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In vitro phenotypic screening of 7-chloro-4-amino(oxy)quinoline derivatives as putative anti-*Trypanosoma cruzi* agents

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ABSTRACT

In this study, a series of 22 pre-synthesized 7-chloro-4-amino(oxy)quinoline derivatives was assayed in vitro as potential antichagasic agents. A primary screening against *Trypanosoma cruzi* epimastigotes and a non-specific cytotoxicity assay on murine fibroblasts were simultaneously performed, resulting quinolines **3**, **7** and **12** with great selectivity (SI) on the extracellular parasite (SI_{7}, SI_{3}, SI_{12} and SI_{BZ} >9.44). Therefore, the activity of these derivatives was evaluated on intracellular amastigotes, achieving derivative **7** the best SI (SI = 12.73). These results, supported by the in silico prediction of a good oral bioavailability and a suitable risk profile, propose the 4-amino-7-chloroquinoline scaffold as a potential template for designing trypanocidal prototypes.

Although more than a century has elapsed since the discovery of Chagas disease (American trypanosomiasis), the etiological treatment of a neglected tropical disease that is currently endemic in poor rural areas of 21 countries along Central and South America is still unsatisfactory.[1] The parasitic illness caused by the hemoflagellate *Trypanosoma cruzi* and endemically transmitted by triatomine vectors, has in the last years emerged in non-endemic countries outside Latin America since the increase of international migrations and the existence of non-vectorial mechanisms of transmission (e.g., congenital route),[2,3] infecting this way about 10 million people worldwide.[1] Focusing on the chemotherapy, nifurtimox (a 5-nitrofuran) and benznidazole (a 2-nitroimidazole), the only two drugs up to now commercialized for the specific treatment of Chagas disease, were introduced more than forty years ago and their accessibility to patients has been discontinued through this time.[4] Although they are effective in the early stages of the trypanosomiasis, both display a limited activity during the chronic infection.[5] Moreover, the toxicity associated to these drugs[6] and the existence of *T. cruzi* strains naturally resistant to them,[7] also hinder the effectiveness of these treatments. The epidemiological data, together with the lack of either a suitable chemotherapy or a vaccine, supports the priority in the development of new prototypes of anti-*T. cruzi* agents.

In the present work, a first series of 16 different 7-chloroquinoline molecules substituted at the C-4 position by either benzylamino fragment or *N*-(aminoalkyl)-1,3-thiazolidin-4-one moiety, previously synthesized and tested against *Plasmodium falciparum*,[8] has been evaluated as potential antichagasic compounds according to the trypanocidal activity exhibited by some thiazolidine derivatives[9,10] and diverse aminoquinolines.[11,12] Concretely, the biological properties associated to the substituted 1,3-thiazolidin-4-ones have led to the introduction of this structure whether in several antimicrobial agents[13] as well as in trypanocidal prototypes with promising in vitro activity.[14,15] Nevertheless, data of neither 7-chloroquinoline-thiazolidinone derivatives (called chloroquine hybrids) nor other 4-arylaminomethylquinolines tested on *T. cruzi* models have been found in literature. Likewise, a second series of 4-aryloxy-7-chloroquinoline molecules[16] also assessed as antimalarial drug prototypes (data not published), has been in vitro evaluated as potential trypanocidal agents.

Both series of compounds were synthesized by employing straightforward and efficient procedures.[8,16] Benzylamino or aryloxy fragments were connected to the 4,7-chloroquinoline ring (DCQ) by nucleophilic substitution S_NAr using commercial benzylamines or substituted phenols to give compounds **1**–**4** and compounds **17**–**22**, respectively. Chloroquinoline–thiazolidinone hybrids **5**–**16** were assembled via a synthetic two-step protocol that includes the preparation of diamines based on DCQ ring through nucleophilic substitution of DCQ and α,ω-diaminoalkanes

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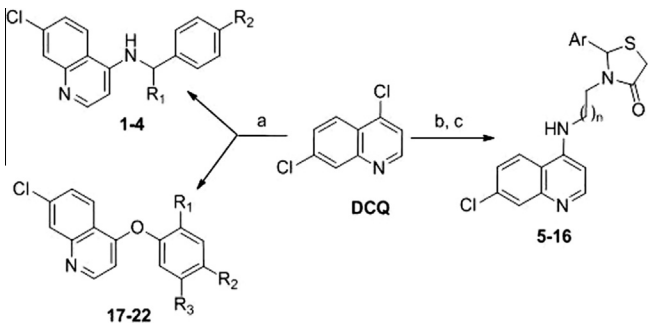
$NH_2(CH_2)_nCH_2NH_2$ (n = 1–3) and the one-pot three component reaction of these diamines, diverse (hetero)aromatic aldehydes and α-mercaptoacetic acid with ratios 1:2.5:2.5 respectively, in dry acetonitrile (reflux, 12 h) to get solid products, which can be filtered and recrystallized in ethanol from the reaction mixture (Scheme 1).

All 7-chloro-4-amino(oxy)quinoline derivatives **1**–**22** were purified by column chromatography and obtained as stable powdered substances, which were fully characterized and their chemical purity corroborated by the analysis of spectroscopic methods (i.e., IR, ^{1}H NMR, ^{13}C NMR and GC–MS), and agree with previous published data.[8,16]

The 22 synthesized quinolines have been distributed in two groups according to their chemical structure, mainly based on the chemical nature of the C-4 substituent attached to the quinoline nucleus. Group 1 includes the simple 4-*N*-benzylamino-7-chloroquinolines **1**–**4** and the 7-chloroquinoline-1, 3-thiazolidin-4-one conjugates **5**–**16** (Fig. 1). Group 2 includes 4-aryloxyquinoline derivatives (compounds **17**–**22**) (Fig. 2).

Molecular design was achieved based on structure–activity relationships (SAR) studies and virtual screening analysis reported in literature. The pre-screening for *hit* identification from libraries of synthetic compounds was based on the oral bioavailability estimated using the Lipinski's rules concepts,[17] through the analysis of the rule of five by employing the free online software Molinspiration (http://www.molinspiration.com/services/). The principal molecular properties, including the number of hydrogen donors (*n*NHOH), number of hydrogen acceptors (*n*NO), number of rotable bonds (*n*RB), molecular weight (MW) and lipophilicity (Log*P*) were calculated. The topological surface area (TPSA),[18] another recognized parameter for the membrane permeation and prerequisite for the bioavailability, was also considered (Table S1, Supplementary material).

According to this analysis, the vast majority of the quinoline derivatives did not present any violation for the oral activity and therefore, are expected to display high bioavailability. Only two 4-aryloxyquinolines (compounds **21** and **22**) violated Lipinski's rule exhibiting Log*P* values higher than 5.0. In fact, the Log*P* values estimated for compounds **1**–**20** (Log*P* 3.3–4.6) predict an auspicious entry into the parasitic cell by penetrating across biological membranes.[19,20] However, the antichagasic reference drugs (nifurtimox and benznidazole) are considerably more hydrophilic molecules exhibiting Log*P* below 1.0 value. Likewise, the TPSA parameters obtained show acceptable values (24.919–74.960 Å^2) (Table S1, Supplementary material), signifying TPSA values lower than 142 Å^2 a good membrane permeability and lower than 60 Å^2 a good penetration through the blood–brain barrier.[21]



Scheme 1. Reagents and conditions: (a) DCQ (4,7-dichloroquinoline) (2.5 mmol), N-benzylamine or phenols (5.10 mmol), and K_2CO_3 (5.01 mmol), DMF, 140 °C, 10 h; (b) DCQ (20.2 mmol), $NH_2(CH_2)_nCH_2NH_2$ (101 mmol), 80 °C for 1 h, 140–150 °C for 6–7 h; (c) diamines based on DCQ, ArCHO, $HSCH_2COOH$, PhMe, reflux for 1 h.

Regarding the trypanocidal activity of the 7-chloroquinoline derivatives, quinolines **3**, **7** and **12** proved to be the most active compounds over *T. cruzi* epimastigotes (Table 1). These results, together with the Log*P* values predicted, confirm the importance of an appropriate lipophilicity that allows these derivatives to access into the parasitic cell[19,22] and consequently, interact with the hydrophobic binding site of an enzyme or receptor.[23] Conversely, the presence of 4-hydroxy-3-methoxyphenyl (compounds **13**–**15**) or 3-hydroxy-4-methoxyphenyl radicals (compound **16**) on the thiazolidinone-based hybrids, seems to decrease these Log*P* values below the estimated average (4.095) and to significantly modify the activity of these derivatives on the extracellular parasite when compared with the unsubstituted phenyl ones (compounds **11** and **12**). However, the *para* introduction of hydroxyl groups on the phenyl ring (compounds **13**–**15**) not only turns this series into the less active compounds from the first group,[24] but also into the less harmful for fibroblasts. Otherwise, such an effect was not observed when the phenolic hydroxyl group is introduced in *meta* position (compound **16**) (Table 1). Details of the growth inhibition assay on *T. cruzi* epimastigotes are described in the Supplementary material.

Since the severe side effects frequently suffered by treated Chagas patients[25,26] often lead to a lack of treatment adherence, indirectly encouraging the development of *T. cruzi* resistance towards nifurtimox and benznidazole,[27] other molecular descriptors pointing to the presence of structural fragments generally responsible for mutagenic, tumorigenic, irritant or reproductive effects[28] were also evaluated. The toxicity risk profile assessment was performed employing the OSIRIS free software (http://www.organic-chemistry.org/prog/peo)[29] and prediction results were valued and color-coded, showing those properties with high risks of undesired effects in red (e.g., mutagenicity or poor intestinal absorption), whereas a green color indicates drug-conform behavior. Virtually exploring the potential toxicity associated, all the synthesized 7-chloroquinoline derivatives represent low risks, with the exception of compounds **17**–**20**, in which the introduction of the formyl group as fragment in the structure induces a negative effect over the safety of these molecules. Even though, the fragments and topology of both reference compounds present potential risk as well (Table S2, Supplementary material).

Besides, the in vitro unspecific cytotoxicity of all these quinoline derivatives was simultaneously evaluated on NCTC-929 fibroblasts (Experimental procedures are detailed in the Supplementary material). It was specially noticed that compounds previously defined as cytotoxic on J774 murine macrophages (**3**, **7** and **8**), did not show such an obvious effect on NCTC-929 fibroblasts and HepG2.[8] Moreover, these compounds also achieved remarkable selectivity indexes on epimastigotes (SI >5) (Table 1). This different cytotoxicity could occur as a result of a higher drug intake by macrophages (phagocytic cell line) compared with that of fibroblasts and hepatocytes (both non-phagocytic cell lines).[30] Moreover, several studies reviewed in[31] lead to the idea that the process of autophagy observed in mammalian cells, and usually involved in survival pathways, can also act as a non-apoptotic mechanism of cell death. In fact, the toxicity of chloroquine on both mouse macrophages and L-strain fibroblasts by inducing autophagy has been studied in detail, being the toxic effect of the antimalarial drug more uniform for the first mammalian cell line,[32,33] what also explains the differences registered in the toxicity caused by our chloroquine derivatives on NCTC-929 fibroblasts (clone of strain L).

Finally, we also used the OSIRIS program (http://www.organic-chemistry.org/prog/peo) for the prediction of drug-score and drug-likeness parameters. According to this analysis, compounds **1**–**16** revealed promising values when compared with both reference drugs. Nevertheless, compounds **17**–**22** proved to be the less drug-like structures among the quinoline derivatives synthesized,

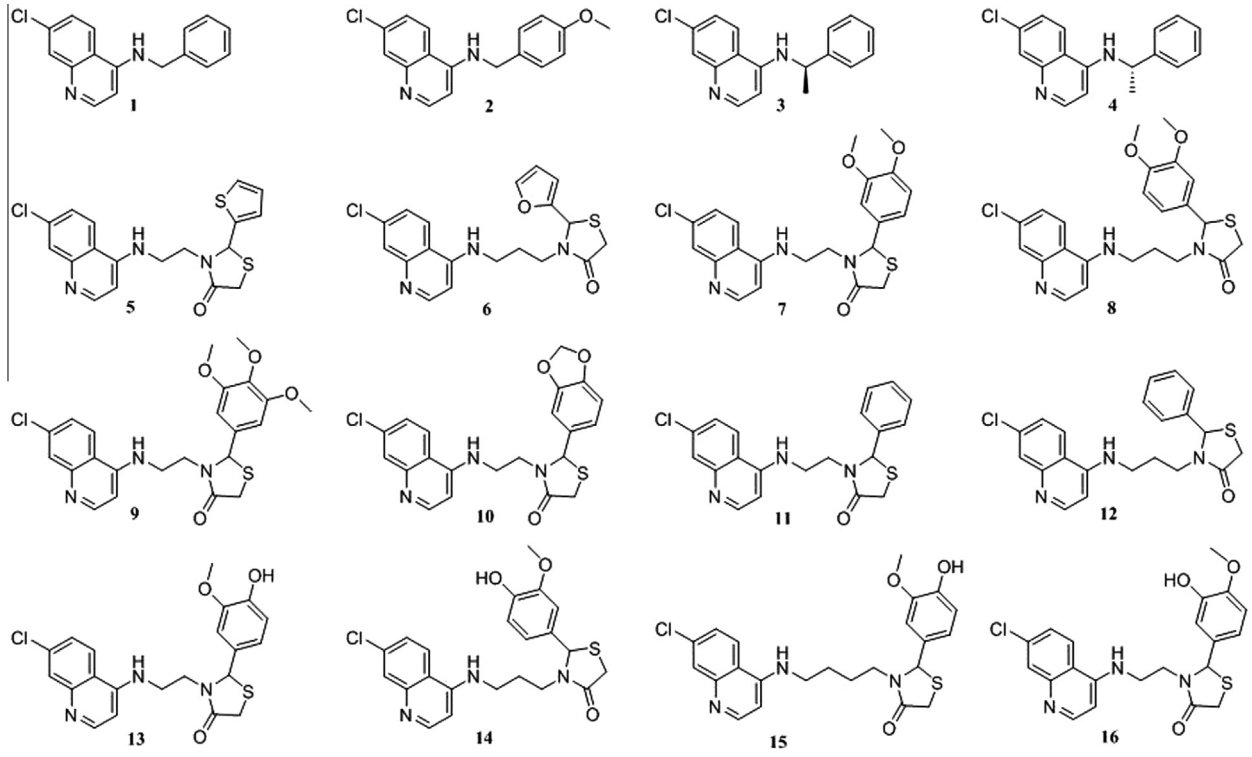


Figure 1. Chemical structures of 4-*N*-benzylamino-7-chloroquinolines (compounds **1**–**4)** and 7-chloroquinoline-1,3-thiazolidin-4-one conjugates (compounds **5**–**16**) (Group 1).

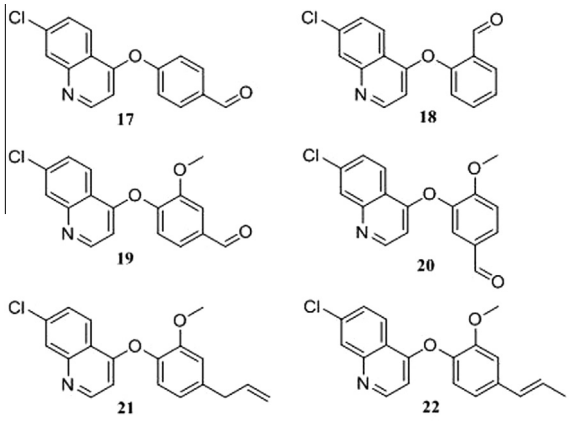


Figure 2. Chemical structures of 4-aryloxy-7-chloroquinoline molecules (Group 2).

likewise the antichagasic drug benznidazole (Fig. S1, Supplementary material).

Some antimalarial agents, such as quinacrine, display notable in vitro activity against the causative agent of Chagas disease as trypanothione reductase (TR) inhibitors, by interacting with the enzyme active site through a ring nitrogen atom, a chlorine atom, a methoxy group and a planar aromatic system.[34] According to our results, those 4-aryloxyquinolines derived from (iso)vainillin cores (compounds **19** and **20**) displayed an important improvement in the activity profile on epimastigotes (IC_{50} ca. 12–13 μM) compared with their respective derivatives with methoxy groups absence (compounds **17** and **18**) (IC_{50} >110 μM) (Table 1). This fact, together with the SAR approaches aforementioned, suggests TR as a presumed target molecule for these derivatives. Moreover, the most active compounds of this second group correspond to the 4-aryloxyquinolines **21** and **22**, in which (iso)eugenol moieties with a three carbon alkenyl side chain are incorporated (Table 1), as well as other quinolines previously assayed on *T. cruzi*.[35] On

Table 1
Antiepimastigote activity (%AE) and unspecific cytotoxicity on NCTC-929 fibroblasts (%C) of 7-chloroquinoline derivatives (**1**–**16**) and 4-aryloxyquinoline derivatives (**17**–**22**) expressed as the percentage of growth inhibition at the highest concentration tested,[a] IC_{50} (μM) and LC_{50}[b] (μM), respectively

Compound	%AE [c]	IC_{50} (μM)	%C [c]	LC_{50} (μM)	SI
1	95.08	6.55	71.43	2.28	3.71
2	96.73	5.93	89.20	14.81	2.50
3	99.37	1.09	69.39	42.78	39.33
4	100	6.76	89.76	7.90	1.17
5	96.39	7.38	82.06	16.49	2.24
6	97.77	7.15	85.59	23.55	3.29
7	99.83	1.90	74.50	120.65	63.52
8	99.77	<8	74.87	41.53	>5.19
9	96.34	ND[d]	68.19	178.49	ND
10	84.35	6.84	ND	19.49	2.85
11	89.14	7.02	92.41	11.18	1.59
12	91.13	<1	89.85	14.06	>14.06
13	87.34	>32	60.55	215.54	<6.74
14	54.29	235.89	ND	ND	ND
15	ND	69.66	9.00	>256	>3.67
16	93.07	4.34	88.80	24.46	5.63
17	57.83	ND[d]	63.05	218.20	ND
18	94.40	113.22	86.23	153.57	1.36
19	97.28	13.18	87.44	25.65	1.95
20	96.92	12.50	88.09	26.74	2.14
21	99.22	2.94	ND	12.00	2.94
22	89.95	6.17	ND	ND	ND
Nifurtimox	100	3.61	26.83	>256	>70.91
Benznidazole	82.01	27.12	17.38	>256	>9.44

Selectivity indexes (SI) for epimastigotes were also estimated.
[a] 256 μM for both experiments.
[b] Estimated by plotting drug concentrations versus percentages of antiepimastigote activity and unspecific cytotoxicity, respectively.
[c] Each concentration was tested by triplicate. SD less than 10% in all cases.
[d] Not determined, because of solubility problems.

Table 2
Antiamastigote activity of promising derivatives expressed as the percentage of growth inhibition (%AA) at the selected concentrations[a,b] and IC_{50}[c] (μM)

Compound	32 μM	16 μM	8 μM	4 μM	2 μM	1 μM	0.5 μM	IC_{50} (μM)	SI
3	–	–	–	41.93	29.89	–	–	5.34	8.01
7	–	100	38.67	29.95	–	–	–	9.48	12.73
12	–	–	–	33.15	26.65	20.24	–	6.61	2.13
Benznidazole	100	100	96.63	74.71	64.37	55.64	41.64	0.80	>320
Nifurtimox	100	100	100	100	100	100	96.54	<0.50	>512

Selectivity indexes (SI) for amastigotes were also estimated.
[a] According to the criteria established in Ref. 30.
[b] Each concentration was tested by triplicate. SD less than 10% in all cases.
[c] Extrapolated by plotting drug concentrations versus percentages of antiamastigote activity.

the other hand, the low SI shown by derivatives **17**–**21**, allow speculating about the absence of *T. cruzi* specific mechanisms of action, assuming that the presence of methoxy groups on the phenyl ring (compounds **19**–**22**) causes an increase in the cytotoxicity of these derivatives (Table 1). However, some tricyclic heterocycle-based drugs with great affinity to *T. cruzi* TR (i.e., some phenothiazines) also exert other effects such as an anti-calmodulin action or a mitochondrial disruption in epimastigotes and trypomastigotes,[36,37] not only generating these damages upon the parasite, but also interfering in vitro on the viability of mammalian cultured cells.[38,39]

The kind of phenotypic screening followed in this study enables the identification of chemical protoypes with activity on multiple parasite proteins or biochemical pathways, by the exposition of whole organisms to the compounds.[40] The existence of an intracellular epimastigote-like form as an intermediate stage within the mammalian host, morphologically and biochemically similar to the *bona fide* one,[41–43] supports the preliminary screening of trypanocidal compounds on the non-infectious stage of the parasite. However, the sensitivity of the extracellular forms (both epimastigotes and trypomastigotes) to drugs is usually higher than that of the intracellular parasite and therefore, a growth inhibition assay over intracellular amastigotes must be carried out in order to avoid any false positive found during the epimastigote susceptibility assay.[44,45] The great capability of *T. cruzi* to invade and replicate it as amastigote inside a wide variety of mammalian cells, allows to employ several cell lines to reproduce in vitro the intracellular cycle of the parasite.[46,47] Our research group has great experience in developing this kind of pharmacological assays by using either phagocytic[45,48] or non-phagocytic mammalian cells.[22,49] However, previous studies have revealed diverse effects displayed by chloroquine that interfere with the intracellular cycle of *T. cruzi*, affecting both parasite and target cell. In fact, Hrabák et al.[50] suggested that chloroquine irreversibly blocks the NO production in murine peritoneal macrophages and therefore, increases the rate of infected cells by *T. cruzi*. Moreover, Stecconi-Silva et al.[51] proposed a different behavior throughout the parasite kinetics in chloroquine-treated Vero cells infected with trypomastigotes. Considering these findings, together with the scaffolds selected to test and the relative cytotoxicity on J774 macrophages we previously reported,[8] NCTC-929 fibroblasts were here selected as the preferred host cell for the amastigote susceptibility assay (see the Suplementary material for the experimental procedures).

The activity on the intracellular parasite was only determined for compounds that after the primary screening resulted at least as selective as the cut-off estimated for benznidazole (SI >10). According to this, only three derivatives (**3**, **7** and **12**) from the initial 22 molecules were tested on intracellular amastigotes, by applying the methodology we previously reported.[22] As Table 2 reflects, derivative **3** achieved the best trypanocidal profile on amastigotes (IC_{50} = 5.34 μM). Similar results were reported by Blackie et al.[52] since the most active compound of a series of ferrocenic 4-aminoquinoline ureas resulted by the introduction of a benzyl moiety as a secondary amine substituent. Indeed, we have recently proposed the outstanding influence that benzylamino fragments have over the effectiveness of trypanocidal prototypes against the intracellular stage of *T. cruzi*,[53] as this moiety is also present in the reference drug benznidazole. Additionally, the higher Log*P* values for these three derivatives are (Table S1, Supplementary material), the more active on the intracellular parasite they become (IC_{50} value on amastigotes compound **3** < compound **12** < compound **7**), bearing out the positive impact that relative lipophilicity has over biological activity. Actually, these three quinolines showed good correlation between their theoretical Log*P* values and the trypanocidal activity displayed on the intracellular parasite (r^2 = 0.9885). However, epimastigotes were more sensitive to these three derivatives than intracellular amastigotes, as demonstrated by their IC_{50} values (Tables 1 and 2), suggesting that the host cell may function as a barrier to these molecules.[30] In spite of the fact that these three molecules displayed outstanding IC_{50} values on the intracellular parasite, resulting derivative **7** the most selective one (SI = 12.73), no compound more active than benznidazole (IC_{50} = 0.80 μM and SI >320) and nifurtimox (IC_{50} <0.50 μM and SI >512) was found.

The preliminary in vitro results compiled in the present work propose the antiplasmodial 4-amino-7-chloroquinoline scaffold, with derivative **7** (SI for epimastigotes = 63.52, SI for amastigotes = 12.73) as prototype of anti-*T. cruzi* agents, supported by the in silico prediction of a good oral bioavailability and a suitable risk profile. However, the low selectivity displayed by some chloroquinoline derivatives, prompts us to optimize the lead compounds of this series in order to ameliorate their unspecific cytotoxicity over *T. cruzi* host cells and therefore, to enhance their potential antichagasic effectiveness. Finally, further kinetic assays directed to explore the putative cellular target for this series are required in order to assert the hypothesis here presented.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.071.

References and notes

1. WHO *Working to Overcome the Global Impact of Neglected Tropical Diseases*; World Health Organization: Geneva, 2010.
2. Oliveira, I.; Torrico, F.; Muñoz, J.; Gascón, J. *Exp. Rev. Anti Infect. Ther.* **2010**, *8*, 945.
3. Schmunis, G. A.; Yadon, Z. E. *Acta Trop.* **2010**, *115*, 14.
4. Coura, J. R.; Dias, J. C. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 549.
5. Urbina, J. A. *Acta Trop.* **2010**, *115*, 55.
6. Castro, J. A.; de Mecca, M. M.; Bartel, L. C. *Hum. Exp. Toxicol.* **2006**, *25*, 471.
7. Murta, S. M.; Gazzinelli, R. T.; Brener, Z.; Romanha, A. J. *Mol. Biochem. Parasitol.* **1998**, *93*, 203.
8. Rojas Ruiz, F. A.; García-Sánchez, R. N.; Villabona Estupiñan, S.; Gómez-Barrio, A.; Torres Amado, D. F.; Martín Pérez-Solórzano, B.; Nogal-Ruiz, J. J.; Martínez-Fernández, A. R.; Kouznetsov, V. V. *Bioorg. Med. Chem.* **2011**, *19*, 4562.
9. Joyeau, R.; Maoulida, C.; Guillet, C.; Frappier, F.; Teixeira, A. R.; Schrével, J.; Santana, J.; Grellier, P. *Eur. J. Med. Chem.* **2000**, *35*, 257.
10. Magdaleno, A.; Ahn, I. Y.; Paes, L. S.; Silber, A. M. *PLoS One* **2009**, *4*, e4534.
11. Kinnamon, K. E.; Poon, B. T.; Hanson, W. L.; Waits, V. B. *Ann. Trop. Med. Parasitol.* **1997**, *91*, 147.
12. Yardley, V.; Gamarro, F.; Croft, S. L. *Antimicrob. Agents Chemother.* **2010**, *54*, 5356.
13. Jain, A. K.; Vaidya, A.; Ravichandran, V.; Kashaw, S. K.; Agrawal, R. K. *Bioorg. Med. Chem.* **2012**, *20*, 3378.
14. Kouznetsov, V.; Rodríguez, W.; Stashenko, E.; Ochoa, C.; Vega, C.; Rolón, M.; Montero Pereira, D.; Escario, J. A.; Gómez-Barrio, A. *J. Heterocycl. Chem.* **2004**, *41*, 995.
15. Pizzo, C.; Saiz, C.; Talevi, A.; Gavernet, L.; Palestro, P.; Bellera, C.; Blanch, L. B.; Benítez, D.; Cazullo, J. J.; Chidichimo, A.; Wipf, P.; Mahler, S. G. *Chem. Biol. Drug Des.* **2011**, *77*, 166.
16. Bueno, J.; Rojas Ruiz, F. A.; Villabona Estupiñán, S.; Kouznetsov, V. V. *Lett. Drug Des. Discovery* **2012**, *9*, 126.
17. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.
18. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
19. Aguirre, G.; Cerecetto, H.; Di Maio, R.; González, M.; Alfaro, M. A.; Jaso, A.; Zarranz, B.; Ortega, M. A.; Aldana, I.; Monge-Vega, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3835.
20. Vega, M. C.; Rolón, M.; Montero-Torres, A.; Fonseca-Berzal, C.; Escario, J. A.; Gómez-Barrio, A.; Gálvez, J.; Marrero-Ponce, Y.; Arán, V. J. *Eur. J. Med. Chem.* **2012**, *58*, 214.
21. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
22. Fonseca-Berzal, C.; Merchán Arenas, D. R.; Romero Bohórquez, A. R.; Escario, J. A.; Kouznetsov, V. V.; Gómez-Barrio, A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4851.
23. Paula, F. R.; Jorge, S. D.; de Almeida, L. V.; Pasqualoto, K. F.; Tavares, L. C. *Bioorg. Med. Chem.* **2009**, *17*, 2673.
24. da Silva, R. B.; Loback, V. B.; Salomão, K.; de Castro, S. L.; Wardell, J. L.; Wardell, S. M.; Costa, T. E.; Penido, C.; Henriques, Md.; Carvalho, S. A.; da Silva, E. F.; Fraga, C. A. *Molecules* **2013**, *18*, 3445.
25. Castro, J. A.; Díaz de Toranzo, E. G. *Biomed. Environ. Sci.* **1988**, *1*, 19.
26. Viotti, R.; Vigliano, C.; Lococo, B.; Álvarez, M. G.; Petti, M.; Bertocchi, G.; Armenti, A. *Exp. Rev. Anti Infect. Ther.* **2009**, *7*, 157.
27. Wilkinson, S. R.; Kelly, J. M. *Expert Rev. Mol. Med.* **2009**, *11*, e31.
28. El-Azab, A. S.; Al-Omar, M. A.; Alaa, A. M.; Naglaa, A.-A.; Magda, A.-A.; Abdulaziz, El-S. .; Aleisa, M.; Sayed-Ahmed, M.; Abdel-Hamide, S. G. *Eur. J. Med. Chem.* **2010**, *45*, 4188.
29. Chohan, Z. H.; Youssoufi, M. H.; Jarrahpour, A.; Ben Hadda, T. *Eur. J. Med. Chem.* **2010**, *45*, 1189.
30. Rolón, M.; Seco, E. M.; Vega, C.; Nogal, J. J.; Escario, J. A.; Gómez-Barrio, A.; Malpartida, F. *Int. J. Antimicrob. Agents* **2006**, *28*, 104.
31. Edinger, A. L.; Thompson, C. B. *Curr. Opin. Cell Biol.* **2004**, *16*, 663.
32. Fedorko, M. E.; Hirsch, J. G.; Cohn, Z. A. *J. Cell Biol.* **1968**, *38*, 377.
33. Fedorko, M. E.; Hirsch, J. G.; Cohn, Z. A. *J. Cell Biol.* **1968**, *38*, 392.
34. Bonse, S.; Santelli-Rouvier, C.; Barbe, J.; Krauth-Siegel, R. L. *J. Med. Chem.* **1999**, *42*, 5448.
35. Fakhfakh, M. A.; Fournet, A.; Prina, E.; Mouscadet, J.-F.; Franck, X.; Hocquemiller, R.; Figadère, B. *Bioorg. Med. Chem.* **2003**, *11*, 5013.
36. Lacuara, J. L.; de Barioglio, S. L.; de Oliva, P. P.; Bernacchi, A. S.; de Culasso, A. F.; Castro, J. A.; Franke, B. M.; Franke de Cazzulo, J. J. *Experientia* **1991**, *47*, 612.
37. Rivarola, H. W.; Paglini-Oliva, P. A. *Curr. Drug Targets Cardiovasc. Haematol. Disord.* **2002**, *2*, 43.
38. Dudani, A. K.; Gupta, R. S. *Tissue Cell* **1987**, *19*, 183.
39. Abdel-Razaq, W.; Kendall, D. A.; Bates, T. E. *Neurochem. Res.* **2011**, *36*, 327.
40. Sykes, M. L.; Avery, V. M. *J. Med. Chem.* **2013**, *56*, 7727.
41. Faucher, J. F.; Baltz, T.; Petry, K. G. *Parasitol. Res.* **1995**, *81*, 441.
42. Almeida-de-Faria, M.; Freymüller, E.; Colli, W.; Alves, M. J. *Exp. Parasitol.* **1999**, *92*, 263.
43. Tyler, K. M.; Engman, D. M. *Int. J. Parasitol.* **2001**, *31*, 472.
44. Martínez-Díaz, R. A.; Escario, J. A.; Nogal-Ruiz, J. J.; Gómez-Barrio, A. *J. Clin. Pharm. Ther.* **2000**, *25*, 43.
45. Muelas, S.; Di Maio, R.; Cerecetto, H.; Seoane, G.; Ochoa, C.; Escario, J. A.; Gómez-Barrio, A. *Folia Parasitol.* **2001**, *48*, 105.
46. McCabe, R. E.; Remington, J. S.; Araujo, F. G. *Infect. Immun.* **1984**, *46*, 372.
47. Martinez-Diaz, R. A.; Escario, J. A.; Nogal-Ruiz, J. J.; Gómez-Barrio, A. *Mem. Inst. Oswaldo Cruz* **2001**, *96*, 53.
48. Muelas, S.; Suárez, M.; Pérez, R.; Rodríguez, H.; Ochoa, C.; Escario, J. A.; Gómez-Barrio, A. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 269.
49. Saraiva, J.; Vega, C.; Rolón, M.; da Silva, R.; Andrade e Silva, M. L.; Donate, P. M.; Bastos, J. K.; Gómez-Barrio, A.; de Albuquerque, S. *Parasitol. Res.* **2007**, *100*, 791.
50. Hrabák, A.; Sefrioui, H.; Vercruysse, V.; Temesi, A.; Bajor, T.; Vray, V. *Eur. J. Pharmacol.* **1998**, *354*, 83.
51. Stecconi-Silva, R. B.; Andreoli, W. K.; Mortara, R. A. *Mem. Inst. Oswaldo Cruz* **2003**, *98*, 953.
52. Blackie, M. A. L.; Saravanamuthu, A.; Fairlamb, A. H.; Chibale, K. *ARKIVOC* **2008**, *6*, 52.
53. Fonseca-Berzal, C.; Escario, J. A.; Arán, V. J.; Gómez-Barrio, A. *Parasitol. Res* **2014**. http://dx.doi.org/10.1007/s00436-013-3740-5.