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# Role of the Hydrophobic Moiety of Tumor Promoters. Synthesis and Activity of 2-Alkylated Benzolactams

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**Abstract**—The size and position of a hydrophobic moiety on a benzolactam skeleton, which reproduces the active conformation and biological activity of teleocidins, play an important role in the appearance of the activity. Compounds with alkyl groups of various sizes and shapes at the 2-position of benzolactam were synthesized. Structure–activity results indicate that a hydrophobic substituent at the C-2 position plays a critical role in the appearance of biological activities, as in the case of substitution at C-9.


(−)-Benzolactam-V8-310 ((−)-BL-V8-310, **1**)[1–3] with an 8-membered lactam ring and a benzene ring instead of the 9-membered lactam and the indole ring of tumor-promoting teleocidins (e.g. teleocidin B-4, **2**)[4], reproduces the active ring conformation and biological activity of teleocidins. The hydrophobic moiety on the aromatic ring of BL-V8s, as in teleocidins,[5,6] plays a critical role in increasing the biological potency. We have reported the synthesis and biological activity of 9-alkylated BL-V8s.[7] Among the BL-V8s, substitution of a C10–C14 linear alkyl chain at the 9-position of the aromatic nucleus is optimum for the appearance of biological activity,[7] though substitution of a C8–C16 cyclic alkyl group, or even a bulky 1,2-dicarba-*closo*-dodecaboran-1-yl group at the 9-position retains almost the same activity.[8] This suggests that the hydrophobic alkyl group on BL-V8s is folded when the molecule binds to a receptor. Diterpene ester tumor promoters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA, **3**) and 3-tetradecanoylingenol (3-TI, **4**), which are biologically identical, have different skeletons with hydrophobic esters at different positions on the molecules. Thus, it seems likely that a large, oriented hydrophobic region on the molecule plays a critical role in the appearance of biological activities. We have designed and synthesized a benzolactam with a *n*-decyl group at the 2-position (BL-8-C10, **5d**), which exhibits potent binding affinity to protein kinase Cδ (PKCδ).[9] However, there is a difference between the potent $K_i$ value of **5d** toward PKC and its relatively weak $EC_{50}$ value for HL-60 differentiation, so **5d** may be useful as a probe for examination of the mechanism of the biological effects of binding of TPA-type tumor promoters to PKC. These results led us to synthesize and biologically evaluate benzolactams (BLs) bearing alkyl groups of various sizes and shapes at the 2-position (**5b**–**5l**).

Although 2-substituted BL-8s can be synthesized from racemic 2-(methylamino)phenylalaninol using optically active triflate of benzyl α-hydroxyalkanoate as a hydrophobic component, which is obtained by resolution of α-aminoalkanoic acid, in a manner similar to that used for **5d**,[9] we decided to introduce racemic hydrophobic components into (*S*)-*N*-Boc-2-(methylamino)phenylalaninol (**6a**) followed by diastereomeric separation for convenience in the preparation of many derivatives. The key compound **6a** was prepared starting from (*S*)-phenylalaninol by means of a modified procedure previously reported.[10] The hydrophobic components, the triflates of the benzyl α-hydroxyalkanoates **7**, were prepared as follows. The appropriate alkanoic acid ethyl ester **8** (Scheme 1) was converted to the silyl enol ether, which was oxidized with lead tetraacetate and treated with HF-pyridine to give the α-acetoxyalkanoic acid ethyl ester **9** (41–91%).[11] Hydrolysis of the two ester groups of **9** followed by esterification afforded benzyl α-hydroxyalkanoates **10** (72–94%). Treatment of **10** with triflic anhydride gave the triflate **7** (86–99%). Reaction of the amine **6a** with the triflate **7** gave diastereomeric esters **11** (65–83%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters **12**

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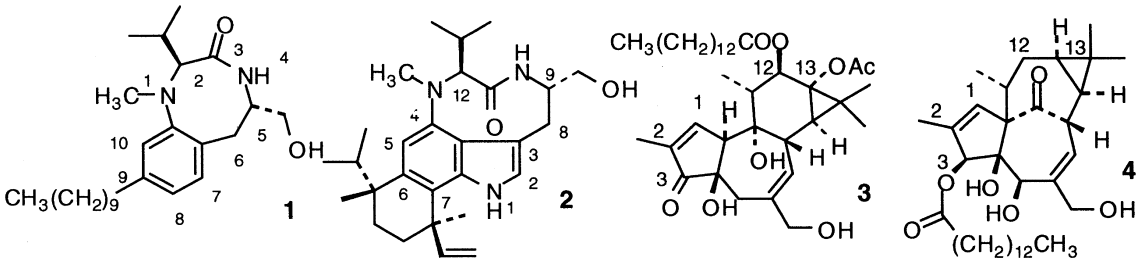
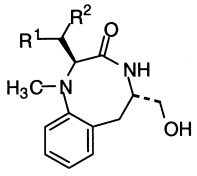
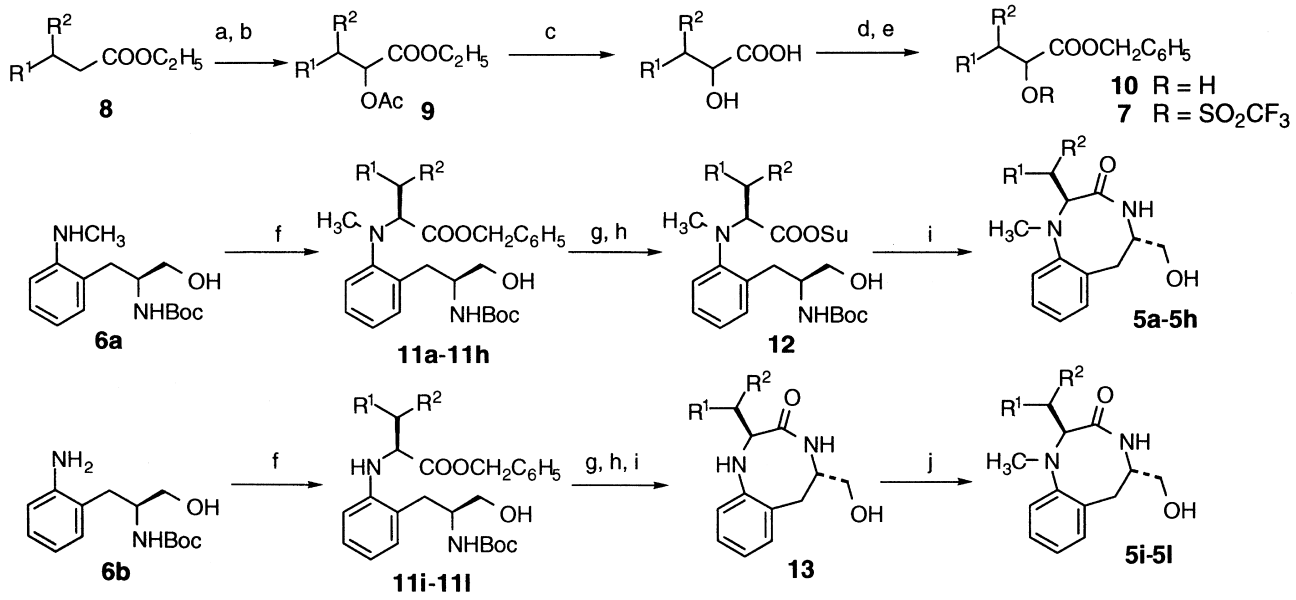




| | $R^1$ | $R^2$ |
|---|---|---|
| **5a** | $CH_3$ | $CH_3$ |
| **5b** | $CH_3(CH_2)_4$ | H |
| **5c** | $CH_3(CH_2)_6$ | H |
| **5d** | $CH_3(CH_2)_8$ | H |
| **5e** | $CH_3(CH_2)_{10}$ | H |
| **5f** | $CH_3(CH_2)_{12}$ | H |
| **5g** | $CH_3(CH_2)_{18}$ | H |
| **5h** | $CH_3(CH_2)_3$ | $CH_3(CH_2)_3$ |
| **5i** | $CH_3(CH_2)_5$ | $CH_3(CH_2)_5$ |
| **5j** | $CH_3(CH_2)_7$ | $CH_3(CH_2)_7$ |
| **5k** | $-(CH_2)_{11}-$ | |
| **5l** | $-(CH_2)_{15}-$ | |

(84–99%). After removal of the Boc group using $CF_3COOH$, cyclization was carried out under dilute conditions to give **5** (25–31%) and the epimer (19–29%), which were isolated at this stage. In the case of reaction of **6a** with the triflate of a secondary alcohol, the reaction rate was slow and decomposition of the triflate was observed. Therefore, the secondary triflate **7i–7l** was reacted with the primary amine **6b** to give diastereomeric esters **11i–11l** (38–70%). After the conversion of **11i–11l** to the activated ester (70–90%), removal of the Boc group using $CF_3COOH$ followed by ring closure gave the lactam **13** (31–44%) and the epimer (24–40%), which were isolated at this stage. The lactam **13** was methylated with $CH_3I$ in MeOH, to give **5i–5l** (24–71%). The structures of the BL-8s (**5b–5l**) were confirmed by a broad array of spectroscopic and analytical data including $^1H$ NMR, $^{13}C$ NMR, HRMS and elemental analysis. The conformational structures of **5b–5l** were confirmed to be twist form, which has been established in the cases of **1**[1] and **5d**,[9] based on the similarity in the $^1H$ NMR spectral data and nuclear Overhauser effect (NOE) experiments.

The biological activities of the BL-8s (**5b–5l**) were examined by means of two bioassays related to in vivo tumor promotion. One of them is induction of growth inhibition of human promyelocytic leukemia cells (HL-60).[12,13] The growth-inhibitory activity of the BL-8s with a linear alkyl substituent at the 2-position (**5b–5l**) is shown in Figure 1 (left). Insertion of $(CH_2)_2$ units in the alkyl chain systematically increased the activity, i.e. the activity increased in the order of C6 (**5b**) < C8 (**5c**) < C10 (**5d**) < C12 (**5e**) = C14 (**5f**). The optimum length of the linear alkyl chain was between C12 (**5e**) and C14 (**5f**). Further introduction of $(CH_2)_2$ units (C20 (**5g**)) caused a decrease of the activity. Figure 1 (right) shows the activity of the BL-8s with a secondary alkyl substituent (**5h–5j**) or a cyclic alkyl substituent (**5k, 5l**) at the 2-position. The activity tended to increase in order of length and ring size.



**Scheme 1.** Synthesis of benzolactams (**5**). Key: (a) 1. LDA/THF-toluene, 2. TMSCl; (b) 1. $Pb(OAc)_4/CH_2Cl_2$, 2. HF-pyridine; (c) $K_2CO_3/H_2O$–$CH_3OH$; (d) $C_6H_5CH_2OH$, DBU/benzene; (e) $Tf_2O$, 2,6-lutidine/$CH_2Cl_2$; (f) **7**, 2,6-lutidine/$CH_2ClCH_2Cl$; (g) $H_2$, Pd–C/EtOH; (h) *N*-hydroxysuccinimide, DCC/$CH_3CN$; (i) 1. $CF_3COOH/CH_2Cl_2$, 2. $NaHCO_3/CH_3COOEt$; (j) $CH_3I$, $NaHCO_3/CH_3OH$.

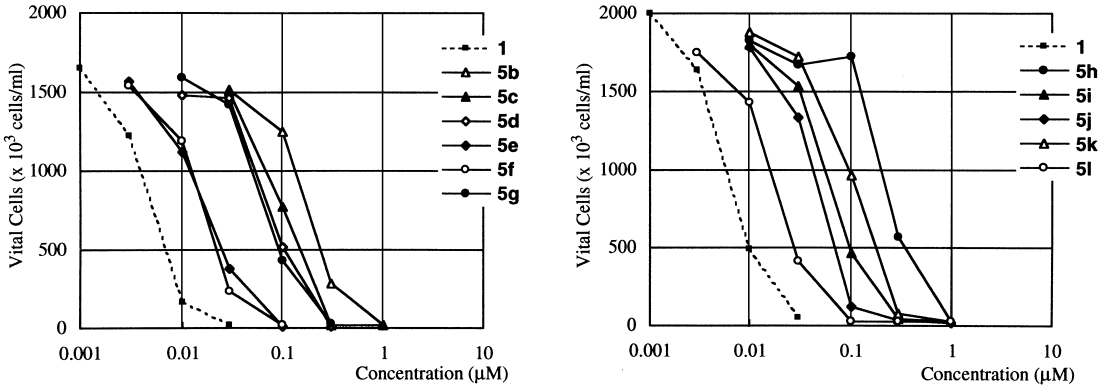



**Figure 1.** Growth inhibition of HL-60 cells. Left: for compounds **1** and **5b**–**5g**. Right: for compounds **1** and **5h**–**5l**.

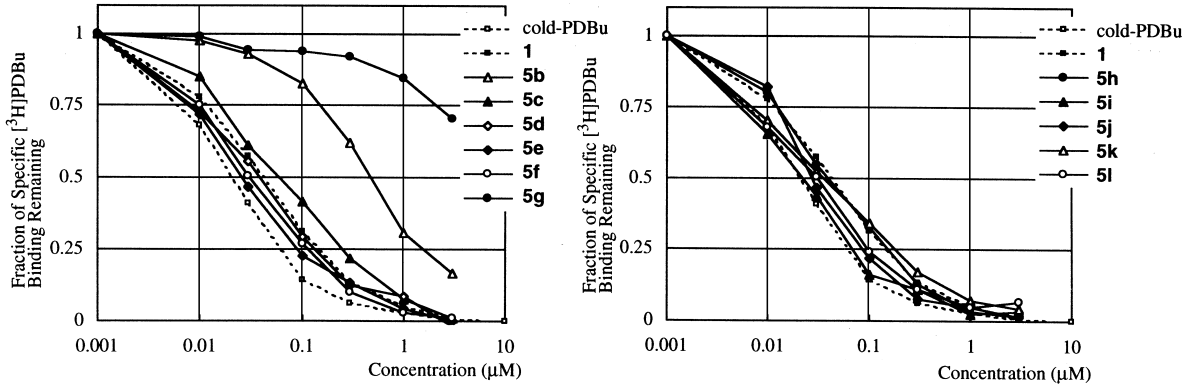



**Figure 2.** Inhibition of [$^3$H]PDBu (30 nM) binding with PKCδ. Left: for compounds **1** and **5b**–**5g**. Right: for compounds **1** and **5h**–**5l**.

Assays of inhibition of [$^3$H]PDBu binding ($K_d = 0.76$ nM) to human recombinant PKCδ (purchased from PanVera Co. Ltd.) were done as previously described.[9,14] The results for the BL-8s with a linear alkyl substituent at the 2-position (**5b**–**5g**) are shown in Figure 2 (left). Although benzolactam with a smaller alkyl group (**5b**) exhibited weak affinity to PKCδ, the compounds with a C8–C14 group (**5c**–**5f**) exhibited potent affinity comparable to that of cold PDBu. However, the introduction of a C20 alkyl chain (**5g**) caused a significant decrease of the activity. On the other hand, BL-8s with a secondary alkyl substituent (**5h**–**5j**) or a cyclic alkyl substituent (**5k**, **5l**) at the 2-position exhibited similar activity to the most active **5d**–**5f** (Fig. 2 right).

A tendency for an increase of the biological activity upon addition of an appropriate hydrophobic moiety has been observed in the case of the growth-inhibitory activity and PKCδ binding potency of the BL-V8s with a linear alkyl substituent at the 9-position.[7] These results indicate a common role of the hydrophobic alkyl chains at the 2- and 9-position of benzolactams. The same tendency has been reported in the case of diterpene ester tumor promoters; i.e. in the two-stage carcinogenesis test of 12-*O*-acylated phorbol-13-acetates on mouse skin.[15] However, the PKCδ binding potency of BL-8s (**5**) is generally higher than that of BL-V8s (**1**), and the HL-60 growth-inhibitory activity of **5** is generally lower than that of BL-V8s (**1**). We have conducted docking simulation of teleocidin-benzolactams[9] to the X-ray structure[16] of PKCδ. The hydrogen-bonding pattern of **5** would be the same as that of **1**, because of the similarity of the ligid skeletal structure. Therefore, the difference in the activities of **1** and **5** can be interpreted in terms of the difference of the hydrophobic moieties. On the other hand, the difference in PKCδ binding potency between **5d**–**5f** ($K_i = 0.8$–$2$ nM) and **5g** ($K_i > 1$ μM) is particularly striking, although this phenomenon can not be explained by docking simulation to the PKC structure. Also, the difference between the $K_i > 1$ μM of **5g** and its $ED_{50}$ of 70 nM foreffect on HL-60 differentiation suggests that target site for the HL-60 cell effect is not PKCδ. The same tendency has been reported in the case of the irritating activity of 3-acylated ingenols on mouse ear[17] and the protein kinase C binding affinity of synthetic 12-*O*-acylated-13-deacetoxy-11-demethylphorbols.[18] Modification of the hydrophobic moiety of benzolactams can afford a selective modulator. The present findings should be helpful in the design of further compounds as biological tools for analyzing the mechanism of tumor promotion.

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## References and Notes

1. Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841–1855.
2. Endo, Y.; Ohno, M.; Hirano, M.; Takeda, M.; Itai, A.; Shudo, K. *BioMed. Chem. Lett.* **1994**, *4*, 491–494.
3. Ohno, M.; Endo, Y.; Hirano, M.; Itai, A.; Shudo, K. *Tetrahedron Lett.* **1993**, *34*, 8119–8122.
4. Fujiki, H.; Mori, M.; Nakayasu, M.; Terada, M.; Sugimura, T.; Moore, R. E. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3872–3876.
5. Endo, Y.; Sato, Y.; Shudo, K. *Tetrahedron* **1987**, *43*, 2241–2247.
6. Irie, K.; Hayashi, H.; Arai, M.; Koshimizu, K. *Agric. Biol. Chem.* **1986**, *50*, 2679–2680.
7. Endo, Y.; Yamaguchi, M.; Hirano, M.; Shudo, K. *Chem. Pharm. Bull.* **1996**, *44*, 1138–1140.
8. Endo, Y.; Yoshimi, T.; Kimura, K.; Itai, A. *BioMed. Chem. Lett.* **1999**, *9*, 2561–2564.
9. Endo, Y.; Takehana, S.; Ohno, M.; Driedger, P. E.; Stabel, S.; Mizutani, M. Y.; Tomioka, N.; Itai, A.; Shudo, K. *J. Med. Chem.* **1998**, *41*, 1476–1496.
10. Kozikowski, A. P.; Wang, S.; Ma, D.; Yao, J.; Ahmad, S.; Glazer, R. I.; Bogi, K.; Acs, P.; Modarres, S.; Lewis, N. E.; Blumberg, P. M. *J. Med. Chem.* **1997**, *40*, 1316–1326.
11. Rubottom, G. M.; Gruber, J. M.; Marrero, G. R.; Juve, H. D., Jr.; Kim, C. W. *J. Org. Chem.* **1983**, *48*, 4940–4944.
12. Fujiki, H.; Mori, M.; Nakayasu, M.; Terada, M.; Sugimura, T. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 976–983.
13. Huberman, E.; Callaham, M. F. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 1293–1297.
14. Driedger, P. E.; Stabel, S.; Frith, D.; Horgan, T. J. *Receptors and Channels* **1994**, *2*, 99–106.
15. Hecker, E. *Cancer Res.* **1968**, *28*, 2338–2349.
16. Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917–924.
17. Hecker, E.; Adolf, W.; Hergenhahn, M.; Schmidt, R.; Sorg, B. *Cellular Interactions by Environmental Tumor Promoters*; Fujiki, H. et al., Eds.; Japan Sci. Soc. Press, Tokyo/VNU Science Press, Utrecht, 1984, pp. 3–36.
18. Sugita, K.; Sawada, D.; Sodeoka, M.; Sasai, H.; Shibasaki, M. *Chem. Pharm. Bull.* **1996**, *44*, 463–465.