Rational Design, Synthesis, and Evaluation of a New Type of PKC Inhibitor

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Protein kinase C (PKC), a serine- and threonine-phosphorylating enzyme, is thought to play central roles in cellular signal transductions.[2] To clarify its physiological roles and to serve, potentially, as an important compound in anticancer therapies, a specific PKC inhibitor would be of great use. PKC has a catalytic site that phosphorylates substrate proteins and a regulatory site that controls its kinase activity. Since the catalytic site shares many features with the catalytic sites of other kinases, such as protein kinase A (PKA), it appears that a specific PKC inhibitor targeting the regulatory site would be advantageous in terms of kinase selectivity and/or PKC subtype selectivities. Most of the potent PKC inhibitors, such as H7[3] and staurosporine,[4] bind to the catalytic site in competition with ATP, and thus these inhibitors have the potential to inhibit other kinases. Although some PKC inhibitors have been reported to act at the regulatory site,[5−7] their mode of interaction with PKC is not fully understood. The following description of the rational design, synthesis, and evaluation of a regulatory site-inhibitor of PKC is therefore presented as a novel approach.

In the inactive state, PKC resides in the cytosol, and the regulatory site of PKC interacts with the catalytic site through the pseudosubstrate sequences, preventing the access of substrates to the catalytic site. PKC is believed to be activated by its translocation to the membrane and the subsequent conformational change caused by the binding of phosphatidyl-L-serine (L-PS) and *sn*-1,2-diacylglycerol (DAG), an endogenous PKC activator, to the cysteine rich domain (CRD)[8,9] in its regulatory site. Many structurally diverse agonists have been reported, such as phorbol 12-myristate 13-acetate (PMA), ingenol, aplysiatoxin, bryostatin, teleocidin, and their derivatives. However, to our knowledge, there is no mention in the literature of a DAG antagonist. This is probably because the binding of these agonists is highly synergetic to the interaction of PKC with a membrane containing L-PS (Figure 1).

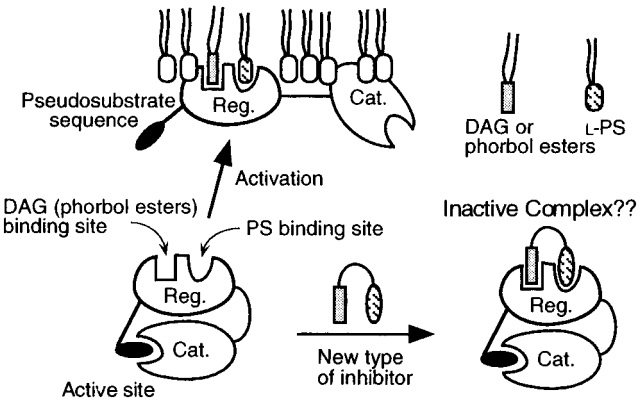

Figure 1. Strategy for the development of novel PKC inhibitor.

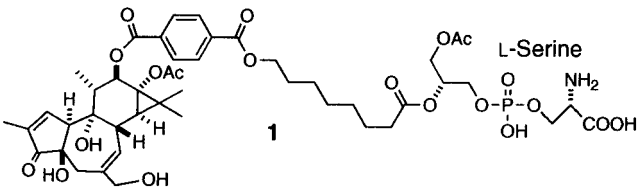

Figure 2. Phorbol ester-phosphatidyl-L-serine hybrid molecule (PEPS).

We focused on this unique property of PKC activation. If a molecule capable of blocking the binding sites of both DAG (phorbol ester) and L-PS in CRD were made available, it would likely prevent the interaction with the membrane and keep PKC inactive in the cytosol (Figure 1). To this end, we designed the phorbol ester-phosphatidyl-L-serine hybrid molecule (PEPS) **1** (Figure 2). Although the exact L-PS binding sites have not been identified, the design of compound **1** was based on the results of the reported photoaffinity labeling experiments using [20-^{3}H]-phorbol 12-azidobenzoate derivatives.[10,11]

Synthesis of PEPS (**1**) was achieved as shown in Scheme 1. 1,2:5,6-*O*-Diisopropylidene-D-mannitol **2** was converted to **3** in five steps.[12] Removal of an allyl group, reduction, and protection of the resulting primary alcohol gave **4**. The triphenylmethyl group of **4** was converted to an acetyl group, and debenzylation gave alcohol **5**. The compound **5** and phosphoramidite **6**[13] were treated with tetrazole in anhydrous THF, and oxidation of the resulting phosphine gave the phosphotriester **7**. Deprotection of the silyl ether gave the L-PS portion **8**. The coupling of the L-PS portion and the phorbol ester **9**, which was synthesized from phorbol in four steps,[14] was achieved using Yamaguchi's method.[15]

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(2) (a) *Protein Kinase C Current Concepts and Future Perspectives*; Lester, D. S., Epand, R. M., Eds.; Ellis Horwood Ltd.: West Sussex, 1992. (b) Nishizuka, Y. *Science* **1992**, *258*, 607 and references therein.

(3) Hidaka, H.; Inagaki, M.; Kawamoto, S.; Sasaki, Y. *Biochemistry* **1984**, *23*, 5036.

(4) (a) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397. (b) Bit, R. A.; Davis, P. D.; Elliott, L. H.; Harris, W.; Hill, C. H.; Keech, E.; Kumar, H.; Lawton, G.; Maw, A.; Nixon, J. S.; Vesey, D. R.; Wadsworth, J.; Wilkinson, S. E. *J. Med. Chem.* **1993**, *36*, 21 and references therein.

(5) Calphostin C is reported to inhibit PKC light dependently, although the mechanism is unknown: Bruns, R. F.; Miller, F. D.; Merriman, R. L.; Howbert, J. J.; Heath, W. F.; Kobayashi, E.; Takahashi, I.; Tamaoki, T.; Nakano, H. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 288 and references therein.

(6) (a) Hannun, Y. A.; Loomis, C. R.; Merill, A. H., Jr.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604. (b) Sozzani, S.; Agwu, D. E.; McCall, C. E.; O'Flaherty, J. T.; Schmitt, J. D.; Kent, J. D.; McPhail, L. C. *J. Biol. Chem.* **1992**, *267*, 20481. (c) Benna, J. E.; Hakim, J.; Labro, M.-T. *Biochem. Pharmacol.* **1992**, *43*, 527. It is possible that these cationic amphipathic compounds inhibit PKC activities by neutralizing the negative phospholipids; see chapter 3.6 in ref 2a.

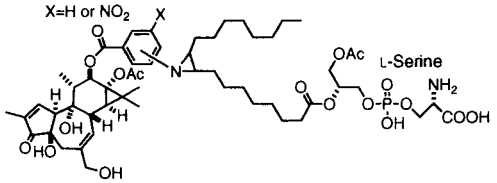
(7) Translocation inhibitor peptides were reported to interact with the regulatory domain of PKC: Yedovitzky, M.; Mochly-Rosen, D.; Johnson, J. A.; Gray, M. O.; Ron, D.; Abramovitch, E.; Cerasi, E.; Nesher, R. *J. Biol. Chem.* **1997**, *272*, 1417.

(8) Binding of phorbol esters to CRD, which consists of about 50 amino acid residues, is L-PS dependent, see: Wender, P. A.; Irie, K.; Miller, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 239 and references therein.

(9) The NMR and X-ray analyses of the structure of CRD in the absence of phospholipids have been reported, see: Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917 and references therein.

(10) (a) Delclos, K. B.; Yeh, E.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3054. (b) Schmidt, R.; Heck, K.; Sorg, B.; Hecker, E. *Cancer Lett.* **1985**, *26*, 97.

(11) We have designed the linker of PEPS (**1**) based on the assumption that the nitrene generated by the photolysis reacts with the double bond in the oleoyl group in L-PS as shown below.



(12) Hébert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777.

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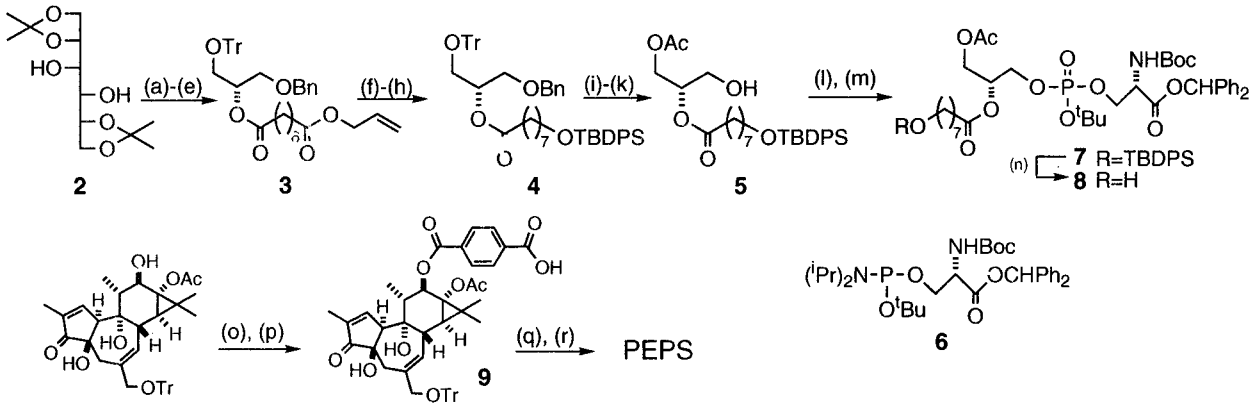
Scheme 1. Synthesis of the Phorbol Ester-Phosphatidyl-L-serine Hybrid Molecule (PEPS)



[a] (a) IO_4^- resin, BH_4^- resin; (b) BnBr, NaH; (c) 1 N − HCl; 73% (three steps); (d) TrCl, Et_3N, DMAP; 94%; (e) 7-allyloxycarbonylheptanoic acid, DCC, DMAP; 97%; (f) $Pd(PPh_3)_4$, morpholine; 92%; (g) BH_3·THF; 92%; (h) TBDPSCl, imidazole; 98%; (i) HCO_2H; 72%; (j) Ac_2O, Py; 95%; (k) Pd−C, H_2; 93%; (l) **6**, tetrazole; 89%; (m) *m*CPBA, $NaHCO_3$; 76%; (n) HF−Py; 41%; (o) 4-allyloxycarbonylbenzoic acid, DCC, DMAP; 95%; (p) $Pd(PPh_3)_4$, morpholine; 93%; (q) 2,4,6-trichlorobenzoyl chloride, Et_3N; then **8**, DMAP; 69%; (r) anisole in TFA; 76%.

Finally, deprotection under acidic conditions gave the target molecule PEPS (**1**).

The PKC-binding affinity of PEPS (**1**) was first examined by its competitive inhibition of the binding of tritium-labeled phorbol 12,13-dibutyrate ([^{3}H]PDBu) to PKC (mixture of α, β, and γ isozymes from rat brain).[16] PEPS (**1**) exhibits significant binding to PKC, although it is about 10-fold weaker than PMA under these conditions. Evaluation of the inhibiting effect of PEPS (**1**) on PKC activity was then carried out by comparison of the catalytic activities of PKC stimulated by PMA (10 nM) in both the absence and presence of PEPS (**1**) according to the method of Bell et al. with minor modifications.[17] As shown in Figure 3 (lane 1), PEPS (**1**) significantly inhibited PKC activity. A phorbol ester derivative **10**, which lacked the L-PS portion, did not inhibit PKC activation (lane 2), indicating that the L-PS portion of PEPS is essential to the inhibition. The inhibition of pure PKC isozymes (human, recombinant) was also evaluated. PEPS (**1**) showed significant inhibition of the PKC α and βI (lanes 3, 4, 5, 7, and 8). To further clarify the importance of specific interaction of the L-PS portion, the inhibition of PKC α and βI by compound **11**,[18] which had a D-PS component in place of L-PS, was examined.[19] The compound **11** was found to be a significantly less potent inhibitor than PEPS (**1**) (lanes 6 and 9). This result suggests that recognition of PS region of PEPS by PKC is stereospecific and that the inhibition of PKC by PEPS is dependent upon the interaction of PKC at both the phorbol and L-PS sites of PEPS.

PEPS (**1**) displayed an ability to act alone as a partial agonist for PKC, probably because its hydrophobic element still had some affinity for the membrane.[20] Attempts to suppress this undesired interaction and increase the binding affinity of PEPS (**1**) for PKC are now in progress.

In summary, this is the first report on a rationally designed phorbol ester derivative showing PKC inhibition. Although PEPS (**1**) would require significant structural modification for therapeutic applications, the concepts demonstrated herein should pave the way for further design of novel PKC inhibitors.

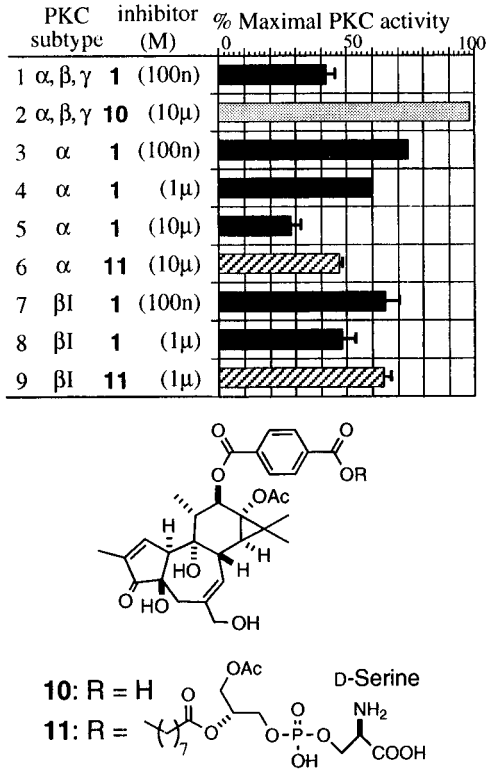


Figure 3. Inhibition of PKC activities by PEPS (**1**). PKC activities in the presence of 10 nM PMA and indicated concentration of inhibitors dispersed in Triton X-100-L-PS mixed micelles were measured.

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Supporting Information Available: Experimental procedures, ^{1}H, ^{13}C NMR, IR, mass spectral data for the products, experimental procedures for biological evaluation of the products, and modeling of the CRD-PEPS (**1**) complex (46 pages). See any current masthead page for ordering and Internet access instructions.

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(13) Kitas, E. A.; Knorr, R.; Trzeciak, A.; Bannwarth, W. *Helv. Chim. Acta* **1991**, *74*, 1314 and references therein.
(14) Sorg, B.; Fürstenberger, G.; Berry, D. L.; Hecker, E.; Marks, F. *J. Lipid. Res.* **1982**, *23*, 443.
(15) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
(16) Tanaka, Y.; Miyake, R.; Kikkawa, U.; Nishizuka, Y. *J. Biochem.* **1986**, *99*, 257.
(17) Hannun, Y. A.; Loomis, C. R.; Bell, R. M. *J. Biol. Chem.* **1985**, *260*, 10039.
(18) Compound **11** was synthesized using the same techniques as for PEPS (**1**) except that D-serine was used instead of L-serine.
(19) Studies using phospholipid analogues have revealed that only L-PS can fully support PKC activity with a high degree of stereospecificity, whereas D-PS only partially supports the activity. See: Lee, M.-H.; Bell, R. M. *J. Biol. Chem.* **1989**, *264*, 14797.
(20) See Supporting Information. Presence of added PS is necessary for activation of PKC by PEPS (**1**), as is the case with other phorbol esters.