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Novel lead structures for antimalarial farnesyltransferase inhibitors

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Through the combination of nitrophenylfurylacryloyl moiety which has been designed to occupy an aryl binding site of farnesyltransferase with several AA(X)-peptidomimetic substructures some novel farnesyltransferase inhibitors were obtained. Evaluation of their antimalarial activity and some initial modifications yielded a 4-benzophenone- and a sulfonamid-based novel lead for antimalarial farnesyltransferase inhibitors.

1. Introduction

Farnesyltransferase is the crucial enzyme of the post-translational modification of several proteins 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 proteins with a carboxy-terminal 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). Inhibitors of farnesyltransferase have been developed as cancer therapeutics of which several are in advanced stages of clinical trials (Cox and Der 2002; Purcell and Donehower 2002).

In addition to mammals, farnesyltransferases were also identified in pathogenic protozoa including *Plasmodium falciparum*, the causative agent of malaria tropica (Chakrabarti et al. 1998; Chakrabarti et al. 2002). Malaria tropica is the most important protozoa caused disease accounting for 2 to 3 million death cases each year. Because of the increasing spread of malaria parasites resistant to chloroquine and other commonly used antimalarials there is an urgent need for new therapeutics (Sachs and Malaney 2002; Ridley 2002).

We have designed the nitrophenylfurylacryloyl moiety as a substructure for our 2,5-diaminobenzophenone-based farnesyltransferase inhibitors capable of occupying an aryl binding site of farnesyltransferase (Böhm et al. 2001). Subsequently, we combined the nitrophenylfurylacryloyl moiety with several other peptidomimetic substructures mimicking the AA(X)-portion of the CAAX-consensus sequence of farnesylated peptides. Thereby, we obtained the novel farnesyltransferase inhibitors **3–7** (Sakowski et al. 2002). In this study, we combined the nitrophenylfurylacryloyl moiety with some additional peptidomimetic substructures and addressed the question whether these compounds may serve as novel lead structures for antimalarial farnesyltransferase inhibitors.

2. Investigations, results and discussion

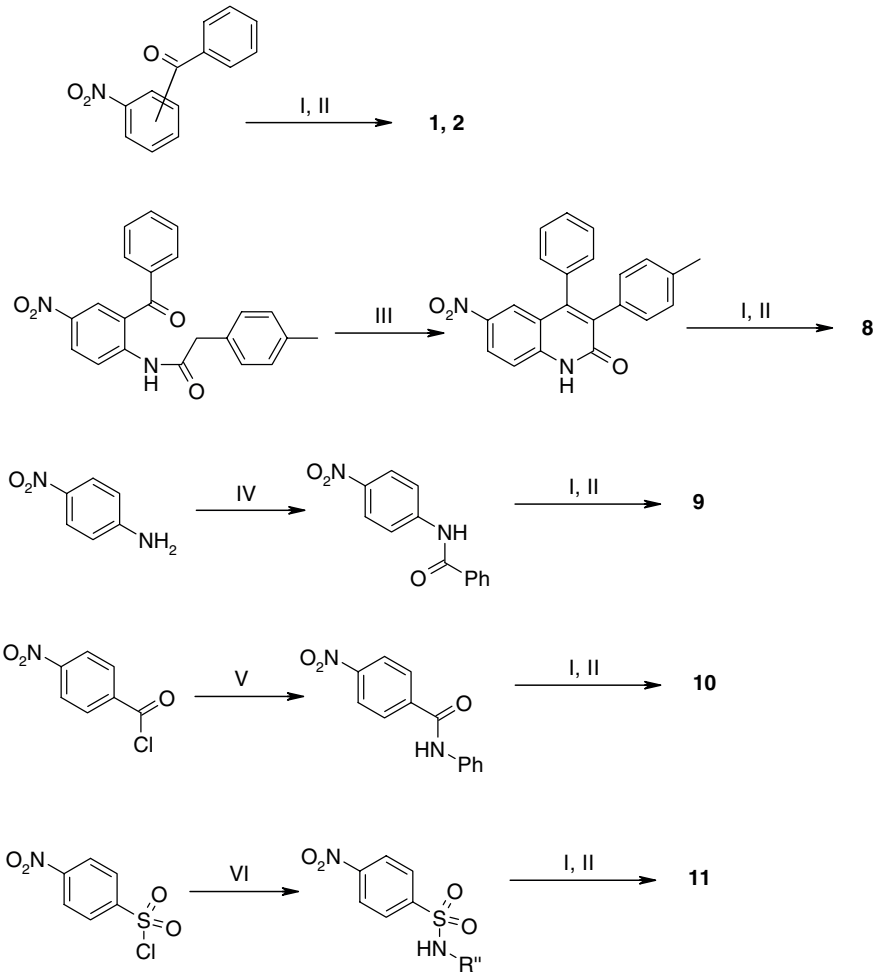
The synthesis of inhibitors **3–7** and their farnesyltransferase inhibitory activity has been described elsewhere (Sakowski et al. 2002). The final step in the preparation of all other compounds was the amide formation between nitrophenylfurylacryloyl chloride (Böhm et al. 2001) and the amino group of the appropriate peptidomimetic substructure. This amino group has been obtained in every case through tin-II-chloride reduction of the corresponding nitro compounds. The starting compounds for the inhibitors **1** and **2** (2- and 3-nitrobenzophenone) are commercially available. 6-Nitro-4-phenyl-3-tolyl-2(1*H*)-quinolinone was obtained through base-catalyzed intramolecular condensation from 5-nitro-2-tolylacetylaminobenzophenone (Sakowski et al. 2001). The reaction of benzoyl chloride with 4-nitroaniline yielded the intermediate for the preparation of inhibitors **9**. Key intermediates for the remaining inhibitors were obtained form the reaction of 4-nitrobenzoyl chloride and 4-nitrobenzenesulfonic acid chloride, respectively with appropriate amines (Scheme 1).

The farnesyltransferase inhibitory activity of the Compounds was determined using the fluorescence enhancement assay described by Pompliano et al. (1992). The assay employes 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 (Chakrabarti et al. 2002). Thus, compounds **1–11** were assayed in a growth inhibition assay against the intraerythrocytic forms of *P. falciparum* strain Dd2. The growth of the parasites was monitored through the incorporation of tritium labeled hypoxanthine Dd2 (Desjardins et al. 1979; Trager and Jensen 1976; Ancelin et al. 1998).

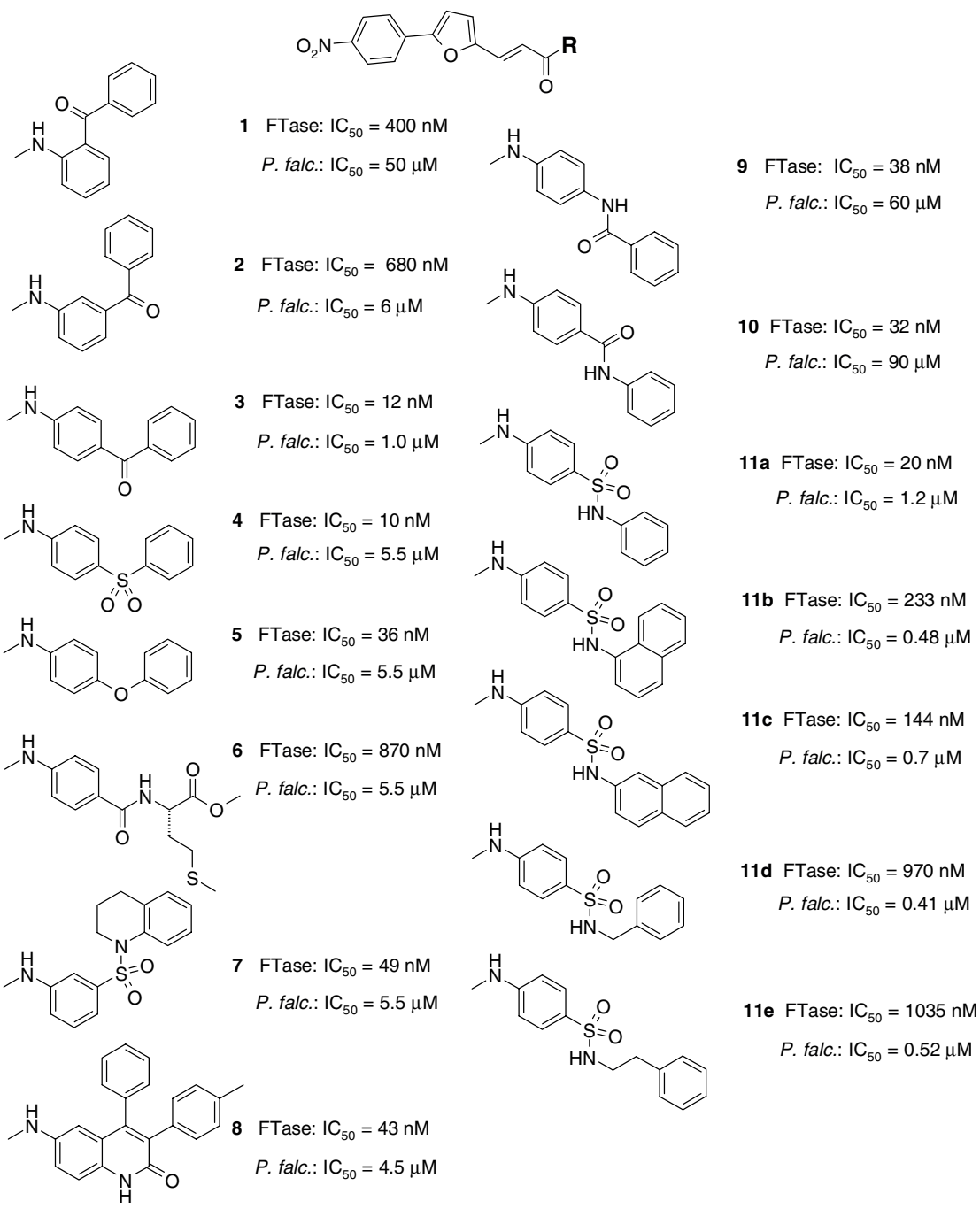
In a previous study, we obtained the novel farnesyltransferase inhibitors **3–7** through the combination of the nitro-

Scheme



(I) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2 h; (II) 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride, toluene/dioxane, reflux, 2 h; (III) NaOH, THF, reflux, 1 h; (IV) benzoyl chloride, toluene/dioxane, reflux, 2 h; (V) aniline, toluene/dioxane, reflux, 2 h; (VI) R″-NH_2, triethylamine or *N*-methylmorpholine, CH_2Cl_2, rt, overnight.

phenylfurylacryloyl moiety and different AA(X)-peptidomimetic substructures. These inhibitors **3–7** displayed high activity against farnesyltransferase (inhibitor **6** is a membrane permeable methyl ester prodrug; the parent acid has an IC_{50}-value of 90 nM) (Sakowski et al. 2002). From these 4 inhibitors, the 4-benzophenone compound **3** displayed the highest activity against intraerythrocytic forms of *P. falciparum* with an IC_{50}-value of 1 μM while the remaining inhibitors **4–7** were less active ($IC_{50} = 5.5$ μM). Lower antimalarial activity ($IC_{50} = 4.5$ μM) was also found for the novel quinolone-based inhibitor **8** which displays high farnesyltransferase inhibitory activity ($IC_{50} = 43$ nM). The regioisomers **1** and **2** of the 4-benzophenone-based inhibitor **3**, displayed considerably reduced activity against farnesyltransferase and the growth of malaria parasites. In a following step, the carbonyl linker of inhibitor **3** was replaced by an amide linkage, since such simple carboamides are more readily accessible from commercially available starting materials. Unfortunately, this modification (inhibitors **9**, **10**) proved to be vastly detrimental to antimalarial activity (IC_{50}-values 60 and 90 μM, respectively), although these inhibitors preserved their high farnesyltransferase inhibitory activity (IC_{50}-values 38 and 32 nM, respectively). In contrast, the replacement of the sulfonyl linker in inhibitor **4** by a sulfonamide yielded a compound (**11a**) with improved antimalarial activity ($IC_{50} = 1.2$ μM) and similar activity against farnesyltransferase ($IC_{50} = 20$ nM). Therefore, this inhibitor was selected for further modification to evaluate its usefulness as a novel lead structure for antimalarial farnesyltransferase inhibitors. Indeed, enlargement of the *N*-aryl residue or introduction of a methylene or ethylene spacer between the sulfonamid nitrogen and the phenyl residue resulted in inhibitors **11b–e** with enhanced antimalarial activity (IC_{50}-values between 0.7 and 0.41 μM), while the farnesyltransferase activity was reduced in comparison to inhibitor **11a**. The most active antimalarial agent of this series is the benzylamide **11d** with an IC_{50}-value of 410 nM. Interestingly, this compound displays comparatively low activity against yeast farnesyltransferase ($IC_{50} = 970$ nM), thus providing some argument for the possibility for a specific antimalarial farnesyltransferase inhibitor. The same trend is visible for inhibitor **11e** which displays an IC_{50} of 520 nM against cultured parasites, while it has an IC_{50} of 1035 nM against the yeast enzyme. These data demonstrate that by variation of the peptidomimetic substructure of farnesyltransferase inhibitors new compounds with high antimalarial activity can be obtained. In the series of sulfonamide-based inhibitors there were two examples in which the activity against cultured malaria parasites was higher



than against isolated recombinant yeast farnesyltransferase thus providing evidence for the feasibility to develop specific antimalarial farnesyltransferase inhibitors.

3. Experimental

3.1. Preparation

^{1}H 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) from Merck.

3.1.1. General procedure 1: Activation of various acids as acid chlorides and reaction with aromatic amines

The various carboxylic acids were dissolved in dichloromethane and 0.2 mL oxalylchloride per mmol acid was added. The mixture was stirred for 2 h and the volatiles were evaporated *in vacuo*. The resulting acyl chlorides were dissolved in 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* and the crude products were purified by recrystallisation.

3.1.2. General procedure 2: Reduction of aromatic nitro compounds

Aromatic nitro compounds were dissolved in EtOAc (50–100 mL) and $SnCl_2 \times 2H_2O$ (1.125 g per mmol nitro compound) was added. The mixture was heated under reflux for 2 h. Then, $NaHCO_3$-solution was added until pH 7–8 was reached and the organic layer was separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. Then, the solvent was removed *in vacuo* to obtain the crude products.

3.1.3. General procedure 3: Reaction of various amines with 4-nitrobenzenesulfonylchloride

The amines were dissolved in dichloromethane, cooled to 0 °C and triethylamine (0.125 ml per mmol amine) or *N*-methylmorpholine (0.24 ml per

mmol amine) was added. 4-Nitrobenzenesulfonylchloride was dissolved in dichloromethane and added to the solution. After stirring over night at room temperature the solution was washed with hydrochloric acid (10%), saturated $NaHCO_3$-solution and brine. The organic layer was dried over $MgSO_4$. Then, the solvent was removed *in vacuo* to obtain the crude products.

3.1.4. (E)-N-(2-Benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**1**)

According to general procedure 1 from 2-aminobenzophenone and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 54%; m.p. 211 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3444, 1621, 1599, 1583, 1514, 1448, 1332, 1263, 1203, 1163, 853, 753, 700. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.61 (d, J = 16 Hz, 1 H), 6.67–7.19 (m, 3 H), 7.43–7.47 (m, 2 H), 7.47 (d, J = 16 Hz, 1 H), 7.55–7.67 (m, 5 H), 7.81–7.83 (m, 2 H), 8.21–8.23 (m, 2 H), 8.76–8.78 (m, 1 H), 11.19 (s, 1 H). MS (EI): m/z (%) = 438 (3, M^+), 408 (32), 212 (100).

3.1.5. (E)-N-(3-Benzoylphenyl)-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**2**)

According to general procedure 1 from 3-aminobenzophenone and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 63%; m.p. 216 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3378, 1683, 1653, 1624, 1598, 1544, 1514, 1331, 1285, 1245, 1107, 854. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.80 (d, J = 16 Hz, 1 H), 7.05–7.06 (m, 1 H), 7.42–7.77 (m, 7 H and d, J = 16 Hz, 1 H), 8.00–8.12 (m, 4 H), 8.31–8.33 (m), 8.75–8.77 (m, 1 H), 10.46 (s, 1 H). MS (EI): m/z (%) = 438 (17, M^+), 242 (100), 196 (28).

3.1.6. 6-Nitro-4-phenyl-3-tolyl-2(1H)-quinolinone

N-(2-Benzoyl-4-nitrophenyl)-4-methylphenyl acetic acid amide was boiled in a mixture of 0.1 N NaOH and tetrahydrofurane. After cooling, water and conc. HCl were added, until pH 1 was reached. The aqueous phase was extracted with EtOAc for three times. The combined organic layers were washed with brine and dried over $MgSO_4$. Then, the solvent was removed *in vacuo* to obtain the crude product. Yield 48%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.26 (s, 3 H), 7.00–7.18 (m, 7 H), 7.26–7.27 (m, 3 H), 8.02–8.09 (m, 2 H), 12.47 (s, 1 H).

3.1.7. 6-Amino-4-phenyl-3-tolyl-2(1H)-quinolinone

According to general procedure 2 from 6-nitro-4-phenyl-3-tolyl-2(1*H*)-quinolinone. Yield 87%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.16 (s, 3 H), 4.79 (s, 2 H), 6.17–6.18 (m, 1 H), 6.81–6.84 (m, 1 H), 6.89–6.90 (m, 4 H), 7.06–7.14 (m, 3 H), 7.20–7.28 (m, 3 H), 11.36 (s, 1 H).

3.1.8. 3-[5-(4-Nitrophenyl)-2-furyl]-N-[4-phenyl-3-tolyl-1,2-dihydro-2-oxo-6-quinolinyl]acrylic acid amide (**8**)

According to general procedure 1 from 6-amino-4-phenyl-3-tolyl-2(1*H*)-quinolinone and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride Yield 56%; m.p. > 260 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3448, 1641, 1600, 1561, 1551, 1516, 1498, 1333. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.19 (s, 3 H), 6.72 (d, J = 16 Hz, 1 H), 6.93–6.99 (m, 5 H), 7.14–7.16 (m, 2 H), 7.27–7.41 (m, 6 H and d, J = 16 Hz, 1 H), 7.94–7.99 (m, 3 H), 8.29–8.31 (m, 2 H), 10.19 (s, 1 H), 11.88 (s, 1 H). MS (EI): m/z (%) = 567 (1, M^+), 381 (11), 352 (13), 351 (14), 327 (23), 326 (100).

3.1.9. N-(4-Nitrophenyl)benzamide

According to general procedure 1 from 4-nitroaniline and benzoylchloride. Yield 50%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.51–7.58 (m, 2 H), 7.62–7.65 (m, 1 H), 7.96–8.09 (m, 4 H), 8.24–8.28 (m, 2 H), 10.75 (s, 1 H).

3.1.10. N-(4-Aminophenyl)benzamide

According to general procedure 2 from *N*-(4-nitrophenyl)benzamide. Yield 94%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.85 (s, 2 H), 6.53–6.55 (m, 2 H), 7.34–7.36 (m, 2 H), 7.45–7.52 (m, 3 H), 7.89–7.95 (m, 2 H), 9.75 (s, 1 H).

3.1.11. (E)-N-[4-(Benzoylamino)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]-acrylic acid amide (**9**)

According to general procedure 1 from *N*-(4-aminophenyl)benzamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 45%; m.p. > 260 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3348, 1654, 1626, 1600, 1548, 1515, 1404, 1332, 1108, 715. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.82 (d, J = 16 Hz, 1 H), 7.01–7.02 (m, 1 H), 7.27–7.30 (m, 2 H), 7.39–7.57 (m, 4 H and d, J = 16 Hz, 1 H), 7.62–7.71 (m, 2 H), 7.80–7.83 (m, 2 H), 7.93–7.99 (m, 3 H), 8.28–8.30 (m, 1 H), 10.12 (s, 1 H), 10.26 (s, 1 H). MS (EI): m/z (%) = 453 (27, M^+), 242 (63), 212 (80),105 (100).

3.1.12. 4-Nitro-N-phenylbenzamide

According to general procedure 1 from aniline and 4-nitrobenzoylchloride. Yield 81%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.12–7.16 (m, 1 H), 7.35–7.39 (m, 2 H), 7.76–7.79 (m, 2 H), 8.17–8.21 (m, 2 H), 8.35–8.38 (m, 2 H), 10.49 (s, 1 H).

3.1.13. 4-Amino-N-phenylbenzamide

According to general procedure 2 from 4-nitro-*N*-phenylbenzamide. Yield 68%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 5.67 (s, 2 H), 6.57–6.60 (m, 2 H), 7.00–7.04 (m, 1 H), 7.27–7.29 (m, 2 H), 7.67–7.73 (m, 4 H), 9.69 (s, 1 H).

3.1.14. (E)-N-[4-(Phenylaminocarbonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**10**)

According to general procedure 1 from 4-amino-*N*-phenylbenzamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 84%; m.p. > 259 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3422, 1658, 1622, 1599, 1528, 1508, 1332, 1246. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.79 (d, J = 16 Hz, 1 H), 7.01–7.04 (m, 2 H), 7.26–7.42 (m, 3 H and d, J = 16 Hz, 1 H), 7.66–7.97 (m, 8 H), 8.27–8.29 (m, 2 H), 9.98 (s, 1 H), 10.46 (s, 1 H). MS (EI): m/z (%) = 453 (56, M^+), 361 (58), 242 (94), 212 (58), 146 (28), 120 (100).

3.1.15. 4-Nitro-N-phenylbenzenesulfonamide

According to general procedure 3 from aniline and 4-nitrobenzenesulfonylchloride. Yield 20%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.03–7.22 (m, 5 H), 7.94–7.97 (m, 2 H), 8.321–8.33 (m, 2 H), 10.45 (s, 1 H).

3.1.16. 4-Amino-N-phenylbenzenesulfonamide

According to general procedure 2 from 4-nitro-*N*-phenylbenzenesulfonamide. Yield 95%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 5.84 (s, 2 H), 6.51–6.53 (m, 2 H), 6.93–6.96 (m, 1 H), 7.03–7.14 (m, 4 H), 7.35–7.37 (m, 2 H), 9.70 (s, 1 H).

3.1.17. (E)-N-[4-(Phenylaminosulfonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**11a**)

According to general procedure 1 from 4-amino-*N*-phenylbenzenesulfonamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 35%; m.p. > 260 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 1617, 1598, 1529, 1497, 1334, 1157. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.78 (d, J = 16 Hz, 1 H), 6.99–7.23 (m, 6 H), 7.41–7.50 (m, 1 H and d, J = 16 Hz, 1 H), 7.02–7.72 (m, 2 H), 7.80–7.82 (m, 2 H), 7.99–8.01 (m, 2H), 8.31–8.33 (m, 2 H), 10.06 (s, 1 H), 10,56 (s, 1 H). MS (EI): m/z (%) = 489 (9, M^+), 248 (91), 242 (49), 156 (100), 108 (16), 93 (20).

3.1.18. N-1-Naphthyl-4-nitrobenzenesulfonamide

According to general procedure 3 from 1-naphthylamine and 4-nitrobenzenesulfonylchloride. Yield 25%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.14–7.16 (m, 1 H), 7.38–7.49 (m, 3 H), 7.80–7.98 (m, 5 H), 8.30–8.32 (m, 2 H), 10.49 (s, 1 H).

3.1.19. 4-Amino-N-1-naphthylbenzenesulfonamide

According to general procedure 2 from *N*-1-naphthyl-4-nitrobenzenesulfonamide. Yield 98%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 5.76 (s, 2 H), 6.44–6.47 (m, 2 H), 7.13–8.08 (m, 9 H), 9.58 (s, 1 H).

3.1.20. (E)-N-[4-(1-Naphthylaminosulfonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**11b**)

According to general procedure 1 from 4-amino-1-*N*-naphthylbenzenesulfonamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 27%; m.p. 287 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3330, 1666, 1653, 1596, 1508, 1403, 1334, 1248, 1184, 1151, 1110, 1094, 853, 800, 776, 753. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.49 (d, J = 16 Hz, 1 H), 7.07–7.18 (m, 2 H), 7.38–7.46 (m, 4 H), 7.49 (d, J = 16 Hz, 1 H), 7.65–7.88 (m, 6 H), 8.00–8.08 (m, 3 H), 8.27–8.24 (m, 2 H), 10.02 (s, 1 H), 10.54 (s, 1 H). MS (EI): m/z (%) = 539 (2, M^+), 384 (33), 298 (66), 259 (8), 242 (67), 234 (21), 156 (20), 143 (100), 142 (53), 115 (45), 93 (23), 92 (11).

3.1.21. N-2-Naphthyl-4-nitrobenzenesulfonamide

According to general procedure 3 from 2-naphthylamine and 4-nitrobenzenesulfonylchloride. Yield 76%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.27–7.31 (m, 1 H), 7.37–7.46 (m, 2 H), 7.58 – 7.59 (m, 1 H), 7.76–7.80 (m, 3 H), 8.03–8.06 (m, 2 H), 8.33–8.35 (m, 2 H), 10.80 (s, 1 H).

3.1.22. 4-Amino-N-2-naphthylbenzenesulfonamide

According to general procedure 2 from *N*-2-naphthyl-4-nitrobenzenesulfonamide. Yield 100%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 5.94 (s, 2 H), 6.48–6.52 (m, 2 H), 7.26–7.51 (m, 6 H), 7.72–7.77 (m, 3 H), 10.11 (s, 1 H).

3.1.23. (E)-N-[4-(2-Naphthylaminosulfonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**11c**)

According to general procedure 1 from 4-amino-2-*N*-naphthylbenzenesulfonamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 58%;

m.p. 263 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3357, 3190, 2922, 1662, 1614, 1594, 1518, 1465, 1400, 1359, 1332, 1294, 1240, 1198, 1161, 1104, 1026, 967, 958, 923, 854, 789, 753. ^{1}H NMR (DMSO-d_6): δ (ppm) = 6.76 (d, J = 16 Hz, 1 H), 7.07–7.09 (m, 1 H), 7.29–7.56 (m, 5 H and d, J = 16 Hz, 1 H), 7.76–8.00 (m, 7 H), 8.00–8.03 (m, 2 H), 8.33–8.35 (m, 2 H), 10.45 (s, 1 H), 10.64 (s, 1 H). MS (ESI): m/z (%) = 539 (100, M^+), 231 (43), 189 (39), 161 (76), 149 (72).

3.1.24. N-Benzyl-4-nitrobenzenesulfonamide

According to general procedure 3 from benzylamine and 4-nitrobenzenesulfonylchloride. Yield 59%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.04 (s, 2 H), 7.15–7.23 (m, 5 H), 7.94–7.98 (m, 2 H), 8.28–8.32 (m, 2 H), 10.42 (s, 1 H).

3.1.25. 4-Amino-N-benzylbenzenesulfonamide

According to general procedure 2 from *N*-benzyl-4-nitrobenzenesulfonamide. Yield 92%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.85 (s, 2 H), 5.77 (s, 2 H), 6.56–6.59 (m, 2 H), 7.16–7.25 (m, 4 H), 7.39–7.49 (m, 3 H), 9.87 (s, 1 H).

3.1.26. (E)-N-[4-(Benzylaminosulfonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**11d**)

According to general procedure 1 from 4-amino-2-*N*-benzylbenzenesulfonamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 73%; m.p. 241 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3350, 1691, 1627, 1594, 1536, 1514, 1402, 1331, 1296, 1247, 1197, 1152, 1107, 855, 835, 800, 753. ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.94 (s, 2 H), 6.80–6.83 (m, 1 H and d, J = 16 Hz, 1 H), 7.20–7.26 (m, 4 H), 7.40–7.42 (m, 2 H), 7.45–8.00 (m, 6 H and d, J = 16 Hz, 1 H), 8.29–8.31 (m, 2 H), 9.88 (s, 1 H), 10.54 (s, 1 H). MS (EI): m/z (%) = 503 (16, M^+), 262 (17), 243 (16), 106 (21), 93 (12).

3.1.27. 4-Nitro-N-phenethylsulfonamide

According to general procedure 3 from phenethylamine and 4-nitrobenzenesulfonylchloride. Yield 82%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.69 (t, J = 7 Hz, 2 H), 3.05 (t, J = 7 Hz, 2 H), 7.14–7.18 (m, 3 H), 7.23–7.26 (m, 2 H), 7.99–8.02 (m, 2 H), 8.10 (s, 1 H), 8.36–8.39 (m, 2 H).

3.1.28. 4-Amino-N-phenethylbenzenesulfonamide

According to general procedure 2 from 4-nitro-*N*-phenethylbenzenesulfonamide. Yield 100%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.64 (t, J = 7 Hz, 2 H), 2.85 (t, J = 7 Hz, 2 H), 5.92 (s, 2 H), 6.59–6.61 (m, 2 H), 7.13–7.27 (m, 5 H and s, 1 H), 7.39–7.44 (m, 2 H).

3.1.29. (E)-N-[4-(Phenethylaminosulfonyl)phenyl]-3-[5-(4-nitrophenyl)-2-furyl]acrylic acid amide (**11e**)

According to general procedure 1 from 4-amino-2-*N*-phenethylbenzenesulfonamide and 3-[5-(4-nitrophenyl)-2-furyl]acrylic acid chloride. Yield 48%; m.p. 249 °C. IR (KBr): $\tilde{\nu}$ (cm^{-1}) 3370, 2924, 1679, 1624, 1596, 1528, 1401, 1333, 1246, 1152, 1095, 974, 834, 752. ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.67 (t, J = 7 Hz, 2 H), 2.94 (t, J = 7 Hz, 2 H), 6.83 (d, J = 16 Hz, 1 H), 7.11–7.28 (m, 6 H), 7.48 (s, 1 H), 7.49 (d, J = 16 Hz, 1 H), 7.61–7.64 (m, 1 H), 7.74–7.76 (m, 2 H), 7.88–7.90 (m, 2 H), 8.03–8.05 (m, 2 H), 8.35–8.38 (m, 2 H), 10.71 (s, 1 H). MS (ESI): m/z (%) = 518 (45, $[M + H]^+$), 437 (48), 393 (56), 391 (33), 349 (48), 305 (59), 242 (84), 139 (100).

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 according to 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 thermostatted 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. 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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