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3,5-Bis(benzylidene)-4-piperidones and related *N*-acyl analogs: A novel cluster of antimalarials targeting the liver stage of *Plasmodium falciparum*

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ABSTRACT

Drug resistance is a major challenge in antimalarial chemotherapy. In addition, a complete cure of malaria requires intervention at various stages in the development of the parasite within the host. There are only a few antimalarials that target the liver stage of the *Plasmodium* species which is an essential part of the life cycle of the malarial parasite. We report a series of antimalarial 3,5-bis(benzylidene)-4-piperidones and related *N*-acyl analogs **1**–**5**, a number of which exhibit potent in vitro growth-inhibiting properties towards drug-sensitive D6 and drug-resistant C235 strains of *Plasmodium falciparum* as well as inhibiting the liver stage development of the malarial life cycle. The compounds **2b** (IC_{50}: 165 ng/mL), **3b** (IC_{50}: 186 ng/mL), **5c** (IC_{50}: 159 ng/mL) and **5d** (IC_{50}: 93.5 ng/mL) emerged as lead molecules that inhibit liver stage *Plasmodium berghei* and are significantly more potent than chloroquine (IC_{50}: >2000 ng/mL) and mefloquine (IC_{50}: >2000 ng/mL) in this screen. All the compounds that showed potent inhibitory activity against the *P. berghei* liver stage were nontoxic to human HepG2 liver cells (IC_{50}: >2000 ng/mL). The compounds **5a** and **5b** exhibit comparable metabolic stability as chloroquine and mefloquine in human plasma and the most potent compound **5d** demonstrated suitable permeability characteristics using the MDCK monolayer. These results emphasize the value of 3,5-bis(benzylidene)-4-piperidones as novel antimalarials for further drug development.

1. Introduction

Malaria is a major health problem. Current drug treatment is hampered by the emergence of drug-resistant strains of the malarial parasite that has put an enormous burden on public health. In 2012 no less than 219 million cases of this disease were recorded along with approximately 660,000 deaths.[1] This problem is caused by various members of the *Plasmodium* species. In particular, *Plasmodium falciparum* is the most pathogenic and lethal strain of malaria, which has developed resistance against chloroquine and other antimalarials. The malarial parasite has a complex life cycle and in order to eradicate the disease, every developmental stage of the parasite should be targeted for treatment. Human malarial infection starts with a one-time asymptomatic liver stage followed by a cyclic symptomatic blood-stage. Thus, targeting liver-stage development of the parasite offers advantages for two reasons. First, blocking the clinically silent liver stage leads to the arrest of parasitic transmission and second, relatively small numbers of parasites are present at these stages which are easy to treat compared to the blood-stage. Most currently available medications target the blood stage except a few drugs, for example, primaquine and atovaquone which target the liver stage of the parasite. Consequently the discoveries of novel compounds that are effective against drug-resistant (DR) strains of malarial parasites and target liver stages of the parasitic development are urgently required.

A recent study from our laboratory revealed that a cytotoxic agent **1a** displays a positive tropism for red blood cells in which the malarial parasite spends some of its life cycle.[2] Evaluation of **1a** against the drug-sensitive D6 and drug-resistant C235 strains of *P. falciparum* revealed the compound to have IC_{50} values of 366 and 1210 ng/mL, respectively.[2] Compounds in series **1**–**5** have been shown to possess potent cytotoxic properties towards a number of human tumour cell lines.[3] In view of the recent observation that a number of anticancer drugs display potent antimalarial properties,[4,5] our interest was directed towards investigating the antimalarial properties of other analogs of **1a**. Furthermore, the compounds in series **1**–**5** were designed as thiol alkylators,[3] and

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in part at least, the mode of action of certain antimalarials is via interactions with critical cellular thiols in the parasite.[6,7]

The objectives of the current investigation are fourfold. First, evaluation of the antimalarial properties of structurally related analogs of **1a** with the aim of obtaining lead compounds for further development. Second, an important consideration is to find if these compounds are effective against drug-resistant (DR) strains of *P. falciparum*. Third, do these compounds target liver stage development of the parasites? Fourth, the evaluation of permeability characteristics and metabolic stabilities of representative promising lead antimalarial agents.

2. Results

The synthesis of the compounds in series **1**–**5** were undertaken by a literature procedure[3] which is outlined in Scheme 1. The olefinic groups in series **1**–**5** are considered to be the sites of interactions with cellular thiols. The *N*-acyl groups in series **1**–**4** allow the possibility of hydrogen and van der Waals bonds to form between these groups and complementary sites in biomacromolecules. In addition, all of the compounds are capable of forming ion pairs between the nitrogen atoms in the side chain (series **1**–**4**) or the piperidine ring (series **5**) and cellular constituents.

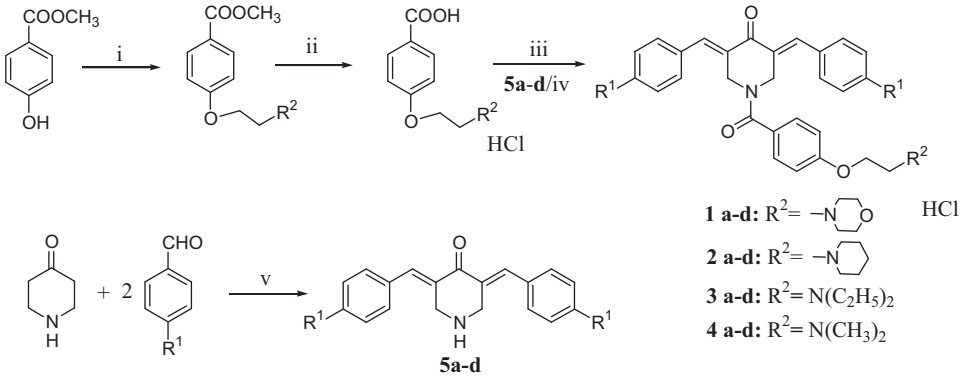
All of the compounds in series **1**–**5** were evaluated against the D6 (drug sensitive) and C235 (drug resistant) strains of *P. falciparum*. In addition, the compounds that displayed promising activities against C235 stains of *P. falciparum* were examined for activity against the liver stage of malaria by testing for inhibition of liver stage development and also for cytotoxicity against human HepG2 liver cells (the ILSDA and MTT cytotoxicity assays are described in the Section 5). These data are presented in Table 1. Two promising compounds of the series **5** were examined for their permeability and stability characteristics and these results are summarized in Table 2.

3. Discussion

The compounds in series **1**–**5** were evaluated against the drug sensitive D6 strain of *P. falciparum* and the results are presented in Table 1. A compound displaying an IC_{50} value of less than 2000 ng/mL was considered an active compound. All of the analogs have IC_{50} values which are below 2000 ng/mL except for **2a**, **3a** and **4c**. Thus 85% of the compounds in series **1**–**5** have antimalarial properties. These molecules are structurally divergent from existing therapies for treating malaria. The most potent compounds with IC_{50} figures of 250 ng/mL or less are **2b**, **3b** and **5a**–**d**. The 4-piperidone with the lowest IC_{50} value (37.5 ng/mL) is **5b** with one-fifth of the potency of both chloroquine and mefloquine. Thus, in the future the following structural modifications should be undertaken. First, the preparation of analogs of **2b**, **3b** and **5b** should be made in which the nitro substituents are placed in the 2 and 3 positions of the aryl rings along with compounds containing multiple nitro groups. Aryl nitro groups can be converted into reactive metabolites which can induce oxidative stress.[8] These reactions may well be important contributors to the observed antimalarial activity. On the other hand, the nitro group and related metabolites may cause unwanted toxicity although in the present case compounds containing the nitro groups, that is, **1b**, **2b**, **3b**, **4b** and **5b**, have IC_{50} values in excess of 2000 ng/mL towards HepG2 liver cells vide infra. The nitro group is strongly electron-withdrawing, for example, the Hammett σ_p constant is 0.78.[9] Hence, the nitro group should be replaced by other electron-withdrawing substituents such as trifluoromethyl and cyano with σ_p values of 0.54 and 0.66, respectively.[10] Furthermore, the 4-nitrophenyl group should be replaced by a 4-pyridyl ring since in addition to eliminating the nitro group, the heterocycle is more electron-withdrawing; the σ_p figures of the 4-nitrophenyl and 4-pyridyl rings being 0.26 and 0.44, respectively.[11]

In addition to potency towards drug sensitive parasites, an important feature of candidate antimalarials is their ability to inhibit the growth of drug resistant strains of *Plasmodium*. Accordingly all of the compounds in series **1**–**5** were evaluated against the drug-resistant C235 strain of *P. falciparum* and the results are presented in Table 1. For 70% of these compounds, the IC_{50} values are less than 2000 ng/mL. The average IC_{50} values of the compounds in series **1**–**5** are >1435, >935, >753, >1234 and 154 ng/mL, respectively, indicating the need for analog development of series **5** in particular. The most potent compound is **5b** with a lower IC_{50} value than **5a**, **c**, and **d** and chloroquine. Among the 4-nitro analogs in series **1**–**5**, two structure–activity relationships are revealed. First, the placement of bulky acyl groups onto the piperidine ring of **5b** led to **1b**, **2b**, **3b** and **4b** with reduced potencies towards C235 parasites. Second, among the amides containing a 4-nitro aryl substituent namely **1b**, **2b**, **3b** and **4b**, the diethylamino analog **3b** has the preferred basic group.

Ideally promising antimalarials should display the same potencies towards drug-sensitive (DS) and drug-resistant (DR) parasites, that is, there should be no evidence of drug resistance to the compounds. Consequently R/S ratios were computed and are presented in Table 1. The compounds which are active towards both DS and DR strains of *P. falciparum* are **1a**, **b**, **2b**–**d**, **3b**–**d**, **4a** and **b** and **5a**–**d**. With the exception of **1a**, **c**, and **d**, these compounds do not require statistically significant greater concentrations to inhibit the growth of DR than DS parasites. Thus, many of the compounds



Scheme 1. Synthesis of series **1**–**5**. The aryl substituents are as follows: (a) R^1 = Cl; (b) R^1 = NO_2; (c) R^1 = CH_3; (d) R^1 = H. The following reagents were used in the syntheses namely (i) $ClCH_2CH_2R_2{\cdot}HCl/K_2CO_3$; (ii) NaOH/HCl; (iii) $SOCl_2$; (iv) Et_3N; (v) HCl/CH_3COOH.