Tetrahedron Letters 50 (2009) 3609–3612

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Asymmetric synthesis of isobenzofuranone derivatives and their unique character as protein kinase Cα (PKCα) activators

Go Hirai [a], Yosuke Ogoshi [b], Megumi Ohkubo [a,b], Yuki Tamura [a], Toru Watanabe [a,b], Tadashi Shimizu [a], Mikiko Sodeoka [a,*]

[a] Synthetic Organic Chemistry Laboratory, Advanced Science Institute, RIKEN, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan
[b] Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1, Katahira, Sendai, Miyagi 980-8577, Japan

ARTICLE INFO

Article history:
Received 15 January 2009
Revised 9 March 2009
Accepted 10 March 2009
Available online 16 March 2009

Keywords:
Protein kinase Cα
Asymmetric dihydroxylation
Isobenzofuranone
Bleb-forming assay

ABSTRACT

Efficient enantio-selective synthesis of conformationally constrained diacylglycerol analogues, 7-substituted isobenzofuranone derivatives, originally developed by us as PKCα ligands, was achieved by asymmetric dihydroxylation and γ-lactone formation via *ortho*-lithiation and carboxylation. A series of derivatives having straight and/or branched side chains were synthesized and evaluated, and low-nanomolar-concentration affinity ligands and highly potent PKCα activators were found among them. These potent ligands induced phenotypic change of K562 cells, which is characteristic of PKC activators.

1,2-Diacylglycerol (DAG), which is generated via enzymatic hydrolysis of various phospholipids, is one of the most important second messengers. It is an activator of protein kinase C (PKC). PKC isozymes are known to play important roles in intracellular signal transduction for cellular events, such as proliferation, differentiation, and apoptosis.[1] They are divided into three classes, conventional PKCs (cPKCs: α, βI/βII, γ), novel PKCs (nPKCs: δ, ε, η, θ), and atypical PKCs (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. C1 domain is the binding site of DAG. The activation mechanism of cPKCs was proposed to be as follows. The cPKCs initially bind to the membrane via Ca^{2+}-dependent binding of the C2 domain, and then binding of DAG on the lipid membrane to C1 domain allows release of a pseudo-substrate region from the active site in the kinase domain and stabilizes the active form of PKCs. However, the molecular mechanism of PKC activation is not yet understood in detail.

Several complex natural products, such as phorbol esters (PMA and PDBu, Fig. 1A), bryostatin, aplysiatoxins, and tereocidins, are also known to be C1 domain ligands and strong agonists of cPKCs and nPKCs.[2] These natural products are valuable tools in cancer research and in the development of anticancer therapeutics,[3] because with the exception of bryostatin, they show tumor-promoting activity. In contrast, bryostatin is an anticancer agent.[4] Therefore, much effort has been made to create analogues of these natural products; indeed, several of the analogues obtained so far have interesting biological activities.[5] On the other hand, the nontumor promoting physiological ligand DAG is also of interest. However, DAG is difficult to utilize as a template for further structural modification because of its structural flexibility and lability under biological conditions. Conformationally constrained analogues of DAG are therefore promising candidates as DAG surrogates for uncovering the roles of C1 domain-containing proteins.[6] Among such DAG analogues, monocyclic γ-lactones (DAG-lactone derivatives) have been extensively studied by Marquez and co-workers.[7] Independently, we have also developed bicyclic isobenzofuranone derivatives as C1 domain ligands of PKCα.[8] Our initial structure–activity relationship (SAR) studies showed that chirality is important, and (*R*)-isobenzofuranone derivatives are better ligands than the (*S*)-isomers. It was also shown that bulkiness of the acyl side chain is important for PKC affinity, and (*R*)-pivaloyl derivative **1** was found to be a potent ligand for PKCα (Fig. 1B, K_i = 122 nM).[8a] Here we report a novel enantioselective synthetic route to isobenzofuranone derivatives having straight and/or branched side chains. The activities of these compounds were evaluated at the enzyme and cellular levels.

As reported previously,[8c] a binding model of **1** with PKCαC1B domain suggested that an acyl side chain carbonyl group would form a hydrogen bond with Gly124 in a similar manner to the 3-carbonyl group of phorbol ester. The side chain of the acyl group would be located between Leu121 and Leu125, and the alkyl chain on the benzene ring would be buried in the phospholipid bilayer,

* Corresponding author. Fax: +81 48 462 4666.
E-mail address: sodeoka@riken.jp (M. Sodeoka).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.03.069

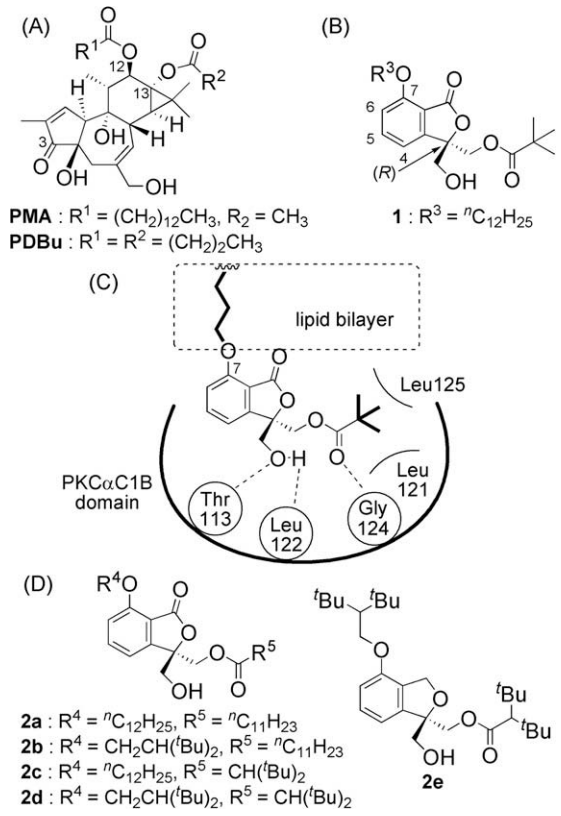
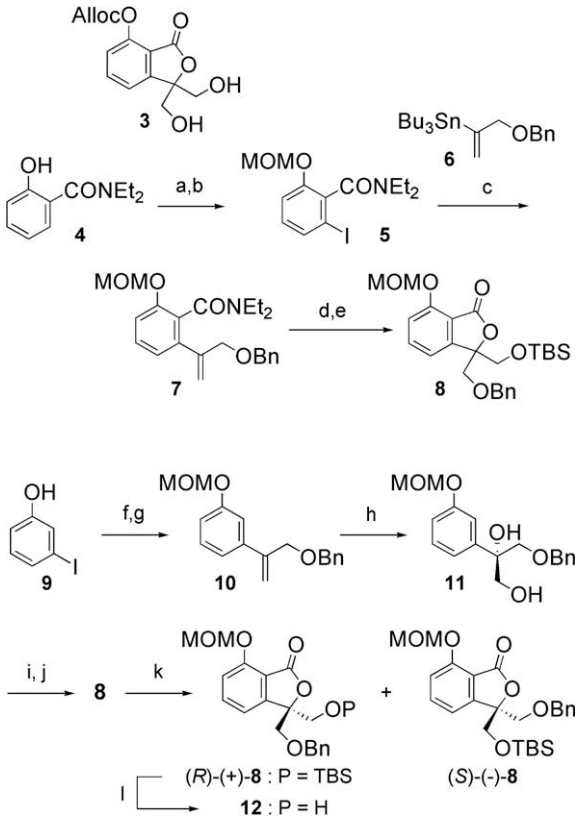


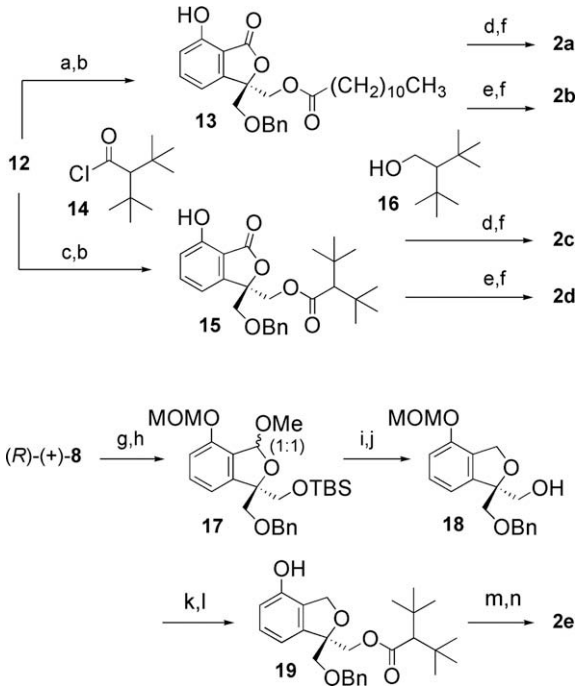
Figure 1. (A) Structure of phorbol esters. (B) Structures of 7-substituted isobenzofuranone derivatives **1**. (C) Binding model of the complex of PKCαC1B domain with **1** and representative interaction between them. (D) Structures of designed isobenzofuranones **2a**–**e**.



Scheme 1. Asymmetric synthesis of (*R*)-(+)-**8**. Reagents and conditions: (a) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, rt (98%); (b) $^{s}BuLi$, THF, −78 °C, then I_2, rt (85%); (c) **6**, $PdCl_2(PPh_3)_2$, LiCl, DMF, 70 °C (65%); (d) AD-mix-β, $^{t}BuOH–H_2O$, $CH_3SO_2NH_2$, 0 °C; (e) TBSCl, Imidazole, DMF, rt (54%, 2 steps, 13% ee); (f) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, rt (95%); (g) **6**, $PdCl_2(PPh_3)_2$, LiCl, DMF, 90 °C (93%); (h) AD-mix-α, $^{t}BuOH–H_2O$, 0 °C (95%, 78% ee); (i) TBSCl, imidazole, DMF, rt, (75%); (j) $^{n}BuLi$ (10 equiv), hexane, rt, then CO_2 (dry ice), rt (80%, 78% ee); (k) separation by HPLC (CHIRALPAK AD-H, hexane/$^{i}PrOH$ = 9:1) ((*R*)-(+)-**8**, 78%; (*S*)-(−)-**8**, 4%); (l) TBAF, THF, rt, (95%).

like the acyl side chain at C12 of phorbol ester (Fig. 1C). Previous SAR studies[8a] indicated that shape of the hydrophobic acyl group is important. Cyclohexylcarbonyl derivative showed comparable affinity to the pivaloyl derivative **1**, but too bulky 3,5-di(*tert*-butyl)benzoyl derivative showed negligible binding with PKC. Based on our binding model, we designed derivatives **2c** and **2d** having a 2,2-di(*tert*-butyl)acetyl group, which is expected to fit into the space between the two Leu side chains (Fig. 1D).[9] Recently, we also found that the direction of the hydrophobic long alkyl chain on the benzene ring of isobenzofuranone derivatives was important for PKCα activation. Interestingly, 7- or 6-substituted isobenzofuranone derivatives activate PKCα strongly, but 4-substituted derivatives do not activate it.[8c] These facts suggest that a long alkyl chain would be important for stabilization of the membrane-bound active conformer. Furthermore, in this model, the lactone carbonyl group would not participate in the interaction with C1 domain of PKCα (Fig. 1C), but might contribute to the interaction with phospholipids and, consequently, to the binding and activation of PKCα. Thus, to examine the roles of the straight alkyl side chain and lactone carbonyl group, we also planned to synthesize **2a**, **2b**, and **2e** (Fig. 1D).

Since an achiral isobenzofuranone diol **3** was the key intermediate of the previous synthesis,[8a] we first tried an enzyme-catalyzed enantioselective mono-acylation/hydrolysis approach, but this was not successful. Therefore we needed to develop a novel synthetic route. We planned to use asymmetric dihydroxylation of α-arylstyrene derivative **7** as a key step for creating the chiral tertiary alcohol.[10] Intermediate **7** was prepared from salicylic acid diethylamide (**4**) by protection with MOM group, selective *ortho*-lithiation followed by reaction with iodine, and Stille coupling with **6** (Scheme 1). Dihydroxylation of **7** using AD-mix-β was carried out and the desired γ-lactone was formed simultaneously. Protection of the newly generated primary alcohol as TBS ether gave **8**. But, unfortunately and unexpectedly, the enantioselectivity was disappointing. The optical purity of **8** was only 13% ee even when the dihydroxylation was conducted in the presence of $MeSO_2NH_2$.[11] The reason for this low enantioselectivity is unclear, but might be related to the participation of the bulky diethylaminocarbonyl group at the *ortho*-position.[12] To solve this problem, we next tried asymmetric dihydroxylation of **10**. After conversion of commercially available *m*-iodophenol (**9**) to its MOM-ether, an alkenyl group was introduced by means of coupling reaction with vinylstannane **6** to give the α-benzyloxymethylstyrene derivative **10** in good yield. Asymmetric dihydroxylation of **10** using AD-mix-α provided the desired diol **11** (78% ee), and the resulting primary alcohol was converted into the corresponding TBS ether. Regioselective *ortho*-lithiation and carboxylation were conducted with excess *n*-butyllithium and CO_2 to give the γ-lactone **8** without loss of enantio-purity.[13] Optically pure (*R*)-(+)-**8** was obtained by HPLC purification using a chiral phase column, and the absolute stereochemistry was determined after conversion to **1**. The common intermediate **12** for the preparation of **2a**–**d** was obtained by removal of the TBS group. Further transformation to **2a**–**d** was conducted by conventional methods (Scheme 2). Acylation of primary alcohol with lauroyl chloride or acid chloride **14**,[14] followed by treatment with TMSBr, afforded phenol **13** or **15** in good yield. After alkylation of the phenolic hydroxyl group via Mitsunobu reaction using dodecanol or alcohol **16**,[14] synthesis of **2a**–**d** was completed by hydrogenolysis to remove benzyl protection. To re-



Scheme 2. Synthesis of **2a–e**. Reagents and conditions: (a) $CH_3(CH_2)_{10}COCl$, pyridine, DMAP, rt (91%); (b) TMSBr, CH_2Cl_2, −30 °C (for **13**, 98%; for **15**, 95%); (c) **14**, pyridine, DMAP, 90 °C (60%); (d) 1-dodecanol, PPh_3, DEAD, THF, rt; (e) **16**, PPh_3, DEAD, THF, rt; (f) $Pd(OH)_2/C$, H_2, MeOH, rt (for **2a**, quant.; for **2b**, 76%; for **2c**, 40%; for **2d**, 60%, 2 steps); (g) DIBAL-H, CH_2Cl_2, −78 °C (88%); (h) CSA, $CH(OMe)_3$/CH_2Cl_2 = 1:1, rt (88%); (i) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C; (j) TBAF, THF, rt (86%, 2 steps); (k) **14**, pyridine, DMAP, 90 °C (70%); (l) TMSBr, CH_2Cl_2, −30 °C (72%); (m) **16**, PPh_3, DEAD, THF, rt; (n) $Pd(OH)_2/C$, H_2, MeOH, rt (13%, 2 steps).

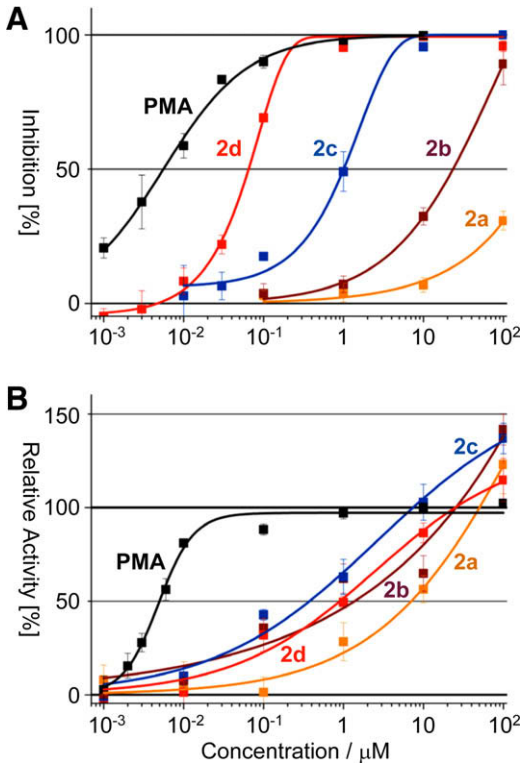


Figure 2. Evaluation of the activity of the phorbol ester PMA and isobenzofuranones (**2a–d**). (A) Dose-dependent inhibition of [^{3}H]PDBu binding to PKCα. (B) Relative activity of PKCα (100% = activity with 10 μM PMA).

move the lactone carbonyl group of the key intermediate, (*R*)-(+)-**8** was first reduced with DIBAL, and the resulting hemiacetal was converted into methyl acetal **17**. Further reduction was conducted with the Et_3SiH–$BF_3{\cdot}OEt_2$ system. After removal of the TBS group, isobenzofuran **18** was obtained in good yield. Acylation with **14** and deprotection of the MOM group afforded phenol **19**. Although the yield was unsatisfactory because of the absence of the lactone carbonyl group, synthesis of the desired **2e** was achieved by the introduction of the bulky alkyl group onto phenol and hydrogenolysis of the benzyl group.[15]

The binding affinity toward PKCα of these compounds was assessed by competitive inhibition of the binding of [^{3}H]PDBu in the presence of phosphatidyl-L-serine (PS) vesicles (Fig. 2A).[16,17] Compound **2d** (Clog*P* = 7.43) with two bulky alkyl chains was found to show the highest binding affinity (K_i = 2.1 nM), and the affinity decreased dramatically in the order of **2c**, **2b**, and **2a**. The K_i values were 43 nM (**2c**, Clog*P* = 9.14), 755 nM (**2b**, Clog*P* = 9.23), and >3000 nM (**2a**, Clog*P* = 10.9), respectively. These results indicated that the branched hydrophobic group greatly improved binding to PKCα, as we had expected, but its contribution is much larger when it is introduced into the acyl group (**2c**) than at the 7-position (**2b**).

Next, PKCα activator potency was measured by comparison of phosphorylation levels in the absence and presence of the isobenzofuranone derivatives **2a–d** using a fluorescence-labeled peptide substrate (Fig. 2B).[16] As expected, all four compounds showed strong, dose-dependent activation of PKCα. However, activation levels of PKCα induced by these compounds were not correlated with the binding affinity. Relative activity of **2d**, which is the strongest binder of PKCα, was slightly lower than that of **2c**. These results indicated that branched alkyl chains in **2d** were less effective for the induction of PKCα activation, though they contributed to strong binding of **2d** to PKCα. In contrast, long straight alkyl chains at the C7 position participated in strong activation of PKCα, and **2c** was the most potent activator of PKCα. As mentioned above, we recently showed the importance of the direction of the alkyl chain on the benzene ring for PKCα activation.[8c] In this case, all four compounds have the hydrophobic alkyl group at C7, and are expected to interact with fatty acid chains in PS lipid (Fig. 1C). But, it is likely that a straight alkyl chain would have a strong anchoring effect, fixing the PKCα-**2c** complex to the lipid bilayer, resulting in the stabilization of active PKCα. Since the anchoring effect of the branched alkyl group seemed to be weaker than that of the straight chain, the direction of the interaction of PKCα-**2d** complex with PS lipid would be less effectively fixed. Therefore, the population of active PKCα would be smaller, and the activation ability of **2d** would be lower than that of **2c**.

Comparison of the results of the binding (Fig. 2A) and activation assays (Fig. 2B) indicated that, in the case of the phorbol ester PMA, both binding and activation of PKCα were saturated over the concentration of 1 μM, and no further increase in the activation level was observed. In contrast, relative activities induced by the isobenzofuranone derivative **2a–d** at 100 μM were higher than the maximum activity induced by PMA. Although binding of **2d** and **2c** seems to reach the maximum level at 1 and 10 μM, respectively, the PKCα activation levels continued to increase at concentrations over 10 μM. Moreover, significant activation was observed with the weaker binders **2a** and **2b** at concentrations at which negligible inhibition of [^{3}H]PDBu binding by these compounds was observed. These results could be explained by different relative affinities of the ligand molecules for the C1A and C1B domains. Cho et al. reported that phorbol esters such as PDBu and PMA have strong preference for the C1B domain of PKCα.[18] Based on their results, it is considered that [^{3}H]PDBu would bind only to the C1B domain under our binding assay conditions, suggesting that only the binding affinity to the C1B domain of PKCα can be estimated by this assay. Therefore, we believe that **2a** and **2b** may bind preferentially to the

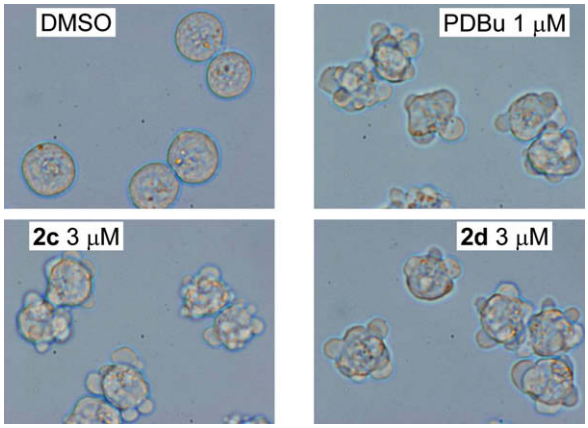


Figure 3. Bleb-forming of K562 cells induced by PKCα activators.

C1A domain and thus can activate PKCα even at concentrations at which negligible binding was observed in the binding assay.

Although **2d** having the two branched side chains was the strongest binder to PKCα, the binding affinity of **2e** (Clog$P$ = 8.67) to PKCα was dramatically lower than that of **2d**, and the K_i value is 704 nM.[19] Moreover, induction of phosphorylation activity of PKCα by **2e** was also decreased, and the relative activity was about 60% even at 100 μM. These facts indicate that the lactone carbonyl group is also essential for strong binding and activation of PKCα.[20,21]

Finally, the activity of these isobenzofuranone derivatives was evaluated at the cellular level by using the bleb-forming assay of K562 cells.[22] As reported, typical bleb formation was observed on treatment of K562 cells with PDBu (1 μM, Fig. 3). As expected, the strong ligands **2c** and **2d** caused similar phenotypic change at 3 μM, whereas **2a**, **2b**, and **2e** were inactive even at 10 μM. In case of **2a** and **2b**, strong activation of PKCα was observed at this concentration, but low membrane permeability or instability of the linear ester group under biological conditions might be a reason for the ineffectiveness of these compounds at cell level.

In conclusion, we have developed an asymmetric synthetic route to isobenzofuranone derivatives. Among them, the derivatives **2c** and **2d,** having a branched acyl side chain, were found to be affinity ligands of PKCα at low nanomolar concentrations. Activation of PKCα induced by these derivatives was also evaluated, and the results indicated the importance of a straight alkyl chain for strong activation of PKCα. The lactone carbonyl group was also important. Moreover, **2c** and **2d** showed remarkable bleb-forming activity in K562 cells, suggesting that these derivatives may be useful as cell-permeable DAG analogues for research at the cellular level. Further study on the biological activities of these derivatives is under way.

Acknowledgments

We thank Ms. Rie Onose, Dr. Hideaki Kakeya, and Professor Hiroyuki Osada (RIKEN) for technical advice about bleb-forming assay.

References and notes

1. (a) Nishizuka, Y. *Science* **1992**, *258*, 607–614; (b) Yang, C.; Kazanietz, M. G. *Trends Pharmacol. Sci.* **2003**, *24*, 602–608; (c) Newton, A. C. *Chem. Rev.* **2001**, *101*, 2353–2364.
2. (a) Kishi, Y.; Rando, R. R. *Acc. Chem. Res.* **1998**, *31*, 163–172; (b) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. *J. Biol. Chem.* **1982**, *257*, 7847–7851; (c) Fujiki, H.; Tanaka, Y.; Miyake, R.; Kikkawa, U.; Nishizuka, Y.; Sugimura, T. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 339–343; (d) Smith, J. B.; Smith, L.; Pettit, G. R. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 939–945; (e) Berkow, R. L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109–1116.
3. Teicher, B. A. *Clin. Cancer Res.* **2006**, *12*, 5336–5345.
4. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.
5. Selected recent reports: (a) Yanagita, R. C.; Nakagawa, Y.; Yamanaka, N.; Kashiwagi, K.; Saito, N.; Irie, K. *J. Med. Chem.* **2008**, *51*, 46–56; (b) Wender, P. A.; Verma, V. A. *Org. Lett.* **2008**, *10*, 3331–3334; (c) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *J. Am. Chem. Soc.* **2008**, *130*, 6660–6661; (d) Mach, U. R.; Lewin, N. E.; Blumberg, P. M.; Kozikowski, A. P. *ChemMedChem* **2006**, *1*, 307–314; (e) Bertolini, T. M.; Giorgione, J.; Harvey, D. F.; Newton, A. C. *J. Org. Chem.* **2003**, *68*, 5028–5036.
6. Marquez, V. E.; Blumberg, P. M. *Acc. Chem. Res.* **2003**, *36*, 434–443.
7. Selected recent reports: (a) El Kazzouli, S.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2008**, *51*, 5371–5386. and references therein; (b) Lee, J.; Lee, J.-H.; Kim, S. Y.; Perry, N. A.; Lewin, N. E.; Ayres, J. A.; Blumberg, P. M. *Bioorg. Med. Chem.* **2006**, *14*, 2022–2031.
8. (a) Baba, Y.; Ogoshi, Y.; Hirai, G.; Yanagisawa, T.; Nagamatsu, K.; Mayumi, S.; Hashimoto, Y.; Sodeoka, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2963–2967; (b) Baba, Y.; Mayumi, S.; Hirai, G.; Kawasaki, H.; Ogoshi, Y.; Yanagisawa, T.; Hashimoto, Y.; Sodeoka, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2969–2972; (c) Hirai, G.; Shimizu, T.; Watanabe, T.; Ogoshi, Y.; Ohkubo, M.; Sodeoka, M. *ChemMedChem* **2007**, *2*, 1006–1009.
9. Marquez and co-workers reported that DAG and DAG–lactone derivatives with bulky hydrophobic substituents were potent ligands with low nanomolar binding affinity. See: (a) Nacro, K.; Sigano, D. M.; Yan, S.; Nicklaus, M. C.; Pearce, L. L.; Lewin, N. E.; Garfield, S. H.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2001**, *44*, 1892–1904; (b) Nacro, K.; Bienfait, B.; Lee, J.; Han, K.-C.; Kang, J.-H.; Benzaria, S.; Lewin, N. E.; Bhattacharyya, D. K.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2000**, *43*, 921–944.
10. Wang, Z.-M.; Sharpless, K. B. *Synlett* **1993**, 603–604.
11. Bennani, Y. L.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 2079–2082.
12. A carboxymethyl group at the *ortho*-position did not disturb asymmetric dihydroxylation: Ohzeki, T.; Mori, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2240–2244; Moderate ee was observed in the case of a highly substituted styrene derivative: Ohzeki, T.; Mori, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2584–2590.
13. (a) Uemura, M.; Tokuyama, S.; Sakan, T. *Chem. Lett.* **1975**, 1195–1198; (b) Trost, B. M.; Rivers, G. T.; Gold, J. M. *J. Org. Chem.* **1980**, *45*, 1835–1838.
14. Olah, G. A.; Wu, A.; Farooq, O. *Synthesis* **1989**, 566–567.
15. The complete characterization for all new compounds has been done using NMR and MALDI-TOFMS.
16. Assay protocol is described in Ref. 8c.
17. Tanaka, Y.; Miyake, R.; Kikkawa, U.; Nishizuka, Y. *J. Biochem.* **1986**, *99*, 257–261.
18. Ananthanarayanan, B.; Stahelin, R. V.; Digman, M. A.; Cho, W. *J. Biol. Chem.* **2003**, *278*, 46886–46894.
19. This K_i value was determined in a different assay system: Lewin, N. E.; Blumberg, P. M. *Methods Mol. Biol.* **2003**, *233*, 129–156. The K_i value of **2d** determined with this system was 11.2 nM.
20. There are two possible reasons for the observed low affinity of **2e** to PKCα. Based on our proposed binding model (Fig. 1C), the lactone carbonyl group would not contribute to the direct interaction with PKCα C1B domain; however, this carbonyl group may participate in the interaction with a head group of the phospholipids (PS) and contributes to the binding through stabilization of the ligand–PKC–PS complex.[21] Another possibility is that **2d** has an alternative binding mode, in which the lactone carbonyl group instead of the side chain carbonyl group makes critical hydrogen bond with Gly124. If this is the case, the hydrogen bonding with Gly124 would be missing in the compound **2e** causing drastic decrease in affinity to PKCα.
21. The importance of the both carbonyl groups in DAG–lactone was discussed in the following literature: Kang, J.-H.; Peach, M. L.; Pu, Y.; Lewin, N. E.; Nicklaus, M. C.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2005**, *48*, 5738–5748.
22. Osada, H.; Magae, J.; Watanabe, C.; Isono, K. *J. Antibiot.* **1988**, *41*, 925–931.