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BINDING SELECTIVITY OF 1- OR 12- SUBSTITUTED INDOLACTAM DERIVATIVES FOR PROTEIN KINASE C ISOZYMES

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Abstract – The selectivity with which 1- or 12-substituted analogues of indolactam-V (**1**) bind protein kinase C (PKC) isozymes was examined. Moderate selectivity for novel PKC isozymes over conventional PKC isozymes was observed in the case of indolactam-*n*V (**13**) and indolactam-L (**14**) without an α-branched side chain at position 12. The introduction of a bulky isopropyl group to position 1 of **1** drastically increased the selectivity for novel PKC isozymes.

INTRODUCTION

Protein kinase C (PKC) comprises a family of serine/threonine kinases involved in various cellular events such as differentiation, proliferation and apoptosis.[1] The PKC family consists of 10 isozymes that are subdivided into 3 classes (Figure 1) based on their structure and mechanism of activation.[2] Conventional PKC isozymes (cPKCs: α, βI, βII, γ) and novel PKC isozymes (nPKCs: δ, ε, η, θ) contain tandem C1 domains (C1A, C1B), which play a critical role in the activation by endogenous

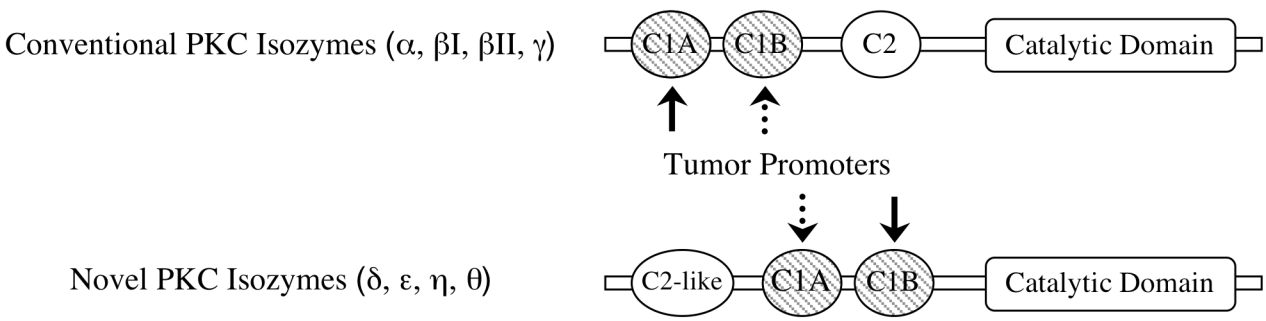
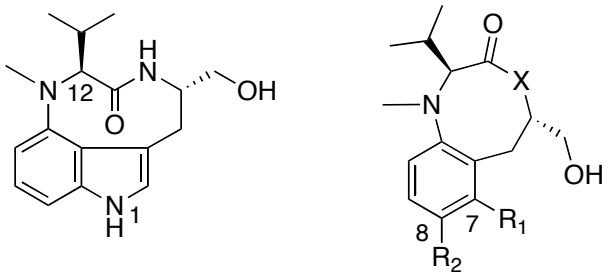


Figure 1. PKC isozymes. Solid arrows indicate main tumor promoter-binding domains.



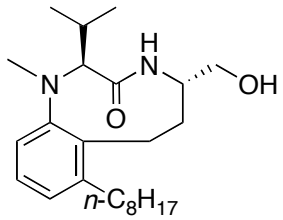
indolactam-V (**1**)

X = NH, R_1, R_2 = H (**2**)

X = NH, R_1 = H, R_2 = *n*-decynyl (**3**)

X = NH, R_1 = *n*-decyl, R_2 = H (**4**)

X = O, R_1 = H, R_2 = *n*-decyl (**5**)



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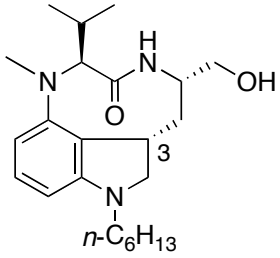

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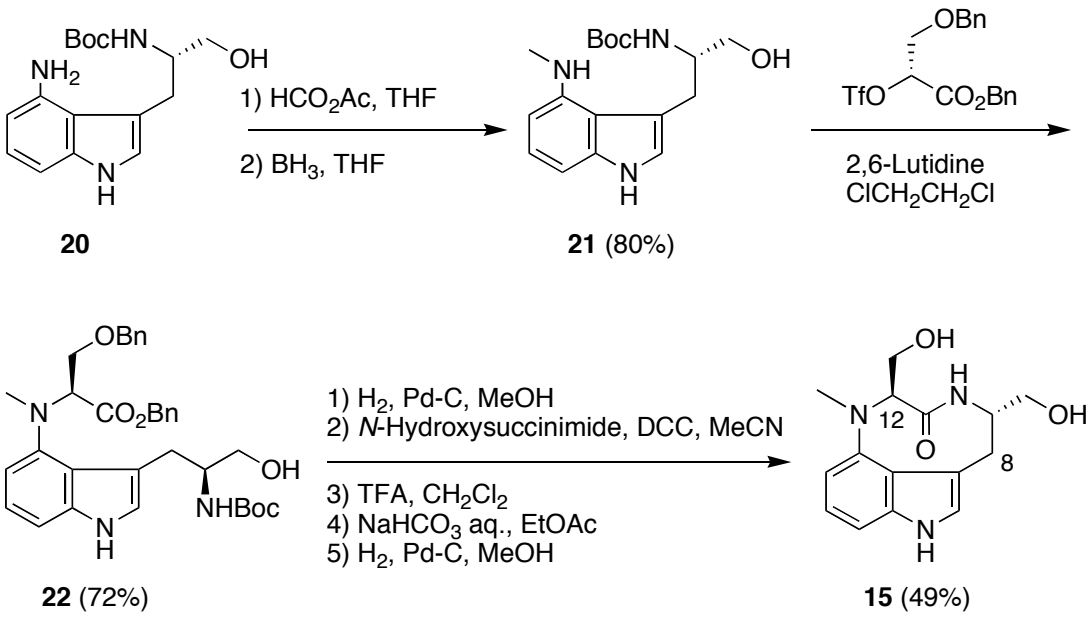
Figure 2. Structures of indolactam-V and PKC isozyme-selective analogues.[9-12]

1,2-diacylglycerol (DAG). In addition to DAG, the binding of calcium ion to the C2 domain also evokes the activation of cPKCs, while the C2-like domains in nPKCs lack the ability to bind calcium ion. Atypical PKCs (ζ, λ/ι) bind neither DAG nor calcium ion. Since tumor promoters such as phorbol esters and teleocidins bind to the C1 domains in a manner similar to DAG, cPKCs and nPKCs are main targets of tumor promoters.[3]

Although the precise mechanism of tumor promotion is not fully understood, recent studies have revealed that several nPKCs play a key role. Skin tumor promotion was suppressed in both PKCδ and PKCε transgenic mice,[4,5] and knockout of PKCη in mice resulted in enhancement of tumor promotion.[6] On the other hand, PKCε overexpression caused the development of carcinoma.[5] Since phorbol esters and teleocidins activate all cPKCs and nPKCs non-selectively, the development of analogues of tumor promoters with selectivity for nPKCs is strongly desired for further analysis of the mechanism of tumor promotion.

Indolactam-V (**1**)[7] and benzolactam-V8 (**2**)[8] have received much attention as lead compounds for the development of new agents with selectivity for nPKCs because of their simple structures and ease of derivatization (Figure 2). Hitherto, several compounds with selectivity for certain PKC isozymes have been developed. Compound **3** showed some cPKC selectivity[9] and **4** showed slight nPKC selectivity.[10] Compounds **5** and **6** exhibited marked selectivity for nPKCs over cPKCs.[11] The (3*S*)-1-hexyl indoline derivative of **1** (**7**) also exhibited moderate nPKC selectivity.[12] However, these modifications on the indole ring often reduced the absolute ability to bind PKC, that would make it difficult to adopt these



Scheme 1. Synthesis of indolactam-S (**15**).

compounds for *in vivo* analysis of PKC function. We tried to explore nPKC-selective analogues with an intact indolactam skeleton. Previous structure-activity studies of **1** revealed that substitution of the isopropyl group at position 12 with hydrophobic groups and the introduction of alkyl groups to position 1 of **1** increased the affinity for PKC.[13-15] However, the profiles of 1- or 12-substituted analogues of **1** remain unknown because their binding to PKC was evaluated using a mixture of several isozymes. Recently, we established a PKC C1 peptide library for the rapid screening of new PKC isozyme selective compounds.[16] Using this library, the profiles of 1- or 12-substituted analogues of **1** were examined.

RESULTS AND DISCUSSION

The 12-substituted analogues of **1** with hydrophobic groups (**8** – **14**) were prepared by microbial conversion using *Streptomyces blastmyceticum* as reported previously (Figure 3).[14] However, those with hydrophilic groups could not be obtained by this method. In order to examine the effects of a hydrophilic group at position 12 of **1** on both PKC binding and isozyme selectivity, indolactam-S (**15**) with a hydroxymethyl group at position 12 was separately synthesized by the method of Endo *et al.*[8,17] and Kogan *et al.*[18] with slight modifications (Scheme 1).

Monomethylation of **20** gave **21** (80% in two steps), which was then treated with D-serine-derived triflate (72%) prepared from *O*-benzyl-D-serine by the method of Kogan *et al.*[18] The nine-membered lactam ring was constructed by the activated ester method,[8] and cleavage of the benzyl ether gave **15** (49% in five steps), whose conformation was analyzed by NMR spectroscopy. Indolactam-V (**1**) exists in an equilibrium of two stable conformers in a solution at room temperature: an active twist form with the

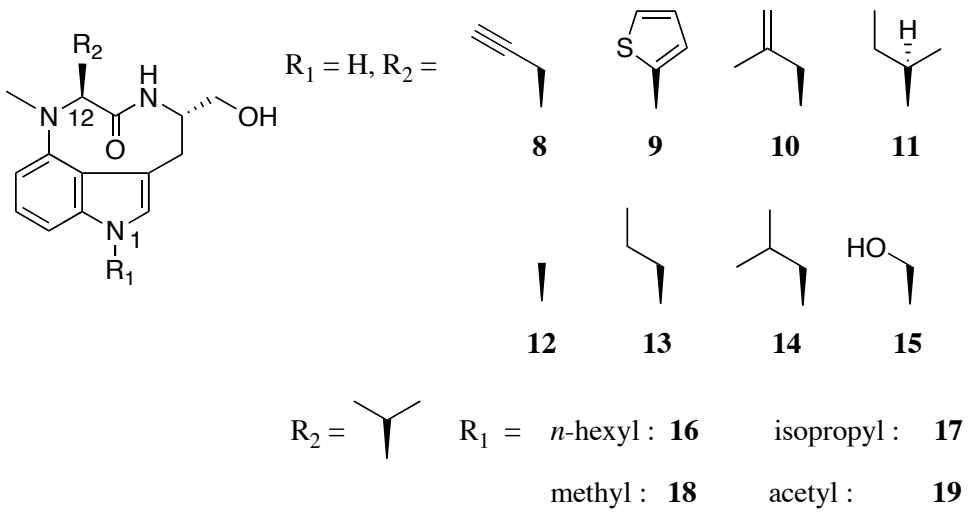


Figure 3. Structures of 1- or 12-substituted indolactam derivatives.

Table 1. K_i values for inhibition of the specific binding of [^{3}H]PDBu by 12-substituted derivatives (**8** - **15**) of indolactam-V (**1**).

PKC C1 peptide	K_i (nM)								
	1[a]	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
α-C1A	21 (1.0)[b]	84 (3.9)	34 (8.5)	35 (7.0)	17 (3.3)	390 (22)	110 (5)	63 (4.8)	ND[c]
β-C1A	19 (4.5)	89 (15)	25 (6.4)	31 (4.3)	13 (2.2)	292 (1)	100 (0.1)	87 (3.7)	ND
γ-C1A	89 (3.9)	948 (77)	94 (5.4)	152 (3)	56 (2.5)	1800 (3)	120 (8)	90 (7.2)	ND
δ-C1B	11 (0.6)	46 (5.7)	4.5 (0.1)	5.1 (0.8)	2.8 (0.2)	81 (3.3)	7.1 (0.1)	5.8 (0.8)	ND
ε-C1B	7.7 (1.2)	80 (17)	2.9 (0.8)	4.3 (0.5)	2.4 (0.2)	32 (2.7)	7.3 (0.5)	4.1 (0.3)	ND
η-C1B	5.5 (0.6)	16 (1.8)	3.2 (0.7)	2.6 (0.1)	1.4 (0.1)	36 (2.7)	4.4 (0.1)	2.5 (0.3)	ND
θ-C1B	8.7 (1.2)	36 (1.0)	4.4 (0.5)	5.5 (0.4)	2.7 (0.2)	41 (2.0)	7.2 (0.2)	4.0 (0.1)	ND

[a] Cited from Ref. 22.
[b] Standard deviation of triplicate experiments
[c] Specific binding of [^{3}H]PDBu was not inhibited by **15** even at 10 μM

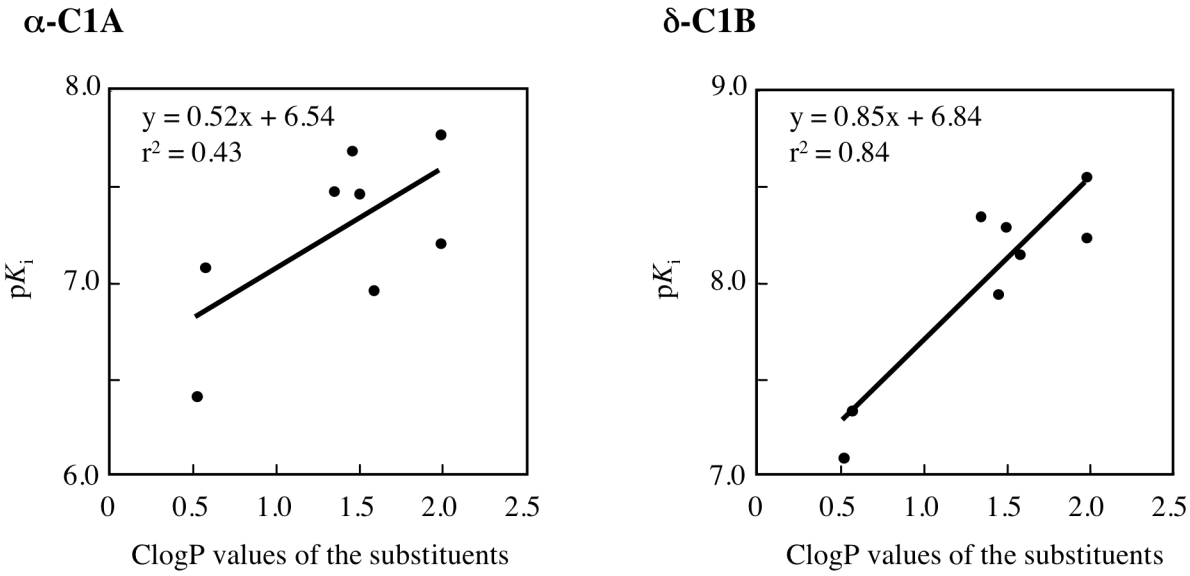
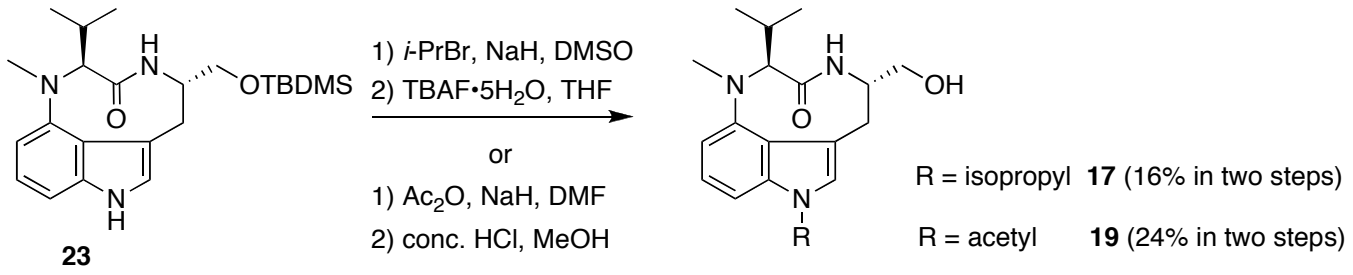


Figure 4. Relationship between the ClogP values of the substituents of the indolactam derivatives and log $1/K_i$ (pK_i). The ClogP values of the substituents of **1** and 12-substituted indolactam (**8** - **14**) derivatives were plotted against the pK_i of those compounds for α-C1A and δ-C1B. The ClogP values were calculated using the ClogP module in ChemDraw Ultra 8.0 (Cambridge Soft).

cis-amide and an inactive sofa form with the *trans*-amide.[8,17,19] Compound **15** existed in a single conformation in CD_3OD, and a significant NOESY cross-peak was observed between hydrogen atoms at positions 8 (δ 3.07) and 12 (δ 4.58), which is characteristic for the twist form.[17] These results indicate that the conformation of **15** was similar to the twist form of **1** with PKC binding ability.

The affinity of these derivatives for PKC isozymes was evaluated by inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to each PKC C1 peptide.[20] Recent investigations suggest that C1A domains in cPKCs are mainly involved in phorbol ester binding and translocation from the cytosol to plasma membrane, while C1B domains in nPKCs play a critical role in these phenomena.[21] The binding affinity was, therefore, measured using the C1A peptides of cPKCs and the C1B peptides of nPKCs. Table 1 shows the inhibition constants (K_i) of a series of the 12-substituted derivatives. For nPKCs (δ, ε, η, θ), the more hydrophobic analogues showed higher affinity, and hydrophilic **15** did not bind at all. In contrast, a significant correlation between the binding affinity and hydrophobicity of the 12-substituents was not observed in cPKCs (α, β, γ) (Figure 4). All analogues except for **11** showed less affinity for cPKCs than did **1**, suggesting their increased selectivity for nPKCs. The highest selectivity was observed in **13** and **14** with alkyl substituents containing no α-branching, being about 25 - 35 times selective for nPKCs over cPKCs. Docking simulation of indolactam-V (**1**) with the PKCδ C1B domain suggested that the isopropyl group at position 12 of **1** might be close to the side chain of Leu24 of the PKCδ C1B domain.[23] PKCα- and β-C1A domains have aromatic Phe residues at position 24, while other C1 domains have aliphatic Leu or Ile residues at position 24. This difference might



Scheme 2. Synthesis of **17** and **19**.

result in the relatively low affinity of **13** and **14** for α- and β-C1A.

Next, we examined the effects of substituents at position 1 on the selectivity for PKC isozymes. We previously reported that the 1-*n*-hexyl analogue of **1** (**16**) showed moderate selectivity (4 - 80 fold) for nPKCs.[12] Since steric and/or hydrophobic factors of the *n*-hexyl group seemed important to the affinity for nPKCs, we evaluated the PKC isozyme-selectivity profiles of a series of 1-substituted indolactam derivatives with a bulky isopropyl group (**17**), a less-hydrophobic methyl group (**18**),[15] and a hydrophilic acetyl group (**19**). Compounds **17** and **19** were synthesized from 14-*O*-TBDMS-indolactam-V (**23**)[24] by conventional methods (Scheme 2).

Table 2. K_i values for inhibition of the specific binding of [^{3}H]PDBu by the 1-substituted derivatives (**16** - **19**) and indolactam-V (**1**).

PKC C1 peptide	K_i (nM)				
	16[a] (*n*-hexyl)	**17** (isopropyl)	**18** (methyl)	**19** (acetyl)	**1**[b]
α-C1A	5.8 (1.1)[c]	2600 (90)	140 (7)	700 (90)	21 (1.0)
β-C1A	9.8 (1.6)	3600 (320)	140 (18)	1100 (50)	19 (4.5)
γ-C1A	18 (2.4)	6000 (260)	220 (4)	2400 (260)	89 (3.9)
δ-C1B	0.22 (0.04)	32 (1.1)	5.4 (0.7)	94 (4)	11 (0.6)
ε-C1B	0.47 (0.12)	42 (1)	6.5 (0.4)	220 (13)	7.7 (1.2)
η-C1B	0.34 (0.11)	8.7 (0.6)	2.4 (0.2)	78 (4.6)	5.5 (0.6)
θ-C1B	1.41 (0.03)	25 (0.9)	3.7 (0.4)	120 (14)	8.7 (1.2)

[a] Cited from Ref. 12.
[b] Cited from Ref. 22.
[c] Standard deviation of triplicate experiments.

K_i values of 1-substituted analogues for PKC C1 peptides are summarized in Table 2. Similar to the disappearance of the PKC-binding ability of **15**, introduction of the hydrophilic acetyl group to position 1 of **1** caused a significant reduction in the ability to bind PKC. The 1-methyl derivative (**18**) showed about 2 times higher affinity for nPKCs and 3–7 times lower affinity for cPKCs than **1**. The selectivity for nPKCs of **18** was comparable to that of the 1-*n*-hexyl derivative (**16**). Marked selectivity was observed for the 1-isopropyl derivative (**17**); the K_i value of **17** for η-C1B was about 400 - 700 times smaller than that for the C1A peptides of cPKCs. It is noteworthy that the selectivity of **17** was comparable to that of previously reported nPKC-selective analogues (**5** - **7**), but that **17** showed significantly higher affinity for nPKCs.

Recently, we have revealed that the indole ring of **1** could be involved in the CH/π interaction[25] with the hydrogen atom at position 4 of Pro-11 of the PKCδ C1B domain (Figure 5).[26] The CH/π interaction is an attractive molecular force occurring between CH groups and π-electron systems, and plays important roles in protein folding, substrate recognition, and molecular assemblies.[25] Although Pro-11 is conserved among all PKC C1 domains, there might be a difference in the spatial position of Pro-11 among PKC C1 domains. We assumed that the bulky 1-isopropyl group of **17** might severely inhibit the CH/π interaction, especially in cPKC, resulting in the marked selectivity of **17** for nPKCs.

To test this hypothesis, we evaluated the CH/π interaction of **1** or **17** with δ-C1B or γ-C1A using mutant C1 peptides, in which Pro-11 was replaced with 4,4-difluoro-Pro (dfP). Since a fluorine atom has greater electronegativity than but a similar van der Waals radius to a hydrogen atom, the fluorine substitution could inhibit the CH/π interaction.[27] K_i values of **1** and **17** for the mutant C1 peptides (P11dfP) are listed in Table 3. Compound **17** showed more than 10 times less affinity for δ-C1B(P11dfP) than the wild-type δ-C1B, suggesting **17** as well as **1** to be involved in the CH/π interaction with Pro-11 of δ-C1B. In contrast, **17** showed an affinity for γ-C1A(P11dfP) that was only

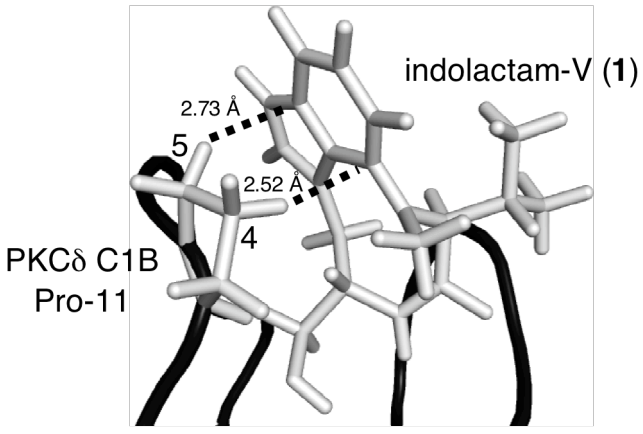


Figure 5. The predicted binding mode of indolactam-V (**1**) with PKCδ C1B domain.[26] Solid lines represent the CH/π interaction.

Table 3. K_i values of **1** and **17** for wild-type and fluorine-substituted C1 peptides of PKC δ and γ.

	K_i (nM)					
Comopunds	δ-C1B	δ-C1B (P11dfP)	ratio	γ-C1A	γ-C1A (P11dfP)	raito
1	11 (0.6) [a]	130 [b] (10)	11.8	89 (3.9)	690 (73)	7.8
17	32 (1.1)	570 (27)	17.8	6000 (260)	19600 (600)	3.3

[a] Standard deviation of triplicate experiments.
[b] Cited from Ref. 26.

about 3 times lower than that for wild-type γ-C1A, whereas **1** showed about 8 times less affinity for the mutant γ-C1A than wild-type γ-C1A. Although the ratio of K_i values of **17** for wild-type and mutant γ-C1A might not be precise because of its weak affinities, the difference between the CH/π interaction of **1** and **17** with γ-C1A seems significant. These results suggest that steric hindrance of the isopropyl group at position 1 of **17** and γ-C1A might have selectively lowered the CH/π interaction between the indole ring of **17** and γ-C1A.

In conclusion, effects of the substituents at position 1 or 12 of indolactam-V (**1**) on PKC isozyme-selectivity profiles can be summarized as follows. Among the 12-substitued analogues examined in this study, indolactam-*n*V (**13**) and indolactam-L (**14**) without an α-branched side chain showed moderate selectivity for nPKCs. The 1-substituted analogues of **1** exhibited much higher selectivity. Notably, **17** showed marked selectivity with greater binding ability than the previous nPKC selective-analogues (**5** - **7**). The nPKC selectivity of **17** might be partly ascribable to selective reduction of the CH/π interaction between the indole ring of **17** and cPKC C1A domains. Compound **17** could be a promising probe with which to analyze the function of nPKCs.

EXPERIMENTAL

General remarks

The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; Digital Polarimeter, Jasco DIP-1000; ^{1}H and ^{13}C NMR, JEOL ECP500 and Bruker AVANCE400 (ref. TMS); HPLC, Waters Model 600E with a Model 2487 UV detector; (HR) EI-MS, JEOL JMS-600H. HPLC was carried out on a YMC packed SH-342-5 (ODS, 20 mm i.d. × 150 mm) column (Yamamura Chemical Laboratory). Wakogel C-200 (silica gel, Wako Pure Chemical Industries) was used for column chromatography. [^{3}H]PDBu (16.3 Ci/mmol) was purchased from PerkinElmer Life Sciences Research

Products. All other chemicals and reagents were purchased from chemical companies and used without further purification.

Synthesis of 12-substituted indolactam derivatives (8 - 14)

Compounds **8** - **14** were obtained by microbial conversion of their seco compounds.[28] Spectroscopic data on **11** - **14** were reported previously.[28] Cyclization yields of each precurser of **8**, **9** and **10** were 6.8, 3.0 and 2.9%, respectively. $[\alpha]_D$ of **9** could not be measured because of its small amount.

Compound 8 : $[\alpha]_D$ −385° (*c* = 0.081, MeOH, 19.0 °C); UV λ_{max} (MeOH) nm (ε) 298 (7,800), 286 (7,500), 228 (30,700); ^{1}H NMR δ (CD_3OD, 0.008 M, 400 MHz, twist only) ppm: 2.04 (1H, t, *J* = 2.4 Hz), 2.50 (1H, m), 2.79 (3H, s), 2.80 (1H, m), 3.03 (1H, br.d, *J* = 17.4 Hz), 3.12 (1H, dd, *J* = 17.4, 4.0 Hz), 3.51 (1H, dd, *J* = 11.3, 8.9 Hz), 3.65 (1H, dd, *J* = 11.3, 4.6 Hz), 4.54 (1H, m), 4.90 (1H, t, *J* = 7.0 Hz), 6.56 (1H, d, *J* = 6.7 Hz), 6.96 (3H, m); HR-EI-MS *m/z*: 297.1472 (M^+, calcd for $C_{17}H_{19}N_3O_2$, 297.1478).

Compound 9 : UV λ_{max} (MeOH) nm (ε) 296 (6,600), 287 (6,500), 227 (30,000); ^{1}H NMR δ (CD_3OD, 0.006 M, 400 MHz, twist only) ppm: 2.65 (3H, s), 3.13 (1H, dd, *J* = 17.1, 4.3 Hz), 3.24 (1H, dd, *J* = 17.1, 4.9 Hz), 3.64 (1H, dd, *J* = 11.0, 7.3 Hz), 3.74 (1H, dd, *J* = 11.0, 5.2 Hz), 5.04 (1H, m), 5.84 (1H, s), 6.52 (1H, d, *J* = 7.9 Hz), 6.82 (1H, d, *J* = 7.9 Hz), 6.93 (1H, dd, *J* = 4.9, 3.7 Hz), 7.00 (1H, t, *J* = 7.9 Hz), 7.02 (1H, d, *J* = 7.6 Hz), 7.36 (1H, d, *J* = 4.9 Hz); HR-EI-MS *m/z*: 341.1199 (M^+, calcd for $C_{18}H_{27}N_3O_2S$, 341.1198).

Compound 10 : $[\alpha]_D$ −424° (*c* = 0.87, MeOH, 25.0 °C); UV λ_{max} (MeOH) nm (ε) 299 (6,900), 289 (6,500), 228 (25,300); ^{1}H NMR δ ($CDCl_3$, 0.084 M, 400 MHz, twist only) ppm: 1.42 (3H, s), 2.42 (1H, dd, *J* = 14.7, 5.5 Hz), 2.68 (1H, dd, *J* = 12.7, 8.2 Hz), 2.85 (3H, s), 3.08 (1H, d, *J* = 17.1 Hz), 3.17 (1H, dd, *J* = 17.1, 4.0 Hz), 3.58 (1H, dd, *J* = 11.6, 7.3 Hz), 3.78 (1H, dd, *J* = 11.6, 3.7 Hz), 4.58 (1H, m), 4.67 (2H, d, *J* = 7.3 Hz), 4.93 (1H, dd, *J* = 7.9, 5.8 Hz), 6.52 (1H, d, *J* = 7.0 Hz), 6.88 (1H, br.s), 6.96 (1H, d, *J* = 7.3 Hz), 7.04 (1H, t, *J* = 7.9 Hz), 7.87 (1H, br.s); HR-EI-MS *m/z*: 313.1792 (M^+, calcd for $C_{18}H_{23}N_3O_2$, 313.1790).

Synthesis of indolactam-S (15)

To a solution of **20** (558 mg, 1.83 mmol) prepared from L-tryptophan methyl ester[17,18] in THF (1.8 mL) was added acetic formic anhydride (1.35 mL) at 0 ºC. The mixture was stirred at 0 ºC for 1 h and poured into saturated aqueous K_2CO_3 and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give an amide. To a solution of the amide in THF (19 mL) was added dropwise 1.0 M BH_3 in THF solution (6.9 mL) at 0 ºC, and the reaction mixture was stirred for 15 min at 0 ºC, then warmed to rt and stirred for a further 5 h. The reaction was quenched by the addition of 10% aqueous citric acid (3 mL), and the mixture was extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and

concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **21** (464 mg, 1.45 mmol, 80% in two steps). Compound **21**: $[\alpha]_D$ –13.4° (*c* = 0.92, MeOH, 28.2 °C); UV λ_{max} (MeOH) nm (ε) 299 (5,340), 278 (5,650), 251 (2,360), 226 (29,200); ^{1}H NMR δ ($CDCl_3$, 0.094 M, 400 MHz, 297 K) ppm: 1.46 (9H, s), 2.95 (4H, m), 3.22 (1H, dd, *J* = 14.5, 4.0 Hz), 3.53 (2H, m), 3.75 (1H, m), 4.09 (1H, br.s), 5.37 (1H, d, *J* = 6.2 Hz), 6.32 (1H, d, *J* = 7.9 Hz), 6.80 (1H, d, *J* = 7.9 Hz), 6.84 (1H, s), 7.07 (1H, t, *J* = 7.9 Hz), 8.08 (1H, br.s); ^{13}C NMR δ ($CDCl_3$, 0.063 M, 100 MHz, 298 K) ppm: 28.43, 28.69, 31.03, 54.36, 62.51, 79.52, 100.80, 102.27, 111.39, 116.26, 121.71, 123.40, 137.59, 143.73, 156.31; HR-EI-MS *m*/*z*: 319.1891 (M^+, calcd for $C_{17}H_{25}N_3O_3$, 319.1896).

A mixture of **21** (120 mg, 0.37 mmol), 2,6-lutidine (87.5 μL, 0.75 mmol) and D-serine-derived triflate (220 mg, 0.53 mmol) in 1,2-dichloroethane (1.2 mL) was refluxed at 70 ºC for 2 h and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **22** (158 mg, 0.27 mmol, 72%). Compound **22**: $[\alpha]_D$ –3.94° (*c* = 0.46, MeOH, 31.2 °C); UV λ_{max} (MeOH) nm (ε) 287 (7,300), 226 (30,300); ^{1}H NMR δ ($CDCl_3$, 0.027 M, 500 MHz, 313 K) ppm: 1.36 (9H, s), 2.98 (4H, m), 3.17 (1H, m), 3.46 (2H, m), 3.64 (1H, m), 3.88 (2H, m), 4.26 (1H, t, *J* = 5.7 Hz), 4.48 (2H, br.s), 5.06 (2H, br.s), 6.83 (1H, d, *J* = 7.6 Hz), 7.01 (2H, m), 7.11 (3H, m), 7.24-7.32 (8H, m), 8.23 (1H, br.s); HR-FAB-MS *m*/*z*: 588.3112 (MH^+, calcd for $C_{34}H_{42}N_3O_6$, 588.3074).

A mixture of **22** (73 mg, 0.124 mmol) and 10% Pd-C (7.3 mg) in MeOH (2.0 mL) was vigorously stirred under 1 atom of H_2 at rt for 1.5 h. The reaction mixture was filtered, and the filtrate was concentrated to give the crude carboxylic acid. To a mixture of the carboxylic acid and *N*-hydroxysuccinimide (28.5 mg, 0.25 mmol) in MeCN (1.0 mL) was added a solution of DCC (38.3 mg, 0.19 mmol) in MeCN (1.0 mL) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was filtered and then concentrated. The residue was dissolved in $CHCl_3$ and the solution was washed with water. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography on Wakogel C-200 using $CHCl_3$ and increasing amounts of MeOH to give the activated ester. A mixture of the activated ester and TFA (1.5 mL) in CH_2Cl_2 (3.5 mL) was stirred for 1.25 h at 0 °C. The solvent was then removed *in vacuo* to give the crude amine. To a solution of the amine in EtOAc (7 mL) was added saturated aqueous $NaHCO_3$ (1.2 mL). The reaction mixture was refluxed for 1.5 h. After cooling to rt, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on Wakogel C-200 using $CHCl_3$ and increasing amounts of MeOH to give a lactam. A mixture of the lactam and 10% Pd-C (14.5 mg) in MeOH (1.0 mL) was vigorously stirred under 1 atom of H_2 at rt for 5 h. The reaction mixture was filtered, and the filtrate was concentrated. The residue was purified by HPLC on YMC SH-342-5 using 40% MeOH to give **15** (5.8 mg, 0.020 mmol, 49% in

five steps). Compound **15**: $[\alpha]_D$ –251° (*c* = 0.29, MeOH, 31.2 °C); UV λ_{max} (MeOH) nm (ε) 296 (7,170), 283 (7,080), 228 (28,300); ^{1}H NMR δ (CD_3OD, 0.039 M, 400 MHz, 297 K, twist only) ppm: 2.86 (3H, s), 3.07 (2H, m), 3.52 (1H, dd, *J* = 11.2, 8.5 Hz), 3.66 (1H, dd, *J* = 11.2, 4.5 Hz), 3.87 (1H, dd, *J* = 11.3, 5.6 Hz), 4.13 (1H, dd, *J* = 11.3, 6.9 Hz), 4.58 (1H, t, *J* = 6.2 Hz), 4.74 (1H, m), 6.63 (1H, t, *J* = 4.0 Hz), 6.94 (1H, s), 6.98 (2H, m); ^{13}C NMR δ (CD_3OD, 0.039 M, 125 MHz, 298 K) ppm: 34.04, 34.91, 56.77, 59.81, 65.42, 70.09, 107.37, 109.11, 114.15, 121.69, 122.87, 123.59, 140.88, 147.65, 175.79; HR-FAB-MS *m/z*: 290.1501 (MH^+, calcd for $C_{15}H_{20}N_3O_3$, 290.1505).

Synthesis of 17

To a solution of 14-*O*-TBDMS-indolactam-V (**23**)[24] (0.073 mmol) in dry DMSO (0.3 mL) was added NaH (3.2 g, 0.08 mmol, 60% dispersion in mineral oil) and the mixture was stirred at rt for 30 min. Isopropyl bromide (7.53 μL, 0.08 mmol) was then added and the reaction mixture was stirred for 2 h. The mixture was poured into crushed ice and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give 1-*N*-isopropyl-14-*O*-TBDMS-indolactam-V, which was treated with TBAF·$5H_2O$ (31.8 mg, 0.1 mmol) in THF (0.2 mL). After stirring for 20 min at rt, the reaction mixture was poured into water and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc, followed by HPLC on YMC SH-342-5 using 75% MeOH to give **17** (4.1 mg, 0.012 mmol, 16% in two steps). Compound **17**: $[\alpha]_D$ –165° (*c* = 0.14, MeOH, 15.7 °C); UV λ_{max} (MeOH) nm (ε) 307 (9,420), 230 (29,200); ^{1}H NMR δ ($CDCl_3$, 0.024 M, 500 MHz, 295 K, twist:sofa = 4.3:1) ppm for the twist form: 0.64 (3H, d, *J* = 6.8 Hz), 0.93 (3H, d, *J* = 6.4 Hz), 1.45 (3H, d, *J* = 6.6 Hz), 1.53 (3H, d, *J* = 6.6 Hz), 2.60 (1H, m), 2.62 (1H, m), 2.91 (3H, s), 3.01 (1H, dd, *J* = 17.4, 3.7 Hz), 3.18 (1H, d, *J* = 17.4 Hz), 3.55 (1H, m), 3.74 (1H, m), 4.31 (1H, m), 4.40 (1H, d, *J* = 10.2 Hz), 4.59 (1H, quintet, *J* = 6.6 Hz), 6.50 (1H, d, *J* = 7.4 Hz), 6.89 (1H, d, *J* = 7.9 Hz), 6.89 (1H, d, *J* = 1.0 Hz), 7.07 (1H, t, *J* = 8.0 Hz), 7.10 (1H, br.s); for the sofa form: 0.94 (3H, d, *J* = 6.1 Hz), 1.24 (3H, d, *J* = 6.2 Hz), 1.51 (3H, d, *J* = 6.8 Hz), 1.52 (3H, d, *J* = 6.1 Hz), 2.40 (1H, m), 2.74 (3H, s), 2.81 (1H, dd, *J* = 14.5, 1.6 Hz), 2.98 (1H, d, *J* = 11.0 Hz), 3.10 (1H, dd, *J* = 14.5, 4.7 Hz), 3.40 (1H, m), 3.47 (1H, m), 4.44 (1H, m), 4.65 (1H, quintet, *J* = 6.8 Hz), 4.74 (1H, br.d, *J* = 11.5 Hz), 7.03 (1H, dd, *J* = 7.3, 1.0 Hz), 7.03 (1H, s), 7.18 (1H, t, *J* = 7.4 Hz), 7.27 (1H, d, *J* = 7.2 Hz); ^{13}C NMR δ ($CDCl_3$, 0.024 M, 125 MHz, 295 K) ppm for the twist form: 19.49, 21.60, 22.52, 22.78, 28.56, 33.08, 34.21, 46.64, 55.92, 65.15, 71.10, 102.23, 106.05, 113.00, 118.38, 120.84, 122.03, 138.83, 147.83, 174.01; HR-EI-MS *m/z*: 343.2249 (M^+, calcd for $C_{20}H_{29}N_3O_2$, 343.2260).

Synthesis of 19

To a solution of **23** (480.5 mg, 1.16 mmol) in DMSO (4 mL) was added NaH (70 mg, 2.3 mmol, 60% dispersion in mineral oil) and the mixture was stirred at 0 ºC for 10 min. Acetic anhydride (2.4 mL) in DMF (2 mL) was added to this suspension, which was stirred for 30 min at 0 ºC. The reaction mixture was poured into water and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give 1-acetyl-14-*O*-TBDMS-indolactam-V[24] (235 mg, 0.51 mmol, 44%). A solution of this compound (20.4 mg, 0.045 mmol) and concd HCl (5 μL) in MeOH (0.45 mL) was stirred at rt for 40 min. After removal of the solvent, the residue was diluted with water and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc, followed by HPLC on YMC SH-342-5 using 70% MeOH to give **19** (8.3 mg, 0.024 mmol, 54%). Compound **19**: $[\alpha]_D$ −178° (*c* = 0.19, MeOH, 21.2 °C); UV λ_{max} (MeOH) nm (ε) 335 (5460), 239 (17400); ^{1}H NMR δ ($CDCl_3$, 0.048 M, 400 MHz, 297 K, twist:sofa = 9.9:1) ppm for the twist form: 0.63 (3H, d, *J* = 6.7 Hz), 0.93 (3H, d, *J* = 6.3 Hz), 2.58 (1H, m), 2.60 (3H, s), 2.91 (3H, s), 3.07 (1H, dd, *J* = 17.7, 3.7 Hz), 3.14 (1H, br.d, *J* = 17.7 Hz), 3.28 (1H, m), 3.60 (1H, m), 3.77 (1H, m), 4.18 (1H, d, *J* = 10.1 Hz), 4.30 (1H, m), 6.76 (1H, d, *J* = 7.8 Hz), 7.14 (1H, s), 7.23 (1H, t, *J* = 8.1 Hz), 7.65 (1H, br.s), 8.09 (1H, d, *J* = 8.2 Hz); for the sofa form: 0.93 (3H, d, *J* = 6.3 Hz), 1.25 (3H, d, *J* = 6.5 Hz), 1.74 (1H, m), 2.39 (1H, m), 2.63 (3H, s), 2.74 (3H, s), 2.82 (1H, d, *J* = 14.3 Hz), 2.96 (1H, d, *J* = 11.0 Hz), 3.04 (1H, m), 3.45 (2H, m), 4.49 (1H, m), 4.49 (1H, br.s), 7.20 (1H, d, *J* = 7.8 Hz), 7.26 (1H, s), 7.34 (1H, t, *J* = 8.0 Hz), 8.46 (1H, d, *J* = 8.2 Hz); ^{13}C NMR δ ($CDCl_3$, 0.024 M, 125 MHz, 295 K) ppm for the twist form: 19.64, 21.65, 24.22, 28.60, 33.32, 33.82, 54.29, 65.01, 72.17, 109.35, 111.74, 120.19, 120.47, 122.26, 126.06, 138.88, 147.35, 168.12, 174.27; HR-EI-MS *m/z*: 343.1900 (M^+, calcd for $C_{19}H_5N_3O_3$, 343.1896).

Inhibition of specific [^{3}H]PDBu binding to PKC C1 peptides

The binding of [^{3}H]PDBu to the PKC C1 peptides was evaluated using the procedure of Sharkey and Blumberg[20] with modifications as reported previously[16] with 50 mM Tris - maleate buffer (pH 7.4 at 4 ºC), 10 - 40 nM of PKC C1 peptide, 20 nM [^{3}H]PDBu (16.3 Ci/mmol), 50 mg/mL of 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, 3 mg/mL of bovine γ-globulin, and various concentrations of inhibitor. Binding affinity was evaluated based on the concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, IC_{50}, which was calculated with PriProbit 1.63 software.[29] The inhibition constant, K_i, was calculated using the method of Sharkey and Blumberg.[20]

Synthesis of γ-C1A(P11dfP)

γ-C1A(P11dfP) was produced by solid-phase Fmoc synthesis as reported previously.[26] The K_d value of [^{3}H]PDBu for γ-C1A(P11dfP) in the presence of 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine was 5.1 nM.

The identity of the peptide was confirmed by MALDI-TOF-MS as reported previously.[16] The purity of γ-C1A(P11dfP) was confirmed by HPLC (>98%). MALDI-TOF-MS of γ-C1A(P11dfP), average molecular mass: 6133.10 (MH^+, calcd. 6132.61).

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