Antiplasmodial and Leishmanicidal Activities of 2-Cyano-3-(4-phenylpiperazine-1-carboxamido) Quinoxaline 1,4-Dioxide Derivatives

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Abstract: Malaria and leishmaniasis are two of the World’s most important tropical parasitic diseases. Thirteen new 2-cyano-3-(4-phenylpiperazine-1-carboxamido) quinoxaline 1,4-dioxide derivatives (CPCQs) were synthesized and evaluated for their in vitro antimalarial and antileishmanial activity against erythrocytic forms of Plasmodium falciparum and axenic forms of Leishmania infantum. Their toxicity against VERO cells (normal monkey kidney cells) was also assessed. None of the tested compounds was efficient against Plasmodium, but two of them showed good activity against Leishmania. Toxicity on VERO was correlated with leishmanicidal properties.

Keywords: quinoxaline; piperazine; Plasmodium; Leishmania; VERO
1. Introduction

Malaria is a major public health problem in more than 90 countries, affecting 40% of the World’s poorest population. Mortality due to malaria is estimated to be over 1 million deaths annually and this situation is worsened by the spread of drug-resistant strains of the parasite. Therefore, new effective and affordable antimalarial agents are urgently needed [1,2].

Leishmaniasis threatens approximately 350 million people and almost 12 million people are currently infected with the disease. The emergence of resistant parasites, the high cost and toxicity of current treatments call for the discovery of new drugs [3,4].

Quinoxalines, also known as 1,4-benzodiazines, are aromatic bicycles that present two nitrogen atoms on positions 1 and 4, described as bioisosteres of quinoline, naphtyl and some other heteroaromatic rings including pirazine [5].

Quinoxaline derivatives are of great interest as antimycobacterial, anti-inflammatory, anticancer and antiparasitic agents. More specifically, their 1,4-di-N-oxides are considered to be particularly important because they are responsible for a resulting increase in various biological properties [6].

As a result of different research projects, our group synthesized different series of quinoxaline 1,4-di-N-oxide derivatives, with a great variety of substituents in positions 2, 3, 6 and 7 [7–15]. With the aim of improving their pharmacological properties, we synthesized compounds with a carbonitrile group in position 2, thereby enhancing their antiparasitic activity. We also added an amine group in position 3 in order to link together new molecules, leading to interesting activities [10,16].

*In silico* studies showed that piperazine derivatives could target *Plasmodium* plasmepsin II enzyme. We synthesized phenylpiperazines derivatives that were active against *Plasmodium falciparum* [17]. In this context, we have now synthesized thirteen new 3-amino-1,4-di-N-oxide quinoxaline-2-carbonitrile derivatives linked with phenyl piperazines analogs (Figure 1) and investigated their *in vitro* activity and toxicity against *Plasmodium falciparum* Colombian FCR-3 strain and *Leishmania infantum* [18].

![Figure 1](image-url)  
**Figure 1.** Design of new CPCQs as potential drugs against *P. falciparum* and *L. infantum*.

2. Results and Discussion

2.1. Chemistry

We prepared thirteen new 2-cyano-3-(4-phenylpiperazine-1-carboxamido) quinoxaline 1,4-dioxide derivatives (CPCQs; Scheme 1). The benzofuroxane starting compounds, (BFX, I), were prepared
using previously described methods [19,20]. The 3-amine-1,4-di-N-oxide quinoxaline-2-carbonitrile derivatives (cyanoamines, II) were obtained from the corresponding BFX by the Beirut reaction with malononitrile, using N,N-dimethylformamide (DMF) as solvent and triethylamine as catalyst [21].

Scheme 1. Synthetic route to CPCQs 1–13.

The method for synthesizing the final compounds consisted of two steps; the first one was the reaction of cyanoamines with an excess of commercially available 1,1'-carbonyldiimidazole (CDI) in order to obtain the intermediates 3a–m that can react in the second step with the phenylpiperazines, thus affording the CPCQs [22].

2.2. Pharmacology and Structure-Activity Relationship

With regard to antiplasmodial activity shown in Table 1, halogen groups in R⁷ and/or R⁶ increase the activity as shown in previous series of quinoxaline 1,4-di-N-oxide derivatives [16]. Compounds with CF₃ or CH₃O groups at R₄' show comparable activity, but higher activity than those which have F in that position. In relation with position R₂', the NO₂ group does not increase the activity.

With regard to leishmanicidal activity shown in Table 1, among the most active compounds, 5 and 8 were also among the most cytotoxic compounds against VERO cells. Interestingly, the presence of halogen groups in R⁷ and/or R⁶, responsible for increasing anti-malarial activity, also increased leishmanicidal activity. Moreover, the compounds without NO₂ group in R₂' showed considerably higher activity. Compounds with CF₃ or F in R₄' show similar activity, but higher activity than those with CH₃O in this position.
Table 1. Biological characterization of the thirteen new quinoxaline 1,4-di-N-oxides.

| Compd. | MW  | R⁶ | R⁷ | R²¹ | R⁴ | IC₅₀ (µM)ᵃ | IC₅₀ (µM)ᵇ | CC₅₀ (µM)ᶜ | SIᵈ |
|--------|-----|----|----|-----|----|------------|------------|------------|-----|
| 1      | 537.5 | H  | Cl | NO₂ | CF₃ | 24.5       | 21.8       | 7.0        | 0.3 |
| 2      | 517   | H  | CH₃| NO₂| CF₃ | 44.7       | 36.3       | 17.7       | 0.5 |
| 3      | 521   | H  | F  | NO₂| CF₃ | 14.6       | 41.1       | 11.0       | 0.3 |
| 4      | 572   | Cl | Cl | NO₂| CF₃ | 13.9       | 22.7       | 1.6        | 0.1 |
| 5      | 492.5 | H  | Cl | H  | CF₃ | 18.6       | 7.6        | 6.4        | 0.8 |
| 6      | 472   | H  | CH₃| H  | CF₃ | 30.5       | 23.3       | 12.1       | 0.5 |
| 7      | 476   | H  | F  | H  | CF₃ | 30.9       | 28.8       | 12.2       | 0.4 |
| 8      | 527   | Cl | Cl | H  | CF₃ | 18.5       | 5.7        | 2.2        | 0.4 |
| 9      | 422   | H  | CH₃| H  | F   | 36.3       | 23.0       | 24.1       | 1.1 |
| 10     | 426   | H  | F  | H  | F   | 34.3       | 31.3       | 24.3       | 0.8 |
| 11     | 454.5 | H  | Cl | H  | CH₃O| 12.8       | 18.8       | 47.5       | 2.5 |
| 12     | 434   | H  | CH₃| H  | CH₃O| 30.4       | 30.0       | 183.5      | 6.1 |
| 13     | 489   | Cl | Cl | H  | CH₃O| 26.1       | 10.9       | 14.0       | 1.3 |
| CQ     | 320   |    |    |    |     | 0.1        |            |            |     |
| DOX    | 543.5 |    |    |    |     | 6.4        | 0.4        | >10        |     |

Selectivity index (SI): DT₅₀ drug/IC₅₀ drug. MW: molecular weight; ᵃ IC₅₀ against *P. falciparum* FCR-3; ᵇ IC₅₀ against *L. Infantum*; ᶜ Cytotoxicity in VERO cells; ᵈ Selectivity index.

3. Experimental

3.1. Chemical Synthesis

3.1.1. General Remarks

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance (¹H-NMR), melting point and elemental microanalyses (CHN). Alugram SIL G/UV254 (0.2 mm layer, Macherey-Nagel GmbH & Co. KG., Düren, Germany) was used for TLC and silica gel 60 (0.040–0.063 mm, Merck, Darmstadt, Germany) was used for Flash Column Chromatography. The ¹H-NMR spectra were recorded on a Bruker 400 Ultrashield instrument (400 MHz, Bruker, Billerica, MA, USA), using TMS as internal standard and with DMSO-*d₆* as solvent; the chemical shifts are reported in ppm (δ) and coupling constants (J) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet), dd (double doublet) and m (multiplet). The IR spectra were recorded on a Nicolet Nexus FTIR (Thermo, Madison, WI, USA) in KBr pellets. Elemental microanalyses were obtained on a CHN-900 Elemental Analyzer (Leco, Tres Cantos, Spain) from vacuum-dried samples. The analytical results for C, H and N, were within ± 0.5 of the theoretical
values. Chemicals were purchased from Panreac Química S.A. (Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Spain), Acros Organics (Janssen Pharmaceutical, Geel, Belgium) and Lancaster (Bischheim-Strasbourg, France).

3.1.2. General Procedure for the Synthesis of Cyanoamines II

Malononitrile (18.0 mmol) was added to a solution of the appropriate benzofuroxane (I, 15.0 mmol) in DMF (10 mL). The mixture was allowed to stand at 0 °C. Triethylamine (1.5 mL) was added dropwise, and the reaction mixture was stirred at room temperature in darkness for 1 day. The resulting precipitate was filtered off and washed by adding diethyl ether, affording the target compound. The obtained red solid was used in the next step without further purification [10]. The yield of this reaction depends on the substituents in position 5 and 6 in the benzofuroxane.

3.1.3. General Procedure for the Synthesis of 2-Cyano-3-(4-phenylpiperazine-1-carboxamido) Quinoxaline 1,4-Dioxide (CPCQs)

The corresponding cyanoamine (5.0 mmol) was reacted with a slight excess (1.5 equiv.) of 1,1'-carbonyldiimidazole (CDI) in dry tetrahydrofuran (40 mL) during 3 hours at 50 °C. The intermediate (2.5 mmol) was stirred with an excess of phenylpiperazine (1.2 equiv.) in chloroform during 3 h at 50 °C and under nitrogen atmosphere. Next, the solvent was removed in vacuo and the solid was collected and purified by column chromatography using dichloromethane/methanol (9:1). Finally, the solvent was removed in vacuo and the solid precipitated with cold diethyl ether, and filtered off to obtain a solid [22].

7-Chloro-2-cyano-3-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (1). Yield 30%; $^1$H-NMR δ ppm: 8.30 (s, 1H, H$_{11'}$); 8.24 (d, 2H, H$_{2''}$, J$_{2''-3''}$ = 2.44 Hz); 8.22 (dd, 1H, H$_{9}$, J$_{9-8}$ = 2.73 Hz, J$_{9-9'}$ = 3.84 Hz); 8.19 (dd, 2H, H$_{6}$, J$_{6-2''}$ = 2.96 Hz, J$_{6-5'}$ = 3.70 Hz); 8.11 (s, NH); 7.96 (dd, 2H, H$_{3}$, J$_{3-5'}$ = 8.95 Hz, J$_{3-2''}$ = 9.02 Hz); 7.90 (d, 1H, H$_{8}$, J$_{8-9}$ = 2.01 Hz); 7.88 (d, 1H, H$_{8}$, J$_{8-6}$ = 2.68 Hz); 7.62 (d, 1H, H$_{6}$, J$_{6-5'}$ = 9.27 Hz); 7.54 (d, 1H, H$_{5}$, J$_{5-6}$ = 8.40 Hz); 7.49 (dd, 2H, H$_{5}$, J$_{5-3'}$ = 4.21 Hz, J$_{5-6'}$ = 8.77 Hz). IR $\nu$ cm$^{-1}$: 3,105 (w, NH); 2,211 (w, C≡N); 1,533 (s, C=O); 1,326 (s, N$^{+}$O$^{-}$). Anal. Calc. for C$_{21}$H$_{15}$N$_{7}$O$_{5}$F$_{3}$Cl: C: 46.88%; H: 2.79%; N: 18.23%. Found: C: 46.74%; H: 2.94%; N: 18.18%.

2-Cyano-7-methyl-3-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (2). Yield 50%; $^1$H-NMR δ ppm: 8.21 (d, 1H, H$_{5}$, J$_{5-6}$ = 3.85 Hz); 8.20 (d, 1H, H$_{8}$, J$_{8-6}$ = 1.66 Hz); 8.17 (s, 1H, NH); 7.91 (d, 1H, H$_{6}$, J$_{6-5}$ = 2.04 Hz); 7.88 (d, 1H, H$_{8}$, J$_{8-9}$ = 2.43 Hz); 7.51 (s, 1H, H$_{11}$); 7.49 (s, 1H, H$_{9}$); 3.69 (d, 2H, H$_{2}$, J$_{2-3}$ = 2.66 Hz); 3.25 (dd, 2H, H$_{6}$, J$_{6-5}$ = 2.65 Hz, J$_{6-2''}$ = 3.11 Hz); 3.11 (d, 2H, H$_{5}$, J$_{5-2''}$ = 5.27 Hz); 2.56 (dd, 2H, H$_{5}$, J$_{5-3'}$ = 2.79 Hz, J$_{5-6'}$ = 3.40 Hz); 2.54 (s, 3H, CH$_{3}$-C$_{7}$). IR $\nu$ cm$^{-1}$: 3,098 (w, NH); 2,231 (w, C≡N); 1,549 (s, C=O); 1,328 (s, N$^{+}$O$^{-}$). Anal. Calc. for C$_{22}$H$_{18}$N$_{7}$O$_{5}$F$_{3}$Cl: C: 51.06%; H: 3.48%; N: 18.95%. Found: C: 51.28%; H: 3.46%; N: 19.03%.

2-Cyano-7-fluoro-3-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (3). Yield 15%; $^1$H-NMR δ ppm: 8.38 (dd, 2H, H$_{3}$, J$_{3-5'}$ = 4.94 Hz, J$_{3-2''}$ = 9.74 Hz); 8.20
(dd, 2H, H2', J2'−6' = 2.68 Hz, J2'−3 = 3.37 Hz); 8.13 (d, 1H, H1'); 1.79 Hz); 8.11 (dd, 1H, H9, J8−9 = 1.42 Hz, J9−8 = 2.01 Hz); 7.90 (d, 2H, H5', J5'−6' = 2.64 Hz); 7.88 (dd, 2H, H6, J6−2' = 2.81 Hz, J6−5 = 1.47 Hz); 7.69 (dd, 1H, H8, J6−8 = 5.87 Hz, J5−5 = 9.23 Hz); 7.51 (d, 1H, H8, J8−6 = 2.62 Hz); 7.49 (d, 1H, H8, J8−9 = 2.67 Hz); 7.44 (d, 1H, H5, J5−6 = 2.13 Hz); 3.55 (s, 1H). IR ν cm−1: 3,108 (w, NH); 2,362 (w, C=N); 1,533 (s, C=O); 1,321 (s, N'O). Anal. Calc. for C21H15N7O5F4: C: 48.36%; H: 2.87%; N: 18.80%. Found: C: 47.92%; H: 2.94%; N: 18.31%.

6-7-Dichloro-2-cyano-3-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (4). Yield 20%; 1H-NMR δ ppm: 8.37 (d, 1H, H1'); 1.47 Hz); 8.31 (s, NH); 8.25 (d, 1H, H8, J5−8 = 1.98 Hz); 8.18 (d, 1H, H8, J5−8 = 2.41 Hz); 7.95 (dd, 1H, H9, J8−9 = 3.31 Hz, J9−8 = 9.84 Hz); 7.88 (d, 2H, H2', J2'−3 = 6.93 Hz); 7.73 (d, 2H, H6, J6−5 = 1.89 Hz); 7.51 (m, 2H, H5); 3.59 (d, 2H, H3, J3−2 = 4.38 Hz); 1.09 (t, 1H, H9, J8−9 = 6.73 Hz, J8−6 = 6.73 Hz). IR ν cm−1: 3,104 (w, NH); 2,240 (w, C=N); 1,532 (s, C=O); 1,330 (s, N'O). Anal. Calc. for C21H14N7O5F3Cl2: C: 44.05%; H: 2.44%; N: 17.13%. Found: C: 43.87%; H: 2.50%; N: 17.36%.

7-Chloro-2-cyano-3-(4-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (5). Yield 65%; 1H-NMR δ ppm: 8.13 (t, 1H, H9); 7.99 (t, 1H, H8, J5−9 = 9.06 Hz, J5−12 = 9.06 Hz); 7.78 (dd, 1H, H12, J12−8 = 3.86 Hz, J12−11 = 9.00 Hz); 7.62 (dd, 2H, H6, J6−2' = 1.21 Hz, J6−5 = 8.84 Hz); 7.58 (s, NH); 7.54 (d, 2H, H3, J3−2 = 8.50 Hz); 7.50 (dd, 1H, H11, J11−12 = 2.30 Hz, J11−12 = 19.19 Hz); 7.41 (dd, 1H, H6, J6−8 = 8.44 Hz, J6−5 = 9.06 Hz); 7.10 (dd, 2H, H5, J5−3 = 8.39 Hz, J5−6 = 9.06 Hz); 6.74 (d, 1H, H5, J5−6 = 7.39 Hz); 3.57 (d, 1H, H8, J8−6 = 1.47 Hz); 3.10 (d, 2H, H2', J2'−3 = 5.43 Hz). IR ν cm−1: 3,107 (w, NH); 2,234 (w, C=N); 1,524 (s, C=O); 1,333 (s, N'O). Anal. Calc. for C21H16N6O4F3Cl: C: 51.16%; H: 3.24%; N: 17.05%. Found: C: 50.85%; H: 3.12%; N: 16.93%.

2-Cyano-7-methyl-3-(4-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (6). Yield 23%; 1H-NMR δ ppm: 8.24 (d, 1H, H5, J5−6 = 8.08 Hz); 8.17 (d, 1H, H6, J5−6 = 6.82 Hz); 8.09 (s, 1H, H11); 8.02 (s, 1H, H9); 7.94 (s, NH); 7.82 (d, 1H, H8, J6−9 = 9.78 Hz); 7.54 (d, 2H, H2, J2−3 = 2.85 Hz); 7.53 (d, 2H, H3, J3−2 = 4.83 Hz); 7.39 (d, 1H, H12, J12−11 = 4.54 Hz); 7.32 (s, 1H, H5); 7.12 (d, 2H, H3, J3−2 = 2.91 Hz); 7.10 (d, 2H, H6, J6−5 = 1.88 Hz); 2.44 (s, 3H, CH3-C). IR ν cm−1: 3,082 (w, NH); 2,233 (w, C=N); 1,547 (s, C=O); 1,329 (s, N'O). Anal. Calc. for C22H19N6O3F3: C: 55.93%; H: 4.02%; N: 17.79%. Found: C: 55.67%; H: 3.88%; N: 18.27%.

2-Cyano-7-fluoro-3-(4-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (7). Yield 31%; 1H-NMR δ ppm: 7.89 (dd, 1H, H5, J5−8 = 2.57 Hz, J5−6 = 8.98 Hz); 7.71 (dd, 1H, H6, J6−8 = 2.75 Hz, J5−6 = 7.96 Hz); 7.67 (s, 1H, H8); 7.52 (dd, 2H, H6, J6−2' = 7.83 Hz, J6−5 = 9.14 Hz); 7.45 (s, 2H, H2'); 7.38 (d, 1H, H9, J9−8 = 8.55 Hz); 7.10 (dd, 2H, H5, J5−3 = 8.94 Hz, J5−6 = 9.57 Hz); 6.97 (d, 1H, H11, J11−12 = 9.11 Hz); 6.74 (dd, 2H, H3, J3−2 = 7.94 Hz, J3−2 = 9.35 Hz); 3.70 (s, NH); 2.98 (d, 1H, H12, J12−11 = 7.19 Hz); 2.59 (d, 1H, H6, J6−9 = 6.56 Hz). IR ν cm−1: 3,106 (w, NH); 2,232 (w, C=N); 1,532 (s, C=O); 1,332 (s, N'O). Anal. Calc. for C21H16N6O3F4: C: 52.94%; H: 3.36%; N: 17.64%. Found: C: 52.53%; H: 3.32%; N: 17.76%.

6,7-Dichloro-2-cyano-3-(4-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamido) quinoxaline 1,4-dioxide (8). Yield 17%; 1H-NMR δ ppm: 8.31 (s, 1H, H3); 7.88 (s, 1H, H8); 7.73 (d, 2H, H6,
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\[ J_{g-s} = 1.75 \text{ Hz}; J_{s-g} = 3.60 \text{ Hz}, J_{s-g'} = 9.32 \text{ Hz}; J_{s'} = 2.11 \text{ Hz} \]

2-Cyano-3-(4-(4-fluorophenyl)piperazine-1-carboxamido)-7-methylquinoxaline 1,4-dioxide (9). Yield 79\%; \(^1\)H-NMR \(\delta\) ppm: 8.23 (d, 1H, H\(_5\), J\(_{g-s}\) = 8.39 Hz); 8.16 (s, 1H, H\(_6\)); 7.94 (s, 1H, H\(_{11}\)); 7.81 (d, 1H, H\(_6\), J\(_{g-s}\) = 8.46 Hz); 7.52 (d, 1H, H\(_8\), J\(_{g-s}\) = 8.36 Hz); 7.39 (s, 1H, H\(_8\)); 7.17 (d, 1H, H\(_{12}\), J\(_{g-s}\) = 8.17 Hz); 7.08 (d, 2H, H\(_2\), J\(_{g-s}\) = 6.02 Hz); 7.06 (d, 2H, H\(_6\), J\(_{g-s}\) = 7.13 Hz); 7.01 (d, 2H, H\(_3\), J\(_{g-s}\) = 4.73 Hz); 6.99 (d, 2H, H\(_5\), J\(_{g-s}\) = 6.17 Hz); 3.19 (s, 3H, CH\(_3\)C\(_7\)). IR v cm\(^{-1}\): 3,079 (w, NH); 2,231 (w, C=\(\equiv\)N); 1,538 (s, C=O); 1,344 (s, N\(^{+}\)O\(^-\)). Anal. Calc. for C\(_{21}\)H\(_{19}\)N\(_6\)O\(_3\)F: C: 59.71\%; H: 4.50\%; N: 19.90\%. Found: C: 59.80\%; H: 4.55\%; N: 19.51\%.

2-Cyano-3-(4-(4-fluorophenyl)piperazine-1-carboxamido)quinoxaline 1,4-dioxide (10). Yield 23\%; \(^1\)H-NMR \(\delta\) ppm: 8.41 (d, 1H, H\(_5\), J\(_{g-s}\) = 5.14 Hz); 8.38 (d, 1H, H\(_6\), J\(_{g-s}\) = 5.96 Hz); 8.14 (s, 1H, H\(_9\)); 8.12 (s, 1H, H\(_{11}\)); 7.91 (t, 2H, H\(_5\), J\(_{g-s}\) = 8.92 Hz, J\(_{g-s}\) = 8.92 Hz); 7.45 (s, 1H, H\(_8\)); 7.10 (s, 1H, H\(_8\)); 7.07 (s, 2H, H\(_2\)); 7.05 (s, 1H, H\(_{12}\)); 7.01 (d, 2H, H\(_3\), J\(_{g-s}\) = 3.69 Hz); 7.00 (d, 2H, H\(_5\), J\(_{g-s}\) = 3.25 Hz); 6.99 (s, 1H, NH). IR v cm\(^{-1}\): 3,109 (w, NH); 2,231 (w, C=\(\equiv\)N); 1,534 (s, C=O); 1,354 (s, N\(^{+}\)O\(^-\)). Anal. Calc. for C\(_{20}\)H\(_{16}\)N\(_6\)O\(_3\)F\(_2\): C: 56.33\%; H: 3.75\%; N: 19.71\%. Found: C: 55.84\%; H: 3.68\%; N: 19.53\%.

7-Chloro-2-cyano-3-(4-(4-methoxyphenyl)piperazine-1-carboxamido)quinoxaline 1,4-dioxide (11). Yield 12\%; \(^1\)H-NMR \(\delta\) ppm: 8.24 (s, 1H, H\(_8\)); 8.19 (d, 1H, H\(_6\), J\(_{g-s}\) = 8.47 Hz); 8.13 (d, 1H, H\(_5\), J\(_{g-s}\) = 8.63 Hz); 8.07 (s, 1H, NH); 7.90 (d, 1H, H\(_9\), J\(_{g-s}\) = 7.83 Hz); 7.63 (d, 1H, H\(_{12}\), J\(_{g-s}\) = 6.55 Hz); 7.58 (s, 1H, H\(_{11}\)); 7.53 (d, 1H, H\(_8\), J\(_{g-s}\) = 8.73 Hz); 6.93 (d, 2H, H\(_6\), J\(_{g-s}\) = 6.87 Hz); 6.91 (d, 2H, H\(_2\), J\(_{g-s}\) = 3.39 Hz); 6.84 (d, 2H, H\(_3\), J\(_{g-s}\) = 7.41 Hz); 6.82 (d, 2H, H\(_5\), J\(_{g-s}\) = 6.48 Hz); 3.63 (s, 3H, CH\(_3\)O). IR v cm\(^{-1}\): 3,115 (w, NH); 2,228 (w, C=\(\equiv\)N); 1,540 (s, C=O); 1,351 (s, N\(^{+}\)O\(^-\)). Anal. Calc. for C\(_{21}\)H\(_{19}\)N\(_6\)O\(_4\)Cl: C: 55.44\%; H: 4.18\%; N: 18.48\%. Found: C: 54.97\%; H: 3.95\%; N: 18.21\%.

2-Cyano-3-(4-(4-methoxyphenyl)piperazine-1-carboxamido)-7-methylenquinoline 1,4-dioxide (12). Yield 32\%; \(^1\)H-NMR \(\delta\) ppm: 8.02 (d, 1H, H\(_5\), J\(_{g-s}\) = 8.69 Hz); 7.94 (d, 1H, H\(_6\), J\(_{g-s}\) = 2.45 Hz); 7.61 (dd, 1H, H\(_{12}\), J\(_{g-s}\) = 1.78 Hz, J\(_{g-s}\) = 8.64 Hz); 7.52 (d, 1H, H\(_6\), J\(_{g-s}\) = 8.61 Hz); 7.39 (d, 2H, H\(_5\), J\(_{g-s}\) = 3.60 Hz); 7.32 (s, 2H, H\(_6\)); 7.27 (dd, 1H, H\(_8\), J\(_{g-s}\) = 1.61 Hz, J\(_{g-s}\) = 8.82 Hz); 6.92 (d, 1H, H\(_9\), J\(_{g-s}\) = 8.44 Hz); 6.84 (d, 2H, H\(_3\), J\(_{g-s}\) = 8.67 Hz); 3.69 (t, 3H, CH\(_3\)O, J\(_{g-s}\) = 3.39 Hz); 3.62 (s, 2H, H\(_2\)); 3.13 (s, 1H, NH); 2.55 (s, 3H, CH\(_3\)C\(_7\)); 1.23 (s, 1H, H\(_{11}\)). IR v cm\(^{-1}\): 3,120 (w, NH); 2,230 (w, C=\(\equiv\)N); 1,546 (s, C=O); 1,328 (s, N\(^{+}\)O\(^-\)). Anal. Calc. for C\(_{22}\)H\(_{22}\)N\(_6\)O\(_4\): C: 60.82\%; H: 5.06\%; N: 19.35\%. Found: C: 61.20\%; H: 4.61\%; N: 19.84\%.

6,7-Dichloro-2-cyano-3-(4-(4-methoxyphenyl)piperazine-1-carboxamido)quinoxaline 1,4-dioxide (13). Yield 11\%; \(^1\)H-NMR \(\delta\) ppm: 8.46 (d, 1H, H\(_8\), J\(_{g-s}\) = 8.85 Hz); 8.29 (s, 1H, NH); 8.19 (d, 1H, H\(_{12}\), J\(_{g-s}\) = 1.33 Hz); 7.87 (s, 1H, H\(_8\)); 7.73 (d, 2H, H\(_6\), J\(_{g-s}\) = 2.11 Hz); 6.94 (s, 1H, H\(_3\)); 6.91 (s, 1H,
H9'); 6.85 (s, 1H, H11'); 6.83 (s, 2H, H2'); 3.69 (s, 3H, CH3O); 3.18 (d, 2H, H3', $J_{3'-2'}$ = 3.00 Hz); 3.17 (d, 2H, H5', $J_{5'-6'}$ = 2.12 Hz). IR ν cm$^{-1}$: 3,115 (w, NH); 2,229 (w, C≡N); 1,542 (s, C=O); 1,352 (s, N$^+$O$^-$).

Anal. Calc. for C$_{21}$H$_{18}$N$_6$O$_4$Cl$_2$: C: 51.53%; H: 3.68%; N: 17.17%. Found: C: 51.05%; H: 3.49%; N: 17.02%.

3.2. Pharmacology

3.2.1. In Vitro Antiplasmodial Drug Assay

Chloroquine resistant FCR-3 strain of *P. falciparum* was cultivated at 37 °C in a 5% CO$_2$ environment on glucose-enriched RPMI 1640 medium supplemented with gentamicin 0.1 mg/mL and 10% heat-inactivated A+ human serum, as previously described [23]. The drugs, dissolved in dimethylsulfoxide (DMSO), were added at final concentrations ranging from 250 to 0.1 µM. The final DMSO concentration was never greater than 0.1%. *In vitro* antimalarial activity was measured using the [3H]-hypoxanthine (MP Biomedicals, Santa Ana, CA, USA) incorporation assay [24]. Briefly, 250 µL of total culture medium with the diluted drug and the suspension of human red blood cell in medium (A$^+$ group, 5% haematocrit) with 1% parasitaemia, were placed into the wells of 96-well microtitre plates. After 48 h of incubation at 37 °C in a 5% O$_2$, 5% CO$_2$ and 90% N$_2$ atmosphere. On the third day of the test, radioactivity was assessed. All experiments were performed in triplicate. Results were expressed as the concentration resulting in 50% inhibition (IC$_{50}$) which was calculated by a nonlinear regression logistic dose response model; the mean IC$_{50}$ values and standard deviation for each compound was calculated.

3.2.2. In Vitro Cytotoxicity

Toxicity was determined using Vero cells (normal monkey kidney cells) cultured under the same conditions as *P. falciparum*, except for the replacement of 5% human serum with 10% fetal calf serum. After the addition of compounds at increasing concentrations, cell growth was measured by [3H]-hypoxanthine incorporation after a 48-hour incubation period and then compared with a control sample [25].

3.2.3. In Vitro Antileishmanial Drug Assay

Leishmanicidal activity was determined on axenic cultures of *L. infantum* amastigotes. In order to estimate the 50% inhibitory concentration (IC$_{50}$) of the drugs, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micromethod was used as previously described [26].

Briefly, Leishmania strain was maintained in promastigote stage in a biphasic medium (blood agar with 0.89% NaCl, pH 7.4) at 24 °C, with sub-passage every 3–4 days. Promastigotes ($5 \times 10^6$ parasites) were then transferred in M199 medium supplemented with 10% fetal bovine serum, pH 7.4. After 4 days, exponential phase promastigotes were centrifuged for 10 min at 1,500 g and 4 °C. The supernatant was discarded and replaced by fresh M199 medium supplemented with 20% FBS, pH 5.5. Axenic amastigotes transformation was then induced by increasing the temperature to 34 °C. Drugs were then tested at increasing concentrations.
4. Conclusions

All the tested compounds were almost 100 times less active against Plasmodium than chloroquine. Consequently they did not deserve further examination as antimalarials. Against Leishmania compounds 5 and 8 showed good activity and the most cytotoxic compound is four times less toxic than the reference drug. Unfortunately, these compounds show a low selectivity index. In this context, we suggest that synthesis should be focused on compounds with halogens at R⁷ and/or R⁶ in order to improve activity and lower toxicity.

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*Sample Availability:* Samples of the compounds are available from the authors.

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