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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF FLUORINE-SUBSTITUTED (-)-INDOLACTAM-V, THE CORE STRUCTURE OF TUMOR PROMOTER TELEOCIDINS

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Abstract: To investigate the conformation-activity relationship, new indolactam derivatives with a fluorine atom at position 5 or 6 were synthesized. (-)-5-Fluoroindolactam-V (2) existing mainly as the sofa conformer in solution was far less biologically active than (-)-6-fluoroindolactam-V (3) existing mainly as the twist conformer. This suggests that the sofa conformation of (-)-indolactam-V (1) is not a biologically active form.

Teleocidins (for example, teleocidin A-1 and teleocidin B-4)[1-3] are potent skin tumor promoters[4] produced by actinomycetes. Teleocidins and their core structure (-)-indolactam-V (1)[5,6] exist as two stable conformers, a twist and a sofa form, in solution at room temperature.[7] Although recent structure-activity studies using a number of indolactam derivatives[8-13] have revealed the essential structural factors for tumor-promoting activity, the active conformation of teleocidins remains unclear. To solve this problem, conformationally restricted analogues of teleocidins are indispensable. We have previously reported that 5-substituted indolactam derivatives such as (-)-5-acetyl- and (-)-5-chloroindolactam-V exist exclusively as the sofa conformer.[14] However, the biological activities of these derivatives are expected to reflect both the conformational change and the steric effect at position 5 of the indole ring. To estimate more exactly the activity of the sofa conformer, it is necessary to minimize the steric, electronic, and hydrophobic effects of the substituent at position 5. The most promising candidate is (-)-5-fluoroindolactam-V (2). This communication describes the synthesis and biological activities of (-)-5-fluoroindolactam-V (2) along with those of (-)-6-fluoroindolactam-V (3) as a positive control.

Since most fluorinating agents are explosive, toxic, or unstable materials which require special equipment and techniques, and since the locus of electrophilic substitution reactions on (-)-indolactam-V (1) is position 7,[14] we abandoned the route of the direct fluorination of 1. We have recently shown that the microbial conversion of *seco*-compounds (*N*-methyl-L-amino acidyl-L-tryptophanol) of indolactams is a simple and convenient method to prepare various indolactam analogues.[15] Therefore, we tried to expand this microbial conversion to substituted *N*-methyl-L-valyl-L-tryptophanols; 2 and 3 were synthesized from the corresponding fluorinated DL-tryptophans using the microbial conversion as a key step.

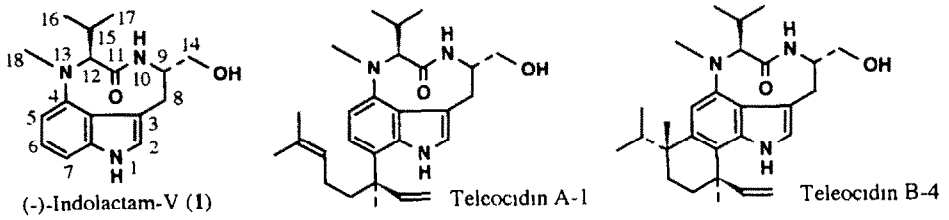
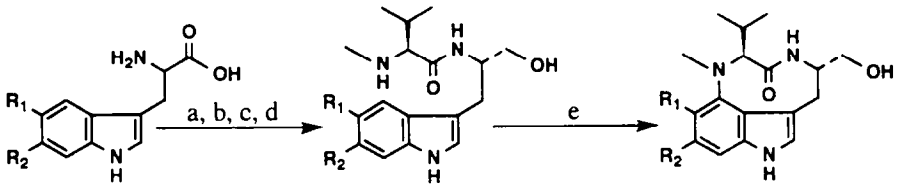


Figure 1 Naturally-occurring teleocidin-related compounds.



a) $SOCl_2$, MeOH, reflux b) $LiAlH_4$, THF, reflux c) Boc-*N*-methyl-L-valine, DCC, HONSu, THF
d) i) 1M HCl/AcOH ii) NaOH, H_2O, MeOH e) *S. blastmyceticum* NA34-17, 36hr

Figure 2 Synthesis of fluorine-substituted indolactams (**2** and **3**).

Table 1. Several characteristic signals of the ^1H NMR spectra of (-)-indolactam-V and its fluorine-substituted analogues in $CDCl_3$ (400MHz)

Position	δ (multiplicity, *J* in Hz)			
	(-)-Indolactam-V (**1**)		(-)-5-Fluoroindolactam-V (**2**)	(-)-6-Fluoroindolactam-V (**3**)
	twist conformer[a]	sofa conformer[a]	sofa conformer[b]	twist conformer[c]
1	8.03 (br.s)	8.35 (br.s)	8.27 (br.s)	7.98 (br.s)
10	7.36 (br.s)	4.74 (d, *J*=11.6)	4.72 (d, *J*=11.0)	7.18 (br.s)
12	4.40 (d, *J*=10.1)	2.99 (d, *J*=11.0)	2.90 (d, *J*=11.0)	4.40 (d, *J*=10.4)
14	3.57 (dd, *J*=11.3, 8.5)	3.41 (dd, *J*=11.0, 7.0)	3.43 (m)	3.57 (dd, *J*=11.3, 8.5)
	3.74 (dd, *J*=11.3, 3.7)	3.46 (dd, *J*=11.0, 6.4)		3.75 (dd, *J*=11.3, 3.7)
15	2.60 (m)	2.40 (m)	2.46 (m)	2.59 (m)
16	0.63# (d, *J*=6.7)	0.94+ (d, *J*=6.4)	0.94§ (d, *J*=6.7)	0.64* (d, *J*=6.7)
17	0.93# (d, *J*=6.4)	1.25+ (d, *J*=6.4)	1.26§ (dd, *J*=6.7, 3.4)	0.93* (d, *J*=6.4)
18	2.92 (s)	2.75 (s)	2.76 (s)	2.88 (s)

[a]sofa:twist = 1:4.5 (0.033M, 27°C). [b]sofa > 95% (0.013M, 27°C). [c]sofa:twist = 1:15 (0.015M, 27°C).
[d]Assignments bearing the same symbol may be reversed.

The *seco*-compounds, 5-fluoro- or 6-fluoro-*N*-methyl-L-valyl-L-tryptophanol (**4**, **5**),[16] were synthesized from Boc-*N*-methyl-L-valine and DL-5- or DL-6-fluorotryptophan by the method reported previously.[15] Compounds **4** and **5** were then converted to **2** and **3**[17] at 6.7% and 8.1% yield, respectively, by *Streptoverticillium blastmyceticum* NA34-17 (Fig. 2).[6,18] This is the first report of synthesis of 6-substituted indolactams. Since the orientation of the electrophilic substitution reaction on **1** is position 7,[14] this microbial conversion would be a simple and convenient method to prepare a series of 6-substituted indolactams; in fact, we have also succeeded to synthesize (-)-6-methylindolactam-V by a similar method (data not shown). The ^1H NMR spectra indicated that **2** and **3** existed mainly as the sofa and the twist conformer, respectively (Table 1). This was determined by several characteristic signals of the two conformers.[7] The sign of $[\alpha]_D$ of **2** was opposite to that of **3**,[17] indicating the existence of a large difference in the tertiary structure between them.

Table 2. Biological activities of (-)-indolactam-V and fluorine-substituted analogues

Compound	Inhibition of specific [^{3}H]TPA binding[a]	Incorporation of $^{32}P_i$ into phospholipids of HeLa cells[b] relative cpm/mg of protein				
	ID_{50} (log1/M)	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	$10^{-4.5}$M
DMSO (control)[c]	———			1.00 (0.06)[d]		
(-)-Indolactam-V (1)	5.97 (0.10)	1.04 (0.05)	1.36 (0.22)	4.69 (0.49)	7.82 (0.37)	NT[e]
(-)-5-Fluoro-indolactam-V (2)	4.41 (0.14)	NT	1.29 (0.07)	1.28 (0.06)	3.57 (0.33)	5.32 (0.38)
(-)-6-Fluoro-indolactam-V (3)	5.85 (0.01)	1.18 (0.13)	1.78 (0.01)	7.43 (0.20)	6.87 (0.00)	NT

[a]This inhibition assay was carried out by the cold acetone filter method.[19] [b]This assay was done by the method reported previously[20] with slight modifications.[13] [c]0.2% DMSO. [d]Standard deviation. [e]Not tested.

The tumor-promoting activity of these compounds was examined by two *in vitro* bioassays: binding affinity to the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) receptor in the mouse epidermal particulate fraction and stimulation of the incorporation of radioactive inorganic phosphate ($^{32}P_i$) into phospholipids of HeLa cells. Table 2 summarizes the results of these assays. The binding affinity of (-)-5-fluoroindolactam-V was about 1/40 of that of (-)-indolactam-V and about 1/30 of that of (-)-6-fluoroindolactam-V. (-)-Indolactam-V had a maximum increase of $^{32}P_i$ incorporation at 10^{-5}M and (-)-6-fluoroindolactam-V at 10^{-6}M. By contrast, (-)-5-fluoro-indolactam-V did not maximally increase the $^{32}P_i$ incorporation even at $10^{-4.5}$M. These two biological activities correlated very well for each derivative.

The sofa form has been hypothesized to be very close to the active conformation of teleocidins from the results of the superposition of teleocidins and phorbol esters[21] and molecular dynamics calculations.[22] However, our present findings suggest that the sofa conformation of (-)-indolactam-V is far from the biologically active form because (-)-5-fluoroindolactam-V existing mainly as the sofa form was biologically nearly inactive. A similar conclusion has been recently reported by Kozikowski *et al.*[23] and Ohno *et al.*[24] using newly designed benzolactams, indolactam analogues without pyrrole moiety. A high level of the activities of (-)-6-fluoroindolactam-V existing mainly as the twist form seems to indicate that the active conformation is the twist form. However, according to Kawai *et al.*[22] at least ten conformations of indolactams should be considered as candidates for the active conformation because of the low energy barrier among them. In conclusion, the present study excluded the possibility of the sofa conformer as the biologically active form. Further synthetic studies of other conformationally restricted analogues are necessary to determine the active conformation of teleocidins.

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16. 5-Fluoro-*N*-methyl-L-valyl-L-tryptophanol (**4**): $[\alpha]_D$ -17.0° (*c*=1.06, MeOH, 23°C). UV λ_{max} (MeOH) nm (ε): 287(4,200), 222.5 (16,100). ^{1}H NMR δ (CD_3OD, 0.10M) ppm: 0.85 (3H, d, *J*=7.0Hz), 0.87 (3H, d, *J*=6.7Hz), 1.78 (1H, m), 2.03 (3H, s), 2.65 (1H, d, *J*=6.4Hz), 2.85 (1H, dd, *J*=14.7, 8.2Hz), 3.03 (1H, dd, *J*=14.7, 6.1Hz), 3.57 (1H, dd, *J*=10.7, 5.5Hz), 3.61 (1H, dd, *J*=10.7, 4.9Hz), 4.28 (1H, m), 6.82 (1H, dt, *J*=9.2, 2.4Hz), 7.15 (1H, s), 7.25 (1H, dd, *J*=8.9, 4.3Hz), 7.31 (1H, dd, *J*=10.1, 2.4Hz). HR-EIMS *m/z*: 321.1832 (M^+, calcd. for $C_{17}H_{24}N_3O_2F$, 321.1852). 6-Fluoro-*N*-methyl-L-valyl-L-tryptophanol (**5**): $[\alpha]_D$ -35.6° (*c*=0.96, MeOH, 21°C). UV λ_{max} (MeOH) nm (ε): 283 (3,900), 220 (22,400). ^{1}H NMR δ (CD_3OD, 0.06M) ppm: 0.85 (3H, d, *J*=6.4Hz), 0.87 (3H, d, *J*=6.4Hz), 1.78 (1H, m), 2.04 (3H, s), 2.66 (1H, d, *J*=6.4Hz), 2.87 (1H, dd, *J*=14.7, 8.5Hz), 3.05 (1H, dd, *J*=14.7, 6.1Hz), 3.57 (1H, dd, *J*=11.0, 5.5Hz), 3.61 (1H, dd, *J*=11.0, 5.5Hz), 4.29 (1H, m), 6.78 (1H, ddd, *J*=9.8, 8.5, 2.4Hz), 6.99 (1H, dd, *J*=9.8, 2.4Hz), 7.08 (1H, s), 7.58 (1H, dd, *J*=8.5, 5.2Hz). HR-EIMS *m/z*: 321.1823 (M^+, calcd. for $C_{17}H_{24}N_3O_2F$, 321.1852).
17. (-)-5-Fluoroindolactam-V (**2**): $[\alpha]_D$ +71.7° (*c*=0.10, MeOH, 26°C). UV λ_{max} (MeOH) nm (ε): 299 (7,600), 289 (8,100), 223.5 (25,800). ^{1}H NMR δ ($CDCl_3$, 0.013M, sofa > 95%) ppm: sofa conformer; 0.94 (3H, d, *J*=6.7Hz), 1.26 (3H, dd, *J*=6.7, 3.4Hz), 2.46 (1H, m), 2.76 (3H, s), 2.82 (1H, dd, *J*=14.7, 1.5Hz), 2.90 (1H, d, *J*=11.0Hz), 3.10 (1H, dd, *J*=14.3, 4.3Hz), 3.43 (2H, m), 4.47 (1H, m), 4.72 (1H, d, *J*=11.0Hz), 7.01 (1H, dd, *J*=10.1, 8.6 Hz), 7.07 (1H, d, *J*=2.4Hz), 7.19 (1H, dd, *J*=8.6, 4.0Hz), 8.27 (1H, br.s). HR-EIMS *m/z*: 319.1693 (M^+, calcd. for $C_{17}H_{22}N_3O_2F$, 319.1696). (-)-6-Fluoroindolactam-V (**3**): $[\alpha]_D$ -386.0° (*c*=0.11, MeOH, 18°C). UV λ_{max} (MeOH) nm (ε): 292 (11,000), 227 (28,000). ^{1}H NMR δ ($CDCl_3$, 0.015M, sofa:twist = 1:15) ppm: twist conformer; 0.64 (3H, d, *J*=6.7Hz), 0.93 (3H, d, *J*=6.4Hz), 2.59 (1H, m), 2.88 (3H, s), 3.01 (1H, dd, *J*=17.4, 3.7Hz), 3.15 (1H, br.d, *J*=17.4Hz), 3.57 (1H, dd, *J*=11.3, 8.5Hz), 3.75 (1H, dd, *J*=11.3, 3.7Hz), 4.22 (1H, br.s), 4.40 (1H, d, *J*=10.4Hz), 6.28 (1H, dd, *J*=12.2, 2.1Hz), 6.57 (1H, dd, *J*=8.5, 2.1Hz), 6.86 (1H, s), 7.18 (1H, br. s), 7.98 (1H, br. s). HR-EIMS *m/z*: 319.1683 (M^+, calcd. for $C_{17}H_{22}N_3O_2F$, 319.1696).
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