ROLE OF THE HYDROPHOBIC MOIETY OF TUMOR PROMOTERS. SYNTHESIS AND ACTIVITY OF BENZOLACTAMS WITH ALKYL SUBSTITUENTS AT VARIOUS POSITIONS

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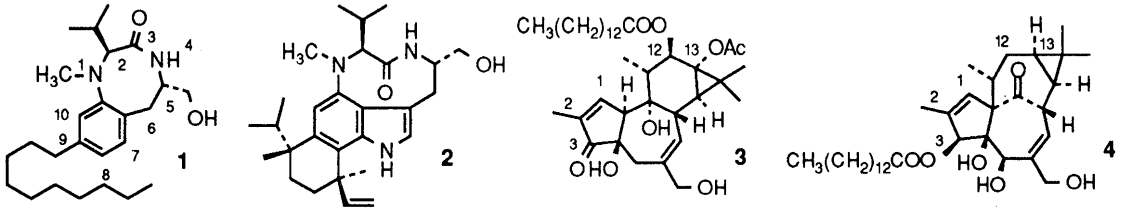
We synthesized benzolactams with hydrophobic substituents at various positions as analogs of (-)-benzolactam-V8-310 ((-)-BL-V8-310, **1**) which reproduces the active conformation and biological activity of teleocidins. Structure-activity data indicate that the existence of a hydrophobic region between C-2 and C-9, and the steric factor at C-8 play critical roles in the appearance of biological activities.

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

(-)-Benzolactam-V8-310 ((-)-BL-V8-310, **1**) 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**), reproduces the active ring conformation and biological activity of teleocidins.[1] The hydrophobic moiety on the aromatic ring of BL-V8s, as in teleocidins,[2] plays a critical role in increasing the biological potency. Recently, we have reported the synthesis and biological activity of 9-alkylated BL-V8s.[3] 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, though substitution of a C8-C16 cyclic alkyl group, or even a bulky adamantanemethyl group at the 9-position retains almost the same activity. 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.[4] We report herein the synthesis and activity of benzolactams (BLs) with alkyl groups at various positions on the molecules.



For the purpose of characterizing the hydrophobic region, (-)-BL-8-C10 (**5**) with a decyl group at the 2-position, (-)-BL-V8-210 (**6**) with a decyl group at the 8-position and (-)-BL-V8-23TM (**7**) were designed. The designed BLs were synthesized as shown in the Chart. In the synthesis of **5**, the *n*-decylglycine unit is introduced as the triflate[5] of benzyl *R*-α-hydroxy-n-dodecanoate (**8**) which was prepared as follows. Racemic *n*-decylglycine (**9**) was optically resolved by stereoselective hydrolysis of the *N*-chloroacetate **10** using amino acylase (y: 35%) according to Mori and Otsuka.[6] After hydrolysis of *R*-**10**, conversion of the amino group of *R*-**9** into a hydroxy group via the diazonium ion followed by esterification with benzyl alcohol-$SOCl_2$ gave benzyl *R*-α-hydroxy-*n*-dodecanoate (**11**, 53%). Treatment of **11** with triflic anhydride gave the triflate (**8**, 74%). Substitution of *N*-Boc-2-methylaminophenylalaninol (**12**)[1] with the triflate **8** gave **13** (80%). Deprotection of the benzyl ester, formation of an activated ester, deprotection of the *N*-Boc group and cyclization afforded **5** (27%) and its epimer (24%). Synthesis of **6** was performed from 3-carbomethoxy-4-nitrobenzoic acid (**14**). Reduction of **14** with borane dimethylsulfide complex followed by oxidation with PCC gave 3-carbomethoxy-4-nitrobenzaldehyde (**15**) (86%). Reaction of the aldehyde with nonyl phosphonium ylide gave **16** (84%), which was converted to the tosylate **17** by hydride-reduction and tosylation (63%). Condensation of **17** with

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diethyl acetamidomalonate gave **18** (77%). Conversion of **18** into (-)-BL-V8-210 (**6**) was performed (11 steps, total yield 11%) in a manner similar to that used for (-)-BL-V8-310 (**1**).[1] Synthesis of **7** was performed from 1,2,3,4-tetrahydro-1,1,4,4,7-pentamethylnaphthalene (**19**), prepared by Friedel-Crafts alkylation of toluene with 2,5-dichloro-2,5- dimethylhexane. Nitration of **19** followed by benzylic bromination gave the nitro bromide **20** (51%). Condensation of **20** with diethyl acetamidomalonate gave **21** (72%). Conversion of **21** into (-)-BL-V8-23TM (**7**) was performed (11 steps, total yield 4 %) in a manner similar to that used for (-)-BL-V8-310.[1] The conformational structures of the BLs (**5**, **6**, **7**)[7] were confirmed to take the twist form which has been established in the case of **1**,[1] on the basis of the similarity of the ^{1}H-NMR spectral data and nuclear Overhauser effect (NOE) experiments.

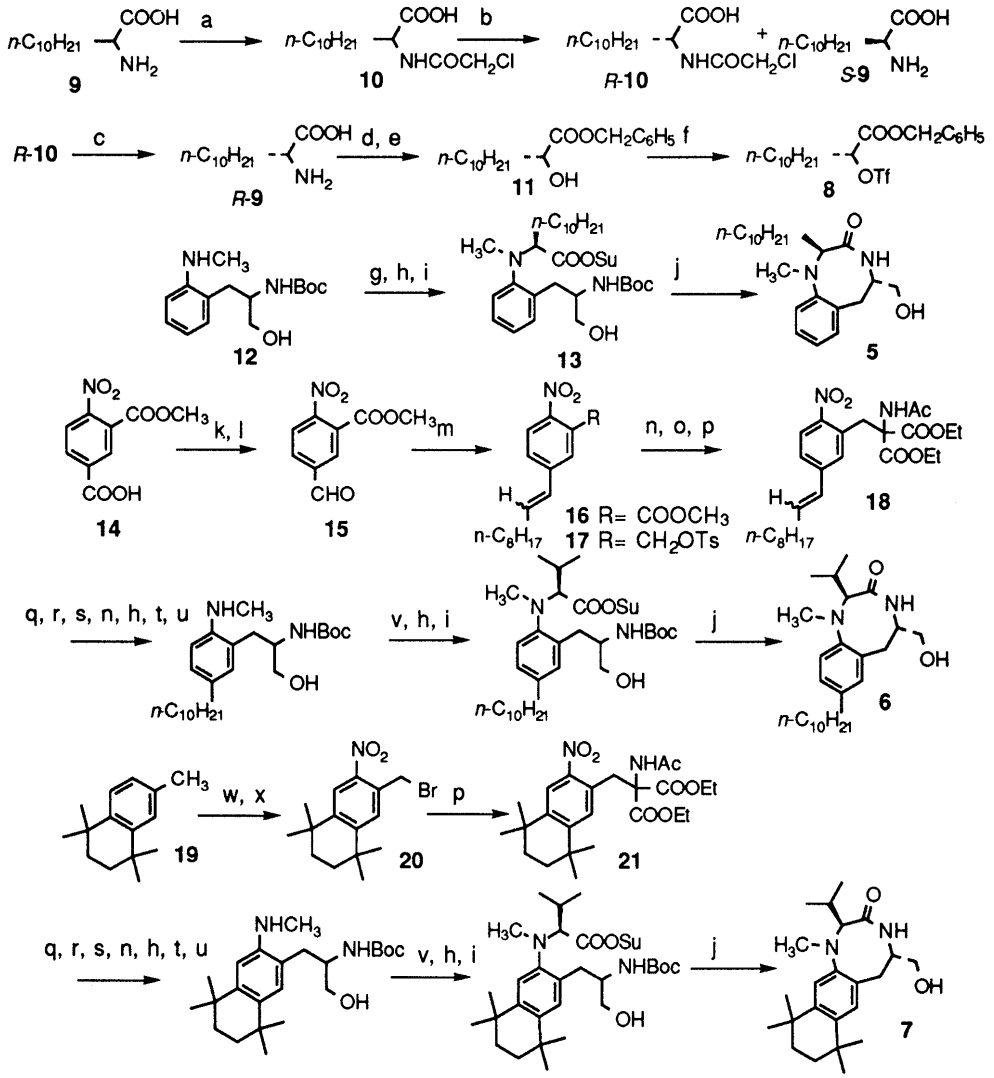


Chart. Synthesis of Benzolactams (**5**, **6** and **7**). Key: a) $ClCH_2COCl$, NaOH/ H_2O; b) *Aspergillus* amino acylase, $CoCl_2$, pH 7.26; c) HCl/ H_2O; d) $NaNO_2$, H_2SO_4/ H_2O; e) $C_6H_5CH_2OH$, $SOCl_2$; f) Tf_2O, 2,6-lutidine/ CH_2Cl_2; g), **8**, 2,6-lutidine/ CH_2ClCH_2Cl; h) H_2, Pd-C/ EtOH; i) *N*-hydroxysuccinimide, DCC/ CH_3CN; j) CF_3COOH/ CH_2Cl_2 then $NaHCO_3$aq/ CH_3COOEt; k) BH_3-$S(CH_3)_2$/ THF; l) PCC/ CH_2Cl_2; m) $C_9H_{19}P^+Br^-$, n-BuLi/ THF; n) $LiBH_4$/ THF; o) NaH, TsCl/ toluene; p) $CH_3CONHCH(COOC_2H_5)_2$, NaH/ DMF; q) HCl, AcOH; r) $SOCl_2$/ EtOH; s) Boc_2O/ CH_2Cl_2; t) HCOOH, Ac_2O; u) BH_3/ THF; v) trifrate of benzyl *R*-α-hydroxyisovalerate, 2,6-lutidine/ CH_2ClCH_2Cl; w) HNO_3, H_2SO_4; x) NBS/ CCl_4, hv.

One of the most specific and sensitive biological activities of the TPA-type tumor promoters is induction of growth inhibition and differentiation to monocytes of human promyelocytic leukemia cells (HL-60).[8] The growth inhibition activity of the BL-Vs with alkyl groups at various positions (**1**, **5**-**7**) is shown in Figure 1. In spite of switching of the decyl group from C-9 to C-2, (-)-BL-8-C10 (**5**) showed distinct, though decreased activity. The activity is 50 times stronger than that of BL-V8 (**22**) which lacks a hydrophobic group at C-9. This suggests that the hydrophobic region at C-2 and C-9 has a common function in the appearance of the activity. Unexpectedly, switching of the decyl group from C-9 to the neighboring C-8 also caused a distinct decrease of the activity, *i.e.* the activity of

(-)-BL-V8-210 (**6**) is 30 times weaker than that of **1**. Further introduction of a bulky substituent at C-8 caused a significant decrease of the activity, i.e., the activity of (-)-BL-V8-23TM (**7**) is 1000 times weaker than that of **1** and almost the same as that of **22**.

Assay of inhibition of [^{3}H]PDBu binding (K_d= 2.90 nM) and the determination of dissociation constants from binding curve IC_{50} values were done as previously described.[9] The results are shown in Fig. 2. The difference in PKCδ binding potency between **1** (K_i= 5.56 nM) and **7** (K_i= 5150 nM) is particularly striking. On the other hand, the tetramethyltetramethylene analog of IL-V (**23**) has been shown to possess activity comparable to that of TPA by PKC binding assay.[10] These BLs (**1**, **5**-**7**) and teleocidins exist in the same conformational state, which means that the hydrophilic functional groups are arranged in appropriate positions for binding to the receptor. Therefore, the significant difference in the activity caused by the substituent clearly indicates that a steric factor that disfavors binding to the receptor exists around the 8-position of BLs. This is apparently the first observation of an effect on PKC binding attributable to essentially steric changes in the hydrophobic alkyl portion of a PKC ligand. Also, the difference between the K_i= 2.96 nM of **5** and its ED_{50} of 200 nM for HL-60 differentiation suggests that target site for the HL-60 cell effect is not PKCδ. Additional details of this phenomenon includig examination of PKC isotype selectivity will be reported in due course.

Fig. 1. Growth Inhibition of HL-60 Cells

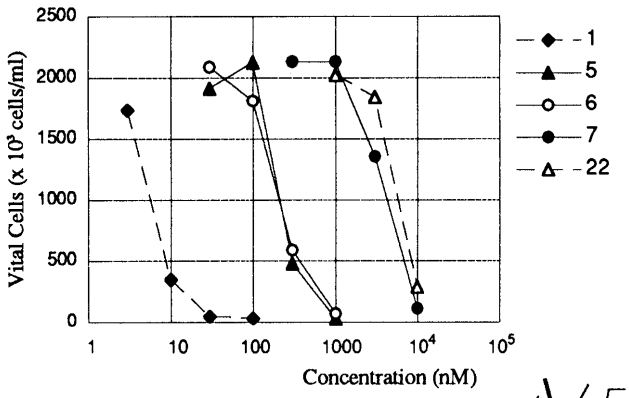
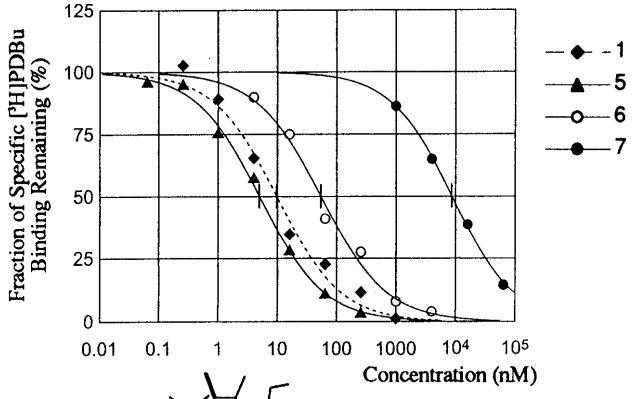
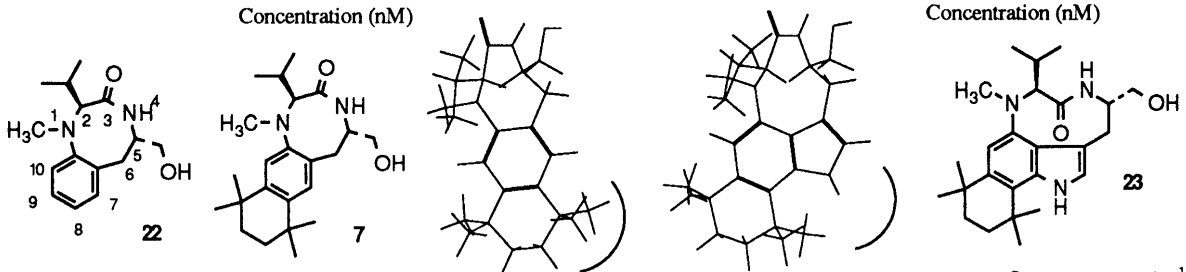


Fig. 2. Inhibition of Specific PDBu Binding with Rat PKCδ





Recently, a crystallographic study revealed a direct interaction of phorbol 13-acetate with PKCδ Cys2 domain.[11] However, the role of the flexible hydrophobic ester chain of phorbol esters is still not clear. An approach to this problem would be to examine the binding mode of teleocidins, which are biologically identical to phorbol esters, with PKCδ. The present findings should be helpful in the design of compounds as biological tools for analyzing the mechanism of tumor promotion, or of antagonists.

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