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Design and synthesis of protein kinase Cα activators based on 'out of pocket' interactions

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ABSTRACT

Novel types of PKCα activators based on isobenzofuranone bearing a *myo*-inositol moiety were designed and synthesized. The derivatives with bulky substituents on the *myo*-inositol moiety significantly activated PKCα, but their binding sites were not the same as that of phorbol ester.

Protein kinase C (PKC) isozymes are known to play important roles in intracellular signal transduction pathways associated with proliferation, differentiation, and apoptosis.[1] The mammalian PKC family comprises 11 isozymes grouped into three classes, known as conventional PKCs (cPKCs: α, βI/βII, γ), novel PKCs (nPKCs: δ, ε, η, θ), and atypical PKCs (aPKCs: ζ, τ/λ). PKCs have a kinase domain that phosphorylates their substrate proteins and regulatory domains that control the kinase activity. Regulatory domains of cPKCs are composed of the membrane targeting domains, two C1 domains (C1A and C1B) and a C2 domain, and an autoinhibitory pseudo-substrate sequence. The activity of cPKCs is regulated by activating ligands, such as 1,2-diacylglycerol (DAG), phosphatidylserine (PS), and calcium ion. Although the molecular mechanism of cPKCs activation has not been fully clarified, it has been proposed that cPKCs initially binds to the membrane via Ca^{2+}-dependent interaction of PS with the C2 domain. Further binding of activating ligands to the C1 domain on the lipid bilayer allows release of the pseudo-substrate sequence from the active site in the kinase domain and stabilizes the active conformer of cPKCs.

It is well-known that various natural products, such as phorbol esters (PMA and PDBu, Fig. 1A), bryostatins, aplysiatoxin, and teleocidin, also bind to the C1 domain and activate cPKCs and nPKCs.[2] Since many of these compounds have strong tumor-promoting activity, PKC inhibitors have attracted attention as anti-cancer drug candidates.[3] On the other hand, PKC activators, such as bryostatins, also show anti-cancer activity and have recently been suggested to be candidate therapeutic agents for Alzheimer's disease.[4] Therefore, various analogues of these complex natural PKC activators have been synthesized.[5] As simpler compounds, DAG-based C1 domain ligands were developed by Marquez and co-workers.[6] We also reported conformationally constrained DAG-based isobenzofuranone (IB) derivatives such as **1** (Fig. 1A) as C1 domain ligands of PKCα[7] and revealed the importance of the phenolic alkyl chain at the C7 position for PKCα activation.[8] Further SAR studies resulted in development of the more potent PKCα activator **2**.[9]

During the course of SAR studies, we found that PKCα activator **3** having a straight alkyl chain on the acyl group of IB showed weak competitive binding against [^{3}H]PDBu, but exhibited significant PKCα activating ability.[9] According to Cho and co-workers,[10] phorbol esters bind preferably to the C1B domain and DAGs bind to the C1A domain in PKCα. These results suggested that the DAG-like molecule **3** is likely to bind to the C1A domain to activate PKCα, because **3** showed weak binding affinity toward the phorbol ester binding domain (Phorbol BD). On the other hand, Irie and Blumberg reported that phorbol esters binds to their chemically synthesized or GST-fusion C1A and C1B domains with similar affinities.[11] Therefore the possibility that **3** might bind to an unknown binding site on PKCα other than C1 domains could not be ruled out. In either case, to further analyze the molecular mechanism of PKCα activation, a series of potent PKCα activators with different selectivity to each ligand-binding site would be required. We have already reported a highly potent PKCα activator with high affinity for the Phorbol BD.[9] Here, we report a novel class of PKCα activators having negligible affinity for the Phorbol BD.

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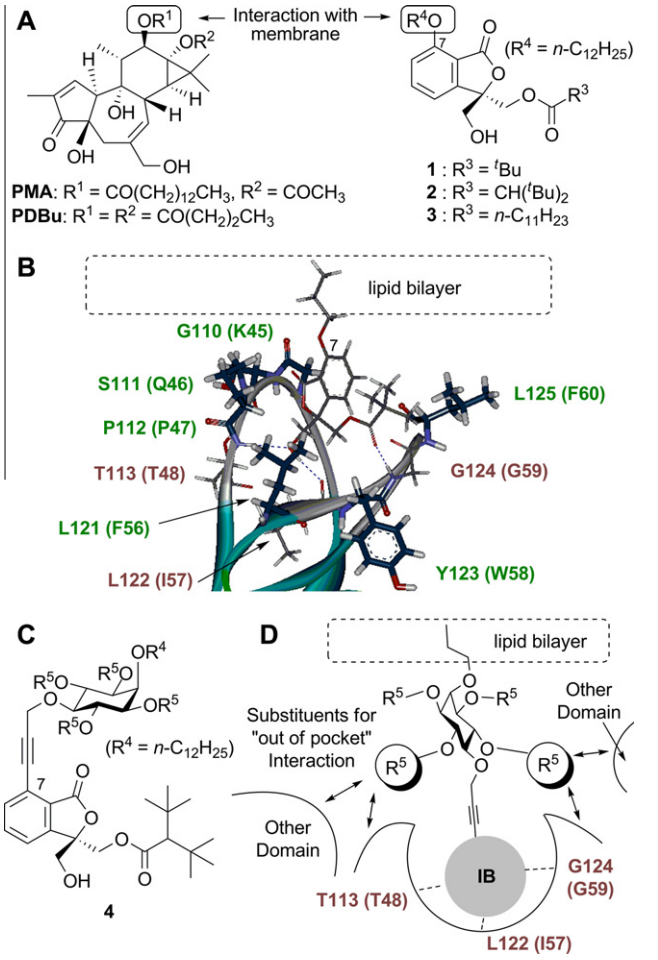
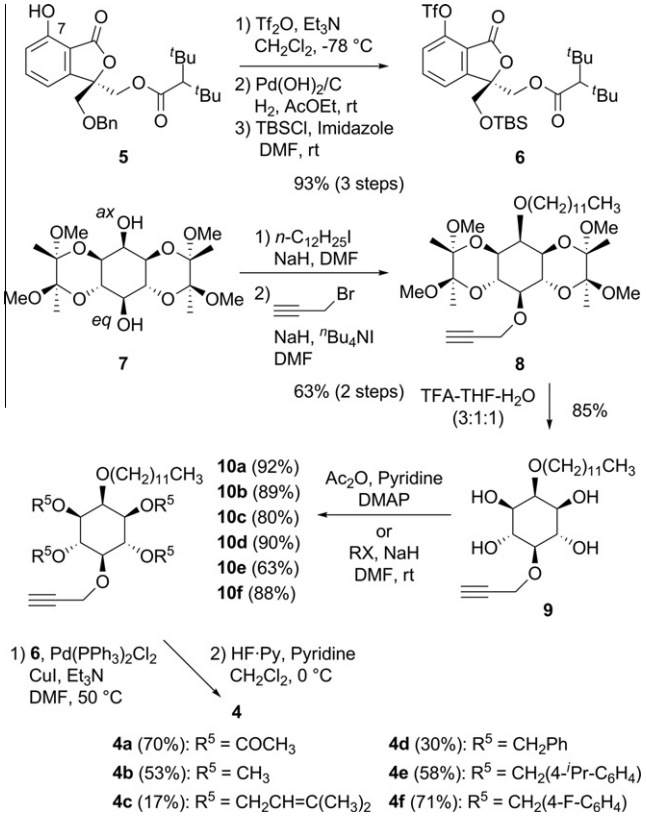


Figure 1. (A) Structures of phorbol esters and isobenzofuranone (IB) derivatives **1**–**3**. (B) Binding model of **1** (short chain analogue: $R^4 = C_3H_7$) with PKCαC1B domain. Essential amino acids for binding to the C1B domain are indicated in brown. Outer amino acids around the binding site are indicated in green. Amino acids in parentheses are the corresponding amino acids of the C1A domain. (C) Structure of designed PKCα activator **4**. (D) Schematic illustration of the concept of 'out of pocket' interaction.

To design new C1A domain ligand molecules, we postulated that the core structure of IB derivatives is able to bind to both C1A and C1B domains, and modification of the side chains could change the affinity towards each domain. Since the much weaker PKCα activating ability of **3** than that of **2** suggested insufficient binding affinity of **3** to its binding site, we selected the high affinity phorbol BD ligand **2** as a parent compound. The isobenzofuranone structure is expected to interact with the binding pocket by forming hydrogen bonds with key inner amino acids, which are quite similar between the two domains (for C1A: Thr48, Ile57, and Gly59; for C1B: Thr113, Leu122, and Gly124). In contrast, the outer amino acids around the binding sites of the two C1 domains are significantly different (Fig. 1B). Therefore, to develop a C1A-selective ligand, we focused on the outer amino acids, and designed compound **4** (Fig. 1C). Although **2** has high affinity for the C1B domain, we anticipated that the 'out of pocket' interaction between the substituents R^5 on the inositol moiety and the outer amino acid residues would discriminate the two C1 domains and influence the selectivity (Fig. 1D). Furthermore, in case of whole PKCα, interaction of the substitutents R^5 with the other domains such as C2 domain may influence the binding to each C1 domains. *myo*-Inositol structure was chosen as the recognition moiety for 'out of pocket' interaction because of its stability and C_2-symmetric structure, and was introduced at the C7 position of **2** via a rigid alkyne linker. A



Scheme 1. Synthesis of IB derivatives **4a**–**4f**.

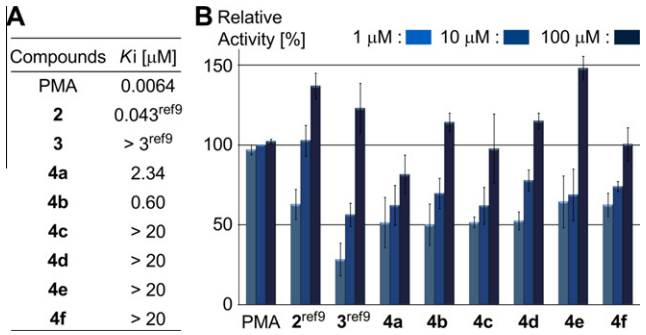


Figure 2. (A) K_i values of compounds estimated from competitive inhibition of [^{3}H]PDBu binding to PKCα. (B) Relative activity of PKCα in the presence of the indicated compounds (0% = phosphorylation level without activating ligand; 100% = phosphorylation level with 10 μM PMA).

long alkyl chain at the C7 position would be important for the interaction of ligand-C1 domain complex with PS-containing lipid bilayer to stabilize the active conformer of PKCα, and thus such a chain was introduced at the axial hydroxyl group of the inositol moiety.

Synthesis of **4** is illustrated in Scheme 1. Since we have already established methodology for asymmetric synthesis of **5**,[9] transformation of **5** to the corresponding triflate **6** was performed via a routine three-step sequence of trifluoromethanesulfonylation, hydrogenolysis of benzyl ether, and re-protection of the resulting primary alcohol with a TBS group. Synthesis of the recognition moiety began from the known C_2-symmetric *myo*-inositol derivative **7**.[12] Alkylation of the axial secondary alcohol of **7** with a straight C12 chain, followed by etherification with propargyl bromide, gave fully protected inositol derivative **8** in good yield. After

removal of the cyclic acetal of **8**, introduction of various groups into tetraol **9** by acetylation (**10a**) or alkylation with MeI (**10b**), prenyl bromide (**10c**), or benzyl bromides (**10d**–**10f**) afforded **10a**–**10f**. Connection of alkynes **10a**–**10f** with triflate **6** were investigated utilizing Hagiwara–Sonogashira conditions.[13] Although only 29% yield of the desired coupling product was obtained in the case of **10c**, probably due to the presence of the prenyl ether moiety, all other coupling reactions proceeded smoothly in 60% to quantitative yield. Finally, removal of the TBS group gave IB derivatives **4a**–**4f**.

The binding affinity of these compounds was evaluated by assay of competitive inhibition of the binding of [^{3}H]PDBu to whole PKCα in the presence of PS vesicles.[14] As summarized in Fig. 2A, variation of substituent R^5 on the inositol moiety resulted in different binding profiles toward the phorbol BD. As reported before, **2** showed very strong inhibition of [^{3}H]PDBu binding (K_i = 43 nM), while compounds **4a** and **4b** with small substituents R^5 on the inositol moiety showed modest inhibition, with K_i values of 2.34 μM and 598 nM, respectively. These results indicated that the existence of the *myo*-inositol moiety affected the binding affinity for the phorbol BD, nevertheless **4a** and **4b** still showed significant binding. In contrast, **4c**–**4f** showed weaker inhibition even at high concentration (K_i >20 μM), indicating that these derivatives possessed negligible binding affinity for the phorbol BD. Because the binding ability of prenyl derivative **4c** and benzyl derivatives **4d**–**4f** was dramatically reduced compared to that of **4a** and **4b**, the size of R^5 seems to be critical for the inhibition of [^{3}H]PDBu binding, as we expected.

As mentioned above, at least one of the phorbol BD is the C1B domain. Thus these results of binding assay suggest that **4a** and **4b** have binding ability to the C1B domain but **4c**–**4f** do not. To speculate the origin of this selectivity, a binding model of **4b** with the C1B domain of PKCα was constructed based on the binding model of **1** shown in Figure 1B, in which we postulated the IB skeleton of **4b** would bind to the ligand binding site and form hydrogen bonds similar to compound **1**. The substituent at C7 and the *t*-Bu group of R^3 of **1** were replaced by alkyne-inositol moiety **10b** and CH(*t*-Bu)$_2$ group, and molecular dynamics and energy minimization calculations were performed by Discovery Studio 2.5. As shown in Figure 3A, introduction of the bulky hydrophobic CH(*t*-Bu)$_2$ group would result in the stabilization of the complex of IB derivatives with the C1B domain, in which the CH(*t*-Bu)$_2$ group effectively interacted with Leu121 and Leu125 (Fig. 3A right). Moreover, one of the R^5 substituents on the inositol moiety would be located around Pro112 and appear to contribute to 'out of pocket' interaction with the C1B domain (Fig. 3A). However, in case of bulky R^5 substituents such as the benzyl group, this 'out of pocket' interaction would be unfavorable. Namely, the steric repulsion between bulky R^5 substituents and Pro112 might prevent these compounds from interacting with the C1B domain (Fig. 3B). On the other hand, the C1B domain would exist between the C1A and the C2 domain, and tight interaction with these domains was proposed.[15–17] These reports suggested that neighboring C1A and C2 domains would be located around the binding site of phorbol ester on the C1B domain. Thus there is another possibility that IB derivatives having bulky R^5 substituents could not bind to C1B domain due to the steric repulsion with neighboring domains (Fig. 3B).

Finally, PKCα activator potency was assessed by comparison of the phosphorylation levels in the absence and presence of IB derivatives **4a**–**4f**, using a fluorescence-labeled peptide substrate

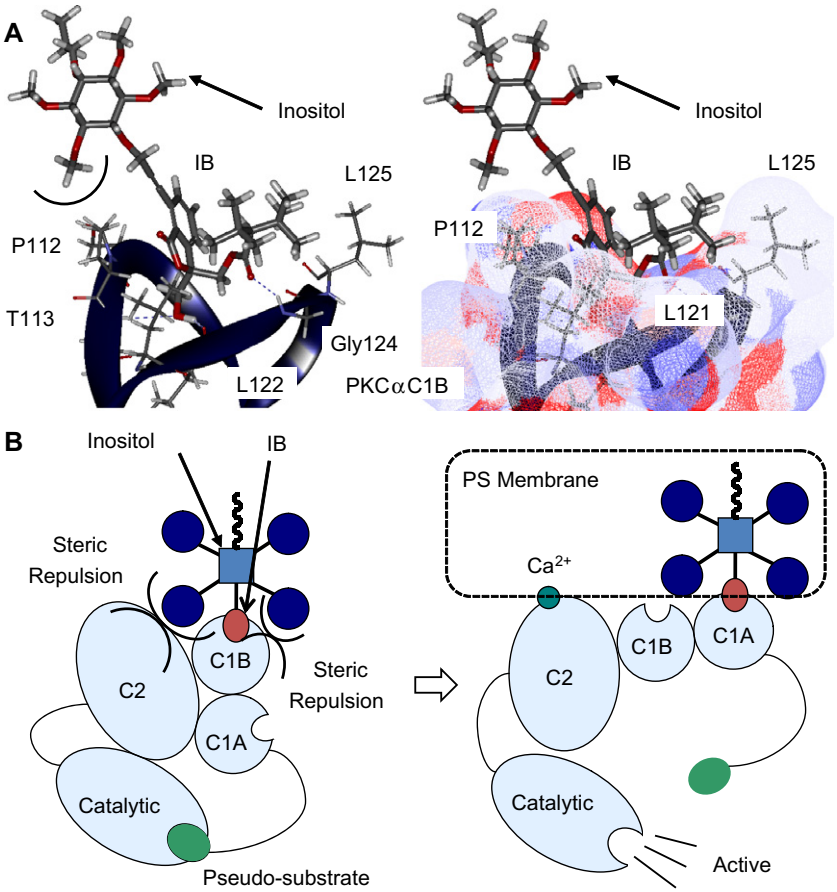


Figure 3. (A) Binding model of compound **4b** with PKCα (left: ribbon model; right: surface model). (B) Hypothetical model for the decrease of binding affinity of IB derivatives with bulky substituents to phorbol ester binding site and for origin of their activation potency.

(Fig. 2B).[8] As expected, all the new IB derivatives were found to induce PKCα activation, suggesting that the inositol moiety of IB derivatives does not disturb the stabilization of the active conformer of PKCα induced by IB derivatives. Importantly, although compounds **4c**–**4f** showed negligible inhibition of [^{3}H]PDBu binding to PKCα, these derivatives were able to activate PKCα. These facts indicate that **4c**–**4f** would stabilize the active conformer by the binding to a particular site on PKCα other than phorbol BD. Although further investigation should be required, we speculate that these molecules would bind to C1A domain and activate PKCα in view of the DAG-based structure of these molecules and Cho's report (Fig. 3B).[18] The PKCα activation levels in the presence of 1 μM **4c**–**4f** were comparable or slightly lower than that with the strong phorbol BD binder **2**. However, they were much higher than that with **3**, and **4e** was found to be the most potent among the synthesized compounds. Thus, compound **4e** should be useful as a probe molecule to elucidate the mechanism of PKCα activation.

In conclusion, we have developed a new class of PKCα activators which do not show binding affinity for the phorbol BD. Few PKCα activators with selective binding affinity for the C1A domain are known,[11,19] and therefore these new activators are expected to be useful tools in PKCα research if these molecules selectively bind to C1A domain. In particular, because there is still controversial over the binding selectivity of the physiological ligand DAG and the strong tumor promotor phorbol ester to the C1A and C1B domains, it is of interest to elucidate the binding site of **4e** to understand the physiological and non-physiological activation modes of PKCα. Efforts along this line are in progress.

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Supplementary data

Supplementary data (^{1}H NMR and HRMS data of compounds **4a**–**f** and methods for the evaluation of the biochemical activity of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.108.

References and notes

1. (a) Nishizuka, Y. *Science* **1992**, *258*, 607; (b) Yang, C.; Kazanietz, M. G. *Trends Pharmacol. Sci.* **2003**, *24*, 602; (c) Newton, A. C. *Chem. Rev.* **2001**, *101*, 2353.
2. (a) Kishi, Y.; Rando, R. R. *Acc. Chem. Res.* **1998**, *31*, 163; (b) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. *J. Biol. Chem.* **1982**, *257*, 7847; (c) Fujiki, H.; Tanaka, Y.; Miyake, R.; Kikkawa, U.; Nishizuka, Y.; Sugimura, T. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 339; (d) Smith, J. B.; Smith, L.; Pettit, G. R. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 939; (e) Berkow, R. L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109.
3. Mackay, H. J.; Twelves, C. J. *Nat. Rev. Cancer* **2007**, *7*, 554.
4. (a) Etcheberrigaray, R.; Tan, M.; Dewachter, I.; Kuipéri, C.; Van der Auwera, I. V.; Wera, S.; Qiao, L.; Bank, B.; Nelson, T. J.; Kozikowski, A. P.; Van Leuven, F.; Alkon, D. L. *PNAS* **2004**, *101*, 11141; (b) Khan, T. K.; Nelson, T. J.; Verma, V. A.; Wender, P. A.; Alkon, D. L. *Neurobiol. Dis.* **2009**, *34*, 332.
5. Selected recent reports: (a) Nakagawa, Y.; Yanagita, R. C.; Hamada, N.; Murakami, A.; Takahashi, H.; Saito, N.; Nagai, H.; Irie, K. *J. Am. Chem. Soc.* **2009**, *131*, 7573; (b) Wender, P. A.; Verma, V. A. *Org. Lett.* **2008**, *10*, 3331; (c) Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Peach, M. L.; Blumberg, P. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 4580; (d) Mach, U. R.; Lewin, N. E.; Blumberg, P. M.; Kozikowski, A. P. *ChemMedChem* **2006**, *1*, 307; (e) Bertolini, T. M.; Giorgione, J.; Harvey, D. F.; Newton, A. C. *J. Org. Chem.* **2003**, *68*, 5028.
6. Selected recent reports: (a) Raifman, O.; Kolusheva, S.; Comin, M. J.; Kedei, N.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E.; Jelinek, R. *FEBS J.* **2010**, *277*, 233; (b) Comin, M. J.; Czifra, G.; Kedei, N.; Telek, A.; Lewin, N. E.; Kolusheva, S.; Velasquez, J. F.; Kobylarz, R.; Jelinek, R.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2009**, *52*, 3274.
7. (a) Baba, Y.; Ogoshi, Y.; Hirai, G.; Yanagisawa, T.; Nagamatsu, K.; Mayumi, S.; Hashimoto, Y.; Sodeoka, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2963; (b) Baba, Y.; Mayumi, S.; Hirai, G.; Kawasaki, H.; Ogoshi, Y.; Yanagisawa, T.; Hashimoto, Y.; Sodeoka, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2969.
8. Hirai, G.; Shimizu, T.; Watanabe, T.; Ogoshi, Y.; Ohkubo, M.; Sodeoka, M. *ChemMedChem* **2007**, *2*, 1006.
9. Hirai, G.; Ogoshi, Y.; Ohkubo, M.; Tamura, Y.; Watanabe, T.; Shimizu, T.; Sodeoka, M. *Tetrahedron Lett.* **2009**, *50*, 3609.
10. Ananthanarayanan, B.; Stahelin, R. V.; Digman, M. A.; Cho, W. *J. Biol. Chem.* **2003**, *278*, 46886.
11. (a) Irie, K.; Nakagawa, Y.; Ohigashi, H. *Chem. Rec.* **2005**, *5*, 185; (b) Pu, Y.; Perry, N. A.; Yang, D.; Lewin, N. E.; Kedei, N.; Braun, D. C.; Choi, S. H.; Blumberg, P. M.; Garfield, S. H.; Stone, J. C.; Duan, D.; Marquez, V. E. *J. Biol. Chem.* **2005**, *280*, 27329.
12. Riley, A. M.; Jenkins, D. J.; Potter, B. V. L. *Carbohydr. Res.* **1998**, *314*, 277.
13. Murty, K. V. S. N.; Vasella, A. *Helv. Chim. Acta* **2001**, *84*, 939.
14. Lewin, N. E.; Blumberg, P. M. *Methods Mol. Biol.* **2003**, *233*, 129.
15. (a) Conesa-Zamora, P.; Gómez-Fernández, J. C.; Corbalán-García, S. *Biochim. Biophys. Acta* **2000**, *1487*, 246; (b) Bittova, L.; Stahelin, R. V.; Cho, W. *J. Biol. Chem.* **2001**, *276*, 4218; (c) Stahelin, R. V.; Wang, J.; Blatner, N. R.; Rafter, J. D.; Murray, D.; Cho, W. *J. Biol. Chem.* **2005**, *280*, 36452; (d) Slater, S. J.; Seiz, J. L.; Cook, A. C.; Buzas, C. J.; Malinowski, S. A.; Kershner, J. L.; Stagliano, B. A.; Stubbs, C. D. *J. Biol. Chem.* **2002**, *277*, 15277.
16. The structure of PKCα has been proposed. See: Srinivasan, N.; Bax, B.; Blundell, T. L.; Parker, P. J. *Proteins: Struct. Funct. Genet.* **1996**, *26*, 217.
17. Recently crystal structure of large part of PKC βII (C1B, C2, and catalytic domain) was reported, and interesting activation mechanism was also proposed. In this structure, it was indicated that C1B domain would be surrounded by C1A, C2, and catalytic domain. See: Leonard, T. A.; Różycki, B.; Saidi, L. F.; Hummer, G.; Hurley, J. H. *Cell* **2011**, *144*, 55–66.
18. As far as we know, there is no direct method to estimate the binding affinity toward C1A domain using whole PKCα.
19. Selected Paper: Irie, K.; Masuda, A.; Shindo, M.; Nakagawa, Y.; Ohigashi, H. *Bioorg. Med. Chem.* **2004**, *12*, 4575.