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Synthesis of 7,8-Disubstituted Benzolactam-V8 and Its Binding to Protein Kinase C

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Abstract—7-Methoxy-8-decynyl-benzolactam-V8 **4** is synthesized using a catalytic asymmetric alkylation reaction as a key step. This compound shows potent activity to three PKC isozymes tested (K_i = 45.6, 91.1, and 121.3 nM to PKCα, δ, and ε, respectively), indicating that introduction of a suitable substituent at the 7-position of 8-decynyl-benzolactam-V8 only slightly reduces the PKC binding affinity.

PKCs are a growing family of isozymes involved in a wide variety of cellular processes.[1] Marked differences in tissue distribution and substrate specificities have suggested that these isozymes may play the different roles in physiological and pathophysiological processes.[1,2] The isozyme-specific modulators are highly required in identifying these different roles, especially in vivo.[1,2] However, although several isozyme-selective inhibitors for PKCs have been developed in recent years,[3-6] few isozyme-selective activators have been reported up to now.[7] The teleocidins are a class of natural products that were found to have potent activity for PKCs but with little selectivity.[8] Endo and coworkers reported that benzolactam-V8 **1a** (Fig. 1), a *twist*-like conformation mimic of indolactam is still a potent activator to PKCs.[9] We felt that this compound is a good lead compound for developing isozyme-selective activators owing to its simplicity. In a previous report,[10] we have mentioned that if an acetylene chain is placed at the 8-position of benzolactam-V8, the generated compound **2a** had improved isozyme-selectivity in either activation or down-regulation to PKCs, while analogue **2b** with a saturated chain at the 8-position of benzolactam-V8 did not show marked isoform-selectivity. Further studies have shown that **2a** had marked antiproliferative activity against two breast carcinoma cell lines. These results implied that the substituted groups at the aromatic ring of the benzolactam-V8s might play some roles to their isoform-selectivity. Encouraged by these results, we designed two new analogues **3** and **4**, in which a methoxy group was introduced at the 7-position

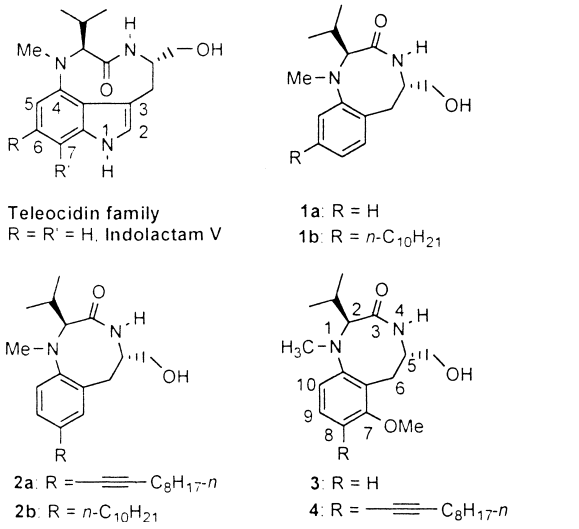

Figure 1. Structures of teleocidin family and benzolactam-V8 analogues.

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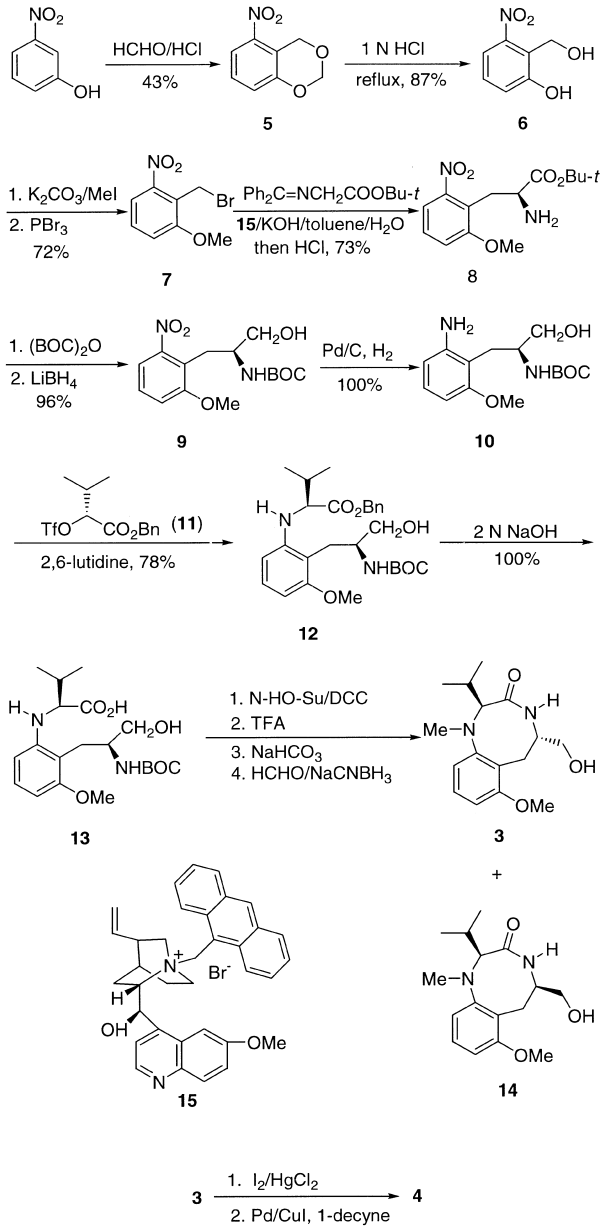
of **1a** and **2a**, respectively, in order to check if a 7-substituted group could change the activity or selectivity of this class of compounds to PKCs.

Our synthesis for **3** and **4** is outlined in Scheme 1. 5-Nitro-1,3-benzodioxane **5**, prepared from 3-nitrophenol according to the known procedure[11] with an improved yield, was refluxed in 1 N HCl for 48 h to provide alcohol **6** (87% yield based on about 30% starting material recovery). Methylation of phenol **6** with iodomethane under the assistance of K_2CO_3 followed by bromination of the primary alcohol afforded bromide **7**. Next, we tried to use a newly reported method to introduce the chiral phenylalanine moiety.[12] As a result, treatment of **7** with Schiff base derived from *tert*-butyl glycinate[12] under asymmetric phase transfer condition (catalyst: cinchonidine-derived salt **15**) gave the coupling product, which was hydrolyzed with hydrochloric acid to produce the desired amino ester **8**. In our laboratory, this reaction was carried out in a scale of more than 20 g and therefore proven to be a very practical procedure. Because it was not easy to determine the enantiomeric purity of the resultant α-amino acid derivative and the absolute configuration of the major enantiomer at this stage, we planned to solve this problem by transforming **8** into the target molecules. Thus, *N*-protection of **8** with Boc and reduction of ester with lithium borohydride afforded alcohol **9**. Hydrogenation of **9** catalyzed by Pd/C released amine **10**, which was coupled with D-valine-derived triflate **11** to afford the substitution product **12**. After hydrolysis of **12** with 2 N NaOH, the generated acid **13** was cyclized via the activated ester approach[9,10] to give two lactams, which were subjected to reductive methylation to provide separable **3** and **14** in a ratio of 3/1. By this result we concluded that the ee value of the asymmetric alkylation step (from **7** to **8**) was about 75%. The stereochemistry of **3**[13] and **14**[13] was confirmed by comparing their spectra with (2*S*,5*S*)- and (2*S*,5*R*)-benzolactam-V8s.[10,11] Therefore, we concluded that the configuration of the major enantiomer in step alkylation should be *S*, which is consistent with Lygo's report.[12] In addition, by NOE studies and comparison of the chemical shifts of **3** with those of (2*S*,5*S*)-benzolactam-V8, it was found that compound **3** displayed the *twist*-conformation in solution. It was notable that if the cyclization method mediated by DPPA in the transformation of **12** to **3** was used, a quite lower yield (23% yield) was obtained. This result gave an additional example to demonstrate that the activated ester approach was a general method for synthesizing this class of lactams although the DPPA method gave a high yield in some cases.[14] Finally, iodination of **3** assisted by mercury(II) chloride followed by palladium-catalyzed coupling reaction with 1-decyne produced **4**[15] in 75% yield.



Scheme 1.

Compounds **3** and **4** have been evaluated for their ability to displace phorbol 12,13-dibutyrate (PDBU) binding from recombinant PKCα.[5a] K_i values for **3**, **4**, benzolactam-V8 and 8-decynyl benzolactam-V8 were 7162, 45.6, 334 and 15 nM, respectively. This meant that **4** was 3-fold less potent than 8-decynyl benzolactam-V8, while **3** was 22-fold less potent than benzolactam-V8. It indicated that introduction of a suitable substituent at the 7-position of 8-decynyl benzolactam-V8 only slightly reduced its activity. In addition, compound **4** was found to have potent binding affinities to PKCδ and ε (K_i = 91.1 and 121.3 nM, respectively), which implied that this analogue had similar but poorer isoform-selectivity in comparison with 8-decynyl-benzolactam-V8. Further development of more analogues using this methodology, as well as their isoform-selectivity studies are in progress.

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15. Selected data for **4**: $[\alpha]_D^{22}$ −286 (*c* 0.08, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.4 Hz, 1H), 6.93 (br s, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 3.83 (s, 3H), 3.70–3.49 (m, 3H), 3.43 (d, *J* = 8.7 Hz, 1H), 3.21 (d, *J* = 17.6 Hz, 1H), 2.75 (s, 3H), 2.72 (dd, *J* = 17.5, 5.8 Hz, 1H), 2.40 (m, 3H), 1.60 (m, 2H), 1.23 (m, 10H), 1.02 (d, *J* = 6.3 Hz, 3H), 0.84 (t, *J* = 6.4 Hz, 3H), 0.76 (d, *J* = 6.3 Hz, 3H); HRMS found *m*/*z* 428.3021; $C_{26}H_{40}N_2O_3$ requires 428.3042.