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Non-thiol Farnesyltransferase Inhibitors: *N*-(4-Acylamino-3-benzoylphenyl)-4-nitrocinnamic Acid Amides

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Abstract—We have developed the 4-nitrocinnamoyl substituted benzophenone **4a** as a novel non-thiol farnesyltransferase inhibitor. Replacement of the *p*-tolyl moiety of our initial lead structure **4a** by different *para* and *ortho* substituted phenyl residues as well as by 1-naphthyl resulted in derivatives with considerably enhanced activity displaying IC_{50} values between 42 and 52 nM. These compounds represent novel, readily accessible non-thiol farnesyltransferase inhibitors being more active than the corresponding thiol-containing analogues.

Introduction

Inhibition of farnesyltransferase has received considerable interest in recent years as a strategy for the development of novel potential anti-cancer drugs.[1–6] Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesylpyrophosphate to the thiol of a cysteine side chain of proteins which bear the CAAX-tetrapeptide sequence (C: cysteine, A: aliphatic amino acid, X: serine or methionine) at their C-terminus.[7,8] The rationale for using inhibitors of farnesyltransferase as anti-cancer agents stems from the observation that farnesylation is a pre-requisite for the transforming activity of oncogenic Ras which is found in approximately 30% of all cancers in humans. However, there is accumulating evidence that prevention of Ras farnesylation may not be the crucial cellular event responsible for the antiproliferative effect of farnesyltransferase inhibitors.[9] Focus has shifted to the prenylation of RhoB, another member of the class of small GTPases which is involved in receptor trafficking.[10,11] Disregarding of the unresolved mechanism by which farnesyltransferase inhibitors exert their antiproliferative effects, the efficacy of these compounds and their low toxicity has been demonstrated[12] and, therefore, administration of such compounds is regarded as a major strategy emerging in cancer therapy.

Most inhibitors described in literature are peptidomimetics resembling the CAAX-tetrapeptide recognition sequence of farnesylated proteins. The majority of these CAAX-peptidomimetics exhibit a free thiol group[1–6] which is believed to coordinate the enzyme-bound zinc ion as it has been shown for the native peptide substrate.[13] However, free thiols are associated with several adverse drug effects[14] and, therefore, the development of farnesyltransferase inhibitors is clearly directed towards the so-called non-thiol farnesyltransferase inhibitors. The most frequently used replacement for cysteine are nitrogen-containing heterocycles. The ring nitrogen is supposed to coordinate to the enzyme-bound zinc similar to the cysteine thiol group.[15] However, it has been shown that nitrogen heterocycles can be replaced by aryl residues lacking the ability to coordinate metal atoms without loosing too much of their farnesyltransferase inhibitory activity.[16,17] Therefore, the existence of at least one hitherto unknown aryl binding region in the farnesyltransferase's active site has been postulated.[18,19]

Using our benzophenone-based AAX-peptidomimetic scaffold,[20] we have introduced *para*-substituted cinnamoyl moieties as cysteine replacements designed to utilize one of these aryl binding sites. Early investigations on the structure–activity relationships of this novel class of non-thiol farnesyltransferase inhibitors revealed the nitrocinnamoyl-substituted benzophenone **4a** as a particular potent congener.[21] In this study, we addressed

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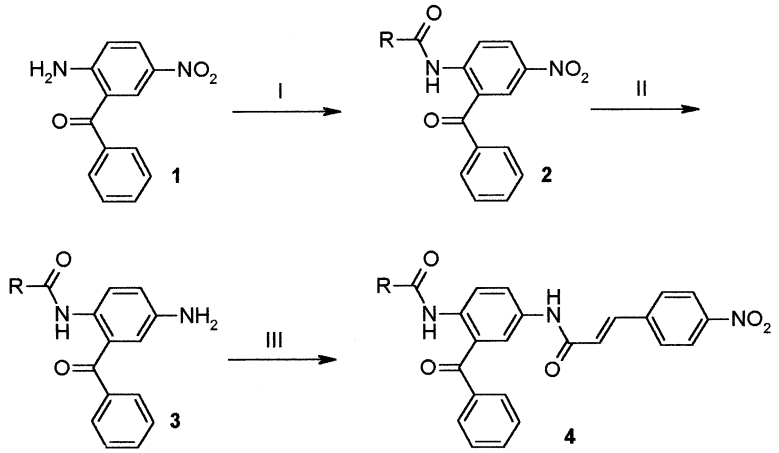
the question on how the replacement of the *p*-tolylacetyl residue at the 2-amino group of the AAX-peptidomimetic benzophenone core structure of **4a** would influence farnesyltrasferase inhibitory activity.

Chemistry

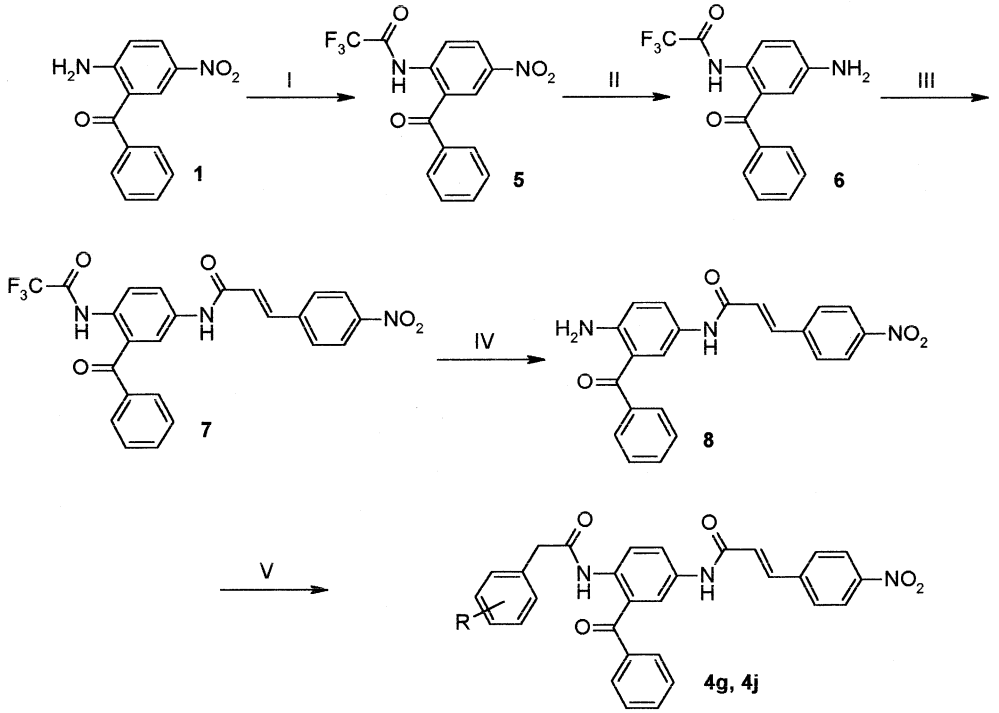
Synthesis of most of the target compounds **4** was accomplished by acylation of the appropriate 2-acylamino-5-aminobenzophenones **3** using 4-nitrocinnamic acid chloride. Intermediates **3** were prepared from 2-amino-5-nitrobenzophenone **1** as described previously[22] (Scheme 1). However, since acylation of **1** by 2- or 4-trifluoromethylphenylacetic acid chloride failed, an alternative route had to be developed for the preparation of compounds **4g** and **4j** (Scheme 2). First, the 2-amino group of **1** was protected as trifluoroacetamide (**5**). After reduction of the 5-nitro group, the resulting amine **6** was acylated by 4-nitrocinnamic acid chloride. After removal of the protective group from **7**, the resulting intermediate **8** could by acylated by 2- and 4-trifluoromethylphenylacetic acid chloride, respectively, yielding compounds **4g** and **4j**.

Farnesyltransferase Inhibition Assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano.[23] The assay employed yeast farnesyltransferase (FTase) fused to glutathione *S*-transferase at



Scheme 1. (i) R–CO–Cl, toluene/dioxane, reflux, 2 h; (ii) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2 h; (iii) 4-nitrocinnamic acid chloride, toluene/dioxane, reflux, 2 h.



Scheme 2. (i) TFAA, DCM/pyridine, 0 °C, 2 h; (ii) $SnCl_2 \times 2H_2O$, EtOAc, reflux 2 h; (iii) 4-nitrocinnamic acid chloride, toluene/dioxane, reflux, 2 h; (iv) K_2CO_3, dioxane/H_2O, reflux, 3 h; (v) R–C_6H_4–CH_2–COCl, toluene/dioxane, reflux, 2 h.

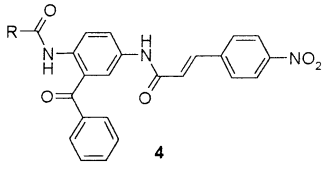
the N-terminus of the β-subunit.[24] Farnesylpyrophosphate and the dansylated pentapeptide Ds-Gly-CysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol, the dansyl residue is placed in a lipophilic environment. The resulting enhancement of fluorescence at 505 nm is used to monitor the enzyme reaction.

Results and Discussion

We have developed a novel class of non-thiol farnesyltransferase inhibitors in which a cinnamoyl moiety has been introduced as cysteine replacement designed to fit into a recently discovered aryl binding site next to the farnesyltransferase's active site. From early investigations on the structure activity relationships of these inhibitors, the *p*-nitrocinnamoyl derivative **4a** turned out to be the most active example.[21] In this study we addressed the question of how variations of the acyl moiety at the 2-amino group of the AAX-peptidomimetic benzophenone core structure would influence farnesyltransferase inhibitory activity of these compounds (Table 1). Thus, we were aiming at developing more potent inhibitors. Shifting the methyl group of the lead structure **4a** from the *para* into the *meta* (**4b**: $IC_{50}=88$ nM) or *ortho* position (**4c**: $IC_{50}=66$ nM) resulted in an enhancement of inhibitory potency. From several derivatives with different substituents in the *para* position, the methoxy (**4d**: $IC_{50}=925$ nM) and the chloro substitutions (**4e**: $IC_{50}=875$ nM) yielded compounds with a considerable drop in activity. In contrast, the *para* bromo (**4f**: $IC_{50}=46$ nM) and trifluoromethyl (**4g**: $IC_{50}=50$ nM) substituted derivatives displayed farnesyltransferase inhibitory activity with IC_{50} values equal to or less than 50 nM. The same was true for the bulky phenyl residue (**4h**: $IC_{50}=44$ nM). In case of the methyl residue, shifting this substituent from *para* into the *meta* or *ortho* position resulted in an increased activity. However, this effect could not seen with the bromo or the trifluoromethyl residue. While the *ortho* bromo derivative (**4i**: $IC_{50}=65$ nM) was less active than its *para* analogue **4f**, the trifluoromethyl group in the *ortho* position produced a slightly more active inhibitor (**4j**: $IC_{50}=42$ nM) than the para substituted derivative **4g**. With compounds **4k** and **4l**, the phenyl residue was replaced by a naphthyl moiety. Here, the position by which the naphthyl is attached turned out to be crucial for farnesyltransferase inhibitory activity. While the compound with attachment at C-1 of the naphthyl group (**4k**: $IC_{50}=68$ nM) displayed considerable activity, the attachment at C-2 (**4l**: $IC_{50}=950$ nM) led to a significant drop in activity. The same was found for the analogues compounds **4m** ($IC_{50}=52$ nM) and **4n** ($IC_{50}=210$ nM) in which the methylene spacer between the aryl moiety and the amide group had been omitted.

In summary, replacement of the *p*-tolyl moiety of our initial lead structure by different *para* and *ortho* substituted phenyl residues as well as by 1-naphthyl resulted in derivatives with considerably enhanced activity displaying IC_{50} values between 42 and 52 nM. These compounds represent novel, readily accessible non-thiol farnesyltransferase inhibitors being more active than the corresponding thiol-containing analogues.

Table 1. Farnesyltransferase inhibitory activity of compounds **4a–n**



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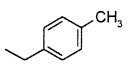
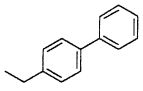
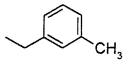
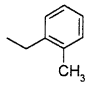
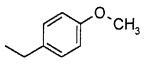
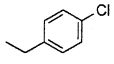
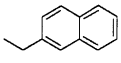
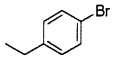
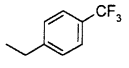
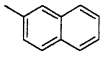
Compd	R	IC_{50} (nM)	Compd	R	IC_{50} (nM)
4a		235±20	**4h**		44±5
4b		88±18	**4i**		65±4
4c		66±4	**4j**		42±2
4d		975±65	**4k**		68±4
4e		825±5	**4l**		950±50
4f		46±4	**4m**		52±5
4g		50±3	**4n**		210±30

Experimental

^1H and ^{13}C NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer. Mass spectra were obtained with a Vacuum Generators VG 7070 H using a Vector 1 data acquisition system from Teknivent or a AutoSpec mass spectrometer from Micromass. IR spectra were recorded on a Nicolet 510P FT-IR-spectrometer. Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic and from a Hewlett Packard CHN-analyzer type 185. Melting points were obtained with a Leitz-microscope and are uncorrected. Column chromatagraphy was carried out using silica gel 60 (0.062–0.200 mm) from Merck. The preparation of the following compounds has been described: **4a**,[25] **2b-f**, **2h**, **2k-m**, **3b-f**, **3h**, **3k-m.**[22]

General procedure 1: acylation of aromatic amines by acyl chlorides

Appropriate acyl chlorides were dissolved in toluene or dioxane (approx. 10 mL) and added to a solution of the appropriate aromatic amine in hot toluene (approx. 50 mL). The mixtures were heated under reflux for 2 h.

Then, the solvent was removed in vacuo to give the crude products.

Acyl chlorides not commercially available were prepared form the corresponding carboxylic acids. These 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 residue obtained was used for the acylation step as described above.

***N*-[3-Benzoyl-4-[(3-methylphenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4b).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(3-methylphenyl)acetamide (344 mg, 1.0 mmol) according to general procedure 1. Yield 478 mg (92%); mp 219 °C. IR (KBr): ν = 3310, 3065, 1685, 1667, 1638, 1595, 1553, 1513, 1403, 1341 1290 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.22 (s, 3H), 3.36 (s, 2H), 6.91 (m, 2H), 6.99 (m, 1H), 7.10 (m, 1H), 7.47 (m, 2H), 7.60 (m, 3H), 7.66 (m, 3H), 7.76 (m, 1H), 7.83 (m, 2H), 7.87 (m, 1H), 8.24 (m, 2H), 10.03 (s, 1H), 10.40 (s, 1H). MS (EI): *m*/*z* 519 (29) [M^+], 44 (100), 73 (86), 105 (67), 212 (65), 387 (55), 129 (49), 414 (29). Anal. calcd for $C_{31}H_{25}N_3O_5$: C, 71.67; H, 4.85; N, 8.09; found: C, 71.43; H, 4.86; N, 7.99.

***N*-[3-Benzoyl-4-[(2-methylphenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4c).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(2-methylphenyl)acetamide (344 mg, 1.0 mmol) according to general procedure 1. Yield 500 mg (96%); mp 260 °C. IR (KBr): ν = 3434, 3071, 1685, 1666, 1634, 1596, 1544, 1505, 1401, 1339, 1290 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.13 (s, 3H), 3.44 (s, 2H), 6.91 (d, *J* = 16 Hz, 1H), 7.03 (m, 2H), 7.09 (m, 2H), 7.49 (m, 2H), 7.62 (m, 2H), 7.66 (m, 3H), 7.76 (m, 1H), 7.84 (m, 2H), 7.88 (m, 1H), 8.25 (m, 2H), 10.05 (s, 1H), 10.41 (s, 1H). MS (EI): *m*/*z* 519 (53) [M^+], 105 (100), 146 (75), 212 (64), 387 (63), 211 (57). Anal. calcd for $C_{31}H_{25}N_3O_5$: C, 71.67; H, 4.85; N, 8.09; found: C, 71.49; H, 4.89; N, 8.02.

***N*-[3-Benzoyl-4-[(4-methoxyphenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4d).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methoxyphenyl)acetamide (360 mg, 1.0 mmol) according to general procedure 1. Yield: 416 mg (78%); mp 214 °C. IR (KBr): ν = 3421, 3070, 2840, 1665, 1633, 1597, 1551, 1511, 1402, 1340, 1291 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.32 (s, 2H), 3.69 (s, 3H), 6.77 (m, 2H), 6.91 (d, J = 16 Hz, 1H), 7.01 (m, 2H), 7.47 (m, 2H), 7.60 (m, 2H), 7.66 (m, 2H), 7.75 (m, 1H), 7.82 (m, 3H), 7.86 (m, 1H), 8.23 (m, 2H), 10.00 (s, 1H), 10.73 (s, 1H). MS (EI): *m*/*z* 535 (7) [M^+], 121 (100), 387 (96), 212 (58), 211 (44), 77 (30), 105 (33), 414 (17). Anal. calcd for $C_{31}H_{25}N_3O_5$: C, 69.52; H, 4.71; N, 7.85; found: C, 69.39; H, 4.83; N, 7.51.

***N*-[3-Benzoyl-4-[(4-chlorophenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4e).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-(4-chlorophenyl)acetamide (364 mg, 1.0 mmol) according to general procedure 1. Yield 437 mg (81%); mp: 224 °C. IR (KBr): ν = 3322, 3070, 2925, 1687, 1633, 1597, 1552, 1512, 1403, 1341, 1291 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.41 (s, 2H), 6.93 (d, *J* = 16 Hz, 1H), 7.11 (m, 2H), 7.27 (m, 2H), 7.47 (m, 2H), 7.59 (m, 2H), 7.63 (m, 1H), 7.66 (m, 2H), 7.77 (m, 1H), 7.84 (m, 2H), 7.89 (m, 1H), 8.25 (m, 2H), 10.06 (s, 1H), 10.39 (s, 1H). MS (EI): *m*/*z* 539 (21), 146 (100), 212 (86), 121 (58), 77 (57), 105 (57), 387 (56), 91 (49), 414 (30). Anal. calcd for $C_{30}H_{22}ClN_3O_5$: C, 66.73; H, 4.11; N, 7.78; found: C, 66.43; H, 4.03; N, 7.78.

***N*-[3-Benzoyl-4-[(4-bromophenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4f).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-bromophenyl)acetamide (409 mg, 1.0 mmol) according to general procedure 1. Yield 0.427 g (73%); mp 231 °C. IR (KBr): ν = 3398, 1668, 1635, 1596, 1553, 1513, 1403, 1341, 1290 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.40 (s, 2H), 6.94 (d, *J* = 16 Hz, 1H), 7.05 (m, 2H), 7.42 (m, 2H), 7.48 (m, 2H), 7.57 (m, 1H), 7.64 (m, 2H), 7.67 (m, 2H), 7.78 (m, 1H), 7.85 (m, 2H), 7.90 (m, 1H), 8.26 (m, 2H), 10.10 (s, 1H), 10.43 (s, 1H). MS (EI): *m*/*z* 583 (51) [M^+], 585 (15, $M^+ + 2$), 146 (100), 212 (72), 387 (43), 105 (33), 175 (31), 414 (21). Anal. calcd for $C_{30}H_{22}BrN_3O_5$: C, 61.66; H, 3.79; N, 7.19; found: C, 61.58; H, 3.87; N, 7.18.

***N*-(2-Benzoyl-4-nitrophenyl)trifluoroacetamide (5).** 2-Amino-5-nitrobenzophenone (1.2 g, 5 mmol) was dissolved in a mixture of dry dichloromethane (50 mL) and dry pyridine (4.5 mL). The solution was cooled to 0 °C and trifluoroacetic anhydride (0.75 mL) was added. The mixture was left to warm to room temperature for 2 h. Then, the solution was diluted with dichloromethane and washed with water, brine, satd $NaHCO_3$ solution and dried over $MgSO_4$. The residue obtained after removal of the solvent was recrystallized from EtOH. Yield 1.420 g (83%); mp 135 °C. IR (KBr): ν = 3432, 1735, 1643, 1619, 1587, 1558, 1522, 1448, 1417, 1353, 1324 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.56 (m, 2H), 7.71 (m, 3H), 8.50 (m, 1H), 8.57 (m, 1H), 8.87 (m, 1H), 12.27 (s, 1H). MS (EI): *m*/*z* 105 (100), 77 (69), 338 (55) [M^+], 269 (34), 191 (32), 145 (16), 339 (10), 139 (8), 106 (8), 241 (7). Anal. calcd for $C_{15}H_9F_3N_2O_4$: C, 53.27; H, 2.68; N, 8.28; found: C, 53.03; H, 2.91; N, 8.28.

***N*-(4-Amino-2-benzoylphenyl)trifluoroacetamide (6).** To a solution of the *N*-(2-benzoyl-4-nitrophenyl)trifluoroacetamide (**5**) (1.400 g, 4.1 mmol) in EtOAc (25 mL) $SnCl_2 \times 2H_2O$ (4.62 g) was added. Then, the solution was refluxed for 2 h. The cooled solution was diluted with water and the pH was adjusted to 7–8 by addition of sat. $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc (3 × 100–200 mL) and the combined organic extracts were thoroughly washed with brine and dried over $MgSO_4$. The product obtained after the removal of the solvent was used without further purification. Yield 1.205 g (95%); mp 108 °C. IR (KBr): ν = 3445, 3389, 3058, 2975, 2874, 1714, 1653, 1595, 1539, 1438, 1329 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.83 (m, 1H), 6.87 (m, 1H), 7.43 (m, 2H), 7.55 (m, 1H), 7.65 (m, 2H), 8.32 (m, 1H), 11.47 (s, 1H). MS (EI): *m*/*z* 308

(100) [M^+], 105 (91), 77 (54), 211 (29), 161 (21), 239 (18), 309 (17), 106 (12), 210 (10), 78 (10). $C_{15}H_{11}F_3N_2O_2$

***N*-[3-Benzoyl-4-(trifluoroacetylamino)phenyl]-4-nitrocinnamic acid amide (7).** From 4-nitrocinnamoyl chloride (633 mg, 3.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-trifluoroacetamide (**6**) (925 mg, 3.0 mmol) according to general procedure 1. Yield 1.228 g (82%); mp 210 °C. IR (KBr): ν = 3396, 1725, 1685, 1635, 1598, 1558, 1522, 1448, 1408, 1342 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.94 (d, J = 16 Hz, 1H), 7.52 (m, 3H), 7.65 (m, 1H), 7.69 (m, 3H), 7.83 (m, 1H), 7.87 (m, 2H), 7.99 (m, 1H), 8.25 (m, 2H), 10.59 (s, 1H), 11.31 (s, 1H). MS (EI): *m*/*z* 59 (100), 72 (46), 69 (39), 73 (35), 83 (27), 105 (24), 483 (10) [M^+]. Anal. calcd for $C_{24}H_{16}F_3N_3O_5$: C, 59.63; H, 3.34; N, 8.69; found: C, 59.83; H, 3.59; N, 8.95.

***N*-(4-Amino-3-benzoylphenyl)-4-nitrocinnamic acid amide (8).** *N*-[3-Benzoyl-4-(trifluoroacetylamino)phenyl]-4-nitrocinnamic acid amide (**7**) (1.162 g, 2.4 mmol) was dissolved in a 1:1 mixture of dioxane/satd K_2CO_2 solution (30 mL) and refluxed for 3 h. Then, the solution was diluted with water and extracted with EtOAc (3 × 50 mL). The combined organic extracts were thoroughly washed with water, brine and dried over $MgSO_4$. The residue obtained after removal of the solvent was recrystallized from toluene. Yield 0.790 g (85%); mp 242 °C. IR (KBr): ν = 3397, 1725, 1685, 1635, 1598, 1556, 1519, 1448, 1411, 1342 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.93 (d, J = 16 Hz, 1H), 7.56 (m, 2H), 7.63 (m, 3H), 7.71 (m, 1H), 7.79 (m, 1H), 7.86 (m, 2H), 8.28 (m, 2H), 10.03 (s, 1H). MS (EI): *m*/*z* 387 (100) [M^+], 211 (50), 212 (46), 388 (25), 176 (11), 193 (10), 105 (6), 213 (6), 102 (6), 77 (5), 166 (5), 130 (4).

***N*-[3-Benzoyl-4-[(4-trifluormethylphenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4g).** From 2-(4-trifluoromethylphenyl)acetyl chloride (0.156 g, 0.7 mmol) and *N*-(4-amino-3-benzoylphenyl)-4-nitrocinnamic acid amide (**8**) (271 mg, 0.7 mmol) according to general procedure 1. Yield 0.293 g (73%); mp 231 °C. IR (KBr): ν = 3439, 3072, 2924, 1685, 1668, 1633, 1597, 1550, 1514, 1448, 1403, 1341, 1291 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.50 (s, 2H), 6.93 (d, *J* = 16 Hz, 1H), 7.29 (m, 2H), 7.43 (m, 2H), 7.54 (m, 4H), 7.64 (m, 3H), 7.74 (m, 1H), 7.83 (m, 2H), 7.88 (m, 1H), 8.23 (m, 2H), 10.11 (s, 1H), 10.42 (s, 1H). MS (EI): *m*/*z* 146 (100), 573 (51) [M^+], 212 (42), 211 (41), 176 (40), 387 (28), 159 (28), 118 (23), 398 (19), 129 (19), 105 (18), 574 (18). Anal. calcd for $C_{31}H_{22}F_3N_3O_5$: C: 64.92; H: 3.82; N: 7.33; found: C: 64.79; H: 3.98; N: 7.38.

***N*-[3-Benzoyl-4-(4-biphenylylacetylamino)phenyl]-4-nitrocinnamic acid amide (4h).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-biphenylyl)acetamide (406 mg, 1.0 mmol) according to general procedure 1. Yield 420 mg (72%); mp 239 °C. IR (KBr): ν = 3427, 3066, 1686, 1658, 1636, 1599, 1554, 1516, 1401, 1339, 1287 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.94 (s, 1H), 6.87 (d, *J* = 16 Hz, 1H), 7.07 (m, 4H), 7.13 (m, 2H), 7.19 (m, 4H), 7.38 (m, 2H), 7.53 (m, 1H), 7.57 (m, 1H), 7.61 (m, 2H), 7.71 (m, 1H), 7.77 (m, 1H), 7.79 (m, 1H), 7.82 (m, 1H), 8.19 (m, 2H), 10.22 (s, 1H), 10.37 (s, 1H). MS (EI): *m*/*z* 581 (29) [M^+], 414 (100), 182 (32), 105 (29), 415 (24), 265 (22). Anal. calcd for $C_{36}H_{27}N_3O_5$: C, 74.34; H, 4.68; N, 7.22; found: C, 74.15; H, 4.81; N, 7.28.

***N*-(2-Benzoyl-4-nitrophenyl)-2-(2-bromophenyl)acetamide (2i).** From 2-amino-5-nitrobenzophenone (0.969 g, 4.0 mmol) and 2-(2-bromophenyl)acetyl chloride (0.935 g, 4.0 mmol) according to general procedure 1. The product crystallized upon cooling and was recrystallized from EtOH. Yield 1.023 g (58%); mp 96 °C. IR (KBr): ν = 3203, 3108, 1701, 1642, 1615, 1597, 1579, 1539, 1506, 1442, 1411, 1348 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.96 (s, 2H), 7.23 (m, 1H), 7.37 (m, 1H), 7.41 (m, 1H), 7.52 (m, 2H), 7.60 (m, 1H), 7.66 (m, 3H), 8.38 (m, 1H), 8.42 (m, 1H), 8.89 (m, 1H), 11.01 (s, 1H). MS (EI): *m*/*z* 359 (100), 269 (43), 169 (32), 171 (32), 105 (29), 191 (26), 77 (22), 438 (0.5) [M^+], 440 (0.5, $M^+ + 2$). Anal. calcd for $C_{21}H_{15}BrN_2O_4$: C, 57.42; H, 3.44; N, 6.38; found: C, 57.54; H, 3.56; N, 6.58.

***N*-(4-Amino-2-benzoylphenyl)-2-(2-bromophenyl)acetamide (3i).** As described for compound **6** from *N*-(2-benzoyl-4-nitrophenyl)-2-(2-bromophenyl)acetamide (**2m**) (1.008 g, 2.3 mmol) and $SnCl_2 \times 2H_2O$ (2.58 g). Yield 0.847 g (87%); Oil. IR (KBr): ν = 3438, 3357, 3282, 1658, 1594, 1559, 1518, 1471, 1446, 1434, 1325 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.83 (s, 4H), 6.73(d, *J* = 3 Hz, 1H), 6.85 (dd, *J* = 3, 7 Hz, 1H), 7.15 (m, 1H), 7.31 (m, 1H), 7.36 (m, 1H), 7.44 (m, 2H), 7.56 (m, 2H), 7.65 (m, 2H), 8.26 (d, *J* = 9 Hz, 1H), 10.04 (s, 1H). MS (EI): *m*/*z* 212 (100), 408 (70), 410 (70), 211 (50), 409 (17) [M^+], 41 (16), 239 (16), 213 (15), 105 (14), 169 (9), 171 (8), 210 (8).

***N*-[3-Benzoyl-4-[(2-bromophenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4i).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-(2-bromophenyl)acetamide (**3m**) (409 mg, 1.0 mmol) according to general procedure 1. Yield 403 mg (69%); mp 248 °C. IR (KBr): ν = 3432, 1686, 1631, 1596, 1541, 1507, 1443, 1403, 1340 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.57 (s, 2H), 6.91 (d, *J* = 16 Hz, 1H), 7.16 (m, 2H), 7.26 (m, 2H), 7.51 (m, 3H), 7.62 (m, 1H), 7.66 (m, 4H), 7.77 (m, 1H), 7.82 (m, 2H), 7.88 (m, 1H), 8.23 (m, 2H), 10.08 (s, 1H), 10.39 (s, 1H). MS (EI): *m*/*z* 44 (100), 73 (76), 69 (56), 71 (43), 129 (38), 585 (8, $M^+ + 2$), 583 (8) [M^+]. Anal. calcd for $C_{30}H_{22}BrN_3O_5$: C: 61.66; H: 3.79; N: 7.19; found: C: 62.06; H: 3.94; N: 7.21.

***N*-[3-Benzoyl-4-[(2-trifluormethylphenyl)acetylamino]phenyl]-4-nitrocinnamic acid amide (4j).** From (2-trifluoromethylphenyl)acetyl chloride (0.156 g, 0.7 mmol) and *N*-(4-amino-3-benzoylphenyl)-4-nitrocinnamic acid amide (**8**) (271 mg, 0.7 mmol) according to general procedure 1. Yield 0.271 g (72%); mp 224 °C. IR (KBr): ν = 3431, 1686, 1665, 1637, 1597, 1559, 1512, 1403, 1342, 1317, 1292 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.64 (s, 2H), 6.94 (d, *J* = 16 Hz, 1H), 7.28 (m, 1H), 7.45 (m, 1H), 7.52 (m, 2H), 7.57 (m, 1H), 7.65 (m, 3H), 7.69 (m, 2H), 7.80 (m, 1H), 7.86 (m, 2H), 7.91 (m, 1H), 8.27 (m, 2H), 10.10 (s, 1H), 10.42 (s, 1H). MS (EI): *m*/*z* 146 (100), 212 (60), 73 (60), 84 (53), 211 (51), 159 (41), 105 (41), 91 (38), 129

(35), 573 (27) [M^+]. Anal. calcd for $C_{31}H_{22}F_3N_3O_5$: C: 64.92; H: 3.82; N: 7.33; found: C: 64.79; H: 4.19; N: 7.26.

***N*-[3-Benzoyl-4-(1-naphthylacetylamino)phenyl]-4-nitrocinnamic acid amide (4k).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-1-naphthylacetamide (380 mg, 1.0 mmol) according to general procedure 1. Yield 494 mg (89%); mp 229 °C. IR (KBr): ν = 3420, 3062, 1663, 1597, 1557, 1512, 1402, 1341, 1237 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.92 (s, 2H), 6.92 (*d*, *J* = 16 Hz, 1H), 7.31 (m, 1H), 7.40 (m, 1H), 7.48 (m, 4H), 7.61 (m, 2H), 7.69 (m, 3H), 7.81 (m, 2H), 7.84 (m, 2H), 7.88 (m, 2H), 7.93 (m, 1H), 8.25 (m, 2H), 10.19 (s, 1H), 10.40 (s, 1H). MS (EI): *m*/*z* 555 (11) [M^+], 44 (100), 387 (86), 73 (81), 141 (70), 129 (54), 256 (51), 212 (43), 414 (36), 105 (29). Anal. calcd for $C_{34}H_{25}N_3O_5$: C, 73.50; H, 4.54; N, 7.56; found: C, 73.29; H, 4.60; N, 7.44.

***N*-[3-Benzoyl-4-(2-naphthylacetylamino)phenyl]-4-nitrocinnamic acid amide (4l).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-naphthylacetamide (380 mg, 1.0 mmol). Yield 272 mg (49%); mp 225 °C. IR (KBr): ν = 3297, 3063, 1686, 1663, 1595, 1550, 1507, 1402, 1341, 1290 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.57 (s, 2H), 6.91 (d, *J* = 16 Hz, 1H), 7.24 (m, 1H), 7.44 (m, 4H), 7.55 (m, 1H), 7.60 (m, 3H), 7.65 (m, 2H), 7.76 (m, 3H), 7.82 (m, 3H), 7.87 (m, 1H), 8.23 (m, 2H), 10.12 (s, 1H), 10.39 (s, 1H). MS (EI): *m*/*z* 555 (9) [M^+], 387 (100), 212 (98), 211 (71), 141 (51), 105 (33), 238 (27), 414 (21). Anal. calcd for $C_{34}H_{25}N_3O_5$: C, 73.50; H, 4.54; N, 7.56; found: C, 73.20; H, 4.69; N, 7.55.

***N*-[3-Benzoyl-4-(1-naphthoylamino)phenyl]-4-nitrocinnamic acid amide (4m).** From 4-nitrocinnamoyl chloride (0.211 g, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-1-naphthoyl amide (0.366 g, 1.0 mmol) according to general procedure 1. Yield 0.380 g (70%); mp 230 °C. IR (KBr): ν = 3427, 1680, 1633, 1596, 1514, 1402, 1341, 1292 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.96 (d, *J* = 16 Hz, 1H), 7.28 (m, 1H), 7.48 (m, 2H), 7.51 (m, 3H), 7.62 (m, 1H), 7.67 (m, 2H), 7.77 (m, 2H), 7.86 (m, 3H), 7.92 (m, 1H), 7.98 (m, 2H), 7.99 (m, 1H), 8.25 (m, 2H), 10.47 (s, 1H), 10.57 (s, 1H). MS (EI): *m*/*z* 541 (16) [M^+], 155 (100), 127 (56), 63 (33), 366 (10). Anal. calcd for $C_{33}H_{23}N_3O_5$: C, 73.19; H, 4.28; N, 7.76; found: C, 72.92; H, 4.46; N, 7.51.

***N*-(2-Benzoyl-4-nitrophenyl)-2-naphthoyl amide (2n).** From 2-amino-5-nitrobenzophenone (0.726 g, 3.0 mmol) and 2-naphthoyl chloride (0.570 g, 3.0 mmol) according to general procedure 1. The products crystallized upon cooling and were recrystallized from EtOH. Yield 0.475 g (40%); mp 211 °C. IR (KBr): ν = 3447, 3219, 1695, 1630, 1579, 1559, 1546, 1509, 1470, 1426, 1340, 1285 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.51 (m, 4H), 7.61 (m, 1H), 7.68 (m, 2H), 7.79 (m, 1H), 7.91 (m, 1H), 7.97 (m, 1H), 8.03 (m, 1H), 8.42 (m, 1H), 8.50 (m, 1H), 8.53 (m, 1H), 9.11 (m, 1H), 12.34 (s, 1H). MS (EI): *m*/*z* 155 (100), 396 (41) [M^+], 156 (13), 397 (11), 366 (4), 291 (3), 127 (2), 398 (1), 367 (1), 157 (1). Anal. calcd for $C_{24}H_{16}N_2O_4$: C: 72.72; H: 4.07; N: 7.07; found: C: 72.55; H: 4.22; N: 6.99.

***N*-[(4-Amino-2-benzoyl)phenyl]-2-naphthoyl amide (3n).** As described for compound **6** from *N*-(2-benzoyl-4-nitrophenyl)-2-naphthoyl amide (**2b**) (0.450 g, 1.1 mmol) and $SnCl_2 \times 2H_2O$ (1.25 g). Yield 0.385 g (95%); mp 81 °C. IR (KBr): ν = 3433, 3233, 1661, 1620, 1595, 1529, 1506, 1433, 1321, 1305 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.63 (s, 2H), 6.84 (m, 1H), 6.94 (m, 1H), 7.43 (m, 2H), 7.50 (m, 3H), 7.68 (m, 2H), 7.81 (m, 1H), 7.87 (m, 1H), 7.94 (m, 1H), 8.00 (m, 1H), 8.47 (m, 1H), 8.617 (m, 1H), 11.53 (s, 1H). MS (EI): *m*/*z* 155 (100), 127 (71), 366 (43) [M^+], 59 (42), 41 (25), 43 (23), 57 (23), 44 (22), 55 (21), 771 (19), 105 (18), 211 (15). $C_{24}H_{18}N_2O_2$

***N*-[3-Benzoyl-4-(2-naphthoylamino)phenyl]-4-nitrocinnamic acid amide (4n).** From 4-nitrocinnamoyl chloride (211 mg, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-2-naphthoyl amide (366 mg, 1.0 mmol) according to general procedure 1. Yield: 108 mg (20%); mp 262 °C. IR (KBr): ν = 3339, 3059, 1665, 1631, 1596, 1550, 1517, 1403, 1341, 1295 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.98 (d, J = 16 Hz, 1H), 7.49 (m, 2H), 7.59 (m, 3H), 7.68 (d, *J* = 16 Hz, 1H), 7.76 (m, 3H), 7.86 (m, 3H), 7.91 (m, 1H), 7.97 (m, 5H), 8.22 (m, 1H), 8.27 (m, 2H), 10.51 (s, 1H), 10.72 (s, 1H). MS (EI): *m*/*z* 541 (22) [M^+], 155 (100), 127 (46), 73 (32), 366 (10). Anal. calcd for $C_{33}H_{23}N_3O_5$: C, 73.19; H, 4.28; N, 7.76; found: C, 73.19; H, 4.27; N, 7.66.

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.[24] 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.[23] 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. 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.

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