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Antiplasmodial activity of 3-trifluoromethyl-2-carbonylquinoxaline di-*N*-oxide derivatives

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*The in vitro antiplasmodial activity of some 3-trifluoromethyl-2-carbonylquinoxaline di-N-oxide derivatives is reported. The evaluation was performed on cultures of FcB1 strain (chloroquine-resistant) of P. falciparum and the most interesting compounds were then evaluated on MCF7 tumor cells in order to evaluate an index of selectivity. The 7-methyl (**2b**, **4b**, **5b**, **6b**) and nonsubstituted (**3c**, **4c**, **5c**) quinoxaline 1,4-dioxide derivatives presented the best level of activity.*

Uniterms
- Quinoxaline
- Antimalarial
- Chloroquine
- MCF7

INTRODUCTION

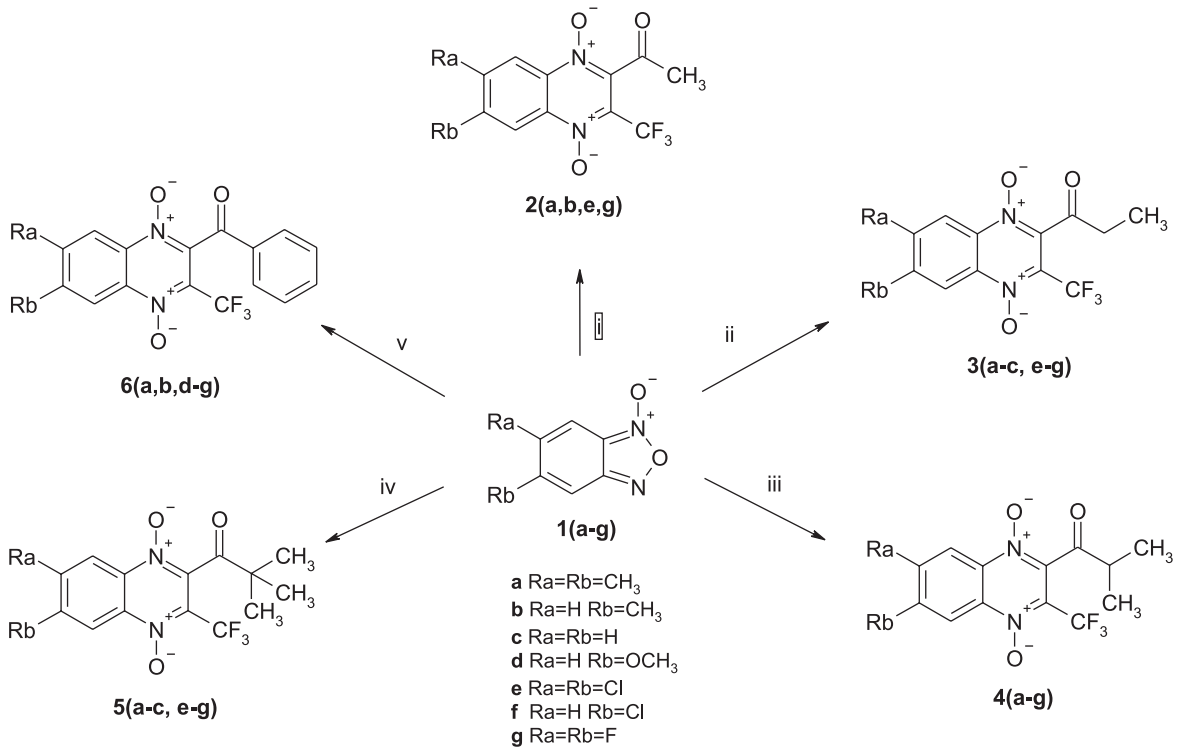
Malaria remains one of the most widespread health threats in the tropics with 300 to 500 million cases every year and two million deaths (Greenwood *et al*., 2005). The major concern in the treatment of this disease is the possible growing resistance of *Plasmodium falciparum* to the limited arsenal of antimalarial drugs. Chloroquine, a traditional old antimalarial drug, is believed to exert its activity by inhibiting hemozoin formation in the digestive vacuole of the malaria parasite, but the mechanism of *Plasmodium falciparum* resistant to chloroquine remains unknown. Recently, some pyrrolo-quinoxaline derivatives have shown antimalarial activity correlated with a high inhibitory of ß-hematin formation (Guillon *et al*., 2004), suggesting a bioisosteric relationship between the quinoline and pyrrolo-quinoxaline rings.

Based on these statements and the urgent need to develop new antimalarial drug candidates, we researched the *in vitro* antimalarial activity of lead compounds in our library containing the quinoxaline di-*N*-oxide subunit (Scheme 1), which were previously shown to have good antituberculosis activity (Zarranz *et al*., 2003; Jaso *et al*., 2003).

MATERIAL AND METHODS

Chemistry

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The 1H NMR spectra were recorded on a Bruker AC-200E instrument (200 MHz) and Bruker 400 Ultrashield® (400 MHz), using TMS as the internal standard and with DMSO-d_6 and $CDCl_3$ as the solvent; the chemical shifts are reported in ppm (d) and coupling constant (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 Perkin Elmer 1600 FTIR (Norwalk, CT, USA) using KBr pellets; the frequencies are expressed in cm^{-1}.



SCHEME 1. [a]Reagents**:** (i) 1,1,1-trifluoro-2,4-pentanedione, (ii) 1,1,1-trifluoro-2,4-hexanedione, (iii) 1,1,1-trifluoro-5-methyl-2,4-hexanedione, (iv) 1,1,1-trifluoro-5,5-dimethyl-2,4-hexanedione and (v) 4,4,4-trifluoro-1-phenyl-1,3-butanedione; in dry chloroform.

Elemental microanalyses were obtained on an Elemental Analyzer (Carlo Erba 1106, Milan, Italy) from vacuum-dried samples. The analytical results for C, H, and N were within ± 0.4 of the theoretical values.

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). HPLC conditions: Column Nova Pack C18 60 A 4 mm (3.9×150 mm); mobile phase: acetonitrile/water (60:40); flux: 1 mL/min.

Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceutical 3[a], 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

Synthesis of 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethyl-quinoxaline 1,4-di-*N*-oxide derivatives

The compounds under study were previously synthesized and reported by our group (Zarranz *et al*., 2004). Syntheses of the compounds were carried out by the classical Beirut reaction (Scheme 1). The appropriate benzofuroxane and the corresponding 1-(alkyl/phenyl)-4,4,4-trifluoromethyl-β-diketone were dissolved in dry chloroform in the presence of triethylamine, which acted as the catalyst. When the reaction ended, the solvent was evaporated to dryness, and a yellow crude solid or brown oil was obtained. Each compound was purified by either recrystallization or flash chromatography. The starting compounds, 5-substituted or 5,6-disubstituted benzofuroxanes, **1(a–g)**, were obtained by previously described methods (Scheme 1) (Ortega *et al*., 2002).

Antiplasmodial Drug Assay

In vitro activity against *P. falciparum* (chloroquine-resistant strain FcB1 was evaluated by a micromethod, using the lactate dehydrogenase (LDH) enzyme of *P. falciparum* (5).

Erythrocytes infected with *P. falciparum*, from parasite cultures, were resuspended in the complete culture medium at a haematocrit of 1.5% and distributed into 96-well, microtitre plates (200 µL/well). For each assay, the parasite culture was incubated with the drug for 48 h in 5% CO_2, at 95% relative humidity, and then frozen until the biochemical assay could be run.

A 20 µL subsample of the contents of each well was mixed with 100 µL of a substrate solution containing 20 mg sodium L-lactate (Sigma), 5.5 mg TRIS (Sigma) and 3.7 mg 3-acetyl pyridine adenine dinucleotide (APAD; Sigma)/mL. After incubation for 30 min at 37 °C, 25 µL of a mixture of NBT (1.6 mg/mL; Sigma) and PES (0,1 mg/mL, Sigma) were added to each well. After another 35 min of incubation, the formation of the reduced form of APAD was measured at 650 nm, using a spectrophotometer (Polarstar BMG). IC_{50} values were determined graphically in concentration versus inhibition percent curves.

Cytotoxicity Tests

General Procedures. Human breast adenocarcinoma (MCF-7) cells were cultured in DMEM culture medium containing L-glutamine (2 mM) (Biowittaker), supplied with 5% of fetal calf serum (FCS) (Sigma), and incubated under standard conditions (37 °C, 5% CO_2). The cells were trypsinized, resuspended in DMEM containing 5% FCS, and seeded (20000 cells/well) in 96-well plates. Cell viability was evaluated measuring the activity of the mitochondrial enzyme succinate dehydrogenase. This test used sodium 3,3-[1(1-phenyl amino carboxyl)-3-4-tetrazolium *bis* (4-methoxy-6 nitro)] benzene sulfonic acid hydrate] (XTT) (Sigma) as substrate, which was converted to a formazan product, detected spectrophotometrically at 450 nm.

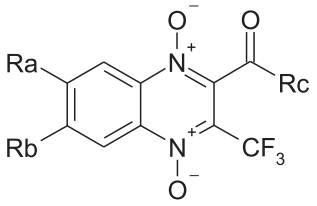
Screening Test. The cells were treated for 48 h with drug concentrations ranging from 1 to 100 µg/mL. After 48 h of drug exposure, the medium was replaced by 50 µL of a XTT solution (0.5 mg/mL), and the cells were incubated for 180 min. The IC_{50} values were determined graphically in concentration versus inhibition percent curves (Tabbi *et al.,* 2002).

RESULTS AND DISCUSSION

The 3-trifluoromethyl-2-carbonylquinoxaline di-*N*-oxide derivatives were initially assayed in order to determine their ability to inhibit *P. falciparum* (chloroquine resistant) (Delhaes *et al*., 1999; Tabbi *et al*., 2002) and the results are shown in table 1. Interesting, compounds **3c**, **4b**, **4c**, **5b**, **5c** and **6b** demonstrated a satisfactory inhibition with IC_{50} values of 2.4, 1.9, 2.0, 2.1, 1.2 and 1.7 µM, respectively. Contrary to the works of Guillon and co-workers (Guillon *et al*., 2004), the antiplasmodial activity of derivatives **3c**, **4b**, **4c**, **5b**, **5c** and **6b** does not appear to interfere with β-hematin formation (data not shown).

In order to determine the potential cytotoxic profile of quinoxaline di-*N*-oxide derivatives and establish the selectivity index, compounds **3c**, **4b**, **4c**, **5b**, **5c** and **6b** were evaluated on MCF7 tumor cells. The selectivity index (SI) was defined as the ratio of the IC_{50} value for the human cells (MCF7 cells) to the IC_{50} for the *P. falciparum* strain under study. The IC_{50} values for the tumor cells are as follows: 13.9 µM for **3c**, 10.8 µM for **4b**, 11.3 µM for **4c**, and 18.9 µM for **5b**, all of which have shown the best

TABLE I - *In vitro* sensitivity of chloroquine-resistant strain (FcB1) of *P. falciparum* and *in vitro* cytotoxicity on MCF7 cells.



Compd.	Ra	Rb	Rc	IC_{50} (µM)	
				FcB1	MCF7
2a	CH_3	CH_3	CH_3	24	ND
2b	CH_3	H	CH_3	3.1	ND
2e	Cl	Cl	CH_3	>30	ND
2g	F	F	CH_3	23.7	ND
3a	CH_3	CH_3	CH_2CH_3	16.5	ND
3b	CH_3	H	CH_2CH_3	15.3	17.6
3c	H	H	CH_2CH_3	2.4	14.0
3e	Cl	Cl	CH_2CH_3	>30	ND
3f	H	Cl	CH_2CH_3	3.1	11.8
3g	F	F	CH_2CH_3	>30	ND
4a	CH_3	CH_3	$CH(CH_3)_2$	23.7	ND
4b	CH_3	H	$CH(CH_3)_2$	1.9	10.8
4c	H	H	$CH(CH_3)_2$	2.0	11.3
4d	OCH_3	H	$CH(CH_3)_2$	12.1	ND
4e	Cl	Cl	$CH(CH_3)_2$	21.9	ND
4f	Cl	H	$CH(CH_3)_2$	17.9	ND
4g	F	F	$CH(CH_3)_2$	>30	ND
5a	CH_3	CH_3	$C(CH_3)_3$	26.9	ND
5b	CH_3	H	$C(CH_3)_3$	2.1	18.9
5c	H	H	$C(CH_3)_3$	1.2	8.9
5e	Cl	Cl	$C(CH_3)_3$	>30	ND
5f	Cl	H	$C(CH_3)_3$	13.7	ND
5g	F	F	$C(CH_3)_3$	>30	ND
6a	CH_3	CH_3	C_6H_5	2.7	ND
6b	CH_3	H	C_6H_5	1.7	10.0
6d	OCH_3	H	C_6H_5	23.6	ND
6e	Cl	Cl	C_6H_5	11.4	ND
6f	Cl	H	C_6H_5	2.9	ND
6g	F	F	C_6H_5	>30	ND
Chloroquine sulfate (Sigma)				0.43	ND

ND: no data.

selectivity index, with a value of 9. An IC_{50} value of 8.9 μM was found for **5c** and 10.0 μM was the value found for compound **6b**. These results clearly demonstrate poor selectivity indexes, ranging between 1 and 9, which do not justify *in vivo* evaluation of the compounds.

In summary, the screening of the *in vitro* antimalarial activity of 3-trifluoromethyl-2-carbonylquinoxaline di-*N*-oxide derivatives using cultures of FcB1 strain of *P. falciparum* (Chloroquine-resistant) has permitted the identification of compounds with expressive activity, although less potent than the standard chloroquine. However, considering the originality of this structural pattern as an antimalarial lead compound, the results described in this paper can contribute to the designing of new antimalarial drug candidates that could represent a promising alternative for treating this negligent disease. Some structural requirements for the antiplasmodial activity could be identified, such as the necessity of oxygenation at the **N-1** and **N-4** of quinoxaline ring, because no activity was found for the mono and di-reductive compounds. Furthermore, the best results were found for 7-methyl (**2b**, **4b**, **5b**, **6b**) and non-substituted (**3c**, **4c**, **5c**) quinoxaline 1,4-dioxide compounds, while the double pattern of substitution at phenyl ring of quinoxaline subunit resulted in a loss of activity (*e.g.* **2e**). The substituents of the carbonyl group did not greatly influence the antimalarial activity, and consequently, the considerable steric volume can be well tolerated. Based on the aforementioned, this structural data is being used for the optimization of this bioactive series.

This paper describes the antiplasmodial activities of a novel structure pattern of potential antimalarial drug candidates and their cytotoxic profiles measured in MCF7 tumor cells bioassay. The quinoxaline di-*N*-oxide derivatives **3c**, **4b**, **4c**, **5b**, **5c** and **6b**, with the best antiplasmodial activity, were demonstrated to possess a lower selectivity index, ranging between 1 and 9; this suggests a nonselective cytotoxic profile. A preliminary study of the structure-activity relationship (SAR) taken from this work is being used in the optimization of this bioactive series.

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RESUMO

Atividade antimalárica de derivados di-*N*-óxido de 3-trifluorometil-2-carbonilquinoxalina

*Neste artigo descreve-se a atividade anti-Plasmodium falciparum de derivados 3-trifluorometil-2-carbonilquinoxalinas di-N-óxidos (**2a-6g**). A avaliação das propriedades farmacológicas dos derivados **2a-6g** foi realizada em modelo in vitro de inibição de cepas P. falciparum FcB1 (cloroquina resistente) em cultura celular, e sobre culturas de células tumorais MCF7, com a finalidade de estabelecer o índice de seletividade para os compostos mais promissores. Os derivados 7-metil* (**2b**, **4b**, **5b**, **6b**) *e não-substituído* (**3c**, **4c**, **5c**) *apresentaram o melhor perfil de atividade.*

UNITERMOS: Quinoxalina. Antimalarial. Cloroquina. MCF7.

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