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Synthesis and structure–activity relationship of disubstituted benzamides as a novel class of antimalarial agents

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ABSTRACT

Malaria is a devastating world health problem. Using a compound library screening approach, we identified a novel series of disubstituted benzamide compounds with significant activity against malaria strains 3D7 and K1. These compounds represent a new antimalarial molecular scaffold exemplified by compound **1**, which demonstrated EC_{50} values of 60 and 430 nM against strains 3D7 and K1, respectively. Herein we report our findings on the efficient synthesis, structure–activity relationships, and biological activity of this new class of antimalarial agents.

Malaria is the world's most deadly parasitic disease. It is caused by a variety of plasmodial species, but only four species (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium* malariae) are implicated in human disease,[1] the most virulent being *P. falciparum*. Malaria is endemic to 109 nations, most located in Africa. This infectious disease disproportionately affects poor communities that have inadequate vector control; it remains a leading cause of death of young children.[2]

Despite the great world health impact of malaria, only a limited number of therapeutic agents are available. Furthermore, the lack of scaffold diversity poses a threat, should multi-drug–resistant strains emerge. The current antimalarial arsenal comprises five classes of compounds, the first two of which are chemically related: (1) 4-aminoquinolines (chloroquine, quinine), (2) 8-aminoquinolines (primaquine), (3) proguanil, (4) atovaquone, and (5) artemisinin derivatives.[2–4] The only combination regimen currently available is artemisinin-based.[1] *P. falciparum* is reported to be susceptible only to artemisinin-based drugs, and resistance can be expected to emerge with the increased use of artemisinin derivatives.[3–14] Therefore, new antimalarial agents are urgently needed. Here we report our studies on the efficient synthesis, structure–activity relationships, and biological activity of a new class of disubstituted benzamide analogs (analogs **1–3**, Fig. 1) which show promising antimalarial properties.

A recent screen of a small library of two distinctive classes of compounds, isoquinoline/phenethyl acetamide—type compounds[15] and disubstituted benzamide/aryl ether—type compounds (Fig. 1), revealed compounds **1** and **4** to represent a novel antimalarial scaffold class whose mechanism of action may differ from those of current agents, as it occupies a different chemical space. All compounds were tested against both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) parasites by following a known protocol.[5–8] The heat map corresponding to our antimalarial results and the cytotoxicity of these compounds to Raji, HepG2, HEK293, and BJ cells can be found in Figure 4 **a**nd Supplementary Table 1. As depicted in Fig. 4, there was a pattern of antimalarial activity in the order of compounds **1** > **2** > **3**, with compound **1** showing the greatest potency. The overall biological results obtained for the isoquinoline and phenyl acetamides were not optimal since they showed poor selectivity (cytotoxicity vs parasite). However, compound **34** stood out from the group, with EC_{50} values of 0.664 μM for 3D7and 0.822 μM for K1. Compound **34** showed low cytotoxicity, but it was not further derivatized, as its isomers **31**, **32**, and **33** showed weaker antimalarial properties. The inhibition potency of compounds **1** and **4** did not differ substantially between 3D7 (EC_{50}: 0.0604 and 0.1738 μM, respectively) and K1 (EC_{50}: 0.4377 and 0.9953 μM, respectively). Compounds **2** and **5** showed some promising results (EC_{50} <1.8 μM), but inhibited only the K1 parasite and displayed a modest therapeutic index (Fig. 4). With this information in hand, we proceeded to develop a focus library from hit compound **1**, the optimal lead compound for analysis of structure–activity relationship (SAR).

The benzamide scaffold of compound **1** is composed of an alkyl substituent and a benzylic substituent with an ether group. We selected the mono-ether linker carrying a carboxylic acid to serve as a handle for future pull-down experiments and to enhance the solubility properties of these disubstituted benzamides. When we assessed the length of the ether linker, it was found that either a short ether linker (2–3 carbons) or a longer linker (>5 carbons)

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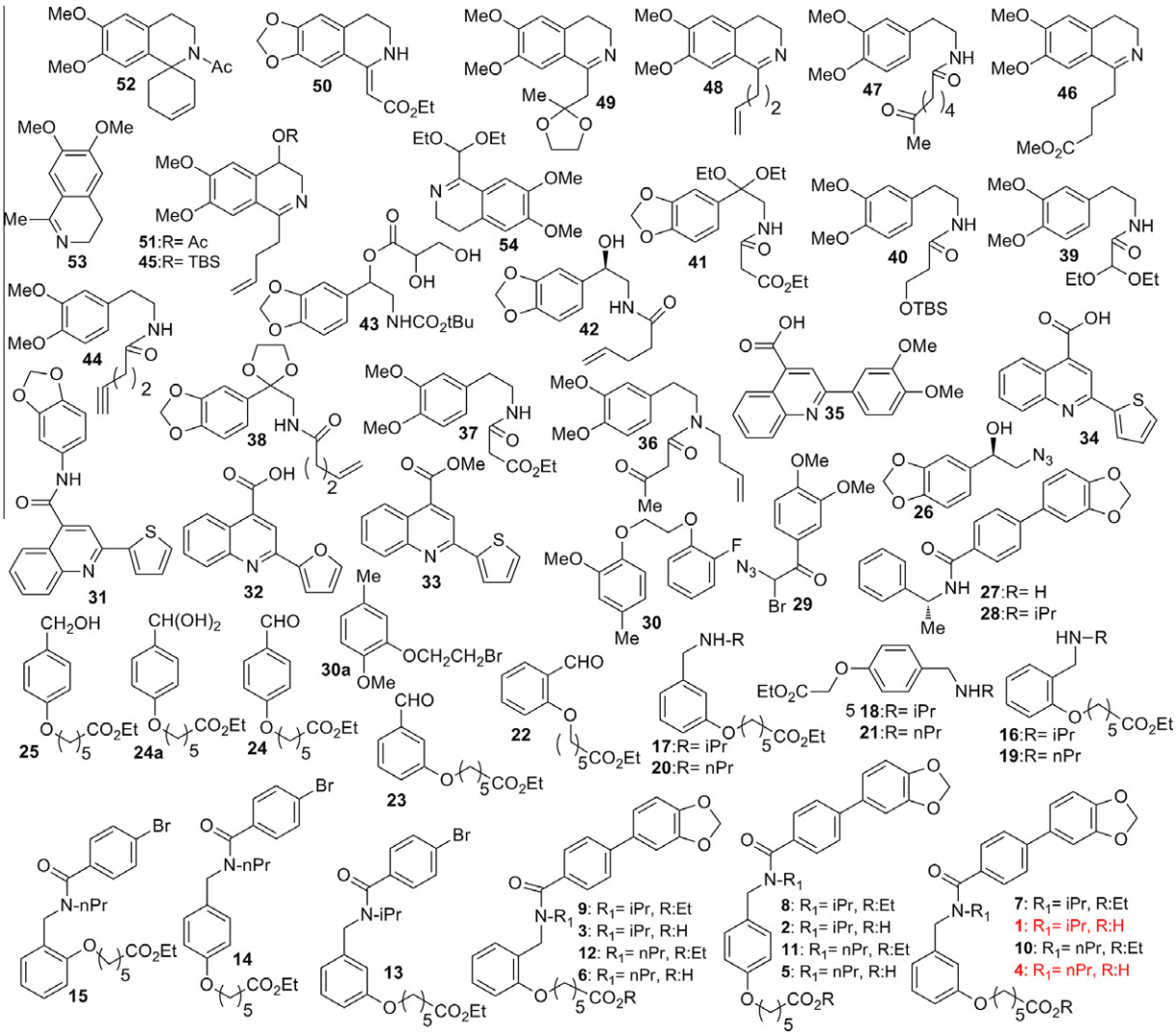


Figure 1. Focused library of disubstituted benzamides and isoquinoline/phenyl acetamides.

afforded unsatisfactory chemical yields so the linear 5-carbon ether linker was selected for further SAR studies due to good physical properties such as solubility. The alkyl group on the amide was also evaluated and the results showed that a bulky side chain was optimal. Amongst the studied alkyl groups, *n*-butyl, *n*-propyl, and isopropyl amine, the isopropyl group was the best candidate. As observed in our initial screen, compound **1** was approximately three times more potent against 3D7 malarial strain than its isomer **4**. The meta-linked ether position in these disubstituted benzamides also provided greater antimalarial activity than their corresponding *para*/*ortho* isomers (**1**, **2,** and **3**). Together, these disubstituted benzamide isomers provided an activity profile on two variables; the ether linker size, and the substitution pattern on the phenyl ring, but the role of the functional group on the benzamide ring remained to be investigated. The main objective was then to identify the steric and electronic factors of the aromatic ring that might be responsible for the observed antimalarial activity. Compound **23** was selected as the core to be derivatized.

Efficient synthesis of the disubstituted benzamide scaffold series was designed from commercially available *meta*-hydroxybenzaldehyde (the method could also be used with *para*/*ortho* isomers). Figure 2 outlines our key synthetic disconnections, which consisted of four synthetic maneuvers: Williamson etherification, reductive alkylation, amide formation, and Suzuki coupling, utilizing

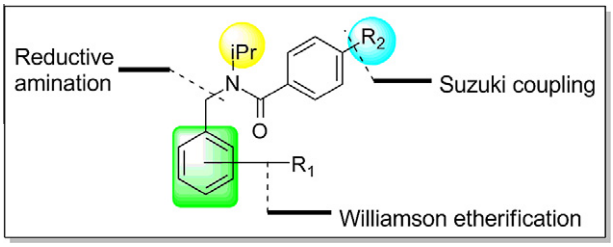
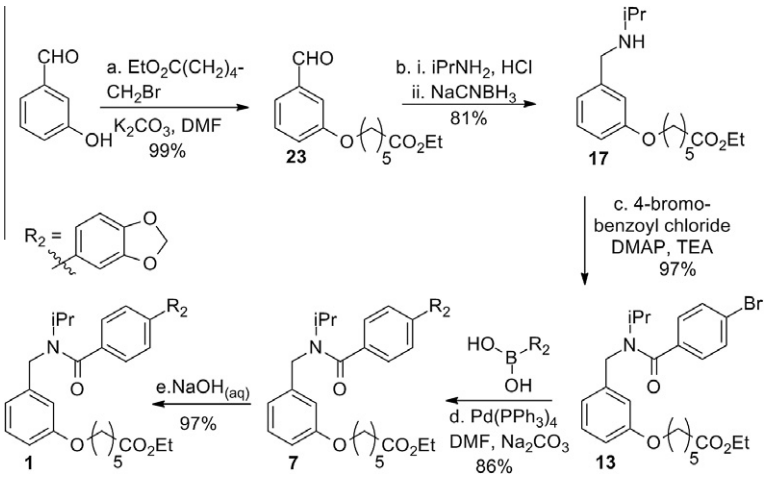


Figure 2. General synthetic disconnections.

minimal purification procedures. Parallel synthesis solution-phase chemistry was utilized, as it offers simple and rapid synthesis of this type of disubstituted benzamide.

Scheme 1 depicts our general synthetic approach. Synthesis commenced with *m*-hydroxybenzaldehyde, which was etherified using conventional Williamson etherification conditions[16] (1.5 equiv $BrCH_2CO_2Me$, 1.3 equiv K_2CO_3, DMF, 90 °C, 4 h, 98%), affording compound **23** almost quantitatively. The reaction mixture was filtered, washed, and used without further purification. The corresponding aldehyde **23** was treated with isopropyl amine in the presence of catalytic acid (4 N HCl/EtOAc, 23 °C, 1.5 h), and reducing agent ($NaCNBH_3$ or $NaBH_4$) was then directly added.[17]



Scheme 1. Efficient general synthesis of disubstituted benzamides **1–7**.

The crude reaction mixture was treated with 4-bromobenzoyl chloride, TEA, and catalytic DMAP in DCM to afford compound **13** in excellent yield. Compound **13** was dissolved in DMF, treated with Na_2CO_3 and tetrakis(triphenylphosphine)palladium, and degassed, and the corresponding boronic acid was added. The reaction mixture was heated to 50 °C for 8 h to afford compound **7** (86% yield after silica gel purification). Saponification of compound **7** with NaOH produced compound **1** (97% yield). Compound **13** was synthesized in multigram scale to allow development of its derivatives (shown in Fig. 3).

In our follow-up focused library (Fig. 3), a broad range of both electronic and steric factors affecting the benzamide aromatic ring of lead compound **1** were evaluated. The chemical yields of the Suzuki coupling reaction and hydrolysis ranged from modest to good. The Suzuki reactions that provided compounds **7c′–e′** and **7j′** were less efficient, and large quantities of starting materials were recovered; changes in the catalyst, solvent, and/or overall conditions did not improve reactivity. Hydrolysis reactions of the heterocycles (**7f′–h′**) were impeded by competing decomposition under basic conditions.

Heat maps of the antimalarial activity of compounds **1–54** and **1a–7p′** are shown with their cytotoxicity profiles in Figure 4. The results indicate that the nature of the benzamide aromatic ring influences its antimalarial properties; for example, **1a** inhibited the K1, but not the 3D7, strain. Addition of moderately small groups, halogens, or bulky groups to the benzamide ring, such as phenyl, naphthyl, heterocycles (furan, thiophene, oxadiazole, indoles and their corresponding sulfonyl counterparts) did not substantially affect the antimalarial profile (EC_{50}: >13 or 15 μM). Some compounds, such as **1v**, and **1l**, showed good activity (EC_{50}: 0.0175 and 0.15015 μM, respectively) against K1, but not against 3D7. Interestingly, compound **1l** differed from our lead compound **1** in the size of the ether ring size (dioxole vs dioxine), indicating the importance of spatial factors around the benzamide

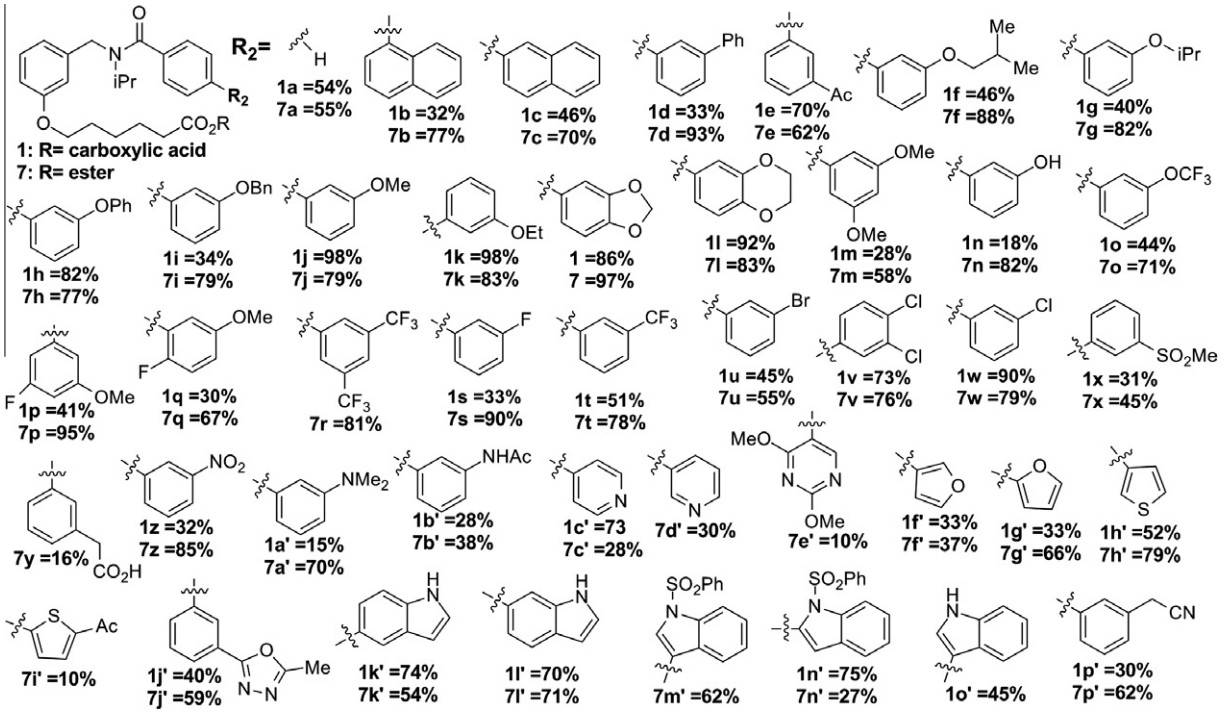


Figure 3. Chemical yields for Suzuki and saponification reactions of disubstituted benzamides **1–7p′**.

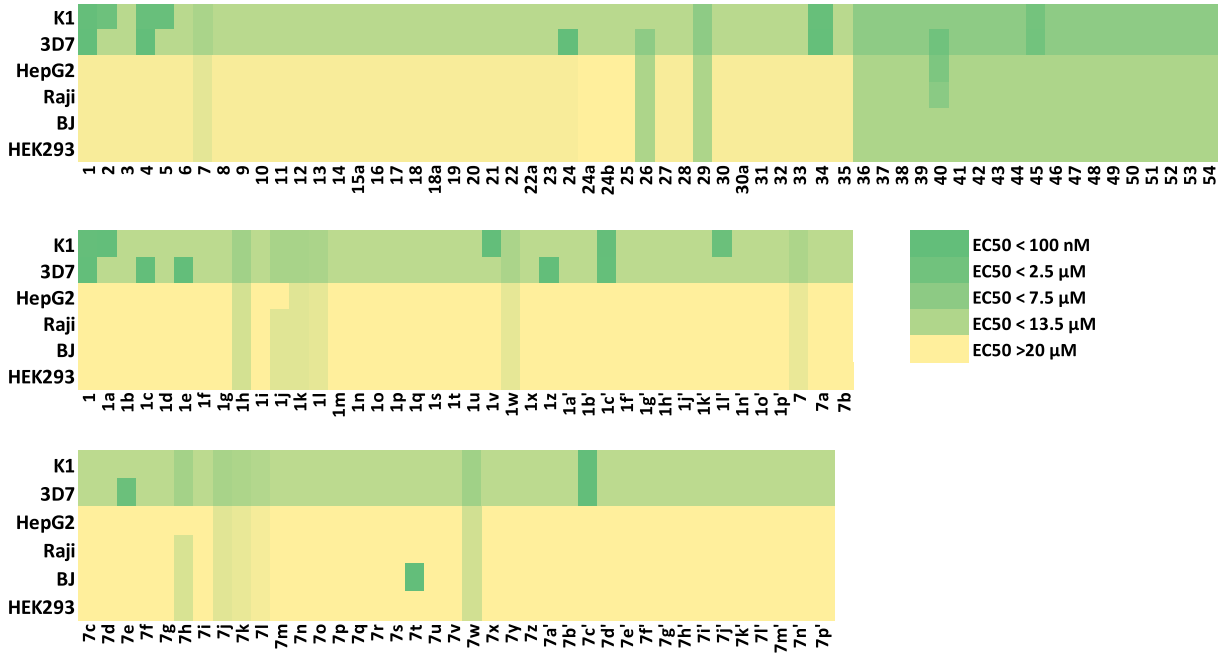


Figure 4. Antimalarial activity and cytotoxicity of compounds **1–54** and **1a–7p′**.

ring. Gratifyingly, this new compound library provided compound **7c′**, whose antimalarial activity was the most promising lead of all our compounds (EC_{50}: 0.800 nM) against both K1 and 3D7. This compound also showed no considerable cytotoxicity at the tested concentrations (EC_{50}: > 26.0417 µM). The 4-phenylpyridyl group (**7c′**) appeared to contribute to the antimalarial activity, as its 3-phenylpyridyl isomer (**7d′**) showed no significant activity.

In summary, an efficient synthetic route to a new class of disubstituted benzamides has been developed which show significant antimalarial properties. Due to the high potency of compounds **1**, **4**, and **7c′** against the 3D7 and K1 plasmodium strains, and their low toxicity in the tested mammalian cells, these compounds provide promising leads as antimalarial therapeutic agents. Our SAR studies indicated that the functional groups on the benzamide ring are, to some extent, responsible for the compounds′ antimalarial potency and selectivity. These results warrant future studies to elucidate the mechanism of action of this novel series of disubstituted benzamides.

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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. 05.124.

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