Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 785–792

BIOORGANIC & MEDICINAL CHEMISTRY

Discovery of a Novel Lead Structure for Anti-Malarials

Jochen Wiesner,[b] Pia Wißner,[a] Hans-Martin Dahse,[c] Hassan Jomaa[b] and Martin Schlitzer[a,*]

[a]*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany*
[b]*Biochemisches Institut der Universitätsklinik Gießen, Friedrichstraße 24, D-35249 Gießen, Germany*
[c]*Hans-Knöll-Institut für Naturstoff-Forschung e. V., Beutenbergstraße 11, D-07745 Jena, Germany*

Received 30 August 2000; accepted 2 November 2000

Abstract—From a library of 61 compounds available from former studies 2,5-bis-acylaminobenzophenone **7p** was identified as a lead structure for a novel class of anti-malaria agents active against multi-resistant *Plasmodium falciparum* strain Dd2. Some structural modifications of this initial lead demonstrated the potential for further improvement of the anti-plasmodial activity of this novel class of anti-malarials.

Introduction

Malaria is one of the most threatening tropical diseases causing between 1.5 and 2.7 million fatal cases per year, 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 *P. 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]

We have assayed a small library of altogether 61 compounds which were available from different studies[2] for potential activity against *P. falciparum*.

Chemistry

The synthesis of the library compounds has been described before.[2] The synthesis of the derivatives of the initial lead compound **7p** is outlined in Scheme 1. Briefly, appropriate 4-nitroaniline derivatives **12** and **14** have been acylated using phenylacetyl and acetyl chloride, respectively, in boiling toluene or toluene/dioxane. The nitro groups of the resulting amides have been reduced to amino functions using tin-II-chloride as reductive agent. Finally, the newly formed amino groups of **13** and **16** were acylated with 3-phenylpropionyl chloride. The cinnamic acid derivatives **11a–e** have been prepared from **17**[2e] and the corresponding cinnamic acids which have been prepared as described.[2b]

Biological testing

Compounds were evaluated for their inhibitory activity against intraerythrocytic forms of *P. falciparum* strains Dd2 and 3D7 using a semi-automated microdilution assay as described.[3] 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 percent inhibition in comparison to an untreated control) was determined at test compound concentrations of 100, 10 and 1.0 μM. For selected compounds IC_{50} values were determined. All values are estimated to be correct within ±30%.

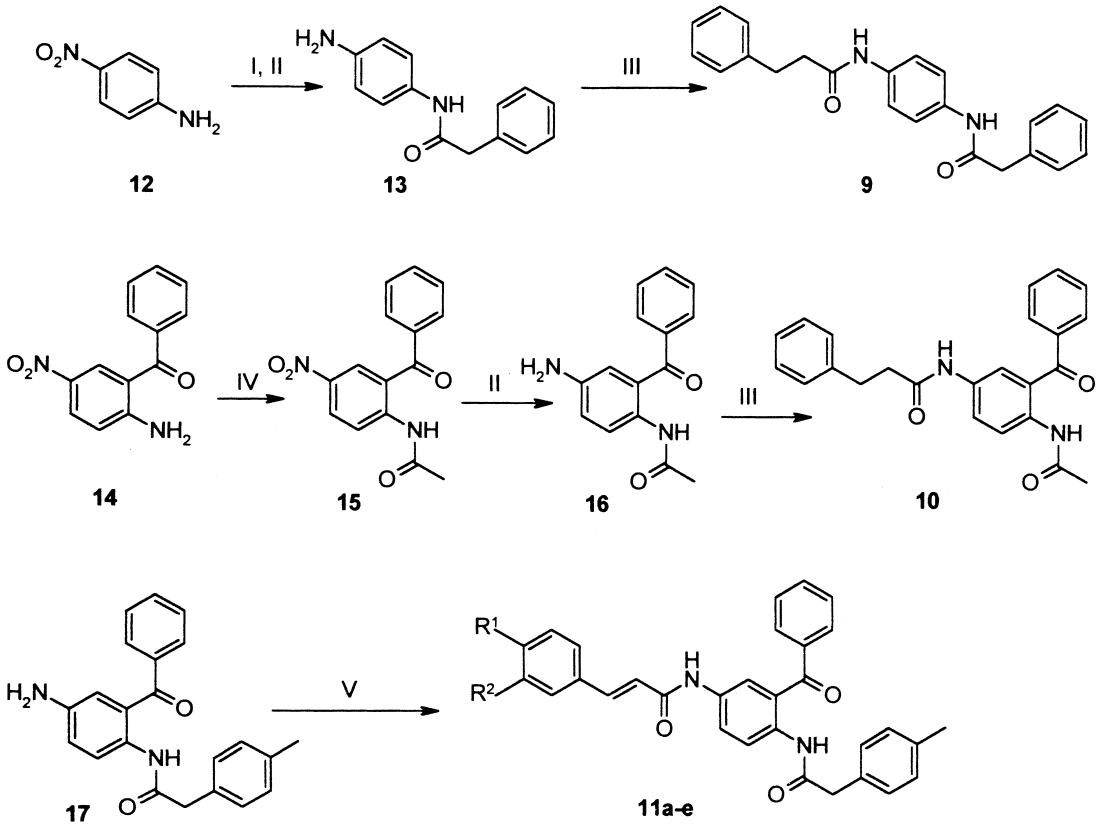
Compounds **7p** and **11a** were assayed against cell lines K-562 (human chronic myeloid leukaemic cell line), THP-1 (human acute monocytic leukaemic cell line), L-929 (mouse fibroblast cell line) and HeLa (human cervix carcinoma cell line) for their antiproliferative and cytotoxic effects. The cells were incubated with 10 concentrations of the test compounds ranging from 50 to 0.1 μg/mL.

Results and Discussion

A library of 61 compounds of different structural classes (Figs 1–3) which were available from other studies[2] were

*Corresponding author. Tel.: +49-6421-28-25825; fax: +49-6421-28-27052; e-mail: schlitze@mailer.uni-marburg.de

0968-0896/01/$ - see front matter
PII: S0968-0896(00)00296-0



Scheme 1. (I) Ph–CH_2–COCl, toluene, 2 h, reflux; (II) $SnCl_2 \times 2H_2O$, ethyl acetate, 2 h, reflux; (III) Ph-CH_2-CH_2-COCl, toluene, 2 h, reflux; (IV) H_3C-COCl, toluene, 2 h, reflux; $R^1R^2C_6H_3$–HC=CH–COCl, toluene/dioxane, 2 h, reflux.

assayed for their inhibitory activity against *P. falciparum* (Table 1). Forty seven of these compounds showed an inhibition of *P. falciparum* growth at a concentration of 100 μM. However, only seven compounds which are distributed over all structural classes retained significant inhibitory activity when assayed at a concentration of 10 μM. No rationale can be provided for the distribution of the activity within the library. Compound **7p**, which inhibited the growth of *P. falciparum* by 93% at a concentration of 10 μM, failed to inhibit the growth of the parasites at 1.0 μM. Nevertheless, compound **7p** appears to be the most active compound of the whole library and therefore, and because of its chemical structure which allows a variety of structural modifications, it was further evaluated as a potential lead structure for novel anti-malaria agents. First, an exact IC_{50} value was determined. The value of 2.7 μM against the wild strain 3D7 seems not to be particularily impressive if compared to the values of some standard drugs (Table 3). However, compound **7p** retained its activity if assayed against the multi-resistant strain Dd2, while most of the standard therapeutics showed a marked reduction in activity.

Next, some drastical structural modifications were performed with compound **7p** (Fig. 4). Each of the three derivatives (**8**–**10**) is lacking one of the substituents of the central benzene core. As shown in Table 2, all three modifications resulted in a marked decrease in anti-plasmodial activity demonstrating that the complete structure is necessary for activity. This argues for a rather specific interaction of **7p** with a molecular target although this target has yet to be determined. As all three substituents of the central benzene have been demonstrated to be important for activity, all three positions are open for structural variations to possibly improve anti-plasmodial activity. In the first line, we decided to concentrate our efforts on the acylamino substituent in the 5-position. We replaced the 3-phenyl-propionyl residue in **7p** by some substituted cinnamic acid derivatives (Fig. 4, Table 2). In this relatively small series of cinnamic acid derivatives, clear structure–activity relationships can be delineated. The 4-phenyl and the 4-benzyloxy derivative showed a significant improvement in activity compared to the 3-phenylpropionyl derivative **7p**, while the other two showed no improvement or were even less active. For **11a** and **11c**, exact IC_{50} values were determined (Table 3). Both compounds are approximately 5-fold more active than compound **7p** initially identified by random screen. Although their IC_{50} values of 650 and 500 nM, respectively, are still 10-fold higher than the values of some active anti-malarials as for example mefloquine (see Table 3), the improvement in activity upon structural modification demonstrates that this class of compounds possesses a potential for further development.

Altogether, the activity against a multi-resistant strain of *P. falciparum* and the potential for further improvement of activity by structural modifications demonstrated by compounds **11a** and **11c** qualifies compound **7p** as a lead structure for a novel class of anti-malarial agents.

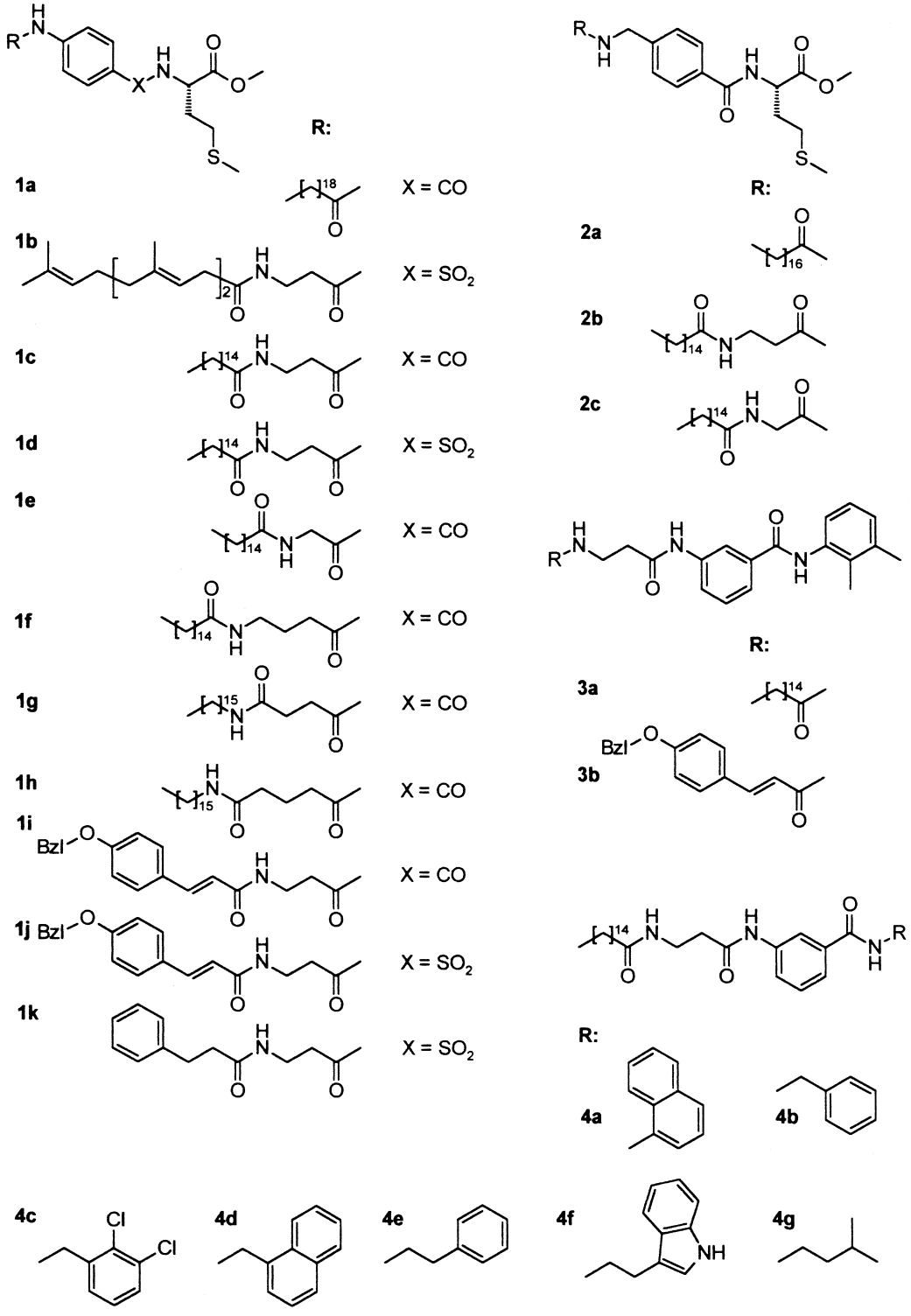


Figure 1. Structures of the library compounds.

One major concern in this stage of development was that the activity of the compounds investigated against *P. falciparum* may be due to an unspecific cytotoxicity. Therefore, compounds **7p** and **11a** were assayed for potential cytotoxic effects against some cultured cell lines (Table 4). Indeed, compound **7p** turned out to be cytotoxic but the 5-fold more active compound **11a** showed no cytotoxic activity at concentrations up to 180 μM which is more than 300 times the concentration at which the compound inhibits the growth of malaria parasites. Therefore, it has been demonstrated that the inhibitory activity against *P. falciparum* can be separated from cytotoxicity by structural modifications which at the same time improve anti-plasmodial efficacy.

In summary, we report a novel lead structure for anti-malaria agents with interesting activity against a multi-resistant strain of *P. falciparum*. This class of com-

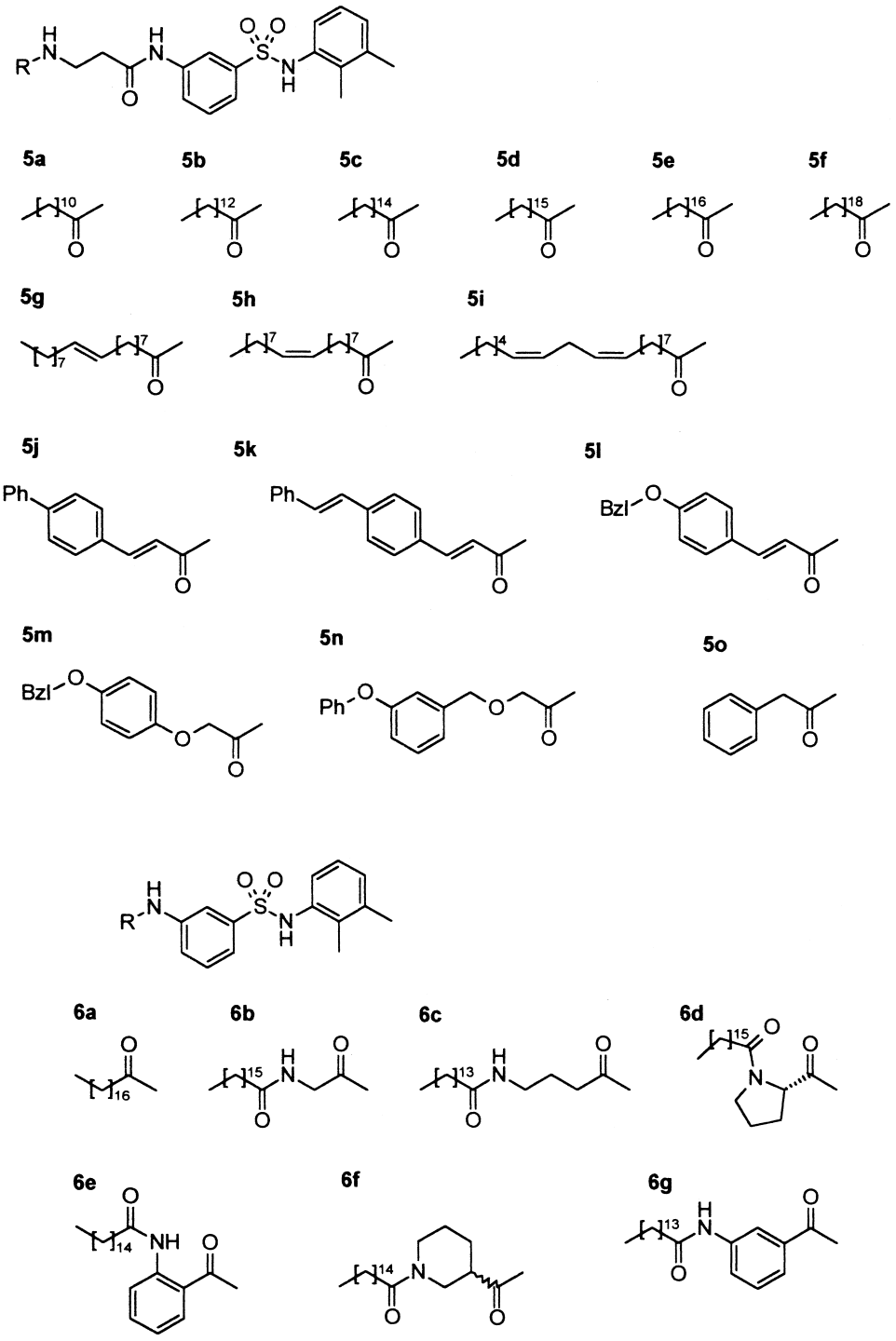


Figure 2. Structures of the library compounds, continued.

pounds possesses a potential for further development which will be necessary to obtain useful anti-malarials. Progress will be reported in due course.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer. Mass spectra were obtained with a Vacuum Generators VG 7070 H using a Vector 1 data acquisition system from Teknivent or a AutoSpec mass spectrometer from Micromass. IR spectra were recorded on a Nicolet 510P FT-IR-spectrometer. Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic and from a Hewlett Packard CHN-analyzer type 185 and are within ±0.4% of the theoretical values. Melting points were obtained with a Leitz-microscope and are uncorrected. Column chromatography was carried out using silica gel 60 (0.062–0.200 mm) from Merck. Medium

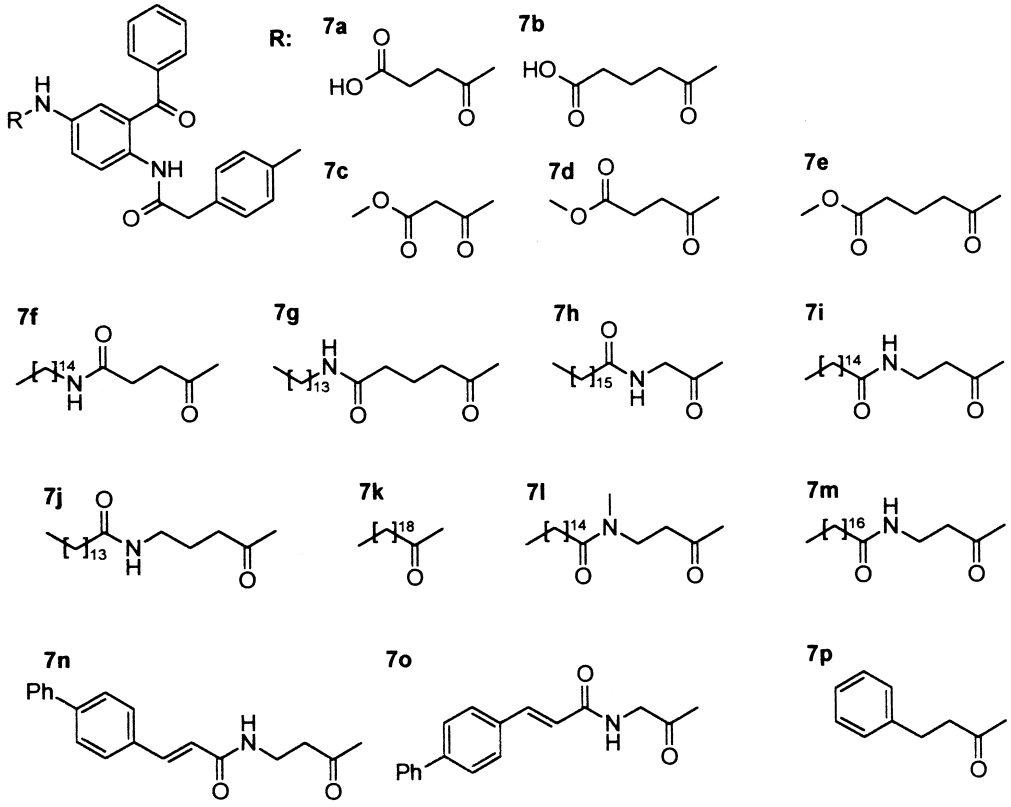


Figure 3. Structures of the library compounds, continued.

pressure liquid chromatography (MPLC) was performed using a pump type 688 from Büchi and a column of 3.5 cm diameter and 45 cm length filled with silica gel 60 (0.015–0.040 mm) from Merck.

General procedure for the acylation of aromatic amines (procedure 1)

The various carboxylic acids were dissolved in toluene and 0.1 mL $SOCl_2$ per mmol acid was added. The mixture was heated under reflux for 2 h and the volatiles were evaporated in vacuo. The resulting acyl chlorides were dissolved in toluene or dioxane (approx. 10 mL) and added to a solution of the appropriate aromatic amine in hot toluene (approx. 50 mL). The mixtures were heated under reflux for 2 h. Then, the solvent was removed in vacuo to give the crude products.

***N*-[4-(Phenylacetylamino)phenyl]-3-phenylpropionic acid amide 9.** 4-Nitroaniline **12** (690 mg, 5 mmol) was acylated by phenylacetyl chloride (773 mg, 5 mmol) according to general procedure 1. Yield: 870 mg (68%). ^{1}H NMR ($CDCl_3$): $\delta = 3.79$ (s, 2H), 7.34 (m, 2H), 7.41 (m, 1H), 7.43 (m, 2H), 7.60 (m, 2H), 8.16 (m, 2H).

The product from the step described above was dissolved in ethyl acetate (50 mL) and $SnCl_2 \times 2H_2O$ (3.83 g) was added. After 2 h under reflux, the solution was diluted with water and the pH was adjusted to 8 by the addition of satd $NaHCO_3$ solution. The aqueous mixture was extracted with ethyl acetate (3×100 mL) and the combined organic extracts were washed with brine. The ethyl acetate phase was dried over $MgSO_4$ and evaporated to dryness. The product **13** (384 mg, 1.7 mmol, 50%) was used without further purification for the next acylation step according to general procedure 1 employing 3-phenylpropionic acid chloride (286 mg, 1.7 mmol). Purification: recrystallization from toluene. Yield: 444 mg (73%); mp 223 °C. IR (KBr): $\nu = 3250$, 3060, 1700, 1670, 1600, 1555, 1505 cm^{-1}. ^{1}H NMR ($CDCl_3$): $\delta = 2.59$ (t, $J = 8$ Hz, 2H), 2.90 (t, $J = 8$ Hz, 2H), 3.60 (s, 2H), 7.10–7.32 (m, 10H), 7.48 (m, 4H), 9.72 (s, 1H), 9.96 (s, 1H). MS: m/z (%) 358 (100) [M^+], 226 (41), 108 (41). Anal. calcd for $C_{23}H_{22}N_2O_2$ (358.44): C, 77.07; H, 6.19; N, 7.82; found: C, 77.38; H, 6.56; N, 8.11.

***N*-(4-Acetylamino-3-benzoylphenyl)propionic acid amide 10.** 2-Amino-5-nitrobenzophenone **14** (1.21 g, 5 mmol) was acylated by acetylchloride (393 mg, 5 mmol) according to general procedure 1. Yield: 978 mg (68%). The product **15** was dissolved in ethyl acetate (50 mL) and $SnCl_2 \times 2H_2O$ (3.83 g) was added. After 2 h under reflux, the solution was diluted with water and the pH was adjusted to 8 by the addition of satd $NaHCO_3$ solution. The aqueous mixture was extracted with ethyl acetate (3×100 mL) and the combined organic extracts were washed with brine. The ethyl acetate phase was dried over $MgSO_4$ and evaporated to dryness. The product **16** (381 mg, 1.5 mmol, 44%) was used without further purification for the next acylation step according to general procedure 1 employing 3-phenylpropionic acid chloride (252 mg, 1.5 mmol). Purification: recrystallization from toluene. Yield: 301 mg (52%); mp 178 °C. IR (KBr): $\nu = 3255$, 3060, 1665, 1595, 1555 cm^{-1}. ^{1}H NMR ($CDCl_3$):

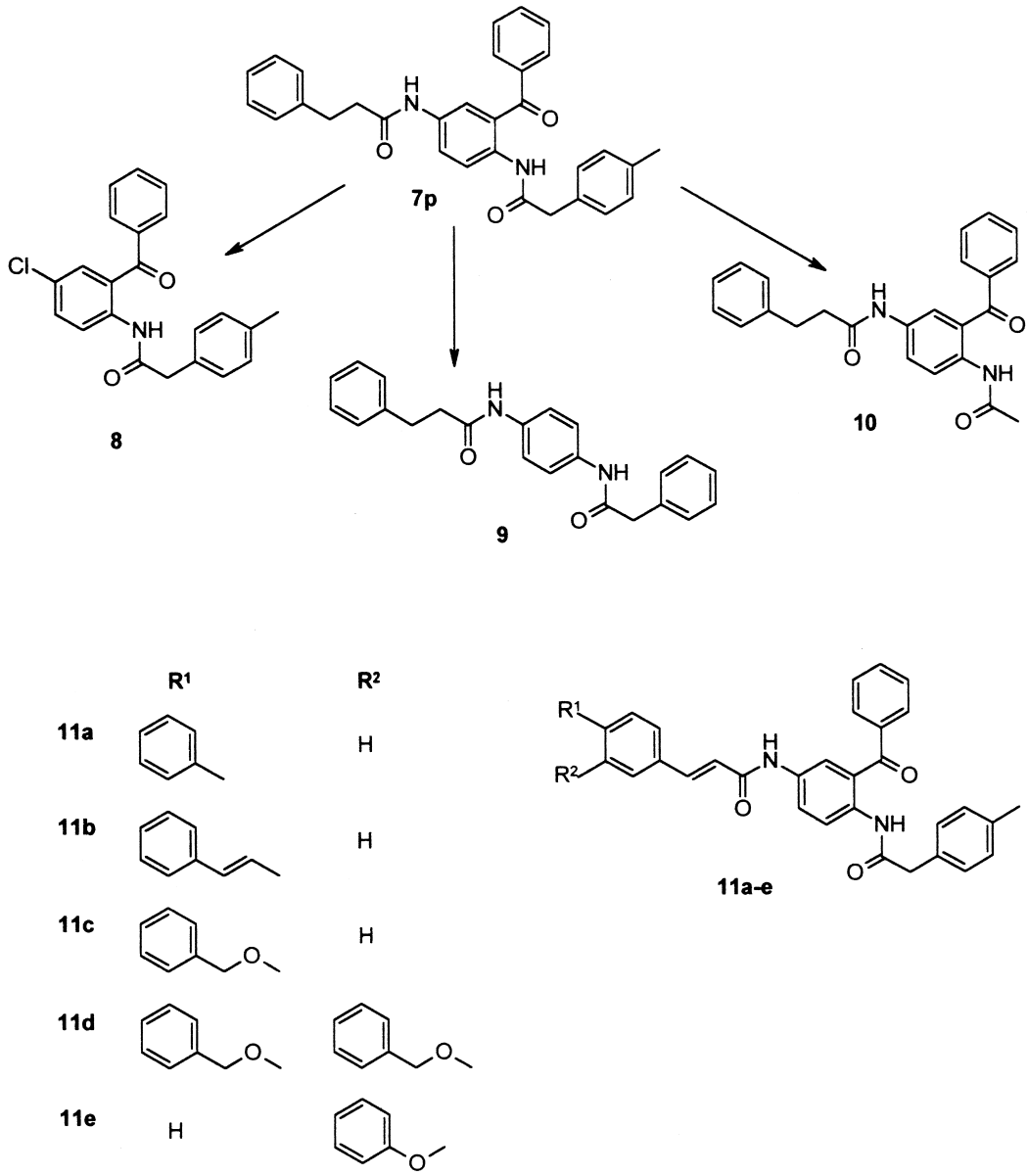


Figure 4. Structural variations of lead compound **7p** and cinnamic acid amides **11a**–**e**.

δ = 2.19 (s, 3H), 2.60 (t, J = 8 Hz, 2H), 2.99 (t, J = 8 Hz, 2H), 7.19 (m, 4H), 7.25 (m, 2H), 7.40 (m, 1H), 7.49 (m, 2H), 7.61 (m, 1H), 7.72 (m, 2H), 7.81 (m, 1H), 8.49 (m, 1H), 10.56 (s, 1H). MS: m/z (%) 386 (100) [M^+], 344 (29), 254 (23), 212 (52). Anal. calcd for $C_{24}H_{22}N_2O_3$ (386.45): C, 74.59; H, 5.74; N, 7.29; found: C, 74.50; H, 5.57; N, 7.76.

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-4-phenylcinnamic acid amide 11a.** From 4-phenylcinnamic acid (0.270 g, 1.2 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamid **17** (0.344 g, 1.0 mmol) according to general procedure 1. Purification: recrystallization from toluene. Yield: 456 mg (83%); mp 200 °C. IR (KBr): ν = 3445, 1700, 1685, 1655, 1640, 1550, 1505 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.24 (s, 3H), 3.60 (s, 2H), 6.42 (d, J = 16 Hz, 1H), 7.08 (m, 2H), 7.16 (m, 3H), 7.28 (m,1H), 7.38 (m, 5H), 7.48 (m, 6H), 7.61 (m, 3H), 7.96 (m, 1H), 8.43 (m, 1H), 10.46 (s, 1H). MS: m/z = 550 (98) [M^+], 207 (100). Anal. calcd for $C_{37}H_{30}N_2O_3$ (550.66) C, 80.70; H, 5.49; N, 5.09; found C, 80.53; H, 5.36; N, 5.12.

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-4-styrylcinnamic acid amide 11b.** From 4-styrylcinnamic acid (0.360 g, 1.5 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamid **17** (0.413 g, 1.2 mmol) according to general procedure 1. Purification: recrystallization from acetone/*n*-hexane. Yield: 437 mg (63%); mp 163 °C. IR (KBr): ν = 3310, 3025, 2930, 1650, 1600, 1560, 1510 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.31, (s, 3H), 3.67, (s, 2H), 6.47 (d, J = 16 Hz, 1H), 7.07 (s, 1H), 7.11 (s, 1H), 7.13–7.15 (m, 2H), 7.22–7.23 (m, 2H), 7.25–7.28 (m, 1H), 7.33–7.39 (m, 4H), 7.42–7.45 (m, 4H), 7.49–7.51 (m, 2H), 7.52–7.56 (m, 2H), 7.64 (d, J = 16 Hz, 1H), 7.69–7.70 (m, 2H), 7.96 (s, br, 1H), 8.06 (s, br, 1H), 8.49

Table 1. Activity of library compounds **1**–**7** against *P. falciparum* Dd2 strain

Compound	% Inhibition[a] at	
	100 μM	10 μM
1a	96	0
1b	0	0
1c	89	0
1d	0	0
1e	30	0
1f	58	0
1g	0	0
1h	78	0
1i	50	0
1j	38	0
1k	0	0
2a	0	0
2b	57	0
2c	99	84
3a	76	0
3b	83	0
4a	91	0
4b	17	0
4c	0	0
4d	0	0
4e	73	0
4f	93	63
4g	0	0
5a	98	0
5b	76	0
5c	98	0
5d	98	0
5e	59	0
5f	96	0
5g	91	0
5h	98	0
5i	97	36
5j	98	0
5k	89	0
5l	nd[b]	0
5m	99	0
5n	99	0
5o	99	0
6a	32	0
6b	92	0
6c	98	0
6d	98	0
6e	67	0
6f	98	33
6g	53	0
7a	38	0
7b	59	0
7c	95	19
7d	29	0
7e	98	0
7f	nd	0
7g	96	0
7h	83	0
7i	nd	0
7j	93	0
7k	99	0
7l	nd	0
7m	0	0
7n	nd	0
7o	89	67
7p	99	93

[a]None of the compounds was active at 1.0 μM.
[b]nd: not determined.

(d, $J = 9$ Hz, 1H), 10.53 (s, br, 1H). MS: *m*/*z* (%) 576 (80) [M^+], 577 (35), 574 (22), 344 (37), 233 (100). Anal. calcd for $C_{39}H_{32}N_2O_3$ (576.69): C, 81.21; H, 5.60; N, 4.86; found: C, 81.21; H, 5.63; N, 4.92.

Table 2. Activity of derivatives **8**–**11** of the lead structure **7p** against *P. falciparum* Dd2 strain

Compound	% Inhibition at		
	100 μM	10 μM	1.0 μM
7p	99	93	0
8	92	0	0
9	0	0	0
10	95	0	0
11a	99	99	96
11b	99	99	0
11c	97	96	85
11d	98	36	0
11e	99	99	0

Table 3. IC_{50} values (μM) of selected compounds and some standard anti-malarials

Compound	*P. falciparum* strain	
	3D7	Dd2
7p	2.7	2.7
11a	0.49	0.65
11c	0.40	0.50
Chloroquine	0.022	0.17
Quinine	0.10	0.38
Mefloquine	0.024	0.057
Pyrimethamine	0.003	2.5
Cycloguanil	0.008	2.2

Table 4. Cytotoxic and anti-proliferative effect of compounds **7p** and **11a**

Compound	IC_{50} (μM)		GI_{50} (μM)	
	L929	HeLa	K-562	THP-1
7p	17.8	36.9	13.6	14.6
11a	> 180	> 180	> 180	> 180

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-4-benzyloxycinnamic acid amide 11c.** From 4-benzyloxycinnamic acid (0.381 g, 1.5 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamid **17** (0.413 g, 1.2 mmol) according to general procedure 1. Purification: recrystallization from toluene. Yield: 482 mg (69%); mp 183 °C. IR (KBr): ν = 3285, 1675, 1600, 1540 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.31 (s, 3H), 3.67 (s, 2H), 5.06 (s, 2H), 6.33 (d, $J = 16$ Hz, 1H), 6.90–6.93 (m, 2H), 7.14–7.16 (m, 2H), 7.22–7.26 (m, 2H), 7.31–7.46 (m, 9H), 7.53–7.54 (m, 2H), 7.60 (d, $J = 16$ Hz, 1H), 7.67–7.72 (m, 2H), 7.78 (s, br, 1H), 8.03 (s, br, 1H), 8.49 (m, 1H), 10.52 (s, br, 1H). MS: *m*/*z* (%) 580 (0.3) [M^+], 581 (81), 345 (55), 237 (100). Anal. calcd for $C_{38}H_{32}N_2O_4$ (580.72): C, 78.60; H, 5.55; N, 4.82; found: C, 78.85; H, 5.59; N, 4.94.

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-3,4-bis-(benzyloxy)cinnamic acid amide 11d.** From 3,4-bis-(benzyloxy)cinnamic acid (0.541 g, 1.5 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamid **17** (0.413 g, 1.2 mmol) according to general procedure 1. Purification: MPLC ethyl acetate *n*-hexane, 1:1. Yield: 502 mg (61%); mp 102 °C. IR (KBr): ν = 3265, 3030,

1665, 1630, 1595, 1550, 1505 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.31$, (s, 3H), 3.67, (s, 2H), 5.09 (s, 2H), 5.15 (s, 2H), 6.25 (d, $J = 16$ Hz, 1H), 6.85–6.86 (m, 1H), 6.96–7.00 (m, 1H), 7.02–7.03 (m, 1H), 7.14–7.15 (m, 2H), 7.22–7.45 (m, 14H), 7.50–7.53 (m, 3H), 7.67–7.69 (m, 3H), 8.00 (s, br, 1H), 8.49 (m, 1H), 10.53 (s, br, 1H). MS: m/z (%) 686 (10) [M^+], 344 (12), 129 (25), 105 (41), 91 (100), 57 (48), 44 (78). Anal. calcd for $C_{45}H_{38}N_2O_5$ (686.85): C, 78.69; H, 5.59; N, 4.08; found: C, 78.53; H, 5.61; N, 4.28.

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-3-phenoxycinammic acid amide 11d.** From 3-phenoxycinnamic acid (0.360 g, 1.5 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamid **17** (0.413 g, 1.2 mmol) according to general procedure 1. Purification: MPLC ethyl acetate *n*-hexane, 1:1. Yield: 428 mg (63%); mp 133 °C. IR (KBr): $\nu = 3240$, 3085, 1670, 1640, 1595, 1580, 1550 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta = 2.31$, (s, 3H), 3.67, (s, 2H), 6.38 (d, $J = 16$ Hz, 1H), 6.93–7.01 (m, 3H), 7.08–7.16 (m, 5H), 7.21–7.35 (m, 5H), 7.42–7.46 (m, 2H), 7.51–7.61 (m, 3H), 7.64–7.69 (m, 3H), 7.99 (s, br, 1H), 8.49 (d, $J = 9$ Hz, 1H), 10.51 (s, br, 1H). MS: m/z (%) 566 (100) [M^+], 567 (42), 434 (31), 344 (31), 223 (35), 212 (30). Anal. calcd for $C_{37}H_{30}N_2O_4$ (566.69): C, 78.42; H, 5.35; N, 4.94; found: C, 78.20; H, 5.31; N, 5.22.

In vitro measurement of *P. falciparum* parasite growth inhibition

Compounds were tested by a semiautomated microdilution assay against intraerythrocytic forms of *P. falciparum*.[3a] The *P. falciparum* strains Dd2 and 3D7 were cultivated by a modification of the method described by Trager and Jensen.[3b] 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 (final DMSO concentrations $\leq$1%). 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.[3c] After the addition of 0.8 μCi [^3H]-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 measured using a β-counter (Matrix 9600, Packard).

Cytotoxicity assay

Cells of established suspended cell lines K-562, THP-1, and L-929 were cultured in RPMI 1640 medium (Gibco BRL 42402-016), supplemented with 100 U/mL penicillin G sodium salt and 100 μg/mL streptomycin sulfate (Gibco BRL 15140-114) and 10% heat inactivated fetal bovine serum (Gibco BRL 10500-064), and L-glutamine (Gibco BRL 25030-024) at 37 °C in high density polyethylene flasks (Nunc 156340). HeLa cells were grown in RPMI 1640 culture medium (Gibco BRL 21875-034) supplemented with 100 U/mL penicillin G sodium salt and 100 μg/mL streptomycin sulfate (Gibco BRL 15140-114), 10% heat inactivated fetal bovine serum (Gibco BRL 10500-064), and 10 mL/L non-essential amino acid (Gibco BRL 11140-035) at 37 °C in high density polyethylene flasks (Nunc 156340).

For each experiment with K-562 and THP-1 cells, approximately 10,000 cells were seeded with 0.1 mL RPMI 1640 culture medium (Gibco BRL 21875-34) but without HEPES, into 96-well microplates (Nunc 163320). The adherent cells of L-929 and HeLa were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA. L929 and HeLa cells were seeded with approximately 10,000 cells per 0.1 mL RPMI 1640 per well of the 96-well microplates. The plates were previously prepared with ten dilutions of the test compounds in 0.1 mL RPMI 1640. The cells were incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2.

Suspension cultures of K-562 and THP-1 cells in microplates were analysed by an electronic cell analyser system CASY1 (Schärfe, Reutlingen, Germany). The principle of measurement and evaluation of data were described.[4] The 0.2 mL content of each well in the microplate was diluted 1:50 with CASYTON (Schärfe). Every count/mL was automatically calculated from the arithmetic mean of three successive counts of 0.4 mL each. From the dose response curves the GI_{50} values (concentration which inhibited cell growth by 50%) were calculated with the software for data evaluation CASYSTAT (Schärfe). The monolayer of the adherent cell lines L-929 and HeLa were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gentle washing the stain was eluted by 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 630 nm in a Dynatech MR 7000 microplate reader. Comparison of different values was performed with Microsoft EXCEL.

Acknowledgements

M. Schlitzer thanks Professor Dr. W. Hanefeld for generous support and Ms. K. Burk and Mr. G. Hohage for technical assistance.

References

1. Wirth, D. F. *Ann. Rep. Med. Chem.* **1999**, *34*, 349.
2. (a) Schlitzer, M.; Sattler, I. *Angew. Chem., Int. Ed. (Engl.)* **1999**, *38*, 2032. (b) Schlitzer, M.; Böhm, M.; Dahse, H.-M.; Sattler, I. *Bioorg. Med. Chem.* **2000**, *8*, 1991. (c) Schlitzer, M.; Böhm, M.; Sattler, I. *Bioorg. Med. Chem.* **2000**, *8*, 2399. (d) Schlitzer, M.; Sattler, I. *Pharm. Pharmacol. Commun.* **2000**, *5*, 117. (e) Schlitzer, M.; Sattler, I. *Eur. J. Med. Chem.* **2000**, *35*, 721.
3. (a) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710. (b) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673. (c) Ancelin, M. L.; Calas, M.; Bompart, J.; Cordina, G.; Martin, D.; Bari, M. B.; Jei, T.; Druilhe, P.; Vial, H. J. *Blood* **1998**, *91*, 1.
4. Winkelmeier, P.; Glauner, B.; Lindl, T. *ATLA* **1993**, *21*, 269.