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Investigating the activity of quinine analogues versus chloroquine resistant *Plasmodium falciparum*

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ABSTRACT

Plasmodium falciparum, the deadliest malarial parasite species, has developed resistance against nearly all man-made antimalarial drugs within the past century. However, quinine (QN), the first antimalarial drug, remains efficacious worldwide. Some chloroquine resistant (CQR) *P. falciparum* strains or isolates show mild cross resistance to QN, but many do not. Further optimization of QN may provide a well-tolerated therapy with improved activity versus CQR malaria. Thus, using the Heck reaction, we have pursued a structure–activity relationship study, including vinyl group modifications of QN. Certain derivatives show good antiplasmodial activity in QN-resistant and QN-sensitive strains, with lower IC_{50} values relative to QN.

1. Introduction

Malaria remains one of the deadliest infectious diseases, ranking in the top five.[1] Five *Plasmodium* species cause the disease in humans, with *Plasmodium falciparum* causing the greatest mortality. Currently, about half of the world's population is at risk of contracting this disease. In 2010, more than 200 million malaria cases led to an estimated 781,000 deaths, with nearly 85% of those being children in Africa.

Nearly four centuries ago, a treatment for malaria was discovered in the bark of the *Cinchona* tree.[2] Quinine (QN, Fig. 1), the most abundant *Cinchona* alkaloid, was the only known antimalarial drug for over 300 years. Widespread use of QN ceased in the mid-twentieth century upon the introduction of the more practical and less expensive chloroquine[3] (CQ, Fig. 1). Parasite resistance to CQ and related synthetic QN derivatives, such as mefloquine (MQ, Fig. 1), began to appear within a few decades of introduction. More recently, artemisinin (ART, Fig. 1) derivatives have gained favor as replacement therapy; however, the cost of these ART-based combination therapies (ACTs) is comparatively high relative to CQ and QN. Also, based on the history of *P. falciparum* drug resistance, it is no surprise that resistance to ART and MQ-ART combination therapies has begun to emerge in Southeast Asia.[4,5]

Remarkably, resistance to QN remains relatively low despite almost 400 years of use, and QN is currently a recommended therapy for CQ- and ART-resistant *P. falciparum*. Low parasite resistance to QN observed today is often attributed to its low frequency of use. However, QN has an impressive track record considering that most antimalarial drugs encounter parasite resistance within a few years of introduction. Low parasite resistance to QN might also suggest a privileged natural structure which is difficult for the parasite to circumvent. While the search for novel drug targets and new lead structures for the treatment of malaria is a critical venture, a complimentary strategy is to utilize and further develop lead structures from nature with proven attractive properties.

Identifying antimalarial compounds with novel mechanisms of action is of increasing importance to combat the increased drug failures linked to parasite resistance.[6,7] The synthesis of QN derivatives, a strategy that led to the discovery of CQ, has been largely abandoned by most laboratories[8] in part because of toxicity concerns and reaction complexity. Yet, modification of the *Cinchona* alkaloids was one of the most successful strategies for antimalarial drug development. QN has been the target of numerous synthetic endeavors, but its total synthesis remains laborious. In terms of QN modifications, researchers have primarily focused on substitution around the quinoline moiety, as alteration of the hydroxyl and quinuclidine structure appeared detrimental for antimalarial activity.[9] An alternative point of functionalization is the terminal alkene. Modifications involving this functional group have been pursued,[10–12] often during catalyst development for asymmetric reactions.[13–16] Attempts to tailor biological activity by alkene derivatization are less common.[17,18] Bhattacharjee et al. have put forth a catalyst™-generated binding model that indicates the

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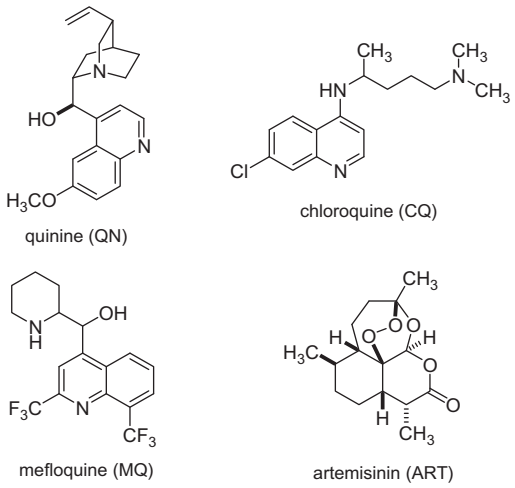


Figure 1. Chemical structures of active antimalarial drugs.

alkene portion may be important for QN's activity, despite being spatially distant from the remaining functional groups.[19] However, Alumasa et al.[9] recently showed the alkene functionality to be non-essential for binding to free heme, the putative drug target of QN and other quinoline antimalarials. Binding to free heme within the parasite digestive vacuole (DV) is believed to inhibit the parasite's ability to sequester toxic ferriprotoporphyrin IX (FPIX) heme into nontoxic hemozoin (Hz).[9,20–23]

Although historically less popular than derivatization of CQ and other amino quinolines,[8] further optimization of QN could provide a rapid avenue for the development of potent, inexpensive antimalarial drugs with good activity versus CQ resistant (CQR) malaria. In addition, recent data suggests that simplified quinoline antimalarial drugs and QN operate by different mechanisms.[9,24–26] In light of these data, we have considered new modifications to QN that further explore structure–function relationships. In this work, we modify the quinuclidine alkene of the *Cinchona* alkaloids by Heck reaction[27,28] to search for derivatives with improved activity versus CQR malaria. Several derivatives exhibited improved activity versus CQR malaria relative to QN, with IC_{50} <200 nM. We also inspected whether improved activity relative to QN correlated with ability to inhibit Hz crystallization.

2. Materials and methods

2.1. General

Anhydrous toluene was purchased from EMD Chemicals and purged with Argon for a minimum of 15 min prior to use. *Cinchona* alkaloids quinine (QN), quinidine (QD), cinchonine (CN), and cinchonidine (CD) were purchased from Alfa Aesar and used without further purification despite containing up to 5% of the corresponding dihydro derivatives. All other reagents were obtained from commercial sources and used without further modification.

2.2. General synthetic method for *Cinchona* alkaloid derivatives—Synthesis of QN-1

The reaction was conducted in oven and flame dried glassware under an inert atmosphere of argon. Quinine (81.1 mg, 0.25 mmol), palladium(II) acetate (2.8 mg, 0.0125 mmol), and triphenylphosphine (6.6 mg, 0.025 mmol) were all weighed out on the bench top and placed into a reaction vial. Bromobenzene (53.0 μL, 0.50 mmol) and dry toluene (1 mL) were added to the reaction vial by syringes. TEA (69.7 μL, 0.50 mmol) was added last, dropwise, into the reaction vial via syringe. The vial was then sealed with a Teflon-cap and stirred at 110 °C for 24 h under argon. Contents were allowed to cool to room temperature, followed by filtration through a cotton plug. The solid was washed with DCM and the filtrate was concentrated under reduced pressure. The product was purified by silica gel flash column chromatography using gradient solvent systems of DCM and MeOH. Fractions containing the product were combined and concentrated under reduced pressure to give a 66.1 mg (66% yield) of slightly yellow solid. IR: 2943, 1620, 1508, 1240 cm^{-1}. HRMS: Calculated for $C_{26}H_{28}N_2O_2$: 401.2224 ($M+H^+$), found 401.2220 ($M+H^+$). ^1H NMR ($CDCl_3$): δ 8.63 ppm (1H, d, J = 4 Hz), 7.95 (1H, d, J = 9.2), 7.54 (1H, d, J = 4.4), 7.29 (1H, dd, J = 2.0, 9.2), 7.24 (5H, m), 7.18 (1H, m), 6.36 (1H, d, J = 16.0), 6.10 (1H, dd, J = 8.0, 16.0), 5.76 (1H, s), 3.88 (3H, s), 3.65 (1H, m), 3.26 (2H, m), 2.78 (2H, m), 2.54 (1H, m), 1.95 (1H, m), 1.88 (2H, m), 1.62 (2H, m). ^{13}C NMR ($CDCl_3$): δ 157.9 ppm, 147.5, 147.3, 143.8, 137.0, 132.5, 131.2, 130.6, 128.5, 127.3, 126.4, 126.0, 121.6, 118.6, 101.2, 70.4, 60.2, 57.2, 56.1, 43.3, 39.1, 28.1, 26.9, 21.0.

2.3. Antiplasmodial screens

All *P. falciparum* strains were maintained using the method of Trager and Jensen[29] with minor modification. Briefly, cultures were maintained under a 5% CO_2/5% O_2/90% N_2 atmosphere at 2% hematocrit and 1–2% parasitemia in RPMI 1640 supplemented with 10% O^+ human serum, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; pH 7.4), 24 mM $NaHCO_3$, 11 mM glucose, 0.75 mM hypoxanthine, and 20 μg/L gentamycin. Regular media changes were performed every 48 h.

Antiplasmodial growth assays were performed against the chloroquine sensitive (CQS) HB3 strain and the CQR Dd2 strain of *P. falciparum*, as previously reported,[30] with minor modification. Typically, compounds were dissolved in DMSO, with further dilution of these stocks performed with complete media. Drug-media solutions (100 μL) were transferred to 96-well plates which were then pre-warmed to 37 °C prior to the addition of culture. Ring stage-synchronized culture was utilized for the assays,[31] final 1% parasitemia, 2% hematocrit. The plates were transferred to an airtight chamber gassed with 5% CO_2/5% O_2/90% N_2 and incubated at 37 °C. After 72 h, 50 μL of 10X SYBR Green I dye (diluted using complete media from a 10,000× DMSO stock) was added, plates incubated for an additional 1 h at 37 °C to allow DNA intercalation, and fluorescence measured at 530 nm (490 nm excitation) using a Spectra GeminiEM plate reader (Molecular Devices). IC_{50} values from three replicates were averaged and are reported ± S.E.M. In these assays, QN was included as a control. For IC_{50} calculations, data analysis was performed using SigmaPlot 10.0 software after downloading data in Excel format.

2.4. β-Hematin crystallization inhibition

β-Hematin crystal growth inhibition in the presence of lipid catalyst was assessed using a modified 96-well plate high throughput assay.[32] Briefly, the assay uses physiologic temperature and lipid catalyst, and relies on the differential solubility properties of crystalline and non-crystalline forms of FPIX in 2.5% SDS (86.7 mM) and alkaline bicarbonate buffer (0.1 M, pH 9.1). Hemin was dissolved in 0.1 M NaOH to 2 mM. 10 μL was then transferred to 96-well plate wells, followed by propionate buffer (180 μL at desired pH) and 10 μL of sonicated phosphatidyl choline suspension (10 mg/mL). Drugs were added, plates mixed, wrapped in plastic wrap, and incubated at 37 °C for 16 h. The assay was terminated by adding 100 μL of a solution of SDS dissolved in 0.1 M bicarbonate buffer (pH 9.1, final concentration/well of SDS 2.5% (w/v) or 86.7 mM). The well contents were gently mixed and the plate

incubated at room temperature for 10 min to allow undissolved Hz crystals to settle. A 50 μL aliquot from each well was then transferred to a second plate pre-loaded with 200 μL of SDS solution (2.5% [w/v] or 86.7 mM) in 0.1 M bicarbonate buffer (pH 9.1). Absorbance of non-crystallized heme was recorded at 405 nm with a 96-well plate adapted ELx800 BioTek absorbance microplate reader. Standard curves (known [heme] in the same solvent as assay conditions) were generated for each assay. Percent Hz formed was computed using Excel and SigmaPlot 10.0 software.

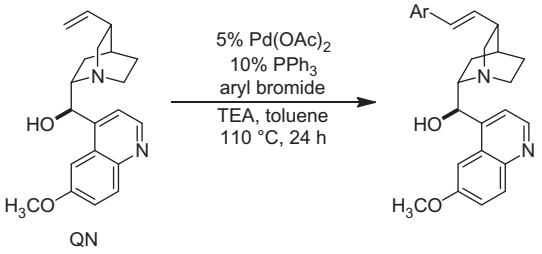
3. Results

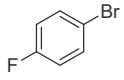
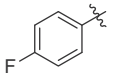
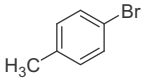
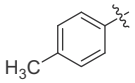
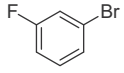
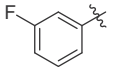
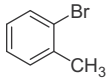
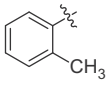
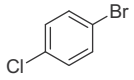
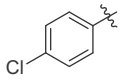
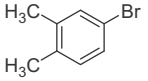
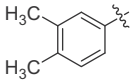
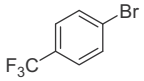
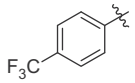
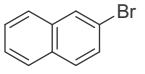
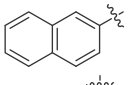
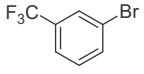
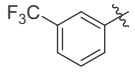
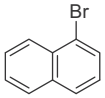
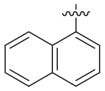
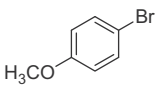
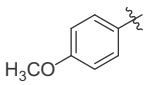
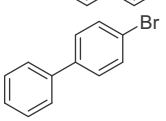
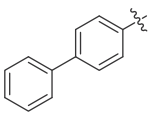
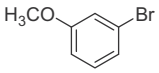
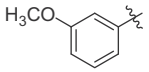
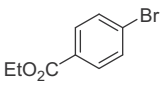
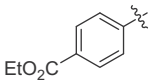
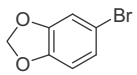
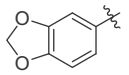
3.1. Compound synthesis

The Heck reaction of a cinchonidine derivative has been reported,[16] but no conditions have been reported for QN. A short catalyst screen for optimal conversion was conducted which led to standard conditions for the Heck reaction between QN and bromobenzene (Table 1). The initial set of compounds was synthesized using the QN core. Aryl bromides were favored as coupling partners because of starting material availability, even though aryl iodides also participated in coupling under these reaction conditions. Complete conversion to product, which occurred over 24 h, was required to simplify purification by eliminating co-elution of the starting alkaloid. The use of toluene as a solvent aided in purification because the triethylamine hydrochloride could be removed via filtration. All quinine derivatives were isolated as the free base in moderate to excellent yield. The variation in percent yields is more indicative of the challenge associated with isolating pure product than any appreciable side reactions. The reaction was tolerant of a variety of substitutions around the aryl bromide, including substitution at the *ortho* position (**QN-3** and **QN-6**). Both electron poor (**QN-8** to **QN-13**) and electron rich (**QN-14** to **QN-16**) functional groups performed equally well in the cross coupling reaction.

Following the synthesis of QN derivatives, the remaining *Cinchona* alkaloids (Fig. 2) were similarly modified with select aryl bromides (Fig. 3). Reactions of the remaining alkaloids were analogous to those involving QN; however, in some cases the product was insoluble in toluene. In these cases, instead of filtration, the entire reaction mixture required silica gel chromatography. We

Table 1
Quinine derivatives synthesized by the Heck reaction[a]



Compound #	Aryl bromide	Ar	Yield[b] (%)	Compound #	Aryl bromide	Ar	Yield[b] (%)
QN-1			66	**QN-9**			57
QN-2			82	**QN-10**			51
QN-3			63	**QN-11**			53
QN-4			74	**QN-12**			61
QN-5			70	**QN-13**			66
QN-6			67	**QN-14**			59
QN-7			62	**QN-15**			60
QN-8			89	**QN-16**			58

[a] Reactions were performed on a 0.25 mmol scale with quinine (1 equiv), aryl bromide (2 equiv), triethyl amine (2 equiv), palladium (II) acetate (0.05 equiv), and triphenylphosphine (0.1 equiv) in toluene (0.25 M) for 24 h at reflux.
[b] Average isolated yields for 2 or more runs.

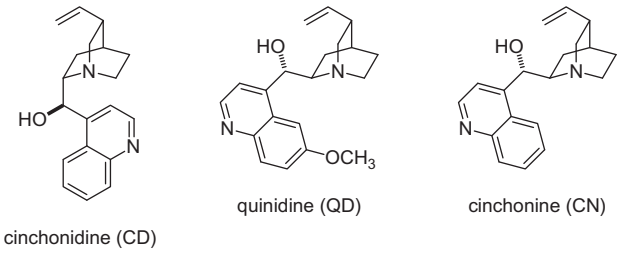


Figure 2. Stereoisomeric *Cinchona* alkaloids.

also observed some isolated derivatives to be the hydrobromide salt following purification. Again, yields were generally satisfactory and reproducible, but varied as we focused on alkaloid purity.

3.2. Antiplasmodial screen results

The initial screen of sixteen QN derivatives identified only four compounds that exhibited IC_{50} data comparable to unmodified QN against HB3 (Table 2). The fact that a majority of our synthetic QN derivatives had decreased activity versus the CQS/QNS HB3 strain indicates that only specific alterations to the alkene are tolerated. QN derivatives bearing phenyl (**QN-7**), ethyl ester (**QN-8**), and trifluoromethyl (**QN-12**) substitutions *para* to the alkene attachment site showed good activity versus QNS strain HB3, with a fourth compound (**QN-10**), containing a meta-fluoro substituent, also active. The first three showed good activity versus QNR strain Dd2, with **QN-7** more potent than QN. Thus, simple alkene modifications to QN can improve activity versus QNR strains of *P. falciparum* malaria.

Table 2
IC_{50} data[a,b] for select QN derivatives versus QNS (strain HB3) and QNR (strain Dd2) *P. falciparum*

Entry	Compound #	HB3 IC_{50} (nM)	Dd2 IC_{50} (nM)
1	**QN**	81 ± 8	320 ± 50
2	**QN-1**	>500	>500
3	**QN-2**	>500	>500
4	**QN-3**	>500	>500
5	**QN-5**	>500	>1500
6	**QN-6**	>500	>500
7	**QN-7**	128 ± 5	184 ± 7
8	**QN-8**	428 ± 4	318 ± 2
9	**QN-9**	>500	>500
10	**QN-10**	145 ± 4	733 ± 9
11	**QN-11**	>500	>500
12	**QN-12**	427 ± 4	485 ± 6
13	**QN-13**	>500	>500
14	**QN-14**	>500	>500
15	**QN-15**	>500	>500
16	**QN-16**	>500	>500

[a] IC_{50} values are an average of triplicate measurements and reported ± S.E.M.
[b] Derivatives with low activity (>500 nM) were not quantified for IC_{50}.

To test whether this trend continued for other *Cinchona* alkaloids,[33–35] derivatives containing similar modifications to the CD,

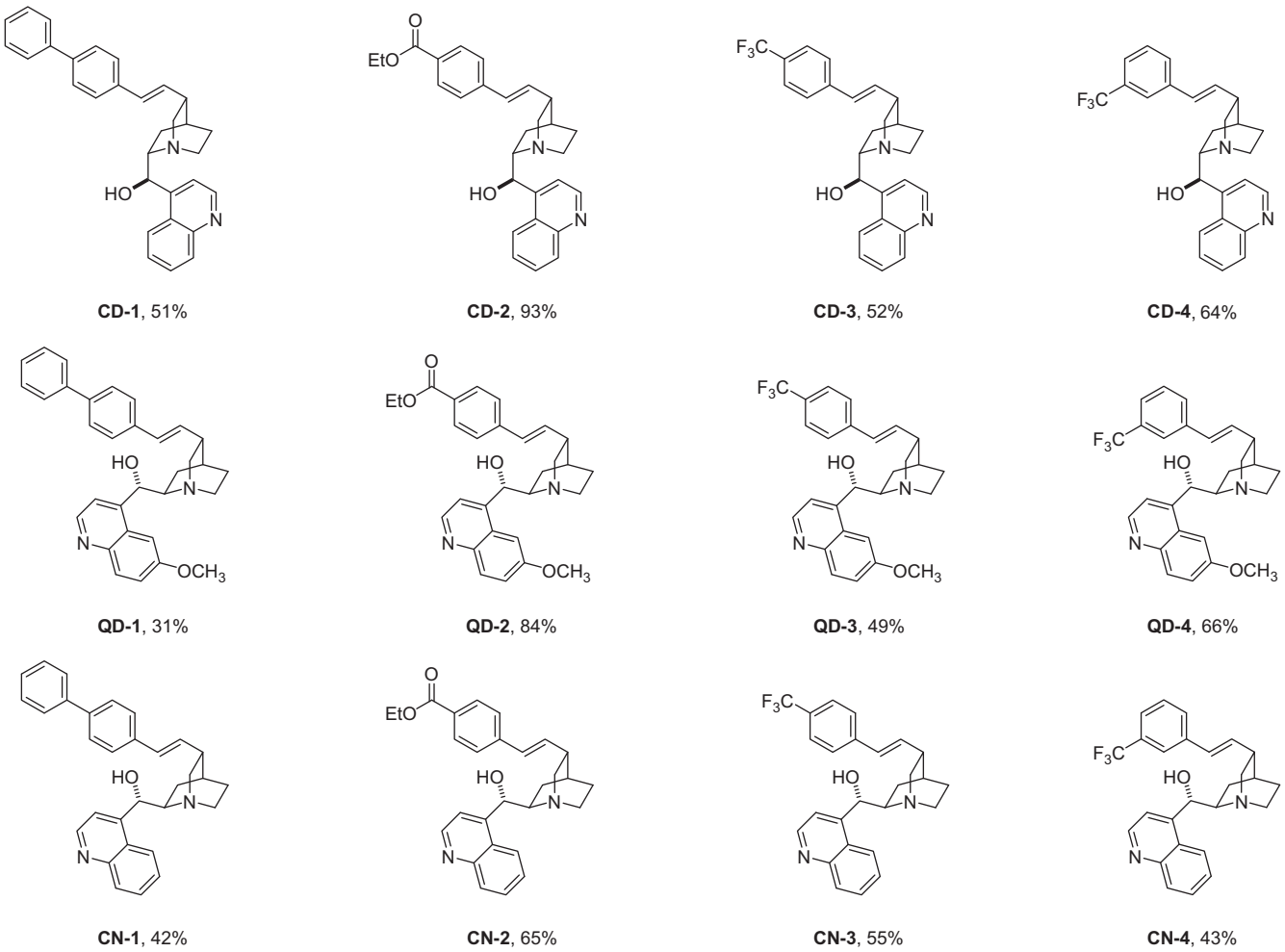


Figure 3. Structures and yields of *Cinchona* alkaloid derivatives synthesized by the Heck reaction.

Table 3
IC_{50} data[a] for *Cinchona* alkaloid derivatives against *P. falciparum*

Entry	Compound ID	HB3 IC_{50} (nM)	Dd2 IC_{50} (nM)
1	**CD**	70 ± 8	207 ± 11
2	**QD**	18 ± 2	90 ± 8
3	**CN**	22 ± 6	82 ± 8
4	**CD-1**	127 ± 4	176 ± 2
5	**CD-2**	590 ± 17	198 ± 5
6	**CD-3**	297 ± 13	223 ± 8
7	**CD-4**	394 ± 5	928 ± 6
8	**QD-1**	123 ± 1	202 ± 7
9	**QD-2**	120 ± 6	203 ± 10
10	**QD-3**	165 ± 5	2350 ± 4
11	**QD-4**	98 ± 3	182 ± 12
12	**CN-1**	90 ± 3	243 ± 2
13	**CN-2**	144 ± 1	263 ± 4
14	**CN-3**	66 ± 8	983 ± 2
15	**CN-4**	154 ± 1	146 ± 2

[a] IC_{50} values are an average of triplicate measurements and reported ± S.E.M.

QD, and CN cores (Fig. 3) were synthesized. With the exception of **QD-3**, QD derivatives exhibited IC_{50}s about 2-fold higher than QD versus strain Dd2 but about 6-fold higher versus strain HB3 (Table 3, entries 8–11). The trend for CN was similar; IC_{50} values were about 2.5-fold higher versus strain Dd2 and about 6-fold higher versus strain HB3 (entries 12–15). A notable exception was **CN-3**, which had the most potent activity versus HB3 but was one of the least potent versus Dd2. Data for the CD derivatives was more variable, but **CD-1**, **CD-2**, and **CD-3** were comparable to CD versus Dd2 (entries 4–6). With the exception of **CD-1**, all CD derivatives were significantly less active than the parent versus HB3.

3.3. β-Hematin crystal growth inhibition data

A select set of the most active and least active compounds were screened for their ability to inhibit Hz formation in vitro at pH 5.2 and 5.6 (Table 4). Measurement at both pH 5.2 and 5.6 may mimic physiological conditions, since different DV pH for sensitive (HB3) versus resistant (Dd2) strains has been measured in some studies.[36] All analogs exhibited Hz inhibition similar to QN at pH 5.6 with the exception of **CD-1**, which is closer to CD in its Hz inhibitory potency. It should also be noted that **CD-1** did not achieve >50% Hz crystal growth inhibition at pH 5.2 despite several trials, making IC_{50} determination impossible at this pH. Interestingly, derivatives that were either active or inactive in antiplasmodial growth inhibition assays were active inhibitors of Hz formation (Fig. 4), similar to other trends observed recently with CQ derivatives.[37]

Table 4
β-Hematin inhibitory IC_{50} data[a] for select *Cinchona* alkaloids at pH 5.2 and 5.6

Entry	Compound ID	pH 5.2 IC_{50} (μM)	pH 5.6 IC_{50} (μM)
1	**QN**	291 ± 1	34 ± 1
2	**CQ**	49 ± 1	33 ± 2
3	**QD**	130 ± 3	23 ± 2
4	**CN**	370 ± 12	170 ± 9
5	**CD**	321 ± 14	142 ± 11
6	**QN-8**	54 ± 4	40 ± 3
7	**QN-9**	44 ± 5	23 ± 2
8	**QN-12**	100 ± 15	46 ± 2
9	**QN-13**	71 ± 3	52 ± 2
10	**QD-2**	81 ± 1	40 ± 0.2
11	**CD-1**	—	116 ± 1
12	**CD-4**	75 ± 10	70 ± 9
13	**CN-2**	66 ± 10	50 ± 1
14	**CN-4**	63 ± 5	39 ± 4

[a] IC_{50} values are an average of three independent measurements and reported ± S.E.M.

4. Discussion

Some trends emerge when considering the structure–activity relationships of these *Cinchona* derivatives. Modification of all four *Cinchona* alkaloids with aromatics containing *para*-substituted withdrawing groups was generally beneficial. A *para*-phenyl (**QN-7**, **CD-1**, **QD-1**, and **CN-1**) or *para*-ester (**QN-8**, **CD-2**, **QD-2**, and **CN-2**) aryl modification resulted in improved activity versus the QNR Dd2 strain, relative to QN. The noticeable outliers to this trend were derivatives containing either *para*- or *meta*-trifluoromethyl substituted aryls (**CD-4**, **QD-3**, and **CN-3**). While activity for the QD, CN, and CD derivatives were, in most cases, improved relative to QN, in general they were at best comparable to the parent drug. CN has the same absolute configuration as QD and is more active relative to CD, which has the same configuration as QN, similarly, QD is known to be more active than QN.[33] These trends are reflected in the series of derivatives; however, CD compounds exhibited, on average, slightly improved activity over CN compounds versus QNR strain Dd2 (Table 3).

Based on a recent QN-heme binding model presented by Alumasa et al.[9] the lack of correspondence with Hz inhibitory activity is perhaps supported by the fact that the quinuclidine alkene does not appear to play a role in complex formation.[9] That is, further derivatization at the alkene would not be expected to add to or alter Hz inhibition activity. The explanation for the differential antiplasmodial activity of *ortho*/*para* versus *meta* substituted derivatives could lie in steric and/or electronic interaction with non-heme drug targets, such as the *P. falciparum* chloroquine resistance transporter (PfCRT), multidrug resistance transporter (PfMDR1), or sodium-proton exchanger (PfNHE).[38–43] These possibilities might also account for the lack of correlation between the two sets of IC_{50} values. Indeed, derivatives **QD-2**, **CD-1**, and **CN-4** exhibit significantly decreased Hz inhibition at pH 5.2 but improved antiplasmodial activity against Dd2, suggesting alternative targets may be responsible. It has been recently shown that both PfCRT and PfMDR1 protein likely bind QN,[43–45] and that deletion of the *pfcrt* gene is not possible for parasite red cell stages, arguing PfCRT function is essential for cell survival.[44] It should also be noted that Hz inhibition occurs at drug concentrations closer to cytotoxic doses and not to the cytostatic dosages employed in the antiplasmodial screens.[46] Perhaps FPIX is an important *Cinchona* alkaloid target at cytostatic concentrations, with an alterna-

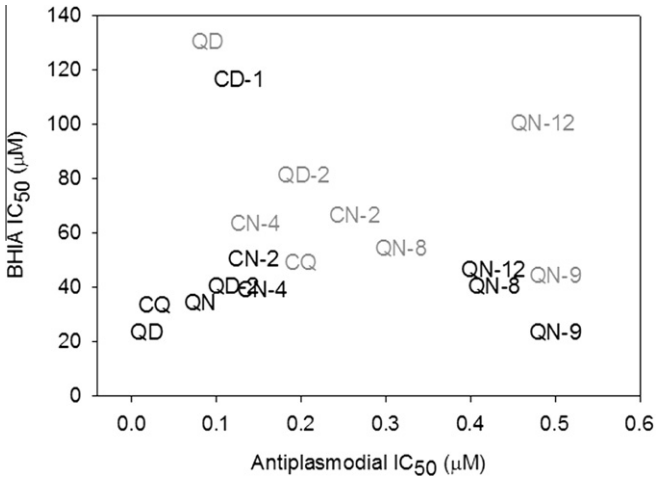


Figure 4. Plot of β-hematin inhibitory IC_{50} versus antiplasmodial IC_{50}. Black symbols correspond to BHIA IC_{50} at pH 5.6 and antiplasmodial IC_{50} against HB3. Grey symbols correspond to BHIA IC_{50} at pH 5.2 and antiplasmodial IC_{50} against Dd2. The IC_{50} value for QN at pH 5.2 is omitted for clarity. QN series derivatives that were not active antiplasmodial agents (and therefore do not have an antiplasmodial IC_{50}) are also not included.

tive mechanism of action relevant at cytocidal concentration. The fact that QN and CD are slightly poorer antimalarial drugs relative to QD and CN (matched stereochemical pairs) but that CN and CD are poorer Hz inhibitors relative to QN and QD (matched constitutional isomeric pairs) supports this dose dependency. Additional experiments will address these points.

Acknowledgments

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Supplementary data

Supplementary data (detailed experimental procedures and characterization data for all unknown compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.03.042.

References and notes

1. World Health Organization (WHO 2010 Report: http://www.who.int/malaria/publications/atoz/9789241564106/en/index.html).
2. Meshnick, S.; Dobson, M. In *Antimalarial Chemotherapy Mechanism of Action Resistance and New Directions in Drug Discovery*; Rosenthal, P., Ed.; Humana: Totowa, NJ, 2001; pp 15–26.
3. Rathore, D.; McCutchan, T. F.; Sullivan, M.; Kumar, S. *Expert Opin. Investig. Drugs* **2005**, *14*, 871.
4. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, K.; Socheat, D.; White, N. J. *New Engl. J. Med.* **2009**, *36*, 455.
5. Lim, P.; Wongsrichanalai, C.; Chim, P.; Khim, N.; Kim, S.; Chy, S.; Sem, R.; Nhem, S.; Yi, P.; Duong, S.; Bouth, D. M.; Genton, B.; Beck, H.-P.; Gobert, J. G.; Rogers, W. O.; Coppee, J.-Y.; Fandeur, T.; Mercereau-Puijalon, O.; Ringwald, P.; Le Bras, J.; Ariey, F. *Antimicrob. Agents Chemother.* **2010**, *54*, 2135.
6. Gamo, F. J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J. L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305.
7. Rottmann, M.; McNamara, C.; Yeung, B. K. S.; Lee, M. C. S.; Zou, B.; Russell, B.; Seitz, P.; Plouffe, D. M.; Dharia, N. V.; Tan, J.; Cohen, S. B.; Spencer, K. R.; Gonzalez-Paez, G. E.; Lakshminarayana, S. B.; Goh, A.; Suwanarusk, R.; Jegla, T.; Schmitt, E. K.; Beck, H. P.; Brun, R.; Nosten, F.; Renia, L.; Dartois, V.; Keller, T. H.; Fidock, D. A.; Winzeler, E. A.; Diagana, T. T. *Science* **2010**, *329*, 1175.
8. Kumar, V.; Mahajan, A.; Chibale, K. *Bioorg. Med. Chem.* **2009**, *17*, 2236.
9. Alumasa, J. N.; Gorka, A. P.; Casabianca, L. B.; Comstock, E.; de Dios, A. C.; Roepe, P. D. *J. Inorg. Biochem.* **2011**, *105*, 467.
10. Hoffmann, H. M. R.; Frackenpohl, J. Organic Chemistry of Cinchona Alkaloids. In *Cinchona Alkaloids in Synthesis and Catalysis, Ligands, Immobilization and* In Song, C. E., Ed.; Wiley-GmbH & Co. KGaA: Weinheim, 2009; pp 361–418.
11. Langer, F.; Schwink, L.; Devasagayaraj, A.; Chavant, P. Y.; Knochel, P. *J. Org. Chem.* **1996**, *61*, 8229.
12. Lambers, M.; Beijer, F. H.; Padron, J. M.; Toth, I.; de Vries, J. G. *J. Org. Chem.* **2002**, *67*, 5022.
13. Arrington, M. P.; Bennani, Y. L.; Gobel, T.; Walsh, P.; Zhao, S. H.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 7375.
14. Fache, F.; Piva, O. *Tetrahedron Lett.* **2001**, *42*, 5655.
15. Merschaert, A.; Delbeke, P.; Daloze, D.; Dive, G. *Tetrahedron Lett.* **2004**, *45*, 4697.
16. Ma, B.; Parkinson, J. L.; Castle, S. L. *Tetrahedron Lett.* **2007**, *48*, 2083.
17. Walsh, J. J.; Coughlana, D.; Heneghan, N.; Gaynor, C.; Bell, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3599.
18. Garner, A. L.; Koide, K. *Org. Lett.* **2007**, *9*, 5235.
19. Bhattacharjee, A. K.; Hartell, M. G.; Nichols, D. A.; Hicks, R. P.; Stanton, B.; van Hamont, J. E.; Milhous, W. K. *Eur. J. Med. Chem.* **2004**, *39*, 59.
20. Egan, T. J.; Ncokazi, K. K. *J. Inorg. Biochem.* **2005**, *99*, 1532.
21. Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727.
22. Egan, T. J.; Ross, D. C.; Adams, P. A. *FEBS Lett.* **1994**, *352*, 54.
23. Slater, A. F.; Cerami, A. *Nature* **1992**, *355*, 167.
24. Casabianca, L. B.; Kallgren, J. B.; Natarajan, J. K.; Alumasa, J. N.; Roepe, P. D.; de Dios, A. C. *J. Inorg. Biochem.* **2009**, *103*, 745.
25. Casbianca, L. B.; An, D.; Natarajan, J. K.; Alumasa, J. N.; Roepe, P. D.; Wolf, C.; de Dios, A. C. *Inorg. Chem.* **2008**, *47*, 6077.
26. Leed, A.; BuBay, K.; Ursos, L. M.; Sears, D.; de Dios, A. C.; Roepe, P. D. *Biochemistry* **2002**, *41*, 10245.
27. Heck, R. F. *J. Am. Chem. Soc.* **1968**, *90*, 5518.
28. Crisp, G. T. *Chem. Soc. Rev.* **1998**, *27*, 427.
29. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
30. Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D. *Antimicrob. Agents Chemother.* **2004**, *48*, 1807.
31. Gligorijevic, B.; Purdy, K.; Elliot, D. A.; Cooper, R. A.; Roepe, P. D. *Mol. Biochem. Pharmacol.* **2008**, *159*, 7.
32. Alumasa, J. N.; Gorka, A. P.; Nickley, K. B.; Brower, J. P.; Sherlach, K. S.; de Dios, A. C.; Roepe, P. D. Unpublished results.
33. Warhurst, D. C.; Craig, J. C.; Adagu, I. S.; Meyer, D. J.; Lee, S. Y. *Malaria J.* **2003**, *2*, 1.
34. Cooper, R. A.; Ferdig, M. T.; Su, X. Z.; Ursos, L. M.; Mu, J.; Nomura, T.; Fujioka, H.; Fidock, D. A.; Roepe, P. D.; Wellems, T. E. *Mol. Pharmacol.* **2002**, *61*, 35.
35. Karle, J. M.; Karle, I. L.; Gerena, L.; Milhous, W. K. *Antimicrob. Agents Chemother.* **1992**, *36*, 1538.
36. Bennett, T. N.; Kosar, A. D.; Ursos, L. M.; Dzekunov, S.; Singh Sidhu, A. B.; Fidock, D. A.; Roepe, P. D. *Mol. Biochem. Parasitol.* **2004**, *133*, 99.
37. Natarajan, J. K.; Alumasa, J. N.; Yearick, K.; Ekoue-Kovi, K.; Casabianca, L. B.; de Dios, A. C.; Wolf, C.; Roepe, P. D. *J. Med. Chem.* **2008**, *51*, 3466.
38. Ferdig, M. T.; Cooper, R. A.; Mu, J.; Deng, B.; Joy, D. A.; Su, X. Z.; Wellems, T. E. *Mol. Microbiol.* **2004**, *52*, 985.
39. Singh Sidhu, A. B.; Valderramos, S. G.; Fidock, D. A. *Mol. Microbiol.* **2005**, *57*, 913.
40. Singh Sidhu, A. B.; Uhlemann, A. C.; Valderramos, S. G.; Valderramos, J. C.; Krishna, S.; Fidock, D. A. *J. Infect. Dis.* **2006**, *194*, 528.
41. Bennett, T. N.; Patel, J.; Ferdig, M. T.; Roepe, P. D. *Mol. Biochem. Parasitol.* **2007**, *153*, 48.
42. Nkrumah, L. J.; Riegelhaupt, P. M.; Moura, P.; Johnson, D. J.; Patel, J.; Hayton, K.; Ferdig, M. T.; Akabas, M. H.; Fidock, D. A. *Mol. Biochem. Parasitol.* **2009**, *165*, 122.
43. Pleeter, P.; Lekostaj, J. K.; Roepe, P. D. *Mol. Biochem. Parasitol.* **2010**, *173*, 158.
44. Lekostaj, J. K.; Natarajan, J. K.; Paguio, M. F.; Wolf, C.; Roepe, P. D. *Biochemistry* **2008**, *47*, 10394.
45. Waller, K. L.; Muhle, R. A.; Ursos, L. M. B.; Verdier-Pinard, D.; Fujioka, H.; Roepe, P. D.; Fidock, D. A. *J. Biol. Chem.* **2003**, *278*, 33593.
46. Paguio, M. F.; Bogle, K. L.; Roepe, P. D. *Mol. Biochem. Parasitol.* **2011**, *178*, 1.