Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 13, pp. 1633-1636, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00206-1

Preparation and Antitumor Activity of [*N*-alkyl-Ala^2]RA-VII, Antitumor Cyclic Hexapeptide[1]

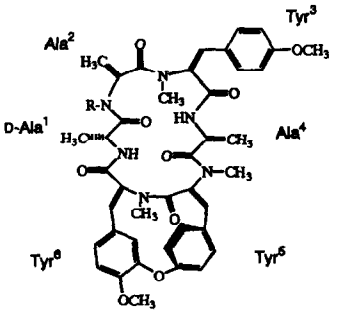
Yukio Hitotsuyanagi, Jin Suzuki, Koichi Takeya, and Hideji Itokawa*

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432-1, Hachioji 192-03, Tokyo, Japan

Abstract. A number of [*N*-alky-Ala^2]RA-VII derivatives have been prepared from RA-VII (**1**) and evaluated for *in vitro* antitumor activity against P388 and KB cells, and partly for *in vivo* anti-P388 activity. All analogues retained significant cytotoxicity, and *N*-prenyl derivative (**2j**) showed more promising *in vivo* activity than **1**.

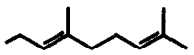
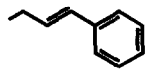
RAs, antitumor cyclic hexapeptides, have been isolated from Japanese and Chinese medicinal plants of the genuses *Rubia akane* and *R. cordifolia* (Rubiaceae), and particular interest has centered on the unique bicyclic structure and antitumor activity of them.[2] RA-VII (**1**), the most potent congener of RAs is now under clinical trial in Japan as an anticancer agent.[3] **1** is potentially promising anticancer agent, but its rather high toxicity restricted the doses and schedule for administration.[3] To find a lower toxic alternative, we have undertaken chemical modification of **1**. Derivatization of **1** has been difficult thus far due to the lack of a suitable functional structure, and only *O*-acylation or *O*-alkylation of the phenolic hydroxyl group of RA-V (Tyr^6-de-*O*-methylRA-VII) has been extensively studied.[4] Another possible part for modifications seemed to be the amide nitrogen of Ala^2, at which a methyl group could be introduced by excess amount of methyl iodide and KF-alumina.[5] Recently we found that under the phase transfer conditions the amide nitrogen is effectively alkylated with *N,N*-dialkylaminoethyl chloride without any racemization of the constituting amino acid residues.[6] By using various alkylating agent, this method is found to be applicable to prepare the corresponding *N*-alkylated derivatives. In this report we will describe the preparation and biological evaluation of a number of [*N*-alkyl-Ala^2]RA-VII derivatives.

In the presence of phase transfer catalyst (tetrabutylammonium bromide), **1** was readily alkylated with two equivalents of allyl bromide-type reagent (allyl, crotyl, 2-pentenyl, prenyl and geranyl bromide) and 50% NaOH to afford **2f-h, j** and **k** in good yields (Table. 1).[7] However in case of the methallyl derivative (**2i**), the yield was low (31%), which may be attributed to a use of methallyl chloride instead of the bromide. Such less efficacy of the chlorides was observed for the introduction of cinnamyl group; substitution of cinnamyl bromide by a chloride caused reduction of the yield from 96 to 44%. Direct introduction of saturated alkyl groups using alkyl iodides was limited to methyl (**2a**) and ethyl (**2b**) analogues; propyl,



1: R = H
2: R = alkyl

Table 1. Cytotoxicity of RA-VII (1) and its Derivatives 2a - l against P388 and KB Cells[9]

#	R	Yield (%)[a]	Cytotoxicity (IC_{50})[b] P388	KB
1 (RA-VII)	H	—	0.0013	0.0023
2a	CH_3	97	0.0012	0.0077
2b		85	0.035	0.035
2c		— (84)[c]	0.0032	0.0097
2d		— (75)[c]	0.010	0.024
2e		— (88)[c]	0.018	0.063
2f		98	0.015	0.013
2g		95	0.0076	0.018
2h		98	0.010	0.022
2i		31	0.0090	0.027
2j		98	0.0058	0.0064
2k		96	0.044	0.062
2l		96	0.0094	0.030

[a]Yields refer to the N-alkylation.

[b]μg/mL.

[c]Yields refer to the catalytic hydrogenation of the corresponding olefin analogues. See text.

Table 2. Antitumor Activity of Compounds **1**, **2g**, **2h**, **2j** and **2k** against P388 Leukemia in Mice[9]

	T/C (%)[b]						
# / dose[a]	0.4	0.8	1.6	3.13	6.25	12.5	25.0
1 (RA-VII)	144	144	152	163	toxic		
2g	112		127		155		
2h	113		125		155		
2j	123		132		160	174	
2k	119		131		148		164

[a] Dose administered i.p. on days 1 - 5 (mg/kg/day). [b] T/C (%) = (mean survival time of tested mice)/ (mean survival time of control mice) x 100.

butyl and pentyl iodide did not react at all. Thus, *N*-propyl (**2c**), *N*-butyl (**2d**) and *N*-pentyl (**2e**) derivatives were prepared by the catalytic hydrogenation (H_2, 10% Pd/C, EtOH, r.t.) of **2f**, **2g** and **2h**, respectively.[8]

In Table 1 the activities of all of the derivatives are recorded as IC_{50} values (μg/mL) against the murine lymphocytic leukemia (P388) and human epidermoid carcinoma of the nasopharynx (KB) cells, and **1** and **2a** were reevaluated for comparison. All new compounds retain cytotoxicity, and the potencies of which vary depending on the alkyl group, but clear structure-activity relationships were not observed. In the case of saturated straight chain analogues except *N*-ethyl one (**2b**), cytotoxicity against KB cell decreases as the chain lengthens (**1** > **2a** > **2c** > **2d** > **2e**), and the same tendency was also observed for the unsaturated analogues (**2f** > **2g** > **2h**). The shape of the alkyl group might influence the activity in some cases; the branched chain analogue **2j** is 2-3 times more potent than the straight chain analogue (**2h**). Interestingly, *N*-geranyl (**2k**) and *N*-cinnamyl (**2l**) derivatives retain fairly potent cytotoxicity, which suggests that such a sterically large functional group at this position is compatible with activity.

Preliminary *in vivo* evaluation was conducted for **2g**, **2h**, **2j** and **2k** by using P388 leukemia in mice (Table 2). These compounds expressed significant antitumor activity at a toxic (6.25 mg/kg/day) or a higher dose level for **1**. These data suggest that the *in vivo* activity parallel the cytotoxicity. **2j** showed more promising antitumor activity than **1** in terms of maximum T/C value, which ensures the further derivatization of **1** to be worth while.

In conclusion, alkylation of the Ala^2 amide nitrogen of **1** constitutes possible approach for the biologically promising RA analogues. Further chemical modification of **1** on this line is in progress in our laboratory.

Acknowledgment. We would like to thank Dr. Shiro Nakaike and Dr. Masaharu Tamai of Taisho Pharmaceutical Co., Ltd. for the biological evaluations.

References and Notes

1. Studies on RA derivatives. 6. Part 5: Itokawa, H.; Kondo, K.; Hitotsuyanagi, Y.; Isomura, M.; Takeya, K. *Chem. Pharm. Bull.*, **1993**, *42*, 1402.

2. *Isolation:* Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Iitaka, Y. *Chem. Pharm. Bull,* **1983**, *31*, 1424; Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Mihashi, S. Hamanaka, T. *ibid.*, **1986**, *34*, 3762. *Synthesis:* a) Inaba, T.; Umezawa I.; Yuasa, M.; Inoue, T.; Mihashi, S.; Itokawa, H.; Ogura, K. *J. Org. Chem.*, **1987**, *52*, 2957. b) Boger, D. L.; Yohannes, D. *J. Am. Chem. Soc*, **1991**, *113*, 1427; Boger, D. L.; Yohannes, D.; Zhou, J.; Patane, M. A. *ibid.*, **1993**, *115*, 3420.
3. 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.
4. Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Serizawa, N.; Hamanaka, T.; Mihashi, S. *Chem. Pharm. Bull.*, **1984**, *32*, 3216.
5. Itokawa, H.; Sonobe, T.;. Serizawa, N.; Saitou, S. Japan Patent Kokai, 36648 (1984); Morita, H.; Kondo, K.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H.; Tomioka, N.; Itai, A.; Iitaka, Y. *Tetrahedron*, **1991**, *47*, 2757.
6. Itokawa, H.; Suzuki, J.; Hitotsuyanagi, Y.; Kondo, K.; Takeya, K. *Chemistry Lett.*, **1993**, 695.
7 A typical procedure for the alkylation is described for the preparation of **2h**: To a solution of **1** (200.5 mg, 0.260 mmol), 1-bromo-2-pentene (65 μL, 0.55 mmol) and tetrabutylammonium bromide (42.3 mg, 0.131 mmol) in CH_2Cl_2 (8 mL) was added 50% aqueous NaOH (2 mL). After vigorous stirring for 48 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed successively with H_2O (1 x 5 mL), 1 N HCl (1 x 7 mL) and brine (1 x 8 mL), and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2-EtOAc-MeOH, 12:2:1) to afford **2h** (214.2 mg, 98%) as a colorless powder.
8. All new compounds were characterized by ^1H, ^{13}C NMR and High-resolution mass spectra. Melting points (from MeOH) and optical rotations (in $CHCl_3$) for new compounds: **2b**: mp 196-197 °C, $[\alpha]^{25}_D$ -142.9° (*c* 0.11); **2c**: mp >300 °C, $[\alpha]^{25}_D$ -164.4° (*c* 0.13); **2d**: mp 286-287 °C, $[\alpha]^{25}_D$ -152.8° (*c* 0.10); **2e**: mp 197-198 °C, $[\alpha]^{25}_D$ -157.0° (*c* 0.12); **2f**: mp 289-290 °C, $[\alpha]^{25}_D$ -185.3° (*c* 0.14); **2g**: mp 194-197 °C, $[\alpha]^{25}_D$ -171.3° (*c* 0.13); **2h**: mp 209-211 °C, $[\alpha]^{25}_D$ -171.6° (*c* 0.16); **2i**: mp 206-209 °C, $[\alpha]^{25}_D$ -178.3° (*c* 0.14); **2j**: mp 263-264 °C, $[\alpha]^{25}_D$ -157.6° (*c* 0.19); **2k**: mp 159-161 °C, $[\alpha]^{25}_D$ -174.9° (*c* 0.14); **2l**: mp 167-169 °C, $[\alpha]^{25}_D$ -149.2° (*c* 0.13).
9. Descriptions of the protocol for these experiments have been previously described in detail. See, reference 1 of this letter.

(*Received in USA* 16 *February* 1994; *accepted* 24 *May* 1994)