Diversity-Oriented Synthesis-Facilitated Medicinal Chemistry: Toward the Development of Novel Antimalarial Agents

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Diversity-Oriented Synthesis-Facilitated Medicinal Chemistry: Toward the Development of Novel Antimalarial Agents

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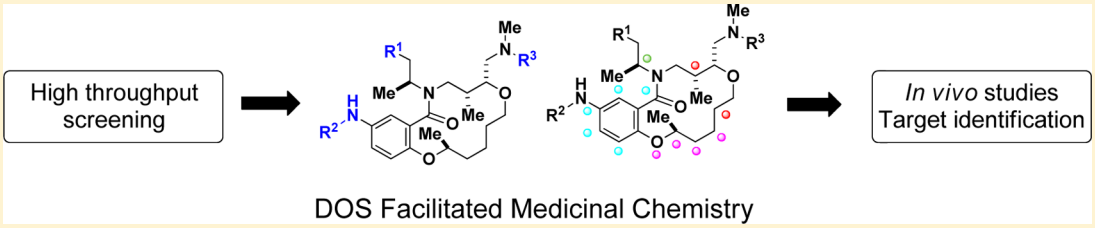
Supporting Information



ABSTRACT: Here, we describe medicinal chemistry that was accelerated by a diversity-oriented synthesis (DOS) pathway, and *in vivo* studies of our previously reported macrocyclic antimalarial agent that derived from the synthetic pathway. Structure–activity relationships that focused on both appendage and skeletal features yielded a nanomolar inhibitor of *P. falciparum* asexual blood-stage growth with improved solubility and microsomal stability and reduced hERG binding. The build/couple/pair (B/C/P) synthetic strategy, used in the preparation of the original screening library, facilitated medicinal chemistry optimization of the antimalarial lead.

INTRODUCTION

Malaria is a major public health threat responsible for high mortality and morbidity burdens in endemic countries.[1] The disease in humans is caused by five species of mosquito-borne *Plasmodium* parasites, with *P. falciparum* being the most virulent and deadly.[2] Resistance has become a major challenge, and recently artemisinin and its derivatives, such as artesunate, the mainstays of malaria treatment,[3] have shown reduced clinical efficacy to populations at the Cambodia–Thai border,[4,5] indicating that it may only be a matter of time before these too become ineffective. Given the lack of an effective vaccine,[6] the discovery of safe and effective antimalarial therapeutics is urgent. While the search for antimalarials is centuries old,[7] no new class of antimalarial has been introduced into clinical practice since 1996.[8] Given that resistance to the traditional chemotypes is an increasing problem, there is a pressing need for the discovery of new classes of compounds, with unique core structures, more likely to have novel mechanisms of action.[9] We have developed a screening collection of 100,000 diverse small molecules that combine the complexity of natural products and the efficiency of high-throughput synthesis[10−12] and subsequently reported the performance of a small subset of this collection (~8,000 compounds) in the phenotypic screening of *P. falciparum* blood-stage parasites.[13] Given the need for new antimalarial chemotypes, we sought to develop upon our previous reported inhibitor of the viability of *P. falciparum*. Our approach, outlined in Figure 1, uses a "build/couple/pair" (B/C/P) strategy of DOS,[14] originally developed during library synthesis,[10] to manipulate almost every position of the reported 14-membered macrocycle, allowing us to investigate the biological relevance of most core atoms. This report describes the DOS-facilitated medicinal chemistry and subsequent *in vivo* studies of antimalarial macrocycles.

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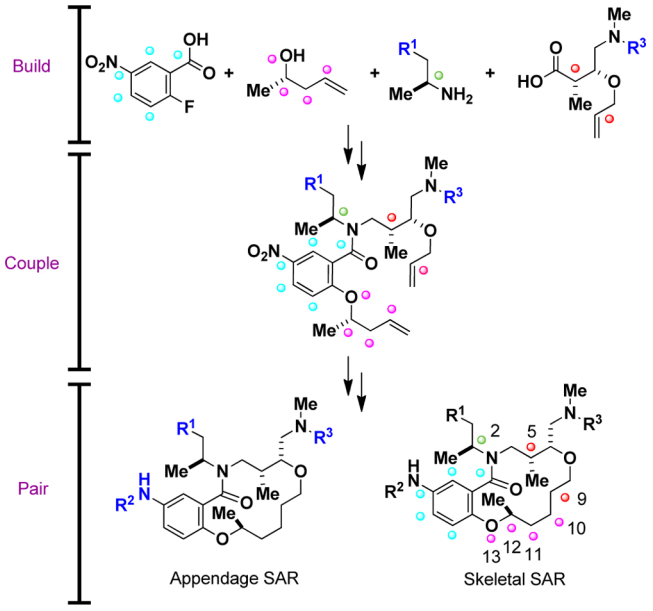


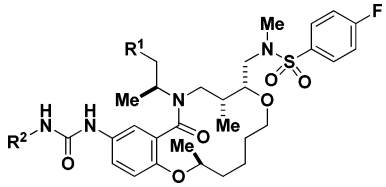
Figure 1. Overview of the B/C/P strategy showing points of manipulation and appendage sites.

RESULTS

We have previously reported the 14-membered macrolactam **2** with potent antimalarial activity against both wild type 3D7 and multidrug-resistant Dd2 strains (Table 1) along with initial ADME data, such as plasma protein binding (PPB, 99% bound).[13] Further analysis of this compound revealed a potential hERG liability (95% displacement of control ([^{3}H]-astemizole) at 10 μM of **2**; see SI), poor mouse microsomal stability (<5% remaining after 1 h), and low solubility in the PBS medium (<5 μM). Given that these ADME properties can lead to developmental issues such as low bioavailability and high-dose administrations in the case of insolubility, our goal was to increase microsomal stability, address the potential cardiotoxicity (hERG) liability, and obtain a compound with PBS solubility at least >20 μM while maintaining potency. Macrolactam **2** contains three appendage-diversification sites, namely (1) the exocyclic dimethyl amine (Table 1, R^1), (2) the exocyclic sulfonamide, and (3) the urea at the aniline nitrogen (Table 1, R^2). Our strategy sought to explore two of these sites while taking advantage of the modularity of the B/C/P process to investigate almost every atom in our lead structure. By systematically accessing each fragment of **2**, we aimed to identify the minimum pharmacophore required for activity. These analogs were accessed through the pathways illustrated in Figure 2. The modular nature of the pathway enables changes to be introduced into the final analogs by varying individual "building blocks". Several features of these medicinal chemistry efforts are worth noting: (1) the introduction of heteroatoms within the aromatic ring; (2) removal of both methyl groups within the macrocycle; and (3) systematic variation of the ring size from a 14-membered through an 8-membered ring.

We initially focused on two of the three appendage sites, since we had previously established that the sulfonamide was essential for antimalarial activity.[13] We hypothesized that attenuation of the nitrogen pK_a at R^1, structural changes to both R^1 and R^2, and the introduction of functionality to lower log *D* would impart more favorable DMPK. The *p*-fluorophenyl urea **3** had similar potency to the parent (**2**) but lacked microsomal stability (<5% remaining after 1 h) (Table 1). Solubilizing groups such as the oxetane (**4**) had a dramatic effect on PBS solubility (>100 μM), and while **4** was approximately 60-fold less potent than **2**, it still showed good

Table 1. Key Physicochemical Properties from SAR Studies of the R^1 and R^2 Positions of the 14-Membered Macrocycle



Compound	R^1	R^2	Dd2, GI_{50} (nM)	Solubility (PBS, μM)	hERG[a]	LogD (HPLC)	Microsome Stability[c]	Compound	R^1	R^2	Dd2, GI_{50} (nM)	Solubility (PBS, μM)	hERG[a]	LogD (HPLC)	Microsome Stability[c]
2	NMe_2		0.54	<5	95[b]	4.1	<5	8	H		22	<5	0	4.0	<5
3	NMe_2		1.4	<5	-	3.5	<5	9	NEt_2		5	8	-	3.1	5
4	NMe_2		32	>100	-	1.1	8	10	$N(O)Me_2$		160	>100	-	1.5	35
5	NMe_2		4.2	>100	13	2.9	8	11	$NHCH_2C(CH_3)_3$		14	7	-	3.8	5
6	NHMe		320	>100	-	1.7	51	12	$NHCH_2CF_3$		9	-	15	3.9	<5
7	OH		240	44	11	2.9	<5	13			105	29	1	3.5	<5

[a]% displacement control at 10 μM, assay run by Cerep. [b]Assay run by Ricera under identical conditions; see SI for further details. [c]Mouse, % remaining at 1 h. See SI for further ADME details. GI_{50} is the concentration at 50% of maximal inhibition of cell proliferation.

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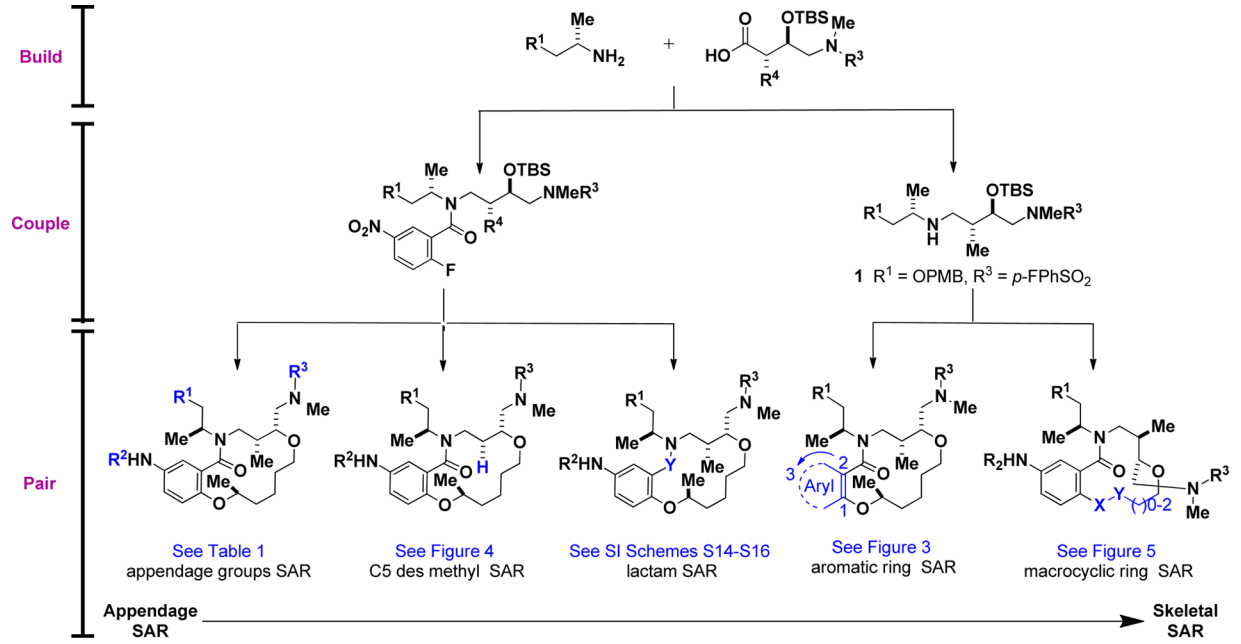

Figure 2. Build/couple/pair pathway for achieving appendage and skeletal SAR.

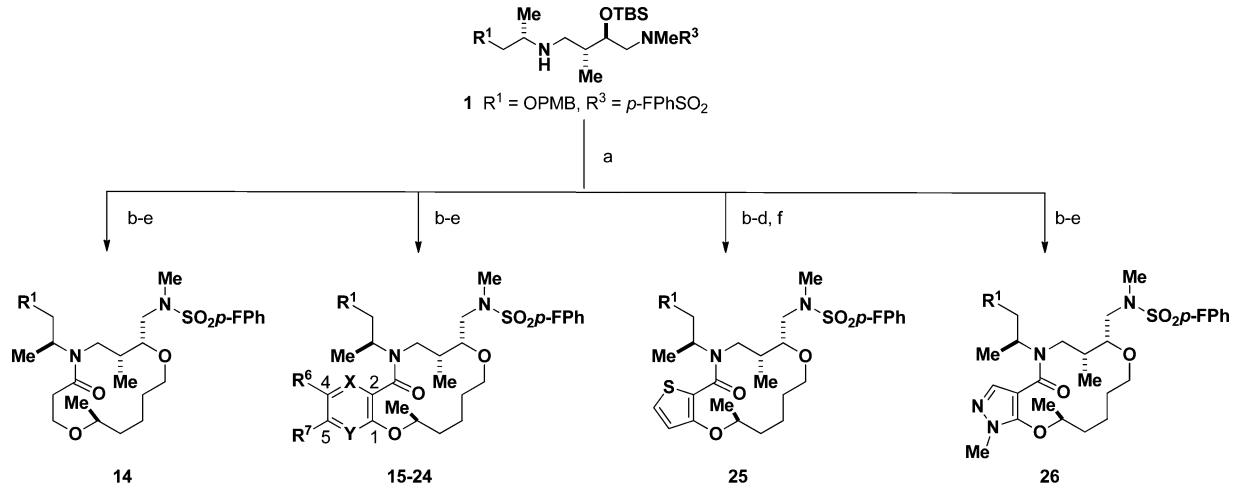

Figure 3. General synthesis of the *des*-urea series of compounds (See SI Schemes S3−S7 for expanded schemes). Reagents and conditions: (a) PyBOP/BOPCl, DIEA, Ar/AlkylCO$_2$H; (b) TBAF; (c) NaH, allylBr; (d) Grubbs−Hoveyda catalyst II; (e) H_2, 10% Pd/C; (f) Pd(OH)$_2$, H_2.

activity (GI_{50} = 32 nM). We reasoned that replacement of the phenyl urea with the 3,5-dimethylisoxazole urea derivative would improve solubility through the introduction of heteroatoms and the disruption of planarity.[15] This afforded the more soluble derivative **5** (PBS solubility >100 μM) with good potency (GI_{50} = 4.2 nM). Further profiling of **5** also showed significantly lowered hERG binding relative to the parent (13% displacement of control) and reduced plasma protein binding (8% free fraction in mouse plasma).

We then investigated substituents on R^1 while keeping R^2 constant. The previously prepared alcohol analogue **7**[13] gave diminished potency (GI_{50} = 240 nM) and microsomal stability (<5% remaining), as did the isopropyl analogue **8** (in regard to microsomal stability). The steric bulk of the amine functionality was increased with the diethylamine and neopentane analogues (**9** and **11**, respectively); however, this did not significantly improve microsomal stability. Reducing the basicity of the amine functionality as in the trifluoroethylamine **12** and morpholine derivative **13** resulted in active compounds (GI_{50} = 9 and 105 nM, respectively) but with similar microsomal stability to **2** (<5% remaining). Both the *N*-oxide amine **10** and the *mono*-methyl amine analog **6** did show improved solubility (>100 μM) and microsomal stability (35% and 51% remaining, respectively) along with a lower log *D*, but at the expense of potency (GI_{50} = 160 and 320 nM, respectively).

These results demonstrate our ability to improve PBS solubility and reduce hERG binding while maintaining acceptable activity through modification of different appendage groups. Although **5** is a viable candidate for *in vivo* efficacy studies, we sought to improve its microsomal stability by focusing on modulation of the core molecule itself. Metabolite identification studies on **5** and **6** revealed that both compounds were extensively metabolized *via* *N*-demethylation at R^1, hydroxylation, and combined events. In the case of **5**, six major metabolites were identified, and while the exact point of hydroxylation could not be determined, at least one

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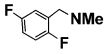
hydroxylation appeared to take place within the core molecule (see SI Figure S2).

We first directed our attention to the removal of *N,N′*-diaryl urea (Figure 3, Table 2 and SI Schemes S3–S7) to identify the minimum pharmacophore. This was achieved using a series of alkene containing *des*-urea acids in the "coupling" phase of the B/C/P pathway. Coupling linear amine **1** followed by deprotection of the secondary alcohol and subsequent allylation gave the ring closing metathesis (RCM) precursor. RCM was efficiently achieved for most substrates to give compounds **14**–**26**.

Table 2. SAR of the *des*-Urea Series of Compounds[b]

Compound	R^1	R^5	R^6	X	Y	Dd2, GI_{50} (nM)	Solubility (PBS, μM)	Microsome Stability[a]
14	OPMB	-	-	-	-	3410	<5	-
15	OPMB	F	H	CH	CH	162	<5	-
16	OPMB	H	F	CH	CH	38	<5	-
17	OPMB	H	CF_3	CH	CH	48	<5	-
18	OPMB	H	H	N	CH	403	<5	-
19	OPMB	H	H	CH	N	59	<5	-
20	OPMB	H	H	N	N	214	<5	-
21	OPMB	F	H	CH	N	162	<5	-
22	NMe_2	H	H	CH	N	231	>100	<5%
23	NEt_2	H	H	CH	N	79	>100	<5%
24		H	H	CH	N	10	<5	-
25	OPMB	-	-	-	-	481	<5	-
26	OPMB	-	-	-	-	375	15	-

[a]Mouse, % remaining at 1 h. [b]GI_{50} is the concentration at 50% of maximal inhibition of cell proliferation. See SI for further ADME details.

As shown in Table 2, removal of the aromatic group (**14**) (see SI Scheme S3 for expanded synthetic route) led to an inactive substrate (GI_{50} = 3410 nM). Replacement of the *N,N′*-diaryl urea at the 4-position with a fluorine atom (**15**) or CF_3 group (result not shown) also resulted in a loss in potency (GI_{50} = 162 and 138 nM, respectively).[16] The 5-fluoro isomer **16** showed good activity, as did the corresponding 5-trifluoromethyl analog **17** (GI_{50} = 38 and 48 nM, respectively). We prepared both the 3- and 6-pyridyl analogues (**18** and **19**) (see SI Scheme S4 for expanded synthetic route), and while the former provided a less active compound, the 6-pyridyl macrocycle provided good potency (GI_{50} = 59 nM). Building on this result, both pyrazine **20** and substituted pyridine **21** were prepared but proved less potent than **19** (GI_{50} = 214 and 162 nM, respectively). Replacement of the aromatic derivative with five-membered aromatic fragments was also investigated; however, both thiophene **25** and pyrazole **26** obviated much of the activity. With both the pyridyl and fluoro-substituted analogs showing similar potency within the *des*-urea series, the pyridyl series was pursued due to its perceived physicochemical property advantage. Removal of the OPMB group in **19** and installation of the dimethylamine group (**22**) did not preserve potency (GI_{50} = 231 nM), as was the case with the *N,N′*-diaryl urea series; however, it did afford excellent solubility (>100 μM). A number of derivatives of the 6-pyridyl macrocycle were prepared. Here, we found that the (2,5-difluorobenzyl)-methanamine analogue **24** was highly potent (GI_{50} = 10 nM) but lacked PBS solubility (<5 μM). Further profiling of both **22** and **23** showed no improvement in microsomal stability (<5% remaining). With reduced potency and no improvement in microsomal stability, we decided to shift our focus back to lead **5** rather than pursue the *des*-urea series further.

We investigated the aliphatic portion of the macrocycle with the continued aim of establishing a minimum pharmacophore (Figures 4 and 5 and Table 3). The methyl substituents at C-5

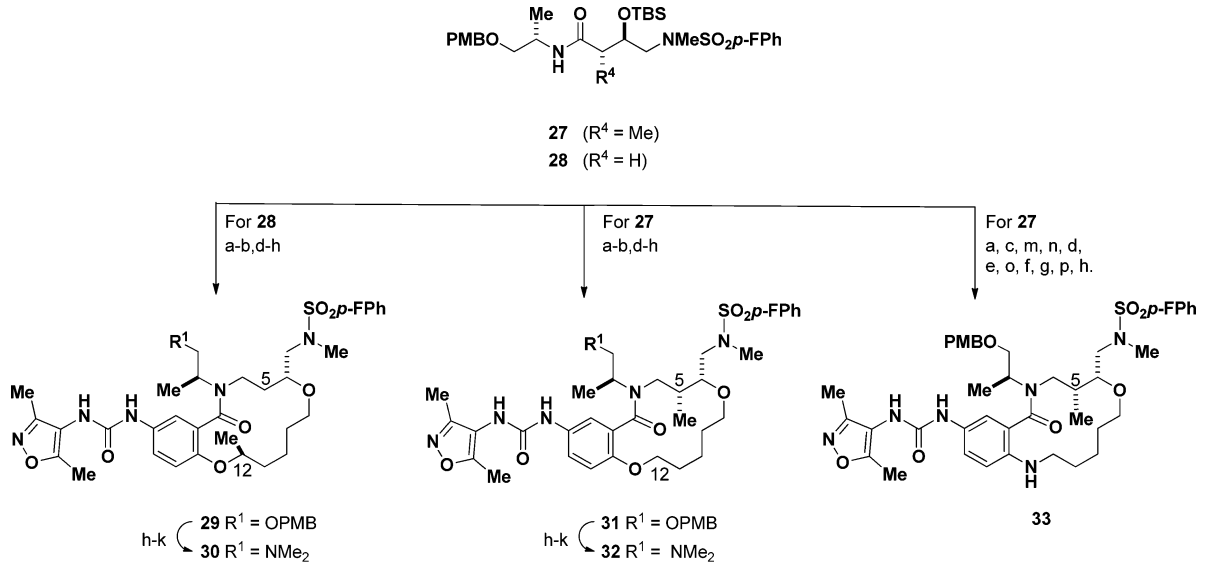


Figure 4. Synthesis of C-5 and C-12 *des*-methyl analogues. Reagents and conditions: (a) BH_3–SMe_2; (b) PyBOP, DIEA, 2-alkoxy-5-nitrobenzoic acid; (c) PyBOP, DIEA, 2-fluoro-5-nitrobenzoic acid; (d) TBAF; (e) NaH, allyl-Br; (f) Grubbs–Hoveyda catalyst II; (g) H_2, 10% Pd/C; (h) ArNCO; (i) DDQ; (j) DPPA, DBU, THF; (k) PPh_3, H_2O; (l) CH_2O, $MgSO_4$, H_2O; then $NaBH(OAc)_3$; (m) DIEA, but-3-en-1-amine; (n) di-*tert*-butyl dicarbonate, DMAP, THF, reflux; (o) TBS-OTf, lutidine then HF-pyridine; (p) $SnCl_2$–$2H_2O$.

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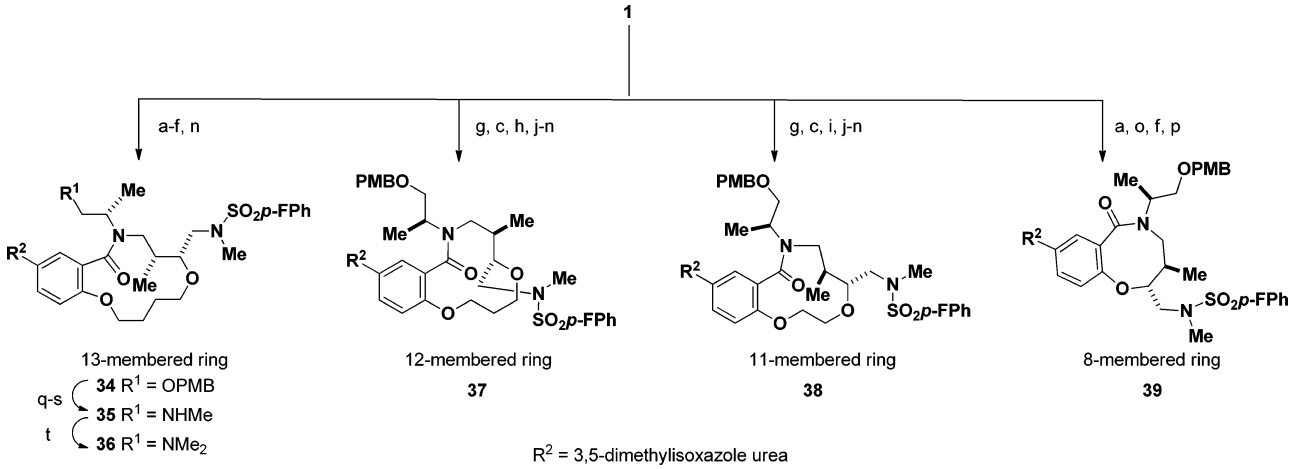
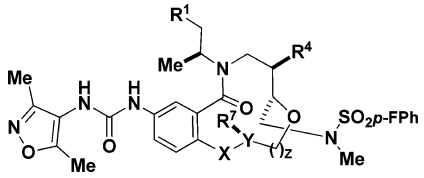


Figure 5. Synthesis of the macrocycle ring size analogues. Reagents and conditions: (a) PyBOP, DIEA, 2-fluoro-5-nitrobenzoic acid; (b) NaH, prop-2-en-1-ol; (c) TBAF; (d) NaH, allylBr; (e) Grubbs I; (f) H_2, 10% Pd/C; (g) Boc_2O, NEt_3; (h) Cs_2CO_3, methyl acrylate; (i) NaHMDS, methyl 2-bromoacetate; (j) DIBAL; (k) *tert*-butyl 2-fluoro-5-nitrobenzoate, TBAF; (l) TBS-OTf, lutidine then HF-pyridine; (m) DIEA, BOP-Cl; (n) $SnCl_2$ then ArNCO; (o) CsF; (p) ArNCO; (q) DDQ; (r) DIAD, PPh_3, o-NsNHMe; (s) K_2CO_3, PhSH; (t) CH_2O, $MgSO_4$, H_2O; then $NaBH(OAc)_3$.

Table 3. SAR of the Macrocycle Ring Size, *des*-Methyl and Heteroatom Exchange Analogues



Cmpd	R^1	R^4	R^7	X	Y	Z	ring size	Dd2, GI_{50}[a] (nM)	Solubility (PBS, μM)	Microsome stability[b]
29	OPMB	H	Me	O	CH	4	14	20	<5	–
30	NMe_2	H	Me	O	CH	4	14	71	99	2
31	OPMB	Me	H	O	CH	4	14	6	–	–
32	NMe_2	Me	H	O	CH	4	14	111	>100	–
33	OPMB	Me	H	NH	CH	4	14	76	<5	–
34	OPMB	Me	H	O	CH	3	13	17	<5	17
35	NHMe	Me	H	O	CH	3	13	624	>100	65
36	NMe_2	Me	H	O	CH	3	13	91	>100	15
37	OPMB	Me	H	O	CH	2	12	767	<5	–
38	OPMB	Me	H	O	CH	1	11	2970	6	–
39	OPMB	Me	–	–	–	0	8	>5000	–	–
40	OPMB	Me	Me	O	CH	4	14	0.88	<5	–

[a]GI_{50} is the concentration at 50% of maximal inhibition of cell proliferation. See SI for further ADME details. [b]Mouse, % remaining at 1 h.

and C-12 (Figure 4 and Table 3) were probed, with focus on the latter, as its removal would simplify the chemistry in the investigation of macrocycle ring size. While HTS included all 16 stereoisomers of our original hit,[13] the effect of removing individual stereogenic centers had only been investigated with respect to the excocyclic C-2 methyl group, which lost approximately 5-fold potency upon deletion.[13] Similar potency was observed between **31** and **40** (GI_{50} = 6 nM and 0.88 nM, respectively), suggesting that the C-12 methyl is not essential in this series. Removal of the C-5 methyl group (**29**) resulted in a slight loss in activity (GI_{50} = 20 nM). The influence of the aryl ether moiety was then tested by changing the O-13 oxygen for the nitrogen analogue (**33**), which showed diminished potency (GI_{50} = 76 nM) and no improvement in solubility (<5 μM). Interestingly, attempted macrocyclization leading to **33** gave no desired product when the N-13 nitrogen heteroatom was protected as *N*-Boc, but the RCM reaction proceeded efficiently with the compound lacking this protecting group (see SI Scheme S10). We then systematically reduced the size of the 14-membered macrocycle **31** to 13-, 12-, 11-, and 8-membered ring analogs (Figure 5 and Table 3). As the synthesis of 9- and 10-membered macrocycles would require the preparation of peroxides or acetals, these analogues were omitted from the study.

The 13-membered ring analog **34** was prepared from linear amine **1** using the RCM reaction (Figure 5, SI Scheme S11). In this case, macrocyclization was not as efficient as the 14-membered analog and the transformation was best accomplished using the first-generation Grubbs catalyst. Preparation of the 12- and 11-membered lactams was achieved by either Michael addition or alkylation of **1** with methyl acrylate (SI Scheme S12) or 2-bromoacetate, respectively (Figure 5). Both analogues contained methyl esters that were reduced to the corresponding alcohols followed by intermolecular SnAr reactions with *tert*-butyl 2-fluoro-5-nitrobenzoate to install the aromatic esters. Simultaneous deprotection of the ester and amine protecting groups followed by intramolecular coupling yielded the 12- and 11-membered macrocycles (**37** and **38**).

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The synthesis of 8-membered ring analog **39** followed the method reported by Marcaurelle et al.[10] A clear SAR trend emerged from analogs **34**−**39** with potency decreasing with the size of the macrocycle ring (Table 3). While the complete removal of the aliphatic backbone obviated all activity (**39**, GI_{50} >5000 nM), potency was maintained in the 13-membered analogue (**34**, GI_{50} = 17 nM). Similar to previous experiments, analogs **29**, **31**, and **34** were converted to the dimethyl amine analogs at R^1 (**30**, **32**, and **36**, respectively) to assess potency, solubility, and microsomal stability. All three analogs showed excellent solubility, as expected, along with somewhat reduced potency relative to their respective PMB protected analogs. Unfortunately, microsomal stability was still low, with only the 13-membered ring **36** showing modest gains in microsomal stability (15% remaining). While greater microsomal stability was observed in the NHMe analogue **35** (65% remaining), this was accompanied by a significant loss in potency (GI_{50} = 624 nM).

Through systematic chemistry efforts, we were able to develop SAR within lead antimalarial compound **2**. This included removal of the urea and methyl substituents within the ring, macrocyclic ring contraction, and variation at three different appendage sites. After identifying analogs that showed improved activity/PK profiles along with improved hERG profiles, we investigated the *in vivo* activity of **5** by intraperitoneal dosing in a *P. berghei* malaria mouse model. Our goal was to have *in vivo* exposure exceeding three times the GI_{50} of the unbound compound (not bound to plasma proteins) at its lowest concentration (i.e., exposure >3 × GI_{50} × f_u). Intraperitoneal administration of macrocyle **5** at 20 mg/kg showed that sufficient exposure was observed for approximately 5 h (see SI Figures S3 and S4). This suggested that it would be possible to achieve our goal at higher concentrations with intraperitoneal administration. In the *P. berghei* malaria mouse model, animals were infected and administered a total of seven 100 mg/kg intraperitoneal doses every 12 h over 3 days. On day four, parasitemia was assessed and **5** produced a 2-fold reduction in total parasitemia (p = 0.02). This is a significant reduction but not to the levels typically seen with standard of care antimalarials such as chloroquine or artesunate (e.g., artesunate can give parasitemia reductions of 97%). Plasma samples taken 30 min after the final dose on day 3 showed low exposure of the compound (300 ng/mL of **5** in plasma in this study compared with 768 ng/mL 2 h after IP dosing at 20 mg/kg observed during PK study), suggesting that, upon dose escalation, compound pharmacokinetics did not scale. Based on this data, the lack of efficacy in the *in vivo* assay most likely speaks to the poor exposure of **5** rather than on the mechanism of action of the compound, on which we comment below.

The goal of our study was to use a diverse screening collection to discover antimalarial chemotypes with new mechanisms of action. In a separate manuscript we discuss the use of resistance selection to identify the target of **5** as the reductase Qi center of cytochrome *b* (cytb).[17] While inhibition of cytochrome *b* at Qo has been well studied and is the mechanism of action of the widely used antimalarial drug atovaquone, inhibition of cytb at Qi has been significantly less studied.[18] An appealing concept is that, if used in combination, Qi and Qo site inhibitors would likely lessen the emergence of resistance. It is conceivable that mutations at both Qi and Qo sites of the same target would come at a severe cost to cell fitness and would be unlikely to be tolerated. Given this, Qi site inhibitors acting synergistically with Qo inhibitors have the potential to be powerful antimalarial agents.

DISCUSSION AND CONCLUSION

In this study we prepared analogues with improved potency, solubility, hERG binding, and modestly improved microsomal stability relative to our initial lead using DOS enabled medicinal chemistry. This allowed for the ready manipulation of our lead antimalarial. While *in vivo* efficacy studies gave only moderate reductions in parasitemia, we speculate that this is a result of insufficient exposure and is not a reflection of the mechanism of action of this compound. Furthermore, this study has led to the discovery of potent inhibitors of cytochrome *b* that do not inhibit this complex through the Qo binding site, but through the comparatively less studied Qi site, and represents a new chemotype and tool to target this promising antimalarial mechanism of action. These studies encouraged us to expand our studies to the entire Broad Institute collection of DOS-derived compounds, which has yielded a rich collection of novel antimalarial compounds acting through varied mechanisms. We are finding that the lessons gained from the medicinal chemistry studies reported here have been of value as we advance novel antimalarial agents using this overall drug-discovery strategy.

ASSOCIATED CONTENT

Supporting Information

Procedures and references for synthesis of compounds **1**−**39** in addition to NMR data and protocols for in vivo studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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