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Non-peptidic, Non-prenylic Bisubstrate Farnesyltransferase Inhibitors, 4. Effect on Farnesyltransferase Inhibitory Activity of Conformational Restrictions in the Central Group

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Abstract

We have recently described non-peptidic, non-prenylic bisubstrate analogues as novel farnesyltransferase inhibitors comprising three modules–a farnesylmimetic, a linker and an AAX-peptidomimetic substructure. In this study we replaced the originally used β-alanyl linker by several aliphatic and cyclic amino acids to investigate the effects on inhibitory potential of the stereochemistry of this central group. Whereas replacement of β-alanine by glycine did not affect inhibitory activity, all other modifications resulted in reduced activity. This result, which will be helpful for further development, shows that the bioactive conformation is none of those fixed by the rigid linkers.

Cancer is caused by stepwise accumulation of mutations that affect growth control, differentiation and cell survival (McCormick 1999). Ras proteins play a central role in the signal transduction cascades controlling these processes (Macara et al 1996; Gomez et al 1998). Mutated forms of Ras, which are constitutively active, are found in 30% (approx.) of all cancers in man. Several post-transformational modifications occur before Ras acquires its full biological activity. The crucial step is transfer of a farnesyl residue from farnesylpyrophosphate to the thiol of a cysteine side-chain of the C-terminal CAAX-tetrapeptide sequence (C: cysteine, A: aliphatic amino acid, X: serine or methionine) catalysed by the enzyme farnesyltransferase (Zhang & Casey 1996). Therefore, inhibition of farnesyltransferase has received considerable interest in recent years as a strategy for the development of novel potential cancer therapeutics (Leonard 1997; Quian et al 1997; Sebti & Hamilton 1998). However, there is accumulating evidence that Ras might not be the only substrate of farnesyltransferase involved in oncogenesis (Cox & Der 1997; Du et al 1999).

Irrespective of the unresolved issue of the mechanism by which farnesyltransferase inhibitors exert their antiproliferative effects, the efficacy of these compounds and their low toxicity have been demonstrated and they are, therefore, regarded as a major emerging strategy in cancer therapy.

We have previously described novel types of bisubstrate analogue farnesyltransferase inhibitor (**1**; Figure 1) (Schlitzer & Sattler 1999a). These inhibitors are different from the few known bisubstrate analogues in having no peptidic or prenylic substructures. They are composed of three modular building blocks–a AAX-peptidomimetic, a linker or central group and a farnesylmimetic (Figure 1). We have shown that replacement of the amide group which connects the farnesylmimetic to the linker by an ethylene bridge considerably reduces the inhibitory activity of farnesyltransferase (Schlitzer & Sattler unpublished results).

We suggested that the carbonyl oxygen interacts with the active site of the farnesyltransferase and that this is important for the binding affinity. Because the position of this carbonyl group relative to the peptidomimetic substructure is governed by the length and conformational flexibility of the linking substructure, we have prepared derivatives of **1** in and that this β-alanyl linker is replaced by other building blocks. It was hoped the use of rigid cyclic structures would provide information about the bioactive conformation of **1** (N-{2-[3-(2,3-dimethyl}phenylaminosulphonyl)phenylaminocarbonyl]ethyl}hexadecanoic acid amide).

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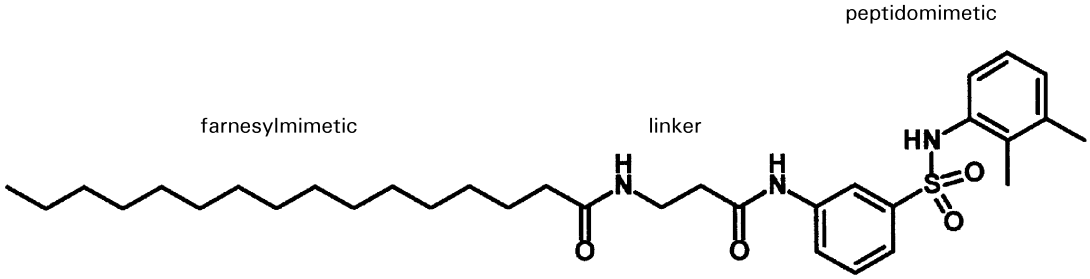

Figure 1. The modular composition of bisubstrate analogue farnesyltransferase inhibitor **1**.

Material and Methods

Chemistry

^{1}H and ^{13}C NMR spectra were recorded on Jeol JMN-GX-400 and JMN-LA-500 spectrometers. Mass spectra were acquired with a Vacuum Generators VG 7070H spectrometer and Vector 1 data acquisition system from Tecnivent, or with an AutoSpec mass spectrometer from Micromass. IR spectra were recorded on a Nicolet 510P FTIR spectrometer. Microanalysis was performed with a CH analyser according to Dr Salzer from Labormatic, and with a Hewlett–Packard type 185 CHN analyser. Column chromatography was performed on silica gel 60 (0.062–0.200 mm) from Merck. Compound **2** Schlitzer & Sattler 1999; was prepared as described elsewhere (3-amino-N-(2,3-dimethylphenylbenzene sulphonanide) Schlitzer et al 1999).

General procedure for acylation of amines by mixed anhydride activation (Procedure 1)

The appropriate carboxylic acid was dissolved in dry dimethylformamide (DMF) in a flame-dried flask under an atmosphere of argon. After addition of *N*-methylmorpholine (0.25 mL (mmol acid)$^{-1}$) the solution was cooled to -15°C and isobutyl chloroformate (0.13 mL (mmol acid)$^{-1}$) was added. A solution of the amine component (1 equiv.) in dry DMF was added after 5 min. When a hydrochloride was used as the amine component, additional *N*-methylmorpholine (0.25 mL mmol^{-1}) was added. The mixture was left to warm to room temperature overnight and then poured into brine (400–800 mL). If a solid precipitate formed, this was collected by vacuum filtration 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 0.67 M citric acid, saturated $NaHCO_3$ solution and brine and dried with $MgSO_4$. The residue obtained after removal of the solvent was purified by flash chromatography.

General procedure for N-boc-deprotection (Procedure 2)

N-Boc derivatives and HCl gas 4M HCl were dissolved in dioxane (10 mL mmol^{-1}) and the solution was stirred for 2 h at room temperature. After addition of diethyl ether the volatile compounds were distilled in-vacuo into a flask immersed in liquid N_2. The solid residue was used without further purification.

General procedure for the preparation of the N-acyl amino acids (Procedure 3)

The amino acid and K_2CO_3 (430 mg (mmol amino acid)$^{-1}$) were dissolved in water (40 mL) and cooled to 0°C. A solution of the appropriate acyl chloride (1 equiv.) in acetone (40 mL) was added dropwise. Stirring was continued for 1 h at 0°C and 1 h at room temperature. Most of the acetone was then removed by rotary evaporation and the resulting aqueous solution was acidified with conc. hydrochloric acid to pH 1. The precipitate was collected, washed with water and dried. The products were used without further purification.

*2-(tert-Butyloxycarbonylamino)-N-[3-(2,3-dimethylphenylaminosulphonyl)phenyl]acetic acid amide (**11**).* Compound **2** (1.66 g, 6.0 mmol) was acylated with N-boc-glycine (1.05 g, 6.0 mmol) according to procedure 1. Yield 1.67 g (65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (9H, m), 1.97 (3H, s), 2.17 (3H, s), 3.94 (2H, m), 6.85–7.10 (5H, m), 7.29 (2H, m), 8.05 (1H, m), 8.80 (1H, m); ESIMS: $m/z = 434$ $[M + H]^+$; HR-ESIMS: exact mass calculated for $C_{21}H_{27}N_3O_5S$ $[M + H]^+$ 434.1749; found 434.1706.

*N-{[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]methyl}heptadecanoic acid amide (**3**).* Compound **11** (866 mg, 2 mmol) was deprotected according to procedure 2 and acylated with heptadecanoic acid (541 mg, 2 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (3 : 2) as eluent yielded 1060 mg

(91%), mp 64°C. IR: $\nu = 2920$, 2850, 1715, 1645 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.84 (3H, m), 1.22 (26H, m), 1.48 (2H, m), 1.95 (3H, s), 2.15 (5H, m), 3.84 (2H, d, J = 6 Hz), 6.70 (1H, m), 6.92 (1H, m), 6.98 (1H, m), 7.28 (1H, m), 7.45 (1H, m), 7.77 (1H, m), 7.99 (1H, m), 8.02 (1H, m), 9.47 (1H, s), 10.15 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ 14.1, 14.3, 20.3, 22.3, 24.8, 25.4, 28.8, 28.9, 28.95, 29.0, 29.1, 29.2, 29.3, 31.5, 34.0, 35.5, 43.3, 117.4, 121.5, 125.0, 125.6, 132.6, 134.0, 134.8, 137.7, 139.5, 168.6, 172.0; EIMS: m/z = 585 $[M]^+$; HR-EIMS: exact mass calculated for $C_{33}H_{51}N_3O_4S$ $[M]^+$ 585.3600; found 585.3601.

*4-(tert-Butyloxycarbonylamino)-N-[3-(2,3-dimethylphenylaminosulphonyl)phenyl]butyric acid amide (**12**).* Compound **2** (690 mg, 2.5 mmol) was acylated with N-boc-4-aminobutyric acid according to procedure 1. Yield 875 mg (76%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, m), 1.83 (2H, m), 1.98 (3H, s), 2.19 (3H, s), 2.38 (2H, m), 3.19 (2H, m), 6.78 (1H, m), 6.96 (1H, m), 7.03 (1H, m), 7.31 (1H, m), 7.52 (1H, m), 7.81 (1H, m), 8.10 (1H, m); EIMS: m/z = 461 $[M]^+$; HR-EIMS: exact mass calculated for $C_{23}H_{31}N_3O_5S$ $[M]^+$ 461.1984; found 461.1980.

*N-{3-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]propyl}pentadecanoic acid amide (**4**).* Compound **12** (507 mg, 1.1 mmol) was deprotected according to procedure 2 and acylated with pentadecanoic acid (266 mg, 1.1 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (3 : 2) as eluent yielded 200 mg (31%), mp 45°C. IR: $\nu = 2925$, 2855, 1645, 1545 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.83 (3H, m), 1.22 (22H, m), 1.46 (2H, m), 1.68 (2H, m), 1.95 (3H, s), 2.02 (2H, m), 2.15 (3H, s), 2.28 (2H, m), 3.05 (2H, m), 6.71 (1H, m), 6.92 (1H, m), 6.97 (1H, m), 7.26 (1H, m), 7.41 (1H, m), 7.64 (1H, m), 7.77 (1H, m), 8.00 (1H, m), 9.42 (1H, s), 10.06 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ 13.6, 13.8, 19.8, 21.8, 24.9, 25.0, 28.3, 28.4, 28.5, 28.6, 28.7, 31.0, 33.7, 35.3, 37.8, 116.8, 120.7, 122.3, 124.4, 125.1, 127.7, 129.1, 133.4, 134.5, 137.2, 139.5, 141.1, 171.1, 171.9; EIMS: m/z = 585 $[M]^+$; HR-EIMS: exact mass calculated for $C_{33}H_{51}N_3O_4S$ $[M]^+$ 585.3600; found 585.3609; elemental analysis calculated for $C_{33}H_{51}N_3O_4S$ (585) C, 67.66; H, 8.77; N, 7.17; found: C, 67.76; H, 8.90; N, 7.29.

*2-(N-tert-Butyloxycarbonyl-N-methyl-amino)-N-[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-acetic acid amide (**13**).* Compound **2** (414 mg 1.5 mmol) was acylated with N-boc-*N*-methylglycine (284 mg, 1.5 mmol) according to procedure 1. Yield 520 mg (78%). ^1H NMR (500 MHz, $CDCl_3$): δ 1.38 (9H, m), 1.94 (3H, s), 2.19 (3H, s), 2.98 (3H, s), 3.91 (2H, s), 6.82–6.95 (5H, m), 7.86 (1H, s).

*N-{[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]methyl}-N-methylheptadecanoic acid amide (**5**).* Compound **13** (520 mg, 1.17 mmol) was deprotected according to procedure 2 and acylated with heptadecanoic acid (316 mg, 1.17 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (3:2) then ethyl acetate as eluents yielded 320 mg (46%), mp 58°C. IR: $\nu = 2925$, 2855, 1690, 1630 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.80 (3H, m), 1.20 (26H, m), 1.55 (2H, m), 2.01 (3H, s), 2.16 (3H, s), 2.35 (2H, m), 3.15 (3H, s), 4.10 (2H, s), 6.64 (1H, m), 6.92 (1H, m), 6.80 (2H, m), 6.92 (1H, m), 7.15 (2H, m), 7.68 (1H, m), 7.76 (1H, m), 9.08 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ 14.1, 20.6, 22.7, 24.8, 29.3, 29.4, 29.5, 29.6, 29.65, 29.7, 31.9, 33.3, 54.0, 118.0, 122.9, 123.5, 124.1, 125.8, 128.5, 129.3, 132.8, 134.0, 138.0, 138.5, 140.4, 168.0, 175.5; EIMS: m/z = 599 $[M]^+$; elemental analysis calculated for $C_{34}H_{53}N_3O_4S$ (599.88) C, 68.08; H, 8.91; N, 7.00; found: C, 68.00; H, 8.75; N, 6.88.

*N-Methyl-N-palmitoyl-β-alanine (**14**).* *N*-Methyl-β-alanine nitrile (0.94 mL, 10 mmol) was heated under reflux for 2 h in a mixture of ethanol (25 mL), water (5 mL) and KOH (3.2 g). After evaporation of the solvents the remaining residue was dissolved in water (35 mL) and acylated with palmitoyl chloride according to procedure 3 to yield 2.22 g (65%), mp 64°C. IR: $\nu = 2920$, 2850, 1710, 1605 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.83 (3H, m), 1.22 (24H, m), 1.46 (2H, m), 2.17 (2H, m), 2.37 (2H, m), 2.92 (3H, s), 3.43 (2H, s); EIMS: m/z = 341 $[M]^+$.

*N-{2-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]ethyl}-N-methylhexadecanoic acid amide (**6**).* Compound **2** (414 mg, 1.5 mmol) was acylated with **14** (512 mg, 1.5 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (3 : 2) as eluent yielded 284 mg (32%), mp 57°C. IR: $\nu = 2925$, 2850, 1705, 1630 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.80 (3H, m), 1.21 (24H, m), 1.50 (2H, m), 1.95 (3H, s), 2.14 (3H, s), 2.24 (2H, m), 2.65 (2H, m), 2.99 (3H, s), 3.67 (2H, m), 6.68 (1H, m), 6.90 (3H, m), 7.25 (2H, m), 7.74 (1H, m), 8.12 (1H, s), 9.57 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ

13.9, 14.1, 20.6, 22.7, 25.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 33.6, 36.1, 36.4, 44.3, 118.1, 122.7. 123.2, 123.9, 125.9, 128.3, 129.3, 131.8, 134.1, 137.9, 139.3, 140.3, 169.4, 175.0; EIMS: m/z = 599 $[M]^+$; elemental analysis calculated for $C_{34}H_{53}N_3O_4S$ (599.88) C, 68.08; H, 8.91; N, 7.00; found: C, 68.21; H, 8.81; N, 7.33.

*N-Heptadecanoylproline (**15**).* Proline (575 mg, 5 mmol) was acylated with heptadecanoyl chloride (1.44 g, 5 mmol) according to procedure 3 to yield 1.7 g (93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, m), 1.21 (26H, m), 1.65 (2H, m), 2.02 (2H, s), 2.33 (2H, m), 2.46 (2H, m), 3.45 (1H, M), 3.56 (1H, m), 4.58 (1H, m).

*N,N-{1-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]tetramethylene}-heptadecanoic acid amide (**7**).* Compound **2** (552 mg, 2.0 mmol) was acylated with **15** (734 mg, 2.0 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent yielded 360 mg (29%), mp 45°C. IR: ν = 2925, 2855, 1680, 1620 cm^{-1}; ^{1}H NMR (500 MHz, d_6-DMSO): δ 0.83 (3H, m), 1.22 (26H, m), 1.46 (2H, m), 1.87 (2H, m), 1.96 (3H, s), 2.10 (2H, m), 2.16 (3H, s), 2.25 (2H, m), 3.49 (1H, m), 3.54 (1H, m), 4.38 (1H, m), 6.70 (1H, m), 6.92 (3H, m), 6.98 (1H, m), 7.38 (1H, m), 7.44 (1H, m), 7.78 (1H, m), 8.00 (1H, m), 9.43 (1H, s), 10.14 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ 14.2, 14.4, 20.4, 22.3, 24.5, 24.7, 28.9, 29.0, 29.1, 29.2, 29.26, 29.3, 29.7, 31.6, 34.0, 47.2, 60.3, 117.5, 121.4, 123.0, 125.0, 125.6, 128.4, 129.7, 134.1, 135.0, 137.8, 141.6, 171.3, 171.5; EIMS: m/z = 625 $[M]^+$; elemental analysis calculated for $C_{36}H_{55}N_3O_4S$ (625.92) C, 69.08; H, 8.86; N, 6.71; found: C, 69.05; H, 8.60; N, 6.80.

*N-[3-(2,3-Dimethylphenylaminosulphonyl)phenyl]-2-aminobenzoic acid amide (**16**).* 2-Nitrobenzoyl chloride (930 mg, 5 mmol) was added to a solution of **2** (1.38 g, 5 mmol) in a mixture of toluene (100 mL) and dioxane (20 mL) and heated to reflux. After 2 h the solvents were evaporated to furnish a yellow foam. Yield 2.1 g (95%). IR: ν = 1665, 1530, 1350 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.88 (3H, s), 2.12 (3H, s), 6.53 (1H, m), 6.84 (1H, m), 6.91 (1H, m), 6.93 (1H, m), 7.10 (1H, m), 7.18 (1H, m), 7.25 (1H, m), 7.32 (1H, m), 7.54 (2H, m), 7.62 (1H, m), 7.77 (1H, s), 8.00 (2H, m), 8.29 (1H, s).

Tin(II)chloride dihydrate (5.34 g) was added to a solution of this foam in ethyl acetate (70 mL). After 2 h under reflux the mixture was poured into water (300 mL) and the pH was adjusted to 7–8 by addition of saturated $NaHCO_3$ solution The mixture was extracted with ethyl acetate (3 × 200 mL). The combined extracts were washed with brine, dried with $MgSO_4$ and evaporated to yield **16** as a yellow foam. Yield 1.7 g (91%). IR: ν = 3360, 1660, 1550 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.88 (3H, s), 2.12 (3H, s), 6.53 (1H, m), 6.84 (1H, m), 6.91 (1H, m), 6.93 (1H, m), 7.10 (1H, m), 7.18 (1H, m), 7.25 (1H, m), 7.32 (1H, m), 7.54 (2H, m), 7.62 (1H, m), 7.77 (1H, s), 8.00 (2H, m), 8.29 (1H, s).

*N-{2-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]phenyl}hexadecanoic acid amide (**8**).* Palmitoyl chloride (0.76 mL) was added to a solution of **16** (1.0 g, 2.5 mmol) and *N*-methylmorpholine (0.6 mL) in dichloromethane (30 mL). After stirring overnight at room temperature the mixture was diluted with dichloromethane and washed with 1 M hydrochloric acid, saturated $NaHCO_3$ solution and water and dried with $MgSO_4$. The residue obtained after evaporation of the solvent was purified by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent to yield 1.12 g (71%), mp 105°C. IR: ν = 3235, 2920, 2850, 1660, 1585 cm^{-1}; ^{1}H NMR (500 MHz, d_6-DMSO): δ 0.84 (3H, m), 1.21 (24H, m), 1.54 (2H, m), 1.99 (3H, s), 2.14 (3H, s), 2.27 (2H, m), 6.77 (1H, m), 6.93 (1H, m), 6.97 (1H, m), 7.19 (1H, m), 7.38 (1H, m), 7.49 (2H, m), 7.73 (1H, m), 7.91 (1H, m), 8.10 (1H, m), 8.16 (1H, m); ^{13}C NMR (125 MHz, d_6-DMSO): δ 13.6, 13.8, 19.8, 21.8, 24.3, 24.7, 28.3, 28.4, 28.5, 28.6, 28.7, 28.8, 32.0, 33.5, 37.2, 118.2, 121.6. 121.7, 122.8, 123.8, 124.4, 125.1, 127.7, 128.5, 129.0, 131.5, 133.4, 134.5, 137.2, 137.6, 139.8, 141.1, 166.9, 174.1; EIMS: m/z = 633 $[M]^+$; elemental analysis calculated for $C_{37}H_{51}N_3O_4S$ (633.90) C, 70.11; H, 8.11; N, 6.63; found: C, 70.43; H, 9.18; N, 6.29.

*N-Palmitoylpiperidine-3-carboxylic acid (**17**).* Piperidine-3-carboxylic acid (1.29 g, 10 mmol) was acylated with palmitoyl chloride (3.0 mL, 10 mmol) according to procedure 3 to yield 3.0 g (86%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 0.84 (3H, m), 1.23 (24H, m), 1.45 (2H, m), 1.55 (1H, m), 1.65 (1H, m), 1.90 (1H, m), 2.28 (2H, m), 2.41 (1H, m), 2.69 (1H, m), 3.18 (1H, m), 3.28 (1H, m), 3.72 (1H, m), 3.81 (1H, m).

*N,N-{2-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]pentamethylene}hexadecanoic acid amide (**9**).* Compound **2** (414 mg, 1.5 mmol) was acylated with **17** (479 mg, 1.5 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent

yielded 290 mg (31%), mp 104°C. IR: $\nu = 2925$, 1700, 1605 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 0.85 (3H, m), 1.23 (24H, m), 1.54 (3H, m), 1.66 (1H, m), 1.90 (1H, m), 1.98 (3H, s), 2.07 (1H, m), 2.18 (3H, s), 2.31 (2H, m), 2.60 (1H, m), 3.43 (1H, m), 3.52 (1H, m), 3.75 (1H, m), 3.97 (1H, m), 6.76 (1H, m), 6.95 (3H, m), 7.29 (2H, m), 7.81 (1H, m), 8.12 (1H, s), 9.14 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9, 14.1, 14.2, 20.6, 22.7, 24.6, 25.4, 26.9, 29.3, 29.4, 29.5, 29.6, 29.65, 29.7, 31.9, 33.3, 43.4, 44.0, 46.8, 118.1, 119.9, 122.6, 123.4, 123.9, 125.8, 128.3, 129.3, 132.1, 134.0, 137.9, 139.2, 140.3, 171.1, 173.0; EIMS: m/z = 625 $[M]^+$; HR-EIMS: exact mass calculated for $C_{36}H_{55}N_3O_4S$ $[M]^+$ 625.3913; found 625.3926; elemental analysis calculated for $C_{36}H_{55}N_3O_4S$ (625.92) C, 69.08; H, 8.86; N, 6.71; found: C, 69.29; H, 8.75; N, 6.89.

*3-Pentadecanoylaminobenzoic acid (**18**).* 3-Aminobenzoic acid (1.37 g, 10 mmol) was acylated with pentadecanoyl chloride (2.6 g, 10 mmol) according to procedure 3 to yield 3.13 g (87%). ^1H NMR (400 MHz, d_6-DMSO): δ 0.84 (3H, m), 1.23 (22H, m), 1.59 (2H, m), 2.31 (2H, m), 7.37 (1H, m), 7.57 (1H, m), 7.80 (1H, m), 8.18 (1H, m), 9.83 (1H, s)

*N-{3-[3-(2,3-Dimethylphenylaminosulphonyl)phenylaminocarbonyl]phenyl}pentadecanoic acid amide (**10**).* Compound **2** (552 mg, 2.0 mmol) was acylated with **18** (722 mg, 2.0 mmol) according to procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent yielded 582 mg (47%), mp 176°C. IR: $\nu = 2925$, 2850, 1650 cm^{-1}; ^1H NMR (500 MHz, d_6-DMSO): δ 0.84 (3H, m), 1.23 (22H, m), 1.60 (2H, m), 1.99 (3H, s), 2.17 (3H, s), 2.31 (2H, m), 6.75 (1H, m), 6.95 (1H, m), 7.36 (1H, m), 7.43 (1H, m), 7.51 (1H, m), 7.60 (1H, m), 7.80 (1H, m), 7.96 (1H, m), 8.10 (1H, m), 8.23 (1H, m), 9.48 (1H, s), 9.97 (1H, s), 10.44 (1H, s); ^{13}C NMR (125 MHz, d_6-DMSO): δ 14.0, 14.1, 20.1, 22.1, 25.1, 28.7, 28.8, 28.9, 29.0, 29.1, 31.3, 36.4, 118.3, 118.8, 119.8, 121.7, 123.0, 123.8, 124.8, 125.4, 128.1, 128.7, 129.4, 133.9, 134.7, 135.2, 137.6, 139.6, 139.8, 141.3, 165.9,

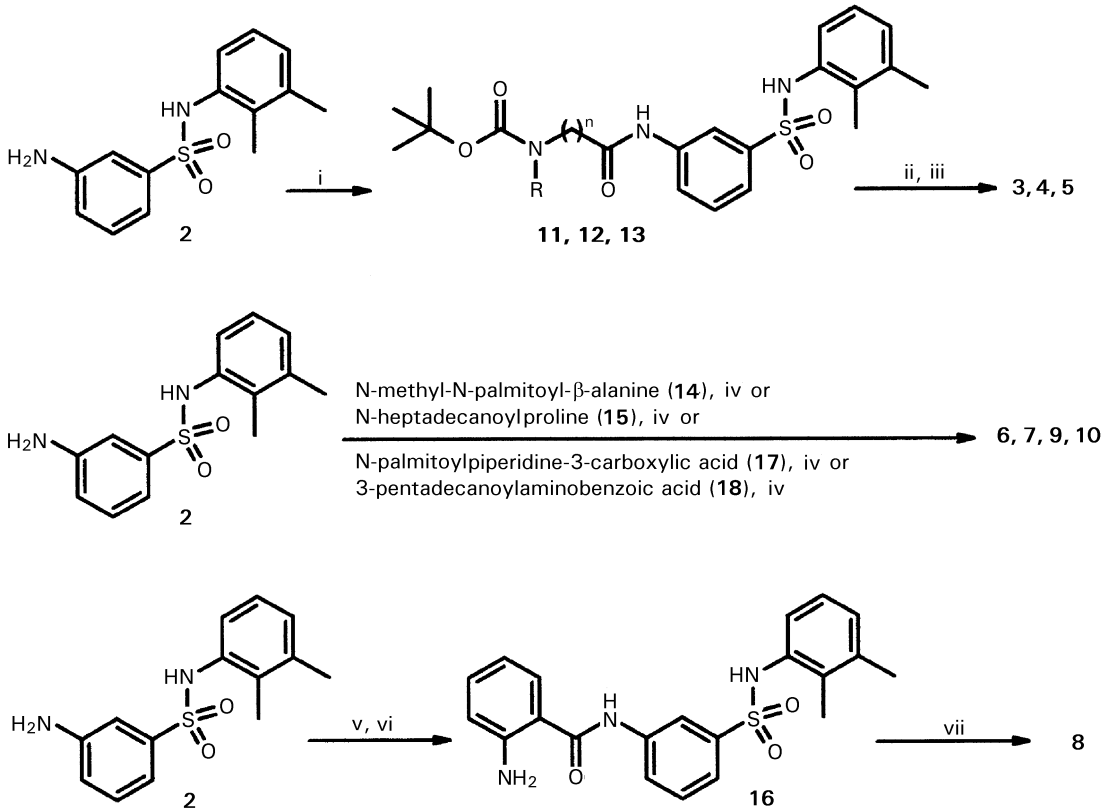

Figure 2. Synthesis of compounds **3**–**10**: (i) N-Boc-amino acid, isobutyl chloroformate, *N*-methylmorpholine, dimethylformamide, −15°C, 5 min, then **2**, −15°C→room temperature, overnight; (ii) 4 M HCl in dioxane, room temperature, 2 h; (iii) RCOOH, isobutyl chloroformate, *N*-methylmorpholine, dimethylformamide, −15°C, 5 min, then deprotected **11**, **12** or **13**, −15°C → room temperature, overnight; (iv) isobutyl chloroformate, *N*-methylmorpholine, dimethylformamide, −15°C, 5 min, then **2**, −15°C→room temperature, overnight; (v) 2-nitrobenzoic acid chloride, *N*-methylmorpholine, dimethylformamide, 0°C → room temperature, overnight; (vi) $SnCl_2 \cdot 2H_2O$, ethyl acetate, reflux, 2 h; (vii) palmitoyl chloride, *N*-methylmorpholine, dichloromethane, 0°C→room temperature, overnight.

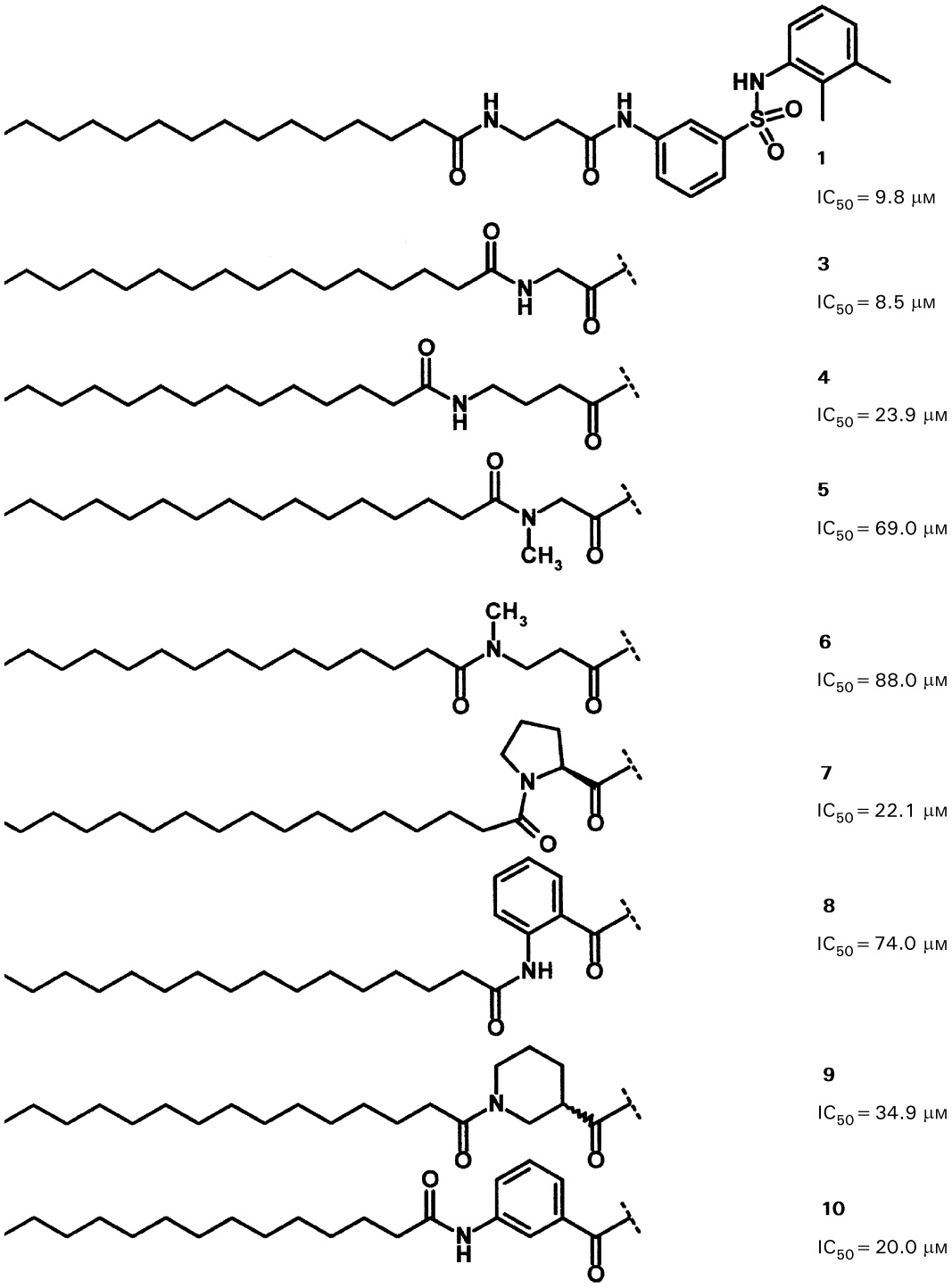

Figure 3. Structures and farnesyltransferase inhibitory activity of compounds **3**–**10**.

171.6; EIMS: m/z = 619 $[M]^+$; elemental analysis calculated for $C_{36}H_{49}N_3O_4S$ (619.87) C, 69.76; H, 7.97; N, 6.78; found: C, 69.89; H, 8.03; N, 6.70.

Enzyme preparation

Yeast farnesyltransferase (FTase) was used as a fusion to glutathione *S*-transferase at the N-terminus of the subunit. FTase was expressed in *Escherichia coli* DH5α grown in LB media containing ampicillin and chloramphenicol for co-expression of pGEX-DPR1 and pBC-RAM2 for FTase production (Del Villar et al 1997). 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 elsewhere (Pompliano et al 1992). FPP was obtained as a solution of the ammonium salt in methanol–10 mM aqueous NH_4Cl (7 : 3) from Sigma–Aldrich. Dansyl-Gly-Cys-Val-leu-Ser (Ds-GCVLS) was custom synthesized by ZMBH, Heidelberg, Germany. The assay mixture (100 L) contained 50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 10 μM, $ZnCl_2$, 5 mM DTT (dithiothreitol), 7 μM Ds-GCVLS, 20 μM FPP, 5 nmol (approx.) yeast GST-Ftase, and 1% of different concentrations of the test compounds dissolved in dimethylsulphoxide (DMSO). The progress of the enzyme reaction was followed by monitoring the enhancement of 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. IC50 values (concentrations resulting in 50% inhibition) were calculated from the initial velocity of three independent measurements of four or five different concentrations of inhibitor.

Results and Discussion

The preparation of the target structures was accomplished as outlined in Figure 2. The inhibitory activity against yeast farnesyltransferase (Del Villar et al 1997) was determined as described elsewhere (Pompliano et al 1992). The structures and results are shown in Figure 3. The length of the original β-alanyl linker was either reduced or increased by one methylene unit. Whereas the shorter glycine derivative **3** was as active (approx.) as the lead structure **1**, the longer γ-aminobutyric acid derivative **4** was half as active as **1** as an inhibitor of farnesyltransferase. Therefore, glycine and β-alanine were selected as linkers to determine how an additional substituent on the amide nitrogen would influence the inhibitory potency of the compounds. In both instances (**5**, **6**) N-methylation resulted in a considerable drop in activity. A possible interpretation that the free NH function is necessary for activity and acts, for instance, as a hydrogen-bond donor, does not hold true because compounds **7** and **9**, also lacking a free NH function, are significantly more active than **5** or **6**. The distinct reduction in activity observed with **5** and **6** can be more readily attributed to a conformational effect than to the loss of a hydrogen-bond donor.

With the use of rigid ring systems possible conformations of the linkers glycine, β-alanine and γ-aminobutyric acid can be frozen. Replacement of the glycine by proline prevents any rotation around the Cα–N bond present in glycine. This obviously forces the system into a conformation different from the bioactive conformation, because activity is reduced to less than half. Compounds **8** and **9** represent the *syn* and *anti* conformations, respectively, of β-alanine. Both compounds are significantly less active than the β-alanyl derivative **1**. The more pronounced drop in activity of **8** is not entirely unexpected, because **8** represents the *syn* conformation which is very unlikely to be adopted by **1**. In addition to their fixing the conformation, the cyclic linkers have a second effect which must be considered. Because they are more spacious than the acyclic linkers the loss of activity could also be attributed to simple steric hindrance, although there is evidence (Ciccarone et al 1999) that the stereochemistry of that part of the farnesyltransferases active site can accommodate the cyclic systems used in this study. In conclusion, this study will be helpful for further development because it shows that none of the conformations fixed by the different linkers represents the bioactive conformation of our bisubstrate analogue farnesyltransferase inhibitors.

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