Vicente, E; Charnaud, S; Bongard, E; Villar, R; Burguete, A; Solano, B; Ancizu, S; Prez-Silanes, S; Aldana, I; Vivas, L; Monge, A (2008) Synthesis and antiplasmodial activity of 3-furyl and 3-thienylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives. Molecules, 13 (1). pp. 69-77. ISSN 1420-3049 DOI: https://doi.org/10.3390/molecules13010069

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Synthesis and Antiplasmodial Activity of 3-Furyl and 3-Thienylquinoxaline-2-carbonitrile 1,4-Di-N-oxide Derivatives

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† Initial data presented at the 11th International Electronic Conference on Synthetic Organic Chemistry, ECSOC-11, http://www.usc.es/congresos/ecsoc/11/ECSOC11.htm, 1-30 November 2007, paper c006

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Received: 18 December 2007 / Accepted: 11 January 2008 / Published: 17 January 2008

Abstract: The aim of this study was to identify new compounds active against Plasmodium falciparum based on our previous research carried out on 3-phenylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives. Twelve compounds were synthesized and evaluated for antimalarial activity. Eight of them showed an IC$_{50} < 1$ µM against the 3D7 strain. Derivative 1 demonstrated high potency (IC$_{50}= 0.63$ µM) and good selectivity (SI=10.35), thereby becoming a new lead-compound.

Keywords: Quinoxaline, N-oxides, malaria, antiplasmodial, P. falciparum.
Introduction

Malaria is by far the world’s most important tropical parasitic disease. Mortality, currently estimated at over a million people per year, has risen in recent years, probably due to increasing resistance to antimalarial medicines. It exacts a heavy toll of illness and death - especially amongst children and pregnant women. It also poses a risk to travellers and immigrants, with imported cases increasing in non-endemic areas [1]. A major drawback in the chemotherapy of malaria is the rapid and widespread development of drug resistance in Plasmodia to most existing antimalarials [2, 3]. This is the reason research was aimed at the development of new antimalarials [4].

Based on activity results, our group had previously synthesized 3-phenylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives [5], and in attempts to establish the structural requirements for inhibition of P. falciparum, we decided to synthesize new compound series with a structural modifications from our previous lead-compounds (Figure 1). The change that was carried out was the substitution of the phenyl subunit in position 3 by 2-furyl (series A) or 2-thienyl (series B) moieties, following the classical notion of bioisosteric replacement used in Medicinal Chemistry [6], in order to determine the influence of the size and nature of aromatics rings in this position.

Figure 1. Design of new derivatives as antimalarial drugs from our previous lead.

Results and Discussion

The most potent quinoxaline derivative from our previous in vitro studies was 3-(4'-chlorophenyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide [5] which was subjected to a structural change in order to obtain new active compounds: replacement of the benzene in position 3 of the quinoxaline subunit by a heteroaromatic 5-membered 2-furan or 2-thiophene ring.

Twelve new compounds were thus synthesized and evaluated for antimalarial activity against Plasmodium falciparum. The modified quinoxaline 1,4-di-N-oxide derivatives 1-12 were evaluated against the CQ (chloroquine)-sensitive 3D7 strain and the results are listed in Table 1. Compounds were selected for further assays if their IC50 values were less than 1 μM when tested against said 3D7 strain. The chosen compounds were then tested against K1 strain (CQ-resistant) and their cytotoxicities were determined in mammalian KB cells (Table 1).
Upon carrying out the biosteric replacement of phenyl subunit with a heteroaromatic 5-membered ring, the importance of the heteroatom is clearly observed; only two of the 2-thienyl derivatives (series B) showed an IC₅₀ <1 µM, whereas all of the 2-furyl derivatives (series A) show IC₅₀ values ranging from 0.49 to 0.93 µM.

All of the derivatives that were tested showed superior activity towards the K1 strain, in comparison with chloroquine (IC₅₀(1-6)<IC₅₀(CQ)). In this way, the 3-(2'-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives appear to be novel and promising antimalarial candidates.

The activities of these derivatives are also affected by the different substituents on the quinoxaline nucleus: regarding the R7 position, it has been observed that the presence of a trifluoromethyl (5, 11) or a methoxy (6, 12) group results in two of the most potent compounds in each series. The 7-chloro derivatives 3, 9 show contrasted effects: while the furyl derivative 3 is the most potent compound against 3D7 and the second most active against K1, the thienyl derivative 9 was inactive towards 3D7.

Unfortunately, the most active compounds against *P. falciparum* (3, 5 and 6 in series A and 11 and 12 in series B) are also cytotoxic in KB cells, with IC₅₀ values lower than 4 µM. The most cytotoxic derivatives are 5, 11, which include a CF₃ group in position R7 of the quinoxaline ring, and the least cytotoxic compound is the 3-furyl derivative 1 nonsubstituted in the R7 position.

**Table 1.** Structures, *in vitro* activity against *P. falciparum* (3D7 and K1 strains) and cytotoxicity in mammalian KB cells.

| Comp. | R7 | X  | 3D7 IC₅₀ (µM)ᵃ | K1 IC₅₀ (µM)ᵃ | KB IC₅₀ (µM)ᵃ |
|-------|----|----|----------------|---------------|--------------|
| 1     | H  | O  | 0.75±0.31      | 0.63±0.18     | 6.52±0.86    |
| 2     | F  | O  | 0.77±0.22      | 0.59±0.06     | 5.59±0.44    |
| 3     | Cl | O  | 0.49±0.28      | 0.34±0.04     | 3.48±0.78    |
| 4     | CH₃| O  | 0.93±0.41      | 0.56±0.01     | 4.38±0.50    |
| 5     | CF₃| O  | 0.53±0.25      | 0.37±0.12     | 3.00±0.43    |
| 6     | OCH₃| O | 0.63±0.14      | 0.28±0.07     | 3.17±0.67    |
| 7     | H  | S  | 2.97±0.33      | NDᵇ          | ND           |
| 8     | F  | S  | >10            | ND           | ND           |
| 9     | Cl | S  | 7.41±0.20      | ND           | ND           |
| 10    | CH₃| S  | 2.86±0.46      | ND           | ND           |
| 11    | CF₃| S  | 0.89±0.36      | ND           | 1.80±0.47    |
| 12    | OCH₃| S | 0.80±0.10      | ND           | 3.80±0.22    |
| CQᶜ   |    |    | 0.0135         | 0.682         | 110          |

ᵃ Figures are the mean IC₅₀ values (µM) ± standard derivation from independent experiments each performed in triplicate.

ᵇ No data

c Chloroquine is used as a standard drug.
As observed in Table 1, all of the 3-furyl derivatives showed a tendency towards lower IC₅₀ values against the K1 strain than against the 3D7 one. The most interesting results were obtained from calculating the Resistance and Selectivity Indexes (RI and SI) of selected compounds (IC₅₀ < 1 µM), as illustrated in Table 2. While chloroquine shows an IC₅₀ 50 times higher in the K1 strain than in the 3D7 strain (RI = 50), we obtained RI values lower than 1 for all of our most active compounds (Table 2). This fact suggests low levels of cross-resistance to CQ [7].

Table 2. Resistance index (RI) and Selectivity indexes (SI).

| Comp. | RI² (K1/3D7) | SIb 3D7 | SIb K1 |
|-------|--------------|---------|--------|
| 1     | 0.84         | 8.69    | 10.35  |
| 2     | 0.76         | 7.26    | 9.47   |
| 3     | 0.69         | 7.10    | 10.24  |
| 4     | 0.60         | 4.81    | 7.82   |
| 5     | 0.71         | 5.66    | 8.11   |
| 6     | 0.44         | 5.03    | 11.32  |
| CQ²   | 50.52        | 8150    | 161    |

²Resistance Index. calculated as (3D7 IC₅₀)/(K1 IC₅₀).
²Selectivity Index calculated as (KB IC₅₀)/(P. falciparum IC₅₀).
²Chloroquine is used as a standard drug.

With regard to the selectivity of the compounds, the SI value required for a compound to be selected for in vivo assays is 10 [8]. Four of the tested derivatives (1, 2, 3 and 6) showed a SI value close to the established cut-off with respect to the K1 strain; in addition, the 3-(2’-furyl) derivative 1, nonsubstituted in the R7 position, was the most selective compound with respect to the 3D7 strain. The potency, low cytotoxicity and selectivity of 3-(2’-furyl)quinoxaline 1,4-di-N-oxide (1) make it valid lead for synthesizing new compounds that may possess better activity.

Conclusions

Screening of the in vitro antimalarial activity of these novel series has evidenced that the 3-(2’-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives 1-6 appear to be novel and promising antimalarial candidates, improving upon the activity of chloroquine against the K1 strain. The nonsubstituted derivative in position R7 is especially noteworthy, as it showed the lowest cytotoxicity and the highest SI values.

Experimental

General

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), infrared (IR), nuclear magnetic resonance (¹H-NMR), mass spectra (MS) and elemental microanalysis (CHN). Alugram SIL G/UV254 (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG.
Postfach 101352. D-52313 Düren, Germany) was used for Thin Layer Chromatography and Silica gel 60 (0.040-0.063 mm) for Column flash Chromatography (Merck). The $^1$H-NMR spectra were recorded on a Bruker 400 Ultrashield instrument (400 MHz), using TMS as the internal standard and with CDCl$_3$ as the 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), d (doublet), t (triplet), q (quadruplet), dd (double doublet) and m (multiplet). The IR spectra were performed on a Thermo Nicolet Nexus FTIR (Madison, USA) in KBr pellets; the frequencies are expressed in cm$^{-1}$. The mass spectra were measured on an Agilent Technologies Model MSD/DS 5973N (mod. G2577A) mass spectrometer with direct insertion probe (DIP) (Waldbonn, Germany) and the ionization method was electron impact (EI, 70 eV). Elemental microanalyses were obtained on an Elemental Analyzer (Leco CHN-900, Tres Cantos, Madrid, Spain). The analytical results for C, H, and N, were within ± 0.4 of the theoretical values. Chemicals were purchased from Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceuticaal 3a, 2440 Geel, Belgium) and Lancaster (Bischheim-Strasbourg, France).

Synthesis of 3-(2’furyl) and 3-(2’-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives 1-12

The required 3-arylquinoxaline-2-carbonitrile derivatives were obtained by the classical Beirut reaction [9]. The method used for their synthesis has been reported previously [5, 10]. The appropriate benzofuroxan and the corresponding arylacetonitrile were dissolved in dry dichloromethane in the presence of triethylamine, which acted as the catalyst.

3-(2’-Furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (1). IR $\nu$/cm$^{-1}$: 2233, 1336; $^1$H-NMR δ/ppm: 6.80 (td, $J=1.77$, 3.65 Hz, 1H, H4), 7.86 (d, $J=0.74$ Hz, 1H, H3), 7.91 (tdd, $J=1.40$, 7.13, 8.61 Hz, 1H, H7), 8.01 (tdd, $J=1.52$, 7.11, 8.63 Hz, 1H, H6), 8.38 (dd, $J=0.90$, 3.64 Hz, 1H, H5), 8.62 (d, $J=8.58$ Hz, 1H, H8), 8.70 (d, $J=8.64$ Hz, 1H, H5); Anal. calcd. for C$_{13}$H$_7$N$_3$O$_3$: C, 61.66; H, 2.79; N, 16.59. Found: C, 61.67; H, 2.86; N, 16.92; MS m/z (%): 253 ([M]+, 51), 237 (25), 191 (52), 175 (36), 105 (100).

7-Fluoro-3-(2’-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (2). IR $\nu$/cm$^{-1}$: 2238, 1327; $^1$H-NMR δ/ppm: 6.81 (td, $J=1.75$, 3.70 Hz, 1H, H4), 7.73 (ddd, $J=2.77$, 7.26, 9.96 Hz, 1H, H3), 7.87 (dd, $J=0.73$, 1.69 Hz, 1H, H5), 8.28 (dd, $J=2.69$, 7.97 Hz, 1H, H8), 8.35 (dd, $J=0.71$, 3.66 Hz, 1H, H5), 8.73 (dd, $J=4.94$, 9.45 Hz, 1H, H3); Anal. calcd. for C$_{13}$H$_6$FN$_3$O$_3$: C, 57.57; H, 2.23; N, 15.49. Found: C, 57.77; H, 2.21; N, 15.87.

7-Chloro-3-(2’-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (3). IR $\nu$/cm$^{-1}$: 2226, 1323; $^1$H-NMR δ/ppm: 6.81 (td, $J=1.78$, 3.66 Hz, 1H, H4), 7.87 (dd, $J=0.77$, 1.69 Hz, 1H, H3), 7.92 (dd, $J=2.21$, 9.18 Hz, 1H, H5), 8.37 (dd, $J=0.75$, 3.66 Hz, 1H, H5), 8.60 (dd, $J=0.45$, 2.20 Hz, 1H, H8), 8.64 (d, $J=9.18$ Hz, 1H, H3); Anal. calcd. for C$_{13}$H$_6$ClN$_3$O$_3$: C, 54.28; H, 2.10; N, 14.61. Found: C, 54.30; H, 2.29; N, 14.74; MS m/z (%): 287 ([M]+, 100), 271 (30).
7-Methyl-3-(2'-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (4). IR υ/cm⁻¹: 2226, 1330; ¹H-NMR δ/ppm: 2.67 (s, 3H, C7'-CH₃), 6.79 (td, J=0.60, 2.57 Hz, 1H, H₄'), 7.81 (dd, J=1.82, 8.81 Hz, 1H, H₈), 7.87 (dd, J=0.78, 1.72 Hz, 1H, H₃'), 8.34 (dd, J=0.72, 3.63 Hz, 1H, H₅'), 8.40 (s, 1H, H₈), 8.58 (d, J=8.80 Hz, 1H, H₃); Anal. calcd. for C₁₄H₉N₃O₃ (267.25): C, 62.92; H, 3.39; N, 15.72. Found: C, 62.54; H, 3.48; N, 15.77; MS m/z (%): 267 ([M⁺], 100), 251 (21).

7-Trifluoromethyl-3-(2'-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (5). IR υ/cm⁻¹: 2232, 1342, 1318; ¹H-NMR δ/ppm: 6.83 (td, J=1.67, 3.65 Hz, 1H, H₄'), 7.90 (dd, J=0.73, 1.57 Hz, 1H, H₃'), 8.17 (dd, J=0.68, 1.67 Hz, 1H, H₆), 8.45 (dd, J=0.58, 3.62 Hz, 1H, H₅'), 8.83 (d, J=8.99 Hz, 1H, H₈), 8.91 (s, 1H, H₈); Anal. calcd. for C₁₄H₆F₃N₃O₃ (321.22): C, 52.35; H, 1.88; N, 13.08. Found: C, 52.53; H, 1.90; N, 12.90.

7-Methoxy-3-(2'-furyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (6). IR υ/cm⁻¹: 2226, 1329; ¹H-NMR δ/ppm: 4.06 (s, 3H, C7'-OCH₃), 6.77 (td, J=1.75, 3.63 Hz, 1H, H₄'), 7.57 (dd, J=2.74, 9.52 Hz, 1H, H₈), 7.83 (dd, J=0.68, 1.67 Hz, 1H, H₃'), 8.28 (dd, J=1.32, 8.59 Hz, 1H, H₆), 8.69 (dd, J=0.96, 4.26 Hz, 1H, H₅'), 8.74 (dd, J=1.22, 8.63 Hz, 1H, H₃); Anal. calcd. for C₁₄H₉O₃N (283.25): C, 59.37; H, 3.20; N, 14.84. Found: C, 59.14; H, 3.27; N, 15.00; MS m/z (%): 283 ([M⁺], 100), 267 (26).

3-(2'-Thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (7). IR υ/cm⁻¹: 2225, 1352, 1322; ¹H-NMR δ/ppm: 7.38 (td, J=4.29, 5.10 Hz, 1H, H₄'), 7.87 (dd, J=0.96, 5.12 Hz, 1H, H₆), 7.92 (dd, J=1.28, 7.10, 8.48 Hz, 1H, H₇), 8.03 (ddd, J=1.28, 7.10, 8.48 Hz, 1H, H₆), 8.61 (dd, J=1.32, 8.59 Hz, 1H, H₈), 8.69 (dd, J=0.96, 4.26 Hz, 1H, H₅'), 8.74 (dd, J=1.22, 8.63 Hz, 1H, H₃); Anal. calcd. for C₁₃H₇N₃O₂S (269.28): C, 57.99; H, 2.62; N, 15.60. Found: C, 57.72; H, 2.63; N, 15.75; MS m/z (%): 269 ([M⁺], 100), 253 (43), 224 (54).

7-Fluoro-3-(2'-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (8). IR υ/cm⁻¹: 2219, 1354, 1312; ¹H-NMR δ/ppm: 7.39 (td, J=4.74, 5.10 Hz, 1H, H₄'), 7.76 (ddd, J=2.74, 7.26, 9.91 Hz, 1H, H₆), 7.89 (dd, J=0.89, 5.12 Hz, 1H, H₃'), 8.27 (dd, J=2.23, 7.90 Hz, 1H, H₆), 8.66 (dd, J=0.80, 4.30 Hz, 1H, H₅'), 8.77 (dd, J=4.92, 9.50 Hz, 1H, H₃); Anal. calcd. for C₁₃H₆F₃N₃O₂S (287.27): C, 54.35; H, 2.11; N, 14.63. Found: C, 54.59; H, 2.14; N, 15.00.

7-Chloro-3-(2'-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (9). IR υ/cm⁻¹: 2228, 1353, 1311; ¹H-NMR δ/ppm: 7.39 (m, 1H, H₄'), 7.89 (dd, J=1.00, 5.51 Hz, 1H, H₆), 7.94 (dd, J=2.23, 9.21 Hz, 1H, H₆), 8.59 (d, J=2.21 Hz, 1H, H₃), 8.68 (d, J=9.17 Hz, 1H, H₆), 8.69 (dd, J=0.98, 4.27 Hz, 1H, H₅); Anal. calcd. for C₁₃H₆ClN₃O₂S (303.73): C, 51.41; H, 1.99; N, 13.83. Found: C, 51.26; H, 2.08; N, 14.14; MS m/z (%): 303 ([M⁺], 99), 287 (53), 273 (45), 258 (100).

7-Methyl-3-(2'-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (10). IR υ/cm⁻¹: 2232, 1343, 1318; ¹H-NMR δ/ppm: 2.67 (s, 3H, C7'-CH₃), 7.37 (td, J=4.34, 5.04 Hz, 1H, H₄'), 7.83 (dd, J=1.43, 8.72 Hz, 1H, H₈), 7.86 (dd, J=0.87, 4.25 Hz, 1H, H₃'), 8.39 (s, 1H, H₈), 8.61 (d, J=8.81 Hz, 1H, H₆), 8.66 (dd, J=0.90, 4.24 Hz, 1H, H₅'); Anal. calcd. for C₁₄H₉N₃O₂S (283.31): C, 59.35; H, 3.20; N, 14.83. Found: C, 59.32; H, 3.16; N, 14.52; MS m/z (%): 283 ([M⁺], 100), 267 (41), 238 (54).
7-Trifluoromethyl-3-(2′-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (11). IR υ/cm⁻¹: 2230, 1354, 1314; ¹H-NMR δ/ppm: 7.42 (td, J=4.32, 5.16 Hz, 1H, H₄'), 7.93 (dd, J=0.95, 5.09 Hz, 1H, H₃'), 8.20 (dd, J=1.83, 9.03 Hz, 1H, H₆), 8.76 (dd, J=0.93, 4.28 Hz, 1H, H₅), 8.87 (d, J=8.97 Hz, 1H, H₃), 8.90 (s, 1H, H₈); Anal. calcd. for C₁₄H₆F₃N₃O₂S (337.28): C, 49.86; H, 1.79; N, 12.46. Found: C, 50.04; H, 1.84; N, 12.82.

7-Methoxy-3-(2′-thienyl)quinoxaline-2-carbonitrile 1,4-di-N-oxide (12). IR υ/cm⁻¹: 2231, 1351, 1313; ¹H-NMR δ/ppm: 4.07 (s, 3H, C₇′-OCH₃), 7.35 (td, J=4.23, 5.14 Hz, 1H, H₄), 7.59 (dd, J=2.75, 9.50 Hz, 1H, H₆), 7.83 (dd, J=1.03, 5.13 Hz, 1H, H₃), 7.88 (d, J=2.64, 1H, H₅), 8.60 (dd, J=1.01, 4.23 Hz, 1H, H₅'), 8.63 (d, J=9.52 Hz, 1H, H₆); Anal. calcd. for C₁₄H₉N₃O₃S (299.31): C, 56.18; H, 3.03; N, 14.04. Found: C, 56.08; H, 3.27; N, 14.19; MS m/z (%): 299 ([M⁺], 26), 285 (63), 256 (24), 167 (39), 111 (100).

Plasmodium falciparum in vitro culture and parasite growth inhibition assays

In vitro evaluation of the antimalarial activity was carried out at the London School of Hygiene and Tropical Medicine. Biological tests were performed according to the previously described method [11]. All parasite clones, isolates and strains were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, Virginia, USA). Strains/isolates used in this study were: the drug sensitive 3D7 clone of the NF54 isolate (unknown origin); and the chloroquine, pyrimethamine and cycloguanyl resistant K1 strain (Thailand). In vitro culture of P. falciparum was carried out following standard methods [12] with modifications as described [8]. In vitro parasite growth inhibition was assessed by the incorporation of [³H] hypoxanthine based on the method used by Desjardins [13] and modified as described [11]. Briefly, stock drug solutions were dissolved in 100% dimethylsulfoxide (Sigma, Dorset, UK) and 50 μL of a 3-fold dilution series (10.0, 3.33, 1.11, 0.370, 0.124, and 0.0412 μg/mL) of the drugs prepared in assay medium [RPMI 1640 supplemented with 0.5% Albumax II (Invitrogen), 0.2% w/v glucose, 0.03% L-glutamine, and 5 μM hypoxanthine] added to each well of 96-well plates in triplicate. Fifty microlitres of asynchronous (65–75% ring stage) P. falciparum culture (0.5% parasitemia) or uninfected erythrocytes (blank) were added to each well reaching a final volume of 100 μL per well, a final hematocrit of 2.5% and final dimethylsulfoxide concentrations ≤0.01%. Plates were incubated at 37 °C in 5% CO₂, and 95% air mixture for 24 h, at which point [³H]hypoxanthine (Perkin-Elmer, Hounslow, UK, 10 μL) was added to each well (0.2 μCi/well). After an additional 24 h incubation period, the experiment was terminated by placing the plates in a -80 °C freezer. Plates were thawed and harvested onto glass fibre filter mats using a 96-well cell harvester (Harvester 96, Tomtec, Oxon, UK) and left to dry. After the addition of MeltiLex solid scintillant (Perkin–Elmer, Hounslow, UK) the incorporated radioactivity was counted using a Wallac 1450 Betalux scintillation counter (Wallac). Data acquired by the Wallac BetaLux scintillation counter were exported into a MICROSOFT EXCEL spreadsheet (Microsoft), and the IC₅₀ values of each drug were calculated by using XLFit line fitting software (ID Business Solutions, UK). Chloroquine diphosphate, as a standard drug, and control wells with untreated infected and uninfected erythrocytes were included in all assays. Generally, compounds showing a IC₅₀ value greater than 2 μM in 3D7 strain are not further evaluated.
Concurrent with the determination of IC$_{50}$ in K1 strain, compounds were tested for cytotoxicity (IC$_{50}$) in KB cells at concentrations less than or equal to 100 µg/mL. After 72 hours of exposure, viability is assessed on the basis of the Alamar Blue method (Accumed International, USA) as previously described [8, 11]. Briefly, microtiter plates were seeded at a density of 4·10$^4$ KB cells/mL in RPMI 1640 culture medium supplemented with 10% heat-inactivated foetal calf serum (complete medium, Seralab). Plates were incubated at 37°C, 5% CO$_2$, 95% air mixture for 24 h followed by compound addition to triplicate wells in a dilution series in complete medium. The positive control drug was podophyllotoxin (Sigma). Plates were incubated for a further 72 h followed by the addition of 10 μL of Alamar-Blue (AccuMed International) to each well and incubation for 2–4 h at 37 °C, 5% CO$_2$, 95% air mixture. Fluorescence emission at 585 nm was measured in a Spectramax Gemini plate reader (Molecular Devices) after excitation at 530 nm. IC$_{50}$ values were calculated using the XLFit (ID Business Solutions, UK) line fitting software. The Selectivity Index [(SI=(KB IC$_{50}$)/(3D7 or K1 IC$_{50}$)] was also determined; it is considered significant when SI >10.

Acknowledgements

We thank the Ministerio de Educación y Ciencia (Grant AP2003-2175 to Esther Vicente) and the University of Navarra (PiUNA project).

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