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Novel diaryl ureas with efficacy in a mouse model of malaria

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ABSTRACT

Exploration of triclosan analogs has led to novel diaryl ureas with significant potency against in vitro cultures of drug-resistant and drug-sensitive strains of the human malaria parasite *Plasmodium falciparum*. Compound **18** demonstrated EC_{50} values of 37 and 55 nM versus in vitro cultured parasite strains and promising in vivo efficacy in a *Plasmodium berghei* antimalarial mouse model, with >50% survival at day 31 post-treatment when administered subcutaneously at 256 mg/kg. This series of compounds provides a chemical scaffold of novel architecture, as validated by cheminformatics analysis, to pursue antimalarial drug discovery efforts.

2Traditional treatments for *Plasmodium falciparum* malaria such as chloroquine and pyrimethamine-sulfadoxine have in many areas succumbed to drug resistance, and evidence now suggests emerging resistance to the new first-line artemisinins in Western Cambodia.[1] Infection is rampant, resulting in ~650,000 deaths per year.[2] Clearly, a need exists for novel therapies that are orally bioavailable, economical, and safe and whose activities are not compromised by existing resistance.[3–5]

Our laboratories have been interested in fatty acid biosynthesis in both *Plasmodium* spp. and *Mycobacterium tuberculosis*. Initial investigations into the mycobacterial target of the front-line drug isoniazid (INH) led to the discovery that the INH–NAD adduct binds the enoyl acyl carrier protein reductase InhA (also known as FabI or ENR).[6] This sparked efforts by us and others to study triclosan-inspired small molecule inhibitors of InhA that do not require KatG activation of INH for *M. tuberculosis*,[7] and that inhibit the *P. falciparum* homolog (PfFabI, previously termed PfENR) in biochemical assays with purified enzyme.[8–10] Interestingly, the plasmodial FabI is not essential for the intra-erythrocytic stage of the parasite and instead is required for normal progression of the liver-stage of infection.[11,12]

Efforts to optimize the antimalarial efficacy of small molecule triclosan analogs began with the hypothesis that the phenol could be replaced by organic functionality capable of maintaining some of the hydrogen-bonding interactions that have been shown crystallographically to be important for enzyme inhibition.[13] The key interactions in this case involve the PfFabI Tyr-277 phenol and the 2′-OH of the ribose unit of the bound NAD^+ co-factor. The compounds (Table 1) were prepared via adaptation of standard synthetic methods common to diaryl ether assembly[14] and feature a variety of 1-substituents, ranging from an ether and a nitrile to carboxylic acid derivatives, amines, sulfonamides, and ureas. Triclosan methyl ether was prepared by methylation of the parent with methyl iodide in the presence of potassium carbonate to afford **1**. The synthesis of triclosan 1-substituted analogs relied significantly on the preparation of anilines **5a** and **5b** (Scheme 1). The anilines were synthesized beginning with the coupling of the respective *o*-chloronitrobenzene with 2,4-dichlorophenol in DMSO. The resulting diaryl ethers underwent reduction to afford anilines **5a** and **b**. Aniline **5a** was carried in three straightforward steps, involving diazotization, the Sandmeyer reaction, and the Rosenmund-von Braun reaction,[15] to benzonitrile **2**. The 1-cyano

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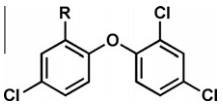
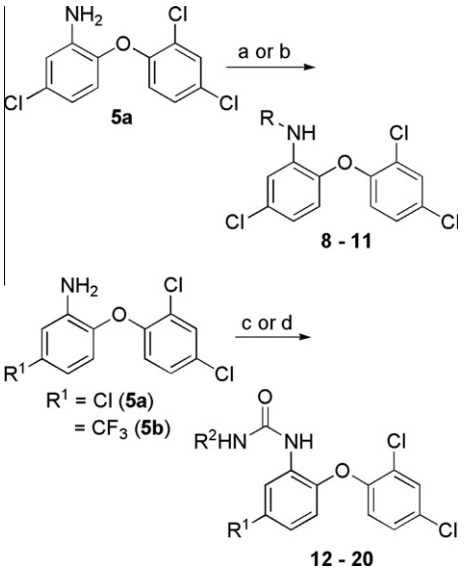
Table 1
In vitro activities of triclosan derivatives with a 1-substituent against cultured *P. falciparum* strains



Compound	R	EC_{50}(μM) 3D7	EC_{50}(μM) Dd2
Triclosan	OH	2.9	3.8
1	OCH_3	100	76
2	CN	20	43
3	COOH	>150	>150
4	$CONH_2$	73	60
5a	NH_2	52	55
6	CH_2NH_2	2.3	4.3
7	$C(CH_3)_2NH_2$	7.2	1.8
8	$NHSO_2Me$	38	60
9	$NHSO_2Ph$	4.7	5.1
10	NHAc	33	48
11	NHBz	9.7	9.4
12	$NHC(O)NH_2$	18	18
13	NHC(O)NHPh	0.73	1.2

triclosan **2** was then hydrolyzed to afford amide **4** and carboxylic acid **3**. Reduction of **2** with lithium aluminum hydride afforded aminomethyl **6**. Utilization of Ciganek's methylorganocerium reagent,[16] generated in situ from cerium(III) chloride and 1 equiv of methyllithium reduced **2** to α,α-dimethylamine **7**. Aniline **5a** provided a starting point for the synthesis of a limited set of sulfonamide and amide analogs **8**–**11** via reaction with a sulfonyl chloride, anhydride or acid chloride under traditional conditions (Scheme 2). Two routes to ureas were devised, involving either direct reaction of aniline **5a** or **5b** with a commercial isocyanate or activation with triphosgene followed by mixing with a commercial amine. Two simple urea analogs **12** and **13** were initially made.

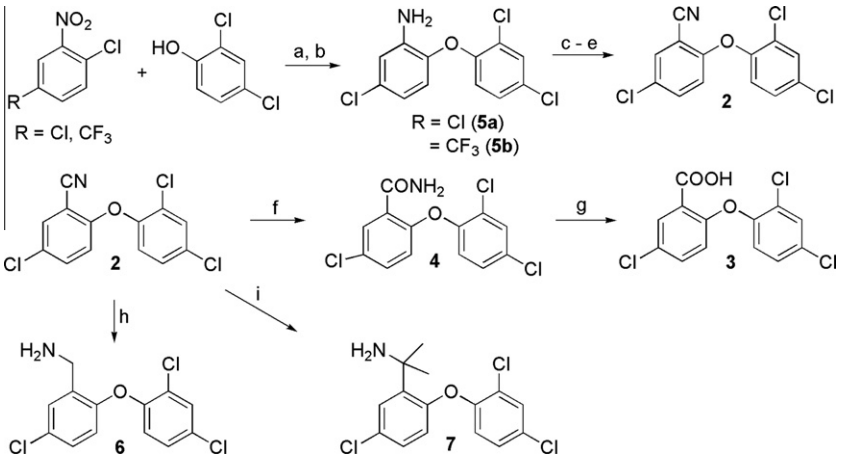
While none of the phenol replacements displayed significant activity (IC_{50} >10 μM) in a PfFabI inhibition assay,[13] both aminomethyl **6** and diaryl urea **13** demonstrated efficacy in growth inhibition assays[17] with cultured 3D7 (drug-sensitive) and Dd2 (resistant to chloroquine and pyrimethamine–sulfadoxine) *P. falciparum* strains. Molecular modeling studies rationalized how the loss of hydrogen-bonding (perhaps through both donating and accepting) upon replacement of the 1-OH may lead to abrogation of potent binding to, and hence reduced inhibition of PfFabI. Chemical inspection of **6** and **13** led to the prioritization of the diaryl urea for optimization based on its superior whole-cell efficacy and the potential to readily explore a range of aryl substituents, not belonging to the diaryl ether subunit.



Scheme 2. Synthesis of various 1-substituted triclosan amides, sulfonamides, and ureas. Reagents and conditions: (a) RSO_2Cl, NEt_3, DCM; (b) Ac_2O or PhC(O)Cl and NEt_3, DCM; (c) (i) $(Cl_3CO)_2CO$, NEt_3, DCM, −78 °C to rt; (ii) R^2NH_2; (d) R^2NCO, tol.

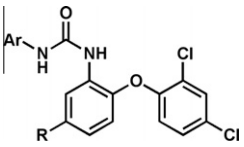
A set of follow-up diaryl ureas (Scheme 2, Table 2) was prepared to probe the structure–activity relationship pertinent to growth inhibition of in vitro-cultured *P. falciparum*, regardless of molecular target. It is clear from a select subset of analogs that the substitution pattern on the non-diaryl ether aryl moiety affected antimalarial activity against both strains of *P. falciparum*. In particular, the 3-position favored electron-withdrawing groups such as trifluoromethyl or cyano. The 4-position also preferred electron-withdrawing groups such as cyano, nitro, fluoro, and chloro. This was exemplified in the most potent compounds in this series: **18** (3-CF_3-4-ClC_6H_3), **21** (3-CF_3-4-$NO_2C_6H_3$), and **24** (3,4-diCNC_6H_3).

The in vivo activity of a subset of the urea derivatives was assessed utilizing the *Plasmodium berghei* rodent malaria model (Table 2).[18] Briefly, ~5 weeks old CD-1 mice (Charles River Laboratories) were infected intraperitoneally with 10^6 *P. berghei* (KBG-173 line) parasitized red blood cells on day 0. Drug dosing (bid, divided dose) was initiated on day 3 and continued on days 4 and 5. Subcutaneous administration was achieved using a peanut oil suspension of the compound. Activity was defined by the fractional survival at day 31. The infected control mice survived an



Scheme 1. Synthesis of various 1-substituted triclosan derivatives. Reagents and conditions: (a) KOH, DMSO, 100 °C; (b) H_2, Ra-Ni, EtOH; (c) *t*-BuONO, $BF_3{\cdot}Et_2O$, THF, 0 °C; (d) NaI, acetone; (e) CuCN, DMF, 150 °C; (f) NaOH, H_2O_2, EtOH, 50 °C; (g) 6 N $HCl_{(aq)}$, 2-methoxyethanol, 80 °C.

Table 2
Profile of diaryl ureas tested for in vitro activity against cultured *P. falciparum* strains and in vivo efficacy in a *P. berghei* mouse model



Compound	Ar	R	EC_{50} (μM) 3D7	EC_{50} (μM) Dd2	SC dose (mg/kg)	$F_{Survival}$ @ 31d[a,b,c]
14	1-Naphthyl	CF_3	1.2	0.81	256	NES
15	2-Naphthyl	CF_3	0.61	0.41	256	NES
16	4-ClPh	Cl	2.0	5.0	256	NES
17	3,4-Cl_2Ph	Cl	1.9	2.5	256	NES
18	3-CF_3-4-ClPh	CF_3	0.037	0.055	256	4/7
18					128	EXT
19	3-CF_3-4-FPh	CF_3	0.13	0.15	256	1/7
19					128	2/7
20	3-CF_3-4-OMePh	CF_3	0.18	0.18	nd[d]	nd
					nd	nd
21	3-CF_3-4-NO_2Ph	CF_3	0.072	0.072	nd	nd
22	3-CF_3-4-NH_2Ph	CF_3	1.5	3.6	nd	nd
23	3-CF_3-4-CNPh	CF_3	0.13	0.075	nd	nd
24	3,4-CN_2Ph	CF_3	0.081	0.14	nd	nd
25	3,4-Cl_2Ph	CF_3	0.20	0.20	nd	nd

[a] $F_{Survival}$ = proportion of animals living at day 31.
[b] NES = no extension of survival beyond infected control animals (8 days).
[c] EXT = extension of survival beyond infected control animals (8 days), but no survival at day 31.
[d] nd = not determined.

average of 8 days whereas non-infected control mice survived the entire 31 days of the study. Compounds with comparatively lower in vitro activity, such as **14**–**17**, failed to exhibit an extension of survival beyond the control animals. **19**, exhibiting better whole-cell efficacy, allowed survival of 2 of 7 and 1 of 7 animals at 31 days post-infection, at the 128 and 256 mg/kg doses, respectively. Diaryl urea **18**, displaying the most potent whole-cell efficacy to date in this family, when dosed at 128 mg/kg, enabled extension of survival of the infected mice beyond the control. Promisingly, dosing at 256 mg/kg of **18** demonstrated 4 of 7 animals surviving 31 days post-infection. While many factors contribute to the in vivo efficacy of a small molecule antimalarial, it is clear that the diaryl ureas' ability to inhibit the growth of *P. falciparum* in vitro was correlated with their efficacy in an in vivo *P. berghei* mouse model of infection.

It is interesting to note that phenoxy-substituted ureas have been previously reported as potent inhibitors of *Plasmodium* spp., based on solely in vitro data. These include the compound WR268961 (Supplementary data Fig. S1),[19] where the urea linkage is *para*- with respect to the oxygen of the diaryl ether unit instead of *ortho*- as in **13**–**25**. WR268961 abrogated parasite growth with an EC_{50} = 87 nM (W2 strain) and 460 nM (D6 strain), and modestly inhibited the *P. falciparum* cysteine protease plasmepsin 2 (PfPM2; IC_{50} = 17 μM). The diaryl ureas presented herein, however, do not appear to significantly target the plasmepsins as they equally inhibit the growth of both wild type and knockouts of PfPM1 through 4 (see Supplementary data Table S1), attained via a genetic disruption methodology in the Dd2 background.[20] GlaxoSmithKline disclosed the whole-cell efficacy of screening hit TCMDC-139010 (Supplementary data Fig. S1; XC_{50} = 930 nM vs 3D7 strain), but without information concerning the biochemical target.[4] We also reported in 2005 the preparation of triclosan-based 4′-ureas (Supplementary data Fig. S1) that were less potent against cultured parasite (EC_{50} values of ~100 μM) than **12**–**25**, but exhibited IC_{50} values of ~100 nM against purified PfFabI assayed in vitro.[14] More generally, the diaryl urea class of small molecules has been previously reported in the literature to exhibit potent efficacy against cultured parasites[21–25] but without definitive biological target identification. This chemotype was also found amongst hits against *P. falciparum* dihydroorotate dehydrogenase[26] that lacked whole-cell activity.

In order to more quantitatively compare the diaryl ureas reported herein with those disclosed in the literature as antimalarials, we leveraged a total of thirty-four diaryl ureas generated in our laboratories (Supplementary data Table 1) to generate a common features pharmacophore (Accelrys Discovery Studio 2.5.5) with five hydrophobic features and two hydrogen bond acceptors. Figure 1 depicts the top 3 active compounds that mapped to it. This pharmacophore is also able to select 23 compounds out of the 451 with the diaryl urea substructure present in the GSK library of antimalarial hits[4] (up to 100 conformers per molecule generated using the FAST algorithm in CAESAR). Slight variants on the diaryl urea scaffold, such as TCMDC-140251 (Supplementary data Fig. S1; XC_{50} = 190 nM against 3D7 strain) containing aryl and 1-indolinyl moieties, mapped well to the model. Interestingly, TCMDC-139010 exhibited a poor fit because it lacked three of the hydrophobic features. The pharmacophore differs from that constructed by Zhang et al. which contained 2–3 hydrophobic features and 2–3 hydrogen bond acceptors.[25] Not surprisingly, the 3 most active diaryl ureas from the paper by Zhang et al. failed to map to our pharmacophore; most likely, the 4-aminoquinaldine-derived diaryl ureas present different features than the triclosan-derived diaryl ureas. Distinctions in the arrangement of hydrophobic and hydrogen bonding features in the diaryl ureas from Zhang et al. and in this study may enable these molecules to target different proteins in *Plasmodium falciparum*. The pharmacophore developed in this study may be leveraged to search other databases (e.g. compound vendor libraries and approved drugs) to identify novel compounds with antimalarial activity.

A novel class of diaryl ureas derived initially from triclosan has been disclosed with regard to their potent in vitro efficacy against cultured drug-sensitive and drug-resistant strains of *P. falciparum*. Importantly, family members such as **18** demonstrate promising *in vivo* activity in a *P. berghei* mouse model of infection. Further investigation of the structure–activity relationship of these triclosan derivatives is necessary to further improve their antimalarial

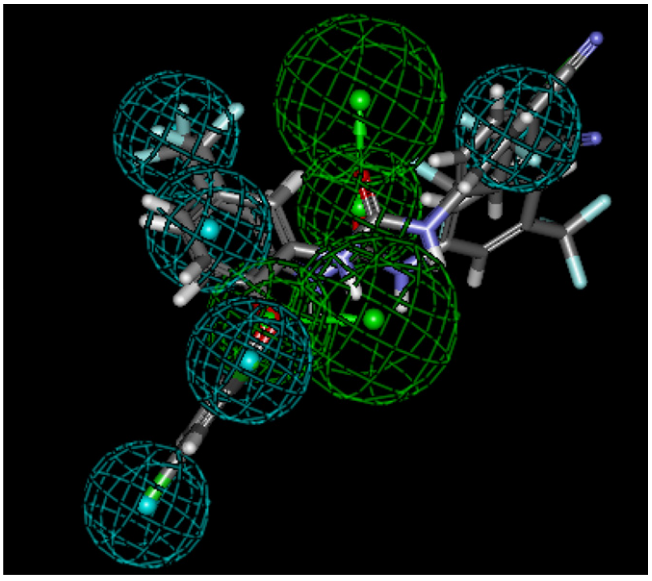


Figure 1. Common features pharmacophore for triclosan-inspired diaryl ureas showing the top 3 active molecules aligned to the 5 hydrophobic features (cyan) and 2 hydrogen bond acceptors (green).

activity, in addition to their pharmacokinetic profiles. Biological studies are also important to determine the molecular target(s) of these potent compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.022.

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