RELEVANCE OF THE 1,2,4-TRIOXANE RING IN THE LEISHMANICIDAL ACTIVITY OF ARTEMISININ AND DERIVATIVES: A COMPUTATIONAL STUDY

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ABSTRACT

In this manuscript, one of the approaches that have been used in teaching Computational Chemistry to undergraduate chemistry students is reported. The topic Neglected Tropical Diseases (NTDs) was used as a motivating element, a disturbing subject in several regions of the world, including Brazil. Artemisinin (**1**) and derivatives (**2** and **3**) from the literature, with different degrees of leishmanicidal activity, are computationally investigated to unravel the relevance of the 1,2,4-trioxane ring in its biological actions. Initially, different theoretical approaches were investigated to establish the most adequate theory/atomic basis (B3LYP/6-31G**) for the development of the study. Subsequently, molecular electrostatic potential (MEP) and molecular orbitals (MOs) approximations were used in an assumption to identify key structural features of compounds necessary for their leishmanicidal activities, as well as to investigate likely interactions with the receptor in a biological process. In addition, to discover the best geometry involving the studied ligand-receptor complexes and to help in the investigation of the theme on screen, simulations of interactions between the endoperoxide bridge region of the 1,2,4-trioxane ring of the compounds and the Fe^{2+} ion of the receptor (heme) were performed. The investigation with MEP showed that the compounds (**1** and **2**), which have the 1,2,4-trioxane ring, can undergo electrophilic attack in this region, mainly due to the existence of the endoperoxide bridge, unlike the compound (**3**) which missed this bridge. The scrutiny carried out with the MOs, was anchored in information from the literature, indicating that the HOMO of the compounds (**1** and **2**) can interact with the heme in the occurrence of a biological

process. Besides that, the study of ligand-heme interaction, in addition to indicating specific interactions between the endoperoxide bridge of the 1,2,4-trioxane ring and Fe^{2+} heme, corroborated the finding that the loss of this bridge directly influences the loss of the leishmanicidal activity of one of the derivatives (**3**). The motivating topic NTDs, along with the topics Methodology, Results and Discussion, as well as the Conclusions and References presented in this manuscript constitute a robust exercise that can help in the development of competence in Computational Chemistry in Chemical Education (undergraduate and postgraduate). *[African Journal of Chemical Education—AJCE 14(3), July 2024]*

INTRODUCTION

According to the World Health Organization (WHO) [1], leishmaniasis is caused by a parasitic protozoan of more than 20 species of Leishmania, with more than 90 species of sandflies known to be transmitters of Leishmania parasites. Among the main forms of the disease, there is the visceral leishmaniasis, caused by *L. donovani*, fatal if not treated, and characterized by irregular episodes of fever, weight loss, enlargement of the spleen and liver and anemia [1]. The literature has reports on artemisinin and derivatives with antimalarial activity linked to a possible leishmanicidal activity. In all reported compounds, the importance of the endoperoxide bridge O1-O2 in the leishmanicidal activity is evident and the mechanism of action of these compounds must be similar to that of artemisinin in *Plasmodium falciparum* [2, 3].

Artemisinin derivatives are considered promising compounds in the treatment of *L. donovani,* and their leishmanicidal activity varies with changes in their molecular structures, without loss of this ability when the 1,2,4-trioxane ring (-O1-O2-C3-O13 -C12-C12a-) is preserved during these modifications [2, 3]. However, the loss of the oxygen atom from the endoperoxide bridge leads, inexorably, to the inactivity of these derivatives [2, 3].

This reporter outlines one of the approaches that our Research Group [4] has been developing in the teaching of Computational Chemistry to assist undergraduate students in Chemistry at the

Federal University of Pará, Brazil. The proposal is anchored in the motivating element [5] NTDs, a theme that permeates several regions of the globe, including Brazil [1].

Artemisinin **1**, IC_{50} = 124 μM, and two (2) derivatives from the literature: **2**, IC_{50} = 0.3 μM, and **3** (8-deoxyartemisinin), inactive, were investigated in order to unravel the relevance of ring 1, 2,4-trioxane on the leishmanicidal activity of these compounds. At first, the investigation identified the B3LYP/6-31G** approximation as the most adequate to describe the geometric parameters in the region of the 1,2,4-trioxane ring of artemisinin. Next, with the B3LYP/6-31G** approximation, the electrostatic potential (MEP) and molecular orbitals of **1, 2**, and **3** were calculated, Fig 1. Subsequently, calculations of ligand-receptor (heme) interactions were performed, and MEP maps, molecular orbitals, and ligand-heme interactions were scrutinized.

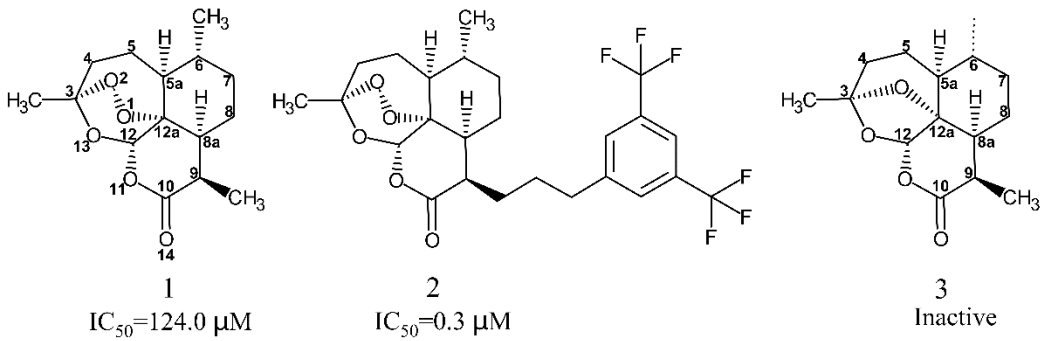

Fig 1 2D structures of compounds **1** (artemisinin), **2**, and 3 (8-deoxyartemisinin)

COMPUTATIONAL

Compounds studied and theoretical approaches to establish theory/set of atomic basis suitable for investigation

The 2D structures of compounds **1** (artemisinin), **2**, and **3** (8-deoxyartemisinin) investigated, as well as the values of leishmanicidal activities (IC_{50}) are shown in Fig 1, with the adopted numbering being the same in the atoms that form the rings in the three compounds. Initially, the 3D structure of artemisinin, encoded in CCDC-691593, [6] was optimized completely with different levels of the atomic basis theory/set available in the Gaussian software package [7]: Austin method model 1 (AM1) [8], Parametric Method Number 3 (PM3) [9], Hartree-Fock Method (HF) [10], Becke's Three-Parameter Hybrid Method [11], Lee-Yang-Parr (LYP) [12] and Standard Basis Sets

[13], all available in the same software package. The parameters obtained were compared with experimental data from the literature [14, 15].

*MEP and Molecular Orbitals of compounds **1** (artemisinin), **2,** and **3** (8-deoxyartemisinin)*

With the geometry of compound **1** (artemisinin) optimized, the structures of **2** and **3** (8-deoxyartemisinin) were built, fully optimized, and MEP and molecular orbital calculations were conducted for the three (3) investigated molecules.

Physically, the electrostatic potential means the description of the surroundings of a molecule, and with it, the main key characteristics that determine the occurrence or not of a recognition of one molecule by another are sought. Furthermore, this tool has been shown to be an effective means of analyzing and elucidating molecular recognition processes [16, 17].

Calculations of the electrostatic potential V(**r)** produced by a molecule at some point r were conducted using the equation below (Eq. 1) [18] and were also computed from the electron density, with the MEP maps displayed with the help of the Molekel program [19].

$$\mathrm{V}(\mathbf{r}) = \sum_{\alpha} \frac{Z_{\alpha}}{|\mathbf{R}_{\alpha} - \mathrm{r}|} - \int \frac{\rho(\boldsymbol{r}')\mathrm{d}\mathbf{r}}{|\mathbf{r}' - \mathbf{r}|} \qquad (1)$$

In Eq. 1, Z_α is the charge of the nucleus α, located in R_α and $\rho(r')$ is the electron density of the molecule. The sign of V(**r)** in a given region will depend on the positive contributions of nuclei and the negative contributions of electrons [16-18].

Molecular orbitals, in processes involving the interaction of small molecules with biological targets, often contribute to the biological activities of these molecules through reactions involving charge transfer, and it is therefore relevant to qualify them with one of the important properties (descriptors) in the mechanism of action of these molecules. In this scrutiny, the molecular orbitals were obtained in the LCAO-MO approximation given by the expression.

$$\psi_\mu = \sum_i c_{\mu i}\phi_i \qquad (2)$$

where ϕ_i represents a complete set of orbitals of the component atoms of these molecules [10, 20, 21].

*Interaction between **1** (artemisinin), **2**, and **3** (8-deoxyartemisinin) and heme*

The study of ligand-receptor interaction (molecular docking) seeks to understand in detail how a small molecule (ligand) interacts in the binding region with a macromolecule (receptor), generating information that facilitates the understanding of the biological activity of that molecule. Furthermore, it allows visualizing and quantifying, respectively, the way in which the two molecules

interact and the interaction. The process begins when the ligand seeks its best position to fit in the receptor's active site, generating a certain number of conformational possibilities in search of the best ligand-receptor anchorage [22-25]. Ligand-receptor molecular recognition involves different stages of conformational accommodation, resulting in favorable enthalpy and entropic mode of interaction. The processes involved can be estimated by Eq. 3, through the Gibbs binding free energy (ΔG); which is related to the inhibition constant (K_i), determined experimentally. In Eq. 3, ΔH is the enthalpy change, T is the absolute temperature, ΔS is the entropy change, and R is the universal gas constant,

$$\Delta G = \Delta H - T\Delta S = RTlnK_i \quad (3)$$

In receptor ligand investigation, heme [26, 27] was used as a receptor for compounds **1** (artemisinin), **2** and **3** (8-deoxyartemisinin). Fig 2 shows the 2D (a) and 3D (b) structures of heme. The 3D structure was taken from the RCSB Protein Data Bank (PDB), 1A6M [27], which contains only the A chain. The heme consists of the Fe in the center of the protoporphyrin and for the most realistic description of the biological environment in the ligand-heme interaction, the histidine residue was left attached to the Fe^{2+}. Calculations of the ligand-receptor interaction were performed using the AutoDock program [28] and the procedure conditions are the same throughout the scrutiny of the ligand-heme interaction, i.e., a box of 40 units was used in each direction.

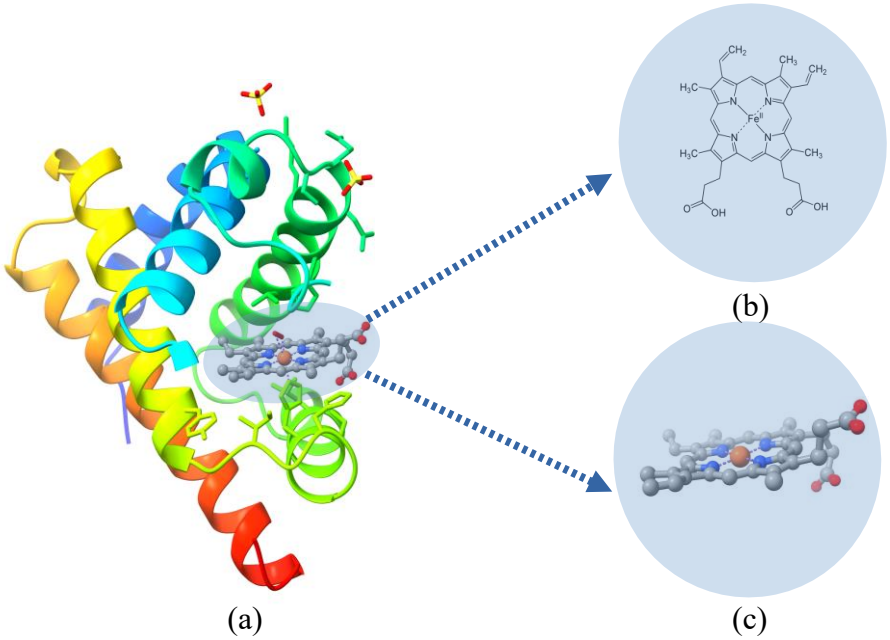

Fig 2 Region of hemoglobin containing the active site of the receptor highlighted in blue (a), 2D (b) and 3D (c) structures of the heme receptor

All of the calculations in the investigation were performed on the lowest energy conformation of each molecule on a Linux Mint platform running on a Phenom FX8320 processor with 8 gb of RAM.

RESULTS AND DISCUSSION

Theoretical approach/atomic basis set selected for investigation

Table 1 shows the theoretical and experimental geometric parameters [14, 15] for the 1,2,4-trioxane ring of compound 1 (artemisinin).

Table 1. Experimental and theoretical geometrical parameters of the 1,2,4-trioxane ring for compound 1 (artemisinin)

Geometrical parameters	Theoretical level										Experimental	
	HF/ 3-21G	HF/ 6-31G	HF/ 6-31G*	HF/ 6-31G**	B3LYP/ 3-21G	B3LYP/ 6-31G	B3LYP/ 6-31G*	B3LYP/ 6-31G**	AM1	PM3	[14]	[15]
Bond length (Å)												
C12a-O1	1.471	1.469	1.429	1.429	1.504	1.499	1.455	1.455	1.468	1.426	1.450(4)	1.462(3)
O1-O2	1.462	1.447	1.390	1.390	1.524	1.524	1.460	1.460	1.289	1.544	1.474(4)	1.469(2)
O2-C3	1.441	1.436	1.396	1.396	1.456	1.452	1.414	1.414	1.447	1.406	1.418(4)	1.416(3)
C3-O13	1.436	1.435	1.408	1.408	1.473	1.473	1.441	1.441	1.427	1.428	1.451(4)	1.445(3)
O13-C12	1.408	1.403	1.376	1.376	1.431	1.426	1.376	1.376	1.416	1.403	1.388(4)	1.380(3)
C12-C12a	1.529	1.529	1.533	1.32	1.532	1.532	1.538	1.539	1.537	1.555	1.528(5)	1.523(2)
Bond angle (degree)												
C12a-O1- O2	111.3	113.2	112.7	112.7	109.6	111.4	111.6	111.6	113.7	112.2	111.5(2)	111.1(1)
O1-O2-C3	107.1	108.8	109.5	109.5	105.6	107.3	108.2	108.3	112.5	110.3	117.7(2)	108.1(2)
O2-C3-013	107.3	106.8	107.8	107.8	108.2	107.7	108.5	108.5	103.6	104.8	107.1(2)	106.6(2)
C3-O13- C12	115.7	117.3	115.3	115.3	113.2	115.0	114.1	114.1	115.5	116.0	113.6(3)	114.2(2)
O13-C12- C12a	112.1	112.9	112.3	112.2	113.3	113.6	113.3	113.3	113.5	115.2	114.7(2)	114.5(2)
C12-C12a- O1	111.6	110.9	110.6	110.6	112.4	111,7	111.3	111.3	111.1	113.2	111.1(2)	110.7(2)
Torsion angle (degree)												
C12a-O1-O2-C3	50.3	46.7	48.7	48.7	51.1	48.9	47.9	47.9	47.1	35.6	47.7(3)	47.8(2)
O1-O2-C3-O13	-74.7	-74.9	-73.4	-73.4	-76.6	-73.5	-74.0	-73.0	-77.8	-75.3	-75.5(3)	-75.5(2)
O2-C3-O13-C12	32.3	33.4	31.1	31.1	33.8	35.0	32.9	32.9	42.1	52.7	36.3(4)	36.0(2)
C3-O13-C12-C12a	28.3	25.2	27.4	27.4	29.0	26.3	27.3	27.3	11.4	2.8	24.7(4)	25.3(2)
O13-C12-C12a-O1	-50.9	-49.4	50.1	-50.1	-52.7	-51.2	-51.1	-51.2	-41.8	-40.5	-50.8(4)	-51.3(2)
C12-C12a-O1-O2	10.0	12.5	10.5	10.9	9.60	12.8	11.7	11.7	12.0	20.0	12.2(3)	12.6(2)

As one can see, the bond lengths are generally well described with all levels of theory, however the endoperoxide bridge, crucial to the leishmanicidal activity of the compounds, is best described, in general, at the B3LYP/6-31G* levels and B3LYP/6-31G**. In addition, all theoretical bond angles are very close to experimental data [14, 15]. For the torsion angle of the twisted boat conformation (O1-O2-C3-O13) adopted in compound **1** (artemisinin), the theory at all levels shows very good description when compared to the experimental values. The best description of the endoperoxide bridge, combined with the good description of the bond angles and the twisted boat conformation, as well as the need for an atomic basis with sufficient robustness to best describe the bulky substituent $–(CH_2)_3$-C_6H_4(3,5 $–CF_3$) in C9 in compound **2**, led to the choice of the theoretical approximation/atomic basis set B3LYP/6-31G** as the most adequate in the development of the investigation.

*MEP maps, molecular orbitals, and ligand-heme interactions for compounds **1** (artemisinin), **2**, and **3** (8-deoxyartemisinin)*

In Fig 3 (a-d) the 2D structures (a), the MEP maps (b) – kcal/mol, the highest occupied molecular orbital – HOMO (c), and the interactions with heme (d) – kcal/mol, for compounds 1 (artemisinin), **2**, and **3** (8-deoxyartemisinin) are shown.

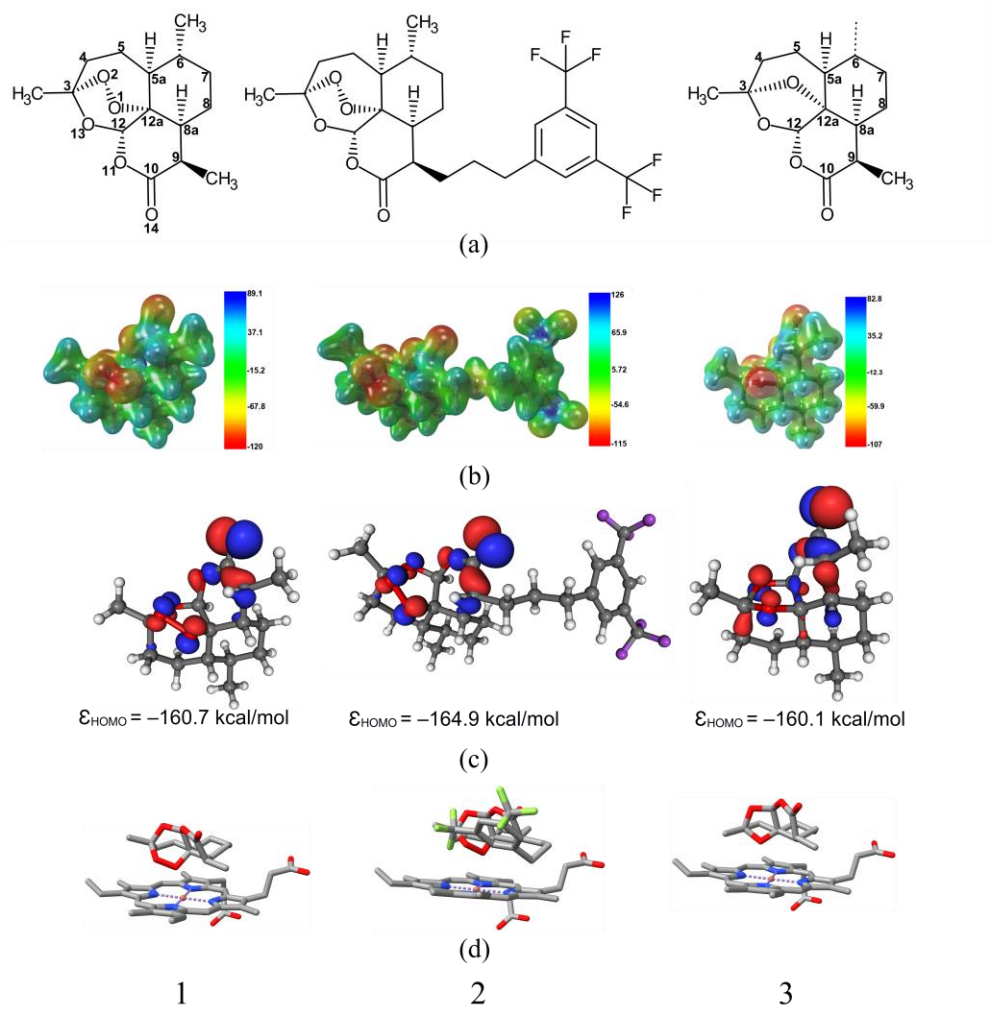

Fig 3 2D structure (a), MEP maps - kcal/mol- (b), front view of the endoperoxide bridge (-O1-O2-) for compounds **1** (artemisinin) and **2** and the oxygen atom resulting from the loss of this function for compound 3 (8-deoxyaretemisinin), lobes for the HOMO orbital - **1** (artemisinin), **2**, and **3** (8-deoxyartemisinin – (c), (positive lobes are colored in blue and negative lobes are colored in red), and ligand-heme interactions - (kcal/mol – (d). Atom colors: gray (carbon); blue (nitrogen); red (oxygen); and green (fluoride)

According to Fig 3b, in the regions colored in red (atoms: O1, O2, O11, O13 and O14), yellow and green (atoms: O1-C12a, O2-C3, C3-O13, O13-C12, O11- C12, O11-C10, C10-O14), with negative values for MEP in the ranges: -120 to slightly > -15.2 kcal/mol (**1**) and -115 to slightly > -54.6 kcal/mol (**2**), the 1,2,4-trioxane ring stands out (more negative values located on the O1 and O2 atoms of the endoperoxide bridge), indicating its importance in a possible electrophilic attack by a biological target. For derivative **3**, the negative MEP values are in the range -107 to slightly > -12.3 kcal/mol. In addition, the loss of the O1-O2 endoperoxide bridge and, consequently, the absence of the 1,2,4-trioxane ring in this derivative highlights the impossibility of electrophilic attack by a biological target that is favorable to its leishmanicidal activity.

The literature has highlighted the importance of HOMO in the leishmanicidal activity of artemisinin and derivatives [29]. Therefore, the suggestion of a mechanism of action in *L. donnovani* similar to that in *P. falciparum* [2, 3] may indicate the HOMO of compounds **1** (artemisinin) and **2** as the favorable OM in the formation of the complex with heme in the biological process. The distinction in the description between the most active compound (**2**) and the least active (**1**) becomes clear when we compare the positioning of this orbital in atoms of the two compounds, which can be seen in Fig 3c. The HOMO lobes are mainly positioned on atoms of the 1,2,4-trioxane ring and on atoms of the C10-containing ring in the least active (**1**) and most active (**2**) compounds, respectively.

However, the most active compound (**2**) presents these lobes more prominent. Furthermore, the existence of corresponding hydrogen atoms, especially in the region of the ring containing C10 of this compound, may contribute to the direction of some of these lobes towards the possible position of the heme, indicating the importance of C-H..π [30] and other orbital interactions with the receptor's delocated system. The absence of the endoperoxide bridge in compound **3** probably induces complexation with heme through π bonds that do not adjust properly and, as a result, result in the loss of leishmanicidal activity.

The interaction of each ligand with Fe^{2+} of heme, according to Fig 3d, occurs at distances d(FeO1) = 2.61 and d(FeO2) = 3.70 Å for compound **1** (artemisinin) and d(FeO1) = 2.54 and d(FeO2) = 3.30 Å for compound **2**, showing that the approximation between the ligand and the heme occurs preferentially between the Fe^{2+} and O1 atoms in the two compounds. Additionally, these values show that the best ligand-heme fit oriented by the $–(CH_2)_3$-C_6H_4(3,5-CF_3) substituent at C9 substantially contributes to the leishmanicidal activity of compound **2**. For compound **3**, the absence of the ring 1,2,4-trioxane resulting from the loss of the endoperoxide bridge necessarily conditions the approach of the remaining oxygen to Fe^{2+}, with a value of d(Fe^{2+}O) = 3.20 Å, reflecting the loss of the leishmanicidal activity of this compound.

CONCLUSIONS

Artemisinin and derivatives were studied computationally to investigate the relevance of the 1,2,4-trioxane ring in their leishmanicidal activities. The comparison of the geometric parameters in the 1,2,4-trioxane ring region obtained at various levels of theory and with experimental data, considering the best description of the endoperoxide bridge, combined with the good description of the bond angles and the twisted boat conformation, as well as the presence of the substituent at position C9, -$(CH_2)_3$-C_6H_4(3,5-CF_3), in derivative **2**, indicated the B3LYP/6-31G** approximation as the most appropriate for the investigation.

Scrutiny of the compounds through MEP showed that compounds **1** (artemisinin) and **2**, due to having the 1,2,4-trioxane ring, can suffer attacks by nucleophiles, especially in the region of the endoperoxide bridge, by a biological target.

Scrutiny of the OMs of the three compounds, anchored by results reported in the literature [29] and the mechanism of action of artemisinins and derivatives in P. falciparum [2, 3] may indicate the HOMO as a likely region of ligand-heme interaction in a biological process , with the distinction in the description between the most active (**2**), least active (1) and inactive (**3**) compound becoming clear by comparing the positioning of this orbital in atoms of these compounds, that is, the HOMO lobes are positioned mainly in 1,2,4-trioxane ring atoms and C10-containing ring atoms in

compounds (**1** and **2**). However, the most active compound (**2**) showed these lobes more prominent. Furthermore, the existence of corresponding hydrogen atoms, mainly in the region of the ring containing C10 of this compound, may contribute to the direction of some of these lobes towards the possible position of the heme, indicating the importance of C-H..π [30] and other orbital interactions with the receptor's delocalized system.

The investigation of the ligand-heme interaction made it possible to identify specific interactions between the endoperoxide bridge of the 1,2,4-trioxane ring and the Fe^{2+} of the receptor, and indicated that the absence of the endoperoxide bridge by derivative **3** directly reflects on its loss of leishmanicidal activity.

Finally, it was inferred that the substituent, $-(CH_2)_3-C_6H_4(3,5-CF_3)$, in the C9 position of compound **2**, leads to better orientation in the binding of the ligand to the heme and, consequently, to a substantial improvement in the leishmanicidal activity of this derivative.

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