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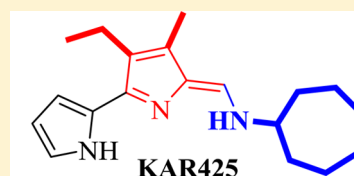
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Synthesis and Structure–Activity Relationships of Tambjamins and B-Ring Functionalized Prodiginines as Potent Antimalarials

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Supporting Information

ABSTRACT: Synthesis and antimalarial activity of 94 novel bipyrrrole tambjamins (TAs) and a library of B-ring functionalized tripyrrrole prodiginines (PGs) against a panel of *Plasmodium falciparum* strains are described. The activity and structure–activity relationships demonstrate that the ring-C of PGs can be replaced by an alkylamine, providing for TAs with retained/enhanced potency. Furthermore, ring-B of PGs/TAs can be substituted with short alkyl substitutions at either 4-position (replacement of OMe) or 3- and 4-positions without impacting potency. Eight representative TAs and two PGs have been evaluated for antimalarial activity against multidrug-resistant *P. yoelii* in mice in the dose range of 5–100 mg/kg × 4 days by oral administration. The KAR425 TA offered greater efficacy than previously observed for any PG, providing 100% protection to malaria-infected mice until day 28 at doses of 25 and 50 mg/kg × 4 days, and was also curative in this model in a single oral dose (80 mg/kg). This study presents the first account of antimalarial activity in tambjamins.



IC₅₀ (nM) = 62 (D6), 55 (Dd2), 60 (7G8)
Cytotoxicity (IC₅₀) = 19200 nM (HepG2)
ED₅₀ < 2.5 mg/kg/day; **cLogP** = 2.7
NRD = 25 and 50 mg/kg × 4 days by oral
Curative in a single oral dose (80 mg/kg)

INTRODUCTION

Malaria is a global parasitic infectious disease caused by *Plasmodium* parasites, among which *Plasmodium falciparum* (Pf) is the most dangerous one, with the highest rates of complications and mortality. It has been estimated that 584 000 people died from this disease in 2013, and the burden is heaviest in the African region, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, who account for 78% of all deaths.¹ On the heels of the global spread of chloroquine-resistant *P. falciparum* (CQ^RPf), resistance has also quickly developed to a variety of quinoline analogues, to antifolates, to inhibitors of electron transport, and perhaps most ominously, now to artemisinin.^{2,3} Therefore, novel medicinal agents are urgently needed to overcome the emergence and spread of resistance.

Prodiginines (PGs, **1a–c**), tambjamins (TAs, **2a–b**), and modified prodiginines (streptorubin B (**3a**), metacycloprodiginine (**3b**), and marineosins (**4** and **5**)) belong to a family of pyrrolypyrromethene (PPM) alkaloids (Figure 1) derived from bacterial and marine sources.^{4–7} These structurally distinctive natural products can be envisioned to arise via a bifurcated process from a common precursor, 4-methoxy-2,2'-bipyrrrole-5-carboxaldehyde (MBC; **6**, Figure 1), and the corresponding alkylpyrrole and/or alkylamine.^{7–10} The natural and synthetic PPM products are undergoing intense scrutiny in the medicinal chemistry because of both their wide range of biological activities and modes of action (antimicrobial,^{11–14} immunosuppressive,^{15–22} antitumor,^{11,12,23} anticancer,^{6,24–30} antimalarial,^{7,31–38} transmembrane anion transport,^{28–30,39–45} and DNA intercalation^{46,47}). Certain PGs and TAs have also been observed to bind duplex DNA and can cleave this biomolecule in the presence of Cu(II).^{4,48} Some of these compounds have

shown clinical potential, and in particular, PG analogue GX15-070 has completed phase II clinical trials for the treatment of small cell lung cancer and is engaged in multiple clinical trials for the treatment of other cancer conditions.^{49,50}

As a part of an ongoing interest in developing new anti-parasitic agents, we reisolated the natural PGs **1a** and **3a** from *Streptomyces coelicolor* M511, and **3b** from *S. longisporus* *ruber* (Figure 1).^{51,52} These natural PGs exhibited great potency with very low IC₅₀ values against *P. falciparum* strains, a potency only slightly more than chloroquine (CQ).³⁷ The natural PG **3b** provided an excellent in vivo efficacy against multidrug-resistant *P. yoelii* in mice by oral route, and it was curative in this model at 100 mg kg⁻¹ day⁻¹, and three of four mice were cured. These data provided the first demonstration of oral effectiveness of PGs.³⁷ Recently we also have isolated the modified prodiginines, marineosins (**5**), and their pathway intermediates 23-hydroxyundecylprodiginine (**1b**), 23-ketoundecylprodiginine (**1c**), and premarineosin (**4**) through heterologous expression of the entire *mar* gene cluster and/or gene replacement mutants in a heterologous host, *S. venezuelae*.⁷ Of these, the compound **4** antimalarial activity compares favorably with the most potent naturally occurring PGs and CQ.

The structural and functional diversity and promising antimalarial activity of these natural PGs and marineosins spurred us to synthesize various analogues of these lead molecules to obtain more active compounds. We recently reported the antimalarial activity of a large library of synthetic PGs.^{37,38} This work has shown that a terminal nonalkylated pyrrole

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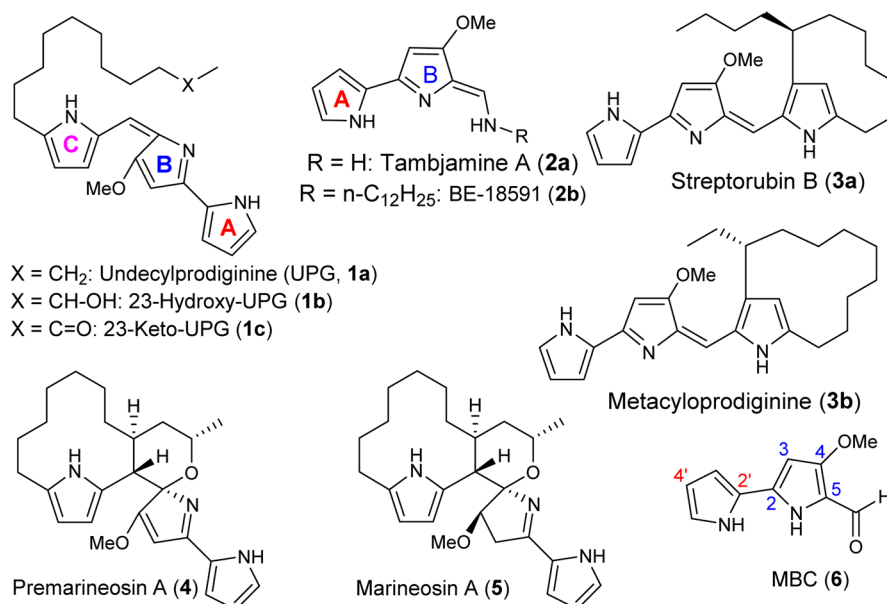


Figure 1. Structures of PPM natural products (**1–5**) and their common biosynthetic precursor (**6**).

(ring-A) and 3,5-dialkyl substitutions on the other terminal alkylated pyrrole (ring-C) of a natural tripyrrole PGs core structure are crucial for the potent antimalarial activity. A number of the synthetic PGs were effective at lower concentrations ($\text{IC}_{50} = 0.9\text{--}16.0$ nM) against *P. falciparum* strains, and their potency was more than the natural PGs and CQ. However, preliminary in vitro assays indicate concerns associated with the toxicity of PGs.

Our work on the potent antimalarial activity of PGs^{37,38} to date has been limited to SAR studies of A- and C-ring functionalized PGs. With a few exceptions,^{20,24,25,30,38} there have been no reports of a comprehensive series of TAs and B-ring functionalized PGs being prepared and evaluated for biological activities. In particular, the antimalarial activities of the TAs have not been reported to the best of our knowledge. These toxicity concerns for PGs and the intriguing biological activities of these PPM scaffolds have spurred us to expand the structural and functional diversity. Therefore, we have undertaken syntheses of novel TAs and B-ring functionalized PGs for enhanced antimalarial activity and reduced toxicity. To that end, we have developed new methods for the synthesis of various 2,2'-bipyrrrole-5-carboxaldehydes⁵³ and utilized in the generation of the novel TAs and B-ring functionalized PGs. Here we report the synthesis and structure–activity relationships (SARs) of TAs and B-ring functionalized PGs. The results show TAs with impressive in vitro potency and low toxicity and demonstrate that a tripyrrole structure is not required for activity. Furthermore, evidence of in vivo efficacy with TAs, including curative efficacy in mice after oral administration, is reported.

RESULTS AND DISCUSSION

Chemistry. The key precursors **6–43**, which are involved in the synthesis of prodiginines (PGs) and tambjamines (TAs) (Scheme 10), are depicted in Figures 2 and 3. By use of literature methodologies, MBC (**6**) and analogue **21** were prepared from readily available 4-methoxy-3-pyrrolin-2-one in two steps⁵⁴ and 2,2'-bipyrrrole-5-carboxaldehydes **7**, **8**, and **10–18** were synthesized by our recent methods.⁵³ The

syntheses of various new pyrrole carboxaldehydes **9**, **19**, **20**, and **22–39** are outlined in Schemes 1–9.

Synthesis of 4-(4-Chlorophenyl)-[2,2'-bipyrrrole]-5-carboxaldehyde (9**).** Synthesis of the aryl substituted 3-pyrrolin-2-one **48**, a key synthon in the synthesis of bipyrrrole-carboxaldehyde **9**, began with the coupling of Boc-glycine (**44**) with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) to afford the acylated Meldrum's acid, which was further converted into the desired intermediate **45** by an intramolecular cyclization and a subsequent decarboxylation (Scheme 1).⁵⁵ The compound **45** was treated with *p*-toluenesulfonyl chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) to give the tosylated product **46**, in 89% yield, which was further subjected to Suzuki coupling reaction with 4-chlorophenylboronic acid to give the *N*-Boc-4-aryl-3-pyrrolin-2-one **47**. The desired 4-aryl-3-pyrrolin-2-one **48** was obtained in excellent yield by deprotection of the *N*-Boc group with trifluoroacetic acid.⁵⁶ By use of the reported Vilsmeier formylation method,⁵⁴ **48** was then smoothly transformed to 5-bromo-3-(4-chlorophenyl)-pyrrole-2-carboxaldehyde **49**, which when further subjected to Suzuki coupling with *N*-Boc-2-pyrroleboronic acid followed by deprotection of the *N*-Boc group gave the desired 2,2'-bipyrrrole-5-carboxaldehyde **9** in 59% yield (Scheme 1).⁵³

Synthesis of 2,2'-Bipyrrrole-5-carboxaldehyde (19**).** In 1988, Borger and Patel synthesized the 2,2'-bipyrrrole-5-carboxaldehyde (**19**) in seven steps.¹² In this work, we successfully accomplished **19** in two one-pot sequences from easily available pyrrole (**50**), as shown in Scheme 2. To that end, compound **50** was consecutively treated with *N*-chlorosuccinimide (NCS) and Vilsmeier reagent (POCl_3/DMF , in situ generation) under controlled temperatures to obtain the 5-chloro-pyrrole-2-carboxaldehyde (**51**) in good yield.⁵⁷ The Suzuki cross-coupling of **51** with *N*-Boc-2-pyrroleboronic acid followed by deprotection of the *N*-Boc group provided the desired bipyrrrolecarboxaldehyde **19** in 45% isolated yield (Scheme 2).

Synthesis of 3-(Pyrrol-2-yl)-4,5,6,7-tetrahydroisoindole-1-carboxaldehyde (20**).** The key intermediate **53** was prepared via BartoneZard's method, using 1-nitro-1-cyclohexene (**52**) as a starting material (Scheme 3).^{53,58,59} Upon treatment with NaOH in ethylene glycol under reflux, **53** was

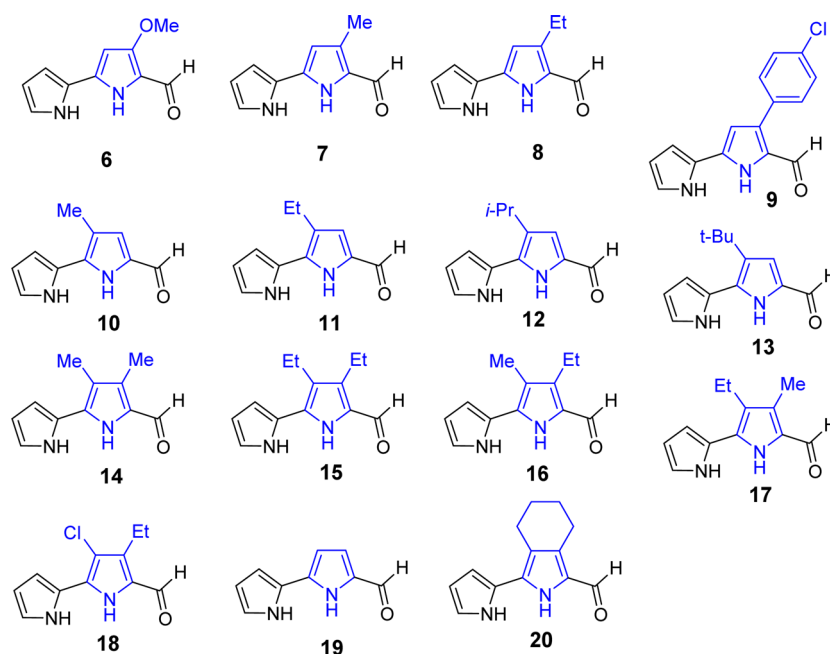


Figure 2. Key precursors (6–20) for the synthesis of B-ring functionalized PGs and TAs.

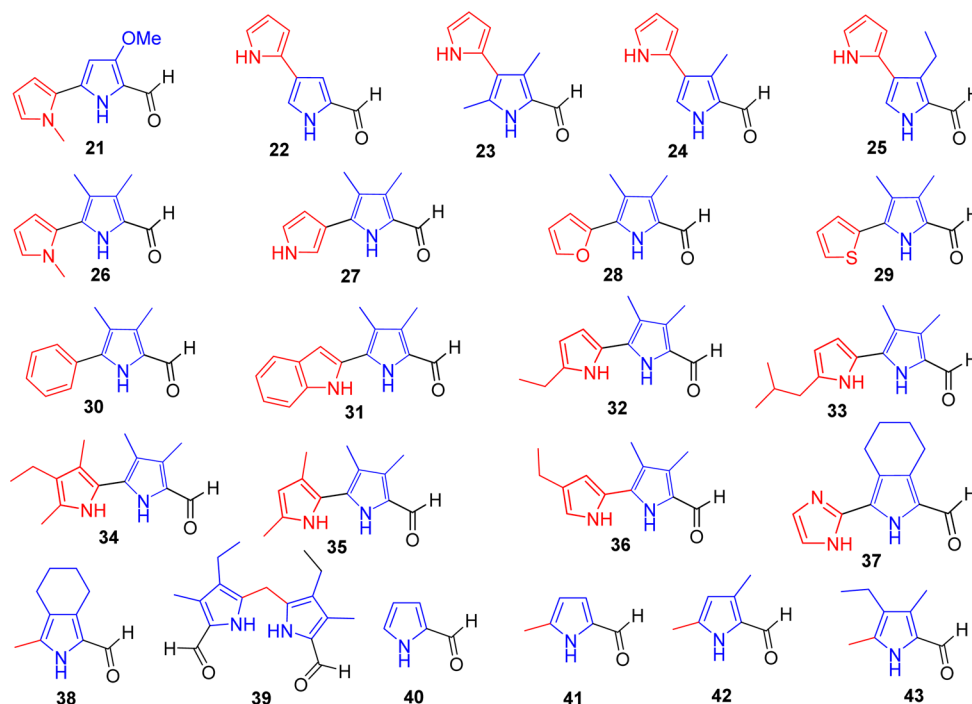
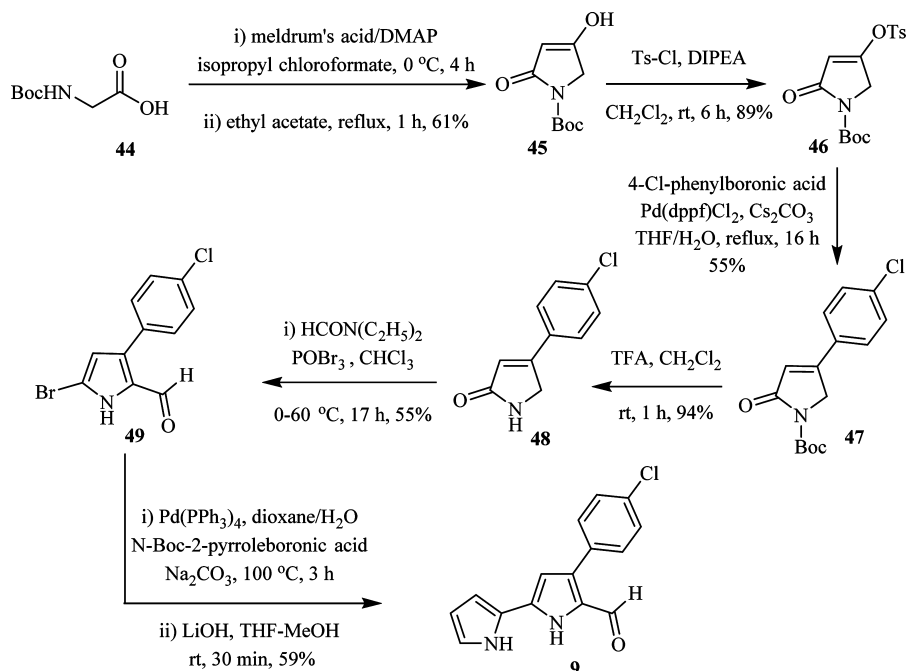


Figure 3. Key precursors (21–43) for the synthesis of A- and B-ring functionalized PGs and TAs.

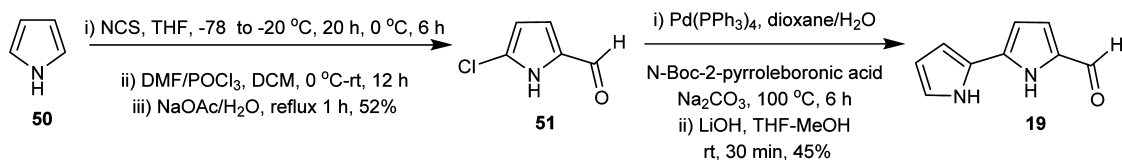
smoothly converted to 4,5,6,7-tetrahydroisindole (54) in 90% yield by successive hydrolysis and decarboxylation of the ester group.⁵³ Using the standard Vilsmeier formylation method, 54 was then transformed to 4,5,6,7-tetrahydroisindole-1-carboxaldehyde (55), which when further treated with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH)⁵³ in THF at -78 °C to room temperature provided the 3-bromo-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (56). Subsequently, Suzuki cross-coupling reaction between 56 and *N*-Boc-2-pyrroleboronic acid and further deprotection of the *N*-Boc group led to the desired bipyrrolecarboxaldehyde 20 in good yield (Scheme 3).

Synthesis of Isomeric [2,3'-Bipyrrole]-5'-carboxaldehydes (22–25). To investigate the ring-A positional effect on antimalarial activity, the isomeric bipyrrolecarboxaldehydes 22–25 were prepared, as shown in Scheme 4. Pyrrole-2-carboxaldehyde (40) and 3,5-dimethylpyrrole-2-carboxaldehyde (42) were obtained from commercial sources, and the 3-methylpyrrole-2-carboxaldehyde (57) and 3-ethylpyrrole-2-carboxaldehyde (58) were prepared according to our reported procedures.⁵³ These pyrrole-2-carboxaldehydes were then converted into the corresponding 4-bromopyrrole-2-carboxaldehydes 59–62 via a regioselective bromination at 4-position using DBDMH in THF in good yields (Scheme 4).⁵³ These

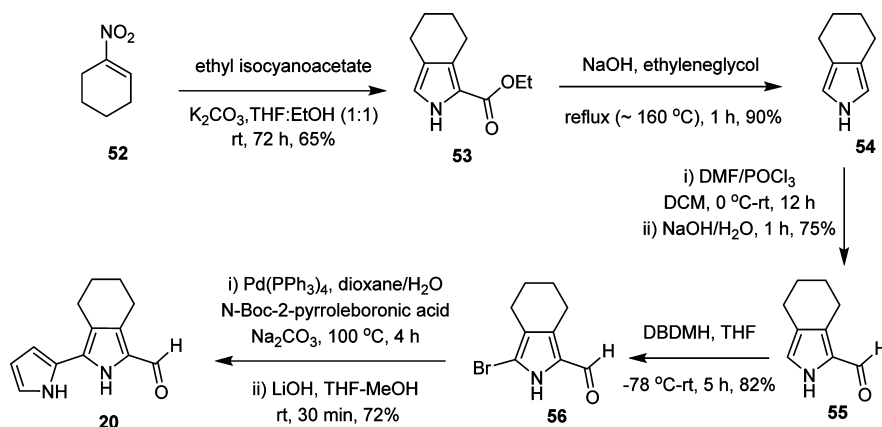
Scheme 1. Synthesis of 4-(4-Chlorophenyl)-[2,2'-bipyrrole]-5-carboxaldehyde (9)



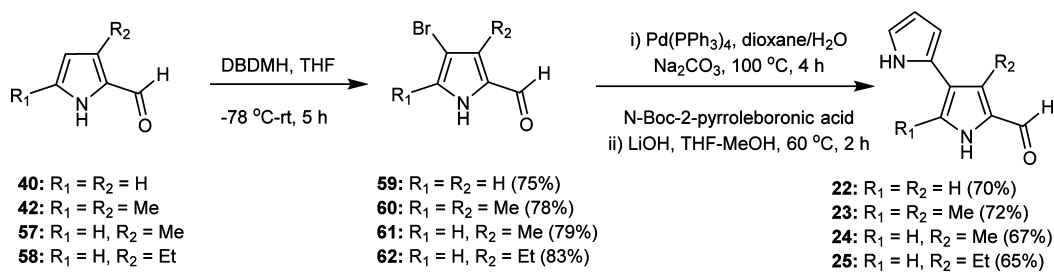
Scheme 2. Synthesis of 2,2'-Bipyrrole-5-carboxaldehyde (19)



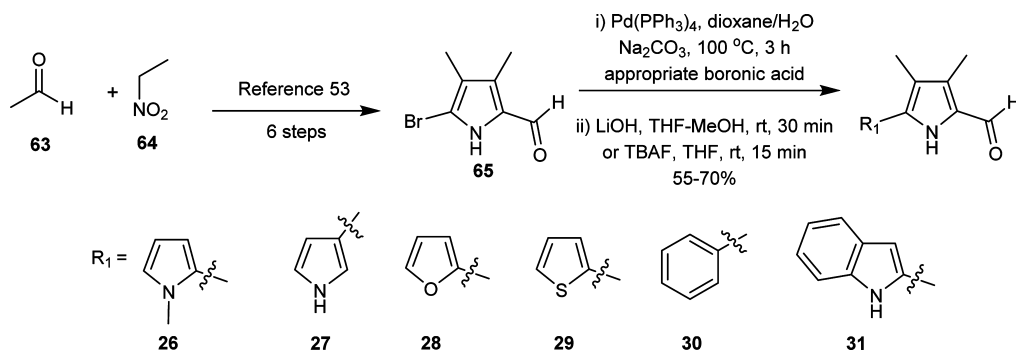
Scheme 3. Synthesis of 3-(Pyrrol-2-yl)-4,5,6,7-tetrahydroisoindole-1-carboxaldehyde (20)



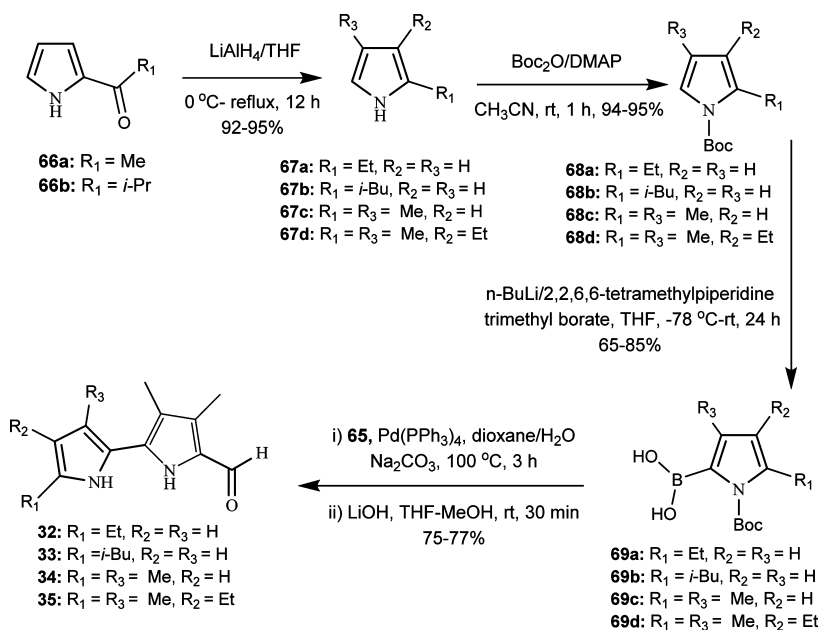
Scheme 4. Synthesis of Isomeric [2,3'-Bipyrrole]-5'-carboxaldehydes (22–25)



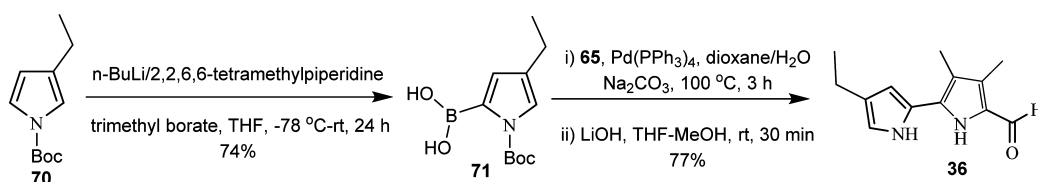
Scheme 5. Synthesis of MBC's analogues containing heteroaryl/aryl groups in the place of ring-A (26–31)



Scheme 6. Synthesis of 3,4-Dimethyl-[2,2'-bipyrrole]-5-carboxaldehydes Where the Ring-A Contains C-Alkyl Groups (32–35)



Scheme 7. Synthesis of 4'-Ethyl-3,4-dimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (36)



4-bromopyrrole-2-carboxaldehydes **59–62** were further subjected to Suzuki coupling reaction with *N*-Boc-2-pyrroleboronic acid, and a subsequent treatment with LiOH in THF/MeOH (1:1) at 60 °C resulted in the desired isomeric bipyrrole-carboxaldehydes **22–25** (Scheme 4).

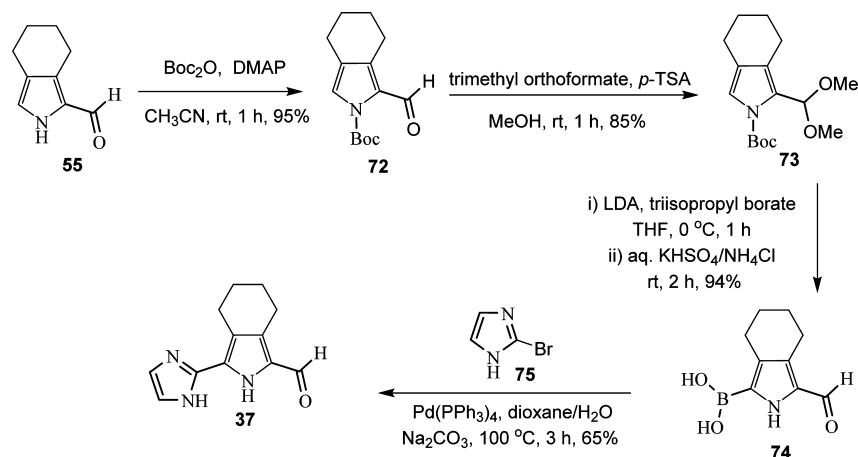
Synthesis of MBC's Analogues (26–31) Containing Heteroaryl/Aryl Groups in the Place of Ring-A. To probe the exact role of the 2-pyrrolyl moiety (ring-A) on activity, we have prepared various key carboxaldehyde precursors **26–31** in which the ring-A is completely replaced by various heterocycles and/or aryl moieties and the ring-B is substituted with short alkyl groups (Scheme 5). The 5-bromo-3,4-dimethylpyrrole-2-carboxaldehyde (**65**) was prepared in six steps according to the literature methods from acetaldehyde (**63**) and nitroethane (**64**),⁵³ and it was subsequently subjected to Suzuki coupling reaction with various boronic acids, and further deprotection of

the Boc/TIPS group led to the corresponding carboxaldehydes **26–31** (Scheme 5).

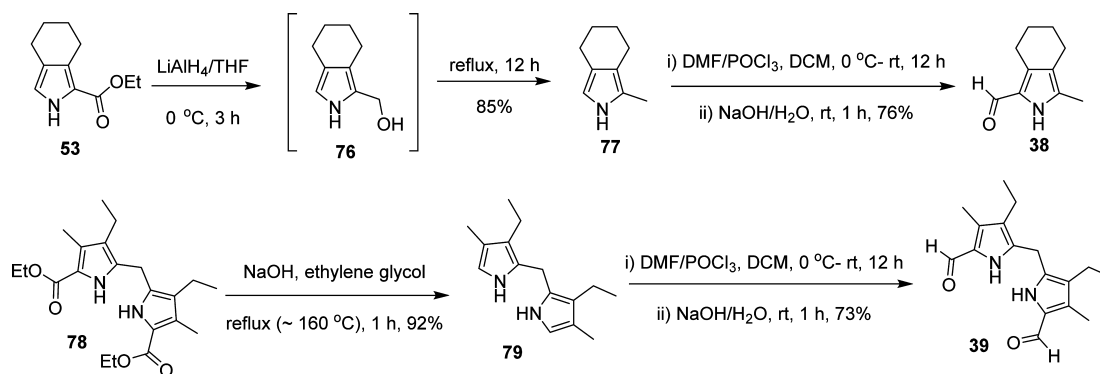
Synthesis of 3,4-Dimethyl-[2,2'-bipyrrole]-5-carboxaldehydes Where Ring-A Contains C-Alkyl Groups (32–36).

To investigate the effect of the ring-A alkyl substituents pattern on potency, we have prepared various alkylated bipyrrole-carboxaldehyde precursors **32–36**, as shown in Schemes 6 and 7. The 2-acetylpyrrole (**66a**), 2,4-dimethylpyrrole (**67c**), and 3-ethyl-2,4-dimethylpyrrole (**67d**) were obtained from commercial sources, and the 2-isobutylpyrrole (**66b**) was prepared according to the literature methods.⁶⁰ The compounds **66a** and **66b** were then converted into the corresponding 2-alkylpyrroles **67a** and **67b**, respectively, using LiAlH₄ in THF under reflux (Scheme 6).⁶¹ By use of standard procedures, the *N*-Boc-protected pyrroles **68a–d** were prepared in excellent yields from **67a–d** using di-*tert*-butyl dicarbonate (Boc₂O) in

Scheme 8. Synthesis of 3-(Imidazol-2-yl)-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (37)



Scheme 9. Synthesis of 3-Methyl-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (38) and 5,5'-Methylenebis(4-ethyl-3-methylpyrrole-2-carboxaldehyde) (39)



the presence of 4-dimethylaminopyridine (DMAP), and subsequently these were converted into the corresponding 5-alkyl-(1-*tert*-butoxycarbonylpyrrol-2-yl)boronic acids **69a–d**.⁶² The resultant boronic acids **69a–d** were carried forward into the Suzuki coupling reaction with **65** without further purification to afford their corresponding [2,2'-bipyrrrole]-5-carboxaldehydes **32–35** in good yields (Scheme 6).

We have also developed a simple and convenient method for the synthesis of *N*-Boc-4-ethyl-2-pyrrolboronic acid (**71**) via a regioselective boronylation of *N*-Boc-3-ethylpyrrole (**70**),⁵³ using *n*-BuLi/2,2,6,6-tetramethylpiperidine and trimethyl borate (Scheme 7, Experimental Section). Further investigations to expand the substrate scope of the regioselective boronylation as well as mechanistic studies are underway in our laboratory. Finally the 4'-ethyl-3,4-dimethyl-[2,2'-bipyrrrole]-5-carboxaldehyde (**36**) was prepared in good yield via Suzuki coupling of **65** with boronic acid **71**, followed by the deprotection of *N*-Boc group with LiOH (Scheme 7). The final compound **36** was fully characterized by extensive 2D NMR analysis (see Supporting Information).

Synthesis of 3-(Imidazol-2-yl)-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (37). To investigate the role of ring-A with an extra nitrogen atom on potency, we have replaced the ring-A by imidazole moiety, as in **37** (Scheme 8). The *N*-Boc-pyrrole **72** was prepared in 95% yield from compound **55** using $\text{Boc}_2\text{O}/\text{DMAP}$, and subsequently the aldehyde group was protected by trimethyl orthoformate under acidic conditions to obtain the desired intermediate **73**. The compound **73** was

further reacted with triisopropyl borate/LDA in THF and followed by aqueous solution of $\text{KHSO}_4/\text{NH}_4\text{Cl}$ at room temperature to provide the desired boronic acid **74** in excellent yield.⁵³ Finally, the Suzuki cross-coupling reaction between **74** and 2-bromoimidazole (**75**) and subsequent deprotection of the *N*-Boc group led to the desired carboxaldehyde **37** in 65% isolated yield (Scheme 8).

Synthesis of 3-Methyl-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (38) and 5,5'-Methylenebis(4-ethyl-3-methylpyrrole-2-carboxaldehyde) (39). We wanted to study the analogues of TAs without ring-A; therefore, two representative pyrrole aldehydes **38** and **39** (Scheme 9) were synthesized. Initially, 1-methyl-4,5,6,7-tetrahydroisindole (**77**) was synthesized from ethyl-4,5,6,7-tetrahydroisindole-1-carboxylate (**53**) via an unstable intermediate **76**, using LiAlH_4 in THF at 0 °C to room temperature in 85% isolated yield. The resultant alkyrrrole **77** was further converted to 3-methyl-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (**38**) by Vilsmeier reagent (POCl_3/DMF) (Scheme 9). Conversely, the bis(3-ethyl-4-methylpyrrol-2-yl)methane (**79**) was prepared from diethyl-5,5'-methylenebis(4-ethyl-3-methyl-2-pyrrolicarboxylate) (**78**) in excellent yields via a successive hydrolysis and a decarboxylation of the ester groups. Further Vilsmeier formylation of **79** provided the desired dicarboxaldehyde **39** in 73% isolated yield (Scheme 9).

Synthesis of Novel PGs (85–98) and TAs (99–187). By use of our standardized procedures, the mono- and dialkyl/alkylaryl pyrroles **80–84** were synthesized (Figure 4).³⁷ The acid-catalyzed

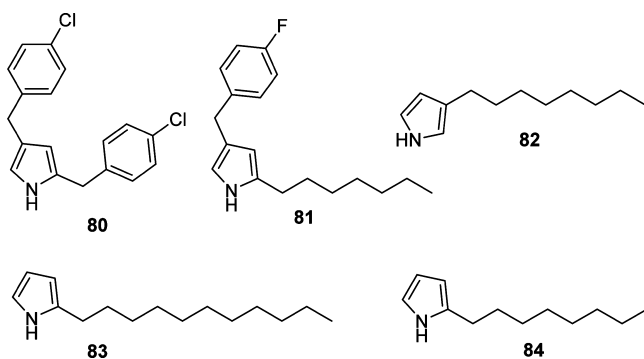


Figure 4. Potential substrates (80–84) for the synthesis of PGs.

condensation of either the alkylpyrroles 80–84 or the commercially available alkyl/arylamines with various bipyrrrole-carboxaldehydes and analogues 6–43 provided the desired PGs 85, 86, 88–98 and TAs 99–187, respectively, in good to excellent isolated yields (Scheme 10). The PG 85 was further treated with MeI/NaH in DMF to provide the *N,N*-dimethyl PG 87 in 85% isolated yield (Scheme 10).

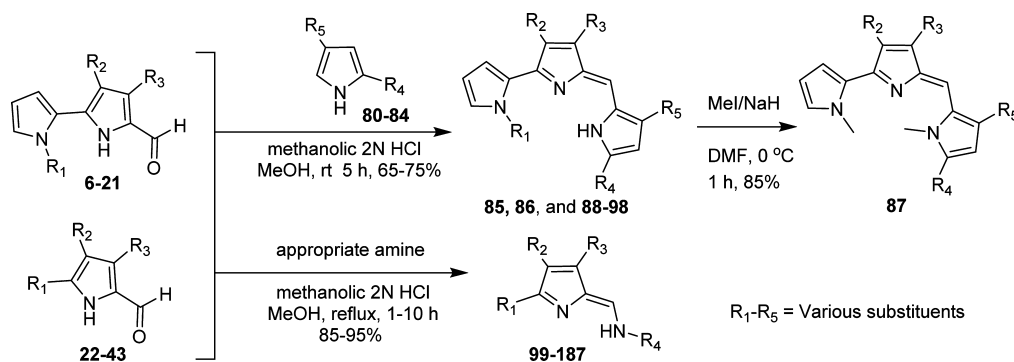
Synthesis of TA-like Analogues (190, 191, and 194–196). Distinct syntheses were designed and executed to obtain a different class of TA-like analogues 190, 191, and 194–196, in which the crucial ring-B of TAs is completely replaced by an alkylamide/amine linkage (Scheme 11). To that end, compound 188 was synthesized via a standard condensation method (EDCI/DMAP) from 44 and 1-adamantylamine in 85% yield. Removal of the Boc group of 188 by trifluoroacetic acid/water (1:1) provided the intermediate 189 in good yield,⁶³ which was further utilized in a condensation reaction with pyrrole-2-carboxylic acid to furnish the desired product 190. Treatment of 190 with LiAlH₄ in THF at 0 °C to reflux conditions gave the 191 in 82% isolated yield (Scheme 11). Conversely, analogues 194–196 were also synthesized, as shown in Scheme 11. The pyrrole-2-carboxaldehyde (40) was subjected to Horner–Wadsworth–Emmons (HWE) reaction with methyl diethylphosphonoacetate in the presence of NaH to obtain the methyl-3-(pyrrol-2-yl)acrylate (192),^{64,65} which when hydrolyzed under basic (LiOH·H₂O) conditions, furnished the 2-pyrrolylacrylic acid 193. Condensation of 193 with 189 in the presence of EDCI/DMAP led to the corresponding condensed product 194, which was further treated with NaBH₄/NiCl₂·6H₂O to give the saturated product 195. Treatment of 195 with LiAlH₄ in THF at 0 °C to reflux conditions provided the desired product 196 in 78% yields (Scheme 11).

Biological Activity. In this work, the structure–activity relationships (SARs) focused on various substitutions and positions of the ring-A and -B and the nature of the alkylamines of TAs, and ring-B of PGs. Specifically, the modifications to the ring-B of TAs and PGs were designed in order to understand the structural requirements, as well as the necessity of the ring-B for the potent antimalarial activity. We have synthesized various series of novel TAs and B-ring functionalized PGs and evaluated for antimalarial activity against the chloroquine-sensitive (CQ^S) D6 and the chloroquine-resistant (CQ^R) Dd2 and 7G8 strains of *Pf* with chloroquine (CQ) as a reference drug.^{66,67} In parallel, the cytotoxicity of the most potent antimalarial PGs and TAs (IC₅₀ < 250 nM) was tested against hepatocellular HepG2 cancer cell line using mefloquine (MQ) as a control drug (see Tables 1–6).^{68,69}

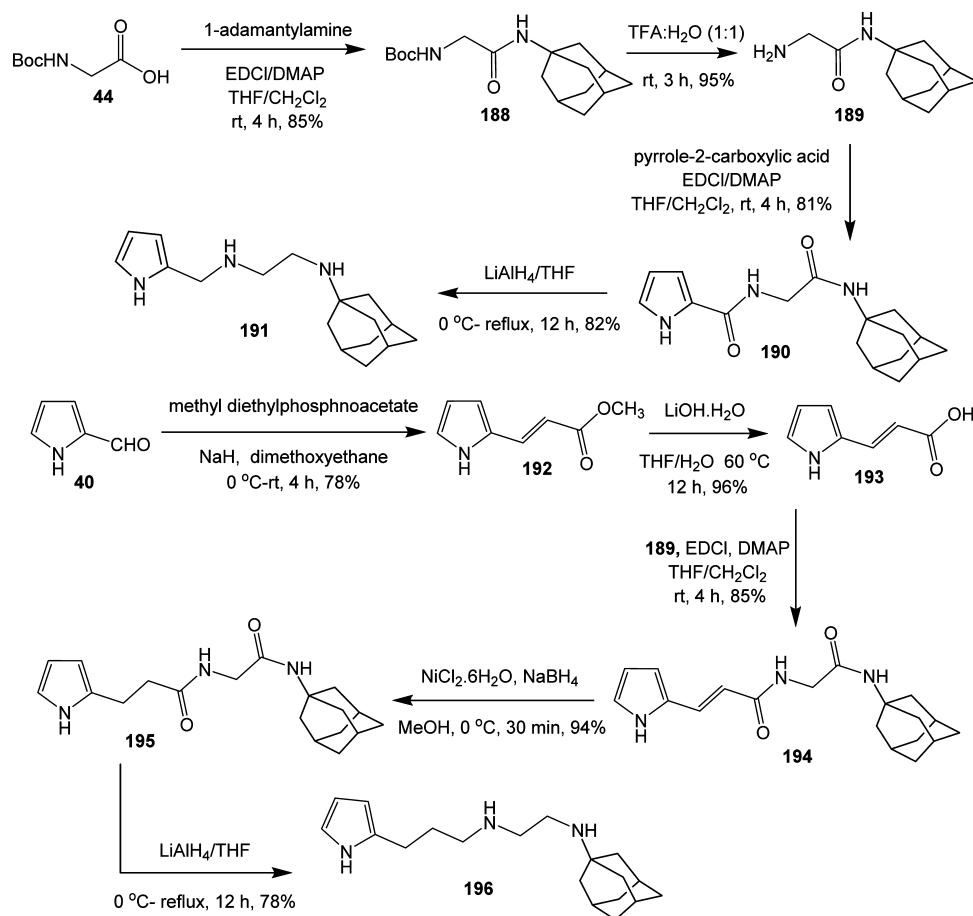
In Vitro Antimalarial Activity of PGs (85–98). In our previous work, synthetic PG 85 had shown an excellent potency against *Pf* strains D6 (CQ^S) and Dd2 (CQ^R) with great IC₅₀ values (Table 1) and had the most favorable profile: 92% parasite reduction at 5 mg kg⁻¹ day⁻¹, 100% reduction at 25 mg kg⁻¹ day⁻¹ in a *P. yoelii* murine patent infection without any evident weight loss or clinical overt toxicity.³⁷ To explore the *N*-alkyl effect on potency, initially we synthesized two *N*-methylated analogues 86 and 87 of the 85 (Table 1). These compounds 86 and 87 led to a large decrease in the antimalarial activity (IC₅₀ > 2250 nM) against three *Pf* strains D6, Dd2, and 7G8, demonstrating that both pyrrole NH groups (ring-A and -C) of the PGs are required for potent antimalarial activity, supporting our previous findings.³⁸ To investigate the importance of the methoxy group (OMe) on ring-B, two analogues 88 and 89, in which the OMe group is replaced by 4-chlorophenyl moiety and hydrogen (complete removal of OMe), respectively, were prepared and examined for in vitro antimalarial activity. A dramatic loss of potency was observed for both compounds 88 and 89, which have an IC₅₀ of >2500 nM against all tested *Pf* strains (Table 1). Interestingly, while replacing the OMe group by ethyl unit as in 90 also led to the reduced potency (90 IC₅₀ = 101 nM versus 1a IC₅₀ = 7.2 nM against D6), the reduction was modest (14-fold). This result demonstrated that a short aliphatic substitution at 4-position on the ring-B could replace the OMe group and retain activity. Together, these results highlighted the importance of the OMe or short alkyl group on the ring-B of PGs for potent antimalarial activity, supporting our previous findings.³⁸

We next investigated whether substitutions at 2 and 3 positions of the ring-B are tolerated. A series of novel B-ring functionalized PGs 91–98, in which the ring-B is substituted with

Scheme 10. Synthesis of Novel PGs (85–98) and TAs (99–187)



Scheme 11. Synthesis of Novel Analogues (190, 191, and 194–196)



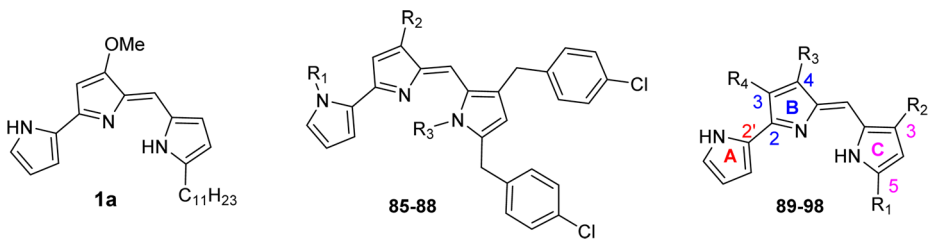
either mono- and/or disubstituents at 3- and 4-positions, were generated and examined for their *in vitro* antimalarial activity (Table 1). A significant loss of potency ($IC_{50} > 1500$ nM) was observed for **91** and **92**, containing an isopropyl and *tert*-butyl groups, respectively, at 3-position on the ring-B. The adverse effect of the substitutions at 3-position on the ring-B was further confirmed by the introduction of the chloro (Cl) substitution at 3-position of **90**, as with the analogue **93**, which had an IC_{50} of >2500 nM against all strains (**90** IC_{50} = 101 nM versus **93** IC_{50} > 2500 nM), suggesting that the rigid bulky substitutions or chlorine moiety (EWG) at 3-position are not preferred (Table 1). To further investigate the impact of the short alkyl substituents at both the 3- and 4-positions on ring-B, a set of mixed analogues **94–98**, which contain the 3-ethyl/4-methyl groups on the ring-B, was examined. Analogues **94** and **95**, which have monoalkyl groups at 5-position of the ring-C, showed a roughly 20-fold drop in activity as compared to undecylprodiginine (**1a**) (Table 1). Conversely, the analogue **96** containing a monoalkyl group at 3-position on the ring-C, showed higher potency (3-fold) than **95** against all tested *Pf* strains, while it had 9-fold lesser potency than the corresponding OMe group containing analogue (IC_{50} = 4.6 nM against D6³⁷). Interestingly, the analogue **97**, which has 3-alkyl and 5-alkylaryl substituents on the ring-C, was equipotent to the **85**, while the analogue **98**, which has 3,5-dialkylaryl substituents on ring-C, showed ~ 5 -fold lower potency when compared to the corresponding OMe group containing analogue **85** (Table 1). Again these results are consistent and support the findings that the 3,5-disubstitutions on ring-C are very important for potent

activity.³⁷ In summary, these SAR analyses of the ring-B functionalized PGs demonstrate that the short alkyl substitutions are well tolerated at 3/4-positions on the ring-B.

In Vitro Antimalarial Activity of 4-Substituted B-Ring Functionalized TAs (99–129). Having determined the substituents impact on the antimalarial activity of the PGs (through this and previous work^{37,38}), we subsequently tested a hypothesis that the complete replacement of the right-hand-side alkylated pyrrole (ring-C) of PGs by alkylamines, providing the TAs, might represent an opportunity to make potent and selective antimalarials with the desired “druglike” properties. Specifically, lower molecular weight (MWT) and lipophilic properties ($\log P$) are the two key characteristics that determine adsorption, distribution, metabolism, excretion, and toxicity (ADMET) liabilities, with some ADMET parameters depending more on MWT and some on $\log P$.⁷⁰ Subsequent TA analogues **99–129** (Table 2), which have lower MWT (<400) and $cLogP$ (<4.2 , except **114**), were generated to obtain a SAR for the alkylamines in the place of ring-C and substituents at 4-position on the ring-B.

Initially, a series of new TAs **99–112**, which have various alkyl/aryl amines in the place of ring-C and the OMe group at the 4-position on the ring-B (as in natural products), were synthesized and evaluated for their *in vitro* antimalarial activity against *Pf* strains, and the results are shown in Table 2. TAs **99–102** containing the *n*-alkylamines in the place of ring-C exhibited good activity against all *Pf* strains; specifically, analogues **100** and **102** showed the highest potencies ($IC_{50} < 50$ nM) (Table 2). To probe the effect of cycloalkylamines in the place

Table 1. In Vitro Antimalarial Activity and Cytotoxicity of PGs (85–98)



compd	R ₁	R ₂	R ₃	R ₄	antimalarial activity (IC ₅₀ in nM) ^a			cytotoxicity (IC ₅₀ in nM) ^a HepG2	SI ^b (D6)	cLogP ^c
					D6	Dd2	7G8			
85	H	OMe	H		6.1	4.8	5.5	>250000	>40983	4.8
86	Me	OMe	H		2250	>2500	>2500	nt ^d		5.1
87	Me	OMe	Me		>2500	>2500	>2500	nt		5.3
88	H	4-ClC ₆ H ₄	H		> 2500	>2500	>2500	nt		7.7
89	<i>n</i> -C ₁₁ H ₂₃	H	H	H	>2500	>2500	>2500	nt		5.2
90	<i>n</i> -C ₁₁ H ₂₃	H	Et	H	101	66	51	18939	187	5.7
91	<i>n</i> -C ₁₁ H ₂₃	H	H	<i>i</i> -Pr	1586	1500	>2500	nt		6.3
92	<i>n</i> -C ₁₁ H ₂₃	H	H	<i>t</i> -Bu	>2500	>2500	>2500	nt		6.7
93	<i>n</i> -C ₁₁ H ₂₃	H	Et	Cl	>2500	>2500	>2500	nt		5.8
94	<i>n</i> -C ₁₁ H ₂₃	H	Me	Et	162	190	145	62000	383	6.1
95	<i>n</i> -C ₈ H ₁₇	H	Me	Et	127	216	132	71000	559	4.8
96	H	<i>n</i> -C ₈ H ₁₇	Me	Et	41	53	61	57200	1395	4.9
97	<i>n</i> -C ₇ H ₁₅	4-FC ₆ H ₄ CH ₂	Me	Et	6.5	7.0	5.9	82024	12619	6.7
98	4-ClC ₆ H ₄ CH ₂	4-ClC ₆ H ₄ CH ₂	Me	Et	28	42	42	30600	1093	6.7
1a					7.2	7.5	7.0	nt		4.2
CQ					13	115	130	nt		3.7
MQ					nt	nt	nt	21800		5.3

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate ($\pm 10\%$). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6). ^ccLogP values were calculated using ChemBioDraw Ultra software (version 14). ^dnt = not tested.

of ring-C/*n*-alkylamines on activity, we synthesized another set of TAs **103–109** (Table 2). Of these cycloalkylated TAs, analogues **108** and **109**, which have the cyclooctylamine and 1-adamantylamine moieties, respectively, were the most potent antimalarial candidates (**108** IC₅₀ < 7.1 nM and **109** IC₅₀ < 3.8 nM against all tested *Pf* strains; see Table 2) with good selectivity, and these results are more comparable to the potent PG **85** (IC₅₀ < 6.1 nM) and the natural PG **1a** (IC₅₀ < 7.5 nM). These results clearly demonstrated that the elongation of the cycloalkyl ring size (from cyclopropyl, **103**, IC₅₀ = 2500 nM to 1-adamantyl, **109**, IC₅₀ < 3.1 nM) led to an increase in activity (Table 2 and Figure 5). The greatest loss of potency (IC₅₀ > 2500 nM) was observed in **110**, in which ring-C is replaced by piperidine moiety, suggesting that the free NH is required for the potent antimalarial activity. Replacement of cyclohexyl moiety with benzylpiperidine as with **111** led to slightly reduced potency (**106** IC₅₀ = 49 nM versus **111** IC₅₀ = 127 nM against D6). The analogue **112**, which contain a 4-chloroaniline in the place of ring-C showed the moderate activity (Table 2). These results unequivocally demonstrate that the ring-C of PGs can be replaced by alkylamines, providing the novel TAs with retained and/or enhanced antimalarial and cytotoxic properties.

To investigate the importance of the OMe group on ring-B of TAs, another set of TAs **113–119**, in which the OMe group is replaced by 4-chlorophenyl moiety, was generated and examined for their in vitro antimalarial activity (Table 2). In vitro analysis of the activity of these compounds **113–119**

against *Pf*, demonstrated activity (IC₅₀ > 250 nM) significantly diminished when compared to the corresponding OMe group containing TAs (**100**, **102**, and **105–109**). This work suggested that the bulky aromatic substitution at 4-position on the ring-B had an adverse effect on antimalarial activity. Interestingly the replacement of the OMe group with short alkyl substituents (methyl/ethyl) also reduced the potency of the compounds **120–122**, **124**, and **125** (IC₅₀ > 250 nM) (Table 2). Conversely, the adamantyl analogues **123** and **126**, in which the OMe group is replaced by methyl and ethyl groups on the ring-B, respectively, showed a substantially higher potency against D6 strain (**109** IC₅₀ = 3.1 nM versus **123** IC₅₀ = 1.3 nM, **126** IC₅₀ = 2.5 nM) with great selectivity. Complete removal of the OMe group on ring-B as with the analogues **127–129** resulted in the total loss of activity (**127**, **128**, IC₅₀ > 2500 nM vs **107** IC₅₀ = 23 nM, **108** IC₅₀ = 4.8 nM, and **129** IC₅₀ = 341 nM vs **109** IC₅₀ = 3.1 nM, **123** IC₅₀ = 1.3 nM, **126** IC₅₀ = 2.5 nM against D6). Together, these results again demonstrate that the substituents at 4-position on the ring-B have an important role in potent antimalarial activity, and the OMe group can be replaced by short alkyl substituents (methyl/ethyl) when 1-adamantylamine exists in the place of ring-C.

In Vitro Antimalarial Activity of 3-Substituted B-Ring Functionalized TAs (130–141). Having established the substitution pattern at 4-position on the ring-B and the terminal alkylamines (cycloheptyl-, cyclooctyl-, and 1-adamantylamines) as optimal, we then examined the effects of substitution pattern at 3-position, where the 4-position is vacant on the ring-B of

Table 2. In Vitro Antimalarial Activity and Cytotoxicity of 4-Substituted B-Ring Functionalized TAs (99–129)

antimalarial activity
(IC₅₀ in nM)^a

compd	R ₁	R ₂	antimalarial activity (IC ₅₀ in nM) ^a			cytotoxicity (IC ₅₀ in nM) ^a HepG2	SI ^b (D6)	cLogP ^c
			D6	Dd2	7G8			
99	OMe	n-C ₄ H ₉	210	159	74.6	23000	109	0.08
100	OMe	n-C ₆ H ₁₃	34	37	25	26700	785	0.9
101	OMe	n-C ₈ H ₁₇	345	177	69	nt ^d	-	1.7
102	OMe	n-C ₁₁ H ₂₃	55	53	23	9800	178	3.0
103	OMe		2400	2500	946	nt	-	-0.9
104	OMe		591	497	156	nt	-	-0.4
105	OMe		68	84	45	30500	448	-0.03
106	OMe		49	71	30	15000	306	0.4
107	OMe		23	34	15	10100	439	0.8
108	OMe		4.8	7.1	7.5	9700	2021	1.2
109	OMe		3.1	2.6	3.8	3300	1064	0.7
110	OMe		> 2500	> 2500	> 2500	nt	-	-0.05
111	OMe		127	244	207	> 250000	> 1968	0.5
112	OMe	4-ClC ₆ H ₄	255	368	314	nt	-	1.1
113	4-ClC ₆ H ₄	n-C ₆ H ₁₃	1129	> 2500	564	nt	-	3.8
114	4-ClC ₆ H ₄	n-C ₁₁ H ₂₃	664	> 2500	663	nt	-	5.9
115	4-ClC ₆ H ₄		1218	> 2500	510	nt	-	2.9
116	4-ClC ₆ H ₄		1025	> 2500	415	nt	-	3.3
117	4-ClC ₆ H ₄		963	1250	348	nt	-	3.7
118	4-ClC ₆ H ₄		832	1135	316	nt	-	4.1
119	4-ClC ₆ H ₄		> 250	> 250	126	nt	-	3.6
120	Me	n-C ₁₁ H ₂₃	1167	1469	515	nt	-	4.2
121	Me		> 250	> 250	> 250	nt	-	2.0
122	Me		> 250	> 250	> 250	nt	-	2.4
123	Me		1.3	15	4.3	6900	5308	1.8
124	Et		> 250	> 250	> 250	nt	-	2.4
125	Et		> 250	> 250	> 250	nt	-	2.8
126	Et		2.5	16	7.7	6100	2440	2.2
127	H		> 2500	> 2500	> 2500	nt	-	1.8
128	H		> 2500	> 2500	> 2500	nt	-	2.2
129	H		341	295	235	70000	205	1.6
CQ			13	115	130	nt	-	3.7
MQ			nt	nt	nt	21800	-	5.3

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6). ^ccLogP values were calculated using ChemBioDraw Ultra software (version 14), ^dnt = not tested.

the TAs (Table 3). To that end, we generated a series of novel TAs 130–141, in which the 3-position on the ring-B is

occupied with alkyl groups and screened for their antimalarial activity against *Pf* strains (Table 3). The greatest loss of potency

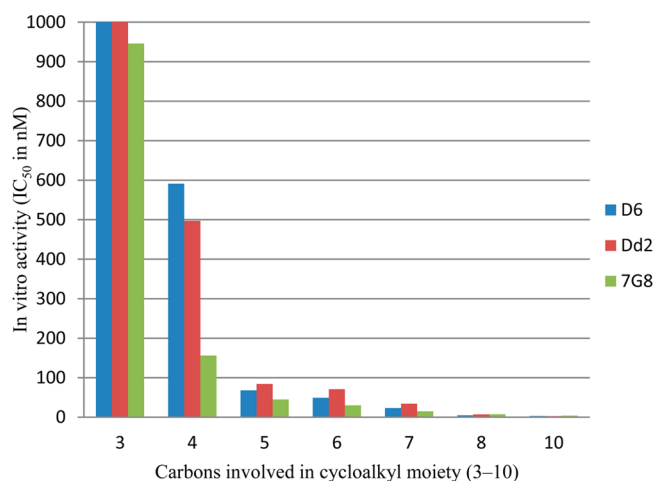


Figure 5. SAR of TAs (103–109) containing various cycloalkyl groups and in vitro antimalarial activity against *Pf* strains D6, Dd2, and 7G8.

was observed when the short alkyl (methyl/ethyl) groups move from 4-position (121–126, Table 2) to the 3-position (130–141, Table 3). Moreover, the adamantyl analogues 132 and 135 showed a significant decline in activity (132 IC₅₀ = 106 nM vs 123 IC₅₀ = 1.3 nM, and 135 IC₅₀ = 117 nM vs 126 IC₅₀ = 2.5 nM against D6), and the analogue 141 had an

almost total loss of activity (IC₅₀ > 2500 nM). The one exception is the adamantyl analogue 138, containing an isopropyl group at 3-position on the ring-B, which showed the better potency (IC₅₀ < 30 nM) against all tested *Pf* strains with good selectivity. These results show that generally alkyl substitutions at 3-position versus the 4-position adversely affect the potency irrespective of the terminal alkylamines.

In Vitro Antimalarial Activity of 3,4-Disubstituted B-Ring Functionalized TAs (142–165). Exploration of the SARs around the ring-B of TAs indicated that the substitutions at 4-position were greatly favored compared to the 3-position (Tables 2 and 3). This finding is exemplified by the poor activity of the 3-substituted analogues (130–141) with the exception of 138. We next investigated whether substitutions at both the 3- and 4-positions are tolerated. We synthesized a series of 3,4-disubstituted B-ring functionalized TAs 142–149, which have 3-ethyl and 4-methyl groups on the ring-B (Table 4). Of these 3,4-disubstituted TAs, analogues 142–144, 148, and 149 with an *n*-alkyl, cyclopropyl, benzylpiperidine, and morpholine moieties, respectively, showed diminished activity (Table 4). Conversely, the analogues 145 and 146, which have cycloheptyl and cyclooctyl moieties, respectively, showed the highest potencies (Table 4) compared with those of the corresponding 3- and 4-monoalkyl substituted analogues (see Tables 2 and 3). Significantly, the adamantyl analogue 147 showed comparable

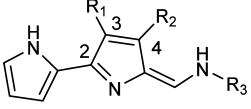
Table 3. In Vitro Antimalarial Activity and Cytotoxicity of 3-Substituted B-Ring Functionalized TAs (130–141)






















antimalarial activity
(IC₅₀ in nM)^a

compd	R ₁	R ₂	antimalarial activity (IC ₅₀ in nM) ^a			cytotoxicity (IC ₅₀ in nM) ^a HepG2	SI ^b (D6)	cLogP ^c
			D6	Dd2	7G8			
130	Me		2107	> 2500	2147	nt ^d	-	2.1
131	Me		1376	> 250	1778	nt	-	2.5
132	Me		106	170	95	30000	283	2.0
133	Et		1305	523	1456	nt	-	2.5
134	Et		1276	> 250	1326	nt	-	3.0
135	Et		117	45	90	15200	130	2.4
136	<i>i</i> -Pr		> 2500	1968	1980	nt	-	2.9
137	<i>i</i> -Pr		1079	665	1480	nt	-	3.3
138	<i>i</i> -Pr		26	20	31	18500	711	2.7
139	<i>t</i> -Bu		> 2500	> 2500	> 2500	nt	-	3.3
140	<i>t</i> -Bu		> 2500	> 2500	> 2500	nt	-	3.8
141	<i>t</i> -Bu		> 2500	> 2500	> 2500	nt	-	3.2
CQ			13	115	130	nt	-	3.7
MQ			nt	nt	nt	21800	-	5.3

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6). ^ccLogP values were calculated using ChemBioDraw Ultra software (version 14). ^dnt = not tested.

Table 4. In Vitro Antimalarial Activity and Cytotoxicity of 3,4-Disubstituted B-Ring Functionalized TAs (142–165)



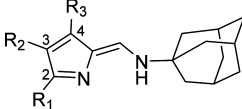
compd	R ₁	R ₂	R ₃	antimalarial activity (IC ₅₀ in nM) ^a			cytotoxicity (IC ₅₀ in nM) ^a HepG2	SI ^b (D6)	cLogP ^c
				D6	Dd2	7G8			
142	Et	Me	n-C ₄ H ₉	883	680	260	nt ^d	-	2.0
143	Et	Me	n-C ₈ H ₁₇	1166	633	244	nt	-	3.7
144	Et	Me		> 2500	2047	2500	nt	-	1.1
145	Et	Me		62	55	60	19200	310	2.7
146	Et	Me		56	60	75	18900	337	3.1
147	Et	Me		5.5	4.3	3.6	3300	600	2.6
148	Et	Me		> 2500	1576	855	nt	-	2.4
149	Et	Me		> 2500	> 2500	> 2500	nt	-	0.3
150	Me	Et		150	200	117	15800	105	2.7
151	Me	Et		111	201	128	23900	215	3.1
152	Me	Et		19	14	14	4500	237	2.6
153	Me	Me		60	38	47	21300	355	2.3
154	Me	Me		56	31	45	18100	323	2.7
155	Me	Me		2.4	1.7	1.5	6400	2667	2.2
156	Et	Et		54	30	88	16900	313	3.1
157	Et	Et		39	26	58	13000	333	3.6
158	Et	Et		1.6	1.0	2.5	3900	2437	3.0
159	-(CH ₂ -CH ₂) ₂ -			35	39	23	6200	177	2.6
160	-(CH ₂ -CH ₂) ₂ -			32	37	22	4600	144	3.1
161	-(CH ₂ -CH ₂) ₂ -			6.1	7.5	2.8	2700	442	2.5
162	Cl	Et	t-Bu	1217	> 2500	> 2500	nt	-	1.7
163	Cl	Et		> 2500	> 2500	> 2500	nt	-	2.4
164	Cl	Et		> 2500	> 2500	> 2500	nt	-	2.8
165	Cl	Et		2300	> 2500	2250	nt	-	2.3
CQ				13	115	130	nt	-	3.7
MQ				nt	nt	nt	21800	-	5.3


















^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate ($\pm 10\%$). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6). ^ccLogP values were calculated using ChemBioDraw Ultra software (version 14). ^dnt = not tested.

potency to that of the corresponding 4-alkyl/methoxy substituted analogues (147 IC₅₀ = 5.5 nM versus 109 IC₅₀ = 3.1 nM, 123 IC₅₀ = 1.3 nM, 126 IC₅₀ = 2.5 nM against D6), and this potency is 5- to 20-fold greater than the corresponding 3-alkyl substituted analogues (147 IC₅₀ = 5.5 nM versus 132 IC₅₀ = 106 nM, 135 IC₅₀ = 117 nM, 138 IC₅₀ = 26 nM against D6). Interchange of the methyl and ethyl groups between 3- and 4-positions on the ring-B as in 150–152 resulted in a ~2-fold decrease in potency (IC₅₀ of 150–152 vs IC₅₀ of 145–147). We were encouraged that the short alkyl substitutions at both the 3- and 4-positions on the ring-B were well tolerated with comparable and/or enhanced activities. This allowed for a

variety of different analogues to be synthesized with representative examples (153–165, Table 4). The analogues 153, 154, 156, 157, 159, and 160, which contain the same alkyl groups (methyl/ethyl/-(CH₂-CH₂)₂-) at both 3- and 4-positions on the ring-B, and cycloheptyl/cyclooctylamines in the place of ring-C were shown to have comparable and/or greater potency to the dissimilar alkyl groups at both 3- and 4-positions containing TAs. Significantly, the adamantyl analogues 155 (IC₅₀ < 2.4 nM), 158 (IC₅₀ < 2.5 nM), and 161 (IC₅₀ < 7.5 nM) showed enhanced (2- to 8-fold) or comparable potency against all tested Pf strains when compared to 147 (IC₅₀ < 5.5 nM) and 152 (IC₅₀ < 19 nM). The biggest potency loss occurred

Table 5. In Vitro Antimalarial Activity and Cytotoxicity of A- and B-Ring Functionalized TAs (166–187)



compd	R ₁	R ₂	R ₃	antimalarial activity (IC ₅₀ in nM) ^a			cytotoxicity (IC ₅₀ in nM) ^a HepG2	SI ^b (D6)	cLogP ^c
				D6	Dd2	7G8			
166	H		H	> 2500	> 2500	> 2500	nt ^d	-	1.3
167	H		Me	> 2500	> 2500	> 2500	nt	-	1.4
168	H		Et	> 2500	1233	> 2500	nt	-	1.9
169	Me		Me	1418	1736	2005	nt	-	1.6
170		Me	Me	250	328	215	nt	-	2.1
171		Me	Me	647	1716	415	nt	-	2.2
172		Me	Me	415	273	2282	nt	-	3.6
173		Me	Me	1141	831	>2500	nt	-	3.6
174		Me	Me	318	388	161	nt	-	3.2
175		-(CH ₂ -CH ₂) ₂ -		1335	1103	946	nt	-	1.9
176		Me	Me	> 2500	> 2500	> 2500	nt	-	2.4
177		Me	Me	2.1	2.3	0.5	3600	1714	3.0
178		Me	Me	< 2.5	< 2.5	< 2.5	1235	> 494	3.7
179		Me	Me	4.8	4.0	2.8	3825	797	3.1
180		Me	Me	27	75	12	17920	664	3.0
181		Me	Me	58	92	48	21323	368	3.9
182	H	H	H	> 2500	> 2500	> 2500	nt	-	1.2
183	Me	H	H	2100	1682	> 2500	nt	-	1.3
184	Me	H	Me	315	268	399	nt	-	1.5
185	Me	Et	Me	33	80	33	29900	906	2.2
186	Me	-(CH ₂ -CH ₂) ₂ -		61	64	60	5430	89	2.2
187		Et	Me	> 2500	> 2500	> 2500	nt	-	4.5
CQ				13	115	130	nt	-	3.7
MQ				nt	nt	nt	21000	-	5.3

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate ($\pm 10\%$). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6). ^ccLogP values were calculated using ChemBioDraw Ultra software (version 14). ^dnt = not tested.

(IC₅₀ > 2250 nM) when we introduced a chlorine atom at 3-position on the ring-B as in **162–165** (IC₅₀ of **156–158** vs **163–165**, Table 4), and it is consistent with the observation that the chlorine atom (EWG) has an adverse effect at 3-position on the ring-B of PGs. Collectively, from the monoalkylated (Tables 2 and 3) and 3,4-dialkylated TAs (Table 4) the data clearly showed that the 3,4-disubstituted TAs containing

cycloheptyl/cyclooctyl groups have significantly improved potency than the corresponding monoalkylated TAs (Tables 2 and 3), and these potencies were comparable to the corresponding OMe group containing analogues (Table 2). Notably, all the adamantyl analogues, which have short (alkyl/methoxy) groups at 4-position (Table 2) and dialkyl groups at 3/4-

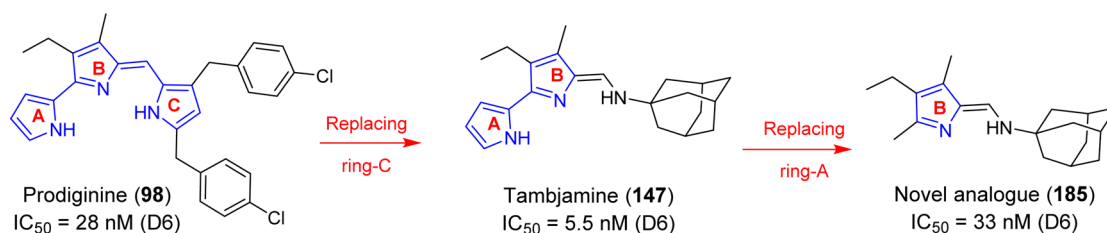


Figure 6. Structure pruning approach of the lead PG compounds (**98**).

Table 6. In Vitro Antimalarial Activity of TA-like Analogues (**190**, **191**, and **194–196**)

compd	linkage	antimalarial activity (IC ₅₀ in nM) ^a			cLogP ^b
		D6	Dd2	7G8	
190		> 2500	> 2500	> 2500	0.4
191		> 2500	> 2500	> 2500	1.6
194		> 2500	> 2500	> 2500	0.7
195		> 2500	> 2500	> 2500	0.6
196		> 2500	> 2500	> 2500	2.1
CQ	-	13	115	130	3.7

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate ($\pm 10\%$). In order to compare results run on different days and with different batches of each stain, CQ was run as a positive control. All results obtained were “normalized” to the CQ values of 13 nM for D6, 115 nM for Dd2, and 130 nM for 7G8. ^bcLogP values were calculated using ChemBioDraw Ultra software (version 14).

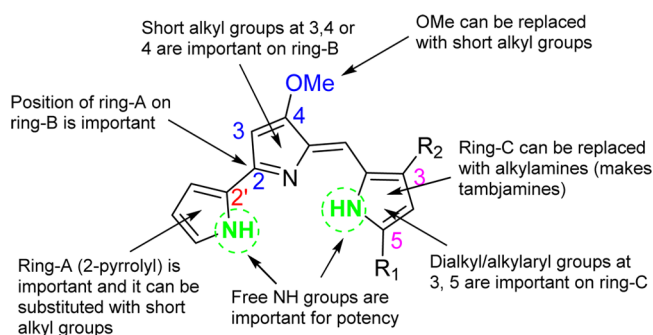


Figure 7. Summary of SAR analysis of PGs and TAs.

positions (Table 4) on ring-B, showed the greatest activity with good selectivity.

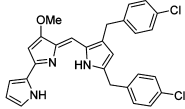
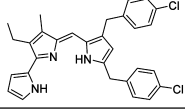
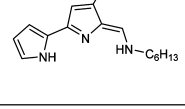
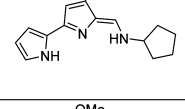
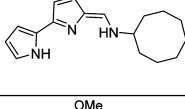
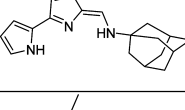
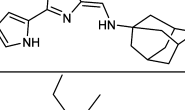
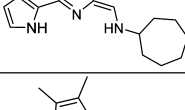
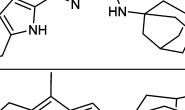
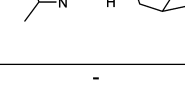
In Vitro Antimalarial Activity of A- and B-Ring Functionalized TAs (166–187). After establishing the substitutions pattern at 3- and 4-positions on the ring-B of TAs, we investigated the importance of positioning of the ring-A at 2-position on the ring-B of TAs (Table 5) by keeping the 1-adamantylamine as an active pharmacophore for all analogues. The TAs **166–169**, in which the ring-A (2-pyrrolyl

moiety) is shifted from 2- to 3-position on the ring-B and are isomeric to **129**, **123**, **126**, and **155** (Tables 2 and 4), respectively, were synthesized and tested against *Pf* strains (Table 5). It is noteworthy that the potency was significantly declined against all tested *Pf* strains after shifting the ring-A from 2- to 3-position (**166–168** IC₅₀ > 2500 nM vs **123** IC₅₀ = 1.3 nM, **126** IC₅₀ = 2.5 nM, **129** IC₅₀ = 341 nM, and **169** IC₅₀ = 1418 nM vs **155** IC₅₀ < 2.5 nM, against D6, Tables 2, 4, and 5). The importance of the location of nitrogen within ring-A was analyzed by moving from the 2'-position to the 3'-position (Figure 1 and Table 5), where compound **170** showed a roughly 100-fold drop in activity (**170** IC₅₀ = 250 nM vs **155** IC₅₀ < 2.5 nM, against D6, Tables 4 and 5). We also looked at the alternatives to the ring-A at 2 position of the ring-B. Replacement of the ring-A (2-pyrrolyl) by various 2-heteroaryl/phenyl moieties (compounds **171–175**) resulted in a decrease in antimalarial activity (IC₅₀ of **171–175** vs **155** and **161**). Notably, our previous SAR investigations revealed that the ring-A (2-pyrrolyl moiety) of PGs provides optimal activity,^{37,38} and the current results also suggest the importance of the ring-A of TAs for the potent activity. Alkylation (methylation) on the NH group of the ring-A as in **176** resulted in a large decrease in potency (**176** IC₅₀ > 2500 nM vs **155** IC₅₀ < 2.5 nM), suggesting that the pyrrole NH (ring-A) of the TAs is important for potent antimalarial activity. Conversely, the analogues **177–181**, which contain C-alkyl moieties on the ring-A, retained the potency against all tested *Pf* strains, suggesting that the alkyl groups are well tolerated on the ring-A.

To further investigate the exact role of the ring-A of TAs on potency, a set of mixed alkylated analogues **182–186**, in which the ring-A is completely removed from the core moiety of TAs, were examined. Complete removal of the substitutions on the ring-B dramatically reduced the potency of the compound **182** (IC₅₀ > 2500 nM). Incorporation of the substitutions into the ring-B as in **183–186** (from mono- to trialkyl) resulted in a large increase in potency (Table 5), whereas the dimer **187** of the **185** showed the poorest activity. It is noteworthy that the analogues **185** and **186**, which contain a monopyrrole with trialkyl substituents and an enamine moiety, showed the comparable potency to that of the corresponding bipyrrole TAs. These results demonstrated that the ring-A is not essential for the antimalarial activity, but both the trialkylated monopyrrole and enamine moiety are important. In summary, structure pruning of PGs has shown that in vitro potency can be retained and/or enhanced when moving from a tripyrrole (PGs) to bipyrrole (TAs) and even to a monopyrrole as shown in Figure 6.

In Vitro Antimalarial Activity of TA-like Analogues (190, 191, and 194–196), in Which the Ring-B Is Replaced by an Alkylamide/Amine Linkage. Our detailed SAR explorations around the ring-A and -B and nature of alkylamines of TAs led to a robust understanding of the structural features that

Table 7. In Vivo Antimalarial Efficacy of PGs and TAs in a Murine *P. yoelii*

compd	compd code names	structure	dose (mg/kg × 4 days)	% suppression of parasitaemia on day 5 ^b	ED ₅₀ (mg/kg/day)
control	-	-	PEG-400	-	-
85^a	KAR71		5	90	2.8
			25	100	
			50	100	
			100	100	
98	KAR276		5	66	< 5
			25	100	
			50	100	
100	KAR458		5	90	< 5
			25	93	
			50	96	
			100	100	
105	KAR383		5	92	< 5
			25	94	
			50	96	
			100	100	
108	KAR457		5	93	< 5
			25	94	
			50	99	
			100	100	
109	KAR422		5	0	84
			25	27	
			50	38	
			100	97	
123	KAR790		5	30	20
			25	77	
			50	100	
145	KAR425		5	100	< 2.5
			25	100 ^c	
			50	100 ^c	
			100	100	
177	KAR767		5	7	5.5
			25	100	
			50	100	
			100	100	
185	KAR765		5	24	45
			25	17	
			50	67	
			100	100	
CQ	-	-	1	65	2.2
			4	94	
			16	100	
			64	100	

^aPrevious lead compound. ^b% suppression of parasitemia = 100 × [(parasitemia control group) – (parasitemia treated group)]/parasitemia control group. ^cProvided cures (100% protection to malaria-infected mice).

are required for potent antimalarial activity. We also sought to explore whether any linkage (total replacement of ring-B) between two of the most active pharmacophores (i.e., 2-pyrrolyl and 1-adamantyl moieties) is tolerated. A set of novel TA-like analogues **190**, **191**, and **194–196**, in which ring-B is completely replaced by an alkylamide/amine linkage, were generated and screened for their antimalarial activity against *Pf*

strains (Table 6). None of these analogues showed activity (IC₅₀ > 2500 nM, Table 6). These data confirmed that the ring-B between ring-A and alkylamine plays an important role in the antimalarial activity of TAs and PGs as well.

In Vivo Efficacy Studies in Mice Models. Given the attractive antiplasmodial activity of several PGs and TAs against CQ^S-D6, CQ^R-Dd2, and 7G8 strains of *P. falciparum* along with

favorable toxicological properties against hepatocellular HepG2 cancer cell line and lower MWT and lipophilic properties, an in vivo proof of concept study in a murine *P. yoelii* model was undertaken with the most potent and selective analogues **98**, **100**, **105**, **108**, **109**, **123**, **145**, **177**, and **185**, using side by side comparison with our previous lead PG **85**³⁷ and CQ as a reference drug (Table 7). In vivo efficacy was determined in a murine *P. yoelii* model,^{71,72} in which animals were randomly placed in groups of four and administered test drugs in the range of 5–100 mg/kg by oral gavage on 4 sequential days following the day of inoculation. The in vivo data are expressed as ED₅₀ values and reflect the dose (estimated from dose–response curves) for suppression of parasitemia by 50% relative to vehicle-only controls as assessed on day 5 of each study. In these experiments, the animals with parasitemia either on day 5 or later were euthanized and the parasitemia free animals were kept in observation until day 28. Drug treated animals that were parasitemia free on day 28 of the experiment are defined as “cures”, and the amount of drug that was needed to achieve a cure is referred to as the “nonrecrudescence dose” (NRD).

Following four once-daily doses of PGs **85** and **98** at 5 mg/kg, each reduced parasitemia by a 90% and 66% on day 5, respectively, and parasitemia free animals were observed at 25 and 50–100 mg/kg; however, none of these animals were cured, while the CQ was also not curative in this model even at doses as high as 64 mg kg⁻¹ day⁻¹ (Table 7). The TA analogues **100**, **105**, and **108** each reduced parasitemia >90% after 5, 25, and 50 mg/kg × 4 days dosing, and at the higher dose (100 mg/kg × 4 days) these reduced parasitemia 100% on day 5. Intriguingly, the TA **109** with good in vitro potency showed much less efficacy with an ED₅₀ value of 84 mg kg⁻¹ day⁻¹, which may relate to low aqueous solubility and/or poor oral bioavailability (Table 7). Interestingly the analogue **123**, in which the methyl group of ring-B is replaced the OMe group of **109**, showed improved efficacy at all doses; specifically 100% reduction was observed at 50 mg/kg × 4 days on day 5. Of these TAs, the analogue **145** with 3-ethyl/4-methyl substitution pattern on the ring-B and the cycloheptylamine in the place of ring-C provided an excellent in vivo efficacy against *P. yoelii* in mice with an ED₅₀ value of < 2.5 mg kg⁻¹ day⁻¹, and it cleared all parasitemia on day 5 after dosing 5 mg/kg to 100 mg/kg × 4 days. Indeed, the compound **145** provided parasite-free cures on day 28 (100% protection to malaria-infected mice) at 25 and 50 mg kg⁻¹ day⁻¹, without evident weight loss and toxicity. In separate experiments, a single oral dose (80 mg/kg) of KAR425 (**145**) was also used. The preliminary experiments demonstrated that the KAR425 is also curative in this model and two of four mice were cured with no obvious signs of toxicity or behavior change; higher dose studies are underway in our laboratories. The analogues **177** and **185** showed 100% parasitemia reduction on day 5 after 25–100 mg/kg and 100 mg/kg dosing, respectively; however these were not curative in this model.

CONCLUSIONS

We report here the synthesis and antimalarial activity of the novel class of potent tambjamins (TAs) and B-ring functionalized prodiginines (PGs). The compounds were synthesized via simple and inexpensive chemical procedures using easily available building blocks to respond to the demand for low-cost novel antimalarial agents. When compared to tripyrrole PGs,^{37,38} these bipyrrole TAs exhibited marked improvements with regard to the color properties, in vitro potency, selectivity, and in vivo efficacy. Several key findings emerged from these studies: (i)

the alkylated pyrrole (ring-C) can be replaced by an alkyl/cycloalkylamine, providing for TAs with retained and/or enhanced antimalarial activity; (ii) the OMe group at the 4-position on the ring-B, between ring-A and ring-C/alkylamine of PGs/TAs, can be replaced with short alkyl substitutions at either 4-position or 3- and 4-positions without impacting in vitro potency; (iii) the 2-pyrrolyl moiety (ring-A) must be linked at 2-position on the ring-B for potency, and it can be substituted with alkyl groups (see Figure 7). In addition, these analogues are equally effective against *P. falciparum* pansensitive D6 and MDR Dd2 and 7G8 strains. Some of these analogues have shown very promising in vivo efficacy in mice; specifically, the KAR425 (**145**) TA offered greater efficacy than previously observed for any tripyrrole PG, providing 100% protection to malaria-infected mice until day 28 at doses of 25 and 50 mg/kg × 4 days, and was also curative in this model in a single oral dose (80 mg/kg). In our overall study, the KAR425 stands out as an excellent lead compound, with low molecular weight (<300), good lipophilic profile (cLogP < 2.7), oral efficacy, and no obvious signs of toxicity or behavior change. Detailed lead optimization, pharmacology, safety, and modes of action studies of the KAR425 will be studied in our laboratories in due course to produce the antimalarial candidates for full preclinical studies.

EXPERIMENTAL SECTION

General. NMR spectra were recorded on Bruker AMX-400 and AMX-600, spectrometers at 400, 600 MHz (¹H) and 100, 150 MHz (¹³C). Experiments were recorded in CDCl₃, CD₃OD, acetone-*d*₆, and DMSO-*d*₆ at 25 °C. Chemical shifts are given in parts per million (ppm) downfield from internal standard Me₄Si (TMS). HRMS (ESI) were recorded on a high-resolution (30000) thermo LTQ-Orbitrap Discovery hybrid mass spectrometer (San Jose, CA). Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions that required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of argon/nitrogen. Chromatography was executed on CombiFlash Rf 200 instrument, using silica gel (230–400 mesh) and/or neutral alumina as the stationary phase and 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 CH₃OH/CH₃CN/H₂O (40%/10%/50%) to CH₃OH (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.

Synthesis of 4-Hydroxy-2-oxo-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butyl Ester (45**).** To a stirred solution of *N*-(*tert*-butoxycarbonyl)glycine (**44**; 5.0 g, 28.57 mmol) in 90 mL of anhydrous CH₂Cl₂ (DCM) were added Meldrum's acid (4.93 g, 34.28 mmol) and 4-dimethylaminopyridine (DMAP; 8.71 g, 71.42 mmol) under an argon atmosphere at 0 °C. A solution of isopropyl chloroformate (42.85 mL, 42.85 mmol, 1 N in toluene) was added dropwise, and the reaction mixture was stirred for 4 h at 0 °C. The reaction mixture was diluted with DCM (100 mL), washed with 15% KHSO₄ (2 × 70 mL), and organic layer was dried over Na₂SO₄, and the solvent was evaporated under reduced pressure to give the acylated Meldrum's acid. This material was then refluxed in ethyl acetate (600 mL) for 1 h, and the solvent was evaporated under reduced pressure, and the product was recrystallized from ethyl acetate to give the desired product **45** (3.46 g, 61%) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.13 (br s, 1H), 4.88 (s, 1H), 4.14 (s, 2H), 1.44 (s, 9H); HRMS (ESI) calcd for C₉H₁₃NaNO₄ (M + Na)⁺ 222.0737, found 222.0740.

Synthesis of 2-Oxo-4-(toluene-4-sulfonyloxy)-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butyl Ester (46**).** To a stirred solution of **45** (3.4 g, 17.08 mmol) in anhydrous CH₂Cl₂ (150 mL) were added *p*-toluenesulfonyl chloride (3.24 g, 17.08 mmol) and DIPEA (4.4 g, 34.17 mmol). The resulting reaction mixture was stirred

for 6 h at 25 °C. Then the reaction mixture was washed with 5% HCl (2 × 25 mL), brine and dried over anhydrous Na₂SO₄. The organic solvent was removed under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford **46** (5.37 g, 89%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.86 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 5.75 (s, 1H), 4.22 (d, *J* = 1.2 Hz, 2H), 2.50 (s, 3H), 1.52 (s, 9H); HRMS (ESI) calcd for C₁₆H₁₉NaNO₆S (M + Na)⁺ 376.0825, found 376.0830.

Synthesis of 4-(4-Chlorophenyl)-2-oxo-2,5-dihydropyrrole-1-carboxylic Acid tert-Butyl Ester (47). To a degassed stirred solution of **46** (4.0 g, 11.33 mmol) and 4-chlorophenylboronic acid (2.65 g, 17.0 mmol) in 100 mL of THF at room temperature were added Pd(dppf)Cl₂ (410 mg, 0.56 mmol) and a solution of cesium carbonate (11.05 g, 34.0 mmol) in water (15 mL). The reaction mixture was stirred at 25 °C for 1 h and then heated to reflux for 16 h. The reaction mixture was filtered through Celite and washed with ethyl acetate (400 mL). The organic layer was washed with saturated sodium bicarbonate (2 × 75 mL) and brine and dried over anhydrous Na₂SO₄. Then the organic solution was concentrated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **47** (1.82 g, 55%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.50 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 6.42 (t, *J* = 1.5 Hz, 1H), 4.68 (d, *J* = 1.5 Hz, 2H), 1.61 (s, 9H); HRMS (ESI) calcd for C₁₅H₁₆NaClNO₃ (M + Na)⁺ 316.0711, found 316.0713.

Synthesis of 4-(4-Chlorophenyl)-1,5-dihydropyrrol-2-one (48). To a stirred solution of **47** (1.8 g, 6.14 mmol) in anhydrous CH₂Cl₂ (25 mL) was added dropwise TFA (2.8 g, 24.57 mmol). The reaction mixture was stirred for an additional hour at 25 °C. The solvent was evaporated under reduced pressure, and the crude material was then dissolved in ethyl acetate (200 mL). The organic layer was washed with 5% NaHCO₃ and brine and dried over anhydrous Na₂SO₄. The organic solvent was evaporated under reduced pressure and the solid material was washed with CH₂Cl₂ to afford the pure product **48** (1.14 g, 94%) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.15 (br s, 1H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 2H), 6.50 (t, *J* = 1.5 Hz, 1H), 4.30 (s, 2H); HRMS (ESI) calcd for C₁₀H₉ClNO (M + H)⁺ 194.0367, found 194.0372.

Synthesis of 5-Bromo-3-(4-chlorophenyl)pyrrole-2-carboxaldehyde (49). To a stirred solution of diethylformamide (DEF; 1.57 g, 15.54 mmol) in anhydrous chloroform (10 mL) at 0 °C was added dropwise a solution of phosphorus oxybromide (POBr₃; 3.62 g, 12.95 mmol) in chloroform (10 mL). The resulting thick suspension was stirred at 0 °C for 30 min to obtain the Vilsmeier complex as a solid. After the sample was dried in vacuo for 20 min, chloroform (50 mL) was added to the solid and the reaction mixture was cooled to 0 °C. The compound **48** (1.0 g, 5.18 mmol) was added portionwise, and the reaction mixture was warmed to room temperature and then heated at 70 °C for 16 h. The reaction mixture was poured onto ice-water (75 mL), and the pH of the aqueous solution was adjusted to pH 9–10 by treatment with 5 N NaOH. Dichloromethane (100 mL) was added to the resulting precipitate, and the mixture was filtered through Celite. The two layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (3 × 100 mL). The organic layers were combined, washed with brine, and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was passed through a silica gel, with ethyl acetate/hexanes as eluent, to afford the pure **49** (806 mg, 55%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 10.05 (br s, 1H), 9.49 (s, 1H), 7.49–7.40 (m, 4H), 6.42 (d, *J* = 2.6 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 178.2, 137.1, 134.5, 131.5, 130.9, 130.3, 129.9, 129.1, 128.9, 113.6, 113.1; HRMS (ESI) calcd for C₁₁H₈BrClNO (M + H)⁺ 283.9472, found 283.9484.

Representative Procedure for the Synthesis of 4-(4-Chlorophenyl)-[2,2']bipyrrolyl-5-carboxaldehyde (9). To a degassed stirred solution of **49** (1.0 g, 3.53 mmol) and *N*-Boc-2-pyrroleboronic acid (1.11 g, 5.30 mmol) in 10% water/dioxane (50 mL) were added Pd(PPh₃)₄ (204 mg, 0.17 mmol) and Na₂CO₃ (749 mg, 7.06 mmol). The reaction mixture was stirred for 3 h at 100 °C and poured onto water (100 mL). The pH of the solution was lowered to pH 7 with 2 N HCl and extracted with ethyl acetate (3 × 75 mL). The combined

organic layers were washed with water and brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the residue was dissolved in methanol (25 mL) and evaporated the solvent to remove the volatile B(OMe)₃. This was then dissolved in THF (10 mL), and LiOH (850 mg, 35.33 mmol) in methanol (10 mL) was added dropwise under an argon atmosphere at room temperature. The resulting reaction mixture was stirred at room temperature for 30 min. On completion of the reaction, the solvent was removed under reduced pressure. The resulting solid was picked up with ethyl acetate (200 mL), washed with water and brine, and dried over anhydrous Na₂SO₄. The organic solvent was removed under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure **9** (562 mg, 59%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 12.09 (br s, 1H), 11.31 (br s, 1H), 9.46 (s, 1H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 6.93 (m, 1H), 6.81 (m, 1H), 6.72 (d, *J* = 2.5 Hz, 1H), 6.14 (m, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 177.1, 135.6, 133.6, 132.6, 132.3, 130.5 (2C), 128.7 (2C), 127.5, 123.0, 120.2, 109.3, 108.1, 106.4; HRMS (ESI) calcd for C₁₅H₁₂ClN₂O (M + H)⁺ 271.0633, found 271.0639.

Synthesis of 5-Chloropyrrole-2-carboxaldehyde (51). To a stirred solution of pyrrole (**50**; 5.0 g, 74.62 mmol) in 200 mL of dry THF was added *N*-chlorosuccinimide (NCS; 9.92 g, 74.62 mmol) under an argon atmosphere at –78 °C. The reaction mixture was stirred for an additional 4 h at the same temperature and placed at –20 °C for overnight. To the reaction mixture was added dropwise Vilsmeier reagent (149.25 mmol, in situ generation from POCl₃/DMF, 0 °C, 1 h) in 100 mL of DCM at –20 °C. The reaction mixture was stirred for 10 h while it was allowed to warm to room temperature. The solvent was removed under reduced pressure, and 100 mL of water was added. To the stirred mixture, sodium hydroxide (2 N, 100 mL) was added slowly, and the reaction mixture was allowed to stir for 1 h at room temperature. Ethyl acetate (300 mL) was added to the resulting precipitate, the two layers were separated, and the aqueous layer was further extracted with ethyl acetate (2 × 100 mL). The organic layers were combined, washed with brine, and dried over anhydrous Na₂SO₄. The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **51** (3.46 g, 36%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 12.28 (br s, 1H), 9.31 (s, 1H), 6.85 (dd, *J* = 2.3, 4.0 Hz, 1H), 6.14 (dd, *J* = 2.3, 4.0 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 178.2, 131.9, 126.0, 122.4, 110.0; HRMS (ESI) calcd for C₅H₃ClNO (M + H)⁺ 130.0054, found 130.0055.

Synthesis of [2,2'-Bipyrrole]-5-carboxaldehyde (19). Compound **19** (558 mg, 45%) was synthesized by the same procedure as described for **9**. ¹H NMR (CDCl₃, 400 MHz) δ 11.98 (br s, 1H), 11.24 (br s, 1H), 9.35 (s, 1H), 7.00 (dd, *J* = 2.3, 3.9 Hz, 1H), 6.89 (m, 1H), 6.73 (m, 1H), 6.54 (dd, *J* = 2.3, 3.9 Hz, 1H), 6.12 (m, 1H); HRMS (ESI) calcd for C₉H₉N₂O (M + H)⁺ 161.0709, found 161.0713.

Synthesis of Ethyl 4,5,6,7-Tetrahydroisindole-1-carboxylate (53). To a stirred solution of **52** (5.0 g, 39.37 mmol) and ethyl isocynoacetate (5.33 g, 47.24 mmol) in 1:1 mixture of THF and ethanol (100 mL) was added portionwise anhydrous potassium carbonate (10.86 g, 78.74 mmol). The reaction mixture was then stirred at room temperature for 3 days. The mixture was poured into water (100 mL), acidified to pH 5 with 2 N HCl, and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **53** (4.93 g, 65%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 9.28 (br s, 1H), 6.67 (d, *J* = 2.9 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 2.85 (t, *J* = 5.8 Hz, 2H), 2.57 (t, *J* = 6.0 Hz, 2H), 1.77 (m, 4H), 1.38 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) calcd for C₁₁H₁₆NO₂ (M + H)⁺ 194.1176, found 194.1184.

Synthesis of 4,5,6,7-Tetrahydroisindole (54). Sodium hydroxide (1.47 g, 36.71 mmol) was added to a solution of **53** (3.8 g, 18.35 mmol) in anhydrous ethylene glycol (20 mL) under an argon atmosphere at room temperature, and the reaction mixture was heated to reflux and stirred at refluxing temperature for an hour. After cooling to room temperature,

the reaction mixture was taken up in *n*-hexane, washed with water and dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure afforded the **54** (2.0 g, 90%) as a white solid that was directly used in the next step without further purification. ¹H NMR (CDCl₃, 400 MHz) δ 7.92 (br s, 1H), 6.53 (d, *J* = 2.6 Hz, 2H), 2.67 (m, 4H), 1.80 (m, 4H); HRMS (ESI) calcd for C₈H₁₂N (M + H)⁺ 122.0964, found 122.0969.

Representative Procedure for the Synthesis of 4,5,6,7-Tetrahydroisindole-1-carboxaldehyde (55) by Standard Vilsmeier Conditions. Phosphorus oxychloride (POCl₃; 5.05 g, 33.05 mmol) was added dropwise to dimethylformamide (DMF; 2.41 g, 33.05 mmol) at 0 °C. The resulting solution was stirred at 0 °C until the formation of the Vilsmeier complex as a solid. After the solid was dried in vacuo for 20 min, dichloromethane (50 mL) was added to the solid and the reaction mixture was cooled to 0 °C. A solution of **54** (2.0 g, 16.52 mmol) in DCM (50 mL) was added dropwise, and the reaction mixture was warmed to room temperature and then stirred for 10 h. After removal of all solvent under vacuo, the residue was mixed with water (100 mL). To the stirred mixture, sodium hydroxide (5.28 g, 132.23 mmol) was added slowly, and the reaction mixture was allowed to stir for 1 h at room temperature. Ethyl acetate (200 mL) was added to the resulting precipitate, the two layers were separated, and the aqueous layer was further extracted with ethyl acetate (2 × 50 mL). The organic layers were combined, washed with brine, and dried over anhydrous Na₂SO₄. The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **55** (1.84 g, 75%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 10.23 (br s, 1H), 9.51 (s, 1H), 6.87 (d, *J* = 2.8 Hz, 1H), 2.86 (t, *J* = 5.9 Hz, 2H), 2.55 (t, *J* = 6.0 Hz, 2H), 1.80 (m, 4H); HRMS (ESI) calcd for C₉H₁₂NO (M + H)⁺ 150.0913, found 150.0920.

Representative Procedure for the Synthesis of 3-Bromo-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (56). To a stirred solution of **55** (2.0 g, 13.42 mmol) in THF (100 mL) was added portionwise DBDMH (1.90 g, 6.71 mmol) in a period of 10 min at -78 °C. Then the reaction mixture was stirred for 5 h while it was allowed to warm to room temperature. The reaction was quenched with 5% aqueous KHSO₄ solution and extracted with ethyl acetate (3 × 75 mL). The combined organic layers were washed with brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **56** (2.48 g, 82%). ¹H NMR (CDCl₃, 400 MHz) δ 10.60 (br s, 1H), 9.41 (s, 1H), 2.83 (m, 2H), 2.42 (m, 2H), 1.77 (m, 4H); ¹³C NMR (CDCl₃, 100 MHz) δ 175.7, 134.7, 128.9, 122.7, 110.5, 22.8, 22.6, 21.3, 21.0; HRMS (ESI) calcd for C₉H₁₁BrNO (M + H)⁺ 228.0019, found 228.0031.

Synthesis of 3-(Pyrrol-2-yl)-4,5,6,7-tetrahydroisindole-1-carboxaldehyde (20). Compound **20** (682 mg, 72%) was synthesized by the same procedure as described for **9**. ¹H NMR (DMSO-*d*₆ + CDCl₃, 400 MHz) δ 10.64 (br s, 1H), 10.35 (br s, 1H), 8.77 (s, 1H), 6.21 (s, 1H), 5.81 (s, 1H), 5.56 (s, 1H), 2.19 (s, 2H), 1.98 (s, 2H), 1.16 (m, 4H); ¹³C NMR (DMSO-*d*₆ + CDCl₃, 100 MHz) δ 173.0, 133.1, 128.8, 125.6, 122.5, 117.9, 116.7, 108.2, 107.5, 21.9, 21.5, 21.1, 19.6; HRMS (ESI) calcd for C₁₃H₁₃N₂O (M + H)⁺ 215.1179, found 215.1188.

Synthesis of Compounds 59–62. Compounds **59** (1.36 g, 75%), **60** (1.27 g, 78%), **61** (1.35 g, 79%), and **62** (1.35 g, 83%) were synthesized by the same procedure as described for **56**.

4-Bromopyrrole-2-carboxaldehyde (59). ¹H NMR (CDCl₃, 400 MHz) δ 10.16 (br s, 1H), 9.49 (d, *J* = 1.0 Hz, 1H), 7.15 (m, 1H), 7.00 (m, 1H); HRMS (ESI) calcd for C₅H₃BrNO (M + H)⁺ 173.9549, found 173.9555.

4-Bromo-3,5-dimethylpyrrole-2-carboxaldehyde (60). ¹H NMR (CDCl₃, 400 MHz) δ 10.82 (br s, 1H), 9.45 (s, 1H), 2.36 (s, 3H), 2.28 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 176.2, 137.0, 133.0, 127.7, 101.5, 12.2, 10.0; HRMS (ESI) calcd for C₇H₉BrNO (M + H)⁺ 201.9862, found 201.9871.

4-Bromo-3-methylpyrrole-2-carboxaldehyde (61). ¹H NMR (acetone-*d*₆, 400 MHz) δ 11.17 (br s, 1H), 9.69 (d, *J* = 0.7 Hz, 1H),

7.25 (d, *J* = 3.2 Hz, 1H), 2.32 (s, 3H); HRMS (ESI) calcd for C₆H₇BrNO (M + H)⁺ 187.9705, found 187.9711.

4-Bromo-3-ethylpyrrole-2-carboxaldehyde (62). ¹H NMR (CDCl₃, 400 MHz) δ 10.08 (br s, 1H), 9.34 (s, 1H), 7.20 (d, *J* = 2.6 Hz, 1H), 2.47 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H); HRMS (ESI) calcd for C₇H₉BrNO (M + H)⁺ 201.9862, found 201.9869.

Synthesis of 22–25. Compounds **22** (647 mg, 70%), **23** (673 mg, 72%), **24** (623 mg, 67%), and **25** (608 mg, 65%) were synthesized by the same procedure as described for **9**, with modification of the reaction conditions for deprotection of *N*-Boc group. The crude material was dissolved in THF (10 mL), and LiOH (10 equiv) in methanol (10 mL) was added dropwise under an argon atmosphere. The resulting mixture was stirred at 60 °C for 2 h.

[2,3'-Bipyrrole]-5'-carboxaldehyde (22). ¹H NMR (CD₃OD, 400 MHz) δ 9.43 (d, *J* = 1.0 Hz, 1H), 7.37 (dd, *J* = 1.6, 2.5 Hz, 1H), 7.15 (d, *J* = 1.6 Hz, 1H), 6.70 (dd, *J* = 1.5, 2.7 Hz, 1H), 6.23 (dd, *J* = 1.5, 3.4 Hz, 1H), 6.10 (dd, *J* = 2.7, 3.4 Hz, 1H); ¹³C NMR (CD₃OD, 100 MHz) δ 180.7, 134.4, 127.7, 123.8, 122.6, 118.1, 117.2, 109.4, 104.7; HRMS (ESI) calcd for C₉H₉N₂O (M + H)⁺ 161.0709, found 161.0713. Note: Two NH protons are not appearing under these conditions.

2',4'-Dimethyl-[2,3'-bipyrrole]-5'-carboxaldehyde (23). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.68 (br s, 1H), 10.59 (br s, 1H), 9.51 (s, 1H), 6.76 (br s, 1H), 6.08 (br s, 1H), 5.94 (br s, 1H), 2.28 (s, 3H), 2.24 (s, 3H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 176.4, 135.2, 129.8, 127.7, 124.2, 117.3, 117.1, 107.9, 107.0, 12.1, 9.5; HRMS (ESI) calcd for C₁₁H₁₃N₂O (M + H)⁺ 189.1022, found 189.1026.

4'-Methyl-[2,3'-bipyrrole]-5'-carboxaldehyde (24). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.78 (br s, 1H), 10.78 (br s, 1H), 9.64 (s, 1H), 7.33 (d, *J* = 3.0 Hz, 1H), 6.71 (dd, *J* = 1.8, 2.4 Hz, 1H), 6.10 (dd, *J* = 1.8, 3.0 Hz, 1H), 6.07 (dd, *J* = 2.4, 3.0 Hz, 1H), 2.41 (s, 3H); ¹³C NMR (DMSO-*d*₆ + CDCl₃, 100 MHz) δ 176.2, 128.7, 125.8, 124.5, 122.1, 118.1, 115.6, 107.1, 104.0, 8.9; HRMS (ESI) calcd for C₁₀H₁₁N₂O (M + H)⁺ 175.0866, found 175.0871.

4'-Ethyl-[2,3'-bipyrrole]-5'-carboxaldehyde (25). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 9.69 (s, 1H), 7.40 (d, *J* = 2.7 Hz, 1H), 6.70 (dd, *J* = 1.7, 2.7 Hz, 1H), 6.25 (d, *J* = 3.2 Hz, 1H), 6.16 (dd, *J* = 2.7, 3.2 Hz, 1H), 2.70 (q, *J* = 7.3 Hz, 2H), 1.12 (t, *J* = 7.3 Hz, 3H); HRMS (ESI) calcd for C₁₁H₁₃N₂O (M + H)⁺ 189.1022, found 189.1027. Note: Two NH protons are not appearing under these conditions.

Synthesis of 26–31. Compounds **26** (276 mg, 55%), **27** (266 mg, 57%), **28** (296 mg, 63%), **29** (346 mg, 68%), **30** (346 mg, 67%), and **31** (385 mg, 65%) were synthesized by the same procedure as described for **9** with modification of the reaction conditions for the deprotection of *N*-triisopropylsilyl group. The crude material was dissolved in THF (10 mL), and TBAF (2 equiv) was added dropwise under an argon atmosphere. The resulting mixture was stirred at room temperature for 15 min.

1',3,4-Trimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (26). ¹H NMR (CDCl₃, 400 MHz) δ 9.62 (s, 1H), 8.84 (br s, 1H), 6.77 (dd, *J* = 1.8, 2.4 Hz, 1H), 6.28 (dd, *J* = 1.8, 3.7 Hz, 1H), 6.23 (dd, *J* = 2.4, 3.7 Hz, 1H), 3.61 (s, 3H), 2.33 (s, 3H), 2.02 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 176.8, 131.8, 129.6, 129.0, 124.3, 124.2, 120.4, 111.3, 108.4, 34.8, 9.6, 9.0; HRMS (ESI) calcd for C₁₂H₁₄NaN₂O (M + Na)⁺ 225.0998, found 225.1006.

3,4-Dimethyl-[2,3'-bipyrrole]-5-carboxaldehyde (27). ¹H NMR (CDCl₃, 400 MHz) δ 9.52 (s, 1H), 9.10 (br s, 1H), 8.64 (br s, 1H), 7.12 (m, 1H), 6.89 (m, 1H), 6.48 (m, 1H), 2.31 (s, 3H), 2.15 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 175.4, 134.3, 133.3, 127.9, 119.1, 117.1, 116.6, 115.5, 106.9, 9.8, 8.9; HRMS (ESI) calcd for C₁₁H₁₂NaN₂O (M + Na)⁺ 189.1022, found 189.1028.

5-(Furan-2-yl)-3,4-dimethylpyrrole-2-carboxaldehyde (28). ¹H NMR (CDCl₃, 400 MHz) δ 9.63 (s, 1H), 9.52 (br s, 1H), 7.48 (dd, *J* = 1.6, 2.8 Hz, 1H), 6.64 (dd, *J* = 1.6, 3.6 Hz, 1H), 6.52 (dd, *J* = 2.8, 3.6 Hz, 1H), 2.30 (s, 3H), 2.17 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 176.8, 146.5, 142.1, 132.1, 128.6, 128.0, 117.9, 111.9, 107.8, 9.6, 8.6; HRMS (ESI) calcd for C₁₁H₁₁NaN₂O (M + Na)⁺ 212.0682, found 212.0689.

3,4-Dimethyl-5-(thiophen-2-yl)pyrrole-2-carboxaldehyde (29). ^1H NMR (CDCl_3 , 400 MHz) δ 9.63 (s, 1H), 9.53 (br s, 1H), 7.37 (dd, $J = 1.6, 2.7$ Hz, 1H), 7.34 (dd, $J = 1.6, 3.5$ Hz, 1H), 7.13 (dd, $J = 2.7, 3.5$ Hz, 1H), 2.33 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.8, 133.6, 132.6, 131.4, 128.9, 127.8, 125.7, 124.2, 118.9, 9.9, 8.9; HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{11}\text{N}\text{NaNO}_3$ ($\text{M} + \text{Na}$) $^+$ 228.0454, found 228.0459.

3,4-Dimethyl-5-phenylpyrrole-2-carboxaldehyde (30). ^1H NMR (CDCl_3 , 400 MHz) δ 9.64 (s, 1H), 9.49 (br s, 1H), 7.52 (m, 2H), 7.46 (m, 2H), 7.39 (m, 1H), 2.34 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.9, 137.0, 132.6, 131.7, 129.0, 128.9 (2C), 128.2, 127.8 (2C), 118.6, 9.8, 9.0; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{13}\text{N}\text{NaNO}_3$ ($\text{M} + \text{Na}$) $^+$ 222.0889, found 222.0897.

5-(Indol-2-yl)-3,4-dimethylpyrrole-2-carboxaldehyde (31). ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 11.43 (br s, 2H), 9.62 (s, 1H), 7.44 (m, 2H), 7.09 (m, 2H), 6.82 (s, 1H), 2.29 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($\text{CDCl}_3 + \text{DMSO}-d_6$, 100 MHz) δ 177.1, 136.1, 130.9, 129.6, 129.2, 128.8, 128.3, 122.1, 120.2, 119.6, 118.4, 111.2, 101.5, 10.0, 8.5; HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 239.1179, found 239.1188.

Representative Procedure for the Synthesis of 2-Ethylpyrrole (67a). To a stirred suspension of LiAlH_4 (3.49 g, 91.74 mmol) in dry THF (50 mL) was added dropwise **66a** (5.0 g, 45.87 mmol) in THF (50 mL) at 0 °C. Then the resulting solution was heated to reflux overnight. The reaction was quenched with saturated solution of sodium sulfate. The insoluble solid was filtered off and washed with DCM (100 mL). Then the combined organic solution was concentrated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **67a** (4.0 g, 92%).

2-Isobutylpyrrole (67b). Yield: 4.26 g, 95%. HRMS (ESI) calcd for $\text{C}_8\text{H}_{14}\text{N}$ ($\text{M} + \text{H}$) $^+$ 124.1121, found 124.1126.

Representative Procedure for the Synthesis of tert-Butyl 2-Ethylpyrrole-1-carboxylate (68a). 4-Dimethylaminopyridine (DMAP; 257 mg, 2.10 mmol) was added to a stirred solution of **67a** (2.0 g, 21.05 mmol) and di-tert-butyl dicarbonate (Boc_2O ; 6.23 g, 27.36 mmol) in acetonitrile (50 mL), and the reaction was left to stir for 1 h at room temperature. Dichloromethane (150 mL) was added, and the solution was washed with water and brine and dried over anhydrous Na_2SO_4 . The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure **68a** (3.90 g, 95%). HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{18}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 196.1332, found 196.1335.

tert-Butyl 2-Isobutylpyrrole-1-carboxylate (68b). Yield: 3.40 g, 94%. ^1H NMR (CDCl_3 , 400 MHz) δ 7.21 (dd, $J = 1.6, 2.4$ Hz, 1H), 6.09 (dd, $J = 1.6, 3.6$ Hz, 1H), 5.95 (dd, $J = 2.4, 3.6$ Hz, 1H), 2.73 (d, $J = 7.0$ Hz, 2H), 1.93 (m, 1H), 1.61 (s, 9H), 0.94 (d, $J = 6.3$ Hz, 6H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 149.6, 135.1, 120.9, 112.3, 109.7, 83.1, 37.8, 28.0 (3C), 27.7, 22.5 (2C); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{22}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 224.1645, found 224.1649.

tert-Butyl 2,4-Dimethylpyrrole-1-carboxylate (68c). Yield: 3.77 g, 92%. ^1H NMR (CDCl_3 , 400 MHz) δ 6.94 (s, 1H), 5.80 (s, 1H), 2.41 (s, 3H), 2.02 (s, 3H), 1.60 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 149.6, 131.6, 120.4, 117.5, 114.2, 82.8, 28.1 (3C), 15.4, 11.7; HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{18}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 196.1332, found 196.1339.

tert-Butyl 3-Ethyl-2,4-dimethylpyrrole-1-carboxylate (68d). Yield: 3.26 g, 90%. ^1H NMR (CDCl_3 , 400 MHz) δ 6.96 (s, 1H), 2.38 (q, $J = 7.6$ Hz, 2H), 2.37 (s, 3H), 2.01 (s, 3H), 1.61 (s, 9H), 1.07 (t, $J = 7.6$ Hz, 3H); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{22}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 224.1645, found 224.1653.

Representative Procedure for the Synthesis of (1-(tert-Butoxycarbonyl)-5-ethylpyrrol-2-yl)boronic Acid (69a). To a stirred solution of 2,2,6,6-tetramethylpiperidine (2.60 g, 18.46 mmol) in dry THF (50 mL) was added dropwise *n*-BuLi (1.6 M in pentane, 12.5 mL, 20.0 mmol) under an argon atmosphere at -78 °C. The reaction mixture was allowed to warm to 0 °C and maintained at that temperature for 30 min. After cooling again to -78 °C, a solution of **68a** (3.0 g, 15.38 mmol) in THF (10 mL) was added. The reaction mixture was stirred for 2 h at -78 °C prior to the addition of trimethyl borate (7.92 g, 76.92 mmol). The solution was allowed to react at

ambient temperature overnight. The reaction mixture was diluted with EtOAc (200 mL), washed with water and brine solution, and dried over anhydrous Na_2SO_4 . The solvent was removed by rotary evaporation to furnish the desired product **69a** (3.12 g, 85%) as a brown solid. The product **69a** was carried forward into the next reaction without further purification. The products **69b** (1.82 g, 76%), **69c** (1.59 g, 65%), and **69d** (1.62 g, 68%) were also carried forward into the next reaction without further purification.

Synthesis of 32–35. Compounds **32** (403 mg, 75%), **33** (467 mg, 77%), **34** (1.59 g, 57%), and **35** (1.62 g, 55%) were synthesized by the same procedure as described for **9**.

5'-Ethyl-3,4-dimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (32). ^1H NMR ($\text{DMSO}-d_6$, 600 MHz) δ 10.99 (s, 1H), 10.94 (br s, 1H), 9.46 (s, 1H), 6.35 (br s, 1H), 5.89 (br s, 1H), 2.61 (br s, 2H), 2.22 (s, 3H), 2.06 (s, 3H), 1.21 (br s, 3H); ^{13}C NMR ($\text{CDCl}_3 + \text{DMSO}-d_6$, 150 MHz) δ 175.0, 135.6, 131.8, 130.9, 127.5, 122.0, 115.3, 108.9, 106.0, 20.3, 13.6, 9.9, 8.9; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 217.1335, found 217.1348.

5'-Isobutyl-3,4-dimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (33). ^1H NMR ($\text{CDCl}_3 + \text{DMSO}-d_6$, 400 MHz) δ 11.18 (br s, 1H), 10.92 (br s, 1H), 9.24 (s, 1H), 6.40 (br s, 1H), 5.85 (s, 1H), 2.41 (d, $J = 7.1$ Hz, 2H), 2.17 (s, 3H), 2.04 (s, 3H), 1.86 (m, 1H), 0.85 (d, $J = 6.4$ Hz, 6H); ^{13}C NMR ($\text{CDCl}_3 + \text{DMSO}-d_6$, 150 MHz) δ 173.6, 134.4, 134.2, 133.3, 127.5, 122.3, 116.8, 110.2, 107.9, 37.3, 29.1, 22.4 (2C), 10.3, 8.7; HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{21}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 245.1648, found 245.1660.

3,3',4,5'-Tetramethyl-[2,2'-bipyrrole]-5-carboxaldehyde (34). ^1H NMR (CDCl_3 , 400 MHz) δ 9.52 (s, 1H), 9.24 (br s, 1H), 8.40 (br s, 1H), 5.84 (d, $J = 2.6$ Hz, 1H), 2.31 (s, 6H), 2.16 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 174.2, 130.4, 127.4, 127.2, 117.0, 116.7, 116.6, 107.7 (2C), 11.6, 11.1, 8.6, 7.8; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 217.1335, found 217.1348.

4'-Ethyl-3,3',4,5'-tetramethyl-[2,2'-bipyrrole]-5-carboxaldehyde (35). HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{21}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 245.1648, found 245.1656.

Synthesis of (1-(tert-Butoxycarbonyl)-4-ethylpyrrol-2-yl)boronic Acid (71). Compound **71** (1.81 g, 74%) was synthesized by the same procedure as described for **69a**. The product **71** was carried forward into the next reaction without further purification.

Synthesis of 4'-Ethyl-3,4-dimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (36). Compound **36** (413 mg, 77%) was synthesized by the same procedure as described for **9**. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 11.26 (br s, 1H), 10.75 (br s, 1H), 9.48 (s, 1H), 6.70 (s, 1H), 6.35 (s, 1H), 2.45 (q, $J = 7.5$ Hz, 2H), 2.22 (s, 3H), 2.07 (s, 3H), 1.15 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ 173.4, 130.8, 129.8, 126.3, 125.6, 122.2, 122.0, 114.6, 107.4, 18.6, 14.0, 8.9, 7.3; HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 217.1335, found 217.1346.

Synthesis of tert-Butyl 1-Formyl-4,5,6,7-tetrahydroisindole-2-carboxylate (72). Compound **72** (3.17 g, 95%) was synthesized by the same procedure as described for **68a**. ^1H NMR (CDCl_3 , 400 MHz) δ 10.38 (s, 1H), 7.14 (s, 1H), 2.88 (t, $J = 5.8$ Hz, 2H), 2.52 (t, $J = 5.6$ Hz, 2H), 1.74 (m, 4H), 1.69 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 183.4, 148.7, 137.3, 129.1, 123.8, 122.7, 84.8, 28.0 (3C), 24.2, 22.7, 22.6, 21.5; HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{19}\text{NaNO}_3$ ($\text{M} + \text{Na}$) $^+$ 272.1257, found 272.1263.

Synthesis of tert-Butyl 1-(Dimethoxymethyl)-4,5,6,7-tetrahydroisindole-2-carboxylate (73). A solution of aldehyde **72** (2.0 g, 8.03 mmol), trimethyl orthoformate (1.70 g, 16.06 mmol), and a catalytic amount (30 mg) of *p*-toluenesulfonic acid (PTSA) in MeOH (20 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with Et₂O (200 mL) and washed with a solution of NaHCO_3 . The organic layer was washed with water and dried over anhydrous Na_2SO_4 . The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **73** (2.01 g, 85%). HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{26}\text{NO}_4$ ($\text{M} + \text{H}$) $^+$ 296.1856, found 296.1863.

Synthesis of (3-Formyl-4,5,6,7-tetrahydroisindol-1-yl)boronic Acid (74). To a stirred solution of **73** (1.2 g, 4.06 mmol) in THF (10 mL) was added triisopropyl borate (1.14 g, 6.10 mmol). The solution was cooled to 0–5 °C in an ice bath, and lithium diisopropylamide (LDA; 2 N, 4 mL, 8.13 mmol) was added over

20 min, and stirring was continued for an additional hour. The saturated ammonium chloride (5 mL) and 10% aqueous potassium bisulfate solution (50 mL) were added to adjust to pH 2, followed by stirring at room temperature for 2 h. The reaction mixture was diluted with EtOAc (200 mL), washed with brine solution, and dried over anhydrous Na_2SO_4 . The solvent was removed by rotary evaporation to furnish the desired product **74** (738 mg, 94%) as an orange solid. The product **74** was carried forward into the next reaction without further purification.

Synthesis of 3-(Imidazol-2-yl)-4,5,6,7-tetrahydroisoindole-1-carboxaldehyde (37). Compound **37** (478 mg, 65%) was synthesized by the same procedure as described for **9**. ^1H NMR (DMSO- d_6 , 400 MHz) δ 12.02 (s, 1H), 11.68 (br s, 1H), 9.53 (s, 1H), 7.26 (br s, 1H), 7.07 (br s, 1H), 2.79 (m, 4H), 1.72 (m, 4H); ^{13}C NMR (CDCl_3 + DMSO- d_6 , 100 MHz) δ 176.9, 139.6, 132.5, 129.3, 127.3, 126.2, 120.7, 117.2, 22.8, 22.6, 22.3, 20.9; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}$ ($\text{M} + \text{H}$) $^+$ 216.1131, found 216.1136.

Synthesis of 1-Methyl-4,5,6,7-tetrahydroisoindole (77). To a stirred suspension of LiAlH_4 (1.57 g, 41.45 mmol) in dry THF (50 mL) was added dropwise **53** (2.0 g, 10.36 mmol) in THF (50 mL) at 0 °C. Then the resulting solution was stirred at the same temperature for an additional 3 h and heated to reflux overnight. The reaction was quenched with saturated solution of sodium sulfate. The insoluble solid was filtered off and washed with DCM (100 mL). Then the combined organic solution was concentrated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **77** (1.19 g, 85%). ^1H NMR (CDCl_3 , 400 MHz) δ 7.61 (br s, 1H), 6.47 (d, $J = 2.8$ Hz, 1H), 2.71 (t, $J = 5.8$ Hz, 2H), 2.60 (t, $J = 6.0$ Hz, 2H), 1.90 (s, 3H), 1.87 (m, 4H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 121.9, 120.1, 115.5, 110.7, 24.2 (2C), 22.3, 21.5, 10.9; HRMS (ESI) calcd for $\text{C}_9\text{H}_{14}\text{N}$ ($\text{M} + \text{H}$) $^+$ 136.1121, found 136.1117.

Synthesis of 3-Methyl-4,5,6,7-tetrahydroisoindole-1-carboxaldehyde (38). Compound **38** (917 mg, 76%) was synthesized by the same procedure as described for **55**. ^1H NMR (CDCl_3 , 400 MHz) δ 10.10 (br s, 1H), 9.37 (s, 1H), 2.82 (t, $J = 5.8$ Hz, 2H), 2.41 (t, $J = 6.0$ Hz, 2H), 2.23 (s, 3H), 1.78 (m, 4H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.0, 135.4, 135.1, 126.5, 120.2, 23.3, 22.8, 21.0, 20.8, 11.3; HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{14}\text{NO}$ ($\text{M} + \text{H}$) $^+$ 164.1070, found 164.1065.

Synthesis of Bis(3-ethyl-4-methyl-1H-pyrrol-2-yl)methane (79). Compound **79** (1.13 g, 92%) was synthesized by the same procedure as described for **54**. ^1H NMR (CDCl_3 , 400 MHz) δ 7.25 (br s, 2H), 6.26 (t, $J = 1.2$ Hz, 2H), 3.73 (s, 2H), 2.36 (q, $J = 7.5$ Hz, 4H), 1.97 (s, 6H), 1.02 (t, $J = 7.5$ Hz, 6H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{23}\text{N}_2$ ($\text{M} + \text{H}$) $^+$ 231.1856, found 231.1861.

Synthesis of 5,5'-Methylenebis(4-ethyl-3-methyl-1H-pyrrole-2-carbaldehyde) (39). Compound **39** (907 mg, 73%) was synthesized by the same procedure as described for **55**. ^1H NMR (CDCl_3 , 400 MHz) δ 11.46 (br s, 2H), 9.48 (s, 2H), 3.86 (s, 2H), 2.31 (q, $J = 7.4$ Hz, 4H), 2.18 (s, 6H), 0.81 (t, $J = 7.5$ Hz, 6H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.5, 134.2, 130.0, 127.9, 123.7, 22.4, 16.3, 14.9, 8.4 (dimer); HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{23}\text{N}_2\text{O}_2$ ($\text{M} + \text{H}$) $^+$ 287.1786, found 287.1782.

Representative Procedure for the Synthesis of Prodiginine (85). To a stirred solution of **6** (250 mg, 1.31 mmol) and 2,4-dialkylpyrrole (**80**; 829 mg, 2.63 mmol) in anhydrous methanol (50 mL) was added methanolic 2 N HCl (catalytic amount). The resulting brightly colored solution was stirred for 5 h at room temperature. The methanol was removed under reduced pressure and the product was chromatographed on neutral alumina, with ethyl acetate/hexanes as eluent, to afford the desired prodiginine analogue **85**-HCl (468 mg, 68%) as a bright red compound. ^1H NMR (CDCl_3 , 400 MHz) δ 12.85 (br s, 1H), 12.81 (br s, 1H), 12.65 (br s, 1H), 7.30 (d, $J = 8.1$ Hz, 2H), 7.26 (m, 5H), 7.06 (d, $J = 8.1$ Hz, 2H), 7.01 (s, 1H), 6.97 (m, 1H), 6.38 (m, 1H), 6.09 (d, $J = 1.9$ Hz, 1H), 5.87 (d, $J = 1.6$ Hz, 1H), 4.23 (s, 2H), 4.00 (s, 3H), 3.93 (s, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 166.2, 149.1, 148.8, 141.0, 138.3, 136.4, 132.5, 132.2, 130.5 (2C), 129.8 (2C), 128.7 (4C), 127.9, 123.9, 122.1, 121.7, 118.3, 113.4, 112.9, 112.2, 93.1, 58.9, 33.8, 31.9; HRMS (ESI) calcd for

$\text{C}_{28}\text{H}_{24}\text{Cl}_2\text{N}_3\text{O}$ ($\text{M} + \text{H}$) $^+$ 488.1291, found 488.1284; IR (KBr) ν_{max} 3320, 3010, 2845, 1510, 1045, 742 cm^{-1} .

Synthesis of 5'-((3,5-Bis(4-chlorobenzyl)-1-methylpyrrol-2-yl)methylene)-4'-methoxy-1-methyl-2,2'-bipyrrole (87). To a stirred solution of prodiginine **85** (50 mg, 0.10 mmol) in DMF (10 mL) was added NaH (10 mg, 0.41 mmol) at 0 °C. The resulting bright red suspension was stirred for 10 min, and methyl iodide (58 mg, 0.41 mmol) was added at 0 °C and stirred for additional 30 min. The reaction mixture was warmed to room temperature and gradually poured into ice cold water and extracted with ethyl acetate (3 \times 30 mL). The combined organic layers were washed with water and dried over anhydrous Na_2SO_4 . The solvent was evaporated under reduced pressure and the product was chromatographed on neutral alumina, with ethyl acetate/hexanes as eluent, to afford the desired prodiginine **87** (46 mg, 85%). ^1H NMR (CDCl_3 , 400 MHz) δ 7.24 (d, $J = 8.3$ Hz, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 7.11 (d, $J = 8.4$ Hz, 2H), 7.06 (d, $J = 8.3$ Hz, 2H), 6.84 (s, 1H), 6.74 (br s, 1H), 6.68 (dd, $J = 1.5$, 3.8 Hz, 1H), 6.17 (dd, $J = 2.6$, 3.7 Hz, 1H), 5.92 (s, 1H), 5.75 (s, 1H), 4.25 (s, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 3.89 (s, 2H), 3.63 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 167.8, 161.2, 142.1, 140.7, 136.9, 132.4, 131.6, 130.1 (3C), 129.8 (2C), 129.3, 128.8, 128.7 (3C), 127.8 (2C), 127.2, 115.3, 113.5, 111.7, 108.4, 96.9, 58.4, 37.5, 33.0, 32.7, 29.7; HRMS (ESI) calcd for $\text{C}_{30}\text{H}_{28}\text{Cl}_2\text{N}_3\text{O}$ ($\text{M} + \text{H}$) $^+$ 516.1604, found 516.1607.

Representative Procedure for the Synthesis of Tambjamine (99). To a stirred solution of **6** (100 mg, 0.52 mmol) and *n*-butylamine (77 mg, 1.05 mmol) in anhydrous methanol (10 mL) was added methanolic 2 N HCl (catalytic amount). The resulting pale yellow colored solution was stirred at refluxing temperature for 5 h, and the solvent was removed under reduced pressure. The crude solid was dissolved in EtOAc (50 mL) and washed with 2 N HCl (2 \times 10 mL). The organic layer was dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and the product was chromatographed on neutral alumina, with ethyl acetate/hexanes as eluent, to afford the desired tambjamine **99** (117 mg, 91%) as a yellow solid. ^1H NMR (CDCl_3 , 400 MHz) δ 7.26 (s, 1H), 6.98 (dd, $J = 1.3$, 2.7 Hz, 1H), 6.67 (dd, $J = 1.3$, 3.6 Hz, 1H), 6.20 (dd, $J = 2.7$, 3.6 Hz, 1H), 5.87 (s, 1H), 3.84 (s, 3H), 3.41 (t, $J = 7.1$ Hz, 2H), 1.67 (m, 2H), 1.37 (m, 2H), 0.89 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 163.7, 142.2, 140.3, 124.0, 122.7, 113.1, 110.8, 110.7, 91.1, 58.5, 50.7, 32.2, 19.7, 13.6; HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{20}\text{N}_3\text{O}$ ($\text{M} + \text{H}$) $^+$ 246.1601, found 246.1605. IR (KBr) ν_{max} 3299, 2936, 1420, 1175, 722 cm^{-1} .

Representative Procedure for the Synthesis of *N*-(Adamantan-1-yl)-2-((oxoboranyl)methylene)aminoacetamide (188). To a stirred solution of **44** (2.0 g, 11.43 mmol) in a mixture of THF (25 mL) and CH_2Cl_2 (25 mL) were added 1-adamantylamine (2.07 g, 13.71 mmol), DMAP (348 mg, 2.85 mmol), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI, 2.62 g, 13.71 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction was quenched with saturated aqueous NH_4Cl solution (50 mL) and extracted with ethyl acetate (3 \times 100 mL). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and the product was chromatographed on neutral alumina, with ethyl acetate/hexanes as eluent, to afford the desired product **188** (2.99 g, 85%). ^1H NMR (CDCl_3 , 400 MHz) δ 6.01 (br s, 1H), 5.52 (br s, 1H), 3.64 (d, $J = 4.7$ Hz, 2H), 2.03 (m, 3H), 1.96 (d, $J = 2.9$ Hz, 6H), 1.64 (m, 6H), 1.41 (s, 9H); HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{29}\text{N}_2\text{O}_3$ ($\text{M} + \text{H}$) $^+$ 309.2173, found 309.2180.

Representative Procedure for the Synthesis of *N*-(Adamantan-1-yl)-2-aminoacetamide (189). Compound **188** (2.5 g, 8.11 mmol) was dissolved in 20 mL of trifluoroacetic acid/water (1:1) and stirred at room temperature for 3 h. The reaction mixture was neutralized with 2 N NaOH and extracted with ethyl acetate (3 \times 100 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure to obtain the pure product **189** (1.60 g, 95%) as a white solid. ^1H NMR (CDCl_3 , 400 MHz) δ 6.88 (br s, 1H), 3.20 (s, 2H), 2.06 (br s, 3H), 1.97 (d, $J = 2.8$ Hz, 6H), 1.70 (m, 6H); HRMS (ESI) calcd for

$C_{12}H_{21}N_2O$ ($M + H$)⁺ 209.1648, found 209.1646. Note: Two NH protons are not appearing under these conditions.

Synthesis of *N*-(2-((Adamantan-1-yl)amino)-2-oxoethyl)pyrrole-2-carboxamide (190). Compound 190 (1.09 g, 81%) was synthesized by the same procedure as described for 188. ¹H NMR (CDCl₃, 400 MHz) δ 9.83 (br s, 1H), 7.21 (t, *J* = 5.3 Hz, 1H), 6.85 (m, 1H), 6.69 (m, 1H), 6.16 (m, 1H), 6.08 (s, 1H), 3.90 (d, *J* = 5.3 Hz, 2H), 1.99 (br s, 3H), 1.92 (d, *J* = 2.6 Hz, 6H), 1.59 (m, 6H); ¹³C NMR (CDCl₃ + CD₃OD, 100 MHz) δ 169.1, 162.4, 124.9, 122.2, 111.2, 109.5, 51.0, 43.2, 42.2 (3C), 36.2 (3C), 29.4 (3C); HRMS (ESI) calcd for C₁₇H₂₃NaN₃O₂ ($M + Na$)⁺ 324.1682, found 324.1693.

Synthesis of *N*¹-((Pyrrol-2-yl)methyl)-*N*²-(adamantan-1-yl)ethane-1,2-diamine (191). Compound 191 (186 mg, 82%) was synthesized by the same procedure as described for 67a. ¹H NMR (CDCl₃, 400 MHz) δ 9.52 (br s, 1H), 6.74 (dd, *J* = 1.9, 2.7 Hz, 1H), 6.11 (dd, *J* = 3.0, 5.6 Hz, 1H), 6.02 (d, *J* = 1.9 Hz, 1H), 3.80 (s, 2H), 3.00 (br s, 2H), 2.76 (m, 4H), 2.08 (br s, 3H), 1.69–1.60 (m, 12H); ¹³C NMR (CDCl₃, 100 MHz) δ 130.1, 117.5, 107.8, 106.4, 51.5, 48.9, 46.1, 42.1 (3C), 39.4, 36.5 (3C), 29.5 (3C); HRMS (ESI) calcd for C₁₇H₂₈N₃ ($M + H$)⁺ 274.2278, found 274.2287.

Synthesis of 3-(Pyrrol-2-yl)acrylic Acid Methyl Ester (192). To a stirred suspension of NaH (910 mg, 37.89 mmol) in 50 mL of anhydrous dimethoxyethane at 0 °C was added dropwise methyl diethylphosphonoacetate (7.96 g, 37.89 mmol). The reaction mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature. Pyrrole-2-carboxaldehyde (40; 3.0 g, 31.58 mmol) was added, and the reaction mixture was stirred for additional 4 h. The reaction was quenched with ice–water and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product 192 (3.72 g, 78%). *R_f* value of the product 192 is similar to the starting material 40; the visualization of the product was black spot on TLC after applying the iodine vapor. ¹H NMR (CDCl₃, 600 MHz) δ 9.26 (br s, 1H), 7.61 (d, *J* = 15.9 Hz, 1H), 6.94 (d, *J* = 1.4 Hz, 1H), 6.58 (s, 1H), 6.29 (d, *J* = 3.6 Hz, 1H), 6.10 (d, *J* = 15.9 Hz, 1H), 3.80 (s, 3H). HRMS (ESI) calcd for C₈H₁₀NO₂ ($M + H$)⁺ 152.0706, found 152.0710.

Synthesis of 3-(Pyrrol-2-yl)acrylic Acid (193). To a stirred suspension of 192 (2.0 g, 13.24 mmol) in a mixture of THF (50 mL) and water (60 mL) was added LiOH·H₂O (1.66 g, 39.73 mmol). The reaction mixture was stirred at 60 °C for 12 h after which it was cooled to 0 °C and washed with ethyl acetate (3 × 30 mL). The aqueous layer was carefully acidified to pH 2 with 2 N HCl and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give the pure product 193 (1.74 g, 96%). ¹H NMR (CD₃OD, 600 MHz) δ 7.51 (d, *J* = 15.8 Hz, 1H), 6.91 (dd, *J* = 1.1, 2.3 Hz, 1H), 6.49 (dd, *J* = 1.1, 3.5 Hz, 1H), 6.18 (dd, *J* = 2.2, 4.9 Hz, 1H), 6.04 (d, *J* = 15.8 Hz, 1H); HRMS (ESI) calcd for C₇H₇NaNO₂ ($M + Na$)⁺ 160.0369, found 160.0363. Note: NH and COOH protons are not appearing under these conditions.

Synthesis of *N*-(2-Adamantan-1-yl)amino)-2-oxoethyl)-3-(pyrrol-2-yl)acrylamide (194). Compound 194 (2.02 g, 85%) was synthesized by the same procedure as described for 188. ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.34 (s, 1H), 7.95 (t, *J* = 5.8 Hz, 1H), 7.27 (d, *J* = 15.7 Hz, 1H), 7.26 (s, 1H), 6.91 (dd, *J* = 2.4, 3.7 Hz, 1H), 6.41 (s, 1H), 6.26 (d, *J* = 15.7 Hz, 1H), 6.12 (dd, *J* = 2.4, 5.6 Hz, 1H), 3.71 (d, *J* = 5.8 Hz, 2H), 1.99 (s, 3H), 1.92 (d, *J* = 2.7 Hz, 6H), 1.61 (m, 6H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 167.9, 165.9, 129.8, 128.5, 121.6, 114.9, 111.5, 109.5, 50.7, 42.5, 41.0 (3C), 36.0 (3C), 28.8 (3C); HRMS (ESI) calcd for C₁₉H₂₅NaN₃O₂ ($M + Na$)⁺ 350.1839, found 350.1853.

Representative Procedure for the Synthesis of 195. To a stirred solution of 194 (500 mg, 1.52 mmol) in methanol (10 mL) at room temperature was added NiCl₂·6H₂O (180 mg, 0.76 mmol). When the clear solution acquired a greenish color, the whole reaction mixture was brought to 0 °C and NaBH₄ (85 mg, 2.29 mmol) was added portionwise. The black reaction mixture was stirred for 30 min at 0 °C, and the solvent was removed under reduced pressure.

The crude product was dissolved in ethyl acetate (50 mL) and treated with aqueous NH₄Cl (2 × 10 mL). The organic layer was washed with brine and dried over anhydrous Na₂SO₄. The organic solvent was evaporated under reduced pressure and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product 195 (473 mg, 94%) as a white solid. ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.50 (s, 1H), 7.97 (t, *J* = 5.8 Hz, 1H), 7.24 (s, 1H), 6.55 (dd, *J* = 2.3, 3.9 Hz, 1H), 5.86 (dd, *J* = 2.6, 5.4 Hz, 1H), 5.72 (s, 1H), 3.62 (d, *J* = 5.8 Hz, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.00 (br s, 3H), 1.91 (d, *J* = 2.6 Hz, 6H), 1.60 (br s, 6H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 171.8, 167.9, 130.8, 116.0, 107.0, 104.2, 50.7, 42.3, 41.0 (3C), 35.9 (3C), 35.3, 28.8 (3C), 23.2; HRMS (ESI) calcd for C₁₉H₂₈N₃O₂ ($M + H$)⁺ 330.2176, found 330.2169.

Synthesis of *N*¹-(3-(Pyrrol-2-yl)propyl)-*N*²-(adamantan-1-yl)ethane-1,2-diamine (196). Compound 196 (178 mg, 78%) was synthesized by the same procedure as described for 67a. ¹H NMR (CDCl₃, 400 MHz) δ 9.28 (br s, 1H), 6.68 (s, 1H), 6.12 (t, *J* = 2.8 Hz, 1H), 5.92 (m, 1H), 2.78–2.66 (m, 8H), 2.16 (br s, 2H), 2.09 (br s, 3H), 1.83 (m, 2H), 1.86–1.67 (m, 12H); ¹³C NMR (CDCl₃, 100 MHz) δ 132.1, 116.2, 107.9, 104.9, 50.1, 50.0, 48.9, 42.6 (3C), 39.6, 36.7 (3C), 29.6 (4C), 25.6; HRMS (ESI) calcd for C₁₉H₃₂N₃ ($M + H$)⁺ 302.2591, found 302.2587.

In Vitro Antimalarial Activity: *P. falciparum* Growth Inhibition. In vitro antimalarial activity was determined by the malaria SYBR Green I-based fluorescence (MSF) assay described previously⁶⁶ with minor modifications as previously described,⁶⁷ and expressed as the compound concentration inhibiting growth by 50% (IC₅₀).

HepG2 Cytotoxicity Assay. Drugs were dissolved in DMSO to make 10 mM stock solutions. Human hepatocarcinoma cells (HepG2) were maintained on RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO₂ atmosphere. Cells were seeded at a density of 2 × 10⁴ per well in 96-well flat-bottom tissue culture plates containing complete medium in a total volume of 160 μL/well. The cells were allowed to attach at 37 °C overnight. On the following day, drug solutions (40 μL/well) were serially diluted with complete culture medium across the plate. The plates were then incubated at 37 °C and 5% CO₂ for another 24–36 h. Afterward, the medium was aspirated and replaced with complete RPMI medium (200 μL/well), and the plates were incubated for an additional 24 h at 37 °C and 5% CO₂. 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 3 h. After this period, fluorescence in each well, indicative of cellular redox activity, was measured in a Gemini EM plate reader with excitation wavelength at 560 nm and emission wavelength at 590 nm.^{68,69} IC₅₀ values were determined by nonlinear regression analysis of logistic concentration–fluorescence intensity curves (GraphPad Prism software).

In Vivo Efficacy against Murine Malaria. The in vivo activity of selected PGs and TAs was assessed against the blood stages using a modified 4-day test.^{71,72} The 4- to 5-week-old female CF1 mice (Charles River Laboratories) were infected intravenously with 2.5 × 10⁵ *P. yoelii* (Kenya strain, MR4 MRA-428) parasitized erythrocytes from a donor animal. Drug administration commenced the day after the animals were inoculated (day 1). The test compounds were dissolved in PEG-400 and administered by oral gavage once daily for 4 successive days; chloroquine phosphate was used as a positive control. Blood for blood film analysis and body weights were obtained on the day following the last dose and then at weekly intervals through day 28. Blood films were Giemsa stained and examined microscopically to determine the levels of parasitemia. These blood samples were collected from the tail vein with the aid of a syringe needle. All mice were observed daily to assess their clinical signs, which were recorded. Animals with observable parasitemia following the experiment were euthanized; animals cleared of parasites from their bloodstream were observed daily with assessment of parasitemia performed weekly until day 28, at which point we score the animal(s) as cured of infection, and the animals were euthanized. All treated mice with a negative smear on day 28 were considered cured (100% protection). ED₅₀ values (mg kg⁻¹ day⁻¹)

were derived graphically from the dose required to reduce parasite burden by 50% relative to drug-free controls.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00560.

Molecular formula strings (XLSX)

Structural characterization data and spectra (NMR, and HRMS) of all final compounds (PDF)

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Notes

United States provisional patent application has been filed by the Portland State University to protect the intellectual property described in this report.

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CQ^RPf, chloroquine-resistant *P. falciparum*; CQ^R, chloroquine-resistant; CQ^S, chloroquine-sensitive; Pf, *Plasmodium falciparum*; PG, prodiginine; TA, tambjamine; PPM, pyrrolypyrromethene; SAR, structure–activity relationship; CQ, chloroquine; MQ, mefloquine; IC₅₀, half maximal inhibitory concentration; nM, nanomolar; MDR, multidrug-resistant; ADMET, adsorption, distribution, metabolism, excretion, and toxicity; ED₅₀, median effective dose; NRD, nonrecrudescence dose (the amount of drug needed for 100% protection to malaria-infected mice until day 28)

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