***In vitro* evaluation of arylsubstituted imidazoles derivatives as antiprotozoal agents and docking studies on Sterol 14 α -demethylase (CYP51) from *Trypanosoma cruzi*, *Leishmania infantum* and *Trypanosoma brucei*.**

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Abstract.

There is an urgent need to discover and develop new drugs to combat parasitic diseases as Chagas disease (*Trypanosoma cruzi*), Sleeping sickness (*Trypanosoma brucei*) and Leishmaniasis (*Leishmania spp*). These diseases are considered among the thirteen most unattended diseases worldwide according to the WHO. In the present work, the synthesis of 14 arylsubstituted imidazoles and its molecular docking onto sterol 14 α -demethylase (CYP51) was executed. In addition, the compounds, antiprotozoal activity against *T. brucei*, *T. cruzi*, *T.b. rhodesiense* and *L. infantum* was evaluated. *In vitro* antiparasitic results of the arylsubstituted imidazoles against *T. brucei*, *T. cruzi*, *T.b. rhodesiense*, *L. infantum* indicated that all samples from arylsubstituted imidazole compounds presented interesting antiparasitic activity to various extent. The ligands **5a**, **5c**, **5e**, **5f**, **5g**, **5i** and **5j** exhibited strong activity against *T. brucei*, *T. cruzi*, *T.b. rhodesiense* and *L. infantum* with IC₅₀ values ranging from 0.86 to 10.23 μ M. Most samples were cytotoxic against

24 MRC-5 cell lines ($1.12 < CC_{50} < 51.09 \mu M$) and only ligand **5c** showed a good selectivity against
25 all tested parasites. According to the results of the molecular docking, the aromatic substituents in
26 positions 1, 4 and 5 have mainly stabilizing hydrophobic interactions with the enzyme matrix, while
27 the oxygen from NO_2 , SO_3H and OH groups interacts with the Fe^{2+} ion of the Heme group.

28 **Keywords.**

29 arylsusbstituted imidazoles, *Trypanosoma cruzi*, *Trypanosoma brucei*, *Trypanosoma b. rhodesiense*,
30 *Leishmania infantum*, *in vitro* evaluation, molecular docking

31

32 **Introduction.**

33 *Trypanosomatidae* form a family of unicellular eukaryotic parasites from the order *Kinetoplastida*,
34 phylum *Euglenozoa*, supergroup *Excavates*. Many human pathogens possess a number of species
35 of kinds *Leishmania* and *Trypanosoma*. The life cycles of these organisms turn out to be very
36 complex, constantly moving between insects and mammalian hosts. (Guedes da Silva et al. 2015,
37 Lepesheva et al. 2015).

38 *Leishmaniasis* is common disease in many continents, each year 20 000 to 30 000 deaths and almost
39 1 million of new cases are reported, arriving to report oneself around 12 million infected people.
40 (Lepesheva et al. 2015). Caused by unicellular eukaryotic organisms from genus *Leishmania*
41 (*Trypanosomatidae* family). Analogous to other protozoan parasites, *Leishmania* has a complex life
42 cycle, transmitted via the bite of female Phlebotomine sandflies. The parasite exist in two forms
43 namely *promastigote* in sandfly and *amastigote* in human macrophage (Bates 2007, Rodríguez et
44 al. 2006). It is exhibited in three clinical form visceral leishmaniasis (*L. donovani*, *L. infantum* / *L.*
45 *chagasi*), also known as kala-azar, cutaneous leishmaniasis caused by *Leishmania major*, *L.*

46 *donovani*, *Leishmania tropica*, and *Leishmania aethiopica*; and muco-cutaneous leishmaniasis
47 caused by *Leishmania braziliensis*.

48 American trypanosomiasis, more known as Chagas disease, is usually transmitted by the bites of
49 triatomine insects, also known as “kissing bugs.”, these insect or their feces carry the flagellate
50 parasite *Trypanosoma cruzi* (*T. cruzi*) and can also contaminates foods such as fruits juices, resulting
51 in foodborne. It is an endemic disease in Central and South America, a few cases occur occasionally
52 in the southern US. This disease has become a growing problem in non-endemic areas, where
53 infections may not be recognized by many people, and where the few effective drugs may not be
54 generally available. (Lepesheva et al. 2015).

55 Human African trypanosomiasis, known commonly as sleeping sickness, is caused by infection with
56 protozoans of the genus *Trypanosoma*. It is transmitted by their only vector tsetse fly (*Glossina*
57 *genus*) bites, which have acquired their infection from human or other animals that contain in her
58 organism other human parasites (WHO 2017). This disease has two clinical manifestations,
59 *Trypanosoma brucei gambiense* (*T.b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T.b.*
60 *rhodesiense*). The *T.b. gambiense* causes a more chronic infection that is responsible for over 90
61 percent of cases, whereas *T.b. rhodesiense* causes an acute infection that is responsible for a smaller
62 proportion of the overall Human African trypanosomiasis disease burden. The disease develops
63 rapidly and invades the central nervous system (WHO 2017).

64 The patient may exhibit some of the following symptoms: fevers, skin eruptions, irregular febrile
65 episodes, headaches, malaise, exhaustion, anorexia, extreme thirst, muscle and joint pains, anaemia,
66 rash, coma and ultimately death. Sleeping sickness is endemic in 36 Sub-Saharan African countries,
67 with an 85 percent case reduction since year 2000 and less than 3000 cases in year 2015 a record
68 low with around >60 million people are at risk (WHO 2017).

Sterol 14 α -demethylase (CYP51, EC.1.14.13.70) has been found as a vital target for the development of anti-fungal, anti-leishmania, anti-trypanosoma drugs and also for the design of cholesterol-lowering drugs. Crystal structures of protozoan sterol 14 α -demethylases provide an opportunity for structure-directed development of such inhibitors. The enzyme is responsible for catalyzing the elimination of the alpha-methyl group of the sterol, which leads to the formation of cholesterol in vertebrates, the well-known ergosterol in fungi and several ergosterol alkylated derivatives (phytosterol) for plants and protozoa. (Buckner and Urbina 2012). The production of ergosterol in fungus and protozoa, unlike mammals, is lethal when it accumulates, affecting cytokinesis, stopping cell growth to cause death of the cell membrane. (Roberts et al. 2003). Extensive work is performed already considering CYP51 as target. Silva et al. (Guedes et al. 2017) have analyzed the effectiveness of two classes of drugs using CYP51: **VNI** and **VFV**. Schel et al. (Schel et al. 2016), have demonstrated the anti-fungal activity of **VT-1161** and **VT-1129**, among many other application reports with the use of CYP51 target (Singh et al. 2015, Anusha et al. 2015). Here, we report the synthesis, *in vitro* antiprotozoal activity, cytotoxicity of 14 arylsubstituted imidazoles, as well as its molecular docking onto sterol 14 α -demethylase (CYP51). We have chosen the CYP51 target because all analyzed compounds possess the principal pharmacophore features of CYP51 inhibitors reported by Vita et al., where they state that an active compound must contain a heterocycle with a nitrogen able to interact with the Fe atom present in the Heme group and having two hydrophobic groups near to it. (De Vita et al. 2016).

Materials and Methods.

General procedure for the Synthesis of substituted imidazoles

The arylsubstituted imidazoles were obtained by reaction of aromatic aldehydes, primary amines, benzils and ammonium acetate (**Figure 1**) catalyzed by boric acid (Shelke et al. 2009). All reagents used had commercial grade. Melting points were determined in open capillaries using a Digital Melting Point apparatus WRS-2A. ^1H -NMR and ^{13}C -NMR spectra were recorded with a Bruker DRX-600 spectrometer obtained in DMSO- d_6 and MeOD solutions. The FT-IR spectra were recorded on a Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). The reaction occurred at the maximum energy area in the reaction vessel, where was slightly lower than the level of the water and the temperature was controlled at 60°C .

Figure 1. Synthesis of arylsubstituted imidazoles.

Reaction conditions: aldehyde **1** (1 mmol), amine **2** (1 mmol), ammonium acetate **3** (1.5 mmol), benzil **4** (1 mmol), H_3BO_3 (5 mol %) and Propylene glycol (15 mL) heating under reflux for 6 h.

The products (**5a-n**) in **Table 1** were confirmed by the analysis of spectra ^1H -NMR, ^{13}C -NMR, FT-IR, mass spectra and the melting points obtained for each compounds.

Table 1. Chemical structure of substituted imidazole.

Entry	Compound	R	R	R ₁	R ₂
1	5a	Ph	Ph	Ph-CH ₂	p-N(CH ₃) ₂
2	5b	Ph	Ph	Ph-CH ₂	p-OH
3	5c	Ph	Ph	Ph-CH ₂	H
4	5d	Ph	Ph	Ph-CH ₂	p-OCH ₃
5	5e	Ph	Ph	m-CH ₃ -C ₆ H ₅ -	m-NO ₂
6	5f	Ph	Ph	o-CH ₃ -C ₆ H ₅ -	p-NO ₂
7	5g	Ph	Ph	m-CH ₃ -C ₆ H ₅ -	p-NO ₂
8	5h	Ph	Ph	p-CH ₃ -C ₆ H ₅ -	p-NO ₂
9	5i	Ph	Ph	p-SO ₃ H-C ₆ H ₅	p-N(CH ₃) ₂
10	5j	Ph	Ph	-	p-N(CH ₃) ₂
11	5k	Ph	Ph	Fu-CH ₂	4-OH, 3-CH ₃ O
12	5l	Fu	Fu	-	4-OH, 3-CH ₃ O
13	5m	Fu	Fu	-	C ₆ H ₅
14	5n	Ph	Ph	Fu-CH ₂	p-OH-C ₆ H ₅

Ph: Phenyl; Fu: Furyl

108

109 ***Antiparasitic activity.***

110 All compounds were *in vitro* tested against *Trypanosoma cruzi* Tulahuen CL2, β -galactosidase
 111 strain (nifurtimox-sensitive), *Leishmania infantum* (MHOM/MABE/67 amastigotes) and
 112 *Trypanosoma brucei* (Squib-427 strain, suramin-sensitive) and *Trypanosoma b. rhodesiense* (strain
 113 STIB-900).

114

115 Compound stock solutions were prepared in DMSO at 20 mM. The compounds were serially pre-
116 diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized
117 water to assure a final in-test DMSO concentration of <1%. Test plates are identical for all screens
118 and produced as a single batch. The results were expressed as % reduction in parasite burdens
119 compared to control wells and an IC₅₀ (50% inhibitory concentration) was calculated. The *in vitro*
120 screening was carried out according to the previously described procedure (Cos et al. 2006).

121 *Trypanosoma cruzi*, Tulahuen CL2, β galactosidase strain (nifurtimox-sensitive) was used. The
122 strain was maintained on MRC-5_{SV2} (human lung fibroblast) cells in MEM medium, supplemented
123 with 200 mM L-glutamine, 16.5 mM NaHCO₃, and 5% inactivated fetal calf serum. All cultures
124 and assays were conducted at 37°C under an atmosphere of 5% CO₂. Assays were performed in
125 sterile 96-well microtiter plates, each well containing 10 μ l of the watery compound dilutions
126 together with 190 μ l of MRC-5 cell/parasite inoculum ($2 \cdot 10^4$ cells/ml + $2 \cdot 10^5$ parasites/ml). Parasite
127 growth was compared to untreated-infected controls (100% growth) and non-infected controls (0%
128 growth) after 7 days incubation. Parasite burdens were assessed after adding the substrate CPRG
129 (chlorophenolred β -D-galactopyranoside): 50 μ l/well of a stock solution containing 15.2 mg CPRG
130 + 250 μ l Nonidet in 100 ml PBS. The change in color was measured at 540 nm after 4 hours
131 incubation at 37 °C. **Benznidazole** was included as reference drug. The test compound is classified
132 as inactive when the IC₅₀ is higher than 30 μ M. When IC₅₀ lies between 30 and 5 μ M, the compound
133 is regarded as being moderate active. When the IC₅₀ is lower than 5 μ M, the compound is classified
134 as highly active on the condition that it also demonstrates selective action (absence of cytotoxicity).

135 *Trypanosoma brucei brucei* Squib 427 strain (**suramin**-sensitive) and *Trypanosoma brucei*
136 *rhodesiense* (strain STIB-900) were used for screening. The strains are maintained in Hirumi (HMI-
137 9) medium, supplemented with 10% inactivated fetal calf serum. All cultures and assays were

138 conducted at 37°C under an atmosphere of 5% CO₂. Assays were performed in sterile 96-well
139 microtiter plates, each well containing 10 µl of the compound dilutions together with 190 µl of the
140 parasite suspension (1.5x10⁴ parasites/well – *T.b. brucei* and 4x10³ parasites/well – *T.b.*
141 *rhodesiense*). Parasite growth was compared to untreated-infected (100% parasite growth) and
142 uninfected controls (0% growth). After 3 days incubation, parasite growth was assessed
143 fluorimetrically after addition of 50ml resazurin per well. After 6 hours (*T.b. rhodesiense*) or 24
144 hours (*T.b. brucei*) at 37°C, fluorescence was measured (λ_{ex} 550 nm, λ_{em} 590 nm). **Suramin** (*T.b.*
145 *brucei* and *T.b. rhodesiense*) was included as the reference drugs. The compound is classified as
146 inactive when the IC₅₀ is higher than 5 µM. When IC₅₀ lies between 5 and 1 µM, the compound is
147 regarded as being moderate active. When the IC₅₀ is lower than 1 µM, the compound is classified
148 as highly active on the condition that it also demonstrates selective action (absence of cytotoxicity).

149 *Leishmania infantum* MHOM/MA(BE)/67 was maintained in the Golden Hamster (*Mesocricetus*
150 *auratus*) and spleen amastigotes were collected for infection. Primary peritoneal mouse
151 macrophages (PMM) were used as host cells and were collected 2 days after peritoneal stimulation
152 with a 2% potato starch suspension. Assays were performed in 96-well microtiter plates, each well
153 containing 10 µl of the compound dilutions together with 190 µl of macrophage-parasite inoculum
154 (3.10⁵ cells + 3.10⁶ parasites/well // RPMI-1640 + 5% FCSi). After 5 days incubation, total
155 amastigote burdens was microscopically assessed after Giemsa staining. **Miltefosine** was included
156 as reference drug. A test compound is classified as inactive when the IC₅₀ is higher than 30 µM.
157 When IC₅₀ lies between 30 and 10 µM, the compound is regarded as moderately active. If the IC₅₀
158 is lower than 10 µM, the compound is classified as highly active on the condition that it also
159 demonstrates selective action (absence of cytotoxicity against primary peritoneal macrophages).

160

161 ***In vitro* cytotoxicity evaluation on human fibroblasts (MRC-5 cell line).**

162 MRC-5_{SV2} cells were cultured in MEM + Earl's salts-medium, supplemented with L-glutamine,
163 NaHCO₃ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37°C under
164 an atmosphere of 5% CO₂. Assays were performed in 96-well microtiter plates, each well containing
165 10 µl of the watery compound dilutions together with 190 µl of MRC-5_{SV2} inoculum (1.5x10⁵
166 cells/ml). Cell growth was compared to untreated-control wells (100% cell growth) and medium-
167 control wells (0% cell growth). After 3 days incubation, cell viability was assessed fluorimetrically
168 after addition of resazurin (λ_{ex} 550 nm, λ_{em} 590 nm). **Tamoxifen** was used as positive control. The
169 compound is classified non-toxic when the IC₅₀ is higher than 30 µM. Between 30 and 10 µM, the
170 compound is regarded as moderately toxic. When the IC₅₀ is lower than 10 µM, the compound is
171 classified as highly toxic.

172 ***Molecular docking.***

173 ***Ligands and proteins preparation.***

174 Molecular modelling was performed using the High-performance computing capabilities of the
175 Cluster of Chemistry Department of University of Oriente running the Linux operating system
176 Debian 8.0 distribution. The pdb file was prepared using the software UCSF Chimera molecular
177 graphic system, version 1.10.227 (Pettersen et al. 2004). Cytochrome P450 14 α -sterol demethylase,
178 CYP51, of the species *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* and *Leishmania infantum*
179 were selected: CYP51 of *Trypanosoma cruzi* in complex with an inhibitor **VNF** ((4-(4-
180 chlorophenyl)-N-[2-(1H-imidazol-1-yl)-1-phenylethyl]benzamide) - PDB ID: **3KSW** (CYP51 - *T.*
181 *cruzi*) (Lepesheva et al. 2010) with a resolution of 3.05 Å, CYP51 of *Trypanosoma brucei* (*T.*
182 *brucei*) bound to (S)-N-(3-(1H-indol-3-yl)-1-oxo-1-(pyridin-4-ylamino)propan-2-yl)-3,3'-difluoro-
183 (1,1'-biphenyl)-4- carboxamide – PDB ID: **4BJK** (CYP51 - *T. brucei*) (Choi et al. 2013) with a

resolution of 2.67 Å and CYP51 of *Leishmania infantum* (*L. infantum*) in complex with fluconazole - PDB ID: **3L4D** (*CYP51* - *L. infantum*) (Hargrove et al. 2011) with resolution of 2.75 Å. The structures **3L4D** and **4BJK** contained identical domains; we selected chain A for the docking experiments. Structure **3KSW** contained only one chain. Finally, the resulting prepared 3D structure of the proteins was saved as PDB file, using AutodockTools 1.5.6 (Sanner 1999). Kollman united atom charges, solvation parameters and polar hydrogens were added to the receptor. Since the ligands are not peptides, Gasteiger charges were assigned and non-polar hydrogens were merged. ChemBioDraw (Evans 2014) was used to draw and design the structures of arylsubstituted imidazoles (**Figure 1**) taking into account reference Planche et al. (2009). ChemBio3D Ultra 12.0 (Evans 2014) was used to optimize the geometry, running a MMFF94 energy minimization of the 3D structures. The Gasteiger charge calculation method (Gasteiger and Marsili 1980) was used and partial charges were added to the ligand atoms prior to docking, via AutodockTools.

Identification of binding site residues.

The binding site residues for *CYP51* - *T. cruzi*, *CYP51* - *T. brucei* and *CYP51* - *L. infantum* were identified from the analysis of 3KSW, 4BJK and 3L4D available in the RCSB Protein Data Bank. *CYP51* - *L. infantum* in the active site involves the amino acids Leu355, Met357, Leu358, Met459, Val212, Phe104, Met105, Tyr115, Ala114, Phe109, Gly282, Met283, Phe289, Leu129, Ala290 and Val460 (Hargrove et al. 2011), *CYP51* - *T. cruzi* involves the amino acids Tyr103, Met106, Phe110, Tyr116, Leu127, Ala297, Phe290, Ala291, Thr295, Leu356, Leu357, Met358, Met360, Val461, Met460 and Ala287 (Lepesheva et al. 2010) and *CYP51* - *T. brucei* involves the amino acids Tyr116, Tyr103, Met106, Phe105, Phe290, Ala287 and Phe110 (Choi et al. 2013) according to the references.

208

209 ***Grid box preparation and docking.***

210 Docking experiment were performed between arylsubstituted imidazoles and *CYP51 - T. cruzi* and
 211 *CYP51 - L. infantum* proteins. AutoDock requires pre-calculated grid maps, one for each atom type,
 212 present in the ligand being docked, as it stores the potential energy arising from the interaction with
 213 the macromolecule. This grid must surround the region of interest (active site) in the macromolecule.
 214 Grid box parameters (**Table 2**) were set by using AutoDockTools (ADT), a free graphic user
 215 interface of MGL software packages (version 1.5.6rc3) (Sanner 1999).

216

217 The molecular docking program AutoDock version 4.2 (Sanner 1999) was employed to perform the
 218 docking experiment. The Lamarckian Genetic Algorithm was used during the process to explore the
 219 best conformational space for the ligand with a population size of 50 individuals. The maximum
 220 number of generations and evaluations were set at 27000 and 2500000, respectively. Other
 221 parameters were set as default. After complete execution of Autodock, fifty conformations of the
 222 ligands in complex with the receptor were obtained, which were ranked based on binding energy
 223 and inhibition constant (K_i).

224 **Table 2.** Grid box parameters selected for the target enzymes.

Enzyme	x, y, z			Size (points)	Spacing (Å)
	coordinates of center of				
	box				
<i>CYP51 - T. cruzi</i>	2.48	26.996	22.191	50 x 50 x 50	0.375
<i>CYP51 - L. infantum</i>	31.917	-28.96	-1.658	50 x 50 x 50	0.375
<i>CYP51 - T. brucei</i>	26.642	58.216	26.493	50 x 50 x 50	0.375

225

226 To check the docking procedure and given the similar structure (imidazole and triazole rings)
227 between the compounds here reported we choose known CYP51 inhibitors, **VNF** ((R)-N-(2-(1H-
228 imidazol-1-yl)-1-phenylethyl)-4'-chloro-[1,1'-biphenyl]-4-carboxamide) to *CYP51* - *T. cruzi*
229 (Friggeri et al. 2014), Ketoconazole (**KET**) for *CYP51* - *T. Brucei* (Hargrove et al. 2012) and **VFV**
230 ((R)-N-(1-(3,4'-difluorobiphenyl-4-yl)-2-(1H-imidazol-1-yl)ethyl)-4-(5-phenyl-1,3,4-oxadiazol-
231 yl)benza-mide) for *CYP51* - *L. infantum* (Lepesheva et al. 2015), which were rebuilt and redocked
232 (**Figure 2**).

233

234 **Figure 2.** Structure of control compounds **VNF** (A), **KET** (B) and **VFV** (C).

235

236 **Results and Discussion.**

237 *Synthesis and characterization of arylsubstituted imidazoles.*

238 Since arylsubstituted imidazoles have become increasingly useful and important in the
239 pharmaceutical field, the development of clean, high-yielding, and environmentally friendly
240 synthetic approaches are still desirable and much in demand. The synthesized imidazole derivatives
241 were characterized after recrystallization from an appropriate solvent by recording their ¹H-NMR,
242 ¹³C-NMR, FT-IR and MS spectra. The results of the synthesis are summarized in **Table 3**.

243

Table 3. Physical data and yields of arylsubstituted imidazoles.

Product	R	R	R ₁	R ₂	Yield ^a (%)	M.P. (°C) (Observed)	M.P. (°C) (Literature)
5a	Ph	Ph	Ph-CH ₂	p-N(CH ₃) ₂	89	151-153	150–152 ^b
5b	Ph	Ph	Ph-CH ₂	p-OH	88	136-139	135-138 ^c
5c	Ph	Ph	Ph-CH ₂	H	90	158-160	155-158 ^c
5d	Ph	Ph	Ph-CH ₂	p-OCH ₃	91	165-168	167-169 ^c
5e	Ph	Ph	m-CH ₃ -C ₆ H ₅ -	m-NO ₂	89	165-166	-
5f	Ph	Ph	o-CH ₃ -C ₆ H ₅ -	p-NO ₂	86	235-236	-
5g	Ph	Ph	m-CH ₃ -C ₆ H ₅ -	p-NO ₂	89	221-223	-
5h	Ph	Ph	p-CH ₃ -C ₆ H ₅ -	p-NO ₂	88	219-222	219-220 ^b
5i	Ph	Ph	p-SO ₃ H-C ₆ H ₅	p-N(CH ₃) ₂	88	109-112	-
5j	Ph	Ph	-	p-N(CH ₃) ₂	84	255-257	256–259 ^d
5k	Ph	Ph	Fu-CH ₂	4-OH, 3-CH ₃ O	87	244-245	-
5l	Fu	Fu	-	4-OH, 3-CH ₃ O	83	189-190	-
5m	Fu	Fu	-	C ₆ H ₅	82	196-198	197–198 ^e
5n	Ph	Ph	Fu-CH ₂	p-OH-C ₆ H ₅	89	155-158	-

^a Indicated yields refer to isolated products. ^b Kantevari et al. 2006. ^c Khalafi-Nezhad et al. 2016. ^d

Eidi et al. 2016. ^e Kang et al. 2011.

According to the results shown in **Table 3**, all synthesized compounds have a similar melting point and good yield compared with the reference.

250 ***Spectral data results.***

251 **(5a)** 4-(1-benzyl-4,5-diphenyl-1H-imidazol-2-yl)-N,N-dimethylaniline. **MW:** 429.57 **mp:**151-
252 153°C (Reported: 150–152°C (Kantevari et al. 2006)). **¹H-NMR** (600MHz, DMSO-*d*₆): δ ppm 8.10-
253 7.18 (m, 19H, Ar-H), 5.12 (s, 2H, CH₂), 3.34 (s, 6H, CH₃×2). **¹³C-NMR** (150MHz, DMSO-*d*₆): δ
254 ppm 155.2, 150.42, 141.3, 137.78, 131.51, 129.38, 128.94, 128.75, 128.60, 128.49, 128.35, 127.48,
255 126.38, 125.61, 118.42, 111.99(C_{arom}), 47.68 (CH₂), 41.02 (CH₃×2). **Mass Spectrum (ES+).** 430.23
256 [M+H]⁺, 387.18, 340.18, 297.13.

257 **(5b)** 4-(1-benzyl-4,5-diphenyl-1H-imidazol-2-yl) phenol. **MW:**402.49 **mp:**136-139°C (Reported:
258 135-138 (Khalafi-Nezhad et al. 2016)). **¹H-NMR** (600MHz, DMSO-*d*₆): δ ppm 9.76 (s, 1H, OH),
259 6.74-7.46 (m, 19H), 5.11 (s, 2H, CH₂), **¹³C-NMR** (150MHz, DMSO-*d*₆): δ ppm 158.04, 153.84,
260 141.40, 137.57, 136.52, 134.75, 130.88, 130.07, 129.63, 128.80, 128.55, 128.10, 127.18, 126.10,
261 125.70, 121.57, 115.46 (C_{arom}), 47.63(CH₂) **Mass Spectrum (ES+).** 403.18 [M+H]⁺, 313.13,
262 297.14.

263 **(5c)** 1-benzyl-2,4,5-triphenyl-1H-imidazole **MW:**386.50 **mp:** 158-160°C (Reported: 155-158
264 (Khalafi-Nezhad et al. 2016)). **¹H-NMR** (600MHz, DMSO-*d*₆): δ ppm 6.75-7.69 (m, 20H, Ar-H),
265 5.13 (s, 2H, CH₂). **¹³C-NMR** (150MHz, DMSO-*d*₆) δ ppm 153.04, 141.33, 137.20, 137.36, 133.58,
266 131.12, 130.43, 129.7, 129.10, 127.49, 126.94, 126.18, 125.67, 125.26 (C_{arom}), 47.73 (CH₂). **Mass**
267 **Spectrum (ES+).** 387.18 [M+H]⁺ 297.13.

268 **(5d)** 1-benzyl-2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazole **MW:**416.52 **mp:**165-168°C
269 (Reported: 167-169 (Khalafi-Nezhad et al. 2016)). **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 6.75-
270 8.01 (m, 17H, Ar-H), 5.65 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). **¹³C-NMR** (150MHz, DMSO-*d*₆):
271 159.66, 153.70, 147.20, 141.30, 138.82, 137.49, 134.30, 130.70, 129.80, 129.60, 128.59, 127.66,

272 125.63, 123.20, 114.15(C_{arom}), 55.26(CH₃), 47.66(CH₂). **Mass Spectrum (ES+)**. 417.19 [M+H]⁺
273 327.14, 221.14.

274 **(5e)** 2-(3-nitrophenyl)-4,5-diphenyl-1-(m-tolyl)-1H-imidazole **MW**:431.50 **mp**:165-166°C
275 (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 7.10-8.80 (m, 18H, Ar-
276 H), 2.35 (s, 3H, CH₃) **¹³C-NMR** (150MHz, DMSO-*d*₆): 149.80, 148.73, 146.04, 145.00, 143.97,
277 139.15, 138.12, 136.04, 134.86, 132.72, 131.00, 130.08, 129.99, 129.57, 127.83, 126.07, 123.16,
278 122.19, 118.82, 115.25(C_{arom}), 21.4 (CH₃). **IR (KBr)**: ν_{max}3058(C=C_{arom}), 169(C=N), 1575(N-O),
279 1460 y 1502(C=C_{arom}), 1335 (C-NO₂). δ_{max} 853(C-N_{Ar-NO₂}), 710 y 810(C-H_{m-sust}) cm⁻¹. **Mass**
280 **Spectrum (ES+)**. 432.17 [M+H]⁺ 418.19, 342.12, 297.13, 221.13, 179.08.

281 **(5f)** 2-(4-nitrophenyl)-4,5-diphenyl-1-(o-tolyl)-1H-imidazole **MW**:431.50 **mp**:235-236°C
282 (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 7.12-8.69 (m, 18H, Ar-
283 H), 2.33 (s, 3H, CH₃) **¹³C-NMR** (150MHz, DMSO-*d*₆): 149.63, 148.89, 144.32, 141.74, 137.36,
284 135.60, 132.30, 131.72, 130.36, 129.65, 129.53, 127.68, 126.93, 126.65, 124.10, 122.36,
285 117.76(C_{arom}), 17.57(CH₃) **IR (KBr)**: ν_{max}3080(C=C_{arom}), 1685(C=N), 1580(N-O), 1500(C=C_{arom}),
286 1345(C-NO₂). δ_{max} 860(C-N_{Ar-NO₂}), 740(C-H_{o-sust}) cm⁻¹. **Mass Spectrum (ES+)**. 432.17 [M+H]⁺
287 387.18, 342.12, 297.14.

288 **(5g)** 2-(4-nitrophenyl)-4,5-diphenyl-1-(m-tolyl)-1H-imidazole **MW**:431.50 **mp**: 221-223°C
289 (Reported: none reported found) **¹H-NMR** (600MHz, MeOH-*d*₄): δ_H ppm 7.33-8.31 (m, 12H, Ar-
290 H), 2.21 (s, 3H, CH₃) **¹³C-NMR** (150MHz, DMSO-*d*₆): 149.63, 148.89, 144.32, 141.74, 137.36,
291 135.60, 132.30, 131.72, 130.36, 129.65, 129.53, 127.68, 126.93, 126.65, 124.10, 122.36,
292 117.76(C_{arom}), 17.57(CH₃) **IR (KBr)**: ν_{max} 3058 (C=C_{arom}), 1650 (C=N), 1599 (C=C_{arom}), 1335 (C-
293 NO₂), 853 (C-N_{Ar-NO₂}), 767 (C-H_{m-sust}) cm⁻¹. **Mass Spectrum (ES+)**. 432.17 [M+H]⁺ 342.12,
294 179.09, 149.02.

295 (5h) 2-(4-nitrophenyl)-4,5-diphenyl-1-(p-tolyl)-1H-imidazole **mp**: 219-222°C (Reported: 219-
296 220°C (Kantevari et al. 2006)). **MW**:431.50 **¹H-NMR** (600MHz, MeOH-*d*₄): δ_H ppm 7.32-8.21 (m,
297 18H, Ar-H), 2.14 (s, 3H, CH₃) **¹³C-NMR** (150MHz, MeOH-*d*₄): δ_H ppm, 148.81 145.56, 139.16,
298 137.06, 133.29, 130.39, 129.72, 129.61, 129.52, 129.43, 128.92, 128.89, 127.28, 126.82, 125.21,
299 124.35, (C_{arom}). 22.15(CH₃) **IR (KBr)**: ν_{max} 3050 (C=C_{arom}), 1615 (C=N), 1500 (C=C_{arom}), 1338 (C-
300 NO₂), δ_{max} 860 (C-N_{Ar}-NO₂), 840 (C-H_{p-sust}) cm⁻¹. **Mass Spectrum (ES+)**. 432.17 [M+H]⁺ 342.12,
301 301.14, 179.09, 149.02, 137.08.

302 (5i) 4-(2-(4-(dimethylamino)phenyl)-4,5-diphenyl-1H-imidazol-1-yl)benzenesulfonic acid
303 **MW**:495.60 **mp**: 109-112°C (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H
304 ppm 9.66 (s, 1H, OH), 6.47-7.97(m, 18H, Ar-H), 2.99 (s, 6H, CH₃×2). **¹³C-NMR** (150MHz, DMSO-
305 *d*₆): 151.35, 145.87, 143.10, 139.60, 136.40, 130.69, 130.09, 128.72, 128.53, 128.31, 127.40,
306 126.88, 126.3, 126.21, 113.93, 112.49 (C_{arom}), 40.01 (CH₃×2) ppm **Mass Spectrum (ES-)**. 495.14
307 [M-H]⁻, 338.17, 214.01, 172, 155.

308 (5j) 4-(4,5-diphenyl-1H-imidazol-2-yl)-N,N-dimethylaniline **MW**:339.44 **mp**: 255-257°C
309 (Reported: 256–259°C (Eidi et al. 2016)) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 12.36 (s, 1H,
310 NH), 6.80-7.97 (m, 14H, Ar-H), 2.99 (s, 6H, CH₃×2). **¹³C-NMR** (150MHz, DMSO-*d*₆): δ_H ppm
311 154.21, 150.36, 146.80, 128.44, 127.84, 126.56, 118.60, 112.03, 111.11(C_{arom}), 43.45 (CH₃×2).
312 **Mass Spectrum (ES+)**. 340.18 [M+H]⁺, 297.14

313 (5k) 4-(1-(furan-2-ylmethyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol **MW**:422.48 **mp**:
314 244-245°C (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 9.28 (s, 1H,
315 OH), 6.85-7.72 (m, 16H, Ar-H, Furanyl), 4.46 (s, 2H, CH₂), 3.83 (s, 3H, CH₃). **¹³C-NMR** (150MHz,
316 DMSO-*d*₆): δ_H ppm 148.72, 147.77, 147.22, 146.08, 142.06, 138.62, 128.50, 128.74, 127.82,

127.52, 127.15, 121.77, 118.52, 115.70, 112.08, 110.92, 105.42, 109.46(C_{arom}), 55.79 (CH₃), 40.01 (CH₂). **Mass Spectrum (ES+)**. 423.17 [M+H]⁺, 343.14, 297.14

(5l) 4-(4,5-di(furan-2-yl)-1H-imidazol-2-yl)-3-methoxyphenol **MW**:276.30 **mp**:189-190°C (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 3.87 (s, 3H, CH₃), 9.33 (s, 1H, OH), 6.57-7.81 (m, 9H, Ar-H, Furanyl), 12.62 (s, 1H, NH). **¹³C-NMR** (150MHz, DMSO-*d*₆): δ_H ppm 144.71, 142.46, 141.80, 129.33, 128.97, 121.29, 120.91, 118.91, 115.67, 112.81, 111.45, 108.18, 106.79(C_{arom}), 55.84(CH₃). **Mass Spectrum (ES+)**. 323.10 [M+H]⁺, 293.09, 201.08

(5m) 4,5-di(furan-2-yl)-2-phenyl-1H-imidazole **MW**:276.30 **mp**:196-198 (Reported: 197–198 °C (Kang et al. 2011)) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 6.58-8.10 (m, 11H, Ar-H, Furanyl), 12.87 (s, 1H, NH). **¹³C-NMR** (150MHz, DMSO-*d*₆): δ_H ppm 149.34, 146.32, 144.48, 142.69, 141.94, 129.89, 128.78, 119.42, 111.96, 106.98(C_{arom}) **Mass Spectrum (ES+)**. 277.09 [M+H]⁺, 201.07

(5n) 4-(4,5-di(furan-2-yl)-1-(furan-2-ylmethyl)-1H-imidazol-2-yl)phenol **MW**:392.46 **mp**:155-158 (Reported: none reported found) **¹H-NMR** (600MHz, DMSO-*d*₆): δ_H ppm 9.79 (s, 1H, Ar-OH), 3.42 (s, 2H, CH₂), 6.87-7.92 (m, 13H, Ar-H, Furanyl) **¹³C-NMR** (150MHz, DMSO-*d*₆): δ_H ppm 158.50, 157.91, 153.67, 151.30, 146.19, 142.9, 142.1, 130.75, 128.48, 127.80, 126.98, 121.73, 115.96, 107.70(C_{arom}), 37.80 (CH₂). **Mass Spectrum (ES+)**. 373.12 [M+H]⁺ 293.09, 277.10

Antiprotozoal and Cytotoxic Effects.

The antiparasitic activities and the cytotoxicity of the test and reference compounds are listed in **Table 4**. All compounds were tested *in vitro* against the tripomastigotes stage of *T.b. brucei* and *T.b. rhodesiense* and amastigote stage of *T. cruzi* and *L. infantum*. The cytotoxicity of all arylsubstituted imidazoles against MRC-5 cell lines (human lung fibroblasts) was evaluated and the

339 selectivity indices (SI) ($SI = CC_{50} \text{ MRC-5 cells} / IC_{50} \text{ protozoa}$) were calculated (**Table 5**) (Ehata et
340 al. 2012).

341

342 **Table 4.** Antitrypanosomal, antileishmanial (IC₅₀, μM±SD) and cytotoxic (MRC-5) (CC₅₀,
343 μM±SD) activities of arylsubstituted imidazoles. Biological experiments were performed in
344 duplicate.

Products	MRC-5	T.b. brucei	T.b. rhod.	T. cruzi	L. infantum
5a	1.56±0.2	3.59±0.41	0.86±0.06	1.09±0.01	1.02±0.02
5b	>64.0	39.85±5.13	37.11±3.88	35.12±5.88	>64.0
5c	38.62±5.33	3.36±0.34	3.77±0.29	4.07±0.51	10.23±1.16
5d	>64.0	35.21±7.21	44.11±4.99	34.13±4.87	>64.0
5e	2.29±0.31	2.11±0.11	2.24±0.31	2.07±0.24	2.07±0.21
5f	2.03±0.14	4.21±0.29	5.17±0.56	1.93±0.23	8.20±0.81
5g	1.12±0.06	2.29±0.21	3.19±0.31	1.80±0.43	1.99±0.19
5h	51.09±4.76	72.22±9.15	14.56±2.67	17.66±3.2	40.89±6.21
5i	4.66±0.32	3.75±0.33	3.29±0.33	4.31±0.51	4.11±0.51
5j	4.11±0.42	4.37±0.43	4.37±0.44	0.96±0.03	4.07±0.42
5k	3.67±0.12	12.33±2.34	12.09±1.33	3.78±0.02	16.44±1.77
5l	13.22±1.22	32.00±4.12	11.99±1.87	18.19±0.20	64.99±8.81
5m	14.67±1.11	35.11±5.89	15.53±2.19	16.99±1.77	48.23±5.51
5n	3.23±0.33	16.23±1.78	24.89±4.12	3.81±0.45	25.47±2.45
Tamoxifen	10.2±1.44				
Suramin		0.03±0.01	0.04±0.01		
Benznidazole				2.89±0.56	
Miltefosine					10.07±4.23

345 T. cruzi: *Trypanosoma cruzi*, L. infantum: *Leishmania infantum*, T.b. brucei: *Trypanosoma b. brucei*, T.b. rhod.:
346 *Trypanosoma b. rhodesiense*, MRC-5: Human fetal lung fibroblast cell line.

347 The imidazoles **5a**, **5c**, **5e**, **5f**, **5g**, **5i**, **5j**, **5k** and **5n** exhibited IC₅₀ values lower than 5 µM against
348 *T. cruzi*. The ligands **5l**, **5m** and **5h** had a moderate activity with IC₅₀ values of 18.19, 16.99 and
349 17.66 µM, respectively. Only the ligands **5d** and **5b**, both with similar structure (**Table 1**) substituted
350 in position two of the arylsubstituted imidazole by a hydroxyl (OH) and a methoxy (OCH₃), showed
351 a low activity. The most active compounds **5a**, **5f**, **5g** and **5j** showed lower IC₅₀ values than the
352 reference compound benznidazole (**Table 4**). However, only compound **5c** with an IC₅₀ of 4.07 µM
353 presented a good selectivity toward *T. cruzi* with a SI of 9.49 (**Table 5**).

354 On the other hand, against *T.b. brucei* and *T.b. rhodesiense*, the active compounds were **5a**, **5c**, **5e**,
355 **5f**, **5g**, **5i** and **5j**. The most active compound against *T.b. brucei* was **5e** (IC₅₀ = 2.11 µM), while for
356 *T.b. rhodesiense* the most active compound was **5a** with an IC₅₀ = 0.86 µM. Both compounds were
357 less active than the reference compounds **suramin** for *T.b. brucei* (0.03 µM) and *T.b. Rhodesiense*
358 (0.04 µM). For *T.b. rhodesiense*, the ligands with a good activity were **5h**, **5k**, **5l** and **5m**, with IC₅₀
359 values between 11.99 and 15.53 µM. Ligands **5k** and **5l** have a similar structure in position two of
360 the imidazoles with a phenyl group joined to a hydroxyl (4-OH) substituent and a methoxyl (3-
361 CH₃O) group.

362 None of studied arylsubstituted imidazoles showed a higher activity than the reference drug **suramin**
363 towards *T.b. rhodesiense*, demonstrating the lower sensitivity for this class of compounds (De Vita
364 et al. 2016). Only the ligand **5a** (IC₅₀ = 0.86 µM) possessed an IC₅₀ value comparable with the
365 control drug **suramin**.

366 Compounds **5a**, **5c**, **5e**, **5f**, **5g**, **5i** and **5j** presented similar antileishmanial activity compared with
367 the control drug **miltefosine**, but they showed a pronounced cytotoxicity on MRC-5 cells (**Table**
368 **4**), indicating the lack of selectivity towards *L. infantum* (**Table 5**).

369 Compound **5c** had the highest selectivity to the Trypanosoma family with a selectivity index (SI) of
 370 9.49, 11.5 and 10.2 for *T. cruzi*, *T.b. brucei* and *T.b. rhodesiense*, respectively (**Table 5**).

371

372 **Table 5.** Selectivity index (SI) of the arylsubstituted imidazole from *T. cruzi*, *L. infantum*, *T.b.*
 373 *Brucei* and *T.b. rhodesiense*.

Products	MRC-5/ <i>T.b. brucei</i>	MRC-5/ <i>T.b. rhod.</i>	MRC-5/ <i>T. cruzi</i>	MRC-5/ <i>L. infantum</i>
5a	0.43	1.81	1.43	1.53
5b	>1.61	>1.72	>1.82	>1
5c	11.5	10.2	9.49	3.78
5d	>1.81	>1.45	>1.87	>1
5e	1.09	1.02	1.11	1.11
5f	0.48	0.39	1.05	0.24
5g	0.49	0.35	0.62	0.56
5h	0.71	3.50	2.89	1.25
5i	1.24	1.42	1.08	1.13
5j	0.94	0.94	4.28	1
5k	0.29	0.30	0.97	0.22
5l	0.41	1.10	0.73	0.20
5m	0.42	0.94	0.86	0.30
5n	0.20	0.13	0.85	0.13

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376 ***Molecular docking.***

377 The procedure adopted for the molecular docking was used for all designed arylsubstituted
378 imidazoles (**Table 1**) with each target selected. The docked poses for each of the compounds have
379 been evaluated and the pose with the lowest binding free energy and inhibition constant was thereby
380 chosen (**Table 6**).

381

Table 6. Binding energy and inhibition constant of the arylsubstituted imidazoles and control CYP51 inhibitors **VNF**, **KET** and **VFV**.

Test Comp.	<i>CYP51 - T. cruzi</i>		<i>CYP51 - L. infantum</i>		<i>CYP51 - T. brucei</i>	
	ΔG	Ki	ΔG	Ki	ΔG	Ki
	(kcal/mol)	(nM)	(kcal/mol)	(nM)	(kcal/mol)	(nM)
5a	-9.91	54.32	-11.13	7.00	-10.58	17.68
5b	-9.98	48.54	-10.37	25.10	-9.84	61.38
5c	-9.61	90.61	-10.74	13.31	-9.76	69.82
5d	-10.41	23.31	-10.95	9.46	-9.97	49.08
5e	-13.32	0.17	-12.88	0.36	-14.47	0.02
5f	-13.63	0.10	-9.22	175.52	-14.75	0.02
5g	-13.80	0.08	-9.40	128.58	-14.01	0.05
5h	-13.49	0.13	-8.44	649.09	-12.56	0.62
5i	-14.54	0.02	-6.39	20800	-10.58	17.55
5j	-9.48	112.65	-9.84	61.34	-8.68	437.39
5k	-9.25	167.21	-10.61	16.18	-10.15	36.02
5l	-7.24	4950	-7.75	2080	-8.43	665.25
5m	-7.31	4370	-8.19	1000	-9.07	226.01
5n	-9.59	93.98	-10.26	29.97	-9.83	62.73
VNF	-9.65	85.03	-	-	-	-
VFV	-	-	-10.29	28.60	-	-
KET	-	-	-	-	-11.37	4.60

385 The lowest binding free energy (i.e. best docking score) and inhibition constant indicates the highest
386 ligand/protein affinity. The docking studies were done in comparison with control drugs (i.e. known
387 inhibitors of the target enzymes). The control drugs were redocked with each protein target chosen,
388 **VNF** for *CYP51* - *T. cruzi*, **VFV** for *CYP51* - *L. infantum* and **KET** for *CYP51* - *T. brucei*.

389 As shown in **Table 6** the binding energies (ΔG) and inhibition constant (K_i) of the control drug
390 **VNF** in the docking with *CYP51* - *T. cruzi*, was found to be -9.65 kcal/mol and 85.03 nM,
391 respectively. However, the value for almost designed imidazoles were found to be higher than the
392 **VNF** control, with ΔG value between -9.25 to -14.54 kcal/mol and low values of K_i . The ligand **5i**
393 shows the better values (ΔG = -14.54 kcal/mol and K_i = 0.02 nM) and a similar conformation
394 compared with the **VNF** control (**Figure 3A**), other ligands showed better values and only the
395 ligands **5l** and **5m** showed not accepted values of binding energies and inhibition constant compared
396 with the control.

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Figure 3. Superimposition of the best poses of the ligand + control drugs for *CYP51* - *T. cruzi* in (A) **5i** (tan) + **VNF** (sky blue) and (B) **5f** (tan) + **KET** (sky blue) for *CYP51* - *T. brucei* included in the binding pocket.

In the case of *CYP51* - *T. brucei* the binding energy (ΔG) of the control drug ketoconazole was -11.37 kcal/mol ($K_i = 4.60$ nM), exceeded only by the ligands **5e**, **5f**, **5g** and **5h** with a binding energy of -14.47, -14.75, -14.01 and -12.56 kcal/mol, respectively and K_i values very low compared with the **KET** control. The best ligand **5f** showed similar conformation respect to **KET** control (**Figure 3B**) and the ligands **5e**, **5g** and **5h** presented similar values (ΔG and K_i) to ligand **5f**, due to the same interaction with the Heme group through the oxygen presented in the NO_2 group (**Figure 4**)

Figure 4. Similar interaction between ligands **5e**, **5f**, **5g** and **5h** with the enzyme *CYP51 - T. brucei*.

For enzyme *CYP51* - *L. infantum* with the control drug **VFV** the binding energy was found to be -10.29 kcal/mol with a K_i = 28.60 nM. The values of **VFV** are only exceeded by ligands **5a**, **5b**, **5c**, **5d**, **5e**, **5k** were found to have -11.13, -10.37, -10.74, -10.95, -12.88 and -10.61, respectively for the binding energy and K_i values very low respect to the **VFV** control (**Table 6**). The best ligand was **5e** and we can observe in **Figure 5** the superimposition between **VFV** and **5e**, where both ligands interacted directly with the Heme group.

Figure 5. Superimposition of the best pose of the **5e** (tan) + **VFV** (sky blue) included in the binding pocket of *CYP51* - *L. infantum*

It should be noted that ligand **5e** appeared to bind in the three receptor enzymes (*CYP51* - *T. cruzi*, *CYP51* - *L. infantum*, *CYP51* - *T. brucei*) and in all cases this ligand had lower ΔG values when compared to the control drugs. Some similar behavior occurs with the ligands **5a** and **5k** binding to *CYP51* - *L. infantum* and *CYP51* - *T. brucei* and **5f**, **5g**, **5h** and **5i** binding with *CYP51* - *T. cruzi* and *CYP51* - *T. brucei*. All of them show better binding values than the control drugs. Our molecular docking studies revealed that all compounds (**5a-n**) in one way or another interacted with the 14 α -

448 demethylase enzyme for each species (*CYP51* - *T. cruzi*, *CYP51* - *L. infantum*, *CYP51* - *T. brucei*)
 449 by hydrophobic interactions or azole - Heme coordination and π - π and π - cation interactions.
 450 This study with *CYP51* - *T. cruzi* demonstrated that in some ligands (**5a**, **5b**, **5c**, **5d**, **5j**, **5k**, **5m** and
 451 **5n**) there are hydrophobic interaction with the Heme group (**Figure 6**).
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 454
 455 **Figure 6.** Docked structures of compound **5d** in *CYP51* - *T. cruzi*; A. Two-dimensional
 456 representations of interaction by the Ligplot software (Wallace et al. 1996), B. Three-dimensional
 457 representation of the interaction the ligand (tan) and Heme group (cyan).
 458 We see also hydrogen bond interactions with amino acid residue Arg361 (**5b**, **5d** and **5n**). All ligands
 459 showed hydrophobic interaction with Ala291, Ala287, Phe290, Phe110, Met106, Tyr116, Tyr103,

460 Pro99, His420, Leu356 and Thr295. They shared some residues Ala291, Ala287, Phe290, Phe110,
461 Met106, Tyr116, Tyr103, Leu356 and Thr295 involved in the active site according to Lepesheva et
462 al. (2010). The ligands **5e**, **5f**, **5g** and **5h** showed an electronic interaction between the NO₂ group
463 (electron pair of oxygen O33) and the Fe²⁺ of Hem488 group (**Figure 7**), however for ligand **5i** and
464 **5l** the interaction with the Hem488 is with SO₃H (electron pair of O20) for **5i** and OCH₃ (electron
465 pair of O25) for **5l** (**Figure 8**).

466 The ligands **5e**, **5f**, **5g** and **5h** also showed the interaction with the heme group. We could observe
467 the hydrophobic interaction with Ala291, Ala287, Phe290, Phe110, Met106, Tyr116, Tyr103,
468 Pro99, His420, Leu356 and Thr295 similar as seen with the ligands **5a**, **5b**, **5c**, **5d**, **5j**, **5k**, **5m** and
469 **5n** where they share the same residues involved in the active site.

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Figure 7. Docked structures of compound **5g** in the *CYP51* - *T. cruzi*; **A.** 2D interaction by the Ligplot software, **B.** 3D representation of the interaction the ligand (tan) and the heme group (cyan).

Figure 8. Docked structures of compounds **5i** (A) and **5l** (B) in the *CYP51* - *T. cruzi* pocket represented using the Ligplot software.

495 In the case of *CYP51* - *L. infantum* only the ligands **5e**, **5f**, **5g** and **5h**, showed interaction between
 496 one of the NO₂ group's oxygens (O32) with the heme group (**Figure 9A**) and in the ligand **5i**
 497 complex the interaction was with the SO₃H group's oxygen atoms (**Figure 9B**).

506 **Figure 9.** Docked structures of compounds **5g** (A) and **5i** (B) in the *CYP51* - *L. infantum* pocket
 507 represented using the Ligplot software.

508 On other hand the ligands **5a**, **5b**, **5c**, **5d**, **5j**, **5k**, **5l**, **5m** and **5n** were found to have hydrophobic
 509 interactions with Met359, Val212, Pro209, Me459, Val356, Met357, Val460, Thr294, Ala290,
 510 Leu355, Phe109, Met105, Phe289, Tyr115, Tyr102, Phe104 and Hem481 (**Figure 10**). These

ligands share some residues Leu355, Met357, Val212, Phe104, Met105, Tyr115, Phe109 and Ala290 involved in the active site according to Hargrove et al. (2011).

Figure 10. Docked structures of compound **5b** in the *CYP51* - *L. infantum*; **A.** 2D interaction by Ligplot software, **B.** 3D representation of the interaction the ligand (tan) and Heme group (cyan)

Compounds **5a**, **5b**, **5c**, **5d**, **5i**, **5j**, **5k**, **5l** and **5m** were found to establish hydrophobic interactions with *CYP51* - *T. brucei* (**Figure 11**) that involved the residues Val461, Ala291, Phe290, Thr295, His294, Phe105, Tyr103, Met106, Tyr116, Leu127, Ala115, Ala287, Phe110, Hem1450 and His294. All those compounds (**5a**, **5b**, **5c**, **5d**, **5i**, **5j**, **5k**, **5l** and **5m**) share some residues: Tyr116, Tyr103, Met106, Phe105, Phe290, Ala287 and Phe110 involved in the active site (Choi et al. 2013).

On other hand the ligands **5e**, **5f**, **5g**, **5h** and **5n** were observed to interact with the Heme group trough the NO₂ function for **5e**, **5f**, **5g**, **5h** and with OH for **5n** (**Figure 12**).

These results reveal that the position of the substituent in the phenyl joined to the imidazole ring in the four positions affected strongly the orientation and the subsequently interaction of the imidazoles with the Heme iron atom in the binding site of the enzyme.

Figure 11. Docked structures of compound **5a** in the *CYP51 - T. brucei*; **A.** 2D interaction by Ligplot software, **B.** 3D representation of the interaction the ligand (tan) and Heme group (cyan)

(A)

(B)

Figure 12. Docked structures of compounds **5f** (A) and **5n** (B) in the *CYP51* - *T. brucei* pocket represented using the Ligplot software.

Conclusions.

In conclusion, the present investigation has described the chemical synthesis and antiprotozoal, cytotoxic activities of several arylsubstituted imidazoles as well as their molecular docking in the active site of CYP51 of those protozoa. The reported results showed that all samples possessed interesting *in vitro* antiprotozoal activity at different extents. The ligand **5c** was found to be non-toxic according to the study conducted with the MRC-5 cell line and the best selectivity index against *T. cruzi*, *T.b. brucei* and *T.b. rhodesiense*. The main interactions, studied by molecular docking, are hydrophobic between the substituents in positions 1, 4 and 5 of the imidazolic rings and residues in the active site. Electrostatic interactions were identified between oxygen fundamentally present in NO₂, SO₃H and OH groups and the Fe²⁺ ion of the Heme group. The reported results can partly justify and support the possible use of some of these imidazoles for the treatment of parasitic diseases such as sleeping sickness, leishmaniasis and in some extent American trypanosomiasis named Chagas disease, with no apparent toxic effects in patients.

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