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Design, synthesis and in vitro antimalarial activity of an acylhydrazone library

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Abstract—A library of acylhydrazone iron chelators was synthesized and tested for its ability to inhibit the growth of a chloroquine-resistant strain of *Plasmodium falciparum*. Some of these new compounds are significantly more active than desferrioxamine **DFO**, the iron chelator in widespread clinical use and also than the most effective chelators.

Almost one-half of the world's population is exposed to the burden of malaria, a disease which is responsible for the death of about 2 million people every year. The spread of multidrug-resistant *Plasmodium falciparum* has highlighted the urgent need to discover new antimalarial drugs, preferably those affordable to developing countries where malaria is prevalent.[1,2]

Iron was identified as an essential nutrient for the development of the parasite, many enzymes of the plasmodial metabolic pathways (δ-aminolevulinate synthase, responsible for haem's de novo synthesis,[3] or ribonucleotide reductase, involved in DNA synthesis[4]) depend on the presence of this element. For this reason, iron chelators gained a respectable, although still undeveloped, place among compounds presenting antimalarial activity. Several possible sources for iron acquisition have been postulated: host transferrin in the plasma, host erythrocyte ferritin or, even, host haemoglobin.[5]

Several classes of chelators have been shown to suppress the growth of *P. falciparum* in erythrocytes in vitro: hydroxamate siderophores (**DFO** for instance, Fig. 1) and derivatives, catecholamide and catecholate siderophores, α-ketohydroxypyridinones, dihydroxycoumarins, acylhydrazones and aminophenols.[5] The problems with these classes of compounds are side effects due to toxicity.[6]

Nevertheless, **DFO** has been extensively used for the treatment of Fe overload disease,[7] has a detectable antimalarial activity in humans,[8,9] but has not found a role in the clinical treatment of malaria, probably due to short plasma half-life, lack of oral activity and high cost.[7]

The antimalarial activity of **DFO** prompted several authors[10,11] to study three aroylhydrazone Fe chelators, salicylaldehyde isonicotinoyl hydrazone (SIH, **1**), 2-hydroxy-1-naphthylaldehyde *m*-fluorobenzoyl hydrazone (HNFBH, **2**) and pyridoxal isonicotinoyl hydrazone (PIH, **3**) (Fig. 1). As these compounds were orally effective and inexpensive, the results were promising, the hydrazones **1** and **2** providing the best results. An important prerequisite of an iron-chelating drug as an antimalarial is a high affinity for iron. The affinity constant of acylhydrazones[12] for iron(III) is about 10^{28}.

Another study pointed out the interest of those compounds together with their thiosemicarbazone analogues.[13]

Some derivatives of these compounds are proteinase inhibitors with antiparasitic activity against *Trypanosoma brucei*[14] and Nifurtimox, a furyl hydrazone, has been commercialised for the treatment of Chaga's disease.

Keywords: Antimalarial; Hydrazone; Library; Iron chelator.
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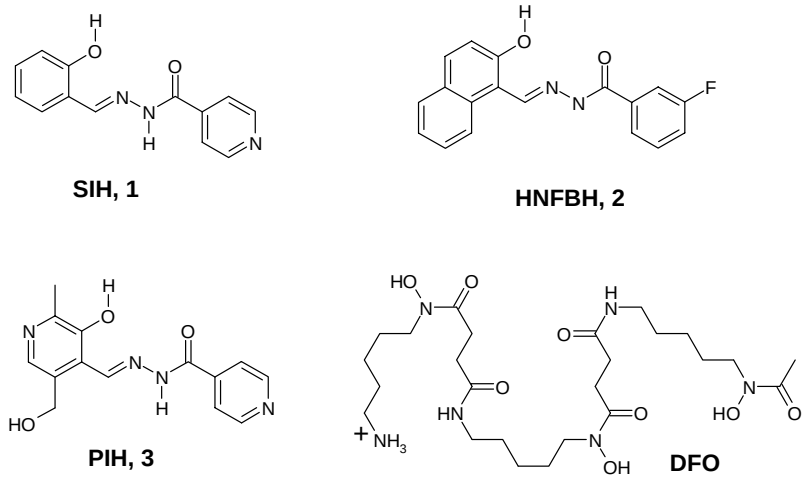
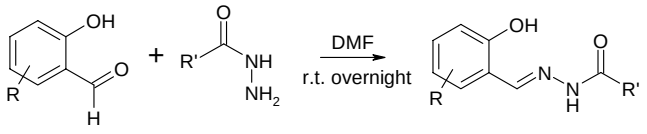


Figure 1. Structures of **DFO** and acylhydrazones **1**, **2** and **3**.

A library of 156 acylhydrazones was designed in order to evaluate the potential of this class of compounds as antimalarials and to study more in detail the structure–activity relationships. The library's diversity was ensured by the large number of commercially available aldehydes and hydrazides. This allowed the study of SAR rules, influence of the compounds' accumulation in the parasite food vacuole and of the lipophilicity. To allow a comparison with previous studies, the library also contained compound **1**. As one of the major problems with malaria is the development of resistant strains, our efforts were focused towards FcB1, a chloroquine (CQ)-resistant strain.

The acylhydrazones were synthesized in deepwell plates at 10 μmol scale according to Scheme 1. The purity was assessed by HPLC and all the mass spectra were consistent with the anticipated product structure. Out of the total 156 compounds of the library, 153 were obtained with a purity over 85% and were submitted to biological activity evaluation (Fig. 2). The experimental procedure



Scheme 1. Synthesis of acylhydrazones.

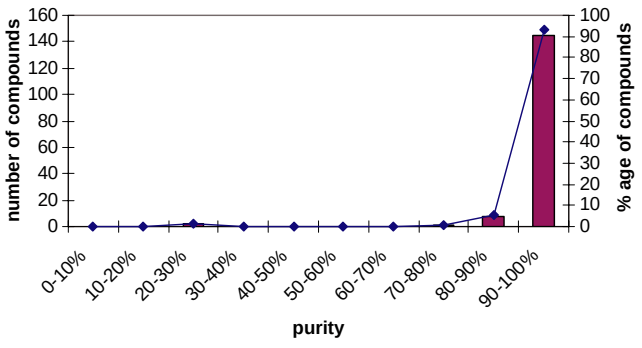


Figure 2. Quality control of the library.

is included in the reference section, together with the quality control (QC) results of the most active compounds.[15]

Antimalarial activity and cytotoxicity. The crude compounds were tested for their ability to inhibit parasite growth (CQ-resistant strain FcB1, IC_{50} CQ = 126 nM)[16] at 1 μM (Table 1). Compounds displaying inhibition of parasite growth by more than 50% were directly submitted to further pharmacological characterisation (IC_{50}, Table 2) as their purity was over 95%.

Most active compounds were submitted to a cytotoxicity test (CC_{50}) on a human diploid embryonic lung cell line (MRC-5) using the colorimetric MTT assay[17] (Table 2).

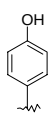
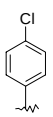
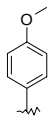
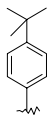
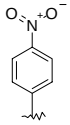
In vitro inhibition of β-hematin formation. Compounds were tested for their ability to inhibit formation of β-hematin (the synthetic equivalent of hemozoin) induced by 1-monooleoyl glycerol (MOG)[18,19] (Table 2).

Introduction of a variety of substituents either on the aldehyde or on the hydrazone moiety provided compounds with modest or low inhibition percentage of parasite growth at 1 μM (Table 1), except for 11 compounds (compounds **2**–**12**). Reference compound **1** is representative of the results with 22% of growth inhibition.

Very low inhibition percentages were obtained in the case of methylhydrazide (0–34%) or benzylhydrazide (0–45%) whatever the structure of aldehyde partner. The introduction of polar or ionizable groups led to no improvement, either on hydrazide partner (pyridine, phenol substituent), or on the aldehyde moiety (3, 4 or 5-hydroxy substituent). The highest inhibition was obtained for aromatic partners, substituted by *tert*-butyl, chloro, nitro, methyl or methoxy groups. The results suggested the importance of rather hydrophobic or bulky substituents.

Compounds displaying inhibition of parasite growth by more than 50% were submitted to an IC_{50} evaluation

Table 1. Antimalarial activity on CQ-resistant strain FcB1 (inhibition of parasite growth at 1 μM)

R	R′: 											CH3–
H	21%	27%	**1**: 22%	35%	26%	13%	11%	25%	14%	43%	4%	0%
Naphthyl	42%	26%	28%	36%	31%	14%	5%	24%	30%	35%	48%	11%
3-OH	20%	19%	11%	30%	16%	29%	20%	13%	21%	41%	24%	29%
3-OMe	41%	30%	43%	10%	32%	16%	16%	36%	37%	39%	32%	33%
4-OH	13%	0%	nd[a]	14%	23%	13%	15%	16%	29%	37%	18%	nd[a]
4-OMe	45%	41%	35%	32%	38%	24%	14%	8%	9%	43%	45%	4%
4-NEt_2	23%	nd[a]	18%	**2**: **60**%	2%	37%	12%	45%	39%	**3**: **73**%	**4**: **69**%	34%
5-OH	21%	4%	26%	0%	7%	19%	4%	25%	39%	38%	28%	25%
5-OMe	33%	45%	34%	38%	24%	24%	20%	24%	18%	47%	14%	3%
5-Me	37%	19%	8%	49%	33%	23%	24%	**5**: **52**%	28%	**6**: **65**%	46%	32%
5-*t*Bu	14%	14%	40%	**7**: **59**%	32%	**8**: **52**%	31%	**9**: **69**%	**10**: **53**%	48%	**11**: **81**%	32%
5-Br	35%	33%	29%	47%	21%	38%	10%	36%	29%	**12**: **52**%	17%	17%
5-NO_2	16%	0%	15%	21%	19%	21%	4%	31%	26%	36%	31%	22%

[a] Not determined.

Table 2. Biological activity of the 11 most active compounds

	R	R′	Antimalarial activity IC_{50}[a] (μM)	CC_{50}[a] (μM) (MRC5 cells)	Inhibition of β-hematin formation IC_{50}[a] (μM)	VAR × E+3
CQ	—	—	0.126 ± 0.005	50 ± 4	60 ± 10	54
2	4-NEt_2	Phenyl	1.1 ± 0.3	1.1 ± 0.1	50 ≪ 100	68
3	4-NEt_2	4-*tert*-Butyl-phenyl	0.9 ± 0.1	1.1 ± 0.1	>100	120
4	4-NEt_2	4-Nitro-phenyl	1.0 ± 0.1	2.5 ± 0.7	>100	86
5	5-Me	4-Chloro-phenyl	1.9 ± 0.1	>100	50 ≪ 100	59
6	5-Me	4-*tert*-Butyl-phenyl	2.2 ± 0.2	16.3 ± 2.6	50 ≪ 100	60
7	5-*t*Bu	Phenyl	1.3 ± 0.2	2.6 ± 0.8	>100	71
8	5-*t*Bu	2-Thienyl	1.6 ± 0.2	12 ± 4	50 ≪ 100	60
9	5-*t*Bu	4-Chloro-phenyl	2.9 ± 0.6	3.6 ± 0.8	>100	59
10	5-*t*Bu	4-Methoxy-phenyl	2.3 ± 0.3	>100	>100	60
11	5-*t*Bu	4-Nitro-phenyl	1.6 ± 0.1	1.1 ± 0.2	50 ≪ 100	57
12	5-Br	4-*tert*-Butyl-phenyl	1.7 ± 0.4	>100	>100	46

[a] Mean IC_{50} ± SD values for two to three independent experiments are shown.

(Table 2). Highest inhibition was obtained for 2-hydroxy-4-diethylaminobenzaldehyde 4-*tert*-butyl-benzoyl hydrazone **3** with an IC_{50} of 900 nM. But the range of IC_{50} is not large enough to draw some rules.

The partition coefficient log *D* at pH 7.4 was evaluated in silico[20] to determine the influence of lipophilicity on the antimalarial activity. As expected, the 11 most active compounds provided the highest values, between 3.5 and 5.2. This direct correlation of a compound lipophilicity with its antimalarial activity has already been experimentally demonstrated in the case of reversed siderophores.[11,21]

Host haemoglobin is degraded in the food vacuole of the parasite, and haem liberated in this process is polymerized to hemozoin.

In order to evaluate the ability of the component to accumulate in the parasite food vacuole, in silico calculations of vacuolar accumulation ratios (VAR) were carried out based on a weak-base model.[20,22] A wide range of accumulation ratios were obtained (from 640 to 3.6×10^9, see supplementary materials). As expected, compounds with phenol groups as substituents provided better VAR values. Compounds presenting the highest activity accumulate in the same range as CQ.

CQ inhibits the polymerization process, thus, leading to poisoning of the parasite. **DFO** seems to have an opposite behaviour.[24] We have tested the ability of the compounds to inhibit this process of detoxification of haem (Table 2) to get some information on the mechanism of action. Even if the compounds are known as inhibitors via direct coordination of iron(III), the mechanism will be different from CQ which inhibits the crystallisation of the haem to form the hemozoin (and its equivalent β-hematin) through π–π stacking. The most active compounds are equivalent or less potent inhibitors than CQ and the best inhibitors (2 compounds provided an IC_{50} of about 20 μM) are totally inactive on parasite growth (see supplementary materials). The inhibition of this process does not seem to be the base of the antimalarial activity of this class of compounds. It can be notified that no significant red blood cells (RBC) haemolysis was recorded after 48 h of incubation with the more active compound at a concentration totally inhibiting the parasite growth (20 μM), as well as no alteration of the RBC morphology was observed by phase contrast microscopy suggesting that the inhibition of growth

could not be attributed to indirect effects due to modifications of the host cell membrane.

The average cytotoxicities of acylhydrazones upon MRC-5 cells extended from 1 μM to more than 100 μM (Table 2). Three compounds showed a reproducible lack of toxicity at 100 μM: 2-hydroxy-5-methyl-benzaldehyde 4-chloro-benzoyl hydrazone **5**, 2-hydroxy-5-*tert*-butyl benzaldehyde 4-methoxy-benzoyl hydrazone **10** and 2-hydroxy-5-bromobenzaldehyde 4-*tert*-butyl-benzoyl hydrazone **12**. All the other compounds provided a selectivity index (ratio CC_{50}/IC_{50}) between 1 and 16, too low for a potential development as drug candidates.

A library of 153 acylhydrazones was synthesized with high purity. Though the activity is in the micromolar range against *P. falciparum* growth, these compounds have the highest antimalarial activity of this class of iron chelators. As three of the compounds present no detectable toxicity, further studies of their biological activity are in progress. From a mechanistic point of view, as ribonucleotide reductase is a target for HNFBH **2**,[25] an investigation of the potency of this library on this enzyme's activity is also intended.

Acknowledgments

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Supplementary data

QC results, inhibition of haem polymerization, VAR in silico calculations for the entire library. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.09.058.

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15. In deepwell plates, 100 μL of aldehyde (0.1 M in DMF) was added to 50 μL of hydrazide (0.2 M in DMF). The mixture was stirred at room temperature overnight. Five microlitres was removed for QC analysis. Reaction plates and QC plates were evaporated in a Genevac EZ2 evaporator. QC aliquots were solubilized with 5 μL DMF and 200 μL CH_3CN before HPLC and MALDI/TOF analysis.

Compound	t_R (min)	Purity (%)	*m/z* $[M+H]^+$ found (calcd)
2	5.29	97	312.2 (311.16)
3	7.18	96	368.2 (367.23)
4	5.90	>99	357.1 (356.15)
5	7.60	99	289.1 (288.07)
6	8.56	>99	311.2 (310.17)
7	8.14	>99	297.1 (296.15)
8	8.05	>99	303.1 (302.11)
9	8.74	>99	331.1 (330.11)
10	8.20	>99	327.1 (326.16)
11	8.41	>99	342.1 (341.14)
12	9.05	95	375.1–377.4 (374.06–376.06)

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20. Values of Log*D* and Log*P* at pH 5.0 and pH 7.4 were calculated using ACD/pK_a DB software from Advanced Chemistry Development Inc., Toronto, Canada.
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22. Vacuolar accumulation ratios (VARs) were calculated from the equation below:

$$\mathrm{VAR} = \frac{1 + \sum_{n=1}^{4}\sum_{i=1}^{n} 10^{\mathrm{p}K_{\mathrm{ai}}\text{-}\mathrm{pH_v}}}{1 + \sum_{n=1}^{4}\sum_{i=1}^{n} 10^{\mathrm{p}K_{\mathrm{ai}}\text{-}\mathrm{pH_0}}},$$

where pH_v = pH inside the vacuole (assumed to be pH 5.0) pH_0 = pH externally (assumed to be pH 7.4). This equation proceeds from a derivation of the Henderson–Hasselbach equation, based on predicted values of drug pK_a accord-

ing to previous works of Hawley et al.[23] Values of pK_a were calculated using ACD/pK_a DB software from Advanced Chemistry Development Inc., Toronto, Canada.

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