Communication

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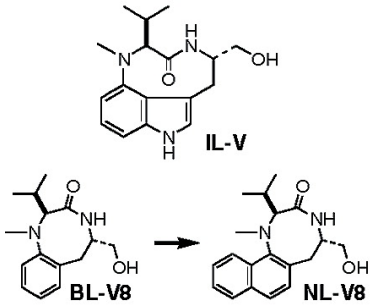

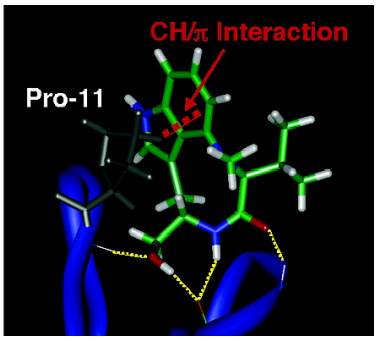

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Indolactam-V Is Involved in the CH/π Interaction with Pro-11 of the PKCδ C1B Domain: Application for the Structural Optimization of the PKCδ Ligand

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Protein kinase C (PKC) isozymes are the main receptors of tumor promoters such as phorbol esters and teleocidins.[1] PKC isozymes are subdivided into three classes: conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ϵ, η, θ), and atypical PKCs (λ/ι, ζ).[2,3] Tumor promoters bind to the C1A and/or C1B domains of conventional and novel PKCs. Since tumor promoters are structurally quite different from each other, the binding mode of each tumor promoter with each C1 domain of the PKC isozymes has attracted much attention from medicinal chemists. X-ray analysis of the crystal structure of the isolated PKCδ C1B domain in complex with phorbol 13-acetate revealed how the phorbol ester binds to the PKCδ C1B domain.[4] A docking simulation of indolactam-V (IL-V, Figure 1),[5,6] the core structure of teleocidins, with the PKCδ C1B domain suggested that the hydroxyl group, amide carbonyl group, and amide hydrogen of IL-V are involved in hydrogen bonding with Thr-12, Leu-21, and Gly-23 of the PKCδ C1B domain.[7,8] A similar hydrogen bond network was observed in the docking model of the simplified analogue of IL-V, benzolactam-V8 (BL-V8),[9] in which the indole ring of IL-V is replaced with a benzene ring.[7] Several mutational studies supported these models and revealed that Pro-11, which is conserved among PKC isozymes, plays important roles in IL-V binding.[8] However, it remains unclear whether Pro-11 mutation causes a conformational change in the binding site or loss of the hydrophobic interaction with IL-V. Moreover, these models cannot clearly explain the fact that the binding affinity of IL-V for PKCδ is more than 20 times higher than that of BL-V8.

To interpret the large difference in the binding affinity between IL-V and BL-V8, we wanted to provide evidence that IL-V may be involved in the CH/π interaction[10] with Pro-11 of the PKCδ C1B domain, whereas BL-V8 may not. The results suggested that lack of this CH/π interaction might be the main reason for the low binding affinity of BL-V8. The structural optimization of BL-V8 is also described as an application of this concept to the design of a potent PKCδ ligand.

To predict the amino acid residues arising from PKCδ interacting with the aromatic rings of IL-V and BL-V8, docking simulations of IL-V and BL-V8 with the crystal structure of the PKCδ C1B domain[4] were performed using the FlexX program[11] (Figure 2). The resultant docking models were quite similar to those reported by Endo et al.[7] and Wang et al.[8] The aromatic rings of both ligands were close to the hydrogen atom at position 5 of Pro-11 of the PKCδ C1B domain (2.7 Å). On the other hand, the distance between the aromatic ring of each ligand and the hydrogen atom at position 4 was quite different (2.5 Å for IL-V and 3.3 Å for BL-V8). Since a hydrogen atom and an aromatic ring are involved in the CH/π interaction only on the condition that their distance is within 3.05 Å,[10] these results suggest that IL-V may be involved in the CH/π interaction with the hydrogen atoms at positions 4 and 5 of Pro-11 of the PKCδ C1B domain, but BL-V8 may interact only with the hydrogen atom at position 5.

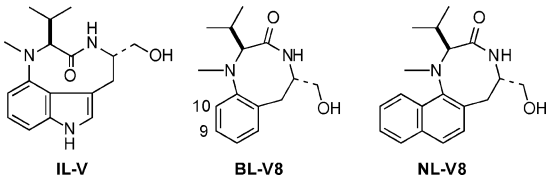

Figure 1. Structures of IL-V, BL-V8, and NL-V8.

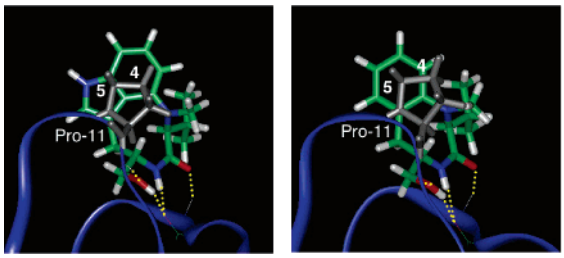

Figure 2. Docking models of IL-V (left) and BL-V8 (right) with the PKCδ C1B domain. Yellow dotted lines represent hydrogen bonds.

Substitution of the hydrogen atom involved in the CH/π interaction with a fluorine atom is one of the reliable methods to evaluate the CH/π interaction.[12] Since a fluorine atom has an extremely higher electronegativity but a similar van der Waals radius compared to that of a hydrogen atom, substitution with a fluorine atom can inhibit the CH/π interaction of the target hydrogen atom without causing a significant conformational change of the whole protein. To examine the existence and importance of the CH/π interaction in the binding of IL-V and BL-V8 to the PKCδ C1B domain, a mutant peptide of the PKCδ C1B domain, in which Pro-11 was replaced by 4,4-difluoro-Pro,[13] was prepared by solid-phase Fmoc synthesis, as reported previously.[14] The binding affinities of IL-V and BL-V8 for the wild-type and the mutant peptides of the PKCδ C1B domain, designated as δ-C1B and δ-C1B(P11dfP), respectively, were evaluated by the inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to these peptides by the method reported previously.[15] Table 1 shows the inhibition constants (K_i) of IL-V and BL-V8 for δ-C1B and δ-C1B-(P11dfP).

IL-V showed about a 10 times lower affinity for δ-C1B(P11dfP) compared to that for δ-C1B. In contrast, the binding affinity of BL-V8 for δ-C1B(P11dfP) was almost equal to that for δ-C1B. These results strongly suggest that IL-V could be involved in the CH/π interaction with the hydrogen atom at position 4 of Pro-11, and the low binding affinity of BL-V8 could be mainly ascribable to the absence of this CH/π interaction.

On the basis of these results, the structural optimization of BL-V8 was carried out. The benzene part of the indole ring of IL-V

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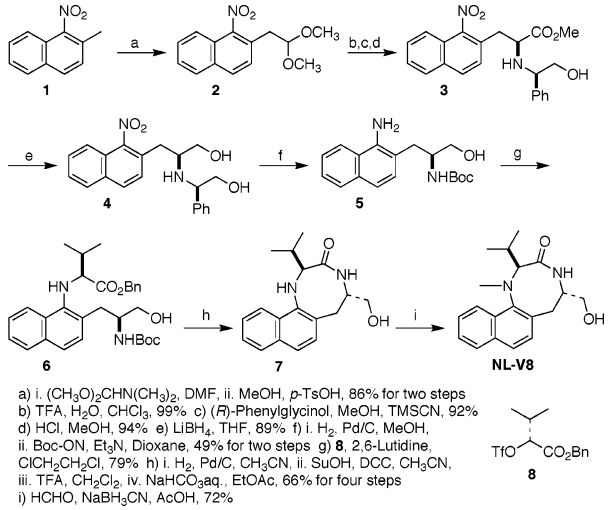
Table 1. K_i Values (nM) of the Inhibition of [^{3}H]PDBu Binding by IL-V, BL-V8, and NL-V8[a]

peptides	IL-V	BL-V8	NL-V8
δ-C1B	11.4 (1.0)[b]	414 (28)	44.1 (4.8)
δ-C1B(P11dfP)	131 (9.5)	436 (41)	139 (20)

[a] The K_d values for δ-C1B and δ-C1B(P11dfP) were 0.53 and 3.5 nM, respectively. Although these values were slightly different from each other, a significant conformational change by the mutation would not possibly occur since the K_i values of BL-V8 for both peptides were almost similar. [b] Standard deviation of at least two separate experiments.

Scheme 1. Synthesis of NL-V8



was deduced to interact with the hydrogen atom at position 4 of Pro-11 from the docking model (Figure 2). Most of the benzene part of IL-V is located outside positions 9 and 10 of the benzene ring of BL-V8 when the lactam rings of both compounds are superimposed.[7] Since the addition of another benzene ring to positions 9 and 10 of BL-V8 was deduced to amplify the binding affinity to PKCδ by the CH/π interaction, naphtholactam-V8 (NL-V8) was designed (Figure 1).

NL-V8 was synthesized from 1-methyl-2-nitronaphthalene (**1**), as shown in Scheme 1. Treatment of **1** with dimethylformamide dimethylacetal followed by methanolysis of an imine gave a dimethylacetal (**2**). After hydrolysis of the acetal, the asymmetric Strecker reaction[16] was applied to the resulting aldehyde to give an (*R*)-amino nitrile diastereoselectively. The nitrile group was converted to the methyl ester (**3**), which was then reduced with lithium borohydride. Reduction of the nitro group and removal of the phenylethanol group of **4** were achieved by hydrogenation. The resulting amino group was protected with a Boc group to give an amino alcohol (**5**). After the S_N2 substitution of **5** with the valine-derived triflate (**8**),[17] the eight-membered lactam (**7**) was formed by the method of Endo et al.[9] Reductive methylation of **7** gave NL-V8 at a total yield of 12%.

The binding affinity of NL-V8 for δ-C1B was about 10 times higher than that of BL-V8 (Table 1). A similar result was observed for whole PKCδ; the K_i value of NL-V8 for PKCδ was 147 $\pm$ 18 nM, which was about 12 times smaller than that of BL-V8 (1700 nM), reported by Endo et al.[7] On the other hand, NL-V8 showed an affinity to δ-C1B(P11dfP) about 4 times lower than that for δ-C1B. These results indicate that the higher affinity of NL-V8 compared to that of BL-V8 could be partially attributed to the CH/π interaction between the additional benzene ring and the hydrogen atom at position 4 of Pro-11.

Our present data provide evidence that the CH/π interaction plays a pivotal role in the binding of IL-V and its analogues to the PKCδ C1B domain. It was also shown that the binding affinity of BL-V8 could be enhanced by the effective formation of the CH/π interaction. The information presented in this communication is useful for the verification of the docking model of IL-V with the PKCδ C1B domain and for the rational design of new potent ligands for PKCδ, which has a tumor suppresser role.[18]

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Supporting Information Available: Modeling methods 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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