Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b00560 • Publication Date (Web): 25 Aug 2015

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

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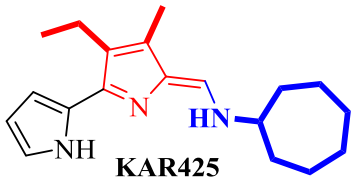

KAR425

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

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

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ABSTRACT

Synthesis and antimalarial activity of 94 novel bipyrrole tambjamines (TAs) and a library of B-ring functionalized tripyrrole 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 either at 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 that 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 tambjamines.

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 there are 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^R*Pf*), 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**), tambjamines (TAs, **2a–b**), and modified prodiginines (streptorubin B (**3a**), metacycloprodiginine (**3b**) and marineosins (**4** and **5**)) belong to a family of pyrrolylpyrromethene (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′-bipyrrole-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]

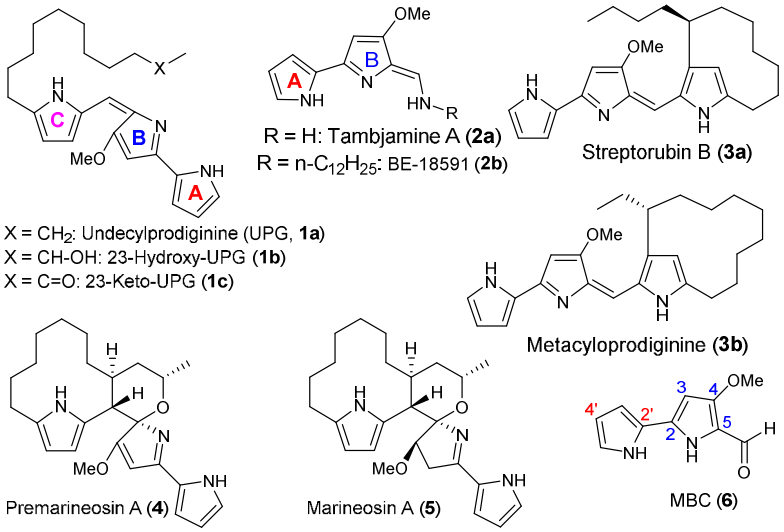


Figure 1. Structures of PPM natural products (**1**–**5**) and their common biosynthetic precursor (**6**)

As a part of an ongoing interest in developing new antiparasitic agents, we reisolated the natural PGs **1a**, and **3a** from *Streptomyces coelicolor* M511, and **3b** from *S. longisporusruber* (Figure 1).[51,52] These natural PGs exhibited great potency with very low IC_{50} 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/day, and three of four mice were cured. This 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 (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 (IC_{50} = 0.9–16.0 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 have 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′-bipyrrole-5-carboxaldehydes,[53] 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 steps[54] and 2,2′-bipyrrole-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.

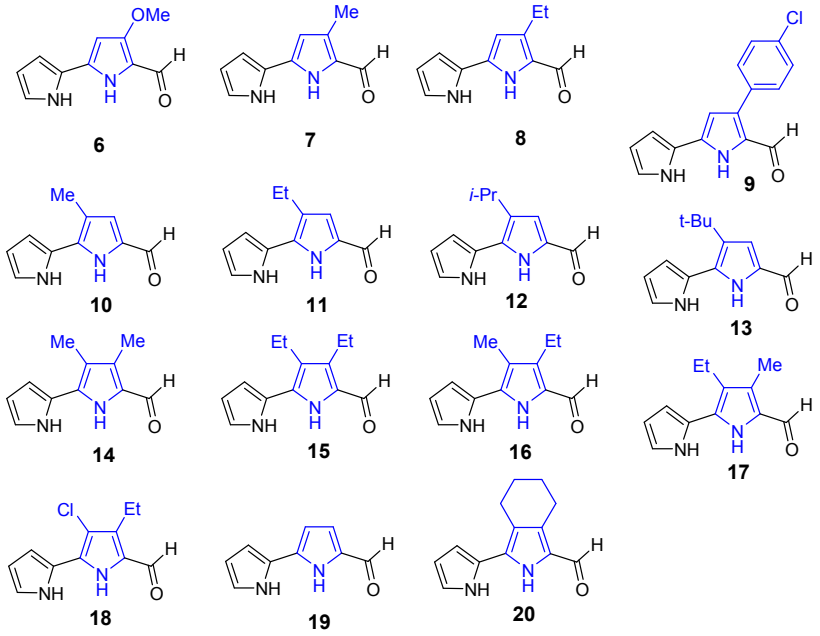


Figure 2. Key precursors (**6**–**20**) for the synthesis of B-ring functionalized PGs and TAs

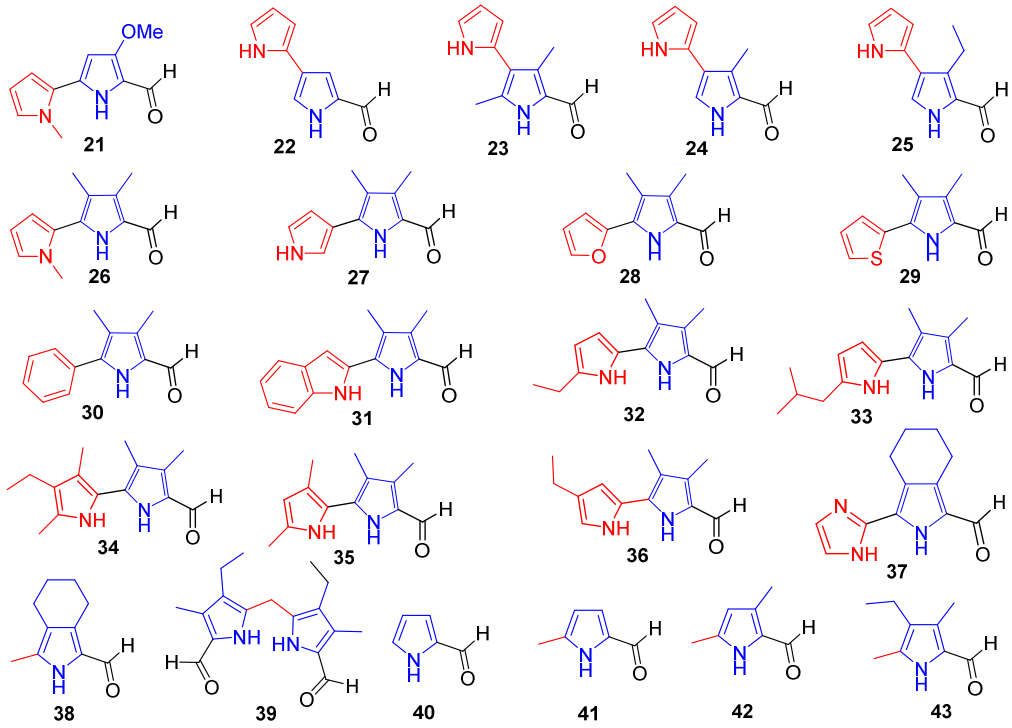
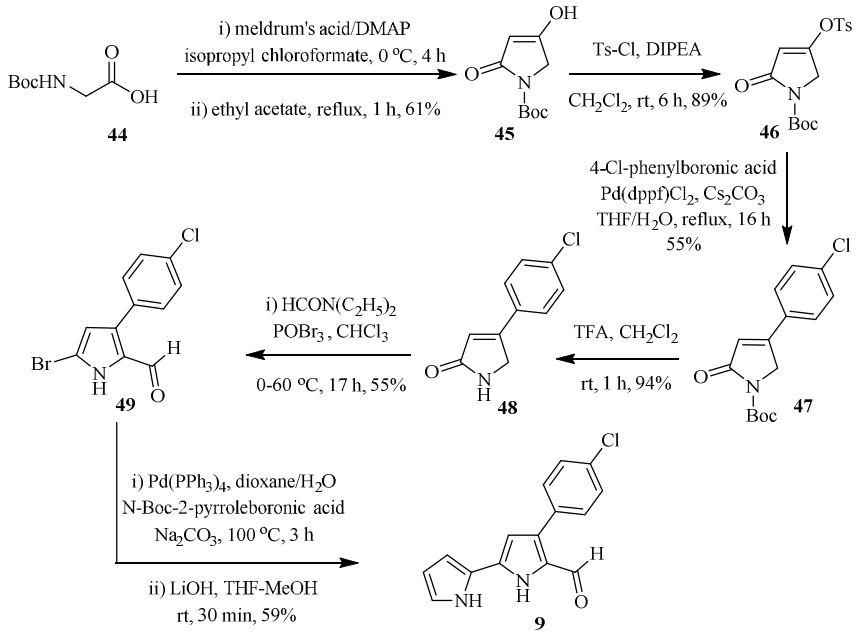


Figure 3. Key precursors (**21**–**43**) for the synthesis of A- and B-ring functionalized PGs and TAs

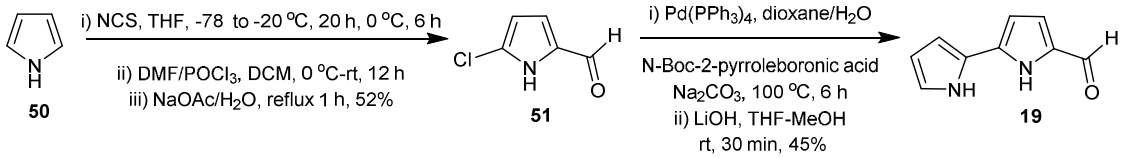
Synthesis of 4-(4-chlorophenyl)-[2,2′-bipyrrole]-5-carboxaldehyde (9). Synthesis of the aryl substituted 3-pyrrolin-2-one **48**, a key synthon in the synthesis of bipyrrole-carboxaldehyde **9**, was began with the coupling of Boc-glycine (**44**) with 2,2-dimethyl-1,3-dioxane-4,6-dione (meldrum′s acid) 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 of **47** with trifluoroacetic acid.[56] Using 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′-bipyrrole-5-carboxaldehyde **9**, in 59% yield (Scheme 1).[53]



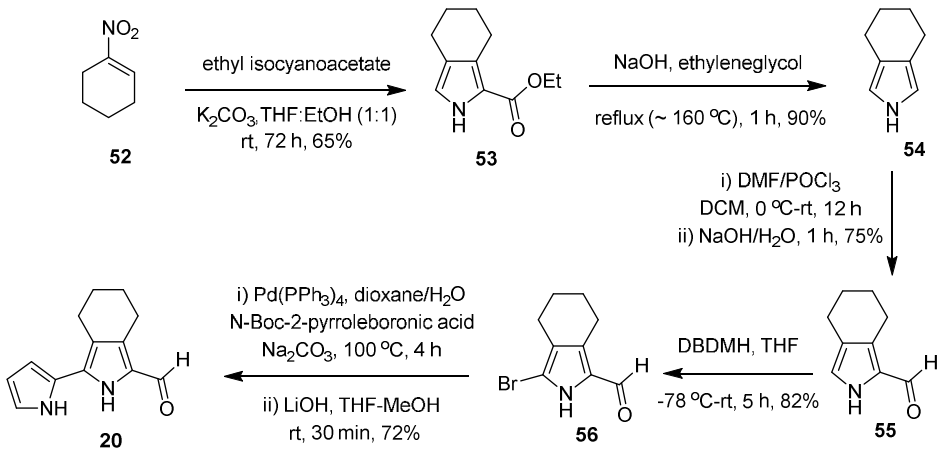
Scheme 1. Synthesis of 4-(4-chlorophenyl)-[2,2′-bipyrrole]-5-carboxaldehyde (**9**)

Synthesis of 2,2′-bipyrrole-5-carboxaldehyde (19). In 1988, Borger and Patel synthesized the 2,2′-bipyrrole-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 bipyrrole-carboxaldehyde **19** in 45% isolated yield (Scheme 2).



Scheme 2. Synthesis of 2,2′-bipyrrole-5-carboxaldehyde (**19**)

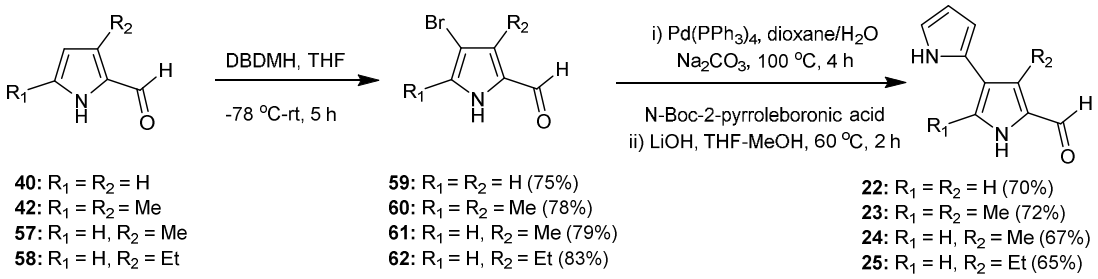
Synthesis of 3-(pyrrol-2-yl)-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (20). The key intermediate **53** was prepared via BartoneZard's method, using 1-nitro-1-cyclohexene (**52**) as a starting material (Scheme 3).[53,58,59] Upon treating with NaOH in ethylene glycol under reflux, **53** was smoothly converted to 4,5,6,7-tetrahydro-isoindole (**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-tetrahydro-isoindole-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-tetrahydro-isoindole-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 bipyrrole-carboxaldehyde **20** in good yield (Scheme 3).



Scheme 3. Synthesis of 3-(pyrrol-2-yl)-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (**20**)

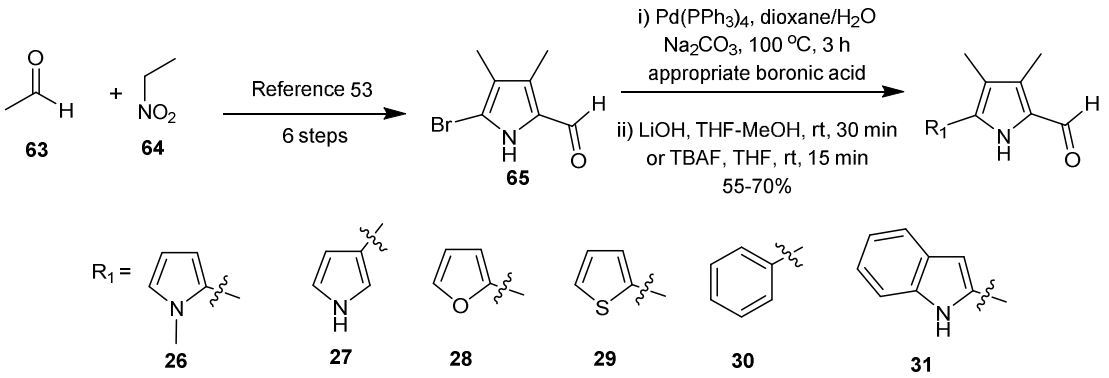
Synthesis of isomeric [2,3′-bipyrrole]-5′-carboxaldehydes (22–25). To investigate the ring-A positional effect on antimalarial activity, the isomeric bipyrrole-carboxaldehydes **22**–**25** were prepared, as shown in Scheme 4. Pyrrole-2-carboxaldehyde (**40**) and 3,5-dimethyl-pyrrole-2-carboxaldehyde (**42**) were obtained from commercial sources, and the 3-methyl-pyrrole-2-carboxaldehyde (**57**) and 3-ethyl-pyrrole-2-carboxaldehyde (**58**) were prepared according to our

reported procedures.[53] These pyrrole-2-carboxaldehydes were then converted into the corresponding 4-bromo-pyrrole-2-carboxaldehydes **59**–**62**, via a regioselective bromination at 4-position using DBDMH in THF in good yields (Scheme 4).[53] These 4-bromo-pyrrole-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 ^{o}C, resulted in the desired isomeric bipyrrole-caraboxaldehydes **22**–**25** (Scheme 4).



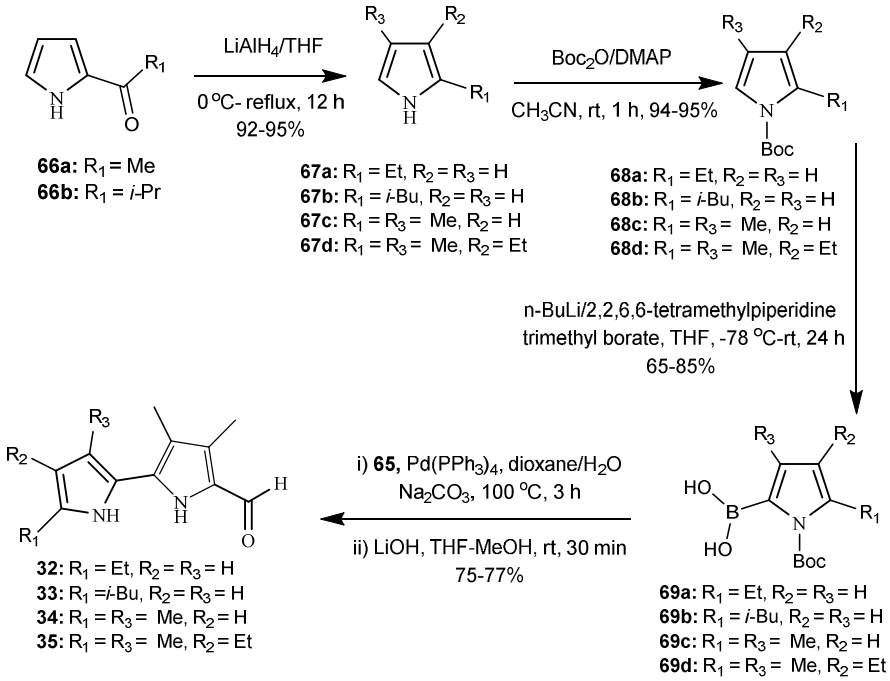
Scheme-4. Synthesis of isomeric [2,3′-bipyrrole]-5′-carboxaldehydes (**22**–**25**)

Synthesis of MBC′s analogues (26–31) containing herteroaryl/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-dimethyl-pyrrole-2-carboxaldehyde (**65**) was prepared in 6 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).



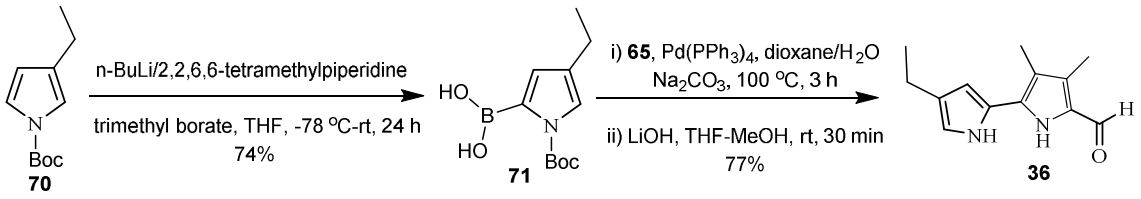
Scheme-5. Synthesis of MBC′s analogues containing heteroaryl/aryl groups in the place of ring-A (**26–31**)

Synthesis of 3,4-dimethyl-[2,2′-bipyrrole]-5-carboxaldehydes where the 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-acetyl-pyrrole (**66a**), 2,4-dimethylpyrrole (**67c**), and 3-ethyl-2,4-dimethylpyrrole (**67d**) were obtained from commercial sources, and the 2-isobutyryl-pyrrole (**66b**) was prepared according to the literature methods.[60] The compounds **66a** and **66b** were then converted into the corresponding 2-alkyl-pyrroles **67a** and **67b**, respectively, using $LiAlH_4$ in THF under reflux (Scheme 6).[61] By using standard procedures, the *N*-Boc-protected pyrroles **68a**–**68d** were prepared in excellent yields from **67a**–**67d** using di-tert-butyl dicarbonate (Boc_2O) in the presence of 4-(dimethyl amino)pyridine (DMAP), and subsequently these were converted into the corresponding 5-alkyl-(1-tert-butoxycarbonylpyrrol-2-yl)boronic acids **69a**–**69d**.[62] The resultant boronic acids **69a**–**69d** were carried forward into the Suzuki-coupling reaction with **65** without further purification to afford their corresponding [2,2′-bipyrrole]-5-carboxaldehydes **32–35** in good yields (Scheme 6).



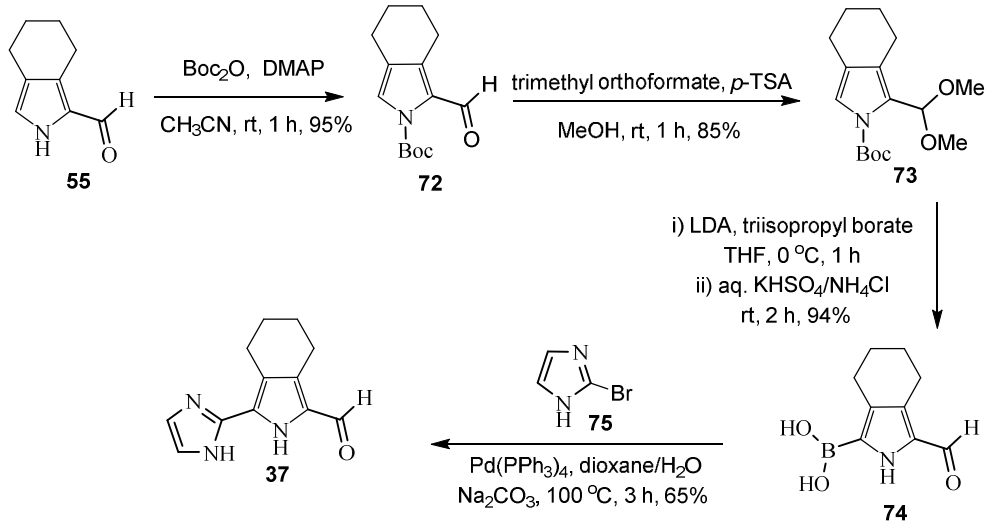
Scheme-6. Synthesis of 3,4-dimethyl-[2,2′-bipyrrole]-5-carboxaldehydes where the ring-A contains C-alkyl groups (**32**–**35**)

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-ethyl-pyrrole (**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′-bipyrrole]-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 Supplementary Information).



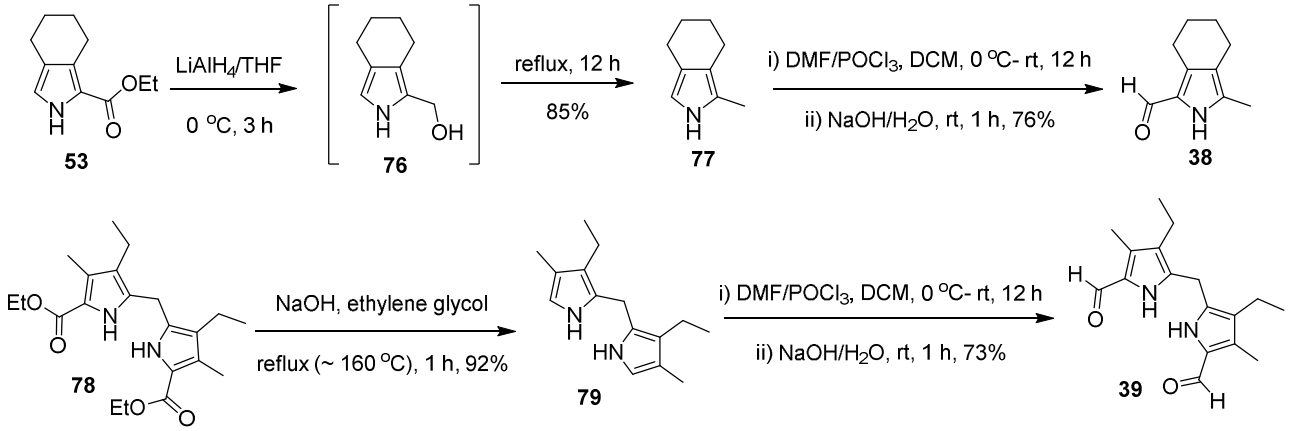
Scheme 7. Synthesis of 4′-ethyl-3,4-dimethyl-[2,2′-bipyrrole]-5-carboxaldehyde (**36**)

Synthesis of 3-(imidazol-2-yl)-4,5,6,7-tetrahydro-isoindole-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 Boc_2O/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 $KHSO_4/NH_4Cl$ at room temperature to provide the desired boronic acid **74** in excellent yield.[53] Finally, the Suzuki cross-coupling reaction between **74** and 2-bromo-imidazole (**75**), and subsequent deprotection of the *N*-Boc group led to the desired carboxaldehyde **37** in 65% isolated yield (Scheme 8).



Scheme 8. Synthesis of 3-(imidazol-2-yl)-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (**37**)

Synthesis of 3-methyl-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (38) and 5,5′-methylenebis(4-ethyl-3-methyl-pyrrole-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-tetrahydro-isoindole (**77**) was synthesized from ethyl-4,5,6,7-tetrahydro-isoindole-1-carboxylate (**53**) via an unstable intermediate **76**, using $LiAlH_4$ in THF at 0 ^oC to room temperature in 85% isolated yield. The resultant alkyl-pyrrole **77** was further converted to 3-methyl-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (**38**) by Vilsmeier reagent ($POCl_3$/DMF) (Scheme 9). Conversely, the bis(3-ethyl-4-methyl-pyrrol-2-yl)methane (**79**) was prepared from diethyl-5,5′-methylenebis(4-ethyl-3-methyl-2-pyrrolecarboxylate) (**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).



Scheme 9. Synthesis of 3-methyl-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (**38**) and 5,5'-methylenebis(4-ethyl-3-methyl-pyrrole-2-carboxaldehyde) (**39**)

Synthesis of novel PGs (85–98) and TAs (99–187). By using our standardized procedures, the mono- and dialkyl/alkylaryl pyrroles **80**–**84** were synthesized (Figure 4).[37] The acid-catalyzed condensation of either the alkyl pyrroles **80**–**84** or the commercially available alkyl/arylamines with various bipyrrole-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).

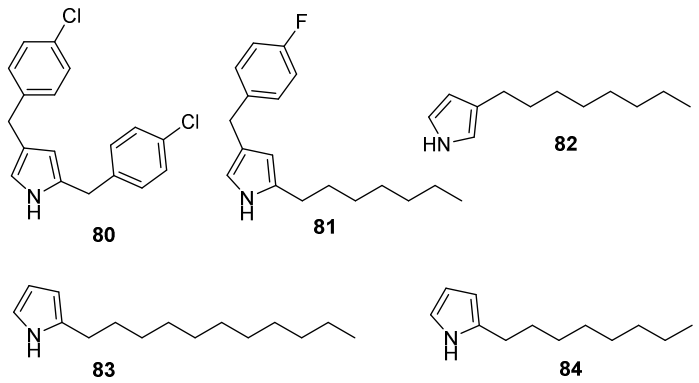
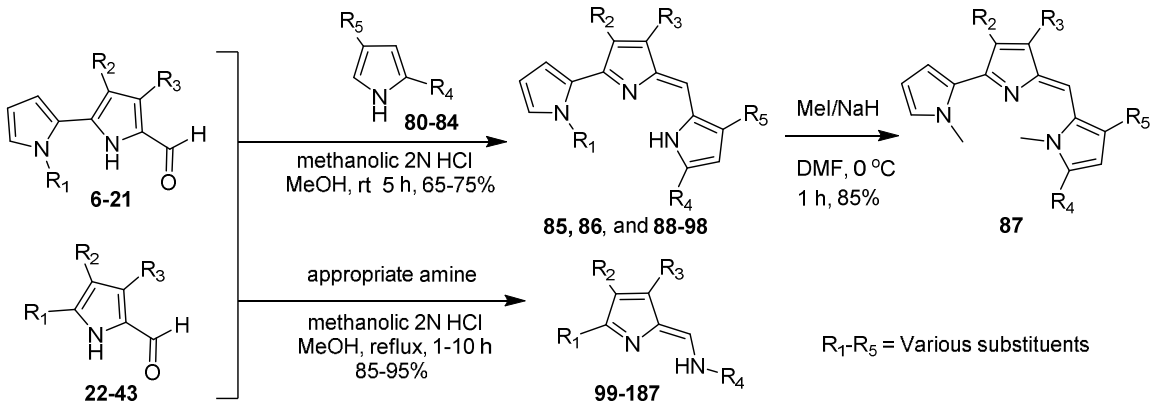


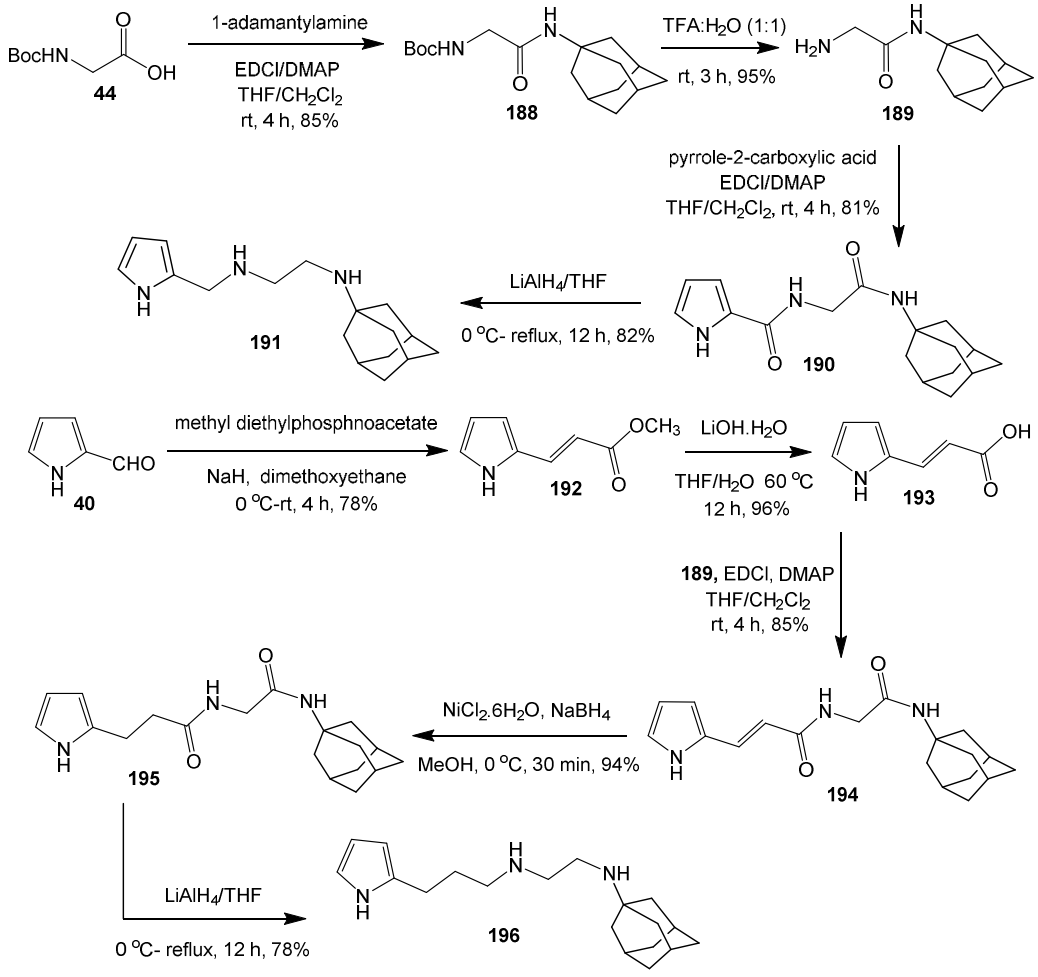
Figure 4. Potential substrates (**80–84**) for the synthesis of PGs



Scheme-10. Synthesis of novel PGs (**85–98**) and TAs (**99–187**)

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 (EDCl/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 $LiAlH_4$ in THF at 0 ^oC 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 ($LiOH.H_2O$) conditions, furnished the 2-pyrrolyl acrylic acid **193**. Condensation of **193** with **189** in the presence of EDCl/DMAP led to the corresponding condensed product **194**, which was further treated with $NaBH_4/NiCl_2.6H_2O$ to give the saturated product **195**. Treatment of **195** with $LiAlH_4$ in THF at 0 ^oC to reflux conditions provided the desired product **196** in 78% yields (Scheme 11).



Scheme 11. Synthesis of novel analogues (**190**, **191** and **194–196**)

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 (IC_{50} < 250 nM) was tested against hepatocellular HepG2 cancer cell line using mefloquine (MQ) as a control drug (see Tables 1–6).[68,69]

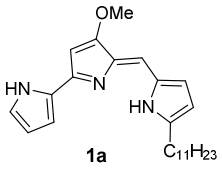
In Vitro Antimalarial Activity of PGs (85–98). In our previous work, synthetic PG **85** had shown an excellent potency against *Pf* strains D6 (CQ^S) and Dd2 (CQ^R) with great IC_{50} values (Table 1), and had the most favorable profile: 92% parasite reduction at 5 mg/kg/day, 100% reduction at 25 mg/kg/day 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 (IC_{50} > 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 and that support 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 IC_{50} of > 2500 nM against

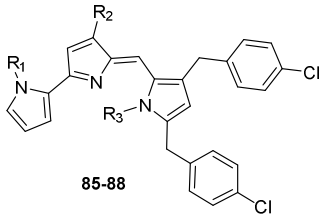
all tested *Pf* strains (Table 1). Interestingly, while replacing the OMe group by ethyl unit as in **90** also led to the reduced potency (**90**: IC_{50} =101 nM versus **1a**: IC_{50} = 7.2 nM against D6), the reduction was modest (14-fold). This result demonstrated that a short aliphatic substitution 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, support our previous findings.[38]

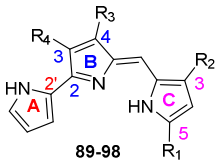
We next investigated whether substitutions at 2 and 3 positions of the ring B are tolerated. A series of novel B-ring functionalized PGs **91**−**98**, in which the ring-B is substituted with either mono- and/or di-substituents at 3- and 4-positions, were generated and examined for their in vitro antimalarial 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 mono-alkyl 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 D6[37]). Interestingly, the analogue **97**, which has 3-alkyl and 5-alkylaryl

substituents on the ring-C, showed 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.

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] D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)	cLogP[c]
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_6H_4	H	-	> 2500	> 2500	> 2500	nt	-	7.7
89	n-$C_{11}H_{23}$	H	H	H	> 2500	> 2500	> 2500	nt	-	5.2
90	n-$C_{11}H_{23}$	H	Et	H	101	66	51	18939	187	5.7
91	n-$C_{11}H_{23}$	H	H	*i*-Pr	1586	1500	> 2500	nt	-	6.3
92	n-$C_{11}H_{23}$	H	H	t-Bu	> 2500	> 2500	> 2500	nt	-	6.7
93	n-$C_{11}H_{23}$	H	Et	Cl	> 2500	> 2500	> 2500	nt	-	5.8
94	n-$C_{11}H_{23}$	H	Me	Et	162	190	145	62000	383	6.1
95	n-C_8H_{17}	H	Me	Et	127	216	132	71000	559	4.8
96	H	n-C_8H_{17}	Me	Et	41	53	61	57200	1395	4.9
97	n-C_7H_{15}	4-$FC_6H_4CH_2$	Me	Et	6.5	7.0	5.9	82024	12619	6.7

98	4-$ClC_6H_4CH_2$	4-$ClC_6H_4CH_2$	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

[a]IC_{50} 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.
[b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6)

[c]cLogP values were calculated using ChemBioDraw Ultra software (version 14), [d]nt = not tested

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 (LogP) 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 LogP.[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.

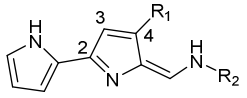
Initially, a series of new TAs **99–113**, which have various alkyl/arylamines in the place of ring-C and the OMe group at the 4-position on the ring-B (as in natural products), were synthesized and evaluated for their in vitro antimalarial activity against *Pf* strains and the results are shown in Table 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 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**: IC_{50} < 7.1 nM, and **109**: IC_{50} < 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** (IC_{50} < 4.5 nM), and the natural PG **1a** (IC_{50} < 7.0 nM). These results, clearly demonstrated that the elongation of the cycloalkyl ring size (from cyclopropyl, **103**: IC_{50} = 2500 nM to 1-adamantyl, **109**: IC_{50} < 3.1 nM) lead to an increase in activity (Table 2 and Figure 5). The greatest loss of potency (IC_{50} > 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:** IC_{50} = 49 nM versus **111:** IC_{50} = 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 unequivocal 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.

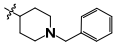
To investigate the importance of the OMe group on ring-B of TAs, another set of TAs **113**–**119**, in which the OMe group is replaced by 4-chlorophenyl moiety, was generated and examined for their in vitro antimalarial activity (Table 2). In vitro analysis of the activity of these compounds **113**–**119** against *Pf,* demonstrated activity (IC_{50} > 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**

(IC_{50} > 250 nM) (Table 2). Conversely, the adamantly 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:** IC_{50} = 3.1 nM, versus **123:** IC_{50} = 1.3 nM, **126:** IC_{50} = 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**: IC_{50} > 2500 nM *vs* **107:** IC_{50} = 23 nM, **108**: IC_{50} = 4.8 nM, and **129:** IC_{50} = 341 nM *vs* **109:** IC_{50} = 3.1 nM, **123:** IC_{50} = 1.3 nM, **126:** IC_{50} = 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.

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



compd	R_1	R_2	antimalarial activity (IC_{50} in nM)[a] D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)	cLogP[c]
99	OMe	n-C_4H_9	210	159	74.6	23000	109	0.08
100	OMe	n-C_6H_{13}	34	37	25	26700	785	0.9
101	OMe	n-C_8H_{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_6H_4	255	368	314	nt	-	1.1
113	4-ClC_6H_4	n-C_6H_{13}	1129	> 2500	564	nt	-	3.8
114	4-ClC_6H_4	n-$C_{11}H_{23}$	664	> 2500	663	nt	-	5.9
115	4-ClC_6H_4		1218	> 2500	510	nt	-	2.9
116	4-ClC_6H_4		1025	> 2500	415	nt	-	3.3
117	4-ClC_6H_4		963	1250	348	nt	-	3.7
118	4-ClC_6H_4		832	1135	316	nt	-	4.1
119	4-ClC_6H_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

[a]IC_{50} 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.
[b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6)
[c]cLogP values were calculated using ChemBioDraw Ultra software (version 14), [d]nt = not tested

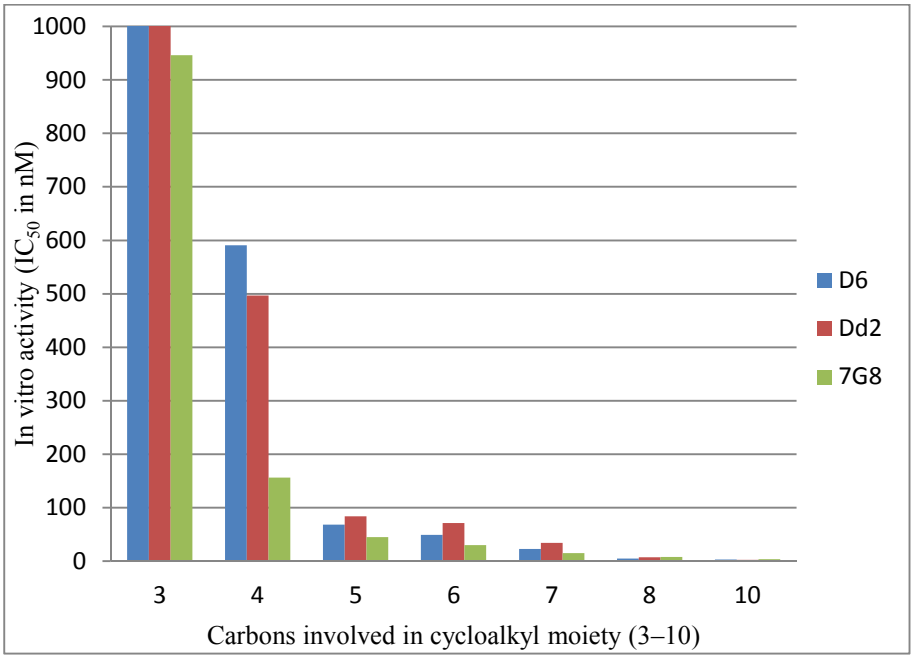
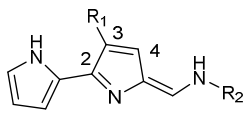


Figure 5. SAR of TAs (**103–109**) containing various cycloalkyl groups and in vitro antimalarial activity against *Pf* strains D6, Dd2, and 7G8

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 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 was observed when the short alkyl (methyl/ethyl) groups moving from 4-position (**121–126**, Table 2) to the 3-position (**130–141**, Table 3). Moreover, the adamantly analogues **132** and **135**, showed a significant decline in activity (**132:** IC_{50} = 106 nM *vs* **123:** IC_{50} = 1.3 nM; and **135:** IC_{50} = 117 nM, *vs* **126:** IC_{50} = 2.5 nM against D6), and the analogue **141**, had an almost total loss of activity (IC_{50} > 2500 nM). The one exception is the adamantly analogue **138**, containing an isopropyl group at 3-position on the ring-B, which showed the better potency (IC_{50} < 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 affects the potency irrespective of the terminal alkylamines.

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



compd	R_1	R_2	antimalarial activity (IC_{50} in nM)[a] D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)	cLogP[c]
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*-Pr		> 2500	1968	1980	nt	-	2.9

137	*i*-Pr		1079	665	1480	nt	-	3.3
138	*i*-Pr		26	20	31	18500	711	2.7
139	t-Bu		> 2500	> 2500	> 2500	nt	-	3.3
140	t-Bu		> 2500	> 2500	> 2500	nt	-	3.8
141	t-Bu		> 2500	> 2500	> 2500	nt	-	3.2
CQ			13	115	130	nt	-	3.7
MQ			nt	nt	nt	21800	-	5.3

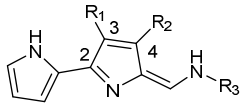
[a]IC_{50} values are the average of at least three determinations, each carried out in triplicate (± 10%). In order to compare results run on different days, and with different batches of each stain; CQ was run as a positive control. All results obtained were 'normalized' to the CQ values of 13 nM for D6, 115 nM for Dd2 and 130 nM for 7G8.
[b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6)

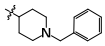
[c]cLogP values were calculated using ChemBioDraw Ultra software (version 14), [d]nt = not tested

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 the diminished activity (Table 4). Conversely, the analogues **145** and **146**, which have cycloheptyl and cyclooctyl moieties, respectively, showed the highest potencies (Table 4) than those of the corresponding 3- and 4-monoalkyl substituted analogues (see Tables 2 and 3). Significantly, the adamantly analogue **147**, showed comparable potency to that of the corresponding 4-alkyl/methoxy substituted analogues (**147:** IC_{50} = 5.5 nM versus **109:** IC_{50} = 3.1 nM, **123:** IC_{50} = 1.3 nM, **126:** IC_{50} = 2.5 nM against D6), and this potency is 5–20-fold greater

than the corresponding 3-alkyl substituted analogues (**147:** IC_{50} = 5.5 nM versus **132:** IC_{50} = 106 nM, **135:** IC_{50} = 117 nM, **138:** IC_{50} = 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 (IC_{50} of **150**–**152** *vs* IC_{50} 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/-$(CH_2$-$CH_2)_2$-) at both 3- and 4-positions on the ring-B, and cycloheptyl/cyclooctylamines in the place of ring-C, were shown comparable and/or greater potency to the dissimilar alkyl groups at both 3- and 4-positions containing TAs. Significantly, the adamantly analogues **155** (IC_{50} < 2.4 nM), **158** (IC_{50} < 2.5 nM), and **161** (IC_{50} < 7.5 nM) showed enhanced (2–8-fold) or comparable potency against all tested *Pf* strains when compared to **147** (IC_{50} < 5.5 nM) and **152** (IC_{50} < 19 nM). The biggest potency loss occurred (IC_{50} > 2250 nM) when we introduced a chlorine atom at 3-position on the ring-B as in **162–165** (IC_{50} 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-positions (Table 4) on ring-B, showed the greatest activity with good selectivity.

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] D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)	cLogP[c]
142	Et	Me	n-C_4H_9	883	680	260	nt[d]	-	2.0
143	Et	Me	n-C_8H_{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	$-(CH_2\text{-}CH_2)_2-$			32	37	22	4600	144	3.1
161	$-(CH_2\text{-}CH_2)_2-$			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

[a]IC_{50} 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.
[b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6)

[c]cLogP values were calculated using ChemBioDraw Ultra software (version 14), [d]nt = not tested

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-adamantlyamine 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:** IC_{50} > 2500 nM *vs* **123:** IC_{50} = 1.3 nM, **126:** IC_{50} = 2.5 nM, **129:** IC_{50} = 341 nM, and **169:** IC_{50} = 1418 nM vs **155:** IC_{50} < 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:** IC_{50} = 250 nM vs **155:** IC_{50} < 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 (IC_{50} 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:** IC_{50} > 2500 nM vs **155:** IC_{50} < 2.5 nM), suggest 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** (IC_{50} > 2500 nM). Incorporation of the substitutions into the ring-B as in **183–186** (from mono- to tri-alkyl) 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.

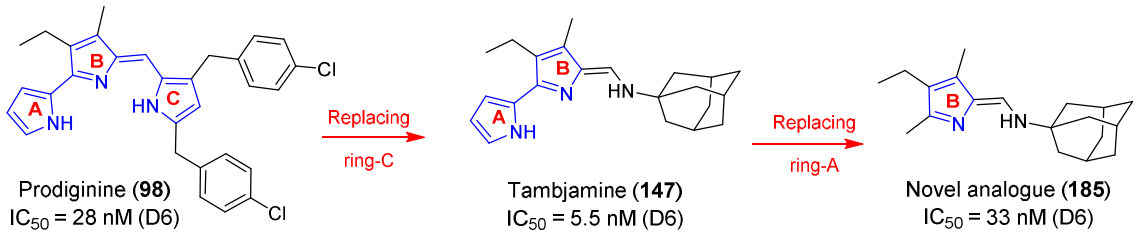
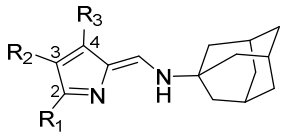
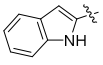
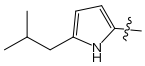
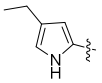
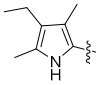
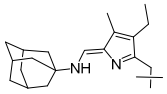


Figure 6. Structure pruning approach of the lead PG compounds (**98**)

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] D6	Dd2	7G8	cytotoxicity (IC_{50} in nM)[a] HepG2	SI[b] (D6)	cLogP[c]
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

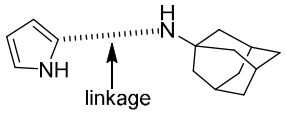
[a]IC_{50} values are the average of at least three determinations, each carried out in triplicate (± 10%). In order to compare results run on different days, and with different batches of each stain; CQ was run as a positive control. All results obtained were 'normalized' to the CQ values of 13 nM for D6, 115 nM for Dd2 and 130 nM for 7G8.
[b]SI (selectivity index) = IC_{50} (cytotoxicity)/IC_{50} (D6)

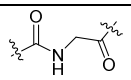
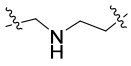
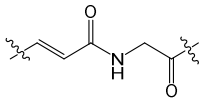
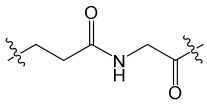
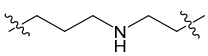
[c]cLogP values were calculated using ChemBioDraw Ultra software (version 14), [d]nt = not tested

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 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 (IC_{50} > 2500 nM, Table 6). This 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.

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



		antimalarial activity (IC_{50} in nM)[a]			
compd	linkage	D6	Dd2	7G8	cLogP[b]
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

[a]IC_{50} 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.
[b]cLogP values were calculated using ChemBioDraw Ultra software (version 14)

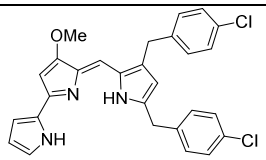
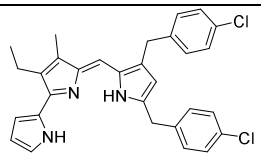
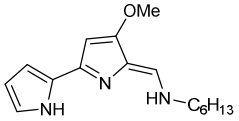
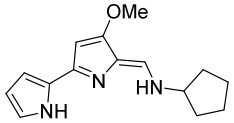
In Vivo Efficacy Studies in Mice Models. Given the attractive antiplasmodial activity of several PGs and TAs against CQ^S-D6, CQ^R-Dd2, and 7G8 strains of *P. falciparum* along with favourable toxicological properties against hepatocellular HepG2 cancer cell line and lower

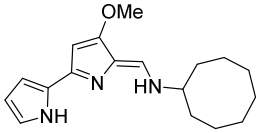
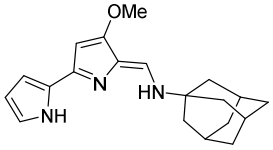
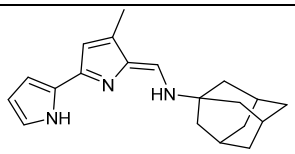
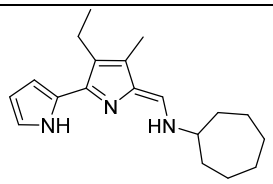
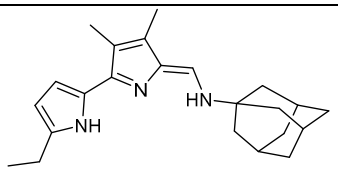
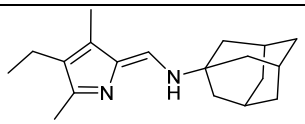
MWT and lipophilic properties, an in vivo proof of concept study in a murine *P. yoelii* model was undertaken with the most potent and selective analogues **98**, **100**, **105**, **108**, **109**, **123**, **145**, **177**, and **185**, using side by side comparison with our previous lead PG **85**[37] 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 range of 5 mg/kg to 100 mg/kg by oral gavage on four sequential days following the day of inoculation. The in vivo data are expressed as ED_{50} 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 latter 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/day (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 ED_{50} value of 84 mg/kg/day, 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 ED_{50} value of < 2.5 mg/kg/day, 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/day, 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 and further 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.

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	

[a]previous lead compound,[37] [b] % suppression of parasitemia = 100 × parasitemia control group−parasitemia treated group/parasitemia control group, [c]provided cures (100% protection to malaria-infected mice)

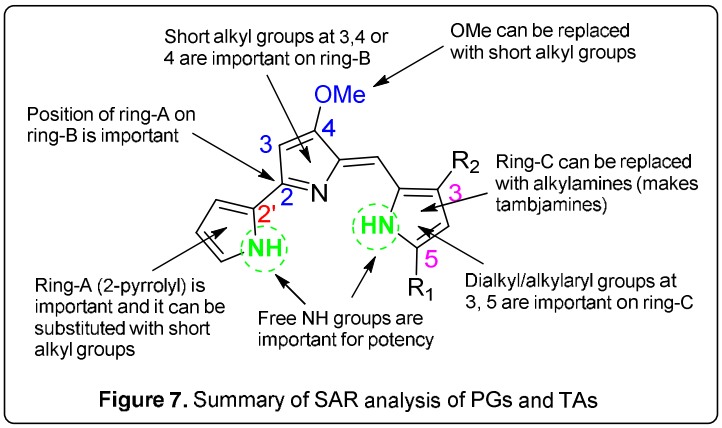


Figure 7. Summary of SAR analysis of PGs and TAs

CONCLUSIONS

We report here the synthesis and antimalarial activity of the novel class of potent tambjamines (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 either at 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 that previously observed for any tripyrrole PG, providing 100% protection to malaria-infected mice until day 28 at doses of 25 and 50 mg/kg x 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 (^{1}H), and 100, 150 MHz (^{13}C). Experiments were recorded in $CDCl_3$, CD_3OD, acetone-d_6 and DMSO-d_6 at 25 °C. Chemical shifts are given in parts per million (ppm) downfield from internal standard Me_4Si (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 which required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of argon/nitrogen. Chromatography was executed on Combi*Flash*® 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 × 250 mm) with a linear elution gradient ranging from $CH_3OH/CH_3CN/H_2O$ (40%/10%/50%) to CH_3OH (100%) in 0.15% trifluoroacetic acid at a flow rate of 1 mL/min. A purity of ≥ 95% has been established for all tested compounds.

Synthesis of 4-Hydroxy-2-oxo-2,5-dihydro-pyrrole-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 CH_2Cl_2 (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% $KHSO_4$ (2 × 70 mL), and organic layer was dried over Na_2SO_4, 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. ^{1}H 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 $C_9H_{13}NaNO_4$ $(M + Na)^+$ 222.0737, found 222.0740.

Synthessis 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 CH_2Cl_2 (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 Na_2SO_4. 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 **46** (5.37 g, 89%) as a white solid. ^{1}H NMR ($CDCl_3$, 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 $C_{16}H_{19}NaNO_6S$ $(M + Na)^+$ 376.0825, found 376.0830.

Synthesis of 4-(4-Chloro-phenyl)-2-oxo-2,5-dihydro-pyrrole-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)Cl_2 (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 Na_2SO_4. 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. ^{1}H NMR ($CDCl_3$, 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 $C_{15}H_{16}NaClNO_3$ $(M + Na)^+$ 316.0711, found 316.0713.

Synthesis of 4-(4-Chloro-phenyl)-1,5-dihydro-pyrrol-2-one (48). To a stirred solution of **47** (1.8 g, 6.14 mmol) in anhydrous CH_2Cl_2 (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% $NaHCO_3$, and brine and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure and the solid material was washed with CH_2Cl_2, to afford the pure product **48** (1.14 g, 94%) as a white solid. ^{1}H 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 $C_{10}H_9ClNO$ $(M + H)^+$ 194.0367, found 194.0372.

Synthesis of 5-Bromo-3-(4-chloro-phenyl)-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 ($POBr_3$; 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 CH_2Cl_2 (3 × 100 mL). The organic layers were combined, washed with brine and dried over anhydrous Na_2SO_4. 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. ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{11}H_8BrClNO$ $(M + H)^+$ 283.9472, found 283.9484.

Representative Procedure for the Synthesis of 4-(4-Chloro-phenyl)-[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 $Pd(PPh_3)_4$ (204 mg, 0.17 mmol) and Na_2CO_3 (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 Na_2SO_4. 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 Na_2SO_4. 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%). ^{1}H 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); ^{13}C 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 $C_{15}H_{12}ClN_2O$ $(M + H)^+$ 271.0633, found 271.0639.

Synthesis of 5-Chloro-pyrrole-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 $POCl_3$/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 added 100 mL of water. 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 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 desired product **51** (3.46 g, 36%) as a white solid. ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.2, 131.9, 126.0, 122.4, 110.0; HRMS (ESI) calcd for C_5H_5ClNO $(M + H)^+$ 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**. ^{1}H NMR ($CDCl_3$, 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 $C_9H_9N_2O$ $(M + H)^+$ 161.0709, found 161.0713.

Synthesis of Ethyl 4,5,6,7-tetrahydro-isoindole-1-carboxylate (53). To a stirred solution of **52** (5.0 g, 39.37 mmol) and ethyl isocyanoacetate (5.33 g, 47.24 mmol) in 1:1 mixture of THF and ethanol (100 mL) was added portion-wise 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 Na_2SO_4. 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. ^{1}H NMR ($CDCl_3$, 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 $C_{11}H_{16}NO_2$ $(M + H)^+$ 194.1176, found 194.1184.

Synthesis of 4,5,6,7-Tetrahydro-isoindole (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 Na_2SO_4. 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. ^{1}H NMR ($CDCl_3$, 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 $C_8H_{12}N$ $(M + H)^+$ 122.0964, found 122.0969.

Representative Procedure for the Synthesis of 4,5,6,7-Tetrahydro-isoindole-1-carboxaldehyde (55) by Standard Vilsmeier Conditions. Phosphorus oxychloride ($POCl_3$; 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 removing 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 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 desired product **55** (1.84 g, 75%) as a white solid. ^{1}H NMR ($CDCl_3$, 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 $C_9H_{12}NO$ $(M + H)^+$ 150.0913, found 150.0920.

Representative Procedure for the Synthesis of 3-Bromo-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (56). To a stirred solution of **55** (2.0 g, 13.42 mmol) in THF (100 mL) was added portion-wise 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 $KHSO_4$ solution, and extracted with ethyl acetate (3 × 75 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. 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%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.60 (br s, 1H), 9.41 (s, 1H), 2.83 (m, 2H), 2.42 (m, 2H), 1.77 (m, 4H); ^{13}C NMR ($CDCl_3$, 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 $C_9H_{11}BrNO$ $(M + H)^+$ 228.0019, found 228.0031.

Synthesis of 3-(Pyrrol-2-yl)-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (20). Compound **20** (682 mg, 72%) was synthesized by the same procedure as described for **9**. ^{1}H NMR (DMSO-d_6 + $CDCl_3$, 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$, 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 $C_{13}H_{15}N_2O$ $(M + H)^+$ 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-Bromo-pyrrole-2-carboxaldehyde (**59**).* ^{1}H NMR ($CDCl_3$, 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 C_5H_5BrNO $(M + H)^+$ 173.9549, found 173.9555.

*4-Bromo-3,5-dimethyl-pyrrole-2-carboxaldehyde (**60**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.82 (br s, 1H), 9.45 (s, 1H), 2.36 (s, 3H), 2.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.2, 137.0, 133.0, 127.7, 101.5, 12.2, 10.0; HRMS (ESI) calcd for C_7H_9BrNO $(M + H)^+$ 201.9862, found 201.9871.

*4-Bromo-3-methyl-pyrrole-2-carboxaldehyde (**61**).* ^{1}H NMR (acetone-*d6*, 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 C_6H_7BrNO $(M + H)^+$ 187.9705, found 187.9711.

*4-Bromo-3-ethyl-pyrrole-2-carboxaldehyde (**62**).* ^{1}H NMR ($CDCl_3$, 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 C_7H_9BrNO $(M + H)^+$ 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 modifying 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**).* ^{1}H NMR (CD_3OD, 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); ^{13}C NMR (CD_3OD, 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 $C_9H_9N_2O$ $(M + H)^+$ 161.0709, found 161.0713. *Note. Two NH protons are not appering under these conditions.*

*2',4'-Dimethyl-[2,3'-bipyrrole]-5'-carboxaldehyde (**23**).* ^{1}H 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); ^{13}C 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 $C_{11}H_{13}N_2O$ $(M + H)^+$ 189.1022, found 189.1026.

*4'-Methyl-[2,3'-bipyrrole]-5'-carboxaldehyde (**24**).* ^{1}H 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$, 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 $C_{10}H_{11}N_2O$ $(M + H)^+$ 175.0866, found 175.0871.

*4'-Ethyl-[2,3'-bipyrrole]-5'-carboxaldehyde (**25**).* ^{1}H 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 $C_{11}H_{13}N_2O$ $(M + H)^+$ 189.1022, found 189.1027. *Note. Two NH protons are not appering 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 modifying 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**).* ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{12}H_{14}NaN_2O$ $(M + Na)^+$ 225.0998, found 225.1006.

*3,4-Dimethyl-[2,3'-bipyrrole]-5-carboxaldehyde (**27**).* ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{11}H_{12}NaN_2O$ $(M + Na)^+$ 189.1022, found 189.1028.

*5-(Furan-2-yl)-3,4-dimethyl-pyrrole-2-carboxaldehyde (**28**).* ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{11}H_{11}NaN_2O$ $(M + Na)^+$ 212.0682, found 212.0689.

*3,4-Dimethyl-5-(thiophen-2-yl)-pyrrole-2-carboxaldehyde (**29**).* ^{1}H 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 $C_{11}H_{11}NaNOS$ $(M + Na)^+$ 228.0454, found 228.0459.

*3,4-Dimethyl-5-phenyl-pyrrole-2-carboxaldehyde (**30**).* ^{1}H 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 $C_{13}H_{13}NaNO$ $(M + Na)^+$ 222.0889, found 222.0897.

*5-(Indol-2-yl)-3,4-dimethyl-pyrrole-2-carboxaldehyde (**31**).* ^{1}H NMR (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 ($CDCl_3$ + 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 $C_{15}H_{15}N_2O$ $(M + H)^+$ 239.1179, found 239.1188.

Representative Procedure for the Synthesis of 2-Ethyl-pyrrole (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 filtrated 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-Isobutyl-pyrrole (**67b**).* (4.26 g, 95%); HRMS (ESI) calcd for $C_8H_{14}N$ $(M + H)^+$ 124.1121, found 124.1126.

Representative Procedure for the Synthesis of tert-Butyl 2-ethyl-pyrrole-1-carboxylate (68a). 4-(Dimethyl-amino)pyridine (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 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 $C_{11}H_{18}NO_2$ $(M + H)^+$ 196.1332, found 196.1335.

*tert-Butyl 2-isobutyl-pyrrole-1-carboxylate (**68b**).* (3.40 g, 94%), ^{1}H 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 $C_{13}H_{22}NO_2$ $(M + H)^+$ 224.1645, found 224.1649.

*tert-Butyl 2,4-dimethyl-pyrrole-1-carboxylate (**68c**).* (3.77 g, 92%), ^{1}H 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 $C_{11}H_{18}NO_2$ $(M + H)^+$ 196.1332, found 196.1339.

*tert-Butyl 3-ethyl-2,4-dimethyl-pyrrole-1-carboxylate (**68d**).* (3.26 g, 90%), ^{1}H 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 $C_{13}H_{22}NO_2$ $(M + H)^+$ 224.1645, found 224.1653.

Representative Procedure for the Synthesis of (1-(tert-Butoxycarbonyl)-5-ethyl-pyrrol-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**).* ^{1}H NMR (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 ($CDCl_3$ + 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 $C_{13}H_{17}N_2O$ $(M + H)^+$ 217.1335, found 217.1348.

*5'-Isobutyl-3,4-dimethyl-[2,2'-bipyrrole]-5-carboxaldehyde (**33**).* ^{1}H NMR ($CDCl_3$ + 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 ($CDCl_3$ + 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 $C_{15}H_{21}N_2O$ $(M + H)^+$ 245.1648, found 245.1660.

*3,3',4,5'-Tetramethyl-[2,2'-bipyrrole]-5-carbaldehyde (**34**).* ^{1}H 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 $C_{13}H_{17}N_2O$ $(M + H)^+$ 217.1335, found 217.1348.

*4'-Ethyl-3,3',4,5'-tetramethyl-[2,2'-bipyrrole]-5-carboxaldehyde (**35**).* HRMS (ESI) calcd for $C_{15}H_{21}N_2O$ $(M + H)^+$ 245.1648, found 245.1656.

Synthesis of (1-(tert-Butoxycarbonyl)-4-ethyl-pyrrol-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**. ^{1}H NMR (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 (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 $C_{13}H_{17}N_2O$ $(M + H)^+$ 217.1335, found 217.1346.

Synthesis of tert-Butyl 1-formyl-4,5,6,7-tetrahydro-isoindole-2-carboxylate (72). Compound **72** (3.17 g, 95%) was synthesized by the same procedure as described for **68a**. ^{1}H 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 $C_{14}H_{19}NaNO_3$ $(M + Na)^+$ 272.1257, found 272.1263.

Synthesis of tert-Butyl 1-(dimethoxymethyl)-4,5,6,7-tetrahydro-isoindole-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 Et_2O (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 $C_{16}H_{26}NO_4$ $(M + H)^+$ 296.1856, found 296.1863.

Synthesis of (3-Formyl-4,5,6,7-tetrahydro-isoindol-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 the 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-tetrahydro-isoindole-1-carboxaldehyde (37)*. Compound **37** (478 mg, 65%) was synthesized by the same procedure as described for **9**. ^{1}H 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 $C_{12}H_{14}N_3O$ $(M + H)^+$ 216.1131, found 216.1136.

Synthesis of 1-Methyl-4,5,6,7-tetrahydro-isoindole (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 same temperature for additional 3 h and heated to reflux overnight. The reaction was quenched with saturated solution of sodium sulfate. The insoluble solid was filtrated 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%). ^{1}H 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 $C_9H_{14}N$ $(M + H)^+$ 136.1121, found 136.1117.

Synthesis of 3-Methyl-4,5,6,7-tetrahydro-isoindole-1-carboxaldehyde (38). Compound **38** (917 mg, 76%) was synthesized by the same procedure as described for **55**. ^{1}H 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 $C_{10}H_{14}NO$ $(M + H)^+$ 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**. ^{1}H 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 $C_{15}H_{23}N_2$ $(M + H)^+$ 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**. ^{1}H 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 $C_{17}H_{23}N_2O2$ $(M + H)^+$ 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 colored

compound. ^{1}H 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 $C_{28}H_{24}Cl_2N_3O$ $(M + H)^+$ 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-methyl-pyrrol-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 × 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%). ^{1}H 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 $C_{30}H_{28}Cl_2N_3O$ $(M + H)^+$ 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 × 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. ^{1}H 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 $C_{14}H_{20}N_3O$ $(M + H)^+$ 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)amino)acetamide (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-dimethylamino-propyl)-N′-ethylcarbodiimide hydrochloride (EDCl, 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 × 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%).^{1}H 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 $C_{17}H_{29}N_2O_3$ $(M + H)^+$ 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 × 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. ^{1}H 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 appering 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**. ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$ + CD_3OD, 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 $C_{17}H_{23}NaN_3O_2$ $(M + Na)^+$ 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**. ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{17}H_{28}N_3$ $(M + H)^+$ 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 a methyl diethylphosphnoacetate (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 Na_2SO_4. 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. ^{1}H NMR ($CDCl_3$, 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 $C_8H_{10}NO_2$ $(M + H)^+$ 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 $LiOH.H_2O$ (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 Na_2SO_4, and concentrated under reduced pressure to give the pure product **193** (1.74 g 96%). ^{1}H NMR (CD_3OD, 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 $C_7H_7NaNO_2$ $(M + Na)^+$ 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**. ^{1}H 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); ^{13}C 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 $C_{19}H_{25}NaN_3O_2$ $(M + Na)^+$ 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 $NiCl_2.6H_2O$ (180 mg, 0.76 mmol). When the clear solution acquired a greenish color, the whole reaction mixture was brought to 0 °C and $NaBH_4$ (85 mg, 2.29 mmol) was added portion-wise. The black colored 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 NH_4Cl (2 × 10 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. 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. ^{1}H 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); ^{13}C 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 $C_{19}H_{28}N_3O_2$ $(M + H)^+$ 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**. ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{19}H_{32}N_3$ $(M + H)^+$ 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 previously[66] with minor modifications as previously described,[67] and expressed as the compound concentration inhibiting growth by 50% (IC_{50}).

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% CO_2 atmosphere. Cells were seeded at a density of 2×10^4 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% CO_2 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% CO_2. An aliquot of a stock solution of resazurin (Alamar Blue, prepared in 1 × PBS) was then added at 20 μL per well (final concentration 10 μM), and the plates were returned to the incubator for 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] IC_{50} 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] A 4- to 5-week-old female CF1 mice (Charles River Laboratories) were infected intravenously with 2.5×10^5 *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 four 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). ED_{50} values (mg/kg/day) were derived graphically from the dose required to reduce parasite burden by 50% relative to drug-free controls.

ASSOCIATED CONTENT

ACKNOWLEDGMENT. This work was supported by a grant from the National Institutes for Health (GM077147).

NOTES

The authors declare no competing financial interest.

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

Supporting Information. Structural characterization data and spectra (NMR, and HRMS) of all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED. CQ^{R}*Pf*, chloroquine-resistant *P. falciparum*; CQ^{R}, chloroquine-resistant; CQ^{S}, chloroquine-sensitive; *Pf, Plasmodium falciparum;* PGs, prodiginines: TAs, tambjamines; PPM, pyrrolylpyrromethene; SAR, structure-activity relationship; CQ, chloroquine; MQ, mefloquine; IC_{50}; half maximal inhibitory concentration; nM, nanomolar; MDR, multidrug-resistant; ADMET, adsorption, distribution, metabolism, excretion and toxicity; ED_{50}, median effective dose; NRD, non-recrudescence dose (the amount of drug needed for 100% protection to malaria-infected mice until day 28).

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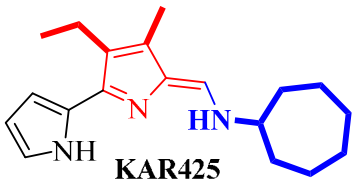

KAR425

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