de *Trypanosoma cruzi*. Los principales resultados demostraron la formación de complejos enzimáticos de considerable estabilidad para los compuestos strictosidina (ΔG = -11,23 Kcal.mol^{-1}), ajmalicina (ΔG = -9,59 Kcal.mol^{-1}) y serpentina (ΔG = -9,28 Kcal.mol^{-1}). Tales resultados demostraron una buena actividad inhibitoria enzimática de cruzain. Las predicciones a través de ADMET y PASS mostraron resultados prometedores para las tasas de absorción y la biodisponibilidad. Por lo tanto, las moléculas investigadas se consideraron prometedoras en la prospección de nuevos fármacos antichagásicos.
Palabras clave: Trypanosoma cruzi. Catharanthus roseus. Anclaje molecular.

1. Introduction

The Neglected Tropical Diseases (NTDs) are pathologies caused by infectious agents (viruses, bacteria, protozoa, parasites) and that manifest in tropical and subtropical low-developed countries, with poor sanitation (Vanderslott, 2019; Engels & Zhou, 2020). Among the NTDs, chagas disease stands out, an infection caused by the protozoan *Trypanosoma cruzi*.

Chagas disease has an acute phase that lasts about 2 months and may or may not be symptomatic, and a chronic phase that can be indeterminate, cardiac or digestive, whose host takes 10 to 30 years to experience symptoms (Guarner, 2019; Chao, Leone & Vigliano, 2020). This pathology becomes even more important in the current pandemic scenario since cardiovascular diseases are factors that can worsen the symptoms of COVID-19 (Williamson et al., 2020). The main vectors of this disease are triatomine insects, but it can also be spread congenitally and orally through food contaminated with the protozoan (Honorato et al., 2021; Klein et al., 2021; Monsalve-Lara et al., 2021). It is estimated that there are at least 1 million people infected with *Trypanosoma cruzi* in Brazil (Brasil, 2019).

Currently, the drugs used for the treatment of chagas disease are nifurtimox and benznidazole (Berenstein et al., 2021; Losada et al., 2021) and in Brazil the use of nifurtimox has been discontinued since 1980 (Coura & Castro, 2002). The mechanism of action of benznidazole is not yet fully understood, but the most accepted hypothesis is that this drug is metabolized by T. cruzi enzymes and converted into a highly reactive product that interferes with the protozoan's antioxidant system, allowing it to undergo oxidative stress (Losada et al., 2021; Caldas, Santos & Novaes, 2019).

These drugs cause adverse reactions, such as anorexia, depression, sleep loss, gastritis, nausea, vomiting, migraine and hepatitis. In addition, when the disease is in the chronic phase, the effectiveness of these drugs is almost nil (Berenstein et al., 2021; Losada et al., 2021). In addition, they are contraindicated in pregnant patients and/or with cardiac, renal and hepatic respiratory failure (Coura & De castro, 2002). Therefore, there is a need to develop drugs with antichagasic properties.

One of the obstacles in the search for more effective drugs against Chagas disease is the high investment and too much research time on a laboratory scale. In this sense, computational chemistry becomes a valuable tool in the pharmaceutical area since it helps to increase the effectiveness and decrease the time and research costs (Fan et al., 2019). Molecular docking (Jiao et al., 2021) is one of the main computational techniques used in pharmaceutical research, whose objective is to analyze

the interactions between the ligand (drug) and the receptor (pathogen protein) to find the best conformation in terms of energy through in silico calculations. The best ligand-receptor complex will be the one with the lowest interaction energy (Pinzi & Rastelli, 2019).

Cruzain is the main cysteine protease of *Trypanosoma cruzi* and is present throughout the life cycle of the protozoan. This enzyme has the function of degrading other proteins through hydrolysis and, therefore, participates in the processes of invasion of host cells, in the nutrition and development of the protozoan and in the destruction of immune cells (Alvarez, et al., 2021). In this context, cruzain becomes an excellent pharmacological target for the development of antichagasic drugs.

The study of substances from plants is of great scientific interest, especially in Brazil, given that it has a huge variety of plants with medicinal properties (Ferreira et al., 2019). *Catharanthus roseus* is an herbaceous plant belonging to the *Apocynaceae* family, found in tropical and subtropical regions worldwide (Das et al., 2020; Das & Sharangi, 2017). It has about 130 terpenoid indole alkaloids widely used in the treatment of diabetes, asthma, depression, high blood pressure, cancer, diarrhea, gastritis, loss of appetite, insomnia, muscle pain, in addition to antibacterial and pesticide properties (Das & Sharangi, 2017; Das et al., 2020).

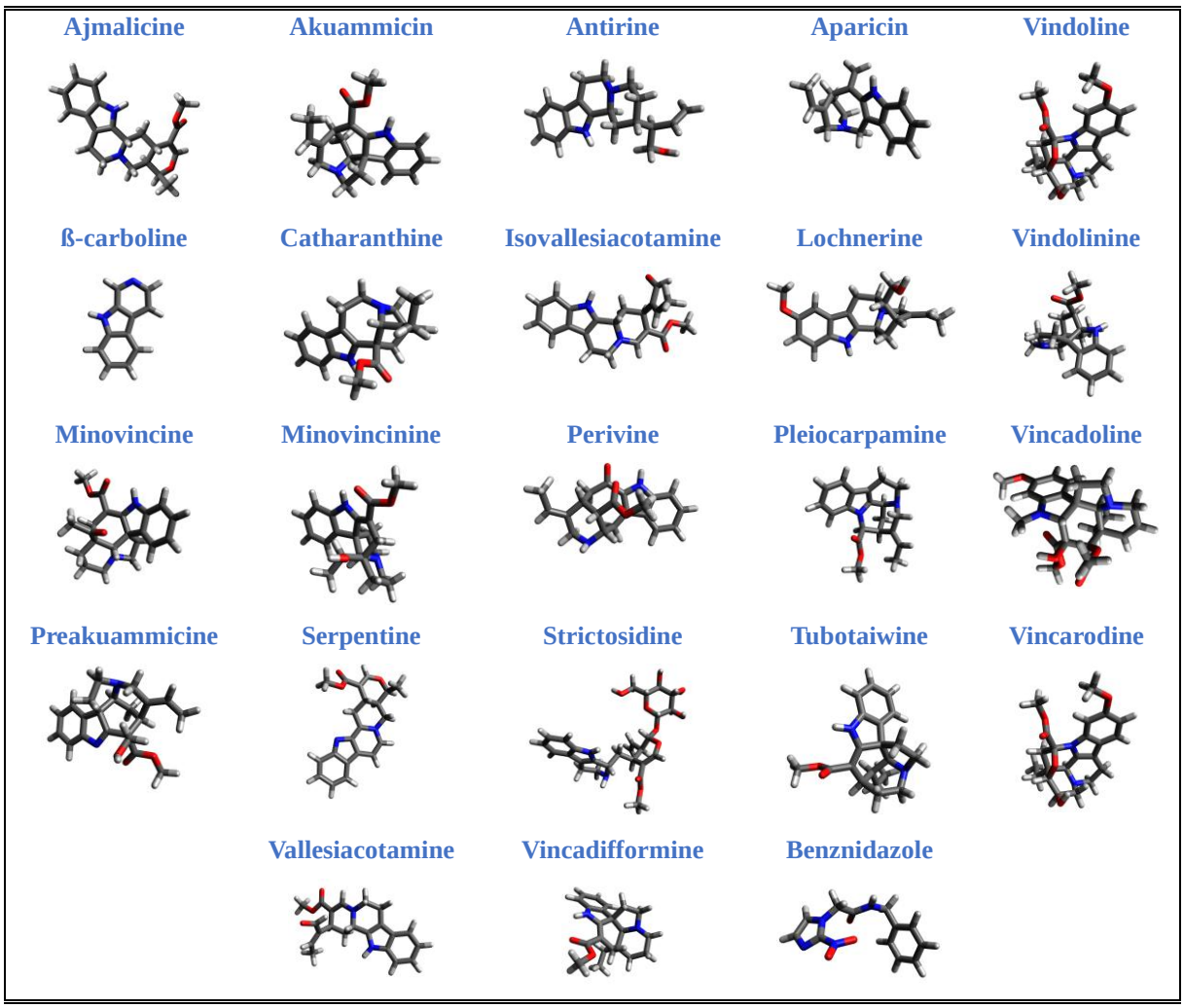
In this paper was carried out molecular anchoring studies of alkaloids derived from *Catharanthus roseus* in relation to the active site of *Trypanosoma cruzi*, the cruzain cysteine protease, in order to evaluate the prospect of new drugs with inhibitory activity against chagas disease. As support, the pharmacokinetic properties of the ligands via ADMET were also investigated.

2. Methodology

2.1 3D structures of receptor and ligands

The crystal structure of cruzain (PDB ID: 3LXS) and its respective three-dimensional coordinates were obtained from the Protein Data Bank (PDB) (Berman, 2000). The 3D structures of the alkaloids ajmalicine, akuammycin, antirine, aparicin, vindoline, ß-carboline, catharanthine, isovallesiacotamine, lochnerine, vindolinine, minovincine, minovincinine, perivine, pleiocarpamine, vincadoline, preakuammycine, serpentine, strictosidine, tubotaiwine, vincarodine, vallesiacotamine, vincadiformine (Heijden et al., 2004) and the drug benznidazole were obtained from PubChem (Kim et al., 2021). The three-dimensional structures found are listed in Figure 1.

Figure 1. Three-dimensional structures of alkaloids derived from *Catharanthus roseus* and the drug benznidazole.



Source: Own Authorship.

2.2 Molecular Docking Simulations

Autodock 4.2 software was used to perform all molecular docking procedures (Goodsell et al., 1996; Morris et al., 2008; Goodsell, 2009). Cruzain and ligand setups were prepared for Molecular Docking simulations using the AutoDock Tools (ADT) version 1.5.6 program (Sanner, 1999). For molecular anchoring, each ligand *setup* was considered flexible, while the cruzain receptor was made rigid. After the addition of all the hydrogens, the partial Gasteiger charges were calculated (Gasteiger & Marsili, 1980).

The literature reveals that the cruzain Gln19 amino acid residue presents an interesting pattern of interaction, which can act as a receptor site (Chen et al., 2010). Thus, the grid box was centered on the amino acid Gln19 with coordinates x = 53.843, y = 6.032 and z = 19.604, with a cubic size of 60x60x60 points, spacing of 0.375Å and numerical number equal to 100 (RAMOS et al., 2012). The Lamarckian Genetic Algorithm (LGA) for global research and the pseudo-Solis and Wets methods for local research (LS) were applied to the Molecular Docking research (Morris et al., 1998; Solis & Wets, 1981).

The other parameters associated with the docking were defined from default values. The conformations resulting from the docking process were organized into families according to the RMSD – Root Mean Square Deviation. Regarding the choice of the best conformations for the discussion of the results, the criterion of lowest coupling conformation of the lowest combined energy cluster was applied. Hydrophobic interactions and hydrogen bonding were analyzed using Ligplot+ version

2.2 software (Laskowski & Swindells, 2011). The docking of the drug benznidazole served as a parameter for the discussions of the other docking obtained.

2.3 Pre ADMET and PASS Online Study

The study of the properties of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) is essential in the prospection of new drugs because it allows a preliminary evaluation of the pharmacokinetics of a compound before in vitro tests (Khaldan et al., 2021). The activity spectrum prediction for substances (PASS) evaluates the biological potential of a molecule in the human organism. To carry out the respective studies, the online servers PreADMET (*https://preadmet.bmdrc.kr/)* and PASS Online (Filimonov et al., 2014) were used.

3. Results and Discussion

3.1 Molecular Docking

Molecular Docking is an indispensable tool for predicting interactions between possible drugs and biomolecules of pathogens. In summary, this technique seeks the lowest energy condition during the interaction between a ligand and a given protein active site (Pinzi & Rastelli, 2019; Jiao et al., 2021). In other words, the technique reveals possible enzyme inhibitors from the formation of complexes of considerable stability. Through Anchoring it was possible to obtain the binding energies ($\Delta G_{binding}$) and the inhibition constants (K*i*) which are parameters of great importance for the evaluation of the complexes obtained between the derivatives of *Catharanthus roseus* and cruzain.

Minimization of the Gibbs energy is of great importance for the formation of a stable ligand-receptor complex. The inhibition constant reflects the energy barrier between reactants and products. The lower the K_i value, the lower the energy barrier for the formation of the complex and, therefore, the more effective the ligand becomes in relation to the inhibitory capacity (Costa et al., 2021). The results of the binding Gibbs energies, as well as the values of the enzyme inhibition constants for the studied systems are shown in Table 1.

According to the results obtained, the most stable complexes were between cruzain and the ligands strictosidine (ΔG = -11.23 Kcal.mol^{-1}), ajmalicine (ΔG = -9.59 Kcal.mol^{-1}) and serpentine (ΔG = -9.28 Kcal.mol^{-1}). These same systems presented the smallest inhibition constant. Therefore, they form more stable complex systems.

The presence of hydrophobic groups such as the aromatic rings present in the investigated ligands favors the disturbance of solvent water molecules, increasing the entropy of the system and, consequently, decreasing the gibbs energy (Gupt et al., 2018; Guerra, 2019).

Hydrophobic and hydrogen interactions for the three best complex systems obtained, in addition to the complex generated with the drug benznidazole, were analyzed according to the LigPlot+ diagram. Table 2 describes the types of interactions for said complexes. Figures 2 to 5 illustrate cruzain and the respective ligands anchored in the protein active site, in addition to LigPlot+ diagrams describing the hydrophobic and hydrogen interactions in the generated complexes. It is noted that for the cruzain complexes formed with the ligands strictosidine, ajmalicine and serpentine, they showed different types of interactions, either hydrophobic or even hydrogen. It was observed that the greater the number of interactions, the greater the affinity of the inhibitor for the enzymatic active site.

Table 1. Values of the enzyme inhibition constants and binding energy related to the interaction between the derivatives of *Catharanthus roseus* and cruzain, an enzyme of *Trypanosoma cruzi*.

Ligands	$\Delta G_{binding}$ (Kcal.mol^{-1})	K_i x 10^{-9} (mol.L^{-1})	Ligands	$\Delta G_{binding}$ (Kcal.mol^{-1})	K_i x 10^{-6} (mol.L^{-1})
Benznidazole	-7,08	6,51	Vincadifformine	-8,11	1,13
Strictosidine	-11,23	5,90	Antirine	-8,06	1,23
Ajmalicine	-9,59	93,17	Tubotaiwine	-7,94	1,50
Serpentine	-9,28	157,53	Pleiocarpamine	-7,94	1,51
Akuammicine	-8,94	278,06	Vindoline	-7,92	1,56
Vincarodine	-8,86	320,12	Minovincine	-7,73	2,16
Isovallesiacotamine	-8,58	514,62	Vindolinine	-7,63	2,57
Vallesiacotamine	-8,55	539,51	Catarantine	-7,49	3,24
Minovincinine	-8,32	796,98	Lochnerine	-7,05	6,79
Preakuammicine	-8,26	885,35	Aparicine	-7,03	7,08
Perivine	-8,22	941,78	ß-carboline	-6,63	13,72

Source: Own Authorship.

A very interesting result resulted from the fact that the aforementioned ligands, in addition to the drug benznidazole itself, interact via hydrogen bonding with the Gln19 residue, the same residue adopted as the target site in the *in silico* simulations. In other words, this strongly corroborates the assertion that this, in fact, is the enzymatic active site. Furthermore, it was observed that the interaction energies of the ligands stritosidine, ajmalicine and serpentine were much lower than the interaction energy obtained by the drug benznidazole (ΔG = -7.08 Kcal.mol^{-1}) anchored at the same site.

Table 2. Hydrophobic and hydrogen interactions regarding the most stable complex systems between *Catharanthus roseus* derivatives and cruzain.

Ligands	Type of interaction	
	Hydrophobic	**Hydrogen**
Strictosidine	Asp18, Gly20, Gln21, Gly23, Met145, Trp184, Thr185	Gln19, Cys25, Asp161, His162
Ajmalicine	Asp18, Ser183, Thr185, Gln187, Trp188	Gln19, Gly20, Trp184
Serpentine	Asp18, Gly20, Gly23, Cys25, Ala141, Ser142, Met145, Asp161, His162, Trp184	Gln19
Benznidazole	Asp18, Gly20, Met145, His162	Gln19, Cys25, Trp184

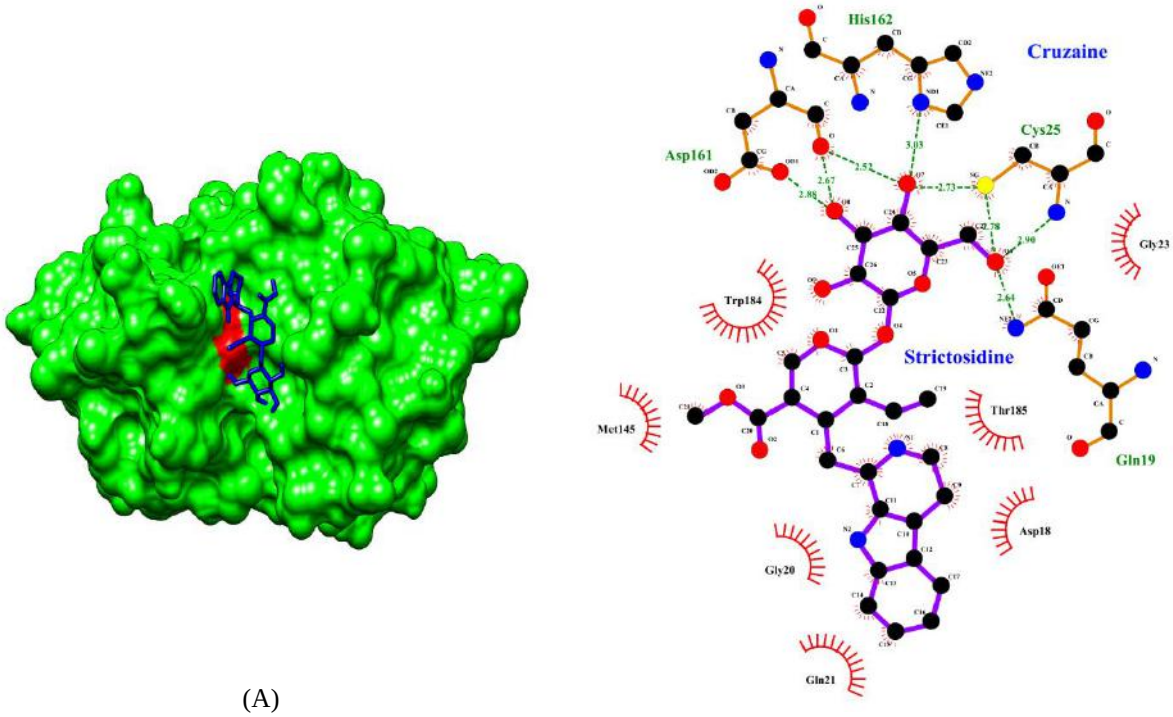
Source: Own Authorship.

A more detailed analysis of Table 1 reveals that most compounds derived from *Catharanthus roseus* investigated in this work have, theoretically, greater inhibitory capacity than the current drug used in the treatment of Chagas disease. In particular, strictosidine stands out, which, in addition to lower binding energy, also has a lower inhibition constant than benznidazole.

In addition to the hydrogen interactions between the three ligands and the amino acid Gln19, we have other interactions of relevant interest. The Asp18 amino acid interacted hydrophobically with the three ligands. The Gly20 residue

interacted hydrophobically with strictosidine and serpentine and by hydrogen interaction with ajmalicine. The Cys25 residue interacted by hydrogen bonding with strictosidine and by hydrophobic interaction with serpentine. The amino acids Asp161 and His162 interacted by hydrogen bonding with strictosidine and hydrophobically with Serpentine. The amino acid Trp184 interacted by hydrogen bonding with ajmalicine and by hydrophobic interaction with strictosidine and serpentine. The amino acids Gly23 and Met145 interacted hydrophobically with Serpentine and by hydrogen interaction with strictosidine.

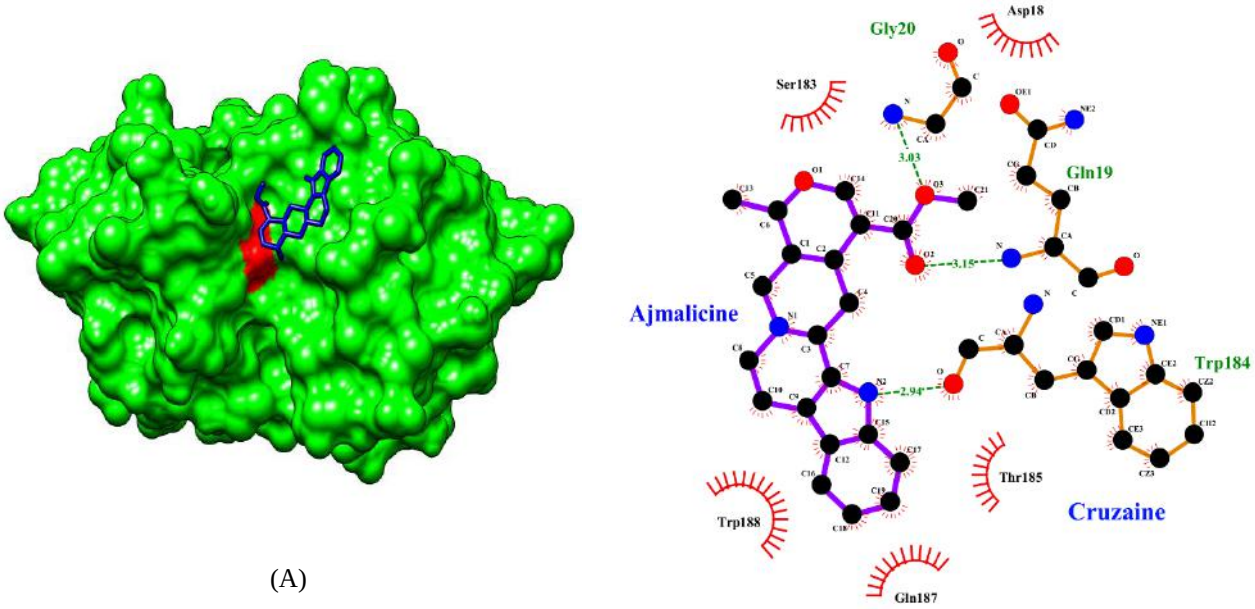
Figure 2. (A) Cruzain (green surface), strictosidine ligand (blue) and Gln19 amino acid (red); (B) LigPlot+ diagram hydrophobic and hydrogen interactions between strictosidine and the active site of cruzain.



(A) (B)

Source: Own Authorship.

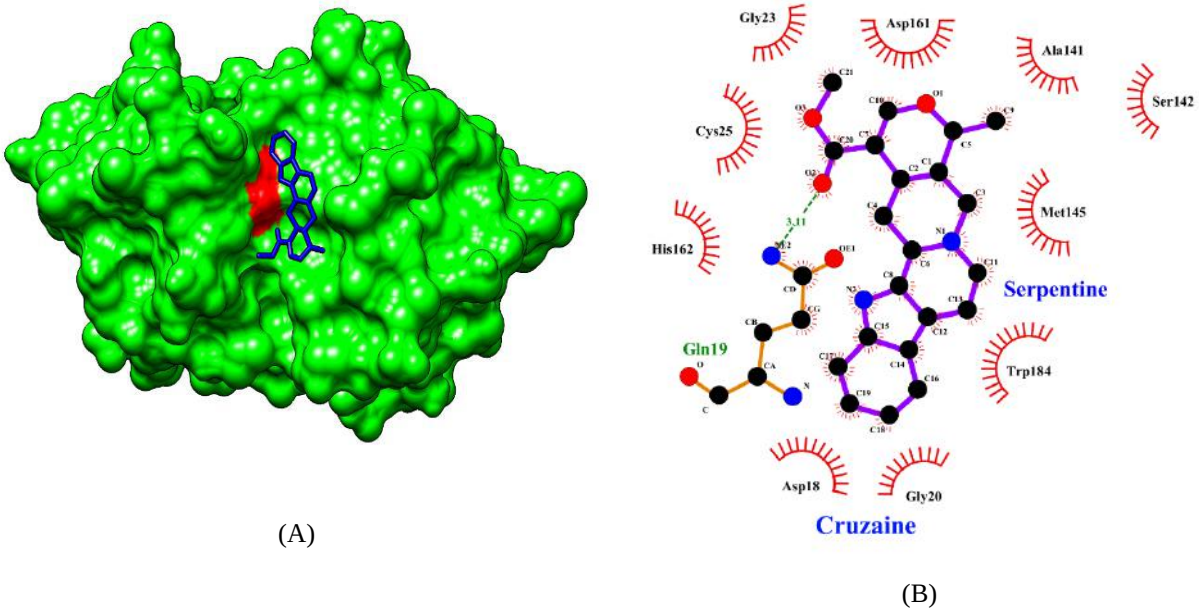
Figure 3. (A) Cruzain (green surface), ajmalicine ligand (blue) and Gln19 amino acid (red); (B) LigPlot+ diagram hydrophobic and hydrogen interactions between ajmalicine and the cruzain active site.



(A) (B)

Source: Own Authorship.

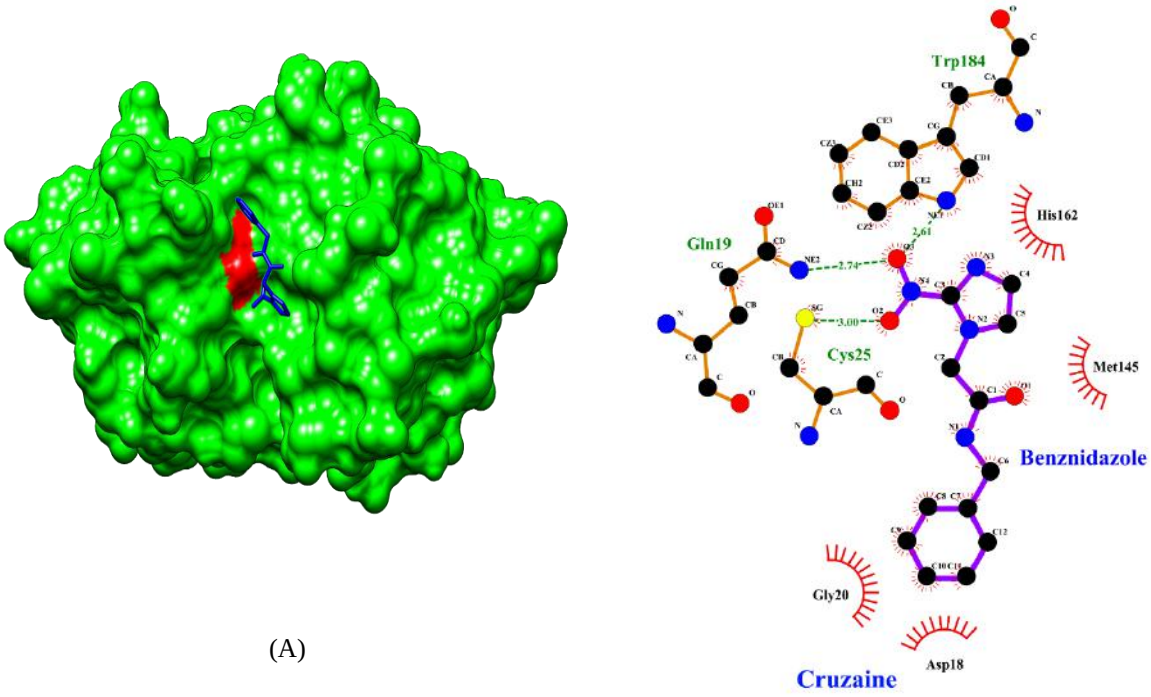
Figure 4. (A) Cruzain (green surface), serpentine ligand (blue) and Gln19 amino acid (red); (B) LigPlot+ diagram hydrophobic and hydrogen interactions between serpentine and cruzain active site.



(A) (B)

Source: Own Authorship.

Figure 5. (A) Cruzain (green surface), drug benznidazole (blue) and amino acid Gln19 (red); (B) LigPlot+ diagram hydrophobic and hydrogen interactions between benznidazole and the cruzain active site.



(A) (B)

Source: Own Authorship.

3.2 Drug-Likeness and ADMET predictions

The "Drug-likeness" (Similarity with Drugs) parameters consist of a qualitative analysis used in the design of new drugs. These parameters seek to qualify drug candidates in terms of similarity to pre-existing drugs (Jia et al., 2019). Some of these parameters were investigated in relation to the ligands with the greatest enzymatic inhibitory effect on cruzain and are illustrated in Table 3.

The parameter CMC-like – Comprehensive Medicinal Chemistry, aims to facilitate the development of new drugs from predictions of biological and pharmacological properties of molecules of interest. These predictions have as a reference the similarity between molecular structures whose properties are already known and available in a database. Thus, the drug candidate must be within the Qualification Range of each property: Lipophilicity (logP) between -0.4 and 5.6; Molecular Weight between 160 and 480; Molar refractivity between 40 and 130 and Number of Atoms between 20 and 70 (Ghose, Viswanadhan & wendoloski, 1999). Through the CMC database, strictosidine was not qualified for this rule, however, ajmalicine and serpentine were qualified.

The Lead like rule is used to classify compounds with defined physicochemical properties, that can be manipulated and optimized to become drug-like using the following parameter: affinity << 0.1 μM; molecular mass >> 350; and logP ≥ 3 (Pascolutti & Quinn, 2014; Teague et al., 1999). The three compounds violated this rule.

The MDDR like rule – MACCS II Drug Data Report, indicates how similar the studied ligands are to other drugs. To be considered as similar to Pharmaceuticals, the molecule must have: number of rings greater than or equal to 3; number of rigid connections greater than or equal to 18; number of free rotating connections greater than or equal to 6 (Oprea, 2000). The three studied structures demonstrated to have average structures in relation to the parameters of the MDDR rule.

The Rule_of_Five parameter (rule of 5) indicates the bioavailability of the drug in the human body through 5 parameters: the number of hydrogen bond donors less than 5; the number of hydrogen bond acceptors less than 10; the

molecular mass less than 500; lipophilicity lower than 5. If at least 2 parameters are not met, the drug candidate will probably not be well absorbed in the human body, making its bioavailability difficult (Lipinski et al., 2001). Ajmalicine and serpentine parameters were within the ideal limit, however the rule of 5 was violated with the strictosidine parameters.

The WDI - World Drug Index rule, indicates how similar the drug candidate is to the drugs present in the World Drug Index using the following parameters: molecular mass must be equal to or less than 550; number of hydrogen bond acceptors less than or equal to 9 and number of hydrogen bond acceptors less than or equal to 5 (Brown; Hassan & Waldman, 2001). For this rule, no violations were observed for ajmalicine and serpentine, however the rule was violated for strictosidine.

Table 3. Drug-likeness (Similarity with Drugs), ADME (Absorption, Distribution, Metabolism and Excretion) and toxicity parameters for the strictosidine, ajmalicine and serpentine ligands.

Parameters	Strictosidine	Ajmalicine	Serpentine
Drug- likeness			
CMC like	Not Qualified	Qualified	Qualified
Lead like	Violated	violated	violated
MDDR like	Average Structure	Average Structure	Average Structure
Rule_of_Five	Violated	Suitable	Suitable
WDI like rule	Out of 90% cut	Within the 90% cut	Within the 90% cut
ADME			
BBB	0.0410562	1.98978	0.477075
Caco2	5.65588	39.4694	43.0589
MDCK	0.0575701	27.7786	2.16471
HIA%	59.70	93.31	97.46
Pure water solubility (mg/L)	317.289	184.109	576.34
Skin Permeability (Log Kp)	-4.79082	-4.37806	-4.43562
PPB%	44.99	55.51	53.82
Toxicidade			
Ames test	Mutagenic	Mutagenic	Mutagenic
Carcino Mouse	Non Carcinogenic	Non Carcinogenic	Carcinogenic
Carcino Rat	Non Carcinogenic	Non Carcinogenic	Carcinogenic
Risk hER inib	High Risk	Medium Risk	Medium Risk

Source: Own Authorship.

The ADME study aims to describe the bioavailability of a particular compound with pharmacological properties. The parameters investigated are Absorption, Distribution, Metabolism and Excretion. Such parameters directly influence the pharmacological performance of compounds (Fedi et al., 2021). The ADME results for the ligands with the greatest enzymatic inhibitory effect on cruzain are shown in Table 3.

The BBB - Blood Brain Barrier parameter, indicates the degree of absorption that the Blood-Brain Barrier will likely have, in that: if the value is greater than 2.0, there will be high absorption to the central nervous system; if the value is between 2.0 and 0.1 the absorption will be medium and if the value is less than 0.1 the absorption will be low (MA, CHEN & YANG, 2005). While stritosidine showed a low degree of absorption, ajmalicine and serpentine showed medium level absorption.

Caco-2 cells (Cancer coli-2) are of great value in in vitro studies in order to understand the mechanism of drug absorption and metabolism in the large intestine. This study seeks to find more effective oral administration mechanisms

(WANG et al, 2020). The Caco-2 parameter indicates the degree of absorption according to the following values: if it is less than 4, it will be of low absorption; if the value is between 4 and 70, it will be of medium absorption and if it is greater than 70, it will be of high absorption (Yazdanian et al, 1998). It was observed that the three molecules obtained medium absorption.

MDCK – Mandi-Darby Canine Kidney refers to Mandi-Darby canine kidney cells whose lifespan is shorter than Caco-2 cells, serving as a tool for a rapid investigation of permeability (Wadapurkar et al., 2018). The degree of permeability is evaluated in three classes: if the value is less than 25, the molecule will have low permeability; if the value between 25 and 500, the molecule will have medium permeability and if above 500, the molecule will have high permeability. Only ajmalicine had medium permeability while strictosidine and serpentine had low permeability.

The parameter HIA – Human Intestinal Absorption, indicates the degree of absorption of the studied molecule in the human intestine, in which: low absorption if the value is less than 20%; moderate absorption if between 20% and 70% and good absorption if greater than 70% (Yee, 1997). Ajmalicine and serpentine showed a moderate degree of absorption while strictosidine showed low absorption.

The solubility in pure water, calculated by the parameter Pure Water Solubility, of a drug is of paramount importance because it directly influences its pharmacokinetics, therefore, a high solubility is indispensable for an effective therapeutic effect in the body (Uspenskaya et al., 2021). Serpentine had the highest solubility followed by strictosidine and ajmalicine.

The Skin Permeability parameter indicates the drug's ability to be absorbed through the skin. This parameter is important in the prospection of intradermal drugs, to predict possible exposure to toxic compounds or accidental absorption of drugs during their handling. The negative value of the Skin Permeability parameter indicates that the drug will be impermeable by the skin. The three compounds studied showed favorable values (Potts & Guy, 1992).

The ability of a drug to bind to plasma proteins is calculated by the parameter PPB - Plasma Protein Binding. A strong interaction between the drug and these proteins decreases its effectiveness because its availability in the body is compromised. PPB values above 90% indicate a strong binding between the studied compound and plasma proteins (Yun et al., 2021). PPB values below 90% of the three compounds studied showed weak interaction with plasma proteins.

Other tests of relevant interest are the toxicity tests that describe predictions about the mutagenic and carcinogenic capacities of certain compounds as a function of their molecular structure (Rim, 2020). The results of the toxicity tests for the ligands with the greatest enzymatic inhibitory effect on cruzain are shown in Table 3.

The Ames test evaluates the ability of a drug to cause mutations in DNA (mutagenicity) and has become an indispensable tool in studies of medicines, pesticides, cosmetics, samples of products found in the environment. (Smith et al., 2021). The ability of the drug to cause carcinomas in rats and mice (carcinogenicity) was also evaluated. The three compounds were shown to be mutagenic, according to the Ames test. Strictosidine and ajmalicine did not show carcinogenic activities in rats and mice, unlike serpentine, which showed a positive result.

The hERG - human ether-à-go-go-related gene (human ether-à-go-go-related gene) is a potassium ion channel that acts on the repolarization of the cardiac action potential and its inhibition can cause the syndrome of long QT, a side effect that leads to tachycardia (Asai et al., 2021). The three compounds studied had an average risk of hERG inhibition.

3.3 PASS Online Prediction

The Prediction of Activity Spectra for Substances (PASS) predicts the Probability of Activity (P_a) and Probability of Inactivity (P_i) of a substance on a given property. The ability of the studied compound to exhibit a certain property experimentally is classified as very likely (when $P_a > 0.7$), probable (when $0.5 > P_a > 0.7$) and unlikely (when $P_a < 0, 5$) (Matin et al., 2020). The results evaluated via PASS Online for the ligands with the greatest enzymatic inhibitory effect on cruzain are shown in Table 4.

Regarding the biological activities in which the Probability of Activity is greater than the Probability of Inactivity ($P_a > P_i$), the compound stritosidine showed potential as an antiprotozoal of the *Leishmania* genus, vasodilator, anti-inflammatory, antibacterial, antioxidant, antimetastatic.

Table 4. Bioactivity parameters via PASS Online for the ligands strictosidine, ajmalicine and serpentine.

Bioactivity							
Pa	**Strictosidine**	**Ajmalicine**	**Serpentine**	***Pi***	**Strictosidine**	**Ajmalicine**	**Serpentine**
A	0,860	0,402	-	A	0,004	0,048	-
B	0,739	0,230	0,317	B	0,006	0,119	0,065
C	0,636	-	-	C	0,025	-	-
D	0,420	-	-	D	0,026	-	-
E	0,369	-	-	E	0,015	-	-
F	0,382	0,206	-	F	0,052	0,151	-
G	-	0,462	0,479	G	-	0,017	0,015
H	-	0,422	0,374	H	-	0,035	0,046
I	-	0,228	0,215	I	-	0,051	0,056

A: Antiprotozoal - Leishmania; B: Vasodilator; C: Anti-inflammatory; D: Antibacterial; E: Antioxidant; F: Antimetastatic; G: Alzheimer's Treatment; H: Antiarrhythmic; I: Antihypertensive. Source: Own Authorship.

Ajmalicine showed potential as a treatment in Alzheimer's disease, antihypertensive, antiprotozoal of the genus Leishmania, antimetastatic, antiarrhythmic. Serpentine showed potential as an antihypertensive, vasodilator, antiarrhythmic.

4. Conclusion

Through the in silico study of molecular docking, the interaction energies (ΔG) between several compounds derived from *Catharanthus roseus* and cruzain, an enzyme of *Trypanosoma cruzi*, were obtained. Among the main ligands studied, strictosidine, ajmalicine and serpentine stand out, whose complexes obtained were of greater stability, and these also showed greater stability than the current drug, benznidazole, used in the treatment of chagas disease.

A study of molecular dynamics would allow obtaining other information of scientific relevance, among which the time of interaction of the ligand in the active site of the protein stands out, so that in this technique, the target protein is considered flexible, approaching the simulation of a real biological system. The results obtained in the PreADMET and PASS studies do not rule out the use of these molecules as potential drugs, one must emphasize the complexity of a real human organism in terms of chemical interactions with such compounds, which may differ from the predicted results.

A posteriori, in vitro analyzes are fundamental in order to experimentally evaluate the enzymatic inhibitory behavior. Finally, it is noteworthy that the present work opens a direction for the prospection of new antichagasic drugs.

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