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Design, synthesis, and in vitro antiprotozoal, antimycobacterial activities of *N*-{2-[(7-chloroquinolin-4-yl)amino]ethyl}ureas ☆

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ABSTRACT

We have synthesized a new series of quinoline tripartite hybrids from chloroquine, ethambutol, and isoxyl drugs, using a short synthetic route. Compounds **1–8** were tested in vitro against five protozoa (*Giardia intestinalis*, *Trichomonas vaginalis, Entamoeba histolytic*a, *Leishmania mexicana* and *Trypanosoma cruzi*) and *Mycobacterium tuberculosis. N*-(4-Butoxyphenyl)-*N*′-{2-[(7-chloroquinolin-4-yl)amino]ethyl}urea (**6**) was the most active compound against all parasites tested. Compound **6** was 670 times more active than metronidazole, against *G. intestinalis.* It was as active as pentamidine against *L. mexicana*, and it was two-fold more potent than ethambutol and isoxyl versus *M. tuberculosis*. This compound could be considered as a new broad spectrum antimicrobial agent.

1. Introduction

Protozoan diseases are a main problem in developing countries, affecting hundreds of millions people and animals around the world.[1] Since protozoa are eukaryotic, they share many common features with their mammalian host, making the development of effective and selective drugs a hard job. The chemotherapy against diseases such as giardiasis, amoebiasis, trichomoniasis, leishmaniasis, and trypanosomiasis is limited by the existence of only a few drugs in the market, most of which are of low efficacy, showing toxic side effects, and frequently lead to the appearance of resistant strains.[2] This reflects the need to continue searching for new and better antiprotozoal drugs.[3]

On the other hand, tuberculosis (TB) is one of the most common infectious diseases known by the mankind. Around 32% of the world's population is infected by *Mycobacterium tuberculosis*, the main causal agent of TB. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to the first-line drugs: isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide.[4]

Isoxyl and phenylthiourea derivatives,[5] have shown considerable antimycobacterial activity, however, the clinical use of isoxyl was discontinued, apparently because of the poor bioavailability of the highly non-polar product.[6]

Quinoline derivatives are a class of bioactive heterocycles against *Plasmodium falciparum* and *Leishmania mexicana*.[7–9] Quinine, chloroquine (CQ), and mefloquine, are mainly used as antimalarial drugs.[10] However, they possess moderate biological activity against *M. tuberculosis* (MIC 25–100 μg/mL). Recent investigations have reported the activity of some quinolines against *M. tuberculosis* with MIC's of 3.12–6.25 μg/mL (Fig. 1).[10–12]

Due to the antimalarial activity is known for this kind of heterocycles, we have investigated the broad antiparasitic spectrum of quinolines against other protozoa and mycobacteria. As a part of our search for basic information about the structural requirements for new antiinfective molecules, we have synthesized a series of novel chloroquine–ethambutol–isoxyl tripartite hybrids **1**–**8** (Fig. 2), according to de Souza et al. approach.[10] The in vitro antiparasitic activity of these compounds on intestinal unicellular parasites (*Giardia intestinalis* and *Entamoeba histolytica*), a urogenital tract parasite (*Trichomonas vaginalis*) and kinetoplastid

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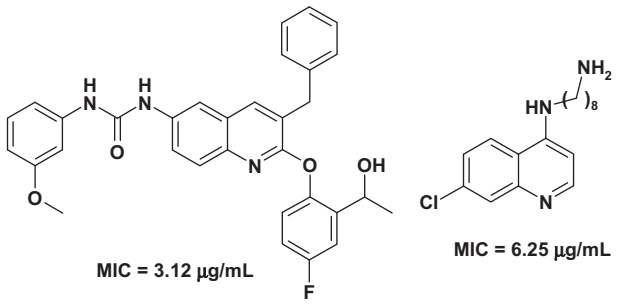
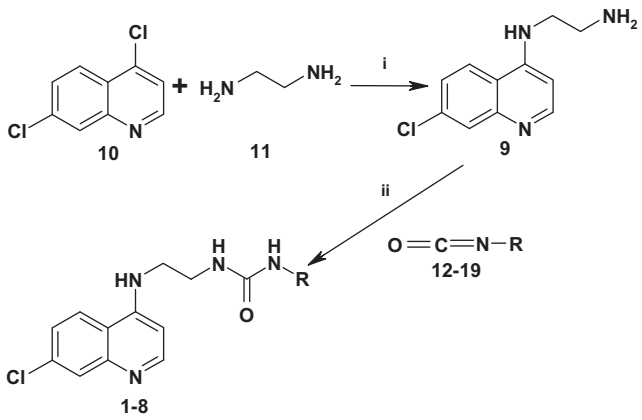


Figure 1. Antimycobacterial quinolines.[10,11]

parasites such as *Trypanosoma cruzi* and *L. mexicana* is reported in this paper. Additionally, we also report the biocide effect against *M. tuberculosis.*



Scheme 1. Reagents and conditions: (i) 6 equiv of **11**, 80 °C; (ii) Et_3N, CH_3CN.

2. Results and discussion

2.1. Drug design of derivates 1–8

Compounds **1**–**8** were designed on the basis of the structure of antiprotozoal drug chloroquine (CQ), and the antimycobacterial drugs ethambutol (ETH) and isoxyl (ISO). The hybridization of these three pharmacophores (7-chloroquinoline, ethylenediamine spacer and phenylurea as thiourea bioisostere) leading the title compounds (Fig. 2). As defined by Viegas-Junior et al. molecular hybridization is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric subunits in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.[13] Recently, Saadeh et al. have published a novel hybrid antiparasitic molecule, based on the structures of metronidazole and chloroquine.[14]

2.2. Chemistry

Compounds **1–8** were prepared starting from 4,7-dichloroquinoline (**10**). It was reacted with an excess of ethylenediamine (**11**), through an addition-elimination mechanism to give *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**9**). The last compound was then reacted with adequate isocyanate derivatives **12–19** under inert atmosphere, triethylamine and acetonitrile as solvent (Scheme 1). Compounds were purified by recrystallization. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data.

In the nuclear magnetic resonance spectra (^{1}H NMR; δ ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants.

In compounds **3–8**, the 4-substituted phenylurea region of the ^{1}H NMR spectrum contained an A_2B_2 pattern signals ranging from δ 7.25–7.64 ppm, attributable to H-2′ and H-6′, whereas displacements of H-3′ and H-5′ ranging from 6.77 to 8.14 ppm. We also observed in all compounds a characteristic pattern for 7-chloroquinoline core: a doublet signal ranging in δ 8.34–8.37 ppm, attributable to H-2, with *ortho* coupling constant (J = 5.2–5.4 Hz); a doublet signal ranging from 6.52 to 6.60 ppm, assigned to H-3, with J_o = 5.4–5.6 Hz. A third doublet signal in 8.15–8.37 ppm, with J_o = 8.0–9.2 Hz, given to H-5. A doublet of doublets appears ranging from 7.25 to 7.64 ppm, belongs to H-6 with *ortho* and *meta* coupling constants, and the last doublet signal in 7.77–7.79 ppm, attributable to H-8.

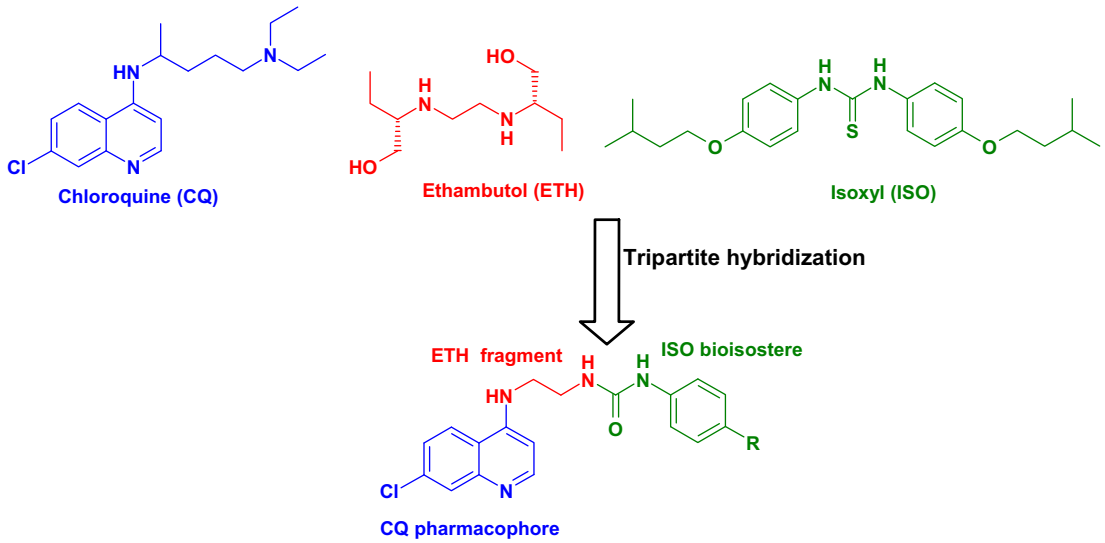


Figure 2. Compounds used as lead and drug design of **1–8** through tripartite molecular hybridization.

2.3. Biological activities

2.3.1. In vitro antiprotozoal effects

The new tripartite hybrids **1**–**8** were tested in vitro as antiprotozoal agents. Biological assays results against the five protozoa tested are summarized in Table 1. Comparison was made among new compounds and the antiprotozoal drug of choice: metronidazole, against *G. intestinalis*, *E. histolytica*, and *T. vaginalis*. In order to compare bioactivities, chloroquine (CQ) and precursor **9** were also tested. In vitro susceptibility assays were performed using a method previously described.[15,16]

In general, all the screened compounds showed high bioactivity (IC_{50} <1.8 μM) against *G. intestinalis,* being more potent than metronidazole (IC_{50} = 5.36 μM). Compound **6**, with a 4-butoxyphenyl substituent, showed nanomolar potency (IC_{50} = 8 nM). It was 670-times more active than metronidazole, 50-fold more potent than CQ, and 156-fold more active than precursor **9**.

Against *T. vaginalis*, only compounds **3** and **6** showed high bioactivity (<10 μM). However, none of them were more active than metronidazole. In comparison, CQ and precursor **9** had low potencies, with IC_{50} >20 μM.

In vitro antiamoebic activity exhibited by compounds **1–3**, **5**, and **6** was acceptable. All of them showed high bioactivity in the range of 3.63–6.93 μM. Again, compound **6** was the most active of the series against *E. histolytica.* It was three-times more active than CQ, four-times more potent than precursor **9**, but fourfold less potent than metronidazole.

For *L. mexicana*, only compound **6** was as active as pentamidine (second-line antileishmanial drug). The rest of compounds were inactive against this kinetoplastid parasite.

In vitro antichagasic activity of compounds **3**, **5**–**7** was moderated (50 μM), whereas benznidazole (first-line antichagasic drug) showed an IC_{50} = 34.38 μM.

With these results, we could conclude that compounds **1**–**8** displayed a selective toxicity against *G. intestinalis* over the other unicellular parasites tested.

2.3.2. In vitro antimycobacterial effect

Following the Microplate Alamar Blue Assay (MABA),[17] tripartite hybrids **1**–**8** were tested in vitro for their antimycobacterial activity against *M. tuberculosis* strain $H_{37}Rv$ (ATCC 27294). Results revealed that compounds **3** and **6** exhibited high mycobactericidal activity. Among others, these structures were found to be the most potent compounds with MIC's of 4 and 2 μg/mL, respectively. Compound **6** was twofold more potent than reference drugs ethambutol and isoxyl (MIC 4 μg/mL), whereas compound **3** was as active as these two antimycobacterial drugs (Table 2). Compounds **3** and **6** have a high *C* log *P* value (4.60 and 5.16, respectively), leading us to the hypothesis that an increase of lipophilicity could improve antimycobacterial activity, in agreement with recent literature reports.[18–20]

2.3.3. In vitro cytotoxic effect

The most active compound (**6**) was evaluated for its toxicity against mammalian VERO cell line, showing an CC_{50} of 5 μM. The selectivity index (SI) of the compound, defined as the ratio of cytotoxicity to biological activity (SI = CC_{50} VERO cells/IC_{50} parasites) was calculated. It is generally considered that biological efficacy is not due to in vitro cytotoxicity when SI ⩾10. Compound **6** showed a nanomolar giardicidal effect, having a selectivity index of 625.

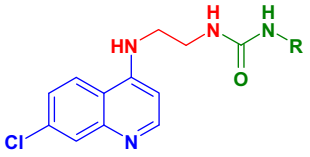
3. Conclusion

We have synthesized and screened the in vitro antiprotozoal and antimycobacterial activities of new tripartite hybrids from

Table 2
In vitro antimycobacterial activity of **1**–**8**

Compd	R	MIC (μg/mL) *M. tuberculosis* $H_{37}Rv$
1	Cyclohexyl	8
2	Ph	8
3	4-ClPh	4
4	4-FPh	8
5	4-EtOPh	8
6	4-BuOPh	2
7	4-NO_2Ph	8
8	4-MeOPh	8
9	—	8
CQ	—	>8
Ethambutol	—	4
Isoxyl		4

Table 1
In vitro antiparasitic activity of quinoline ureas (**1**–**8**)



Compd	R	IC_{50} (μM)				
		G. intestinalis	*T. vaginalis*	*E. histolytica*	*L. mexicana*	*T. cruzi*
1	Cyclohexyl	1.647	66.668	6.933	>50	>50
2	Ph	1.171	45.974	5.370	>50	>50
3	4-ClPh	0.267	9.589	6.866	50	50
4	4-FPh	0.927	23.212	17.664	>50	>50
5	4-EtOPh	0.339	30.128	5.934	50	50
6	4-BuOPh	0.008	8.444	3.636	9.90	50
7	4-NO_2Ph	1.748	12.026	10.563	50	50
8	4-MeOPh	0.595	21.738	10.678	>50	>50
9	—	1.253	22.986	14.597	>50	>50
CQ	—	0.401	30.000	9.579	>50	>50
Metronidazole	—	5.360	0.290	0.770	—	—
Pentamidine	—	—	—	—	13.32	—
Benznidazole	—	—	—	—	—	34.38

pharmacophores 7-chloroquinoline (CQ), ethylenediamine spacer (ETH) and phenylurea as thiourea bioisostere (ISO). This study demonstrated that the hybridization approach have generated a new antiparasitic and mycobactericidal scaffold. The obtained results are very promising since many of the compounds showed activity comparable with the current used antiprotozoal drugs metronidazole and pentamidine, whereas compound **6** exhibited even higher bioactivity, especially towards *G. intestinalis*, *L. mexicana*, and *M. tuberculosis*. Further optimization and pharmacokinetic characterization of this series are in progress in our laboratory.

4. Experimental

4.1. Instruments

Melting points were determined on an EZ-Melt MPA120 automated melting point apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated Silica Gel 60 F254 plates (E. Merck). ^{1}H NMR spectra were recorded on a Varian INOVA 400 (400 MHz) and ^{13}C NMR (100 MHz) instruments, and Varian Mercury 200 instrument. Chemical shifts are given in ppm relative to tetramethylsilane (Me_4Si, $\delta = 0$) in DMSO-d_6; J values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br s, broad signal. MS and HRMS were recorded on a JEOL JMS-700 spectrometer by Electron Impact or Fast Atom Bombarded [(FAB$^+$)]. The *C* log *P* values were obtained using ACD/labs software v.4.5.

4.2. Synthesis of *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (9)

A mixture of 4,7-dichloroquinoline (3.0 g, 0.0151 mol) and ethylenediamine (5.45 g, 6.08 mL, 0.0909 mol, 6 equiv), was stirred under reflux for 3 h. After cooling to room temperature, the reaction was verted under water–ice mixture. The precipitate formed was filtered off, and the residue washed with cold water. Compound was recrystallized from ethanol to give 3.13 g (94%), mp 143.5–145.4 °C (lit. 145–147 °C).[10]

4.3. General method of synthesis of *N*-{2-[(7-chloroquinolin-4-yl)amino]ethyl}-*N*′-arilureas (1–8)

To a solution of *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (0.3 g, 0.0013 mol) in dry acetonitrile (5 mL), was added appropriate isocyanate (0.0014 mol, 1.1 equiv) in dry acetonitrile dropwise at 0 °C, and stirred at room temperature under nitrogen atmosphere for 2–4 h. Solvent was removed in vacuo, and the residue was suspended in water. The white precipitate was filtered and dried. Crude compounds were purified by recrystallization.

4.3.1. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-cyclohexylurea (1)

Recrystallized from ethanol. Yield: 88%, mp: 210–212 °C. ^{1}H NMR (200 MHz, DMSO) δ: 8.83 (1H, d, J_o = 5.2, H_2); 8.16 (1H, d, J_o = 8.8, H_5); 7.78 (1H, d, J_m = 2.2, H_8); 7.47 (1H, dd, J_o = 9.2, J_m = 2.8, H_6); 6.52 (1H, d, J_o = 5.6, H_3); 5.98 (2H, s, NH); 3.32 (4H, s, $H_{9,10}$); 1.67 (4H, m, $H_{2'-6'}$); 1.55 (4H, m, $H_{4'}$); 1.15 (4H, m, $H_{3'-5'}$) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 158.8 (CO), 152.5 (C-2), 150.8 (C-8a), 149.9 (C-4), 134.0 (C-7), 128.2 (C-8), 124.7 (C-6), 124.4 (C-5), 117.9 (C-4a), 99.3 (C-3), 48.6 (C-1′), 44.6 (C-9), 40.6 (C-10), 33.9 (C-2′-6′), 25.2 (C-3′–5′), 20.02 (C-4′) ppm; MS/FAB$^+$: *m/z* 347 (M+H$^+$). HRMS (FAB$^+$): *m/z* 347.1619 [M+H]$^+$ (Calcd for $C_{18}H_{24}ClN_4OH^+$ 347.1639).

4.3.2. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-phenylurea (2)

Recrystallized from ethanol. Yield: 77%, mp: 208–212 °C. ^{1}H NMR (200 MHz, DMSO) δ: 8.34 (1H, d, J_o = 5.2, H_2); 8.15 (1H, d, J_o = 9.2, H_5); 7.77 (1H, d, J_m = 2.0, H_8); 7.42 (1H, dd, J_o = 9.0, J_m = 1.8, H_6); 7.34 (2H, m, J_o = 8, $H_{2'-6'}$); 7.19 (2H, t, J_o = 7.8, $H_{3'-5'}$) 6.87 (1H, t, J_o = 7.6, $H_{4'}$); 6.57 (1H, d, J_o = 5.6, H_3); 6.37 (1H, s, NH); 3.71 (4H, s, $H_{9,10}$) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 151.72 (C-2), 150.75 (C-4), 150.27 (CO), 148.45 (C-8a), 139.90 (C-1′), 133.59 (C-7), 128.62 (C-3′–5′), 126.99 (C-8), 124.26 (C-6), 117.95 (C-2′–6′), 117.16 (C-4a), 98.61 (C-3), 42.99 (C-9), 37.70 (C-10) ppm; MS/FAB$^+$: *m/z* 341 (M+H$^+$). HRMS (FAB$^+$): *m/z* 341.1172 [M+H]$^+$ (Calcd for $C_{18}H_{18}ClN_4OH^+$ 341.1169).

4.3.3. *N*-(4-Chlorophenyl)-*N*′-{2-[(7-chloroquinolin-4-yl)amino]-ethyl}urea (3)

Recrystallized from acetonitrile–methanol. Yield: 32%, mp: 228–230 °C 1H NMR (200 MHz, DMSO) δ: 8.40 (1H, d, J_o = 5.4, H_2); 8.23 (1H, d, J_o = 9.2, H_5); 7.79 (1H, d, J_m = 2.2, H_8); 7.47 (1H, dd, J_o = 8.0, J_m = 2.2, H_6); 7.43 (2H, m, J_o = 8.4, $H_{2'-6'}$); 7.27 (2H, t, J_o = 9.2, $H_{3'-5'}$), 6.58 (1H, d, J_o = 5.4, H_3); 6.37 (1H, s, NH); 3.37 (4H, s, $H_{9,10}$) ppm. ^{13}C NMR. (50 MHz, DMSO) δ: 156.13 (C-2), 152. 55 (C-8a), 150.73 (CO), 149.64 (C-4), 140.00 (C-1′), 134.06 (C-7), 129.09 (C-8), 128.18 (C-3′–5′), 126.00 (C-4′), 124.78 (C-6), 124.54 (C-5) 119.43 (C-2′–6′), 118.05 (C-4a), 99.38 (C-3), 43.60 (C-9), 40.63 (C-10) ppm; MS/FAB$^+$: *m/z* 375 (M+H$^+$). HRMS (FAB$^+$): *m/z* 375.0800 [M+H]$^+$ (Calcd for $C_{18}H_{17}Cl_2N_4OH^+$ 375.0779).

4.3.4. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-(4-fluorophenyl)urea (4)

Recrystallized from ethanol. Yield: 50%, mp: 220–221 °C. ^{1}H NMR (500 MHz, DMSO) δ: 8.40 (1H, d, J_o = 5.4, H_2); 8.23 (1H, d, J_o = 9.2, H_5); 7.79 (1H, d, J_m = 2.2, H_8); 7.47 (1H, dd, J_o = 9.2, J_m = 2.2, H_6); 7.40 (2H, m, J_o = 9.0, $H_{2'-6'}$); 7.06 (2H, t, J_o = 9.2, $H_{3'-5'}$), 6.57 (1H, d, J_o = 5.4, H_3); 6.37 (1H, s, NH); 3.29 (4H, s, $H_{9,10}$) ppm. ^{13}C NMR. (125 MHz, DMSO) δ: 157.47 (*J* = 235.6, C–F); 156.28 (CO), 152.51 (C-2), 150.66 (C-4), 149.59 (C-8a), 137.29 (C-1′), 133.94 (C-7), 128.10 (C-8), 124.66 (C-6), 124.46 (C-5), 119.97 (*J* = 6.68, C-2′–6′), 117.95 (C-4a) 115.64 (*J* = 25.74, C-3′–5′), 99.25 (C-3), 40.36 (C-9), 39.70 (C-10) ppm. MS/FAB$^+$: *m/z* 359 (M+H$^+$). HRMS (FAB$^+$): *m/z* 359.1059 [M+H]$^+$ (Calcd for $C_{18}H_{17}ClFN_4OH^+$ 359.1075).

4.3.5. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-(4-ethoxyphenyl)urea (5)

Recrystallized from ethanol. Yield: 88%, mp: 213–216 °C. ^{1}H NMR (200 MHz, DMSO) δ: 8.40 (1H, d, J_o = 5.2, H_2); 8.37 (1H, d, J_o = 8.0, H_5); 7.79 (1H, d, J_m = 2.2, H_8); 7.46 (1H, dd, J_o = 8.0, J_m = 2.4, H_6); 7.28 (2H, m, J_o = 8.8, $H_{2'-6'}$); 6.79 (2H, t, J_o = 8.8, $H_{3'-5'}$), 6.57 (1H, d, J_o = 5.4, H_3); 6.50 (1H, s, NH); 3.37 (4H, s, $H_{9,10}$), 3.93 (2H, c, CH_2O), 1.28 (3H, t, CH_3) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 155.91 (C-4′), 153.18 (C-2), 151. 84 (C-4), 150.03 (CO), 148.93 (C-8a), 133.47 (C-7), 133.23 (C-1′), 127.47 (C-8), 124.08 (C-6), 123.83 (C-2′–6′), 119.65 (C-3′–5′), 117.37 (C-4a) 114.37 (C-5), 98.67 (C-3), 63.02 (OCH_2), 43.19 (C-9), 40.34 (C-10), 14.76 (CH_3) ppm; MS/FAB$^+$: *m/z* 385 (M+H$^+$). HRMS (FAB$^+$): *m/z* 385.1415 [M+H]$^+$ (Calcd for $C_{20}H_{22}ClN_4O_2H^+$ 385.1431).

4.3.6. *N*-(4-Butoxyphenyl)-*N*′-{2-[(7-chloroquinolin-4-yl)amino]-ethyl}urea (6)

Recrystallized from ethanol. Yield: 92%, mp: 202–204 °C. ^{1}H NMR (200 MHz, DMSO) δ: 8.37 (1H, d, J_o = 5.4, H_2); 8.19 (1H, d, J_o = 9.0, H_5); 7.79 (1H, d, J_m = 2.2, H_8); 7.43 (1H, dd, J_o = 8.0, J_m = 1.4, H_6); 7.25 (2H, m, J_o = 8.8, $H_{2'-6'}$); 6.77 (2H, t, J_o = 8.8, $H_{3'-5'}$), 6.55 (1H, d, J_o = 5.4, H_3); 6.25 (1H, s, NH); 3.30 (4H, s,

$H_{9,10}$), 3.84 (2H, t, CH_2O), 1.60 (2H, c, CH_2-2″), 1.37 (2H, q, CH_2-3″), 0.92 (3H, t, CH_3″) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 156.55 (C-4′), 154.02 (C-2), 152. 55 (C-4), 150.73 (CO), 149.64 (C-8a), 133.94 (C-7), 133.94(C-1′), 128.18 (C-8), 124.78 (C-6), 124.54 (C-5), 120.30 (C-2′–6′), 117.99 (C-4a) 115.08 (C-3′–5′), 99.32 (C-3), 67.85 (OCH_2), 43.90 (C-9), 39.35 (C-10), 31.69 (CH_2-2″), 19.47 (CH_2-3″), 14.44 (CH_3) ppm; MS/FAB$^+$: *m/z* 413 (M+H$^+$). HRMS (FAB$^+$): *m/z* 413.1780 [M+H]$^+$ (Calcd for $C_{22}H_{26}ClN_4O_2H^+$ 413.1744).

4.3.7. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-(4-nitrophenyl)urea (7)

Recrystallized from acetonitrile-methanol. Yield: 70%, mp: 223–224 °C. ^1H NMR (200 MHz, DMSO) δ: 8.41 (1H, d, J_o = 5.4, H_2); 8.23 (1H, d, J_o = 9.2, H_5), 8.14 (2H, t, J_o = 8.8, $H_{3′,5′}$) 7.79 (1H, d, J_m = 1.8, H_8); 7.46 (1H, dd, J_o = 8.8, J_m = 1.8, H_6); 7.64 (2H, m, J_o = 8.8, $H_{2′-6′}$), 6.67 (1H, s, NH); 6.60 (1H, d, J_o = 5.4, H_3); 3.39 (4H, s, $H_{9,10}$) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 154.71 (C-2), 154.02 (C-4), 149. 98 (CO), 148.92 (C-8), 146.95 (C-1′), 140.34 (C-4′), 133.94 (C-7), 127.43 (C-8), 125.00 (C-2′–6′), 124.00 (C-6), 123.82 (C-5), 117.34 (C-4a) 116.82 (C-3′–5′), 98.63 (C-3), 41.26 (C-9), 40.31 (C-10) ppm; MS/FAB$^+$: *m/z* 386 (M+H$^+$). HRMS (FAB$^+$): *m/z* 386.1020 [M+H]$^+$ (Calcd for $C_{18}H_{17}ClN_5O_3H^+$ 386.1020).

4.3.8. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-*N*′-(4-methoxyphenyl)urea (8)

Recrystallized from acetonitrile–ethanol. Yield: 76%, mp: 216–219 °C. ^1H NMR (200 MHz, DMSO) δ: 8.42 (1H, d, J_o = 5.4, H_2); 8.22 (1H, d, J_o = 9.2, H_5), 7.79 (1H, d, J_m = 2.2), 7.79 (1H, d, J_m = 1.8, H_8), 7.46 (1H, dd, J_o = 8.8, J_m = 2.2, H_6); 7.29 (2H, m, J_o = 9.2, $H_{2′-6′}$), 6.81 (2H, t, J_o = 8.8, $H_{3′-5′}$), 6.28 (1H, s, NH); 6.58 (1H, d, J_o = 5.4, H_3); 3.39 (4H, s, $H_{9,10}$) ppm. ^{13}C NMR (50 MHz, DMSO) δ: 155.91 (C-4′), 153.97 (C-2), 151. 84 (C-4), 150.09 (CO), 148.93 (C-8), 133.35 (C-7), 133.35 (C-1′), 127.47 (C-8), 124.08 (C-6), 123.83 (C-5), 119.65 (C-2′–6′), 117.35 (C-4a), 113.37 (C-3′–5′), 98.67 (C-3), 55.08 (CH_3O) 43.19 (C-9), 40.71 (C-10) ppm; MS/FAB$^+$: *m/z* 371 (M+H$^+$). HRMS (FAB$^+$): *m/z* 371.1273 [M+H]$^+$ (Calcd for $C_{19}H_{20}ClN_4O_2H^+$ 371.1275).

4.4. Biological assays

4.4.1. In vitro giardicidal, amebicidal and trichomonicidal assay

G. intestinalis strain IMSS:0696:1 was cultured in TYI-S-33 modified medium, supplemented with 10% calf serum and bovine bile. *T. vaginalis* strain GT3 and *E. histolytica* HM1-IMSS were cultured in TYI-S-33 medium, supplemented with 10% bovine serum. In vitro susceptibility assays were performed using a method previously described.[15,16] Briefly: 4×10^4 trophozoites of *Giardia intestinalis* or *T. vaginalis* or *E. histolytica* were incubated for 48 h at 37 °C with increasing concentrations of synthesized compounds, chloroquine, **9**, and metronidazole. As the negative control, trophozoites were incubated in culture medium with DMSO used in the experiments. After the incubation, trophozoites were washed and subcultured for another 48 h in fresh medium alone. At the end of this period, trophozoites were counted and the 50% inhibitory concentration (IC_{50}) was calculated by probit analysis. Experiments were carried out in triplicate and repeated at least twice.

4.4.2. In vitro antileishmanial and trypanocidal assay[21]

The growth inhibition test was performed on promastigotes of *L. mexicana* (MHOM/MX/ISETGS; clinical strain originally isolated from a patient with diffuse cutaneous leishmaniasis) and epimastigotes of *T. cruzi* (MHOM/MX/1994/Ninoa; clinical strain originally isolated from a patient with the disease in acute phase). Parasites were cultivated at 26 °C in Schneider's drosophila medium, supplemented with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 μg/mL). Biological assays were performed in 96-well plates and all compounds were evaluated in duplicate. Compounds were solubilized in DMSO and diluted in a liquid medium. A mixture of 100 μL of compounds solution and 100 μL of culture medium containing 10,000 *Leishmania* promastigotes or 20,000 *T. cruzi* epimastigotes was added to obtain concentrations of 10, 5, 2.5, 1.25 μg/mL. Benznidazole (first-line antichagasic drug) and pentamidine (second-line antileishmanial drug) were used as positive controls. Cultures containing parasites without compound solution were also included. The plate was incubated at 26 °C for 72 h and the leishmanicidal and trypanocidal activity of compounds were determined by direct count of parasites in a Neubauer chamber.[22] The concentration required to inhibit 50% of the parasites grow (IC_{50}) was calculated by probit analysis.

4.4.3. Anti-*Mycobacterium* tuberculosis assay[17,18]

M. tuberculosis $H_{37}Rv$ (ATTC 27294) was used in the present study, which is sensitive to all five first-line antituberculosis drugs (STR, INH, RIF, ETH and PYR). Mycobacteria was cultured at 37 °C and 5% CO_2 atmosphere in Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC enrichment (oleic acid–albumin–dextrose–catalase) until the logarithmic phase of growth was reached. The inoculum for the assay was prepared by diluting a logarithmically growing culture to match the McFarland 1 turbidity standard and then further diluting this to 1:50 with Middlebrook 7H9 broth to obtain a concentration of 6×10^6 colony forming units/mL. The working suspension was prepared just before inoculation. The antibacterial activity of compounds against *M. tuberculosis* was tested using the modified MABA. The concentrations for compounds ranged from 8.000 μg/mL to 0.016 μg/mL. ETH was included as positive internal standard. All evaluations were carried out in triplicate.

4.4.4. Cytotoxicity on VERO cell line

The cytotoxicity assay was performed as reported previously,[16] where 1.5×10^4 viable cells from the VERO cell line were seeded in a 96-well plate and incubated for 24–48 h. VERO cells were grown in DMEM media supplemented with 10% (v/v) fetal bovine serum with 100 UI/mL penicillin and 100 mg/mL streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. When cells reached >80% confluence, the medium was replaced and the cells were treated with the compounds at 6.25, 12.5, 25, 50, and 100 μg/mL dissolved in DMSO at a maximum concentration of 0.05%. After 72 h of incubation, 10 μL of a 0.005% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. The medium was removed and the formazan, a product generated by the activity of dehydrogenases in cells, was dissolved in acidified isopropanol (0.4 N HCl). The amount of MTT-formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 540 nm using a Bio-assay reader. Metronidazole was used as a positive control, whereas untreated cells were used as negative controls. The concentration of the compound that killed 50% of the cells (CC_{50}) was calculated by GraphPad Prism 4 software. All determinations were performed in triplicate.[16]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.008. These data include MOL files and InChiKeys of the most important compounds described in this article.

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