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A palladium catalyzed route to a benzofuran analogue of indolactam V (ILV): effects on PKC isotype selectivity

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Abstract: A novel palladium catalyzed synthesis of the benzofuran analogue **11** of *n*-hexyl-ILV and details of its isoform selectivity are described. Of considerable interest is the unexpected finding that this subtle structural change leads to a compound that is more like phorbol 12,13-dibutyrate (PDBu) and less like *n*-octyl-ILV in its pattern of activity.

Molecular cloning analysis has revealed that the calcium/phospholipid-dependent protein kinase C (PKC) is comprised of a family of at least ten individual isoforms. According to their structural organization and their sensitivity to Ca^{2+}, these isoforms have been classified into three groups: the classical PKCs (α, βI, βII, and γ) require Ca^{2+} for full activation, while the new PKCs (δ, ε, η, and θ) and atypical PKCs (ζ, λ) are Ca^{2+} independent.[1] It is believed that each of these PKC isoforms may play a specific role in cell type-specific processes like endocytosis, secretion, growth, or differentiation.[1, 2] While much attention has focused on the elucidation of the roles of the individual PKC isoforms in the past few years, this research has been hindered by the lack of small molecules that can selectively activate or inhibit either the individual isoforms or a small group of these isoforms.[1, 2]

Physiologically, PKC is activated by diacyl glycerols (DAGs), which result from the agonist-induced hydrolysis of membrane phospholipids.[1,3] The DAG drives the translocation of the inactive, cytoplasmic PKC to the membrane, where it interacts with other phospholipids such as phosphatidylserine and becomes fully activated. Several complex natural products and their derivatives like the phorbol esters, bryostatins, and teleocidins including indolactam V (ILV) can mimic DAG to activate PKC at low concentrations.[4] However, unlike DAG, these molecules can cause depletion, or down-regulation, of cellular PKC through its prolonged activation.[4]

Preliminary studies have shown that these ligands exhibit some differential isoform selectivity for binding *in vitro* and cause differential isoform translocation and down-regulation *in vivo*.[5, 6] For example, the mezerein analogue thymeleatoxin showed 20-fold lower affinity for PKCε than for PKCβ. In contrast, phorbol 12,13-dibutyrate (PDBu) showed a 5-fold spread in affinities and DAG only a 1.7-fold difference between isoforms.[6] The discovery of other isoform-selective agents thus appears worthwhile in further defining the roles of the PKC isoforms, and as well in generating new agents for cancer chemotherapy.[7,8]

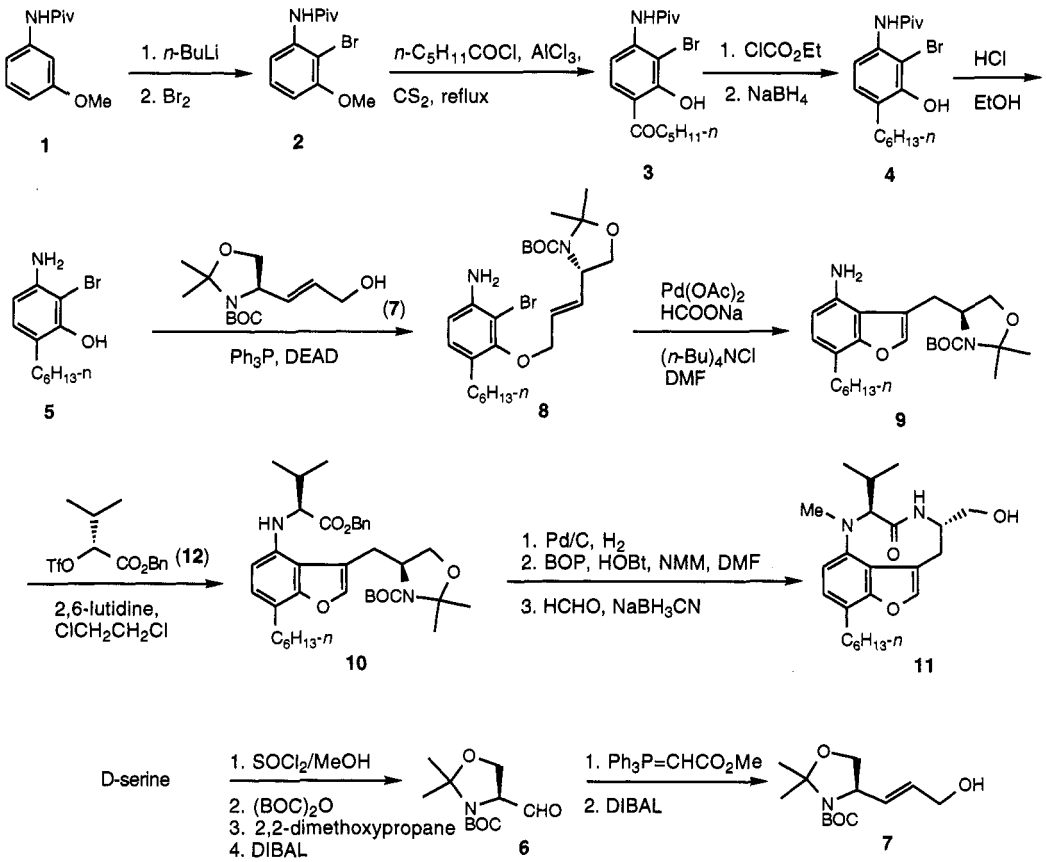
In comparison with the phorbol esters, little information is available regarding the isoform selectivity of the teleocidin family. Blumberg has reported recently that 7-*n*-octyl-indolactam V exhibits little if any selectivity for the isoforms tested.[6] In order to probe the possibility of developing isotype selective agents based on the ILV structure,[9] we sought to explore replacement of the indole nucleus by a benzofuran ring.[10] Herein we describe a novel synthesis of the benzofuran analogue **11** of *n*-hexyl-ILV and its biological evaluation.[11]

As detailed in the accompanying Scheme I, this benzofuran analogue was assembled enantioselectively starting from the *N*-pivaloyl derivative of *m*-anisidine **1**, which was converted to the bromide **2** through a directed ortho-metalation reaction.[12] The Friedel-Crafts reaction of **2** with *n*-hexanoyl chloride at 70 °C for 2 days accompanied by *O*-demethylation afforded ketone **3** in 41% yield. After failure to reduce the acyl group to an alkyl group by use of H_2/catalyst or LAH/$AlCl_3$, we found that treatment of **3** with ethyl chloroformate followed by reduction with $NaBH_4$ gave the desired product **4** in excellent yield.[13] The pivaloyl protecting group was then removed by refluxing **4** in HCl/ethanol to afford **5**. This anisidine derivative was reacted with the D-serine derived allyl alcohol **7** under Mitsunobu conditions to produce **8** in 75% yield. Synthesis of the allyl alcohol **7** ($[\alpha]^{22}_D$ = +23.1°, *c* 0.12, EtOAc) was carried out starting from aldehyde **6**[14] by the standard sequence of operations detailed at the bottom of Scheme I. Subjection of the ether derivative **8** to Larock's intramolecular cyclization protocol employing Jeffrey's palladium catalyst system and sodium formate as the reducing agent led in turn to the benzofuran **9** ($[\alpha]^{22}_D$ = -23.3°, *c* 0.12, EtOAc) in 83% yield.[15] Next, the reaction of **9** with the D-valine derived triflate **12** was carried out under basic conditions to afford compound **10** ($[\alpha]^{22}_D$ = -42.7°, *c* 1.14, EtOAc).[16] Lastly, the three protecting groups of **10** were removed in one pot by Pd/C catalyzed hydrogenation and hydrolysis with a 1:1 mixture of concentrated HCl and ethanol. The resulting amino acid hydrochloride was immediately treated with BOP, HOBt, and *N*-methylmorpholine in DMF to generate the lactam ring, and then the amine nitrogen was *N*-methylated to furnish **11** ($[\alpha]^{22}_D$ = -175.8°, *c* 0.24, EtOAc).

Compound **11** was evaluated for its ability to inhibit [^{3}H]PDBu binding to recombinant PKCα, PKCβ, PKCγ, PKCδ, and PKCε employing the same experimental protocol described elsewhere.[6] We found that **11** exhibited modestly weaker affinity for PKCδ (12.3 ± 3.4 nM), PKCε (8.0 ± 2.1 nM), and PKCγ (6.4 ± 0.5 nM) than for PKCα (4.0 ± 0.7 nM) and PKCβI (2.3 ± 0.5 nM, n = 6). Relative affinities are shown in graphical form in Figure 1. These results indicate that this new analogue exhibits a pattern of activity which is modestly more like that for PDBu and less like that for 7-*n*-octyl-ILV and ILV.[6] Compound **11** was also shown to stimulate PKC enzymatic activity to the same maximal extent as PDBu. Our idea of pursuing further structural modifications of ILV in order to obtain agents exhibiting improved levels of isoform selectivity thus appears to be reasonably well founded.

The above findings reveal that the absence of the indole NH does not have a deleterious effect on the ability of the benzofuran analogue to activate PKC. Moreover, our findings reveal that the ring heteroatom of the ILV analogues plays a role in the recognition of these molecules by the different isoforms of PKC. While this result is somewhat unexpected, and may be related to differences in H bond donor/acceptor character of N and O, a more complete understanding of the subtleties of the

Scheme I. A Palladium Catalyzed Route to a Benzofuran Analogue of ILV.



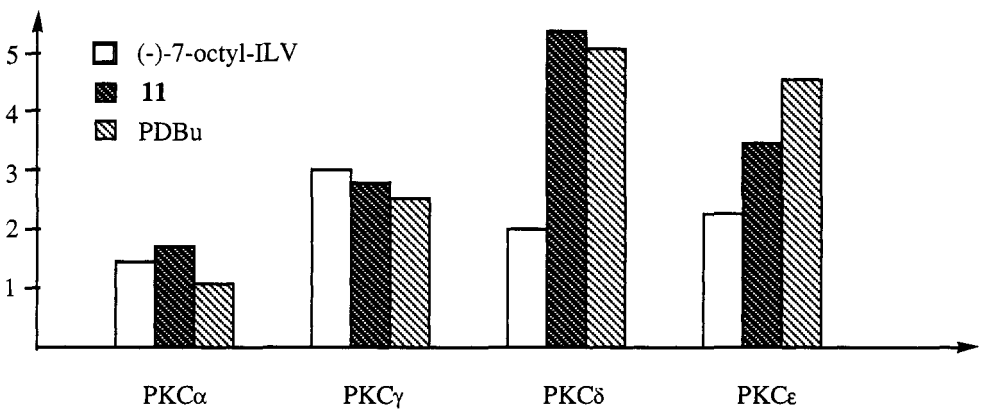


Fig. 1 Relative affinities of PKC ligands for the different PKC isoforms. Affinities of the different ligands for each PKC isoform are expressed as the ratio to those for PKCβI.

molecular recognition process at the atomic level will require detailed NMR/x-ray studies of the individual complexes formed from these small molecules and the various isotypes of PKC.[6]

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