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Non-Thiol Farnesyltransferase Inhibitors: Structure–Activity Relationships of Benzophenone-Based Bisubstrate Analogue Farnesyltransferase Inhibitors

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Abstract—Investigations on the structure–activity relationships of benzophenone-based bisubstrate analogue farnesyltransferase inhibitors yielded a bisubstrate analogue farnesyltransferase inhibitor lacking any prenylic or peptidic substructures with nanomolar activity. This represents a considerable progress in comparison to those non-prenylic, non-peptidic bisubstrate analogue farnesyltransferase inhibitors we have described before which utilized AAX-peptidomimetic substructures different from the benzophenone since those inhibitors displayed activity only in the micromolar range.

Introduction

Inhibition of farnesyltransferase has become a major strategy for the development of novel potential anticancer drugs.[1–6] Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesylpyrophosphate to the thiol of a cysteine side chain of proteins bearing C-terminal the CAAX-tetrapeptide sequence (C: cysteine, A: aliphatic amino acid, X: serine or methionine).[7,8] Farnesylation is a prerequisite 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 the unresolved mechanism of action of farnesyltransferase inhibitors, the efficacy of these compounds and their low toxicity has been demonstrated.[12]

Most inhibitors described in literature are peptidomimetics resembling the CAAX-tetrapeptide recognition sequence of farnesylated proteins. The majority of these CAAX-peptidomimetics exhibits 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.

We have replaced the thiol in a benzophenone-based CAAX-peptidomimetic farnesyltransferase inhibitor[15] by a carboxylic acid moiety (e.g., **1**; Fig. 1). This modification resulted in a marked drop in inhibitory potency.[16] Obviously, we lost a lot of affinity upon the replacement of the thiol by a carboxyl group. We rationalized that addition of a lipophilic moiety to the terminal carboxyl group, which presumably occupies considerable portions of that region in farnesyltransferase's active site which nomally harbours the farnesyl residue, should regain at least some of the lost affinity. The additional hydrophobic interactions should enhance the overall binding energy. Thus, we transformed our peptidomimetic inhibitors into so-called bisubstrate analogues, compounds displaying molecular features of both, the peptidic and the prenylic substrate. Indeed, transformation of the carboxylic acid derivatives into bisubstrate analogues (e.g., **2**; Fig. 1) resulted in a regaining of inhibitory activity. Here, we report some further investigations of the structure–activity relationships of this class of benzophenone-based bisubstrate farnesyltransferase inhibitors.

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Chemistry

Synthesis of the target compounds **3** and **7–10** was accomplished by acylation of the appropriate 2-acylamino-5-aminobenzophenones **11**[15] by *N*-palmitoyl-β-alanine,[17] *N*-methyl-*N*-palmitoyl-β-alanine[18] and *N*-stearoyl-β-alanine,[19] respectively, using mixed anhydride activation. Intermediates **12** were prepared from 2-amino-5-nitrobenzophenone **11a** using *N*-Boc-glycine and *N*-Boc-γ-aminobutyric acid, respectively. Acidic removal of the *N*-protective groups yielded the amino acid amides **13** which were acylated yielding the target compounds **4** and **5**. Acylation of **11a** by arachidic acid, activated as mixed anhydride, gave the target compound **6**.

Farnesyltransferase inhibition assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described by Pompliano.[20] The assay employes yeast farnesyltransferase (FTase) fused to glutathione *S*-transferase at the N-terminus of the β-subunit.[21] Farnesylpyrophosphate and the dansylated pentapeptide Ds-Gly-CysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol, the dansyl residue is placed into a lipophilic environment. The resulting enhancement of fluorescence at 505 nm is used to monitor the enzyme reaction.

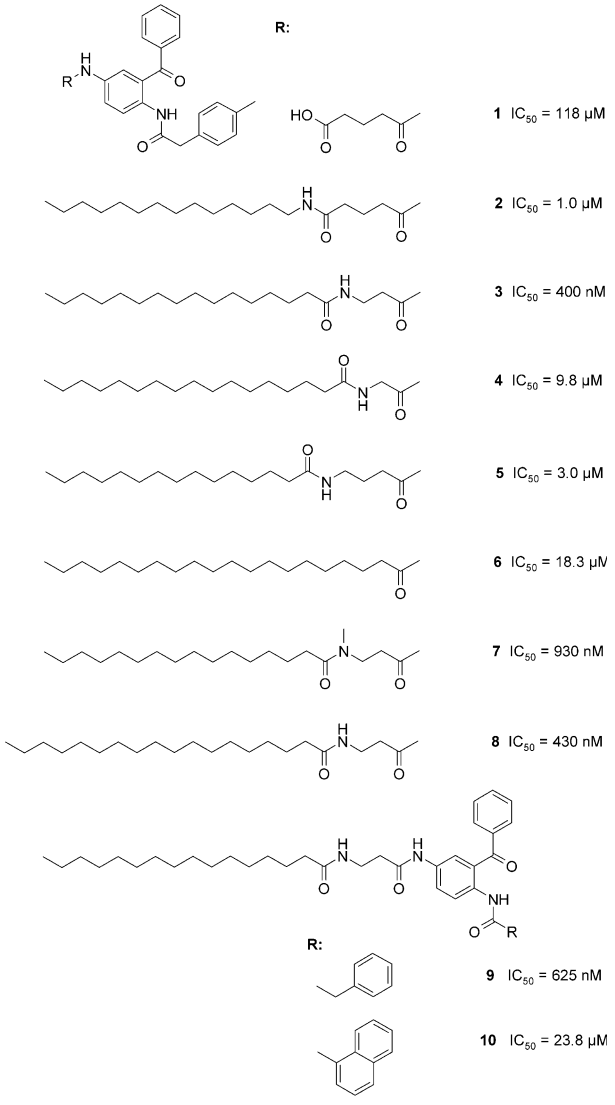


Figure 1. Stuctures and farnesyltransferase inhibitory activity of inhibitors **1–10**.

Results and Discussion

Starting from the benzophenone-based bisubstrate analogue **2** as lead structure (Fig. 1), we first inverted the sequence of the two elements of the amide moiety connecting the alkyl chain to the remainder of the molecule from amide nitrogen→carbonyl group to carbonyl group→amide nitrogen. The resulting inhibitor **3** displayed an IC_{50} value of 400 nM and is therefore 2.5-fold more active than the lead structure **2**. In our binding model of compound **2**, we suggest the 2-acylaminobenzophenone moiety occupies the peptide-binding site of the farnesyltransferase while the alkyl chain of the terminal palmitoyl residue is located the farnesyl binding cleft. In this model, the carbonyl oxygen of the amide linkage connecting the β-alanyl to the palmitoyl moiety should be coordinated to the enzym-bound zinc. This binding model is supported by an alignment of inhibitor **3** with the CAAX-peptide and the farnesylpyrophosphate which enzyme bound conformations were taken form the crystal structure of a ternary complex of farnesyltransferase, a farnesylpyrophosphate analogue and *N*-acetyl-Cys-Val-Ile-selenoMetOH (PDB 1QBQ).[22] Hereby, the program SEAL[23] tries to superimpose the molecules in such a way that their physicochemical properties (steric, electrostatic, hydrophobic and hydrogen bond donor/acceptor) show the best fit. The so generated alignment (Fig. 2) shows the 2-acylaminobenzophenone peptidomimetic substructure remarkable well fitted to the Val-Ile-selenoMetOH tripeptide as well as the superimposition of the eight terminal palmitoyl carbons with the farnesyl residue. The carbonyl oxygen of the β-alanyl→palmitoyl amide

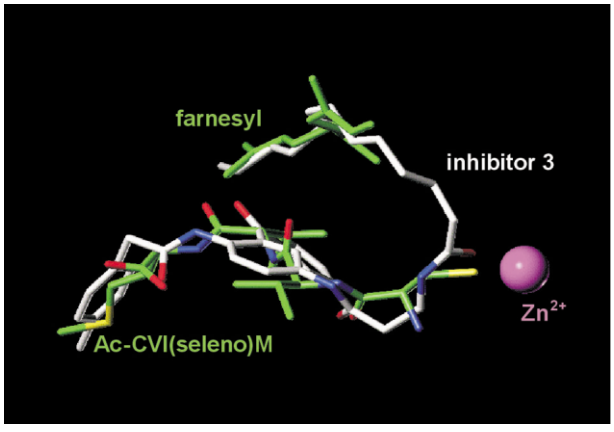


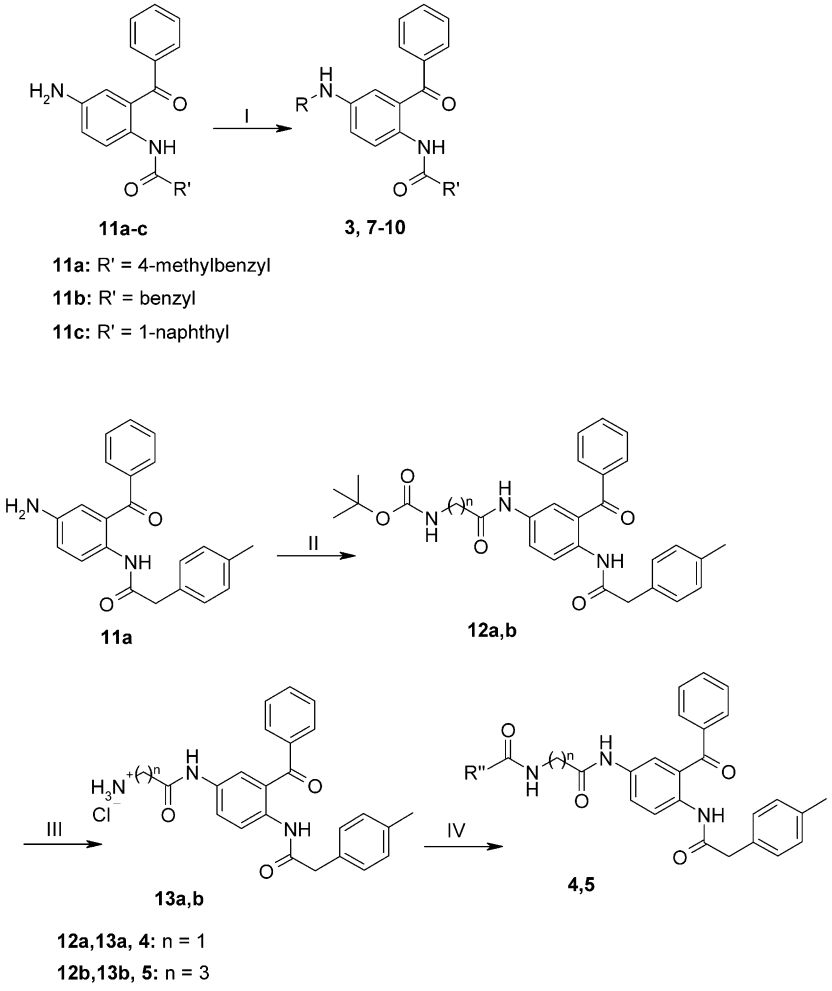
Figure 2. SEAL superimposition of the enzyme bound conformation of N-acetyl-Cys-Val-Ile-selenoMetOH, the farnesyl residue of farnesylpyrophosphate and the bisubstrate inhibitor **3**. Colors: carbon: white (**3**), light green [*N*-Ac-CVI(seleno)M and farnesyl]; nitrogen: blue; oxygen: red; sulfur: yellow. The zinc ion is shown as a magenta sphere.

linkage is shown to be coordinated to the enzyme bound zinc (Scheme 1).

Since the carbonyl oxygen–zinc interaction is supposed to be important for the overall binding affinity, the position of the terminal amide moiety in relation to the peptidomimetic substructure is assumed to be crucial. Indeed, reduction or enlargement of the distance between the benzophenone core and the alkanoyl residue by replacing of the central β-alanine with glycine (inhibitor **4**) and γ-aminobutyric acid (inhibitor **5**), respectively, resulted in a noteable reduction in farnesyltransferase inhibitory activity (**4**: $IC_{50} = 9.8$ μM; **5**: $IC_{50} = 3.0$ μM). Our binding model is further supported by the even more pronounced reduction in activity observed upon the replacement of the amide moiety by an ethylene linker (inhibitor **6**: $IC_{50} = 18.3$ μM), since this compound in not capable of forming the carbonyl oxygen–zinc interaction any longer. In contrast to the amide carbonyl, the free NH function seems to be of minor importance as methylation of this moiety results only in a 2-fold reduction in activity (inhibitor **7**: $IC_{50} = 930$ nM). Elongation of the terminal alkanoyl residue by two methylene units (inhibitor **8**: $IC_{50} = 430$ nM) showed no visible effect on the activity.

With the last two modifications, the 2-aminoacyl residue of the benzophenone core structure has been varied. Replacement of the *p*-tolyl residue of inhibitor **3** by a phenyl group resulted in a slight reduction in activity (inhibitor **9**: $IC_{50} = 650$ nM). The 1-naphthyl derivative **10** is considerably less active ($IC_{50} = 23.8$ μM). These changes in activity upon variation of the 2-acylamino substituent mirror the results obtained with the cysteine-containing benzophenone inhibitors,[15] although the effects are more pronounced in case of the bisubstrate analogues.

The investigations on the structure–activity relationships of benzophenone-based bisubstrate analogue farnesyltransferase inhibitors described here yielded a bisubstrate analogue farnesyltransferase inhibitor lacking any prenylic or peptidic substructures with nanomolar activity. This represents a considerable progress in comparison to those non-prenylic, non-peptidic bisubstrate analogue farnesyltransferase inhibitors we



Scheme 1. (I) *N*-Palmitoyl-β-alanine or *N*-methyl-*N*-palmitoyl-β-alanine or *N*-stearoyl-β-alanine, isobutyl chloroformate, *N*-methylmorpholine, DMF, −15 °C, 5 min, then add **11**, DMF, −15 °C to rt, overnight; (II) *N*-Boc-glycine or *N*-Boc-γ-aminobutyric acid, isobutyl chloroformate, *N*-methylmorpholine, DMF, −15 °C, 5 min, then add **11a**, DMF, −15 °C to rt, overnight; (III) HCl 4 M in dioxane, rt, 1 h; (IV) H_3C–$(CH_2)_{15}$–COOH or H_3C–$(CH_2)_{13}$–COOH, isobutyl chloroformate, *N*-methylmorpholine, DMF, −15 °C, 5 min, then add **13**, *N*-methylmorpholine, DMF, −15 °C to rt, overnight.

have described before[18,24–27] which utilized AAX-peptidomimetic substructures different from the benzophenone since those inhibitors displayed activity only in the micromolar range.

Experimental

^{1}H 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.

General procedure: amide formation using acids activated as mixed anhydrides

The appropriate acid was dissolved in a sufficient amount of dry DMF in a flame dried flask under an atmosphere of Ar. After addition of *N*-methylmorpholine (NMM) (0.25 mL per mmol acid) the solution was cooled to −15 °C and isobutyl chloroformate (0.13 mL per mmol acid) was added. A solution of the amine component (1 equivalent) in dry DMF was added after 5 min. In case the amine component was employed as a hydrochloride, additional NMM (0.25 mL per mmole) was added. The mixture was allowed to warm up to room temperature overnight and then poured into brine (400–800 mL). In case a solid precipitate was formed, this was collected by suction and thoroughly washed with water. Otherwise, the aqueous mixture was extracted with ethyl acetate (3×100 mL) and the combined organic extracts were washed successively with 2 N citric acid, satd $NaHCO_3$ soln and brine, and dried with $MgSO_4$. The residue obtained after removal of the solvent was purified by recrystallisation or flash chromatography.

***N*-[3-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenylamino]-3-oxopropyl]hexadecanoic acid amide (3).** From *N*-palmitoyl-β-alanine (0.270 g, 0.82 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (0.282 g, 0.82 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.405 g (62%); mp 119 °C. IR (KBr): ν 3299, 2923, 2853, 1652, 1545 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.81 (t, J=7 Hz, 3H), 1.18 (m, 24H), 1.46 (m, 2H), 2.03 (t, J=8 Hz, 2H), 2.27 (s, 3H), 2.49 (t, J=6 Hz, 2H), 3.47 (m, 2H), 3.61 (s, 2H), 6.06 (m, 1H), 7.10 (m, 2H), 7.18 (m, 2H), 7.41 (m, 2H), 7.55 (m, 2H), 7.62 (m, 2H), 7.75 (m, 1H), 8.18 (s, 1H), 8.45 (m, 2H), 10.43 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 25.6, 29.2, 29.3, 29.35, 29.46, 29.58, 29.62, 29.65, 29.68, 31.9, 35.2, 36.7, 37.0, 45.0, 122.4, 124.2, 124.3, 125.3, 128.3, 129.3, 129.6, 130.0, 131.1, 132.4, 132.7, 136.0, 137.0, 138.0, 169.7, 170.2, 174.0, 198.5. MS (EI): m/z 653 (6, M^+), 521 (5), 256 (6), 212 (10). Anal. calcd for $C_{41}H_{55}N_3O_4$: C, 75.31; H, 8.48; N, 6.43; found: C, 75.12; H, 8.38; N, 6.48.

***N*-[2-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-N^{α}-*tert*-butyloxycarbonylglycine amide (12a).** From *N*-Boc-glycine (0.700 g, 4.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (1.376 g, 4.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 2:3. Yield: 1.20 g (60%); mp 115 °C. IR (KBr): ν 3341, 2956, 2872, 1731, 1682, 1628, 1546 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 2.26 (s, 3H), 3.61 (s, 2H), 3.78 (d, J=6 Hz, 2H), 5.24 (m, 1H), 7.09 (m, 2H), 7.16 (m, 3H), 7.38 (m, 3H), 7.52 (m, 1H), 7.61 (m, 2H), 7.76 (m, 1H), 8.42 (m, 1H), 10.49 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 21.1, 28.2, 44.9, 45.2, 80.8, 122.4, 124.2, 124.5, 125.2, 128.3, 129.3, 129.6, 130.0, 131.1, 132.0, 132.7, 136.4, 137.0, 138.0, 160.8, 167.9, 170.3, 198.5. MS (EI): m/z 444 (100), 312 (92), 283 (43), 256 (30), 238 (58). Anal. calcd for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.38; found: C, 69.30; H, 6.27; N, 8.48.

***N*-[2-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenylamino]-2-oxoethyl]heptadecanoic acid amide (4).** *N*-[2-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-N^{α}-*tert*-butyloxycarbonylglycine amide (**12a**) (0.50 g, 1.0 mmol) was stirred in a 4 N solution of HCl (g) in dioxane for 1 h. After the volatiles were removed in vacuo, the residue (**13a**) was used without further purification for the reaction with heptadecanoic acid (0.270 g, 1.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.415 g (63%); mp 46 °C. IR (KBr): ν 3303, 2921, 2852, 1647, 1557 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.81 (t, J=7 Hz, 3H), 1.21 (m, 26H), 1.47 (m, 2H), 2.09 (t, J=7 Hz, 2H), 2.26 (s, 3H), 3.61 (s, 2H), 4.00 (d, J=5 Hz, 2H), 6.44 (m, 1H), 7.09 (m, 2H), 7.18 (m, 2H), 7.39 (m, 2H), 7.50 (m, 2H), 7.62 (m, 2H), 7.79 (m, 1H), 8.43 (m, 1H), 8.96 (s, 1H), 10.41 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 24.8, 25.5, 29.1, 29.2, 29.3, 29.4, 29.6, 29.65, 29.7, 31.9, 36.3, 44.4, 45.0, 122.4, 124.1, 124.2, 125.1, 128.3, 129.3, 129.6, 130.1, 131.1, 132.3, 132.7, 136.3, 137.0, 138.0, 167.2, 170.3, 174.4, 198.5. MS (EI): m/z 653 (49, M^+), 652 (100), 650 (38), 521 (34), 344 (93), 213 (31), 212 (94). Anal. calcd for $C_{41}H_{55}N_3O_4$: C, 75.31; H, 8.48; N, 6.43; found: C, 75.34; H, 8.79; N, 6.50.

***N*-[2-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-N^{γ}-*tert*-butyloxycarbonyl-γ-aminobutyric acid amide (12b).** From *N*-Boc-γ-aminobutyric acid (0.406 g, 2.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (0.688 g, 2.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 2:3. Yield: 0.675 g (65%); mp 65 °C. IR (KBr): ν 3307, 2925, 2855, 1667, 1558 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 9H), 1.73 (m, 2H), 2.24 (m, 2H), 2.25 (s, 3H), 3.12 (m, 2H), 3.60 (s, 2H), 7.08 (m, 2H), 7.18 (m, 3H), 7.38 (m, 2H)), 7.48 (m, 1H), 7.63 (m, 3H), 7.88 (m, 1H), 8.42 (m, 1H), 9.08 (s, 1H), 10.46 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 21.7, 22.6, 28.3, 34.3, 39.1, 45.0, 79.9, 122.3, 124.1, 124.5, 124.7, 125.0,

127.3, 128.3, 129.3, 129.6, 130.0, 131.2, 132.0, 132.6, 133.2, 136.9, 138.0, 157.3, 171.2, 171.5, 198.8. MS (EI): *m*/*z* 516 (21), 441 (27). Anal. calcd for $C_{31}H_{35}N_3O_5$: C, 70.30; H, 6.66; N, 7.93; found: C, 70.69; H, 6.99; N, 7.86.

***N*-[4-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenylamino]-4-oxobutyl]pentadecanoic acid amide (5).** From *N*-[2-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]-N^{γ}-*tert*-butyloxycarbonyl-γ-aminobutyric acid amide (**12b**) (1.06 g, 2.0 mmol) and pentadecanoic acid (0.484 g, 2.0 mmol) as described for **4**. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.560 g (43%); mp 115 °C. IR (KBr): ν 3297, 2922, 2852, 1645, 1549 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.81 (t, $J=7$ Hz, 3H), 1.20 (m, 22H), 1.50 (m, 2H), 1.75 (m, 2H), 2.07 (t, $J=8$ Hz, 2H), 2.21 (m, 2H)), 2.26 (s, 3H), 3.24 (m, 2H), 3.60 (s, 2H), 5.80 (m, 1H), 7.10 (m, 2H), 7.16 (m, 2H), 7.40 (m, 2H), 7.49 (m, 1H), 7.55 (m, 1H), 7.65 (m, 2H), 7.96 (m, 1H), 8.43 (m, 1H), 9.20 (s, 1H), 10.47 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 25.8, 26.6, 29.27, 29.3, 29.34, 29.5 29.6, 29.7, 31.9, 34.6, 36.8, 38.5, 45.0, 122.3, 124.1, 124.4, 125.2, 128.3, 129.3, 129.6, 130.1, 132.6, 133.1, 136.0, 137.0, 138.1, 170.1, 171.1, 174.7, 198.8. MS (EI): *m*/*z* 653 (18, M^+), 346 (15), 311 (20), 212 (21), 140 (64), 127 (100). Anal. calcd for $C_{41}H_{55}N_3O_4$: C, 75.31; H, 8.48; N, 6.43; found: C, 75.54; H, 8.06; N, 6.62.

***N*-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenyl]arachidic acid amide (6).** From arachinic acid (0.312 g, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (0.344 g, 1.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 2:3. Yield: 0.476 g (74%); mp 102 °C. IR (KBr): ν 3444, 2920, 2851, 1685, 1657 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.81 (t, $J=7$ Hz, 3H), 1.16–1.23 (m, 32H), 1.58 (m, 2H), 2.20 (t, $J=8$ Hz, 2H), 2.66 (s, 3H), 3.61 (s, 2H), 7.09 (m, 2H), 7.16 (m, 3H), 7.41 (m, 3H), 7.52 (m, 1H), 7.63 (m, 2H), 7.79 (m, 1H), 8.44 (m, 1H), 10.43 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 25.5, 29.2, 29.3, 29.4, 29.5, 29.6, 29.65, 29.7, 31.9, 37.6, 45.0, 122.4, 124.3, 125.1, 128.3, 129.3, 129.6, 130.0, 131.1, 132.7, 136.2, 137.0, 138.0, 171.4, 174.0, 198.6. MS (EI): *m*/*z* 638 (100, M^+), 533 (39), 506 (68), 212 (31). Anal. calcd for $C_{42}H_{59}N_2O_3$: C, 78.95; H, 9.15; N, 4.38; found: C, 78.62; H, 8.78; N, 4.73.

***N*-[3-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenylamino]-3-oxopropyl]-*N*-methylhexadecanoic acid amide (7).** From *N*-methyl-*N*-palmitoyl-β-alanine (0.341 g, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (0.344 g, 1.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.386 g (58%); oil. ^1H NMR ($CDCl_3$): δ 0.81 (t, $J=7$ Hz, 3H), 1.19 (m, 24H), 1.44 (m, 2H), 2.19 (t, $J=8$ Hz, 2H), 2.27 (s, 3H), 2.56 (m, 2H), 2.94 (s, 3H), 3.59 (m, 2H), 3.61 (s, 2H), 7.09 (m, 2H), 7.17 (m, 2H), 7.40 (m, 2H), 7.50 (m, 1H), 7.58 (m, 1H), 7.64 (m, 2H), 7.88 (m, 1H), 8.44 (m, 1H), 9.34 (s, 1H), 10.49 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 24.9, 29.3, 29.4, 29.5, 29.6, 29.64, 29.7, 31.9, 33.5, 35.9, 36.2, 44.1, 45.1, 122.2, 124.0, 124.5, 125.4, 128.2, 129.3, 129.6, 130.0, 131.3, 132.6, 132.9, 136.2, 136.9, 138.1, 168.9, 170.1, 174.8, 198.7. MS (EI): *m*/*z* 666 (100, M^+), 535 (32), 327 (38), 266 (39). Anal. calcd for $C_{42}H_{58}N_3O_4$: C, 75.41; H, 8.74; N, 6.28; found: C, 75.18; H, 8.57; N, 6.45.

***N*-[3-[3-Benzoyl-4-[(4-methylphenyl)acetylamino]phenylamino]-3-oxopropyl]octadecanoic acid amide (8).** From *N*-stearoyl-β-alanine (0.355 g, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)-(4-methylphenyl)acetamide (**11a**) (0.344 g, 1.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.435 g (64%); mp 95 °C. IR (KBr): ν 3300, 3057, 2920, 2851, 1649, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.80 (t, $J=7$ Hz, 3H), 1.17 (m, 28H), 1.41 (m, 2H), 1.99 (t, $J=7$ Hz, 2H), 2.25 (s, 3H), 2.43 (m, 2H), 3.40 (m, 2H), 3.58 (s, 2H), 6.28 (m, 1H), 7.08 (m, 2H), 7.14 (m, 2H), 7.37 (m, 2H), 7.46 (m, 1H), 7.51 (m, 1H), 7.60 (m, 2H), 7.78 (m, 1H), 8.36 (m, 1H), 8.92 (s, 2H), 10.38 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 21.1, 22.7, 25.7, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 35.2, 35.4., 36.4, 36.6, 44.9, 117.3, 117.4, 122.4, 124.2, 124.3, 125.2, 126.2, 126.3, 128.1, 128.2, 128.3 129.1, 129.2, 129.6, 130.0, 131.1, 131.3, 132.6, 132.9, 136.0, 137.0, 138.0, 170.2, 173.9, 174.0, 198.4. MS (EI): *m*/*z* 681 (53, M^+), 549 (73), 344 (31), 212 (100). Anal. calcd for $C_{43}H_{59}N_3O_4$: C, 75.73; H, 8.72; N, 6.16; found: C, 75.68; H, 8.70; N, 6.55.

***N*-[3-[3-Benzoyl-4-(phenylacetylamino)phenylamino]-3-oxopropyl]hexadecanoic acid amide (9).** From *N*-palmitoyl-β-alanine (0.490 g, 1.5 mmol) and *N*-(4-amino-2-benzoylphenyl)phenylacetamide (**11b**) (0.495 g, 1.5 mmol) according to general procedure. Purification: column chromatography: (1) ethyl acetate/*n*-hexane 2:3, (2) ethyl acetate. Yield: 0.85 g (88%); mp 117 °C. IR (KBr): ν 3310, 2920, 2850, 1640, 1550 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.81 (t, $J=7$ Hz, 3H), 1.17 (m, 24H), 1.45 (m, 2H), 2.02 (t, $J=7$ Hz, 2H), 2.46 (m, 2H), 3.44 (m, 2H), 3.65 (s, 2H), 6.12 (m, 1H), 7.23 (m, 1H), 7.29 (m, 3H), 7.39 (m, 2H), 7.48–7.57 (m, 2H), 7.62 (m, 2H), 7.77 (m, 1H), 8.43 (m, 2H), 10.45 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 25.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.62, 29.7, 31.9, 35.2, 36.7, 36.9, 45.4, 122.4, 124.2, 124.3, 125.3, 127.4, 128.3, 128.9, 129.4, 130.0, 132.6, 132.7, 134.2, 136.2, 138.0, 169.7, 170.0, 174.0, 198.6. MS (EI): *m*/*z* 639 (100, M^+), 330 (69), 312 (67), 212 (73). Anal. calcd for $C_{40}H_{53}N_3O_4$: C, 75.08; H, 8.35; N, 6.57; found: C, 74.77; H, 8.38; N, 6.89.

***N*-[3-[3-Benzoyl-4-(1-naphthoylamino)phenylamino]-3-oxopropyl]hexadecanoic acid amide (10).** From *N*-palmitoyl-β-alanine (0.327 g, 1.0 mmol) and *N*-(4-amino-2-benzoylphenyl)naphthaline1-carbonic acid amide (**11c**) (0.36 g, 1.0 mmol) according to general procedure. Purification: column chromatography ethyl acetate/*n*-hexane 3:2. Yield: 0.350 g (52%); mp 52 °C. IR (KBr): ν 3411, 2925, 2853, 1645, 1550 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.80 (t, $J=7$ Hz, 3H), 1.16 (m, 24H), 1.53 (m, 2H), 2.08 (t, $J=8$ Hz, 2H), 2.54 (m, 2H), 3.51 (m, 2H), 6.08 (m, 1H), 7.42 - 7.55 (m, 6H), 7.68 (m, 3H), 7.80 (m, 2H), 8.20 (s, 1H), 8.48 (m, 2H), 8.82 (m, 1H), 11.19 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.2, 22.8, 25.8, 29.3, 29.41, 29.43,

29.6, 29.70, 29.72, 29.77, 32.0, 35.3, 36.8, 37.3, 122.5, 124.2, 124.9, 125.6, 125.7, 125.8, 126.6, 127.4, 128.0, 128.5, 130.2, 131.5, 132.6, 132.9, 134.0, 134.1, 137.0, 138.2, 168.0, 169.7, 174.2, 199.1. MS (EI): *m*/*z* 675 (49, M^+), 420 (57), 366 (42), 155 (100). Anal. calcd for $C_{43}H_{53}N_3O_4$: C, 76.41; H, 7.90; N, 6.22; found: C, 76.21; H, 7.57; N, 6.18.

Enzyme preparation

Yeast farnesyltransferase was used as a fusionprotein to glutathione *S*-transferase at the N-terminus of the -subunit. Farnesyltransferase was expressed in *Escherichia coli* DH5 grown in LB media containing ampicillin and chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for farnesyltransferase production.[21] 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.[20] 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 L 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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