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Letter

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Potent Antimalarial Activity of Two Arenes linked with Triamine, Designed to Have Multiple Interactions with Heme.

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KEYWORDS: Antimalarial, heme, hemozoin, molecular recognition, heme detoxification protein.

ABSTRACT: Based on the idea that compounds designed to exhibit high affinity for heme would block hemozoin formation, a critical heme-detoxification process for malarial parasites, we synthesized a series of compounds with two π-conjugated moieties at terminal amino groups of triamine. These compounds exhibited moderate to high antimalarial activities towards both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum in vitro*. In a *P. berghei*-infected mouse model, **3a** and **12a** showed potent antimalarial activities compared to artesunate, as well as a prolonged duration of antimalarial effect. We found a good correlation between protective activity against hemin degradation and antimalarial activity. Compounds **8b** and **3a** strongly inhibited hemozoin formation catalyzed by heme detoxification protein.

Malaria is a serious and sometimes fatal disease caused by several species of parasite of the genus *Plasmodium* that are transmitted by mosquitos of the *Anopheles* genus.[1] About 200 million people develop malaria and almost 429,000 people die every year.[1] Relapse after treatment often occurs, and moreover, resistance to chloroquine, formerly a gold-standard antimalarial drug, has become epidemic.[1] Global warming is expected to expand the range of countries at risk. Therefore, development of new drugs effective against resistant *Plasmodium falciparum* is important.

The malaria parasite must escape the toxicity of free heme when it lives within the erythrocyte during its blood stage and digests hemoglobin, which contain large amounts of heme in the active site of hemoglobin.[2] To achieve this, heme molecules are bound to each other under acidic conditions in the parasites' vacuoles to form a heme assembly named hemozoin (Hz), which is harmless to the parasite, but harmful to humans. This Hz formation is considered to proceed via hemin dimerization (Figure 1: hemin dimer) mediated by proteins such as heme detoxification protein (HDP)[3-5] and also via a self-assembly process (Figure 1),[2, 6] although the parasite factors contributing to Hz formation remain a subject of debate. Quinoline-containing antimalarial drugs such as chloroquine are considered to block the fastest growing face of the Hz crystal by interacting with the surface or with free heme.[2, 7-9] The quinoline ring would interact with heme through π–π stacking forces; in addition, there would be an electrostatic interaction between the ammonium group of the drug and the carboxylate group of the heme.[2] However, known antimalarial drugs have not been specifically designed with this mechanism in mind. Here, we designed new quinolinic compounds intended to interact strongly with heme.

The synthesized compounds indeed showed potent antimalarial activity towards both a chloroquine-sensitive and a resistant *P. falciparum in vitro*.

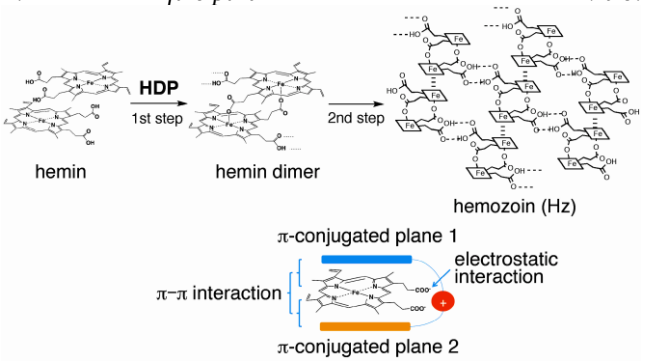
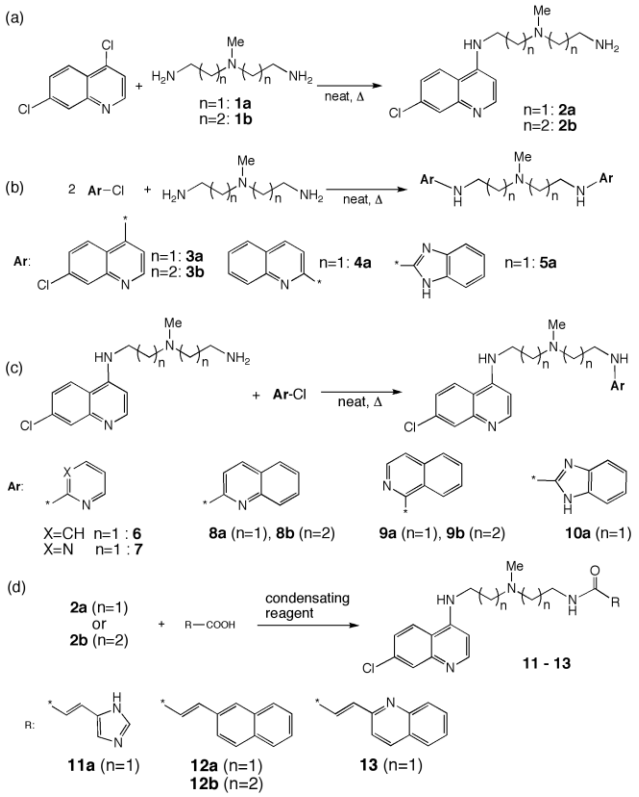


Figure 1. Hemozoin (Hz) formation, and molecular design of inhibitors of hemin self-assembly as candidate antimalarial agents.

π-Electron-conjugated planar molecules interact with heme via π–π stacking, so molecules with two planar moieties should have greater affinity for heme than those with just one. As shown in Figure 1, we designed compounds with two condensed heteroaromatic rings conjugated with terminal amino groups of N^{n+3}-methyl[1, n+3, 2n+5]triazaheptane (n=1: **1a**) or triazanonane (n=2: **1b**). The compounds bearing two planar scaffolds are expected to interact with heme by pinching (Figure 1). Some symmetric bis-quinoline-type compounds have already been reported to have relatively potent antimalarial activity, including towards chloroquine-resistant strains.[10-21] However, compounds designed to form simultaneous π–π interactions with two bicyclic aromatics as well as electrostatic interaction with heme in a host-guest manner have not been reported, although several compounds were designed considering π–π interaction with heme.[16, 22-24] Also, reported molecules bearing two planar scaffolds are mostly symmetric, probably for reasons of synthetic convenience.[25] We synthesized various molecules, including asymmetric ones, based on the molecular design described above. Here we report that these compounds bearing two planar scaffolds show potent antimalarial activity *in vitro* and *in vivo*, and also exhibit protective activity against hemin degradation and HDP-inhibitory activity.

Scheme 1. Synthetic scheme of two planar scaffolds linked



with triamines (**3**-**10**) and α-acyltriamine-type compounds **11**-**13**

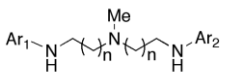
Triamines **1a** and **1b** (Scheme 1a) having two primary amino groups and one tertiary amino group were adopted as basic skeletons and two planar moieties were attached to the primary amino groups of the triamines. The tertiary amino group takes ammonium form in the vacuoles of malaria parasites (pH ~5) and should interact strongly with carboxylates of hemin through Coulombic force.

We prepared 4-(7-chloroquinolyl)-bearing triamines **2a** and **2b** as common intermediates (Scheme 1a) by the reaction of triamines **1a** or **1b** with 4,7-dichloroquinoline. Symmetric compounds **3a**, **3b**, **4a** and **5a** with two planar moieties were simply prepared as shown in Scheme 1b (Ar series). Compound **3a** was originally developed by us as an antimalarial agent,[26] and was recently reported to be a potent inhibitor of autophagy.[27]

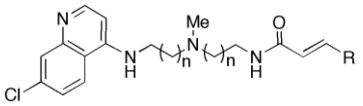
Next, two series of compounds having asymmetric structure were prepared (Schemes 1c, 1d and 1e). Only a few asymmetric quinolinic compounds have previously been reported.[28-31] We mainly adopted 7-chloroquinoline-attached triamines **2a** and **2b** as common structures. The primary amino group on **2** was directly conjugated with chlorinated Ar to afford compounds **6** – **10** (Schemes 1c). Carboxylic acid-bearing π-conjugates planar molecules were condensed with compound **2a** or **2b** to afford **11** - **13** having an amide bond (Schemes 1d and 1e).

All the compounds having two π-conjugated planes (**3**-**13**) were evaluated *in vitro* for antimalarial activity against the K1 (chloroquine-resistant) and FCR-3 (chloroquine-sensitive) strains of *P. falciparum* according to the procedure reported in the literature, and the data are presented in Table 1 (triamine-type compounds and α-acyltriamine-type compounds). Compounds not having a 7-chloroquinolin-4-yl group generally showed almost no antimalarial activity (**4a** and **5a**). These results indicate the importance of the 7-chloroquinolin-4-yl group for the activity. Compounds with a monocyclic aromatic group, **6** and **7**, showed poor antimalarial activities towards both strains. On the other hand, most of the compounds having a bicyclic aromatic group (**3**, **8**, **9**, **12**) exhibited much higher activity than the monocyclic compounds. Especially, **3a**, **3b**, **Nor-3a**, **8b**, **12a** and **12b** showed potent activity against both strains. It is noteworthy that the 6 compounds were about 10 times more effective against the chloroquine-resistant strain (K1) than chloroquine itself. There was little difference in inhibitory activity or toxicity between **3a** and **Nor-3a**. Compound **8b** showed potent activity against FCR-3 strain (EC_{50}: 0.92 nM), although its structure resembles that of **3b** (EC_{50}: 97 nM). The toxicity of the compounds toward human cells was also evaluated using MRC-5 cells (human embryonic lung-derived fibroblast cells). In general, triazaheptane-type compounds (n=1) were less toxic than triazanonane-type ones (n=2). Compound **12a** showed low toxicity to human cells and its selective toxicity (for K1 versus MRC-5) was the highest among the compounds tested. We prepared analog of **12a** for structure optimization and evaluated their antimalarial activity (**13**) (Table 1). However, the activity and selectivity did not exceed those of **12a**.

Table 1. *In vitro* Antimalarial activity and cytotoxicity of triamine-type compounds **3**-**10** and α-acyltriamine-type compounds **11**-**13**



compounds **3**-**10**



compounds **11**-**13**

Comp.	Ar_1	Ar_2	n	IC_{50} (nM) K1	IC_{50} (nM) FCR-3	Cytotoxicity MRC-5 (nM)	Selectivity Index MRC-5/K1
3a	CQ	CQ	1	45	20	8800	200
Nor-3a	CQ	CQ	1	44	37	14000	320
3b	CQ	CQ	2	21	97	1400	66
4a	Q	Q	1	18000	21000	310000	17
5a	BzIm	BzIm	2	19000	17300	90000	4.7
6	CQ	Py	1	1100	510	470000	430
7	CQ	Pym	1	750	210	30000	40
8a	CQ	Q	1	370	29	9500	36
8b	CQ	Q	2	37	0.92	2200	61
9a	CQ	IQ	1	270	24	9500	36
10a	CQ	BzIm	1	3100	230	26000	8.5
11a	R=4-imidazolyl		1	18000	750	> 250000	> 14
12a	2-naphthyl		1	28	12	34000	1200
12b	2-naphthyl		2	10	4.9	1400	140
13	2-quinolinyl		1	870	76	5300	6.0
chloroquin				570	46	57000	100

CQ: 7-chloro-4-quinolyl; Q: 2-quinolyl; BzIm: 2-benzimidazolyl; Py: 2-pyridyl; Pym: 2-pyrimidyl; IQ: 1-isoquinolyl. **Nor-3a**: *N*-demethylated form of **3a**.

We examined the *in vivo* antimalarial activity of compounds **3a** and **12a**, which had shown especially good results *in vitro*. Compounds **3a** and **12a** were orally (p.o.) or subcutaneously (s.c.) administered to *P. berghei*-infected mice. Artesunate, which is one of principal clinically used antimalarial drugs, was tested as a positive control. Both **3a** (both p.o. and s.c.) and **12a** (s.c. only) (30 mg/kg/day, 4 times) completely eliminated the malaria parasites from the mouse body (Table 2). On the other hand, a few parasites still remained in the case of artesunate. That is to say, the parasite-inhibitory effects of **3a** and **12a** are almost equivalent to that of artesunate, since the molar amounts of **3a** (68.3 μmol/kg) and **12a** (65.3 μmol/kg) used were larger than that of artesunate (39.0 μmol/kg). The reported *in vivo* therapeutic efficacy of chloroquine seems to be better than those of **3a** and **12a**.[32] However, **3a** and **12a** would be superior to chloroquine against chloroquine-resistant strains (the *P. berghei* strain is chloroquine-sensitive).

Table 2. *In vivo* antimalarial activity of **3a**, **12a** and artesunate on *P. berghei*-infected mice

Comp.	Dosage (mg/kg), route	Inhibition (%)	Survival (%)
3a	30 x 4, s.c.	100	>284.6*
3a	30 x 4, p.o.	99.5	115.4
12a	30 x 4, s.c.	100	223.1
12a	30 x 4, p.o.	23.5	100
artesunate	15 x 4, p.o.	95.2	102.6

>284.6*:one mice of **3a** treatment group was still survive without malaria parasites on day 35.

We also examined the duration of the pharmacological effect of the test compounds by observing prolongation of life of mice after the end of administration (Table 2, Figure S1). Mean of survival days in control group were 7.8 days and artesunate-, **3a**-, and **12a**-administered group (p.o. treatment) were dead within 9 days. On the other hand, administration of **3a** or **12a** (s.c. treatment) prolonged the lives of the mice by 15-35 days. Especially, subcutaneously inject in to mice of **3a**, one mice of them was survive without malaria parasite in the mice. This result indicates that **3a** and **12a** are superior to artesunate not only in pharmacological activity but also in the duration of antimalarial effect. Compounds **3a** and **12a** are thus considered promising lead compounds for drugs to prevent malarial relapse.

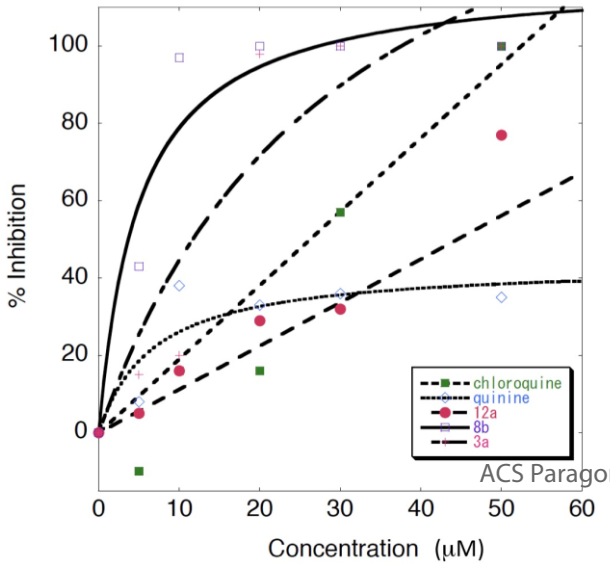
The Clog P values of chloroquine (free base) and primaquine are reported to be 5.06 and 2.6, respectively.[33] Calculated Clog P values of **3a**, **8b**, **12a** (free base) were 5.09, 5.08 and 5.36,[34] which are slightly higher than that of chloroquine. However, at least each aliphatic amino group of the prepared compounds should be protonated under physiological conditions. For example, the Clog P value of mono-protonated **12a** was calculated to be 2.2, which is an appropriate value from the viewpoint of bioavailability.

ITC is an excellent method for evaluation of affinity between two compounds, because the binding constant, two thermodynamic parameters and binding ratio can be obtained simultaneously. The interactions of chloroquine with hemin[35] or hemin μ-oxo dimer[36-37] have been determined by ITC. Here, we examined the affinity between the synthesized compounds and hemin by using ITC after adjusting the pH to 5.2, which is close to that of the malaria parasites' vacuoles.[38] Table S1 (Supporting Information) shows the results. Contrary to our expectation, the differences among **12a**, **8b** and chloroquine in terms of binding constant, ΔH and $T\Delta S$ were not large. There did not appear to be a clear relationship between the antimalarial activity and the binding constant.

Hemin reacts with H_2O_2 to form hydroxyl radical (•OH) and oxo-Fe^{IV} heme via O-O bond homolysis, and •OH and other oxy radical species attack the hemin, causing degradation accompanied by

bleaching.[39] Therefore, we used the H_2O_2-mediated hemin degradation system to evaluate the protective effect of quinolinic compounds at pH 5.2.[3] As shown in Figure 2, chloroquine had moderate protective effect and quinine showed almost no effect. In contrast, compounds having two π-conjugated planar moieties had much higher protective activities than chloroquine. These results strongly suggest that these molecules have high affinity for hemin. The order of the inhibitory potency is as follows: **12a** > **8b** > **3a**> chloroquine >> quinine.

DFT calculation was carried out for antimalarial compound–hematin (HO^- coordinated ferric heme) complex in order to clarify the correlation between complex formation equilibrium and antimalarial activity. We chose compounds **8a** and **8b** for comparison and a hematin (dianion form) with one **8a** or **8b** molecule (dication form) was set for calculation. Geometry optimization (function set: ωB97X-D / 6-31G*) successfully converged in both cases (Table S3). Calculated formed heat in complexation of hematin with **8b** was more than that with **8a** with shorter methylene chains (0.0324 Hartree (85.0 kJ/mol) difference). This result matches that antimalarial activity of **8b** was much higher than that of **8a**. The optimized shape of hematin-8b complex looks like pincer type one (Figure S3). Next, we examined the complexation with hemin dimer (β-hematin). Calculation in the case of hemin dimer **8b** successfully converged (Table S4), but calculation in the case of **8a** did not converge in spite of several trials. As shown in Figure S3, the optimized structure of hemin dimer **8b** complex was greatly transformed from the original form of hemin dimer. This complexation would contribute to the prevention of hemozoin formation.



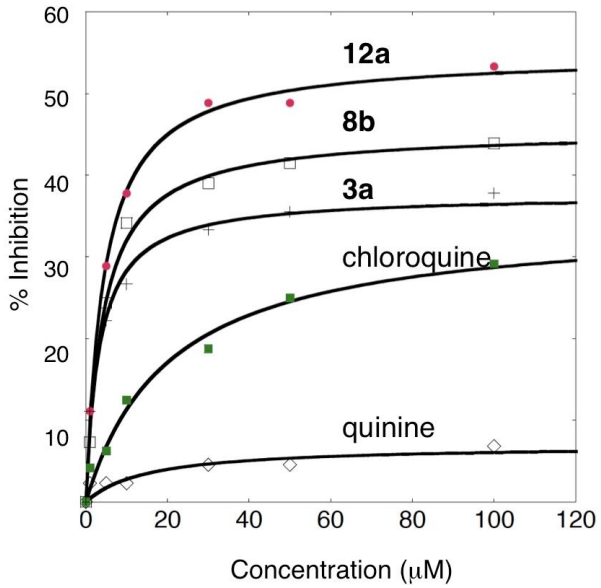


Figure 2. Protective effect of antimalarial compounds against degradation of hemin by H_2O_2.

HDP has a critical role in dimerization of hemin in the food vacuoles of malaria parasites to generate hemozoin.[3, 40-41] Therefore, we next examined the inhibitory effect of our compounds on the production of hemozoin by HDP. HDP was obtained by expression in *Escherichia coli* and subsequent purification. The amount of HDP-produced hemozoin

Figure 3. Inhibitory effect of antimalarial compounds on hemozoin formation catalyzed by heme detoxification protein (HDP). IC_{50} against FCR-3 strain: Compound **3a** (20 nM), **8b** (0.92 nM), **12a** (12 nM), chloroquine (46 nM).

was determined by absorption spectrometry of an alkaline solution of isolated hemozoin. Concentration-dependent inhibition by the test compounds is shown in Figure 3. Compounds **8b** and **3a** were much more potent inhibitors than quinine and chloroquine, and **8b** showed very high inhibitory activity at low concentration. Hence, the extremely high antimalarial activity (FCR-3) of **8b** may be explained mainly in terms of this potent HDP inhibition. Compound **12a** was the most potent protector of hemin decomposition by H_2O_2, whereas it was only an intermediate-strength inhibitor of HDP-catalyzed hemozoin formation. These results strongly suggest that the antimalarial effect of the compounds we developed is due not only to interference with the self-assembly of heme dimer by direct interaction with hemin, but also to inhibition of heme dimerization by HDP. Hence, the antimalarial activity of the developed compounds involves at least these two mechanisms.

In summary, we designed and synthesized a new series of antimalarial compounds based on the idea that high affinity for heme would block hemozoin polymer formation in malaria parasites. Compounds with a 7-chloroquinolin-4-yl group and another π-conjugated moiety at the two terminal amino groups of triamine **1a** or **1b** generally exhibited moderate to high antimalarial activities in vitro. Especially, **3a**, **3b**, **Nor-3a**, **8b**, **12a** and **12b** had potent activities, with EC_{50} toward a chloroquine-resistant strain (K1) reaching the 10^{-8} M level. It is well established that 4-amino-7-chloroquinolines bearing a basic side-chain are strongly active

against malaria parasites. However, there has been no explicit attempt to design molecules with two π-stacking groups that can pincer heme. On the other hand, there is recent evidence that quinoline-type drugs may inhibit Hz formation by binding the surfaces of the crystal.[9] An alternative mechanism has also been suggested, i.e., that these compounds could bind to more than one surface site on the Hz crystal. Compounds **3a** and **12a** showed more potent *in vivo* antimalarial activity than artesunate in s.c. treatment, and provided longer-lasting protection against *P. berghei*-infected mice. The plot of protective activity against hemin degradation by H_2O_2 versus antimalarial activity showed a good correlation, supporting the validity of our molecular design concept. However, the order of inhibitory activity on HDP-catalyzed hemozoin formation was somewhat different from that of protective activity against hemin degradation. This result indicates that the effect on HDP catalysis is also significant. These findings results should contribute to the development of antimalarial drugs with excellent activity against drug-resistant malaria.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental data of synthesis, experimental methods of assays, ITC, Suppressing effect of **3a** or **12a**, curve fitting data for Figure 2(a), DFT calculations (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

ITC: isothermal titration calorimetry. DFT: density functional theory.

REFERENCES

1. Centers for Disease Control and Prevention (CDC) http://www.cdc.gov/malaria/.
2. Weissbuch, I.; Leiserowitz, L., Interplay Between Malaria, Crystalline Hemozoin Formation, and Antimalarial Drug Action and Design. *Chem. Rev.* **2008,** *108* (11), 4899-4914.
3. Jani, D.; Nagarkatti, R.; Beatty, W.; Angel, R.; Slebodnick, C.; Andersen, J.; Kumar, S.; Rathore, D., HDP—A Novel Heme Detoxification Protein from the Malaria Parasite. *PLoS Pathog.* **2008,** *4* (4), e1000053.
4. Chugh, M.; Sundararaman, V.; Kumar, S.; Reddy, V. S.; Siddiqui, W. A.; Stuart, K. D.; Malhotra, P., Protein complex directs hemoglobin-to-hemozoin formation in <em>Plasmodium falciparum</em&gt. *Proc. Natl. Acad. Sci. USA* **2013,** *110* (14), 5392.
5. Egan, T. J., Recent advances in understanding the mechanism of hemozoin (malaria pigment) formation. *J. Inorg. Biochem.* **2008,** *102* (5), 1288-1299.
6. Kumar, S.; Guha, M.; Choubey, V.; Maity, P.; Bandyopadhyay, U., Antimalarial drugs inhibiting hemozoin (β-hematin) formation: A mechanistic update. *Life Sci.* **2007,** *80* (9), 813-828.
7. Buller, R.; Peterson, M. L.; Almarsson, Ö.; Leiserowitz, L., Quinoline Binding Site on Malaria Pigment Crystal: A Rational Pathway for Antimalaria Drug Design. *Cryst. Growth Des.* **2002,** *2* (6), 553-562.
8. Ketchum, M. A.; Lee, A. M.; Vekilov, P. G.; Rimer, J. D., Biomimetic Assay for Hematin Crystallization Inhibitors: A New Platform To Screen Antimalarial Drugs. *Cryst. Growth Des.* **2017,** *17* (1), 197-206.
9. Olafson, K. N.; Nguyen, T. Q.; Rimer, J. D.; Vekilov, P. G., Antimalarials inhibit hematin crystallization by unique drug–surface site interactions. *Proc. Natl. Acad. Sci. USA* **2017,** *114* (29), 7531.
10. Raynes, K., Bisquinoline antimalarials: their role in malaria chemotherapy. *Int. J. Parasitol.* **1999,** *29* (3), 367-379.
11. Davis, T.; Hung, T.-Y.; Sim, I.-K.; Karunajeewa, H.; Ilett, K., *Piperaquine: A Resurgent Antimalarial Drug*. 2005; Vol. 65, p 75-87.
12. Raynes, K.; Galatis, D.; Cowman, A. F.; Tilley, L.; Deady, L. W., Synthesis and Activity of Some Antimalarial Bisquinolines. *J. Med. Chem.* **1995,** *38* (1), 204-206.
13. Vennerstrom, J. L.; Ellis, W. Y.; Ager, A. L.; Andersen, S. L.; Gerena, L.; Milhous, W. K., Bisquinolines. 1. N,N-bis(7-chloroquinolin-4-yl)alkanediamines with potential against chloroquine-resistant malaria. *J. Med. Chem.* **1992,** *35* (11), 2129-2134.
14. Vennerstrom, J. L.; Ager, A. L.; Dorn, A.; Andersen, S. L.; Gerena, L.; Ridley, R. G.; Milhous, W. K., Bisquinolines. 2. Antimalarial N,N-Bis(7-chloroquinolin-4-yl)heteroalkanediamines. *J. Med. Chem.* **1998,** *41* (22), 4360-4364.
15. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Lemière, P.; Mouray, E.; Davioud-Charvet, E.; Sergheraert, C., Antiplasmodial Activity and Cytotoxicity of Bis-, Tris-, and Tetraquinolines with Linear or Cyclic Amino Linkers. *J. Med. Chem.* **2001,** *44* (11), 1658-1665.
16. Dascombe, M. J.; Drew, M. G. B.; Morris, H.; Wilairat, P.; Auparakkitanon, S.; Moule, W. A.; Alizadeh-Shekalgourabi, S.; Evans, P. G.; Lloyd, M.; Dyas, A. M.; Carr, P.; Ismail, F. M. D., Mapping Antimalarial Pharmacophores as a Useful Tool for the Rapid Discovery of Drugs Effective in Vivo: Design, Construction, Characterization, and Pharmacology of Metaquine. *J. Med. Chem.* **2005,** *48* (17), 5423-5436.
17. Aguiar, A. C. C.; Santos, R. d. M.; Figueiredo, F. J. B.; Cortopassi, W. A.; Pimentel, A. S.; França, T. C. C.; Meneghetti, M. R.; Krettli, A. U., Antimalarial Activity and Mechanisms of Action of Two Novel 4-Aminoquinolines against Chloroquine-Resistant Parasites. *PLoS One* **2012,** *7* (5), e37259.

18. van Heerden, L.; Cloete, T. T.; Breytenbach, J. W.; de Kock, C.; Smith, P. J.; Breytenbach, J. C.; N'Da, D. D., Synthesis and in vitro antimalarial activity of a series of bisquinoline and bispyrrolo[1,2a]quinoxaline compounds. *Eur. J. Med. Chem.* **2012,** *55*, 335-345.

19. Ayad, F.; Tilley, L.; Deady, L. W., Synthesis, antimalarial activity and inhibition of haem detoxification of novel bisquinolines. *Bioorg. Med. Chem. Lett.* **2001,** *11* (16), 2075-2077.

20. Ismail, F. M. D.; Dascombe, M. J.; Carr, P.; MÉRette, S. A. M.; Rouault, P., Novel Aryl-bis-quinolines with Antimalarial Activity In-vivo. *J. Pharm. Pharmacol.* **1998,** *50* (5), 483-492.

21. Ismail, F. M. D.; Dascombe, M. J.; Carr, P.; North, S. E., An Exploration of the Structure-activity Relationships of 4 − Aminoquinolines: Novel Antimalarials with Activity In-vivo. *J. Pharm. Pharmacol.* **1996,** *48* (8), 841-850.

22. Joshi, M. C.; Okombo, J.; Nsumiwa, S.; Ndove, J.; Taylor, D.; Wiesner, L.; Hunter, R.; Chibale, K.; Egan, T. J., 4-Aminoquinoline Antimalarials Containing a Benzylmethylpyridylmethylamine Group Are Active against Drug Resistant Plasmodium falciparum and Exhibit Oral Activity in Mice. *J. Med. Chem.* **2017,** *60* (24), 10245-10256.

23. Gorka, A. P.; de Dios, A.; Roepe, P. D., Quinoline Drug–Heme Interactions and Implications for Antimalarial Cytostatic versus Cytocidal Activities. *J. Med. Chem.* **2013,** *56* (13), 5231-5246.

24. Warhurst, D. C.; Craig, J. C.; Adagu, I. S.; Guy, R. K.; Madrid, P. B.; Fivelman, Q. L., Activity of piperaquine and other 4-aminoquinoline antiplasmodial drugs against chloroquine-sensitive and resistant blood-stages of Plasmodium falciparum: Role of β-haematin inhibition and drug concentration in vacuolar water- and lipid-phases. *Biochem. Pharmacol.* **2007,** *73* (12), 1910-1926.

25. Mishra, M.; Mishra, V. K.; Kashaw, V.; Iyer, A. K.; Kashaw, S. K., Comprehensive review on various strategies for antimalarial drug discovery. *Eur. J. Med. Chem.* **2017,** *125*, 1300-1320.

26. Higuchi, T.; Omiya, H.; Umezawa, N.; Kim, H. S.; Wataya, Y., Compound with antimalarial activity and antimalarial drug containing the same as active ingredient. Google Patents: Eur Patent Appl 07737389.2, Publ WO 2007/097450 (30.08.2007 Gazette 2007/35). 2007.

27. McAfee, Q.; Zhang, Z.; Samanta, A.; Levi, S. M.; Ma, X.-H.; Piao, S.; Lynch, J. P.; Uehara, T.; Sepulveda, A. R.; Davis, L. E.; Winkler, J. D.; Amaravadi, R. K., Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc. Natl. Acad. Sci. USA* **2012,** *109* (21), 8253.

28. Kumar, A.; Srivastava, K.; Raja Kumar, S.; Puri, S. K.; Chauhan, P. M. S., Synthesis of new 4-aminoquinolines and quinoline–acridine hybrids as antimalarial agents. *Bioorg. Med. Chem. Lett.* **2010,** *20* (23), 7059-7063.

29. Lödige, M.; Lewis, M. D.; Paulsen, E. S.; Esch, H. L.; Pradel, G.; Lehmann, L.; Brun, R.; Bringmann, G.; Mueller, A.-K., A primaquine–chloroquine hybrid with dual activity against Plasmodium liver and blood stages. *Int. J. Med. Microbiol.* **2013,** *303* (8), 539-547.

30. Kondaparla, S.; Agarwal, P.; Srivastava, K.; Puri, S. K.; Katti, S. B., Design, synthesis and in vitro antiplasmodial activity of some bisquinolines against chloroquine-resistant strain. *Chem. Biol. Drug Des.* **2017,** *89* (6), 901-906.

31. Singh, K.; Kaur, H.; Smith, P.; de Kock, C.; Chibale, K.; Balzarini, J., Quinoline–Pyrimidine Hybrids: Synthesis, Antiplasmodial Activity, SAR, and Mode of Action Studies. *J. Med. Chem.* **2014,** *57* (2), 435-448.

32. Basilico, N.; Parapini, S.; Sparatore, A.; Romeo, S.; Misiano, P.; Vivas, L.; Yardley, V.; Croft, L. S.; Habluetzel, A.; Lucantoni, L.; Renia, L.; Russell, B.; Suwanarusk, R.; Nosten, F.; Dondio, G.; Bigogno, C.; Jabes, D.; Taramelli, D., In Vivo and In Vitro Activities and ADME-Tox Profile of a Quinolizidine-Modified 4-Aminoquinoline: A Potent Anti-P. falciparum and Anti-P. vivax Blood-Stage Antimalarial. *Molecules* **2017,** *22* (12).

33. Wan, H.; Åhman, M.; Holmén, A. G., Relationship between Brain Tissue Partitioning and Microemulsion Retention Factors of CNS Drugs. *J. Med. Chem.* **2009,** *52* (6), 1693-1700.

34. http://www.molinspiration.com/cgi-bin/properties.

35. Vippagunta, S. R.; Dorn, A.; Bubendorf, A.; Ridley, R. G.; Vennerstrom, J. L., Deferoxamine: Stimulation of hematin polymerization and antagonism of its inhibition by chloroquine. *Biochem. Pharmacol.* **1999,** *58* (5), 817-824.

36. Vippagunta, S. R.; Dorn, A.; Matile, H.; Bhattacharjee, A. K.; Karle, J. M.; Ellis, W. Y.; Ridley, R. G.; Vennerstrom, J. L., Structural Specificity of Chloroquine−Hematin Binding Related to Inhibition of Hematin Polymerization and Parasite Growth. *J. Med. Chem.* **1999,** *42* (22), 4630-4639.

37. Vippagunta, S. R.; Dorn, A.; Ridley, R. G.; Vennerstrom, J. L., Characterization of chloroquine-hematin μ-oxo dimer binding by isothermal titration calorimetry. *Biochim. Biophys. Acta* **2000,** *1475* (2), 133-140.

38. Krogstad, D. J.; Schlesinger, P. H.; Gluzman, I. Y., Antimalarials increase vesicle pH in Plasmodium falciparum. *J. Cell Biol.* **1985,** *101* (6), 2302.

39. Mullié, C.; Jonet, A.; Desgrouas, C.; Taudon, N.; Sonnet, P., Differences in anti-malarial activity of 4-aminoalcohol quinoline enantiomers and investigation of the presumed underlying mechanism of action. *Malar. J.* **2012,** *11* (1), 65.

40. Nakatani, K.; Ishikawa, H.; Aono, S.; Mizutani, Y., Heme-binding properties of heme detoxification protein from Plasmodium falciparum. *Biochem. Biophys. Res. Commun.* **2013,** *439* (4), 477-480.

41. Nakatani, K.; Ishikawa, H.; Aono, S.; Mizutani, Y., Identification of Essential Histidine Residues Involved in Heme Binding and Hemozoin Formation in Heme Detoxification Protein from Plasmodium falciparum. *Sci. Rep.* **2014,** *4*, 6137.

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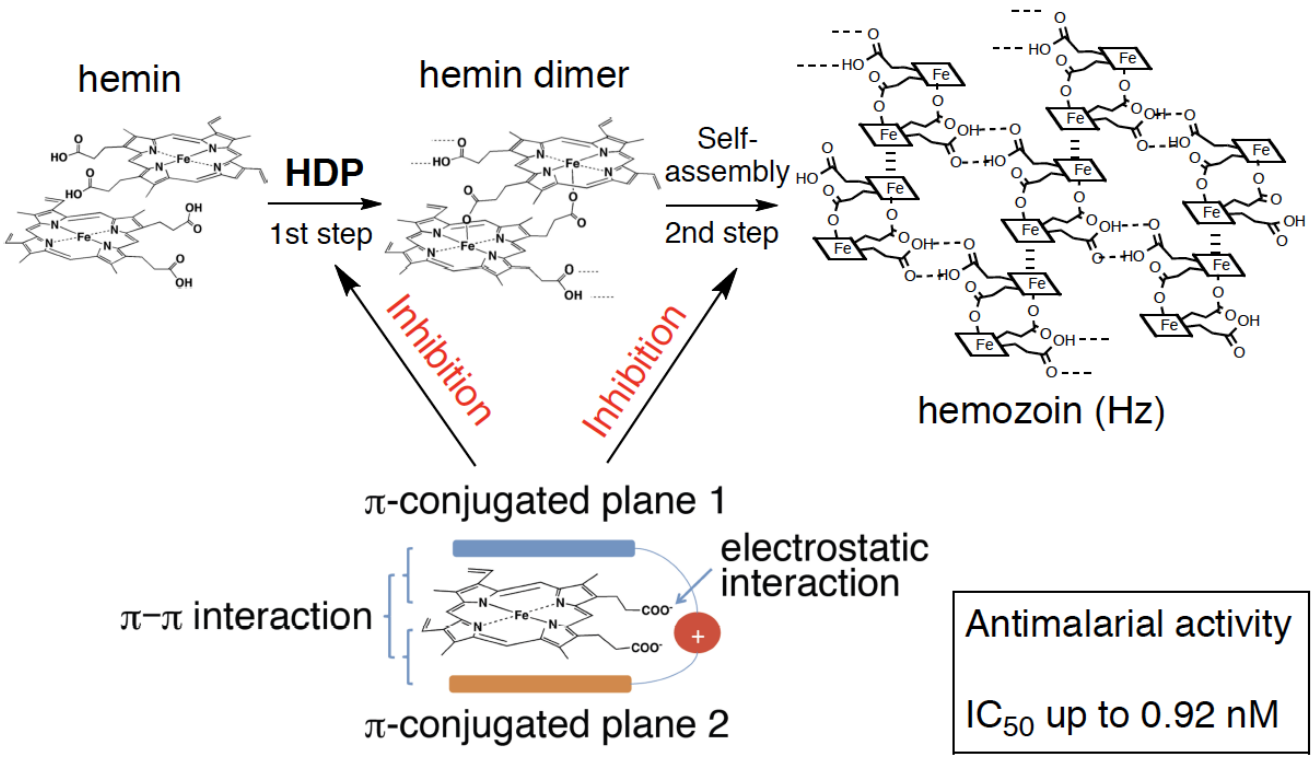

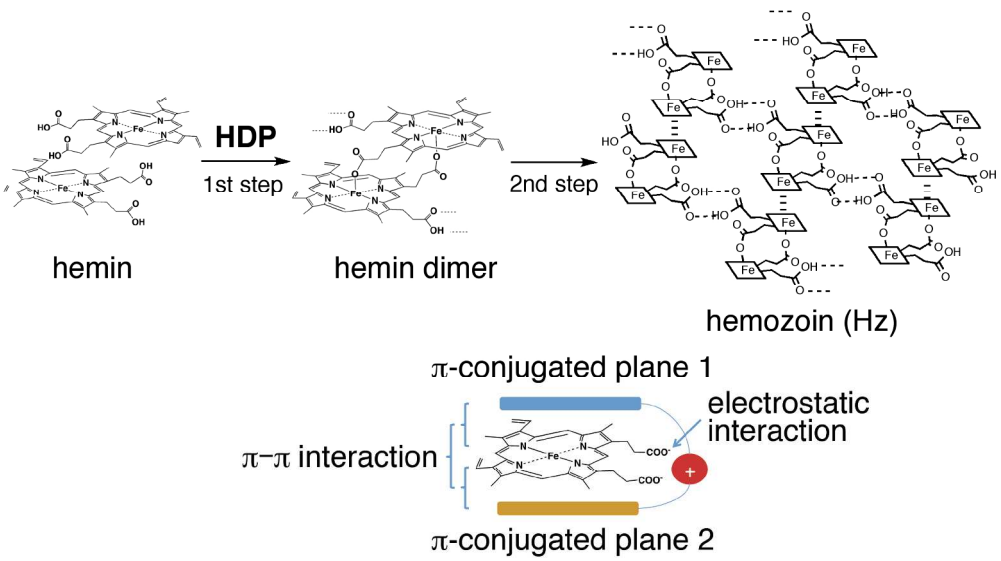

Figure 1. Hemozoin (Hz) formation, and molecular design of inhibitors of hemin self-assembly as candidate antimalarial agents.

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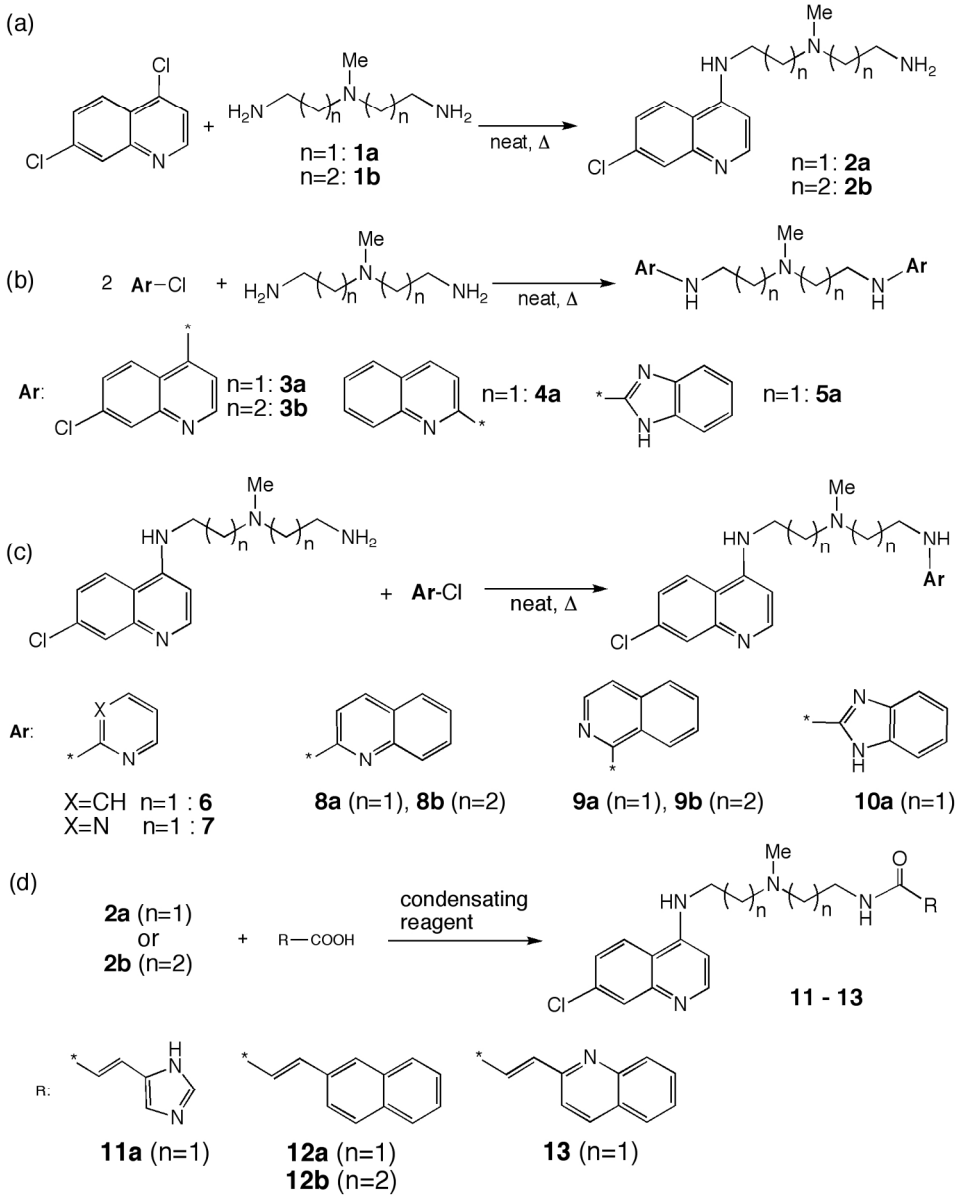

Scheme 1. Synthetic scheme of bis-planar triamines (**3**-**10**) and α-acyltriamine-type compounds **11**-**13**.

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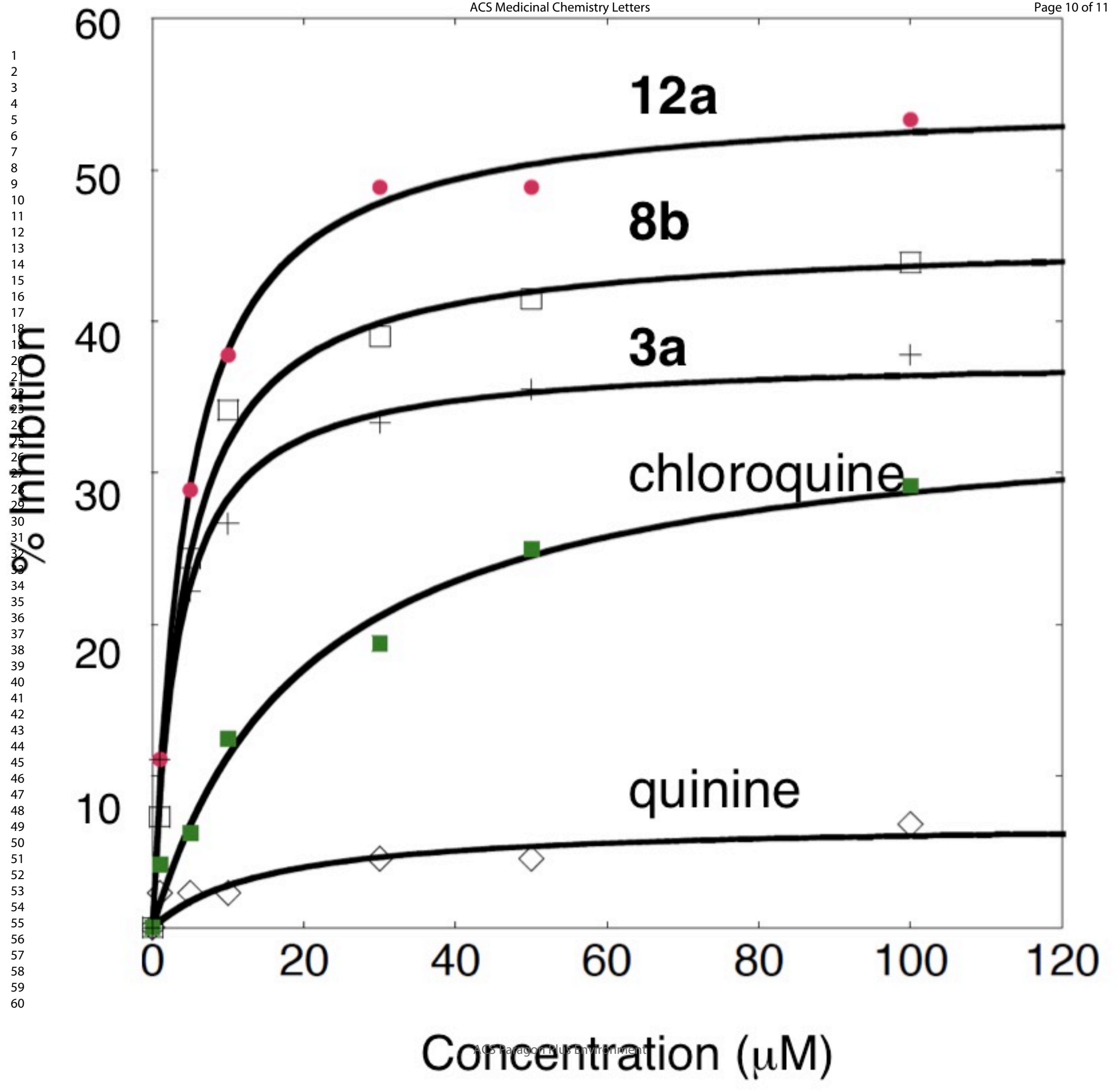

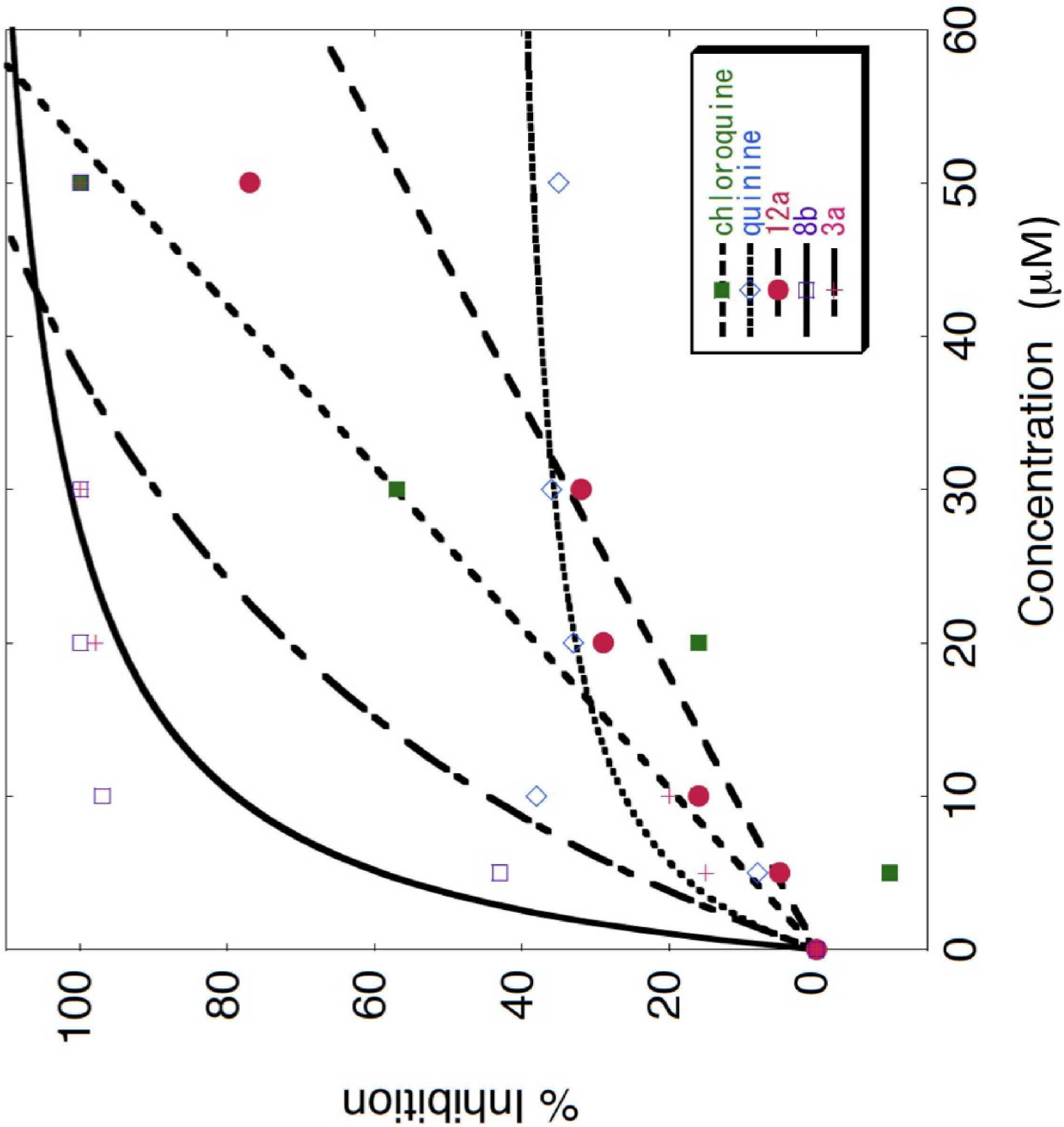
% Inhibition
100
80
60
40
20
0
Concentration (μM)
0
10
20
30
40
50
60
chloroquine
quinine
12a
8b
3a