Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2399–2406

BIOORGANIC & MEDICINAL CHEMISTRY

Non-Peptidic, Non-Prenylic Bisubstrate Farnesyltransferase Inhibitors. Part 3: Structural Requirements of the Central Moiety for Farnesyltransferase Inhibitory Activity

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Received 14 June 1999; accepted 13 May 2000

Abstract—Recently, we have described non-peptidic, non-prenylic bisubstrate analogues as a novel type of farnesyltransferase inhibitor composed of a farnesyl-mimetic, a linker and an AAX-peptidomimetic substructure. With this study, we showed that the amide function connecting the farnesyl-mimetic and the linking substructures of our inhibitors is crucial for their activity. We suggest that the amide is bound to the essential zinc ion in the farnesyltransferases active center. We identified succinic and glutaric acid, respectively, in addition to the initially used β-alanyl moiety as suitable linking structures. Glycine can also be used in this function provided the distance between the α-amide group and the center of the peptidomimetic substructure is enlarged by introduction of an additional methylene unit into the peptidomimetic substructure.

Introduction

Ras proteins serve as molecular switches in the signal transduction cascade which controls cell differentiation and proliferation. Point mutations in the ras oncogene yield ras proteins locked in their GTP-bound active state. In approximately 30% of all human cancers, including 90% of pancreatic, 50% of colon, and 50% of thyroid tumors, these oncogenic ras proteins are found. To perform both their normal and their oncogenic activity, ras proteins must be farnesylated. The C-terminal CAAX amino acid sequence (C: cysteine, A: aliphatic amino acid, X: methionine or serine) of the ras protein is recognized by the enzyme farnesyltransferase (FTase) and farnesylated at the cysteine thiol function. In this reaction farnesylpyrophosphate (FPP) is serving as the prenyl group donor.[1]

Inhibition of the farnesylation reaction reverses the transformation caused by oncogenic ras. Therefore, inhibition of the farnesyltransferase was identified as a promising target in cancer therapy. Most known farnesyltransferase inhibitors are peptidomimetic analogues of the CAAX recognition sequence. Stable non-substrate analogues of the second substrate, farnesylpyrophosphate, are much less common. A third approach for inhibitor design is bisubstrate analogues: molecules that contain structural elements of both substrates, the CAAX-tetrapeptide and the farnesylpyrophosphate.[2]

We have recently reported the development of a novel type of bisubstrate analogue inhibitor of the farnesyltransferase.[3] These novel compounds (e.g., **1**) are conveniently composed of three modular building blocks: (1) a farnesyl-mimetic, (2) a linker and (3) an AAX- or AA-peptidomimetic, thus representing novel non-peptidic, non-prenylic bisubstrate analogue farnesyltransferase inhibitors. In contrast to other bisubstrates, our compounds lack peptidic substructures which are sensitive towards hydrolytic or enzymatic degradation and they lack the farnesyl residue which is susceptible towards oxidation due to its allylic positions.

Recent crystallographic studies provide considerable insight into the structure of a ternary FTase–FPP–CAAX complex.[4] The CAAX tetrapeptide has been shown to bind in an extended conformation with its thiol sulfur coordinated to the zinc ion located in the FTase active site[4] as has been postulated[5] (Fig. 1(a)). We imagine that our bisubstrate inhibitors should occupy considerable portions of the peptide as well as the farnesyl binding regions in the FTase active center (Fig. 1(b)). We suggest that our bisubstrate analogue inhibitors are inserted

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0968-0896/00/$ - see front matter
PII: S0968-0896(00)00173-5

into the farnesyltransferase, in a way that the carbonyl oxygen of the amide group which connects the farnesyl surrogate to the linking structure is coordinated to the zinc ion in the active center. This interaction should be crucial for the inhibitory activity of our inhibitors. Therefore, the length and the nature of the linking structure which governs the position of this presumably essential carbonyl group should be crucial for the inhibitory activity of our bisubstrate inhibitors. In the present study we chose compound **1** as a lead and replaced the β-alanyl linker by various moieties (Figure 2, Table 1). These variations were accompanied by minor variations of the overall dimensions of the molecules. In a previous study,[6] we have shown that minor variations in the length of the farnesyl-mimetic do not change the inhibitory activity significantly so that differences in the inhibitory activity can be assigned to the variations of the linking moiety.

Chemistry

The synthesis of the target compounds **2**–**10** is outlined in Scheme 1. Compound **2** was obtained from 4-aminobenzoylmethionine methyl ester **11**[7] and arachidic acid, which was activated with *iso*-butyl chloroformate as a mixed anhydride. Compound **10** was obtained in the same way using palmitoyl-*N*-methyl-β-alanine instead of arachidic acid. For the synthesis of **3** and **4**, **11** was acylated with *N*-Boc-glycine and *N*-Boc-γ-aminobutyric acid, respectively, using mixed anhydride activation. The resulting amides **12** and **13** were deprotected with HCl in dioxane and acylated with palmitic acid chloride. Heating of **11** with succinic and glutaric anhydride, respectively, in toluene/dioxane yielded the acids **14** and **15**, which were coupled with palmityl amine to the target compounds **5** and **6**, using the PyBOP methodology. Compound **16** was obtained from 4-(*tert*-butyloxycarbonylaminomethyl)benzoic acid[8] and methionine methyl ester hydrochloride. Compound **16** was deprotected and acylated with stearoyl chloride yielding **7**. Compounds **8** and **9** were obtained from deprotected **16** using the same sequence as described for the synthesis of **3**.

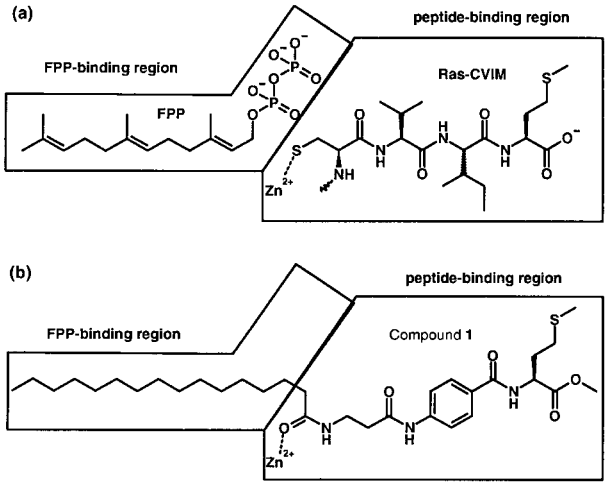


Figure 1. Schematic model of the FTase active center with: (a) the natural substrates; (b) compound **1** in the suggested binding mode.

Table 1. Farnesyltransferase inhibition of compounds **1**–**10**

Compound	$IC_{50} \pm SD$ (μM)
1	3.7±0.7
2	>100
3	12.0±1.1
4	13.1±1.0
5	5.7±0.5
6	5.4±0.1
7	90±5
8	7.5±0.3
9	7.3±0.3
10	>100

Farnesyltransferase inhibition assay

The inhibitory activity of the inhibitors was determined using the fluorescence enhancement assay as described

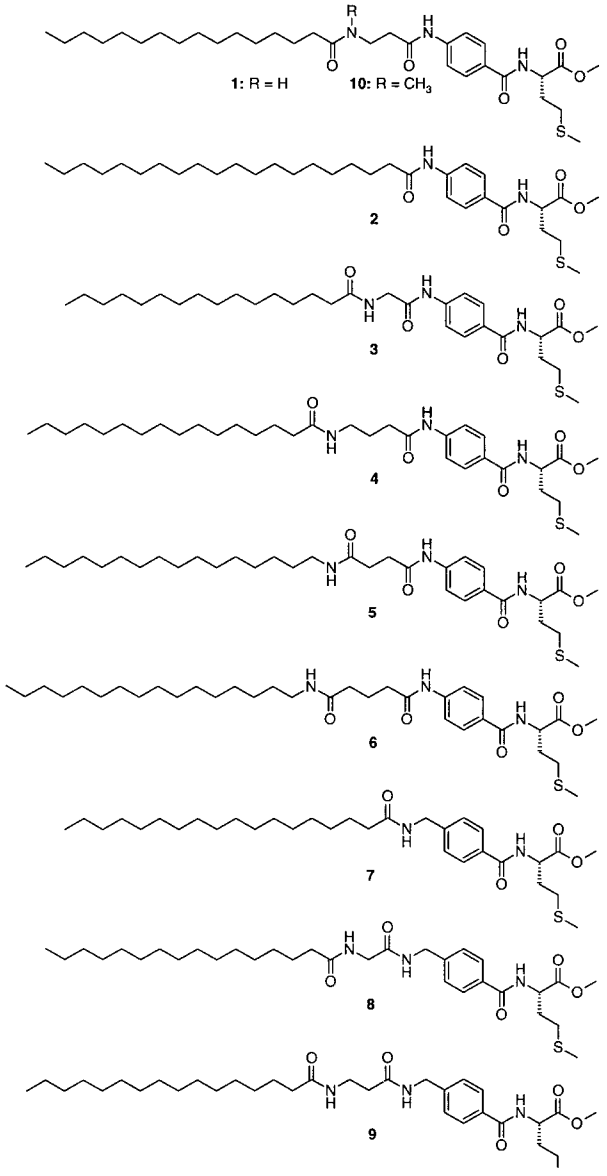
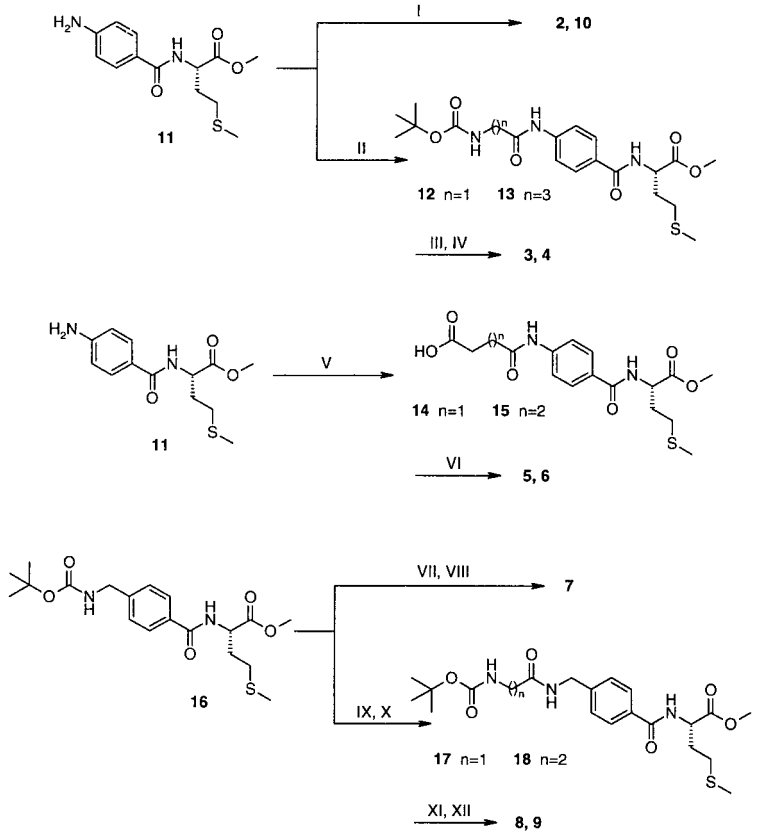


Figure 2. Structures of the lead compound **1** and the novel inhibitors **2**–**10**.



Scheme 1. (I) (a) Arachidic acid or *N*-palmitoyl-*N*-methyl-β-alanine, *i*-BuOCOCl, NMM, DMF, −15 °C, 5 min, (b) add **11**, DMF to (a) −15 °C→rt, overnight; (II) (a) *N*-Boc-glycine or *N*-Boc-γ-aminobutyric acid, *i*-BuOCOCl, NMM, DMF, −15 °C, 5 min, (b) add **11**, DMF to (a), −15 °C→rt, overnight; (III) 4 N HCl/dioxane, rt, 2 h; (IV) palmitoyl chloride, NMM, CH_2Cl_2, 0 °C→rt, overnight; (V) succinic anhydride or glutaric anhydride, toluene/dioxane, 80 °C, 3 h; (VI) hexadecylamine, PyBOP, DIPEA, DMF, rt, overnight; (VII) 4 N HCl/dioxane, rt, 2 h; (VIII) stearoyl chloride, NMM, CH_2Cl_2, 0 °C→rt, overnight; (IX) 4 N HCl/dioxane, rt, 2 h; (X) (a) *N*-Boc-glycine or *N*-Boc-β-alanine, *i*-BuOCOCl, NMM, DMF, −15 °C, 5 min, (b) add product from preceding step, NMM, DMF to (a), −15 °C→rt, overnight; (XI) 4 N HCl/dioxane, rt, 2 h; (XII) palmitoyl chloride, NMM, CH_2Cl_2, 0 °C→rt, overnight.

by Pompliano.[9] The assay employed yeast farnesyltransferase (FTase) fused to glutathione *S*-transferase at the N-terminus of the β-subunit.[10] Farnesylpyrophosphate and the dansylated pentapeptide Ds-GlyCysValLeuSer were used as substrates. Upon farnesylation of the cysteine thiol the dansyl residue is placed in a lipophilic environment which results in an enhancement of fluorescence at 505 nm which is used to monitor the enzyme reaction.

Results and Discussion

In our inhibitor binding model (Fig. 1(b)), the amide oxygen should exert a crucial interaction with the zinc ion located in the active center of the FTase. Therefore, a molecule in which this amide function is replaced by a structure unable to act as a π-donor like an ethylene group should show a considerable decrease in farnesyltransferase inhibitory activity. To study this hypothesis, we prepared compound **2** (Fig. 2), which has the same overall dimensions as our lead structure **1** but lacks the amide function in question. As shown in Table 1, **2** is at least two orders of magnitude less active against farnesyltransferase than **1**. This strongly supports our picture of how our molecules are bound to the enzyme's active center.

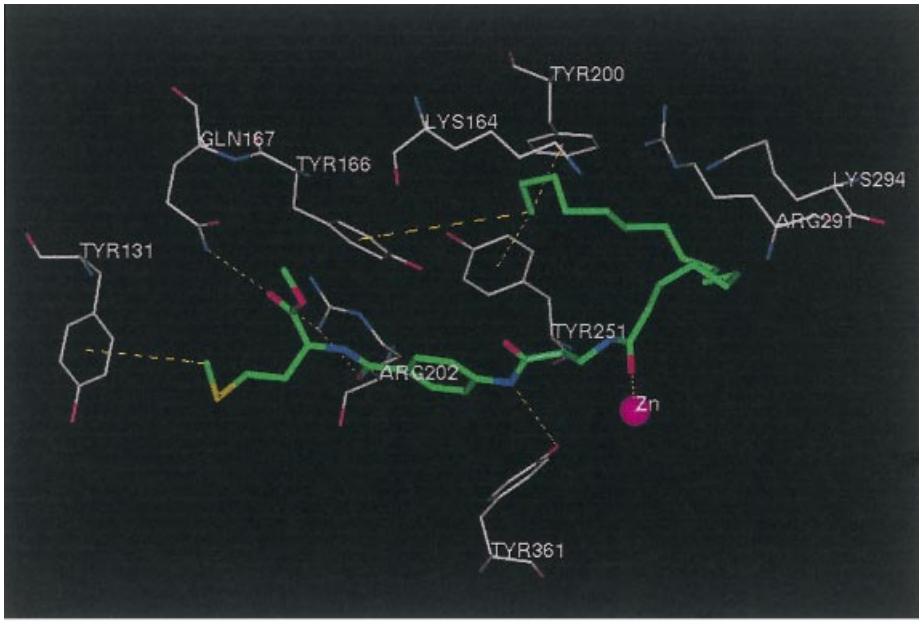
For the initial design of **1** as well as for other bisubstrate analogue inhibitors, we chose β-alanine as the linking moiety because the distance between its carbonyl carbon and its nitrogen resembles the distance between the carbonyl carbon and the thiol sulfur in cysteine.[3,6] After showing the importance of the amide group, we next changed its position by replacing the β-alanyl moiety by glycine (**3**) and γ-aminobutyric acid (**4**), respectively, thereby reducing or increasing the distance between the amide group and the AAM-peptidomimetic substructure by one methylene unit. Both variations led to a threefold reduction in inhibitory activity.

We next inverted the amide structure by replacing the amino acid linkers by dicarboxylic acids succinic (**5**) and glutaric acid (**6**), respectively. In both cases, this resulted in an approximately 2-fold reduction in inhibitory activity, compared to the lead compound **1**, but an improvement in activity if compared to compounds **3** and **4**.

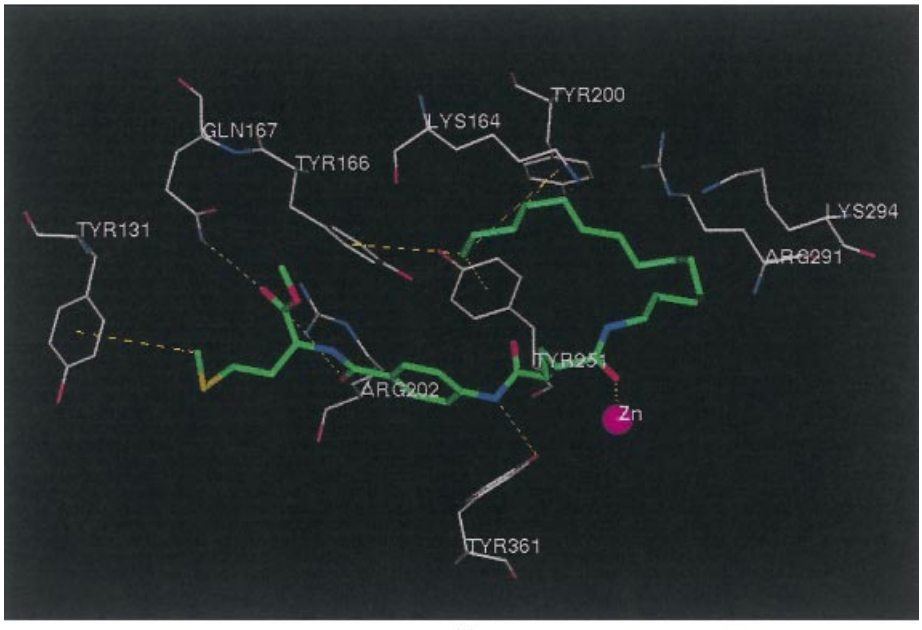
Finally, we introduced a methylene unit into the AAM-peptidomimetic substructure by replacing the 4-aminobenzoyl moiety by an 4-aminomethylbenzoyl structure. The leftmost amide group in the *N*-stearoylaminomethylbenzoyl derivative **7** is obviously still too close to the center of the AAM-peptidomimetic substructure to

produce a relevant inhibitory activity against farnesyltransferase although the activity of this compound is somewhat higher than that of **2**. Reintroduction of glycine and β-alanine as linkers (compounds **8** and **9**) improved the inhibitory activity significantly; both compounds being almost as potent inhibitors as our lead structure **1**. In addition to the secondary amides investigated so far we have prepared the tertiary amide derivative **10**. In comparison to the lead structure **1**, compound **10** shows a distinct decrease in inhibitory activity which may be due to a dislodgement of the carbonyl oxygen from the enzyme-bound zinc because of steric reasons.

Recently, we reported[6] the docking of compound **1** into the active site using the coordinates of the published[4] crystal structure of a ternary complex of farnesyltransferase, a farnesylpyrophosphate analogue and *N*-Ac-Cys-Val-Ile-selenoMetOH. Flexible docking was performed using the program FlexX.[11] The C-terminal methionine was used as starting fragment and placed at the same position as the seleno-methionine of *N*-Ac-Cys-Val-Ile-selenoMetOH in the crystal structure. Then, the docking program placed the remainder of the molecule fragment by fragment into the active site searching for favorable interactions between the ligand and the amino acid side chains while avoiding steric overlaps.



(a)



(b)

Figure 3. (a) Interactions between **1** and amino acid side chains in the active site; (b) Interactions between **6** and amino acid side chains in the active site.

Figure 3(a) shows interactions between **1** and amino acid side chains in the active site. One key interaction calculated by FlexX is the interaction between the carbonyl oxygen of the palmitoyl residue and the enzyme-bound zinc, as we suggested above. In the present study we performed additional docking runs with compounds **2** and **6**. Not surprisingly, no interaction between **2** and the enzyme-bound zinc could be found by FlexX. This easily explains the drop in activity of at least two orders of magnitude. Interactions calculated between **6** and the amino acid side chains of the farnesyltransferase are basically the same as for **1** (Fig. 3(b)). Hydrophobic interactions are found between the methionine side chain of **6** and Tyr 131α. The C-terminal carbonyl group of **6** is hydrogen-bonded to Gln 167α and to the guanidino group of Arg 202β, which also forms a second hydrogen bond to the carbonyl of the central aminobenzoyl moiety. Another hydrogen bond is formed between the hydroxy group of Tyr 361β and the amide nitrogen in the 4-position of the aminobenzoyl moiety. The docking program calculates again an interaction between the terminal carbonyl group of the glutaryl linker and the zinc. The central part of the alkyl chain is placed close to the side chains of Lys 164α, Lys 294β and Arg 291β, which form the positively charged cleft binding the pyrophosphate portion of FPP.[4] Interactions are found between the terminal carbons of the alkyl chain hydrophobic and Tyr 166α, Tyr 200α and Tyr 251β, which form a part of the prenyl binding cleft.[4]

In summary, structure–activity relationships and docking experiments provide strong evidence for the initial hypothesis that the amide structure connecting the farnesyl mimetic and the linking substructures is crucial for the inhibitory activity of our novel type of bisubstrate analogue farnesyltransferase inhibitors. Variations of the position and inversion of the amide group showed distinct influence on the inhibitory activity. We assign the observed difference in inhibitory activity to the variations of the amide group position, since in a previous study on the structure–activity relationships of our bisubstrate inhibitors we have shown that variations of the chain length of the farnesyl mimetic to the extent done here do not produce significant effects on the inhibitory activity.[6] This study left our initially chosen linker β-alanine as the best linking structure but shows that in addition to that moiety succinic and glutaric acids can be used for that position. A tolerable reduction in activity is also observed upon the replacement of β-alanine by glycine if an additional methylene unit is introduced in the AAX-peptidomimetic substructure. As they open a broader selection of building blocks our linking substructure can be chosen from, the reported results will be applied for our ongoing research on these novel bisubstrate analogue farnesyltransferase inhibitors.

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 an 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 procedures for amide bond formation

Procedure A: mixed anhydride method. 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 equiv) in dry DMF was added after 5 min. When the amine component was employed as a hydrochloride, additional NMM (0.25 mL per mmol) 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 recrystallization or flash chromatography.

Procedure B: PyBOP method. One equiv of the appropriate acid, hexadecylamine and PyBOP (520 mg per mmol acid), respectively, were dissolved in dry DMF in a flame dried flask. After addition of diisopropylethylamine (0.65 mL per mmol acid) the mixture was stirred at room temperature overnight. Then, the mixture was poured into brine (400–800 mL). 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 flash chromatography.

Procedure C: acid chloride method. To a solution of the appropriate amine hydrochloride in a sufficient amount of dry CH_2Cl_2 and *N*-methylmorpholine (0.24 mL per mmol amine), 1 equiv of the appropriate acid chloride was added at 0 °C. Stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2 and 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 flash chromatography.

General procedure for the cleavage of *N*-Boc protective groups

The *N*-Boc derivatives were dissolved in 4 N HCl in dioxane (10 mL per mmol) and stirred for 2 h at room temperature. After addition of diethyl ether, the volatiles were removed in vacuo. The residue was used without further purification.

***N*-(4-Arachidoylaminobenzoyl)methionine methyl ester (2).** From arachidic acid (313 mg, 1 mmol) and **11** (282 mg, 1 mmol) using procedure A. Purification: flash chromatography (ethyl acetate:hexane, 3:2). Yield: 385 mg (67%); mp 52 °C; IR (KBr) ν 3440, 2920, 2850, 1740, 1710, 1625 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.85 (m, 3H), 1.18–1.25 (m, 32H), 1.46 (m, 2H), 1.98–2.06 (m, 5H), 2.54 (m, 2H), 3.64 (s, 3H), 4.52 (m, 1H), 5.61 (s, 1H), 6.56 (m, 2H), 7.61 (m, 2H), 8.20 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.5, 16.2, 22.6, 29.4, 29.5, 30.8, 30.9, 32.2, 37.0, 52.2, 52.3, 113.0, 120.1, 129.6, 153.3, 167.6, 168.0, 168.3. MS (EI): *m*/*z* 339 (9), 312 (18), 100 (27), 87 (100). Anal. calcd for $C_{33}H_{56}N_2O_4S$: C, 68.71; H, 9.78; N, 4.86; S, 5.56; found: C, 68.83; H, 10.09; N, 4.70; S, 5.88.

***N*-[4-(*tert*-Butyloxycarbonylaminoacetylamino)benzoyl]-methionine methyl ester (12).** From *N*-Boc-glycine (875 mg, 5 mmol) and **11** (1.41 g, 5 mmol) using procedure A. Yield: 1.76 g (99%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.08 (s, 3H), 2.12–2.15 (m, 1H), 2.21–2.29 (m, 1H), 2.58 (m, 2H), 3.76 (s, 3H), 3.82–3.95 (m, 2H), 4.90 (m, 1H), 7.39 (m, 1H), 7.50 (m, 1H), 7.67–7.74 (m, 2H).

***N*-[4-(Hexadecanoylaminoacetylamino)benzoyl]methionine methyl ester (3).** Compound **12** (660 mg, 1.5 mmol) was deprotected as described and coupled with palmitoyl chloride (0.46 mL, 1.5 mmol) using procedure C. Purification: flash chromatography (ethyl acetate: hexane, 3:2). Yield: 800 mg (92%); mp 97 °C. IR (KBr) ν 3400, 3315, 2920, 2850, 1745, 1695, 1640, 1605 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.85 (t, J = 7 Hz, 3H), 1.21–1.24 (m, 24H), 1.51 (m, 2H), 2.06–2.08 (m, 5H), 2.15 (t, J = 7 Hz, 2H), 2.52–2.60 (m, 2H), 3.65 (s, 3H), 3.88 (d, J = 5 Hz, 2H), 4.58 (m, 1H), 7.65–7.68 (m, 2H), 7.83–7.86 (m, 2H), 8.07 (m, 1H), 8.59 (d, J = 7 Hz, 1H), 10.14 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.5, 15.2, 22.6, 25.7, 29.2, 29.4, 29.5, 29.6, 30.5, 30.8, 31.8, 35.7, 43.2, 52.2, 52.5, 118.8, 128.7, 129.0, 142.3, 166.7, 168.9, 173.0, 173.3. MS (EI): *m*/*z* 577 (6, M^+), 415 (40), 208 (25), 146 (28), 120 (100). Anal. calcd for $C_{31}H_{51}N_3O_5S$: C, 64.44; H, 8.89; N, 7.27; S, 5.55; found: C, 64.79; H, 8.91; N, 7.15; S, 5.83.

***N*-{4-[(4-*tert*-Butyloxycarbonylamino)butanoylamino]-benzoyl}methionine methyl ester (13).** From *N*-Boc-γ-aminobutyric acid (508 mg, 2.5 mmol) and **11** (705 mg, 2.5 mmol) using procedure A. Yield: 900 mg (75%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.80 (m, 2H), 2.08–2.12 (m, 5H), 2.38 (m, 2H), 2.56 (m, 2H), 3.14 (m, 2H), 3.75 (s, 3H), 4.87 (m, 1H), 6.61 (m, 1H), 7.60–7.76 (m, 3H).

***N*-{4-[(4-Hexadecanoylamino)butanoylamino]benzoyl}-methionine methyl ester (4).** Compound **13** (900 mg, 1.88 mmol) was deprotected as described and coupled with palmitoyl chloride (0.58 mL, 1.88 mmol) using procedure C. Purification: flash chromatography (ethyl acetate:hexane, 3:2). Yield: 978 mg (86%); mp 81 °C. IR (KBr) ν 3320, 2915, 2850, 1745, 1640 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.76 (t, J = 7 Hz, 3H), 1.09–1.17 (m, 24H), 1.40 (m, 2H), 1.62 (m, 2H), 1.93–1.97 (m, 7H), 2.24 (t, J = 7 Hz, 2H), 2.45 (m, 2H), 2.99 (m, 2H), 3.55 (s, 3H), 4.48 (m, 1H), 7.56–7.58 (m, 2H), 7.73–7.75 (m, 2H), 8.48 (m, 1H), 10.00 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.4, 16.1, 23.5, 26.6, 26.8, 30.2, 30.3, 30.4, 30.45, 30.5, 31.4, 31.7, 32.7, 35.4, 37.0, 53.1, 53.4, 119.6, 128.8, 129.6, 143.6, 167.6, 172.8, 173.6, 174.0. MS (EI) *m*/*z* 605 (20, M^+), 545 (38), 531 (100), 443 (96). Anal. calcd for $C_{33}H_{55}N_3O_5S$: C, 65.45; H, 9.09; N, 6.94; found: C, 65.50; H, 8.67; N, 6.84.

3-{{*N*-{4-[*N*-(1-Methoxycarbonyl-3-methylthiopropyl)-carbamoyl]phenyl}carbamoyl}}-propionic acid (14). Compound **11** (564 mg, 2 mmol) was dissolved in a hot mixture of toluene (50 mL) and dioxane (10 mL). Then, succinic anhydride (200 mg, 2 mmol) dissolved in dioxane (10 mL) was added and the mixture was kept at 80 °C for 3 h. Most of the solvent was removed in vacuo and pentane was added carefully to the remaining solution. The solution was kept at room temperature until crystallization occurred. Yield: 600 mg (78%). ^1H NMR (400 MHz, DMSO-d_6) δ 2.02–2.06 (m, 5H), 2.48–2.62 (m, 6H), 3.64 (s, 3H), 4.56 (m, 1H), 7.66 (m, 2H), 7.83 (m, 2H), 8.57 (d, J = 7 Hz, 1H), 10.16 (s, 1H).

***N*-{4-[(3-Hexadecylaminocarbonylpropanoyl)amino]benzoyl}methionine methyl ester (5).** From **14** (382 mg, 1 mmol) and hexadecylamine (241 mg, 1 mmol) using procedure B. Purification: flash chromatography (1. ethyl acetate:hexane, 3:2; 2. ethyl acetate). Yield 310 mg (51%); mp 163 °C. IR (KBr) ν 3315, 2920, 2850, 1750, 1665, 1640, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.77 (t, J = 7 Hz, 1.15 (s, 26H), 1.28 (m, 2H), 1.96–1.99 (m, 5H), 2.31 (m, 2H), 2.46–2.49 (m, 4H), 2.94 (m, 2H), 3.57 (s, 3H), 4.48 (m, 1H), 7.58 (m, 2H), 7.69 (m, 1H), 7.75 (m, 2H), 8.49 (m, 1H), 10.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.8, 14.5, 21.9, 26.3, 28.5, 28.6, 28.8, 28.9, 29.0, 30.1, 31.1, 31.7, 38.4, 51.5, 51.8, 117.9, 127.7, 128.2, 142.1, 166.0, 170.7, 170.8, 172.4. MS (EI) *m*/*z* 605 (0.1, M^+), 323 (35), 120 (64), 100 (100). Anal. calcd for $C_{33}H_{55}N_3O_5S$: C, 65.45; H, 9.09; N, 6.94; found: C, 65.32; H, 9.20; N, 6.54.

4-{{*N*-{4-[*N*-(1-Methoxycarbonyl-3-methylthiopropyl)-carbamoyl]phenyl}carbamoyl}}butyric acid (15). Compound **15** was prepared as described for **14** from **11** (564 mg, 2 mmol) and glutaric anhydride (228 mg, 2 mmol). Yield 749 mg (95%). ^1H NMR (400 MHz, DMSO-d_6) δ 1.84 (m, 2H), 2.02–2.06 (m, 5H), 2.28 (m, 2H), 2.38 (m, 2H), 2.52–2.62 (m, 2H), 3.65 (s, 3H), 4.58 (m, 1H), 7.67 (m, 2H), 7.83 (m, 2H), 8.57 (m, 1H), 10.10 (s, 1H).

***N*-{4-[(4-Hexadecylaminocarbonylbutanoyl)amino]benzoyl}methionine methyl ester (6).** From **15** (396 mg, 1 mmol) and hexadecylamine (241 mg, 1 mmol) using procedure B. Purification: flash chromatography (1. ethyl acetate:hexane, 3:2; 2. ethyl acetate). Yield 338 mg (55%); mp 134 °C. IR (KBr) ν 3305, 2920, 2850, 1740, 1665, 1640, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.76 (t, J = 7 Hz, 3H), 1.12–1.18 (m, 26H), 1.28 (m, 2H), 1.72 (m, 2H), 1.96–1.98 (m, 5H), 2.03 (m, 2H), 2.24 (m, 2H), 2.46 (m, 2H), 2.93 (m, 2H), 3.56 (s, 3H), 4.48 (m, 1H), 7.58 (m, 2H), 7.60 (m, 1H), 7.74 (m, 2H), 8.48 (d, J = 7 Hz, 1H), 9.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.8, 14.5, 21.0, 21.9, 26.3, 28.5, 28.6, 28.8, 28.9, 29.0,

29.8, 30.1, 31.1, 34.6, 35.7, 38.3, 38.9, 51.5, 51.8, 118.0, 128.2, 142.1, 166.0, 171.1, 172.4. MS (EI) *m*/*z* 619 (4, M^+), 545 (13), 457 (19), 339 (22), 338 (100), 120 (65). Anal. calcd for $C_{34}H_{57}N_3O_5S$: C, 65.91; H, 9.20; N, 6.78; found: C, 65.49; H, 9.32; N, 6.82.

***N*-(4-*tert*-Butyloxycarbonylaminomethylbenzoyl)methionine methyl ester (16).** From 4-*tert*-butyloxycarbonylaminobenzoic acid[8] (2.2 g, 10 mmol) and methionine methyl ester hydrochloride (2.0 g, 10 mmol) using procedure A. Yield 2.2 g (55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.07–2.13 (m, 1H), 2.09 (s, 3H), 2.26 (m, 1H), 2.55 (m, 2H), 3.77 (s, 3H), 4.33 (d, $J=6$ Hz, 2H), 4.91 (m, 1H), 6.92 (d, $J=6$ Hz, 1H), 7.32–7.34 (m, 2H), 7.74–7.76 (m, 2H).

***N*-(4-Octadecanoylaminomethylbenzoyl)methionine methyl ester (7).** Compound **16** (594 mg, 1.5) was deprotected as described and coupled with stearoyl chloride (450 mg, 1.5 mmol) using procedure C. Purification: recrystallization from ethyl acetate. Yield: 440 mg (52%); mp 67 °C. IR (KBr) ν 3305, 2920, 2850, 1740, 1700, 1640 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (m, 3H), 1.24 (m, 28H), 1.52 (m, 2H), 1.98–2.05 (m, 5H), 2.15 (m, 2H), 2.58 (m, 2H), 3.65 (s, 3H), 4.31 (m, 2H), 4.58 (m, 1H), 7.32 (m, 2H), 7.84 (m, 2H). MS (EI) *m*/*z* 562 (10, M^+), 488 (100), 400 (61). Anal. calcd for $C_{32}H_{54}N_2O_4S$: C, 68.28; H, 9.67; N, 5.69; S, 5.69; found: C, 68.06; H, 10.47; N, 5.22; S, 5.38.

***N*-[4-(*tert*-Butyloxycarbonylaminoacetylaminomethyl)benzoyl]methionine methyl ester (17).** Compound **16** (660 mg, 1.67 mmol) was deprotected as described and coupled with *N*-Boc-glycine (292 mg, 1.67 mmol) using procedure A. Yield: 600 mg (79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (m, 9H), 2.02–2.10 (m, 5H), 2.57 (m, 2H), 3.68 (s, 3H), 3.82 (d, $J=6$ Hz, 2H), 4.47 (d, $J=6$ Hz, 2H), 4.90 (m, 1H), 7.30 (m, 2H), 7.74 (m, 2H).

***N*-[4-(Hexadecanoylaminoacetylaminomethyl)benzoyl]methionine methyl ester (8).** Compound **17** (600 mg, 1.32 mmol) was deprotected as described and coupled with palmitoyl chloride (0.4 mL, 1.32 mmol) using procedure C. Purification: flash chromatography (ethyl acetate:hexane, 3:2). Yield 548 mg (70%); mp 94 °C. IR (KBr) ν 3315, 2920, 2850, 1745, 1640 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 (t, $J=7$ Hz, 3H), 1.13–1.19 (m, 24H), 1.96–2.02 (m, 5H), 2.04 (m, 2H), 2.45–2.53 (m, 2H), 3.56 (s, 3H), 3.63 (d, $J=6$ Hz, 2H), 4.24 (d, $J=6$ Hz, 2H), 4.49 (m, 1H), 7.25 (m, 2H), 7.74 (m, 2H), 7.90 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.6, 14.2, 21.7, 24.7, 24.9, 28.3, 28.4, 28.5, 28.6. 28.65, 28.7, 29.6, 29.9, 30.9, 34.9, 41.4, 41.8, 51.3, 51.6, 126.5, 127.1, 131.8, 142.8, 166.1, 169.9, 172.1, 172.3. MS (EI) *m*/*z* 591, (10, M^+), 517 (50), 296 (30), 118 (37). Anal. calcd for $C_{32}H_{53}N_3O_5S$: C, 64.97; H, 8.96; N, 7.10; found: C, 64.53; H, 9.34; N, 7.52.

***N*-{4-[3-(*tert*-Butyloxycarbonylamino)propanoylaminomethyl]benzoyl}methionine methyl ester (18).** Compound **16** (1.19 g, 3 mmol) was deprotected as described and coupled with *N*-Boc-β-alanine (567 mg, 3 mmol) using procedure A. Yield 1.05 g (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (m, 9H), 2.07–2.13 (m, 5H), 2.44 (m, 2H), 2.54 (m, 2H), 3.38 (m, 2H), 3.76 (s, 3H), 4.44 (d, $J=6$ Hz, 2H), 4.88 (m, 1H), 7.30 (m, 2H), 7.73 (m, 2H).

***N*-{4-[3-(Hexadecanoylamino)propanoylaminomethyl]benzoyl}methionine methyl ester (9).** Compound **18** (500 mg, 1.07 mmol) was deprotected as described and coupled with palmitoyl chloride (0.34 mL, 1.07 mmol) using procedure C. Purification: flash chromatography (1. ethyl acetate:hexane, 3:2; 2. ethyl acetate). Yield 356 mg (55%); mp 147 °C. IR (KBr) ν 3300, 2920, 2850, 1745, 1640 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 (t, $J=7$ Hz, 3H), 1.37 (m, 24H), 1.91–1.98 (m, 5H), 2.23 (m, 2H), 2.41–2.55 (m, 4H), 3.16 (m, 2H), 3.56 (s, 3H), 4.22 (d, $J=6$ Hz, 2H), 4.51 (m, 1H), 7.24 (m, 2H), 7.69 (m, 1H), 7.74 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.8, 14.5, 22.0, 28.6, 28.7, 28.8, 28.9, 29.0, 29.9, 30.1, 31.2, 35.2, 35.3, 35.4, 41.7, 51.6, 51.8, 126.8, 127.4, 143.1, 166.4, 170.4, 172.0, 172.3. MS (EI) *m*/*z* 605 (1, M^+), 531 (15), 324 (67). Anal. calcd for $C_{33}H_{55}N_3O_5S$: C, 65.45; H, 9.09; N, 6.94; found: C, 65.52; H, 8.83; N, 7.11.

***N*-{4-{3-[*N*-(Hexadecanoyl)-*N*-methylamino]propanoylamin}benzoyl}methionine methyl ester (10).** Compound **10** was prepared from *N*-palmitoyl-*N*-methyl-β-alanine (512 mg, 1.5 mmol) and **11** (423 mg, 1.5 mmol) using procedure A. Purification: flash chromatography (ethyl acetate). Yield 116 mg (13%); oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (t, $J=7$ Hz, 3H), 1.17 (m, 24H), 1.56 (m, 2H), 2.04 (m, 5H), 2.28 (m, 2H), 2.52 (m, 2H), 2.67 (m, 2H), 3.00 (s, 3H), 3.69 (m, 3H), 3.72 (s, 3H), 4.85 (m, 1H), 6.85 (d, $J=8$ Hz, 1H), 7.66 (m, 2H), 7.70 (m, 2H), 9.49 (s, 1H). MS (EI) *m*/*z* 605 (5, M^+), 525 (15, 215 (16). HRMS calcd for $C_{33}H_{55}N_3O_5S$: 605.3862; found: 605.3850.

Farnesyltransferase assay

Yeast farnesyltransferase (FTase) fused to glutathione *S*-transferase at the N-terminus of the β-subunit was expressed in *Escherichia coli* DH5α grown in LB media containing ampicillin and additional chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for FTase production.[10] The enzyme was purified by standard protocol using glutathione–agarose beads for selective binding of the target proteins. FPP was obtained as ammonium salt solution in methanol:10 mM aq NH_4Cl (7:3) from Sigma-Aldrich. Dansyl-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 DTT, 7 μM Ds-GCVLS, 20 μM FPP and approx. 5 nmol yeast GST–FTase and 1% of various concentrations of the test compounds dissolved in DMSO. The progress of the enzyme reaction was followed by 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}s were calculated from initial velocity of three independent measurements of each inhibitor concentration and expressed as mean ± SD.

Acknowledgements

The pGEX-DPR1 and pBC-RAM2 plasmids were kindly provided by Prof. F. Tamanoi (UCLA). M. Schlitzer thanks Ms. K. Burk, Ms. S. Eilers and Ms. K. Sellinger for technical assistance. I. Sattler wishes to thank Prof. Dr. S. Grabley and PD Dr. R. Thiericke for generous support and Ms. S. Egner for excellent technical assistance.

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