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Structure-activity relationships of novel anti-malarial agents part 8. Effect of different central aryls in biarylacryloylaminobenzophenones on antimalarial activity

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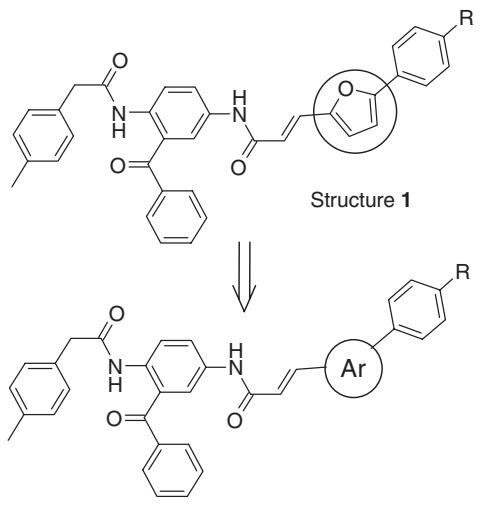
Replacement of the 2,5-disubstituted furyl residue present in the known antimalarial agents **8** by other aryl residues resulted in a more or less reduced antimalarial activity in most cases. The only exemption was the 2,4-thienylene compound **11a** displaying activity with an IC_{50} value of 120 nM. In conclusion, the 2,5-furylene compound **8e** remains to represent the most active antimalarial agent in this series of farnesyltransferase inhibitors.

1. Introduction

With respect to 300 to 500 million clinical cases of malaria every year and 1 to 3 million deaths mainly caused by *Plasmodium falciparum* strains resistant to the commonly used drugs the development of novel antimalaria agents is an important task of medicinal chemistry [1, 2].
In previous studies we have described the development of antimalarial agents based on a novel class of farnesyltransferase inhibitors [3] as exemplified by structure **1** with the 3-(5-aryl-2-furyl)acryloyl moiety being a common structural element of these compounds [4–8]. In this study we have addressed the question how the replacement of the central furyl ring by other ring systems leading to an overall similar geometry for the biarylacryloyl moiety would influence antimalarial activity (Scheme 1).

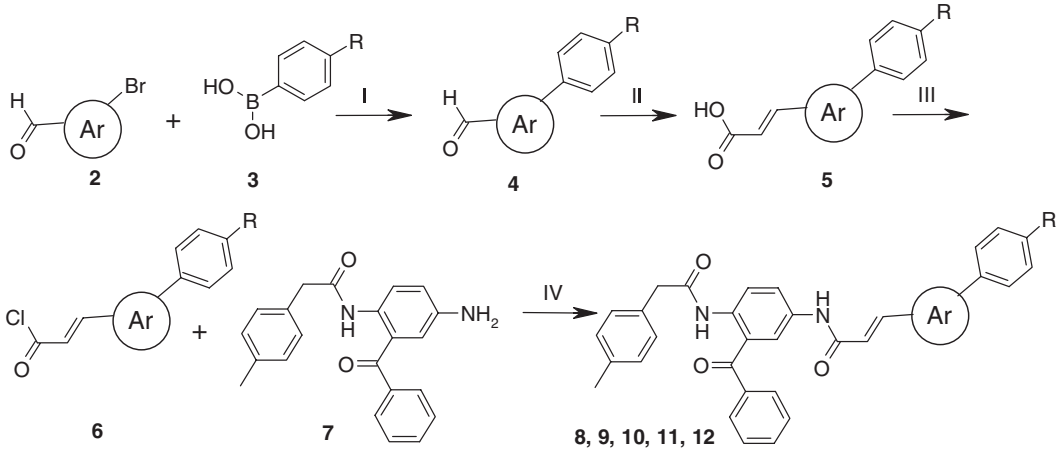
Scheme 1



2. Investigations, results and discussion

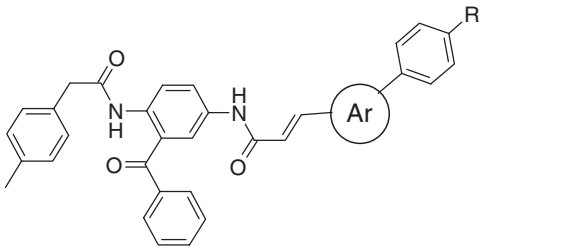
Key intermediates for the synthesis of the target compounds were the biarylaldehydes **4** which were prepared by Suzuki-coupling (modified from [9]) from corresponding bromoarylaldehydes **2** and the appropriate phenylboronic acids **3**. The biarylaldehydes **4** were then transformed into the corresponding 3-biarylacrylic acids **5**, which were activated as acid chlorides **6** and reacted with 5-amino-2-tolylacetylaminobenzophenone **7** [10, 11] (Scheme 2).
Compounds **8**–**12** were assayed for their inhibitory activity against intraerythrocytic forms of *P. falciparum* strain Dd2 using a semi-automated microdilution assay [12, 13]. The growth of the parasites was monitored through the incorporation of tritium labeled hypoxanthine. Comparability of different experiments was ensured by a concurrent assay of standard compounds. The Dd2 strain used for the inhibition assays is resistant to several commonly used anti-malarial drugs (chloroquine, cycloguanile and pyrimethamine) (Table).
Comparing the antimalarial activity of the compounds bearing the different central aryl moieties the following SAR can be deduced. Replacement of the central 2,5-furylene (**8a**–**e**) by its sulphur analogue (**9a**–**e**) resulted in a decreased activity, independent of residue R. In contrast, comparison of the 2,4-disubstituted furyl (**10a**–**e**) and thienyl compounds (**11a**–**e**) revealed superior activity of the sulphur containing heterocycle with all but the methylsulphonyl residue. When the two oxygen-containing heterocycles are compared to each other the 2,5-furylene compounds are generally more active with the exemption of the compounds with R = H. Here, the 2,4-furylene **10a** compound is twice as active as the 2,5-furylene **8a**. When the sulphur containing compounds are compared to each other, the situation is different and the 2,4-isomers are more active than the 2,5-thienylenes. So, in the series of the 2,5-substituted heterocyles the furylene derivatives are

Scheme 2



(I) $(Ph_3P)_4Pd$, K_2CO_3, toluene/ethanol/water, 5 h, reflux; (II) malonic acid, pyridine/piperidine, 2 h, reflux; (III) oxalyl chloride, toluene, 2 h, reflux; (IV) toluene/dioxane, 2 h, reflux.

Table: Anti-malarial activity (nM) of compounds 8–12[a]



R					
−H	**8a:** 415	**9a:** 700	**10a:** 280	**11a:** 120	**12a:** 1200
$-CH_3$	**8b:** 120	**9b:** 8000	**10b:** 1100	**11b:** 370	**12b:** 740
$-CF_3$	**8c:** 77	**9c:** 8500	**10c:** 5600	**11c:** 1200	**12c:** 1000
$-O-CH_3$	**8d:** 130	**9d:** 800	**10d:** 740	**11d:** 360	12d: 740
$-SO_2-CH_3$	**8e:** 37	**9e:** 180	**10e:** 76	**11e:** 180	**12e:** 200

[a] Activity was assayed by measuring radioactive hypoxanthine uptake by the multi-resistant *P. falciparum* strain Dd2. IC_{50} values (nM) for standard antimalarials were: chloroquine, 170; pyrimethamine, 2500; cycloguanile, 2200; quinine, 380; lumefantrine, 30; artemisinin, 18

the more active ones whereas in the series of the 2,4-disubstituted heterocyles the thienylene derivatives are more active. In direct comparison of the 2,5-furylene derivatives **8a**–**e** to the 2,4-thienylene derivatives **11a**–**e**, most furylene derivatives are more active than the 2,4-thienylenes. Only **11a** in which R = H is more active than the oxygene analogue **8a**. The activity of the 1,3-phenylene derivatives **12a**–**e** in comparison to the heterocyclic derivatives depends strongly on the residue R. No general trend can be outlined apart from the fact that the phenylene derivatives **12a**–**e** seem to provide no advantage over the most active heterocyclic derivatives.

In summary, a more or less pronounced reduction in antimalarial activity is observed when the 2,5-disubstituted furyl residue present in the antimalarial agents **8** is replaced by other aryl residues. An exemption of this trend is seen comparing compounds **8a**–**12a** carrying an unsubstituted terminal phenyl residue. In this series, the 2,4-disubstituted furyl (**10a**) and thienyl (**11a**) derivatives are 2–4fold more active than the original 2,5-furylene compound **8a** with the thienylene compound **11a** displaying considerable activity with an IC_{50} value of 120 nM. In conclusion, the 2,5-furylene compound **12e** seems to remain the most interesting lead compound with respect to the future development of antimalarial agents based on farnesyl transferase inhibitors.

3. Experimental

3.1. General procedure for the preparation of phenylarylcarbaldehyde derivatives by Suzuki-coupling of substituted benzeneboronic acids with bromoarylcarbaldehydes

All solutions have to be oxygen free and the reaction has to be done under an argon atmosphere. The bromoarylcarbaldehyde (1 mmol) and the appropriate benzeneboronic acid (1–1.2 mmol) were dissolved or suspended in a mixture of ethanol, toluene and a saturated aqueous Na_2CO_3 solution (15/15/10 ml). Tetrakistriphenylphosphinepalladium(0) (50 mg) was added and the reaction mixture was heated under reflux for 5 h. After cooling, the mixture was extracted three times with dichloromethane. The organic layers were combined and dried over anhydrous $MgSO_4$. Solvents were evaporated and the resulting residue was purified by flash chromatography. Target compounds **8** were structurally characterized by IR, ^1H NMR and MS and gave microanalysis within ± 0.4% of the theoretical values.

3.2. General procedure for the preparation of E-3-(phenylaryl)acrylic acid derivatives from phenylarylcarbaldehydes and malonic acid

The aldehyde was dissolved in a mixture of pyridine (5 ml) and piperidine (0.2 ml). Malonic acid (125 mg per mmol aldehyde) was added and the mixture was heated under reflux for 2 h. The cooled reaction mixture was poured into a mixture of water, conc. HCl and ice (60/60/60 ml). The resulting solids were isolated and purified by recrystallisation.

3.3. General procedure for the preparation of E-N-[3-benzoyl-4-(4-tolylacetylamino)phenyl]-3-(phenylaryl)acrylic acid amide derivatives from E-3-(phenylaryl)acrylic acids and N-(4-amino-2-benzoylphenyl)-4-tolylacetic acid amide

The appropriate E-3-(phenylaryl)acrylic acid was dissolved or suspended in a small amount of dichloromethane. After addition of oxalyl chloride (0.2 ml per mmol acid) the mixture was stirred at room temperature for 2 h. Volatiles were evaporated in vacuo and the resulting residue was dissolved in hot toluene. This solution was added to a solution of *N*-(4-amino-2-benzoylphenyl)-4-tolylacetic acid amide in hot toluene. The resulting mixture was heated under reflux for 2 h. The solvent was evaporated and the obtained residue was purified by recrystallisation or flash chromatography.

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

Compounds were tested by a semiautomated microdilution assay against intraerythrocytic forms of *P. falciparum* [12]. The *P. falciparum* strain Dd2

was cultivated by a modification of the method described by Trager and Jensen [13]. 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 $\leq 1\%$) In order to avoid a loss of lipophilic test compounds by adsorbance to the plastic material used for the assay, complete culture medium containing erythrocytes was used to dilute the DMSO stock solutions. 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 [14]. 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 measured using a β-counter (Matrix 9600, Packard).

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