Pharm. Pharmacol. Commun. 2000, 6: 507–512
Received May 3, 2000
Accepted August 3, 2000

Non-Peptidic, Non-Prenylic Bisubstrate Farnesyltransferase Inhibitors. Effect of a Carboxyl Group at the Central Group on Farnesyltransferase Inhibitory Activity

MARTIN SCHLITZER AND ISABEL SATTLER*

*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg and *Hans-Knöll-Institut für Naturstoff-Forschung e. V., Beutenbergstraße 11, D-07745, Jena, Germany*

Abstract

We recently described non-peptidic, non-prenylic bisubstrate analogues as novel farnesyltransferase inhibitors comprising three modules—a farnesyl-mimetic, a linker and an AAX-peptidomimetic substructure. In this study, we replaced the originally used β-alanyl linker with aminomalonic, aspartic and glutamic acid, respectively, to introduce a second functional group capable of complexing the essential zinc ion, located in the active site of farnesyltransferase.

Apart from aminomalonic acid, all moieties showed reduced inhibitory activity. Interestingly, the benzyl esters of the aspartic and glutamic acid derivatives were more active than the free acids.

The results provide further evidence for an additional lipophilic binding cleft in the active site of farnesyltransferase.

Cancer is caused by a 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 that control these processes (Macara et al 1996; Gomez et al 1998). Mutated forms of Ras, which are constitutively active, are found in approximately 30% of all cancers in man. Several post-transformational modifications occur before Ras acquires its full biological activity. The critical step is the 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, the inhibition of farnesyltransferase has received considerable interest in the recent years as a strategy for the development of novel potential cancer therapeutics (Leonard 1997; Qian et al 1997; Sebti & Hamilton 1998). However, there is increasing evidence that Ras may not be the only substrate of farnesyltransferase involved in oncogenesis (Cox & Der 1997; Du et al 1999). Irrespective of the mechanisms by which farnesyltransferase inhibitors exert their antiproliferative effects, the efficacy of these compounds and their low toxicity has been demonstrated (Oliff 1999), and they are, therefore, regarded as a major new approach in cancer therapy.

Correspondence: M. Schlitzer, Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany.

We have previously described novel types of bisubstrate analogue farnesyltransferase inhibitors (**1**; Figure 2) (Schlitzer & Sattler 1999). These inhibitors are different from the few known bisubstrate analogues in that they do not have peptidic or prenylic substructures. They are composed of three modular building blocks—an AAX-peptidomimetic, a linker or central moiety and a farnesylmimetic (Figure 2). We demonstrated that the replacement of the amide moiety which connects the farnesylmimetic to the linker by an ethylene bridge considerably reduces the farnesyltransferase inhibitory activity (Schlitzer et al 2000). We suggest that the carbonyl oxygen interacts with the farnesyltransferase active site and that this interaction is important for the binding affinity. This interaction might be enhanced by introducing a carboxylic acid into the central moiety as a second function capable of complexing the

essential zinc ion, located in the active site of farnesyltransferase. Therefore, we prepared analogues of **1** in which the central β-alanyl moiety was replaced by aminomalonic, aspartic and glutamic acid, respectively.

Material and Methods

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol JMN-GX-400 and Jeol JMN-LA-500 spectrometers. Mass spectra were acquired 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 a Hewlett Packard CHN-analyser (type 185). Column chromatography was performed on silica gel 60 (0·062–0·200 mm) from Merck. *N*-(2-(3-(2,3-Dimethylphenylaminosulphonyl)-phenylaminocarbonyl)ethyl)hexadecanoic acid amide (**1**), 3-amino-*N*-(2,3-dimethyl-phenyl)benzenesulphonamide (**2**) (Schlitzer & Sattler 1999; Schlitzer et al 1999) and *N*-Boc-2-aminomalonic acid monoethyl ester (Kawai et al 1982) were prepared as described elsewhere.

General procedure for the acylation of amines using 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^{-1}) the solution was cooled to -15°C and isobutyl chloroformate (0·13 mL mmol^{-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). 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 the N*-Boc-deprotection (Procedure 2)*

The *N*-Boc derivatives were dissolved in dioxane (10 mL mmol^{-1}) supplied with 4 M HCl and stirred for 2 h at room temperature. After addition of diethylether 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 saponification of esters **4a**, **6a** and **8a** (Procedure 3)*

To a solution of esters **4a**, **6a** and **8a**, respectively, in dimethoxyethane (15 mL), 1 M LiOH solution (15 mL) was added and the mixture was stirred at room temperature until the reaction was complete (TLC). The volatile compounds were removed in-vacuo and the residue was dissolved in water (20 mL). This solution was extracted with ethyl acetate. The organic phase was discarded. The aqueous solution was adjusted to pH 1 with concentrated HCl and extracted three times with ethyl acetate. The combined organic extracts were dried with $MgSO_4$ and evaporated to dryness.

*2-(tert-Butyloxycarbonylamino)-2-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]acetic acid ethyl ester (**3**)*

Compound **2** (1·38g, 5·0 mmol) was acylated with *N*-Boc-2-aminomalonic acid monoethyl ester (1·24 g, 5·0 mmol) according to Procedure 1. Yield 2·41 g (95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0·92 (3H, m, O-CH_2-CH_3), 1·46 (9H, m, t-Bu-H), 1·96 (3H, s, Ph-CH_3), 2·20 (3H, s, Ph-CH_3), 4·02 (1H, m, malonyl-α-H), 4·25 (2H, m, O-CH_2-CH_3), 6·20 (1H, s, NH), 6·78 (1H, m, aryl-H), 6·96–7·01 (4H, m, aryl-H), 7·04 (1H, m, aryl-H), 7·16 (1H, m, aryl-H).

*2-(Heptadecanoylamino)-2-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]acetic acid ethyl ester (**4a**)*

Compound **3** (1010 mg, 2 mmol) was deprotected according to Procedure 2 and acylated by heptadecanoic acid (541 mg, 2 mmol) according to Procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent yielded 1260 mg (96%);^{1}H NMR (400 MHz, d_6-DMSO): δ 0·85 (6H, m, heptadecanoyl-CH_3, O-CH_2-CH_3), 1·22 (26H, m, heptadecanoyl-CH_2), 1·46 (2H, m, heptadecanoyl-CH_2), 1·94 (3H, s, Ph-CH_3), 2·15 (3H, m, Ph-CH_3), 2·25 (2H, m, heptadecanoyl-CH_2), 3·93 (1H, m, malonyl-α-H), 4·15 (2H, m, O-CH_2-CH_3), 6·70 (1H, m, aryl-H), 6·92 (1H, m, aryl-H), 6·98 (1H, m, aryl-H), 7·26 (1H, m, aryl-H), 7·42 (1H, m, aryl-H), 7·78 (1H, m, aryl-H), 8·00 (1H, m, aryl-H), 9·44 (1H, s, NH), 10·04 (1H, s, NH); ^{13}C NMR (125 MHz, d_6-DMSO): δ 13·9, 14·0, 14·1, 18·7, 18·8, 20·2, 22·2, 25·1, 25·2, 27·4, 28·8, 28·9,

29·0, 29·1, 29·2, 31·4, 31·4 and 34·7 (alkyl-C), 56·1 (malonyl-α-C), 61·7 (O-*C*H_2-CH_3), 117·0, 121·0, 122·6, 124·9, 124·4, 128·2, 129·5, 133·8, 134·7, 137·6, 139·9 and 141·3 (aryl-C), 166·6, 171·8 and 172·7 (CO).

*2-(Heptadecanoylamino)-2-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]acetic acid (**4b**)*

Compound **4a** (210 mg, 0·32 mmol) was deprotected according to Procedure 3. Yield 180 mg (89%); mp 94°C. IR: $\nu = 3340$, 3285, 2920, 2850, 1765, 1695, 1545 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO): δ 0·84 (3H, m, heptadecanoyl-CH_3), 1·25 (26H, m, heptadecanoyl-CH_2), 1·48 (2H, m, heptadecanoyl-CH_2), 1·96 (3H, s, Ph-CH_3), 2·16 (3H, m, Ph-CH_3), 2·27 (2H, m, heptadecanoyl-CH_2), 4·88 (1H, m, malonyl-α-H), 6·71 (1H, m, aryl-H), 6·93 (1H, m, aryl-H), 6·97 (1H, m, aryl-H), 7·27 (1H, m, aryl-H), 7·43 (1H, m, aryl-H), 7·77 (1H, m, aryl-H), 8·00 (1H, m, aryl-H), 8·25 (1H, m, NH), 9·40 (1H, s, NH); ^{13}C NMR (100 MHz, d_6-DMSO): δ 13·6, 13·8, 19·8, 21·8· 24·7, 28·4, 28·5, 28·7, 31·0, 34·4 and 36·3 (alkyl-C), 56·0 (malonyl-α-C), 116·8, 124·5, 125·1, 127·8, 129·1, 137·2, 139·6 and 141·0 (aryl-C), 167·9, 171·4 and 172·3 (CO); HR-ESIMS: exact mass calculated for $C_{34}H_{52}N_3O_6S$ $[M+H]^+$, 630·3577; found 630·3574.

*2(S)-2-(tert-Butyloxycarbonylamino)-3-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]propionic acid benzyl ester (**5**)*

Compound **2** (1·66 g, 6·0 mmol) was acylated with *N*-Boc-L-aspartic acid-1-benzyl ester (1·81 g, 6·0 mmol) according to Procedure 1. Yield 2·86 g (95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1·32 (9H, m, t-Bu-H), 1·90 (3H, s, Ph-CH_3), 2·11 (3H, s, Ph-CH_3), 2·79–2·88 (2H, m, Asp-β-H), 4·56 (1H, m, Asp-α-H), 5·10 (2H, m, Bzl-CH_2), 6·81–6·95 (3H, m, aryl-H), 7·06–7·13 (1H, m, aryl-H), 7·18–7·26 (6H, m, aryl-H), 7·84 (2H, m, aryl-H).

*2(S)-2-(Hexadecanoylamino)-3-[[3-(2,3-dimethylphenylaminosuphonyl)phenyl]-aminocarbonyl]propionic acid benzyl ester (**6a**)*

Compound **5** (871 mg, 1·5 mmol) was deprotected according to Procedure 2 and acylated by palmitic acid (386 mg, 1·5 mmol) according to Procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (3 : 2) as eluent yielded 1040 mg (96%); ^{1}H NMR (400 MHz, d_6-DMSO): δ 0·83 (3H, m, hexadecanoyl-CH_3), 1·21 (24H, m, hexadecanoyl-CH_2), 1·45 (2H, m, hexadecanoyl-CH_2), 1·94 (3H, s, Ph-CH_3), 2·06 (2H, m, hexadecanoyl-CH_2), 2·15 (3H, s, Ph-CH_3), 2·74 (1H, m, Asp-β-H), 2·89 (1H, m, Asp-β-H), 4·70 (1H, m, Asp-α-H), 5·08 (2H, s, Bzl-CH_2), 6·70 (1H, m, aryl-H), 6·92 (1H, m, aryl-H), 6·98 (1H, m, aryl-H), 7·22 (1H, m, aryl-H), 7·28 (5H, m, aryl-H), 7·41 (1H, m, aryl-H), 7·74 (1H, m, aryl-H), 8·03 (1H, m, aryl-H), 8·25 (1H, m, NH), 9·04 (1H, s, NH), 10·24 (1H, s, NH); ^{13}C NMR (125 MHz, d_6-DMSO): δ 13·8, 13·9, 20·0, 20·1, 21·9, 24·4, 25·1, 28·4, 28·5, 28·6, 28·7, 28·8, 28·85, 28·9, 31·1, 33·6 and 34·9 (alkyl-C), 48·7 (Asp-α-C), 65·9 (Bzl-*C*H_2), 116·0, 116·8, 119·9, 121·1, 121·8, 122·4, 124·5, 125·2, 127·4, 127·7, 127·9, 128·1, 129·3, 133·6, 134·5, 134·6, 135·8, 137·3, 139·4, 139·9 and 141·2 (aryl-C), 168·0, 170·8 and 171·9 (CO).

*2(S)-2-(Hexadecanoylamino)-3-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]propionic acid (**6b**)*

Compound **6a** (145 mg, 0·2 mmol) was deprotected according to Procedure 3. Yield 120 mg (95%); mp 128°C. IR: $\nu = 3370$, 3250, 2920, 2860, 1720, 1650 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO): δ 0·83 (3H, m, hexadecanoyl-CH_3), 1·22 (24H, m, hexadecanoyl-CH_2), 1·47 (2H, m, hexadecanoyl-CH_2), 1·96 (3H, s, Ph-CH_3), 2·06 (2H, m, hexadecanoyl-CH_2), 2·15 (3H, s, Ph-CH_3), 2·70 (1H, m, Asp-β-H), 2·83 (1H, m, Asp-β-H), 4·60 (1H, m, Asp-α-H), 6·72 (1H, m, aryl-H), 6·92 (1H, m, aryl-H), 6·98 (1H, m, aryl-H), 7·20 (1H, m, aryl-H), 7·43 (1H, m, aryl-H), 7·75 (1H, m, aryl-H), 8·03 (1H, m, aryl-H), 9·00 (1H, s, NH), 9·45 (1H, s, NH), 10·17 (1H, s, NH), 11·97 (1H, s br, COOH); ^{13}C NMR (125 MHz, d_6-DMSO): δ 13·8, 13·9, 20·0, 20·1, 20·6, 20·9, 21·9, 24·4, 25·1, 28·4, 28·5, 28·6, 28·7, 28·75, 28·8, 28·9, 31·1, 33·5 and 35·0 (alkyl-C), 48·5, 116·0, 116·8, 124·4, 125·2, 127·6, 129·3, 137·1, 139·5 and 141·1 (aryl-C), 168·2, 170·9 and 172·2 (CO); HR-ESIMS: exact mass calculated for $C_{34}H_{52}N_3O_6S$ $[M+H]^+$, 630·3577; found 630·3580; elemental analysis calculated for $C_{34}H_{51}N_3O_6S$ (629·87) C, 64·84; H, 8·16; N, 6·67; found C, 64·48; H, 8·43; N, 6·63.

*2(S)-2-(tert-Butyloxycarbonylamino)-4-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]butyric acid benzyl ester (**7**)*

Compound **2** (552 mg, 2·0 mmol) was acylated with *N*-Boc-L-glutamic acid-1-benzyl ester (674 mg, 2·0 mmol) according to Procedure 1. Yield 1·03 g (87%).^{1}H NMR (500 MHz, $CDCl_3$): δ 1·36 (9H, m, t-Bu-H), 1·92 (3H, s, Ph-CH_3), 2·15 (3H, s, Ph-CH_3), 2·20 (2H, m, Glu-β-H), 2·36 (2H, m, Glu-γ-H), 4·30 (1H, m, Glu-α-H), 5·10 (2H, m, Bzl-CH_2),

6·82 (1H, m, aryl-H), 6·88–7·00 (5H, m, aryl-H), 7·11 (1H, m, aryl-H), 7·27 (5H, m, aryl-H).

*2(S)-2-(Pentadecanoylamino)-4-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]butyric acid benzyl ester (**8a**)*

Compound **7** (300 mg, 0·5 mmol) was deprotected according to Procedure 2 and acylated by pentadecanoic acid (121 mg, 0·5 mmol) according to Procedure 1. Purification by flash chromatography with ethyl acetate–*n*-hexane (2 : 3) as eluent yielded 260 mg (72%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0·81 (3H, m, pentadecanoyl-CH_3), 1·19 (22H, m, pentadecanoyl-CH_2), 1·52 (2H, m, pentadecanoyl-CH_2), 1·94 (3H, s, Ph-CH_3), 2·12 (3H, s, Ph-CH_3), 2·16 (2H, m, Glu-β-H), 2·23 (2H, m, pentadecanoyl-CH_2), 2·37 (2H, m, Glu-γ-H), 4·58 (1H, m, Glu-α-H), 5·08 (2H, s, Bzl-CH_2), 6·40 (1H, m, NH), 6·64 (1H, m, aryl-H), 6·90 (3H, m, aryl-H), 7·24 (6H, m, aryl-H), 7·78 (1H, m, aryl-H), 8·07 (1H, m, aryl-H), 9·21 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13·9, 14·1, 20·6, 22·7, 25·6, 29·2, 29·3, 29·4, 29·5, 29·6, 31·9, 33·4, 33·9 and 36·7 (alkyl-C), 51·8 (Glu-α-C), 67·7 (Bzl-*C*H$_2$), 117·9, 123·4, 123·8, 125·9, 128·4, 128·5, 128·7, 129·4, 134·0, 134·8, 137·9 and 140·3 (aryl-C). 170·9, 171·7 and 174·6 (CO).

*2(S)-2-(Pentadecanoylamino)-4-[[3-(2,3-dimethylphenylaminosulphonyl)phenyl]-aminocarbonyl]butyric acid (**8b**)*

Compound **8a** (80 mg, 0·11 mmol) was deprotected according to Procedure 3. Yield 60 mg (87%); mp 170°C. IR: ν = 3365, 3285, 2925, 2855, 1705, 1600, 1545 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO): δ 0·84 (3H, m, pentadecanoyl-CH_3), 1·23 (22H, m, pentadecanoyl-CH_2), 1·47 (2H, m, pentadecanoyl-CH_2), 1·96 (3H, s, Ph-CH_3), 2·09 (2H, m, Glu-β-H), 2·15 (3H, s, Ph-CH_3), 2·24 (2H, m, pentadecanoyl-CH_2), 2·38 (2H, m, Glu-γ-H), 4·23 (1H, m, Glu-α-H), 6·71 (1H, m, aryl-H), 6·92 (1H, m, aryl-H), 6·98 (1H, m, aryl-H), 7·27 (1H, m, aryl-H), 7·42 (1H, m, aryl-H), 7·75 (1H, m, aryl-H), 7·90 (1H, m, NH), 8·01 (1H, m, aryl-H), 9·40 (1H, s, NH), 10·09 (1H, s, NH); HR-ESIMS: exact mass calculated for $C_{34}H_{52}N_3O_6S$ $[M+H]^+$, 630·3577; found 630·3570.

Enzyme preparation

Yeast farnesyltransferase was used as a fusion to glutathione *S*-transferase at the *N*-terminus of the β-subunit. Farnesyltransferase was expressed in *Escherichia coli* DH5α grown in LB media containing ampicillin and chloramphenicol for the co-expression of pGEX-DPR1 and pBC-RAM2 plasmids for farnesyltransferase 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.

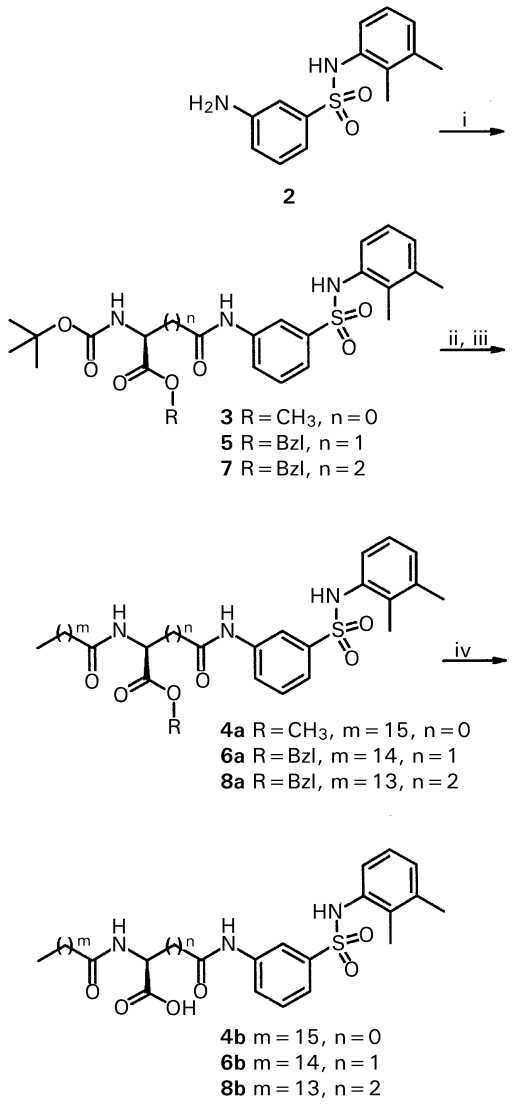

Figure 1. Synthesis of compounds **4**, **6** and **8**. i. *N*-Boc-amino acid mono ester, isobutyl chloroformate, NMM, DMF, −15°C, 5 min, then **2**, −15°C, room temp., overnight; ii. 4 M HCl/dioxane, room temp., 2 h; (iii) $H_3C(CH_2)_n$-COOH, isobutyl chloroformate, NMM, DMF, −15°C, 5 min, then deprotected **3**, **5** or **7**, −15°C, room temp., overnight; and iv. 1 M LiOH, DME, room temp.

Dansyl-GlyCysValLeuSer (Ds-GCVLS) was synthesized by ZMBH, Heidelberg, Germany. The assay mixture (100 μL) contained 50 mM Tris/HCl pH 7·4, 5 mM $MgCl_2$, 10 μM, $ZnCl_2$, 5 mM dithiothreitol (DTT), 7 μM Ds-GCVLS, 20 μM FPP and approximately 5 nmol yeast GST-farnesyltransferase and 1% of various concentrations of the test compounds dissolved in DMSO. The progress of the enzyme reaction was followed by monitoring the enhancement of the fluorescence emission at 505 nm (excitation 340 nm). The reaction was started by addition of the enzyme and run in a Quartz cuvette at 30°C. Fluorescence emission was recorded with a Perkin Elmer LS50B spectrometer. IC50 values (concentrations resulting in 50% inhibition) were calculated from initial velocity of three independent measurements of four to five different concentrations of inhibitor.

Results and Discussion

The preparation of the target structures was achieved as outlined in Figure 1. The inhibitory activity against yeast farnesyltransferase (Del Villar et al 1997) was determined as described elsewhere (Pompliano et al 1992). The structures and IC50 values are shown in Figure 2. Only the

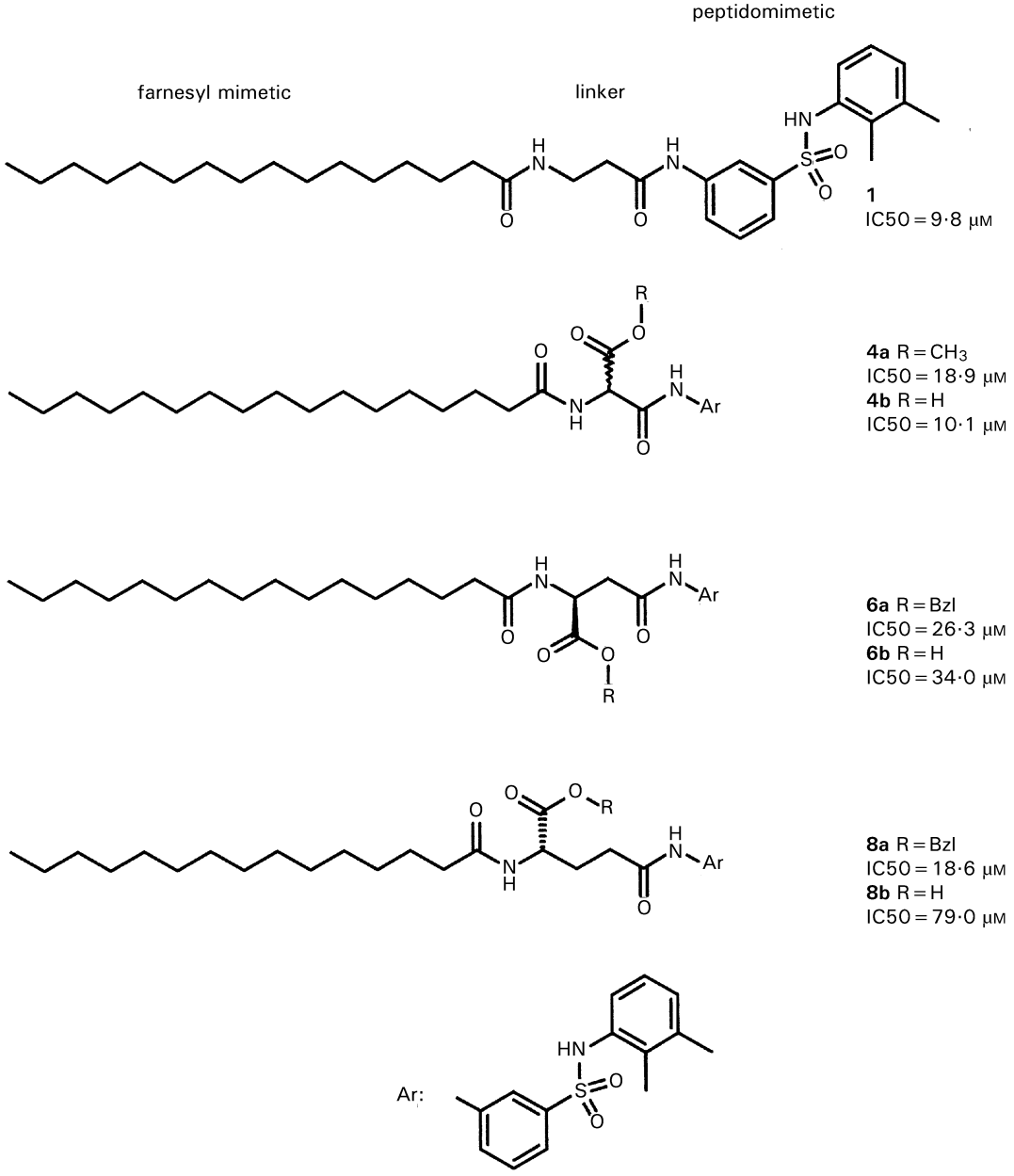

Figure 2. Structure and farnesyltransferase inhibitory activity (IC50) of compounds **1**, **4**, **6** and **8**.

aminomalonic acid derivative **4b** was approximately as active as the lead compound **1**. The aspartic and the glutamic acid derivatives **6b** and **8b** showed a marked decrease in farnesyltransferase inhibitory activity. This effect was much more pronounced in the larger glutamic acid derivative **8b**. None of the three additional carboxyl groups were in the correct position to enhance the interaction of the inhibitors with the enzyme-bound zinc. In contrast, the carboxy groups of compounds **6b** and **8b** reduced the affinity of the inhibitors towards the enzyme.

Interestingly, the benzylesters **6a** and **8a** were more active than the free carboxylic acids. Again, this effect was more pronounced with the larger glutamic acid derivative **8a**. Obviously, the presence of the bulky lipophilic benzyl residue attenuates the detrimental effect of the carboxyl group on farnesyltransferase inhibitory activity. This provides further evidence for the existence of a novel aromatic binding region in the farnesyltransferase active site which has been recently postulated (Breslin et al 1998). Further exploration of this lipophilic binding region for inhibitor design is necessary.

Acknowledgements

The pGEX-DPR1 and pBC-RAM2 plasmids were kindly provided by Professor F. Tamanoi (UCLA). M. Schlitzer wishes to thank Ms K. Burk for technical assistance and the German Pharmaceutical Society for financial support. I. Sattler wishes to thank Professor S. Grabley and Dr R. Thiericke for generous support and Ms S. Egner for excellent technical assistance.

References

Breslin, M. J., deSolms, J., Giuliani, E. A., Stokker, G. E., Graham, S. L., Pompliano, D. L., Mosser, S. D., Hamilton, K. A. Hutchinson, J. H. (1998) Potent, non-thiol inhibitors of farnesyltransferase. Bioorg. Med. Chem. Lett. 8: 3311–3316

Cox, A. D., Der, C. J. (1997) Farnesyltransferase inhibitors and cancer treatment: targeting simply Ras? Biochim. Biophys. Acta 1333: F51–F71

Del Villar, K., Mitsuzawa, H., Yang, W., Sattler, I., Tamanoi, F. (1997) Amino acid substitutions that convert the protein substrate specificity of farneslytransferase to that of geranylgeranyltransferase type I. J. Biol. Chem. 272: 680–687

Du, W., Lebowitz, P. F., Prendergast, G. C. (1999) Cell growth inhibition by farnesyltransferase inhibitors is mediated by gain of geranylgeranylated RhoB. Mol. Cell. Biol. 19: 1831–1840

Gomez, J., Martinez-A, C., Gonzalez, A., Rebollo, A. (1998) Dual role of Ras and Rho proteins: at the cutting edge of life and death. Immunol. Cell Biol. 76: 125–134

Kawai, M., Nyfeler, R., Berman, J. M., Goodman, M. (1982) Peptide sweeteners. 5. Side-chain homologues relating zwitterionic and trifluoroacetylated amino acid anilide and dipeptide sweeteners. J. Med. Chem. 25: 397–402

Leonard, D. M. (1997) Ras farnesyltransferase: a new therapeutic target. J. Med. Chem. 40: 2971–2990

Macara, I. G., Lounsbury, K. M., Richards, S. A., McKiernan, C., Bar-Sari, D. (1996) The Ras superfamily of GTPases. FASEB J. 10: 625–630

McCormick, K. G. (1999) Signalling networks that cause cancer. Trends Cell Biol. 9: M53–M56

Pompliano, D. L., Gomez, R. P., Anthony, N. J. (1992) Intramolecular fluorescence enhancement: a continuous assay of Ras farnesyl:protein transferase. J. Am. Chem. Soc. 114: 7945–7946

Oliff, A. (1999) Farnesyltransferase inhibitors: targeting the molecular basis of cancer. Biochim. Biophys. Acta 1423: C19–C30

Qian, Y., Sebti, S. M., Hamilton, A. D. (1997) Farnesyltransferase as a target for anticancer drug design. Biopolymers 43: 25–41

Schlitzer, M., Sattler, I. (1999) Design, synthesis and evaluation of novel modular bisubstrate analogue inhibitors of farnesyltransferase. Angew. Chem. Int. Ed. 38: 2032–2034

Schlitzer, M., Sattler, I., Dahse, H.-M. (1999) Different amino acid replacements in CAAX-tetrapeptide based farnesyltransferase inhibitors. Arch. Pharm. Med. Chem. 332: 124–132

Schlitzer, M., Böhm, M., Sattler, I. (2000) Non-peptidic, non-prenylic bisubstrate farnesyltransferase inhibitors 3. Structural requirements of the central moiety for farnesyltransferase inhibitory activity. Bioorg. Med. Chem. In press.

Sebti, S. M., Hamilton, A. D. (1998) New approaches to anticancer drug design based on the inhibition of farnesyltransferase. Drug Discov. Today 3: 26–32

Zhang, F. L., Casey, P. J. (1996) Protein prenylation: molecular mechanism and functional consequences. Annu. Rev. Biochem. 65: 241–269