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Antimalarial Activity of Natural and Synthetic Prodiginines

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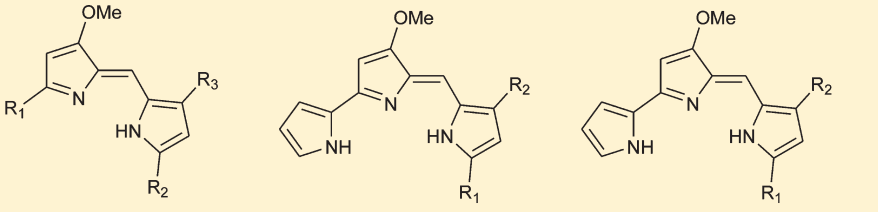
Supporting Information

ABSTRACT:



Prodiginines are a family of linear and cyclic oligopyrrole red-pigmented compounds. Herein we describe the in vitro antimalarial activity of four natural (IC_{50} = 1.7−8.0 nM) and three sets of synthetic prodiginines against *Plasmodium falciparum*. Set 1 compounds replaced the terminal nonalkylated pyrrole ring of natural prodiginines and had diminished activity (IC_{50} > 2920 nM). Set 2 and set 3 prodiginines were monosubstituted or disubstituted at either the 3 or 5 position of the right-hand terminal pyrrole, respectively. Potent in vitro activity (IC_{50} = 0.9−16.0 nM) was observed using alkyl or aryl substituents. Metacycloprodiginine and more potent synthetic analogues were evaluated in a *P. yoelii* murine patent infection using oral administration. Each analogue reduced parasitemia by more than 90% after 25 (mg/kg)/day dosing and in some cases provided a cure. The most favorable profile was 92% parasite reduction at 5 (mg/kg)/day, and 100% reduction at 25 (mg/kg)/day without any evident weight loses or clinical overt toxicity.

INTRODUCTION

Malaria remains a major global health threat and an enormous economic burden to disease-endemic nations. Despite increased attention and new commitment to malaria eradication, the disease causes more than a million deaths each year, and drug resistance to each new chemotherapeutic approach has developed, most notably in *Plasmodium falciparum*, the parasite causing nearly all malaria-related deaths.[1] On the heels of the global spread of chloroquine-resistant (CQR) *P. falciparum*, resistance has also quickly developed to a variety quinoline analogues, to antifolates, to inhibitors of electron transport, and perhaps most ominously, now to artemisinin.[2a,b] It is evident that the search for effective novel antimalarial compounds must be comprehensive and must include explorations of chemotypes distinct from the prototypes in clinical use.

Prodiginines are a family of linear and cyclic oligopyrrole red-pigmented compounds with antibacterial,[3] anticancer,[4] and immunosuppressive activity,[5] produced by actinomycetes and other eubacteria. Some prodiginines induce apoptotic effects, breaking genomic deoxyribonucleic acid (DNA) strands.[6] The prodiginines are also shown to have potent in vitro activity against *Plasmodium* species, at much lower concentrations than seen with mammalian cells.[7a−e] Despite potent and selective in vitro activity against *P. falciparum,* the reported efficacy of prodiginines in vivo has been incomplete, associated with toxicity, or lacking altogether, discouraging their further consideration as antimalarial candidates.

On careful review, however, the previous studies are each limited in the identification or variety of compounds tested, by the lack of in vivo correlation studies, or by the route of drug administration in the animals studied. These limitations leave open the question of whether or not there is suitable antimalarial activity among these compounds. Furthermore, previous studies have been limited to naturally occurring prodiginines, leaving open the possibility that some synthetic analogues may have improved in vitro activity or in vivo efficacy or reduced toxicity. We therefore undertook a more comprehensive assessment of

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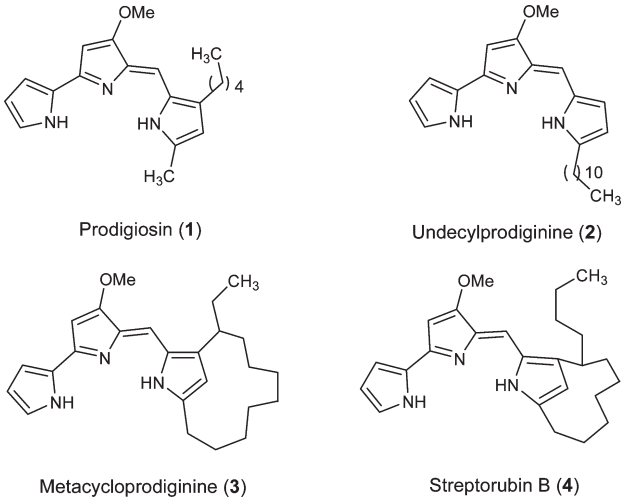
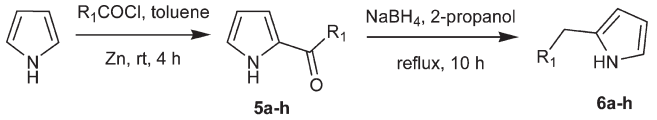


Figure 1. Naturally occurring alkyl prodiginines (**1**−**4**).

Scheme 1. Synthesis of 2-Alkylated Pyrroles (6a−h)



R_1 = n-$C_{10}H_{21}$ = **a**; C_2H_5 = **b**; n-C_3H_7 = **c**; n-C_5H_{11}= **d**

n-C_7H_{15} = **e**; n-$C_{15}H_{31}$= **f**; n-$C_{10}H_{20}NH_2$ = **g**; $C_2H_4CO_2H$= **h**

the antiplasmodial activity of prodiginines, initially reassessing the activity of four naturally occurring prodiginines **1**−**4** (Figure 1) and subsequently assessing a series of synthetic analogues. Here we report these results, including impressive in vitro potency, structure−activity relationship (SAR), and evidence of in vivo efficacy, including curative efficacy in mice after oral administration.

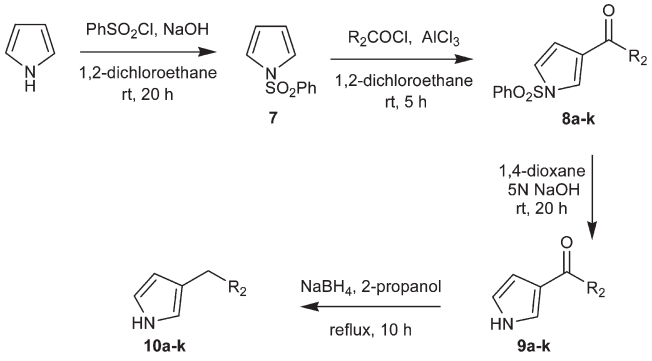
RESULTS AND DISCUSSION

Synthesis. The synthesis of various 2-alkylpyrrole segments **6a**−**h** (Scheme 1) was initiated by converting the readily accessible pyrrole to the corresponding 2-acylpyrroles **5a**−**h**.[8] These acylpyrroles were then smoothly converted to **6a**−**h** using an excess of $NaBH_4$ in 2-propanol under reflux.[9]

Synthesis of 3-alkylpyrrole segments **10a**−**k** (Scheme 2) used the same pyrrole starting material, which treated with phenylsulfonyl chloride in the presence of NaOH provided a *N*-phenylsulfonylpyrrole (**7**).[10] Use of $AlCl_3$ permitted the regioselective acylation of **7** at the 3 position with the acyl chlorides to obtain the *N*-phenylsulfonyl-3-acylpyrroles **8a**−**k**, which were subsequently hydrolyzed under basic (5 N NaOH) conditions to give 3-acylpyrroles **9a**−**k**.[11] In the final step **9a**−**k** were reduced to **10a**−**k** using $NaBH_4$ under reflux.[9]

By use of literature methodologies, the key intermediates involved in the synthesis of prodiginines, 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**13**) and its analogues **14**−**17**, were prepared via intermediate **12** from the commercially available 4-methoxy-3-pyrrolin-2-one (**11**) (Scheme 3).[12] Acid-catalyzed condensation of appropriate alkylpyrrole segments (2,4-dimethylpyrrole, **6a**−**h**, and **10a**−**k**) with **13**−**17** (Scheme 4)[13]

Scheme 2. Synthesis of 3-Alkylated Pyrroles (10a−k)



R_2 = *i*Pr = **a**; n-C_3H_7 = **b**; n-C_5H_{11} = **c**; n-C_7H_{15} = **d**; C_9H_{19} = **e**; n-$C_{15}H_{31}$= **f**

C_6H_5 = **g**; 4-$OCH_3C_6H_5$ = **h**; 4-ClC_6H_5 = **i**; 4-BrC_6H_5 = **j**; 2-naphthyl = **k**

gave the desired prodiginine analogues **18**−**25** (Table 1) and **26**−**43** (Tables 2 and 3).

A new synthetic strategy, outlined in Scheme 5, was used to synthesize 3,5-disubstituted prodiginine analogues **46**−**55** (Table 2) and **56**−**75** (Table 3). The 2,4-disubstituted pyrroles **44** were synthesized from the corresponding 2- or 3-acylpyrroles (**5** or **9**) with their corresponding acyl chlorides and $AlCl_3$.[14] Subsequent reduction with $NaBH_4$ gave the desired 2,4-disubstituted pyrroles **45**.[9] Acid-catalyzed condensation of these **45** with **13** provided the desired 3,5-disubstituted prodiginine analogues **46**−**75** (Tables 2 and 3).[13]

Biological Activity. The natural and synthetic prodiginines were assayed for their in vitro antimalarial activity against *P. falciparum* pansensitive D6 with chloroquine (CQ) as a reference drug. A direct comparative assessment of the antimalarial activity of natural products prodigiosin (**1**), undecylprodiginine (**2**), metacycloprodiginine (**3**), and streptorubin B (**4**) (Figure 1) revealed they all had remarkably potent activity with very low IC_{50} values (8, 7.7, 1.7, and 7.8 nM, respectively) against *P. falciparum* strain D6 and were slightly more active than CQ (11 nM). The natural prodiginine **3** was consistently observed to be the most potent of the four natural products and demonstrated that changes in the alkyl component of prodiginines affected the in vitro antimalarial activity. On the basis of these observations, a series of new analogues of prodiginines with similar core structure were generated.

In the early stages, a set of analogues **18**−**25** (Table 1), in which an indole, phenyl, thiophen, and furan replaced the terminal nonalkylated pyrrole ring (left-hand side) of the core moiety, were generated. In vitro analysis of the activity of these compounds against *P. falciparum* pansensitive D6 demonstrated activity (IC_{50} > 2920 nM) significantly diminished relative to that of the natural prodiginines **1**−**4** (IC_{50} = 1.7−8.0 nM). This work suggested that the left-hand side pyrrole ring of the prodiginines is important for the potent antimalarial activity. We subsequently tested a hypothesis that the alkyl chain on right-hand side pyrrole of the prodiginines might represent an opportunity to make potent and selective antimalarials with the desired "druglike" properties. Subsequent analogues were synthesized (Schemes 4 and 5) to obtain a SAR for the alkyl groups at the terminal alkylated pyrrole of the prodiginines.

One set of these analogues **26**−**38** (Table 2) and **39**−**43** (Table 3) were monosubstituted and contained different alkyl

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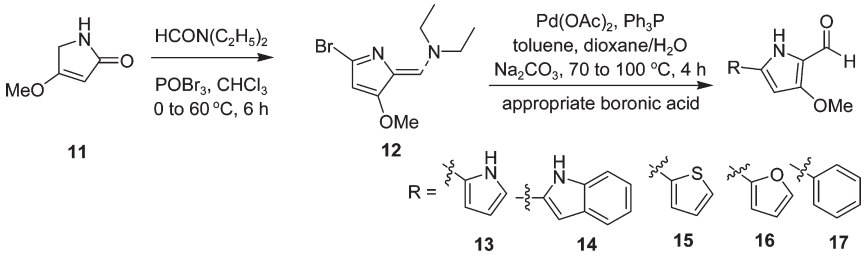
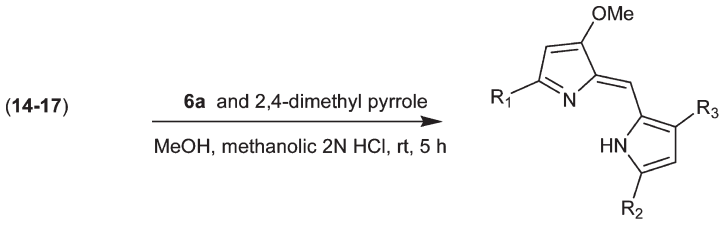
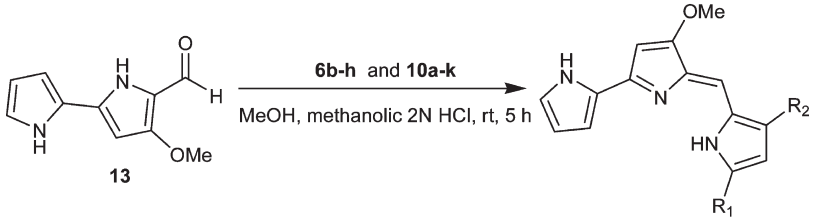
Scheme 3. Synthesis of 4-Methoxy-2,2′-bipyrrole-5-carbaldehyde (13) and Its Analogues (14−17)



Scheme 4. Synthesis of Various Prodiginine Analogues (18−43)



R_1 = Phenyl, 2-heteroaryl groups; R_2, R_3 = CH_3 or R_2 = n-$C_{11}H_{23}$, R_3 = H (**18-25**)



R_1 = Alkyl groups, R_2 = H (**26-31**); R_1 = H; R_2 = Alkyl/aryl groups (**32-43**)

chains or aryl groups at either the 3 or 5 position of the terminal pyrrole ring (right-hand side) (Scheme 4). These compounds were screened for their in vitro antimalarial activity against *P. falciparum* strain D6, and the results are shown in Tables 2 and 3. Of the monoalkylated analogues, compounds **26**, **27**, **31**, **32**, and **38** showed the poorest activity with IC_{50} values above 1700 nM against D6. Compounds **28**, **30**, and **33** all exhibited slightly better activity with IC_{50} values 375, 300, and 460 nM, respectively, against D6. Analogues **29**, **34**, **35**, **36**, and **37** were the most potent of the monoalkylated prodiginine analogues with IC_{50} values of 80, 80, 28, 4.6, and 8.0 nM, respectively, against D6. These observations and the activity of the natural product **2** demonstrate that the C_6-C_{11} alkyl chain length at either the 3 or 5 position of the right-hand side pyrrole ring is required for optimal activity of monoalkylated prodiginines. The presence of either an amine group (see **31** IC_{50} = 1700 nM (D6) versus **2** IC_{50} = 7.7 nM (D6)) or methyl carboxylate (see **32** IC_{50} = 4500 nM (D6) versus **34** IC_{50} = 80 nM (D6)) at the end of the alkyl chain leads to a decrease in activity. The 3-aryl monosubstituted prodiginine analogues **39**−**43** (Table 3) exhibited moderate inhibitory activity with IC_{50} values of 83, 170, 65, 90, and 56 nM, respectively, against D6. While the presence of a halogen substituent appeared to lead to an increase in activity (see **41** IC_{50} = 65 nM (D6) versus **39** IC_{50} = 83 nM (D6) and **40** IC_{50} = 170 nM (D6)), the activity was poorer than that seen for the more potent monoalkylated prodiginines (such as **2** and **35**−**37**).

The final set of prodiginine analogues generated had substituents at both positions 3 and 5 of the terminal pyrrole ring (right-hand side). The substituents were two alkyl chains (**46**−**55**), an alkyl chain and an aryl group (**56**−**65**), or two aryl groups (**66**−**75**). These compounds **46**−**75** (Tables 2 and 3) were screened for in vitro animalarial activity against *P. falciparum* strain D6. Among the dialkylated analogues, **49**, **50**, and **51** showed potent in vitro antimalarial activity with IC_{50} values of 1.7, 1.7, and 2.1 nM, respectively (Table 2), against D6. Other dialkylated prodiginines **47**, **48**, **52**, **53**, and **55** also exhibited excellent activity with IC_{50} values of 4.5, 2.9, 4.9, 6.2, and 5.3 nM, respectively, against D6 and demonstrated that they are more active than the monoalkylated prodiginines (**2** and **26**−**38**). The two exceptions were analogues **46** (IC_{50} = 8900 nM) and **54** (IC_{50} = 92 nM), which contained a total alkyl chain length of 2 and 16 carbons, respectively, on the right-hand side pyrrole ring of the core moiety. From both the mono- and dialkylated series the data clearly show that prodiginines containing a combined C_8-C_{15} alkyl chain length had the most potent in vitro antimalarial activity (Figure 2).

The analogues **56**−**65**, which contain one alkyl and one aryl substituents on the right-hand pyrrole ring were also tested for in

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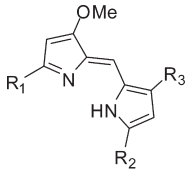
Table 1. In Vitro Antimalarial Activity of Prodiginine Analogues Where the Nonalkylated Terminal Pyrrole (Left-Hand Side) Has Been Substituted



18-25

Compd	R_1	R_2	R_3	IC_{50} (nM)		Cytotoxicity (nM)
				D6	Dd2	
CQ	-	-	-	11	135	16000
18		CH_3	CH_3	4250	4950	< 125
19		n-$C_{11}H_{23}$	H	4060	6150	3410
20		n-$C_{11}H_{23}$	H	10470	15750	3580
21		CH_3	CH_3	19410	13640	7950
22		n-$C_{11}H_{23}$	H	2920	3810	980
23		CH_3	CH_3	> 25000	> 25000	3530
24		n-$C_{11}H_{23}$	H	5940	7770	2120
25		CH_3	CH_3	> 25000	> 25000	3890

vitro antimalarial activity against *P. falciparum* strain D6. Among these analogues, **62** (IC_{50} = 0.9 nM) and **63** (IC_{50} = 1.3 nM) (Table 3) were the most potent and comparable to the most potent dialkylated prodiginines **49**−**51** and the natural prodiginine **3** (IC_{50} = 1.7 nM). In this series (**56**−**65**), all compounds with the exception of **60** (IC_{50} = 16.0 nM) were extremely potent (IC_{50} < 6.3 nM), significantly more than the corresponding analogues **39**−**43** which have an aryl substituent but lack the alkyl substituent. The in vitro activity results of chloro (**56**−**61**), fluoro (**62** and **63**), and bromo (**64** and **65**) substituted analogues of prodiginine indicated that the bromo substituted analogues **64** and **65** were slightly less potent than chloro and fluoro substituted analogues (Table 3).

The final set of analogues **66**−**75** contains two aryl substituent groups on the right-hand side pyrrole ring. In this set, most of the compounds (**66**, **67**, **69**−**71**, **74**, and **75**) exhibited potent activity (IC_{50} < 8.3 nM). The exceptions were **68** (IC_{50} = 14.0 nM), which contains a bromine atom on both aryl substituents, and **72** (IC_{50} = 12.6 nM) and **73** (IC_{50} = 14.7 nM) in which each aryl group is dihalogenated (Table 3). A comparison of the most potent compounds in this series with analogues **47**−**65** revealed that the in vitro activity for prodiginines bearing two aryl substituents is slightly less than for disubsititued prodiginines in which either one or both of the substituents are alkyl chains. That said, a general pattern of more potent in vitro activity for disubstutued prodignines compared to monosubstituted prodiginines was observed.

The majority of the new synthetic prodiginines were also tested alongside CQ against the multidrug-resistant (MDR) Dd2. In all cases there was no significant change in the activity of each analogue between the D6 and Dd2 (Tables 1−3), indicating no cross-resistance with CQ. The most potent synthetic prodiginines were active in the 1 nM range against Dd2, compared to 135 nM for CQ.

A number of the synthetic prodiginines were tested against a number of mammalin cell lines, and IC_{50} in the 500−2000 nM range was not observed (data not shown). A general assay with mitogen stimulated murine splenic lymphocytes was used to evaluate all the synthetic prodiginines (Tables 1−3) and to determine if synthetic changes led to reduced toxicity.[15] The majority of the synthetic prodiginines exhibited much greater toxicity (IC_{50} < 125 nM) in this particular assay. There were prodiginines that had slightly less toxicity (1000−4000 nM), but these were in all cases also associated with markedly reduced activitiy against *P. falciparum* and were still more toxic than CQ (16 000 nM). Thus, by use of these particular in vitro assays, the antimalarial activity and toxicity for the current set of synthetic prodiginines exhibit comparable SAR.

The in vivo antimalarial efficacy of the natural prodiginine **3** and a selection of the more potent synthetic analogues were evaluated in a *P. yoelii* murine patent infection. In initial proof-of-principle testing, administration of 25 (mg/kg)/day **3** by gavage using a caprylic/capric triglyceride vehicle resulted in greater than 99% reduction in parasitemia (0.28% vs 54.1% in

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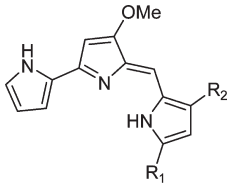
Table 2. In Vitro Antimalarial Activity of Prodiginines Containing Alkyl Substituents at the 3 and 5 Positions of the Terminal Pyrrole Ring[a]



26-38 and **46-55**

Compd	R_1	R_2	IC_{50} (nM)		Cytotoxicity (nM)
			D6	Dd2	
CQ	-	-	11	135	16057
26	n-C_3H_7	H	2300	N.D.	N.D.
27	n-C_4H_9	H	1780	1590	< 125
28	n-C_6H_{13}	H	375	450	< 125
29	n-C_8H_{17}	H	80	130	< 125
30	n-$C_{16}H_{33}$	H	300	400	< 125
31	n-$C_{11}H_{22}NH_2$	H	1700	N.D.	N.D.
32	H	$(CH_2)_3COOCH_3$	4500	N.D	N.D.
33	H	$CH_2CH(CH_3)_2$	460	230	< 125
34	H	n-C_4H_9	80	18	< 125
35	H	n-C_6H_{13}	28	7	< 125
36	H	n-C_8H_{17}	4.6	1.8	< 125
37	H	n-$C_{10}H_{21}$	8.0	10	< 125
38	H	n-$C_{16}H_{33}$	> 25000	> 25000	3628
46	CH_3	CH_3	8900	8130	< 125
47	n-C_6H_{13}	n-C_3H_7	4.5	4.0	< 125
48	n-C_8H_{17}	n-C_3H_7	2.9	2.7	< 125
49	n-C_3H_7		1.7	1.3	< 125
50	n-C_6H_{13}	n-C_6H_{13}	1.7	1.1	< 125
51	n-C_7H_{15}	n-C_6H_{13}	2.1	1.2	< 125
52	n-C_6H_{13}	n-C_8H_{17}	4.9	2.0	< 125
53	n-C_7H_{15}	n-C_8H_{17}	6.2	2.9	3906
54	n-C_8H_{17}	n-C_8H_{17}	92	129	31250
55			5.3	3.5	< 125

[a] N.D.: not determined.

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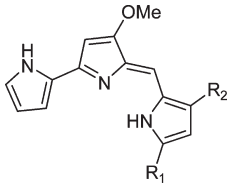
Table 3. In Vitro Antimalarial Activity of Prodiginines Containing Aryl Substituents at Either the 3 Position or the 3 and 5 Positions of the Terminal Pyrrole Ring[a]



39-43 and **56-75**

Compd	R_1	R_2	IC_{50} (nM)		Cytotoxicity (nM)
			D6	Dd2	
CQ	-	-	11	135	16057
39	H	$C_6H_5CH_2$	83	86	< 125
40	H	4-$OCH_3C_6H_4CH_2$	170	156	< 125
41	H	4-$ClC_6H_4CH_2$	65	81	< 125
42	H	4-$BrC_6H_4CH_2$	90	108	< 125
43	H	2-NaphthylCH_2	56	N.D.	N.D.
56	C_2H_5	4-$ClC_6H_4CH_2$	6.3	6.2	< 125
57	n-C_3H_7	4-$ClC_6H_4CH_2$	3.0	2.6	< 125
58	n-C_6H_{13}	4-$ClC_6H_4CH_2$	2.0	1.8	< 125
59	n-C_7H_{15}	4-$ClC_6H_4CH_2$	2.8	2.2	< 125
60	n-C_8H_{17}	4-$ClC_6H_4CH_2$	16.0	12.0	1105
61	4-$ClC_6H_4CH_2$		3.9	2.9	1007
62	n-C_6H_{13}	4-$FC_6H_4CH_2$	0.9	0.9	1462
63	n-C_8H_{17}	4-$FC_6H_4CH_2$	1.3	1.2	< 125
64	n-C_6H_{13}	4-$BrC_6H_4CH_2$	2.9	2.8	< 125
65	n-C_8H_{17}	4-$BrC_6H_4CH_2$	4.0	2.9	< 125
66	4-$ClC_6H_4CH_2$	4-$ClC_6H_4CH_2$	6.1	4.8	< 125
67	4-$FC_6H_4CH_2$	4-$FC_6H_4CH_2$	5.6	5.7	< 125
68	4-$BrC_6H_4CH_2$	4-$BrC_6H_4CH_2$	14.0	11.0	< 125
69	4-$FC_6H_4CH_2$	4-$ClC_6H_4CH_2$	6.1	6.1	< 125
70	4-$BrC_6H_4CH_2$	4-$ClC_6H_4CH_2$	8.3	7.7	< 125
71	4-$BrC_6H_4CH_2$	4-$FC_6H_4CH_2$	5.7	5.1	< 125
72	2,4-$Cl_2C_6H_3CH_2$	2,4-$Cl_2C_6H_3CH_2$	12.6	11.0	< 125
73	2,6-$F_2C_6H_3CH_2$	2,6-$F_2C_6H_3CH_2$	14.7	18.3	< 125
74	3-$FC_6H_4CH_2$	3-$FC_6H_4CH_2$	5.1	6.7	< 125
75	2-$ClC_6H_4CH_2$	2-$ClC_6H_4CH_2$	3.6	4.9	< 125

[a] N.D.: not determined.

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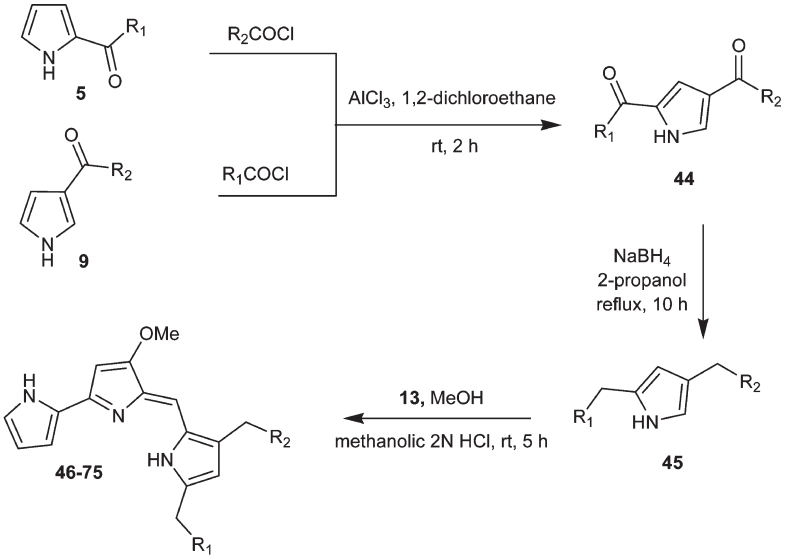
Scheme 5. Synthesis of 2,4-Disubstitued Pyrroles (45) and the Corresponding Prodiginine Analogues (46−75)



R_1, R_2 = Alkyl groups (**46-55**); R_1 = Alkyl/aryl; R_2 = Aryl/alkyl (**56-65**); R_1, R_2 = Aryl groups (**66-75**)

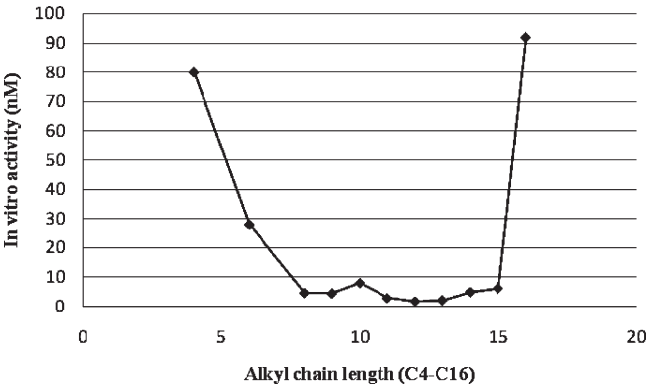


Figure 2. SAR of combined alkyl chain length for mono- and dialkylated prodiginines and in vitro antimalarial activity against D6 strain of *P. falciparum*.

controls). At 100 (mg/kg)/day parasite clearance was complete and was accompanied by temporary weight loss, and three of four mice were cured. These data provided the first demonstration of oral effectiveness of prodiginines. Equally important, unlike previous reports of parenteral dosing, efficacy after gavage dosing was observed to be without evident toxicity. These findings indicated that antiplasmodial oral efficacy might be achievable and led to similar in vivo testing of some of the more potent synthetic prodiginines. Examples of impressive potency against murine malaria were observed and are briefly summarized here (Table 4). Analogues **48**, **49**, **57**, **58**, and **66** each reduced parasitemia by more than 90%; the exceptions were **41** (38%) and **60** (72%) after 5 (mg/kg)/day dosing, without any evidence of weight loss or toxicity. **41**, **48**, **49**, **57**, **58**, **60**, and **66** each reduced parasitemia by ≥90% after 25 (mg/kg)/day dosing; however, weight loss was observed during the dosing course with a few analogues (Table 4). However, the weight reduction was temporary and for unknown reasons (including dehydration, fasting of tested mice, or toxicity

Table 4. In Vivo Antimalarial Efficacy of Prodiginine Analogues in *P. yoelii* Murine Infection

compd	dose, (mg/kg)/day	no. of treated mice	% group mean weight, day 3 vs day 0	% parasitemia reduction vs controls, day3
controls		5	100.8	0
41	5	5	101.2	38
41	25	5	105.0	90
48	5	5	100.1	90
48	25	4	92.4	100
49	5	5	101.5	97
49	25	4	84.5	100
57	5	5	102.9	94
57	25	5	87.6	100
58	5	5	100.3	99
58	25	4	96.0	100
60	5	5	100.2	72
60	25	3	93.4	100
66	5	5	107.5	92
66	25	5	102.5	100

at the higher dose). While an exhaustive in vivo analysis of prodiginine analogues was not conducted, the temporary weight loss was primarily associated with analogues with alkyl substituents. The prodiginines **41** and **66** by contrast have no alkyl substituents and have one or two aryl substituents. Of these compounds tested, **66** had the most favorable profile: 92% parasite reduction at 5 (mg/kg)/day, 100% reduction at 25 (mg/kg)/day without any evident weight loss or clinical overt toxicity. Although testing consisted only of equidose comparisons between compounds, without full dose ranging or efforts to define curative dose levels, it is worth mentioning that there was one cure each in the **60** and **58** treated groups (25 (mg/kg)/day).

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CONCLUSIONS

Although natural prodiginines **3**[7d] and heptylprodigiosin (**76**)[7e,16] have demonstrated remarkable in vitro potency against growth of *P. falciparum*, the mode of action is unknown, and a SAR has not been reported. Herein, we have prepared a set of prodiginine analogues via simple and efficient pathways from commercially available inexpensive starting materials and reagents. These standardized protocols can be used to generate a large number of prodiginines in a gram scale from a single batch. This current work has suggested that the nonalkylated terminal pyrrole (left-hand side) of the natural prodiginines is required for in vitro activity but that the substituents at the alkylated terminal pyrole (right-hand side) can be varied. Specifically in vitro activity comparable or better ($IC_{50} \leq 1$ nM) than most of the natural prodiginines was observed for analogues with either alkyl or aryl substituents at both the 3 and 5 positions of the right-hand side pyrrole, In addition these synthetic prodiginines are equally effective against *P. falciparum* pansensitive D6 and MDR Dd2.

Previous results of in vivo testing, have also cast doubt on the suitability of prodiginines as antimalarial candidates. Against *P. berghei* in mice, subcutaneous injection of natural prodiginines **1**,[7a] **76**,[7e] **2**, **3**, butylcycloheptylprodiginine (**77**), and cyclononylprodiginine (**78**)[7b,16] have each been shown to extend survival, however, either without cure or at doses resulting in more toxic deaths than cures. Natural prodiginine **1** was shown to be completely ineffective for antimalarial activity, even at toxic doses, for oral treatment of Rhesus monkeys infected with *P. cynomolgi*.[7c] In the current study it has been demonstrated for the first time that prodiginines can be administered orally and produce marked parasite clearance, including cures in some cases, without evident weight lose and toxicity. Nonetheless, preliminary in vitro assays indicate concerns associated with the toxicity of prodiginines, and the quest for analogues with improved antimalarial activity but reduced toxicity continues.

EXPERIMENTAL SECTION

Purification of Natural Prodiginines (1−4). Natural product **1** was purchased from the developmental therapeutics program NCI/NIH. By use of minor modifications to literature procedures, **2** and **4** were isolated from *S. coelicolor* M511[17] and **3** was isolated from *S. longisporus ruber*.[13a]

General. ^{1}H NMR spectra were recorded with a Bruker AMX-400 MHz spectrometer using $CDCl_3$. ^{13}C NMR spectra were recorded at 100 MHz with $CDCl_3$. Chemical shifts are reported as values in ppm relative to $CHCl_3$ (7.26) in $CDCl_3$, and TMS was used as internal standard. HRMS (ESI) data were recorded on a high-resolution (30 000) thermo LTQ-Orbitrap Discovery hybrid mass spectrometer (San Jose, CA). Reagents were from Aldrich and used as supplied. Unless otherwise noted, reactions that required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of argon/nitrogen. Chromatography was executed with silica gel (230−400 mesh) and neutral alumina using mixtures of ethyl acetate and hexane as eluents. Analytical HPLC analyses were performed on a Supelco Discovery HS C18 column (4.6 mm × 250 mm) with a linear elution gradient ranging from $CH_3OH/CH_3CN/H_2O$ (40%/10%/50%) to CH_3OH (100%) in 0.15% trifluoroacetic acid at a flow rate of 1 mL/min. A purity of >95% has been established for all tested compounds with the exception of **25**, **26**, and **33**, which achieved 93%, 81%, and 86%, respectively.

Representative Procedure for the Synthesis of 1-(1*H*-Pyrrol-2-yl)octan-1-one (5e). A mixture of pyrrole (2.0 g, 29.8 mmol), octanoyl chloride (7.8 g, 44.7 mmol), and zinc powder (3.88 g, 59.7 mmol) in toluene (50 mL) was stirred at room temperature for 4 h. The reaction mixture was quenched with saturated sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was chromatographed on silica gel to afford the title compound **5e** (3.16 g, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.71 (br s, 1H), 7.02 (m, 1H), 6.91 (m, 1H), 6.27 (m, 1H), 2.75 (t, J = 7.5 Hz, 2H), 1.70 (m, 2H), 1.33 (m, 8H), 0.92 (t, J = 7.1 Hz, 3H).

Representative Procedure for the Synthesis of 2-Octyl-1*H*-pyrrole (6e). To a stirred solution of **5e** (1.0 g, 5.18 mmol) in 150 mL of 2-propanol (IPA) at 25 °C was added slowly sodium borohydride ($NaBH_4$) (1.34 g, 36.26 mmol), and the reaction mixture was heated at reflux for 10 h. The hot reaction mixture was poured into 150 mL of ice−water, and the solution was acidified with 10% aqueous HCl. The suspension was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel to afford the title compound **6e** (714 mg, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (br s, 1H), 6.56 (m, 1H), 6.04 (m, 1H), 5.83 (m, 1H), 2.50 (t, J = 7.6 Hz, 2H), 1.53 (m, 2H), 1.28 (m, 10H), 0.79 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.9, 116.0, 108.2, 104.8, 31.9, 29.7, 29.5, 29.4, 29.3, 27.8, 22.7, 14.1.

Synthesis of Compound 7. Pyrrole (10 g, 149 mmol) was added to a well-agitated suspension of NaOH (17.9 g, 447 mmol) in 100 mL of 1,2-dichloroethane. This mixture was then cooled to 0 °C and stirred for 10 min. Then a solution of phenylsulfonyl chloride (31.5 g, 173 mmol) in 20 mL of 1,2-dichloroethane was added dropwise over a period of 20 min. Thirty minutes after the completion of addition, the mixture was allowed to come to room temperature and left stirring overnight. The reaction was quenched by pouring the mixture into 300 mL of distilled water. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic extract was washed with distilled water to neutrality and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo gave **7** as a white crystalline solid (21.6 g, 70%). This solid was subsequently washed with hot hexane to give greater than 65% overall yield.

Representative Procedure for the Synthesis of (1-Benzenesulfonyl-1*H*-pyrrol-3-yl)-(4-chlorophenyl)methanone (8i). To a stirred suspension of anhydrous $AlC1_3$ (3.85 g, 29 mmol) in 50 mL of 1,2-dichloroethane at 25 °C was added slowly octanoyl chloride (5.0 g, 27 mmol). The resulting solution was stirred at 25 °C for 10 min. A solution of **7** (5.0 g, 24 mmol) in 10 mL of 1,2-dichloroethane was added slowly, and the mixture was stirred at 25 °C for 5 h. The reaction was quenched with ice and water, and the product was extracted into dichloromethane (3 × 100 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to afford crude product **8i** (8.0 g, 100%) as a white solid. The crude product was used for the next step without further purification.

Representative Procedure for the Hydrolysis of 8i. A solution of **8i** (8.0 g, 24 mmol) in 100 mL of 1,4-dioxane was stirred with 100 mL of 5 N NaOH at 25 °C for 20 h. The organic layer was collected, and the aqueous layer was thoroughly extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give (4-chlorophenyl)-(1*H*-pyrrol-3-yl)methanone (**9i**) as a solid material (4.6 g, 100%).

Synthesis of (5-Bromo-3-methoxypyrrol-2-ylidenemethyl)diethylamine (12). To a mixture of diethylformamide (2.68 g, 26.5 mmol) and chloroform (10 mL) at 0 °C was added

dropwise a solution of phosphorus oxybromide (6.32 g, 22.1 mmol) in chloroform (10 mL). The resulting suspension was stirred at 0 °C for 30 min, and the solvent was removed by rotary evaporation to obtain the Vilsmeier complex as a white solid. After the sample was dried in vacuo for 20 min, chloroform (10 mL) was added to the solid and the mixture was cooled to 0 °C. A solution of 4-methoxy-3-pyrrolin-2-one (**11**) (1.0 g, 8.8 mmol) in chloroform (20 mL) was added dropwise, and the mixture was warmed to room temperature and then heated at 60 °C for 5 h. The mixture was poured onto ice−water (75 mL), and the pH of the aqueous solution was adjusted to pH 7−8 by treatment with 2 N NaOH. Ethyl acetate (40 mL) was added to the resulting precipitate, and the mixture was filtered over Celite to remove the black solid containing phosphorus salts. The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with brine (3 × 200 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent was removed by rotary evaporation to furnish the crude bromoenamine **12**. The residue was filtered over a pad of silica gel (50 mL) using a 10% EtOAC/hexanes as eluent to obtain the enamine as an oil, which upon drying in vacuo led to a solid (1.64 g, 72%). ^{1}H NMR ($CDCl_3$, 100 MHz) δ 6.90 (s, 1H), 5.50 (s, 1H), 4.05 (q, J = 7.1 Hz, 2H), 3.65 (s, 2H), 3.30 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ165.3, 138.7, 133.5, 120.8, 96.4, 58.0, 51.2, 44.5, 18.3, 21.2.

Representative Procedure for the Preparation of Compound 13. To a degassed solution of toluene (5 mL) were added $Pd(OAc)_2$ (303 mg, 1.3 mmol) and PPh_3 (1.6 g, 6.1 mmol). The mixture immediately turned bright yellow and was stirred at 70 °C for 30 min under N_2. A solution of **12** (3.5 g, 13.5 mmol) and *N*-Boc-2-pyrroleboronic acid (3.72 g, 17.6 mmol) in 10% water/dioxane (50 mL) was degassed and purged with N_2. The solution was transferred to a suspension of $Pd(PPh_3)_4$ in toluene followed by the addition of Na_2CO_3 (4.31 g, 40.6 mmol). The mixture was stirred for 3 h at 100 °C and then poured into water (100 mL). The pH of the solution was lowered to pH 7 with 2 N HCl. The brown precipitate was recovered by filtration over a fritted disk funnel and washed with water and then acetone. The yellow solid was washed with 10 mL of $CHCl_3$ and then Et_2O (2 × 10 mL). The desired product **13** was obtained as a yellow solid and used without further purification (2.32 g, 90%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.40 (br s, 1H), 11.20 (br s, 1H), 9.30 (s, 1H), 6.90 (t, J = 4.4 Hz, 1H), 6.75 (s, 1H), 6.25 (d, J = 4.4 Hz, 1H), 6.10 (d, J = 4.4 Hz, 1H), 3.80 (s, 3H).

Representative Procedure for the Synthesis of 4′-Methoxy-5′-(3-octyl-1*H*-pyrrol-2-ylmethylene)-1*H*,5′*H*-[2,2′]bipyrrolyl (36). To a stirred suspension of **13** (220 mg, 1.15 mmol) and compound **10d** (269 mg, 1.5 mmol) in anhydrous methanol (10 mL) was added methanolic 2 N HCl (catalytic amount). The resulting bright colored solution was stirred for 5 h at room temperature. The methanol was removed under reduced pressure. The crude solid was dissolved in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ solution (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was chromatographed on neutral alumina as stationary phase and hexane−ethyl acetate as mobile phase to afford the final prodiginine analogue **36** (256 mg, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (s, 1H), 6.77 (m, 1H), 6.72 (dd, J = 1.2, 2.4 Hz, 1H), 6.60 (d, J = 2.4 Hz, 1H), 6.21 (dd, J = 0.9, 2.7 Hz, 1H), 6.06 (s, 1H), 5.97 (d, J = 2.5 Hz, 1H), 4.00 (s, 3H), 2.58 (t, J = 7.6 Hz, 2H), 1.57 (m, 2H), 1.28 (m, 10H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.5, 159.8, 139.8, 134.6, 128.5, 126.8, 125.3, 122.5, 113.4, 112.6, 110.4, 110.2, 95.1, 58.4, 31.9, 31.4, 29.5 (2C), 29.3, 26.1, 22.7, 14.1. HRMS (ESI) calcd for $C_{22}H_{29}N_3O$ $(M + H)^+$, 352.2383; found, 352.2387. Most of these prodiginine analogues were in the form of HCl salt (addition of ethereal HCl to the pure prodiginine analogue).

Representative Procedure for the Synthesis of 1-(5-Octanoyl-1*H*-pyrrol-3-yl)octan-1-one (44). To a stirred suspension of anhydrous $AlCl_3$ (1.34 g, 10.1 mmol) in 30 mL of 1,2-dichloroethane at 25 °C was added slowly octanoyl chloride (1.34 g, 8.1 mmol). The resulting solution was stirred at 25 °C for 10 min. A solution of **9i** (1.3 g, 6.7 mmol) in 10 mL of 1,2-dichloroethane was added slowly, and the mixture was stirred at 25 °C for 2 h. The reaction was quenched with ice and water, and the product was extracted into dichloromethane (3 × 50 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to afford **44** (2.14 g, 100%) as a white solid. The crude product was washed with hot hexane to give greater than 96% of overall yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.26 (br s, 1H), 7.60 (dd, J = 1.5, 1.9 Hz, 1H), 7.32 (dd, J = 0.9, 1.5 Hz, 1H), 2.78 (m, 4H), 1.72 (m, 4H), 1.38−1.25 (m, 16H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.1, 192.2, 132.6, 127.5, 127.2, 115.2, 39.8, 38.1, 31.7 (2C), 29.4, 29.3, 29.1, 29.0, 25.1, 24.7 (2C), 22.6, 14.1 (2C).

Representative Procedure for the Synthesis of 2,4-Dioctyl-1*H*-pyrrole (45). To a stirred solution of **44** (1.3 g, 4.0 mmol) in 150 mL of 2-propanol at 25 °C was added slowly sodium borohydride (2.11 g, 57.0 mmol), and the reaction mixture was heated at reflux for 10 h. The hot reaction mixture was poured into 150 mL of ice−water, and the solution was acidified with 10% aqueous HCl. The suspension was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give **45**, and the crude product was chromatographed on neutral alumina to afford the title compound **45** (865 mg, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (br s, 1H), 7.40 (s, 1H), 5.76 (s, 1H), 2.53 (t, J = 7.6 Hz, 2H), 2.41 (t, J = 7.6 Hz, 2H), 1.57 (m, 4H), 1.33 (m, 18H), 0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.8, 124.9, 112.8, 105.4, 31.9 (2C), 31.2, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.9, 27.4, 27.2, 22.7 (2C), 14.1 (2C).

Representative Procedure for the Synthesis of 4′-Methoxy-5′-(5-octyl-3-propyl-1*H*-pyrrol-2-ylmethylene)-1*H*,5′*H*-[2,2′]bipyrrolyl, Hydrochloride (48). To a stirred suspension of **13** (100 mg, 0.52 mmol) and compound **45** (151 mg, 0.68 mmol) in anhydrous methanol (10 mL) was added methanolic 2 N HCl (Catalytic amount). The resulting bright colored solution was stirred for 5 h at room temperature. The methanol was removed under reduced pressure. The crude solid was dissolved in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ solution (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was chromatographed on neutral alumina as stationary phase and hexane−ethyl acetate as mobile phase to afford the final prodiginine analogue **48** (143 mg, 69%). Most of these prodiginine analogues were in the form of HCl salt (addition of ethereal HCl to the pure prodiginine analogue). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.79-12.60 (3 br s, 3H), 7.20 (m, 1H), 7.04 (s, 1H), 6.90 (m, 1H), 6.33 (m, 1H), 6.08 (br s, 2H), 4.01 (s, 3H), 2.92 (t, J = 7.7 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 1.75 (m, 2H), 1.64 (m, 2H), 1.41−1.31 (m, 10H), 0.97 (t, J = 7.3 Hz, 3H), 0.87 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6, 153.4, 147.4, 145.5, 126.7, 124.2, 122.3, 120.1, 116.7, 113.2, 112.7, 111.6, 92.7, 58.7, 31.9, 29.4, 29.3, 29.2, 29.1, 28.4, 28.3, 24.2, 22.7, 14.1, 13.9. HRMS (ESI) calcd for $C_{25}H_{36}N_3O$ $(M + H)^+$, 394.2853; found, 394.2853.

In Vitro Antimalarial Activity: *P. falciparum* Growth Inhibition. *P. falciparum* pansensitive D6 and MDR Dd2 (parasites MRA-285, MRA-156, ATCC, Manassas, VA) were cultivated under standard conditions, as previously described,[18] and in vitro susceptibility to test compounds was determined by measuring growth after 72 h in the presence of varying concentrations of test compound or CQ (positive control) or no drug; each condition was done in quadruplicate. Growth inhibition was determined using the SYBR Green I based fluorescence assay[19] with minor modifications as previously described[20] and expressed as the compound concentration inhibiting growth by 50% (IC_{50}).

In Vivo Antimalarial Efficacy: *P. yoelii* Murine Patent Infection. Female outbred CF-1 mice (Charles River, Wilmington, MA) were injected intravenously via tail vein with 5×10^5 erythrocytes infected with *P. yoelii* Kenya (parasite MRA-428, ATCC, Manassas, VA), and groups of five mice were then treated with test compound or vehicle only, once each day for 3 days beginning 24 h after infection. All compounds were administered by gavage in caprylic/capric triglyceride vehicle (Miglyol 812, Sasol, Hamburg, Germany). One day after the final dose, thin blood smears were made from all mice, stained using a modified Giemsa method, and parasitemia ((infected erythrocytes/total erythrocytes) $\times$ 100) was determined using microscopy and compared to untreated controls. Mice without detectable parasitemia were observed and monitored by serial examination of blood smears. Infected mice were euthanized; those remaining parasite-free for 28 days were considered cured.

Alamar Blue Assay for Mammalian Cell Viability. The general cytotoxic effects of prodiginine analogues on host cells were assessed by a functional assay as described previously[15] using murine splenic lymphocytes induced to proliferate and differentiate by concanavalin A. Splenic lymphocytes isolated from C57BL/6J mice were washed twice in RPMI 1640 medium and resuspended in complete RPMI containing 10% fetal bovine serum (FBS), 50 μg/mL penicillin/streptomycin, 50 μM β-mercaptoethanol, and 1 μg/mL concanavalin A. Cells (100 μL/well) were then seeded into 96-well flat-bottom tissue culture plates containing drug solutions (100 μL) serially diluted with complete culture medium to a final cell density of 2×10^5 per well. The plates were then incubated for 72 h in a humidified atmosphere at 37 °C and 5% CO_2. An aliquot of a stock solution of resazurin (Alamar Blue, prepared in 1× PBS) was then added at 20 μL per well (final concentration, 10 μM), and the plates were returned to the incubator for another 24 h. After this period, the fluorescence in each well was measured in a Gemini EM plate reader with an excitation wavelength of 560 nm and an emission wavelength of 590 nm. IC_{50} values were determined by nonlinear regression analysis of logistic concentration−fluorescence intensity curves (GraphPad Prism software).

■ ASSOCIATED CONTENT

Supporting Information. Compounds characterization data and spectra (NMR and HRMS) of all the prodiginine analogues associated with this article. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

CQR, chloroquine-resistant; DNA, deoxyribonucleic acid; SAR, structure−activity relationship; CQ, chloroquine; IC_{50}, half maximal inhibitory concentration; nM, nanomolar; MDR, multidrug-resistant

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