Studies on *Rubia akane* (RA) Derivatives. Part 7.[1] Thioamide Analogues of RAs: Antitumour Cyclic Hexapeptides

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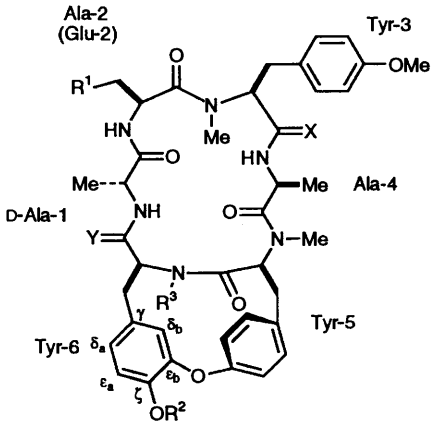
[Tyr-3-ψ(CS-NH)-Ala-4]RA-VII **3**, [Tyr-3-ψ(CS-NH)-Ala-4; Tyr-6-ψ(CS-NH)-D-Ala-1]RA-VII **4**, [Tyr-3-ψ(CS-NH)-Ala-4]RA-V **7** and [Tyr-3-ψ(CS-NH)-Ala-4]RA-X methyl ester **8** have been prepared from corresponding RAs, and they showed more promising *in vitro* antitumour activity than the parent compounds.

The cyclic hexapeptide RA-VII **1** isolated from the roots of *Rubia akane* and *R. cordifolia* (Rubiaceae) has attracted much attention because of its potent antitumour activity and its unique structure incorporating an unusual cycloisodityrosine moiety.[2] Peptide **1** is the most potent and abundant (as the result of the isolation process when *O*-methylation of the RA-V **2** present occurs) congener among RAs and is now undergoing clinical evaluation in Japan as an anticancer agent.[3] However, the amount of **1** and **2** present in the plant is small (total ~0.01% of dry roots), so the problem of supply is one of the obstacles to its widespread clinical use. A solution would be to develop a more potent alternative for **1** obtainable through a minimum of chemical manipulations. The lack of proper functional groups in **1** restricts a normal approach such as acylation or alkylation. Thus we have focused our attention on the thioamidation of the backbone peptide bond of **1**. Substitution of a thioamide for a peptide bond is considered to be an 'isosteric replacement' since the structure of the thiopeptide unit is very similar to the peptide bond. Such modifications may alter the biological profile, as is known for leucine[4] and cyclic[5] enkephalin, GHRP[6] and cyclosporin A[7] analogues, and in some cases a particular peptide bond is stabilized against proteases when it is replaced by a thiopeptide.[8]

Treatment of **1** with 2 mol equiv. of Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione][9] in dioxane at 50 °C for 72 h afforded a mono- **3** and a di-thionated product **4** in 80 and 3% yields, respectively, the structures of which were suggested by their molecular ion peaks in their mass spectra [*m/z* 786.3432 (M^+) and 803.3277 $(M + 1)^+$] and characteristic low-field resonances (δ 197.54, and 197.21 and 202.81, respectively) assignable to thiopeptides in ^{13}C NMR. Further spectroscopic analysis of **3** and **4** using a combination of 2D NMR (H–H COSY, HMBC, HMQC and NOESYPH) techniques revealed the thioamide positions and characterized them as [Tyr-3-ψ(CS-NH)-Ala-4]RA-VII and [Tyr-3-ψ(CS-NH)-Ala-4; Tyr-6-ψ(CS-NH)-D-Ala-1]RA-VII, respectively.† The positions where the thionation occurs can be explained by considering the solution conformation of **1**.[10] The carbonyl groups of the residues Tyr-3 and Tyr-6 do not participate in internal hydrogen bonding and are structurally the more reactive amides.

The *in vitro* antitumour activities of the analogues were evaluated against murine lymphotic leukaemia (P388) and human epidermoid carcinoma of the nasopharynx (KB) cells and the results are summarized in Table 1.‡ To our delight the activity of **3** was double that of **1**, and **4** retained activity. The most potent (twice as active as **1** against L1210 cells) analogue of **1** known is N^{29}-desmethyl RA-VII **5** which is only accessible by multi-step total synthesis.[11] The activity of **3** is comparable to that of **5**. [Tyr-3-ψ(CS-NH)-Ala-4]RA-V **7** and [Tyr-3-ψ(CS-NH)-Ala-4]RA-X methyl ester **8**,§ prepared in the same manner



1 $R^1 = H$, $R^2 = R^3 = Me$, X = Y = O
2 $R^1 = R^2 = H$, $R^3 = Me$, X = Y = O
3 $R^1 = H$, $R^2 = R^3 = Me$, X = S, Y = O
4 $R^1 = H$, $R^2 = R^3 = Me$, X = Y = S
5 $R^1 = R^3 = H$, $R^2 = Me$, X = Y = O
6 $R^1 = CH_2CO_2Me$, $R^2 = R^3 = Me$, X = Y = O
7 $R^1 = R^2 = H$, $R^3 = Me$, X = S, Y = O
8 $R^1 = CH_2CO_2Me$, $R^2 = R^3 = Me$, X = S, Y = O

Table 1 *In vitro* antitumour activity of RAs and their thioamide analogues against P388 and KB cells

	IC_{50} (μg cm^{-3})	
Entry	P388	KB
1	0.001 3	0.002 3
2	0.002 7	0.003 8
3	0.000 58	0.001 1
4	0.001 7	0.001 7
6	0.034	0.030
7	0.000 88	0.001 5
8	0.004 1	0.007 8

† The application of Lawesson's reagent to the thioamidation of polypeptides and cyclic peptides is generally less practical owing to the problems with the regioselectivity and yields.[5,7,17,18]

‡ The protocol for these experiments has been previously described in detail, see ref. 14.

§ The yields of **7** and **8** are 71 and 77%, respectively. In the case of **7** chloroform was used as solvent, which proved to be essential for a better result. Data for **7**: m.p. 214–216 °C (from EtOAc); $[\alpha]_D^{25}$ −211.8 (*c* 0.14, $CHCl_3$) [HR-FAB-MS; Found: (M + 1), 773.3323. Calc. for $C_{40}H_{49}N_6O_8S$ (*M* + 1), 773.3333]. Data for **8**: m.p. 196–199 °C (from EtOAc); $[\alpha]_D^{25}$ −205.2 (*c* 0.29, $CHCl_3$) [HR-FAB-MS; Found: (M + 1), 859.3759. Calc. for $C_{44}H_{55}N_6O_{10}S$ (*M* + 1), 859.3700].

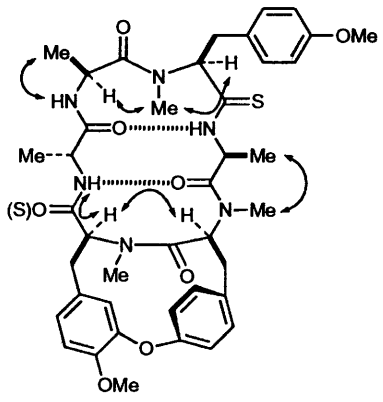


Fig. 1 Selected NOESY correlations for the major conformers of **3** and **4** in $CDCl_3$

from **2** and RA-X methyl ester **6**, a possible prodrug of water-soluble RAs,[12] also showed twofold and four- to eight-fold more potent activity than **2** and **6**, respectively. We have recently pointed out that in RAs the conformation of the 18-membered macro ring structure is important for the antitumour activity[13] and the major conformer in $CDCl_3$, characterized by the *trans* amide bond between Ala-2 and Tyr-3, is at least in part responsible for the activity.[14] The NOESYPH correlations (Fig. 1) and ^1H NMR coupling constants obtained for **3** and **4** are almost identical with those of **1**,[10] suggesting that these thionated analogues retain the bioactive conformation. The increased activity might be attributed to the more rigid bioactive conformation caused by replacement of a peptide bond with a thioamide which will enhance the hydrogen bonding between D-Ala-1 C*O* and Ala-4 N*H* and will restrict amide rotation between the Tyr-3 and the Ala-4 residues.* As thioamide bonds can be converted into other bondings such as in desoxopeptide[15] and thioimidate,[16] further backbone transformations of **1** may be possible. Since all the analogues reported here showed enhanced activity,† thioamidation of the Tyr-3 carbonyl group of RAs constitutes a ready access to more promising analogues.

Experimental

Thioamidation of RA-VII **1**.—A solution of **1** (100.8 mg, 0.13 mmol) in 1,4-dioxane (3 cm^3) was treated with Lawesson's reagent (109.1 mg, 0.27 mmol) at 50 °C for 72 h. Water (3 cm^3) was added to the solution, and the whole was stirred at room temperature for 12 h. The solution was evaporated to dryness. The residue was dissolved in dichloromethane and the solution was washed with potassium hydroxide (2 mol dm^{-3}) and water, dried, and then evaporated to dryness. Chromatography on alumina with dichloromethane–ethyl acetate–methanol (12:2:1) as the eluent yielded a mixture of **3** and **4**, which was separated by silica gel column chromatography with dichloromethane–methanol (30:1) to afford cyclic peptides **3** (82.5 mg, 80%) and **4** (2.7 mg, 3%).

3: M.p. 222–225 °C (from EtOAc); $[\alpha]_D^{25}$ −233.2 (*c* 0.15, $CHCl_3$);‡ (HR-EI-MS Found: M, 786.3432. Calc. for $C_{41}H_{50}N_6O_8S$, *M*, 786.3411); λ_{max}(EtOH)/nm 205 (log ε/dm^3 mol^{-1} cm^{-1} 4.73), 224sh (4.47) and 272 (4.15); ν_{max}(KBr)/cm^{-1} 3319, 2934, 1668, 1641 and 1515; δ_H(400 MHz; $CDCl_3$; major conformer; Me_3Si; *J*/Hz) 1.24 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.29 (3 H, d, *J* 7.0, D-Ala-1 β-H_3), 1.33 (3 H, d, *J* 6.9, Ala-2 β-H_3), 2.64 (1 H, dd, *J* 11.5, 3.3, Tyr-5 β-H_a), 2.66 (3 H, s, Tyr-6 NMe), 2.85 (3 H, s, Tyr-3 NMe), 2.97 (1 H, dd, *J* 18.0, 3.7, Tyr-6 β-H_b), 3.09 (1 H, dd, *J* 18.0, 11.9, Tyr-6 β-H_a), 3.19 (3 H, s, Tyr-5 NMe), 3.52 (1 H, dd, *J* 13.3, 10.9, Tyr-3 β-H_a), 3.64 (1 H, dd, *J* 11.4, 11.4, Tyr-5 β-H_b), 3.79 (3 H, s, Tyr-3 OMe), 3.83 (1 H, dd, *J* 10.9, 4.5, Tyr-3 α-H), 3.89 (1 H, dd, *J* 13.3, 4.5, Tyr-3 β-H_b), 3.93 (3 H, s, Tyr-6 OMe), 4.31 (1 H, d, *J* 2.1, Tyr-6 δ-H_b), 4.33 (1 H, qd, *J* 7.0, 6.9, D-Ala-1 α-H), 4.50 (1 H, dd, *J* 11.9, 3.7, Tyr-6 α-H), 4.94 (1 H, dq, *J* 9.0, 6.9, Ala-2 α-H), 5.31 (1 H, dq, *J* 7.2, 6.7, Ala-4 α-H), 5.36 (1 H, dd, *J* 11.4, 3.3, Tyr-5 α-H), 6.15 (1 H, d, *J* 9.0, Ala-2 NH), 6.35 (1 H, d, *J* 6.9, D-Ala-1 NH), 6.58 (1 H, dd, *J* 8.4, 2.1, Tyr-6 δ-H_a), 6.80 (1 H, d, *J* 8.4, Tyr-6 ε-H_a), 6.83 (2 H, d-like, *J* 8.6, Tyr-3 ε-H_2), 6.88 (1 H, dd, *J* 8.4, 2.3, Tyr-5 ε-H_a), 7.06 (2 H, d-like, *J* 8.6, Tyr-3 δ-H_2), 7.21 (1 H, dd, *J* 8.3, 2.3, Tyr-5 ε-H_b), 7.27 (1 H, dd, *J* 8.4, 2.2, Tyr-5 δ-H_a), 7.43 (1 H, dd, *J* 8.3, 2.2, Tyr-5 δ-H_b) and 8.51 (1 H, d, *J* 7.2, Ala-4 NH); δ_C(100 MHz; $CDCl_3$; major conformer; Me_4Si) D-Ala-1 (20.75β, 47.91α, 171.94 CO), Ala-2 (17.06β, 44.35α, 172.44 CO), Tyr-3 (36.13β, 40.19 NMe, 55.26 OMe, 74.24α, 114.04ε, 130.29δ, 130.74γ, 158.49ζ, 197.54 CS), Ala-4 (16.66β, 51.39α, 171.27 CO), Tyr-5 (30.50 NMe, 36.97β, 53.75α, 124.36ε_a, 125.91ε_b, 131.08δ_b, 132.89δ_a, 134.69γ, 158.32ζ, 169.55 CO) and Tyr-6 (29.14 NMe, 35.31β, 56.17 OMe, 57.53α, 112.34ε_a, 113.41δ_b, 120.80δ_a, 128.05γ, 146.50ζ, 153.15ε_b, 170.40 CO).

4: M.p. 206–208 °C (from EtOAc); $[\alpha]_D^{25}$ −129.3 (*c* 0.12, $CHCl_3$) [HR-FAB-MS Found (M + 1), 803.3277. Calc. for $C_{41}H_{51}N_6O_7S_2$ (*M* + 1), 803.3261]; λ_{max}(EtOH)/nm 205 (log ε/dm^3 mol^{-1} cm^{-1} 4.76) 225sh (4.50) and 272 (4.38); ν_{max}(KBr)/cm^{-1} 3319, 2933, 1636, 1586 and 1514; δ_H(400 MHz; $CDCl_3$; major conformer; Me_4Si; *J*/Hz) 1.26 (3 H, d, *J* 6.7, Ala-4 β-H_3), 1.33 (3 H, d, *J* 6.8, Ala-2 β-H_3), 1.37 (3 H, d, *J* 6.9, D-Ala-1 β-H_3), 2.64 (1 H, dd, *J* 11.4, 3.4, Tyr-5 β-H_a), 2.74 (3 H, s, Tyr-6 NMe), 2.86 (3 H, s, Tyr-3 NMe), 2.83 (1 H, dd, *J* 18.0, 3.3, Tyr-6 β-H_b), 3.12 (1 H, dd, *J* 18.0, 11.7, Tyr-6 β-H_a), 3.16 (3 H, s, Tyr-5 NMe), 3.57 (1 H, dd, *J* 14.7, 12.4, Tyr-3 β-H_a), 3.62 (1 H, dd, *J* 11.4, 11.4, Tyr-5 β-H_b), 3.80 (3 H, s, Tyr-3 OMe), 3.83–3.92 (2 H, m, Tyr-3 α-H, β-H_b), 3.95 (3 H, s, Tyr-6 OMe), 4.33 (1 H, d, *J* 2.1, Tyr-6 δ-H_b), 4.82 (1 H, qd, *J* 6.9, 6.7, D-Ala-1 α-H), 4.66 (1 H, dd, *J* 11.7, 3.3, Tyr-6 α-H), 5.03 (1 H, dq, *J* 9.5, 6.8, Ala-2 α-H), 5.16 (1 H, qd, *J* 6.7, 6.6, Ala-4 α-H), 5.34 (1 H, dd, *J* 11.4, 3.4, Tyr-5 α-H), 6.16 (1 H, d, *J* 9.5, Ala-2 NH), 6.56 (1 H, dd, *J* 8.4, 2.1, Tyr-6 δ-H_a), 6.81 (1 H, d, *J* 8.4, Tyr-6 ε-H_a), 6.84 (2 H, d-like, *J* 8.6, Tyr-3 ε-H_2), 6.91 (1 H, dd, *J* 8.4, 2.4, Tyr-5 ε-H_a), 7.08 (2 H, d-like, *J* 8.6, Tyr-3 δ-H_2), 7.23 (1 H, dd, *J* 8.3, 2.4, Tyr-5 ε-H_b), 7.28 (1 H, dd, *J* 8.4, 2.2, Tyr-5 δ-H_a), 7.48 (1 H, dd, *J* 8.3, 2.2 Tyr-5 δ-H_b), 7.98 (1 H, d, *J* 6.7, D-Ala-1 NH) and 8.73 (1 H, d, *J* 6.6, Ala-4 NH); δ_C(100 MHz; $CDCl_3$, major conformer) D-Ala-1 (18.07β, 53.70α, 171.35 CO), Ala-2 (17.31β, 44.45α, 172.81 CO), Tyr-3 (36.09β, 40.18 NMe, 55.30 OMe, 75.03α, 114.08ε, 130.28δ, 130.69γ, 158.56ζ, 197.21 CS), Ala-4 (16.94β, 51.57α, 171.23 CO), Tyr-5 (30.42 NMe, 37.46β, 53.82α, 124.28ε_a, 126.01ε_b, 131.34δ_b, 132.76δ_a, 134.74γ, 158.21ζ, 169.83 CO) and Tyr-6 (29.99 NMe, 38.14β, 56.22 OMe, 64.13α, 112.43ε_a, 113.53δ_b, 120.52δ_b, 128.33γ, 146.48ζ, 153.19ε_b, 202.81 CS).

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* Rotation about the thioamide bond is *ca.* 3 kcal mol^{-1} (1 cal = 4.183 J) more difficult than rotation about a peptide bond.[19]

† [Tyr-3-ψ-(CS-NH)-Ala-4; *N*-allyl-Ala-2]RA-VII, [Tyr-3-ψ(CS-NH)-Ala-4; *N*-crotyl-Ala-2]RA-VII and [Tyr-3-ψ-(CS-NH)-Ala-4; *N*-pent-2-enyl-Ala-2]RA-VII prepared in the same manner showed two- to four-fold more potent *in vitro* antitumour activity against P388 cells than the parent analogues. A preliminary *in vivo* study against P388 leukaemia in mice showed *T*/*C* = 152% for **3** at the 0.4 mg kg^{-1} day^{-1} level (*cf.* *T*/*C* = 144% for **1**).

‡ $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

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