Structure-Based Design of a New Class of Protein Kinase C Modulators

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Protein kinase C (PKC) is a ubiquitous diacylglycerol (DAG)-activated signal transducing enzyme system that is coupled to diverse biological events including regulation of ion channels, neurotransmitter release, growth and differentiation, apoptosis, and neuronal plasticity.[1] Apart from diacylglycerol,[2] the endogenous activator of PKC, several complex natural products and their analogues such as the phorbol esters, bryostatin, teleocidin, and indolactam V (ILV) can mimic DAG to activate PKC at low concentrations.[3−5] Synthesis,[6−8] molecular modeling,[6,9−11] and structure−activity relationships[12,13] of ILV and its analogues have been reported. The available X-ray structure of PKCδ in complex with phorbol 13-acetate[10] provides information invaluable to the design of new classes of PKC modulators. This paper presents the first example of the structure-based design, synthesis, and biological activities of certain γ-lactams as novel mimics of ILV.

The search for a simpler structural template that retains PKC-activating properties was driven largely by our desire to work with a compound that was readily amenable to modification so that ultimately we can discover isozyme selective modulators for the DAG superfamily. On the basis of the X-ray structure of PKCδ CRD2 (cysteine-rich domain) in complex with phorbol 13-acetate, we have determined how the high-affinity ligands ILV and the eight-membered ring benzolactam bind through a combination of molecular modeling and site-directed mutagenesis studies.[6] To both simplify and to rigidify the ILV structure, conceptually we considered linking C-9 and N-13, as these atoms are close to one another in ILV's twist conformation (Figure 1a), to arrive at the pyrrolidone derivatives **6** (Figure 2). In order for this type of compound to interact efficiently with PKC, modeling studies revealed that the isopropyl and phenyl groups must be cis oriented and trans to the hydroxymethyl group. With this stereochemistry, the pyrrolidone is capable of engaging in the same hydrogen-bond network to PKC as identified for ILV (Figure 1). Its isopropyl group interacts with the side chain of Leu 24, thereby mimicking the isopropyl group of ILV. Also, its phenyl group is parallel to Pro 11, thus allowing for strong hydrophobic interactions. However, the important interaction of the *N*-methyl group of ILV with Pro 11 and Leu 20 is absent (the absence of this group in ILV results in a 100-fold reduction in potency[11]). In addition, the optimal water solubility values (log WS)[14] can be adjusted by introduction of an appropriate substituent. This side chain generally enhances a ligand's binding affinity through interaction with the lipid membrane.

The phenyl-bearing pyrrolidone **6a** was synthesized starting from L-glutamic acid[15] via **1** (Scheme 1). Copper-catalyzed conjugate addition of PhMgBr to **1** furnished **2a**. Subsequent aldol condensation with acetone gave rise to the corresponding tertiary alcohol **3a** which was dehydrated by the Burgess reagent to afford a mixture of two olefins. Isomerization of the nonconjugated olefin with DBU provided conjugated lactam **4a** as a single compound. A hydroxyl-directed hydrogenation[16] over 10% Pd/C was carried out after removal of the silyl group. Last, deprotection of Boc group with TFA yielded **6a**. By using *p*-BrPhMgBr in the conjugate addition step, and then at the stage of **4** performing a palladium-catalyzed coupling reaction with 1-nonyne,[6] we acquired access to the pyrrolidone **6b** possessing a hydrophobic alkyl residue. The results obtained with **6a** and **6b** (Table 1) suggested that our design concepts were correct. However, additional modifications were clearly needed to obtain compounds of nanomolar affinity.

Molecular modeling suggested that replacement of phenyl by α-naphthyl can partially compensate for the absence of ILV's *N*-methyl group in our γ-lactams. Attempts to synthesize these naphthylpyrrolidone derivatives by the conjugate addition strategy of Scheme 1 failed. Instead, as shown in Scheme 2, D-serine methyl ester hydrochloride was acylated with α-bromoisovaleryl chloride, and the hydroxyl and amido groups were protected by reaction with 2,2-dimethoxypropane to provide **8**. The ester was then reduced to aldehyde by DIBAL-H treatment, followed by addition of α-naphthylmagnesium bromide. Perruthenate-catalyzed oxidation of the resulting secondary alcohol furnished the ketone **9a**. Its SmI_2-mediated ring closure gave only a single diastereoisomer **10a**, with the stereochemistry being confirmed by X-ray diffraction. The Barton deoxygenation served to remove the tertiary alcohol and to invert the C-4′ stereocenter, thereby delivering the desired *cis*-C-3′/C-4′ stereochemistry as evidenced by the shielding of the methine proton belonging to the isopropyl group in **12** to the extent of 0.8 ppm compared to that in **10**. After deprotection by transketalization with ethanedithiol in the presence of $BF_3 \cdot Et_2O$, the resulting product **13a** was tested and found to exhibit a 23-fold improvement in activity compared to **6a**.

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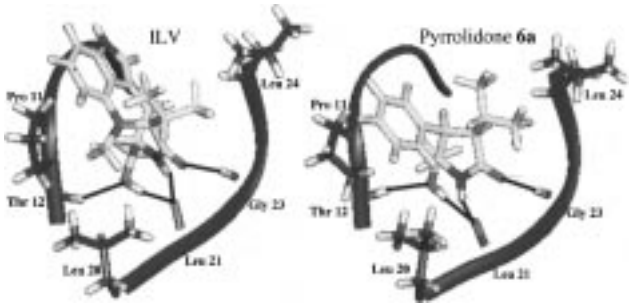


Figure 1. The overall features of the binding model for the ILV (left) and pyrrolidone derivative **6a** (right) in complex with PKCδ CRD2.

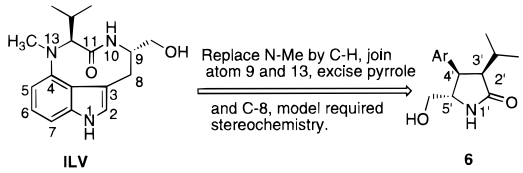
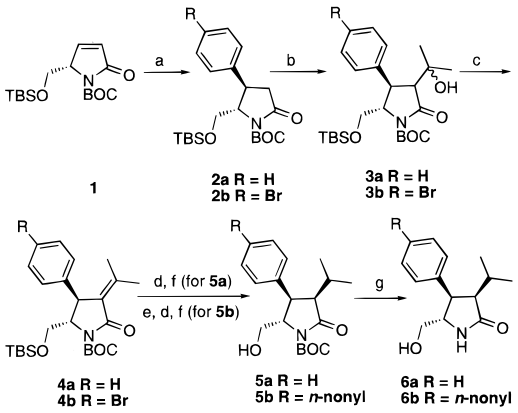


Figure 2. Strategy for simplification of the ILV structure.

Scheme 1[a]



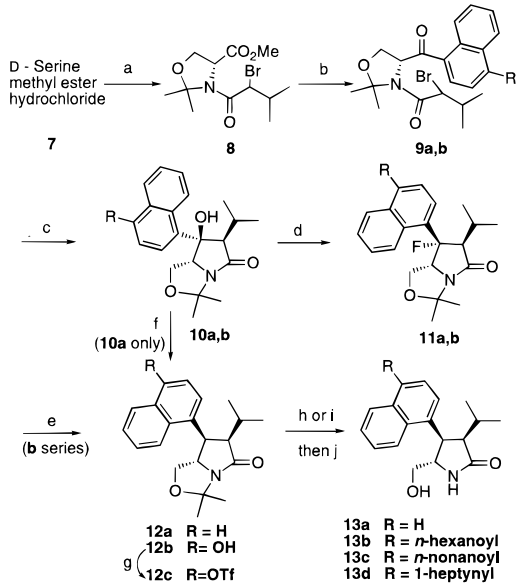
[a] Key: (a) *p*-R-C_6H_4MgBr, CuI (cat.), TMSCl, HMPA, −20 °C, 70%; (b) $LiN(TMS)_2$, $Mg(ClO_4)_2$ (2 equiv), acetone, THF, 84%; (c) 1. $Et_3N^+SO_2N^-CO_2Me$, C_6H_6, 60 °C; 2. DBU, toluene, reflux, 93% (2 steps); (d) Bu_4NF, THF, 96%; (e) 1-nonyne, $PdCl_2(PPh_3)_2$ (cat.), CuI (cat.), Et_3N, DMF, 80 °C, 85%; (f) H_2, 10% Pd/C, EtOH, 100%; (g) CF_3COOH, CH_2Cl_2, 94%.

Table 1. Log WS Values and K_i Values for the Inhibition of [^{3}H]PDBu Binding from Recombinant PKCα by the Compounds Tested

	log WS	$K_i \pm$ SEM		log WS	$K_i \pm$ SEM
6a	0.5	129 ± 13 μM	**13b**	−3.5	506 ± 38 nM
6b	−3.8	2.29 ± 0.15 μM	**13c**	−4.5	296 ± 12 nM
13a	−1.3	5.50 ± 0.94 μM	**13d**	−4.9	1.50 ± 0.18 μM

At this stage, it was appropriate to introduce a hydrophobic side chain likely to lead to a further enhancement in activity. The 4-benzyloxy-substituted α-naphthyl Grignard reagent was used with the notion to employ the oxygen substitutent to introduce various side chains later in the synthesis. The Barton reaction on intermediate **10b**, however, failed to give the product of inverted C-4′ configuration. In contrast, fluorination of the tertiary alcohol with DAST gave rise to compound **11b**, possessing the naphthyl and isopropyl groups in a *cis*-relationship. Debenzylation/defluorination of **11b** was successfully achieved by hydrogenation over 5% Pd/C in methanol affording **12b**. In the ^{1}H NMR spectrum, the methine proton of the isopropyl group in **12b** remained shielded by the naphthyl ring, thus supporting the *cis*-relationship of the isopropyl and naphthyl substituents. Subsequently, derivatives **13b**−**d** were synthesized as shown in Scheme 2.

Scheme 2[a]



[a] Key: (for compounds **9**−**11**, **a** series, R = H; **b** series, R = OBn). (a) 1. α-bromoisovaleryl chloride, Et_3N (2 equiv), $CHCl_3$, 23 °C, 80%; 2. $Me_2C(OMe)_2$, PPTS (cat.), toluene, reflux, 77%; (b) 1. (*i*-Bu)$_2$AlH, CH_2Cl_2, −70 °C, 87%; 2. Grignard reagent (2 equiv), THF, −78 to 0 °C, 74% (**9a**), 55% (**9b**); 3, NMO, TPAP (cat.), 4 Å M.S., CH_2Cl_2, 23 °C, 85%; (c) SmI_2 (3 equiv), THF−HMPA, $FeCl_3$ (cat.), 23 °C, 91% (**10a**), 80% (**10b**); (d) DAST (2 equiv), CH_2Cl_2, −78 °C, 80%; (e) H_2, MeOH, 5% Pd/C, 90%; (f) 1. NaH, CS_2, MeI; 2. Bu_3SnH, AIBN (cat.), benzene, reflux, 35%; (g) Tf_2O, 2,6-lutidine, CH_2Cl_2, 75%; (h) acyl chloride, pyridine, 23 °C, 80−93%; (i) 1-heptyne, $PdCl_2(PPh_3)_2$ (0.1 equiv), CuI (cat.), Et_3N, DMF, 60 °C, 61%; (j) 1,2-ethanedithiol (10 equiv), $BF_3{\cdot}Et_2O$ (2 equiv), CH_2Cl_2, 23 °C, 60−85%.

Compounds **6** and **13** have been evaluated for their ability to displace phorbol 12,13-dibutyrate (PDBU) binding from recombinant PKCα (Table 1). As is apparent, the introduction of a lipophilic side chain improves the potency. Compound **6b** is 56-fold more potent than **6a**, and **13c** is 18-fold more potent than **13a**. Comparison of **6a** with **13a** reveals the importance of the hydrophobic interactions of the extra aromatic ring in partially compensating for the absence of the *N*-methyl group present in ILV. Compound **13c**, the most active compound in this series of γ-lactams, is 435-fold more potent than the prototype **6a**.

Further efforts aimed at improving compound potency as well as an investigation of their selectivity for specific members of the DAG superfamily are underway.

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Supporting Information Available: Spectral data for compounds **2**−**13**, X-ray data for **10a** and **11a**, hydrogen-bond parameters, hydrophobic interaction analysis, and biological methods (39 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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