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New potent imidazoisoquinolinone derivatives as anti-*Trypanosoma cruzi* agents: Biological evaluation and structure–activity relationships

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ABSTRACT

A series of novel benzoimidazo and *N*-aryl-5-oxo-imidazo[1,2-*b*]isoquinoline-10-carbothioamides was developed. All the compounds were evaluated for their in vitro action against the epimastigote form of *Trypanosoma cruzi*. Four of them showed higher activity than Nifurtimox. Their unspecific cytotoxicity was evaluated using HeLa and L6 cells, being non-toxic at concentrations at least 15 and 200 times higher than that of *T. cruzi* IC_{50}. To gain insight into the mechanism of action, their DNA binding properties and reactivity with glutathione were studied, and QSAR study was performed.

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1. Introduction

Chagas' disease or American Trypanosomiasis is a parasitic disease that constitutes an important health problem in Central and South America and affects around 20 million people living in poorly constructed homes in rural areas. It is estimated that 50,000 infected people die each year.[1] The causative agent of this disease is a haemoflagellate protozoan, *Trypanosoma cruzi* (*T. cruzi*), which enters the human body through damaged skin.

Despite significant progress in the understanding of the biochemistry and physiology of its etiological agent, current specific treatments are usually effective only in the acute stage. Once the disease has progressed to the later stages, there is no effective cure and severe side effects arise in association with long-term treatments.

There are two drugs available for the etiological treatment of Chagas' disease: Nifurtimox (Nfx) and benznidazole (Bz). Nfx is a nitrofurane and Bz is a nitroimidazole compound.[2] The use of these drugs to treat the acute phase of the disease is widely accepted. However, their use in the treatment of the chronic phase is controversial. The undesirable side effects of both drugs are a major drawback in their use, frequently forcing the physician to stop the treatment.

The development of safer and more efficient therapeutic anti-*Trypanosoma cruzi* compounds continues to be a major goal in trypanocidal chemotherapy.

In this regard, agents based on different strategies, such as inhibition of specific parasite enzymes, actions on parasite DNA and oxidative stress damage, have been described.[3] A variety of lead compounds, including acridines, phenothiazines, benzoazepines and pyridoquinolines show trypanocidal activities.[4]

Isoquinolines generally constitute an important branch of heterocyclic compounds and are currently used against parasitic infections.[5] Imidazoisoquinolinones are valuable substrates for the synthesis of potentially biologically active compounds with structural features different from those of the existing drugs. Recently, we have described the synthesis of a series of imidazoisoquinolinones C-10 and N-1 substituted,[6] which possess a planar structure with an heterocyclic scaffold present in antiprotozoal agents.

In this work, we describe the synthesis and in vitro anti-*T. cruzi* proliferative activity of a series of imidazoisoquinolinones arylthioamides. A structure-activity relationship analysis is also discussed in order to identify the structural requirements for optimum activity.

In an attempt to gain better insight into the trypanocidal mechanism of action of these compounds, DNA affinity and redox metabolism were evaluated.

2. Methods and results

2.1. Synthesis

Derivatives **2–9** were prepared by a reaction between arylisothiocyanates and 2,3-dihydroimidazo[1,2-*b*]isoquinoline-5 (1*H*)-one

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1.[6] The arylisothiocyanates were prepared according to the procedures described in the literature in two steps[7] with minor modifications. Initially, we obtained the different 1,3-diarylthioureas from the corresponding anilines substituted and carbon disulfide and assayed different basic catalysts (potassium hydroxide, pyridine, 4-(dimethylamino) pyridine (DMAP)) in order to diminish the time of the reaction. DMAP was effective in 18 h as compared to other catalysts, which were effective in 2 days.[8] In the second step, by reaction of 1,3-diarylthioureas with sulfuric acid and acetic anhydride, we obtained the corresponding isothiocyanates[9] (Scheme 1) by isolating them by steam distillation. All the precursor compounds were identified by spectroscopy methods and are in agreement with those described in the literature.

Therefore, coupling of compound **1** with isothiocyanates afforded the C-10 substituted final compounds **2–9** (Scheme 1). Reaction time varied from 6 to 72 h depending on the substitution of the isothiocyanates used and the yields were poor. Thus, to obtain better yields, we used DMAP in concentrations equimolar. In the case of 3-trifluormethylphenylisothiocyanate no reaction was observed.

Compound **6** was treated with hydrogen peroxide at 0–5 °C to obtain compound **10** to compare its biological activity to that of sulfur compounds. Furthermore, we synthesized derivative **11** by acylation with acetyl chloride and DMAP as catalyst to determine whether the substitution in N-1 was necessary to show activity.

Compound **12** was synthesized in order to introduce a new aromatic moiety to enlarge compound **1** scaffold. For this, we used an efficient and environmentally friendly synthetic method for the synthesis under microwave irradiation, in which homophthalic acid and 2-aminoaniline directly reacted on a silica gel support under solvent-free conditions. Experiments were carried out using a domestic microwave oven modified with a reflux condenser. Nevertheless, after varying time and power irradiation, products of decomposition were obtained. Then, the synthesis was carried out using equimolar concentrations of reagents and drops of DMF as solvent. This allowed a greater heating in the reaction medium and thus, the possibility to obtain the product in much reduced reaction times, in a cleaner reaction and with excellent yields.

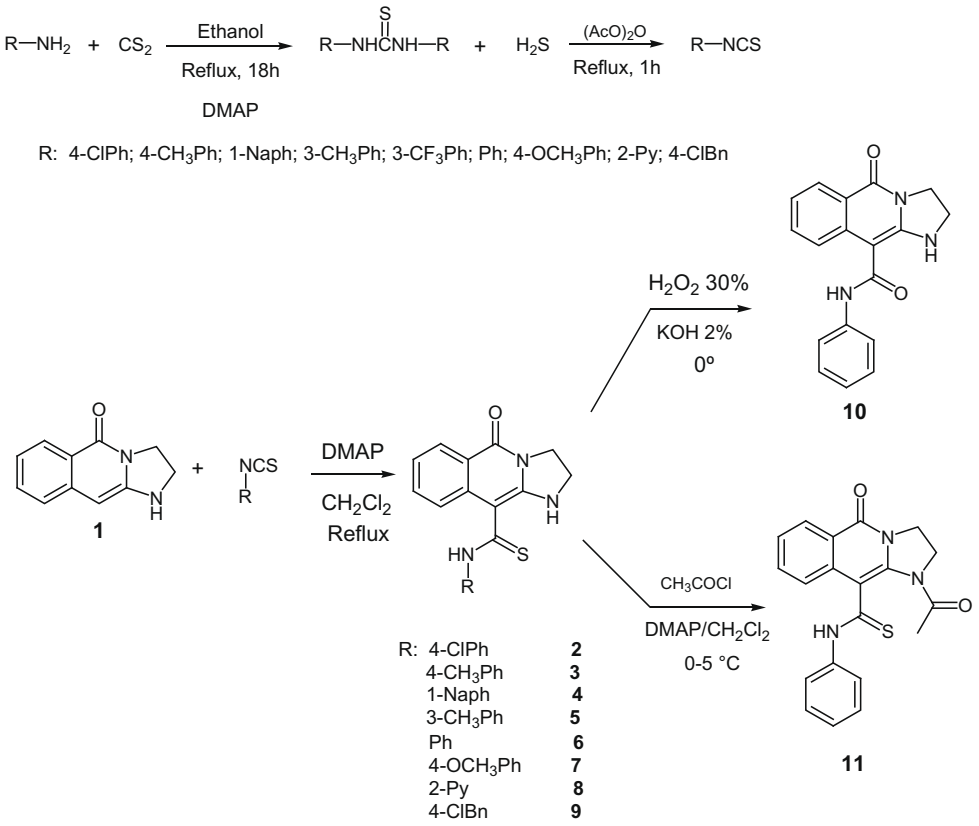
Finally, coupling of compound **12** with phenylisothiocyanate with DMAP as catalyst afforded the C-10 substituted final compound **13** (Scheme 2).

All the products were fully characterized by ^{1}H and ^{13}C NMR, MS and IR (see Section 5).

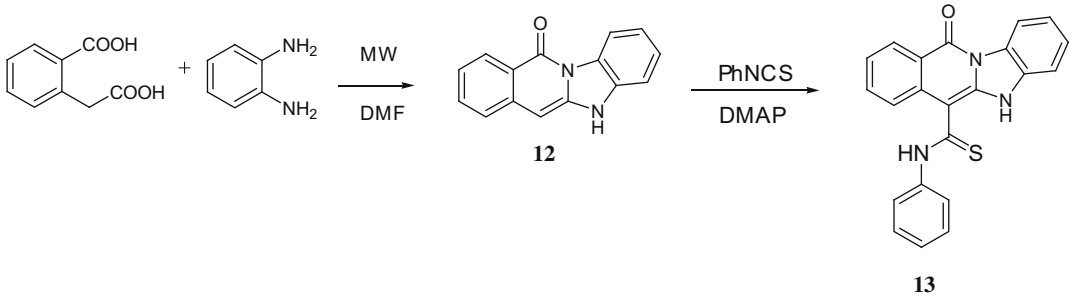
2.2. Biological characterization

2.2.1. Antitrypanosomal activities

The existence of the epimastigote form of *T. cruzi* as an obligate intracellular stage in mammals has been revised and confirmed recently.[10] Therefore, compounds were tested in vitro against the epimastigote form of the parasite.[11] As a first screening, the ability of developed derivatives to inhibit the growth of the epimastigote form of *T. cruzi* (Tulahuen 2 strain) was evaluated at 25 μM. Besides, the IC_{50} concentrations (50% inhibitory dose) were determined for the derivatives **1–13** (Table 1). Parasites were grown in the presence of the compound for 5 days, and the percentage of growth inhibition was determined against control (no drug added to the medium). Nifurtimox (Nfx) was used as the reference trypanocidal drug, as explained in Section 5. Imidazoisoquinolinones **2**, **3**, **6**, **7** and **13** showed either good or excellent activity against the epimastigote form. Compounds **2** and **3** exhibited remarkable trypanocidal activity and were 15- and 8-fold more potent than Nfx, respectively. Differently, compounds **1** and **12** did not show antichagasic activity.



Scheme 1. (a) Synthesis of isothiocyanates and (b) synthesis of compounds **2–11**.



Scheme 2. Synthesis of compounds **12** and **13**.

Table 1
In vitro activity of imidazo and benzoimidazoisoquinolinones derivatives and Nfx on *T. cruzi*

Compound	IC_{50} epimastigote[a] (μM)	IC_{50} HeLa (μM)	SI[b]
1	>25	>100	—
2	0.5	>90	>180
3	1	>100	>100
4	25	Nt[c]	—
5	20	>52.5	—
6	3	>100	>33
7	12.3	22	1.7
8	30	>100	—
9	35	>90	—
10	30	>100	—
11	43	Nt	—
12	>25	>100	—
13	6.4	98	15
Nfx	7.7	12	1.5

[a] IC_{50}: concentration that produces 50% inhibitory effect.
[b] SI: selectivity index = % IC_{50Hela}/% $IC_{50epimastigote}$.
[c] Not tested.

2.2.2. Antiparasitic activity

Four of the synthesized products were selected for a primary in vitro screening at Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland). Compounds **1**, **2**, **10** and **13** were tested in vitro against *Plasmodium falciparum*, *Leishmania donovani*, *Leishmania infantum* and *Trypanosoma b. rhodesiense*, causative agents of malaria, leishmaniasis and sleeping sickness, respectively, and their cytotoxicity was tested on rat L6 myoblasts. The activity data are given as μg/mL concentration that produces 50% inhibition (IC_{50}) in the assays used and are shown in Table 2.

Compound **1** presented selective and good activity against *T. b. rhodesiense*. The remaining compounds did not exhibit antiparasitic activity on the protozoa evaluated. In addition, compound **1** was evaluated against *Onchocerca gutturosa,* the causative agent of onchocercasis, and found to be inactive (Table 3).

2.2.3. Unspecific cytotoxicity

Cytotoxicity of the best trypanocidal compounds against mammalian cells was evaluated in vitro at 4, 20, and 100 μM, using cultured human cervix adenocarcinoma (HeLa) cells. Nfx was included as trypanocidal reference. We found that none of the compounds evaluated was cytotoxic at any of the concentrations assayed, and that derivative **7** was slightly toxic (Table 1).

Table 2
Antiprotozoal activity of compounds **1**, **2**, **10** and **13** (IC_{50} values are given in μg/mL)[a]

Compound	*P. falciparum*	*L. infantum*	*T. b rhodesiense*	Cytotoxicity L-6
Ref. drugs	Chloroquina 0.026	Pentostam 2.4	Suramin 0.13	Tamoxifen 4.9
1	>10	>20.7	1.20	>100
2	>22.7	>22.7	>22.7	>22.8
10	>19.5	>19.5	>19.5	>19.6
13	>19.5	>19.5	>22.7	>22.8

[a] IC_{50} = 50% growth inhibition.

Table 3
Antiprotozoal activity (*Onchocerca gutturosa*) of compound **1**

Compound	Mot Redn[a] (%)	MTT Redn[b] (%)	Activity score
Amocarzine (positive control)	85.71	48.57	2
1	14.29	0.00	1

[a] Motility score (mean % reduction at 120 h).
[b] MTT colorimetry (mean inhibition of formazan formation).

2.3. Study of anti-*T. cruzi* mechanism of action

2.3.1. DNA binding properties

To gain insight into the trypanocidal mechanism of action of these compounds, DNA affinity was evaluated.[12]

All the final products were tested for their ability to bind DNA. The binding capacity of these compounds was evaluated by measuring the hypochromic and bathochromic effects of their absorbance in the UV spectra.[13] The standard experiment was enhanced by means of a slow rotation of DNA-drug mixture stirring, in a 5:1 ratio for 24 h. The procedure was validated by repeating assays with well-known intercalating agents (m-AMSA and mitoxantrone) and a compound which binds closely in the minor groove (bis-benzimide, Hoechst No. 33258). The degree of interaction was expressed by the ratio between the final absorbance area after 24 h (a_{24}) and that of the compound at the same concentration (a_0), centred at maximal absorbance. Values of 1 indicated a total lack of affinity and value of 0 showed that the whole compound was bound to DNA. The a_{24}/a_0 coefficient values of compounds **1–13** are between 0.80 and 0.95. The DNA binding assay showed that the compounds tested are poor DNA ligands with low affinity, thus suggesting that DNA interaction is probably not the mechanism of action of the imidazoisoquinolinone derivatives.

2.3.2. Reactivity with glutathione

In order to confirm the capacity of the new imidazoisoquinolinones to react with thiols in the reductive metabolism of the parasite, Glutathione (GSH) was used as a model assay.[14] The reaction between compound **2** and GSH was followed spectroscopically in the UV–visible region. The results are shown in Figure 1. Clearly, it was confirmed that the thioamide derivative **2** reacted with this biological thiol. This compound was able to react with GSH at 28 °C and after 2 min of incubation showed hypochromic effect. After 2 h of incubation, it exhibited bathochromic and hyperchromic effects. Taking into account the UV-signal shifts, the GSH reacted mainly via a redox pathway, producing Glutathione disulfide (GSSG) and the corresponding reduced derivative from compound **2,** 10-((4-

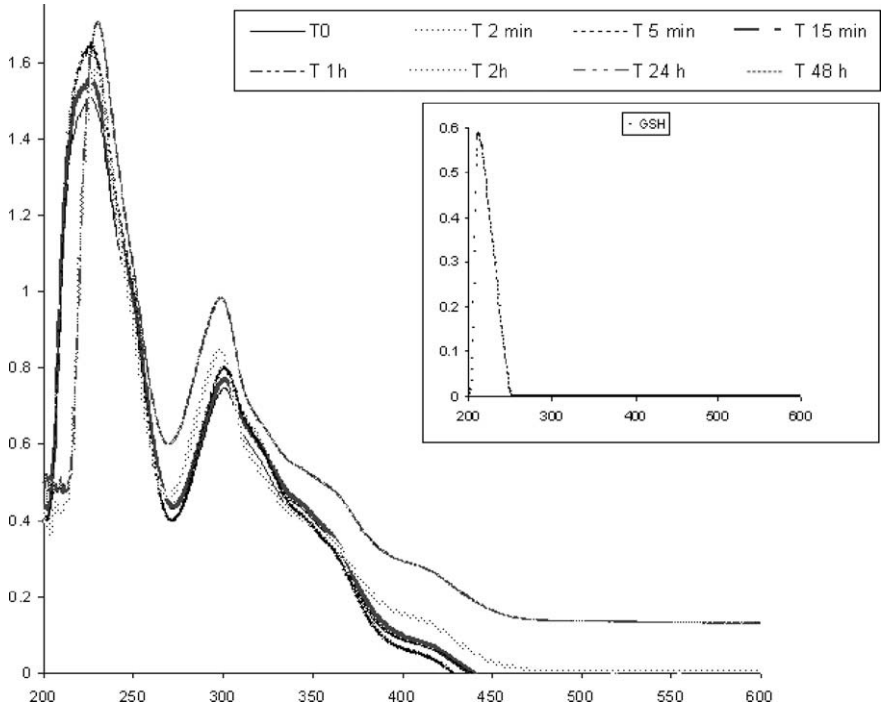


Figure 1. Studies of the reaction between compound **2** and GSH using UV–vis spectroscopy.

Chlorophenylamino)methyl)-2,3-dihydroimidazo[1,2-*b*]isoquinolin-5(1*H*)-one (**2a**).

2.4. Structure–activity relationship

Molecular modelling studies were performed on the developed imidazoisoquinolinones derivatives by calculating the stereoelectronic properties in order to understand the mechanism of action. These properties were determined using DFT/B3LYP calculations.[15,16] A detailed conformational search for each of the molecules was performed, using MM methods, to find the minimum energy and highest abundance conformer. The geometry of this conformer was fully optimized by applying B3LYP/6-31G*, and density functional calculation was performed in the gas phase. The properties determined and examined in this study were volume, total energy, solvation (water), heat formation energy, magnitude of dipolar moment, HOMO's and LUMO's energies, gap (E_{LUMO}–E_{HOMO}) and the logarithm of the partition coefficient of the non-ionized molecules (log*P*). Theoretical log*P* (*c*log*P*) was calculated using the Villar method, implemented in PC SPARTAŃ 02 package at the B3LYP/6-31G* density functional. In the equations and models, *n* represents the number of data points, r^2 the correlation coefficient, *s* the standard deviation of the regression equation, the *F* value is related to the *F*-statistic analysis (Fischer test) and r^2 adj defines the cross-validated correlation coefficient. The activity used in the structure–activity relationship studies was the inhibitory effect (compounds **1**–**13**) on the growth of *T. cruzi* (Tulahuen strains) expressed as 50% growth inhibition on day 5 (% IC_{50}). We used $\log_{10}$ (IC_{50}) values as the dependent variables in the linearization procedure. First, one-variable and multivariable regressions between these activities and the physicochemical properties (calculated descriptors) were studied. Only structure–activity models having a value of r^2 adj above 0.5 were considered. The models are shown in Table 4 and indicate that Eq. 2 is the best one-variable relationship obtained where activity is correlated with heat formation (HF).[17] This result could be modestly improved by including a lipophilic descriptor in the analysis (Eq. 3), however, this showed that the *c*log*P* term did not lead to significant increase in the r^2 correlation coefficient. The best equation was obtained when we analysed the correlation between activity, the independent heat formation (HF) the magnitude of dipolar moment μ (Table 4, Eq. 4). Besides, the correlation matrix for the physicochemical descriptors used was performed and no cross-relations between the descriptors used in each equation were obtained.[18] These parameters are therefore orthogonal, a fact that affords their use in the multilinear regression procedure.[19]

3. Discussion

New imidazoisoquinolinone derivatives were synthesized by a simple methodology and in good yields. Compound **12** was synthesized under microwave irradiation and was obtained in higher

Table 4
QSAR study

Eq	Statistic parameter						Expression
	r^2 adj	r^2	*s*	*p*	*F* value	*n*	
1	0.680	0.655	0.4120	0.002	79.72	11	$Log(1/IC_{50}) = 2.26\ (\pm0.32) - 0.38(\pm0.09)\mu$
2	0.801	0.849	0.2873	0.000001	44.98	11	$Log(1/IC_{50}) = 1.44(\pm0.11) - 0.19(\pm0.02)HF$
3	0.771	0.8627	0.2928	0.0001	22.00	11	$Log(1/IC_{50}) = 1.75\ (\pm0.38) - 0.17(\pm0.02)HF - 0.10\ (\pm0.12)\log P$
4	0.899	0.921	0.2212	0.00001	120.34	11	$Log(1/IC_{50}) = 1.82\ (\pm0.17) - 0.14(\pm0.02)HF - 0.41\ (\pm0.05)\mu$

n, represents the number of data points; r^2, the correlation coefficient; *s*, the standard deviation of the regression equation; *F* value is related to the *F*-statistic analysis (Fischer test) and r^2 adj defines the cross-validated correlation coefficient.

Table 5
Assessment of Lipinski's rules and TPSA values for imidazoisoquinolinones derivatives

Compound	Lipinski rules					TPSA[b,c]
	clog P[a]	No. of H-bond donor[b]	No. of H-bond acceptors[a]	Mol. Wt.	No. of criteria met	
Regla	<5	⩽5	⩽10	<500	At least 3	
2	3.77	2	5	355.9	All	50.6
3	3.44	2	5	335.4	All	50.6
6	3.13	2	5	321.4	All	50.6
7	2.89	2	6	351.4	All	64.7
13	4.11	2	4	369.4	All	50.6
Nfx	8.51	0	8	287.0	3	108.7

Nfx: nifurtimox.
[a] Theorical Log*P* (*clog P*) was calculated using Villar method, at AM1 semiempirical. method, implemented in Spartań02, 1.0.1 version, suite of programs.
[b] http://www.molinspiration.com/cgi-bin/properties.
[c] TPSA, topological polar surface area.

yield (85%) and a cleaner reaction than conventional heating. In addition, the reaction time was reduced from 4 h to 3 min.

The results indicate that the in vitro activity of the imidazoisoquinolinones studied against *T. cruzi* epimastigotes is superior, in most cases, to Nfx, the drug used for treatment of this disease. In particular, derivatives **2, 3**, **6**, **7** and **13** displayed low micromolar IC_{50} values (Table 1), with an adequate selective index.

In order to establish the key structural scaffold for the activity of these compounds, we selected compound **6** and carried out a variation of the individual substituents in this lead structure**.** The structure–activity relationship (SAR) studies indicated that functionality at position 4 of the phenyl group was not essential for good activity. However, the 4-chloro (**2**) and 4-methyl (**3**) group yielded the most potent compounds in this series. Replacement of the 4-methyl group with a 4-methoxy group resulted in a decrease in the inhibitory activity. Introduction of a methyl substituent at position 3 of the phenyl group (**5**) resulted in a significant decrease in potency. Interestingly, acylation of N-1 in compound **11** completely abolished the activity. This result suggests that NH should be free for binding through a hydrogen bond to a target receptor.

In addition, only the change of sulfur atom (**6**) by oxygen atom (**10**) abolished the antichagasic activity.

Furthermore, the insertion of a methylene group (**9**) resulted in a significant decrease in potency. No further improvement was observed when the phenyl group was replaced by a pyridine or naphthyl group. This result shows that phenylthioamide moiety is essential for antichagasic activity.

When we included a benzene ring at position 2, 3 in compound **6** the activity was decreased 2-fold; however, derivative **13** exhibited better activity than Nifurtimox.

QSAR studies demonstrated the relevance of the dipolar moment module and the heat formation for trypanocidal activity on the Tulahuen strain. The relationship between activity and dipolar moment could indicate the electronic requirements of the derivatives to have optimal interaction with target biomolecules. In this study, it was observed that the activity of the compounds increases as heat formation increases.

From the DNA binding study, it is possible to conclude that the imidazoisoquinolinone derivatives are not an appropriate ligand (in structure and function) for interaction with DNA. On the other hand, the reactivity with the glutathione assay showed that compound **2** can react with GSH in a redox process.

It is well-known that GSH is one of the essential precursors of Trypanothione ($T(SH)_2$) synthesis in the parasite cells. In addition, *T. cruzi* has much lower GSH levels than those of the mammalian host. Therefore, in parasites, the depletion of GSH levels and consequently of $T(SH)_2$ levels, could be more dangerous than the GSH decrease in mammals. While in mammals GSH synthesis can be inhibited up to 80–90% without evidence of toxicity, in *T. cruzi* this situation aggravates the precarious defence against oxidative stress.[20] Accordingly, in mammalian cells, the effects of derivative **2** could be precluded by the enzyme Glutathione reductase (GR) maintaining GSH levels. On the contrary, in the parasitic cells, compound **2** may decrease GSH levels irreversibly because TR is unable to reduce GSSG effectively, although another possibility is that GSSG might deplete $T(SH)_2$, Tryparedoxin (Tpx), and/or TR as a result of chemical[21] or biochemical pathways.[22,23]

On the other hand, if we consider the potential use of imidazoisoquinolinone derivatives as drugs, an adequate in vivo behaviour according to Lipinski's rules[24] and their topological polar surface area (TPSA) should be present.[25] Lipinski has described the desired ranges for certain properties thought to be important for drug bioavailability and absorption. These are: *clog P* <5, number of hydrogen bond donor ⩽5, number of hydrogen bond acceptor ⩽5 and molecular weight <500. A compound that fulfils at least three out of the four criteria is said to adhere to Lipinskís 'rule of 5'. Besides, TPSA is correlated with various types of drug transport properties including intestinal absorption, blood-brain barrier penetration, and Caco-2 cell permeability. Table 5 lists such physicochemical properties for the trypanocidal imidazoisoquinolinone derivatives. All of the potent antiparasitic imidazoisoquinolinones presented herein are fully compatible with Lipinskís rule and possess better TPSA than Nifurtimox.

4. Conclusion

The new imidazoisoquinolinone derivatives developed in this study are in vitro active against *T. cruzi* epimastigotes. In general, the toxic effects of the parasite are not associated with mammal cytotoxicity in HeLa cells. Derivative **2**, with a 4-chlorophenylthioamide substituent, possesses the best activity (IC_{50} 0.5 μM). These results provide supporting evidence to stimulate further in vivo studies of these compounds in appropriate animal models of Chagas' disease.

5. Experimental

5.1. Chemistry

Melting points were determined in a capillary with an Electrothermal 9100 SERIES-Digital apparatus and are uncorrected. IR spectra were recorded with a FT Perkin Elmer Spectrum One from KBr discs. UV spectra were measured with a Jasco V-570 UV/vis/NIR spectrophotometer. ^{1}H NMR (300 MHz) spectra were obtained with a Bruker spectrometer at room temperature with tetramethylsilane as internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hertz. Elemental analysis was carried out in our laboratories with a Coleman Analyser.

Microwave-assisted reaction carried out in a household MW oven (BGH-QUICK chef 15240). The apparatus was modified for laboratory application with an external reflux condenser.

5.1.1. General procedure for the preparation of 1, 3-diarylthioureas

A mixture of corresponding aniline (25 mmol), CS_2 (2.4 mL, 40 mmol) and DMAP (0.3 g, 2.5 mmol) in ethanol absolute was heated at reflux for 18 h. Then the mixture was allowed to cool to room temperature and the product was collected and washed with ethanol. Physical properties of the product obtained were according to literature data.[26]

5.1.2. General procedure for the preparation of aryl, naphthyl, pyrimidylisothiocyanates

The corresponding 1,3-diarylthioureas (2.4 g, 8 mmol) was dissolved in anhydride acetic (5 mL) and was heated at reflux for 1 h. The product was isolated by steam distillation, the organic layer was separated, dried ($MgSO_4$), filtered and purified by simple distillation. Physical properties of the product obtained were according to literature data.[9]

5.1.3. General procedure for the synthesis of compounds 2–9 and 13

A mixture of compound **1**[6] (0.5 g, 2.7 mmol), the corresponding isothiocyanate (2.7 mmol), DMAP (0.6 g, 4.9 mmol) and CH_2Cl_2 (10 mL) was stirred at reflux for 6–24 h. Then the mixture was allowed to room temperature and the crystalline solid was collected, washed with ethanol and dried in vacuum.

5.1.4. *N*-(4-Chlorophenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1, 2-*b*]isoquinolin-10-carbothioamide (2)

Reaction time: 6 h, yield: 0.52 g, 55%, mp 207–209 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.74 (s, 1H, N*H* (thioamide)), 8.02 (d, 1H, H-6, J = 7.6 Hz), 7.86 (s, 1H, NH, exchangeable with D_2O), 7.43–7.50 (m, 6H, H-Ar), 7.19–7.30 (m, 1H, H-Ar), 4.17 (t, 2H, H-3, J = 8.8 Hz), 3.98 (t, 2H, H-2, J = 8.7 Hz). IR ν (KBr): 3274 (N–H thioamide), 3240 (N–H), 1699 (C=O), 1548 (N–CO), 1520, 1297 and 1080 (C=S), 886 and 767 (C–H Ar) cm^{-1}. Anal. Calcd for $C_{18}H_{14}ClN_3OS$: C, 60.76; H, 3.97; N, 11.81. Found: C, 60.41; H, 3.59; N, 11.96.

5.1.5. *N*-(4-Methylphenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1, 2-*b*]isoquinolin-10-carbothioamide (3)

Reaction time: 6 h, yield: 0.46 g, 51%, mp 210–212 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.64 (s, 1H, N*H* (thioamide)), 8.03 (d, 1H, H-6, J = 6.0 Hz), 7.74 (s, 1H, NH, exchangeable with D_2O), 7.08–7.28 (m, 7H, H-Ar), 4.17 (t, 2H, H-3, J = 8.4 Hz), 3.66 (t, 2H, H-2 J = 8.3 Hz), 2.32 (s, 3H, CH_3). IR ν (KBr): 3370 (N–H thioamide), 3240 (N–H), 1701 (C=O), 1568 (N–CO), 1519, 1297 and 1044 (C=S), 882 and 768 (C–H Ar) cm^{-1}. Anal. Calcd for $C_{19}H_{17}N_3OS$: C, 68.03; H, 5.11; N, 12.53. Found: C, 68.42; H, 4.71; N, 12.13.

5.1.6. *N*-(1-Naphtalenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1, 2-*b*]isoquinolin-10-carbothioamide (4)

Reaction time: 3 h, yield: 0.49 g, 49%, mp: 189–191 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.29 (s, 1H, N*H* thioamide), 8.12 (d, 1H, H-6, J = 8.2 Hz), 7.89–7.99 (m, 3H, H-Ar), 7.64 (s, 1H, NH, exchangeable with D_2O), 7.52–7.59 (m, 5H, H-Ar), 7.43 (dd, 1H, H-Ar, J = 7.3 Hz, J = 1.2 Hz), 7.35 (dt, 1H, H-Ar, J = 6.4 Hz, J = 1.4 Hz), 4.52 (t, 2H, H-3, J = 8.2 Hz), 4.20 (t, 2H, H-2, J = 8.5 Hz). IR ν (KBr): 3372 (N–H thioamide), 3229 (N–H), 1700 (C=O), 1599 (N–CO), 1519, 1297 and 1044 (C=S), 882, 768 (C–H Ar) cm^{-1}. Anal. Calcd for $C_{22}H_{17}N_3OS$: C, 71.14; H, 4.61; N, 11.31. Found: C, 70.88; H, 5.01; N, 11.11.

5.1.7. *N*-(3-Methylphenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1, 2-*b*]isoquinolin-10-carbothioamide (5)

Reaction time: 5 h, yield: 0.65 g, 72%, mp: 246–248 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.45 (s, 1H, N*H* (thioamide)), 8.03 (d, 1H, H-6, J = 6.0 Hz), 7.60 (s, 1H, NH, exchangeable with D_2O), 7.48–7.54 (m, 2H, H-Ar), 7.08–7.28 (m, 5H, H-Ar), 4.22 (t, 2H, H-3, J = 8.8 Hz), 3.89 (t, 2H, H-2, J = 8.8 Hz), 2.32 (s, 3H, CH_3). IR ν (KBr): 3377 (N–H thioamide), 3240 (N–H), 1698 (C=O), 1560 (N–CO), 1540, 1299 and 1044 (C=S), 890, 805 and 765 (C–H Ar) cm^{-1}. Anal. Calcd for $C_{19}H_{17}N_3OS$: C, 68.03; H, 5.11; N, 12.53. Found: C, 68.29; H, 5.31; N, 12.33.

5.1.8. *N*-Phenyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*b*] isoquinolin-10-carbothioamide (6)

Reaction time: 3 h, yield: 0.39 g, 45%, mp: 228–230 °C (mp lit.[27] 221–222 °C). ^{1}H NMR (DMSO-d_6) δ (ppm): 11.75 (s, 1H, N*H* (thioamide)), 8.02 (d, 1H, H-6, J = 7.7 Hz), 7.89 (s, 1H, NH), 7.39–7.5 (m, 7 H, H-Ar), 7.08–7.13 (m, 1H, H-Ar), 4.14 (t, 2H, H-3, J = 8.5 Hz), 3.66 (t, 2H, H-3, J = 8.5 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 210.5 (C=S), 159.1 (C=O), 149.2 (C-10a), 144.0 (C-Ar), 133.3 (C-Ar), 132.4 (C-Ar), 128.4 (C-Ar), 126.7 (C-Ar), 122.9 (C-Ar), 121.0 (C-Ar), 119.7 (C-Ar), 110.6 (C-10), 50.0 (C-3), 43.9 (C-2). IR ν (KBr): 3375 (N–H thioamide), 3219 (N–H), 1644 (C=O), 1568 (N–CO), 1519, 1293 and 1086 (C=S), 760 and 668 (C–H Ar) cm^{-1}. m/z (%): 322.05 ([M+1]$^+$, 9.96), 321.05 (M$^+$, 47.58), 288.10 (M$^+$-SH, 100), 211.15 (M$^+$−33−C_6H_5, 17.62), 186.15 (M$^+$−CSNHC$_6$H$_5$, 71.77), 135 (C_6H_5NHCS, 73.22), 77.04 ([C_6H_5]$^+$, 74.90). Anal. Calcd for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07. Found: C, 67.81; H, 5.05l; N, 13.44.

5.1.9. *N*-(4-Methoxyphenyl)-5-oxo-1,2,3,5-tetrahydroimidazo [1,2-*b*]isoquinolin-10-carbothioamide (7)

Reaction time: 9 h, yield: 0.57 g, 60%, mp: 182–184 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.60 (s, 1H, N*H* (thioamide)), 8.03 (d, 1H, H-6, J = 6.0 Hz), 7.80 (s, 1H, NH, exchangeable with D_2O), 7.08–7.28 (m, 7H, H-Ar), 4.24 (t, 2H, H-3, J = 8.4 Hz), 3.60 (t, 2H, H-2, J = 8.2 Hz), 3.45 (s, 3H, OCH_3). Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96. Found: C, 65.12; H, 5.16; N, 11.74.

5.1.10. 5-Oxo-*N*-(2-pyridinyl)-1,2,3,5-tetrahydroimidazo[1, 2-*b*]isoquinolin-10-carbothioamide (8)

Reaction time: 4 h, yield: 0.28 g, 32%, mp: 209–210 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.60 (s, 1H, N*H* (thioamide), 8.43 (d, 1H, α-H-pyridin, J = 5.7 Hz), 8.07 (d, 1H, H-6, J = 7.1 Hz), 7.82 (s, 1H, NH, exchangeable with D_2O), 7.20–7.38 (m, 2H, H-Ar), 7.08–7.15 (m, 4H, H-Ar), 4.12 (t, 2H, H-3, J = 8.9 Hz), 3.90 (t, 2H, H-2, J = 8.8 Hz). Anal. Calcd for $C_{17}H_{14}N_4OS$: C, 63.33; H, 4.38; N, 17.38. Found: C, 63.47; H, 3.99; N, 17.19.

5.1.11. *N*-(4-Chlorobenzyl)-5-oxo-1,2,3,5-tetrahydroimidazo [1,2-*b*]isoquinolin-10-carbothioamide (9)

Reaction time: 4 h, yield: 0.29 g, 29%, mp: 267–269 °C (d). ^{1}H NMR (DMSO-d_6) δ (ppm): 11.20 (s, 1H, N*H* (thioamide)), 8.04 (d, 1H, H-6, J = 6.9 Hz), 7.59–7.68 (m, 4H, H-Ar), 7.20–7.38 (m, 3H, H-Ar), 7.02 (s, 1H, NH, exchangeable with D_2O), 4.15 (t, 2H, H-3, J = 9.1 Hz), 3.91 (s, 2H, CH_2), 3.88 (t, 2H, H-2 J = 9.0 Hz). Anal. Calcd for $C_{19}H_{16}ClN_3OS$: C, 61.70; H, 4.36; N, 11.36. Found: C, 61.35; H, 4.18; N, 11.55.

5.1.12. 5-Oxo-*N*-phenyl-1,2,3,5-tetrahydroimidazo[1,2-*b*] isoquinolin-10-carboxamide (10)

To a stirred solution of compound **6** (0.15 g, 0.46 mmol) and KOH (2%) (1.5 mL), in ethanol (3 mL), 0.11 mL H_2O_2 (30%) was slowly added. The temperature was maintained at 0 °C for 15 min. and then warmed to room temperature for 30 min. The yellow crystalline solid was collected, washed with benzene and dried in vacuum. Yield 63 mg, 45%, mp >320 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.1 (s, 1H, N*H* (amide)), 8.0 (d, 1H, H-6, J = 8.2 Hz), 7.69 (d, 2H, H-Ar, J = 7.7 Hz), 7.47 (s, 1H, NH, exchangeable with

D_2O), 7.28 (t, 1H, H-Ar, J = 7.9 Hz), 6.9–7.07 (m, 5H, H-Ar), 4.09 (t, 2H, H-3, J = 8.4 Hz), 3.70 (t, 2H, H-2, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 162.3 (C=O), 159.1 (C=O), 150.0 (C-10a), 140.8 (C-Ar), 133.1 (C-Ar), 131.1 (C-Ar), 128.9 (C-Ar), 127.0 (C-Ar), 122.5 (C-Ar), 119.7 (C-Ar), 118.0 (C-Ar), 111.0 (C-10), 51.4 (C-3), 44.1 (C-2). IR ν (KBr): 3393 (N–H), 1658 (C=O), 1616 (C=O), 1598 (N–CO), 756 and 617 (C–H Ar) cm^{-1}. EM m/z (%): 306.10 ([M+1]$^+$, 10.61), 305.00 (M$^+$, 31.60), 212.45 (M$^+$-$NH_2C_6H_5$, 100), 186.10 (M$^+$-$CONHC_6H_5$, 31.89), 93.10 ($C_6H_5NH_2^+$, 30.96), 77.05 ([C_6H_5]$^+$, 20.51). Anal. Calcd for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.44; H, 4.73; N, 14.00.

5.1.13. 1-Acetyl-5-oxo-*N*-phenyl-1,2,3,5-tetrahydroimidazo[1,2-*b*]isoquinolin-10-carbothioamide (11)

A mixture of compound **6** (0.15 g, 0.46 mmol), DMAP (56 mg, 0.46 mmol) and CH_2Cl_2 as a solvent was stirred. After 5 min, the acetyl chloride (0.15 mL, 3.5 mmol) was added over a period of several minutes and the mixture was stirred at 0–5 °C for 2 h. The organic layer was washed with H_2O (20 mL) and then with K_2CO_3 (10%) (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated under pressure, the residue was recrystallized with ethanol. Yield 0.23 g, 56%, mp 94–96 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 9.98 (s, 1H, N*H* (amide)), 8.51 (dd, 1H, H-6, J = 7.9 Hz, J = 1.3 Hz), 8.03 (dt, 1H, H-Ar, J = 7.2 Hz, J = 1.3 Hz), 7.83–7.92 (m, 1H, H-Ar), 7.63–7.71 (m, 4H, H-Ar), 7.40 (d, 2H, H-Ar, J = 7.2 Hz), 4.93 (m, 2H, H-3), 4.68 (m, 2H, H-2), 2.60 (s, 3H, C*H*$_3$). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.71; N, 11.76. Found: C, 66.18; H, 4.58; N, 11.22.

5.1.14. Benzo [4,5] imidazo [1,2-*b*]isoquinolin-5(1*H*)-11-one (12)

A mixture of homophthalic acid (5 g, 24 mmol), *o*-phenylendiamine (2.5 g, 24 mmol), and 0.1 mL of DMF was introduced into bottom flask and subjected to microwave irradiation at 480 W for 10 min. The reaction was monitored by thin layer chromatography. After complete conversion the reaction mixture the product was recrystallized with ethanol to yield **2** (5.96 g, 85%) mp >330 °C (mp lit.:[28] 324–326 °C). ^{1}H NMR (DMSO-d_6) δ (ppm): 11.87 (s, 1H, N–H, exchangeable with D_2O), 8.62 (d, 1H, H-10, J = 7.4 Hz), 8.26 (dt, 1H, H-Ar, J = 7.4 Hz, J = 1.2 Hz, J = 0.5 Hz), 7.58–7.62 (m, 2H, H-Ar), 7.32–7.42 (m, 2H, H-Ar), 7.16–7.25 (m, 2H, H-Ar), 6.35 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6) δ (ppm): 159.03 (C-11), 141.43 (C-5), 138.69 (C-Ar), 133.19 (C-Ar), 131.9 (C-Ar), 127.89 (C-Ar), 126.9 (C-Ar), 126.05 (C-Ar), 124.74 (C-Ar), 121.43 (C-Ar), 120.03 (C-Ar), 117.42 (C-Ar), 115.79(C-Ar), 109.22 (C-Ar), 78.61 (C-6). IR ν (KBr): 3135 (N–H), 1679 (C=O), 1512 (N–CO), 780 and 680 cm^{-1}. Anal. Calcd for $C_{15}H_{10}N_2O$: C, 76.91; H, 4.30; N, 11.96. Found: C, 76.56; H, 4.69; N, 12.07.

5.1.15. *N*-Phenyl-11-oxo-benzo[4,5]imidazo[1,2-*b*]isoquinolin-5(1*H*)-6-carbothioamide (13)

Reaction time: 6 h, yield: 0.59 g, 60%, mp >300 °C. ^{1}H NMR ($CDCl_3$) (ppm): 11.80 (s, 1H, NH thioamide), 8.60 (d, 1H, H-10, J = 7.9 Hz), 8.18–8.28 (m, 5H, H-Ar), 7.59–7.73 (m, 3H, H-Ar), 7.32–7.39 (m, 2H, H-Ar), 7.19–7.25 (m, 3H, 2H-Ar, NH, exchangeable with D_2O). IR ν (KBr): 3375 (N–H thioamide), 3258 (N–H), 1699 (C=O), 1559 (N–CO), 1520, 1213 and 1080 (C=S), 890, 760 and 668 (C–H Ar) cm^{-1}. Anal. Calcd for $C_{22}H_{15}N_3OS$: C, 71.52; H, 4.09; N, 11.37. Found: C, 71.49; H, 3.78; N, 11.41.

5.2. In vitro anti-*T. cruzi* activity (Epimastigote Form)

Trypanosoma cruzi epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-tryptose) complemented with 5% fetal calf serum. Cells from 5-day-old culture (stationary phase) were inoculated to 50 mL of fresh culture medium to give an initial concentration of 1 × 10^6 cells/mL. Cell growth was followed by measuring the absorbance of the culture at 600 nm every day. Before inoculation, the medium was supplemented with the indicated amount of the studied compound from a stock solution in DMSO. The final concentration of DMSO in the culture medium never exceeded 0.4%, and the control was run in the presence of 0.4% DMSO and in the absence of compound. No effect on epimatigotes growth was observed by the presence of up to 1% DMSO in the culture medium. The percentage of inhibition was calculated as follows: $I\% = \{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\} \times 100$, where $A_p = A_{600}$ of the culture containing the drug at day 5; $A_{0p} = A_{600}$ of the culture containing the compound just after the addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any compound (control) at day 5; $A_{0c} = A_{600}$ in the absence of the compound at day 0. To determine IC_{50} values, 50% inhibitory concentrations, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. At day 5, the absorbance of the culture was measured and related to the control. The IC_{50} value was taken as the concentration of compound needed to reduce the absorbance ratio to 50%.

5.3. Antiparasitic activity[29]

The evaluation of antiparasitic[29] activity was performed at Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland)[1], following the well-known in vitro disease-oriented primary antiparasitc screening against *P. falciparum*, *L. donovani*, *L. infantum*, *T. b. rhodesiense*, and *O. gutturosa*. For antimalarial activity, K1 strain was used. If the IC_{50} is >5 μg/mL, the compound is classified as inactive. If the IC_{50} is 0.5–5 μg/mL, the compound is classified as moderately active. If the IC_{50} is <0.5 μg/mL, the compound is classified as active. For sleeping sickness, if the IC_{50} is >16 μg/mL, the compound is classified as inactive. If the IC_{50} is between 1 and 16 μg/mL, the compound is classified as moderately active. If the IC_{50} is <1 μg/mL, the compound is classified as active. For Leishmania activity, if the IC_{50} is >16 μg/mL, the compound is classified as inactive. If the IC_{50} is between 1 and 16 μg/mL, the compound is classified as moderately active. If the IC_{50} is <1 μg/mL, the compound is classified as active. For *O. gutturosa* a test compound is considered active if there is 50% or greater reduction in motility score and/or 50% or greater inhibition of formazan formation compared to untreated controls. Compounds are classified as moderately active if there is a 50–75% reduction in motility and/or inhibition of formazan, or highly active at 76–100%.

5.4. Reactivity with glutathione[14]

Solution A: GSH (15.6 mM) in phosphate buffer (0.1 M Na_2HPO_4, 1.5 mM EDTA, pH 7.4). Solution B: studied compound **2** (3 mM) in anhydrous MeOH. The reaction was started by mixing solution A (500 μL) (GSH final concentration 1 mM) with solution B (500 μL) (studied compound final concentration 500 μM) in phosphate buffer (1000 μL). The reaction mixture was maintained at 28 °C. At variable times (0, 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h and 6 h) the reactions were quenched by cooling, at 0 °C, and the UV–vis spectra were acquired between 200 and 600 nm. As negative controls, GSH and each compound were incubated at 28 °C in phosphate buffer.

Compound 10-((4-Chlorophenylamino)methyl)-2,3-dihydroimidazo[1,2-*b*]isoquinolin-5(1*H*)-one (**2a**) was isolated from the reaction mixture after 6 h and purified by column chromatography (SiO_2, petroleum ether/EtOAc (7:3)). ^{1}H NMR (DMSO-d_6) δ (ppm): 8.02 (dd, 1H, H-Ar, J = 7.92, 0.93 *Hz*), 7.59–7.62 (dd, 2H, H-Ar, J = 6.75 *Hz*), 7.45–7.47 (m, 1H, H-Ar), 7.31–7.28 (m, 2H, H-Ar), 7.03–7.05 (m, 1H, H-Ar), 6.98 (s, 1H, N–H, exchangeable with D_2O), 5.20 (s, 1H, NH, exchangeable with D_2O), 4.11 (t, 2H, H-3, J = 8.3 Hz), 3.62 (m, 4H, H-2, CH_2). Anal. Calcd for $C_{18}H_{16}ClN_3O$: C, 66.36; H, 4.95; N, 12.90. Found: C, 66.21; H, 5.01; N, 12.85.

5.5. DNA affinity assay

DNA solution: Calf thymus DNA (12.5 mg) was slowly magnetically stirred in Tris–HCl buffer 10 mM, pH 7.4 (5 mL), for 24 h at 4 °C. 0.6 mL was taken from this solution and diluted to 25 mL with the same buffer.

The test compound solution was prepared at 10^{-4} M concentration using a minimal volume of ethanol and then diluted adding water to a concentration of 2×10^{-5} M. 3 mL sample of this solution was mixed with 3 mL of the DNA solution. The mixture was slowly rotated during 24 h and, then, its UV spectra were recorded at 20 °C using a 1 cm cell.

5.6. Cytotoxic activity

The HeLa cells were cultured under standard culture conditions at 37 °C and 5% CO_2 atmosphere in a humidified incubator in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 UI/mL penicillin and 100 mg/L streptomycin. Cells 9×10^{-5} cell/mL were seeded in 96 multiwell plates (Falcon) and after 48 h different concentrations of compound **2** (4, 20 and 100 μM) were added. After 24 h of incubation, cells were washed twice with PBS and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) from a stock solution (5 mg/mL) was added at a final concentration of 0.5 mg/mL. Plates were incubated for 4 h at 37 °C. Finally, blue precipitates were dissolved in 0.1 mL isopropanol/HCl (300:1) and cells were incubated 1 h a 37 °C. Samples were read on a plate reader (Spectra CountTM BS 10001) at 570 nm. The values of absorbance showed a good correlation with viable cell counts using trypan blue. Values from blank plates containing only medium and reagents were subtracted from the values of the samples. Cell survival percentage (%SP) was expressed as percentage of the control cells. All MTT assays were repeated at least three times by using four samples per assay.

The evaluation of cytotoxicity activity with L-6 cells was performed at Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland).[1]

5.7. QSAR studies

Quantum-chemical semiempirical AM1 calculations were performed for the lowest energy conformation of the compounds using the Spartan'02 suite of programs. The compounds were built with standard bond lengths and angles using the Spartan'02 1.0.1 version, and the geometry of each molecule was fully optimized by applying the semiempirical AM1 method in gas phase from the most stable conformer obtained using molecular mechanics (MMFF) methods. Then, a single point calculation using DFT/6-31G* was used. Lipophilic properties of the compounds were included into the analyses, as clog*P* (log*P* calculated by Villar method, AM1).

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18. Squared correlation matrix of descriptors used in the QSAR study

μ	1		
Hf	0.04	1	
clogP	0.13	0.06	1
r^2	μ	Hf	clogP

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