Non-Thiol Farnesyltransferase Inhibitors: Utilization of the Far Aryl Binding Site by Arylthienylacryloyl-aminobenzophenones

Andreas Mitsch[b], Mirko Altenkämper[a], Isabel Sattler[c], and Martin Schlitzer[a]

[a] Institut für Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, München, Germany
[b] Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marburg, Germany
[c] Hans-Knöll-Institut für Naturstoff-Forschung e.V., Jena, Germany

We recently described two novel aryl binding sites of farnesyltransferase. The 4- and 5-arylsubstituted thienylacryloyl moieties turned out as appropriate substituents for our benzophenone-based AAX-peptidomimetic capable for occupying the far aryl binding site.

Keywords: Non-thiol farnesyltransferase inhibitors; Structure-activity relationships; Aryl binding site

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Introduction

Farnesyltransferase catalyzes the covalent modification of proteins carrying the CAAX-sequence at their C-terminus by the transfer of a farnesyl residue from farnesylpyrophosphate to the thiol of a cysteine side chain. In the CAAX sequence, C represents a cysteine which side chain is farnesylated, A amino acids which normally, but not necessarily, carry aliphatic side chains, and X mostly methionine or serine [1, 2].

Farnesyltransferase is one of the major targets in the development of novel anticancer drugs, since several farnesylated proteins are involved in intracellular signal transduction. Farnesyltransferase inhibitors are in advanced stages of clinical trials for the therapy of different types of cancer [3–12].

Current farnesyltransferase inhibitors are lacking the free thiol of early inhibitors because of adverse drug effects associated with free thiols [13]. Most of these so-called non-thiol farnesyltransferase inhibitors have nitrogen-containing heterocycles. Here, the ring nitrogen coordinates the enzyme-bound zinc similarly to the cysteine thiol group [14]. However, nitrogen heterocycles can be replaced by aryl residues lacking the ability to coordinate metal atoms, without loosing too much of their farnesyltransferase inhibitory activity [15, 16]. Therefore, the existence of two aryl binding regions in the farnesyltransferase's active site has been postulated [17, 18].

Correspondence: Martin Schlitzer, Department für Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstraße 5–13 D-81377 München, Germany. Phone: +49 89 2180-77804, Fax: +49 89 2180-79992, martin.schlitzer@cup.uni-muenchen.de

Based on docking studies and GRID analyses, we have located two different aryl binding clefts in farnesyltransferase's active site which we suggest to be the postulated aryl binding regions [19]. One of these regions, which we called the "far aryl binding site", is targeted in the present study.

Chemistry

The key intermediates for the synthesis of the target compounds **11** and **12** were the 4- and 5-arylthiophene-2-aldehydes **4** and **5**, prepared via *Suzuki* coupling (modified from [20]) from 4- and 5-bromothiophene-2-aldehyde **1** and **2** and the appropriate aryl boronic acids **3**. The thiophene-2-aldehydes **4** and **5** were then transformed into the corresponding 3-biarylacrylic acids **6** and **7** via *Knoevenagel* condensation. The 3-biarylacrylic acids **6** and **7** were activated as acid chlorides **8** and **9** and reacted with 5-amino-2-tolylacetylaminobenzophenone **10** [21] as described previously [19] (Scheme 1). In the same way 3-biphenylyl derivatives **13** were prepared starting from 3-bromobenzaldehyde and appropriate boronic acids. Bromothiophene derivatives **15** and **16** were obtained from 4- and 5-bromothiophene-2-aldehyde, respectively. The inhibitor **12b** was prepared from compound **16** and 4-methylbenzeneboronic acid.

Farnesyltransferase inhibition assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano [22]. The assay employs yeast farnesyltransferase (FTase) fused to Glutathione *S*-transferase at the N-terminus of the β-subunit [23]. Farnesylpyrophosphate and the dansylated pentapeptide Ds-GlyCysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol the dan-

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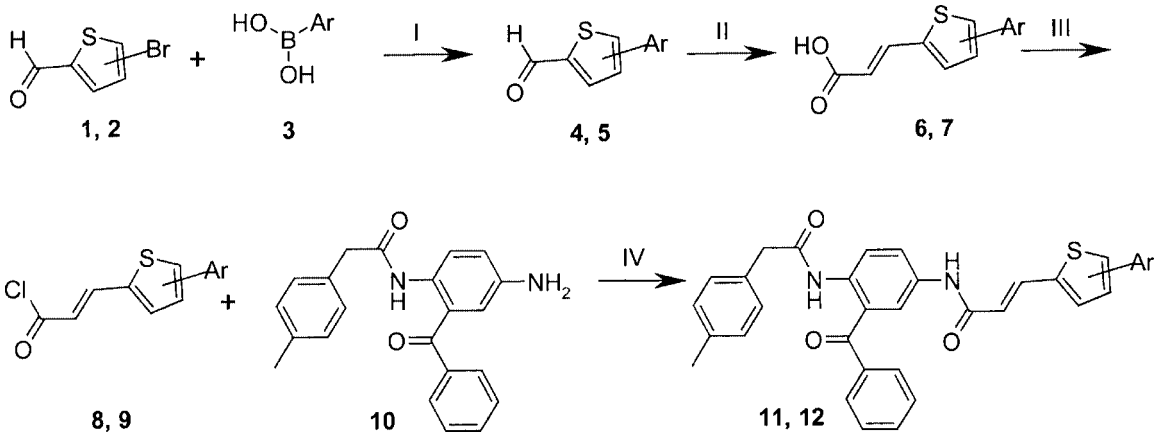
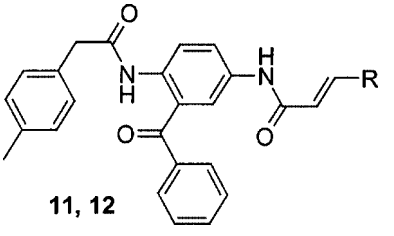
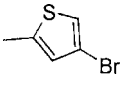
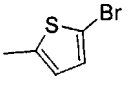
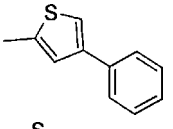
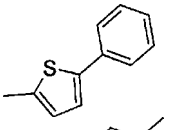
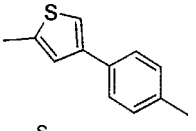
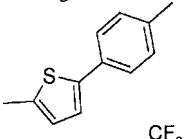
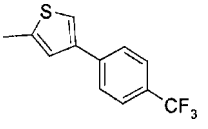
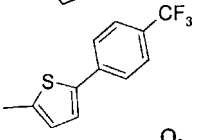
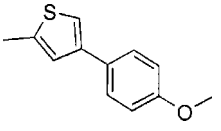
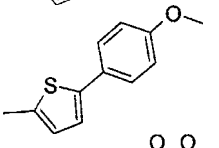
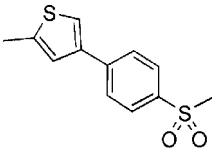
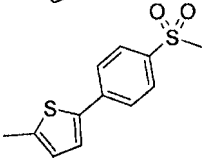


Scheme 1. Synthesis of inhibitors **11** and **12**. (I) $(Ph_3P)_4Pd$, K_2CO_3, toluene/ethanol/water, 5 h, reflux; (II) malonic acid, pyridine/piperidine, 2 h, reflux; (III) thionyl chloride, toluene, 2 h, reflux; (IV) toluene/dioxane, 2 h, reflux.

Table 1. Structure and farnesyltransferase inhibitory activity of 4-arylthienyl derivatives **11a**–**e** and 4-arylthienyl derivatives **12a**–**e**.



Compd.	R	IC_{50} [nM]	Compd.	R	IC_{50} [nM]
15		50 ± 8	**16**		185 ± 21
11a		50 ± 10	**12a**		8 ± 4
11b		10 ± 3	**12b**		20 ± 3
11c		40 ± 6	**12c**		18 ± 5
11d		145 ± 10	**12d**		10 ± 1
11e		800 ± 80	**12e**		24 ± 10

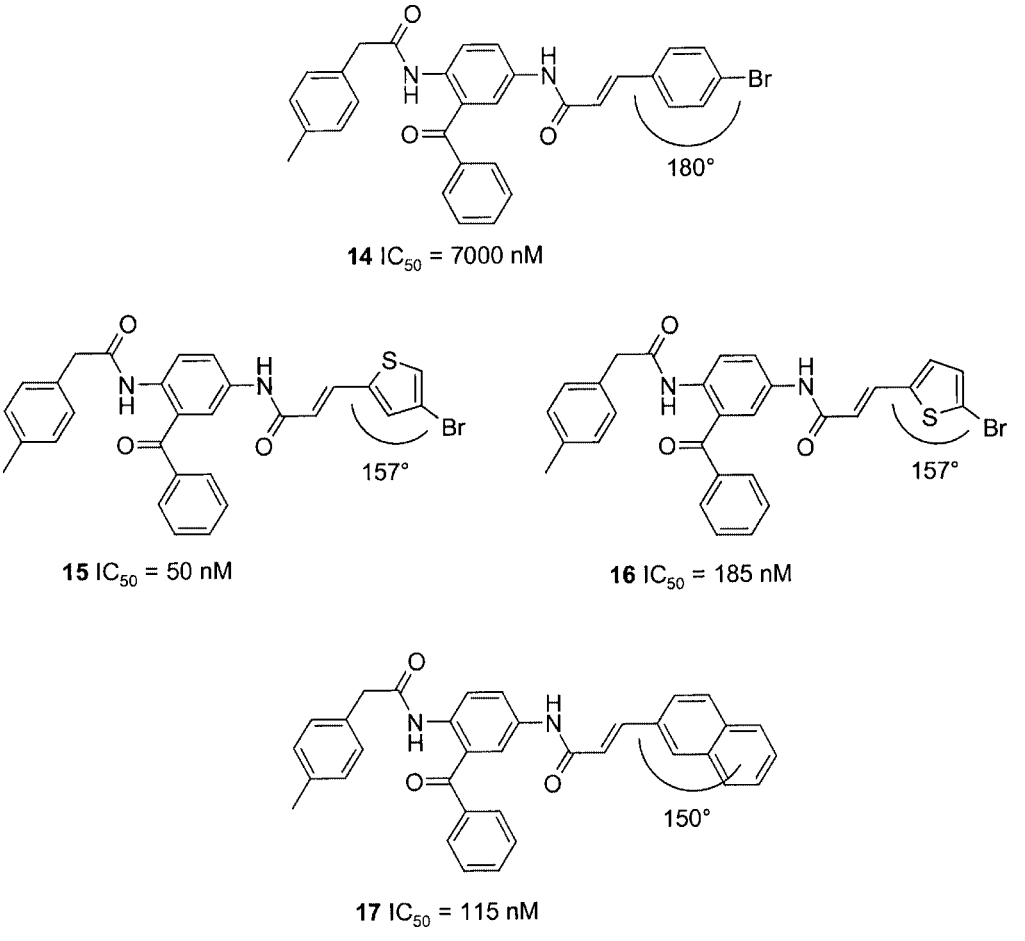


Figure 1. Comparison of the geometry of selected FTase inhibitors (see text).

syl residue is placed in a lipophilic environment which results in an enhancement of fluorescence at 505 nm which is used to monitor the enzyme's reaction.

Results and discussion

In the course of our studies towards the design of novel non-thiol farnesyltransferase inhibitors using the far aryl binding site we tested a series of cinnamic acid derivatives. For instance, the bromocinnamic acid derivative **14** [24] displayed a comparatively low inhibitory activity (Figure 1). Thiophene is generally accepted as a bioisosteric replacement of benzene, therefore we used this moiety to address the influence of the replacement on the farnesyltransferase activity. Surprisingly, at first sight the 4- and 5-bromothiophene derivatives **15** and **16** turned out to be significantly more active than the cinnamic acid derivative **14** with IC_{50} values of 50 and 185 nM, respectively. However, as depicted in Figure 1, the angles between the vinyl-aryl bond and the particular substituent are considerably different. For example, the cinnamic acid derivative shows an angle of 180° compared to only 157° for the thiophenes. The similar activity of the 2-naphthyl substituted inhibitor **17** [24] fits nicely into this picture. Here, the angle between the vinyl-2-naphthyl bond and the center of the outmost ring amounts to 150° (Figure 1). Obviously, a curved structural geometry seems to be more suited to place the lipophilic bromo substituent into deeper regions of the far aryl binding site than the linear orientation of the para substituted cinnamoyl derivative **14**. Encouraged by this observation we assumed that larger lipophilic substituents at the particular positions of the thiophene ring could occupy the far aryl binding site to an even greater extent and, therefore, might lead to more potent inhibitors. Indeed, all 5-aryl substituted thiophene derivatives **12a**−**e** (see Table 1) proved to be considerably more active than the corresponding bromothiophene **16**, with IC_{50} values between 8 and 24 nM. Notably all five compounds displayed virtually the same inhibitory activity, in spite of carrying substituents with different electronic properties (e.g. −OMe vs. $-SO_2Me$) and to a lower extent also spatial requirements (e.g. −H vs. −OMe). In contrast, marked differences in activity are visible in the series of the

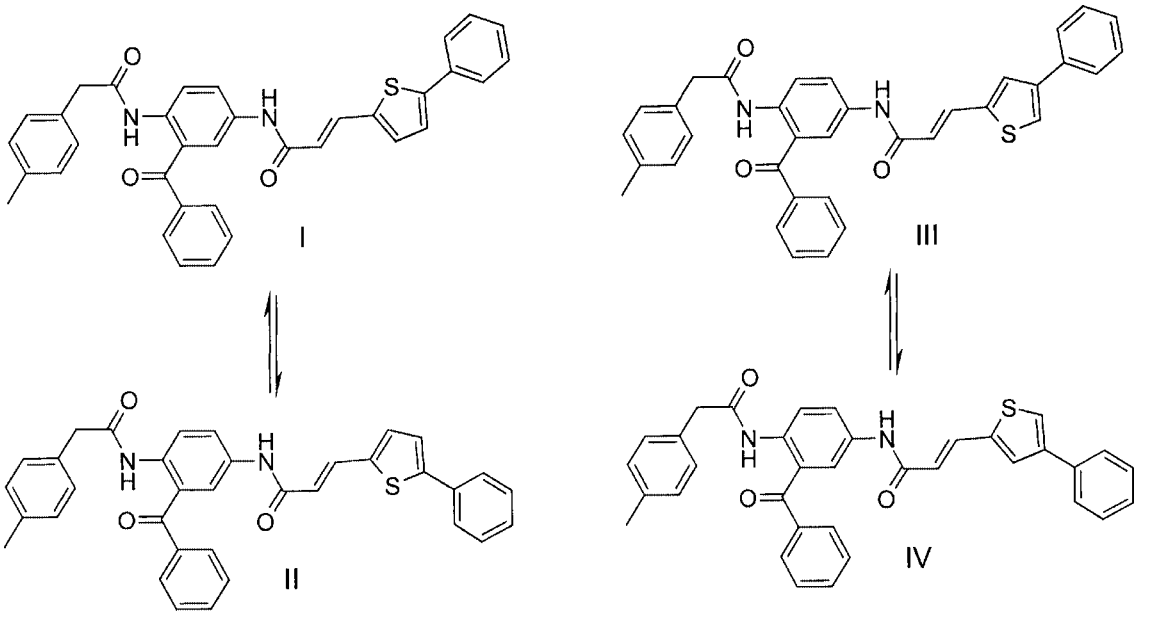


Figure 2. Conformations of 4- and 5-arylthienyl derivatives obtained through rotation of the acryloyl-thienyl bond. Individual regioisomers can have identical aryl orientations but different thienyl sulfur positions (I and III; II and IV), or identical thienyl sulfur positions but different aryl orientations (I and IV, II and III), which accounts for a distinct structure-activity relationship.

4-aryl substituted thiophene derivatives **11a**–**e** (see Table 1) with IC_{50} values ranging from 10 to 800 nM, depending on the particular substituent. As shown in Figure 2, both the 5- and 4-isomers can be drawn in a conformation with identical orientation of the aryl substituent but different positions of the thiophene sulfur. Obviously, the relative position of the thiophene sulfur is important. On the other side, sketching both regioisomers in a conformation with the thiophene sulfur in the same position results in different orientations of the aryl residues, which accounts for a distinct structure-activity relationship.

The activity values of the 3- and 4-biphenylyl derivatives **13** (Table 2) provide further support for our hypothesis showing that a biaryl moiety with a bent shape fits properly into the far aryl binding site. Accordingly, the linear 4-biphenylyl derivative **13a** [24] displays activity only in the micromolar range and is therefore as weakly active as the 4-bromocinnamic acid derivative **14**. The 3-biphenylyl derivatives **13b**–**f** are significantly more active than the 4-biphenylyl **13a** but also considerably less active than the 5-arylthiophene derivatives **12** and most of the 4-arylthiophene derivatives **11**. The angle between the vinyl aryl bond and the outermost phenyl residue for the 3-biphenylyl derivatives is 120° which is obviously too small to fit optimally into the far aryl binding site.

In summary, appropriate substituted cinnamoyl residues proved to be suitable for occupying the far aryl binding site, leading to potent non-thiol benzophenone-based farnesyltransferase inhibitors.

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Experimental

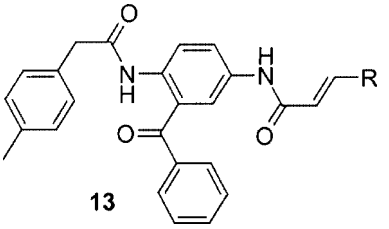
Chemistry

^{1}H-NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer (Jeol USA Inc., Peabody, MA, USA). Mass spectra were obtained with a Vacuum Generator VG 7070 H (Vacuum Generators, Manchester, UK) using a Vector 1 data acquisition system from Teknivent (Teknivent Corp., Maryland Heights, MO, USA) or a AutoSpec mass spectrometer from Micromass (Micromass, Manchester, UK). IR spectra were recorded on a Nicolet 510P FT-IR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA). Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic, and from a Hewlett-Packard CHN analyzer type 185 (Hewlett-Packard, Palo Alto, CA, USA). Melting points were obtained with a Leitz microscope (Leitz, Wetzlar, Germany) and are uncorrected. Column chromatography was carried out using silica gel 60 (0.062-0.200 mm) from Merck (Merck AG, Darmstadt, Germany).

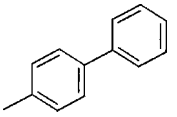
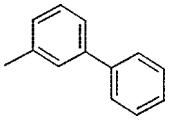
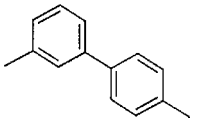
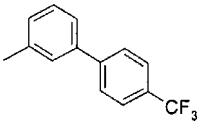
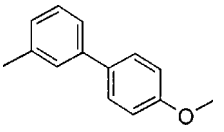
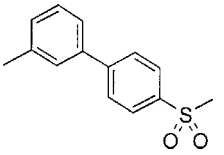
General procedure 1: Formation of 4- and 5-arylthiophencarbaldehydes via biaryl coupling

Bromothiophenecarbaldehyde and 1.2 equivalents of the benzeneboronic acid derivative were dissolved or suspended in a mixture of 30 mL toluene, 15 mL ethanol and 30 mL of an aqueous solution of potassium carbonate (1 M) under an argon atmosphere. After addition of 50 mg tetrakis(triphenylphosphine)palladium(0)

Table 2. Structure and farnesyltransferase inhibitory activity of 3-biphenylyl derivatives **13a**–**e**.



13

Compd.	R	IC_{50} [nM]
13a [24]		4600 ± 400
13b		265 ± 35
13c		50 ± 12
13d		435 ± 58
13e		300 ± 40
13f		900 ± 80

and 25 mg [1,1'-bis(diphenylphosphine)ferrocene]palladium(II) chloride per mmol bromothiophenecarbaldehyde the mixture was heated under reflux for 5 h. After cooling, the mixture was extracted with dichloromethane for three times. The organic layers were combined, dried over anhydrous magnesium sulfate and the solvent was evaporated *in vacuo*.

*4-Phenylthiophene-2-carbaldehyde (**4a**)*

From benzeneboronic acid (366 mg, 3 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 441 mg (78%). ^{1}H NMR ($CDCl_3$): δ 7.28 (m, 1H), 7.36 (m, 2H), 7.52 (m, 2H), 7.80 (m, 1H), 7.98 (m, 1H), 9.90 (s, 1H).

*4-(4-Methylphenyl)thiophene-2-carbaldehyde (**4b**)*

From 4-methylbenzeneboronic acid (170 mg, 1.25 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 224 mg (88%). ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H), 7.18 (m, 2H), 7.41 (m, 2H), 7.74 (m, 1H), 7.95 (m, 1H), 9.89 (s, 1H).

*4-(4-Trifluoromethylphenyl)thiophene-2-carbaldehyde (**4c**)*

From 4-trifluoromethylbenzeneboronic acid (378 mg, 2 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 461 mg (90%). ^{1}H NMR ($CDCl_3$): δ 7.63 (m, 4H), 7.86 (m, 1H), 7.98 (m, 1H), 9.92 (s, 1H).

*4-(4-Methoxyphenyl)thiophene-2-carbaldehyde (**4d**)*

From 4-methoxybenzeneboronic acid (300 mg, 2 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 337 mg (77%). ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H), 6.89 (m, 2H), 7.44 (m, 2H), 7.67 (m, 1H), 7.90 (m, 1H), 9.88 (s, 1H).

*4-(4-Methylsulfonylphenyl)thiophene-2-carbaldehyde (**4e**)*

From 4-methylsulfonylbenzeneboronic acid (480 mg, 2.4 mmol) according to general procedure 1. Purification: column chromatography (EtOAc:n-hexane 3:2). Yield: 415 mg (78%). ^{1}H NMR ($CDCl_3$): δ 3.08 (s, 3H), 7.77 (m, 2H), 7.98 (m, 2H), 8.03 (m, 1H), 8.06 (m, 1H), 9.99 (s, 1H).

*5-Phenylthiophene-2-carbaldehyde (**5a**)*

From benzeneboronic acid (366 mg, 3 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 428 mg (76%). ^{1}H NMR ($CDCl_3$): δ 7.36 (m, 3H), 7.38 (m, 2H), 7.62 (m, 1H), 7.70 (m, 1H), 9.83 (s, 1H).

*5-(4-Trifluoromethylphenyl)thiophene-2-carbaldehyde (**5c**)*

From 4-trifluoromethylbenzeneboronic acid (141 mg, 0.75 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 85 mg (44%). ^{1}H NMR ($CDCl_3$): δ 7.42 (m, 1H), 7.64 (m, 2H), 7.71 (m, 3H), 9.86 (s, 1H).

*5-(4-Methoxyphenyl)thiophene-2-carbaldehyde (**5d**)*

From 4-methoxybenzeneboronic acid (300 mg, 2 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 345 mg (79%). ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H), 6.87 (m, 2H), 7.14 (m, 1H), 7.53 (m, 2H), 7.71 (m, 1H), 9.78 (s, 1H).

*5-(4-Methylsulfonylphenyl)thiophene-2-carbaldehyde (**5e**)*

From 4-methylsulfonylbenzeneboronic acid (480 mg, 2.4 mmol) according to general procedure 1. Purification: column chromatography (EtOAc:n-hexane 3:2). Yield: 441 mg (83%). ^{1}H NMR ($CDCl_3$): δ 3.09 (s, 3H), 7.52 (m, 1H), 7.79 (m, 1H), 7.85 (m, 2H), 8.00 (m, 2H), 9.93 (s, 1H).

Biphenyl-3-carbaldehyde

From benzeneboronic acid (488 mg, 4 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 627 mg (86%). ^{1}H NMR ($CDCl_3$): δ 7.28–7.34 (m, 1H), 7.36–7.44 (m, 2H), 7.51–7.70 (m, 3H), 7.78 (m, 2H), 8.02 (m, 1H), 10.00 (s, 1H).

4'-Methyl-biphenyl-3-carbaldehyde

From 4-methylbenzeneboronic acid (544 mg, 4 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 552 mg (67%). ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 7.22–7.31 (m, 2H), 7.40–7.61 (m, 3H), 7.82 (m, 2H), 8.08 (m, 1H), 10.08 (s, 1H).

4'-Methoxy-biphenyl-3-carbaldehyde

From 4-methoxybenzeneboronic acid (547 mg, 3.6 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 505 mg (66%). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 6.99 (m, 2H), 7.52–7.60 (m, 3H), 7.80 (m, 2H), 8.06 (m, 1H), 10.08 (s, 1H).

4′-(Trifluoromethyl)-biphenyl-3-carbaldehyde

From 4-trifluoromethylbenzeneboronic acid (760 mg, 4 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 772 mg (77%). ^{1}H NMR ($CDCl_3$): δ 7.57−7.72 (m, 5H), 7.78−7.86 (m, 2H), 8.00 (m, 1H), 10.03 (s, 1H).

4′-(Methylsulfonyl)-biphenyl-3-carbaldehyde

From 4-(methylsulfonyl)benzeneboronic acid (400 mg, 2 mmol) according to general procedure 1. Purification: column chromatography (dichloromethane). Yield: 396 mg (76%). ^{1}H NMR ($CDCl_3$): δ 3.11 (m, 3H), 7.43−7.70 (m, 2H), 7.80−7.95 (m, 3H), 8.01−8.13 (m, 3H), 10.10 (s, 1H).

General procedure 2: Formation of biarylylacrylic acids via Knoevenagel condensation

The aromatic aldehyde was dissolved in a mixture of 5 mL pyridine and 0.2 mL piperidine. After addition of 125 mg malonic acid per mmol aldehyde the mixture was heated under reflux for 2 h. After cooling, this mixture was poured into a mixture of 60 mL water, 60 mL ice and 60 mL concentrated hydrochloric acid to yield a solid.

*3-[4-Phenyl-2-thienyl]acrylic acid (**6a**)*

From 4-phenylthiophene-2-carbaldehyde (414 mg, 2.2 mmol) according to general procedure 2. Yield: 461 mg (91%). ^{1}H NMR (DMSO d_6): δ 6.24 (d, J = 16 Hz, 1H), 7.30 (m, 1H), 7.42 (m, 2H), 7.69 (m, 2H), 7.72 (d, J = 16 Hz, 1H), 7.95 (m, 1H), 8.00 (m, 1H).

*3-[4-(4-Methylphenyl)-2-thienyl]acrylic acid (**6b**)*

From 4-(4-methylphenyl)thiophene-2-carbaldehyde (202 mg, 1 mmol) according to general procedure 2. Yield: 219 mg (90%). ^{1}H NMR (DMSO d_6): δ 2.31 (s, 3H), 6.23 (d, J = 16 Hz, 1H), 7.22 (m, 2H), 7.59 (m, 2H), 7.72 (d, J = 16 Hz, 1H), 7.93 (m, 2H).

*3-[4-(4-Trifluoromethylphenyl)-2-thienyl]acrylic acid (**6c**)*

From 4-(4-trifluoromethylphenyl)thiophene-2-carbaldehyde (205 mg, 0.8 mmol) according to general procedure 2. Yield: 488 mg (84%). ^{1}H NMR (DMSO d_6): δ 6.27 (d, J = 16 Hz, 1H), 7.72 (d, J = 16 Hz, 1H), 7.75 (m, 2H), 7.91 (m, 2H), 8.03 (m, 1H), 8.18 (m, 1H).

*3-[4-(4-Methoxyphenyl)-2-thienyl]acrylic acid (**6d**)*

From 4-(4-methoxyphenyl)thiophene-2-carbaldehyde (327 mg, 1.5 mmol) according to general procedure 2. Yield: 338 mg (78%). ^{1}H NMR (DMSO d_6): δ 3.74 (s, 3H), 6.19 (d, J = 16 Hz, 1H), 6.93 (m, 2H), 7.59 (m, 2H), 7.69 (d, J = 16 Hz, 1H), 7.83 (m, 1H), 7.86 (m, 1H).

*3-[4-(4-Methylsulfonylphenyl)-2-thienyl]acrylic acid (**6e**)*

From 4-(4-methylsulfonylphenyl)thiophene-2-carbaldehyde (400 mg, 1.5 mmol) according to general procedure 2. Yield: 345 mg (93%). ^{1}H NMR (DMSO d_6): δ 3.22 (s, 3H), 6.29 (d, J = 16 Hz, 1H), 7.73 (d, J = 16 Hz, 1H), 7.95 (m, 4H), 8.06 (m, 1H), 8.23 (m, 1H).

*3-[5-Phenyl-2-thienyl]acrylic acid (**7a**)*

From 5-phenylthiophene-2-carbaldehyde (414 mg, 2.2 mmol) according to general procedure 2. Yield: 443 mg (88%). ^{1}H NMR (DMSO d_6): δ 6.16 (d, J = 16 Hz, 1H), 7.42 (m, 1H), 7.49 (m, 2H), 7.53 (m, 1H), 7.68 (m, 2H), 8.00 (m, 1H), 8.87 (m, 1H).

*3-[5-(4-Trifluoromethylphenyl)-2-thienyl]acrylic acid (**7c**)*

From 5-(4-trifluoromethylphenyl)thiophene-2-carbaldehyde (85 mg, 0.33 mmol) according to general procedure 2. Yield: 90 mg (94%). ^{1}H NMR (DMSO d_6): δ 6.22 (d, J = 16 Hz, 1H), 7.56 (m, 1H), 7.71 (m, 1H), 7.75 (m, 1H), 7.78 (m, 1H), 7.84−7.96 (m, 3H), 8.83 (m, 1H).

*3-[5-(4-Methoxyphenyl)-2-thienyl]acrylic acid (**7d**)*

From 5-(4-methoxyphenyl)thiophene-2-carbaldehyde (327 mg, 1.5 mmol) according to general procedure 2. Yield: 386 mg (89%). ^{1}H NMR (DMSO d_6): δ 3.78 (s, 3H), 6.10 (d, J = 16 Hz, 1H), 6.99 (m, 2H), 7.43 (m, 2H), 7.62 (m, 2H), 7.69 (d, J = 16 Hz, 1H).

*3-[5-(4-Methylsulfonylphenyl)-2-thienyl]acrylic acid (**7e**)*

From 5-(4-methylsulfonylphenyl)thiophene-2-carbaldehyde (400 mg, 1.5 mmol) according to general procedure 2. Yield: 291 mg (63%). ^{1}H NMR (DMSO d_6): δ 3.28 (s, 3H), 6.26 (d, J = 16 Hz, 1H), 7.59 (m, 1H), 7.76 (m, 2H), 7.97 (m, 4H).

3-Biphenylylacrylic acid

From biphenyl-3-carbaldehyde (619 mg, 3.4 mmol) according to general procedure 2. Yield: 695 mg (91%). ^{1}H NMR (DMSO d_6): δ 6.65 (d, J = 16 Hz, 1H), 7.37−7.54 (m, 4 H), 7.67−7.77 (m, 5H), 7.92−7.98 (m, 1H).

3-(4′-Methyl-biphenyl-3-yl)acrylic acid

From 4′-methyl-biphenyl-3-carbaldehyde (620 mg, 2.6 mmol) according to general procedure 2. Yield: 535 mg (85%). ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 6.50 (d, J = 16 Hz, 1H), 7.24 (m, 2H), 7.44−7.53 (m, 4H), 7.61 (m, 1H), 7.74−7.87 (m, 2H).

3-(4′-Methoxy-biphenyl-3-yl)acrylic acid

From 4′-methoxy-biphenyl-3-carbaldehyde (488 mg, 2.3 mmol) according to general procedure 2. Yield: 506 mg (87%). ^{1}H NMR (DMSO d_6): δ 3.81 (s, 3H), 6.64 (d, J = 16 Hz, 1H), 7.03−7.08 (m, 2H), 7.45 (m, 1H), 7.62−7.68 (m, 5H), 7.93 (m, 1H).

3-(4′-Trifluoromethyl-biphenyl-3-yl)acrylic acid

From 4′-trifluoromethyl-biphenyl-3-carbaldehyde (750 mg, 3 mmol) according to general procedure 2. Yield: 740 mg (84%). ^{1}H NMR (DMSO d_6): δ 6.69 (d, J = 16 Hz, 1H), 7.54−7.88 (m, 6H), 7.98 (m, 3H), 8.08 (m, 1H).

3-(4′-Methylsulfonyl-biphenyl-3-yl)acrylic acid

From 4′-methylsulfonyl-biphenyl-3-carbaldehyde (390 mg, 1.5 mmol) according to general procedure 2. Yield: 393 mg (86%). ^{1}H NMR (DMSO d_6): δ 3.27 (s, 3H), 6.68 (d, J = 16 Hz, 1H), 7.55−7.65 (m, 1H), 7.67 (d, J = 16 Hz, 1H), 7.76−7.82 (m, 2H), 8.00−8.28 (m, 5H).

*General procedure 3: Preparation of target compounds **11**−**13***

Acrylic 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 5-aminobenzophenone derivative **10** in hot toluene (approx. 50 mL). The mixtures were heated under reflux for 2 h. Then, the solvent was evaporated *in vacuo* to give the crude products.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-(4-phenyl-2-thienyl) acrylic acid amide (**11a**)*

From 3-[4-phenyl-2-thienyl]acrylic acid (230 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from ethanol/toluene. Yield: 200 mg (36%). Mp 179°C. IR (KBr): ν = 3283, 3114, 1675, 1656, 1636, 1615, 1509, 1292, 1237, 1172, 751 cm^{-1}. ^{1}H NMR (DMSO d_6): δ 2.24 (s, 3H), 3.34 (s, 2H), 6.55 (d, J = 16 Hz, 1H), 6.98 (m, 2H), 7.03 (m, 2H), 7.31 (m, 1H), 7.42 (m, 2H), 7.49 (m, 2H), 7.55 (m, 1H), 7.63 (m, 1H), 7.67−7.71 (m, 5H), 7.75 (m, 1H), 7.86 (m, 2H), 7.94 (m, 1H), 10.09 (s, 1H), 10.33 (s, 1H). MS (EI): m/z = 103 (21), 213 (95), 344 (100), 424 (20), 556 (57) M^+. Anal. calcd for $C_{35}H_{28}N_2O_3S$: C, 75.52; H, 5.07; N, 5.03; S, 5.76; found: C, 75.27; H, 5.19; N, 5.12; S, 5.83.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[4-(4-methylphenyl)-2-thienyl]acrylic acid amide (**11b**)*

From 3-[4-(4-methylphenyl)-2-thienyl]acrylic acid (219 mg, 0.9 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 367 mg (72%). Mp 218 °C. IR (KBr): ν = 3371, 1677, 1653, 1631, 1554, 1508, 1402 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.20 (s, 3H), 2.27 (s, 3H), 3.30 (s, 2H), 6.50 (d, J = 16 Hz, 1H), 6.94 (m, 2H), 7.00 (s, 2H), 7.18 (s, 2H), 7.45 (m, 2H), 7.51−7.61 (m, 4H), 7.64 (m, 2H), 7.71 (m, 2H), 7.79 (m, 1H), 7.82 (m, 2H), 10.06 (s, 1H), 10.29 (s, 1H). MS (EI): *m/z* = 43 (100), 55 (91), 69 (56), 83 (35), 256 (29), 570 (7) M^+. Anal. calcd for $C_{36}H_{30}N_2O_3S$: C, 75.76; H, 5.30; N, 4.91; S, 5.62; found: C, 75.41; H, 5.25; N, 4.94; S, 5.84.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[4-(4-trifluoromethylphenyl)-2-thienyl]acrylic acid amide (**11c**)*

From 3-[4-(4-trifluoromethylphenyl)-2-thienyl]acrylic acid (289 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 360 mg (58%). Mp 179 °C. IR (KBr): ν = 3347, 1672, 1653, 1617, 1559, 1507, 1327 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.25 (s, 3H), 3.35 (s, 2H), 6.59 (d, J = 16 Hz, 1H), 6.98 (m, 2H), 7.04 (m, 2H), 7.49 (m, 2H), 5.57 (m, 1H), 7.64 (m, 1H), 7.68 (m, 2H), 7.73−7.79 (m, 4H), 7.87 (m, 1H), 7.94 (m, 3H), 8.15 (m, 1H), 10.11 (s, 1H), 10.36 (s, 1H). MS (EI): *m/z* = 212 (56), 281 (80), 344 (59), 492 (45), 624 (100) M^+. Anal. calcd for $C_{36}H_{27}F_3N_2O_3S$: C, 69.22; H, 4.36; N, 4.48; S, 5.13; found: C, 69.07; H, 4.47; N, 4.59; S, 5.27.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[4-(4-methoxyphenyl)-2-thienyl]acrylic acid amide (**11d**)*

From 3-[4-(4-methoxyphenyl)-2-thienyl]acrylic acid (260 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 328 mg (56%). Mp 182 °C. IR (KBr): ν = 3430, 2925, 1667, 1614, 1550, 1509, 1251 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.23 (s, 3H), 3.34 (s, 2H), 3.75 (s, 3H), 6.52 (d, J = 16 Hz, 1H), 6.96 (m, 4H), 7.03 (m, 2H), 7.48 (m, 2H), 7.54 (m, 1H), 7.62 (m, 3H), 7.67 (m, 3H), 7.74 (m, 1H), 7.80 (m, 2H), 7.85 (m, 1H), 10.08 (s, 1H), 10.32 (s, 1H). MS (EI): *m/z* = 243 (21), 326 (100), 586 (1) M^+. Anal. calcd for $C_{36}H_{30}N_2O_4S$: C, 73.70; H, 5.15; N, 4.77; S, 5.46; found: C, 73.36; H, 5.29; N, 4.96; S, 5.14.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[4-(4-methylsulfonylphenyl)-2-thienyl]acrylic acid amide (**11e**)*

From 3-[4-(4-methylsulfonylphenyl)-2-thienyl]acrylic acid (308 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from toluene/dioxan. Yield: 342 mg (60%). Mp 220 °C. IR (KBr): ν = 3422, 1673, 1633, 1555, 1510, 1141 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 3.24 (s, 3H), 3.36 (s, 2H), 6.60 (d, J = 16 Hz, 1H), 6.99 (m, 2H), 7.02−7.07 (m, 2H), 7.51 (m, 4H), 7.57−7.73 (m, 4H), 7.77 (m, 1H), 7.87 (m, 1H), 7.96−8.01 (m, 4H), 8.20 (m, 1H), 10.09 (s, 1H), 10.36 (s, 1H). MS (EI): *m/z* = 212 (99), 291 (81), 326 (61), 344 (100), 634 (23) M^+. Anal. calcd for $C_{36}H_{30}N_2O_5S_2$: C, 68.12; H, 4.76; N, 4.41; S, 10.10; found: C, 68.02; H, 4.92; N, 4.44; S, 9.83.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-(5-phenyl-2-thienyl)acrylic acid amide (**12a**)*

From 3-[5-phenyl-2-thienyl]acrylic acid (230 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 258 mg (46%). Mp 172 °C. IR (KBr): ν = 3436, 1653, 1560, 1543, 1507, 1400 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.20 (s, 3H), 3.31 (s, 2H), 6.49 (d, J = 16 Hz, 1H), 6.93 (m, 2H), 7.00 (m, 2H), 7.31 (m, 1H), 7.38−7.47 (m, 5H), 7.51 (m, 2H), 7.59 (m, 2H), 7.63−7.66 (m, 4H), 7.72 (m, 1H), 7.82 (m, 1H), 10.06 (s, 1H), 10.32 (s, 1H). MS (EI): *m/z* = 213 (100), 344 (38), 557 $[M+H]^+$. Anal. calcd for $C_{35}H_{28}N_2O_3S$: C, 75.52; H, 5.07; N, 5.03; S, 5.76; found: C, 75.42; H, 5.07; N, 5.24; S, 5.62.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[5-(4-methylphenyl)-2-thienyl]acrylic acid amide (**12b**)*

From *N*-[3-benzoyl-4-(4-tolylacetylamino)phenyl]-3-(5-bromo-2-thienyl)acrylic acid amide **15** (440 mg, 0.75 mmol) according to general procedure 1. Purification: column chromatography with dichloromethane to wash out side products and EtOAc to elute the product. Yield: 286 mg (67%). Mp 204 °C. IR (KBr): ν = 3388, 3296, 3029, 1642, 1614, 1498 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.18 (s, 3H), 2.31 (s, 3H), 3.27 (s, 2H), 6.44 (d, J = 16 Hz, 1H), 6.92 (m, 4H), 7.13 (m, 2H), 7.23 (m, 2H), 7.27−7.36 (m, 5H), 7.42 (m, 2H), 7.54 (m, 2H), 7.61 (d, J = 16 Hz, 1H), 7.89 (m, 1H), 10.11 (s, 1H), 11.93 (s, 1H). MS (EI): *m/z* = 44 (81), 227 (100), 326 (64), 552 (42), 570 (0.3) M^+. Anal. calcd for $C_{36}H_{30}N_2O_3S$: C, 75.76; H, 5.30; N, 4.91; S, 5.62; found: C, 75.93; H, 5.36; N, 5.02; S, 5.68.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[5-(4-trifluoromethylphenyl)-2-thienyl]acrylic acid amide (**12c**)*

From 3-[5-(4-trifluoromethylphenyl)-2-thienyl]acrylic acid (86 mg, 0.3 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 73 mg (35%). Mp 240 °C. IR (KBr): ν = 3242, 1662, 1614, 1540, 1509, 1327 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.25 (s, 3H), 3.35 (s, 2H), 6.58 (d, J = 16 Hz, 1H), 6.98 (m, 2H), 7.04(m, 2H), 7.49 (m, 3H), 7.56 (m, 1H), 7.63 (m, 1H), 7.69 (m, 3H), 7.73 m, 1H), 7.76 (m, 2H), 7.79 (m, 1H), 7.87 (m, 1H), 7.91 (m, 2H), 10.11 (s, 1H), 10.39 (s, 1H). MS (EI): *m/z* = 281 (100), 326 (95), 344 (20), 607 (22), 624 (30) M^+. Anal. calcd for $C_{36}H_{27}F_3N_2O_3S$: C, 69.22; H, 4.36; N, 4.48; S, 5.13; found: C, 69.07; H, 4.47; N, 4.59; S, 5.36.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[5-(4-methoxyphenyl)-2-thienyl]acrylic acid amide (**12d**)*

From 3-[5-(4-methoxyphenyl)-2-thienyl]acrylic acid (260 mg, 1 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 407 mg (69%). Mp 178 °C. IR (KBr): ν = 3241, 3028, 1666, 1645, 1605, 1550, 1506, 1253, 1177 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.24 (s, 3H), 3.36 (s, 2H), 3.78 (s, 3H), 6.46 (d, J = 16 Hz, 1H), 6.96−7.04 (m, 6H), 7.39 (m, 2H), 7.48 (m, 2H), 7.54 (m, 1H), 7.60−7.66 (m, 4H), 7.67 (m, 2H), 7.74 (m, 1H), 7.85 (m, 1H), 10.08 (s, 1H), 10.30 (s, 1H). MS (EI): *m/z* = 212 (42), 243 (100), 326 (26), 344 (55), 586 (24) M^+. Anal. calcd for $C_{36}H_{30}N_2O_4S$: C, 73.70; H, 5.15; N, 4.77; S, 5.46; found: C, 73.57; H, 5.13; N, 4.98; S, 5.25.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-[5-(4-methylsulfonylphenyl)-2-thienyl]acrylic acid amide (**12e**)*

From 3-[5-(4-methylsulfonylphenyl)-2-thienyl]acrylic acid (231 mg, 0.75 mmol) according to general procedure 3. Purification: recrystallization from toluene/dioxan. Yield: 273 mg (63%). Mp 266 °C. IR (KBr): ν = 3396, 2927, 1560, 1507, 1152 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 3.26 (s, 3H), 3.36 (s, 2H), 6.60 (d, J = 16 Hz, 1H), 6.99 (m, 2H), 7.05 (m, 2H), 7.48−7.52 (m, 4H), 7.58 (m, 2H), 7.63−7.71 (m, 2H), 7.75 (m, 2H), 7.88 (m, 1H), 7.95 (m, 4H), 10.09 (s, 1H), 10.37 (s, 1H). MS (EI): *m/z* = 212 (76), 291 (80), 326 (58), 344 (100), 634 (16) M^+. Anal. calcd for $C_{36}H_{30}N_2O_5S_2$: C, 68.12; H, 4.76; N, 4.41; S, 10.10; found: C, 67.97; H, 4.70; N, 4.57; S, 10.31.

*N-[3-Benzoyl-4-(2-p-tolylacetylamino)phenyl]-3-biphenyl-3-yl acrylic acid amide (**13b**)*

From 3-biphenylylacrylic acid (673 mg, 3 mmol) according to general procedure 3. Purification: recrystallization from toluene. Yield: 991 mg (60%). Mp 183 °C. IR (KBr): ν = 1685, 1659, 1645, 1552, 1503, 1399 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.24 (s, 3H), 3.35 (s, 2H), 6.84 (d, *J* = 16 Hz, 1H), 6.97−7.04 (m, 4H), 7.38−7.70 (m, 15H), 7.60 (m, 1H), 7.87 (m, 2H), 10.07 (s, 1H), 10.30 (s, 1H). MS (EI): *m/z* = 207 (25), 344 (25), 418 (21), 550 (100) M^+, 551 (42). Anal.

calcd for $C_{37}H_{30}N_2O_3$: C, 80.70; H, 5.49; N, 5.09; found: C, 80.77; H, 5.44; N, 5.17.

*N-[3-Benzoyl-4-(2-p-tolylacetylamino)phenyl]-3-(4′-methyl-biphenyl-3-yl)acrylic acid amide (**13c**)*

From 3-(4′-methyl-biphenyl-3-yl) acrylic acid (673 mg, 3 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 723 mg (43%). Mp 195°C. IR (KBr): ν = 1551, 1504, 1399, 1215, 1183, 969 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 2.36 (s, 3H), 3.37 (s, 2H), 6.85 (d, *J* = 16 Hz, 1H), 7.01−7.07 (m, 4H), 7.28 (m, 2H), 7.51−7.69 (m, 12H), 7.70 (m, 1H), 7.78−7.92 (m, 2H), 10.10 (s, 1H), 10.33 (s, 1H). MS (EI): *m/z* = 212 (32), 221 (49), 344 (37), 564 (100) M^+, 565 (45). Anal. calcd for $C_{38}H_{32}N_2O_3$: C, 80.83; H, 5.71; N, 4.96; found: C, 80.47; H, 5.68; N, 5.37.

*N-[3-Benzoyl-4-(2-p-tolylacetylamino)phenyl]-3-(4′-trifluoro-methyl-biphenyl-3-yl)acrylic acid amide (**13d**)*

From 3-(4′-trifluoromethyl-biphenyl-3-yl)acrylic acid (730 mg, 2.5 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 868 mg (56%). Mp 218°C. IR (KBr): ν = 1659, 1552, 1503, 1399, 1329, 1124 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 3.37 (s, 2H), 6.87 (d, *J* = 16 Hz, 1H), 6.99−7.07 (m, 4H), 7.49−7.71 (m, 9H), 7.76−7.85 (m, 4H), 7.89−7.96 (m, 4H), 10.10 (s, 1H), 10.36 (s, 1H). MS (EI): *m/z* = 212 (21), 486 (39), 513 (24), 618 (100) M^+, 619 (45). Anal. calcd for $C_{38}H_{29}F_3N_2O_3$: C, 73.78; H, 4.72; N, 4.53; found: C, 73.42; H, 4.95; N, 4.89.

*N-[3-Benzoyl-4-(2-p-tolylacetylamino)phenyl]-3-(4′-methoxy-biphenyl-3-yl) acrylic acid amide (**13e**)*

From 3-(4′-methoxy-biphenyl-3-yl)acrylic acid (483 mg, 1.9 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 817 mg (73%). Mp 184°C. IR (KBr): ν = 1684, 1660, 1644, 1632, 1553, 1504, 1399, 1340, 1247 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 3.28 (s, 2H), 3.81 (s, 3H), 6.84 (d, *J* = 16 Hz, 1H), 6.99 (m, 5H), 7.47−7.55 (m, 3H), 7.58−7.71 (m, 10H), 7.78 (m, 1H), 7.84−7.91 (m, 2H), 10.09 (s, 1H), 10.32 (s, 1H). MS (EI): *m/z* = 237 (57), 344 (46), 579 (28), 580 (100) M^+, 581 (45). Anal. calcd for $C_{38}H_{32}N_2O_4$: C, 78.60; H, 5.55; N, 4.82; found: C, 78.59; H, 5.57; N, 4.78.

*N-[3-Benzoyl-4-(2-p-tolylacetylamino)phenyl]-3-(4′-methyl-sulfonyl-biphenyl-3-yl)acrylic acid amide (**13f**)*

From 3-(4′-methylsulfonyl-biphenyl-3-yl)acrylic acid (363 mg, 1.2 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 440 mg (58%). Mp 154°C. IR (KBr): ν = 1667, 1541, 1500, 1314, 1149 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.26 (s, 3H), 3.28 (s, 3H), 3.37 (s, 2H), 6.88 (d, *J* = 16 Hz, 1H), 6.99 (m, 2H), 7.02 (m, 2H), 7.49−7.90 (m, 11H), 7.92−7.98 (m, 1H), 7.99−8.04 (m, 5H), 10.10 (s, 1H), 10.36 (s, 1H). MS (EI): *m/z* = 285 (11), 344 (8), 496 (9), 523 (6), 628 (9) M^+. Anal. calcd for $C_{38}H_{32}N_2O_5S$: C, 72.59; H, 5.13; N, 4.46; S, 5.10; found: C, 72.66; H, 5.22; N, 4.88; S, 4.81.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-(4-bromo-2-thienyl)acrylic acid amide (**15**)*

From 3-(4-bromo-2-thienyl)acrylic acid (1.4 g, 6 mmol) according to general procedure 3. Purification: recrystallization from ethanol/EtOAc. Yield: 2.16 g (64%). Mp 220°C. IR (KBr): ν = 3303, 3109, 3057, 2921, 1673, 1628, 1555, 1509, 1403 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.23 (s, 3H), 3.34 (s, 2H), 6.54 (d, J = 16 Hz, 1H), 6.96 (m, 2H), 7.03 (m, 2H), 7.47 (m, 3H), 7.54 (m, 1H), 7.60−7.67 (m, 4H), 7.73 (m, 2H), 7.84 (m, 1H), 10.09 (s, 1H), 10.34 (s, 1H). MS (EI): *m/z* = 105 (98), 212 (100), 344 (37), 428 (40), 558 (55) M^+, 560 (60) M^+. Anal. calcd for $C_{29}H_{23}BrN_2O_3S$: C, 62.26; H, 4.14; N, 5.01; S, 5.73; found: C, 62.30; H, 4.34; N, 5.05; S, 5.47.

*N-[3-Benzoyl-4-(4-tolylacetylamino)phenyl]-3-(5-bromo-2-thienyl)acrylic acid amide (**16**)*

From 3-(5-bromo-2-thienyl)acrylic acid (699 mg, 3 mmol) according to general procedure 3. Purification: recrystallization from ethanol. Yield: 894 mg (53%). Mp 182°C. IR (KBr): ν = 3328, 1671, 1654, 1620, 1594, 1553, 1510, 1405 cm^{-1}. ^1H NMR (DMSO d_6): δ 2.23 (s, 3H), 3.33 (s, 2H), 6.42 (d, J = 16 Hz, 1H), 6.96 (m, 2H), 7.02 (m, 2H), 7.25 (m, 2H), 7.48 (m, 2H), 7.54 (m, 1H), 7.60−7.67 (m, 4H), 7.72 (m, 1H), 7.82−7.85 (m, 1H), 10.08 (s, 1H), 10.31 (s, 1H). MS (EI): *m/z* = 212 (100), 215 (38), 217 (38), 344 (98), 558 (60) M^+, 560 (65) M^+. Anal. calcd for $C_{29}H_{23}BrN_2O_3S$: C, 62.26; H, 4.14; N, 5.01; S, 5.73; found: C, 62.16; H, 4.10; N, 5.12; S, 5.53.

Pharmacology

Enzyme preparation

Yeast farnesyltransferase was used as a fusion protein 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 [23]. The enzyme was purified by standard procedures with glutathione-agarose beads for selective binding of the target protein.

Farnesyltransferase assay

The assay was conducted as described [22]. Farnesylpyrophosphate (FPP) was obtained as a solution of the ammonium salt in methanol 10 mM aqueous NH_4Cl (7:3) from Sigma-Aldrich. Dansyl-GlyCysValLeuSer (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 the enzyme and run in a Quartz cuvette thermostatted at 30°C. Fluorescence emission was recorded with a Perkin Elmer LS50B spectrometer (Perkin Elmer, Shelton, CT, USA). 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.

References

[1] F. L. Zhang, P. J. Casey, *Annu. Rev. Biochem.* **1996**, *65*, 241−269.

[2] H.-W. Fu, P. J. Casey, *Rec. Prog. Hormon Res.* **1999**, *54*, 315−343.

[3] F. Tamanoi, C.-L. Gau, C. Jiang, H. Edamatsu, J. Kato-Stankiewicz, *Cell Mol. Life Sci.* **2001**, *58*, 1636−1649.

[4] G. C. Prendergast, N. Rane, *Expert Opin. Investig. Drugs* **2001**, *10,* 2105−2116.

[5] M. Crul, G. J. de Klerk, J. H. Beijnen, J. H. M. Schellens, *Anti-Cancer Drugs* **2001**, *12*, 163−184.

[6] W. T. Purcell, R. C. Donehower, *Curr. Oncol. Rep.* **2002**, *4*, 29−36.

[7] A. D. Cox, C. J. Der, *Curr. Opin. Pharmacol.* **2002**, *2*, 388−393.

[8] A. Wittinghofer, H. Waldmann, *Angew. Chem.* **2000**, 112, 4360−4383; *Angew. Chem. Int. Ed.* **2000**, *39*, 4192−4214.

[9] E. K. Rowinsky, A. Patnaik, *Emerging Drugs* **2000**, *5*, 161−199.

[10] S. M. Sebti, A. D. Hamilton, *Oncogene* **2000**, *19*, 6584−6593.

[11] A. D. Cox, *Drugs* **2001**, *61*, 723−732.

[12] I. M. Bell, *Exp. Opin. Therp. Patents* **2000**, *10*, 1813−1831.

[13] *Martindale The Extra Pharmacopeia*, 31st ed. Reynolds, J. E. F. Ed.; Royal Pharmaceutical Society of Great Britain: London, 1996; p. 821.

[14] J. T. Hunt, V. G. Lee, K. Leftheris, B. Seizinger, J. Carboni, J. Mabus, C. Ricca, N. Yan, V. Manne, *J. Med. Chem.* **1996**, *39*, 353-358.

[15] S. J. O'Connor, K. J. Barr, L. Wang, B. K. Sorensen, A. S. Tasker, H. Sham, A.-C. Ng, J. Cohen, E. Devine, S. Cherian, B. Saeed, H. Zhang, J. Y. Lee, R. Warner, S. Tahir, P. Kovar, P. Ewing, J. Alder, M. Mitten, J. Leal, K. Marsh, J. Bauch, D. J. Hoffman, S. M. Sebti, S. H. Rosenberg, *J. Med. Chem.* **1999**, *42*, 3701−3710.

[16] D. J. Augeri, D. Janowick, D. Kalvin, G. Sullivan, J. Larsen, D. Dickman, H. Ding, J. Cohen, J. Lee, R. Warner, P. Kovar, S. Cherian, B. Saeed, H. Zhang, S. Tahir, S.-C. Ng, H. Sham, S. H. Rosenberg, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1069−1074.

[17] M. J. Breslin, J. deSolms, E. A. Giuliani, G. E. Stokker, S. L. Graham, D. L. Pompliano, S. D. Mosser, K. A. Hamilton, J. H. Hutchinson, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3311−3316.

[18] T. M. Ciccarone, S. C. MacTough, T. M. Williams, C. J. Dinsmore, T. J. O'Neill, D. Shah, J. C. Culberson, K. S. Koblan, N. E. Kohl, J. B. Gibbs, A. I. Oliff, S. L. Graham, G. D. Hartman, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1991−1996.

[19] M. Böhm, A. Mitsch, P. Wißner, I. Sattler, M. Schlitzer, *J. Med. Chem.* **2001**, *44*, 3117−3124.

[20] S. S. Shin, M.-S.Noh, Y. J. Byun, J. K. Choi, J. Y. Kim, K. M. Lim, J.-Y. Ha, J. K. Kim, C. H. Lee, S. Chung, *Bioog. Med. Chem. Lett.* **2001**, *11*, 165.

[21] J. Sakowski, M. Böhm, I. Sattler, H.-M. Dahse, M. Schlitzer, *J. Med. Chem.* **2001**, *44*, 2886−2899.

[22] D. L. Pompliano, R. P. Gomez, N. J. Anthony, *J. Am. Chem. Soc.* **1992**, *114*, 7945−7946.

[23] K. Del Villar, H. Mitsuzawa, W. Yang, I. Sattler, F. Tamanoi, *J. Biol. Chem.* **1997**, *272*, 680−687.

[24] A. Mitsch, P. Wißner, M. Böhm, K. Silber, G. Klebe, I. Sattler, M. Schlitzer, *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 493−501.