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Structure-activity relationships of novel anti-malarial agents: 1. arylacyl and cyclohexylacyl derivatives of 5-amino-2-tolylacetylaminobenzophenone

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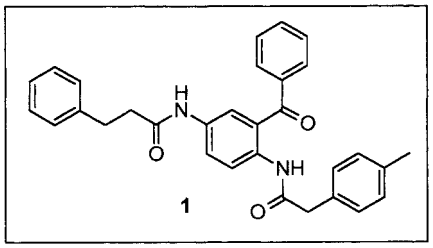
We have identified compound **1** as a lead structure of a novel class of anti-malarial agents. Here, we report on our continuing studies towards the establishment of structure-activity relationships by varying the terminal phenyl moiety and the alkyl linker of the acyl substituent at the 5-amino function of the benzophenone core structure. Most of the derivatives of our lead structure **1** essentially display the same anti-plasmodial activity as the lead.

1. Introduction

Malaria is one of the most threatening tropical diseases causing between 1.5 and 2.7 million fatal cases per year. These occur particularly among children and women, primarily in Africa [1]. Nearly all fatal cases are caused by *Plasmodium falciparum*, the causative agent of *Malaria tropica*. This is largely due to the widespread emergence of *Plasmodium falciparum* strains which are resistant to presently available drugs. Therefore, there is an urgent need for new agents active against multi-resistant *Plasmodium* strains [1].

By random screen of a compound library we have identified compound **1** as a lead structure for a novel class of anti-malarial agents [2]. We have demonstrated that all three substituents at the central benzene core are essential for anti-plasmodial activity. Here, we continued our studies towards the establishment of structure-activity relationships by varying the acyl substituent at the 5-amino function of the benzophenone core structure.

The preparation of the structural variants **2**–**5** of our lead structure **1** will be described elsewhere [3].



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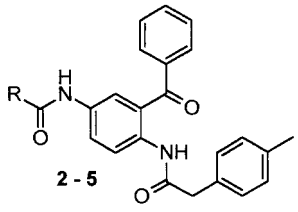

2 - 5

R:

2a

2b

2c

2d

2e

R:

3a

3b

3c

3d

2. Investigations, results and discussion

Compounds were evaluated for their inhibitory activity against intraerythrocytic forms of multi-resistant *P. falciparum* strain Dd2 using a semi-automated microdilution assay as described [4–6]. The growth of the parasites was monitored through the incorporation of tritium-labeled hypoxanthine. For initial assessment of the anti-plasmodial activity of the test compounds, growth inhibition (expressed as % inhibition in comparison to an untreated control) was determined at test compound concentrations of 100 μM, 10 μM and 1.0 μM (Table). Since no improvement in activity compared to the lead structure could be recorded, no IC_{50}-values were determined.

In the first series of derivatives (**2a**–**e**) of the lead structure **1**, the distance between the terminal phenyl moiety and the benzophenone core structure was either shortened (**2a, b**) or enlarged (**2c**–**e**). Apart from the phenylacetic

R:

4a

4b

4c

4d

4e

4f

4g

4h

R:

5a

5b

5c

Table: Anti-plasmodial activity of compounds 1–5

Compd.	% Inhibition[a] at		
	100 μM	10 μM	1.0 μM
1[b]	94	91	0
2a	96	77	0
2b	94	0	0
2c	97	95	0
2d	97	94	0
2e	96	92	0
3a	98	87	0
3b	98	93	0
3c	98	93	9
3d	98	99	0
4a	97	91	0
4b	97	93	0
4c	96	93	0
4d	96	0	0
4e	86	0	0
4f	99	38	0
4g	96	35	0
4h	98	99	0
5a	93	65	0
5b	61	39	0
5c	98	0	0

[a] values are estimated to be correct within ± 30%
[b] IC_{50} (**1**) = 2.7 μM; IC_{50} (chloroquine) = 0.17 μM; IC_{50} (quinine) = 0.38 μM; IC_{50} (mefloquine) = 0.057 μM; IC_{50} (pyrimethamine) = 2.5 μM [2]

acid derivative **2b** which is considerably less active than the lead structure **1**, no significant effect of this type of structural modification on the anti-plasmodial activity could be recorded. The same holds true for the replacement of the phenyl residue by a cyclohexyl moiety as done in the compound series **3a**–**d**. With the next series of compounds (**4a**–**h**) structural variations were concentrated on the alkyl linker between the terminal phenyl and the amide moiety connecting this substituent to the benzophenone core. Again, most of the derivatives essentially displayed the same anti-plasmodial activity as the lead structure **1**. Considerably less active than the lead are the 4′-nitro substituted thioether derivative **4d** and the amide derivative **4e**. In case it had been active, this amide derivative would have been of particular value since it would have opened an easy access to a variety of structural variants prepared from amino acids and benzoic acid derivatives. In the last series of compounds (**5a**–**c**) of this study, the phenyl residue of **2a** was replaced by more bulky aryl residues. Again, these derivatives displayed no improved activity.

In summary, most of the derivatives of our lead structure **1** display essentially the same anti-plasmodial acitvity as the lead. No improvement in activity could be obtained by the structural modifications investigated in this study. Therefore, work on this particular type of structural variants of our lead compounds was discontinued, as meanwhile different modifications yielded much more potent anti-malarials as will be reported in due course.

3. Experimental

Compounds were tested by a semi-automated microdilution assay against intraerythrocytic forms of *P. falciparum* [4]. The *P. falciparum* strain Dd2 was cultivated by a modification of the method described by Trager and Jensen [5]. The culture medium consisted of RPMI 1640 supplemented with 10% human type 0^+ serum and 25 mM HEPES. Human type 0^+ erythrocytes served as host cells. The cultures were kept at 37 °C in an atmosphere of 5% O_2, 3% CO_2, and 92% N_2.

Drug testing was carried out in 96-well microtiter plates. The compounds were dissolved in DMSO (10 mM) and prediluted in complete culture medium. Infected erythrocytes (200 μl per well, with 2% hematocrit and 0.4% parasitemia, predominantly ring-stage parasites) were incubated in duplicate with a serial dilution of the drugs for 48 h [6]. After the addition of 0.8 μCi [^{3}H]-hypoxanthine in 50 μl medium per well, the plates were further incubated for 24 h. Cells were collected on glass fiber filters with a cell harvester (Micromate 196, Packard) and incorporated radioactivity was measured using a β-counter (Matrix 9600, Packard).

References

1 Wirth, D. F.: Ann. Rep. Med. Chem. **34**, 349 (1999)
2 Wiesner, J.; Wißner, P.; Dahse, H.-M.; Jomaa, H.; Schlitzer, M.: Bioorg. Med. Chem., in press
3 Mitsch, A.; Wißner, P.; Sattler, I.; Schlitzer, M.: Arch. Pharm. Pharm. Med. Chem., submitted for publication
4 Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D.: Antimicrob. Agents Chermother. **16**, 710 (1979)
5 Trager, W.; Jensen, J. B.: Science **193**, 673 (1976)
6 Ancelin, M. L.; Calas, M., Bompart, J.; Cordina, G.; Martin, D.; Bari, M. B.; Jei, T.; Druilhe, P.; Vial, H. J.: Blood **91**, 1 (1998)

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