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# SYNTHESIS AND PROTEIN KINASE C BINDING ACTIVITY OF BENZOLACTAM-V7

Dawei Ma*[a], Guoqiang Wang[a], Shaomeng Wang[b], Alan P. Kozikowski*[b], Nancy E. Lewin[c] and Peter M. Blumberg[c]

[a]*State Key Laboratory of Bio-organic and Natural Product Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, China,* [b]*Drug Discovery Laboratory, , Institute for Cognitive & Computational Sciences, Georgetown University Medical Center, 3970 Reservoir Road, N.W., Washington, DC 20007-2197, and* [c]*National Cancer Institute, Bethesda, MD 20892*

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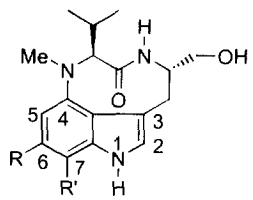
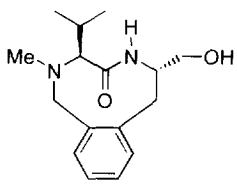
**Abstract**: Benzolactam-V7 (**3a**), a simplified analogues of (-)-indolactam-V with *twist*-form conformation, was synthesized and evaluated as a new protein kinase C modulator. Both **3a** and its-7-substituted analogue **3c** showed weak binding activity to displace PDBU binding from recombinant PKCα. 

The teleocidin family of molecules has continued to fascinate both synthetic chemists and medicinal chemists over the past two decades as a consequence of their ability to bind to the regulatory domain of protein kinase C (PKC) and to activate this important signal transducing enzyme. Since the overactivation of this enzyme system can lead to its downregulation, potent activators of PKC have actually been shown to possess anticancer activity.[1] By $^1$H NMR studies, these compounds were found to exist in two conformational states, namely the *twist* conformer (with a *cis*-amide bond) and the *sofa* conformer (with a *trans*-amide bond) in solution.[2] For some time, the conformational state of these molecules that was required for PKC activation remained open to question.[1-3] To probe the active conformation, some simplified benzolactams that might possess more defined conformations or even a single conformation were investigated.[4] We first reported a 9-membered benzolactam analogue of (-)-indolactam-V (ILV), compound **1**, that possesses a sofa-like conformation. This compound proved to be relatively inactive in binding to PKC.[4a] Later, Endo found that certain 8-membered ring analogues such as benzolactam-V8 (**2**) that could only exist in a *twist* conformation was a weak PKC activator.[4b,4c] These results implied that a *twist*-like conformation of the lactam ring of the teleocidin family is a requirement for PKC activation.

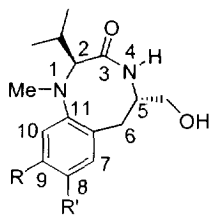
**Scheme 1**



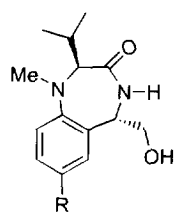
**Teleocidin family**
R = R' =H, (-)-**Indolactam-V (ILV)**
*sofa + twist*



**1**
*sofa*
inactive



**2**
R = R' = H, **benzolactam-V8**
*twist*
active



**3**
*cis*-amide bond
activity?

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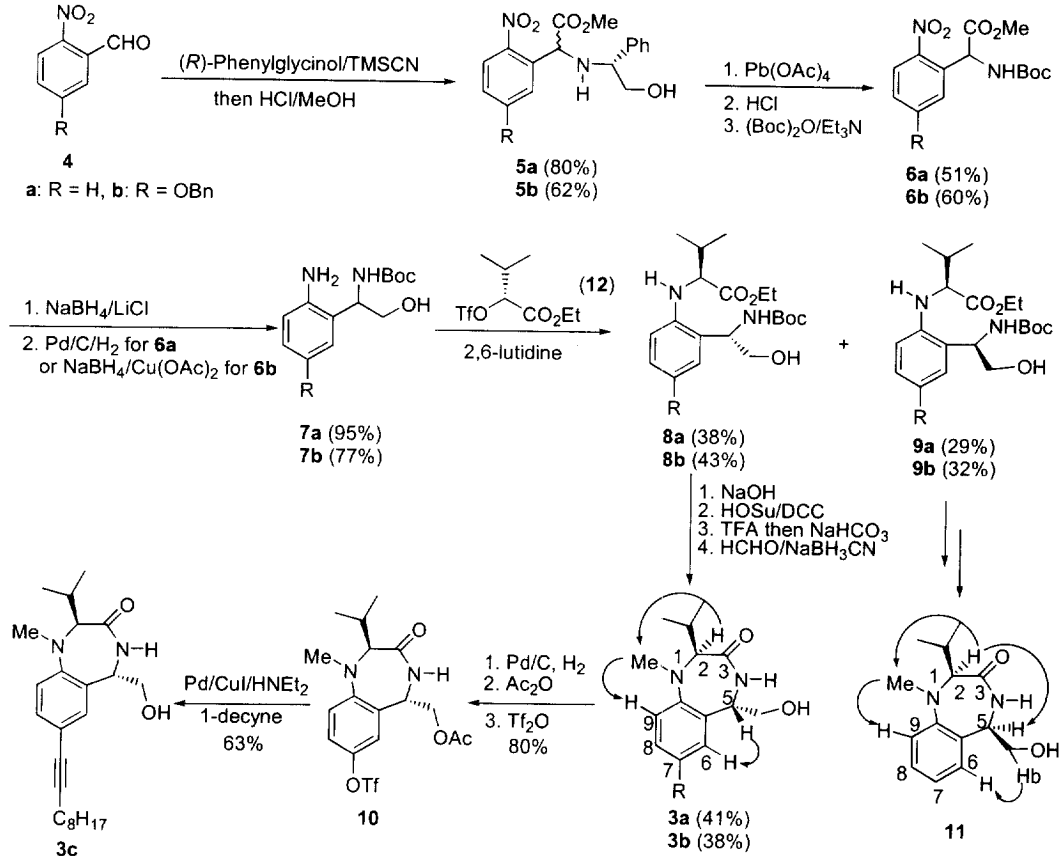
From molecular modeling studies we found that the 7-membered lactam **3a,** which of necessity must contain a *cis*-amide bond like benzolactam-V8. It thus became of interest to synthesize and evaluate these analogues as possible PKC modulators. The studies thus undertaken are reported here.[5]

**Scheme 2**



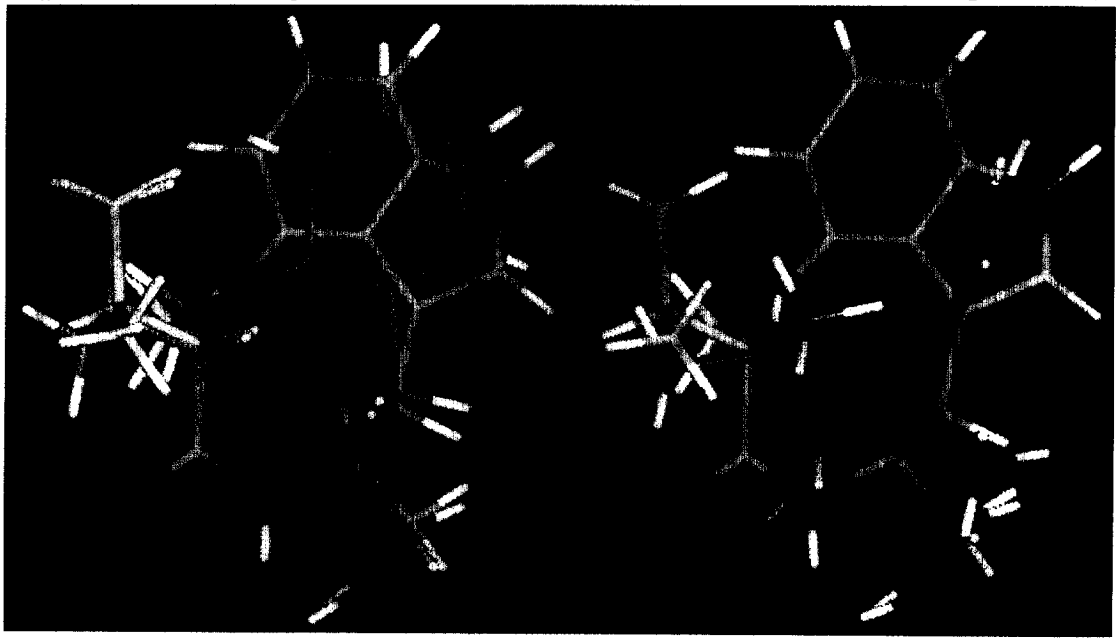

Our syntheses for the 7-membered lactam analogue **3** are detailed in Scheme 2. A similar strategy with the syntheses of benzolactam-V8 analogues was used.[5] The first problem was building an *ortho*-substituted phenylglycinol. Starting from *o*-nitrobenzaldehyde, amino ester **5a** was obtained by a typical Strecker reaction followed by methanolysis.[6] Initially, it was expected to get **5a** in higher diastereoselectivity through the chemical induction of (*R*)-phenylglycinol. Although good diastereoselectivity (about 3/1) could be observed by $^1$H NMR after quenching the reaction, two diastereomers were obtained in almost 1/1 ratio after purification by column chromatography. This disappointed result might come from the quick racemization of **5a** during purification because of high electron-withdrawing effect of the nitro group. Next, the chiral auxiliary of **5a** was removed by the oxidation with small excess of $Pb(OAc)_4$ to afford an amino ester, which was protected with a Boc group to give the ester **6a**. After reduction of the ester group of **6a** to alcohol using $NaBH_4$/LiCl in THF/MeOH, hydrogenation catalyzed by Pd/C was carried out to provide the aniline **7a**. Subjected **7a** to an $S_N2$ reaction with the valine-derived triflate **12** afforded a mixture of diastereomers **8a** and **9a**, which could be easily separated by column chromatography. Because it was not so easy to identify the detailed stereochemistry of both isomers, we

transformed both of them to the corresponding lactams by the known method. Thus, **3a** (41% overall yield) and **11** (39% overall yield) were obtained from **8a** and **9a** respectively. The NOE correlations of **3a**[7] and **11**[8] were shown in Scheme 2. Marked NOEs between H2 and H5, Hb and H6, H2 and 1-Me, 1-Me and H9 were observed in compound **11** and it was therefore concluded that the configuration of **11** is 2*S*,5*R*. In compound **3a**, only NOEs between H5 and H6, H2 and 1-Me, 1Me and H9 were observed, which implied that the configuration of **3a** is 2*S*,5*S*.

Because the direct introduction of a side chain substituent into **3a** (such as iodination[5a]) was found to be difficult, we decided to synthesize the 7-substituted benzolactam-V7 by starting from an aldehyde bearing an appropriate substituent *meta* to the aldehyde group. Accordingly, the aldehyde **4b**, which was prepared from 3-hydroxybenzaldehyde *via* nitration and protection, was selected as our starting material. Through a similar reaction sequence as used in the synthesis of **3a** except for reduction of **6b** to **7b** using $NaBH_4/CuSO_4$,[9] 7-benzoxy-benzolactam-V7 (**3b**) was prepared in a 4.7% overall yield. After protection of the hydroxy group as its acetate and removal of the benzyl protecting group by hydrogenation, the generated phenol was converted to its triflate, which was coupled with 1-decyne to afford **3c**.[10]

Compounds **3a** and **3c** have been evaluated for their ability to displace phorbol 12,13-dibutyrate (PDBU) binding from recombinant PKCα.[5a] $K_i$ values for ILV, benzolactam-V7's (**3a**, **3c**), benzolactam-V8 and 8-decynyl benzolactam-V8 were 11 nM, 127 μM, 21μM, 334 nM and 15 nM, while (2S,5R)-isomer **11** did not show binding activity at 300 μM.

**Figure 1**. Molecular modeling studies for ILV and its related analogues (left: benzolactam-V8 + ILV, right: **3a** + ILV)



As is apparent, compound **3a** is about 500-fold and 11500-fold less potent than benzolactam-V8 and ILV respectively, while **3c** is about 1400-fold less potent than 8-decynyl-benzolactam-V8. The biological results of **3a** and **3c** was explained by our molecular modeling studies. As can be seen from Figure 1, the benzolactam-V8 adopts a twist conformation and has an excellent overlap to the twist conformation of ILV. In sharp contrast, the 7-membered lactam **3a** adopts a conformation very different from the twist conformation. Although its key hydrogen

bonding groups can achieve a reasonably good overlap with those of ILV and benzolactam-V8, two crucial hydrophobic groups, namely, the *N*-methyl group and the phenyl group have a very bad overlap with the corresponding groups in ILV and benzolactam-V8. The *N*-methyl group in the 7-membered lactam **3a** resides on the opposite side as compared to that in ILV and benzolactam-V8 and the phenyl ring is perpendicular to the indole ring in ILV. It has been shown previously that both of these groups are important to the binding affinity of ILV and benzolactam-V8.[5a,11] Our docking simulation studies also confirmed that compound **3a** indeed is unable to achieve optimal interactions with the PKC CDR (data not shown). Taken together, our results suggest that the three dimensional spatial arrangement of these crucial hydrogen bonding and hydrophobic groups play a crucial role to the PKC binding affinity of ILV and its related analogs.

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7. **3a**: $[\alpha]_D^{20}$ +101.7 (*c* 0.75, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.23 (t, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 4.25 (m, 1H), 3.94 (dd, *J* = 10.6, 8.4 Hz, 1H), 3.63 (dd, *J* = 10.9, 4.4 Hz, 1H), 3.44 (d, *J* = 6.5 Hz, 1H), 2.88 (s, 3H), 1.87 (m, 1H), 1.87 (d, *J* = 6.9 Hz, 3H), 0.61 (d, *J* = 7.0 Hz, 3H). HRMS found *m/z* 248.1519 ($M^+$), $C_{14}H_{20}N_2O_2$ requires 248.1525.
8. **11**: $[\alpha]_D^{20}$ +8.2 (*c* 0.5, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 2H), 6.91 (t, *J* = 7.8 Hz, 1H), 4.91 (br s, 1H), 4.20 (m, 2H), 3.67 (d, *J* = 5.5 Hz, 1H), 2.90 (s, 3H), 2.10 (m, 1H), 1.04 (d, *J* = 7.0 Hz, 3H), 0.56 (d, *J* = 7.1 Hz, 3H). HRMS found *m/z* 248.1521 ($M^+$), $C_{14}H_{20}N_2O_2$ requires 248.1525.
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10. **3c**: $[\alpha]_D^{20}$ +97 (*c* 0.34, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.15 (d, *J* = 1.9 Hz, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 4.30 (m, 1H), 3.92 (dd, *J* = 10.6, 8.3 Hz, 1H), 3.70 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.52 (d, *J* = 6.9 Hz, 1H), 2.95 (s, 3H), 2.40 (t, *J* = 7.1 Hz, 2H), 1.91 (m, 1H), 1.65-1.28 (m, 12H), 0.90 (m, 6H), 0.71 (d, *J* = 6.3 Hz, 3H); HRMS found *m/z* 384.2746 ($M^+$), $C_{24}H_{36}N_2O_2$ requires 384.2777.
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