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Antimalarial activity of 4-(5-trifluoromethyl-1*H*-pyrazol-1-yl)-chloroquine analogues

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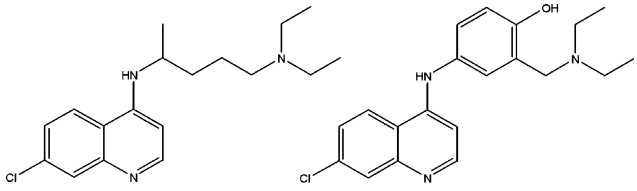
Abstract—The antimalarial activity of chloroquine-pyrazole analogues, synthesized from the reaction of 1,1,1-trifluoro-4-methoxy-3-alken-2-ones with 4-hydrazino-7-chloroquinoline, has been evaluated in vitro against a chloroquine resistant *Plasmodium falciparum* clone. Parasite growth in the presence of the test drugs was measured by incorporation of [^{3}H]hypoxanthine in comparison to controls with no drugs. All but one of the eight (4,5-dihydropyrazol-1-yl) chloroquine **2** derivatives tested showed a significant activity in vitro, thus, are a promising new class of antimalarials. The three most active ones were also tested in vivo against *Plasmodium berghei* in mice. However, the (pyrazol-1-yl) chloroquine **3** derivatives were mostly inactive, suggesting that the aromatic functionality of the pyrazole ring was critical.

Malaria remains one of the most important diseases of human with over half of the world population at risk of infection. It affects mainly those living in tropical and subtropical areas with an incidence of 500 million cases per year globally.[1] Despite the importance of this disease, treatment investment and malaria control are modest, especially in Africa where it kills over 2.5 million children per year, according to the World Health Organization data.[2] In Brazil, it is estimated that 500,000 cases occur annually, mainly in the Amazon Rain Forest.[3]



Chloroquine (CQ) Amodiaquine (AQ)

Figure 1. Structures of chloroquine and amodiaquine, the drugs most frequently used to treat malaria.

Chloroquine (CQ) and other quinoline antimalarials have been mainstays of malaria chemotherapy for much of the past 40 years (Fig. 1). The success of these drugs was based on limited host toxicity especially of CQ, ease of use, with very few side effects, low cost and effective synthesis. The inhibition of parasite growth by CQ and related antimalarials is caused by formation of a complex with hematin (Fe(III)FPIX), a toxic lytic moiety that must be sequestered as an inert pigment, the hemozoin.[4]

The use of such drugs has been seriously eroded in recent years, mainly as a result of the development of parasite resistance to CQ.[5] The molecular basis for CQ resistance is not fully understood. However, it is clear

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that the resistance is a consequence of decreased accumulation of the drug in the parasite, due to enhanced efflux, reduced uptake or a combination of both.[6] Several studies indicate that point mutations in the multidrug resistance 1 (*pfmdr1*),[7] candidate (*cg2*)[8] and CQ resistance transporter (*pfcrt*) gene[9] are involved in the mechanism of resistance. Recently, it was also demonstrated that resistance to mefloquine in Southeast Asia was due to an increase in the gene copy numbers of *pfmdr1*.[10]

It has been shown that close analogues of CQ and CQ-derivatives are active against CQ-resistant parasites,[11] suggesting that the mechanism of resistance does not involve any changes to their targets but involves a compound specific resistance.

Pyrazoles and 4,5-dihydropyrazoles are important biological agents with a wide range of pharmaceutical (anti-inflammatory, antifungal, antibacterial, antitumor and antiviral) and agrochemical activities.[12,13]

The aim of this study was to assess in vitro activity of pyrazole chloroquinoline analogues against *Plasmodium falciparum* and in vivo activity against *Plasmodium berghei*. The parasite clone W2, chloroquine resistant, was used[14] for the in vitro tests, as described in our previous work,[15] and the activity compared to that of CQ, used in parallel in each test.

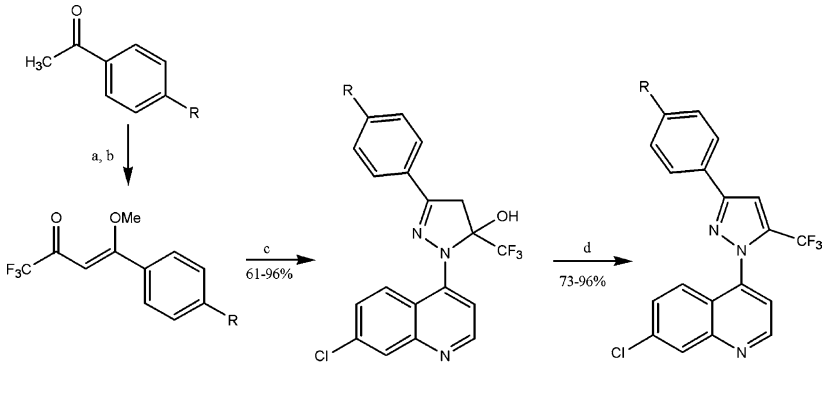
Parasites were cultured with human erythrocytes (blood group O^+) at 5% haematocrit in RPMI 1640 supplemented with 10% human plasma as previously described.[16] Molecules were solubilized in ethanol prior to in vitro tests. The antiparasitic effects of the molecules were measured by the [^{3}H]hypoxanthine incorporation assay.[17] Briefly, trophozoite stages in sorbitol-synchronized blood [18] were cultured at 2% parasitaemia and 2.5% haematocrit, in the presence of the test molecules (at various concentrations), diluted with culture medium (RPMI 1640) without hypoxanthine; a chloroquine control (as a reference antimalarial drug) was used in each experiment. Inhibition of parasite growth was evaluated through the levels of [^{3}H]–hypoxanthine incorporation plotted to generate dose–response curves. The half-maximal inhibitory response (IC_{50}) compared with parasite growth in the drug-free controls was estimated by curve fitting using a software program [Microcal, Origin Software (Northampton, MA, USA)].

The synthesis of 1,1,1-trifluoro-4-methoxy-3-alken-2-ones **1a**–**h** was performed as previously reported.[19] The reaction of ketones with trimethyl orthoformate in the presence of *p*-toluenosulfonic acid delivered the respective acetals. The intermediate acetals react with

Table 1. Antimalarial activity of 4-(5-trifluoromethyl-5-hydroxy-4,5-dihydropyrazol-1-yl)-7- chloroquinolines **2a**–**h** and 4-(pyrazole-1-yl)-7-chloroquinolines **3a**–**j** against *Plasmodium falciparum* W2 clone in vitro

Compound	IC_{50}[a] (μg/mL)
2a	1.39
2b	3.04
2c	2.13
2d	1.69
2e	1.55
2f	>50.0
2g	5.71
2h	2.12
Chloroquin	0.19
3a	9.53
3b	>50
3c	27.62
3d	>50
3e	>50
3f	>50
3g	>50
3h	18.53
3i	4.29
3j	>50
Chloroquine	0.22

[a] The IC_{50} represents concentration inhibitory dose of the parasite growth in relation to control cultures with no drugs.



	a	b	c	d	e	f	g	h
R	H	Me	F	Cl	Br	OMe	NO_2	phenyl

Scheme 1. Reagents and conditions: (a) TsOH, $HC(OCH_3)_3$, MeOH, rt, 24 h; (b) $(CF_3CO)_2O$, Py, $CHCl_3$, 0–45 °C, 16 h; (c) 4-hydrazine-7-chloroquinoline, MeOH, 68 °C, 15–30 min; (d) AcOH, reflux, 4–10 h.

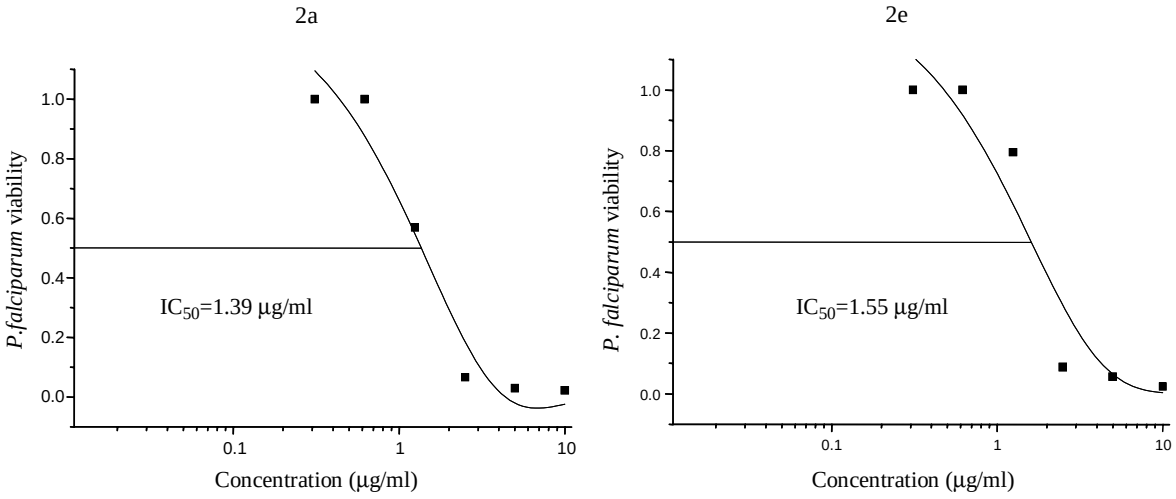
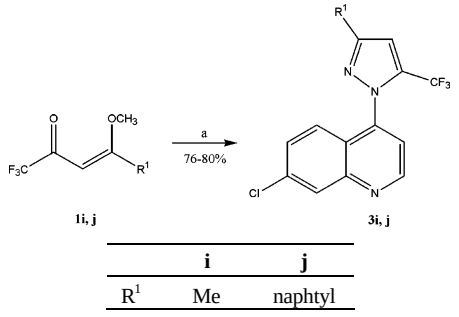


Figure 2. Dose–response curves of 4-(3-substituted-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl)-7-chloroquinolines analogues **2a** and **e** against *Plasmodium falciparum* in vitro.



	i	j
R^1	Me	naphtyl

Scheme 2. Reagents and conditions: (a) 4-hydrazine-7-chloroquinoline, MeOH, 68 °C, 15–30 min.

trifluoroacetic anhydride in pyridine using chloroform as solvent to obtain compounds **1a**–**h**. The cyclocondensation reaction of 1,3-dielectrophilic compounds **1a**–**h** with 4-hydrazino-7-chloroquinoline was carried out in methanol for 15–30 min under reflux to give the 4-(5-hydroxy-4,5-dihydropyrazol-1-yl)-7-chloroquinolines **2a**–**h** in good yields (Scheme 1).[20,21] Compounds **2a**–**h** were converted to the respective aromatic system **3a**–**h** by treatment of **2a**–**h** with acetic acid at reflux for 4 h in 73–96% yields (Scheme 1).[20,22] Both analytical and spectral data (^{1}H and ^{13}C NMR) of all compounds are in full agreement with the proposed structure.[23,24]

Table 1 shows the antimalarial activities of all compounds expressed as 50% inhibitory concentration (IC_{50}) of *P. falciparum* growth.

All (4,5-dihydropyrazol-1-yl) chloroquine derivatives **2a**–**h** but one (**2f**, inactive) inhibited growth of *P. falciparum* in vitro at concentrations of micromolar range (Table 1). The compound **2a** that has no substituent group (R = H) was the most active one (IC_{50} = 1.39 µg/mL) (Fig. 2). The halogen substituted compounds **2c**–**e** have also shown good activity based on the low IC_{50} values. The compound **2g**, that has a strong electron-withdrawing group (R = NO_2), showed a little decrease of antimalarial activity (IC_{50} = 5.71 µg/mL). Even the bulky biphenyl compound **2h** was active (IC_{50} = 2.12 µg/mL). Despite the fact that the more active compounds in vitro are at least 10- to 15 times less active than chloroquine, their IC_{50} values are in the micromolar concentration range comparable to recently reported results.[25]

As shown for compound **2f**, the presence of a strong electron-releasing (R = OMe) group results in complete loss

Table 2. Antimalarial activity of 4-(4,5-dihydropyrazol-1-yl)-7-chloroquinolines **2a**, **d** and **e** against *Plasmodium berghei* in Balb/c mice infected with blood parasites, treated by oral or subcutaneous routes (20 mg/kg)

Compound	Route of treatment	% parasitaemia mean ± SD	Reduction of parasite growth[a] (%)
2a	Oral	8.7 ± 5.8	0
	Subcutaneous	11.2 ± 2.5	0
2d	Oral	11.4 ± 5.4	0
	Subcutaneous	3.9 ± 3.5	47
2e	Oral	9.7 ± 3.5	0
	Subcutaneous	1.3 ± 0.9	82
Chloroquine[b]	Oral	3.4 ± 4.6	53
	Subcutaneous	0 ± 0	100
Non-treated	—	7.3 ± 4.0	0

[a] Calculated in comparison to non-treated mice (6 per group), at day 7 after parasite inoculation.
[b] Dose of 15 mg/kg.

of activity at the maximum concentration (50 μg/mL) used.

The aromatic functionality of pyrazole ring is critical since water elimination of 5-hydroxy-4,5-dihydropyrazoles **2a**–**h** results in a significant drop in potency for all pyrazoles **3**, including **3a** (IC_{50} = 9.53 μg/mL), **3c** (IC_{50} = 27.62 μg/mL) and **3h** (IC_{50} = 18.53 μg/mL). For compounds **3b**,**d**,**e**,**f** and **g,** the IC_{50} values were higher than 50 μg/mL, thus considered inactive.

The 3-methyl and 3-naphthyl pyrazole derivatives **3i** and **j** were pursued in an attempt to gain insight into the importance of 3-substituent group at pyrazole ring (Scheme 2).[20] The introduction of a methyl group instead of an aryl group had an increasing effect on antimalarial activity (IC_{50} = 4.29 μg/mL), however lower than that of 3-aryl-5-hydroxy-4,5-dihydopyrazole series. The compound with the bulky group R^1 = naphthyl (**3j**) was inactive at high concentration (50 μg/mL). No traces of the corresponding intermediate 5-hydroxy-4,5-dihydropyrazoles are detected in these reactions.

The most effective in vitro analogues (**2a**, **d** and **e**) were selected for in vivo tests against NK-65 strain of *P. berghei* in infected mice.[26] Orally, compounds **2a**, **d** and **e** did not inhibit the parasite growth at the dose of 20 mg/kg (Table 2). However, after subcutaneous injection, **2a** was still inactive and compounds **2d** and **e** inhibited parasite growth by 47% and 82%, respectively. Chloroquine controls inhibited parasite growth by 53% (oral route) and 100% (subcutaneous route).

Assayed as antimalarial for the first time, these 4-(5-trifluoromethyl-5-hydroxy-4,5-dihydropyrazol-1-yl)-7- chloroquinolines **2d** and **e** have exhibited promising antimalarial activity in vitro and in vivo.

Acknowledgments

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21. General procedure for the preparation of 4-(3-substituted-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl)-7-chloroquinolines (**2a**–**h**): to a magnetic stirred solution of **1a**–**h** (1 mmol) in MeOH (10 mL), 7-chloro-4-hydrazinoquinoline (1 mmol) was added at room temperature. The mixture was stirred under reflux (64 °C) for 15–30 min. The solvent was evaporated under reduced pressure and the solid products **2a**–**h** were recrystallized from a mixture of acetone and water 5:1.
22. General procedure for the preparation of 4-(3-substituted-5-trifluoromethyl-1*H*-pyrazol-1-yl)-7-chloroquinolines (**3a**–**h**): to a magnetic stirred pure acetic acid (10 mL), compounds **2a**–**h** (1 mmol) were added at room temperature. The mixture was stirred under reflux (116 °C) for 4–10 h. The solvent was evaporated under reduced pressure and the solid products **3** were recrystallized from a mixture of ethanol and water 5:1.
23. Selected data for compound 4-[3-(4-bromophenyl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl]-7-chloroquinoline **2e**: mp 220–222 °C. ^{1}H NMR (200 MHz, DMSO) (pyrazol) δ = 8.75 (s, OH); 4.05 (d, J = 18.5, Ha); 3.68 (d, J = 18.5, Hb); (quinolyl) δ = 8.85 (d, H2, J = 4.9); 8.34 (d, H8, J = 9.1); 8.06 (d, H6, J = 2.2); 7.79 (d, H5, J = 5.0); 7.60 (d, H3, J = 2.3); (phenyl) δ = 7.63–7.62 (m, 4 H). ^{13}C NMR (50 MHz, DMSO) (pyrazol) δ = 148.1 (C3); 124.9 (q, J_{CF} = 285.3, CF_3); 94.1 (q, $^2J_{CF}$ = 31.7, C5); 43.5 (C4); (quinolyl) δ = 151.8 (C2); 149,7 (C8a); 146.2 (C4); 133.8 (C7); 130.0 (C8); 127.7 (C6); 126.1 (C5);

122.1 (C4a); 113.9 (C3); (phenyl) δ = 131.7; 127.6; 127.3; 122.9 (6C).

24. Selected data for compound 4-[3-(4-bromophenyl)-5-trifluoromethyl-1*H*-pyrazol-1-yl]-7-chloroquinoline **3e**: mp 162–164 °C. ^{1}H NMR (200 MHz, DMSO) (phenyl pyrazol) δ = 7.96–7.91; 7.72–7.68 (m, 5 H, H4 + 4 H_{Ar}); (quinolyl) δ = 9.24 (d, H2, J = 4.4); 8.33 (d, H8, J = 2.0); 7.99 (d, H6); 7.75 (d, H5, J = 2.0); 7.50 (d, H3, J = 8.9). ^{13}C NMR (50 MHz, DMSO) (pyrazol) δ = 151.2 (C3); 134.5 (q, $^{2}J_{CF}$ = 39.5, C5) 121.7 (J_{CF} = 269.1, CF_3); 107.2 (C4); (quinolyl) δ = 152.3 (C2); 149,0 (C8a); 142.1 (C4); 135.4 (C7); 129.9 (C8); 128.0 (C6); 124.5 (C5); 122.3 (C4a); 120.0 (C3); (phenyl) δ = 131.8; 129.3; 127.7; 124.9 (6C).
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26. The suppressive Peters test[27] as modified was used. Briefly, adult Swiss outbred mice, 20 ± 2 g weight, inoculated by intraperitoneal route with *P. berghei*-infected red blood cells (1×10^5), were daily treated by oral or subcutaneous route, during 4 days. The samples were dissolved in an aqueous solution of DMSO 0.02% (v:v) and this solution was, later, diluted to final volume with saline and given in a 200 μL volume per animal. Two control groups (n5) were used each time, one treated with non-curative doses of chloroquine (⩽25 mg/kg), one untreated or treated with saline, as specified in the results. On day 5 after parasite inoculation blood smears were prepared, methanol fixed, stained with Giemsa and microscopically examined by counting parasitaemia in 1000 up to 6000 erythrocytes. Overall mortality was daily monitored until all controls died. The inhibition of parasite growth in the drug-treated groups was calculated in relation to non-treated controls.[28]
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