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NOVEL WATER-SOLUBLE ANALOGUES RETAINING POTENT ANTITUMOR ACTIVITY OF RA-VII, A CYCLIC HEXAPEPTIDE FROM *RUBIA* PLANTS[1]

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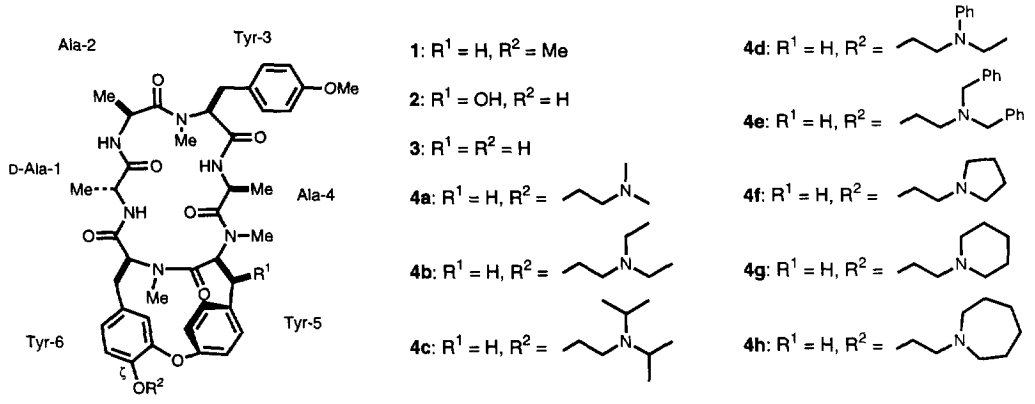
Abstract: Deoxybouvardin (**3**) was reacted with 2-dialkylaminoethyl chloride salts to produce analogues **4a–h**. All analogues retained antitumor activity against P388 leukemia in mice, and analogue **4c** showed more promising antitumor activity than RA-VII (**1**) against the P388 leukemia, B16 melanoma and colon adenocarcinoma 26 murine tumor models. The hydrochloride salt of **4c** is soluble in water.

RA-VII (**1**)[2] and bouvardin (NSC 259968, **2**)[3] are a class of bicyclic hexapeptides obtained from Rubiaceous plants, *Rubia akane* and *Bouvardia ternifolia*, respectively. These peptides showed potent antitumor activity, and their mode of action is considered to be inhibition of protein synthesis through binding to eukaryotic 80S ribosomes.[4] Although peptide **1** has already been evaluated clinically,[5] its one major drawback is low water solubility which makes its intravenous infusion difficult. Previous efforts towards producing water-soluble analogues of these peptides addressed glycosylation of the phenolic hydroxyl group of deoxybouvardin[3] (= RA-V, **3**),[6] substitution of the Ala-2 residue of **1** by aspartic acid,[7] and introduction of a 2-dialkylaminoethyl group at the Ala-2 amide nitrogen atom,[8] but none of these methods retained potent antitumor activity comparable to that of the parent peptides. The structure–activity relationship studies of *O*-alkyl and *O*-acyl analogues of peptide **3** at the ζ position of the Tyr-6 residue showed that a medium to long alkyl/acyl chain (up to nine carbon atoms) at this position is compatible with the potent antitumor activity.[9] This fact led us to synthesize analogues possessing a 2-dialkylaminoethyl group at this position. Such functionality mimics an alkyl chain in length and would enhance water solubility through acid salt formation. We describe herein the preparation and preliminary biological evaluation of these analogues.

Reaction of peptide **3** with 2-dimethylaminoethyl chloride hydrochloride (1.5 equiv.) and potassium carbonate (4 equiv.) in *N,N*-dimethylformamide at room temperature for 60 h produced analogue **4a** in 49% yield. By using various 2-dialkylaminoethyl chloride salts under the same reaction conditions, analogues **4b–h** were synthesized.[10]



Analogues **4a–h** were evaluated both *in vitro* and *in vivo* using several tumor models, and RA-VII (**1**) was also evaluated for comparison. Table 1 shows the cytotoxicity of analogues **4a–h** and compound **1**. All analogues retained fairly potent cytotoxicity against human epidermoid carcinoma of nasopharynx (KB) cells. *In vivo* antitumor activity of these analogues against P388 mouse leukemia is summarized in Table 2. P388 cells were inoculated intraperitoneally on day 0 into CDF_1 female mice (8 weeks old, n = 8), and the mice were treated with samples intraperitoneally on days 1–5. Antitumor activity was evaluated based on increase in life span. Analogues **4a** and **4c** showed significant antitumor activity, and the *T/C* values of the latter exceeded that of peptide **1** at all dose levels tested. The activity of other analogues was weak. They did not show effective (*T/C* ≥ 125%) activity at the lowest dose level tested of 0.4 mg/kg/day.

Table 1 Cytotoxicity of RA-VII (1) and Analogues 4a–h against KB, L1210 and KATO-III Cells[a]

Comp.	KB	L1210	KATO-III
1	0.0023[b]	0.0012	0.0029
4a	0.025	0.018	0.079
4b	0.010		
4c	0.0046	0.0045	0.0015
4d	0.0099		
4e	0.059		
4f	0.019		
4g	0.0087		
4h	0.0076		

[a] IC_{50}, μg/mL. [b] Reference 13.

Table 2 Antitumor Activity of RA-VII (1) and Analogues 4a–h against P388 Leukemia in Mice

		T/C (%)				
Comp.	Dose[a] /	0.4	0.8	1.6	3.13	6.25
1[b]		144	144	152	163	Toxic
4a		127		145		153
4b		116		124		136
4c		151		157		170
4d		116		133		150
4e		105		119		134
4f		112		124		134
4g		115		123		127
4h		112		123		137

[a] Dose in mg/kg given i.p. on days 1–5; n = 8. *T/C* (%), Percentage of the median survival time of treated groups (*T*) to that of controls (*C*); 125% or above considered active.
[b] Reference 13.

Since analogues **4a** and **4c** were seen from these experiments to possess promising antitumor activity, further biological tests were performed on these two compounds and peptide **1**. These peptides also showed potent cytotoxicity against L1210 leukemia and KATO-III gastric carcinoma cells (Table 1). Table 3 shows the antitumor activity of compounds **1**, **4a** and **4c** against B16 melanoma. B16 melanoma cells were inoculated subcutaneously on day 0 to BDF_1 female mice (15 weeks old, n = 6), and the mice were treated with compounds **1**, **4a** and **4c** intravenously on days 4–8. Antitumor activity was evaluated based on tumor growth inhibition. Analogues **4a** and **4c** showed activity of the *T/C* values of 40.8% and 32.1% on day 11 at doses of 6.25 and 3.13 mg/kg/day, respectively, while peptide **1** did not show the effective ($T/C \leq 42\%$) activity in this experiment.

Antitumor activity of compounds **1**, **4a** and **4c** against the colon adenocarcinoma 26 (Colon 26) tumor model is summarized in Table 4. CDF_1 female mice (6 weeks old, n = 8) implanted subcutaneously with Colon 26 cells on day 0 were treated with compounds **1**, **4a** and **4c** intravenously on days 3–7. Antitumor activity was evaluated based on tumor growth inhibition. Although compounds **1** and **4a** did not show activity, exceeding the critical *T/C* value of 42%, analogue **4c** showed effective activity of the *T/C* values of 41.5% on day 8 at a dose of 1.6 mg/kg/day and of 34.5% and 38.5% on days 8 and 10, respectively, at 3.13 mg/kg/day level.

The preliminary biological evaluation described here of the novel analogues of peptide **1** showed that analogue **4c** possesses as potent as, and even more potent antitumor activity than peptide **1**. As expected, analogue **4c** formed hydrochloride salt **4c•HCl** which is more than 250 times more soluble in water than peptide **1**.[10,11] Although the quantity of such peptides in the *Rubia* plants is small (*ca.* 0.01% of dry roots), peptides **1** and **3** are the most abundant congeners among them, and the latter is easily converted from peptide **1**.[12] Since analogue **4c** is readily accessible from peptide **3** (Y. 72–93%), the hydrochloride salt **4c•HCl** or the other salts of **4c** would be considered as a promising water-soluble alternative to peptide **1**. Further biological evaluation of analogue **4c** and its salt **4c•HCl** is in progress and will be disclosed in due course.

Table 3 Antitumor Activity of Compounds 1, 4a and 4c against B16 Melanoma in Mice

Comp.	Dose[a]	*T/C* (%) Day 8	Day 11	Day 13
1	1.6	65.8	62.4	85.1
	3.13	60.0	55.5	49.9
4a	3.13	77.2	67.9	140.5
	6.25	57.0	40.8	65.7
4c	1.6	55.9	43.3	69.7
	3.13[b]	57.5	32.1	46.2

[a] Dose in mg/kg given i.v. on days 4–8; n = 6. *T/C* (%); Percentage of the tumor volume of treated groups (*T*) to that of control groups (*C*); 42% or below considered active. [b] n = 5.

Table 4 Antitumor Activity of Compounds 1, 4a and 4c against Colon 26 Cells in Mice

Comp.	Dose[a]	*T/C* (%) Day 8	Day 10	Day 12
1	1.6	55.8	72.8	88.0
	3.13	48.0	60.9	85.6
	6.25	49.0	50.3	78.0
4a	1.6	84.1	76.7	102.5
	3.13	54.4	55.8 (7/8)[b]	101.5 (6/8)[b]
	6.25	48.1	70.0	125.5 (7/8)[b]
4c	0.8	42.1	83.4	136.3
	1.6	41.5	57.1	143.1
	3.13	34.5	38.5	99.6

[a] Dose in mg/kg given i.v. on days 3–7; n = 8. *T/C* (%); Percentage of the tumor volume of treated groups (*T*) to that of comtrol groups (*C*); 42% or below considered active. [b] Number of surviving mice/number of mice tested.

References and notes

1. Studies on *Rubia akane* (RA) derivatives. Part 11. Part 10: Hitotsuyanagi, Y.; Matsumoto, Y.; Sasaki, S.; Suzuki, J.; Takeya, K.; Yamaguchi, K.; Itokawa, H. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1749–1755.
2. (a) Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1424–1427. (b) Itokawa, H.; Takeya, K.; Mori, N.; Hamanaka, T.; Sonobe, T.: Mihara, K. *Chem. Pharm. Bull.* **1984**, *32*, 284–290.
3. (a) Jolad, S. D.; Hoffmann, J. J.; Torrance, S. J.; Wiedhopf, R. M.; Cole, J. R.; Arora, S. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040–8044. (b) Bates, R. B.; Cole, J. R.; Hoffmann, J. J.; Kriek, G. R.; Linz, G. S.; Torrance, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1343–1347.
4. (a) Zalacaín, M.; Zaera, E.; Vázquez. D.; Jiménez, A. *FEBS Lett.* **1982**, *148*, 95–97. (b) Sirdeshpande, B. V.; Toogood, P. L. *Bioorg. Chem.* **1995**, *23*, 460–470.
5. (a) Majima, H.; Tsukagoshi, S.; Furue, H.; Suminaga, M.; Sakamoto, K.; Wakabayashi, R.; Kishino, S.; Niitani, H.; Murata, A.; Genma, A.; Nukariya, N.; Uematsu, K.; Furuta, T.; Kurihara, M.; Yoshida, F.; Isomura, S.; Takemoto, T.; Hirashima, M.; Izumi, T.; Nakao, I.; Ohashi, Y.; Ito, K.; Asai, R. *Jpn. J. Cancer Chemother.* **1993**, *20*, 67–78. (b) Yoshida, F.; Asai, R.; Majima, H.; Tsukagoshi, S., Furue, H.; Suminaga, M.; Sakamoto, K.; Niitani, H.; Murata, A.; Kurihara, M.; Izumi, T.; Nakao, I.; Ohashi,Y.; Ito, K. *Jpn. J. Cancer Chemother.* **1994**, *21*, 199–207.
6. Itokawa, H. U.S. US 4,725,578, 1988; *Chem. Abstr.* **1988**, *108*, 205102e. The *O*-glucoside of deoxybouvardin has also been isolated from a natural source: Morita, H.; Yamamiya, T.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1992**, *40*, 1352–1354.
7. Itokawa, H.; Kondo, K.; Hitotsuyanagi, Y.; Isomura, M.; Takeya, K. *Chem. Pharm. Bull.* **1993**, *41*, 1402–1410.
8. Itokawa, H.; Suzuki, J.; Hitotsuyanagi, Y.; Kondo, K.; Takeya, K. *Chem. Lett.* **1993**, 695–696.
9. Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Serisawa, N.; Hamanaka, T.; Mihashi, S. *Chem. Pharm. Bull.* **1984**, *32*, 3216–3226.
10. **4a**: mp 220–222 °C, $[\alpha]^{26}_D$ –205.0° (c, 0.21, $CHCl_3$); **4b**: mp 235–236 °C, $[\alpha]^{27}_D$ –194.3° (c, 0.58, $CHCl_3$); **4c**: mp 223–226 °C (decomp.), $[\alpha]^{27}_D$ –182.9° (c, 0.19, $CHCl_3$); **4d**: mp 191–194 °C, $[\alpha]^{27}_D$ –182.7° (c, 0.24, $CHCl_3$); **4e**: mp 163–165 °C, $[\alpha]^{27}_D$ –167.7° (c, 0.34, $CHCl_3$); **4f**: mp 210–213, $[\alpha]^{27}_D$ –198.7° (c, 0.17, $CHCl_3$); **4g**: mp 239–241 °C, $[\alpha]^{28}_D$ –189.6° (c, 0.37, $CHCl_3$); **4h**: mp 226–229 °C, $[\alpha]^{27}_D$ –166.8° (c, 0.29, $CHCl_3$); **4c•HCl**: mp 218–221 °C (decomp.), $[\alpha]^{23}_D$ –209.5° (c, 0.49, H_2O).
11. Solubility of compounds **1**, **4a**, **4c** and **4c•HCl** in water at 25 °C is 0.07, 0.39, 0.23 and >19.59 mg/mL, respectively.
12. (a) Inaba, T.; Umezawa, I.; Yuasa, M.; Inoue, T.; Mihashi, S.; Itokawa, H.; Ogura, K. *J. Org. Chem.* **1987**, *52*, 2957–2958. (b) Inoue, T.; Inaba, T.; Umezawa, I.; Yuasa, M.; Itokawa, H.; Ogura, K.; Komatsu, K.; Hara, H.; Hoshino, O. *Chem. Pharm. Bull.* **1995**, *43*, 1325–1335.
13. Itokawa, H.; Kondo, K.; Hitotsuyanagi, Y.; Nakamura, A.; Morita, H.; Takeya, K. *Chem. Pharm. Bull.* **1993**, *41*, 1266–1269.

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