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2-(Aminoacylamino)benzophenones: farnesyltransferase inhibition and antimalarial activity

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The use of amino acids as acyl substitutents at the 2-amino group of our benzophenone core structure yielded compounds with mainly good to moderate farnesyltransferase inhibitory and moderate antimalarial activity. However, these farnesyltransferase inhibitors display some degree of selectivity towards malarial parasites since there was no cytotoxic activity observed at 70–80 μM.

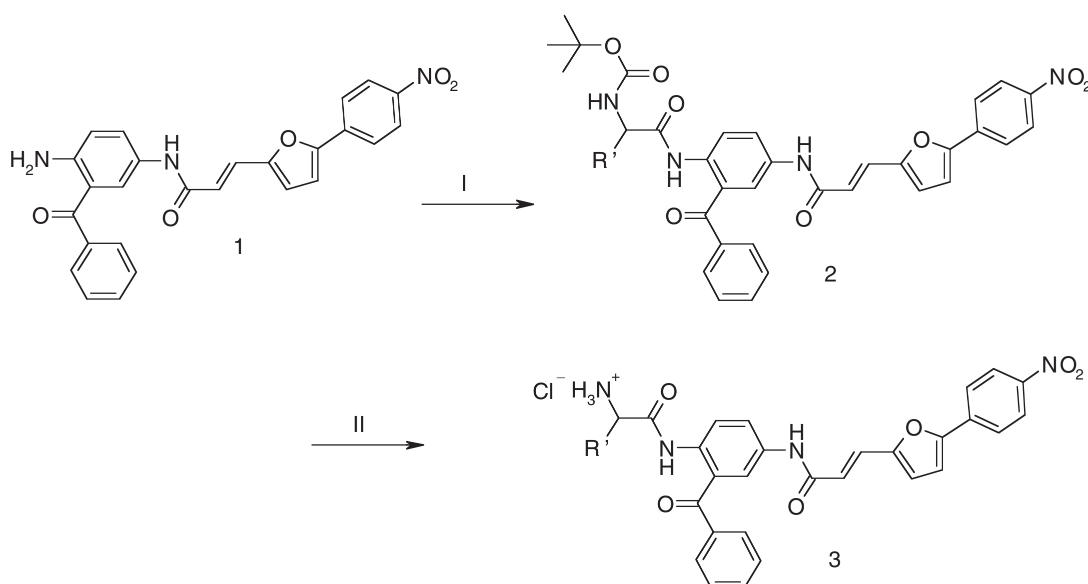
1. Introduction

Malaria is one the world's most serious infectious diseases since approximately 40% of the world population lives in areas with malaria risk, and 2 to 3 million people die each year from malaria. The situation is worsened through the increasing spread of *Plasmodium falciparum* (the causative parasite of malaria tropica) resistant to chloroquine and other commonly used antimalarials (Sachs and Malaney 2002; Ridley 2002). Farnesyltransferase is generally accepted as a novel target in the development of novel antimalarial agents (Medicines for Malaria Venture – annual report 2003).

Farnesyltransferase takes part in the post-translational modification of numerous proteins most of them involved in intracellular signal transduction. Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesyl pyrophosphate to the thiol of a cysteine side chain of the protein substrate characterized by a carboxy-terminal consensus sequence, the so-called CAAX box (C, cysteine; A, amino acid with aliphatic side chain; X, serine or methionine) (Fu and Casey 1999; Wittinghofer and Waldmann 2000; Bell 2000).

We have developed a novel class of farnesyltransferase inhibitors based on a benzophenone scaffold (Schlitzer 2002). A number of these farnesyltransferase inhibitors

Scheme



(I) *N*-boc amino acid, $POCl_3$, pyridine, 0 °C, 30 min; (II) HCl/dioxane, RT, 2 h

displayed interesting antimalarial activity (Wiesner et al. 2003a, 2003b, 2004). In course of our studies towards the establishment of structure activity relationships of this type of compounds we introduced various amino acid residues at the 2-amino group of the benzophenone core to address the influence of this structural variation on farnesyltransferase inhibition and antimalarial activity.

2. Investigations, results and discussion

The amino acid derivatives **3** were prepared from a common intermediate **1** whose synthesis has been described (Sakowski et al. 2002; Kettler et al. 2003). Appropriate *N*-boc protected amino acids were activated using phosphorous oxychloride in pyridine (Rijkers et al. 1995) and reacted with the intermediate **1** to yield the boc-protected amino acyl derivatives **2**. The *N*-boc protected amino acids were either commercially available or as in the case of *p*-trifluoromethylphenylalanine derivative prepared from commercially available *p*-trifluoromethylphenylalanine (Keller et al. 1990). However, these intermediates could not be isolated in every case. With several compounds the *N*-deprotection occurred with the acylation step. In all other cases the last step in the synthesis was the acidic removal of the boc-protective group from the corresponding intermediates **2** (Scheme).

The farnesyltransferase inhibitory activity of the compounds was determined using the fluorescence enhancement assay as described by Pompliano et al. (1992). The assay employs yeast farnesyltransferase (FTase) fused to Glutathione *S*-transferase at the N-terminus of the β-subunit (Del Villar et al. 1997). The heterologous expression of the farnesyltransferase genes from *P. falciparum* has not been achieved so far and, therefore, no recombinant enzyme is available for routine screening (Chakrabarti et al. 2002).

Farnesylpyrophosphate and the dansylated pentapeptide Ds-GlyCysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol, the dansyl residue is placed into a lipophilic environment. The resulting enhancement of fluorescence at 505 nm is used to monitor the enzyme reaction.

Compounds were assayed for their inhibitory activity against intraerythrocytic forms of *P. falciparum* strain Dd2 using a semi-automated microdilution assay (Desjardins et al. 1979; Trager and Jensen 1976; Ancelin et al. 1998). The growth of the parasites was monitored through the incorporation of tritium labeled hypoxanthine. Comparability of different experiments was ensured by 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).

Cytotoxicity of selected compounds was evaluated against HeLa cells. Viability of the cells was determined after 72 h incubation period using methylene blue staining and photometric evaluation.

The use of different amino acids as acyl substituents at the 2-amino group of our benzophenone core structure yielded compounds with mainly good to moderate farnesyltransferase inhibitory activity (Table). Only the *S*-methionine derivative **3i** is markedly less active with an IC_{50}-value of more than 1 μM. Some stereo-differentiation of the activity is visible but this effect is not much pronounced. This holds also true for the activity differences between the boc-protected intermediates **2** and the free amino acid derivatives **3**. There are some indications that boc-protected amino acid derivatives and the free amino acid derivatives bind in different modes to the farnesyltransferase but due to the large number of rotatable bonds no unambiguous results were obtained from flexible docking of such compounds (Kettler et al. 2004). Antimalarial activity of the inhibitors is varying with IC_{50}-values between 0.35 μM and 7 μM with the majority of the compounds displaying IC_{50}-values around 2 μM (Table) which makes them notably weaker antimalarial agents than a number of benzophenone-based farnesyltransferase inhibitors we have described before. Again, no clear structure-activity relationships are deducible and no clear correlation between farnesyltransferase inhibition and antimalarial activity is visible. In spite that there is no data concerning the activity of the compounds against plasmodial farnesyltransferase due to the unavailability of this enzyme, we address these activity differences not mainly to different susceptibility of the enzymes of different species. We base this conclusion on a homology model recently presented (Kettler et al. 2004) which shows rather few structural differences between both enzymes as far as the active side is concerned which should not affect binding of this type of inhibitors significantly. Most probably differences in the penetration of different molecules into the parasite are the main cause of different activities. It is important to note that none of the compounds displayed significant cytotoxicity even at concentrations higher than 70–80 μM (Table) indicating that in spite of comparably low antimalarial activity there is a marked difference in the susceptibility of the intraerythrocytal form of *P. falciparum* and cultured human cells. Thus, these farnesyltransferase inhibitors display some degree of selectivity towards malarial parasites which is one of the main issues of antiparasitic drug development. Therefore, the results described here are of considerable value for the development of *P. falciaprum* selective farnesyltransferase inhibitors.

3. Experimental

3.1. Synthesis of the compounds

^{1}H- and ^{13}C NMR spectra were recorded on a Jeol Lambda 500 delta, a Jeol JNM-GX-400, a Jeol Eclipse 500 and a Jeol Eclipse 400 spectrometer. Mass spectra were obtained with a Vacuum Generator VG 7070 H using a Vector 1 data acquisition system from Teknivent, an AutoSpec mass spectrometer from Micromass, an API 2000 LC/MS/MS-system of PE SCIEX using Analyst 1.2 of Applied Biosystems/MDS SCIEX and on a MStation JMS 700 of Jeol using Jeol Mass Data System MS-MP 9021D 2.30. IR spectra were recorded on a Nicolet 510P FT-IR-spectrometer and a Jasco FT/IR-410 FT-IR-spectrometer. Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic, from a Hewlett-Packard CHN-analyzer type 185 and from a Vario EL of Elementar and are within ± 0.4% of the calculated values. Melting points were obtained with a Reichert Austria microscope and are uncorrected. Column chromatography was carried out using silica gel 60 (0.062–0.200 mm) from Macherey-Nagel and silica gel 60 (0.040–0.063) of Merck. The preparation of compound **1** has already been described (Sakowski et al. 2002; Kettler et al. 2003)

3.1.1. General procedure 1: Activation of N-boc amino acids with $POCl_3$ and reaction with intermediate 1

A solution of the N-boc amino acid and the intermediate **1** (1 equivalent of each) were dissolved in dried pyridine (about 3–5 ml per mmol amino acid). After cooling the solution to −15 °C, $POCl_3$ (0.1 ml per mmol amino acid) was added drop wise. After 30 min at −15 °C, the mixture was poured into 200 ml of an ice-water-mixture and extracted with ethyl acetate for three times. The combined organic layers were washed with brine and dried over Na_2SO_4. Then, the solvent was removed *in vacuo* followed by recrystallization of the crude product.

3.1.2. General procedure 2: Acidic removal of the boc-protective group

The boc-protected compound **2** is dissolved or suspended in a saturated solution of HCl in dioxane (10–20 ml per mmol of the boc-protected compound) and stirred at room temperature for 2 h. After removing the HCl-dioxane-solution *in vacuo* the remaining crude product was washed with acetone several times.

Table: Farnesyltransferase inhibition, antimalarial activity[a,b] and cytotoxicity[c] of compounds 2 and 3

Compd.	R	IC_{50} FTase (nM)	IC_{50} *P.fal.* (nM)	CC_{50} (μM)
2a		230 ± 25	900	> 71
3a		210 ± 15	1200	79
2b		68 ± 11	420	> 71
3b		116 ± 21	580	> 78
2c		283 ± 18	3150	–
3c		95 ± 10	710	–
2d		148 ± 45	7000	–
3d		60 ± 15	1900	> 74.5
2e		106 ± 28	2600	–
3e		70 ± 13	650	> 74.5
3f		360 ± 50	1700	> 83
3g		503 ± 50	1800	47.8
3h		151 ± 30	1900	> 83
2i		391 ± 33	1700	–
3i		1776 ± 300	2800	> 81
2j		330 ± 28	350	–
3j		496 ± 52	1800	> 81
3k		582 ± 47	1800	–

[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.
[b] Antimalarial activity of compounds **2a–c** and **3a–c** has been published in (Wiesner et al. 2003b).
[c] Assayed against HeLa cells.

The following boc-protected compounds **2** were prepared from intermediate **1** and the appropriate *N*-boc amino acids according to General Procedure **1**. When necessary boc-protected compounds **2** were deprotected according to General Procedure 2 to the final compounds **3**:

3.1.3. (E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonylphenylalanine amide (**2a**)

Purification: recrystallization from toluene; yield 81%; m.p. 198 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3427, 2976, 1685, 1628, 1598, 1517, 1404, 1332, 1247, 1166, 1108, 1028, 853, 752, 701. ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.25 (s, 9 H), 2.60 (m, 1 H), 2.79 (m, 1 H), 4.16 (m, 1 H), 6.77 (d, J = 16 Hz, 1 H), 7.02–7.21 (m, 8 H), 7.41–7.71 (m, 8 H), 7.93 (s, 1 H), 7.99 (m, 2 H), 8.30 (m, 2 H), 10.40 (s, 2 H). MS (EI): m/z (%) = 700 (1, M^+), 256 (59), 242 (98), 129 (59), 73 (100), 69 (82).
$C_{40}H_{36}N_4O_8$ (700.8)

3.1.4. (E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)phenylalanine amide hydrochloride (**3a**)

Yield 65%; m.p. 179 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3439, 2926, 1653, 1625, 1599, 1517, 1406, 1332, 1251, 1110, 1027, 852, 751, 702. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.67 (m, 1 H), 2.78 (m, 1 H), 4.09 (m, 1 H), 6.78 (d, J = 16 Hz, 1 H), 6.99 (m, 1 H), 7.19 (m, 1 H), 7.22 (m, 3 H), 7.37 (m, 2 H), 7.48 (m, 1 H), 7.50 (m, 2 H), 7.61 (m, 1 H), 7.72 (m, 1 H), 7.75 (m, 2 H), 7.82 (m, 1 H), 7.96 (m, 2 H), 8.11 (m, 2 H), 8.27 (m, 3 H), 10.49 (s, 2 H), 10.56 (s, 1 H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 36.03, 53.72, 112.75, 117.21, 119.79, 120.80, 121.75, 124.40, 124.42, 124.56, 126.63, 127.00, 128.29, 128.42, 129.44, 129.83, 129.91, 132.01, 132.86, 134.79, 135.05, 136.18, 136.63, 146.27, 152.14, 152.33, 163.23, 166.71, 194.13. MS (EI): m/z (%) = 242 (33), 119 (52), 118 (38), 117 (100).
$C_{35}H_{29}ClN_4O_6$ (637.1)

3.1.5. (E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonylphenylalanine amide (**2b**)

Purification: recrystallization from ethanol; yield 76%; m.p. 210 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3357, 1685, 1628, 1599, 1516, 1455, 1403, 1367,

1332, 1292, 1248, 1198, 1166, 1108, 1028, 968, 853, 752, 701. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.26 (s, 9 H), 2.60 (m, 1 H), 2.72 (m, 1 H), 4.15 (m, 1 H), 6.76 (d, J = 16 Hz, 1 H), 7.03–7.24 (m, 8 H), 7.41–7.87 (m, 7 H and d, J = 16 Hz, 1 H), 7.92 (s, 1 H), 7.99–8.02 (m, 2 H), 8.31–8.33 (m, 2 H), 10.40 (s, 1 H), 10.41 (s, 1 H). MS (FAB): m/z (%) = 700 (39, M$^+$), 645 (3), 601 (45), 509 (5), 480 (8), 454 (100), 438 (8).
$C_{40}H_{36}N_4O_8$ (700.8)

3.1.6. (E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)phenylalanine amide hydrochloride (**3b**)

Yield 58%; m.p. 195 °C. IR (KBr): ṽ (cm^{-1}) = 3374, 1684, 1653, 1625, 1598, 1559, 1507, 1403, 1332, 1247, 1197, 1108, 751, 701. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 2.71 (m, 1 H), 2.80 (m, 1 H), 4.16 (m, 1 H), 6.81 (d, J = 16 Hz, 1 H), 7.03–7.04 (m, 1 H), 7.21–7.66 (m, 10 H and d, J = 16 Hz), 7.76–8.01 (m, 6 H), 8.17 (s, 3 H), 8.32–8.34 (m, 3 H), 10.58 (s, 1 H), 10.70 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 36.11, 53.79, 112.83, 117.33, 119.88, 120.82, 121.83, 124.47, 124.50, 124.66, 126.74, 127.09, 128.37, 128.51, 129.49, 129.88, 129.98, 132.10, 132.95, 134.84, 135.12, 136.24, 136.69, 146.37, 152.23, 152.39, 163.29, 166.78, 194.22. MS (ESI): m/z (%) = 601 (44, [M$_{base}$ + H]$^+$), 583 (100), 488 (3), 454 (9), 382 (5), 342 (6), 271 (9), 217 (6), 194 (6), 171 (4), 120 (9), 105 (49), 83 (14).
$C_{35}H_{29}ClN_4O_6$ (637.1)

3.1.7. (E-RS)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonyl-4-trifluoromethylphenylalanine amide (**2c**)

Purification: recrystallization from ethanol; yield 73%; m.p. 229 °C. IR (KBr): ṽ (cm^{-1}) = 3305, 2981, 2933, 1725, 1691, 1677, 1628, 1599, 1557, 1514, 1444, 1331, 1289, 1164, 1110, 852, 795, 752. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.25 (s, 9 H), 2.59 (m, 1 H), 2.75 (m, 1 H), 4.02 (m, 1 H), 6.80 (d, J = 16 Hz, 1 H), 7.06 (m, 1 H), 7.23 (m, 1 H), 7.44 (m, 2 H), 7.46 (m, 2 H), 7.54 (m, 2 H), 7.65 (m, 3 H), 7.74 (m, 2 H), 7.90 (m, 1 H), 7.96 (m, 1 H), 8.02 (m, 2 H), 8.35 (m, 2 H), 8.60 (m, 1 H), 10.53 (s, 1 H), 10.54 (s, 1 H). MS (FAB): m/z (%) = 768 (10, [M + H]$^+$), 669 (20), 454 (65), 438 (11), 242 (100), 188 (67).
$C_{41}H_{35}F_3N_4O_8$ (768.8)

3.1.8. (E-RS)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-4-trifluoromethylphenylalanine amide hydrochloride (**3c**)

In contrast to General Procedure 2, a reaction time of two days was needed to complete deprotection. Yield 8%; m.p. 241 °C. IR (KBr): ṽ (cm^{-1}) = 3370, 2929, 1682, 1626, 1599, 1546, 1496, 1404, 1330, 1245, 1123, 852, 784, 752. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 2.73 (m, 1 H), 2.88 (m, 1 H), 4.20 (m, 1 H), 6.83 (d, J = 16 Hz, 1 H), 7.07 (m, 1 H), 7.43 (d, J = 16 Hz, 1 H), 7.47 (m 2 H), 7.51 (m, 2 H), 7.53 (m, 2 H), 7.68 (m, 3 H), 7.80 (m, 2 H), 7.83 (m, 1 H), 7.98 (m, 1 H), 8.02 (m, 2 H), 8.21 (s, 3 H), 8.34 (m, 2 H), 10.65 (s, 1 H), 10.88 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 36.15, 53.26, 112.85, 115.11, 117.45, 119.87, 120.67, 121.83, 124.48, 124.53, 124.59, 124.95, 125.38, 126.85, 129.67, 129.98, 130.38, 132.33, 132.85, 135.13, 136.36, 136.64, 139.70, 146.39, 152.27, 152.35, 163.28, 166.54, 194.26. MS (FAB): m/z (%) = 669 (88, M$_{base}$$^+$), 454 (43), 307 (100), 289 (65), 212 (19), 188 (60).
$C_{36}H_{28}ClF_3N_4O_6$ (705.1)

3.1.9. (E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonyl-4-chlorophenylalanine amide (**2d**)

Purification: recrystallization from ethanol; yield 72%; m.p. 230 °C. IR (KBr): ṽ (cm^{-1}) = 3355, 1682, 1658, 1628, 1596, 1553, 1521, 1446, 1405, 1367, 1330, 1291, 1275, 1249, 1222, 1203, 1165, 1108, 1093, 969, 852, 749. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.22 (s, 9 H), 2.49 (m, 1 H), 2.60 (m, 1 H), 4.10 (t, J = 7 Hz, 1 H), 6.73 (d, J = 16 Hz, 1 H), 7.04–7.05 (m, 1 H), 7.16–7.23 (m, 3 H), 7.29–7.31 (m, 2 H), 7.39 (d, J = 16 Hz, 1 H), 7.43–7.44 (m, 1 H), 7.50–7.54 (m, 2 H), 7.61–7.64 (m, 1 H), 7.70–7.72 (m, 2 H), 7.77–7.79 (m, 1 H), 7.86 (s, 1 H), 7.94–7.95 (m, 1 H), 7.97–8.00 (m, 2 H), 8.31–8.33 (m, 2 H), 10.46 (s, 1 H), 10.48 (s, 1 H). MS (FAB): m/z (%) = 737 (15, [M + H]$^+$), 736 (12, M$^+$), 735 (52, [M + H]$^+$), 734 (30, M$^+$), 637 (15), 635 (52), 455 (36), 454 (64), 453 (15), 242 (100).
$C_{40}H_{35}ClN_4O_8$ (735.2)

3.1.10. (E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-4-chlorophenylalanine amide hydrochloride (**3d**)

Yield 62%; m.p. 189 °C. IR (KBr): ṽ (cm^{-1}) = 3434, 1680, 1625, 1598, 1554, 1510, 1404, 1331, 1291, 1246, 1108, 852. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 2.60 (m, 1 H), 2.76 (m, 1 H), 4.15 (s, 1 H), 6.81 (d, J = 16 Hz, 1 H), 7.01–7.02 (m, 1 H), 7.28–7.32 (m, 4 H), 7.36–7.51 (m, 4 H and d, J = 16 Hz, 1 H), 7.59–7.62 (m, 1 H), 7.74–7.80 (m, 3 H), 7.91–7.93 (m, 1 H), 7.96–7.98 (m, 2 H), 8.19 (s, 3 H), 8.28–8.30 (m, 2 H), 10.71 (s, 1 H), 11.03 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 35.19, 53.62, 112.82, 117.30, 119.81, 120.82, 121.78, 124.46, 124.49, 124.68, 126.71, 128.35, 128.42, 129.80, 129.95, 131.43, 131.90, 132.18, 132.92, 133.86, 135.12, 136.25, 136.66, 146.36, 152.21, 152.38, 163.27, 166.58, 194.19. MS (FAB): m/z (%) = 637 (43, M$_{base}$$^+$), 635 (100, M$_{base}$$^+$), 509 (10), 454 (60), 212 (27).
$C_{36}H_{28}Cl_2N_4O_6$ (671.5)

3.1.11. (E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonyl-4-chlorophenylalanine amide (**2e**)

Purification: recrystallization from ethanol; yield 53%; m.p. 230 °C. IR (KBr): ṽ (cm^{-1}) = 3355, 1680, 1658, 1628, 1596, 1554, 1518, 1446, 1406, 1367, 1330, 1291, 1248, 1222, 1164, 1107, 1093, 969, 852, 749. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.22 (s, 9 H), 2.49 (m, 1 H), 2.63 (m, 1 H), 4.12 (t, J = 7 Hz, 1 H), 6.75 (d, J = 16 Hz, 1 H), 7.03–7.04 (m, 1 H), 7.15–7.22 (m, 3 H), 7.27–7.30 (m, 2 H), 7.39 (d, J = 16 Hz, 1 H), 7.43–7.44 (m, 1 H), 7.49–7.53 (m, 2 H), 7.59–7.63 (m, 1 H), 7.69–7.71 (m, 2 H), 7.75–7.78 (m, 1 H), 7.85 (s, 1 H), 7.91–7.94 (m, 1 H), 7.98–8.00 (m, 2 H), 8.31–8.33 (m, 2 H), 10.48 (s, 1 H), 10.50 (s, 1 H). MS (FAB): m/z (%) = 737 (15, [M + H]$^+$), 736 (21, M$^+$), 735 (30, [M + H]$^+$), 734 (28, M$^+$), 637 (11), 635 (30), 455 (26), 454 (79), 453 (13), 242 (100).
$C_{40}H_{35}ClN_4O_8$ (735.2)

3.1.12. (E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-4-chlorophenylalanine amide hydrochloride (**3e**)

Yield 50%; m.p. 189 °C. IR (KBr): ṽ (cm^{-1}) = 3433, 1700, 1679, 1626, 1598, 1556, 1510, 1403, 1332, 1290, 1251, 1196, 1108, 851, 753. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 2.63 (m, 1 H), 2.79 (m, 1 H), 4.18 (m, 1 H), 6.84 (d, J = 16 Hz, 1 H), 7.04–7.05 (m, 1 H), 7.31–7.35 (m, 4 H), 7.39–7.54 (m, 4 H and d, J = 16 Hz, 1 H), 7.62–7.65 (m, 1 H), 7.77–7.79 (m, 2 H), 7.82–7.83 (m, 1 H), 7.94–7.96 (m, 1 H), 7.99–8.01 (m, 2 H), 8.22 (s, 3 H), 8.31–8.33 (m, 2 H), 10.74 (s, 1 H), 11.07 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 35.32, 53.63, 112.83, 117.32, 119.83, 120.83, 121.80, 124.46, 124.50, 124.69, 126.72, 128.36, 128.43, 129.81, 129.96, 131.44, 131.91, 132.20, 132.93, 133.88, 135.12, 136.26, 136.67, 146.37, 152.22, 152.39, 163.28, 166.59, 194.20. MS (FAB): m/z (%) = 637 (31, M$_{base}$$^+$), 635 (100, M$_{base}$$^+$), 454 (53).
$C_{36}H_{28}Cl_2N_4O_6$ (671.5)

3.1.13. (E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)leucine amide hydrochloride (**3f**)

Yield 60%; m.p. 198 °C. IR (KBr): ṽ (cm^{-1}) = 3375, 2958, 1684, 1653, 1628, 1598, 1550, 1513, 1405, 1333, 1291, 1247, 1196, 852. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 0.79 (d, J = 6 Hz, 3 H), 0.80 (d, J = 6 Hz, 3 H), 1.00 (m, 1 H), 1.19 (m, 1 H), 1.50 (sept, J = 6 Hz, 1 H), 3.80 (s, 1 H), 6.83 (d, J = 16 Hz, 1 H), 7.05–7.06 (m, 1 H), 7.41 (d, J = 16 Hz, 1 H), 7.44–7.52 (m, 4 H), 7.62–7.65 (m, 1 H), 7.71–7.72 (m, 2 H), 7.81–7.82 (m, 1 H), 7.95–8.01 (m, 3 H), 8.20 (s, 3 H), 8.32–8.34 (m, 2 H), 10.70 (s, 2 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 23.43 (2 CH_3), 23.80, 39.92, 51.48, 113.46, 118.03, 120.25, 121.37, 122.32, 125.09, 125.14, 125.34, 127.38, 129.00, 130.30, 130.38, 133.18, 133.63, 135.71, 137.07, 137.22, 147.00, 152.85, 152.99, 163.88, 168.38, 194.66. MS (FAB): m/z (%) = 567 (96, M$_{base}$$^+$), 454 (60), 453 (26), 227 (100).
$C_{32}H_{31}ClN_4O_6$ (603.1)

3.1.14. (E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)leucine amide hydrochloride (**3g**)

Yield 59%; m.p. 198 °C. IR (KBr): ṽ (cm^{-1}) = 3372, 2958, 1684, 1628, 1597, 1550, 1512, 1404, 1333, 1291, 1247, 852. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 0.78 (d, J = 6 Hz, 3 H), 0.79 (d, J = 6 Hz, 3 H), 1.00 (m, 1 H), 1.19 (m, 1 H), 1.50 (sept, J = 6 Hz, 1 H), 3.81 (s, 1 H), 6.84 (d, J = 16 Hz, 1 H), 7.05–7.06 (m, 1 H), 7.40 (d, J = 16 Hz, 1 H), 7.43–7.53 (m, 4 H), 7.62–7.63 (m, 1 H), 7.71–7.73 (m, 2 H), 7.82–7.83 (m, 1 H), 7.98–7.97 (m, 3 H), 8.22 (s, 3 H), 8.31–8.33 (m, 2 H), 10.71 (s, 1 H), 10.72 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 23.29, 23.68, 39.83, 51.38, 113.33, 117.86, 120.14, 121.30, 122.20, 124.97, 125.01, 125.22, 127.24, 128.87, 130.21, 130.26, 133.06, 133.49, 135.62, 136.95, 137.13, 146.87, 152.73, 152.88, 163.77, 168.26, 194.75. MS (FAB): m/z (%) = 567 (100, M$_{base}$$^+$), 454 (35), 453 (10), 242 (29).
$C_{32}H_{31}ClN_4O_6$ (603.1)

3.1.15. (E-2S, 3S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)isoleucine amide hydrochloride (**3h**)

Yield 60%; m.p. 196 °C. IR (KBr): ṽ (cm^{-1}) = 3433, 2964, 2928, 1679, 1628, 1598, 1550, 1512, 1403, 1332, 1291, 1246, 1197, 1107, 851. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 0.79 (m, 3H,), 0.82 (s, 3 H), 1.03 (m, 1 H), 1.41 (m, 1 H), 1.77 (s, 1 H), 3.87 (s, 1 H), 6.83 (d, J = 16 Hz, 1 H), 7.03–7.04 (m, 1 H), 7.37 (d, J = 16 Hz, 1 H), 7.44–7.55 (m, 4 H), 7.62–7.67 (m, 1 H), 7.78–7.81 (m, 3 H), 7.91–7.94 (m, 1 H), 7.98–8.00 (m, 2 H), 8.26 (s, 3 H), 8.30–8.33 (m, 2 H), 10.74 (s, 1 H), 10.86 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 11.95, 14.55, 24.36, 36.90, 57.25, 113.48,

117.99, 119.84, 121.42, 122.02, 125.06, 125.13, 127.28, 128.98, 130.38, 130.65, 132.98, 133.60, 135.71, 136.58, 137.00, 146.94, 152.80, 152.97, 163.86, 167.22, 194.57. MS (FAB): m/z (%) = 567 (100, M_{base}^+), 454 (9), 353 (6).
$C_{32}H_{31}ClN_4O_6$ (603.1)

3.1.16. *(E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonylmethionine amide* (**2i**)

Purification: recrystallization from ethanol : ethyl acetate (3 : 1); yield 92%; m.p. 162 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3274, 1711, 1681, 1663, 1639, 1626, 1598, 1556, 1502, 1436, 1394, 1367, 1332, 1284, 1246, 1200, 1162, 1108, 1032, 969, 851, 752. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.33 (s, 9 H), 1.61 (m, 2 H), 1.99 (s, 3 H), 2.39 (m, 2 H), 4.00 (m, 1 H), 6.75 (d, J = 16 Hz, 1 H), 7.04–7.05 (m, 1 H), 7.24–7.29 (m, 1 H), 7.35–7.55 (m, 3 H and d, J = 16 Hz, 1 H), 7.64–7.80 (m, 3 H), 7.87 (s, 1 H), 7.93–7.98 (m, 2 H), 8.31–8.33 (m, 2 H), 8.56–8.57 (m, 2 H), 10.50 (s, 1 H), 10.51 (s, 1 H). MS (FAB): m/z (%) = 685 (33, [M + H]$^+$), 684 (23, M$^+$), 629 (7), 586 (17), 585 (43), 554 (14), 455 (30), 454 (100), 453 (28), 242 (97), 212 (44).
$C_{36}H_3N_4O_8S$ (684.8)

3.1.17. *(E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)methionine amide hydrochloride* (**3i**)

Yield 30%; m.p. 179 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 2915, 1696, 1627, 1597, 1508, 1331, 1290, 1231, 1108, 962, 850. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.75 (m, 2 H), 2.02 (s, 3 H), 2.38 (m, 2 H), 3.79 (m, 1 H), 6.83 (d, J = 16 Hz, 1 H), 7.03–7.04 (m, 1 H), 7.38–7.54 (m, 4 H and d, J = 16 Hz, 1 H), 7.63–7.65 (m, 1 H), 7.71–7.81 (m, 3 H), 7.93–8.01 (m, 3 H), 8.30–8.32 (m, 2 H), 8.35 (s, 3 H), 10.73 (s, 1 H), 10.93 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 14.96, 26.56, 30.98, 52.28, 113.45, 117.95, 120.22, 121.43, 122.27, 125.07, 125.12, 125.35, 127.32, 128.96, 130.29, 130.51, 133.12, 133.64, 135.73, 136.92, 137.14, 146.97, 152.83, 152.99, 163.89, 167.47, 194.72. MS (FAB): m/z (%) = 585 (100, M_{base}^+), 454 (39).
$C_{31}H_{28}ClN_4O_6S$ (621.1)

3.1.18. *(E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)-N-tert.-butoxycarbonylmethionine amide* (**2j**)

Purification: recrystallization from ethanol; yield 85%; m.p. 162 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3275, 1711, 1681, 1663, 1639, 1625, 1598, 1553, 1503, 1436, 1332, 1284, 1246, 1199, 1162, 1108, 967, 851. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.33 (s, 9 H), 1.60 (m, 2 H), 2.00 (s, 3 H), 2.40 (m, 2 H), 4.00 (m, 1 H), 6.75 (d, J = 16 Hz, 1 H), 7.04–7.05 (m, 1 H), 7.26–7.56 (m, 4 H and d, J = 16 Hz, 1 H), 7.62–7.81 (m, 3 H), 7.87 (s, 1 H), 7.91–8.00 (m, 2 H), 8.31–8.34 (m, 2 H), 8.56–8.57 (m, 2 H), 10.51 (s, 1 H), 10.52 (s, 1 H). MS (FAB): m/z (%) = 685 (17, [M + H]$^+$), 684 (14, M$^+$), 586 (13), 585 (33), 554 (11), 455 (23), 454 (91), 453 (24), 242 (100), 212 (44).
$C_{36}H_3N_4O_8S$ (684.8)

3.1.19. *(E-R)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)methionine amide hydrochloride (3j).*

Yield 40%; m.p. 179 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 2920, 1695, 1623, 1596, 1509, 1331, 1292, 1235, 1107, 964, 850. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.73 (m, 2 H), 2.02 (s, 3 H), 2.38 (m, 2 H), 3.97 (m, 1 H), 6.84 (d, J = 16 Hz, 1 H), 7.03–7.04 (m, 1 H), 7.38 –7.52 (m, 4 H and d, J = 16 Hz, 1 H), 7.62–7.81 (m, 4 H), 7.93–8.00 (m, 3 H), 8.30–8.32 (m, 2 H), 8.34 (s, 3 H), 10.74 (s, 1 H), 10.95 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 14.96, 28.57, 30.99, 52.28, 113.45, 117.93, 120.21, 121.46, 122.28, 124.95, 125.12, 125.34, 127.31, 128.96, 130.30, 130.51, 133.12, 133.63, 135.73, 136.92, 137.14, 146.97, 152.82, 153.00, 163.89, 167.48, 194.72. MS (FAB): m/z (%) = 585 (100, M_{base}^+), 454 (38), 366 (41), 344 (56).
$C_{31}H_{28}ClN_4O_6S$ (621.1)

3.1.20. *(E-S)-N-[2-Benzoyl-4-{3-[5-(4-nitrophenyl)-2-furyl]acryloyl-amino}phenyl)tryptophane amide hydrochloride* (**3k**)

Yield 48%; m.p. 220 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3288, 2920, 2853, 1683, 1597, 1512, 1404, 1331, 1291, 1253, 1117, 745. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 2.75 (m, 1 H), 2.82 (m, 1 H), 4.08 (s, 1 H), 6.86 (d, J = 16 Hz, 1 H), 6.97–7.22 (m, 4 H), 7.31–7.85 (m, 9 H and d, J = 16 Hz, 1 H), 7.95–8.05 (m, 3 H), 8.11 (s, 3 H), 8.30–8.34 (m, 2 H), 8.89–8.91 (m, 1 H), 10.78 (s, 1 H), 10.98 (s, 1 H), 11.08 (s, 1 H). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 27.05, 53.45, 107.20, 112.02, 113.49, 118.03, 118.97, 119.24, 120.44, 121.43, 121.71, 122.41, 125.16, 125.72, 127.58, 128.98, 129.26, 130.58, 132.84, 133.57, 135.74, 136.91, 137.29, 143.01, 145.99, 146.98, 152.83, 153.00, 163.90, 167.82, 194.82. MS (FAB): m/z (%) = 640 (94, M_{base}^+), 563 (15), 510 (15), 454 (26), 453 (22), 359 (31), 233 (100).
$C_{37}H_{29}ClN_5O_6$ (676.1)

3.2. *Enzyme preparation*

Yeast farnesyltransferase was used as a fusionprotein to Glutathione S-transferase at the N-terminus of the β-subunit. Farnesyltransferase was expressed in *Escherichia coli* DH5α grown in LB media containing ampicillin and chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for farnesyltransferase production (Del Villar 1997). The enzyme was purified by standard procedures with glutathione-agarose beads for selective binding of the target protein.

3.3. *Farnesyltransferase assay*

The assay was conducted as described (Pompliano et al. 1992). Farnesylpyrophosphate (FPP) was obtained as a solution of the ammonium salt in methanol-10 mM aqueous NH_4Cl (7 : 3) from Sigma-Aldrich. Dansyl-Gly-CysValLeuSer (Ds-GCVLS) was custom synthesized by ZMBH, Heidelberg, Germany. The assay mixture (100 μL volume) contained 50 mM Tris/HCl pH 7.4, 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM dithiothreitol (DTT), 7 μM Ds-GCVLS, 20 μM FPP and 5 nmol (approx.) yeast GST-farnesyltransferase and 1% of various concentrations of the test compounds dissolved in dimethylsulfoxide (DMSO). The progress of the enzyme reaction was followed by monitoring the enhancement of the fluorescence emission at 505 nm (excitation 340 nm). The reaction was started by addition of FPP and run in a Quartz cuvette thermostattated at 37 °C. Fluorescence emission was recorded with a Perkin Elmer LS50B spectrometer. IC_{50} values (concentrations resulting in 50% inhibition) were calculated from initial velocity of three independent measurements of four to five different concentrations of the respective inhibitor.

3.4. *Cytotoxicity assay*

HeLa (DSM ACC 57) cells were grown in RPMI 1640 culture medium (GIBCO BRL 21875–034) supplemented with 25 μg/ml gentamicin sulfate (BioWhittaker 17-528Z), and 10% heat inactivated fetal bovine serum (GIBCO BRL 10500-064) at 37 °C in high density polyethylene flasks (NUNC 156340). The test substances are dissolved in DMSO (10 mg/ml) before being diluted in the cell culture medium (1 : 200). The adherent HeLa cells were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG L2163). For each experiment approximately 10,000 cells were seeded with 0.1 ml RPMI 1640 (GIBCO BRL 21875-034), containing 25 μg/ml gentamicin sulfate (BioWhittaker 17-528Z), but without HEPES, per well of the 96-well microplates (NUNC 167008). For the cytotoxic assay HeLa cells were preincubated for 48 h without the test substances. The dilutions of the test substances were carried out carefully on the monolayers of HeLa cells after the preincubation time. Cells of HeLa were incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2. The adherent HeLa cells were fixed by 25% glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing the stain was eluted by 0.2 ml of 0.33 N HCl in the wells. The optical densities were measured at 660 nm in a SUNRISE microplate reader (TECAN). Comparisons of the different values were performed with software Magellan (TECAN).

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

Compounds were tested by a semiautomated microdilution assay against intraerythrocytic forms of *P. falciparum* (Desjardins et al. 1979). The *P. falciparum* strain Dd2 was cultivated by a modification of the method described by Trager and Jensen (1976). 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 ≤ 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 (Ancelin 1998). 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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