Diversity-Oriented Synthesis Yields a Novel Lead for the Treatment of Malaria

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Ⓢ *Supporting Information*

ABSTRACT: Here, we describe the discovery of a novel antimalarial agent using phenotypic screening of *Plasmodium falciparum* asexual blood-stage parasites. Screening a novel compound collection created using diversity-oriented synthesis (DOS) led to the initial hit. Structure−activity relationships guided the synthesis of compounds having improved potency and water solubility, yielding a subnanomolar inhibitor of parasite asexual blood-stage growth. Optimized compound 27 has an excellent off-target activity profile in erythrocyte lysis and HepG2 assays and is stable in human plasma. This compound is available via the molecular libraries probe production centers network (MLPCN) and is designated ML238.

ML238
Blood-stage Malaria (Dd2, GI_{50}) = 0.54 nM
Erythrocyte Lysis (EC_{50}) = >40 μM

KEYWORDS: *diversity-oriented synthesis, malaria, macrocycle, high-throughput screening, phenotypic screen, infectious disease, molecular libraries probe production centers, stereochemical structure−activity relationships*
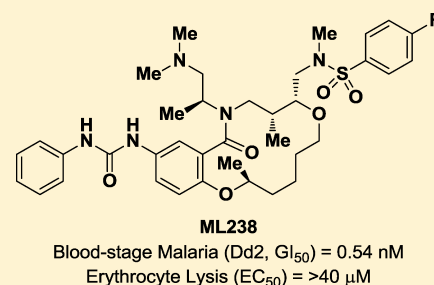
Malaria afflicts approximately 225 million people and leads to ∼780 000 deaths per year.[1,2] The apicomplexan *Plasmodium* parasite responsible for this condition is contracted via *Anopheles* mosquito bites and lives a part of its life cycle within this vector.[3] Endemic areas are limited to the tropics, where continuous mosquito breeding is possible. Several species of *Plasmodium* can infect mammalian hosts, and *Plasmodium falciparum* is the most prevalent cause of fatal outcomes in malaria patients.[4]

Chemotherapy over a period of decades has likely contributed to the high degree of genetic diversity within these parasites.[5] The emergence of resistance to antimalarial drugs has led to poor treatment outcomes.[6] The current best practice is a combination therapy with the artemisinin family of antimalarial agents.[7] In addition to drug resistance, malaria is prevalent in underdeveloped nations with poor infrastructures, high population

densities, and insufficient health care funding. These factors have combined to present a challenging clinical environment.[8] Combination therapies have been used to mitigate the rate of resistance in several diseases.[9] As such, new small molecules with novel mechanism(s) of action have the greatest potential to enable a successful path to the treatment of drug-resistant malarial infections.[10,11]

Diversity-oriented synthesis (DOS)[12−14] yields small molecules having diverse stereochemistries and skeletons. Short, modular synthetic pathways, for example, using the build/couple/pair (B/C/P) strategy, facilitate downstream efforts using medicinal chemistry. These novel compounds effectively
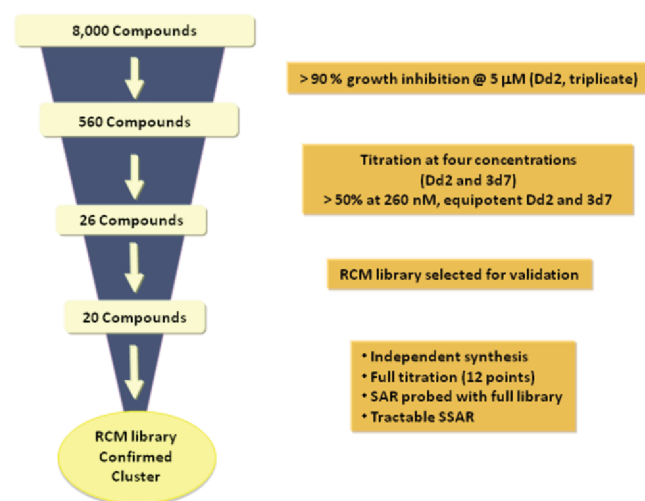
Figure 1. Graphical depiction of the HTS triage.

HTS Hit Compound 1

Stereochemistry: *SRRS*

Dd2 (GI$_{50}$): 120 nM

3d7 (GI$_{50}$): 120 nM

Hemolysis (EC$_{50}$): >40 μM

Solubility (water): <0.5 μM

Stereochemistry (C$_2$C$_5$C$_6$C$_{12}$)

RSSR	SRRS	RSRR	SRSS
SSSS	RRRR	SSSR	RRRS
RSSS	SRRR	SSRS	RRSR
SSRR	RRSS	RSRS	SRSR

0   50   100
% inhibition at 280 nM
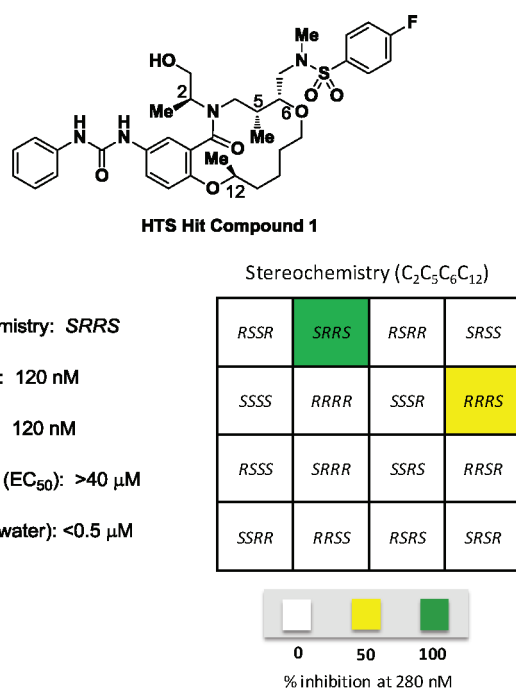
Figure 2. Structure of lead compound **1**, potency in two strains of blood-stage malaria parasites, and the SSAR of the hit.

complement traditional screening collections.[15−21] Our recent efforts[22] have focused on incorporating both structure−activity relationships (SAR) and stereochemical structure−activity relationships (SSAR) into the library design, thereby enabling quick prioritization of hit clusters from primary screens. Coupling this initial SAR with the ability to synthesize analogues rapidly using modular synthetic pathways provides an efficient process to pursue hits identified using high-throughput screening (HTS). These principles were highlighted recently by an aldol-based B/C/P strategy, resulting in compounds having medium-sized and macrocyclic rings derived from a common linear intermediate.[23,24] This report describes an HTS that yielded novel compounds having antimalarial activity and subsequent medicinal chemistry that yielded a promising and novel subnanomolar inhibitor of the major etiologic agent of severe malaria, *P. falciparum*.

Table 1. SARs Derived from the Broad Compound Collection

Compound	R^1	R^2	Dd2 GI$_{50}$ (nM)
1			120
2			160
3			350
4			2600
5			1100
6			400
7			240
8			>5000
9			2400

We initiated the search for novel antimalarial agents via HTS of an 8000-membered DOS 'informer' collection in a phenotypic blood-stage malaria assay.[25] This representative set of compounds was computationally selected for maximum diversity representing all libraries in our collection while maintaining the full complement of all possible stereoisomers of each compound. HTS was conducted in triplicate at a 5 μM compound concentration using a growth inhibition assay (72 h, DAPI) with
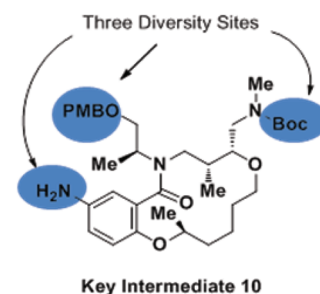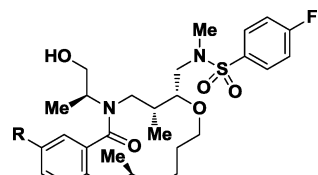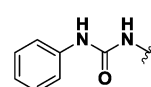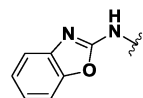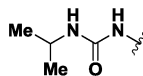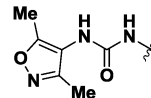

Figure 3. Preparation of derivatives in the solution phase.

Table 2. Initial SARs of the Aryl Substituent

Compound	R	Dd2 GI$_{50}$ (nM)	Solubility (water, μM)
1		120	<0.5
11	H$_2$N	990	-
12		580	<0.5
6		400	2.2
7		240	45

Table 3. Initial SARs at the Amine Substituent

Compound	R	Dd2 GI$_{50}$ (nM)	Solubility (water, μM)
1		120	<0.5
13		1100	<0.5
14		877	<0.5
15		>5000	1.6
16		4300	<0.5
17		1400	12
18		>5000	1.2
19		>5000	280
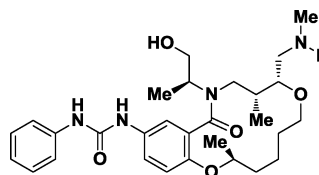
multidrug-resistant Dd2 *P. falciparum* parasites (Figure 1). A total of 560 compounds showed greater than 90% growth inhibition, and titration at a four concentrations further narrowed the list to 26 candidates (>50% inhibition at 280 nM). Of these, 20 compounds were from the 'ring-closing metathesis' (RCM) library, and this scaffold was selected for further studies.[26]
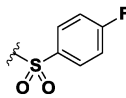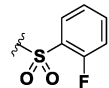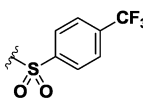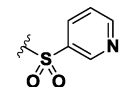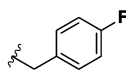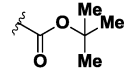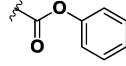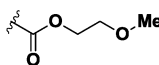
The most active hit (compound **1**, Figure 2) was independently synthesized and iteratively titrated in a 12-point assay to confirm a potency (GI$_{50}$) of 120 nM against Dd2 intraerythrocytic parasites (GI$_{50}$ values were obtained with Dd2 parasites unless otherwise specified). This lead has a similar potency in the drug-sensitive 3d7 parasite strain and does not cause hemolysis of erythrocytes at up to 40 μM concentration; unfortunately, it is largely insoluble in aqueous solution (<0.5 μM in water). The stereochemical SAR (SSAR) analysis of all 16 possible isomers of compound **1** demonstrates that interesting biological activity is predominantly located in two stereoisomeric compounds that are epimeric outside the macrocyclic ring (C$_2$, Figure 2).

In the first round of analogue testing, we searched the Broad Institute chemical compound collection for analogues of hit **1** that had the same *SRRS* configuration (C$_2$C$_5$C$_6$C$_{12}$) and retained one of the two (phenyl urea or 4-fluorophenyl sulfonamide) side chain diversity elements. This exercise resulted in more than 50 available analogues. The results of selected titrations are reported in Table 1. The first five structures show changes to the amine side chain. Alternatively substituted and unsubstituted sulfonamides **2** and **3** showed similar potency to the lead (GI$_{50}$ = 160 and 350 nM, respectively), while the corresponding phenyl amide **4** (GI$_{50}$ = 2600 nM) and benzyl amine **5** (GI$_{50}$ = 1100 nM) demonstrated a loss in potency.

Alteration of the urea substituent from phenyl (**1**, L/D = 120 nM) to isopropyl (**6**, GI$_{50}$ = 400 nM) or 2,4-dimethylisoxazolyl (**7**, GI$_{50}$ = 240 nM) afforded compounds with somewhat diminished potency. Formal truncation of the urea to the dimethylaniline **8**
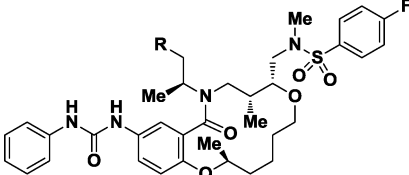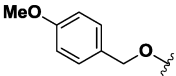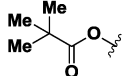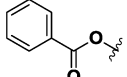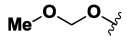
(GI$_{50}$ = >5000 nM) led to an inactive substrate, while the corresponding phenyl-sulfonamide **9** (GI$_{50}$ = 2400 nM) also provided a less biologically interesting substrate. The presence of both reasonable SAR via the presence of close structural analogues in the compound collection combined with promising SSAR prompted additional investigation into this lead.

Independent preparation of interesting compounds and the synthesis of additional derivatives closely followed the strategy originally reported by Marcaurelle et al.[24] These syntheses were performed in the solution phase. Protected intermediate **10** was converted into the requisite derivatives via iterative capping and deprotection steps (Figure 3). When the aniline group required masking, a 9-fluorenylmethyloxycarbonyl (Fmoc) group was used. Details of the synthetic steps used to prepare these compounds can be found in the Supporting Information.

Our preliminary studies of the SAR of this novel lead revolved around monitoring potency in the growth inhibition assay while improving aqueous solubility by the addition of polar functional groups and attempting to remove hydrophobic bulk. We first directed our attention to the *N,N'*-diaryl urea (Table 2). Formal truncation of the urea group provides the corresponding aniline **11** (GI$_{50}$ = 990 nM). Aniline **11**, heterocyclic aminobenzoxazole **12** (GI$_{50}$ = 580 nM), and the isopropyl urea **6** (GI$_{50}$ = 400 nM) led to modest decreases in potency. We followed on the observation that the isopropyl
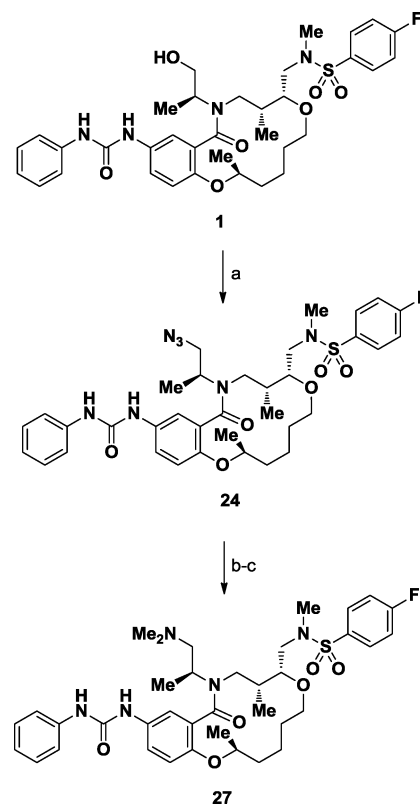

Table 4. Initial SARs at the Amide Substitutent

Compound	R	Dd2 GI$_{50}$ (nM)	Solubility (water, μM)
1	HO	120	<0.5
20	MeO-(benzyl)-O	0.18	<0.5
21	Me,Me,Me-C(=O)O	7.3	<0.5
22	(phenyl)C(=O)O	15	<0.5
23	Me-O-CH$_2$-O	8.2	<0.5
24	N$_3$	3.2	<0.5
25	H$_2$N	370	270
26	Me-NH	79	400
27	Me$_2$N	0.54	120

Scheme 1. Synthesis of Probe 27 from Initial Lead 1^a

^{a}Reagents and conditions: (a) DPPA, DBU, THF, 87%. (b) PPh$_3$, H$_2$O, THF, 76%. (c) CH$_2$O, MgSO$_4$, H$_2$O, DCM; then NaBH(OAc)$_3$, 87%.

urea **6** showed a modest increase in water solubility (water solubility =2.2 μM) by independent preparation of the heterocyclic and substituted 4-amino-3,5-dimethylisoxazolyl urea **7**. This afforded a soluble derivative (water solubility = 45 μM) with some loss of potency (GI$_{50}$ = 240 nM).

We next optimized the amine side chain for potency and solubility (Table 3). The regioisomeric 2-fluorophenylsulfon-amide **13** has lower potency (GI$_{50}$ = 1100 nM) when contrasted with the parent, and the 4-trifluoromethyl derivative **14** showed no improvement (GI$_{50}$ = 880 nM). Hetereocycles such as 3-pyridylsulfonamide **15** were not tolerated (GI$_{50}$ > 5000 nM), and benzylic amine **16** also showed a lack of potency in the primary phenotypic assay (GI$_{50}$ = 4300 nM). Carbamate derivatives such as *tert*-butyl (**17**), phenyl (**18**), and methox-yethoxy (**19**) showed improvements in solubility but lost biological activity (all GI$_{50}$ > 1400 nM).

Exploration of alternate substituents of the amide group began serendipitously with the submission of protected derivatives to the growth inhibition assay (Table 4). The *para*-methoxybenzyl (PMB) ether **20** showed subnanomolar potency (GI$_{50}$ = 0.18 nM). To confirm this result, other less lipophilic groups were appended to the alcohol. Pivalate (**21**, GI$_{50}$ = 7.3 nM), benzoyl (**22**, GI$_{50}$ = 15 nM), and methoxymethyl (**23**, GI$_{50}$ = 8.2 nM) showed similar but somewhat diminished improvements in potency. Unfortunately, none of these compounds were soluble in water.

Displacement of the sterically hindered hydroxyl group within **1** with azide under Mitsunobu conditions afforded both a potent derivative (**24**, GI$_{50}$ = 3.2 nM) and access to nitrogen-based

functional groups expected to improve water solubility (Scheme 1). Reduction provided soluble primary amine **25** (GI$_{50}$ = 370 nM, water solubility = 268 μM). The corresponding monomethylamine **26** showed no improvements over its parent (GI$_{50}$ = 79 nM, water solubility = 401 μM), while dimethylamino derivative **27** demonstrated both subnanomolar potency (GI$_{50}$ = 0.54 nM) and solubility in water (water solubility = 120 μM).

With this encouraging result in hand, we revisited the alternate stereochemical series on the C$_2$ carbon located outside the macrocycle originally noted in the HTS screen (Table 5). Independent synthesis and titration of our original HTS hits showed that the two epimeric alcohols **1** and **28** had similar potency (GI$_{50}$ = 120 and 62 nM, respectively), while desmethyl ethanol derivative **30** lost some activity (GI$_{50}$ = 630 nM). As previously noted, the addition of a PMB ether in the *S*-series resulted in a 3−4 log improvement in potency (**20**, GI$_{50}$ = 0.18 nM, vida supra). When the same formal transformation was performed on the epimeric (*R*) diastereomer and desmethyl examples, no correspondingly large potency improvements were observed (**29**, GI$_{50}$ = 36 nM; and **31**, GI$_{50}$ = 55 nM). Confident that we were operating on the optimal stereoisomer, we nominated **27** as the Molecular Libraries Probe Production Centers Network (MLPCN) probe (ML238) and submitted it for further testing.

Compound **27** has subnanomolar activity in two *P. falciparum* strains, and inhibition of parasite growth is not time dependent (contrasting 48−96 h time points, Table 6). The compound is more potent than chloroquine or artesunate and is similar in potency to atovoquone (GI$_{50}$ = 0.54 nM, all three known anti-malarial agents were used as controls). This compound is
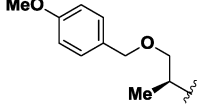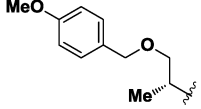
Table 5. SSARs at the Amide Substitutent
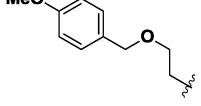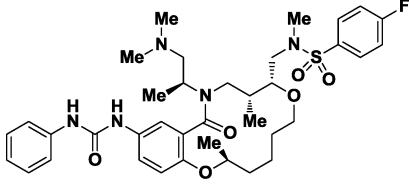
Compound	C$_2$ Stereochemistry (Amide Sidechain)	R	Dd2 GI$_{50}$ (nM)
1	S		120
20	S		0.18
28	R		62
29	R		36
30	Desmethyl		640
31	Desmethyl		55

Table 6. Profile of Malaria Probe 27 (ML238)

27 (ML238)

assay	result
live/dead (Dd2, GI$_{50}$, 72 h)	0.54 nM
live/dead (3d7, GI$_{50}$, 96 h)	0.54 nM
live/dead (3d7, GI$_{50}$, 48 h)	3.6 nM
solubility (water)	120 μM
erythrocyte lysis	>40 μM
HepG2	>30 μM
plasma protein binding (human)	99%
plasma stability (human, 5 h)	98%

soluble in water (120 μM), and nontoxic to both erythrocytes (>40 μM) and HepG2 cells (>30 μM). In the presence of human plasma, it is highly protein-bound (99%) and stable (98% remaining at 5 h).

Studies of the mechanism of action of this new class of antimalarial agent are underway.[27] Secondary assays have confirmed that representative compounds from this chemical series are not inhibitors of dihydroorotate dehydrogenase (DHODH) enzymes (human and parasite enzymes >30 μM).[28,29] This compound (27) is available via the MLPCN and is designated ML238.

ASSOCIATED CONTENT

S Supporting Information

Experimental procedures and characterization of new chemical entities. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

B/C/P, build/couple/pair; DBU; 1,8-diazobicyclo[5.4.0]undec-7-ene; DCM; dichloromethane; DHODH, dihydroorotate dehydrogenase; DAPI, 4′,6-diamidino-2-phenylindole; DOS, diversity oriented synthesis; DPPA; diphenylphosphoryl azide; FMOC, 9-fluorenylmethyloxycarbonyl; HTS, high-throughput screening; GI$_{50}$, phenotypic assay GI$_{50}$ values utilizing Dd2 parasites; MLPCN, Molecular Libraries Probe Production Centers Network; PMB, *para*-methoxybenzyl; RCM, ring-closing metathesis; SAR, structure−activity relationships; SSAR, stereochemical structure−activity relationship

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