ROLE OF THE HYDROPHOBIC MOIETY OF TUMOR PROMOTERS. SYNTHESIS AND ACTIVITY OF 9-ALKYLATED BENZOLACTAMS

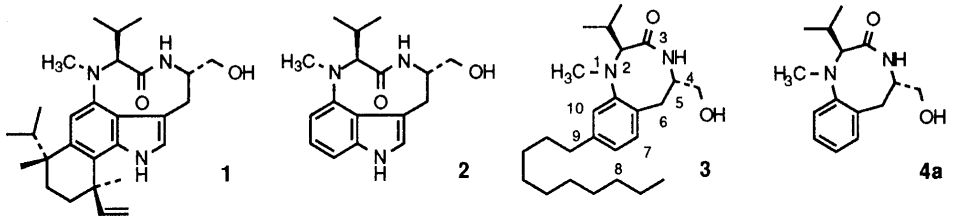
Yasuyuki ENDO,* Mitsuhiro YAMAGUCHI, Masaaki HIRANO and Koichi SHUDO

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

(-)-Benzolactam-V8-310 reproduces the active conformation and biological activity of teleocidins. Various sizes and shapes of alkyl groups on aromatic nucleus of BL-V8 were synthesized as optically pure forms. Structure-activity results indicate that hydrophobic substituents at C-9 position plays a critical role for the appearance of biological activities.

KEY WORDS tumor promoter; teleocidin; benzolactam; hydrophobic interaction; structure-activity relation

Teleocidins[1] (e.g. teleocidin B-4 (**1**)) and their active core structure (indolactam-V(IL-V, **2**))[2] are known to exist in an equilibrium between at least two conformational states, the twist and sofa forms, in solution.[3] (-)-Benzolactam-V8-310 ((-)-BL-V8-310, **3**) is a conformationally restricted, designed molecule which has a simplified linear alkyl group and a benzene ring instead of the indole ring and terpenoid side chain of teleocidins.[4] (-)-BL-V8-310 (**3**), which exists only in the twist form with cis-amide in solution, exhibits biological activity at 10^{-8} M concentration in the HL-60 cell growth inhibition assay. It is only 10 times less potent than teleocidin B-4, and is 30 times more potent than IL-V in the HL-60 assay. Therefore, we concluded that the twist form of teleocidin is the biologically active form. This finding appears to solve the conformational flexibility problem that has bedeviled structure-activity studies of teleocidins.



The relative position of the hydrogen-bonding functional groups of teleocidins required for the appearance of biological activity has been established by the above findings. Although hydrogen bonding is an important factor for *recognition* of a biologically active molecule at a receptor, hydrophobic interaction is also important for *stability* of binding to the receptor. In fact, the hydrophobic moiety on the indole ring of teleocidins plays a critical role in increasing the biological potency. The activity of **1** is 100-500 times higher than that of IL-V (**2**), which lacks the hydrophobic moiety.[5] In the case of benzolactam, the activity of BL-V8 (**4a**), which lacks the hydrophobic moiety, is 1000 times weaker than that of BL-V8-310 (**3**).[4] The benzolactams are useful compounds in the development of strategies for analyzing the mechanism of tumor promotion. Accordingly, it is important to find the optimum size of the hydrophobic moiety. We report herein the synthesis and activity of BL-V8s with alkyl groups of various size and shape on the aromatic nucleus.

Although 9-substituted BL-V8s can be synthesized in a manner similar to that used for BL-V8-310,[4] we decided to introduce hydrophobic substituents by use of the Heck reaction with 9-bromobenzolactam-V8 (**4b**) for convenience in the preparation of many derivatives. The key compound, **4b**, was prepared starting from 4-bromo-2-nitrobenzyl bromide[6] (**5**) as shown in the Chart. The benzyl bromide reacted with diethyl acetamidomalonate in DMF to afford the diester **6** (y: q). Mild alkaline hydrolysis followed by decarboxylation gave (4-bromo-2-nitro)-N-acetyl- phenylalanine ethyl ester. Acid-catalyzed removal of the acetyl group yielded an amine,[7] which was protected by the Boc group to give **7** (43%). Reduction of the ester group of **7** using $LiBH_4$ in THF (90 %), followed by reduction of the aromatic nitro group by catalytic hydrogenation (69 %), yielded the aminoalcohol **8**. Reaction of **8** with the triflate of methyl *R*-α-hydroxyisovalerate[8] gave diastereomeric esters **9** (95 %). After hydrolysis of the methyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters **10** (68%). After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **11** (31 %) and the epimer (37 %), which were isolated at this stage. The lactam **11** was methylated with CH_3I in MeOH, to give 9-bromo-BL-V8 (**4b**, 81 %).

* To whom correspondence should be addressed.

Coupling of **4b** with the terminal alkyne catalyzed by bis(triphenylphosphine)palladium(II) dichloride and copper(I) iodide in diethylamine gave **12d-12j** (70-90 %). 9-Ethynyl-BL-V8 (**12c**) was prepared by the coupling of **4b** with trimethylsilylacetylene, followed by alkaline hydrolysis. Catalytic hydrogenation of **12** yielded the 9-linear alkyl-BL-V8 (**4c-4j**). 9-Cycloalkyl-BL-V8 (**4k-4n**) was also prepared from **4b** by the similar procedure (40-80%). Coupling of **4b** with methyleneadamantane[9] catalyzed by palladium acetate(II), tris(*o*-tolyl)phosphine and triethylamine in CH_3CN, followed by hydrogenation, yielded 9-(2-adamantanemethyl)-BL-V8 (**4o**). BL-V8, having a polar functional group in the alkyl substituent, was also prepared. TBDMS-protected 9-bromo-BL-V8 (**15**) reacted with propargyl alcohol, and catalytically hydrogenated to give 9-(3-hydroxypropyl)-BL-V8 (**16**). Esterification of **16** with caproyl chloride, followed by deprotection, gave the ester **4p**. The conformational structures of the BL-V8s (**4c-4p**) were confirmed to be twist form, which has been established in the cases of **3**(**4f**) and **4a**,[4] based on the similarity in the ^{1}H-NMR spectral data and nuclear Overhauser effect (NOE) experiments.

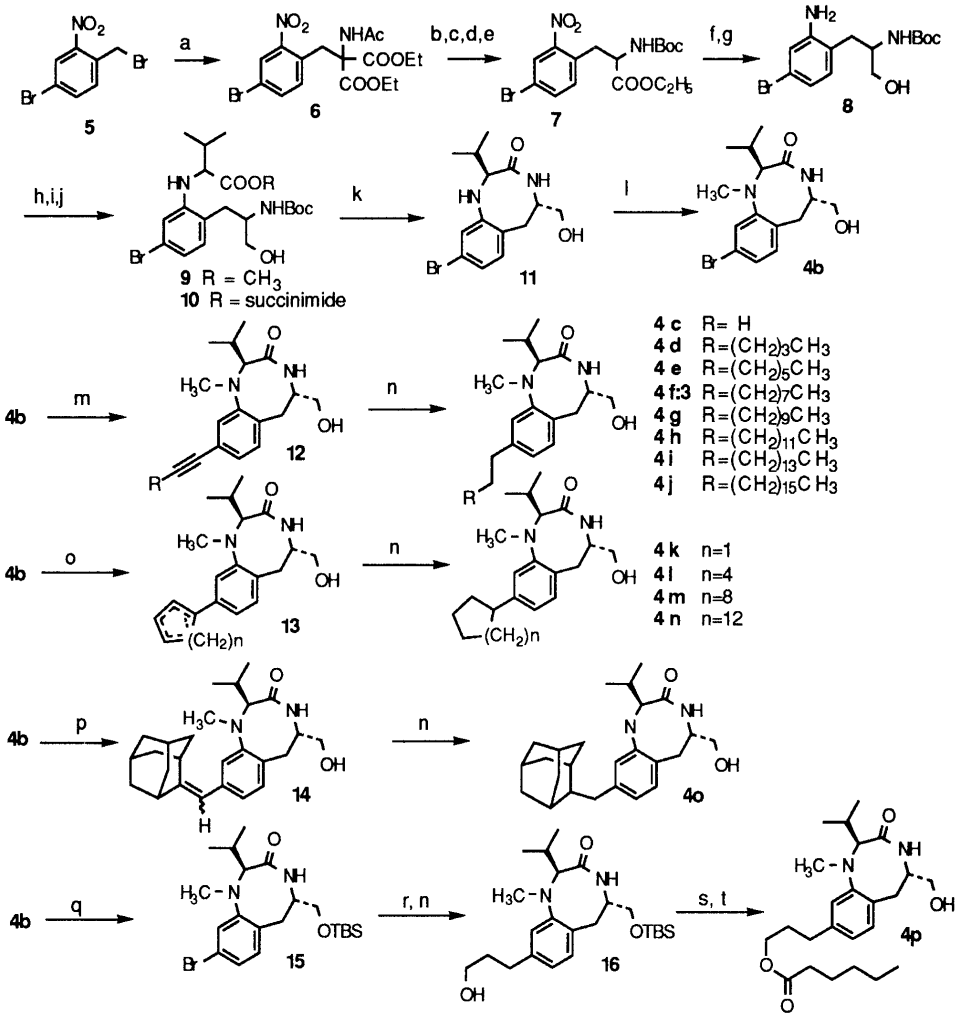


Chart. Synthesis of BL-V8s with 9-Alkyl Substituents. Key: a) $CH_3CONHCH(COOC_2H_5)_2$, NaH/ DMF b) KOH/EtOH-THF c) CH_3COOEt, heat d) HCl-EtOH e) Boc_2O/ CH_2Cl_2 f) $LiBH_4$/ THF g) H_2, PtO_2/ EtOH h) trifrate of methyl *R*-α-hydroxyisovalerate, 2,6-lutidine/ CH_2ClCH_2Cl i) KOH/ H_2O-CH_3OH j) *N*-hydroxysuccinimide, DCC/ CH_3CN k) CF_3COOH/ CH_2Cl_2 then $NaHCO_3$aq/ CH_3COOEt l) CH_3I, $NaHCO_3$/ CH_3OH m) 1-alkyne, $(PPh_3)_2PdCl_2$,CuI/ $NHEt_2$ n) H_2, Pd-C/ EtOH o) cycloalkene, $(PPh_3)_2PdCl_2$,CuI/ $NHEt_2$ p) methyleneadamantane, $Pd(OAc)_2$, P(*o*-MePh)$_3$,Et_3N/ CH_3CN q) TBDMSCl, imidazole/ DMF r) propargyl alcohol, $(PPh_3)_2PdCl_2$,CuI/ $NHEt_2$ s) caproyl chloride, pyridine/ CH_2Cl_2 t) n-$Bu_4N^+F^-$/ THF

One of the most important, sensitive and specific biological activities of the TPA-type tumor promoters is induction of growth inhibition, cell adhesion and differentiation to monocytes of human promyelocytic leukemia cells (HL-60).[10] The growth-inhibitory activity of the BL-V8s with a linear alkyl substituent at the 9-position (**4a**, **4c-4j**) is shown in Fig 1. Insertion of $(CH_2)_2$ units in the side chain systematically increased the activity, i. e., the activity increased in the order of C2 (**4c**)< C6 (**4d**)< C8 (**4e**)< C10 (**4f**). The optimum length of the linear alkyl chain was between C10 (**4f**), C12 (**4g**) and C14 (**4h**). Further introduction of $(CH_2)_2$ units (C16 (**4i**), C18 (**4j**)) caused a decrease of the activity. The same tendency has been reported in the case of the diterpene ester tumor promoters; i.e.,

Fig. 1. Growth Inhibition of HL-60 Cells by BL-V8 with Linear Alkyl Chain

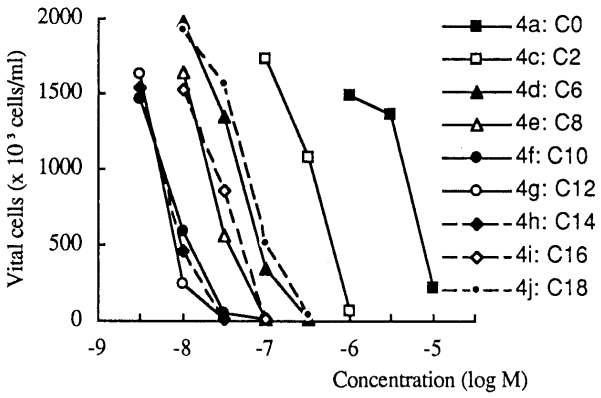
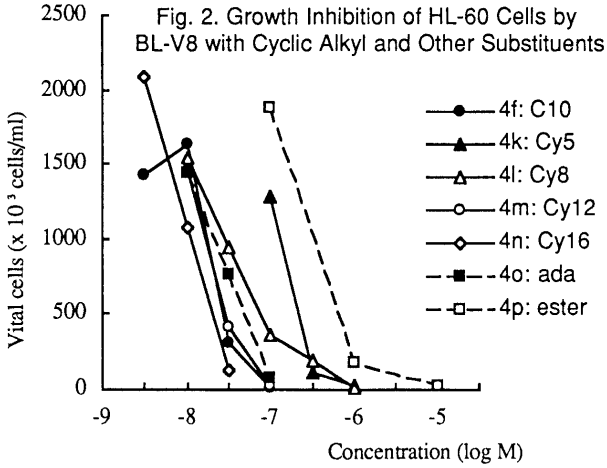


Fig. 2. Growth Inhibition of HL-60 Cells by BL-V8 with Cyclic Alkyl and Other Substituents



two-stage carcinogenesis test of 12-*O*-acylated phorbol-13-acetates on mouse skin,[11] protein kinase C binding affinity of synthetic 12-*O*-acylated-13-deacetoxy-11-demethylphorbols,[12] and irritating activity of 3-acylated ingenols on mouse ear.[13] These results indicate a common role of the hydrophobic alkyl chains of teleocidin-benzolactams and the diterpene ester tumor promoters. Figure 2 shows the activity of the BL-V8s with a cyclic alkyl substituent (**4k-4n**), a bulky substituent (**4o**) and a polar substituent (**4p**) at the 9-position. The activity increased in order of the ring size; C12 (**4m**) and C16 (**4n**) were exhibited the same activity as BL-V8-310 (**4f**). Substitution of the adamantanemethyl group (**4o**) did not decrease the activity. It seems likely that the linear alkyl substituents on BL-V8 are folded when the molecule binds to a receptor. On the other hand, insertion of the polar ester group in the alkyl chain (**4p**) significantly decreased the activity. Apparently hydrophobic interaction is important for stability of binding to a receptor. Recently, a crystallographic study revealed direct interaction of phorbol 13-acetate with protein kinase C (PKCδ) cys2 domain.[14] However, the role of the flexible hydrophobic ester chain of phorbol esters is still not clear. To approach this problem, it is of interest to study the mode of binding to PKCδ of teleocidins, which are biologically identical to phorbol esters. The present findings should be helpful in the design of further compounds as biological tools for analyzing the mechanism of tumor promotion.

REREERENCES AND NOTES

1) Fujiki H., Mori M., Nakayasu M., Terada M., Sugimura T., Moore R. E. *Proc. Natl. Acad. Sci. USA.*, **78**, 3872-3876 (1981).
2) Endo Y., Shudo K., Okamoto T., *Chem. Pharm. Bull.*, **30**, 3457-3460 (1982); Irie K., Hagiwara N., Koshimizu K., *Tetrahedron*, **43**, 5251-5260 (1987).
3) Endo Y., Shudo K., Itai A., Hasegawa M., Sakai S., *Tetrahedron*, **42**, 5905-5924 (1986).
4) Ohno M., Endo Y., Hirano M., Itai A., Shudo K., *Tetrahedron Lett.*, **34**, 8119-8122 (1993); Endo Y., Ohno M., Hirano M., Takeda M., Itai A., Shudo K., *BioMed. Chem. Lett.*, **4**, 491-494 (1994); Endo Y., Ohno M., Hirano M., Itai A., Shudo K., *J. Am. Chem. Soc.*, **118**, 1841-1855 (1996).
5) Endo Y., Sato Y., Shudo K., *Tetrahedron*, **43**, 2241-2247 (1987); Irie K., Hayashi H., Arai M., Koshimizu K., *Agric. Biol. Chem.*, **50**, 2679-2680 (1986); Irie K., Hagiwara N., Tokuda H., Koshimizu K., *Carcinogenesis*, **8**, 547-552 (1987); Webb II R. R., Venuti M. C., Eigenbrot C., *J. Org. Chem.*, **56**, 4706-4713 (1991).
6) Rinehart K. L., Kobayashi J., Harbour G. C., Gilmore J., Mascal M., Holt T. G., Shield L. S., Lafargue F. L., *J. Am. Chem. Soc.*, **109**, 3378-3387 (1987); McCord T. J., Kelley D. H., Rabon J. A., Foyt D. C., Davis A. L., *J. Heterocycl. Chem.*, **9**, 119-122 (1972).
7) The transformation of **6** to **7** by the method used for BL-V8-310 (HCl-AcOH, heat) or the method used for IL-V (KOHaq, heat then HClaq, heat) gave a complex mixture, probably due to the decomposition of the *m*-nitrobromobenzene residue
8) In the first attempt at synthesis, substitution of the *N*-methyl derivative of **8** with the triflate was very slow and the hydrolysis did not proceed effectively.
9) Kuthan J., Palecek J., Musil L., *Coll. Czech. Chem. Commun.*, **38**, 3491-3495 (1973).
10) Fujiki H, Mori M., Nakayasu M., Terada M., Sugimura T., *Biochem. Biophys. Res. Commun.*, **90**, 976-983 (1979); Huberman E., Callaham M. F., *Proc. Natl. Acad. Sci. USA.*, **76**, 1293-1297 (1979).
11) Hecker E., *Cancer Res.*, **28**, 2338-2349 (1968).
12) Sugita K., Sawada D., Sodeoka M., Sasai H., Shibasaki M., *Chem. Pharm. Bull.*, **44**, 463-465 (1996).
13) Hecker E., Adolf W., Hergenhahn M., Schmidt R., Sorg B., Cellular Interactions by Environmental Tumor Promoters, eds. Fujiki H. *et al.*, Japan Sci. Soc. Press, Tokyo/ VNU Science Press, Utrecht, 1984, pp. 3-36.
14) Zhang G., Kazanietz M. G., Blumberg P. M., Hurley J. H., *Cell*, **81**, 917-924 (1995).

(Received March 6, 1996; accepted April 1, 1996)