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Synthesis and PKC Isozyme Surrogate Binding of Indothiolactam-V, A New Thioamide Analogue of Tumor Promoting Indolactam-V

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Abstract—To investigate the role of the amide group of (−)-indolactam-V (**1**) on PKC binding, we synthesized (−)-indothiolactam-V (**2**), a new thioamide analogue of **1**, by microbial conversion using *Streptomyces blastmyceticum*. Compounds **2** and **1** showed similar binding affinities to conventional PKCs but **2** had lower affinities to novel PKCs, suggesting that novel PKCs recognize amide modifications more effectively than conventional PKCs. © 2000 Elsevier Science Ltd. All rights reserved.

The potent tumor promoting teleocidins[1] activate protein kinase C (PKC),[2] a crucial enzyme involved in cellular signal transduction. (−)-Indolactam-V (**1**),[3,4] which represents the basic biologically active subunit of the teleocidins, also exhibits activity as a tumor promoter and PKC activator (Fig. 1). Structure–activity studies of over 100 indolactam derivatives have served to identify most structural features of the indolactams that are required for tumor promotion and PKC binding except for the role of the amide subunit.[5] Conformational studies led to the finding that **1** exists as two stable conformers in solution at room temperature:[6] the active twist conformer with a *cis* amide geometry and the inactive sofa conformer with a *trans* amide geometry.[7,8] This finding further indicates that both the amide hydrogen and the carbonyl group of **1** could interact with PKCδ as suggested independently by computational docking studies[9,10] based on the crystal structure of PKCδ-C1B domain with phorbol-13-acetate as a ligand.[11] However, the role of the amide hydrogen and carbonyl group has not been determined for solution structures. Moreover, the role of the amide in binding to other PKC isozymes, a matter of considerable importance in the design of PKC selective activators, has not been examined since its modification is synthetically quite difficult and such modifications could significantly perturb the conformational preferences of the system. Previous investigations unrelated to indolactam-V revealed that a thioamide group, while isosteric with an amide group,[12,13] possesses a different hydrogen bonding ability due to the lower electronegativity of the sulfur atom.[14,15] These findings coupled with the ease with which thioamides can be synthesized from amides with Lawesson's reagent,[16] prompted us to synthesize (−)-indothiolactam-V (**2**), a new thioamide analogue of **1**, and examine its conformation and binding affinity to all PKC isozyme C1 domains.

We attempted at first the direct conversion from **1** to **2** by Lawesson's reagent. After protecting the hydroxyl group of **1** with an acetyl group, the corresponding acetyl derivative was treated with Lawesson's reagent. However, the reaction did not proceed at all even at 120 °C. The bulky isopropyl group at position 12 and the compact lactam ring structure of **1** presumably serve to sterically block reaction at the carbonyl site. We previously developed a new strategy to prepare a variety of indolactam derivatives from their *seco*-compounds (*N*-methyl-L-amino acidyl-L-tryptophanols) by the microbial conversion with teleocidin-producing *Streptomyces blastmyceticum* NA34-17.[17] Since the carbonyl of *N*-methyl-L-valyl-L-tryptophanol, the non-cyclic form of **1**, is less sterically hindered than that of **1**, we set out to prepare the thioamide first and then use a microbial conversion for the synthesis of **2**.

Thioamide **7**, the *seco*-analogue of **2**, was synthesized from L-tryptophan methyl ester (**3**) as shown in Scheme 1.

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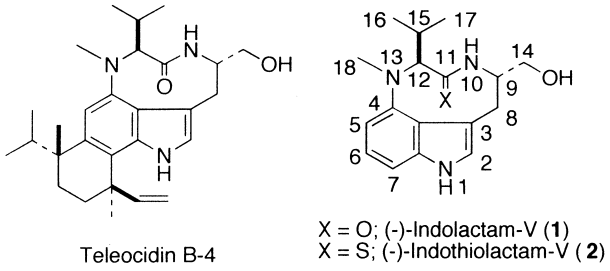
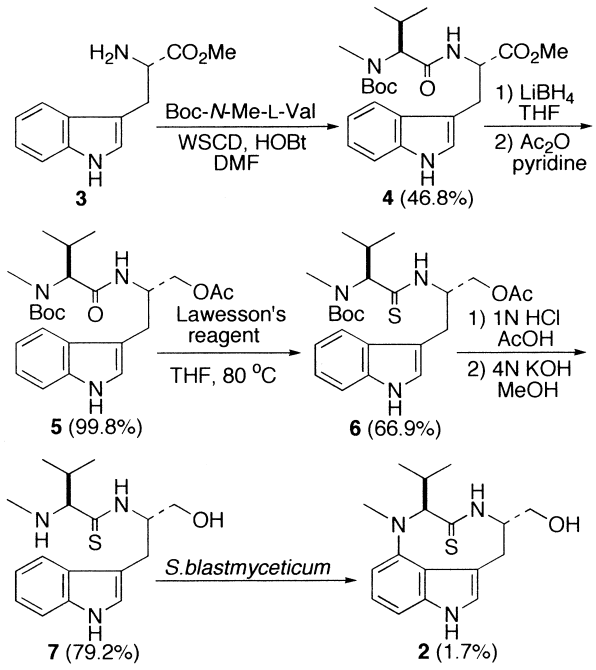


Figure 1. Structures of teleocidin B-4 and indolactams.



Scheme 1. Synthesis of (−)-indothiolactam-V (**2**).

Compound **3** was condensed with Boc-*N*-methyl-L-valine to give **4** in a 46.8% yield. After reduction of the methyl ester, the resultant hydroxyl group was protected with an acetyl group to give **5** in a 99.8% yield. Significantly, conversion of **5** to the thioamide was accomplished with the Lawesson's reagent in a 66.9% yield. This high yield strongly suggests that the lactam ring of **1** is the main reason for the low reactivity of its carbonyl group. Deprotection of the Boc group under acidic conditions followed by alkaline hydrolysis gave the *seco*-compound **7** in a 79.2% yield.[18] The feeding experiment with **7** using *S. blastmyceticum* NA34-17 was carried out as reported previously.[17] (−)-Indothiolactam-V (**2**)[19] was obtained in a 1.7% yield. This low yield might be due to modification or decomposition of **7** during the incubation step since several unusual metabolites deduced to be derived from **7** were detected. While the yield for this step is low, the overall route serves to provide effective access to key compounds needed to investigate PKC recognition at the amide site.

The ^{1}H NMR spectrum of (−)-indothiolactam-V (**2**) in deuterochloroform showed that **2** existed as two conformers at room temperature (major:minor = 1:0.4). The signals of the major and minor conformers of **2** were very close to those of the twist and the sofa form of **1**, respectively, except for the signals at positions 10, 12, and 15 which were shifted downfield due to substitution with the amide group by the thioamide group (Table 1). In addition, a remarkable NOE enhancement between H-8b (δ 3.26) and H-12 (δ 4.64) characteristic of the twist form of **1**[6] was observed in the major conformer of **2**, while a significant NOE between H-10 (δ 6.35) and H-12 (δ 3.22) was detected in the minor conformer of **2** analogous to that found in the sofa form of **1**. These results indicate that the two conformers of **2** are almost exactly the same as those of **1**.

The binding affinities of **2** to the PKC C1 domains were evaluated by inhibition of the specific binding of [^{3}H]phorbol-12,13-dibutyrate (PDBu) to these C1 domains as reported by Sharkey and Blumberg.[20] Tumor promoters like phorbol esters and indolactams activate PKC by binding to the cysteine-rich C1 domains designated as C1A and C1B.[21] Since the C1 domains are zinc fingers consisting of ca. 50 amino acids, we have synthesized individual C1A and C1B domains of all conventional PKCs (PKCα, β, γ) and novel PKCs (PKCδ, ε, η, θ) by the solid-phase synthesis and measured the dissociation constants (K_d) using [^{3}H]PDBu.[22,23] All C1B peptides were folded upon zinc treatment to afford PKC C1 domain surrogates which as required for surrogates exhibit PDBu binding affinities comparable to the native PKC isozymes. Although the PDBu binding affinities of the C1A peptides were observed to be weak under the previous assay conditions (30 °C incubation),[22] we have recently found that some peptides (γ-C1A, γ-C1B, ε-C1A and η-C1A) suffered from the temperature dependent inactivation.[24] To circumvent this problem, the dissociation constants (K_d) of [^{3}H]PDBu for the temperature sensitive peptides were successfully determined at lower temperature (4 °C incubation).[24] The other C1A peptides whose PDBu binding could not be detected previously are α-C1A and β-C1A. Recent investigation revealed that the C1A and C1B domains of PKCα play equivalent roles for translocation in response to tumor promoters, suggesting that C1A of PKCα should potently bind to PDBu.[25] Since the solubility of α-C1A and β-C1A peptides was extremely low compared with other C1A peptides, 10 residues from both the N- and C-terminus of α-C1A and β-C1A were added to achieve satisfactory solubility. The 72-mer α-C1A-long (27–98) and β-C1A-long (27–98) bound to PDBu with high affinity, giving K_d values with [^{3}H] PDBu at 4 °C of 1.1 and 1.3 nM, respectively. Using the PKC C1 peptides under the new assay conditions, the concentration required to cause 50% inhibition of the specific [^{3}H]PDBu binding, IC_{50}, was determined.[20] Table 2 summarizes the K_i values of **1** and **2** calculated from the IC_{50} values and the corresponding revised K_d values of [^{3}H]PDBu at 4°C incubation.

(−)-Indolactam-V (**1**) exhibited potent binding affinities to all C1B peptides of novel PKCs, but quite low affinities to the corresponding C1A peptides, indicating that the major binding site in novel PKCs of **1** is C1B. In contrast, the binding affinities of **1** to the C1A peptides of conventional PKCs were relatively high compared

Table 1. ^{1}H NMR spectra of (−)-indolactam-V (**1**) and (−)-indothiolactam-V(**2**) in deuterochloroform (500 MHz, 300 K)

No.	δ (Multiplicity, *J* in Hz)			
	(−)-Indolactam-V (**1**)		(−)-Indothiolactam-V (**2**)	
	Twist[a]	Sofa[a]	Major[b]	Minor[b]
1	7.99 (br. s)	8.27 (br. s)	8.02 (br. s)	8.33 (br. s)
2	6.89 (s)	7.06 (s)	6.89 (s)	7.07 (s)
5	6.51 (d, *J* = 7.5)	7.06 (d, *J* = 8.2)	6.51 (d, *J* = 7.9)	7.10 (d, *J* = 7.8)
6	7.06 (t, *J* = 7.5)	7.17 (t, *J* = 8.2)	7.07 (t, *J* = 7.9)	7.19 (t, *J* = 7.8)
7	6.91 (d, *J* = 7.5)	7.28 (d, *J* = 8.2)	6.89 (d, *J* = 7.9)	7.29 (d, *J* = 7.8)
8a	3.00 (dd, *J* = 17.4, 3.8)	2.83 (d, *J* = 14.0)	3.01 (dd, *J* = 17.4, 3.4)	2.78 (dd, *J* = 14.5, 1.9)
8b	3.20 (d, *J* = 17.4)	3.11 (dd, *J* = 14.0, 4.8)	3.26 (d, *J* = 17.4)	3.36 (dd, *J* = 14.5, 4.4)
9	4.30 (m)	4.46 (m)	4.51 (m)	4.51 (m)
10	6.59 (br. s)	4.72 (d, *J* = 10.8)	8.75 (br. s)	6.35 (d, *J* = 10.0)
12	4.39 (d, *J* = 10.2)	2.99 (d, *J* = 10.8)	4.64 (d, *J* = 10.8)	3.22 (d, *J* = 10.6)
14a	3.54 (m)	3.44 (m)	3.61 (m)	3.56 (m)
14b	3.74 (m)	3.44 (m)	3.78 (m)	3.56 (m)
15	2.62 (m)	2.40 (m)	2.96 (m)	2.70 (m)
16	0.93 (d, *J* = 6.4)	1.25 (d, *J* = 7.1)	1.00 (d, *J* = 6.3)	1.29 (d, *J* = 6.7)
17	0.63 (d, *J* = 6.8)	0.94 (d, *J* = 7.1)	0.62 (d, *J* = 6.7)	1.01 (d, *J* = 6.4)
18	2.92 (s)	2.75 (s)	3.02 (s)	2.74 (s)

[a]Twist:sofa = 1.0:0.4 (0.004 M).
[b]Major:minor = 1.0:0.4 (0.064 M).

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

PKC C1 peptide	K_i (nM)		K_d (nM)
	(−)-Indolactam-V (**1**)	(−)-Indothiolactam-V (**2**)	PDBu
α-C1A (72-mer)[a]	126.9 (13.4)[b]	261.5 (9.7)	1.12 (0.04)
α-C1B	4000 (870)	2140 (380)	7.44 (0.27)
β-C1A (72-mer)[a]	173.5 (17.7)	179.5 (7.6)	1.31 (0.26)
β-C1B	135.6 (4.4)	225.9 (1.6)	1.34 (0.41)
γ-C1A	137.9 (13.5)	295.7 (5.7)	1.50 (0.57)
γ-C1B	212.6 (5.0)	288.1 (9.8)	1.19 (0.29)
δ-C1A	1900 (190)	2950 (990)	51.9 (15.6)
δ-C1B	8.3 (1.1)	67.5 (6.6)	0.53 (0.14)
ε-C1A	4110 (50)	3860 (750)	5.60 (0.62)
ε-C1B	7.7 (1.2)	41.5 (2.4)	0.81 (0.03)
η-C1A	3770 (480)	2160 (440)	4.30 (0.18)
η-C1B	5.5 (0.6)	20.6 (2.4)	0.45 (0.12)
θ-C1A	NT	NT	>200
θ-C1B	8.7 (1.2)	61.6 (7.3)	0.72 (0.14)

[a]Ten residues from both N- and C-terminus of the previous α-C1A and β-C1A were elongated since the solubility of the original 52-mer peptides was extremely low.
[b]Standard deviation of at least two separate experiments.

with the corresponding C1B peptides. Both C1 peptides of PKCβ and PKCγ exhibited significant binding to **1**.

(−)-Indothiolactam-V (**2**) showed a C1 domain selectivity similar to **1**. However, the binding affinities of **2** to the C1B domains of novel PKCs were significantly lower (5–8 times) than those of **1**, while those of **2** to the C1 peptides of conventional PKCs were similar to each other. It is known that the sulfur atom of a thioamide is a weaker hydrogen bond acceptor compared with the oxygen atom of an amide, but the donor ability of the adjacent NH is enhanced due to the increased polarity and acidity.[14,15] Since the three dimensional structures and ratios of the two conformers of **2** were quite similar to those of **1**, the binding modes of **1** and **2** to each C1 peptide are expected to be similar. Based on the assumption that both the carbonyl group and the hydrogen of the amide group of **1** interact with all PKC C1 domains like PKCδ-C1B,[9,10] the present results suggest that novel PKCs recognize the amide carbonyl group of **1** more strongly than conventional PKCs.

However, it is also possible that the hydrogen bond formed between the thioamide hydrogen of **2** and novel PKC C1 peptides is perturbed by the larger sulfur atom and the longer thiocarbonyl bond. To investigate this possibility, we recently synthesized indolactone-V, the ester analogue of **1**.[26] However, indolactone-V existed in only the inactive sofa conformer, precluding a determination of whether the amide hydrogen of **1** interacts with PKC C1 domains. Synthesis of a new twist-restricted analogue of indolactone-V is in progress.

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References and Notes

1. Fujiki, H.; Sugiyama, T. *Adv. Cancer. Res.* **1987**, *49*, 223.
2. Nishizuka, Y. *Nature* **1984**, *308*, 693.
3. Endo, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1982**, *30*, 3457.
4. Irie, K.; Hirota, M.; Hagiwara, N.; Koshimizu, K.; Hayashi, H.; Marao, S.; Tokuda, H.; Ito, Y. *Agric. Biol. Chem.* **1984**, *48*, 1269.
5. Irie, K.; Koshimizu, K. *Comments Agric. Food Chem.* **1993**, *3*, 1.
6. Endo, Y.; Shudo, K.; Itai, A.; Hasegawa, M.; Sakai, S. *Tetrahedron* **1986**, *42*, 5905.
7. Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841.
8. Irie, K.; Isaka, T.; Iwata, Y.; Yanai, Y.; Nakamura, Y.; Koizumi, F.; Ohigashi, H.; Wender, P. A.; Satomi, Y.; Nishino, H. *J. Am. Chem. Soc.* **1996**, *118*, 10733.
9. Endo, Y.; Takehara, S.; Ohno, M.; Driedger, P. E.; Stabel, S.; Mizutani, M. Y.; Tomioka, N.; Itai, A.; Shudo, K. *J. Med. Chem.* **1998**, *41*, 1476.
10. Wang, S.; Liu, M.; Lewin, N. E.; Lorenzo, P. S.; Bhattacharrya, D.; Qiao, L.; Kozikowski, A. P.; Blumberg, P. M. *J. Med. Chem.* **1999**, *42*, 3436.
11. Zhang, G.; Kazanietz, G. M.; Blumberg, M. P.; Hurley, H. J. *Cell* **1995**, *81*, 917.
12. La Cour, M. F. T.; Hausen, S. A. H.; Clausen, K.; Lawesson, O. S. *Int. J. Pept. Protein Res.* **1983**, *22*, 509.
13. La Cour, M. F. T. *Int. J. Pept. Protein Res.* **1987**, *30*, 564.
14. Dudek, P. E.; Dudek, G. J. *J. Org. Chem.* **1967**, *32*, 823.
15. Ramakrishnan, C.; Prasad, N. *Int. J. Protein Res.* **1971**, *3*, 209.
16. Clausen, K.; Thorsen, M.; Lawesson, O. S. *Tetrahedron* **1983**, *20*, 3635.
17. Irie, K.; Iguchi, M.; Oda, T.; Suzuki, Y.; Ohigashi, H.; Koshimizu, K.; Hayashi, H.; Arai, M.; Nishino, H. *Tetrahedron* **1995**, *51*, 6255.
18. Compound **7**: $[\alpha]_D$ −23.0° (c = 0.44, MeOH, 32.5°C); UV λ_{max} (MeOH) nm (ε) 221 (25,700), 271 (10,400); ^{1}H NMR (500 MHz, CD_3OD, 0.063 M, 27 °C) δ ppm: 0.84 (3H, d, J = 6.8 Hz), 0.92 (3H, d, J = 6.9 Hz), 2.01 (1H, m), 2.06 (3H, s), 2.96 (1H, d, J = 6.5 Hz), 3.08 (1H, dd, J = 14.7, 7.1 Hz), 3.16 (1H, dd, J = 14.7, 7.7 Hz), 3.66 (1H, dd, J = 11.0, 5.0 Hz), 3.73 (1H, dd, J = 11.0, 4.7 Hz), 5.01 (1H, m), 7.00 (1H, t, J = 7.0 Hz), 7.07 (1H, t, J = 7.0 Hz), 7.12 (1H, s), 7.30 (1H, d, J = 7.0 Hz), 7.75 (1H, d, J = 7.0 Hz); HR-EIMS m/z: 319.1730 (M^+ calcd for $C_{17}H_{25}N_3OS$, 319.1718).
19. (−)-Indothiolactam-V (**2**): $[\alpha]_D$ −274.4° (c = 0.54, MeOH, 32.5 °C); UV λ_{max} (MeOH) nm (ε) 226 (29,200), 288 (17,300); ^{13}C NMR (125 MHz, $CDCl_3$, 0.050 M, 27 °C) δ ppm for the twist conformer: 19.66, 22.79, 31.45, 32.38, 33.26, 58.79, 64.25, 71.92, 103.92, 106.02, 114.10, 117.21, 121.32, 123.14, 139.44, 148.14, 206.09: δ ppm for the sofa conformer: 19.79, 20.06, 26.13, 27.22, 35.73, 59.68, 62.96, 82.21, 109.32, 110.38, 123.46, 123.55, 124.77, 127.60, 139.32, 145.08, 204.03; HR-EIMS m/z: 317.1564 (M^+ calcd for $C_{17}H_{23}N_3OS$, 317.1562).
20. Sharkey, N. A.; Blumberg, P. M. *Cancer Res.* **1985**, *45*, 19.
21. Hurley, J. H.; Newton, A. C.; Parker, P. J.; Blumberg, P. M.; Nishizuka, Y. *Protein Science* **1997**, *6*, 477.
22. Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U. *J. Am. Chem. Soc.* **1998**, *120*, 9159.
23. Wender, P. A.; Irie, K.; Miller, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 239.
24. Irie, K.; Nakahara, A.; Ohigashi, H.; Fukuda, H.; Wender, P. A.; Konishi, H.; Kikkawa, U. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2487.
25. Bögi, K.; Lorenzo, P. S.; Acs, P.; Szallasi, Z.; Wagner, G. S.; Blumberg, P. M. *FEBS Lett.* **1999**, *456*, 27.
26. Nakagawa, Y.; Irie, K.; Nakamura, Y.; Ohigashi, H.; Hayashi, H. *Biosci. Biotech. Biochem.* **1997**, *61*, 1415.