Studies on *Rubia akane* (RA) derivatives. Part 8.[1] Design, syntheses and antitumour activity of cyclic hexapeptide RA analogues possessing an alkyl substituent on the Tyr-3 aromatic ring

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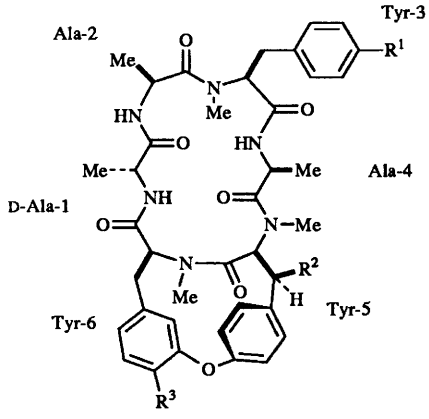
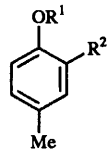
The effective conversion of RA-VII 1 into the naturally less-accessible RA-II 4 has been devised through boron tribromide bis-O-demethylation and successive selective partial O-methylation using diazo(trimethylsilyl)methane. The *O*-triflate 11 prepared from RA-II 4 was subjected to cross-coupling reaction with alkylstannanes to produce analogues 12, 13 and 15, while compounds 13 and 15 were later converted into analogues 14 and 16, respectively. Analogues 12–16 showed antitumour activity against P-388 leukaemia both *in vitro* and *in vivo*.

Introduction

RA-VII **1**[2] and bouvardin (NSC 259968) **2**[3] are a class of antitumour bicyclic hexapeptides isolated from *Rubiaceae* plants. Owing to their promising antitumour activity in addition to their unique mode of action—inhibition of protein synthesis through interaction with eukaryotic 80 S ribosomes[4]—peptide **1** is currently undergoing clinical trials in Japan as an anticancer agent.[5] Their unique cycloisodityrosine structure has attracted much attention from synthetic chemists,[6] and two total syntheses of compound **1** have been accomplished.[7,8] A previous structure–activity relation study[9] and biotransformations[10,11] of these peptides revealed that the methoxy group of the Tyr-3 residue is very important for such activity. When this methoxy group is substituted by a hydrogen atom (*e.g.* compound **3**) or a hydroxy group (*e.g.* RA-II **4**), their cytotoxicity is reduced by ~ 100 to 1000 times.[9] Also, this O-demethylation has been identified as a metabolic pathway for these peptides.[5a,10,11] In spite of such importance, no effort has been made thus far to modify the substituents of Tyr-3 because of the difficulties associated with the selective manipulation of this residue. We proposed that substitution of this methoxy group by an alkyl group, especially a sterically very similar ethyl group, would produce an analogue which possesses metabolic stability and might express more pronounced *in vivo* antitumour activity. We report here on the syntheses of these analogues and their *in vitro* and *in vivo* antitumour activities.





1; $R^1 = R^3 = OMe$, $R^2 = H$

2; $R^1 = OMe$, $R^2 = R^3 = OH$

3; $R^1 = R^2 = H$, $R^3 = OMe$

4; $R^1 = OH$, $R^2 = H$, $R^3 = OMe$

5; $R^1 = OMe$, $R^2 = H$, $R^3 = OH$

6; $R^1 = R^3 = OH$, $R^2 = H$

11; $R^1 = OSO_2CF_3$, $R^2 = H$, $R^3 = OMe$

12; $R^1 = Me$, $R^2 = H$, $R^3 = OMe$

13; $R^1 = vinyl$, $R^2 = H$, $R^3 = OMe$

14; $R^1 = Et$, $R^2 = H$, $R^3 = OMe$

15; $R^1 = allyl$, $R^2 = H$, $R^3 = OMe$

16; $R^1 = Pr$, $R^2 = H$, $R^3 = OMe$

7; $R^1 = R^2 = H$

8; $R^1 = H$, $R^2 = OMe$

9; $R^1 = Me$, $R^2 = H$

10; $R^1 = Me$, $R^2 = OMe$

Results and discussion

We considered that RA-II **4**,[12] possessing a hydroxy group at the ζ position of the Tyr-3 residue, would be a suitable precursor for these transformations. However, because of the very small amount (0.000 025% of the dry roots of *Rubia cordifolia*) of compound **4** in the plant, an alternative access route from the more available RA-VII **1** or RA-V **5** (total ~0.01%) had to be devised. We first examined the selective O-demethylation at the Tyr-3 residue of RA-VII **1** under various conditions. However, the use of Lewis acid reagents (*e.g.* $AlCl_3$, BCl_3, BBr_3 or BI_3) under selected conditions gave mainly RA-V **5**, and an excess of these reagents resulted in di-O-demethylated product **6**. The addition of nucleophiles such as ethanethiol did not yield favourable selectivity. Other O-demethylating reagents such as iodotrimethylsilane resulted in decomposition of the substrate. We next attempted the selective Tyr-6 O-methylation of the di-*O*-nor derivative **6** which was effectively obtained after slightly modifying previous conditions[9] (7 mole equivalents of boron tribromide in dichlorometh-

ane) in 74% yield. Initial attempts using iodomethane or dimethyl sulfate with potassium carbonate or sodium hydride under various conditions yielded little or non-selective O-methylation. However, we found that diazo(trimethylsilyl)methane under modified Shioiri conditions[13] afforded the desired product RA-II **4** predominantly, with optimised conditions. The best result was obtained when compound **6** was treated with 1.7 mol equiv. of diazo(trimethylsilyl)methane in the presence of 4-(dimethylamino)pyridine (DMAP) (1.7 mol equiv.) in acetonitrile–methanol (10:1) at room temperature for 21 h which produced RA-II **4**, RA-VII **1** and RA-V **5** in yields of 68, 19 and 4%, respectively.† Although this selectivity of O-methylation is rather unexpected, the reaction appears to have some generalities. When equimolar amounts of 4-methylphenol **7** and 2-methoxy-4-methylphenol **8** were treated with 1.7 mol equiv. of diazo(trimethylsilyl)methane under the same conditions, yields of 38% for 4-methylanisole **9** and 60% of 3,4-dimethoxytoluene **10** were obtained, and 51% of substrate **7** and 31% of substrate **8** was recovered. The more pronounced selectivity observed for compound **6** might be attributed to the strained 14-membered ring structure which deforms the Tyr-6 aromatic ring and would enhance the reactivity of the hydroxy group attached to this ring.

Introduction of the alkyl group was conducted through cross-coupling reactions[14] of various alkylstannanes with RA-II *O*-triflate **11** which was prepared by the reaction of RA-II **4** with *N*-phenyltrifluoromethanesulfonimide in quantitative yield. Compound **11** was first treated with tetramethyltin in the presence of dichlorobis(triphenylphosphine)palladium(II) and lithium chloride to produce the methyl analogue **12** in 82% yield. However, attempted direct introduction of an ethyl group using tetraethyltin under the same conditions was unsuccessful. Thus, the more reactive tributylvinyltin was used to produce the vinyl analogue **13** (87%), which was hydrogenated over palladium on carbon to furnish the ethyl analogue **14** in 92% yield. Similarly, the allyl analogue **15** was prepared (78%) from compound **11** using allyltributyltin, and was then converted into the propyl analogue **16** in 82% yield.

Cytotoxicity and *in vivo* antitumour activity of the prepared compounds including resynthesized analogue **3** for comparison were evaluated using P-388 leukaemia cells, and the results are summarised in Table 1. The prepared alkyl analogues **12–16** are less cytotoxic than RA-VII **1**, but more than ten times as toxic as the unsubstituted analogue **3**. *in vivo* Antitumour activity (P-388 cells) almost parallels the cytotoxicity. Although all alkyl analogues **12–16** showed antitumour activity, none of them exceeded the activity of RA-VII **1**. The unsubstituted analogue **3** showed no activity. These results suggest that the alkyl group at position ζ on the Tyr-3 residue produces a similar effect on the activity as a methoxy group, and this appendage site is responsible for the potentiation of the activity. In spite of the close structural similarities in steric bulkiness between an ethyl group and a methoxy group, the inferior activity of compound **15** compared with RA-VII **1** might be explained by the differences between their electric field potential around this region and/or different metabolic pathways *in vivo*.‡

Experimental

Organic solutions, dried over Na_2SO_4, were evaporated under an aspirator vacuum with a rotary evaporator. Medium-pressure liquid chromatography (MPLC) was performed with a Kusano C.I.G. system. Dichloromethane was distilled from calcium hydride. Mps were taken on a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a JASCO A-302 spectrophotometer. NMR spectra were measured on a Bruker AM 400 spectrometer. ^{1}H Chemical shifts are referenced in $CDCl_3$ to residual $CHCl_3$ (7.26 ppm); ^{13}C chemical shifts are referenced to the solvent ($CDCl_3$, 77.03). *J* Values are given in Hz. Mass spectra were taken using a VG AutoSpecE spectrometer.

Table 1 Cytotoxicity and *in vivo* antitumour activity of RA analogues against P-388 leukaemia

Compound	Cytotoxicity IC_{50}[a]	Antitumour activity (*t*/*c* %) Dose[b] 0.4	1.6	6.25
1[c]	0.0013	144	152	Toxic
3	0.22[c]	92	100	101
12	0.018	105	121	149
13	0.013	100	110	127
14	0.0072	108	120	151
15	0.0039	102	130	130
16	0.020	109	121	149

[a] μg cm^{-3}. [b] mg kg^{-1}. [c] Ref. 9.

O-Methylation of di-*O*-norRA-VII 6 with diazo(trimethylsilyl)-methane

To a solution of compound **6** (65.3 mg, 0.088 mmol) and DMAP (18.3 mg, 0.15 mmol) in acetonitrile–MeOH (9:1; 1 cm^3) was added a 2 mol dm^{-3} solution of diazo(trimethylsilyl)-methane in hexane (0.075 cm^3, 0.15 mmol), and the mixture was stirred at room temperature for 21 h. Acetic acid (0.5 cm^3) was added to the mixture, which was then concentrated under reduced pressure. The residue was chromatographed on a silica gel column with CH_2Cl_2–MeOH (10:1) as eluent and then separated by MPLC with CH_2Cl_2–EtOAc–MeOH (12:2:1) to give RA-VII **1** (13.2 mg, 19%), RA-V **5** (2.6 mg, 4%) and RA-II **4** (45.3 mg, 68%). Compounds **1**, **4** and **5** were confirmed by direct comparison with authentic samples.

O-Methylation of the phenols 7 and 8 using diazo(trimethylsilyl)methane

To a solution of 4-methylphenol **7** (108 mg, 1.0 mmol), 2-methoxy-4-methylphenol **8** (138 mg, 1.0 mmol) and DMAP (208 mg, 1.7 mmol) in acetonitrile–MeOH (9:1; 20 cm^3) was added a 2 mol dm^{-3} solution of diazo(trimethylsilyl)methane in hexane (0.85 cm^3, 1.7 mmol), and the mixture was stirred at room temperature for 24 h. Acetic acid (2 cm^3) was added to the mixture, which was then concentrated under slightly reduced pressure. The residue was chromatographed on silica gel (MPLC) with CH_2Cl_2–hexane (1:1) as eluent and then with hexane–CH_2Cl_2–tetrahydrofuran (THF) (15:2:1), to provide 4-methylanisole **9** (46.4 mg, 38%) and 3,4-dimethoxytoluene **10** (91.6 mg, 60%), and 51% (54.7 mg) of initial substrate **7** and 31% (43.2 mg) of initial substrate **8** were recovered. The structures of the obtained materials were confirmed by comparison of their ^{1}H NMR (400 MHz) spectra and TLC mobility with those of authentic samples.

RA-II *O*-triflate 11

A mixture of RA-II **4** (80.5 mg, 0.11 mmol), *N*-phenyltrifluoromethanesulfonimide (189.6 mg, 0.53 mmol), Et_3N (0.074 cm^3, 0.53 mmol) and CH_2Cl_2 (4 cm^3) was stirred at room temperature for 26 h. The mixture was diluted with CH_2Cl_2, washed successively with water, 1 mol dm^{-3} HCl and brine, and dried over Na_2SO_4. The solvent was evaporated off

† Although we have disclosed the predominant formation of RA-II **4** using diazomethane in a previous communication,[7] the results are inconsistent and less reproducible due to the difficulty in controlling the stoichiometry of the reagent.

‡ Although the 14-membered cycloisodityrosine moiety (Tyr-5 and Tyr-6) has been proposed to be the pharmacophore for this class of antitumour agents,[8b,15] this work suggests that the Tyr-3 residue is also very important for the activity both *in vitro* and *in vivo*.

under reduced pressure to leave a residue, which was purified using MPLC (SiO_2) with CH_2Cl_2–AcOEt–MeOH (15:2:1) as eluent to give the *triflate* **11** as an amorphous powder (94.0 mg, 99%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3425, 3040, 1680, 1640, 1510, 1425, 1270 and 1150; δ_H(400 MHz; $CDCl_3$; major conformer) 1.10 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.31 (3 H, d, *J* 7.0, D-Ala-1 β-H_3), 1.32 (3 H, d, *J* 7.0, Ala-2 β-H_3), 2.64 (1 H, dd, *J* 11.3 and 3.1, Tyr-5 β-H^a), 2.69 (3 H, s, Tyr-6 NMe), 2.92 (3 H, s, Tyr-3 NMe), 2.95 (1 H, dd, *J* 18.0 and 3.8, Tyr-6 β-H^b), 3.10 (1 H, dd, *J* 18.0 and 11.8, Tyr-6 β-H^a), 3.12 (3 H, s, Tyr-5 NMe), 3.45 (1 H, dd, *J* 13.9 and 10.6, Tyr-3 β-H^a), 3.50 (1 H, dd, *J* 13.9 and 5.0, Tyr-3 β-H^b), 3.62 (1 H, dd, *J* 10.6 and 5.0, Tyr-3 α-H), 3.67 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H^b), 3.93 (3 H, s, Tyr-6 OMe), 4.34 (1 H, d, *J* 2.0, Tyr-6 δ-H^b), 4.37 (1 H, qd, *J* 7.0 and 6.8, D-Ala-1 α-H), 4.54 (1 H, dd, *J* 11.8 and 3.8, Tyr-6 α-H), 4.75 (1 H, dq, *J* 7.6 and 6.7, Ala-4 α-H), 4.84 (1 H, dq, *J* 8.2 and 7.0, Ala-2 α-H), 5.41 (1 H, dd, *J* 11.3 and 3.1, Tyr-5 α-H), 6.45 (1 H, d, *J* 6.8, D-Ala-1 NH), 6.56 (1 H, d, *J* 8.2, Ala-2 NH), 6.57 (1 H, dd, *J* 8.4 and 2.0, Tyr-6 δ-H^a), 6.74 (1 H, d, *J* 7.6, Ala-4 NH), 6.80 (1 H, d, *J* 8.4, Tyr-6 ε-H), 6.87 (1 H, dd, *J* 8.4 and 2.4, Tyr-5 ε-H^a), 7.18–7.28 (6 H, m, Tyr-3 δ- and ε-H_2, and Tyr-5 δ-H^a and ε-H^b) and 7.41 (1 H, dd, *J* 8.4 and 2.2, Tyr-5 δ-H^b); δ_C(100 MHz; major conformer) D-Ala-1 (20.59β, 47.53α, 170.63 CO), Ala-2 (16.30β, 44.36α, 172.78 CO), Tyr-3 (33.08β, 39.69 NMe, 67.70α, 118.59 CF_3, 121.36ε, 130.97δ, 139.57γ, 148.19ζ, 167.52 CO), Ala-4 (18.25β, 46.32α, 172.15 CO), Tyr-5 (30.42 NMe, 36.79β, 54.22α, 124.08ε^a, 125.80ε^b, 130.82δ^b, 132.62δ^a, 135.05γ, 158.09ζ, 169.20 CO) and Tyr-6 (29.18 NMe, 35.37β, 56.02 OMe, 57.22α, 112.24ε^a, 113.27δ^b, 120.84δ^a, 128.00γ, 146.38ζ, 152.96ε^b, 171.41 CO); *m/z* 889 ($[M + 1]^+$, 10%) and 121 (100); HR-FAB-MS [Found: (M + H), 889.3066. $C_{41}H_{48}F_3N_6O_{11}S$ (M + H) requires *m/z*, 889.3054].

[*N*,ζ-Dimethylphenylalanine-3]RA-VII 12

To a solution of triflate **11** (74.8 mg, 0.084 mmol) in dimethylformamide (DMF) (1.2 cm^3) were added lithium chloride (36.0 mg, 0.85 mmol), dichlorobis(triphenylphosphine)palladium(II) (29.5 mg, 0.04 mmol) and tetramethyltin (0.058 cm^3, 0.42 mmol), and the mixture was stirred at 80 °C for 47 h. The mixture was diluted with CH_2Cl_2, passed through a Celite pad, washed with saturated aq. NaCl, and dried over Na_2SO_4. The solvent was evaporated off under reduced pressure to leave a residue, which was purified using MPLC (SiO_2) with CH_2Cl_2–AcOEt–MeOH (15:2:1) as eluent to give compound **12** as a crystalline powder (52.3 mg, 82%); mp > 300 °C (from MeOH); $[\alpha]_D$ −209.8 (*c* 0.49, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3390, 3320, 2940, 1670, 1620, 1500, 1445, 1410, 1265, 1210, 1130 and 1100; δ_H(400 MHz; $CDCl_3$; major conformer) 1.11 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.30 (3 H, d, *J* 6.9, D-Ala-1 β-H_3), 1.35 (3 H, d, *J* 6.9, Ala-2 β-H_3), 2.32 (3 H, s, Tyr-3 η-H_3), 2.64 (1 H, dd, *J* 11.3, 3.0, Tyr-5 β-H^a), 2.69 (3 H, s, Tyr-6 NMe), 2.84 (3 H, s, Tyr-3 NMe), 2.95 (1 H, dd, *J* 18.6 and 3.9, Tyr-6 β-H^b), 3.09 (1 H, dd, *J* 18.6 and 11.9, Tyr-6 β-H^a), 3.12 (3 H, s, Tyr-5 NMe), 3.35 (1 H, dd, *J* 12.9 and 10.8, Tyr-3 β-H^a), 3.39 (1 H, dd, *J* 12.9 and 4.9, Tyr-3 β-H^b), 3.60 (1 H dd, *J* 10.8 and 4.9, Tyr-3 α-H), 3.67 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H), 3.93 (3 H, s, Tyr-6 OMe), 4.34 (1 H, d, *J* 2.0, Tyr-6 δ-H^b), 4.36 (1 H, dq, *J* 7.1 and 6.9, D-Ala-1 α-H), 4.54 (1 H, dd, *J* 11.9 and 3.9, Tyr-6 α-H), 4.75 (1 H, dq, *J* 7.6 and 6.7, Ala-4 α-H), 4.84 (1 H, dq, *J* 8.4 and 6.9, Ala-2 α-H), 5.42 (1 H, dd, *J* 11.3 and 3.0, Tyr-5 α-H), 6.40 (1 H, d, *J* 8.4, Ala-2 NH), 6.44 (1 H, d, *J* 7.1, D-Ala-1 NH), 6.57 (1 H, dd, *J* 8.4 and 2.0, Tyr-6 δ-H^a), 6.71 (1 H, d, *J* 7.6, Ala-4 NH), 6.80 (1 H, d, *J* 8.4, Tyr-6 ε-H), 6.87 (1 H, dd, *J* 8.4 and 2.4, Tyr-5 ε-H^a), 7.01 (2 H, d-like, *J* 7.8, Tyr-3 ε- or δ-H_2), 7.09 (2 H, d-like, *J* 7.8, Tyr-3 δ- or ε-H_2), 7.20 (1 H, dd, *J* 8.4 and 2.4, Tyr-5 ε-H^b), 7.26 (1 H, dd, *J* 8.4 and 2.2, Tyr-5 δ-H^a) and 7.42 (1 H, dd, *J* 8.4 and 2.2, Tyr-5 δ-H^b); δ_C(100 MHz; $CDCl_3$; major conformer) D-Ala-1 (20.62β, 47.67α, 170.61 CO), Ala-2 (16.41β, 44.43α, 172.51 CO), Tyr-3 (20.95η, 33.00β, 39.72 NMe, 68.20α, 129.09ε, 129.20δ, 135.53ζ, 136.07γ, 168.08 CO), Ala-4 (18.36β, 46.31α, 172.19 CO), Tyr-5 (30.44 NMe, 36.85β, 54.21α, 124.11ε^a, 125.82ε^b, 130.90δ^b, 132.69δ^a, 135.13γ, 158.12ζ, 169.25 CO) and Tyr-6 (29.23 NMe, 35.43β, 56.08 OMe, 57.27α, 112.26ε^a, 113.33δ^b, 120.86δ^a, 128.12γ, 146.41ζ, 153.02ε^b, 171.65 CO); HR-FAB-MS [Found: (M + H), 755.3807. $C_{41}H_{51}N_6O_8$ (M + H) requires *m/z*, 755.3768].

[*N*-Methyl-ζ-vinylphenylalanine-3]RA-VII 13

To a solution of triflate **11** (62.3 mg, 0.070 mmol) in DMF (1.6 cm^3) were added lithium chloride (30.6 mg, 0.72 mmol), dichlorobis(triphenylphosphine)palladium(II) (49.3 mg, 0.070 mmol) and tributyl(vinyl)tin (0.154 cm^3, 0.53 mmol), and the mixture was stirred at 80 °C for 49 h. The mixture was then diluted with CH_2Cl_2, passed through a Celite pad, washed with saturated aq. NaCl, and dried over Na_2SO_4. The solvent was evaporated off under reduced pressure to leave a residue, which was purified using MPLC (SiO_2) with CH_2Cl_2–AcOEt–MeOH (15:2:1) as eluent to give compound **13** as a crystalline powder (47.0 mg, 87%); mp 257–261 °C (from MeOH); $[\alpha]_D$ −215.3 (*c* 0.71, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3390, 3310, 2940, 1670, 1625, 1510, 1445, 1410, 1265, 1210, 1135 and 1100; δ_H(400 MHz; $CDCl_3$; major conformer) 1.08 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.28 (3 H, d, *J* 6.7, D-Ala-1 β-H_3), 1.33 (3 H, d, *J* 6.8, Ala-2 β-H_3), 2.63 (1 H, dd, *J* 11.3 and 2.5, Tyr-5 β-H^a), 2.68 (3 H, d, Tyr-6 NMe), 2.85 (3 H, s, Tyr-3 NMe), 2.94 (1 H, m, Tyr-6 β-H^b), 3.07 (1 H, m, Tyr-6 β-H^a), 3.10 (3 H, s, Tyr-5 NMe), 3.31–3.43 (2 H, m, Tyr-3 β-H_2), 3.61 (1 H, dd, *J* 11.1 and 5.3, Tyr-3 α-H), 3.66 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H^b), 3.91 (3 H, s, Tyr-6 OMe), 4.32 (1 H, s-like, Tyr-6 δ-H^b), 4.39 (1 H, dq, *J* 6.7 and 6.7, D-Ala-1 α-H), 4.54 (1 H, dd, *J* 11.8 and 3.6, Tyr-6 α-H), 4.75 (1 H, dq, *J* 7.7 and 6.7, Ala-4 α-H), 4.79 (1 H, m, Ala-2 α-H), 5.21 (1 H, dd, *J* 11.0 and 1.4, Tyr-3 θ-H^a), 5.40 (1 H, dd, *J* 11.3 and 2.5, Tyr-5 α-H), 5.70 (1 H, dd, *J* 17.6 and 1.4, Tyr-3 θ-H^b), 6.49 (1 H, d, *J* 6.7, D-Ala-1 NH), 6.56 (2 H, d-like, *J* 8.3, Tyr-6 ε-H and Ala-2 NH), 6.67 (1 H, ddd, *J* 17.6, 11.0 and 1.4, Tyr-3 η-H), 6.73 (1 H, d, *J* 7.7, Ala-4 NH), 6.78 (1 H, dd, *J* 8.4 and 1.8, Tyr-6 δ-H^a), 6.85 (1 H, m, Tyr-5 ε-H^a), 7.08 (2 H, d-like, *J* 8.0, Tyr-3 δ-H_2), 7.19 (1 H, m, Tyr-5 ε-H^b), 7.24 (1 H, m, Tyr-5 δ-H^a), 7.32 (2 H, d-like, *J* 8.0, Tyr-3 ε-H_2) and 7.40 (1 H, m, Tyr-5 δ-H^b); δ_C(100 MHz, major conformer) D-Ala-1 (20.62β, 47.76α, 170.67 CO), Ala-2 (16.49β, 44.50α, 172.64 CO), Tyr-3 (33.32β, 39.80 NMe, 68.16α, 113.53θ, 126.41ε, 129.44δ, 136.07γ, 136.38η, 138.50ζ, 167.94 CO), Ala-4 (18.40β, 46.39α, 172.24 CO), Tyr-5 (30.48 NMe, 36.92β, 54.27α, 124.18ε^a, 125.87ε^b, 130.92δ^b, 132.73δ^a, 135.15γ, 158.22ζ, 169.30 CO) and Tyr-6 (29.26 NMe, 35.49β, 56.16 OMe, 57.36α, 112.40ε^a, 113.44δ^b, 120.91δ^a, 128.21γ, 146.51ζ, 153.13ε^b, 171.68 CO); HR-FAB-MS [Found: (M + H), 767.3807. $C_{42}H_{51}N_6O_8$ (M + H) requires *m/z*, 767.3768].

[ζ-Ethyl-*N*-methylphenylalanine-3]RA-VII 14

Palladium (10%) on activated carbon (61 mg) was added to a solution of the styrene **13** (60.3 mg, 0.079 mmol) in EtOH (15 cm^3), and the mixture was stirred vigorously at room temperature for 1 h under hydrogen. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (MPLC) with CH_2Cl_2–AcOEt–MeOH (12:2:1) to provide *compound* **14** as a crystalline powder (55.8 mg, 92%); mp 234–236 °C (from MeOH); $[\alpha]_D$ −208.1 (*c* 0.54, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3400, 3330, 2950, 1670, 1620, 1510, 1445, 1410, 1265, 1210, 1130 and 1100; δ_H(400 MHz; $CDCl_3$; major conformer) 1.10 (3 H, d, *J* 6.6, Ala-4 β-H_3), 1.21 (3 H, t, *J* 7.6, Tyr-3 θ-H_3), 1.30 (3 H, d, *J* 6.9, D-Ala-1 β-H_3), 1.35 (3 H, d, *J* 6.9, Ala-2 β-H_3), 2.59 (2 H, q, *J* 7.6, Tyr-3 η-H_2), 2.66 (1 H, dd, *J* 11.3 and 2.9, Tyr-5 β-H^a), 2.68 (3 H, s, Tyr-6 NMe), 2.83 (3 H, s, Tyr-3, NMe), 2.94 (1 H, dd, *J* 18.3 and 3.8, Tyr-6 β-H^b), 3.09 (1 H, dd, *J* 18.3 and 11.9, Tyr-6 β-H^a), 3.12 (3 H, s, Tyr-5 NMe), 3.34 (1 H, dd, *J* 13.8 and 10.9, Tyr-3 β-H^a), 3.39 (1 H, dd, *J* 13.8 and 4.7, Tyr-3 β-H^b),

3.60 (1 H, dd, *J* 10.9 and 4.7, Tyr-3 α-H), 3.67 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H^b), 3.92 (3 H, s, Tyr-6 OMe), 4.33 (1 H, *J* 1.8, Tyr-6 δ-H^b), 4.37 (1 H, qd, *J* 6.9 and 6.7, D-Ala-1 α-H), 4.54 (1 H, dd, *J* 11.9 and 3.8, Tyr-6 α-H), 4.75 (1 H, *J* 7.6 and 6.6, Ala-4 α-H), 4.83 (1 H, dq, *J* 8.0 and 6.9 Ala-2 α-H), 5.41 (1 H, dd, *J* 11.3 and 2.9, Tyr-5 α-H), 6.45 (1 H, d, *J* 6.7, D-Ala-1 NH), 6.52 (1 H, d, *J* 8.0 Ala-2 NH), 6.57 (1 H, dd, *J* 8.4 and 1.8, Tyr-6 δ-H^a), 6.71 (1 H, d, *J* 7.6, Ala-4 NH), 6.79 (1 H, d, *J* 8.4, Tyr-6 ε-H), 6.86 (1 H, dd, *J* 8.4 and 2.3, Tyr-5 ε-H^a), 7.04 (2 H, d-like, *J* 7.8, Tyr-3 δ- or ε-H_2), 7.11 (2 H, d-like, *J* 7.8, Tyr-3 ε- or δ-H_2), 7.20 (1 H, dd, *J* 8.4 and 2.3, Tyr-5 ε-H^b), 7.26 (1 H, dd, *J* 8.4 and 2.1, Tyr-5 δ-H^a) and 7.42 (1 H, dd, *J* 8.4 and 2.1, Tyr-5 δ-H^b); δ_C (100 MHz; $CDCl_3$; major conformer) D-Ala-1 (20.68β, 47.83α, 170.69 CO), Ala-2 (16.58β, 44.53α, 172.54 CO), Tyr-3 (15.62θ, 28.45η, 33.12β, 39.73 NMe, 68.33α, 128.08ε, 129.23δ, 135.92γ, 142.67ζ, 168.04 CO), Ala-4 (18.47β, 46.41α, 172.25 CO), Tyr-5 (30.51 NMe, 36.97β, 54.25α, 124.22ε^a, 125.90ε^b, 130.98δ^b, 132.78δ^a, 135.17γ, 158.23ζ, 169.33 CO) and Tyr-6 (29.28 NMe, 35.50β, 56.16 OMe, 57.37α, 112.32ε^a, 113.40δ^b, 120.92δ^a, 128.18γ, 146.52ζ, 153.13ε^b, 171.77 CO); HR-FAB-MS [Found: (M + H), 769.3947. $C_{42}H_{53}N_6O_8$ (M + H) requires *m*/*z*, 769.3925].

[ζ-Allyl-*N*-methylphenylalanine-3]RA-VII 15

To a solution of triflate **11** (48.3 mg, 0.054 mmol) in DMF (1 cm^3) were added lithium chloride (24.0 mg, 0.57 mmol), dichlorobis(triphenylphosphine)palladium(II) (39.0 mg, 0.056 mmol) and allyltributyltin (0.17 cm^3, 0.55 mmol), and the mixture was stirred at 80 °C for 55 h. The mixture was diluted with CH_2Cl_2, passed through a Celite pad, washed with saturated aq. NaCl, and dried over Na_2SO_4. The solvent was evaporated off under reduced pressure to leave a residue, which was purified using MPLC (SiO_2) with CH_2Cl_2–AcOEt–MeOH (12:2:1) as eluent to give *compound* **15** as a crystalline powder (33.2 mg, 78%), mp 224–227 °C (from MeOH); $[\alpha]_D$ −159.7 (*c* 0.50, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3390, 3310, 2940, 1665, 1625, 1510, 1500, 1440, 1410, 1265, 1200, 1160, 1135 and 1100; δ_H(400 MHz; $CDCl_3$; major conformer) 1.09 (3 H, d, *J* 6.9, Ala-4 β-H_3), 1.29 (3 H, d, *J* 6.9, D-Ala-1 β-H_3), 1.34 (3 H, d, *J* 6.9, Ala-2 β-H_3), 2.65 (1 H, dd, *J* 11.3 and 3.0, Tyr-5 β-H^a), 2.68 (3 H, s, Tyr-6 NMe), 2.83 (3 H, s, Tyr-3 NMe), 2.95 (1 H, dd, *J* 18.0 and 3.7, Tyr-6 β-H^b), 3.08 (1 H, dd, *J* 18.0 and 11.9, Tyr-6 β-H^a), 3.11 (3 H, s, Tyr-5 NMe), 3.30–3.41 (4 H, m, Tyr-3 β- and η-H_2), 3.60 (1 H, dd, *J* 10.8 and 4.7, Tyr-3 α-H), 3.66 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H^b), 3.92 (3 H, s, Tyr-6 OMe), 4.33 (1 H, d, *J* 1.7, Tyr-6 δ-H^b), 4.38 (1 H, qd, *J* 6.9 and 6.7, D-Ala-1 α-H), 4.54 (1 H, dd, *J* 11.9 and 3.7, Tyr-6 α-H), 4.76 (1 H, dq, *J* 7.6 and 6.9, Ala-4 α-H), 4.82 (1 H, dq, *J* 7.9 and 6.9, Ala-2 α-H), 4.99–5.07 (2 H, m, Tyr-3 ι-H_2), 5.41 (1 H, dd, *J* 11.4 and 3.0, Tyr-5 α-H), 5.94 (1 H, m, Tyr-3 θ-H), 6.44 (1 H, d, *J* 6.7, D-Ala-1 NH), 6.51 (1 H, d, *J* 7.9, Ala-2 NH), 6.57 (1 H, dd, *J* 8.4 and 1.7, Tyr-6 δ-H^a), 6.71 (1 H, d, *J* 7.6, Ala-4 NH), 6.79 (1 H, d, *J* 8.4, Tyr-6 ε-H), 6.86 (1 H, dd, *J* 8.4 and 2.3, Tyr-5 ε-H^a), 7.04 (2 H, d-like, *J* 7.8, Tyr-3 ε- or δ-H_2), 7.09 (2 H, d-like, *J* 7.8, Tyr-3 δ- or ε-H_2), 7.20 (1 H, dd, *J* 8.4 and 2.3, Tyr-5 ε-H^b), 7.25 (1 H, dd, *J* 8.4 and 2.1, Tyr-5 δ-H^a) and 7.41 (1 H, dd, *J* 8.4 and 2.1, Tyr-5 δ-H^b); δ_C (100 MHz; major conformer) D-Ala-1 (20.68β, 47.77α, 170.66 CO), Ala-2 (16.57β, 44.49α, 172.47 CO), Tyr-3 (33.12β, 39.73η and NMe, 68.26α, 115.75ι, 128.82ε, 129.32δ, 136.48γ, 137.36θ, 138.39ζ, 167.98 CO), Ala-4 (18.45β, 46.37α, 172.21 CO), Tyr-5 (30.48 NMe, 36.94β, 54.22α, 124.18ε^a, 125.86ε^b, 130.96δ^b, 132.75δ^a, 135.16γ, 158.18ζ, 169.29 CO) and Tyr-6 (29.25 NMe, 35.48β, 56.13 OMe, 57.33α, 112.29ε^a, 113.37δ^b, 120.89δ^a, 128.14γ, 146.48ζ, 153.09ε^b, 171.71 CO); HR-FAB-MS [Found: (M + H), 781.3937. $C_{43}H_{53}N_6O_8$ (M + H) requires *m*/*z*, 781.3925].

[*N*-Methyl-ζ-propylphenylalanine-3]RA-VII 16

Palladium (10%) on activated carbon (31 mg) was added to a solution of compound **15** (30.3 mg, 0.039 mmol) in EtOH (8 cm^3), and the mixture was stirred vigorously at room temperature for 1.5 h under hydrogen. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (MPLC) with CH_2Cl_2–AcOEt–MeOH (12:2:1) as eluent to provide compound **16** as a crystalline powder (25.0 mg, 82%), mp 235–238 °C (from MeOH); $[\alpha]_D$ −200.3 (*c* 0.51, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3390, 3320, 2940, 1670, 1620, 1510, 1440, 1405, 1265, 1130 and 1100; δ_H(400 MHz; $CDCl_3$; major conformer) 0.90 (3 H, t, *J* 7.3, Tyr-3 ι-H_3), 1.09 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.29 (3 H, d, *J* 6.9, D-Ala-1 β-H_3), 1.34 (3 H, d, *J* 6.9, Ala-2 β-H_3), 1.61 (2 H, m, Tyr-3 θ-H_2), 2.55 (2 H, t-like, *J* 7.5, Tyr-3 η-H_2), 2.64 (1 H, dd, *J* 11.3 and 3.0, Tyr-5 β-H^a), 2.68 (3 H, s, Tyr-6 NMe), 2.83 (3 H, s, Tyr-3 NMe), 2.95 (1 H, dd, *J* 17.8 and 3.7, Tyr-6 β-H^b), 3.08 (1 H, dd, *J* 17.8 and 12.0, Tyr-6 β-H^a), 3.11 (3 H, s, Tyr-5 NMe), 3.34 (1 H, dd, *J* 13.9 and 10.7, Tyr-3 β-H^a), 3.38 (1 H, dd, *J* 13.9 and 5.0, Tyr-3 β-H^b), 3.61 (1 H, dd, *J* 10.7 and 5.0, Tyr-3 α-H), 3.66 (1 H, dd, *J* 11.3 and 11.3, Tyr-5 β-H^b), 3.92 (3 H, s, Tyr-6 OMe), 4.34 (1 H, d, *J* 2.0, Tyr-6 δ-H^b), 4.39 (1 H, dq, *J* 6.9 and 6.9, D-Ala-1 α-H), 4.55 (1 H, dd, *J* 12.0 and 3.7, Tyr-6 α-H), 4.75 (1 H, dq, *J* 7.6 and 6.7, Ala-4 α-H), 4.82 (1 H, dq, *J* 8.1 and 6.9, Ala-2 α-H), 5.41 (1 H, dd, *J* 11.3 and 3.0, Tyr-5 α-H), 6.52 (1 H, d, *J* 6.9, D-Ala-1 NH), 6.56 (1 H, d, *J* 8.1, Ala-2 NH), 6.57 (1 H, dd, *J* 8.3 and 2.0, Tyr-6 δ-H^a), 6.72 (1 H, d, *J* 7.6, Ala-4 NH), 6.79 (1 H, d, *J* 8.3, Tyr-6 ε-H), 6.86 (1 H, dd, *J* 8.4 and 2.4, Tyr-5 ε-H^a), 7.03 (2 H, d-like, *J* 7.9, Tyr-3 ε- or δ-H_2), 7.08 (2 H, d-like, *J* 7.9, Tyr-3 δ- or ε-H_2), 7.20 (1 H, dd, *J* 8.4 and 2.4, Tyr-5 ε-H^b), 7.25 (1 H, dd, *J* 8.4 and 2.2, Tyr-5 δ-H^a) and 7.41 (1 H, dd, *J* 8.4 and 2.2, Tyr-5 δ-H^b); δ_C(100 MHz; $CDCl_3$; major conformer) D-Ala-1 (20.67β, 47.75α, 170.71 CO), Ala-2 (16.39β, 44.50α, 172.86 CO), Tyr-3 (13.69ι, 33.08β, 37.57η, 39.77 NMe, 68.37α, 128.70ε, 129.11δ, 135.79γ, 141.07ζ, 167.97 CO), Ala-4 (18.36β, 46.41α, 172.35 CO), Tyr-5 (30.51 NMe, 36.91β, 54.25α, 124.20ε^a, 125.88ε^b, 130.92δ^b, 132.77δ^a, 135.13γ, 158.21ζ, 169.30 CO) and Tyr-6 (29.29 NMe, 35.48β, 56.14 OMe, 57.33α, 112.29ε^a, 113.42δ^b, 120.91δ^a, 128.20γ, 146.49ζ, 153.08ε^b, 171.71 CO); HR-FAB-MS [Found: (M + H), 783.4090. $C_{43}H_{55}N_6O_8$ (M + H) requires *m*/*z*, 783.4081].

Cell survival by MTT assay

MTT colorimetric assay was performed in a 96-well plate.[16] The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product which can be measured spectrophotometrically. Mouse P-388 leukaemia cells (2 × 10^4 cells cm^{-3}) were inoculated in each well with 0.1 cm^3 of RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Flow Laboratories, UK), 100 units cm^{-3} of penicillin and 100 μg cm^{-3} of streptomycin. After overnight incubation (37 °C; 5% CO_2), sample solution (0.1 cm^3) was added to each well and the plates were incubated for 2 days. Then MTT (0.05 cm^3) (200 μg cm^{-3} PBS) was added to each well and the plates were incubated for a further 4 h. The resulting formazan was dissolved in Me_2SO (0.15 cm^3). The plates were placed on a plate shaker for 5 min and read immediately at 540 nm. The IC_{50} (μg cm^{-3})-value was defined as that concentration of sample which caused 50% reduction of growth in sample-treated cells, with respect to the controls. The IC_{50}-value was calculated by using the probit test.

***in vivo* Antitumour activity**

P-388 murine leukaemia cells (1 × 10^6 cells) were inoculated i.p. into female CDF_1 mice (6–7 weeks old, control *n* = 16; test *n* = 8) on day 0. Samples, suspended in 0.5% gum arabic–saline solution, were administered i.p. on days 1–5. The antitumour activity was estimated according to the NCI tumour panel screening method.[17]

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