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Synthesis and Modeling Study of (2*S*,5*R*,6*R*)- and (2*S*,5*R*,6*S*)-6-Hydroxy-8-(1-decynyl)-benzolactam-V8 as Protein Kinase C Modulators

Zhi-Liang Wei,[†] Sukumar Sakamuri,[†] Pavel A. Petukhov,[†] Clifford George,[‡] Nancy E. Lewin,[§] Peter M. Blumberg,[§] and Alan P. Kozikowski*,[†]

Drug Discovery Program, Department of Neurology, Georgetown University Medical Center, 3900 Reservoir Road, NW, Washington, DC 20007, Naval Research Laboratory, 4555 Overlook Ave., SW, Washington, DC 20375, and Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, Maryland 20892

kozikowa@georgetown.edu

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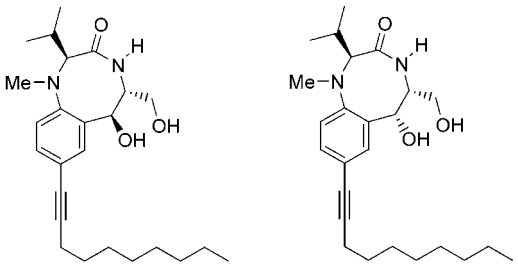
ABSTRACT



Both (2*S*,5*R*,6*R*)- and (2*S*,5*R*,6*S*)-6-hydroxy-8-(1-decynyl)benzolactam-V8 were designed and synthesized as PKC modulators. Biological assays reveal the (6*R*)-ligand to be 20-fold more potent than its (6*S*)-counterpart in binding to PKCα.

Protein kinase C (PKC) is a phospholipid-dependent serine/threonine kinase family consisting of at least 11 closely related isozymes that mediate a wide range of cellular signal transduction processes.[1] However, complete details of functional significance of each PKC isozyme in these cellular events and in tumor promotion are not fully understood, which is attributable in part to the lack of isozyme-selective modulators. The discovery of isozyme-selective activators and inhibitors of PKC is crucial to both dissecting and manipulating their physiological pathways. The teleocidins, as well as analogues of indolactam V (ILV) (Figure 1), are a class of natural products that show high affinity for PKC but possess little isozyme selectivity.[2] The teleocidins thus serve as important lead structures in the search for isozyme-selective modulators of PKC.[3] Through studies of the active conformation of ILV, benzolactam-V8 (**1**), a molecule whose constrained eight-membered lactam is forced to adopt the active twist conformation, was designed by Endo and co-workers.[4] Such benzolactams provide a synthetically more accessible core structure in the search for isozyme-selective

[†] Georgetown University Medical Center.
[‡] Naval Research Laboratory.
[§] National Cancer Institute.

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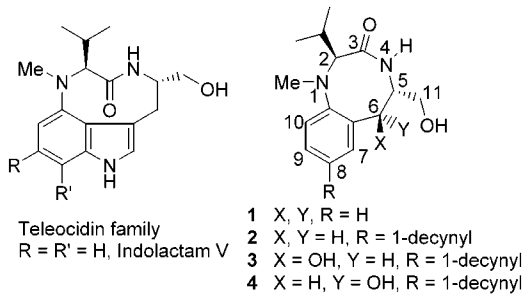


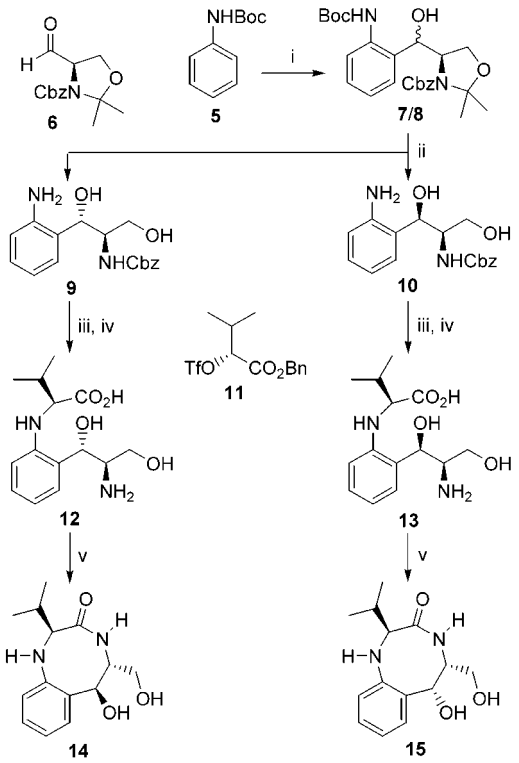
Figure 1.

PKC modulators. In connection with such efforts, we reported that 8-(1-decynyl)benzolactam-V8 (**2**) exhibited improved potency and selectivity for the classical isozymes.[5] These results prompted us to synthesize additional benzolactam-V8 analogues bearing substituents that may confer enhanced isozyme selectivity.[6]

The X-ray crystal structure of the complex of the C1b activator-binding domain of PKCδ with phorbol 13-acetate has revealed how this phorbol ester binds to PKC.[7] Although the structure is incomplete from the standpoint of revealing interactions with lipids, it nonetheless opens the possibility for the rational design of selective PKC modulators through the evaluation of their binding to the homologous PKC isozymes using computer assisted docking methods.[5,8] Herein we describe the synthesis and modeling of the 6-hydroxyl-substituted benzolactam-V8 analogues **3** and **4** as PKC modulators. These compounds were designed with the idea that the extra hydrogen bond donor group might enhance PKC affinity and selectivity.

The ortho functionalization of *N*-(*tert*-butoxycarbonyl)-aniline was used as the key strategy in our synthesis. Thus, ortho metalation of **5** with excess *tert*-butyllithium at low temperature provided the corresponding dilithio intermediate,[9] which was treated with the aldehyde **6**[8c] to give rise to a mixture of the alcohols **7**/**8**. The diastereoisomers of **7** and **8** could not be separated efficiently by flash column chromatography. Acid hydrolysis of **7**/**8** by treatment with 1 M HCl in THF at 70 °C yielded **9** and **10** in a ratio of ca. 1.3:1, which were readily separated chromatographically. The absolute configuration of the newly generated hydroxyl group of the two isomers was assigned by the X-ray crystallographic analysis of **9**. Coupling of the amines **9** and **10** with D-valine-derived triflate **11**[10] followed by simultaneous removal of the benzyl ester and *N*-Cbz protecting groups by catalytic hydrogenation provided the amino acids **12** and **13**, respectively (Scheme 1).

Scheme 1[a]



[a] (i) *t*-BuLi (2.2 equiv), THF, −78 to −20 °C, 2 h, then **6**, −20 °C, 5 h, 31%. (ii) 1 M HCl, THF, 70 °C, 5 h, 87%. (iii) **11**, 2,6-lutidine, 1,2-dichloroethane, 70 °C, 72 h, 65−67%. (iv) 10% Pd−C, H_2 (1 atm), ethanol, rt, 24 h, 100%. (v) See Table 1.

With the amino acids **12** and **13** in hand, their lactamization to **14** and **15** was investigated. Two procedures, BOP/HOBt/NMM/DMF and DPPA/Et_3N/DMF, were examined for this cyclization reaction. The results are summarized in Table 1. The *erythro*-isomer **12** could be readily lactamized

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Table 1. Lactamization of Amino Acids **12** and **13**

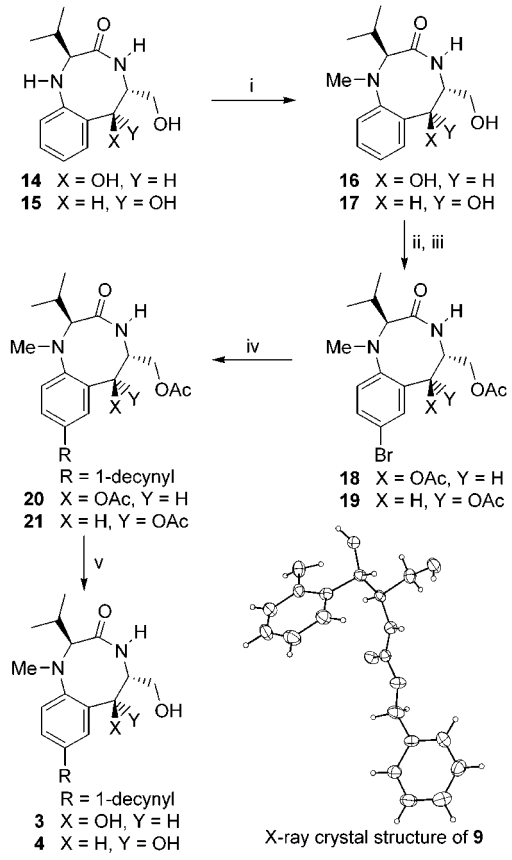
entry	substrate	conditions[a]	product	yield[b]
1	**12**	A	**14**	91%
2	**12**	B	**14**	75%
3	**13**	A	**15**	18%
4	**13**	B	**15**	39%

[a] Condition A: BOP, HOBt, NMM, DMF, rt, 72 h; condition B: DPPA, Et_3N, DMF, rt, 48 h. BOP, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; HOBt, hydroxybenzotriazole; NMM, *N*-methylmorpholine; DPPA, diphenylphosphoryl azide. [b] Isolated yield.

by use of either the BOP or DPPA reagent to afford **14** in good yield, although higher yields were achieved by using the BOP reagent (entries 1 and 2). However, only low to moderate yields were achieved in the lactamization of the *threo*-isomer **13** to **15** (entries 3 and 4). The lower yields in this cyclization reaction may reflect increased steric hindrance in the transition state.

Completion of the synthesis of (6*S*)-hydroxy-benzolactam-V8 (**3**) is outlined in Scheme 2. Reductive methylation of **14** afforded the (6*S*)-hydroxylated parent benzolactam-V8 (**16**). Treatment of **16** with Ac_2O and pyridine followed by bromination at room temperature using benzyltrimethylammonium tribromide (BTMA Br_3)[11] gave **18** in high yield. The latter was coupled with 1-decyne in the presence of $PdCl_2(PPh_3)_2$ and CuI followed by removal of the acetate groups with 2 M NaOH in methanol to afford (2*S*,5*R*,6*S*)-6-hydroxy-8-(1-decynyl)benzolactam-V8 (**3**).

Scheme 2[a]



[a] (i) HCHO, HOAc, $NaCNBH_3$, CH_3CN, 0 °C, 1 h, 89−90%. (ii) Ac_2O, pyridine, Et_3N, rt, 12 h, 81−83%. (iii) BTMA Br_3, CH_2Cl_2, MeOH, $CaCO_3$, rt, 2 h, 89−90%. (iv) $PdCl_2(PPh_3)_2$, CuI, 1-decyne, Bu_4NI, Et_3N, DMF, 70 °C, 72 h, 69−75%. (v) 2 M NaOH, MeOH, rt, 30 min, 88−93%.

In the same manner as described above, the other isomer, (2*S*,5*R*,6*R*)-6-hydroxy-8-(1-decynyl)benzolactam-V8 (**4**), was synthesized from **15** (Scheme 2).

Compounds **3** and **4** have been evaluated for their ability to displace phorbol 12,13-dibutyrate (PDBu) binding from recombinant PKCα. The K_i values for these analogues were found to be 1571 ± 147 nM and 76.1 ± 3.8 nM, respectively, while the K_i value for **2** was 14.7 ± 1.3 nM. While both of the hydoxylated benzolactams were unexpectedly found to be less potent than the parent compound, the (6*R*)-isomer is 20-fold more potent than the (6*S*)-isomer.

To understand these results, the ligands **2**, **3**, and **4** were docked to the PKCα C1b domain using the FlexX[12] module in SYBYL. Loop A comprising amino acids Thr 108, Tyr 109, Ser 110, Ser 111, Pro 112, Thr 113, and Phe 114 and loop B comprising amino acids Leu 121, Leu 122, Tyr 123, Gly 124, Leu 125, Ile 126, His 127, and Gln 128 were used to create the binding site for the docking experiments. The poses of ligands **2**, **3**, and **4** ranked as best are shown in Figure 2.

The molecular modeling and docking studies reveal that all ligands **2**, **3**, and **4** are able to form a network of hydrogen bonds with the PKCα C1b domain (Table 2 and Figure 2).

Table 2. Hydrogen Bond Distance (Å) between Acceptor and Hydrogen Atoms in PKCα C1b Domain and Ligands **2**, **3**, and **4**

	2	**3**	**4**
$r(O_{6-OH}\cdots NH2_{Gln128})$	n/a[a]		1.92
$r(H_{6-OH}\cdots CO_{Gly124})$	n/a[a]		1.88
$r(H_{6-OH}\cdots CO_{Tyr109})$	n/a[a]	1.85	
$r(H_{11-OH}\cdots CO_{Tyr109})$	1.75	1.95	2.29
$r(H_{11-OH}\cdots CO_{Ser111})$		2.11	1.60
$r(H_{4-NH}\cdots CO_{Leu122})$	2.15	2.38	1.83
$r(O_{3-CO}\cdots NH_{Gly124})$	1.64	1.75	1.77

[a] n/a, not applicable, group absent in the ligand.

The amido group in all three ligands forms two hydrogen bonds with Leu 122 and Gly 124. The primary hydroxyl group at C-11 in ligands **3** and **4**, acting as a hydrogen bond donor, forms two hydrogen bonds with Tyr 109 and Ser 111, although in ligand **2** it participates in only one interaction with Tyr 109. The (6*S*)-hydroxyl group in ligand **3** forms only one hydrogen bond with Tyr 109, whereas the (6*R*)-hydroxyl group in ligand **4** forms two hydrogen bonds with

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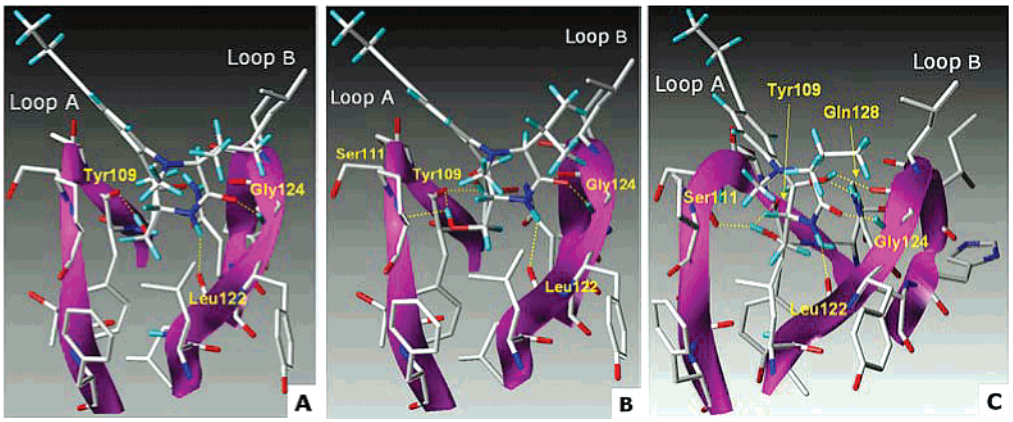

Figure 2. Hydrogen bonding interactions between PKCα C1b domain and the best docked poses of **2** (A), **3** (B), and **4** (C).

Gly 124 and Gln 128. While ligand **3** forms five hydrogen bonds with PKCα and ligand **4** forms six, this difference alone is unlikely to explain the poorer binding affinity of **3**. The lower activity of **3** may also arise from the ability of its hydroxyl groups to engage in intramolecular hydrogen bond formation in the binding conformation ($r[H_{11-OH}\cdots O_{6-OH}]$ = 1.85 Å, Figure 2B). Consequently, this would make the intermolecular interactions of these groups less favorable. On the other hand, the lower activity of **4** compared to the activity of **2** may originate from additional bulk and unfavorable electrostatic interactions as well as desolvation factors due to the presence of the 6-hydroxyl group. The surface of the binding site in the PKCα C1b domain is negatively charged (Figure 3A), thus resulting in an unfavorable interaction with the oxygen atom of the 6-hydroxyl group in **4** (note the additional blue, negatively charged spot in Figure 3C compared to Figure 3B).

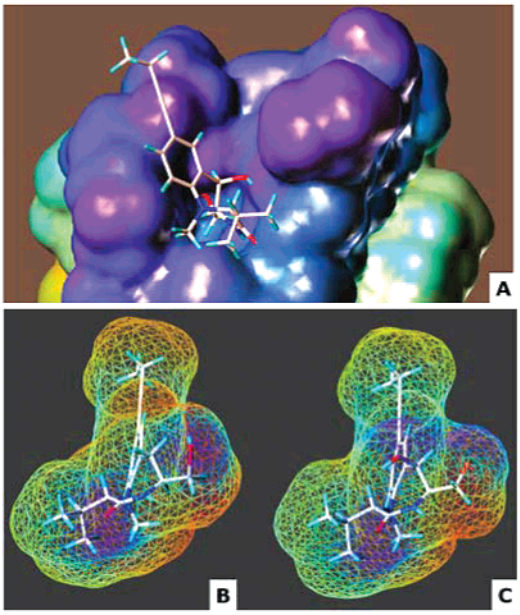

Figure 3. Electron density surface of the PKCα C1b domain mapped by electrostatic potential, docked ligand **4** depicted by capped sticks (A); electron density surfaces of **2** (B) and **4** (C) mapped by electrostatic potential (blue is negative and red is positive).

In summary, we have described an efficient route to the 6-hydroxylated benzolactam-V8 isomers **3** and **4**. Biological assays reveal that the (6*R*)-isomer shows higher affinity for PKCα than the (6*S*)-isomer, a result that is rationalized by our modeling studies.

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Supporting Information Available: Modeling methods, crystal data for compound **9**, and detailed experimental procedures with spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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