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Tambjamines as Fast-Acting Multistage Antimalarials

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Detailed biological experimental procedures, and ^{1}H NMR spectra and HRMS of all target compounds and HPLC chromatograms of the key compounds (PDF).

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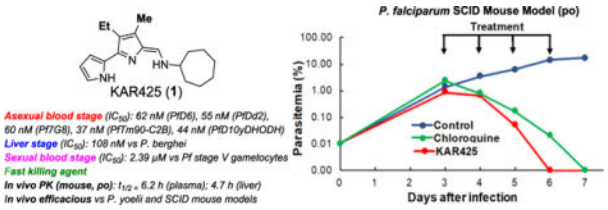
Abstract

Well-tolerated and novel antimalarials that can combat multiple stages of the parasite life cycle are desirable but challenging to discover and develop. Herein, we report results for natural product inspired novel tambjamine antimalarials. We show that they are potent against liver, asexual erythrocytic, and sexual erythrocytic parasite life cycle stages. Notably, our lead candidate **1** (KAR425) displays excellent oral efficacy with complete clearance of parasites within 72 h of treatment in the humanized *Plasmodium falciparum* (NOD-scid) mouse model at 50 mg/kg × 4 days. Profiling of compound **1** demonstrated a fast *in vitro* killing profile. In addition, several other tambjamine analogues cured erythrocytic *Plasmodium yoelii* infections after oral doses of 30 and 50 mg/kg × 4 days in a murine model while exhibiting good safety and metabolic profiles. This study presents the first account of multiple-stage antiplasmodial activities with rapid killing profile in the tambjamine family.

Graphical Abstract



Keywords

antimalarials; antiplasmodial; fast-acting; multistage; natural products; tambjamines

Malaria is estimated to cause over 200 million clinical cases and claim over 600,000 lives each year, mostly in children under the age of five.[1] Full or partial drug resistance to current therapies, specifically quinolines, antifolates, artemisinin derivatives, and other artemisinin partner drugs, is of great concern.[2–6] With increasing multi-drug resistance (MDR) to currently available antimalarials and the spread of insecticide-resistant vectors,[7] there is an urgent need for novel, affordable, effective, and well-tolerated drugs for the prevention and treatment of malaria.

Over the past several decades, natural products had an extensive history as pioneering agents for drug development.[8–10] In particular, many of the promising antimalarials known to date, such as quinine, chloroquine (CQ), artemisinin (ART), dihydroartemisinin (DHA), artemether and artesunate (AS), are natural products or their derivatives. Recently, we discovered and developed tambjamine (TA) and prodigiosin/prodiginine (PG) natural and synthetic products as a novel class of orally efficacious antimalarial agents.[11–14] Our previous work showed that many of the natural and synthetic TAs and PGs were equally effective against a panel of *P. falciparum* pan-sensitive and MDR strains at low nanomolar concentrations, suggesting the potential to discover new drugs to treat malaria. A number of these novel TAs and PGs provided curative *in vivo* efficacy against murine *Plasmodium yoelii* malaria, with 25 or 50 mg/kg administered daily for 4 days.[11, 14] Notably, a TA analogue, KAR425 (**1**, Scheme 1), provided cure (with day 28 follow-up) in 2/4 mice with a single oral dose of 80 mg/kg.[11] In addition, structure-activity relationship (SAR) explorations of this novel class of scaffolds led us to a robust understanding of the structural features that are required for potent antiplasmodial activity with a feasible therapeutic index (TI).

The intriguing structural features and promising antiplasmodial activities of these novel PG and TA chemotypes against asexual blood-stage malaria parasites spurred us to investigate activity against other life-cycle stages. In the current work, we report the antiplasmodial activities of selected lead TAs against liver-stage and sexual blood-stage malaria parasites. In addition, we report extensive metabolic and pharmacokinetic (PK) profiles, *in vivo* efficacy in two different mouse models, *ex vivo* activity against *P. falciparum* clinical isolates, *in vitro* parasite killing rates, and safety profiles of the lead TAs.

RESULTS AND DISCUSSION

Chemistry.

Target TAs **1**–**14** were synthesized in good to excellent yields as previously described in our publications.[11, 15] The general synthesis of key bipyrrole-carboxaldehydes **III** and TAs **1**–**14** using various substituted 5-bromo-pyrrole-2-carboxaldehydes **I**, commercially available *N*-Boc-2-pyrroleboronic acids (**II**), and alkyl/cycloalkyl amines **IV** is outlined in Scheme 1.

In Vitro Liver-Stage Antiplasmodial Activity against *Plasmodium berghei*.

The *in vitro* liver-stage activity of four selected TAs **1**–**4**, which previously showed good potency against asexual blood-stage malaria parasites,[11] was assessed utilizing luciferase-expressing *P. berghei* sporozoite infected human hepatocyte HepG2 cells,[16] and the results are summarized in Table 1. The selection of these promising TAs was based on the diversity of chemical structures (Scheme 1) and their potency against blood-stage malaria parasites. Significantly, all of the tested TAs exhibited potent activity against liver-stage parasites at low concentrations (IC_{50} = 0.0167–0.108 μM vs *P. berghei*).

In Vitro Antiplasmodial Activity against Sexual Blood-Stage Parasites.

To assess the transmission blocking potential of TAs, compounds **1**–**4** were tested with a gametocytocidal assay using late-stage gametocytes, and the *P. falciparum* Dual Gamete

Formation Assay (*Pf* DGFA) which tests the functionality of male and female gametocytes by measuring their ability to form male and female gametes.[17, 18] All of the tested TAs inhibited the growth of *P. falciparum* stage V gametocytes at low micromolar concentrations (IC_{50} = 1.41–2.55 μM). Interestingly, after 24 h preincubation with mature gametocytes, TAs **2** and **4** moderately inhibited both male and female gamete formation at 1.0 μM (**2**: 50.5% vs male gametes, 50.0% vs female gametes; **4**: 84.2% vs male gametes, 39.5% vs female gametes), as shown in Table S1, supporting information. In a subsequent dose-response evaluation, these two TAs inhibited male gamete formation from the gametocyte stage with low micromolar IC_{50} values (**2**: IC_{50} = 1.04 μM and **4**: IC_{50} = 0.721 μM) (Table 1), suggesting that the TA chemotype is an inhibitor of the functional viability of male gametocytes with little activity against females. These results indicated that TAs not only exhibited promising asexual blood[11, 14] and liver (Table 1) stage antiplasmodial activities, but in addition demonstrated gametocyte-targeting activity indicative of blocking transmission to the mosquito. Synthesis and evaluation of a large library of novel TAs against blood and liver-stage parasites are currently in progress.

In Vitro Parasite Killing Rate.

The parasite killing profile of our lead candidate **1** was assessed using a double-colorimetric FACS *in vitro* parasite reduction ratio (PRR) assay with erythrocytes infected with *P. falciparum* 3D7 strain,[19] in the presence of drugs at a concentration corresponding to 10 × IC_{50} values. Samples of parasites were taken from compound treated cultures after 24 and 48 h. These PRR results demonstrated that compound **1** displayed a significant effect over parasite viability comparable to fast-acting antimalarial drugs CQ and AS (Figure 1). Our preliminary investigations unequivocally demonstrated that TA chemotype has the potential to kill the malaria parasite with highly-likely a fast rate.

In Vivo Asexual Blood-Stage Efficacy in the *P. falciparum* SCID Mouse Model.

Given the rapid killing profile and excellent *in vivo* blood-stage efficacy (ED_{50} = 0.09 mg/kg/d and ED_{90} = 1.1 mg/kg/d, Table 2) of compound **1** in both 4-day and 1-day rodent models against *P. yoelii*,[11] it was also evaluated in a 4-day test using a humanized mouse *P. falciparum* model.[20] Mice were infected at day 0 with *P. falciparum* strain $3D7^{0087/N9}$, and 3 days after infection, compound **1** was dosed (50 mg/kg) once a day for 4 consecutive days. Notably, at day 6 post-infection, compound **1** showed >99.9% parasitemia reduction compared to untreated control mice (Figure 2A), with the rate of *in vivo* parasite clearance faster than that of the reference drug CQ. One of the two mice in the treated group showed some adverse findings (including apathetic, balance impaired and diarrhea) 2 h after the first dose, so that the experiment was continued with only one mouse through day 7 without any adverse findings. The exact reason for adverse findings in one of the SCID mouse was unknow; however, it could be possible drug toxicity specifically to an immunocompromised mouse, or due to other routine manipulation and/or handling. It is noteworthy that no adverse findings were observed in any CF1 mice (n = 4) treated with compound **1** in the erythrocytic *P. yoelii* mouse model at various doses (Table 2).[11] In parallel, the blood concentration profile of compound **1** was also measured (Figure 2B). Collectively, from our previous work[11] and this study, TA analogue **1** demonstrated excellent *in vivo* efficacy in

both *P. yoelii* and humanized *P. falciparum* mouse models, and the efficacy of **1** was greater than that of CQ in both models.

In Vivo Asexual Blood-Stage Efficacy in the *P. yoelii* Mouse Model.

To identify more efficacious and safer antimalarial agents in the TA family, several TAs **5**–**13** that are structurally close to our lead candidate **1** (Scheme 1) were also evaluated in both 4-day and 1-day rodent models against *P. yoelii*[11, 14, 21], and the results are summarized in Table 2. TAs **5**–**13** were tested in this mouse model at 10, 25, 30 and 50 mg/kg once daily × 4 days of oral treatment. The animals were considered cured if they survived 28 days after the infection without detectable bloodstream parasites. Interestingly, most of the tested TAs exhibited excellent efficacy at 25, 30 and 50 mg/kg × 4 days of oral treatment with low ED_{50} and ED_{90} values (Table 2). Of these TAs, compounds **8**–**10** and **13** were curative (4/4 mice cured) at 50 mg/kg × 4 days of oral treatment (Table 2). Notably, two TAs **8** and **9** bearing ethyl substituents at the 3- and 4-positions on ring-B and 1-adamantyl and cyclooctyl moieties at the terminal amine, respectively, provided 100% protection (4/4 mice cured) at both 30 and 50 mg/kg × 4 days treatment. Most significantly, compound **8** provided complete protection with a single oral dose of 80 mg/kg (4/4 mice cured; Table 2). It is noteworthy that the *in vivo* asexual blood-stage antimalarial efficacy of **8** was superior to that of compound **1**. Collectively, the *in vivo* results and the SAR observations for these TAs demonstrated that the di-alkyl substitutions, specifically ethyl groups at the 3- and 4-positions on ring-B and large cycloalkyl moieties on the terminal amine of the TA scaffold (Scheme 1) have positive impacts on *in vivo* efficacy. During the *in vivo* experiments, all mice were observed daily for mortality/morbidity and clinical signs of toxicity. No overt clinical toxicity or behavioral changes were observed in any of the mice treated with these TAs (Table 2).

Ex Vivo Antimalarial Activity against Clinical Isolates.

Four selected TAs **1**, **2**, **5** and **14** were evaluated against *P. falciparum* clinical isolates collected from malaria patients in Uganda using an *ex vivo* IC_{50} assay.[22, 23] Interestingly, all of these tested TAs exhibited excellent activity, with the IC_{50} values ranging from 14.6 to 1236 nM for **1** (geometric mean = 114 nM; 95% CI, 83.9–154; $N = 43$); 31.3 to 138 nM for **2** (geometric mean = 60.7 nM; 95% CI, 55.1–66.9; $N = 52$); 15.7 to 74.5 nM for **5** (geometric mean = 30.1 nM; 95% CI, 26.8–33.7; $N = 53$); and 37.3 to 418 nM for **14** (geometric mean = 98.9 nM; 95% CI, 85.1–115; $N = 50$) (Figure 3). The IC_{50} values were also compared with various control antimalarial drugs (Figure 3). The potency of these TAs against *P. falciparum* clinical isolates was slightly diminished as compared to the *in vitro* activity against *P. falciparum* pan-sensitive D6 and MDR Dd2 strains (Table 5 and published work[11]).

In Vitro Metabolic Stability, and Solubility Assessments.

The *in vitro* metabolic stability of TAs **1**–**13** was assessed by measuring the disappearance of the parent compounds after incubation with pooled human liver microsomes (HLM) and mouse liver microsomes (MLM) using well-established methods.[24, 25] Notably, the majority of the TAs showed an excellent metabolic profile in HLM (CL_{int} = 11.3–34.0 μL/min/mg),

however, they displayed only moderate stability in MLM (CL_{int} >56.5 μL/min/mg) (Table 3). Interestingly, TA **12** showed excellent metabolic profile in both HLM and MLM (HLM CL_{int} = 29.1 μL/min/mg vs MLM CL_{int} = 12.8 μL/min/mg). The aqueous solubility of TAs **1**–**13** was also measured in PBS medium at pH 7.4, and these results demonstrated moderate to good aqueous solubility (Table 3).

In Vitro Permeability Assessments:

The bidirectional permeability coefficient (P_{app}) and efflux ratio of the TAs **1**–**13** was also determined in MDCK MDR1 cells at pH 7.4.[26] Majority of the TAs **1**–**10** displayed a low P_{app} in both directions, in particular, TAs **11**–**13** with a cyclohexyl ring fused with ring-B of TA scaffold (Scheme 1), were not permeable (Table 3), indicating a possible low permeability classification, and potentially these compounds are a P-gp substrates, which decrease absorbency of these TA compounds.

Metabolic Profiling and Identification.

To further understand the microsomal clearance of the TAs, two representative but structurally distinct TAs, **2** and **9**, were investigated to establish metabolic profiles generated in the presence of HLM and MLM *in vitro*. TAs **2** and **9** were incubated with HLM and MLM for 30 min and possible metabolites were identified by LC-MS analyses at four different time points (0, 5, 15, and 30 min). It is noteworthy that these TAs were metabolized, mostly via desaturation, likely at the terminal cycloalkyl amine and hydroxylation on both the ring-A and ring-B, which appear to be the major sites for oxidative metabolism (Figure 4). LC-MS analysis demonstrated the production of a desaturation metabolite (**2-M1**), and two hydroxylated metabolites (**2-M2** and **2-M3**) with different retention times as major metabolites when **2** was incubated with both HLM and MLM (Top panel, Figure 4). It appears that compound **2**, which is lacking the substitutions at the 3-position of ring-B, was rapidly metabolized as evidenced by its high metabolic instability profiles in both HLM (CL_{int} = 105 μL/min/mg) and MLM (CL_{int} = 101 μL/min/mg) (Table 3). Similarly, a desaturation metabolite (**9-M1**), and three hydroxylated metabolites (**9-M2**, **9-M3** and **9-M4**) with different retention times were identified (bottom panel, Figure 4) with compound **9**. Notably, compound **9**, with ethyl substitution at the 3-position of ring-B, exhibited an increased metabolic stability in HLM (CL_{int} = 24.4 μL/min/mg) as compared to compound **2**, lacking that substitution, suggesting that the substitution at the 3-position of ring-B plays a crucial role in enhancing the HLM stability of the TA molecules. Collectively, these data indicated that substitutions at both the 3-, and 4-positions of ring-B within the TA core are important for the enhancement of liver microsomal stability. Further structural optimizations are in progress to make novel TAs containing substitutions on both ring-A and ring-B, aiming for enhanced metabolic stability while maintaining antiplasmodial activity. In addition, the synthesis, antiplasmodial activity, and toxicity studies of these metabolites are currently underway.

Biological and Chemical Stability Assessments.

Biological stability of lead compound **1** was conducted in mouse, human and rat plasma. After incubation with mouse, human and rat plasma for 120 min, compound **1** showed

moderate stability in the following order rat > mouse > human (Figure 5). Conversely, the chemical stability of compound **1** was examined in 0.1 M phosphate buffer solution at pH 7.4 and pH 2.0 at 37 ℃ for 120 min. This data demonstrated that the compound **1** is highly likely stable at pH 2.0, however, approximately 50% degradation was observed at pH 7.4 within 120 min (Figure 5).

In Vivo Pharmacokinetic Analysis.

An *in vivo* oral exposure pharmacokinetic (PK) study of several TAs was conducted following a single intragastric (po) administration in mice and/or rats at 40 mg/kg, with blood and liver samples taken at the following time points: 0, 0.5, 1, 2, 4, 8, 24, 30, 48, 54, and 72 hr.[27, 28] The key PK parameters of these compounds in both plasma and liver are summarized in Table 4. Significantly, all of these TAs showed a long half-life ($t_{1/2}$ > 5.6 h for plasma and > 4.7 h for liver) in both mice and rats. Overall, the preliminary PK data for these TAs indicates that the observed single oral dose efficacy might be the result of the combination of a fast-killing rate and a long elimination half-life in both plasma and liver.

In Vitro Cytotoxicity.

In vitro general cytotoxicity was tested for all of these TAs **1**–**14** using human hepatic HepG2 cells, and reported in our previous publication.[11] Inhibition of mammalian cells occurred only at very high relative concentrations with many of these TAs (Table 3).

In Vitro Mutagenicity.

Given the promising antiplasmodial activities of TAs, lead candidate **1** was investigated for mutagenicity using the Ames assay[29, 30] (EPBI Inc) at concentrations up to 10 μM, with and without S9 metabolic activation, against *Salmonella typhimurium* TA100 and T98 strains. Results were negative: there was no increase over the background reversion rate with compound **1**, suggesting low risk of mutagenicity with this TA class of compounds.

In Vitro Cardiotoxicity.

The *in vitro* effect of the lead candidate **1** on the hERG (human-ether-a-go-go-related gene) potassium channel current expressed in mammalian cells was evaluated using the QPatch automated patch-clamp system (Sophion, Denmark). Compound **1** demonstrated an hERG inhibition level considerably much lower (IC_{50} = 3.17±0.52 μM) than the positive control (verapamil: IC_{50} = 0.68±0.16 μM), suggesting a moderate cardiotoxicity risk is associated with this compound. Further structural optimizations are in progress to decrease the hERG IC_{20} and the ratio of free hERG IC_{50} to free predicted C_{max} in humans will be simulated for any advanced compound to properly characterize the risk.

In Vitro Cross-Resistance Studies.

To gain insight into the mechanism(s) of the TAs, we investigated compounds **1**–**4** for cross-resistance pattern using the additional *P. falciparum* strains (Tm90-C2B, an ATQ resistant clinical isolate,[31] and D10yDHODH[32], a transgenic parasite that is resistant to inhibitors of mitochondrial electron transport). It is noteworthy that the tested TAs were almost equally

effective against *P. falciparum* pan-sensitive and MDR strains across the entire test panel (Table 5). Further mechanistic studies are in progress.

CONCLUSIONS

We have discovered and developed a natural product inspired novel TA chemotype with multiple-stage antimalarial activities. In particular, the lack of any cross-resistance against a large panel of MDR *P. falciparum* strains and equipotency on liver-stage suggests that the TA chemotype operates through a unique mechanism distinct from that of other antimalarials currently in use on in the global portfolio. Selected TAs exhibited significant *in vitro* potency against liver-stage parasites (*P. berghei*) and reduced potency on late-stage *P. falciparum* gametocytes. In addition, our lead candidate **1** demonstrated excellent *in vivo* efficacy against both the *P. yoelii* and humanized *P. falciparum* mouse models, and this compound stood out as a fast-killing antiplasmodial agent. On the other hand, several other TAs **8**–**10**, and **13** have shown very promising *in vivo* efficacy, and their efficacy was comparable and/or enhanced compared to compound **1**. Outstandingly, compound **8** provided 100% cure (4/4 mice cured) in malaria-infected mice against *P. yoelii* at doses of 30 and 50 mg/kg × 4 days, and 80 mg/kg as a single dose.

Overall, our lead TA candidates meet the following key criteria: (i) novel chemotype as compared to standard antimalarials; (ii) equally effective against asexual blood-stage pan-sensitive and MDR *P. falciparum* parasites at low concentrations with an excellent therapeutic index; (iii) effective against liver-stage *P. berghei* parasites; (iv) effective against sexual blood-stage *P. falciparum* gametocytes; (v) excellent potency against clinical isolates; (vi) *in vivo* curative efficacy via oral administration in both erythrocytic *P. yoelii* and humanized *P. falciparum* mouse models; (vii) complete *in vivo* cure via a single oral dose in erythrocytic *P. yoelii* rodent model; (viii) fast-acting; (ix) acceptable tolerability in rodent efficacy studies, *in vitro* metabolic stability consistent with a long rodent oral half-life, acceptable solubility for oral dosing and *in vivo* oral PK profiles with rapid absorption; and (x) synthetically accessible with low-cost of production. Further studies are required to understand the fraction absorbed, the volume of distribution, the *in vitro* and *in vivo* correlation of clearance, and oral bioavailability in different species, along with the prediction of human clearance, volume of distribution and dose. Our current structural optimization studies are in progress to produce lead TA candidates that demonstrate enhanced efficacy, safety, solubility, and metabolic/PK profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Brief Synopsis:

Herein, we report results for natural products inspired novel tambjamine antimalarials that are potent against liver, asexual erythrocytic, and sexual erythrocytic parasite life cycle stages. Notably, our lead candidate KAR425 (**1**) demonstrated excellent *in vivo* efficacy in different mouse models, and has the potential to kill the malaria parasite with a fast rate. Our overarching goal is to develop novel, potent, well-tolerated, and inexpensive antimalarials for both prevention and treatment of malaria, thus supporting world-wide elimination of the disease.

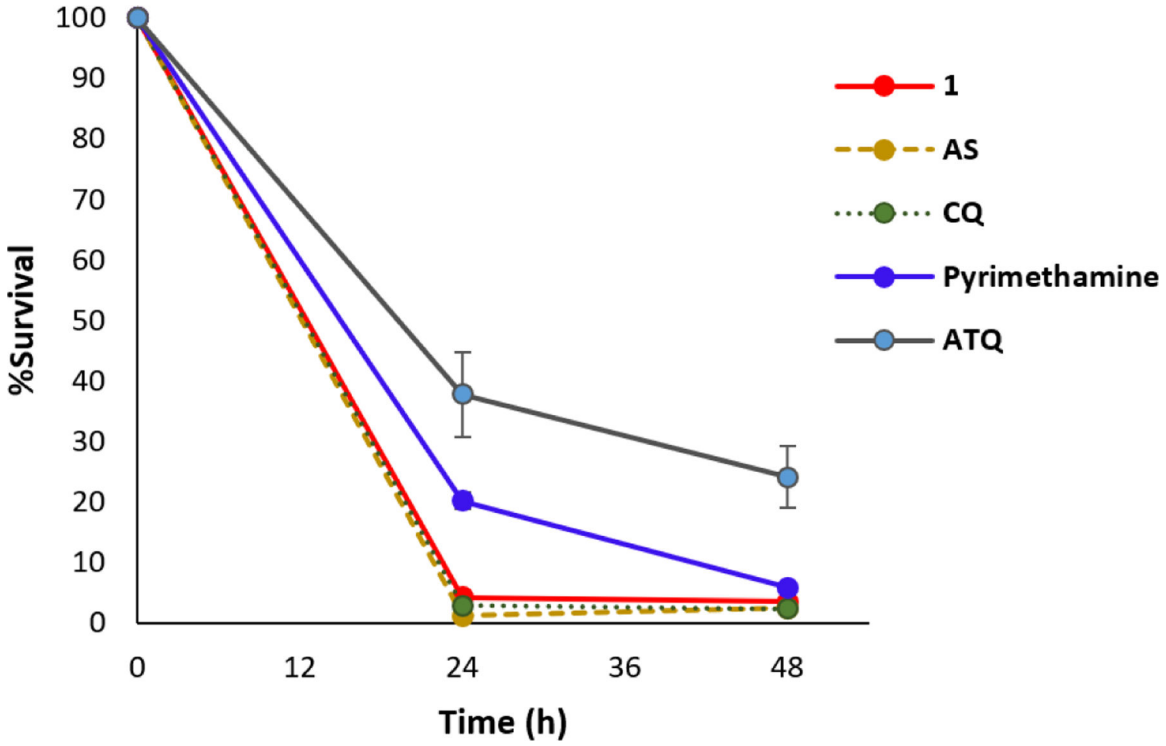


Figure 1.
Percentage of parasite survival after **1** treatment and standard antimalarials.

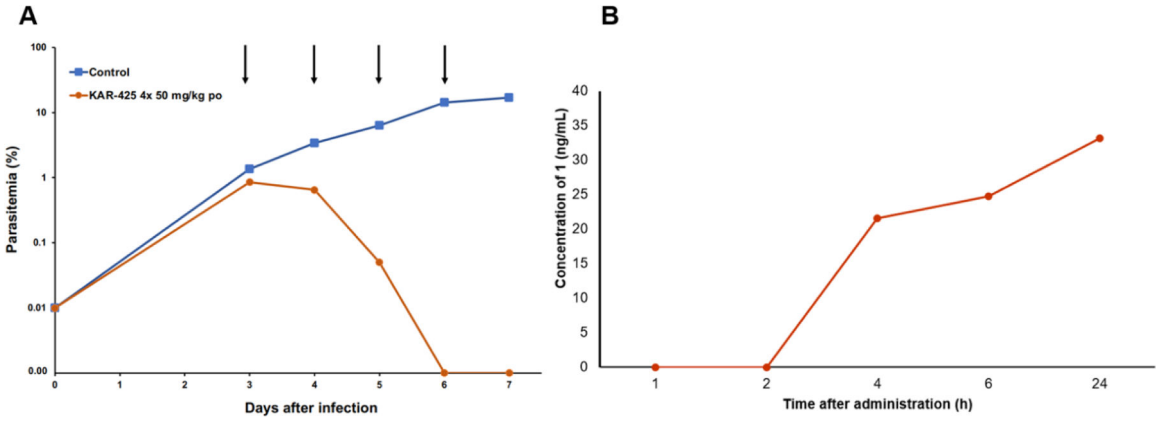


Figure 2.

(**A**) In vivo efficacy of **1** (KAR425) against *P. falciparum Pf3D7*$^{0087/N9}$. The arrows indicated the days of treatment. The efficacy estimated was expressed as the reduction (in %) of parasitemia at day 7 after infection (n=1 mouse per dose of **1**) as compared to the untreated control group (n=4). (**B**) Blood concentration profile of **1** after oral administration in mice.

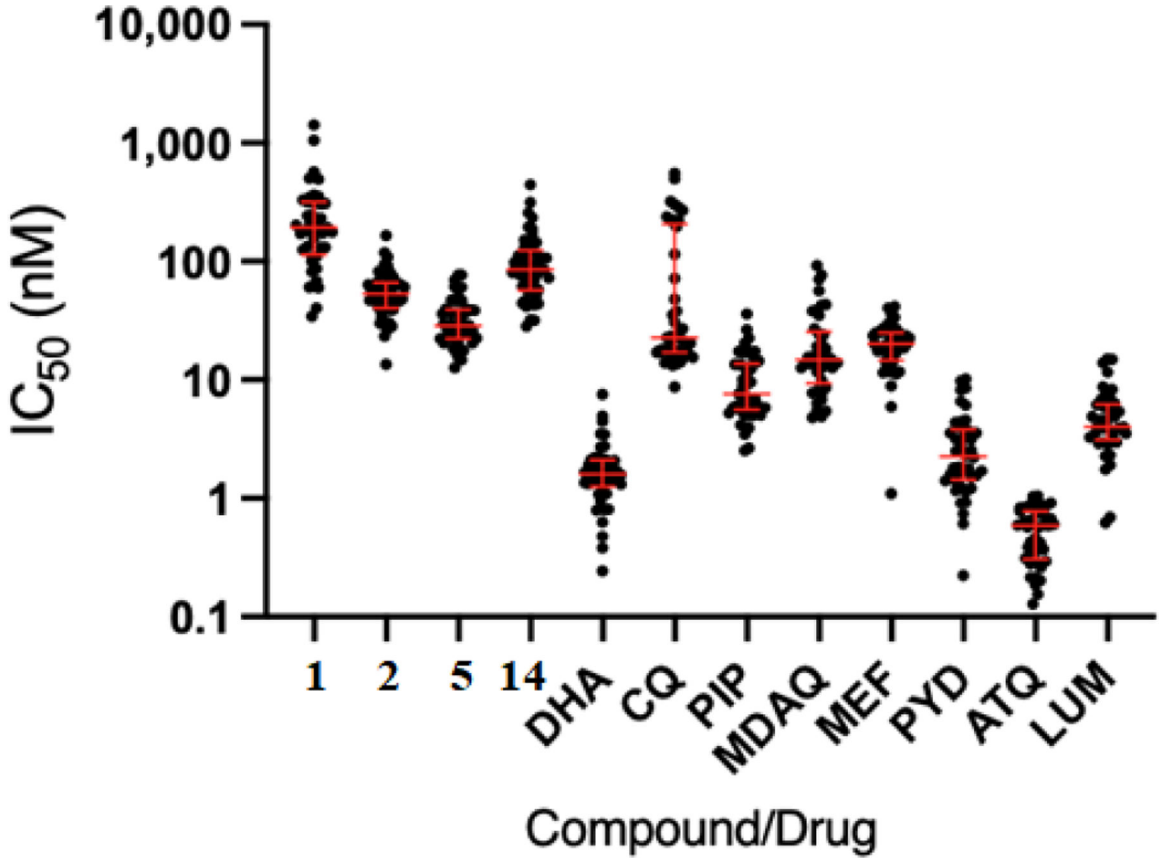


Figure 3.

Ex vivo activity of **1**, **2**, **5** and **14** and control antimalarial drugs against *P. falciparum* clinical isolates in Uganda. Data are presented as geometric mean ± 95% CI. DHA, dihydroartemisinin; CQ, chloroquine; PIP, piperaquine; MDAQ, monodesethyl-amodiaquine; MEF, mefloquine; PYD, pyronaridine; ATQ, atovaquone; LUM, lumefantrine.

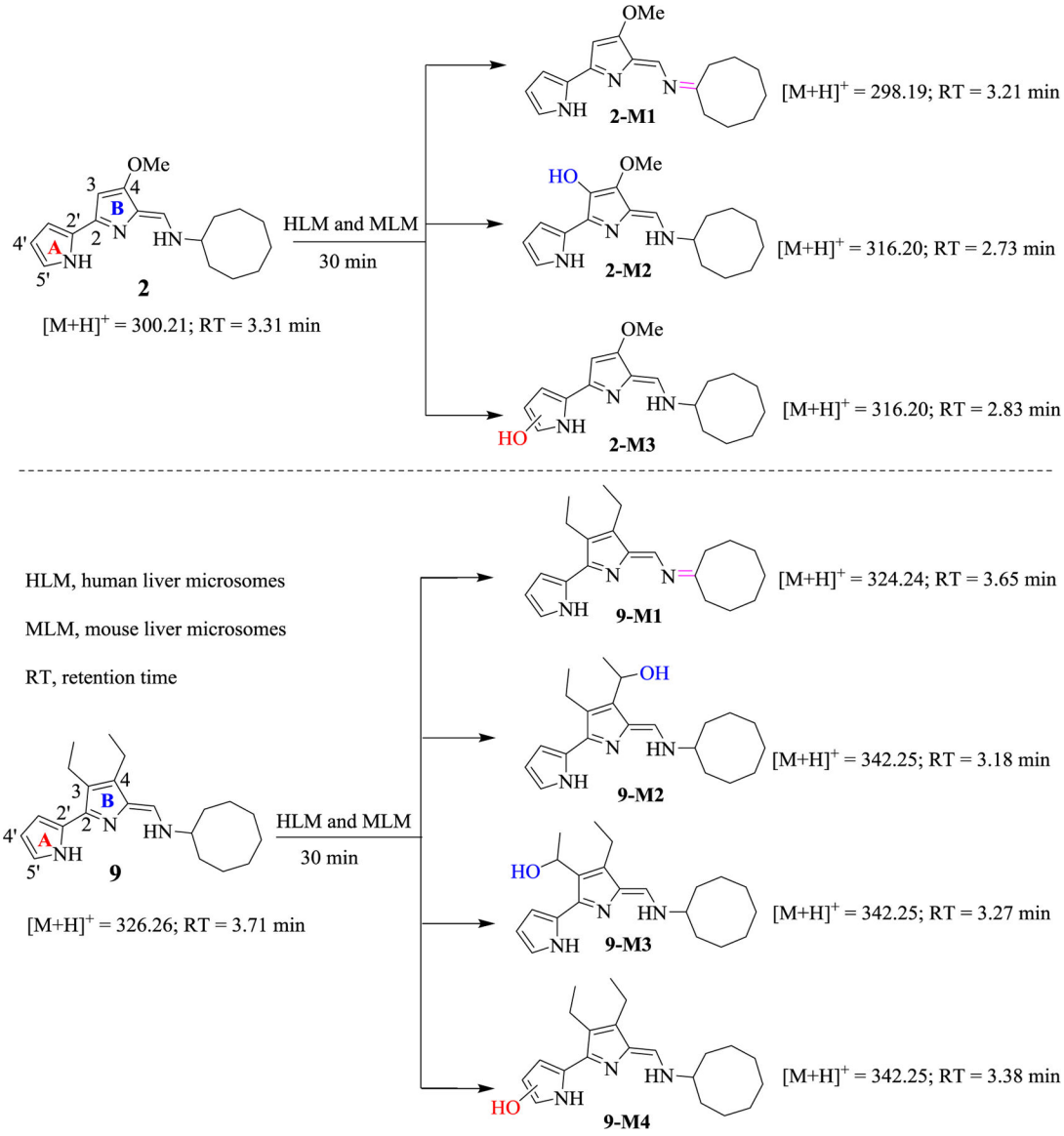


Figure 4.

Metabolite profiles of representative TAs **2** and **9** in human and mouse liver microsomes.

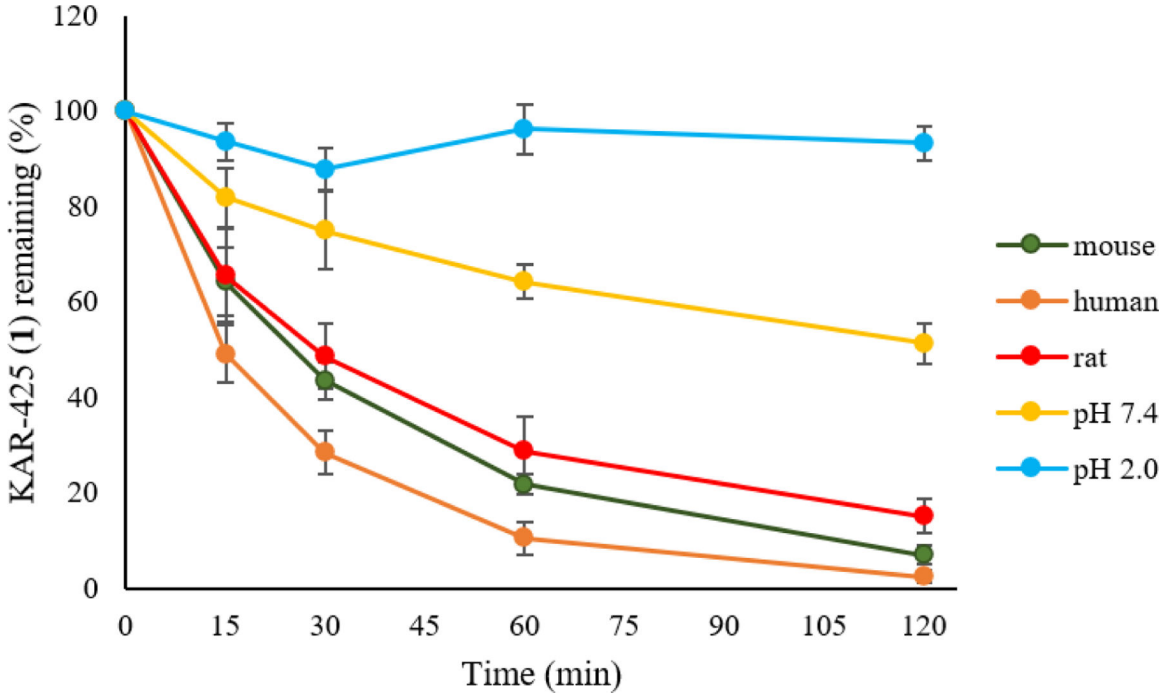
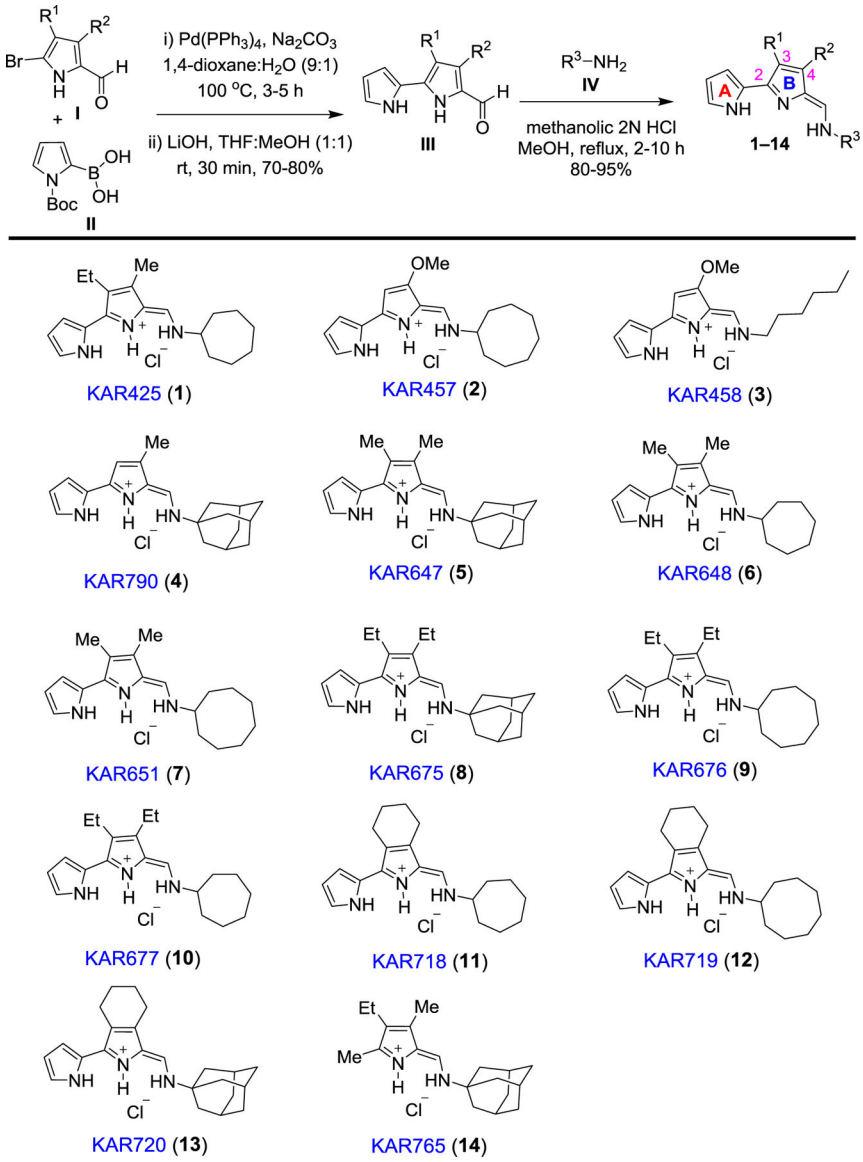


Figure 5.
Biological and chemical stability of compound **1**.



Scheme 1.

General Synthetic Route and Chemical Structures of Target TAs **1**–**14**.

Table 1.

In Vitro Asexual Blood-Stage, Liver-Stage and Sexual Blood-Stage Antiplasmodial Activities of Selected TAs.

				sexual blood-stage vs.		
compound	**code name**	**asexual blood-stage vs. *P. falciparum* D6 IC_{50} (μM)[a]**	**liver-stage vs. *P. berghei* IC_{50} (μM)**	***P. falciparum* stage V gametocytes IC_{50} (μM)**	***P. falciparum* DGFA IC_{50} (μM)**	
					male	**female**
1	KAR425	0.0620	0.108	2.39	IA[b]	IA
2	KAR457	0.00480	0.0190	1.41	1.04	IA
3	KAR458	0.0340	0.0167	2.55	IA	IA
4	KAR790	0.0013	0.0230	2.45	0.721	IA
CQ		0.0150	-	-	-	-
ATQ		0.0001	0.0065	-	-	-
methylene blue		-	-	0.143	-	-

[a] potency against D6 was reported in our previous publication[11];

[b] IA, inactive

Table 2.

In Vivo Antimalarial Blood-Stage Efficacy of Selected TAs in the *P. yoelii* Mouse Model.

		***in vivo* %parasitemia reduction vs. controls on day 5**						
compound	**code name**	**10 mg/kg/4d**	**25 mg/kg/4d**	**30 mg/kg/4d**	**50 mg/kg/4d**	**80 mg/kg/d**	**ED_{50} mg/kg/d**	**ED_{90} mg/kg/d**
1	KAR425[a]	100 (0/4)	100 (4/4)[b]	-	100 (4/4)[b]	100 (2/4)	0.09	1.1
5	KAR647	60.6	100 (3/4)	100 (3/4)	100 (3/4)	-	8.6	20.6
6	KAR648	68.1	100 (0/4)	100 (2/4)	100 (3/4)	-	5.0	24.1
7	KAR651	77.2	100 (0/4)	100 (1/4)	100 (3/4)	-	4.5	23.0
8	KAR675	82.5	100 (3/4)	100 (4/4)	100 (4/4)	100 (4/4)	3.8	6.0
9	KAR676	55.5	100 (1/4)	100 (4/4)	100 (4/4)	100 (1/4)	9.3	20.1
10	KAR677	43.5	91.4	100 (3/4)	100 (4/4)	100 (0/4)	10.9	25.6
11	KAR718	71.4	100 (0/4)	100 (1/4)	100 (3/4)	-	4.1	17.9
12	KAR719	80.7	100 (1/4)	100 (1/4)	100 (1/4)	-	4.8	18.6
13	KAR720	77.6	100 (0/4)	100 (3/4)	100 (4/4)	100 (2/4)	5.2	19.6
CQ	-		100 (0/4)	-	100 (0/4)	-	1.5	3.3
PEG-400	-	0	0	0	0	0	-	-

[a] in vivo efficacy of compound **1** against P. yoelii was reported in our previous publication[11];

[b] number of cured mice/treated mice on day 28 are within parentheses.

Table 3.

In Vitro Microsomal Metabolic Stability, Permeability, Solubility, and Cytotoxicity of TAs.

		intrinsic clearance CL_{int} (μL/min/mg) vs			permeability in MDCK MDR1 cells			
compound	code name	HLM[a]	MLM[b]	solubility (μM)	P_{app}A-B ($\times 10^{-6}$ cm/s)	P_{app}B-A ($\times 10^{-6}$ cm/s)	efflux ratio[c]	cytotoxicity vs HepG2 IC_{50} (nM)[d]
1	KAR425	79.3	136	80.1	1.127	0.311	0.276	19,200
2	KAR457	105	101	227	0.723	1.347	1.864	9700
3	KAR458	82.1	139	239	1.208	1.758	1.456	26,700
4	KAR790	34.0	69.5	190	1.932	2.541	1.315	6900
5	KAR647	15.2	67.5	54.8	0.402	0.091	0.226	6400
6	KAR648	30.7	123	107	0.877	0.945	1.077	21,300
7	KAR651	18.1	127	132	0.758	0.255	0.337	18,100
8	KAR675	11.3	86.4	<3.12	0.466	0.133	0.284	3900
9	KAR676	24.4	113	52.2	0.194	0.071	0.366	13,000
10	KAR677	16.2	132	30.4	0.715	0.138	0.193	16,900
11	KAR718	22.5	143	51.7	0.000	0.227	0.000	6200
12	KAR719	29.1	12.8	7.91	0.000	0.055	0.000	4600
13	KAR720	14.8	56.5	29.4	0.000	0.000	0.000	2700

[a] HLM, human liver microsomes;

[b] MLM, mouse liver microsomes;

[c] efflux ratio = P_{app}B-A/P_{app}A-B;

[d] cytotoxicity vs HepG2 cells was reported in our previous publication.[11]

Table 4.

Total Compound Concentrations and Key PK Parameters of Selected TAs in Liver and Plasma Following Single Oral Dose of 40 mg/kg Administration in Rats/Mice.

compound	code name	species	matrix	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{last} (ng.h/mL)	AUC_{extrap} (%) (ng.h/mL)	AUC_{inf} (ng.h/mL)
1	KAR425	mice	liver	1301	24.0	4.70	44,875	-	45,076
			plasma	65.0	1.00	6.20	2295	-	2544
2	KAR457	rats	liver	843	1.25	8.40	5596	26.3	-
			plasma	21.0	0.50	32.8	147	49.6	-
3	KAR458	rats	liver	912	3.75	29.2	6728	38.4	-
			plasma	172	0.75	26.3	354	34.6	-
4	KAR790	rats	liver	398	1.00	24.1	5190	44.7	-
			plasma	17.3	1.25	5.60	128	12.4	-
6	KAR648	mice	liver	1195	7.00	9.50	28,483	2.97	293,564
			plasma	14.1	0.50	24.7	230	45.1	420
9	KAR676	mice	liver	3790	1.00	9.32	38,841	5.55	41,122
			plasma	100.3	30.0	13.0	1930	12.3	2200
11	KAR718	mice	liver	644	24	6.73	30,452	0.15	30,497
			plasma	30.3	1.00	>50	1248	-	-

C_{max}: maximum plasma or hepatic concentration; T_{max}: time to C_{max}; AUC_{last}: area under the concentration-time curve from 0 up to the last sampling time at which a quantifiable concentration is found; $t_{1/2}$: apparent elimination half-life; AUC_{extrap}: percentage of the AUC extrapolated from the last observed time point; AUC_{inf}: area under the concentration-time curve from 0 up to infinity.

Table 5.

In Vitro Antiplasmodial Activity of against a Diverse Panel of *P. falciparum* Strains.

		in vitro* asexual blood-stage activity: IC_{50} (nM) vs. *P. falciparum				
compound	**code name**	**D6[a]**	**Dd2[a]**	**7G8[a]**	**Tm90-C2B**	**D10yDHODH**
1	KAR425	62±7.5	55±5.8	60±7.1	37±3.5	44±4.9
2	KAR457	4.8±0.4	7.1±0.8	7.5±0.6	2.3±0.1	2.8±0.2
3	KAR458	34±3.5	37±4.1	25±2.9	21±1.5	69±7.8
4	KAR790	1.3±0.1	1.5±0.2	4.3±0.5	2.0±0.3	6.7±0.8
CQ	-	15±1.8	163±15	171±19	208±25	10.0±0.9
ATQ	-	0.10±0.02	0.10±0.03	0.20±0.05	8256±690	>25000

D6: originally isolated from Sierra Leone and sensitive to all studied antimalarials, including CQ, quinine, pyrimethamine, and sulfadoxine; Dd2: a clone derived from a southeast Asia isolated after selection for resistance to mefloquine. The Dd2 strain is resistance to CQ, quinine, mefloquine, and pyrimethamine; 7G8: originally isolated from Brazil and resistant to CQ and quinine; Tm90-C2B: originally isolated from a Thai patient with recrudescence after treatment with ATQ, and shown to be resistant to ATQ; D10yDHODH: transgenic parasites that are resistant to inhibitors of mitochondrial electron transport; ATQ: Atovaquone; CQ: Chloroquine. Results are presented as mean ± SEM (n=3);

[a]potency against D6, Dd2, 7G8 was reported in our previous publication[11]