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Anti-*Plasmodium* activity of imidazole–dioxolane compounds

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Abstract—A series of imidazole–dioxolane compounds, which we hypothesize should bind to heme and thus interfere with heme catabolism in the parasite, were assayed for inhibitory activity in *Plasmodium falciparum* cultures and the results were compared to those obtained with Chinese hamster ovary (CHO) cells. The majority of the compounds displayed a similar ratio of inhibitory activity in the two culture systems; however, a number of the compounds tested showed promising anti-*Plasmodium* activity. The mechanism of action of these compounds remains unclear, however their inability to act synergistically with chloroquine suggests that, if they are inhibiting heme detoxification, they do so in a manner that does not complement the action of chloroquine.

Malaria accounts for an estimated 300–500 million cases and up to 2.7 million deaths annually,[1] particularly among young children.[2–4] Despite optimistic predictions of the eventual control and eradication of malaria made in the past, the spread of drug-resistant parasites, particularly those resistant to chloroquine, has led to a resurgence of the disease.[5–9] *Plasmodium falciparum*, the species responsible for the majority of malaria-related deaths in humans, digests the hemoglobin present in an erythrocyte, and while it is able to safely export a majority of the liberated amino acids[10] this process also creates a source of heme that must be detoxified to prevent the destabilization of membranes and inhibition of enzymes that will lead to parasite death.[11] The degradation of hemoglobin liberates free heme (or hemin)[12] which can be oxidized to hematin (ferriprotoporphyrin IX). Free hematin is toxic to the parasite and can interfere with cellular metabolism by enzyme inhibition, membrane peroxidation, and also through the generation of oxidative free radicals.[13] Despite the lack of a parasite-encoded heme oxygenase enzyme (HO, EC 1.14.99.3),[14,15] other methods of heme catabolism in *P. falciparum* are utilized in this parasite. Through the formation of a complex with malarial histidine-rich protein (HRP), some (30–50%) of the free hematin in the food vacuoles of plasmodial species is sequestered into the non-toxic crystal hemozoin.[5] In this process, several units of hematin are linked via carboxylate–Fe(III) and carboxylic acid–carboxylic acid interactions forming an insoluble aggregate. The remaining free heme can also be efficiently decomposed by complexation with reduced glutathione (GSH) once it passes through the membrane of food vacuoles and into the parasite cytosol.[16,17] The aggregation into hemozoin is a primary method of detoxification and has been used as a target for drug therapy.[18–20] Many antimalarial compounds such as quinine, chloroquine, and other 4-aminoquinolines work by stabilizing (associating with) hematin or a hematin derivative (a μ-oxo-dimer), thus suppressing the hematin aggregation process which forms hemozoin.[21]

Antifungal agents such as econazole, ketoconazole, miconazole, and clotrimazole have been reported[22] to inhibit the growth of *P. falciparum* through an interaction with hematin. Each of these compounds is a 1-substituted imidazole; ketoconazole also incorporates a 1,3-dioxolane ring. In detailed studies,[23] it was determined that the axial coordination of the imidazole moieties of two clotrimazole molecules to the heme iron formed a stable heme–$(clotrimazole)_2$ complex which

Keywords: Antimalarial; *Plasmodium falciparum*; Imidazole–dioxolane compounds.
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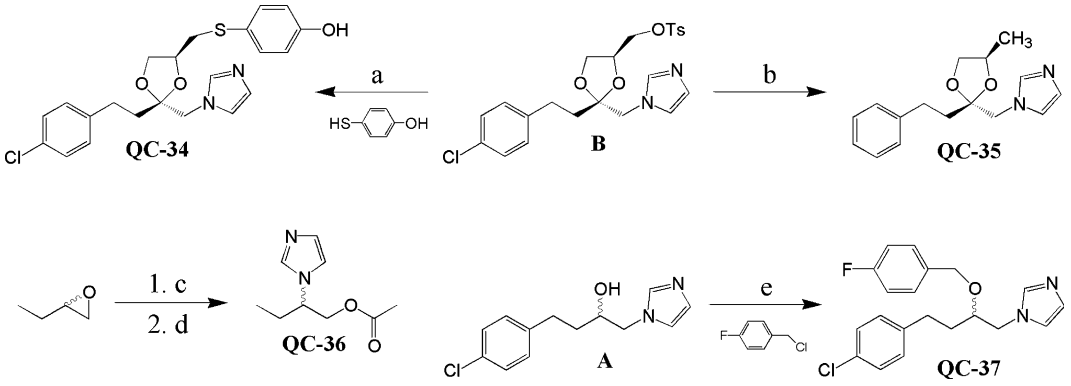
prevents heme association into hemozoin by HRP, in addition to inhibiting completely GSH-dependent heme degradation. Under the assumption that other 1-substituted imidazole compounds should bind to heme and thus also interfere with heme catabolism in the parasite, we have studied a series of imidazole–dioxolane compounds with respect to anti-*Plasmodium* activity in *P. falciparum* cultures. A number of compounds tested showed good anti-*Plasmodium* activity, however the mechanism involved in their action remains to be defined.

The synthesis of compounds **QC-1**, **-3**, **-4**–**7**, **-9**–**12**, **-14**, and **-16**–**24** has been described previously;[24] the synthesis of compounds **QC-34**–**37** is reported here (see Scheme 1),[25] and the synthesis of the remaining compounds will be reported separately. The racemic alcohol 4-(4-chlorophenyl)-1-(1*H*-imidazol-1-yl)butan-2-ol (**A**) was prepared from 4-chlorobenzyl chloride[26] and the tosylate (2*R*,4*S*)-2-[2-(4-chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-4-[(*p*-toluenesulfonyloxy)methyl]-1,3-dioxolane (**B**) was prepared as previously reported.[24] Both **QC-34** and **QC-35** were prepared from tosylate **B**; the nucleophilic displacement reaction with 4-mercaptophenol afforded **QC-34**, whereas the complete reduction of tosylate **B** using an excess of $LiAlH_4$ gave **QC-35**. **QC-36** was prepared by the nucleophilic ring opening of 1,2-epoxybutane with imidazole, followed by acid-catalyzed transesterification of the resulting alcohol with ethyl acetate; the ring opening of 1,2-epoxybutane gave preponderately 1-(1*H*-imidazol-1-yl)butan-2-ol. **QC-37** was prepared simply by the alkylation of the racemic alcohol **A** with 4-fluorobenzyl chloride. All compounds were isolated/characterized as hydrochloride salts, with the exception of compounds **QC-6**, **-16**, **-17**, **-21**, **-34**, and **-36**.

The antimalarial activity of the QC compounds was determined in quadruplicate using *P. falciparum* cultures,[27] whereas the general toxicity of the QC compounds was determined also in quadruplicate using Chinese hamster ovary (CHO) cell cultures.[28] The IC_{50} values of the compounds listed in Table 1 were determined, and a selectivity index was calculated by dividing the IC_{50}values obtained in CHO cell cultures by the IC_{50} values obtained in *P. falciparum* cultures. A ratio that was greater than unity indicates that a compound was preferentially toxic to *P. falciparum* parasites (Table 1). Further, the relative toxicity of these compounds was compared graphically by plotting the IC_{50} values obtained in *P. falciparum* cultures versus the values obtained in CHO cell cultures (Fig. 1). The majority of the data points in Figure 1 appear to fall parallel to and about 1 log-unit above a diagonal line that represents unity, suggesting that there is a common toxicity to both CHO and *P. falciparum* cultures. The data points for six of the compounds fell significantly above the diagonal (about 2 log-units), indicating that the compounds were preferentially toxic to *P. falciparum* cultures. Examination of *P. falciparum* cultures exposed to inhibitory concentrations of compounds **QC-5**, **-18**, **-34**, **-35**, and **-36** indicated that the parasites present in the cultures had formed compacted structures that lacked hemozoin and had not progressed beyond the ring stage of development (the results from **QC-5** are shown in Fig. 2). We hypothesized that 1-substituted imidazole compounds should bind to heme and therefore interfere with heme catabolism in the parasite. This mechanism of action is similar to that of chloroquine,[21] hence the effects of the four most selective QC compounds (**QC-34**, **-35**, **-36**, and **-5**) in the presence of chloroquine were investigated. The presence of varying amounts of the QC compounds had an additive rather than synergistic effect on the action of chloroquine (results for **QC-34** are shown in Fig. 3), suggesting that, if these compounds inhibited heme detoxification, they did not do so by inhibiting a biochemical process that complements the action of chloroquine (see also Ref. 29).

Virtually all of the compounds tested were found to be selectively toxic to *Plasmodium*, however, only a few were highly selective for *P. falciparum*. It is recognized that five of these compounds do not contain the 1,3-dioxolane ring. Interestingly, **QC-9**, **-10**, and **-33** were least potent against *P. falciparum* cultures, and **QC-37** was only modestly selective. In contrast, **QC-36** proved to be highly selective for *P. falciparum* cultures. These compounds, that lack the dioxolane moiety, provide other avenues worthy of investigation and will be the subject of a separate study. Most of the compounds



Scheme 1. Reagents and conditions: (a) Cs_2CO_3, acetone, reflux, 8.5 h; (b) $LiAlH_4$, THF, reflux, 9 h; (c) imidazole, NaH, DMF, 130 °C, 1 h; (d) EtOAc, HCl, heat; (e) NaH, THF, reflux, 1.5 h.

Table 1. Inhibitory activity of imidazole–dioxolane compounds in CHO cell and *Plasmodium falciparum* cultures

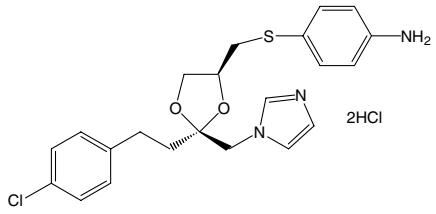
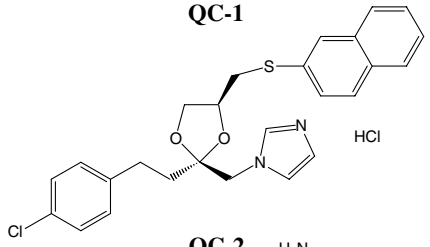
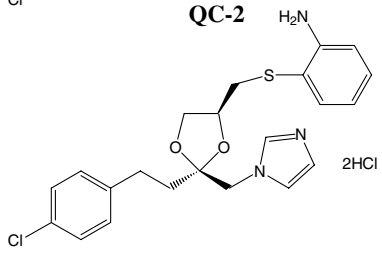
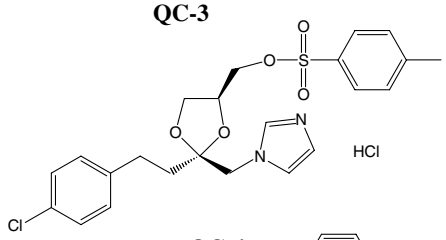
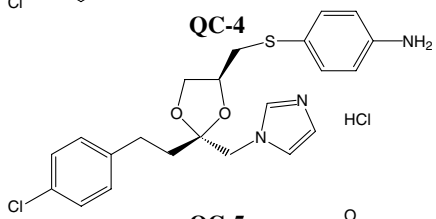
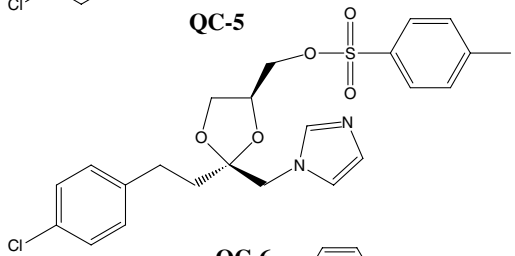
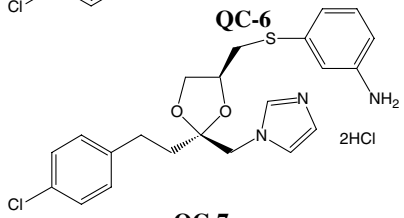
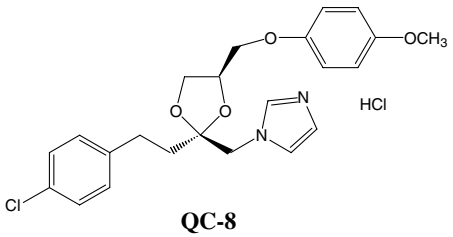
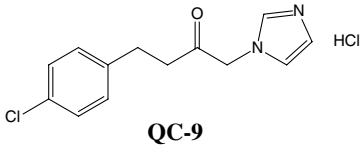
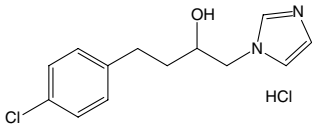
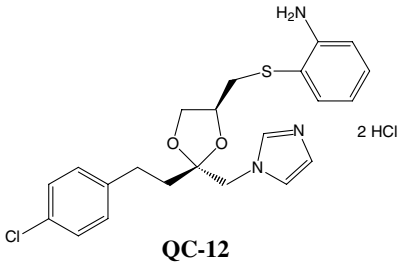
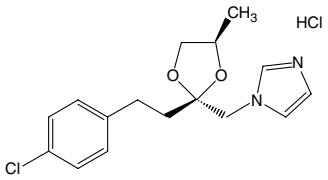
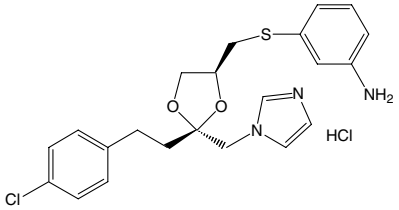
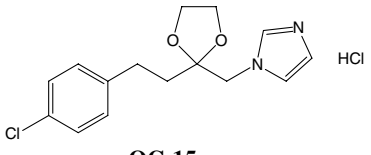
Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-1**	0.8 ± 0.1	13 ± 5	16.3
 **QC-2**	1.2 ± 0.2	9 ± 1	7.5
 **QC-3**	0.6 ± 0.1	6 ± 1	10
 **QC-4**	0.67 ± 0.05	7 ± 1	10.4
 **QC-5**	1.3 ± 0.1	374 ± 49	287.7
 **QC-6**	1.2 ± 0.2	3.8 ± 0.9	3.2
 **QC-7**	0.3 ± 0.1	18 ± 6	60

Table 1 (*continued*)

Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-8**	0.8 ± 0.1	15 ± 5	18.8
 **QC-9**	25 ± 2	168 ± 13	6.7
 **QC-10**	70 ± 19	314 ± 36	4.5
 **QC-12**	3.9 ± 0.5	16 ± 2	4.1
 **QC-13**	7 ± 1	51 ± 3	7.3
 **QC-14**	2.0 ± 0.7	10 ± 4	5
 **QC-15**	9 ± 2	84 ± 9	9.3

(*continued on next page*)

Table 1 (*continued*)

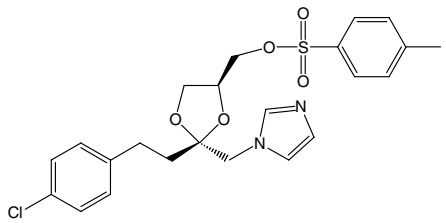
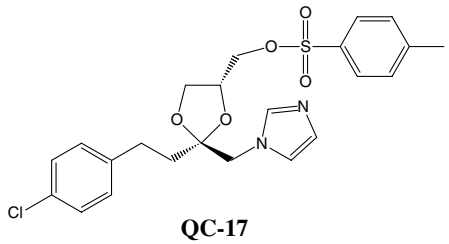
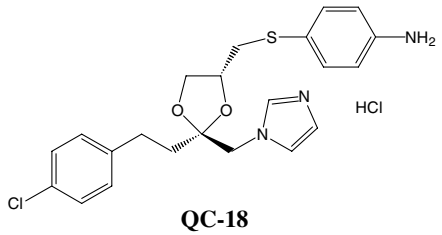
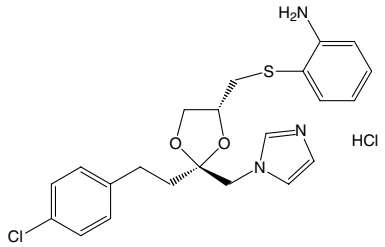
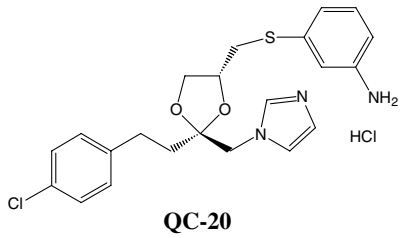
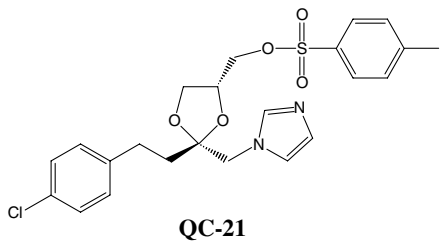
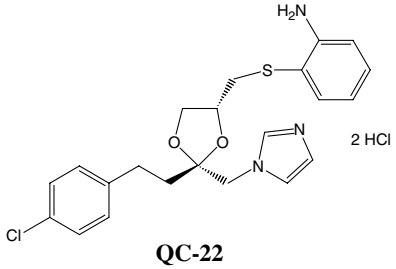
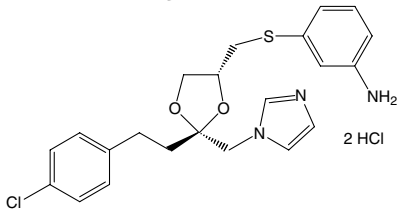
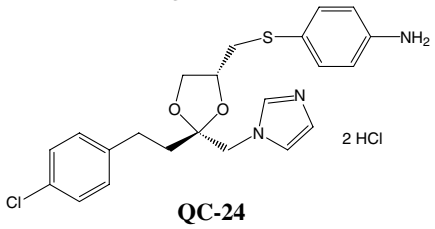
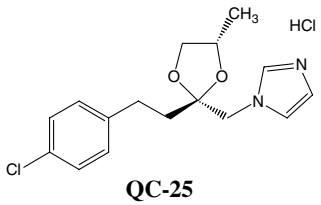
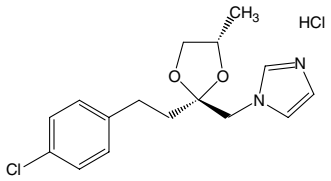
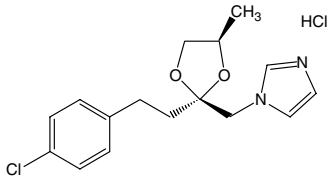
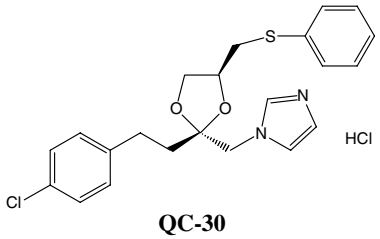
Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-16**	2.9 ± 0.2	8 ± 2	2.8
 **QC-17**	2.8 ± 0.2	13 ± 3	4.6
 **QC-18**	3.0 ± 0.8	254 ± 34	84.7
 **QC-19**	3.3 ± 0.6	6.6 ± 0.1	2
 **QC-20**	2.5 ± 0.3	9.2 ± 0.7	3.7
 **QC-21**	1.9 ± 0.3	5.7 ± 0.4	3

Table 1 (*continued*)

Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-22**	2.3 ± 0.3	11 ± 1	4.8
 **QC-23**	1.2 ± 0.1	9 ± 1	7.5
 **QC-24**	2.7 ± 0.6	6 ± 2	2.2
 **QC-25**	4.1 ± 0.4	39 ± 7	9.5
 **QC-26**	18 ± 2	82 ± 16	4.6
 **QC-27**	23 ± 4	102 ± 25	4.4
 **QC-30**	3.3 ± 0.3	7 ± 4	2.1

(*continued on next page*)

Table 1 (*continued*)

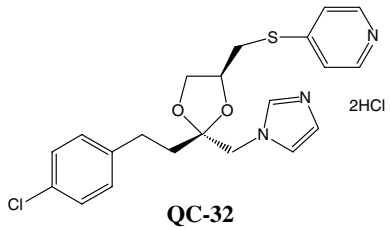
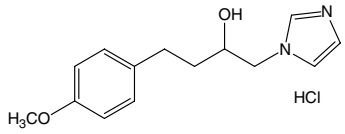
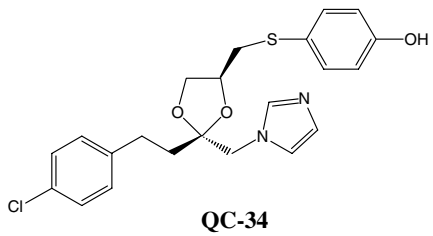
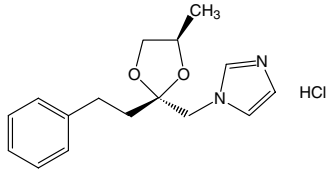
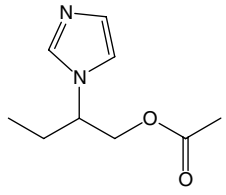
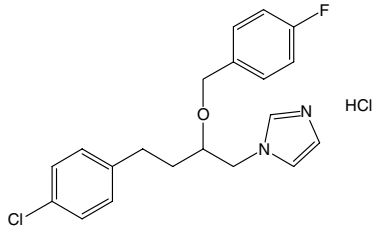
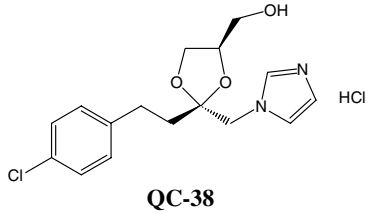
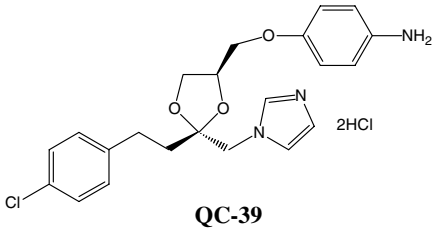
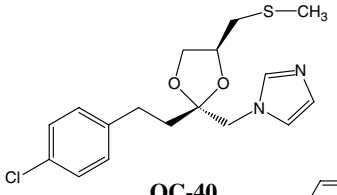
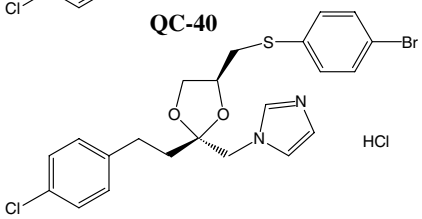
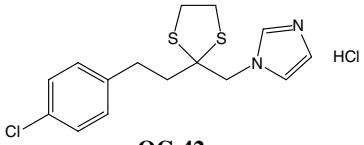
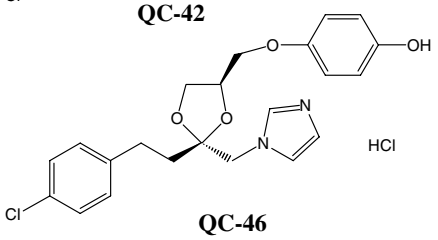
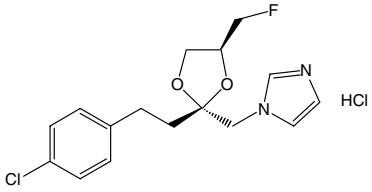
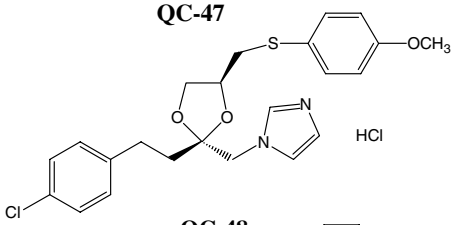
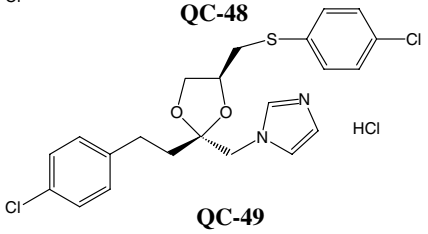
Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-32**	4.9 ± 0.3	27 ± 12	5.5
 **QC-33**	38 ± 6	116 ± 66	3.1
 **QC-34**	0.06 ± 0.03	10.9 ± 2.5	181.6
 **QC-35**	0.34 ± 0.02	668 ± 378	2226.7
 **QC-36**	8.3 ± 0.8	1848 ± 437	222.7
 **QC-37**	0.8 ± 0.1	5.3 ± 0.5	6.6
 **QC-38**	9 ± 2	227 ± 14	25.2

Table 1 (*continued*)

Structure	IC_{50} (μM)		IC_{50} CHO/IC_{50} *P. falciparum*
	P. falciparum	CHO cells	
 **QC-39**	1.3 ± 0.2	14 ± 1	10.8
 **QC-40**	2.1± 0.4	9.3 ± 0.8	4.7
 **QC-41**	0.9 ± 0.2	12 ± 3	13.3
 **QC-42**	2.3 ± 0.6	25 ± 6	10.9
 **QC-46**	1.1 ± 0.1	0.9 ± 0.3	0.8
 **QC-47**	20 ± 3	24 ± 4	1.2
 **QC-48**	3.5 ± 0.2	3.7 ± 0.5	1.1
 **QC-49**	2.5 ± 0.3	3.3 ± 0.4	1.3

Values represent means of four independent determinations with the standard deviation indicated.

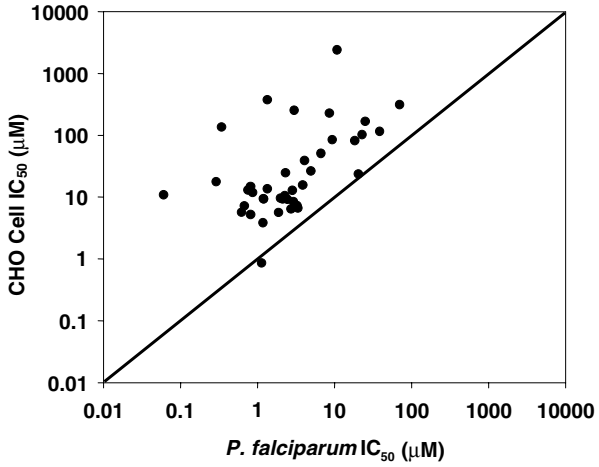


Figure 1. log–log plot of the IC_{50} values obtained for the compounds in *Plasmodium falciparum* cultures (*X* axis) versus CHO cell cultures (*Y* axis). Points above the diagonal represent compounds that selectively inhibit *Plasmodium falciparum* cultures.

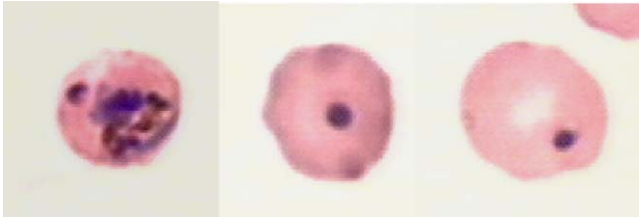


Figure 2. The inhibition of *Plasmodium falciparum* cultures with chloroquine or **QC-5**. *Plasmodium falciparum* line ItG was cultured and used for viability assays as described in the text. Untreated parasite cultures (left panel) developed normally and produced visible hemozoin crystals. Cultures were treated with either 250 nM chloroquine (center panel) or 15 μM **QC-5** (right panel) and incubated for 48 h. Treated cultures failed to develop past the ring stage of their intra-erythrocytic cycle.

were active in both cultures in the low micromolar range and therefore show low selectivity. Selectivity comes about either because of low inhibitory activity in CHO cell cultures, as for example in the case of **QC-5**, **QC-18**, and **QC-36**, or alternatively because of increased potency in *P. falciparum* cultures, as for example in the case of **QC-34** and **QC-35**. The overall examination of the results in Table 1 clearly suggests that the combination of 1,3-dioxolane and imidazole moieties has the potential to bestow significant anti-*P. falciparum* activity. Indeed, **QC-35** displayed both high potency in *P. falciparum* cultures (<1 μM) and low CHO cell inhibitory activity (>100 μM).

The mechanism by which these agents kill *P. falciparum* cultures is not immediately clear. The importance of the stereochemistry of the compounds, for instance, as in **QC-1** versus **QC-18**, and the morphology of the parasites present in treated cultures (Fig. 2) are consistent with the hypothesis that some aspect of an enzymatic heme detoxification process is being targeted. However, if heme detoxicification is being inhibited or the heme-dependent hemolysis is being enhanced[23] it would be expected that these agents might display either additive or synergistic behavior with chloroquine. For example, Srivastava et al.[29] have suggested that inhibiting alternative pathways of heme detoxification[15,16] or export[30] might have a synergistic effect with chloroquine since this would increase the amount of heme in the chloroquine-sensitive pathway. The linear relationship between the concentration of **QC-34** and the IC_{90} for chloroquine shown in the isobologram in Figure 3 demonstrates the apparent lack of synergy between chloroquine and the QC compounds studied. This observation suggests that the QC compounds cannot be used to potentiate the effects of chloroquine by promoting the effects of oxidative stress present in a parasitized erythrocyte.[30] An initial determination of the effect

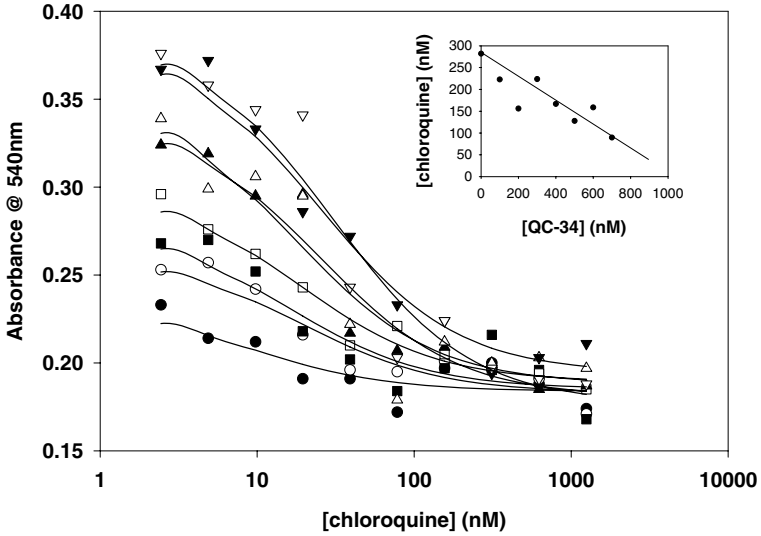


Figure 3. The interaction of chloroquine and **QC-34** in *Plasmodium falciparum* cultures. The effect of increasing concentrations of compound **QC-34** in the presence of varying amounts of chloroquine was determined. Varying concentrations of **QC-34** (700 nM ●, 600 nM ○, 500 nM ■, 400 nM □, 300 nM △, 200 nM ▲, 100 nM ∇, and 0 nM ▼) were added and the corresponding IC_{90} values for chloroquine were calculated. An isobologram (inset) plotted for 90% inhibition supports a lack of synergy between the two compounds.

of **QC-34** and **QC-35** on chloroquine, and mefloquine-resistant *P. falciparum* cultures suggests that these compounds have similar activities in these lines.

It should be noted that most of the compounds in Table 1 have been found[24] to display inhibitory activity against heme oxygenase, an enzyme that is not present in *P. falciparum*.[14,15,31] Consequently, their use as antimalarials might be viewed with caution since infection of the host with *Plasmodium* leads to several conditions in which an active heme oxygenase may be a potential benefit.[32,33] Thus, the incorporation of selectivity is a target for the future design of useful antimalarial compounds.

Acknowledgments

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25. Synthetic procedures and characterization of the compounds outlined in Scheme 1: **(2*R*,4S)-2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-4-[{(4-hydroxyphenyl)thio}methyl]-1,3-dioxolane** (**QC-34**). To a solution of tosylate **B**[24] (100 mg, 0.21 mmol) in acetone (4 mL) were added cesium carbonate (137 mg, 0.42 mmol) and 4-mercaptophenol (90 mg, 0.71 mmol). The mixture was heated at reflux temperature for 8.5 h and then filtered. The filter cake was washed with acetone and ethyl acetate, and the filtrate and washings were concentrated to a yellow residue. Flash chromatography on silica gel (EtOAc) followed by recrystallization from acetone gave 60 mg (0.14 mmol, 67%) of **QC-34** as a white solid: R_f = 0.15 (EtOAc); mp 153–154 °C; $[\alpha]_D^{22}$ −11.5 (*c* 0.70, CD_3OD); ^1H NMR (400 MHz, CD_3OD): δ 1.84–1.89 (m, 2H), 2.60–2.76 (m, 2H), 2.83 (dd, *J* = 13.8, 7.0 Hz, 1H), 2.98 (dd, *J* = 13.6, 5.6 Hz, 1H), 3.54 (t, *J* = 7.8 Hz, 1H), 3.59–3.66 (m, 1H), 3.88 (dd, *J* = 7.8, 5.8 Hz, 1H), 4.12 (s, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 6.91 (s, 1H), 7.08 (s, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.59 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 29.8, 39.4, 39.6, 53.3, 71.2, 77.9, 110.6, 117.2, 122.5, 125.0, 128.4, 129.5, 131.0, 132.7, 135.4, 139.8, 141.8, 158.7; HRMS (ES) $[M+H]^+$ Calcd for $C_{22}H_{24}ClN_2O_3S$: 431.1196. Found: 431.1198; Anal. Calcd for $C_{22}H_{23}ClN_2O_3S$: C, 61.31; H, 5.38; N, 6.50. Found: C, 61.10; H, 5.34; N, 6.66. **1-Acetoxy-2-(1*H*-imidazol-1-yl)butane (QC-36) and 1-(1*H*-imidazol-1-yl)butan-2-ol hydrochloride**. To a suspension of NaH (1.33 g, 55.47 mmol) in DMF (20 mL) was added imidazole (3.77 g, 55.47 mmol). After the evolution of gas had subsided, 1,2-epoxybutane (3.50 g, 48.5 mmol) was added dropwise, and the reaction mixture was stirred at rt for one week and then at 130 °C for 1 h. The mixture was concentrated and the brown residue was washed with Et_2O (3×). The residue was then dissolved in boiling acetone and the mixture filtered hot, and the filtrate concentrated. The residue was treated with boiling acidic (HCl) EtOAc for a few minutes, and the mixture was then cooled in the freezer. The mixture was filtered and the filtrate was concentrated to an impure dark oil (2.25 g) which was fractionated by flash column chromatography on silica gel (EtOAc) giving preponderately 1-(1*H*-imidazol-1-yl)butan-2-ol (R_f = 0.16) and also 370 mg (2.03 mmol, 4%) of 1-acetoxy-2-(1*H*-imidazol-1-yl)butane (**QC-36**) as a clear oil: R_f = 0.22 (EtOAc); ^1H NMR (400 MHz, D_2O): δ 0.87 (t, *J* = 7.4 Hz, 3H), 1.44–1.63 (m, 2H), 1.99 (s, 3H), 4.08 (dd, *J* = 14.8, 8.0 Hz, 1H), 4.22 (dd, *J* = 14.8, 2.8 Hz, 1H), 4.95–5.02 (m, 1H), 6.94 (s, 1H), 7.10 (s, 1H), 7.61 (s, 1H); ^{13}C NMR (100 MHz, D_2O + CD_3OD): δ 9.7, 21.2, 25.1, 50.3, 76.1, 121.8, 128.7, 139.3, 174.3; HRMS (ES) $[M+H]^+$ Calcd for $C_9H_{15}N_2O_2$: 183.1133. Found: 183.1138. All of the 1-(1*H*-imidazol-1-yl)butan-2-ol isolated above was dissolved in EtOH (5 mL) and to this solution was added a solution of 37% aqueous HCl dropwise until the pH of the solution was acidic. The solution was concentrated, and the residue was dried under high vacuum and recrystallized from acetone to give 1.41 g (7.98 mmol, 16%) of 1-(1*H*-imidazol-1-yl)butan-2-ol hydrochloride as a hygroscopic white solid: R_f = 0.45 (EtOH); ^1H NMR (400 MHz, D_2O): δ 0.98 (t, *J* = 7.4 Hz, 3H), 1.42–1.54 (m, 1H), 1.55–1.66 (m, 1H),

3.87–3.96 (m, 1H), 4.15 (dd, J = 14.2, 8.2 Hz, 1H), 4.38 (dd, J = 14.0, 2.8 Hz, 1H), 7.48 (s, 1H), 7.52 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 9.3, 26.7, 54.6, 71.4, 119.8, 122.8, 135.3; HRMS (ES) $[M+H]^+$ Calcd for $C_7H_{13}N_2O$: 141.1027. Found: 141.1022; Anal. Calcd for $C_7H_{13}ClN_2O$: C, 47.60; H, 7.42; N, 15.86. Found: C, 47.54; H, 7.21; N, 16.02. **(2*R*,4*R*)-2-(2-Phenylethyl)-2-[(1*H*-imidazol-1-yl)methyl]-4-methyl-1,3-dioxolane hydrochloride** (**QC-35**). To a solution of tosylate **B**[24] (215 mg, 0.45 mmol) in THF (4 mL) was added powdered lithium aluminum hydride (102 mg, 2.69 mmol). The mixture was heated at reflux temperature for 9 h and then carefully quenched with H_2O. The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed sequentially with a saturated aqueous solution of Na_2CO_3, and H_2O, and then dried ($MgSO_4$). The solution was concentrated to a yellow oil, which was purified by flash chromatography on silica gel (EtOAc) to give the free base (90 mg, 0.33 mmol) as an oil (R_f = 0.22, EtOAc). To a solution of this oil in hot 2-propanol (1 mL) was added a solution of 37% aqueous HCl (40 mg, 0.41 mmol) in 2-propanol (2 mL). The mixture was concentrated and dried under high vacuum. The residue was recrystallized from 2-propanol–Et_2O to give 90 mg (0.29 mmol,64%) of **QC-35** as a white solid: R_f = 0.02 (EtOAc); mp 163–164 °C; $[\alpha]_D^{23}$ −17.0° (c 0.83, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD): δ 1.25 (d, J = 6.0 Hz, 3H), 1.91–2.00 (m, 2H), 2.70–2.82 (m, 2H), 3.48 (t, J = 8.4 Hz, 1H), 3.62–3.70 (m, 1H), 4.04 (dd, J = 8.0, 5.6 Hz, 1H), 4.41 (s, 2H), 7.15–7.29 (m, 5H), 7.52 (s, 1H), 7.59 (s, 1H), 8.83 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 18.1, 30.5, 39.7, 55.0, 73.0, 75.3, 109.3, 120.5, 125.1, 127.1, 129.3, 129.6, 137.7, 142.7; HRMS (ES) $[M+H]^+$ Calcd for $C_{16}H_{21}N_2O_2$: 273.1603. Found: 273.1609; Anal. Calcd for $C_{16}H_{21}ClN_2O_2$: N, 9.07. Found: N, 8.81. **4-(4-Chlorophenyl)-2-(4-fluorobenzyloxy)-1-(1*H*-imidazol-1-yl)butane hydrochloride** (**QC-37**). To a solution of alcohol **A**[26] (70 mg, 0.28 mmol) in THF (2 mL) was added a suspension of NaH (12 mg, 0.50 mmol) in THF (1 mL). The mixture was stirred at rt for 1 h and then a solution of 4-fluorobenzyl chloride (44 mg, 0.30 mmol) in THF (1 mL) was added. The mixture was stirred at rt for 24 h, heated at reflux temperature for 1.5 h, and then concentrated. After dilution with H_2O, the mixture was extracted with EtOAc (3×) and the combined organic extracts were washed once with H_2O, dried ($MgSO_4$), and concentrated. The resulting residue was purified by flash column chromatography on silica gel (EtOAc) to give the free base (50 mg, 0.14 mmol) as an oil ($R_f \approx 0.2$, EtOAc). To a solution of this oil in hot 2-propanol (1 mL) was added a solution of 37% aqueous HCl (16 mg, 0.16 mmol) in 2-propanol (1 mL). The mixture was concentrated and dried under high vacuum. The residue was recrystallized from 2-propanol–Et_2O to give 40 mg (0.10 mmol, 36%) of **QC-37** as a white solid: R_f = 0.18 (EtOAc); mp 125–127 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.80–1.94 (m, 2H), 2.74 (t, J = 7.8 Hz, 2H), 3.76–3.82 (m, 1H), 4.29 (dd, J = 14.4, 7.6 Hz, 1H), 4.38 (d, J = 11.6 Hz, 1H), 4.48–4.53 (m, 1H), 4.54 (d, J = 11.6 Hz, 1H), 7.00–7.06 (m, 2H), 7.17–7.23 (m, 4H), 7.28 (d, J = 8.4 Hz, 2H), 7.51 (s, 1H), 7.56 (s, 1H), 8.85 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 31.3, 34.2, 53.2, 71.8, 77.4, 116.2 (d, J_{CF} = 21.6 Hz), 120.9, 124.1, 129.6, 131.0, 131.2 (d, J_{CF} = 8.2 Hz), 132.9,135.1 (d, J_{CF} = 3.0 Hz), 137.1, 141.5, 163.9 (d, J_{CF} = 245.2 Hz); HRMS (ES) $[M+H]^+$ Calcd for $C_{20}H_{21}ClFN_2O$: 359.1326. Found: 359.1330; Anal. Calcd for $C_{20}H_{21}Cl_2FN_2O$: N, 7.09. Found: N, 6.93.

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27. In vitro *P. falciparum* activity assay: *P. falciparum* cultures were grown in A+ blood obtained by venipuncture of volunteers. Cultures of the laboratory line ItG were maintained by the method of Trager and Jensen[34] using RPMI 1640 supplemented with 10% human serum and 50 μM hypoxanthine (RPMI-A). In vitro *P. falciparum* susceptibility testing was performed using an LDH enzyme assay specific to LDH found in *Plasmodium* (pLDH).[35–37] Briefly, compounds to be tested were dissolved in DMSO at a final concentration of 10 mg/mL and were then serially diluted in duplicate in a 96-well plate to produce a compound gradient with twofold dilutions. Fifty microliters of parasite culture (5% hematocrit, 2% parasitemia from non-synchronous cultures) were added to each well and the plates were then incubated at 37 °C in an atmosphere of 95% N_2, 3% CO_2, and 2% O_2 for 72 h. The contents of the wells were then resuspended in 100 μL of tissue culture media and a 15-μL sample was removed and added to 100 μL of pLDH enzyme assay mixture.[37] After 1 h, the absorbance of the wells at 540 nm was determined using a microplate reader (BioRad, Mississauga, ON). The IC_{50} values of individual compounds were determined using a non-linear regression analysis of the data[38] using the computer program SigmaPlot (Jandel Scientific). The IC_{50} values represent means ± standard deviation of four independent assays.
28. In vitro CHO cell activity assay: CHO cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 25 mM HEPES, and gentamicin. Cells were grown to 50% confluency in 96-well plates prior to the addition of individual samples in DMSO. After 24 h, the viability of the cells was determined by adding 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO) in 100 μL of culture media. The plates were incubated for a further 30 min and then the media were removed and 100 μL of DMSO were added and the absorbance at 540 nm was read.[39] The IC_{50} values of individual compounds were determined using a non-linear regression analysis of the data as in Ref. 27.
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