Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2037–2045

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Evaluation of Homofarnesoyl-Substituted CAAX-Peptidomimetics as Farnesyltransferase Inhibitors and Antiproliferative Agents

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Received 8 March 1999; accepted 3 May 1999

Abstract—Several CAAX-peptidomimetics were linked to homofarnesoic acid via a β-alanyl spacer with the intention to obtain a novel type of bisubstrate analogue farnesyltransferase inhibitors. However, the compounds were found to be only weakly active in the farnesyltransferase inhibition assay. Nevertheless, they displayed antiproliferative activity against different tumor cell lines in the low micromolar range. Replacement of the β-alanine moiety by aspartic acid-1-methyl ester resulted in a compound which inhibited the farnesyltransferase with an IC_{50} of 860 nM. The corresponding free acid showed a eightfold loss in activity ($IC_{50} = 6.9$ μM).

Introduction

Ras proteins are members of the family of small guanyl nucleotide binding proteins. They 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 mutated ras proteins are found. To perform both, their normal and their oncogenic functions, ras proteins must undergo a series of post-translational modifications. First, a 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 farnesyl pyrophosphate (FPP) is serving as the prenyl group donor (Fig. 1). After proteolytic removal of the AAX tripeptide, the resulting C-terminal *S*-farnesyl cysteine is reversibly converted into its methyl ester. From these post-translational modification events only the farnesylation is obligatory for ras activity (see reviews[1–8]).

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. Intense efforts of several groups have resulted in a large number of different FTase inhibitors.[1–8] Most groups used the CAAX recognition motif as a template for their inhibitors. In comparison to CAAX mimetics obtained by this approach, stable non-substrate analogues of the second substrate farnesylpyrophosphate are much less common.[1–8] Since the ras farnesylation is a bisubstrate reaction and therefore two binding domains for two different substrates have to exist in the enzyme in close proximity, an interesting approach is the development of bisubstrate analogues: molecules that display structural features of both substrates, the CAAX tetrapeptide and the farnesyl pyrophosphate. One example of these bisubstrate analogues is **1** which is 100-fold more active ($IC_{50} = 0.033$ μM) than its parent tetrapeptide CVLS ($IC_{50} = 4$ μM).[2] Representative examples of the few published bisubstrate analogues (**1**–**4**)[9–11] are shown in Chart 1.

Inhibitors **1**–**4** contain an unmodified AAX tripeptide motif and the 'original' farnesyl residue. These bisubstrate analogues differ only in the nature of the linker connecting the two parts of the molecule. We intended to explore whether it is possible to develop novel bisubstrate analogue inhibitors of farnesyltransferase by replacing the peptidic structures by non-peptidic CAAX

Key words: Antiproliferative agents; bisubstrate analogues; farnesyltransferase inhibitors; peptidomimetics; ras.
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0968-0896/99/$ - see front matter
PII: S0968-0896(99)00165-0

surrogates. From six CAAX and CAA-peptidomimetics previously described[12–16] we used the AAX- and AA-mimetic substructures, respectively, for our envisioned bisubstrate analogues. These were linked via β-alanine with homofarnesoic acid which functioned as the lipophilic part of our target structures. We chose homofarnesoic acid because in a series of farnesylpyrophosphate-based inhibitors of farnesyltransferase the homofarnesoyl derivative turned out to be a more active antagonist of FPP than the corresponding farnesoyl derivative.[17]

Chemistry

The amino acid 2,3-dimethylanilides **6** and **12** have been prepared from 2,3-dimethylaniline and the appropriate *N*-protected amino acid (**5** and **11**). Either acidic or hydrogenolytic removal of the *N*-protective group and subsequent acylation with *N*-Boc-β-alanine, activated as mixed anhydride, yielded compounds **8** and **14**, respectively. The *N*-Boc protective groups were removed with HCl in dioxane and the resulting hydrochlorides **9** and **15** were coupled with homofarnesoic acid using PyBOP methodology (Scheme 1).

Homofarnesoic acid was prepared as described[17] in two steps from farnesyl bromide. The nitrobenzamides **18**, **29** and nitrobenzenesulfonamides **19**, **30**, respectively, were prepared by reaction of the appropriate nitrobenzoic acid chloride and nitrobenzenesulfonyl chloride with 2,3-dimethylaniline **28** and methionine methyl ester hydrochloride **17**, respectively. Reduction of the nitro group using tin(II)chloride dihydrate in boiling ethyl acetate[18] yielded the corresponding aminobenzene derivatives **20**, **21**, **31** and **32**. Addition of *N*-Boc-β-alanine, acidic removal of the protective group and subsequent acylation with homofarnesoic acid as described above completed the synthesis (Scheme 2). In case of the methionine derivatives **26b** and **27b**, the final step of the synthesis was the alkaline hydrolysis of the corresponding methyl esters **26a** and **27a**. The aspartic acid derivative **39** was prepared by the addition of *N*-Z-aspartic acid-1-methyl ester to **32**. After hydrogenolytic removal of the *N*-protective group, acylation with homofarnesoic acid to **41a** was carried out as described above. The synthesis was completed by the saponification of the methyl ester yielding the target compound **41b** (Scheme 3).

Farnesyltransferase inhibition assay

The inhibitory activity of the potential synthetic inhibitors was determined using the fluorescence assay as described by Pompliano.[12] The assay employed yeast farnesyltransferase (FTase) fused to Glutathione *S*-transferase at the *N*-terminus of the β-subunit.[20] Farnesylpyrophosphate and the dansylated pentapeptide DsGlyCysValLeuSer 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.

Antiproliferation assay

The target compounds were tested against cell lines K-562 (chronic myeloid leukaemic cell line),[21] THP-1 (acute monocytic leukaemic cell line),[22] and HL-60 (acute myeloid leukaemic cell line)[23] for their antiproliferative effects. The cells were incubated with different concentrations of the test compounds and an automatic volumetric cell analysis was carried out during the logarithmic phase of cell proliferation.

Results and Discussion

The results of the farnesyltansferase inhibiton assay are displayed in Table 1; unfortunately, the N^{β}-homofarnesoyl-β-alanine amides were only weakly active. Most of the compounds inhibited the enzyme less than 50% at 100 μM, the highest concentration assayed. In case of the methionine derivatives the presence of the

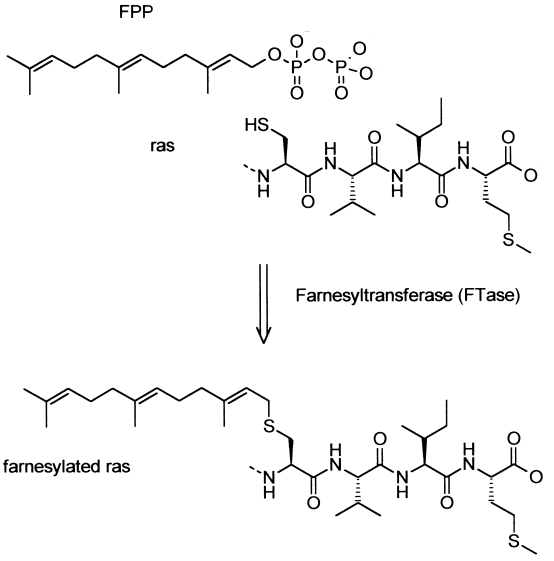


Figure 1. Farnesylation of ras proteins.

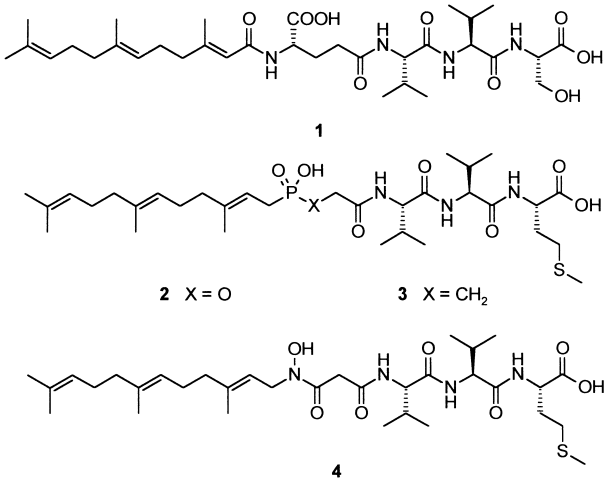
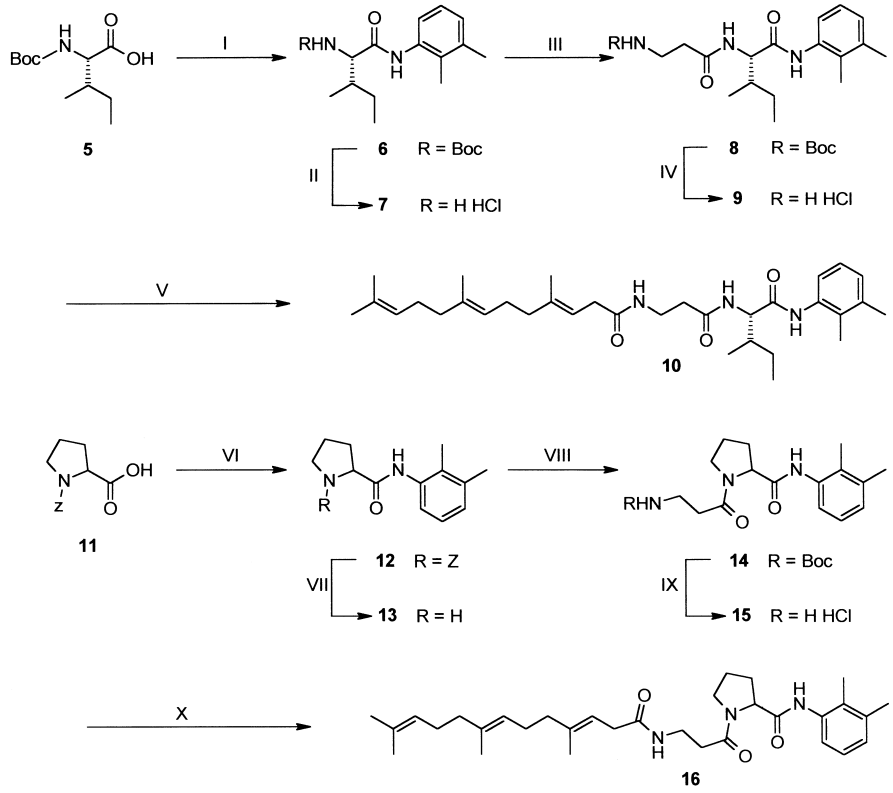


Chart 1. Literature known bisubstrate analogues.

carboxyl group either as its methyl ester as in **26a** and **27a** or as a sodium salt as in **26b** and **27b** made no significant difference in activity. Only the isoleucine derivative **10** displayed a somewhat higher activity. β-alanine was chosen as the linker connecting the lipohilic and the peptidomimetic substructures of our target molecules because the distance between its carbonyl carbon and its nitrogen is the same as the distance between the carbonyl carbon and the thiol sulfur in cysteine. However, the introduction of a methoxycarbonyl group into the linking structure by replacement of the β-alanine by aspartic acid-1-methyl ester (compound **41a**) resulted in a dramatic enhancement in the farnesyltransferase inhibitory activity. One could speculate that the side chain ester carbonyl group exerts a much stronger interaction with the crucial zinc atom in the farnesyltransferases catalytic center than the amide moiety of the other compounds. This interaction obviously adds a considerable amount of binding affinity which might be responsible for the improved inhibitory activity. Surprisingly, saponification of **41a** to the corresponding free acid **41b** led to a eightfold reduction of the inhibitory activity. The compounds were applied to an antiproliferation assay using three different tumor cell lines. Because compounds carrying free carboxyl functions usually show poor membrane penetration,

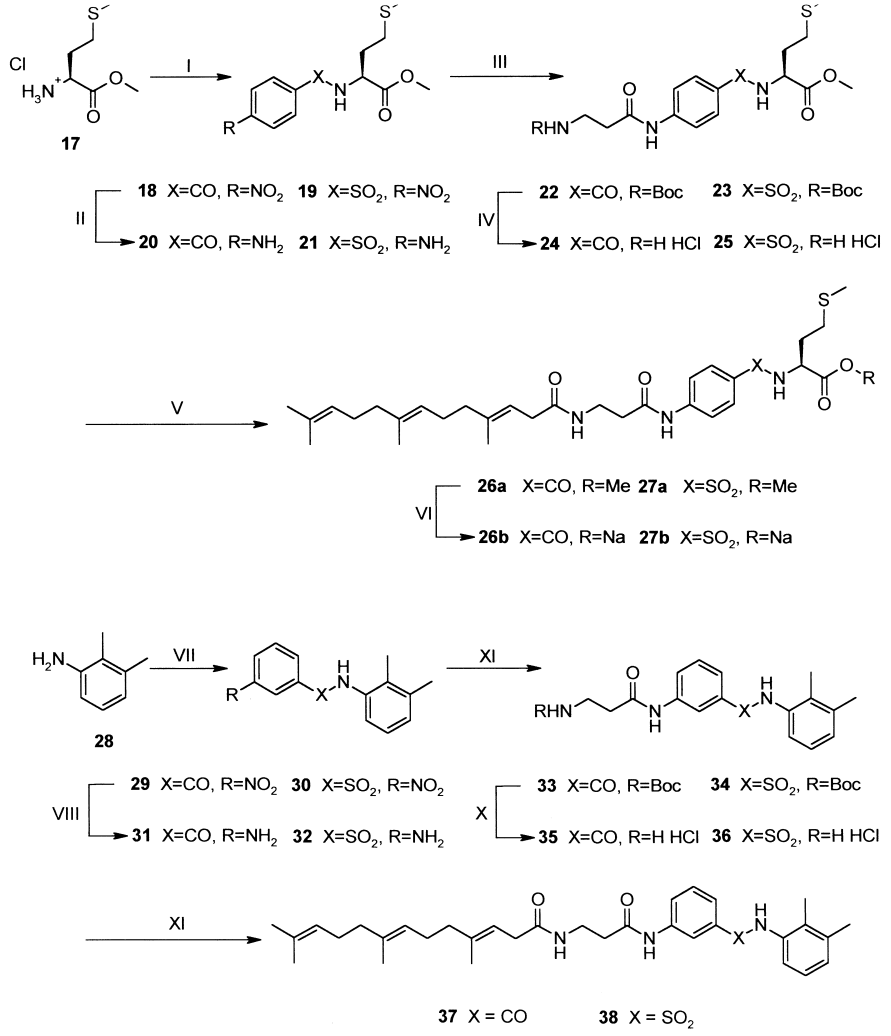


Scheme 1. I. (a) isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) 2,3-dimethylaniline, DMF, −15°C → rt, overnight; II. 4 N HCl/dioxane, rt, 2 h; III. (a) *N*-Boc-β-alanine, isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) add **7**, NMM, DMF to (a), −15°C → rt, overnight; IV. 4 N HCl/dioxane, rt, 2 h; V. homofarnesoic acid, PyBOP, DIPEA, DMF, rt, overnight; VI. (a) isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) 2,3-dimethylaniline, DMF, −15°C → rt, overnight; VII. Pd/C, H2, EtOH, rt, 2 h; VIII. (a) *N*-Boc-β-alanine, isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) add **13**, NMM, DMF to (a), −15°C → rt, overnight; IX. 4 N HCl/dioxane, rt, 2 h; X. homofarnesoic acid, PyBOP, DIPEA, DMF, rt, overnight.

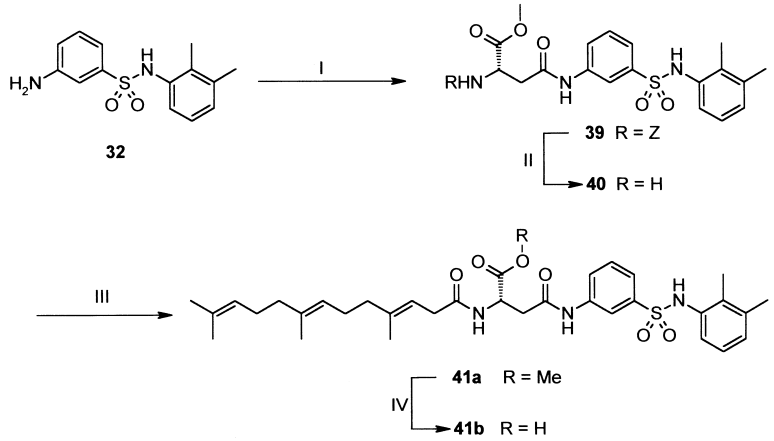
Table 1. Farnesyltransferase inhibitory and antiproliferative activity of homofarnesoyl substituted CAA(X) mimetics

Compound	FTase inhibition	Antiproliferative activity [IC_{50} (μM)]		
		HL-60	THP-1	K-562
10	77% at 100 μM	31.4	>93	55.2
16	0% at 100 μM	74.2	>96	>96
26a	35% at 100 μM	14.7	13.3	27.1
26b	0% at 100 μM	nd[a]	nd	nd
27a	21% at 100 μM	6.5	22.2	12.2
27b	5% at 100 μM	nd	nd	nd
37	16% at 100 μM	11.2	14.7	15.8
38	22% at 100 μM	5.3	8.5	6.9
41a	$IC_{50} = 860 \pm 52$ nM	60.7	>78	>78
41b	$IC_{50} = 6.9 \pm 0.9$ μM	nd	nd	nd

[a] nd: not determined.



Scheme 2. I. 4-Nitrobenzoic acid chloride or 4-nitrobenzenesulfonic acid chloride, NMM, CH_2Cl_2, 0°C → rt, overnight; II. $SnCl_2 \times H_2O$, EtOAc, reflux, 2 h; III. (a) *N*-Boc-β-alanine, isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) add **20** or **21**, DMF to (a), −15°C → rt, overnight; IV. 4 N HCl/dioxane, rt, 2 h; V. homofarnesoic acid, PyBOP, DIPEA, DMF, rt, overnight; VI. 1 N NaOH, THF/MeOH, rt; VII. 3-nitrobenzoic acid chloride or 3-nitrobenzenesulfonic acid chloride, NMM, CH_2Cl_2, 0°C → rt, overnight; VIII. $SnCl_2 \times 2H_2O$, EtOAc, reflux, 2 h; IX. (a) *N*-Boc-β-alanine, isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) add **31** or **32**, DMF to (a), −15°C → rt, overnight; X. 4 N HCl/dioxane, rt, 2 h; XI. homofarnesoic acid, PyBOP, DIPEA, DMF, rt, overnight.



Scheme 3. I. (a) *N*-Z-aspartic acid-1-methyl ester, isobutyl chloroformate, NMM, DMF, −15°C, 5 min; (b) add **32**, DMF to (a), −15°C → rt, overnight; II. H2, Pd/C, EtOAc, rt; III. homofarnesoic acid, PyBOP, DIPEA, DMF, rt, overnight; IV. (a) 1 N LiOH, DME, rt; (b) HCl.

compounds **26b**, **27b** and **41b** were excluded from this assay. The isoleucine derivative **10** and the proline derivative **16** showed no significant effect in the antiproliferation assay. In contrast, the benzoic acid derivatives **26a** and **37** and the benzenesulfonic acid derivatives **27a** and **38** showed a strong antiproliferative activity against all three cell lines. Highest activity was found with the sulfonamide **38**, which inhibited the proliferation of all cell lines in low micromolar doses. Replacement of the sulfonamide moiety of **38** by an amide as in **37** resulted in a decrease of activity with the sulfonamide **38** being approximately twice as active as the corresponding amide **37**. With the methionine derivatives **26a** and **27a**, the effect of the replacement of the amide by a sulfonamide is not that obvious. While the sulfonamide derivative **27a** is roughly twice as active against the HL-60 and the K-562 cells as the amide derivative **26a**, this ratio is reversed for the THP-1 cells. Interestingly, the presence of a carbonyl group in the linker substructure as in **41a** which resulted in a significant increase in the farnesyltransferase inhibition had a detrimental effect on the antiproliferative activity. Since compounds **26a**, **27a**, **37** and **38** display considerable antiproliferative activity but do not inhibit farnesyltransferase, and in contrast, **41a** is a good farnesyltransferase inhibitor but does not have an antiproliferative effect, it can be concluded that ras inhibition is most likely not responsible for the observed antiproliferative activity. The mechanism by which the observed activity is exerted, is open for speculation. It has been shown, that another farnesylated molecule, *S*-farnesylthioacetic acid, induces apoptosis in HL-60 cell. The authors suggest that this may be caused by an interference of *S*-farnesylthioacetic acid with a specific recognition of isoprenylated protein moieties.[24] There is accumulating evidence that isoprenoid moieties of certain proteins could play an important role in protein–protein interactions.[25–30] In fact, an isoprenoid acceptor structure has been identified in rho-GDI, the guanosine diphosphate dissociation inhibitor of rho proteins.[31] Inhibition of such interactions has been demonstrated with lipids[32] and prenylated molecules.[33,34] Because of their chemical structure, our molecules might well be able to interrupt isoprenoid-mediated protein—protein interactions.

In summary, with compounds **41a,b** we have obtained a new lead structure for bisubstrate analogue farnesyltransferase inhibitors. This compounds are different from known bisubstrate analogues in having the peptidic moiety replaced by a peptidomimetic structure. Our ongoing research is directed to further improvement of the inhibitory activity of this novel class of farnesyltransferase inhibitors. Special attention will be paid to the replacement of the homofarnesoyl residue by other non-prenylic lipophilic structures. In addition, the peptidomimetic moiety will be replaced by building blocks which structural relation to the farnesyltransferase substrate is even less pronounced. Very recently, we reported the first results of this strategy.[35,36]

Furthermore, a novel potent class of antiproliferative agents as exemplified by compound **38** was discovered. Investigations addressing structure–activity relationships and the mode of action of these agents are under way.

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 Tecnivent 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. Column chromatography was carried out using silica gel 60 (0.062—0.200 mm) from Merck.

General protocol for the preparation of nitrobenzamides 18, 29 and nitrobenzenesulfonamides 19 and 30. To a solution of 2,3-dimethylaniline or methionine methyl ester hydrochloride in a sufficient amount of dry CH_2Cl_2 and *N*-methylmorpholine (0.24 mL per mmol amine) 1 equiv of the appropriate nitrobenzoic acid or nitrobenzenesulfonamide, respectively 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, sat. $NaHCO_3$-solution and brine and dried with $MgSO_4$. The product obtained after the removal of the solvent was used without further purification or characterization.

General protocol for the reduction of nitrobenzene derivatives 18, 19, 29 and 30 to the corresponding amines 20, 21, 31 and 32. To a solution of the nitro derivative in EtOAc (approx 5 mL per mmol) $SnCl_2 \times H_2O$ (1.125 g per mmol nitro compound) was added. The soln. was refluxed for 2 h. The cooled solution was diluted with water and the pH was adjusted to 7–8 by addition of satd. $NaHCO_3$-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 products obtained after the removal of the solvent were used without further purification or characterization.

General protocol for the coupling of N-protected amino acids with various amines using mixed anhydride activation

Protocol 1. The N-protected amino 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 EtOAc (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.

General protocol for the *N*-Boc-deprotection

Protocol 2. 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 diethylether the volatiles were distilled in vacuo into a flask immersed in liquid N_2. The solid residue was used without further purification.

General protocol for the coupling of homofarnesoic acid with β-alanine amides using PyBOP methology

Protocol 3. One equivalent of homofarnesoic acid, the β-alanine amide and PyBOP (520 mg per mmol homofarnesoic acid), respectively, were dissolved in dry DMF in a flame dried flask. After addition of diisopropylethylamine (0.65 mL per mmol homofarnesoic 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 EtOAc (3×100 mL) and the combined organic extracts were washed successively with 2 N citric acid, satd $NaHCO_3$-solution and brine and dried with $MgSO_4$. The residue obtained after removal of the solvent was purified by flash chromatography.

***N*-*tert*-Butyloxy-*N*-(2,3-dimethylphenyl)isoleucine amide 6**. Compound **6** was prepared from Boc-Ile-OH (**5**) following protocol 1.

***N*-Benzyloxy-*N*-(2,3-dimethylphenyl)proline amide 12**. Compound **12** was prepared from Z-Pro-OH (**11**) following protocol 1.

***N*-(3-*tert*-Butyloxycarbonylaminopropionyl)-*N*-(2,3-dimethylphenyl)isoleucine amide 8**. Compound **6** (668 mg, 2 mmol) was deprotected following protocol 2 and coupled with *N*-Boc-β-alanine following protocol 1. Yield: 668 mg (82%); mp 204°C (EtOAc/MeOH). IR (KBr) ν 3330, 3295, 2965, 2935, 1690, 1645 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (t, $J=7$ Hz, 3H), 1.01 (d, $J=6$ Hz, 3H), 1.20 (m, 1H), 1.40 (s, 9H), 1.60 (m, 1H), 2.00 (m, 1H), 2.08 (s, 3H), 2.24 (s, 3H), 2.45 (m, 2H), 3.38 (m, 2H), 4.41 (m, 1H), 5.09 (s, 1H), 6.49 (m, 1H), 6.98 (m, 1H), 7.03 (m, 1H), 7.37 (m, 1H), 7.74 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 13.8, 15.7, 20.5, 25.1, 28.3, 36.3, 36.7, 58.5, 79.4, 122.1, 125.8, 127.6, 129.4, 134.8, 137.5, 156.0, 169.6, 171.9. MS *m*/*z* (%) 405 (1) [M+], 121 (100). Anal. calcd for $C_{22}H_{35}N_3O_4$ (405.54): C, 65.16; H, 8.70; found: C, 65.58; H, 9.08.

***N*-(3-*tert*-Butyloxycarbonylaminopropionyl)-*N*-(2,3-dimethylphenyl)proline amide 14**. Compound **12** (915 mg, 2.6 mmol) was dissolved in EtOH and stirred after addition of Pd/C (10%) under an atmosphere of H_2 for 2 h at room temperature. The solution was filtered through Celite and silica gel. The residue obtained after removal of the solvent was coupled with *N*-Boc-β-alanine following protocol 1. Compound **14** was purified by flash chromatography (EtOAc). Yield: 993 mg (98%); mp 65°C. IR (KBr) ν 3305, 2975, 1700, 1640 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.70 (m, 1H), 1.85 (m, 1H), 2.05 (m, 1H), 2.15 (s, 3H), 2.28 (s, 3H), 2.56 (t, $J=6$ Hz, 2H), 2.60–2.64 (m, 1H), 3.41–3.48 (m, 3H), 3.52 (m, 1H), 4.82 (d, $J=8$ Hz, 1H), 5.28 (s, 1H), 6.95–6.97 (m, 1H), 7.06–7.09 (m, 1H), 7.66–7.68 (m, 1H), 9.03 (s, 1H); ^{13}C NMR (CDCl3) δ 13.7, 20.6, 25.0, 26.8, 28.4, 34.8, 36.0, 47.5, 60.3, 79.3, 120.9, 125.7, 126.6, 127.5, 135.9, 137.1, 155.9, 168.9, 172.5. MS *m*/*z* (%) 389 (3) [M+], 142 (25), 70 (100). Anal. calcd for $C_{21}H_{31}N_3O_4$ (389.49): C, 64.76; H, 8.02; N, 10.79; found: C, 64.39; H, 8.09; N, 10.67.

***N*-[4-(3-*tert*-Butyloxycarbonylaminopropionyl)aminobenzoyl]methionine methyl ester 22**. Compound **20** (530 mg, 1.88 mmol) was coupled with *N*-Boc-β-alanine following protocol 1. Yield: 546 mg (64%); mp 148°C (EtOAc). IR (KBr) ν 3310, 2980, 1750, 1635, 1600 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.38 (s, 9H), 2.06 (m, 5H), 2.49–2.59 (m, 4H), 3.23 (m, 2H), 3.65 (s, 3H), 4.57 (m, 1H), 6.82 (s, 1H), 7.66–7.68 (m, 2H), 7.82–7.85 (m, 2H), 8.58 (d, $J=8$ Hz, 1H), 10.12 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.5, 28.2, 29.9, 30.1, 36.3, 36.8, 51.6, 51.8, 118.1, 127.9, 128.3, 142.0, 155.5, 166.1, 169.8, 172.4. MS *m*/*z* (%) 453 (2) [M+], 305, (62), 279 (64), 217 (100), 120 (82). Anal. calcd for $C_{21}H_{31}N_3O_6S$ (453.55): C, 55.61; H, 6.89; N, 9.26; found: C, 55.78; H, 6.80; N, 9.05.

***N*-[4-(3-*tert*-Butyloxycarbonylaminopropionyl)aminophenylsulfonyl]methionine methyl ester 23**. Compound **21** (795 mg, 2.5 mmol) was coupled with *N*-Boc-β-alanine following protocol 1. Yield: 860 mg (70%); mp 145°C (EtOAc/*n*-hexane). IR (KBr) ν 3360, 3270, 2980, 1745, 1680 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.86–1.95 (m, 1H), 2.00–2.05 (m, 1H), 2.06 (s, 3H), 2.56 (m, 2H), 2.65 (m, 2H), 3.51 (m, 2H), 3.55 (s, 3H), 4.07 (m, 1H), 5.19 (m, 1H), 5.52 (d, $J=8$ Hz, 1H), 7.70–7.72 (m, 2H), 7.76–7.78 (m, 2H) 8.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.3, 28.4, 29.6, 32.5, 36.3, 38.2, 52.7, 54.6, 80.1, 119.3, 128.4, 134.0, 142.3, 156.6, 170.1, 171.8. MS *m*/*z* (%) 313 (12), 164 (51), 162 (100). Anal. calcd for $C_{20}H_{31}N_3O_7S_2$ (489.60): C, 49.06; H, 6.38; N, 8.58; found: C, 48.70; H, 6.15; N, 8.42.

3-[(3-*tert*-Butyloxycarbonylaminopropionyl)amino]-*N*-(2,3-dimethylphenyl)-benzamide 33. Compound **31** (480 mg, 2 mmol) was coupled with *N*-Boc-β-alanine following protocol 1. Compound **33** was purified by flash chromatography EtOAc. Yield: 428 mg (52%); mp 159°C (toluene). IR (KBr) ν 3295, 1710, 1685, 1650, 1590, 1430, 1280, 1170 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.35 (s, 9H), 2.09 (s, 3H), 2.27 (s, 3H), 2.48 (m, 2H), 3.23 (m, 2H), 6.80 (s, 1H), 7.06–7.13 (m, 3H), 7.42 (m, 1H), 7.64 (m, 1H), 7.81 (m, 1H), 8.11 (m, 1H), 9.84 (s, 1H), 10.06 (s, 1H). MS *m*/*z* (%) 411 (6) [M+], 311 (91), 283 (65), 217 (65), 191 (100). Anal. calcd for $C_{23}H_{29}N_3O_4$

(411.50): C, 67.13; H, 7.10; N, 10.21; found: C, 67.20; H, 7.05; N, 10.15.

3-(*tert*-Butyloxycarbonylamino)-*N*-[3-(2,3-dimethylphenylaminosulfonyl)phenyl]propionamide 34. Compound **32** (610 mg, 2.2 mmol) was coupled with *N*-Boc-β-alanine following protocol 1. Compound **34** was purified by flash chromatography (EtOAc:*n*-hexane, 3:2). Yield: 340 mg (35%); mp 124°C. IR (KBr) ν 3330, 2975, 1685 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.37 (s, 9H), 1.96 (s, 3H), 2.17 (s, 3H), 2.49 (m, 2H), 3.21 (m, 2H), 5.47 (s, 1H), 6.70–6.79 (m, 2H), 6.91–7.01 (m, 2H), 7.21–7.29 (m, 1H), 7.41–7.46 (m, 1H), 7.76 (m, 1H), 8.01 (s, 1H), 9.49 (s, 1H), 10.16 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 15.6, 20.7, 28.8, 36.8, 37.2, 78.0, 117.3, 121.8, 123.1, 125.7, 126.3, 128.0. 130.0, 134.7, 138.1, 140.2, 141.7, 152.8, 156.0, 170.4. MS *m*/*z* (%) 447 (0.1) [M^+], 373 (33), 276 (66), 120 (100). Anal. calcd for $C_{22}H_{29}N_3O_5S$ (447.55): C, 59.04; H, 6.53; N, 9.39; found: C, 59.07; H, 6.10; N, 9.72.

2-(2*S*)-(Benzyloxycarbonylamino)-3-[3-(2,3-dimethylphenylaminosulfonyl)phenylcarbamoyl]propionic acid methyl ester 39. Compound **32** (552 mg, 2 mmol) was coupled with *N*-benzyloxycarbonyl-aspartic acid-1-methyl ester (562 mg, 2 mmol) following protocol 1. Compound **39** was purified by flash chromatography (EtOAc:*n*-hexane, 3:2). Yield: 605 mg (56%); mp 59°C. ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.18 (s, 3H), 2.97 (m, 1H), 3.14 (m, 1H), 3.74 (s, 3H), 4.66 (m, 1H), 5.08 (s, 2H), 6.10 (d, $J=8$ Hz, 1H), 6.78 (s, 1H), 6.92 (m, 2H), 6.98 (m, 1H), 7.29 (m, 6H), 7.86 (s, 2H), 8.26 (s, 1H). ESI–MS *m*/*z* 540 $[M+H]^+$, 562 $[M+Na]^+$. ESI–HRMS calcd for $C_{27}H_{30}N_3O_7S$ $[M+H]^+$: 540.180448; found: 540.181993; calcd for $C_{27}H_{29}NaN_3O_7S$ $[M+Na]^+$: 562.162392; found: 562.162391.

(*E*,*E*)-*N*-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)-aminopropionyl]-*N*-(2,3-dimethylphenyl)isoleucine amide 10. Compound **8** (465 mg, 1.2 mmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Yield: 320 mg (50%); mp 148°C (EtOAc:MeOH:*n*-pentane). IR (KBr) ν 3275, 3065, 2965, 1655, 1640 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.85 (t, $J=7$ Hz, 3H), 0.92 (d, $J=7$ Hz, 3H), 1.18 (m, 2H), 1.54 (s, 8H), 1.62 (s, 3H), 1.65 (s, 1H), 1.80–1.90 (m, 2H), 1.91–2.04 (m, 7H), 2.04 (s, 3H), 2.23 (s, 3H), 2.32–2.37 (m, 2H), 2.77 (d, $J=7$ Hz, 2H), 3.22–3.38 (m, 2H), 4.36 (m, 1H), 5.07 (m, 2H), 5.23 (t, $J=7$ Hz, 1H), 6.99–7.08 (m, 3H), 7.65 (m, 1H), 7.99 (d, $J=8$ Hz, 1H), 9.40 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 10.9, 13.8, 15.4, 15.6, 16.0, 17.4, 19.9, 24.4, 25.9, 26.0, 26.1, 35.1, 35.4, 36.4, 37.9, 38.9, 57.3, 117.9, 123.5, 123.7, 123.8, 124.0, 124.9, 126.8, 131.1, 135.8, 136.7, 170.1, 170.5. MS *m*/*z* (%) 537 (10) [M^+], 304 (14), 121 (100). HRMS calcd for $C_{33}H_{51}N_3O_3$ (537.79): 537.3930; found: 537.3927.

(*E*,*E*)-*N*-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)-aminopropionyl]-*N*-(2,3-dimethylphenyl)proline amide 16. Compound **14** (466 mg, 1.2 mmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Compound **16** was purified by flash chromatography (acetone:*n*-hexane, 2:1). Yield: 135 mg (22%); mp 54°C. IR (KBr) ν 3300, 2970, 1655, 1535 cm–1. ^1H NMR ($CDCl_3$) δ 1.59 (s, 8H), 1.67 (s, 3H), 1.75 (s, 1H), 1.81–191 (m, 1H), 1.95–2.11 (m, 10H), 2.15 (s, 3H), 2.28 (s, 3H), 2.52–2.62 (m, 2H), 2.92 (d, $J=7$ Hz, 2H), 3.52 (m, 1H), 3.54–3.62 (m, 3H), 4.77 (d, $J=7$ Hz, 1H), 5.08 (m, 2H), 5.25 (m, 1H), 6.49 (m, 1H), 6.95–6.97 (m, 1H), 7.07 (m, 1H), 7.60–7.62 (m, 1H), 8.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.6, 16.0, 16.2, 17.7, 20.6, 23.4, 25.0, 25.6, 26.7, 26.9, 34.3, 35.0, 35.9, 39.6, 39.7, 47.6, 60.4, 116.3, 116.9, 121.0, 123.1, 124.2, 125.7, 126.8, 128.1, 131.3, 135.8, 137.1, 141.0, 169.0, 171.6, 172.4. MS *m*/*z* (%) 521 (13) [M^+], 452 (119, 219 (21), 70 (100). HRMS calcd for $C_{32}H_{47}N_3O_3$ (521.74): 521.3617; found: 521.3598.

(*E*,*E*)-*N*-{4-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)aminopropionyl]-aminobenzoyl}methionine methyl ester 26a. Compound **22** (545 mg, 1.2 mmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Compound **26** was purified by flash chromatography (EtOAc). Yield: 50 mg (7%); mp 110°C. IR (KBr) ν 3260, 3080, 2920, 1750, 1635 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.52 (s, 8H), 1.60 (s, 3H), 1.89–2.10 (m, 13H), 2.19 (m, 1H), 2.50–2.58 (m, 4H), 2.90 (d, $J=7$ Hz, 2H), 3.50 (m, 2H), 3.71 (s, 3H), 4.84 (m, 1H), 5.00 (t, $J=7$ Hz, 2H), 5.20 (t, $J=7$ Hz, 1H), 6.51 (t, $J=5$ Hz, 1H), 7.01 (d, $J=8$ Hz, 1H), 7.59–7.62 (m, 2H), 7.68–7.71 (m, 2H), 9.18 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.5, 16.0, 16.2, 17.6, 25.6, 26.3, 26.7, 30.1, 31.4, 35.5, 35.8, 37.0, 39.5, 39.6, 52.1, 52.6, 115.9, 119.1, 123.6, 124.2, 128.1, 128.7, 131.3, 135.5, 141.7, 141.8, 166.6, 170.1, 172.5, 172.6. MS *m*/*z* (%) 585 (37) [M^+], 286 (33), 217 (31), 191 (33), 120 (100). HRMS calcd for $C_{32}H_{47}N_3O_5S$ (585.80): 585.3236; found: 585.3245.

(*E*,*E*)-*N*-{4-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)aminopropionyl]-aminobenzoyl}methionine, sodium salt 26b. Compound **26** (130 mg, 0.22 mmol) was dissolved in a 1:1 mixture of THF and MeOH (15 mL) and stirred after additon of 1 N NaOH (0.24 mL, 0.24 mmol) at room temperature until TLC analysis indicated completion of the reaction. Then the solvents were removed in vacuo. Yield: 128 mg (98%); mp 160°C. IR (KBr) ν 3300, 2920, 1605 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.56 (s, 8H), 1.62 (s, 3H), 1.90–1.96 (m, 4H), 2.01 (m, 9H), 2.50 (m, 4H), 2.80 (md, $J=7$ Hz, 2H), 3.30 (m, 2H), 4.00 (m, 1H), 5.06 (m, 2H), 5.24 (m, 1H), 7.68–7.74 (m, 4H), 10.32 (s, 1H). ESI-MS *m*/*z* (%) 572 $[M+2H-Na]^+$. ESI-HRMS calcd for $C_{31}H_{46}N_3O_5S$: 572.315819; found: 572.313579.

(*E*,*E*)-*N*-{4-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)aminopropionyl]-aminophenylsulfonyl}methionine methyl ester 27a. Compound **23** (400 mg, 0.8 mmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Compound **27** was purified by flash chromatography (EtOAc). Yield: 165 mg (33%); mp 90°C. IR (KBr) ν 3265, 2920, 1735, 1680, 1645 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.53 (s, 8H), 1.61 (s, 3H), 1.75 (s, 1H), 1.80–2.04 (m, 13H), 2.49 (m, 2H), 2.61 (t, $J=6$ Hz, 3H), 2.91 (d, $J=7$ Hz, 2H), 3.52 (m, 2H), 4.00 (m, 1H), 5.01 (t, $J=7$ Hz, 2H), 5.21 (t, $J=7$

Hz, 1H), 5.47 (d, $J=9$ Hz, 1H), 6.44 (t, $J=6$ Hz, 1H), 7.69 (m, 4H), 9.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.3, 16.0, 16.2, 17.7, 25.7, 26.3, 26.7, 29.6, 32.5, 35.6, 35.8, 37.5, 39.5, 39.7, 52.7, 54.6, 115.8, 119.3, 123.5, 124.2, 128.4, 131.4, 134.0, 135.7, 142.2, 142.6, 170.1, 171.9, 172.8. MS *m/z* (%) 621 (28) [M^+], 552 (23), 260 (50), 162 (100). Anal. calcd for $C_{31}H_{47}N_3O_6S_2$ (621.85): C, 59.88; H, 7.62; N, 6.76 ; found: C, 60.04; H, 7.61; N, 7.16.

(*E*,*E*)-*N*-{4-[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl) aminopropionyl] - aminophenylsulfonyl} methionine, sodium salt 27b. Compound **27** (120 mg, 0.19 mmol) was dissolved in a 1:1 mixture of THF and MeOH (15 mL) and stirred after additon of 1 N NaOH (0.2 mL, 0.2 mmol) at room termperature until TLC analysis indicated completion of the reaction. Then, the solvents were removed in vacuo. Yield: 118 mg (99%); mp 248°C. IR (KBr) ν 3310, 2920, 1660 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.55 (s, 8H), 1.63 (s, 3H), 1.71 (m, 1H), 1.87–2.08 (m, 13H), 2.30 (m, 2H), 2.39–2.50 (m, 2H), 2.79 (d, $J=7$ Hz, 2H), 3.18 (m, 1H), 3.29 (m, 2H), 5.10 (m, 2H), 5.27 (m, 1H), 7.41 (s, 4H). ESI-MS *m/z* (%) 652 (100) [$M-H+2Na]^+$, 674 (27) [$M-2H+3Na]^+$. ESI-HRMS calcd for $C_{30}H_{45}NaN_3O_6S_2$ [$M+Na]^+$: 630.264750; found: 630.264572; calcd for $C_{30}H_{44}Na_2N_3O_6S_2$ [$M-H+2Na]^+$: 652.246695; found: 652.247846.

3-{[3-(4,8,12-Trimethyl-1-oxo-3,7,11-tridecatrienyl)aminopropionyl]amino} - *N*-(2,3-dimethylphenyl)benzamide 37. Compound **33** (575 mg, 1.4 mmmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Compound **37** was purified by flash chromatography (EtOAc:*n*-hexane, 3:2). Yield: 68 mg (11%); mp 88°C. IR (KBr) ν 3390, 3300, 2920, 1655 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 8H), 1.61 (s, 1H), 1.66 (s, 3H), 1.94–1.97 (m, 2H), 2.02–2.09 (m, 6H), 2.20 (s, 3H), 2.30 (s, 3H), 2.57 (t, $J=6$ Hz, 2H), 2.95 (d, $J=7$ Hz, 2H), 3.55 (m, 2H), 5.07 (t, $J=7$ Hz, 2H), 5.27 (t, $J=7$ Hz, 1H), 6.34 (t, $J=6$ Hz, 1H), 7.06 (m, 1H), 7.12 (m, 1H), 7.40 (m, 1H), 7.51 (m, 1H), 7.59 (m, 1H), 7.76 (s, 1H), 7.81 (m, 1H), 8.10 (s, 1H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 16.0, 16.3, 17.7, 20.5, 25.7, 26.4, 26.8, 35.6, 35.9, 37.2, 39.6, 39.7, 116.1, 118.4, 122.5, 122.6, 123.0, 123.7, 124.3, 126.0, 127.9, 129.5, 130.1, 131.4, 135.3, 135.6, 137.7, 138.9, 141.7, 165.7, 170.2, 172.3. MS *m/z* (%) 544 (39) [M^+], 543 (100), 120 (63). HRMS calcd for $C_{34}H_{45}N_3O_3$ (543.75): 543.346093; found: 543.349899.

***N*-{2-[3-(2,3-Dimethylphenylaminosulfonyl)phenylaminocarbonyl]ethyl} - 4,8,12 - trimethyl - 3,7,11 - tridecatrienoic amide 38**. Compound **34** (340 mg, 0.76 mmol) was deprotected following protocol 2 and coupled with homofarnesoic acid following protocol 3. Compound **38** was purified by flash chromatography (EtOAc:*n*-hexane, 3:2). Yield: 105 mg (24%); mp 105°C. IR (KBr) ν 3350, 2925, 1690, 1640, 1600 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 8H), 1.59 (s, 3H), 1.68 (s, 1H), 1.84–2.05 (m, 8H), 1.97 (s, 3H), 2.13 (s, 3H), 2.59 (t, $J=6$ Hz, 2H), 2.88 (d, $J=7$ Hz, 2H), 3.32 (m, 2H), 4.97–5.05 (m, 2H), 5.16 (m, 1H), 6.49 (t, $J=6$ Hz, 1H), 6.88–6.94 (m, 4H), 7.22–7.27 (m, 2H), 7.81 (m, 1H), 8.05 (s, 1H), 9.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 16.2, 17.7, 20.6, 25.7, 26.4, 26.7, 35.6, 35.8, 37.2, 39.6, 39.7, 115.9, 117.8, 120.6, 123.5, 123.6, 123.8, 124.2, 15.8, 128.5, 129.4, 131.4, 132.3, 133.9, 135.6, 138.0, 139.1, 140.3, 141.9, 170.2, 172.7. MS *m/z* (%) 579 (16) [M^+], 330 (14), 119 (100). Anal. calcd for $C_{33}H_{45}N_3O_4S$ (579.80): C, 68.36; H, 7.82; N, 7.25; found: C, 68.50; H, 8.04; N, 7.95. HRMS calcd for $C_{33}H_{45}N_3O_4S$: 579.313079; found: 579.310120.

3-[3-(2,3-Dimethylphenylaminosulfonyl)phenylcarbamoyl]-2-(2S)-(4,8,12-trimethyl-3,7,11-tridecatrienoylamino)propionic acid methyl ester 41a. Compound **39** (485 mg, 0.9 mmol) was dissolved in EtOAc (20 mL) and stirred after addition of Pd/C (10%) under an atmosphere of H_2 for 2 h at room temperature. The solution was filtered through Celite and silica gel. The residue obtained after removal of the solvent was coupled with homofarnesoic acid (223 mg, 0.89 mmol) following protocol 3. Compound **41a** was purified by flash-chomatography (EtOAc:hexane, 3:2). Yield: 128 mg (22%); mp 41°C. IR (KBr) ν 2925, 1750, 1720, 1545 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.57 (m, 8H), 1.66 (m, 5H), 1.92–2.03 (m, 9H), 2.04 (s, 3H), 2.20 (s, 3H), 2.98 (m, 2H), 3.04–3.15 (m, 2H), 3.76 (s, 3H), 4.79 (m, 1H), 5.08 (m, 2H), 5.30 (m, 1H), 6.89–7.03 (m, 4H), 7.10, (m, 1H) 7.30 (m, 2H), 7.93 (m, 1H), 8.91 (m, 1H). ESI-MS *m/z* 638 [$M+H]^+$, 660 [$M+Na]^+$. ESI-HRMS calcd for $C_{35}H_{48}N_3O_6S$ [$M+H]^+$: 638.326384; found: 638.333211; calcd for $C_{35}H_{47}NaN_3O_6S$ [$M+Na]^+$: 660.308328; found: 660.311051.

3-[3-(2,3-Dimethylphenylaminosulfonyl)phenylcarbamoyl]-2-(2S)-(4,8,12-trimethyl-3,7,11-tridecatrienoylamino)propionic acid 41b. To a solution of **41** (120 mg, 0.19 mmol) in DME (15 mL) 1 N LiOH-solution (15 mL) was added and the mixture was stirred at room temperature until the reaction was complete (TLC). Then, the volatiles were removed in vacuo and the residue was dissolved in water (20 mL). This solution was extracted with EtOAc. The organic phase was discarded. The aqueous solution was adjusted to pH 1 with concd HCl and extracted three times with EtOAc. The combined organic extracts were dried with $MgSO_4$ and evaporated to dryness. Yield: 52 mg (47%); mp 64°C. IR (KBr) ν 2925, 1720, 1545 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.54 (m, 8H), 1.62 (m, 5H), 1.90–2.05 (m, 9H), 1.95 (s, 3H), 2.15 (s, 3H), 2.86 (m, 2H), 4.58 (m, 1H), 5.05 (m, 2H), 5.23 (m, 1H), 6.70 (m, 1H), 6.92 (m, 1H), 6.92 (m, 1H), 6.98 (m, 1H), 7.27 (m, 1H), 7.43 (m, 1H), 7.75 (m, 1H), 8.03 (m, 1H), 9.47 (s, 1H). ESI–MS *m/z* 624 [$M+H]^+$, 646 [$M+Na]^+$. ESI-HRMS calcd for $C_{34}H_{46}N_3O_6S$ [$M+H]^+$: 624.310734; found: 624.302916; calcd for $C_{34}H_{45}NaN_3O_6S$ [$M+Na]^+$: 646.292678; found: 646.292515.

Farnesyltransferase assay. The assay was carried out as described.[19] The assay mixture (100 μL volume) contained 50 mM Tris/HCl pH 7.4, 5 mM $MgCl_2$, 10 μM, ZnCl2, 5 mM DTT, 7 μM Ds-GCVLS, 20 μM FPP and 5 nmol GST-FTase[20] 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 460 nm (excitation: 320 nm). The slight deviation from the emission maximum (505 nm) was chosen for technical reasons. Fluorescence emission was recorded with BMG Polarstar microplate reader. IC_{50}s were calculated from initial velocity of three independent measurements of each inhibitor concentration and expressed as mean ± SD.

Cells and culture conditions. Established suspended human leukaemic cell lines K-562 cells (chronic myeloid leukaemic cell line), HL-60 cells (acute myeloid leukaemic cell line) and THP-1 cells (acute monocytic leukaemic cell line) were cultured in RPMI 1640 medium (Gibco, cat.-no. 15140-114), supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FBS.

Test conditions. For each experiment, approximately 10,000 cells were seeded with 0.1 mL culture medium, containing sodium bicarbonate, but without HEPES, into 96-well microplates (NUNC). The plates were previously prepared with dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 of test substances in 0.1 mL medium. The cells were incubated for 72 h at 37°C in a humidified atmosphere and 5% CO_2.

Methods of evaluation. The 0.2 mL content of each well in the microplate was diluted 1/50 with CASYTON. Suspension cultures of K-562, HL-60, and THP-1 cells in microplates were analysed by an electronic cell analyser system CASY1. Every count/mL was automatically calculated from the arithmetic mean of three successive counts of 0.4 mL each. The IC_{50} value was defined as being where the concentration–response curve intersected the 50% line, determined by means of the cell counts/mL, compared to control. The essential parameters for the estimation of growth inhibition and for changes in diameter distribution curves are expressed as diagrams.

Acknowledgements

The pGEX-DPR1 and pBC-RAM2 plasmides were kindly provided by Professor F. Tamanoi (UCLA). M. Schlitzer wishes to thank Professor W. Hanefeld for generous support. I. Sattler wishes to thank Professor Dr. S. Grabley and PD Dr. R. Thiericke for generous support and S. Egner for technical assistance.

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