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Letter

Malaria Box-Inspired Discovery of *N*-Aminoalkyl-β-carboline-3-carboxamides, a Novel Orally Active Class of Antimalarials

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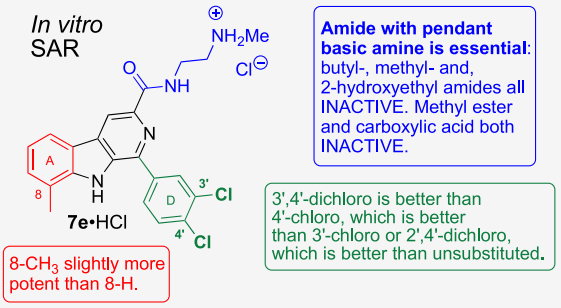
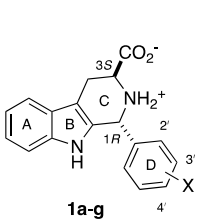
ABSTRACT: Virtual ligand screening of a publicly available database of antimalarial hits using a pharmacophore derived from antimalarial MMV008138 identified TCMDC-140230, a tetrahydro-β-carboline amide, as worthy of exploration. All four stereoisomers of this structure were synthesized, but none potently inhibited growth of the malaria parasite *Plasmodium falciparum*. Interestingly, **7e**, a minor byproduct of these syntheses, proved to be potent *in vitro* against *P. falciparum* and was orally efficacious (40 mg/kg) in an *in vivo* mouse model of malaria.

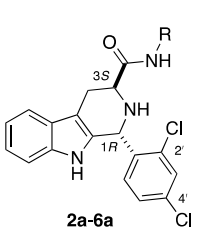


KEYWORDS: *Drug discovery, Plasmodium, synthesis, DMPK, in vivo*

Worldwide, malaria is one of the deadliest diseases, causing more than 600,000 deaths in 2020.[1] In our search for new and safe drugs that can kill resistant strains of *Plasmodium falciparum* parasites, we were drawn to the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway for synthesis of isoprenoid precursors, since this pathway is not present in humans.[2] Using the isopentenyl pyrophosphate (IPP) chemical rescue approach[3] to screen the 400-compound Malaria Box,[4] only MMV008138 (**1a**, Figure 1) was found to be an inhibitor of this pathway.[5] Other researchers determined that **1a** inhibits IspD, the third enzyme in the MEP pathway.[6,7] We subsequently synthesized and assayed dozens of analogs of **1a** that differed in stereochemistry and substitution of the B-, C-, and D-rings.[8−10] From these studies a clear picture emerged: (1*R*,3*S*)- stereochemistry, 2′,4′-halogen disubstitution of the D-ring (e.g., **1a**−**d**), and a 3-carboxylic acid or methyl amide (e.g., **1a** and **3a**) were required for antimalarial potency. However, to date none of these potent *Pf*IspD inhibitors has shown oral efficacy in mice models of malaria.



	X	Dd2 strain Growth inhibition EC_{50}^{72h} (nM)
1a	2′,4′-Cl_2	250 ± 70
1b	2′-Cl,4′-F	433 ± 55
1c	2′-Cl,4′-Br	320 ± 60
1d	2-Br′,4′-Cl	360 ± 40
1e	3′,4′-Cl_2	>10,000
1f	4′-H	>10,000
1g	4′-Cl	1,170 ± 60



	R	
2a	H	1,200 ± 100
3a	Me	190 ± 30
4a	Et	~5,000
5a	Bu	>10,000
6a	*i*-Pr	~10,000

Figure 1. MEP pathway inhibiting antimalarials based on the MMV008138 (**1a**) scaffold.[8−10]

Since **1a**, as a member of the 400-compound Malaria Box, was selected from a much larger initial hit set (20K compounds with 3D7 strain half-maximal effective concentration (EC_{50}) < 1 μM),[4] we wondered if other structurally related compounds were present in the initial set. We thus performed virtual ligand screening (VLS) of the GlaxoSmithKline (GSK) portion of this larger collection (~13K compounds),[11] using a 3D pharmacophore derived from **1a** and 92 close analogs. From this exercise the compound TCMDC-140230 emerged (Figure 2).

Several features of this compound stand out; in addition to the undisclosed stereochemistry, 3′,4′-dichloro substitution and the large amide substituent were not suggestive of good

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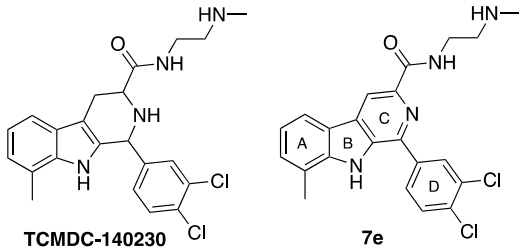


Figure 2. VLS hit TCMDC-140230 and derived lead **7e**.

antimalarial potency within the established pharmacophore of **1a** (Figure 1, cf. **1e** vs **1a**; **4a**−**6a** vs **3a**). As described below, the stereoisomers of tetrahydro-β-carboline TCMDC-140230 proved to be dead ends, but the β-carboline analog **7e** (tested as the HCl salt, Table 1) was found to have excellent antimalarial activity *in vitro* and *in vivo*.

Table 1. *In Vitro P. falciparum* Growth Inhibition of 1a, 13e Stereoisomers, and 7e·HCl

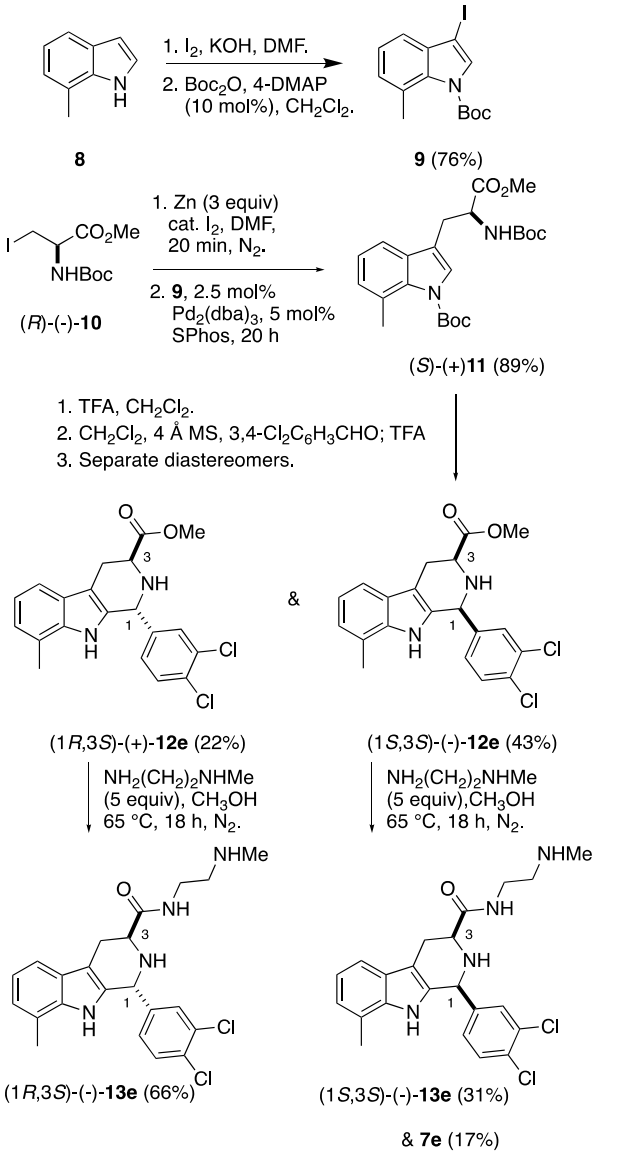
Compound	EC_{50} (nM)[a]	IPP rescue[b]
1a	250 ± 70[c]	100% @ 2.5 μM
(1*R*,3*S*)-(−)-**13e**	1,950 ± 240	50% @ 5 μM
(1*S*,3*S*)-(−)-**13e**	1,300 ± 100	nd
(1*S*,3*R*)-(+)-**13e**	3,690 ± 310	nd
(1*R*,3*R*)-(+)-**13e**	1,430 ± 70	nd
7e·HCl	108 ± 7	0% @ 2.5 μM

[a]Growth inhibition (72 h) of asexual blood stages determined using SYBR Green I assay. Values represent average ± SEM from at least three independent biological replicates. The Dd2 strain is resistant to chloroquine, pyrimethamine, and mefloquine. [b]Recovery of parasite growth in the presence of 200 μM IPP, at the indicated concentration of the test compound (Supporting Information); nd designates "not determined". [c]Reported previously.[8]

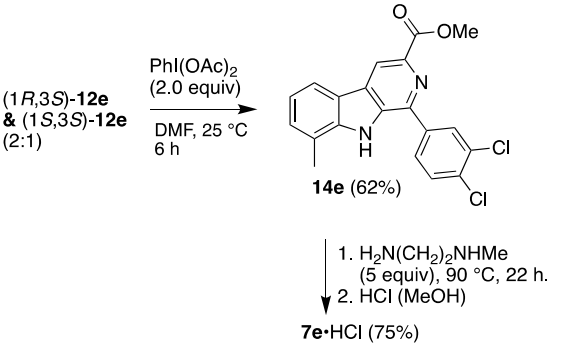
Since stereochemistry proved so crucial to the antimalarial activity of **1a**, we undertook the enantioselective synthesis of the four stereoisomers of TCMDC-140230. 7-Methylindole **8** was converted to **9**, which then underwent Negishi coupling with the organozinc reagent derived from *N*-Boc-iodoalanine methyl ester (*R*)-**10**,[12] affording the protected 7-methyltryptophan ester (*S*)-**11**. Deprotection, followed by Pictet−Spengler reaction[8] with 3,4-dichlorobenzaldhyde afforded (1*R*,3*S*)- and (1*S*,3*S*)-**12e** (Scheme 1). The relative configuration of the **12e** diastereomers was assigned on the basis of the $^{1}H-^{1}H$ coupling constants.[13] Conversion to the amides (1*R*,3*S*)- and (1*S*,3*S*)-**13e** was achieved with *N*-methylethylene diamine in MeOH at reflux under nitrogen. The remaining enantiomeric pair of diastereomers of **13e** was then prepared from (*S*)-**10** (Supporting Information). Interestingly, in the amidation of (1*S*,3*S*)- and (1*R*,3*R*)-**12e**, oxidized byproduct **7e** was isolated in 14−17% yield (Scheme 1 and Supporting Information). To confirm its structure, **7e** was independently prepared by oxidation of the mixture of the *trans*- and *cis*-esters **12e** to β-carboline **14e**, using $PhI(OAc)_2$[14] (Scheme 2). Amidation of **14e** in neat amine and HCl treatment yielded **7e**·HCl in 75% yield. With these compounds in hand, we examined their activity against asexual blood stage *P. falciparum* (Table 1).

None of the stereoisomers of tetrahydro-β-carboline **13e** potently inhibited the growth of *P. falciparum*. Since (1*R*,3*S*)-**13e** has the same configuration of **1a**, the ability of IPP supplementation (200 μM) to reverse growth inhibition was

Scheme 1. Synthesis of the Stereoisomers of VLS Hit TCMDC-140230 and Serendipitous Isolation of 7e



Scheme 2. Independent Synthesis of 7e·HCl



assessed; the 50% rescue seen at 5 μM drug suggested this stereoisomer is a weak MEP pathway inhibitor. Fortuitously, the β-carboline amide **7e**, isolated initially as a byproduct, did potently inhibit parasite growth with EC_{50} = 108 ± 7 nM. Interestingly, supplementation with IPP did not rescue parasite

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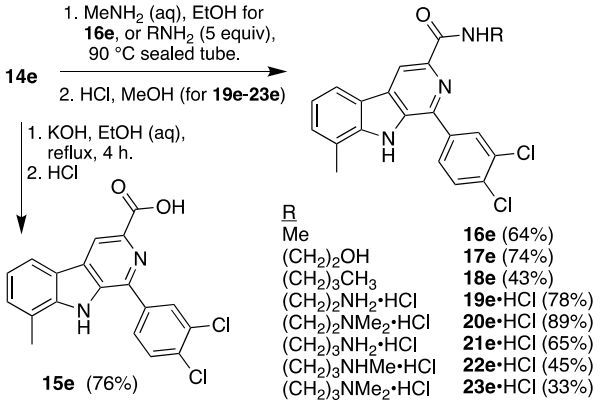
growth. Thus, the antimalarial activity of **7e**·HCl is not related to inhibition of the MEP pathway.

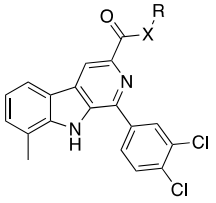
We then prepared a range of analogs of **7e** that differed in the C3 carbonyl substituent (Scheme 3).

Scheme 3. Synthesis of Acid and Amide Variants of 7e



Hydrolysis of **14e** afforded carboxylic acid **15e**; amidation of **14e** yielded neutral amides **16e**–**18e** and amides **19e**–**23e**, which resemble **7e** in having a pendant basic amine. These were tested for growth inhibition of *P. falciparum*, and a clear pattern emerged (Table 2). As can be seen, ester **14e** and acid

Table 2. *In Vitro P. falciparum* Growth Inhibition of Acid and Amide Variants of 7e



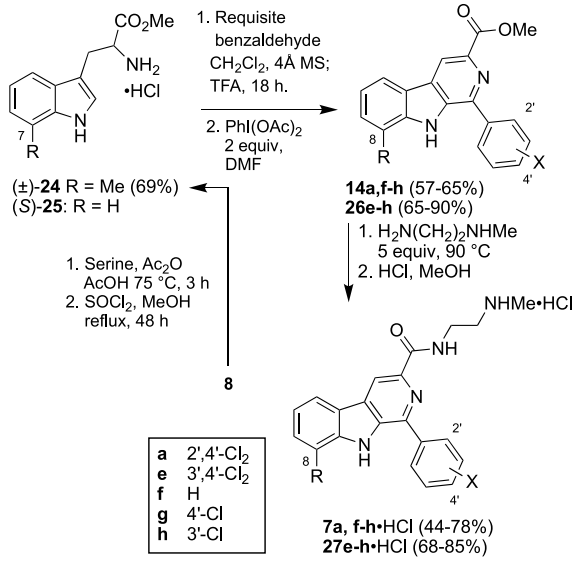
Compound	X	R	EC_{50} (nM)[a]
7e·HCl	NH	$(CH_2)_2NHMe$·HCl	108 ± 7
14e	O	Me	>10,000
15e	O	H	>10,000
16e	NH	Me	>10,000
17e	NH	$(CH_2)_2OH$	3,270 ± 40
18e	NH	$(CH_2)_3CH_3$	1,020 ± 20
19e·HCl	NH	$(CH_2)_2NH_2$·HCl	263 ± 25
20e·HCl	NH	$(CH_2)_2NMe_2$·HCl	264 ± 26
21e·HCl	NH	$(CH_2)_3NH_2$·HCl	726 ± 57
22e·HCl	NH	$(CH_2)_3NHMe$·HCl	217 ± 28
23e·HCl	NH	$(CH_2)_3NMe_2$·HCl	240 ± 23

[a]Growth inhibition (72 h) of asexual blood stages determined using SYBR Green I assay. Values represent average ± SEM from at least three independent biological replicates. The Dd2 strain is resistant to chloroquine, pyrimethamine, and mefloquine.

15e did not inhibit growth at the highest concentration tested, and only amides with a pendant basic amine (**7e** and **19e**–**23e**) had submicromolar potency. Within the limited set of compounds explored, it appears that the *N*-(2-methylamino)-ethyl amide (i.e., **7e**) confers the best antimalarial potency.

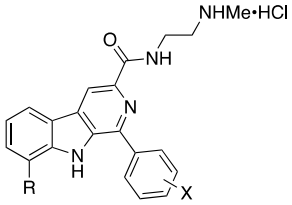
We thus prepared a set of analogs of **7e**·HCl that differed in the 8-substituent of the A-ring and in the substitution of the D-ring (Scheme 4).

Scheme 4. Synthesis of A- and D-Ring Variants of 7e·HCl



Racemic 7-methyl tryptophan methyl ester (±)-**24** was prepared from **8** by adapting a literature method;[15] it and (*S*)-**25** were subjected to Pictet–Spengler reaction with the requisite benzaldehydes; oxidation with $PhI(OAc)_2$ yielded the corresponding *β*-carboline methyl esters **14a,f–h** and **26e–h**. Amidation and treatment with methanolic HCl afforded the desired A- and D-ring variants of **7e**·HCl. The antimalarial potencies of these compounds and **7e**·HCl are shown in Table 3.

Table 3. Antimalarial Potencies of 7e·HCl and Its A-/D-Ring Variants



Compound	R	X	EC_{50} (nM)[a]
7a·HCl	CH_3	2′,4′-Cl_2	583 ± 91
7e·HCl	CH_3	3′,4′-Cl_2	108 ± 7
7f·HCl	CH_3	H	4,000 ± 900
7g·HCl	CH_3	4′-Cl	274 ± 12
7h·HCl	CH_3	3′-Cl	342 ± 7
27e·HCl	H	3′,4′-Cl_2	227 ± 33
27f·HCl	H	H	2,500–5,000
27g·HCl	H	4′-Cl	496 ± 60
27h·HCl	H	3′-Cl	641 ± 11

[a]Growth inhibition (72 h) of asexual blood stages determined using SYBR Green I assay. Values represent average ± SEM from at least three independent biological replicates. The Dd2 strain is resistant to chloroquine, pyrimethamine, and mefloquine.

As can be seen, 3′,4′-Cl_2 substitution of the D-ring (i.e., **7e**·HCl and **27e**·HCl) affords the best potency in both the R = CH_3 and R= H series. Unsubstituted analogs **7f**·HCl and **27f**·HCl are approximately 10-fold less potent than **7e**·HCl and **27e**·HCl. Single Cl-substitutions in the 4′- or 3′-positions (e.g., **7g,h**·HCl /**27g,h**·HCl) recover some potency relative to the

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unsubstituted analogs **7f**·HCl/**27f**·HCl but are less potent than 3′,4′-Cl_2 analogs **7e**·HCl/**27e**·HCl. Compound **7a**·HCl provides one example of 2′,4′-Cl_2 disubstitution (cf. *Pf*IspD inhibitor **1a**), but it is less potent than **7e**·HCl. Lastly, in this small series it appears that 8-methyl substitution may confer some advantage for *in vitro* potency (cf. **7e**·HCl and **27e**·HCl).

Since **7e**·HCl emerged as the best compound, we evaluated it for potency against a number of different strains of *P. falciparum* (Table 4).

Table 4. *In Vitro* Antimalarial and ADME-Tox Data, Physicochemical Parameters, and *In Vivo* PK Data for 7e·HCl

Entry	Assay	Value
1	Dd2 EC_{50}^{72h} (nM)[a,b]	108 ± 7
2	3D7 EC_{50}^{72h} (nM)[a,c]	53 ± 7
3	Dd2-KAE609^R EC_{50}^{72h} (nM)[a,d]	101 ± 5
4	W2 EC_{50}^{72h} (nM)[a,e]	100 ± 8
5	4G EC_{50}^{72h} (nM)[a,f]	110 ± 17
6	3D7 ^{3}H−H IC_{50}^{48h} (nM)[g]	70 ± 10
7	3D7 LDH IC_{50}^{72h} (nM)[h]	117 ± 11
8	HEK293 CC_{50} (nM)	32,000 ± 4,000
9	hPHep CC_{50}^{96h} (nM)[i]	8,500 ± 1,900
10	*E. coli* MIC (nM)	>250,000
11	hERG IC_{50} (nM)	510
12	Mini-Ames Panel	negative
13	MW (g/mol, free base)	427
14	*t*PSA (Å^2)	65.5
15	Log D (pH 7.4)[j]	3.3
16	PBS Solubility (μM, pH 7.4)	9.5
17	PPB (mouse)	99.49%
18	Mouse plasma stability	100% at 1 h
19	Mouse microsomal $t_{1/2}$ (min)	110
20	Cl_{int} (mouse μg/mL/min)	12.6
21	%F (rat)[k]	40%
22	C_{max} (nM, oral 40 mg/kg, rat)[k]	800 ± 25
23	Rat plasma $t_{1/2}$ (h)[k]	8
24	V_{dss} (L/kg, rat)[k]	21.8

[a]Growth inhibition determined using the SYBR Green I assay for all EC_{50} determinations. Values represent average ± SEM from at least three independent biological replicates. [b]Resistant to chloroquine (CQ), pyrimethamine (PY), and mefloquine (MQ). [c]Sensitive to CQ, PY, and MQ. [d]KAE609-resistant strain, courtesy of E. Winzeler. [e]Resistant to CQ, quinine, PY, cycloguanil, and sulfadoxine. [f]Dihydroartemisinin- and CQ-resistant field strain, courtesy of D. E. Kyle. [g][^{3}H]hypoxanthine incorporation assay. [h]Lactate dehydrogenase activity. [i]Primary human hepatocytes. [j]Experimentally determined. [k]See Supporting Information for details.

Compound **7e**·HCl had similar EC_{50} values against the susceptible 3D7 strain and a number of resistant strains of *P. falciparum* (Table 4, entries 1−5). Thus, **7e**·HCl is apparently not subject to the resistance mechanisms of a number of antimalarials such as chloroquine, mefloquine, quinine, pyrimethamine, cycloguanil, sulfadoxazine, KAE609, and dihydroartemisinin. Furthermore, the antimalarial potency against the 3D7 strain was similar when assessed for DNA replication, purine biosynthesis, and metabolic activity (Table 4, entries 2, 6, and 7). **7e**·HCl was also not generally cytotoxic, as demonstrated by 78- to 320-fold higher half-maximal cytotoxic concentration (CC_{50}) values against mammalian cells and no effect at 250 μM against human microbiome constituent *E. coli* (Table 4, entries 8−10). The compound showed a hERG liability, but it was not mutagenic in a mini-Ames panel (Table 4, entries 11−12). The physicochemical properties of **7e**·HCl are suggestive of acceptable oral bioavailability (Table 4, entries 13−15). The compound is highly plasma-protein-bound but has excellent plasma and microsomal stability and, consequently, low Cl_{int} (Table 4, entries 17−20). In rat, **7e**·HCl has moderate oral bioavailability (40%) and a C_{max} of 800 ± 25 nM (at 40 mg/kg po); the long plasma half-life (8 h) and relatively high volume of distribution (21.8 L/kg) are also positive features for an antimalarial candidate (Table 4, entries 21−24).

With this data in hand, the *in vivo* antimalarial activity of **7e**·HCl was assessed in a *P. berghei* model of murine malaria (Figure 3).

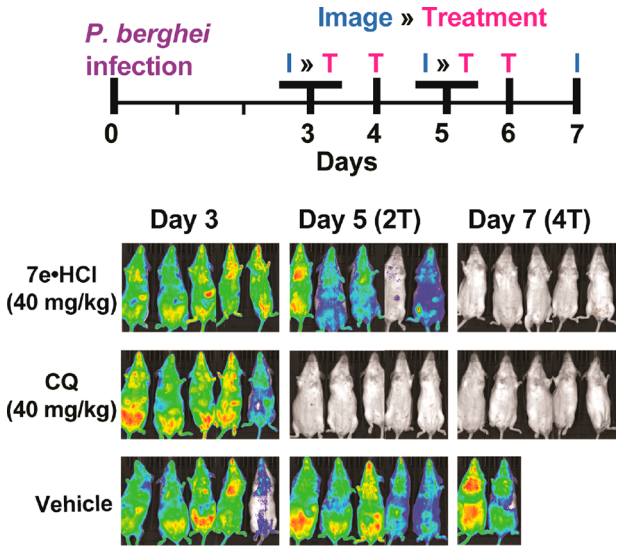

Figure 3. **7e**·HCl (40 mg/kg/day) has oral efficacy in *P. berghei*-infected mice. A transgenic *P. berghei* ANKA strain (l676m1cl1 line, PbGFP-Luccon) was used, that expresses a fusion GFP (mutant 3) and firefly luciferase (LucIAV). CQ is chloroquine. Note: three of the five vehicle-treated mice died before Day 7.

Mice were infected on Day 0 with 10^3 *P. berghei*-infected erythrocytes and treated for 4 days starting on Day 3 after inoculation with *P. berghei*. Mice were imaged (luminescence) before dosing on Days 3 and 5; final images were taken on Day 7. As can be seen in Figure 3, at 40 mg/kg (po), **7e**·HCl substantially reduced parasitemia in 3−4 doses. At the same dose, chloroquine cleared infection in 1−2 doses. No signs of toxicity (lethargy, gait, weight loss, ruffled fur, and differences in behavior) were observed in any of the groups. Results were reproduced in two independent trials with independently synthesized samples of **7e**·HCl at 40 mg/kg. Note that no reduction in parasitemia was seen at 20 mg/kg, using the same dosing/imaging protocol shown in Figure 3. Thus, further increases in *in vitro* protency will be needed. Nevertheless, to the best of our knowledge, this is the first example of oral antimalarial efficacy within the β-carboline (as opposed to the tetrahydro-β-carboline) scaffold. In addition to its good druglike properties and low general toxicity, **7e**·HCl has a number of other promising characteristics, which we will disclose in due course.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00663.

Synthetic procedures, tabulations of analytical data, and NMR spectra of all tested compounds; *in vitro* growth inhibition procedures; details on the ADME-Tox assays, pharmacokinetics, and *in vivo* efficacy studies (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.

Notes

The authors declare no competing financial interest.

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