4-Aryl Pyrrolidines as Novel Orally Efficacious Antimalarial Agents. Part 2: 2-Aryl-N-(4-arylpyrrolidin-3-yl)acetamides

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Supporting Information

ABSTRACT: Malaria is caused by infection from the *Plasmodium* parasite and kills hundreds of thousands of people every year. Emergence of new drug resistant strains of *Plasmodium* demands identification of new drugs with novel chemotypes and mechanisms of action. As a follow up to our evaluation of 4-aryl-N-benzylpyrrolidine-3-carboxamides as novel pyrrolidine-based antimalarial agents, we describe herein the structure–activity relationships of the reversed amide homologues 2-aryl-N-(4-arylpyrrolidin-3-yl)acetamides. Unlike their carboxamide homologues, acetamide pyrrolidines do not require a third chiral center to be potent inhibitors of *P. falciparum* and have good pharmacokinetic properties and improved oral efficacy in a mouse model of malaria. Compound (–)-32a (CWHM-1552) has an *in vitro* IC₅₀ of 51 nM in the *P. falciparum* 3D7 assay and an *in vivo* ED₉₀ of <10 mg/kg/day and ED₉₉ of 30 mg/kg/day in a murine *P. chabaudi* model. Remarkably, the absolute stereochemical preference for this acetamide series (3S,4R) is opposite of that determined for the homologous carboxamide series. Lead compounds for this class have modest affinities for the hERG channel and inhibit CYP 3A4. Additional optimization is needed in order to eliminate these undesired properties from this otherwise promising series of antimalarial compounds.

KEYWORDS: Aspartic protease inhibitors, pyrrolidines, antiplasmodial, antimalarial

The World Health Organization reports that there were approximately ~219 million new cases of malaria resulting in an estimated 435,000 deaths in 2017.¹ Given the persistent emergence of drug resistant strains of *Plasmodium falciparum*, the most deadly of the species causing malaria, there is an ongoing need to identify novel chemotypes for new antimalarial drugs. In the preceding paper,² we described the evaluation of pyrrolidine carboxamides as a novel class of antimalarial compounds with the potential for optimization as drug candidates (Figure 1). That work led to the discovery of CWHM-1008 (1), which is a potent inhibitor of *P. falciparum* growth in both the drug sensitive 3D7 and resistant Dd2 strains and is orally efficacious in a mouse model of malaria (ED₉₉ ≈ 30 mg/kg/day, p.o., q.d.).

In our quest to identify related aryl pyrrolidines with an improved oral efficacy, we synthesized a set of pyrrolidine acetamides in which the carboxamide from the previous work is “reversed” (Figure 1). Prototypical compounds from our previous work included (±)-2 and (±)-3. These compounds

Figure 1. Evaluation of 4-aryl pyrrolidines as novel antimalarials.

Received: March 20, 2019

Accepted: May 28, 2019

Published: May 28, 2019

have respective IC_{50} potency values in a SYBR Green *Pf* 3D7 assay³ of 190 and 250 nM, highlighting the need for a third chiral center for good potency. Herein, we describe the synthesis and structure–activity relationship (SAR) studies for 4-aryl pyrrolidine acetamides that do not rely on a third chiral center for good potency and *in vivo* efficacy and pharmacokinetic (PK) properties.

Results and Discussion. *Synthesis.* Direct acetamide analogs of ($\pm$)-**2** were synthesized as racemic mixtures according to the route illustrated in Scheme 1. Aldehyde **4**

Scheme 1. Synthesis of Racemic Acetamide Analogs^a

^aReagents and conditions: (a) nitromethane, NaOH, EtOH; (b) Ac_2O , DMAP, DCM; (c) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine, TFA, DCM; (d) Fe, NH_4Cl , EtOH, H_2O ; (e) 2-(4-(dimethylamino)phenyl)acetic acid, HATU, TEA, DCM; (f) Pd/C, ammonium formate, MeOH.

was then condensed with nitromethane and NaOH followed by dehydration with acetic anhydride to give the nitro olefin **5**. The azomethine ylide generated from *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine was reacted with the nitro olefin **5** to stereoselectively give the *trans*-3 + 2 cycloaddition product^{4,5} pyrrolidine intermediate ($\pm$)-**6**. The nitro group was reduced with iron to furnish the 3,4-*trans*-pyrrolidinyllamine intermediate ($\pm$)-**7a**. Amide coupling followed by deprotection of the *N*-benzylpyrrolidine using transfer hydrogenation conditions with ammonium formate and Pd/C provided the target analog ($\pm$)-**9** as a racemic mixture. Additional analogs ($\pm$)-**10**–**23** were synthesized in the same manner from their corresponding aryl aldehydes and aryl acetic acids (see Supporting Information).

Acetamide Aryl Ring SAR. The SAR for the terminal acetamide aryl ring is shown in Table 1. The reversed amide tended to be equipotent to slightly more potent than the direct amide comparators (e.g., compare **9** to **2** and **10** to **3**). The SAR for the acetamides was similar to the carboxamides in our previous work.² Unadorned phenyl (**11**) was 20-fold less active than dimethylaniline **9**. Methoxy **12** and methyl **13** analogs were modestly less potent than the dimethyl amine analog **9**. Removing the methyl groups from the aniline moiety led to a 30-fold loss in potency (**14**). Replacement of the aniline N atom with CH resulted in a 6-fold loss in potency (**15**). Extension of the dimethylaniline ring by one methylene led to a 14-fold loss in potency (**16**). Moving the dimethylamino, methoxy, and methyl groups to the meta position gave more modest 2-fold losses in potency (**17**–**19**). Replacing the

Table 1. Acetamide Aryl Ring SAR

Compound	R	X	<i>Pf</i> 3D7 IC_{50} , nM ^a
($\pm$)- 9	4-Me ₂ NPh-	CH	96 ± 10
($\pm$)- 10	4-Me ₂ NPh-	N	210 ± 20
($\pm$)- 11	Ph-	CH	2,000 ± 64
($\pm$)- 12	4-MeOPh-	CH	350 ± 17
($\pm$)- 13	4-MePh-	CH	230 ± 38
($\pm$)- 14	4-H ₂ NPh-	CH	2,900 ± 150
($\pm$)- 15	4- <i>i</i> -Pr-Ph-	CH	600 ± 140
($\pm$)- 16	4-Me ₂ NPhCH ₂ -	CH	1,400 ± 29
($\pm$)- 17	3-Me ₂ NPh-	CH	230 ± 36
($\pm$)- 18	3-MeOPh-	CH	760 ± 39
($\pm$)- 19	3-MePh-	CH	370 ± 20
($\pm$)- 20		CH	3,700 ± 270
($\pm$)- 21		CH	1,600 ± 250
($\pm$)- 22		CH	2,200 ± 180
($\pm$)- 23		CH	3,600 ± 250

^a IC_{50} values are given as average potency values in the *Pf* 3D7 assay ± SD ($n \geq 3$). Standards chloroquine and artemisinin have IC_{50} values of 54 ± 0.4 and 33 ± 0.6 nM, respectively.

phenyl ring with a pyridine (**20**–**23**) resulted in larger reductions in potency.

Synthesis of Single Enantiomers. Single enantiomers were prepared as illustrated in Scheme 2. Intermediate ($\pm$)-**7** was coupled with naproxen (**24**) to provide diastereoisomers that were resolved by silica gel chromatography as (–)-**25a** and (+)-**25b**. Independently, the diastereomers were hydrolyzed in a three-step process by first acylation to the Boc amide **26a** followed by displacement of naproxen with hydrazine and finally deprotection of the Boc group with trifluoroacetic acid to give the aminopyrrolidine as a single enantiomer (**28a**). Amide coupling and deprotection then furnished the target pyrrolidines as single enantiomers (–)-**30a** and (+)-**30b**. Analogs **31**–**44** were prepared by the analogous procedure. Absolute stereochemistry was determined as (3*S*,4*R*)-configuration by small molecule X-ray powder diffraction with (–)-**32a** (see Supporting Information). Absolute stereochemical assignment was made for remaining analogs based on their optical rotations with negative rotations being assigned the (3*S*,4*R*)-configuration and positive rotations being assigned the (3*R*,4*S*)-configuration.

Stereochemistry and 4-Aryl Ring SAR. The (+)- and (–)-single enantiomers of **30**–**44** were assayed in the *Pf* 3D7 assay (Table 2). In our previous work with the carboxamide

Scheme 2. Synthesis of single enantiomers.^a

^aReagents and conditions: (a) HATU, TEA, DCM; (b) silica gel chromatography; (c) Boc_2O , DMAP, THF; (d) hydrazine hydrate, MeOH; (e) TFA, DCM; (f) 2-(4-(dimethylamino)phenyl)acetic acid, HATU, TEA, DCM; (g) Pd/C, ammonium formate, MeOH.

pyrrolidines, we had found that halo- and haloalkyl- phenyl and pyridyl groups tended to be favored aryl groups in the 4-position. In particular, CF_3 and CF_2CH_3 were optimal substituents. As illustrated with 30–32, the (–)-enantiomers are 7- to 13-fold more potent than the (+)-enantiomers. This is particularly remarkable since the preferred (3*S*,4*R*)-configuration for these acetamide pyrrolidines is the opposite of the (+)-(3*R*,4*S*)-configuration that is preferred for the carboxamide pyrrolidines in our previous work.²

Since the (–)-enantiomer was clearly more potent than the (+)-enantiomer, we focused our SAR studies on the (–)-enantiomer (Table 2). In this series of compounds, 4- SF_5 (31a) was equipotent to 4- CF_3 (30a) and only modestly less potent than the CF_2CH_3 (32a), which was the most potent compound we identified at 51 nM. CF_2CF_3 , *t*-Bu, and Br (33–35) were all potent in the 120–140 nM range. Increasing the size of the 4-substituent to Ph, however, led to large drops in potency (36). Similar to the carboxamide series, 3-substitution with Cl and SF_5 was tolerated with no detrimental effects (37–38). It is notable that SF_5 is ~5-fold more potent in this acetamide series than the carboxamide series.

As observed in the carboxamide series, heterocycles such as pyridines, benzothiophenes, and benzofurans are tolerated. Di- and pentafluoro ethylpyridines (39–40) are among the most potent compounds we have assayed (IC_{50} = 60 and 63 nM, respectively). Unsubstituted benzothiophene and -furan were potent (41–42; IC_{50} = 140–150 nM), but substitution with small halogens led to reductions in potency (43–44).

We also evaluated methylation of both the amide (45) and pyrrolidine (46) (Figure 2). Methylation of the amide led to less than 2-fold reduction in potency (compare 45 to 30a). However, methylation of the pyrrolidine led to a more substantial 6-fold reduction of potency (compare 46 to 10).

Potency against the Drug-Resistant Dd2 Strain of *P. falciparum*. Lead compounds 30a, 31a, and 32a were profiled in extensive side-by-side studies in the multidrug resistant Dd2 strain of *P. falciparum* (Table 3). All three compounds have equivalent potency against the *Pf* 3D7 strain and the multidrug resistant Dd2.

Inhibition of Aspartic Proteases. Since our original hypothesis was that these pyrrolidines might be aspartic protease inhibitors,² we profiled a select set of three

compounds for inhibition of human β -secretase (BACE1), *Pf* plasmepsin II (PM-II), and *Pf* plasmepsin IV (PM-IV) enzymes. However, none of the three compounds inhibited these aspartic proteases (data not shown). To date, we have not identified a biomolecular target for these pyrrolidines, and we cannot rule out inhibition of other *Plasmodium* aspartic proteases.

In Vitro Safety Profiling. In our previous work, we identified the potential for binding the hERG channel.² To address this potential safety concern, we evaluated three lead compounds in this series for hERG binding in a competitive binding assay (Table 4). Compounds tested have binding affinities for hERG ranging from 2 to 5 μM , giving modest 28- to 55-fold hERG/3D7 selectivity ratios. We also evaluated these same three compounds for inhibition of a panel of five human CYPs. While the three compounds had minimal to no inhibition of CYPs 1A2, 2D6, 2C9, and 2C19, they did moderately inhibit CYP 3A4 with IC_{50} values from 871 to 2500 nM. Cytotoxicity in HepG2 cells indicated no cytotoxicity at 5 μM (see Supporting Information). Compound 32a was nontoxic at 50 μM , a selectivity index of nearly 1000-fold. However, additional optimization work will need to be done in order to reduce hERG binding and CYP 3A4 inhibition.

In Vivo Pharmacokinetics. Compounds 30a, 31a, and 32a were selected for mouse PK studies. The compounds were dosed as a single cassette by i.v. administration. All three compounds were found to have respectable half-lives (2.7 to 7.0 h) and low clearance in mice (Table 5).

32a Is Orally Efficacious in a Mouse Model of Malaria. In order to determine if the acetamides were orally efficacious, lead compounds 30a, 31a, and 32a were evaluated in the *P. chabaudi* murine Peters 4-day suppressive test.^{3,6} NIH mice were inoculated with parasitized red blood cells. After 4 h, the compounds were dosed orally once daily for 4 days. Parasitemia levels were determined 24 h after the last treatment (Table 6 and Figure S2). We initially tested all three compounds at 30 mg/kg/day alongside CQ and carboxamide 1 and found all three compounds to fully inhibit parasitemia at 99.9% at 30 mg/kg/day, while carboxamide 1 exhibited 92.2% inhibition in this experiment (see Supporting Information). Since our target ED_{99} was 10 mg/kg/day, we elected to evaluate all three compounds at 10 and 32a at 3, 10, and 30 mg/kg/day. Only the 30 mg/kg/day dose of 32a gave

Table 2. Pyrrolidine Aryl Ring SAR

Compound	Ar	<i>Pf</i> 3D7 IC ₅₀ , nM ^a
(-)-30a	(<i>R</i>)-Ph-4-CF ₃	120 ± 20
(+)-30b	(<i>S</i>)-Ph-4-CF ₃	860 ± 42
(-)-31a	(<i>R</i>)-Ph-4-SF ₅	91 ± 7
(+)-31b	(<i>S</i>)-Ph-4-SF ₅	1,200 ± 190
(-)-32a	(<i>R</i>)-Ph-4-CF ₂ CH ₃	51 ± 8
(+)-32b	(<i>S</i>)-Ph-4-CF ₂ CH ₃	440 ± 33
(-)-33	(<i>R</i>)-Ph-4-CF ₂ CF ₃	140 ± 19
(-)-34	(<i>R</i>)-Ph-4- <i>t</i> -Bu	120 ± 26
(-)-35	(<i>R</i>)-Ph-4-Br	140 ± 9
(-)-36	(<i>R</i>)-Ph-4-Ph	4,600 ± 340
(-)-37	(<i>R</i>)-Ph-3-Cl-4-CF ₃	120 ± 22
(-)-38	(<i>R</i>)-Ph-3-SF ₅	59 ± 10
(-)-39		60 ± 3
(-)-40		63 ± 13
(-)-41		150 ± 13
(-)-42		140 ± 22
(-)-43		270 ± 31
(-)-44		1,000 ± 240

^aIC₅₀ values are given as average potency values in the *Pf* 3D7 assay ± SD (*n* ≥ 3).

Figure 2. Methylated analogs. IC₅₀ values are given as average potency values in the *Pf* 3D7 assay (*n* ≥ 3).Table 3. *In Vitro* Potency against *Pf* Dd2 Strain

Compound	<i>Pf</i> 3D7 IC ₅₀ , nM (<i>n</i>)	<i>Pf</i> Dd2 IC ₅₀ , nM (<i>n</i>)
30a	120 ± 20 (3)	78 ± 9 (5)
31a	91 ± 7 (6)	89 ± 9 (5)
32a	51 ± 8 (7)	53 ± 4 (5)

99.9% inhibition and 94% at 10 mg/kg/day. The dose–response data allow us to approximate an ED₉₀ of <10 mg/kg/day

and an ED₉₉ of 30 mg/kg/day. Compounds 30a and 31a did reduce parasitemia at 10 mg/kg/day, but not to the same level as 32a.

Compound concentrations in the plasma were determined at 1, 6, and 24 h post dose on the last day of treatment. Compound concentrations of 32a in the plasma at 24 h remained within 2-fold of the *Pf* 3D7 IC₅₀ (51 nM) only for the most fully efficacious dose of 30 mg/kg/day. At 10 mg/kg/day, compound concentrations at 24 h post dose were 6- to 30-fold lower than their respective *Pf* 3D7 IC₅₀ values. Compound 32a narrowly misses our goal of *in vitro* EC₅₀ < 10 nM but satisfies our goal of *in vivo* ED₉₀ < 10 mg/kg/day.

Conclusions. 4-Aryl 3-acetamide pyrrolidines are potent inhibitors of *P. falciparum* and have good pharmacokinetic properties and oral efficacy in a mouse model of malaria. Compound 32a (CWHM-1552) has an *in vitro* IC₅₀ of 51 nM in the *Pf* 3D7 assay and an *in vivo* ED₉₀ of <10 mg/kg/day and ED₉₉ of 30 mg/kg/day. Surprisingly, the absolute stereochemical preference for this acetamide series is opposite of that for the related carboxamide series we previously described. Unfortunately, the lead compounds for this class have modest affinities for the hERG channel (single digit micromolar) and inhibit CYP 3A4 at submicromolar levels. Additional optimization is needed in order to eliminate these undesired properties from this otherwise promising series of antimalarial compounds.

EXPERIMENTAL SECTION

(±)-(3*R*,4*S*)-1-Benzyl-3-nitro-4-(4-(trifluoromethyl)phenyl)pyrrolidine (6). To a stirred solution of 5 (3.06 g, 14.1 mmol) in dichloromethane (50 mL) was added *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)-benzylamine (4.01 g, 16.9 mmol). The resulting mixture was cooled to 0 °C, and a solution of TFA (0.001 mL, 0.1 equiv) in dichloromethane (1.0 mL) was added dropwise. The reaction mixture was allowed to warm to room temp and stirred for 16 h. The solvent was removed by evaporation *in vacuo*, and the resulting oil was purified by flash column chromatography (petroleum ether/ethyl acetate: 80/20) to give the title compound (3.5 g, 72% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 7.60 (d, *J* = 8.0, 2H), 7.45 (d, *J* = 8.0, 2H), 7.35 (m, 5H), 4.94 (br.s, 1H), 4.06 (br.s, 1H), 3.77 (s, 2H), 3.40 (br.s, 1H), 3.29 (m, 2H), 2.77 (br.s, 1H).

(±)-(3*R*,4*S*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-amine (7). To a flask was added (±)-6 (3.5 g, 10 mmol), ammonium chloride (5.35 g, 100 mmol), iron (0.9 g, 50 mmol), EtOH (40 mL), and water (10 mL). The reaction flask was replaced with argon three times and then heated at 80 °C for 4 h. The reaction was cooled to room temp and filtered through Celite, rinsing with ethyl acetate. The ethyl acetate layer was washed using additional water, then brine, dried over sodium sulfate, and concentrated under vacuum to give 2.56 g (80% yield) of the title compound as a brown solid. The product was then used in the next step without further purification. MS: *m* + 1 = 321.5.

(-)-(5*S*)-*N*-((3*S*,4*R*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-2-(6-methoxynaphthalen-2-yl)propanamide (25a) and (+)-(5*S*)-*N*-((3*R*,4*S*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-2-(6-methoxynaphthalen-2-yl)propanamide (25b). To a suspension of (±)-7 (2.56 g, 8.0 mmol), naproxen (1.7 g, 9.6 mmol), and HATU (3.65 g, 9.6 mmol) in dichloromethane (50 mL) was added triethylamine (2.0 mL, 16.0 mmol). The reaction mixture was stirred at room temperature for 16 h. The mixture was diluted with dichloromethane (50 mL) and washed with sat. sodium carbonate solution then brine, dried over sodium sulfate, filtered, mixed with silica gel, and concentrated. The residue was purified by flash column chromatography (DCM/ethyl acetate: 20/1).

The first eluting compound was (-)-(5*S*)-*N*-((3*S*,4*R*)-1-benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-2-(6-methoxynaphthalen-

Table 4. *In Vitro* Safety Profiling Data^a

Compound	Pf 3D7 IC ₅₀ nM	hERG IC ₅₀ nM	hERG/3D7 Ratio	CYP 3A4 IC ₅₀ nM	CYP 1A2 IC ₅₀ nM	CYP 2D6 IC ₅₀ nM	CYP 2C9 IC ₅₀ nM	CYP 2C19 IC ₅₀ nM
30a	120	5,910	49	871	>100,000	24,500	98,100	45,400
31a	91	2,572	28	159	>100,000	90,020	36,600	29,400
32a	51	2,809	55	2,500	>100,000	40,600	>100,000	49,700

^aPf = *P. falciparum*; IC₅₀ = inhibitory concentration at 50%.Table 5. Mouse Pharmacokinetic Data^a

Compound	<i>t</i> _{1/2} , h	CL _z , mL/min/kg	V _z , L/kg
30a	5.6	7.9	4.2
31a	7.0	10.5	6.2
32a	2.7	13.8	2.1

^aCompounds were dosed by IV cassette at 2 mg/kg/day to male KM mice (*n* = 6). CL_z = apparent rate of clearance; V_z = apparent volume of distribution.

2-yl)propanamide (**25a**), which was obtained as a white solid (1.1 g, 27% yield). TLC *R*_f = 0.55 (DCM/ethyl acetate: 10/1). [α]_D²⁰ –42.1 (*c* 0.58, MeOH). MS: *m* + 1 = 533.1. ¹H NMR (500 MHz, CDCl₃) δ ppm 7.71–7.67 (m, 3H), 7.50 (d, *J* = 7.05, 2H), 7.32 (m, 8H), 7.16 (dd, *J* = 9.0, 2.0, 1H), 7.12 (s, 1H), 4.60 (br.s, 1H), 3.93 (s, 3H), 3.88 (br.s, 1H), 3.83 (br.s, 1H), 3.67 (q, *J* = 7.0, 1H), 3.44 (br.s, 2H), 3.05 (br.s, 1H), 2.91 (br.s, 1H), 2.69 (br.s, 1H), 1.54 (d, *J* = 7.5, 3H).

The second eluting compound was diastereomer (+)-(S)-N-((3*R*,4*S*)-1-benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-2-(6-methoxynaphthalen-2-yl)propanamide (**25b**), which was obtained as a white solid (1.3 g, 31% yield). TLC *R*_f = 0.45 (DCM/ethyl acetate: 10/1). [α]_D²⁰ +99.1 (*c* 0.21, MeOH). MS: *m* + 1 = 533.2. ¹H NMR (500 MHz, CDCl₃) δ ppm 7.65 (dd, *J* = 8.5, 4.5, 2H), 7.60 (s, 1H), 7.38 (d, *J* = 8.0, 2H), 7.32 (m, 6H), 7.12 (m, 4H), 4.58 (br, 1H), 3.92 (s, 3H), 3.78 (br, 2H), 3.65 (q, *J* = 7.0, 1H), 3.26 (t, *J* = 8.5, 1H), 3.15 (br, 1H), 3.04 (t, *J* = 9.0, 1H), 2.90 (br, 1H), 2.60 (t, *J* = 9.0, 1H), 1.51 (d, *J* = 7.0, 3H).

tert-Butyl (3*S*,4*R*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-ylcarbamate (27a). To a suspension of (–)-**25a** (1.1 g, 2.1 mmol) and DMAP (303 mg, 2.5 mmol) in THF (25 mL) was added di-*tert*-butyl dicarbonate (1.5 mL, 6.3 mmol). The reaction mixture was heated to reflux for 4 h. After the solution was cooled to room temp, MeOH (25 mL) and hydrazine (0.32 mL, 10.5 mmol) were added, and the mixture was stirred at room temp for 12 h. The solvent was removed by evaporation in vacuo, and the resulting oil was purified by flash column chromatography (petroleum ether/ethyl acetate: 60/40) to give the title compound (600 mg, 68% yield). MS: *m* + 1 = 421.3. ¹H NMR (400 MHz, CDCl₃) δ ppm 7.39 (d, *J* = 4.4, 2H), 7.33 (d, *J* = 4.4, 2H), 7.28 (m, 5H), 4.93 (br.s, 1H), 4.21 (br.s, 1H), 3.65 (s, 2H), 3.16 (br.s, 2H), 2.97 (t, *J* = 8.4, 1H), 2.69 (br.s, 1H), 2.50 (br.s, 1H), 1.41 (br.s, 9H).

(3*S*,4*R*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-amine (28a). To a suspension of **27a** (600 mg, 1.42 mmol) in dichloromethane (2.0 mL) was added TFA (2.0 mL), and the mixture

was stirred at room temp for 2 h. The solvent was removed in vacuo. The residue was taken up in dichloromethane and washed with saturated sodium carbonate solution followed by saturated sodium chloride. The organic phase was dried over sodium sulfate, filtered, and concentrated under vacuum to give 430 mg (95% yield) of the title compound as a colorless oil. The product was then used in the next step without further purification. MS: *m* + 1 = 321.5.

N-((3*S*,4*R*)-1-Benzyl-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-2-(4-(dimethylamino)phenyl)acetamide (29a). To a suspension of **28a** (430 mg, 1.34 mmol), 2-(4-(dimethylamino)phenyl)acetic acid (288 mg, 1.61 mmol), and HATU (611 mg, 1.61 mmol) in dichloromethane (10 mL) was added triethylamine (0.37 mL, 2.68 mmol). The reaction mixture was stirred at room temp for 16 h. The mixture was diluted with dichloromethane (50 mL) and washed with saturated sodium carbonate solution then brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate: 1/1) to give 483 mg (75% yield) of the title compound as a white solid. MS: *m* + 1 = 482.2.

(–)-2-(4-(Dimethylamino)phenyl)-N-((3*S*,4*R*)-4-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)acetamide (30a). To a flask was added **29a** (483 mg, 1.0 mmol), ammonium formate (315 mg, 5.0 mmol), Pd/C (50 mg, 10%), and MeOH (10 mL). The reaction flask was flushed with argon three times and then heated at 70 °C for 30 min. The reaction was cooled to room temp and filtered through Celite, rinsing with MeOH. The solvent was removed by evaporation in vacuo, and the residue was purified by flash column chromatography (DCM/NH₃ in MeOH: 20/1) to give the title compound 234 mg (60% yield). [α]_D²⁰ –58.2 (*c* 0.22, MeOH). MS: *m* + 1 = 392.6. ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.19 (d, *J* = 7.5, 1H), 7.62 (d, *J* = 8.0, 2H), 7.47 (d, *J* = 8.0, 2H), 6.97 (d, *J* = 8.5, 2H), 6.61 (d, *J* = 9.0, 2H), 4.16 (m, 1H), 3.21 (d, *J* = 3.0, 2H), 3.15 (m, 3H), 2.84 (s, 6H), 2.80 (dd, *J* = 11.2, 8.0, 1H), 2.60 (dd, *J* = 10.8, 6.4, 1H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm 171.0, 149.6, 148.1, 129.7, 128.7, 127.4 (q, *J* = 31.3), 125.5 (t, *J* = 3.8), 124.9 (q, *J* = 270.0), 124.4, 112.8, 58.4, 53.9, 53.5, 51.6, 42.0, 40.7. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₂₁H₂₅F₃N₃O 392.1944; found 392.1962.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.9b00123.

Table 6. *In Vivo* Efficacy of Acetamide Pyrrolidines in *P. chabaudi* ASS Infected Mice

Oral Dose (qd)	% Inh. of Growth ^a	Plasma Compound Concentration (nM) ^b		
		1 h	6 h	24 h
30a, 10 mg/kg/day	61.2 ± 6.6	255 ± 60	181 ± 55	7.4 ± 4.2
31a, 10 mg/kg/day	65.3 ± 11.3	129 ± 22	119 ± 56	14.9 ± 7.0
32a, 3 mg/kg/day	34.0 ± 16.7	114 ± 21	34.1 ± 5.0	6.6 ± 2.0
32a, 10 mg/kg/day	94.1 ± 1.5	389 ± 148	48.9 ± 4.0	1.7 ± 0.3
32a, 30 mg/kg/day	99.9 ± 0.0	1310 ± 420	1150 ± 500	28.5 ± 20.4
CQ, 10 mg/kg/day	99.9 ± 1.1	80.7 ± 9.6	83.4 ± 8.9	68.6 ± 12.0

^aInhibition of parasitemia after 4 days of qd oral dosing. ^bPlasma compound concentrations were determined at the designated hour post dosing on day 4. *n* = 6 animals/group. nd = not determined.

Synthetic procedures and characterization data for compounds **5**, **8–23**, **30b**, and **31–46**, ^1H NMR spectra for (–)-**30a**, (–)-**31a**, and (–)-**32a**, X-ray crystallographic data for **32a**, and biological assays (PDF)

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Author Contributions

M.J.M. designed compounds. J.L., Z.L., and H.M. synthesized compounds. L.D., D.A., S.Z., and X.Li tested compounds for antimalarial activity. X.Liu determined PK of compounds. Y.L. determined absolute stereochemistry of compounds. Y.H. and Z.T. determined the hERG, CYP, and cytotoxicity of compounds. M.J.M., X.C., and M.D.T. designed and directed the experiments. M.J.M. and J.L. wrote the manuscript. All authors have approved of the final version of the manuscript. All authors have given approval to the final version of the manuscript.

Funding

Research reported in this publication conducted at Saint Louis University was supported by Saint Louis University and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R01AI106498. Research reported in this publication conducted at the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, was supported by Bureau of Science and Information Technology of Guangzhou Municipality Grant number 2009Z1-E841 and Natural Science Foundation of China (NSFC) and by the Ministry of Sciences and Technology Key Program (No. 2016YFE0107300). The content is solely the responsibility of the authors and does not necessarily represent the official views of Saint Louis University, the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, the Natural Science Foundation of China, or the National Institutes of Health.

Notes

The authors declare the following competing financial interest(s): X.C., L.D., J.L., H.M., M.D.T., and M.J.M. are inventors on a patent application filed by Legion Pharma claiming compounds in this manuscript. M.D.T. owns stock in Legion Pharma.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Bryan Yeung for helpful discussions.

ABBREVIATIONS

Pf, *Plasmodium falciparum*; PM, plasmepsin; SI, selectivity index; CQ, chloroquine; mg/kg/day, milligrams per kilogram

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