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Synthesis, conformation and PKC isozyme surrogate binding of indolinelactam-Vs, new conformationally restricted analogues of (−)-indolactam-V

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Abstract—New conformationally restricted analogues of tumor promoter (−)-indolactam-V (**1**), indolinelactam-Vs (**8**, **11**) and their hexyl derivatives at position 1 or 7 (**9**, **10**, **12**, **13**), were synthesized from **1**. (3*R*)-Indolinelactam-V (**8**) adopted a conformation similar to the twist form of **1** with a *cis* amide, while the conformation of (3*S*)-indolinelactam-V (**11**) was close to that of the sofa form of **1** with a *trans* amide. 7-Hexyl derivatives of **8** and **11** (**10**, **13**) showed binding affinities for C1 domains of protein kinase C (PKC) isozymes compared to **1**, but exhibited little selectivity among these PKC isozymes. However, introduction of the hexyl group at position 1 of **8** and **11** significantly enhanced their binding selectivity for novel PKC isozymes. The best selectivity for novel PKC isozymes was observed in (3*S*)-1-hexylindolinelactam-V (**12**) with a sofa-like conformation. These results suggest that a sofa-restricted analogue of **1** with a hydrophobic chain at an appropriate position would be a promising lead for designing agents with a high selectivity for novel PKC isozymes.

1. Introduction

Protein kinase C (PKC) isozymes are serine/threonine-specific protein kinases involved in a variety of cellular functions such as gene expression, growth, differentiation and apoptosis.[1] PKC isozymes are subdivided into three groups; conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ϵ, η, θ) and atypical PKCs (ζ, ι/λ) (Fig. 1).[2,3] Conventional and novel PKCs contain two cysteine-rich C1 domains (C1A, C1B), both of which bind tumor promoters such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA)[4] and teleocidin B-4.[5] Recent investigations have suggested that novel PKCs (δ, ϵ, η) are involved in mouse skin tumor promotion[6–9] and that the C1B domains of these PKC isozymes are the main targets of tumor promoters.[10,11] Design of new agents with high selectivity for C1B domains of novel PKCs is thus indispensable in elucidating the precise mechanism of skin-tumor promotion.

The naturally occurring tumor promoter (−)-indolactam-V (**1**)[12,13] is a promising lead compound for such agents since it has a simple structure and shows a binding preference for C1B domains over C1A domains of novel PKCs (Fig. 2).[14] (−)-Indolactam-V (**1**) exists as two stable conformers in solution at room temperature; the twist form with a *cis* amide geometry and the sofa form with a *trans* amide geometry.[15] We have recently found that the sofa-restricted analogue of **1** (**5**), not the twist-restricted analogue (**4**),[16] shows significant selectivity for novel PKCs.[14] These results suggest that the conformation of **1** plays a crucial role in its binding selectivity for PKC isozymes.

(3*R*)- and (3*S*)-2-Oxyindolactam-V (**6**, **7**),[17,18] isolated from the culture broth of *Streptomyces blastmyceticum* NA34-17 adopted slightly different conformations from both the twist and the sofa forms of **1**. However, these compounds were inactive in several in vitro bioassays that correlate with in vivo tumor promotion probably due to the steric hindrance between the carbonyl group at position 2 and receptors such as PKC isozymes.[19,20] (3*R*)- and (3*S*)-Indolinelactam-Vs (**8**, **11**), which lack a carbonyl group at position 2, might be new conformationally restricted analogues of **1** and may exhibit different binding selectivity for PKC isozymes from that of **1**, **4** and **5**. We report here the synthesis, conformation and PKC isozyme surrogate binding of indolinelactam-Vs (**8**, **11**) and their hexyl derivatives at positions 1 and 7 (**9**, **10**, **12**, **13**) (Fig. 3).

Keywords: (−)-Indolactam-V; Indolinelactam-V; Phorbol ester; Protein kinase C; Tumor promoter.
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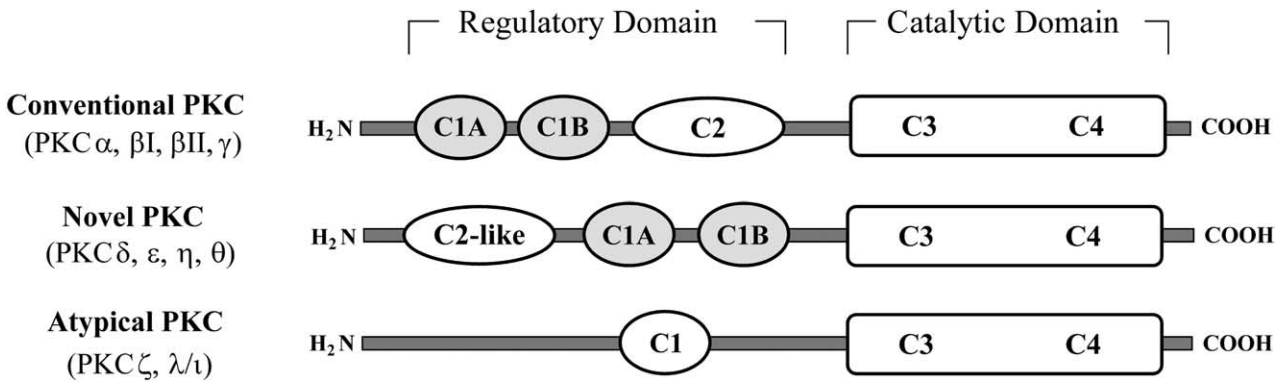
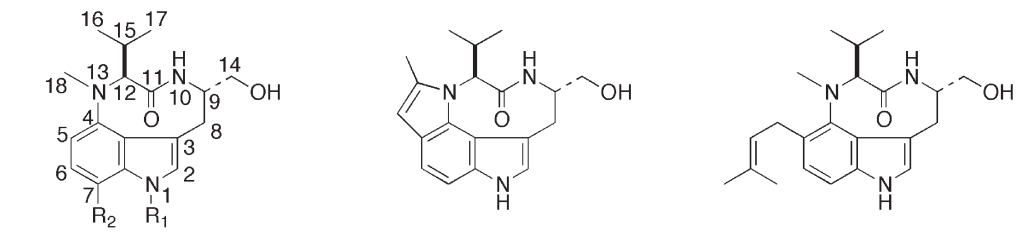


Figure 1. Structures of PKC isozymes.

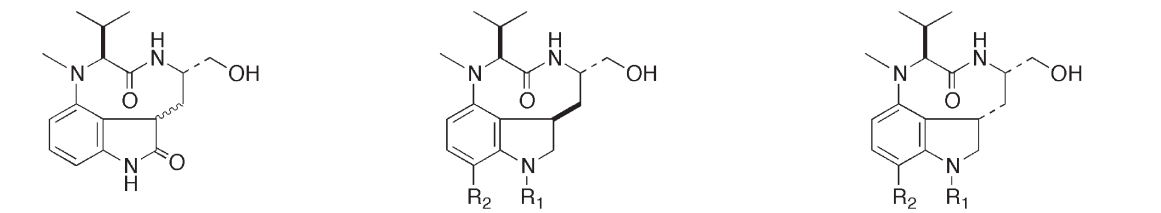


$R_1 = R_2 = H$; (-)-Indolactam-V (**1**)
$R_1 = n\text{-}C_6H_{13}$, $R_2 = H$;
(-)-1-Hexylindolactam-V (**2**)
$R_1 = H$, $R_2 = n\text{-}C_6H_{13}$;
(-)-7-Hexylindolactam-V (**3**)

Twist-restricted analogue (**4**)

Sofa-restricted analogue (**5**)

Figure 2. Structures of indolactam forms.



(3*R*)-2-Oxyindolactam-V (**6**)
(3*S*)-2-Oxyindolactam-V (**7**)

$R_1 = R_2 = H$; (3*R*)-Indolinelactam-V (**8**)
$R_1 = n\text{-}C_6H_{13}$, $R_2 = H$;
(3*R*)-1-Hexylindolinelactam-V (**9**)
$R_1 = H$, $R_2 = n\text{-}C_6H_{13}$;
(3*R*)-7-Hexylindolinelactam-V (**10**)

$R_1 = R_2 = H$; (3*S*)-Indolinelactam-V (**11**)
$R_1 = n\text{-}C_6H_{13}$, $R_2 = H$;
(3*S*)-1-Hexylindolinelactam-V (**12**)
$R_1 = H$, $R_2 = n\text{-}C_6H_{13}$;
(3*S*)-7-Hexylindolinelactam-V (**13**)

Figure 3. Structures of 2-oxyindolactam-Vs (**6**,**7**) and indolinelactam-Vs (**8**–**13**).

2. Results and discussion

(3*R*)- and (3*S*)-Indolinelactam-Vs (**8**, **11**) were synthesized from (−)-indolactam-V (**1**). Reduction of the indole ring of **1** proceeded easily with sodium cyanoborohydride in acetic acid[21] to give two diastereomers **8** and **11** (31 and 20%, respectively). The ^{1}H NMR spectra of **8** and **11** in $CDCl_3$ showed that each compound existed as a single conformer at room temperature and at −40 °C (Table 1). A significant NOE interaction between H-3 (δ 3.84) and H-12 (δ 4.37) was observed in **8**, suggesting that **8** is the (3*R*) isomer. The downfield shift of the ^{1}H NMR signals H-10 (δ 7.31) and H-12 (δ 4.37), and the lack of an NOE interaction between these protons, are characteristic of the twist form of **1**[15] and indicate that **8** adopts the twist-like conformation with a *cis* amide. On the other hand, **11** was the (3*S*) isomer because of a significant NOE interaction between H-3 (δ 3.17) and H-9 (δ 4.25) protons. As observed in the sofa form of **1**,[15] the ^{1}H NMR signals for H-10 (δ 4.92) and H-12 (δ 3.08) in **11** shifted upfield and an NOE interaction was observed between them. These results indicate that the conformation of **11** is close to the sofa form of **1** with a *trans* amide.

To determine the conformations of **8** and **11** precisely, we performed conformational studies using molecular mechanics and quantum mechanics calculations. The initial structure of **8** was calculated by MM2 with the distances between H-3 and H-12, and between H-9 and H-18 fixed at 2 Å in accordance with the NOE data. The resultant structure was optimized by PM3. The initial structure of **11** was similarly determined with the three NOE interactions between H-3 and H-9, H-3 and H-18, and H-10 and

Table 1. ^{1}H NMR data of (−)-indolactam-V (**1**) and (3*R*)- and (3*S*)-indolinelactam-Vs (**8**, **11**) in $CDCl_3$ (500 MHz, 300 K)

No.	δ (multiplicity, *J* in Hz)			
	(−)-Indolactam-V (**1**)		(3*R*)-Indolinelactam-V (**8**)	(3*S*)-Indolinelactam-V (**11**)
	Twist[a]	Sofa[a]	Twist-like[b]	Sofa-like[c]
1	7.99 (br. s)	8.27 (br. s)	3.75 (br. s)	3.73 (br. s)
2	6.89 (s)	7.06 (s)	3.18 (dd, *J*=8.9, 0.9)	3.21 (d, *J*=8.4)
			3.77 (t, *J*=8.9)	3.52 (dd, *J*=8.4, 6.6)
3			3.84 (m)	3.17 (m)
5	6.51 (d, *J*=7.5)	7.06 (d, *J*=8.2)	6.23 (d, *J*=7.9)	6.52 (d, *J*=7.8)
6	7.06 (t, *J*=7.5)	7.17 (t, *J*=8.2)	6.98 (t, *J*=7.9)	7.02 (t, *J*=7.8)
7	6.91 (d, *J*=7.5)	7.28 (d, *J*=8.2)	6.21 (d, *J*=7.9)	6.48 (d, *J*=7.8)
8	3.00 (dd, *J*=17.4, 3.8)	2.83 (d, *J*=14.0)	1.70 (dt, *J*=12.6, 2.0)	1.87 (dt, *J*=12.2, 1.8)
	3.20 (d, *J*=17.4)	3.11 (dd, *J*=14.0, 4.8)	1.92 (dt, *J*=12.6, 4.6)	2.06 (q, *J*=12.2)
9	4.30 (m)	4.46 (m)	4.01 (m)	4.25 (m)
10	6.59 (br. s)	4.72 (d, *J*=10.8)	7.31 (d, *J*=6.2)	4.92 (br.s)
12	4.39 (d, *J*=10.2)	2.99 (d, *J*=10.8)	4.37 (d, *J*=8.6)	3.08 (d, *J*=10.7)
14	3.54 (m)	3.44 (m)	3.43 (m)	3.37 (m)
	3.74 (m)	3.44 (m)	3.64 (m)	3.56 (m)
15	2.62 (m)	2.40 (m)	2.43 (m)	2.26 (m)
16	0.93 (d, *J*=6.4)	1.25 (d, *J*=7.1)	1.10 (d, *J*=6.4)	1.14 (d, *J*=6.7)
17	0.63 (d, *J*=6.8)	0.94 (d, *J*=7.1)	0.90 (d, *J*=6.8)	0.94 (d, *J*=6.5)
18	2.92 (s)	2.75 (s)	2.84 (s)	2.62 (s)

[a] Twist: Sofa=1.0: 0.4 (0.004 M).
[b] 0.079 M.
[c] 0.046 M.

H-12 used to restrict the distance between these proton pairs to 2 Å. Further optimization of these initial structures was carried out by a Hartree–Fock calculation with the 6-31G* basis set to give the optimized structures of **8** and **11** as shown in Figure 4. The conformations of **8** and **11** resembled the twist and the sofa forms of **1**, respectively. However, the spatial arrangement of the methylene group at position 8 in **8** and **11** was quite different from that of the twist and the

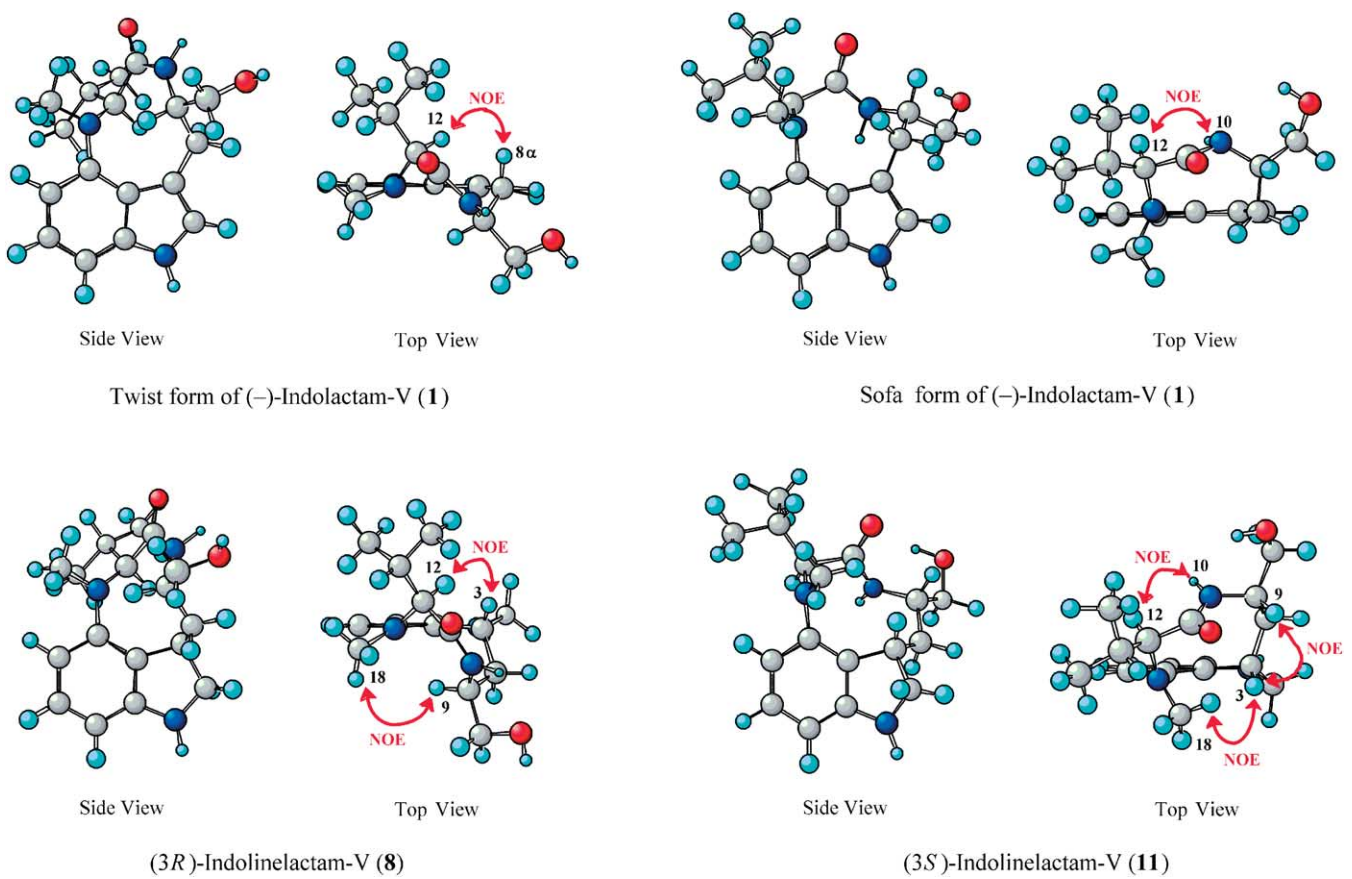


Figure 4. Stable conformations of twist (upper left) and sofa (upper right) form of (-)-indolactam-V (**1**), (3*R*)-indolinelactam-V (**8**, lower left) and (3*S*)-indolinelactam-V (**11**, lower right). The initial structures were determined by MM2 and PM3 calculations based on the indicated NOE interactions. Optimization was carried out by a Hartree-Fock calculation with the 6-31G* basis set.

Table 2. K_i Values for inhibition of the specific binding of [^{3}H]PDBu by (−)-indolactam-V (**1**), the twist- and sofa-restricted analogues of **1** (**4**, **5**), and (3*R*)- and (3*S*)-indolinelactam-Vs (**8**, **11**)

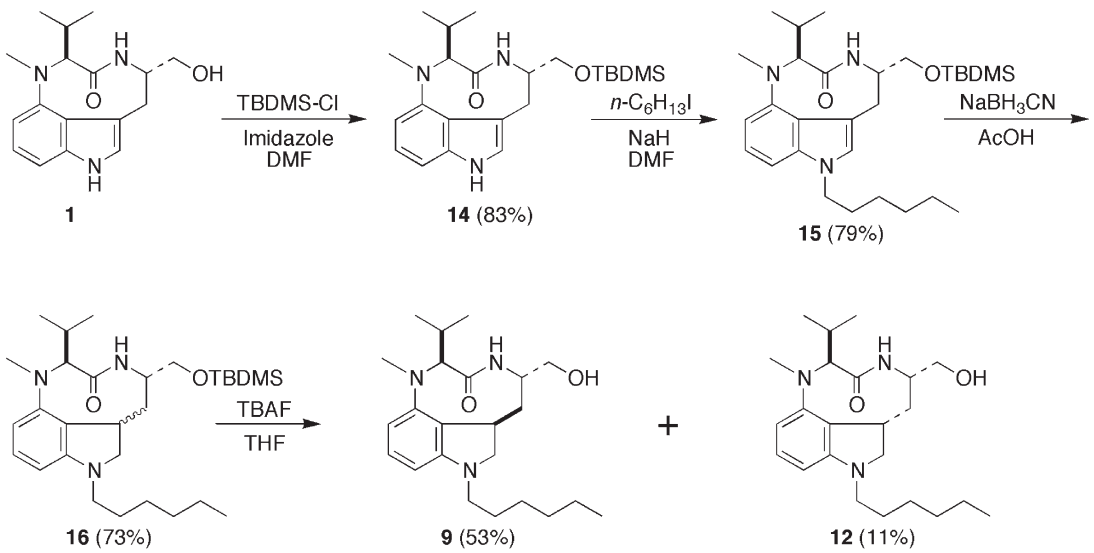
PKC C1 peptide	K_i (nM)				
	1[a]	**4**[a]	**5**[a]	**8**	**11**
Conventional PKC					
α-C1A (72-mer)[b]	21 (1.0)[c]	39 (3.9)	6000 (730)	13,000 (2500)	ND[d]
α-C1B	4000 (870)	4500 (1200)	11,000 (900)	>10,000	ND
β-C1A (72-mer)	19 (4.5)	25 (2.6)	8200 (830)	8900 (1800)	ND
β-C1B	140 (4.4)	330 (21)	321 (25)	16,000 (2500)	ND
γ-C1A	140 (14)	110 (8.0)	12,000 (2400)	>10,000	ND
γ-C1B	210 (5.0)	270 (26)	790 (140)	17,000 (3800)	ND
Novel PKC					
δ-C1A	1900 (190)	2900 (570)	23,000 (4300)	>10,000	ND
δ-C1B	8.3 (1.1)	31 (6.6)	21 (4.4)	660 (45)	5000 (960)
ϵ-C1A	4100 (50)	2500 (850)	15,000 (3100)	8200 (2100)	ND
ϵ-C1B	7.7 (1.2)	29 (4.1)	12 (2.4)	920 (190)	7700 (2200)
η-C1A	3800 (480)	1300 (2.0)	8600 (1400)	11,000 (730)	ND
η-C1B	5.5 (0.6)	14 (3.2)	6.2 (0.4)	310 (42)	2200 (400)
θ-C1A	NT[e]	NT	NT	NT	NT
θ-C1B	8.7 (1.2)	43 (6.8)	26 (2.2)	400 (52)	7700 (600)

[a] These data are cited from Ref. 14.
[b] Ten residues from both N and C-termini of the previous α-C1A and β-C1A were elongated as the solubility of the original 52-mer peptides was extremely low.
[c] Standard deviation of at least two separate experiments.
[d] Not detected.
[e] Not tested. The K_d value of [^{3}H]PDBu to θ-C1A could not be measured because of its very weak binding affinity.

sofa forms of **1**, respectively. These results indicate that **8** and **11** are new conformationally restricted analogues of **1**.

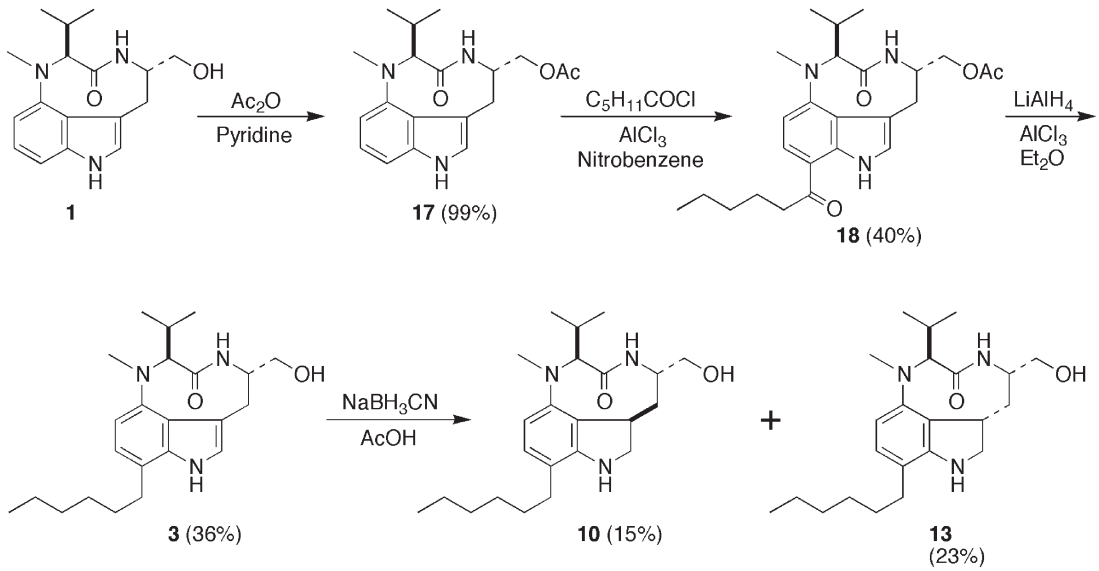
Binding affinities of **8** and **11** for PKC isozyme C1 domains were evaluated by inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to the synthetic C1 peptides (about 50–70 amino acids) of all PKC isozymes as reported previously.[11,22,23] PKC C1 peptides exhibit PDBu binding affinities comparable to the whole PKC isozymes enabling evaluation of PKC isozyme selectivity, as well as C1 domain selectivity of PKC C1 domain-binding compounds. Using the PKC C1 peptides, the concentration required to cause 50% inhibition of the [^{3}H]PDBu binding (IC_{50}) was measured. The binding affinities of **8** and **11** for each PKC C1 peptide were expressed as K_i values calculated from the IC_{50} and the K_d value of [^{3}H]PDBu as reported by Sharkey and Blumberg.[23] Table 2 summarizes the K_i values of **8** and **11** along with those of (−)-indolactam-V (**1**) and its conformationally restricted analogues (**4**, **5**).

(3*R*)- and (3*S*)-Indolinelactam-Vs (**8**, **11**) showed quite low binding affinities for all PKC C1 peptides compared with those of **1**, **4** and **5**. The binding affinities of **8** for almost all PKC C1 peptides were more than 50-fold lower than those of **1**, and **11** exhibited little binding affinity for all PKC C1 peptides. Since introduction of a substituent at position 2 in **1** abolished the tumor-promoting activity due to steric hindrance,[24] the quite low PKC binding affinities of **8** and **11** might be due to the steric hindrance between the two hydrogen atoms at position 2 and the C1 peptides of PKC isozymes. Interestingly, the selectivity of **8** for the C1B peptides of novel PKCs (δ, ϵ, η, θ), relative to both C1



1 **14** (83%) **15** (79%) **16** (73%) **9** (53%) **12** (11%)

Scheme 1. Synthesis of 1-hexylindolinelactam-Vs (**9**,**12**).



Scheme 2. Synthesis of 7-hexylindolinelactam-Vs (**10**,**13**).

peptides of conventional PKCs (α, β, γ), was considerably higher than that of **1** and **4**. This result was unexpected since the conformation of **8** has many points of similarity to that of **4**, which showed low selectivity for the C1B peptides of novel PKCs relative to the C1A peptides of conventional PKCs. It is not clear whether the conformational difference between **4** and **8**, such as the spatial arrangement of the methylene group at position 8, or the possible steric hindrance of hydrogen atoms at position 2, produces the binding selectivity of **8**. However, **8** could be a lead compound for a new agent that selectively binds to the C1B domains of novel PKCs.

Since introduction of a hydrophobic alkyl chain at position 1 or 7 in **1** dramatically increases its binding affinity for phorbol ester receptors such as PKC isozymes,[24] we attempted to enhance the PKC binding ability of **8** and **11** by introducing a hexyl group at position 1 or 7. The 1-hexyl derivatives of **8** and **11** (**9**, **12**) were synthesized from (−)-indolactam-V (**1**) in four steps as shown in Scheme 1. After protection of the hydroxyl group of **1** with a *tert*-butyldimethylsilyl (TBDMS) group (83%), a hexyl group was introduced at position 1 by S_N2 reaction of **14** with 1-iodohexane (79%). The indole ring of **15** was reduced with sodium cyanoborohydride followed by removal of the TBDMS group with tetrabutylammonium fluoride (TBAF) to give (3*R*)-1-hexylindolinelactam-V (**9**, 57%) and its (3*S*)-isomer (**12**, 11%). Synthesis of the 7-hexyl derivatives (**10**, **13**) is shown in Scheme 2. 7-Hexylindolactam-V (**3**)[25] was synthesized in three steps (14% from **1**) according to our previously reported method.[24] Hydride reduction of **3** was then carried out to give the desired two diastereomers (15% for **10**, 23% for **13**). Proton NMR spectroscopy showed that each of the 1- and 7-hexyl derivatives of **8** and **11** existed as a single conformer in $CDCl_3$ at room temperature and at −40 °C. Conformer analysis similar to that mentioned above indicated that the conformations of **9** and **10** were almost the same as the twist-like form of **8**. On the other hand, the calculated conformation of **12** and **13** was the sofa-like form similar to that of **11**. These results indicate that introduction of the hexyl group at position 1 or 7 does not influence the conformations of **8** and **11**.

Recent investigations suggest that the C1A domain of conventional PKCs (α, β, γ) is mainly involved in phorbol ester binding and translocation from the cytosol to plasma membrane,[26,27] whereas the C1B domain of novel PKCs (δ, ϵ, η, θ) plays a predominant role in translocation in response to TPA.[28] Our binding study using PKC C1 peptides also supports these results; the major binding site

Table 3. K_i Values for inhibition of the specific binding of [^{3}H]PDBu by 1-hexyl derivatives

PKC C1 peptide	K_i (nM)		
	(−)-1-Hexylindolactam-V (**2**)	(3*R*)-1-Hexylindolinelactam-V (**9**)	(3*S*)-1-Hexylindolinelactam-V (**12**)
Conventional PKC			
α-C1A (72-mer)	5.8 (1.1)[a]	170 (25)	550 (44)
α-C1A (72-mer)	9.8 (1.6)	240 (14)	1200 (27)
γ-C1A	18 (2.4)	570 (90)	1800 (290)
Novel PKC			
δ-C1B	0.22 (0.04)	4.7 (0.5)	16 (1.5)
ϵ-C1B	0.47 (0.12)	7.0 (0.8)	14 (0.9)
η-C1B	0.34 (0.11)	4.9 (0.8)	12 (2.0)
θ-C1B	1.41 (0.03)	7.0 (0.6)	12 (1.3)

[a] Standard deviation of at least two separate experiments.

Table 4. K_i Values for inhibition of the specific binding of [^{3}H]PDBu by 7-hexyl derivatives

PKC C1 peptide	K_i (nM)		
	(−)-7-Hexylindolactam-V (**3**)	(3*R*)-7-Hexylindolinelactam-V (**10**)	(3*S*)-7-Hexylindolinelactam-V (**13**)
Conventional PKC			
α-C1A (72-mer)	1.2 (0.1)[a]	15 (1.2)	65 (14)
β-C1A (72-mer)	2.5 (0.1)	35 (5.4)	91 (17)
γ-C1A	2.6 (0.2)	89 (12)	136 (29)
Novel PKC			
δ-C1B	0.21 (0.02)	4.7 (0.2)	12 (0.5)
ϵ-C1B	0.36 (0.07)	7.0 (0.8)	13 (1.3)
η-C1B	0.18 (0.05)	2.9 (0.3)	13 (3.1)
θ-C1B	0.59 (0.05)	6.1 (0.9)	13 (1.4)

[a] Standard deviation of at least two separate experiments.

of (−)-indolactam-V (**1**) is the C1A domain for conventional PKCs and the C1B domain for novel PKCs.[14] Thus, binding affinities of **9**, **10**, **12** and **13**, along with (−)-1- and (−)-7-hexylindolactam-Vs (**2**, **3**), were evaluated for C1A peptides of conventional PKCs and C1B peptides of novel PKCs (Tables 3 and 4). The binding affinities of 7-hexyl derivatives (**3**, **10**, **13**) for all PKC C1 peptides dramatically increased compared with those of the corresponding core compounds (**1**, **8**, **11**). Unexpectedly, the selectivity of **10** for novel PKCs was lower than that of **8**, and **13** did not show significant selectivity, regardless of its sofa-like conformation. This conformation is similar to **5**, which showed increased selectivity for novel PKCs. In contrast to the 7-hexyl derivatives, the selectivity for novel PKCs was improved in the 1-hexyl derivatives. These derivatives (**2**, **9**, **12**) showed lower binding affinities for conventional PKCs compared with the corresponding 7-hexyl derivatives (**3**, **10**, **13**), but similar binding affinities for novel PKCs. These results suggest that for indolactam derivatives the position of the hydrophobic chain is more important than the conformation for exhibiting novel PKC selectivity. However, **12**, which has a sofa-like conformation, showed the highest selectivity for novel PKC C1B peptides; the binding affinities of **12** for PKCη- and θ-C1B peptides were approximately 50-fold and more than 100-fold higher than those for C1A peptides of PKCα, β and γ, respectively. Since the sofa-restricted form of **1** (**5**) bound selectively to novel PKCs, sofa analogues of **1** with a hydrophobic chain at an appropriate position may be promising agents that exhibit high selectivity not only for novel PKCs but also among novel PKCs.

3. Conclusions

We have synthesized (3*R*)- and (3*S*)-indolinelactam-Vs (**8**, **11**) along with their hexyl derivatives (**9**, **10**, **12**, **13**) as new conformationally restricted analogues of (−)-indolactam-V (**1**) in order to find new lead compounds with a high selectivity for novel PKC isozymes. (3*R*)-Indolinelactam-Vs (**8**–**10**) adopted a conformation similar to the twist form of **1** with a *cis* amide, and the conformation of the (3*S*)-isomers (**11**–**13**) was close to that of the sofa form with a *trans* amide. Although the binding affinities of **8** and **11** to PKC C1 peptides were far less than those of **1**, the introduction of a hexyl group at position 1 or 7 resulted in PKC binding affinities comparable to those of **1**. Corresponding to the structure–activity studies of **1**,[24] 7-hexyl derivatives (**10**, **13**) bound to conventional PKCs (α, β, γ) more strongly than 1-hexyl derivatives (**9**, **12**) probably due to steric hindrance between the hexyl group at position 1 and conventional PKCs. However, a similar effect was not observed in novel PKCs (δ, ϵ, η, θ); the binding affinities of 1- and 7-hexyl derivatives of **8** and **11** for novel PKC C1B peptides were similar to each other. (3*S*)-1-Hexylindolinelactam-V (**12**), with a sofa-like conformation, exhibited about 50–300 fold greater selectivity for C1B domains of novel PKCs than for C1A domains of conventional PKCs, indicating that sofa-restricted analogues of **1** with a hydrophobic chain at an appropriate position, such as **5** and **12**, would be likely lead compounds for the rational design of novel PKC selective modulators. The present results provide a basis for the synthesis and exploitation of such compounds as medicinal agents.

4. Experimental

4.1. General remarks

The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; Digital Polarimeter, Jasco DIP-1000; ^{1}H NMR, Bruker ARX500 (ref. TMS); HPLC, Waters Model 600E with Model 2487 UV detector; (HR) EI-MS, JOEL JMS-600H. HPLC was carried out on a YMC packed SH-342-5 (ODS, 20 mm i.d. ×250 mm) column (Yamamura Chemical Laboratory). Wako C-200 gel (silica gel, Wako Pure Chemical Industries) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography. [^{3}H]PDBu (17.0 Ci/mol) was purchased from PerkinElmer Life Sciences Research Products. All other chemicals and reagents were purchased from chemical companies and used without further purification.

4.1.1. Synthesis of (3*R*)- and (3*S*)-indolinelactam-Vs (8, 11). To a solution of (−)-indolactam-V (**1**, 48.8 mg, 162 μmol) in acetic acid (1 ml) was added $NaCNBH_3$ (25 mg, 397 μmol) at room temperature. After stirring for 1.5 h, another $NaCNBH_3$ (25 mg, 397 μmol) was added and the reaction mixture was stirred for another 2.5 h. The reaction was quenched by the addition of H_2O (1 ml) and the mixture was poured into saturated $NaHCO_3$ aq (20 ml), followed by extraction with EtOAc. The EtOAc layer was

washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by HPLC on YMC SH-342-5 using 25% CH_3CN to give **8** (15.1 mg, 50 μmol, 31%) and **11** (9.6 mg, 32 μmol, 20%). Compound **8**: $[\alpha]_D$ −478.0° (*c*=0.700, MeOH, 302.5 K); UV λ_{max} (MeOH) nm (ϵ): 303 (2000), 238 (24,000); ^{13}C NMR δ ($CDCl_3$, 0.079 M, 500 MHz, 300 K) ppm: 19.65, 21.85, 30.50, 34.87, 39.30, 40.12, 52.87, 53.95, 66.25, 68.16, 102.02, 107.87, 117.97, 129.23, 150.85, 152.83, 175.70; HR-EI-MS *m*/*z*: 303.1937 (M^+, calcd for $C_{17}H_{25}N_3O_2$, 303.1947). Compound **11**: $[\alpha]_D$ +146.0° (*c*=0.477, MeOH, 300.2 K); UV λ_{max} (MeOH) nm (ϵ): 298 (2600), 244 (9500); ^{13}C NMR δ ($CDCl_3$, 0.046 M, 500 MHz, 300 K) ppm: 19.06, 19.87, 24.11, 34.77, 40.29, 40.58, 54.43, 57.22, 66.11, 76.10, 106.68, 119.35, 129.04, 130.39, 150.87, 152.46, 170.88; HR-EI-MS *m*/*z*: 303.1918 (M^+, calcd for $C_{17}H_{25}N_3O_2$, 303.1947).

4.1.2. Synthesis of (3*R*)- and (3*S*)-1-hexylindolinelactam-Vs (9, 12). To a mixture of (−)-indolactam-V (**1**, 44.0 mg, 146 μmol) and imidazole (30.0 mg, 441 μmol) in dry DMF (0.5 ml) was added TBDMS-Cl (24.1 mg, 160 μmol) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was poured into H_2O 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 Wako gel C-200 using hexane and increasing amounts of EtOAc to give **14** (50.3 mg, 121 μmol, 83%).

NaH in oil (11.6 mg, 290 μmol) was washed with hexane and suspended in dry DMF (0.5 ml) under an Ar atmosphere To this suspension was added **14** (50.3 mg, 121 μmol) in dry DMF (0.5 ml) at 0 °C. After stirring for 10 min, 1-iodohexane (21.4 μl, 145 μmol) was added dropwise and the reaction mixture was stirred for 1 h at 0 °C. The mixture was poured into H_2O (20 ml) 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 Wako gel C-200 using hexane and increasing amounts of EtOAc to give **15** (47.4 mg, 95 μmol, 79%). Compound **15**: $[\alpha]_D$ −107.0° (*c*=0.188, MeOH, 298.3 K); UV λ_{max} (MeOH) nm (ϵ): 307 (8200), 230 (25,000); ^1H NMR δ ($CDCl_3$, 0.068 M, 500 MHz, 300 K, twist:sofa=3.3:1) ppm for twist conformer: 0.03 (3H, s), 0.05 (3H, s), 0.63 (3H, d, *J*=6.8 Hz), 0.87 (9H, s), 0.88 (3H, t, *J*=7.0 Hz), 0.92 (3H, d, *J*=6.4 Hz), 1.30–1.36 (6H, m), 1.81 (2H, m), 2.61 (1H, m), 2.87 (1H, dd, *J*=17.4, 3.5 Hz), 2.91 (3H, s), 3.14 (1H, d, *J*=17.4 Hz), 3.45 (1H, dd, *J*=10.1, 9.8 Hz), 3.63 (1H, dd, *J*=10.1, 4.3 Hz), 3.98 (2H, t, *J*=7.4 Hz), 4.21 (1H, m), 4.38 (1H, d, *J*=10.2 Hz), 6.16 (1H, br.s), 6.49 (1H, d, *J*=7.9 Hz), 6.76 (1H, s), 6.84 (1H, *J*=7.9 Hz), 7.07 (1H, t, *J*=7.1 Hz); HR-EI-MS *m*/*z*: 499.3574 (M^+, calcd for $C_{29}H_{49}N_3O_2Si$, 499.3594).

To a solution of **15** (39.1 mg, 78 μmol) in acetic acid (0.5 ml) was added $NaCNBH_3$ (9.0 mg, 140 μmol) at room temperature. After stirring for 1.5 h additional $NaCNBH_3$ (9.0 mg, 140 μmol) was added and the reaction mixture was stirred for another 2.5 h. The reaction was quenched by the addition of H_2O (1 ml) and the mixture was poured into saturated $NaHCO_3$ aq (20 ml) 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 Wako gel C-200 using hexane and increasing amounts of EtOAc to give **16** (28.5 mg, 56.9 μmol, 73%) as a mixture of two diastereomers.

TBAF·$5H_2O$ (108 mg, 338 μmol) was added to **16** (28.5 mg, 56.9 μmol) in THF (1.4 ml) at 0 °C. After stirring for 40 min at 0 °C, the reaction mixture was poured into 5% $KHSO_4$ aq and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by HPLC on YMC SH-342-5 using 80% MeOH to give **9** (11.7 mg, 30.2 μmol, 53%) and **12** (2.5 mg, 6.5 μmol, 11%). Compound **9**: $[\alpha]_D$ −435.0° (*c*=0.435, MeOH, 301.2 K); UV λ_{max} (MeOH) nm (ϵ): 310 (2500), 246 (30,000); ^1H NMR δ ($CDCl_3$, 0.060 M, 500 MHz, 300 K) ppm: 0.90 (3H, t, *J*=6.8 Hz), 0.91 (3H, d, *J*=6.8 Hz), 1.10 (3H, d, *J*=6.4 Hz), 1.34–1.37 (6H, m), 1.55 (2H, m), 1.63 (1H, t, *J*=12.2 Hz), 1.92 (1H, dt, *J*=12.2, 4.6 Hz), 2.43 (1H, m), 2.83 (3H, s), 2.88 (1H, m), 3.13 (1H, d, *J*=8.6 Hz), 3.23 (1H, m), 3.43 (1H, t, *J*=8.6 Hz), 3.44 (1H, m), 3.50 (1H, t, *J*=6.0 Hz), 3.65 (1H, m), 3.73 (1H, m), 4.02 (1H, m), 4.36 (1H, d, *J*=8.4 Hz), 6.05 (1H, d, *J*=8.0 Hz), 6.17 (1H, d, *J*=8.0 Hz), 7.02 (1H, t, *J*=8.0 Hz), 7.41 (1H, d, *J*=6.4 Hz); ^{13}C NMR δ ($CDCl_3$, 0.060 M, 125 MHz, 300 K) ppm: 14.07, 19.68, 21.84, 22.66, 26.85, 27.31, 30.60, 31.70, 35.03, 38.34, 39.52, 48.47, 54.00, 58.63, 66.25, 68.27, 99.45, 107.04, 118.06, 129.18, 150.64, 153.71, 175.92; HR-EI-MS *m*/*z*: 387.2884 (M^+, calcd for $C_{23}H_{37}N_3O_2$, 387.2886). Compound **12**: $[\alpha]_D$ +215.0° (*c*=0.052, MeOH, 300.8 K); UV λ_{max} (MeOH) nm (ϵ): 307 (2800), 259 (13,900); ^1H NMR δ ($CDCl_3$, 0.013 M, 500 MHz, 300 K) ppm: 0.90 (3H, t, *J*=6.6 Hz), 0.95 (3H, d, *J*=6.5 Hz), 1.14 (3H, d, *J*=6.7 Hz), 1.32–1.37 (6H, m), 1.56 (2H, m), 1.88 (1H, dd, *J*=14.1, 2.5 Hz), 2.03 (1H, q, *J*=13.2 Hz), 2.26 (1H, m), 2.62 (3H, s), 2.76 (1H, m), 3.11 (1H, d, *J*=10.7 Hz), 3.14 (3H, m), 3.22 (1H, m), 3.41 (1H, m), 3.58 (1H, m), 4.24 (1H, m), 4.85 (1H, br.s), 6.29 (1H, d, *J*=7.8 Hz), 6.44 (1H, d, *J*=7.8 Hz), 7.06 (1H, t, *J*=7.8 Hz); ^{13}C NMR δ ($CDCl_3$, 0.013 M, 125 MHz, 300 K) ppm: 14.06, 19.07, 19.85, 22.63, 24.15, 26.91, 27.16, 31.67, 34.88, 39.07, 41.05, 49.15, 54.83, 62.44, 66.30, 76.05, 104.25, 117.95, 129.13, 130.46, 150.51, 153.52, 171.33; HR-EI-MS *m*/*z*: 387.2868 (M^+, calcd for $C_{23}H_{37}N_3O_2$, 387.2886).

4.1.3. Synthesis of (3*R*)- and (3*S*)-7-hexylindolinelactam-Vs (10, 13). (−)-7-Hexylindolactam-V (**3**)[25] was treated in a manner similar to that described for the synthesis of **9** and **12** to give **10** (2.5 mg, 6.5 μmol, 15%) and **13** (3.8 mg, 9.8 μmol, 23%). Compound **10**: $[\alpha]_D$ −397.0° (*c*=0.165, MeOH, 299.4 K); UV λ_{max} (MeOH) nm (ϵ): 305 (3000), 237 (26,000); ^1H NMR δ ($CDCl_3$, 0.013 M, 500 MHz, 300 K) ppm: 0.89 (3H, t, *J*=6.8 Hz), 0.92 (3H, d, *J*=6.8 Hz), 1.10 (3H, d, *J*=6.4 Hz), 1.32 (4H, m), 1.37 (2H, m), 1.57 (2H, m), 1.70 (1H, t, *J*=12.2 Hz), 1.89 (1H, dt, *J*=12.2, 4.5 Hz), 2.36 (2H, t, *J*=7.8 Hz), 2.45 (1H, m), 2.84 (3H, s), 2.97 (1H, br.s), 3.22 (1H, d, *J*=8.9 Hz), 3.44 (1H, m), 3.58 (1H, br.s), 3.64 (1H, m), 3.78 (1H, t, *J*=8.9 Hz), 3.83 (1H, m), 4.04 (1H, m), 4.33 (1H, d, *J*=8.4 Hz), 6.22 (1H, d, *J*=8.2 Hz), 6.84 (1H, d, *J*=8.2 Hz), 6.97 (1H, d, *J*=6.5 Hz); ^{13}C NMR δ ($CDCl_3$, 0.013 M, 125 MHz, 300 K) ppm: 14.12, 19.72, 21.83, 22.67, 29.07, 29.46, 30.67, 30.81, 31.77, 35.07, 39.47, 40.14, 52.88, 53.81, 66.31, 68.26, 108.08, 116.07, 118.11, 128.58, 148.80, 150.55, 175.71; HR-EI-MS *m*/*z*: 387.2889 (M^+, calcd for $C_{23}H_{37}N_3O_2$,

387.2886). Compound **13**: $[\alpha]_D$ +114.0° (c=0.151, MeOH, 300.4 K); UV λ_{max} (MeOH) nm (ϵ): 298 (3300), 243 (9700); ^{1}H NMR δ ($CDCl_3$, 0.020 M, 500 MHz, 300 K) ppm: 0.89 (3H, t, J=6.8 Hz), 0.94 (3H, d, J=6.5 Hz), 1.14 (3H, d, J=6.7 Hz), 1.32 (4H, m), 1.36 (2H, m), 1.56 (2H, m), 1.89 (1H, dd, J=13.2, 2.2 Hz), 2.06 (1H, q, J=13.2 Hz), 2.24 (1H, m), 2.40 (2H, t, J=7.8 Hz), 2.62 (3H, s), 3.06 (1H, d, J=10.6 Hz), 3.18 (1H, m), 3.25 (1H, d, J=8.3 Hz), 3.42 (1H, m), 3.52 (1H, t, J=7.4 Hz), 3.58 (1H, m), 3.60 (1H, br.s), 4.25 (1H, m), 4.62 (1H, br.s), 6.49 (1H, d, J=8.0 Hz), 6.87 (1H, d, J=8.0 Hz); ^{13}C NMR δ ($CDCl_3$, 0.020 M, 125 MHz, 300 K) ppm: 14.11, 19.08, 19.85, 22.65, 24.16, 29.01, 29.36, 30.91, 31.74, 34.82, 40.59 (two carbon signals overlapped), 54.55, 57.17, 66.24, 76.25, 119.18, 120.89, 128.89, 129.81, 148.43, 150.04, 171.10; HR-EI-MS m/z: 387.2884 (M^+, calcd for $C_{23}H_{37}N_3O_2$, 387.2886).

4.2. Conformer analysis

The most stable conformations of indolinelactam-Vs (**8**–**13**) were estimated by the Chem 3D (Cambridge Soft) and AMOSS-H11 (NEC quantum chemistry group) programs. The initial structures were calculated by molecular mechanics calculation using MM2 theory with the distance between two protons (H-3 and H-12, and H-9 and H-18 for **8**–**10**, H-3 and H-9 protons, H-3 and H-18 protons and H-10 and H-12 protons for **11**–**13**) fixed at 2 Å congruent with NOE data. The resultant structures were optimized by a semiempirical quantum mechanics calculation using PM3 theory. Further optimization of these calculated structures was carried out by ab initio molecular orbital schemes using a Hartree–Fock theory with the 6-31G* basis set to give the most stable conformers.

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

The [^{3}H]PDBu binding to the PKC isozyme C1 peptides was evaluated using the procedure of Sharkey and Blumberg[23] with modifications as reported previously[11] under the following conditions: 50 mM Tris–maleate buffer (pH 7.4 at 4 °C), 5–20 nM a PKC isozyme C1 peptide, 20–40 nM [^{3}H]PDBu (17.0 Ci/mmol), 50 μg/ml 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, 3 mg/ml bovine γ-globulin, and various concentrations of an inhibitor. Binding affinity was evaluated by the concentration required to cause 50% inhibition of the specific [^{3}H]PDBu binding, IC_{50}, which was calculated by a computer program (SAS) with a probit procedure. The binding constant, K_i, was calculated using the method of Sharkey and Blumberg.[23]

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