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Synthesis and antimalarial activity of novel chiral and achiral benzenesulfonamides bearing 1, 3, 4-oxadiazole moieties

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Abstract

A series of new benzenesulfonamides, most of which are chiral, incorporating 1, 3, 4-oxadiazole and amino acid moieties have been synthesized. Some of these compounds were screened for antimalarial activity and also evaluated for their ability to inhibit hem polymerization. The electrophoretic analysis indicated that one compound was effective in inhibiting the degradation of hemoglobin. The synthesized compounds were tested in mice infected with *Plasmodium berghei*. These derivatives have the potential for the development of novel antimalarial lead compounds.

Keywords: *Chiral benzenesulfonamides, 1,3,4-oxadiazole, antimalarial activity, parasitaemia, electrophoretic analysis*

Introduction

Malaria is one of the most widespread infectious diseases in the world. The disease affects approximately 500 million people worldwide, causing 2.5 million people die annually, mainly children in African countries [1]. Four protozoan species infect humans, with *Plasmodium falciparum,* the most virulent human malaria parasite, being responsible for the majority of deaths [1].

Further complicating this grim scenario is the emergence of widespread resistance to the available antimalarial drugs accompanied by a worldwide resurgence of malaria, requiring the development of new drugs to combat this disease. Medium or long acting sulfonamides have been used clinically as antimalarial agents, particularly sulfadiazine and sulfadoxine. However each is much more effective when given in combination with pyrimethamine or trimethoprim [2]. Apart from their previous and more recently published antimalarial activity reports [3], sulfonamides have extensively been documented for their wide variety of pharmacological activities such as antimicrobial, insulin-releasing antidiabetic, carbonic anhydrase inhibitory, anti-HIV, high ceiling diuretic, antithyroid and antitumor [4–5]. Sildenafil citrate, another sulfonamide derivative, was approved as the first drug for treating male erectile dysfunction (ED) [6] and a second generation antimitotic sulfonamide, ER-34410, evolved again from a scaffold of antibacterial-like sulfonamides, being 2- to 3-fold more potent than E7010 in a panel of various human tumor cell lines *in vitro*, and unlike E7010, can be administered intravenously [7,8]. Therefore, the sulfonamide moiety is a crucial functionality because of its wide variety of pharmacological activities. In addition to various pharmacological applications of 2,5-disubstituted-1,3, 4- oxadiazoles [9–13], the 2-phenyl -5- (trichloromethyl)-1,3,4-oxadiazole has been reported [14] as a potent antimalarial agent.

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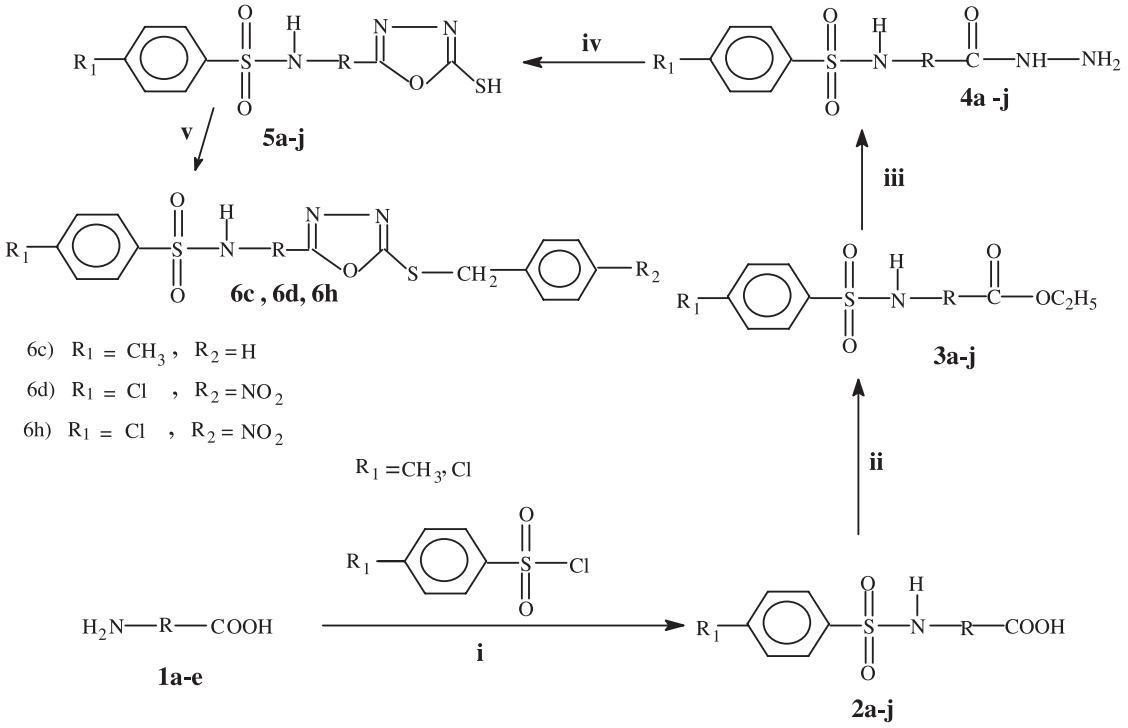
Many benzenesulfonamide derivatives with biological activity also require a free —SH/mercaptoaryl group in order to show an enhanced antiviral or anti-carbonic anhydrase activity [15–17]. Accordingly, our design and synthetic strategy (Scheme 1) for the synthesis of novel chiral and achiral compounds incorporating sulfonamide, 1,3,4-oxadiazole, and free mercapto (—SH) entities in one molecule is based on these observations and hypotheses. Accordingly, the compounds designed and reported here have been evaluated for antimalarial activity.

Materials & methods

Chemistry

Experimental protocols. Melting points were determined on a Gallenkamp digital melting point apparatus and are uncorrected. Optical rotation data were recorded on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr disc on a FT-IR model FTS 3000 MX spectrometer. Elemental analysis was performed on a Carlo Erba 1106 elemental analyzer. ^{1}H NMR (400 and 500 MHz) spectra were recorded on a



6c) $R_1 = CH_3$, $R_2 = H$

6d) $R_1 = Cl$, $R_2 = NO_2$

6h) $R_1 = Cl$, $R_2 = NO_2$

$R_1 = CH_3$, Cl

1a) R = $-(CH_2)_3-$

b) R= —CH(CH$_2$CH$_3$)— (D)

c) R= —CH(CH(CH$_3$)$_2$)— (L)

d) R= —CH(CH$_3$)— (D)

e) R= —CH((CH$_2$)$_4$NH$_2$.HCl)— (L)

a) R = $-(CH_2)_3-$, $R_1 = CH_3$ (b) $R_1 = Cl$

c) R= —CH(CH$_2$CH$_3$)— (D), $R_1 = CH_3$ (d) $R_1 = Cl$

e) R = —CH(CH(CH$_3$)$_2$)— (L), $R_1 = CH_3$ (f) $R_1 = Cl$

g) R= —CH(CH$_3$)— (D), $R_1 = CH_3$ (h) $R_1 = Cl$

i) R= —CH((CH$_2$)$_4$NH—SO$_2$—C$_6$H$_4$(4R$_1$))— (L), $R_1 = CH_3$ (j) $R_1 = Cl$

Scheme 1. (i) NaOH (5% aqueous), ether, stirring at RT, 6 h (ii) H_2SO_4 / ethanol, refluxing, 11 h (iii) NH_2-NH_2.H_2O / ethanol (absolute), refluxing, 9 h (iv) CS_2 / KOH, ethanol, refluxing, 16-17 h (v) Et_3N, DMAP, CHCl3 (dry), benzyl bromide or *p*-nitrobenzyl bromide, stirring at 30–70°C, 5—7 h.

Bruker NMR spectrophotometer. The chemical shifts of proton signals are in parts per million (ppm) downfield from tetramethylsilane (TMS) as an internal standard. EI-MS spectra were recorded on MAT 312 and MAT 311A mass spectrometers. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} aluminum sheets (Merck). Intermediate compounds **2a—j** of amino acids **1a—e** and their corresponding esters **3a—j** were prepared following an established literature procedure [20a,b]. Compound **2e** (yield 72%, m.p. 149°C, lit. 149°C) is has been reported in the literature [20c].

A number of methods have been reported in the literature for the synthesis of benezenesulfonamides by using carboxylic acids/hydrazides [18] and sulfonic acids/sulfonylchlorides [19] as starting compounds. We report here a novel and cost-effective approach for preparing such compounds, using different chiral and achiral amino acids as starting material (Scheme 1). The advantages of this method include ready availability of starting material, high yields of the final products (sulfonamides), a free mercapto group at position-5 of the oxadiazole ring, and most importantly the incorporation of chiral centre for the possible stereo-selectivity in the various applications of such compounds (e.g. as antimalarial agents). Benzyl and *p*-nitrobenzyl derivatives **6c, 6d** and **6 h** have been prepared from the corresponding free thiols **5c, 5d** and **5h,** using benzyl bromide and *p*-nitrobenzyl bromide (Scheme 1). Amino acids **1a—e** were converted to their corresponding sulfonamides **2a—j** by reaction with 4-methylbenzenesulfonyl chloride or 4-chlorobenzenesulfonyl chloride in alkaline medium (Scheme 1) using the standard literature method [20a]. The carboxylic acid group of these sulfonamides **2a—j** was esterified with ethanol in acidic medium [20b], the esters **3a—j** thus obtained were reacted with hydrazine hydrate (80%) to furnish the corresponding hydrazides **4a—j** in good yields [21]. The sulfonamides bearing the 2,5-disubstituted- 1, 3, 4-oxadiazole moiety, **5a—j,** were then prepared by the reaction of the hydrazides **4a—j** with carbon disulphide and potassium hydroxide [22]. Mass spectroscopy (see experimental protocol) showed that the 2,5-disubstituted 1,3,4-oxadiazoles **5** are formed by the elimination of H_2S from the carbohydrazides **4** and CS_2. Structures of all the synthesized compounds were confirmed by micro analysis, optical rotation, IR, ^{1}H-NMR and mass spectral data. The synthesis of compounds **4b, 4e, 4f, 4g, 5b, 5e, 5f** and **5g** has been reported earlier by our group.

*General procedure for the synthesis of 4 – (4–methyl/chlorophenylsulfonamido) alkane hydrazide **4a—j**.* A mixture of **3** (10 mmole) and hydrazine hydrate (80%) in absolute ethanol (50 mL) was refluxed for 9h. The excess solvent was distilled off and residue was filtered, washed with water and recrystallized from 70% aqueous ethanol. Pure products **4a—j** were collected in 71–89% yields.

*4–(4 – Methylphenylsulfonamido)butane hydrazide **4a**.* Recrystallized from 70% aqueous ethanol, microcrystals, M.p. = 92–94 °C,Yield = 76%. IR (KBr) (ν, cm^{-1}) 3316, 3286 (NH), 1651 (C = O), 1365 (SO_2), 1147 (SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ = 1.65–1.72 (m, 2H, CH_2), 2.5 (t, 2H, CH_2, J = 7.4 Hz), 2.7 (t, 2H, CH_2, J = 6.6 Hz), 2.39 (s, 3H, CH_3), 7.35 (d, 2H, ArH, J = 8.0 Hz), 7.7 (d, 2H, ArH, J = 8.0 Hz), 9.3 (bs, 2H, NH_2), 10.5 (bs, 1H, NH). EI-MS(%): 271(M^+,2.4),198(20.6), 184 (12.2), 171 (6.9), 155 (66), 91 (100), 87 (4.2), 71 (2.1)

*4– (4 – Chlorophenylsulfonamido)butane hydrazide **4b**.* Recrystallized from 70% aqueous ethanol, crystalline, M.p. 91–93°C, Yield = 75%. IR (KBr)(ν, cm^{-1}) 3377, 3285(NH), 1671(C=O), 1376 and 1155(SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ 1.66–1.75(m, 2H, CH_2), 2.55(t, 2H, CH_2, J = 7.4 Hz), 2.73 (t, 2H, CH_2, J = 6.7 Hz), 7.45(d, 2H, ArH, J = 8.0 Hz), 7.89(d, 2H, ArH, J = 8.0 Hz), 9.61(bs, 2H, NH), 11.29(bs, 1H, NH). EI-MS (%): 291 (M^+, 19), 175(100), 111(87), 76(9), 57(11).

*2– (4 – Methylphenylsulfonamido)butane hydrazide **4c**.* Recrystallized from 70% aqueous ethanol, microcrystals, M.p. = 146–148°C, Yield = 83%. IR (KBr)(ν, cm^{-1}) 3342, 3286 (NH), 1653 (C=O), 1361 (SO_2), 1149 (SO_2).^{1}H NMR (400 MHZ, Acetone-d_6): δ = 0.95 (dd, 3H, CH_3, J = 7.4 Hz, J = 7.4 Hz), 1.81–1.89 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 4.42 (dd, H, CH, J = 7.5 Hz), 7.33 (d, 2H, ArH, J = 8.1 Hz), 7.76 (d, 2H, ArH, J = 8.0 Hz), 9.52 (bs, 2H, NH_2), 10.70 (bs, 1H, NH). EI-MS (%): 271(M^+, 12), 198 (23), 184 (11), 171 (6), 155 (69), 116 (4), 91 (100), 87 (5), 85 (3), 71 (4), 57 (9).

*2– (4 – Chlorophenylsulfonamido)butane hydrazide **4d**.* Recrystallized from 70% aqueous ethanol, crystalline, M.p. = 155–157°C, Yield = 85%. IR (KBr)(ν, cm^{-1}) 3339, 3292(NH), 1653(C=O), 1365 and 1149(SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ 0.97(dd, 3H, CH_3, J = 7.2 Hz, J = 7.2 Hz), 1.81–1.90(m, 2H, CH_2), 4.38(t, 1H, CH, J = 7.5 Hz), 7.65(d, 2H, ArH, J = 8.0 Hz), 8.03(d, 2H, ArH, J = 8.0 Hz), 9.76(bs, 2H, NH), 11.27(bs, 1H, NH). EI-MS (%): 291 (M^+, 14), 175(25), 111(100), 76(19), 57(9).

*2– (4 – Chlorolphenylsulfonamido)propane hydrazide **4h**.* Recrystallized from 70% aqueous ethanol, microcrystals, M.p. = 157–159°C, Yield = 89%. IR (KBr)(ν, cm^{-1}) 3375, 3281 (NH), 1671 (C=O), 1369 (SO_2), 1165 (SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ = 1.25 (d, 3H, CH_3, J = 7.3 Hz),

4.73–4.81 (m, 1H, *CH), 7.55 (d, 2H, ArH, J = 8), 7.80 (d, 2H, ArH, J = 8 Hz), 9.12 (bs, 2H, NH_2), 9.47 (bs, 1H, NH), 10.38 (bs, 1H, NH). EI-MS(%): 277 (M^+, 4), 218 (100), 175 (95), 142 (14), 111 (69),102(3), 99 (33), 76 (3), 72 (33), 56 (5).

2,6-Bis(4-methylphenylsulfonamido]hexane hydrazide ***4i***. Recrystallized from 70% aqueous ethanol, microcrystals, M.p. = 125–127°C, Yield = 77%. IR (KBr)(ν, cm^{-1}) 3319, 3299 (NH), 1671 (C=O), 1341 (SO_2), 1166 (SO_2).^{1}H NMR (400 MHZ, Acetone-d_6): δ = 1.79–1.85 (m, 6H, $3CH_2$), 2.38 (s, 6H, $2CH_3$), 2.58 (td, 2H, CH_2, J = 7.5 Hz, J = 4.1 Hz), 4.39 (dd, 1H, CH, J = 7.5 Hz, J = 7.4 Hz), 7.33 (d, 4H, ArH, J = 8.4 Hz), 7.67 (d, 4H, ArH, J = 8.4 Hz), 9.61 (bs, 2H, NH_2), 10.53 (bs, 2H, NH). EI-MS (%): 313 (M^+– 155, 4), 91 (100), Anal. Calc. for $C_{20}H_{28}O_5N_4S_2$ (468.5878): C, 51.26; H, 6.02; N, 11.96; S, 13.68. Found: C, 51.03; H, 5.91; N, 11.93; S, 13.61%.

2,6-Bis(4-Chlorophenylsulfonamido]hexane hydrazide ***4j***. Recrystallized from 70% aqueous ethanol, microcrystals, M.p. = 135–137°C, Yield = 71%. IR (KBr)(ν, cm^{-1}) 3309, 3286 (NH), 1676 (C=O), 1375 (SO_2), 1146 (SO_2).^{1}H NMR (400 MHZ, Acetone-d_6): δ = 1.84–2.01 (m, 6H, $3CH_2$), 2.65 (td, 2H, CH_2, J = 7.6 Hz, J = 4.1 Hz), 4.42 (dd, 1H, CH, J = 7.5 Hz, J = 7.6 Hz), 7.55 (d, 4H, ArH, J = 8.0 Hz), 7.91 (d, 4H, ArH, J = 8.0 Hz), 9.75 (bs, 2H, NH_2), 11.21 (bs, 2H, NH). EI-MS (%): 334 (M^+– 175, 3), 175 (100), Anal. Calc. for $C_{18}H_{22}O_5N_4S_2Cl_2$(509.424): C, 42.44; H, 4.35; N, 11.11; S, 12.59. Found: C, 42.47; H, 4.37; N, 11.31; S, 12.28%.

General procedure for the synthesis of 4-methyl/chloro-N-[3 or 1-(5-mercapto-1,3,4-oxadiazol-2-yl) alkyl]benzenesulfonamide ***5a—j***. The compound **4** (5.5 mmole) was dissolved in 80 mL absolute ethanol and 6.6 mmol of carbon disulphide was added followed by 5.5 mmol of potassium hydroxide dissolved in 10 mL of water. The reaction mixture was stirred for 15 min and then refluxed for 16.5 h. The progress of the reaction was monitored by TLC. The excess ethanol was distilled off and the reaction mixture was diluted with water and acidified with 4N HCl to pH 2–3 (Congo Red). The solid obtained was filtered, washed with water and recrystallized from 60% aqueous ethanol. Pure product **5a—j** was collected in 80–91% yield.

4-Methyl-N-[(3-(5-mercapto-1,3,4-oxadiazol-2-yl) propyl]benzenesulfonamide ***5a***. Recrystallized from 60% aqueous ethanol, Microcrystals, M.p. = 145–147°C, Yield = 85%. IR (KBr)(ν, cm^{-1}): 3206 (NH), 2564 (SH), 1589 (C=N), 1379 (SO_2), 1179 (SO_2), 1285 (C=S). ^{1}H NMR (400 MHZ, Acetone-d_6): δ = 1.89–1.96 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.80 (t, 2H, CH_2, J = 7.4 Hz), 3.04 (t, 2H, CH_2, J = 6.6 Hz), 7.22 (bs, 1H, NH, exchangeable with D_2O), 7.38 (d, 2H, ArH, J = 8.10 Hz), 7.72 (d, 2H, ArH, J = 8.2 Hz), 12.80 (bs,1H, NH + SH). EI-MS(%): 313(M^+, 10), 240(7.2), 186(1.42), 155(49), 129(43), 91 (100), 65 (30). Anal. Calc. for $C_{12}H_{15}O_3N_3S_2$ (313.388): C, 45.99; H, 4.82; N, 13.41; S, 20.46. Found: C, 45.71; H, 4.70; N, 13.22; S, 20.71%.

4-Chloro-N-[3-(5-mercapto-1,3,4-oxadiazol-2-yl) propyl]benzenesulfonamide ***5b***. Recrystallized from 60% aqueous ethanol, powder, M.p. = 171–172°C, Yield = 80%. IR (KBr)(ν, cm^{-1}) 3287(−NH), 2545(− C=N), 1375 and 1165(−SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ1.92–1.98 (m, 2H, CH_2), 2.80 (t, 2H, CH_2, J = 7.5 Hz), 3.09 (t, 2H, CH_2, J = 6.7 Hz), 7.25 (bs, 1H, NH, exchangeable with D_2O), 7.63(d, 2H, ArH, J = 8.1 Hz), 7.87 (d, 2H, ArH, J = 8.2 Hz), 12.80 (bs,1H, NH + SH). EI-MS (%): 335(M^+ + 2), 333 (M^+, 7), 260(14), 177(25), 175(69), 158(10), 141(13), 129 (91), 113(32), 112(10), 111(100), 98(24), 76(11), 75(48), 69(29), 56(22), 55(16). Anal. Calc. for $C_{11}H_{12}O_3N_3S_2Cl$ (333.7921): C, 39.58; H, 3.62; N, 12.59; S, 19.21. Found: C, 39.68; H, 3.74; N, 12.77; S, 19.20%.

4-Methyl-N-[1-(5-mercapto-1,3,4-oxadiazol-2-yl) propyl]benzenesulfonamide ***5c***. Recrystallized from 60% aqueous ethanol, crystalline, $[\alpha]_D^{20} = +36.32$ (C = 1.01 g/100 cm^3, acetone). M.p. = 181–182 ^{0}C, Yield = 87%. IR (KBr)(ν, cm^{-1}) 3266 (NH), 2565 (SH), 1582 (C = N), 1356 (SO_2), 1145 (SO_2), 689 (C(S). ^{1}H NMR (400 MHZ, Acetone-d_6): δ = 0.92 (dd, 3H, CH_3, J = 7.4 Hz, J = 7.4 Hz), 1.82-1.90 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 4.38 (dd, H, CH, J = 7.5 Hz, J 7.6 Hz), 7.21 (bs, 1H, NH, exchangeable with D_2O), 7.32 (d, 2H, ArH, J = 8.1 Hz), 7.66 (d, 2H, ArH, J = 8.2 Hz), 12.75 (bs,1H, NH + SH). EI-MS(%): 313 (M^+,11), 249(7), 212(9), 155(58), 147(5), 91(100), 65 (26). Anal. Calculated for $C_{12}H_{15}O_3N_3O_3S_2$ (313.388): C, 45.99; H, 4.82; N, 13.41; S, 20.46. Found: C, 45.81; H, 4.56; N, 13.26; S, 20.61%.

4-Chloro-N-[1-(5-mercapto-1,3,4-oxadiazol-2-yl) propyl]benzenesulfonamide ***5d***. Recrystallized from 60% aqueous ethanol, microcrystals, $[\alpha]_D^{20} = +38.12$ (C = 1.05 g/100 cm^3, acetone). M.p. = 193–195 ^{0}C, Yield = 81%. IR (KBr)(ν, cm^{-1}) 3283(−NH), 2555(− SH), 1589(− C=N), 1365 and 1149(−SO_2). ^{1}H NMR (500 MHZ, Acetone-d_6): δ 0.98 (t, 3H, CH_3, J = 7.4 Hz), 1.82–1.91 (m, 2H, CH_2), 4.39 (dd, H, CH, J = 7.8 Hz, J = 7.5 Hz,), 7.24 (bs, 1H, NH, exchangeable with D_2O), 7.66 (d, 2H, ArH, J = 8.0 Hz), 7.87 (d, 2H, ArH, J = 8.0 Hz) 12.89(bs,1H, NH + SH). EI-MS (%): 333 (M^+, 11),

260(4), 177(15), 175(67), 129(81), 111(100), 76(11), 69(21). Anal. Calcd for $C_{11}H_{12}O_3N_3S_2Cl$ (333.7921): C, 39.58; H, 3.62; N, 12.59; S, 19.21. Found: C, 39.57; H, 3.65; N, 12.20; S, 19.46%.

4-Chloro-N-[1-(5-mercapto-1,3,4-oxadiazol-2-yl) ethyl]benzenesulfonamide ***5h***. Recrystallized from 60% aqueous ethanol, powder, $[\alpha]_D^{20} = +42.45$ (C = 1.04 g/100 cm^3, acetone) M.p. = 172–174 ^{0}C, Yield = 91%. IR (KBr)(ν, cm^{-1}) 3285(−NH), 2550 (−SH), 1609(− C = N), 1356 and 1149(−SO_2), 669 (C-S). ^{1}H NMR (400 MHZ, Acetone-d_6): δ 1.51 (d, 3H, CH_3 J = 7.0 Hz), 4.65–4.73 (m, 1H, CH), 7.23 (bs, 1H, NH, exchangeable with D_2O), 7.58 (d, 2H, ArH, J = 8.0 Hz), 7.83 (d, 2H, ArH, J = 8.0 Hz) 12.86(bs,1H, NH + SH). EI-MS (%): 319 (M^+, 2), 244(10), 218(20), 177(39), 175(100), 144(38), 139 (29) 111(87), 75(21). Anal. Calcd for $C_{10}H_{10}O_3N_3S_2$ Cl (319.779): C, 37.56; H, 3.15; N, 13.14; S, 20.05. Found: C, 37.74; H, 2.88; N, 13.10; S, 20.23%.

2-[1,5-Bis(4-methylphenylsulfonamido)]pentyl-5-mercapto-1,3,4-oxadiazole ***5i***. Recrystallized from 60% aqueous ethanol, Microcrystals, M.p. = 147–149°C, Yield = 89%. IR (KBr)(ν, cm^{-1}) 3299(NH), 1597(C=N), 1321and 1161(SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ 1.37–1.48(m, 4H, 3CH_2), 1.78(dd, 2H, CH_2, J = 7.5 Hz, J = 7.5 Hz), 2.37(s, 3H, CH_3), 2.41(s, 3H, CH_3), 2.79(dd, 2H, CH_2, J = 6.6 Hz, J = 6.6 Hz), 4.38(dd, 1H, CH, J = 7.5 Hz, J = 7.5 Hz), 7.21 (bs, 2H, NH, exchangeable with D_2O), 7.32(d, 2H, ArH, J = 7.8 Hz), 7.37(d, 2H, ArH, J = 7.9 Hz), 7.65(d, 2H, ArH, J = 8.0 Hz), 7.74(d, 2H, ArH, J = 8.0 Hz), 12.85 (bs,1H, NH + SH). EI-MS (%) 381(11), 284(19), 170(10), 91(100), 76(9), 75(17). Anal. Calcd for $C_{21}H_{26}O_5N_4S_3$ (510.662): C, 49.39; H, 5.13; N, 10.97; S, 17.84. Found: C, 49.28; H, 5.13; N, 11.27; S, 18.50%.

2-[1,5-Bis(4-chlorophenylsulfonamido)]pentyl-5-mercapto-1,3,4-oxadiazole ***5j***. Recrystallized from 60% aqueous ethanol, Microcrystals, M.p. = 153–155°C, Yield = 81%. IR (KBr)(ν, cm^{-1}) 3286(NH), 1607(C=N), 1327and 1165(SO_2). ^{1}H NMR (400 MHZ, Acetone-d_6): δ 1.39–1.53(m, 4H, 3CH_2), 1.75(dd, 2H, CH_2, J = 7.5 Hz, J = 7.5 Hz), 2.79(dd, 2H, CH_2, J = 6.6 Hz, J = 6.6 Hz), 4.39(dd, 1H, CH, J = 7.3 Hz, J = 7.3 Hz), 7.21 (bs, 2H, NH, exchangeable with D_2O), 7.52(d, 2H, ArH, J = 7.8 Hz), 7.57(d, 2H, ArH, J = 7.9 Hz), 7.79(d, 2H, ArH, J = 8.0 Hz), 7.82(d, 2H, ArH, J = 8.0 Hz), 12.91 (bs,1H, NH + SH). EI-MS (%) 421(2), 324(11), 175(100), 111(81),76(15),75(7), Anal. Calcd for $C_{19}H_{20}O_5N_4S_3Cl_2$ (551.479): C, 41.38; H, 3.66; N, 10.16; S, 17.44 Found: C, 41.18; H, 3.69; N, 10.47; S, 17.30%.

General procedure for the synthesis of derivatives of 4-methyl/chloro-N-[1-(5-mercapto-1,3,4-oxadiazol-2-yl) alkyl]benzenesulfonamide ***(6c, 6d and 6h)***. 0.75 mmol (250 mg) of compound **5**, 0.22 mmol (0.3 mL) of Et3N and a catalytic amount (25 mg) of DMAP were stirred in 25mL of dry CHCl3 for 15 min. 0.8 mmole of benzyl bromide/*p*-nitrobenzylbromide was added and the mixture was stirred for 5–7 h at 30–70°C. The reaction mixture was washed with dilute HCl, brine, water and dried over Na_2SO_4 (anhydrous). The excess solvent was distilled off and product was recrystallized from 65% aqueous ethanol.

N-[1-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)propyl]-4-methylbenzenesulfonamide ***6c***. Recrystallized from 65% aqueous ethanol, Prisms, $[\alpha]_D^{20} = +24.52$ (C = 0.54 g/100 cm^3, acetone); M.p. = 113–1150C, Yield = 91%.IR (KBr)(ν, cm^{-1}) 3256(−NH), 1602(− C = N), 1345 and 1155(−SO_2) 687(C − S).^{1}H NMR (400 MHZ, Acetone-d6): δ 0.88 (dd, 3H, CH3, J = 7.5 Hz, J = 7.4 Hz), 1.75–1.80(m, 2H, CH2), 2.39(s, 3H,CH_3), 4.17 (s, 2H, CH_2), 4.28 (dd, H, CH, J = 7.8 Hz, J = 7.6 Hz,), 7.18 (bs, 1H, NH, exchangeable with D_2O), 7.23–7.31 (m, 5H, ArH), 7.35 (d, 2H, ArH, J = 8.0 Hz), 7.67 (d, 2H, ArH, J = 8.4 Hz). EI-MS (%): 403 (M^+, 19), 91(100). Anal. Calcd for $C_{19}H_{21}O_3N_3S_2$ (403.515): C, 56.56; H, 5.25; N, 10.41; S, 15.89. Found: C, 56.36; H, 5.37; N, 10.38; S, 15.57%.

N-[1-(5-(4-nitrobenzylthio)-1,3,4-oxadiazol-2-yl) propyl]-4-chlorobenzenesulfonamide ***6d***. Recrystallized from 65% aqueous ethanol, Microcrystals, $[\alpha]_D^{20} = +21.39$ (C = 0.63 g/100 cm^3, acetone); M.p = 116–117°C, Yield = 75%.IR (KBr)(ν, cm^{-1}) 3289(−NH), 1592(− C = N), 1371 and 1166(−SO_2) 679(C–S). ^{1}H NMR (400 MHZ, Acetone-d6): δ 1.51 (d, 3H, CH_3 J = 7.0 Hz), 4.35 (s, 2H, CH_2), 4.64–4.71(m, 1H, CH), 7.22 (bs, 1H, NH, exchangeable with D_2O), 7.34 (d, 2H, ArH, J = 8.2 Hz), 7.56 (d, 2H, ArH, J = 8.4 Hz), 7.66 (d, 2H, ArH, J = 8.4 Hz), 8.17(d, 2H, ArH, J = 8.0 Hz). EI-MS (%): 454 (M^+, 7), 175(100). Anal. Calcd for $C_{17}H_{15}O_5N_4S_2$ Cl (454.904): C, 44.61; H, 3.32; N, 12.32; S, 14.11. Found: C, 44.56; H, 3.37; N, 12.68; S, 14.07%.

N-[1-(5-(4-nitrobenzylthio)-1,3,4-oxadiazol-2-yl) ethyl]-4-chlorobenzenesulfonamide ***6h***. Recrystallized from 65% aqueous ethanol, Microcrystals, $[\alpha]_D^{20} = +34.62$ (C = 1.00 g/100 cm^3, acetone); M.p. = 121–122°C, Yield = 79%.IR (KBr)(ν, cm^{-1}) 3286(−NH), 1595(− C = N), 1375 and 1166(−SO_2), 689(C − S). ^{1}H NMR (400 MHZ, Acetone − d6): δ 0.87 (dd, 3H, CH_3 J = 7.4 Hz, J = 7.4 Hz), 1.79 − 1.84(m, 2H, CH_2), 4.38 (s, 2H, CH_2), 4.50 (dd, H, CH J = 7.6 Hz, J = 7.6 Hz,), 7.20

(bs, 1H, NH, exchangeable with D_2O), 7.33 (d, 2H, ArH, J = 8.1 Hz), 7.54 (d, 2H, ArH, J = 8.2 Hz), 7.64 (d, 2H, ArH, J = 8.4 Hz), 8.14 (d, 2H, ArH, J = 8.4 Hz). EI-MS (%): 468 (M^+, 11), 175(100). Anal. Calcd for $C_{18}H_{17}O_5N_4S_2$ Cl (468.931): C, 46.10; H, 3.65; N, 11.95; S, 13.67. Found: C, 46.36; H, 3.38; N, 11.88; S, 13.29%.

Antimalarial assays

Parasite and experimental host. Male NIH mice, weighing 18–22 g were maintained on a commercial pellet diet and housed under conditions approved by the Ethics Committee. *Plasmodium berghei* (ANKA strain), a rodent malaria parasite, was used for infection. Mice were infected by i.p. passage of 1×10^6 infected erythrocytes diluted in phosphate buffered saline solution (PBS, 10 mM, pH 7.4, 0,1 mL). Parasitemia was monitored by microscopic examination of Giemsa stained smears.

Parasite extracts. Blood of infected animals, at a high level of parasitemia (30–50%), was collected by cardiac puncture with a heparinized syringe and the blood pool was centrifuged (500 × g × 10 min, 4°C). Plasma and buffy coats were removed and the red blood cells (RBC) pellet was washed twice with chilled PBS-Glucose (5.4%). The washed RBC pellet was centrifuged on a discontinuous percoll gradient (80–70% percoll in PBS-Glucose, 20000 × g × 30 min × 4°C) [25]. The upper band (mature forms) was removed by aspiration, collected in Eppendorf tubes and washed twice with chilled PBS-Glucose and the infected erythrocytes were lysed with the non-ionic detergent saponin (0.1% in PBS × 10 min). Cold PBS (1 mL) was added and the samples were centrifuged (13000 × g × 5 min, 4°C) to remove erythrocyte cytoplasm content (including erythrocyte hemoglobin). The free parasites were mixed with PBS-Glucose (5.4%), and subjected to three freeze-thaw cycles (−70 °C / +37 °C). The final homogenate was used in the hemoglobin hydrolysis inhibition assay [26].

Mice native hemoglobin. Native hemoglobin from non-infected mice was obtained by treating one volume of pellet erythrocytes with two volumes of water. The resulting solution was used as the substrate in the inhibition of the hemoglobin hydrolysis assay.

Inhibition of hemoglobin hydrolysis assay. The proteolytic effect of the parasite extract on the native mice hemoglobin was assayed using a 96-well tissue culture plate (Greiner Bio-One). The assay mixture contained: mice native hemoglobin (10 μL), parasite extract (50 μL), GSH (10 μL, 10 μM), and acetate buffer (0.2 M, pH 5.4) to a final volume of 100 μL. The compounds (50 mM) were incorporated in the incubation mixture dissolved in DMSO. The incubations were carried out at 37 °C for 18 h and the reactions were stopped by addition of reduced sample buffer. The degree of digestion was evaluated electrophoretically by SDS-PAGE [26] and the intact globin bands were analysed by densitometry. A DMSO control was electrophoresced at the same time.

4-Day suppressive test. NIH mice (18–23 g) were infected i.p. (using caudal vein) with 10^6 infected red blood cells with *Plasmodium berghei*. Two hours after infection, treatment began with the best compounds tested in the hypoxanthine assay. These were dissolved in DMSO (0.1 M), diluted with Saline-Tween 20 solution (2%). Each compound (20 mg/kg) was administered once by i.p. for 4 days. At day four, the parasitemia was counted by examination of Giemsa stained smears. Chloroquine (25 mg/Kg) was used as a positive control. The survival time beyond the control group (without drug treatment) was recorded. The results were expressed as percentage of parasitaemia in relation to the control (% of parasitemia) and percentage of survival mice [27].

Data analysis. Data were statistically analyzed using t-tests for specific group comparisons, assuming 95% of confidence according to Graph Pad Prism 3.02.

Inhibition of hem crystallization. The hem polymerization assay of compounds **4d, 4e, 4 h, 5a–i, 6c–d,** and **6h** was performed according to the reported procedure [23]. The results are shown in Table I. Briefly, a solution of haemin chloride (*50* μL, 4 mM), dissolved in DMSO (5.2 mg/mL), was distributed in 96-well micro plates. Different concentrations (*100-5* mM) of the compounds dissolved in DMSO, were added in triplicate in test wells (*50* μL) with a final concentration of 25-1, 25 mM/well. Controls contained either water (*50* μL) or DMSO (*50* μL). β-Hematin formation was initiated by the addition of acetate buffer (*100* μL 0.2 M, pH 4.4). Plates were incubated at 37°C for 48 h, carefully centrifuged for 15 min (4000 RPM, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with 200 μL of DMSO and finally, dissolved in 200 μL of NaOH (0.2 N). The solublized aggregates were further diluted at a ratio of 1:2, with NaOH (0.1N). The absorbances were recorded at 405 nm (Microplate Reader, BIORAD-550). The results are expressed as a percentage of inhibition of flavoprotein

Table I. Effects of compounds on the hem crystallization and hemoglobin proteolysis.

Compound	% IHC*	% IHbP*
4d	< 5	35.30 ± 1.90
4e	< 5	18.22 ± 1.04
4h	< 5	54.15 ± 2.26
5a	< 5	29.08 ± 3.55
5b	< 5	< 10
5c	< 5	36.05 ± 1.55
5d	< 5	35.30 ± 1.90
5e	< 5	27.54 ± 1.51
5f	< 5	28.45 ± 0.78
5g	< 5	38.16 ± 3.12
5h	< 5	34.15 ± 1.82
5i	< 5	25.33 ± 0.94
6c	< 5	28.45 ± 0.78
6d	< 5	27.54 ± 0.82
6h	< 5	12.16 ± 2.56
CQ	86.6 ± 2.75	NA

*IHC: Inhibition of hem crystallization. IHbP: Inhibition of hemoglobin proteolysis.

(FP) polymerization. The results are reported in Table 1. Compound **4h** showed a moderate decrease in the parasitaemia levels at 4th day post-infection, however, this did not cure the infected animals (Figure 1). This could be due to bioavailability problems since this compound is not water-soluble. It is necessary to assay this drug with a different dose-regimen in further experiments.

Results and discussion

To evaluate the antimalarial activity, we selected and tested the ability of the novel compounds **4d–e, 4h, 5a–i, 6c, 6d** and **6h** to inhibit hem polymerization, since hem can polymerize spontaneously in the food vacuole of the parasite under acid and low oxygen conditions [23]. Therefore, each compound was tested for its inhibition of globins proteolysis, in an *in vitro* assay which uses a trophozoite-rich extract to digest the native hemoglobin of mice. The electrophoretic analysis indicated that within a total of 15 compounds studied, **4h** was a potent inhibitor of the degradation of hemoglobin with an inhibition value of 54.14% (intact band at 14.4 kDa). Whereas, other compounds showed weak inhibition (< 50%) due to the presence of some intact hemoglobin (Table 1). The active compound **4h** (>50% inhibition of hemoglobin proteolysis), was tested in mice infected with *Plasmodium berghei* (ANKA strain, Figure 1), a chloroquine-susceptible strain of murine malaria. The mice were treated with compounds (chloroquine or the active ones, at 20 mg/kg, ip once daily) for 4 consecutive days (days 0–4 post infection, Figure 1), and their survival times and parasitaemias on the fourth day were monitored and compared with control mice receiving saline (untreated mice). Thus, chiral benzenesulfonamides and their derivatives, especially the compound **4h,** reported here may be considered as very interesting lead molecules for the possible design of novel selective antimalarial drugs.

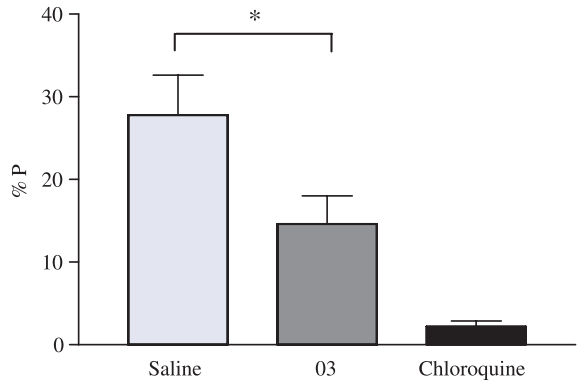


Figure 1. Parasitaemias at 4th day post-infection (%P). Mice were infected with 1x10^7 infected red blood cells and treatment was administered 2 hours after infection (20 mg/kg) every 24 h for 4 consecutive days. Results are expressed as the media ± SEM. * $p < 0.05$. n = 6. Compound **03 = 4h**.

The structures of the synthesized compounds are supported by physical, optical rotation data (for chiral compounds), micro analytical data, IR, ^{1}H NMR and mass spectral data. The IR spectrum of the representative sulfonamide **5h,** revealed the presence of characteristic bands for -NH at 3285 cm^{-1}, and 2550 cm^{-1} for -SH in addition to the -SO2 functional group. In the mass spectrum of **5h** the molecular ion peak was observed at m/z 319 (M^+, 2). In the ^{1}H NMR spectrum of **5h,** measured in acetone-d6, the following important signals were observed: δ = 1.51 (d, 3H, CH_3, J = 7.0 Hz), 4.65–4.73 (m, 1H, CH), 7.23 (bs, 1H, NH, replaceable with D_2O), 7.58 (d, 2H, ArH, J = 8.0 Hz), 7.83 (d, 2H, ArH, J = 8.0 Hz) and 12.86 (bs, 1H, NH + SH) with $[\acute{\alpha}]_D^{20} = +42.45^{o}$(1.04 g / 100 cm^3 of acetone). Spectral, optical and crystal structure data confirmed the presence of only one enantiomer in the case of chiral compounds. Sulfonamides **5a, 5c–e, 5h–j, 6c–d** and **6h** are new compounds not reported previously in the literature. None of these compounds has been investigated earlier for their antimalarial activities.

Conclusion

A series of new benzenesulfonamides, most of which are chiral, incorporating a 1, 3, 4-oxadiazole and selected amino acid entities have been synthesized using a cost-effective novel approach starting with known reagents and amino acids, via the ester and carbohydrazide intermediate, followed by cyclization with carbon disulfide. Some of these compounds have been investigated for their antimalarial activity. Compound **4h** was found to be a potent inhibitor of the degradation of hemoglobin from *Plasmodium berghei* (ANKA strain). It is worthwhile to note that

none of these compounds has been investigated earlier for their antimalarial activities, therefore, they may be considered as very interesting lead molecules for the possible design of novel selective antimalarial drugs.

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