13. Tumor Promoting Activities of New Synthetic Analogues of Teleocidin^{†)}

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The tumor promoter, teleocidin is a mixture of two teleocidin A-isomers and four teleocidin B-isomers (Fujiki and Sugimura, 1983). All six isomers bind in a similar way to the phorbol ester receptor in a particulate fraction of mouse cell membranes and have the same specific activities for inducing several biological activities. All but one of these isomers, which was not obtained in sufficient quantity, were recently shown to be potent tumor promoters in mouse skin (Fujiki *et al.*, 1984a).

The active teleocidin isomers all contain the structure, (–)-indolactam-V, the name of which is based on its constituents, L-tryptophan with the lactam ring structure and L-valine (Fig. 1). Among synthetic (±)-indolactam-Vs and (±)-*epi*-indolactam-Vs, (Endo *et al.*, 1984), only (–)-indolactam-V bound to the phorbol ester receptor of cell membranes and induced both ornithine decarboxylase activity (ODC) in mouse skin and adhesion of HL-60 cells (Fujiki *et al.*, 1984b). Since (+)-indolactam-V is biologically inactive, the racemic mixture of (±)-indolactam-Vs was found to be slightly less active than (–)-indolactam-V (Table I).

On the basis of the basic common structure of (–)-indolactam-V, racemic mixtures of N-prenyl-(±)-indolactam-Vs and of N-geranyl-(±)-indolactam-Vs were synthesized (Fig. 1). This paper reports the effects of new synthetic analogues of teleocidin, such as (±)-indolactam-Vs, N-prenyl-(±)-indolactam-Vs and N-geranyl-(±)-indolactam-Vs in comparison with those of teleocidin A. (±)-Indolactam-Vs had the weakest activity of these four compounds in tests on induction of ODC, adhesion of HL-60 cells and inhibition of specific binding of ³H-12-O-tetradecanoylphorbol-13-acetate (TPA) to a mouse particulate fraction (Table I). N-Geranyl-(±)-indolactam-Vs was more effective than N-prenyl-(±)-indolactam-Vs. The results indicate that a large hydrophobic domain attached to the (–)-indolactam-V molecule is important for induction of biological activities. However, there was a big difference in the biological activities of N-geranyl-(±)-indolactam-Vs and teleocidin A, although the hydrophobic domains

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(-)-Indolactam-V

N-Prenyl-(±)-indolactam-Vs

Teleocidin A

N-Geranyl-(±)-indolactam-Vs

Fig. 1. Structures of new synthetic analogues of teleocidin, (-)-indolactam-V, N-prenyl-(±)-indolactam-Vs and N-geranyl-(±)-indolactam-Vs, and of teleocidin A.

Table I. Effects of new synthetic analogues of teleocidin and teleocidin A

	Irritation	Induction of ODC	Adhesion of HL-60 cells	Inhibition of specific binding of ^3H -TPA	Tumor incidence in week 30
	1 μg	nmol CO_2/mg protein/5.0 μg compound	ED_{50} ng/ml	ED_{50} nM	(%)
(-)-Indolactam-V	—	0.43	28	1,100	29
(±)-Indolactam-Vs ((±)-IL-Vs)	—	0.46	640	3,600	20
N-Prenyl-(±)-IL-Vs	—	0.41	100	850	—
N-Geranyl-(±)-IL-Vs	+	1.06	52	280	20
Teleocidin A	###	3.65	1.4	7.5	87

in the geranyl group and in the monoterpenoid chain of teleocidin A are almost the same.

The tumor promoting activity of N-geranyl-(±)-indolactam-Vs in mouse skin

was determined by our experimental procedure (Fujiki *et al.*, 1981). The skin of the back of mice was treated with a single dose of 100 μg of 7,12-dimethylbenz(a)anthracene (DMBA) and from one week later with 5.0 μg of N-geranyl-($\pm$)-indolactam-Vs twice a week until week 30.

Previously, we had difficulty in obtaining sufficient synthetic (–)-indolactam-V, and so in a preliminary experiment only 5 mice were treated with DMBA and then 20 μg of ($\pm$)-indolactam-Vs. Of these 5 mice, one was found to have a tumor in week 36 (Fujiki *et al.*, 1984a). Recently, a method was developed for obtaining large quantities of (–)-indolactam-V from the culture broth of *Streptovercillium blastmyceticum* as a possible precursor of teleocidin B (Irie *et al.*, 1984). Therefore, we could test the tumor promoting activity of (–)-indolactam-V, not its racemic mixture. In this experiment, we used 12.5 μg of (–)-indolactam-V per application, because this amount was almost equivalent to 5.0 μg of N-geranyl-($\pm$)-indolactam-Vs in terms of potency of ODC induction in mouse skin. The percentages of tumor bearing mice in the groups treated with DMBA plus N-geranyl-($\pm$)-indolactam-Vs and DMBA plus (–)-indolactam-V were 20% and 29%, respectively, in week 30 (Table I). Since in groups treated with DMBA plus 2.5 μg of teleocidin A tumors developed in 80 to 90% of the mice, the tumor promoting activities of the two synthetic analogues of teleocidin were weak and compatible with the low potency of the above short-term tests.

The finding that the natural product, teleocidin A is a much stronger tumor promoter than N-geranyl-($\pm$)-indolactam-Vs, makes us interested in investigating the tumor promoting activity of the other new compound, the geranyl group of which attaches to C-7 of (–)-indolactam-V. The present approach using synthetic analogues in combination with organic chemistry, seems valuable in studies on the key to tumor promotion and prevention of tumor promotion.

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