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Short communication

Synthesis and antimalarial activities of some furoxan sulfones and related furazans

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Abstract

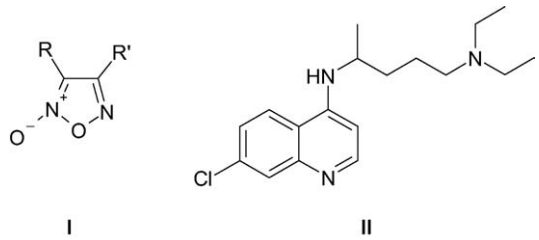
Furoxan derivatives bearing a sulfone moiety at position 3 or 4 were synthesized and tested for their antimalarial action on the chloroquine-sensitive D10 and the chloroquine-resistent W2 strains of *Plasmodium falciparum*. The furazan analogues were considered for comparison. The most active compounds were the products in which the $-SO_2R$ groups are at the 3-position of the furoxan system. These latter substances displayed an antimalarial activity in the μM range, possibly related in part to their ability to release NO.

Keywords: Plasmodium falciparum; Nitric oxide; Antimalarial; Furoxanyl sulfones

1. Introduction

Furoxan ring (1,2,5-oxadiazole 2-oxide) **I** (Chart 1), is an heterocyclic system known to chemists thanks to its intriguing chemistry and an argument on its structure [1]. Furoxan derivatives have recently received particular attention in view of their ability to release nitric oxide (NO) [2]. In pH 7.4 buffered water solution at 37 °C, the release of NO requires the presence of thiol cofactors. Several of these products were studied as cardiovascular agents principally for their vasodilating properties [3,4].

Endogenous NO is a potent antimicrobial agent. Together with reactive oxygen intermediates (ROI), NO is one of the toxic mediators released by activated macrophages against pathogens. NO-mediated cellular toxicity is due to the generation of reactive species and/or inhibition of essential enzymes [5]. In particular *S*-nitrosilation of cysteine containing proteins seems to be a widespread mechanism for the antiparasitic effect of NO [6]. A number of NO-donors, namely molecules able to release NO in physiological conditions,



I II

Chart 1.

proved to inhibit the catalytic activity of cysteine proteases [6]. There are scattered reports in literature that exogenous NO also displays cytotoxic and cytostatic effects against viruses and microbial agents [7] including protozoa [6]. The NO-donors *S*-nitroso glutathione (GSNO) and *S*-nitroso-L-cysteine (SNOCys) kill *Plasmodium falciparum* [8]. Their 50% inhibitory concentrations (IC_{50}) were found to be very close, approximately 40 μM. A number of furoxan derivatives proved to display antimicrobial activity [4]. Some of them inhibited in vitro the growth of a number of protozoa including *T. vaginalis*, *E. histolytica* and *T. cruzi* [4]. In this last case electrochemical studies suggested that trypanosoma cell damage could be caused by oxidative stress associated

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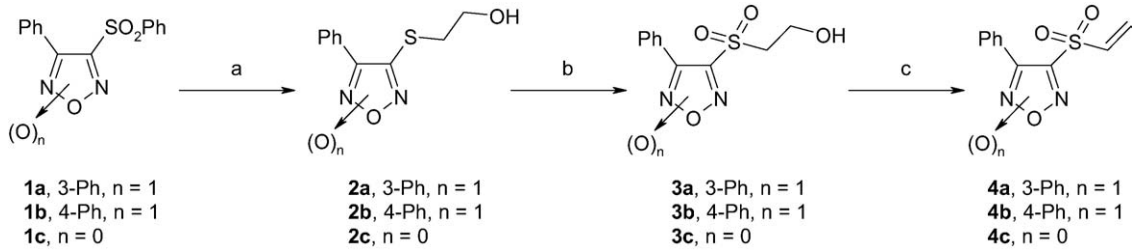
with the presence of the N^+–O^- moiety [9]. A benzofuroxanyl thiourea derivative was evaluated as inhibitor of tripanosome cysteine proteinases displaying interesting activity. In addition, 4-(phenylsulfonyl)-3-(((2-dimethylamino)-ethyl)thio)furoxan oxalate proved to be able to inhibit the activity of the HIV-1 reverse transcriptase, an enzyme containing cysteine residue(s) [4], and *Leishmania infantum* cysteine proteinase [10]. Until now, the potential antimalarial activity of the furoxans has not been evaluated in spite of an urgent need for new antimalarials because of the widespread resistance of malaria parasites to conventional drugs, such as chloroquine (CQ) **II** (Chart 1) [11].

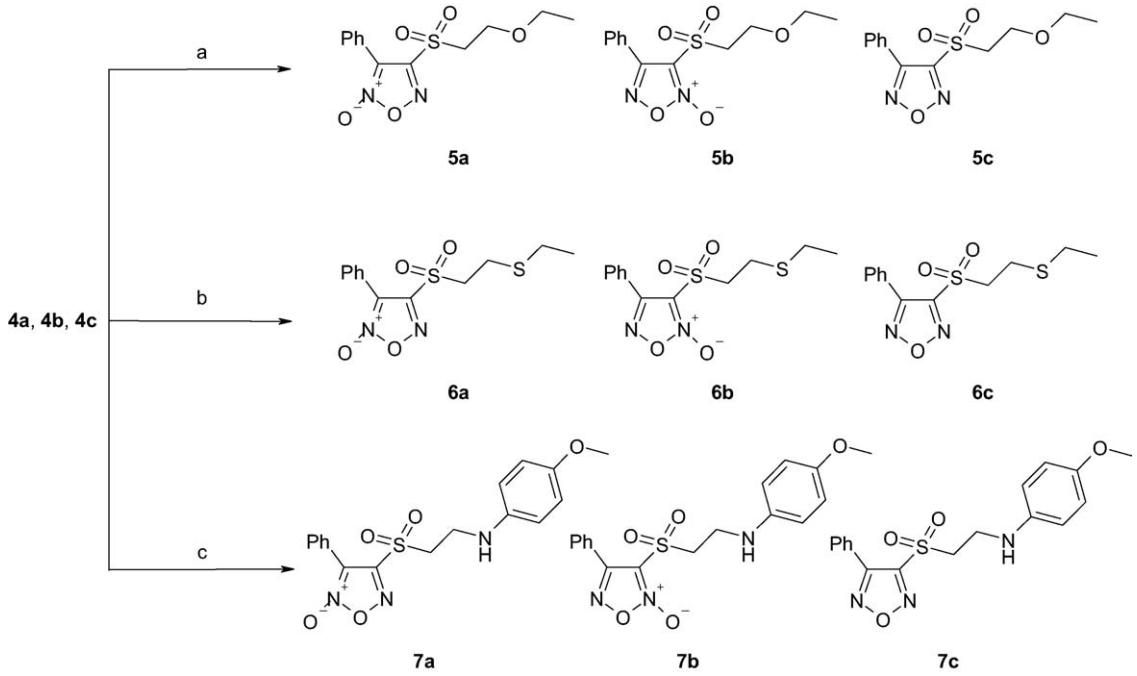
In the context of our research on the furoxan pharmacochemistry, we decided to study selected derivatives of this heterocyclic system as potential antimalarial compounds. In this paper, we report the synthesis of new furoxan sulfones derivatives (**4a**, **4b**–**7a**, **7b**) and preliminary results of their in vitro activity against *P. falciparum*, the aetiological agent of the most deadly form of human malaria. The antimalarial activity of the sulfones used as intermediates for the preparation of the new compounds was also evaluated (derivatives **1a**, **1b**, **3a**, **3b**) [12]. The choice of this kind of products is connected not only with their potential ability to release NO [13,14] and to induce oxidant stress owing to the presence of the N^+–O^- moiety [9] but also to their capacity of undergoing nucleophilic attack by a number of nucleophiles, which includes the thiol group present in cysteine proteinases, with consequent displacement of the SO_2R groups (Scheme 1) [15] or a prevalent addition on the vinyl moiety in the case of the derivatives **4a**, **4b** (Scheme 2). The furazan analogues (series **c**), deprived of the N^+–O^- moiety, but able to react with thiol groups (see Schemes) [15], were also included for comparison.

2. Results and discussion

For the preparation of the final vinyl sulfones **4a**, **4b, 4c** we started from the corresponding thioethers **2a**, **2b**, **2c** (Scheme 1). The intermediates **2a**, **2b** were prepared through nucleophilic substitution of the phenylsulfonyl group in **1a**, **1b** by 2-mercaptoethanol, using a procedure we have already described [12]. The intermediate **2c** was synthesized in a similar manner from the parent phenylsulfonylfurazan derivative



Scheme 1. a) 2-Mercaptoethanol, 50% aq. NaOH, THF for **2a**, **2c**; 2-mercaptoethanol, MeO^-Na^+, –10 °C for **2b**; b) MCPBA, CH_2Cl_2, 0 °C to r.t.; c) MsCl, TEA, CH_2Cl_2, 0 °C.



Scheme 2. a) EtOH, EtO^-Na^+, THF, –45 °C; b) EtSH, TEA, THF, 0 °C; c) *p*-anisidine, THF, r.t.

1c. The thioethers were transformed into the corresponding sulfones **3a, 3b**, **3c** by action of *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2. These last products were dehydrated in CH_2Cl_2 to the final vinyl sulfones, by action of mesyl chloride (MsCl) in the presence of triethylamine (TEA). In order to obtain differently substituted alkylsulfonyl furoxan and furazan derivatives, these Michael-type systems were let to react with three different models of nucleofiles, namely *p*-anisidine, ethanol and ethanethiol (Scheme 2). These reagents contain as nucleophilic center a N, O, S atom, respectively, and are characterized by having different nucleophilic power. *p*-Anisidine was chosen as nitrogen nucleophile also in view of the facility to isolate the final amines **7a**, **7b**, **7c**. These products display low basicity (e.g. **7c** pK_a = 2.19 ± 0.03) and consequently they exist at physiological pH prevalently in the uncharged form.

The reactions with ethanol were run in THF at –45 °C, in the presence of catalytic amount of sodium ethoxide to give in fairly good yields the corresponding ethers **5a**, **5b**, **5c**. Similarly both the reactions in THF with ethanethiol at 0 °C, in the presence of TEA, and the reactions in THF with *p*-anisidine at room temperature, gave in very good yields the expected sulfur **6a**, **6b**, **6c**, and nitrogen **7a**, **7b**, **7c** adducts. The high reactivity of the furoxanyl and furazanyl vinyl sulfones towards the nucleophilic agents makes these products useful scaffolds for the synthesis of a variety of sulfones containing either the furoxan or the furazan system. The ability of the furoxan sulfones to produce nitrite, which is the principal oxidative product of nitric oxide in aerobic water solution, under the action of an excess of cysteine (5:1) was evaluated by the Griess reaction according to a procedure previously described [12]. The results are collected in Table 1. Analysis of the data shows that the 3-RSO_2-furoxan derivatives are more potent NO-donors than the corresponding 4-RSO_2-isomers.

To ascertain their antimalarial activity, all of the sulfones were screened in vitro against the chloroquine-sensitive (CQ-S), D10, and the chloroquine-resistant (CQ-R), W2 strain of *P. falciparum*. The antiplasmodial activities are expressed as 50% inhibitory concentrations (IC_{50}) in the two *P. falciparum* strains and are compared to that of CQ (Table 1). The products in which the R–SO_2-group is at the 3-position of the furoxan system (series **b**) displayed antimalarial activity in the low micromolar range, without significant differences between the CQ-R or CQ-S strain. These compounds proved to be more potent than the corresponding 3-phenyl isomers (series **a**), which, in turn, were more potent than the furazan analogues (series **c**). They also appear to be more active than the NO-donors GSNO and SNOcys. Within each series, the vinyl derivatives, **4a**, **4b**, **4c** were the least active products.

The furoxan derivatives belonging to series **b** also had significant NO releasing properties with respect to the furoxans of series **a**. This statement, in connection with the minor activity of the furazan analogues deprived of the ability to release NO, could suggest nitric oxide played a role in the mechanism of parasite death. Additional studies are necessary to confirm this possibility since higher NO-release of these products might parallel their higher ability to induce oxidative stress and/or to undergo nucleophilic substitutions.

Table 1
In vitro antimalarial activity of furoxanyl and furazanyl sulfones and nitrite production (% NO_2^- mol/mol) by furoxan derivatives

Compound	% NO_2^- ± S.E.	D10 IC_{50} (μM)[a]	W2 IC_{50} (μM)[a]
1a	< 1	6.67 ± 2.53	7.04 ± 2.11
1b	10.3 ± 0.1	3.76 ± 0.33	3.07 ± 0.89
1c	///	35.87 ± 16.59	32.86 ± 9.74
3a	< 1	41.54 ± 15.12	40.36 ± 14.83
3b	9.64 ± 0.21	4.41 ± 1.61	3.03 ± 0.85
3c	///	> 78.65	> 78.65
4a	< 1	56.13 ± 12.29	50.74 ± 10.98
4b	10.5 ± 0.8	22.04 ± 2.97	18.31 ± 4.12
4c	///	76.66 ± 11.30	70.85 ± 12.91
5a	< 1	22.79 ± 4.46	17.22 ± 4.19
5b	10.0 ± 0.5	5.36 ± 1.94	5.33 ± 0.20
5c	///	> 70.84	> 70.84
6a	< 1	9.67 ± 4.01	7.79 ± 4.38
6b	10.0 ± 0.4	4.23 ± 1.68	3.59 ± 0.89
6c	///	59.79 ± 10.22	55.93 ± 15.72
7a HCl	< 1	14.08 ± 3.37	12.82 ± 2.59
7b HCl	20.8 ± 0.7	4.73 ± 0.92	4.47 ± 0.41
7c	///	42.76 ± 13.13	37.98 ± 11.71
Chloroquine	///	0.025 ± 0.011	0.35 ± 0.17

[a] Compounds were tested against CQ-S, D10, and CQ-R, W2 strains of *P. falciparum* using the pLDH method. All values are mean ± S.D. of three different experiments conducted in duplicate.

3. Experimental protocols

3.1. Chemistry

Melting points were measured with a capillary apparatus (Stuart Smp 3) and are uncorrected. All the compounds were routinely checked by IR (FT-IR Thermo-Nicolet Avatar), ^{1}H-and ^{13}C-NMR (Bruker Avance 300 and Jeol ECP300) and mass spectrometry (Finnigan-Mat TSQ-700). The following abbreviations were used to indicate the peak multiplicity: *s* = singlet; *d* = doublet; *t* = triplet; *m* = multiplet. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM); thin layer chromatography (TLC) was carried out on 5 × 20 cm plates with a layer thickness of 0.25 mm using the indicated eluents. Petroleum ether 40–60 °C (PE) was used as a co-eluent. Anhydrous magnesium sulfate was used as drying agent for the organic phases. Analysis (C, H, N) of the new compounds was performed by REDOX (Monza) and the results are within ± 0.4%. Structures **1a** [16], **1b** [16], **1c** [16], **2a** [12], **2b** [12], were synthesized according to methods described in the literature. Tetrahydrofuran (THF) was distilled immediately before use from Na and benzophenone under a positive atmosphere of N_2. All reactions were carried out three times without any attempt to optimize the yields.

*3.1.1. 2-[(4-Phenylfurazan-3-yl)thio]ethanol (**2c**)*

The title compound was prepared in the same manner as **2a**. Eluent (PE/EtOAc 80:20 v/v). Yield 98%. ^{1}H-NMR

($CDCl_3$) δ 3.42 (*t*, 2H, –OCH_2CH_2S–), 3.97 (*t*, 2H, –OCH_2CH_2S–), 7.45–7.81 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 35.5 (–OCH_2CH_2S–), 60.5 (–OCH_2CH_2S–), 125.1 (C*i* Ph), 128.1, 129.1 (C*o*, C*m* Ph), 130.9 (C*p* Ph), 151.1 (C4 Furaz.), 152.6 (C3 Furaz.).

*3.1.2. General procedure for the preparation of **3a, 3b, 3c***

To a solution of 2-[(3-phenylfuroxan-4-yl)thio]ethanol (**2a**) (6.10 g, 25.63 mmol) in CH_2Cl_2 (60 ml), kept at 0 °C, MCPBA (14.31 g, 64.08 mmol) was added. The reaction was allowed to reach room temperature (r.t.) and was completed in 20 h. The mixture was filtered under vacuum and the precipitate was washed with CH_2Cl_2. To the filtrate were added ice and NaOH 2 M until pH 8–9, after separation the aqueous layer was extracted with CH_2Cl_2 (60 ml). The organic layers were dried and evaporated. The crude product was purified by flash chromatography.

*3.1.2.1. 2-[(3-Phenylfuroxan-4-yl)sulfonyl]ethanol (**3a**).* Eluent (PE/EtOAc 70:30 v/v). Yield 69%. M.p. 62–63 °C (from EtOAc/PE, Ref. [12] 62–63 °C).

*3.1.2.2. 2-[(4-Phenylfuroxan-3-yl)sulfonyl]ethanol (**3b**).* Eluent (CH_2Cl_2). Yield 80%. M.p. 79–81 °C (from Et_2O/PE, Ref. [12] 80–81 °C).

*3.1.2.3. 2-[(4-Phenylfurazan-3-yl)sulfonyl]ethanol (**3c**).* Eluent (PE/EtOAc 70:30 v/v). Yield 78%. M.p. 60.5–62 °C (from EtOAc/PE, Ref. [12] 60.5–62 °C).

*3.1.3. General procedure for the preparation of **4a, 4b, 4c***

To a stirred solution of 2-[(3-phenylfuroxan-4-yl)sulfonyl]ethanol (**3a**) (0.84 g, 3.11 mmol) in CH_2Cl_2 (10 ml), kept at 0 °C, TEA (1.08 ml, 7.76 mmol) and MsCl (0.29 ml, 3.72 mmol) were added. After 10 min the reaction was completed. The mixture was washed with a saturated solution of NH_4Cl (5 ml), dried and evaporated. The crude product was purified by flash chromatography.

*3.1.3.1. 3-Phenyl-4-(vinylsulfonyl)furoxan (**4a**).* Eluent (PE/EtOAc 90:10 v/v). Yield 94%. M.p. 98.5–99.5 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 6.45 (*m*, 1H, –SO_2CH=CH_aH_b, $^3J_{HH}$ = 9.8 Hz), 6.65 (*m*, 1H, –SO_2CH=C$H_a$$H_b$, $^3J_{HH}$ = 16.5 Hz), 6.90–6.98 (*m*, 1H, –SO_2C*H*=CH_aH_b), 7.54–7.92 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 112.0 (C3 Furox.), 120.2 (C*i* Ph), 128.8, 129.1 (C*o*, C*m* Ph), 131.6 (C*p* Ph), 134.4 (–SO_2CH=*C*H_2), 135.3 (–SO_2*C*H=CH_2), 157.9 (C4 Furox.); MS (EI) *m/z* 252 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{10}H_8N_2O_4S$) C, H, N.

*3.1.3.2. 4-Phenyl-3-(vinylsulfonyl)furoxan (**4b**).* Eluent (PE/EtOAc 95:5 v/v). Yield 88%. M.p. 94–95 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 6.41 (*m*, 1H, –SO_2CH=CH_aH_b, $^3J_{HH}$ = 9.4 Hz), 6.65 (*m*, 1H, –SO_2CH=C$H_a$$H_b$, $^3J_{HH}$ = 16.3 Hz), 6.74–6.80 (*m*, 1H, –SO_2C*H*=CH_aH_b), 7.50–7.75 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 116.6 (C3 Furox.), 124.6 (C*i* Ph), 128.7, 129.5 (C*o*, C*m* Ph), 131.7 (C*p* Ph), 133.5 (–SO_2CH=*C*H_2), 135.6 (–SO_2*C*H=CH_2), 154.7 (C4 Furox.); MS (EI) *m/z* 252 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{10}H_8N_2O_4S$) C, H, N.

*3.1.3.3. 3-Phenyl-4-(vinylsulfonyl)furazan (**4c**).* Eluent (PE/EtOAc 90:10 v/v). Yield 79%. M.p. 56.4–57.2 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 6.43–6.46 (*m*, 1H, –SO_2CH=CH_aH_b, $^3J_{HH}$ = 9.9 Hz), 6.65–6.71 (*m*, 1H, –SO_2CH=C$H_a$$H_b$, $^3J_{HH}$ = 17.3 Hz), 7.00–7.09 (*m*, 1H, –SO_2C*H*=CH_aH_b), 7.55–7.99 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ, 121.7 (C*i* Ph), 127.8, 128.0 (C*o*, C*m* Ph), 130.4 (C*p* Ph), 132.6 (–SO_2CH=*C*H_2), 134.2 (–SO_2*C*H=CH_2), 150.9 (C3 Furaz.), 154.0 (C4 Furaz.); MS (EI) *m/z* 236 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{10}H_8N_2O_3S$) C, H, N.

*3.1.4. General procedure for the preparation of **5a, 5b, 5c***

To a solution of 3-phenyl-4-(vinylsulfonyl)furoxan (**4a**) (200 mg; 0.79 mmol) in dry THF (5 ml), kept under inert atmosphere at –45 °C, a solution of sodium ethoxide 0.636 M (440 µl; 0.4 eq) was slowly added. At the end of the addition the reaction was completed. The mixture was washed with a saturated solution of NH_4Cl (5 ml), dried and evaporated. The crude product was purified by flash chromatography.

*3.1.4.1. 4-[(2-Ethoxyethyl)sulfonyl]-3-phenylfuroxan (**5a**).* Eluent (PE/EtOAc 80:20 v/v). Yield 63%. M.p. 66–67 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 1.06 (*t*, 3H, –OCH_2CH_3), 3.38 (*q*, 2H, –OCH_2CH_3), 3.50 (*t*, 2H, –$SO_2CH_2CH_2$–), 3.84 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.54–7.86 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.8 (–OCH_2CH_3), 55.1 (–OCH_2CH_3), 63.3 (–$SO_2CH_2CH_2$–), 66.9 (–$SO_2CH_2CH_2$–), 112.4 (C3 Furox.), 120.6 (C*i* Ph), 129.2 (C*o*, C*m* Ph, overlapped signals), 131.6 (C*p* Ph), 157.7 (C4 Furox.); MS (EI) *m/z* 298 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_5S$) C, H, N.

*3.1.4.2. 3-[(2-Ethoxyethyl)sulfonyl]-4-phenylfuroxan (**5b**).* Eluent (PE/EtOAc 90:10 v/v). Yield 51%. M.p. 58.6–59.6 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 1.01 (*t*, 3H, –OCH_2CH_3), 3.34 (*q*, 2H, –OCH_2CH_3), 3.58 (*t*, 2H, –$SO_2CH_2CH_2$–), 3.78 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.49–7.74 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.8 (–OCH_2CH_3), 53.4 (–OCH_2CH_3), 63.7 (–$SO_2CH_2CH_2$–), 67.1 (–$SO_2CH_2CH_2$–), 118.0 (C3 Furox.), 125.0 (C*i* Ph), 128.7, 129.6 (C*o*, C*m* Ph), 131.6 (C*p* Ph), 155.1 (C4 Furox.); MS (EI) *m/z* 298 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_5S$) C, H, N.

*3.1.4.3. 3-[(2-Ethoxyethyl)sulfonyl]-4-phenylfurazan (**5c**).* Eluent (PE/EtOAc 80:20 v/v). Yield 63%. M.p. 35–36 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 0.98 (*t*, 3H, –OCH_2CH_3), 3.33 (*q*, 2H, –OCH_2CH_3), 3.64 (*t*, 2H,

–$SO_2CH_2CH_2$–), 3.86 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.54–7.92 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.9 (–OCH_2CH_3), 56.6 (–OCH_2CH_3), 63.5 (–$SO_2CH_2CH_2$–), 67.1 (–$SO_2CH_2CH_2$–), 123.7 (C*i* Ph), 129.4, 129.9 (C*o*, C*m* Ph), 131.9 (C*p* Ph), 152.9 (C4 Furaz.), 155.6 (C3 Furaz.); MS (EI) *m/z* 282 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_4S$) C, H, N.

3.1.5. General procedure for the preparation of ***6a, 6b, 6c***

To a solution of 3-phenyl-4-(vinylsulfonyl)furoxan (**4a**) (200 mg; 0.79 mmol) in dry THF (3 ml), kept under inert atmosphere at 0 °C, ethanethiol (117 μl; 1.58 mmol) and TEA (220 μl; 1.58 mmol) were added. At the end of the addition the reaction was completed. The mixture was washed with a saturated solution of NH_4Cl (5 ml) and then extracted with EtOAc (2 × 10 ml). The combined organic layers were dried and evaporated. The crude product was purified by flash chromatography.

3.1.5.1. 4-{[2-(Ethylthio)ethyl]sulfonyl}-3-phenylfuroxan ***(6a)****.* Eluent (PE/EtOAc 95:5 v/v). Yield 95%. M.p. 70–71 °C (from EtOAc/PE). ^{1}H-NMR ($CDCl_3$) δ 1.27 (*t*, 3H, –SCH_2CH_3), 2.58 (*q*, 2H, –SCH_2CH_3), 3.02 (*t*, 2H, –$SO_2CH_2CH_2$–), 3.77 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.54-7.91 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.8 (–SCH_2CH_3), 24.0 (–SCH_2CH_3), 26.5 (–$SO_2CH_2CH_2$–), 54.9 (–$SO_2CH_2CH_2$–), 112.0 (C3 Furox.), 120.5 (C*i* Ph), 129.2, 129.6 (C*o*, C*m* Ph), 132.1 (C*p* Ph), 157.6 (C4 Furox.); MS (CI) *m/z* 315 $(M + 1)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_4S_2$) C, H, N.

3.1.5.2. 3-{[2-(Ethylthio)ethyl]sulfonyl}-4-phenylfuroxan ***(6b)****.* Eluent (PE/EtOAc 95:5 v/v). Yield 95%. M.p. 73.5–74.5 °C (from EtOAc/PE). ^{1}H-NMR ($CDCl_3$) δ 1.22 (*t*, 3H, –SCH_2CH_3), 2.52 (*q*, 2H, –SCH_2CH_3), 2.88 (*t*, 2H, –$SO_2CH_2CH_2$–), 3.66 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.50-7.75 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.5 (–SCH_2CH_3), 23.7 (–SCH_2CH_3), 26.4 (–$SO_2CH_2CH_2$–), 53.3 (–$SO_2CH_2CH_2$–), 117.1 (C3 Furox.), 124.6 (C*i* Ph), 128.8, 129.7 (C*o*, C*m* Ph), 131.9 (C*p* Ph), 155.2 (C4 Furox.); MS (CI) *m/z* 315 $(M + 1)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_4S_2$) C, H, N.

3.1.5.3. 3-{[2-(Ethylthio)ethyl]sulfonyl}-4-phenylfurazan ***(6c)****.* Eluent (PE/EtOAc 95:5 v/v). Yield 95%. M.p. 33.5–34.5 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 1.25 (*t*, 3H, –SCH_2CH_3), 2.57 (*q*, 2H, –SCH_2CH_3), 3.01 (*t*, 2H, –$SO_2CH_2CH_2$–), 3.83 (*t*, 2H, –$SO_2CH_2CH_2$–), 7.50–7.96 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 14.5 (–SCH_2CH_3), 23.6 (–SCH_2CH_3), 26.1 (–$SO_2CH_2CH_2$–), 56.3 (–$SO_2CH_2CH_2$–), 122.9 (C*i* Ph), 129.2, 129.4 (C*o*, C*m* Ph), 131.8 (C*p* Ph), 152.5 (C4 Furaz.), 154.4 (C3 Furaz.); MS (EI) *m/z* 298 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_3S_2$) C, H, N.

3.1.6. General procedure for the preparation of ***7a, 7b, 7c***

p-Anisidine (195 mg; 1.58 mmol) was added to a solution of 3-phenyl-4-(vinylsulfonyl)furoxan (**4a**) (200 mg; 0.79 mmol) in dry THF (3 ml). The reaction was completed after 1.45 h. The mixture was concentrated under reduced pressure and the residue was treated with EtOAc (15 ml). To the resulting solution oxalic acid 0.5 M in EtOAc (4 ml) and then water (10 ml) were added. After separation the organic layer was dried and evaporated. The crude product was purified by flash chromatography.

*3.1.6.1. 4-Methoxy-*N*-{2-[(3-phenylfuroxan-4-yl)sulfonyl]-ethyl}aniline (****7a****).* Eluent (PE/EtOAc 90:10 v/v). Yield 88%. ^{1}H-NMR ($CDCl_3$) δ 3.62–3.75 (*m*, 7H, –$SO_2CH_2CH_2$–, –OCH_3, overlapped signals), 6.47 (2H, $A_2'B_2'$ system), 6.77 (2H, $A_2'B_2'$ system), 7.48–7.73 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 38.5 (–$SO_2CH_2CH_2$–), 53.0 (–$SO_2CH_2CH_2$–), 55.9 (–OCH_3), 114.6, 115.3 (C*o*, C*m* $PhOCH_3$), 112.2 (C3 Furox.), 120.1 (C*i* Ph), 128.9, 129.2 (C*o*, C*m* Ph), 131.8 (C*p* Ph), 139.6 (C*p* $PhOCH_3$), 153.2 (C*i* $PhOCH_3$), 157.6 (C4 Furox.); MS (EI) *m/z* 375 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{17}H_{17}N_3O_5S$) C, H, N. For the pharmacological tests the product was treated with Et_2O/HCl to give the corresponding hydrochloride. Yield 81%. M.p. 148 °C dec (from dry EtOAc).

*3.1.6.2. 4-Methoxy-*N*-{2-[(4-phenylfuroxan-3-yl)sulfonyl]-ethyl}aniline (****7b****).* Eluent (PE/EtOAc 90:10 v/v). Yield 98%. ^{1}H-NMR ($CDCl_3$) δ 3.54 (*m*, 4H, –$SO_2CH_2CH_2$–), 3.74 (*s*, 3H, –OCH_3), 6.41 (2H, $A_2'B_2'$ system), 6.72 (2H, $A_2'B_2'$ system), 7.40–7.57 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 38.8 (–$SO_2CH_2CH_2$–), 52.7 (–$SO_2CH_2CH_2$–), 55.8 (–OCH_3), 114.8, 115.2 (C*o*, C*m* $PhOCH_3$), 117.3 (C3 Furox.), 124.6 (C*i* Ph), 128.7, 129.6 (C*o*, C*m* Ph), 131.7 (C*p* Ph), 139.6 (C*p* $PhOCH_3$), 153.4 (C*i* $PhOCH_3$), 154.8 (C4 Furox.); MS (EI) *m/z* 375 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{17}H_{17}N_3O_5S$) C, H, N. For the pharmacological tests the product was treated with Et_2O/HCl to give the corresponding hydrochloride. Yield 80%. M.p. 166–168 °C dec (from dry MeOH).

*3.1.6.3. 4-Methoxy-*N*-{2-[(4-phenylfurazan-3-yl)sulfonyl]-ethyl}aniline (****7c****).* Eluent (PE/EtOAc 90:10 v/v). Yield 93%. M.p. 79–80 °C (from diisopropylether). ^{1}H-NMR ($CDCl_3$) δ 3.66–3.79 (*m*, 7H, –$SO_2CH_2CH_2$–, –OCH_3, overlapped signals), 6.53 (2H, $A_2'B_2'$ system), 6.78 (2H, $A_2'B_2'$ system), 7.49–7.90 (*m*, 5H, *Ph*); ^{13}C-NMR ($CDCl_3$) δ 38.6 (–$SO_2CH_2CH_2$–), 54.6 (–$SO_2CH_2CH_2$–), 55.7 (–OCH_3), 114.8, 115.1 (C*o*, C*m* $PhOCH_3$), 122.9 (C*i* Ph), 129.1, 129.4 (C*o*, C*m* Ph), 131.8 (C*p* Ph), 139.5 (C*p* $PhOCH_3$), 153.2 (C*i* $PhOCH_3$), 152.4 (C4 Furaz.), 154.8 (C3 Furaz.); MS (EI) *m/z* 359 $(M)^+$; drying conditions: r.t.; 24 h, pressure < 10 mmHg. Anal. ($C_{12}H_{14}N_2O_3S_2$) C, H, N.

3.1.7. NO-release: detection of nitrite

A solution of the appropriate compound (20 μL, 10^{-2} M) in dimethyl sulfoxide (DMSO) was added to 1980 μl of 50 mM phosphate buffer (pH 7.4) in the presence of 0.5 mM L-cysteine. The final concentration of drug was 10^{-4} M. After

1 h at 37 °C, 1 ml of the reaction mixture was treated with 250 μl of Griess reagent [sulfanilamide (4 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 ml) in distilled water (final volume: 100 ml)]. After 10 min at room temperature, the absorbance was measured at 540 nm (Shimadzu UV-2501PC spectrophotometer). 10–70 nmol ml^{-1} sodium nitrite standard solutions were used for the calibration curve [14]. The yields in nitrite was expressed as % NO_2^- (mol/mol) ± S.E.

3.2. Pharmacology

3.2.1. Parasite cultures

P. falciparum cultures were carried out according to Trager and Jensen's with slight modifications [17]. The CQ-sensitive, strain D10 and the CQ-resistant, strain W2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (Gibco BRL, $NaHCO_3$ 24 mM) medium with the addition of 10% heat-inactivated A-positive human plasma, 20 mM Hepes (Biological Industries, Kibbutz, Israel), 2 mM Glutammine (Biological Industries, Kibbutz, Israel). All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, 94% N_2.

3.2.2. Parasite growth and drug susceptibility assay

Compounds were dissolved in either water (chloroquine) or DMSO and then diluted with a medium to achieve the required concentrations (final DMSO concentration < 1%, which is non-toxic to the parasite). Drugs were placed in 96 wells flat-bottom microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD_{650}) by measuring the activity of the parasite lactate dehydrogenase (LDH), according to a modified version of Makler's method in control and drug-treated cultures [18,19]. Antimalarial activity is expressed as the 50% inhibitory concentrations (IC_{50}); each IC_{50} value is the mean and standard deviation of at least three separate experiments performed in duplicate.

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