Eur. J. Med. Chem. 36 (2001) 555–560

PII: S0223-5234(01)01245-4/SCO

Short communication

Synthesis of quinolinyl chalcones and evaluation of their antimalarial activity

José N. Domínguez[a,*], Jaime E. Charris[a], Gricela Lobo[a], Neira Gamboa de Domínguez[b], María M. Moreno[b], Flavia Riggione[c], Erlinda Sanchez[d], Jed Olson[e], Philip J. Rosenthal[e]

[a]Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Caracas 1051, Venezuela
[b]Laboratorio de Bioquímica, Facultad de Farmacia, Universidad Central de Venezuela, Caracas 1051, Venezuela
[c]Laboratorio de Investigación en Malaria, Facultad de Medicina, Universidad Central de Venezuela, Caracas 1051, Venezuela
[d]Laboratorio de Inmunoparasitología Biomed, Universidad de Carabobo, Maracay, Venezuela
[e]Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, USA

Received 15 December 2000; accepted 30 May 2001

Abstract – Quinolinyl chalcones were synthesized and evaluated for their inhibition of the *Plasmodium falciparum* cystein protease falcipain and their activity against cultured *P. falciparum* parasites. They were also tested for in vivo efficacy in a rodent *P. berghei* model. Their activity against falcipain and as antimalarials was moderate, but antimalarial activity was probably not due to the inhibition of falcipain and may follow a different mechanism. 1-(2,4-Dichlorophenyl)-3-[3-(2-chloro-6,7-dimethoxiquinolinyl)]-2-propen-1-one 3j was the most promising compound among those here reported (IC_{50} 19.0 μM). © 2001 Éditions scientifiques et médicales Elsevier SAS

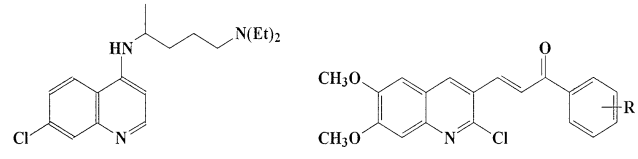
antimalarial / falcipain / synthesis / chalcones / P. berghei / P. falciparum

1. Introduction

Malaria affects over one hundred countries in the world, and the prevalence of this disease has been escalating at an alarming rate. An estimated 300 to 500 million cases each year cause 1.5 at 2.7 million deaths, more than 90% in children under five years of age [1]. The emergence of drug resistance continues to be a serious global problem. Chloroquine **1** was until recently the drug of choice, but resistance has now spread to all major malaria endemic regions [2]. New antimalarial drugs are needed and drug development efforts are ongoing [3–5]. Recently we have reported the synthesis and the biological evaluation of some antimalarial compounds [6–10]. However, more research is needed, and additional agents are required to replace available drugs whose efficacy is limited by resistance [11–13]. In this work, we report the synthesis and biological activity against a chloroquine resistant strain of *Plasmodium falciparum* of some quinolinyl chalcones **3a**–**l**. We studied the effects of these inhibitors against the *P. falciparum* cysteine protease falcipain and also two of them **3b**, **3l** were tested for in vivo efficacy in a rodent *P. berghei* model (see *figure 1*).

2. Chemistry

The strategy employed to prepare these compounds was based on the Claisen-Schmidt condensation [14].



1 3a-l

Figure 1. Compounds 1 and 3a–l.

* Correspondence and reprints
E-mail address: dominguj@camelot.rect.ucv.ve (J.N. Domínguez).

Table I. Antimalarial activity against *P. falciparum* of quinolinyl chalcones

No	R	IC_{50}[a] μM	IC_{50}[b] μM
3a[c]	H	20	[d]
3b[c]	4-Me	50	175
3c	4-Br	70	[d]
3d	4-Cl	[e]	[d]
3e	2,4-OMe	40	[d]
3f	2,5-OMe	40	132
3g	2,6-OMe	100	[d]
3h	3,4-OMe	>100	[d]
3i	3,4,5-OMe	>100	[d]
3j	2,4-Cl	>100	19.0
3k	2,5-Cl	>100	48.6
3l	3,4-Cl	>100	[d]

[a] IC_{50} for inhibition of falcipain activity, measured as the hydrolysis of Z–Phe–Arg–AMC.
[b] IC_{50} ^{3}[H] hypoxanthine incorporated extrapolated from curves of percent activity vs. concentration.
[c] Ref.14.
[d] Inhibition >200 μM.
[e] Not tested.

Commercially available acetophenones were reacted with 2-chloro-6,7-dimethoxy-3-formylquinoline **2** [15] in the presence of sodium methoxide to yield the quinolinyl chalcone derivatives **3a**–**l**. It is interesting to note that in the ^{1}H NMR Spectra of these compounds, the protons of the αβ unsaturated system absorbed as two doublets around 7.5 ppm for H_α and 8.1 ppm for H_β, with a coupling constant between $J = 15$–16 Hz for their trans isomer. The rest of protons appeared in the expected region. Additional support for the structure of these compounds was obtained from ^{13}C NMR at 189 ppm (CO). The molecular ion observed in the mass spectrum for all compounds confirmed their molecular weights. The fragmentation routes involved the C–Cl bond breaking from the 2-chloroquinoline moiety, which afforded the base peaks for all compounds (*table I*).

3. Biological results and discussion

Chalcones have previously been shown to inhibit the *P. falciparum* cystein protease falcipain [11]. The predicted binding of the quinolinyl chalcones with the falcipain active site was not as strong as for other chalcones, and potencies were somewhat lower than those seen for related compounds [11]. The most active quinolinyl chalcones tested in this study inhibited the activity of falcipain at mid-micromolar concentrations (*table I*). The quinolinyl chalcones were tested for inhibition of hypoxanthine uptake by a culture chloroquine resistant strain of *P. falciparum*. Compound **3j** (IC_{50} 19.0 μM) was the most potent of the compounds (*table I*).

The in vivo activity of compounds **3b** and **3l** against malaria parasites was tested by determining their ability to protect mice from the lethal effects of *P. berghei* infection. When these compounds were injected intraperitonealy at 10 and 20 mg/Kg once a day every day, they reduced the level of parasitemia in mice infected with *P. bergehei* and influenced the course of a well established infection in mice (*figure 2*).

Control mice died between day 6 and day 8 post-infection, but the **3b** and **3l** treated mice survived until day 11 post-infection. These compounds are able to reduce the parasitemia, however, the parasites were not completely cleared. Presumably, another scheme of administration of the drugs could be required for best results.

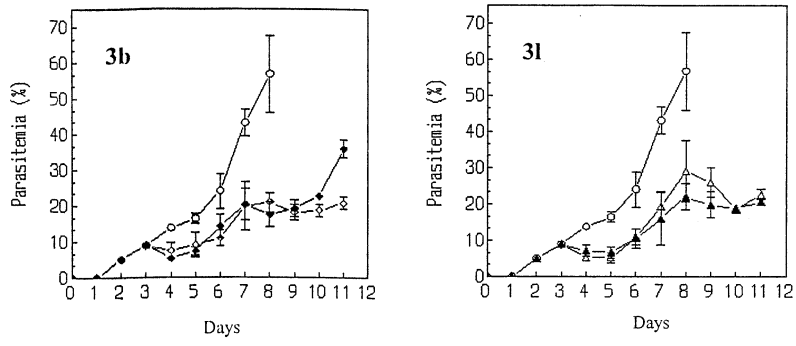


Figure 2. Effects of compounds 3b and 3l on the level of parasitemia in mice parasitized by P. berghei. The compounds were given intraperitonealy at 10 mg/Kg (◇) and 20 mg/Kg (▲) daily over day 3 after infection. The control animal (○) received 0.1 ml of the vehicle. Each point represents the mean±standard mean of the mean for five mice.

The mechanism of action of the quinolinyl chalcones is unclear, as activity did not correlate with the inhibition of falcipain. Most likely, as was the case with a series of phenothiazines [8], although some of the quinolinyl chalcones inhibited falcipain, their most potent antimalarial effects was independent of the inhibition of this enzyme. In any case, it is evident from our results that the substituted group in the benzoyl ring plays a significant role in determining the antimalarial activity of the tested quinolinyl chalcones, and this is in agreement with our previously reported findings [6]. Future efforts will focus on the examination of other derivatives of this general class of compounds.

4. Experimental

4.1. 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. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Jeol Eclipse 270 M Hz spectrometer and are reported in ppm downfield from TMS as internal standard. Mass spectra were performed in a Hewlet Packard model 5995/Gas Chromatograph ionization energy 70 eV. Elemental analyses were performed by C.S.I.C., Madrid, Spain and were within ± 0.4% of predicted values for all compounds.

4.1.1. General procedure for the synthesis of 1-(phenylsubstituted)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3a–l**

A mixture of 2-chloro-6,7-dimethoxy-3-formylquinoline 0.25 g (1 mmol), the respective acetophenone (1 mmol), and sodium methoxide (catalytic) in methanol (4 ml) was stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with water and recrystallized from DMF–H_2O or EtOH–H_2O.

4.1.1.1. 1-Phenyl-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3a** [14]

NMR $CDCl_3$: δ 4.00 (s, 6H, OMe), 7.06(s, 1H, H_5), 7.33(s, 1H, H_8), 7.51–7.59 (m, 4H, Ar, H_α), 8.03 (m, 2H, Ar), 8.19 (d, 1H, $J = 15.8$, H_β), 8.31 (s,1H, H_4).

4.1.1.2. 1-(4-Methylphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3b** [14]

NMR $CDCl_3$: δ 2. 4 (s, 3H, Me), 4.0 (s, 6H, OMe), 7.06(s, 1H, H_5), 7.2 (d, 2H, $J = 8.12$ Hz, 2′ and 6′), 7.31 (s, 1H, H_8,), 7.5 (d, 1H, $J = 15.8$ Hz, H_α), 7.9 (d, 2H, H_β $J = 8.12$ Hz, 3′, 5′), 8.1 (d, 1H, $J = 15.9$ Hz, H_β); 8.3 (s, 1H, H_4).

4.1.1.3. 1-(4-Bromophenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3c**

Yield 92% (DMF–H_2O); mp 244–245 °C; IR (KBr) cm^{-1}: 1638(CO), 1577(C=C). ^{1}H NMR $CDCl_3$: δ 4.02(s, 6H, OMe), 7.06(s, 1H, H_5), 7.33(s, 1H, H_8), 7.52(d, 1H, H_α $J = 15.7$ Hz), 7.63(d, 2H, $J = 8.39$ Hz, Ar), 7.96 (d, 2H, $J = 8.42$ Hz, Ar), 8.29(d, 1H, H_β $J = 15.7$ Hz), 8.32(s,1H, H_4). ^{13}C NMR: δ 56.2, 56.9, 104.6, 107.1, 122.5, 124.6, 126.1, 128.5, 130.3, 132.1, 134.5, 136.7, 140.6, 145.9, 148.7, 150.9, 155.2, 189.7. Mass spectrum (EI, m/z). 433/434 (M^+, 16%), 398/399 (M^+–Cl, 100%). Anal. $C_{20}H_{15}BrClNO_3$: C, 55.52; H, 3.49; N, 3.24. Found: C, 55.37; H, 3.49; N, 3.29%.

4.1.1.4. 1-(4-Chlorophenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3d**

Yield 84% (DMF–H_2O); mp 228–230 °C; IR (KBr) cm^{-1}: 1633(CO), 1581(C=C); ^{1}H NMR $CDCl_3$: δ 4.02(s, 6H, OMe), 7.08(s, 1H, H_5), 7.34(s, 1H, H_8), 7.46(d, 2H, $J = 8.66$ Hz, Ar), 7.52(d, 1H, Hα $J = 15.83$ Hz), 7.98(d, 2H, $J = 8.66$ Hz, Ar), 8.19(d, 2H, Hβ $J = 15.83$ Hz), 8.33(s, 1H, H_4). ^{13}C NMR: 56.2, 56.9, 104.6, 107.1, 122.5, 124.6, 126.0, 128.5, 130.7, 132.0, 134.5, 136.7, 140.6, 145.9, 148.7, 150.9, 155.2, 189.7. Mass spectrum (EI, m/z). 388/389 (M^+, 6%), 353/354 (M^+–Cl, 100%). Anal. $C_{20}H_{15}Cl_2NO_3$: C, 61.87; H, 3.89; N, 3.61. Found: C, 61.85; H, 3.75; N, 3.53%.

4.1.1.5. 1-(2,4-Dimethoxyphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one **3e**

Yield 85% (DMF–H_2O); mp 246 °C; IR (KBr) cm^{-1}: 1654(CO), 1593(C=C). ^{1}H NMR $CDCl_3$: δ 3.89(s, 3H, OMe), 3.91(s, 3H, OMe), 4.02(s, 6H, OMe), 6.59(m, 1H, Ar), 7.06(s, 1H, H_5), 7.35(s, 1H, H_8), 7.50(d, 1H, Hα $J = 15.7$ Hz), 7.83(m, 2H, Ar), 7.90(d, 1H, Hβ $J = 15.8$ Hz), 8.29(s, 1H, H_4). ^{13}C NMR: 55.9, 56.1, 56.3, 56.76, 104.98, 107.32, 122.81, 125.36, 125.83, 128.86, 128.94, 133.15, 134.54, 138.02, 140.8 148.7, 154.7, 155.2, 189.8. Mass spectrum (EI, m/z). 353/355 (M^+–2OCH_3, 3%), 318/319 (M^+–2OCH_3–Cl, 100%). Anal. $C_{22}H_{20}ClNO_5$:

C, 63.85; H, 4.87; N, 3.39. Found: C, 64.01; H, 4.93; N, 3.37%.

4.1.1.6. 1-(2,5-Dimethoxyphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3f***

Yield 90% (DMF–H_2O); mp 150 °C; IR (KBr) cm^{-1}: 1651(CO), 1609(C=C). ^1H NMR $CDCl_3$: δ 3.86(s, 6H, OMe), 4.02(s, 6H, OMe); 7.03(m, 2H, Ar), 7.12(s, 1H, H_5), 7.26(m, 1H, Ar), 7.36(s, 1H, H_8), 7.49(d, 1H, H_α $J = 16$ Hz), 7.98(d, 1H, H_β $J = 16$ Hz), 8.3(s, 1H, H_4). ^{13}C NMR: 56.0, 56.2, 56.3, 56.7, 105.1, 107.8, 122.8, 125.4, 125.8, 128.9, 128.9, 129.0, 133.2, 134.5, 138.0, 140.2, 148.7, 154.8, 155.2, 189.8. Mass spectrum (EI, m/z). 413/414 (M^+, 13%), 378/379 (M^+–Cl, 100%). Anal. $C_{22}H_{20}ClNO_5$: C, 63.85; H, 4.87; N, 3.39. Found: C, 63.90; H, 4.87; N, 3.42%.

4.1.1.7. 1-(2,6-Dimethoxyphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3g***

Yield 78% (DMF–H_2O); mp 218–220 °C; IR (KBr) cm^{-1}: 1648(CO), 1580(C=C). ^1H NMR $CDCl_3$: δ 3.87(s, 6H, OMe), 4.02(s, 6H, OMe), 6.62(d, 2H, $J = 8.4$ Hz, Ar), 6.97(d, 1H, H_α $J = 16$ Hz), 7.04(s, 1H, H_5), 7.29(s, 1H, H_8), 7.34(d, 1H, $J = 8.4$ Ar), 7.75(d, 1H, H_β $J = 16$ Hz), 8.26(s, 1H, H_4). ^{13}C NMR: 56.2, 56.2, 56.3, 56.6, 105.2, 107.8, 122.8, 125.4, 125.8, 128.9, 128.9, 129.0, 133.2, 134.5, 138.0, 140.2, 148.7, 154.8, 155.2, 189.8. Mass spectrum (EI, m/z). 413/414 (M^+, 5%), 378/379 (M^+–Cl, 100%). Anal. $C_{22}H_{20}ClNO_5$: C, 63.85; H, 4.87; N, 3.39. Found: C, 63.85; H, 4.75; N, 3.39%.

4.1.1.8. 1-(3,4-Dimethoxyphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3h***

Yield 85% (DMF–H_2O); mp 220–222 °C; IR (KBr) cm^{-1}: 1648(CO), 1574(C=C). ^1H NMR $CDCl_3$: δ 3.97(s, 6H, OMe), 4.02(s, 6H, OMe), 6.91(d, 1H, $J = 8.4$ Hz, $H_{5'}$), 7.08(s, 1H, H5), 7.34(s, 1H, H_8), 7.5(d, 1H, H_α $J = 15.9$ Hz), 7.63(d, 1H, $J = 1.73$ Hz, $H_{2'}$), 7.69(dd, 1H, H6′ $J_1 = 1.98$, J_2: 8.16 Hz), 8.16(d, 1H, H_β $J = 15.8$ Hz), 8.32(s, 1H, H_4). ^{13}C NMR: 55.9, 55.9, 56.0, 56.2, 105.1, 107.4, 110.1, 111.1, 122.9, 123.5, 125.2, 126.1, 131.2, 134.5, 139.4, 145.7, 148.7, 149.7, 151.0, 153.9, 154.7, 188.8; Mass spectrum (EI, m/z). 413/414 (M^+, 7%), 378/379 (M^+–Cl, 100%). Anal. $C_{22}H_{20}ClNO_5$: C, 63.85; H, 4.87; N, 3.39. Found: C, 63.83; H, 4.80; N, 3.39%.

4.1.1.9. 1-(3,4,5-Trimethoxyphenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3i***

Yield 93% (DMF–H_2O); mp 200–202 °C; IR (KBr) cm^{-1}: 1648(CO), 1574(C=C). ^1H NMR $CDCl_3$: δ 3.9–4.0(3s, 15H, OMe), 7.07(s, 1H, H_5), 7.36(s, 1H, H_8), 7.49(d, 1H, H_α $J = 16$ Hz), 7.91(s, 2H, Ar), 8.18(d, 1H, H_β $J = 16$ Hz), 8.30(s, 1H, H_4). ^{13}C NMR: 55.9, 56.0, 56.1, 56.3, 105.1, 107.3, 123.3, 125.9, 131.7, 134.7, 136.7, 140.2, 143.6, 148.7, 151.0, 153.6, 155.2, 189.7. Mass spectrum (EI, m/z). 444/445 (M^+, 9%), 409/410 (M^+–Cl, 100%); Anal. $C_{23}H_{22}ClNO_6$: C, 62.24; H, 4.99; N, 3.16. Found: C, 62.56; H, 5.03; N, 3.12%.

4.1.1.10. 1-(2,4-Dichlorophenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3j***

Yield 76% (EtOH–H_2O); mp 210 °C; IR (KBr) cm^{-1}: 1657(CO), 1574(C=C). ^1H NMR $CDCl_3$: δ 4.06(s, 6H, OMe), 7.06(s, 1H, H_5), 7.17(d, 1H, H_α $J = 15.7$ Hz), 7.32(s, 1H, H_8), 7.38(m, 1H, $H_{3'}$), 7.48(m, 2H, $H_{5',6'}$), 7.87(d, 1H, H_β $J = 15.7$ Hz), 8.29(s, 1H, H_4). ^{13}C NMR: 56.1, 56.2, 105.2, 107.3, 122.9, 125.0, 127.7, 128.4, 130.5, 130.9, 132.7, 134.7, 137.3, 141.7, 146.1, 148.7, 151.1, 155.0, 160.7, 191.5. Mass spectrum (EI, m/z). 421/424 (M^+, 3%), 386/388 (M^+–Cl, 100%). Anal. $C_{20}H_{14}Cl_3NO_3$: C, 55.77; H, 5.15; N, 3.25. Found: C, 55.76; H, 5.13; N, 3.23%.

4.1.1.11. 1-(2,5-Dichlorophenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3k***

Yield 68% (EtOH–H_2O); mp 246–248 °C; IR (KBr) cm^{-1}: 1651(CO), 1574(C=C). ^1H NMR $CDCl_3$: δ 4.01(s, 3H, OMe), 4.02(s, 3H, OMe), 7.06(s, 1H, H_5), 7.15(d, 1H, H_α $J = 16.08$ Hz), 7.32(s, 1H, H_8), 7.40(m, 2H, $H_{3',4'}$),7.49(m, 1H, $H_{6'}$), 7.93(d, 1H, H_β $J = 16.08$ Hz), 8.29(s, 1H, H_4). ^{13}C NMR: 56.1, 56.2, 105.9, 107.5, 122.7, 124.9, 128.1, 129.6, 129.8, 131.8, 131.9, 133.2, 134.6, 138.2, 142.4, 146.2, 148.8, 151.1, 155.0, 192.4. Mass spectrum (EI, m/z). 421/424 (M^+, 6%), 386/388 (M^+–Cl, 100%). Anal. $C_{20}H_{14}Cl_3NO_3$: C, 55.77; H, 5.15; N, 3.25. Found: C, 55.83; H, 4.93; N, 3.12%.

4.1.1.12. 1-(3,4-Dichlorophenyl)-3-[3-(2-chloro-6,7-dimethoxyquinolinyl)]-2-propen-1-one ***3l***

Yield 81% (EtOH–H_2O); mp 250–251 °C; IR (KBr) cm^{-1}: 1657(CO), 1584(C=C). ^1H NMR $CDCl_3$: δ 4.02(s, 3H, OMe), 4.03(s, 3H, OMe), 7.10(s, 1H, H_α $J = 15.58$ Hz), 7.60(d, 1H, $H_{5'}$ $J_1 = 8.41$ Hz), 7.85(dd, 1H, $H_{6'}$ $J_1 = 8.41$, $J_2 = 1.98$ Hz), 8.11(d, 1H, $H_{2'}$ $J_2 = 1.92$ Hz), 8.22(d, 1H, H_β $J = 15.83$ Hz), 8.35(s, 1H, H_4). ^{13}C NMR: 56.1, 56.2, 105.2, 107.4, 122.8, 124.0, 125.7, 127.9, 130.8, 131.1, 133.4, 134.67, 137.7, 138.2, 141.4, 146.2, 148.6, 151.5, 155.3, 188.2. Mass spectrum (EI, m/z). 422/424 (M^+, 5%), 386/388 (M^+–Cl, 100%). Anal. $C_{20}H_{14}Cl_3NO_3$: C, 55.77; H, 5.15; N, 3.25. Found: C, 55.77; H, 5.14; N, 3.19%.

4.2. Biological assays

4.2.1. Assay of protease activity

Assays of the hydrolysis of the fluorogenic substrate Z–Phe–Arg–AMC, (Enzyme Systems Products, Livermore, CA), by soluble parasite extracts containing falcipain were performed as previously described [16]. For all assays, 0.4 nM falcipain was incubated with 10 mM dithiothreitol and quinolinyl-chalcones added from 100 × stocks (in DMSO) in 0.1 M sodium acetate, pH 5.5, for 30 min at room temperature before the Z–Phe–Arg–AMC substrate (final concentration 50 μM) was added. Fluorescence caused by the cleavage of the substrate (excitation 380 nm, absorbance 460 nm) was then monitored continuously over 30 min. The rate of hydrolysis of substrate (increase in fluorescence over time) in the presence of quinolinyl chalcone inhibitors was compared with the rate of hydrolysis in controls incubated with an equivalent volume of DMSO. In each experiment, multiple concentrations of quinolinyl chalcones were evaluated in duplicate or triplicate, and IC_{50} values were extrapolated from curves of percent control activity over concentration.

4.2.2. In vitro assay of field isolates

The detailed protocol for in vitro antimalarial testing has been published previously [6]. Briefly, drug effects on *P. falciparum* proliferation were measured as inhibition of ^{3}H-hypoxanthine (New England Nuclear, Boston, MA) incorporation into nucleic acids, using the method described by Desjardin et al. [17]. All compounds were dissolved in 10% (DMSO), subsequently diluted with RPMI 1640 medium and tested at 1×10^{-6} M in two parallel columns of a flat-bottomed 96-well microtitre plate (Falcon plastics, Oxnard, CA). The final concentration of isotope in the medium was 2 $\mu Ci/ml^{-1}$. The parasite strain studied was FCB1, a chloroquine-resistant strain (IC_{50} for hypoxanthine uptake 80 nM) that was originally obtained from a Colombian patient. Two hundred microlitres of parasite culture were added to each well of the microculture plates containing the drugs and the isotope. Plates were incubated at 37 °C in candle jars for 24 and 48 h. Just before reinvasion erythrocytes were frozen at −20 °C. Cells were lysed in an automatic cell harvester (LKB, Wallac oy, Finland). The cells were retained on a glass filter (pore size 1 μm; LKB Wallac oy, Finland), and the radioactivity incorporated in DNA was measured in a beta counter (Beta Plate, LKB, Wallac oy, Finland). Control wells containing infected erythrocytes without drugs and the same concentration of DMSO were included. The final concentration of DMSO never exceeded 1% (v/v). DMSO alone had no effect on the proliferation of parasites. The 50% inhibitory concentration (IC_{50}) was defined as the concentration corresponding to 50% inhibition of control uptake of ^{3}H-hypoxanthine.

4.2.3. In vivo antimalarial studies

We selected for this study compounds **3b** and **3l** due to their preliminary results as inhibitors of hemoglobin degradation (unpublished results).

Parasite and animal. P. berghei (ANKA strain) was used in this study. This strain is lethal to infected mice within 6 and 8 days, male mice (Balb-c, age 8 weeks, body weight, 20–23 g) were used throughout the study.

Test procedure. Animals were inoculated intravenously (caudal vein) with 10^{6} parasitized erythrocytes in 0.1 ml . These parasitized erythrocytes were obtained from the blood of highly infected mice (average, 30% raising parasitemia), blood was diluted in 0.9% NaCl to give 10^{7} parasites per ml . The compounds **3b** and **3l** were first dissolved in DMSO and was then diluted in Tween 20 – normal saline solution (2% Tween 20). The animals ($n = 5$) were injected intraperitonealy at dosages of 10 and 20 mg/kg of body weight given once a day every day, starting day 3 after infection. Each day thin blood smears were made from the tail blood of mice and the level of parasitemia in mice was assessed by examination of Giemsa stained smears.

4.3. Acknowledgments

We thank the IIF grant 02-2000 and CYTED for their facilities, CDCH-UCV, grants No. 06-30-4544-99, 06-30-4590-2000, CONICIT grant No. LAB-97000665 and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases for their financial support. We thank Dr Carmen Ochoa de Ocariz (C.S.I.C., Madrid, Spain) for microanalysis and Mr. Rafael Paredes for his technical assistance.

References

[1] Weekly Epidemiological Record, W.H.O. 72 (No I, II, III) (1997) 269–292.
[2] Winstaley P.A., Parasitol. Today 16 (2000) 146–153.
[3] Chen C., Tang L.H., Chun J., Trans. R. Trop. Med. Hyg. 86 (1992) 7–10.
[4] Kesten S.J., Degnan M.J., Hung J., McNamara D.J., Ortwine D.F., Uhlendorf S.E., Werbel L.M., J. Med. Chem. 35 (1992) 3429–3447.

[5] Vennestrom J.L., Ellis W.Y., Ager A.L. Jr, Andersen S.L., Gerena L., Milhous W.K., J. Med. Chem. 35 (1992) 2129–2134.

[6] Domínguez J., Basante W., Charris J., Riggione F., Farmaco 51 (1996) 407–412.

[7] Domínguez J., Charris J., Iarruso L., López S., Lobo G., Riggione F., Farmaco 51 (1996) 781–784.

[8] Domínguez J., Lopez S., Charris J., Iarruso L., Lobo G., Semenov A., Olsen P.J., Rosenthal P.J., J. Med. Chem. 40 (1997) 2726–2732.

[9] Domínguez J., Charris J., Basante W., Mendez B., Magn. Reson. Chem. 36 (1998) 454–456.

[10] Charris J., Domínguez J., Lobo G., Riggione F., J. Pharm. Pharmacol. Commun. 5 (1999) 107–110.

[11] Li R., Kenyon G.L., Cohen F.E., Chen X., Gong B., Domínguez J., Davidson E., Kurzban G., Miller R., Nuzum E.O., Rosenthal P.J., McKerrow J.H., J. Med. Chem. 38 (1995) 5031–5037.

[12] Chen M., Theander T.G., Christensen S.B., Hviid L., Zhai L., Kharazmi A., Antimicrob. Agents Chemother. 38 (1994) 1470–1475.

[13] Nielsen S.F., Christensen S.B., Cruciani G., Kharazmi A., Liljefors T., J. Med. Chem. 41 (1998) 4819–4832.

[14] Herencia F., Ferrándiz M.L., Ubeda A., Domínguez J.N., Charris J.E., Lobo G., Alcaraz M.J., Bioorg. Med. Chem. Lett. 8 (1998) 1169–1174.

[15] Meth-Cohn, O., Narine, B., Tarnowski, B., J.C.S. Perkin I., (1981) 1520–1530

[16] Rosenthal P.J., Olson J.E., Lee G.K., Palmer J.T., Klaus J.L., Rasnick D., Antimicrob. Agents Chemother. 40 (1996) 1600–1603.

[17] Desjardin R.E., Confield C.J., Hagnes J.D., Chulay J.D., Antimicrob. Agents Chemother. 16 (1979) 710–718.