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Il Farmaco 60 (2005) 307–311
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Synthesis and antimalarial activity of sulfonamide chalcone derivatives

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Received 29 November 2004; received in revised form 20 January 2005; accepted 22 January 2005

Available online 16 March 2005

Abstract

A series of sulfonamide chalcone derivatives were synthesized and investigated for their abilities to inhibit β-hematin formation in vitro and their activity against cultured *Plasmodium falciparum* parasites. Inhibition of β-hematin formation was minimal in the aromatic ring of the chalcone moiety as it appeared for compounds 4b, 4d-f, and greatest with compounds 4g (IC_{50} 0.48 μM) and 4k (IC_{50} 0.50 μM) with a substitution of 3,4,5-trimethoxyl and 3-pyridinyl, respectively. In this study, the most active compound resulted 1[4'-N(2'',5''-dichlorophenyl) sulfonyl-amidephenyl]-3-(4-methylphenyl)-2-propen-1-one 4i, effective as antimalarial by the inhibition of cultured *P. falciparum* parasites (1 μM). These studies open up the novel possibility of development of sulfonamide derivatives as antimalarials that target β-hematin formation and the inhibition of the development of cultured *P. falciparum* parasites, which should help delay the rapid onset of resistance to drugs acting at only a single site. Results with these assays suggest that chalcones exert their antimalarial activity via multiple mechanisms.

Keywords: Antimalarial; Synthesis; Sulfonamide; Chalcone; β-hematin

1. Introduction

Malaria is one of the most important infectious disease problems of humans, particularly in tropical regions of the world, with over 275 million new cases annually and mortality reaching 2 million, especially among children in Africa [1]. Control of this debilitating disease has been severely compromised by the development in malaria parasites of resistance to nearly all antimalarial drugs used for prophylaxis and treatment, particularly in *Plasmodium falciparum*, the most virulent of the four species infecting humans. Thus, there is a compelling and urgent need for new antimalarials with modes of action different from those of existing ones in order to replace those that are becoming obsolete and to identify new drug targets [2]. Chloroquine has recently been shown to inhibit hemozoin formation within the parasite food vacuole [3]. This process is also thought to be the molecular target of other quinoline antimalarials [4]. Hemozoin was originally considered to be formed by the polymerization of heme [5], but it has now been demonstrated to be a crystalline cyclic dimer of ferriprotoporphyrin IX [6]. Thus, hemozoin synthesis, a process unique to the malaria parasite, offers a logical and valuable potential target for new antimalarial drug development. New drugs that attack the same vital target of chloroquine but that are not subject to the same resistance mechanism would be highly desirable. Varied biological activities have been attributed to sulfonamide compounds, including fungicidal, bactericidal and nematicidal activities [7]. Novel sulfonamide derivatives having CNS (Central Nervous System Disease) activity, processes for their preparation and their use as medicaments are disclosed [8]. In this study, a series of sulfonamide chalcones derivatives were investigated for their abilities to inhibit β-hematin formation in vitro and the inhibition of the development of cultured *P. falciparum* parasites.

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doi:10.1016/j.farmac.2005.01.005

2. Experimental

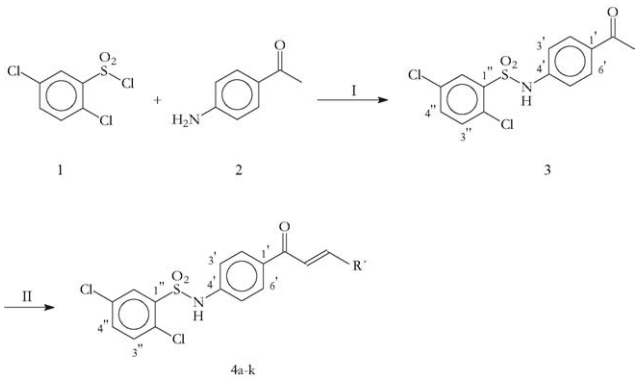
2.1. Chemistry

Melting points were determined in a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer and are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL GSX (270 MHz) spectrometer; chemical shifts are expressed in δ (ppm) relative to tetramethylsilane are given. All the exchangeable protons were confirmed by addition of D_2O. Mass spectral data were obtained with a Varian CP3800 model coupled with Saturn 2000/Gas Chromatograph ionization energy 70 eV, using CIMS (Chemical Ionization Mass Spectrometry). Elemental analyses were performed by Atlantic Microlab; Norcross, GA, USA, results were within ± 0.4% of predicted values for all compounds. All solvents were dried and distilled under nitrogen atmosphere. Analytical TLC was carried out on precoated plates (silica gel 60, F_{254}) and visualized with UV light. The progress of the reactions was monitored by TLC with ethyl acetate: hexane (1:1 v/v) as eluant.

We report a facile synthesis of several sulfonamide compounds containing a chalcone moiety starting from commercial and available materials (Scheme 1). The starting materials 4-aminoacetophenone and 2, 5-dichlorobenzene sulfonyl chloride **1** were reacted at room temperature to yield the corresponding sulfonamide **3**, which was used in one-step Claisen-Schmidt condensations with substituted aromatic aldehydes to obtain sulfonamide chalcone derivatives 4a–k. The formation of the α,β unsaturated ketones almost always yielded the trans alkene (E-form) as judged by ^1H NMR spectroscopy, which have been fully characterized by analytical and spectral data.

2.1.1. Synthesis of 4'-N [(2'', 5''-dichlorophenyl) sulfonyl-amide] acetophenone 3

The compound **3** was prepared according to a previously described procedure with some modifications [9]. A mixture of 4-aminoacetophenone **2** (1 mmol) and recently distillated 2, 5-dichlorobenzene sulfonyl chloride (1 mmol) was dissolved in 5 ml of chloroform. The solution was stirred at room temperature for 3–6 h. The resulting precipitate was washed with acetone and then filtered off; the crude material obtained was recrystallized in acetonitrile to give brown crystals. mp: 232–233 °C. Yield: 57%. IR 3216 (NH); 1667 (CO); 1584 (Ar); 1337, 1270 (SO_2).^1H NMR: (DMSO-d_6) 2.49 (s, 3H, MeCO); 7.21 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.91 Hz); 7.69 (d, 1H, $H_{3''}$, J = 8.41 Hz); 7.77 (dd, 1H, $H_{4''}$, $J_{4''\text{-}3''}$ = 8.41 Hz; $J_{4''\text{-}6''}$ = 2.47 Hz); 7.86 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.91 Hz); 8.10 (d, 1H, $H_{6''}$, J = 2.47 Hz); 11.38 (s, 1H, NH).



Scheme 1. Synthesis of sulfonamide chalcone derivatives.

2.1.2. General procedure for E-2[4'-N (2'',5''-dichlorophenyl) sulfonylamide] chalcones derivatives 4a–k

Mixture of 4'-*N*[(2'',5''-dichlorophenyl)sulfonylamide]acetophenone (1 mmol), substituted aldehydes (1 mmol) and 2.5 mmol of pulverized sodium hydroxide dissolved in dry methanol (5 ml). The reaction mixture was stirred at room temperature for 8–12 h, the resulting precipitates were filtered off and recrystallized in ethanol, yields 25–89%.

2.1.3. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3-(2,4-dimethoxyphenyl)-2-propen-1-one 4a

Mp: 210–212 °C. Yield: 89%. IR 3424 (NH); 1638 (CO); 1555 (Ar); 1338, 1290 (SO_2). ^1H-NMR: (DMSO-d_6) 3.83 (s, 3H, OMe); 3.88 (s, 3H, OMe); 6.63 (s, 1H, H_3); 7.24 (d, 2H, H_5; H_6, J = 8.64 Hz); 7.67 (d, 1H, H_α, J = 15.82 Hz); 7.69 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.64 Hz); 7.77 (dd, 1H, $H_{4''}$, $J_{4''\text{-}3''}$ = 8.40 Hz; $J_{4''\text{-}6''}$ = 2.70 Hz); 7.87 (d, 1H, $H_{3''}$, J = 8.40 Hz); 7.93 (d, 1H, H_β, J = 15.82 Hz); 8.01 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.64 Hz); 8.11 (d, 1H, $H_{6''}$, J = 2.70 Hz), 11.37 (s, 1H, NH); CIMS(m/z): 493 [M+1]; Anal. Calculated: C, 56.11%; H, 3.89%; N, 2.84%. Found: C, 56.82% H, 4.19%; N, 2.43%.

2.1.4. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(4-fluorophenyl)-2-propen-1-one 4b

Mp: 205–206 °C. Yield: 51%. IR 3424 (NH); 1638 (CO); 1555 (C=C Ar); 1338, 1290 (SO_2). ^1H NMR: (DMSO-d_6) 7.00 (d, 2H, H_3 and H_5, J = 8.91 Hz); 7.24 (d, 2H, H_2 and H_6, J = 8.91 Hz); 7.68–7.83 (m, 6H, $H_{3''}$; $H_{4''}$; $H_{3'}$ and $H_{5'}$; H_α, H_β); 8.06 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.64 Hz); 8.13 (d, 1H, $H_{6''}$, J = 2.24 Hz); 11.43 (s, 1H, NH); CIMS(m/z): 451 [M+1]; Anal. Calculated: C, 56.01%; H, 3.13%; N, 3.11%. Found: C, 55.99%; H, 3.57%; N, 3.88%.

2.1.5. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(3,4-methylendioxi-phenyl)-2-propen-1-one 4c

Mp: 254–256°°C. Yield: 58%. cm^{-1} 3504 (NH); 1651 (CO); 1542 (Ar); 1306, 1245 (SO_2). ^1H NMR: (DMSO-d_6) 6.08 (s, 2H, $-OCH_2O-$); 6.82 (d, 1H, H_5, J = 8.91 Hz); 6.95 (d, 1H, H_6, J = 8.91 Hz); 7.24 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.64 Hz); 7.45–7.59 (m, 2H, $H_{3''}$; $H_{4''}$); 7.68 (d, 1H, H_α, J = 15.34 Hz); 7.73 (d, 1H, H_β, J = 15.34 Hz); 7.83 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.64 Hz); 7.93 (br s, 1H, $H_{6''}$); ^{13}C NMR: δ 102.03 ($-OCH_2O-$); 107.31 (C_5); 109.00 (C_2); 120.37 ($C_{3'\text{-}5'}$); 121.06 (C_α); 125.70 ($C_{4''}$); 127.10 ($C_{2''}$); 129.84

($C_{5''}$); 130.21 ($C_{2'-6'}$); 130.39 ($C_{6''}$); 131.27 ($C_{3''}$); 131.46 ($C_{1'}$); 133.40 (C_1); 141.96 ($C_{4'}$); 146.10 (C_β); 148.56 (C_3); 149.52 (C_4); 156.22 ($C_{6''}$); 186.92 (CO); CIMS(m/z): 477 [M+1]; Anal. Calculated: C, 55.47%; H, 3.17%; N, 2.94%. Found: C, 55.88%; H, 3.94%; N, 2.10%.

2.1.6. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(4-chlorophenyl)-2-propen-1-one 4d

Mp: 270–274°°C. Yield: 76%. IR 3656 (NH); 1648 (CO); 1600 (Ar); 1338, 1274 (SO_2). ^{1}H NMR (DMSO-d_6) 6.94 (d, 4H, $H_{3'}$ and $H_{5'}$; H_3 and H_5, J = 8.18 Hz); 7.47–7.52 (m, 4H, $H_{3''}$; $H_{4''}$; H_2 and H_6); 7.58 (d, 1H, H_α, J = 15.58 Hz); 7.85–7.93 (m, 3H, H_β, $H_{2'}$ and $H_{6'}$); 7.98 (br s, 1H, $H_{6''}$); ^{13}C NMR: δ 119.86 ($C_{3'-5'}$); 123.68 (C_α); 128.43 ($C_{4''}$); 129.41 (C_{3-5}); 130.25 ($C_{6''}$); 130.63 ($C_{2'-6'}$); 130.82 (C_{2-6}); 131.85 ($C_{3''}$); 132.35 ($C_{4''}$); 133.66 ($C_{1'}$); 134.57 (C_1); 135.08 (C_4); 140.99 ($C_{4'}$); 187.06 (CO); CIMS(m/z): 468 [M+1]; Anal. Calculated: C, 54.04%; H, 3.02%; N, 3.00%. Found: C, 53.77%; H, 3.84%; N, 2.88%.

2.1.7. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(2,4-dichlorophenyl)-2-propen-1-one 4e

Mp: 216–217 °C. Yield: 56%. IR 3488 (NH); 1651 (CO); 1603 (Ar); 1306, 1254 (SO_2). ^{1}H NMR: (DMSO-d_6) 7.25 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.39 Hz); 7.55 (dd, 1H, H_5; JH_{5-6} = 8.56 Hz; JH_{5-3} = 1.49 Hz); 7.70 (s, 1H, H_3); 7.70 (d, 2H, $H_{3''}$; $H_{4''}$, J = 8.67 Hz); 7.73 (d, 1H, H_α, J = 14.85 Hz); 7.93 (d, 1H, H_6, J = 8.56 Hz); 8.11 (d, 1H, H_β, J = 14.85 Hz); 8.13 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.39 Hz); 8.21 (d, 1H, $H_{6''}$, J = 8.39 Hz); 11.49 (s, 1H, NH); CIMS(m/z): 502 [M+1]; Anal. Calculated: C, 50.32%; H, 2.61%; N, 2.79%. Found: C, 50.14%; H, 3.02%; N, 3.10%.

2.1.8. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(2,4-difluorophenyl)-2-propen-1-one 4f

Mp: 192–194 °C. Yield: 83%. IR 3536 (NH); 1651 (CO); 1603 (Ar); 1344, 1267 (SO_2). ^{1}H-NMR: (DMSO-d_6) 7.24 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.67 Hz); 7.66–7.73 (m, 4H, $H_{3''}$; $H_{4''}$; H_α; H_5); 7.80–7.91 (m, 2H, H_β; H_6); 7.96–8.17 (m, 4H, H_3; $H_{6''}$; $H_{2'}$ and $H_{6'}$); 11.43 (s, 1H, NH); ^{13}C NMR: δ 118,40 ($C_{3'-5'}$); 121.63 (C_α); 130.13 ($C_{4''}$); 130.45 ($C_{5''}$); 130.58 ($C_{6''}$); 130.75 ($C_{3''}$); 130.91 ($C_{1'}$); 131.38 ($C_{2'-6'}$); 132.87 (C_3); 134.31 (C_5); 135.23 (C_6); 138.50 ($C_{4'}$); 142.14 (C_β); 163.20 (C_4); 163.37 (C_2); 187.80 (CO); CIMS(m/z): 469 [M+1]; Anal. Calculated: C, 53.86%; H, 2.80%; N, 2.99%. Found: C, 54.65%; H, 3.15%; N, 3.26%.

2.1.9. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3(3,4,5-trimethoxy-phenyl)-2-propen-1-one 4 g

Mp: 248–252 °C. Yield: 82%. IR 3472 (NH); 1651 (CO); 1587 (Ar); 1274 (SO_2). ^{1}H-NMR: (DMSO-d_6) 3.69 (s, 3H, 4-OMe); 3.84 (s, 6H, 3,5-diOMe); 6.84 (s, 2H, H_2 and H_6); 7.15 (s wide, 2H, $H_{3'}$ and $H_{5'}$); 7.44 (s, 2H, $H_{3''}$ and $H_{4''}$); 7.53 (d, 1H, H_α, J = 15.58 Hz); 7.81 (d, 1H, H_β, J = 15.58 Hz); 7.87 (s, 2H, $H_{2'}$ and $H_{6'}$); 7.93 (s, 1H, $H_{6''}$); ^{13}C NMR: δ 56.62 (3,5-OMe); 60.74 (4-OMe); 106.51 (C_{2-6}); 120.47 ($C_{3'-5'}$); 122.24 (C_α); 127.09 ($C_{2''}$); 129.84 ($C_{5''}$); 130.18 ($C_{2'-6'}$); 130.58 ($C_{3''}$); 131.13 ($C_{1''}$); 131.52 ($C_{1'}$); 133.48 (C_1); 142.72 (C_4); 145.50 (C_β); 153.56 (C_{3-5}); 156.38 ($C_{6''}$); 187.32 (CO); CIMS(m/z): 523 [M+1]; Anal. Calculated: C, 55.18%; H, 4.05%; N, 2.68%. Found: C, 55.99%; H, 4.74%; N, 2.33%.

2.1.10. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3-phenyl-2-propen-1-one 4h

Mp: 140–141 °C. Yield: 53%. IR 3232 (NH); 1664 (CO); 1594 (Ar); 1338, 1274 (SO_2). ^{1}H NMR: (DMSO-d_6) 7.43-7.70 (m, 6H, H_3 and H_5; H_2 and H_6; H_4; H_α); 7.56 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.67 Hz); 7.66 (d, 2H, $H_{3''}$; $H_{4''}$, J = 8.64 Hz); 7.69 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.67 Hz); 7.89–7.94 (m, 2H, H_β; $H_{6''}$); CIMS(m/z): 433 [M+1]; Anal. Calculated: C, 58.34%; H, 3.50%; N, 3.24%. Found: C, 57.87%; H, 3.03%; N, 2.97%.

2.1.11. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3-(4-methylphenyl)-2-propen-1-one 4i

Mp: 179–180 °C. Yield: 25%. IR 3440 (NH); 1654 (CO); 1597 (Ar); 1328, 1283 (SO_2). ^{1}H-NMR: (DMSO-d_6) 7.28 (d, 2H, H_3 and H_5, J = 8.15 Hz); 7.36 (d, 2H, H_2 and H_6, J = 8.15 Hz); 7.37 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.40 Hz); 7.73 (d, 1H, H_α, J = 16.15 Hz); 7.80 (d, 1H, $H_{3''}$, J = 8.15 Hz); 7.87 (d, 1H, $H_{4''}$, J = 8.15 Hz); 7.92 (d, 1H, H_β, J = 16.15 Hz); 8.23 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.40 Hz); 8.63 (s, 1H, $H_{6''}$); CIMS(m/z): 447 [M+1]; Anal. Calculated: C, 59.20%; H, 3.84%; N, 3.14%. Found: C, 58.90%; H, 3.58%; N, 3.76%.

2.1.12. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3-(4-methoxyphenyl)-2-propen-1-one 4j

Mp: 180–182 °C. Yield: 53%. IR 3440 (NH); 1667 (CO); 1597 (Ar); 1341, 1270 (SO_2). ^{1}H NMR: (DMSO-d_6) 3.86 (s, 3H, 4-OMe); 6.99 (d, 2H, H_3 and H_5, J = 8.15 Hz); 7.63–7.74 (m, 5H, $H_{3'}$ and $H_{5'}$; H_α; H_2 and H_6); 7.78–7.88 (m, 3H, $H_{3''}$; $H_{4''}$; H_β); 8.02 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.15 Hz); 8.09 (d, 1H, $H_{6''}$, J = 1.49 Hz); ^{13}C NMR: δ 55.88 (4-OMe); 114.95 (C_{3-5}); 118.48 ($C_{3'-5'}$); 120.78 (C_α); 130.11 ($C_{4''}$); 130.43 (C_{2-6}); 130.66 ($C_{3''}$); 131.23 ($C_{2'-6'}$); 132.75 ($C_{1''}$); 134.22 ($C_{1'}$); 134.96 (C_1); 143.01 ($C_{4'}$); 144.01 (C_β); 161.88 (C_4); 188.03 (CO); CIMS(m/z): 463 [M+1]; Anal. Calculated: C, 57.15%; H, 3.71%; N, 3.03%. Found: C, 56.52%; H, 4.23%; N, 3.65%.

2.1.13. 1[4'-N(2'',5''-dichlorophenyl)sulfonylamidephenyl]-3-(3-pyridinyl)-2-propen-1-one 4k

Mp: 266–268 °C. Yield: 53%. IR 3456 (NH); 1651 (CO); 1590 (Ar); 1302, 1270 (SO). ^{1}H-NMR: (DMSO-d_6) 6.84 (d, 2H, $H_{3'}$ and $H_{5'}$, J = 7.43 Hz); 7.43–7.47 (m, 2H, $H_{3''}$; $H_{4''}$); 7.60 (d, 1H, H_α, J = 15.58 Hz); 7.85 (d, 2H, $H_{2'}$ and $H_{6'}$, J = 7.43 Hz); 7.94 (d, 1H, $H_{6''}$, J = 1.46 Hz); 7.80 (d, 1H, H_β, J = 15.58 Hz); 8.30 (dd, 1H, H_4, JH_{4-5} = 7.91 Hz; JH_{4-2} = 1.22 Hz); 8.56 (d, 1H, H_2, J = 1.22 Hz); 8.95 (s, 1H, NH); ^{13}C NMR: δ 120.47 ($C_{3'-5'}$); 124.52 (C_5); 124.95 (C_α); 126.63 (C_5); 130.19 ($C_{2''}$); 130.65 ($C_{2'-6'}$); 131.38 (C_1);

131.50 ($C_{3'}$); 133.46 ($C_{5'}$); 135.38 ($C_{4'}$); 138.47 ($C_{1'}$); 145.87 ($C_{4'}$); 150.47 (C_{β}); 150.95 (C_2); 156.69 (C_6); 186.68 (CO); CIMS(m/z): 434 [M+1]; Anal. Calculated: C, 55.44%; H, 3.26%; N, 6.47%. Found: C, 55.12%; H, 3.53%; N, 7.01%.

2.2. Pharmacology

2.2.1. Inhibition of hemozoin formation in vitro

The hemozoin formation assay was performed according to Baelmans et al. [10]. Briefly, a solution of hemin chloride (50 μl, 4 mM), dissolved in DMSO (5.2 mg/ml), was distributed in 96-well micro plates. Different concentrations (1–100 mM) of the compounds, dissolved in DMSO, were added in triplicate in test wells (50 μl) with a final concentration of 1.25 μM–25 mM/well. Controls contained either water or DMSO. Hemozoin 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 to allow completion of the reaction and centrifuged (4000 RPM × 15 min, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 μl) and finally dissolved in NaOH (200 μl, 0.2 N). The solubilized aggregates were further diluted 1:2 with NaOH (0.1 N) and absorbance recorded at 405 nm (Micro Plate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of hemozoin formation.

2.2.2. Parasite inhibition flow cytometry

Effects of inhibitors on parasite development were determined as follows. Sorbitol synchronized, 0.1% parasitemia, ring stage *P. falciparum* strain W2 parasites were cultured under the atmosphere of 3% O_2, 6% CO_2 and 91% N_2 in RPMI-1640 medium supplemented with 10% human serum in the presence of inhibitors for 48 h without media change. Inhibitors were added from 1000× DMSO stocks.

After 48 h, the culture medium was removed and replaced with 1% formaldehyde in PBS pH 7.4 for an additional 48 h at room temperature to fix cells. Fixed parasites were transferred into 0.1% Triton-X-100 in PBS containing 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter vs. fluorescence) acquired on a FACS sort flow cytometer using CellQuest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percentage control parasitemia over inhibitor concentration using Graph Pad Prism software [11].Table 1.

3. Results and discussion

Previous report has showed that chalcones exhibited potent antimalarial activities [12–14]. Eleven analogs of sulfonamide chalcones derivatives were tested for their effects as inhibitors of β-hematin formation and their activity against cultured *P. falciparum* parasites (Table 1). An in vitro assay was used to assess the abilities of the sulfonamide chalcones

Table 1
Effects of compounds 4a–k on inhibition of β-hematin formation and their activity against cultured *P. falciparum* parasites

Compound	R´	% Inhibition of heme formation [a]	IC_{50} (μM) [b]	FACSμM [IC_{50}] [c]
4a	2,4-di$OMeC_6H_3$	91.39 ± 2.2	8.65	> 10
4b	4-FC_6H_4	< 5		> 10
4c	3,4-$OCH_2OC_6H_3$	86.31 ± 1.76	14.48	> 10
4d	4-ClC_6H_4	< 5		> 10
4e	2,4-diClC_6H_3	< 5		> 10
4f	2,4-diFC_6H_3	81.33 ± 3.89	0.67	> 10
4 g	3,4,5-tri$OMeC_6H_2$	91.7 ± 3.51	0.48	> 10
4h	C_6H_5	< 5		> 10
4i	4-MeC_6H_4	< 5		1
4j	4-$OMeC_6H_4$	< 5		> 10
4k	3-C_5H_4N	92.00 ± 2.70	0.50	> 10
Chloroquine		84.16 ± 3.9	1.33	

[a] Percentage of inhibition.
[b] IC_{50} of β-hematin formation. The results are expressed by the media ± SEM.
[c] IC_{50} values (μM) for tested compounds as determined by flow cytometry as inhibitors of cultured *P. falciparum* parasites.

compounds to inhibit β-hematin formation. In that assay, hemin was allowed to form β-hematin under acidic conditions. Of the eleven sulfonamide analogs tested, six showed no measurable activity, two with a reasonable activity and compounds 4f, 4 g and 4k inhibited β-hematin formation with an IC_{50} better than chloroquine (Table). The presence of an alkoxyl group as substituents in the aromatic ring with a good antimalarial activity has been reported previously [15,16] and we found that mono, di and trimethoxyl-substituted groups in the aromatic ring (4j, 4c, 4 g) give rise to higher potencies and this appeared to be favorable for this activity, since most of compounds possessing this group showed measurable levels of inhibition of β-hematin formation, regardless of the nature of the substitutions in the aromatic ring. However compounds 4f and 4k played an important role and maintained different substituents groups in the aromatic ring referring to 2, 4-difluoro and 3-pyridinyl with an excellent activity in inhibiting β-hematin formation, apparently due to electron-donating properties, yielding the most interesting compounds. In general, we can speculate that electron-withdrawing groups on the substituted aromatic ring for these sulfonamide chalcones should favor the Michael addition to an available nucleophilic side chain on the enzyme.

The substituted trimethoxyl aromatic compound 4 g being the most active as antimalarial analog evaluated (IC_{50}, 0.48 μM), compared to a chloroquine (IC_{50} 1.33 μM). Having only one Cl, F, Me, or OMe substituents in the aromatic ring (compounds 4d, 4b, 4i and 4j) did not improve the activity of the analog compared with that of the corresponding disubstituted, trisubstituted and pyridinyl analogs 4a, 4c, 4f and 4 g. However, the poor antimalarial activities of the 4b, 4d, 4e, and 4 h-4j derivatives of sulfonamide chalcones could be attributed to the fact that they are not strongly basic, a property required for accumulation in the malaria parasite acidic food vacuole in which hemozoin formation takes place

[17]. We observed a potent inhibition on development of cultured parasites for compound 4i (Table 1) but without any correlation related with inhibition of heme formation. Therefore, the inhibition of heme formation does not guarantee antimalarial activity.

To summarize, results for the series of alkoxylated and halogenated compounds with respect to their differences in activity, it might be due basically for resonance and inductive effects reason. Apparently, it seems to be that resonance factor predominates among them and may have something to do with the active site of the enzyme. However, we noticed some differences to each of halogenated compounds, and it is clear that predominance of the electronegative effect of difluoride may cause stronger chemical interaction in the site of biological substrate. Replacement of a pyridinyl moiety by a phenyl group, antimalarial activity was decreased drastically as seen by comparison of 4 h with 4k and this kind of compound referring to nitrogen-containing heterocyclic resembled pretty much with the nitrogen of chloroquine and may be somewhat concentrated in the food vacuoles of malaria parasites for a given result [18]. These results offer new possibilities for further improvements in the antimalarial performance of sulfonamide chalcones derivatives. Although this initial study involved only a limited number of compounds, it has provided structure-activity relationships that are well worth studying further.

Antimalarials in this study should be inexpensive to produce using the convenient chemistry that we have developed; cost of production is a critically important consideration if the resulting compounds are ever to be developed into therapeutic agents. We will further direct efforts to find even more potent antimalarials in the future.

Acknowledgements

We thank Mr. Angel Salazar for his technical assistance. This work was supported by Consejo de Desarrollo Científico y Humanístico (CDCH) Universidad Central de Venezuela (Grants PG 0630-5126-2.003 and PG 0630-5125-2.003), the National Institutes of Health (Grants AI 35800 and AI 35707) and the Medicines for Malaria Venture. PJR is a Doris Duke distinguished clinical scientist.

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